Exobiology
Abstracts of Selected Proposals
(NGH17ZDA001N-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. 150 proposals were received in response to this opportunity. On March 27, 2018, 30 proposals were selected for funding.

Lisa Allen/J Craig Venter Institute, Inc.
Elucidating The Role Of Viruses In Shaping Microbial Adaptation And Evolutionary Trajectories In The Subseafloor Of Deep-Sea Hydrothermal Vents

Viruses are ubiquitous in the world’s oceans, playing an essential role in biogeochemical processes while representing a vast source of genetic diversity. Yet we still know little about natural viral communities. Hydrothermal systems have been prevalent on Earth since the planet was formed and its hypothesized that life may have originated and evolved near deep-sea hydrothermal vents, making them the most ancient continuously inhabited ecosystems on the planet. Extant organisms living in these analogues of early habitats may still harbor genetic and metabolic characteristics of early life. Viruses play a critical role in the ultimate survival of microbes by mediating host functional diversification and enhancing adaptability via horizontal gene transfer and lysogeny, a replication strategy enabling the virus to incorporate its genome into their hosts (prophage). In this project, we will examine the lifestyle and impact of viruses on microbial communities in the subseafloor of deep-sea hydrothermal vents. We will investigate DNA and RNA viruses, the latter comprising the most ancient group of viruses on our planet. Our research plan will determine infection dynamics and interrogate host associations, both of which are known to lead to physiological alterations and wide-ranging evolutionary trajectories. Specific goals we plan to address include, i) Determine phage host range and specificity to diverse microbial host isolates from deep-sea hydrothermal vents ii) Determine role of vent viruses in microbial host evolution and ecology at deep-sea hydrothermal vents iii) Identify novel RNA viruses from deep-sea hydrothermal vents. Elucidation of these goals will allow us to better constrain the impact of viral communities in the subseafloor and examine the nature of virus-host interactions as models for understanding early life.

We will use viral infection studies to experimentally investigate isolates from a phylogenetically diverse collection of cultured vent microbes, including both Bacteria and Archaea. The existing culture collection will facilitate testing host-ranges of viral isolates, indicating possible evolutionary breakpoints of viral transfer. Cultured isolates will be interrogated using prophage induction assays and challenge experiments followed by sequencing and microscopy. To identify the host range/specificity of phage isolated, phage will be mixed within and between related host microbial genera. Increases in phage titers will be measured to evaluate amplification during infection. Genomic-based
technologies of viruses and microbes will be leveraged to interrogate viruses, their hosts, and interactions. We will computationally interrogate omics information for evidence of viral-mediated cellular gene transfer and lysogenic conversion, and host immunity markers resulting from past infection. Finally, RNA viruses, including retroviruses, will be characterized via homology binning and phylogenetics from community metagenomic, transcriptomic data.

The study of extremophilic organisms and their metabolic pathways, habitats, and distribution is essential to understanding the origin and evolution of life on Earth, the main goal of NASA’s Exobiology and Evolutionary Biology program. Viruses are important evolutionary drivers of natural microbial communities, and examinations of viral communities in the rock-hosted oceanic subseafloor are just beginning that hold great promise for revealing new information about remote life on Earth and the possibilities for life on other planetary bodies. Outreach activities include training undergraduate students at a small liberal arts college in cutting-edge genomics and bioinformatics techniques and developing an extreme virus module for elementary school students.

Dale Andersen/SETI Institute
Investigating Taphonomic Processes under Cold and Dry Conditions Using Sediment Samples from Untersee Oasis, Antarctica

Science Goals & Objectives
The goal of this project is to understand the preservation potential of molecular biosignatures within lacustrine sediments on early Mars. Using laboratory analyses, the principal objective will be to determine the rates of degradation for molecular biosignatures in cold, dry environments using sediment samples obtained from Lake Untersee, a closed-basin, perennially ice-covered Antarctic lake and an adjacent paleo-lake basin.

Understanding the taphonomy of molecular biosignatures in cold, dry, once aqueous environments is critical for developing strategies to search for evidence of life on Mars - where mean annual temperatures may have been well below freezing, even during the planet's presumed warm and wet period - and also has implications for the search for life on Icy Moons. Perennially ice-covered lakes are rare on Earth and their cold and dry paleo-counterparts are especially few. Hyperarid biomarker preservation from such deposits remain largely understudied.

In temperate, hyperarid soils, labile lipid biomarker structures are well preserved over millions of years. However, it is still not known if fragile, information-rich biomolecules such as DNA would survive over geological timescales under optimal conditions. In many environments, it is often exceedingly difficult to know the diversity and abundance of the source biomass for preserved biomarkers, which leads to difficulty in measuring
the rate of degradation. These uncertainties can be addressed by studying sediments from the Untersee Oasis.

Located in Queen Maud Land, Antarctica, Lake Untersee has a local climate that is dominated by intense evaporation and sublimation. Its ecology is dominated by prokaryotic microorganisms that form benthic microbial mats with a range of morphologies including pinnacles and large, conical structures. Adjacent to Lake Untersee is an ice-free region that contains a paleo-lake basin. This basin, and its shorelines contain lacustrine clays and desiccated microbial mats that once resided beneath the ice-cover of a lake that reached depths of 50-60 m. Samples collected in 2016 and now at NASA Ames Research Center, range from modern microbial mats to those that have been exposed to cold desiccation during recent decades, to those that have been desiccated for up to 10,000 years. This range of sample exposure ages will allow us to explore the effect of cold and dry taphonomic processes on biomarker degradation. While this is relatively brief from a geological perspective, it allows us to understand the early stages of biomarker diagenesis, during which time most degradation occurs.

Hypotheses & Measurements
We propose to measure the abundance and structural integrity of range of biomolecules in terms of stability: DNA, proteins, and lipids. We hypothesize that DNA will be significantly more degraded than proteins and that proteins will be preferentially lost in comparison to more recalcitrant lipids. By comparing the abundance of these biomolecules in the freeze-dried mats of various ages to the modern mats in Lake Untersee, we can measure the rate of degradation with increasing exposure age.

Significance and Relevance
With this project, we seek to fill an important knowledge gap by investigating the early diagenesis and the preservation potential of molecular biomarkers in an excellent planetary analog environment found in Antarctica. This project responds to and is relevant to NASA Exobiology themes "Early Evolution of Life and the Biosphere" and "Biosignatures and Life Elsewhere", Key Research Questions about Biosignatures (NASA Astrobiology Strategy 2015) and is directly relevant to Goal 7 of the Astrobiology Road Map: Determine how to recognize signatures of life on other worlds and on early Earth.

Ahmed Badran/The Broad Institute, Inc.
Design and Experimental Evolution of a Protoribosome

Background: The central dogma is quintessential to life on Earth, enabling living systems to carry out information processing, metabolism, reproduction and homeostasis. RNA, a uniquely information-dense and catalytic biomolecule, sits at the center of this requirement for life in all known biological systems, and might have been responsible for the transition from simple chemical to complex biological systems. Among RNA components defining the central dogma, there is perhaps none more important than the
ribosome, which serves as a cellular nexus of information transfer, resource allocation and growth modulation. Conservation of the ribosome is used to trace evolutionary trajectories across the tree of life from LUCA, yet there is little evidence to support rRNA-based phylogenetic reconstructions of intermediate versions of the translational apparatus. Methodologies that enable the reconstruction and experimental validation of ancestral ribosome intermediates along plausible evolutionary trajectories to its current form would transform our understanding of the origin of life on earth, elucidate the evolution of modern ribosomes, and potentially inform routes for translation elsewhere in the cosmos.

Major Goals: Historically, origins-of-life researchers have focused on a ‘bottom-up’ approach to the prediction of intermediate pre-biotic biomolecules, in which artificial chemical systems are constructed in attempts to recreate conditions on the early Earth. Conversely, a ‘top-down’ approach, in which existing biological systems are reduced to their underlying components, would inform the functional and minimal components that may have existed in a prebiotic era. We propose to take a top-down approach to the minimization of the ribosome within the functionality-relevant environment of the cell. Such a combination of theoretical and experimental analysis would inform the chemical functionality defining translation. However, studies aimed at probing cellular translation have rarely accessed such a detailed understanding of the minimal requirements for translation. This is a direct consequence of difficulties associated with ribosome manipulation in vivo, as translation cannot be easily uncoupled from cellular viability or overall fitness. Thus, our proposed work requires the removal of confounding attributes regulating cellular viability that may be incorrectly assumed to influence the requirements for this biomolecular activity.

Specific Aims: To achieve these goals, we propose to integrate synthetic biology, computational and phylogenetic rRNA analysis, and evolutionary principles to inform plausible and testable minimal ribosome intermediates en route to the extant translation machinery of the cell:

1. **Aim I**: construction of a defined sensor that recapitulates ribosomal activity in E. coli as a modular and orthogonal platform by uncoupling translation from host viability.
2. **Aim II**: application of this sensor platform towards the in vivo analysis of the effects of rRNA truncations and/or mutations on translation of a singular synthetic mRNA transcript.
3. **Aim III**: optimization of minimized rRNA variants using phage-assisted continuous evolution (PACE) to reveal compensatory mutations that enhance activity.

Conclusion: Our proposed work aims to overcome challenges associated with ribosomal manipulation in vivo and provides an innovative framework for the derivation of minimized ribosomes. While this is an ambitious integration of evolutionary principles and synthetic biology to illuminate the origins of translation, we hope that it will supplement the existing (but incomplete) molecular record of ribosomal evolution and provide novel insight into potential proto-ribosomal genotypes and phenotypes. Our proposed work would mark the first major effort to minimize this complex
macromolecular machine, providing experimental evidence for a potential route towards
the development of life of Earth.

**Brad Bebout/NASA Ames Research Center**

**Nitrogen Transformations In Photosynthetic Microbial Mats: Implications For
Microbial Evolution On Earth And The Search For Life Elsewhere**

Nitrogen (N) is an element necessary for all life on Earth. There is good evidence that N
has controlled the production of biomass on Earth over geologic time. Microbial mats are
laminated communities of microorganisms present in many modern ‘extreme’
environments, but are also one of the most ancient ecosystems on Earth. They are
recognized as communities within which much microbial diversity has evolved over
geologic time, and are targets of great astrobiological interest in our search for life
elsewhere. Microbial mats are well known for being constrained by their access to N;
they exhibit high rates of N fixation by which they obtain significant N for growth, but at
great metabolic cost. With the exception of the study of N fixation, the N cycle in
microbial mats has received very little study. The fate of the N fixed by mats, relative
amounts retained and lost, and the pathways through which N moves, have not been
elucidated.

The overall objective of the work proposed here is to elevate our understanding of the N
cycle in photosynthetic microbial mats through the construction of complete N cycles in
these otherwise well-studied, important model microbial systems while employing state-
of-the-art molecular ecological techniques to investigate the underlying molecular
framework.

The work proposed here will result in:

1) A complete biogeochemical characterization of the N cycle. Of particular importance
will be the quantification of relatively understudied and recently described N
transformations.
2) A comprehensive molecular characterization and phylogenetic analysis via marker-
gene analysis, metagenomics, metatranscriptomics, and targeted amplicon sequencing of
known N-cycling genes.
3) An experimental test of the potential of microbial mat organic matter to record a
stable-isotopic signature indicative of environmental conditions present at specific times
in the evolution of the biosphere.

