Below are the abstracts of proposals selected for funding for the Exobiology program. Principal Investigator (PI) name, institution, and proposal title are also included. 156 proposals were received in response to this opportunity. On October 26, 2018, 24 proposals were selected for funding.

Kristin Bergmann/Massachusetts Institute of Technology
The Thermal Maturity of Neoproterozoic Strata: Carbonate Clumped Isotope Thermometry and Biomarker Analyses

Science Goals and Objectives:
The Neoproterozoic Era (541-1000 Ma) was a transitional time in the history of life that included both the emergence of early animals and other complex eukaryotes in the midst of Cryogenian climate perturbations (635-850 Ma). However, the specifics of both the climate record and the evolutionary tempo of eukaryotic diversification remain poorly resolved. First, multiple aspects of the climate record could be better known. Perhaps most importantly, temperature constraints across the background climate and before, during and after the 'snowball earth' glacial perturbations will address decades long uncertainties about the Precambrian climate. Second, while great strides have occurred with the fossil and biomarker record of life, high resolution records to define the tempo of evolution are rare. We propose a holistic study of the thermal maturity of shallowly buried Neoproterozoic strata globally to assess the best sites able to provide a paired high resolution clumped isotope and biomarker record through the Neoproterozoic.

Hypotheses and Measurements:
We will pair carbonate clumped isotopic analysis with biomarker analyses and burial history modeling to assess the thermal maturity of key Neoproterozoic strata. We will: A) assess the overprint of diagenesis using paired calcite and dolomite samples from Neoproterozoic carbonates through petrography, phase-specific carbonate clumped isotope thermometry analysis and Secondary Ion Mass Spectrometry (SIMS) analysis of $\delta^{13}C$ and $\delta^{18}O$ variability across 10s of microns, B) use biomarkers and synchrotron-based microanalysis to assess the preservation of biomarkers and diversity of eukaryotes in a range of depositional environments. In this proposed work, we will leverage insights gained from our work on the regional variability of thermal maturity in Oman and NE Svalbard and test the potential of a range of other shallowly buried sites.

Pairing biomarker results with carbonate clumped isotope (47) thermometry has not been done to date, but offers a path forward to better understand both the organic and inorganic records preserved in ancient rock successions. Our preliminary results from NE Svalbard and Oman indicate that regional differences in thermal history lead to varying degrees of recrystallization, solid state reordering, and biomarker destruction. We will
expand on our existing burial history model to utilize the degree of mismatch between calcite and dolomite clumped isotope temperatures and the biomarker preservation to pinpoint thermal maturity. With our three-part approach using clumped isotope thermometry, biomarker analysis, and modeling we aim to assess the potential of different regions to determine how temperature co-varied with the evolution of complex life.

Significance and Relevance:

We expect that our investigation of the thermal maturity of shallowly buried Neoproterozoic strata and the insights we will gain on climate perturbations and associated eukaryotic expansion will significantly contribute to the broader Astrobiology community. This proposed work falls within the scope of the Exobiology program element subsection on "Evolution of Advanced Life" due to its focus on habitats for early animal life associated with Neoproterozoic climate perturbations. In addition, the solicitation specifically highlights 'snowball earth' events. Concerted investigation of this time period promises to transform our understanding of the factors driving the expansion of complex life on Earth.

Debashish Bhattacharya/Rutgers University, New Brunswick
Elucidating the Transition to Eukaryotic Phototrophy

Key central objectives: Plastid primary endosymbiosis occurred ca. 1.6 billion years ago and involved the capture and retention of a free-living cyanobacterium by a single-celled protist, giving rise to the canonical photosynthetic organelle in algae and plants. Plastid origin is one of the most important innovations in the history of our planet, setting the stage for the establishment and diversification of multicellular eukaryotes, including humans. A massive effort has been expended on elucidating plastid origin, function, and its impacts on global primary productivity and geochemical cycles. In addition, ozone, derived from the biological production of oxygen is considered a key marker of life on Earth-like exoplanets. Past work in this field has focused on the primary plastid containing Archaeplastida (red, green [including plants], and glaucophyte algae) and other taxa that gained this organelle through serial (eukaryotic) endosymbiosis (e.g., diatoms, dinoflagellates). What these valuable models cannot provide however, due to the ancient derivation of their plastid, is insights into critical earlier stages of organellogenesis. During this phase, biotic interactions and genetic innovations evolved to stabilize, integrate and regulate the association between the host and the oxygen evolving, potentially toxic (because of reactive oxygen species production) endosymbiont that was compromised by gene loss due to Muller's ratchet. Here, we propose to fill a wide gap in our knowledge of early eukaryote evolution by studying the origin of primary plastids in the only non-Archaeplastida model available. This is the lineage of thecate amoebae, Paulinella, which gained its plastid (the chromatophore) ca. 100 million years ago from an alpha-cyanobacterium. We have generated significant genomic, transcriptomic, metagenomic, and physiological data from this lineage and are now
poised to elucidate innovations that enabled the crucial transition from heterotrophy to phototrophy.

Methods/techniques proposed: Our inter-disciplinary approach has three specific aims: 1) Use transcriptomic (RNA-seq, RT-qPCR) methods to study Paulinella gene expression under a 12h light:12h dark cycle using low and high light levels and nutrient deprivation conditions to identify genes (both known and novel) involved in coordination of photosynthesis and stress responses. 2) Generate a genome-scale metabolic network (GEM) model for Paulinella in order to gain a systems-level understanding of chromatophore integration. This work will be based on the existing GEM for the chromatophore, which will guide our analyses of probable metabolic fluxes associated with high light, low light, and nutrient deprivation. 3) Use a heterologous expression system (Synechococcus PCC7002) for functional studies of high light- and nutrient deprivation-induced genes identified in our preliminary data as well as from planned experiments.

Significance to NASA objectives: The major outcomes of our study will be a detailed understanding of genetic innovations that underlie the transition to phototrophy, an understanding of how biotic interactions may support plastid and host health, the generation of a GEM model for Paulinella that provides a valuable community resource to drive future science, and functional data for novel genes important for fitness when the cells experience stress resulting from high light or nutrient deprivation. These goals fit well with the scope of the NASA C.5 Exobiology Program that aims to understand the origin, evolution, distribution, and future of life in the Universe. In particular, our work addresses the research emphasis, Evolution of Advanced Life, because it directly examines processes associated with endosymbiosis. The proposed research is also integral to the emphasis Early Evolution of Life and the Biosphere because it investigates the development of key biological processes (photosynthesis) and their environmental impact.

Christopher Carr/Massachusetts Institute of Technology
Microbial Functional and Evolutionary Adaptations to Aridity

Hyper-arid and perchlorate-rich regions of the Atacama Desert, Chile, represent some of the best terrestrial analogs of Mars. Prior studies support the presence of indigenous microbial communities that are periodically active during favorable conditions. The Chilean Cost Range and Andes mountains create a rain shadow with aridity levels along one transect varying from arid to hyper-arid (20 mm/yr to <1 mm/yr). Such precipitation differences exert a strong control over surface, soil, and biomass; in the driest regions the relative humidity at 1 m depth is around 14%, on par with measurements made by the Mars Science Laboratory on Mars.

Desiccation tolerance, or xerotolerance is closely related to resistance to ionizing radiation (IR), and indeed, the latter may result from selection for the former. However,
archaeal and bacterial responses to desiccation are diverse, understudied, and not always concordant with IR resistance. Adaptation mechanisms include dormancy, formation of protective barriers or cell membrane alterations, molecular means to preserve activity or conversely induce vitrification, or mechanisms to reduce reactive oxygen species (ROS) production or improve ROS scavenging.

Hypothesis: We hypothesize that adaptation to aridity and IR imposes costs that select for a slow-growth lifestyle. This may limit such organisms’ ability to respond to benign conditions, while also helping match growth rates to limited nutrient supply in harsh environments.

Objectives: We propose to build on prior systematic sampling along variable aridity transects in the Atacama, including extensive prior characterization of soil parameters, ribosomal (16S) community characterization, and cultivation work. First (1a), we propose to provide a comprehensive view of microbial community structure, function, and activity along these transects. Second (1b), we will confirm aridity-associated responses and in situ activity via a focused field study. We will (2a) refine mechanisms of xerotolerance using isolate studies and (2b) disentangle xerotolerance and IR resistance, integrating genomic, transcriptomic, physical, chemical, and physiological responses.

