Jake Bailey/University of Minnesota  
**Investigating The Dissolution Of Carbonate Rocks By Marine Lithotrophic Bacteria: From Biogeochemistry To Biosignatures**

The dissolution of carbonate rocks by acid-producing microbial processes in poorly-buffered non-marine settings is a topic of ongoing research. However, few studies have addressed the dissolution of carbonate rocks under buffered marine conditions. We have observed indicators of corrosion that correlate with the presence of sulfide-oxidizing bacteria on carbonate rock samples that were recovered from marine methane seeps. The end-product of sulfide-oxidation under aerobic conditions is sulfuric acid, which could lead to carbonate dissolution if proton release is concentrated at the mineral surface. However, other dissolution mechanisms are also possible.

The work proposed here will investigate microbial processes that could result in the dissolution of carbonate rocks under marine conditions. The proposed research will use an experimental approach, in addition to the characterization of natural samples, to address the following questions: 1) What are the processes and rates of carbonate dissolution associated with bacterial sulfide-oxidation under carbonate-buffered conditions? 2) Do these chemoautotrophs benefit from carbonate dissolution? 3) Does microbial dissolution of carbonate rocks produce features that can be preserved in the rock record and/or be used as biosignatures?

The rate of carbonate dissolution will be determined using laboratory strains, which will be incubated in a biofilm reactor in the presence of select carbonate and control minerals. Dissolution rates will be determined by mass loss of the carbonates and the release of calcium ions into solution. The production and localization of acidic conditions by biofilms will be examined using a non-invasive pH imaging system. Alternative dissolution mechanisms will be tested using ion chromatography analyses of organic acids and through the use of CO2-producing heterotrophic control experiments.

We will address the likelihood for carbonate dissolution to benefit attached bacteria by incubating our test strains and control strains in a biofilm reactor time series experiment in order to quantify attachment and growth on different test minerals. Stable isotope labeling using the natural 13C depletion of methane-derived carbonates will be used to track rock-derived inorganic carbon fixed into cell biomass.
A subset of rock slides and chips from the above experiments will serve as the subject of our third area of inquiry, biosignatures. Experimental rock surfaces will be characterized before and after dissolution experiments using scanning electron microscopy, XRCT, Raman spectroscopy, and scanning probe microscopy to characterize dissolution and alteration features, as well as characterizing associations between biological material and the dissolution features. The volume and morphology of the dissolution features will be characterized in detail using XRCT and scanning probe microscopy and compared with similar features in modern methane seep carbonates as well as Paleocene-age seep carbonates.

Our proposed research is relevant to NASA’s Astrobiology Roadmap because: 1) understanding the mechanisms and rates of biogeochemical fluxes in the carbon cycle is essential for understanding the coevolution of the geosphere and biosphere (NAI Goal 4); 2) this study may provide insights into early and contemporary life under alkaline vent conditions where bioavailable inorganic carbon can be limiting (NAI Goal 3); and 3) our investigation of the possibility of a dissolution biosignature will further the objective of the Astrobiology Roadmap Goal 7, which is to determine how to recognize signatures of life on other worlds and on the early Earth.

**Jason Barnes/University of Idaho**

**Obliquity Stability of Potentially Habitable Worlds**

We propose to explore the long-term obliquity variations of plausible habitable planets in order to assess the potential for climatic stability in extrasolar planetary systems over astrobiologically important timescales. Because the Moon's gravity stabilizes Earth's obliquity to vary by just +/-1.3 degrees, it has been thought that the presence of a large moon would be required for a planet to maintain climatic stability and thereby to remain habitable over evolutionary timescales. The full problem, however, is more complex. The intricate frequency structure of a planetary system affects obliquity variations of planets, and thus for some planets in some systems a moon can stabilize obliquity, while for other planets a moon would destabilize obliquity. We will investigate the obliquity variations for 8 different potentially habitable worlds in the absence of a large moon. Our previous work on moonless Earths showed that perhaps as high as 80-90% of hypothetical moonless Earth's might maintain restricted obliquities even without the Moon. This work would extend our previous investigation by the inclusion of early Venus, the 6-planet Kepler-11 system, and artificial systems generated by planetary formation algorithms in order to more broadly evaluate the long-term climatic stability of habitable planets in general.
Andrew Becker/University of Washington  
Exploring the Critical Radius Between mini-Neptunes and super-Earths using Kepler

We propose to combine a Bayesian reanalysis of short-period Kepler exoplanet transits with standard models of tidal theory in order to identify the planetary radius that separates rocky and gaseous exoplanets. We exploit the conventional assumption that gaseous planets dissipate orders of magnitude less tidal energy than rocky planets, leading to the expectation that the latter will be on circular orbits out to larger orbital periods. Preliminary dynamical simulations show that short period (2-10 days) gaseous bodies should be found with eccentricities near their primordial value, but rocky bodies are preferentially found at low eccentricity due to tidal circularization. Thus, a study of the eccentricities of short-period planets can constrain the planetary radius of this transition. The identification of the boundary between rocky and gaseous bodies, independently from mass measurements, is vital for understanding the planetary conditions needed to support life.

A lower limit to the orbital eccentricity can be calculated by comparing the difference between the modeled transit duration and the transit duration that would be seen if the orbit were circular. To assess this difference, we analyze Kepler lightcurves using a purely geometric model that includes no assumptions about the orbital dynamics. We cast our measurement of minimum eccentricity in terms of two model parameters, whose posterior distributions we explore using Markov Chain Monte Carlo methods, and two physical parameters (e.g. stellar mass and radius) that must be estimated from other means. We have run a suite of simulations using a grid in transit depth, stellar brightness (lightcurve signal-to-noise), and the number of transits included in the model to gauge our sensitivity to these model parameters. We validated this method on the confirmed exoplanet system Kepler 62-b, and successfully recovered the published results and expected parameter uncertainties. We propose here to extend this analysis to an ensemble of 1100+ KOIs that have been selected based upon their Kepler-reported periods and planetary radius. This reanalysis will enable the first measurements of the boundary between gaseous and rocky exoplanets, as well as of tidal dissipation as a function of planetary radius.

This project spans the fields of high-performance computation, statistical modeling of experimental data, and celestial mechanics, which will make it a valuable contribution to the field of exoplanet studies. We will release code and data using open-source collaboration tools, and help to guide the adoption of reproducible research standards by releasing interactive analysis packages as part of our publication process.
A central question facing NASA as it designs missions to search for life in the cosmos asks how life might be recognized if it were actually encountered. In 2007, NASA and the National Academies published a report that expanded the conceptual horizons of this search beyond replicates of standard terran biology [Baross, Benner, et al., 2007]. This Weird Life Report noted that alternative molecular biology might not only be possible, but might also be more likely than the standard, three biopolymer (DNA-RNA-protein) molecular biology that is universal on Earth. In particular, it appears that natural history, instead of inventing protein to replace RNA as a catalytic biopolymer, might have simply increased the number of independently replicating nucleobases on RNA, added protein-like functionality to these, and used the then-emerging ribonucleotide reductase to make the corresponding DNA parts, perhaps later engineering the DNA parts to make them better suited for genetics.

Indeed, some surviving features of modern molecular biology suggest that Earthbiota actually started down this path towards an alternative biochemistry. Many RNA nucleotides in the oldest RNA molecules (tRNA, rRNA) carry functionality similar to the side chains on proteins, including amines, carboxylates, thiols, and heterocycles. The thymine in DNA, presumably recruited from uracil in a pre-existing RNA, was methylated to convert an RNA nucleobase into something better suited for genetics. C-glycosides, joining nucleobases to ribose by a C-C bond, are today biosynthesized by Earthbiota; one (pseudouridine) is widespread in RNA.

Rather than inventing the ribosome, if natural history had simply made just one more effort by altering the hydrogen bonding groups on the nucleotides to create additional base pairing patterns, terran life might have been avoided the need to invent ribosomes, and spared the continuing expense of translation machinery, which consumes half of the resources of a bacterial cell. Instead, it could be supporting Darwinian evolution with just two-biopolymers, a DNA-like biopolymer with an expanded set of nucleobases optimized for genetics, and an RNA-like biopolymer with many functionalized nucleobases needed for catalysis.

This work will place experiments behind this hypothesis, important in a field (weird life) that is largely speculative. We will actually implement a two-biopolymer encoded catalytic system based on this hypothesis for what might have been. This will involve:

1. Synthesizing genetic components for various forms of artificially expanded genetic information systems (AEGIS) for the alternative DNA (aDNA), and characterize aDNA.

2. Synthesizing functionalized components for various forms of an AEGIS for the alternative RNA (aRNA), and characterize aRNA.
3. Establishing polymerases and reverse transcriptases that allow in vitro selection (SELEX) to work in this two-biopolymer system, and demonstrate aDNA-aRNA SELEX.

4. Establish Lamarckian evolution with this system, where a successfully selected catalytic aRNA feeds its information back to the genetic system.

This project is significant on at least three levels. First, it will deliver insight into the feasibility of a two-biopolymer life form. Further, if things go well, it will initiate a new episode in synthetic biology based on such a life form, allowing us to study a second example of life. Third, as this alternative molecular biology seems to be just as evolvable on Earth-like planets as modern terran molecular biology, it may well be the kind of system encountered by NASA missions to Earth-like planets, such as Mars.

