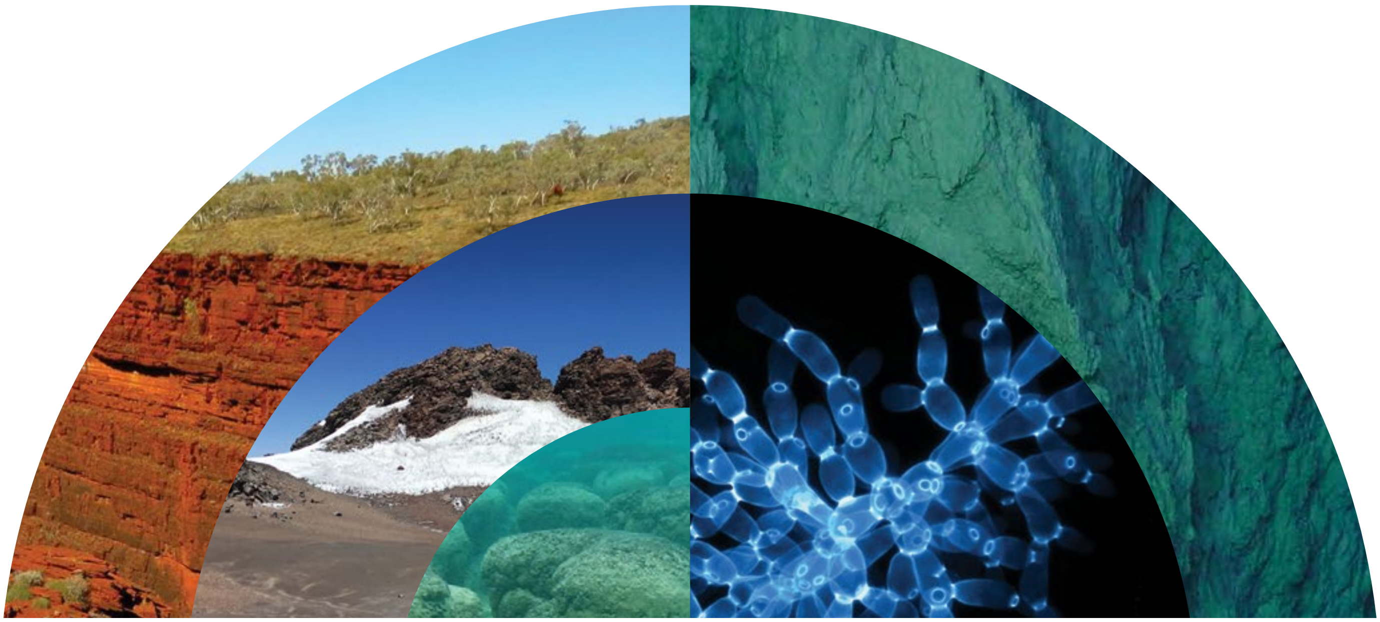
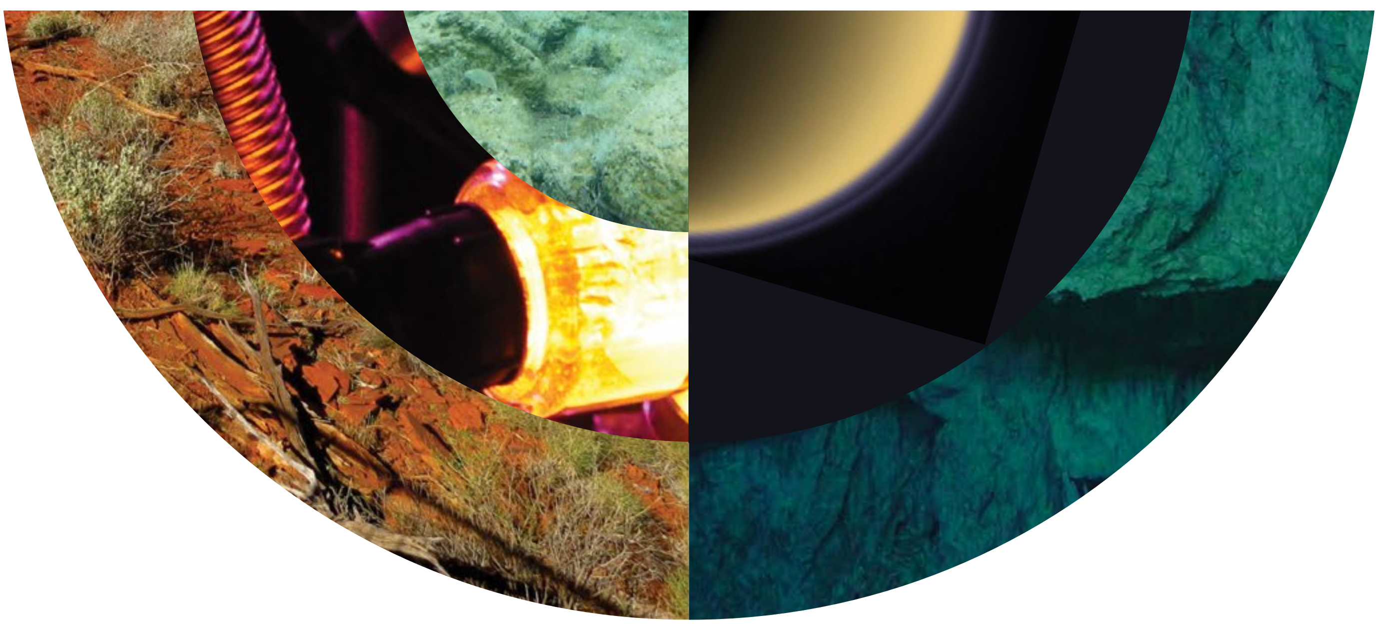
National Aeronautics and Space Administration



# NASA Astrobiology Institute
## 2018 Annual Science Report



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# 2018 at the NAI

In 2018, the NASA Astrobiology Program announced a plan to transition to a new structure of Research Coordination Networks, RCNs, and simultaneously planned the termination of the NASA Astrobiology Institute to occur in December 2019. The NAI, a then-new virtual institute concept, was originally envisioned as a 20-year experiment whose goals were to:

- Carry out, support and catalyze collaborative, interdisciplinary research
- Train the next generation of astrobiology researchers
- Provide scientific and technical leadership on astrobiology investigations for current and future space missions
- Explore new approaches using modern information technology to conduct interdisciplinary and collaborative research amongst widely-distributed investigators
- Support learners of all ages by implementing formal, informal, and higher education programming and public outreach

In the following 2018 annual report, the current NAI teams amply demonstrate their efforts to contribute to these goals and advance the science of astrobiology across a broad suite of interdisciplinary efforts to address significant questions in astrobiology. The 10 current teams, representing the 7th and 8th Cooperative Agreement Notices (CANs) span topics in astrobiology from the evolution of prebiotic chemical complexity to the evolution of biocomplexity and multicellularity, to exploring the habitability of ocean worlds and searching for the fingerprints of life.

In support of training the next generation of astrobiologists, in 2018 the NAI supported the travel of 10 early career scientists to conduct collaborative research with members of the astrobiology community, partnered with the American Philosophical Society to provide funding for 10 graduate students and postdoctoral scholars to conduct field research in astrobiology, from locations in Yellowstone National Park to the Atacama Desert, Chile to Iceland. Through the NAI-sponsored Astrobiology Graduate Conference, AbGradCon, 96 graduate students and recent postdoctoral fellows spent a week sharing oral and poster presentations and forming lasting connections. The NAI partnered with its international partner at the Centro de Astrobiologia for the 18th annual International Summer School in Astrobiology in Santander Spain, with 36 students participating in a week of lectures, projects and field expeditions.

Over the past 20 years, the NAI has pioneered virtual mechanisms to provide information about the Institute's teams activities, initiating and expanding Workshops Without Walls and All Access Events, which have now become well established, and common across NASA.

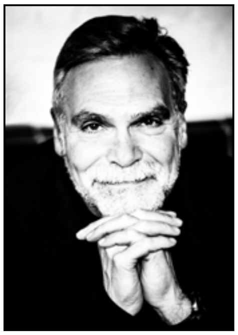
Plans are in place for a series of 20th anniversary events which were kicked off with a symposium at Georgia Tech in September 2018, celebrating the NAI's birthday. In 2019, a series of workshops without walls and in-person sessions are being scheduled. Please check https://nai.nasa.gov/seminars/ for the latest details. We also invite you to share any information, reflections, documents or artifacts of the NAI to support our archiving efforts.

## NAI 2018 Teams

In 2018, the NAI consisted of 10 competitively selected Teams, including 631 researchers distributed across 152 institutions. The Teams are supported through cooperative agreements between NASA and the Teams' institutions, and led by a Principal Investigator.

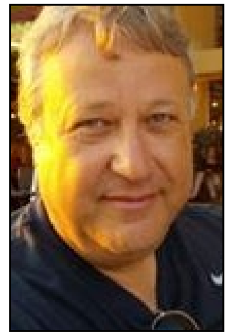
### CAN 7 (2015 – 2019)

**Scott Sandford**
Evolution of Prebiotic Chemistry and the Organic Inventory of Protoplanetary Disks
NASA Ames Research Center



**Frank Rosenzweig**
Reliving the Past: Experimental Evolution of Major Transitions
Georgia Institute of Technology

**Michael Mumma**
Origin and Evolution of Organics and Water in Planetary Systems
NASA Goddard Space Flight Center



**Isik Kanik**
Icy Worlds: Astrobiology at the Water-Rock Interface and Beyond
NASA Jet Propulsion Laboratory

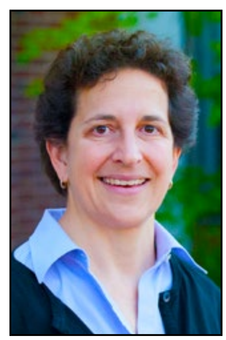
**Nathalie Cabrol**
Changing Planetary Environments and the Fingerprints of Life
SETI Institute

**Timothy Lyons**
Alternative Earths
University of California, Riverside

**Alexis Templeton**
Rock Powered Life
University of Colorado, Boulder

### CAN 8 (2018 – 2023)

**Rosaly Lopes**
Habitability of Hydrocarbon Worlds: Titan and Beyond
NASA Jet Propulsion Laboratory



**Katherine Freeman**
The Origins of Molecules in Diverse Space and Planetary Environments and Their Intramolecular Isotope Signatures
Pennsylvania State University

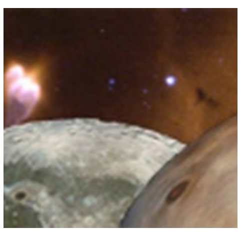
**Paul Falkowski**
ENIGMA: Evolution of Nanomachines in Geospheres and Microbial Ancestors
Rutgers University

**Evolution of Prebiotic Chemistry Complexity and the Organic Inventory of Protoplanetary Disks and Premordial Planets - NASA Ames Research Center**

The NAI Ames team seeks a greater understanding of chemical processes at every stage in the evolution of organic chemical complexity, from quiescent regions of dense molecular clouds, through all stages of cloud collapse, protostellar disk, and planet formation, and ultimately to the materials that rain down on planets, and understanding how these depend on environmental parameters like the ambient radiation field and the abundance of $H_2O$. This team is structured as an integrated, coherent program of astrochemical experiments, quantum chemical computations, disk modeling, and observations of astronomical sources.

**Reliving the Past: Experimental Evolution of Major Transitions - Georgia Institute of Technology**

The Georgia Institute of Technology team (previously based at the University of Montana, Missoula) has assembled an interdisciplinary group of investigators to address, using experimental microbial genomics, this overarching question: What forces bring about major transitions in the evolution of biocomplexity? The Georgia Tech team is organized around five questions related to major transitions in the history of life: (1) How do enzymes and metabolic networks evolve? (2) How did the eukaryotic cell come to be, specifically the cell that contained a mitochondrion? (3) How do symbioses arise? (4) How does multicellularity evolve? and (5) How do pleiotropy, epistasis and mutation rate constrain the evolution of novel traits?



**Origin and Evolution of Organics and Water in Planetary Systems - NASA Goddard Space Flight Center**

The central question being addressed by the Goddard Center for Astrobiology is: Did delivery of exogenous organics and water enable the emergence and evolution of life? In short: Why is Earth wet and alive? The approach being used to answer this central question includes an integrated program of (a) pan-spectral astronomical observations of comets, circumstellar disks, and exoplanet environments, (b) models of dynamical transport in the early Solar System, (c) laboratory studies of extraterrestrial material, and (d) realistic laboratory and numerical simulations of inaccessible cosmic environments. Synergistic integration of these areas is essential for testing whether delivery of the building blocks of life – exogenous water and prebiotic organics– enabled the emergence and development of the biosphere.


**Icy Worlds: Astrobiology at the Water-Rock Interface and Beyond - Jet Propulsion Laboratory**

Astrobiology at water-rock interfaces found on icy bodies (e.g., Europa, Enceladus and Ganymede) in our Solar System (and beyond) is the unifying theme of the JPL Icy Worlds team. We are pursuing an interdisciplinary and highly synergistic combination of experimental, theoretical, and field-based lines of inquiry focused on answering a single compelling question in astrobiology: How can geochemical disequilibria drive the emergence of metabolism and ultimately generate observable signatures on icy worlds? The JPL Icy Worlds teams examine bio-geochemical/bio-geophysical interactions taking place between rock/water/ice interfaces in these environments to better understand and constrain the many ways in which icy worlds may provide habitable niches and how we may be able to identify them.

**Changing Planetary Environments and the Fingerprints of Life - SETI Institute**

The SETI Institute team is developing a roadmap to biosignature exploration in support of NASA's decadal plan for the search for life on Mars – with the Mars 2020 mission providing the first opportunity to investigate the question of past life on Mars. In an ancient Mars environment that may have once either supported life as we know it, or sustained pre-biological processes leading to an origin of life, the Mars 2020 mission is expected to be a Curiosity-class rover that will cache samples for return to Earth at a later date. The SETI team will address the overall question "How do we identify and cache the most valuable samples?" The three fundamental sub-elements of the SETI team's research focuses on (1) where to search for the right rocks on Mars, (2) what to search for, and (3) how to search for them.

**Alternative Earths: Explaining Persistent Inhabitation on a Dynamic Early Earth - UC Riverside**
The Alternative Earths Team main research question is: How has Earth remained persistently inhabited through most of its dynamic history, and how do those varying states of inhabitation manifest in the atmosphere? From this question emerges the team's key goal: to characterize Earth's early oxygen history, its atmospheric evolution more generally, and the coupled drivers and consequences of this record. At its core, the UC Riverside team is structured around plans for a comprehensive deconstruction of the geologic record from the earliest biological production of oxygen to its permanent accumulation in large amounts almost three billion years later.

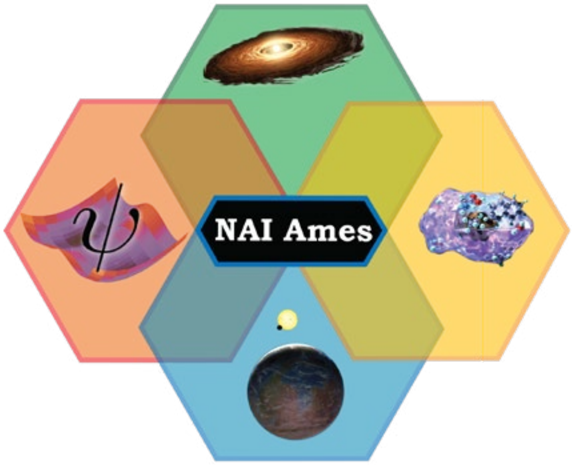
**Rock-Powered Life: Revealing Mechanisms of Energy Flow From the Lithosphere to the Biosphere - University of Colorado, Boulder**
Members of the University of Colorado Rock-Powered Life team have come together to address this question: How do the mechanisms of low temperature water/rock reactions control the distribution, activity, and biochemistry of life in rock-hosted systems? The central research areas are: (1) Defining the pathways that control how energy is released from ultramafic rocks as they react with low-temperature fluids, (2) Identifying and interpreting the process rates and ecology in systems undergoing water/rock reaction, (3) Characterizing microbial communities within rock-hosted ecosystems and evaluating their metabolic activities, (4) Quantifying the geochemical and mineralogical progression of serpentinization reactions in the presence and absence of biology, and (5) Developing and testing predictive models of biological habitability during water/rock interaction.

**Habitability of Hydrocarbon Worlds: Titan and Beyond - Jet Propulsion Laboratory**
The single compelling question for this research is: What habitable environments exist on Titan and what resulting potential biosignatures should we look for? The specific objectives to be addressed by this NAI team are: (1) Determine the pathways for organic materials to be transported (and modified) from the atmosphere to surface and eventually to the subsurface ocean (the most likely habitable environment), (2) Determine whether the physical and chemical processes in the ocean create stable habitable environments, (3) Determine what biosignatures may be produced if the ocean is inhabited, and (4) Determine how biosignatures can be transported from the ocean to the surface and atmosphere and be recognized at the surface and in the atmosphere.

**The Origins of Molecules in Diverse Space and Planetary Environments and Their Intramolecular Isotope Signatures - Pennsylvania State University**
The NAI Penn State team seeks to discover and document isotope patterns in organic molecules found in meteorites, dissolved in deep Earth fluids, from individual living organisms, within microbial ecosystems, and in organics associated with minerals and ice. Employing advanced computational tools and a rich observation portfolio, they will build a predictive understanding of how abiotic and biotic processes and environments are encoded in the isotopes of simple to complex organic compounds. Their work will lead to new understanding of organics and the isotopes they carry from space and planetary environments, in metabolic systems and modern biotic communities, and over Earth's history.

**ENIGMA: Evolution of Nanomachines in Geospheres and Microbial Ancestors - Rutgers University**
The research program of the NAI Rutgers University team is focused on a single, compelling question in astrobiology: How did proteins evolve to become the catalysts of life on Earth? The ENIGMA research program is focused on understanding the evolution of protein nanomachines, particularly those that are involved in electron transfer and redox processes. It seeks to understand the origin of catalysis, the evolution of protein structures in microbial ancestors, and the co-evolution of proteins and the geosphere through geologic time. ENIGMA has three integrated research themes: (1) Synthesis and Function of Nanomachines in the Origin of Life, (2) Increasing Complexity of Nanomachines in Microbial Ancestors, and (3) Co-Evolution of Nanomachines and the Geosphere.

# 2018 Team Reports




# The Evolution of Prebiotic Chemical Complexity
## and the
# Organic Inventory of Protoplanetary Disk and Primordial Planets

**Lead Institution:**
**NASA Ames Research Center**

## Team Overview

**Principal Investigator:**
Scott Sandford

The Evolution of Prebiotic Chemical Complexity and the Organic Inventory of Protoplanetary Disks and Primordial Planets Team seeks a greater understanding of the chemical processes occurring at every stage in the evolution of organic chemical complexity, from quiescent regions of dense molecular clouds, through all stages of disk and planet formation, and ultimately to the materials that rain down on planets. The effort is an integrated, coherent program involving the interaction of closely linked research projects on:

- Modeling and Observations of Protoplanetary Disks
- Modeling and Observations of Exoplanets
- Laboratory Studies of Gas-Grain Chemistry
- Laboratory Studies of Ice Photolysis
- Computational Quantum Chemistry

These projects interact closely with each other such that each benefits from advances made in the others and helps to guide future work. For example, the modeling of the chemistry that takes place in protostellar disks is assisted by inputs provided by spectral, physical, and chemical properties of molecules determined by the laboratory and computational projects. In turn, the models serve to provides guidance for key areas of future computational and laboratory work. Similarly, the computational studies can be used to help interpret laboratory results and extend them to additional materials or environments, while the laboratory results can provide confirmation of computational reaction paths.

**Team Website:** http://amesteam.arc.nasa.gov

## 2018 Executive Summary

During 2018, our team made significant progress on all aspects of our combined research. Highlights are described below; more details reside in the individual project report sections.

**Disk Modeling –** We completed the addition of gas–grain chemistry to our disk physical structure model. This model is an original application of a 3-phase gas–grain network in disks, with distinct gas, grain surface, and bulk mantle phases. We find that in the inner disk, warmer dust grains coupled with photodissociation increases mobility of radicals that form complex molecules. In the outer disk, hydrogenation dominates and species like water, methane, and methanol dominate. These results are being used to generate chemical networks for inclusion in a 2-D ice transport model. Additionally, we are incorporating the full chemical network into a model to study the chemistry of comets.

**Exoplanets –** We published a decade-long Doppler velocity campaign monitoring the nearby M-dwarf Gliese 876 and its remarkable planetary system and showed the system behaves like a damped compound pendulum. The appearance of *'Oumuamua*, the first macroscopic object of clearly interstellar origin detected within our Solar System, motivated us to develop dynamical and thermal models to explain its behavior and assess prospects for impactor missions to reconnoiter future objects of this type. We published an overview of Spitzer's infrared orbital phase curves showing that they can be explained within a 4-parameter framework. We also demonstrated how measuring spin obliquities of exoplanets can provide key discriminants between *in situ* and migration hypotheses for short-period exoplanet formation.

**Laboratory Studies of Gas–Grain and Ice Photolysis Chemistry –** Recent laboratory experiments of UV irradiation of ices containing $H_2O$, $CH_3OH$, CO, and $CO_2$ demonstrated the formation of multiple sugar derivatives (including ribose) and several deoxysugar derivatives including 2-deoxyribose, the sugar of DNA (Nuevo *et al*. 2018; Figure 1). We addressed the formation of nucleobases in purine-containing ices and the formation of variants of hexamethylenetetramine (HMT) in ices containing H, C, N, and O. Diffuse reflectance infrared spectroscopy was used to characterize mineral analogs used in studies of organic–mineral interactions (i.e. gas–grain chemistry) and we investigated their role in the hydrogenation of polycyclic aromatic hydrocarbons (PAHs). Through mineral contact alone, the PAHs were shown to hydrogenate over time without additional energy input (Figure 2) and this was confirmed via isotopic substitution studies.

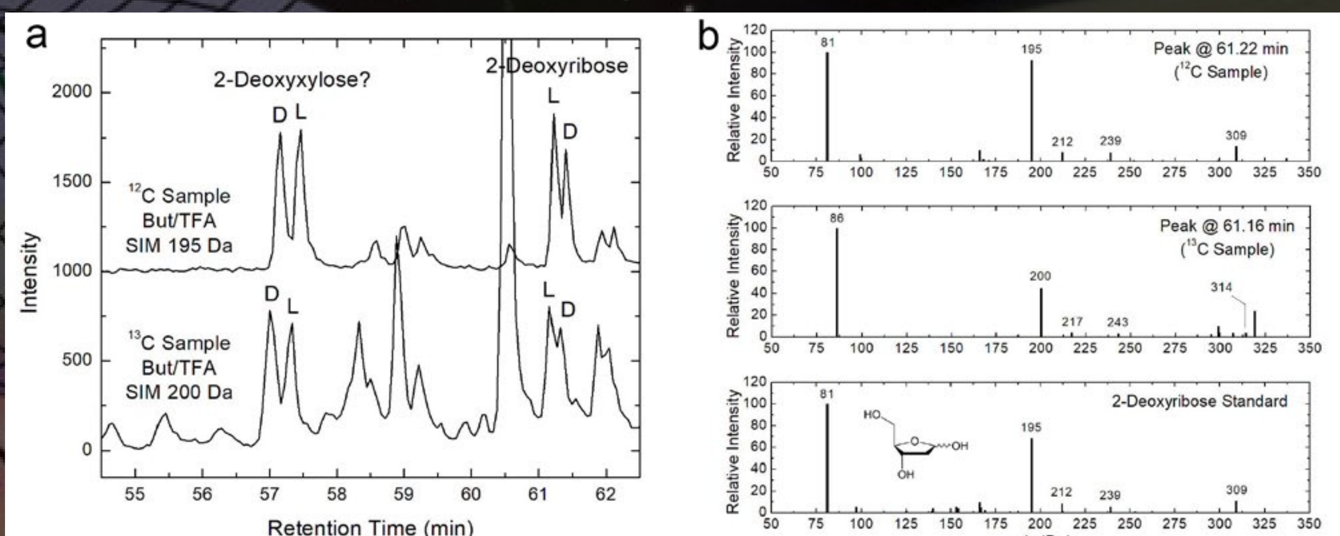



Figure 1. Identification of 2-deoxyribose in ice photolysis residues. (a) Single-ion monitoring chromatograms of residues produced from the UV irradiation of $H_2O$:$CH_3OH$ (2:1) ice mixtures ($^{12}$C sample, m/z = 195 Da) and $H_2O$:$^{13}CH_3OH$ (2:1) ($^{13}$C sample, 200 Da) ice mixtures after derivatization with (+)-2-butanol/TFAA. The peaks around 57.0 and 57.3 min are tentatively assigned to 2-deoxyxylose. Intensities are offset for clarity. (b) From top to bottom, mass spectra of the peaks assigned to 2-deoxyribose in the regular residue, the $^{13}$C-labelled residue, and a standard. (From Nuevo *et al.* 2018.)

**Computational Quantum Chemistry –** We executed quantum calculations on the formation of functionalized HMT molecules and their vibrational and rotational data. We also characterized the energetic, spectroscopic, and physical properties of isomers of azirine and diazirine. Growth of PAH molecules and the formation of nitrogenated heterocyclic molecules were also studied. Our collaboration with the Dutch Astrochemistry Network (DAN II) resulted in multiple publications comparing computed anharmonic vibrational spectra of hydrogenated and methylated PAHs with experiments, and created theoretical methods allowing for the construction of unprecedented fully anharmonic cascade emission spectra of PAHs (Figure 3). Finally, we predicted the rovibrational spectra and spectroscopic constants of small molecules for interpreting high-resolution astronomical observations.

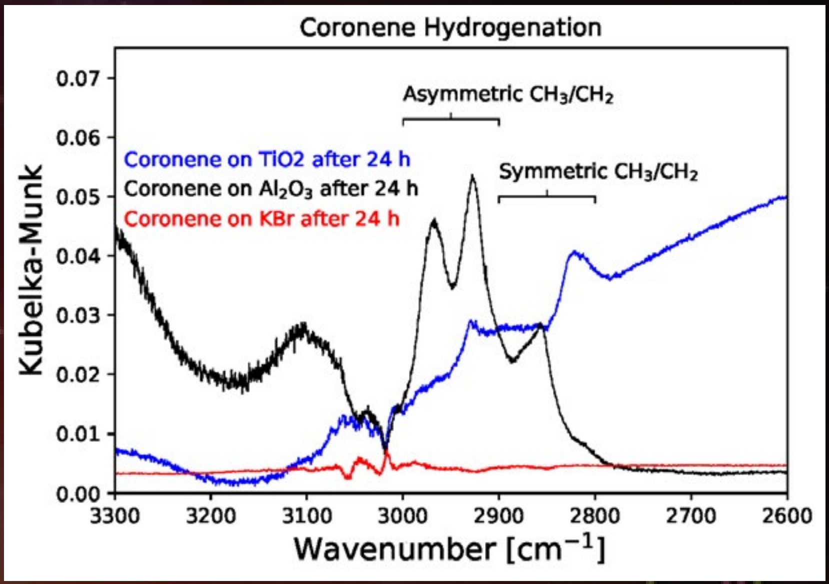



Figure 2. Coronene ($C_{24}H_{12}$) and minerals mixed under vacuum. Aliphatic bands near 2965, 2925, and 2850 $cm^{-1}$ grow with time without any energy input. They also seem to be affected by the catalyzing surface. Credit: NASA

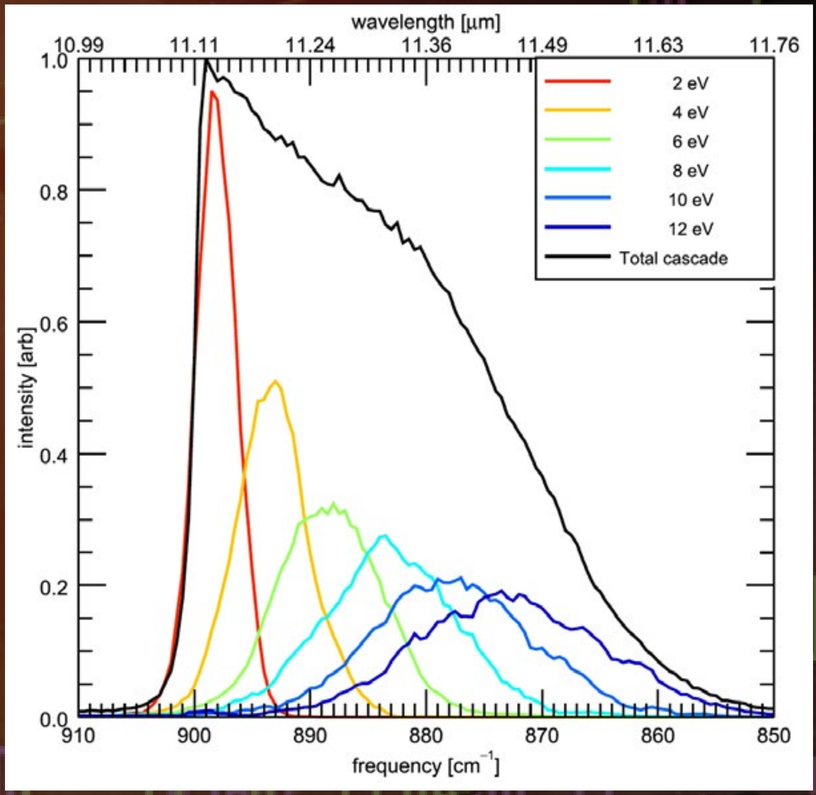



Figure 3. The distribution of IR photon emission probabilities at various internal energies along the cascade of a C–H out-of-plane bending mode of tetracene (colors), with the resulting IR cascade spectrum (black). Computation of these cascades yields spectra that can be directly compared to the spectra actually observed by astronomers (From Mackie *et al*. 2018). Credit: NASA

# Project Reports

## Modeling and Observations of Protoplanetary Disks

We have completed the major task of adding gas–grain chemistry to our detailed, disk physical structure models, which includes heating, cooling, irradiation and dust dynamics. This model is one of the first applications of a 3-phase gas–grain network in disks, with distinct gas (Phase 1), grain surface (Phase 2) and bulk mantle (phase 3) phases. Compared to existing 2-phase models (gas and dust) in the literature, our 3-phase model is more accurate in considering desorption only for the surface species, while the bulk is still chemically active with diffusion driven by water. Molecules here can also undergo photo-dissociation. Our models find that in the inner disk, warmer dust grain temperatures coupled with cosmic ray induced photodissociation lead to increased mobility of radicals that form complex molecules. In the outer disk, where hydrogenation dominates due to the cold dust grain temperatures, the most abundant species are water ice, methane, and species like methanol (Figure 4). More complex molecules are typically low in abundance (Ruaud & Gorti, 2019, in preparation). The results of this detailed chemical analysis are being used to generate simpler chemical networks for inclusion in a 2-D ice transport model that is being developed in parallel.

Based on our earlier work on modeling CO gas emission from debris disks (Hales *et al.* 2019), where we found that the CO is likely being produced by outgassing of comets during collisions in the late stages, we are investigating the chemistry of comets with flow dynamics. Using a smaller chemical network, we have incorporated advection in the model for comparison with observations. This has been tested for CO and its isotope emission. Currently, we are working on incorporating the full chemical network into this model for comparisons with cometary observations. This work is still in progress.

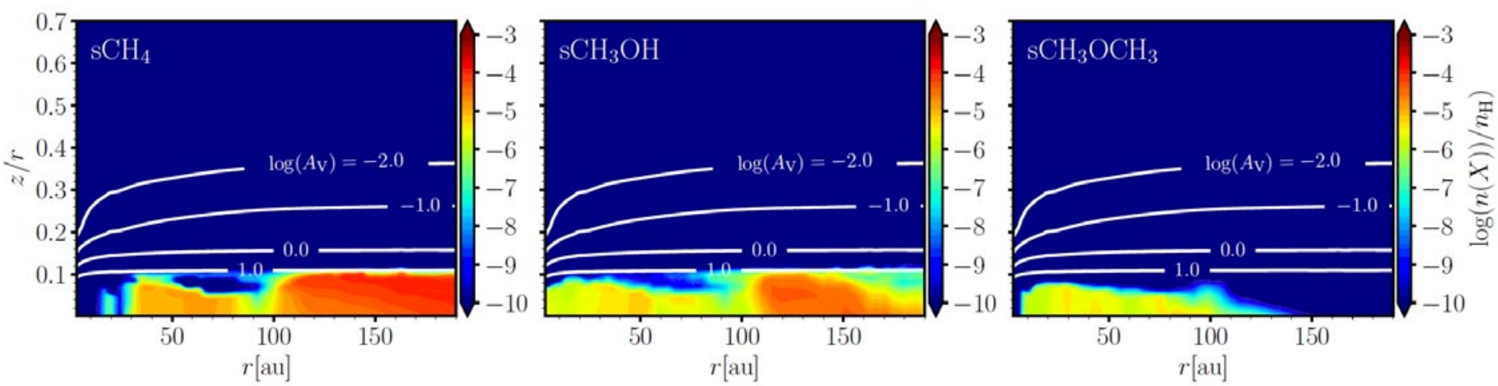



Figure. 4: Abundance maps of ices on grain surfaces are shown as a function of disk radius and disk height (z/r) for one of the disk models. Methane and methanol ices are seen to be more abundant in the outer disk due to efficient hydrogenation while more complex organics (represented here by $CH_3OCH_3$) are abundant only in the inner disk. Credit: NASA

## Exoplanet Studies

Our effort to detect and characterize extrasolar planets made excellent progress in 2018. We published a decade-long precision Doppler velocity campaign monitoring the nearby M-dwarf Gliese 876 and its remarkable planetary system (Millholland *et al.* 2018). We showed that, while there are hints of a low-mass terrestrial planet in this star's habitable zone, statistical analysis rules out a detection to publishable significance. The high quality of the data allowed remarkable conclusions regarding the system's configuration. It behaves like a highly damped compound pendulum, providing important clues to the dissipative processes at work in sculpting the formation of planetary systems.

One of the most surprising recent astronomical stories was the appearance of 'Oumuamua, the first macroscopic object of clearly interstellar origin to be detected within our Solar System. 'Oumuamua's light curve and spectrum indicated that it was highly elongated and reddish. It showed no sign of a dust coma, yet it displayed non-gravitational accelerations typical of Solar System comets.

We developed detailed dynamical and thermal models to explain 'Oumuamua's behavior and assessed the prospects for low delta-V impactor missions to reconnoiter future-arriving objects of this type (Seligman & Laughlin 2018; Figure 5). Interstellar objects represent an extraordinary opportunity to assess how planet formation proceeds.

We also focused on the physical atmospheric structure, and the global appearance, of extrasolar planets. These properties can be mapped from data obtained by the Spitzer Space telescope and the future JWST. We published a detailed overview of all of Spitzer's infrared orbital phase curves, showing that the extant data can be explained within a 4-parameter framework (Adams & Laughlin 2018). We also demonstrated how Spitzer and JWST can measure spin obliquities of extrasolar planets (Millholland & Laughlin 2018), which will provide a key discriminant between the *in situ* and migration hypotheses to describe the formation of short-period exoplanets.

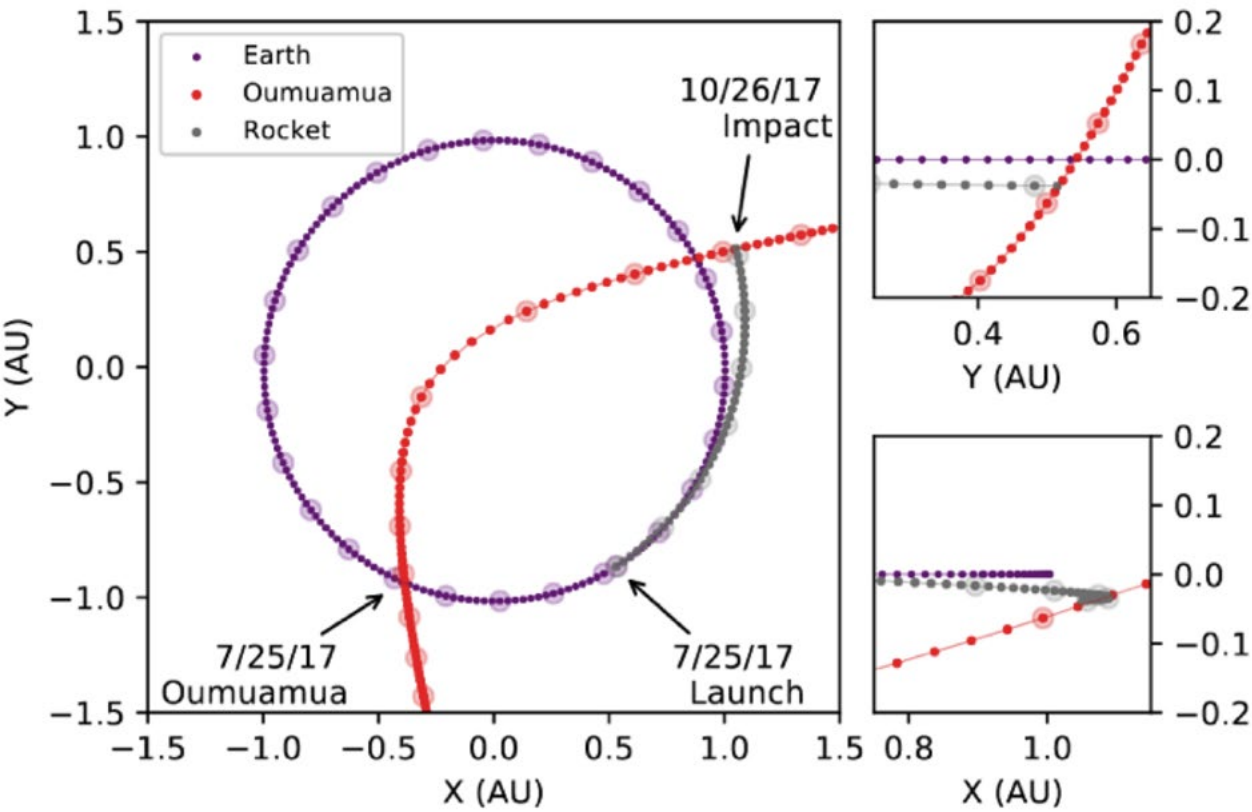
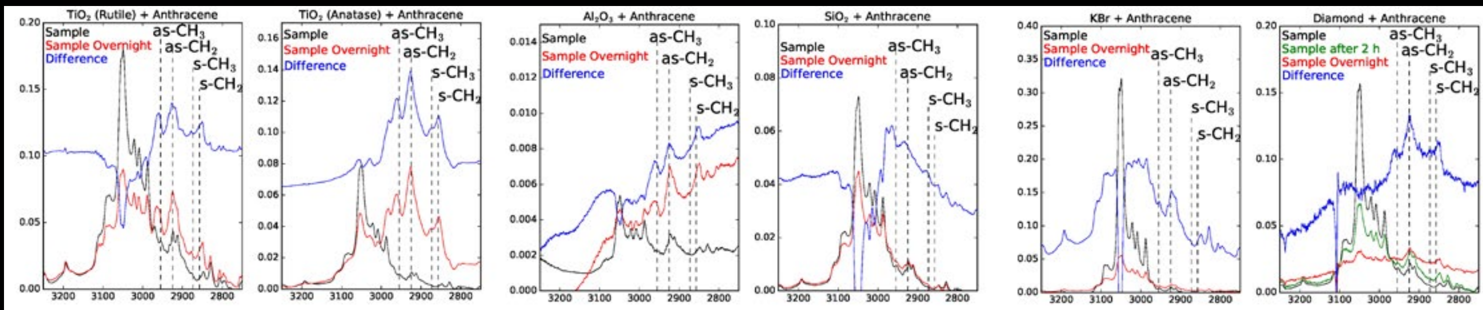



Figure 5. Trajectory of the minimum delta-V interception mission that could have been launched to 'Oumuamua had it been detected on its inbound trajectory into the solar system. In Seligman & Laughlin (2018), we calculate that the LSST telescope (currently under construction) will be capable of detecting several interstellar objects for which a similar mission profile will be possible. Interception of a planetesimal from an exoplanetary system would allow us to put the contents of our own Solar System's planets into their galactic context. Credit: Seligman and Laughlin (2018)

## Laboratory Studies of Gas-Grain Chemistry




We characterized mineral analogs via diffuse reflectance infrared spectroscopy (DRIFTS) for use in studies of organic-mineral interactions (i.e., gas-grain chemistry). Analogs of aluminum oxide ($Al_2O_3$), silicon dioxide ($SiO_2$), titanium dioxide ($TiO_2$), and diamond were investigated to understand their role in the hydrogenation of polycyclic aromatic hydrocarbons (PAHs) (e.g. anthracene and coronene). The studies were conducted under vacuum and at room temperature. Just by being in contact with the minerals, the PAHs were hydrogenated over time, without the need for an additional energy. The growth of the aliphatic bands is easily seen in the IR spectra (Figure 6). These results were confirmed via isotopic substitution studies, which revealed the exchange of hydrogen and deuterium in the anthracene structure (Figure 7). These experiments were repeated on coronene, with similar results. However, the position of the hydrogenation of coronene differed with the mineral substrate. The participation of the mineral substrate in the hydrogenation was confirmed using control experiments using only KBr and the PAH.

Figure 6. Anthracene mixed with (from left to right) $TiO_2$, $Al_2O_3$, $SiO_2$, KBr, and diamond dust analogs. Mid-IR DRIFTS spectra show how the C–H stretching region changes (aromatic bands decrease while aliphatic bands increase) without UV irradiation when anthracene is mixed with each substrate. The rate of alteration is mineral dependent. Credit: NASA

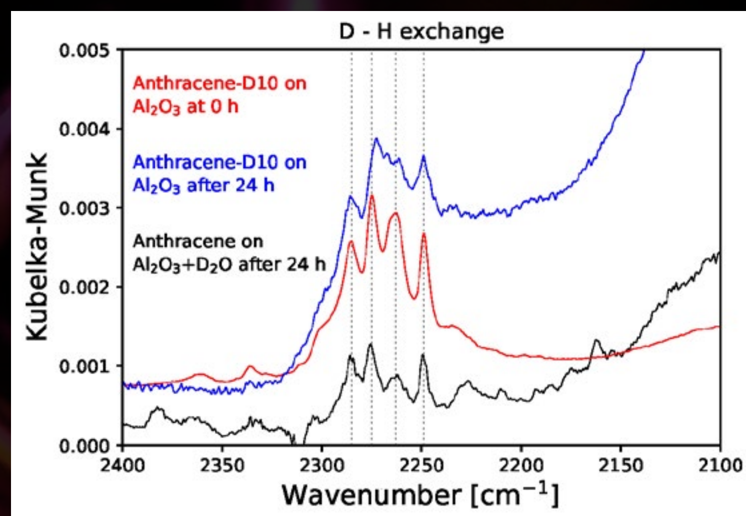



Figure 7. Anthracene and anthracene-$D_{10}$ (fully deuterated) on $Al_2O_3$. After mixing anthracene and $Al_2O_3$ soaked in $D_2O$, we were able to see the production of deuterated anthracene, suggesting an exchange between H and D in the anthracene structure. Credit: NASA

Ms. Julie Korsmeyer interned in our laboratory during the 2018 summer, investigating the photochemistry of the PAH anthracoronene (coronene with an anthracene molecule attached) in water ice. This work built on our past research with the photolysis of coronene and anthracene in water ice, to see if UV photolysis added functional groups (i.e. hydrogen, oxygen, or hydroxyl) to either the coronene or the anthracene portions of the molecule or both. Overall, the photochemistry of the anthracoronene molecule seems to be different from either of the parent PAH molecules. Ms. Korsmeyer's research was used for her Senior Thesis at the W. Keck Science Department at Scripps College. The work is currently in preparation for publication.

In 2019, we will be undergoing a major renovation greatly expanding our capabilities to include high-energy electron irradiation and Raman spectroscopy.

## Laboratory Studies of Ice Photochemistry

Recent experiments in which UV irradiation of $H_2{}^{18}O:CH_3OH$ ice mixtures show that the sugar derivatives (including ribose) produced in these experiments do not form via a pure formose reaction, as previously suggested, but rather through a multitude of pathways involving radicals and ions. This result will be included in an upcoming publication. Moreover, ongoing additional experiments which contain CO and/or $CO_2$ in addition to $CH_3OH$ in starting ices show that the distribution of sugar derivatives in the resulting residues more closely resembles the distribution found in meteorites. Finally, similar experiments led to the formation of several deoxysugar derivatives including 2-deoxyribose, the sugar of DNA (Nuevo *et al.* 2018).

Following the experiments that demonstrated the formation of adenine, guanine, and other functionalized purines from the UV photoprocessing of purine in simple $H_2O:NH_3$ ice mixtures (Materese *et al.* 2017), we published a second paper describing the photochemistry of purine in more astrophysically relevant ice mixtures ($H_2O:CH_3OH:CH_4:NH_3$; Materese *et al.* 2018). Results indicate that the production of purine derivatives (including nucleobases) is very sensitive to the concentration of $CH_3OH$ in the initial ices.

Finally, the experiments involving UV irradiation of purine in astrophysical ices (Materese *et al.* 2018) led to the discovery in the GC-MS chromatograms of the residues of an intense peak with distinctive fragmentation patterns in the GC-MS chromatograms of residues. Additional experiments using isotopically labeled ices ($^{13}C$, $^{15}N$, and $^{18}O$; Figure 8) allowed us to assign this peak to hexamethylenetetramine (HMT) with a $CH_2OH$ group attached to a carbon atom (HMT-methanol). *Ab initio* computations of the infrared spectra of HMT and HMT-methanol in collaboration with the Quantum Chemistry group showed strong bands associated with the high symmetry of HMT, suggesting that HMT-substituted compounds may be detectable in space (Materese *et al.* 2019). Calculations of infrared spectra for several other HMT-substituted compounds are currently in progress.

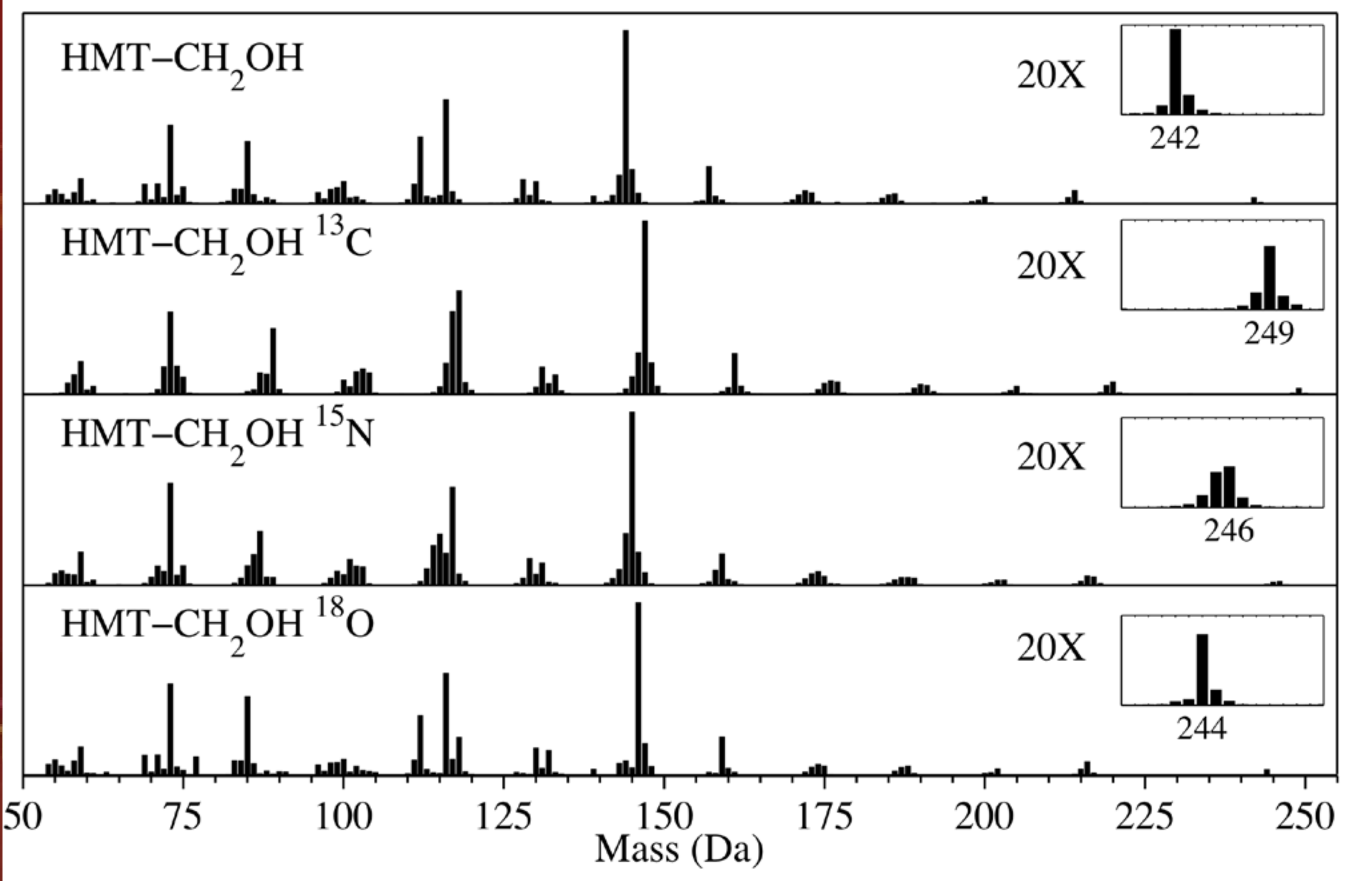



Figure 8. Mass spectra of the normal, $^{13}C$, $^{15}N$, and $^{18}O$ isotopic variants (from top to bottom) of the peaks at ~10.7 min observed in the GC-MS chromatograms of residues derivatized with BSTFA and assigned to HMT-methanol ($C_7H_{14}N_4O$) (from Materese *et al.* 2019).

## Computational Quantum Chemistry

Laboratory irradiation of astrophysical ice analogs containing $H_2O$, $CH_3OH$, CO, and $NH_3$ yields hexamethylenetetramine-methanol (HMT-methanol; $C_7N_4H1_4O$). Infrared spectra for HMT and HMT-methanol were computed using quantum chemistry methods and showed good agreement with observed vibrational spectra (Figure 9) and rotational constants for HMT. HMT-methanol may represent an abundant member of a family of functionalized HMT molecules (for which vibrational and rotational data have been computed) that may be present in astrophysical environments. One manuscript is under consideration (*ApJ*) and another is in preparation.

