Exobiology
Abstracts of Selected Proposals
(NNH20ZDA001N-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. 157 proposals were received in response to this opportunity. On October 15, 2020, 14 proposals were selected for full funding and 9 for partial funding.

Eric Anslyn / University Of Texas, Austin
Exploring Alternatives to the Current DNA-RNA-Protein Basis for Life. An Examination of TORC-Based Templation and Replication

The NASA exobiology program seeks to explore alternatives to the current DNA-RNA-protein basis for life. This is especially important because we really only have one data point for what life looks like, and only a few data points for what biological information storage and replication look like. While there have been some attempts to create synthetic systems capable of sequence-specific replication, these have mostly been based on nucleobases, or are dimeric systems with little information content. Interestingly, these exceptions seem to imply that nucleic acids are somehow special, since the other replicators do not reproduce sequence nor do they allow for mutational substitution. In order to generate an information rich system capable of sequence-specific replication and mutation, we propose to develop a new kind of replicator, one that contains unnatural monomers capable of complementary Dynamic Covalent Bond (DCB) formation, which may ultimately be capable of selection and evolution. The difference between information rich hydrogen-bonding and DCB formation will likely mean that the replication properties will be substantively different than those in the living systems we currently know. This new data point for replication and evolution should inform not only the search for new materials on Earth, but also how we look for life in the stars. Further, due to the fact that the bonds exchange more rapidly at elevated temperatures, DCBs are a potential chemical means to store sequence information on hot planets, where the non-covalent interactions used on earth would not be as efficient. While the basis for life on earth is nucleic acids, the complementary shape and hydrogen bonding patterns of the nucleobases are not the only way to generate complementary pairs. For example, our group has created a series of four pairs of nucleophile/electrophile partners, all of which participate in reversible covalent-bonding, and we have shown that the dynamic exchange of partners are complementary and show no cross-reactivity. Thus, these Tunable Orthogonal Reversible Covalent (TORC) binding pairs are predicted to have a fidelity of information transfer higher than nucleic acids. The goals of our proposed NASA project will be to explore information transfer, fidelity of replication, and tuning the kinetics of replication. We intend to create macrocycles of TORC-mers via standard chemical design strategies, optimize templation of a complementary copy, dissociation of the template/copy complex, and create rounds of templating/copying, thus optimizing replication. Two systems are envisioned for replication, the first being peptide coupling

that is irreversible, while the second is olefin metathesis. The monomers participate in dynamic bonding while the metathesis catalyst acts as a polymerase. In addition, metathesis introduces the ability for error correction, also allowing the system to evolve to the most efficient replicators. PCR-like thermal cycling should lead to rounds of replication with either system. The broader impact, that goes beyond the basic science exploits, derives from two undergraduate laboratories ran by Dr. Anslyn, directed at freshman chemistry and sophormore organic chemistry students. These research labs, of approximately 30 students each, adopt projects related to the funded projects on-going in the Anslyn labs, and thus both labs will be directly involved in this NASA project, exposing around 60 undergraduates to this project. In summary, this NASA project presents questions of whether chemical replication can be created via dynamic covalent bonding pairs rather than the hydrogen bonding/base-stacking that nucleic acids use. Because TORC bonds exchange rapidly at elevated temperatures, they could be used to transfer sequence information on hot planets. And lastly, we address the use of organometallic catalysis for coupling monomers, which mimics the possibility of metals and minerals to catalyze ligations.

Joan Bernhard / Woods Hole Oceanographic Institution
The Metabolic Innovations of Benthic Protists in Response to Anoxia: Foraminifera As Models

The overall goal of this project is to advance our understanding of the evolution of eukaryotic life on Earth by examining extant eukaryotic taxa that live in an analog environment for early Earth conditions, and how symbioses and metabolic pathways used within these holobionts permit inhabitation by eukaryotes of a wide variety of environmental conditions, including sulfidic, anoxic, and hypoxic sediment pore waters. Many eukaryotes, especially protists, are known for forming symbioses as well as harboring organelles from other eukaryotes; such associations may bolster the host s independence from oxygen. The full extent of protistan physiological capabilities is far beyond present understanding. The under-appreciated protists represent successful life forms in anoxic habitats that can bring a level of sophistication to their adaptive strategies, in that they have organelles and/or microbial partners that can bolster their ecological reach. Further, when considering the complexities permitted with protist-prokaryote symbioses, the possible physiological permutations are immense. For these reasons, the study of anaerobic protists may be key to the search for more complex microbial life beyond Earth.

Foraminifera (hereafter forams) are a successful and diverse protistan group that evolved in the Precambrian. Certain foram species thrive near anoxia; others in euxinia (sulfide enriched anoxia). Most of these have the ability to denitrify, an anaerobic respiration process, although debate exists whether this is bona fide eukaryotic denitrification or from prokaryotic associates. As with many other protists, details of foram metabolic innovations are largely unknown.

California s Santa Barbara Basin (SBB), with its well-developed, relatively stable chemocline, creates stagnant bottom waters that can be considered an Ocean Worlds analog. While forams are common worldwide, SBB sediments support abundant forams, even in the most sulfidic zones, where they can dominate eukaryotic biomass. Three species of SBB forams will be used as models because each has different cellular ultrastructure, where two have endobionts of different morphologies; the third is kleptoplastidic (harboring chloroplasts) despite inhabiting sediments far below the classic photic zone. On two short research cruises, five hypotheses will be tested to achieve the project s objectives, which are to: 1) assess the transcriptional activities of the three target holobionts in their natural habitat using specimens preserved in situ on the seafloor; 2) obtain draft genomes of the three target foraminifera and their associated symbionts to elucidate metabolic potential of partners; 3) assess differences in selected metabolic activities thought to be relevant to these symbioses, under low oxygen vs. euxinic conditions; 4) localize the site of selected metabolic activities within foram-symbiont holobionts using correlative TEM-nanoSIMS and IRMS approaches; and 5) assess metabolic activities and potential exchanges between holobiont partners that underpin holobiont survival under anoxia.

David Catling / University Of Washington, Seattle
Biogeochemical Studies of the Early Earth and Implications for Life Elsewhere

The environmental conditions on the early Earth are essential for understanding the evolution of early life and the eventual advent of advanced life. However, there are very few direct constraints of basic environmental variables for the early Earth, such as partial pressures of some key atmospheric gases and even the pH of the ocean and surface waters. We propose to add new, data-based constraints to the early Earth s environment and put those in the context of early Earth biogeochemistry and early Earth analogs orbiting other stars. Our work will help the potential detection of habitable planets and life elsewhere.

We propose 3 tasks. Task 1 will involve new work using the oxidation state of iron-rich micrometeorites found in the rock record to constrain atmospheric CO2 levels on the Earth through time. In preliminary published work, we showed that high levels of CO2 can oxidize micrometeorites when they melted as they entered the early Earth s atmosphere. We constrained CO2 for one time in the Archean using a kinetic model, but the constraint had large uncertainty of on CO2 of 6-100%. The uncertainty arises for two reasons: very few micrometeorites and an uncertain reaction rate between CO2 and molten Fe from lack of experimental data. To resolve these issues, in Task 1A, we will extract new micrometeorites from Precambrian rocks (including from a ~635 Ma cap carbonate associated with the termination of a Snowball Earth); in Task 1B, we will conduct lab measurements of liquid Fe oxidation under appropriate CO2 conditions for micrometeorite entry; and, in Task 1C, we will compute new constraints on CO2 from the Archean to Neoproterozoic.

Task 2 will newly determine a time series of ocean pH inferred from 12 marine carbonates, spread from the Archean to Neoproterozoic. In preliminary work, we have developed a boron isotope measurement method for marine carbonates and applied it to 2.6 Ga carbonates. A new, preliminary boron isotope cycle model allows us to infer ocean pH from Precambrian boron isotope data. We will combine our new boron isotope data with our numerical analysis to estimate seawater pH values through time. The pH is related to the atmospheric CO2 levels. Together Tasks 1 and 2 will fill an important gap in knowledge by newly constraining the carbon cycle through time for the early Earth.