This is ambitious for a NASA Exobiology project. Accordingly, resources provided by NASA will be leveraged by spin-offs that apply this system outside of exobiology. These spin-offs give this project a fourth level of significance, one that is important to the continuing health of NASA itself.

Alexander Bradley/Washington University
Coevolution Of Sulfate Reducer Biosignatures And The Redox State Of Early Earth

The goal of our proposed project is to characterize, at the cellular level, the mechanistic basis for sulfur isotope fractionation during microbial sulfate reduction (MSR), and to understand how this may have evolved over Earth history.

This work seeks to tie the molecular record of living microorganisms through their DNA and protein sequences and functions to the geological record of sulfur isotopes. Pairing these records is a major objective of research into Early Evolution of Life and the Biosphere. We intend to study the physiology of sulfate reducing microorganisms, and the phylogeny of the key genes encoding this physiology, with the goal of gaining a better understanding of the original nature of sulfate reduction and an understanding of the mechanistic basis for MSR. We anticipate this will allow us to make more informed interpretations of the sulfur isotope record preserved in sedimentary minerals from early Earth.

We focus on three major aspects of microbial sulfate reduction: i) the role of the enzymes that microbes use to import sulfur into their cells; ii) the importance of intracellular oxidation state; and iii) the role of the enzymatic subunit DsrC, which catalyzes the final transformation of sulfur to sulfide.

1. What is the relationship between the sulfate affinity constant (KS) of sulfate reducer cells, and the fractionation of sulfur isotopes at varying sulfate concentrations? Sulfur isotope geochemists have frequently suggested that sulfur isotope fractionations above 5 permil are only expressed above a threshold concentration of sulfate, typically
assumed to be about 200 micromolar. Recent results (see below) have shown that in some strains fractionation is less than 5 permil, and correlates with sulfate concentrations, far above 200 micromolar. The relationship between sulfate concentration and fractionation is likely to be highly dependent on the enzymes cells use to transport sulfate into the cell, and the affinity of these enzymes for sulfate. We intend to test the sulfate affinity (Ks) of cells expressing a variety of sulfate transport enzymes, measure their expressed isotope fractionation, and examine the evolutionary relationship of these enzymes.

2. What is the cellular oxidation state of organisms performing MSR under differential environmental redox conditions, and what is the mechanistic relationship to sulfur isotope fractionation?

The most recent metabolic model for sulfur isotope fractionation suggests that a key control on expressed sulfur isotope fractionation is the availability of electrons vs. that of sulfite to the enzyme complex DsrAB (Dissimilatory sulfite reductase, subunits A & B). The relative availability of electron donors vs. acceptors relates to the oxidation state of the cell, and may relate to other cellular parameters such as sulfate reduction rate that are known to correlate with fractionation. We will directly test the hypothesis that intracellular oxidation state correlates with sulfur isotope fractionation, and determine whether this offers a proxy for environmental oxidation state.

3. How does the relative expression and redox state of DsrC affect whole-cell sulfur isotope fractionation?

Much as the flux of sulfate into the cell may effect sulfur isotope fractionation, the reactions producing sulfur molecules that flux out of the cell are also significant. DsrC is a redox-active enzyme responsible for withdrawing reduced sulfur from the DsrAB complex, assisting in the release of sulfide from the cell, and carrying oxidation power from the cytoplasm to membrane-bound proteins. Changes in the expression or cellular oxidation state of DsrC are expected to have isotope consequences. We will test our isotopic model by both observing and inducing changes in DsrC expression and measuring isotope fractionation.

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Aaron Burton/NASA/Johnson Space Center
A Highly Sensitive Instrument To Measure Enantiomeric Excesses Of Meteoric Hydroxy Acids

Modern biology uses L-amino acids almost exclusively (homochirality). However, without a chiral driving force, amino acid-forming reactions produce a 50/50 (racemic) mixture. A central question to Astrobiology, then, is how the transition from the abiotic synthesis of racemic mixtures of chiral (handed) molecules to the homochirality essential for life occurred. Meteorites may hold the answer to this question, since they have preserved a record of the chemistry that took place prior to the origin of life on Earth. Carbon-rich meteorites contain a wealth of organic compounds, including many
important to biochemistry, which contributed to the prebiotic inventory available for the origin of life. Amino acids, the monomers of proteins, have long been a focus of analyses of carbonaceous meteorites. Enantiomeric biases of extraterrestrial origin up to 60/40 (L/D) for the biologically rare amino acid isovaline in meteorites are now well-known (Burton et al. 2013; Glavin and Dworkin 2009; Pizzarello and Cronin 2000). In addition, there have been recent reports of enantiomeric biases approaching 80/20 (L/D) that were argued to be extraterrestrial in origin for proteinaceous amino acids including aspartic acid and isoleucine (Glavin et al. 2012; Pizzarello et al. 2012). The chiral driving force leading to these excesses is unknown, although several hypotheses exist (Glavin and Dworkin 2009; de Marcellus et al. 2011; Pizzarello and Groy 2011). Also unknown is whether any other types of organic molecules are present in enantiomeric excess (ee) in meteorites. This motivates our search for tracer molecules to better constrain the location and timing of the chiral enrichment.

Arguably the most relevant set of tracers for understanding the origin of ee in the alpha-amino acids mentioned above is the alpha-hydroxy acids, which can form through the same Strecker-cyanohydrin synthesis mechanism believed to dominate the formation of alpha-amino acids found in meteorites (Peltzer et al. 1984; Peltzer and Bada 1978). Measuring the ee of 2-hydroxy-2-methylbutanoic acid (designated hy-isovaline) is of particular importance because it is the structural analog of isovaline and should have been formed from the same Strecker synthesis precursor and in the same environment, and therefore may mirror the isovaline ee (Figure 1). To truly understand the significance of the ee comparisons, however, it is critical to couple the ee measurements with a thorough comparison of the structural diversity and isotopic ratios of hy-droxy and amino acids to confirm their assumed shared chemical history. We have been funded to develop extraction, processing, LC-FD/ToF-MS, and GC-isotopic ratio mass spectrometry methods to measure the isomeric, enantiomeric, and isotopic compositions of hy-isovaline and other hydroxy acids in carbonaceous chondrites. Comparing these data to those of the analogous amino acids in the same meteorites will illuminate the relationship between ee in hydroxy and amino acids, clarify the links in their formation chemistry, and help evaluate the plausibility of various chiral forces during synthesis and / or parent-body processing. Accordingly, we request support for the acquisition of a LC-FD/ToF-MS instrument at the NASA Johnson Space Center that will give PI Burton more direct control of the research progress and augment the types of analyses that can be employed for the funded work.

Michael Callahan/Astrochemistry Laboratory
Exploring the Fate of Heterocyclic Compounds in Complex Prebiotic Mixtures

A long-standing problem in prebiotic chemistry is a general lack of robust and/or convincing prebiotic syntheses of genetic macromolecules. Proposed early Earth alternatives to DNA and RNA include peptide nucleic acids (PNA), threose nucleic acids (TNA), and glycol nucleic acids (GNA). However, none of these alternative nucleic acid molecules have been demonstrably produced prebiotically. In order to bridge the gap
between prebiotic chemistry and the RNA World, a primitive nucleic acid should have a robust prebiotic synthesis. Here, we propose to explore the prebiotic fate of nitrogen-containing heterocyclic compounds (nucleobases and nucleobase analogs). To this end, we will react nitrogen heterocycles with complex mixtures of prebiotic organic compounds, formed principally using a spark discharge. We will use the results to evaluate whether the reactions found would effectively remove particular nitrogen heterocycles from the pool of early Earth compounds or result in steps toward possible alternative nucleic acid polymers.

Our preliminary results show that 5-hydroxymethyluracil (HMU), a uracil analog, and isoxanthopterin, a guanine analog, both form cyanide adducts with the complex spark mixture, and HMU forms several other adducts with the spark. Some of these products include HMU dimers with products of the spark discharge forming a bridge between residues. Additionally, HMU was found to self-polymerize in solution to structures as large as heptamers.

Our first task of this proposal is to survey a wide range of heterocyclic compounds for reactivity with the products of a spark discharge (with and without UV photochemical activation of the heterocycles). Task 2 is a focused investigation on the reactive nitrogen heterocycles (and adducts) found in Task 1 by further reacting the heterocycles with the products of spark discharge performed under a variety of different conditions. During Task 3, we will evaluate the prebiotic reactions identified and generalize the lessons learned about the robust plausibly prebiotic reactions identified.

In all cases, the proposed research is based on state-of-the-art analytical chemistry. We will be using a combination of liquid chromatography, UV and fluorescence spectroscopy, ultrahigh resolution-high mass accuracy Orbitrap mass spectrometry, and NMR spectroscopy.

John Chaput/The Biodesign Institute at ASU
The Emergence of Protein Folds

Modern proteins evolved from a small number of primordial folds, but it is not known how these molecules evolved into complex structures capable of supporting life on Earth. One could imagine that protein evolution proceeded through a series of discrete chemical steps in which random pools of sequences gave rise to relatively simple protein folds that were initially quite small (<100 amino acid residues), but over time recombined in various ways to adopt larger structures that were better equipped to support the needs of a primordial metabolism. Understanding the evolutionary pathway that allowed simple protein folds to evolve into larger, more complex structures is a grand challenge in evolutionary biology.

