Exobiology
Abstracts of Selected Proposals
(NNH19ZDA001N-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. 159 proposals were received in response to this opportunity. On November 22, 2019, 17 proposals were selected for funding.

James Farquhar/University of Maryland, College Park
Tracing Sulfur Isotope Signatures in Early-Earth Analog Systems with Microbes and MIF

Archean sedimentary sulfide preserves a range of mass-dependent and mass-independent fractionations for sulfur isotopes with positive and negative ´34S and □33S, and a longstanding debate focuses on how these ranges were produced. While the ´34S variability can be partially attributed to mass-dependent processes associated with microbial sulfate reduction, these □33S fractionations cannot be explained as the sole products from a homogenous ocean with either negative (or positive) □33S. Variation of □33S and covariation between ´34S and □33S, result from a more complex connection between mass-dependent and mass-independent fractionation processes. It has been suggested that trapping these isotopic variations in sulfide minerals is a natural consequences of pyrite formation in the presence of elemental sulfur. According to this hypothesis, variable contributions from sulfate (with negative □33S) and elemental sulfur (with positive □33S) are facilitated by processes involving polysulfide; which forms via the reaction between microbial sulfide and elemental sulfur. Polysulfide modulates both the sign and amplitude of sulfide □33S and its relationship to ´34S in pyrite forming environments. Importantly, understanding the nature of such transfer is relevant to trace MIF back to originating reactions, to detect evidence for early sulfur metabolisms on Earth and on other planets where similar MIF/MDF signatures and ecosystems existed, and ultimately to develop strategies that quantify the role of S metabolism in early environments.

For the first task, we propose a series of microcosm experiments designed to reproduce key aspects of the hypothesis regarding Archean pyrite formation in the presence of elemental sulfur and sulfate with a different mass-independent composition. Proposed experiments will culture the autotrophic sulfate reducer Desulfobacterium autotrophicum HRM2 to test whether model systems with microbial sulfide, elemental sulfur, and Fe(II) generate results permissive of predictions related to these pathways for transfer of mass-independent sulfur to iron sulfides and to the isotopic variation that is seen in the Archean sulfur isotope record. For the second task we will develop □36S as a tool to expand the application of sulfur isotopes. This is facilitated by new high mass resolution mass spectrometry capabilities available at the University of Maryland to measure the 36S

isotopic compositions (a Nu Panorama that will be installed in Fall 2019). We will focus on reference materials to allow other laboratories to address issues of scale compression with the standard gas source mass spectrometers. We will also conduct analyses of previously studied samples from culture experiments to test calibrations of 36S for the sulfate reduction metabolism made in previous studies. The rationale for this developmental work is to develop a tool that can be used for probing evolution of post Archean systems with sulfide oxidation and sulfate reduction and the microbial populations that inhabit them.

The research is broadly relevant to the following three goals of the Astrobiology Program: Early life and increasing complexity (via the 36S development); Co-evolution of life and the physical environment (via the proposed experiments that test hypotheses about the role of sulfur metabolisms in establishing chemical pathways that yield the signatures that are observed in the geological record); and Identifying, exploring, and characterizing environments for habitability and biosignatures (via the way that mass-dependent biosignatures are intertwined with S-MIF photochemical signatures of primitive atmospheres to yield characteristic isotopic fingerprints of sulfur metabolisms).

Jamie Foster/University Of Florida, Gainsville
Unearthing the Role of Microbial Dark Matter in Ecosystem Structure and Function

For billions of years microbes and their metabolic activities have been shaping Earth s physical, chemical and mineralogical landscapes to maintain a habitable world. Microbes comprise 60% of the planet s biomass, yet most of the estimated 10^12 microbial species remain uncharacterized. Beyond a basic understanding of microbial species and their genomes, we currently have an incomplete understanding of the roles microbes and their gene products play in ecosystem processes. Elucidating the importance of these unknown taxa, colloquially referred to as microbial dark matter , requires moving beyond deep sequencing and necessitates a systems biology approach to integrate of all levels of biological information to more accurately predict the emergent properties of ecosystems and prioritize unknown biological entities for further study.

Specifically, in this study we propose to develop a computational pipeline to address the following question: what role, if any, does microbial dark matter play in the organization and formation of complex interactive structured communities? To address this question we propose to use microbialite-forming microbial mats as the target ecosystem for this project. Microbialites are carbonate build-ups that form as a result of the metabolic activities of benthic microbial communities. They are an ideal model system to target for the proposed work plan because they essentially represent an ecosystem in the palm of your hand and numerous amplicon and meta-omic datasets are currently available. Using this system we propose the following specific objectives:

Objective 1: Characterize the importance of unknown taxa on microbial community structure using network analysis. We will generate ecosystem-level networks incorporating SSU rRNA gene reads derived from previously generated metagenomic datasets. We anticipate that network-based analyses will uncover hubs of new sub-assemblages of species, and genes that appear to be playing critical roles in microbialite ecosystem function.

Objective 2: Reconstruct the genetic content of the hubs of unknown taxa using whole metagenome sequencing to identify and characterize novel taxa. We will use ProxiMeta Hi-C sequencing technology that separates and assembles genomes from mixed populations by trapping proximal DNA interactions in intact cells. This approach will enable us to generate hundreds of metagenome-assembled genomes (MAGs). We will then use the hubs identified in Objective 1 to probe the MAGs to identify the functional genes associated with the uncharacterized taxa.

Objective 3: Enrich and isolate the hubs of unknown taxa using magnetic quantum dots conjugated to DNA probes. To complement Objective 2 we propose to extract living representatives of the network hubs of unknown taxa from the microbial community using magnetic nanoparticles that have been conjugated to DNA probes (e.g. 16S/18S rRNA). The unknown taxa can be tagged with the magnetic quantum dots without killing the cells. The tagged cells can then be isolated using a permanent magnet and either undergo cultivation or sequencing of the uncharacterized taxa.

Identifying these unknown aspects of microbial life, or microbial dark matter , represents a grand challenge for Astrobiology. Underpinning NASA s overarching mission to find and understand life beyond the biosphere is a systematic effort to utilize Earth as a model system to derive generalizable principles about how life originates and evolves. We assert that the tools and pipelines developed as part of this project will be applicable to a broad range of environments and ecosystems to help unearth the microbial dark matter thereby fundamentally transforming our understanding of Earth-based ecosystems and potentially improving our search parameters for life beyond our biosphere.

