Below are the abstracts of proposals selected for funding for the Astrobiology: Exobiology and Evolutionary Biology program. Principal Investigator (PI) name, institution, and proposal title are also included. 103 proposals were received in response to this opportunity, and 23 were selected for funding.

Ariel D. Anbar/Arizona State University
In search of early oxygen: Investigation of redox sensitive metal abundances and isotopes in Neoarchean drill cores

We propose to characterize element abundances and isotopes in two Neoarchean drill cores to investigate the oxidation state of the environment ~ 2.5 billion years ago. Our goal is to determine if transient or low-level quantities of O2 were present in the environment before the Great Oxidation Event (G.O.E.). The presence or absence of O2 at this time has bearing on the relationship between the timing of the evolution of oxygenic photosynthesis and the rise of atmospheric O2 - a relationship that will inform the eventual interpretation of spectra from extrasolar Earth-like planets.

The proposed work builds directly on prior research on one of the two cores conducted by the PI, Co-I and others with support from the NASA Astrobiology program. In that study, summarized here, high-resolution chemostratigraphy revealed an episode of enrichment of the redox-sensitive transition metals Mo and Re in pyritic shales from the late Archean Mt. McRae Shale, Western Australia. Correlations with organic carbon and Re-Os data indicated these metals were derived from contemporaneous seawater. We proposed that Mo and Re were supplied to Archean oceans by oxidative weathering of crustal sulfide minerals and, hence, that small but significant amounts of O2 existed in the environment > 50 Ma before the start of the G.O.E.

We now propose to conduct similar analyses on a drill core that samples pyritic shales from the late Archean Klein Naute Formation, S. Africa, believed to be a time-correlative deposit with the Mt. McRae Shale. We also propose to conduct Fe, Mo, U and S isotope analyses and to characterize sedimentary Fe speciation in both cores. The proposed research will test key aspects of the O2 interpretation as well as alternative hypotheses, and will help determine if the event recorded in the Mt. McRae Shale was of local or global extent.
The recent reports of hypersaline paleo-environments on Mars, as well as measurements of methane in that planet's atmosphere, have underscored the need to evaluate the importance of biological (as opposed to geological) trace gas production and consumption. Modern hypersaline microbial mat communities, (thought to be analogous to those present on the early Earth at a period of time when Mars was experiencing very similar environmental conditions), have been shown to produce methane. However, very little is known about the physical and/or biological controls imposed upon the rates at which methane, and other important trace gases, are produced and consumed in these environments. In studying methane production in the Guerrero Negro hypersaline ecosystem, Baja California Mexico, we have measured high concentrations of methane in bubbles of gas produced both in the sediments underlying microbial mats (including one site where methane constitutes over 20% by volume of the bubbles), as well as in areas not colonized by microbial mats. The carbon isotopic signature (delta13C ratio) of the methane in the bubbles exhibited an extremely wide range of values, from ca. -75 per mil to ca. -25 per mil, potentially complicating interpretations of biogenicity. Further work is needed to understand the isotopic signature of released methane and the possible biological and chemical oxidation in these environments. The isotopic ratio of hydrogen in methane (the D/H ratio) in combination with the carbon isotopic signature, and concentrations of C1 to C3 gases generally produced by thermogenic methane formation are all necessary for an unequivocal determination of biogenicity of methane, including Martian methane, as was recently reviewed by Allen et al. (2006a, 2006b). The capability for delta13C and D/H measurements will be present on Mars in the Mars Science Laboratory (MSL), and will be used to evaluate biogenicity. However, few measurements of this type have been made, even on Earth, and we are aware of no data from hypersaline environments.

We propose an intensive investigation of methanogenesis in hypersaline environments, including environments containing sulfate minerals similar to those reported on Mars. We will combine isotopic measurements of methane with measurements of its concentration and rates of production. We will identify methanogenic microorganisms, and pathways used to by them to produce and/or oxidize methane because these biogeochemical processes produce diagnostic isotopic signatures in methane that is released from these environments. This work will lead to a better understanding of the processes producing and consuming methane in Mars analogue environments and provide a framework within which MSL results, as well as results from other missions, such as Terrestrial Planet Finder, may be interpreted.
Bill Casey/University of California
What are the Mg Isotopic-Signals for Photosynthesis?

We propose research to assess whether Mg-isotope geochemistry can be used to detect the signal of photosynthesis, which addresses goals in the Astrobiology program to identify the signals for early life. We recently discovered that biosynthesis of chlorophyll-a in Synechococcus elongatus, a unicellular cyanobacterium, induces a distinct Mg-isotope fractionation. Magnesium extracted from chlorophyll-a is depleted in the heavier isotopes, relative to magnesium in the culture media. This fractionation may be common to all photosynthetic organisms, and, thus, global in extent. We propose a series of tasks to establish this fact.

The research is guided by two hypotheses. First, is the Mg-isotope fractionation occurring during the insertion of Mg(II) into the Protoporphyrin IX molecule, which is a tetrapyrrole that is common to the synthetic pathways for both chlorophyll and heme? To test this hypothesis, we propose to show that Mg-isotopic fractionation is insensitive to the taxonomy of the organism in a series of lab and field measurements where the pigments are separated and the Mg-isotopic compositions compared. We are also examining whether the recycling of Mg in photo-damaged chlorophyll allows for isotopic equilibrium to be established.

Our second hypothesis is that the pool of metal for pigment synthesis is recycled in magnesium-poor environments. The record of Mg-isotopes in chlorophyll in such an environment would then reflect Rayleigh distillation of the bioavailable magnesium isotopes, since chlorophyll biosynthesis favors lighter isotopes over heavy isotopes of magnesium. If so, then we expect to see different Mg-isotope values, and ranges of values, in environments that differ only in magnesium bioavailability.