We hypothesize that early protein folds (proto-proteins) with simple domain structures nucleated the evolution of diverse protein structures by stabilizing the folding of larger
proteins. This hypothesis suggests that the total structural diversity that we observe today in nature may have originated from a small number of distinct protoprotein folds. We recently explored this possibility by evolving a small (80-amino acid) de novo generated ATP-binding protein into a new size-expanded protein of 160 amino acids. This technological advance, which is a major leap forward in de novo protein evolution, yielded numerous sequences, suggesting that many distinct solutions exist to the problem of how a small protein fold can be expanded into a much larger protein domain. While the architecture of these proteins is not yet known, we have identified at least one protein that is monomeric and adopts a discrete fold after expression and purification in Escherichia coli. Given the large number of sequences that remain in the pool, we plan to test our hypothesis by examining the level of structural diversity that can emerge from a model proto-protein system.

We propose to evaluate the diversity of protein architectures that can emerge from a model proto-protein. To achieve this goal, we propose the following specific aims:

Specific Aim 1: Solve the three-dimensional structure of DX-19.3 a model protoprotein created by de novo protein evolution.

Specific Aim 2: Expand our pool of model proto-proteins by de novo evolution.

Specific Aim 3: Characterize the structural diversity of the pool of proto-proteins.

The results from this proposed research are expected to yield insight into the origin and evolution of proteins on the primitive Earth and help generate experimental information concerning the possible emergence of life elsewhere in the universe.

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**Ken Cullings/NASA/Ames Research Center**

**New Lineages of Microbial Endosymbionts of the Basidiomycete Extremophile Pisolithus; Early Origins, Evolution and Biogeography**

We propose to investigate the diversity and evolution of a new endosymbiotic relationship that we have recently discovered between prokaryotic partners and a eukaryotic extremophile. In our preliminary work (see Preliminary Data section for details) we have identified chemoautotrophic Archaea residing within tissues of a fungal sulfur extremophile, Pisolithus. We have also revealed the existence of a previously unknown lineage within the broad domain Archaea that is equivalent in stature to the Crenarchaeota, Euryarchaeota, and Korarchaeota, and has a phylogenetic origin at the base of the broad Archaea clade. In this study we will fully investigate the diversity and evolution of this new, ancient lineage and the diversity and evolution of the microbes comprising our newly-discovered endosymbiosis. Pisolithus exists in thermal soils in only two places on Earth, Yellowstone and New Zealand (20). Thus, to fully and completely investigate the phylogenetic origin, diversity and evolution of these endosymbionts we must sample in both locales.

We are asking three broad questions: 1) What are the origins and diversity of Archaeal and bacterial endosymbionts residing within Pisolithus individuals, on both local (within
a single thermal area) and regional (amongst thermal soils in Yellowstone) and international (between Yellowstone and New Zealand) scales? 2) How does host anatomy and hence redox state of sulfur within the Pisolithus fruiting bodies influence or drive endosymbiont diversity and phylogeny? and 3) Do endosymbionts exist in Pisolithus populations without any obvious energetic need or are the endosymbiotic associations indicated by our preliminary data universal amongst Pisolithus populations?

To answer these questions we will be investigating endosymbiont diversity across several scales using PCR amplification, sequencing and phylogenetic analysis of 16S ribosomal DNA from within the tissues of Pisolithus individuals using taxon-specific primers, fluorescent in situ hybridization to confirm the sequence data and also to localize the endosymbionts within tissue types of individual fruiting bodies, and culturing to attempt to obtain individuals for more detailed physiological studies.

This project fits directly with The Astrobiology: Exobiology and Evolutionary Biology goal to understand the origin, evolution, distribution, and future of life in the Universe, by performing research on the origin and early evolution of life, the potential of life to adapt to different environments. Further, this work fits directly with the goals of research into the goals of Exobiology section on Early Evolution of Life and the Biosphere to (ii) understand the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments and (vii) study the coevolution of microbial communities, and the interactions within such communities, that drive major geochemical cycles, including the processes through which new species are added to extant communities.

Our preliminary data suggest that the outcomes from this project will add exciting new information regarding origin, evolution and factors influencing diversification not only of novel species within known groups of Archaea and Bacteria, but will also provide a once in a lifetime opportunity to address these concepts in the context of discovery of an entirely new Phylum in a novel early Earth analog environment.

David DesMarais/NASA Ames Research Center
Transformations and Fates of Lipid Biomarkers in Microbial Mat Ecosystems

This project links the biomarkers in a living cyanobacterial mat ecosystem to their diagenetic transformations and ultimately to their incorporation into refractory macromolecules that have high geological preservation potential. This work would enhance interpretations of ketogens from ancient benthic ecosystems, including stromatolites. The study addresses key categories of mat biomarkers that represent major microbial lineages: (1) Intact polar lipids (IPL) are valuable biomarkers for living biota. (2) Hopanoids and hopanes are among the most abundant chemical fossil biomarkers and are widely distributed amongst bacteria. Methylated hopanols are probably biomarkers for specific bacteria. (3) Sterols are eukaryotic biomarkers and require O2 for their synthesis, therefore the ancient sterane record helps to chart the impacts of oxygenic
photosynthesis. Modified sterols might be indicators of early fauna hosted in ancient mats. (4) Exopolysaccharides (EPS) are invoked as major contributors to the geologic preservation of mat biosignatures. (5) Molecular carbon isotopic patterns can identify carbon sources and pathways in complex ecosystems.

The Guerrero Negro microbial mats are a rich source of organic materials represented in the ancient sedimentary record. Organic analysis is a core element of work required to relate modern microbial ecosystems to geologic records of Earth’s early biosphere, Lipid biomarkers are central to establishing these relationships.

A comparative study of an important analytical approach to analysis of ancient kerogen, hydropyrolysis to that of more conventional GC/MS analysis is a main element of our parent award. That work will establish a basis for comparing modern and ancient, and provide an additional level of understanding to the complexity of paleoecosystems. Essential to fully realize the potential of this study is the purchase of a modern 5977A GC/MSD. This instrument provides a greater range of mass fragment identification necessary for modern techniques, is extremely sensitive and will allow detection and identification of minor biomarker molecules, is designed for ease of maintenance, limited down time and analysis of dirty environmental samples and is a proven robust design.

Mary Droser/University of California, Riverside
Catching the 'Second Wave' of the Ediacara Biota: Assessing the Role of Environment, Ecology and Diagenesis

The soft-bodied Ediacara Biota comprises Earth’s oldest complex ecosystem and represents the earliest pre-Cambrian radiation of animals on this planet, recorded in globally distributed deposits of terminal Ediacaran age. These morphologically bizarre fossils of enigmatic phylogenetic affinity are preserved in situ and characterized by exceptional preservational fidelity, such that these assemblages have been likened to census populations or geologic snapshots. Although the phylogenetic associations of many of these taxa remain unresolved, they are characterized by considerable morphological complexity and likely include metazoans (e.g. sponges and stem-group bilaterians). Previous efforts have focused largely on description of individual specimens, with the object of relating Ediacara taxa to Phanerozoic clades. However, the habitats in which Ediacara communities lived and the depositional and diagenetic factors responsible for their preservation in the fossil record remain subjects of considerable debate.

Ediacara fossil assemblages are typically considered to represent a single, though highly variable biota, consisting of three major, temporally-disparate faunal assemblages (commonly referred to as the Avalon, White Sea and Nama Assemblages [Waggoner, 2003]). Considerably greater morphological complexity and taxonomic diversity of body fossils, trace fossils and textured organic surfaces (TOS) are associated with the younger (White Sea and Nama) assemblages (Narbonne, 2005; Gehling & Droser, 2009).
Interpretations of the difference between these later, second wave Assemblages and the preceding Avalon Assemblage are far from uniform, ranging from biogeographic provincialism to evolutionary succession to facies-specific preservational filters (Grazhdankin, 2004; cf. Narbonne, 2005). Moreover, these assemblages occur in very different depositional environments: the Avalon Assemblage is known largely from deeper water facies, whereas the White Sea and Nama Assemblages occur in shallower water facies.

We will test the hypothesis that environment exercised a major control upon the diversification of Ediacara faunas and was instrumental in triggering a second-phase radiation, represented by the increased complexity and disparity of the White Sea and Nama Assemblages, associated with the advent of matground-adapted ecosystems and colonization of shallower water marine settings. Interpreting the environments of the Ediacara assemblages is an essential prerequisite for 1) reconstructing the habitat and ecology of Ediacara communities; 2) deciphering the circumstances responsible for their geologically sudden appearance and disappearance and variable distribution within the fossil record; and 3) elucidating their evolutionary significance in the radiation of complex, eukaryotic life on Earth.