Azirine and diazirine are nitrogenated cyclic molecules, a class of organic compounds of astrobiological interest. Energetic, spectroscopic, and physical properties of the isomers of azirine and diazirine were characterized using quantum chemistry. Nitrogenated cyclic molecules would mark a milestone in the search for biologically relevant molecules if identified in the interstellar medium (ISM). A paper about the results obtained for azirine was published (Figure 10), and another one about the results obtained for diazirine is in preparation.

Growth of medium to large PAH molecules was studied using *ab initio* molecular dynamics simulations. To understand the formation of nitrogenated heterocyclic molecules under astrophysical conditions, *ab initio* trajectory calculations were performed on [*x*HCCH + *y*HCN]+ (*x*,*y*=1,2,3) clusters. Two manuscripts are in preparation.

Our collaboration with the DAN II was productive with four peer-reviewed publications. The first two reported the comparison of computed anharmonic vibrational spectra

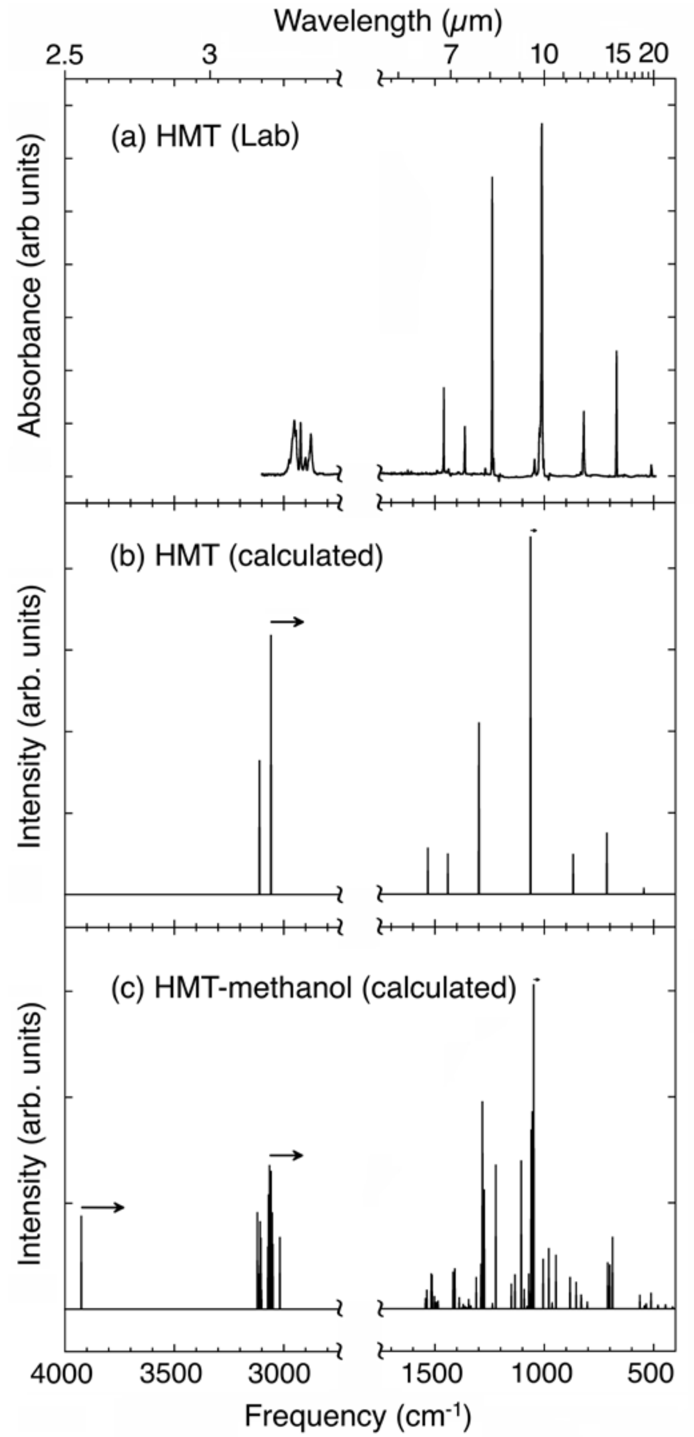



Figure 9. Experimental and DFT computed infrared spectra of HMT and HMT-methanol. Credit: NASA

with high-resolution experiments for hydrogenated and methylated PAHs, the first to include aliphatic C–H stretches. The final two reported development of theoretical methods wherein libraries of anharmonic temperature-dependent spectra are computed, taking resonances fully into account, and culminating for the first time in the construction of fully anharmonic cascade emission spectra of PAHs, i.e., the spectra observed by astronomers.

Through collaborations with Drs. Fortenberry (Univ. Mississippi) and Schaefer (Univ. Georgia), we predicted the rovibrational spectra and spectroscopic constants of small molecules for interpreting high-resolution astronomical observations, and used similar data to analyze SOFIA EXES spectra.

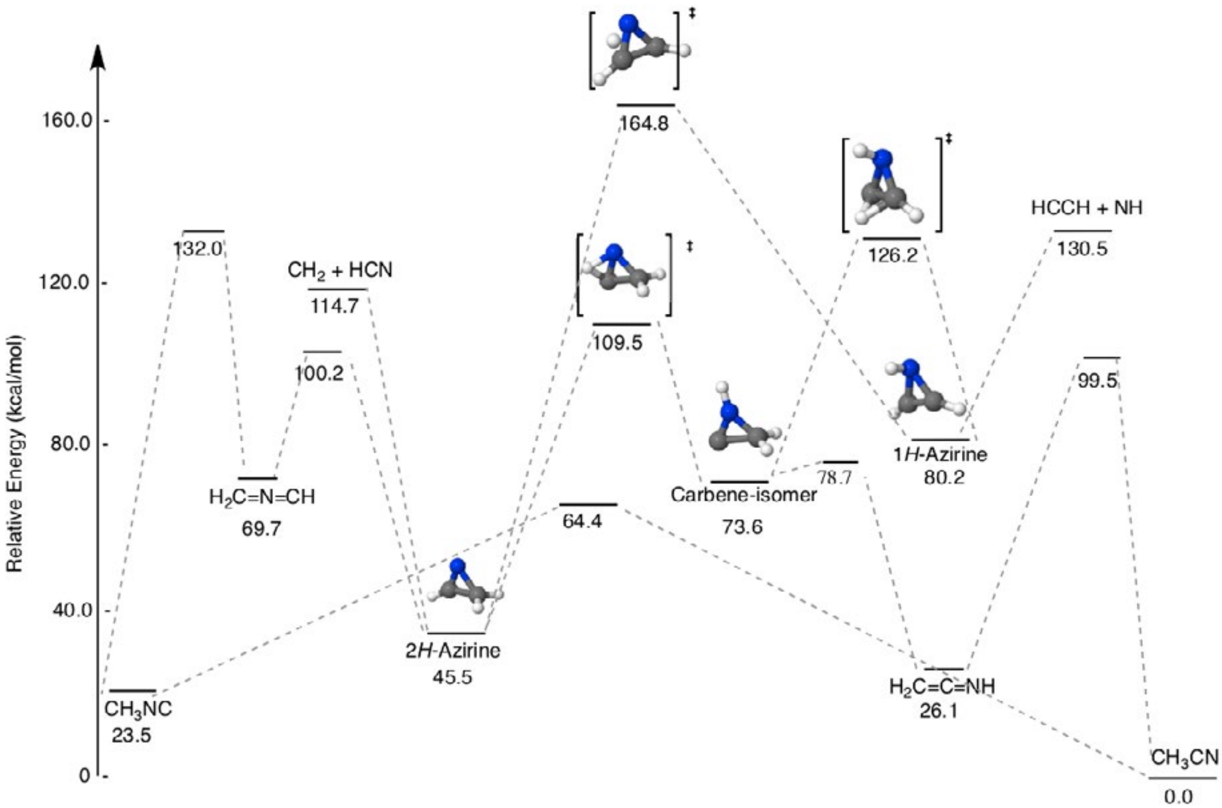



Figure 10. Energy diagram of linear and cyclic isomers of $C_2H_3N$ (azirine) on the singles surface. Credit: NASA

# Flight Mission Involvement

### NASA's OSIRIS-REx Asteroid Sample Return Mission

Dr. Sandford is a Co-Investigator on NASA's OSIRIS-REx (Origins, Spectral Interpretation, Resource Identification, Security, Regolith Explorer) asteroid sample return mission, which launched on September 8, 2016. The OSIRIS-REx spacecraft has arrived at its target asteroid 101955 Bennu and is currently involved in reconnaissance of the asteroid in preparation for a future collection of samples from the asteroid's surface. These samples will be returned to Earth for study in 2025. Dr. Sandford plays a number of roles on the mission and will participate in the study of the returned samples, with an emphasis on the analyses of any organic materials they contain.

### JAXA's Hayabusa2 Asteroid Sample Return Mission

Dr. Sandford is member of the team of researchers who will study insoluble organics found in samples returned to Earth from asteroid Ryugu by JAXA's Hayabusa2 spacecraft. The spacecraft is currently at the target asteroid and will return collected samples to Earth for study in December 2020.

### OREOCube

Dr. Mattioda is one of the senior scientists focusing on molecular spectroscopy and experimental development, with the OREOCube mission. He is involved with the preparation of flight samples for the mission as well as conducting the ground control studies in his laboratory and the analysis of the resulting spectroscopic data from the spacecraft.

### EXOCube

Dr. Mattioda a senior scientist for EXOCube, with an emphasis on the molecular spectroscopy, polycyclic aromatic hydrocarbon molecules (PAHs) as well as other organic molecules. Dr. Mattioda is involved in the planning, instrument design, sample preparation, and analysis of the resulting data.

### ESA Exobiology Facility for the ISS

The European Space Agency announced the start of activities for Phase A/B of the Exobiology Facility destined for installation on the International Space Station (ISS). Team Member Andrew Mattioda is a senior science team member on both OREOCube and Exocube which are two of the three experimental facilities that will comprise this new facility. The facility is slated to be installed on the ISS by early 2020.

## Team Members

**Scott Sandford**
Partha Bera
Gustavo Cruz-Diaz
Uma Gorti
Martin Head-Gordon
Josie Hendrix
Gregory Laughlin
Timothy Lee
Christopher Materese
Andrew Mattioda
Michel Nuevo
Maxime Ruaud
Tamar Stein
Xander Tielens

# The Evolution of Prebiotic Chemical Complexity and the Organic Inventory of Protoplanetary Disk and Primordial Planets: 2018 Publications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Mackie, Cameron J. (2018). The Anharmonic Infrared Spectra of Polycyclic Aromatic Hydrocarbons. A Ph.D. thesis successfully defended by Cameron J. Mackie on 29 March 2018, Leiden Observatory, Leiden University.

Mackie, C. J., Candian, A., Huang, X., Maltseva, E., Petrignani, A., Oomens, J., Buma, W. J., Lee, T. J., Tielens, A. G. G. M. (2018). The anharmonic quartic force field infrared spectra of hydrogenated and methylated PAHs. *Physical Chemistry Chemical Physics* 2(20): 1189-1197. DOI: 10.1039/C7CP06546A

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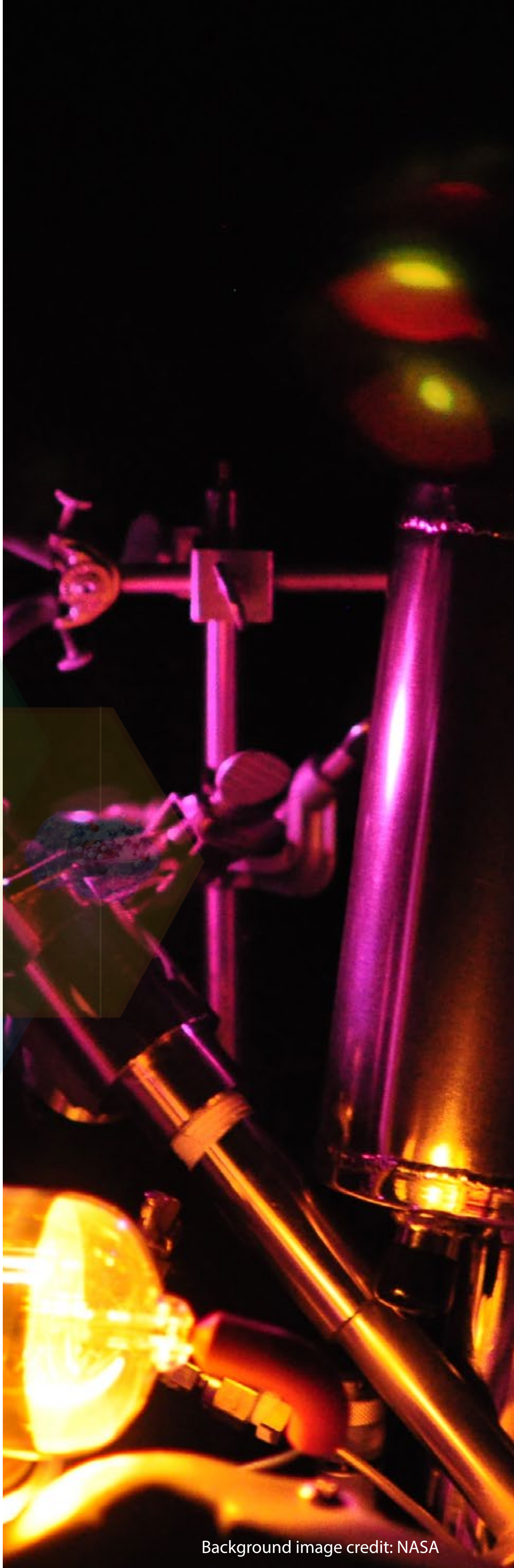
*NAI supported, but not explicitly acknowledged

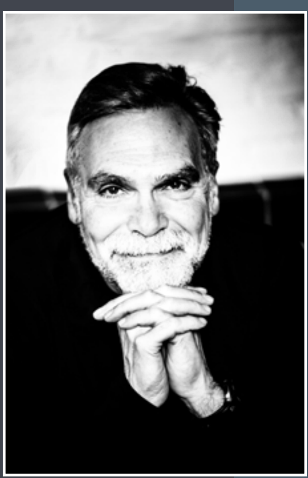


Background image credit: NASA

# Reliving the Past:
# Experimental Evolution of Major Transitions

**Lead Institution:**
**Georgia Institute of Technology**

## Team Overview



**Principal Investigator:**
Frank Rosenzweig

Darwin's *Origin of Species* concludes with a hymn to biocomplexity teeming on an English hillside. The hymn's most penetrating verse is "these elaborately constructed forms, so different from each other, and dependent upon each other, had all been produced by laws acting around us. "To delve into these laws, to understand how differences are selected for and how interdependence is enforced remains biology's grandest challenge. Our team seeks to meet this challenge by "reliving the past" using experimental evolution, which enables us to discern evolution's causes as well as its consequences, and to discover why evolution takes certain paths and not others. By tackling five specific questions, we seek to illuminate what drove the major transitions that led to evolution of complex life on our home planet:

- How do new enzymes and metabolic networks evolve?
- How did the eukaryotic cell come to be?
- How do symbioses arise?
- How does multicellularity evolve?
- How do history, gene interactions and mutation rate constrain innovation?

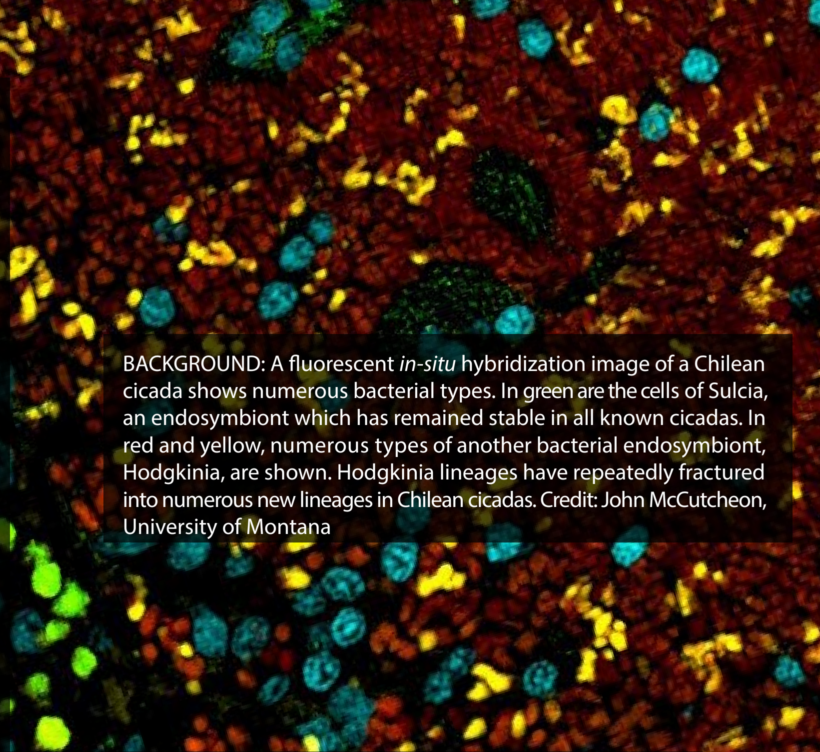
We seek general principles likely to govern the emergence of complexity wherever life exists. Our enterprise falls squarely within Astrobiology, the study of the origins, evolution, distribution, and future of life in the universe, and addresses the fundamental question: How does life begin and evolve?

## 2018 Executive Summary

Major transitions in the history of life occurred when simple subunits coalesced to form autonomous, interdependent self-replicating wholes, producing quantum leaps in biocomplexity. Our team integrates theory with comparative and evolutionary genomics to explore how structurally complex life arose on Earth. We tackle questions like *How do metabolic networks come to be? How do symbioses arise? How did multicellularity originate?* What attributes of the hereditary apparatus control the pace of evolutionary innovation? If we view "life as a self-sustaining chemical system capable of Darwinian evolution" (NASA's working definition of life), then the answers to such questions should apply wherever life has arisen in the Cosmos.

Georgia Tech's Reliving the Past team enjoyed a successful 2018. In terms of scholarly output, we published or currently have in press 30 peer-reviewed papers, including reports in high-impact journals such as Nature Ecology & Evolution and PNAS. Multiple reports attracted media coverage, e.g. John McCutcheon's work on how sap-feeding insects like Cicadas sustain fungal and bacterial endosymbionts was featured in *The Atlantic* (theatlantic.com/science/archive/2018/06/how-to-tame-a-zombie-fungus/562544/). Team members gave more than 40 invited seminars and engaged in a broad range of synergistic activities including, a Visiting Professorship at the École Normale Supérieure (Ratcliff), the EvolvingSTEM evolution-in-action curriculum for high school seniors (Cooper), and serving as a faculty mentor for Engineers Without Borders to Georgia Tech students in Nicaragua (Gerrish). We provided peer review on scores of manuscripts, served on editorial boards for *BMC Microbiology, J Biological Chemistry, Genome Biology Evolution, Microbial Cell, and Biology Letters*, and on panels for NASA, NSF and NIH. The Reliving the Past team hosted high school and undergraduate trainees, pre- and post-doctoral fellows including NASA Postdoctoral Program (NPP) recipients Amanda Garcia, Peter Conlin, and Caroline Turner, Ford Fellow Kennda Lynch, and NSF Graduate Research Fellowships Program (GRFP) fellow Jordan Gulli. Reliving the Past's broader impacts were felt in Education/Public Outreach activities, ranging from televised lectures to high school students, in-classroom and in-lab mentorship of high school teachers, and public, streamed lectures/interviews on the Origin of Life. Among the most noteworthy of our E/PO activities was AbGradCon 2018 (astrobiology.nasa.gov/news/abgradcon-2018/). Early career organizers raised $43,000 to leverage NAI's $77,000 investment. The scientific program attracted 96 participants from 9 different countries, while the writing workshop attracted 25 participants.

Our annual Reliving the Past team meeting coincided with the Fall NAI Executive Council in-person meeting. These two events coalesced in a widely-attended public symposium: "A 20th Birthday Celebration of NASA Astrobiology Institute." Speakers included Georgia Tech faculty leading NASA-supported Centers for Chemical Evolution (Nick Hud), Origin of Life (Loren Williams), Life Detection (Britney Schmidt) and P-STAR (Amanda Stockton), as well as Co-Is of the UC-Riverside and SETI NAI teams: Jen Glass, Chris Reinhard and James Wray. The concluding session featured NPP fellows supported by the Reliving the Past CAN-7 and other Astrobiology-related programs at Georgia Tech (GT). This convocation sparked three major GT initiatives. Tech's NPP Fellows created an "Exploration and Origins" working group (astrobiology.gatech.edu/), and organized an international symposium on "Evolution of Complex Life" (eclife.biosci.gatech.edu/our-sponsors/). Finally, a team of GT Astrobiology faculty led by Martha Grover and supported by NAI members Jen Glass, Chris Reinhard and Frank Rosenzweig began work on an "Origins and Exploration" NSF Research Traineeship proposal submitted 2/9/19.



BACKGROUND: A fluorescent *in-situ* hybridization image of a Chilean cicada shows numerous bacterial types. In green are the cells of Sulcia, an endosymbiont which has remained stable in all known cicadas. In red and yellow, numerous types of another bacterial endosymbiont, Hodgkinia, are shown. Hodgkinia lineages have repeatedly fractured into numerous new lineages in Chilean cicadas. Credit: John McCutcheon, University of Montana

Reliving the Past researchers have made substantial progress towards addressing the questions articulated in our original proposal, and are also pursuing new opportunities. For example, Shelley Copley's group at Colorado uses laboratory evolution to investigate how new proteins arise by gene duplication and divergence, a process that has been responsible for much of the diversification of life on earth. Shelley's students study this process in *Escherichia coli* using a system in which a promiscuous enzyme that has multiple weak secondary activities is recruited to replace an essential enzyme that has been inactivated. Their recent work published in *PLoS Genetics* (Kristofich *et al.* 2018) showed that this duplication and divergence is inevitably accompanied by fitness-enhancing mutations elsewhere in the genome, thus the canonical model for evolution of new proteins must be expanded to accommodate this bigger picture.

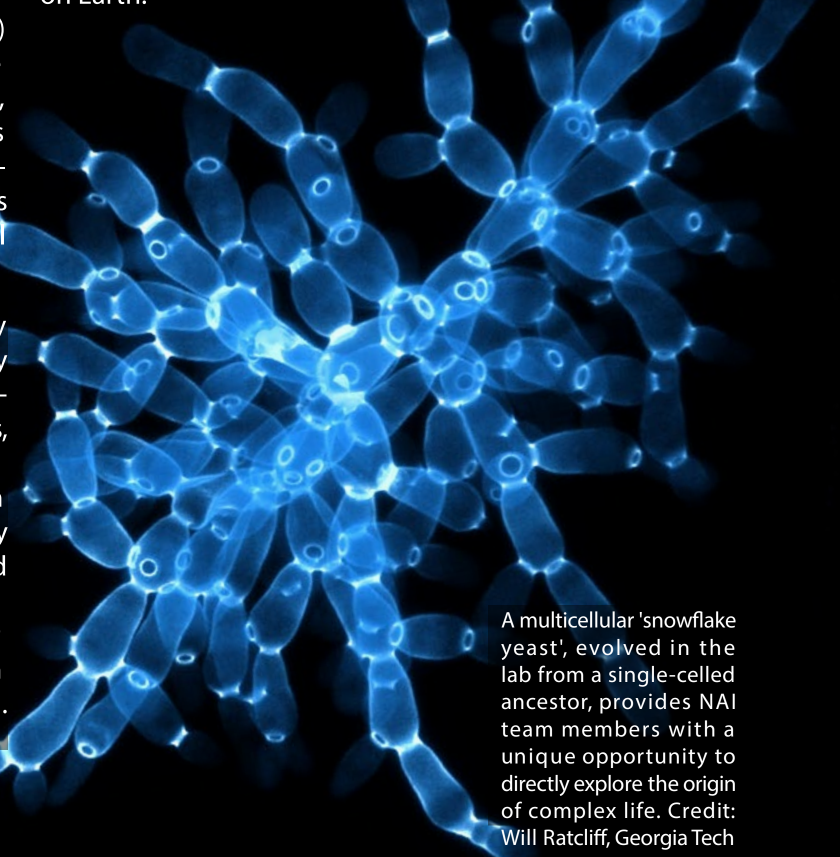
Scott Miller's group at Montana has shown how both newly-duplicated genes (Gallagher & Miller 2018) and the persistence of ancient genomic rearrangements (Sano *et al.* 2018) help cyanobacteria adapt to astrobiology-relevant environments like geothermal springs. They have also begun to study endosymbiosis as a way to illuminate early evolution of the eukaryotes, among whose hallmark features are membrane-bound organelles. Specifically, Scott's team is investigating a newly-described mutualism between the diatom *Rhopalodia gibba* and its cyanobacterial endosymbiont, which is in the process of becoming a nitrogen-fixing organelle.

John McCutcheon's group at Montana also studies symbiosis as a way to gain insight into the origin of organelles. Their model is the metabolic interdependence between sap-feeding insects and their microbial endosymbionts. Like mitochondria and plastids, endosymbionts are transmitted from parent to offspring. John's students have shown that endosymbiont genomes frequently become fragmented, such that the capacity for amino acid biosynthesis ends up being distributed among many genomes, eukaryotic and prokaryotic. In landmark papers published this year in *PNAS* (Łukasik *et al.* 2018; Matsuura *et al.* 2018), they showed that the fragmentation process has occurred many times in different cicada species, and that these independent events evolve to very different genomic outcomes despite starting from exactly the same starting point. They further showed that in many Japanese cicada species the fragmenting cicada endosymbiont has been replaced by a (previously pathogenic) fungus. These papers support the hypothesis that genome fragmentation among endosymbionts is non-adaptive, and perhaps even maladaptive, for the cicada host. John's work therefore provides compelling evidence that evolution does not invariably improve species, and discredits the intuition that "all is for the best in the best of all possible worlds."

Vaughn Cooper and his students at Pittsburgh study how bacterial growth in biofilms can generate diversity and multicellular behavior, and have shown that the incidence of each critically depends upon available resources, intra- and inter-specific competition, and the mutation rate. Georgia Tech Co-Is Will Ratcliff and Matthew Herron are also concerned with how primitive multicellularity arises from unicellular ancestors, and in 2018 reported a number of important findings related to this issue. Together, they developed a theory demonstrating that heritability of collective-level traits is often high, even in the early stages of a major evolutionary transition. Herron further showed that multicellularity can drive the evolution of anisogamy, which in turn sets the stage for the evolution of sexual dimorphism.

The evolution of multicellularity set the stage for an incredible increase in the diversity, size and complexity of life on Earth, especially among plants, animals, and fungi. The success of these complex organisms depends on their internal diversity of cell types and their ability to assemble these varied pieces into coherent bodies. However, nascent multicellular organisms lack the regulatory networks that guide development in complex organisms, leaving them vulnerable to stochastic effects. Ratcliff and his collaborators reported in Nature Physics a surprising, previously uninvestigated roadblock to multicellular complexity: mechanical stress from stochastic growth can break intercellular bonds, thus fracturing multicellular groups (Jacobeen *et al.* 2018). These internal and external forces represent new evolutionary challenges, as they act on length scales too long to be relevant for single cells. Thus, the evolutionary origins of multicellularity require biophysical innovation alongside biological innovation, making this process as much about physics as it is about biology.

In conclusion, whether measured as scholarly output, media coverage, invited seminars, synergistic activities, leadership, or research progress, the Reliving the Past team enjoyed a highly successful Year 4. We expect to achieve even more in our final year, and leave the NAI program with a legacy of achievement in the fields of experimental and comparative genomics sure to advance understanding of the early–and continuing–evolution of complex life on Earth.



A multicellular 'snowflake yeast', evolved in the lab from a single-celled ancestor, provides NAI team members with a unique opportunity to directly explore the origin of complex life. Credit: Will Ratcliff, Georgia Tech

## Project Reports

### Shelley Copley, University of Colorado

### Gene Duplication and Divergence: The Bigger Picture

New enzymes often evolve by gene duplication and divergence from promiscuous activities of enzymes that normally serve other functions. Previous studies have focused on the gene undergoing divergence, but have not considered how mutations elsewhere in the genome might contribute to fitness when a new enzyme is needed. We are examining the interplay of various types of mutations during evolution of an enzyme to replace ArgC, which is required for arginine synthesis in *E. coli*. ProA, which is involved in proline synthesis, has a very inefficient promiscuous ArgC activity. A mutation that changes Glu383 to Ala provides enough ArgC activity to support slow growth on glucose. (We refer to E383A ProA as ProA*.)

We evolved 8 cultures of Δ*argC proA* E. coli* that also had a previously identified promoter mutation in glucose + proline to select for cells with improved arginine synthesis. *proA** amplified up to 20-fold in each population (Fig. 1). In population 3, copy number declined to two as a consequence of a mutation that changes Phe372 to Leu and increases ArgC activity by 3.6-fold relative to ProA*.

Notably, fitness increased substantially even in the absence of a *proA** mutation. We identified genes that were mutated in multiple populations. Mutations upstream of *argB* increase production of ArgB, which generates the substrate for ProA* (Fig. 2), by 3-8-fold. Increasing ArgB levels likely increases the concentration of the substrate for ProA* and pushes material through this slow step in arginine synthesis. Eight mutations were observed in carbamoyl phosphate synthase, which produces an intermediate in arginine and pyrimidine biosynthesis. One mutation increases catalytic activity; the others either decrease inhibition by UMP or increase activation by ornithine (Figs. 2 and 3). Each mutation should increase enzymatic activity in cells. Increasing the level of carbamoyl phosphate should help sweep ornithine, which may be in short supply due to the inefficiency of ProA*, through to citrulline and thereby enhance arginine production.

These findings demonstrate that the process of evolution of a new enzyme by gene duplication and divergence is inextricably intertwined with mutations elsewhere in the genome that improve fitness by other mechanisms.

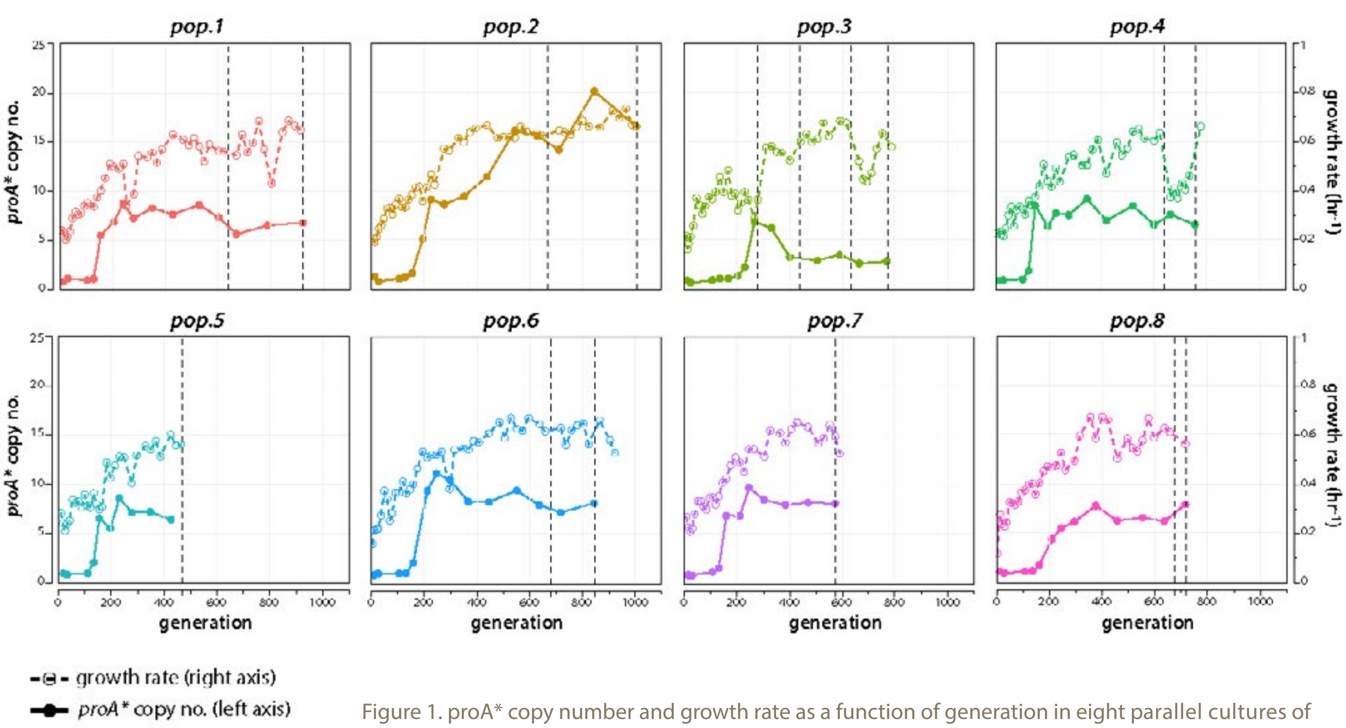



Figure 1. proA* copy number and growth rate as a function of generation in eight parallel cultures of ΔargC proA* *E. coli* evolved in a turbidostat. Dashed vertical lines indicate points at which samples for population genome sequencing were taken. Credit: Shelley Copley, University of Colorado

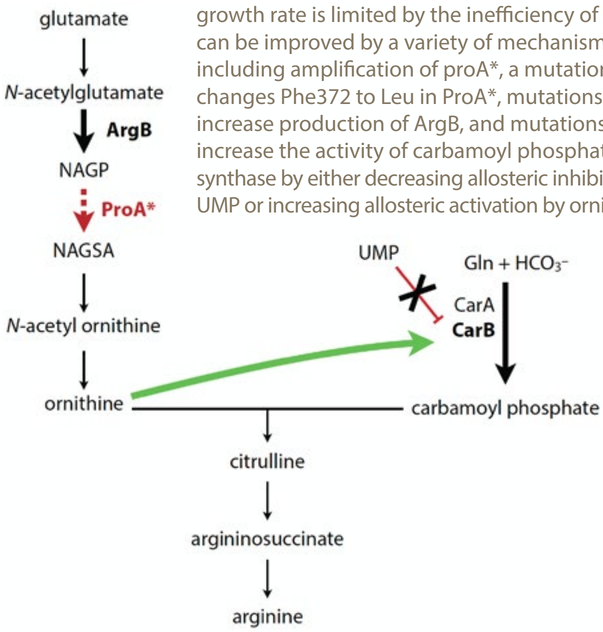



Figure 2. Fitness of ΔargC proA* *E. coli*, whose growth rate is limited by the inefficiency of ProA*, can be improved by a variety of mechanisms, including amplification of proA*, a mutation that changes Phe372 to Leu in ProA*, mutations that increase production of ArgB, and mutations that increase the activity of carbamoyl phosphate synthase by either decreasing allosteric inhibition by UMP or increasing allosteric activation by ornithine.

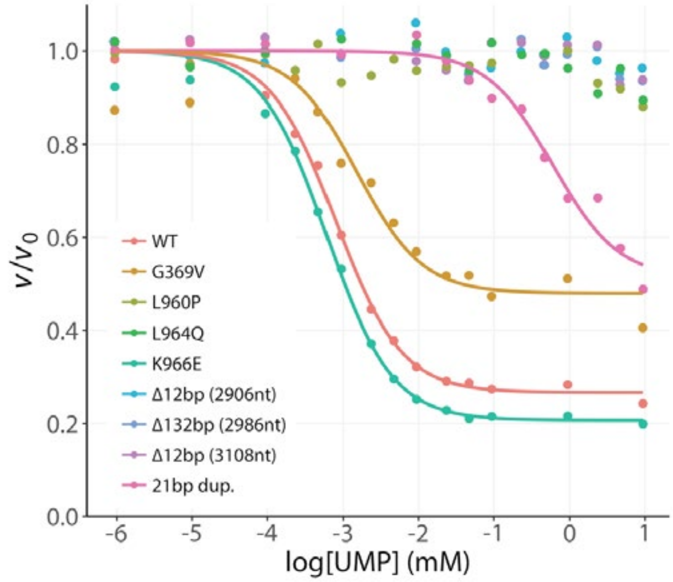



Figure 3. Seven mutations observed in evolved strains of ΔargC proA* *E. coli* decrease inhibition of carbamoyl phosphate synthase by UMP. Credit: Shelley Copley, University of Colorado

## Gavin Sherlock, Stanford University

### Contingency and the Rate of Evolutionary Change

We are investigating the effect of *historical contingency,* investigating how existing mutations constrain and/or affect future evolution. We selected 3 adaptive lineages, each of which have a single beneficial mutation, affecting either the Ras/Protein Kinase A signaling pathway, or the Tor signaling pathway. We lineage-tagged these strains, to track the emergence of beneficial mutations, and evolved them for 168 generations. We found that the rate at which adaptive individuals increase in frequency within the population is lower for the Ras pathway mutants, compared to the Tor pathway mutant. Both Ras pathway mutants have a higher fitness than the Tor pathway mutant (compared to wild-type), suggesting that higher fitness mutants adapt more slowly when they are subsequently evolved. We also tracked the rate at which diploids arise in the populations, and find that a substantial fraction of the adapted individuals in each population (likely the vast majority) are more fit due to diploidy. We have selected haploid clones from these evolution trials for whole genome sequencing. In adaptive clones derived from a founding lineage with a gain of function mutation in adenylate cyclase (*CYR1*), we observed three independent missense mutations in the gene that encodes gamma glutamylcysteine synthetase (*GSH1*), which is the first step of glutathione synthesis. We hypothesize that these are gain-of-function mutations, which would result in more glutathione, leading to greater protection from damage from oxidative stress. We are sequencing ~70 adaptive clones from the other two founding genotypes to determine whether they also have mutations in *GSH1*, or whether

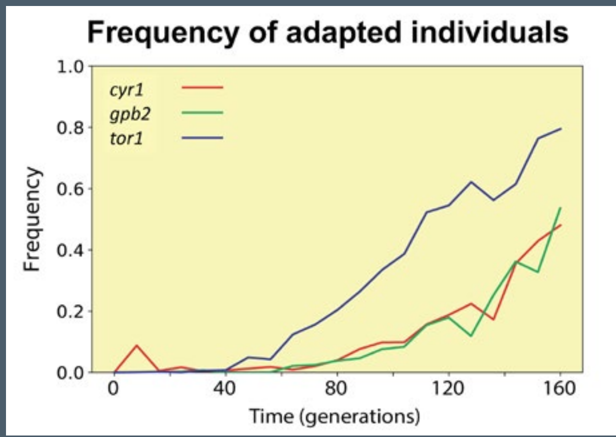



Figure 4. The fraction of adaptive individuals, as determined by lineage tracking, for second step evolution of founder clones already containing a single adaptive mutation. Credit: Gavin Sherlock, Stanford University

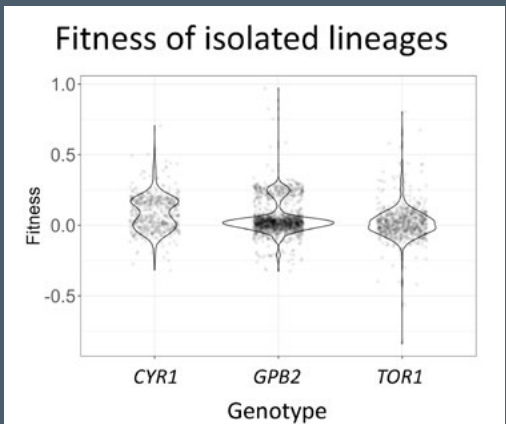



Figure 5. The figure shows the fraction of the population that experiences adaptive increase over time, for each of the founding genotypes. By the end of the evolution trial, ~80% of the tor1 founded population is adaptive, while ~60% of the cyr1 and gpb2 population is adaptive. Credit: Gavin Sherlock, Stanford University

they have taken different evolutionary paths. Finally, we have remeasured fitness for the isolated clones, and are in the process of completing an additional replicate of those fitness measurements. The initial data suggest that GPB2-derived clones have larger fitness gains than for the other mutant backgrounds.

## Paul Sniegowski, University of Pennsylvania

### The Evolution of Evolution: How Variability in Repair and Recombination Control the Pace and Path of Adaptation

The Sniegowski group continues to advance our understanding of how mutation rate, as well as specific types of mutation control the pace of evolutionary change and the options open to that process. In all species, "mutation rate" is a balance between how often and how seriously DNA is damaged, and how often and how well it is repaired. Using the bacterium *E. coli*, Paul's team, working with Kathleen Sprouffske and others, demonstrated that high mutation rates limit the rate of evolution (Sprouffske *et al.* 2018); then, in follow-up work with Eugene Raynes and Daniel Weinreich, they explored the effects of population size and migration on the success of mutator alleles (Raynes *et al*. 2018; Raynes *et al*. in press). Theory developed by NAI Co-I Philip Gerrish and Sniegowski graduate student Ben Galeota-Sprung, reveals that natural selection alone produces conditions that favor genetic recombination in finite populations (Gerrish *et al.*, in prep). Using the yeast *S. cerevisiae*, Ben has also analyzed the contributions of lethal and highly deleterious mutations to the selective cost of mismatch repair deficiency (ΔMSH2). Earlier efforts failed to capture the contributions of lethal and highly deleterious mutations to the mutator fitness. Ben took a two-pronged approach to account for these contributions. First, using direct microscopic observation of individual yeast cells (Figure 6), he estimated the difference in lethal mutation rates in wild-type and mutator yeast strains. Second, using high-through put analyses of competitive fitness in large numbers of random clones isolated from evolving experimental yeast populations, he estimated the contributions of highly deleterious mutations to the fitness of mutators. Paul's team has therefore been able to show that the combined effects of lethal and highly deleterious mutations are sufficient to account for the immediate selective cost of mutators (Figure 7; Galeota-Sprung, in prep).

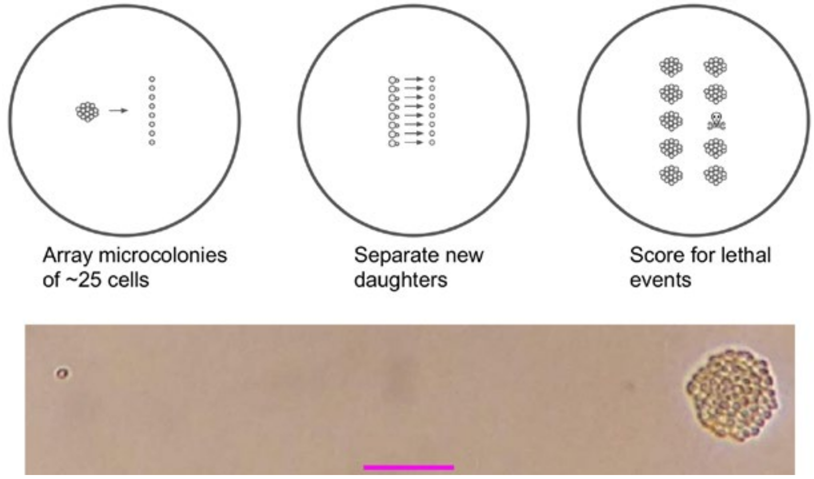



Figure 6. Direct observation and quantification of lethal mutations in yeast (*Saccharomyces cerevisiae*). Individual cells are arrayed on an agar plate using a micromanipulator. Daughter cells are separated from mother cells; success of mother and daughter cells in dividing and forming micro-colonies is observed directly in wild-type and mutator (ΔMSH2) backgrounds. The photograph shows a representative example of a lethal mutation, with the cell on the left failing to form a microcolony after separation. Scale bar = 40 µm. Credit: Paul Sniegowski, University of Pennsylvania

| | Wild-type | | Mutator | | |
|---|---|---|---|---|---|
| | Count | Freq | Count | Freq | p-val |
| Both OK | 2235 | | 2145 | | |
| Lethal | 14 | 0.0062 | 33 | 0.015 | 0.005 |

Difference: 0.9%

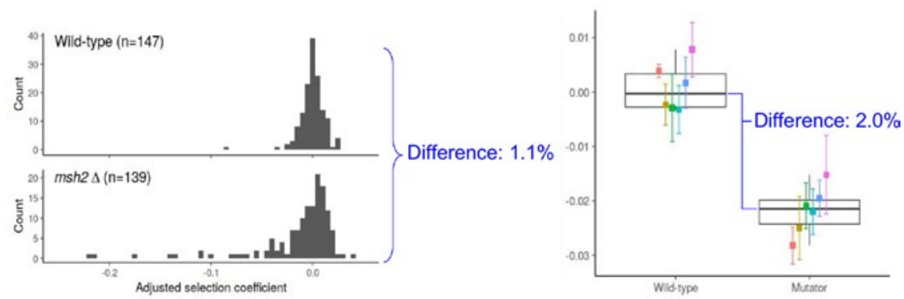



Figure 7. Contributions of lethal (top panel; estimated as in Figure 1) and highly deleterious mutations (left panel) summed together are sufficient to account for the previously observed immediate fitness cost of ΔMSH2 mutators (right panel) in yeast. Effects of highly deleterious mutations were quantified using high-throughput fitness assays competing random clones from a newly founded evolving population against a fluorescently labeled reference strain. Graphs compare wild-type and ΔMSH2 yeast and show that the fitness contributions of lethal (0.9% difference) and sublethal (1.1% difference) mutations summed account for the fitness difference of 2% observed between wild-type and mutator strains. Credit: Paul Sniegowski, University of Pennsylvania

**Outreach**: As dean of Penn's undergraduate College of Arts and Sciences, Sniegowski oversees the College's curricular and advising outreach efforts, in the Penn First Plus program, which began in 2018, and is dedicated to recruitment and success of first-generation and URM students across the university (See https://penntoday.upenn.edu/news/penn-first-plus-expands-programs-and-support-first-generation-students).

## Betul Kacar, University of Arizona

### Reconstruction of Ancestral Metabolic Enzymes: Insights from a Precambrian Metalloprotein

The study of earliest life requires a synthesis of varying lines of evidence to constrain the timing and relationships between environment, organisms, and the molecular catalysts that drive biogeochemical cycles. Geochemical lines of evidence, in the form of isotopic signatures consistent with biological cycling, are scarce in rocks older than 3.5 Ga, and are not unambiguously biogenic. Geological evidence that constrains environmental parameters (e.g., temperature, atmospheric content) thus play an important role in understanding the environmental capacity for and drivers of biological evolution. The relationship between trace metal availability and metalloenzyme evolution has been similarly investigated, for example by tracking the diversification of metal-binding protein phenotypes over geologic timescales. These investigations open up the question as to whether our model of the early Earth environment is resolved enough to make inferences about causative relationships to biological evolution on organismal and molecular scales, whether these connections can be used to make reasonable estimates of timing and calibration. Nitrogenase (NifHDK protein), a metalloprotein, is just one such example of a significant biological innovation, whose evolution may have been modulated by the changing geochemical environment over geologic timescales. To further evaluate ancient nitrogenase function and metal-binding behavior, we reconstructed ancestral sequences of the nitrogenase NifHDK protein subunits and modeled the structural active site pocket volume of inferred ancestors, previously suggested to correlate with differential metal binding. We reconstructed a maximum likelihood NifHDK phylogeny from 284 modern nitrogenase protein sequences, including 28 alternative nitrogenases and 16 uncharacterized nitrogenases, the latter for which metal-binding and precise functional character have not been experimentally determined. Maximum likelihood ancestral sequences were inferred for well-supported ancestral nodes within this lineage across five evolutionary models. In addition, 100 Bayesian sequence variants were randomly sampled from the site posterior to create a probability distributions of each maximum likelihood ancestor. Structural homology models for each of 5,045 inferred ancestral sequences and 66 representative modern sequences were then generated to calculate active site pocket volumes. We find that, though modern sequence features are well correlated with distinct evolutionary clades, pocket volume is not strongly associated with the metal dependency of modern nitrogenases (Figure 8). Our phylogenetic and structural analyses suggest that ancestors of uncharacterized and alternative nitrogenases may not have bound a Mo-cofactor.

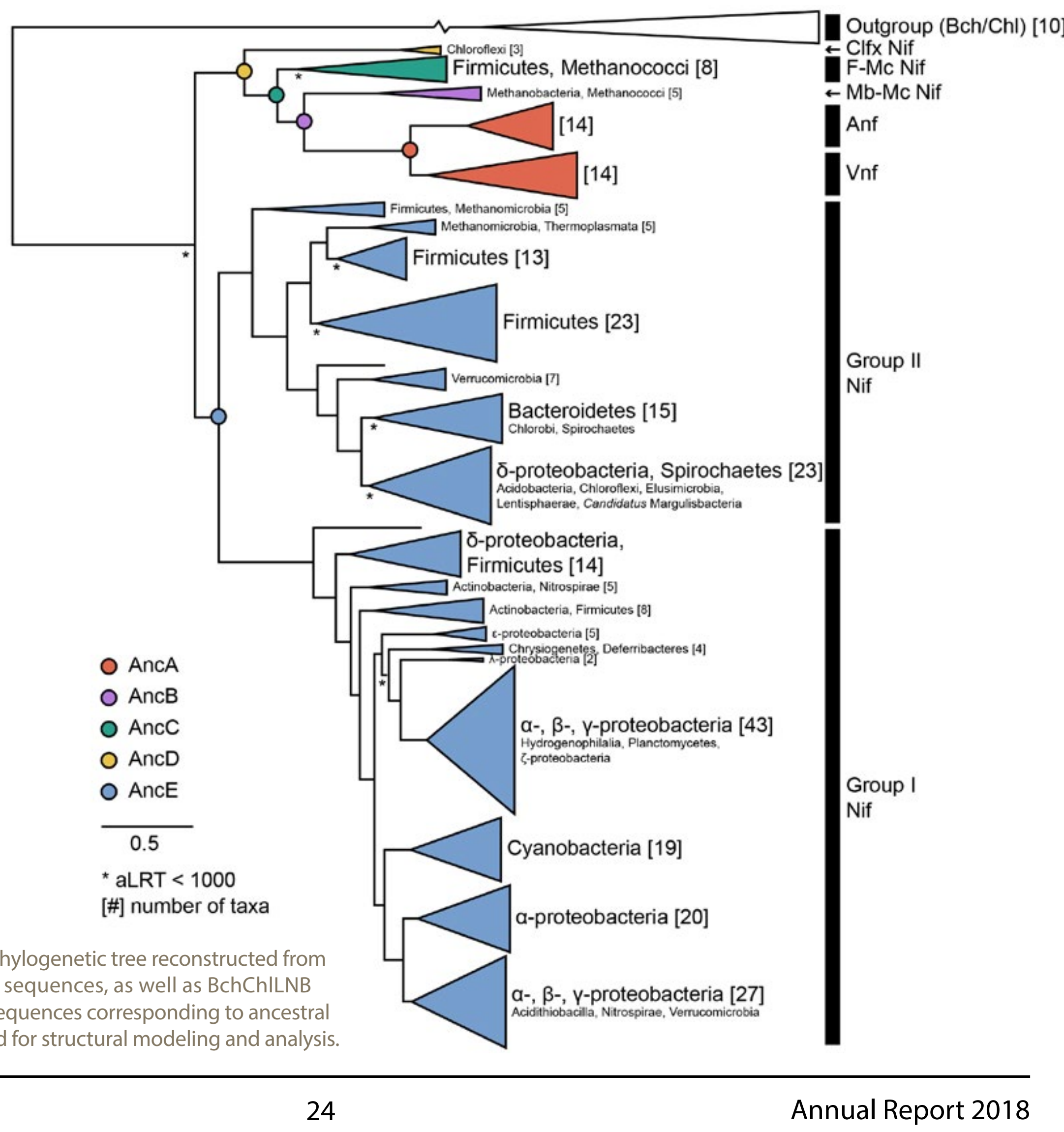



Figure 8. Maximum likelihood phylogenetic tree reconstructed from concatenated Nif/Anf/VnfHDK sequences, as well as BchChlLNB outgroup sequences. Inferred sequences corresponding to ancestral nodes AncA-AncE were targeted for structural modeling and analysis.