Adam Frank/University Of Rochester
Characterizing Atmospheric Technosignatures

We will produce the first entries in a library of spectroscopic signs of extraterrestrial technology (technosignatures") and develop a theoretical and modeling framework for populating the library. Our long-term goal is to use this library to develop a comparative framework for evaluating both the strength and non-ambiguity of proposed non-radio technosignatures in exoplanetary spectroscopy from NASA missions such as JWST, as they begin to search for signs of life in transmission and, eventually, reflection spectra of exoplanets. In this initial proposal we will begin with two technosignatures which been proposed, but not fully evaluated in a comparative and comprehensive fashion, in the literature. We will first calculate modeled spectra of atmospheres with a range of

organofluorine compounds as an example of an atmospheric technosignature . Next we will calculate modeled spectra displaying a silicon edge" associated with large-scale surface deployment of photovoltaic arrays as an example of a surface technosignature" These two modeling efforts will establish best practices for catalog development that can be used in future comprehensive efforts.

Trinity Hamilton/University Of Minnesota
The Distribution and Activity of Metabolically Diverse Anoxygenic Phototrophs across Geochemical Gradients in Hydrothermal Systems

GOALS AND OBJECTIVES
The ability to harvest light and convert it to chemical energy represents one of the most elegant and complex biological innovations to date. The prevalence of extant cyanobacteria (oxygenic phototrophs) in alkaline hydrothermal ecosystems (below 72°C) coupled to recent reports of putative microbially-related structures in terrestrial hot springs that date back as far as 3.5 Ga suggests niche space for terrestrial thermophilic phototrophs early in the evolution of life on Earth. These observations coupled to the data indicating anoxygenic photosynthesis emerged as much as one billion years prior to oxygenic photosynthesis suggest early evolving anoxygenic phototrophs could have thrived in terrestrial environments, particularly hydrothermal springs, on an early Earth characterized by a reducing atmosphere. However, there is a paucity of data on the physiology and extent of anoxygenic phototrophs in modern hydrothermal systems and the physiology of the first anoxygenic phototrophs remains unknown.

The goals of the research proposed here are to address these gaps in knowledge by constraining the taxonomy, physiology, and activity of anoxygenic phototrophs across pH and temperature space in Yellowstone National Park (YNP) and to examine the evolutionary history of acidophily and thermophily in anoxygenic phototrophs.

APPROACH AND METHODOLOGY
To accomplish these goals, we will use stable isotope probing to isolate labeled RNA and identify active phototrophs (both photoautorophs and photoheterotrophs) across the geochemical landscape in YNP hot springs. Because genome sequences of thermophilic and acidophilic bacterial phototrophs are rare compared to genomes of mesophiles, we will perform metagenomic sequencing and examine the metabolic potential in genomes of anoxygenic phototrophs across geochemical gradients to facilitate phylogenomic analyses. We will also characterize the physicochemical attributes of geothermal features to identify abiotic factors, in addition to temperature, pH and sulfide, that may limit anoxygenic phototrophs. Collectively, this data will constrain the distribution and activity of metabolically diverse anoxygenic phototrophs across geochemical gradients and provide a framework for unravelling the ecophysiology of anoxygenic phototrophy throughout Earth s history.

RELEVANCE TO THE EXOBIOLOGY PROGRAM
The proposed research is relevant to the scope of NASA s Exobiology program including to understand the origin, evolution, distribution, and future of life in the Universe . Photosynthesis is constrained to temperature and pH limits not observed for chemotrophs and the proposed studies would examine the physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments . Our sequencing data will be interrogated to 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 . Photosynthesis revolutionized Earth s atmosphere and terrestrial surface, thus our studies will inform the development of key biological processes and their environmental impact . The work will also address Goals 4 and 5 of the Astrobiology Strategy. Our in situ studies will help constrain the environmental limits of anoxygenic photosynthesis, an evolutionary innovation that pre-dated oxygenic photosynthesis, which modified environmental conditions on a planetary scale and ultimately lead to the emergence of more complex life, while our phylogenomic approaches will inform studies of the evolutionary mechanisms that limit life and the acclimatization of life.

Nicholas Hud/Georgia Tech Research Corporation
Exploration of Potential Informational Biopolymers of Early Life on Earth and Elsewhere

Living organisms on Earth utilize very large molecules, or polymers, for making structures and for carrying out the chemical reactions that are essential for survival, replication, and continued evolution. Present-day life utilizes DNA polymers for the storage of genetic information and protein polymers for catalyzing chemical reactions. A longstanding and influential theory for the nature of early life proposes that RNA, a polymer closely related to DNA, was originally used by life for storing genetic information and for catalyzing chemical reactions, before the emergence of DNA and proteins. Evidence put forth in support of this RNA World hypothesis includes the ability of RNA to catalyze a considerable range of chemical reactions and the ever-expanding list of processes that are known to be controlled by RNA in current biology. However, the proposal the RNA was actually the first polymer of life is complicated by the fact that demonstrating a plausible prebiotic synthesis of RNA polymers has remained elusive goal. Accordingly, it has been proposed that RNA is actually a product of evolution, and that a more ancient polymer preceded RNA, what has been called proto-RNA. Based on a number of considerations, we have identified a set of plausibly prebiotic molecules that are strong candidates for the building blocks of early genetic polymers of life on Earth and for polymers that might be utilized by alternative forms of life elsewhere in the universe. We will study the environmental conditions that promote the polymerization of these putative proto-RNA building, as well as the conditions that promote the cleavage (hydrolysis) of these polymers. Our goal is to determine if these relatively simple molecules can spontaneously give rise to polymers that exhibit

properties, including a rudimentary form of evolution, that would be expected for the earliest polymers of life.

Methods and Techniques:
Polymers of our putative proto-RNA building blocks will be produced using model prebiotic reactions and by synthetic organic methods. These polymers will be analyzed using a number of chemical techniques, including NMR spectroscopy, mass spectrometry, circular dichroism, dynamic light scattering, and UV/MS-monitored HPLC.

