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Linking Our Origins to Our Future

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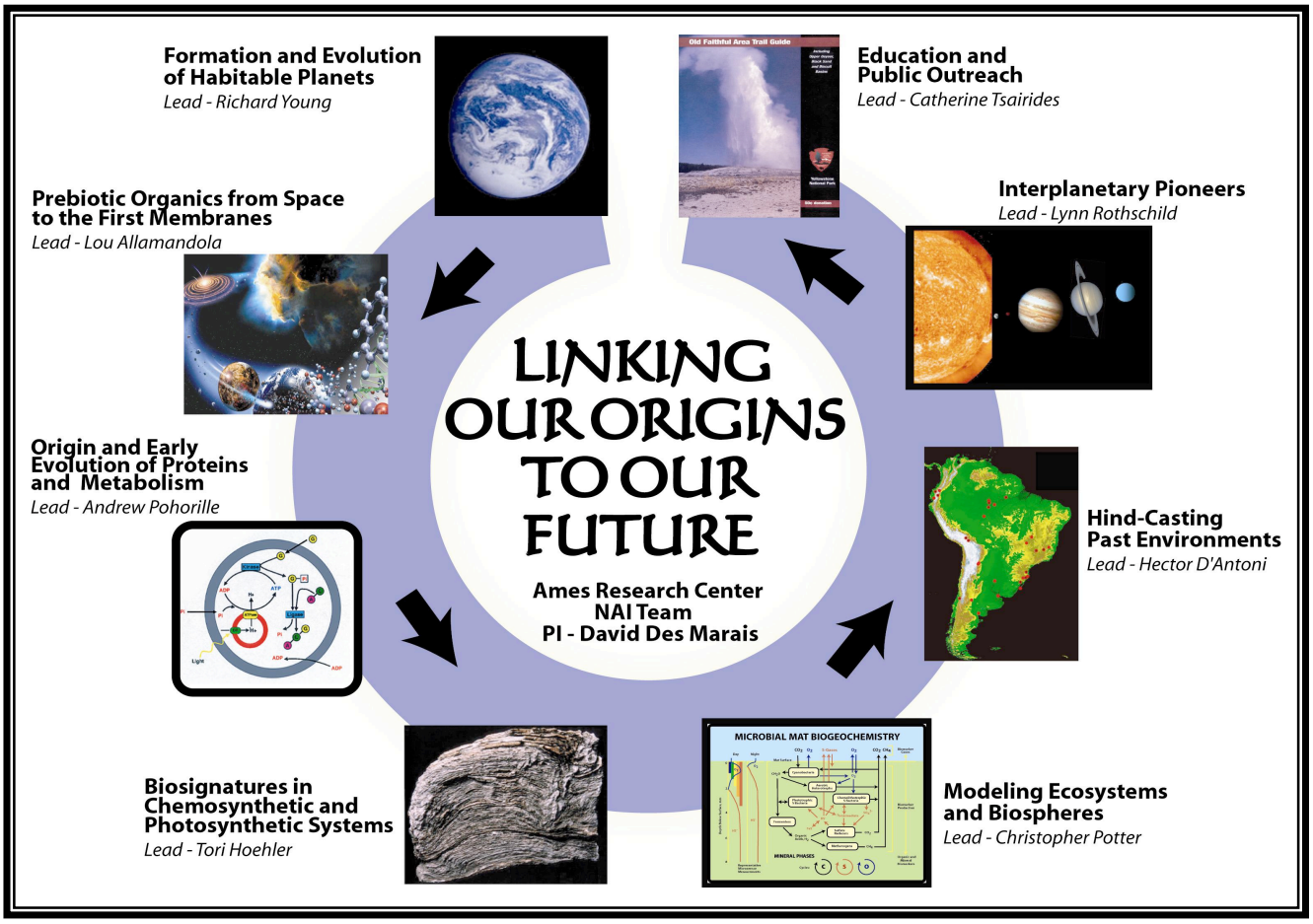

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1.0 EXECUTIVE SUMMARY

Ames Research Center proposes a coordinated program that integrates a broad, interdisciplinary investigation of the origins, evolution, and future of habitable environments and life, with a parallel, high-impact, education and public outreach effort. Tracing a path from interstellar materials to inhabited worlds and beyond, our research will link together investigations of the formation, evolution, and climates of habitable planets; the roles of interstellar chemistry in supplying potential biological precursors to these worlds; the origins and nature of metabolism in the first cells; the impact of established biospheres on planetary chemistry and climate, emphasizing the formation of detectable biosignatures; the response of vegetation to regional climate change; and the potential for life to transcend planetary boundaries through transfer between habitable worlds. Our program for education and public outreach captures these themes and builds around the expertise and enthusiasm of the Ames team to develop an engaging and informative package that will be disseminated to national- and international-scale audiences. This will be achieved through partnerships with the California Academy of Sciences, Yellowstone National Park, New York Hall of Science, and several K-14 educational organizations. Strong conceptual and functional links to multiple NASA missions provide context, motivation, and funding leverage for our research component, along with resource- and audience-sharing opportunities for our education and public outreach component. The proposed program is highly relevant to the goals of the NASA Astrobiology Institute in terms of basic science, mission support, training, education, and public outreach as specified by the goals and objectives of the recently updated Astrobiology Roadmap and NASA Strategic Plan.

The proposed research organizes multiple disciplines into complementary lines of investigation designed to understand the *context for life*, the *origins of life and its impact on the planetary environment*, and the *future of life in changing environments*. The research is broad-based, addressing *all seven goals of the new Astrobiology Roadmap,* and is formulated to address specific near-term objectives in the Roadmap in ways that link these objectives and help to unify astrobiology.

We begin with a multifaceted investigation of the formation, evolution, and climatology of habitable planets. Because extrasolar planets that host surface biospheres are the most likely to be detected by remote spectroscopic search, we focus on terrestrial (rocky) planets where liquid water is stable at the surface, and examine a critical subset of the processes that affect planetary habitability. The research objectives are to understand: how protoplanetary disks evolve and form terrestrial planets; what kinds of planetary systems are likely to harbor terrestrial planets; how volatiles are delivered to terrestrial planets by impacting planetesimals, and how impacts affect the climatology of terrestrial planets; the particular evolutionary paths of terrestrial planets that result in habitability; and how external characteristics, such as orbital eccentricity, and internal factors, such as atmospheric circulation, affect the habitability of terrestrial planets.

We will link these studies of habitability to two major initiatives in prebiotic organic cosmochemistry. Building on our previous NAI-sponsored work that demonstrated the formation of complex and potentially protobiological organics under simulated interstellar conditions, we propose to trace, spectroscopically and chemically, the cosmic evolution of organic molecules from the interstellar medium to protoplanetary disks, planetesimals, and finally onto habitable bodies. We also propose to examine the abiotic mechanisms of primitive membrane formation under the primordial conditions of a habitable planet. Both initiatives will couple spectral and chemical studies of laboratory simulations with astronomical observations and analyses of meteorites and comet dust returned by the Stardust mission.

A third facet of our investigation will address the origin of metabolism in the earliest ancestors of cells by testing the hypothesis that proteins might have arisen and initially evolved in the absence of a genome. In our prior NAI-sponsored research, we selected for the first time a functional protein from a library of random amino acid sequences using a novel *in vitro* evolution technique. We propose to evolve several proteins capable of performing functions that might have been important for early metabolism, such as synthesis of biopolymers and transport of ions across membranous cell walls. We will also estimate the frequency of finding a functional prebiotic protein among random protein sequences that might have formed spontaneously. On the basis of these experiments, we will examine the evolutionary potential of an ensemble of proteins through theoretical and computational modeling.

A fourth element of our study focuses on how to detect life once it has taken hold on a planet, by characterizing the major factors that govern the formation of potentially diagnostic biosignatures in microbial ecosystems. Two ecosystem types will be studied for their particular relevance to astrobiological searches for life (e.g., via Mars or Terrestrial Planet Finder (TPF) missions, respectively): rock-hosted ecosystems in ophiolite springs, as a potential analog for past or present

subsurface life within our solar system, and photosynthetic microbial mats, as the type of biosphere that could be remotely detected on extrasolar planets. Work in the springs will examine how microorganisms might leave residual biosignatures by affecting the formation of aqueous alteration minerals, and how biological energy requirements define an "energetically habitable zone" for chemotrophic life. Studies of microbial mats will focus on elucidating the pathways by which photosynthetic productivity is transformed into volatile biosignatures that could be distinguished in the atmospheres of distant planets.

We will extend the ecosystem-level studies of photosynthetic microbial mats to a planetary scale by refining and evaluating quantitative models that simulate energy relationships, biogeochemical cycling, trace gas exchange, and biodiversity in these systems. Global-scale fluxes of O_2, CO_2, CH_4, H_2S, and other reduced species will be estimated by combining algorithms for the production of trace gas biosignatures with process-level metabolic information from model simulation runs. The ability of the model to explore scenarios and assess implications of experimental findings will complement the field-based measurements, and will begin to assess the consequences of billions of years of ecological change driven by environmental forcing.

We will examine the effects of climate variability on a vegetation-rich biosphere over intermediate time scales, using South American ecosystems as a model. Our past NAI-funded work demonstrated a strong correlation between vegetation changes at 32 South American sites and variations in sea-surface temperature over a 12-year period, related to the El Niño Southern Oscillation (ENSO). We propose to continue this analysis using information from the MODIS and ETM+ sensors onboard more recent satellites, and to use it to predict backward, or hindcast, vegetation assemblages. We will incorporate fossil pollen profiles to extend this hindcasting back 15,000 years, and to assess whether ENSO has caused previously unknown changes in vegetation communities.

Finally, we will assess the potential for life to move beyond its planet of origin, as a potentially important component in the evolution of life in our own solar system. We propose to address natural transport, such as on a meteorite, where survivors must withstand radiation, desiccation, and time in transit. We will identify organisms and ecosystems that are likely to withstand such rigors, and examine the mechanisms for their survival in laboratory experiments and in a space simulator. We will fly these organisms and ecosystems in low Earth orbit (e.g., ESA's EXPOSE facility on the ISS) to test their resistance to the space environment.

The strong mission relevance of our proposal, combined with the direct participation of several team members in the planning and execution of NASA missions, places the Ames team in a position to influence strongly the astrobiology content of ongoing and future missions. Studies of planet formation and habitability will benefit the SIRTF, Kepler, Eddington and TPF missions. Studies of cosmic ices and organics will be synergistic with SOFIA, the Stardust mission, and the proposed Astrobiology Explorer (ABE). Studies of microbial biosignatures will benefit MER, future Mars missions, and TPF. Five of the investigators on this proposal serve as PI or CoIs on NASA missions that could reach fruition during the span of this proposal, and seven are involved in instrument development and observing strategies.

We will continue to serve the needs and interests of the nation's educators, students and public through a high-impact education and public outreach program. Specifically, we propose to partner with the California Academy of Sciences (CAS), Yellowstone National Park (YNP), and the New York Hall of Science to develop new astrobiology workshops, activities, exhibits, and other products for the public. CAS has chosen to utilize astrobiology to link its natural history museum, planetarium, and aquarium under the theme, "Earth and its Place in the Universe." As part of our partnership, Ames personnel will serve directly on the CAS design and exhibit development teams. Ames and CAS will also facilitate interactions between researchers and educators in order to develop inquiry-based programs and activities for K-14 students. Our partnership with YNP, which began as part of our previous NAI work, combines a large annual visitation with a highly effective venue for conveying astrobiology-related content. Specifically, the thermal springs that abound in YNP support concepts related to the early evolution of life on Earth, and to the search for evidence of Martian habitability or inhabitance. With material input from the Ames team, YNP will introduce astrobiology content into trailside interpretive signs, brochures, and the Yellowstone Resources and Issues Guide. Ultimately, astrobiology will be integrated into permanent exhibits for the major visitor centers. By harnessing the E/PO expertise and resources of these organizations, and by accessing the large and diverse audiences they draw, Ames is poised to engage a truly broad cross-section of the public in astrobiology outreach activities. We will extend this impact to the professional level by engaging graduate students and postdoctoral associates in the proposed research activities, through the teaching of undergraduate courses in astrobiology at

Stanford University and local community colleges, and through the sponsorship of workshops that will help introduce student researchers to astrobiology.

The Ames team combines a wealth of experience in astrobiology research and outreach activities with an unusually high degree of functional involvement in NASA missions, ongoing and planned collaborations in NAI focus groups, and a strong institutional backing that leave us well suited to contribute as integral and vital members of the NAI. During the previous term of funding, the Ames team made important advances in each of the research areas proposed here, generating more than 200 peer-reviewed research articles. The proposed efforts build on these successes and will thus be leveraged in a conceptual sense. Through their roles as members of NASA committees, as leaders in professional journals and societies, and as pioneers in astrobiology-related educational and training activities, Ames team members are also poised to broaden the impact of astrobiology in the professional community and to engage the next generation of researchers that are so critical to the long-tem prosperity of the field. Similarly, the national attention drawn by the success of our existing education and outreach program has enabled us to engage and partner with high visibility organizations such as CAS and YNP, which will afford an opportunity to bring astrobiology to a truly broad and diverse public audience.

Our proposed research and E/PO efforts are strongly supported by the facilities, programs, and direct contributions offered by Ames Research Center. Ames will contribute six work-years of civil service (CS) time, as well as CS travel support, to each year of this proposed work. The direct contribution of civil service salaries and travel support exceeds $4 million over the proposed performance period. In further support of this and related research, the Astrobiology and Space Research Directorate will add one permanent staff position during the proposed grant period. Major laboratory complexes for astrochemistry, biogeochemistry and electron microscopy, plus several smaller laboratories, will be provided for the research. Ames also maintains the largest computational facilities devoted to NASA research, facilities that will directly support the proposed theoretical research in astronomy, planetary science and molecular biology. Interactions with the Ames Education Office will enrich the team's education and public outreach efforts. Our program is also leveraged financially by individual commitments from Ames team members, who are variously contributing unsalaried time, resources and technical support derived from complementary funded projects, and research infrastructure developed with previous NAI and other funding.

The interconnected nature of the Ames team as a whole will benefit from a management structure and style that is informed by our previous NAI experience. The Principal Investigator will chair a steering committee comprised of the lead Co-Investigators who will supervise each of the seven investigations and the E/PO program. Monthly team meetings and periodic workshops will further integrate the effort and embrace the lessons learned during prior NAI experience. For example, monthly presentations of biological themes will be paired with those of related astrochemical or planetary themes. Also, discussions of science tasks will be paired with those related to training, education, and public outreach, thus further strengthening team collaboration. We will link to the broader research community through our proposed planetary science workshops and training opportunities.

We have participated actively in NAI focus groups, and will continue to do so in the next round of membership. For example, Investigations 1 and 2 will work with the proposed Astronomy and Astronomical Biosignatures focus groups. Investigations 4 and 5 will participate in the Mars, Ecogenomics, and proposed Astronomical Biosignatures focus groups. Investigations 6 and 7 will work with other NAI teams to develop groups addressing the future of life. Through these interactions with other NAI member institutions, we will continue our role in helping to forge the interdisciplinary connections that will enhance the overall viability and impact of the Institute.

3.0 RESEARCH AND MANAGEMENT PLAN

Introduction and Approach

Background

Ames Research Center proposes a broad interdisciplinary effort to understand life and habitable planetary environments in the context of their origins, evolution, and future. To encompass these themes fully, we must understand the functional links that draw a continuous thread through interstellar chemistry and physics, planet system formation, development of habitability, origins of metabolism, and evolution and persistence of life. The title of this proposal, "Linking our Origins to our Future", reflects our focus on elucidating these links. These links are further strengthened by conceptual connections and functional collaborations with other NAI members, involvement with other NASA research and analysis programs in Astrobiology, Exobiology, Solar System Exploration, Astrophysics, Earth Sciences, and Fundamental Biology, and strong associations with key astrobiology-related flight missions and technology development activities.

Research Overview

This section is organized around three scientific themes, dealing with the *Context for life*, the *Origins and early evolution of life and its biosignatures*, and the *Future of life in changing environments.* The research is broad, addressing *all seven goals of the new Astrobiology Roadmap,* but it is also focused: the research investigations address specific near-term objectives in the Roadmap in ways that strengthen the linkages between these objectives and thus help to unify the new discipline of astrobiology.

Context for life. The elemental building blocks that form the basis for life as we know it are common throughout the universe, but the occurrence of conditions suitable for the origins and sustenance of life is much less common and therefore might greatly limit the distribution of life. Any assessment of the universality of life must therefore include an analysis of the factors that allow life to begin and continue, hence *the context for life.* This research broadly addresses Goal 1 of the new Astrobiology Roadmap, "Understand the nature and distribution of habitable environments in the Universe." In support of Goal 3, "Understand how life emerges from cosmic and planetary precursors," this research also addresses Objective 3.1, "Characterize the cosmic and endogenous sources of matter…for potentially habitable environments..."

Investigation 1 proposes a coordinated research program on the formation, evolution, and climatology of habitable planets. We seek to understand how protoplanetary disks evolve and form terrestrial planets and the kinds of planetary systems that are likely to harbor habitable worlds. We will further examine how particular variables that shape the climates of extrasolar terrestrial planets might affect their habitability, using Mars, Venus, and Earth as model systems.

Investigation 2 proposes initiatives in prebiotic, organic cosmochemistry, work that is intimately bound with *Investigation 1*. We will trace, spectroscopically and chemically, the cosmic evolution of organic molecules from the interstellar medium to protoplanetary disks, planetesimals, and finally onto habitable bodies. We will explore primitive, abiotic membrane formation under the primordial conditions of a habitable planet. Both initiatives will rely on spectral and chemical studies in the laboratory informed by astronomical observations and extraterrestrial sample analysis of meteorites and comet dust returned by the Stardust mission.

These investigations will be coordinated in ways that are both novel and highly significant. For example, the dynamics of the protoplanetary disk are rigorously determined by the chemical composition of the gas. Much of the gas in the nebula is produced initially from the evaporation of the feedstock interstellar ice that forms the protoplanetary disk. Methanol, not methane, has been observed to be the most abundant simple carbon compound frozen in these ices, and also in the warm gas characteristic of star forming regions. This makes an enormous difference in the dynamical and chemical nature of nebulae, a feature that can be captured in the dynamical models of *Investigation 1* as the chemical simulations of *Investigation 2* progress.

Origins and early evolution of life and its biosignatures. The actual origins of life on Earth remain elusive, but assessments of potential mechanisms for creating catalytic and genetic functions may help to constrain this process. We can ultimately help to constrain the timing of life's origins on Earth, and its frequency in nearby planetary systems, by learning to recognize biosignatures that are discernable in deep time and space. Our work in these areas addresses Astrobiology Roadmap Objective 3.2, "Origins and evolution of functional biomolecules," Objective 6.1, "Environmental changes and the cycling of elements by the biota, communities, and ecosystems," and all of Goal 7, "Determine how to recognize signatures of life on other worlds and on early Earth":

Investigation 3 addresses the origins of metabolism in the earliest ancestors of cells by testing the hypothesis that proteins might have arisen and initially evolved in the absence of a genome. We will evolve several proteins capable

of performing functions that might have been important for early metabolism, such as synthesis of biopolymers and transport of ions across membranous cell walls. We will estimate the frequency of finding a functional prebiotic protein among random spontaneously formed protein sequences. We will examine the evolutionary potential of an ensemble of proteins using theoretical and computational modeling techniques. Conceptually, this investigation continues the line of research in *Investigation 2* by aiming to explain how biological precursors of life could be utilized in the formation of living systems.

Investigation 4 seeks to characterize the major factors that govern the formation of potentially diagnostic biosignatures in microbial ecosystems. We will assess the energetic factors that constrain the occurrence of microbes in rock-hosted ecosystems, and assess the potential for preserving biosignatures within them through deposition of aqueous alteration minerals. We also will elucidate the pathways by which photosynthetic productivity is transformed into potentially useful biosignatures (e.g., biogenic gases in the atmosphere) in phototrophic microbial mat communities, and understand the controls thereon.

Investigation 5 will refine and evaluate numerical models that simulate energy relationships, biogeochemical cycling, trace gas exchange, and biodiversity in the same microbial mat ecosystems. The goal is to extrapolate biosignatures from early Earth ecosystems to the scale of a planetary biosphere and, ultimately, over time scales of billions of years.

Studies of biosignature gas formation in photosynthetic ecosystems in *Investigation 4* will yield a conceptual basis and experimental data for the model developed in *Investigation 5*. In turn, the model will ultimately help to scale the ecosystem-level measurements of *Investigation 4* up to planetary scales. *Investigation 4* will also develop the concept of "energetic habitability", which may help to refine our understanding of the *context for life.*

The future of life in changing environments. The final two investigations address Roadmap Goal 6, "Understand the principles that will shape the future of life, both on Earth and beyond." Patterns observed in terrestrial ecosystems during recent millennia can offer clues regarding future ecosystem responses to change. The survival of microbial life as it moves beyond its planet of origin is relevant both to future scenarios of human exploration and to natural interplanetary transfers of life throughout solar system history.

Investigation 6 will examine fossil pollen and historical sea surface temperatures in order to define some of the environmental drivers that shaped extant ecosystems in the past, as a basis for understanding potential future changes. This study will discern whether the El Niño Southern Oscillation caused some past, previously unknown changes in plant communities. Similar changes might be anticipated in the future.

Investigation 7 considers how terrestrial life might survive in space during interplanetary transport. In space, biota will encounter environments with increased radiation and very low water potentials. *Investigation 7* proposes to study how these factors affect selected biological systems. The findings will be used to create descriptive and predictive ecological models for life under a range of extraterrestrial conditions.

3.1 Investigation 1 Formation and Evolution of Habitable Planets

3.1.1 Objectives and Significance of Research

Central to the context for life is the formation and evolution of habitable planets. Here we define habitable planet in the "classical" sense, meaning a planet with an atmosphere having liquid water on the surface. This is appropriate, because extrasolar planets on which liquid water and life are present at the surface should be observable spectroscopically in a search for evidence of life (Leger, 1993; Angel & Woolf, 1996), whereas subsurface biospheres may not be detectable.

The study of habitable planets directly addresses goals and objectives in the Astrobiology Roadmap (see Section 3.1.3), and is especially timely now. From ground based observations over 100 extrasolar planets have been discovered to date. It is now known that extrasolar planetary systems exist, as opposed to single planets. Within a few years space based missions using transit photometry, such as the Kepler Mission to be launched in 2007, will show whether terrestrial type (predominantly rocky), in addition to jovian type (gas giant), planets are ubiquitous in extrasolar planetary systems. Furthermore, for all detected planets including terrestrial-sized planets, the transit observations will allow determination of planet radius, orbital semi-major axis, and under the most optimal circumstances, orbital eccentricity. An additional important scientific objective of the Kepler Mission is to determine whether a detected planet is within the habitable zone of the parent star, where habitable zone is defined as that region of space surrounding a star

within which liquid water could exist at a planet's surface.

Well founded theoretical models now exist regarding planet formation, protoplanetary nebula evolution, and planetary system dynamics. There has also been progress in understanding the factors important for determining the habitable zone around a particular star. Thus, for the first time, there is now the opportunity to meaningfully combine observational and theoretical efforts in order to quantitatively evaluate the frequency and characteristics of extrasolar planets, and to evaluate whether or not they are habitable. A major objective of the proposed work is to conduct theoretical studies of terrestrial planets which will be directly relevant to the planning of, and scientific data interpretation for, missions such as Kepler and more advanced future missions, such as the planned Terrestrial Planet Finder Mission (TPF), which will be capable of obtaining spectral information of the atmospheres.

Habitable planets, in the sense we have defined habitable planets, must be terrestrial planets. We propose a multifaceted, interconnected research program that addresses the formation, evolution, and climatology of terrestrial type planets, including terrestrial planets in our own Solar System, since they provide some guidance for understanding extrasolar terrestrial planets. Obviously we cannot resolve all the questions in their entirety. Instead, the goal is to identify particular key questions, and address those.

Figure 3.1-1 illustrates that the unifying theme of the proposed tasks is planet habitability. There is not necessarily a direct link between each of the processes we propose to address (e.g. protoplanetary disk processes, Section 3.1.2.1, are not directly linked to terrestrial planet climatology, sections 3.1.2.3-5), however, each crucially affects planet habitability. As an example, from only a climatological perspective, solar type stars would be expected to be associated with habitable planets. However if the star formed in a stellar cluster, as many stars do, it is quite possible that the solar star's protoplanetary disk did not last long enough for planets to form at all. Since the density of suitable candidate stars in the Sun's vicinity is an important driver of the TPF mission design, considering just climatological criteria in a search strategy for habitable planets would not be prudent. Elements of the proposed research are relevant to several existing NASA and NSF R&A programs and would complement research being done under those programs. However, the research proposed here is beyond the scope of any single R&A program, and is beyond the interest of any group of R&A programs to coordinate. Hence, the research belongs within the purview of the Astrobiology Institute.

3.1.2 Research Tasks

3.1.2.1 Effects of protoplanetary disk processes on formation of habitable planets

Build On and Extend State of Knowledge

Protoplanetary disk lifetime, and the dynamical and chemical evolution of the disk, control the planetary incubation phase and planet composition, and therefore place constraints on planet formation and the delivery of key volatiles such as water. Without considering these processes, we could be misled into thinking that certain stars had a habitable zone, when in fact disk processes precluded the existence of habitable terrestrial planets.

It is critical to understand disk evaporation rates in the planet-forming region (<30 AU). The presence or absence of significant quantities of gas there has major implications for planet formation and dynamics including: (i) the ability of gas giants to accrete onto rocky cores over ‰ 10^7 years (Pollack et al. 1996; Ikoma et al. 2000); (ii) the extent of planet migration (Lin & Papaloizou 1986; Ward 1997); (iii) the evolution of orbital eccentricities of planetesimals and planets (Tanaka & Ida 1997; Kominami & Ida 2002; Chiang et al. 2002; Goldreich & Sari, 2003). It will become apparent from the discussion in following sections, especially Section 3.1.2.2 which discusses orbital

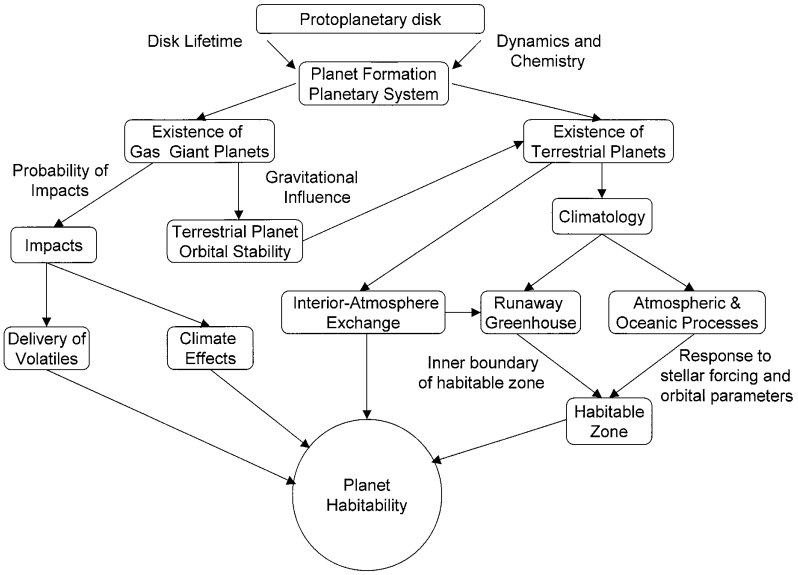


Figure 3.1-1. Illustration of how processes addressed in this proposal act to determine planet habitability. These processes are of course a subset of all processes that have to be ultimately considered, but they are critical ones.

stability and formation of terrestrial planets, and delivery of volatiles to terrestrial planets, that each of the above considerations is ultimately very important for habitable planets. While viscous accretion depletes the inner disk, protostellar winds and photoevaporation disperse the outer disk gas and dust back into the interstellar medium (ISM). Winds and photoevaporation set the ultimate time scale for disk dispersal (cf. Clarke et al. 2001). Beside planet formation, rapid disk dispersal of the outer disk, where H_2O ices form and accumulate into water rich planetesimals, will also affect delivery of volatiles to terrestrial planets (Hollenbach & Adams, 2003).

We propose to calculate the lifetime of protoplanetary disks as a function of stellar mass for stars spanning the entire initial stellar mass function (IMF) in order to evaluate the expected frequency of occurrence of habitable planetary systems. We will also study photoevaporative effects due to star formation in clusters, where most stars are born. This work will leverage from, and be complementary to, work being undertaken by one of us (D. Hollenbach) as part of a Legacy Proposal associated with the Space Infrared Telescope Facility (SIRTF) mission, currently scheduled to be launched in April 2003.

The physical state and chemical composition of forming planets is determined by dynamical and chemical evolution (especially of water) of the protoplanetary nebula. This process is always active in the inner disk and may co-exist with the photoevaporation processes discussed above (Clarke et al. 2001). Temperature and pressure conditions in the early nebula set the rate of chemical reactions, which determines the concentrations and state of water and other volatiles. If disk dynamical and chemical time constants are comparable, the dynamics and chemistry of the disk are tightly coupled to one another, forming a non-linear system that must be treated in a unified and consistent manner. In addition, dynamical accretion in the evolving disk induces spatially inhomogeneous chemical composition that can induce important radial reprocessing effects and species concentration.

Willacy et al. (1998) used a steady state disk model that essentially uncoupled the chemistry and the dynamics. (The chemical model was obtained from the widely used UMIST chemical database.) This work was extended by Willacy and Langer (2000) to include photoprocessing at the nebula margins. Aikawa et al. (1999) also used a steady state disk model and included inward (the only direction allowed by the model) accretion using a step-by-step solution of the chemical kinetic equations. Her model used a chemical database consisting of about 240 species integrated over a period of about 10^6 yrs during which the disk is assumed quiescent. Drouart et al. (1999) and Gail (2001) also investigate uncoupled chemical evolution using steady state disk dynamics. Bockelèe-Morvan et al. (2002) use a sequence of steady state models to study a related problem regarding the formation of silicates. Time dependent dynamics were considered in one recent paper (Wehrsted & Gail, 2002), but they also considered the evolution of silicate dust grains without including chemical reactions. They also used the conventional "alpha viscosity" model (Ruden & Lin, 1986, Ruden & Pollack, 1991) which is not as efficient as the recently proposed "beta viscosity" model of hydrodynamically generated turbulence (Richard & Zahn 1999, Hurè, Richard & Zahn, 2001). While all of these models have greatly contributed to a better understanding of disk dynamics and chemical evolution, none adequately treats the spatially inhomogeneous interactions between dynamics and chemistry. In this proposal, we will take advantage of new turbulence models to study coupled chemical/hydrodynamic processes. The coupled equations will be used to study the transformation of species as they migrate from grain-accreted states in the cold outer nebula to gaseous states in the inner nebula, or visa-versa if the unsteady accretion velocity changes sign.

It should be noted that the grain accreted states just mentioned are ices. These are ices whose IR spectra will be measured and characterized as described in Section 3.2, which deals with pre-biotic organics.

Technical Approach and Methodology

We propose to constrain the lifetime of protoplanetary disks focusing on stellar winds and photoevaporation. For dispersal by winds, we will apply the methods of Canto & Raga (1991) to the mass entrainment that occurs when a wind shears across the surface of disk gas. A mixing layer is created of outwardly flowing gas into which mass flux from the disk gas is inserted at some prescribed fraction of the sound speed, a number theoretically derived but constrained by laboratory experiments. We propose to perform a large parameter (gas surface density distribution, gas temperature distribution, wind mass loss rate and velocity) study to determine when, if ever, wind-stripping is important and how the wind affects the disk structure. This work includes shear physics, and is therefore much more realistic than previous work (e.g., Horedt 1982) which relied on crude energetic arguments and overestimated the dispersal.

In order to solve for photoevaporation at $r < r_g$, where r_g (about 30 AU) is the disk radius at which gas thermal speed equals escape speed from the system, we separate the hydrodynamics from the calculation of the temperature and chemical structure and iterate to achieve consistency. This

technique has been used successfully for photoevaporating clumps (Gorti & Hollenbach 2002). The gas is heated by energetic photons from the central star or a nearby massive star. We will use photodissociation region (PDR) models with both ultraviolet radiation and X-rays (e.g., Hollenbach & Tielens 1999) to calculate the chemical and thermal structure, and a 2-dimensional hydrodynamics code.

To address the dynamical and chemical evolution of the disk, we propose to develop a model that is able to fully couple the physical structure and chemical composition of the disk. We will combine recent work on nebula dynamics by Davis (2002a, 2002b, 2003) with enhanced understanding of mixing processes in the nebula by Richard and Zahn (1999) and Hurè, Richard and Zahn (2001) to produce an efficient nebula, or disk, dynamic transport model. D. Richard (currently at NASA Ames as an NRC Resident Research Associate) will be a key collaborator in developing the dynamical modeling. The chemical network will be similar to that used by Willacy et al. (1998) (see also Markwick et al. 2002). We will collaborate with S. Charnley (SETI Institute, Ames Space Sciences) and A. Markwick (at NASA Ames in 2003-2004 as an NRC Resident Research Associate) who both have extensive experience modeling cosmo- chemical processes. The chemical network will include gas phase two and three body reactions appropriate to the inner disk, and will be augmented with grain adsorption/desorption, and condensation physics models appropriate to the outer disk. A full range of reactions is necessary for an extended disk with extensive radial reprocessing. The particular chemical system we have implemented for our initial studies is a grain chemistry model originally used for molecular cloud physics (Brown & Charnley, 1990) consisting of 66 species and 705 reactions. The coupled chemistry and dynamics will be solved using methods appropriate to such "stiff" systems. In this manner we will be able to follow the coupled physical-chemical hydrodynamics over the full spatial-temporal envelope. An important component of this work will be to focus on chemical processing of water molecules.

3.1.2.2 Effects of planetary system characteristics on formation/existence of habitable planets

Build On and Extend State of Knowledge

The formation of habitable terrestrial planets, and the amount of volatile material they receive (see also Section 3.2), are mainly influenced by the nature of a star's protoplanetary disk, and the characteristics of the giant planets in the system (e.g. Chambers 2000a, 2000b; Jones et al. 2001).

Highly advanced astrobiology missions like TPF will be able to directly detect Earth-sized planets, but in order to efficiently do so, they will need to know where to look, and indeed, the density of suitable candidate stars in the Solar vicinity is a driver of the mission design.

The list of known planet-bearing stars provides an excellent source of candidates. We have unambiguous evidence that the conditions for planet formation were met in these systems, and the example of our own Solar System indicates that the formation of gas giants can be accompanied by the formation of terrestrial planets. One can then ask two interesting questions: First, how many of the known planetary systems admit habitable terrestrial planet orbits which are dynamically viable? Second, given our current understanding of terrestrial planet formation, in what fraction of the aggregate of known extrasolar planetary systems was the formation of habitable terrestrial planets possible? Definitive answers to these questions will require large-scale computation because in general, the gravitational N-body problem is analytically intractable.

To discover whether a particular planetary configuration is stable, one must integrate its equations of motion forward for a reasonable period of time (of order millions of years) and track the planetary excursions (Laughlin & Adams 1999; Rivera & Lissauer 2000; Jones et al. 2001; Marcy et al. 2002; Noble et al. 2002). When a particular system contains only one giant planet, this procedure is straightforward, albeit time-consuming. Menou and Tabachnik (2003), for example, report that time limitations prevented them from systematically evaluating the significant effects of observational uncertainty in the giant planet orbital elements.

When a system contains two or more giant planets, the evaluation of the stability of potential terrestrial planets becomes much more computationally arduous. Radial velocity observations cannot generally determine the planetary orbital inclinations, and so any evaluation of habitability must consider a wide range of mutual inclinations within each observed multiple-planet configuration. That is, for each individual system, one must build up a statistical portrait of likelihood which samples different orbital configurations for a putative habitable planet within the full range of giant planet architectures that are consistent with the observations. Laughlin et al. (2002) have computed the allowed range of mutual inclinations for the 2-planet 47 UMa system, Rivera and Lissauer (2001) have done this for the resonant pair of planets orbiting the star GJ 876, and Lissauer and Rivera (2001) and Chiang et al. (2001) have investigated the range of stable mutual inclinations which are supported by the 3-planet

Upsilon Andromedae system, but to date, the dynamical viability of habitable planets within these and all other multiple-planet systems is unknown when the possibility of mutual inclination is taken into account.

The stability of terrestrial planets in a particular exoplanetary system is a necessary but not sufficient condition for their existence. One must also evaluate whether a particular planetary architecture is likely to allow terrestrial planets to form in the first place. Terrestrial planets have mass and so they modify the strength and location of resonances associated with the giant planets, as Earth and Venus modify the ν_5 resonance in the Solar System (Namouni & Murray, 1999). During the late stages of planetary accretion, many planetary embryos with appreciable mass will be present. Embryos can gravitationally scatter one another into unstable resonance regions, and this greatly enhances the ability of resonances to remove material from a protoplanetary disk (Wetherill 1967). Chambers and Wetherill (2001) have shown that if planetary embryos formed in the asteroid belt of the Solar System, there is a two thirds probability that a combination of gravitational scattering and giant-planet resonances would have removed all embryos from the belt. Similarly, Laughlin et al. (2002) have shown that terrestrial planet formation is extremely unlikely within the habitable zone of the 47 UMa system, even though test-particle orbits within that system are highly stable. We propose to investigate these stability and formation questions using a large set of computations.

Considering now the delivery of volatiles to a planet, current models for protoplanetary disks and planetary accretion indicate that the inner planets of the Solar System formed in an environment that was initially very hot and dry (e.g. Bell et al. 1997). Observed water fractions suggest that solid materials forming in the protoplanetary nebula contained progressively less water with decreasing distance from the Sun. Apparently temperatures were never cold enough for water to condense in the terrestrial planet region and inner asteroid belt.

The two most likely external sources for Earth's water are comets and water-bearing bodies from the asteroid belt (such as the parent bodies of carbonaceous chondrites). Comets are unlikely to have contributed more than a small fraction of Earth's water for two reasons. First, the dynamical collision probability of a comet with Earth is too small, roughly 10^{-6} (Zahnle et al. 1998; Hartmann et al. 2000; Morbidelli et al. 2000). Second, cometary water has a D/H ratio that differs from terrestrial ocean water by a factor of 2 (cf. Lunine et al. 2000).

Conversely, water-bearing asteroids have much higher collision probabilities with Earth, together with a similar D/H ratio to terrestrial water. This suggests that material from the asteroid region was a more likely source of most of Earth's water than comets. Morbidelli et al. (2000) found there is a high probability that Earth could have accreted all its water in one or more collisions with planetary embryos from the asteroid region. Furthermore, Chambers and Wetherill (2001) have shown that the presence of planet embryos in the asteroid region provides a successful mechanism to explain the substantial mass depletion observed in the modern asteroid belt compared to other regions in the Solar System. The success of this model adds weight to the idea that most of Earth's water was delivered in the form of planetary embryos from the asteroid belt. The mass of volatile materials delivered to Earth depends sensitively on the giant planets' orbital eccentricities (Chambers & Cassen 2002), since these control the width and strength of the unstable resonances in the asteroid region. We propose to investigate this dependence in detail, building on the models developed by Morbidelli et al. (2000), Chambers and Wetherill (2001) and Chambers and Cassen (2002).

Technical Approach and Methodology

We propose to address the question of how many of the known planetary systems admit habitable planet orbits which are dynamically stable, as well as to address the question concerning in which systems terrestrial planets are likely to form. The evaluation of terrestrial planet formation and habitability are daunting computational tasks, but they have the advantage of being completely and immediately parallelizable. Vast numbers of individual configurations need to be separately and independently tested: these individual computations can be performed on individual computers. The proposed effort would be analogous to the SETI@Home Project, through which the University of California, Berkeley, SETI team has developed a successful program whereby an individual's home computer, when not in use by the individual, is utilized to analyze immense data sets obtained daily from the Arecibo radio telescope. We propose a similar sort of public program to run the computer codes. Individual simulations (either to evaluate dynamical stability or to test terrestrial planet accretion) involve extremely modest amounts of memory, and only a limited amount of bandwidth is required for transferring data. For both classes of simulations, we can use the public domain code "mercury.f" (Chambers 1999). This code uses the Wisdom-Holman symplectic map when planets or planetesimals are far apart (which allows very fast integration) and incorporates conventional integration to advance the perturbation terms of the Hamiltonian when bodies are close together. This code is very well tested and optimized.

The major task required to implement this project would be to compile mercury.f on various

architectures (i.e. Windows, Linux and the Macintosh OS). Users would then download the executable file appropriate to their own system. This mode of operation would be in the spirit of the SETI@Home Project.

For users who are interested in more interaction, we envision having a website (which could be linked to, or be an outgrowth of the www.transitsearch.org website) in which the integration properties of mercury.f are incorporated into a java code that also contains a detailed user interface. This interface would allow users to design their own habitable-planet configurations within the known planetary system architectures and test them for stability. The java code would likely be slower in performance, but would compensate with a great public outreach functionality.

With regard to volatile inventories of terrestrial planets, we propose to study how widely different the volatile inventories of terrestrial planets will be depending on the characteristics of giant planets in planetary systems. We will concentrate on cases where the giant planets orbit beyond the habitable zone, as in the Solar System. The experience of our own system shows that delivery of volatiles to terrestrial planets is most efficient if volatile bearing bodies exist on orbits interior to the giant planets. Hence, for planets in the habitable zone to receive significant amounts of water and other volatiles, the innermost giant probably needs to have periastron lying beyond 3 AU for a solar mass star, and certainly further out than dynamical-stability considerations alone would suggest. At present there are few if any good candidate extrasolar systems which satisfy this constraint. (Note that orbital eccentricities are not well determined in many cases, so more observations will be required to determine whether some systems could permit life-sustaining planets to form.) However, this situation is likely to change in the next few years, assuming such systems exist, because the time baseline of telescopic surveys for extrasolar planets will be extended.

The proposed work will follow two avenues, each consisting of numerical simulations of the formation of terrestrial planets in extrasolar systems. In each case, the rationale is to assume that giant planets with particular characteristics form in a system, and to then model the likely formation, stability and composition of terrestrial planets in that system, and the amount of volatile material that will be delivered to them. The first approach will make use of an N-body accretion code currently being developed by Chambers for use in a funded project to model the late stages of planetary accretion in the Solar System. A second approach will be based on the Öpik-Arnold scheme pioneered by Wetherill (1967) to model the accretion of the inner planets and asteroids in the Solar System. In the longer term, we propose to further develop the Öpik-Arnold scheme to provide a more realistic treatment of giant-planet perturbations and a crude treatment of secular interactions between planetary embryos (Chambers and Wetherill 1998 have shown that these can play a significant role during planetary accretion). This new code will straddle the gap between the modified Öpik-Arnold scheme and true N-body integrations, providing a more realistic, but fast, treatment of the dynamical evolution of planetary embryos.