Methodology: A diverse array of soil analyses and parameters have already been obtained including organic carbon and nitrogen, delta-13C, water content, carbonate content, dissolved organic carbon and nitrogen, grain size, pore space, cation content (ICP-OES), anion content (ion chromatography), pH, bulk density, electrical conductivity (salinity), aromatic compounds (UV-VIS), dithionite-soluble metal-oxide and -hydroxide compounds, and electron microscopy. We propose to complement these measurements with metagenomic and metatranscriptomic sequencing to characterize taxonomy, function, and activity from existing samples. The confirmatory study will utilize in situ metagenomic (DNA) and RNA sequencing to characterize community without potential storage bias. We will assess xerotolerance of existing and newly generated Atacama isolates and controls through survival assays, transcriptomics, nanoscale imaging (FIB/SEM), chemical characterization (including EPR), and IR resistance.

Significance and Relevance: Our work will help to define, in one of the best analogs of Mars on Earth, the trade-offs microbial communities make to survive in increasingly arid environments. Our comprehensive approach will link microbial community structure, function, and activity to environmental and geological parameters of relevance to habitability and the search for life on Mars and elsewhere. Our work is significant to NASA’s search for life in the universe (Biosignatures and life elsewhere theme) in that it involves biosignature studies of samples from Earth sites thought to be analogues of other planetary environments that might potentially harbor life.
Stromatolites laminated, lithified structures typically built by microbial mats represent the most abundant record of life in the first 7/8ths of Earth history (the Archean and Proterozoic Eons). As macroscopic manifestations of microbial life, they represent a clear target for exobiologic investigation. Despite a long history of study, many strikingly fundamental questions remain with respect to stromatolite formation and their distribution throughout geologic time. Two pertinent questions include: 1) How do microbial mats lithify to become stromatolites (i.e., rocks)? It is still not clear how soft, organic-rich microbial mats transform into hard, lithified stromatolites. 2) What controlled the distribution of stromatolites through time? Stromatolites reached a form diversity peak ca. 1.0 billion years ago, crashed, and became comparatively rare throughout the remaining 600 million years of Earth history. The most common hypothesis the evolution of burrowing/grazing metazoans disrupting the microbial mats is not tenable, as the decline initiated well before the advent of animals.

Here, we propose to investigate how the redox cycling of iron, not previously considered important for the formation of stromatolites, may lead to the lithification of soft microbial mats, and how the changes in the dissolved iron in Archean, Proterozoic, and Phanerozoic oceans may link to the decline in stromatolite abundance and form diversity well before the evolution of animals.

Iron, lithification, ancient ocean chemistry, and mats: Before widespread marine oxygenation, the oxygen produced locally by cyanobacteria in a microbial mat could induce iron oxide precipitation in the mat during sunlight hours. At night, when the mat became anoxic, iron-reducing bacteria, could use the iron oxides to respire, releasing iron into pore waters. During microbial iron reduction, a boost of alkalinity is provided more than any other common metabolism shifting the local geochemical environment in favor of calcium carbonate precipitation in the mat. Testable consequences are evident, including a predicted cyclic iron enrichment within stromatolites formed by this process, and a link between stromatolite abundance/decline and marine iron concentrations through geologic time.

In testing the role of iron redox cycling in the lithification of stromatolites, we may finally understand the decline of stromatolite form diversity/abundance that has remained unresolved since it was recognized.

The novel link between microbial iron reduction and stromatolites will be tested in two ways:
1. By examining iron reduction in modern stromatolites growing in Obsidian Pool Prime (OPP), a siliceous hot spring in Yellowstone National Park. Here, a suite of modern molecular (SSU rRNA, metagenomic/transcriptomic), geochemical, and imaging tools will be used to investigate the importance of iron reduction in a tractable system. Although OPP is a silica spring, the information gained is applicable to other systems,
including carbonate systems. Since the growth rate of the OPP stromatolites is known, the aforementioned approaches will be captured, for the first time, during stromatolite lamination formation/growth.

2. By using micro X-ray Fluorescence techniques to investigate the distribution of iron within a suite of stromatolites through the rise and fall of stromatolite abundance in geologic time, testing the prediction of iron incorporation during lamination formation versus marine iron concentrations. Coincidentally, the decline of stromatolites neatly tracks the loss of the ferruginous oceans, as indicated by Mo and Cr proxies, lending credence to the hypothesis.

Our study would be relevant to two main foci in the Exobiology program 1) Early Evolution of Life and the Biosphere (stromatolites represent the earliest macroscopic evidence of life on Earth) and 2) Biosignature and Life Elsewhere (stromatolites could act as biosignatures for life on early Earth/elsewhere).

Shiladitya DasSarma/University Of Maryland, Baltimore

Halophilic Microbes As Models For Astrobiology: Evolution Of Polyextremophilic Capacity

Salt-loving (halophilic) microorganisms grow in hypersaline environments with multiple extreme conditions. Among halophiles, the most extremophilic are the Haloarchaea which are of additional interest due to their classification as members of Archaea, the third Domain of life. Their study is contributing to the understanding of the nature and characteristics of some of the most primitive and ancient microorganisms on Earth and their polyextreme environments in which they evolved. They have been isolated from extreme environments like Deep Lake Antarctica, Salar de Uyuni Bolivia, and Great Basin deserts of western USA, and survive launches into the stratosphere. These studies are important for guiding our assessment of the potential habitability of Mars, other planets in our Solar System and extrasolar planets detected in the Galaxy. Study of haloarchaeal evolution fits into NASA's 2015 astrobiology strategic priorities, including Evolution of early life and increasing complexity and Co-evolution of life and the environment, and Exobiology research emphasis areas of Early Evolution of Life and the Biosphere and Biosignatures and Life Elsewhere.

The central quality of Haloarchaea relevant for advancement of our understanding of astrobiology is their polyextremophilic character, including tolerance of saturating sodium chloride, intense solar radiation, desiccating conditions, and cycles of freezing and thawing. We are pursuing the understanding of the evolution of these remarkable characteristics on Earth and limits to their polyextremophilic capacity found elsewhere through isolation, metagenomic, and exposure studies in the environment and genomic, bioinformatic, and mutagenic studies in the laboratory. Our recent work has compared a genetically tractable laboratory species, Halobacterium sp. NRC-1, a mesophile from San Francisco Bay, California, to Halorubrum lacusprofundi, a cold-adapted environmental
species from Deep Lake, Antarctica, for mechanisms of tolerance to conditions of Earth's stratosphere and damaging radiation of space. Another significant line of investigation has established an experimental system to investigate the evolution of haloarchaeal protein function in cold, hypersaline conditions.

In the present project, we plan to use our well-developed experimental system to deeply investigate key aspects of the evolution of extremophilic capacity of haloarchaeal microorganisms at the genome and protein levels using a combination of microbiology, genomics, and biochemistry approaches. We plan to address the following questions:

1) At the genomic level, what are the essential evolutionary characteristics and mutations leading to survival of Haloarchaea in multiple extreme conditions in their environment?

2) At the protein level, how do haloarchaeal polyextremophilic enzymes adapt to function in the extreme conditions in their intracellular milieu?

Our studies on the evolution of Haloarchaea will result in better understanding of the limits to polyextremophilic capacity on Earth and the potential habitability of Mars and other planets.

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**Jocelyne DiRuggiero/Johns Hopkins University**

**Roles Of Regulatory Small RNAs (sRNAs) In The Adaptation Of Extremophilic Microbial Communities To Stress And Environmental Changes**

Regulatory small RNAs (sRNAs) represent a major class of regulatory molecules that play large-scale and essential roles in many cellular processes across all domains of life. However, microbial sRNAs have been primarily studied in model organisms and very little is known about the dynamics of sRNA synthesis in natural environments, and the roles of these short transcripts at the community level. The goal of this project is to investigate the roles of regulatory sRNAs in the stress response of extremophile communities and their ability to cope with environmental changes.

The overarching research questions we will address are: do microorganisms activate specific sRNAs when in a community? what are the underlying mechanisms for extremophile microbial communities to adapt to environmental changes? and what are the roles of regulatory sRNAs in this adaptive response at the community-level?

We will use a well-characterized microbial community inhabiting halite nodules (salt rock) from Salar Grande in the Atacama Desert, Chile as our model. These communities are polyextremophilic, and, as such, their study is applicable to a broad range of terrestrial and extraterrestrial environments. This project has 2 major objectives:

In Objective 1, we will sequence the genomes and transcriptomes of isolates and enrichment cultures from Salar Grande halite and characterize their regulatory sRNAs.
using our microbial sRNA identification pipeline. The resulting sRNA catalog will provide a resource for the discovery of community sRNAs in halite metagenomes and will be complementary to the de novo approaches proposed in Objective 2.