Significance to NASA Interests and Programs:
The proposed research is designed &to characterize the overall chemical underpinnings of the processes that lead to the persistence and function of evolvable macromolecular systems. a goal that is explicitly stated, verbatim, as being important to the NASA Exobiology program in the 2015 NASA Astrobiology Strategy.

Daniel Jones/New Mexico Institute Of Mining And Technology
Gypsum-Hosted Biosignatures in Subterranean Chemosynthetic Ecosystems

Gypsum and other hydrated sulfate minerals are widespread on the surface of Mars, and are considered promising targets for the detection of past and potentially present Martian life. However, the timescales, nature and fidelity of biosignature preservation in gypsum are poorly understood. In order to evaluate gypsum as a target for biomarker detection on Mars and other extraterrestrial bodies, we need to evaluate how signatures of life are recorded in gypsum deposits on our home planet.

We propose to take advantage of an exceptional natural experiment in Earth s subsurface to develop and evaluate gypsum-hosted biosignatures associated with microbial chemosynthesis. In sulfidic limestone caves, gypsum deposits form by sulfuric acid corrosion. Initially, gypsum forms as wall residues, coated by acidophilic sulfide-oxidizing microbial communities. These gypsum wall crusts later accumulate on the floor as they thicken and slump to the ground. Gypsum floor deposits can be preserved for millions of years in sulfidic caves, and likely record organic material and other evidence from the extremophilic communities that were present during initial mineral precipitation.

Although these cave deposits are not depositional analogues for Martian sulfates, they offer an exceptional setting for evaluating the preservation potential of microbial biosignatures in acidic gypsum. For example, gypsum formation in sulfidic caves is specifically associated with microbial chemosynthesis, and they do not have inputs of exogenous photosynthesis-derived materials that can overprint chemosynthetic signatures at surface analog study sites. They also form under similar pH conditions to those proposed for Martian sulfates (pH 1-4), so biosignature suites developed in these caves could offer a window into chemosynthetic life under acidic Martian surface conditions

and how we might detect such a suite. And, sulfidic caves also offer a setting where the fate and fidelity of biosignatures in old gypsum deposits can be evaluated within the same system as the extant communities that formed them.

We propose to take advantage of this setting to address two questions:

1. What organic, geochemical, and isotopic biosignatures are associated with gypsum at the time of mineral precipitation, and what is the source of those biosignatures?

2. Which biosignatures persist as the mineral assemblage ages? What is the nature of biosignature changes with time, and how do they relate to gypsum diagenesis?

We will address these questions with a collaborative, multidisciplinary approach in which four investigators from three different institutions apply isotopic, molecular, geochemical, and microbiological tools to evaluate the formation and fate of gypsum-hosted biosignatures. Our field site will be the Frasassi cave system, an active sulfidic cave where modern gypsum crusts are forming near the water table, and in which relict gypsum deposits are preserved in older passages formed between 10,000 and 1 million years ago. First, we will characterize biosignatures associated with extant acidophilic gypsum communities, including hopanoids, other lipid molecules, extremely isotopically light organic nitrogen (´15N < -250, a possible new contextual signature for the extremely acidophilic communities), and other morphological and mineralogical indicators. Then, we will collect samples of relict gypsum deposits preserved in the older cave passages, and compare how organic and isotopic signatures change with the age of the deposit. Our project will provide the astrobiology community with much-needed information on sulfate mineral-hosted biosignatures, and explore the preservation potential of diverse signatures of life in extreme and subsurface environments.

Ramanarayanan Krishnamurthy/Scripps Research Institute
Transitioning from Vesicles to Protocells: Investigating the Physics of Cyclic-Phospholipid Liposomes

On prebiotic earth, the formation of micelles and vesicles is considered an important process setting the physical stage for the chemical origins of life. The creation of a dynamic semi-permeable physical barrier of vesicles is thought to enable separation and concentration of certain classes of molecules, resulting in interactions within enclosed molecules giving rise to emergent properties of polymers. The physical separation of information carrying self-replicating molecules is considered to confer competitive evolutionary advantages. Thus far, vesicles based on carboxylic acids, and their derivatives have been the most investigated systems as plausible prebiotic candidates. But how they can transition via a chemical-evolutionary pathway to the phospholipid-based membranes used in extant biology is not well understood. The search for a cell structure and composition that would have been consistent with the dual requirements of original simplicity and future complexity is an unmet challenge.

We have discovered that single-chain cyclic-phospholipids, which form spontaneously starting from their respective constituents (short chain fatty acids, glycerol and diamidophosphate) under plausible prebiotic aqueous conditions, are capable of forming vesicles. Work in collaboration with Mansy lab shows that these cyclo-phospholipid containing vesicles form, and are stable, over a wide range of pH (4-10) and salt concentrations (2M NaCl and 25 mM MgCl2). They are capable of encapsulating and retaining chemical entities; at the same time, they can be made porous based on their composition and chain lengths of fatty acids. Preliminary data suggests cyclic-phospholipids incorporation increases protocellular stability. This one-pot formation of single chain cyclic-phospholipids alongside fatty acids and their glycerate esters under plausible prebiotic settings, provides a unique opportunity to investigate how and whether vesicles formed from fatty acids and glycerol can transition via cyclic-phospholipids containing vesicles to the modern double-chain phospholipid-based cell structure.
We hypothesize that the spontaneous formation of single chain cyclic-phospholipids and their concurrent incorporation into primitive fatty acid-based vesicles could lead to further reaction of the cyclic-phosphate group with fatty alcohols leading to double-chain phospholipids, and could play a role in facilitating the contiguous chemical evolution of vesicles into functional protocells, towards morphogenesis of phospholipid-based liposomes resembling early functioning cells. We propose to systematically synthesize single-chain cyclic-phospholipids and their double-chain derivatives of various lengths with a view to create vesicles of varying compositions. We will characterize them and investigate their physical properties with respect to (a) their morphology and topologies, (b) equilibrium physical parameters of their formation and stability, and (c) their kinetics of self-assembly and persistence. In addition to studies of ensemble properties, we will also use methods with single-vesicle resolution to understand distributions of physical properties, which may be key in understanding the appearance and evolution of functional features. We aim to gain insights into the dynamic behavior of the library of vesicle-compositions, and develop an understanding that will allow us to manipulate the factors that can control the formation, self-assembly, encapsulation, permeability, growth and division of these vesicles. This information will allow us to identify systems that could morph from the fatty acid-based enclosures to the double-chain phospholipid bilayer liposomes/protocells, which could serve as models of early micro-environments on Earth where organic chemical synthesis and polymerization could occur and provide an understanding towards the origins of the processes giving rise to cellular activities, such as energy transduction and membrane function.