Furthermore, we plan to incorporate advances in models for nebula chemistry and collisional fragmentation into our simulations. As discussed previously, understanding the thermal and chemical evolution of the nebula is vital since these determine the composition of planetesimals as a function of location in the nebula.

One of us (D. Hollenbach) is the director of the Center for Star Formation Studies, a very successful NASA-funded group since 1985 of astrophysicists from the University of California at Berkeley, the University of California at Santa Cruz, and NASA Ames. The Center studies star and planet formation. We propose to initiate a series of one-week biennial summer workshops focused on the research of our Ames astrobiology group. These workshops would bring together the diverse elements of the Ames group, the astrophysicists from the Center, and outside experts in the focused research areas of the Ames group. Both the Ames astrobiology group and the Center astrophysicists would benefit from this mutual interaction, which would stimulate new collaborations, more coherent interdisciplinary work, and cross fertilization of ideas.

3.1.2.3 Atmospheric and oceanic processes and habitable zone

Build On and Extend State of Knowledge

The circulation of, and physical process within, a planetary atmosphere (and ocean if there is one) can have considerable effect on a planet's climatology, and hence influence the extent of the habitable zone. Planets orbiting around cool (M type) stars provide an example where 3-dimensional (3-D) global atmospheric circulation enables a habitable zone to exist where 1-D models predict it would not (Joshi et al. 1997). This example illustrates the fact that a planet whose global mean surface temperature is below freezing might have regions where more clement conditions prevail that could support a regional habitat because of atmospheric circulation.

Furthermore, habitable zone studies have thus far been carried out using 1-D globally-averaged radiative-convective climate models (e.g., Kasting et al. 1993). The inner edge of the habitable zone is determined to a large extent by the loss of water

through photolysis and hydrogen escape. In 1-D models the relative humidity and lapse rates must be specified. In reality, however, the relative humidity, lapse rate, and other quantities are spatially varying and depend on dynamical processes that cannot be modeled in one dimension. We will mention such a situation in connection with the modeling of meridional heat transport as part of the study of potential habitability of planets in Venus-like orbits around stars similar to the Sun in Section 3.1.2.5.

Major advancement in our understanding of internal factors that determine the width of the habitable zone, such as the interaction of cloud microphysics and radiation with circulation, and understanding of external factors, such as parent stellar type and planet orbital characteristics (e.g., orbital eccentricity), will come from full 3-D general circulation models (GCMs) that incorporate important interactions. Computers, modeling algorithms, and analysis tools have advanced to the state where it makes sense to carry out simulations without many of the parameterizations and assumptions that have been used in the past.

Technical Approach and Methodology

We propose to apply a small suite of general circulation models (some available publicly for general scientific research, and one that has been developed on site at the NASA Ames Research Center) that are either simplified (although still 3-D) or fully complex, in order to investigate the habitability of planets. The choice of a particular model will depend on which is better suited for a given application. Members of the team have extensive experience and familiarity with the full suite of models. Our focus here will be on predominantly CO_2/H_2O atmospheres.

The most complex model is based on a new flexible version of the Ames Mars general circulation model (MGCM) (Pollack et al. 1990; Haberle et al. 2003) that has been vastly improved, and now includes a cloud microphysics scheme for both CO_2 and water clouds (Toon et al. 1988), and an updated and more generalized radiation code that can handle cloud multiple scattering in the presence of gaseous absorption for both solar and infrared parts of the spectrum (Toon et al. 1989). This model, for example, will be applied to the study of the climatological effects of impacts on Mars (see Section 3.1.2.4). The second model, which can be run at coarser spatial resolution and somewhat faster time integration because it is a spectral model, is the National Center for Atmospheric Research (NCAR) Community Climate System Model (CCSM) (Kiehl et al. 1996). The third model, ECBILT, (Haarsma et al. 1998) is a highly simplified and adaptive general circulation model that includes a realistic three-layer atmosphere, twelve-layer ocean and a sea-ice model, all with realistic heating in the solar and infrared, and heat exchanges between atmosphere and the underlying surface. The model, because of its simplified parameterizations and time splitting algorithms for the atmosphere and ocean, is very efficient for adaptation to investigate the climate system on long time scales.

Taken together, the models will complement each other, in that the CCSM and ECBILT models can be used relatively quickly to investigate a particular set of realistic climate configurations, while the modified Ames MGCM can be run at higher resolution with a more reasonable and complete physics package, corresponding to an extrasolar terrestrial-like atmosphere that can include, for example, radiative-cloud microphysical interactions which may turn out to be important. It is well known that clouds and their atmospheric radiative interactions are fundamental in understanding terrestrial climatology, but many aspects of cloud microphysics would be more parameterized in the CCSM. However in the MGCM, a detailed treatment of cloud microphysics could be run to corroborate the results of the CCSM, or to suggest how results might be modified.

We have conducted initial numerical experiments using the CCSM in which we have altered the planetary orbital setup (i.e., increased the eccentricity) for a present-day Earth in order to explore the range of seasonal/annual near-surface temperature extremes. Among the possible simulations, it is planned to continue such calculations using more realistic ocean models (our initial calculations used a present-day prescribed sea surface temperature (SST) climatology) so that realistic oceanic heat transport may occur. We also plan to investigate climate extremes using more simplified land-surface prescriptions (e.g., an aqua-planet or an aqua-planet with equatorially confined continents) to assess near-surface temperature extremes for different orbital configurations.

3.1.2.4 Role of impacts in modifying climate of terrestrial, and potentially habitable, planets

Build On and Extend State of Knowledge

Impacts of primitive bodies play an important role in delivering volatiles, such as water, to a planet which are required to make a terrestrial planet habitable. But impacts can also modify a planet's climate in other ways. It is well known that impacts have played a major role in affecting life on Earth, from the moon forming impact more than 4.5 billion years ago, to mass extinctions. Impacts must be considered as a process affecting the habitability of a planet.

As a case study, we propose to investigate the role of impacts in modifying the climate of another terrestrial planet in our solar system, Mars. Recent studies (Segura et al., 2002) of the effects of impacts on Martian climate indicate that, instead of a long lasting wet and warm climate, which has been presumed to have enabled the formation of Martian valley river networks, Mars was instead a cold and dry planet for which impacts were ultimately responsible for forming the observed surface river networks. In this case impacts bring life sustaining volatiles to a planet, but make it difficult for life to exist on the surface.

We envision two mechanisms for river formation. Due to the global distribution of the debris layers, we do not expect that rivers should be located near the craters of the objects that created the rivers. The first mechanism is rainout of the directly injected water from the cooling atmosphere. Global average precipitation rates will exceed 2 m/year depending on impactor size. Flooding and mudslides will reshape the landscape with eroded rivers containing high sediment loads as solids are broken apart by rushing water. The second mechanism is ground water release during the long periods of time the subsurface is above 273 K. Thermal heating from the hot ejecta layer will mobilize ground ice. During the long periods between impacts, vapor diffusion will recharge the groundwater aquifers. It has been suggested that most of the observed valleys were eroded by groundwater, but the absence of a recharge mechanism has frustrated this hypothesis. Impacts induce abundant precipitation that may recharge the aquifers.

Technical Approach and Methodology

The hypothesis that transient rains following impacts formed the Martian river valleys, rather than processes associated with a wet warm ancient Martian climate, has huge implications for life on Mars. The study described in Segura et al. (2002) has several aspects that deserve further work. An observable feature of repeated collisions of large objects should be the multiple ejecta blankets they may have emplaced. Numerous debris layers, contemporaneous with and intermixed with the valley networks, having an apparently subaerial mode of deposition have been identified by Mars Global Surveyor. These layers are precisely what would be expected from impacts of large enough size to generate the valleys through the mechanism we propose. Hence a test of our hypothesis would be careful comparisons of the ages of thick ejecta blankets and the initial formation times of the rivers.

We propose to better determine the origins of the layers of sub-aerial sediments on Mars. Are these from impacts as we hypothesize, or something else? We plan to work with Jen Heldman, a geology graduate student at University of Colorado on this issue. The goal of this project is relatively simple. We will expand on the work of Malin and Edgett (2000) using similar procedures. They reported extensive sub-aerial layers that were placed down at the same time as the Martian river valleys. We suspect these layers are the debris layers from the impacts that formed the craters visible on Mars today. If so, these layers should be present over much of the planet, and not be restricted to basins as would be water deposited layers. In addition the layer thickness should not be random, but instead should be related to the size of the crater and hence follow the impact crater frequency curves. Hence we will trace the layers over a wide range, and use their thickness to show they are derived from cratering.

Second, A. Colaprete and R. Haberle at NASA Ames are funded through the Mars Fundamental Research Program to simulate the aftermath of a large Martian impact with the Ames Mars General Circulation Model (MGCM) (see Section 3.1.2.3). We will collaborate with them, and provide quantitative estimates of the forcing mechanisms, i.e., atmospheric temperature fields, water vapor distribution, and surface temperature distribution as a result of the impact, after solid debris has fallen from the atmosphere. The issue that needs exploration with the MGCM is to understand the hydrological cycle in the aftermath of a large impact. Segura et al. (2002) used a one-dimensional model. Such a model is not adept at removing water vapor from the atmosphere, which is inherently a three-dimensional problem. Hence the one-dimensional model may not properly simulate the greenhouse warming by the water vapor, its latitudinal variations, and the cycling of water in the liquid phase over the planet. However at short times following the impact, horizontal variations in atmospheric properties are not likely to be great, and therefore the 1-D model can be used to provide starting conditions for the MGCM, and that is the procedure we will follow.

3.1.2.5 Runaway greenhouse and inner boundary of habitable zone

Build On and Extend State of Knowledge

The inner boundary of the habitable zone around a star effectively translates into the boundary, interior to which, a planet would experience a runaway greenhouse. This is a process in which there is sufficient positive feedback between planet surface temperature and radiative atmospheric opacity caused by water vapor abundance to produce very high surface temperatures. As more water vapor evaporates into the atmosphere because of increased surface temperature, atmospheric opacity increases, causing still higher surface temperatures, and so on. The surface temperature ultimately "runs away" to such extremely high temperatures that all surface liquid

water evaporates into the atmosphere, with subsequent loss of water from the planet. There is observational evidence for such an event on the terrestrial planet Venus (cf. Donahue et al., 1997). Kasting and Pollack (1983) showed that water could be lost from a planet when the stratosphere of the planet becomes saturated with respect to water vapor, which event occurs at stellar insolations smaller than required for the runaway greenhouse. However, such a planet could still have liquid water on the surface over a geologically significant time, and hence be considered habitable. We propose to study aspects of the runaway greenhouse effect that should give insight into this process in general by considering particular examples of the terrestrial planets in our Solar System.

One of the unexplored issues with Martian impacts, and with planetary formation, is the time scale over which runaway greenhouse atmospheres can occur. The energy input to Mars following a large impact is enough to put it into the runaway energy zone calculated with simple steady state models. Such models have been used to study the accretion of planets. However, Mars does not stay long in this state. How does the planet respond to such impulses? Clearly the planet will begin to runaway, but how long does the impulse have to last for the runaway to go to completion and does it ever sustain itself? The process was not allowed to begin in the simulations conducted by Segura et al. (2002), since the hydrological cycle was suppressed. Indeed the atmosphere plays little role in those calculations. We propose here to include the hydrological cycle in further calculations and to consider time dependent runaway conditions.

Satellite observations over the Earth's oceans may provide useful information on how clouds and atmospheric dynamics influence the greenhouse effect, and hence influence the extent of the habitable zone. Satellite observations over particular locales of the Pacific Ocean show that upwelling long-wave radiative flux at the top of the atmosphere initially increases with sea surface temperature (SST) (Rabbette et al. 2003 and references therein). However, at a certain SST the emitted long-wave flux reaches a maximum and then decreases sharply, coupled with a sharp cutoff to the maximum observed SST (Rabbette et al. 2003). The failure of the emitted long-wave flux to continually increase as SST increases is analogous to the signature of the runaway greenhouse (cf. Ingersoll, 1969; Nakajima et al. 1992; Kasting 1988). We have modeled this phenomenon, and have been able to show how increased atmospheric water vapor over the Pacific Ocean leads to the signature of the runaway greenhouse locally (Rabbette et al. 2003). We propose to further model and investigate this phenomenon in order to better understand the conditions under which a runaway greenhouse occurs, and to identify possible limiting mechanism that might exist, e.g. clouds. Ramanathan and Collins (1991) argue that formation of cirrus clouds is the limiting mechanism, but this view is not universally accepted (cf. Pierrehumbert, 1995).

Venus now lies outside the conventional habitable zone. If Earth were moved to the location of Venus it would experience the runaway greenhouse effect. The surface would become hot enough that carbonate rocks decrepitate and the atmosphere fills with CO_2. Eventually the hydrogen in the water vapor would escape to space, and a planet like Venus would result. It is useful to ask whether any planet suffering Venusian levels of insolation is in fact inevitably uninhabitable.

Venus at present has a surface temperature of 750 K, and is very dry. Yet its albedo is 78% and its effective temperature is actually lower than Earth's. The surface is hot because it is under 90 bars of CO_2. What is curious about the 90 bars is that the CO_2 partial pressure is not very far from equilibrium with carbonate rocks at the surface. $CO_2 + CaSiO_3 <-> CaCO_3 + SiO_2$.

This "Urey" reaction is crucial to regulating CO_2 on Earth. The equilibrium is temperature sensitive, such that at lower temperatures the carbonate rock is favored. If there were substantially less than 90 bars of CO_2 on Venus, the surface today would be cooler and carbonate rocks would be stable, hence most of the CO_2 would be stored as carbonate rock, and there would be a thin mostly nitrogen atmosphere.

Would liquid water be stable at the surface under these conditions? Quite possibly, yes. The runaway greenhouse threshold presumes that the atmosphere is saturated with water above oceans. If there is very little water, and the bulk of the water is cold-trapped in polar caps, it is unclear that the runaway greenhouse threshold should apply. The atmosphere would be dry if there is little open water. The generally very low relative humidity invalidates the assumption that the atmosphere's temperature is controlled by water condensation, and it also limits hydrogen escape. Such a planet would be habitable. We propose to investigate whether a planet in Venus' orbit could become habitable, even if the planet evolved through a runaway greenhouse period.

Technical Approach and Methodology

In order to model the runaway greenhouse associated with Martian impacts, we will use the one-dimensional model of Segura et al. (2002) for these simulations. We will allow clouds to form as water is slowly removed from the atmosphere. We will then allow water which is present on the ground to be recycled into the atmosphere. Much

more water should be placed into the air than is currently allowed in the model in which only impacted injected water is considered. Temperatures should then be hotter, and stay warm longer than in the current model.

With regard to the signature of the runaway greenhouse in the Pacific Ocean, we propose to include the radiative effects of clouds and aerosols in our radiative transfer model to assess how they affect the whole phenomenon, and specifically to assess whether clouds can act to limit the sea surface temperature (SST), as suggested by Ramanathan and Collins (1991). Another study will involve a set of computations to investigate the influence of CO_2 on the magnitude of SST near the SST where outgoing radiative flux at the top of the atmosphere is a maximum. Part of the motivation comes from the observation that maximum SST during the late Cretaceous and Eocene epochs appears to have been several degrees C higher than at present (Pearson et al. 2001), which Pearson et al. suggest may be related to increased levels of CO_2. And of course, CO_2 is now the predominant gas in the atmospheres of Venus and Mars. These studies can be accomplished using appropriate modifications to the radiative transfer code used in Rabbette et al. (2003).

Our proposed research regarding whether Venus might be habitable under different conditions is to investigate four questions. The first part is to understand how relative humidity affects the runaway greenhouse, since all previous studies assumed 100% relative humidity in the lower atmosphere. In this we will build on Nakajima et al. (1992), taking account of the above results of Rabbette et al. (2003) in which vertical distribution of water vapor at less than 100% relative humidity produces the runaway greenhouse signature. The second part is to link the planet's poles to the tropics and consider meridional heat transport. This will be a function of how thick the atmosphere is. At first we will use the simplest model possible to describe meridional transport of heat from the equator to the poles (cf. Gierasch & Toon, 1973). Later, we will make use of the habitable planet general circulation models discussed in Section 3.1.2.3, suitably adjusted to this case, to more accurately describe meridional heat transport. A third issue we will address is the cycling of CO_2 between the mantle and the surface — does mantle CO_2 tend to buffer the atmosphere near the Urey point? Can the mantle store excess CO_2, or must the planet be CO_2-poor from the beginning? In this we will expand on our own previous work (Sleep & Zahnle 2001). A fourth issue to address is the stability of hydrous minerals, and how these affect the budget of surface water, and whether cycling with the mantle is important for the water budget as well. We will then devise evolutionary trajectories for these planets.

3.1.3 Relevance of Proposed Work

The proposed research is directly relevant to Goal 1 given in the CAN and Astrobiology Roadmap, namely to understand habitable planets in the Universe. The proposed research addresses several of the issues described in the Roadmap, specifically, modeling the formation, evolution, and stability of planetary systems that might harbor habitable planets; the delivery of key volatiles, such as water, to potentially habitable planets; planetary processes which affect habitable conditions; the role of impacts on habitability. Portions of the proposed work dealing with the composition of the early atmospheres of Earth and an Earth-like Venus are also relevant for understanding spectra of extrasolar planets which will be obtained by advanced astrobiology missions such as Terrestrial Planet Finder (TPF).

The proposed research will benefit and enhance planned space based astronomical detection and observational astrobiology missions, such as Kepler, Eddington, and TPF, all of which have as goals the detection and characterization of terrestrial type extrasolar planets, and their classification as to habitability. Kepler and Eddinton are scheduled to be launched during the time period covered by this proposal, so the work proposed and its relevance to these missions is very timely.

3.1.4 Key Milestones

Year 1 – Theoretical estimates of disk masses around young stars, mass loss rates, wind velocities and ultraviolet fluxes (Hollenbach); work on developing dynamical and radiative transfer code that will be coupled to nebula chemistry (Davis, Richard); establish public computer network for orbital evolution simulations (Laughlin); begin comparison of ages of Martian ejecta blankets and river networks, and provide post impact initial conditions to climate models (Toon, Zahnle, Colaprete); model effects of relative humidity on runaway greenhouse (Rabbette, McKay, Young, Zahnle); apply CCSM and ECBILT models to study effects of planet orbital parameters (Hollingsworth).

Year 2 - Photoevaporation timescale of circumstellar disks; estimates of viscous accretion timescales and dispersal timescale by protostellar wind (Hollenbach); establish set of chemical reactions to be used in nebula dynamical-chemistry code (Davis, Charnley); implement "mercury.f" orbital dynamics code on public computer network and begin simulations of planet formation and orbital stability (Laughlin); apply Öpik-Arnold scheme to simulations of delivery of volatiles by planetesimals (Chambers, Lissauer); continue

comparison of ages of Martian ejecta blankets and river networks (Toon); incorporate and apply simple models of meridional heat transport to Venus-like planet simulations (Zahnle, Sleep, Toon); begin runaway greenhouse study of post impact conditions on Mars (Toon); investigate effects of clouds on local runaway greenhouse signature over Pacific Ocean (Rabbette, McKay, Young); continue application of CCSM and ECBILT models to study effects of planet orbital parameters (Hollingsworth).

Year 3 - Compute photoevaporation of disks as function of stellar mass and type for single stars spanning the entire initial stellar mass function (IMF) (Hollenbach); begin incorporating the sets of chemical reactions identified in year 2 into the dynamic/radiative code developed in year 1 (Davis, Richard, Charnley); continue simulations of terrestrial planet formation and orbital stability on public computer network established in year 1 (Laughlin); modify and improve Öpik-Arnold scheme to provide a more realistic treatment of giant-planet perturbations and a crude treatment of secular interactions between planetary embryos (Chambers, Lissauer); complete comparison of ages of Martian ejecta blankets and river networks, and continue runaway greenhouse simulations following Martian impacts (Toon, Zahnle); investigate effects of carbon dioxide levels on local runaway greenhouse signature over Pacific Ocean (Rabbette, McKay, Young); refine meridional heat transport calculations for Venus-like planet and begin CO_2 cycling study between mantle and surface (Zahnle, Sleep); compare MGCM with CCSM and ECBILT results for particular cases to assess importance and validity of model parameterizations (Hollingsworth, Young).

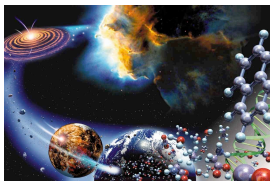
Year 4 - Calculate probability of stars forming in clusters, and probability of occurrence of high mass stars which emit large amounts of UV (Hollenbach); initiate application of coupled dynamical-chemical model to nebula simulations (Davis, Richard, Charnley); continue simulations of terrestrial planet formation and orbital stability on public computer network as number of observed planetary systems increases, and integrate results with Kepler Mission science team activities (Laughlin); apply improved Öpik-Arnold scheme to treatment of giant-planet perturbations and treatment of secular interactions between planetary embryos, and begin to incorporate results from coupled dynamic-chemistry nebula model into simulation of delivery of volatiles (Chambers, Lissauer, Davis); compute hydrogen escape rate for methane rich atmosphere in conjunction with evolution of early Earth's atmosphere (Toon, Zahnle); initiate study of what limits SST in Pacific Ocean near maximum of top of the atmosphere outgoing long-wave radiative flux (Rabbette, McKay, Young); on Venus-like planets investigate stability of hydrous minerals, and how these affect the budget of surface water, and whether cycling with the mantle is important for the water budget (Zahnle, Sleep); study effects of stellar type and orbital parameters on habitable zone (Hollingworth, Young).

Year 5 - Compute lifetime of protoplanetary disks in stellar clusters (Hollenbach); apply coupled dynamical-chemical model to nebula simulations (Davis, Richard, Charnley); continue to integrate results of coupled dynamic-chemistry nebula model to simulations of delivery of volatiles to terrestrial planets (Davis, Chambers, Lissauer); assess habitability of planet in Venus-like orbit after passing through runaway greenhouse phase (Zahnle, Sleep); assessment of habitable zone based on results from all studies (all); joint workshops with Kepler Mission Science Team.

3.1.5 Management Structure and Statements of Contribution

R. Young's (Lead CoI, Ames) responsibility will be to ensure that the research tasks are completed in a timely manner, and contribute his dynamical and climatological expertise to the investigations regarding Mars, Venus, and the Earth.

CoIs **J. Chambers** (SETI Inst.), **J. Lissauer** (Ames), and **G. Laughlin** (UC Santa Cruz) will direct and carry out the proposed simulations of orbit stability for planetary systems and delivery of water to the inner planets by planetary embryos from the asteroid belt. CoI **D. Hollenbach** (Ames) will be principally responsible for carrying out the studies of protoplanetary disk lifetime. CoI **S. Davis** (Ames) will collaborate with **D. Richard** (NRC Research Assoc.) on dynamics and A. J. Markwick (NRC Research Assoc.) and Collaborator S. Charnley (SETI Inst.) to produce a coupled dynamical-chemical model of the solar nebula. CoI **O.B. Toon** (University of Colorado) and CoI **K. Zahnle** (Ames) will be responsible for the studies involving whether impacts created the Martian valley river networks and the runaway greenhouse studies for Mars and Venus-like planets. CoI **M. Rabbette** (Bay Area Environmental Research Inst) will lead the study of the runaway greenhouse effect over the Pacific Ocean, collaborating with R.E. Young and Collaborator C.P. McKay. CoI **N. Sleep** (Stanford University will work with Kevin Zahnle on investigating the potential for habitability of Earth-like planets in Venus like orbits, and will collaborate with O.B. Toon and Kevin Zahnle on the consequences of impacts for Mars. CoI **J. Hollingsworth** will, together with CoI Richard Young and Collaborator Anthony Colaprete, be responsible for carrying out 3-D simulations of the climatology of potentially habitable planets.



3.2 Investigation 2 Prebiotic Organics, From Space to the First Membranes

How did the Earth and life arise? Is there life elsewhere? We hope to bring NASA closer to its goals of answering such questions by using molecules as historical records of the conditions in space when our Solar System formed, and as signs of life beyond Earth. Using organic chemistry as our unifying theme, this investigation spans the cosmochemical context for life, from the origin of molecules in space, through their delivery to planets by meteorites and cometary and asteroidal dust (IDPs), to the self assembly of the first membranes on the early Earth.

We intend to elucidate the role of compounds in space and prebiotic chemistry through a combination of lab work that includes *measuring spectra for comparison to astronomical observations, studying chemical reactions under low temperature space conditions, and exploring the biophysics of simple meteoritic molecules that may have made up the first membranes*. Our lab spectra will allow the identification of species in astronomical objects, and our chemical studies will advance our ability to detect potential biomarkers in extraterrestrial samples or on other planets and judge their significance.

Carbon forms in stars and is ejected at the end of the star's life. Organic compounds arise as a by-product of this stellar death. Following ejection, carbon-containing material disperses into the surrounding diffuse interstellar medium (ISM), where it is modified by different physical and chemical processes. Eventually, much of this material becomes concentrated in dense molecular clouds where, as described in the previous investigation, new stars and planetary systems form. In these dense molecular clouds molecules are modified and new organic compounds form, some of which are of potential prebiotic interest (Sandford 1996; Ehrenfreund & Charnley 2000). Thus, to study the species in these dense clouds is to examine the basic molecules from which planetary systems are made. Through lab spectra we can interpret astronomical observations and identify materials from which planets and perhaps life itself was constructed.

The production of prebiotic molecules in the interstellar medium is of little consequence to the origin of, and search for, life unless they can be delivered intact to habitable planets. This requires that they survive the transition from the dense cloud to protostellar nebula and subsequent incorporation into planetesimals, followed by delivery to a planetary surface (See Fig. 3.2-1). Deuterium isotopic measurements demonstrate that some interstellar organic species do, in fact, survive planetary accretion (Sandford, Bernstein, & Dworkin, 2001 and refs therein).

Meteorites and IDPs bring ~1x10^6 kg/yr organics to Earth (Love & Brownlee 1993), and probably provided orders of magnitude more to the early Earth (Chyba & Sagan 1992). This suggests that this "exogenous" delivery helped to make the early Earth (and other planets) habitable by contributing to our prebiotic chemistry (Oro *et al.* 1990; Chyba & McDonald 1995; Thomas *et al.* 1997).

Thus, the early Earth swept up vast quantities of extraterrestrial organic matter, some of which was created in the interstellar medium. These exogenous molecules include many of prebiotic importance, including functionalized polycyclic aromatic hydrocarbons (PAHs) and amino acids (Cronin & Chang 1993), as well as simple membrane forming compounds (Deamer 1985). Perhaps exogenous materials did more than simply deliver carbon as a starting ingredient for the primordial soup. Rather, the specific molecules may matter, having properties that were of relevance to the rise of life on Earth. We outline below a series of lab experiments to explore this possibility.

First, we propose lab studies of ice spectroscopy and chemistry that allow us to *identify and describe the chemistry of these pre-biotic organic compounds where they form in the cold environments in space.* This will lead to a better understanding of the origin, distribution, and significance of these molecules in meteorites and

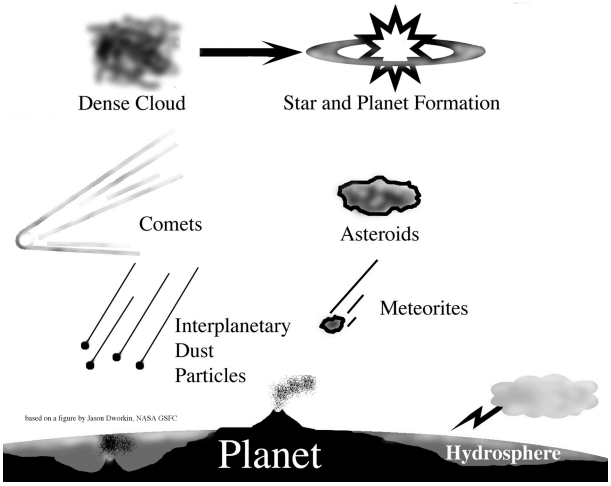


Fig. 3.2-1. Investigation 2 traces our chemical heritage, from the interstellar cloud that made the Solar System to the start of life on Earth. The molecules from which planets and life are composed originate in the interstellar medium. They are modified on incorporation into the solar nebula, and delivered to the surface of planets by meteorites and dust from asteroids and comets.

on other habitable worlds, and will help in the interpretation of potential biomarkers. Second, we propose experiments on synthetic, meteoritic, and model compounds to help us *understand how the first membranes may have arisen.* Membranes are a prerequisite to the maintainance of a chemical gradient, and perhaps the development of self or mutually replicating informational molecules (Szabo et al. 2002).

We are well suited to study extraterrestrial and prebiotic organic compounds, being pioneers in the analysis of mid-IR spectra of interstellar material, having participated in astronomical observations and developed lab techniques for comparison. We have identified interstellar ice constituents, established the ubiquity of PAHs in space (Allamandola et al. 1989, 1999), and constrained the nature of the refractory organic component of the diffuse ISM (Sandford et al. 1995; Pendleton & Allamandola, 2002).

3.2.1 Objectives and Significance of Research

1. Acquire lab spectra of relevant ice molecules under protostellar and outer Solar System conditions so as to improve the identification and our understanding of cosmic ice chemistry. Our work on protostars will inform the chemical and dynamical modeling of Investigation 1, task 1. Our spectra (and optical constants) will be used to fit spectra from ground-based, airborne, and space borne infrared (IR) observatories. This work directly supports NASA missions such as Stratospheric Observatory for Infrared Astronomy (SOFIA), the Space Infrared Telescope Facility (SIRTF), and the Astrobiology Explorer (ABE), and addresses Astrobiology Roadmap Goal 2, directing NASA to "Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System."

2. Perform lab experiments of the radiation processing of PAHs (and amino acid precursors) in interstellar and outer Solar System ice analogs, and characterize the products formed. This will test the potential of ice chemistry to produce meteoritic organic compounds that could help set the chemical stage for the development of life. This work on the formation and survival of prebiotic organics falls under Astrobiology Roadmap Goal 1, which includes study of "The processes by which crucial biologically useful chemicals are carried to a planet and change its level of habitability…" In some cases these molecules could be confused as biomarkers if they are detected in a meteorite or on an extraterrestrial body. The potential of abiotic organics that form on the surface of icy bodies such as Europa to be mistaken as biomarkers correlates to Goal 7 to "Determine how to recognize signatures of life on other worlds."

3. Explore the behavior of pre-biotic compounds that could have served as the first components of membranes. We will analyze molecules from meteorites, our ice processing experiments, and perform studies on off-the-shelf model compounds to put constraints on the earliest membrane systems. We will assess how vesicles made from mixtures of linear acids and PAHs (both simple meteoritic molecules) withstand variation in pH and other parameters. This task is directed at Astrobiology Roadmap Goal 3, which includes studies of "sources of organic compounds for the origin of life" and "origins of cellularity and protobiological systems."

3.2.2. Research Tasks

3.2.2.1 Task 1 - Characterize interstellar, protostellar, and outer Solar System ices.

We propose to measure spectral fingerprints of organic compounds in realistic, lab analogs of interstellar, protostellar, and outer Solar System ices to support numerous NASA projects and missions motivated by a desire to understand the chemical context in which planetary systems and life arise. By facilitating the analysis of the original material in protostellar systems from which planetary systems are made this task feeds into Investigation 1, on nebular processes and habitable planet formation,

Build On and Extend State of Knowledge

Dense interstellar clouds, from which all stars and planetary systems form, attenuate the interstellar radiation, permitting the survival of organic compounds. Mixtures of molecules condense at 10-50 K to form ice mantles on the surfaces of refractory dust grains where they participate in additional chemical reactions. When a new star forms in such a cloud it is called a protostar. Most of what is known about protostars is based on observations of those more massive than our Sun, because they are bright enough to measure. Much less is known about small protostars, and quiescent regions of dense interstellar clouds. By comparing IR spectra of lab ices with absorption spectra of bright protostars, we and others have been able to show that ices around these stars are composed of H_2O, mixed with several percent of simple molecules such as CO, CO_2, CH_3OH, and NH_3 (Gibb et al. 2000; Ehrenfreund & Charnley 2000; Gibb & Whittet 2002). The Astrochemistry Lab Group at Ames has been one of the world's leaders in establishing this field, both observing and identifying the main components of these protostellar ices. Dr. Allamandola, with his collaborators at Leiden University, developed the lab techniques to provide the first refractive indices (*n*'s and *k*'s) of amorphous ices. This approach was applied to realistic mixtures (Hudgins 1993) and that data has been used by us and others to quantify the

abundance of compounds in extraterrestrial ices. Past observations from ground based observatories, the Kuiper Airborne Observatory, and the European Infrared Space Observatory (ISO) led to the detection of the major components of bright protostars, with many minor components and fainter objects left uncharacterized. But this is about to change.

We are approaching an astronomical golden age, with missions directly addressing key astrobiological issues soon to be launched. NASA's IR observatory SIRTF and the airborne IR telescope SOFIA will be flying during the duration of this proposal. The Astrobiology Explorer Mission (ABE), an orbiting IR telescope devoted exclusively to characterizing the evolution of organics in space by measuring their IR spectra to higher levels of sensitivity and resolution than ever before, is now under review. Dr. Sandford, a CoI on this proposal is PI of the ABE mission; Dr. Allamandola is an ABE CoI.

With greater sensitivity and spectral resolution, these missions will make possible observations of new objects and trace species not accessible before. For example, past observations were limited to bright protostars – those producing stars much larger than the Sun. Preliminary data suggest that composition varies with size, and the greater sensitivity of ABE, SOFIA and SIRTF will allow faint objects to be analyzed thoroughly for the first time. For the first time we will directly be able to analyze protostellar environments like the one from which our Solar System arose. These analyses will provide crucial input for the models of planetary system formation described in Investigation 1.

This greater sensitivity also means that new compounds will be detected in the ice. Similarly, better spectra of quiescent regions will reveal the least-processed, coldest, environments. The determination of spectroscopic data described below is essential to maximize NASA's scientific return on its investments in IR missions such as ABE, SOFIA and SIRTF.

We are active CoIs with groups reducing the ISO Spectral database. ISO's archive of mid-IR spectra of the brightest interstellar and extragalactic objects was opened to the public during this past Astrobiology funding cycle. Supported by other funds, we are analyzing this ISO data with our lab spectra, and the results will inform our future work.

Furthermore, longer wavelength (to 5 μm) spectra now achievable from ground-based observatories such as NIRSPEC (a near-infrared echelle spectrograph at Keck) and SpeX (a Medium-Resolution Spectrograph on IRTF) have created new opportunities in the analysis of Solar System ices. Observations of dim Solar System objects have revealed NH_3 on Charon (Brown & Calvin 2000) and CH_3OH on Pholus (Cruikshank 1998). We have a collaboration with Eliot Young of the Southwest Research Institute on an NSF funded project on the interpretation of amino acid precursors (such as HCN, CH_2O, and NH_3) in outer Solar System ices. Our experimental work on these systems will indirectly support NIMS (Galileo) and VIMS (Cassini) missions by providing lab data for comparison, although we are not CoIs on these missions.

All of this new interstellar, protostellar, and outer Solar System data will bring a flood of astronomical spectra of molecules under unusual conditions demanding new reference spectra for interpretation. We propose to extend our historically successful lab spectroscopy program to accommodate this need.

Technical Approach and Methodology

Interpreting astronomical observations requires lab experiments to determine the spectral properties of relevant materials under appropriate conditions, especially refractive indices for light scattering models. We will vapor deposit mixtures of simple ice components (i.e. H_2O, CH_3OH, CO_2 exact components and proportions based on observations) onto a cold substrate. We will measure spectra with an IR spectrometer and ice thickness with a HeNe laser. *From these we will determine refractive indices (n's, k's) of the ice components as a function of concentration and temperature*. The properties of molecules in the ice vary greatly with the conditions; in the solid phase interactions between molecules can be quite strong, causing spectral changes. New spectra are needed each time observations indicate changes, since even if the components are the same, simply changing concentration or temperature can change band profile and strength.

We will also assess effects of radiation processing on the ices. The UV photolysis of CH_3OH in water ice forms HCO, H_2CO, CH_4, and CO_2 and often the spectral appearance of such species formed *in-situ* differs from those vapor deposited. We, and others, have used this behavior to better fit infrared spectra of protostellar objects. By identifying the new photoproducts we made correct predictions and thus discovered new molecules in space. As new more sensitive telescopes produce better spectra of low-mass protostars and quiescent regions we will use the same techniques, exposing to radiation the new mixtures implied by the latest observations, perhaps leading to new astronomical observations. In addition, such lab experiments can constrain chemical models, i.e., by monitoring the photo-destruction rate of the amino acid glycine we were

able to estimate its lifetime in interstellar environments (Ehrenfreund et al. 2001).

Similarly, we will employ lab experiments to determine spectral properties of ices germane to icy outer Solar System bodies to fit reflection spectra. These are primarily ices dominated by water, but the components, types of radiation, and temperatures can be quite different from protostellar ices. For our compositional studies we will focus on objects and spectral regions where there are unidentified features. For example, The NIMS spectra of Callisto and Ganymede display absorptions at 4.57 μm (McCord et al. 1997; 1998) which have been attributed to an organic nitrile (a compound bearing a -C≡N), but the carrier has yet to be identified. Nitriles are a longstanding interest of ours, and we have noted how the IR peak positions vary with the molecular environment (Bernstein et al. 1997; 2000). As above we propose to measure the effects of radiation processing in model Solar System ices i.e., the formation of CO_2 from degradation of exogenous organics on a satellite's icy surface. This portion of the proposed work has recently been partially funded through a grant (from NASA's PG&G program) to study the radiation processing of ices on the icy Galilean satellites.

3.2.2.2 Task 2 - The Connection Between Ice Chemistry and Pre-Biotic Organics Delivered to Earth (and Elsewhere).

We propose to further elucidate the connection between meteoritic pre-biotic compounds and interstellar and protostellar ice chemistry. Understanding the origin of these species is relevant to the first Goal of the Astrobiology Roadmap, which deals with the delivery of prebiotic molecules and their connection to habitability. In addition, inasmuch as some of these molecules are very similar to and can be confused with biomarkers, this work also relates to the second roadmap Goal as well.

Build on and Extend State of Knowledge

In early photochemical studies of interstellar and Solar System ice analogs it was noted that a film of non-volatile organic material sometimes formed (Hagen et al. 1979; Sagan & Khare 1979). It has since become clear that energetic processing of ices at low temperature can create many complex organic molecules from simple starting materials (Bernstein et al. 1995; Gerakines et al. 2000; Khare et al. 2002). The suite of species produced under interstellar ice conditions resembles those found in meteorites. These chemical correspondences, combined with isotopic enrichments typical of low temperature reactions, suggest that some of the complex organic compounds in meteorites may have derived from energetic processing of interstellar or protostellar ice. This source of meteoritic organics from ice complements the paradigmatic pathway to them via reactions in liquid water on the parent asteroid or comet from which the meteorite came (Peltzer et al. 1984). Whereas parent body reactions in liquid water require conditions that are somewhat local, dense molecular cloud ice chemistry is universal, since the material from which all planetary systems are made comes from such clouds. Prebiotic molecules that derive from the ice are ubiquitous, making them relevant to the habitability and prebiotic chemistry of, and recognition of signatures of life on, all solar system bodies.

In order to evaluate the connection between ice photochemistry and the complex organic compounds in carbonaceous meteorites, we have been pursuing two paths in the lab: a reductionist approach studying in great detail the reactions of a single compound in ice at low temperature, and a synthetic method in which we approximate the complexity of extraterrestrial ices with realistic mixtures of several components, expose them to radiation and see what forms. Over the course of our previous NAI funding period, the reductionist and synthetic techniques both yielded significant advances in our understanding of chemical structures and patterns of deuterium enrichment suggestive of ice processing. The former resulted in a good explanation for the chemical groups that decorate meteoritic aromatics (and 6 papers including one in *Science*). The latter produced two papers on ice photochemical syntheses of important pre-biological meteoritic molecules: a paper last year in *Nature* on meteoritic amino acids (Bernstein et al. 2002a), and a paper in *Proc. Nat. Acad. Sci* on simple amphiphiles - membrane forming compounds (Dworkin et al. 2001). The work on amphiphiles especially improved our appreciation of the remarkable complexity that can arise from simple interstellar starting materials. The results from, and proposed future work for, both approaches will now be reviewed in greater detail.

Technical Approach and Methodology

Reactions of Aromatics in Ice

PAHs are observed in emission throughout the universe (Allamandola et al. 1999; Cox & Kessler 1999), and are seen in absorption towards cold dense clouds (Sellgren et al. 1995; Brooke et al. 1999; Chiar et al. 2000; Bregman et al. 2000; Bregman & Temi 2001) where they should condense into ices. PAHs are probably present in cometary ices (Moreels et al. 1994), and are ubiquitous in meteorites and IDPs, so they should be delivered to planetary polar caps and the surfaces of icy bodies in the outer Solar System (such as the Galilean satellites). In all of these environments PAHs would be exposed to UV and cosmic rays while frozen in the ice, but little was known about their reactions in ice at the time we proposed to NAI five years ago.

Given that the D-enrichment of PAHs present in meteorites and comet and asteroidal dust suggest low temperature chemistry (Sandford et al. 2000; Sephton & Gilmour 2000; Sandford, et al. 2001), we proposed and carried out lab experiments on the UV photo-chemistry and MeV proton irradiation of PAHs in ice (Bernstein et al. 1999; 2001; 2002b; 2003). We found that exposure of PAHs to moderate doses of UV radiation in solid H_2O at 10 K, leads to oxidation (alcohol, ketone and ether formation) and hydrogen atom addition (see Fig 3.2-2).

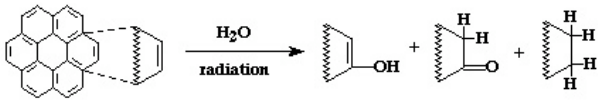


Fig. 3.2-2. A plain PAH, like the kind made at the death of a carbon rich star, is quickly converted to its oxidized and reduced forms when exposed to UV in H_2O ice under interstellar conditions. We proposed that interstellar ice chemistry is a source of these molecules in meteorites. If so, these carbon molecules should be universal, since these dense interstellar clouds are the source of every planetary system.