## Matthew Herron, Georgia Institute of Technology

### De Novo Origins of Multicellularity in the Green Alga, *Chlamydomonas reinihardtii*

The Herron lab published five peer-reviewed papers in 2018 with two others in review or in revision. Co-I Herron presented two invited talks and became a Review Editor for *Frontiers in Plant Science*. Experimental work in the Herron lab showed that the genetic basis for multicellularity in experimentally-evolved *Chlamydomonas reinhardtii* has both lineage-specific and shared features, and that the shared features have more in common with *C. reinhardtii's* relatives among the volvocine algae than with other multicellular green algae or land plants (Fig. 9 from Herron *et al.* 2018). Comparative work in collaboration with Erik Hanschen at the University of Arizona showed that anisogamy, which sets the stage for the evolution of sexual dimorphism, evolved multiple times within the volvocine green algae and is likely driven by the evolution of multicellularity (Hanschen *et al.* 2018). Theoretical work showed that the heritability of collective-level traits is often high even early in a major evolutionary transition, with important implications not only for major transitions but for multi-level selection in general (Fig. 10 from Herron *et al.*, in press). NAI Postdoc Kimberly Chen has made progress toward understanding the genetics underlying the predator-driven transition to a multicellular life cycle in experimentally-evolved *C. reinhardtii*. Postdoc Pedram Samani is finishing an experiment exploring the evolutionary origins of males and females from ancestors lacking distinct sexes. Graduate student Jacob Boswell designed and carried out an experiment testing a new method of cryopreservation for *C. reinhardtii*, and has a first-authored manuscript in review describing this research. Mr. Boswell has graduated from Georgia Tech with a master's degree in bioinformatics. Undergraduate Margrethe Boyd designed and carried out experiments on the motility of experimentally-evolved multicellular *C. reinhardtii* and has published her results in a first-authored paper in *PLoS ONE*. Ms. Boyd is now pursuing her Ph.D. in Biomolecular Engineering at Northwestern University.

| p-values | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3 hours | <0.001 | <0.001 | NS | NS | NS | NS | NS | NS |
| 6 hours | 0.009 | NS | 0.016 | NS | NS | NS | NS | NS |
| 9 hours | NS | 0.003 | NS | NS | NS | NS | NS | NS |
| 12 hours | <0.001 | <0.001 | NS | NS | NS | NS | NS | NS |
| 48 hours | <0.001 | 0.001 | NS | NS | NS | NS | NS | NS |

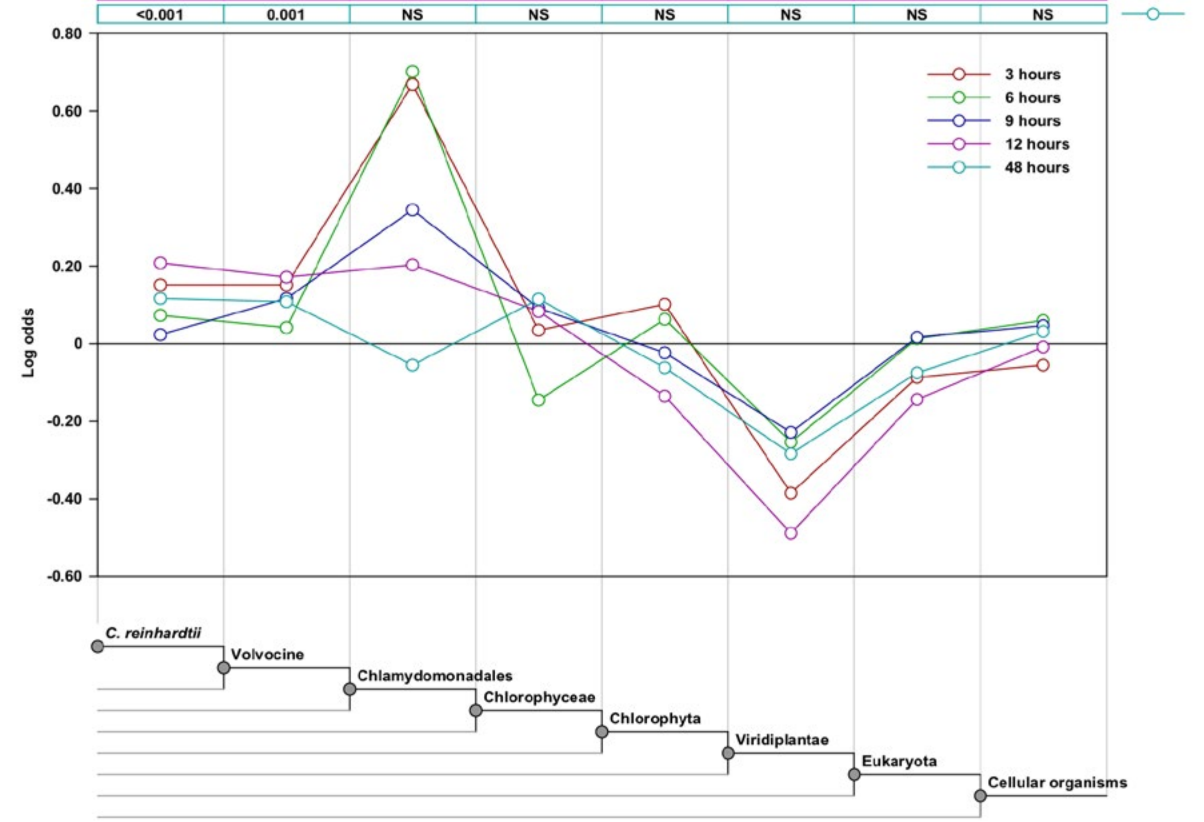



Figure 9. Results of phylostratigraphy analysis of genes differentially expressed between unicellular and multicellular C. reinhardtii. The y-axis represents the log odds of the observed degree of over/underrepresentation relative to genome-wide frequencies. Bonferroni-corrected p-values result from a hypergeometric test (alpha = 0.0025, equivalent to a false discovery rate of 1%) performed in GeneMerge v.1.4. "NS" = not significant (Fig. 3 in Herron *et al*. 2018 R. Soc. Open Sci. 5, 180912.).

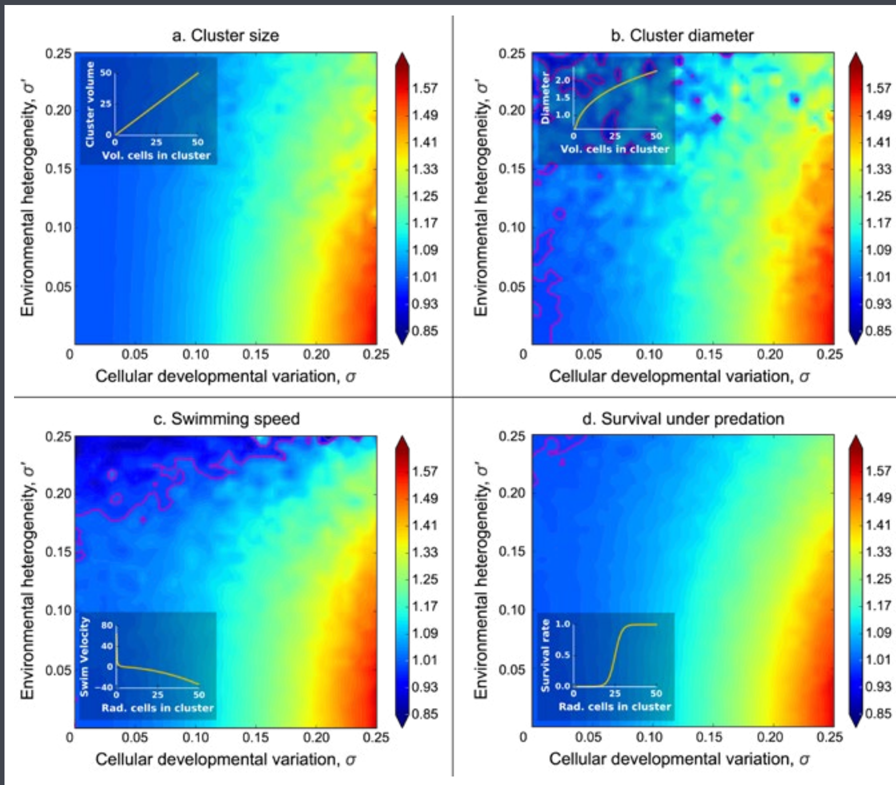
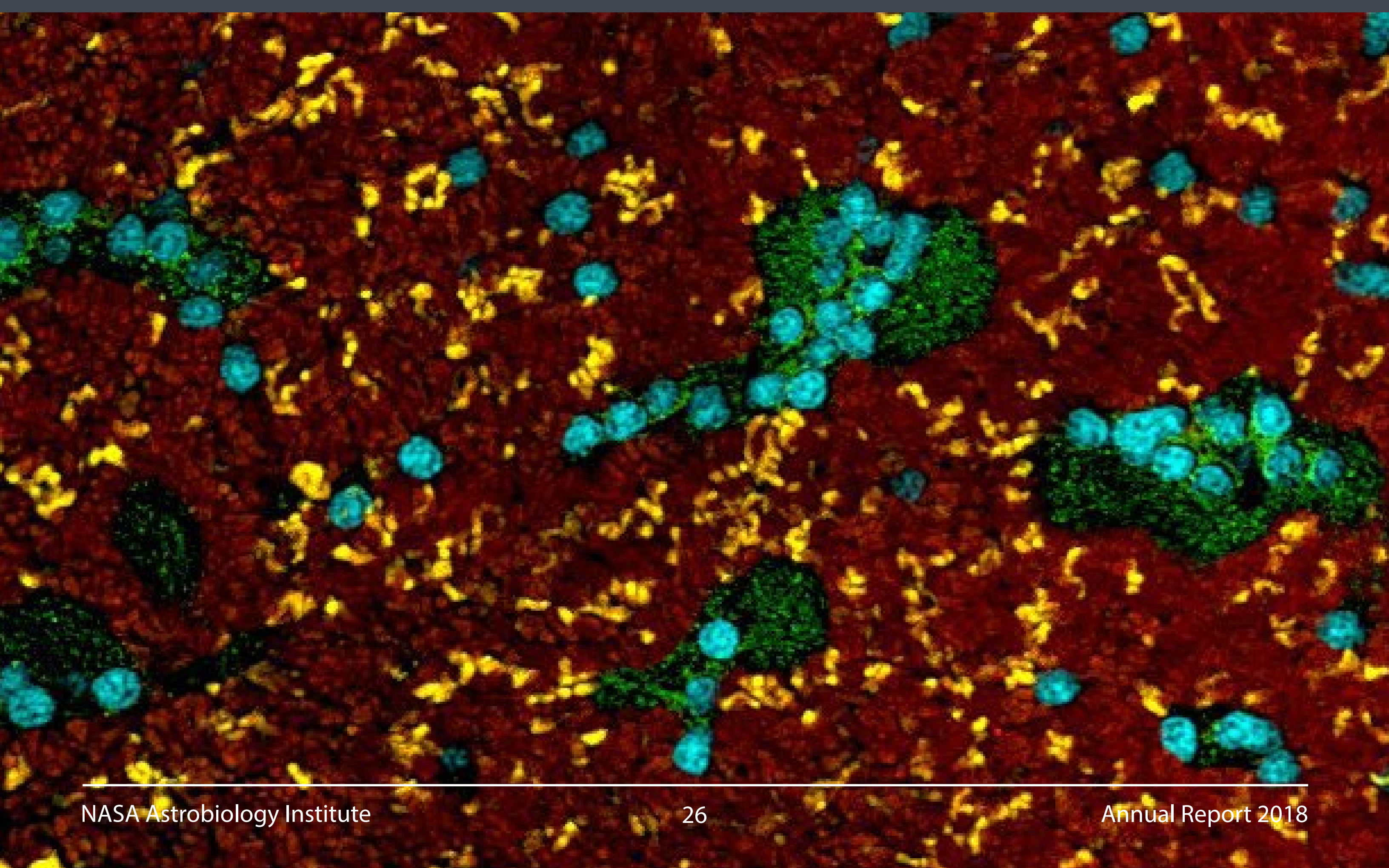



Figure 10. Relative heritability of various collective-level traits to cell-level heritability for size. Here we examine the heritability of four multicellular traits that depend on the size of their constituent cells, relative to cellular heritability for size. The relationship between the size of the cells within collectives and the multicellular trait are shown as insets. We consider three biologically-significant traits with different functions mapping the size of cells within the collective onto collective phenotype. The heritability of collective size (a) and diameter (b) is always higher than cell-level heritability for size, and is maximized when cellular developmental noise is greatest and among-collective environmental effects are smallest (lower right corner). We modeled swimming speed (c) for volvocine green algae. We also considered survival rate under predation as a logistic function of radius (d). Like a and b, collective-level heritability is highest relative to cell-level heritability when environmental heterogeneity is minimal. Pink contours denote relative heritability of 1. In these simulations, we considered 32 cell collectives grown for 7 generations. The colormap denotes collective-level heritability divided by cell-level heritability for size across 1024 σ, σ' combinations (from Herron *et al.* BMC Biol In Press).



# NPP Project Reports

**Caroline Turner, University Pittsburgh;**
**Prof. Vaughn Cooper, Faculty mentor**

## Diversification and Evolution of Synergy in Microbial Biofilms

A fundamental question in evolution is the degree of repeatability of evolution. Recent work has shown that replicate populations of initially identical bacteria evolved in the same environment accumulate mutations in many of the same genes across populations. My work focuses on the repeatability of evolution across different environments, particularly focusing on the evolution of biofilm formation. I have conducted evolution experiments with a factorial design of high- and low-carbon conditions and selection for either planktonic or biofilm modes of growth. In 2018, I published a first-author paper on this experiment in Evolution Letters. We observed higher levels of genetic similarity (as measured by the Bray-Curtis similarity index) between populations that evolved in more similar environments. That paper focused on a single time point, at the end of the experiment. I am now studying how genetic similarity changes over time, using several approaches. I have sequenced samples from additional time points and am examining trajectories of genetic similarity between populations over time. I have also begun a collaboration with Eric Libby, Assistant Professor at Umea University and member of NASA's Laboratory for Agnostic Biosignatures, to model expected patterns in similarity over time, given different distributions of beneficial fitness effects. I am also expanding my analysis of similarity over time to a 60,000-generation evolution experiment with *E. coli*, for which populations have already been sequenced.

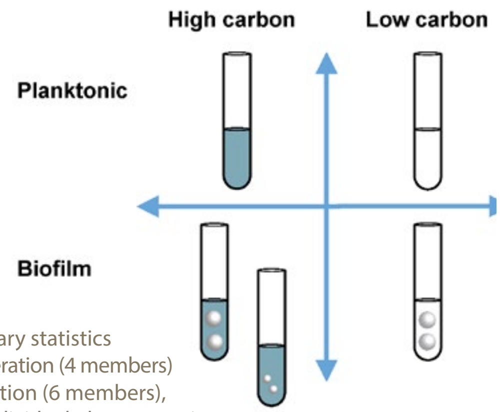



Fig. 11. Set-valued summary statistics between an ancestor generation (4 members) and a descendant generation (6 members), which take the place of individual phenotypes in the set-generalized Price Equation. Fitness is defined between ancestor individuals (dots). Summary statistics for more general correlations can be defined for pairs (links), triples (faces), or higher-ordered sets.

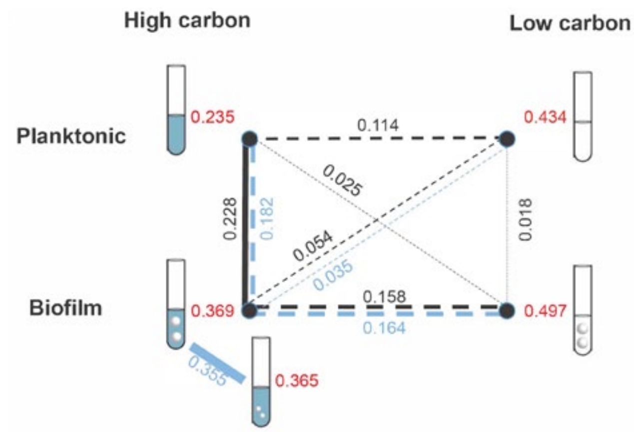



Figure 12. Bray-Curtis similarity was significantly higher within (red) than between (black/blue) treatments. Dashed lines indicate pairs of treatments for which similarity between treatments was significantly lower than similarity within treatments. Between-treatment similarity was significantly higher for pairs of treatments with a shared environmental variable (edges of rectangle) than for pairs that did not share an environmental variable (diagonals of rectangle). Blue lines and numbers indicate comparisons involving the high-carbon, biofilm treatment with small beads. Black lines are used for all other treatment pairs. Credit: Caroline Turner, University of Pittsburgh

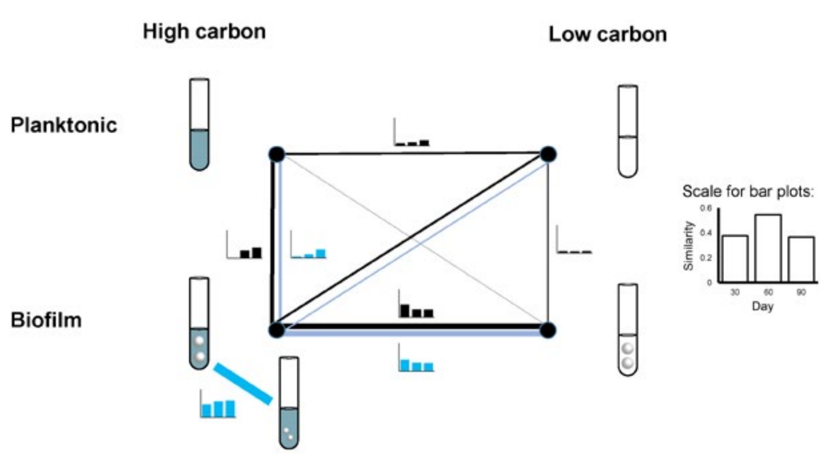



Figure 13: Bray-Curtis similarity over time between populations evolved under similar and dissimilar environmental conditions. Credit: Caroline Turner, University of Pittsburgh

**Peter Conlin, Georgia Tech;**
**Prof. Will Ratcliff, Faculty Mentor**

### Experimentally Investigating the Origin and Consequences of Fitness Decoupling During the Transition to Multicellularity

During the transition to multicellularity, cells evolve from individual organisms in their own right into parts of a new higher-level organism. A hallmark of this process is the evolution of traits that improve fitness in a multicellular context, but are costly in a unicellular context ('fitness decoupling' traits). Our work directly investigates fitness decoupling in a nascent multicellular yeast model system. Preliminary data obtained by reverting multicellular 'snowflake' yeast from various time points back to unicellularity suggests that the longer snowflake yeast evolve as clusters, the lower their single-celled fitness after reversion, a hallmark of fitness decoupling

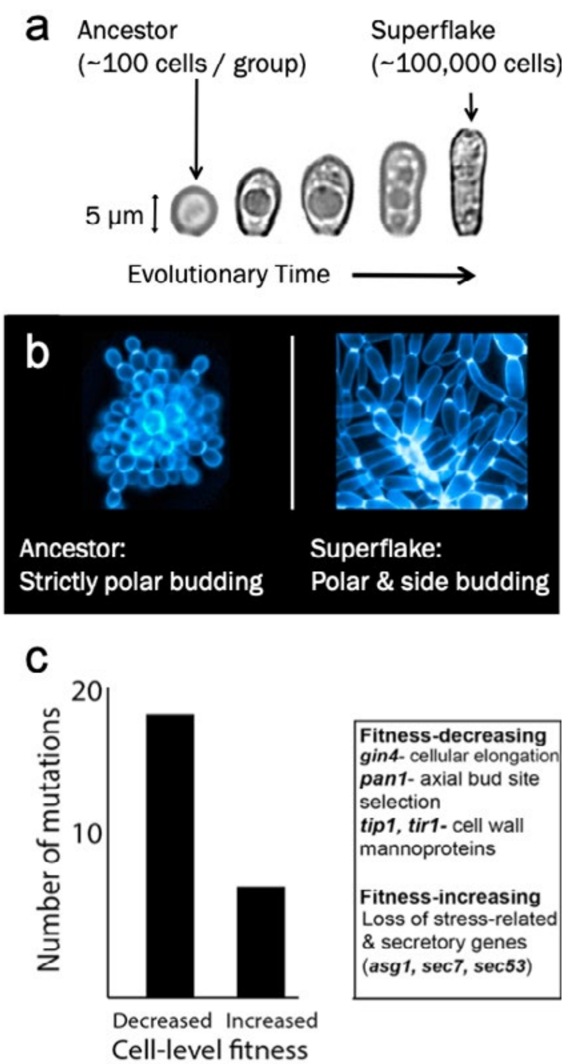



Figure 15. Cellular innovations of superflakes and predicted mutational effects on unicellular fitness. a) Superflakes evolve more elongate cells and b) changes in budding angle from strictly polar to both polar and side budding. c) More than twice the number of mutations found in evolved superflakes result in decreased fitness according to annotations on the Saccharomyces Genome Database. Credit: Peter Conlin

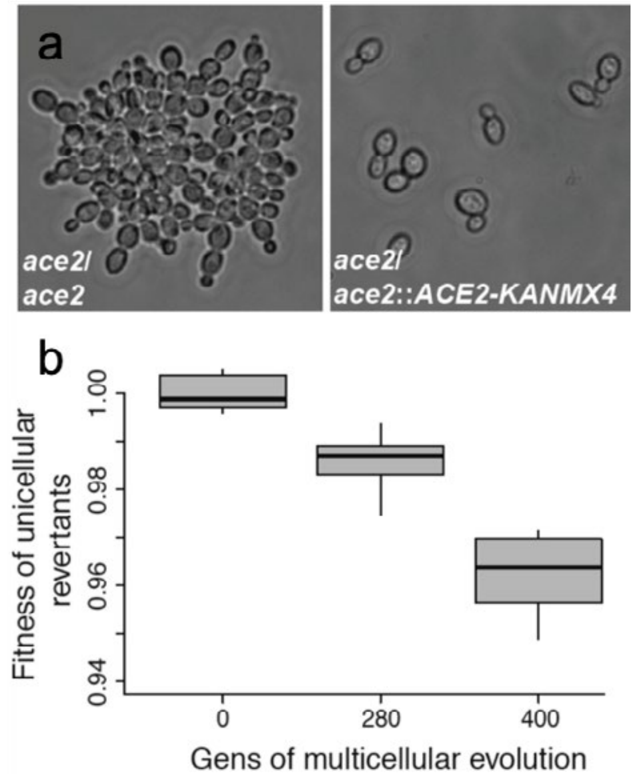



Figure 14. Snowflake yeast genetics. a) Unicellularity is restored via functional complementation with the ancestral ACE2 allele. b) Preliminary evidence of fitness decoupling. The longer snowflake yeast have been evolving, the lower the fitness of unicellular revertants compared to the unicellular ancestor Y55. Credit: Peter Conlin, Georgia Tech

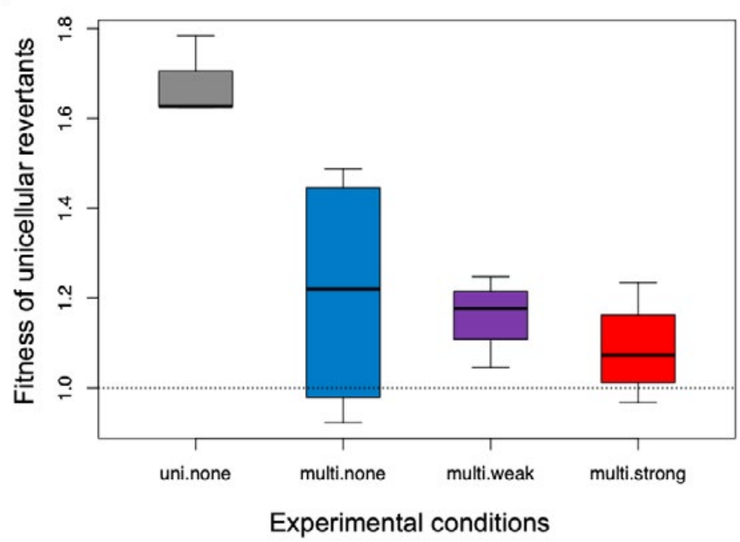


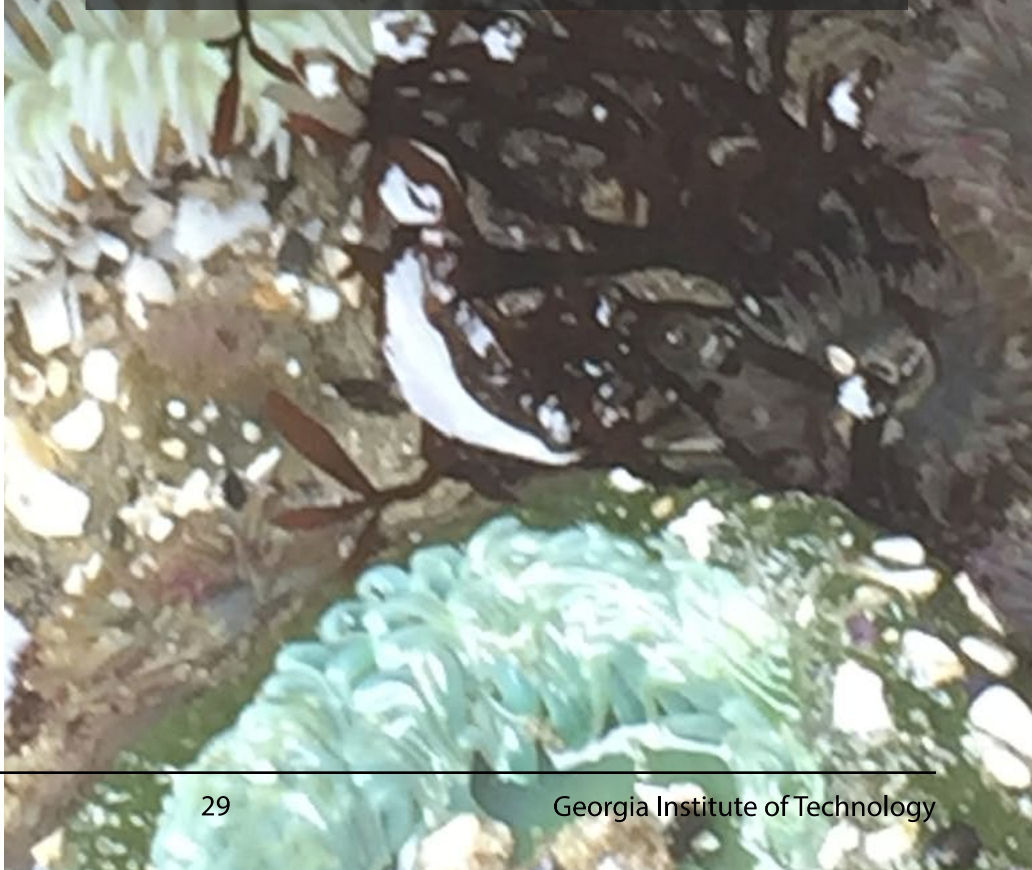

Figure 16. Relative fitness of unicellular revertants. The relative fitness of unicellular revertants is lower than that of the unicellular control, regardless of selection strength. However, average revertant fitness decreases as strength of selection increases. (Data shown for only 4/15 evolved isolates per treatment.) Credit: Peter Conlin

(Figure 14). The first aim of my current research is to determine the genetic basis of two putative decoupling traits (elevated rates of apoptosis and increased cell size), to engineer the causal mutations into unicellular and multicellular contexts, and to measure their fitness effects. To facilitate this, we have constructed stable haploid lines of our ancestral yeast strain to enable backcrossing experiments. Functional annotations of mutations from a separate long-term evolution experiment with snowflake yeast suggest that many more decoupling mutations wait to be discovered (Figure 15). We performed a divergent selection experiment to identify general evolutionary trends and establish the causal basis of fitness decoupling in our system. We found that the strength of selection was negatively correlated with unicellular revertant fitness (Figure 16), but the results are confounded by differences in effective population size. To address this, we developed a network-based simulation model to help understand snowflake yeast population genetics. Next, we will use high-throughput genetic screens to identify the genome-wide spectrum of decoupling mutations. A barcoded plasmid library encoding nearly all the open reading frames in the yeast genome (MoBY-ORF) will be used to mimic gene amplifications and a transposon mutagenesis screen (SATAY) will be used to mimic loss-of-function mutations.

# Field Sites

### California and Oregon (Scott Miller)

Understanding both where new genes come from and the origins of organismal complexity are key goals of astrobiology. Gene duplication is an ancient mechanism that was central to both of these processes during the early evolution of life on Earth. The cyanobacterium Acaryochloris exhibits extraordinary gene duplication dynamics, and we are using this organism to develop a better understanding of the role of gene duplication for the evolution of new functions, complexity, and adaptation to novel environments. Temporal resolution of these dynamics is crucial but currently limited by the poor representation of this group in laboratory culture both within and between populations. To address this need, we have made several targeted field collections along the U. S. Pacific coast, and we are now growing multiple novel *Acaryochloris* strains collected from intertidal populations from California (Shelter Cove) and Oregon (Hug Point). *Acaryochloris* is also unique in its use of the far-red light absorbing Chlorophyll d as its primary pigment in oxygenic photosynthesis. Because the absorptive properties of this novel chlorophyll match the emission of red dwarf (M) stars, it is being used as a model by the VPL NAI (Nancy Kiang, Bob Blankenship and other team members) for understanding the potential long wavelength limits of extrasolar oxygenic photosynthesis. Understanding the diversity of far-red photosynthesis within *Acaryochloris* is essential for this project, and our field work synergistically complements similar efforts to culture *Acaryochloris* from different field sites by the VPL team (Gallagher and Miller, 2018. *Genome Biology Evolution* 6, 1484-1492. https://doi.org/10.1093/gbe/evy099).



**Southeast and Southwest USA and Chile (John McCutcheon)**

Endosymbiosis—the process by which one organism takes up residence in a host cell—is a key driver of organismal complexity. Both the mitochondrion and chloroplast were once free-living bacteria, and their establishment as endosymbiotic organelles played a critical role in the origin of all eukaryotic life. The importance of endosymbiosis is reflected in its inclusion in some of the goals of the NASA astrobiology program. But the ancient nature of organelles makes understanding the process leading to endosymbiosis difficult. Our lab uses a number of insect models to study how bacteria become endosymbionts, and how these processes are both similar and different to those experienced by the classic cellular organelles. These insects have very long term (10-200+ million year) relationships with bacterial endosymbionts that provide key nutrients to their hosts. We use the globally distributed singing cicadas as one of our models, because some of their endosymbionts show patterns of genome instability similar to some mitochondrial genomes. We have active field sites of cicadas from the southwestern and southeastern United States, as well as Chile, because these populations span the range of endosymbiont genome complexity in the insect group. This work, while ongoing, has already provided fundamental insight into the process of endosymbiont genome evolution (Lukasik *et al*., 2018, *PNAS* 115:E226-E235; Łukasik P *et al.* 2018. *J Heredity*, esy068, https://doi.org/10.1093/jhered/esy068).

**Pilot Valley Basin, Utah, USA (Kennda Lynch)**

On Earth, hypersaline ecosystems are known for harboring diverse microbial communities comprised of all three domains of life, yielding novel species of microorganisms, and bearing a breadth of metabolic diversity. From an astrobiological perspective, hypersaline soils/sediments are considered among the prime targets for analog habitability studies on Mars. In particular, ground-water connectivity to lacustrine environments during climate transition is an important factor in the hydrological history of Mars, yet there has been very little research of specifically relevant analog environments. The Pilot Valley basin in northwestern Utah is a hypersaline, groundwater-fed paleolake basin that contains a complex mixture of many of the hydrated minerals detected on Mars. Hence, it is an excellent model for end-stage, near-subsurface habitable zones during Mars' transition from wet to dry. Many putative martian paleolakes could have formed from groundwater upwelling and/or transitioned back to groundwater-dominated systems in the mid-to-late Hesperian/Early Amazonian. Our work in the Pilot Valley Basin, Utah, allows us to research this analog environment to understand the habitability and biosignature preservation potential of end-stage, near-subsurface habitable zones within martian paleolake basins. Our work may also have implications for habitability and detection of near-subsurface extant life. (Lynch, KL *et al*. 2019, A*strobiology*).

## Reliving the Past: 2018 Publications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Herron, M. D., Borin, J. M., Boswell, J. C., Walker, J., Chen, I-C. K., Knox, C. A., Boyd, M., Rosenzweig, F., Ratcliff, W. C. (2019). De novo origins of multicellularity in response to predation. *Scientific Reports*. DOI: 10.1038/s41598-019-39558-8

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Łukasik, P., Nazario, K., Van Leuven, J. T., Campbell, M. A., Meyer, M., Michalik, A., Pessacq, P., Simon, C., Veloso, C., McCutcheon, J. P. (2018). Multiple origins of interdependent endosymbiotic complexes in a genus of cicadas. *Proceedings of the National Academy of Sciences USA* 115(2): E226-E235. DOI: 10.1073/pnas.1712321115

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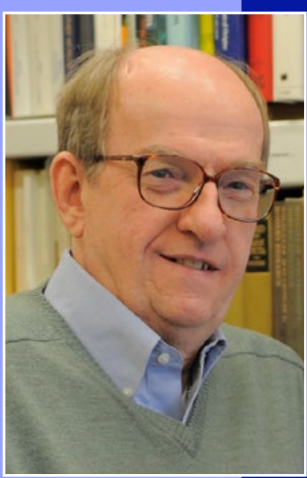
# Origin and Evolution of Organics and Water in Planetary Systems

**Lead Institution:**
**NASA Goddard Space Flight Center**



**Principal Investigator:**
Michael Mumma

## Team Overview

The Goddard Team targets the Origin and Evolution of Organics and Water in Planetary Systems, in short – Why is Earth Wet and Alive? We address this central question through an integrated program of (a) pan-spectral astronomical observations of comets, circumstellar disks, and exoplanet environments, (b) models of chemical evolution and dynamical transport in the early Solar System, (c) laboratory studies of extraterrestrial samples, and (d) realistic laboratory and numerical simulations of inaccessible cosmic environments.

Synergistic integration of these areas is essential for testing whether delivery of life's building blocks – exogenous water and prebiotic organics – enabled the emergence and development of the biosphere. As humankind plans searches for life elsewhere in the Solar System, our team develops (e) instrumental protocols to search for life's fundamental molecules – the informational polymers without which "life as we know it" would not exist.

- From Comets and Asteroids to Planets: Organics as a Key Window into Emergent Earth
- Organic Compounds in Authentic Extraterrestrial Materials: The Ultimate Rosetta Stones
- Laboratory Simulations of Formative Processes in Cosmic Ice and Dust Analogues
- From Molecular Cores to the Protoplanetary Disk: Our Interstellar Organic Heritage
- Analytical Protocols for Detection and Diagnosis of Life's Molecular Compounds

**Team Website:** https://astrobiology.gsfc.nasa.gov

## 2018 Executive Summary

Team members pursued a vigorous and highly productive research program in all five topical areas, conducting many investigations in the laboratory and in the field (mainly astronomical).

*What material was delivered to "barren" Earth?* We sampled material in/ from additional primitive bodies identified as plausible "carriers", and established their compositional diversity – including chemical, isotopic, chiral, and nuclear-spin signatures. We quantified volatile composition and isotopic ratios in four comets, and expanded to 34 comets our taxonomy based on composition. The dynamically new comet C/2013 V5 (Oukaimeden) displayed conspicuous changes in mixing ratios on adjacent nights, suggesting a non-homogeneous volatile composition consistent with differential processing of its constituent ices. In depth analysis of 30 comets revealed evidence of a new class of material in the cometary nucleus with astrobiological significance (Mumma *et al.*, in prep.). Compared with 'normally-active' comets in this group, disrupting comets within 1 au of the Sun were found to be enriched in HCN and $NH_3$ relative to ethane, owing to the dissolution of ammoniated salts. C/2017 E4 was the latest disrupting comet to reveal this effect, and the use of a new spectrometer enriched the scientific return substantially. 2018 also saw near-Earth approaches of two ecliptic comets from the Kuiper Belt, 21P/Giacobini-Zinner and 46P/Wirtanen, rare orbital events that provided bright molecular emissions studied by the team.

GCA scientists expanded their leadership of cometary molecular astronomy with the Atacama Large Millimeter/submillimeter Array (ALMA), pioneering a new technique using autocorrelation data to search for weak molecular emissions in our existing cometary database. This new approach effectively uses ALMA as a large single-dish telescope with a subsequently large increase in sensitivity, at the expense of spatial information on the emission. When applied to our archival data for comet C/2012 S1 (ISON), the resulting HCN spectra permitted measurement of the cometary $^{12}C/^{13}C$ ratio in HCN. This technique will allow searches for weak emission from organic molecules in future comets.

Team scientists in the Astrobiology Analytical Laboratory (AAL) continued to investigate the origin and evolution of meteoritic organic compounds. 2018 highlights include exploration of the correlations of meteoritic amino acids with structurally related species and other organic compounds, such as ketones, aldehydes, and carboxylic acids. AAL scientists have key roles in the new Network for Life Detection (NFoLD)/Laboratory for Agnostic Biosignatures (LAB) program and the Center for Life Detection (CLD). They continue to leverage their expertise and facilities to develop novel instrumentation for future spaceflight, with continuing involvement in multiple NASA flight missions and mission proposals.

Did Earth Receive its Water and Pre-biotic Organics from Comets? From Asteroids?
Image credit: NASA/JPL/USGS

*How was prebiotic matter synthesized and processed in the solar nebula, prior to being incorporated into such carriers?* Team scientists in the Cosmic Dust Laboratory (CDL) study the formation of complex hydrocarbons from gas-phase CO via surface-mediated reactions (SMR) with simple gases ($N_2$ and $H_2$) on almost any grain surface. Rosetta's Cometary Secondary Ion Mass Analyser (COSIMA) team members have been using these carbonaceous products to understand their measurements of C-rich dust found in the coma of comet 67P/Churyumov-Gerasimenko; the only match found to date for the carbonaceous component of dust in 67P is the carbonaceous dust made in the CDL via SMR.

Team scientists in the Cosmic Ice Laboratory (CIL), continued to emphasize the identification and quantification of organic molecules known or suspected to be of astrobiological significance, or related to molecules that are of such significance. Five papers were either accepted or published in 2018. During the 2018 summer program for undergraduate students (see URAA 2018), Ella Mullikin (Wellesley College) conducted experiments in the CIL on the chemistry of cyclic aliphatic organics. They are relevant for terrestrial biology, but little is known of their astrochemistry. Her experiments were the first of their type and are now being prepared for publication. For this work, she was recognized as a John Mather Nobel Scholar In 2018.

*How was prebiotic matter synthesized and processed in the interstellar medium prior to being carried into the solar nebula?* A major focus of this work is to connect interstellar organic chemistry, protoplanetary disk chemistry, and the composition of Solar System bodies. Observational and theoretical studies can elucidate the range of compounds possibly available for prebiotic chemistry or central to it. We continued extensive observational and theoretical analysis of prebiotic molecular complexity in molecular clouds and in protoplanetary disks, attempting to test more complex prebiotic chemistry to link up with the incredibly diverse molecular zoo in carbonaceous chondrites studied by the NAI Goddard team. Using ALMA, we detected the simplest carboxylic acid, HCOOH, in disks for the first time. And, we detected exceptionally strong emission from sugar-related glycolaldehyde in star-forming region NGC 6224I; the presence of many other emission lines suggest that more molecular discoveries await at THz frequencies. Finding new protoplanetary or circumstellar debris disks is central to comparative studies related to the origin and fate of our Planetary System.

The Disk Detective citizen science project achieved a major milestone in 2018. Silverberg, Kuchner *et al.* (2018) reported results from imaging follow-ups to check for contamination of candidate Disks by background objects. Although nearly all (92%) AllWISE-selected infrared excesses are false positives, only 7% of the disk candidates that survive vetting via Disk Detective were rejected as false positives. Of the 244 surviving disks, 213 are new to science and 30 lie within ~125 pc of Earth, making them good candidates for direct-imaging exoplanet searches.

Team Scientist Aki Roberge investigates gas in debris disks such as those in the Beta Pictoris moving group. In 2018, she reported that (startlingly!) the circumstellar gas in the Eta Telescopii disk is quite different from that in Beta Pictoris and may in fact have solar abundances (Youngblood *et al.*, in preparation). Although in the same velocity group and thus having common origins and age, the contrast between these two systems suggests different planetesimal populations as the primary sources of the gas: volatile-rich icy planetesimals (like KBOs) in the case of Beta Pic and volatile-poor rocky planetesimals (like asteroids) in the case of Eta Tel. The nearby debris disks newly identified by Disk Detective may provide lucrative targets for similar studies.

Can we provide new tests of conditions amenable to life elsewhere in our Planetary System? Paganini, Mumma, and collaborators conducted a deep survey at IR wavelengths seeking spectral signatures of $H_2O$ plumes on Europa. A total of 20 half-nights were devoted at Keck-2 for this study; the observations were completed in 2017 and the analysis in 2018; the results were submitted for publication in late 2018 and are now awaiting consideration of our revisions.

Novak, Mumma, Villanueva, and Faggi conducted a deep search for methane, $H_2O$, and HDO on Mars using iSHELL at NASA's IRTF, during early summer in the North (Ls ~120). Analysis is in progress. In related work, Mumma and Villanueva co-authored papers on water and methane on Mars based on data acquired by ExoMars Trace Gas Orbiter. These papers were prepared in late 2018.

Can we define new instrument protocols to extend our knowledge of the complexity of organic compounds in mission targets relevant to astrobiology? We continue to explore the potential application of novel nanopore-based detection and sequencing of informational bio polymers for future missions to Mars and ocean worlds such as Europa or Enceladus. In 2018, Science Associate (and former undergraduate research associate, see URAA 2017) Mark Sutton led the publication of results that characterize and constrain the critical impacts of high-energy ionizing radiation on nanopore operation (Sutton *et al.* 2019). A second GCA undergraduate research associate, Maggie Weng (see URAA 2018), and co-workers explored the tolerance of nanopore sequencing to the range of salt concentrations expected in samples on such missions.

# Project Reports

## From Comets and Asteroids to Planets: Organics and Water as Key Windows into Emergent bio-Earth

Team scientists quantify the diversity of chemical composition among comets, to assess their potential for delivering pre-biotic organic materials and water to young (barren) planets.

Major improvements were made to the emerging taxonomy for comets based on volatile composition. Senior Research Fellow Manuela Lippi and 2016 URAA alumna Maria Camarca compiled an archival database for 60 comets observed with high-resolution IR spectroscopy since 1995, preparing the data for self-consistent re-analysis using current generation computer algorithms. The intent is to remove systematic uncertainties in composition that occurred as analysis algorithms and molecular fluorescence models evolved during this 24-year interval. A manuscript on the first five comets so re-analyzed is nearing submission.

2018 saw near-Earth approaches of two ecliptic comets from the Kuiper Belt. GCA scientists led investigations of 21P/Giacobini-Zinner from July through October, and of 46P/Wirtanen in December and January (2019). 21P is the archetype for comets depleted in the ratio of product species $C_2$ & CN, but its apparition in 2018 was the first to permit comprehensive studies of primary volatiles with modern astronomical spectrometers that might explain the depletion. In 21P (Figure 6), Faggi *et al.* detected $H_2O$, CO, $H_2CO$, $CH_3OH$, $C_2H_2$, $C_2$H6, and HCN, and set sensitive upper limits for $CH_4$. In 46P, detections were even richer owing to the comet's greater brightness. Results for 21P are nearing publication and will appear in year 5 (2019) of this award, as will those for 46P.

DiSanti *et al.* (2018) reported production rates for $H_2O$ and eight trace gases/prebiotic molecules (CO, $H_2CO$, $CH_3OH$, $CH_4$, C2$H_2$, $C_2H_6$, HCN, and $NH_3$) in the dynamically new comet C/2013 V5 (Oukaimeden) over a range in heliocentric distance ($R_h$ = 0.79 – 0.70 au) in 2014. They noted conspicuous changes in mixing ratios on adjacent nights (Figure 7), suggesting a non-homogeneous volatile composition for C/2013 V5 consistent with differential processing of its constituent ices.

Mumma *et al.* used both scheduled and Director's time to observe long period comet C/2017 E4 Lovejoy in April 2017, soon after its discovery (Figure 8), and fortunately so – the comet disrupted completely several weeks later, near 0.5 au heliocentric distance. Nine primary volatiles (same as for C/2013 V5) and three product species were quantified in E4 (Faggi *et al.* 2018). HCN and $NH_3$ were enriched significantly relative to ethane, placing E4 among the group of disrupting comets that display this property. Mumma *et al.* (in prep.) suggest that this behavior stems from disruptive ejection and subsequent heating/dissolution of ammoniated salts, a previously unrecognized fraction of the cometary nucleus that is thermally activated in the coma. If delivered intact with water to young planets, ammoniated salts have major implications for astrobiology.

Team scientists also expanded their leadership of cometary molecular astronomy with the Atacama Large Millimeter/submillimeter Array (ALMA), permitting measurement of the $^{12}C/^{13}C$ ratio in cometary HCN. Details are given in the project report on Interstellar Chemistry, Protoplanetary Disks and Early Solar System Processes.

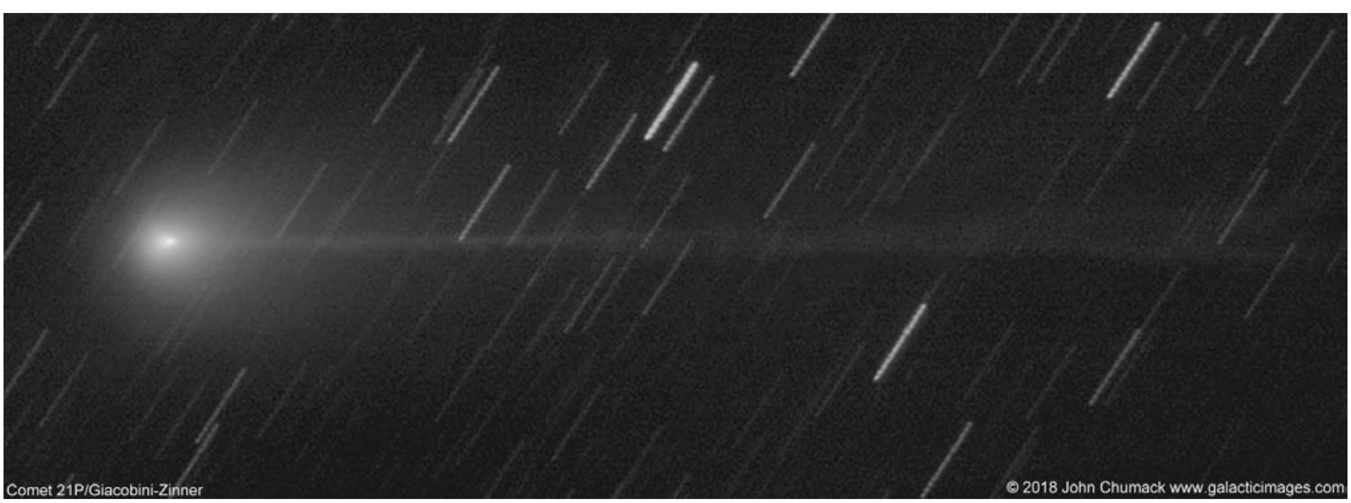



Figure 1. Optical image of ecliptic comet 21P/Giacobini-Zinner. The long thin tail from ionized gas, and lack of a dust tail, mark this comet as gas-rich and dust-poor. The comet survived this apparition. Credit: 2018 John Chumack www.galacticimages.com

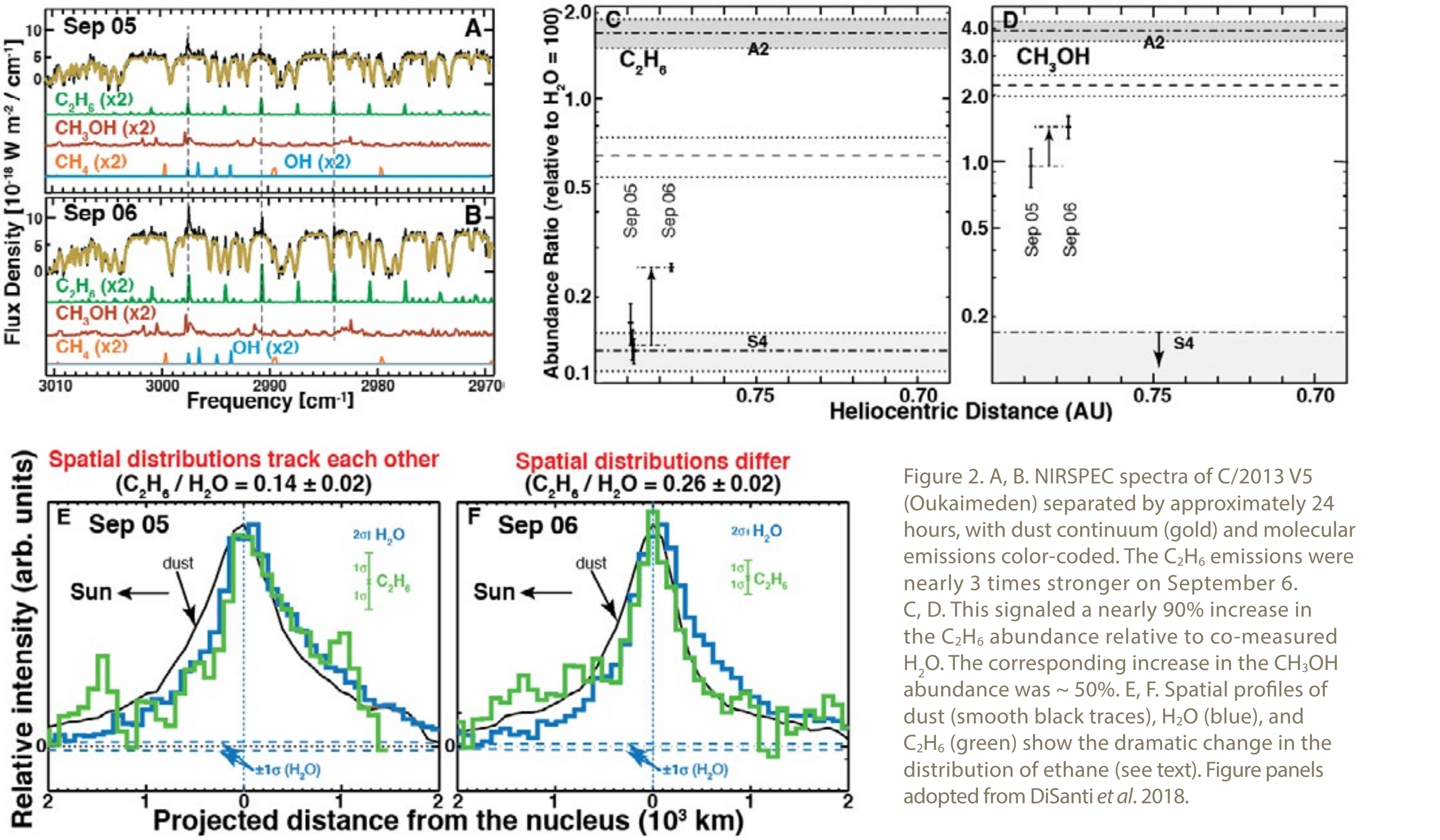



Figure 2. A, B. NIRSPEC spectra of C/2013 V5 (Oukaimeden) separated by approximately 24 hours, with dust continuum (gold) and molecular emissions color-coded. The $C_2H_6$ emissions were nearly 3 times stronger on September 6. C, D. This signaled a nearly 90% increase in the $C_2H_6$ abundance relative to co-measured $H_2O$. The corresponding increase in the $CH_3OH$ abundance was ~ 50%. E, F. Spatial profiles of dust (smooth black traces), $H_2O$ (blue), and $C_2H_6$ (green) show the dramatic change in the distribution of ethane (see text). Figure panels adopted from DiSanti *et al*. 2018.