In Objective 2, we will investigate the molecular and ecological relevance of microbial sRNAs in the stress response of the halite community. To do so, we propose to transplant halite nodules from Salar Grande to several test locations in the desert that have contrasting environmental conditions. We will combine environmental, ecological, and molecular analyses to identify differentially regulated sRNAs between these communities after 1 year. Structure and target prediction of the most differentially expressed community sRNAs will provide the first insights into their roles at the community level. We will build the sRNA identification and target prediction analytical approach developed during this project into an easy-to-use bioinformatics pipeline for the community.

Relevance to NASA: The identification of processes regulated by sRNAs at the community level will further our understanding of co-evolutionary mechanisms of microbial communities and expand our knowledge of the diversity and genomic distributions of microbial sRNAs. Furthermore, understanding RNA’s ecological relevance at the regulatory level, and how it might allow life to survive in extreme environments, can shed light into the early evolution of life and the biosphere and help reconstruct the nature of the most primitive organisms. This project focuses on extremophiles, which are model organisms of possible ecosystems beyond Earth. Therefore, understanding the physiological and molecular adaptations of extremophiles to environmental stress and climate change can also improve predictions of where life might exist elsewhere in the Solar System, and where to search for evidence of it.

Mary Droser/University Of California, Riverside

Did the small inherit the Earth? Analysis of mm-scale Ediacara body and trace fossils (South Australia) with implications for the early evolution of animals.

Newly discovered diverse mm-scale fossils of the Ediacara Biota the earliest fossil evidence of complex, macroscopic communities within the Ediacara Member of South Australia offer the unique opportunity to test whether animal taxa that are abundant today were present prior to the Cambrian explosion of complex animal life. While classic evolutionary biology predicts that complex animals should be preserved within the Ediacara Biota, such taxa have proven difficult to definitively recognize, perhaps as a consequence of their predicted small size. New material from the Ediacara Member the most richly fossiliferous deposit of the Ediacara Biota represents a unique and promising opportunity to identify early animals, including potential bilaterians, in the Precambrian for several reasons: 1) this new fossil material consists of fine-grained sandstone atypical of the Ediacara Member providing the resolution necessary to identify sub-mm to mm-scale potential stem- and crown-group bilaterians; 2) it contains abundant mm-scale body fossils that preliminary analyses indicate share several characters with both modern and
fossil bilaterians; 3) this material preserves abundant Helmithoidichnites, small-scale, bilaterian-produced trace fossils; and 4) it occurs in sedimentary successions that have been extensively studied as part of a previous NASA Exobiology grant, providing a platform for detailed characterization of the paleoenvironmental and preservational context of these small-scale fossils. Additionally, recent advances in digital photography, 3D laser imaging and computer processing software will facilitate investigation of sub-mm to mm-scale fossils to a greater extent than was previously possible.

We propose the study of small-scale Ediacara body and trace fossils preserved in fine-grained sandstones of the Ediacara Member in order to reevaluate a significant but previously intractable question: were animals, including bilaterians, a significant component of the Ediacara Biota? To address this question, we will pursue three main objectives: 1) Determine the morphology and development of new mm-scale body fossils to identify anatomical and behavioral characters indicative of particular phylogenetic affinities; 2) Describe Heliminthoidichnites trace fossils, including the morphology, paleoecological associations and sedimentological distribution of these structures; and 3) Characterize the paleoenvironments in which Ediacara small-scale candidate bilaterians occur and identify the environmental and diagenetic conditions that promoted their preservation.

The results of this work will shed new light on the long-standing question of whether abundant, diverse stem- and/or crown-group bilaterians were present in the Ediacara Biota or whether these early communities were largely composed of extinct failed evolutionary experiments. Regardless, we will gain a comprehensive understanding of the morphology, developmental biology and ecology of some of the first complex, macroscopic organisms preserved in the fossil record and of the environmental settings in which they evolved, with implications for understanding the origins and evolutionary trajectory of complexity on Earth, as well as providing a search image for complex life and the environments that could potentially host it elsewhere in the universe. This will explicitly address the goal of NASA ROSES section C.5 Exobiology aimed at understanding the early evolution of life, the potential of life to adapt to different environments, and the implications for life elsewhere.

Jennifer Glass/Georgia Tech Research Corporation
Microbial Interactions with Methane Clathrate and Implications for Habitability of Icy Moons

Methane clathrates are likely widespread in our solar system, including on icy moons and the Martian subsurface, yet their habitability remains virtually unstudied. The proposed research will investigate interactions between microbial proteins and methane clathrates. Specifically, the proposed work will test the hypothesis that microbes living in methane clathrates encode proteins optimized for clathrate binding, and that these clathrate binding proteins (CBPs) alter the structure, thermodynamics and kinetics of methane clathrates. Our results will contribute to understanding biosignatures of microbial
The proposed research will: (i) heterologously express, purify, assay, and biophysically characterize candidate CBPs; (ii) quantify the effects of CBPs on methane clathrate thermodynamics and kinetics of formation and decomposition in laboratory experiments with pressurized vessels; (iii) solve crystal structures of select CBPs and characterize static interactions with water molecules; (iv) use high-throughput homology modeling, molecular dynamics, and other computations to characterize structures and dynamics of CBPs; (v) create a public database of CBPs. The proposed work is relevant to the Biosignatures and Life Elsewhere Exobiology solicitation research area, specifically establishment of life under conditions prevailing on other planetary bodies and basic research on the formation and retention of biosignatures under non-Earth conditions (e.g., Mars, Europa). Clathrate-binding proteins are a novel example of Co-Evolution of Life and the Physical Environment (NASA Astrobiology Roadmap topic area 4). This work will address topic 4 future research area III How Does Our Ignorance About Microbial Life on Earth Hinder Our Understanding of the Limits to Life? by biochemically verifying the physiological characteristics of uncultured organisms and by directly linking newly discovered genomes to the functional roles the microbes play in a community and/or environment.

**Hilary Hartnett/Arizona State University**

**Experimental Tests For The Origin And Evolution Of Anoxygenic Photosynthesis**

Motivation: A challenge for Exobiology is to explain the evolution of photosynthesis, a complex pathway requiring several cooperative enzymes and the synthesis and use of multiple cofactors. Anoxygenic photosynthesis in particular is important for the early anoxic Earth and for exoplanets, where anoxia might be quite common. If the evolution of photosynthesis via a few 'relatively easy' steps is possible, we might expect to find many worlds with photosynthetic life, whether or not oxygenic photosynthesis ever emerged. Otherwise, we might expect photosynthesis to be rare in the universe.

We propose that a plausible evolutionary intermediate in the development of (anoxygenic) photosynthesis was a chemolithotrophic bacterium that extracted energy from environmental redox gradients. One way to extract more energy from a redox gradient is to insert a photosynthetic reaction center (RC) into the electron transport pathway. This gives a chemolithotroph a competitive advantage, allowing it to use less-reducing donors and/or less-oxidizing acceptors. A shallow gradient or even an uphill electron transfer could be profitable to an organism using light to generate a steeper gradient, thus pumping more protons per electron transported, and making more ATP. In this view, the reaction center allows the development of light-assisted lithotrophy, i.e., photo/chemolithotrophy.

Objectives: There are no known examples of the photo/chemolithotroph we invoke in this evolutionary trajectory. We propose to test this idea by creating such organisms. We will use the primitive phototroph, Heliobacterium modesticaldum, as our starting point,
because it is experimentally much easier to transfer a few genes required to confer lithotrophy rather than the many genes required for phototrophy. We have developed genetic tools to edit the H. modesticaldum chromosome using its endogenous DNA modification systems; this allows us to remove and add genes at will. We propose to break the existing cyclic electron flow pathway by removing a specific enzyme (NADH dehydrogenase) and adding a sulfide:quinone oxidoreductase (SQR) to allow it oxidize sulfide to sulfur. The organism can already reduce protons to molecular hydrogen (H2). Therefore, it should be able to oxidize (external) sulfide and reduce (internal) protons, aided by light, and in the process create a proton electrochemical gradient for ATP synthesis thus, extracting energy from a redox gradient that is energetically unfavorable.

