Exobiology
Abstracts of Selected Proposals
(NNH15ZDA001N-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. 190 proposals were received in response to this opportunity. On March 1, 2016, 31 proposals were selected for funding.

Natalia Artemieva / Planetary Science Institute
Extraterrestrial Cause for Mass Extinctions - Reassessing the Hazard

Objectives: Impacts are extremely hazardous on both a local and global scale, and can occasionally change the course of evolution. The Chicxulub impact caused a global catastrophe and, although the principal drivers for the K-Pg mass extinction remain a matter of some debate, the ejection of dust and climate-active gases (carbon dioxide, sulfur oxides, water vapor, methane) into the atmosphere are likely to have played a significant role. The main goal of the current project is to improve our understanding of large impacts and the Chicxulub impact in particular, using the most advanced hydrocode models and recent improvements in the equations of state of geological materials. Well-known hazardous effects (dust and gas ejection, earthquakes, wildfires) will be re-evaluated, while recently discovered effects (ejecta heating up to vaporization during the re-entry, dramatic long-lasting disturbances in the atmosphere) will be examined for the first time. In order to achieve these goals we will:

1. determine the amount of ejected materials (including their physical state and size-frequency distribution of solid/molten materials) for various impact scenarios (different impact velocities, impact angles and target rocks);
2. quantify the amount of climate active gases released during crater formation and the amount of climate active gases released when high-velocity solid ejecta re-enters the atmosphere;
3. model the spatial distribution of ejecta around the globe;
4. derive scaling laws for estimates of the environmental effects of different projectile sizes and targets (the ocean, crystalline and sedimentary rocks).

Results will be made available through a web-site for use by a wide community of scientists working in climate modeling, for better predictions of the environmental consequences of large impacts.

Instruments and code development: We will use the multi-phase hydrocode SOVA to model impact cratering, plume formation and initial ejecta distribution; then, the so-called dusty-block of the same hydrocode will be used to model ejecta re-entry into the atmosphere. To achieve our objectives we will make some modifications to SOVA to include melting and vaporization of ejecta particles as they re-enter the atmosphere. Finally, we will extend the modeling to longer run times so that we can track ejecta as it

travels around the entire globe and is deposited. Our results will be ground-truthed against available geological and geochemical data at the K-Pg boundary and against observations of plume collapse during the Shoemaker-Levy 9 cometary impacts on Jupiter.

Relevance to Exobiology program. The research is part of the Large scale environmental change and Macro-evolution topic listed in the Exobiology program, saying explicitly Of particular interest are mass extinction events .

Fabia Ursula Battistuzzi / Oakland University
Innovative Molecular Timing Applications to Obtain Accurate Histories of Early Life

Science Goals: When reconstructing evolutionary histories of life on Earth primary sources of information are geologic data, applicable mostly to Phanerozoic life forms, and molecular data for pre-Cambrian life. Timelines inferred from these two sources are generally in agreement but major challenges for key evolutionary steps remain. This is especially true for timelines of early microbial life that, due to the almost complete lack of geologic record, are heavily dependent on molecular-based approaches that often produce contrasting evolutionary histories. To improve the confidence in the estimated timelines it is therefore imperative to (i) reconcile contrasting timelines from molecular data with each other and also with the geologic timeline and (ii) design an objective procedure to identify biases in estimations even in cases when alternative independent evidence is lacking.

Objectives: In response to these needs, here we propose to establish an objective and computationally easy procedure to evaluate the accuracy of published and future timetrees that will lead to more accurate and concordant timelines. Our method investigates the parameters associated with calibration points and evolutionary rate variation among lineages, parameters that are known to affect the accuracy of relaxed molecular clocks. We propose to use a relative framework established from prior-free models (fourth generation molecular clocks) to detect biases in these parameters, which can then be removed to increase accuracy of individual estimates. We show preliminary data from the application of the proposed approach in simulated scenarios and an empirical dataset related to a highly controversial evolutionary event, the timeline of the origin and diversification of animals. The results support the validity of the proposed approach that we plan to apply to hundreds of published timetrees with known disagreements, in an effort to produce an accurate timeline of the evolution of life. The established procedure will be particularly useful in investigations of early microbial life for which molecular data is virtually the only record of their evolutionary history.

Methods: Based on the overall goal of this proposal, we have identified two aims. In aim #1 we will focus on the evaluation of calibration boundaries and their effects on divergence times. This is an applied aim that will first evaluate timetrees from the

literature to identify the most common weaknesses and re-estimate new timetrees with improved parameters and taxonomic sampling. The outcomes of this aim will be the first large-scale assessment of published timetrees and the estimation of new, more accurate, divergence times for a wide range of taxonomic groups focusing on early microbial species. Aim #2 will provide theoretical and computational support for the applied analysis of the previous aim by investigating distributions of rate variation among lineages for different simulated datasets. These distributions will serve as reference to determine patterns of rate variation in empirical data and will provide information to optimize our methodology. The new testing procedure developed in aims #1 and #2 will then be implemented in a new software that will allow experts and non-experts to evaluate the dependency of their estimated divergence times on the chosen parameters.

Significance: We expect this project to be highly significant because, by improving molecular dating methods, it will provide the means to estimate a timeline of the evolution of life that will bring into closer agreement biological, geological, and paleontological views of the conditions on early Earth. Reconstructing a chronology of life on Earth has been a longstanding goal in astrobiology as it empowers researchers to draw inferences on the co-evolution of life and habitable conditions of a planet.

Roger Buick / University of Washington
Sustaining Habitability: Nutrient Cycling on the Early Earth

Goals: The continued evolution of life poses a geochemical conundrum, because it requires steady supplies of phosphorus, nitrogen and trace metals, all nutrients that might have been scarce in the ancient ocean. However, we know little about nutrient availability early in Earth history because few analyses comparing multiple nutrients in similar samples have been performed on Precambrian sedimentary rocks. We propose to address the question of which geochemical constraints had the greatest effect on the habitability of the early Earth by analyzing a diverse suite of Precambrian marine and lacustrine mudrocks and carbonates for the macronutrients N and P and the redox-sensitive micronutrients Cu and Se. We will determine nutrient bio-availability and calculate residence times to see if changes coincided with major evolutionary or environmental events.

Methods: 1. Nitrogen abundances, C/N and isotopic ratios. N is the key limiting nutrient in many parts of the modern ocean and may have been so through much of the mid-Proterozoic when a greater expanse of sulfidic (euxinic) seawater may have inhibited nitrogen fixation and denitrification by removing metabolically essential trace metals. However, an inventory of N abundance and a finely resolved N isotope record have not yet been established across much of the Precambrian. We propose to do this for marine shales from the early Archean into the Paleozoic. We hope to find when the N cycle became aerobic, how much N was buried during episodes of widespread marine anoxia perhaps lowering atmospheric pressure, and whether N was critically limiting or incompletely denitrified in the Mesoproterozoic. 2. Phosphorus levels, speciation and C/P

ratios. The solubility of phosphate minerals is low regardless of redox conditions, which could have affected biological evolution even more than N or trace metal supply. In the same samples, we will analyze P abundance and speciation by a sequential extraction technique modified by addition of high-pressure microwave digestion to account for the abundant acid-resistant aluminophosphate minerals in ancient siliciclastic sedimentary rocks. This will reveal whether there was indeed an Archean P crisis caused by iron deposition and if a mid-Paleoproterozoic peak in atmospheric oxygen was due to global P excess. Carbonate samples will also be analyzed to see if dissolved P was present as phosphate (as now) or as more soluble phosphite. Samples from impact horizons should show if phosphite was derived from meteoritic sources. Comparison of C/P to N isotope ratios over time should record when oceans shifted from P to N limitation. 3. Trace metal abundances and isotopes. The N cycle is regulated by trace metal availability and oceanic redox state. For the former, we will focus on copper that is concentrated in the active site of the enzyme N2OR which controls the last step in denitrification and thus determines whether N2 or N2O is returned to the atmosphere, with greenhouse consequences. We will perform microbiological experiments using various denitrifying microbes under conditions of Cu limitation and surfeit to determine if Cu isotopes are fractionated by denitrifiers and how much it affects the N2/N2O ratio. If a fractionation is found, we will then search for it in mid-Proterozoic rocks. For the latter, we will examine Mesoproterozoic selenium isotopes and abundances as indicators of widespread suboxic environmental conditions that would promote denitrification and thus cause N depletion. Relevance: The project will lead to a greater understanding of how nutrients influenced biological evolution on the early Earth. It will examine nutrient supply across environmental changes, thus adding to our knowledge of how planetary habitability is maintained over long time intervals. As it focuses on the microbial early Earth, it is relevant to finding biosignatures on other planets: i.e. NASA Astrobiology Roadmap Goal 4, Objectives 4.1, 4.2, 4.3 and 7.1.

Donald Burke / University of Missouri
Metabolite-Linked RNA Transcripts

This project investigates cofactor-RNA conjugates to shed light on the origin, early evolution and evolutionary history of life. The biology and biochemistry of this new class of RNAs, recently observed in bacterial RNA fractions, will expand our understanding of how nucleic acids and the small-molecule metabolome work together, how they may shape metabolic flux in modern organisms, and how they may have contributed to catalytic diversity in ancient RNA enzymes. The two aims build upon strong literature precedent and preliminary data, combining innovative experimental and bioinformatics approaches in an integrated examination of a novel class of RNA transcripts.

AIM 1: Bacterial CoA-RNA conjugates. RNA transcripts that carry covalently attached Coenzyme A (CoA) have been unambiguously demonstrated to exist in E. coli and S. venezuelae, but nothing is known about their biological significance. An intriguing possibility - supported by identification of acetyl- and succinyl-CoA ester conjugates to

RNA - is that they are involved in RNA-mediated manipulation of small molecule metabolism. We will establish the identities of CoA-RNA conjugates in multiple bacterial species using an affinity-capture strategy similar to one used recently to identify NAD-RNA conjugates in E. coli. We will also determine sequence and secondary structure requirements for conjugate formation, and use this information to establish phylogenetic distributions of CoA-RNA among sequenced genomes. Our results will provide the first clear picture of this previously unrecognized facet of RNA biology, and they will enable future studies exploring their biological roles. Some of the identified transcripts may represent true 'molecular fossils' held over from the time of an RNA world; others will be 'newer' inventions that illustrate the variety of ways in which organisms exploit RNA transcripts to manipulate small molecule metabolites. Thus, these studies are highly relevant to understanding the evolution of metabolic diversity of extant life on Earth.

AIM 2: Artificial FAD-RNA conjugates. Enzymes enhance the chemical reactivity of cofactors through both covalent and non-covalent interactions. We recently identified RNA aptamers that strongly differentiate between oxidized (FAD) and reduced (FADH2) flavins. We will examine both non-covalent and covalent flavin-RNA complexes, focusing on establishing how their molecular interactions influence intrinsic chemical reactivity of the bound flavin and the structural basis for these influences. Differential binding energy is used by flavoprotein enzymes to perturb intrinsic reactivity (half-reaction reduction potential), thus providing a strong chemical rationale for this approach. The results from this study will provide chemical constraints on how to connect modern RNA-metabolite conjugates with their potential biochemical roles in ancient (and modern?) metabolisms. These studies will address long-standing models for the evolutionary origin of nucleotide cofactors in which these molecules are proposed to have enhanced the catalytic repertoire of ancient ribozymes.