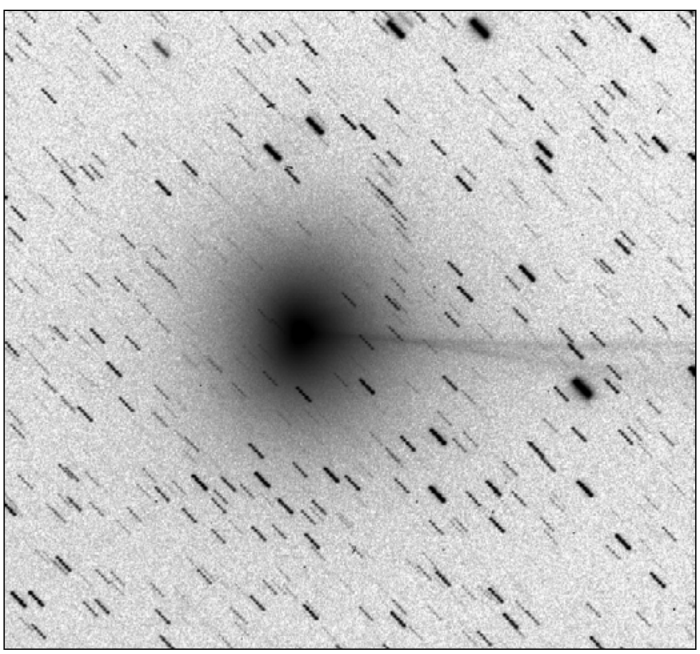


Figure 3. Optical image of Oort Cloud comet, C/2017 E4 (Lovejoy). The comet shows a long narrow (split) tail from ionized gases but a dust tail is missing, reflecting its gas-rich and dust-poor composition. The comet disrupted completely several weeks after this image was taken. Credits: Emmanuel Jehin

## Analysis of Prebiotic Organic Compounds in Astrobiologically Relevant Samples

We continued to investigate the origin and evolution of meteoritic organic compounds. This year, we led the authorship of a book chapter describing the current state of knowledge about meteoritic soluble organic compounds and insoluble organic matter (Figure 1) and participated in workshops and conferences related to this theme. We continued to explore the correlations of meteoritic amino acids with structurally related species and other organic compounds, such as ketones, aldehydes, and carboxylic acids (Figure 2). We published a manuscript on the analysis of mono-carboxylic acids in a series of carbonaceous chondrites, as well as a manuscript examining aldehydes and ketones in the Murchison meteorite; two additional manuscripts studying aldehydes were submitted for publication. We carried out the first amino acid analyses of enstatite meteorite samples. We also continued a variety of collaborations, including one using NASA high-end computing models to explore the degradation of amino acids under meteoritic conditions and another effort aimed at studying organic compounds in micrometeorites.

We have key roles in the new NFoLD/LAB program and the Center for Life Detection (CLD). We continue to leverage our expertise and facilities to develop novel instrumentation for future spaceflight. We continue involvement in multiple NASA flight missions and mission proposals, with key involvement in the OSIRIS-REx arrival at asteroid Bennu and experimental support for the CAESAR mission to comet 67P (Comet Astrobiology Exploration SAmple Return) that was selected for a Phase A study in the New Frontiers 4 competition. We also continue to support the Sample Analysis at Mars (SAM) instrument on the Curiosity rover via laboratory instrument analogs, SAM flight data analysis, and preparation for SAM wet chemistry experiments on Mars.

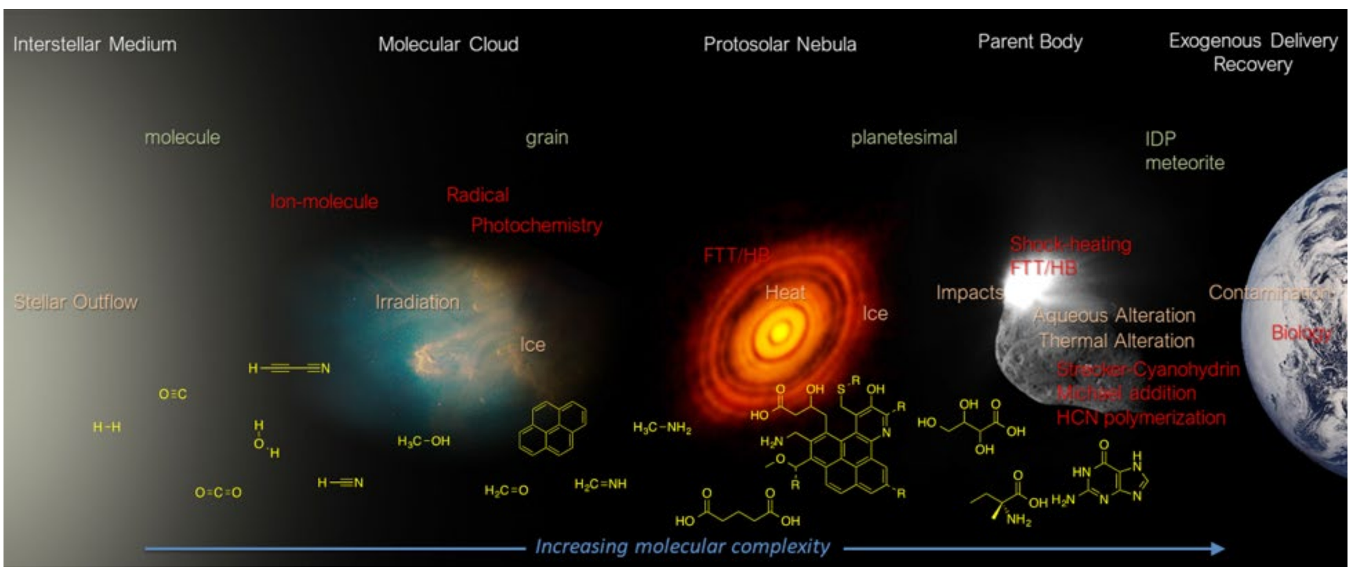



Figure 4. A key goal of this effort is to use meteoritic organic chemistry to better understand how the chemical formation processes that likely occurred within the interstellar medium and on small bodies and planets in our solar system, may have given rise to life on Earth. (Glavin *et al.*, 2018).

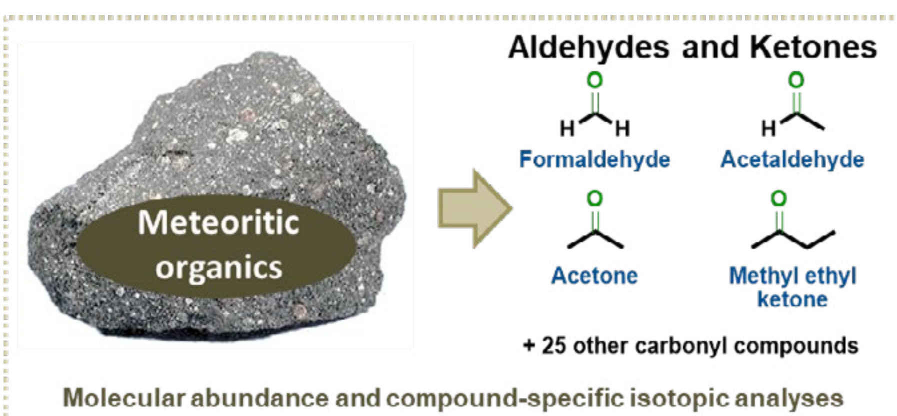



Figure 5. We have developed and applied methods to measure the molecular abundance and isotopic composition of carbonyl compounds in a variety of carbonaceous meteorites. Our work helps to explain the potential chemical pathways and connections between meteoritic organics.

## Interstellar Chemistry, Protoplanetary Disks and Early Solar System Processes

A major focus of this work is to make connections between interstellar organic chemistry, protoplanetary disk chemistry and the composition of Solar System bodies. Observational and theoretical studies can elucidate the range of compounds possibly available for prebiotic chemistry.

The Atacama Large Millimeter/submillimeter Array (ALMA) has proven to be a particularly powerful tool for studies of cometary composition. We have recently pioneered a new technique using ALMA autocorrelation data to search for weak molecular emissions in our existing cometary data (Cordiner *et al.* 2019). We employed ALMA autocorrelation spectra from the main 12 m array (28 active antennas used) to observe extended emission that is resolved out by the interferometer. This new approach effectively uses ALMA as a large single-dish telescope with a subsequently large increase in sensitivity, at the expense of spatial information on the emission. When applied to our archival observations of comet C/2012 S1 (ISON), the resulting HCN spectra are 14-fold more sensitive than the interferometric data and this allows us to detect weak spectral lines that are not otherwise evident in the data. Figure 3 shows that the HCN(J=4-3) emission line in this comet is markedly stronger in the autocorrelation data and that the $H^{13}CN$(J=4-3) line, apparently absent in the interferometric data, is clearly detected and thus allows a measurement of the cometary $^{12}C/^{13}C$ ratio. This technique will allow searches for weak emission from organic molecules in future comets and it can be applied to interferometric observations of any astronomical source that exhibits large-scale molecular emission.

The Blake group has continued its extensive observational and theoretical analysis of prebiotic molecular complexity in molecular clouds and in protoplanetary disks. They endeavored to test more complex prebiotic chemistry to link up with the incredibly diverse molecular zoo in carbonaceous chondrites studied by the NAI GSFC team. They used ALMA to detect the simplest carboxylic acid, HCOOH, in disks for the first time (Favre *et al.* 2018). Deep molecular line surveys of protostellar hot cores are an important observational technique for the discovery of new organic molecules and measurements of stable isotope ratios. In this regard, they made the first line survey aimed at testing the sensitivity of the highest frequency bands of ALMA (Band 10) for such studies. A molecular line survey of NGC 6224I has been published (McGuire *et al.* 2018); the exceptionally strong emission detected from glycolaldehyde, and the presence of many other emission lines, suggests that more molecular discoveries await at THz frequencies.

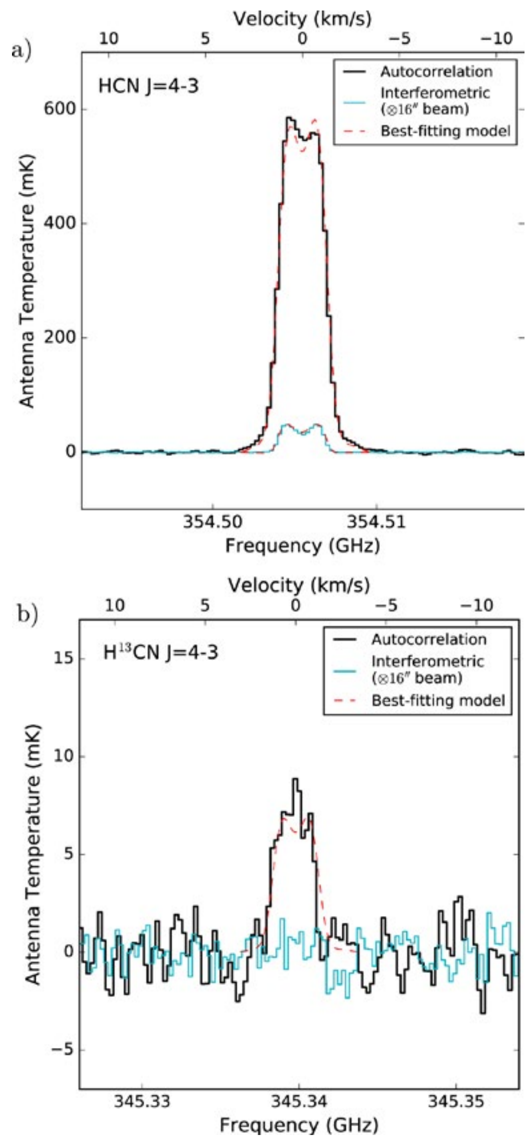



Figure 6. Comparison of ALMA autocorrelation (black) and interferometric (blue) spectra for (a) HCN and (b) $H^{13}CN$ in comet C/2012 S1 (ISON). Best-fitting model spectra are overlaid (dashed red). From Cordiner *et al.* (2019). Credit: Comparison of ALMA autocorrelation (black) and interferometric (blue) spectra for (a) HCN and (b) $H^{13}CN$ in comet C/2012 S1 (ISON). Best-fitting model spectra are overlaid (dashed red). From Cordiner *et al.* (2019).

### Investigations of Cosmic Ice Analogues and Processes

In the Cosmic Ice Laboratory, we continued to emphasize the identification and quantification of organic molecules known or suspected to be of astrobiological significance, or related to molecules that are of such significance. Five papers were either accepted or published in 2018. Two papers concerned radiation-driven oxidation processes, one making inorganic ions and compounds and the other involving alcohols. A third paper covered $H_2S$ and organosulfur compounds (thiols) related to the so-called missing interstellar sulfur problem. Our study of propynal (an interstellar molecule) established infrared spectral assignments, band strengths, and structural changes over temperatures relevant to the interstellar medium, and proposed this molecule as a target for infrared observations. A fifth paper concerned an organic ester, methyl propionate, reporting spectroscopic properties related to two known interstellar organics.

We also continued to educate the next generation of astrobiologists. Co-Investigator Hudson taught the University of Maryland's undergraduate astrobiology course to 60 students, while Co-Investigator Gerakines taught an on-line astrobiology course for a similar number at the University of Alabama at Birmingham. During the 2018 summer program for undergraduate students (see URAA 2018), we mentored Ms. Ella Mullikin (Wellesley College) who conducted experiments on the chemistry of cyclic aliphatic organics. They are relevant for terrestrial biology, but little is known of their astrochemistry. Her experiments were the first of their type and are now being prepared for publication. For this work, she was recognized as a John Mather Nobel Scholar.

Dr. Chris Materese joined us as a new civil servant in the summer of 2018, coming from the NASA Ames astrobiology team. He is continuing our work on the survival of organic molecules, such as nucleobases, on exposure to ionizing radiation.

## Cosmic Dust Lab - Surface Mediated Reactions in the Primitive Solar Nebula

We continue to study the formation of complex hydrocarbons from gas-phase CO and simple gases ($N_2$ and $H_2$) via surface-mediated reactions (SMR) on almost any grain surface. Hydrogen, carbon monoxide and nitrogen are abundant in the primitive solar nebula. Our lab studies show that they react on surfaces of silicate dust and metal grains to produce an abundance of carbon-bearing products including volatile hydrocarbons, amines, alcohols, aldehydes and acids as well as more complex, less volatile species such as carbon nanotubes and other carbonaceous solids. For the primitive solar nebula, surface-mediated reactions might provide a solution for a problem that modern chemical models of nebular processes do not yet address; namely, the conversion of large quantities of CO and carbon dioxide generated by high temperature reactions under oxidizing conditions back into solid carbonaceous species that can be more easily incorporated into planetesimals (see Figure 4). We also found that refractory carbonaceous deposits can catalyze additional surface reactions. We are working to understand the rates and products of such reactions given the large range in time, temperature, pressure, catalyst composition, and secondary reactions that could occur in nebular environments.

Continuing higher temperature experiments use a dedicated system to further investigate the loss of CO with different Fe-based substrates. We are also investigating the 'dusting' or sequestering of iron into the hydrocarbons and the possible formation of iron carbides. These reactions appear to occur over a much wider temperature range than expected ($575K < T < 1200K$).

We are continuing our collaborations with Rosetta's COSIMA team members who have been using our carbonaceous products to understand their measurements of C-rich dust found in the coma of comet 67P/Churyumov-Gerasimenko (presented at the 50th Lunar & Planetary Science Conference, March 2019). While the COSIMA team has analyzed a very wide range of aromatic and aliphatic organic compounds in the laboratory, the only match found to date for the carbonaceous component of dust in 67P is the carbonaceous dust made in our laboratory via SMR. For this effort, we are currently producing dust at low (825K) and high (900K) temperatures with both short (using ~10% of the initial CO) and long runs (using 90% of the initial CO). These runs will begin to illustrate the effects of annealing and secondary reactions on the resulting COSIMA spectra

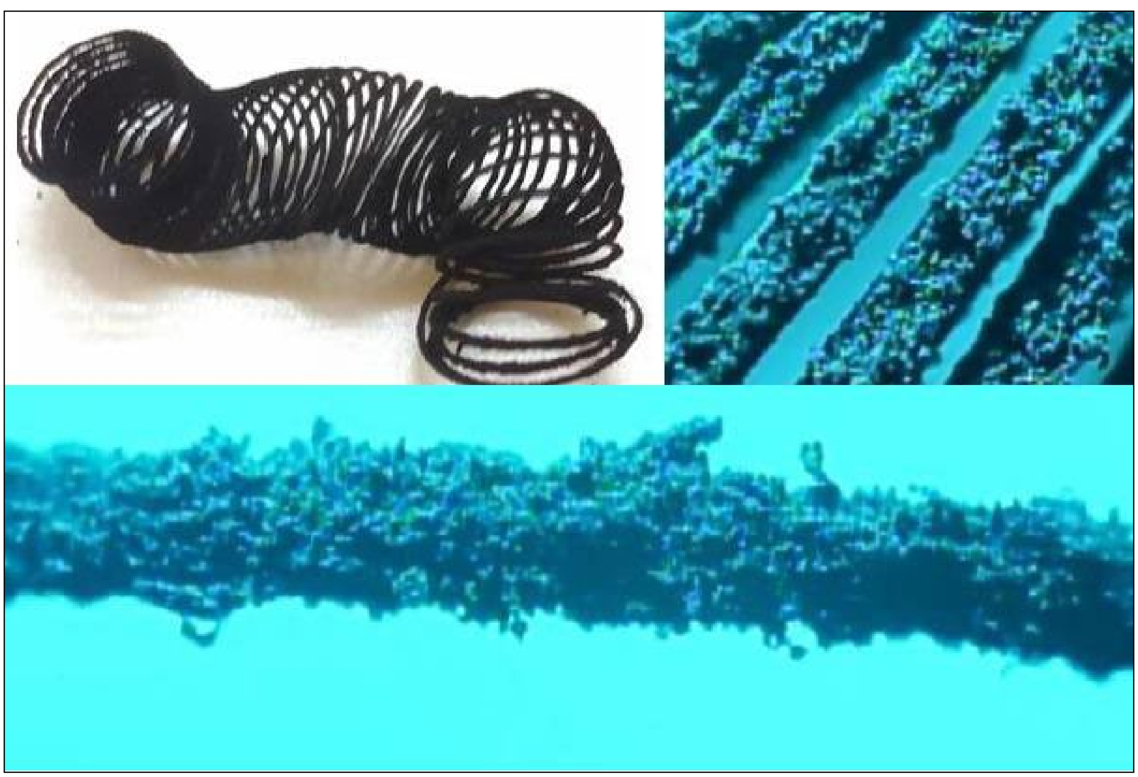


Figure 7. Texture of Carbonaceous Deposits on Iron Wire Catalyst. Top left: Coiled iron wire (~1.5 m long by 0.009" diam.) used to mediate the reaction of $CO + N_2 + H_2$ at temperatures near 900K. Top right: microscopic image of the surface of multiple coils at about 25x magnification illustrates the "uniformly lumpy" growth of the carbon deposits over the coil. Bottom: microscope image at 50x shows the string-like texture of the carbon growth on the iron surface. Credit: Nuth, *et al.* this work.

and may begin to provide constraints on the environmental conditions experienced by dust grains incorporated into 67P.

In 2018, we presented results at several conferences and in peer-reviewed publications. With Dr. Tim McCoy (Smithsonian Museum of Natural History), we discussed size-dependent processes that accrete or affect accreted materials [Nuth *et al.* 2018]. This chapter, included in Elsevier's interdisciplinary book "Primitive Meteorites and Asteroids", addresses possible indicators why the differences between comets and asteroids might result from evolution over billions of years rather than initial accretion processes. Published journal papers reflect the contributions of dust formation within our ALMA collaborations, in particular, our research that silicate grain nucleation occurs over a wide range of stellar radii [Decin *et al.* 2018; Homan *et al.* 2018]. Additionally, multiple Cosmic Dust Lab tours were provided and the dust nucleation generator is always of great interest. We hosted graduate student Sabrina Alam who completed her final Master's degree requirement while focusing on circumstellar outflow dust calculations. We continue to distribute a variety of Cosmic Dust Lab samples to the community for testing and analyses ranging from raw amorphous smokes to metal-dusted organics formed at high temperatures.

## Analytical Protocols in Sequencing Technology

We have continued to develop the potential application of novel nanopore-based detection and sequencing of informational biopolymers on future missions to Mars and ocean worlds such as Europa or Enceladus. The detection of an indigenous polymer with specific information-processing functionality, even if not directly related to DNA, could be a singular biosignature of life that has evolved independently of Earth life. GCA research has recently investigated both the robustness of this technology to space and planetary environmental extremes, and the optimal protocols for successful sequencing with complex samples *in situ*. Science associate (and former GCA undergraduate research associate, see URAA 2017) Mark Sutton has led the publication of results that characterize and constrain the critical impacts of high-energy ionizing radiation on nanopore operation (Sutton *et al.* 2019). The MinION™ device from Oxford Nanopore, Inc. (Figure 5) was found to be robust (able to produce sufficient DNA reads for sequencing) to gamma radiation at levels consistent with a mission to Mars. However selected components and reagents would need to be shielded and/or modified to survive the ~1 kGy levels expected on a mission to Europa. A second GCA undergraduate research associate, Maggie Weng (see URAA 2018), and coworkers explored the tolerance of nanopore sequencing to the range of salt concentrations expected in samples on such missions. The team found evidence that read lengths and other sequencing quality metrics were degraded to varying degrees depending on salinity, salt type, and sample introduction method, suggesting procedures to mitigate signal loss on a future mission (Weng *et al.,* in prep.). The GCA nanopore work is conducted in collaboration with the laboratories of Dr. Sarah Stewart Johnson of Georgetown University and Dr. Aaron Burton of NASA's Johnson Space Center (JSC).

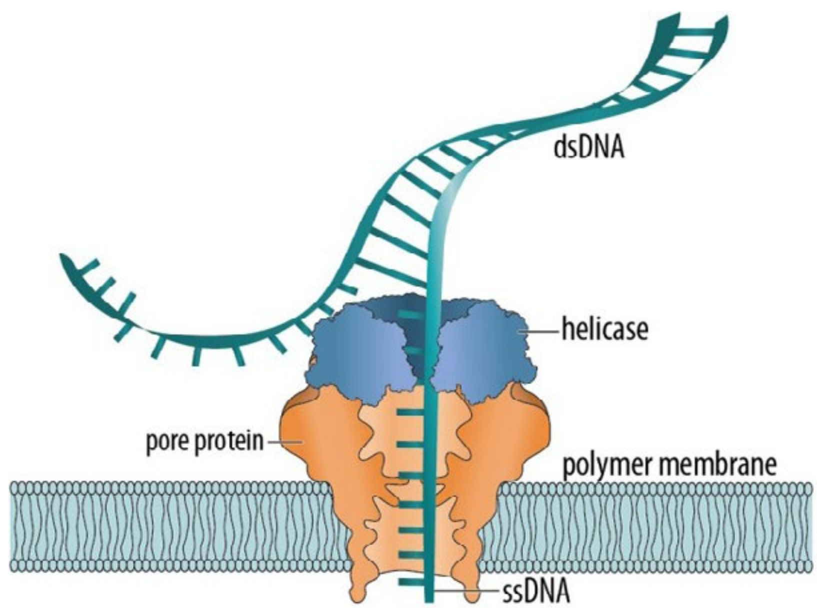



Figure 8. Schematic representation of a DNA molecule translocating a protein nanopore. The double-stranded DNA (dsDNA) is split by a helicase enzyme, allowing only a single strand (ssDNA) to pass while slowing it enough to achieve sufficient resolution for sequencing. Credit: Sutton *et al*., 2019


## Origin and Evolution of Organics and Water in Planetary Systems: 2018 Publications

Anderson, C. M., Nna-Mvondo, D., Samuelson, R. E., McLain, J. L., and Dworkin, J. P. (2018). The SPECTRAL ice chamber: application to Titan's stratospheric ice clouds. *Astrophysical Journal* 865: 62, 12pp. DOI: 10.3847/1538-4357/aadbab

Aponte J. C., Woodward H. K., Abreu N. M., Elsila J. E., Dworkin J. P. (2019). Molecular Distribution, $^{13}$C-Isotope, and Enantiomeric Compositions of Carbonaceous Chondrite Monocarboxylic Acids. *Meteoritics & Planetary Science* 54, Nr 2, 415-430. DOI: 10.1111/maps.13216

Cordiner, M.A., Palmer, M.Y., de Val-Borro, M. *et al*. (2019). ALMA Autocorrelation Spectroscopy of Comets: The HCN/H$^{13}$CN Ratio in Comet C/2012 S1 (ISON). *The Astrophysical Journal Letters* 870, 2, L26, 6pp. DOI: 10.3847/2041-8213/aafb05

Decin, L., Richards, A. M. S., Danilovich, T., Homan, W., and Nuth, J. A. (2018). ALMA spectral line and imaging survey of a low and a high mass-loss rate AGB star between 335 and 362 GHz. *Astronomy & Astrophysics* 615 (A28). DOI: 10.1051/0004-6361/201732216

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Faggi, S., Villanueva, G. L., Mumma, M. J., & Paganini, L. (2018). The Volatile Composition of Comet C/2017 E4 (Lovejoy) before its Disruption, as Revealed by High-resolution Infrared Spectroscopy with iSHELL at the NASA/IRTF. *Astronomical Journal* 156: 68, 17pp. DOI: 10.3847/1538-3881/aace01

Favre, C., Fedele, D., Semenov, D. *et al.* (2018). First Detection of the Simplest Organic Acid in a Protoplanetary Disk. *The Astrophysical Journal Letters* 862, 1, L2, 6pp. DOI: 10.3847/2041-8213/aad046

Friedrich, J. M., McLain, H. L., Dworkin, J. P., Glavin, D. P., Towbin, W. H., Hill, M. and Ebel, D. S. (2018). Effect of polychromatic X-ray microtomography imaging on the amino acid content of the Murchison CM chondrite. *Meteoritics & Planetary Science* 54, Nr 1, 220-228. DOI: 10.1111/maps.13188

Glavin, D. P., C. M. Alexander, J. C. Aponte, *et al.* (2018). Chapter 3 – The Origin and Evolution of Organic Matter in Carbonaceous Chondrites and Links to Their Parent Bodies. *Primitive Meteorites and Asteroids*, Elsevier: 2018; 205-271. DOI: 10.1016/b978-0-12-813325-5.00003-3

Homan, W., Danilovich, T., Decin, L., de Koter, A., Nuth, J., and Van de Sande, M. (2018). ALMA detection of a tentative nearly edge-on rotating disk around the nearby AGB star R Doradus. Astronomy & Astrophysics 614 (A113). DOI: 10.1051/0004-6361/201732246

Hudson, R.L. (2018). $N_2$ Chemistry in Interstellar and Planetary Ices: Radiation-Driven Oxidation. *The Astrophysical Journal* 867, 1- 11. DOI: 10.3847/1538-4357/aae584

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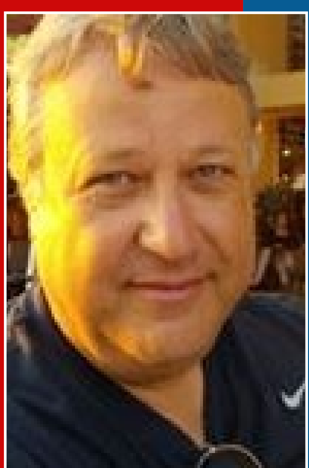
# Icy Worlds: Astrobiology at the Water-Rock Interface and Beyond

**Lead Institution:**
**NASA Jet Propulsion Laboratory**



**Principal Investigator:**
Isik Kanik

## Team Overview

Astrobiology at water-rock interfaces found on icy bodies (e.g., Europa, Enceladus and Ganymede) in our Solar System (and beyond) is the unifying theme for the team's research. In this interdisciplinary research, our Team (The Icy Worlds: Astrobiology at the Water-Rock Interface and Beyond) conducts a highly synergistic combination of experimental, theoretical, and field-based lines of inquiry focused on answering a single compelling question in astrobiology: How can geochemical disequilibria drive the emergence of metabolism and ultimately generate observable signatures on icy worlds? Our team's primary goal is to answer one of the most fundamental questions in all of astrobiology: What geological and hydrologic factors drive chemical disequilibria at water-rock interfaces on Earth and other worlds? Our research encompasses four investigations (INV's):

- What geological and hydrologic factors drive chemical disequilibria at water-rock interfaces on Earth and other worlds?
- Do geoelectrochemical gradients in hydrothermal chimney systems drive prebiotic redox chemistry towards an emergence of metabolism?
- How, where, and for how long might disequilibria exist in icy worlds, and what does that imply in terms of habitability?
- What can observable surface chemical signatures tell us about the habitability of subsurface oceans?

**Team Website:** https://icyworlds.jpl.nasa.gov

## 2018 Executive Summary

In **INV 1**, we continued to work on developing the alkaline vent theory (AVT) and carrying out investigations relevant to the origin of life. Some of the highlights are presented below:

Nine years of continued support through the CAN 5 and CAN 7 NAI funding has culminated in the publication of "*Green rust: The simple organizing 'seed' of all life?*" This paper looks beyond the now relatively well-established initial conditions and the disequilibria required by the earliest living cells (i.e., a potential of one half to one volt, and $10^6$ to $10^{10}$ electrons/cell/sec.). The paper sets a fresh platform for future research that seeks to understand how the external disequilibria are managed by inorganic matter–especially green rust (fougerite), an important iron oxyhydroxide in prebiotic vents–on the way to their animation. The minerals behave as nano-engines and pumps to drive a small number of internally contained disequilibria. This is not just a "metabolism first" requirement–prior conditions include specific physical driving disequilibria, and tensegrity nano-engines that can convert them to specific, endergonically-produced internal disequilibria. At the same time, information transfer in green rust is both digital ($Fe^{II}$ and $Fe^{III}$ sites as oscillating zeros and ones) and analog (through green rust–a double layer hydroxide tensegrity-matrix).

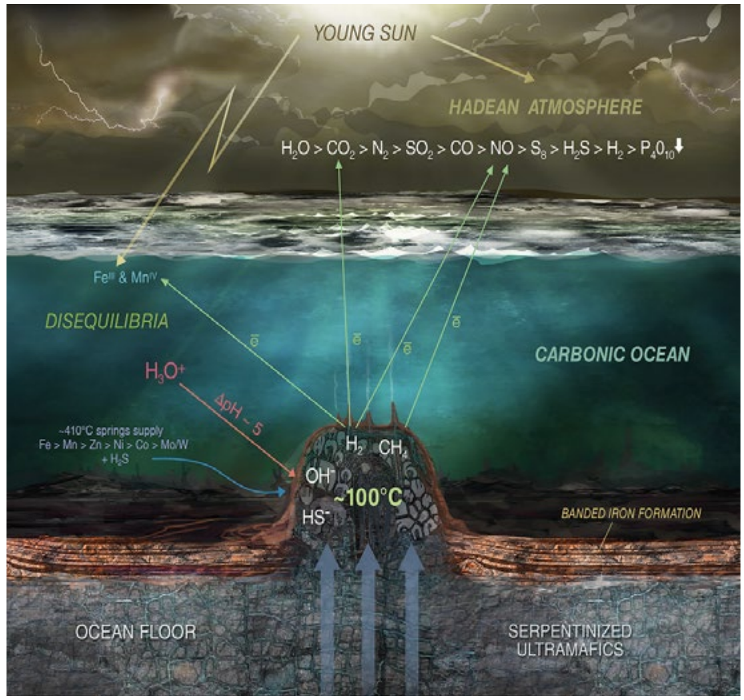



Figure 1. This illustration shows how green rust and Fe-Ni sulfides are precipitated at hydrogen and methane bearing alkaline hydrothermal vent on an early ocean floor. The steep redox and pH gradients drive the synthesis of simple organic molecules from the $CO_2$, $CH_4$ and $NO_3^-$ forced through the green rust interlayers.

**INV 2** researchers have made great progress on various topics relating to emergence of life and metabolism on ocean worlds. Ongoing collaborations with JPL's Electrochemistry group has resulted in a published study (Barge et al., *Astrobiology*, 2018) where fuel cell technology was adapted to simulate the energy produced in hydrothermal systems on ocean worlds. We accomplished this by making "geo-electrodes" out of minerals combined with binding substances to make a device that is in some ways similar to conventional fuel cell membrane electrode assemblies, but can represent electrochemical reactions on mineral surfaces at planetary water-rock interfaces. This work has continued throughout 2018, and we are now working on simulating chondrite-like interiors e.g. of Enceladus, and fabricating new fuel cell materials. Another major area of work centers on continuing investigations of prebiotic organic chemistry in seafloor sediments and hydrothermal chimneys and mounds, particularly focusing on reactions driven by iron hydroxides and iron/nickel sulfides. In iron hydroxide systems, the JPL-Oak Crest team has been testing nitrogen redox chemistry and the reductive amination of carboxylic acids. Regarding nitrogen, we have investigated nitrite-nitrite reactions in various types of lab-synthesized green rust and linked this to the production of $NO_x$ species and ammonia (Baum *et al,* in prep.). The presence of ammonia in vents, either produced *in situ* or by reduction of $NO_x$ species on green rust, can lead to the reductive amination of carboxylic acids to amino acids. We have investigated reaction networks of various carboxylic acids driven by mixed valence iron hydroxides/sulfides, which we have found can drive selectivity toward different products in the system, thus determining which specific amino acids or alpha-hydroxy acids are produced (Figure 2). The distribution of amino acids or other organics that we observe in our simulated prebiotic vent setting is not random and is unexpected given the relative concentrations of precursors. The chemistry of phosphorus in these seafloor systems also remains a focus. Previous work (Barge *et al*. 2014) showed the occurrence of pyrophosphate synthesis in a vent analog experiment, and we are continuing this work (i.e., studying phosphorus adsorption, condensation, and redox) in iron hydroxide/sulfide systems Clearly, reactive minerals formed under anoxic conditions are key to understanding the C, N, and P chemistry as well as energy generation in seafloor/hydrothermal systems on any ocean world. In addition to research progress, we have been active in outreach activities–many undergraduate and graduate students have been involved with Icy Worlds projects this year. Students have presented various posters at national conferences and participated in outreach events at community colleges. We are hosting a sabbatical visitor (Dr. Jason Pagano) in 2018-2019 working on hydrothermal chimneys. Finally, two Icy Worlds students were selected to attend the NAI-CAB International Summer School in Astrobiology.

In Year 4, the **INV 3** group advanced their goals of understanding processes in the interiors of icy ocean worlds. The INV 3 laboratory effort, mainly led by J. Michael Brown's Mineral Physics group at the University of Washington, continued to carry out experiments described in the Icy Worlds Team CAN 7 proposal, and added some new and exciting work. INV researchers finished the planned comprehensive data set for thermodynamic equations of state for salty fluids, using pressure and temperature conditions occurring in the depths of extraterrestrial oceans—both in the solar system and beyond—but not on Earth. This included sound speed data sets with an unprecedented precision of 1 part in 10,000, for systems containing NaCl, $MgSO_4$, $MgCl_2$, and $Na_2SO_4$, respectively, collected mainly by Olivier Bollengier, with support from Evan Abramson, J. Michael Brown, and a team of talented undergraduate students. They are now undertaking measurements in admixtures of these systems as part of a Solar System Workings project made possible by the Icy Worlds NAI.

At JPL, investigators are nearing completion of a new system for measuring sound speeds in simulated ocean world materials, based on the one at the University of Washington. They will begin using this system in FY19 to characterize the same aqueous systems as above, but in the presence of ammonia that is probably present in the oceans of Enceladus and Titan. This work is a companion effort with the Titan NAI team that was selected under CAN 8.

INV 3 Co-I, J.M. Brown, published a paper (Brown, 2018) describing the thermodynamics of our equation of state work, and our approach to solving the inverse problem using local basis functions. This methodology is advantageous and should be preferred to the established damped polynomial methods phase boundaries (Lemmon and Jacobsen, 2005). We are working to demonstrate this through application to the many data sets we now have in hand.

At JPL, S. Vance has completed a 3-year study of icy moon seismology leveraging strategic research and development funds. This effort led directly to four peer-reviewed publications describing the importance of seismology for studying the astrobiology of icy ocean worlds (Vance *et al*., Astrobiology, 2018), models of the internal structures of the five known icy ocean worlds (Vance *et al.* JGR 2018), the propagation of seismic waveforms and naming conventions for different seismic modes (Stähler *et al*. 2018), and the anticipated distribution of seismic signals on Europa (Panning *et al.* 2018). In Vance *et al*. (JGR, 2018), the researchers compiled available equation of state information for ices, fluids, rock, and iron cores, to create the PlanetProfile (PP) Matlab toolbox for computing radial structures, available on github. In that work, they explored multiple geophysical indicators of ocean world composition, thermal state, and interior structure, linking these to the study of planetary habitability through time.

The investigators' key objective in **INV 4** is to create links between chemical species observed on the surface of icy bodies and the characteristics of the underlying ocean. In Year 4, they conducted an investigation which is relevant to Europa in which they studied aqueous Na-Mg-$SO_4$ and Na-Mg-Cl systems via cryogenic differential scanning calorimetry and Raman spectroscopy. Their results suggest that a reduced set of potential icy mineral phases of endogenic origin on Europa's surface may be used to constrain spectral mixing models. They also used a simplified four ionic component Europan ocean (Na, Mg, $SO_4$, Cl) and mapped out the mineral precipitation sequence upon freezing as a function of relative ionic concentration, pH, etc. A 'flow-chart' of the freezing sequence was thus developed and verified experimentally using both published and newly acquired results (Figure 3).

Finally, the INV 4 team has carried out a systematic study to characterize the dehydration rate of fine grained hydrohalite in the near IR spectral range as a function of temperature, UV and electron irradiation energy, as well as ice particle size in order to understand the surface history of Europa.

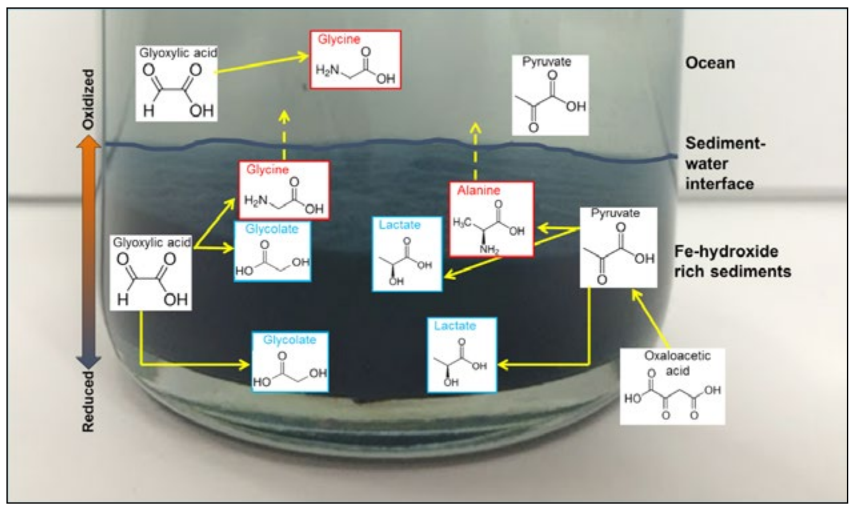



Figure 2. Laboratory simulation of an organic reaction network for a sulfide-free ocean. The sediment-water interface with the Fe-hydroxide rich sediment system is shown.

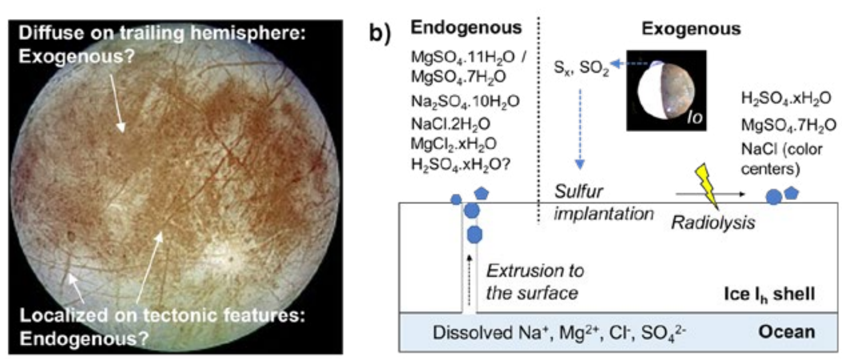



Figure 3. a) False-color Galileo image of Europa's trailing hemisphere highlighting the distribution of non-ice constituents, which appear reddish-brown. b) Schematic illustration of likely composition of non-ice constituents, as well as the processes leading to their presence on the surface. Credit: NASA/JPL/DLR

## INV 1: Energy Production at Water-Rock Interfaces

The submarine Alkaline Hydrothermal Vent (AHV) theory, as it stands at the end of Year 4, focuses on how the hydrous interlayers or channels in green rust, and to a lesser degree in mackinawite ($(Fe,Ni)_9S_8$), likely mediated the imposed proton gradient and, through the bifurcation of electron pairs, effectively stepped up the redox gradients to drive an organic takeover. However, the relative contributions of green rust, mackinawite, and associated minerals, and how they may have cooperated in synthetic biology, have remained partially unresolved. We have significantly revised the AHV theory since it was first proposed, namely, that although we still recognize that the job of life overall is to hydrogenate carbon dioxide, it may be that life first captured both the partially and fully reduced forms of C1 carbon as hydrothermal formate and methane (Russell and Nitschke 2017; Russell *et al*., 2017). Only later (though well before the Last Universal Common Ancestor or LUCA) would life have 'learnt' to reduce $CO_2$ through all the required intermediates for $CO_2$ autotrophy including the requirement for electron bifurcation, and thus to emerge from its mineral placenta (Russell, 2018; Branscomb and Russell, 2018a,b; Baymann *et al.* 2018). Drawing on experimental and theoretical work already accomplished through the NAI Icy Worlds investigations, team member Russell outlined 16 stringent experimental tests of the submarine alkaline hydrothermal vent model to provide a path forward in emergence-of-life research (Russell, 2018). The tests were ordered and tabulated along lines of development toward a 'ligand-accelerated' autocatalytic cycle (Figure 4).

The table itself was built on empirical evidence (Russell, 2018). It assumed that the redox-active, yet physically resilient mineral green rust (~$[Fe^{2+}_{6x} Fe^{3+}_{6(1-x)}O_{12}H_{2(7-3x)}]^{2+}\cdot[CO_2{-}_3\cdot 3H_2O]^{2-}$), could act as a protobiological disequilbrium-conversion engine (Russell, 2017, 2018; Russell *et al*., 2017). As shown in Figure 4, alkaline hydrothermal and acidulous ocean fluids, driven between the layered pliable redox-iron hydroxide boundaries of green rust dosed with Ni, Co, and Mo, and supported by iron sulfides. Such a mechanism could, theoretically, have provided (1) the conditions for the condensation of phosphate to pyrophosphate driven by a steep proton gradient, (2) have enabled the reduction of $CO_2$ to formate or carbon monoxide, (3) oxidized hydrogen and methane to methyl groups to react with formate or CO and thereby, (4) produced acetate and pyruvate, (5) reduced nitrate, (6) aminated carboxylic acids to the simple amino acids, (7) polymerized these acids to heterochiral peptides (8) at which point, such peptides would sequester metal and phosphate anionic clusters, and thereby (9) allow repetition of the cycle ever more efficiently. The activity of the water generated in these proto-biosynthetic reactions may have been kept low in the hydrous innards of green rust and mackinawite through exosmosis to the salty surrounds (Russell, 2018).

**Status of each protometabolic step as numbered in accompanying Figure 4**: Step numbers 1, 2, 4, 5, 9, 12, 15 and 16 have been previously serendipitously demonstrated by others, step numbers 1, 2, 9, 11, 13 and 14 have been experimentally demonstrated through the

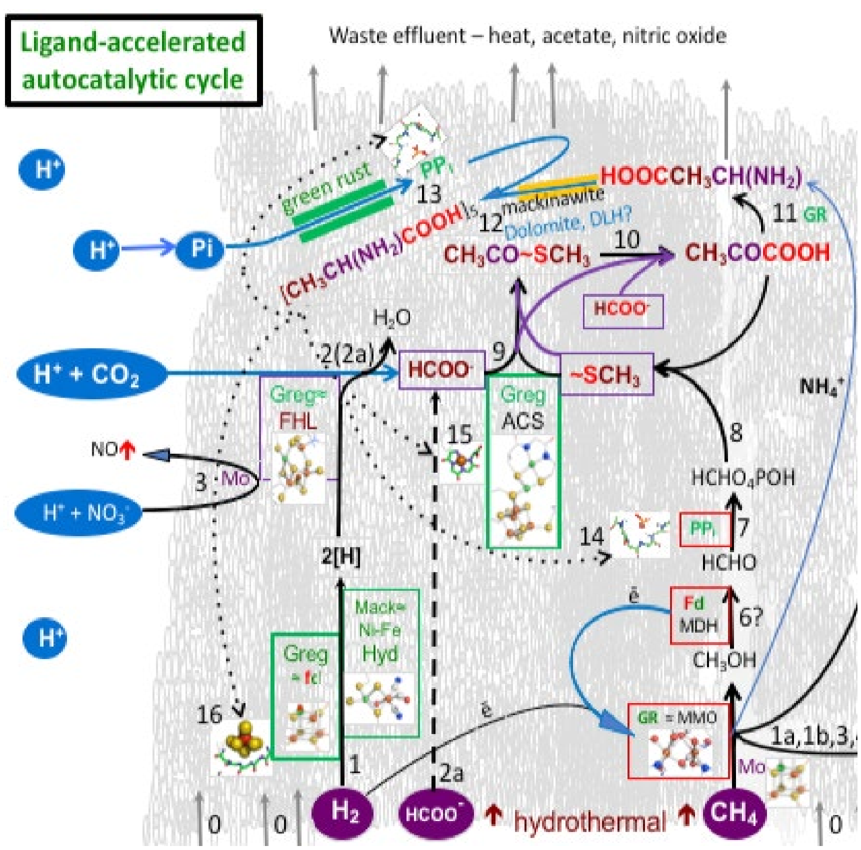



Figure 4. A green rust ramjet reducing nitrate to ammonia and then aminating pyruvate to polyalanine (Russell and Nitschke, 2017; Branscomb *et al*., 2017; Flores *et al*., 2017).

efforts of this NAI and our collaborators (Wong *et al.*, 2017, and the remainder of results to be written up and submitted in the coming year). Step numbers 3, 6, 7, 8 and 10 are as yet undemonstrated and remain to be tested. If these 5 steps can be experimentally demonstrated in the next year by the Icy Worlds' team, or independently in the years to come, then we foresee that such processes could have resulted in the germination and first flowering of the organic evolutionary tree as it emerged from the hydroponically-fertilized green rust seed and evolved toward the LUCA.

The fundamental assumption is that the protonically- and electronically-powered nano-engines (including the disequilibrium conversions needed to drive those endergonic reactions), that are required to produce life's many processors and its superstructures today, were initially co-opted from iron oxyhydroxides and sulfides, then dosed with transition metals and phosphate, and finally precipitated at the submarine alkaline vent.

## INV 2: From Geochemistry to Biochemistry

INV 2 focuses on experimentally simulating and characterizing the geological disequilibria and catalytic minerals generated in hydrothermal systems.