We will integrate biogeochemical measurements with a multifaceted molecular biology
approach. We will quantitatively describe the N cycle in these mats (sources, sinks, and
internal transformations). Molecular ecological studies will enable assessing microbial
community diversity, composition, and functional-gene potential and expression levels
through metagenomics and metatranscriptomics, as well as provide a comprehensive
phylogenetic analysis of microbial mat-derived N-cycling genes through targeted
amplicon sequencing allowing us to investigate the hypothesis that key features of the modern biological N cycle evolved in microbial mat systems. Experimental manipulations will identify controls on the rates of N transformations, which we will be able to relate to any corresponding shifts in the distributions and expression levels of genes responsible for these transformations. We will directly test if N-isotopic fractionation patterns within microbial mats are affected by external environmental changes. This work will allow us to begin disentangling N-isotopic fractionation patterns in support of interpreting fossilized microbial mats (stromatolites) from Earth's past, and potentially elsewhere (e.g., Mars, Europa, and Enceladus).

Within the Exobiology Program Element, this proposed work is directly in support of goals within the section "Early evolution of life and the biosphere", most specifically goal (ii): "understand the phylogeny and physiology of microorganisms whose characteristics may reflect the nature of primitive environments". The work is also specifically relevant to "Biosignatures and Life Elsewhere" through measurements of biosignature gas emissions and the stable isotopic composition of organic matter produced by mats. The work represents a logical continuation of the application of state-of-the-art tools in molecular ecology to understand the evolution of life on Earth, and to inform our search for life elsewhere through the understanding of the co-evolution of life and its planetary environment.

Kathleen Benison/West Virginia University

Preservation And Detection Of Extremophiles In Mars-Analog Halite And Gypsum

Science Goals and Objectives. Many sediments and sedimentary rocks on Mars have similar mineral assemblages, sedimentary characteristics, and diagenetic features as modern and ancient red bed and evaporite sequences on Earth. Acid brine lake systems on cratonic rocks in Western Australia and at an active volcanic complex in northern Chile have pHs as low as 1.4, salinities up to 10 times saltier than seawater, complex aqueous compositions, and precipitate halite, gypsum, iron oxides, alunite, jarosite, opalline silica, and clay minerals. Despite extreme chemistry and low water activity, recent and ongoing studies have documented diverse communities of extremophilic microorganisms, many of which are novel, living in these acid brine lake waters and groundwaters. Are these microorganisms preserved in the mineral and rock record here? Preliminary investigation suggests that the rapid growth of minerals from acid brines causes halite and gypsum to trap microorganisms and organic compounds as solid inclusions and within fluid inclusions. However, further study is needed to fully identify the modes of preservation of biological material, as well as test and refine detection methods. The primary goal of this proposed research is to use microscopy and spectroscopy to characterize the microbial community preserved within halite and gypsum from modern acid brine lakes in Western Australia and Chile. Secondary goals of the project include: (1) investigating Permo-Triassic (~250 Ma) acid lake halite and gypsum for any preserved microorganisms and/or organic compounds; (2) documenting the preservation mode in relation to in situ or reworked halite and/or gypsum, as well as
the role of associated iron oxide and/or clay mineral coatings; and (3) comparing biological matter in modern and ancient acid lake halite and gypsum to evaluate whether microbes and organic compounds can remain preserved through deep time.

Methodology. This proposed research is built on the foundation of a long-term and comprehensive study of acid saline lakes and associated environments. PI Benison has expertise in geology, geochemistry, and geomicrobiology of acid brine lake and groundwater environments, including study of over 60 salt lakes in southern Western Australia and two salars in northern Chile. Funding is requested for a two-year project to support laboratory analyses of modern halite and gypsum previously collected from Western Australia and Chile, as well as on Permian and Triassic acid lake halite in previously sampled cores from the subsurface of Kansas and Northern Ireland. We plan to: (1) conduct petrographic investigations, using transmitted, reflected, polarized, and UV visible light sources, at 6.3 - 2000 x magnification to document optical properties of suspect microorganisms and organic compounds; (2) use laser Raman spectroscopy to identify chemical compositions of organic material found with microscopy; and (3) test the feasibility of imaging organic material in halite and gypsum, as well as in associated siliciclastics, with scanning electron microscopy.

Relevance to Solicitation. It is now widely accepted that acid brines existed on Mars. Several martian localities have similar sedimentological, mineralogical, and geochemical characteristics as acid saline lake systems in Western Australia and Chile, as well as in Permo-Triassic red bed and evaporite deposits. Thus, this proposed research will meet NASA’s Exobiology program goal of understanding the distribution of life in the Universe. In particular, the proposed research has clear implications for the search for evidence of ancient and extant life on Mars (MEPAG Objectives A and B). This research will expand the understanding for the capacity of evaporite minerals to host microfossils and other organic material. Results will contribute directly to planning efforts to analyze for and recognize biosignatures in martian samples, both in situ and via sample return.

Steven Benner/Foundation For Applied Molecular Evolution
Organic-Mineral Interfaces in the Origin of Life

This proposal most directly relates to the Planetary Science/Exobiology goals that seek to understand the origin of life. It ties as well to the broader exobiology program in the Benner lab, which exploits models for origins to guide the search for Darwinism in the Solar System, constructs "alien" genetic systems to understand the range of possible biosignatures, and models the evolution of terran life working back in time to define an "end goal" for origins work. Thus, this work will have impact beyond simply elaborating an important model for the origins of life. Indeed, some of chemistry that we develop could be occurring on Mars today.

The experimental work is organized around the "RNA First" model for the origin of Darwinism. It builds on the "Discontinuous Synthesis Model" (DSM) for the prebiotic
formation of RNA. The DSM, in turn, is set in an explicit model for early planetary history, including a model for forming the "late veneer", models for the redox potentials of the mantle and atmosphere at the time when life emerged, and models for minerals available in such environments. We then identify individual challenges remaining in the DSM that must be resolved before it can be accepted by the community as one possible solution to the "origins" conundrum. We then propose experiments, with alternatives to manage pitfalls, to resolve them. The work benefits from seed money provided by the Templeton Foundation, which (together with NASA support) led to multiple discoveries that will be developed. We discovered:

(1) A spectrum of carbonate, sulfate, and silicate minerals that adsorb and stabilize RNA, often with a Periodic Table trend; one chiral mineral (quartz) even distinguishes homochiral D-RNA and L-RNA. This discovery will be developed by studying other chiral minerals and mineral surfaces, quantitatively comparing RNA binding by competition experiments, and moving from binary minerals to ternary minerals in realistically complex geo-environments.

(2) Volcanic sulfur dioxide provides a simple way to get stable reservoirs of formaldehyde and other simple carbohydrates; these complement borate-carbohydrate minerals that allow advanced species to accumulate in useful amounts. This work will quantitate equilibrium constants to assess the value of sulfonates in managing "tar" problems in carbohydrate prebiotic chemistry, assess their compatibility with other mineral species in realistic geo-environments, and possibly rescue proposals (e.g. of Sutherland) that require large amounts of these.

(3) We hypothesize that molybdenum (+6) can complete the management of the "tar" problem for carbohydrates; experiments now show that it was available in the Hadean. We will assess its performance in realistically complex mineral environments, determine its compatibility with phosphate, borate, and other mineral anions, and study its redox interaction with ferrous, sulfite, and other reduced species.

(4) Mineral-based processes using the Krishnamurthy polyphosphate amide allow formation of glycosidic bonds of nucleoside phosphates in desert environments; borate-mediated rearrangements give nucleoside 5'-phosphates. We will assess three approaches to make activated nucleoside phosphates available for oligomeric RNA formation, including alternative oxidation states of sulfur.

We will then do "big picture" experiments, illustrating how Loeb's "silent discharge" in a post-veneer atmosphere can produce sufficient HCN, HNCNH, and other products that, if rained into physical rock containing olivine, tourmaline, and other mineral species, generate an effluent that can lead to the formation of RNA after draining into a dry space under a CO2 atmosphere. Consistent with a NASA budget, this will start with a synthetic effluent with nucleobases likely made in geothermal regions exposed to formamide, cyanamide, and cyanoacetylene, with RNA products stabilized by absorption on minerals.
Life on Clays: Evaluating Fe(II)-Smectites as Electron Donors on the Early Earth and on Other Planetary Bodies

Trioctahedral Fe(II) smectites are dominant basalt alteration products under anoxic conditions. On modern Earth, the top 600 m of oceanic crust contains 6-10 vol.% Fe(II) smectites, with shallow portions partially oxidized through seawater interaction. Prior to oxygenation of Earth's atmosphere and oceans, such clays would also have occurred in soils on land and as detrital minerals in riverine, lacustrine, and marine settings. In marine environments alone the estimated crustal pool of Fe(II) in smectites is two orders of magnitude larger than the dissolved Fe(II) pool predicted for Archaean seawater.

Fe(II) smectites have recently been identified on Mars in ~3.5 Ga mudstones deposited in a redox-stratified lake in Gale Crater. In addition, these likely occur throughout the solar system wherever water alters mafic rocks, including on subsurface oceans on icy moons like Europa and Enceladus. The role that Fe(II) smectites play in biogeochemical iron cycling has been largely unexplored to date because their modern occurrence is restricted to subsurface, anoxic settings. Trioctahedral Fe(II) smectites are thus a massive, yet under-examined, Fe(II) pool potentially available for use as electron donors in early microbial metabolisms and on other habitable planetary bodies. It remains unclear whether chemolithotrophic or photoferrotrophic microorganisms are capable of using trioctahedral Fe(II) smectites as electron donors, what enzymatic mechanisms are involved in this oxidation, how the chemical and structural properties of these smectites affect their viability as electron donors, and whether such processes leave signatures in the rock record. We propose to assess the ability of iron-oxidizing bacteria to use trioctahedral Fe(II) smectites as electron donors and to identify the mineralogical and chemical signatures of such metabolic activity. Specific objectives include to: (1) Constrain the chemical and biological mechanisms of trioctahedral Fe(II) smectite oxidation by chemolithotrophs and photoferrotrophs; (2) Identify the mineralogical products of microbial and abiotic Fe(II) smectite oxidation; and (3) Assess trace metal signatures of microbial oxidation of Fe(II) smectites. A series of Fe(II) smectites will be synthesized, with a subset containing oxidizable [V(III), Mn(I), Co(II)] and non-oxidizable [Ni(II), Cu(II), Zn(II)] trace metals. Known cultures of chemolithotrophic and photoferrotrophic iron oxidizing bacteria will be used to investigate microbial oxidation of Fe(II) smectites. Diffraction, imaging, and spectroscopic techniques will determine how Fe oxidation state and smectite structure is altered by microbial and abiotic oxidation. Transcriptomic and RT-qPCR experiments will assess the enzymatic mechanisms involved in aqueous Fe(II) versus smectite Fe(II) oxidation. Collectively, these measurements will provide an understanding of the biological, mechanistic, and structural controls on microbial Fe(II) smectite oxidation. The products of smectite oxidation and the effect of microbial oxidation on the distribution and oxidation state of structurally-bound trace metals will be determined to assess potential mineralogical and chemical signatures. The proposed research will reveal whether an abundant mineral host of Fe(II) on the surface of the early Earth that also occurs widely on other planetary bodies can be used as an electron donor in diverse metabolisms involved in iron cycling.
The proposed research addresses the goal of the Exobiology program in the focus area "Early Evolution of Life and the Biosphere" by examining biological utilization of mineral forms of the redox-active element iron expected to have been common on the early Earth. The planned project also addresses the goals of the focus area "Biosignatures and Life Elsewhere" by characterizing detectable mineralogical and compositional signatures associated with microbial oxidation of clays on the early Earth and other planetary bodies.