We will employ a multi-scale approach by means of sedimentological, geochemical and paleoecological proxies. This work will build upon previous study of Ediacara fossils by refining and testing fauna-lithofacies models (Gehling & Droser, 2013) both regionally (South Australia) and globally (western US). Sedimentology will be characterized at the unit-, outcrop- and micro-scale in order to develop process-driven models for reconstructing habitats and interpreting fossil preservation. Associations of particular fossils, TOS and lithofacies will be characterized in order to capture not only fauna-facies relationships but also the role of a TOS in the ecology, habitat and preservation of Ediacara macrofauna. Previous paleoenvironmental interpretations (e.g. Retallack, 2012; Gehling & Droser, 2013) will be tested, via mapping and U-Pb/Pb-Pb dating of iron oxide phases, to distinguish endmember (terrestrial vs. marine) predictions for depositional setting. Models for diagenetic pathways, particularly the role of early-stage silicification, will be investigated by means of Ge/Si ratios, silicon isotopes and experimental studies.

Jamie Foster/University of Florida
Biodiversity, Functional Genomics, And Carbonate Microstructure: An Integrated Approach To Defining The Stromatolite Microbiome

Stromatolites are quintessential geomicrobiological ecosystems with a fossil record that extends back 3.5 billion years. These long-lived features are sedimentary structures formed as a result of the synergy between microbial metabolisms and the environment. Ancient stromatolites formed massive carbonate reefs comparable in size to modern coral reefs, dominating life on our planet for 80% of Earth history. Modern stromatolites, in contrast, are relatively rare, but have been found in diverse habitats including freshwater,
marine and hypersaline environments. Although considerable progress has been made in identifying key metabolisms associated with modern stromatolites, there is a gap in knowledge regarding the stromatolite microbiome (i.e., totality of microbes, genomes and environmental interactions) and the degree to which the core microbiome is conserved in space and time.

Our central hypothesis is that stromatolites, regardless of biogeography, have a core microbiome that influences the extent of carbonate deposition within the microbial communities. The target communities for this study are the marine stromatolites from the Exuma Cays in The Bahamas and the hypersaline stromatolites from Hamelin Pool in Shark Bay, Western Australia. The stromatolites in both of these environments grow through similar mechanisms, such as trapping and binding of sediments as well as biologically influenced carbonate precipitation. Our preliminary data suggest that the stromatolite-forming microbial mats of both habitats are amenable to the molecular approaches outlined in this proposal. The rationale for our proposed approach is that despite several independent analyses of the microbial diversity in stromatolites worldwide, comprehensive comparative molecular analysis of the functional genes associated with the metagenomes of modern stromatolites is lacking. Bahamian and Shark Bay stromatolites are targeted for this research as they represent the two most widely studied modern stromatolite ecosystems.

The proposed research will characterize the microbiome of Bahamian and Shark Bay stromatolites and delineate those conserved molecular pathways leading to carbonate deposition with the following specific objectives:

Objective 1: Generate amplicon libraries to the small subunit ribosomal RNA gene for bacteria, archaea, and eukaryotes of each stromatolite locality;

Objective 2: Compare the metagenomes and functional gene complexity using massively parallel DNA sequencing within each stromatolite locality; and

Objective 3: Integrate molecular analyses with detailed studies of stromatolite microstructure and stable isotopes of carbonate precipitates.

The proposed work has is highly relevant to the field of Astrobiology. By understanding whether there are global patterns of biodiversity and functional metabolisms that are shared between modern stromatolite ecosystems we can begin to understand the environmental and molecular constraints on the processes of stromatolite formation and growth.
Transitioning from an RNA World: The Origins of the Protein Synthesis Machinery

The primary objective of the proposed top down project is to determine if a small pseudosymmetric RNA encompassing the peptidyl transferase center of the modern ribosome can catalyze peptide synthesis. Initially, a 244 base RNA containing the essence of the modern ribosome's peptidyl transferase center will be synthesized. The ability of this RNA to catalyze the synthesis of peptides from mononucleotide and trinucleotide substrates carrying activated amino acids will be determined. Parallel experiments with a larger RNA that encompasses all of Domain V in the 23S rRNA will also be conducted. Substrate size may have an important effect on substrate residence time on the RNA surface. Thus, substrates containing 1, 2 or 3 nucleotide residues will be employed in the assays. Assay conditions will also take into account the likely greatly reduced activity of the naked RNAs relative to the modern ribosome. Thus, in addition to standard conditions, extended reaction times of days and even weeks will be considered. Also in light of the likely nature of the prebiotic world, anaerobic assays will be conducted where ferrous iron can replace magnesium in stabilizing the RNA constructs. Similarly, near freezing incubation temperatures may serve to increase residence times of substrates on the RNA surface and thereby facilitate peptide bond formation. If the RNAs do not show activity small unstructured peptide oligomers will be added to attempt to stabilize the RNA and alternative RNAs will be made. When synthesis is successfully demonstrated, efforts will focus on properties of the reaction such as rate and length of the peptides produced and how these outcomes might be changed by for example by different amino acid choices. The successful demonstration of peptide synthesis will strengthen the RNA World hypothesis and provide new direction for studies relating to Astrobiology Roadmap Objective 3.2: The Origins and Evolution of Functional Biomolecules.

Evolutionary History of the Translation Machinery

A major commonality of life as we know it is the couple between translation and transcription as mediated by the genetic code. Efforts to define the properties of the last universal common ancestor (LUCA) of Earth life consistently conclude that a well-developed nearly modern translation apparatus was already present. If we can elucidate the essence of the evolutionary history of the translation machinery this will provide a window into the pre-LUCA World. As a result we will also obtain insight to the origins of the genetic code and likely chirality as well. It is hypothesized here that substantial, though necessarily incomplete evidence, relating to the origins and early development of the translation machinery and its relation to other core cellular processes continues to exist in the primary sequences, three-dimensional folding and functional interactions of the various macromolecules involved in the modern ribosome. The proposed work will seek to extract and interpret this information and to develop a time line of major events leading up to the modern translation machinery. We will determine the relative age of
various ribosomal components and subsystems and where possible how they relate to other processes. For example, we will seek to better define the major locations where movement occurs in order to understand the interdependence of different ribosome regions. We also will examine RNA pores to attempt to understand how peptidyl transferase center of the ribosome may have evolved. The proposed effort addresses multiple themes in the 2008 Astrobiology Roadmap primarily relating to Goal 3, http://astrobiology.arc.nasa.gov/roadmap/g3.html, the Origins of Life.

Jennifer Glass/Georgia Institute of Technology
Characterization Of Microbes Mediating Anaerobic Oxidation Of Methane Coupled To Iron Reduction From An Ancient Ocean Analogue

Early microbial life evolved in a biosphere rich in iron and methane, and low in sulfate and oxygen. Prior to the Great Oxidation Event (~2.4 billion years ago), the major sink for methane is postulated to have been microbial anaerobic oxidation of methane (AOM). Microbes that coupled AOM to Fe(III) reduction (hereafter abbreviated Fe-AOM) may have comprised a significant portion of biomass in the anoxic ocean due to the scarcity of alternative electron acceptors (i.e. sulfate, nitrate). Recently, geochemical evidence for Fe-AOM has been discovered in numerous modern environments. However the microorganisms catalyzing Fe-AOM remain completely unknown. We propose to enrich, isolate and characterize these enigmatic microbes using existing enrichment cultures from (1) ferruginous Lake Matano, Indonesia, an Archean ocean analogue, (2) Bay of Bothnia, Sweden, and (3) sediment samples to be collected from coastal Georgia, USA. Previous geochemical evidence suggests that Fe-AOM is occurring at all three of these sites. Our first objective is to enrich Fe-AOM microbes using batch enrichments amended with soluble and solid Fe(III). Fe-AOM activity will be monitored by measurements of Fe(II) production and 14CH4 conversion to dissolved inorganic 14C-labeled carbon. Our second objective is to determine the phylogenetic identity and spatial organization of Fe-AOM microbe(s) using microautoradiography (MAR), fluorescent in situ hybridization (FISH), stable isotope (13CH4) DNA probing, and amplicon pyrosequencing. Our final objective is to characterize the metabolic pathways utilized by Fe-AOM microbes in pure or nearly-pure isolates grown in continuous bioreactors using (meta)genomics and (meta)transcriptomics. The proposed work is relevant to the Exobiology solicitation research emphasis Early Evolution of Life and the Biosphere as it will determine the nature and metabolic machinery of some of the most primitive microorganisms and thus contribute to improved geochemical models of the ferruginous, methane-rich ocean in which these microbes evolved.
Biogenic Iron Oxide Transformations By Thermophilic And Mesophilic Iron-Reducing Microbes

Life in our solar system beyond Earth, past or present, is likely to be microbial, but finding distinct evidence of that life is challenging because all organic signatures of that life may have degraded, leaving behind only mineral evidence. Microorganisms that pass electrons to Fe$^{3+}$ oxide minerals and reduce them exogenously to other iron oxide minerals are among the more favorable organisms to study for microbial mineral biosignatures. They are endolithic and transform minerals independent of both light and oxygen, and include hyperthermophilic archaea from deep-sea hydrothermal vents and hot springs. Thus, they are representative of potentially ancient metabolisms associated with geothermal environments, and their growth and iron production can be modeled quantitatively. There is significant evidence that extraterrestrial systems have hosted hydrothermal systems. Therefore, there is good rationale for understanding mineral assemblages produced by iron-reducing microorganisms from hydrothermal environments for the search for life beyond Earth.

