Exobiology
Abstracts of Selected Proposals
(NNH16ZDA001N-EXO)

Below are the abstracts of proposals selected for funding for the Exobiology program. Principal Investigator (PI) name, institution, and proposal title are also included. 173 proposals were received in response to this opportunity. On April 7, 2017, 24 for selected for full funding, 2 for partial funding, and 1 PME proposals were selected for funding.

Vladimir Airapetian/Catholic University Of America
Prebiotic Chemistry of the Young Earth and Mars From Theoretical and Experimental Studies

Objectives. Kepler has revealed frequent superflares on solar-type stars, providing a mechanism by which host stars may have profound effects on the physical and chemical evolution of exoplanetary atmospheres. What effect do extreme energy fluxes from eruptive events during early stellar evolution together with UV light have on prebiotic chemistry, in particular on the production of small molecule precursors currently thought necessary for nucleoside synthesis, and by consequence the origin of life on primitive planets? This may be important in terrestrial planetary atmospheres, where species such as molecular nitrogen and carbon dioxide would require high energy irradiation in order for them to be recombined into reactive species such as HCN and NOx, the former of which is considered crucial for nucleoside formation an outstanding challenge in the field.

Our proposal aims to address these major questions by performing computational and experimental studies on the effects of ionizing radiation initiated by energetic eruptive events including superflares from young stars and associated particle events on the prebiotic chemistry of the early Earth and its twins. First, we will calibrate atmospheric photo-colissional chemical models using experimental characterization of gas and aqueous phase chemistry. Second, we will characterize the processes of nitrogen fixation and the chain of reactions producing NOx, N2O, HCN and other N-containing species in a gas- and later liquid-phase that may eventually form organic molecules of varying degrees of complexity especially those relevant in the prebiotic synthesis of nucleosides, notably RNA.

Methodology. We will employ our 1D photo-collisional atmospheric chemistry model to compute the chemical formation rates of complex organic molecules including O- and N-containing hydrocarbons in the gas phase. These simulations will allow to us investigate the viable photochemical mechanisms for nitrogen and oxygen fixation into complex organic molecules in the atmospheres of early Earth and Mars. These simulations will provide guidance in experiments that explore the steady-state gas composition of N2/CO2/H2O gas mixtures, alone and in combination with minor amounts of CO or

CH_4, exposed to a variety of energy sources including particles, electric discharges and UV light, as well as the nature of the water soluble species when generated in the presence of an aqueous phase held at various pH values that simulate primitive planetary standing water bodies. Experimental studies will be conducted by flowing 0.1 to 1 atm total pressure gas mixtures containing various partial pressures of CO_2 and N_2 together with admixtures of lesser amounts of H_2O, CO, H_2 or CH_4. These will be irradiated by a proton gun, UV lamp and electric discharge. Steady-state gas compostions will be measured downstream from the reaction chamber using a residual gas analyzer. Soluble species will be measured using ion chromatography, direct infusion time-of-flight mass spectrometry, spectroscopically using NMR, and chemically with various assays.

Relevance to Exobiology Program. The proposed studies will provide significant implications for the conditions setting stage for the origin of life on Earth, the resolution of the FYS on the early Earth/Mars and habitability conditions on terrestrial planets around young stars. As a source of prebiotic nitrogen this work is relevant to the research area of Prebiotic Evolution . Abiotic nitrogen fixation is one of the planetary and molecular processes that set the physical and chemical conditions within which living systems may have arisen by helping to understand how planetary nitrogen became available for use in the origin and early evolution of life. Our understanding of the global scale of nitrogen fixation will help model early environments on the Earth in which organic chemical synthesis could occur, and may shed light on the prebiotic synthesis of nucleosides like RNA.

Jennifer Biddle/University Of Delaware
Our Archaeal Cousins: Evolution And Function Of Eukaryotic Homologs In Sedimentary Archaea

Our archaeal cousins: evolution and function of eukaryotic homologs in sedimentary archaea Jennifer F. Biddle and Rosa León-Zayas Scientific and Technical Summary

Exploring the origin and evolution of life is a theme of the NASA Exobiology program, and recent clues to the ancestry of eukaryotes have surfaced from genomic investigations into sedimentary archaeal communities. Genomes of Lokiarchaeota and Thorarchaeota, along with additional deep branching archaeal relatives, share genomic signatures with eukaryotes and are phylogenetically implicated as the nearest neighbors of the branch of life which evolved into multicellular organisms. A better understanding and groundtruthing of this view of the tree of life is needed, including proof beyond genomes.

Currently, the Lokiarchaeota and Thorarchaeota are known only from metagenomic datasets. These deeply branching archaeal genomes house eukaryotic signatures, such as Gtg/Rag family GTPases, actin homologs and synthesis of ether-linked and fatty acid ester linked membranes with bacterial/eukaryotic G3P stereochemistry. These features are suggested, by functional and phylogenetic inference,

to link the archaeal branch of life to the eukaryotic one. Additional genomes of these archaea will better elucidate shared pathways with the eukaryotes, providing clearer phylogenetic interpretations to the relationship between the groups. We hypothesize that additional genomic analysis will help to determine the directionality of transfer of these genes, including genes shared with the bacterial domain, and also further constrain the amount of genetic overlap between domains. We currently have deeply branching archaeal genomes from these groups, recovered from the marine sedimentary deep biosphere, and with existing data will harvest eukaryotic and bacterial homologs for in-depth phylogenetic study. Additionally, we will develop microcosms from anaerobic marine sediments and detect the expression of these eukaryotic homologs within these archaeal relatives using metagenomics and metatranscriptomics. Our ultimate goal will be to visualize expressed homologs within archaeal cells, using fluorescent in-situ hybridization techniques for both ribosomal signatures and mRNA of eukaryotic homologs.

Our research will provide conclusive evidence that these archaeal cousins do express eukaryotic homologs, and our methodology will allow for interpretation into their involvement in cell cycles. Using multiple datasets, we will have the ability to assess the diversity of eukyarotic homolog signatures and better develop our understanding of the biodiversity and phylogenetic extent of homolog evolution in archaea and eukaryotes. This project will lead to a better understanding of the evolution of complex life by examining our single celled cousins, the deeply branching archaea.

Management Summary

Samples will be collected and data will be generated, with all information stored in appropriate data repositories such as Dryad and NCBI. PI Biddle will oversee the project and participate in data generation, analysis and publication. Postdoctoral associate León-Zayas will generate, analyze and publish data.

Dennis Bong/Ohio State University
Triazine-Driven Synthesis Of Macromolecules Under Prebiotically Plausible Conditions

OBJECTIVES

The overall objective of this application is to demonstrate that synthesis of macromolecules can be achieved using only prebiotic materials: triazines, SH2, amino acids, formaldehyde and sugars. The central hypothesis of this application is that triazines served as precursors to the native nucleobases. Our rationale for this hypothesis is as follows: 1) triazines are readily formed from prebiotic conditions; 2) triazines have a high propensity for assembly; 3) triazines are functionally and structurally compatible with DNA and RNA molecules in extant biology. Nucleobase-recognition is required for biosynthesis of sugars, lipids, proteins and nucleic acids. The central goal of this proposal is to explore the possibility that triazines can fulfill the nucleobase role in recognition-driven synthesis of macromolecules. Notably, prebiotically plausible macromolecules

that interface with extant nucleic acids are thought to be central in the origin of life. We will test our hypothesis of triazine function and by doing so, accomplish our central goal via the following three specific objectives:

1. Synthesis of activated triazine and amino acid monomers.
The working hypothesis of this objective is that the materials available in the prebiotic world were sufficient for the generation of macromolecules through monomer activation. Neither triazine nor amino acids and sugars are sufficiently reactive to undergo efficient oligomerization without activation. The goal of this objective is to develop a prebiotic synthesis of thioester-activated amino acids and triazine-glycoconjugates. Triazine and carboxylic acid activation will be pursued using prebiotically plausible sulfur, mineral and formaldehyde chemistry.

2. Templated oligomerization of triazine and amino acids.
The working hypothesis of this objective is that the self and pair-wise assembling properties of triazines would enable the oligomerization of activated monomers even at low overall concentration. The goal of this objective is the quantitative evaluation of triazine assembly-directed oligomer synthesis. In particular, oligomerization of a binary mixture of hydrophobic and polar amino acids will be studied to examine the influence and emergence of amphipathic character during the synthesis of peptide oligomers in water.

3. DNA and RNA templated synthesis of peptide and triazine macromolecules.
The working hypothesis of this objective is that the triazine assembly-templated chemistry of prebiotically-activated monomers will be compatible with extant biomacromolecules such as DNA and RNA. The goal of this objective is to find conditions for DNA and RNA templated oligomerization of prebiotically activated monomers. The successful completion of this goal would support the notion that triazines could serve as a chemical intermediary between the prebiotic and RNA worlds.

SIGNIFICANCE
This proposal directly addresses Program Element subheading Prebiotic Evolution: determining what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere, including alternatives to the current DNA-RNA-protein basis for life. The objectives are framed by a hypothesis that triazines were functional precursors to the native bases; extension of our hypothesis suggests that triazines could likewise direct coupling of biologically important monomers. This proposal will focus on the use of triazine assembly-motifs to drive the formation of amino acid and sugar-derived polymers under prebiotically relevant, enzyme-free conditions. There are no alternative Program Elements better suited to this proposal.

Christine Foreman/Montana State University, Bozeman
SLICE: Spectral Signatures of Life in Ice

One of the most exciting challenges of our times is the identification of life on other planets. With this goal in mind the search for water on Mars, as well as its habitability for living organisms, is a focal point of Astrobiological research. The 2015 Report on Mars Science Goals from the MEPAG Goals Committee set a priority upon determining if environments with high potential for current habitability and expression of biosignatures contain evidence of extant life.