Photosynthesis accounts for ~100 gigatons of carbon per year on Earth, which is fundamental to all geochemical cycles. Magnesium is the metal center in the chlorophyll molecule and thus is central to the history and geochemistry of life on the planet, and perhaps others.

Steven Charnley/S E T I Institute
Interstellar Precursors of Meteoritic Organics -
A Theoretical Study

It is proposed to continue theoretical studies of organic astrochemistry. The principal goals of this project are to identify interstellar molecules in the soluble organic extracts of carbonaceous meteorites, and to quantify the viability of the many interstellar organics which have been proposed as precursors of more complex meteoritic molecules formed by aqueous alteration chemistry. This will be done by chemical kinetic modeling of gas and solid phase processes, as constrained by comparison with the complex molecular inventory of meteorites and molecular clouds.

Stochastic simulations will be performed to investigate pathways to molecular complexity through cold atom addition reactions on the surfaces of dust grains. The expected isotopic fractionation patterns (e.g. in D and $^\{13\}CS$) for the putative
interstellar precursor molecules required by processes such as the Strecker synthesis will be computed and compared to the meteoritic data. The interstellar production of amino acids, in gas phase syntheses driven by ice evaporation in warm protostellar environments, will be evaluated. The isotopic fractionation patterns in large interstellar organic compounds as a function of synthesis route will also be calculated and compared to recent meteoritic analyses. The modeling effort will employ experimental data on surface processes and ion-molecule reactions provided by our collaborators. It is also proposed to undertake a modest observing program to search for new organics predicted by our theoretical work.

The proposed research meshes with NASA's strategic goals, and those of the Exobiology and Evolutionary Biology Program. The results of this study will allow us to quantify the relative contribution, direct or indirect, of various interstellar chemical processes to specific classes of meteoritic organics. This will enable us to assess their general relevance for providing materials capable of initiating prebiotic chemistry in planetary systems.

Samy El-Shall/Virginia Commonwealth University

New Pathways for the Formation of Complex Organics and Prebiotic Synthesis in the Gas Phase, in Nano Clusters, and on Dust Grain Surfaces

The proposed research is focused on the physical and chemical processes that can lead to the formation of bio-forming polyatomic molecules through gas phase ion-molecule reactions, intracluster reactions, and surface reactions catalyzed by dust grains. The overall objective is to understand the general physical and chemical principles underlying the origin and early evolution of life. To understand how life can begin on a habitable planet such as the Earth, it is essential to know what organic compounds were likely to have been available. Among the key questions we plan to address in this proposal are: (1) how are simple organic compounds assembled into more complex molecular systems? and (2) what are the essential processes and pathways by which complex systems can develop those basic properties that are critical to life’s origins?

The proposed project involves the study of the polycyclic aromatic hydrocarbons (PAHs) that are present in interstellar clouds as evidenced by spectroscopy and meteorites. The PAH molecules are readily ionized and their large surfaces provide strong bonding for interstellar species such alkynes (acetylene and polyacetylenes) and polar molecules (formaldehyde, formic acid, HCN, cyanoacetylenes). The PAH surfaces can serve as catalysts for the formation of complex prebiotic organics. The thermochemistry, kinetics, and temperature effects of the gas phase and cluster reactions will be investigated using the mass-selected ion mobility technique. In the nano clusters’ regime, we will study the photochemical reactions of the PAHs within clusters of interstellar molecules including water clusters.

As to reactions on grain surfaces, we will use the LVCC method (Laser Vaporization/Controlled Condensation) to generate highly porous dust-like nanoparticles that model cosmic dust, and study their catalytic and photocatalytic reactions. Building blocks of amino acids such as HCN, ammonia, ketones and aldehydes will be adsorbed on the nanoparticle grains covered with water ice. The porosity and large surface areas of
the grains create little nanovessels in which the organic molecules can pool. In the presence of ultraviolet photons, the organics can be polymerized to form more complex molecules. We will study the effects of UV radiation on the catalytic properties of the grains, and the resultant product distribution. Reaction products will be detected using temperature-programmed desorption and mass spectrometry (TPD/MS) from surface-adsorbed gas targets that have been exposed to the UV radiation. The results of proposed work will allow one to assess the relative importance of gas phase, clusters, and grain catalyzed reactions in the formation of complex organics and in prebiotic synthesis under interstellar conditions. This will contribute to the development of new models that provide more accurate predictions of interstellar molecular abundances. This proposal is directly related to the goals of the NASA Astrobiology Program concerning the formation of complex organic molecules in space and their delivery to planetary surfaces, and investigating models of early environments in which organic chemical synthesis could occur.

Friedemann Freund/SETI Institute
Hydrogen in the Rock Column

This proposal addresses the presence of hydrogen in the rock column. Hydrogen is an energy source for micro-organisms, especially for methanogens. The deep environment may have served as refuge for early Life in the aftermath of large impacts or during global glaciation episodes. Microbial communities in deep-seated rocks need an energy source and food. While it is well-known that hydrogen can be generated by the reduction of water through oxidation of transition metal cations, for instance Fe$^{2+}$ in mafic and ultramafic rocks, a much more broadly distributed hydrogen source seems to exist in the top 20-30 km of the rock column.