Task 3 involves global carbon cycle and atmospheric modeling to put our new empirical data into the context, including that of life on other planets. In Task 3A, using our carbon cycle model, we will estimate limits on pCH4 through time given the new pCO2 and pH data. If high CH4 supported warm surface temperatures in addition to CO2, then lower pCO2 would allow higher seawater pH. We will consider our results applied to Earth-like exoplanets because biogenic CH4 in background CO2 creates major chemical disequilibrium of the Archean Earth, which is a remotely detectable biosignature. We will assess the extent to which our new data would make an Archean Earth analog distinct. In Task 3B, we will use a modified version of our carbon cycle model to simulate the time-dependent evolution of pCO2 and ocean pH of Snowball Earth, particularly deglaciation to compare with our new cap carbonate data from Tasks 1-2. Current theory of post-glacial melt timescales of ~2 kyr conflicts with magnetic reversal evidence in cap carbonates of ~1 Myr, suggesting an incomplete understanding that we hope to resolve.

The overall results of this work will be relevant to the Exobiology call for studies concerning early Earth environments and life: possible chemical evolution relevant to the distribution of life and biosignatures and the impact of planetary processes ("Snowball Earth" events, rapid climate change, etc.) on the large-scale evolution of life on Earth . More generally, proposed work will support an understanding of the planetary co-evolution of life and environment in the context of NASA s goal of detecting life elsewhere.

Shelley Copley / University Of Colorado, Boulder
Confronting A Conundrum: The Prevalence of Loss-of-Function Mutations During Adaptive Evolution

Microbial populations since the LUCA have evolved to colonize habitats ranging from the dry valleys of Antarctica to sea-floor hydrothermal vents to the guts of mammals. Microbial evolution continues today as humans release pharmaceuticals, pesticides, solvents, explosives, and industrial and household chemicals that can serve as new sources of carbon, nitrogen or phosphorus for hungry microbes in soil and water.

Experimental evolution studies have shown that loss-of-function (LOF) mutations that re-wire metabolic and/or regulatory networks are the most common mechanism for microbial adaptation to environmental challenges. (We will define LOF mutations as

those that either abrogate or diminish function.) LOF mutations have been shown to contribute to increased virulence, resistance to an antibiotic or pathogen, increased growth rate on a particular carbon source, and recovery after deletion of an essential gene.

It is not surprising that LOF mutations are more common than gain-of-function (GOF) mutations; there are so many more ways to break a gene than to confer a new function. Pre-mature stop codons, insertions, deletions and non-synonymous point mutations can decrease protein stability, prevent intermolecular interactions, or disrupt catalytic function. In contrast, acquiring a new function typically requires very specific changes in a limited number of amino acids.

LOF mutations may also dominate early in adaptation because they provide an immediate large benefit due to alterations in gene expression and/or metabolism. In contrast, GOF mutations may initially provide small fitness improvements. This supposition is supported by many enzyme engineering experiments; it usually takes several mutations to produce an enzyme with catalytic capabilities comparable to those of extant enzymes. GOF mutations may also be rare during adaptation because mutations that confer a new function may damage an original function. The net effect of such trade-offs depends upon the strength of selection for both the new and original functions.

The prevalence of LOF mutations in experimental evolution presents an existential problem: how could life have evolved to occupy essentially every habitat on Earth if most adaptive mutations cause LOF?

We will examine two possible solutions to this conundrum. First, fluctuating environmental conditions may select against clones that have adapted by LOF mutations and select for those that have acquired gain-of-function (GOF) mutations, or at least less-damaging LOF mutations. Second, early LOF mutations may buy time , allowing survival until a better solution to the environmental challenge is found. In this case, reversion, compensatory mutations or repair via horizontal gene transfer could restore the functions of damaged proteins.

We will address these possibilities using a model system in which metabolic innovation is required for survival. E. coli lacking pdxB cannot make the essential cofactor pyridoxal 5-phosphate (PLP). However, prolonged incubation in glucose leads to emergence of variants that do grow, albeit slowly. These variants acquire additional mutations that restore robust growth within 150 generations. PLP synthesis is reconstituted by a novel pathway patched together from four promiscuous enzyme activities that feeds material into the normal pathway downstream of the block. Notably, nearly all of the mutations in the adapted clones from our initial experiments cause LOF. This system provides an excellent model for examination of how LOF mutations are either avoided during evolution of a new pathway, or repaired after an efficient new pathway has emerged.

This project responds to the goal of the NASA Exobiology program to understand the evolution of life, and specifically to the goal of investigating the evolution of genes and pathways.

Dionysios Foustoukos / Carnegie Institution Of Washington
Physiological Adaptations In Hydrogenotrophic Bacteria At Extreme Pressures

Proposed research targets two questions: (i) how does life evolve under high-pressure and energy-limited conditions? and (ii) what is the role of virus-host interactions for the evolution of deep-branching extremophiles found in deep-sea hydrothermal vents? Our goal is to integrate microbial physiology and functions with ecosystem dynamics to describe piezophilic growth and phage-host interactions in extremophilic biospheres.

We aim to constrain: i) the physiological adaptation of extremophiles to the pressure and nutrient levels found at deep-sea hydrothermal vents, and ii) phage-host interactions and coevolution. We will employ a novel anaerobic, chemolithoautotrophic and thermophilic Epsilonproteobacterium (Nautilia strain PV-1) that exhibits piezophilic growth. The genome of strain PV-1 carries a complete prophage.

Task 1: How do piezophiles adapt to pressure gradients present at deep-sea and subsurface environments? We hypothesize that the membrane lipids saturation in Nautilia strain PV-1 decreases as the hydrostatic pressure increases. We will determine the changes in the membrane lipid composition (fatty acids and polar lipids) of strain PV-1 during growth at different pressures.

Task 2: What is the role of environmental DNA uptake as an adaptation mechanism to pressure and nutrient-limitation stresses? We hypothesize that low and/or high pressure and nutrient availability regimes differentially affect the expression of DNA uptake genes in Nautilia strain PV-1 when exposed to exogenous DNA. We propose experiments to assess the level of expression of competence-related genes during the uptake of free DNA in strain PV-1.

Task 3: We hypothesize that the life cycle of the prophage hosted in Nautilia strain PV-1 is affected by pressure and nutrient conditions that differ from those for optimum growth. We aim to: (i) investigate the pressure adaptation that triggers the lytic cycle of the bacteriophage; (ii) identify the nutrient regimes that trigger lytic cycle in PV-1; and (iii) investigate the ability of the PV-1 phage to infect its relatives in the order Nautiliales.

Methods: We will utilize a novel high-pressure/temperature bioreactor that allows for the continuous culturing of microorganisms at conditions that resemble deep-sea hydrothermal vent habitats (<120 °C, <50 MPa). Our continuous culture approach provides a unique opportunity to study microbial physiology by adjusting pressures dynamically and to monitor the changes in the proteome during transition between pressures. By maintaining the cultures in a steady state, we can accumulate biomass to

levels sufficient for quantitative proteomic analysis. We will: (i) integrate proteomic analysis with qRT-PCR to quantify gene expressions related environmental DNA uptake; (ii) perform lipid analyses to assess membrane fluidity, and (iii) investigate phage production and cell lysis.

Training: We will support the academic development of a postdoctoral researcher and high-school student interns. The postdoctoral researcher will receive training in all aspects of the proposed study (e.g., high-pressure culturing, proteomic analyses, chemical analyses, phage induction). Students will present their work at a special EPL/CIW Summer Internship Symposium.

Relevance: This proposal is relevant to the Exobiology program and more specifically to the Early Evolution of Life and the Biosphere area of research. Our results will contribute to the goals of the RCNs: (i) Network for Ocean Worlds by advancing studies to understand life in relevant ocean world analogues and beyond and (ii) From Early Cells to Multicellularity through targeted exploration of the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments, as well as testing the original nature of&membrane function.

Nir Goldman / Lawrence Livermore National Laboratory/Dept of Energy
Impact Synthesis of Prebiotic Organophosphoric Compounds in Schreibersite Materials

This proposal is focused on understanding the role of schreibersite catalytic mixtures in impact synthesis of phosphorylated organics on early Earth and other icy interstellar bodies. We will conduct high-throughput quantum simulations of aqueous solutions in the presence of the phosphide mineral schreibersite to near chemical equilibrium time-scales in order to determine its effect on the self-assembly of simple precursors into more complex biomaterials. Our work will sample an ensemble of high pressure, high temperature events corresponding to impact of astrophysical materials on planetary surfaces. These data are necessary in order to create a fundamental picture of the production of organophosphoric compounds such as nucleotides from raw materials that likely appeared during the period of Late Heavy Bombardment on early Earth. Schreibersite could have acted as both a source of elemental phosphorus and as a catalyst for lowering energetic barriers for organic chemical reactivity, and extreme thermodynamic conditions can act as a driving factor in creating more complicated chemical compounds with carbon phosphorus bonds.