We will test the growth of the engineered organisms under different conditions and quantify their ability to (1) use sulfide (S-2) as electron donor, (2) reduce protons to H2, (3) extract energy in the process, and (4) fix CO2. We will also test the idea that autotrophy can be conferred by adding ATP-citrate lyase, which is the only enzyme missing from the reverse TCA cycle in H. modesticaldum. We can make several combinations of these genetic modifications in order to construct multiple possible evolutionary intermediates. Lastly, we will assess the isotope signatures associated with the new sulfide oxidation, proton reduction, and CO2 fixation pathways and compare them with known metabolic fractionations and with the geologic records.

Significance: This work is relevant for NASA Exobiology because it directly investigates two research topics in the 2015 Astrobiology Strategy: Early Life and Increasing Complexity and Co-evolution of Life and the Physical Environment. Specifically, we will experimentally explore the origin and evolution of a key metabolic strategy, anoxygenic phototrophy, in the context of environmental redox gradients. Our results will provide insight into the potential biosignatures of the metabolic strategies we explore in the engineered H. modesticaldum strains. The results will have implications for understanding the evolution of life on the early Earth and for the diversity of biosignatures that might result from novel metabolisms.

Roland Hatzenpichler/Montana State University, Bozeman
Ecophysiology Of Uncultured Archaea In Geothermal Features Of Yellowstone National Park

Geothermal sites harbor some of the most enigmatic microbes on our planet. Through the rise of massive parallel gene sequencing technologies we have become aware of the large microbial diversity in geothermal features, many of which previously had been thought to be dominated by a few highly abundant but taxonomically and metabolically redundant taxa. While often highly abundant in these geothermal habitats most lineages have resisted cultivation attempts - and thus a more thorough physiological characterization - for decades. Because of this, not much is known about their metabolic function, ecological niches, biogeochemical roles, and environmental adaptations. Fortunately, new
approaches targeted at the individual cell level can be used to study these questions without the need of lengthy (and often eventually unsuccessful) cultivation attempts. We are currently generating metagenomes of five geochemically distinct hot springs in Yellowstone National Park in which several previously acknowledged, but yet experimentally uncharacterized archaeal phyla are present. According to our current data, high priority targets include members of the archaeal phyla Aig-, Bathy-, Kor-, and Thaumarchaeota, YNPFFA archaea as well as a yet undescribed phylum abundant in some of our study sites. However, genomic analyses might reveal additional yet unidentified targets that could also be targeted in our study. We also have generated exhaustive geochemistry data to help us in our future analysis (~45 different factors were measured). We are proposing to genomically characterize the above enigmatic archaea and use cutting edge, non-destructive methodologies to characterize their in situ physiology and metabolic interactions with other community members.

Our main objectives are (i) to genomically characterize several uncultured archaeal clades present in our geothermally heated sites, (ii) to identify specific energy, carbon, and nitrogen sources through wet lab experiments, and (iii) to identify the most important geochemical drivers of metabolic activity that govern the interaction of these archaea with their environment and other community members. Our eventual, long-term goal is to exploit the knowledge gained in this project in future, more refined experiments, including the design of novel enrichment media to bring some of these archaea into culture (successful isolation is beyond this proposal).

We propose to achieve these goals by experimentally testing genomic hypotheses created during the first year of our project by two single cell resolving approaches in the following two years. The first method, bioorthogonal labeling of active cells has been developed by the PI and has recently been used by his group very successfully on extremophiles from Yellowstone hot springs. In this project, we will use bioorthogonal labeling to determine under which conditions uncultured archaea are active in their natural habitat. These include most importantly specific temperature and pH regimes, dependency on trace metals or vitamins, and sources of bio-elements. By a combination of bioorthogonal labeling and isotope probing we will then reveal specific sources of energy, carbon and nitrogen for high interest targets. Cells will be taxonomically identified in the natural hot spring samples using fluorescence in situ hybridization probes, and assimilation of isotope labeled substrate uptake be visualized by single cell resolved, non-destructive Confocal Raman micro-spectroscopy.

Our work directly addresses several key questions in exobiology: (i) the physiology and ecology of extremophiles, which are understood to be among the most ancient lifeforms on our planet, (ii) the coevolution of life and the physical environment, (iii) the interactions of specific microbes with the broader biological community, and (iv) a better understanding of the diversification of the tree of life and the relationship between archaea and early eukaryotic cells.
The proposed research is part of a NASA-sponsored effort to understand the origin of life on Earth, which will guide the search for life elsewhere in the universe. The research pertains to a form of RNA-based life that likely existed during Earth’s early history, as a predecessor to current DNA-RNA-protein-based life. Building on progress made over the prior grant period, the central aim of the proposed research is to construct RNA-based life in the laboratory, as represented by populations of RNA enzymes that catalyze their own replication and undergo Darwinian evolution in a self-sustained and open-ended manner. Most of these efforts will focus on an RNA polymerase ribozyme that is now able to synthesize functional RNAs and to catalyze the exponential amplification of short RNAs. The enzyme is not yet able to synthesize itself, but that goal is likely to be achievable with additional optimization, primarily by employing in vitro evolution methods to enhance the rate, fidelity, and sequence generality of the polymerase.

Although the aim is to synthesize the polymerase in its entirety, a divide-and-conquer approach also will be pursued, whereby the enzyme will be divided into fragments that can assemble non-covalently to form a catalytic complex, with each of the fragments (and their complements) synthesized by the assembled complex. This approach may reflect what transpired on the primitive Earth, but also explores a general principle for how Darwinian systems might arise. The divide-and-conquer approach has already been used for the polymerase ribozyme to synthesize its evolutionary ancestor, an RNA ligase ribozyme, which can be divided into fragments that are synthesized separately and can assemble non-covalently to form a functional catalyst. This approach will be extended to the synthesis of larger and more complex RNA enzymes, including the polymerase itself. A surprising discovery during the prior grant period was that highly optimized forms of the RNA polymerase ribozyme also can function as a reverse transcriptase, copying RNA templates to complementary DNA products. This activity would have been crucial for the transition from RNA to DNA genomes during the early history of life. More recently it was shown that the polymerase can copy DNA templates to RNA products and, at lower efficiency, copy DNA templates to DNA products. Furthermore, RNA templates can be copied to yield various polynucleotide analogs, most notably threose nucleic acid (TNA), which has been suggested as a possible predecessor to RNA. These activities will be optimized using in vitro evolution to generate a family of polymerase ribozymes that can both amplify and transcribe macromolecular information in various forms. Although life on Earth came to adopt RNA, and ultimately DNA, as the genetic material, a broader view is required when considering the possibility of life elsewhere.

Once a system is in hand for the self-sustained evolution of RNA, it will be possible to conduct open-ended RNA evolution experiments, both as a working model of RNA-based life and as a means to explore the diversification of RNA function. The promise of such experiments was examined previously using a self-replicating ligase ribozyme, which could be made to adapt to different selection constraints. However, that system has a very limited capacity to evolve and is not capable of inventing novel function. In contrast, the polymerase enzyme supports much higher information content among the
RNAs being replicated. It is already capable of synthesizing complex functional RNAs with good fidelity, and its capabilities will be enhanced through further optimization. Ultimately, when the enzyme has the ability to generate copies of itself, the system will become self-optimizing, both for what it evolves and for its capacity to evolve. It has the potential to capture the defining feature of life and to provide the first example of a living system outside of terrestrial biology.

Nina Lanza/Los Alamos National Security, LLC
Discovering Biosignatures In Manganese Deposits On Mars With Rover Payload Instruments

The newly discovered high concentrations of manganese on Mars indicate past episodes of strongly oxidizing conditions within an aqueous environment. On Earth, such simultaneous conditions are almost always both habitable (potentially supportive of life) and inhabited by microbes. Given its close association with life and habitable environments on Earth, manganese has long been considered a principal biosignature for Mars. However, we do not yet understand the unique Mn signatures that can distinguish Mn-rich deposits as biogenic in origin (i.e., produced by life) from altered, abiogenic Mn deposits in rover payload instrument data. While distinguishing between biogenic and abiogenic manganese deposits is possible on Earth with a full suite of terrestrial laboratory instruments, the analytical techniques available on the surface of Mars are far more constrained. A primary goal of the Mars 2020 rover mission is to identify samples containing potential biosignatures for future sample return; thus, it is of the utmost importance that biosignatures that are detectable with the Mars 2020 instrument payload be identified prior to landing on Mars.