Cryoconite holes on Earth are mini-entrained ecosystems, found in the ablation zone of glaciers worldwide that provide conditions by which subsurface liquid water can exist in spite of otherwise hostile environmental conditions. Providing a refuge for microbial life, these holes are made up of sediments, biota, and organic material. The McMurdo Dry Valleys have long been recognized among the best terrestrial analogs for conditions on Mars, with cryoconite holes being the proposed habitat for life on other planets. The present study proposes the existence of cryoconite hole glacial like features on Mars that could harbor ancient, buried life or microenvironments. Our hypothesis is supported by recent findings indicating the presence of liquid water patches on deposited mineral grains (Kereszturi and Rivera-Valentin, 2012) that reach temperatures up to -38°C (Kieffer et al., 2006) on Mars southern polar region, potentially providing conditions similar to those present in cryoconite holes on Earth.

One of the most promising methods of searching for life on other planets/moons has come from the interpretation of hyperspectral images. Although hyperspectral images received from rovers and satellites are promising, albeit confounding, the validation of reliable biomarkers remains ongoing. The hyperspectral and associated measurements we propose to collect will be used to support the analysis of data collected by the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM), the OMEGA spectrometer on the Mars Express ESA mission and the THEMIS instrument on the MARS Odyssey mission through, for example, spectral unmixing and endmembers analysis. By studying the terrestrial analogs of cryoconite holes in Antarctica, we can isolate and culture cryoconite organisms, determine their spectral signatures through in-situ and laboratory hyperspectral measurements and develop a spectral library of biosignatures. In this context, cryoconite holes represent a unique environment on Earth that resembles life on Mars. Consequently, our project directly addresses several of the main program elements of the new Astrobiology Strategy (2015) namely, early life and increasing complexity, co-evolution of life and the physical environment and identifying, exploring, and characterizing environments for habitability and biosignatures.

With this research we propose three broad goals: 1) characterize the in situ physical, biological and geochemical characteristics of cryoconite holes from two glaciers in the Taylor Valley, Antarctica, and relate them to in situ, unmanned aerial vehicle (UAV) and helicopter-based hyperspectral measurements; 2) complement the library of in situ spectral/physical properties data from field measurements with spectral measurements of simulated cryoconite hole parameters under controlled laboratory settings; and 3) apply

these spectral libraries to hyperspectral data collected over Mars for mapping the potential existence of spectral signs of life in Martian ice. Using hyperspectral imaging to investigate the accurate detection of biosignatures in cryoconite holes, we will provide the exobiology community with a crucial tool for interpreting hyperspectral data collected over Mars or other extraterrestrial bodies, build spectral libraries of selected materials for which future missions can search, and help facilitate site selection for future missions.

Steven Harris/University Of Nebraska, Lincoln
The Life History and Systems Biology of Fungal-Algal Mutualisms

Steven D. Harris, University of Nebraska
The life history and systems biology of fungal-algal mutualisms
Extremophiles are widely recognized as organisms that can live in environments that are inhospitable to most forms of life. These environments often exhibit extremes of temperature as well as water availability, and may also feature unusually high levels of radiation, metals, acids, and other toxic compounds. Although the best studied examples of extremophiles are prokaryotic, an increasingly diverse array of eukaryotic fungi have been found that thrive in extreme cold, dry, salty, or acidic habitats. Extremophilic fungi possess a number of adaptations that enable the colonization of harsh environments, including the accumulation of protective pigments, simplified cellular morphologies, and very slow growth. Nevertheless, there is much that remains to be understood about the life history and systems biology of these fungi. We propose to address this problem by testing the hypothesis that extremophilic fungi engage in mutualistic associations with algae (or cyanobacteria) to support their colonization of extreme habitats. In particular, these transient symbioses provide an accessible source of nutrition and may also contribute to stress tolerance. We will test this hypothesis by pursuing two specific objectives. First,

we will use image analysis and microscopy to determine the relative timing of events underlying the mutualistic association of the extremophile fungus Exophiala dermatitidis with the alga Chlorella sorokiniana. Transcriptional profiling and biochemical analyses will be used to investigate the responses of each partner as they interact. Second, we will employ culture-based and meta-genomic approaches to assess the diversity of fungi and algae that colonize rock surfaces in a boreal biome (pre-Cambrian shield). Isolated fungi and algae will be systematically tested for the capacity to engage in mutualistic associations.

Our proposed research falls within the scope of the NASA Exobiology program emphasis area Early Evolution of Life and the Biosphere . For example, our results will provide insight into the physiology of extremophilic microorganisms that are adapted to environments that share features in common with early Earth (and perhaps other planets). Notably, we expect to understand how the cellular systems of interacting fungi and algae evolve in response to these environments. In addition, our results will also shed light on

how the composition of microbial communities and their constituent interactions impacts the geochemistry of rock surfaces.

For our first objective, we have a developed a protocol for the successful co-cultivation of E. dermatitidis with C. sorokiniana. RNA-Seq will be used to generate transcriptional profiles at multiple stages of co-cultivation. These profiles will be correlated with microscopic analysis of fungal morphology as well as biochemical analysis of fungal lipids. In total, we expect these experiments to provide important insight into the strategies employed by extremophile fungi to acquire nutrients in environments that are representative of early Earth. For our second objective, we will sample exposed rock outcrops at three specific locations in the pre-Cambrian shield of Canada. A routine culture-based selection will be used to recover extremophilic fungi and algae (or cyanobacteria) from rock surfaces as well as from pulverized fragments. DNA sequence analysis will be used to establish the identity of each isolate. In parallel, DNA extracted from pulverized rocks will be sequenced to provide a meta-genomic overview of fungal and algal communities. Up to 50 distinct fungal and algal (or cyanobacterial) isolates will be co-cultivated using a microtiter plate format to assess their capacity to engage in mutualistic interactions based on accumulation of biomass and physical association.

Roland Hatzenpichler/Montana State University, Bozeman
Ecophysiology, Cell Differentiation, And Genomics Of Multicellular Magnetotactic Bacteria

Understanding the origin, evolution, and organization of multicellularity has been identified as a key objective by the astrobiology community and NASA. Past studies on the transition from single cells to multicellular entities mostly focused on volvocine algae and early radiating animal taxa as experimental systems. While multicellular forms of bacteria exist, their organization appears comparatively simple and the phenomenon seems to occur only as an adaptation to changing environmental conditions or as a single step in a complex life cycle.

A group of delta-Proteobacteria termed multicellular magnetotactic bacteria (MMB) constitutes the only known exception to this view. MMB are mono-species consortia that are comprised of 10-60 cells arranged in symmetry around a central a-cellular compartment. The life cycle of MMB has no known unicellular stage. Division occurs by separation of a MMB into two apparently identical daughter consortia, while disaggregated cells rapidly lose viability. These characteristics render MMB the only identified bacteria with an obligate multicellular lifestyle and make them a prime subject for the study of the early evolution of advanced life, most importantly the origins of and environmental factors driving multicellularity as well as the cellular organization of complex life. While several genera exhibiting this life-strategy exist in the environment, we are largely ignorant of the biology of these uncultured bacteria.

The most important goals of this project are

(i) to understand the ecophysiology of MMB, determine substrates that sustain their metabolism, and identify the geochemical parameters that drive their ecology;

(ii) to determine whether MMB consortia exhibit cellular cooperation or metabolic division of labor, two key characteristics of multicellular life; and

(iii) to develop hypotheses on which genetic traits and evolutionary events might have been involved in the development of multicellularity in MMB.

This project addresses NASA goals 4 and 5, in particular objectives 4.2. Foundations of complex life, 5.1. Environment-dependent, molecular evolution in microorganisms, and 5.2. Co-evolution of microbial communities.

We propose to study the biology of MMB via a combination of cutting edge molecular biological approaches that do not depend on the availability of a pure culture. Relying on the magnetotactic properties of MMB, we will use metagenomics targeted at magnetically enriched consortia to obtain draft genomes from three different locations with the states of California and Massachusetts. By comparing the genomes of unicellular relatives as well as other microbes capable of multicellular growth to those of MMB we will identify potential factors underlying their multicellular lifestyle. These include, for example, candidate molecules involved in inter-cellular communication and cell adhesion that might be involved in shaping consortia morphology, as well as proteins potentially involved in transcriptional control and regulation, processes that have been shown to be of importance in other multicellular organisms.

The genomes will be used to formulate hypotheses of potential energy-conserving metabolisms. These will be tested in situ using a combination of bioorthogonal non-canonical amino acid tagging (BONCAT) in combination with fluorescence microscopy as well as stable isotope labeling in combination with nano-scale secondary ion mass spectrometry. These experiments will reveal whether all cells within a consortium are equally metabolically active or individual cells are differentiated and exhibit metabolic division of labor. BONCAT will further be used to test how specific geochemical parameters (e.g. temperature, nitrogen source, trace metals availability) influence MMB activity and growth. The results of these experiments will guide us in the development of novel enrichment media to ultimately bring MMB into culture.

Eric Hayden/Boise State University
RNA "Sea-Scapes": Fitness landscapes with a changing environment

GOALS
The RNA world hypothesis predicts that functional RNA molecules played a central role in the origins and early evolution of life. To critically evaluate this scenario, we need to understand the capacity of populations of RNA molecules to evolve specific adaptations. Currently, the most thorough approach is to evaluate vast libraries of sequences using

next generation sequencing. By assigning a relative activity (fitness) to each sequence, it is possible to construct empirical fitness landscapes. These landscapes can be imagined in three dimensions, with the x-y-plane representing nucleotide changes (genotype space), and the z-axis representing RNA fitness. However, for a given RNA sequence, this fitness value will change if the conditions of the experiment change (environmental change). Importantly, environmental changes may promote adaptation, for example, if low fitness valleys become crossable when the landscape changes. In the absence of modern cellular life, changes in the physicochemical environment would have been constant and un-buffered. Despite the importance of environmental fluctuations for RNA fitness landscapes, the relationship between fitness landscapes and changes in the environment remains understudied. Here, we propose to evaluate changes in RNA fitness landscapes across chemical gradients. We will utilize next generation sequencing as a high-throughput biochemical assay. We will spread some of the throughput over multiple points on an environmental gradient. We will use non-linear curve fitting to parameterize the environmental response of RNA sequence libraries, and use computational approaches evaluate how these environmental responses can limit, or promote, RNA adaptations, under different evolutionary scenarios, such as fluctuating environments. This research will advance our ability to critically evaluate the capacity of RNA centered life to adapt to new and changing environments.