Jay Lennon/Indiana University
Energy Limitation and the Evolution of Microbial Dormancy

Objectives: Microorganisms have played a fundamental role in developing and sustaining Earth s biosphere. Ever since the planet cooled and life first arose, microorganisms have been challenged by extreme and fluctuating environmental conditions that are generally unfavorable for growth and reproduction. Dormancy is a universal strategy that allows

organisms to persist over time by entering a reversible state of reduced metabolic activity. In this proposal, we focus on the evolutionary implications of dormancy by studying spore-forming Bacillus and related taxa under energy limitation. Despite being an ancient and complex trait originating billions of years ago, we have documented that sporulation can rapidly be lost due to relaxed selection. The first objective of our proposed research is to quantify the bioenergetic cost of making a spore. Second, we will investigate the causes and consequences of spore loss in environments with varying degrees of energy limitation. Last, we will assess the prevalence of sporulation decay in a range of natural ecosystems and test whether this is associated with increased rates of evolution and diversification.

Approach: We will use a combination of theory, laboratory experiments, and environmental metagenomics to understand the maintenance of sporulation under contrasting energy regimes. First, using a bottom-up bioenergetic approach, we will quantify the gene-, transcript-, and protein-level costs of making a spore. This information will then be incorporated into mechanistic models that will generate testable predictions about the conditions that favor the retention vs. loss of sporulation. Second, we will conduct experimental evolution trials where replicate Bacillus populations will be challenged by energy limitation via the passaging of cells every 1-, 10-, or 100-days. In addition to quantifying the phenotypic loss of sporulation using flow cytometric assays, we will measure the progression of genomic decay using high-throughput population sequencing. Simultaneously, we will track the fate of genetically engineered sporulation-deficient strains of Bacillus to understand how the loss of a complex trait affects subsequent refinement and parallel evolution. Last, to determine whether or not patterns in laboratory studies are common in nature, we will survey biosignatures of sporulation in ~8,000 environmental metagenomes collected from a range of ecosystems and test whether the decay of spore-formation corresponds with accelerated rates of evolution over deep time.

Significance: Our team s cross disciplinary research will inform a number of scientific fields including astrobiology, evolutionary biology, and environmental microbiology. The proposed work will develop and test a general theory of dormancy and evolution by studying microbial populations that are faced with energy limitation, a constraint that has influenced the abundance, productivity, and diversity of life throughout Earth s history. We focus on Bacillus not only because it is a genetically tractable model and relevant to the functioning of contemporary ecosystems, but also because sporulation is a complex and conserved trait that is important for advancing our understanding of early Earth evolution. Endospores are some of the most biologically inert forms of life. They have been resurrected from ancient materials that are millions of years old, pushing the limits of organismal lifespan. Evolutionary insight into sporulation and alternative mechanisms of dormancy will aid in understanding long-term survival of microbial populations through deep time, including periods associated with mass extinction events. By identify biosignatures of important persistence traits, our findings will also be relevant to planetary protection of future NASA missions along with the goal of detecting life elsewhere in the universe.

Vince LiCata/Louisiana State University and A&M College
Salty and Cold: Protein Function and Stability in the Cryosphere

This proposal asks how DNA binding proteins thermodynamically function under conditions of extreme cold and high salt in the cryosphere. Psychromonas ingrahaii is a psychrophlic bacterium that lives in liquid brine veins at -2 to -20 degrees Celsius in Arctic sea ice. This proposal will investigate the thermodynamic and functional properties of the DNA polymerase I from P. ingrahamii, with the goal of identifying the energy management and thermodynamic behaviors that allow this enzyme to remain stable and bind to DNA under such extremely cold and salty conditions. The thermodynamics of protein folding and DNA binding of P. ingrahamii polymerase will be examined as a function of temperature, high salt, and natural osmolytes (compatible solutes) in order to determine the functional landscape and the thermodynamic driving forces that allow such psychrophilic polymerases to function under such environmental conditions. The project also aims to structurally characterize the protein.

While the enzymology of a number of cold-adapted enzymes have been characterized, the thermodynamics of protein function at low temperature is nearly uncharted territory. One actually cannot extrapolate existing data on the thermodynamics of DNA binding or stability on any previously studied polymerase or DNA-binding protein and arrive at reasonable thermodynamic predictions for psychrophilic polymerases. Especially with regards to protein-DNA interactions, extrapolation of any existing binding data on any DNA binding protein predicts binding affinities in the sub-millimolar range for DNA binding under the physiological conditions in Arctic sea ice. Such affinities would be far too low to sustain normal polymerase function, yet P. ingrahamii is known to replicate and repair its DNA under such physiological conditions. These apparently conflicting facts predict that the temperature and salt dependencies of DNA binding by P. ingrahamii DNA polymerase will differ significantly from the patterns thus far characterized for mesophilic, thermophilic, and low-salt environment DNA binding proteins. Similarly, extrapolations of existing stability data on other psychrophilic proteins predict that the folding and stability of P. ingrahamii Pol I will be negligible under physiological conditions, yet the protein functions well and is not predicted to be intrinsically disordered. The proposed studies are thus predicted to reveal novel molecular thermodynamic signatures and combinations of thermodynamic driving forces for DNA binding and protein stability (i.e., combinations of free energy, enthalpy, entropy, and heat capacity, as well as linkages to salt, osmolytes, and other solution variables) that allow proteins to function under extreme conditions in the Earth's biosphere, and that are predicted to exist elsewhere in our solar system.