In this proposal, we seek to search for and understand spectral signatures in a broad range of bioreduced oxides from various iron-reducing microbes. We also seek to explore the conditions that control the growth of these microbes and to provide clues to formation environments and mineral products that will endure in the geologic record. Accordingly, this research has three goals:

1. We will grow a variety of iron reducers (freshwater vs. marine, mesophilic vs. thermophilic) to characterize the rates and constraints of microbial mineral transformations. We will vary temperature, pH, chlorinity, and mineral substrates and measure growth rates, cell yields based on Fe$^{2+}$ produced, and the cell-specific rates of Fe$^{2+}$ production for modeling purposes.

2. We will characterize mineral starting and end products from bioreduction growth using reflectance, Raman and Mössbauer spectroscopies. We will build a spectral library of these minerals produced biotically and abiotically so we can relate spectral features to specific coordination polyhedra in the mineral structures.

3. We will evaluate the mineralogical (crystallographic) constraints on the ability of microbes to bioreduce iron. There are many known iron oxides with identical or very similar compositions but slightly difference crystal structures. We will characterize the size and geometries of Fe sites in oxide and oxyhydroxide minerals and relate them to specific spectral characteristics to link spectral characteristics with minerals that grow in known conditions.

This research is directly applicable to the goals of the Astrobiology: Exobiology and Evolutionary Biology program. Specifically, as it relates to Early Evolution of Life and the Biosphere, our study will determine the nature of the most primitive organisms and the environment in which they evolved. It will help us understand the physiology of
microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments (goal ii), determine the original nature of biological energy transduction via iron reduction (goal iii), and investigate the development of key biological processes and their environmental impact (goal iv). Through this approach, we will also specifically address goals 5 and 7 in NASA's 2008 Astrobiology Roadmap, namely:

"understand the environmental limits of life, determine the biochemical and molecular mechanisms that control and limit evolution, metabolic diversity, and acclimation of life (Goal 5); and

"determine how to recognize signatures of life on other worlds and on early Earth (Goal 7).

Gerald Joyce/The Scripps Research Institute
Evolution of Catalytic RNA and the Origins of Life

This proposal describes work that is part of a larger NASA-sponsored effort to understand the origin of life on Earth, which will guide the search for life elsewhere in the universe. It pertains to a form of RNA-based life that likely existed during Earth's early history, as a predecessor to the current DNA-RNA-protein-based life. The proposed research aims to construct RNA-based life in the laboratory as represented by populations of RNA enzymes that catalyze their own replication and undergo Darwinian evolution in a self-sustained manner. The research also seeks to understand molecular processes that are fundamental to the origin of life on Earth and elsewhere.

During the course of prior NASA-sponsored research, our laboratory developed the first example of an RNA enzyme that catalyzes its own replication and can transmit heritable information from parent to progeny molecules. This system involves pairs of RNA enzymes that catalyze each other's synthesis by joining together component oligonucleotide building blocks. Each building block can have many alternative compositions and can be joined in many different combinations. In addition, new variants can arise through recombination during the course of replication. Variants that replicate most efficiently grow to dominate the population until new, more advantageous variants arise, resulting in an ongoing Darwinian battle for survival.

The proposed research aims to increase the complexity of the present RNA-based evolving system so that it can bring about the invention of novel function. Large, heterogeneous populations of self-replicating RNAs will be challenged to adapt to various environmental conditions, resulting in the emergence of novel functional traits that confer selective advantage. As the environmental conditions change, further adaptations will arise through evolutionary processes that can be continued indefinitely. This work has the potential to provide the first example of a living system outside of
terrestrial biology. It also will shed light on the chemical basis of life and on the presumed ancestor of contemporary life on Earth.

Niles Lehman/Portland State University
How Game Theory Applied To Chemistry Can Inform The Origins Of Life

The transition from non-life to life, regardless of where it occurs, must have involved a competition among information-bearing molecules for scarce resources. This process is at the root of natural selection, and is the driver of evolutionary innovation. The RNA World paradigm has been used as a model system for understanding this transition for a variety of reasons, one of which being that it is experimentally tractable in the laboratory. While a wide variety of chemical and evolutionary studies have been performed with RNA and analogous nucleic acids, none of them has fully and specifically addressed the origin of selection in the absence of fully formed catalytic replicase enzymes. Game theory, developed in the early 1950s, has the potential to do this. The current proposal will, for the first time, exploit the principles of game theory as applied to prebiotic chemistry to shed light on the chemical underpinnings of biology.

Game theory is a branch of mathematics that is the study of the rational behavior of decision makers whose decisions affect each other. It was originally designed to address problems in economics, but has since been applied to evolutionary theory by removing rationality and considering instead the dynamics of how populations of genotypes interact in an evolutionary setting. The results from these studies have been extremely powerful in predicting the outcomes of bouts of natural selection. Nevertheless, this concept has not been applied at the chemical level, in a situation where alternative chemical structures (e.g., different RNAs) are interacting under selection. Thus its utility in prebiotic chemistry has not been explored, despite the fact that this is precisely where it is needed to develop a model of how RNA self-replication originated.

To apply game theory at the chemical level we will use fragments of RNA that can self-assemble and replicate based on trans-esterification chemistry (recombination) and that rely on a three-nucleotide base-pairing process that confers specificity. These fragments will initially be designed from the Azoarcus group I intron self-assembly system that we have developed from previous Exobiology funding. Using pair-wise combinations of different genotypes, we will create a series of payoff matrices, which are 2 x 2 tables of kinetic rate constants that depict what happens when various RNA sequences compete against one another. Because there are four cells in a payoff matrix (the basic data form in game theory), we will assign the observed rate constants to the parameters a, b, c, and d, and then determine the rank order of these parameters. There are 24 possible orderings of these parameters, and the order determines the type of game being played, such as mutual cooperation, the Prisoner's Dilemma, Hawk-Dove, etc. We will run 2-player, 3-player, and multiplayer games in both single reaction vessels and in a serial-dilution format. By changing genotypes and environmental conditions systematically, we will be
able to elucidate the kinetic features of chemical interactions that lead to evolutionary
dynamics, and thus hopefully reveal a key feature of how life originated.

The goal of this work is to tie kinetic parameters directly to selection. These experiments
will thereby examine the power and relevance of game theoretic principles to the origins
of genetic information on the early Earth and elsewhere in the universe – a clear objective
of NASA’s mission. We can accomplish this project with one post-doc and one
undergraduate student at Portland State assisting the PI in three years’ time.

Timothy Magnuson/Idaho State University
Origins of Microbial Iron Respiration

NASA’s current palette of astrobiology research includes studies on extreme
environments around the world, with the goal of characterizing unique modes of
microbial life that might be similar to those found on other worlds such as Mars,
Saturnian Moons, and even newly discovered exoplanets that are in the Goldilocks zone
of other suns and thus capable of harboring at least simple microbial life forms. In our
previous participation in the NASA Spaceward Bound Idaho expeditions, we explored
unique environments in south-central Idaho, and discovered potential study sites with
several extremes: temperature, pH, radiation, and presence of a variety of metals,
including iron. These hot spring systems undoubtedly harbors microbes that can serve as
new and novel model systems for the study of iron transformation, a potentially
universal bioenergetics process common on other planets, and recognized as one of the
earliest forms of biological energy generation on early Earth. These unique systems has
never before been described in terms of geochemistry and microbiology, and thus
represents a unique environment for the study of heat, pH, and metal- and radiation-
resistant microorganisms and communities. Currently, only a few model systems
(mesophilic and neutrophilic iron transforming microbes) have been examined in detail,
while the vast number of microbial species (including thermophiles, acidophiles, and
other extremophiles) using iron in bioenergetics has been largely ignored. The proposed
research will answer several questions, including 1) Do extremophiles possess similar
biochemical mechanisms for iron-based energetics that mesophiles employ, 2) What
mechanisms arose on Earth during evolution under extreme conditions, based on exotic
Earth microbes, and 3) How does this information translate to prediction of iron-based
bioenergetics strategies on other worlds. We will use novel iron-transforming
extremophiles and characterization of electron transport components involved with iron
transformation, and comparison of these components to the current state of knowledge.
We will also employ cultivation-independent approaches such as metagenomics and
metaproteomics to characterize the microbial communities in a radioactive thermal
environment, which will reveal distribution and activity of iron-based bioenergetic
strategies. This project is an ideal fit for the Exobiology program, as it addresses
fundamental questions about the evolution of primitive electron transport and respiration
systems in iron transforming extremophiles. We will use this project as a platform for
further Idaho Spaceward Bound expeditions, undergraduate, graduate, and postdoctoral
training, and public outreach and education about exobiology and the search for life in the universe.