The objective of this proposal is to determine what chemical and mineralogical signatures can uniquely identify Mn-rich materials as biological in origin. If these signatures are identified on Mars, they will provide a clear sign that microbial life has acted in and on the martian environment. The recent discovery of high Mn materials in both Gale and Endeavor craters by the Curiosity and Opportunity rovers and the discovery of Mn-oxides within the Black Beauty martian meteorite suggests that Mn-rich materials may be more widespread on Mars than previously known, and such materials may be present in the as-yet unselected landing site for the Mars 2020 rover. Given that a major goal of the Mars 2020 rover mission is to determine whether life ever existed on Mars, it is imperative that we be able to recognize biogenic Mn minerals with rover payload instruments.

A key component of this proposal is laboratory experiments designed to interpret trace element abundance and mineralogy to conclusively identify Mn-related biosignatures acquired from Mars rover instruments. We will examine Mn-rich materials in detail using gold-standard laboratory techniques to elucidate the signatures of these materials with laser-induced breakdown spectroscopy (LIBS), which is currently on the Curiosity Mars rover payload (ChemCam), and Raman spectroscopy. While ChemCam is limited to LIBS, the Mars 2020 rover SuperCam instrument will combine LIBS and Raman
spectroscopy, and the Mars 2020 SHERLOC instrument will provide additional UV Raman spectroscopy. By developing a thorough understanding of Mn biosignatures in the laboratory, we can remove significant ambiguities that exist in data collected on Mars with measurements from a limited instrument suite and can optimize data acquisition and targeting for the Mars 2020 rover to identify and interpret specific Mn-related biosignatures should they be present on Mars.

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**Jared Leadbetter/California Institute of Technology**

**Closing A Critical Gap In The Manganese Cycle: Discovery, Cultivation, And Genomics Of The First Bacteria To Oxidize Mn(II) As An Energy Source For Growth**

A century ago the concept of lithotrophy, the generation of energy from inorganic substrates, developed via studies on microbial oxidations of NH3, H2S, and Fe(II). Each of these involves unique biocatalysts integrated into specialized energy pathways, often coupled to CO2 fixation into biomass. In both modern and ancient environments, these metabolisms have underpinned global biogeochemical cycles of N, S, Fe, and C, in large part contributing to habitability as we know it. Despite recognition of the importance of lithotrophy, large gaps remain in our knowledge of some biogeochemical cycles where lithotrophic metabolisms presumably have played a role. Chief among these is the Mn cycle. Mysterious Mn(IV) oxide nodules decorate the ocean floor, and similarly enigmatic Mn ore deposits are found near the Black Sea (20 Ma) and the GOE-associated Kalahari Fields (2.2 Ga). How did they arise? Thermodynamic calculations have long predicted that Mn(II) oxidation could sustain microbial growth, but until now, no organisms have been reported to do so. Some bacteria are known to slowly oxidize small amounts of Mn(II), but in these cases, energy is not harvested.

The PI has recently cultured the first known bacteria exhibiting unambiguous Mn(II)-oxidation dependent growth, i.e. in a defined environment devoid of other energy substrates or organic C. Reciprocally, rapid oxidation is dependent on the bacteria being viable, producing macroscopic Mn(IV) precipitates. The culture is stable, can be serially propagated, and has been refined to a 2-species consortium. Organism A, not yet grown alone, is novel, being only very distantly related to any previously cultivated organism described. Organism B has been isolated on another substrate, but doesn't oxidize Mn alone. The complete, refined genome sequences for both species of the consortium have recently been resolved. Preliminary analysis reveals that both species might fix CO2, and that both encode unusual e-transport gene clusters. A broad spectrum of analyses on the biology and minerology is now possible.

**OBJECTIVES/APPROACHES.** A combination of isotopic, genomic, transcriptomic, and evolutionary bioinformatic approaches will be applied to: 1) Determine if Mn(II) oxidation is coupled to CO2 fixation by the consortium; and if so, by which species. 2) Thoroughly evaluate the species' genomes for candidate determinants of Mn(II) oxidation. Mn(II) lithotrophs must solve key biochemical challenges necessitating significant novelties in electron transport, e.g. activating lousy, high potential electrons for respiration and CO2 fixation, and dealing
with the production of a metal oxide solid that must either be transported or generated outside of the cell. 3) Analyze the transcriptomes of Strain A grown in the Mn(II) oxidizing consortium with Strain B, and the latter grown alone on several alternative substrates. Comparative analysis will reveal genes uniquely expressed during Mn(II) oxidation. 4) Perform evolutionary and phylogenetic analyses on candidate genes putatively underlying the novel physiology. SIGNIFICANCE. This new line of inquiry will provide key insights into the molecular basis of a long-sought lithotrophic metabolism, providing a foundation for future studies. Knowledge generated through this work ultimately promises to inform the interpretation of large Mn(IV) oxide deposition events on the early Earth. This research is specifically responsive to the Early Evolution of Life and the Biosphere (points ii, iv, and vii) emphasis of this call, as well as the 2015 Astrobiology Strategy, sections 4.4.III and 7.1.IV (pp 70; 81-88, 147). Lastly, the reduction of Mn(IV) as a growth-promoting respiratory oxidant by anaerobes, discovered 30 years ago, intersects with and is relevant to other biogeochemical cycles. Implicit to the findings underlying this proposal: a novel, complete and heretofore unrecognized microbial-energetics-driven Mn redox cycle must now be entertained.

James Lyons/Arizona State University

Experiments On Small Isotopic Sulfur Molecules To Understand The Origin Of Sulfur Mass-Independent Fractionation In The Early Earth Atmosphere

The objectives of this research are to understand the chemical or photochemical mechanism by which sulfur isotope mass-independent fractionation (S-MIF) signatures were created in the ancient Earth atmosphere. The record of S-MIF signatures in Archean rocks is our best proxy for the rise of O2 about 2.4 Gyr ago. Since the discovery of S-MIF signatures in ancient rocks in 2000, most research into the S-MIF mechanism has focused on SO2 photolysis. It is becoming apparent that SO2 is probably not the origin of S-MIF. Here, we propose experiments to study photochemical fractionation in other small sulfur molecules likely to have been present in a pre-oxygenated Earth atmosphere, including SO, S, S2, and S4. These sulfur species are not relevant to the modern Earth atmosphere, although they are present in the modern Venus atmosphere. We are measuring cross sections and reaction products for the sulfur isotopologues of these molecules in order to identify possible S-MIF processes.

We use several techniques to analyze SO, S, S2, and S4. We will measure SO and S2 isotopic absorption cross sections using the Soleil synchrotron in France. This synchrotron has the best UV Fourier transform spectrometer available today for photon wavelengths < 300 nm. We generate SO and S2 by RF discharge in SO2 and H2S, respectively, and then we measure the UV absorption spectrum in the SO and S2. We will also use UV photofragment instruments at the University of California, Davis (UCD) to measure isotope fractionation in S atoms produced from SO photodissociation. Velocity-map imaging in a time-of-flight mass spectrometer will be used to elucidate the exact state parameters for the product S atoms, allowing us to fully characterize the abundances of isotopic S atoms produced during photolysis of SO, a process that would
have occurred in the ancient atmosphere. Finally, we will use a heated quartz flow tube to study the isotopic properties of \( \text{S}_2 \) self-reaction to form \( \text{S}_4 \). The products will be ionized by lasers and counted by time-of-flight mass spectrometry. Photochemical models will be used to synthesize these laboratory results into existing early Earth model atmospheres.

The proposed work lies within the research area "Early evolution of life and the biosphere" in the Exobiology and Evolutionary Biology Program. The rise of atmospheric \( \text{O}_2 \) was a major event in the history of life and environments on Earth. Our research will help to elucidate the operation of the ancient sulfur cycle, and provide a framework for understanding exactly how S-MIF signatures constraint atmospheric \( \text{O}_2 \) abundance.

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**Ulrich Muller/University Of California, San Diego**  
**Fragmented And More Frequent Ribozymes For An RNA World**

Introduction: The overall objective of the proposed research is to investigate how an early life form could have emerged on Early Earth, or other planets. The focus is on the RNA world hypothesis, which states that today's DNA/RNA/protein life forms were preceded by organisms using RNA as genome and as only genome-encoded catalyst. It is currently unclear how such an RNA world organism could have functioned, and how it could have emerged from a prebiotic environment.

Key objectives of the proposed research: The proposed research will study what conditions could make catalytic RNAs (ribozymes) likely to emerge from a prebiotic environment. Long RNA polymers are unlikely to have existed in a prebiotic environment, and shorter RNA oligomers were much more frequent. Two strategies will be studied that could have led to ribozymes from short oligomers: The assembly of larger ribozymes from short RNA oligomers, and the size reduction of ribozymes by fine-tuning RNA duplex stability.