George Cooper/NASA Ames Research Center
The Possible Role Of Radiation-Magnetism In The Creation Of Prebiotic Enantiomer Excesses In Organic Compounds

Chiral molecules are likely to have been important early in the history of life on Earth because biological polymers (e.g., proteins and nucleic acids) are homochiral: their monomers consist of only one of the two possible enantiomers. However, naturally occurring ‘common’ abiogenic synthetic processes that take place today or in the early solar system were generally assumed to produce only racemic mixtures, which are equal amounts of enantiomers. The fact that life on Earth has strict homochirality might imply that, very early, there were ubiquitous and determining physical forces that directed the direction of enantiomer excesses in organic biopolymers (e.g., they preferentially aided the synthesis of "L" amino acids and "D" sugars). However, the overwhelming majority of experiments that attempt to create enantiomer excesses begin with pre-made (pre-synthesized) pairs of enantiomers, e.g., the slight preferential destruction of one enantiomer by right or left circularly polarized light (Flores, Bonner, and Massey 1977, JACS). Likewise, research on combined magnetism plus radiation (photo-magnetic) effects has so far been applied (mostly theoretically) to inorganic complexes (also assuming pre-existing enantiomers), resulting in the production of very small (~ 10-4) excesses between enantiomers (Rikken and Raupach, 2000, Nature).

However, we propose that photo-magnetic effects during actual synthetic reactions (formose-like reactions) might be capable of producing significant enantiomer excesses. The products of these reactions would be biologically relevant organic compounds: such reactions may have been significant on the early Earth and other solar system bodies. Preliminary results give hints of this possibility. This proposed work would utilize laboratory simulations, organic analyses and computational chemistry (magnetic effects on organic compounds) to investigate the scenario that radiation and magnetism may have been involved in the early generation of enantiomer excesses likely required for subsequent life. The major analytical techniques will include chiral analyses by gas chromatography-mass spectrometry (GC-MS), liquid chromatography-MS and polarimetry. Equipment for simulation experiments will include lamps and magnets of various intensities. The goals of this proposal are highly relevant to those of NASA and the Exobiology Program. One of the emphases of the Exobiology Program is "Prebiotic Evolution": ..."A major objective is determining what chemical systems could have served as precursors of metabolic and replicating systems on Earth and elsewhere,...". It
is very possible that prebiotic homochirality, or at least significant enantiomer excesses, might have been a necessary precursor of nucleic acids. In laboratory model systems, nucleic acid polymers do not form to a significant extent if their monomer building blocks are racemic, e.g., G. Joyce et al., 1984, PNAS. Such results might imply that replicating molecules (as we know them) required near-homochiral building blocks. Our proposed research may at least reveal a possible mechanism of creating enantiomer excesses in such precursors during the actual synthesis of compounds from realistic and widely available prebiotic molecules.

**Charles Danko/Cornell University**

**Pol II Pausing As A Milestone On The Road To Complex Animals**

The focus of this proposal is on the evolution of ‘pausing’, one of the regulatory stages during transcription by RNA Polymerase II (Pol II) that has so far been identified only in animals. Transcription is regulated at early stages in all eukaryotic organisms, including opening chromatin at the proximal promoter and forming the pre-initiation complex. In Drosophila and mammals, Pol II pauses near the transcription start site and awaits a signal to ‘release’ into productive elongation. Our work has demonstrated that the rate at which Pol II is released from a paused state is an active target of gene regulation by transcription factors. Therefore pausing adds a layer of regulatory complexity that has not been reported outside of animal organisms.

Here we propose to investigate the evolution of pausing in organisms at the base of the animal phylogeny. We present a specific hypothesis for how pausing evolved by collapsing an ancestral step necessary for transcription in all eukaryotes to a focal pause always occurring in the same location along the DNA sequence. By having a focal pause that occurs in the same location, transcription factors were able to efficiently catalyze the release of paused Pol II. This allowed release from pause to become a step during mRNA production that is actively regulated by cells. Our proposal to investigate this hypothesis will provide mechanistic insights into how pausing evolved in eukaryotic species, revealing how evolution added a step in complexity to an essential biological process.

**Objective 1:** Determine the architecture of transcriptional pause in a diverse group of animal and unicellular organisms. We know virtually nothing about pausing outside of a few model organisms. Here our goal is to measure the distribution of Pol II in a diverse group of organisms using Precision Run-On and Sequencing (PRO-seq). Paused RNA polymerase leaves a characteristic peak in the distribution of Pol II immediately downstream of the transcription start site, which can be easily recognized in PRO-seq data. PRO-seq data will be integrated with the Assay for Transposase Accessible Chromatin (ATAC-seq) to investigate the interdependence between the pause and surrounding nucleosomes. This will be the first functional characterization of pausing in deeply diverged organisms at the base of the animal phylogeny.
Objective 2: Test the hypothesis that pausing allows cells to tailor the rate of release at specific genes. All eukaryotic species transition Pol II from an inefficient to a fully transcriptionally competent elongation complex. Our hypothesis is that species without a pause make this transition in an approximately uniformly way across the genome, whereas species with a pause tune the rate of transition at each gene over a dynamic range of >100-1000-fold. Here we propose to test this prediction directly by using the small molecule flavopiridol and analyzing transcription in cells using PRO-seq.

Objective 3: Test the hypothesis that focal pausing is required for regulation by transcription factors in animals. We recently found that heat shock factor 1 (HSF1), a transcription factor that is critical for heat stress, activates gene expression by orders of magnitude exclusively by releasing paused Pol II. We will use this system to ask whether HSF1 is able to regulate gene expression in a Drosophila model after depleting paused Pol II. This experiment will reveal whether a focal pause is necessary for active regulation by transcription factors.

Our project will advance the goals of the NASA Exobiology program by determining when pausing evolved and by providing the mechanistic details of how this process developed into an active target of regulation observed in extant animals. Thus, our proposal addresses the biological factors essential to multicellular life and the evolution of multicellularity on Earth.

Alan Decho/University of South Carolina

Cooperation And Adaptability In Microbial Mats From Extreme Environments: Quorum Sensing And Its Relation To Early Life On Earth And Elsewhere

For billions of years, Bacteria and Archaea have formed organized communities in microbial mats. Such cooperation among microbes has played determinative roles in the persistence of life, and provides insight into how life on Earth and elsewhere may respond to and evolve in extreme conditions.

A key cooperative mechanism among cells in high density habitats is quorum sensing (QS) using cell-to-cell signals. Genetic evidence suggests QS evolved 3.4 GYA, and was likely important during life’s early evolution when extreme environments prevailed. However, QS has not been explored in an astrobiology context, nor in astrobiologically relevant extreme environments, where deep branching microbial lineages thrive. This paucity of data presents a critical knowledge gap.

We propose to investigate QS in microbial mats in hypersaline systems and lava caves, both analogs of habitats believed to have been present on early Earth and Mars; both also host deep-branching Archaea and Bacteria. Our main hypothesis is that QS signaling contributes to microbial-survival and resiliency under multiple extreme conditions. We will focus specifically on genetic components of QS, and how they drive gene expression during changing extreme conditions. Doing so will establish a solid foundation for
understanding QS in mats in extreme environments, analogs of which thrived in early life on Earth. QS is a fundamental life process leading to higher organization and also a critical pathway through which microbes tolerate and adapt to environmental extremes similar to those that prevailed on early Earth and Mars.

Objective 1: QS occurs in extreme environments: hypersaline mats and Hawaiian lava caves. H1: QS occurs among phylogenetically diverse Bacteria and Archaea in extreme environments. Acylhomoserine lactones (AHLs) are well-studied signaling molecules that confer important metabolic properties on microbes, but their diversity and activities in microbes in extreme environments are poorly understood. AHL signaling comprises a conserved AHL and a receptor protein (i.e., luxR homolog). Our preliminary data show the presence of AHLs in hypersaline mats. However, additional work is needed to determine if AHL-based QS systems are common in microbial communities in extreme environments. We will investigate genetic bases of AHL-QS and phylogenetic diversities of luxR homologs as possible mechanisms of adaptability in these communities.

Objective 2: QS confers and enhances survivability under changing extremes in early Earth- and early Mars-like environments. H2: Both the types of signals used by Archaea and Bacteria and resulting gene expression, change as conditions (salinity, desiccation, UV) become more extreme. We posit from our preliminary results that certain signals are less susceptible to degradation than shorter-chain counterparts. We thus predict that cells will utilize more-resilient signals under harsh environmental conditions. This in turn will test the potential for life to adapt to changing environmental extremes, and its implications for life elsewhere. To do so, we will determine changes in levels of gene expression, concentrations and types of AHLs in natural, multi-species mats, and in isolates exposed to varying extreme environmental conditions (high UV CO2, desiccation, salinity) in both a Planetary Environmental Liquid Simulator (PELS) and controlled atmosphere chamber.

Objective 3: AHL signals persist over time through extreme environmental changes. H3: QS signals are preserved under extreme conditions (high salinity, desiccation, UV). This contributes to recovery and persistence of cooperative processes in mats. Our preliminary studies show that extracellular osmolytes protect AHLs in natural hypersaline mats during desiccation and UV exposure. When more favorable conditions return, this may elicit community-wide recovery rather than just individual cell survival, and enhance community survival over long periods.

---

Christopher Fedo/University Of Tennessee, Knoxville

Soil Development, Braidplain Deposition, And Potential Terrestrial Colonization In The Cambrian Of Eastern California

Before the proliferation of vascular plants in the Devonian, and accompanying sediment stabilization provided by roots, extensive braidplains covered Earth's continents. Such braidplains could have been continental in scale and so have no true modern analog, yet
should represent the dominant fluvial style for the majority of Earth history, as well as being a potentially important environment early in the history of Mars. During the Precambrian, the evolution of life on Earth saw the development of microbial organisms in marine and then likely subaerial settings. A detailed understanding of terrestrial and associated transitional braided systems provides context for the diverse and extreme environmental conditions that microbial life experienced in soils and within non-marine environments. While there is ample evidence that braidplain systems dominated the pre-Devonian continental surfaces, the detailed morphology of these systems is complex and overlooked. Recent studies have noted an increasing diversity of subenvironments comprising ancient braidplains, including evidence for aeolian dune fields, floodplains, overflow channels, and areas of soil development in what was originally interpreted as sheeted sandstones representing a single flooding event. Interfingering deposits that represent low-energy environments, such as ponds of standing water, suggest that conditions capable of supporting microbial communities could have existed on land. The middle member of the Wood Canyon Formation (mmWCF), a trough cross-beded sandstone interpreted as a paired braidplain and braid delta that drained the Laurentian interior during the earliest Cambrian, is well exposed in southeastern California. In cratonic sections along the southwestern Laurentian margin, the unit rests nonconformably on an exceptionally well-developed bedrock paleosol. The large-scale sedimentology and sequence stratigraphy of the unit is well documented, but fine-scale sedimentology and stratigraphy with the goal of interpreting the wide range of depositional subenvironments noted above has not been conducted. Prior studies have identified and initially characterized the paleosol in terms of mineralogy and geochemistry, as well as measured stratigraphic columns in the mmWCF, but as a result of irregular channel geometry and commonly steep topography, bedding is extremely difficult to trace laterally and sections can only be correlated at a coarse scale.