The proposed project addresses several NASA objectives and interests in the area of Early Evolution of Life and the Biosphere, including: 1) what is the potential of life to adapt to different environments, especially extreme environments (such as extreme cold and high salt), 2) understanding the physiology of extremophiles whose characteristics may reflect the nature of primitive environments (such as Snowball Earth), and 3)

environment-dependent molecular evolution in microorganisms (specifically: retention of evolved molecular resistance to primitive environmental stresses). Further, because cold, saline oceans are predicted to exist on Europa, Enceladus, Ganymede, and now possibly even the south-pole of Mars, this research also addresses the NASA objective of examining evolutionary adaptation processes on Earth that may have relevance to other planetary environments. The proposal team is also committed to public outreach activities for communicating astrobiological research.

Rachel Martin/University Of California, Irvine
Molecular Evolution and Minimal Functional Requirements of Extremophilic Proteases

Over the last two decades, evidence has accumulated for the presence of liquid water on several moons within our solar system. The Cassini spacecraft has observed aqueous geysers on Enceladus, and more recent data are consistent with the existence of a subsurface ocean. The solution conditions on Enceladus appear to be strongly basic (pH 11-12), possibly similar to serpentinite environments on Earth, in which hydrogen gas is used by microbes such as methanogens. Silica grains consistent with the existence of hydrothermal vents deep below the surface of Enceladus have been discovered, suggesting that both hot and cold environments may exist there. Similar observations have been made for Europa, where an icy crust protects a liquid ocean warmed by tidal heating. Recent data from the Hubble telescope also suggest a subsurface saline ocean on Ganymede. Taken together, these data clearly indicate that liquid water, considered to be a requirement for life, is not as rare as previously thought, and that if extraterrestrial life exists in our solar system, it is likely to be in the form of extremophilic microbes.

This project will center on the molecular evolution of protein adaptations enabling such organisms to thrive under conditions similar to those on Enceladus and Europa, with a focus on the adaptations of extremophile proteases. Proteases are chosen in particular because they represent a universal class of enzymes that is required to sustain life. They play critical roles in single-celled organisms, such as nutrient processing and defensive responses, but are also required for functions specific to more complex organisms, including tissue development and remodeling, immune function, and programmed cell death. Our proposal responds to the following two research emphases:

Early Evolution of Life and the Biosphere and Biosignatures, and Life Elsewhere: Understanding the evolution of proteases that are optimized function in cold and alkaline environments is critical for understanding how microbes could have evolved in the environment of Earth s early biosphere, and could potentially thrive in environments such as those predicted to exist on Enceladus. A detailed study of the parameters within which proteases can function will enable us to place bounds on what is possible for these universal biomolecules.

In our prior research, we have conducted genome-wide surveys of particular classes of enzymes (e.g. aspartic proteases, papain-type cysteine proteases). Our approach uses computational methods to identify genes and gene products, followed by molecular modeling and detailed in silico assays of functional properties (e.g., flexibility, thermal stability, and substrate affinity). In this project, we will build on this work by: (1) expanding the scope of our current techniques to detect complex patterns associated with protein adaptation, using recent developments in machine learning and Bayesian nonparametrics; (2) applying these techniques to the comparative analysis of a matched sample of currently sequenced psychrophilic, alkaliphilic, halophilic, and control (mesophilic/neutrophilic) microbes; (3) identifying adaptive trends in function-relevant protein structure across environmental categories; (4) validating these inferred trends by expressing selected extremophilic proteins for detailed characterization. Functional biochemical assays will be performed, and the structures solved either by high-resolution X-ray crystallography or NMR.

Sune Nielsen/Woods Hole Oceanographic Institution
Thallium Isotope Fractionation during Sorption to Manganese Oxides: Calibration of a Novel Ocean Redox Paleo-proxy

The oxygen content of the ocean has been one of the primary factors controlling the origin, evolution and diversification of life on Earth. It is difficult to directly study past marine oxygen levels, however, due to a lack of quantitative means of reconstructing oxygen concentrations in seawater. Manganese (Mn) oxide precipitation in the ocean strongly depends on the presence of oxygen because the oxidation of Mn(II) to Mn(IV) requires at least micromolar levels of oxygen. Yet, directly studying Mn oxide minerals within the rock record is complicated by their alteration and dissolution during sediment diagenesis and metamorphism.

Recent studies have shown that the thallium (Tl) isotope composition of seawater depends critically on the global burial rate of Mn oxides due to strong adsorption and associated isotope fractionation of Tl between seawater and Mn oxides. In addition, several studies have found that the marine Tl isotope composition has varied considerably and systematically during climatic events associated with changes in ocean redox at least since the late Archean. While the recorded Tl isotope variations most likely have to do with Mn oxide burial in marine sediments, the exact reasons for these changes remain elusive largely due to a gap in the knowledge of how Tl and its isotopes are partitioned into Mn oxides. This proposal seeks to quantify the Tl flux and isotope composition associated with sorption to Mn oxides by way of laboratory experiments as well as measurements of natural sediments. To do so, this research will couple mineralogical and isotopic analysis to determine what controls the isotope composition of Tl associated with Fe-Mn minerals and what is the magnitude and isotope composition of Tl flux into natural ferromanganese deposits. The overarching hypothesis is that Mn oxide structure is a dominant control on the Tl isotopic composition within marine sediments. In particular, it is predicted that only the Mn oxide hexagonal birnessite has the capacity to fractionate

Tl isotopes due to its ability to cause surface oxidation of univalent to trivalent Tl during sorption, while other Mn oxides such as triclinic birnessite and todorokite do not have the same oxidative capacity and should not cause Tl isotope fractionation. Thus the marine Tl isotope cycle should be controlled by these mineral-specific isotope fractionation factors. A series of laboratory experiments will be performed whereby aqueous univalent Tl is sorbed onto different Mn oxide minerals. In addition to determination of Tl isotope fractionation factors between solid and liquid phases, the Tl oxidation state in the Mn oxides will be monitored by way of X-ray absorption spectroscopy, which will provide a direct test of whether Tl oxidation is required in order to produce significant Tl isotope fractionation. Other experimental series where temperature, salinity, experiment duration and Tl concentration are varied will reveal the dependence of Tl isotope fractionation on different external parameters and it is anticipated that the experimental data will provide a molecular understanding of Tl isotope fractionation during sorption to Mn oxides. These laboratory experiments will help inform Tl isotope and oxidation state analyses of Mn oxides in natural abyssal clays. Abyssal clays are the major location of Mn oxide precipitation in the oceans and therefore these analyses will yield a new and more accurate estimate of the global Tl flux and isotope composition into Mn oxides. With this detailed knowledge and improved understanding of the modern marine Tl isotopic cycle, it will be possible to construct more accurate models that can explain the seawater Tl isotope variation already observed in the terrestrial rock record and ultimately validate Tl isotopes as a valuable paleoceanographic tool.