INV 2 group members have develop several new experimental systems and designs to facilitate low temperature prebiotic hydrothermal experimentation (Figure 5). On the early Earth, the sediments surrounding a vent as well as the hydrothermal mound itself would have been composed of highly reactive, metastable iron minerals. We have simulated these by precipitating simulated iron hydroxide sediments in the lab and reacting them with geochemical C and N precursors. We find that; (1) amino acids and alpha-hydroxy-acids (AHA's) are abiotically synthesized from carboxylic acid precursors in iron mineral systems over several days (a manuscript is in review about pyruvate reactions and another in prep about mixed carboxylic acid systems), (2) the liquid phase products are stable and detectable, that is, a fraction of the organics are incorporated into the mineral solid phase and are challenging to detect or analyze, and (3) the distributions of amino acids and AHA's produced in a system are not only proportional to the relative concentration of precursors but are a function of the particular geochemical conditions. In a system containing partially oxidized iron (oxy) hydroxide or sulfide minerals plus organic precursors and an ammonia source, it is likely that amino acids (and AHA's) will be formed in detectable amounts, with distributions that are related to the geochemistry of the environment. The redox chemistry of nitrogen in hydrothermal iron mineral systems is being studied by other INV 2 researchers, and will link early Earth atmospheric and ocean chemistry of N species with mineral-driven reactions that could be occurring in the water column, sediments, or hydrothermal mounds.

Other important facets of hydrothermal geochemistry are related to the electrically conductive minerals that comprise vent chimneys, for example sulfides. We have made progress in simulating the electrically linked redox reactions that could occur in a vent in fuel cell experiments in collaboration with JPL's Electrochemical Technologies group, leveraging topical research and technology development funds for studies of mineral-driven iron and sulfur redox chemistry in ocean world hydrothermal vents. We have also developed new experimental setups to incorporate the effects of hydrothermal temperature gradients, which are significant in affecting the mineralogy, catalytic reactivity, and thermoelectric properties of chimneys.

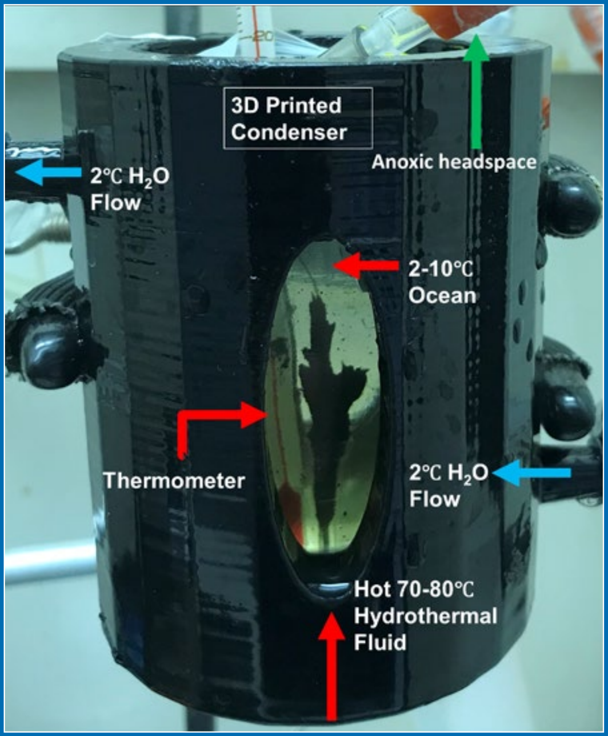


Figure 5. Apparatus for growing simulated hydrothermal chimneys in a temperature gradient similar to a natural vent. Credit: N. Hermis, G. LeBlanc, L. Barge

## INV3: Characterization of Ocean Worlds and Implications for Habitability

In year 4, Investigators of the INV 3 group advanced their goal of understanding processes in the interiors of icy ocean worlds. This activity included progress in experiments gathering fundamental properties, incorporating those properties into models, and communicating their results.

Our Co-Is at the University of Washington Brown Lab conducted some new and exciting work and finished the planned comprehensive data set for thermodynamic equations of state for salty fluids under pressures and temperatures occurring in the depths of extraterrestrial oceans—both in the solar system and beyond—but that do not occur on Earth (see Figure 6). This included sound speed data sets with unprecedented precision of 1 part in 10,000, for systems containing NaCl, $MgSO_4$, $MgCl_2$, and $Na_2SO_4$, individually. We are now undertaking measurements in admixtures of these systems as part of a Solar System Workings project made possible by the Icy Worlds NAI. At JPL, we are nearing completion of a new system for measuring sound speeds in simulated ocean world materials, based on the system at the University of Washington. We will begin using this system in FY19 to characterize the same aqueous systems as above, but in the presence of ammonia that is probably present in the oceans of Enceladus and Titan.

Sound speeds are a sensitive measure of Gibbs energies and chemical potentials, and are our main tool for elucidating the chemistry of icy ocean worlds. However, obtaining these other properties requires additional information—integrating constants—to anchor the derived thermodynamic surfaces in pressure, temperature, and relative to other phases and materials. NPP Baptiste Journaux and continuing UW postdoc Olivier Bollengier have been addressing the phase boundary problem through their ongoing work using diamond anvil cells. Journaux built a Peltier temperature control device, and created a new experiment tracking the melting curves of different types of water ice (III, V, VI, and VII) expected in ocean worlds in the

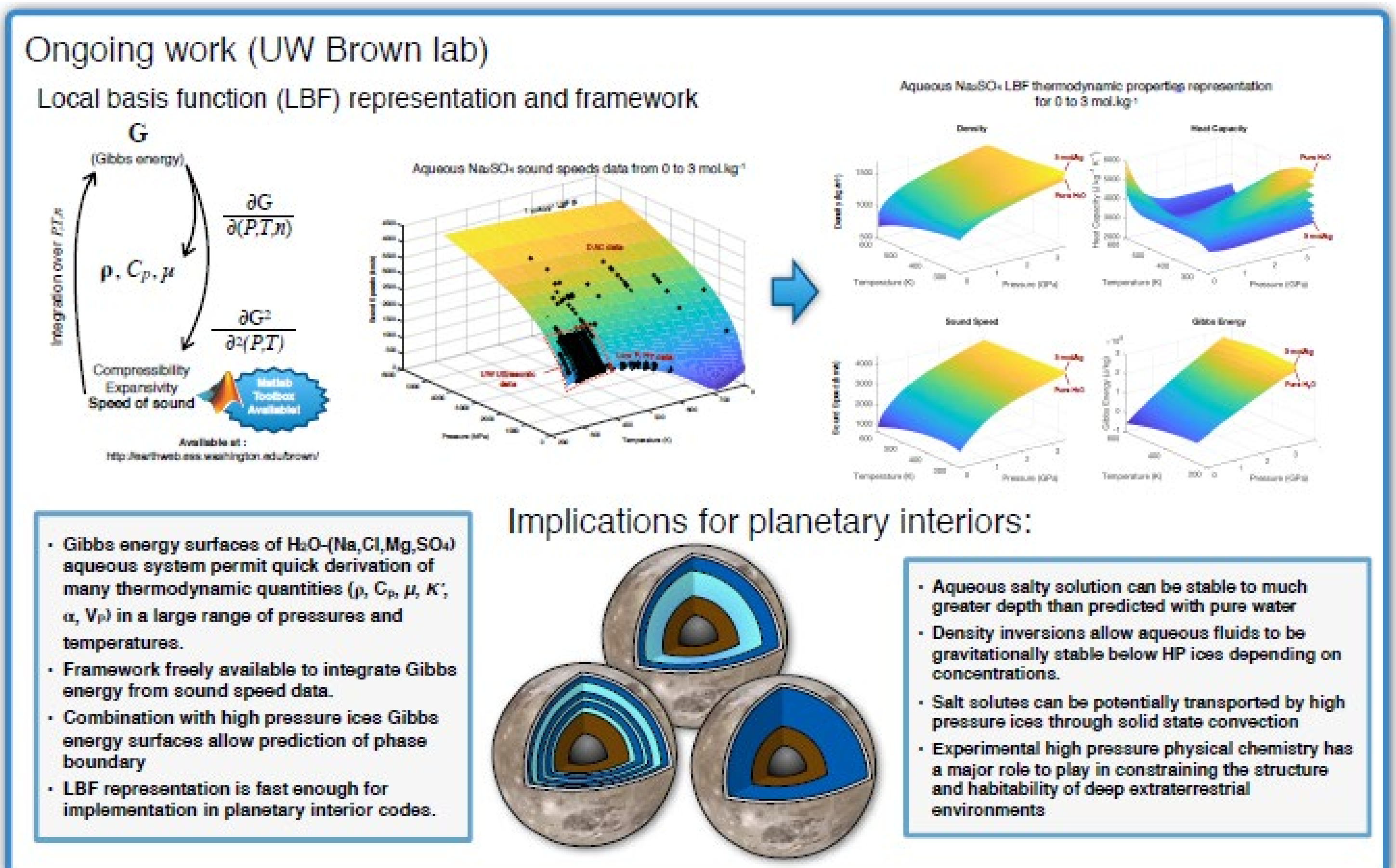



Figure 6. Co-Investigators at the UW Brown Lab conducted some new and exciting work and finished the planned comprehensive data set for thermodynamic equations of state for salty fluids under pressures and temperatures occurring in the depths of extraterrestrial oceans.

solar system and beyond. Working with undergraduate Jason Ott, they also observed the first confirmed density inversion in ice VII, in the presence of $Na_2SO_4$ (Figures 7 and 8). That is, at high concentrations of salt and at sufficient pressure, the fluid becomes denser than the ice, which floats to the top of the diamond anvil cell.

Journaux was awarded beam time at the European Synchrotron Research Facility (ESRF) in 2017 to conduct x-ray diffraction studies of high-pressure ices. This work revealed that incorporated $MgSO_4$ and NaCl salts increase the specific volume of ice VI. Journaux will return to the ESRF in November of 2018 under beam time awarded for follow-up studies.

J.M. Brown published a paper (2018) describing the thermodynamics of our equation of state work, and our approach to solving the inverse problem using local basis functions. The Matlab toolbox for generating equations of state, available through github, uses techniques that are standard in geophysical inverse theory (e.g., in seismology) to facilitate smooth fitting of the data surfaces, easily incorporating new datasets without altering the fitting parameters in other regions of phase space. This methodology is advantageous and, we argue, should be preferred to the well-established damped polynomial methods phase boundaries (Lemmon and Jacobsen, 2005). We are working to demonstrate this through application to the many data sets we have in hand.

At JPL, S. Vance completed a 3-year study of icy moon seismology leveraging strategic research and development funds. This effort led directly to four peer-reviewed publications describing the importance of seismology for studying the astrobiology of icy ocean worlds (Vance et al., Astrobiology, 2018), models of the internal structures of the five known icy ocean worlds (Vance *et al*. JGR 2018), the propagation of seismic waveforms and naming conventions for different seismic modes (Stähler *et al*. 2018), and the anticipated distribution of seismic signals on Europa (Panning *et al*. 2018). In Vance *et al*. (JGR, 2018), we compiled available equations of state information

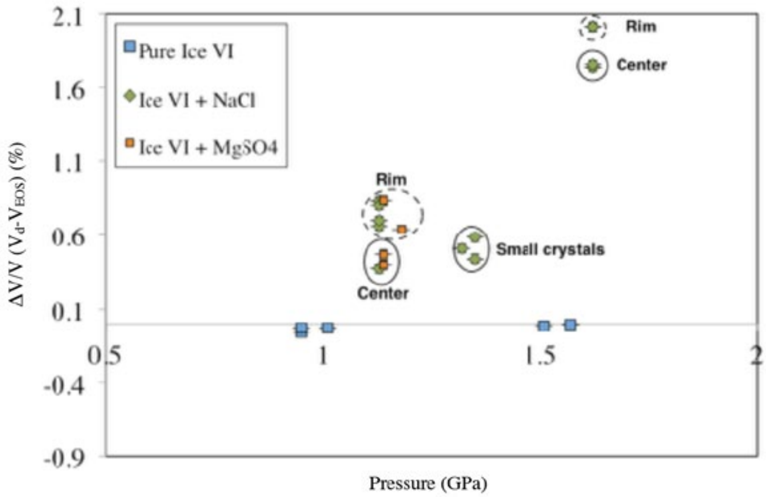



Figure 7. The first confirmed density inversion in ice VII, in the presence of $Na_2SO_4$ is observed as shown.

for ices, fluids, rock, and iron cores, to create the PlanetProfile (PP) Matlab toolbox for computing radial structures, available on github. In that work, we explored multiple geophysical indicators of ocean world composition, thermal state, and interior structure, linking these to the study of planetary habitability through time.

## INV 4: Observable Chemical Signatures on Icy Worlds

In order to better understand **the possible** links between chemical species observed on the surface of Europa and the characteristics of the underlying ocean, we carried out several laboratory investigations. In order to shed some light on the question of whether or not the composition of icy minerals forms from freezing of brines on Europa's surface, we conducted the following experimental investigations. First, we conducted an investigation of the aqueous Na-Mg-$SO_4$ and Na-Mg-Cl systems via cryogenic differential scanning calorimetry and Raman spectroscopy. Only single cation salts have been observed, namely mirabilite and meridianiite/epsomite for the $SO_4$-bearing solutions, and hydrohalite and a magnesium chloride hydrate for the Cl-bearing solutions. A cryogenic X-ray diffraction study is currently underway to further investigate these chemical systems and confirm the results. These results suggest a reduced set of potential icy mineral phases of endogenic origin on Europa's surface, which may be used to constrain spectral mixing models. Further, unambiguous detection of multiple-cation-bearing salts on the surface might indicate their formation via exogenic processes.

Restricting ourselves to a simplified four ionic component (Na, Mg, $SO_4$, Cl) Europan ocean, we used chemical divide modelling to map out the mineral precipitation sequence upon freezing as a function of relative ionic concentration, pH, etc. A 'flow-chart' of the freezing sequence was developed and verified experimentally using both published and newly acquired results (Figure 8). In performing this exercise, we are able to begin making meaningful links between observations of the surface chemistry and the chemical environment of the internal ocean.

Finally, we have embarked on a systematic study to characterize the dehydration rate of fine grained hydrohalite in the near IR spectral range as a function of temperature, UV and electron irradiation energy, as well as ice particle size. The results will help to infer the surface history of Europa by comparing remote sensing data from future Europa Clipper instruments to a systematic catalog of laboratory analogs.

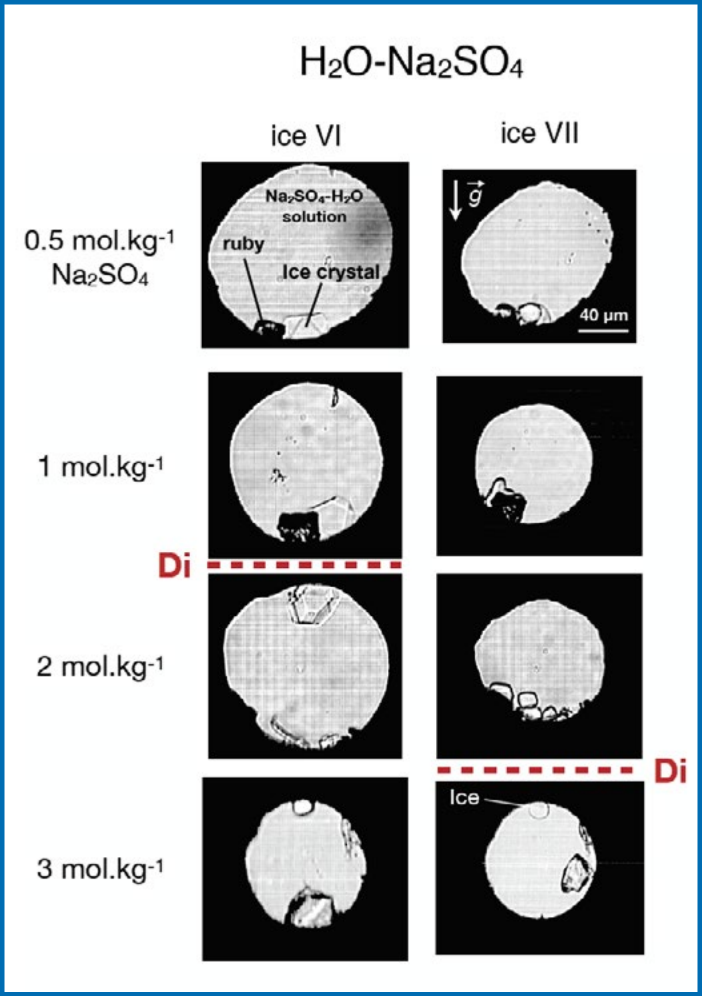



Figure 8. The first confirmed density inversion in ice VII, in the presence of $Na_2SO_4$.

# NPP Reports

**Name:** Baptiste Journaux, Ph.D.
**Team:** Icy worlds, NAI Titan team, Mineral Physics lab, University of Washington - Seattle
**Project Title:** Comprehensive thermodynamics of aqueous solutions and ice for understanding the habitability of extraterrestrial oceans.

My NPP project was aimed at measuring accurate phase equilibria in the $H_2O$-(Na, Cl, Mg, $SO_4$) system from 300 to 500K and 0.5 to 5 GPa. More than 500 data points were measured, exceeding the initial goals in terms of data accuracy and density. The measurement of liquidus curves and eutectic points allowed me to construct the phase diagrams of these systems, at pressures and temperatures relevant for large icy moons and water-rich exoplanets. I was also able to refine the *in situ* observation of aqueous fluid and high-pressure ices density inversions, and successfully compare these with computed densities of high-pressure ices and aqueous solution density.

PVT data points for high pressure water ices II, III, V and VI were measured *in situ* using Synchrotron X-Ray diffraction during two experimental campaigns in February and November of 2018 at the European Synchrotron Radiation Facility in Grenoble, France. The first accurate and comprehensive equations of state were refined for all these ice polymorphs. A thermodynamic model of phase boundaries, including melting curves, was then derived as a function of pressure, temperature and aqueous solution composition of NaCl, $MgSO_4$ and $Na_2SO_4$, that are comparable with *in situ* lab measurements.

In parallel with these experiments, I developed a new diamond anvil cell design (modified from the BX90 type), and a new cryostat apparatus that enables conduct of high-pressure measurements down to 220 K. High pressure data at low temperature on ice and in aqueous systems is directly applicable to icy world habitats and available data are still very sparse in that pressure range. The new apparati developed are allowing us to address this lack of data, and that will help constrain the habitability of deep oceans.

A critical effort was also mounted to implement those new experimental results into the aqueous system thermodynamical representation developed at UW and with the planetary interior models developed at JPL by S. Vance, C. Sotin and K. Kalousova, and at LPGN in Nantes, France, by G. Tobie. I spent a month in France in June, 2018, helping G. Tobie, K. Kalousova and my advisor, M. Brown, implement our thermodynamic representation into geodynamic numerical models.

*In situ* observations of density inversions between ice VI and salty aqueous fluids as a function of composition, and illustration of the relation with the new phase diagrams. Such data are required in order to predict the stability of fluid pockets in between or beneath a high pressure ice mantle and their ability to migrate through it. (Credit: B. Journaux)

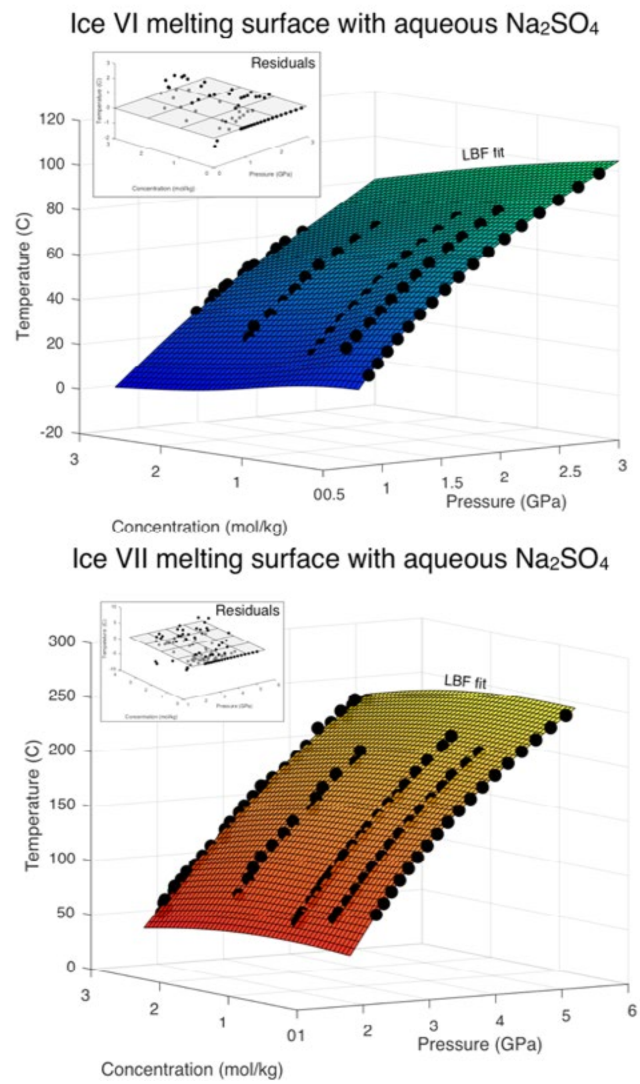



Figure 9. Example of melting point data taken for ice VI and ice VII in the $H_2O$-$Na_2SO_4$ system, using the local basis function (LBF) fit. These data are the basis of the following analysis that includes studying non-ideal fluid mixing properties, prediction of the eutectic compositions, and comparison with the phase equilibria predicted by the Gibbs free energy approach. (Credit: B. Journaux)

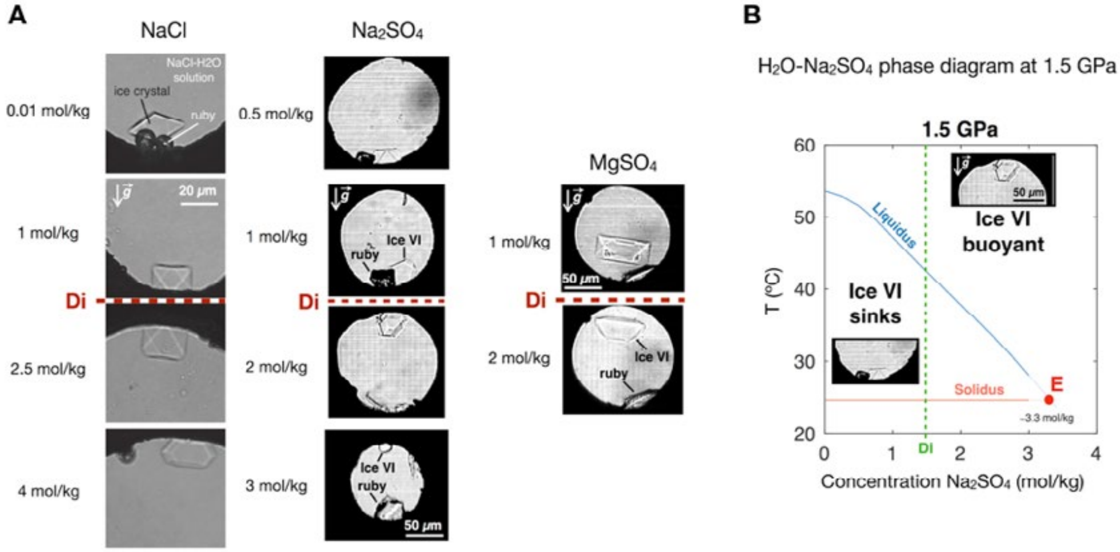



Figure 10. A) *In situ* observations of the density inversions between ice VI and different concentrations of salty aqueous fluids. Dashed lines show when the density inversion (Di) is observed in these different systems. B) Example of the new phase diagram derived from the melting curve and eutectic data, showing the predicted composition of the eutectic in the $H_2O$-$Na_2SO_4$ system at 1.5 GPa. The observed density inversion is also reported here to illustrate the complex phase thermodynamic and buoyancy behavior.

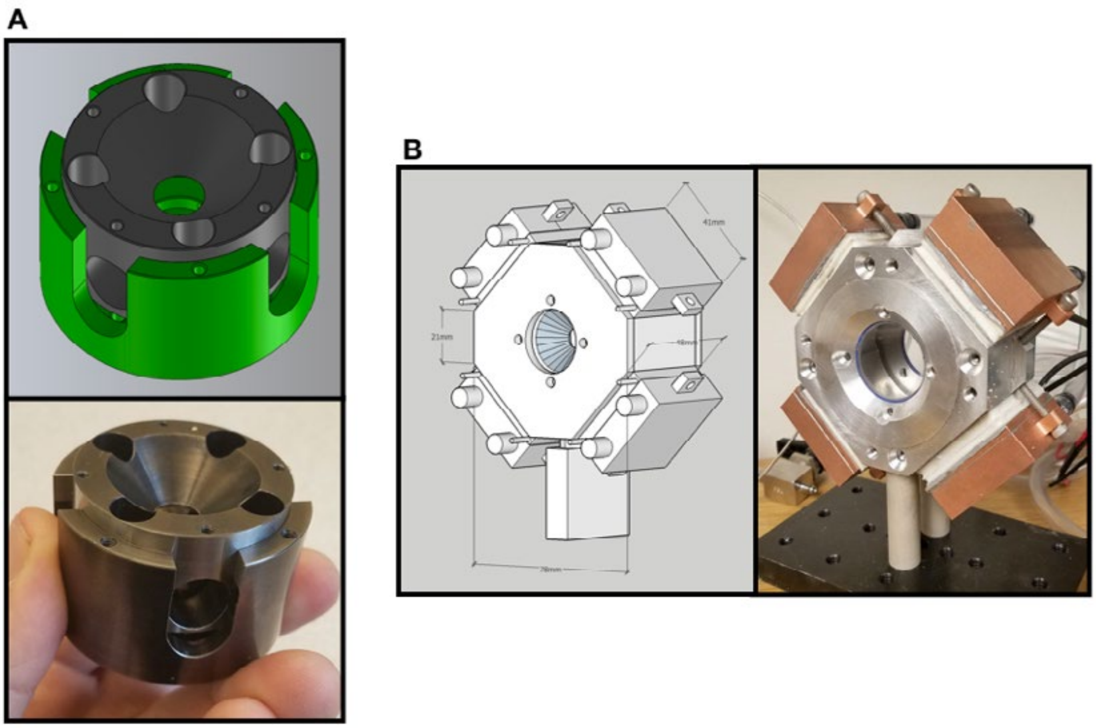



Figure 11. A) Computer generated technical drawing and photograph of the final diamond anvil cell after machining and heat treatment. B) Technical drawing and photograph of the cryostat apparatus, showing the large opening allowing important optical and X-ray access to the sample.

**Collaborations, Extended Scientific Directions, Flight Mission Involvement**

Over the past few years Icy World's INV 2 investigators have formed a strong collaboration with researchers in the University of Tulsa Chemistry department to develop 3D printable experimental setups in which to simulate prebiotic vent systems. Hydrothermal simulations are often expensive and complicated to set up in a lab, and the chimney growth experiment that the Icy Worlds team has developed has been in demand by other labs to test various permutations of the system. One of our near-term goals is to release a 'blueprint' for a 3D printable device that will allow other labs to run similar experiments in realistic alkaline hydrothermal temperature gradients.

Other significant collaborations this year include: INV 2 hosted two NAI Early Career Collaboration Award recipients, a graduate student from U. of Tulsa (to test methods for analyzing redox mechanisms in iron hydroxide systems) and a graduate student from Penn State (for studies of organic compartmentalization in hydrothermal chimneys). In 2018-2019 we are hosting a sabbatical visitor (Dr. Jason Pagano from Saginaw Valley State University) who is working on organic incorporation within hydrothermal chimneys; various graduate and undergraduate students working with INV 2 researchers have presented at conferences; and INV 2 researchers and students have participated in many outreach activities.

Recent work by the Icy World's INV 3 investigators on seismic and other geophysical investigations of habitability had numerous influences on NASA's outlook for future missions. Our work was included in a white paper for the NRC Astrobiology Future report. We also advocated for a sensitive seismometer on the model payload for the Europa Lander concept. Mark Panning joined our team at JPL, and continues to aid in developing concepts for future planetary seismology. Based on work with coauthor and INV3 Co-I Bruce Bills, Vance advocated successfully for the Europa Clipper to adopt a broader definition of ocean salinity. The associated paper (Vance *et al.,* JGR, 2018 see the publication list for a complete citation) led to a NESSF grant for UW Physics graduate student Marshall Styczinski, who worked with Vance and Bills as a summer student.

At JPL, INV 3 researchers are nearing completion of a new system for measuring sound speeds in simulated ocean world materials, based on the one at the University of Washington. They will begin using this system in FY19 to characterize the same aqueous systems as above, but in the presence of ammonia that is probably present in the oceans of Enceladus and Titan.

# Icy Worlds: 2018 Publications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## Team Members

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Jason Goodman
Robert Hodyss
Paul Johnson
Jeffrey Kargel
Jun Kimura
Giles Marion
Victoria Meadows
Kenneth Nealson
Robert Pappalardo
Michael Russell
Takazo Shibuya
Christophe Sotin
Shino Suzuki
Ken Takai
Steve Vance
David VanderVelde


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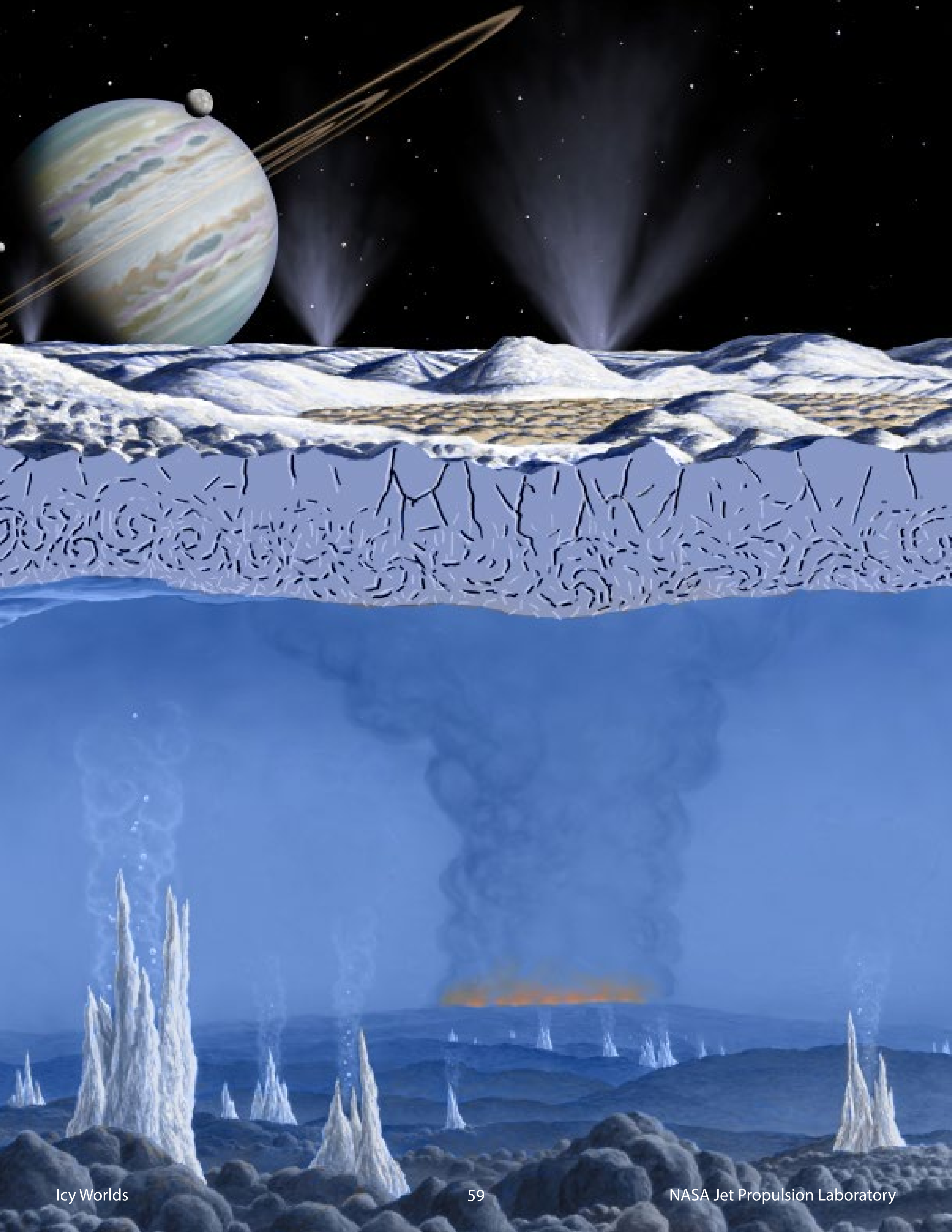


# Habitability of Hydrocarbon Worlds: Titan and Beyond

**Lead Institution:**
**NASA Jet Propulsion Laboratory**

**Principal Investigator:**
Rosaly Lopes

## Team Overview

Our team explores the potential biochemical pathways for organic materials extending from the atmosphere down to the potentially habitable ocean and for any extant chemical biosignatures to ascend from the ocean to the surface and atmosphere. The goals of the team are to:

(i) Determine the pathways for organic materials to be transported (and modified) from the atmosphere to surface and eventually to the subsurface ocean (the most likely habitable environment),
(ii) Determine whether the physical and chemical processes in the ocean create stable habitable environments,
(iii) Determine what biosignatures may be produced if the ocean is inhabited, and
(iv) Determine how biosignatures can be transported from the ocean to the surface and atmosphere and be recognized at the surface and in the atmosphere.

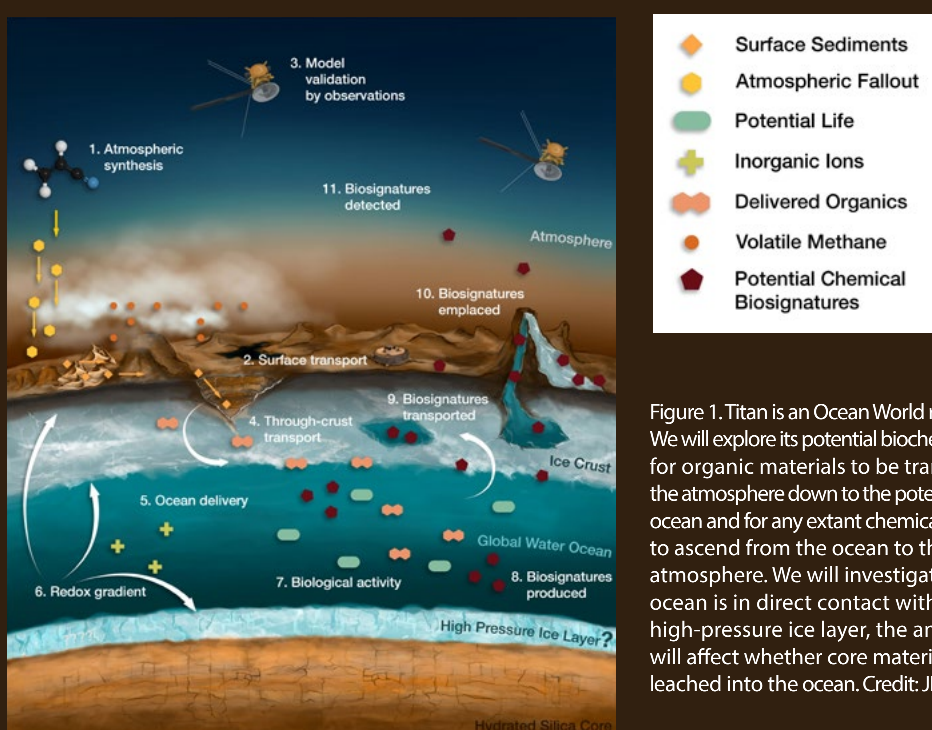
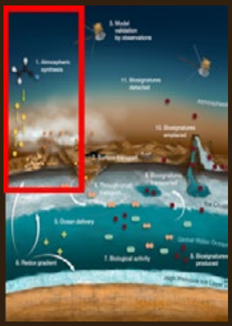


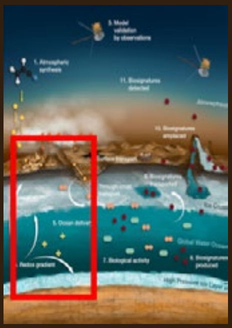

Figure 1. Titan is an Ocean World rich in organics. We will explore its potential biochemical pathways for organic materials to be transported from the atmosphere down to the potentially habitable ocean and for any extant chemical biosignatures to ascend from the ocean to the surface and atmosphere. We will investigate whether the ocean is in direct contact with the core or a high-pressure ice layer, the answer to which will affect whether core materials are directly leached into the ocean. Credit: JPL/A. Karagiotas

**Objective 1: Transfer of Organics from Atmosphere to Ocean** (Lead: Steve Vance).
The goal of Objective 1 is to determine pathways for organic materials to be transported and modified from Titan's atmosphere to the surface and eventually to the subsurface ocean (the most likely habitable environment).



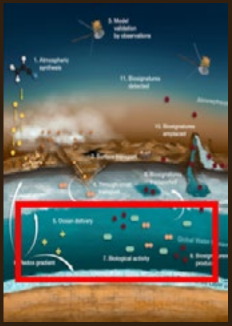
**Investigation 1.1:** Atmospheric Chemistry and Dynamics (Lead: Conor Nixon)
**Investigation 1.2:** Molecular Transport across Titan's Surface: (Lead: Alex Hayes)
**Investigation 1.3:** Molecular pathways: Surface to ocean (Lead: Christophe Sotin)
**Investigation 1.4:** Habitats resulting from molecular transport (Lead: Steve Vance)

**Objective 2: Ocean Conditions and Habitability** (Lead: R. Hodyss)
The goal of Objective 2 is to determine whether the physical and chemical properties in Titan's ocean can lead to the creation of stable habitable environments. Two investigations are designed to determine whether the physical and chemical processes in the ocean create stable, habitable environments.



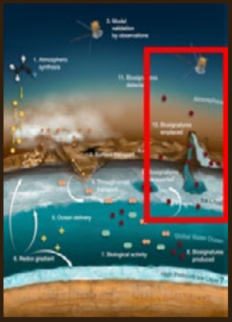
**Investigation 2.1:** Ocean Habitats (Lead: Chris Glein)
**Investigation 2.2:** Ocean Organic Alteration (Lead: Rob Hodyss)

**Objective 3: Oceanic Biosignatures** (Lead: D'Arcy Meyer-Dombard)
The goal of Objective 3 is to determine what biosignatures might be produced if Titan's subsurface is inhabited. We examine whether life could survive and build biomass in Titan's subsurface ocean conditions. We focus on understanding the physiological changes of hypothetical microbes, and the resulting chemical biosignatures to a high-pressure and low-temperature environment.



**Investigation 3.1:** Oceanic Biotic Survivability and Growth (Lead: D'Arcy Meyer-Dombard)
**Investigation 3.2:** Oceanic Biosignatures (Lead: Fabien Kenig)

**Objective 4: Transfer of Organics from Ocean to Surface** (Lead: Sarah Fagents)
The goal of Objective 4 is to determine how biosignatures can be transported from the ocean to the surface and atmosphere and be recognizable at the surface and in the atmosphere.



**Investigation 4.1:** Molecular Pathways: Ocean to Surface (Lead: Sarah Fagents)
**Investigation 4.2:** Molecular Alteration During Transport (Lead: Rob Hodyss)
**Investigation 4.3:** Habitats Resulting from Molecular Transport (Lead: Steve Vance)
**Investigation 4.4:** Biosignature Detection (Lead: Mike Malaska)

**Connection to Astrobiology:** We will address the **"Enhance NASA's missions"** goal by identifying possible biosignatures on Titan. We will **"Foster Interdisciplinary Science"** by bringing laboratory and theory to understand Titan observational results. Our tasks are relevant to two major topics identified in the 2015 Astrobiology Strategy document: **Identifying, exploring, and characterizing environments for habitability and biosignatures (Chapter 5)** and **Constructing Habitable Worlds (Chapter 6)**. In particular, we will most directly address the following questions: 5.1. ***How can we assess habitability on different scales?*** The regional and local chemical or geological telltales of life and habitability, either at Titan's surface or in the atmosphere, are unknown, but are unlikely to be produced in place in a single step, or to be found in pristine form, unaltered by their surroundings or their transport pathways. For an observatory, orbiter, or *in situ* mission to detect, confirm, and characterize habitable environments on Titan, understanding of the interrelationships of regions on Titan, how they respond to Titan's seasonal cycle, and how they have changed through time are required. 5.2. ***How can we enhance the utility of biosignatures to search for life in the solar system and beyond?*** *In situ* or remote investigations must be prepared to look for multiple types of biosignatures: stable isotopes; chemistry; organic matter; minerals; microscopic and macroscopic structures; temporal variability; and surface or atmospheric reflectance, emission, absorption. In contrast to Mars, Titan may have less similar signatures when compared to Earth. 5.3. ***How can we identify habitable environments and search for life within the solar system?*** We seek to understand the processes of degradation or preservation of physical, biogeochemical, and isotopic biosignatures on Titan, in order to refine future exploration. Our investigations chart a course for using the recent trove of Titan science data to understand the context for possible life, and for identifying signatures of life and its environments for Earth observatories or future robotic missions to seek.

# 2018 Executive Summary

We are a new team, funded in mid-2018. We have made progress mostly in the investigations in Objective 1 (Transfer of organics from Atmosphere to Ocean), in agreement with our proposed schedule. We held our first in-person team meeting in January 2019, at the Jet Propulsion Laboratory.

Unlike other ocean worlds in our solar system, Titan is known to have an abundance and great variety of organic molecules that are continuously produced in its atmosphere and transported across its surface. Titan's atmosphere is thus a source of potential nutrients and chemical energy for life in its interior. Investigation 1.1 focuses on improving our knowledge of the chemical content of the atmosphere, contributing to the surface inventory required for Investigation 1.2, which addresses molecular transport across the surface.

Investigation 1.1 consists of numerical modeling of chemistry and transport from the surface to ~1350 km, and validation of the model using observational data. We highlight here the retrieval of abundances of hydrocarbon and nitrile species in Titan's upper atmosphere from Cassini UVIS, which will be used to obtain reliable profiles of atmospheric composition, allowing exploration of Titan's upper atmosphere over season, latitude, and longitude.

On the observational side, we proposed to use all available datasets to measure Titan's molecular inventory, including Cassini's Composite Infrared Spectrometer (CIRS) observations and sub-mm spectra from ALMA. Using ALMA, we have measured distinct spatial variations in Titan's trace gas species created through the photodissociation of $CH_4$ and $N_2$. The minor constituents visible with ALMA include potentially important building blocks for larger biomolecules, such as proposed 'azotosomes' made of spherical agglomerations of acrylonitrile ($CH_2CHCN$). We have also made the first detection of mono-deuterated methane ($CH_3D$) in the sub-millimeter wavelength range, which provides a method for investigating latitudinal variations of Titan's $CH_4$ abundance. We have also measured spatial and seasonal variations in $C_3H_x$ hydrocarbon abundance in Titan's stratosphere using data from Cassini CIRS. We have made the first measured abundance profiles of propene ($CH_3CH=CH_2$) on Titan from radiative transfer modeling, and compared our measurements to predictions derived from several photochemical models. Additionally, using newly corrected line data, we determined an updated upper limit for allene ($H_2C=C=CH_2$). These measurements will further constrain photochemical models by refining reaction rates and the transport of these gases throughout Titan's atmosphere.

Investigation 1.2 consists of developing a landscape evolution model to understand how sediments are transported across the surface of Titan to identify likely regions where materials of astrobiological interest may collect (e.g., large deposits composed of HCN sediments), and comparing results with Cassini Visible and Infrared Mapping Spectrometer (VIMS) data. The analysis of VIMS data enables the extraction of pure surface albedos using a radiative transfer code to determine the contributions of atmospheric haze. We have investigated the characteristics of a selection of small northern lakes that have raised ramparts around their perimeters. We provide two plausible theories for the formation of these unique structures, one of which suggests that liquid material could percolate into the subsurface, indicating a connection of the surface with the subsurface/crust. Our work has also generated an updated estimate of the total amounts and locations of organic deposits on Titan, which will help constrain the lifetime of deposits on the surface.

A recent publication by Miller *et al.* uses geochemical constraints to propose a new model for the origin of Titan's atmosphere. The central argument is that Titan should have accreted abundant organic material from cometary building blocks. The implication of producing Titan's atmosphere from organics is that the core would have been warm, thus outgassing from the core could have delivered volatiles and light organics to the subsurface ocean. If this is the case, then Titan's ocean could receive organics from two potential sources: photochemistry above, and core processing below. This work was featured in a number of news articles such as https://www.sciencenews.org/article/titan-oddly-thick-atmosphere-may-come-cooked-organic-compounds

Our team members are active in the planetary community and convened three relevant sessions at the Fall 2018 AGU (American Geophysical Union) annual conference. We are currently convening sessions for 2019 meetings. A key component of our work is education and outreach. Co-I M. Boryta, a Professor at Mount San Antonio Community College, is in the process of identifying suitable students to become summer interns at JPL to help with our project. We have also reached students by giving seminars at universities, and the public by giving public talks and interviews (e.g. http://www.hawaiinewsnow.com/story/38436846/nasa). The University of Illinois in Chicago issued a press release (https://today.uic.edu/uics-mission-to-model-life-on-saturns-moon-in-the-lab) about this work. PI R. Lopes was honored with the Ambassador Award from the AGU in 2018.

# Project Reports

## Transfer of Organics from Atmosphere to Ocean

**Investigation 1.1:** Atmospheric Chemistry and Dynamics (Lead: Conor Nixon): What is the chemical content of the atmosphere and its contribution to the surface inventory? This investigation focuses on improving our knowledge of the chemical content of the atmosphere, which contributes to the surface inventory required for Investigation 1.2.

ALMA Observations and results (A. Thelen, C. Nixon, M. A. Cordiner): To study Titan's atmospheric chemistry and circulation beyond the end of the Cassini era, we use the Atacama Large Millimeter/submillimeter Array (ALMA), which offers unprecedented views of Titan from the ground. Utilizing frequent flux calibration measurements of Titan and dedicated observations during the summer equinox, in tandem with a targeted Cassini flyby, we have measured distinct spatial variations in Titan's trace gas species created through the photodissociation of $CH_4$ and $N_2$ (Figure 2 A, B). The minor constituents visible with ALMA include potentially important building blocks for larger biomolecules, such as 'azotosomes' made of acrylonitrile ($CH_2$=CHCN). We have also made the first detection of mono-deuterated methane ($CH_3D$) in the sub-millimeter range, which provides a method of investigating latitudinal variations of Titan's $CH_4$ abundance (Figure 2 C). Results are published in Thelen *et al.* (2019) and were presented at the 2018 Fall AGU and DPS (Division of Planetary Sciences) meetings.

Retrieval of Abundances of Hydrocarbon and Nitrile Species in Titan's Upper Atmosphere from Cassini UVIS (S. Fan, Y. Yung): An innovative analytic method was applied to Titan occultation measurements obtained by the Cassini UVIS experiment. To illustrate the methodology, an occultation observation made during flyby T52 was analyzed, when the Cassini spacecraft had insufficient attitude control. For this reason, analysis of data to date was limited to only three occultations out of tens of valid Cassini UVIS observations. The new approach corrects for the effect of pointing drift by forward modeling the Cassini/UVIS instrument response function with the pointing drift value obtained from the SPICE C-kernel along the spectral dimension. The Markov Chain Monte-Carlo method is used to retrieve the line-of-sight abundances of eleven species ($CH_4$, $C_2H_2$, $C_2H_4$, $C_2H_6$, $C_4H_2$, $C_6H_6$, HCN, $C_2N_2$, $HC_3N$, $C_6N_2$ and haze particles) in the spectral vector fitting process. For $C_2H_6$, $C_2N_2$ and $C_6N_2$ only upper limits are obtained over a wide range of altitudes. This is the first time that abundances of major hydrocarbon and nitrile species throughout the Titan upper and middle atmosphere have been derived from the T52 occultation (Figure 3), which was not previously examined because of pointing motion. With this new method, all of the occultations obtained over the entire Cassini mission, with rare exceptions, could yield reliable profiles of atmospheric composition, allowing exploration of Titan's upper atmosphere over season, latitude, and longitude. A paper has been submitted to Earth and Space Science and is currently under review (S. Fan, D. E. Shemansky, C. Li, P. Gao, L. Wan and Y. L. Yung: Retrievals of Abundances of Hydrocarbons and Nitrile Species in Titan's upper Atmosphere).