Methods / techniques used in the proposed research: The proposed research will use an in vitro selection system we previously established in our lab to identify ribozymes under different conditions, and to identify ribozymes composed of multiple short strands. High Throughput Sequencing (HTS) analysis will be used for the analysis of the outcome of the in vitro selections. RNA biochemistry and Molecular Biology techniques will be used throughout the selections, and the analysis of resulting ribozymes. Those methods include PCR (polymerase chain reaction), in vitro transcription, purification by denaturing polyacrylamide gel electrophoresis (PAGE), cloning and sequencing, radiolabeling of RNA molecules, and quantitative analysis of the radiolabeled reaction products.

Perceived significance of the proposed work to the objectives of the research solicitation and to NASA interests and programs in general: The proposed research is significant for NASA's research emphasis 'Prebiotic Evolution' in at least three ways. First, the results of all three aims could identify new types of catalytic RNAs that would have been more
likely to arise in a prebiotic environment. This is important for NASA's objective to determine "what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere". Second, the in vitro selections at lower temperature will explore whether lower temperatures could favor the emergence of RNA catalysts. This will report whether a 'cold RNA world' would have helped its emergence from a prebiotic scenario, in line with NASA's goal to explore the "physical and chemical conditions within which living systems may have arisen." Third, the long-term goal of the proposed research is to generate a self-replicating and evolving system of nucleic acids in the lab. Therefore, in the long term the results of the proposed research address the question "what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere".

Beth Orcutt/Bigelow Laboratory For Ocean Sciences
Sorting Out Active Vs. Inactive Microbes In Subsurface Oceanic Crust Icy World Analogs

One of the biggest challenges in the translation of environmental microbiology research to understanding early-life, and the co-evolution of life and the environment, is untangling the functional potential of the vast suite of uncultivated microorganisms that represent the majority of microbial communities. While recent advances in DNA sequencing metagenomic and single cell genomic techniques have revolutionized the ability to uncover genomic information from these groups, linkage of this information to function in the environment is tenuous. This challenge is magnified in low-energy analog environments, where measuring rates of activity is difficult due to detection limits.

The goal of this project is to tackle this challenge through application of nascent single-cell-level fluorescent substrate labeling techniques that identify active cells using flow cytometric sorting and genomic sequencing in a relevant analog system. The project will take advantage of samples-of-opportunity to be collected from the subsurface environment of Earth's oceanic crust (and Icy Worlds analog), where fluid-rock reactions in mafic rocks support an energy-limited chemolithotrophic ecosystem. Specifically, this project will leverage an NSF-funded expedition in 2019 to subseafloor observatories on the eastern flank of the Juan de Fuca Ridge to enable in situ experimentation and collection of pristine samples. Live microbial cells in this high-pressure, thermophilic (64°C), anoxic system will be selected through incorporation of fluorogenic substrates inside the cell, enabling sorting of these cells from the bulk community through flow cytometry. Various metabolisms including carbon monoxide, iron, and sulfur oxidation will be explored through enrichment experiments pathways of relevance to constraining chemolithotrophy on extraterrestrial targets like Europa and Enceladus. Preliminary experiments conducted with similar subsurface ocean crust samples from a different system confirm that cells in low biomass, low energy systems can be labeled in this way, and that these cells are amenable to downstream genome sequencing.
If successful, results of this project will significantly advance understanding of the specific functional potential and viability of various members of low-energy subsurface analog microbial communities, regardless of whether they are dominant or rare members of the population. This would establish a revolutionary approach to connecting physiology to genomic information, which has applications beyond Exobiology. Confirming the application of this method in a remote analog system would impact NASA mission interests seeking to identify methods for biosignature detection and possible scenarios for experimentation on future rovers to identify life.

The proposed project is relevant to specific research emphases in this solicitation as well as several of the Major Topics in the NASA Astrobiology Strategy 2015. By sorting active cells from ocean crust analog environments, and then being able to directly link cell physiology with genomic information, this project directly addresses the solicitation interests in understanding the phylogeny and physiology of extremophile microorganisms that may reflect the nature of primitive environments and the co-evolution of these microbial communities that drive the biogeochemical processes in these analog environments, which is likewise directly relevant to themes in the Major Topic Co-Evolution of life and the physical environment. Moreover, by focusing on energy limited analog systems relevant to NASA’s interest in Icy Worlds, this research directly contributes to themes in the Major Topic Early life and increasing complexity.

Chris Reinhard/Georgia Tech Research Corporation
Upside-Down Biospheres and the Remote Detectability of Life on Reducing Planets

The astrobiology community is currently on the verge of the first detailed characterization of habitable extrasolar planets. Large space-based telescope missions currently in their science definition phase will directly image terrestrial exoplanets at UV to near-IR wavelengths, while future ground-based observatories will also be able to spectrally characterize the atmospheres of small planets around the very nearest stars. Indeed, for the foreseeable future our only accessible method for detecting life or even fully characterizing habitability beyond Earth will likely be deciphering the chemistry of exoplanetary atmospheres.

As we develop a search strategy for life beyond Earth, the evolutionary history of our own planet provides a powerful natural lab for examining the processes that promote the emergence and maintenance of atmospheric biosignatures. However, the chemistry of Earth’s ocean-atmosphere system is regulated by a vast network of microbial metabolisms linked by competition for substrates, syntrophic cooperation, and production of toxic waste products. In addition, the array of basic metabolic pathways and major processes structuring the nature of their connectivity have not been static through Earth’s history, nor are they expected to be generally applicable to habitable Earth-like planets. For example, the processes involved in the direct and indirect metabolic consumption of photosynthetically produced O2 form a critical network regulating the major biosignature gases in Earth’s atmosphere. More generally, habitable
planets hosting bacterial biospheres that do not produce or consume molecular oxygen (O2) reducing or anoxic worlds may be common throughout the Universe. Indeed, this planetary state may have dominated much of Earth’s earliest history.

Our motivation, though multi-faceted in practice, can be conceptually distilled to a single basic question:

What metabolic networks promote the emergence and maintenance of atmospheric biosignatures on primitively reducing planets?

We will provide fundamental new constraints on this question by developing a new ecophysiological module for use in large-scale planetary system models. This module will contain a suite of primitive and derived microbial metabolisms relevant for a wide range of habitable planets, and will be designed for flexible integration across a range of platforms. As proof of concept, we will embed this module in a simple coupled ocean-atmosphere photochemical model (ATMOS) and in an Earth system model of intermediate complexity (cGENIE) and provide the first detailed reconstruction of Earth’s upside-down biosphere during the Hadean and early Archean Eons.

According to Program Element C.5 of NASA’s Research Opportunities in Space and Earth Sciences (ROSES), 2015, the overarching goal of the Exobiology program is: [T]o understand the origin, evolution, distribution, and future of life in the Universe [&] in the context of NASA’s ongoing exploration of our stellar neighborhood and the identification of biosignatures for in situ and remote sensing applications. The research proposed here will be designed to provide significant steps forward in our predictive understanding of the links between microbial metabolism and atmospheric biosignatures, rendering it germane to both the overarching goals of the Exobiology program and many of the major foci delineated in the most recent NASA Astrobiology Strategic Plan, in particular Section 3.4 (Early life and increasing complexity), Section 4.4 (Co-evolution of life and the physical environment), and Section 5.4 (Identifying, exploring, and characterizing environments for habitability and biosignatures).

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Tyler Robinson/Northern Arizona University
Back to Basics: Assessing Earth’s Atmospheric Biosignatures from Space and Across Time

Exciting results from NASA's Kepler mission indicate that as much as 100% of Sun-like stars have Earth-sized planets orbiting within their Habitable Zone. Substantial recent progress has been made in understanding how spectroscopic observations of such planets can be used to reveal atmospheric composition. Simultaneously, new results have revealed how atmospheric composition can indicate signs of chemical disequilibrium (which can be a basic signature of life). We propose to pair these two approaches by building a first of its kind tool for detecting biosignature gases and signs of atmospheric chemical disequilibrium for exoplanets. Our novel inverse model for detecting key
signatures of life will then be applied to both real and simulated observations of Earth. Finally, using an existing modeling suite, we will generate and interpret simulated observations of Earth through time relevant to under-study NASA mission concepts to enable an understanding of how easy (or hard) it would be for these future missions to detect diverse signs of life for distant Earth-twins at key stages of geological, biological, and atmospheric evolution.