Shuhei Ono/Massachusetts Institute of Technology
Photochemistry And Spectroscopy Of Sulfur Dioxide, Sulfur Monoxide And Elemental Sulfur As Source Reactions For Archean Sulfur Mass-Independent Isotope Fractionation

Oxygenation of the atmosphere at ca. 2.4 Ga (Giga annum before present) is the milestone event in the evolutionary history of the Earth. The event brought the Earth out of Prokaryotes world, set the stage for diversification of Eukaryotes, and led to the later advent of complex lifeforms. Sulfur isotope mass-independent fractionation (S-MIF) provides the most compelling evidence for the model of an early anoxic atmosphere. S-MIF refers to anomalous sulfur isotopes abundances that do not follow traditional law of quantum mechanics of stable isotope fractionation. The S-MIF is thought to originate from ultraviolet light photolysis of SO2. Several hypotheses have been proposed, however, for the physical origin of S-MIF. Archean S-MIF records follow very specific isotope systematics, and we have demonstrated that S-MIF from SO2 photochemistry also follows certain systematics as a function of UV spectrum (i.e., band system), temperature, and pressure. The project proposed herein built upon our previous research, and aims to identify Archean source reaction and gain a mechanistic understanding of S-MIF to develop a high fidelity model that can be used to accurately predict S-MIF pattern for atmospherically relevant temperature, pressure and pSO2 conditions.

We will approach this problem by combination of laboratory photochemistry experiments and chirped-pulse mm-wave spectroscopy of SO2, SO and elemental sulfur. Our flow through photochemical reactor is designed to test a much wider range of pSO2 (down to 0.01 mbar or lower), temperature (down to -50°C), and pressure (0.1 bar or lower) conditions compared to previous studies by us and others. The mm-wave spectroscopy will be used to identify weak forbidden ultraviolet transitions in congested spectrum of SO2 and SO. These perturbation-facilitated transitions populate excited electronic states into which transitions from the ground state are nominally forbidden by spin and/or symmetry selection rules. We hypothesize that because these nominally dark states will have longer radiative lifetimes, they will be subject to further excitation by UV radiation into dissociative states or chemical reaction with other molecules, and contribute to MIF. Because characterization of one perturbation between one pair of electronic-vibrational states can give complete and reliable information about all other perturbations between vibration-rotation levels of the two electronic states, we can construct a global model to predict S-MIF pattern for our experiments as well as for atmospherically relevant conditions.

By identifying the origin of S-MIF and to be able to predict isotope systematics in atmospherically relevant conditions, this proposed research aims to constrain not only the chemistry but also the UV transparency, pressure and temperature of early Earths.
atmosphere. Short wavelength UV would have been significantly attenuated by organic haze particles if early Earth's atmosphere was not only anoxic but reducing. Temperature and pressure of early atmosphere are subject of debate but crucial in understanding the environmental context for early Earth's lifeforms, and the co-evolution of biosphere and atmosphere. Similar chemistry may also be applied to early Mars, where the recent Curiosity mission has identified abundant sulfate minerals of unknown origin (http://www.nasa.gov/mission_pages/msl/). Therefore, the proposed research is designed to contribute to the research emphasis of the Exobiology Program: early Evolution of Life and the Biosphere, and linked to Goal 4 of the Astrobiology Roadmap, understand how past life on Earth interacted with its changing planetary and solar system environment.

Ronald Oremland/US Geological Survey

Light Hydrocarbon Metabolism as a Basis For Microbial Life and its Detection in Oxygen-Free Settings: Mars and the Planet(Oids) of the Outer Solar System.

The notion that life may have gained a foothold elsewhere in the universe has long been a topic of fascination to scientists and the lay public alike. Indeed, there is a possibility that microbial life exists on some of the neighboring planet(o)ids of our Solar System beyond Earth's orbit. This would most likely be in locations that allow for the presence of liquid water, such as in or beneath the regolith of Mars, or underneath the ice layers of satellites of Jupiter and Saturn. For life to survive under these conditions it would need to be anaerobic, as there is little sunlight to sustain oxygenic photosynthesis, and no significant molecular oxygen present. Life would also be constrained by other physical realities including the presence of dissolved toxic elements and low water activities. In the latter instance, liquid water in these locales would most plausibly be in the form of dense brines owing to evap-concentrative (e.g., Mars) or cryo-concentrative (e.g., Europa, Ganymede, Enceladus) processes or from the deliquescence of hygroscopic salts. We make the fundamental assumption that if microbial life arose, it would by adaptation surmount these barriers as it has in many of Earth's most extreme environments. Nonetheless, the basic question remains of how such life would gain sustenance from its surroundings? In the absence of any photosynthetic carbon fixation and photosynthate release, what would these microbes eat? Many of the outer planets and their satellites have abundant methane and other gaseous hydrocarbons, either as atmospheric components or entrapped beneath their surfaces as gases dissolved in brines. Acetylene, a highly reactive molecule, also occurs commonly (as do ethylene and ethane) when formed by atmospheric photo-catalytic reactions of the aforesaid methane. These C2 hydrocarbons may also arise from internal pyrolysis of entrained larger organic molecules derived from meteorites and comets. It has been established that certain terrestrial (i.e., Earth) bacteria ferment acetylene gas into more common metabolites (e.g., acetaldehyde, ethanol, acetate) initially via the enzyme acetylene hydratase. Methane however is a more difficult nut to crack in the absence of oxygen. Although terrestrial anaerobic oxidation of methane (AOM) has been linked to dissimilatory sulfate reduction, it is unlikely that this occurs in high-density brines due to severe energy
constraints on sulfate-reduction and other low energy-yielding processes (e.g., reverse methanogenesis). Such high-density brines are most likely to occur beneath the regolith of Mars (or around near-surface perchlorate salt crystals) or below the ice layers of Jovian satellites. In this proposal we intend to pursue further investigations focused on how metabolism of these two specific gases, acetylene and methane, by Earth-based microorganisms including those inhabiting hypersaline environments, could serve as plausible models for the sustenance of microbial life that may be encountered in the outer Solar System. In the case of methane (as well as ethylene and ethane) we will investigate whether strong oxidants known (or hypothesized) to occur on these planet(oids) (e.g., perchlorate) may ultimately serve as a source of molecular oxygen by which a conventional aerobic methanotrophy may proceed. For acetylene, we propose to extend our research using established and newly isolated cultures to investigate the stable C isotopic fractionation involved in the fermentation of this gas. Such information would establish a logical experimental means by which to determine if life exists, for example, beneath the ice of Enceladus. This can be achieved by collection of the products of acetylene fermentation entrained in the volatile materials spewed from the satellite's vaporous plumes and jets, and determination of their 12C/13C carbon-isotope (e.g., 13C-acetate) ratios relative to the collected, residual pool of unreacted acetylene (e.g., 13C-acetylene).

Victoria Orphan/California Institute of Technology
Assay Of Dissimilatory Sulfate Reduction Enzymes In-Vitro And Analysis Of Sulfur Isotope Fractionation

Sulfur isotopes have a long history of use as measures to gauge the occurrence and extent of biogeochemical processes on Earth, and the advent of modern techniques including multiple isotope measurements and secondary isotope mass spectrometry indicate that much knowledge remains to be obtained through their analysis. A significant portion of microbial sulfur isotope fractionation occurs through the process of dissimilatory sulfate reduction (DSR); however very little data are available which describe the extent and variability of sulfur isotope fractionation at the level of the individual enzymes which carry out intracellular sulfur conversions. The specific aim of this proposal is to define isotopic fractionation values for enzymes involved in DSR and in doing so, better constrain and calibrate isotope fractionation values in the rock record. We propose to investigate the enzymes that catalyze the reactions DSR through two complimentary approaches: 1) comprehensive bioinformatics approaches including sequence phylogeny, structure prediction, and the identification of amino acid motifs/signatures which may impart unique reaction characteristics, and 2) the isolation of representative members of these enzymes families through heterologous expression, and their assay in a purified, cell free in vitro setting. Assay products will be subjected to sulfur isotope analysis by mass spectrometry. This approach will for the first time give values for the discrete steps of microbial sulfate reduction and mark a significant advance in understanding the individual factors contributing to biological sulfur isotope fractionation. In addition, it will make possible investigation of the variability of these isotope effects by studying
enzymes derived different taxonomic lineages. The results will supplement existing models of sulfur isotope fractionation, allow for a more clear description of the evolution and distribution of anaerobic sulfate respiration throughout Earth history, and furnish new insights into the catalytic nature of the enzymes involved in microbial sulfate reduction. The paired approach of biochemical investigation coupled to geochemically relevant isotope fractionation directly addresses the NASA Research Announcement Astrobiology: Exobiology and Evolutionary Biology Solicitation, where The opportunity is taken to investigate two natural repositories of evolutionary history available on Earth: the molecular record in living organisms and the geological record.

Matthew Pasek/University of South Florida
Phosphorus Geochemistry and Cosmochemistry: Extraterrestrial Influences on the Origin and Evolution of Life

Phosphorus (P) is cosmically the limiting nutrient for biologic systems. Understanding the geochemical and cosmochemical sources of P on the early Earth in turn shows pathways leading to the incorporation of P into the prebiotic compounds that led to the origin of life. Our research has shown that reduced oxidation state P compounds such as phosphite and the mineral schreibersite may have comprised a substantial portion of the total P inventory of the early earth. These compounds are capable of reacting with water, oxidants, and organic compounds to provide potentially prebiotic P compounds. We propose to continue investigating the effects of meteorite bombardment on terrestrial P chemistry through the first two billion years of Earth’s geologic history, and will extend these studies to other planets and moons, specifically Europa. This objective will be accomplished through a series of modeling, and analyses of natural and experimental samples.