The project is highly relevant to NASA's Exobiology and Evolutionary Biology program and to the NRA's Early Evolution of Life and the Biosphere area of emphasis. We anticipate that the project will provide critical advances related to understanding small molecule metabolism and RNA biology in modern organisms, and to understanding RNA's ability to provide metabolic catalysis in an RNA world. Thus, these studies are highly relevant to five specific areas of interest to NASA: 1) early evolution of life and the nature of the most primitive organisms; 2) the transition to modern biochemistry; 3) evolution of genes, pathways, and microbial species; 4) the repertoire of demonstrated RNA functions; and 5) construction of artificial chemical systems to test hypotheses regarding the original nature of key biological processes.

Irene Chen / University of California, Santa Barbara
The Evolvability of Ribozymes for the Genetic Code

Motivation: Ribozymes are believed to have been crucial molecules for early life and may have been the earliest evolvable molecules. However, we have little quantitative

insight into their evolvability, with respect to de novo emergence, attainment of the optimal sequence, and evolution of new functions.

Objectives: We propose to study fitness landscapes (complete descriptions of the sequence-activity relationship) for ribozymes, using methods already developed in our lab. Our studies are focused on aminoacylation activity, which forms the chemical basis for the genetic code. In Objective 1, we will map the complete fitness landscape of aminoacylation ribozymes using a tyrosine analog as substrate. This will yield quantitative information about the de novo evolvability and optimization potential of these ribozymes. In Objective 2, we will study the protocellular environment as a possible enhancer of RNA activity and therefore evolvability. In Objective 3, we will focus on a second fitness landscape for aminoacylation ribozymes, using a different substrate (phenylalanine analog). This will enable us to determine the potential for this type of ribozyme to evolve to adopt a new substrate.

Impact: The results from these objectives will help us understand, at a quantitative level, several fundamental issues about ribozyme evolvability. These are: the probability of emergence of ribozymes for aminoacylation, how well natural selection could optimize these ribozymes, how susceptible the evolution of ribozyme sequences would be to chance events, and whether an ancestral ribozyme would be likely to give rise to ribozymes performing different functions.

Relevance: These issues are relevant to the NASA Exobiology Program in the Prebiotic Evolution emphasis, and lie directly within Goal 3 of the 2008 Astrobiology Roadmap examining the origins and evolution of functional biomolecules.

Paul Falkowski / Rutgers University

The Chemical Transformation of Minerals by Light and the Evolution of Prebiotic Metabolism

Objective: Life on Earth is driven by electron transfer reactions far from thermodynamic equilibrium. Overwhelmingly, the primary source of energy for these reactions is light, which is converted to chemical bond energy in photosynthetic organisms. Our research focuses on the reactions of light with primordial minerals that supported the evolution of autocatalytic processes that led to the earliest forms of prebiotic metabolism.

Background: It has long been known that chemisorbed molecules on mineral surfaces can undergo bond rearrangements by photochemical reactions. The absorption of photons by minerals can promote endergonic reactions that are thermodynamically unfavorable and not thermally accessible on Earth's surface (i.e., water splitting: 2H2O ’ O2 + 2H2), as well as catalyze exergonic reactions that are kinetically inhibited (i.e., CO + ½ O2 ’ CO2). We seek to understand how light-driven redox processes on mineral surfaces could have contributed to prebiotic syntheses of gaseous products as well as simple organic molecules, both of which potentially were substrates for prebiological metabolism.

Photogeochemical reactions almost certainly had a profound effect on the net redox state of the Earth by the creation of oxygen species and loss of hydrogen to space. However, there have been no systematic studies on the photoreactivity of minerals present on Earth in its early history and thus their importance to prebiotic and global geochemical processes is largely unknown.

Approach: We propose to screen an initial set of target minerals to experimentally identify photogeochemical processes. Targets will be selected from the putative 420 early Archean minerals described by Hazen. Several of these minerals contain functional groups that have been incorporated into biological catalysts. We will focus on those minerals that were abundant early in Earth's history and potentially in the evolution of extra-solar terrestrial planets. Experimentally, we employ density functional theory to predict antibonding orbitals from which low energy electron transfer reactions are probable. The products of these reactions (e.g. H2) potentially were substrates for the earliest prebiotic metabolism.

We will characterize photochemical properties of minerals that potentially support electron transfer reactions within boundaries of reasonable photon energies from a young star in the main sequence. Light driven interfacial redox reactions typically consist of several steps that occur on the time scale from femto- to milliseconds. The overall photochemical efficiency of the reaction depends on the relative rates of physical and chemical decay from the initial excited state. The partitioning between the competing kinetic pathways is primarily determined by the lifetime of the initial charge-separated state and the concentration of the small molecule substrate on the surface of the mineral. In order to fully understand and quantify the impact of such kinetic chains on the chemical and redox landscape of Earth's surface in the prebiotic era, we will focus on the lifetimes and identities of the key reactive excited states which may catalyze subsequent transformations of increasing complexity and selectivity.

Significance: The proposed research provides a unique window to potential planetary-scale chemical characteristics that could arise from purely abiotic photochemical reactions. Our integrated approach is specifically designed to understand photochemical processes on mineral surfaces that potentially yield gaseous products far from thermodynamic equilibrium. We suggest that these underappreciated photogeochemical reactions were critical in altering the geochemistry of Earth prior to the evolution of life, and therefore an understanding of these processes will help in identifying signatures of life on terrestrial exoplanets in the habitable zone.

Woodward Fischer / California Institute of Technology
Insights Into Pre-Phototrophic Progenitors of Oxygenic Cyanobacteria Via Analysis of Closely Related Non-Phototrophs

Oxygenic photosynthesis is perhaps the most transformative bioenergetic innovation in the history of life, as the activity that fundamentally changed the planet. The carbon cycle

was stimulated by an increase in primary productivity, resulting in cascades of evolutionary innovations. O2 became widely available for utilization as an oxidant in energy metabolism and in diverse anabolic and catabolic pathways. Geochemical cycles were also greatly altered, leading to the accumulation of other oxidants in the atmosphere and oceans. The availability of O2 ultimately led to aerobic respiration, eukaryotic endosymbiosis, and complex multicellularity. Not surprisingly, the presence of abundant atmospheric O2 forms part of our search image for life on planets outside of our solar system.

All evidence supports the theory that oxygenic photosynthesis evolved but once, in an anoxygenic ancestor of modern Cyanobacteria. However, details of the evolutionary steps taken during the rise of oxygenic photosynthesis remain a mystery. A better understanding of the natural history of the phylum marks a path forward. Are lineages of bacteria that share the same pre-phototrophic ancestor as the Cyanobacteria extant today? Might behavior of these cells inform the physiological platform upon which oxygenic phototrophy evolved?

Environmental sequencing efforts illustrate that Cyanobacteria are more diverse than historically appreciated. Several uncharacterized clades of deep branching forms exist; moreover, these occur in perennially dark and often anoxic environments, which suggests that the corresponding organisms are not oxyphototrophs (1). Recent genome analyses on uncultivated representatives of one of these dark cyanobacteria clades (termed Melainabacteria) revealed no genes associated with photosynthesis (2,3); nearly all lacked genes for aerobic or anaerobic respiration. Thus, Melainabacteria and other newly identified clades of dark cyanobacteria provide important insights into the organisms from which oxygenic photosynthesis arose.

Pilot research at Caltech identified an environment wherein several different lineages of dark cyanobacteria are reliably and reproducibly residing: the complex, fermentation-driven gut microbial communities of termites. This model environment is particularly attractive for laboratory-based studies because it is a microliter in volume, well-bounded, available in large numbers of replicates, and straightforward to maintain in the laboratory. Pilot experiments reveal that a single termite hindgut contains at least four independent clades of the dark cyanobacteria - the highest and deepest diversity observed in any environment to date. We propose a detailed and multidisciplinary study of these dark cyanobacteria, using environmental perturbations, comparative meta-genomics and transcriptomics, FISH and other microscale localization approaches, as well as, physiological lines. Comparative gene-based analyses of dark cyanobacterial diversity in different termite species representing distinct insect family lineages will also be performed, offering a view into evolutionary rates and patterns of diversification in dark cyanobacteria [i.e. in the context of the 100+ million year fossil record of termites and other metadata on the hosts (4)].

The proposed scope fits several program objectives. For example, Early Evolution of Life and the Biosphere aims to understand the phylogeny and physiology of microorganisms...whose characteristics may reflect the nature of primitive environments,

investigate the development of key biological processes and their environmental impact, and investigate the evolution of genes, pathways, and microbial species subject to long-term environmental change.

1. F. Warnecke et al., Nature, 450, 560-565 (2007).
2. S. C. Di Rienzi et al., eLife 2:e01102 (2013).
3. R. M. Soo et al., Genome Biol Evol 6, 1031 (2014).
4. X. Zhang, J.R. Leadbetter (2012), mBio, 3:e00223-12 (2012).

Morteza Gharib / California Institute of Technology

Microbial Residue and Compounds of Life from Enceladus (MIRACLE)

There is increasing evidence that Saturn's moon Enceladus possesses all of the required ingredients for life: a subsurface liquid ocean, a source of heat (geothermal), and all chemical building blocks (with the possible exception of phosphorus). Enceladus has thus become a major target for astrobiology mission design studies. Because of the low escape velocity of this moon and the icy plumes that escape from its surface, a flyby mission to capture plume material is a viable approach; this material may be analyzed in orbit or returned to Earth. However, the survivability of organisms and biosignatures in this captured material remains completely unknown. Such data would have key implications for how captured material should be studied to identify biosignatures, whether the material must be returned to Earth, and what the planetary protection requirements for returned material will be. The possibility of forward contamination also needs to be addressed. The hypothesis of this proposal is that extremophilic microorganisms and spores of the type most likely to exist on Enceladus may be captured in a flyby mission in a way such that key chemical and morphological biosignatures are preserved. Using a high velocity water jet, we will simulate the plumes of Enceladus and Europa with or without seeding with bacteria and/or bacterial spores. Survivability of organisms and molecules in the plumes alone will be analyzed by light and electron microscopy, polymerase chain reaction (PCR), and mass spectrometry. The first year of the work will focus on development of the water jet system to create ice particles at different velocities and flow rates. In the second year we will focus on observing survival in the plumes alone, without accounting for capture. In the last year, we will quantify the speeds at which key biomarkers disappear by flash heating ice from the jet to temperatures consistent with their capture energies in order to determine the implications for life detection of different capture speeds. Because microorganisms on Enceladus are likely to be methanogens, the selected organisms for the test will be methanogens as well as other selected extremophiles. This proposal directly addresses the NASA research emphasis on Biosignatures and Life Elsewhere. It identifies the conditions under which biosignatures may be preserved, which is relevant to design of any astrobiology mission, particularly those involving sample return.