METHODOLOGY

As a model system, we will use group I ribozymes. These ribozyme can catalyze a reverse-splicing reaction that results in a substrate sequence becoming attached to its 3 end. We will use this attached sequence to select molecules that have reacted. By counting the population frequency of a specific sequence before and after selection, we will calculate ribozyme fitness. For our model environmental gradient, we will use magnesium ion concentration. Group I ribozymes show a sigmoidal response to increasing Mg2+ concentration that can be fit by the Hill equation. Different variants show magnesium dependent responses that differ in both the inflection point and the hill coefficient, but previous work has been limited to a few sequences. We will construct a library of sequence neighbors, and determine the relative activity of each sequence under 10 different magnesium concentrations that cover the expected response curves. For each sequence variant, we will fit the Mg2+ response curve to the hill equation and extract the fit parameters. We will then map these parameters back to sequence space and determine the change in magnesium dependence caused by changes in the nucleotide sequence. We develop evolutionary simulations to predict how populations of RNA molecules would adapt to constant or fluctuating magnesium levels.

RELEVANCE The proposed research will help understand the origin, evolution, distribution, and future of life in the Universe , which is the goal of the NASA Exobiology program (Appendix C.5). RNA molecules are believed to play a central role in the origins and early evolution of life. As such, the proposed experiments will help elucidate the ability of RNA centered life to adapt to new environments or environmental fluctuations. The ribozymes studied are used as a model of molecules that may have contributed to the origins and early evolution of life. In turn, the research will also help

understand how the environment can constrain and promote the ability of RNA centered life to adapt.

Brian Hedlund/University Of Nevada, Las Vegas
Functional Analysis Of Abundant Candidate Microbial Phyla In Geothermal Springs

Recent advancements in metagenomics and single-cell genomics have enabled unprecedented access to genomes from candidate (yet-uncultivated) phyla of bacteria and archaea, including those inhabiting extreme and early Earth-like systems. These data have enabled insights into the biology of these organisms, including their catabolic and anabolic potential, molecular adaptations to life in extreme environments, and insights into the early diversification of microbial lineages. However, the predicted metabolic functions of these organisms and their genes remain largely untested. The central goal of this proposal is to leverage a foundation of genomic exploration in Great Boiling Spring (GBS), NV, to assess metabolic functions of thermophiles representing candidate phyla of Bacteria and Archaea and their roles in major biogeochemical cycles. We have recently described the first substantial genomic data for the candidate bacterial phyla Atribacteria (OP9), Calescamantes (EM19), and Fervidibacteria (OctSpA1-106), and Kryptonia. To address the metabolic functions of these and other yet-uncultivated major lineages, we will use a combination of targeted and broad-based approaches. Objective 1: Assess C and N assimilatory metabolisms in specific targeted taxa in microcosms and enrichment cultures using (a) stable-isotope probing (SIP), fluorescence in situ hybridization and NanoSIMS imaging (FISH-SIMS) and (b) in situ transcription of genes predicted to be involved in H, C, N, and S cycling, by quantitative reverse-transcriptase PCR. Objective 2: Determine metabolic function of abundant community members using broad-based approaches such as (a) 16S rRNA SIP with a custom phylogenetic microarray (Chip-SIP) to assess C and N assimilation in microcosms and enrichment cultures, (b) whole-community transcriptomics, and (c) whole-community proteomics. Ultimately, the combination of both targeted and untargeted approaches will provide robust evidence of the ecological roles of several yet-uncultivated microbial phyla in GBS and beyond.

This work addresses the research emphasis entitled Early Evolution of Life and the Biosphere within the Exobiology program. Specifically, this work is necessary to understand the&physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments and to study the coevolution of microbial communities, and the interactions within such communities, that drive major biogeochemical cycles . This work also addresses several goals outlined in the Astrobiology Roadmap, particularly Goals 4 and 5. By studying the functions of candidate phyla that may play critical roles in biogeochemical cycles at or near their high-temperature limits, this work will better position the research community to understand the evolutionary mechanisms and environmental limits of life and determine the molecular, genetic, and biochemical mechanisms that control and limit evolution,

metabolic diversity, and acclimatization of life . Finally, since genomic explorations of yet-uncultivated archaea are providing insight into the early diversification of the tree of life and impacting models of the evolution of the first eukaryotic cells, understanding the functions novel archaea is relevant to NASA's goal to understand the evolution of advanced life .

Reggie Hudson/NASA Goddard Space Flight Center
The Survival of Potential Biomarkers in the Subsurfaces of Icy Planetary Bodies Exposed to Ionizing Radiation

Organic molecules on the surface of a planetary body are exposed to and modified by incident ultraviolet rays (UV) and high-energy ionizing radiation. These same molecules are much more likely to survive in a planetary subsurface at depths beyond the reach of UV photons and accessible only by galactic cosmic rays such as MeV protons. Therefore, a study of the radiation chemistry of organic molecules in the solid state is needed in order to plan for and to understand the results from future NASA missions to sample the subsurface regions of bodies such as Europa, Mars, and comets. We propose to examine the stability of selected organic molecules, those that could be considered potential biomarkers, under conditions relevant to these cold subsurfaces by performing proton irradiations of representative organics diluted in ices made of H2O and CO2 and by in situ monitoring of the survival rate of each organic using infrared spectroscopy.

This work is motivated by both past, current, and future NASA missions. Specifically, the non-detection of organic molecules by the Viking and subsequent missions to Mars suggests that the survival rates of organics are raised if they are protected from UV light, such as in the Martian subsurface. On Europa, the UV flux is much smaller than at Mars, but the high flux by keV magnetospheric particles from Jupiter will rapidly destroy traces of surface organics. Again, these same molecules will survive much longer beneath surface ices. Similar arguments apply to the Saturnian satellites, such as Enceladus, and to comets.

Our use of in-situ solid-phase analytical techniques is almost unique in the radiation-astrochemical literature. Its chief advantage is that it removes the uncertainties in determining whether reactants, such as amino acids or other potential biomarkers, are destroyed during a low-temperature (e.g., 100 K) radiolysis of an ice or during the subsequent warming of the sample to room temperature (e.g., 300 K) for chemical analysis. Moreover, our proof-of-concept work for these laboratory methods is complete, and we already have published several papers on the survival of amino acids (Gerakines et al., 2012; Gerakines and Hudson, 2013; Gerakines and Hudson, 2015). We now have the capability to expand our study to the survival of more-complex amino acids, as well as nucleobases, in irradiated ices.

This work is relevant to the Exobiology Program in that it seeks to understand the survival of biosignatures in planetary environments of interest to current and future NASA missions.

Gerakines, P. A., Hudson, R. L., Moore, M. H., Bell, J.-L., 2012. In-situ measurements of the radiation stability of amino acids at 15-140 K. Icarus 220, 647-659.
Gerakines, P. A., Hudson, R. L., 2013. Glycine's radiolytic destruction in ices: first in-situ laboratory measurements for Mars, Astrobiology 13, 647-655.
Gerakines, P. A., Hudson, R. L., 2015. "The radiation stability of glycine in solid CO2 In situ laboratory measurements with app

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Matthew Hurtgen/Northwestern University, Evanston
The Strontium Isotope Composition of Neoproterozoic Carbonates: Implications for the Carbon Cycle and the Evolution of the Biosphere

Objective:
The Neoproterozoic Era (1000-542 Ma) was a time period of extreme chemical, biological and climatic change ultimately culminating in environmental conditions capable of sustaining animals. These changes include massive perturbations to the geochemical cycles of carbon and sulfur (Halverson et al., 2005, Hurtgen et al., 2006, Fike et al., 2006), multiple global-scale glaciations in the Cryogenian period (~720-635 Ma; Hoffman et al., 1998, Macdonald et al., 2010), and an oxygenation event towards the close of the era (~580 Ma) that may be linked to the evolution and diversification of Metazoa (Fike et al., 2006, Canfield et al., 2007, McFadden et al., 2008, Scott et al. 2008, Sahoo et al., 2012). The carbon isotope record of marine carbonates tracks Neoproterozoic glacial events in the form of large negative carbon isotope anomalies that punctuate sustained intervals of high values. The mechanisms responsible for carbon isotope variation through this time interval remain controversial. The research objective of this proposal is to reconstruct the stable and radiogenic strontium and calcium isotope composition of Neoproterozoic carbonates in order to better understand changes in global carbonate burial rates and their relationship to massive environmental change.

Approach/Methodology:
The radiogenic strontium isotope ratio (87Sr/86Sr) of seawater represents a balance between radiogenic strontium inputs from continental weathering and non-radiogenic strontium derived from hydrothermal alteration of oceanic crust. Therefore, the 87Sr/86Sr evolution of seawater has become an important tool in reconstructing long-term changes in the balance between weathering and hydrothermal inputs to the ocean. While the radiogenic strontium isotope system offers a valuable tool for constraining Sr input fluxes to the ocean, it is not useful in assessing strontium output fluxes, which occurs primarily via marine carbonate burial. However, recent work has shown that modern marine carbonates (both biotic and abiotic forms) preferentially incorporate lighter Sr isotopes relative to seawater. Therefore, unlike the 87Sr/86Sr ratio of seawater, the stable strontium isotope composition of seawater is sensitive to fluctuations in global

carbonate burial rates. Therefore, we propose to measure the stable Sr isotope composition of marine carbonates deposited prior to and in the aftermath of Neoproterozoic Snowball Earth events in order to better understand changes in global carbonate burial rates across critical intervals of carbon isotope change.