Spatial and seasonal variations in $C_3H_x$ hydrocarbon abundance in Titan's Stratosphere from Cassini CIRS observations (N. Lombardo, C. Nixon, P. Irwin): Of the $C_3H_x$ hydrocarbons, propane ($C_3H_8$) and propyne (methylacetylene, $CH_3C_2H$) were first detected in Titan's atmosphere during the Voyager 1 flyby in 1980. Propene (propylene, $C_3H_6$) was first detected in 2013 with data from the Composite InfraRed Spectrometer (CIRS) instrument on Cassini. We have made the first measured abundance profiles of propene on Titan from radiative transfer modeling, and compare our measurements to predictions

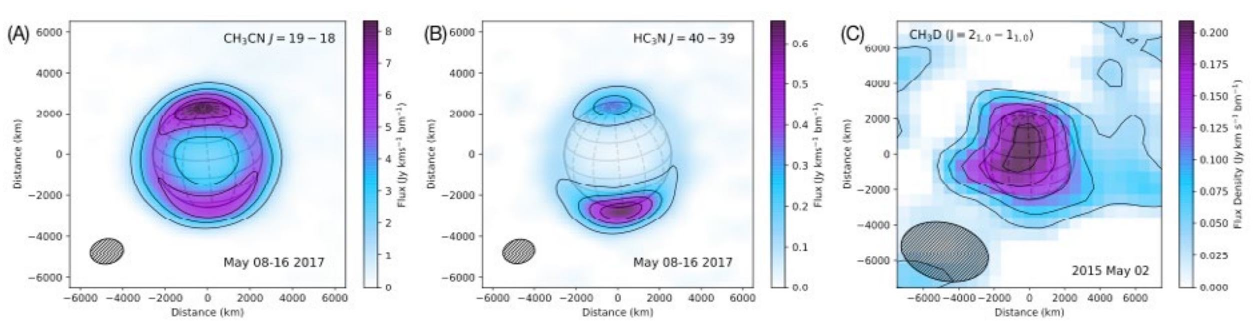



Figure 2. Integrated flux maps of Titan from ALMA observations from Cordiner *et al.* (in prep. 2019 A, B) and Thelen *et al.* (in prep. 2019 C). Contours in maps of $CH_3CN$ (A) and $HC_3N$ (B) are in intervals of Fm/5, where Fm is the maximum flux of each image cube. $CH_3D$ (C) map contours are in 1-sigma levels. The ALMA resolution element is shown as a hashed ellipse in the lower left of each image. Titan's longitude and latitude lines are shown as gray dashed and solid lines, in increments of 30 degrees and 22.5 degrees, respectively. Credit: NASA GSFC

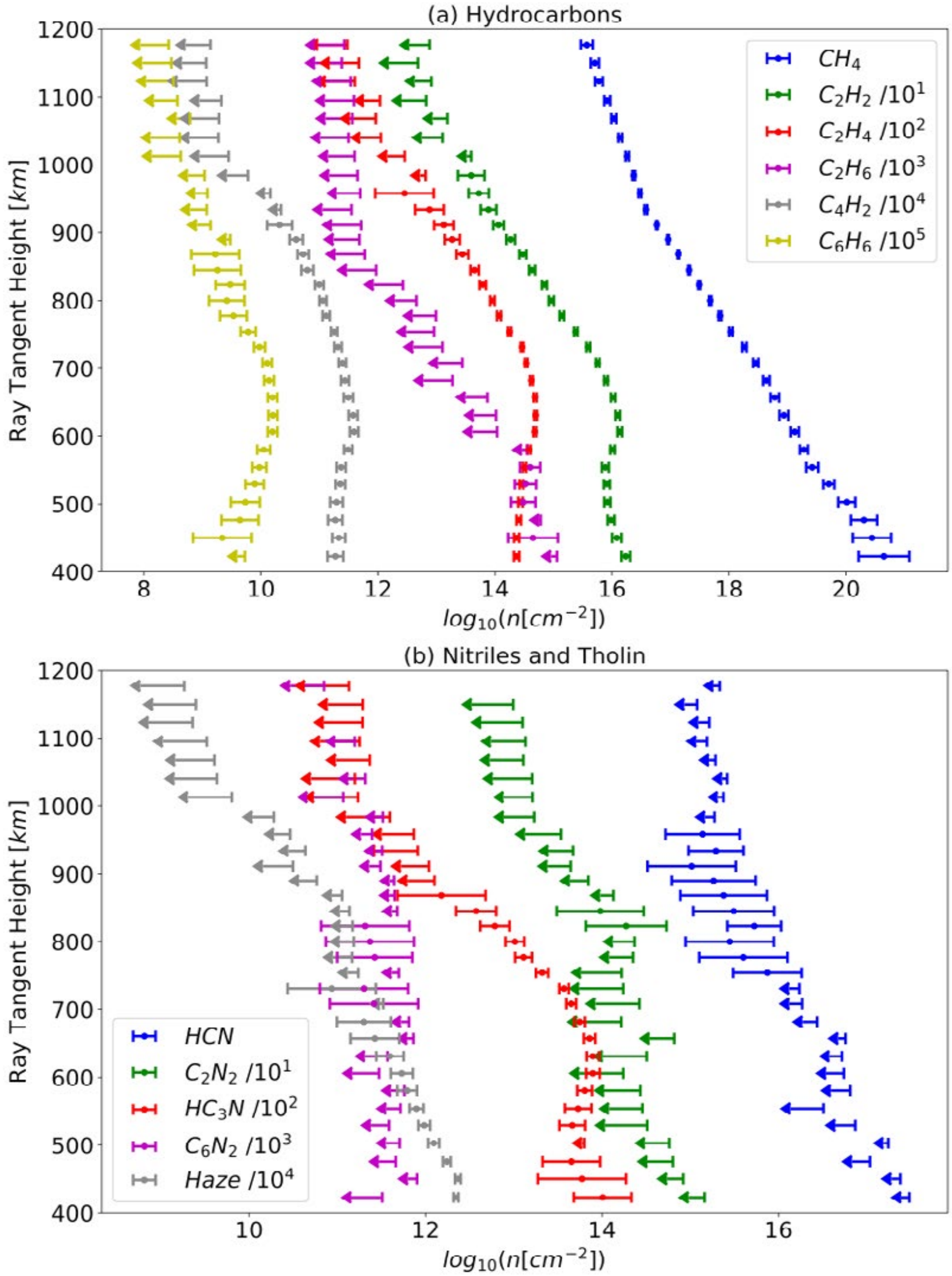



Figure 3. Vertical profiles of the logarithm of LOS abundances retrieved from T52 occultation observations. Some species are offset by a few orders of magnitude for the purpose of presentation. Points with error bars denote well-constrained values, while arrows denote upper limits. Haze particles are assumed to be 12.5 nm spheres with the same optical properties as their laboratory analog "tholin" (Khare *et al.* 1984).

derived from several photochemical models. Near the equator, propene is observed to have a peak abundance of 10 ppbv at a pressure of 0.2 mbar. Several photochemical models predict the amount at this pressure to be in the range 0.3–1 ppbv and also show a local minimum near 0.2 mbar which we do not see in our measurements. We also see that propene follows a different latitudinal trend than the other $C_3$ molecules. While both propane and propyne concentrate near the winter pole, transported via a global convective cell, propene is the more abundant of the two above the equator. Additionally, using newly corrected line data, we determined an updated upper limit for allene (propadiene, $CH_2{=}C{=}CH_2$, the isomer of propyne $(CH_3CH)$). We find a 3-σ upper limit mixing ratio of $2.5 \times 10^{-9}$ within 30° of the equator. The measurements will further constrain photochemical models by refining reaction rates and the transport of these gases throughout Titan's atmosphere. Results were published in Lombardo *et al.* (2019).

**Investigation 1.2:** Molecular Transport across Titan's Surface (Lead: Alex Hayes): How are molecules transported across the surface and deposited/modified? We develop landscape evolution models to predict organic deposit locations across the surface.

To identify locations of astrobiological interest, we need to understand the production, transport, and modification of organic materials across Titan's surface. We will accomplish this by constructing a general, parametrized model that will describe the pathways that route and modify organic sediments throughout Titan's atmosphere-surface-subsurface system. The model will be driven by inputs that include atmospheric organic production (Investigation 1.1), wind and precipitation patterns from a general circulation model, and laboratory experiments to determine solubilities of organic molecules in Titan's hydrocarbon liquids. Regional-scale landscape evolution models will be run to ensure that the necessary physics is properly parametrized for the Titan environment. To validate the model, we will compare simulated landscapes to Cassini-Huygens data. Our end result will be landscape evolution models that identify likely regions where materials of astrobiological interest collect (e.g., large deposits composed of HCN sediments), that will serve as inputs for Inv. 1.3 and also Inv. 4.4.

<u>Progress on Investigation 1.2: Landscape Evolution (A. Hayes *et al.*)</u>:Postdoc Sam Birch is being partially funded at Cornell to work on this task, and is collaborating with researcher Orkan Urmuhan at the SETI Institute. The DELIM code has been re-written from Fortran into Matlab and is being optimized to run faster. A new routine in the model accounts for all flow paths across the landscape, generating a map that we can use to route sediment and fluids. This has given us a significant increase in computational speed and will also permit us to later parallelize the code.

<u>Surface composition (A. Solomonidou *et al.*)</u>: A geologic map of the major surface units on Titan has been completed and the paper submitted to Nature Astronomy and is currently under review (Lopes *et al.*: A Global Geomorphologic Map of Saturn's Moon Titan). New analyses of Cassini-Huygens data on surface composition will serve as a validation of the landscape evolution model. We have followed on from the previous work (Solomonidou *et al.,* 2018) which investigated Titan's low-latitude and mid-latitude surface using spectro-imaging near-infrared data from Cassini VIMS (Visual and Infrared Mapping Spectrometer)and showed a latitudinal dependence of Titan's surface composition, with water ice being the major constituent at latitudes beyond 30°N and 30°S, while Titan's equatorial region appears to be dominated partly by a tholin-like material or by another very dark unknown material. We are now expanding the analysis to higher latitudes, where suitable VIMS data are scarcer. In particular, we investigated the characteristics of a selection of Titan's small northern lakes that have raised ramparts around their perimeters using Cassini VIMS and RADAR (Radio Detection and Ranging) data. Ramparts are radar-bright mounds that extend from the shores of some lakes out for up to tens of kilometers (Figure 4). A paper was submitted to Icarus and is currently under review (Solomonidou *et al.*: Spectral and emissivity analysis of the raised ramparts around Titan's northern lakes), in which we report that the raised ramparts exhibit spectral and emissivity characteristics that

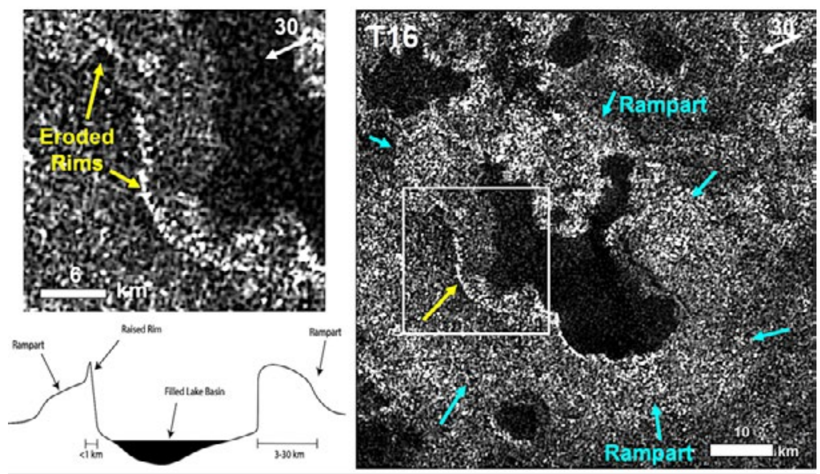



Figure 4. Top right: SAR (Synthetic-aperture radar) image of Viedma Lacus. Cyan arrows denote portions of the perimeter of the rampart feature, a SAR-bright apron that encloses nearly the entire lake. Yellow arrows denote portions of the raised rim. Top Left: The raised rim portion of the lake perimeter, denoted by the white box in the right image. The rim appears eroded in multiple sections. Bottom left: Conceptual model of a lake with a rampart and rim (not to scale). Rims are confined to within ~1 km of the lake, and form higher slopes, while ramparts enclose the lake and form broader (up to 10's of km) mounds. Credit: NASA/JPL/A. Solomonidou/M. Malaska

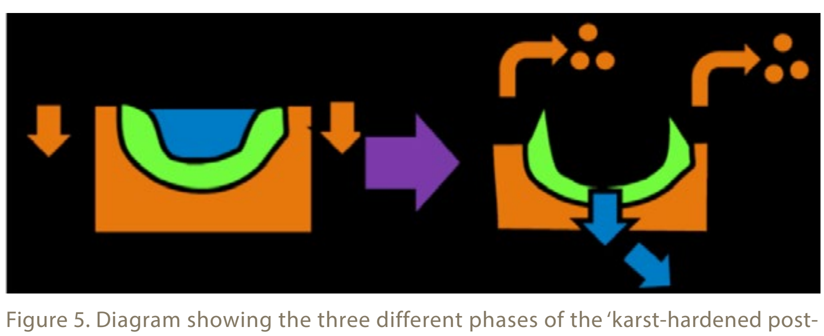


Figure 5. Diagram showing the three different phases of the 'karst-hardened post-deflation remnant' theory: accumulation, case hardening, and deflation that form the raised ramparts (green), on top of the crust (orange) after the lake liquid (blue) percolates into the subsurface. Credit: A.Solomonidou/M.Malaska

are distinct from the surrounding terrain but are similar to those of the floors of empty lakes. This suggests that both units are made or at least covered by the same material, possibly indicating a connection in their formation. We provide two plausible theories for the formation of these unique structures, both of which present estimated relative ages for the different regions of interest based on possible emplacement and layering, thus providing information about the relationship between the atmosphere and the surface and the atmospheric deposition rates (Figure 5). In addition, one of the formation theory mechanisms suggests that liquid material could percolate into the subsurface, indicating a connection of the surface with the subsurface/crust. This investigation is significant because it provides insights into the formation of the unique features of raised ramparts and by extension to the nature of Titan's surface. This work is part of investigations 1.2 and 4.4 as it provides clues to the composition of the surface in addition to geodynamics.

Other work on Investigation 1.2 has generated an updated estimate of the total amounts and locations of organic deposits on Titan, which will help constrain the lifetime of deposits on the surface. In addition, the comparison between the predicted and observed solid amounts and ethane amounts can show potential sequestration which in turn can imply transport processes to crustal reservoirs. A publication in final stages of preparation, led by Deputy PI M. Malaska, describes the labyrinth terrains as important repositories of organic material. Observed characteristics of dissection of these canyonlands will directly feed into landscape evolution models.

**Investigation 1.3:** Molecular Pathways: Surface to Ocean (Lead: Christophe Sotin): What are the pathways for molecules to be transported from the surface to the ocean? We investigate the mechanisms by which material on the surface can be brought to the ocean. We model tectonic overturn in the ~50-80 km thick icy lithosphere and per form laboratory experiments to examine the chemistry in water ice/organic mixtures under T/P in during transport.

Progress in Investigation 1.3: We have acquired and installed a Supercritical Fluid Technologies Phase Monitor II (Figure 6). This instrument is comprised of a high pressure (to 70 MPa), temperature-controlled cell with video monitoring, to enable experiments on the solubility of small organics in liquid hydrocarbons. These experiments will help us constrain the amount of materials that can be transported through Titan's crust. We are beginning preliminary experiments with the instrument utilizing supercritical $CO_2$, followed by butane/acetonitrile. We started modeling convection processes in the icy crust that overlays the ocean. We first run 1D scaling law models to investigate the effect of a layer of clathrates which has a thermal conductivity 10 times smaller than ice at very low temperatures. In parallel, we are setting up a two phase code that includes several complexities including the composite creep law for ice.

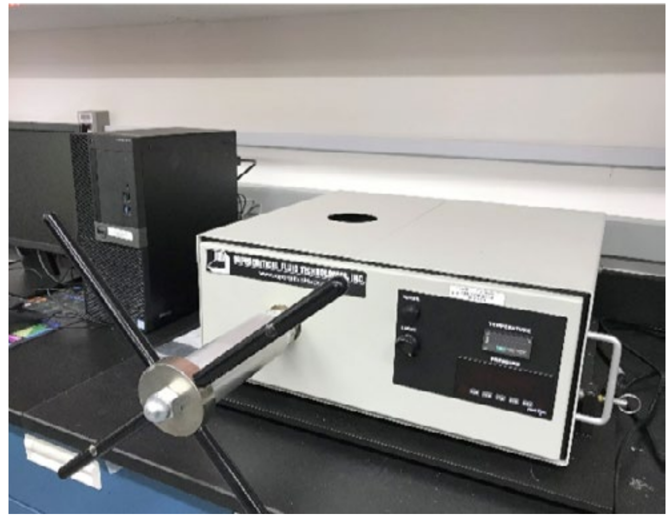


Figure 6. The Supercritical Phase Monitor II installed in the Cryogenic Chemistry Lab. Credit: R. Hodyss

**Investigation 1.4:** Habitats resulting from molecular transport (Lead: Steve Vance): Does organic transport to the subsurface ocean result in habitable environments along the pathway? The culmination of Objective 1 will be to assess how habitable environments might occur in regions in Titan's lithosphere where liquid water is scarce, as materials from different regions are combined and transformed by natural processes.

Progress in Investigation 1.4: A major part of this effort is to constrain the fluxes of materials into Titan's ocean. It seems likely that hydrocarbons at Titan's surface are also present in its ocean, either from transport through the ice or decay of organics that formed in Titan's ocean long ago. To constrain the inventories of dissolved hydrocarbons in Titan's ocean, we first set out to create geochemical tools for computing their stability in water. Co-I Sugata Tan has completed data assimilation for adding the aqueous solubility of small hydrocarbons ($CH_4$, $C_2H_6$, $C_3H_8$, $C_2H_4$, $C_2H_2$) to the CRYOCHEM sofware package. This work has improved the existing parameterizations for the aqueous solubility of both $CH_4$ and $C_2H_6$.

B. Journaux, and S. Vance conducted a synchrotron experiment at the European Synchrotron in late November to study high-pressure ice structures and equilibrium with salts and $CO_2$. This work is important for understanding the varying phase changes and layers that could exist in Titan's interior ice shells. The presence of these layers, and melting within them due to impurities, may aid or inhibit transport of organics to the subsurface ocean. During the synchrotron work, Vance incorporated Journaux's updated equations of state for water ice (Journaux *et al.* in prep) and NaCl (Bollengier *et al.* in prep) into the PlanetProfile model.

Team members presented preliminary results at the recent 2018 Fall American Geophysical Union meeting in Washington, DC. Steve Vance presented updated interior structure models for Titan using thermodynamics developed by our team. J. Lunine presented on Structure I-II clathrate behavior that might aid Titan's outgassing and could provide a sink for hydrogen.

## Ocean Conditions and Habitability

**Investigation 2.1:** Ocean Habitats (Lead: Chris Glein) What are the possible habitable environments in the subsurface ocean, and what are the conditions in these environments? In this investigation, we use modeling and lab experiments to determine if likely fluxes of reductants and oxidants could be present at different interfaces in the interior, and are suitable to support Earth-like life.

Modeling of Melting and Water Transport (K. Kalousova, C. Sotin): We are investigating the conditions for melting and water transport through the high-pressure (HP) ice layer of Titan using a numerical model of two-phase convection. We find that liquids can be generated at the interface between Titan's silicate core and its HP ice layer. Due to their lower density, they could be transported into the adjacent upper ocean by the hot upwellings. This process constitutes a possible means of transporting volatiles such as 40Ar, which is abundant in Titan's atmosphere, from the core into the ocean. Effect of salts and ammonia will be investigated in the next step. We will use a similar numerical approach to investigate the transport processes through Titan's outer Ice I crust with a particular emphasis on the effect of hydrocarbon clathrates (Investigations 1.3 and 4.1).

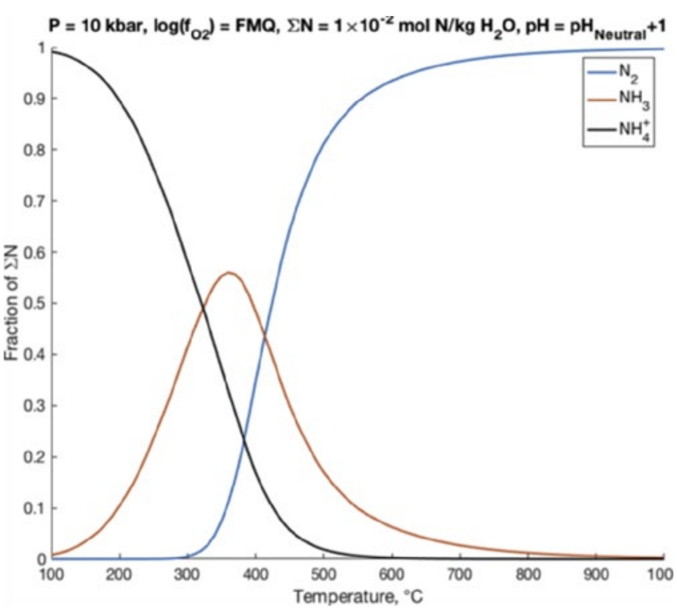



Figure 7. A significant fraction of Titan's $N_2$ can be generated in the rocky core under plausible geochemical conditions of temperature, pH, and oxidation state (Miller *et al.*, 2019).

Modeling of breakdown of primordial organic materials in Titan's core (Lead: C. Glein): A key objective in Inv. 2.1 on Ocean Habitats is to determine how much the core has exchanged materials with the liquid water ocean. Co-I Glein made substantial progress on this issue by coauthoring a new paper with his former postdoc that relates the breakdown of primordial organic materials in Titan's core into volatile species that could have contributed to the formation of Titan's atmosphere (Miller *et al.*, 2019). This novel idea helps to advance the discussion on the origin of Titan's atmosphere, a long-standing problem in planetary science. The paper presents the first thermodynamic calculations of nitrogen speciation in Titan's core (e.g., Figure 7), an advancement made possible by the Deep Earth Water model. In addition to this work, Glein performed kinetic calculations suggesting that radiolysis of water in the ocean could provide sources of chemical energy for possible life (Glein *et al.*, 2018: $H_2$ production from radiolysis in a subsurface ocean on Titan, Goldschmidt Conference, held August 12-17, 2018 in Boston, MA).

**Investigation 2.2:** Ocean Organic Alteration (Lead: Rob Hodyss): How are organic molecules altered in the subsurface ocean? How are organic molecules altered in the subsurface ocean? Results of Inv. 1 will provide a suite of organic molecules that can reach the ocean. These molecules will be evaluated in terms of their solubility, reactivity with water, and interaction with ice and minerals to determine their fate in the ocean.

Progress in Investigation 2.2: We have begun to compile reaction rates and product distributions from the literature on the hydrolysis of acetonitrile and acetylene. We have also set up the GC/MS system to be used for the hydrolysis experiments, and determined the proper column type and derivatization reagent for the experiments. We will use a standard 30 m DB-Wax column with MTBSTFA (methyltributylsilyi-N-methyltrifluoroacetamide) derivatization. This will allow for the analysis of the expected alcohol and amine products of the hydrolysis reactions.

## Oceanic Biosignatures

**Investigation 3.1:** Oceanic Biotic Survivability and Growth (Lead: D'Arcy Meyer-Dombard): Determine if microorganisms can survive and grow in Titan subsurface habitable zones. We seed experiments with samples (and thus organisms) from several appropriate Earth analog systems. The enrichment cultures can then be tested with a wide range of Titan boundary conditions.

**Investigation 3.2:** Oceanic Biosignatures (Lead: Fabien Kenig): What biosignatures could be produced in Titan's subsurface habitable zones? We use the experiments conducted in Inv. 3.1 to determine the isotopic and molecular biomarkers that result from the enrichment cultures, and understand the biochemical basis of their production.

Progress report for Objective 3: Investigation 3.1 begins with identifying potential piezophiles and hyperpiezophiles that can be used in the 'Adaptive Laboratory Evolution'(ALE) protocol. This protocol will slowly train strains of Bacteria and Archaea to grow at temperatures and pressures that are relevant to Titan's subsurface ocean environments. We have targeted strains that are know to require higher pressure and/or lower temperatures for growth, or that originated from Earth analog environments where higher pressures and lower temperatures are found. We have started by purchasing strains of Bacteria and Archaea that are available in culture collections such as the Japan Collection of Microorganisms (JCM) and the German collection Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). We are also obtaining strains from private laboratory collections, and using samples obtained from relevant Earth analog environments to enrich for extremophiles. Two undergraduates are working to prepare the necessary growth media for growing these strains, under the direction of our graduate student, Judy Malas. After successful propagation of these strains, we will begin the ALE process to train them to grow at low temperatures.

Investigation 3.2 is currently in the development stage. In the first year of the project, we are building and testing the very high pressure growth chamber that will be needed to achieve growth of organisms at the pressures relevant to Titan's subsurface. This is in progress. In addition, we have recruited a promising postdoctoral researcher, Olivier Bollengier, who is an expert in high pressure experiments. Olivier will begin working with us at UIC in May 2019, after his current postdoctoral appointment is finished. He will assist with the design and completion of the very high pressure chamber, and learn laboratory techniques associated with microbial culturing.

## Transfer of Organics from Ocean to Surface

Objective 4 examines the upwards transport of biosignature-hosting fluids from the ocean to the surface (Inv. 4.1), their possible chemical modification (Inv. 4.2) and formation of habitable niches (Inv. 4.3) along the way, as well as the requirements for detection of biosignatures at the surface and in the atmosphere (Inv. 4.4). Work on Objective 4 relies on results of previous objectives before it can get fully underway. The investigations are described below:

**Investigation 4.1:** Molecular pathways: Ocean to surface (Lead: Sarah Fagents): What are the mechanisms for upward transport of biosignatures from the ocean to the surface? We model upward transport of convecting ice and/or aqueous solutions through the ice crust.

**Investigation 4.2:** Molecular Alteration During Transport (Lead: Rob Hodyss): How Are Molecules Altered on Transport from the Subsurface Ocean to the Surface? We examine the chemistry that occurs as ocean fluids are subjected to the T/P conditions in the subsurface during transport to surface.

**Investigation 4.3:** Habitats Resulting from Molecular Transport (Lead: Steve Vance): Does transport to the surface result in habitable environments along the pathway? Are there signatures of these environments on the surface or in the atmosphere? We use results from Inv. 2.1 to consider the sources and sinks of oxidants specific to hydrocarbon-water chemistry.

**Investigation 4.4:** Biosignature Detection (Lead: Mike Malaska): How would we detect biosignatures that reach the surface and atmosphere? We will acquire laboratory near-infrared reflectance spectra of putative ocean fluids, both with and without biosignatures, in order to ascertain detectability. We will also acquire spectra of the chemical signatures resulting from ocean fluid/organic interactions studied in Inv. 4.2. These spectra will provide baseline measurements that may be useful to target selection in future missions.

Progress report for Objective 4: We have identified a number of potential mechanisms for fluid transport within Titan's ice shell, including fracturing from the base of the ice shell, tidally-induced fracturing at the surface, and the development of fluid reservoirs within the ice shell as a result, say, of thermal upwelling from the ice-ocean interface or of intrusive cryomagmatism. These mechanisms will be examined in the context of their compatibility with models of convection within the ductile ice layer (Investigation 1.3), and will provide constraints on the flux of biosignature-hosting fluids to the surface. Investigation 4.1 lead Fagents (University of Hawaii) will be joined by a postdoctoral fellow (L. Schurmeier) in June 2019 to begin an investigation into the mechanical nature of the ice shell (the depth to the brittle–ductile transition exploring the range of plausible model parameters), as well as examining the formation mechanisms and residence times of fluid reservoirs in the ice shell. A graduate student will join the group in Fall 2019. We have a publication in preparation, a review chapter on cryovolcanism led by Objective lead S. Fagents, to be submitted to the book Planetary Volcanism Across the Solar System (T.K.P. Gregg, R.M.C. Lopes, S.A. Fagents, eds.), Elsevier. This book chapter will review models of cryomagma transport and serves as a starting point for Investigation 4.1.

In Investigation 4.2, we will examine the chemistry and partitioning that occurs as ocean fluids are transported to the surface. There are two major tasks, the determination of solubilities for the major ocean constituents (Task 4.2.1), and experiments on the reaction of ammonia with hydrogen cyanide to form prebiotic organic molecules (Task 4.2.2). Both tasks are scheduled for the 4th and 5th year of the project. However, we have already begun proof-of concept experiments relating to Task 4.2.2.

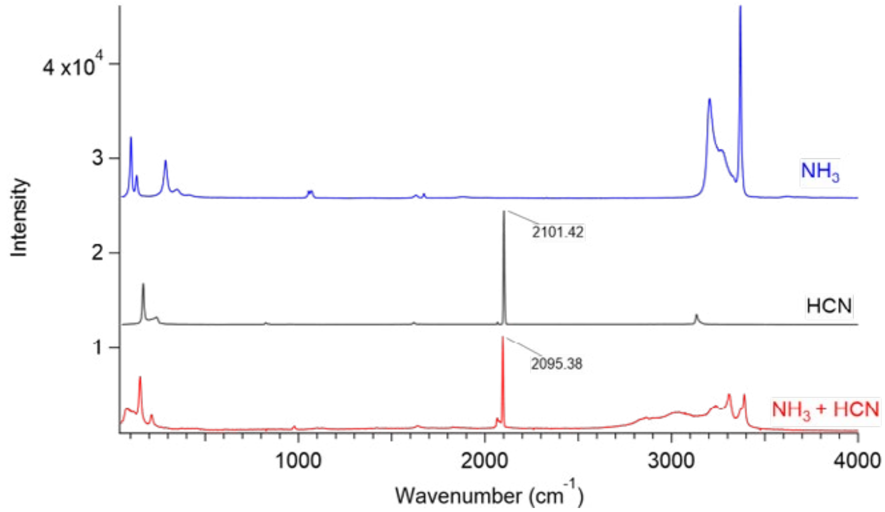



Figure 8. Raman spectroscopy reveals the formation of ammonium cyanide, a precursor to more complex prebiotic reaction, in the reaction between hydrogen cyanide and ammonia at cryogenic temperatures.

We conducted initial experiments on the reaction of ammonia with hydrogen cyanide to form ammonium cyanide at cryogenic temperatures. Figure 8 shows Raman spectra of pure ammonia, pure hydrogen cyanide, and a sequentially deposited film of hydrogen cyanide followed by ammonia. All spectra were acquired at 100 K. In the mixed film, new, broad bands appearing at 3050 $cm^{-1}$ and 2850 $cm^{-1}$ are consistent with the formation of the ammonium ion ($NH_4^+$). The shift in the position of the C-N stretch from 2101 $cm^{-1}$ in the pure HCN film to 2095 $cm^{-1}$, consistent with the formation of $CN^-$. These experiments indicate the formation of $NH_4CN$ at low temperature, and validate our methods for studying this reaction.

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Khare, B. N., Sagan, C., Arakawa, E. T., Suits, F., Callcott, T. A., & Williams, M. W. (1984). Optical constants of organic tholins produced in a simulated Titanian atmosphere: from soft X-ray to microwave frequencies. Icarus, 60, 127-137. DOI: 10.1016/0019-1035(84)90142-8

Solomonidou, A.; A. Coustenis, R.M.C. Lopes, M. J. Malaska, S. Rodriguez, P. Drossart, C. Elachi, B. Schmitt, S. Philippe, M. Janssen, M. Hirtzig, S. Wall, C. Sotin, K. Lawrence, N. Altobelli, E. Bratsolis, J. Radebaugh, K. Stephan, R.H. Brown, S. Le Mouélic, A. Le Gall, E.V. Villanueva, J. F. Brossier, A.A. Bloom, O. Witasse, C. Matsoukas, A. (2018). Schoenfeld. The Spectral Nature of Titan's Major Geomorphological Units: Constraints on Surface Composition. Journal of Geophysical Research: Planets, 123. https://doi.org/10.1002/2017JE005477

# Flight Mission Involvement

**Europa Clipper**
Team Member: Steve Vance
Involvement: Science team member

**JUpiter ICy moons Explorer (JUICE)**
Team Member: Rosaly Lopes
Involvement: Co-I on JANUS camera

**Mars 2020**
Team Member: Rob Hodyss
Involvement: Science team member (PIXL)

**Cassini** (close out phase)
Team Members: Rosaly Lopes,
Mike Malaska, Alex Hayes
Involvement: RADAR associate team members; Lopes also Investigation Scientist

## Team Members

**Rosaly Lopes**
Michael Malaska
Jack Beauchamp
Sam Birch
Olivier Bollengier
Mark Boryta
J. Michael Brown
Martin Cordiner
Alvaro P. Crosta
Mohit M. Daswani
Sarah Fagents
Chris Glein
Alex Hayes
Rob Hodyss
Pat Irwin
Paul Johnson
Baptiste Journaux
Klara Kalousova
Isik Kanik
Jeff Kargel
Fabien Kenig
Jonathan Lunine
D'Arcy Meyer-Dombard
Claire Newman
Conor Nixon
Florian Schwandner
Anezina Solomonidou
Christophe Sotin
Sugata Tan
Nick Teanby
Alexis Templeton
Alexander Thelen
Orkan Umurhan
Steve Vance
Karen Willacy
Veronique Vuitton
Catherine Walker
Yuk Yung

# Habitability of Hydrocarbon Worlds: Titan and Beyond: 2018 Publications


Lombardo, N. A., Nixon, C. A., Achterberg, R. K., Jolly, A., Sung, K., Irwin, P. G. J., Flasar, F. M. (2019). Spatial and seasonal variations in $C_3H_x$ hydrocarbon abundance in Titan's stratosphere from Cassini CIRS observations. *Icarus* 317, 454-469. DOI: 10.1016/j.icarus.2018.08.027

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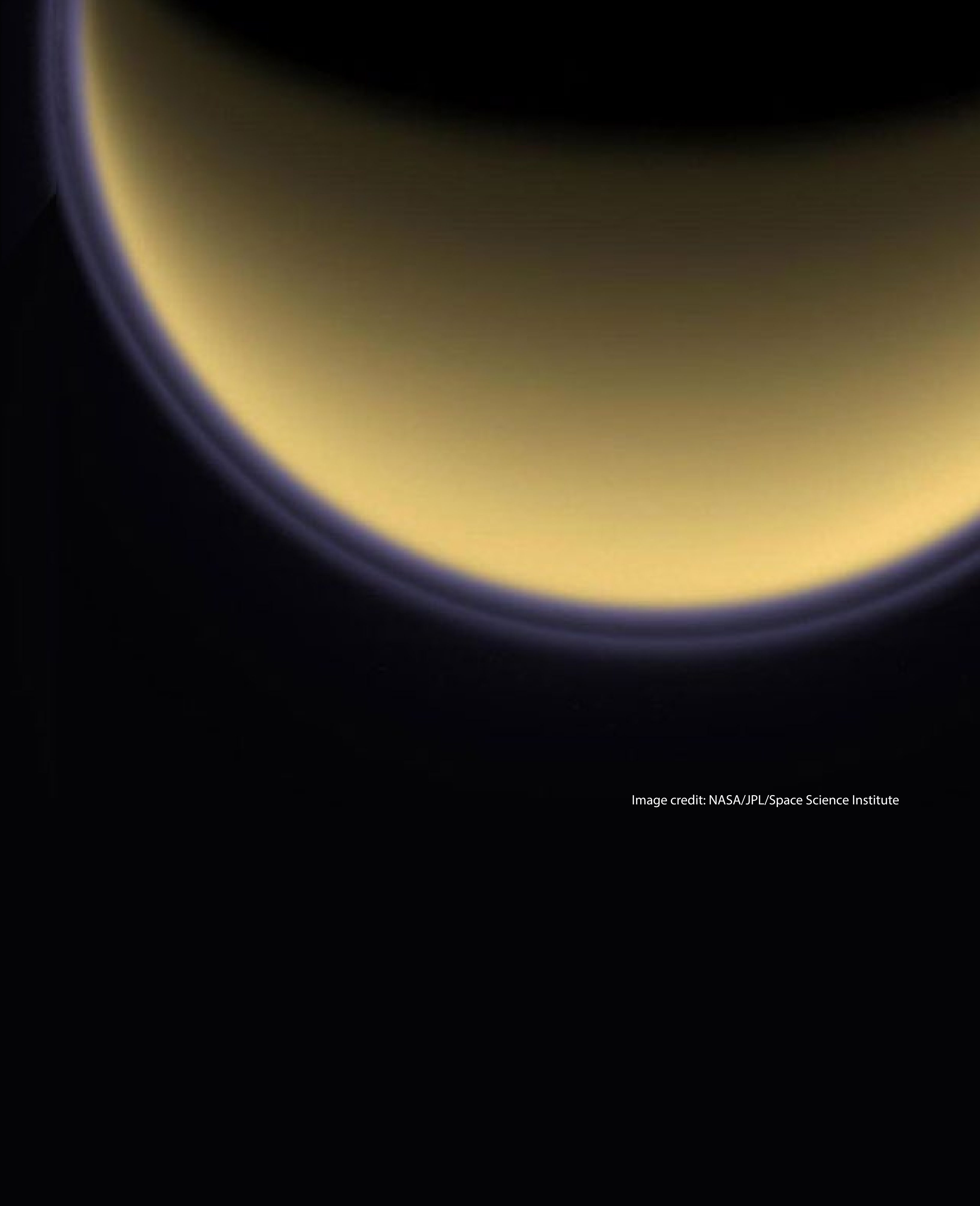


Image credit: NASA/JPL/Space Science Institute

Astrobiology
Center for Isotopologue Research

ACIR

Penn State-Caltech-Lehigh-UTEP
Nantes-ELSI-UCR-Goddard

# The Origins of Molecules in Diverse Space and Planetary Environments and Their Intramolecular Isotope Signatures

**Lead Institution:**
**Pennsylvania State University**

**Principal Investigator:**
Katherine Freeman

## Team Overview

The Astrobiology Center for Isotopologue Research (ACIR) brings together an international team of top scientists and cutting-edge observational and computational tools in order to find out: *how do the abundance and positions of isotopes within molecules explicitly reveal the origins and history of organic compounds?* We seek predictive understanding of how substrates, processes, and environments are encoded in the isotopes of organic compounds in diverse matrices, environments, and organisms. Observations will inform innovative models, and together will lead to new understanding of organics and the isotopes they carry from space and planetary environments, in metabolic systems and biotic communities, and over Earth's history.

ACIR research will support numerous goals in the Astrobiology Science Strategy (Hays *et al*., 2015), and the research portfolio focused on organics amongst other NAI teams. Our studies seek to inform scientific planning for sample return missions and to create a path toward isotope- and isotopologue-ready instruments for future solar system missions. The biological, geological, and chemical signals encoded within isotopologues have the potential to transform how we study organic biosignatures, which are central to multiple NASA Planetary Science and NASA Strategic objectives related to life signatures.

ACIR Research Themes:

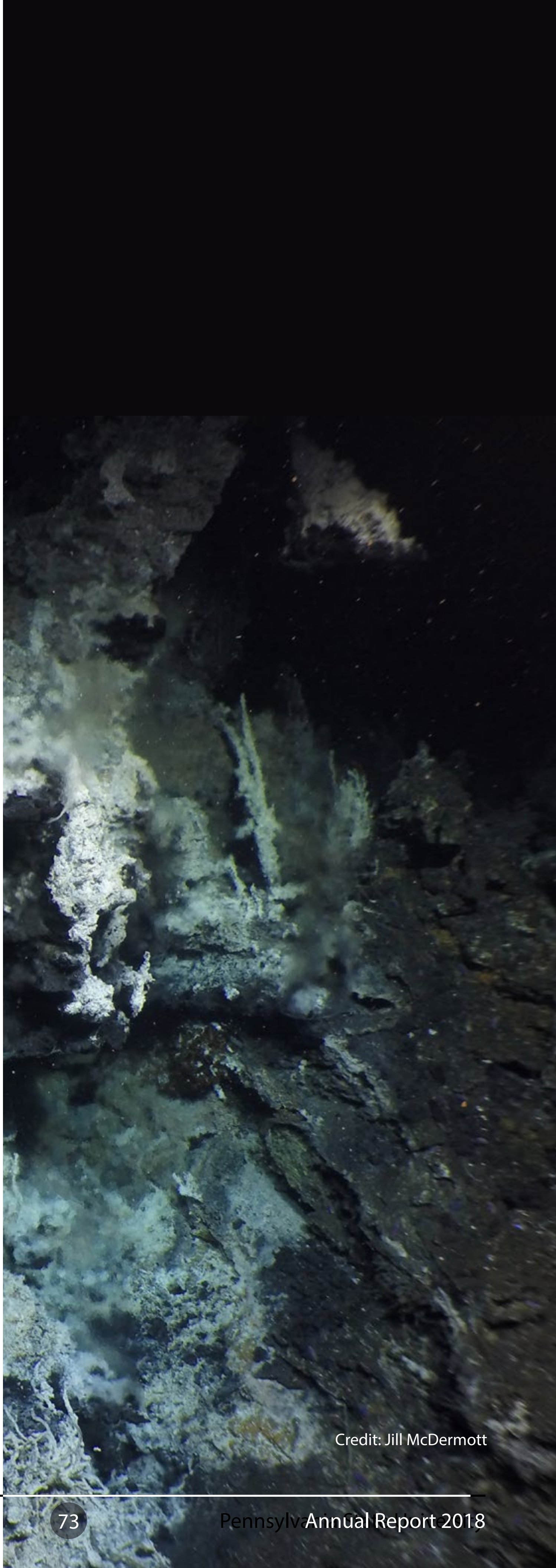
- Meteorites and Cosmochemistry
- Methane Biogeochemistry
- Organics in Earth Fluids
- Organics, Ice, and Minerals
- Biochemicals
- Predictive Models
- Ancient Life and Earth
- Analytical Capacity

## 2018 Executive Summary

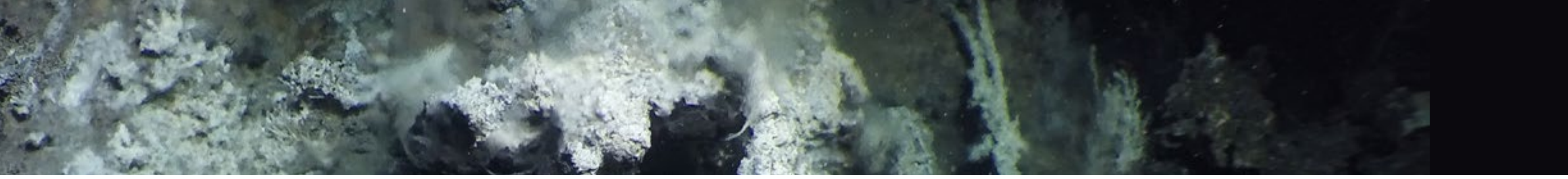
The Astrobiology Center for Isotopologue Research (ACIR) is a globally distributed team, including eight institutions across 17 time zones in France, the U.S., and Japan. Thus, a strong sense of community via shared science is a key goal, which we have fostered by video conferences and small team collaborations. In addition, the center hosted team gatherings and meals at professional meetings, including the Goldschmidt and American Chemical Society (ACS) conferences in Boston, and at the American Geophysical Union (AGU) meeting in Washington, D.C. Since the start of our funding in mid 2018, we have worked together to bring in new postdocs and students, advance computational and analytical methods, and kick off our isotopologue studies.

ACIR's science spans organic signatures derived from beyond the solar system, from life and from the chemistry of living organisms, from deep within the earth, and over the expanse of geologic time. Our cross-connected studies invoke abiotic physical and chemical processes, the biogeochemistry of microbial ecosystems, and the dynamics of biochemistry within individual organisms. We have initiated isotopologue studies to discern thermal imprints on isotope patterns on organic compounds in mantle fluids from the Pescadero Basin, and started novel isotope studies of biomarkers from the Ediacaran. Researchers at all institutions are investigating biochemical imprints on isotopes within amino acids and organic acids.

To initiate scientific exploration of organic isotopo logues, team members collectively established a list of compounds to compare and refine analytical and computational tools. Targets include low molecular weight organic acids and fatty acids (important in deep earth fluids and in biochemistry), amino acids for computational and biochemical investigations (including alanine, aspartic acid, cysteine, methionine, serine, and glutamic acid), and the nucleic bases uracil and thymine. Using DFT (i.e. Discrete Fourier Transform) computational tools, our collaborative efforts investigated bond energy controls on the isotopologues of alkanes, aromatic, and amino acid compounds and are now being used to probe potential position-specific isotope fractionation during organic interactions with mineral and ice surfaces. These modeled fractionation studies provide a framework for laboratory experiments of phase partition and isotope measurements via NMR and IRMS.



Credit: Jill McDermott



Caltech members made significant progress in orbitrap measurements using an electrospray ionization inlet and the power of tandem quadrupole and Orbitrap mass analyzers. Based on this, they developed a successful strategy for position-specific analyses of methionine, an approach now being applied to serine. The Tokyo team developed preparation-scale isolation of various organic acids, and they can now achieve position-specific analyses for mg quantities of pure compounds by $^{13}C$ nuclear magnetic resonance (NMR) analysis. At Penn State, team members have been guiding renovations for a dedicated lab space that is slated to be completed by Fall 2019 and will house existing nano, pico, and conventional molecular IRMS instruments and new LC/MS and Orbitrap MS to support of ACIR research.

We are proud to report two members of our team were recognized for their innovative scientific contributions:

- Naohiro Yoshida: AGU Fellow
- Alex Sessions: John Hayes Award, the Geochemical Society

## Team Members

**Katherine Freeman**
Serge Akoka
Allison Baczynski
Jason Boettger
Jennifer Eigenbrode
John Eiler
Allison Fox
Alexis Gilbert
Christopher House
Andrew Hyde
Maxime Julien
James Kubicki
Max Lloyd
Gordon Love
Jill McDermott
Alexandra Phillips
Gerald Remaud
Alexander Sessions
Heath Watts
Elise Wilkes
Fenfang Wu
Hui Yang
Naohiro Yoshida
Zhidan Zhang

# Project Reports

## Organics in Earth Fluids

ACIR members Gilbert (Tokyo Tech) and McDermmott (Lehigh University) initiated isotopic studies of organics carried by deep Earth fluids. We are trying to determine the characteristic isotopic signatures of abiotic processes, and potentially answer the question, "What is the influence of mixing or overprinting by biotic processes?" The Tokyo Tech team began investigations of position-specific $^{13}C$ isotope fractionation factors thermogenic formation, thermal degradation, and biological degradation. The anaerobic oxidation of propane leads to a specific $^{13}C$-enrichment at the central ($CH_2$) position. This signature distinctly provides evidence of biodegradation in settings where identification by other means is difficult, such as the subsurface (Gilbert *et al.*, *PNAS*, revision submitted). These results show that the position-specific approach will be useful to decipher the biogeochemistry of hydrothermal fluids.

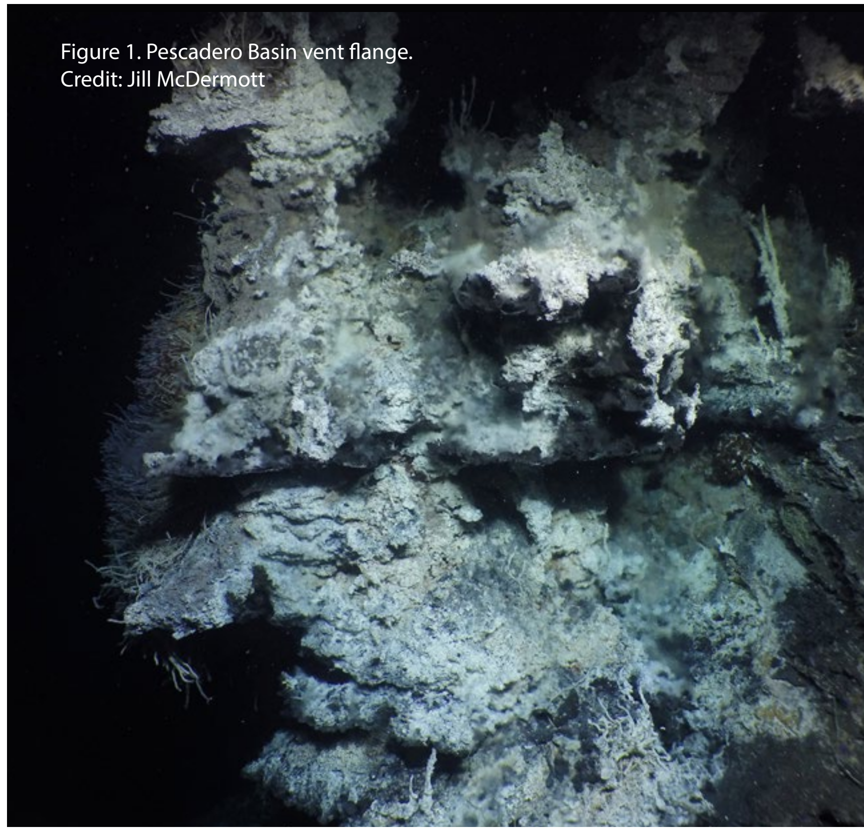
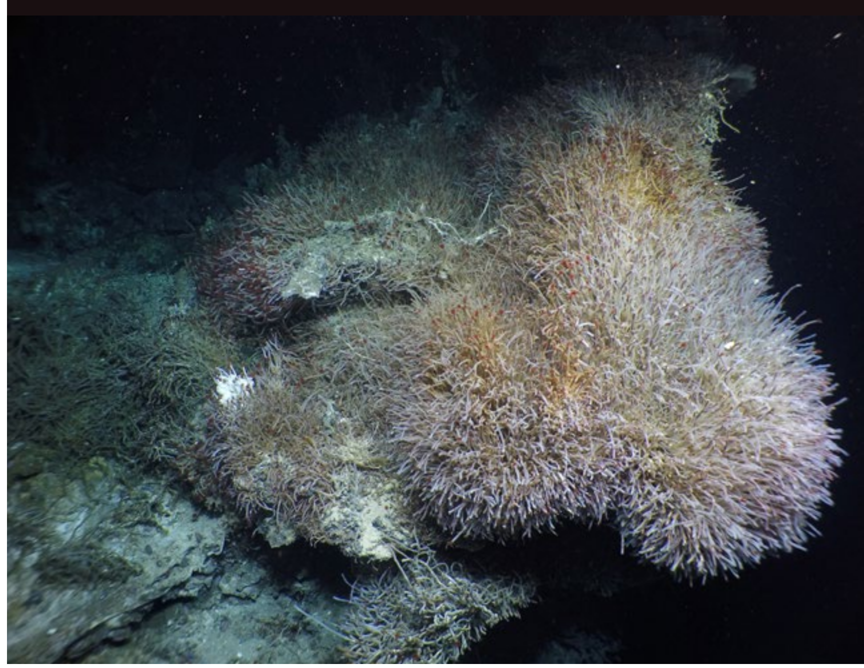


Figure 1. Pescadero Basin vent flange.
Credit: Jill McDermott

The Lehigh team investigated natural hydrothermal vent fluid samples collected from ~3670 m depth at Pescadero Basin, among the deepest known hydrothermal vent systems in the Pacific Ocean. These fluids contain high concentrations of dissolved $CH_4$, $C_{2+}$ n-alkanes, organic acids, and $H_2$ due to interaction with overlying organic-rich sediment cover, and possible reaction with serpentinized and/or basaltic basement. In this system, clumped isotopes of $CH_4$ and alkane-alkene equilibria work as geo-thermometers, and this work was presented by McDermott at the American Geophysical Union Fall Meeting. Future studies of Pescadero samples will focus on isolation of acetate for position-specific isotope analysis to elucidate sources for small carboxylic acids. We are planning new field campaigns to collect fluid and volatile samples from legacy diamond drill boreholes at the Soudan Iron Mine, within the Neo-archean (~2.7 Ga) Wawa-Abitibi Subprovince of the Canadian Shield. Isotopologue studies aim to decipher the sources and fate of organic components within the isolated underground aquifer in order to define the constraints on habitability. The McDermott lab is presently recruiting a postdoctoral researcher and a doctoral student.