David Jablonski / University of Chicago
Multidimensional Analysis of the End-Cretaceous Extinction and Its Consequences: Form, Function, and Taxonomic Diversity

Science goals: One of the most dramatic instances of extraterrestrial influences on evolution is the K-Pg bolide impact at the end of the Cretaceous Period ~66 Myr ago. Nearly all paleontological research on this event and its aftermath has focused on just one dimension of biodiversity - the number of species or higher taxa. Other macroevolutionary currencies, such as (a) morphological diversity (the variety of forms within and among clades) and (b) functional diversity (the different ways organisms make a living), have been neglected, even though they are only loosely correlated with taxonomic diversity, and are essential for understanding the structure and function of past and present-day biotas. We propose to remedy this situation by expanding our existing, spatially explicit taxonomic databases on marine bivalves to include morphological and functional information, providing a foundation for analyzing extinction and recovery associated with the K-Pg event. We will test (i) the strength of the associations of functional and morphological attributes, compared to geographic range size as another key factor, with survivorship at the K-Pg event, and (ii) how the K-Pg impact affected the early Cenozoic, and longer-term, morphological and functional diversity of marine bivalves.

Methods: Augmenting our georeferenced, global data on Maastrichtian, Paleocene, and Recent marine bivalve species, we will collect new morphological data from museum collections, monographic illustrations, taxonomic descriptions and online images. We will construct multi-dimensional morphological spaces (a shape space based on shell outlines and a complementary trait space based on body size, shell thickness, and ornamentation) and a functional space based on four major ecological axes derived from feeding, relation to the substratum, attachment mode, and mobility (inferred from standard aspects of shell form and phylogenetic position). We will apply logistic regression and model-selection approaches to bivalve genera (averaging their species) to test if K-Pg changes in the occupation of bivalve morphological spaces and functional space are better explained by selection against positions in those spaces, against taxa having small geographic ranges, or a combination of those. Preliminary work suggests that some traits were lost or marginalized owing to indirect, hitchhiking effects: they were not necessarily disadvantageous during the mass extinction, but were tied to other features that increased extinction vulnerability, specifically narrow geographic range. We will then compare the deployment of K-Pg survivors in those spaces to the late Paleocene fauna as the recovery pulse was waning, and compare both distributions to the Recent biota, to evaluate which changes represent lasting effects, which faded immediately downstream of the impact, and which faded after the Paleocene. Complete Cenozoic histories of two of the most diverse clades will serve as first-order guides to post-Paleocene events.

Relevance: This work is directly related to the NASA research area described as Large scale environmental change and Macro-evolution, in which mass extinction events are of particular interest. The research will address, from a novel perspective, the effects of

the K-Pg impact on the large-scale evolution of a group that has become a model system by virtue of its preservation potential and well-characterized taxonomic diversity patterns in ancient and Recent seas. The result will be a new view of how catastrophic extinctions and recoveries reshape biodiversity - not just the victims and survivors, but how the morphological and functional diversity of the global bivalve biota was changed, temporarily or permanently.

Qusheng Jin / University of Oregon
Habitability: From Methanogens to Minimum Energy Flux of Life

Objective:
The concept of habitability constrains the possible distribution of life on the Earth and other planets. In qualitative terms, a habitable environment must deliver energy at levels above the threshold and at rates greater than the minimum flux required by life. The threshold energy of life is well understood at a mechanistic level, and its magnitude is highly constrained by theoretical, laboratory, and environmental studies. In comparison, our understanding of life's energy flux requirement has lagged behind: to date, we lack a comprehensive mechanistic model of the energy flux requirement; previous laboratory culture studies and field observations yield energy flux requirements over several orders of magnitude. The objective of the proposed work is to develop a robust theoretical framework for quantifying biological energy flux requirements in very-low energy settings, as in Earth's subsurface environments and likely on other planets.

Methods:
We propose to study the energy flux requirement of life by simulating the metabolism of model organisms under variable energy fluxes. Our modeling approach differs from current metabolic modeling methods in that (1) it breaks cell metabolism into the networks of energy conservation, biosynthesis, and maintenance, (2) it explicitly models biomolecular attrition, membrane leakage, and other potential contributors to the energy flux requirement as a function of environmental conditions, and (3) it describes the enzymatic reactions in the networks of energy conservation and maintenance as kinetic processes, while simulating the biosynthesis network using flux balance analysis. In this way, our modeling approach combines a rigorous treatment of the thermodynamics and kinetics of energy conservation and transduction (our principal focus) with a tractable and computationally manageable treatment of the biosynthesis network.

The proposed effort consists of three main tasks: the development of kinetic network models for hydrogenotrophic methanogenesis and cellular maintenance, and flux balance network model for biosynthesis; the implementation, verification, and validation of the models; and numerical experimentation and exploration of low-energy metabolism using the validated models. Our effort will take advantage of the fact that the models can be driven by energy fluxes that range from those commonly employed in laboratory culture experiments to those characteristic of low-energy natural settings. The former will allow us to ground-truth the models against an extensive body of laboratory studies on

hydrogenotrophic methanogens under variable physicochemical conditions. The latter enables a mechanistic-level exploration of biological energy flux requirements under environmental conditions and at physiological states that cannot presently be recreated in the lab and are difficult to observe in nature.

Significance:
The proposed study will bear both theoretical and practical significance in astrobiology. First, this study directly addresses the concept of habitability of both terrestrial and extraterrestrial environments, an important attempt to move beyond the binary, water-driven approach to habitability, toward multi-dimensional and quantitative metrics. Second, this study will develop a new simulation tool for investigating life in low-energy environments, a condition that is challenging to study directly in nature and cannot be faithfully reproduced in laboratory experiments. Last, the results of this study will provide a mechanistic and quantitative understanding of maintenance reactions, which will unify our view of energy flux requirements across a spectrum of energetic conditions, from oligotrophic to eutrophic.

Soon Sam Kim / Jet Propulsion Laboratory
Catalytic Binuclear Manganese Centers as Biosignatures for Martian Exobiology

In order to succeed in life detection on Mars, we need at least 2 capabilities: 1) unambiguous biosignatures that are robust and stable over geological time under Martian conditions, and 2) a technology that will allow us to detect such biosignatures. Both requirements are achieved in this proposed concept. Using an isotopic signature of sulfur (´34S), Dr. Tornos (Centro de Astrobiologia, Madrid, Spain, no-funding needed) showed that the Tharsis sulfide deposits (355 My) in southern Iberia were the result of metabolism of ancient microbes. Preliminary Electron Paramagnetic Resonance (EPR) spectra of these samples obtained by Kim (PI) indicated possible presence of binuclear manganese centers [Mn(II)-Mn(II)]. The EPR signatures are unique and distinct from monomeric Mn ions, and if confirmed by the proposed experiments, could be interpreted as potent biosignatures, since the binuclear Mn-Mn system is unique in biology, surviving after the decay of organic manifolds and yet distinguishable from inorganic counterparts. These Mn-Mn complexes are the active centers (SoxB) of sulfur oxidizing (Sox) enzyme that provide microbes their capabilities. On Earth, sulfur is an important element for life as part of oxidation and reduction reactions associated with microbial activity (sulfur breathing bacteria). There are more than 120 different bacterial taxa known to oxidize sulfur using this enzyme. In the Martian regolith, Mn and S are relatively abundant elements (0.45%, 7.2% respectively), compared to the meager concentrations (0.1% and 0.03% respectively) in the Earth's crust. Thus, it is reasonable to assume that life on Mars, if it ever existed, evolved in a similar fashion to that on Earth, depositing manganoenzymes that can be used as potent biosignatures. EPR has proven to be the most sensitive technique to characterize binuclear Mn-Mn sites. In view of findings by Phoenix Mars Scout Lander, such manganese biominerals have a far better

chance of survival on the Martian surface than do more conventional organic fossils that could be easily destroyed by interaction with soil perchlorates (ClO4-).
Under this proposal, we will use samples from a broad range of sulfur deposits as well as contemporary field samples, laboratory grown SoxB proteins and their degradation products to confirm and assess the stability of binuclear manganese EPR signatures. We will characterize them by EPR at lower temperatures, e.g., 20 K, 30 K and 50K. Through the work, we will have a better definition of the structural parameters and confirm the binuclear nature of EPR for biosignatures. Through collaboration with Dr. Tornos, we will have access to samples of sulfur deposits of Tharsis as well as other relevant localities. JPL has a Bruker EPR spectrometer (Elexys 500E) equipped with Oxford Cryogenic apparatus for the low temperature work.

This proposal directly addresses objectives of the Exobiology NRA: Biosignatures and Life Elsewhere that focuses on relating the origin of life on Earth to the potential for the origin and establishment of life on other planetary bodies, and research on the formation and retention of biosignatures under non-Earth conditions (e.g., Mars, Europa) as well as Biosignature studies of samples from Earth sites analogues of other planetary environments that might potentially harbor life in the search for life in the Universe. It also satisfies two major goals of Astrobiology Roadmap, 2008 : Goal 2, Determine any past or present habitable environments, signs of life elsewhere in our Solar system, and Goal 7, Determine how to recognize signatures of life on other worlds and on early Earth. Identify biosignatures that can reveal and characterize past or present life in ancient samples from Earth. We have assembled a best team of experts for the execution of the proposed task.

Gary King / Louisiana State University
Carbon Monoxide Oxidation by Extreme Halophiles as a Model for Exobiological Energy Metabolism

Scientific goals and objectives. This proposal will address core goals of NASA's exobiology program through research that emphasizes a novel microbial process- carbon monoxide (CO) oxidation under oxic, sub-oxic and anoxic conditions by members of the Euryarchaeota, including extreme halophiles and Archaeoglobus fulgidus. The major research goals include:

(1) determine the capacity of euryarchaeal CO oxidizers to use levels of CO present in terrestrial and extraterrestrial atmospheres as sources of energy for survival or growth and as a function of various electron acceptors and critical environmental variables (e.g. water potential);

(2) determine the diversity of euryarchaeal CO oxidizers and controls of diversity in geochemically distinct hypersaline systems;

(3) develop a model for the origin and evolution of molybdenum-dependent carbon monoxide dehydrogenase, the key enzyme used by CO-oxidizing haloarchaea.

Several specific objectives will be pursued in order to accomplish the research goals:

Objective 1. Assess the capacity of A. fulgidus to use CO for growth and maintenance as a function of CO concentration and electron acceptor availability, including use of (per)chlorate.

Objective 2. Determine the capacity of Type 2 CO-oxidizing haloarchaea to use atmospheric CO as an energy source for survival under conditions relevant for Mars and other systems.

Objective 3. Assess the phylogenetic diversity of Type 2 CO-oxidizing haloarchaea in hypersaline systems and potential controls of their diversity.

Objective 4. Develop a database of new Type 2 CO-oxidizing haloarchaeal Mo-CODH genes to assess their origin and evolutionary history through phylogenetic analyses.