The proposed study will focus on developing three central objectives. (1) The proposal will focus on the mineralogy, geochemistry, and textural characteristics of paleosols because soil development is greatly catalyzed by microbial processing. A prime target will be the bedrock paleosol upon which the terrestrial mmWCF rests, but research will also be aimed at identifying intraformational weathering profiles developed on sedimentary strata. Research will require field work, microscopic analysis, and geochemical analysis. (2) The detailed inner workings of fluvial transport processes and recognition of associated terrestrial facies will be examined in bed-by-bed detail by combining conventional field techniques and potential modern analog sites, with the development of three-dimensional, sub-centimeter scale, digital outcrop models. In a pilot study we have conducted to demonstrate this approach, thousands of low-altitude, high-resolution images were taken using an unmanned aerial vehicle and then transformed into a model using PIX4D photogrammetry software. We are presently analyzing the model using 3D visualization software. (3) Given the environments of deposition, we plan to complete an exhaustive search in the field for macroscopic evidence of terrestrial life, from both microbiological (e.g., microbially induced sedimentary structures) and macrobiological (trace fossils) perspectives.
James Holden/University Of Massachusetts, Amherst  
Fe(III) Oxide Reduction By A Hyperthermophilic Crenarchaeon: Novel Mechanisms And Detection

Two Exobiology Program goals, as described in the ROSES 2017 NASA Research Announcement, are to understand 'Early Evolution of Life and the Biosphere' and 'Biosignatures and Life Elsewhere'. In keeping with these goals, this proposal addresses two central research questions: 1) how can a hyperthermophilic crenarchaeon gain energy on its cytoplasmic membrane by respiring an insoluble iron oxide mineral, and 2) does this organism produce cytochromes or minerals during iron reduction that provide a unique spectral biosignature? The goals of the project are to improve our understanding of the origin and core components of microbial iron reduction, model the physiological mechanism of electron transfer from cells to iron oxide minerals, expand the suite of possible spectral biosignatures, improve available detection using spacecraft-relevant instrumentation, and increase the computational robustness of biosignature interpretation.

This project uses the hyperthermophilic, H2-oxidizing crenarchaeon and iron reducer Pyrodictium delaneyi as its model organism. P. delaneyi was isolated from a deep-sea hydrothermal vent 'black smoker' chimney. The organism and environment are representative of early life on Earth and models for potential life beyond Earth. The P. delaneyi genome encodes four novel respiratory complexes that are absent in other archaeal families. It lacks the genes for other known archaeal iron reduction mechanisms but has 15 genes for novel c-type cytochrome proteins. Multiheme c-type cytochromes are generally required for microbial iron reduction.

The project has three themes:
1. Physiological mechanism of iron reduction. We will identify the respiratory complex(es) used by P. delaneyi to reduce the iron oxide mineral ferrihydrite to magnetite.
2. Spectral differentiation of hyperthermophiles. We will characterize the spectral properties of P. delaneyi biomass grown on ferrihydrite relative to its growth on nitrate and to other hyperthermophiles.
3. Spectral differentiation of minerals. We will identify the Fe(II) minerals formed by P. delaneyi during iron respiration relative to those formed by other iron-reducing hyperthermophiles and fortuitously by non-iron-reducing hyperthermophiles.

P. delaneyi will be grown on ferrihydrite and nitrate. Differential gene and protein expression will identify iron-respiration specific protein complexes using RNA-Seq, proteomics, cytochrome c heme staining, and peptide mass fingerprinting. Some hyperthermophiles possess different types and proportions of light-absorbing pigments (e.g., cytochromes, coenzyme F420) that may change with metabolism. Therefore, visible-to-near infrared and mid-infrared reflectance and Raman spectroscopies will be used to differentiate P. delaneyi biomass grown on iron and nitrate as well as the biomasses of other hyperthermophiles (e.g., Pyrobaeculum, Thermococcus, Methanocaldococcus). These spectroscopies will likewise be used to differentiate the
reduced iron mineral products of P. delaneyi grown on ferrihydrite with those of other iron-reducing hyperthermophiles and with non-iron reducing hyperthermophiles that fortuitously reduce ferrihydrite when grown in its presence.

This project fulfills many of the Exobiology Program's stated goals, namely to "1) understand the phylogeny and physiology of microorganisms, including extremophiles, whose characteristics may reflect the nature of primitive environments, 2) determine the original nature of biological energy transduction, membrane function, and information processing, to test hypotheses regarding the original nature of key biological processes, and 3) investigate the development of key biological processes and their environmental impact." It also serves the goals of the 2015 NASA Astrobiology strategy document, including Co-evolution of Life and the Physical Environment; and Identifying, Exploring, and Characterizing Environments for Habitability and Biosignatures.

Jena Johnson/University Of Michigan, Ann Arbor
Ancient Iron Silicates: Deciphering Mineral Clues Of Early Life

Evidence for the origin and activities of early microbial life on Earth remain controversial, obscuring our understanding of how life evolved on our planet and impeding our ability to detect the existence of extraterrestrial life. Currently, chemically-precipitated rocks preserve the richest information recording the biogeochemistry of life on ancient Earth. If we can establish a mechanistic understanding of how the presence, activities, and metabolic capabilities of life lead to the chemical precipitation of authigenic minerals, then it will be possible to interpret minerals as proxies for life in the geologic record of Earth and other planetary bodies.

Recent discoveries have placed clay minerals, and iron silicates in particular, into a position of great importance for both deciphering early life on Earth and identifying life on other planets. A prominent example of chemically-precipitated rocks on Earth are the ancient Banded Iron Formations (BIFs), iron- and silica-rich rocks that are found globally from deep marine Precambrian records. Nano-inclusions of iron(II)-rich silicate minerals were recently discovered in well-preserved cherts from Australian and South African BIFs and are compelling new evidence that iron silicates were actually a primary mineral precipitating in the early ocean, demanding fresh studies into the formation of iron-rich clays. With our evolving view of the original BIF minerals, we may be missing signals of life in the geologic record for over two billion years of Earth’s history. Investigating the formation pathways of iron silicate minerals and the effects of biological mediation would also have major implications for determining signals of extraterrestrial life. Other planetoid bodies are thought to currently contain, or have previously hosted, environments where mafic and ultramafic rocks interact with anoxic water, yielding ferruginous and silica-rich fluids. Indeed, abundant iron-rich silicates have been reported on Mars from ancient Noachian terrains over 3.5 billion years old. Yet to be able to accurately probe for signals of life’s activities in these potential extraterrestrial habitats, we need to fully understand the impact of biology on the chemistry of clay minerals.
We intend to perform laboratory studies to probe the pathways and products of iron silicate precipitation in ferruginous, silica-rich systems, and test the effects of the microbial life which may inhabit such environments. We will:

1) Synthesize low-temperature iron silicate precipitates from ferrous, silica-rich fluids with and without Fe(II)-oxidizing microbes (photoferrotrophs and microaerophilic bacteria) and dead biomass
2) Simulate early diagenesis of the initial phases, including incubations with Fe(III)-reducing microbes
3) Characterize the full suite of initial, diagenetically-altered, and hydrothermally-crystallized precipitates for elemental chemistry, mineralogy, and Fe-redox state

We will develop a rigorous understanding of what specific iron silicate chemistry, structure, and Fe redox state signifies for the environmental and microbiological conditions of formation. It is critical to document the route of formation and stabilization of precipitates to identify whether these processes are happening today or in the past on Earth or other planetary bodies. This mechanistic understanding will enable us to decipher whether cellular surfaces or iron metabolisms have influenced iron clay precipitation in examples like iron silicate inclusions in ancient BIFs on Earth and iron-rich clays on Mars, addressing the Exobiology Program Goals: Early Evolution of Life and the Biosphere and Biosignatures and Life Elsewhere.

Karen Junge/University Of Washington, Seattle

Using Proteome Dynamics of Psychrophilic Bacteria to Decipher Metabolic Strategies and Protein Signatures Indicative of Sustained Life in Ice

Liquid water is essential to life on Earth; however, most planets and moons in our solar system have surface temperatures well below the freezing point of pure water. Europa, Enceladus, Ceres, Titan, and other icy bodies are targets for astrobiological investigation. Mars is presently cold and dry, but orbital observations have identified flowing liquid water and there is abundant evidence for past habitable environments. Earth may also have undergone a series of global glaciations (Snowball Earth events) in its early history. The abundance of icy conditions in our solar system suggests that life in frozen environments may provide answers to questions about the origin, evolution, and ultimate fate of microbial cells and their biosignatures.

Bacteria that are growing, metabolically active, and/or surviving in the presence of ice may have characteristics that reflect the evolution and nature of primitive life. These bacteria are interesting to astrobiology, polar ecology, and cryopreservation, but their ability to function at temperatures below -5 deg C is poorly understood. We have recently shown that the marine psychrophile Colwellia psychrerythraea str. 34H [Cp34H] has distinct shifts in protein expression and metabolic activity at -10 deg C using proteomic mass spectrometry (Nunn et al., 2015). Preliminary work in our laboratories reveal that
this organism is capable of growing in nutrient broth with Mars analog brine containing perchlorate.

The objectives of the proposed research are to:
1) Devise three low-temperature liquid-water environments that mimic the known chemistry of brines: i) in sea ice on modern Earth, ii) on Snowball Earth, and iii) on Mars;
2) Measure microbial growth rate, metabolic activity, ability to survive while inactive, and longevity for psychrophiles in these three environments;
3) Reveal proteomic biosignatures for growth, activity, and survival strategies, and understand key molecular responses of life in these three environments.

Methodology: We propose a 3-year project to perform novel proteomic examinations on a suite of three psychrophiles (Cp34H, Psychrobacter sp. str. 7E, and a model halophile) held for 12 months at subzero temperatures (-1, -5, -10, -30, -50, and -70 deg C; representative of ice above and below the eutectic of seawater and putative Mars brines), in comparison to optimal growth temperature benchmark data (8 deg C). Bacteria will be introduced to samples representative of modern sea ice, cold sea ice hypothesized to occur on Snowball Earth, and ice on Mars. We will spike cultures with 3H-Leucine, 3H-Thymidine, or 13C-Leucine incubated at specified temperatures, and sample through time to track metabolic activities, viability, cell death, and proteome changes. We expect that novel 13C-Leucine tracking and global proteome response quantifications using mass spectrometry will allow for the identification and monitoring of individual, newly synthesized proteins, and will provide dominant biomarkers for growth and/or survival.

This research has value to upcoming space-exploration life detection missions. Identification of proteins newly synthesized in low-temperature environments, or proteins indicative of long-term ice survival, will provide biosignatures to target when exploring life in low-temperature ecosystems relevant to future exploration. In addition, understanding key metabolic strategies for long-term survival in ice will provide clues on early evolution and survival of life as Earth underwent extensive glaciation during the Neoproterozoic Era. This proposal directly addresses the goals of ROSES 2017 and the NASA Exobiology Program solicitation to address "the potential for the origin and establishment of life under conditions prevailing on other planetary bodies and basic research on the formation and retention of biosignatures under non-Earth conditions."

Ramanarayanan Krishnamurthy/Scripps Research Institute
Cyanide Mediated Systems Chemistry - Towards Proto-metabolic Pathways

Of all the plausible prebiotic source molecules, cyanide and aldehydes are considered the simplest and widely prevalent. The reactions of cyanide and simple aldehydes, in the context of prebiotic chemistry, are proposed to have played a major role in accumulating important prebiologically relevant building blocks (nucleobases, sugars, hydroxy acids, fatty acids and amino acids). However, further chemistries of the thus produced
primordial building blocks are usually dealt separately, in isolation from the very prebiotic molecular source molecules they were produced from, which is highly unrealistic. Systems chemistry is an important concept that has to be reckoned with in prebiotic chemistry, especially given the heterogeneous product distribution that results in almost all of the prebiotic reactions (for example the formose reaction and HCN polymerization to name a few).

We hypothesize that under systems chemistry scenarios the source prebiotic molecules (such as cyanide and formaldehyde) would also interact with their respective reaction products, and lead to productive primordial reaction pathways. Specifically, we hypothesize that the transformations of simple aldehydes and alpha-hydroxy keto acids by their interaction with cyanide (in the presence and absence of amino compounds) would lead to alternative and extant biogenic molecules. We propose to systematically investigate the scope of this cyanide mediated systems chemistry with a view to developing an inventory of systems chemistry reactions (such as the reactions in a reductive citric acid cycle) that can become self-sustaining given a continuous supply of prebiotic source molecules. This would set the stage for systems chemistry to transform into proto-metabolic pathways.