Figure 2. Tubeworms in the Pescadero Basin.
Credit: Jill McDermott



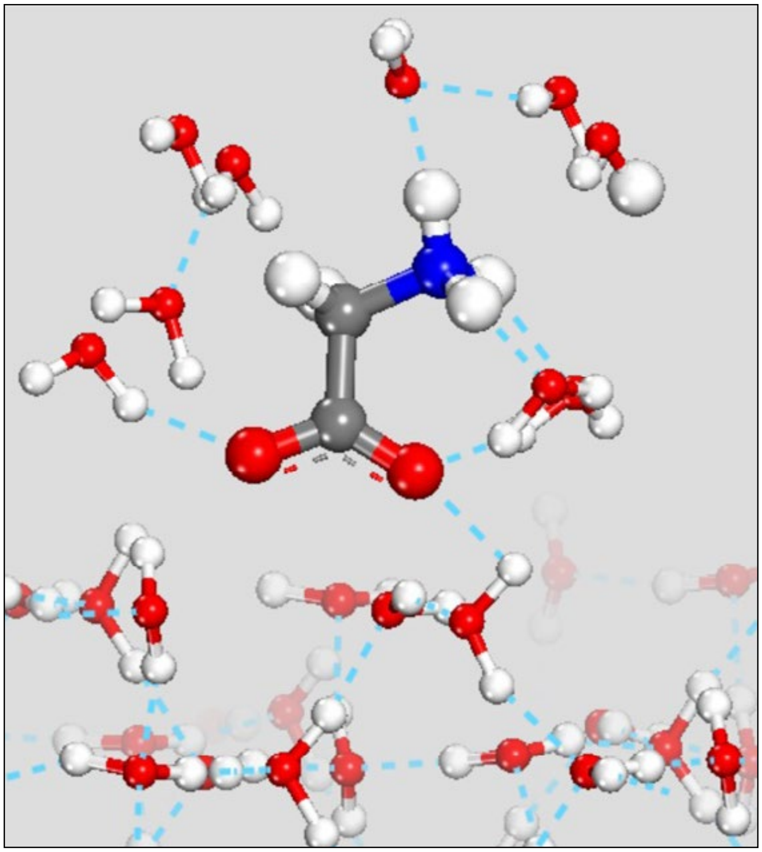


Figure 3. Initial model of solvated glycine zwitterion on (100) surface of cubic ice. Model constructed with Materials Studio 2016 (Accelrys Inc., San Diego CA). Credit: James Kubicki

## Organics, Ice, and Minerals

We are investigating isotopic fractionation during sorption between organic compounds and mineral and ice surfaces. This work has implications for the preservation of biosignature compounds in planetary environments, including Mars soils and on Icy Worlds. Many organic-mineral interactions involve polar functional groups, although a significant portion of preserved biomarker compounds are nonpolar as well. Numerous prior studies indicate changes in the electronic environment associated with sorption that may shift bond energies enough to cause an observed isotope effect, although position-specific patterns have not been studied in detail.

Penn State Graduate student Allison Fox has been modeling the distribution of H and C isotopes within a suite of polar, aromatic, and non-polar compounds on mineral and water ice surfaces with DFT calculations. Her work has been assisted by ACIR Postdocs Heath Watts and Jason Boettger, and is collaborative between Freeman (PSU) and Kubick (University of Texas, El Paso). In the models, heavier isotopes tend to be favored at molecular sites closer to a mineral surface, where electron density is greatest. The electronic environment alters the strength of bonds within the molecule as it nears the surface. Bond strength is shifted as a result of field effects that slightly polarize the molecule during interaction between functional groups and polar sites on the surface. This influence is small for C isotopes, especially in non-polar compounds, and more pronounced for H isotopes. Ongoing work has focused on developing experimental methods to evaluate the H and C isotopes within sorbed molecules using both IRMS methods at Penn State, with follow up position-specific measurements with isotope-NMR in Nantes.

## Biochemicals

We seek to understand how key biosynthetic pathways imprint isotopes at specific sites within biochemicals. Our long-range goal is to use this information to discern biological signatures in modern, ancient, and potentially, space or planetary environments. ACIR collaborative studies have begun this ambitious research theme with studies of organic acids and amino acids.

Both Tokyo Tech and Penn State teams are working to set up sample preparative scale high performance liquid chromatography (HPLC) to isolate compounds prior to intramolecular isotope measurements. The Tokyo team is developing protocols for the separation, purification and analysis of fatty acids and organic acids, with successful isolation of hundreds of mg of pure compounds needed for isotopic $^{13}C$ NMR analysis. This will enable studies of fatty acids in different vegetal oils (coconut, corn, sunflower) to compare their $^{13}C$ intramolecular patterns. Team members at Tokyo have also set up large scale culture experiments for microorganisms in order to be able to collect enough material for isotopic $^{13}C$ NMR analysis.

At Caltech, graduate student Alexandra Phillips joined the team to work on compound-specific $\delta^{34}S$ measurements of the amino acids cysteine and methionine. Her sensitive method has enabled analyses of sulfur in amino acids from fish, plants, and bacteria and to explore natural variability. Postdoc Elise Wilkes, supported by an Agouron Institute Fellowship, arrived at Caltech in October and has begun work on position-specific $\delta^{13}C$ measurements of serine. Her tests of fragmentation patterns for derivatives confirmed that N-trifluoroacetyl and methyl ester derivatives are the most useful for our purposes. She is now defining the 'contribution matrix' which relates the contributions of individual molecular C positions to the various fragments we measure (Fig. 4, panel D). This is done using standards with a 100% C-13 label at specific positions.

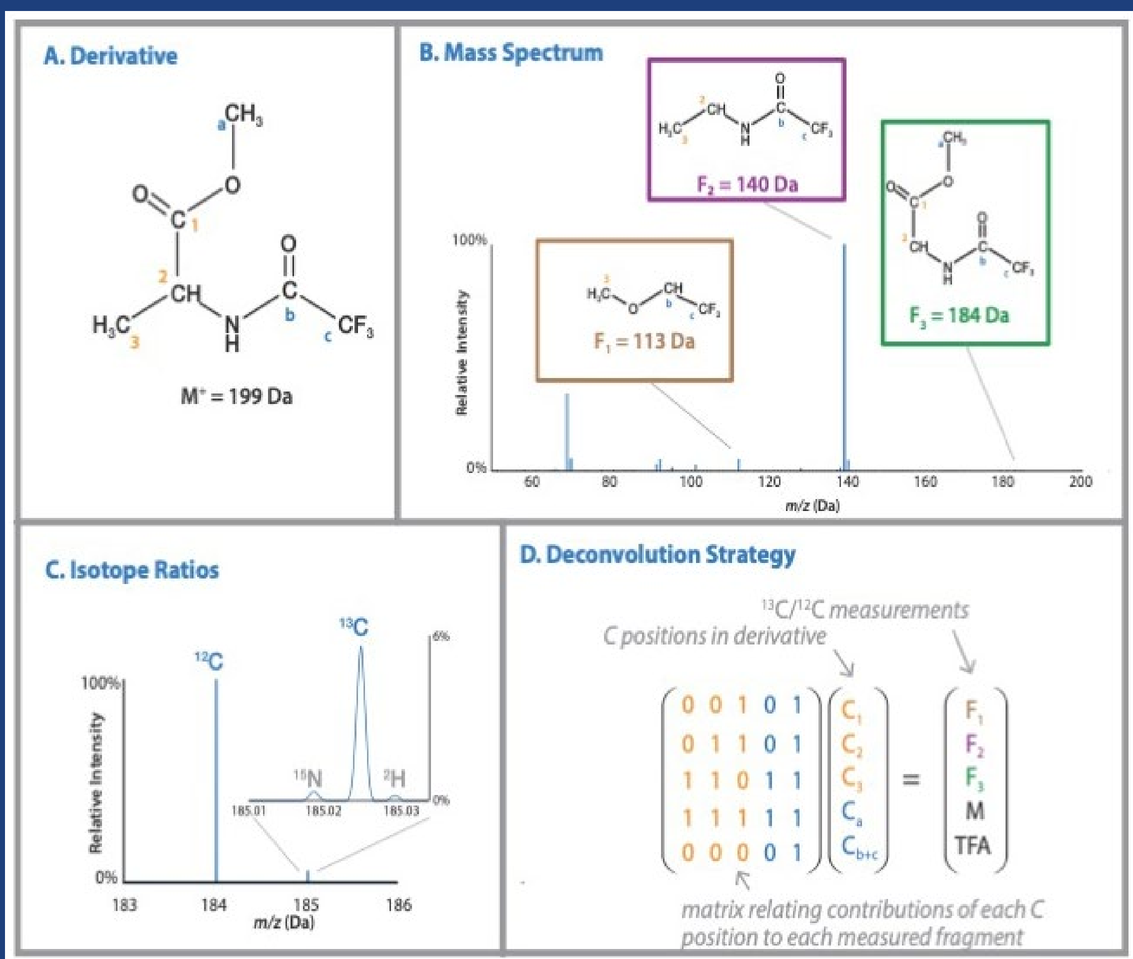



Figure 4. Demonstration of the position-specific C-13 strategy already developed in alanine, and which we are applying to serine. Position-specific measurements of serine and alanine may encode a signal of photorespiration. Credit: Alex Sessions

**Predictive Models**

Several new collaborations within our community, led by Kubicki, have employed Density Functional Theory (DFT) methods to create the electronic structure of molecules and track bond energy influences on isotope patterns for a suite of target compounds. The goal of these molecular models is to lay the ground work for subsequent experimental studies of their reactivity in physical/chemical and biotic systems.

We started with modelling isotopic fractionations in amino acids. We derive the equilibrium isotopic fractionation factors from 1000ln(β) values. This exploratory work will help us develop a guide to the level of differences that we should expect and which chemical factors will influence fractionation. The following are plots of 1000ln(β) versus various parameters for atoms in several amino acids. These values come from DFT molecular models at the B97D/6-311++G(d,p) level. Differences in 1000ln(β) between two atoms represent the (approximate) difference in δ between those two atoms at equilibrium, in per-mil (‰) units.

So far, we have determined gas-phase fractionations, which are less computationally demanding. Aqueous fractionations will likely differ significantly, but that will also require significantly more computational resources. Notably, zwitterionic species were unstable in the gas phase and underwent proton transfer to form neutral molecules. From our studies so far, we found protonation favors the heavy isotope for N, O, and S sites. For N and O, after controlling for protonation, lighter isotopes were slightly favored in negative-charged amino acids. Further, stronger charges tend to favor $^{13}C$ in carboxyl sites and in carbons adjacent to the carboxyl site (alpha carbons). Finally, oxidation state strongly influenced the extent to which $^{13}C$ is favored in sites within amino acid compounds.

## Analytical Capacity

The entire ACIR team is working to build analytical precision, sensitivity and overall capacity for isotopologue research in order to benefit the larger scientific community.

Analytical development has begun with the recruitment of students, and collective selection of suitable target molecules and standards. Analyses of standards will involve multiple methods, including NMR-based carbon isotopic analyses, chemical degradation methods, and novel Orbitrap methods. To enable accurate and standardized position-specific isotope analyses of organic molecules, we will first evaluate the accuracy of isotopic $^{13}C$ NMR with vinyl acetate, a molecule which PSIA (Position Specific Isotope Analysis) can be easily conducted by chemical degradation methods. This allows isotopic $^{13}C$ NMR to be a "reference" method to calibrate data from other techniques. We have initially targeted amino acids alanine, serine, glutamate, and aspartate for PSIA standards. In this regard, we have also determined kinetic isotope effects associated with the decarboxylation of amino acids with ninhydrin, a key step in order to establish $^{13}C$ PSIA of amino acids.

We are also starting to develop PSIA methods for the nucleic bases uracil and thymine. Such an approach could provide three PSIA values representing the pyrimidine carbon from aspartate, the pyrimidine carbon from $CO_2$, and the methyl carbon derived from methylene tetrahydrofolate. This project will likely involve both NMR and chemical degradation approaches so that the method can be ultimately applied to low concentrations of microbial RNA and DNA.

Members of ACIR have begun to partner with other investigators to support research in preparation for NASA missions. Freeman is part of a new ICEE2 project that aims to develop a mass spectrometer with small acid isotopologue capabilities for a Europa Lander (Christopher Glein, PI).

e·nig·ma

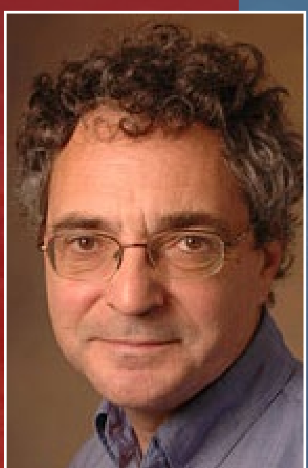
*Evolution of Nanomachines In Geospheres and Microbial Ancestors*

# ENIGMA: Evolution of Nanomachines in Geospheres and Microbial Ancestors

**Lead Institution:**
**Rutgers University**

## Team Overview

Life on Earth is electric. The electronic circuitry is catalyzed by a small subset of proteins which function as sophisticated nanomachines. Currently, very little is known about the origin of these proteins on Earth or their evolution in early microbial life. To fill this knowledge gap, the ENIGMA team is focused on experimental, bioinformatic, and data-driven studies to explore the origin of catalysis, the evolution of protein structures in microbial ancestors, and the co-evolution of proteins and the geosphere. ENIGMA is comprised of three integrated themes.



**Principal Investigator:**
Paul Falkowski

**Theme 1: Synthesis of Nanomachines in the Origin of Life**
**Theme 2: Increasing Complexity of Nanomachines in Microbial Ancestors**
**Theme 3: Co-Evolution of Nanomachines and the Geosphere**

**Theme 1:** Focuses on understanding how complex extant nanomachines that catalyze electron transfer emerged from much simpler prebiotic chemical processes. Two possible biochemical origins scenarios are being explored: on the early Earth at the beginning of the Archean eon, and on other planets where different amino acid alphabets and chemical constraints might likely be present.

**Theme 2:** Examines the emergent complexity of metalloproteins in microbial ancestors. We are developing new computational methods for linking protein structure to the evolution of function, particularly to redox functionality of metal binding proteins.

**Theme 3:** Explores the co-evolution of minerals and proteins through geologic time. We carry out data-driven studies of mineral evolution that document the changes in Earth's mineral diversity and distribution through deep-time, focusing on mineral interactions with the biosphere.

**Team Website:** https://enigma.rutgers.edu/

# 2018 Executive Summary

The ENIGMA team began to recruit post-doctoral fellows and graduate students in April 2018, and by the end of the year eight post-doctoral researchers (six at Rutgers, one at Carnegie Institute of Science and one at UCSD) are engaged and several graduate students have applied to study astrobiology at Rutgers. The post-docs are being cross mentored in the three themes. The entire ENIGMA team met in May 2018 and developed an integrated research plan that is now being implemented.

**Synthesis of Nanomachines in the Origin of Life -** Led by Vikas Nanda (Rutgers), we designed and synthesized several small peptides that incorporate iron-sulfur complexes and are capable of transferring electrons catalytically. One of these, which we call "ambidoxin" contains only twelve amino acids in an alternating L and D structure (Figure 2). The peptide, designed in Nanda's lab by Douglas Pike, an ENIGMA Ph.D. student, and constructed in Paul Falkowski's lab (Rutgers) by John Kim, a post-doc, is remarkably robust; it can undergo hundreds of electron transfer reactions under anoxic conditions. Structural analyses of the metalopeptide was accomplished using both NMR and transient EPR techniques; the former was under the direction of Gaetano (Guy) Montelione (Rutgers). This research was extended to larger peptides that can bind several iron sulfur clusters to form a "wire". The construction of this suite of molecules was accomplished by Andrew Mutter (Rutgers), a post-doc, and the genes for these molecules were expressed in *Escherichia coli* giving rise to novel phenotypes. The expression was accomplished by Ian Campbell, an ENIGMA Ph.D. student working in the laboratory of Joff Silberg (Rice University). With Hagai Raanan (Rutgers), a post-doc working with Nanda and Falkowski, we also have made significant progress in defining a set of primordial metal-protein folds (Raanan, *PNAS* 2018) that likely served as building blocks for complex electron transport pathways critical metabolism.

In cross-theme collaborations, we are using the Yana Bromberg (Rutgers) team's FusionDB platform that contains genome-scale classifications of function to identify how extreme environments may have constrained proteome size and complexity to be smaller and simpler.

With the Hecht group (Princeton), we are examining how readily metalloproteins can emerge by spontaneous

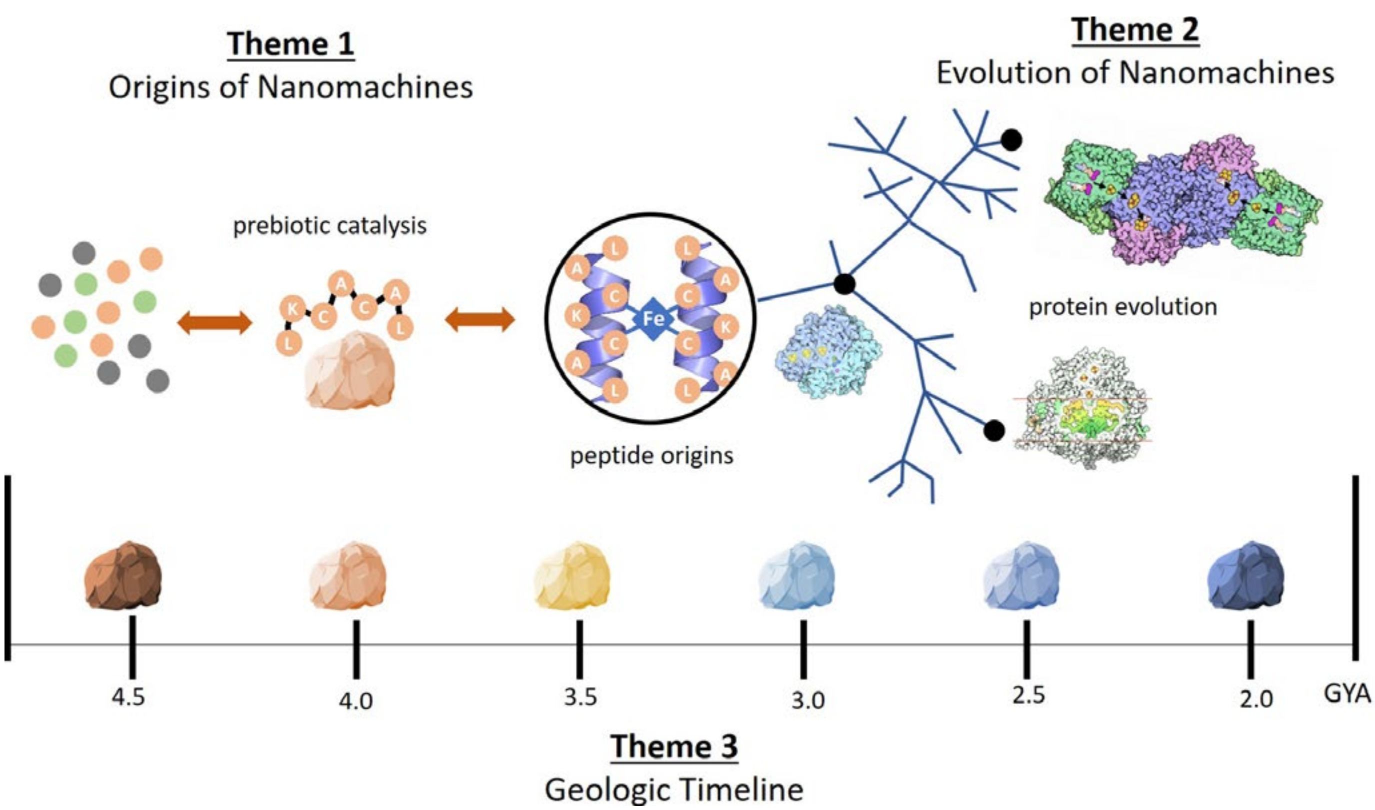



Figure 1. ENIGMA: Evolution of Nanomachines In Geospheres and Microbial Ancestors. This NAI team will explore the catalysis of electron transfer reactions by prebiotic peptides to microbial ancestral enzymes to modern nanomachines, integrated over four and a half billion years of Earth's changing geosphere. Theme 1 focuses on the synthesis and function of the earliest peptides capable of moving electrons on Earth and other planetary bodies. Theme 2 focuses on the evolutionary history of "motifs" in extant protein structures. Theme 3 focuses on how proteins and the geosphere co-evolved through geologic time. Credit: Vikas Nanda

sequence variation in naïve protein libraries. Initial results show that proposed ancient protein folds are capable of iron-sulfur cluster binding. That research suggests that the evolution of metalopeptides/proteins was not a rare event, but rather was probably a common occurrence early in the origin of life, or even prior.

**Increasing Complexity of Nanomachines in Microbial Ancestors -** This year, Yana Bromberg's (Rutgers) group developed a novel method for evaluating protein functional similarity by comparing protein folds. Working with Kenneth McGuinness (Rutgers ENIGMA post-doc), they showed that almost all known metal binding folds are connected in a single network. This analysis suggests a small number of origins for metallopeptide/proteins.

To consider the question of remote protein similarity from the sequence perspective, Diego Ferreiro (University of Buenos Aires) and his group developed an algorithm to search for amino acid patterns in metallo-protein sequences. The results further guide our exploration of functional evolution of proteins.

Akif Tezcan's group at UCSD, including Derek Gagnon (UCSD/ENIGMA post-doc), began engineering redox-active protein-protein interfaces bearing 4Fe-4S clusters.

**Co-evolution of Nanomachines and the Geosphere -** Robert Hazen (Carnegie Institute) and ENIGMA collaborator Shaunna Morrison developed a preliminary list of near-surface minerals present during Earth's Hadean Eon (>4.0 Ga). These minerals were formed by the precipitation of organic crystals prior to the rise of predation by cellular life; large bolide impacts, especially through the generation of hydrothermal systems in circumferential fracture zones; and photo-oxidation of transition metals through abiological redox processes.

Hazen examined how dissolved ions present in an aqueous environment may have affected the adsorption of amino acids onto brucite [Mg(OH)2]. This process is being examined on natural clays by Nathan Yee (Co-I at Rutgers), and serves in molecular self-organization and the assembly of proteins that contain transition metals.

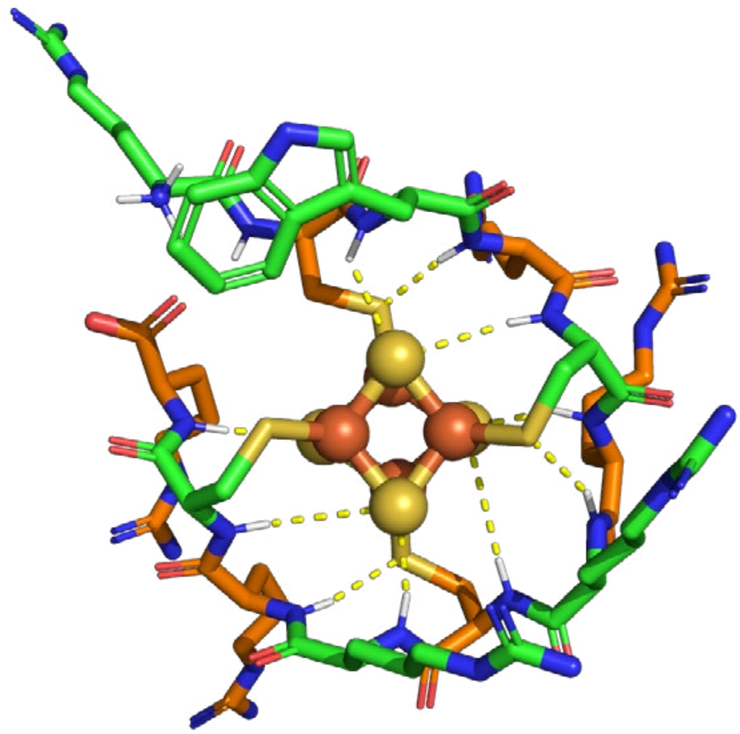


Figure 2. Computationally designed, minimal dodecapeptide structural model of ambidoxin peptide consisting of alternating L and D amino acids that binds a 4Fe–4S cluster through ligand–metal interactions. Credit: Douglas Pike

# Project Reports

## Synthesis of Nanomachines in the Origin of Life

The Vikas Nanda (Rutgers) group is synthesizing models of putative early proteins and studying their function in energy conversion and catalysis. They have made significant progress in defining a set of primordial metal-protein folds (Raanan, *PNAS* 2018) that likely served as building blocks for complex electron transport pathways critical metabolism. The team continues to expand the list of ancient folds and test their function in the laboratory.

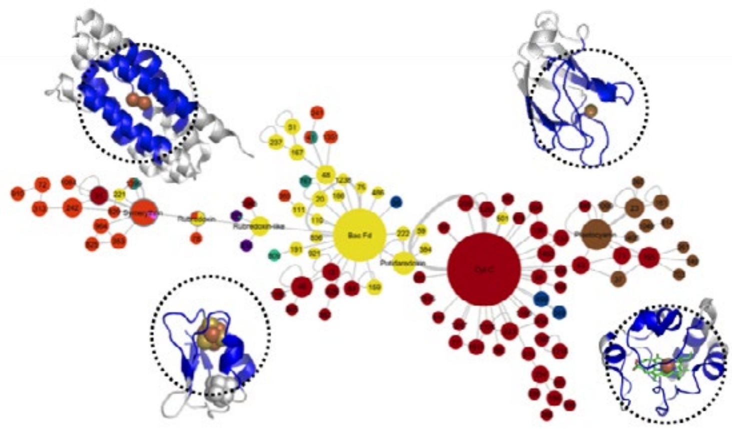


Figure 3. Electronic wiring diagram of early protein building blocks. Credit: Vikas Nanda

Designs based on a primordial ferredoxin fold containing symmetric sequences were synthesized and shown to undergo reversible electron transfer. Several designs also complemented ferredoxin deletions in sulfur reducing bacteria. Biological assays developed at Rice by the Silberg group, set a high bar in the field for characterizing electron transfer of model proteins. This is being submitted for publication.

A minimal 12-residue ferredoxin was designed and synthesized (Kim, *JACS* 2018). Despite its small size, this peptide is able to stably bind metal and reversibly cycle electrons with an efficiency approaching that of modern batteries. This challenges our assumption that early proteins were functionally inferior to modern counterparts. We propose prebiotic chemical selection produced small metallopeptides that were potent starting points for subsequent biochemistry.

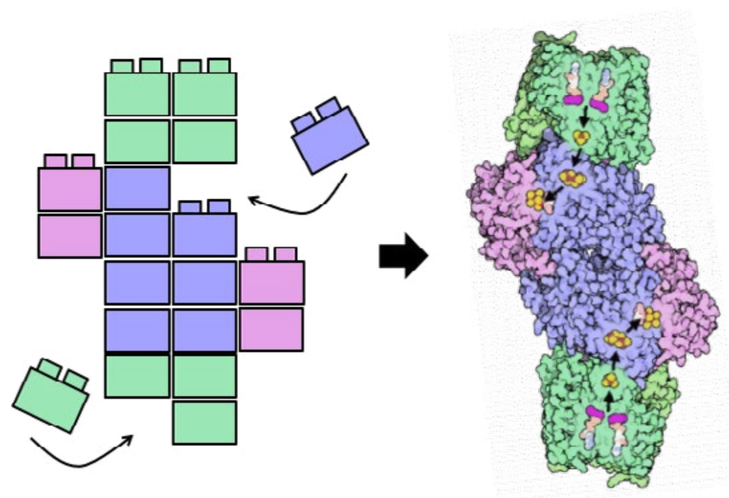


Figure 4. Assembling complex proteins from simpler parts. Credit: Protein Data Bank (www.rcb.org)

With the Hecht group at Princeton, we are examining how readily metalloproteins can emerge by spontaneous sequence variation in naïve protein libraries. Initial results show that proposed ancient protein folds are capable of iron-sulfur cluster binding. This exciting result indicates that the emergence of functional metal-proteins was likely commonplace, rather than a rare evolutionary event.

In cross-theme collaborations, we are using the Bromberg team's FusionDB platform that contains genome-scale classifications of function to identify how extreme environments may have constrained proteome size and complexity to be smaller and simpler. We expect to learn how environment constrains protein evolution and apply these rules to the emergence of early protein networks.

## Increasing Complexity of Nanomachines in Microbial Ancestors

Yana Bromberg's (Rutgers) group developed a method for evaluating protein functional similarity by comparing 3D protein folds. Working with ENIGMA post-doctoral fellow Kenneth McGuinness (Rutgers), they showed that almost all known metal binding folds (vertices) connect into a single network via similarity edges. Fold similarities also correlated with the relative phylogenetic distances between the oldest microbes carrying fold homologs, effectively capturing distant evolutionary relationships. Notably, a fairly small number of microbes contained homologs of almost all available folds. This finding strongly suggested a small number of origins of biological metal binding. The relative timing of spikes in the number of folds identified per microbe also correlated with the likely order of environmental shifts.

To consider the question of remote protein similarity from the sequence perspective, Diego Ferreiro (University of Buenos Aires) and his group developed an algorithm to search for amino acid patterns and groupings of patterns in sequence. The statistics of such pattern occurrence indicate that repeats of short sequences differentiate between natural families and randomized sequence groups. Moreover, matching a small subset of patterns is sufficient to define sequence "familiarity". Additionally, Diego's group had shown that energetic signatures of local (catalytic site and surrounding residues) frustration are evolutionarily more conserved than the protein sequence. This finding may further guide our exploration of functional evolution of proteins.

Furthermore, Akif Tezcan's group (University of California, San Diego), including Derek Gagnon (a new ENIGMA/UCSD post-doc), began engineering redox-active protein-protein interfaces bearing 4Fe-4S clusters. Akif recently developed a new protein engineering approach (MASCoT - metal active sites through covalent tethering), enabling easy access to functional metal centers in protein-protein interfaces. Cytochrome cb562 constructs were created to promote protein dimerization via the formation of 4Fe-4S clusters. Initial experiments show that the clusters may form, but more work is necessary to evaluate and confirm these results.

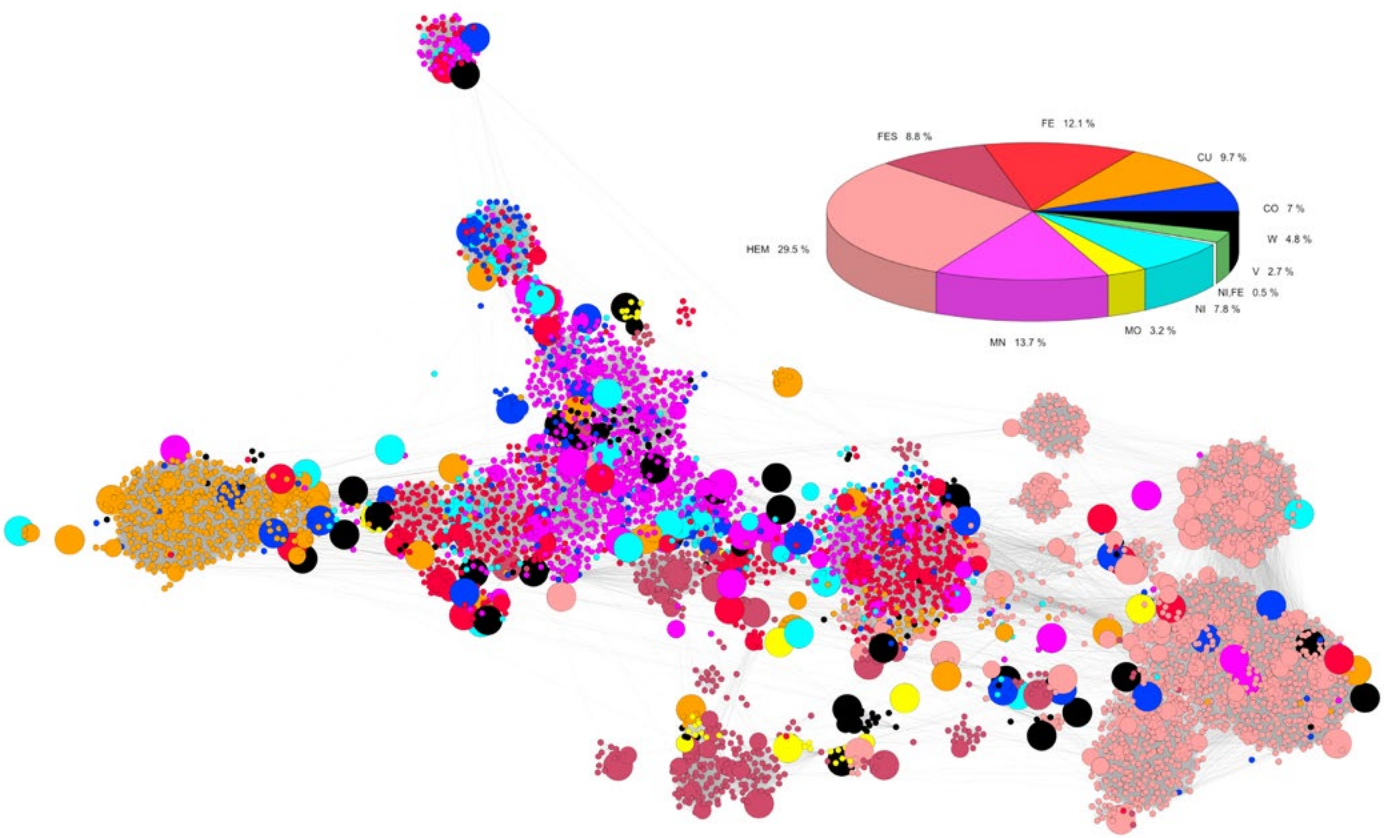



Figure 5. Functional similarity network of all metal-binding spheres in the PDB, colored by primary metal. Larger nodes identify selected representative structures. Pie-chart legend represents the fraction of representatives binding a particular metal ligand. Credit: Yana Bromberg

## Co-evolution of Nanomachines and the Geosphere

Robert Hazen (Carnegie Institute) convened a workshop in Washington DC on March 22-23, 2018 to identify the five "most important reactions" that influence planetary history. This event promoted discussions among scientists with diverse backgrounds in geology, chemistry, biology, and space science. The Earth in Five Reactions Workshop posed two significant challenges: 1) the formulation of a conceptual definition of "reaction," and 2) the identification and ranking of the "most important reactions" in the context of planetary evolution. The main findings and implications of this workshop are reported in Hazen (2018).

Robert Hazen and collaborator Shaunna Morrison developed a preliminary list of near-surface minerals present during Earth's Hadean Eon (>4.0 Ga). These minerals were formed by various processes including: 1) the precipitation of organic crystals prior to the rise of predation by cellular life; 2) large bolide impacts, especially through the generation of hydrothermal systems in circumferential fracture zones; and (3) photo-oxidation of transition metals through abiological redox processes. The results of this analysis are published in Morrison *et al.* (2018).

An important motivation for investigating the diversity and distribution of Hadean minerals is the presumed role of specific minerals in life's geochemical origins. Chemically reactive mineral surface sites on exposed surfaces of common Hadean rock-forming minerals would have adsorbed and concentrated amino acids. Hazen conducted a study to examine how dissolved ions present in an aqueous environment may have affected amino acids adsorption. Adsorption experiments were made with mixtures of the amino acids aspartate, glycine, lysine, leucine, and phenylalanine onto brucite [Mg(OH)2]. When CaCl2 was added, negatively charged amino acids selectively adsorb onto brucite. This process may serve as a key process in molecular self-organization and the assembly of proteins that are composed of metal–ligand complexes. This finding is reported in Estrada *et al.* (2018).

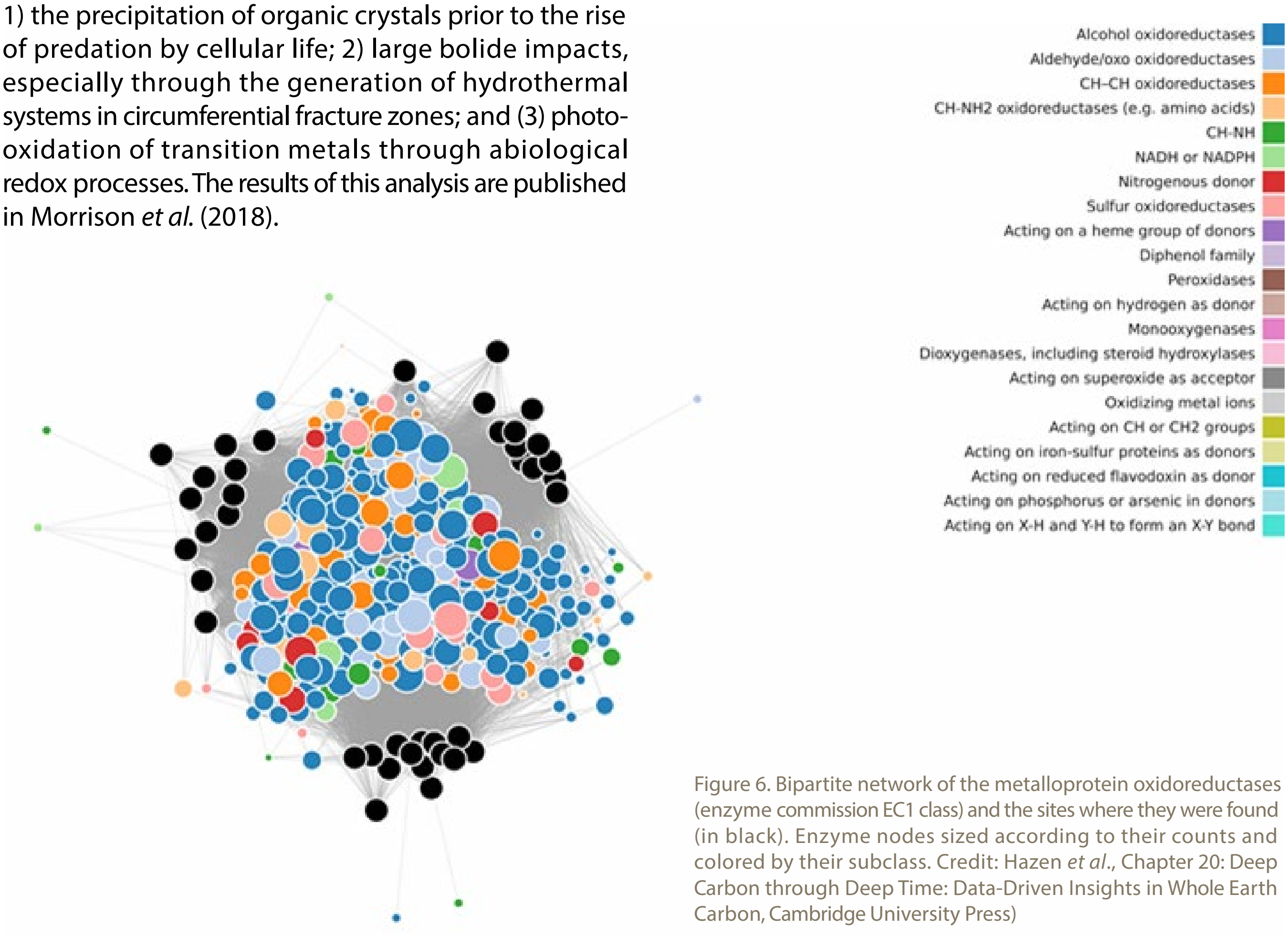



Figure 6. Bipartite network of the metalloprotein oxidoreductases (enzyme commission EC1 class) and the sites where they were found (in black). Enzyme nodes sized according to their counts and colored by their subclass. Credit: Hazen *et al.*, Chapter 20: Deep Carbon through Deep Time: Data-Driven Insights in Whole Earth Carbon, Cambridge University Press)

## ENIGMA Education and Outreach

**Science Communication training for the Next Generation of Scientists:** Janice McDonnell (Rutgers) and her education and outreach team are currently planning a one-day training program (April 2019) for the post-docs and graduate students involved in the ENIGMA project. The workshop, entitled Communication Strategies for Interdisciplinary Learning will provide participants with an overview of learning theory to help improve collaborations within and outside of their discipline. Participants will have the opportunity to practice their science communication skills through participation in a series of Astrobiology community engagement events in New Brunswick, NJ.

**Development and implementation of a series of Community Engagement Events: (Family Science and a Special Interest (SPIN) Club for urban youth**. In May 2019, we will offer two family science programs in two local New Brunswick schools for K-8 students and their families. In fall 2019, we are planning an 8-week ENIGMA SPIN club in collaboration with two local K-8 schools (Greater New Brunswick Charter School and McKinely Ave School, New Brunswick, NJ). We will utilize effective practices from existing high-quality programs like the Science Action Clubs supported through Cal Academy, Northwestern University Science Clubs (Kennedy *et al.,* 2016), and 4-H SPIN Clubs which emphasize STEM learning and building leadership skills. We will model the effective practices/lessons learned from these successful Out-of-School-Time (OST) programs to engage local youth in STEM activities that incorporate art, engineering, and science while contributing to building creative and innovative problem solving skills.

In year 2 of ENIGMA, we plan to build on our science communication experiences and engage a wider science-interested community via videos we create and upload to YouTube and social media. We are currently working with Tilapia Films on the script and conceptual goals. Videos would be added to the successful Tools-of-Science series (toolsofscience.org). Two new videos will be designed to illustrate, in a short and impactful format, the non-linear nature of the scientific process and the creative vision behind ENIGMA's cutting-edge scientific research.

A new Astrobiology course and undergraduate degree minor are currently being developed at Rutgers University.

### Team Members

**Paul Falkowski**
Nidhi Agrawal
Lucia Banci
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Joy Buongiorno
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Saroj Poudel
Hagai Raanan
Jonathan Silberg
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Yuwei Wang
Nathan Yee

## ENIGMA: 2018 Publications


Estrada, C., Sverjensky, D. A., and Hazen, R. M. (2019). Selective Adsorption of Aspartate Facilitated by Calcium on Brucite [Mg(OH)2]. *ACS Earth Space Chem,* 3(1), 1-7. DOI: 10.1021/acsearthspacechem.8b00081*

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# Changing Planetary Environments and the Fingerprints of Life

**Lead Institution:**
**SETI Institute**

**Principal Investigator:**
Nathalie A. Cabrol

## Team Overview

The SETI Institute Changing Planetary Environments and the Fingerprints of Life Team is developing a roadmap to biosignature exploration in support of NASA's decadal plan for the search for life on Mars – with the Mars 2020 mission providing the first opportunity to investigate the question of past life on Mars. In an ancient martian environment that may have once either supported life as we know it, or sustained pre-biological processes leading to an origin of life, the Mars 2020 is expected to be a Curiosity-class rover that will cache samples for return to Earth at a later date. Our Team addresses the overall question "How do we identify and cache the most valuable samples?"

Understanding how a biogeological record was transformed through the loss of atmosphere, increased biologically-damaging ultraviolet radiation, cosmic rays, and chaotically-driven climate changes, we focus on these three themes:

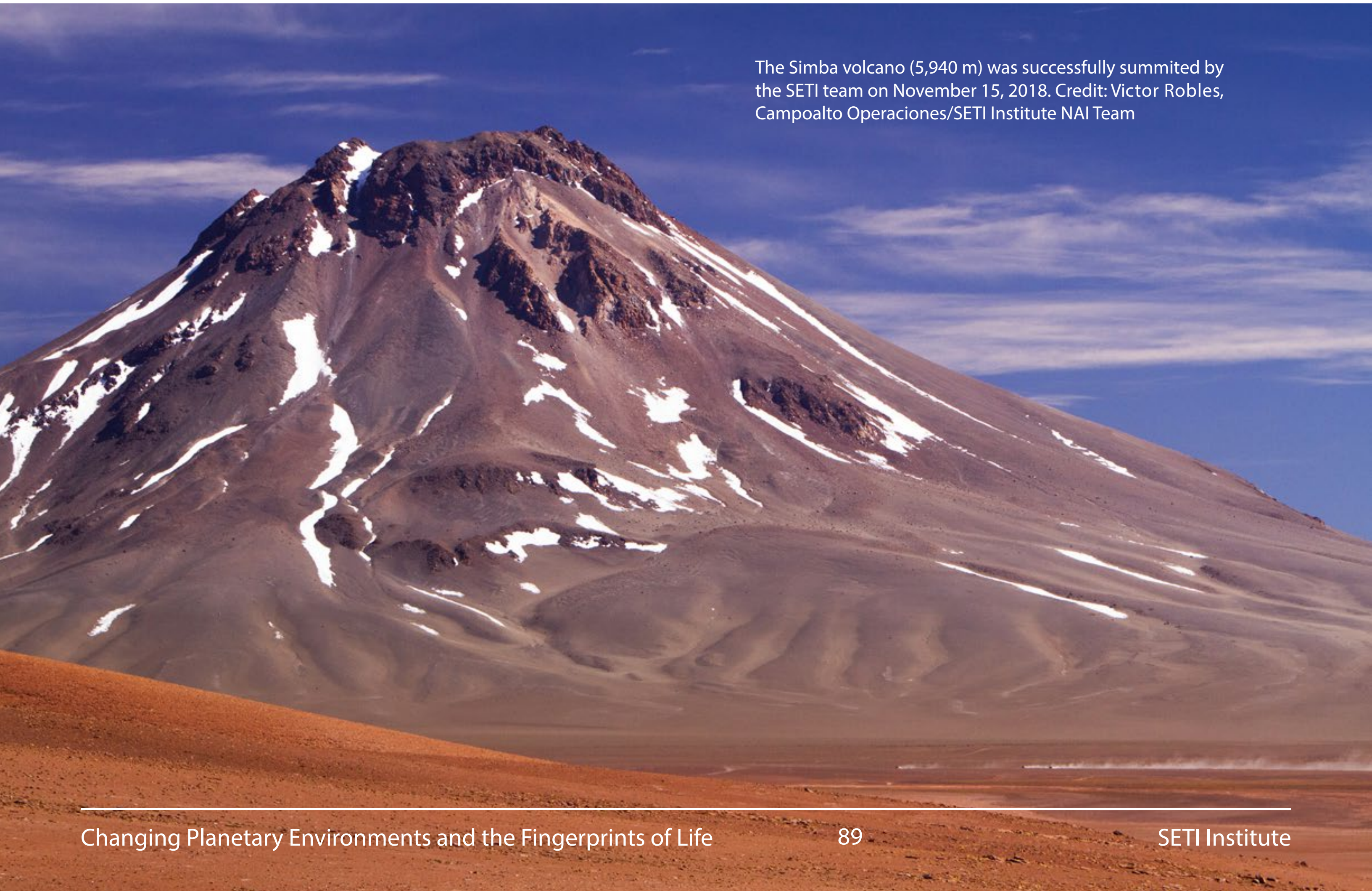
- Where to search on Mars?
- What to search for?
- How to search?

# 2018 Executive Summary

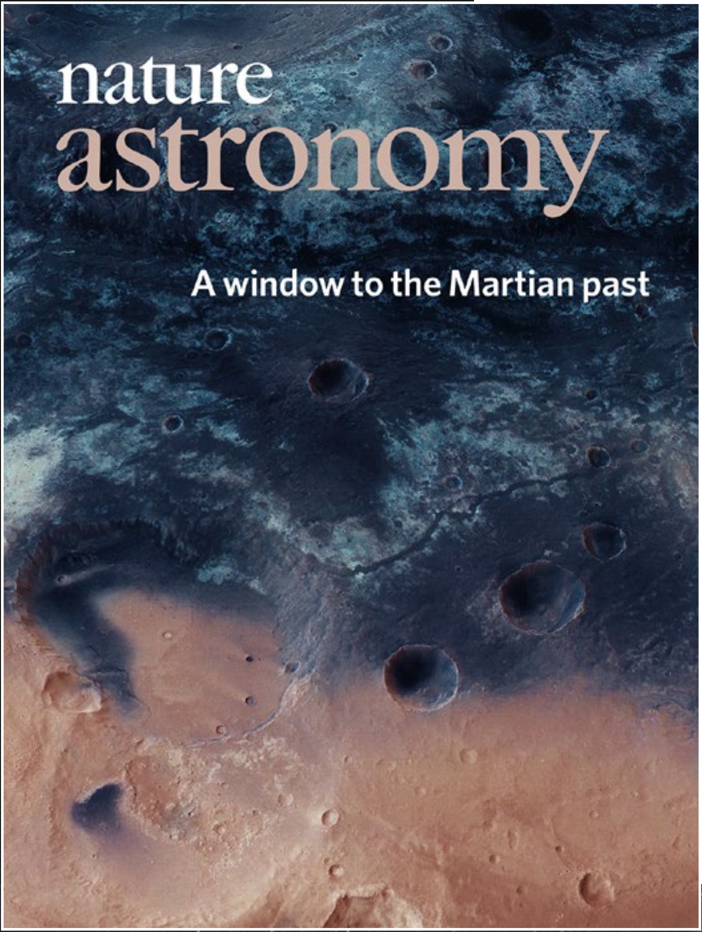
Despite major progress in the past decades, knowledge gaps related to habitable conditions on Mars still constrain our ability to evaluate past and present ecological potential, and possible interactions between environment and life (i.e., coevolution). These gaps include the location of potential biomass, types of potential biosignatures, and detection thresholds and sensitivity. Narrowing these gaps is a major team goal and significant advances were made in 2018. With a Mars 2020 and ExoMars focus on biosignature detection, it is imperative to develop exploration strategies that optimize their chances of success.

Nathalie Cabrol published a theory of coevolution between life and environment on Mars that introduces a biosphere exploration approach and includes the concepts of Mars as a biosphere, polyextreme environments ecosystems, pathways to biological dispersal, biomass repositories, and the spatial and spectral resolution needed for exploration (Cabrol, 2018). Tying to these concepts, our field deployments were designed with two primary objectives: (a) the development of round-robin methods for sample acquisition and analysis; and (b) orbit-to-ground data integration to identify microhabitat location. Data for habitat classification at intermediate scales was obtained using drones and was followed by ecological mapping on the ground. The team developed drone mapping techniques and resulting data products with sub-cm resolution. We found that habitability in ancient lake habitats could not be resolved at levels relevant to current Mars orbital data, but instead required resolution at cm scales or below. Our fieldwork also advanced significantly towards understanding biosignature location and extraction within stratigraphies, that have little, or no biosignature surface expression, which is a critical Mars exploration knowledge gap. Additionally, the characterization and quantification of biosignature preservation potential is currently being performed by our team through lab work on samples returned from the field.