Brief summary of methods. New CO-oxidizing haloarchaeal isolates will be obtained from field sites where CO uptake has been documented using in situ field assays and ex situ analyses. New and existing isolates will be used to assess capacities for CO oxidation under a wide range of conditions that will establish the physiological range of the process, and potential for activity in an extraterrestrial context. These assays will emphasize low CO levels, water stress, varied electron acceptors and persistence. Nextgen sequencing of 16S rRNA genes will be used to determine the diversity of haloarchaeal communities as a whole, and analyses of coxL genes will be used to assess CO-oxidizing haloarchaea. Sequences of CODH genes and various housekeeping genes will be subjected to phylogenetic analyses based on multiple algorithms, the results of which will be used to infer the evolutionary history of Mo-dependent CODHs.

Proposal relevance. The work proposed here is directly relevant for at least two areas of early evolution of life and the biosphere. First, anticipated results will provide novel insights into the ecophysiology and diversity of CO-oxidizing Euryarchaeota, whose characteristics may reflect the nature of early biotic systems on Earth, Mars or elsewhere. Second, the research will investigate the phylogenetic history of an ecologically and biogeochemically important process, CO oxidation, and its environmental impact as determined by interactions between CO-oxidizing halophiles and other halophilic functional groups.

Karen Lloyd / University of Tennessee
Metabolic Flexibility of Low Energy Specialist Methanogens

A major metabolic mode of life that has been hypothesized, but not yet described, is one that allows an organism to harvest life-sustaining energy from either the net forward or the net reverse direction of a single chemical reaction. This, of course, cannot occur in a closed system because it would be a perpetual motion machine, but when external environmental factors alter the relative concentrations of products and reactants, a very small number of metabolic reactions can switch to become exergonic in the reverse direction. What is still a mystery is whether organisms can harness this thermodynamic quirk to survive. The chemical reaction used by hydrogenotrophic methanogenic archaea (Fig. 1), is one of the few metabolic reactions that can undergo exergonic reversals (Hoehler et al. 1994). Given the intriguingly transient appearances of methane on Mars (Webster et al. 2015), discovering and describing of this new metabolic mode on Earth is essential for modeling potential biological contributions to the methane signal.

The main obstacle to describing exergonic metabolic reversals in hydrogenotrophic methanogens is that this process only occurs near thermodynamic equilibrium, so the organisms capable of living on it must survive very low energy yields. Therefore, growth and metabolic signals often approach detection limits, and natural populations can be easily overgrown by more energetic organisms in laboratory enrichments. Our preliminary evidence, however, suggests that we can overcome these obstacles using molecular biological techniques. We propose to A) determine whether natural, low energy, methanogens are phylogenetically and phenotypically distinct from all known cultures of methanogens, B) demonstrate their ability or inability to grow under methanogenesis or methanotrophy, and C) discover the genes responsible for either the metabolic flexibility or the metabolic rigidity that we encounter.

This work is of interest to the NASA exobiology program's research emphasis: Biosignatures and Life Elsewhere ; specifically, the requirement for basic research on the formation and retention of biosignatures under non-Earth conditions (e.g., Mars, Europa) . Recently, transient appearances of methane have been found on Mars. If this methane has supported life at any time in Mars' history, the organisms will probably resemble these elusive low-energy methanogens we propose to study. Our work will provide much needed evidence to support the theory that methanogenesis is a metabolism that can support life in the forward direction or the reverse direction, depending on growth conditions.

Ulrich Muller / University of California San Diego
In Vitro Selection of Ribozymes to Study the Emergence of an RNA World

Science goals and objectives:
The goal of the proposed research is to explore how an RNA world organism may have emerged on early Earth. Two specific questions are addressed. First, how did the

presence of prebiotically formed peptides influence the rise and ability of catalytic RNAs (ribozymes)? Second, how was it possible to generate nucleoside triphosphates (NTPs) from free nucleosides and the prebiotically plausible molecule trimetaphosphate? The objective is to generate and study ribozymes relevant to the RNA world that use prebiotically plausible peptide cofactors, and that generate NTPs. The study of these ribozymes will refine and constrain our understanding of how an RNA world organism could have emerged and functioned on early Earth and on other planets.

Methodology:
In vitro selections from random RNA libraries will be used to identify new ribozymes. For the first question, an in vitro selection will be performed in the presence of peptides. The specific in vitro selection procedure was established and executed in our lab under previous NASA funding. The procedure is very efficient and robust, and therefore ideally suited to study cofactors that influence the activity of ribozymes. For the second question, a new selection procedure will be established and executed to isolate ribozymes that triphosphorylate free nucleosides (as opposed to RNA polymers). This selection is technically more demanding than the first selection but the critical parts of this selection procedure were established in the lab. After both in vitro selections, isolated ribozymes will be characterized and optimized using RNA biochemical techniques and small-scale in vitro selections.

Relevance to the NASA Exobiology program (NNH14ZDA001N-EXO):
Both aims of the proposal are relevant to the research emphasis of "Prebiotic Evolution" because a major research objective of this research emphasis is "determining what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere, including alternatives to the current DNA-RNA-protein basis for life". RNA world organisms fit this definition of "chemical systems [...], including alternatives to the current DNA-RNA-protein basis for life", and the proposed research studies the emergence and function of RNA world organisms.

The two specific aims study details of this topic. The first aim, the in vitro selection of ribozymes that recruit peptides for optimal function, studies how prebiotically formed peptides may have impacted the emergence of catalytic RNAs and an RNA world, and therefore the described chemical systems and alternatives to DNA-RNA-protein basis for life. The second aim, the in vitro selection of ribozymes that generate free nucleoside triphosphates from free nucleosides and trimetaphosphate, studies how an RNA world organism could have used small molecules from its environment to self replicate and establish an energy metabolism. Because the studied nucleoside triphosphates act as energy metabolites in every known living system, and because they would serve as the central molecule for the self-replication of an RTNA world organism, this aim studies a different detail of the described chemical systems that "could have served as precursors of metabolic and replicating systems and alternatives to the current DNA-RNA-protein basis for life".

Dianne Newman / California Institute of Technology
Towards a New Interpretation of Sedimentary Hopanes Using Bradyrhizobium Diazoefficiens as Model System

Hopanes, molecular fossils of bacterial lipids called hopanoids, are abundant biomarkers found in sedimentary rocks. Our ability to interpret them is limited by our knowledge of the function of hopanoids in extant bacteria. Early measurements correlating 2-methyl (2Me) hopanoid production with cyanobacteria gave rise to the inference that 2Me-hopanes were biomarkers of oxygenic photosynthesis. With the discovery that an anoxygenic phototroph produces 2Me-hopanoids and subsequent identification of a C-2 methylase, we found that 2Me-hopanoids originated in the Alphaproteobacteria and were horizontally transferred into the Cyanobacteria well after the invention of oxygenic photosynthesis. These and related studies proved that 2Me-hopanes cannot be used as biomarkers for oxygenic photosynthesis, yet elicited a new interpretation. We now know that methylation is a stress-response that increases the capacity of hopanoids to rigidify membranes. Moreover, today most (2Me)-hopanoid producers live in association with plants. Consistent with this observation, we recently discovered that hopanoids promote symbiotic fitness of Bradyrhizobium diazoefficiens with a tropical legume. In planta, a mutant lacking C35 hopanoids is compromised in nitrogen fixation and grows poorly ex planta under conditions that characterize the plant microenvironment: hypoxic, acidic, hyperosmotic with oxidative stress. A mutant unable to make 2Me-hopanoids also exhibits growth defects under hypoxic and anoxic conditions. Exploring the functions of hopanoids under these conditions will help us understand their roles in diverse contexts, including those captured in the rock record. For instance, peaks in the 2Me-hopane index in Phanerozoic sediments correlate with oceanic anoxic events, and today, an isomer of bacteriohopanetetrol is enriched in hypoxic portions of the marine water column. These and other data suggest that 2Me-hopanoids may have conferred a fitness advantage to ancient bacteria that helped them cope with the stresses associated with anoxia. We propose to test the hypothesis that hopanoids increase stress resistance by decreasing membrane permeability, which is required for efficient metabolisms under hypoxia and anoxia.

Towards this end, we propose to examine effects of specific hopanoids on membrane permeability, assess if changes in membrane biophysical properties due to hopanoids affect core metabolic processes such as respiration and nitrogen fixation, and characterize membrane composition in the presence and absence of hopanoids to identify a larger set of hopanoid functional interactions in an unbiased fashion. Because the biophysical and biochemical functions of hopanoids are likely conserved among bacteria, gaining insight into how hopanoids impact survival of B. diazoefficiens under ecologically-relevant conditions will strengthen our ability to interpret hopane biomarkers in ancient rocks. The microscale is a great equalizer: from a bacterial perspective, it matters less whether a cell is housed within a nodule, embedded within a stromatolite, or attached to particles of marine snow. What matters is the nature of the microenvironment, which can be highly conserved at the scale of the microbe despite different macroscopic contexts. If, as we hypothesize, specific hopanoids impact membrane permeability, thus bolstering core metabolisms (e.g. respiration, nitrogen fixation) under stress (e.g. hypoxia, high

temperature, osmolarity, low pH), we may be able to rationalize correlations between hopane content and paleoenvironmental conditions where independent lines of evidence suggest such stresses existed at the time of hopanoid biosynthesis and the capacity to respire under hypoxia and/or perform nitrogen fixation may have provided a selective advantage.

Alison Olcott Marshall / University of Kansas
Pilot Study: Biomarker Preservation Potential of a Sulfur-Rich Evaporative Mars Analog

Pilot Study: Biomarker Preservation Potential of a Sulfur-rich Evaporative Mars Analog.

Science Goals and Objectives: Hypersaline evaporative environments are a frequently studied Mars analog here on Earth, as there is increasing sedimentological and mineralogical evidence that there were once ephemeral lakes present on the surface of Mars. However, although there has been a great deal of research how these environments preserve the biomarkers of extant microbial communities, there has been very little, if any, research into the geological record of biomarkers in ancient Earth analogs. This gap in knowledge is bad, as if these environments on Mars preserve a record of life, it is more likely to be a record of extinct life than extant life. This pilot study will provide these missing data, by examining the geological record of biomarkers preserved at multiple Mars analog sites. This study will not only increase our understanding of whether these environments can preserve a long-term record of life, but will determine the best practices for maximizing the chances of success when analyzing these types of samples remotely via a rover.

Methodology: I have identified multiple Mars-analogous field localities, sulfur- and iron-rich evaporative environments with mineralogy, sedimentology, and trace element composition similar to what has been described on Mars. These types of environments have not been thoroughly searched for biomarkers, the geological form of biosynthesized molecules. Although the record of ancient life in these rocks has yet to be investigated, I have found microorganisms preserved within gypsum crystals, and carbonaceous microstructures have been described from correlative units. The fact that these rocks are known to have once contained microbial life makes them an excellent target for this study; if biomarkers are not found, it is an indication that this type of environment does not easily preserve them. This proposed project will use the gas chromatography/mass spectroscopy (GC/MS) instrumentation in my lab to search, and if found, to characterize, the preserved biosignatures at each locality. Furthermore, samples will be collected in geological context, so any biomarkers found can be correlated to depositional environment.