The experimental work will involve laboratory measurement of spectra of phosphate and polyphosphate compounds in ices by IR spectroscopy to provide the essential data set for searching for life elsewhere. We shall also characterize how the P redox state is changed during the hydrothermal serpentinization of rocks. Our analyses will employ high-performance liquid chromatography coupled to an inductively coupled plasma mass spectrometer (HPLC-ICP-MS), a method we have been developing at USF, and Raman and Infrared (IR) spectroscopy to determine P redox states, origins, and fates. Finally, we shall model the meteoritic flux of material to the surface of the earth with consideration of ablation, changing atmosphere, angle, and material strength.

Results from these experiments, analyses, and models will identify the geochemical sources of P for the origin of life, including the formation of critical polymers like RNA, and the development of oxidative metabolism. It will also elucidate whether we can detect life on icy moons using by IR signatures of polyphosphates. Our data will also constrain the early geochemistry of this limiting nutrient by elucidating the extent of changes to P redox state by serpentinization. In turn these studies may provide a
geochemical means of tracking extraterrestrial flux and will aid in understanding planetary geochemistry.

The proposed research is highly relevant to the objectives of NASA and the Astrobiology: Exobiology and Evolutionary Biology Program. Our work is particularly relevant to two of the areas of research emphasis: Origin and Early Evolution of Life, and Implications for Life Elsewhere. This research links the formation of reduced P compounds originating from large, frequent impacts to the origin and development of life on Earth, will attempt to determine whether hydrothermal systems can also provide reduced and reactive P, and will seek to provide constraints on life detection elsewhere using polyphosphates and organophosphates. This research will be especially useful for proposed Europa missions, specifically for near-IR compositional analysis of the surface.

Andrew Pohorille/NASA/Ames Research Center
Towards Co-Evolution Of Membrane Proteins And Metabolism

Coupling between oligopeptides or proteins interacting with or lodged in membranes that bound protocells and metabolism that they encapsulated was a factor driving evolution of cellular systems since their inception, even before the emergence of modern biochemistry. To be inheritable, early metabolism must have led to an increased rate of growth and division of vesicles and, similarly, transport through vesicle boundaries must have supplied substrates needed for the nascent metabolism. We argue that peptides participated in mediating both growth and transport since very early stages of cellular evolution. Elucidating the mechanisms by which this happened is the goal of this proposal.

To accomplish this goal, we will investigate how membrane-bound dipeptides, synthesized inside fatty-acid vesicles, stimulate competitive growth of protocells, as recently observed by Adamala and Szostak (Nat Chem, 5, 495-501, 2013). We will also determine whether this is a general, robust phenomenon and how substrates for peptide synthesis could have been supplied. Next, we will study how simple, small peptides, too short to span protocellular walls, could increase delivery rates of ions and building blocks of biopolymers to protocells through creating thinning defects in membranes. Finally, we will identify mechanisms by which the earliest, highly flexible ion channels gained rigidity, which was a necessary condition for improving their function and their further evolution. Taken together with our previous work on ion channels, this study will help to map key steps on an evolutionary path from the simplest, membrane-bound oligopeptides to modern channels, pumps, receptors and transporters that are at the heart of biology, as they provide communication between a cell and its environment, and keep the system far from equilibrium.

We will pursue our objectives using large-scale, atomic-level computer simulations complemented by kinetic modeling. We recently used a similar approach to characterize the earliest ion channels and establish how building blocks of nucleic acids could be
selectively supplied to protocells. We will examine natural or synthetic model systems selected for their significance, relevance to the origins of life, technical feasibility to model and the availability of experimental data needed for validating our simulations. Once validated, not only will this approach provide information about processes of interest that are often difficult to obtain experimentally, but also will allow for generalizing our results in terms of their evolutionary implications for a broad range of similar systems.

The proposed studies directly address Goal 3 of the Astrobiology Roadmap. At their center is early coordination and evolution of key cellular processes. The focus is on membrane peptides of increasing complexity that, through different mechanisms, both facilitated and constrained evolution of protocells. This focus make the proposed work relevant to objectives 3.2 and 3.4 of the Roadmap devoted to the origins and evolution of functional biomolecules and origins of cellularity and protobiological systems, respectively.

Raphael Rosenzweig/University of Montana
Sweet are the Uses of Adversity: Genomic Responses to Stress and Their Implication for Adaptation and the Origin of New Species

INTELLECTUAL MERIT The origin and fate of genetic diversity are central organizing themes in biology, crucial to understanding how life arose and evolved on earth, and how life might arise and evolve on other worlds. Because all life forms encounter severely adverse conditions, it is not surprising that selection has favored the evolution of mechanisms that can increase population variation by adjusting mutation rate. Such innovations complicate the dynamic interplay of genetic variation, selection, and mutation, which together determine the tempo and trajectory of adaptation and the likelihood that new species arise. Stress-related mutations - both those that result in local changes in DNA sequence and those that cause large-scale changes in genome architecture are now known to play decisive roles in the emergence of antibiotic resistance and the progression of cancer. Here, we seek to investigate whether they also play a role in speciation itself.

The goal of our first NASA-Exobiology grant was to study speciation by creating de novo yeast species and determining what genomic changes occurred following the "shock" of interspecific hybridization. We now seek a Successor to that award to follow up on our most recently published work, where we report on an unexpected twist in the drama of adaptation and speciation: the possibility that severe stress specifically elevates rates of genomic rearrangement, creating new variants, some of which are adaptively favored and whose novel genome structures favor reproductive isolation in sympatry. We will investigate this possibility by pursuing three Objectives. First, we will identify, isolate and assay the fitness effects of specific large-scale genomic rearrangements that arise in yeast during prolonged starvation. Second, we will determine the frequency with which these genomic rearrangements arise in starved cultures. Third, we will delineate
the genetic pathways which lead to starvation-induced genomic rearrangement by systematically evaluating genes involved in homologous recombination, nonhomologous end-joining, mismatch repair, environmental signal transduction and retrotransposition. Achieving these objectives will fundamentally advance our understanding of the feedback between genomes and their external environment. Knowing how starving cells undergo genomic changes that make them stress-resistant and knowing the cost of such mutations, will better enable us to predict under what genetic and environmental conditions stress-induced genome rearrangements are likely to be lost to drift, selection or sexual recombination, or to be retained in a reproductively isolated subpopulation that becomes a new species.

Relevance to the NASA Mission This research proposal addresses the first of the three basic questions articulated in the NASA Astrobiology Roadmap, namely "how does life begin and evolve?" Our work will advance two Objectives identified under Roadmap Goal 5: Understand the evolutionary mechanisms and environmental limits of life (OBJECTIVE 5.1-Environment dependent, molecular evolution in microorganisms, and OBJECTIVE 5.3-Biochemical adaptation to extreme environments, (c), explore the biochemical and evolutionary strategies that push the physical-chemical limits of life by reinforcing, replacing, or repairing critical biomolecules (e.g., spore formation, resting stages, or DNA repair). Our project will also help NASA achieve two of its five Strategic Goals, namely: Expand scientific understanding of the Earth and the universe in which we live (#2) and Share the challenges and results of NASA missions to inspire the American public, to encourage scientific literacy, and to foster innovation and a strong national economy (#5). We envision Opportunities for Education and Public Outreach, because elements of our experiments are simple enough to be suitable for teaching evolutionary principles at the community college and secondary school levels.

Burckhard Seelig/University of Minnesota
Linking The Evolution Of Primordial Amino Acid Alphabets To The Structure And Function Of Proteins

All known life today is based on the standard set of twenty amino acids. Conclusive evidence from numerous studies in areas ranging from prebiotic chemistry to evolutionary biology has shown that the modern set of amino acids is a continually evolving work in progress which originated from a smaller number of primordial amino acids. Therefore, proteins employed by early forms of life relied on a significantly reduced chemical diversity of their amino acid building blocks. Although numerous theories have proposed explanations for the nature of these early amino acid alphabets and the gradual addition of later amino acids leading to today's genetic code, experimental data linking these theories to biological functions of the corresponding proteins are missing. This project will use combinatorial chemistry to synthesize libraries of random polypeptides that are based on different subsets of the twenty standard amino acids representing different stages of a plausible history of the alphabet. Individual proteins from those libraries will be examined with a variety of biophysical and
biochemical methods to evaluate their ability to fold into three-dimensional structures, a hallmark of contemporary proteins. Using the investigator's proven expertise, the libraries will also be screened for proteins that exhibit a simple biological function: binding to adenosine triphosphate (ATP) or guanosine triphosphate (GTP). ATP and GTP are the energy currency of all known life forms and are therefore fundamental to metabolism. The goal of this project is to correlate the chemical nature of specific sets of primordial amino acids to their ability to form structured proteins and to enable simple biological functions. By generating tangible empirical data in a field of research that has been largely dominated by theoretical approaches, this proposal has the potential to have a broad impact on our understanding of the history of the standard amino acid alphabet. With the proposed work, we will explore potential scenarios of extinct biochemistry at the origin of protein-based life. Investigating the nature of functional proteins entirely built from early amino acids will help to understand the nature of the most primitive organisms. Furthermore, examining minimal subsets of primordial amino acids for their ability to support functional proteins will help our understanding of environmental requirements conducive to the emergence of protein-based life. Linking a set of amino acids that can be produced abiotically to the appearance of functional proteins can also guide our search for life elsewhere in the universe.