This form of hydrogen is introduced through a solid state redox reaction taking place in the matrix of nominally anhydrous minerals that crystallized or recrystallized in water-laden magmatic or high-grade metamorphic environments. Those minerals invariably incorporate small amounts of water in form of hydroxyl, O$_3$Si-OH. During cooling hydroxyl pairs undergo a redox reaction, in the course of which the protons “rip off” an electron from their oxygens. Two protons turn into hydrogen, while two oxygens change from valence 2- to 1- forming a peroxy link, O$_3$Si-OO-SiO$_3$. Molecular hydrogen is diffusively mobile and can enter the space between grains and dissolve in intergranular water films. It thus becomes accessible to micro-organisms.

We propose a study of the presence of hydrogen in rocks. We shall measure: (i) the slow release of hydrogen after crushing rocks in inert gas using a MIS-FET detector, sensitive over the 0.5-2000 vppm range with 5-10 sec resolution. We shall analyze rocks from mid- to lower-crustal and upper mantle environments; (ii) the near-instant release of hydrogen during fracture of single crystals using a fast mass spectrometer with micro-second time resolution. Combining the two types of measurements (i) and (ii) we expect to obtain information about the amount of hydrogen contained in the rock column and, hence, its availability to deep microbial communities.
This work is central to the Astrobiology Roadmap Goal 4, Objective 4.1 “Earth’s early biosphere”, to Goal 3, Objective 3.1 “Sources of prebiotic materials and catalysts”, and to Goal 2, Objective 2.1 “Mars exploration”. It will provide information about a source of molecular hydrogen that has never before been systematically investigated.

Judith Herzfeld/Brandeis University
Solid State NMR Studies of Prebiotic Scenarios

A variety of intriguing prebiotic scenarios involve systems that are difficult to study by conventional methods. Here we identify several well known systems for which NMR, particularly solid state NMR, can provide unique probes capable of answering unresolved questions.

We propose carrying out definitive studies of two systems for which we have interesting preliminary results. Both involve the spontaneous polymerization of small molecules that would have been ubiquitous in prebiotic environments. In the first case, the question is whether a proposed direct pathway to polypeptides exists. In the second case, we seek to determine what kinds of chemical activity could occur inside the microspherules that are formed. Both of these investigations require probes of amorphous polymers.

We also propose undertaking feasibility and demonstration studies for systems in which minerals have been shown to perform enzyme-like functions. Here we seek to understand how catalytic mineral surfaces interact with and modify bound reactants and products. These studies require probes of immobilized and exchanging substrates.

The insights obtained will contribute directly to the goal of the NASA Astrobiology Program "to understand the pathways and processes leading from the origin of planetary bodies to the origin of life", focusing particularly on "determining what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere". This information is important to inform the pursuit of the NASA objectives to "advance scientific knowledge of the origin and history of the solar system [and] the potential for life elsewhere" and "to discover the origin, structure, evolution and destiny of the universe". In particular, our studies should allow the prebiotic significance of specific molecular processes to be better assessed and could thereby influence what molecules we look for as signs of the habitability of other parts of the universe.

Andrew Knoll/Harvard University
Neoproterozoic evolution and environmental change: Integrated experimental and geological approaches

Sedimentary rocks deposited during the later Neoproterozoic Era (ca. 800-542 million years ago) record the first unambiguous signs of animal life, multiple glaciations of global dimensions, and the oxidation of the deep ocean. Despite recognition that Earth and life changed fundamentally during this interval, much remains to be understood about the interrelationships of and feedbacks between Neoproterozoic biological and
Here we propose to couple detailed chemostratigraphic analyses of paleobiologically informative Neoproterozoic sedimentary successions with geobiological experiments designed to provide empirical calibration of temporal changes in seawater sulfate. The microbiological experiments will employ a novel, continuous flow bioreactor system that will provide quantitative constraints on the relationships among seawater sulfate concentration, microbial activity (and diversity), and sulfur isotope fractionation. Many biochemical reactions discriminate against minor isotopes, resulting in end products that are isotopically distinct relative to the substrate pool. Therefore, all isotopes of sulfur [32S, 33S, 34S, 36S] will be measured in substrates and products. Complementary geochemical analyses will focus on fossiliferous Neoproterozoic successions that record physiologically distinct events in early animal (and algal) evolution; detailed profiles of carbon isotopes (to facilitate correlation with other localities) and iron-speciation chemistry (which provides a proxy for redox conditions in waters overlying the seafloor) will be joined with measurements of the total isotopic composition of sulfur. The proposed research will, thus, marry high resolution chemostratigraphic data and microbial experiments to test hypotheses about the relationship between evolution and environmental history on the one planet where life is known to exist. The sulfur isotopic data will also provide a baseline for interpreting total sulfur isotopic abundances in sedimentary surface minerals collected during sample return missions to Mars.

Niles Lehman/Portland State University

A Self-assembling Collectively Autocatalytic Set of RNA Oligomers

The origins of life required the advent of biological information. Such information could exist in a pool of short linear polymers, such as RNA oligomers synthesized abiotically. We propose to demonstrate that self-replicating RNA molecules can emerge spontaneously via the aggregation of a collectively autocatalytic set found within a pool of random RNA oligomers, each 40 nucleotides or less in length. This would be an empirical demonstration of the ideas proposed by Stuart Kauffman regarding emergence of order from chaos. Our approach is to exploit the intrinsic ability of some RNAs to recombine other RNAs to produce new combinations of sequences.

In publications and preliminary data we have demonstrated that the Azoarcus ribozyme, 198 nucleotides in length, can be fragmented into four pieces that can spontaneously self-assemble into a covalently-contiguous molecule through recombination reactions. These assemblages can self-replicate because they can autocatalytically catalyze further self-assembly reactions. In the current proposal we will extend this methodology by exploring whether smaller and more random fragments in a pool of oligomers can accomplish the same task.