Relevance: This proposed research is directly relevant to the fifth region of interest for this call, Biosignatures and Life Elsewhere, which states [b]iosignature studies of samples from Earth sites thought to be analogues of other planetary environments that

might potentially harbor life will be considered as part of NASA's broader interest in the search for life in the Universe.

Jeremy Owens / Florida State University
Tracking the Initiation of Marine Ocean Oxygenation Through Manganese-Oxide Burial Using Thallium Isotopes

The timing of the initial accumulation of oxygen in the atmosphere and oceans has been debated for the past several decades. Oxygenation of the ocean-atmosphere system is critical because the evolution of life is closely tied to the progressive rise of oxygen. As such, habitability of the early Earth and potentially other planets like Mars is closely linked to the processes and timescales responsible for the rise of oxygen. While great strides have been made in our understanding of atmospheric oxygenation through mass independent sulfur isotope studies, there is still substantial uncertainty with respect to how the initial atmospheric rise in oxygen was translated to the oceans. Were these first 'whiffs' of oxygen limited to a few local environments? Or was ocean oxygenation a widespread global phenomenon? One of the earliest indicators of ocean oxygenation is manganese (Mn), which will oxidize and form Mn-oxides even when minute amounts of oxygen are present in the water column. However, it has been very difficult to unambiguously track the Mn-oxide burial in ancient environments and thereby the initiation of the rise of marine oxygenation.

The proposed project will focus on thallium (Tl) isotopes in the Archean and Proterozoic as a global Mn-oxide fingerprint. The study of Tl isotopes in ancient marine environments is based on the well-known very strong sorption of Tl to Mn-oxides, which requires free oxygen to form. In addition, there is a large Tl isotope fractionation associated with Tl sorption to Mn-oxides, which imparts a specific Tl isotope composition to seawater when Mn-oxides are removed into marine sediments. Given that Tl has a long modern marine residence time, the characteristic Tl isotope fractionation pattern allows Tl isotopes to track the global Mn-oxide burial. Thus Tl isotopes have the potential to track the earliest changes in ocean oxygenation through the burial of Mn-oxides. The utility of Tl isotopes to track Mn-oxide burial is augmented by the fact that the Tl isotopic compositions of all major marine inputs are identical, which means that large changes in terrestrial weathering conditions, hydrothermal fluid or volcanic inputs and dust aerosol deposition do not impart a significant change in the Tl isotope composition of seawater. Hence, if the seawater Tl isotope composition in the past significantly deviated from the input value in the direction opposite to Mn-oxides then it can confidently be related to Mn-oxide burial with little ambiguity.

The modern oceanic Tl isotope signature is faithfully recorded in sediments deposited under euxinic conditions because Tl is quantitatively sequestered from the water column via partitioning into pyrite. This finding ground-truths the preservation of the marine Tl isotope signature and allows for a simple reconstruction of marine Tl isotope compositions in ancient anoxic shales. Thus, we propose to use Tl isotopic measurements

in shales from critical time periods where oxygen has been hypothesized to rise in the ocean, atmosphere or both to better understand the global timing and increased burial of Mn-oxides. Specifically, we will investigate shales ranging in ages from 3.3 to 2.1 billion years to reconstruct the global shifts in Tl isotopes and determine whether Mn-oxide burial took place in the marine environment during these periods. Extracting this information from ancient sediments has the potential to provide crucial information about the links between ocean oxygenation, evolution of life and the impact on global biogeochemical cycles. It is important to note, Tl isotopes are not cycled biologically and the isotopes are unaffected by biotic evolution. Thus they are a valuable bio-independent proxy to track the global changes in ocean oxygenation. The development of a proxy to track Mn-oxide burial in large water basins could potentially be applied to sedimentary environments in other planetary settings like Mars (e.g. future return missions).

Mary Parenteau / SETI Institute
Remotely Detectable Photosynthetic Biosignatures

The study of potential exoplanet biosignatures -- the global impact of life on a planetary environment -- has been informed primarily by the modern Earth, with little yet explored beyond atmospheric O2 from oxygenic photosynthesis and its accompanying planetary surface feature, the vegetation red edge reflectance. However, these biosignatures have only been present for less than half the Earth's history, and recent geochemical evidence suggests that atmospheric O2 may have been at very low - likely undetectable - levels, until 0.8 Ga (Planavsky et al., 2014). Given that our planet was inhabited for very long periods prior to the rise of oxygen, and that a similar period of anoxygenic life may occur on exoplanets, more studies are needed to characterize remotely detectable biosignatures associated with more evolutionarily ancient anoxygenic phototrophs.

The goals of this proposed multidisciplinary study are to measure the surface reflectance spectra of anoxygenic phototrophs, understand the rules that dictate their spectral features so that they can be predicted for exoplanets, and assess their remote detectability as a function of environmental context across a range of spatial scales: (1) local field measurements of environmental samples, (2) regional on modern Earth (continents and oceans) using airborne sensors and Earth-observing satellites, and (3) planetary-scale in exoplanetary disk-averaged spectra under various atmospheres and cloud coverage levels.

Our preliminary measurements show that pure cultures of anoxygenic phototrophs produce NIR edge(s) in their spectral reflectance due to absorption of light by bacteriochlorophyll (Bchl) pigments. We assessed the detectability of these features environmental samples of microbial mats, and observed multiple NIR edges associated with each pigment. We initially expected only to detect the absorption of light by the pigments in the surface layer of the mat. Surprisingly, we detected cyanobacterial Chl a in the surface layer, as well as Bchl c and Bchl a in the anoxygenic underlayers. This suggests that it does not matter who's on top, as we were able to observe pigments through all mat layers due to their different absorption maxima. The presence of multiple

pigments and thus multiple NIR edges could signify layered phototrophic communities and possibly strengthen support for the detection of a surface exoplanet biosignature. In general, the proposed work will inform the search for life on exoplanets at a similar stage of evolution or biogeochemical state as the pre-oxic Earth.

This proposal speaks to the Exobiology solicitation NNH15ZDA001N-EXO research areas of Early Evolution of Life and the Biosphere and Biosignatures and Life Elsewhere. Specifically, the proposed research will provide a new suite of experimentally measured surface spectral biosignatures for remote sensing applications, which will aid in the investigation of the distribution of life in the Universe.

Ann Pearson / Harvard University

Evolution of Sub-Cellular Metabolism in Photosynthetic Eukaryotes: Isotopic Investigation of the Partitioning of Nitrogen Between Chloroplasts and Mitochondria

Science goals: Nitrogen (N) - along with phosphorous (P), iron (Fe), and other trace metals - is a limiting nutrient for algal primary production. Eukaryotic algae contain multiple pathways of nitrogen assimilation and metabolism, relicts of their mitochondrial and chloroplast bacterial symbiotic ancestors. The mechanisms and expression of these pathways may preserve the legacy of metabolic pressures on the nitrogen and carbon metabolisms of photosynthetic taxa as they evolved. This, in turn, may reflect the history of the oxygenation of Earth's surface.

This project will measure the natural distribution of nitrogen isotopes (15/14N ratios) at the sub-cellular level to provide insight into how eukaryotic algae allocate nitrogen under different growth conditions. A central hypothesis is that mitochondrial activity (respiration and/or mixotrophy) is reflected in expression of the glutamate dehydrogenase (GDH) pathway of glutamate synthesis; while chloroplast activity (phototrophy) is reflected in expression of the glutamine/glutamate/alpha-ketoglutarate synthase (GS/GOGAT) pathway. The balance of these processes will determine the sub-cellular allocation of N. This, in turn, will affect the N isotope distributions in amino acids (AAs) and chlorophyll. Because chlorophyll is preserved in ancient sediments, it is critical to understand how its 15/14N ratio is related to the original 15/14N ratio of cellular AAs most specifically to its direct precursor, glutamate. This, in turn, requires an understanding of glutamate N-isotopes at the organelle level.

Methodology: We will use targeted genetic manipulation and associated experimental investigation in the model pennate diatom Phaeodactylum tricornutum; as well as perform experiments with other major algal taxonomic groups such as green algae and dinoflagellates. Targeted sub-cellular proteins from mitochondria and chloroplasts will be purified, digested to amino acids, and natural 15/14N values will be incorporated into an existing model of whole-cell N metabolism. When combined with analysis of the 15/14N ratios of chlorophyll, as well as transcriptomic analyses, we will seek to establish an

index for photosynthetic vs. respiratory N flux. This will allow us to make predictions for how the 15/14N distributions of amino acids and chlorophyll reflect cellular nutrient and energy status in both modern and ancient systems and to examine whether major taxonomic divisions of photosynthetic eukaryotes have different patterns of intracellular N fractionation.

This project specifically is relevant to the following goals of the C.5 Exobiology program:

Early Evolution of Life and the Biospher...iv) investigate the development of key biological processes and their environmental impact; v) investigate the evolution of genes, pathways, and microbial species subject to long-term environmental change... Evolution of Advanced Lif...&biological and environmental factors leading to the origin of eukaryotes and the development of multicellularity...and...studies of the processes associated with endosymbiosis and the origin and early evolution of those biological factors.

The endosymbiotic history of algal photosynthesis reflects the biogeochemical history of Earth's nitrogen cycle. This project will achieve a greater understanding of both subjects, by taking the evolutionary and genetic tools that are used in the study of the evolution of complex life and bringing them together with the compound-specific isotopic analyses that are fundamental to generating deep-time geobiological records. The project represents a novel, interdisciplinary collaboration between a diatom geneticist and a stable isotope geochemist. The proposed research will integrate important current themes in genome biology and biogeochemistry, and a PhD student and a post-doctoral investigator will receive interdisciplinary training in state-of-the-art functional genomics methods and natural-abundance isotope ratio studies.

Noah Planavsky / Yale University
Calibrating the Chromium Isotope System as a Tracer of Atmospheric Oxygenation

The chromium (Cr) isotope system is an emerging proxy that could provide a new view of Earth's oxygenation. There have been two key outcomes from the early stage of Cr isotope work. First, Cr isotopes have been proposed to provide evidence for an early rise of oxygenic photosynthesis and for pulse in Archean atmospheric oxygen 2.95 billion years ago. Secondly, Cr isotopes have been used to argue for low atmospheric oxygen levels in Earth's middle history, with implications for the evolution of complex life. There are limited Cr isotope fractionations in mid-Proterozoic sediments, despite evidence for Cr mobility, suggesting surface oxygen levels were too low to induce significant Cr redox cycling. Both of these conclusions build from a framework developed mostly through modern environmental Cr work and experiments designed with environmental work in mind. Within the current view, it is thought that appreciable Cr isotope variation is possible only if Cr(VI) can be generated, and that Cr isotope signatures are not significantly affected by non-redox processes (e.g., adsorption). However, this

assumption must be fully validated before it can be widely applied to place unambiguous constraints on Earth's early redox evolution. In modern environments, Cr(III) is known to solubilized by many types of organic ligands. This process could have potentially led to the enrichment of Cr and/or isotope variation in the sedimentary rock record. Consistent with this view, in preliminary experimental work for this proposal we found that significant Cr isotope fractionation occurs during the solubilization and mobilization of Cr(III) from solid phases by oxalate ligands and siderophores desferrioaxamine B. This observation challenges the aforementioned assumption that Cr oxidation is a prerequisite for Cr isotope variability. Driven by this preliminary work, we seek to answer the following questions: (1) what are the mechanisms of Cr isotope fractionation during the mobilization of solid-phase Cr(III) by the microbial ligand siderophores? (2) Can different types of siderophores and other types of organic molecules mobilize Cr(III) and induce significant Cr isotope fractionation?