We have promising preliminary results that lend credence to the cyanide mediated systems chemistry hypothesis and approach. We have discovered that cyanide interacts with many small molecules (such as formaldehyde and glyoxylate), gets incorporated and hydrolyzed to yield a suite of hydroxy acids that are constituents of some of the metabolic cycles of extant biology. Moreover, based on the potential to transform such hydroxy acids to keto acids, we propose that interaction of these keto acids with cyanide would open up opportunities (such as reductive chemistries) that were not possible previously in a prebiotic context. Based on this hypothesis, we have developed an efficient conversion of an alpha-keto acid (oxala acetate) to its corresponding alpha hydroxy acid (malate) under potentially prebiotic conditions. This observation also opens up a novel reductive amination process that could convert selected alpha-keto acids to the corresponding alpha-amino acids. The demonstration of many of the key steps of the reductive citric acid cycle, that so far has eluded experimental demonstration, would become possible by employing this cyanide mediated chemical transformation repetitively.

This proposal addresses the prebiotic evolution research emphasis of Exobiolgy, specifically relating to the major objective in determining what chemical systems could have served as precursors of metabolic systems on Earth and/or elsewhere. This proposal also experimentally models a type of early environment on the Earth in which organic chemical synthesis could occur in the context of protometabolic pathways.
Aerosolization of Catalytic RNA for Prebiotic Transport and In Situ Reactivity

Aerosols are colloidal suspensions of solid or liquid particles in air or other gases, formed and emitted into the atmosphere by both natural and anthropogenic processes. Sea Spray Aerosol is generated at the air-water interface of the oceans, while other natural aerosols such as smoke particles are released into the atmosphere via volcanic eruptions and forest fires. Early life may have evolved in the upper atmosphere because of interactions with the ocean.

In 1979 Carl Woese (J Mol Evol 13, 95-101) submitted a critique of the commonly accepted Oparin-Haldane primordial soup model of the origin of life. This critique proposed a greater role than previously imagined for atmospheric aerosols in the transition from prebiotic to biological systems. Woese pointed out that many essential biological reactions are dehydrations, unlikely to occur without catalysis in a bulk aqueous environment, and thus requiring a phase separation. He suggested that the atmospheric droplet phase would have been a potential medium to constrain and promote biochemical evolution. These droplets possess characteristics of ‘membrane’ chemistry, where a relatively hydrophobic phase develops as macromolecules partition at the surface, thus increasing reactivity that would lead to more efficient chemical development.

The unique properties of organic films at the air-water interface of an early ocean would have provided a milieu for biochemical reactions to occur. It is known that organic molecules partition at air-water interfaces, generating a sea surface microlayer film with a relatively hydrophobic phase. Organics generated and/or retained by the system of ocean-atmosphere interaction tend to stay within this cycle once they enter, and would be exposed to widely fluctuating conditions of solar irradiation, temperature, pressure, and humidity, far from equilibrium. Transport of these materials within an aerosol phase would have been potentially significant to prebiotic evolution, and might have been rapid some 4 Gya on the early Earth given its faster rotational speed.

Although aerosol formation at the air-water interface of Earth’s emerging oceans would likely have provided an avenue for the partition and concentration of organic material and relevant prebiotic reactions within an essentially closed phase, this idea has never been empirically coupled with the RNA World concept of the origins of life. The goal of this project will be to explore aerosolization of RNA molecules, testing capacity for both atmospheric transport via aerosols and ribozyme activity in the droplet phase. The ability to aerosolize catalytic RNA within a controlled system will test Woese’s idea of the use of the aerosol medium to concentrate and transport developing prebiotic molecules in the RNA world.

In a two-year pilot study, we propose to accomplish two specific aims: first, demonstrate aerosolization and local transport of RNA; and second, demonstrate transport-dependent ribozyme activity in the aerosol phase. A team of an RNA biochemist (Lehman), an atmospheric chemist (Atkinson), and an aerosol biologist (Rosenstiel) at Portland State...
University have constructed a small (3\(\text{\texttimes}\)3\(\text{\texttimes}\)2\(\text{\texttimes}\)) atmospheric chamber capable of aerosolizing RNA. Various RNA molecules, including the ca. 50-nt fragments of the spontaneously self-assembling Azoarcus ribozyme, will be tested for their abilities to be aerosolized and transported locally in this chamber. Furthermore, catalytic RNA reactions that require the fusion of separate aerosol particles will be assayed by the used of radioactive 32P-labeling and RT-PCR. These experiments can be scaled up at least an order of magnitude in size by the employment of room-sized aerosol chambers available regionally at the Pacific Northwest National Laboratory. These efforts for the first time will test the feasibility of aerosols to transport RNA molecules on the early Earth and explore the possibility of aerosol-based life-like reactions.

**Elena Litchman/Michigan State University**

**Modeling The Evolutionary Emergence Of Diverse Microbial Metabolisms**

The origin of the fundamental metabolic pathways and the subsequent rise of the great metabolic diversity in microbes are major steps in life’s evolution on Earth and, potentially, other habitable planets. Understanding how different metabolisms may arise and what conditions select for what types of metabolic networks is a key question for the origin of life. The evolutionary emergence of diverse metabolisms depends not only on environmental conditions but on the microbial interactions, such as competition and mutualism, as well. So far, the role of changing microbial interactions in the origin of metabolic pathways under dynamic conditions has not been investigated in detail. Here we propose to combine two novel modeling approaches from two disparate disciplines to explore how microbial metabolic networks arise and evolve in dynamic competitive environments. We will embed a recently developed metabolic modeling approach for the elementary flux mode analysis under nonequilibrium conditions (the Dynamic Reduction of Unbalanced Metabolism, DRUM) in an eco-evolutionary modeling framework of trait evolution (Adaptive Dynamics) to investigate how different metabolic networks arise and compete in different environments. The resulting new Evolutionary Systems Biology mathematical framework (evoDRUM) is a powerful tool that will allow extensive explorations of how early metabolisms appeared and were maintained by natural selection and, thus, will be useful for the field of early microbial evolution. EvoDRUM extends and modifies the idea of gathering the evolutionarily possible reactions by defining a large -- ideally universal -- mutation space in which evolution can proceed. In line with the Adaptive Dynamics framework, evolution is driven by a step by step mutant/resident invasion dynamics, with a defined mutation rate. The novelty of the proposed approach is that it investigates the metabolically explicit trait changes and evolution as a result of selection through competitive interactions of different phenotypes. It allows incorporating metabolic accumulation and evolutionary innovations.

We will have four major foci in the proposed research. 1) We will fully develop this novel framework and apply it to several simple metabolic networks, with several resources and temporally fluctuating conditions. In particular, we will investigate how the number of mutations, the mutation rate and neutral mutations, the concentrations of
available resources and their temporal variation affect the metabolic network evolution, 2) We will apply and validate our approach on the genome-scale metabolic network of Escherichia coli. We will investigate the conditions leading to the development of fermentation vs respiration strategies and the diauxic metabolic shifts and the effect of Horizontal Gene Transfer (HGT), corresponding to the addition of big blocks of reactions, on the evolutionary outcome. We will look at the changes in modularity and connectivity in metabolic networks during evolution. 3) We will use our method to understand the evolution of nitrogen fixation in early marine cyanobacteria and their adaptation to different conditions. 4) evoDRUM will be used to study the evolution of simple microbial communities with competitive and mutualistic interactions, first with modified E. coli strains, with a possibility of cross-feeding and, second, with a marine microbial consortium involving nitrogen fixers.

Relevance Statement. The proposed work is directly relevant to the 'Early Evolution of Life and the Biosphere' area of research in this solicitation. It combines for the first time two distinct modeling frameworks to investigate the origin and the diversification of different metabolic pathways in microbes under changing conditions and explores how microbial interactions shape the evolution of diverse metabolisms. This work will help gain insights into the evolution of early metabolic networks in microbes and microbial interactions.

Gordon Love/University Of California, Riverside
Exceptional Preservation Of Ediacaran Organic Biosignatures Yields Novel Insights Into The Marine Environments And Ecology That Hosted Early Multicellular Organisms

We will gain new information regarding the marine environmental conditions and the organic food sources that sustained the enigmatic fauna called the Ediacaran Biota; which were the first macroscopic, multicellular organisms that evolved on our planet. Exceptionally preserved biomarker lipids in thermally immature sedimentary organic matter in 560-540 million-year-old marine rocks from Baltica, together with stable isotopic (C and N) stratigraphic records, will allow us to assess marine microbial communities and modes of primary productivity (bacterial versus algal) in the shallow-marine settings where early animals flourished during an important period with dramatic fluctuations in climatic and environmental conditions. Ediacaran oceans likely maintained strong heterogeneity in chemical conditions including nutrient-poor (oligotrophic) environments that fostered early metazoan evolution, so it is important to consider appropriate rock targets from these settings as well as the more commonly studied eutrophic (productive) basins.

Our study will be based around detailed geochemical analyses of samples from multiple cores from locations across Russia and Ukraine. Mapping of the Ediacaran biomarker assemblages across hundreds of kilometers of epicontinental seaways of Baltica will allow new insights into the responses of biological communities in shallow-marine
environments to changes in the redox and nutrient budget. Detailed organic biomarker analysis will be performed on rock extracts and kerogen pyrolysates using sophisticated multiple reaction monitoring-gas chromatography-mass spectrometry (MRM-GC-MS). This selective and highly sensitive methodology opens up the possibility of monitoring for a large suite of biomarker compounds for assessing biogenic inputs and facilitating more accurate paleoenvironmental reconstructions. This includes quantification of any ancient animal steroids detectable in our Baltica sedimentary rocks using the latest biomarker analysis methods developed in-house, comprising established targets as well as new sponge biomarkers. Various marine depositional settings are represented in our sample selection, including shallow-marine shelves and epicontinental basins. A lipid biomarker scoping study has been performed on samples from a selection of drill cores from Russia and Ukraine. These confirm excellent preservation and syngenicity of organic biomarkers as gauged from the hydrocarbon distributions and the very immature stereochemical signatures, consistent with the mild thermal history of the rocks. Strikingly, the biomarker assemblages encompass an exceptionally wide range of hopane/sterane ratios (1.6 to 119), which is a broad measure of bacterial/eukaryotic source organism contribution. These include some high hopane/sterane ratios (22-119), particularly during the peak in diversity and abundance of Ediacaran fauna, and reveal ancient microbial communities rich in bacteria. A high contribution of bacterial productivity may have bolstered a microbial loop sustaining dissolved organic matter as a viable feeding strategy for Late Ediacaran benthic multicellular organisms. The exceptional preservation of these Proterozoic strata, as confirmed by multiple organic and inorganic proxies, highlights the previously unrecognized and thus untapped potential of preserving organic biological signatures of oligotrophic settings under low thermal stresses despite long burial times.

This program of work is highly relevant to a stated research emphasis of the NASA Exobiology goal, namely Evolution of Advanced Life, which seeks to determine the biological and environmental factors leading to the origin and diversification of eukaryotes and the development of multicellularity on Earth. Additionally, our investigation will expand the existing repertoire of robust chemical biosignatures for primitive animals, through the identification of novel steroid biomarkers for sponges.

---

**Roxana Lupu/Bay Area Environmental Research Institute, Inc.**  
**Impact-generated Miller-Urey Atmospheres and the Emergence of Life on Earth**

Life may have appeared on Earth very early on, following the Moon-forming impact, but the conditions leading to the emergence of the first pre-biotic compounds are still under debate. The famous Miller-Urey experiments and many subsequent ones have shown that discharges in reducing gas mixtures, such as various combinations of H2O, H2, N2, NH3, CH4, CO, CO2 and H2S, can lead to pre-biotic molecules. However, theoretical models and known volcanic gas compositions do not favor early Earth atmospheres as reducing as laboratory experiments. In fact, Urey believed that reduced atmospheres would exist only transiently in the intervals between volatile delivery by asteroids or comets and
hydrogen escape to space, and not as a stable steady state. This hypothesis has never actually been tested.