Stephanie Olson/Purdue University
Assessing Atmospheric Seasonality as an Exoplanet Biosignature

The appearance of habitable and inhabited exoplanets may change seasonally due to changes in the hydrologic cycle (e.g., snow/ice cover, cloud characteristics) and/or biologically modulated changes in atmospheric composition. For example, carbon dioxide rises and falls seasonally due to shifts in the balance of photosynthesis, which consumes carbon dioxide and dominates in the sunny summer, and respiration, which releases carbon dioxide and dominates in the dark winter. Critically, however, we do not yet have a quantitative understanding of how these changes may manifest across the diversity of rocky exoplanets that we expect to find. Moreover, we do not know whether we might leverage these seasonal signals as independent or corroborating evidence for habitability and life or whether these changes might instead pose a challenge for future life detection missions by rendering key biosignatures only intermittently detectable.

Our study addresses this important problem by using a sequence of climate, biogeochemical, photochemical, and spectral models to investigate: (1) seasonal changes in the environment across a broad range of planetary circumstances; (2) how Earth-like life, both past and present, might respond to those seasonal changes; (3) how these seasonal changes may be expressed in atmospheric composition, and (4) how these seasonal changes would impact our characterization of a planet when viewed with future space-based telescopes.

In sum, these tasks will allow us to evaluate whether seasonality arising from life is remotely detectable with space-based telescopes and if it is distinguishable from other seasonal signals that may emerge on habitable planets. This is our primary objective. Additionally, we will determine whether seasonality is likely to introduce false positives or negatives for more conventional biosignature analysis. This is an issue that is relevant to all future attempts to characterize habitable and inhabited exoplanets regardless of whether searching for seasonal biosignatures is a central goal of those missions. Ultimately, our ability to confidently recognize life on a particular planet may depend on repeated observations spanning several orbital phases. Our study will thus inform life detection strategy for future NASA missions and will support NASA's search for life via the Exobiology research emphasis area of Biosignatures and Life Elsewhere.

Richard Remsing/Rutgers University, New Brunswick
Understanding the Molecular Driving Forces for the Chemistry of Weird Life on Titan

Biochemistry on Earth requires water as its solvent. However, many chemical processes occur more easily in organic liquids, and it has been hypothesized that such solvents will facilitate the emergence of non-aqueous or weird life. The Saturnian moon Titan is an ideal candidate for weird life. The Cassini-Huygens mission identified lakes of liquid hydrocarbons (methane/ethane) at low, cryogenic on Titan s surface, in which weird life, or weird prebiotic chemistry, may have evolved. Still, we have little idea where to find such hydrocarbon-based life, ultimately because we do not know how it might function. To understand how weird life may function, we will take cues from life on Earth, namely that fundamental chemical processes like electron and proton transfer reactions underlie terrestrial biochemistries. Moreover, a prevailing theory in terrestrial prebiotic evolution is that mineral interfaces catalyzed essential chemical reactions in a manner similar to biological enzymes. It is natural to assume that non-aqueous life evolved in a similar manner by exploiting the unique chemistry at interfaces, suggesting that one should look for weird life in areas with high concentrations of relevant minerals. But, these relevant minerals must still be identified based on their ability to catalyze the chemistry of weird life. Therefore, finding weird life, or prebiotic chemistry that may lead to weird life, ultimately begins with understanding the fundamental processes that may underlie its biochemistry. However, a foundational understanding of physicochemical processes at Titan conditions is currently lacking. To build such a foundation, we will use molecular theory and computer simulations to uncover the molecular driving forces for fundamental prebiotic chemistry and biochemistry in liquid hydrocarbon solvents and at their interfaces with organic minerals.

We hypothesize that biochemistries evolved in cryogenic, nonpolar liquids require the same fundamental processes as on Earth, but with inverted polarities and a significantly greater role of quantum effects. Biological systems on Earth seemingly exploit the quantum nature of electrons and nuclei when possible, for example, to tune reactivity.

Quantum effects become increasingly important as temperature is lowered, and we expect the low temperatures on Titan to enable weird life to functionally employ quantum effects more efficiently than Earth-life. Moreover, the low surface temperature on Titan will result in minerals largely composed of organic crystals held together by weak hydrogen bonds and van der Waals interactions, different than those on Earth that are stabilized by strong covalent and ionic bonds. We will use molecular simulations to investigate fundamental processes in hydrocarbon mixtures and their interfaces with organic minerals at Titan-like conditions, including electron, proton, and energy transfer. We will additionally connect our results to experiments by predicting reaction kinetics and spectroscopic signatures, which can be measured in laboratory simulations of Titan s chemistry and will aid in identifying surface organics found by planned Titan landers.

The ultimate goal of this work is to identify environments on Titan that are conducive to biochemical processes to aid in the search for non-aqueous life on Titan and other worlds. This work is responsive to the Prebiotic Evolution heading of the Exobiology (C.5) solicitation, which seeks proposals to understand processes that govern the prebiotic evolution of materials necessary for life. This goal also addresses aspects of the Astrobiology Roadmap that seek to determine how life may arise in solvents other than water and how one might identify corresponding bio-signatures. Our end product will be a molecular-scale foundation for understanding fundamental biochemical processes in cryogenic, non-aqueous liquids that will help guide the search for weird life and prebiotic chemistry in the universe.