To explore the link between ice processes and the molecules in meteorites, we examined the UV-induced oxidation of naphthalene (a PAH). We separated isomeric alcohols and napthoquinones using HPLC (Bernstein et al. 2001) and described its regiochemistry (what ends up where). In addition, since deuterium enrichments of extraterrestrial molecules are often taken as the strongest indication of an interstellar heritage, isotopic labeling is an important test of lab experiments. We have used deuterium labeling in PAH-ice experiments in the lab and published papers making specific predictions of where deuterium should be found on meteoritic molecules (Sandford et al. 2000, 2001). *We propose to extend this work to study the regiochemistry of anthracene*, the oxidized variants of which have been observed in the Murchison meteorite (Krishnamurthy et al. 1992).

We have also studied the UV processing of PAHs with common interstellar compounds like NH_3, HCN, CH_4, CH_3OH, and CO_2 in the ice. In addition to the products in Fig 3.2-2 we found the addition of amino, cyano, methyl, methoxy, and carboxylic acid groups (see Fig. 3.2-3).

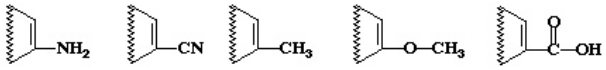


Fig. 3.2-3. Simple interstellar ice compounds add to PAHs to produce a range of functional groups. This is important because it explains a meteoritic molecules. Given that substituted aromatics have been invoked as biomarkers, understanding all sources of such compounds is important for the search for chemical signs of life.

Substituted aromatic species are common in meteorites including ketone (>C=O; Krishnamurthy et al. 1992), acid (-COOH; Sephton et al. 2001), and alkyl (-CH3; Cronin et al. 1988) groups. Evidence from degradation analyses (Hayatsu et al. 1980; Komiya et al. 1993; Sephton et al. 1999) and NMR studies (Gardinier et al. 2000; Cody et al. 2002) suggest meteoritic macromolecular material also contain such functional groups. Similarly, two-step laser mass spectra of IDPs are consistent with alkyl and nitrogen-containing functional groups attached to aromatics (Clemett et al. 1993).

In conjunction with our collaborators at NASA Goddard we have begun to explore simple oxidation of and acid formation (-COOH Fig. 3.2-3) on PAHs by MeV proton irradiation in ice (Bernstein et al. 2003). Comparisons of UV and MeV proton experiments indicate differences between the conditions and mechanisms that give rise to acid formation, but this is not yet understood. We plan to continue this collaboration and work on PAH MeV proton chemistry in the presence of the other common interstellar ice components. *We also propose to study these reactions under conditions germane to Galilean satellites*, investigating the effects of higher temperatures, and low energy (<10 Kev) electrons.

Understanding the chemistry of PAHs bearing side groups is important for astrobiology for a number of reasons. First, to the extent that we understand their formation these molecules have potential as tracers of interstellar chemistry in Solar System materials. Second, given that substituted aromatics not unlike those above were invoked as biomarkers in a Martian meteorite (McKay 1996) understanding non-biological processes that produce them must bear on issues of finding signatures of life.

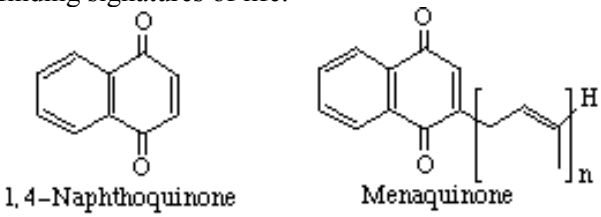


Fig. 3.2-4. The molecule on the left was formed in one of our interstellar ice simulations, the one on the right is a biomarker. The striking similarity of an ice photoproduct to a ubiquitous biochemical suggests that searching for chemical biomarkers on places like Europa will be difficult if we fail to fully understand the non-biotic chemistry first.

Finally, some of the other functionalized PAHs that we have generated look so much like those in living systems that, as compounds exogenously delivered to the early Earth, they may played a role in the evolution of life. For example, 1,4-

napthoquinone is a significant product in our naphthalene/H_2O photolysis experiments. Substituted naphthoquinones, such as menaquinones (see Fig. 3.2-4), perform key biochemical functions (Suttie, 1979), helping or acting as electron transporters and oxidative phosphorylation co-enzymes, even in "primitive" organisms that are often studied by astrobiologists. For example, the Archaea Thermoproteus tenax use menaquinones in the reduction of elemental sulfur to H_2S as their main energy source (Thurl et al. 1985). Perhaps extraterrestrial quinones, which spontaneously partition into model membrane systems (see 3.2.2.3, below) first afforded an evolutionary advantage to early organisms by providing some protection against UV radiation on the early Earth (Wynn-Williams and Edwards 2002), and later were exploited for their capacity to pump protons across bilayers (Deamer 1985).

Synthesis of Complex (Pre-Biotic) Organics

Our lab experiments simulating interstellar environments support the notion that dense molecular clouds permit the synthesis of species far more complex than the simple starting molecules seen in interstellar spectra. In particular, our ISM ice experiments have yielded two classes of compounds that are also present in meteorites and are of interest to astrobiology because of the potential relevance to the origin of life: amino acids (Bernstein et al. 2002a) and amphiphiles (Dworkin et al. 2001).

Many indigenous amino acids have been detected in a number of meteorites, (Cronin & Pizzarello 1983; Cronin & Chang 1993). It has been generally assumed that the amino acids in meteorites formed in *liquid* water (Peltzer 1984) on an asteroid or comet parent-body. However, the water in the Murchison meteorite, for example, was depleted of deuterium (Robert and Epstein 1982), making the distribution of deuterium in organic acids in Murchison difficult to explain (Lerner 1995; 1997). Similarly, occasional but consistent meteoritic excesses of left handed (L), non-terrestrial, amino acids (Cronin & Pizzarello 1997) are difficult to rationalize by liquid water parent-body reactions. Rather this bias towards L has been postulated to arise in space from circularly polarized radiation (Rubenstein et al. 1983; Cronin & Pizzarello 1997; Rubenstein et al. 1999) – essentially, handed molecules from handed light. But the formation, under interstellar conditions, of any kind of amino acids, let alone handed ones, had not been demonstrated.

As part of our previous NAI grant we reported the first interstellar ice photo-synthesis of amino acids (glycine, alanine, and serine) and other meteoritic molecules (Bernstein et al. 2002a; Muñoz-Caro et al. 2002). Careful controls, chirality, and isotopes demonstrate they are not contaminants. We propose to employ partial ^{13}C and ^{15}N isotopic labeling experiments to uncover the mechanism by which these molecules form.

Furthermore, amino acids are of great biological significance, since proteins are made of amino acids. This aspect of our work goes hand in hand with the research presented in Investigation 3 on the polymerization of amino acids leading to enzymes and proteins. Since living systems are made of L amino acids, understanding the origin of the excesses of L amino acids in meteorites relates to astrobiology both in terms of the use of handedness as a sign of life on other planets (Rodier 2002; Brinton et al. 2002) and as a way in which life on Earth was potentially predisposed towards L (Greenberg 1997). *We propose to employ circularly polarized radiation to test whether the meteoritic bias towards L can indeed be explained by a photo-synthetic process from "handed" light.*

While amino acids formed in our interstellar ice synthesis experiments are more complex than the simple one-carbon starting materials, they still are relatively small molecules (75-100 amu). A mass spectrum of trace material from the UV photolysis of the realistic interstellar ice analog H_2O:CH_3OH:CO:NH_3 (100:10:1:1) at 15 K gives an indication of the complexity of the product mixture (see Figure 3.2-5).

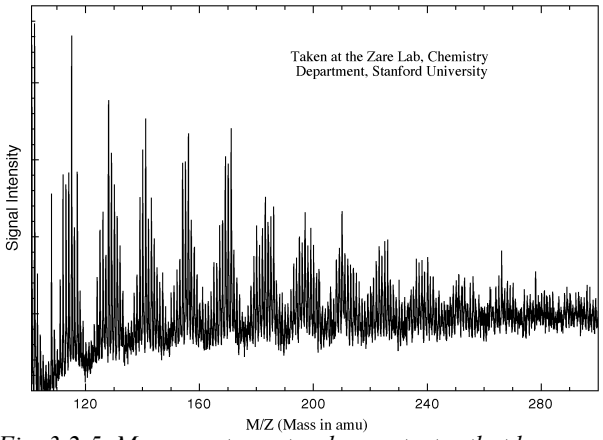


Fig. 3.2-5. Mass spectrometry demonstrates that large molecules can be made from a realistic ice mixture of simple interstellar molecules of no more than one carbon atom each. If the products contain carboxylic acids (as our results below suggest) then these spectra are consistent with organic acids of up to 18 carbon atoms!

These ices begin with only a few simple species but larger ones are produced, with molecules of almost 300 amu made from compounds of only one carbon atom each. Careful control and isotopic labeling experiments demonstrate that these are not contaminants (Dworkin et al. 2001). Little is known about how product formation depends on

conditions or the mechanism. We propose to determine the range of extraterrestrial conditions that can give rise to these compounds by exploring the effects of temperature and composition on the mass spectra. But even more remarkable than the large masses and diversity of these compounds is the capacity of these mixtures to form vesicles (bi-layer spherical structures like membranes). This is also a property shared by chloroform extracts of the carbon-rich Murchison meteorite (Deamer 1985).

3.2.2.3 Task 3 - The First Membranes: Meteoritic and Model Systems that Self-Assemble

We propose to study the structure, stability, permeability and dynamics of vesicles (bi-layer spherical structures like membranes) made of prebiotic compounds, to understand roles they may have played in forming the first cell membranes. We will use primarily off-the-shelf model molecules, but we will also continue to test the amphiphilic capacity of the compounds created in our ice simulations (above). Furthermore, we will closely examine the meteoritic extracts that form these compounds, separating out and testing components for vesicle formation and deuterium enrichment.

Build on and Extend the State of Knowledge

Membranous boundary structures define all life today, and were probably essential for the rise of life (Deamer et al. 2002). Not only are barriers needed to maintain a chemical gradient, but recent work suggests that the isolation they provide was a prerequisite for the development of self-replicating informational molecules, i.e., DNA or RNA (Szabo et al. 2002). In fact, rather than focusing on a single, self-replicating, informational compound, some suggest the origin of life involved the self-assembly of molecular systems within cell-sized environments (Morowitz 1992; Segré et al. 2001).

Amphiphiles - compounds that can self-assemble to form bi-layer structures (see Fig. 3.2-6) - are present in the Murchison meteorite (Deamer 1985) and are produced in our interstellar ice simulation experiments (Dworkin et al. 2001). Whether exogenous or endogenous (or most likely a mixture of the two), such self-assembling molecules probably played a key role in the emergence of life because of the unique advantages they can provide. For example, enclosed vesicular structures are capable of concentrating molecules, overcoming dilution and facilitating interactions that develop or maintain macromolecules. Moreover, since the aromatic components of the prebiotic organic inventory include non-polar pigments (i.e. naphthoquinone, Fig. 3.2-4), they have the potential to capture light energy and screen the interior from damaging UV light when partitioned into the hydrophobic phase of a bi-layer. This emergent set of biophysical properties can only arise from amphiphilic molecules that have the ability to self-assemble into more complex vesicular structures.

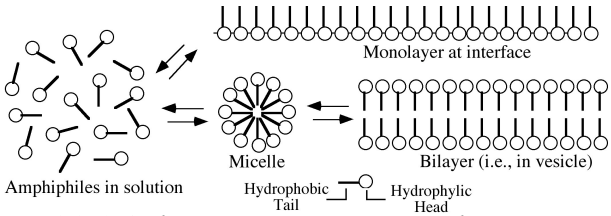


Fig. 3.2-6. At the correct concentration and pH, certain compounds spontaneously self-assemble into bi-layer structures known as vesicles. Mixtures of molecules from the Murchison meteorite and from our ice simulations have this capacity. We suspect long chain linear carboxylic acids are the active molecules in these cases, but little is known about the "prebio"-physics of the vesicles formed by these prebiotic molecules.

Technical Approach and Methodology

We plan a three-pronged approach to advance our understanding of prebiotic amphiphiles. First, we will extend our successful lab work on reproducing the ice chemistry of space environments to assess under what conditions amphiphiles form. Second, we will extract, separate by HPLC, and structurally characterize meteoritic amphiphilic compounds, looking for deuterium enrichments. Of course, meteorites and prebiotic soups have mixtures of compounds, and this leads to potential complications, whereas traditional studies on amphiphiles have been performed on individual (pure) compounds. In attempting to approach realism by using mixtures, a synergistic effect was recently established: the bilayer structures made from mixtures are far more stable than pure compounds, at least for poor amphiphiles (Apel et al, 2002).

These vesicles were made from monocarboxylic acids, which are found in carbonaceous chondrites. Nonanoic acid forms vesicles only at concentrations above 85 mM at pH 6.9 (the pKa of the acid in membranes). However, upon the addition of 10% nonanol, only 20 mM of total acid is necessary to form vesicles, and they are stable up to pH 11 (Apel et al. 2002). Thus, the addition of a small amount of alcohol to the acid creates membranous structures that are stable over a far wider range of conditions, even at a quarter the concentration.

Meteoritic PAHs and their polar derivatives (Mautner et al. 1995) have been shown to partition between membrane bi-layers. In certain lab settings aromatics (such as naphthoquinones Fig. 3.2-4) can generate protons and establish transmembrane pH gradients (Klein & Pilpel 1973; Deamer 1985). However, PAHs have never been studied with prebiotic amphiphiles, like a

carboxylic acids. With their multiple rings and flat structure, PAHs may change the properties of these vesicles, as cholesterol stabilizes biological membranes. We will explore the effect of adding PAHs to prebiotic vesicles, with a special emphasis on changes in stability and permeability to pH gradients. These were presumably the important parameters if such structures were employed by the first organisms.

3.2.3 Description of Instruments and Hardware

The instrumentation and hardware needed to carry the proposed lab work are present at the Astrochemistry Lab at NASA Ames, or our collaborator's labs at UC Santa Cruz, and Stanford. For a detailed description of our equipment see Section 5.2.5.

3.2.4 Relevance of Proposed Work

The work in this module squarely addresses the following Astrobiology Roadmap Goals: 1- including the study of "The processes by which crucial biologically useful chemicals are carried to a planet and change its level of habitability…", 2- "prebiotic chemistry and signs of life elsewhere in our Solar System," 3- "sources of organic compounds for the origin of life and origins of cellularity and protobiological systems," and 7- "Determine how to recognize signatures of life on other worlds." Moreover, our lab spectral data will help fit spectra from NASA's ground-based, airborne, and spaceborne IR observatories. As a result, this work will directly support missions such as the SOFIA, SIRTF, and ABE.

3.2.5 Key Milestones

Year 1 - Use Existing IR database to interpret ISO spectra. Perform IR spectroscopy on outer Solar System ices and a series of naphthalene-H_2O mixtures of known concentration for generation of n's and k's. Begin amino acid ^{13}C partial labeling experiments.

Year 2 - Continue ISO data analysis program. Perform UV photolysis experiments on anthracene-H_2O mixtures and analyze using HPLC. Continue IR spectroscopy on outer Solar System ices for n's and k's. Begin studies of amphiphilic model compounds, mixtures of PAHs and long chain (>9) acids. Continue amino acid ^{13}C partial labeling experiments and begin ^{15}N amino acid partial labeling experiments.

Year 3 - Use lab spectra to interpret data from SIRTF and direct SOFIA mid-IR instrument requirements and first observations. Continue studies of amphiphilic model compounds exploring pH stability. Perform UV photolysis experiments on PAH-H_2O mixtures and other ice components at higher (50-100 K) range. Complete amino acid partial labeling experiments and explore effects of temperature.

Years 4 and 5 – Continue astronomical observations extending to other galaxies. Perform irradiation experiments on PAH-ice mixtures using electrons as radiation source. Test the permeability of the most stable nonanoic acid PAH vesicles, and explore the effects of UV radiation. Perform amino acid experiments with circularly polarized radiation to attempt to generate an enatiomeric excess, this will require exploring different wavelengths.

3.2.6 Management Structure and Statements of Contribution

CoI Dr. **Lou Allamandola** (NASA/Ames) has extensive experience in all the aspects of lab astrophysics and IR observations relevant to this proposal and will participate fully in those areas. He will supervise the overall program.

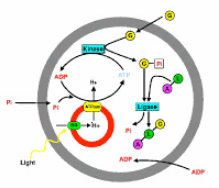
CoI Dr. **Max Bernstein** (SETI and NASA/Ames) is responsible for performing ice processing experiments and analysis of product molecules. He will aid NRC post-doc Charles Apel on the self-organizing behavior of amphiphiles extracted from meteorites and produced synthetically in the lab.

CoI Dr. **Scott Sandford** (NASA/Ames) will aid in the interpretation of the results in the context of comparisons with meteorites and IDPs, especially regarding isotopes. He will also coordinate the lab IR studies with those required for ABE, the proposed MIDEX mission for which he is PI.

Collaborator Prof. **David Deamer** (UC, Santa Cruz) will provide Murchison samples, the use of microscopes, and his expertise for the studies of meteoritic amphiphiles.

Collaborator Prof. **Richard Zare** (Stanford University) and his group will continue to measure mass spectra of molecules made in PAH-ice processing experiments.

Collaborator Dr. **Jason Dworkin** (NASA Goddard), will perform MeV proton irradiations of PAH-ice mixtures and some chromatographic analyses of amino acids.



3.3 Investigation 3 The Origin and Early Evolution of Proteins and Metabolism

3.3.1 Objectives and Significance of Research.

Hypotheses. We propose to study the origin and early evolution of the peptides and proteins that putatively carried out metabolic functions in the ancestors of contemporary cells. There are two competing models that describe the possible

origins of these molecules. According to one model, catalysis of biochemical reactions was initially carried out by RNA enzymes (ribozymes). The transition from "the RNA World" to the modern, protein-dominated biological world was bridged by the evolution of ribozymes that synthesized peptides in a non-coded fashion. This was followed by the coded evolution of short peptides, and then of longer proteins. An alternative model begins with an autocatalytic network of peptides which evolved to a state of increasing metabolic complexity prior to the evolution of RNA-coded information. Although these two models are quite different they share a common feature – both assume the existence of proteins not coded by nucleic acids. To evaluate the validity of this assumption and to distinguish between the two models, it is critical to establish the probability that random peptides can perform protocellular functions, especially those that RNA is unlikely to perform, such as the transport of specific ions across membranes. In order to understand the subsequent evolution towards modern proteins, it is also essential to determine the structure of the first functional peptides and collective properties of networks of chemical reactions catalyzed by these peptides.

Specific Objectives. The proposed work builds on our accomplishments in the previous NAI granting period. For the first time, we were able to select, from a very large population of random-sequence proteins, four families of small peptides with novel amino acid sequences that efficiently bind adenosine triphosphate (ATP) (Keefe & Szostak 2001). From this study, it appears that the emergence of a functional protein from a pool of random sequences is approximately as likely as the emergence of a functional RNA molecule from random RNA sequences (Ellington & Szostak 1990, Bartel & Szostak 1993). This research direction will be continued with the following specific objectives:

1. To obtain the 3-dimensional structure of the ATP-binding protein. This will allow us to determine if the peptide, which has an amino acid sequence unrelated to any known proteins, has a novel structure or, alternatively is folded into an already known structure. As discussed in the next section, the evolutionary implications of these two possibilities are both important and quite different.
2. To reconstruct in the laboratory the emergence of early catalytic functions by constructing for the first time models of protobiologically ubiquitous protein enzymes. We will identify their structures, properties, relationships to known proteins performing similar or different functions, and the ability to evolve to proteins with altered functions.
3. To select peptides that assemble into transmembrane channels capable of transporting ions across membranous cell walls which was, most likely, one of the earliest cellular functions. This function is very difficult (and perhaps impossible) to achieve using RNA molecules because they do not partition into membranes.
4. To examine the evolutionary potential of a collection of proteins in the absence of self-replicating mechanisms. We will determine under what conditions evolution could progress and assess whether these conditions were protobiologically plausible.

The first three goals will be pursued mostly experimentally. At the center of this effort will be *in vitro* selection approaches similar to that applied before to obtain ATP-binding proteins. The last goal will be pursued through theoretical and computational modeling. The experimental and theoretical approaches complement each other. Experiments provide estimates of efficiencies with which non-coded proteins can catalyze biochemical reactions. These efficiencies are critical parameters for modeling non-genomic evolution. Computer modeling will aid interpretation of experimental results in terms of protein structure, evolution and mechanisms of action. Finally, the proposed modeling effort can guide future experiments aimed at demonstrating the emergence of coordination between different metabolic processes using the suite of protein enzymes created in the laboratory.

Significance. If successful, the proposed studies will yield novel, essential information about the origin and evolutionary potential of the earliest biopolymers that facilitated the chemical reactions supporting life. Successful selection of novel enzymes that are not derived from biological proteins could shed new light on the potential of life beyond Earth and open new avenues for biotechnology. Furthermore, the ability to evolve small, proteins with high affinity and specificity for binding small molecules could have applications in biosensor development.

Conceptually, the proposed research is a continuation of Investigation 2, which addresses the origin of the building blocks of both proteins and membranes. It also complements Investigation 1, which deals with physical constraints to habitability, by providing potential biological constraints.

3.3.2 Building on and Extending State of Knowledge

The identity of the earliest biopolymers. In modern organisms, most functions are performed by proteins, which are, in turn, synthesized on an RNA template. The discovery that RNA possesses catalytic capabilities led to a suggestion that the present world of nucleic acids and proteins was preceded by the "RNA World", wherein RNA molecules alone acted as both catalysts of

biochemical reactions and as information storage systems (Gilbert 1986; Gestland et al. 1999). This model is supported by the fact that a variety of RNA enzymes have been created in the laboratory using *in vitro* selection (Wilson & Szostak 1999), and by the recent discovery that the decoding and peptidyl transferase centers of ribosomes are composed entirely of RNA.

The major difficulty with this model is that no efficient, prebiotically relevant means of RNA synthesis has been found. Furthermore, RNA cannot be readily incorporated into membranes to perform functions, which, in modern cells, include the vital functions of energy transduction and transport.

In an alternative view, the first biological macromolecules were proteins. They do not suffer from the same problems as RNA. Specifically, both terrestrial and extraterrestrial sources of protein building blocks - amino acids - have been identified (Chang 1993; Cronin & Chang 1993). A protobiologically plausible mechanism to convert simple molecular precursors that might have been abundant on the early earth into amino acids has been proposed and tested by Weber (1998, 2001). Furthermore, the condensation of amino acids into peptides was demonstrated in a liposome model system (Luisi et al. 2000). In contrast to nucleic acids, simple proteins insert spontaneously into membranes and aggregate into functional assemblies.

The "protein-first" concept, however, encounters serious difficulties of its own. In particular, with only a few exceptions (Severin et al. 1997a; Yao et al. 1998), attempts to create simple, protobiologically relevant proteins possessing catalytic activity have not been successful. Furthermore, since amino acids cannot form base-pairs like nucleic acids it is not clear that peptides alone could transfer information between generations and evolve. Resolving such difficulties will advance our understanding of how protocellular metabolism emerged.

Experimental basis for the "protein-first" concept. Several recent studies demonstrated the novel catalytic and evolutionary potential of simple peptide systems. Ghadiri and co-workers have shown that two activated peptide fragments can be joined (ligated) on a template of a third, longer peptide with a rate enhancement of 10-fold to 4000-fold over the background (Severin et al. 1997a). The system exhibits not only sequence specificity but also selectivity between diastereo-isomers (Saghatelian et al. 2001). In the presence of several different templates this peptide system forms autocatalytic, self-correcting networks (Lee et al. 1997). Chmielewski et al. (Yao et al. 1998) have extended this work by constructing another peptide system capable of auto- and cross-catalysis and of generating self-replicating peptides that were not present in the original mixture. Importantly, these peptide systems do not suffer from a "diversity catastrophe", in which the identity of the original replicator is lost in a sea of novelty, as was expected for systems lacking an accurate self-replicating mechanism (Eigen 1971).

Recently, we have used a novel *in vitro* selection technique (Roberts & Szostak 1997) to select ATP-binding proteins from six trillion random polypeptides (Keefe & Szostak 2001). It yielded four new protein families, each containing proteins with highly similar amino acid sequences, that were unrelated to each other or to anything found in the current protein databases. Because they were selected from a random-sequence library, these proteins can be considered as the best currently available models of protocellular proteins.

Proteins from one family have been characterized in fair detail and appear to form folded structures. The originally selected protein contained 80 amino acids but deletion studies revealed that the minimal binding unit is less than 50 amino acids long and, thus, is the smallest known ATP-binding protein. The proteins are highly selective towards ATP, as they bind neither guanosine triphosphate (GTP) nor cyclic AMP. However, their sequences do not contain any known ATP-binding motifs. To function, they require zinc ions and contain conserved cysteine residues. This suggests that their three-dimensional structures may be similar to those of zinc finger proteins**.**

The emergence of catalytic functions. The three-dimensional structures of the selected ATP-binding proteins remain unknown because they are insufficiently soluble in water for high-resolution structure determination. This information, however, is of utmost interest. If the structure resembled that of zinc fingers, which are one of the most common DNA-binding motifs found in eukaryotic transcription factors, it would mean that a potentially protocellular protein could share a folding pattern with seemingly unrelated proteins from higher organisms, rather than with functionally related proteins. This could inspire a search for a small number of "protocellular folding patterns'' shared by different, protobiological proteins. If the newly selected proteins have a novel fold, this fact may indicate that some original folding patterns were lost during subsequent evolution. This would raise doubts about our ability to elucidate the structure of protocellular proteins by analysis of proteins from contemporary cells. An alternative interpretation of such a finding might be that this folding pattern and perhaps many others were never present among proteins that started the evolutionary pathway on the early Earth. However, they could have been encountered along a different evolutionary pathway on a different habitable

planet. Clearly each possibility has important and different implications to the studies on the origin and early evolution of cellular life on Earth and beyond. The work identified in Objective 1 will advance our knowledge in this direction.

Selection of ATP-binders is only the first step on the road to identifying a suite of possible protobiological proteins that could support metabolism of the simplest protocells. Among them, and of special interest, are enzymes capable of catalyzing the formation of peptide bonds in proteins, and of phosphodiester bonds in nucleic acids. In the work proposed in Objective 2, we will focus on these two enzymes because they would have been necessary for self-reproduction of a protein-based system, and for its evolution to contemporary cells based on both proteins and nucleic acids. With a single exception (Severin et al. 1997a), there are currently no protobiologically relevant models for proteins catalyzing these reactions. One approach to generating simple enzymes with desired functions is by rational design and engineering. This approach, however, has met with little success (Corey & Corey 1996). More promising is an approach based on *in vitro* selection (Roberts & Szostak 1997) since it has already been successfully applied to create novel ATP-binders (Keefe & Szostak 2001). In the proposed work, we will extend its capabilities by generating novel catalysts.

The origin of transmembrane channels. Not all protocellular proteins essential for metabolism were enzymes. Some proteins transported ions, nutrients and waste products across cell walls, transduced environmental signals or captured energy to store in high-energy compounds. In many instances, no means of performing these essential functions that would not require proteins are known, or have even been postulated. All of these "non-enzymatic" proteins are integrated into membranes. Although they can be complex, their transmembrane regions are often remarkably simple. The most common structural motif for these regions is a bundle of α-helices (Montal 1995, Bayley 1999). Many simple, α-helical membrane peptides aggregate spontaneously and form functional complexes, exhibiting sequence-dependent specificity (Akerfeldt 1993, Bayley 1999, Bechinger 2000). These characteristics suggest that the earliest transmembrane proteins might have been built of α-helices (Popot & Engelman 2000, Pohorille et al. 2003).

Transmembrane protein domains reveal considerable variability in their amino acid sequence, even among closely related proteins (Wallin & von Heijne 1998). Such heterogeneity indicates that a highly specific sequence may not have been required for a peptide to function in a protocellular membrane. This, in turn, suggests that the likelihood of finding a transmembrane peptide among non-coded proteins may be relatively high. While this would be a desirable property in early evolution it also poses some difficulties. Structural similarity of transmembrane peptides formed from assemblies of α-helices raises questions about the origin of specificity towards different functions or substrates, which was essential for evolution. For example, channels that transported both positive and negative ions, independent of size, would have been of no utility to a protocell. Specificity must have emerged from sequence-modulated, interhelical associations. However, the number of channel-forming peptides that were ion-selective is not known, and the nature of interactions that drive associations of helices is poorly understood. *In vitro* selection experiments, proposed as Objective 3, can contribute to resolving these issues.

Protein evolution without a genome. Our recent findings that truly different, simple peptides (Keefe & Szostak 2001) can perform the same function (such as ATP binding) provide experimental support for a novel mechanism of early protobiological evolution without a genome. The central concept underlying this mechanism is that the reproduction of cellular functions, without replication of macromolecules, was sufficient for self-maintenance of protocells. The precise transfer of information between successive generations of the earliest protocells was unnecessary and, possibly, undesirable. The key requirement in the initial stage of protocellular evolution was an ability to rapidly explore a large number of protein sequences in order to "discover" a set of molecules capable of supporting self-maintenance and growth of protocells. Undoubtedly, the essential protocellular functions were carried out by molecules not nearly as efficient or as specific as contemporary proteins. Many, potentially unrelated sequences could have performed these functions at an evolutionarily acceptable level. As evolution progressed, proteins must have performed their functions with increasing efficiency and specificity. This, in turn, put additional constraints on protein sequences, and the fraction of proteins capable of performing their functions at the required level decreased. At some point, the likelihood of generating a sufficiently efficient set of proteins through non-coded synthesis was so small that any further evolution required coupling between proteins and the informational polymers. The emergence of such coupling must be postulated in any scenario of the origin of life, whether it starts with RNA or proteins.

Several theoretical studies have addressed a possibility of non-genomic evolution (Kauffman 1993; Dyson 1999; Morowitz 1992, 2000; Segrè & Lancet 1999; Bagley & Farmer 1991; New & Pohorille, 2000). Models developed by Dyson (1982, 1999), Kauffman (1986, 1993) and Lancet

(Segrè et al. 2000, 2001) describe possible transitions between initial, poorly organized catalytic networks and evolved, well-connected catalytic systems. The basic idea behind these approaches is that evolution of networks of chemical transformations does not necessarily require self-replication of biopolymers that catalyze these transformations. In the Dyson "mean field" model (Dyson 1982, 1999), molecules enclosed in a bounded environment exist in either catalytically active or inactive states. An autocatalytic phenomenon is assumed, whereby active molecules activate inactive molecules. Inactivation reactions prevent exponential explosion of active species. Kauffman (1986, 1993) demonstrated that a set of random molecules could replicate in its entirety forming an autocatalytic set, providing that there is sufficient diversity of molecular species. Segrè et al. (2000, 2001) further advanced these concepts and methodologies by introducing the graded catalysis models and demonstrating that populations of molecules with better interconnected catalytic networks emerge as a consequence of compositional mutations. A recent paper by Segrè and Lancet (1999) provides a more complete account of the long history of "metabolism-first" concepts.

Our work, defined as Objective 4, builds on these accomplishments by introducing into the model both constructive and destructive processes in a stochastic manner and focusing on global autocatalytic processes and their diversity. However, it also differs from the previous work in several important respects. Instead of assuming random reaction sets we consider only a suite of protobiologically plausible reactions, which most likely was fairly limited (Weber, 2001). Peptides are explicitly considered as protoenzymes and their catalytic efficiencies are assigned on the basis of biochemical principles and experimental estimates obtained from our current (Keefe & Szostak 2001) and future work. Finally, simulations will be carried out using a novel approach that is appropriate even for very low concentrations of reactants (Gibson & Bruck 2000, New & Pohorille 2000).

3.3.3 Technical Approach and Methodology

3.3.3.1 Determining Structure of the ATP-binding Protein

As already mentioned, our initially evolved ATP-binding protein is not sufficiently soluble at high protein concentrations to allow biophysical studies, presumably due to the low thermodynamic stability of the folded state. More stable mutants of this protein have been recently obtained by continued selection for ATP binding in the presence of guanidine (Chaput & Szostak, to be published). Guanidine is a denaturant of protein structure and, therefore, its presence in solution favors selection of proteins in stable folded states. Some of the variant proteins show increased expression as maltose binding protein (MBP)-fusion proteins in *E. coli,* and some of the purified MBP-fusion proteins show increased solubility. When cleaved with thrombin, the purified free ATP binding protein (ABP) from a subset of the selected clones also remains soluble. This is shown in Fig. 3.3-1. As expected, more of these proteins stay soluble in the presence of ATP, presumably because ATP binding stabilizes the folded state of the protein.

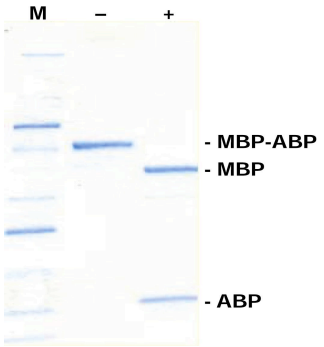


Figure 3.3-1. Purified MBP-ABP fusion protein and corresponding thrombin cleavage products; lower band is ATP binding protein. M, molecular weight markers.

We are currently testing our soluble ATP-binding proteins by size exclusion chromatography (SEC) to establish whether the protein is monomeric or aggregates into multimers. Preliminary results show that at least some of our MBP-fusion proteins exist as monomers. We are currently exploring methods for the preparation and purification of monomeric ATP-binding protein without MBP either by direct expression in *E. coli*, or by thrombin cleavage of purified MBP-ABP fusion protein.

If at least one of our sequence variants remains monomeric at high concentration we will proceed to its physical characterization, initially by recording circular dichroism melting curves in the presence and absence of ATP. These experiments, which can be carried out in samples of ~ 20 µM, will yield evidence for or against a cooperative folding transition and the presence of secondary structure elements (see *e.g.* Oakley and Kim, 1997). If we are able to prepare samples of concentrated protein (~ 0.5 µM, or ~ 5 mg/ml) that remain unaggregated we will proceed to determine the high-resolution, three-dimensional structure of the protein using NMR spectroscopy. This work will be carried out by James Carothers, a graduate student in the Szostak laboratory in collaboration with the laboratory of Gerhard Wagner at Harvard Medical School. The structure will be further

examined in the presence and absence of ATP. We will also compare this structure with other structures available in protein databases. These studies will be carried out collaboratively by the Szostak and Pohorille groups. They will allow us to determine whether the selected protein has a novel folded structure (or, alternatively, which known proteins have related structures) and the mechanism of ATP binding.

If further work is required to increase the solubility of the protein, we will remove regions of the protein that are not essential for its function but might lower its solubility. If these efforts fail to yield a well-behaved sample, we will repeat *in vitro* selection, starting from a mutagenized version of our current best clone. We will use higher concentrations of denaturants to further stabilize the folded state. To improve solubility, we will select against proteins with exposed hydrophobic residues using a hydrophobic chromatographic resin.

As a follow up (and backup) to the above studies, we will take another ATP-binding protein (family C in Keefe & Szostak 2001) through the same path. We already have a version that has been optimized for binding under our standard selection conditions (Cho & Szostak, unpublished), and we are ready to begin selection for a more stable folded state in the presence of denaturants.

From the origin of life standpoint, not only the emergence of functional proteins but also their evolutionary potentials are of interest. We have begun to address this issue by attempting to evolve the ATP-binding protein to a GTP-binding protein. Considering the evolutionary flexibility of biological proteins evidenced by the numerous examples of natural evolution of enzymes that led to alteration of substrate specificity we expected this to be a straightforward task. However, despite several attempts, we were unsuccessful. Without structural information, this negative result is difficult to interpret. Once we can examine the structure of the ATP-binding protein, we might be able to explain the apparent difficulty in accomplishing this seemingly simple evolutionary task. Equipped with this additional knowledge we should be able to carry out evolutionary experiments with greatly improved chances for success. These experiments may begin to answer the question whether some protein frameworks can evolve more easily than others.

3.3.3.2 Selection of Catalytic Proteins

Recently, we have initiated efforts to use the *in vitro* selection technique, which has been successfully used to select ATP-binding proteins, to evolve novel enzymes. However, selection for proteins that catalyze reactions is more complicated than selection for binding proteins because it is difficult to identify successful catalysts once they separate from the reaction product. To deal with this difficulty a number of approaches have been developed for evolving ribozymes. In the approach that appears to be suitable for our purposes, the reverse transcription of the RNA that is attached to the peptide is primed with an oligonucleotide bearing the potential substrate. Then the RNA will hold the substrate in close proximity to the potential catalyst. Subsequent separation of the product of the reaction from the unreacted substrate will reveal the identity of the protein catalyst encoded in the nucleic acid sequence to which it is attached.

Our initial efforts have concentrated on enzymes that catalyze Diels-Alder reactions (a 1,4-addition of a diene and a dienophile to form unsaturated six-membered rings). Since selection of protein enzymes *de novo* has never been accomplished before we have chosen this reaction for the ease and stringency of the selection process rather than for its protobiological significance. A postdoctoral fellow, Burckhard Seelig, who isolated for the first time Diels-Alderase ribozymes composed of unmodified RNA (Seelig & Jaschke 1999) will carry out this work. Furthermore, antibodies that catalyze this condensation reaction have been isolated (Romesberg et al. 1998), suggesting that selections for *de novo* enzymes might also be successful. Specifically, we are selecting for a protein that can accelerate the condensation of an anthracene moiety (the diene, coupled to an oligonucleotide that also serves as a reverse transcriptase (RT) primer) to a biotinylated maleimide (the dienophile, with the biotin serving as a selectable tag). The initial library of random-sequence proteins has been created and selection is in progress. The reaction and selection scheme is illustrated in Fig 3.3-2.

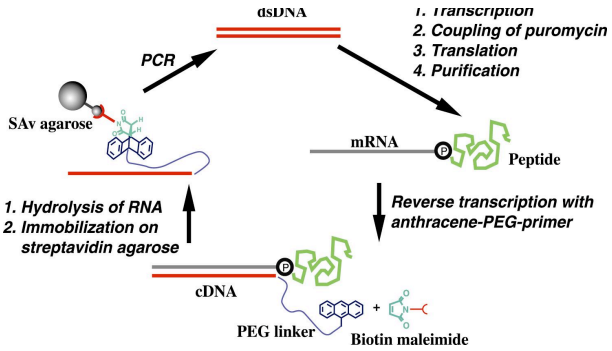


Figure 3.3-2. Selection for a Diels-Alderase Enzyme

We have also developed a plan for an alternative approach, which should allow for searching protein sequence space more effectively, thus increasing the probability of finding enzymes. This plan is based upon our observation, made during the selection of ATP-binders, that most of the functional proteins present in the initial library

have a low probability of folding into the active structure. To counter this effect, we will exploit a new emulsion-based selection technology (Tawfik & Griffiths 1998, 2003), in which DNA templates are compartmentalized into emulsion droplets, followed by transcription and translation, so that several hundred copies of each protein are expressed per template. Even if folding is inefficient, there is a good chance that some molecules will fold properly and exhibit the desired enzyme activity. Furthermore, this approach is intrinsically more suited for enzymatic selections because it allows for multiple catalytic turnover. Even if our current selections yield active Diels-Alderase proteins, we will optimize their activities using the emulsion selection approach.

Once we demonstrate selection of *de novo* enzymes by either of these approaches, we will try to isolate novel enzymes that require more involved selection schemes. We are especially interested in two activities that are relevant to the early evolution of life: a simple RNA polymerase and a simple protein ligase. Previous experience with isolating ribozymes that perform the same functions will be helpful to this end. Ribozymes with limited RNA polymerase activity (Johnston et al. 2001) have been generated by directed evolution of RNA ligase progenitors, which were in turn isolated by *in vitro* selection from large pools of random RNA sequences (Bartel & Szostak 1993). The selection methods required for RNA ligase enrichment are well worked out and are extremely efficient, yielding enrichments approaching 10^6 per round. We plan to use similar procedures in our attempts to isolate protein enzymes capable of accelerating the same chemical steps. If successful we will attempt further directed evolution to generate novel polymerases.

In preparation for this selection, we have already designed and synthesized the substrates required for a ligase selection (see Fig. 3.3-3). Successful ligation of the tag oligonucleotide to the acceptor will allow RT-PCR amplification of the encoding protein ligase sequence. The enriched pool of amplified products will then be used, as usual, as input to the next cycle of selection and amplification.

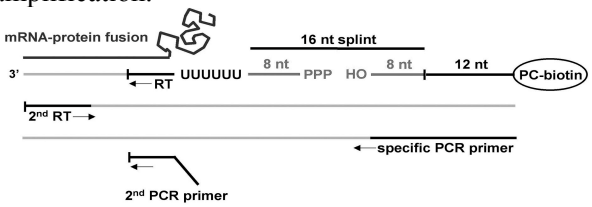


Figure 3.3-3. Selection for an RNA ligase enzyme. The splint and complementary oligonucleotides are composed of RNA; other sequences are DNA. cDNAs that become ligated to the tag oligonucleotide can be enriched both by the biotin and by specific RT-PCR.

Ribozymes capable of catalyzing acyl transferase reactions including peptide synthesis have been isolated (Lohse & Szostak 1996, Zhang & Chech 1997). We propose to isolate novel proteins that accelerate peptide ligation using a scheme analogous to that described above for RNA ligase selection, except that the complementary oligonucleotides will be replaced with peptides similar to those used by Ghadiri et al. (Severin et al. 1997, Lee et al. 1997). The peptide to be ligated onto the cDNA-peptide conjugate will contain affinity tag sequences that allow for selection. In preliminary experiments we will examine various activation chemistries, beginning with the thioester activation used by Ghadiri. However, since this procedure is limited to peptides with a N-terminal cys residue, we plan to identify more general approaches. One possibility is to retain the C-terminal thioester activation, but to select for direct attack by the N-terminal amino group of the reacting peptide, such that the new peptide bond is formed directly, instead of by trans-thioesterification followed by an intramolecular rearrangement. An alternative approach would be to supply a condensing agent, such as a water-soluble carbodiimide, and select for an enzyme that accelerates the resulting peptide ligation.

3.3.3.3 Selection of Transmembrane Ion Channels

To find amphipathic α-helices that self-assemble into channels we will construct libraries of peptides sufficiently long to span the membrane (approximately 23-27 amino acids). We will try both random sequence libraries and a library of sequences in which both hydrophobic and hydrophilic residues are distributed periodically such that they form separate "faces" if a peptide folded to an α-helix. Precise choice of periodicity determines the widths of both faces, which in turn influence how many monomers associate to form the channel. To discriminate against peptides that could act as ionophores (*i.e.* physically carry ions across the membrane) instead of channels, we will include in our sequences a short, terminal segment composed of polar and/or charged amino acids which should "anchor" them to the membrane surface.