The research proposed here includes state of the art quantum simulations of condensed phases and development of long time-scale models for chemical reactivity under extreme conditions. We will leverage our existing expertise with quantum simulation methods and the pre-existing computational facilities available at LLNL. This will give us the unique opportunity to answer long standing questions in Astrobiology regarding the synthesis of potentially life building compounds in aqueous environments on any given icy satellite or

planet. Our research plan supports the NASA Exobiology ROSES 2020 announcement by providing a unique set of theoretical studies to address the listed areas of research emphasis, including Prebiotic Evolution. Our study addresses many of the objectives of Goals #1-4 in the NASA Astrobiology Roadmap.

Maya Gomes / Johns Hopkins University
Do Sulfur Isotope Signatures In Sedimentary Pyrite Record A Major Transition In Earth S Geochemical Cycles Associated With The Rise Of Eukaryotic Algae?

The sulfur isotope record of sedimentary pyrite is an archive of the coupled geochemical cycles of sulfur, carbon, and oxygen - elemental cycles that are used to reconstruct environmental shifts in the atmosphere-ocean system, which often coincide with large-scale evolutionary events. A compilation of pyrite sulfur isotope data from globally distributed sedimentary rocks indicates that the range of sulfur isotope values has increased over Earth history in two major transitions: a first rise to a range of >~200 at ~2.3Ga, and a second rise to a range of >~500 at ~0.7Ga. The first increase has been attributed to a rise in marine sulfate levels as a result of the Great Oxidation Event. Previously, the second rise was attributed to a rise in atmospheric oxygen levels, which enabled an evolutionary radiation in sulfur-oxidizing bacteria that were thought to be required to produce pyrite with very low sulfur isotope values. Subsequent studies proved that this requirement was not necessary, as very low sulfur isotope values could be produced by sulfate reduction alone. Thus, we currently lack consensus about what caused the second rise and if it is necessarily linked with an increase in atmospheric oxygen levels.

We hypothesize that the second rise in the range of sulfur isotope values of sedimentary pyrite during the Neoproterozoic was triggered by a transition in marine primary productivity that affected rates of microbial sulfate reduction. Recent work has argued that eukaryotic algae replaced cyanobacteria as dominant marine primary producers in the Cryogenian Period (0.72-0.64 Ga; Brocks, J.J. et al., Nature, http://dx.doi.org/10.1038/nature23457 (2017)). It has long been known that organic matter type and availability can affect rates of microbial sulfate reduction and sulfur isotope fractionation. However, it is not currently possible to link changes in marine primary producers to the second rise in the range of sedimentary pyrite sulfur isotope values because sulfur isotope fractionation studies have utilized simple sugars (e.g., lactate, malate) rather than the mélange of organic matter produced by different types phototrophic organisms. Recent advances in mass spectrometry have made it possible to resolve complex mixtures of organic matter in natural waters. Our initial results indicate that cyanobacteria and eukaryotic algae biomass differ. We propose to perform experiments to test if sulfur isotope fractionations increase when sulfate reducing microorganisms transition from cyanobacteria to eukaryotic algae biomass, which would support the hypothesis that this transition resulted in an increase in the range of sulfur isotope values of sedimentary pyrite. During these experiments, we will document how complex mixtures of organic matter from cyanobacteria and eukaryotic algae change as

sulfate reduction proceeds and if these consumption patterns vary between microbial communities enriched on the different biomass types. Lastly, we will document rates of organic matter consumption with different biomass types to determine if the transition in marine primary productivity affected rates of organic carbon transformation through sulfate reduction. Thus, the results will enable us to determine: (1) if the rise in sulfur isotope variability of sedimentary pyrite is due to a change in biomass, and (2) if this transition resulted in lower rates of microbial sulfate reduction, resulting in higher rates of organic carbon burial and a rise in atmospheric oxygen levels.

This work is relevant to Exobiology s research area, Large scale environmental change and Macro-evolution . Our experiments will enable us to document the links between the macro-evolution of life on Earth and environmental factors. A key question is if this transition played a role in rising oxygen levels in Earth surface environments. This addresses the Astrobiology strategy question, How have changing patterns of life through time impacted the environment?

Colleen Hansel / Woods Hole Oceanographic Institution
Exploration of Photochemical Processes Involved in the Formation of Manganese Oxides

Manganese (Mn) oxide deposits are observed on Earth globally, and more recently on Mars. Oxidation of Mn(II) to form Mn oxides on modern Earth is believed to be mediated by microbial activity, and requires the presence of molecular oxygen or derivatives thereof, specifically reactive oxygen species (ROS). In turn, observations of Mn oxides in modern, ancient, and extraterrestrial systems are often interpreted as evidence for the presence of molecular oxygen and even life.

Nevertheless, a growing body of evidence suggests that photochemical reactions may exert an important, yet poorly constrained control on Mn(II) oxidation and Mn(III,IV) oxide formation. The aqueous and mineralogical composition of surface environments on Earth and Mars, in fact, have the demonstrated abilities to produce ROS with rates elevated upon UV irradiation. Further, arid soils typical of modern deserts in particular contain elevated levels of photosensitizers, especially nitrate. The potential for these conditions to promote Mn oxidation is amplified when considering the high UV intensity and presence of deep UV (UVC) for modern Mars and early Earth. Thus, we posit that photochemical Mn oxidation may represent an overlooked regulator of Mn oxide formation within modern and past sunlit environments.

The overall goal of this research is to constrain the geochemical conditions where photochemical processes lead to the oxidation of Mn and subsequent formation of Mn oxides, and determine whether these pathways impart distinct structural and isotopic signatures within the oxides. The overall objectives are to: (1) constrain the reaction space within aqueous incubations that support photochemical Mn(II) oxidation and Mn oxide formation under a range of geochemical and irradiance conditions, (2) identify the

role of drying and cyclical humidity changes on photochemical-derived Mn oxide formation at the rock/mineral-water interface, and (3) investigate structural, morphological and isotopic signatures harbored within photochemically produced Mn oxides.

We will initially probe the potential for photochemical Mn oxide formation via lab incubations of aqueous and mineral/rock suspensions under a matrix of conditions varying in photosensitizers (nitrate, minerals), ROS and light scavengers (perchlorate, iron), pH, O2, light composition (+/-UVC), relative humidity, and temperature. We will target conditions representing shallow aqueous and (semi)arid terrestrial environments in the presence and absence of UVC. Reaction conditions that support Mn oxidation will be more intensely interrogated to characterize oxidation rates and identify pathway signatures within mineral products using a suite of techniques, including X-ray diffraction and absorption spectroscopy (mineral structure), electron microscopy (mineral size, morphology), and oxygen isotope ratio mass spectrometry (´18O, D17O). Leveraging these results, we will further constrain geochemical conditions specific to rock varnish by conducting incubations targeting reactions at the rock/mineral-water interface under fluctuating relative humidity conditions, simulating cyclical variations in hydration of reactive desert surfaces. We will also attempt to identity terminal Mn oxidant(s) in the most prolific reaction pathways through targeted chemical and isotope incubations and mineral characterization. Together, these experiments will provide a robust framework for evaluating the fidelity of Mn oxides as reliable paleoredox proxies and/or biosignatures.

James Holden / University Of Massachusetts, Amherst
Competition among Thermophilic Autotrophs in Resource-Limited Environments

One of the goals of the Exobiology Program, as described in the ROSES 2020 NASA Research Announcement, is to understand 'Early Evolution of Life and the Biosphere'. In keeping with this goal, this proposal addresses the central hypothesis that competition between thermophilic, H2-consuming methanogens and sulfur reducers is not based solely on Gibbs energy for a metabolic reaction but is also due to other factors such as cell yield adjustments, stochasticity, and fluid flow geometry. This study is transformative by examining growth efficiency and competition at cellular-to-population scale levels to determine how thermophilic chemoautotrophs organize themselves within geochemical gradients, compete for resources, assemble into complex communities, and impact local biogeochemistry and carbon fixation.

This project uses the thermophilic, H2-oxidizing and sulfur reducing bacterium Desulfurobacterium thermolithotrophum and the hyperthermophilic, H2-oxidizing methanogenic archaeon Methanocaldococcus jannachii as its model organisms. The organisms are among the most common autotrophic thermophiles found in deep-sea hydrothermal vents. The organisms and environment are representative of early life on Earth and models for potential life beyond earth. The genome sequences are available for

both organisms. They use different suites of hydrogenases for H2 oxidation and different CO2 fixation pathways, which may give them a competitive advantage under varying environmental conditions.