Analysis of radiogenic and stable strontium isotope ratios will follow the procedures outlined in Andrews et al. (2016). Briefly, the stable strontium isotope compositions will be measured with an 87Sr 84Sr double-spike method, which was optimized according to the Lehn et al. (2013) Monte Carlo error model. The method accounts for abundance variations of 87Sr. An 87Sr-84Sr double-spike having an 87Sr/84Sr ratio of 0.33 mol/mol is mixed with 450 ng of sample (or standard) Sr to achieve a spike/sample ratio of 0.36 mol/mol. Care will be taken to ensure complete spike-sample equilibration. Presently, NBS 987 yields 88/86Sr = 0.000 ± 0.0040 (2ÃSEM, n = 77), and IAPSO seawater yields 88/86Sr = 0.396 ± 0.0050 (2ÃSEM, n = 54). The standard data correspond to a global, external reproducibility of 0.0200 (2ÃSD), which is the uncertainty assigned to samples.

Relevance to Program Element (Exobiology):
This proposal is directly relevant to the research emphasis of large scale environmental change and Macro-evolution. More specifically, this work seeks to better understand the mechanisms responsible for driving Neoproterozoic carbon cycle perturbations and their relationship to Snowball Earth events, Earth surface oxygenation and the evolution of the biosphere.

Luc Jaeger/University Of California, Santa Barbara
Resurrecting RNA polymerase ribozymes from the ribosome

In nature, RNA and proteins are the materials of choice for evolving complex nanomachines. Although modern living organisms rely heavily on proteins for catalyzing chemical reactions, all encoded proteins are synthesized by the RNA component of the ribosome, one of the most ancient cellular machineries. This strongly corroborates the RNA world hypothesis that proposes that early life was essentially based on RNA as the key informational biopolymer able to replicate the genetic information and enabling the evolution of proto-cells towards the last universal common ancestor (LUCA) of all living organisms. As such, the RNA world likely predated the current biological world based on DNA, RNA and proteins. Nevertheless, molecular traces of the ancestral RNA polymerase ribozyme have remained elusive within living organisms. While tremendous progress has been achieved towards the demonstration of RNA-based polymerization, in vitro selected and evolved RNA polymerase ribozymes are still far from the remarkable efficiency of modern proteinaceous RNA polymerases. Using a combination of RNA rational design, selection and evolution techniques and biochemical characterization approaches, this project aims at isolating complex RNA-based nanomachines with activity and efficiency matching those of modern proteinaceous RNA polymerases. Posited on the hypothesis that the evolution of protein translation was tightly coupled to

the evolution of RNA replication, molecular paleontology of the ribosome has revealed the existence of a remarkable architectural domain that will be used as a structural scaffold for evolving RNA polymerase ribozymes. The early conditions of life on Earth were very different from the present ones and the RNA world and pre-LUCA organisms likely evolved in an anoxic environment with iron and manganese readily soluble in water. Therefore, the retro-synthesis of RNA polymerase ribozymes will be achieved by in vitro evolution experiments performed in presence of iron, manganese and magnesium in anoxic conditions, presupposed to be those prevailing before the emergence of LUCA.

The recovery of efficient RNA-based nanomachines similar to the ancestral RNA replicase represents one of the most formidable challenges for unambiguously validating the RNA world hypothesis. Moreover, the creation of RNA-based nanomachines represents a formidable challenge for which new strategies are needed. Comparison of the outcome of in vitro evolution experiments performed in anoxic versus oxic conditions will be particularly useful for understanding better how the RNA world might have strive before being superseded by an RNA/DNA/protein world. Besides demonstrating the existence of the missing link between the ancestral RNA world and the last universal common ancestor, this project will also provide design principles of biological nanomachines and generate remarkable orthologous molecular tools for enabling the future development of novel technologies. This study will contribute to a better understanding of the origin and evolution of functional biomolecules towards greater complexity. As such, it will have transformative impact in the field of astrobiology as well as in the nascent field of RNA synthetic biology.

Christine Keating/Pennsylvania State University
Pre-cellular compartmentalization for RNA synthesis, structure, and function

A number of theories have been advanced for the origin of life, many of which posit that RNA or RNA-like molecules were both information-carrying and catalytic entities. Ultimately, compartmentalization of these functional molecules was required to form the earliest replicating cells. This proposal seeks to understand general physical and chemical principles that led to the first cells and investigates physical means that may have facilitated the emergence of life through the synthesis of RNA polymers and their localization and catalysis. The proposed work is within the scope of the Exobiology program element (C.5 of ROSES 2016) and specifically the Prebiotic Evolution research emphasis. It addresses Topic 2, Synthesis and Function of Macromolecules in the Origin of Life from the 2015 NASA Astrobiology Strategy. It speaks to key questions regarding early polymers and big picture questions about coupling monomeric building blocks and catalyzing reactions important to early chemical evolution, including self-replication. It also addresses several Key Research Questions, including: What is the Role of Environment? How did Physicochemical Effects Develop Over Time? What are the Advanced Steps of Macromolecular Function?

Our team brings fresh insight by combining expertise in compartmentalization (Keating) and ribozyme chemistry (Bevilacqua). In our current funding, we have generated a series of diverse non-membranous compartments (NMCs) by liquid-liquid phase coexistence in aqueous polymer solutions and used these to compartmentalize nucleotides and RNA, compact RNA structure, and enhance RNA polymerization and catalysis. We discovered that certain NMCs have remarkable properties that should favor the synthesis and function of RNA; these observations underpin the work proposed here.

RESEARCH OBJECTIVES: (1) Understand and control how NMC composition dictates RNA accumulation, dynamics, and structure. (2) NMC-facilitated RNA polymerization and function. (3) NMC-facilitated emergence and evolution of new functional RNAs.

APPROACH: Our NMCs are liquid droplets formed by phase separation in aqueous solutions of one or more macromolecular components. They offer strong solute accumulation and reaction environments distinct from the dilute aqueous surroundings and are feasible on early earth because they occur at low concentrations for many different polymer chemistries. NMC occurrence, composition, and physical properties will be evaluated as a function of their makeup and solution environment (salt, pH, temperature). RNA mobility within the compartments and RNA exchange with the external solution will be determined by diffusion measurements. Partitioning will be assayed by scintillation counting using radiolabeled RNAs, and for individual droplet-level studies by fluorescence using dye-labeled RNAs. RNA synthesis, function, and structure will be tested in the NMCs as well. Synthesis will be assayed by gel electrophoresis of labeled RNAs. Ribozyme function will be tested by standard kinetic assays with radioisotope or FRET labels. Structure of functional RNAs will be assessed at the atomic level by in-line probing or SHAPE; at the base pairing level by fluorescence resonance energy transfer (FRET), which can be done in bulk or at the individual droplet level; and at the global level by hydroxyl radical footprinting, SAXS, and kinetic assays.
SIGNIFICANCE: The work proposed here will establish principles that may have led to the first cells and will elucidate how these protocell compartments would have facilitated the synthesis, structure, and function of RNA.

Sarah Keller/University Of Washington, Seattle
Interactions between fatty acids and building blocks of RNA and peptides; How self-assembly could drive formation of biological polymers and protocells

VISION:
An outstanding question in the origin of life is how protocells composed of a membrane, RNA, and protein arose on the early Earth. Our proposed experiments test key elements of a compelling scenario for the emergence of protocells in which:

- The building blocks of RNA and protein were selected and concentrated via binding to fatty acid aggregates, which self-assemble in water.

- Those bound building blocks stabilized fatty acid membranes against salt-induced flocculation, and the membrane surfaces in turn oriented the building blocks to facilitate their polymerization.

- Membranes that were more stable bound more building blocks, leading to an auto-amplifying system.

- Polymers of the building blocks stabilized membranes more effectively than individual components did, explaining their accumulation prior to the evolution of their complex functions in metabolism and information transfer.

Our team has a history of productive collaboration and has established feasibility of the proposed project. Specifically, we previously showed that the building blocks of RNA, nucleobases and sugars, do indeed bind to membranes and increase membrane stability (PNAS 2013).

CENTRAL OBJECTIVES:
Each of our objectives relates to a theme of broad interest in prebiotic evolution.
(1) Test whether the association of prebiotic fatty acids with nucleobases and ribose facilitates the critical prebiotic reactions of ribonucleotide synthesis and polymerization. (How did RNA arise?)
(2) Test whether amino acids and peptides interact with fatty acid membranes and, if so, whether this interaction facilitates peptide bond formation. (How did proteins arise?)
(3) Test whether bases, sugars, amino acids, nucleotides, and combinations thereof lower the critical vesicle concentration (CVC) of fatty acids, or increase vesicle growth rates. (How did stable membranes arise?)
(4) Test whether dinucleotides and/or dipeptides lower the CVC of a fatty acid or increase the growth rate of vesicles more than unlinked nucleotides or amino acids do. (How were oligonucleotides and peptides selected before they grew long enough to be functional catalysts and carriers of information?)

METHODS:
We will accomplish our objectives using only prebiotically plausible molecules (e.g. decanoic acid) to ensure that our results are directly applicable to the origins of life. We will employ standard in vitro assays listed below as well as novel assays developed by our group.
- For Objective 1: We will test for nucleoside and oligonucleotide formation in the presence of (a) potential prebiotic catalysts (e.g. phosphate, amino acids, and dipeptides), (b) UV radiation, and (c) cyclic dehydration at various temperatures. We will test for successful synthesis using TLC, HPLC, mass spectrometry, and agarose gel electrophoresis.
- For Objective 2: We will use the filtration and flocculation assays we previously developed (PNAS 2013) to evaluate binding of amino acids and peptides to fatty acid vesicles. We will incubate amino acids with vesicles under the conditions as described for Objective 1, and then screen for peptides by TLC and mass spectrometry.

- For Objectives 3 and 4: We will measure CVC using turbidity and pinacyanol chloride absorbance assays, and we will measure growth rates using dynamic light scattering.

SIGNIFICANCE:
This project addresses a central theme of the 2016 NASA Exobiology Program in section C.5 on Prebiotic Evolution. We will investigate the self-assembly of membranes and the building blocks of RNA and protein as a molecular [process] that set the physical and chemical conditions within which living systems may have arisen . Establishing conditions under which the assembled components form polymers will reveal environmental requirements for the emergence of protocells. The project will directly demonstrate a chemical system that could have served as [a] precursor of metabolic and replicating systems on Earth.