Our approach will be similar to the microemulsion selection, described in section 3.3.3.2, but instead of microemulsions we will use vesicles. We will exploit the fact that channel-forming peptides and proteins spontaneously insert into membranes and self-assemble, as discussed in sections 3.3.2. Our first task will be to devise an efficient protocol for the encapsulation of DNA templates into phospholipid vesicles, along with a coupled transcription/translation mix. This will require the efficient and reproducible formation of relatively large vesicles of 0.5 to 2 microns in

diameter to allow expression of multiple copies of single peptides within each vesicle. In parallel, we will begin the validation of a selection method. We will prepare vesicles with or without ionophores, and use fluorescent sensors for ion detection inside the vesicle. Experiments with vesicle populations will confirm changes in ion composition and fluorescent signal output. These data will then be used to devise an effective vesicle sorting procedure by fluorescence activated cell sorting.

If the selection works we will determine the number of monomers forming functional channels. Further, we will examine the stability, specificity and mechanism of action of the selected channels by a combination of systematic mutation studies, model building (Torres *et al.*, 2001) and molecular-level computer simulations (Roux 2001; Pohorille et al. 2003). The Szostak and Pohorille groups will collaborate in this effort.

3.3.3.4 Evolution of Populations of Protocellular Proteins

To examine the evolutionary potential of a non-genomic system, we have developed a simple, computationally tractable model, which is capable of capturing the essential features of the real system. In this model, protocellular walls are permeable to small molecules and amino acids but not to oligopeptides of any length. Within the protocells, chemical reactions are catalyzed by peptides, albeit possibly with low efficiency and specificity. Protocells can grow either by acquiring amphiphilic material from the environment or by producing it internally. Once a protocell reaches sufficient size it can divide, distributing its content between the two "offspring" protocells.

In our model, which is stochastic in nature, the specific identities of the amino acids forming peptides are not considered. Instead, the key quantity is the probability distribution of finding a peptide with a given efficiency of catalyzing a desired function, irrespective of its sequence. In this case, efficiency can be thought of as the inverse of the turnover rate. Biochemical considerations dictate that the efficiencies of short peptides increase only slightly with the length of the polymer. Only when peptides reach lengths sufficient for them to adopt an ordered three-dimensional structure do the average efficiencies increase markedly with length. Increasing the lengths of polymers in which catalytic centers have already been formed produces no significant improvement in catalytic properties. In the current formulation, it is assumed that the catalytic efficiencies of peptides of a given length are distributed normally. Other distributions, such as the decaying exponential or Gram-Charlier (distorted normal), can be implemented.

Considering efficiencies of proteins without explicit reference to their sequences is also motivated by practical reasons. According to the canonical view of the structure-function relationship in proteins, the sequence of amino acids determines the three-dimensional structure of a protein, which, in turn, determines its function (Creighton 1992). In principle, there is good correspondence between sequence and function, which suggests an approach where large libraries of sequences are generated on the computer and then each peptide is assigned function and efficiency based on its sequence. However, despite extensive efforts, the nature of the sequence-function relationship in proteins has not yet been unraveled.

Central to our model of protein evolution is the emergence of protoenzymes forming peptide bonds (ligases). A simple ligase has been developed experimentally by Ghadiri et al. (Severin et al. 1997) and we expect to evolve other ligases, as described in section 3.3.3.2. Most of the peptides generated in the model are disordered. Since the ability to adopt ordered structure is required for efficient catalytic activity these peptides are non-functional or only weakly functional. However, a few of the newly synthesized peptides are better ligases than the peptides that generated them. They, in turn, ligate even more peptide bonds and, by doing so, increase the repertoire of peptides in the protocellular system. As a consequence, the likelihood of finding an even better ligase increases. When two peptides are joined to form a new peptide, the catalytic properties of the product are chosen from a probability distribution contingent upon the properties of the peptides from which the new peptide was formed. This formulation captures the biochemical intuition that when two functional peptides are joined, the catalytic center of the product will be "inherited" from one of the parent peptides (although there is also a finite probability of forming a new catalytic center).

Some of the peptides generated by ligases act as proteases and hydrolyze existing peptide bonds. Peptide bonds in disordered and, therefore, non-functional molecules are more likely to be exposed to the aqueous medium than bonds in structured peptides. Since proteases require water for their function this means that they preferentially destroy non-functional peptides. This property is incorporated into our model. As in the case of ligases, the catalytic efficiencies of protein fragments cleaved by proteases are related to the efficiency of their "parent". The conditional probabilities of ligation and hydrolysis are not independent, however. In real proteins, joining two peptides and then cutting the newly formed bond reproduces the original peptides. If the amino acid sequences are not explicitly considered this property cannot be exactly captured. However, by relating the two conditional probability

distributions by Bayes' Theorem, we can preserve this relationship for *the population* of peptides.

Using Monte Carlo (MC) methods, we have already simulated in detail the behavior of a simple system composed of only ligases and proteases (New & Pohorille 2000). That paper also provides mathematical detail of our model. We found that over a fairly wide range of parameters the number, length and overall catalytic efficiency of peptides in the system increases, and eventually reaches a steady state. The increase is determined by the balance between ligating and proteolytic activities and the bias towards the destruction of unstructured peptides. These conclusions were quite robust with respect to other parameters of the model, including the shape of the probability function.

The simple, two-function model is too restricted to describe the emergence of novelty (emergent properties) in the system. To capture these features and to provide a more biochemically faithful description of possible protein evolution, the current model has to be extended in two directions. First, we assume that some of the newly produced peptides can catalyze reactions other than ligation and hydrolysis. Examples of such reactions are pathways and cycles that lead to the utilization of external energy for activating reactants with high-energy groups (*e.g.* thioesters), synthesis of amino acids, membrane-forming amphiphiles and nucleic acids, and metabolism of small molecules. Several such pathways and cycles have been postulated on experimental grounds (see e.g. Weber 1984, 1991, 1998; Morowitz 2000). They may couple constructively to peptide synthesis, allow for protocellular growth and division and provide links to systems that involve both proteins and nucleic acids.

Second, we will add to the model new features that allow longer peptides to increase not only catalytic efficiency but also specificity. Specificity toward different substrates will be determined from the set of descriptors assigned to each peptide. A similar approach is commonly taken in Qualitative Structure-Activity Relationship (QSAR). Of course, in our case the good descriptors and their relation to functions and specificities are not known, so they will have to be assigned somewhat arbitrarily. However, they could be, in principle, established experimentally. Furthermore, we will systematically investigate the robustness of our results with respect to the mapping between descriptors and specificity. During ligation and hydrolysis, values of the descriptors will propagate according to the same probabilistic rules as catalytic efficiency.

These additions to the model require extensions of the methodology. Specifically, we want to simulate time evolution of a system with many species and many reaction channels. In the most common approach, one assumes that there are sufficiently many molecules of each species that their number can be replaced by continuous variables (concentrations) that vary deterministically over time. This leads to a coupled system of differential equations that are solved numerically. This approach, however, is poorly suited to our systems. Its basic assumptions are not well supported. Furthermore, the approach involves reaction rates that are continuous variables and species that can act either as substrates, products or catalysts of reactions. For these reasons we will use, instead, the recently developed Next Reaction Method, an exact and efficient stochastic algorithm to simulate coupled chemical reactions (Gibson & Bruck 2000; Gillespie 1977). For a system in a given state, the method defines a probabilistic algorithm to answer two questions: (1) which reaction occurs next, and (2) when does it occur? The method also allows for incorporation of other cellular processes such as channel-mediated transport and cell growth and division. This approach can be seamlessly connected with our stochastic model of a protein system.

Our simulations will be aimed at determining conditions that are necessary for evolution of a population of proteins in increasingly complicated systems. Examples of issues that we will focus on are:

(1) What are the frequencies of finding functional peptides that allow for evolution of the system and how do they compare with the frequencies estimated experimentally; (2) How does the balance between constructive and destructive processes (including substrate and product inhibition and possible emergence of useless pathways) influence evolutionary potential of the system; (3) Can we observe self-organized pathways and auto-catalytic cycles and what is the degree of complexity of the system in which they emerge; (4) How compartment-alization of proteins in vesicles influence their evolution; (5) How robust are the results with respect to the change of different parameters of the model?

3.3.4 Relevance to NASA OSS Programs

The proposed work will advance our understanding of the physical and chemical principles underlying the origins of life, as outlined in Goal 3 of the Astrobiology Roadmap. Specifically, it directly addresses Objective 3.4 devoted to investigating the origins and early coordination of key cellular processes such as metabolism, energy transduction and translation. We will follow the main approach outlined in the Roadmap – to create and study artificial chemical systems that undergo natural selection in the laboratory, without regard to how life actually emerged on Earth. Our research will be a step

towards the development of a broader discipline, a "Universal Biology", as described in the Roadmap.

3.3.5 General Plan of Work and Key Milestones

Year 1 - Obtain purified, folded, functional ATP-binding protein. Generate and test the code for computer modeling of populations of peptides.

Year 2 - Characterize first newly evolved enzyme (Diels-Alderase), and begin selection for an RNA ligase enzyme.

Year 3 - Complete high-resolution structure of ATP binding protein and compare with biological protein structures. Use directed evolution to obtain optimized versions of newly selected enzymes. Complete modeling of self-organized pathways and auto-catalytic cycles without considering vesicles in the model.

Year 4 - Evolve novel protein ligase enzymes and channel forming peptides.

Year 5 - Complete structural and biochemical studies of newly evolved RNA and protein ligases, and channel forming peptides. Complete modeling of evolution of early peptides in protocellular environments.

3.3.6 Management Structure and Statement of Contribution

Lead CoI, Dr. **Andrew Pohorille** (NASA-Ames) will be responsible for theoretical work, as outlined in objective 4 and sections 3.3.3.4 and 3.3.3.1. He will share his expertise in ion channels, as needed for accomplishing Objective 3. He will also carry out management and organizational duties associated with this project.

CoI, Dr. **Jack Szostak** (Harvard Medical School) will be responsible for experimental work, as outlined in objectives 1-3 and in sections 3.3.3.1, 3.3.3.2 and 3.3.3.3.

Collaborator, Dr. **Michael Wilson** will participate in designing and analyzing computer simulations outlined in sections 3.3.3.1, 3.3.3.3 and 3.3.3.4.

3.4 Investigation 4 Biosignatures in Chemosynthetic and Photosynthetic Systems

3.4.1 Objectives and Significance of Research

The past or present occurrence of life on an extraterrestrial planet does not ensure our ability to detect it. Recognition of life or its processes requires biosignatures that are clearly discernible against a landscape of abiotic processes, which may vary widely depending on the host environment. *The overarching goal of the work proposed here is to provide astrobiological search strategies and missions with an enhanced understanding of factors that control biosignature formation in terrestrial ecosystems.* Our approach recognizes that astrobiology search targets fall predominantly into two categories: those where detailed observation and/or physical sampling is (or could be) feasible, and those where assessment of biogenicity must be made exclusively via telescopic observation.

The first category (detailed observation and sampling) includes only bodies in our solar system. Presently, Earth is the only planet on which liquid water is stable against the atmosphere, and may therefore be the only one capable of supporting a surface biosphere. The possibility of an extant biosphere on other solar system bodies is thus limited to the subsurface, where water is stable but light is unavailable as an energy source. Metabolism in such environments must limited to non-photosynthetic anaerobic processes than can be supported by the local geochemistry. In this effort, we will explore the biological and biosignature potential of possible subsurface environments on the planets via studies of similar environments on Earth.

The subsurface environments we will study are basaltic and ultramafic (olivine-rich) rocks that contain liquid water. These rock types are (and were) abundant on planetary bodies: the crusts of differentiated bodies (Earth, Mars, Venus, 4 Vesta) contain basaltic and ultramafic rock, and most undifferentiated bodies (chondritic asteroids) are composed entirely of ultramafic rock. Liquid water reacts spontaneously with rocks of this type to generate H_2 (which could fuel microbial metabolism) and the aqueous alteration mineral serpentine (which could preserve biosignatures in a resilient lithified form). Our studies will focus on a series of northern California springs that percolate through ophiolite host rocks (sections of basaltic/ultramafic ocean crust that have been obducted onto land). These springs offer perhaps the best available terrestrial analog for the early and modern Martian crust. Our preliminary studies show that these springs support a microbial community, and likely possess an aqueous chemistry capable of providing energy to non-photosynthetic microbes. *The first major task of this investigation is to examine biosignature formation in this model system, with a primary emphasis on the mineralized component.*

The primary objectives of this task are to:

(i) Determine whether the microbial life in ophiolite-hosted alkaline springs leaves a residual mineral biosignature. We emphasize the mineralized component of this process because, in the astrobiological exploration of Mars, everything

older than a few tens of millions of years (~99% of Mars history) will either be a rock or will only be interpretable in the context of the rocks that contain it.

(ii) Assess the energetic requirements of these biological systems in order to establish boundary conditions on their potential distribution in a planetary subsurface, as a guide to sampling strategies on future life-detection missions.

For bodies beyond our solar system, any assessment of inhabitance must be made telescopically, via a basic analysis of atmospheric chemistry. It is generally believed that photosynthetic biospheres offer the best possibility for telescopic detection because: (a) by harnessing starlight as an energy source, a photosynthetic biosphere can attain a level of productivity orders of magnitude greater than that possible in a non-photosynthetic one; (b) photosynthetic biospheres can drive planetary surface chemistry dramatically away from thermodynamic equilibrium (whereas non-photosynthetic life catalyzes planetary chemistry towards equilibrium), and this disequilibrium condition may itself represent a potential biosignature. While photosynthetic biology is the engine that drives the production of chemical biosignatures, the composition and magnitude of biosignature flux ultimately depend on the specific nature of interactions between photosynthetic and non-photosynthetic elements of the biosphere. These interactions govern, for example, whether photosynthetic productivity is partitioned into volatile versus non-volatile forms, or into compounds that are diagnostically biogenic versus those that are merely ambiguous. As such, they directly impact the detectability of putative extrasolar biospheres. *The second major task of this investigation focuses on examining the nature of interactions between photosynthetic and non-photosynthetic microorganisms in tightly and loosely coupled associations, in order to understand the mechanisms by which photosynthetic productivity is transformed into detectable biosignatures.*

The primary objectives of this task are to:

(i) Determine the ultimate fate of photosynthetic carbon and electrons within several model oxygenic and anoxygenic photosynthetic microbial ecosystems, with particular emphasis on potentially diagnostic and detectable biosignatures;

(ii) Determine the chain of organisms, and organism-organism interactions that are involved in transforming photosynthetic productivity into biosignatures;

(iii) Understand how the community-level mechanisms of biosignature production are influenced by changes in the physico-chemical environment, with particular emphasis on parameters that may vary substantially during planet-biosphere co-evolution.

The anticipated significance of the proposed work lies chiefly in its potential to support the design and interpretation of astrobiology missions with respect to biosignature detection, and in its relevance to understanding the co-evolution of Earth and its biosphere. As such, this module squarely addresses Astrobiology Roadmap Goals 4 and 7. The aqueous mineral alteration studies in Task One will provide a context for interpretation of Mars rover, sample return, and Mars meteorite data. Development and quantification of the energetic habitability concept will establish stringent boundary conditions that may greatly narrow the search parameters for subsurface life on Mars. Collectively, these studies will aid in the exploration of past or presently habitable zones within our solar system (Goal 2). Task Two will directly support the design and interpretation of Terrestrial Planet Finder and related missions by identifying potential chemical search targets and examining the controls on their flux to the atmosphere. In that these targets may also be radiatively- or redox-active, these studies will also address the contribution of biology to long-term chemical and climatic evolution on Earth's surface (Goal 4). The specific emphasis on elucidating and understanding microbe-microbe interactions will help to provide a foundation for exploring Earth's uncharacterized microbial diversity, much of which may be inextricably bound in such associations.

3.4.2 Research Tasks

3.4.2.1 Task 1: Biosignatures in Chemosynthetic Systems

Build On and Extend the State of Knowledge

Earth is presently the only body in the solar system with a habitable surface environment, as defined by the stability of liquid water. Any extant life elsewhere in the solar system must therefore be capable of survival in subsurface niches, where light or other exogenous sources of energy are unavailable. Metabolically, such communities are limited to low energy anaerobic chemical processes sustained by local mineral geochemistry. This limitation on possible biospheres applies to Mars during most of its history and to Earth for some periods of its history (e.g., prior to the advent of photosynthesis, during surface-sterilizing impacts and extreme climate fluctuations). The search for evidence of life in our solar system therefore hinges on our ability to (a) discriminate the biosignatures of the subsurface environment, and (b) understand and quantify the factors that constrain the habitability of these settings.

Despite the importance of chemosynthetic ecosystems, very little is understood about either the microbial ecology of such environments or

their potential to generate detectable biosignatures. There is a lack of demonstrably analogous systems, because most accessible environments on Earth are dominated by photosynthesis or its products. Among several environments proposed as possible examples of chemosynthetic ecosystems (Baross & Deming 1995; Stevens & McKinley 1995; Chapelle et al. 2002), energy is generally hypothesized to derive from the interaction of reducing rocks (e.g., unweathered basalts or ultramafics) with water. However, detailed characterization of these systems is limited by their inaccessibility.

We propose to examine ophiolite-hosted alkaline springs as model systems in which to identify possible mechanisms of biosignature formation, and to quantify the energetic constraints on chemosynthetic communities. The continental borderland of California contains several ophiolite units that are ~150 My old. Springs emanating from these units have a unique geochemistry that has not been studied in the context of microbial ecology or biosignature formation. A predominant type of rock found in ophiolites is peridotite, which is composed almost entirely of the mineral olivine [$(Mg,Fe)SiO_4$]. Olivine is unstable at conditions near the Earth's surface, and reacts with water to form the mineral serpentine along with accessory minerals such as magnetite and brucite: Olivine + H_2O → Serpentine + Magnetite + Brucite + H_2. The "serpentinization" process yields H_2, and fluids emanating from ophiolite terranes are often highly enriched in H_2 as a result (Neal & Stanger 1984). This carries significant implications in the context of mineral-hosted life, because H_2 can be used as metabolic fuel by CO_2-reducing methanogens ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) and a host of other anaerobic microorganisms. Indeed, fluids emanating from serpentinizing terranes often contain high methane concentrations. The strong kinetic inhibition of abiotic methanogenesis during serpentinization (McCollom & Seewald 2001), suggests that much of the methane is biologically-produced. Recently, much interest has been generated by the discovery of large submarine ultramafic bodies in the process of serpentinization (Kelley et al. 2001a; Kelley et al. 2001b). Remarkably, however, the much more easily accessed surface equivalents of these systems (i.e., ophiolite-hosted alkaline springs) are largely unexplored and undocumented with respect to the life that may inhabit them.

The specific mineralogy and aqueous geochemistry of ophiolite-hosted alkaline springs suggests that they represent a particularly compelling analog for potential life-bearing systems on early or modern Mars, and on the pre-photosynthetic Earth. On the early Earth, oceanic-type crust was the principal surface rock type. However, little of this early mafic/ultramafic crust remains in a form that has not been highly altered by the continual resurfacing of the Earth via tectonic processes. Although the Jurassic-Cretaceous age of most ophiolite terranes in the western U.S. post-dates the pre-photosynthetic era of Earth's biosphere, one could argue that these highly accessible pockets of exposed oceanic crust are much more representative of early terrestrial mineralogy than are the slivers of highly metamorphosed rock that survive from that period. The potential importance of rock-hosted subsurface habitats as refugia from surface-sterilizing impacts, high UV fluxes, or extreme climate fluctuations underscores the necessity for understanding their potential to harbor life, even in a solely terrestrial frame of reference.

Most evidence thus far available suggests that ophiolite terranes may also represent the best available terrestrial analog for the early and modern crustal geology of Mars (Longhi et al. 1992; Singer & McSween 1993). The Martian meteorites so far identified are either basalts or more ultramafic rocks (Fisk & Giovannoni 1999) which closely resemble the rocks present in ophiolite sequences. Mars apparently experienced little (if any) plate tectonic activity and little compositional differentiation of its primitive lithosphere. Aqueous activity and presumably aqueous alteration would thus in all likelihood involve the stabilization of mafic and ultramafic rocks, as occurs presently in terrestrial ophiolites. This hypothesis is supported by evidence from Martian meteorites, where the predominant style of aqueous alteration within the Nakhlites (the "N" of SNC Martian meteorites) is that of olivine to phyllosilicates (Treiman & Goodrich 2002; Treiman & Lindstrom 1997; Treiman et al. 1993), in complete analogy to the serpentinization of ophiolites. Due to the similarities of the minerals and processes involved, we anticipate that studies of terrestrial ophiolite-hosted alkaline cold springs may provide significant insight into the nature of aqueous geochemistry on Mars. This is particularly important given the discovery of possibly recent aqueous seeps which suggests that even present conditions might allow for a rock-hosted chemosynthetic biosphere in near-surface regions of the Martian crust. Similarly, areas of the Martian crust that exhibit a history of aqueous alteration (e.g., the "hematite feature") are among the chief targets under consideration as possible MER landing sites. For these reasons, we believe that studies of terrestrial ophiolite-hosted alkaline springs are particularly timely.

Missions designed to search for evidence of life on bodies within our own solar system will benefit materially from input in two key areas: What to look for as evidence of present or past life

(biosignatures) and where to look (habitability). We address these issues with two hypotheses, which are formulated with an emphasis on Mars mission architecture and rock-hosted ecosystems:

Hypothesis 1: The formation of secondary mineral phases during aqueous alteration of ultramafic minerals preserves biosignature information in a highly stable lithified matrix.

Hypothesis 2: The habitability of subsurface environments is constrained by the ability of the mineral matrix to deliver chemical energy (e.g., as H_2) at or above minimum rates and levels required by mechanisms of microbial energy metabolism.

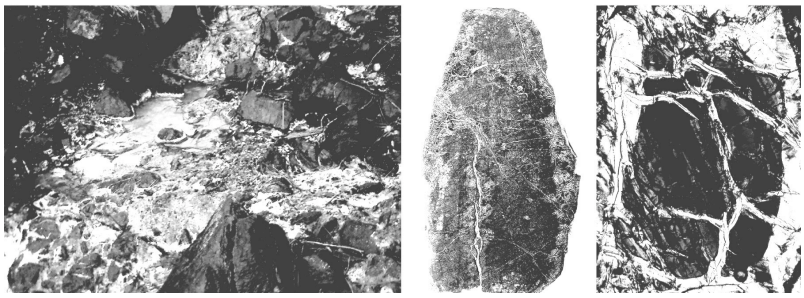


Figure 3.4-1. (a) Complexion Spring, field of view is about 2 meters; (b) hand specimen of ophiolite rock from Complexion spring showing serpentinized edges, 10 cm in width; (c) Optical micrograph of serpentinized ophiolite (crossed polars), showing single crystal of olivine complexly bisected by serpentine alteration, field of view 500 microns.

Technical Approach and Methodology

We propose to address these hypotheses with a combination of field, laboratory, and theoretical work focused on ophiolite-hosted alkaline springs. These springs will provide an excellent means for linking the work of our two hypotheses, and for tying our efforts to Mars missions, for several reasons:

(1) Ophiolite-hosted alkaline springs may be the best available terrestrial analog to the mineralogy and aqueous geochemistry of the Martian crust;

(2) Ophiolite-hosted alkaline springs combine a potential mechanism for preservation of microbial biosignatures (deposition of secondary mineral phases during aqueous alteration) with the presence of active microbial communities (which likely include metabolic types that could be supported by Mars-type aqueous geochemistry);

(3) Comparison of the aqueous geochemistry and energetics of ophiolite-hosted alkaline springs with the structure and activity of the microbial populations they support will provide real-world constraints on the magnitude of geochemical energy transduction that is required to maintain microbial energy metabolism.

Preliminary scouting trips to several locales within the Coast Range Ophiolites (northern California) indicate that Complexion Springs (Peters 1993) embodies each of these advantages, and thus represents an ideal setting in which to examine our hypotheses. Rocks collected at the site are composed primarily of the ultramafic mineral olivine, which has been extensively serpentinized along numerous fracture faces (indicating active deposition of secondary mineral phases; see Figure 3.4-1). Water emanating from this spring is enriched in hydrogen, a potential energy source for chemotrophic metabolism. A preliminary analysis of the 16S-rRNA contents of the spring water by our group indicates the presence of a variety of archaea and bacteria. Analysis of this rock-water system will allow us to simultaneously examine the influence of microbial life on the deposition of aqueous alteration minerals and correlate the community structure with the distribution of chemical potential energy within the spring. The high likelihood that Complexion Springs harbors a community of chemotrophic microorganisms (the only form of life possible in the Martian subsurface) benefits our study in several respects; however, it is noteworthy that neither aspect of the proposed research requires that the community consists exclusively of chemotrophs.

1. Serpentinization as a mechanism of biosignature preservation (addresses Hypothesis 1). We assume that robotic missions to Mars will analyze or bring back rocks and soil, not water or biological samples. We will characterize the mineral and morphological biosignatures that exist within and around ophiolite–hosted alkaline springs. The near certainty that life elsewhere in our solar system is or was exclusively microbial strongly suggests that biosignature detection efforts should be focused on developing highly discriminatory visualization and mineralogical methodologies for use at a microscopic scale. The controversy surrounding the reported presence of "life-like structures" and "mineral biomarkers" in Mars meteorite ALH84001, combined with the demonstration that similar structures and mineralogies can be found in demonstrably abiotic samples, underscores this need. The approach we propose will deploy an extensive array of chemical, mineralogical and morphological techniques (including optical petrography, electron microprobe (EMPA), field emission Scanning Electron Microscopy (FESEM), Analytical Transmission Electron Microscopy (AEM, TEM/STEM), confocal laser Raman microscopy, Atomic Force Microscopy (AFM), Scanning Tunneling Microscopy (STM), micro X-ray Diffraction/X-ray Fluorescence (μXRD/XRF) and others to a Mars-analog system where life is

known to be present, and where the deposition of secondary mineral phases is active. Detection of diagnostic biosignatures through one or more techniques would serve to suggest which particular technologies or methodologies would be most useful for flight or sample-return missions. On the other hand, equivocal or negative results with regard to biosignature detection would provide an independent and objective assessment of putative biosignatures prior to the return of data or samples from extraterrestrial sources.

Microscale characterization of rock samples from ophiolite-hosted alkaline springs. As ultramafic rocks react with water to release hydrogen, secondary sialic mineral phases (e.g., serpentines) are deposited on the active alteration front. Because these phases are precipitated along the surface, where chemotrophic microbes would most likely position themselves, the potential exists for the creation of persistent biosignatures by "entombing" microbial forms into a highly recalcitrant lithic matrix. Alternatively, some microbes actively catalyze the dissolution of substrate minerals in order to increase the energy flux they receive, which can result in the creation of definitively biogenic microscale features within the rock matrix. We will collect rocks from Complexion Springs "fixed" on-site with glutaraldehyde. Bulk mounts, fracture surfaces, thin sections and the like will be prepared. A combination of light microscopy (with DNA staining), mineralogical and micro-morphological techniques will be used to identify regions of (a) previous but not current aqueous alteration, (b) active alteration without the presence of microbes, and (c) aqueous alteration in the presence of microbes. Fracture surfaces will be prepared for FESEM using cold ion-beam deposited Ir to eliminate coating artifacts and careful analyses will be made of the exposed surfaces. Similar studies will be carried out at all scales of resolution using complementary techniques. Polished thin sections will be used for optical microscopy (with fluorescent staining) as well as petrology and Electron Microbprobe analysis. We hope to identify unique morphologies associated with the presence of microbes, as well as to characterize mineral reactants and products associated with the abiotic / microbial serpentinization processes.

Evolution of aqueous alteration fronts. Utilizing rocks collected from Complexion Springs and abiotic equivalents, we will create fresh fracture surfaces and track the evolution of secondary mineral phases in time-course aqueous alteration experiments. Bulk samples will be subjected to aqueous alteration in sealed glass vessels under a variety of conditions (see below); samples will be removed at designated intervals and analyzed through the suite of microscale characterization techniques described above. Preliminary experiments will be conducted to establish an appropriate timescale for subsampling in these time experiments. In even the most gradual weathering processes, we should be able to identify incipient alterations using high resolution imaging techniques. Initially, these time course studies will focus on the alteration process in samples that have been rendered abiotic through a combination of physical sectioning, washing, or dry autoclaving. These studies will establish a baseline for the spectrum of possible morphologies or alteration styles that can generated even in the absence of life. Subsequent experiments will examine samples that are selected specifically for the presence of active microbial populations. These experiments will be conducted under a variety of physical and chemical conditions (temperature, pH, salinity, silica concentration) that (a) represent a range of possible aqueous geochemistries that might be encountered in terrestrial or Martian settings; and (b) will likely affect rates and styles of mineral alteration.

2. Mineral-water interactions as an energy source for microbes (addresses Hypothesis 2). Habitability of a given environment is generally considered to stem from the presence of liquid water (in an appropriate temperature range) and energy that can be used in metabolism. By these criteria, much of the terrestrial and Martian subsurface might be considered habitable. However, it is less frequently considered that, in order to be useful to life, energy must be available at a certain minimum level, and must be delivered at a certain minimum rate. In surface environments, the presence of abundant energy in the form of sunlight or the products of photosynthetic metabolism means that biological energy constraints are seldom tested. Without access to sunlight or O_2, however, the metabolic processes available to subsurface microbes are frequently characterized by inherently low energy yields. For these organisms, relatively minor fluctuations in substrate concentration and flux, or in physical conditions (e.g., temperature), can render an environment energetically uninhabitable. Quantifying these microbial energy requirements, and assessing the potential of various mineral matrices to meet them, would establish further (and far more stringent) boundary conditions on the habitability of the subsurface realm. Our approach will focus on these goals, with specific emphasis on the chemistries and metabolisms that characterize ophiolite springs.

Biological Energy Quantum (BEQ) Requirement. All known mechanisms of biological energy conservation require that free energy must be available at a minimum "quantum" level, below which metabolism is not possible. The energy available for an organism to use in meeting this requirement depends directly on the concentrations

of metabolic products and reactants within the cell (Schink 1997). In some energy-limited ecosystems, the natural ordering of microorganisms into specific geometries relative to substrate sources apparently leads to the near equalization of intra- and extracellular product/reactant concentrations in cells operating at the energetic limits imposed by the BEQ (Hoehler et al. 2001a). In such systems, the magnitude of the BEQ can therefore be determined through measurement of product and reactant concentrations in the bulk extracellular fluid. We will mimic these natural conditions in a series of well-defined culture experiments that will allow us to quantify the BEQ for organisms possessing metabolisms that could be supported in subsurface environments. Our initial (and chief) focus will be on H_2-consuming methanogens, since the presumed ubiquity of both CO_2 and H_2 in the subsurface suggests this process should be considered among the leading candidate metabolisms. Briefly, the culture apparatus will supply H_2 to an active population of microbes by diffusion through a semi-permeable membrane. Colonization of the membrane by microbes (in order to gain the most direct access to substrate H_2) will continue until the flux of H_2 is reduced to levels that are minimally sufficient to meet their energy requirements. Measurement of the residual H_2 partial pressure, along with partial pressures of CO_2 and CH_4 (through periodic or continuous-monitoring GC-based techniques), will allow us to calculate the BEQ for the particular organisms in culture. These experiments will be carried out with varying temperatures, differing strains of methanogens, and ultimately, different types of (possible subsurface) metabolisms, in order to simulate a spectrum of possible subsurface environmental conditions. Where possible, these experiments will utilize microbial strains that are isolated from, or closely related to organisms present in, Complexion Spring (as determined by 16S-rRNA phylogenies).

Maintenance Energy Requirement. A second constraint on microbial metabolism is imposed by the need to obtain energy at a rate that is minimally sufficient to maintain cellular integrity (to "fix" what periodically "goes wrong" in the cell). As with the BEQ, a simple culture apparatus that mimics the natural environment will be used to quantify the maintenance energy requirement. Briefly, this apparatus will consist of small tube filled with culture medium, in which the delivery of substrates occurs solely by diffusion through opposite ends of the apparatus (e.g., H_2 through one end, CO_2 through the other). Knowing the diffusion coefficient of the medium and the flux rates of products and reactants in and out of the apparatus, the energy flux into any zone of the tube can be accurately calculated. In initial experiments, we will allow populations to grow up to a level that is at steady-state with respect to the available energy. Subsequent determination of the total biomass within the system will provide bulk estimates of the maintenance energy requirement, as a function of biomass supported. Once the methodology is well established, we will use physical sectioning or chemical microsensors to quantify energy availability through small regions within the culture, and correlate these with biomass abundance in the same region. Experiments will be conducted over a range of temperatures in order to test a hypothesized temperature dependence of the maintenance energy requirement.

Mineral H_2 Yields. A putative subsurface biosphere would depend extensively on the capacity of mineral-water interactions to deliver energy-bearing substrates (e.g., H_2) at concentrations and rates sufficient to meet the above-determined energy requirements. Minerals with high Fe^{2+} contents (e.g., ultramafic rocks) should be capable of evolving equilibrium H_2 partial pressures that far exceed the BEQ requirement. However, their capacity to support fluxes consistent with the maintenance energy requirement remains uncertain. To help quantify this capacity for mineralogies relevant to Mars exploration, we will examine rates of H_2 evolution during serpentinization of rocks from Complexion Spring. These experiments will entail creating fresh fracture surfaces on rocks collected from the springs, determining the resultant surface area of unreacted mineral, and reacting the sample with water in a sealed gas vessel. H_2 evolution over time will be observed for a variety of temperatures and fluid compositions. We will also track the alteration of the reactive surface by SEM, to determine how rapidly the capacity for H_2 evolution is diminished through deposition of sialic alteration minerals. By normalizing time-dependent H_2 evolution rates to mineral surface area, we will be able to establish the capacity of ultramafic minerals to sustain microbial metabolism under a range of conditions, as determined by their ability to meet the above-determined BEQ and maintenance energy requirements.

Complexion Springs. We will synthesize the results of our BEQ, maintenance energy, and mineral experiments to evolve a predictive capacity with respect to the potential distribution of chemotrophic organisms in rock-hosted environments. This predictive model will be correlated with and tested against the naturally-occurring distribution of chemotrophic energy sources and organisms in Complexion Springs.

We will measure the suite of physical and chemical parameters necessary for calculating the potential energy yields available to several forms of chemotrophic metabolism (e.g., H_2-based methanogenesis and sulfate reduction), using established analytical techniques that can largely be employed on-site. The overall community composition of organisms in the spring water and host rocks will be determined by constructing 16S-rRNA phylogenies through established methods. The results will be screened for the presence or absence of organisms that would appear to be allowed or disallowed on the basis of the experimentally-determined energy requirements, as a partial validation of our predictive model. For chemotrophic metabolisms whose presence is supported by both chemical (energetic) and phylogenetic analyses, we will subsequently seek to confirm the presence of actively metabolizing populations. First, we will determine bulk rates of metabolism in aqueous or mineral samples by applying previously-described radiotracer, stable isotope, or natural-abundance methodologies. Second, utilizing 16S-rRNA sequences from organisms representing the metabolisms of interest, we will design fluorescently-labeled, species-specific nucleic acid probes that will facilitate microscopic visualization of the microscale distribution of organisms in the spring system (through fluorescent in situ hybridization (FISH)). Our chief focus in this regard will be on the aqueous alteration fronts present on mineral surfaces.

3.4.2.2 Task 2: Biosignatures in Photosynthetic Systems

Build On and Extend the State of Knowledge

An astrobiological search for life in star systems other than our own offers the remarkable opportunity to observe potentially dozens of habitable worlds, where any life almost certainly emerged and developed independently from that on Earth. While such a survey could help to address some of the most fundamental questions concerning the frequency or rarity of life on habitable worlds, it will come at the cost of extreme limitations in the data sets that will form the sole basis for determination of habitation. Telescopic observation in mid-IR or visible to near-IR wavelengths can provide basic information about the size, mass, effective temperature, and atmospheric chemistry of extrasolar planets, but the collection of more detailed information falls beyond our current capacities (Des Marais et al. 2002). Characterization of atmospheric chemistry offers a potential indicator of habitation (Beichman et al. 1999), because all known life alters the chemistry of its host environment in the course of energy harvesting and biomass building. For this reason, any search for extrasolar life will depend critically on obtaining a clear understanding of the uniquely biogenic impacts of life processes on planetary chemistry. Specific examples might include gaseous compounds that are only produced by life, or levels of chemical disequilibrium (in reactive pairs such as O_2 + CH_4) that require continuous biological input to sustain.

Because the impact of biology on atmospheric chemistry must always be considered within the context of concurrent abiotic processes, planets harboring photosynthetic biospheres offer a much greater potential for telescopic detection than those with solely non-photosynthetic life (Caroff & Des Marais 2000). Photosynthetic life captures an extra-planetary energy source (starlight), and is thereby capable of greatly increased productivity relative to its non-photosynthetic counterpart (by approximately three orders of magnitude on Earth, for example (Des Marais 1997)). The result is a greatly enhanced biological "signal" relative to the "noise" of abiotic planetary chemistry. Additionally, light-aided biocatalysis can impart a degree of thermodynamic disequilibrium to planetary chemistry that might be considered uniquely biogenic, whereas non-photosynthetic life tends instead to catalyze planetary chemistry towards an equilibrium that would ultimately prevail even in the absence of life (resulting in a signal that is ambiguous with respect to biogenic character).

The capacity of a photosynthetic biosphere to contribute a flux of uniquely biogenic chemistry to the atmosphere of its host planet is constrained by: (i) the magnitude of global productivity by photosynthetic microorganisms, (ii) the specific nature of chemical or microbial processes that transform productivity into gaseous biosignatures, (iii) the sensitivity of these transformation processes to variations in physico-chemical environment. Much work has focused on understanding the global scale controls on past and present photosynthetic productivity. However, little is known about the subsequent microbial transformation of this productivity within tightly-coupled microbial ecosystems, and even less is understood about the potential response of these processes to global geochemical change, such as that occurring on the evolving Earth. Our work will seek to elucidate in detail the mechanisms involved in transforming photosynthetic productivity into potential biosignature compounds, and to understand in more basic terms the influence of a changing environment on those mechanisms.

This investigation will focus primarily on the anaerobic microbial community that is supported by photosynthesis, because these organisms are the most likely source of a distinctly biogenic flux to the atmosphere. Photosynthesizers themselves may produce the volatile and spectroscopically

detectable end-product O_2, but the potential for abiotic photochemical O_2 production makes this signal ambiguous without the contemporaneous presence of reduced volatiles, such as those produced by anaerobic microbial communities (Kasting 1997). Similarly, the primary volatile end-product of aerobic metabolism is CO_2, which yields no evidence of a biogenic origin. Anaerobic metabolism, by contrast, yields a wide array of volatile and spectroscopically-detectable products that, by themselves or in combination with other compounds, may constitute a signal diagnostic of life (Visscher 1996; Visscher et al. 2003). Indeed, any signs of life on Earth during approximately the first billion years of its habitation would have arisen exclusively from anaerobic activity. Our work will examine the liberation of reduced volatiles along with O_2 in order to address both the production of species that are uniquely biogenic in themselves, and also the creation of a "disequilibrium signature" in O_2 / reduced gas pairs.

The transformation of complex organic molecules (such as those produced by photosynthesis) in the absence of O_2 requires the coordinated activity of multiple groups of organisms (Schink 1988), often in close physical association, and the transfer through free solution of a variety of chemical intermediates (Dolfing 1988). Studies of purely anoxic systems, such as organic sediments, indicate that almost every stage of this multi-step process is strongly influenced by micro- and macro-scale variations in population structure and physico-chemical environment, such that the ultimate products of the transformation process (the prospective biosignature compounds) are subject to substantial variability (Boone & Bryant 1980; Wolin & Miller 1982; Lee & Zinder 1988; Hoehler et al. 1994). The same must be true of anaerobic transformation processes within microbial mats, the primary source of biological productivity (and therefore, of biosignature production) for nearly half the history of Earth's biosphere. Yet, the close physical association of photosynthetic and anaerobic organisms in these mats may profoundly affect the composition and flux of fixed carbon between the two groups, and dramatically alter the microchemical environment relative to organic sediments (Cohen et al. 1984; Stal & Caumette 1994). Although the nature of interaction between groups of organisms in microbial mats has been, historically speaking, perhaps the most significant influence on the biological component of Earth's atmospheric chemistry, studies in this area have just begun.

The latter stages of our previous NAI-funded research began to focus on the transformation of photosynthetic productivity, with several significant early findings. The photosynthetic members of microbial mats were shown to deliver a large flux of highly labile organic compounds and hydrogen to the anaerobic community, forming a potential chemical basis for close interactions between these populations (Albert et al. 2000; Hoehler et al. 2001b; Hoehler et al. 2002). Mat communities expressed a distinct interdependence of the microbial carbon and sulfur cycles, which represents a likely source of diagnostically-biogenic organosulfur compounds (Thamdrup et al. 2003; Visscher et al. 2003). Mats also produced appreciable quantities of methane under conditions where traditional microbial ecology principles would predict none (Hoehler et al. 2001b; Bebout et al. 2003). These processes, and the ultimate array of chemical products, exhibited marked dependence on macroenvironmental factors such as light intensity, temperature, and redox state (presence or absence of oxygen, sulfate). The proposed investigation will build on and elaborate each of these findings to create a detailed mechanistic picture of biosignature production in photosynthetic microbial communities. Quantitative mechanistic information, particularly with respect to environmental controls, will foster an ability to expand outward in the application of our findings: initially, to understanding the changing biological input to Earth's atmosphere as the planet's chemistry and solar radiation budget varied over geologic time; subsequently, to providing input for predictive, ecosystem-level models of gaseous efflux (see Investigation 5); and ultimately, to providing a highly generalized observational framework to aid in the design and interpretation of telescopic searches for life on extrasolar worlds.