Raphael Rosenzweig/Georgia Tech Research Corporation
World Enough and Time: Mapping the Martian Adaptive Landscape with a Terran Bacterium

The present-day surface of Mars is a frigid, UV radiation-soaked desert blanketed by a hypobaric atmosphere. While Terran extremophiles, and even some mesophiles can survive these conditions, none can reproduce, which is a prerequisite for adaptive evolution. But what if cells were allowed to evolve under each of these conditions then given time to adapt to others? Could evolution rewire the genetic circuitry of a Terran bacterium so that it could not only survive but also replicate? We propose to evolve Escherichia coli, a model human commensal, under an increasing number and intensity of Mars-relevant stressors: freeze-thaw cycles, UV radiation, desiccation, and hypobaricity. If a Terran microbe can evolve a physiology that enables it to reproduce under these conditions, then it is possible that Martian life, if it ever existed, was able to adaptively evolve when Mars underwent climate change long ago. If so, then near-surface environments such as ephemerally-wetted crater walls may still support microbial life.

We propose evolution experiments to simulate physical conditions in these near-surface environments and gain insight into their habitability. Specifically, we will explore how microbes adapt to physical challenges, which have received less attention than the question of how microbes adapt to chemical challenges such as antibiotics, substrate

limitation, and growth on novel substrates. To follow the adaptive process and to ascertain the fitness of new mutations as they arise, we will employ the molecular barcoding approach pioneered by co-I Sherlock in yeast, which has now been brought to E. coli. Under each Mars-relevant condition we will follow the evolutionary dynamics of ancestral populations consisting of at least 100,000 barcoded lineages, each of which will also carry a second, condition-specific barcode. This approach will enable us to follow adaptive evolution as it occurs under each condition, to determine the distribution of fitness effects among novel mutations, and to isolate adaptive mutants for sequencing. We will then pool adaptive mutants, compete this pool under each Mars-relevant condition, and assess the degree to which mutations underlying adaptation to one Mars-relevant stress facilitate or impede adaptation to another. Finally, we will establish the identity of new mutants that prove resistant to two or more Mars-relevant stresses.

Our research will address four questions: (1) How, mechanistically, might microbes adapt to each of four physical challenges prevailing on the present-day Martian surface? (2) Which of these challenges presents the greatest obstacle to adaptive evolution, and why? (3) If cells can adapt to Mars-like surface conditions, or to a subset of those conditions, do trade-offs appear when cells are cultured under benign conditions? Finally, (4), do we see correlated responses to selection, i.e., are cells adapted to one stress predictably more resistant or more susceptible to others? Answers to these questions respond to 6 Key Questions under 2 of the Major Topics in the current NASA Astrobiology Strategy document: Topic 3 Early Life (i) What can we learn about the evolution of early life from long-term experimental evolution of microbes? (ii) How do environmental conditions affect and constrain evolutionary pathways, influencing, for example, the types of mutations and the rates at which they arise? (iii) How reproducible and diverse are evolutionary pathways, given the same or different conditions? Topic 5 Habitability and Biosignatures (i) How does environmental stress influence the persistence and activity of life? (ii) Are there habitable environments on Mars at present? (iii) How can we better understand the range of parameters that influence habitability? Answers to these questions are also of relevance to the Office of Planetary Protection, as humans and their commensal bacteria now stand on the verge of colonizing the Red Planet.

Thomas Santangelo/Colorado State University
Alternative Strategies of DNA Replication in Environmental Extremes

Modern marine hyperthermophilic archaea share many of the metabolic pathways and energy production systems attributed to early life. In addition to thriving in environments that are inhospitable to most species, members of the anaerobic Thermococcales are also known to survive extended periods (> 1 year) at very low temperatures when drifting on ocean currents to colonize new thermal areas. These oligoploid microbes regularly survive near 100ÚC temperature changes, dramatic differences in salinity, aerobic and anaerobic shifts, and months adrift without access to nutrients. As both genetic context and environmental conditions heavily influence phenotype, the contemporary

Thermococcales provide an ideal, genetically-accessible platform to monitor the influence of mutation rate and DNA replication strategies on the evolution of genomes.

Extant DNA replication is controlled by domain-specific initiator protein complexes that assemble on specific locations in the genome termed replication origins. These complex replication strategies facilitate accurate replication of the genome, but were unlikely present in the first cells. The original strategies for initiating DNA replication likely took advantage of natural intermediates generated during recombination or transcription, but these alternative replication mechanisms even in contemporary cells are error prone and result in rapid evolution. Many questions remain regarding the original nature of DNA replication, the evolutionary consequences of accurate versus error prone mechanisms of replication, and how changing environments control the fidelity of genomic maintenance.

We established that some extremophilic archaea retain and regularly switch between two fundamentally different DNA replicative strategies: origin- versus recombination-dependent replication. We further identified that the switch between these two radically different DNA replication strategies is dictated by changes in environmental conditions, likely fundamentally controlled by changes in the replicative DNA polymerase and recombination factor activity. This perhaps surprising result reveals that extant replicative strategies have not completely replaced primordial systems for initiating DNA replication. Most habitable environments (particularly presumptive non-earth habitats) are volatile, and environmental conditions will dictate general chemistry and mutation rate. Our identification of extant species that regular switch modes of DNA replication due to significant changes in environmental conditions thus provides a unique opportunity to identify how environments shape genomes and evolution.

The hyperthermophilic archaeon Thermococcus kodakarensis regularly uses RDR at elevated temperatures and is not dependent on an initiator protein or replication origin for viability. The genetic accessibility of T. kodakarensis provide a unique platform to investigate primordial aspects of DNA replication in the absence of replication origins and initiator protein assembles. This proposal will use a combination of genetic and biochemical experiments to i) investigate the environmental and molecular mechanisms controlling origin- versus recombination-dependent replication, ii) experimentally probe the fitness advantages provided by replication origins and initiator-proteins, and iii) identify how environmental conditions shape genomes through alternative strategies of DNA replication.

The results obtained will have an immediately impact on the primary goals of the Exobiology Program, with our emphasis focused on the Early Evolution of Life and the Biosphere. By detailing the evolution and activity of information processing systems we will directly address the early evolution of life and test hypotheses regarding the original nature of key biological processes (e.g. genome replication).