We propose three sets experiments to achieve this goal. The first is to break the current Azoarcus RNA system into five or more fragments, such that the average fragment length drops below 40 nucleotides. The second is to select for a shorter version of the Azoarcus ribozyme by deleting large sections of the molecule, targeting a recombinase that is less than 140 nucleotides. The third is to use a novel type of in vitro selection, termed autocatalytic enrichment selection, to bring a pool of RNA oligomers which does not demonstrate detectable self-assembly back across a complexity threshold
to the point where it does self-assemble and self-replicate. We can accomplish these experiments inexpensively with graduate and undergraduate students at Portland State University in three years’ time.

Scot Martin/Harvard University
Prebiotic Metabolic Systems Driven by Colloidal Semiconductor Photocatalysis

The proposed work will test the hypothesis that important reactions of prebiotic metabolic systems can be driven by photocatalysis on colloidal semiconductor particles. How prebiotic metabolic cycles could have developed and been driven in the absence of catalytic networks is an important unanswered question about the origins of life because such prebiotic cycles are considered as necessary platforms in the development of more advanced self-replicating biotic systems. The prototypical model system we propose to employ to evaluate these possibilities is the reductive (reverse) citric acid cycle. This cycle has received much attention in the literature of prebiotic evolution because repeated cycling provides a core mechanism for the synthesis of useful biomolecules from CO2. Colloidal photochemistry, which has received limited attention in the Exobiology community, opens new reaction pathways because of the interactions of excited-state species, and these reaction rates can be very rapid with high yields.

The key questions to be addressed in the proposed work are: (1) What are the rates and the yields of the photoreduction reactions? (2) What is the effect of reaction conditions, including the wavelength of irradiation, pH, temperature, and chemical competitors, on enhancing or constraining the reaction rates and yields? (3) How and why do interactions of aqueous reactants & products with the surface of the solid semiconductor photocatalyst affect the rates and the yields of the reactions? Specifically, we propose to determine the capabilities and limitations of colloidal semiconductor particles to photocatalyze at solar wavelengths the reduction reactions of oxaloacetate to malate, fumarate to succinate, succinate/CO2 to oxoglutarate, oxoglutarate/CO2 to oxalosuccinate, and oxalosuccinate to isocitrate. Photocatalysis provides the free energy for these reactions and, in addition, could promote rapid reactions rates because of high overpotentials. We will initially focus on Fe-doped ZnS colloidal particles, prepared by hydrothermal methods, because of their probable high occurrence in marine environments of the prebiotic Earth and their proven high performance towards CO2 photoreduction.

The impacts of the proposed study, defining the role of colloidal photochemistry in driving prebiotic metabolic cycles, will be forward steps in achieving (1) NASA's Exobiology Program goal of understanding what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere (Astrobiology Roadmap goal 3, objectives 3.1 and 3.2) and (2) NASA's Strategic Objectives of providing a deeper understanding of how early life could have originated.
Thomas McCollom/University of Colorado  
**Experimental Investigation of Prebiotic Organic Geochemistry in Hydrothermal Environments**

Hydrothermal environments have been proposed as locations of abiotic organic synthesis in the early solar system and as a possible site for the origin of life. Interpreting the role of hydrothermal systems in prebiotic chemistry leading to the emergence of life requires comprehensive knowledge of the thermal stability of biomolecules and reaction pathways for organic synthesis under hydrothermal conditions. The proposed research will investigate two aspects of the organic chemistry of hydrothermal environments through laboratory experiments. One set of experiments will evaluate the role of minerals on the reactivity of amino acids in geologic systems. Although there have been numerous previous studies of the decomposition of amino acids in hydrothermal solutions, none of these studies have included mineral reactants and the impact of minerals on reaction rates and pathways has never been evaluated. The proposed research will complete a study of the hydrothermal reactivity of the amino acid norvaline in the presence of minerals begun under a prior Exobiology grant, and then extend the research to investigate the behavior of aspartic acid and alanine. A second set of experiments will investigate the possible role of alkylthiols in abiotic carbon fixation and organic synthesis in sulfur-rich hydrothermal environments. The results of the experiments are expected to improve understanding of the chemical environment from which life emerged on Earth, and that might have supported origins of life elsewhere. In addition, the results will aid in interpreting the source of organic matter in meteorites and other rock samples.

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Stephen Mojzsis/University of Colorado  
**EXPLORING THE LATE HEAVY BOMBARDMENT**  
**EXPLORING THE HADEAN EARTH**

We propose a 3-year project to investigate a record of thermal effects, which include impacts, to the earliest terrestrial crust as recorded by pre-4 Gyr old minerals. We will use these “ground truth” data - verifiable with known examples of shock-metamorphosed samples from terrestrial impacts - as crucial input parameters to existing solar system dynamical models. With this knowledge, we will investigate impact-induced modifications to planetary surfaces (including radiometric age-resetting) with detailed thermal models of rocky planetary crusts. We will use the unique resource of the Hadean zircons to probe the geochemical and dynamical environment of the Hadean Earth’s surface zone, and use this information to place quantitative constraints on habitability.