The research has the following goals:
1. Chemostat experiments coupled with differential gene expression (RNA-Seq) and proteomic (tandem mass tags for isobaric labeling of peptide fragments coupled with mass spectrometry) analyses will determine the effect of varying H2 flux on the growth rate, cell yield, and physiology of the sulfur reducer Desulfurobacterium thermolithotrophum.
2. Competitive coculture growth experiments will determine the effect of H2 and thiosulfate availability and initial cell concentration on the growth rate, cell yield, and physiology (using RNA-Seq and proteomics) of D. thermolithotrophum and the methanogen Methanocaldococcus jannaschii.
3. A reactive transport model will predict the effect of H2, thiosulfate, temperature, initial cell concentration, and residence time on the growth and biogeochemical impact of methanogens and autotrophic sulfur reducers.

This project fulfills many of the Exobiology Program's stated goals, namely to "1) understand the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments, 2) determine the original nature of biological energy transduction, membrane function, and information processing, to test hypotheses regarding the original nature of key biological processes, and 3) investigate the development of key biological processes and their environmental impact."

Christine Keating / Pennsylvania State University
RNA Synthesis, Structure And Function In Pre-Cellular Proto-Cytoplasms Based On Liquid-Liquid Phase Separation

Central Objectives
The proposed research will uncover how proto-cytoplasms favor gain-of-function in prebiotic milieu. Our proto-cytoplasms are coacervate droplets formed by liquid-liquid phase separation. They will host RNAs, which are of special interest owing to their ability to perform catalytic functions in addition to storing information. We will focus on increasingly realistic and therefore messy molecular systems to form the proto-cytoplasms. Multiple classes and sequences of ribozymes will be interrogated with a goal of evaluating the robustness of ribozyme-protocytoplasm compatibility. To accomplish this, millions of ribozyme/proto-cytoplasm combinations will be tested for accumulation and activity, followed by in situ structure on thousands of ribozymes, and dynamics on representative ones. Additionally, we will evaluate how amino acids may have cooperated to protect RNA from degradation in these protocytoplasms, and how accumulated metal ions and basic oligocations chaperoned RNAs through wet-dry cycles. Our driving hypothesis is that many combinations of multicomponent coacervates and

ribozyme sequences are compatible with emergent RNA functions of native ribozyme folding and function. Moreover, we hypothesize that protocytoplasms led to an early cooperation between nucleic acids and amino acids/basic cationic polymers to both protect RNAs and chaperone their function.

Methods/Techniques
Genomics tools of Structure-seq and Twist Ribozyme screening, which use Next-Gen Sequencing (NGS), will test pools containing tens of thousands of candidates under millions of conditions, while mass spectrometric tools of metabolomics will query the molecular composition of coacervates as the complexity of the molecules from which they form increases. This proposal finds its power in the overlap of the latest, multiplexing methods of the 21st century with the conditions that may have existed 4 billion years ago on Earth. It seamlessly blends genomics and metabolomics with biophysics and physical chemistry. This enables discovery portions that require examining enormous combinations and characterization portions that are necessary to understand in a deep and profound way how extendable the ideas are to emergence of life anywhere in the universe.

Significance
We aim to develop a mechanistic understanding that connects coacervate physical chemistry with RNA enzymology, and will test several hypotheses relevant to the solicitation and NASA s interests. We will assess the chemical systems that could have served as precursors of metabolic replicating systems on Earth and elsewhere. Specifically, the proto-cytoplasms and the ribozymes they host are chemical systems that could have been the precursors to extant cells and functioning RNA and protein systems that we now know on Earth. Our tools to test millions of combinations allow us to evaluate robustness in a way that rises above the chemical details of any one system and generalizes to what might have been on Earth or was/is elsewhere in the universe. Moreover, the ability of amino acids and basic oligocations to aid RNA stability and folding, could establish a general way in which chemical systems developed mutualistic relationships that stuck through the history of time. We are sensitive to the specific geochemistry of early Earth and thus address models of early environments on the Earth and how they influenced/aided RNA chemistry and stability. Finally, we address evolutionary driving forces that could have been operative on the early Earth, including the ability of protocytoplasms to chaperone RNA folding and to give rise to cooperatively folding RNAs. The relationships between RNA accumulation and structure-function could have been shaped by the very containers RNA found itself in so as to favor compact and well-folded structures, which are characteristic of functional RNAs such as ribozymes and riboswitches known today.

Konstantinos Konstantinidis / Georgia Tech Research Corporation
How Microbes Adapt to Living in the Upper Atmosphere: Implications for Cloud Formation, and Life During Early Earth and Elsewhere in the Universe

The abundance of microorganisms in the atmosphere can be high enough to absorb solar radiation and modulate the formation and chemical processes in clouds, thereby affecting the hydrological cycle and climate. Several bacterial species are known to serve as efficient ice nuclei based on an excreted protein (InaZ) that can initiate the formation of ice at temperatures as high as -4°C and thus, potentially participate in cloud formation in the atmosphere. Beyond this, very little quantitative understanding exists on the efficiency of ice nuclei (IN) or cloud condensation nuclei (CCN; when ambient temperature is above the freezing point) formation by different microbial species and more importantly, which cell properties control the observed CCN/IN activity. Yet, condensing water vapors around the cell (i.e., CCN activity) was probably one of the very first cellular functions that enabled life during the early history of (hot) Earth. Therefore, identifying these cellular properties and corresponding proteins, and studying their phylogenetic distribution in the tree of life, e.g., how ancestral such proteins may be and how they have evolved through evolutionary history, may provide new insights into early life. Further, how the physiology and CCN/IN activity of a cell changes during environmental transition (e.g., increasing temperatures, greenhouse gases and UV) also remains essentially unknown; yet, these cellular adaptations are presumably important for successful survival in the atmosphere and during climate change as well as for survival in the hot and gaseous primordial soup. How life adapts to living in the atmosphere is not only relevant for early life and bioaerosol-cloud-precipitation-climate interactions but, more importantly, for Exobiology as the atmosphere is one of the most extreme environments on our planet and a good analog for airborne life elsewhere.

This project will address these issues by studying the CCN/IN activities of different cell types collected from the atmosphere as well as their metabolic activities related to the utilization of abundant organic compounds under changing environmental conditions using the advanced instrumentation that we recently developed to measure these activities. For the most promising genetic traits identified such as proteins related to the CCN/IN activity, the evolutionary relationships and distribution in the Tree of Life will be determined to provide new insights into the importance of these traits for early life. Following these laboratory experiments, we will leverage a plethora of archived samples collected on board specialized NASA aircrafts to test our findings from the laboratory in-situ, using culture-independent techniques such as metagenomics (DNA level, who is there) and metabolomics (what compounds they excrete to or utilize from the environment).

To achieve these goals, a combination of time-series atmospheric samples collected across multiple seasons and altitudes, shotgun metagenomics, isolate manipulation studies, and advanced laboratory instrumentation for measuring CCN/IN efficiency of cells will be employed, building upon a substantial body of relevant preliminary results and available infrastructure in the Konstantinidis Lab. The project will provide multifaceted learning experiences for both undergraduate and graduate students at the interface of microbiology and genomics with aerosol science and chemistry. Educational and outreach activities will be organized during local science fairs to disseminate our knowledge to non-experts. This project will contribute to at least two important areas of

the Exobiology program: (ii) to understand the phylogeny and physiology of microorganisms 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.

Charles Liotta / Georgia Tech Research Corporation
The Prebiotic Formation of Linear Sugars and Sugar Acids

The prebiotic formation of sugars, within the context of origins of life scenarios, is both a fundamental and formidable problem that continues to attract possible solutions. Historically, attempts to answer the question of the origin of linear sugars has focused on the formose reaction which involves the polymerization of formaldehyde (a C1 sugar) with glycolaldehyde in the presence of calcium ions under highly alkaline conditions. The perceived likelihood of this reaction occurring abiotically has been further enhanced by observations of formaldehyde in interstellar media suggesting it would be available on the early earth. While the formose reaction has been demonstrated to produce some amount of linear sugars, the utility of the reaction is diminished by formation of accompanying branched products and tars. Even with its many drawbacks, the formose reaction has remained central in the prebiotic formation of sugars. We propose an alternative scenario wherein glyoxylate (carboxylated formaldehyde), is utilized as the primary building block, instead of formaldehyde. It is postulated that through a series of aldol reactions, carbonyl migrations, and decarboxylations, linear sugars and sugar acids can be produced avoiding the branched-sugars and tar formation. We term this reaction sequence the glyoxylose reaction. Broadly, this proposal describes a different approach to the simultaneous formation of linear sugars and sugar acids under plausible prebiotic conditions.