With the broader goal of quantitatively calibrating the Cr isotope system, we will conduct a series of controlled laboratory experiments to explore Cr isotope fractionation during Cr(III) mobilization by different types of organic molecules under varying environmental conditions. Additionally, ab initio calculations will be conducted to calculate the isotope fractionations between these Cr(III) species and used to provide a mechanistic view of Cr isotope fractionation. In the end, experimental and computational results will be combined to provide a framework to estimate fractionations due to complexation of a wide range of organic ligands not yet studied by laboratory experiments. We expect that our results will refine our ability to use the Cr isotope system to track the redox evolution of Earth's surface environment, a goal closely aligned with NASA Exobiology Program's aim to determine[e] the nature of the most primitive organisms, the environment in which they evolved, and the way in which they influenced that environment .

Anna-Louise Reysenbach / Portland State University
Evolution and Diversification of Nanoarchaeota and Korarchaeota

Science Goals and Objectives
Understanding the evolution and diversification of hyperthermophilic Archaea is fundamental to tracing back the physiology and adaptation of early organisms in primitive Earth environments and communities. Significant progress has been made in expanding the known diversity of Archaea through culture-independent sequencing approaches. However, inference of physiological characteristics, adaptation and interspecies interactions is limited and remains speculative if only by sequence gazing. Two lineages of hyperthermophilic Archaea, the deep rooting Korarchaeota and the symbiotic/parasitic Nanoarchaeota have single described species in culture and little additional representation in genomic datasets but represent key taxa for studying archaeal evolution and adaptation.

The scientific goal of this proposal is to expand the repertoire of Nanoarchaeota and Korarchaeota through both culture-independent and organism isolation and physiological

characterization from across distinct marine and terrestrial geothermal environments. Nanoarchaeota that specialize in symbiosis/parasitism on distinct archaeal hosts will provide clues to some of the earliest types of interspecies interaction, mechanisms of intercellular exchange and genomic changes associated with symbiosis/parasitism. Expanding the selection of genomic and physiological information pertaining to Korarchaeota, both from existing Candidatus Korarchaeum cryptofilum and novel marine species, will provide perspectives of the scale and breadth of recent diversification in ancient lineages.

Methodology
Culture independent sequencing of single cell isolates will be used to characterize novel Nanoarchaeota and their associated hosts as well as Korarchaeota from terrestrial geothermal and marine hydrothermal vent samples. Genomic-informed enrichment cultures will serve to narrow in towards organism isolation and physiological characterization through ultrastructural, proteomic and metabolomic analyses of isolates and defined laboratory communities.

Relevance
The work proposed here is relevant to the NASA Exobiology mission to understand the early evolution of life and the biosphere. Korarchaeota is one of the deepest branches in the tree of life, but only a single species can be cultivated at present. Detailed physiological and metabolomic characteristics of Korarchaeota remain unknown but could unlock clues to understanding unique hyperthermophilic metabolic pathways. Nanoarchaeota are the simplest, perhaps earliest types of organisms that have adapted to parasitic or symbiotic life styles, important to the goal of understanding coevolution of microbial communities, the interactions within such communities, mechanisms of intercellular signaling, cellular adhesion control and membrane exchanges, in the context of the origin of advanced life.

Karyn Rogers / Rensselaer Polytechnic Institute
Abiotic RNA Polymerization in Early Earth Environments

The RNA World hypothesis is an active area of investigation in the search for plausible pathways leading to the emergence of life on Earth that may also inform us about extraterrestrial life. One of the most significant challenges to an RNA World is the need for a prebiotically-plausible route to RNA polymerization that is consistent with our current understanding of environmental conditions on early Earth. Although abiotic RNA polymerization has been demonstrated in laboratory experiments, the need in most cases for pre-activated nucleotides and very specific types of (pre-treated) montmorillonite clay catalysts raises concerns about the robustness of these pathways and their feasibility in the uncontrolled and unconstrained environments of prebiotic Earth. This proposal seeks alternative pathways that are more general and robust, based on our current understanding of early Earth environments. Our focus on mineralogy is based on the hypothesis that prebiotic polymerization occurred at the interfaces between aqueous

solutions and solid, rocky surfaces, where minerals were available to facilitate chemical reactions through selective sequestration of reactants, templating of the polymerization products, and catalysis of the polymerization chemistry. Our introduction of pressure as an important experimental variable derives from the rich mineralogy found in high pressure, subsurface environments, and is supported by promising preliminary results. Other considerations, including fluid chemistry, pH, redox potential, and temperature, provide additional prebiotic context for these experiments. New experimental approaches to the analysis of the polymerization reaction products, combined with characterization of the mineral substances and their interactions with the nucleotide reactants and oligomer products, will elucidate the mechanisms that promote surface catalyzed RNA polymerization. This research program will evaluate the potential for an RNA World to be widespread across a variety of early Earth environments, and unravel the mechanisms and environmental conditions that could lead to RNA polymers of sufficient length and appropriate sequence for autocatalysis and self-replication, eventually leading to the emergence of life.

Steven Ruff / Arizona State University
Recognizing Biosignatures in Hot Spring Silica Occurrences on Earth with Applications to Mars

Objectives
Opaline silica occurrences have now been documented on Mars in multiple locations using in situ and orbital remote sensing observations. On Earth, silica deposits formed by precipitation of silica-rich hydrothermal fluids from springs and geysers are widespread and intensively studied. The role of microbes in mediating the precipitation of hydrothermal silica deposits is still a developing area of study. Our objective is to evaluate the role of microbes in siliceous hot spring deposits found at the El Tatio geothermal field in the Atacama Desert of northern Chile. This location, at an elevation of ~4300 m, is unique in having saline springs that precipitate micro-digitate forms composed of opaline silica encrusted in halite, minerals that have been shown to capture and preserve a microbial fossil record. Most importantly in the present context, these deposits provide the best-known morphologic and spectral analog for the opaline silica outcrops discovered with the Spirit rover at Home Plate in Gusev crater. Our goal is to assess the potential biogenicity and preservation potential of the micro-digitate silica structures seen in outcrops at Home Plate using detailed characterization and comparisons to the structures at El Tatio.

Methods/Techniques
We will conduct fieldwork at El Tatio and at a second site in Peru (Calientes) intended to characterize the diversity and formation conditions of the micro-digitate silica structures and expand our sample suite. Various microscopic techniques will be used to characterize the textural, chemical, and mineralogical features of micro-digitate silica structures to understand the potential role of microbes in their formation. Silica sinter samples will be subjected to wind-driven sand abrasion to assess the potential for this process to create or

destroy key features. Infrared spectral characteristics of El Tatio silica sinters and those from Calientes will be measured both before and after sand abrasion, to help interpret infrared spectra of opaline silica materials on Mars. Measurements with a flight-like Alpha Particle X-ray Spectrometer (APXS) will allow, for the first time, the elemental composition of both halite-encrusted and abraded silica sinter samples to be compared with Home Plate silica measured with the Spirit APXS.

Perceived Significance
We expect that results from these tasks will allow for a more critical evaluation of the hypothesis that biosignatures may be present in silica deposits on Mars discovered with the Spirit rover. Our results also could prove useful for assessing the biomediation and preservation potential of similar silica occurrences encountered by future missions. Because of the extraordinary implications of identifying biosignatures on Mars, a commensurate level of effort must be undertaken to support or refute such identification. Our work is intended to be part of that effort.

Everett Shock / Arizona State University
Geochemical and Biomolecular Changes at the Transition to Photosynthesis

Goals & Objectives
The rise of photosynthesis fundamentally changed the trajectory of early biological evolution, caused major realignments of elemental cycles on a planetary scale, and ultimately drove major geochemical transformations of Earth's atmosphere, hydrosphere, and crust. It is presently not known whether the evolution of photosynthesis was a fluke of biochemistry or whether it was enabled by geochemical changes that preceded its emergence. The intact geologic record in ancient rocks may extend back to times when there was no photosynthesis on Earth, but researchers do not yet know what evidence to seek to pin down the historical record of when and how photosynthesis began. At present, there are hundreds of examples of active hot spring ecosystems where easily identified transitions to photosynthesis occur spatially in response to combined temperature and chemical changes. We propose that hot spring ecosystems that host transitions to photosynthesis are ideal environments to test ideas about the geochemical and biomolecular changes that may have permitted the historical rise of photosynthesis. This approach is fundamentally geologic as it derives from the notions that the present is the key to the past, and that various stages of a process distributed spatially can be linked together to reveal its temporal progress - even over geologic time.

Methodology
Fieldwork at Yellowstone since 1999 by the PI and colleagues has yielded a database of geochemical analyses for >1500 hot spring samples. Recently added multiple layers of biomolecular investigations include analyses of genes, proteins, pigments, and lipids. All of these data sources will be merged to guide new field and laboratory investigations of the geochemical and biomolecular changes that occur across the photosynthetic fringe -- the sometimes remarkably narrow zone separating conditions conducive to

photosynthesis from those that are not. The combination of data will allow tests of hypotheses about how geochemistry sets the stage for the transition to photosynthesis, and how biochemistry responds. Research will involve fieldwork, laboratory geochemical and biomolecular analysis of samples, synthesis of new and existing data, and theoretical modeling of relations among geochemical and biomolecular data.

Relevance to the Exobiology Call for Proposals
The proposed research is directly relevant to aspects of the Early Evolution of Life and the Biosphere research emphasis of the Exobiology program. Specifically, the proposed work will combine geochemical and biomolecular data to understand the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments and investigate the development of key biological processes and their environmental impact . More broadly, the rise of photosynthesis triggered the environmental changes earth's surface that ultimately allowed the emergence of complex life, and a fuller understanding of any environmental factors that permit photosynthesis will allow researchers to conduct better-informed exploration of other planets that may support life at their surfaces.

Mark Skidmore / Montana State University
Microbe-Mineral Interactions in Glaciated Basaltic Terrains

Earth, like numerous other planets in our solar system, has experienced numerous intervals of glaciation throughout its history. On Earth, abundant, active, and diverse microbiomes exist beneath ice masses which are supported by mineral-based energy made available through the communition of bedrock. Recent research conducted on ice masses in sedimentary and metasedimentary catchments indicate a primary role for Fe and S in minerals such as pyrite in sustaining microbes in a range of subglacial environments despite pyrite only being of low abundance (1-2 wt%) in the bedrock. However, little is known of the role of mineralogy or of the specific water rock interactions that sustain microbial ecosystems in granitic or basaltic systems.