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Shuhei Ono/Massachusetts Institute of Technology  
**A Multi-Proxy Search for Atmospheric Oxygen in the 2.9 Ga Pongola Supergroup, Southern Africa**

The evolution of oxygenic photosynthesis impacted the early Earth's atmosphere and climate, and left us geochemical signatures in the rock records. We will test our
hypothesis that biological oxygenic photosynthesis emerged as early as ~2.9 Ga and is responsible for triggering the 2.9 Ga glacial by destabilizing a methane-rich greenhouse atmosphere. We will focus on the rocks of the ~2.9 Ga Pongola Supergroup in Southern Africa because they are minimally altered and contain the oldest known record of glacial features, stromatolites with diverse morphologies, and a series of banded iron formations and carbonaceous shales. Previous research by us and others reported relatively small signatures of sulfur-mass-independent fractionation (S-MIF) and distinct negative cerium anomalies from the rocks between 2.76 and 2.92 Ga, pointing to an early oxygenation at this age.

Our principle tool is high precision analysis of all four sulfur isotopes (32S, 33S, 34S and 36S) of sulfide minerals. The S-MIF in Archean rocks is a direct proxy for atmospheric chemistry that is particularly sensitive to the atmospheric oxygen as low as a few ppm levels. We will compare not only the magnitude of S-MIF signatures (D33S) but also the relationship among d34S, D33S, and d36S values in the stratigraphic interval containing glacial records to test if S-MIF records a change in atmospheric chemistry before and after the glacial events. Cerium anomaly in carbonates and banded iron formations, if present, will provide a supporting evidence for water column oxygen. Small-scale (mm-to cm) analysis of multiple-sulfur isotope system, in combination with carbon isotope ratios of organic carbon, will provide new insights about the evolution of early microbial sulfur metabolisms.

The fund requested will support one graduate student at EAPS-MIT. The proposed research is designed to contribute to the research emphasis of the Exobiology Program: early evolution of life and the biosphere.

**Ronald Oremland/US Geological Survey**

**Acetylene as a Substrate for the Development of Anaerobic Microbial Ecosystems on Primordial Earth: Implications for Microbial Life on Planets and Satellites with Jovian/Titan-like Atmospheres.**

Acetylene is present in substantial quantities in the hydrogen-rich, reducing atmospheres of Jovian planets, as well as that of Saturn's moon Titan. In these cases, acetylene arises as a reactive intermediate initiated by the photolysis of the abundant methane also present therein. In contrast, acetylene is only an anthropogenic trace component of the Earth's current atmosphere. Nonetheless, a hydrogen- and methane-rich primordial atmosphere has been hypothesized for that of the early Earth, in part as a means of retaining heat (greenhouse warming) to counter the effects of the sun's lower luminosity ~ 4 Ga years ago. If this was the case, the Earth's early anoxic atmosphere would also have correspondingly rich in acetylene. This poses a biological paradox because acetylene is a potent inhibitor of a number of anaerobic microbial processes thought to have been critical in the development of the primordial microbial ecosystems and the biogeochemical cycles of the early Earth. These include (but are not limited to) methanogenesis, anaerobic methane oxidation, nitrogen fixation, and hydrogen oxidation. Curiously, anaerobic fermentation of acetylene was discovered 25 years ago as a serendipitous consequence of the use of this gas to block N2O reductase activity in
sediment denitrification assays. One microorganism, Pelobacter acetylenicus, has been isolated and shown capable of fermentative growth on acetylene. This it achieves by virtue of its unique low-potential tungsten-containing enzyme, acetylene hydratase, which results in the exothermic formation acetaldehyde. Acetaldehyde subsequently dismutates to ethanol and acetate (plus some hydrogen), substrates that can readily couple with either sulfate reduction or methanogenesis. However, acetylene hydratase is specific for acetylene and will not react with any other compounds, including a number of triply bonded analogs. Thus an additional biological paradox is posed, namely why does acetylene hydratase exist when this gas has no current biological sources and is not present in any reasonable abundance in the Earth's present troposphere? We hypothesize that microbes which possessed acetylene hydratase played a key role in the evolution of the Earth's early biosphere by exploiting a readily fermentable source of carbon ("acetylenic-manna") from the atmosphere (as well as from geothermal and other sources), and in doing so formed protective niches that allowed for other anaerobic processes to flourish within. We hypothesize that the ability to ferment acetylene has been retained by the microbial gene-pool as an evolutionary advantage to exploit the acetylenic-manna during episodes of global anoxia in the Earth's past. In this proposal we will survey a number of diverse anaerobic sediments and soils for their ability to consume acetylene, and will determine the lower substrate affinities of these materials for the gas. We will also employ both classic culturing and modern culture-independent techniques to determine the microbial diversity of acetylene fermenting prokaryotes in nature. The work proposed here will contribute to our understanding of the potential nature of Archaean-era microbial metabolisms and the role of acetylene in the evolution of the biosphere and Earth's early atmosphere. Furthermore, the presence of acetylene in the atmosphere of a planet(oids) could be construed, under certain circumstances, as evidence for an extraterrestrial anaerobic microbial ecosystem. Thus, it is relevant to several of NASA's dominant themes, including the search for signs of extant (or extinct) life outside of the Earth's biological envelope.

Matthew Pasek/University of Arizona

Reduced Phosphorus Assemblages as Sources of Reactive P the Origin and Evolution of Early Life

The proposed research will investigate the origins of phosphorylated biomolecules and the effect meteoritic phosphorus (P) had on early Earth P geochemistry. Phosphorylated biomolecules are ubiquitous in life and are the basis for cellular replication and information storage (RNA and DNA), metabolism (adenosine triphosphate, or ATP), and structure (phospholipids). A rigorous pathway leading to the formation of phosphorylated biomolecules is unknown, yet there is growing evidence that the interaction of meteoritic material with water on the early Earth may have led to their formation (Pasek and Lauretta 2005, Gorrell et al. 2006, Bryant and Kee 2006). Furthermore, recent research has shown that (Fe,Ni)3P corrosion in aqueous solution with organics produces organic P compounds including organophosphates (Pasek et al. 2007). Additionally, recent investigations of bacterial metabolic pathways (White and Metcalf 2004) and geochemistry (Pasek 2006) suggest that the inventory of P on the early Earth may have included a substantial fraction of reduced aqueous P compounds.
However, the stability of these reduced P compounds is unclear and merits further investigation.