Technical Approach and Methodology

Photosynthetic processes on Earth extract electrons from inorganic reductants (e.g., H_2O, H_2S, H_2, Fe^{2+}) and use them to "fix" inorganic carbon (CO_2) into organic compounds. Our overall approach is to track the fate of these electrons and fixed carbon through each stage of the transformation into reduced volatiles (e.g., organosulfur species, methane, methylhalides) or non-volatile end-products. We will characterize the major chemical pools and organisms or organism associations involved in each step of this process in order to identify specific "critical points", where the partitioning of carbon and electrons within the system are influenced by micro- and macro-environmental factors. Our work will be guided by four main hypotheses concerning the nature of these critical points:

Hypothesis 1: The production of hydrogen and highly labile organics by phototrophic members of the community creates a functional link to the anaerobic members that directly affects both the

composition and quantity of biosignature compounds that are ultimately produced.

Hypothesis 2: The flux of photosynthetic electrons into the microbial sulfur cycle strongly controls the composition of gaseous efflux from the system; rising levels of sulfate (such as characterized much of the Archaean Era) shift the flow of electrons from reduced carbon compounds towards more oxidized and often sulfur-bearing compounds.

Hypothesis 3: Conditions of low oxygen (such as prevailed during the first half of Earth history) enhance the flow of photosynthetic productivity to anaerobic microorganisms, and thereby increase the potential efflux of biosignature gases.

Hypothesis 4: The partitioning of photosynthetic productivity into the spectrum of possible end-products is directly dependent on specific physical associations between photosynthetic and non-photosynthetic organisms, and within the population of non-photosynthetic organisms.

Hypotheses will be addressed experimentally by three coordinated efforts:

1. Create a quantitative budget for the fate of photosynthetic fixed carbon and electrons, with specific emphasis on the ultimate partitioning into terminal "biosignature compounds" (addresses Hypotheses 1 and 2). A combination of chemical concentration and flux measurements, radio- and stable-isotope tracer experiments, and natural abundance stable isotope measurements will be employed to quantify the partitioning of photosynthetic resources into various end-products. We anticipate that the major steps involved in the transformation of photosynthetic productivity can be grouped broadly into four coupled processes (see Figure 3.4-2).

Productivity. Rates of carbon and electron fixation by photosynthetic microorganisms will be determined in order to (i) quantify the pool of starting materials available for the ultimate production of reduced volatiles, (ii) provide a simultaneous measure of O_2 production (for use in quantifying the creation of "disequilibrium signatures"), and (iii) provide a normalization factor that will facilitate the scaling of biogas flux measurements up to planetary scales, using published estimates of past and present global productivity. The total influx of reducing equivalents attributable to photosynthesis will be determined at several times of day via oxygen microelectrode measurements employing the dark-shift method (Revsbech et al. 1981). Our previous NAI-supported work experimentally quantified the sensitivity of these rates to light intensity and temperature, allowing us to estimate daily integrated rates of gross photosynthesis by interpolation between measured time points. The total amount of carbon fixed by photosynthetic organisms will be determined by measuring the integrated flux of CO_2 into the system by means of a flux chamber methodology (Canfield & Des Marais 1993; Des Marais 1995). Finally, the daytime accumulation of fixed carbon as storage polysaccharides (SPS) and osmoregulants will also be quantified; we hypothesize that this reservoir serves as the primary source of carbon and electrons to the associated community of anaerobes, via fermentation into highly labile forms.

Fermentation. Our previous NAI work showed that much of the carbon and electrons fixed by photosynthetic organisms during daylight hours is subsequently released at night in the form of organic acids and hydrogen – strongly suggesting that fermentation of fixed-carbon storage products is the primary pathway for mobilization of photosynthetic productivity into the chain of transformation (Albert et al. 2000; Hoehler et al. 2001b). Because these simple fermentation products can be directly utilized by many anaerobic microorganisms, their production may provide a functional link between the phototrophic and non-phototrophic populations in the system (Hoehler et al. 2001b). Fermentation rates will be

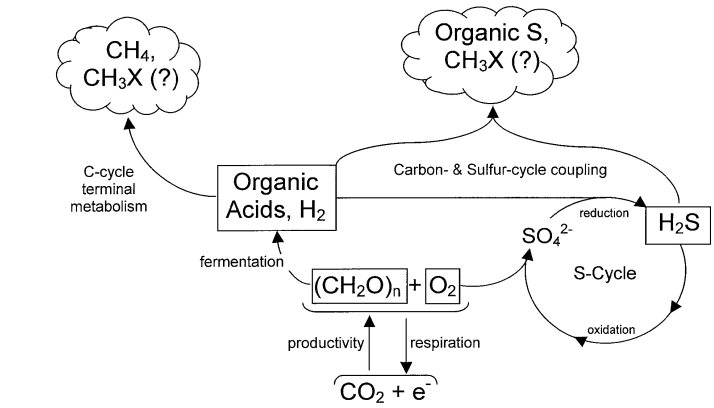


Figure 3.4-2. Major steps in the anaerobic transformation of photosynthetic productivity. Our approach will quantify the flow of carbon and electrons fixed by primary productivity through each of the major processes and reservoirs identified in this diagram. Molecular biology and organic biomarker approaches will be used to characterize the organisms that mediate the transformation process. At major "junction points", where carbon or electrons may have alternate fates (e.g., H_2/Organic Acid or H_2S pools), we will characterize the micro- and macro-environmental factors that regulate their ultimate partitioning into the spectrum of biomarker end-products.

determined by quantifying the time-dependent decrease in SPS content, and the increase in concentrations and fluxes of H_2 and C_1-C_5 organic acids. Measurements will be made under natural conditions and following the inhibition of potential consumption pathways (e.g., oxic respiration, sulfate reduction, or methanogenesis) in order to estimate total gross rates of fermentation.

Electron Transfer to Sulfur Cycle. Due to high oceanic concentrations of sulfate on the modern Earth, and the metabolic accessibility of fermentation end-products to sulfate-reducing bacteria, the sulfur cycle represents the dominant sink for photosynthetic carbon and electrons in marine or hypersaline microbial mats (Jørgensen & Cohen 1977; Canfield & Des Marais,1993). The coupling of carbon and sulfur cycles in phototropic systems carries several important implications with respect to production of biosignatures: (i) electrons that enter the sulfur cycle and are further transformed through oxidative inorganic sulfur metabolism will be rendered largely non-volatile, and therefore insignificant for atmospheric chemistry (this represents a decrease in the maximum possible contribution of photosynthesis to remotely detectable biosignatures); (ii) some of the volatile organic sulfur compounds that result from coupling of the biological sulfur and carbon cycles may be difficult to form abiotically, and might therefore provide a more robust biosignature than volatile carbon compounds (Visscher et al. 2003); (iii) through their own high levels of productivity, organisms associated with an active sulfur cycle can "recycle" energy and electrons back into the pool of storage polysaccharides; (iv) abiotic "sulfurisation" reactions involving sulfide and unsaturated organics can serve to couple carbon and sulfur isotopic information into lasting organic biomarkers, of potential utility in understanding Earth's early history; (v) oceanic sulfate concentrations are believed to have increased more than 100-fold since the Archaean, so that the coupling of the carbon and sulfur cycles (and the importance of (i) and (ii)) may have varied substantially as the Earth system evolved (Habicht & Canfield 1996; Canfield et al. 2000). The primary link between the carbon and sulfur cycles, is the biological reduction of sulfate to sulfide using fermentative electron donors. Rates of sulfate reduction will be quantified by measuring the accumulation of radio-labeled $H_2{}^{35}S$ from $^{35}SO_4{}^{2-}$ (Jørgensen 1978), and by advection-diffusion-reaction modeling of microelectrode-derived H_2S profiles. Subsequent production of organic sulfur compounds from H_2S will be quantified by GC and HPLC techniques (Visscher et al. 2003), and compared to experimentally-determined abiotic reaction rates for these compounds. Incorporation of H_2S into the inorganic sulfur cycle will be quantified by measuring changes in the pools of elemental sulfur, thiosulfate, and sulfate.

Terminal Metabolism. In the final stages of photosynthate transformation, carbon and/or electrons are converted into volatile compounds that can escape the system. These compounds represent the ultimate influence of the photosynthetic system on atmospheric chemistry, and the "fingerprint" by which any determination of biogenicity must be made. We anticipate that methane, organic sulfur compounds, and methylhalides will constitute the major volatile end-products of the transformation of photosynthetic productivity. Release of these compounds from the system will be measured using a flux chamber methodology, and possibly via open-system techniques (bubble flux measurements, open-path laser array system). This methodology will permit easy quantification of shifts in carbon/electron partitioning that result from imposed environmental manipulations.

Overall. The coupled effects of each of these processes will be assessed in isotope-tracing experiments that follow the fate of labeled carbon through the system as a whole. Radio- or stable isotope-labeled CO_2 will be introduced into the system during peak rates of photosynthetic carbon fixation. The techniques described above will be used to quantify the partitioning of labeled material into specific chemical pools at discrete time intervals following its introduction. These experiments will present a time-dependent, whole-system view of photosynthate transformation, and will also aid in identifying the specific groups of organisms involved in the process (see following section).

2. Determine the key organisms and organism-organism interactions that mediate the transformation of photosynthetic productivity (addresses Hypothesis 4).

Molecular methodologies and organic biomarker analyses will be combined with isotope labeling techniques to ascribe phylogenetic identity to the major groups of organisms involved in the transformation process. The application of a culture-independent approach is critical for the systems in this study, because >99% of mat associated microorganisms are phenotypically uncharacterized and have yet to be grown in culture (Amann et al. 1995). In the isotope tracing experiments described above, we will follow the incorporation of labeled compounds into lipid biomarkers (elucidating the involvement of specific functional groups) and into DNA/RNA (elucidating phylogenetic identity). By introducing the label in different chemical forms (representing different stages of the chemical transformation

process), the organisms associated with specific steps or chemical transfers will be individually targeted. Population sizes of key groups of organisms will be assessed by monitoring natural abundances of lipid biomarkers that are labeled in the isotope experiments and ascribed a phylogenetic origin through DNA-based identification methods. These findings will be linked to the rock record of recalcitrant organic biomarkers, which currently provide the best evidence of specific microbial functionality in Earth's deep past.

Using ribosomal RNA phylogenies constructed through our previous and proposed NAI work, and the genetic identities ascribed in the above experiments, we will design group- and species-specific nucleic acid probes for key organisms in the transformation process. Applying these fluorescently-labeled probes in FISH assays will facilitate microscopic visualization of organisms in three dimensions (by laser scanning confocal microscopy), allowing us to infer the specific assemblages or physical juxtapositions of organisms that are involved in chemical transfer (DeLong et al. 1989; Upton et al. 2000). Furthermore combination of FISH with secondary ion mass spectrometry (SIMS) will enable fine scale determination of the assimilation and transfer of specific stable isotope-labeled substrates, confirming which organismal associations are involved with specific chemical transfer processes (Orphan et al. 2001; Orphan et al. 2002). The structure and function of these associations can then be followed through the course of ecophysiological manipulation studies, as described in the following section.

3. Identify the physico-chemical factors that influence the partitioning of photosynthetic resources into different biosignature groups (addresses Hypotheses 2 and 3).

The multi-step nature of photosynthate transformation exposes multiple critical points, where the flow of carbon and electrons may be channeled into differing sets of products or pathways, depending on external forcing factors. Abiotic processes (e.g., in parts of the organic and inorganic sulfur cycle) depend on relative pool sizes among products and reactants, and on the wealth of physico-chemical parameters affecting the thermodynamics and kinetics of these reactions. Biological processes share these chemical sensitivities, and are also influenced by variations in gene expression, population size, and activities of partner organisms (parameters that are, themselves, controlled by physico-chemical variability). Collectively, these effects have a dramatic influence on pathways of organic transformation in purely anoxic systems, and we anticipate that the same will be true of anaerobic processing in phototrophic systems.

Using the results of our coupled biogeochemistry and molecular biology studies, we will identify the major critical points (in terms of organisms or chemical intermediates) in the transformation process. Subsequently, we will isolate these critical points through the use of simplified model systems, enrichment cultures, or FISH-probing, and determine the effects of variable environmental conditions. Our primary goal will be to determine the effects of variable oxygen and sulfate concentrations, since these likely represent the primary species through which Earth's evolving redox state has been "sensed" by biological communities over geologic time. We will examine the influence of variable sulfate concentrations on the balance of oxidizing and reducing metabolism with respect to carbon compounds (e.g., CO_2 versus CH_4 production), and on the degree of coupling between the carbon and sulfur cycles. The sensitivity of sulfur isotope fractionation in various pools to the concentration of sulfate will be determined concurrently as a way to link our studies to the Archaean sulfur isotope record. We will examine the effect of variable atmospheric oxygen levels on the flow of productivity to the anaerobic community (e.g., decreasing the productivity flux due to increased consumption by aerobic respiration or limiting the spatial distribution of anaerobes through toxicity effects). We will also determine the influence of variable O_2 levels on the partitioning of H_2S electrons into oxidative metabolism (chiefly non-volatile products, but potential "recycling" into SPS pool) versus formation of volatile organic species (potential utility as biomarkers). Each of these variables will be studied with respect to chemical cycling and organismal function (at the metabolic, gene expression, and population levels), with results providing a basis for inclusion of environmental forcing parameters in the numerical simulations of Investigation 5.

Field Sites. Our model systems for this work will be the hypersaline microbial mats of Baja California, (which are well-characterized with respect to general chemical function and microbial composition through our previous NAI work) and the thermophilic microbial mats of Yellowstone National Park (YNP). The majority of this work will be performed on samples returned from Baja and maintained in the Ames greenhouse facility, which permits substantial environmental manipulations at the community level. In addition to the primary focus on cyanobacteria-dominated mats, we will undertake parallel studies of photosynthate transformation in mats dominated by eukaryotic photosynthesis, and by anoxygenic photosynthesis. The eukaryotic system (mats of the red alga *Cyanidium* in YNP) will extend our studies to include acidic conditions (pH 0.5-3.5)

and variability in cell structure (eukaryotic versus prokaryotic). The anoxygenic system will embrace a critical period in the co-evolution of Earth and its biosphere – a time with potentially high rates of global productivity, but with insignificant levels of environmental free oxygen. These studies will be based on mats of *Chromatium* and/or *Chloroflexus* found in YNP, and on greenhouse-maintained mats in which oxygenic photosynthesis has been chemically inhibited.

3.4.3 Relevance of Proposed Work

The principal importance of our work in an astrobiology context lies in its intent to provide basic science content relevant to the design and interpretation of search-for-life missions within and outside our solar system. The focus on identifying useful biosignatures in two widely disparate biosphere types directly addresses Roadmap Goal 7. Because our studies are based on terrestrial ecosystems, they are also inherently relevant to understanding the co-evolution of Earth and its biosphere (Roadmap Goal 4). For example, microbial mats represented the principal biologic agents of global geochemical change throughout most of the Precambrian Era. Our studies of the factors that control and channel photosynthetic productivity within these systems will aid in understanding their role in the oxidation of the planet's surface, or in the liberation of climatically-important trace gases. Similarly, our studies of the factors that constrain life in the subsurface realm offer a possible insight into the nature of Earth's earliest biosphere, and of the viability of deep refugia from events that might render the surface uninhabitable. This work also addresses Goal 1 in that it may help to revise our concept of habitability to include stringent, energetic constraints on metabolism.

3.4.4 General Plan of Work and Key Milestones

Year 1 - Basic characterization of ophiolite spring geochemistry and microbial population structure; initial isotope tracing experiments to quantify carbon and electron flow in microbial mats; feed results to the numerical model of Investigation 5 (continued in years 2-5).

Year 2 - Conduct electron microscopy characterizations and experiments on ophiolite samples and begin bioenergetics experiments; lipid biomarker studies in microbial mats and ecophysiology study using mat and sediment communities.

Year 3 - Continue electron microscopy and bioenergetics experiments related to ophiolite springs; long-term manipulation studies of oxygen and sulfate availability in microbial mats.

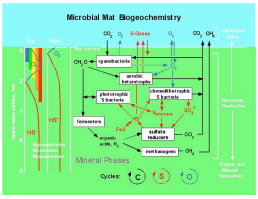
Years 4-5 - Deploy genome specific probes and perform microscopic characterization of organismal associations in both environmental settings; metabolic process measurements in ophiolite springs; continued manipulation and ecophysiology studies in microbial mats, culminating with large-scale group experiments for validating mechanistic models.

3.4.5 Management Structure and Statements of Contribution

Management: **Tori Hoehler** (Ames, Lead CoI) is responsible for overall coordination of the investigation and manages Task 2. **David Blake** (Ames, CoI) manages Task 1.

Science: **Daniel Albert** (U. North Carolina, CoI), a biogeochemist and expert in anaerobic fermentation processes; will characterize the processes related to cycling of organic acids in photosynthetic mats. **David Blake** (Ames, CoI), a leading mineralogist/geologist who has developed mineralogy instrumentation for possible deployment in Mars missions, will be responsible for electron microscopy and petrologic analysis of ophiolite samples. **Donald Canfield** (Odense University, collaborator), a leading biogeochemist who has pioneered the use of sulfur stable isotopes in studying the redox evolution of Archaean Earth, will link studies of sulfur transformations and sulfur isotope chemistry in the photosynthetic mats to the Archaean sulfur isotope record. **Richard Castenholz** (U. Oregon, collaborator), a microbiologist and leading authority on the cyanobacteria, will be responsible for the studies of acidophilic eukaryotic systems of microbial mats; **David Des Marais** (Ames, CoI) a biogeochemist and authority on stable isotope geochemistry, will be responsible for flux experiments and measurements of photosynthetic productivity. **Tori Hoehler** (Ames, Lead CoI), a biogeochemist and leader in applying thermodynamic principles to the ecology of microbial ecosystems, will be responsible for the bioenergetics studies and for measurements of H_2 cycling and terminal metabolism in springs and microbial mats. **Tom McCollom** (U. Colorado, collaborator), a geochemist and expert in the thermodynamics of aqueous geochemistry, will employ computation models to characterize the ophiolite springs with respect to free energy potential. **Linda Jahnke** (Ames, CoI), a microbiologist and expert in lipid biomarker analysis, will be responsible for the studies of lipid biomarkers in the microbial mats. **Victoria Orphan** (Ames, collaborator), a molecular biologist who has pioneered the coupling of microscale biological and geochemical analyses for use in microbial ecology, will be responsible for molecular biology and microscopy in the ophiolite springs and microbial mats. **Mitchell Schulte** (Ames, CoI), a geochemist and leader in modeling the energetics of aqueous geochemical processes, will lead the ophiolite field campaign, and will characterize the aqueous

geochemistry and energetics of these springs. **Roger Summons** (MIT, collaborator), a leader in the field of lipid biomarkers who has established the earliest evidence of oxygenic photosynthesis, will correlate studies of lipid biomarkers in photosynthetic mats with analyses from the Archaean rock record of organic fossils. **Bo Thamdrup** (Odense University, collaborator), a microbial ecologist and expert in microbial sulfur cycling, will characterize the oxidative sulfur cycle, sulfur isotopes, and ecophysiology of photosynthetic mat communities. **Allan Treiman** (LPI, CoI), an authority on Mars meteorites and aqueous alteration processes, will participate in ophiolite-related field work, and characterize samples with respect to petrology and primary and secondary mineralogy. **Pieter Visscher** (U. Conn., CoI), a microbiologist and expert in the ecology of microbial mats, will characterize the photosynthetic mat communities with respect to organic sulfur cycling, methylhalide fluxes, and ecophysiology.



3.5 Investigation 5 Modeling Ecosystems and Biospheres

3.5.1 Objectives and Significance of Research

The creation of a mathematical simulation model of photosynthetic microbial mats is an important stepping stone in our understanding of key biogeochemical cycles that may have altered the atmospheres of early Earth and of other terrestrial planets. A modeling investigation is presented here as a tool to utilize and integrate empirical results from research in Investigation 4 Photosynthetic Systems (of this proposal) into a computational system that can be used to simulate biospheric inputs of trace gases to the atmosphere.

Photosynthetic microbial mats are prokaryotic assemblages that grow from the topmost photic layers of submerged or partially submerged sediment. These microbial communities were abundant in shallow seas surrounding continents during the Precambrian, primarily during the Proterozoic era from about 2.2 billion years onward. (Brock & Madigan 1991; Castenholz 1994). Microbial mats are significant to the field of Astrobiology because of their paleontological importance and because their trace gas emissions to Earth's atmosphere, when modeled, can serve as remote indicators of life (a.k.a., biosignatures, sensu Hoehler et al. 2001b) elsewhere in the solar system. The evolution of cyanobacteria, (the major bacterial guild of these mat ecosystems) with their ability to use water as a source of reducing power, probably caused Earth's biosphere to increase productivity by 2-3 orders of magnitude (Des Marais 1997). Despite their inherently high productivity and dominant role in evolution of the biosphere, many of these cyanobacterial mats are found in environments that today could be considered extreme or stressed, at least with respect to salinity, acidity, temperature, radiation flux, and potential for desiccation.

Microbial mats residing in benthic marine and hypersaline environments may be living analogs to biological communities of early Earth. Thus, they are useful in understanding biotic-atmospheric interactions of early Earth and in identifying biosignatures, particularly for O_2, CO_2, H_2S, and CH_4. Notwithstanding recent advances in understanding how hypersaline microbial mats function (Bebout & Garcia-Pichel 1995; Des Marais 1995; Hoehler et al. 2001b), many uncertainties remain concerning the importance of physical controls, especially the light limitations, energy fluxes, and the chemical environment. These uncertainties have not yet been addressed fully on an ecosystem scale. In past years, numerous field and laboratory experiments have contributed to the body of knowledge on these communities (e.g., Castenholz 1994; Nübel et al. 2001; Overmann & van Gemerden 2000; Weiland et al. 2001). Such experiments are, by nature, limited in their applicability to the study of microbial interactions over a variety of time scales (from hours to years to millennia), and in their capacity to explain complex biogeochemical interactions between (aerobic) primary producers and (anaerobic) consumer and fermenter bacterial groups. Simulation modeling can be used with new experimental results derived by Investigation 4 to explain complex biochemical interactions of photosynthetic microbial ecosystems at progressively larger temporal and spatial scales. Generally, computer modeling is a highly cost-effective means to create a "virtual laboratory", in which we can expand and test understanding of Astrobiology concepts. Models allow us to explore scenarios and assess implications of experimental findings in an efficient manner that can compliment more expensive field-based measurement activities.

The overall objective of this research investigation is to refine and evaluate simulation models of energy relations, biogeochemical cycling, trace gas exchange, and biodiversity in microbial mat ecosystems with the goals of extrapolating biosignatures of early Earth ecosystems to the scale of a planetary biosphere. We will extend the predicted results from our

models over billions of years of ecological change due to environmental forcing conditions.

The significance of the proposed modeling work is to efficiently and comprehensively evaluate the limits of microbial evolution and ecosystem functions in ways that will support NASA missions aimed at explaining biosignatures on distant planets.

Specific Study Objectives:

- Predict (using simulation modeling) and validate (using experimental measurements) biochemical cycles, and biosignature emissions of O_2, CH_4, and reduced S gases from the water surface to the atmosphere above photosynthetic microbial mats.
- Investigate the spectral properties and light use efficiency of microbial mat ecosystems using satellite image analysis and in-situ spectroradiometery.
- Extend the generalized model of photosynthetic microbial mats in hypersaline subtidal environments to simulate other prominent microbial ecosystem types (with their peculiar physical and geochemical properties) that may have influenced the atmosphere of early Earth.
- Incorporate results from studies of diversity in microbial mats into the generalized model of microbial ecosystems and biospheres.
- Discern variation in biosignature emissions due to spatial and seasonal environmental fluctuations of a biosphere.

3.5.2 Research Tasks

Build On and Extend State of Knowledge

We have developed a simulation model called MBGC (Microbial BioGeoChemistry) to infer effects of major environmental controllers on microbial community structure and function (Decker & Potter 2001). Microbial growth, metabolic reaction rates, and mass flows are represented within and between vertical sediment layers of a microbial mat in the hypersaline environment (Figure 3.5-1). In addition, diel cycles are simulated to capture natural variation in environmental boundary conditions, such as light and temperature.

The fundamental structure of our MBGC simulation model follows that of a previous model (de Wit et al. 1995) of population dynamics and biogeochemistry within benthic mats containing cyanobacteria (CYN), purple sulfur bacteria (PSB) and colorless sulfur bacteria (CSB). Specific additions were incorporated recently into our MBGC model in order to (1) represent CYN photosynthesis metabolism operating as a light-driven quantum efficiency function, (2) complete the microbial sulfur cycle, and (3) enable a comparison of the model predictions to hourly field-measured biogeochemical fluxes. More specifically, we have added sulfate-reducing bacteria (SRB) to MBGC, which can consume organic carbon and produce the H_2S oxidized by PSB and CSB. In addition, molecular hydrogen (H_2), decomposition of dead organic matter (DOM), CO_2 fluxes, and realistic (time-varying) temperature and light controls on major metabolic pathways are included in MBGC as extensions of the original de Wit model structure.

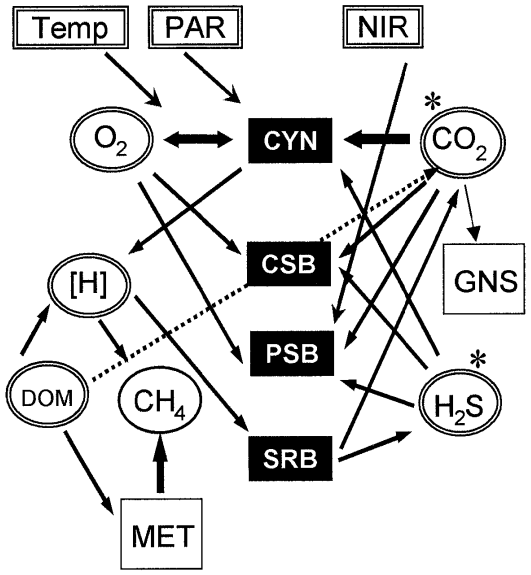


*Figure 3.5-1. Schematic representation of the MBGC model (Decker and Potter, 2002), including bacterial pools (squares), light (PAR, NIR) and temperature input as model drivers, and gas concentration pools (circles). Each vertical layer of the model is comprised of all model pools shown and adjacent layers are coupled by gas fluxes between layers. Fluxes between pools are shown as arrows. Existing components of MBGC are shown as double outline symbols, whereas new components that we are proposing to add are shown as single outline symbols. Key to new component symbols: MET are methanogenic bacteria, GNS are green non-sulfur bacteria, * are isotope tracers.*

MBGC was constructed with several noteworthy initial assumptions. Major bacterial groups compete for, or are inhibited in growth by, metabolic substrates such as O_2 and H_2S. Photosynthetically active radiation (PAR between 400-700 nm) limits the growth of the CYN population, while near infra-red (NIR) radiation limits the growth of PSB population. Mass flows control gas exchange rates between sediment layers. Surface water flow affects O_2 diffusion rates from the mat to the water through the diffusive boundary layer (DBL). Finally, concurrent aerobic chemosynthesis and anoxygenic photosynthesis by PSB is possible, but its occurrence depends on the concentration of bacteriochlorophyll-a (Bchla).

Early results from MBGC simulations show that the model reproduces major diel fluctuations in trace gases in hypersaline subtidal mat layers and into the overlying water column (Figure 3.5-2). To extend our knowledge further of the principal controls over the light/energy relations and biochemical pathways by which these mat ecosystems consume and emit trace gas biosignatures, we are testing various approaches to simulating the net hourly emissions of O_2, CO_2, and H_2S gases to the atmosphere above the mat ecosystem over the course of several days during which actual measurements of these same fluxes have been made.

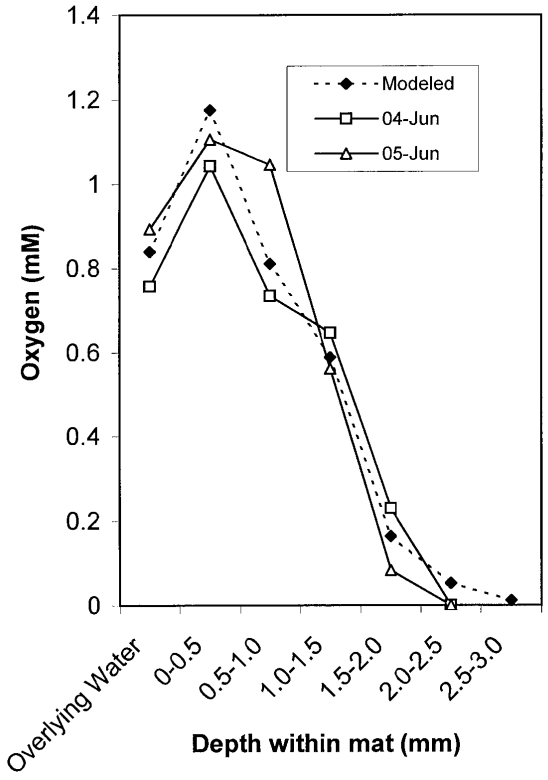


Figure 3.5-2. Oxygen concentration within the top layers of a cyanobacterial mat at mid-day for two dates in June 2001 in salt ponds near Guerrero Negro, Baja (Source: S. R. Miller), compared to results of a MBGC simulation using light and temperature values measured during the same period of the field measurements.

At Baja California salt ponds near Guerrero Negro, Mexico where such diurnal fluxes have been measured by other NAI investigations, we have recently collected near-simultaneous *in situ* spectral data and satellite images of radiation absorption and reflectance of subtidal microbial mat communities. After correction for the absorption of incident solar radiation by the overlying water column and the atmosphere, we can attempt to isolate the absorption spectrum of the mat community to infer spatial variability of PAR and NIR attenuation by the various bacteria groups (Figure 3.5-3).

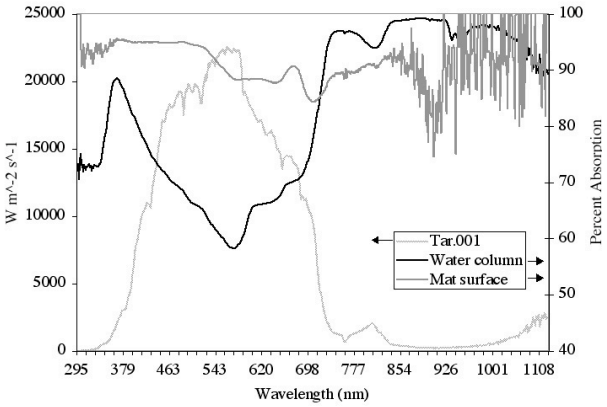


Figure 3.5-3. Spectral results from absorption and reflectance studies of subtidal microbial mat communities in September 2002. The water column was found to absorb 60-75% of incident PAR energy and more than 95% of incident NIR energy entering at the pond surface. The subtidal mat absorbs 85-95% of the residual PAR and NIR energy that is transmitted through the water column from the pond surface. TAR is the solar irradiance input at the water column surface in units of W m^{-2} s^{-1}

In order to further build on and extend state of knowledge of biosignatures from these early Earth ecosystem analogs, we propose to evaluate the following hypotheses within our modeling framework. Our primary means of testing each of these hypotheses will be to compare measured fluxes and pools to new predictions from the MBGC model that is modified in various alternative versions.

- Light use and photosynthetic rates in MBGC mat simulations are strongly influenced by prescribed properties of the overlying water layer and that of its biological constituents.
- The fate of atmospheric carbon fixed in the MBGC mat can be predicted by incorporating fluxes and stocks of fixed C (organic acids) and electrons (via organic acids and H_2) into the existing MBGC simulation model.
- Sulfate reduction in the photic (oxygenated) zone of the photosynthetic MBGC mat can be maintained by simulated pools of SRB that are capable of metabolism in oxic zones of the model ecosystem.
- Methane production rates in the photosynthetic MBGC mat depend upon the presence of simulated pools for both competitive (with SRBs) substrates (e.g., H_2, organic acids) and non-competitive substrates (e.g., osmoregulant such as methylamines) interactions that are sensitive to a variety of environmental conditions.

Technical Approach and Methodology

Ecosystem model development

We will continue to expand and refine the MBGC simulation model of microbial mat ecosystems using all available computer programming tools at NASA Ames. Our technical approach will involve synthesis of measurement data and observations from all published sources and from experiments carried out by our co-investigators on this proposal. This will result in a combined effort to develop new metabolic response algorithms and computation approaches with which to quantify trace gas biosignatures as characteristic exchanges of energy and minerals between the simulated ecosystem and the atmosphere. The modeled gas fluxes will be validated at short time scales (hours to days) and then run over a series of evolutionary scenarios that will represent millions of years of change in planetary climate and geochemical conditions. The MBGC structure will allow us to simulate changes in microbial communities in response to scenarios of changing redox conditions (ambient O_2 levels and geochemical H_2S sources) and predict the outcomes of competition among several major bacterial groups for survival and productivity.

Our overall plan of work is to evaluate MBGC modeling approaches that can accurately simulate the carbon and energy cycles in microbial mats, from the point at which CO_2 is fixed into products of photosynthesis, through decomposition and fermentation of organic acids, and finally to the biogenic emissions of reduced gas forms (biosignatures) such as CH_4 and H_2S. Our MBGC model simulations will be designed to aid our co-investigators in Investigation 4 in understanding the potential fate of carbon that is fixed during daytime photosynthesis and transformed during subsequent dark periods as non-photosynthetic bacteria utilizes this carbon for growth.

It will be necessary to first add several variables to the MBGC model to simulate the metabolism and population growth dynamics of methanogenic bacteria (MET) and green non-sulfur bacteria (GNS). We will also add pools and fluxes to assess the electron budget. We will work closely with co-investigators in Investigation 4 to develop the necessary understanding of metabolic processes of these new MET and GNS pools in the model. We anticipate, for example, the inclusion of both competitive and non-competitive substrate interactions by which growth dynamics of MET populations can be simulated and evaluated.

Secondly, we will add mathematical tracer components to the MBGC model as another computational approach to understanding the potential pathways of decomposition of biogenic organic matter and its eventual emission back to the atmosphere in reduced gas forms. Tracers such as stable carbon isotopes and ^{14}C and $^{35}SO_4^{2-}$ radioactive radioactive markers will be added to the MBGC model in an attempt to replicate the inferred (from measurements) pathways, beginning from initial fixation during CYN photosynthesis, to biogenic emissions of carbon biosignatures such as CO_2, H_2S and CH_4.

Once our MBGC simulations of subtidal hypersaline microbial mats are completed, we will generalize the model to simulate other prominent microbial communities and geochemical environments. Chief among these other microbial communities for MBGC simulations will be inter-tidal marine mats, which experience higher levels of oxygen circulation (by wave action), and green non-sulfur mats, like those that grow commonly in thermophilic environments ranging from 35°C to 72°C. These green sulfur mats may exist either anaerobically via photosynthesis or aerobically by fermentation and use sulfide or H_2 as electron donor for CO_2 fixation.

Satellite Image Analysis

To obtain simultaneous field measurements and satellite images of radiation absorption and reflectance in microbial mat communities, we will first determine optimal days and times to collect spectroradiometry data at selected locations where ongoing NAI investigations of microbial mat biogeochemistry and biosignatures are being conducted. Local meteorological conditions, seasonal sun angles and satellite overpass schedules will be evaluated. These combined data sets will provide the required information to select representative sampling periods for field sampling trips. NASA Earth Observing System (EOS) satellite images will be obtained to coincide as closely as possible to all selected close-range spectral sampling periods.

We will deploy optimal field-based methods for configuration of portable spectroradiometer and GPS equipment used for microbial mat reflectance sampling through the presence of a natural water surface or column. Calibration of close-range spectroradiometer data under variable conditions of light and wind will be evaluated. Field data sets will be returned to NASA Ames for entry into a geographic information system (GIS) and comparison with newly acquired EOS satellite imagery from the same periods of time. We will develop optimal methods for remote sensing calibration and classification of microbial mat communities. Collection of close-range spectroradiometry data, precise GPS coordinates, physical measurements of the surface water, and local meteorology on the same dates as EOS satellite image acquisition will enable us to make substantial corrections (e.g., for atmospheric interference and sun angle effects) that normally

confound calibration and classification procedures for satellite imagery. We will work to produce a new GIS of diversity in microbial mat pigments and surface physical properties, which together regulate microbial gas exchange with the atmosphere and other biogeochemical processes. This GIS will be used to drive the MBGC simulation model of microbial mat ecology and biogeochemistry at multiple spatial scales.

Biosphere model development

We will transfer subsets of trace gas biosignature algorithms and process-level metabolic information from individual ecosystem simulation runs using the MBGC model to extrapolate emission fluxes of O_2, CO_2, H_2S (and other reduced forms of S), and CH_4 to the scale of an entire planetary biosphere. Our technical approach will involve the development of relatively simple zonal biosphere models that can represent geographic (e.g., latitude zonal) and seasonal variability of these emission fluxes, beginning from about 2.5 Gyr ago to the past several million years. This is same approach that was used by Potter et al. (1996, 1998) to construct modern Earth budgets of seasonal trace gas bio-emissions.

We will evaluate our zonal biosphere models that represent geographic and seasonal variability of O_2, CO_2, H_2S, and CH_4 emission fluxes, beginning from about 2.5 Gyr ago to the past several million years. Global microbial sources of these historical gas emissions from shallow aquatic environments will be computed and compared to modern global emission sources. Digital versions of these historical gas emission predictions will be shared with other NAI teams who are interested in coupling biosphere to atmosphere evolution.

Relevance of Proposed Work

The development and refinement of our MBGC model addresses the following Astrobiology Roadmap Goals: (4) to understand how past life on Earth interacted with its changing planetary and Solar System environment, (5) to understand the evolutionary mechanisms and environmental limits of life (specifically with regards to the biochemical limits), (6) to understand the principles that will shape the future of life, both on Earth and beyond (especially Objective 6.1 - Environmental changes and the cycling of elements by the biota, communities, and ecosystems), and (7) - Determine how to recognize signatures of life on other worlds and on early Earth.

3.5.3 General Plan of Work and Key Milestones

Year 1 - Add new MBGC model components and evaluate research hypotheses using measured data from CYN microbial mats. Select new field sites for remote sensing of microbial mat ecosystems.

Year 2 - Evaluate research hypotheses using measured data from several types of microbial mats. Visit new field sites for near (and remote) sensing of microbial mat ecosystems. Submission of journal manuscripts.

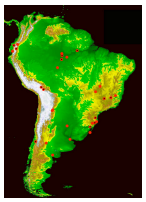
Year 3 - Develop and evaluate new research hypotheses using measured data from several types of microbial mats. Develop first set of biosphere model codes.

Year 4 - Develop and evaluate next set of biosphere model codes. Select and visit new field sites for near (and remote) sensing of microbial mat ecosystems.

Year 5 - Continue as above and finish all research activities with submission of journal manuscripts.

3.5.4 Management Structure and Statements of Contribution

Day-to-day management of the research investigation will be the responsibility of **Christopher Potter** (Lead CoI), a NASA Civil Servant at ARC. Potter is an ecosystem scientist and modeler who will be responsible for coordination and integration of the major components including modeling design, data analysis-interpretation, and dissemination of results through written reports. **Kelly Decker** (CoI) and **Steven Klooster** (CoI) will operate under an existing Memorandum of Understanding (MOU) and the Cooperative Agreement between NASA ARC and the Foundation of California State University at Monterey Bay (FCSUMB). Both Decker and Klooster will be responsible for MBGC model code programming using software available within the Ecosystem Computational Facility (ECF) at NASA ARC. Decker will also be responsible for dissemination of results through written reports. Klooster will also be chiefly responsible for analysis and interpretation of satellite image and spectral data from microbial mat sites.



3.6 Investigation 6 Hind-Casting Past Environments

3.6.1 Objectives and Significance of Research

Our own biosphere has undergone adaptations as the Earth's climate has changed, and we will use this natural "experiment" to understand how environmental drivers affect local and continental-scale ecosystems. Variation in atmospheric circulation patterns has been linked to the El Niño

Southern Oscillation (ENSO), and our previous work has shown that South American vegetation responds to those changes. Further, we have mapped vegetation responses at 8-km resolution. Using proxies in the paleontological record (such as tree rings), we have reconstructed sea surface temperatures back to the mid-13th century, and have proceeded to year 300 B.C. as more data became available. The current investigation will rely upon remote sensing data to expand our (and other) reconstruction to include ecosystem properties during this period. In addition, we propose to use pollen records to "synchronize" our reconstruction with a variety of proxy measurements and interpret behavior of ecosystems over a longer period of time and over a wider variation in environmental conditions.

The limited length of data from instrument records can hinder a detailed understanding of decadal and longer-term land-atmosphere-ocean climate variability. Tree rings, fossil pollen, banded coral, and other proxy data can help resolve the nature of such variability over millennia, much longer than is possible using data from conventional instrumentals.

Strong climate index associations with proxy data are a consequence of the close relationship between the "El Niño" Southern Oscillation (ENSO) or the North Atlantic Oscillation (NAO) and Sea Surface Temperature (SST), precipitation and storm track trajectories worldwide. These extended proxy records can also aid in model construction of historical and biosphere function, an important NASA ESE (Code Y) goal.

For this project, we will use past changes in South American vegetation to predict and demonstrate the evolution of a drying, cooling, and perhaps dying, planet. Specifically, we will ascertain if changes in Advanced Very High Resolution Radiometer Normalized Difference Vegetation Index (AVHRR-NDVI) in South America resulting from "normal", "El Niño", or "La Niña" conditions can be linked to variations in SST. We will also use imagery and data sets from instruments on newer satellites, specifically data from the Enhanced Thematic Mapper Plus (ETM+) instrument onboard Landsat 7 and data from the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra satellite. Previously, a correlation between the 32 South American sites we used and SST data from Ship Track 1 was done yielding an r^2- value of about 0.8; under this task, we will expand our research in this promising line. Changes in atmospheric circulation patterns have been linked to ENSO at a global scale, and we found that South American vegetation responds to those changes. Some such responses may have a proxy version in the paleontological record.

Using the correlation found previously, we will "hind-cast" (as opposed to forecast) changes in AVHRR-NDVI during the last 1,300 year using reconstructed SST data. This will allow us to predict what vegetation communities were present in the past seven plus centuries. Linking AVHRR-NDVI to level "zero" (approximately the present time) of pollen profiles, it is possible to "hind-cast" past changes of NDVI over a much longer time period. Since many pollen profiles reach back to 15,000 years BP, it should be possible to synchronize them with the global C curve, the Lake Vostok temperature curves, and other records. Should we determine a historical C budget, we will need to have C data from the Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA) study and elsewhere. Since time resolution will not allow direct linkages of pollen fluctuations with ENSO events, we will need to use published data and radiocarbon dating to estimate the likelihood of ENSO being the cause of some past vegetation community changes, previously undiscovered or unexplained.