Emily Sessa/University Of Florida, Gainsville
Surviving a Mass Extinction: Lessons from the K-Pg Fern Spike

The K-Pg asteroid impact and its aftermath profoundly disrupted life across the planet. While this event is usually associated with the loss of dinosaurs, its impact on plants, which form the foundation of ecosystems across the globe, was also profound. In north temperate latitudes, regions close to the impact site were denuded of all life, forests were leveled, and four out of five species of plants went extinct (1, 2). Analysis of North American K-Pg sediments indicate that generalist ferns were the first plants to recolonize these sites (1). Over time, this fern spike was noted as a biomarker in K-Pg localities around the globe (3, 4). Surprisingly, the attributes that imparted ferns with such astonishing resilience to stress have not been investigated. This is non-trivial because the immediate post-impact climate was stressful enough to largely eliminate competition from both flowering and non-flowering plants, which today are the dominant plant groups on Earth with respect to species numbers, biomass, and economic significance.

Post-impact devastation and climate disturbance re-shuffled the structure of the planet s vegetation. The impact released megatons of particulates into the atmosphere, along with climate-altering gases from the carbonate and sulfate rich rocks at the impact site (4). Global dimming, cooling, and acid rain characterized an environment that has been described as a nuclear winter (3, 4, 5). There is also evidence for massive fires around the globe (6), which would have added soot and compounded the loss of sunlight. The duration of this post-impact winter is unknown, but the pollen/spore record indicates that ferns were dominant for at least 30,000 years following the strike (3). The goal of the proposed research is to combine fossil and phylogenetic analyses with physiological experiments to understand why ferns responded differently than other plants to the post-impact environment.

Objective 1: Characterize the fern spike phenomenon: We will study microfossil deposits from sediment before and after the K-Pg boundary at Hell Creek fossil localities in Montana, South Dakota, and Wyoming, to identify which fern genera were present before and immediately after the impact. This analysis will form the basis of morphological and phylogenetic analyses to establish their modern counterparts for Objective 2. We will also identify which genera went extinct, and which flowering plants were the first to colonize after the ferns, using the fossil record to understand traits associated with plant extinction and recolonization patterns.

Objective 2: Evaluate the physiological and growth responses of flowering and nonflowering plants and ferns in post-impact, early Paleocene climates: We will grow target species of ferns, gymnosperms, and angiosperms under low light conditions and acid rain; these are the primary environmental stresses thought to have impacted plants following the impact. Control plants will be grown concurrently. The efficiency of carbon and light capture in mature leaves will be evaluated, and all plants will be harvested at the end of the experiment to estimate total biomass and growth rates. Similar experiments will be performed on the free-living gametophyte stage of the life cycle, which is critical to fern sporophyte establishment.

Relevance to NASA: The proposed research is relevant to the Program Element Large scale environmental change and Macro- evolution , as our work will focus on understanding the consequences of mass extinction events, which are specifically mentioned in the solicitation. This work will inform understanding of how plant groups survived in the aftermath of a mass extinction event.

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Paula Welander/Stanford University
Identifying the Biosynthetic Pathway of brGDGT Biomarker Lipids

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are microbial membrane lipids that have been detected in marine, freshwater, and terrestrial environments and found to be preserved in million-year-old sedimentary rocks. These lipids have emerged as potentially robust biomarkers that allow for reconstruction of various ecological parameters including terrestrial input, pH, and temperature over geological timescales. However, the microbes that produce brGDGTs have not yet been discovered and this ambiguity has led to uncertainty in the interpretation of brGDGT signatures.

In addition to their potential role as geological proxies, brGDGTs also represent an exception to the archaeal/bacterial lipid divide. Archaeal membranes are typically composed of isoprenoidal lipids that are ether linked to glycerol-1-phosphate while bacterial membranes are composed of fatty acid lipids ester linked to glycerol-3-phosphate. The brGDGTs are unique because they contain mixture of archaeal and bacterial lipid properties - they are composed of fatty acid chains with cyclopentane moieties that are ether linked to glycerol-3-phosphate. These chemical distinctions have led to numerous studies attempting to identify the bacteria that produce brGDGTs. Thus far, two Acidobacteria have been shown to produce trace amounts of the simplest branched GDGTs, brGDGT I, but the abundance of brGDGTs identified in different ecosystems as well as the diversity of brGDGT structures that have been documented suggest that other extant sources of these lipids must exist.

The overarching goal of this work is to identify brGDGT biosynthesis proteins that we could subsequently use to identify potential bacterial sources of these lipids. Using a targeted bioinformatics approach based on biochemical hypotheses regarding the synthesis of tetraether lipids in archaea and bacteria, we have identified candidate brGDGT biosynthesis genes in bacterial metagenome acquired genomes (MAGs) and environmental metagenomic datasets. We propose to use a combination of heterologous expression and lipid analyses to determine if the proteins encoded by these candidate

genes are indeed brGDGT biosynthesis proteins through the following two research objectives:

1) Candidate brGDGT condensation proteins identified in the Edaphobacter aggregans genome, one of two Acidobacteria shown to produce small amounts of brGDGT I, will be expressed in Bacillus megaterium and analyzed for brGDGT I production.

2) Candidate brGDGT cyclization proteins identified in bacterial MAGs and environmental metagenomes will be synthesized and expressed in an archaeal GDGT cyclization mutant and in an E. aggregans expression system we are developing to determine if any of these candidate proteins are capable of inserting cyclopentane rings into the core GDGT or brGDGT structure.

Uncovering the brGDGT biosynthetic pathway will allow us to identify potential brGDGT producers through genomic and metagenomic analyses and will also enable future studies to culture brGDGT producers and to characterize the physiological proprieties of membranes composed of brGDGTS. Understanding the biosynthesis of unique lipids that challenge the archaeal/bacterial lipid divide, such as the brGDGTs, is essential for properly understanding the chemical composition of early microbial membranes, the evolution of cellular membranes, and the physiological pressures that may have led to the emergence of the membrane structures we observe today in modern organisms. These topics are relevant to the Exobiology theme of Early Evolution of Life and the Biosphere and specifically pertinent to the objectives of (iii) determining the original nature of biological energy transduction, membrane function, and information processing and (v) investigating 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.