We plan a two-stage experimental study of early prebiotic phosphorus geochemistry. Our goals are: 1) To understand the corrosion of schreibersite and their role in pre-biotic chemistry and the origin of life, and 2) To understand the pathways by which reduced phosphorus species degrade through a series of controlled experiments.

Experiments will be prepared and analyzed at the University of Arizona. All samples will be characterized by 31P nuclear magnetic resonance (NMR) spectroscopy, a highly selective, versatile technique capable of rapid and non-destructive identification of aqueous phosphorus species. NMR will provide information of species formed, yields, and rates of reactions. Several samples will be analyzed using electron paramagnetic resonance spectroscopy, which detects free radical species, several of which may be key precursors to biochemical compounds. Finally, several samples will be analyzed by mass spectrometric techniques, providing a rapid detection of trace compounds.

The results of this work will inform chemical pathways that may have lead to the origin of life here on Earth and elsewhere, it will describe the nature of early Earth phosphorus geochemistry and the effects on early microbes, and it will aid in the development of geochemical techniques for the detection of reduced P compounds and biosignatures on other planets, like Mars.

Sandra Pizzarello/Arizona State University
METEORITIC AMINO ACIDS AS ASYMMETRIC CATALYSTS IN PREBIOTIC CHEMISTRY

The non-racemic amino acids of meteorites provide the only natural sample of molecular asymmetry measured so far outside the Biosphere and it is reasonable to suggest that these compounds may have been important to the prebiotic molecular evolution of the early Earth. Much has been learned about meteoritic amino acids through extensive molecular, isotopic, and chiral analyses of the last thirty years. We know, for example, that they were formed abiotically and that their syntheses involved several solar and pre-solar environments, with some showing high deuterium enrichments that relate them to the cold regimes of the interstellar medium. To date, however, several gaps remain in our understanding of the specific syntheses that led to these compounds’ formation as well as of the significance their exogenous delivery might have had for the origin of terrestrial homochirality. Therefore, we propose to continue the studies conducted in this laboratory during previous grant years with the following analytical tasks.

- To constrain the possible origin of meteoritic amino acids by conducting computer-aided ab initio calculation of selected amino acid species.
- To probe further the possible effect of abiotic ee in molecular evolution and expand the prebiotic modeling of catalytic transfer of asymmetry from di-peptides to sugars to include the syntheses of compounds that are important to extant life such as ribose and glucose.
The proposed work has shown much promise in preliminary studies and will expand greatly our understanding of the prebiotic traits in chemical evolution that could foster the establishment of biomolecule precursors. As such, they are directly relevant to the study of the origin of life and the objectives of this NRA.

Andrew Pohorille/NASA Ames Research Center
Mechanisms and early evolution of protocellular transport

This proposal is devoted to investigations of the emergence and early evolution of nutrient transport across cell walls, which was an essential step in the origins of cellular life. We assume that the earliest mechanism of transport was permeation through membranes, and it must have sufficed to deliver the required monomers and charged species to the interior of protocells. We further argue that, once simple proteins became available, some of them were recruited into membranes and self-organized into channels. Despite their simplicity, these channels were capable of mediating transport of ions and small molecules across protocellular walls at greatly increased rates, thus facilitating faster evolution. To test key elements of our hypotheses we will examine simple, molecular models of unassisted and protein-mediated protocellular transport and generalize our results in terms of their implications for the origins of life on Earth and elsewhere in the universe. Our studies will be pursued through computer simulations and validated in laboratory measurements. By combining recent theoretical improvements with advances in massively parallel computing we expect to achieve efficiencies that have not been reached in protobiological simulations.

We propose two specific aims. One is to determine the mechanism and rates of unassisted transport of ribonucleotides, their molecular components and their di- and triphosphate derivatives across membranes formed by fatty acids or phospholipids. Our focus on ribonucleotides is motivated by their role as the building blocks of RNA, which is thought to be the first self-replicating and catalytic polymer. The results will not only help to develop more fully the RNA World hypothesis but will also guide experiments aimed at creating laboratory models of protocells.

Our second aim is to explain how transmembrane channels formed through self-association of small, helical proteins can efficiently mediate transport across protocellular walls, and how the channel size and amino acid sequence influences efficiency and selectivity of transport. As a model, we will use a simple channel formed by small, trichotoxin proteins. We expect that the results of this study combined with our previous work on related systems will allow us to establish the minimal requirements for proteins to mediate selective ion transport, and to develop a comprehensive theory of the earliest evolution of ion channels.

The proposed studies support Goal 3, Subgoal 3C of the NASA Strategic Plan and directly addresses Goal 3 of the Astrobiology Roadmap. By focusing on transport of nutrients, which both facilitated and constrained evolution of protocells, this work is relevant to objectives 3.2 and 3.4 devoted to the origins and evolution of functional biomolecules and origins of cellularity and protobiological systems, respectively.
Mitchell Schulte/University of Missouri - Columbia  
**Experimental determination of the partial molal heat capacities and volumes of aqueous organic compounds**

Studies of chemical processes leading to the origin of life are aided by geochemical models that involve reactions of aqueous organic mixtures. In turn, these models require accurate values of the thermodynamic properties of these compounds as a function of temperature and pressure. Currently, these model calculations depend largely on extrapolated or estimated values of the thermodynamic properties from a sparse experimental database, mostly at low temperatures.