Andrej Luptak / University Of California, Irvine
High-Energy Phosphate In Early Genome Replication And Maintenance

A central topic in the experimental study of the origin of life is the emergence of phenotypes the traits recorded by the very first informational polymers. A key part of the early (as well as modern) metabolism concerns phosphate, which had to have existed when the first evolvable chemical systems could be encoded. Phosphate is not only the building block of RNA and DNA, but also of phospholipids, and a multitude of high-energy metabolic intermediates.

DNA and RNA are replicated using highly evolved polymerase enzymes that utilize nucleotide triphosphates as substrates for templated synthesis of DNA and RNA, yielding a pyrophosphate for each nucleotide incorporated. The pyrophosphate is further degraded to drive the overall reaction forward, because high levels of pyrophosphate can lead to

degradation of the information polymer by the same enzymes. Recently, we showed that modern DNA polymerases can replicate DNA using deoxyribonucleotide diphosphates (as opposed to triphosphates). DNA replication using dNDPs appears to be quite efficient. We also found that the reverse reaction phosphorolysis of DNA to yield dNDPs is slow, but detectable. These measurements, together with the determination of Kms for both dNDPs and phosphate (as well as pyrophosphate), allowed us to conclude that in the presence of modest concentrations (high μM) of dNDPs, the forward reaction (DNA synthesis) is strongly favored over phosphorolysis (degradation of the genome). Part of the reason for this strong forward bias appears to be the insolubility of magnesium phosphate at concentrations that could efficiently drive the reverse reaction. Thus phosphorolysis does not appear to pose a threat to the stability of genomes. On the other hand, the need for the higher-energy triphosphate over diphosphate to replicate genomes is not clear and warrants further investigation, because similar biochemical challenges were undoubtedly faced by the early living systems.

The goal of this proposal is to investigate the role of phosphate, particularly high-energy phosphate, in chemical reactions that are central to genome replication and maintenance. In addition to the role of diphosphate in DNA synthesis we recently turned our attention to the forward and reverse reactions of the RNA polymerization by RNA polymerase ribozymes. These ribozymes were evolved from RNA ligase ribozymes over the past 25 years and some have been shown to produce other functional RNAs. We have started analyzing the activity of the most evolved RNA polymerase ribozymes and have obtained the first evidence of ribozyme-catalyzed pyrophosphorolysis of RNA. We also observed RNA repair pyrophosphorolysis of a chain-terminated RNA primer (containing a 22 32 phosphate, which cannot be extended by these ribozymes) followed by the incorporation of rNTPs. In these situations, simple primer extensions were blocked and the RNA had to be repaired before further replication could take place. With these experiments in place, we can experimentally address the following questions:

Were high-energy metabolic intermediates necessary for the origin of life? Will RNA and DNA repair by polymerases be favored by pyrophosphorolysis over phosphorolysis? We will test these questions by: a) testing the fidelity of the DNA and RNA synthesis using diphosphorylated vs triphosphorylated substrates, and b) measuring the activation energies and KMs of all experimentally accessible reactions to establish their fundamental parameters.
How abundant are ribozymes that utilize di- vs triphosphates for catalysis? We will use in vitro selection to evolve a series of ribozymes that will perform catalysis with di- or tri-phosphorylated substrates. We will perform these selections in direct, head-to-head competition to directly gauge the ribozyme abundance in random pools and thus measure the relative requirement for the high-phosphate substrate.

Timothy McDermott / Montana State University, Bozeman
Environmental Distribution and Evolutionary History of Non-Methanogen Methane Synthesis

Microbial methane production is viewed to be a strictly anaerobic process carried out by a phylogenetically constrained group of archaea colloquially referred to as methanogens. Methanogens are uniformly viewed to be of ancient origins, estimated to have arisen sometime between the Archean to Precambrian at 3.05 4.49 Ga to 1.26 1.31 Ga. It has been suggested that CH4 synthesis during the Archean may explain the faint young Sun paradox , resulting in increased global temperatures. As such, the occurrence and development of biogenic methane synthesis likely impacted the environment of early Earth, hastening the evolution of various life forms. Because of the same greenhouse gas effect, methane critically impacts contemporary Earth s atmospheric chemistry in the context of global warming. Therefore, it is of critical importance to understand sources and sinks of methane in the biosphere.

Over the past four decades, studies from around the globe have documented the occurrence of methane supersaturation in oxygen-saturated aqueous environments. This phenomenon is in contradiction to accepted models of biogenic methane production and thus has been termed the methane paradox by microbiologists and biogeochemists alike. Most studies have focused on marine environments, but recent work involving freshwater bodies has likewise documented this phenomenon.

The PIs have been involved in investigating this phenomenon in Yellowstone Lake (Yellowstone National Park), documenting novel forms of methane synthesis that are neither constrained by redox conditions nor restricted to the archaeal domain. Methane synthesis was found to be catalyzed by an enzyme that is common to all self-replicating lifeforms, aerobic and anaerobic alike. We hypothesize this process likely arose concurrently with, but distinctly separate from, the known and characterized pathway of methylotrophic methanogenesis. As such, it represents an important contribution to Earth s biogeochemical and atmospheric history, and thus is highly relevant to NASA s Exobiology program. More specifically, this project addresses & the development of key biological processes and their environmental impact& and [is] of relevance to environmental change occurring throughout Earth s history . Furthermore, because of the different organisms involved in this process in current day microbial communities, it is reasonable to also hypothesize that this process had a role in the coevolution of microbial communities, and the interactions within such communities, that drive major geochemical cycles and the co-evolution of biology and geochemistry . Lastly, it addresses fundamental innovations in earliest life , common attributes of living systems on Earth , and dynamics [of these innovations] on the subsequent evolution of life .

The nature of our discoveries boldly suggests that this form of biogenic methane synthesis is not restricted to aqueous environments, but probably exists in a wide array of organisms and occurs in all biomes. Our proposal will describe three objectives:

Objective 1) Screen phylogenetically and environmentally diverse plp-aat clones for MeA --> CH4 activity.

Objective 2) Directly assay Exobiology relevant environmental samples for aerobic and anaerobic non-methanogen GB/MeA --> CH_4 activity.

Objective 3) Use the molecular record in living organisms to interpret the evolutionary history for this form of methane synthesis within the general structure of microbial functional evolution.

Aaron Noell / Jet Propulsion Laboratory
Cellular and Complex Organic Biosignature Preservation Potential of Cryogenic Ices

The near-surfaces of ocean worlds in our Solar System are inhospitable places. The combination of, cold, vacuum, UV and particle irradiation and long duration exposure make organism survival improbable. Still, various delivery mechanisms bring material to the near-surfaces of these worlds from the more hospitable regions deeper within their ice shells or in the oceans below that may contain evidence for life. Near-surface delivery is critical, because for practical reason the first landed missions to these surfaces will only be able to access depths on the centimeter scale. The most striking of the possible different types of evidence for life in this case would be cell-like objects with complex organic chemistry. However, very little is known about what the biosignature preservation potential is for cells in an ocean world near-surface environment, under the intense cold, vacuum, and radiation conditions. There have been a number of studies that have looked at the survival of building block organic molecules, such as amino acids, and some biopolymers under cryogenic irradiation, but no work has investigated cells beyond establishing their viability. This is important because cell structures offer potentially much greater shielding, as well as a natural sample concentration mechanism, leading to significantly enhanced preservation compared to bare molecules alone. Determining the biosignature preservation potential of cells will have impacts for how samples are collected and processed in order to maximize life detection potential for future in situ ocean worlds missions.

We will unravel the biosignature preservation potential of cells using a high vacuum chamber equipped with a rotating cryostage to create low temperature ~ 50 150 K surfaces and water ices. This temperature range will allow assessing conditions relevant to bodies like Europa and Enceladus. Irradiation sources include UV from a selection of discharge lamps and an Ar arc lamp, and an electron gun. Both UV and electron sources are capable of producing high intensities to simulate long timescale exposures. Bacillus subtills bacterial spores will be the primary organism irradiated and analyzed given the enhanced preservation possible of resilient cells with multi-layer coats similar to spores. At the completion of irradiation, the substrates with spores will be first observed via microscopy, and then recovered and extracted for analytical chemistry analyses of amino acids, polypeptides/proteins, nucleic acids, and fatty acids.