Fabien Kenig/University Of Illinois, Chicago
fs-LDPI MS mapping of organic compounds in deep time Earth sediments: A tool for determination of the spatial distribution of lipid biosignatures at the micron scale.

Mass spectrometry (MS) imaging will be used to probe Archaean and Proterozoic sedimentary rocks, as well as Proterozoic microbial mats using femtosecond-laser desorption postionization-MS (fs-LDPI-MS) developed by co-PI Luke Hanley. The fs-LDPI-MS is a high lateral resolution MS imaging method allowing depth profiling and mapping of single organic compounds. MS images of the distribution of a given mass/charge ratio (1 -1200 Da) are created by assembling mass spectra collected from individual focused laser spots. The resolution of each laser spot currently reaches 2 microns, allowing the reconstruction of maps of organic compounds with unprecedented resolution.

A major issue of Archean (4 to 2.5 billion years, By) and Proterozoic (2.5 to 0.54 By) research is to determine if lipids extracted from these samples were emplaced within the sediments during deposition (syngenetic) or if these compounds are post-depositional additions. Ancient sediments may also be contaminated during sampling and processing. The spatial distribution of the organic compounds in a sample obtained by fs-LDPI-MS imaging would be diagnostic for the compounds origin. For example, organic compounds found exclusively in a quartz vein but not in the sample groundmass would not be syngenetic but would have been included in the sample during emplacement of that vein (post depositional hydrothermal activity). The spatial information provided by fs-LDPI-MS, in association with contextual mineralogy can be used to constrain the timing of emplacement of organic compounds within the rocks and more confidently determine their biosignature value. Additionally, when used on very well preserved Proterozoic samples, such as rare, immature microbial mats, fs-LDPI-MS would map organic compounds at the micron resolution, providing unique paleobiological information on ancient microbial assemblages.

Preliminary results obtained on Archaean metasediments from the Abitibi greenstone belt (ON, Canada) provided a positive feasibility test, showing that the distribution of organic compounds are limited to specific zones within the samples, tentatively suggesting their emplacement during an Archean hydrothermal phase, post-peak metamorphism. The samples to be analyzed range from the Neoproterozoic to the 2.7 By Archaean and encompass the full range of maturity, immature sediments to lower greenshist metamorphics. All samples to be analyzed have already been collected by the PI or by collaborator J. Brocks (ANU, Australia).

Relevance: The proposed research addresses the Research Emphasis Early Evolution of Life and the Biosphere of the NASA Exobiology Program Element by offering an unambiguous molecular record of lipid biosignatures and their distribution at the micron scale in Proterozoic and Archaean sedimentary rocks. The proposed research also addresses the Research Emphasis Biosignature and Life Elsewhere of the NASA Exobiology Program Element by developing a molecular mapping approach for the enhanced determination of the presence or absence of valid biosignatures in Archaean and Proterozoic rocks. Such an approach, if validated on Earth would turn into an invaluable high-resolution imaging tool upon Mars sample return. The proposed research would address some of the Key Research Questions defined in the Astrobiology Strategy 2015, subsection 5.4 II What contextual information is required to enhance the confidence in interpretation of biosignatures? by obtaining the spatial distribution of molecular components within its mineral context and allowing the distinction between syngenetic compounds, post depositional additions (hydrothermal) and compounds introduced during sampling and analysis. Additionally, the proposed research is a direct response to the need for enhanced technology to improve the detection of biosignatures as outlined on page 118 of the Astrobiology Strategy 2015, Detection Strategies for extinct life, Chemical Signatures.

Armin Kleinboehl/Jet Propulsion Laboratory
HCN Emissions From Explosive Volcanic Eruptions: Evidence For Abiotic Organic Synthesis In Subaerial Volcanic-Hydrothermal Systems

Hydrogen cyanide (HCN) is central to several reaction pathways that can lead to the abiotic formation of simple organic compounds containing nitrogen. For example, the simplest nitrogen-containing base, adenine, is a complex of five HCN molecules. The abiotic formation of HCN is of central interest to astrobiology because it has likely been an important step in the origin of life on the early Earth. Extensive characterization of chemical conditions in submarine hydrothermal environments like the ones at mid-ocean ridges show their HCN-forming potential. In contrast, ubiquitous subaerial hydrothermal environments have received almost no attention. HCN is likely to have been present in such environments on early Earth because it is formed by a variety of chemical processes driven by thermal energy. Further support for organic synthesis comes from nitriles occurring in gases emitted from subaerial >300°C volcanic-hydrothermal systems on modern Earth.

In June 1991 Mt Pinatubo explosively erupted magma and overlying rock, including a long-lived extensive volcanic-hydrothermal system. The ejecta reached stratospheric altitudes. Measurements by the ATMOS instrument on the Atlas-1 Space Shuttle mission and the MkIV balloon interferometer in 1992 detected excess HCN in the middle stratosphere. HCN has no stratospheric sources and a lifetime of several years. We hypothesize that this positive HCN anomaly was injected into the stratosphere by the explosive eruption of Mt Pinatubo. Preliminary analyses of the MkIV data indicate ~3 kilotons of excess HCN injected into the middle stratosphere. It might have formed partially or fully abiotically either in-situ during the eruption, or prior to the eruption in the volcanic-hydrothermal system of Mt Pinatubo, possibly after accumulation. Knowledge of this process would have a significant impact on our understanding of the formation, reservoirs, and persistence of HCN on the early Earth. The serendipitous observation of HCN in the eruption of Mt Pinatubo opens up a rare window that allows us to study this process and estimate its contribution to HCN formation on early Earth by using the HCN observed in the eruption as a proxy.

Our research fits well into the research area prebiotic evolution of the NASA Exobiology call because we try to discern molecular processes that set the physical and chemical conditions within which living systems may have arisen . Our research focuses on two major objectives:

1. Estimate the contribution of subaerial volcanic-hydrothermal systems to the abiotic formation of HCN on early Earth using HCN emitted by the eruption of Mt Pinatubo as a proxy;

2. Identify and quantify processes that lead to the formation of HCN through plume chemistry, evaluate to what extent HCN formation or accumulation occurred in the subaerial volcanic-hydrothermal system, identify possible sources of nitrogen, and draw conclusions on abiotic organic synthesis.

To address objective 1, we will use the ATMOS and MkIV data to precisely quantify the amount of excess HCN in the stratosphere observed in 1992. We will use the Chemistry-Transport model GEOS-Chem to model the stratospheric HCN injection, dispersion and loss, in order to reconstruct the amount present or formed at the time of the eruption. We use this information together with estimates of volcanic activity on early Earth to estimate the contribution of subaerial systems to the abiotic formation of HCN on early Earth. To address objective 2, we will identify processes that can lead to the formation of HCN. We will use a coupled thermochemical equilibrium and kinetics model to simulate the chemistry in the eruption plume. This will allow us to identify processes that lead to the formation of HCN either in-situ or through the accumulation of nitrogen precursors and/or HCN itself, in the subaerial volcanic-hydrothermal system of Mt Pinatubo.

Sarah Maurer/Connecticut State University, New Britain
Energy Transduction And Carbon-Fixation By Membrane-Bound Polycyclic Aromatic Hydrocarbons As An Early Photosynthetic Model

GOALS AND OBJECTIVES:
Many forms of energy transduction on early Earth have been suggested, perhaps most popularly geothermal and electrical synthesis of organic molecules. However, solar energy was likely more abundant and ubiquitous in surface environments, due to the lack of an ozone layer. Additionally, the availability of photoactive compounds, including polycyclic aromatic hydrocarbons (PAHs), is evidenced by their presence in carbonaceous meteorites.

We propose that an early form of photosynthesis using PAHs would have produced reduced carbon from carbon dioxide, by coupling photo-excitation to transmembrane electron transport and the generation of a proton gradient. If successful, this will represent a simple way to generate usable carbon within a cell, and will further our understanding of proto-biological chemical systems that could have preceded the first living organisms.

APPROACH AND METHODOLOGY
The first step is to generate a proto-cell composed of single chain amphiphiles, such as fatty acids, which are considered prebiotic, along with the photosensitizing PAHs. PAHs have been shown to spontaneously insert into fatty acid membranes and enhance stability. Carbon dioxide will be used to generate an oxygen free atmosphere, and act as a reactant. Because it is non-polar, CO2 can easily diffuse through the cell membrane. Lastly, an external electron donor will be added, such as dithiothreitol, less odorous mimic of hydrogen sulfide or other early Earth reductants.

The proto-cellular construct will be exposed to light, where the CO2 will be converted into organic carbon, likely formate, and be trapped inside of the negatively charged membrane. The interior of these cells can also be loaded with a pH-sensitive dye to monitor proton gradient formation. The cells will then be separated from the external products using size exclusion chromatography, and the encapsulated products will be evaluated using gas and/or liquid chromatography, and detected with either mass spectrometry, flame ionization, or as charged aerosols. Additionally, changes in both the amphiphiles and PAHs can be monitored with these methods.

This approach distinguishes itself from other photochemical studies by coupling the following properties: 1) modeling an early Earth environment; 2) using simple amphiphiles as opposed to phospholipids; 3) generating usable carbon for the protocellI. If successful, future work will include conversion of products into larger carbon molecules, like amino acids, or sugars.

SCOPE WITHIN EXOBIOLOGY
This research will enhance our understanding of prebiotic energy transduction including possible molecular processes and chemical conditions that would have promoted

abiogenesis. The model proposed is based on experimental and theoretical models of Earth prior to the formation of life. Additionally, this study will provide a model for early forms of photosynthesis and experimentally investigate its applications and limitations. The conclusions of this research will also contribute to the search for life elsewhere in the universe by demonstrating alternatives to Earth s current bioenergetics.