Model calculations also illustrate the biochemical pathways used by microorganisms that live at conditions on the Earth that are considered extreme. These environmental conditions include elevated temperatures and pressures, very low (or high) values of pH, and metabolism of unusual chemical compounds. The presence of these organisms has led many to speculate that similar organisms may exist or have existed on extraterrestrial objects such as Mars or Europa.

We propose to determine experimentally the thermodynamic properties of aqueous organic compounds at elevated temperatures and pressures. Specifically, we will measure the partial molal heat capacities of aqueous organic compounds as a function of temperature. Our initial efforts will be directed at a number of simple organic compounds that may be considered as precursors to biomolecules (in particular, thiols and organic sulfides) and several important biomolecules (coenzyme M, cysteine, ATP, adenosine). These data will provide direct tests of the estimation methods currently used in theoretical geochemistry to estimate thermodynamic properties. The data will also aid in developing new methods to predict thermodynamic properties for aqueous organic mixtures and help to refine the equations used to calculate reaction properties away from the standard state conditions of 25 C and 1 bar. Lastly, these data are necessary for realistic geochemical models of extreme environments on the Earth, the early Earth and solar system bodies such as Mars and Europa.

Glenn Stark/Wellesley College  
**Absorption measurements of SO2 isotopologues with application to sulfur isotope mass-independent fractionation during SO2 photolysis**

We propose to measure high-resolution absorption cross sections for several isotopologues of SO2 including 33SO2, 34SO2, and 36SO2 from 180 to 220 nm. The measurements will be performed on a vacuum ultraviolet Fourier Transform Spectrometer at Imperial College, London. The data will be at sufficiently high resolution to resolve rotational structure, and will complement existing data for 32SO2. The measured cross section data will be interpreted by radiative transfer calculations in atmospheric photochemical models.
The expected results from this study are: i) measurement of high-resolution absorption cross section data for 33SO2 and 34SO2; ii) measurement of high-resolution cross section data for a mixture of ~ 50% 36SO2 and ~ 50% 32SO2; iii) incorporation of the cross section data for all isotopologues into a photochemical model of a low O2 atmosphere. We will be able to constrain the magnitude of the mass-independent fractionation signature associated with SO2 photodissociation, and possibly the concentration of sulfur in the Archean atmosphere. The cross section data will also be useful in existing photochemical models of the Archean atmosphere, and in constraining ab initio cross section calculations.

A key component of the "Early Evolution of Life and the Biosphere" research area is the geological and geochemical record in rocks. The discovery of sulfur mass-independent fractionation in ancient sedimentary minerals, and the strong likelihood that these signatures arise from atmospheric photochemical processes, implies that a complete understanding of the kinetics of atmospheric sulfur isotopes is essential to extracting the full implications of sulfur mass-independent fractionation to the composition and evolution of the early atmosphere. By measuring and interpreting high-resolution spectra for SO2, a key component of the Archean sulfur cycle, we believe we can contribute significantly to understanding the full implications of sulfur mass-independent fractionation to the environment of the Archean and Paleoproterozoic Earth. The proposed study will contribute to our understanding of the O2 and sulfur composition of the Archean and Paleoproterozoic Earth atmosphere.

Paul Strother/Weston Observatory of Boston College
A Comparative Study of Precambrian and Cambrian Terrestrially-Derived Organic Remains

Palynology, the study of structurally preserved microscopic organic matter, provides a very useful method for the study of ancient ecosystems because this method can recover abundant direct remains of once-living organisms. Spores and cysts of both algal and embryophyte (true land plants) origin can directly trace the history of surface-dwelling subaerial and freshwater aquatic organisms. The proposed research will compare palynomorphs from terrestrial (an near-shore) rocks of Precambrian age with their counterparts from non-marine facies from lower Paleozoic rocks. This study will identify and characterize those components of lower Paleozoic (about 0.5 billion years ago) terrestrial ecosystems that were in existence around 1 billion years ago. This information will be crucial in resolving questions about the evolution of morphological characters leading up to the origin of land plants. There are no records of (macroscopic) plant axes from rocks older than middle Silurian. Yet, the spore record indicates that land plants (embryophytes) originated much earlier by middle Cambrian time, or perhaps earlier. By examining entire assemblages of diverse populations of spores and associated remains from non-marine sediments that clearly predate the origin of land plants, I hope to distinguish those components of the ancient subaerial and terrestrial aquatic flora that are more algal in character from those related to the evolving charophyte to embryophyte lineage. This will be the first study to examine in a comprehensive and comparative way,
the nature of the evolving terrestrial (=subaerial and freshwater aquatic) landscape based on a direct microfossil proxy.

The proposed work involves field collection and chemical maceration of rock samples. The extracted microfossils will be characterized using transmitted light microscopy, scanning electron microscopy and transmitted electron microscopy.

An assessment of the development of non-marine ecosystems can help constrain geochemical (and other) models of planetary development that include atmosphere-organism interactions. In helping to document the water to land transition within the course of plant evolution on Earth, this proposal is relevant to the study of universal evolutionary processes that may apply to the evolution of life on planets in general. Research on Precambrian paleobiology is based largely on microfossils and geochemistry from marine sources rocks. This proposal represents an opportunity to add studies of microfossils that inhabited the Precambrian terrestrial landscape.