3.6.1.2 Build On and Extend State of Knowledge

Numerous proxy climate reconstructions have been developed that are directly or indirectly related to our research area. Examples are the reconstruction of the winter NAO index using tree-ring widths records from eastern North America and Europe (D'Arrigo et al. 1995, 1996; Cook et al. in press). Several other investigators have linked ENSO and North Pacific climate indices with proxy records (Fritts et al. 1979; Cleaveland et al. 1992; Stahle & Cleaveland 1993; Villalba et al. 1997; D'Arrigo et al. 1999; Wiles et al. in press). Banded coral records have the potential to extend the ENSO event record back for more than 100,000 years (Tudhope et al. 2001). Our current collaboration with R. Armstrong (University of Puerto Rico-Mayagüez) is exploring this record in the Caribbean.

Our previous research used South America as an analog of the biosphere of a planet that is cooling and drying, i.e., one that is leaving the "habitable zone" around the star which it orbits (Kasting 1996; D'Antoni 2000). The main features of the current South American vegetation were established after the last Ice Age (~ 10k yrs). As we demonstrated at 8-km resolution, most of the continent vegetation indices are altered by the El Niño Southern Oscillation (ENSO) events, but the largest effects are concentrated in the Northern Amazon basin, the dry forest of Coastal Ecuador, and the Paraná-Plata watershed (D'Antoni et al. 2002). Further, we identified NDVI variation in the area most intensely studied by dendrochronologists in South America. If sustained, the large effects of ENSO must have had a significant role in shaping the modern landscape of the continent. This assumption led us to search for ENSO signals in the paleontological record (see references above). Using available tree-ring

width records for South America (NOAA, Paleoclimate), and modern data for the SST of the Atlantic and Pacific Ocean (NOAA), we generated a multivariate statistical model that showed intriguing trends. We then refined the model using an artificial intelligence model (NeuroShell by Ward Systems Group, Inc.) and produced a reconstruction of the Atlantic and Pacific SST from year 1246 AD through 1995 AD (Fig. 3.6-1) (D'Antoni & Mlinarevic, 2002).

3.6.2 Research Tasks

3.6.2.1 Data Analysis and AI Modeling

For this effort we will use the pollen calibration model [$C_f = T_m P_m$] for pollen studies (Bradley 1999). Here C is climate, T is a transfer function and P is pollen. The subscript *m* denotes "modern" and *f* represents "fossil". We will use simple (linear and non-linear) and multiple linear regression, and regression on principal components to establish the relationships between SST, NDVI and pollen. We will then train an artificial intelligence algorithm to refine our predictions. For tree-ring width data analysis, we will follow the above mentioned literature and the critical suggestions of Bradley (1999). Once our results are available, we will collate and compare all pertinent data sources for ENSO and NAO index reconstruction for the length of time available in the continental proxy records of South America. We will use relationships of the Ames CASA model (Potter et al. 1998; see Investigation 5, this proposal) and other ecosystem models that use NDVI inputs to make gridded predictions of past 20-yr terrestrial carbon budgets, which have been successfully linked to ENSO and NAO in the tropical zones, and globally. We will use gridded historical estimates of global ice cover overlaid to mask out terrestrial productivity in the extra-tropical zone during the last glacial age.

With this integrative approach we expect to bring the contribution of South America to the reconstruction of the Earth's terrestrial biosphere productivity for the past thousands of years, based on synthesis of modern satellite observations, ecosystem models, global climate records, and other proxy data sets from field studies.

3.6.2.2 Fieldwork

Established collaboration with local scientists in South America include field measurements of photosynthesis in the dry tropical forest of Ecuador, in a specific point of the riparian Paraná forest north of Buenos Aires and in the dry "caldén forest" near Santa Rosa. The LBA program has measured photosynthesis in several points of the Amazon basin area and those data are readily available. An area with no significant changes from ENSO to non-ENSO conditions, the Ambato valley and its surroundings in Catamarca (Argentina) will be used as a control. To reinforce the link between tree-ring width and SST, we will encourage the involved scientists (Villalba, and the group from CRICYT) to fill the gap between the end of their tree-ring research and the present day in order to enlarge the time overlap of AVHRR and tree-ring data.

3.6.3 Relevance to NASA's Astrobiology Programs

The Astrobiology Program Goal Number 6 deals with the forces and drivers that have shaped or will shape the environment of Earth. Goal 6.1 deals specifically with the structure of ecosystems due to the cycling of elements by the biota, communities and ecosystems, both present *and past*. Understanding vegetative assemblages in local ecosystems or at the continental level requires an understanding of the environmental drivers (especially rainfall patterns) that caused those assemblages to be there. This work will show how the vegetation of portions of South America has changed over thousands of years and will indicate a possible future of communities, using Earth as an analogue of a drying, cooling, and perhaps dying planet.

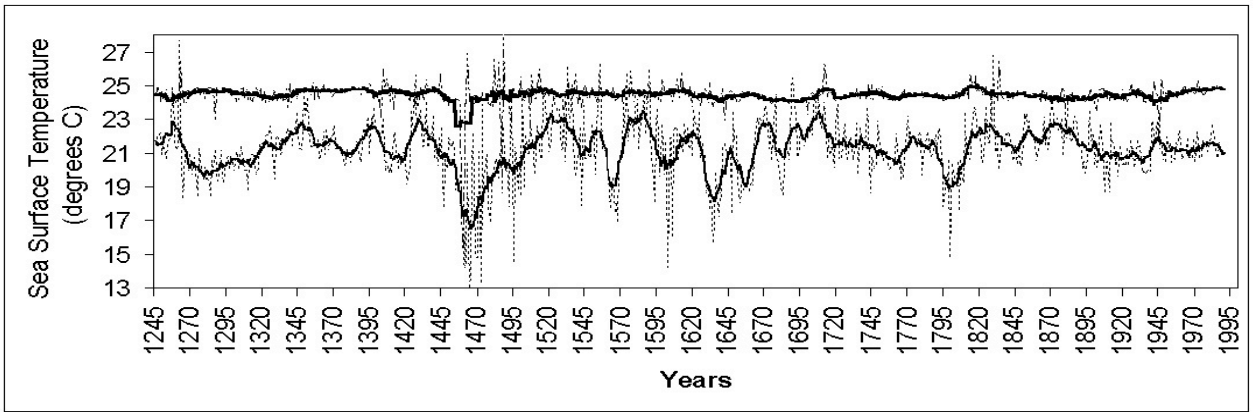


Figure 3.6-1. Reconstruction of past sea surface temperatures (SST) from 1246 AD to 1995 AD. Atlantic SST predictions are shown in the upper two curves with the thinner weight line as the raw prediction; the bold line is the five year smoothed average. Pacific SST predictions are shown in the lower two curves with the thinner weight line as the raw prediction; the bold line is the five year smoothed average.

3.6.4 General Plan of Work and Key Milestones

Due to the nature of this investigation we will need repeated measurements in the field, from year 1 through year 5.

Year 1 - Establish network of collaborators and share protocols for fieldwork. Measurements (see Fieldwork, above) starting in the Austral spring and continuing through summer. A trip to Parana-Plata basin in Argentina and Uruguay is planned. Link field measurements with remote sensing data and SST data. Milestone: identify Y1 data with an analog in our 20-year remote sensing database and with NOAA's SST record. Send or take three photosynthesis meters to S. America.

Year 2 - Repeat measurements in the field. A trip to Catamarca, Argentina is planned. Link field measurements with remote sensing and SST data. Milestone: identify Y2 data with an analog in our 20-year remote sensing database and with NOAA's SST record. Send or take two photosynthesis meters to S. America.

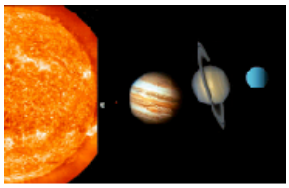
Year 3 - Run test simulations and compare with compiled climate, tree ring and other data. Collate with instrument data where available and tune AI model. Refine simulations. A trip to Calden Forest, Argentina is planned.

Year 4 - Continue refinement of models and incorporation of field and instrument data. Assembled proxy data incorporated into model refinements. A trip to a Nothofagus forest in Argentina and Chile is planned.

Year 5 - Compile all new data, expand database. Repeat as in year 4 and make final simulations. Extrapolations of climate into past done. Identify data with an analog in our 20-year remote sensing database and with NOAA's SST record. Cross-validate our record with all available and pertinent proxy records available. A trip to Parana-Plata basin in Argentina and Uruguay is planned.

3.6.5 Management Structure and Statements of Contributions

Dr. **H. D'Antoni** will supervise work done for this module and report to Dr. Des Marais. He will also work with paleoclimate and paleo-environment data. Dr. **J. Skiles** will be responsible for gathering and analyzing data from the remote sensing instruments mentioned above, overall data analysis, computer simulations and for the area of plant ecology and plant eco-physiology. Our foreign collaborators will contribute the field data they gather in each of the years of the project.



3.7 Investigation 7 Interplanetary Pioneers

Here we focus on life moving beyond its planet of origin, a question of evolutionary interest and because the human exploration of space *is* the movement of life from Earth. Today, our research effort provides a home within NAI for an integrated research program relating to this major area of Astrobiology. With this renewal, *we propose a focused research plan as a nucleus for an expanded emphasis on this area of interplanetary travel of life as the field of Astrobiology matures.*

Moving beyond the planet of origin requires a vehicle for transport, the ability to withstand transport, and the ability to colonize, thrive and ultimately evolve in the new environment. The core of this study will be to identify organisms and ecosystems that are likely to withstand the rigors of space, using as a guiding principle the hypothesis that desiccation resistance and natural exposure to high levels of radiation are good predictors of radiation resistance. Once this work – collecting, testing in the lab and in a space simulator, looking at mechanisms underlying the results – has been established, we will expand to include a flight component and to bring in workers from related fields to study other aspects of natural transport.

As a means of transport the work proposed focuses on natural transport, such as on a meteorite, where the primary factors determining success are finding a suitable vehicle for transport, and the ability of the organisms to withstand space radiation, space vacuum, desiccation, time in transit, and the physical rigors of leaving the parent body and landing on a new one. The product of the probabilities of each factor provides an estimate of the likelihood of success. As a result of this proposed effort we will increase our understanding of several of these parameters.

The proposed work is ambitious, especially for the budget permitted. The funds are primarily for work that takes place under Rothschild or Mancinelli's direction and include supplies, salary and travel, the last of which is crucial to allow the use of the unique facilities of the DLR in Germany, and facilitate collaboration with our European and Australian colleagues. The non-US citizens are not requesting monies. *It is important for the NAI to support the proposed effort because it provides a catalyst and a vehicle for interaction among disparate groups and application of on-going work to a fundamental question in astrobiology.*

This work is clearly connected philosophically with other portions of this proposal, from questions of defining the habitable zone to understanding microbial ecosystems and the biosignatures they can produce. We would anticipate through our monthly group meeting and more frequent informal contacts to form synergistic exchanges with these groups.

3.7.1 Objectives and Significance of Research

- To identify terrestrial organisms and ecosystems that will survive space radiation and vacuum. To assess the mechanisms underlying survival.
- To expand this nucleus to other aspects of transport beyond the planet of origin, focusing on the suitability of meteorites as a vehicle of transport.
- To provide a conceptual framework and collaboration for this new field.
- To use this knowledge to inform related fields such as evolutionary biology, global change, the search for life elsewhere, and planetary protection.

3.7.2 Build On and Extend State of Knowledge

Can life survive beyond its home planet? In attempts to answer this question microbes tested in the space environment as of the beginning of 2003 include *Bacillus subtilis* spores, bacteria, bacteriophage T-1, Tobacco Mosaic Virus, and osmophilic microbes (Horneck & Brack 1992, Horneck 1993; Mancinelli et al. 1998, Nicholson et al. 2000). *B. subtilis* spores will survive for years in space if in a multilayer or mixed with glucose to protect them against high solar UV-radiation flux, but are killed in minutes if exposed in a mono-layer (Horneck 1993; Horneck et al. 1994, Horneck et al. 2001). Viruses lose viability in weeks (reviewed in Horneck & Brack 1992). The halophile *Haloarcula*-G and the cyanobacterium *Synechococcus* (Nägeli) can survive for at least weeks in the space environment (Mancinelli et al. 1998) and probably much longer (Mancinelli, unpublished data). Funded in part by the current NAI grant to NASA Ames, team member Mancinelli and colleagues (Mancinelli et al. 1998) were the first to show that a vegetative cell, in this case a halophile, could survive exposure to the space environment. *We believe that many organisms suitable for space simply have been ignored or not yet isolated. This is the focus of the proposed work.*

Are there mechanisms to transport organisms off their planet of origin and deliver them safely elsewhere? We know that meteorites such as ALH84001 have journeyed from Mars to Earth, and they can contain fissures which should provide a suitable environment for microbes. Modeling studies have suggested that microbes on a planet, such as Mars, could survive the impact and forces needed to cause a meteor to be jettisoned from the planet, escape to space and survive the impact of landing on a planet such as Earth. This pioneering work by team member Horneck and collaborators (Mileikowsky et al. 2000; Horneck et al. 2001) showed a substantial rate of survival of bacterial endospores after a simulated meteorite impact at shock pressures of 32 GPa. Further, spores of *B. subtilis* mixed with minerals representing the mineralogy of generic meteorites survive in the space environment better than unprotected spores (Horneck et al. 2002).

It is easy to envision a scenario where extraterrestrial life within a meteorite could enter microcracks, in the meteorite, seal off a small volume within them, and thus be both protected from the vacuum environment and shielded from the cosmic ray and UV environment. If this is true, the transport of life beyond its planet of origin becomes far more likely.

But surviving in cracks within meteorites may not be easy. Shocks created by hypervelocity impacts induce failure and concentrate heating, particularly along strong impedance mismatches or at point contacts including pre-existing failure planes. This bad news could be good news for organisms. Survivors may find themselves in a temporarily habitable environment. *No one has attempted to place microbes within the naturally occurring cracks and crevices of a natural meteorite and determine their survival during an impact or in space as we are proposing.*

The interplanetary medium poses obstacles to the survival of Earth-based, and presumably, all carbon-based life. Space is extremely cold. It is subject to unfiltered solar radiation, solar wind, and galactic cosmic radiation. It has exceedingly low pressures, and has a much lower gravity than Earth (Rothschild 2003; Horneck 2003, Mileikowsky et al. 2000). Space is a nutritional wasteland with respect to water and organic carbon. The organisms most likely to survive these conditions are microbes, although some seeds, fungi, lichens or invertebrates might be able to make the journey. For microbes, gravity is not an issue, and cold tolerance is widespread among spores.

There are four potential "show-stoppers": a meteorite is not a suitable vehicle for transit, exposure to external radiation or the space vacuum causes death or even loss of reproductive potential, or the time in transit is prohibitively long. We will address the first three here.

During the short term, most damage to microbes exposed to the space environment is due to UV radiation, but heavy ionizing radiation has a greater probability of being lethal (rev. in Horneck et al. 1994). Reactive oxygen species are produced by ionizing radiation during flight, and are an

important component of radiation damage in space (LBNL-40278, 1997). Types of DNA damage due to UV and ionizing radiation in space are, in order of increasing danger to the cell, damage to bases, single strand breaks and double strand breaks (LBNL-40278, 1997).

Desiccation tolerance has been reported for a variety of organisms including bacteria, yeast, lichenized fungi, plants, insects and crustacea (e.g., Clegg 1986; Crowe et al. 1992; Csonka & Hanson 1991; reviewed in Potts 1994). One of the supposed mechanisms of death due to anhydrobiosis (extreme desiccation) in prokaryotes is the dehydration of DNA leading to breakage (Dose et al. 1992; 1991 Dose et al. 1995).

The ability to cope with high salt and/or desiccation appears to be a good predictor of protection from radiation damage as has been shown in microbes on Earth. This is because DNA damage accumulates during desiccation because there is no DNA repair (Setlow 1992). We know that organisms living in evaporitic salt crusts are highly resistant to desiccation, space vacuum and UV radiation through ground (Rothschild 1990; Rothschild et al. 1994) and spaceflight experiments aboard ESA's BIOPAN facility (Mancinelli et al. 1998). Duricrusts, thought to be indicative of salt crusts, were found at both Viking lander sites (Clark 1978, Clark & van Hart 1981). Deposits considered to be salt pans are seen on images of the martian surface (Forsythe & Zimbelman 1995). On any world in which liquid water becomes limited, we would expect salt formations to become an important niche for life.

Of course organisms have evolved mechanisms to avoid or repair damage. Organisms other than archaeal halophiles use organic compounds as osmotica, whereas the halophiles use K+ as their internal osmoticum. Oxidative damage, which results from space radiation as well as occurring on earth during aerobic metabolism (Brawn & Fridovich 1981), may be avoided by detoxification mechanisms such as the enzyme superoxide dismutase. Nearly all these organisms also contain catalase which catalyzes the decomposition of hydrogen peroxide to oxygen and water. Peroxidases are used by some bacteria and protists to decompose hydrogen peroxide to water by oxidizing organic compounds. Moreover, water- and lipid soluble antioxidants such as glutathione or ascorbate, and tocopherol, respectively, scavenge free radicals. Carotenoids are a class of pigments that act as quenchers of single-state oxygen (rev. in Siefermann-Harms 1987). *Understanding mechanisms for survival is important, and will be initiated during the proposed work.*

3.7.3 Technical Approach and Methodology

Our approach will be to identify novel organisms or ecosystems that have the potential to survive interplanetary transfer by bringing them into Rothschild's and Mancinelli's labs at NASA Ames and screening for UV and desiccation resistance. The most promising samples will be further screened in Germany at the DLR space simulators, and, if possible, ultimately flown on the EXPOSE facility on ISS. We will initiate studies of the mechanisms of desiccation and radiation resistance by screening DNA damage, antioxidants and detoxifying enzymes after exposure. To supplement the core project, we will experimentally test the suitability of meteorite cracks to house terrestrial organisms en route to another body.

1. Identify and collect samples that are halophilic, desiccation or radiation resistant from field sites and culture collections. *(Rothschild, Mancinelli, Johansen, Anitori, Kranner)*

Halophilic and desiccation resistant microbial samples. Some samples were collected during the first funding cycle of this project. We will collect additional halophile samples from the salt evaporation ponds of the Cargill company along the southern edge of San Francisco Bay, near NASA-ARC. The ponds range in salinity from sea water (ca. 3%) to saturation (>25%) with a pH range of 6.5-7.2.

To screen and isolate desiccation resistant organisms from the samples, the brine will be washed 3 x by centrifugation. The final pellet will be suspended with sterile 25% NaCl to bring the concentration to ~10^7 cells per µL. Fifty µL aliquots will be pipetted into 96-well microtiter plates and dried for 3 h at 30°C in a forced air incubator. After drying, the plates will be placed in a standard laboratory desiccator at 22°C. Periodically plates will be removed, each well filled with 130 µL of nutrient medium (e.g., OS liquid media, ATTC manual), incubated at 30°C, and checked for growth by monitoring increases in turbidity using a 96-well plate-reading spectrophotometer. Those exhibiting growth and desiccated for the longest period of time will be isolated on agar plates.

Desert crust. Johansen will provide microbiotic crusts from throughout the western United States and western Australia. Available collections include eukaryotic algae from such soils (Chlorophyceae, Tribophyceae, Bacillario-phyceae). The soils range from xeric, hot habitats to those in arid, temperate climates. Cyanobacteria from the crusts are identified in Johansen's lab using DNA sequences from the 16S rDNA and 16S-23S ITS region. Desiccation resistance will be tested in a similar manner to that for halophiles.

Radiation resistant samples. Two types of samples will be tested. *Deinococcus radiodurans* and those from team member Anitori who is

working on a unusual radon-rich hot spring (48-63°C) situated in a region of South Australia's Flinders Ranges which has a long history of hydrothermal activity. Radon-222 is present in gas which bubbles up from the sandy bottom of the spring. The gas has a radiation level of ~29,000 Bq/L; the radiation level in the water is 2000-5800 Bq/L. Paralana is unique in that a flourishing microbiota is present in association with the radon environment (Anitori et al. 2002; Anitori et al. 2003). 16S rRNA studies have indicated that Paralana contains, among others, cyanobacteria (e.g. *Fischerella* spp.), and halophilic Archaea (*Natronococcus xingiangensis*). Desiccation resistance will be determined as for the halophiles.

Lichens: Desert species or high altitude species are well adapted to desiccation and probably quite high UV irradiation. Two desert species, *Ramalina maciformis* (Negev) or *Teloschistes capensis* (Namib) are good candidates. Desiccation resistance will be determined as for the halophiles. Viability will be tested with the Live/Dead stain if that is shown to be appropriate, or by CO_2 exchange. If they recover photosynthetic activity, they are alive.

2. In Mancinelli's lab, expose organisms to increasing doses of UV radiation using a solar simulator. Determine survival. (*Mancinelli, Rothschild, Purcell*)

Organisms exhibiting the highest level of desiccation resistance, as determined from (1), will be prepared for UV radiation resistance as described above for desiccation resistance screening, except instead of being placed in a desiccator the dried samples will be exposed to UV radiation from a deuterium lamp (Oriel model 6316) in Mancinelli's lab. Samples will be collected periodically to determine survival using reproduction ability on the appropriate medium for the organism and Molecular Probe's Live-Dead Stain. Those organisms surviving the highest dose will be grown on the appropriate medium.

3. Expose most promising samples to space simulators at the DLR e.V., Institute of Aerospace Medicine, Radiation Biology Section, Köln, Germany *(Rettberg, Horneck, Rabbow, Mancinelli)*

Organisms exhibiting the highest survival rate for desiccation (1) and UV radiation resistance (2) in the solar simulator will be tested in the space simulators located at the DLR. For example, for halophiles the organisms will be grown to mid-log phase and washed by centrifugation as described previously. After the final wash, the pellet will be suspended in the appropriate wash salt to a dilution equaling 2.5 x 10^8 cells. Forty µL aliquots of the diluted suspension will be placed onto 7 mm diameter quartz discs and dried in forced air incubator at 30°C for three hours.

All of the samples will be exposed to space vacuum, with half of the samples exposed to solar UV-radiation and half kept in darkness shielded from solar UV-radiation. Duplicate samples will be kept in the laboratory to serve as unexposed controls. Periodically (hours, days, weeks, and months) a set will be collected from the space simulation chamber and analyzed for survivability. Live versus dead cells will be monitored using reproductive capacity in the appropriate medium as well as with a dual fluorescent stain from Molecular Probes (Live/Dead viability kit).

Determination of Survivability. Microbial survival will be determined by comparing the number of viable cells in the laboratory controls (i.e., samples not exposed to vacuum desiccation or solar UV radiation) with the number of viable cells recovered from the exposure chambers after completion of the test.

Data Analysis of Survivability. The survivability of the laboratory ground simulation chamber time course experiment samples will be calculated as a percent of the initial number of organisms placed in the flight and ground simulation control chambers. The number of survivors will be compared to the number of survivors from samples stored in the laboratory under ambient conditions. In addition, because samples will be collected periodically from the simulation chambers, their percent survival will be determined as a function of time. The data will then be compared to survival percentages of organisms on past flights and other organisms tested on this flight.

4. Look for mechanistic explanations for radiation or desiccation resistance by assessing DNA damage, antioxidants, detoxification enzymes, and pigmentation before and after exposure to solar simulator at Ames *(Rothschild, Purcell, Kranner, Lancaster)*.

While a complete assessment of mechanisms of radiation or desiccation resistance is out of the scope of the present proposal, several team members have expertise in different aspects of cellular damage, and will apply their expertise to promising samples.

DNA damage (Rothschild). Double and single strand breaks will be determined qualitatively. Double strand breaks will be analyzed by agarose gel electrophoresis with a digital gel documentation system equipped with image analysis software. Breakage of DNA will be determined by loss of high molecular weight DNA. Single strand breaks will be determined similarly, but following denaturing the DNA. If more quantitative data is required, a modification of the familiar "nick-translation" method which omits the nicking step will be used. Incorporation of radioactive nucleotides into the DNA is proportional to the number of single strand breaks. To assess oxidative damage resulting in base

modification, Rothschild's lab will determine the ratio of 8-hydroxy-2'-deoxyguanosine to the unoxidized 2'-deoxyguanosine using reverse phase HPLC with a Coulchem II electrochemical detector. Prior to HPLC analysis, the DNA will be digested with P1 nuclease (1.5 h at 37°C), and incubated in alkaline phosphatase (1 h at 37°C). DNA will be isolated using commercially-available kits such as the Wizard® Genomic DNA Purification Kit (Promega). This technique has been in use in Rothschild's lab since the autumn of 2002.

Antioxidants and plastid pigments (Kranner). HPLC analysis of reduced glutathione (g glutamyl-cysteinyl-glycine; GSH) and glutathione disulfide (GSSG) will be performed according to Kranner (1998). HPLC assays of ascorbate and dehydroascorbate will follow the procedure of Tausz et al. (1996). Tocopherols (□-, □-, □, □-tocopherol) and plastid pigments will be analyzed according to Pfeifhofer et al. (2002).

Detoxifying enzymes (Lancaster). Catalase will be assayed using the Amplex Red Catalase Assay Kit (Molecular Probes: A-22180). The superoxide dismutase (SOD) assays are based on monitoring the degree of the autoxidation of pyrogallo (Marklund 1985). Peroxidase activity in crude cell extract will be assayed using the QuantBlu fluorogenic peroxidase substrate from Pierce Biotechnology. Glutathione reductase activity will be measured by monitoring the decrease in absorbance at 340 nm due to the conversion of NADPH to $NADP^+$. All three assays will be monitored by absorption using a multiwell plate reading spectrophotometer in Rothschild's lab.

With local funds, Rabbow will study the role of exogenous and endogenous protection mechanisms allowing survival of bacterial endospores in outer space, for example, inside of artificial meteorites. Rettberg will study the role of the repair systems in the radiation (UV and ionizing) resistance of various genetically well-defined strains of *Deinococcus radiaodurans* irradiated under the conditions simulating outer space (e.g., vacuum, extreme temperatures) using molecular biology techniques (e.g. DNA chip technology).

5. Prepare for flight experiments as part of ROSE consortium. Fly most promising samples when the external platform is built on ISS. *(Mancinelli, Horneck, Rettberg, Rabbow, and others)*

Candidate organisms will be flown in flight experiments through membership in the ROSE (Response of Organisms to the Space Environment) consortium on The European Space Agency's (ESA) external EXPOSE platform aboard the International Space Station as permitted. *Members of our team have already been selected as PI's through a peer- reviewed process to fly experiments as part of the ROSE consortium.* They are Horneck (SPORES) and Mancinelli (OSMO).

6. Use these results to determine if these organisms are likely to survive in earth orbit, and thus would make a good future candidate for long-term (multi year) space flight. Initiate assessment of meteorites as environment for microbes *(Consolmagno, Rothschild).* Initiate studies of the survival of the most promising organisms during simulated meteoritic impact (*Schultz, Rothschild*). Model potential for such life forms elsewhere *(Raven, Wolstencroft).*

To test the suitability of meteorite crevices for interplanetary transport, Consolmagno will select a typical non-carbonaceous meteorite, and measure its porosity using a large helium pycnometer (described in Consolmagno & Britt 1998). He will image a small sample by SEM backscatter and measure and characterize microcrack porosity. At Ames, the meteorite will be heat sterilized, and a known and easily traceable type of microbe (e.g., *Bacillus subtilis*, *Synechococcus* or yeast) will be introduced into it. The meteorite will be exposed to simulated space conditions, then cored or cut to determine the survival characteristics of the microbes as a function of depth within the rock and time of exposure. Survival will be determined by reproduction as described previously.

The meteorite will be relatively unweathered to ensure unclogged pores (Bland et al. 1998). It will be at least 3 cm in every dimension to provide a significant volume with at least 1 cm of shielding, suggesting a meteorite e~100 g in mass. Fusion crust will be avoided, as it is not typical of the state of the material in space and could affect the effective permeability of the meteorite. Meteorites will be obtained from the Antarctic Meteorite collection maintained at NASA's Johnson Space Center. Meteorites suitable for this work include EET 96 032 (435 g L4, weathering grade A/B, fracture B) and ALH 82 104 (400 g, L5, weathering grade A, fracture A/B.)

The next step is to determine if impacts kill the microbes when the pore spaces are squeezed shut by the shock wave of an impact. Collaborator Schultz will test this by taking meteorite sample impregnated with living microbes and subjecting them to controlled velocity impacts using the Vertical Gun Facility at NASA Ames. Previous experimental studies demonstrated that oblique impacts reduce the peak pressures within the impactor and induce failure prior to penetration into the target (Schultz and Gault 1990). Intact fragments (up to 10-50% the mass of the initial projectile) survive and are sprayed downrange. It is possible for fragments (and contained organisms) to survive collisions long thought to be devastating for transport. Although Schultz has separate funding through Exobiology to assess survival during hypervelocity impact, the

experiments outlined here are distinct, and collaboration with members of the NAI would allow broader applications and involvement.

3.7.4 General Plan of Work and Key Milestones

General plan of work:

- Identify and collect samples likely to withstand space flight.
- Screen for survival after exposure to a solar simulator in Mancinelli's lab, NASA/ARC.
- Expose most promising samples to space simulators at the DLR e.V., Köln, Germany.
- Look for mechanistic explanations for radiation or desiccation resistance.
- Prepare for flight experiments as part of ROSE consortium. Fly most promising samples when the external platform is built on the International Space Station.
- Use these results to determine if these organisms are likely to survive in Earth orbit, and thus would make a good future candidate for long-term space flight.
- Assess meteorites as environment for microbes. Study survival of the most promising organisms during simulated meteoritic impact.
- Model potential for such life forms elsewhere.

Key Milestones

Year 1 - Initial collection and screening of samples using solar simulator at Ames. Survival will be monitored. Test techniques on monitoring DNA damage on new organisms (e.g., DNA isolations). Select and obtain meteorites. Team meeting.

Year 2 - Continue isolation and tests of new model organisms. Initiate studies on mechanisms of survival. Begin tests of promising samples in space simulation facility, DLR. Measure meteorite fissures (Although the EXPOSE first flight is scheduled for October 2004, we anticipate that it will slip by a year).

Year 3 - Continue isolation and tests of new model organisms. Continue experiments on mechanisms of survival. Continue tests of new model organisms. Measure colonization of microbes in meteorite cracks. Begin flight component, Team meeting.

Year 4-5 - Continue research including flight component. Begin work on impact survival of test organisms that show high survival rates. Team meeting year 5.

3.7.5 Management Structure and Statements of Contribution

Rothschild (lead CoI), will be responsible for the overall management of the proposed work. **Mancinelli** (CoI), is the only US PI on the BioPan and ROSE experiments. He will be responsible for the work on prokaryotes, and testing all organisms in the Space Simulation Facility at the DLR. He, along with Horneck, will co-ordinate flight experiments. **Horneck** (CoI), will work with Mancinelli on all experiments conducted in Germany, and be the primary advisor for sample testing in flight simulators, and flight experiments. She will coordinate with ESA on the flight experiment logistics. **Consolmagno** (collaborator), will be responsible for the experiments on the survival of microbes in meteorites. **Johansen** (collaborator), will provide desert crust samples from his collection, and isolated cyanobacteria from microbiotic crusts from the western US and western Australia. **Rabbow** (collaborator), will study the role of exogenous and endogenous protection mechanisms allowing survival of bacterial endospores in outer space, and will be responsible for the work of the SSIOUX group examining this. **Raven** (collaborator), will examine generalized energetic and kinetic constraints on photosynthesis, and to relate these requirements to the range of conditions on other bodies on which life may be possible. **Rettberg** (collaborator), will study the role of the repair systems in the radiation resistance of *D. radiodurans*. **Schultz** (collaborator), will lead the study of the effect of impact on survival of microbes in meteorites. **Wolstencroft** (collaborator), will explore the generalized energetic and kinetic constraints on photosynthesis using a theoretical approach, and relate these requirements to the range of conditions on other planets on which life may be possible. **Anitori**, will be responsible for the isolation and characterization of samples from the Paralana site and Shark Bay. He will provide a link with the Australian Centre for Astrobiology. **Kranner**, will be responsible for work on lichens, antioxidants and plastid pigments.

3.8 Management Plan

Ames fulfills the following three distinct roles in NASA's astrobiology program: (1) management of the Astrobiology Institute; (2) team membership in the Institute, if this proposal is accepted; and (3) non-Institute research and technology development for astrobiology. This proposal deals directly only with (2).

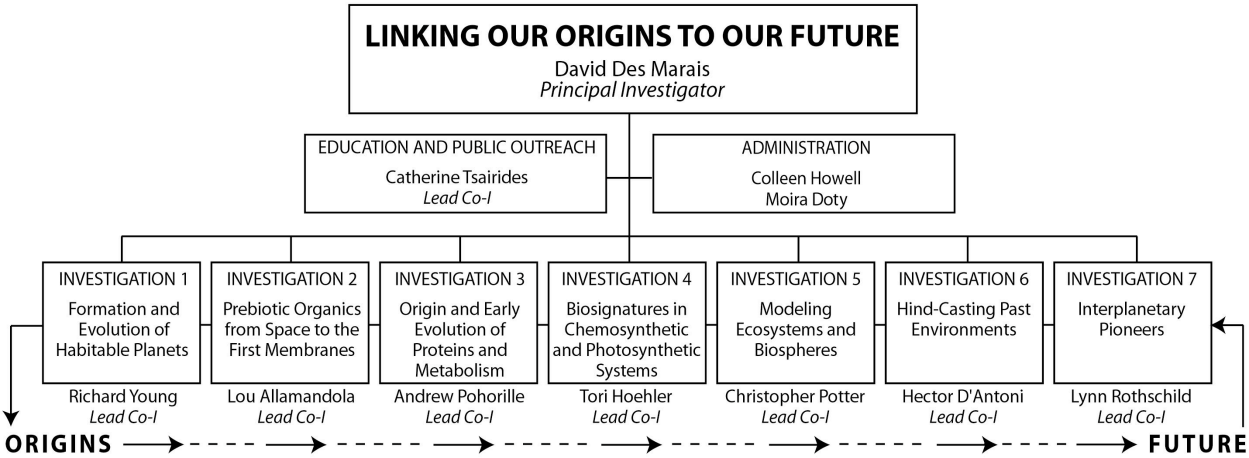
David Des Marais, Principal Investigator (PI), has overall authority and responsibility for this proposed work. To provide overall scientific direction to the effort, he will chair a steering committee comprised of the Co-Investigator (CoI) leads who will supervise each of the seven investigations and the program for education and public outreach. Des Marais will supervise a staff (see Volume II, Section 11.9) that will administer this project, including helping to meet the requirements of the NAI management.

Richard Young, Louis Allamandola, Andrew Pohorille, Tori Hoehler, Christopher Potter, Hector

D'Antoni, Lynn Rothschild, and Catherine Tsairides, each a Co-Investigator (CoI) lead, will lead the seven investigations and the E/PO program. Each CoI lead will be responsible for detailed management of funding and personnel resources, for the laboratories and facilities of his/her investigation. Each CoI lead is also responsible for coordination and integration of the various individuals and institutions that comprise the team and for interacting with each other to identify new research ideas, new areas for collaboration, and exchange of resources, including personnel, among the implementing institutions.

This program will be integrated further through monthly meetings and less frequent workshops that will address selected elements of the program and create opportunities for new collaborations and research directions. Both activities will embrace the lessons learned during our current membership with NAI. For example, monthly presentations of biological themes will be paired with those of related astrochemical or planetary themes. Also, discussions of science tasks will be paired with those related to training, education, and public outreach. These meetings are also modeled after the highly successful Center for Star Formation (CSF), a research effort led by Ames in collaboration with UC Berkeley and UC Santa Cruz. Indeed, CSF will coordinate its own meetings with Investigation 2 of this proposal. To improve participation with our external colleagues at collaborating institutions, we will utilize NetMeeting, which has been adopted recently by NAI.

We have participated actively in NAI focus groups, and will continue to do so in the next round of membership. For example, Investigations 1 and 2 will work with the proposed Astronomy and Astronomical Biosignatures focus groups. Members of Investigation 3 are working with NAI members from the Colorado team and the University of Florida to propose a focus group on the origin and early evolution of self-reproducing systems. Investigations 4 and 5 will participate in the Mars, the Ecogenomics, and the proposed Astronomical Biosignatures focus groups. Investigations 6 and 7 will work with other NAI teams to develop groups addressing the future of life. Through these interactions with other NAI member institutions, we will continue our role in forging the interdisciplinary connections that will contribute to the overall viability of the institute.



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5.0 PLAN FOR STRENGTHENING THE ASTROBIOLOGY COMMUNITY

5.1 Education and Public Outreach

The NASA Ames Astrobiology team is committed to fostering the broad involvement of the science community in Education and Public Outreach (E/PO). Within the outreach program we adhere to the NASA Office of Space Science Education and Public Outreach Criteria and the Origins Roadmap 2003. *The major criteria that the Ames team is focused on are:*

- *The potential for the proposed education and public outreach activity to expand its scope by having an impact beyond the direct beneficiaries*
- *Reaching relatively large audiences*
- *Being suitable for replication or broad dissemination*
- *Drawing on resources beyond those directly requested*

We highlight collaborations the Ames Astrobiology team has initiated with two institutions that serve as portals for large segments of the public to explore science and the excitement of astrobiology. California Academy of Sciences (CAS), located in Golden Gate Park, San Francisco, and Yellowstone National Park (YNP) will provide the team an opportunity to work within a collaborative process. *We have been proactive in seeking high-leverage opportunities to maximize the usefulness and effectiveness of material, programs and services created by NASA missions.* The team will be creating new exhibit plans, products, and activities for CAS, YNP, and the New York Hall of Science. In addition, we will conduct specific activities associated with the Carnegie Institution of Washington, and the Ames Education Office. The exhibits, activities and educational materials developed will be distributed in a coordinated manner across these organizations for maximum impact to amplify the effect of "strengthening" the astrobiology community.

5.1.1 Programs in Astrobiology

Astrobiology at California Academy of Science

Founded in 1853, CAS is the oldest scientific institution in the American West and currently holds one of the largest natural history collections in the world. It is also one of the only such places in the world that houses a natural history museum, planetarium and aquarium together. Thus, it is one of the few institutions devoted to the dissemination of science to the public that has a range of facilities with a breadth that approaches that of the field of astrobiology. CAS is currently planning a $370 M renovation of its existing museum. This will result in more modern facilities redesigned in a manner worthy of their interactive style, so as to engage the museum's many visitors. The renovation represents a remarkable opportunity for the NASA Astrobiology Institute (NAI) Ames team to leverage those funds and convey its science to the public. The community that the NAI will reach through this collaboration is amazing by the standards of E/PO grants of the size of ours. The Ames team will serve on the design team and contribute to the exhibit development team. CAS and Ames education teams will partner researchers with educators incorporating science content with methodological background and training in conducting inquiry based programs and activities. *We will facilitate high quality product and program development by researching product usage and audience needs in conjunction with the high leverage organizations.* The Ames science team will contribute to the development of lessons and materials that will be created and presented to educators during the museum's formal education professional development program. The Astrobiology Educator Guide and the Astro-Venture program will be incorporated into the professional development workshops held at the CAS each summer. In turn, CAS will benefit from its interaction with the Ames team by gaining access to experts who can provide perhaps the only overarching theme capable of connecting CAS's disparate subjects. Moreover, in some cases the specific exhibits planned by CAS center on issues that are synonymous with NASA's main goals. This is exemplified by the exhibits "Earth and its Place in the Universe" and "Water is Life"; the latter funded in large part by a $2.5 M NSF Informal Science Education grant. In both of these exhibits, NASA Astrobiology themes—the role of water, and conditions that allow the origin, development, and persistence of life—are central.

Because the scientists at CAS are oriented towards systematic biology and evolutionary biology they will rely on this partnership to provide input on other scientific concepts and cutting-edge research for exhibit development. With NASA Ames Research Center so close geographically to CAS, we foresee a strong collaborative relationship between Ames and CAS in the development of these ideas. Specifically, we see our expertise as crucial to bringing these projects to their full potential in two ways. First, our team members will be active in ensuring that the exhibits are, and continue to be, scientifically current - a quality that has historically garnered CAS acclaim. Second, we plan to extend their features that focus on Earth environments to include examples from other worlds. Connections to science Investigations 1 and 2 can aid in the continuation of the water theme to put it into the larger context of Solar System history and future, as well as include comparisons to sub-surface oceans on icy satellites of Jupiter, and possible

liquid water on or near the surface of Mars. Another connection can be seen in Investigations 4, 5, and 7 helping to expand the presentation of extremophiles as possibly representative of what life might have looked like in the ancient past, and/or elsewhere. The Ames Astrobiology team has created this formal and informal research education partnership with CAS featuring the topic of astrobiology to bridge the three parts of the museum—natural history, planetarium, and aquarium—together. A diverse suite of programming activities at CAS, YNP, and NYHOS are in development.

- BioForum (lectures for teachers) "Astrobiology: search for life in the universe"
- 3-hour docent training tours on astrobiology for "Earth and Space"
- Planetarium show
- Planetarium lecture series
- Members lecture on NAI research
- Science Now exhibit panel on NAI research
- Science Now web page on NAI research

Timeline:

- 2003 - Scientific content discussions, professional development programs for docents area educators, and research partners
- 2004 - Scientific content discussions with exhibit designers
- 2005 - Production of prototype astrobiology exhibit elements for display in a temporary museum in downtown San Francisco
- 2006 - Evaluation and assessment of design elements
- 2007 - Modify and design astrobiology permanent exhibit elements
- 2008 - Opening of "Earth and its Place in the Universe" and "Water is Life" exhibits including astrobiology elements

Astrobiology at Yellowstone National Park

The Ames/YNP partnership will help the general public understand and appreciate the unique natural resources preserved in YNP. Information on YNP's resources forms the basis for interpretation offered to the general public in the form of exhibits, publications, audiovisual programs, the park's official web site, a variety of ranger-led programs and activities, and its formal education program. YNP provides insights into the origin and evolution of life and the potential for life to exist beyond Earth. The Ames Astrobiology team has forged a partnership with YNP to provide a tiered public engagement program through the Department of Interpretative (DOI) Research at YNP to initiate a focused outreach project that highlights both astrobiology and the work of the NAI in the Park. Specifically, the NASA/Ames Astrobiology team will work with the National Park Service's Division of Interpretation and Education, which bases its operations, programs, and functions on the Long Range Interpretive Plan. This Plan describes YNP's visitor experience goals for the Division of Interpretation based on identification of primary park resources and associated themes, and in conformity with the NPS' s mission, management policies, and other legislative guidance.