Jill Mikucki/University Of Tennessee, Knoxville
ICE-MAMBA Ice-covered Chemosynthetic Ecosystems: Mineral Availability and MicroBiological Accessibility

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Scott Nichols/University Of Denver
Evolution of a Multi-Functional Adhesion Module Necessary for Complex Multicellularity

The evolution of cell-cell adhesion and the assembly of epithelial tissues were intimately coupled to the evolution of complex multicellularity. Epithelia have essential roles, from acting as an environmental barrier and partitioning organisms into discrete compartments, to coordinating morphogenesis during development. We propose to explore the early evolution of epithelia - structures that we predict would be advantageous in complex life forms anywhere to understand innovations in the animal stem-lineage that contributed to the evolution of animal body plans. Defining features of epithelia are the adhesion modules that tether cells to each other, enable folding and bending of sheets and tubes of cells, define apical/basal polarity, and specify gene expression. Our focus is on the cadherin/catenin module (CCM), which forms the molecular foundation of adherens junctions in all studied bilaterian tissues, and is hypothesized to have evolved in concert with animal multicellularity. Formation of these junctions appears to be the earliest event in epithelial morphogenesis, and it serves as a spatial landmark for formation of other types of cell-cell junctions. Experimental disruption of the CCM perturbs epithelial development, integrity, and polarity. Our objective is to examine how an ancient actin-myosin scaffold, common to all eukaryotes, was recruited by the more recently evolved CCM to coordinate cell interactions necessary for multicellularity and tissue diversity. Importantly, this multi-functional module is present in all animal lineages and potentially satisfies all requirements for multicellular adhesion and morphogenesis - cadherins enable adhesion between cells, and catenins bind to the actin scaffold to organize multicellular architecture and can signal to the nucleus to change gene expression. We have a detailed mechanistic understanding of the component parts and function of this adhesion module in bilaterian animals, but the early evolution of this module is far less clear. Our approach is to examine the function of CCM proteins in key non-bilaterian animal lineages, including cnidarians, ctenophores and sponges. Little is known about how they function in these organisms, nor how they evolved to take on novel roles to

enable multicellular complexity. What is the minimal set of adhesion proteins necessary for the coordination of cell-cell adhesion and linkage to the actin cytoskeleton, and are basic functions of this set shared by all animals? The CCM is a likely candidate, and we will test this hypothesis by observing CCM proteins in cells and tissues of non-bilaterian metazoans during morphogenesis, by examining physical interactions between these putative epithelial proteins with comparative biochemistry, and using functional genetics to delete and replace genes putatively involved in tissue morphogenesis and cell-cell adhesion. Other potential cadherin-related proteins, signaling and actin-binding proteins will be identified using bioinformatic dissection of genomes. Results will be integrated with known properties of the cadherin-catenin adhesion module in bilaterians. Our experimental approach is unusually comprehensive and interdisciplinary for broad comparative studies, and involves a diverse team to tackle this problem that comprises evolutionary-developmental biologists (Lowe/Martindale), an evolutionary-cell biologist (Nichols) and a biochemist/structural biologist (Weis). The capacity to develop robust tissues that provide a physical and chemical barrier to the outside environment and facilitate tissue specialization is a basic characteristic of complex life. The work outlined in this proposal addresses fundamental questions about the assembly of a key cellular innovation early in animal evolution epithelia are at the core of hypotheses of the evolution of multicellularity and animal body-plan complexity, and therefore central to the NASA Exobiology mission of understanding advanced life.

Wayne Nicholson/University Of Florida, Gainsville
Evolution Of Microbial Adaptations To Pressure Extremes: From The Deep Ocean To The Upper Atmosphere

During Earth s history, life has faced a succession of environments, each exerting selective pressures to which it must adapt or perish. Earth life has been challenged by gradual and abrupt environmental changes (e.g., variable solar output, bolide impacts, tectonics, volcanism). In turn, life processes have brought about global environmental changes (e.g. nitrogen cycling, oxygenic photosynthesis, CO2 sequestrtion, carbonate deposition). Both abiotic and biotic chemistry is governed by the fundamental physical parameters of pressure (P) and temperature (T). Phylogenetics suggest that the first life may have evolved in anoxic, high-P environments such as occur at modern-day hydrothermal vents. By ~3.5 Gya microbes had migrated to the ocean/air and land/air interfaces, where recent research indicates that by ~2.7 Gya Earth s atmosphere may have actually been thinner than that of present-day Earth. Thus, microbial life has evolved to function at wide extremes of P. Indeed, modern microbes can be found in niches ranging over 6 logs in P, from <10^3 Pa in the upper troposphere to >10^8 Pa in the ocean depths.

How did microbes evolve the ability to function at such a wide range of P's? To study this question, we propose to use strains of the genus Carnobacterium which are psychrotolerant, facultatively anaerobic Gram-positive bacteria that grow at both low- and high-P extremes. PI Nicholson showed that two Siberian permafrost strains

(WN1359, WN1374) and 11 Carnobacterium type species were capable of growth at 0ÚC and 7x10^2 10^5 Pa. Co-I Bartlett has isolated Carnobacterium strain AT7 from 2,500 m depth in the Aleutian trench, which is capable of growth at 1.8ÚC and P from 10^5 6x10^7 Pa. With DOE-JGI (PI) and the Craig Venter Institute (Co-I), we obtained the genome sequences from all 14 Carnobacterium strains. The complete P range for growth is not known for these bacteria, nor is the effect of P on gene expression. Microbes can adapt to changing environments by altering the global pattern of DNA methylation (the methylome), which in turn can influence global gene transcription (the transcriptome). We have obtained 10^5 Pa reference methylome data from all strains sequenced at JGI. To test the influence of P on DNA methylation, we cultivated strain WN1359 at 7x10^2 vs. 10^5 Pa, and discovered dramatic differences in their methylomes. From these observations we have developed the Working Hypothesis that:

"Bacteria adapt to the P environment by altering their methylomes and transcriptomes."
Objectives:

1. Measure growth rates of all strains at 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 Pa (with Co-I Bartlett).

2. Isolate genomic DNA and total RNA from samples capable of growth. Map their methylomes and transcriptomes by Methyl-seq and RNA-seq, respectively (with DOE-JGI).

3. Correlate how methylomes and transcriptomes change in response to P using bioinformatics tools (with DOE-JGI).

4. Identify DNA methyltransferase genes in relevant genomes and inactivate them by site-directed mutagenesis.

5. Measure alterations in growth capabilities of mutants at differing P s (with Co-I Bartlett) and corresponding changes in methylomes and transcriptomes of mutants using Methyl-seq and RNA-seq (with DOE-JGI).

Relevance: This proposal is relevant to NASA Exobiology sections 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 v) investigate the evolution of genes, pathways, and microbial species subject to long-term environmental change relevant to the origin of life on Earth and the search for life elsewhere .

Data Management Plan: This proposal will generate large amounts of -omics data. In Section 4 we present an extensive integrated management plan to archive the data and to make it easily and freely available online to the scientific community and the public.

Mary Parenteau/NASA Ames Research Center
Planetary Major Equipment Request: A Gas Chromatograph Isotope Ratio Mass Spectrometer (GC-IRMS) To Support Isotopic Analyses Of Parent Award "Biosynthesis Of 3-Methylhopanoids By Purple Non-Sulfur Anoxygenic Phototrophs"

All cells are bound by a lipid membrane that separates the interior of the cell from the exterior environment, which is a universal characteristic of all known life on Earth. Some types of membrane lipids are found only in specific groups of organisms, and historically have been used as biomarkers for these groups. The carbon skeletons of branched or cyclic lipids are more resistant to degradation after cell death and can survive in the rock record for billions of years. Such geolipids, and their carbon isotopic composition, provide important fossil information and can help reconstruct the record of past microbial ecosystems on the early Earth, and potentially other planetary bodies.

Previous lipid biomarker work has largely been directed at establishing taxonomic proxies by identifying compounds in pure cultures and environmental samples. But recent molecular work identifying the genes involved in the biosynthesis of geologically relevant lipids such as hopanoids has opened the door to surveying genomic and metagenomic data sets for the ability to synthesize these compounds. This work has revealed a much broader distribution of the genes than was previously realized via the pure culture studies. This discovery has necessitated a shift from taxonomic studies to investigating the cellular function of these compounds. The goal is to link the biological function of the lipid biomarkers to specific environmental conditions or bio(geo)chemical processes. Such a relationship will offer new criteria for interpreting the rock record.

In keeping with the discovery of the wider distribution of certain lipid biomarkers, we have recently found that three purple non-sulfur (PNS) anoxygenic phototrophs isolated from marine, hypersaline, and hot spring environments synthesize 3-methylbacteriohopanepolyols (3-MeBHPs). These compounds were previously thought to be exclusively made by oxygen-requiring methanotrophic and acetic acid bacteria, and have long been considered a signal for methanotrophy in the rock record. However, our cultures are the first example of both the production of 3-MeBHPs by phototrophic bacteria and their production under anoxic conditions. We propose to investigate the cellular function of these compounds in PNS by utilizing a novel combination of molecular genetics, laboratory culture, and environmental studies. We will also perform detailed compound-specific C isotopic studies, as preliminary analyses indicate that the PNS 3-MeBHPs will be distinguishable from methanotrophic 3-MeBHPs in the rock record.

The C isotopic analyses are a main element of our parent award, and the panel evaluation indicated, The C isotopic studies and their application to environmental samples are a major merit of this study. Essential to fully realizing the potential of this approach is the purchase of a Thermo Delta V Plus isotope ratio mass spectrometer (IRMS) with peripherals to enable GC-IRMS and EA-IRMS capabilities. This instrument contains a magnetic sector mass spectrometer, which allows for compound-specific and bulk

isotopic analyses. The modern IRMS is extremely sensitive and would allow detection and identification of minor biomarker molecules, critical in the analysis of environmental samples where biomarker concentration can be very low.

Alexander Pavlov/NASA Goddard Space Flight Center
Exploring destruction of biomolecules in Martian rocks and regolith by Cosmic Rays.