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**Margaret Tolbert/University of Colorado**

**Aerosols and Clouds on Early Earth**

Photochemistry in the atmosphere of the early Earth may have led to the formation of complex organic molecules, similar to those observed on Saturn’s moon Titan. Previous work in our laboratory has used a novel aerosol mass spectrometer (AMS) to probe aerosols formed in a likely post-biotic early Earth atmosphere. We found a myriad of aerosol organic products were produced with complex functional groups beyond simple hydrocarbons. Thus photochemistry in the early Earth atmosphere might have provided complex organics for the sustenance of early life.

Here we propose to extend our previous studies of post-biotic haze formation to conditions more representative of early Earth before the development of life. Little is known about the composition of the early Earth atmosphere so we will consider a range of possible compositions including CH4, N2, H2, SO2, NH3 and H2O. A state-of-the-art high resolution AMS will be used for part of this study. One main aspect of this work will be to determine if an early Earth haze could supply life-building organics to the surface of the Earth.

In addition to studies of aerosol chemical composition, we will also examine how early Earth haze particles might directly and indirectly influence the ancient climate. We will use a novel aerosol cavity ring down spectrometer system to probe how early Earth particles take up water and grow to sizes that efficiently scatter incoming solar light. We will also use this technique to evaluate the potential role of early Earth haze particles as nuclei for cloud condensation. As is the case on current Earth, the direct and indirect effect of aerosols on climate could be very large. Thus our measurements will provide key data needed for improving climate models of the early Earth.
Arthur Weber/NASA Ames Research Center  
Prebiotic synthesis of autocatalytic and self-replicating molecules from sugars as the primary carbon substrate

Summary: Our research objective is to understand and model the chemical processes on the primitive Earth that generated the first replicating molecules and cell-like microstructures involved in the origin of life. Our approach involves (a) investigation of a model origin-of-life process named the Sugar Model that is based on the reaction of sugars with ammonia, and (b) elucidation of the role of chemical constraints on the origin of life. The Sugar Model is a plausible “one-pot” prebiotic process that converts very simple substrates (formaldehyde and ammonia) into a variety of chemical building blocks, catalytic molecules, and energy-rich molecules essential to the origin of life. Recently, we showed that the reactions of the Sugar Model generate cell-like organic microspherules and “nucleic acid-like” molecules. Here we propose to investigate four aspects of the Sugar Model chemistry that are relevant to central questions about the origin of life: (1) the origin of biological asymmetry (homochirality) by examining the stereoselective synthesis of sugars by peptide catalysts, (2) the origin of autocatalytic protocells by incorporating catalytic groups into cell-like organic microspherules, (3) the origin of molecular replication by examining the prebiotic synthesis and properties of “nucleic acid-like” molecules from sugar-ammonia reactions, and (4) the origin of ammonia biosynthesis by investigating the ability of sugars to drive the synthesis of ammonia from nitrite (nitrate). In addition, to develop a better understanding of the chemical constraints that governed the origin of life we plan to estimate the reaction rates of nitrogen-containing organic molecules under mild aqueous conditions.

Our proposed study of prebiotic chemistry involved in the origin of life supports (a) NASA’s Strategic Sub-goal 3C: Advance scientific knowledge of the origin and history of the solar system . . .” which includes understanding “the origin and evolution of the Earth’s biosphere” and (b) NASA’s Astrobiology Program Element objective in Prebiotic Chemistry that seeks to determine “what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere, including alternatives to the current DNA-RNA-protein basis for life.”

Aubrey Zerkle/University of Maryland  
Fractionation of multiple sulfur isotopes during biological sulfur oxidation

The proposed research will focus on collecting sulfur multiple isotope data for laboratory culture experiments with phototrophic and non-phototrophic S oxidizing organisms, and evaluating the experimental results in the context of metabolic fractionation models for these S oxidizing organisms. Our findings will be relevant for studies of sulfur ecosystems that use sulfur cycle models and isotopic data to characterize the transformations and transport of sulfur, and should have specific applications to modern and ancient ecosystems where biological S oxidation plays a role, on earth and elsewhere. In this manner, we anticipate that this research will directly contribute to NASA studies aimed at both: 1) understanding biogeochemical cycling in modern environments and in Earth's early biosphere; and 2) interpretation of biological versus abiotic chemical signatures on earth and other planets.
The primary objectives of the proposed research are:
1) to place basic limits on the magnitude of multiple sulfur isotope fractionations associated with biological S oxidation;
2) to reinforce the understanding of the metabolic steps that control the isotopic fractionations for all four stable isotopes in S oxidation metabolisms; and
3) to quantify the contribution of S oxidation pathways to sulfur isotope signatures produced in models of the sulfur cycle relevant to the early Earth and other planets.

These research objectives will be met by performing laboratory culture studies on phototrophic and non-phototrophic S oxidizing bacteria, and analyzing 33S/32S, 34S/32S, and 36S/32S in the resulting sulfur products. This work builds on a series of studies conducted in collaborations between Co-I Farquhar and other members of the research team involved in this proposal (also including a substantial body of related work on sulfate reduction and sulfur compound disproportionation by Johnston and others), and extends a database of results in progress by PI Zerkle. Laboratory culturing experiments will be conducted in collaboration with researchers at the University of Southern Denmark in the departments of Biology and Biochemistry & Molecular Biology. All analytical instrumentation and protocols for multiple sulfur isotope measurements are well established at the University of Maryland. Isotope measurements are undertaken by converting extracted sulfur to silver sulfide and then fluorinating this to sulfur hexafluoride, which is purified and analyzed using a ThermoFinnigan MAT 253 dual inlet mass spectrometer.