We propose to theoretically investigate how a transient Miller-Urey atmosphere can emerge on Earth due to large impacts during late accretion. This naturally follows the Moon-forming impact and precedes the emergence of life. A large amount of volatiles is thought to have been brought during late accretion, and big impacts with longer cooling times favor the formation of CH4 and NH3. Moreover, post impact chemistry of chondritic material was found to be rich in H2, CH4 and NH3 (Urey 1951, Schaefer and Fegley 2007, Hashimoto et al. 2007). We will analyze a wide range of impactor material, with compositions specific to chondrites, comets, and differentiated planetesimals.

We will link impact-generated thermochemistry, atmospheric photochemistry, and atmospheric radiative transfer to follow the chemical and thermal evolution of atmospheres kick-started by very large impacts. The timescales calculated will be relevant to the formation of aldehydes and HCN (amino acid precursors) in the atmosphere. Our photochemistry model also contains the formation and destruction of organic hazes, while the detailed chemistry may also reveal the presence of some prebiotic reaction catalysts. Our main focus is the Earth's atmosphere prior to the emergence of life, but the generality of our methods also allows us to look at the effects of large impacts on the atmospheres of Mars and Titan. This will provide a more general view on the possible emergence of prebiotic environments in the Solar System.

Our team has an extensive expertise in the chemistry, radiative transfer, and atmospheric kinetic processes relevant to this study. Our recent work has analyzed the emerging atmospheres of terrestrial planets after giant impacts, using a well-established radiative-convective atmospheric structure code, with an extensive opacity database for all relevant molecules, and the chemistry self-consistently calculated for vapor in equilibrium with a magma ocean (Lupu et al. 2014). We have also developed the chemistry code to study the transient impact chemistry and photochemistry, including nearly all the small molecules and free radicals that can be made from H, C, N, O, and S.

The development of the atmospheric code and the atmospheric structure calculations will be performed by R. Lupu, S. Vahidinia and M. Marley at NASA Ames, and by T. Robinson at UCSC. S. Vahidinia will also perform calculations related to haze and cloud aggregate opacities. R. Freedman at SETI Institute will advise on molecular opacity updates. The atmospheric chemistry grids will be provided by B. Fegley at Washington University. The photochemistry and impact chemistry calculations will be performed by K. Zahnle at NASA Ames.

This proposal is relevant to the "prebiotic evolution" part of Exobiology program by looking at "models of early environments on the Earth in which organic chemical synthesis could occur". With the applications to Mars and Titan we will also address the "Biosignatures and Life Elsewhere" goal, "relating what is known about the origin of life on Earth to the potential for the origin and establishment of life under conditions prevailing on other planetary bodies".
Animals can be divided into two broad clades based on their body plans. Over 99% of extant animals belong to a single clade of complex bilaterally symmetrical animals called the Bilateria and 4 remaining early emerging clades are called non-bilaterians. The evolution of complex animals remains an enduring unsolved question in biology, unlikely to be solved by theories invoking environmental changes in Earth's history, and its resolution is critical to understanding the existence of complex life in the Universe. The developmental origins of bilaterality nor increases in genomic complexity, CANNOT be the definitive event that drove the dramatically successful radiation of complex animals because these traits predated the origin of Bilateria. Rather, embryological and molecular studies in bilaterians and non-bilaterians indicate that the critical developmental constraint to bilaterian radiation was a shift in the site of gastrulation from one embryonic pole in pre-bilaterians (e.g. ctenophores and cnidarians) to the opposite pole in virtually all of Bilateria.

Gastrulation was crucial for evolutionary radiation of animals, because it is during this process that embryos acquire cellular and morphological complexity during ontogeny. Gastrulation requires complex molecular interactions between cells from different embryonic regions leading to activation of spatially restricted gene regulatory networks (GRN). These GRNs in turn induce organized tissues with distinct differentiated cell types that have been crucial for the success of Bilateria. In most animals the localized signal for asymmetric activation of GRNs that induce gastrulation can be traced back to the animal-vegetal (AV) axis, a polarity established in the ovum during oogenesis. We propose that a shift in the site of activation of a subset of molecular signaling pathways from the animal to the vegetal pole triggered an axial reorganization that relieved the developmental constraint of generating multiple germ layer cell types from the same spatial location (animal pole) that drove body plan evolution of Bilateria. Our goal is to experimentally reconstruct the mechanism for this axis reorganization in embryos of three phylogenetically pivotal experimental systems.

Our central hypothesis is that critical upstream regulators of cell signaling pathways focused at the animal pole of pre-bilaterians led to gastrulation at that pole, and that a shift of a subset of these regulators to the vegetal pole led to gastrulation at that pole in Bilateria. The central objectives of this proposal are to: 1) To identify and functionally characterize novel mediators of animal-vegetal axis polarity in non-bilaterians, 2) To identify genes that are directly activated or repressed by beta-catenin in sea urchins, Nematostella, and Mnemiopsis to determine the underlying cis-regulatory mechanisms, 3) To experimentally re-engineer the primary egg axis and gastrulation site by manipulating the spatial activation of signaling pathways.
Our research will combine embryo manipulation with modulation of gene expression using mRNA injection, morpholinos, transgenesis and CRISPR/Cas9 in sea urchins (Bilateria) and two prebilaterians (cnidarians and ctenophores). We will also use Co-Immunoprecipitation to identify upstream regulators of these signaling pathways and cell sorting to identify novel modifications of GRNs in embryos with re-engineered primary egg axes.

We aim to experimentally dissect the cellular and molecular constraints to rapid animal body plan diversification which directly addresses the goals of section 3.14.IV of the 2015 Astrobiology Roadmap associated with the Evolution of Advanced Life, the origin and early evolution of those biological factors that are essential to multicellular life, such as developmental programs, intercellular signaling, programmed cell death, the cytoskeleton, cellular adhesion control and differentiation, in the context of the origin of advanced life.

---

**Michele Nishiguchi/New Mexico State University**

**The Ecological Basis For Evolution Of Microbe-Animal Interactions**

One of the most significant and evolutionary relevant challenges in the origins of multicellular eukaryotes is how these organisms survived in a bacterial laden world. Animals and bacteria have evolved together since the appearance of the first eukaryotes through shared ecological niches or symbiotic relationships. Most of these symbiotic relationships are unique in that they allow the exploitation of challenging ecological niches that neither host nor symbiont can survive on their own. In many of these associations the host interacts ‘transiently’ with the symbiont or bacterial consortium, whereas other symbioses are much more stable and specific, reflecting a true physiological or metabolic dependence on each other. In these types of associations, the capabilities of the holobiome (host + symbiont) gives them the ability to form new metabolic machinery that is beneficial to both partners. The proposed work aims at determining the effects of environmental factors on the evolutionary ecology of symbiosis, whether environmental conditions affect the overall fitness of the association, and how a multicellular host’s immune system responds to symbionts that evolved under different environmental conditions. We will determine how hosts respond (their ability to recognize and select beneficial bacteria) to native, non-native, and experimentally evolved bacteria, and whether they are able to differentiate between strains that are less competent than others. We will use the well-established bobtail squid-Vibrio bacteria beneficial association as the symbiosis model to measure how host recognition accommodates changes in the symbiont population, as well as whether Vibrio bacteria that are affected by changes in abiotic (environmental) conditions are less able to colonize native squid hosts. The specific objectives of the proposed research include:

1) Examine whether bacteria evolved under different abiotic conditions are more or less capable of infecting native squid hosts;
2) Measure host immunological response to native, non-native, and experimentally evolved Vibrios to determine how the host immune system recognizes and differentiates closely related symbiotic bacteria;
3) Determine whether specific abiotic variables affect the fitness of the symbiosis by assessing how squid host and introduced Vibrio persist over time.

The methods utilized to complete these objectives will include experimentally evolving Vibrio bacteria under different abiotic conditions (salinity, temperature, UV, and pH) in vitro and subsequently introducing the evolved strains to naive squid hosts to measure symbiont competency and host immune response. We plan to examine any changes in Vibrio phenotype (growth, biofilm formation, host colonization) as well as genotype (through RNA seq and illumina sequencing of targeted symbiotic genes). Symbiotic bacteria that demonstrate phenotypes that are more favorable than others will be screened genetically to determine if specific genes are responsible for both host immune changes as well as the increase in fitness of the symbionts. Likewise, host response and subsequent fitness will be assessed measuring specific immunological components (e.g., pattern recognition receptors and effector molecules) and responses (binding, phagocytosis) to determine if Vibrios that are less competent are recognized and rejected compared to wild type Vibrios.

The work proposed here is relevant to the NASA Exobiology mission to understand the early evolution of multicellular life and the biosphere. Understanding how complex multicellular animals with innate immune systems evolved with bacteria in a beneficial manner will provide insight as to how advanced eukaryotes were able to infiltrate new habitats and acquire novel physiological capabilities due to the association with beneficial microbes. Additionally, this work will focus on how animal evolution has been guided by the presence of bacteria since their origins on early Earth.

Jeremy Owens/Florida State University
Using Vanadium Isotopes To Investigate Small Fluctuations In Early Oceanic Oxygenation: Implications For The Emergence And Evolution Of Early Animals

The redox state of atmosphere-ocean system is strongly coupled to the evolution of the climate that likely controlled biological innovation. The Neoproterozoic era (~1,000-542 Myr ago) was a period of drastic environmental change with at least two low-latitude 'Snowball Earth' glaciations and carbon cycle perturbation as observed in carbon isotope fluctuation. Evidence has shown that the atmospheric oxygen increased from ambiguously low concentrations to near modern levels prior to the Phanerozoic which likely had a major effect on the organic carbon burial, remineralization rate and carbon isotope record. Coupled to this large environmental shift is the observed evolution of complex multicellular life. These broad observations have documented the connections among large scale environmental change, ocean-atmosphere oxygenation and the evolution of the life. Even with an intense dedicated to understanding Earth's oxygenation-timing, initial rise of ocean oxygenation and quantification-remains
ambiguous largely due to proxy limitations. It has been especially difficult to unambiguously track low oxygen environments in the paleo-ocean as current proxies lack the ability to uniquely track non-sulfidic low-oxygen conditions which is critical to unravel the mechanism(s) between atmosphere/ocean oxygenation, emergence and evolution of metazoans.

The proposed project will focus on vanadium (V) isotopes in the neo-Proterozoic as a fingerprint for determining low oxygen, non-sulfidic conditions. The framework for this study is based on our modern V isotope signatures that capture variable bottom water oxygen conditions. We have observed remarkable V isotope variations of modern samples that are related to local bottom water oxygen contents. Importantly, the shift of the V isotope signature starts at suboxic (reduced but none-zero oxygen) conditions. Thus, sedimentary V isotopes, for the first time, have the potential to track changes in marine low oxygen conditions. Moreover, V concentrations begin enriching under low oxygen environments, thus the reductive enrichment of V in marine sediments occurs without the presence of sulfide. Modern observation also documents significantly different V isotope signatures between marine sediments and hydrothermal sediments, thus V isotopes can distinguish the mechanism of enrichment (redox or hydrothermal). Furthermore, given that V has a long modern marine residence time, the characteristic V isotope fractionation pattern has the potential to track the global oxygen content, when combined with other proxies. Thus, combining our current understanding of V systematics a high-resolution ancient study can illuminate sediment burial under low oxygen conditions.