Science Goals and Objectives
Based on the recent results from the MSL and Phoenix missions, the Martian shallow subsurface should be considered as the prime candidate to search for the extraterrestrial biosphere both extinct and extant. Therefore, it is critical to understand the preservation of various biomolecules in the Martian shallow subsurface. Recent experiments and modeling work suggest that long-term exposure to Cosmic Rays (CRs) can destroy organic molecules in the top 2 meters of the Martian surface effectively. MSL and Phoenix also found the ubiquitous presence of perchlorate salts in both surface rocks and regolith. CRs interaction with perchlorates will produce additional oxidants in regolith and rocks and likely further increase the rate of destruction of biomolecules on Mars.

We propose to determine the destruction rates of amino acids and peptides in Martian surface rocks and regolith due to long-term exposure to CRs through both laboratory simulations and numerical modeling. Specifically, we will determine the destruction rates of amino acids and peptides in mineral powders mixed with perchlorates and exposed to gamma, neutron and proton radiation. Experimentally derived radiolysis constants will be then used in conjunction with the Monte Carlo computer code to determine the destruction rates of amino acids and peptides at various depths and during various epochs on Mars.

Methodology
Task 1. Determine the rate of radiolysis of the free amino acids and peptides in typical mineral mixtures observed in Martian surface rocks and regolith. We will prepare mineral mixtures (dry silicates or hydrated silicates) mixed with perchlorates and spiked with trace amounts of amino acids and peptides. Samples will be then exposed to ionizing radiation (gamma, neutron and proton) to dosages up to 2 MGy. After irradiation samples will be analyzed using liquid chromatography mass spectrometry (LC-MS) and the surviving fractions of amino acids and peptides will be determined.

Task 2. Determine the rate of radiolysis of the amino acids in meteorites powders mixed with silicates and perchlorates. Amino acids can be delivered on the surface of Mars with IDPs and meteorites. To simulate destruction of those amino acids in the Martian regolith, we will prepare powder samples from the actual meteorites (e.g. Murchison) mixed with silicates and perchlorates. Irradiated samples will be analyzed as in Task 1.

Task 3. Determine radiation accumulation dosages at various rock/regolith depths and epochs on Mars. Our proposed numerical modeling will include extensive calculations of radiation accumulation rates from different types of CRs as a function of rock/regolith composition using the Monte Carlo computer code (GEANT4). We will also conduct similar calculations assuming various atmospheric columns on Mars to determine how radiation dosages changed through time on earlier Mars.

Task 4. Destruction of biomolecules in Martian rocks and regolith through time. Using radiolysis rates from Tasks 1, 2 we will calculate radiolysis constants for free amino acids and peptides as a function of mineral composition, perchlorates abundance and type of ionizing radiation. We will use these new radiolysis constants and radiation accumulation rates (Task 3) to determine the destruction rates of amino acids and peptides on Mars at various depths and epochs.

Relevance to Exobiology
Our proposal is focused on the survival of biomolecules in the shallow subsurface of Mars. Any search for extant of extinct life on Mars has to account for the degradation of the organic biosignatures due to CRs in surface rocks and regolith. Thus our proposal is directly relevant to the Exobiology s research area: Biosignatures and Life Elsewhere.
Perceived Impact

Our results will help data interpretation of the current MSL mission and the planning of the future landers on Mars as well such as ExoMars, Mars 2020 and a Mars Sample Return.

Andrew Schuerger/University Of Florida, Gainsville
Transcriptomics and Proteomics of Methanogens under Simulated Subsurface Conditions that Mimic Recurring Slope Lineae on Mars

The Curiosity rover has detected background atmospheric CH4 levels of 0.7 ppbv with spikes to 7 ppbv [Webster et al., 2015]. Of the many mechanisms to inject CH4 into the Martian atmosphere (e.g., serpentinization, UV destruction of accreted organics, etc.), methanogenesis is the only biological process proposed [Reid et al., 2006] that might be active in the shallow subsurface on Mars. Also, liquid brines composed of Mg-perchlorate, Mg-chlorate and Na-perchlorate have been detected in active Recurring Slope Lineae (RSL) on steep crater walls on Mars [Ojha et al., 2015]. The experiments outlined herein will establish the low-pressure, low-temperature, low-oxygen (pO2), and high perchlorate and chlorate salt limits for metabolism, growth, and CH4 production of psychrophilic methanogens under simulated Martian conditions. Furthermore, the experiments will characterize the transcriptomic and proteomic alterations in methanogens when grown under conditions that may persist in shallow subsurface RSL brines.

The research will be divided into two sections in which the Schuerger lab will focus on characterizing the low-pressure, low-temperature, low-pO2, and high-salt limits of growth for psychrophilic or psychrotolerant methanogens for which complete genomes exist (e.g., Methanogenium frigidium DSM 16458, Methanococcoides burtonii DSM 6242). The Onstott lab will focus on characterizing the variability of the transcriptomes and proteomes of the methanogens as functions of exposure to increasingly Mars-like conditions testing the low-pressure, low-temperature, low-pO2, and high perchlorate/chlorate salt limits for metabolism and growth.

A Mars Simulation Chamber (Schuerger lab) will be configured with two independent Microbial Assay Chambers (MAC; units on-hand) in which the bulk atmosphere of each MAC unit can be individually sampled with a quadrupole mass-spec residual gas analyzer (RGA; operational; minimum detection of CH4 is 0.5 ppm at 0.7 kPa (7 mbar)) or gas chromatograph (GC; minimum detection of CH4 is 3 ppm a 0.7 kPa). Experiments will focus on the metabolic responses of the methanogens between 5.0 kPa (the current low-pressure growth limit for methanogens [Kral et al., 2011]) and 0.7 kPa (Martian surface pressure).

We will examine the temporal expression of genes using RNA-seq and proteome analyses using LC-MS/MS as the methanogens are subjected to varying pressure, temperature, pO2, and perchlorate/chlorate salt conditions. The assembled mRNA contigs and protein peptides will be mapped to the host genomes and their coverage and abundance measured, respectively. We will use a fluorescent molecular beacon targeting the mcrA mRNA to determine what proportion of cells are actively producing CH4 thereby providing a specific cellular CH4 production rate under the diverse conditions listed above. The combination of these two tools will provide a complete picture of the response of the different methanogen strains to decreasing pressure and temperature, the presence of low pO2, and to elevated perchlorate/chlorate salt levels when compared to control incubations at 0°C and 101.3 kPa in standard liquid media.

The proposal is relevant to the C.5 Exobiology program based on: (1) the research will &seek to determine the biological and environmental factors leading to&the potential distribution of complex life in the Universe, and (2) the research will study the &establishment of life under conditions prevailing on other planetary bodies&; namely the growth of a plausible methanogenic microbial community in active RSL features on Mars.

Robert Stanley/Temple University
DNA Repair under Extreme Conditions - Extended Studies

The Sun emits near-UV and UV light engendering deleterious photochemical reactions. However, heredity is impossible without genetic continuity, leading to the evolution of bacterial DNA repair systems. We propose to extend our comparative analysis of extremophile DNA photolyases from the archaeon hyperthermophile S. solfataricus (SsPL) and the bacterial psychrophile C. psychrerythraea (CpPL). This is a successor request for NNX13AH33G. The research team of Robert Stanley (Temple), Yvonne Gindt (Montclair State) and David Beratan (Duke) will remain the same.

Key Objectives:
I. Compare and contrast mesophilic light-driven enzymatic DNA repair with extremophilic enzymes, extending our early work, to understand how biophysical processes are adapted to extreme environmental conditions.
II. Explore psychrophile substrate promiscuity as a source of greater biosynthetic complexity with a focus on flavin adenine dinucleotide and its analogs.
III. Test the hypothesis that modern PLs evolved from an Ur-PL , whose kernel contains only the FAD cofactor in a single domain protein.
A few highlights from the previous period include:

" The discovery that archaeal hyperthermophilic SsPL is a missing link in the evolution of Photolyases into the blue-light photosensory Cryptochrome (CRY) protein. Eukaryotic CRY performs several light-driven functions depending on the organism (photomotility in plants, etc.).

" The discovery of cofactor promiscuity in psychrophilic CpPL. PLs contain a second cofactor that acts as a light antenna to reduce the conserved catalytic Flavin Adenine Dinucleotide (FAD) molecule. CpPL can take up either a folate or a different flavin (FMN or flavin mononucleotide).

" The temperature dependence of substrate binding and of DNA repair have been measured in mesophilic and hyperthermophilic PLs from near freezing up to the denaturation point of the protein. While the free energy of substrate binding appears independent of temperature, SsPL shows significantly faster repair at all temperatures.

Relevance to NASA Exobiology Mission Goals: This proposal responds to specific mission goals of NASA Exobiology in Appendix C.5 of the 2016 ROSES Solicitation. Our research goals address Aims (iii), and (v) from Early Evolution of Life and the Biosphere (EELB). These include EELB(iii) - determine the original nature of biological energy transduction&to test hypotheses regarding the original nature of key biological processes , and EELB(v) - investigate the evolution of genes, pathways, and microbial species subject to long-term environmental change relevant to the origin of life on Earth and the search for life elsewhere .

Approach and Methodology:
To extend these studies we will perform comparative analyses of photobiological energy transduction, protein:substrate binding release, and biological electron transfer in extremophile DNA photolyases. Photolyase encompasses all basic and critical biochemical processes but has the advantage of being light-driven. This affords a separation of enzymatic events so that the effects of extreme environmental changes can be accurately monitored. Experiments will be performed by the Stanley group using ultrafast laser spectroscopy and complemented by QM/MM simulations by the Beratan group. Stanley and Gindt will execute substrate binding/repair experiments over a wider range of environmental conditions, including temperature, viscosity, and ionic strength to test the functional limits to environmental extremes. Gindt will provide thermodynamic and electrochemical measurements to complement kinetics data taken by the Stanley group. An important complementary aim of this proposal is the study of UV-DNA damage under extreme environmental conditions which will involve effort from the research team as a whole.