The Plan is a living document that is reviewed annually and adjusted to respond to changing park issues, research updates and reports, and visitor needs and studies. Through this partnership the NASA/Ames Astrobiology team will be a national leader in supporting resource education and interpretation. Its collaboration with the Division of Interpretation will provide the park's diverse audience with appropriately designed and developed media and other programs in which astrobiology research is presented. This presentation will be consistent with the goals in the Long Range Interpretive Plan, and it will provide insights into the origin and evolution of life and the potential for life to exist beyond Earth. The many venues we discuss in the Education Plan will be coordinated through the YNP DOI to disseminate information to tourists and web audiences. A major advantage is that YNP interpretive staff provides strong background in the science of education to complement our strengths as scientists. YNP's infrastructure for developing and distributing interpretive materials will help us reach very large audiences with maximum efficiency. For instance, the Resources and Issues Manual is the primary tool by which park managers and interpretive staff learn the information they need to affect decision-making and for interfacing with tourists. YNP has an established system for production and emplacement of signage and trail guides, which reach 1 million visitors per year. YNP will also coordinate all outreach activities associated with the new Old Faithful Visitor Education Center, including not only exhibits, but also educational programs for park visitors of all ages (Junior Ranger programs, curriculum development, etc.). In the last two years the NASA Ames Astrobiology team has initiated collaboration with the YNP Division of Interpretation to find ways to tell the astrobiology story through the many YNP venues. We have been invited to:

- Contribute chapters to the Resources and Issues manual
- Develop wayside signs and edit trail guides
- Participate in planning and development of the new Old Faithful Visitor Education Center (OFVEC)
- Explore opportunities to educate tourists and interpretive staff through informal talks
- Explore new interactive uses of existing child education tools (e.g., Junior Ranger Program)

- Explore how the YNP web site can best be used to communicate information about astrobiology.
- Participate in annual Yellowstone Association Institute formal education program

Timeline:

- 2003 - Revise and publish chapters in Resources and Issues manual; continue development of signage; initiate revision of trail guides; OFVEC proposal writing and planning; dialog about informal talks and web-sites
- 2004 - Progress from initial signs to additional signs; publish revised train guides; OFVEC planning; Junior Ranger Program revision; initiate informal talks
- 2005 - OFVEC exhibit production; informal talks; initiate web-site projects
- 2006 - OFVEC evaluation and revision; complete web-site projects
- 2007 - OFVEC opening

Formal Education Product Development and Dissemination. New York Hall of Sciences (NYHOS), in cooperation with Technical Education Research Center (TERC), and the Ames Astrobiology team will provide content development for the "Search for Extraterrestrial Life" exhibit at the NYHOS. We will participate in the design of the K-14 Discovery Center experience and hands-on activity stations which will deepen the visitors experience of the exhibit. A series of hands-on activity stations and a manual for investigations related to the activity stations that museum staff will use with the formal education student groups that frequent the museum will be developed.

Timeline:

- 2003 - Discovery Station content
- 2004 - Discovery Station Docent manual published

NASA Ames/Lunar and Planetary Institute. *The Professional development standard for teachers of science requires learning essential science content through perspectives and methods of inquiry, science learning experiences for teachers must involve teachers in actively investigating phenomena that can be studied scientifically, interpreting results, and making sense of findings consistent with currently accepted scientific understanding.* A YNP field experience in collaboration with The Lunar and Planetary Institute/Broker Facilitator role with NASA's Office of Space Science utilizing the Space Grant Consortium is a strong partnership that we have entered to provide professional development for 6-12 educators with field site studies. The field site and university connections rotate yearly.

Development and Presentation Team. NAI integrated Outreach team members Ames, MBL, and Penn State have designed and developed the first Astrobiology Short Course (five hours) accepted for presentation at the National Science Teacher's Association Conference where participants interact with scientists and educators from the NASA Astrobiology Institute as they learn about the latest content, research and curricula material *This team will explore new connections within the NAI teams and also continue past relationships.*

Children's book on the Microbial World in collaboration with The Carnegie Institution of Washington. In collaboration with CIW the Ames team will contribute to the creation of a 64 page, full color children's book on the Microbial World with over 70 original illustrations and high quality photographs. The Ames Astrobiology team will develop the teacher's guide and activities-based book with our collaborating partners, align the book to national standards, and participate in the field testing of the product. Research sites such as Yellowstone will serve as context for the story. The activities from this book can be incorporated into the Junior Ranger Program at Yellowstone National Park.

Astro-Venture is an educational, interactive, multimedia web environment focusing on Astrobiology. The Ames Astrobiology team provides content and design input into the storyboard development for the concept. The team participates on the review panel for all modules and lessons for the classroom.

5.1.2 Approach to Communicating Astrobiology

California Academy Of Sciences

CAS offers a variety of formal standards based K-14 workshops from an existing National Science Foundation grant, *meeting the criterion for leveraging non-NASA funds.* By incorporating an active and innovative formal education program with the museum we are targeting the underserved communities around the Bay area. We collaborate on presenting the Astrobiology Educator Guide and Astro-Venture program to the professional educators in the San Francisco City Schools. These programs were designed as interactive inquiry, standards-based activities that cover a range of astrobiology topics. Bio-Forum: high school, middle school, community college, and student science teachers are invited to symposia on a variety of topics of which astrobiology will be a selected topic involving the Ames team researchers as speakers. Continued development of programs for educators will evolve as the research team designs the new exhibits at the Academy.

Professional Development for Scientists. CAS and Ames education teams will work on developing science team partnerships and exhibit presentation material. Resource materials will transfer from the museum floor the formal classroom.

Exhibit Development. Our team will assist in the development the new concept and content for the new planetarium. We will be developing and testing ideas for the new exhibit spaces being created. Our team will prepare breaking mission stories, technology, and images for the news and exhibit program that can be transferred to YNP and the NYHOS.

Museum Tours. Lively and informative expeditions through the exhibition halls are led by museum docents who are education specialists. The Ames Astrobiology team will participate in the continued training of the docents at NASA Ames Research Center in collaboration with the Ames Education Office.

Yellowstone National Park Partnership

Yellowstone's diverse resources, ecological processes, and cultural history provide important opportunities for research and education. The Ames Astrobiology team will be a national leader in resource education and interpretation providing the diverse and sophisticated public with the high level of astrobiology research content incorporated into the plan. The integrated NASA Ames Team participated yearly on site at Yellowstone to develop the formal and informal education plan. The programs described below have been designed and accepted in the plan due to the planning team's collaboration with the national park system, which is opening the door to astrobiology on a significant level as we pursue our goal of inspiring the general public.

Old Faithful Visitor Education Center. The development of exhibits for a new OFVEC offers the NASA Astrobiology lead team an opportunity to work with the National Park Service's Division of Interpretation and an interdisciplinary team of geoscientists including geologists, volcanologists, geothermal specialists, microbiologists, sociologists, educators, exhibit specialists, and interpretive planners. The NASA Ames Astrobiology team will provide information on the astrobiology component of the thermophile story, based on their ongoing scientific research in YNP. Planned products that evolved from the planning committee include:

WaySide Exhibit and Trail Guides. Interpret astrobiology research in YNP through the development of outdoor wayside exhibits. These will be located park wide at sites that best illustrate the most compelling aspects of astrobiology research as it relates to the goals and themes stated in the Long-Range Interpretive Plan.

Expedition Yellowstone. The formal education team from Yellowstone will bring classroom lessons and activities to the outlying school districts that are rural and underserved many of which are Native Americans. The K-14 school districts will also have students transported back into the park for an on-site experience.

Junior Ranger Program. In collaboration with the Junior Ranger Program staff, we will develop a question and answer element or inquiry activity in the Junior Ranger newspaper designed for older children (9-14) and their parents. This activity might logically relate to development of wayside exhibits where children would have to visit a location and provide feedback as part of the overall process presented in the newspaper, leading to their achievement of Junior Ranger status.

Resources and Issues Handbook. We are contributing to the development of an expanded section of the Resources and Issues Handbook. This annually produced publication is a convenient, one volume reference manual used to train the interpreters, educators, and park staff in all offices needing quick and concise information on the full spectrum of YNP's natural and cultural resources, and controversial or critical management issues. Additionally, the Handbook is sold in bookstores at 8 visitor centers, museums, and contact stations in the park. The Ames team will provide astrobiology related content, which will then be passed through the editorial process and included in the Handbook. Sources for detailed/expanded levels of Ames team research will be listed in the Handbook.

Real Time Media. To inform the broad audiences that visit the park virtually we will work with YNP to incorporate remote broadcasts from the research sites, interactive exhibits and educational materials developed by the research team that involves the audience in the experiment.

Informal Public Talks. We will organize "fire-side" chats with the integrated Ames team and the community of astrobiology researchers to take place at YNP for the interpretive rangers and the general public.

Yellowstone Association Institute Course. A three-day field course entitled "Yellowstone's charismatic microorganisms" to interested visitors educators and the general public. The course will interpret microbial resources in YNP and will link them to life history on Earth and Astrobiology.

5.1.3 Audiences

The NASA Public Engagement program aims to include all of the diverse members of the American Public, with their varied backgrounds, wide range of experience and different ages. These large impact collaborative partnerships bring astrobiology to over four and a half million national and international general audiences reaching close to one million directly *inspiring the K-14 population of which half a million are the non traditional underrepresented audiences.* Each year 1.5 million visitors enjoy the many exhibits at CAS. The Education Division reaches over 300,000 more, many of them from urban, disadvantaged backgrounds, through tours, outreach, teacher workshops, lecture series,

museum facilitation, and special classes. Today, as one of the 10 largest natural history museums in the world, CAS brings the message of research to nearly one and a half million visitors each year. At YNP each year 3 million national and international tourists from varied geographic, economic and ethnic backgrounds visit and most of them go to see Old Faithful. The New York Hall of Science Exhibits/Discovery Learning program reaches close to 300,000 visitors in New York, 100,000 of which are underrepresented populations.

5.1.4 Project Impacts and/or Metrics

The Office of Space Science criteria, *which facilitates high quality product and program development by providing evaluation services, and by sharing their collective expertise and best practices.* For the CAS, YNP, and NYHOS large scale E/PO projects and programs three stages of evaluation are part of the assessment process: formative, summative, and field testing. The integrated NASA Ames Team participates yearly on site at Yellowstone to develop the formal and informal education plan. The Division of Interpretation and Education then applies sociological and other visitor survey data to develop multi-faceted, appropriately designed programs and media. The recommendations contained on the Long Range Interpretive Plan are based on appropriate uses of media and programs in relation to subject matter, setting, and visitor behavior patterns and expectations. Field testing will be part of that process with the exhibit and discovery station projects. Our "Summative" evaluation will provide us with the results of the effort: how effective it was, whether it met the stated intentions, whether it had other unanticipated effects. Summative evaluation is a major component of the CAS exhibit so as to publish the lessons learned as we develop future projects for the new museum. Methods of evaluation include focus groups, surveys, observations, follow-up interviews, pre- and post-testing, and many other techniques will be incorporated into our formal education program

5.1.5 Personnel and Their Expertise

Catherine J. Tsairides, Lead CoI, E/PO, will manage all aspects of the project directing the YNP, CAS and NYHOS partnerships. **Linda Young**, CoI, YNP Project and Deputy Chief of Interpretation, Planning and Media, will be responsible for selecting appropriate interpretive media and locations, and where relevant, develop media (content, design, fabrication), install and maintain media over the life-span of the device. Dr. **David Ward**, CoI, Montana State University, as a member of the YNP Old Faithful Visitor Education Center Scientific Advisory Board, will be the team's liaison for planning and developing exhibits in this important new venue. Dr. **Terrence Gosliner**, CoI, California Academy of Sciences, specifically for this project, will work with the integration of scientific content and its dissemination to audiences at the CAS. Dr. **Carol Tang**, CoI, California Academy of Sciences, will be responsible for facilitating the public outreach component at the CAS through exhibit development, lectures, workshops, outreach and professional development.

5.1.6 E/PO Costs

E/PO conference travel expenses, as well as salary for Catherine Tsairides, Lead CoI, will be covered as outlined in the outreach budget (section 11.8). California Academy of Sciences will receive $5K to provide formal astrobiology workshops and printing costs for advertising the programs. Dr. David Ward will receive $5K towards salary costs for the Yellowstone OFVEC Planning Committee, and YNP exhibit development. A Science Writer will receive $12K for content development.

5.2 Additional Elements

Here we summarize how Ames will make specific resources available to support the professional community, as well as the research and training described in this proposal. Some of these resources are offered at no, or reduced, cost to the NASA Astrobiology Institute. Also summarized is the involvement of the Ames team in professional and training activities, and in flight missions. This broad involvement indicates not only the high quality of the Ames team, but also the many important opportunities that Ames team membership in NAI will create for other NAI members and the astrobiology community.

5.2.1 Professional Community

5.2.1.1 Journal Editorships

Members of the Ames proposal team serve the professional research community in a variety of ways. The following team members will continue to serve in editorships and/or editorial boards of professional journals:

- Des Marais, D.: *Astrobiology*, Editorial Board; *Geobiology*, Editorial Board
- Lissauer, J.: *New Astronomy Reviews*, Editor
- Sandford, S.: *Meteoritics and Planetary Science*, Editor
- Schulte, M.: *Geochemical News*, Associate Editor, *Astrobiology*, Editorial Board
- Summons, R.: *Astrobiology*, Editorial Board
- Toon, O.B.: *Journal of Geophysical Research*, Associate Editor
- Treiman, A.: *Meteoritics and Planetary Science,* Associate Editor

5.2.1.2 Professional Workshops

Team members also will organize and participate in workshops that will advance the intellectual

content of the field, as well as contribute to the planning and development of missions.

For example, Dr. D. Des Marais chaired the Blue Dot Workshop (1997) and co-chaired the Pale Blue Dot Workshop II (1999). Each of these events provided the content for substantial NASA Conference Proceedings publications that were published the following year. These workshops identified major issues and approaches that address the search for evidence of life on habitable planets that orbit other stars. Thus they contributed directly to the conceptual development of a search strategy for NASA's Terrestrial Planet Finder mission, which is envisioned to be launched early in the next decade. *We propose to maintain this intellectual discourse by organizing an oral session "Searching for life beyond our solar system," to be held at the American Geophysical Union Fall meeting*, sometime during the proposed period of funding.

Dr. D. Hollenbach is the director of the Center for Star Formation Studies (CSF), a very successful NASA-funded group since 1985 of astrophysicists from the University of California at Berkeley, the University of California at Santa Cruz, and NASA Ames. CSF studies star and planet formation. *We propose to initiate a series of one-week summer workshops biennially focussed on the research of our Ames astrobiology group.* These workshops would bring together the diverse elements of the Ames group, the astrophysicists from CSF, and outside experts in the focussed research areas of the Ames group. The Ames NAI team, CSF astrophysicists, and students from San Francisco Bay area universities and beyond would benefit from this mutual interaction, which will stimulate new collaborations, more coherent interdisciplinary work, and cross fertilization of ideas.

5.2.1.3 NASA Advisory Committees

Team members participate in NASA advisory committees. For example, L. Allamandola recently served on the Origins Subcommittee of the Space Science Advisory Council (SSAC). D. Des Marais is a former member of SSAC itself. Des Marais participated actively on the Project Science Integration Group (PSIG) for the 2009 Mars Science Laboratory (MSL) Mission. Des Marais continues to serve on Mars Exploration Payload Advisory Group (MEPAG) and as an advisor for MSL.

5.2.1.4 Scientific Meeting Organization

Team members contribute to the organization of future scientific meetings. For example, L. Allamandola is a member of the Scientific Organizing Committee for the future international symposium entitled, "The Astrophysics of Dust."

D. Des Marais serves on the steering committee for the international "Earth System Processes II" (ESP2) meeting, which will address the past, present and future Earth processes from a multidisciplinary and interdisciplinary viewpoint, analogous to the field of astrobiology. Indeed at least one session at the ESP2 meeting will specifically address astrobiology.

R. Young is a member of both the Scientific Organizing Committee and the Local Organizing Committee for the 2003 Division of Planetary Sciences Meeting of the American Astronomical Society. He also served on the Scientific Organizing Committee of the Astrobiology Science Conference in 2002.

L. Rothschild is the chair of the organizing committee for the 2004 Astrobiology Science Conference (AbSciCon04), to be held at Ames Research Center. AbSciCon has become a major forum for astrobiology. More than 600 scientists, technologists, students and lay public attended AbSciCon02. D. Des Marais will serve as a member of the scientific organizing committee for AbSciCon04.

5.2.2 Training

The Ames NAI team training effort includes the following activities: (1) participation by students and postgraduate research associates in the proposed research activities, (2) an undergraduate course at Stanford University, (3), a community college course, and (5) astrobiology-related workshops that involve students. These activities will contribute large quantities of novel and current source material for the Ames team's astrobiology education and public outreach program (Section 5.1).

Development of source material both for university and for postgraduate training, as well as K-12 education, will be the responsibility of the Ames scientists and other participants in the training and education and public outreach programs. Translation of source material into curriculum, web pages, public presentations, and other education or public outreach products will be the responsibility of professional educators, curriculum developers and communication specialists working with the scientists.

5.2.2.1 Training by Direct Involvement in the Proposed Research Program

For decades, the basic research program at Ames has substantially involved graduate students, postdoctoral research associates and more-advanced professionals. One example among many is the participation of National Research Council (NRC) Fellows in astrobiology-related research at Ames. *Over the past 10 years, more than 130 NRC Fellows have completed two-year training programs here; more than 50 of these were advised by investigators who are participating in this astrobiology proposal.* A substantial fraction of these former associates are still active in

astrobiology-related activities, as evidenced by the fact that some *20 of them are either investigators or named collaborators on this proposal*. Many former postdoctoral associates are participating in other proposals, and at least two are principal investigators.

This proposal seeks to continue this successful tradition of research training. *It requests support for an average of six graduate students and eight postdoctoral associates per year* (see personnel listings in the detailed budget sheets, Volume II).

Also, David Blake is directing an engineering project for astrobiology, resulting in deliverables for potential direct use in Mars '09 MSL mission technology development with the Harvey Mudd University engineering students and faculty. A new program involving David Blake will include an on site training program in astrobiology technology development for US Air Force Academy cadets

All of the participants that are mentioned above will work alongside NRC Fellows and other students and young professionals who participate in closely related programs at Ames. They will move freely between Ames laboratories and those of its collaborators, and several will work with interdisciplinary teams on field expeditions. They will benefit from the numerous astrobiology seminars and workshops held at Ames. This next generation of astrobiologists will receive truly interdisciplinary training, for they will witness the ongoing development of astrobiology at the place of its birth.

5.2.2.2 Stanford University Astrobiology Course

We propose to continue to sponsor a course on Astrobiology and Space Exploration in the Human Biology Department at Stanford University. The course "Astrobiology and Space Exploration" (Human Biology 107) has been offered at Stanford University for more than a decade by several members of the staff at Ames Research Center. The course has changed its scope and content over the years, and has reflected the latest theory, research and planning in the various fields that now comprise astrobiology. Dr Malcolm M. Cohen, Chief of the Human Information Processing Research Branch at Ames, is currently directing this astrobiology course. Dr. L. Rothschild, a lead CoI of the Ames NAI team, will assume direction of the course in 2004. C. Tsairides wll contribute to the organization and content of this course. Further, she will identify course content that can be utilized in the proposed program for E/PO.

The format of this class consists of seminars and lectures at Stanford, with thirty students per class. The prerequisites are to have had a college level course in biology, chemistry, physics, behavioral science, or mathematics. The course, originally offered in the Department of Aeronautics and Astronautics, and entitled "Colloquium on Life in Space," was moved to the Interdisciplinary Human Biology Program in 1994. Primarily designed for advanced undergraduate students in the sciences, the course emphasizes evolutionary themes that lead from the big bang to the human exploration of space. The goal of the course is to provide a context from which to examine the three fundamental questions of Astrobiology: "What is the origin and history of life? Do life forms exist beyond the Earth? What is the potential for human expansion beyond the home planet?" Space, time, and life itself are examined from a universal perspective, and the place of humanity in the universe is seen to emerge as a unique integration of chance and necessity, of chaos and of order.

ASTROBIOLOGY AND SPACE EXPLORATION
--- Course Schedule for Spring 2003 ---

April 1	M. Cohen: Introduction - Cosmology, Astrobiology, and Space Exploration
April 3	S. Shostak: The Big Bang, Our Universe, and All That Jazz
April 8	J. Cuzzi: Stellar Evolution and Habitable Planets
April 10	L. Allamandola: From Molecules to Microorganisms
April 15	L. Rothschild: Pushing the Envelope for Life; Life at the Edge
April 17	B. Runnegar: Evolution of Life from Microbial to Multicellular
April 22	D. Morrison: When Worlds Collide - Extraterrestrial Threats to Life
April 24	L. Rothschild: Replaying the Tape (Lecture and Class Discussion)
April 29	D. Des Marais: Environments for Life in the Universe
May 1	M. Cohen and E. Holton: Newton, Gravity, and Biology
May 6	C. Chyba: Looking for Life in All the Right Places – Europa, Titan and Elsewhere
May 8	E. Holton: Gravitational Biology and Adaptation to Space
May 13	M. Kliss: Life Support Systems for Space Exploration
May 15	C. McKay: Exploration and Colonization of Mars – Why and How
May 20	M. Cohen: Behavioral Adaptation and Working in Space
May 22	M. Cohen: Group Interaction and Crew Performance
May 27	M. Cohen: Circles in Space - Skylab, Shuttle, Mir and Space Station
May 29	S. Shostak: Search for Extraterrestrial Intelligence
June 3	M. Cohen: The Place of Humanity in the Universe
June 5	T.A.: Final Review (OPTIONAL)
June 6	Staff: Final Examination (12:15 - 3:15 p.m.)

While the course already exists and is available to a few Stanford students, we propose here to widen its impact greatly. As part of this proposal, the lectures will be recorded, edited, and made available on the internet. A web site for the lectures will provide a continuing opportunity for further elaboration and discussion of important issues during the intervals between annual presentations. This electronically based education and outreach effort represents the development of a living electronic textbook on astrobiology that will include an integration of efforts from the life sciences, space sciences, information sciences, and the outside community. These services will be provided as part of Ames' Institutional commitment.

The course Website is http://www.stanford.edu/class/humbio107

The course schedule for Spring 2003 is given below. The course offering for 2004 will emphasize more strongly those aspects of astrobiology addressing the space sciences and Earth system sciences.

5.2.2.3 The Center for Star Formation workshops

The Center for Star Formation workshops that were mentioned earlier under the "Professional Community Section 5.2.2.1, would indeed include graduate students and postdoctoral researchers. Thus the workshops would provide the opportunity to attract promising young researchers to the field of astrobiology from the astrophysics and planetary science communities. It is anticipated that a number of new collaborative research projects involving graduate students and the Ames astrobiology group would emerge from these workshop interactions.

5.2.2.4 The NASA Center for Computational Astrobiology and Fundamental Biology Students Internship Program

The NASA Center for Computational Astrobiology and Fundamental Biology plans to hold its fourth Summer Students Internship Program. Eight students participated in 2000 and also in 2001; 14 students participated in 2002. For 2003, members of the Ames NAI proposal team will once again recruit a group of students with diverse backgrounds interested in applying theoretical and computational methods to biology, chemistry, astrophysics and ecology. This program is open to undergraduate and graduate students who are U.S. citizens or permanent residents. Students will be working on a variety of projects that reflect the breadth of computational astrobiology.

Students will spend ten weeks at Ames working on individual research projects. Before arriving at Ames each student will be assigned a mentor and a project that matches her/his interests and skills. Our goal is to choose projects so that at the end of the internship each participant will accomplish tangible results. In addition, the students will participate in a series of collaborative activities that will expose them to a broad range of science being done at NASA-Ames, including that by the Ames NAI team. At the end of their internship, the students will prepare a publication-style report from their work. As they have in previous courses, these reports can sometimes lead to peer-reviewed publications.

To learn more about NCCAFB see http://cca.arc.nasa.gov.

5.2.2.5 Astrobiology Academy

The NASA Astrobiology Academy is a unique summer institute of higher learning whose goal is to help guide future leaders of the U.S. Space Program. While at Ames for a six-week period, the students participate in individual research guided by world-class investigators at the center, a rigorous lecture series, and a group project that fosters leadership skills and interdisciplinary collaborations. This course provides research opportunities in state-of-the-art astrobiology laboratories coupled with broad-based views into the inner workings of the space program and research missions. The students assigned to a principal investigator will work independently on a technical project of their choice. Several members of the proposed Ames NAI team participate as principal investigators. This mentor-student relationship gives students insight into the challenges and rewards of primary scientific research. Approximately 60% of the typical workweek is dedicated towards this aspect of the Academy. Academy participants also experience other aspects of NASA during field trips to other centers, tours, lectures, etc. A formal lecture series focusing on all the various aspects of astrobiology is coordinated with some of NASA's top scientists. The students participate in a group project that typically involves them designing a scientific and technology plan for a space flight mission relevant to astrobiology. The academy has an Alumni network of over 300 Academy graduates.

5.2.2.6 Linking Life's Origins and Ecology, Ecosystem-to-Biosphere Modeling: Graduate and Upper-division Undergraduate Training and Education and Outreach Component (Lead CoI: Chris Potter, CoI: Kelly Decker)

Objectives. This portion of the proposal explicitly addresses the undergraduate and graduate-level training objective and the objectives of Section 3.4 – 3.5 of the NAI CAN proposal announcement. The simulation model we are developing is well suited for use in a classroom or a computer laboratory. To this end, our overall objective is to create an interactive web page that

can be used for undergraduate and graduate education in courses that discuss microbial ecology, biogeochemistry, ecological simulation modeling, and astrobiology. This type of web-interactive modeling tool has been successfully used to teach principles of ecology and biology within a traditional in-campus context (e.g. Maxwell and Sheley 1997; Voinov 2002) with the aid of a professor. Once the page is constructed, we will have several professors use the tool in the classroom and ask them to help us evaluate the efficacy of the model as a tool for teaching astrobiology, microbial ecology and biogeochemistry.

Model description. The current and proposed model structures are outlined in the introduction to this module. In summary, we propose to adapt our Stella™ model so as to enable biogeochemical simulations of various photosynthetic mat types such as intertidal, stromatolite-forming, and cool thermal (Yellowstone) microbial mats. The adaptations of the model will also incorporate data acquired through partnerships with microbial astrobiologists at Ames and elsewhere.

Web site construction. The web site will be designed around the model and software necessary to use the model. The model was constructed in a Stellaä environment and there is a free Stella Reader available for downloading that allows students to alter the model input, run simulations and display or print output without saving the changes. The web site will include links to the model via this free software program. This is similar to how the model has been made available to several members of the astrobiology community via the use of Science Organizer. The site will also have directions for laboratory exercises that are tailored for different classes and sections of classes (see supplementary information). There will be exercises for grades 9-14 as well as for upper-division and graduate-level students. Instructors can opt to use exercises from the web site or create their own. At each step in each exercise, students can double click on instructions for use of this tool. There will also be a section to offer advice to teachers. At the end of each exercise, we will ask students and teachers to evaluate the web site as a learning exercise (see below).

Relevant disciplines. The first goal of this project is to provide web-integrated exercises for students within astrobiology, microbiology, ecosystem ecology, and ecosystem modeling. Students within these specialized disciplines are at an upper-division level, and can alter the model components that are relevant to their field and choose relevant data to graph. At this level, some or all of the line and box structure of the model itself can be revealed.

A second goal of this project is to develop a menu-driven interface of the model for use by students in grades 9-14. Visually, the model can be simplified for different uses depending on how much the student needs to understand of its inner workings. At the 9-14 grade level, students will be able to alter different portions of the model, and choose to display graphical output via pull-down menus. Additionally, simplified sub-models will be extracted from the mail model to illustrate simple ecophysiological relationships such as oxygen production during photosynthesis of the cyanobacteria. This will be an interactive option on a mat web page that is linked to the Yellowstone page.

Exercise examples. Below are some sample questions that students should be able to answer using the model.

- How might this microbial mat affect the atmosphere?
- How does a change in day length affect the output of O_2? How does it change the distribution of H_2S within the mat?
- How does a change of temperature affect bacterial growth?
- What happens if you remove the cyanobacteria? The sulfur-reducing bacteria?
- What happens if you change reaction rates for: sulfur reduction? Oxygenic photosynthesis? Methanogenesis?

Efficacy testing and feedback. Below are some sample questions that we will ask educators after they use our model. There will be a section on how the web site was used, what was required of the students, and how the teacher assisted. There will be a section on how effective the web instructions were. There will be a section on what the students learned from the experience, and what the shortcomings were. Finally, there will be a section suggesting modifications to the web site.

- For what course did you use this model?
- What did you wish to demonstrate using this model?
- What sort of classroom instruction did you provide to the students in 1) the use of this model and, 2) to help them interpret the output?
- What did you want the students to learn from this exercise?
- What was the model effective in teaching the students?
- What was the model ineffective at teaching the students?

Literature Cited

- Maxwell, B.D. and R.L. Sheley (1997). Noxious weed population dynamics education model. Weed Technology 11:182-88.
- Voinov, A. (2002). Teaching and learning ecological modeling over the web: a collaborative approach. Conservation Ecology 6(1) 10.

5.2.2.7 University of New Mexico Field Course - Minority Involvement

CoI A. Treiman is leading the organization of a grade 6-12 teacher training workshop, for summer 2003, with significant minority service. Treiman is working closely with Dr. Horton Newsom of the University of New Mexico (and other colleagues) to arrange a week-long training experience, focusing on geology and astrobiology, that merges field study in Arizona and northern New Mexico with laboratory/classroom studies of Mars and life in extreme environments. The workshop will last a week, of which three days will be field experience at Grand Canyon, Meteor Crater, Sunset Crater cinder cone, and hot springs of the Jemez Mountains NM. Classroom/lab exercises at the University of New Mexico will cover: processes of erosion, impact, tectonics, and volcanism; remote sensing of biological materials; and the biology of life in extreme environments. The latter exercises will be taught or assisted by other CoIs of this proposal (Hoehler and/or Blake). C. Tsairides, Lead CoI E/PO, will contribute to the preparation and presentation of course materials. The web site announcement of the workshop is at http://www.lpi.usra.edu/education/desert2003.

These workshops provide unique learning experiences for teachers in two respects. First is the close coordination of field study, laboratory study, and classroom exercises. For instance, extremophiles were a focus of last year's teacher training workshop to Yellowstone. The teachers saw extremophiles in their life habitats, and then did a series of lab and classroom experiments (with those same materials) about how these biota could be detected remotely (by reflectance spectra). This work is shown in the web site for the workshop, http://lpi.usra.edu/education/yellowstone2002. The second unique aspect of these workshops is the close association between teachers and active researchers. The teachers learn directly from experts working now in those disciplines, can ask questions at will, and can observe the investigator-oriented mindsets of the researchers. Commonly, the experts disagree, and that can be a learning experience in itself.

The 2003 workshop aims for a significant enrollment of minority teachers, especially Native Americans from Arizona and New Mexico. The organizers will pay registration fees for 5 or 6 Native American teachers, who will be selected through Dr. Newsom's close connections with the 'minority' university program SIPPE. It is hoped that these teachers, who would likely be otherwise unable to attend, will share their perspective on the landscape and its life. We will provide them with a geological and astrobiological perspective, and hope that it will complement their own perspectives to yield a deeper understanding of the importance of place to human and non-human life. We also hope to provide free registration for several teachers from minority-dominated or inner-city school districts in Texas and/or Louisiana.

These teacher-training workshops are positioned to provide a limited number of teachers, 40 for the coming summer, a deep understanding of planetary geology and astrobiology. We expect that participation will strongly influence the teachers' own classes – our evaluations have shown this to be true. We will also ask that the teachers spread their knowledge through their schools and districts via word-of-mouth and tutorials. In this way, we hope to achieve significant leverage from the limited number of teachers that can be served in our workshops.

5.2.2.8 Additional Astrobiology Courses

Several members currently teach university courses related to astrobiology, as follows: Planetary atmospheres and processes (O.B. Toon, Univ. Colo.); geophysics of terrestrial planets (N. Sleep, Stanford Univ.); processes in star and planet formation (G. Laughlin, Univ. of Calif., Santa Cruz); and planetary sciences (J. Lissauer, Stanford Univ.).

5.2.3 Teaming with a Minority Institution

5.2.3.1 Jackson State University

For the last three years Dr. Andrew Pohorille has served as a member (and the chair in 2001) of the External Advisory Board for the Computational Center for Molecular Structure & Interactions NSF CREST Center at Jackson State University. This summer, NASA Center for Computational Astrobiology and Fundamental Biology at Ames will hold its Third Summer Students Internship Program (see Section 5.2.2.4). Dr. Pohorille will recruit a group of students with diverse backgrounds interested in applying theoretical and computational methods to biology, chemistry, astrophysics and ecology. They will be working on a variety of projects that reflect the breadth of computational astrobiology.

5.2.4 Staff

With the exception of students and postdoctoral research associates, the Ames researchers and most of our academic colleagues who will carry out the work proposed are civil servants or tenured faculty who will not charge their time to this proposal. The contribution of civil service salaries and travel support exceeds four million dollars over the proposed performance period.

We will continue to augment this workforce. In the past five year funding period with NAI, *Ames hired three new civil service astrobiologists, two of whom appear as CoIs on this proposal.* To augment further the existing scientific staff needed to carry out our proposed research, the Ames Astrobiology and Space Directorate will hire one new astrobiologist during the proposed grant

period, and fill additional civil service positions in subsequent years as permitted by NASA resources and personnel ceilings. Although it is not assigned a dollar value in our accounting of the Ames contribution, this ongoing, long-term development of our human resources for astrobiology represents a major Ames commitment to this program.

5.2.5 Facilities

Ames Research Center commits unique specialized facilities and equipment in order to strengthen the research effort in the astrobiology community. Major components of this committed resource are listed below.

5.2.5.1 Astrochemistry Laboratory

This laboratory complex in Building N45 will support the spectroscopic and other laboratory measurements crucial to our proposed studies. This laboratory provides the capability to: (1) prepare realistic interstellar and cometary ice analogs under a wide variety of temperature and pressure conditions, (2) subject the analogs to radiation mimicking the interstellar and solar radiation fields, (3) measure the absorption spectra of the analogs from the ultraviolet through the far-infrared at all stages of sample preparation, (4) chemically analyze the photoproducts using techniques like HPLC, MS, GC-MS, chromatography, NMR, etc., and (5) characterize physical and chemical properties of the membranes produced by hydration of the amphiphilic molecules

The equipment needed to carry out items #1, 2, and 3 are in place and routinely operating at NASA-Ames. This equipment now includes two research level Fourier transform infrared spectrometers, two completely computer-controlled UV-Visible, moderate resolution monochromator systems, one Alexandrite laser system capable of continuous tuning from 200 nm to 1 μm for in-situ luminescence studies, five high vacuum sample preparation systems which operate routinely in the 10^{-8} mbar range, and four cryostats. Four of the cryostats are closed cycle helium refrigerators that operate between 10 K and room temperature, the fifth is a helium flow system that operates between 4.2 K and room temperature. Four of the cryostat-vacuum systems are mated to the spectrometers such that complete UV to far-IR spectral coverage from 180 nm to 50 cm^{-1} is available. The fifth cryostat-vacuum system will be devoted to full time residue production if this proposal is funded.

The analytical techniques now available at Ames to use to carry out item #4 are wet chemical analysis, High Performance Liquid Chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), deuterium labeled MS, and IR spectroscopy. The spectroscopic equipment, HPLC, as well as many of the wet chemical analytical tools necessary for this work are on hand in the Astrochemistry Laboratory at Ames while the GC/MS is located in the Life Sciences Laboratory at Ames.

5.2.5.2 The Ames Electron Microscopy Laboratory

The Electron Microscopy Laboratory at Ames Research Center provides a variety of analytical capabilities that will be made available as a remote research facility for members of the Institute (see Section 3) who participate in our proposed research. This facility includes the following instrumentation and equipment: Hitachi S-4000 Field Emission SEM with EDS / digital imaging; JEOL 2000FX Analytical Electron Microscope with EDS / digital imaging; Hitachi H-500H Cryogenic Electron Microscope, with digital imaging; liquid helium, liquid nitrogen, cryotransfer and analytical cold stages; automated Philips/Norelco X-ray Diffractometer; Renishaw Confocal Raman Spectrometer / microscope; Nicolet Nexus 670 FTIR; Oxford TOPS-3 mini-cryo (4 Kelvin) scanning tunneling microscope (STM); Perkin-Elmer Pyris 1 differential scanning calorimeter (DSC); Perkin-Elmer Lambda 900 UV-Vis-NIR spectrophotometer; Perkin-Elmer Pyris 1 thermo-gravimetric analyzer; Agilent HPLC; Digital Instruments Nanoscope IIIA multimode atomic force microscope (AFM); Molecular Imaging Picoscan AFM; Class 100 clean room with laminar flow clean hood; Nikon Microphot FXA petrographic microscope; Nikon SMZ-2 binocular microscope; Gatan dual ion-mill; Sorbol MT-2 microtomes with diamond knives; photographic dark room; VCR Group sample dimpler; VCR Group cold beam ion deposition system ("chrome coater"); Polaron Metal Sputter Coater; and an Edwards vacuum evaporator for metals and carbon.

5.2.5.3 Biogeochemical Laboratories

The biogeochemical laboratories at Ames maintain an array of instrumentation relevant to the chemical and visual characterization of microbial communities, including: gas chromatographs (Hewlett Packard, Inc.; SRI, Inc.; Trace Analytical, Inc.) equipped with FID (hydrocarbon), FPD (sulfur gases), TCD (atmospheric "fixed" gases), HgO-reduction (H_2, CO), and MSD (mass selective) detectors; an ion chromatography system (Dionex, Inc.) suitable for the determination of dissolved ion concentrations at micromolar levels; and a Finnigan-Mat Delta Plus mass spectrometer with GC-combustion interface, suitable for compound-specific analyses of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ at picomolar sample sizes.

Greenhouse Facility. Building N239 at Ames houses a rooftop greenhouse facility capable of maintaining live microbial mats under conditions of irradiance, flow, and substrate supply that

approximate natural environments in the field. This facility was developed during the previous grant period of the Ames NAI team, and represents a valuable heritage for future astrobiological research in microbial ecology (see Section 3). Recent studies demonstrate that the structure of the microbial community and the rates of major biogeochemical processes are retained, relative to field samples, for > 6 months during incubation in the greenhouse. This facility includes a UV-transparent enclosure and also flow boxes (flumes) that can maintain a total of 1.3 m^2 of active microbial mats. One table that contains these flow boxes is also equipped with a computer-controlled XYZ-positioning stage capable of deploying an instrument package (chemical and optical microsensors, cameras) at a desired location on/within the mats, with 10-mm resolution. The positioning stage and instrument package can be operated remotely by collaborators via a web-based interface.

Molecular Biology Facility. We are currently developing a laboratory for genetic analysis and characterization of microbiological samples. The new molecular biology laboratory is equipped with a –80°C So-Low freezer, an MJ Research thermal cycler for PCR, electrophoresis gel rigs, a Fotodyne UV gel imaging system, incubators, and an Eppendorf refrigerated centrifuge with fixed angle rotor.

5.2.5.4 Ames Computational Facilities

Computational Facilities at Ames Research Center continue to enjoy a world-class status. These facilities have long enhanced theoretical research in planetary science and astronomy at Ames (e.g., in support of *Investigations 1 and 2* in this proposal). More recently, they have enhanced research efforts in "Bioinformatics" (related to *Investigation 3*). These capabilities will thus enhance the overall research effort, as indicated in Section 3 of this proposal, with benefits to NAI members of other institutional teams, as the network of collaborations develops. These computational efforts and capabilities are discussed further in section 5.2.7.

5.2.6 Flight Missions

Ames NAI team members are intimately involved in a host of upcoming space flight missions that are centrally important for astrobiology. Below is a short description of each mission, the particular roles played by Ames team members, and how these roles will benefit research in astrobiology.

5.2.6.1 Kepler Mission

Kepler is a recently approved Discovery mission that was conceived and led by an Ames science team with Dr. W. Borucki as Principal Investigator. This mission sets the stage for understanding the abundance and distribution of habitable planets in the Universe. "The scientific goal of the Kepler Mission is to explore the structure and diversity of planetary systems. This is achieved by surveying a large sample of stars to: 1) Determine the frequency of terrestrial and larger planets in or near the habitable zone of a wide variety of spectral types of stars, 2) Determine the distributions of sizes and semi-major axes of these planets, 3) Estimate the frequency and orbital distributions of planets in multiple-stellar systems, 4) Determine the distributions of semi-major axis, albedo, size, mass and density of short-period giant planets, 5) Identify additional members of each photometrically discovered planetary system using complementary techniques; and 6) Determine the properties of those stars that harbor planetary systems. Transits by terrestrial planets produce a fractional change in stellar brightness of 5×10^{-5} to 40×10^{-5} lasting for 2 to 16 hours. The orbit and size of the planets can be calculated from the period and depth of the transit. The Kepler instrument is a 0.95-meter aperture differential photometer with a 105 $degree^2$ field of view. It continuously and simultaneously monitors brightnesses of 100,000 A-K dwarf (main sequence) stars brighter than 14th magnitude. From measurements of the period, change in brightness and known stellar type, the planetary size, orbital size and characteristic temperature are determined. From this the question of whether or not the planet is habitable (not necessarily inhabited) can be answered" (cited from http://www.kepler.arc.nasa.gov/summary.html). Kepler is scheduled to launch during October, 2006.

Ames NAI team member Dr. Jack Lissauer is also a CoI on the Kepler Mission. He is responsible for science planning, scientific data interpretation, and assessment of implications of the data for multiple planet systems. It will soon be possible to combine observational and theoretical efforts for the first time in order to evaluate quantitatively the frequency and characteristics of extrasolar planets, and to evaluate whether or not they are habitable. A major objective of *Investigation 1* is to conduct theoretical studies of terrestrial planets regarding their formation, orbital stability, evolution, and climatology. This research will be directly relevant to interpreting data obtained by Kepler, to be launched in 2007, and more advanced future missions, such as the planned Terrestrial Planet Finder Mission.

Members of the *Investigation 1* and Kepler science teams will co-host a workshop in 2008, the fifth year of this proposed Ames NAI membership. The two teams will share findings of mutual interest that could enhance the science return of the Kepler Mission.