Christopher Reisch / University Of Florida, Gainsville
Understanding the Genetics of Extreme Stress Resistance in Bacteria

Microbes have the capacity to survive under conditions unsuitable for other organisms. For example, we isolated viable bacteria from Earth s stratosphere, an environment in which the hypobaric pressure, low-temperature, UV-radiation spectrum and flux, and low relative humidity represent a combination of extremes that parallel conditions on the surface of Mars. The mechanisms by which bacteria prevent or repair cell damage caused by these extremes are poorly understood. Moreover, the genetic determinants enabling these survival mechanisms are mostly unknown. Several of the stratospheric isolates we propose to study possess very high resistance to shortwave UV radiation and desiccation. The central objective of this work is to identify the genes that enable these bacteria to survive the space-like environment in the stratosphere. We hypothesize that the genetic mechanisms contributing to the resistance of oxidative stress, UV radiation, and desiccation have overlap, and that evolved resistance to one of the stressors fortuitously provides resistance to the other.

To identify the genetic features that are important for resistance to UVC radiation, desiccation, and reactive oxygen species we will use a combination of transcriptomics, genetics, physiology, and directed evolution. Bacteria that are closely related to the stratospheric will be obtained from culture collections and evaluated from resistance to the same stressors. In cases where the culture collection representative is found to be less resistant than their counterparts, we will evolve these cells to increase resistance and identify the genetic changes using whole-genome DNA sequencing. Gene expression will also be studied in these evolved bacteria and the stratospheric isolates to identify genes differentially expressed in response to the stressor. Third, transposon mutants will be constructed and screened to identify genes that are important or detrimental to the stressors. To confirm that the identified genes and mutations identified are important for stress resistance we will use targeted mutagenesis to recapitulate these genetic changes into culture collection bacteria. Lastly, these engineered strains and the evolved strains will be screened for resistance to each of the stressors to investigate whether resistance to one stressor also conveys resistance to other stressors.

The proposed work is highly relevant to NASA s Exobiology Program foci of early evolution of life and the biosphere and biosignatures and life elsewhere . The development of life on early Earth was limited in part because of the same stressors that we propose to investigate (high UV-radiation flux and water stress). Thus, the tolerance mechanisms that allowed early life to migrate on land may be shared with stratospheric extremophiles and enable us to relate what is known about the origin of life on Earth to the potential for the origin and establishment of life under conditions prevailing on other planetary bodies . Furthermore, this work will answer questions relating to 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. According to evolutionary theory, the mechanisms responsible for resistance phenotypes are important drivers of evolution because they enable increased fitness in bacteria. Understanding

whether these mechanisms are the result of subtle genotypic changes, acquiring new genes, or large genetic evolution will inform the likelihood of astrobiologically-relevant phenotypes emerging under exposure to selective pressures.

Annette Rowe / University Of Cincinnati
Understanding the Interactions and Energy Transfer Between the Lithosphere and Biosphere in a Marine-like Terrestrial Serpentinizing System

Despite recent advances in understanding the nature of microbial life in terrestrial serpentinized systems (i.e., The Cedars, Tablelands, Oman), differences in the underlying fluid chemistry between these freshwater environments and their marine counterparts limit the extent to which direct comparisons can be made. Specifically, there is a gap in our quantitative understanding of the role that sulfur and/or methane cycling may play in high pH and high conductivity fluids. These conditions are highly relevant for understanding the potential for life on ocean worlds such as Europa or Enceladus, as they could drive chemolithoauthrophic life (microbes that acquire energy and fix carbon from inorganic sources). We propose to study an unusual terrestrial spring, Aqua de Ney or Ney s Spring in Northern California, which has similar chemistry to marine serpentinized ecosystems, such as Lost City, but is far easier to access. Ney s waters are highly alkaline (pH 12), with high concentrations of reduced inorganic compounds (CH4, S2-, NH3). Our preliminary microbial community analysis at Ney s suggests the presence of sulfur and methane oxidizing organisms, from genera that have not been observed in freshwater serpentinizing environments, but that are present at the Lost City. Our preliminary geochemical data indicates that Ney s has the highest sulfide concentration of a terrestrial serpentinized system (>500ppm), as well as one of the largest methane fluxes observed (>80% methane). We hypothesize that sulfide in this ecosystem is a result of subsurface microbial sulfate reduction (likely coupled to hydrogen oxidation) and that the microbial communities we observed at this site, conserve energy by oxidizing the reduced compounds present in these fluids (i.e., S2- and CH4 acting as primary nutrient sources for chemolithoautotrophic life). The goal of this proposed work is to understand the endemic microbial community, energy utilization and electron flow through food webs, and the role microbe-mineral interactions play (such as, S0 production or oxidation) in supporting life under saline serpentinizing conditions.

Our research objectives are to characterize the microbial and geochemical context of Ney s Spring using integrated geochemical and molecular analyses, in situ and functional microcosm studies, and targeted cultivation of important microbial metabolisms. We will support this effort with an analysis of the fluid and solid phase geochemical environment at Ney s, using a series of samples collected at different times of year (to evaluate wet/dry seasonal influences), with depth in the spring, and over the course of several years. Depth samples for geochemical and microbial analysis will be combined with geochemical profiling electrodes to elucidate natural gradients of sulfide, oxygen, hydrogen and redox. Our work will offer the first cohesive insight into active microbial communities at Ney s Spring, and will further our understanding of the microbial interactions, food webs, and

the biological adaptations that support life in this characteristically marine serpentinizing system. This work will provide experimental data to help answer questions posed at the Lost City, and will provide quantitative data to help extend this understanding to the search for life elsewhere. Understanding microbial life in serpentinizing systems is distinctly relevant to NASA s Exobiology Program as these environments support lithoautotrophic microbial ecosystems that: 1) are an analog for studying early Earth conditions when/where life arose; 2) host a geologic process thought to be occurring (e.g., Enceladus) or have occurred in the past (e.g., Mars) in other bodies of our solar system; and 3) provide broader understanding of the physical boundary conditions of microbial life, such as those of extremely high pH fluids derived from serpentinization.

Daniel Stolper / University of California, Berkeley
New Isotopic Constraints On The Oldest Terrestrial Biosignatures: An Experimental Study On What The Isotopic Composition Of Ancient Organic Carbon Reflects About Early Metabolisms And Environments On Earth

A fundamental question in Earth and planetary science is when and under what conditions the earliest life on Earth evolved and developed. Among the lines of evidence put forward as the earliest evidence of life on Earth, one of the most common and longstanding is based on measurements of 13C/12C ratios (given as d13C) in organic carbon phases preserved in ancient geological materials. Specifically, ancient reduced carbon as old as 4.1 billion years has d13C values less than -200. These values have been widely interpreted to reflect the geological residue of biomass as d13C values overlap with those of modern autotrophs that fix CO2 via the Calvin cycle with the enzyme Rubisco. As such, measurements of d13C values of preserved organic carbon represent an important class of biosignatures on the Earth and beyond for the identification of life and the autotrophic metabolisms used for biosynthesis.

The use of 13C/12C ratios as biosignatures relies on a biochemical understanding of the mechanics of what exactly controls the relative rates of 12C vs. 13C incorporation into biomolecules during carbon fixation. Traditionally, carbon isotopes have mostly been used as a diagnostic measurement for the biogenicity of organic carbon, with the precise value less important than the carbon being light . However, there is also evidence that fractionation factors for carbon fixation have varied substantially over Earth s history and that their magnitude can be modulated by changing environmental conditions. For example, measurements of the d13C of ancient organic carbon on Earth indicate that the isotope effects of carbon fixation changed by perhaps as much as 2x over the past 2.5 billion years. Additionally, a few experiments have indicated that the trace element composition of the liquid medium influences the isotope effects of carbon fixation. For Rubisco, replacement of the Mg in this protein with other divalent cations (e.g. Mn or Ni) yields functional enzymes, but modifies the isotope effect by ±100. Finally, early life is commonly envisioned to have evolved and proliferated in warm (>50°C) conditions, but the role of temperature on the magnitude of carbon isotope effects for Rubisco has only been explored over a limited range of temperature (15-35°C).