To track subtle low oxygen shifts in the Neoproterozoic we will investigate the V isotope signature of shales ranging in ages from ~1,000 to 542 million years. It is important to deconstruct this ~460 million years to capture the expansion and contraction of the local and global redox states of the ocean. In this proposal we seek to better constraint: (1) the redox state of the outer shelf vs. deep basin settings before and during the Cryogenian to better constrain the temporal and spatial (local vs. global) relationships between the expansion of low oxygen regions and the earliest evidence of emerging of multicellular life (earliest record of demosponges), (2) the fluctuation of redox state during the Ediacaran, to test the 'Ocean Oxygenation Events' postulated for the end-Marinoan glaciation. Extracting this information from ancient sediments has the potential to provide crucial information about the links between large scale environmental change (global glacial event), ocean oxygenation, hydrothermal activity and the evolution of animals. Developing V isotope to reconstruct low-oxygen paleo-redox conditions and the relationship to multicellular evolution could potentially be used to search for exoplanet life.
Characterizing The Molecular Mechanisms And The Limits Of Archaeal Gene Transfer Using Haloferax Volcanii As A Model Genetic System

Project Title:
Characterizing the molecular mechanisms and the limits of archaeal gene transfer using Haloferax volcanii as a model genetic system

Background
Bacteria exchange genes by three canonical mechanisms: natural transformation, conjugation and transduction. Archaea undergo tremendous amounts of horizontal gene transfer (HGT), and haloarchaea specifically are well documented to have i) exchanged genes within species at rates that randomize alleles within populations (like sexually reproducing eukaryotes); ii) exchange genes at rates higher than those seen between bacterial model species and genera; iii) have acquired thousands of genes from bacterial sources - e.g., their aerobic respiration comes largely from bacteria. However, the mechanisms for how they do it, and the extent to which they do it are largely uncharacterized. Using the model archaeon Haloferax volcanii, we have previously shown a cell-contact dependent mechanism (called mating) for gene exchange moves >500kb fragments between species at a rate higher than seen in bacterial model organisms. Recently, we have begun to unravel the mechanisms behind gene transfer in H. volcanii. Our goals in this proposal are to identify and characterize genes responsible for HGT in the archaeon H. volcanii; to identify and characterize components of quorum sensing and if it regulates mating; determine rates of HGT within and between haloarchaeal species; and to assess the roles of host ‘immune’ systems on gene transfer.

The specific objectives of the research are:
1) Identify and characterize molecular components necessary for the cell-cell contact mediated gene exchange (mating) in H. volcanii.
2) Identify and characterize components of a homoserine lactone based quorum sensing system in H. volcanii
3) Examine frequencies of H. volcanii for intra- vs. inter-species, and inter-genus exchange for both modes of HGT, and investigate the roles of host ‘immune’ systems (e.g., restriction/modification and CRISPR-Cas), mismatch repair and recombination machinery, which are all known to affect HGT in bacterial model systems, in their ability to increase or decrease those rates in an archaeon.

Summary of methodology
We will primarily use gene knockouts for developing null mutants and then examine them for their ability to affect HGT, using HGT frequency assays. We have already identified several genes that affect HGT, and we will screen a transposon mutant library, and transcriptomes of mating and NT for additional mutants incapable of HGT. Genes identified as affecting HGT will be characterized primarily by site-directed mutagenesis, phylogeny and transcriptional regulation.
Relevance to Exobiology
The goal of research funded by the Exobiology program is to understand the physiology of extremophilic microbes; to investigate key evolutionary mechanisms; to determine the molecular mechanisms that control and limit evolution and metabolic diversity. The work proposed would address these goals by providing insight into the mechanisms of HGT in hypersaline adapted Archaea, and to the features that limit their ability to exchange genes and generate diversity. The research fulfills the NASA Astrobiology roadmap Goal 5, objective 5.1, to experimentally investigate the forces and mechanisms that shape the structure, organization, and plasticity of the microbial genome, and examine how these forces control the genotype-to-phenotype relationship. Gene transfer among and between bacterial and archaeal lineages is the leading process by which ‘prokaryotes’ adapt to their environment and speciate: limits to HGT limit evolution and metabolic diversity. Yet, how archaea do it and what controls and limits their evolution and diversity is largely an unsolved mystery.

Matthew Pasek/University Of South Florida, Tampa
Phosphorylation On Water Worlds As A Consequence Of Phosphite Oxidation

Phosphorylation on water worlds as a consequence of phosphite oxidation

The element phosphorus is critical to modern biochemistry, and presumably would have been important in the early evolution of life. However, phosphorus as phosphate (PO43-) is poorly reactive and minimally soluble, and hence its prebiotic chemistry has been argued to be rather limited in scope. Several prebiotic studies have attempted to replace phosphate with other ions or linkers in nucleic acids, suggesting that the role of phosphate is a post-origin biochemical addition to life.

This work proposes a new hypothesis: that the incorporation of phosphate within biomolecules was a natural consequence of the early geochemistry of the early earth. More specifically, organophosphates were formed by reaction of abundant condensed phosphates formed from oxidation of intrinsic, reduced oxidation state phosphorus. This hypothesis is based on several recent findings coming from our lab. First is a demonstration of phosphorylation using the mineral schreibersite, (Fe,Ni)3P, that produced nucleotides from nucleosides by a "just add water" experiment (Gull et al., Scientific Reports 2015). This phosphorylation demonstrated that phosphorylated products can come about spontaneous from mineral-water-organic interactions. The reaction pathway leading to nucleotides has not yet been determined, but may involve a polyphosphate as a phosphorylating agent. We propose to elucidate the pathways of phosphorylation from reactive P compounds under plausible conditions, building on our prior work, using NMR, Raman, and XRD analyses.

The origin of the phosphorylating agent in the above reactions is probably linked to the redox chemistry of schreibersite. Our group has studied this extensively, and, if polyphosphates are the route of this phosphorylation, Pasek et al. (Ang. Chem. 2008)
provides a likely source from reduced oxidation state phosphorus compounds. This reaction is between reduced P compounds such as phosphite (HPO32-) and hydrogen peroxide, mediated by an iron catalyst. We propose to investigate how reduced oxidation state P compounds generate plausible phosphorylating agents during oxidation.

The origin of the reduced oxidation state phosphorus compounds is likely the most novel part of this proposed work. We have recently demonstrated (Pasek et al. PNAS 2013) that reduce P compounds were present in a 3.52 billion year old carbonate rock. The origin of this phosphite was at the time attributed to meteoritic materials. We now believe that the phosphite originates from natural geochemical reactions occurring on the earth's surface, akin to:

\[ 2\text{FeO} + \text{HPO}_4^{2-} = \text{Fe}_2\text{O}_3 + \text{HPO}_3^{2-} \]

This reaction yields about 0.1% phosphite at equilibrium and 200°C (Herschy et al. in review). If this reaction was active on a large scale through low-temperature diagenesis and burial of sediment, then phosphite could have been a significant fraction of the chemistry of the early oceans. We have initial data that demonstrate reduced P was widespread in the Archean in sedimentary rocks, likely from this pathway, and will investigate other sources as part of this grant. We will explore these processes using NMR, HPLC, Raman, ICP-MS, XRD, and TGA analyses, coupled to modeling.

This work is directly relevant to Exobiology, specifically "Research in the area of prebiotic evolution seeks to understand the planetary and molecular processes that set the physical and chemical conditions within which living systems may have arisen". This work will supplement our Emerging Worlds research (focused on high-temperature reactions) and will focus on how reduced P compounds are formed, how they oxidize, and what the oxidation products can do.

---

**John Perona/Portland State University**

**Biological Sulfur Metabolism On The Ancient Earth**

**Scientific goals and objectives**

A central goal of our research is achieving a deeper understanding of the unusual sulfide-based assimilatory sulfur metabolism that is found among Earth’s most primitive anaerobic organisms. We have discovered four new genes that are conserved among methanogens in highly sulfidic habitats, and have elucidated the physiological or biochemical roles of several of the encoded proteins. Our next objectives involve more intensive structure-function analysis, and investigation of how environmental sulfide is further mobilized for incorporation into key cellular metabolites, RNAs and proteins. The second major goal of the research is to understand how these microbial sulfur assimilation processes on the ancient Earth evolved in concert with changes in the global biogeochemical environment. We focus especially on the sharp increase of oxygen
concentrations in the oceans and atmosphere that occurred following the advent of oxygenic photosynthesis two to three billion years ago.

Approach and methodology

The objectives will be addressed using both experimental and computational tools. Biochemical approaches to characterizing methanogen proteins involved in sulfide uptake and assimilation include X-ray crystallography and enzyme kinetics. These methods will be complemented by other techniques for tracking protein-protein interactions in cells, including endogenous expression of tagged proteins, quantitative affinity purification, and mass spectrometry. To investigate how anaerobic sulfide assimilation processes evolved upon the advent of molecular oxygen on the early Earth, we will employ methods derived from synthetic biology to reconstruct the anaerobic sulfur assimilation apparatus in the common bacterium Escherichia coli, which is able to grow both aerobically and anaerobically.

Relevance to the Exobiology program element

These experiments are highly relevant to this call and to the NASA Astrobiology Roadmap, particularly with respect to understanding how life on Earth and its planetary environment have co-evolved through geologic time. A second theme is to achieve deeper understanding of the evolutionary mechanisms and environmental limits of life. By characterizing novel sulfur metabolic proteins present in still-extant microorganisms that originated as long as 3.5 billion years ago, we will be able to trace evolutionary histories of the key proteins. Molecular adaptations necessary to bridge from the anaerobic to the aerobic Earth should become clear in comparing genes and proteins from organisms that differentiated at particular junctures. Finally, this work will also inform studies of how ancient cells adapted to the increase in oceanic and atmospheric oxygen concentrations, triggering the emergence of the new sulfur acquisition pathways that are present in most organisms today.

Alexandra Pontefract/Massachusetts Institute of Technology
Biosignature Preservation in Sulfate-Dominated Hypersaline Environments

Hypersaline environments are chemically diverse systems that are host to organisms in all domains of life, many with unique adaptations for survival under extreme conditions. Salts have long been documented as being capable of preserving a rich record of biological processes, having obvious astrobiological implications for the search for life beyond Earth: observations of Mars have revealed the existence of ephemeral paleolakes, represented by widespread deposits of sulfate salts, and there is significant evidence for the presence of MgSO4 oceans on Europa. Much of the work into biosignature preservation in hypersaline environments on Earth, however, has focused on NaCl-rich systems, owing to the dominance of this salt type. MgSO4 systems, alternatively, are of interest given the stabilizing nature of the sulfate anion for biological molecules. Ongoing
investigations into a range of sulfate deposits have revealed the preservation of DNA, amino acids, and cells on timescales of thousands to millions of years. Here we investigate the sulfate-dominated systems of the Basque Lakes and Clinton Lake, in British Columbia, Canada and explore the preservation potential of these salts for (geologically-speaking) short-term and long-term biosignatures. This work will explore the interactions between salts and biological molecules, and inform the search for life in these important hypersaline environments. This builds on work that the PI has previously conducted at Spotted Lake in BC.

Goals and Objectives:
1. Constrain the effect of physicochemistry on habitability in MgSO4 environments: Habitability in these systems can be qualified through a measurement of water activity, but this metric alone does not convey the complex effects that salt type, concentration and ionic charge have on the habitability of a system. To elucidate this complexity, we will conduct standard geochemical measurements, along with water activity, chaotropic (destabilizing effects), and ionic strength calculations.

2. Characterize the microbial community in each ephemeral lake environment: We will conduct metagenomic analyses using both Illumina and Nanopore sequencing to elucidate the microbial communities within the salt crusts, water column and soils. We will also sequence from the collected cores to understand how these communities are changing with depth. This data will then be linked to the biosignatures that we identify.