Our proposed work is relevant to both NASA's Strategic Plan 2018 and to the Exobiology Program. We aim to shed light on the various signatures of life that appear in Earth's evolving spectrum, and we will address strategies for observing these biosignatures for Earth-like exoplanets. Both of these goals are relevant to NASA's Strategic Plan as they seek to understand Earth, search for life elsewhere, and to "develop tools for detecting life," all of which are highlighted under Objective 1.1. Our work falls under the "biosignatures and life elsewhere" emphasis of the Exobiology Program, and we aim to develop remote sensing tools for detecting life (which is a topic also highlighted by the Exobiology Program). Finally, our work addresses Goal 5 of NASA's Astrobiology Strategy ("Identifying, Exploring, and Characterizing Environments for Habitability and Biosignatures"). Through our emphasis on understanding how future NASA missions might detect signs of life from Earth-like exoplanets, we address Part IV of Goal 5 ("How can we [. . .] search for life beyond the Solar System?") as well as key exoplanet research questions (e.g., "What future technologies must be developed in order to best characterize exoplanets for [. . .] life?").

Stephen Romaniello/Arizona State University

Oxygen is a fundamental requirement for the development of complex life, and its buildup in the Earth system has been a major focus of geobiological research. Although many studies have focused on the major oxygen pulses that bookend the Proterozoic Eon, fundamental questions persist about global redox during the middle chapters of Earth history. This is problematic because this mid-Proterozoic interval (~1.8 to 0.8 billion years ago) was characterized by a major diversification of early eukaryotes that set the stage for subsequent animal evolution. Existing constraints on atmospheric pO2 range more than two orders of magnitude, hindering our ability to decipher the role that oxygen played in driving key milestones in eukaryotic evolution.

Uranium (U) isotopes can potentially provide critical new constraints because the U isotope composition of seawater which can be recorded in marine carbonates is controlled by the global extent of seafloor anoxia. Development and validation of the U isotope proxy in carbonates was the subject of prior support to our group from NASA Exobiology, and the current proposal is a logical extension of the pioneering work that resulted from that support. Over the past year, our team has amassed a large U isotope dataset for carbonates of mid-Proterozoic age. Surprisingly, U isotope values (0.40±
0.280 [Å] are similar to modern seawater, suggesting that large portions of the mid-Proterozoic oceans might have been oxygenated. However, there are still major gaps in our understanding of the U isotope system that preclude definitive interpretation. U isotope values in this range could also be consistent with a largely suboxic or anoxic, iron-rich (ferruginous) ocean. Unfortunately, existing constraints on U isotope behavior under suboxic and ferruginous conditions are limited to just a few data points.

Here, we propose to systematically investigate U isotope behavior in suboxic and ferruginous settings to refine our ability to interpret our U isotope data. Our proposed work involves: 1) biotic and abiotic U-reduction experiments to constrain fractionation factors associated with a variety of U-reduction pathways in the presence of Ca; 2) comparison of U isotope sediment profiles from two modern suboxic analogues; and 3) water and sediment U isotope measurements from two modern ferruginous environments.

Specifically, we will investigate both microbially-mediated and abiotic U-reduction under a variety of conditions, focusing on how Ca concentrations (which affects U-speciation) and the reductant (H2S, Fe(II)aq, microbial Fe-reducers) influence U isotope fractionation. We will constrain U isotope fraction under suboxic and anoxic conditions using publicly-available ODP cores from two prominent upwelling zones: the Oman and Peru margins. Sediment profiles at these sites record expansion and contraction of the oxygen minimum zone on millennial timescales, allowing us to investigate U isotope behavior under a range of low-oxygen, low-sulfide conditions that existed in these basins over the past 30,000 years. Finally, we will explore U isotope fractionation under ferruginous conditions in two ferruginous lake analogs, Canyon Lake (Michigan) and Kabuno Bay (East Africa). These sites provide an ideal analogue for studying U isotope behavior under ferruginous conditions, which may have been prevalent in the Proterozoic oceans.

This experimental and modern analogue approach will substantially refine our ability to interpret the U isotope record, eliminating critical blind spots in our understanding of U isotope geochemistry. Moreover, when combined with our existing mid-Proterozoic dataset, this study will provide robust new constraints on the redox landscape of the mid-Proterozoic oceans, thus allowing a fundamental re-evaluation of the relationship between oxygenation and evolution on the early Earth.

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Sara Seager/Massachusetts Institute of Technology
A Database Approach to Life's use of Chemical Space for Insight into the Nature and Signatures of Life on Other Worlds

The question of what atmospheric gases indicate the presence of life both on solar system and exoplanets is an old one. To guide new research we have taken a data-driven approach extending beyond simple extrapolation from terrestrial biochemistry, and based on our compilation of three separate yet intertwined databases. The first is a database of all molecules that are volatile and stable at standard temperature and pressure (Seager et
al. 2016). The second is a collection of molecules produced by life (natural products), manually curated from available literature and public data repositories (Petkowski, Bains, Seager, 2018 in press). This database contains genuine natural products (not drug metabolites or synthetic derivatives) and each natural product is connected to species from which the product was isolated. The third database is under construction and is a proxy for hydrolytically stable chemical space, created by combinatorics from seed molecules and filtered by hydrolytic and thermodynamic stability calculations and estimators.

We now have developed technology to describe the structure and thermodynamics of the chemical space from which a biochemistry is selected. This allows us to map the chemical space of life onto an exhaustive space from which biochemistry is selected, and hence identify both the chemistry that life uses and the lacunae that life avoids.

Building on this work, we propose to 1) mine the databases both to find not yet considered potential biosignature gases and explore some of them and 2) map how Earth life explores chemical space by a few concrete examples of chemical functionality life avoids. The goal is to extend concepts of possible chemical evolution relevant to the origin, evolution, and distribution of life.

For the first topic, we consider exoplanets with atmospheres with no molecular oxygen and low to medium host star ultraviolet radiation. This opens the possibility for gases to accumulate to detectable levels, even for molecules rapidly destroyed in Earth's atmosphere, thus expanding the biosignature gases we should be aware of. Preliminary work shows phosphine, isoprene, and halocarbons are worthy for detailed exploration. We will use existing exoplanet atmosphere codes, existing photochemistry codes, and existing molecular line lists or molecule cross sections or estimates.

For the second topic we propose a radical new approach, based on exploring how life avoids or rarely uses certain specific chemical classes of molecules as well as the molecules it does use. Some of the excluded molecular classes are chemically flexible, stable, and have wide chemical and structural functionality. This approach has not been attempted before; instead the community has focused on a wide range of isolated hypotheses as to why life does use certain specific chemical functionalities (e.g. peptide bond, phosphates etc.). We have quantified occurrence rates of different bonds, molecular fragments, and molecules amongst life's products, solidifying known, yet, chemically puzzling gaps amidst the vast diversity of the chemistry of life. In recent work we present a hypothesis for life's near avoidance of N-S containing compounds (Petkowski, Bains, Seager, in press) and the proposed work is to explore additional concrete examples. For this topic we use a custom chemical combinatorics code, a suite of Python tools both custom-made and existing for chemoinformatics, literature review for reaction rates, and experiments involving NMR time course reactivity assays.

The significance of this work is that our new approach may provide fresh insight into life's evolution through chemical space and its origin and early evolution as well as an insight towards possible biochemistries and biosignatures of non-Earth-like life elsewhere.
Science Goals & Objectives
We seek to characterize the molecular and isotopic biosignatures of microbial ecosystems of the Dirty Ice environments of the McMurdo Ice Shelf (MIS) of Antarctica. These are little-studied melt-water ponds, protected from anthropogenic disturbance and with unique environmental constraints. They are near to freezing even in summer, have diverse chemistries, waters of mixed origin and an unusual light regime. They are also oases of microbial biodiversity in a landscape that is otherwise largely lacking liquid water and poorly supportive of life. The primary objectives of our research are to characterize the biosignatures of a suite of MIS ponds, and to determine which have the potential to be predictive for analog environments. These data will inform studies of a variety of ice-bound ecosystems, potentially including those of icy moons. Moreover, the physiography of the McMurdo Dirty Ice system is such that similar environments were likely widespread during the Cryogenian (720 to 635 Ma). The biosignatures we identify could thus have particular impact on our understanding of the persistence and evolution of life through the Snowball Earth glaciations.