We propose to conduct a systematic survey of what controls the carbon isotopic fractionation factor of the most important carbon fixing enzyme on Earth Rubisco in order to interpret what d13C values reveal about the metabolic functions and environmental conditions of ancient life. We propose to study several different Form I Rubiscos prepared from native hosts or heterologous expression systems in E. coli. We will examine, in vitro, the isotopic fractionation factors of this enzyme using standard Rayleigh curves with 13C/12C ratios measured via isotope-ratio mass spectrometry. (1) We will first work with a classic model system spinach Rubisco, which has well-known kinetics and a well-established fractionation factor. We will metalate the enzyme with a suite of common cellular divalent metals (Fe, Mg, Ca, and Mn), manipulate temperature from 10-40°C, and adjust O2 contents to directly quantify how these conditions do or do not change carbon isotope fractionations. (2) Following this we will study such effects for cyanobacterial Rubiscos focusing on S. elongatus, a model cyanobacterium and the thermophilic cyanobacteria Thermosynechococcus to explore higher temperatures (>40°C). (4) Finally, we will study the same effects and isotopic fractionations of ancient, extinct Rubiscos in the laboratory that were thought to exist on the early Earth.

The proposed scope of our proposed fits several Exobiology program objectives. For example, the work will aid in understanding nature and characteristics of Earth s earliest organisms and the environments in which they lived and evolved, and in investigations of the development of biological processes and their environmental impact.

Yuanzhi Tang / Georgia Tech Research Corporation
Understanding the Formation of Manganese Oxides in Low Oxygen Environments

Science goals and objectives:
Oxygenic photosynthesis is undoubtedly one of the most important metabolic innovations that have occurred in Earth s history, but its timing is still poorly constrained. Over the past few years, inorganic geochemical tools have emerged as the primary means of tracking the emergence of oxygenic photosynthesis based on the premise of tracking its metabolic waste product: free oxygen (O2). Due to the significantly higher redox potential of Mn, the presence of widespread Mn oxides is considered to be associated with highly oxidizing aqueous environments and the presence of oxygen. This hypothesis serves as the backbone of the fundamental framework of several newly emerged popular paleo redox proxies, such as the Cr, Tl, and Mo isotope systems. However, additional research is needed to gauge the strength of the fundamental assumption on the correlation between Mn oxides and oxygen. Attempts to understand and quantify the formation of Mn oxides under suboxic or anoxic conditions are lacking, which hinders our ability to accurately apply the Mn redox system and related isotope systems for pinpointing the initial emergence of biological oxygen production on Earth. More specifically, if Mn oxides are able to form under low to no oxygen conditions, understanding the related kinetics, mechanism, and the phase/structure of the Mn oxides can avoid false positive interpretation of oxygenation events.

Our recent study demonstrated the feasibility of direct photochemical oxidation of Mn(II) catalyzed by natural minerals and the formation of Mn oxides at rates comparable or even higher than currently known biotic/abiotic processes. We further revealed considerable Mn(II) oxidation and Mn oxide formation at extremely low oxygen level (~1 ppm). These exciting observations indicate that direct photochemical oxidation of Mn(II) catalyzed by natural minerals is a previously overlooked abiotic pathway that might explain the formation of Mn oxides under varied environments especially those with extremely low oxygen content.

The following objectives will be achieved:
(1) To investigate the photocatalytic oxidation of Mn(II) in the presence of natural minerals in aqueous condition under low oxygen level and varied solution chemistry. (2) To characterize the structure of the photochemically produced Mn oxides. (3) To investigate the photocatalytic oxidation of mineral-surface adsorbed Mn(II) in humid atmosphere with varied oxygen and humidity level.

Approach and methodology:
The photochemical oxidation of Mn(II) catalyzed by natural minerals will be investigated using a selected set of representative photoactive mineral phases, under varied low oxygen level, and varied solution chemistry (fresh water vs seawater). The oxidation rate will be compared with currently known biotic/abiotic processes and extrapolated from laboratory conditions (Xe lamp) to natural sunlight conditions. The morphology and structure of the produced Mn oxides will be characterized using a suite of complementary advanced characterization techniques to reveal the related Mn(II) oxidation and Mn oxide formation mechanisms will be revealed. We will also challenge to investigate the photocatalytic oxidation of adsorbed Mn(II) on hematite surface in humid atmosphere with varied oxygen and humidity levels.

Significance:
The proposed work is significant as it is the first study that systematically investigates the direct photochemical oxidation of Mn(II) catalyzed by natural minerals under low to no oxygen conditions. The obtained knowledge will shift the current paradigm of Mn redox chemistry and allow predictions of Mn oxide formation in a wide range of natural settings, which will ultimately help move forward better utilization of proxy systems for tracking planetary oxygenation history.

Alexis Templeton / University Of Colorado, Boulder
Targeted Life Detection in Subsurface Serpentinites

Water is often stored within the Mg-Fe silicate crusts of rocky bodies within our solar system, such as Earth, Mars, Europa and Enceladus. The hydration of these ultramafic rocks gives rise to redox reactions that produce biologically useful reductants such as H2 and organic compounds, as well as a diversity of secondary minerals dominated by

serpentine. However, although such rock-hosted systems are broadly considered to be habitable, we do not know how life will be distributed within them, which prevents the development of a targeted approach to search for serpentinite-hosted life on other planets. There exists a need to increase our capabilities in recognizing active and fossil biospheres in habitable fractured-rock systems, such as serpentinites, at a variety of scales. We will address these critical knowledge gaps, in order to advance the understanding of rock-hosted systems thought to promote the emergence and evolution of early life on Earth and the potential for similar environments to host life on other planetary bodies such as Mars, Europa and Enceladus.

Through our previous efforts to develop a subsurface observatory in peridotite rocks undergoing active low-temperature serpentinization, we have generated a wealth of data that point to an abundant, diverse and active chemosynthetic microbial biosphere within the Samail Ophiolite, Oman. Our data are primarily derived from fracture waters, and show a differential distribution in diversity and evolutionary adaptations of organisms across contrasting geochemical regimes. Now we have an opportunity to use high-quality core samples to decipher the relationships between metabolic potential and diversification in the context of the localized mineralogy and geochemistry.

We intend to demonstrate when, where and why biological activity is localized to hot spots in the serpentinite subsurface. Specifically, we will quantify the distribution of microbial activity in serpentinite rock cores spanning geochemical and mineralogical gradients. This work will include (1) experimental measurements of the rates of tritium incorporation into biomass to identify hot spots of activity, rates of deuterium incorporation into lipids to quantify turnover times in these hot spots of activity, and rates of C1 compound assimilation/dissimilation to trace some of the dominant metabolic and biosynthetic processes. We will then (2) identify microbe/mineral associations in hot spots through chemical and biological imaging approaches. Lastly, we will (3) determine the metabolic potentials and ongoing diversification of organisms in hot spots via genomic sequencing, using whole-rock approaches to extract DNA for metagenomic analysis, and using purified cell aggregates obtained from fractures for single cell genomics.

The proposed work is directly relevant to NASA s strategic plan to determine the distribution of life on and beyond Earth, by demonstrating how and where microbial life harnesses the energy and nutrients available when reactions occur between rocks and water. Our work will support the Exobiology program interest in defining the range of planetary conditions amenable to life , while also informing our targets and strategies for life detection by identifying where the formation and preservation of biosignatures associated with rock-hosted life is most probable. In addition, our focus on microbial adaptations to actively serpentinizing environments will address open questions regarding the Early Evolution of Life and the Biosphere, specifically by investigat(ing) 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 .

Michael Tice / Texas A & M, College Station
Oxidizing Oases in the Paleoarchean Moodies Group, Barberton Greenstone Belt, South Africa

Objectives: Oxygenic photosynthesis has directly altered the Earth s atmospheric composition, prompting an evolutionary radiation of biosynthetic and metabolic pathways, and ultimately making multicellular life possible. Although atmospheric oxygen levels increased dramatically at 2.4 billion years ago (Ga), the timing of production of oxygen by phototrophs remains poorly constrained. Our primary objectives are 1) to locate the sites of iron oxidation on a 3.2 Ga coastal system preserved in the Paleoarchean Moodies Group of South Africa, and 2) to determine the presence or absence of oxygen production in those sites.

Methods: We will obtain unweathered samples by drilling through terrestrial and shallow-marine sandstone of the Moodies Group in collaboration with an international ICDP project . From these cores, we will select samples and perform detailed petrographic (polarized transmitted and reflected light, cathodoluminescence) and geochemical analyses of redox sensitive grains as well as early cements to document the mineralogy and redox conditions during deposition. Optical petrography will be supplemented by electron microprobe and EDS analyses. Geochemical analyses will include micro-X-ray fluorescence (micro-XRF) spectroscopy, Raman microspectroscopy and mapping, synchrotron X-ray analytical spectroscopy, laser-ablation inductively-coupled plasma mass spectrometry, and in situ Fe, C and O isotope analyses.