3. Assess the preservation potential of biosignatures with differing temporal stabilities in both the aqueous brine phase and mineral evaporites: (a) Short-term Biosignatures: ATP, DNA and amino acids are typically hydrolyzed quickly in aqueous environments, but in salt systems can persist over much longer time periods. We will explore the perseverance of these molecules in the system, utilizing (1) an ATP luminometer in concert with LIVE/DEAD assays, (2) Nanopore technology to characterize DNA degradation in the system, and (3) conduct laboratory experiments to assess amino acid racemization and degradation rates. (b) Long-term Biosignatures: Both lipid biomarkers and sulfur isotopic signals can persist in the rock record for billions of years. Intact polar lipids (IPLs) generally degrade quickly, whereas core lipids are stable over geological timescales. Here we will evaluate the degradation rates of both types in MgSO4 salt systems and how preservation here may differ from freshwater and marine systems. In addition, we will explore the production and preservation of sulfur isotope signals in sulfides and sulfates in salts.

Relevance: This work is relevant to the Exobiology program theme of Biosignatures and Life Elsewhere as it focuses on the generation and preservation of biosignatures with different temporal stabilities in a relevant Mars analog environment. This work also serves topic #5 of the 2015 NASA Astrobiology Strategy for Identifying, Exploring and Characterizing Environments for Habitability and Biosignatures.
Chris Reinhard/Georgia Tech Research Corporation
Upside-Down Biospheres And The Remote Detectability Of Life On Reducing Planets

Upside-down Biospheres and the Remote Detectability of Life on Reducing Planets

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

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

Overview of Research and Basic Methodology - Our motivation, though multi-faceted in practice, can be conceptually distilled to a single basic question:

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

We will provide fundamental new constraints on this question by developing a new ecophysiological module for use in large-scale planetary system models. This module will contain a suite of primitive and derived microbial metabolisms relevant for a wide range of habitable planets, and will be designed for flexible integration across a range of platforms. As proof of concept, we will embed this module in a simple coupled ocean-atmosphere photochemical model (ATMOS) and in an Earth system model of intermediate complexity (cGENIE) and provide the first detailed reconstruction of Earth's 'upside-down biosphere' during the Hadean and early Archean Eons.
Relevance to the Exobiology program - According to Program Element C.5 of NASA's Research Opportunities in Space and Earth Sciences (ROSES), 2015, the overarching goal of the Exobiology program is: "[T]o understand the origin, evolution, distribution, and future of life in the Universe [...] in the context of NASA's ongoing exploration of our stellar neighborhood and the identification of biosignatures for in situ and remote sensing applications". The research proposed here will be designed to provide significant steps forward in our predictive understanding of the links between microbial metabolism and atmospheric biosignatures, rendering it germane to both the overarching goals of the Exobiology program and many of the major foci delineated in the most recent NASA Astrobiology Strategic Plan, in particular Section 3.4 (Early life and increasing complexity), Section 4.4 (Co-evolution of life and the physical environment), and Section 5.4 (Identifying, exploring, and characterizing environments for habitability and biosignatures).

Burckhard Seelig/University Of Minnesota
Evolvability Of Proteins And The Emergence Of New Functions

The vast majority of metabolic functions in contemporary biology is made possible only by the catalytic and regulatory activities of proteins. The corresponding diversity of protein functions that evolved over billions of years is simply astounding. While the result of this evolutionary process can be studied through analyzing modern proteins, the mechanisms that have likely led to the emergence of this abundant variety of protein structures and functions are still poorly understood. We will experimentally study the process of how proteins can acquire novel functions. We will address the two key aspects of this fundamental biological question:

(i) How can an existing protein evolve to adopt novel functions?
(ii) How could the earliest functional proteins have first appeared on the stage of Darwinian evolution?

We anticipate to demonstrate potential scenarios for the emergence of functional proteins that interface with living organisms. In our approach, we will screen combinatorial libraries of tens of millions of randomized proteins for variants with new functions using an in vivo selection technique. We will evaluate and compare two very different protein libraries for their capacity to yield novel proteins that enable bacterial cell growth through new enzymatic or regulatory activities. Our search will begin with nature's most common enzyme fold, the (alpha/beta)8 barrel. This ubiquitous fold is adopted by about 10% of all known enzyme structures, utilized in five of the six enzymatic classes and catalyzes a wide array of different reactions. Despite its dominant presence in biology, it is not fully understood how nature is able to so readily repurpose this fold. We engineered a library containing trillions of randomized yet soluble (alpha/beta)8 barrel variants. A library with such a high complexity and quality is unprecedented. Considering the universal role of this fold among natural enzymes, we are poised to screen our library for emerging de novo catalytic activities. Our second library consists of entirely random
polypeptides of 80 amino acids in length. This library will be used to emulate the conceivable scenario in which the earliest functional proteins originated from mixtures of random-sequence polypeptides. While this is a plausible scenario, experimental data in support are surprisingly sparse. Both libraries will be screened for functional proteins using a bacterial growth selection. This technique employs strains of the model organism Escherichia coli with single-gene deletions rendering them conditionally auxotrophic - capable of growth on nutrient rich medium and incapable of growth on minimal medium. These auxotrophic strains are deficient in a function essential for core metabolism such as a gene necessary for amino acid, nucleotide or cofactor biosynthesis. Each of these strains will be transformed with our protein libraries. Growth of select clones on minimal medium will identify protein variants whose function rescued the wild type phenotype. This new protein function could either effectuate regulatory changes in the metabolic network, or even provide a new catalytic activity. This method allows to test for about 100 different biological functions. In a pilot study, we have already identified artificial proteins from both libraries that compensate for a missing enzyme.

Victor Ugaz/Texas A&M Engineering Experiment Station
Chaotically Mediated Biochemistry and Vesicular Protocell Assembly in Prebiotic Hydrothermal Microenvironments

Key Objectives:
Porous mineral formations near subsea alkaline hydrothermal vents embed chemically rich microenvironments, making them potential hot spots for prebiotic macromolecular synthesis. But synthesis of long-chain molecules needed to support higher order functions in living systems (e.g., polypeptides, proteins, nucleic acids) and their subsequent encapsulation within lipid membrane protocells cannot occur without enrichment of chemical precursors prior to initiating polymerization and vesicular packaging. Identification of a suitable enrichment mechanism has therefore become one of the key unanswered questions in the origin of life. Our team has recently discovered how 3D chaotic thermal convection--flow phenomena that naturally permeate hydrothermal pore networks--supply a robust mechanism for focused chemical accumulation at discrete targeted surface sites [PNAS, 114 (2017): 1275-1280]. We propose fundamental research that builds on these new discoveries to fully elucidate how synchronization of interfacial enrichment with bulk homogenization of chemical species, two distinct processes that are seemingly opposed, can synergistically accelerate reaction kinetics by several orders of magnitude.

Methods and Techniques to Accomplish:
We will perform fundamental coordinated experimental and computational studies to systematically characterize micro-scale 3D flow, chemical species transport, enrichment, biochemical reaction, and vesicular assembly processes within hydrothermal pore environments. A state-of-the-art micro particle image velocimetry approach with thermally induced fluorescent imaging will be employed to quantitatively measure in-pore 3D velocity and temperature distributions with high spatial and temporal resolution.
Results of these studies will enable us to quantitatively identify combinations of porosity, thermal gradient, and surface reactivity conditions that are conducive to protocell formation, and introduce a new in-situ experimental platform capable of directly probing these phenomena in micro-scale alkaline pore-mimicking surroundings. These new insights will lay a foundation to rationally predict how chaotic thermal convection can mediate protocell formation in the prebiotic milieu.

Relevance of Proposed Research:
This research will establish a versatile platform to explore the role of thermal convection in prebiotic scenarios involving surface adsorption, catalysis, polymerization, and assembly. Our preliminary results suggest that chaotic thermal convection may supply a previously unappreciated driving force to orchestrate synthesis of prebiotic chemical precursors critical to the origin of life and their encapsulation in lipid vesicle protocells. These findings are particularly exciting in light of the recent discovery of highly alkaline vent systems (e.g., Lost City vent, mid-Atlantic ridge) that appear to provide ideal microenvironments inherently rich in the dissolved chemical species and catalytically active mineral surface sites that would have been needed to foster emergence of biochemical complexity. These phenomena are also likely to play a pivotal role in exobiological scenarios involving hydrothermal vent systems (e.g., the Jovian moon Europa and the Saturnian moon Enceladus).

More broadly, this research promises to impact a diverse array of processes beyond prebiotic biochemistry that can be catalyzed in hydrothermal microenvironments. Submarine igneous formations, for example, play a key role in geothermal conversion of CO2 into stable carbonates and partial reduction to formate, carbon monoxide, and methane. These reactions are accelerated within the pores of hydrothermal formations, suggesting a compelling role for the thermal convective phenomena described here in governing transport and reaction of CO2 along pathways not captured in existing climate models.

Shuhai Xiao/Virginia Polytechnic Institute & State University

After the "Boring Billion" and Before the "Snowball Earth": Evolutionary Patterns and Innovations in the Tonian Period

The Tonian Period (ca. 1000-720 Ma) follows the so-called "Boring Billion" in the Mesoproterozoic Era and precedes the "snowball Earth" glaciations in the Cryogenian Period. Thus, it holds to key to understand the transition from apparent quiescence in the Mesoproterozoic to climatic catastrophes in the Cryogenian. Emerging geochemical data indicate that the Tonian represents a major transition from predominantly sulfidic to ferruginous mid-depth seaways, although atmospheric pO2 levels in this geological period are highly uncertain and intensely debated. Molecular clock estimates suggest that early animals and other important eukaryotic clades may have diverged in the Tonian Period, raising the intriguing possibility of coupled environmental changes and evolutionary innovations. However, the fossil record of the Tonian Period is rather poor,
critically limiting the capability to resolve current debate on Earth-life evolution during this time period and its astrobiological implications. To fill this knowledge gap, the PI proposes to (1) analyze the global taxonomic and morphological diversity of Tonian eukaryotes and multicellular organisms; and (2) systematically investigate problematic microfossils and macrofossils preserved in the Tonian Huainan, Feishui, and Huaibei groups. The main objectives of the proposed research are (1) to construct and analyze Proterozoic databases of microscopic and macroscopic eukaryotic fossils, (2) to analyse microfossils and macrofossils from the Huainan, Feishui, and Huaibei groups using a combination of microanalytical tools; and (3) to integrate database-based and specimen-based data to test the co-evolution of eukaryotes and multicellular organisms and the physical environments during the Tonian and the Proterozoic. Specifically, the proposed research will test (1) whether eukaryote taxonomic and morphological diversity in the Tonian Period is greater than, comparable to, or lower than in other Neoproterozoic periods; and (2) what eukaryotic and multicellular groups were present in the Tonian Period, and whether they survived the Cryogenian into the Ediacaran Period. The ultimate goal of the proposed research is to illuminate the co-evolution between life and the physical environment before, during, and after Cryogenian snowball Earth glacialiations.

The proposed research consists of database-based and specimen-based analyses. Databases of Proterozoic eukaryotic microfossils and macrofossils will be analyzed using a variety of statistical tools to detect and test major evolutionary patterns and trends of taxonomic diversity and morphological disparity. Fossil specimens will be analyzed using reflected and transmitted light microscopy, scanning and transmission electron microscopy, focused ion beam electron microscopy, energy-dispersive X-ray spectroscopy, X-ray microCT, isotope ratio mass spectrometry, and second ion mass spectroscopy. Analytical results will be integrated to characterize the chemical compositions and microstructures in order to constrain phylogenetic interpretations. Funding for this project will support a post-doctoral fellow, who will be supervised by the PI to carry out the proposed analyses.

The proposed research will provide long-needed data to test competing hypotheses about environmental changes and evolutionary innovations in the Proterozoic. It addresses key questions regarding the co-evolution of life and the physical environment in early Earth history, which is a major research topic identified in NASA’s Astrobiology Strategy. It directly supports two research areas identified in NASA Exobiology Program: (1) evolution of advanced life and (2) large-scale environmental change and macro-evolution.