Crushed basalt, which comprises 5-14 wt % FeO and 45-55 wt % SiO2, has been shown to generate hydrogen (H2) when exposed to water at 30 to 60oC. The H2 produced was suggested to result from the reduction of water by reduced iron. However, recent research by the authors demonstrates significant H2 production following abiotic crushing of a variety of silicate minerals at 0oC and subsequent wetting in a process that simulates glacial communition of silicate bedrock. Despite its high Si content, basalt was not analyzed in our recent study and so rates of abiotic H2 release from crushed, wetted basalt via a Si mineral shearing mechanism at temperatures relevant to subglacial ecosystems (0-1oC) are unknown. Moreover, it is unclear if Si mineral shearing or Fe-based mechanisms are most likely to contribute H2 in basaltic subglacial systems and whether microbial communities present in these systems are founded on H2-based chemolithoautotrophy. This leads to our overarching hypothesis:-

Basalt in subglacial systems, comminuted by glacial grinding and abrasion, liberates H2 abiotically which sustains a diverse and active H2-based chemolithoautotrophic microbial community at ~ 0-1oC.

Our overarching objective is to investigate the production of H2 in subglacial basaltic systems and its role in sustaining chemolithotrophic communities. Specific objectives are to 1. characterize the chemistry of meltwaters in a range glacial catchments in Iceland and determine the capacity for silicate mineral shearing and basalt iron to generate H2; 2. determine rates of biological H2 transformation and CO2 fixation in subglacial sediment communities; 3. quantify enrichment in H2 based metabolism along geochemical gradients using metagenomics and targeted transcriptomics; and 4. model the relationships between abiotic mechanisms of H2 production, process rates, and genetic/transcriptional data. The integration of the proposed data will provide key new insights into the potential for water-rock interactions in supporting microbial life in cold, dark subglacial environments.

The proposed research is relevant to several aspects of NASA's Exobiology Program including Early Evolution of Life and the Biosphere , Large-scale Environmental Change and Macro-evolution and Biosignatures and Life Elsewhere . The advance and retreat of ice during glacial - interglacial cycles throughout Earth's history is thought to have driven large scale shifts in the distribution of taxa to habitats with more stable climatic conditions (refugia); enabling diverse microbial assemblages to persist in subglacial sediments. As Earth has experienced a number of Snowball Earth events, findings from this research will provide key insights into the predominant metabolic processes capable of sustaining Earth's biosphere during widespread glaciation and its potential in driving changes in major geochemical cycles. Finally, microbially driven subglacial weathering of basalts in terrestrial systems may produce biosignatures (e.g., Fe-oxides) that could be relevant for the formation and retention of biosignatures under non-Earth conditions given the presence of basalt in formerly glaciated regions on Mars and other planetary bodies.

David Summers / Ames Research Center
Very Low Temperature Detection of Microbial Metabolism

This project will look at environments where organisms are trying to survive periods of very low metabolic activity. For example trying to survive such events as a snowball Earth . These are likely to be have been environments where low temperatures caused organisms to have low rates of metabolism and subsequent difficulties in synthesizing DNA and repairing damage caused by background radiation.

One of the limiting factors in the survival of "dormant" organisms is repair of damage to various large molecules, such as DNA. Although such damage is universal, a low DNA synthesis/repair rate due to low metabolic activity at low temperature limits the ability of an organism to respond. It had once been assumed that microbes are not metabolically

active when frozen and are unable to repair any damage they suffer below the freezing point of water. More recent work has shown this not to be true. However, if such activity exists, it likely exists at only low levels. To understand life in such environments, we need to be able to detect activity that occurs and understand how it can affect survival.

Radiolabeling of DNA through incorporation of ^{125}I-5-iodo-2'-deoxyuridine (^{125}I-IdU), in place of thymidine, can provide very highly sensitive detection of DNA synthesis and repair, especially through he use of Multiphoton Detection. We can use this sensitivity to measure cellular metabolism and repair at lower levels by observing the incorporation of ^{125}I labeled deoxyuridine into DNA. The objective of this work will be to push the lower temperature limit at which metabolic activity can be detected past those previously observed. Our results will also give a direct comparison to methods based on ^{3}H and ^{14}C labeling methods. We will also study how the lower limits of temperature affects the viability of other model organism and how low temperatures affect a thermophile that encounters temperatures well below optimal growth without encountering a phase change.

By better understanding life on the edge of habitability, we will gain a new understanding of the limits of life, the environments that life can exist in, and how long life can exist in dormant states. This will better inform mission planning on where life might exist, give a better basis for the formulation of life detection experiments, and help formulate mission strategies to detect life and its influences. It can be used as a tool for the detection of organisms, in the search for life and in planetary protection. It can lead to future work to study how organisms survive periods of extreme climate.

Henry Sun / Desert Research Institute
Origin of Radiation Resistance

This proposal will test the hypothesis that extreme radiation resistance in desert microorganisms is functionally related to photochemical oxidation of desiccated cells. Aerobic organisms possess antioxidant enzymes (superoxide dismutase, catalase, peroxidase) that protect metabolically active cells against endogenous reactive oxygen species (ROS) generated from aerobic respiration. The enzymes are useless during dry periods against external, UV-induced ROS. Survival through dry periods requires nonenzymatic antioxidants such as Mn and trehalose. The same antioxidants would also be protective against ionizing radiation. We will test this hypothesis by studying photochemical oxidation and microbial response in the Mojave Desert. We predict that: 1) there is a depth limit beyond which photochemical oxidants no longer reach, 2) topsoil bacteria can survive higher hydrogen peroxide concentrations and higher ionizing radiation doses than bottom-soil bacteria, 3) nonenzymatic antiperoxide activity, but not catalase activity, differs between radiation resistant and sensitive bacteria, and 4) both resistant and sensitive isolates can survive brief dehydration, especially when protected from oxidation. This investigation would shed light on an evolutionary biology enigma:

the widespread presence of radiation resistant organisms on a planet where natural background radiation is low.

David Ward / Montana State University
Comprehensive Comparison of Taxonomic Composition and Metabolic Functioning in Oxygenic and Anoxygenic Microbial Mats of Yellowstone Hot springs

Scientific goals: This proposal focuses on comparing the taxonomic composition and metabolic functioning of (i) a rare, but evolutionarily important, modern laminated microbial mat formed exclusively by anoxygenic photosynthetic microorganisms and (ii) a well-studied mat formed by oxygenic cyanobacteria, in Yellowstone hot springs. The different organisms inhabiting these mats have different pathways for fixing dissolved inorganic carbon (DIC) and thus impart unique carbon isotopic fractionation signatures on mats and biomarkers. Metagenomic analyses will be used to identify the predominant taxa and to infer their functional potential, providing a reference for taxon-specific metatranscriptomic analyses of diel gene expression patterns. Hypotheses formulated from these patterns will be tested using (i) metametabolomic analyses of diel patterns of metabolite abundances in mat biomass and the intercellular milieu, and (ii) metaproteomic analyses coupled with stable-isotope probing (SIP) to associate uptake of 13C-labeled substrates (e.g., DIC, organic compounds) with the specific taxa. Pulse-chase labeling will be used to track exchange of metabolic intermediates among taxa. Our goal is to provide a detailed biochemical model of anoxygenic and oxygenic mats that can guide interpretation of the geochemical signatures that might be associated with the most ancient microbial mats.

Methodology: An anoxygenic mat formed by Chloroflexi and a cyanobacterial mat will be studied. Ultra-deep metagenomic databases for these mats will be produced, assembled and clustered. Metatranscriptomic and metametabolomics analyses will be performed using samples collected over a diel cycle, with concurrent analyses of O2, H2S, H2 and light depth profiles by collaborating microsensor experts. Short-term SIP experiments will be conducted under natural light conditions to assess which taxa incorporate DIC or organic substrates. To track exchange between specific DIC-fixing taxa and heterotrophic taxa, light conditions that favor different phototrophic taxa will be used to pre-label samples and unincorporated substrates will be removed before continued incubation. 13C will be tracked into total biomass, metabolites and specific cellular components, including peptides, proteins, and lipid and pigment biomarkers. All of these methods were developed and proven effective in characterizing the surface layers of oxygenic mats together with co-investigators who are experts on (i) phototroph physiology and metagenomics and metatranscriptomics analyses, (ii) metabolomics/proteomics analyses, and (iii) isotope geochemistry. Phylogenetically intriguing phototrophic and chemotrophic isolates representative of native taxa will be cultivated so that analysis of their cell-component biomarkers and their isotopic fractionations can be also be tested ex situ.

Relevance: Studies of these laminated mats are crucial for gaining insight into the types of microbial communities that might have resembled the Earth's earliest phototrophic mat communities. The development and use of meta-genomics, -transcriptomics, -metabolomics, and combined meta-proteomics-SIP methods have yielded a comprehensive biochemical model for the photic layers of oxygenic mats, in the process revealing unexpected taxa and physiologies. Comparable analyses of the oxygenic mat lower layers and of an anoxygenic mat will provide a thorough, comprehensive understanding of the organisms comprising such communities, their in situ metabolisms, and how these metabolic capabilities influence stable carbon isotopic signatures for comparison to oxygenic mats. Proposed studies directly relate to Exobiology Program central goals to understand the development of key biological processes (anoxygenic and oxygenic photosynthesis), the nature of the most primitive [phototrophic] organisms and the environments in which they evolved and interactions within such communities, that drive major geochemical cycles.

Chenyu Wei / Ames Research Center
Evolutionary Mechanisms of Simple genomes

Objectives: The objective of the proposed study is to shed light on the mechanism of the earliest evolution of genomes. We will determine how polymer length impacts the evolutionary potential of early biopolymers and the emergence of biological functions. The study combines theoretical and experimental methods to understand early evolutionary pathways and thus the possibilities available to early life.

The evolution of functional biopolymers can be described as an exploration of fitness landscapes, defined as the relative ability (fitness) of all possible sequences to carry out a specific function. Natural selection biases this process toward peaks of high fitness and away from valleys of low fitness. Understanding the structure of fitness landscapes, and how simple genomes explore them, is fundamental to understanding early evolution. For landscapes consisting of isolated peaks, evolutionary outcomes will depend on chance events resulting in polymer sequences that are only locally optimized. If, in contrast, the peaks are connected through a large network, new evolutionary solutions will be discovered in a reproducible manner resulting in globally optimized sequences.

Approach: According to theoretical models, fitness landscapes for sufficiently long polymers will consist of highly connected peaks. We will test these models with the aid of high throughput DNA sequencing coupled with in vitro evolution techniques. We will investigate two RNA functions that presumably are represented in fitness landscape with different frequencies and determine how the connectivity of peaks in their fitness landscapes changes with genome length. One function is catalysis of aminoacyl-RNA synthesis, a model reaction for primitive aminoacyl-tRNA synthetases, which can be catalyzed by simple RNA sequences as short as a few nucleotides. For the second function, nuclease activity, the number of catalytic sequences is expected to be substantially smaller, and efficient catalysts might require genomes longer than 20

nucleotides. These studies will allow us to understand how the minimal, functional requirements for both sequence length and structural complexity influence the shape of fitness landscapes.