Linda Sohl/Columbia University
Reconstructions of a Snowball Earth: A Data/Model Perspective

The overlap between the rise of complex metazoan life and the "Snowball Earth" glaciations of the Neoproterozoic Era, ca. 850-635 Ma, raises tantalizing questions about the influence of climatic processes on the evolution of life. Can we relate specific surface conditions—persistence of extreme cold, icehouse/greenhouse transitions—to changes in marine metazoan species diversity, as determined via the fossil record or molecular phylogenetic studies?

Since Hoffman et al. (1998) first put forward the "hard snowball" hypothesis involving complete ocean ice cover, geologists, paleobiologists, and climate modelers have been exploring the feasibility of the hypothesis. Many geologists and paleobiologists have accumulated evidence supporting a "slushball Earth" instead, which allows for substantial ocean ice cover but still permits significant areas of open ocean. Climate modeling studies attempting to reproduce Cryogenian glacial conditions have achieved variable results, though hard snowball solutions as stable climatic states are often favored despite limited support from the geologic record. We suggest that the difficulty in reconciling geological and paleobiological evidence with modeling results stems in part from the fact that most Earth climate models are optimized for modern climate, and thus lack certain capabilities that would better address the otherworldly character of Earth's deep-time paleoclimates.

Here we propose a new investigation of the factors contributing to the Cryogenian glaciations, especially their onset and termination—the environmental events most likely
to influence speciation or trigger extinctions using the NASA Generalized Rocky ExoPlanet (GREP) model, a modified version of GISS ModelE2-R (the fully coupled CMIP5 ocean-atmosphere model) that is especially suited for deep-time Earth paleoclimate. Modifications include alterations to the radiation to accommodate varying atmospheric compositions, especially high-CO2/low-O2 atmospheres; the ability to handle CO2 condensation/sublimation under extreme cold climates; and planetary/astrophysical parameters such as length of day, orbital variations, and solar spectra.

Our team already has experience supplying realistic paleogeographic reconstructions to NASA’s GISS ModelE2-R and can thus explore: 1) the role of continental configuration and topography on the development and maintenance of glaciated land cover (as a check on how well the model can reproduce known surface conditions); 2) the impact of CO2 condensation in polar regions on the acceleration/maintenance of glacial conditions; 3) the ability to transition back to non-glacial states; 4) heat transports via dynamic ocean circulation on the extent of sea ice cover; 5) the role of methane as an ameliorating factor compensating for relatively low estimated atmospheric CO2 levels and reduced solar luminosity in a low-O2 environment; and 6) the contribution of cloud feedbacks to the glaciated state.

The expanded model capabilities, combined with boundary condition constraints from paleogeographic data, will provide the most realistic simulations yet produced of the time periods that fostered the Snowball Earth events. This data-model linkage places an emphasis on discovering what the actual Earth surface conditions were like during the rise of the Neoproterozoic metazoans, rather than limiting our investigation to the ramifications of a particular assumed surface state. Through these investigations, we can set a firm foundation for a systems paleobiology approach to understanding the interplay between life and environment under extreme glacial conditions. This proposal addresses the Exobiology program element, “Evolution of Advanced Life,” and contributes to Goal 4 of the 2008 NASA Astrobiology Roadmap, Understand how life on Earth and its planetary environment have co-evolved through geological time.

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**Terrestrial Impact of Nearby Supernovae**

We will undertake the first realistic assessment of the effects of a nearby supernova upon the Earth, using parameters derived from an event with terrestrial evidence and combining effects of atmospheric ionizations and radiation impact on organisms on the ground and in the ocean.

Supernova (SN) explosions arise from the same star-forming processes that give rise to planetary systems. The ionizing radiation during and immediately after SN explosions poses an ongoing hazard to the biosphere. This extrasolar influence on the planetary conditions for life can be crucially important, but the true threat of nearby supernovae,
and detailed nature of their impact on the biosphere, has not yet been studied in a
systematic way. Past work on understanding nearby SN effects has been approximate.

Evidence exists in the form of 60Fe in sediment cores that a relatively nearby SN close
even to modify surface UV levels, boost muon radiation levels, and possibly modify
climate happened within the last few Myr. Additional evidence, sufficient to make
estimates of the distance of an event 2-3 Myr ago, was announced at the APS April 2013
meeting and further details will be released in fall 2013.

The key question is: What overall impact do we expect from event(s) implied by the
recent ocean core isotopic signal? What is the ionizing photon dose and time history from
a SN explosion, and how does this vary with SN type? What is the dose/history of SN
cosmic rays and their interaction with the heliosphere? What terrestrial effects does the
SN radiation cause?

This issue is clearly related to priorities in this call for research proposals: examine the
response of Earth's biosphere to extraterrestrial events. This includes an evaluation of
environmental factors such as the influence of extraterrestrial processes on the
appearance and evolution of multicellular life. Of particular interest are mass extinction
events. Similar language appears in the 2008 Astrobiology Roadmap: Objective 4.3:
Effects of extraterrestrial events upon the biosphere. Study the short- and long-term
effects of extraterrestrial phenomena such as secular changes in the magnitude and
quality of solar and cosmic radiation...

We will assume photon and cosmic ray spectra from SN shocks, propagate them with
solar and terrestrial magnetic field effects included, and consistently study atmospheric
ionization all for the first time. Based on past extensive work, we will compute the
increased radiation dose of muons on the ground and in the upper kilometer of ocean.
We will apply multifaceted skills, using data on a variety of SNe from NASA space
missions such as Swift, Chandra, GALEX, and Fermi, considerably improving on
existing knowledge. Rather than focus on probable disaster scenarios in the more distant
gologic past, we will study a more recent event based on isotopic evidence, and whose
consequences, being more recent, can be more easily compared with paleontological
evidence (including a moderate extinction event). We will compute the ionization dose
and radiation history for nearby SNe, representing possibilities consistent with data on a
particular end-Pliocene event. We will incorporate effects of arbitrary spectra of CRs on
the Earth's atmosphere, and describe the effects in much more detail than has been
possible before. Treatment of UV effects and direct muon irradiation will be done
together at given times in the progression of supernova effects development.

No detailed, integrated models exist which link all these issues over the whole time
development of a SN event to attack this problem. Our research will fill this gap and
answer these questions. Our study will quantify and link together the terrestrial
atmospheric, environmental, and biospheric response to the photon and CR ionization,
the muon irradiation, and the enhanced UVB flux from ozone depletion due to a specific
nearby SN.
One of the major evolutionary transitions in life’s early history occurred when genes became consolidated into genomes. This has been described as the Darwinian transition from a communal mode of evolution, in which genetic material was freely exchanged, to an individualized one, in which genetic material is primarily vertically inherited. During this transition there was a reorganization of fitness so that selection acting on the level of the gene affected linked genes within the genome. We propose to examine the selective regimes that promote the transition to consolidated genomes by investigating the action of natural selection occurring in extant microbial genomes. In an interdisciplinary collaboration between geochemists, microbiologists, and virologists, we ask how natural selection shapes the archaeal pan-genome in natural populations. To answer this question we address two fundamental aspects of evolutionary change:

"How do genomes change as microorganisms adapt to different environments?"
"What is the primary force that differentiates populations and causes them to diverge?"

Our approach is to examine the genomic changes that result in recent evolutionary divergence between locally adapted microbial populations of the model organism from geothermal hot springs, *S. islandicus*. We predict that strong local selection differentiates local populations. By comparing populations we will be able to analyze footprints of selective forces that differentiate populations. If our hypothesis is correct, these changes will differentially fix genes or alleles in regions of the genome that are highly susceptible to horizontal transfer. Through previous work funded by NASA we have set the stage to address these questions including identifying differentiated populations that correspond to different chemistries of geothermal hot springs, developing genetic tools to test hypotheses in the laboratory, and isolating and characterizing co-evolving viral populations. In this system we propose the following specific objectives:

Objective 1: Identify genetic loci that differentiate ecologically distinct Sulfolobus populations from Yellowstone National Park (YNP).

"Comparative analysis of genome sequences of closely related genomes sampled over time and space from YNP."
"Determining the relationship between the change in frequency of candidate loci over time with biotic and abiotic changes between natural populations"
"Identifying the competitive fitness of different genotypes in common garden experiments in YNP with and without the biotic components."

Objective 2: Experimentally define gene function of candidate loci that differentiate isolated populations.
Testing the function of candidate adaptive loci under laboratory conditions using forward genetic methods.

Experimentally determining immunity and resistance profiles of S. islandicus to viruses.

Addressing these aspects of microbial evolution will make significant contributions to NASA’s objectives concerning both the Early Evolution of Life and the Biosphere and the Evolution of Advanced Life. We expect that the proposed work will contribute to these objectives by: i) developing techniques to link the molecular and geochemical records of life on Earth, ii) elucidating the evolutionary and co-evolutionary processes of speciation and adaptation in microbial populations, while also establishing a timescale on which these processes occur in nature, iii) understanding the unique constraints and adaptations occurring in high temperature geothermal environments that represent conditions on early Earth, and iv) building an evolutionary framework in which to understand the major transition from genes to consolidated genomes.