5.2.6.2 2003 Mars Exploration Rover Mission (MER) Twin Rovers

These spacecraft are the products of a large effort led by Dr. Steve Squyres, the Athena payload team, and JPL. "The scientific objectives of the Mars Exploration Rover mission are the following: 1) Search for and characterize a variety of rocks and soils that hold clues to past water activity. In particular, samples sought will include those that have minerals deposited by water-related processes such as precipitation, evaporation, sedimentary cementation, or hydrothermal activity. 2) Determine the distribution and composition of minerals, rocks, and soils surrounding the landing sites. 3) Determine what geologic processes have shaped the local terrain and influenced the chemistry. Such processes could include water or wind erosion, sedimentation, hydrothermal mechanisms, volcanism, and cratering. 4) Perform "ground truth" — calibration and validation — of surface observations made by Mars orbiter instruments. This will help determine the accuracy and effectiveness of various instruments that survey Martian geology from orbit. 5) Search for iron-containing minerals, identify and quantify relative amounts of specific mineral types that contain water or were formed in water, such as iron-bearing carbonates. 6) Characterize the mineralogy and textures of rocks and soils and determine the processes that created them. 7) Search for geological clues to the environmental conditions that existed when liquid water was present. Assess whether those environments were conducive to life." (cited from http://mars.jpl.nasa.gov/mer) NASA will launch twin rovers to Mars in May and June, 2003, and will land on Mars in January 2004.

D. Des Marais is a member of the MER Science Operations Working Group (SOWG). As such, he will serve operationally as one of the leads of the SOWG Long Term Planning science theme groups (LTPSTG). The LTPSTGs develop and sustain a strategic plan that looks several sols (martian "days") ahead of the current tactical operations. Also, the LTPSTGs supervise and maintain the scientific discourse during the mission, leading to an integration of all-important tactical efforts and the achievement of mission success criteria, as well as the objectives for astrobiology and other key scientific disciplines. The involvement of Des Marais with the MER mission will enable scientific perspectives and priorities developed from the NAI research program to be factored into the MER mission. In turn, the findings and perspectives gained from this mission will help to guide research by the Ames team, as well as NAI, that is related to future initiatives in NASA's Solar System Exploration program.

Several of our proposed investigations will provide basic science in direct support of MER and future Mars missions. The aqueous mineral alteration studies in *Investigation 4* will provide a context for the interpretation of MER data. Similarly, the development and quantification of the concept of energetic habitability by *Investigation 4*, in concert with studies of classical habitability by *Investigation 1* , will establish stringent boundary conditions that may greatly narrow the search parameters for subsurface life on Mars.

5.2.6.3 Mars 2005 Reconnaissance Orbiter, CRISM Spectrometer

The 2005 orbiter mission includes the CRISM remote sensing instrument, developed and managed by the Johns Hopkins University Applied Physics Laboratory. CRISM's mission is the following, "…to find the spectral fingerprints of aqueous and hydrothermal deposits and map the geology, composition and stratigraphy of surface features. The instrument will also watch the seasonal variations in Martian dust and ice aerosols, check the water content in surface materials and collect atmospheric data — leading to new areas of exploration and discovery." (cited from http://crism.jhuapl.edu).

This near-infrared spectrometer will obtain high spatial resolution coverage of the entire planet in the near infrared wavelength. These observations should reveal geological deposits that might have recorded the activity of liquid water and thus the potential for habitable conditions on Mars.

D. Des Marais is a participating scientist for astrobiology on the CRISM instrument team. In this role, he will recommend sites for detailed near-infrared observations, with the intent of identifying and characterizing mineralogical assemblages that indicate the effects of aqueous alteration of the martian crust. The sites of interest would include the martian equivalents of the ophiolite-hosted spring systems to be examined by *Investigation 4* of this proposal. The proposed studies of these spring systems would indeed enhance the prospects for detecting analogous fossil spring systems during the Odyssey Mission.

5.2.6.4 Stratospheric Observer for Infrared Astronomy (SOFIA)

SOFIA is an airborne observatory that will study the universe in the infrared spectral region. SOFIA will serve in the development of observational techniques, of new instrumentation and in the education of young scientists and teachers in the discipline of infrared astronomy. NASA and the DLR, the German Aerospace Center, are creating the airborne observatory - a Boeing 747SP aircraft to accommodate a 2.5-meter reflecting telescope. SOFIA will be the largest airborne observatory in the world, and will make observations that are

impossible for even the largest and highest of ground-based telescopes. SOFIA will be based at NASA's Ames Research Center in California, and is expected to begin flying in 2004.

The expertise of the Ames Astrochemistry Laboratory Group (*Investigation 2)* has been and will continue to be used to develop design specifications for the mid-IR instrument to be flown on SOFIA. Furthermore, our spectral database and experimental capabilities will be used to interpret the mid-IR spectra that will be measured by SOFIA. Mid-IR spectroscopy is particularly suited to probe the prebiotic organic composition of cosmic materials. Thus, this will add to our understanding of the interstellar organic inventory of the Galaxy and sharpen our ability to distinguish between true and false spectroscopic biosignatures.

5.2.6.5 Space Infrared Telescope Facility (SIRTF)

SIRTF is the next in NASA's series of Great Observatories. It will be launched in April, 2003 for a 2.5 to 5 year mission. It consists of a 0.85-meter diameter lightweight telescope and three cryogenically cooled science instruments to provide imaging and spectroscopy from 3 to 180 microns. Incorporating large-format detector arrays and innovative choices in orbit and cryogenic architecture, SIRTF offers orders-of-magnitude improvements in capability over previous infrared telescopes. SIRTF will study phenomena ranging from our Solar System to the distant reaches of the Universe. SIRTF represents an important scientific and technical cornerstone of NASA's Astronomical Search for Origins Program.

The Astrochemistry Laboratory's database (*Investigation 2*) will be used to interpret the mid-IR spectra measured for many deeply embedded objects and PAH emitting objects, both galactic and extragalactic. The extragalactic aspect of this will deepen our understanding of the organic inventory of the cosmos and the evolution of these carbonaceous materials over the past several billion years. The Galactic aspect will reveal the nature of the raw materials that are the building blocks of planetary systems in the Milky Way. The team for *Investigation 2* will be involved in both aspects. The Astrochemistry Lab group has the world's largest database of PAH IR properties taken under realistic interstellar conditions. These will be used to map the distribution of carbon in many, many galaxies for the first time. Within our Galaxy, the infrared database of celestial ice analogs that is maintained by the Astrochemistry Laboratory will be used to interpret observations of dense star and planet forming clouds.

CoI David Hollenbach is also Co-I on the SIRTF Legacy Proposal entitled "Formation and Evolution of Planetary Systems " associated with the SIRTF Mission. His responsibilities include leading the protoplanetary disk gas modeling activity and data reduction effort. SIRTF Legacy projects are large and coherent science projects, not reproducible by any reasonable number or combination of smaller General Observer investigations. They are projects of general and lasting importance to the broad astronomical community with the SIRTF observational data yielding a substantial and coherent database. The selection process for SIRTF Legacy awards was highly competitive, with only 6 investigations selected from 35 proposals.

5.2.6.6 Stardust-Comet Dust Sample Return Mission

Stardust is the first U.S. space mission dedicated solely to the exploration of a comet, and the first robotic mission designed to return extraterrestrial material from outside the orbit of the Moon. The Stardust spacecraft was launched on February 7, 1999. The primary goal of Stardust is to collect dust and carbon-based samples during its closest encounter with Comet Wild 2, a rendezvous scheduled to take place in January 2004. Additionally, the Stardust spacecraft will bring back samples of interstellar dust, including recently discovered dust streaming into our Solar System from the direction of Sagittarius. These materials are believed to consist of ancient pre-solar interstellar grains and remnants from the formation of the Solar System.

S. Sandford, a CoI on this proposal, is also a CoI on the Stardust mission. The Ames Astrochemistry Laboratory played a direct role in development of the properties of the aerogel to be used for sample collection during this mission. We will be involved in sample analysis of the first cometary samples ever studied. For example, we will search for the presence of amino acids, amphiphilic molecules and deuterium enrichment within the organics and compare these with model predictions and the findings from similar studies in meteorites and interplanetary dust particles. These observations are direct extensions of the work proposed under Investigation 2.

5.2.6.7 Astrobiology Explorer (ABE)

ABE is the first space mission solely devoted to addressing key astrobiological questions. ABE is a cooled 60 cm diameter telescope equipped with spectrographs covering the 2.5-20 μm spectral range at a spectral resolution of > 2500, far in excess of any other mid-infrared mission. This will enable ABE, in conjunction with the spectral database available in The Astrochemistry Laboratory group (*Investigation 2 team*) to determine the prebiotic chemical inventory of all major astrobiologically important classes of astronomical objects at all stages of their history.

This comprehensive study will probe chemical composition, tracking the history of carbon from its birth in circumstellar ejecta, its voyage through the diffuse interstellar medium, incorporation in dense clouds and star forming regions, and ending with the composition of objects in the Solar System. ABE will also characterize the organic chemistry and carbonaceous dust composition of many galaxy types for the first time, including Dwarfs, Ellipticals, Disk Starburst, ULIRGS, and galaxies with active nuclei (AGNs).

S. Sandford, a CoI of *Investigation 2* of this proposal, is also PI of the ABE mission. Lou Allamandola is CoI of both proposals. The Ames Astrophysics Laboratory group has played a key role in the concept development, design, and proposal for this mission that has been funded for study. It is among the first MIDEX missions to succeed getting this support on the first attempt. Members of *Investigation 2* are intimately coupled to this mission and will be involved at all stages of its execution.

5.2.6.8 Earth Observing System (EOS)

EOS is the principal science mission of NASA's Earth Science Enterprise (ESE). EOS consists of a series of satellite observing missions, a science component, and a data system that supports a coordinated series of polar-orbiting and low inclination satellites for long-term global observations of the land surface, biosphere, solid Earth, atmosphere, and oceans. *Investigations 5 and 6* propose to make extensive use EOS satellite imagery for astrobiology research. Moreover, the investigators for *Investigation 5* are funded as PIs on current EOS investigation teams for Interdisciplinary Science (IDS) and the Large-Scale Biosphere Atmosphere Experiment in Amazonia (LBA), and can thereby effectively leverage all relevant ESE program support."

5.2.6.9 Future Missions

The New Frontiers Program is a new NASA planetary exploration program consisting of medium class missions ($650M) to be initiated in 2003 as recommended by the 2002 NRC Planetary Science Decadal Report. L. Allamandola, R. E. Young, and K. Zahnle are participating on science planning teams for Comet, Jupiter, and Venus missions that may be conducted under the New Frontiers Program. Their responsibilities include defining science objectives, and determining science instrument requirements.

Comet Surface Sample Return Mission Concept is being developed in response to NASA's New Frontiers program. L. Allamandola is among a small number of individuals who are developing a mission to respond to this flight opportunity. Dr. Joe Ververka of Cornell University is coordinating this effort as its PI. This mission is to return a significant amount of comet surface material, the primordial ingredients of our solar system. If the research proposed in *Investigation 1* is funded, its findings could influence the development of the concepts and capabilities of this mission.

Terrestrial Planet Finder (TPF) *Investigation 4* will directly support the design and interpretation of TPF and related missions (e.g., establishing frequency window, spectral resolution requirements), by identifying candidate search molecules and developing mechanistic models to help understand the controls on the atmospheric abundances of such species.

5.2.7 Information Technology

5.2.7.1 The NASA Center for Computational Astrobiology and Fundamental Biology

The principal objective of the NASA Center for Computational Astrobiology and Fundamental Biology (NCCAFB) at the Ames Research Center is to advance our understanding of the origin, evolution and distribution of life in the Universe, using theoretical and computational tools. NCCAFB, adopting the multidisciplinary spirit of Astrobiology and Fundamental Biology synthesizes diverse methods and viewpoints. The Center draws on scientists with different backgrounds and interests across different organizations at Ames and in other institutions involved in NASA-related research. The Center's capabilities are greatly leveraged by Ames' status as the Lead Center in information technologies. The NCCAFB currently has access to over 4,000 processors on supercomputers at the NASA Advanced Supercomputing Division.

The main goals of NCCAFB are:

- Provide scientific and programmatic leadership in computational astrobiology and fundamental biology.
- Organize an active international community of scientists working in the fields of computational astrobiology and fundamental biology by extensive research collaborations, visiting scholar programs, workshops, seminars, etc.
- Develop and participate in joint research programs with experimental astrobiologists.
- Participate in developing NASA astrobiology-oriented missions and in analyzing data from these missions.
- Promote applications of high performance computing, communication, data analysis, and database management to astrobiology.
- Promote applications of computational astrobiology to other disciplines and to technology transfer to benefit life on earth.
- Examples of astrobiological problems that are being studied by scientists at NCCAFB are:
- Self-organization of organic matter into the simplest life forms phylogenetic relationships between organisms;

- Evolution of metabolisms of simple organisms in ambient and extreme environments;
- Changes in gene expression of terrestrial organisms in the absence of gravity;
- The future habitability of earth, as determined by interactions between biosphere and the chemistry and radiation balance of the atmosphere;
- Design, stability and evolution of systems that support life in space.

More information about NCCAFB may be found at http://cca.arc.nasa.gov

5.2.7.2 NASA Center for Astrobioinformatics

Scientists at Ames are in the final stage of creating a state-of-the-art bioinformatics center for analysis of genomic and proteomic data from modern biological experiments. The capabilities of this center will be greatly enhanced by collaborations with several leading institutions in the country, such as Stanford University, U.C. Berkeley and National Cancer Institute. The resources of the Center and the assistance of professional bioinformatics staff will be made available to all NAI members at no charge.

A variety of research programs within NAI have relied to an increasing extent on obtaining and interpreting genomic and proteomic data. It is expected that this trend will continue. To receive maximum benefit from the data, one must often analyze and characterize data with a variety of computational bioinformatics tools and, in the process, use other, relevant information stored in multiple large databases.

To meet bioinformatics needs, the NASA Center for Computational Astrobiology and Fundamental Biology, based at Ames, has established a NASA Center for Astrobioinformatics, a dedicated facility for genomic analysis and a repository for microarray data. Its main function is to create bioinformatics environment needed for solving NASA-specific biology problems. This environment will consist of tools, databases and knowledge management system suited for NASA goals in astrobiology, space genetics and fundamental biology. It will provide a unified, integrated platform for NASA scientists and their collaborators to share data, information and analyses and, by doing so, greatly improve the capabilities for interdisciplinary, collaborative research. The capabilities of the environment will be further enhanced by developing new tools, adapting to the needs of bioinformatics several sophisticated techniques for data analysis, originally developed at NASA to support other research areas, and by integrating bioinformatics capabilities with massively parallel computational environment at Ames.

Among the capabilities of the Astrobioinformatics Center are:

- A dedicated Linux cluster of 40 CPUs for genomic computations,
- A data repository for genomic and proteomic data,
- A database system, which contains a number of databases that are continuously updated (including a catalogue of microarray data relating to experiments in space and ground-based controls),
- Software for analysis of genomic, proteomic and microarray data,
- Tools for data visualization and manipulation,
- Novel, sophisticated tools for discovery of regulatory networks by combining microarray data with background biological knowledge.

All of the tools offered by the Center are conveniently available though web interface. The Center will become operational by April 2003 and by the end of the year most of its capabilities should be in place.

The capabilities of the Center will be further leveraged by formal partnerships with the Advanced Biomedical Computer Center at the National Cancer Institute and the Center for Biomedical Computation at Stanford University, which are already in place, and by active collaboration with Biostatistics Department and U.C. Berkeley. Several other institutions, including NCBI and U.C. San Francisco expressed interest in developing similar collaborations. The scope of the collaboration includes exchange of tools, joint work on the development of new computational methods and databases and sharing experience with knowledge management techniques.

5.2.8 Linkage to Other Agencies

The programs for research, training, education and public outreach outlined in this proposal involve several outside institutions, including other government agencies and private foundations. Non-university institutions and their staff who are participating in this proposal include the following: Bay Area Environmental Research Institute (CoI M. Rabbette), California Academy of Sciences (CoIs T. Gosliner and C. Tang), Deutsche Zentrum für Luft- und Raumfahrte (CoI G. Horneck and Collaborator P. Rettberg), Flugmedizin RWTH Aachen (Collaborator E. Rabbow), Lunar and Planetary Institute (CoI A. Treiman), National Park Service (CoI L. Young), National Research Council (Collaborator V. Orphan), Royal Observatory, Edinburgh, UK (Collaborator R. Wolstencroft), and SETI Institute (CoIs M. Bernstein and R. Mancinelli).

6.0 FACILITIES AND EQUIPMENT

6.1 Investigation 1 - Formation and Evolution of Habitable Planets

No major special facilities or equipment are required for the proposed research. Commonly available computers and workstations will be sufficient.

6.2 Investigation 2 – Prebiotic Organics, from Space to the First Membranes

Central to this project is the ability to: (1) prepare realistic interstellar and cometary ice analogs under a wide variety of temperature and pressure conditions, (2) subject the analogs to radiation mimicking the interstellar and solar radiation fields, (3) measure the absorption spectra of the analogs from the ultraviolet through the far-infrared at all stages of sample preparation, (4) chemically analyze the photoproducts using techniques like HPLC, MS, GC-MS, chromatography, NMR, etc., and (5) characterize physical and chemical properties of the membranes produced by hydration of the amphiphillic molecules

The equipment needed to carry out items 1, 2, and 3 are in place and routinely operating at NASA-Ames. This equipment and the techniques associated with its use are described in Allamandola et al. (1988). This equipment now includes two research level Fourier transform infrared spectrometers, two completely computer-controlled UV-Visible, moderate resolution monochromator systems, one Alexandrite laser system capable of continuous tuning from 200 nm to 1 μm for in-situ luminescence studies, five high vacuum sample preparation systems which operate routinely in the 10-8 mbar range, and four cryostats. Four of the cryostats are closed cycle helium refrigerators that operate between 10 K and room temperature, the fifth is a helium flow system that operates between 4.2 K and room temperature. Four of the cryostat-vacuum systems are mated to the spectrometers such that complete UV to far-IR spectral coverage from 180 nm to 50 cm-1 is available. The fifth cryostat-vacuum system will be devoted to full time residue production if this proposal is funded.

The analytical techniques we now have available at Ames to use to carry out item 4 are wet chemical analysis, High Performance Liquid Chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), deuterium labeled MS, and IR spectroscopy. The spectroscopic equipment, HPLC, as well as many of the wet chemical analytical tools necessary for this work are on hand in the Astrochemistry Laboratory at Ames while the GC/MS is in the Life Sciences Laboratory at Ames. Our collaboration with Professor Deamer at the University of California at Santa Cruz adds the availability of thin layer chromatography, fluorescence microscopy, and membrane analysis techniques. Our collaboration with Professor R. Zare at Stanford University gives us access to laser desorption mass spectroscopic sample analysis. Thus all is in hand to carry out the work proposed here.

6.3 Investigation 3 - The Origin and Early Evolution of Proteins and Metabolism

Computer facilities in the Pohorille laboratory at NASA-Ames Research Center include a Linux cluster of 20 modern workstations, each containing 2 CPUs, two 4-processor Silicon Graphics Origin 1000 workstation, three single-processor Silicon Graphics graphics workstation, several personal computers, X-window terminals and laser printers. The P.I. also has free access to the graphics facilities in the Computer Graphics Laboratory at Ames.

Advanced simulations will be performed using resources at the Numerical Aerospace Simulation (NAS) Division at NASA-Ames Research Center. NAS is equipped with several SGI massively parallel computers, including one 1024 CPU, one 512 CPU and two 256 CPU machines. The P.I. has free access to these resources.

Department of Molecular Biology Resources at Massachusetts General Hospital:

Laboratory: The Department of Molecular Biology at MGH occupies the upper half (floors 7-12) of the 150,000 square foot Wellman Research Building. Main laboratory facilities are housed on floors 8 through 11, with greenhouses, plant labs, and growth chamber facilities on floors 7 and 12. Typically, each main laboratory floor houses three research groups, placing 30-40 researchers on a floor. Each floor has a full complement of laboratory support spaces—two instrument rooms, warm/cold rooms, a balance and chemical storage room, a large tissue culture facility, electrophoresis and gel rooms, a dark booth and a specialty dark room, and generous fume hood capacity, including an isotope dilution hood. The Szostak laboratory comprises the right half of the 9th floor.

In addition to group- and floor-specific support spaces, each main floor also houses one or more department-wide support facilities. The 8th floor contains an animal storage and prep facility. The main administrative offices, supply area, materials management and purchasing operations, and mail services are found on the 9th floor. Glasswashing, media preparation, and sterilization facilities occupy the north (departmental) section of the 10th floor. These services process all laboratory glassware, plasticware, and pipettes for the Department, as well as supplying a broad range of sterile plates, growth media and buffers. Eleventh floor departmental spaces include the

conference/meeting room. The meeting room is separated from an adjacent, oversized tearoom by a sliding soundproof wall which, when opened, provides 80+ seating for departmental seminars and other social and scientific functions. A tea/conference room, equipped with full kitchen facilities, is also found on each of the four main floors.

Computer: The Department's UNIX and Windows NT servers and workstations form the heart of a sophisticated network of Macintosh and PC computers and printers. The network provides the vehicle for a highly developed package of genetic sequence manipulation and analysis programs. Macintosh & PC computers are available throughout the laboratories, offices, and in tea and conference rooms as well. At least 2 PostScript laser printers & a color printer are available for common use on each floor with additional laser printers available in secretarial offices. Flatbed & 35mm color digitizing scanners, photographic quality color printer, automatic slide maker, and dial-in remote access servers are also available. Full Ethernet communications networks are provided for within the Department and to the greater MGH computer network. All computers within the department are fully connected to the worldwide Internet via a high-speed connection. Interactive molecular design software is also available utilizing a high performance Silicon Graphics computer. All departmental computers are provided with a wide variety of state-of-the-art scientific and office productivity software. The Department maintains a number of internationally used World Wide Web servers including the Arabidopsis and Caenorhadbitis genomes.

Office: A two-person, floor secretarial office and the offices of the three faculty members are found on each of the four main floors.

Other: MGH Core Facility: the oligonucleotide synthesis and oligonucleotide sequencing cores, are housed within and run by the Department. The Core's 3 ABI 377 DNA Sequencers can run a minimum of 1000 samples/week and the Core is in the process of upgrading to a capillary electrophoresis machine which will allow a minimum of 384 samples/8 hr. day The Core's The cores 6 Millipore Expedite workstations allow synthesis of 88 concurrent oligos for a minimal output of 1760/wk. Additional services to all departmental researchers needing peptide synthesis, protein sequencing, and/or amino acid analysis are available within the MGH community.

Major Shared Equipment: The following list is representative of other major scientific equipment distributed throughout the Department: 20 ultracentrifuges, 12 high speed centrifuges, 4 low speed centrifuges, 4 liquid scintillation counters, 2 gamma counters, 4 lyophilizers, computer-driven spectrophotometers (including melting curve capability), dozens of tissue culture and other incubators, several preparative and analytical HPLC's, an FPLC, an analytical microdensitometer, a fluorescence spectrophotometer, ultramicrotome for EM sectioning, Imaging System, Total Array System, Picking and Gridding Q Bot, and a multitude of stereo, dissecting, inverted, and microinjection microscopes. Departmental darkroom facilities are equipped with 2 X-O-Mat processors, an instant slide maker, and an automatic print processor.

Szostak Laboratory: The Szostak laboratory consists of approximately 2000 sf of bench space (20 lab benches), along with additional special purpose rooms and shared instrument space detailed above. The lab contains four chemical hoods suitable for organic syntheses, and equipped with vacuum pumps and standard equipment for organic synthesis. NMR, FTIR, and mass spectrometer facilities are available through Harvard. The lab is well equipped with state of the art instrumentation including two HPLC systems, an automated mini-prep 24 processor, Octane/SSE Dual Computer Molecular Graphics WorkStations, UV-Vis and Fluorescence Spectrometers, Rapid Quench Flow device for fast kinetics, a Biacore X surface plasmon resonance instrument for measuring binding interactions, an instant imager for imaging radioactive gels, and 2 DNA thermal cycler 480 PCR machines. Equipment particularly relevant to this grant proposal includes a Pharmacia FPLC system for protein purification and a Jasco Instruments J-715 CD Spectrometer.

6.4 Investigation 4 - Biosignatures in Chemosynthetic and Photosynthetic Ecosystems

The Ames Electron Microscopy Laboratory houses a broad array of equipment for the micro-scale visual and mineralogical characterization of rock samples. This facility includes the following instrumentation and equipment: Hitachi S-4000 Field Emission SEM with EDS / digital imaging; JEOL 2000FX Analytical Electron Microscope with EDS / digital imaging; Hitachi H-500H Cryogenic Electron Microscope, with digital imaging; liquid helium, liquid nitrogen, cryotransfer and analytical cold stages; automated Philips/Norelco X-ray Diffractometer; Renishaw Confocal Raman Spectrometer / microscope; Nicolet Nexus 670 FTIR; Oxford TOPS-3 mini-cryo (4 Kelvin) scanning tunneling microscope (STM); Perkin-Elmer Pyris 1 differential scanning calorimeter (DSC); Perkin-Elmer Lambda 900 UV-Vis-NIR spectrophotometer; Perkin-Elmer Pyris 1 thermo-gravimetric analyzer; Agilent HPLC; Digital Instruments Nanoscope IIIA multimode atomic force microscope (AFM); Molecular Imaging Picoscan AFM; Class 100 clean room with laminar flow clean hood; Nikon

Microphot FXA petrographic microscope; Nikon SMZ-2 binocular microscope; Gatan dual ion-mill; Sorbol MT-2 microtomes with diamond knives; photographic dark room; VCR Group sample dimpler; VCR Group cold beam ion deposition system ("chrome coater"); Polaron Metal Sputter Coater; and an Edwards vacuum evaporator for metals and carbon.

Biogeochemistry and Microbial Ecology Laboratories at Ames maintain an array of instrumentation relevant to the chemical and visual characterization of microbial communities, including: gas chromatographs (Hewlett Packard, Inc.; SRI, Inc.; Trace Analytical, Inc.) equipped with FID (hydrocarbon), FPD (sulfur gases), TCD (atmospheric "fixed" gases), HgO-reduction (H_2, CO), and MSD (mass selective) detectors; an ion chromatography system (Dionex, Inc.) suitable for the determination of dissolved ion concentrations at micromolar levels; and a Finigan-Mat Delta Plus mass spectrometer with GC-combustion interface, suitable for compound-specific analyses of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ at picomolar sample sizes.

Greenhouse Facility. Building N239 at Ames houses a rooftop greenhouse facility capable of maintaining live microbial mats under conditions of irradiance, flow, and substrate supply that approximate natural environments in the field. Recent studies demonstrate that the structure of the microbial community and the rates of major biogeochemical processes are retained, relative to field samples, for > 6 months during incubation in the greenhouse. This facility includes a UV-transparent enclosure and also flow boxes (flumes) that can maintain a total of 1.3 m^2 of active microbial mats. One table that contains these flow boxes is also equipped with a computer-controlled XYZ-positioning stage capable of deploying an instrument package (chemical and optical microsensors, cameras) at a desired location on/within the mats, with 50-mm vertical and 1-mm horizontal resolution. The positioning stage and instrument package can be operated remotely by collaborators via a web-based interface.

Molecular Biology Facility. We are currently developing a laboratory for genetic analysis and characterization of microbiological samples. The new molecular biology laboratory is equipped with a –80°C So-Low freezer, an MJ Research thermal cycler for PCR, electrophoresis gel rigs, a Fotodyne UV gel imaging system, incubators, and an Eppendorf refrigerated centrifuge with fixed angle rotor.

6.5 Investigation 5 - Modeling Ecosystems and Biospheres

The Ecosystem Computational Facility (ECF) at NASA Ames is well equipped and staffed to support our modeling activities. ECF will supply most of the necessary computational support to the research, including data backup and storage, GIS software support, digital scanner, color printing and film recorder services. Existing equipment in direct support of this research includes several computers hosting systems modeling, GIS, spreadsheet, word-processing, statistics and image processing software (for an updated on-line description, see http://geo.arc.nasa.gov/info/ECF_Description.html). Specific to our research, four workstations, including a 4-CPU Sun Microsystems UltraSPARC server, are available for modeling and image processing tasks, while a fifth system is shared between image processing and GIS tasks. All systems have 24-bit color displays. The primary image processing package is ERDAS IMAGINE, although several other packages (listed below) are also available. Raster imagery from both aircraft and satellite sensors is used extensively in the analysis process, and ranges from standard Landsat and SPOT data to experimental hyperspectral sensors such as AVIRIS. GIS software available includes ARC/INFO (including GRID, TIN, and NETWORK), and GRASS, plus ENVI and Matlab for image analysis. In addition to our own ECF, we periodically have access (albeit not unlimited) to NASA Ames Center-wide facilities for high performance computing. Fees are likely to apply in the future for computing time on these facilities.

6.6 Investigation 6 - Hind-Casting Past Environments

We have access to the Ecosystem Computational Facility (ECF) described in section 6.5. We also have access to laboratories on the ARC center grounds. There, we have glassware, plastic ware and hardware; a microscope workstation, with the following microscopes: 1) Zeiss Photomat light microscope with automatic photomicrography equipment, phase contrast and planapochromatic optic, fluorescence equipment. 2) Zeiss Utraphot II light microscope with zoom attachment and large format photomicrography attachment. 3) American Optics Spencer light microscope with phase contrast optic. 4) Wild Stereo microscope with epi and transillumination and zoom optic. 5) High Resolution Imaging Microscope (HIRIM) with computer for cell-level spectrometry; various balances with precision to 0.01g; a Zeiss adapter 0.5 x for c-mount camera, 1-CCD Pulnix high resolution color video camera with light and speed regulation enabling the video signal to be directed to a monitor, a video printer, a video recorder or to a computer desktop and lap-top computers (Apple and PC), printers, scanners, and access to large format (91 cm) printers; a glasshouse on the building terrace; an Optronics OL-752 portable spectroradiometer, double monochromator with OL 752-O-PMT head; ADC LDC4 gas exchange

(photosynthesis) measurement system and a Minolta SPAD-502 chlorophyll meter with calibration disk.. In addition to the ECF computers and software, we have various Apple and PC personal computers, statistical and plotting software, word processing software, photo-manipulation software.

Laboratory - NASA Ames Research Center:

- Fumes-hoods
- Open growth chamber with light and UV radiation sources, timer for day/night simulation and monitor lights
- Ohaus balances (piezoelectric) (Precision = 0.1g)
- Metler piezoelectric balance (Precision = 0.01g)
- Metler analytic balance
- Electric oven
- Electric furnace
- Centrifuges
- Vortex mixers, hotplates and small equipment
- Glassware, plasticware and hardware
- Freezer
- Refrigerator
- Glasshouse on the building terrace with airflow, temperature, humidity and light controls, telephone line and power connections
- Optronics OL-752 portable spectroradiometer, double monochromator with OL 752-O-PMT head. Spectral range 200-800nm, with OL 752-150 plug-in dual calibration and gain check source module, OL 752-10E spectral irradiance [250-800nm], OL 65A programmable current source, OL 752-50 battery pack, 2-OL 752-51 spare batteries, and OL 752-70 carrying cases
- Minolta SPAD-502 chlorophyll meter with calibration disk
- Microscope workstation, with the following microscopes: 1) Zeiss Photomat light microscope with automatic photomicrography equipment, phase contrast and planapochromatic optic, fluorescence equipment. 2) Zeiss Utraphot II light microscope with zoom attachment and large format photomicrography attachment. 3) American Optics Spencer light microscope with phase contrast optic. 4) Wild Stereo microscope with epi and transillumination and zoom optic. 5) High Resolution Imaging Microscope (HIRIM) with computer for cell-level spectrometry.
- 1-Zeiss adapter 0.5 x for c-mount camera, 1-CCD Pulnix high resolution color video camera with light and speed regulation. Video signal can be directed to the monitor, the video printer, a video recorder or to the computer
- 1-C-mount lens for macro video
- 1-Sony high resolution color monitor
- 1-Sony UP-2200 Color Video Printer
- Statistical and plotting software, word processing software, photo-manipulation software
- Desktop and lap-top computers (Apple and PC), printers, scanners, and access to large format (91 cm) printers
- ADC LDC4 gas exchange (photosynthesis) measurement system

Office: Complete office facilities on Ames' premises.

6.7 Investigation 7 - Interplanetary Pioneers

Rothschild and Mancinelli each have two labs at NASA Ames Research Center. Jointly the labs have all of the basic equipment for studies of microbial physiology and ecology including a Zeiss Axioscope microscope with phase optics, fluorescence, DIC optics and both film and digital cameras for microscopical analysis. Additionally, they have a model Z1 Coulter Counter, a UV/Visible plate reading spectrophotometer and fluorometer (Molecular Devices), glassware, balance, pH meter, microprobes for pH, O_2 and CO_2 measurement, a Li-Cor model LI-185B quantum/ radiometer/ photometer with an spherical sensor, a broad band UVA/UVB meter (Solar Light Company), an underwater temperature sensor, several microfuge centrifuges, a radioisotope hood, anaerobic hood, gas manifolds, incubators, freezers, chromatography refrigerators, and assorted supplies. In addition, their labs have assorted equipment for biochemical and molecular analyses (e.g., speed vac centrifuge, thermal cycler, horizontal and vertical gel electrophoresis equipment, UV and white light boxes and cameras). Centrifuges, autoclaves, combustion oven and drying ovens are all available on the same floor. Rothschild has a EGC environmental chamber to simulate diurnal changes in light and temperature and maintain stock cultures, and Mancinelli has four New Brunswick incubator/shakers set at different temperature and light levels. For computer facilities, Rothschild and Mancinelli have six powerMacs including G4s, printers and scanners, and one PC. Facilities for experiments performed by the collaborators such as isolation of desert varnish and antioxidant analyses are available already in the relevant laboratories.

Specialized equipment includes three unique facilities. A solar simulator is located in Mancinelli's lab at NASA Ames. A space simulation facility at The Institute of Aerospace Medicine at the DLR has nine test beds for extreme environment simulation which can be modularly equipped with ionizing and UV radiation sources, atmosphere control, pumping systems, and temperature control. Depending on the space parameters to be simulated and the duration of the experiment, the most suitable facility will be selected and refurbished. Through a grant to the DLR from ESA the facilities are being

refurbished specifically for this purpose. For example, space simulation facility #2 served as ground control facility for the 9 month long EURECA mission (Horneck et al., 1995). It provides a vacuum up to 10^{-4} Pa, 8 deuterium lamps inserted in the lid to simulate solar UV radiation including the vacuum UV range (>110 nm), a cold plate to duplicate the temperature profile in space, and a sample tray with an optical filtering system to accommodate different biological test systems. Finally, organisms that are selected for flight will be flown in the EXPOSE facility on the outside of the International Space Station, an ESA-built facility designed to test the ability of organisms to survive the space environment. The flight experiments will be flown as part of the already scheduled experiments by two team members: SPORES (PI: G. Horneck) and OSMO (P.I. R.L. Mancinelli), if appropriate. EXPOSE is one of the ESA-built facilities designed to test the ability of organisms to survive the space environment, scheduled for 2004. The laboratory studies will also give rise to new experiment proposals for EXPOSE-follow-on missions.

6.8 Ames Computational Facilities at Ames Research Center continue to enjoy a world-class status. These facilities have long enhanced theoretical research in planetary science and astronomy at Ames (e.g., in support of *Investigations 1 and 2* in this proposal). More recently, they have enhanced research efforts in "Bioinformatics" (related to *Investigation 3*). These capabilities will thus enhance the overall research effort, as indicated in Section 3 of this proposal, with benefits to NAI members of other institutional teams, as the network of collaborations develops.

APPENDIX A - ACRONYMS

16S-rDNA	Small Sub-Unit Ribosomal DNA
16S-rRNA	Small sub-unit Ribosomal Nucleic Acid
ABE	Astrobiology Explorer
ABP	ATP Binding Protein
AFM	Atomic Force Microscopy
AGNs	Active Galactic Nuclei
AMP	Adenosine Monophosphate
AMR	Adaptive Mesh Refinement
ARC	Ames Research Center
ATP	Adenosine Triphosphate
AVHRR	Advanced Very High Resolution Radiometer
Bchla	Bacteriochlorophyll-a
BEQ	Biological Energy Quantum
BioPan	The Biological Spaceflight facility used by ESA for free flyers
C - Terminal	Carbonyl end of a protein or peptide
CAN	Cooperative Agreement Notice
CAS	California Academy of Sciences
CASA	Carnegie Ames Stanford Approach - Biosphere Model
CBMC	Center for Biomedical Computation (Stanford)
CCSM	Community Climate System Model
cDNA	Complementary or Cloned DNA
CREST	Computational Center for Molecular Studies at Jackson State University
CRICyT	Centro Regional de Investigaciones Cientificas y Tecnologias, Argentina
CSB	Colorless Sulfur Bacteria
CSF	Center for Star Formation
CSUMB	California State University at Monterey Bay
CYN	Cyanobacteria
D/H Ratio	Deuterium/Hydrogen Ratio
DBL	Diffusive Boundary Layer
DLR	Deutsche Zentrum für Luft-und Raumfahrt (German Aerospace Center)
DNA	Dioxy Nucleic Acid
DOI	Division of Interpretation
DSC	Differential Scanning Calorimeter
ECBILT	Royal Netherlands Meteorological Institute, Center for Climate Research General Circulation Model
ECF	Ecosystem Computational Facility
EDS	Energy Dispersive X-Ray Spectroscopy
EMPA	Electron Microprobe Analyser
EOS	Earth Observing System
ESA	European Space Agency
ESE	NASA's Earth Science Enterprise
ETM+	Enhanced Thematic Mapper Plus
EURECA	Facility used to house microbe experiments flown on NASA's Long Duration Space Flight Facility.
EXPOSE	Facility used by ESA to expose samples to the space environment on an outside platform of the ISS

FCSUMB	Foundation of California State University at Monterey Bay
FESEM	Field Emission Scanning Electron Microscopy
FID	Flame Ionization Detection
FISH	Fluorescent in situ hybridization
FPD	Flame Photometric Detector
GCM	Gas-Chromatography-Mass Spectrometery (an analytical technique)
GCM	General Circulation Model (Mars)
GIS	Geographic Information System
GNS	Green Non-Sulfur Bacteria
GPS	Global Positioning System
GSH	Glutamyl-Cysteinyl-Glycine
GSSG	Glutathione Disulfide
GTP	Guanosine Triphosphate
HBCU	Historically Black Colleges and Universities
HPLC	High Performance Liquid Chromatography
IDP's	Interplanetary Dust Particles (asteroidal and cometary)
IMF	Initial Stellar Mass Function
IR	Infrared
IRTF	Infrared Telescope Facility (a ground based telescope)
ISM	Interstellar Medium
ISO	European Infrared Space Observatory
ISS	International Space Station
JSC	Johnson Space Center
JWST	Jack Webb Space Telescope
k's	Refractive Indices
LBA	Large Scale Biosphere - Atmosphere Experiment
LPI	Lunar Planetary Institute
MBGC	Microbial Biogeochemistry
MBL	Marine Biological Laboratory
MBP	Maltose Binding Protein
MC	Monte Carlo Method
MER	Mars Exploration Rover
MER-SOWG	Mars Exploration Rover-Science Operation Working Group
MET	Methanogenic Bacteria
MeV	Millions of Electron Volts
MGCM	Mars General Circulation Model
MIDEX	Mid-sized Explorer Mission
MODIS	Moderate Resolution Imaging Spectroradiometer
MOU	Memorandum of Understanding
MS	Mass Spectrometry
MSD	Mass Selective Detectors
M-type	One category of stars, classified according to its range of masses. M-type stars are smaller than our own Sun, a G-star
NAD^+	Nicotine Amide Phosphate
NADPH	Hydrogen Nicotine Amide Phosphate
NAI	NASA Astrobiology Institute
Nakhlites	Class of Meteorite probably of Martian origin
NASA R&A	NASA Research & Analysis Programs
N-body	Three or more gravitational interacting bodies

NCAR	National Center for Atmospheric Research
NCCAFB	NASA Center for Computational and Fundamental Biology
NDVI	Normalized Difference Vegetation Index
NFP	New Frontiers Program
NIMS	Near-Infrared Mapping Spectrometer on the Galileo Spacecraft.
NIR	Near Infra-red radiation
NIRSPEC	Near-Infrared Echelle Spectrograph at Keck
NMR	Nuclear Magnetic Resonance
NOAA	National Oceanic and Atmospheric Administration
NPS	National Park Service
NRC	National Research Council
n's	Refractive indices
NSF	National Science Foundation
N-Terminal	Amino Group end of a peptide chain
OFVEC	Old Faithful Visitor Education Center
PAHs	Polycyclic Aromatic Hydrocarbons
PAR	Photosynthetically Active Radiation
PDR	Photodissociation Region
Promega	Genomic DNA Purification Kit
PSB	Purple Sulfur Bacteria
PSIG	Project Science Integration Group
QSAR	Qualitative Structure - Activity Relationship
RNA	Ribonucleic Acid
ROSE	Response of Organisms to the Space Environment
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscope
SIMS	Secondary Ion Mass Spectrometry
SIRTF	Space Infrared Telescope Facility
SOD	Superoxide Dismutase
SOFIA	Stratospheric Observatory for Infrared Astronomy
SpeX	Medium-Resolution Spectrograph on the IRTF.
SPORES	Experiment using Bacillus Subtilis spores to be flown on the expose facility aboard the Space Station
SRB	Sulfate Reducing Bacteria
SSAC	Space Science Advisory Council
SSIOUX	Space Simulation for Investigating Organics, Evol ution and E xobiology. A series of experiments using the space simulation facilities at the German Aerospace Facility in Cologne, Germany, sponsored by the ESA
SST	Sea Surface Temperature
STELLA	Computer Program used for Modeling
STM	Scanning Tunneling Microscopy
SwRI	South West Research Institute
TCD	Thermal Conductivity detector
TEM	Transmission Electronic Microscopy
TERC	Technical Education Research Center
TPF	Terrestrial Planet Finder
ULIRGS	Ultra Luminour Infrared Galaxies
UMa System	Ursae Majoris Extrasolar System

UMIST	University of Manchester Institute of Science and Technology
UV	Ultra Violet
UV-Vis	Ultra Violet Visible Wavelength Range
VIMS	Visible and Infrared Mapping Spectrometer
μXRD/XRF	Micro X-Ray Deffraction/X-Ray Fluorescence