Kenneth Stedman/Portland State University
The Emergence of Chimeric RNA-DNA Viruses

The Emergence of Chimeric RNA-DNA Viruses.
Viruses are probably as old as life itself and have been involved in most major evolutionary transitions. Currently there are three main groups of known viruses, those that have RNA genomes, those that have DNA genomes, and the retroviruses that use cellular DNA intermediates to replicate their RNA genomes. Until very recently it was thought that viruses did not exchange genetic information between viruses of different groups. However, in 2012 in Boiling Springs Lake (BSL), we discovered the first example of a heretofore undiscovered widespread group of viruses with genomes that apparently arose by an unprecedented RNA-DNA recombination event between 2 completely different classes of viruses, the chimeric viruses (ChiV).

The frequency and mechanism of the recombination generating ChiVs are unclear, but critical for understanding virus evolution, the emergence of new viruses and possibly allow insights into the transition between RNA and DNA in the evolution of cellular life on Earth. We will address the frequency and mechanism of emergence of ChiV formation by pursuing 3 specific aims:

1. Isolate and characterize the ChiV replication-initiation protein, implicated in the RNA-DNA recombination.

2. Isolate and characterize the ChiV capsid protein, a RNA-virus-like capsid protein that encapsidates DNA.

3. Determine the ChiV host, by screening environments known to harbor ChiVs using PACS, Phage-FISH and FACS.

Aim 1: We will clone, express and purify ChiV Rep endonuclease-ligase proteins, which are likely to have the enzymatic activity linking DNA and RNA. Proteins will be expressed and purified using standard recombinant DNA and protein purification techniques. Activity will be tested using sensitive DNA and RNA ligation assays. These results will address the mechanism and frequency of recombination in vitro.

Aim 2: We will clone, express and purify the ChiV capsid protein (CP). The capsid protein is highly conserved in all CHiVs and is clearly monophyletic with Tombusvirus RNA virus capsid proteins. However, CHiVs CPs package DNA. Purified ChiV CP (methods as in Aim 1) will be tested using biophysical tools, including analytical ultra-centrifugation, spectroscopy, small angle neutron scattering, and electron microscopy. Antibodies will be produced for FACS. These results will assess how straightforward it is to change the specificity of a virus CP and provide insights into the frequency of emergence of ChiVs.

Aim 3: To date no ChiV virus or host has been isolated. The largest diversity of ChiV sequences found to date is in freshwater lakes samples. We will screen low-diversity samples with PCR-Activated Cell Sorting (PACS), a cutting-edge extremely high throughput method for separation of virus sequence associated genomes, viruses and hosts for potential ChiV hosts. We will also use Phage-FISH and flow-cytometry (see aim 2) on these samples. We will also use bioinformatics tools to try to identify potential hosts. We will also screen local freshwater lakes for ChiV genomes, virions and hosts. When a host is identified, we will attempt growth, infection and virus characterization. These results will also address the frequency of ChiV formation.
NASA Relevance:

This research seeks to further our fundamental understanding of evolution and the basic biochemical processes that drive it. This research will characterize novel biochemical catalytic functions of proteins. There is a distinct possibility that ChiVs or a similar activity played a role in the transition from the RNA world to DNA-world . These unknown biochemical mechanisms have important implications for the evolution of both pre-biotic and advanced life, making this proposed work directly relevant to two areas of emphasis within the Exobiology program. Furthermore understanding the emergence of new viruses is very important for manned space flight and life support.

David Stillman/Southwest Research Institute
Toward Geophysical Detection of the Biological Modification of Ice

Microorganisms found in glaciers, sea ice and permafrost can remain viable, or even metabolically active, at temperatures well below 0°C. These organisms can live within the salty liquid-vein networks that exist between ice crystals. In this proposal, we will study the extracellular ice binding protein (IBP) that inhibits ice recrystallization from cold-tolerant microorganisms isolated from the basal ice of an Antarctic ice core. Similar

extant life could exist within warm pockets of Europa s ice shell (chaos terrain). Using magnetic resonance (MR) techniques, we have previously shown that this IBP drastically alters liquid-vein networks within ice. Using dielectric spectroscopy (DS) measurements, we have previously shown on recrystallized IBP-free ice that the dielectric properties of ice are dominated by liquid-vein networks.

In this proposal, these two laboratory techniques will conduct measurements on the same samples combining MR diffusion measurements with measurements of the electrical properties for the first time on ice. MR techniques and simple microscopy conducted at Montana State University will provide us with microstructural properties (unfrozen water content, ice-grain size, surface-area-to-volume-ratio, diameter of liquid veins and tortuosity) and imagery of triple junction liquid networks. DS measurements conducted at Southwest Research Institute in Boulder will provide us with broadband complex electrical properties, which can be used to determine the likelihood of a remote geophysical detection of IBP. We will study the detectably of two geophysical techniques (ice penetrating radar and magnetotellurics) that can measure variations of the electrical properties of Europa s ice shell. This project will constitute the first time that MR techniques and DS have been used together to study the microstructural and electrical properties of an icy microbial habitat altered by a biosignature (IBP).

Our primary objective is to determine the effects of IBP on the microstructural properties of liquid-vein networks within ice and quantify the impact of IBP alteration upon remotely detectable electrical properties. Therefore, we seek to address the following science questions: (1) What is the threshold IBP concentration within the liquid-vein network needed to impede grain growth? (2) How do ice s microstructural properties (unfrozen water content, grain diameter, tortuosity), permeability and electrical properties vary with temperature and IBP or salt concentration? (3) Can ice s electrical properties reliably constrain its microstructural properties or permeability? (4) Can ice s microstructural properties be constrained by geophysical techniques?

Our approach to solve these questions follows: (1) Prepare samples with varying concentrations of salt and IBP. (2) Using MR techniques, image and measure the temperature-dependent volume, surface area-to-volume ratio, and tortuosity of liquid-vein networks in lab-frozen ice samples. (3) Using DS, measure the electrical properties of those same samples. (4) Develop models relating the dielectric properties of these ice samples to the MR-derived microstructural properties of the liquid-vein networks. (5) Investigate the habitability of vein networks in ice. (6) As IBP-rich ice inhibits grain growth it should decrease the radar attenuation and conductivity. Therefore, we will evaluate the geophysical detectability of IBP-altered microbial habitats.

This work is directly relevant to the Exobiology program goal to understand the distribution of life in the universe, as we will gain a better understanding of the nature of a microorganism habitat within ice as well as how easily similar habitats could be detected remotely elsewhere in the solar system. Our proposed research is also relevant to the biosignatures area of emphasis, which supports basic research on the formation and retention of biosignatures under extraterrestrial conditions.

Qing-zhu Yin/University Of California, Davis
Chronologic and isotopic constraints on the Great Oxidation Events and evolution of life on Earth

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Xinning Zhang/Princeton University
What Controls The Nitrogen Isotope Fractionation Of N2 Fixation?

The requirement for bioavailable fixed nitrogen is a fundamental constraint for all life on Earth, making biological nitrogen fixation one of the most important evolutionary breakthroughs in the history of our planet. All biological nitrogen fixation is catalyzed by the metalloenzyme nitrogenase in a complex, energy-intensive reaction that reduces atmospheric N2 gas into bioavailable ammonium. Without this process, only a fraction of our biosphere could be sustained, and it is likely to have played a crucial role in the timing and trajectory of the evolution of life. However, the origins of N2 fixation remain elusive, partly due to complexities of interpreting ancient signatures of this life-supporting reaction. The N stable isotope composition (´15N) of biomass provides a powerful tool for reconstructing present and past N cycling but its interpretation hinges on a complete understanding of the isotopic signature of N2 fixation. Recent investigations into the metal cofactor variants of nitrogenase revealed that the Mo-, V-, and Fe-only isoforms of the enzyme impart different isotope fractionations during N2 fixation in vivo and that Mo-nitrogenase can fractionate N in excess of 100 under in vitro conditions. This challenges the traditional view that N2 fixation only imparts small, invariable isotope effects (~0-20) and has led to new estimates that N2 fixation by Mo-nitrogenase must have evolved by 3.2 Ga. Yet, the mechanistic basis for changes in the fractionation of N2 fixation remain largely unknown, preventing a full understanding of the variability of this key parameter in N cycle reconstructions and resulting in large uncertainties in the interpretation of 15N in past and present systems.

Objectives
A better mechanistic understanding of the enzymatic, physiological, and environmental drivers of 15N fractionation during N2 fixation is a crucial prerequisite to reconstructing N cycle processes over the course of Earth history. We will use laboratory and modeling approaches to address 4 aims: 1) Examine the isotope range accessible to understudied nitrogenases in strict anaerobes. 2) Determine the control exerted by the enzyme active site on the expressed isotope effect to evaluate how nitrogenase sequence evolution may have altered fractionation. 3) Determine how the physiological context of N2 fixation (growth rate, energy, Fe availability, light levels, temperature, pH) affects expressed fractionation to evaluate the role of environmental constraints. 4) Develop an organism-level kinetic model for expressed fractionation based on the results from Aims 1 - 3 and published kinetic parameters.

Significance

The proposed research falls in the Early Evolution of Life and the Biosphere category. It seeks to investigate (a) the development of N2 fixation, a key biological process, its environmental impact and expression in geological records (subtopic iv); and (b) the evolution of nitrogenase, the N2 fixing enzyme which has shaped the origin and trajectory of life on Earth (subtopic v).

Experimental Approach

We will draw on our expertise in measuring N isotope effects and in working with a wide range of nitrogen fixing organisms to evaluate N isotope fractionation by less well studied forms of nitrogenase (Cluster III) and the effect of select changes in amino acid sequence in the nitrogenase active site using existing libraries of Mo-nitrogenase mutants of chemoheterotroph Azotobacter vinelandii and cyanobacterium Anabaena. We will apply our expertise in chemically controlled aerobic and anaerobic continuous culturing techniques to modulate environmental and physiological parameters in Azotobacter vinelandii, Anabaena variabilis, and Rhodopseudomonas palustris (model organisms for chemoheterotrophic, oxygenic, and anoxygenic photoautotrophic N2 fixation) to evaluate the role of metabolic rates, enzyme abundance, metal, light limitation, pH, and temperature in controlling expressed nitrogen isotope effects.