For RNAs shorter than 25 nucleotides, the shape of the fitness landscapes will be determined by exhaustively sampling sequence space for multiple lengths. For longer sequences, complete sampling is technically infeasible and an approach that combines experimental and theoretical methods is needed. We will first identify segments of ribozyme sequences that, once modified, are likely to cause large-scale structural changes. This will be done with the aid of state-of-the-art structure prediction software. The chosen segments will be exhaustively randomized and the products will be tested for the required catalytic activity. If the resulting sequences have different structures than the parent sequence, possible evolutionary pathways connecting the initial and final structures will be analyzed with tools of statistical mechanics. Combining exhaustive mapping of landscapes for short RNAs with structure-guided mapping for long RNA will reveal how readily new functional structures of ribozymes can evolve over a wide range of polymer lengths and how likely is the formation of networks that connect functional sequences.

Relevance: This project is highly relevant to NASA s Exobiology and Evolutionary Biology program and the NRA s Prebiotic Evolution area of emphasis. In the proposed research, we examine the nature and mechanisms of the evolution of RNA, which is widely believed to be an early, if not the earliest, genetic and catalytic biomolecule. The project is therefore relevant to Objective 3.2 of the Astrobiology Roadmap, Origins and evolution of functional biomolecules .

Paula Welander / Stanford University

Does the Bacterial Production of the Eukaryotic biomarker Tetrahymanol Impact our Interpretation of Gammacerane Biosignatures in the Geological Record?

Tetrahymanol is a sterol-like polycyclic lipid that was first discovered in the ciliate Tetrahymena pyriformis, a sterol auxotroph that produces tetrahymanol under conditions of sterol starvation. Tetrahymanol is also readily recognized as the biological precursor to gammacerane, a polycyclic hydrocarbon detected in sedimentary rocks dating as far back as the late Proterozoic. Based on the predominant occurrence of tetrahymanol in ciliates and in reducing environments, gammacerane signatures in the rock record are often utilized as geological proxies for both eukaryotic occurrence and conditions of water column stratification (e.g. anoxic, sulfidic) in ancient ecosystems. However, eukaryotic ciliates are not the only biological source of tetrahymanol as it is also a minor component of the lipidome of two bacterial species, Rhodopseudomonas palustris and Bradyrhizobium japonicum. Because very little is known about the physiological aspects of tetrahymanol in bacteria, it is unclear how the occurrence of tetrahymanol production in bacteria affects our interpretation of gammacerane signatures in the rock record.

The major objective of this proposal is to explore the physiological aspects of tetrahymanol in bacteria with the aim of better informing our interpretation of gammacerane biosignatures. Our preliminary work has revealed tetrahymanol production in a third bacterial species, the aerobic methanotroph Methylomicrobium alcaliphilum 20Z. M. alcaliphilum produces significantly more tetrahymanol than R. palustris and B. japonicum. Further, tetrahymanol production in M. alcaliphilum seems to be regulated by environmental factors, similar to what has been observed in ciliates. Using comparative genomics, we have also identified a novel protein specifically required for tetrahymanol biosynthesis. This tetrahymanol synthase is not present in the genomes of tetrahymanol producing eukaryotes, indicating that bacteria and eukaryotes have evolved distinct biochemical mechanisms for producing the same molecule.

We propose to utilize M. alcaliphilum as a model organism to understand tetrahymanol biosynthesis and function in bacteria, and how these mechanisms compare to what is observed in tetrahymanol-producing eukaryotes. Our team will take a multidisciplinary approach combining the expertise of a microbial physiologist, a microbial biochemist, and an organic geochemist to pursue the following research aims:

Specific Aim 1. Physiological experiments under environmental conditions often associated with gammacerane biosignatures - suboxic, hypersaline and warm temperatures - will be carried out to determine if there is a correlation between these conditions and tetrahymanol synthesis in bacteria.

Specific Aim 2. In vivo and in vitro studies will characterize the biochemical properties and potentially novel enzymatic mechanism of tetrahymanol synthesis in bacteria and how it differs from eukaryotes.

Specific Aim 3. Compound specific isotope analysis of tetrahymanol from bacterial and eukaryotic cultures will be undertaken to trace variations in isotopic fractionation due to difference in biosynthetic pathways and metabolism.

These topics are relevant to the Exobiology theme of Early Evolution of Life and the Biosphere; specifically objectives (ii) understand the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments and (v) investigate the evolution of genes, pathways, and microbial species subject to long-term environmental change relevant to the origin of life on Earth and the search for life elsewhere. This work is also relevant to the theme of Large Scale Environmental Change and Macro-evolution as gammacerane is commonly used to trace variations in redox conditions across extreme climatic transitions associated with major perturbation of the carbon cycle and mass extinction (e.g., oceanic anoxic events, the P-T and T-J boundary events).

Loren Williams / Georgia Institute of Technology
Iron, Ancient Biochemistry and the Origin of Life

When life originated and first proliferated, iron (Fe) was abundant, soluble and benign. The Great Oxidation Event (GOE) approximately 2.4 billion years ago introduced the modern conditions of iron scarcity and iron-mediated oxidative damage to biological systems. We have developed a new model for the ancient Earth in which Fe2+ was an essential cofactor for nucleic acids with important roles in RNA folding and catalysis, including RNA-catalyzed protein synthesis and protein-catalyzed nucleic acid processing. In this model, nucleic acid folding and catalysis, as well as protein-mediated polymerization, ligation, cleavage and nucleic acid repair, were mediated by Fe2+ instead of or alongside Mg2+ on the ancient Earth. We postulate that the close association of biological macromolecules with Fe2+ may have been a critical determinant in the establishment and development of the current biological DNA-RNA-protein triumvirate, possibly during an ancient 'RNA world'. We suggest that differences in electronic structure led to increased folding stability and reactivity of Fe2+-RNA versus Mg2+-RNA complexes. Moreover, Fe2+, unlike Mg2+, conferred RNA with the ability to transfer electrons. In sum, this model implies that the catalytic and genetic potential of RNA may have been greater than previously understood.

The model predicts that modern biochemical systems retain latent abilities to revert to primordial Fe2+-associated states when exposed to pre-GOE conditions. Our previous quantum mechanical calculations, along with folding and ribozyme experiments, suggest that Fe2+ can substitute for Mg2+ in a variety of RNA systems. Moreover, stability of folded RNA and rates of ribozyme catalysis both appear greater with Fe2+ as a cofactor than with Mg2+.

In the proposed work, we will revisit ancient Earth biochemistry by recreating anoxic, micromolar Fe2+ conditions in the laboratory. We will (i) compare folding and catalytic efficiencies of biological RNAs in the presence of Mg2+ or Fe2+; (ii) investigate the ability of Fe2+ to substitute as a cofactor in a variety of ancient protein enzymes that transfer information between RNA and DNA; (iii) characterize undiscovered catalytic functionalities of Fe2+-RNA complexes; and (iv) determine if Fe2+ persists as a metal cofactor in extant bacterial ribosomes and mediates information transfer between RNA and protein.

Our proposed work is based on the assumption that the transition from chemistry to early biology, and thereafter to modern biology, can be understood only in the context of relevant ambient geochemical conditions. Experiments will probe the role of Fe2+ in the origins and early evolution of DNA, RNA and protein. Our work provides alternatives to current DNA/RNA/protein basis for life by proposing extended capabilities of early RNA and the subsequent DNA-RNA-protein triumvirate. We are investigating the evolution of genes, pathways, and microbial species relevant to the origin of life on Earth by exploring the long-term transition from the ancient anoxic world to the modern oxic world. The proposed work addresses the 2008 NASA Roadmap (National Aeronautics and Space Administration, 2014) Objective 3.2 Origins and evolution of functional biomolecules ,

which seeks to identify the potential for creating catalytic and genetic functions, by testing new models for the catalytic and genetic potential of RNA. It is also relevant to Objective 4.1 Earth's early biosphere , which seeks to investigate the development of key biological processes during the early history of Earth, by investigating RNA and information-transducing proteins under relevant early Earth conditions.

Loren Williams / Georgia Institute of Technology
Primordial Assembly and Catalysis

The translation system can be viewed as a molecular record of biology's history, a guide to a world of primordial molecules and the origins of life. Protein translation is one of life's most universal and complex processes, and is key to understanding ancient biochemistry. Using translation as a window to peer back in time, Woese and Fox discovered that life on Earth has arisen from three primary lineages. They succeeded in redrawing the long-standing two-lineage tree of life because the translation system has recorded and retained interpretable information on the ancient past.

We propose here an experimentally grounded inquiry that follows logically from our prior NAI-funded work, aimed at retracing the earliest evolution of the ribosome on an early Earth. Ribosome structures have now been solved for bacteria, archaea, protists, fungi, plants, insects, mammals, and most recently, organelles. We believe these data provide a map to the origin of the ribosome, the catalytic macromolecule central to the protein translation system and all life on Earth. We recently proposed an 'Accretion Model' for the correlated emergence and evolution of the large ribosomal subunit (LSU), the small ribosomal subunit (SSU), transfer RNA and messenger RNA. We propose here to test, refine or refute early events in the Accretion Model of ribosome evolution and to investigate stages of ribosome development that eventually result in a complex chemical assembly, of proto-LSU, proto-SSU, proto-mRNA and prototRNA, capable of catalyzing the formation of peptides. Specific goals of the proposed work are to evaluate, in silico and in vitro, a proposed series of rRNA fragments that represent a stepwise model of the first critical phases of ribosome formation. We will i) create, in vitro, ancestral translation system components representing early phases of the Accretion Model; ii) test key predictions of the Accretion Model related to RNA folding, assembly, ligation, chemical stability and catalytic capability of the ancestral ribosome, targeting the earliest phases of emergence; iii) characterize the folding, assembly, chemical stability and catalytic capability of accreting rRNA in a variety of environmental conditions and assess the biophysical and biochemical advantages to rRNA accretion in specific environments. We will also continue our study of ribosome fossils, in silico, to refine and expand evolutionary events as predicted by the Accretion Model. Through the proposed work, we will also attempt to constrain the range of environments plausible for the emergence of a catalytic LSU and to build a proto-LSU by non-enzymatic ligation.

This effort will utilize methods of bioinformatics, biophysics and molecular biology. Model ancestral molecules will be evaluated and iteratively refined by computation and

experiments, including folding, assembly and catalysis assays, and binding assays with peptides and proteins. Genes will be synthesized and expressed as RNA or protein. Folding and assembly experiments will include chemical footprinting, hydrodynamics and spectrophotometry. Catalytic competence will be quantified by the fragment and peroxidase assays, and products analyzed by mass spectrometry. Computation will include Molecular Dynamics and RNA secondary structure prediction (Mfold). In addition, RiboVision, our publically available analytical and visualization tool, will be extended and further developed. RiboVision facilitates understanding the structure, function and evolution of the ribosome, and is already in use by scientists around the world.

The proposed work supports 2008 NASA Astrobiology Roadmap Objectives 3.2 Origins and evolution of functional biomolecules - and 4.1 - Earth's early biosphere.