Hypotheses & Measurements
The environmental constraints of the MIS ponds lead to unique biological communities and, we hypothesize, unique biosignatures. Prior work has shown that the chemistries of the ponds vary widely; primary production is dominated by cyanobacteria coexisting with algal protists; and the communities face perennial cold and potentially limiting light, CO2, and fixed N. Based on these characteristics, we hypothesize a number of likely biosignatures: 1) very high ratio of bacterial/algal lipids, 2) abundant cyanobacterial lipids distinct from those of tropical to temperate mats, 3) lipids reflecting adaptation to perennial cold and freeze/thaw survival, 4) heavy ¹³C and ¹⁵N values reflecting CO2-limitation and N-fixation, respectively; 5) positive organic and/or pyrite ³⁴S values reflecting consumption of marine sulfate in a closed system; 6) variable water and lipid ²H values due to mixing of seawater and glacial ice. More generally, we will search for relationships and correlations among environmental variables, community compositions and their diagnostic lipid and isotopic assemblages to assess the processes influencing biosignature formation, preservation, and destruction. We propose to test these hypotheses by applying state-of-the-art methods of lipid characterization (HPLC-MS-MS, GC-MS) and stable isotope analysis (GC-IRMS, EA-IRMS, and MC-ICP-MS) to samples from 16 ponds and 4 cryoconite pans that were previously collected by PI Summons and collaborators Jungblut and Hawes. No field work is requested by this proposal, although our collaborators will be returning to MIS in 2019 and could collect further samples if needed. Collaborator Jungblut will also be conducting further genomic characterization of splits from the same samples, allowing us to directly compare lipid and stable isotope data with phylogenetic identities.
Significance & Relevance
A prime focus for future research outlined in the NASA Astrobiology Strategy 2015 document is the study of habitability indicators, including biosignatures and their interpretation within planetary and environmental contexts. Our research will characterize the Dirty ice microbial ecosystems, the range of parameters that influence their habitability, and ways in which they may be preserved, detected and characterized in the ancient rock record and, potentially, on other icy worlds. A second focus of the Astrobiology Strategy (Section 4), is the Co-Evolution of Life and the Physical Environment, including major changes in the physical state of the planet such as Snowball Earth episodes with hypothesized links to biological innovation. The biosignatures we seek will provide a direct path to investigating links between the Cryogenian and evolution of complex life.

Wesley Swingley/Northern Illinois University
Chlorophyll D As A Model For Biosignature Evolution

The search for life beyond Earth has focused on planetary bodies within our Solar System. However, the remote detection of life on planets orbiting other stars is fast becoming a reality. The Kepler mission has detected thousands of extrasolar planets, and based on this statistical sampling, researchers now estimate that there are billions of rocky exoplanets in our galaxy alone (e.g. Dressing and Charbonneau, 2015; Silburt et al., 2015). The next step beyond detection is to characterize exoplanet habitability and then search for signs of life using ground- and space-based telescopes. Because these planets are so far away, these signs of life must be large, planetary-scale phenomena that are remotely detectable.

On Earth, our strongest biosignatures are from atmospheric oxygen and the vegetation red edge. The latter is a spectral reflectance feature of plant leaves that is observable through the atmosphere, manifested as absorption of most visible light between ~400 700 nm contrasting in the red with the scattering or reflectance in the near-infrared (NIR). Both of these biosignatures are a consequence of oxygen-producing (oxygenic) photosynthesis originally derived from cyanobacteria more than 2.5 billion years ago. The red wavelength of the red edge is derived from the primary oxygenic photosynthetic pigment, chlorophyll a (Chl a), present in all plants and cyanobacteria, and whose peak absorbance occurs in the red. While many anoxygenic bacteria (that is those that do not produce oxygen as a product of photosynthesis) absorb light well into the NIR, cyanobacteria have developed only two pigments to extend their peak absorption into this range, Chl d and Chl f. It has been presumed that the energetic limitations of splitting water to oxygen is a hard upper constraint for cyanobacterial absorbance.

The cyanobacterium Acaryochloris, with Chl d as its primary pigment, is the only known oxygenc phototroph to use a far-red light-absorbing pigment in its photosynthetic reaction centers, directly powering the conversion of water to oxygen and is, as such, of
profound interest for understanding the low energy limits on this chemical conversion
both on our planet and on potentially habitable exoplanets, particularly those orbiting M
dwarfs that emit light primarily in the NIR. By characterizing the upper wavelength limits
and limitations for Chl d-based photosynthesis in Acaryochloris, we hope to lay the
foundation for constraining photosynthetic biosignatures on exoplanets orbiting cool
cars, and from there generalize for any stellar temperature. This will contribute to a
quantitative theoretical understanding of the energy limitation of this metabolism, which
is needed to assign confidence levels to exoplanet observations seeking signs of life.

We will address our central hypotheses by coupling genomic and biochemical
characterization of Chl d-based photosynthesis in Acaryochloris, with the ultimate goal of
establishing energetic limits on oxygen evolution on Earth as a constraint for assessing
exoplanet biosignatures. To that end, the primary goals of this proposal are to: 1)
characterize the Chl d biosynthetic machinery in Acaryochloris and other cyanobacteria;
2) establish upper absorption wavelength and lower photon irradiance limits on oxygenic
photosynthesis in Acaryochloris, other far-red pigment producing cyanobacteria, and Chl
a-only cyanobacteria; and 3) assess the energetic and physiological trade-offs (if any) of
utilizing far-red light for oxygenic photosynthesis.

Mark Urban/University of Connecticut, Storrs
Overcoming Adaptation: How Colonization, Evolution, And Competition Determine
The Assembly Of Extremophile Communities

Background
Discovering how communities assemble in novel environments is critical to
understanding the evolution of early life and the biosphere. Increasingly, observations,
experiments, and theory suggest that evolution can affect the formation of complex
communities. We developed theory on one such dynamic called the monopolization
effect. A species monopolizes a system if it arrives early, adapts to novel conditions, and
thereby alters the establishment of other species. Importantly, monopolization theory
suggests that the characteristics of remote ecosystems will depend stochastically on the
traits of the first life to evolve or arrive.

The extremophile microbes we study are likely analogues for extra-terrestrial life.
Archaea from the salt extremophile genus Haloferax can survive the extreme high
temperatures (> 50°C) and salty conditions likely found in exposed, low-precipitation
planetary environments. Our work suggests that these extremophiles can adapt quickly to
novel conditions, and not just by mutation: Haloferax can take up DNA directly from
other species (horizontal gene transfer) or from the environment, which can facilitate
their rapid adaptation. Thus, a late-arriving colonizer could cheat monopolization by
taking up DNA from residents, thus facilitating its adaptation and establishment.
Key objectives
1) Does adaptation of a resident species allow it to monopolize a novel environment and decrease the colonization success of future colonizers?
2) Does DNA uptake allow a late-arriving species to adapt and establish despite monopolization?
3) How does genetic background and epistasis influence the role of horizontal gene transfer?
4) How do varying levels of colonization influence community dynamics in multi-patch, linked systems?

Methods summary
We will allow residents (Haloferax volcanii) to adapt to novel environments created by combining different temperatures and salt concentrations. Following different periods of adaptation, we will introduce a second species (Haloferax mediterranei) adapted to standard conditions. We predict that longer periods of adaptation will enhance monopolization by H. volcanii.

Like many early life forms, Haloferax can exchange genetic material. The resident’s adaptations could be passed on to the invader, overcoming the monopolization effect. By using genetic knockouts, we will create treatments with and without horizontal gene transfer. We predict that horizontal gene transfer will allow the late colonist to adapt eventually and overcome monopolization. However, epistatic interactions between the invader’s genes and horizontally transferred genes might reduce their fitness advantage. Therefore, we will contrast the effect of horizontal gene transfer on the establishment of invaders with the same versus a different genetic background. We predict that epistatic interactions from the divergent species’ genome will reduce the advantages of horizontal gene transfer.

Lastly, we will create a system of linked, heterogeneous patches to test how evolution and stochastic colonization affect the entire metacommunity. We predict that lower colonization rates will enhance monopolization of habitats by one species, whereas higher colonization will lead to partitioning of environments by species and greater regional and local diversity.

Significance
This research will directly address three major goals (ii., iv., v., vi.) of the Exobiology emphasis on Early Evolution of Life and the Biosphere, including horizontal gene transfer, the coevolution of microbial communities, the processes by which new species are added to extant communities, the evolution of microbial species subject to long-term environmental change, and development of key biological processes. More generally, research will provide an important test for novel theories about the evolution of communities and ecosystems in remote and extreme environments.