This proposal includes an appended Planetary Major Equipment and Facilities (PMEF) request to replace the micro-XRF instrument used in the project.

Perceived Significance: This project will contribute to the Early Evolution of Life and the Biosphere goal of the Exobiology program by investigating the evolution of photosynthesis and its effect on surface environments. Recent work has suggested that oxygen production began by at least the Mesoarchean without building up in the environment until hundreds of millions of years later. This work will allow us to probe sensitive recorders of oxidation state preserved in sedimentary rocks (including potential fossilized biological sources of oxidants) deposited in 3.22 billion-year-old coastal environments. Studying drill core samples collected from deep below the modern weathering profile will provide material minimally altered by oxidative weathering for constraining early biological and geochemical coevolution. Cores from this project and two other drill sites will be made available to the broader science community through the International Continental Drilling Program following a two-year science moratorium.

This project will also contribute to the Biosignatures and Life Elsewhere goal by developing mineral biosignatures of early oxidation. These results will be highly relevant to the Mars 2020 mission, as the Moodies deltaic sedimentary rocks to be explored are strong partial analogs to Jezero deltaic sedimentary rocks set to be explored by the Perseverance rover. In particular, the source terrains for both the Moodies Group and

Jezero crater deltaic sediments had significant mafic components. Weathering and transport in both locations occurred in anoxic environments, likely under a high UV flux. This combination of critical environmental factors is rare in the surviving record of terrestrial deltas.

Moreover, the workflow for identifying and analyzing potential biosignatures will be similar to that used in the Mars 2020 mission. The geochemical screening tools for these biosignatures (X-ray analytical microscopy and UV and Vis Raman microspectroscopic mapping) will be similar to instruments on Perseverance (PIXL and SHERLOC, respectively). Results from these instruments will be used to sample for further analysis. We anticipate that results will be useful for designing measurements for in situ science and for selecting samples for return. The PI (Tice) is a PIXL Co-I and one of the Co-Is (Czaja) is a Returned Sample Science Participating Scientist.

Elizabeth Trembath-Reichert / Arizona State University
Cold and Dry Limit to Life: Understanding Microbial Activity in Dry Permafrost Samples from the Newly Discovered Elephants Head, Antarctica

Motivation:

Understanding the tolerance of life to low temperature and water availability is key to the search for life on Mars and Icy Worlds. Microbial life has been shown to be active within permafrost soils at temperatures as low as -25°C and ice may improve preservation of molecular biosignatures. While widespread on Mars, dry permafrost overlying ice-rich ground with sublimation driven water exchange is rare on Earth. Until recently, the only known analogous sites on Earth existed in dry permafrost in the high elevations of the McMurdo Dry Valleys, Antarctica . Previous studies have confirmed the presence of microbial life in arid permafrost soils via microscopy or DNA sequencing ; however, limited studies of activity have been conducted. In permafrost soils of University Valley, Antarctica metabolic activity could not be detected with either in situ gas flux measurements, or highly sensitive radiorespiration assays at in situ relevant sub-zero temperatures. Recently, dry permafrost was discovered for the first time outside of the McMurdo Dry Valleys, at a site called Elephants head. In January 2020, we acquired samples from this site (no field work is proposed here), and samples were shipped frozen and are now stored at -20°C at NASA Ames. Subsurface soil moisture and temperature analyses conducted at this site show the conditions thought to be amenable to life are on the order of hours per annum, much less than in University Valley.

Our goal is to understand whether such cold and dry permafrost does indeed represent a natural limit to life on Earth, or whether the results at University Valley are unique. Our objectives are to (1) detect present day biological activity and (2) look for evidence of past activity to understand the preservation potential of biosignatures in dry permafrost and underlying ice cemented permafrost soils.

Approach:

We expect microbial biomass and activity to be extremely low in these samples and therefore propose an integrated approach that combines multiple mechanisms for determination of biomass, diversity and microbial activity. Biomass will be determined by laboratory analyses to quantify the cell density, DNA concentration, and lipid concentration of these samples using methods previously designed for dry permafrost (Goordial et al., 2016) and hyperarid soils (Wilhelm, 2017). Microbial activity will be determined by (1) permafrost-tailored bulk measurement of soils respiration with 14C-acetate (Goordial et al. 2016), (2) low-energy sediment-tailored highly sensitive single-cell measurement of microbial activity and acetate uptake (Trembath-Reichert et al. , 2017), and (3) measuring growth-phase markers of stress in lipid membranes (Wilhelm et al., 2018). Geochemical measurements such as total organic carbon, total organic nitrogen, as well as concentrations of major cations and ions will give context to the microbiological findings.

Significance and Relevance:

This study will determine the potential for active microbial metabolism, observable fluxes of metabolites, and biomarker preservation in dry permafrost soils on Mars to help us understand the requirement for surface liquid water in defining habitable exoplanets. This study specifically addresses objectives from the 2015 NASA Astrobiology Strategy: Are there habitable environments on Mars at present? How can we identify and characterize locations where liquid water currently exists? Are there near-surface habitable environments on Mars? What types of life, and associated metabolisms, might exist in these environments?) and 2018 NAS Astrobiology reports: increased understanding of the nature and detectability of biosignatures, especially for in situ detection of energy-starved or otherwise sparsely distributed forms and Assessing the relative signal-to-noise ratio of each type of population in its given environmental context would help identify corresponding biosignatures that are most relevant and distinctive.

Edward Young / University of California, Los Angeles
Developing Methane Isotopologues as Interplanetary Biosignatures

We propose a program of experiments to evaluate the potential of methane clumped isotope ratios as geochemical and biogeochemical tracers for solar system bodies in general. Multiply substituted isotopologues of methane are well suited as tracers of methane formation pathways in general, and potential biosignatures in particular. They remove the difficulties associated with using bulk carbon and hydrogen isotope ratios on other worlds where the geochemical context necessary for interpreting these ratios are by necessity lacking. However, uncertainties remain about the uniqueness of the isotopologue signatures. We propose a program of experiments that will mitigate (if not eliminate) these uncertainties. Our work will inform future missions about the potential benefits of including in-situ measurements of rare methane isotopologues on Mars,

Enceladus, and other bodies where the origin of methane is a key geochemical and biogeochemical tracer.

We will carry out a series of experiments on microbial methane formation and oxidation and a set of experiments on abiotic synthesis of methane. Our goal is to disambiguate the mass-18 isotopologue signatures of microbial methanogenesis, bacterial oxidation of methane, and abiotic methane formation pathways. This experimental program builds on five years of data collection in natural settings and in laboratory experiments that have shown that the mass-18 isotopologues of methane have great potential as biosignatures if some of the lingering uncertainties about uniqueness of relating their relative abundances to specific pathways can be eliminated.

A primary focus will be on the expression of the so-called combinatorial effect that is a key source of the putative biosignature. In order to quantify the influence of energy flux and reaction rates on the magnitude of this clumping signature of microbial methanogenesis, we will cultivate anaerobic methanogens under different energy fluxes that will vary by a factor of ~50. To quantify the effects of microbial aerobic methane oxidation on methane clumping, we will perform closed-system cultures with several different electron acceptors. The details of fractionation during oxidation are crucial for the fidelity of the mass-18 biosignature.

Abiotic synthesis experiments are aimed at exposing the effects of temperature on kinetic mass-18 isotopologue abundances produced abiotically and on the potential role of the combinatorial effect. We will determine whether or not a false positive biosignature can be produced abiotically and if so, under what conditions.

This proposal is responsive to the Exobiology program as described in ROSES Appendix C.5. In particular, the proposal addresses the scope of the program to understand the distribution of life in the Universe. The proposal seeks to develop a biosignature for eventual deployment to other worlds, including Mars, Enceladus, Titan and elsewhere more generally. In section 1 of Appendix C.5 one of the areas of focus is that of Biosignatures and Life Elsewhere. Under that solicitation studies are sought that constitute basic research on the formation and retention of biosignatures under non-Earth conditions (e.g., Mars, Europa). This is the goal of the present proposal. Our proposal describes the establishment of an isotopic biosignature suitable for application beyond Earth where carbon and hydrogen cycles may not be well understood.