Mars data analysis resulted in new views on how clays formed on early Mars (Bishop *et al.*, 2018). This study brought new insights into the evolution of early Mars climate, and expanded our perspective on where habitats could have formed. Mars data analysis by our team has also led to the discovery of an abundance of new paleolakes in Hellas on Mars (Hargitai *et al.*, 2018).



The Simba volcano (5,940 m) was successfully summited by the SETI team on November 15, 2018. Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team




The resulting publication showed that hydrothermal processes could have generated habitats beyond the period of overall favorable surface conditions of the Noachian in that region.

Our 2018 collaborations were productive and included: 1) Co-organization (with the JPL NAI team) of the Biosignature Detection Session at COSPAR. 2) The selection of a NASA PSTAR icy moon exploration proposal with team members brought together through the NAI Biosignature Detection Working Group and an NAI DDF project. We also welcomed Ph.D. candidate Alberto Candela (Carnegie Mellon University) to the team through a Lewis and Clark grant. Finally, NAI teamwork was brought together through the publication of the book "Habitability and Life on Mars" (Elsevier). Co-edited by Nathalie Cabrol and Edmond Grin (preface by Carl Pilcher), with team member contributions to 7 of the 13 chapters, the book reviews current knowledge of Mars habitability and provides recommendations on how to search for biosignatures.

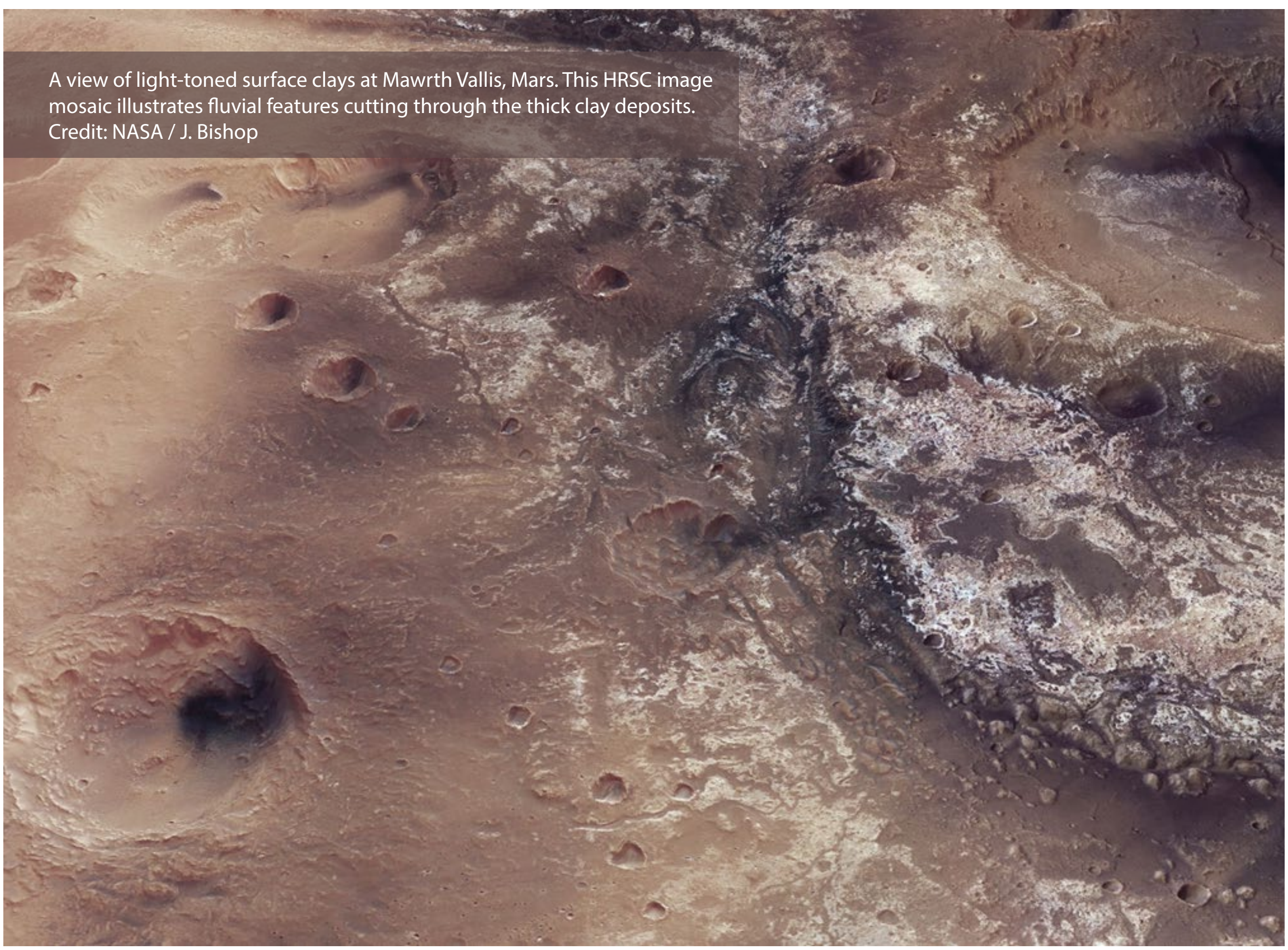
Left: Cover of Nature Astronomy

Star trail above basecamp at Salar de Pajonales, Chilean altiplano (3,500 m).

# Project Reports

## Signatures of Habitability - What and Where to Search?

Surface minerals on Mars constrain our understanding of the aqueous geochemical conditions during their formation and thus guide us to the types of habitable environments once present on Mars. For example, understanding the origin of clay-rich strata and determining the likely conditions controlling formation of salty horizons in these layered clay outcrops enables us to evaluate Mars' past prebiotic and biological potential. The salty soils are of particular interest for biology because of their ability to retain small amounts of liquid water, even in permafrost settings. We have explored occurrences of clay-sulfate outcrops on Mars in orbital CRISM images (J. Bishop, lead) and coordinated these with lab and field investigations. Major results include identification of different environments supporting liquid water on Mars, thus presenting multiple potential ecosystems to be considered. These aqueous settings include: 1) the sulfate minerals jarosite and gypsum embedded in the clay-rich strata at Mawrth Vallis, Mars that resemble salty evaporite deposits on Earth, and 2) possible salt deliquescence mechanisms in Mars analog experiments related to landslides and collapse in terrestrial settings that could be related to surface flows on Mars. Knowledge of sustainable, habitable conditions throughout Mars' history continues to constrain our ability to evaluate Mars' past prebiotic and biological potential. Understanding the duration of surface water activity in the formation of fluvial landforms resulting from paleo microclimates throughout Mars geologic history is central to this debate (V. Gulick, lead). For example, fluvial activity apparently continued periodically in Northeast Hellas throughout Mars' geologic history and resulted in the formation of 33 paleolakes ranging in age from ~3.75 Ga to 0.5 Ga. Several are adjacent to Hadriaca volcano, where persistent, long-duration hydrothermal systems could have provided sustained habitable environments. Deposits within these paleolake depressions and at channel termini may contain the geological and potentially biological record of these environments.



A view of light-toned surface clays at Mawrth Vallis, Mars. This HRSC image mosaic illustrates fluvial features cutting through the thick clay deposits. Credit: NASA / J. Bishop

## Taphonimic Windows and Biosignature Preservation - What, Where, and How to Search?

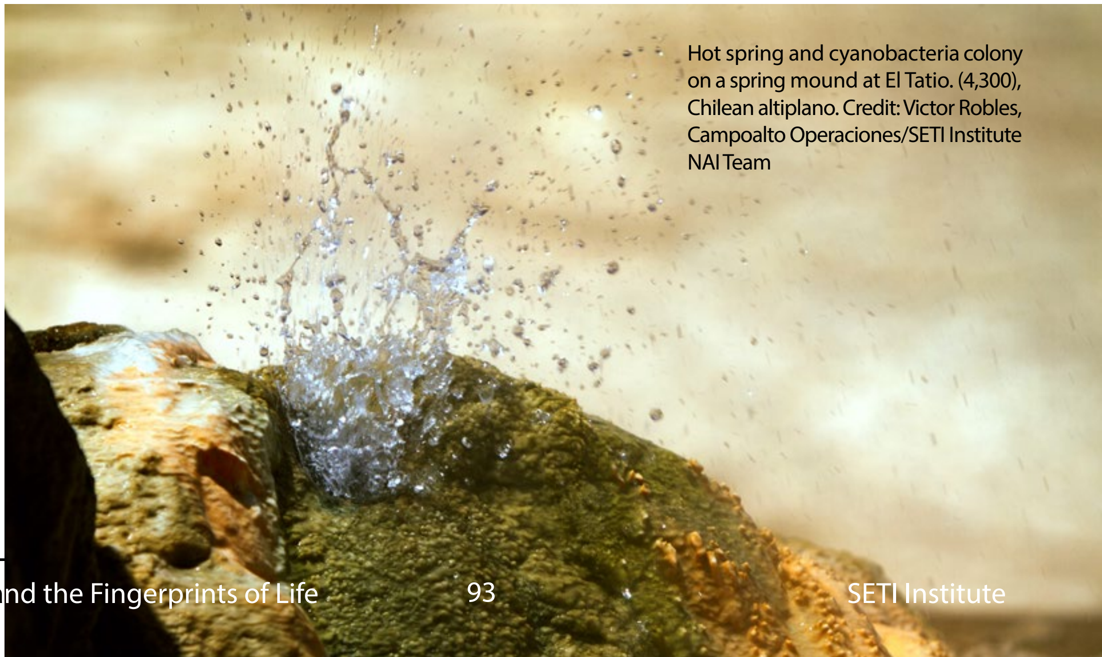
The goal of our Taphonomic Windows and Biosignature Preservation research is to explore analog Mars environments from mesoscale to textural microscale. In 2018, the SETI Institute team performed a major field expedition in the Chilean Altiplano and Andes that included the exploration of five sites representing key terrestrial analogs to martian habitable environments currently explored – or to be visited by upcoming Mars missions. These sites included Salar de Pajonales, where cryptic niches such as salt deposits and rocks represent some of the last microbial refuges in this hyperarid part of the Atacama. Highly adapted to the desert's extreme conditions, endolithic photosynthetic microbial communities include desiccation and radiation resistant bacteria colonizing gypsum evaporite deposits, and represent some of the hardiest microorganisms studied to date. Using drone imagery, the team mapped (at kilometer to centimeter scales) the location and surface area of gypsum salt structures and classified habitats prior to ecological mapping. An understanding of these microbial communities' spatial distribution and biodiversity yields insights into the potential of, and strategies for detecting biosignatures on Mars. At the El Tatio site, microbial biosignature suites preserved in hot spring sinter samples were characterized. The distribution of cellular and extracellular carbonaceous remains in the context of the biofabrics of three types of silica sinter deposits (from active, episodically active, to extinct geyser mounds) illustrates how differences in the biofabric microstructures affect preservation of biofilm relicts. Other sites explored in 2018 included Laguna de Aguas Calientes, and Laguna Lejia (both around 4,300 m elevation). The field expedition also included the successful ascent of the Andes Simba volcano (5,940 m) by the science team, which allowed the return of unique astrobiology samples for extensive laboratory analysis of extreme UV resistant microbial species.

Drone mapping the El Tatio geyser field (Study Site 2). Drones were using to build a database that allows us to integrate information from orbit to the ground and to the lab. This integration will enable us to develop new methods to detect high probability areas for habitability and microhabitats in the near future. Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team

## Environmental Controls on Biosignature Preservation - What, Where, and How to Search?

In this study, our team experimentally examined samples from Mars analog environments both in the field and the laboratory, and at molecular scales. Chemical, morphological, and petrographic methods were used by Co-I N. Hinman to spatially correlate chemistry, fabrics, and mineralogy in quartzose and opaline high-temperature sinters (aka geyserite) from mixed acid-sulfate-chloride and acid-sulfate hot springs collected from Yellowstone National Park. Co-I S. Cady focused on characterizing and correlating microbial biosignature suites preserved in hot spring sinter samples from the Atacama and from Yellowstone National Park, using state-of-the-art mass spectrometry techniques,optical, electron, and infrared microscopy methods, and the LD-Chip method. This research revealed the best approaches to look for microbial biosignatures in these sample types. Activities completed by Co-I J. Farmer include the synthesis of outcrop-scale field observations and petrographic analyses of modern sulfate deposits collected by the SETI Institute field teams. Co-I R. Quinn examined the preservation of amino acids in perchlorate and nitrate samples under Mars-like ionizing radiation conditions. The studies show that both perchlorate and nitrate decompose to form by-products that react with amino acids, suggesting poor preservation in perchlorate rich environments on Mars. Co-I A. Davila has been building a database of the amino acid content in meteorites with the goal of defining an abiotic baseline for amino acid composition in natural samples. The data confirms that the structurally simplest amino acids are significantly overrepresented in abiotic samples, and together they account for >70% of the total amino acid content. On the other hand, samples that contain biogenic amino acids show a more even distribution where the structurally simplest amino acids only represent a small fraction of the total amino acid content. This suggests that amino acid distributions could be used to differentiate biotic from abiotic sources of organics in samples from Mars or from Icy Moons in the outer Solar System.

Pigmented filamentous algae in a hot spring at El Tatio, Chilean altiplano (4,300 m). Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team



Hot spring and cyanobacteria colony on a spring mound at El Tatio. (4,300), Chilean altiplano. Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team

# Field Work

### Salar de Pajonales

Location: Chilean Altiplano (3500 meters elevation) The goal of our field work at Salar de Pajonales, Chile, as well as our other field sites is to understand how to integrate data from orbit, to the ground, and then to the lab, in order to develop new detection methods, exploration strategies, and instruments for Mars use.

### Laguna Aguas Calientes

Location: Chilean Altiplano (4300 meters elevation) Laguna Aguas Calientes is a lake fed by cold hydrothermal springs. The Altiplano is a story about interactions between volcanism and water, and how they mix, and how they generate or destroy habitats as may have also once happened on Mars.

### Laguna Lejia

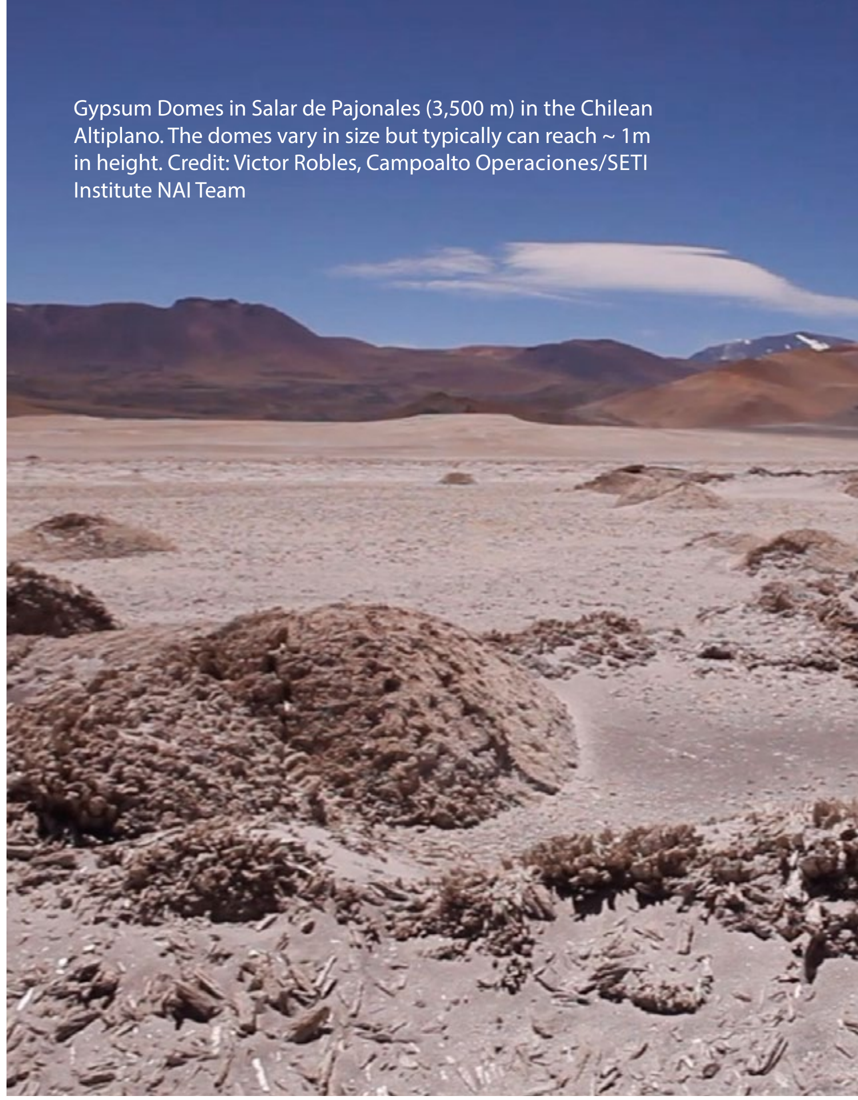
Location: Chilean Altiplano (4300 meters elevation) The outcrops of Laguna Lejia tell the story of two very different lakes, a past deglaciation lake, and its modern remnant which is now evaporating. Characterizing the adaptation of the microorganisms in this environment inform us about how to search for biosignatures on Mars, including what measurement scale and resolution are required for the search.

### Elk Geyser, Yellowstone National Park

A photochemical field study of Elk Geyser documented high and near-sterilizing concentrations of ROS produced during peak natural UV flux followed by lower values during diminished UV flux. Microbes were observed in but not on precipitated opal deposits. This suggests that the microbes may use the precipitates as protection from both UV and harsh chemical conditions. The UV radiation promotes iron oxidation, which possibly leads to formation of recognizable microfossils.

### Timmins, Canada; Lava Beds NM, California; Rio Tinto, Spain

NIR reflectance, Raman, and LIBS instruments were used to explore these sites. This work addresses Mars astrobiology exploration objectives by performing field work and instrumental analyses in acid sulfate environment (Rio Tinto), talc-carbonate deposits in (Timmins) Lava Tubes National Monument (California) as high fidelity analog environments to putative habitable environments on Mars.



Gypsum Domes in Salar de Pajonales (3,500 m) in the Chilean Altiplano. The domes vary in size but typically can reach ~ 1m in height. Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team

Base camp of the SETI Institute NAI team in the High Andes near Laguna Lejia and the Simba volcano (4,300 m elevation). This area was the base of operations for Laguna Aguas Calientes, Laguna Lejia and Simba. Credit: Victor Robles, Campoalto Operaciones/ SETI Institute NAI Team

Laguna Lejia: Its shore shows interbedded sequences of volcanic and lacustrine deposits in which biosignatures have been preserved, allowing the team to test instruments, technology, and sample acquisition methods relevant to Mars exploration. Credit: Victor Robles, Campoalto Operaciones/SETI Institute NAI Team

Summit of the Simba volcano (5,940 m) that the team reached on November 15, 2018. The lake was frozen after several days of winter storm and very cold temperatures (-18C). Microbial organisms, sediments, rocks, and water were collected from the lake and shore. Credit: Michael Phillips, SETI Institute NAI Team/University of Tennessee, Knoxville

## Flight Mission Involvement

**MRO-CRISM**
Team Members: Janice Bishop, James Wray
Involvement: CRISM Co-I, testing new image products, investigating outcrops of aqueous materials

**MRO HIRISE**
Team Member: Virginia Gulick: Co-I
Involvement: Science team member. Leads the fluvial and hydrothermal processes sciences themes

**MSL**
Team Member: Jack Farmer
Involvement: CHEMIN team member and E&PO lead

**Mars Exploration Rover Mission**
Team Members: Jeffrey Moersch, Nathalie Cabrol
Involvement: Participating scientists

**Mars Odyssey Mission**
Team Member: Jeffrey Moersch
Involvement: Participating scientist on Thermal Emission Imaging System

**ExoMars 2020**
Team Member: Pablo Sobron
Involvement: Member of mission science team, Co-I on RLS (Raman Laser Spectroscopy) instrument

**Mars 2020**
Team Member: Pablo Sobron
Involvement: Science team member

**Icebreaker Discovery Mission Concept**
Team Members: Alfonso Davila (Deputy PI), Richard Quinn (Co-I, Instrument Scientist)

# Changing Planetary Environments and the Fingerprints of Life: 2018 Publications

Barge, L. M., Krause, F. C., Jones, J-P., Billings, K., Sobron, P. (2018). Geoelectrodes and Fuel Cells for Simulating Hydrothermal Vent Environments. *Astrobiology* 18(9): 1147–1158. DOI: 10.1089/ast.2017.1707

Bishop, J.L. (2018). Remote detection of phyllosilicates on Mars and implications for climate and habitability. Chapter 3 in: *From Habitability to Life on Mars*. Editors Cabrol, N. A. and E. A. Grin, Elsevier, pp. 37-75. ISBN: 9780128099353

Bishop, J. L., Fairén, A. G., Michalski, J. R., Gago-Duport, L., Baker, L. L., Velbel, M. A., Rampe, E. B. (2018). Surface clay formation during short-term warmer and wetter conditions on a largely cold ancient Mars. *Nature Astronomy* 2: 206–213. DOI: 10.1038/s41550-017-0377-9

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# ALTERNATIVE EARTHS

**Lead Institution:**
**University of California, Riverside**

UCR

**Principal Investigator:**
Timothy Lyons

## Team Overview

A single motivation drives the research of the Alternative Earths Team: How has Earth remained persistently inhabited through most of its dynamic history, and how do those varying states of inhabitation manifest in the atmosphere? It is conceivable that each of Earth's diverse planetary states translates to a particular atmospheric composition that could one day be detected on an exoplanet—and that one of these "Alternative Earths" could help prove the presence of life elsewhere in the universe.

Defining these ancient and possible atmospheric compositions and their potential for remote detectability relies on teamwork among Co-Investigators at UC Riverside (UCR), Yale, Georgia Tech (GT), Arizona State University (ASU), Oregon Health and Science University (OHSU), and the J. Craig Venter Institute (JCVI), as well as with our collaborators at home and abroad. No matter what time slice of Earth history we tackle, our vertically integrated approach spans from a comprehensive deconstruction of the geologic record to a carefully coordinated sequence of modeling efforts to assess our own planet's relevance to exoplanet exploration. These efforts, from empirical evidence to complementary theory, require unique interdisciplinarity that bridges one perspective to the next:

- Composition of the oceans and atmosphere
- Gas fluxes and ecological impacts
- Stability and remote detectability of biosignature gases

**Team Website:** https://astrobiology.ucr.edu

# 2018 Executive Summary

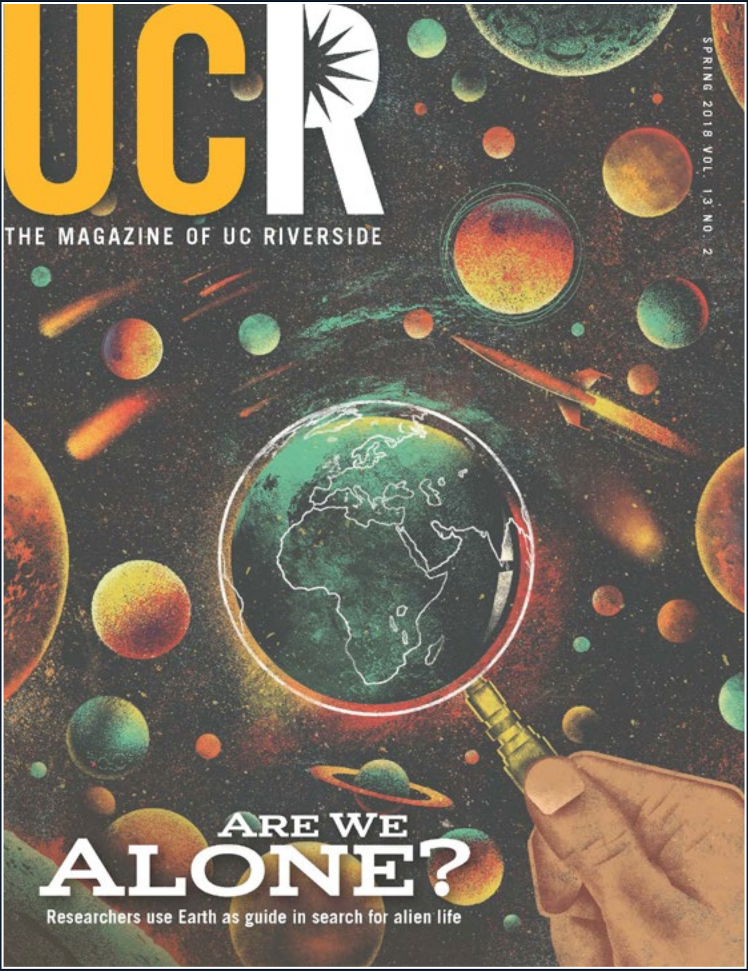
Simply put, the Alternative Earths Team is unraveling the evolving redox state of Earth's early atmosphere as a guide for exoplanet exploration. Redox-sensitive greenhouse gases, for example, can expand the habitable zone well beyond what is predicted from the size of a planet's star and its distance from that energy source alone. Indeed, our team this year proposed a variety of gas-production scenarios that could reconcile how the young Earth remained ice-free when the sun was much dimmer: methane from a novel combination of primitive anoxygenic photosynthesizers (Ozaki *et al*., 2018b); nitrous oxide emanating from iron-rich seas (Stanton *et al*., 2018), and enhanced $CO_2$ production via reverse weathering (Isson and Planavsky, 2018).

Conversely, the absence of obvious biosignature gases such as oxygen does not necessarily mean a planet is sterile: cyanobacteria were producing oxygen on Earth long before it accumulated to remotely detectable concentrations in the atmosphere. Further developing this concept of 'false negative' biosignatures (first articulated in Reinhard *et al*., 2017), the team explored a powerful way to assess exoplanets for inhabitation: observe their atmospheres throughout their orbits, potentially revealing life-driven seasonal changes in biosignature gases over the course of a year (Olson *et al*., 2018b). This study indicates ozone may be the only detectable biosignature on planets like the early Earth, making ultraviolet capability and direct imaging essential on next-generation telescopes—a point the team made in white papers submitted to the National Academy of Sciences Astrobiology and Exoplanet Science solicitations (Schwieterman *et al*., 2018c).

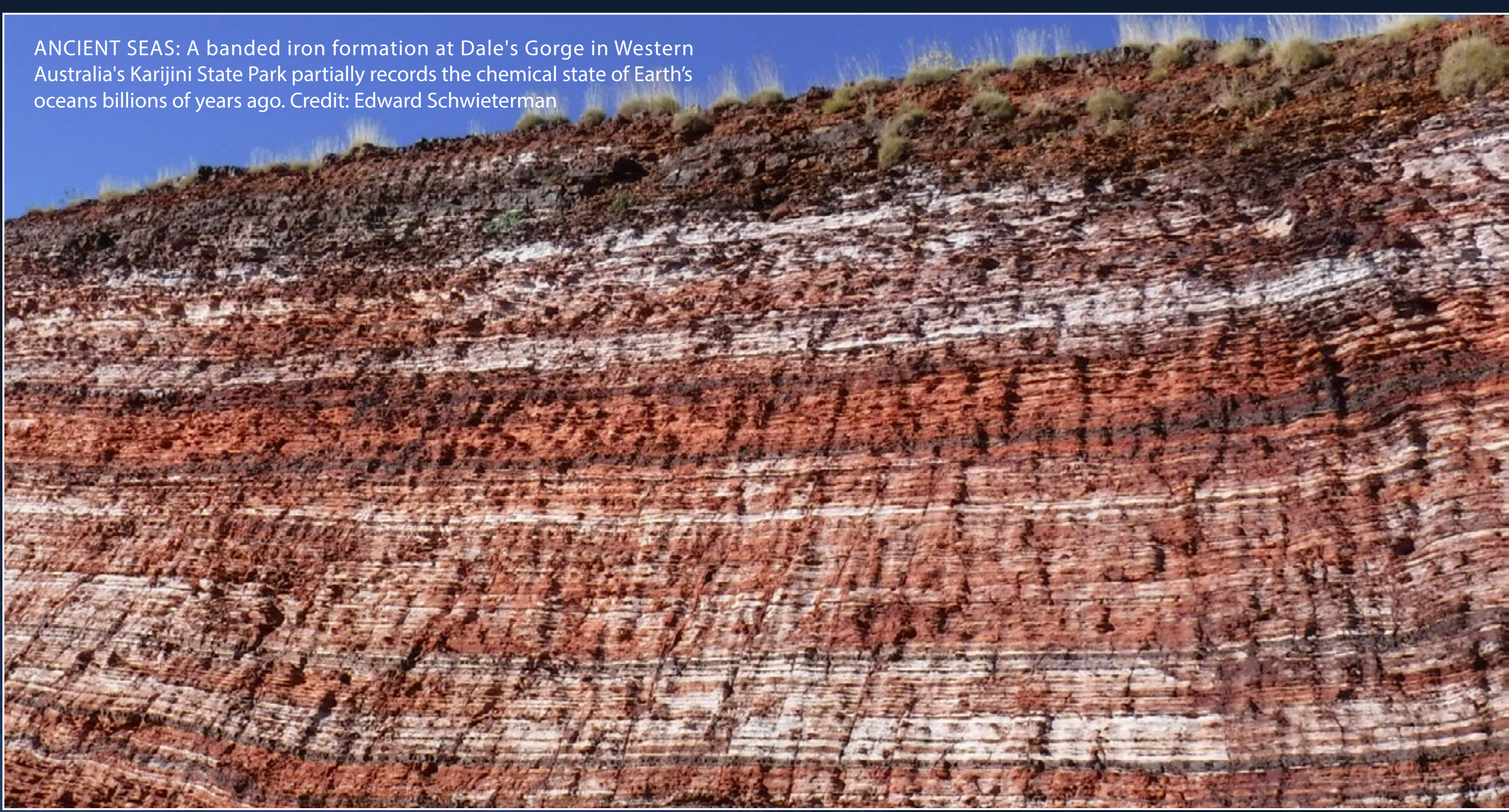
Using Earth's past to guide exoplanet exploration requires a careful vertical integration of research efforts, anchored by the core strengths of our team: development of geochemical proxies that reveal the composition of the ancient oceans and atmosphere. Several geochemical systems—chromium, rhenium, uranium, and zinc—continue to provide new evidence for very low $pO_2$ during much of the Precambrian and even suggest a delayed rise to prominence of eukaryotes (Isson *et al*., 2018) and oxygen fluctuations as a potential trigger for the Cambrian explosion (Wei *et al*., 2018). Another key focus of our team's mission is the role that contingent boundary conditions play in stabilizing the habitability of a planet for long timescales. Perhaps the most striking example in our solar system is plate tectonics, which, based on a new suite of papers from our Tectonics Working Group, may have been fully

EYE ON THE SKY: Earth's atmosphere contains myriad signatures of inhabitation. Credit: NASA operational throughout Earth history and much earlier than most previous estimates (Korenaga 2018a, 2018b; Rosas and Korenaga, 2018).

Collaboration and creative leadership by early career scientists are hallmarks of our team's success. Several papers showcase success at the interface between the Alternative Earths and Virtual Planetary Laboratory NAI teams (e.g., Schwieterman *et al*., 2018a, and Krissansen-Totton *et al.*, 2018), and a graduate student-led paper features our team's synergy with the CAN 6 Foundations of Complex Life NAI team (Zumberge *et al.*, 2018). Graduate students first-authored some of the team's other most important results this year (Diamond *et al*., 2018; Evans *et al.*, 2018; Olson *et al*., 2018b; Stanton *et al*., 2018; Zumberge *et al*., 2018), and NASA Postdoctoral Program fellows Edward Schwieterman (UCR), Kazumi Ozaki (GT), Juan Rosas (Yale), and Nadia Szeinbaum (GT) have brought new areas of modeling and experimentation into the mix (Rosas and Korenaga, 2018; Schwieterman *et al*., 2018a, 2018b, 2018c; Ozaki *et al*., 2018a, 2018b). Our team also works to elevate the profile of the Alternative Earths mission at the regional, national, and international levels through outreach, press coverage, conference organization, public talks, and honors.




ARE WE ALONE? UCR's Alternative Earths mission is the cover story of the May issue of UCR Magazine. Credit: UC Riverside

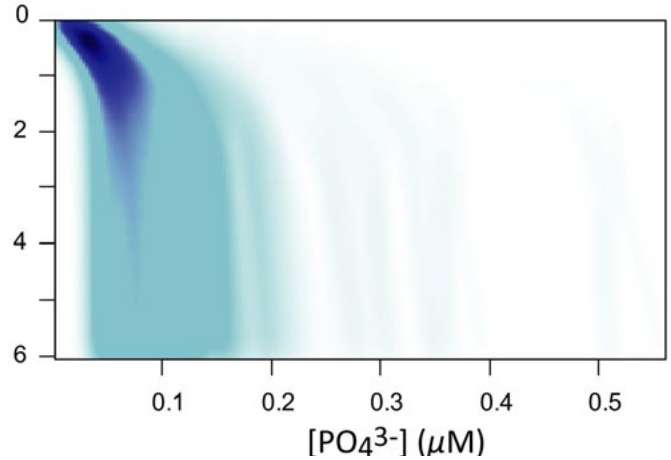


ANCIENT SEAS: A banded iron formation at Dale's Gorge in Western Australia's Karijini State Park partially records the chemical state of Earth's oceans billions of years ago. Credit: Edward Schwieterman

## Project Reports

### Composition of the Oceans and Atmosphere

Several geochemical and molecular systems have led the team to an array of conclusions about atmospheric oxygenation, the rise of eukaryotes, and the onset of plate tectonics. Chromium, rhenium, and uranium analyses continue to provide new evidence for very low $pO_2$ during much of the Precambrian (Cole *et al.*, 2018a; Sheen *et al.*, 2018; Wang *et al.*, 2018). Now we have also identified a compelling mechanism for this protracted oxygenation of the Earth's atmosphere. A statistical analysis of a novel biogeochemical model conducted by NPP Fellow Kazumi Ozaki (GT) and Institutional PI Chris Reinhard (GT) indicates that the rate of net $O_2$ production in mid-Proterozoic oceans (1.8 to 0.8 billion years ago) was ~25% of today's value, owing largely to phosphorus scarcity in the ocean interior (from Ozaki *et al.*, 2018a).

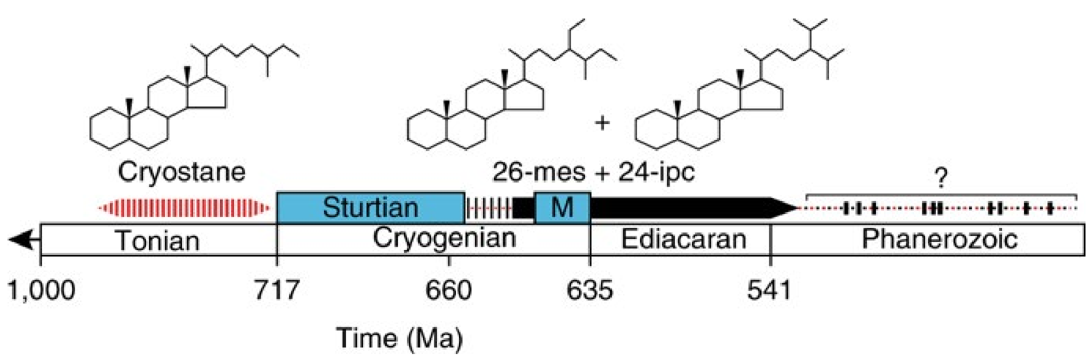



PHOSPHORUS SCARCITY: Probability distribution of phosphate with water depth on the mid-Proterozoic Earth shows a scarcity of this critical nutrient in the ocean interior (Ozaki *et al.*, 2018a).

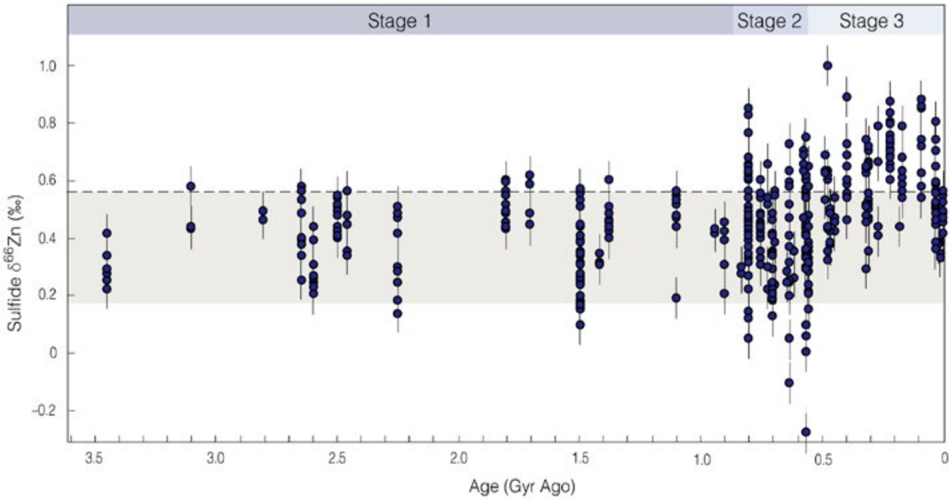
This new evidence for low atmospheric $pO_2$ and a sluggish biosphere during the mid-Proterozoic complements two parallel studies that pinpoint the rise of eukaryotes to sometime after 800 million years ago. Using new sterane biomarker evidence from rocks in South Oman, graduate student Alex Zumberge and his advisor Gordon Love (UCR) led a study strongly suggesting that demosponges, and hence multicellular animals, were prominent in some late Neoproterozoic marine environments at least as far back as 660–635 million years ago. The new molecular fossil, a hydrocarbon skeleton called 26-methylstigmastane (26-mes), is the first animal-specific biomarker detected in the geological record that can be unambiguously linked to compounds known only to be synthesized by certain species of modern sponges called demosponges. This early sponge arose nearly a billion years after the oldest microfossil evidence for the emergence of eukaryotes ~1.7 to 1.6 billion years ago.

Consistent with these organic biomarker records, a new study of sedimentary zinc isotopes offers a potential explanation for the apparent billion-year lag between the emergence of eukaryotes and their rise to ecological prominence. In an unprecedented, extremely labor-intensive analysis of the sedimentary zinc isotope record spanning 3.5 billion years of Earth's history, graduate student Terry Isson (Yale) and several other members of the Alternative Earths team discovered a fundamental shift in the marine zinc cycle ~800 million years ago. At that time, the biosphere transitioned toward much greater efficiency at sequestering organic-derived zinc. A shift toward a more eukaryote-rich global marine ecosystem could explain this jump because eukaryotes have a much higher affinity for zinc relative to prokaryotes (Isson *et al.,* 2018). In other words, these data may be our best smoking gun for the earliest proliferation of eukaryotic (algal) life in the oceans—almost a billion years after the first appearance of eukaryotes.

Analyzing yet another isotopic system across Earth's history, NPP Fellow Juan Rosas and his advisor Jun Korenaga (Yale) published a series of papers suggesting that plate tectonics was likely to have




OLDEST SPONGE: A revised Neoproterozoic–Cambrian timeline showing co-occurrences of 26-mes and 24-ipc sterane biomarkers (from Zumberge *et al.*, 2018).




ZINC ISOTOPES: In this sedimentary zinc (Zn) isotope record spanning 3.5 billion years of Earth's history, we identify three stages within the sulfide record. Stage 2 (the late Neoproterozoic) is a transitional interval during which $\delta^{66}Zn_{sulf}$ values distinctively lighter and heavier than the crustal range are expressed, likely reflecting both the ecological rise of eukaryotes and re-organization of the global biogeochemical Zn cycle (from Isson *et al.*, 2018).

been fully operational since early in the Hadean. Their reconstruction of net growth of continental crust—using geochemical box modeling and a compilation of samarium-neodymium isotope data—predicts rapid crustal growth and recycling in early Earth prior to 3.8 billion years ago (Rosas and Korenaga, 2018). Their new model also successfully reproduced the crust formation age distribution produced by their latest zircon-based estimates of continental growth (Korenaga 2018a).

Korenaga also made important progress on the evolution of continental mantle lithosphere. His global compilation of cratonic mantle xenoliths revealed, for the first time, a smooth secular trend in mantle depletion (Servali and Korenaga, 2018). Overall, this evidence for greater continuity between the Hadean and later worlds fuels intriguing possibilities for how habitability might have arisen and sustained during an interval previously noted for its assumed challenges to life and life-sustaining conditions. The timing and pattern of early continents and the related onset of plate tectonics are among the central remaining issues in studies of early Earth. Assessing the potential importance of analogous tectonics for sustained habitability on exoplanets is paramount.

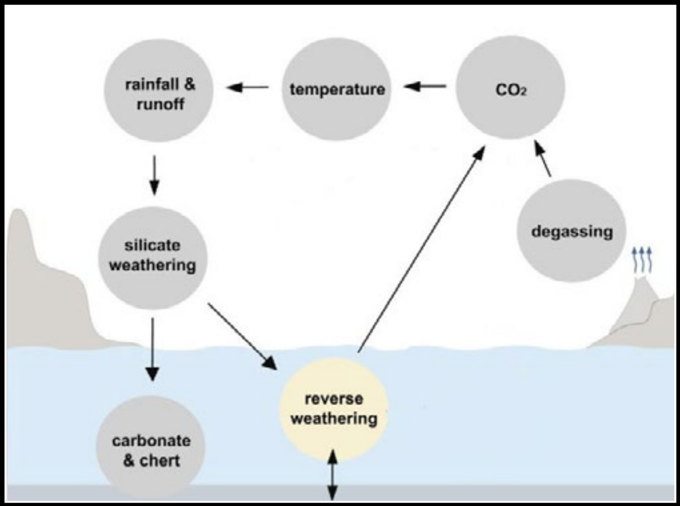
JENNIFER GLASS OF GEORGIA TECH HOLDING STROMATOLITIC IRONSTONE (above and close-up, below). Credit: Georgia Tech / A. Carter

## Gas Fluxes and Ecological Impacts

Understanding how our planet regulates climate in the modern era and in the distant past is critical for explaining the long-term maintenance of planetary habitability on early Earth and worlds beyond our solar system. Three featured studies are helping to reconcile how Earth remained ice-free when the sun was much dimmer. Institutional PI Chris Reinhard (GT), NPP Kazumi Ozaki (GT), and colleagues from Japan developed a comprehensive new model that shows how a combination of two anoxygenic pathways of photosynthesis together could have produced enough methane to counter this faint young sun paradox' on early Earth (Ozaki *et al.*, 2018b). Two other exciting results focused on the greenhouse gases nitrous oxide and $CO_2$.

**Laughing Gas Could Have Helped Warm Early Earth:** Carbon dioxide and methane get partial credit for keeping the early Earth ice free, but established research (including important results from our group, e.g., Olson *et al.*, 2016) suggests that those gases were not always sufficiently abundant to warm the globe on their own. New views on ocean chemistry during Proterozoic Eon, ~2.4-0.5 billion years ago, reveal a new way that nitrous oxide could have helped fill this 'greenhouse gap.' A non-biological process known as chemodenitrification produces nitrous oxide only when seawater is high in dissolved iron and low in oxygen, a condition now widely hypothesized for the Proterozoic oceans. Undergraduate student Chloe Stanton (GT), working with Jennifer Glass (GT), Chris Reinhard (GT), Tim Lyons (UCR), and collaborator Jim Kasting (Penn State), coupled experiments with photochemical modeling to better understand the processes controlling atmospheric $N_2O$ levels during Proterozoic time. In lab experiments testing chemodenitrification, ferrous iron dissolved in seawater reacted with nitrogen molecules to yield unusually high fluxes of nitrous oxide. Plugging these higher fluxes into a photochemical model of the Proterozoic atmosphere, assuming oxygen at only 10 percent of present atmospheric level, yielded concentrations of nitrous oxide 10 times greater than today's—plenty to provide a hearty boost to the greenhouse effect.

**Rethinking Long-Term Controls on Planetary Climate: Reverse Weathering**: Over geologic time, chemical weathering of silicate rocks on Earth removes $CO_2$ from the atmosphere. Graduate student Terry Isson (Yale) has combined both geologic and model data that predict the reverse of this process—that is, when dissolved products of weathering react in the ocean to form clay minerals and return $CO_2$ to the atmosphere—would have been more active on Earth prior to about 500 million years ago. Before the evolution of silica-secreting eukaryotic life, early oceans were more silica rich. Higher silica fueled more rapid rates of clay formation and $CO_2$ production during reverse weathering. This process would have enhanced climate stability by mitigating large $pCO_2$ swings—a critical component of Earth's natural thermostat that dominated the planet's first four billion years.



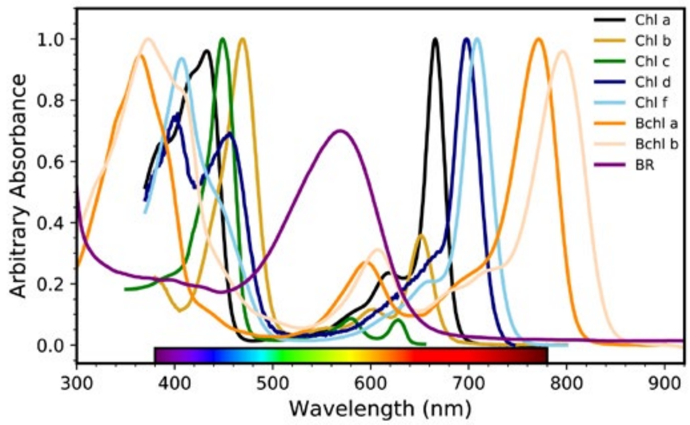

CONCEPTUAL MODEL FOR REVERSE WEATHERING: The return rate of $CO_2$ to the atmosphere was greater when silica content of the oceans was higher, as during the first four billion years of Earth's history (from Isson *et al.*, 2018).

REVERSE WEATHERING IN THE FIELD: On the Arctic island of Svalbard Noah Planavsky (Yale) samples black shales containing clay minerals analyzed in the reverse weathering study. His canine companion is there to alert the team to approaching polar bears. (from Isson and Planavsky, 2018).

## Stability and Remote Detectability of Biosignature Gases

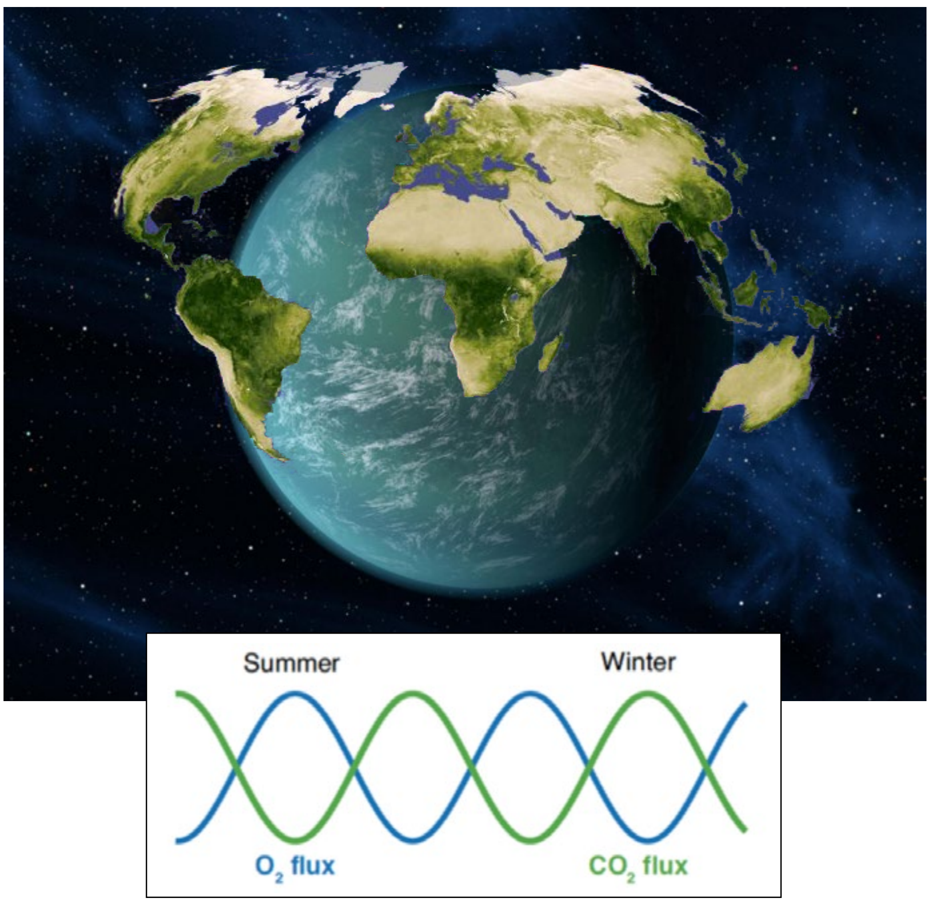
Remote life detection on exoplanets will encompass characterization of both surfaces and atmospheres. One featured study expands the categories of surface signatures (pigments) expected from light-powered organisms beyond those typically considered for photosynthetic organisms. Another makes the case that seasonal signals, including those for ozone, may be the only detectable biosignature on planets like the early Earth, making ultraviolet capability and direct imaging essential on next-generation telescopes:

**Early Evolution of Purple Retinal Pigments:** Retinal pigments present in Haloarchaea illuminate the early evolutionary origins of light-harvesting for energy generation—and may be a signature of life on Earth-like exoplanets. Simple retinal-based, light-harvesting systems such as purple chromoprotein bacteriorhodopsin absorb at complementary wavelengths to the chlorophyll pigments used by photosynthesizers. Alternative Earths postdoctoral fellow Edward Schwieterman (UCR) and his colleague Shiladitya DasSarma (University of Maryland) explored the hypothesis that these simpler light capture systems have an evolutionary origin that predated photosynthesis and examined their applications to remote exoplanet biosignatures (DasSarama and Schwieterman, 2018). Purple retinal pigments absorb light at complementary wavelengths to chlorophyll pigments and power ATP (energy) synthesis by coupling to membrane potential. Their early origins may have influenced the evolution of more complex photosystems on Earth and may create unique spectral signatures on inhabited exoplanets, such as "green-edges" compared to the more familiar vegetation "red-edge."




COMPLEMENTARY PIGMENTS IN PURPLE PONDS: The striking colors of hypersaline ponds are partially due to light-harvesting extremophilic Haloarchaea (top). These microbes have a purple membrane that absorbs light in the green-yellow part of the spectrum (purple line, bottom) compared to chlorophyll and bacteriochlorophyll pigments, which absorb most strongly in the blue and red (from DasSarma and Schwieterman, 2018).

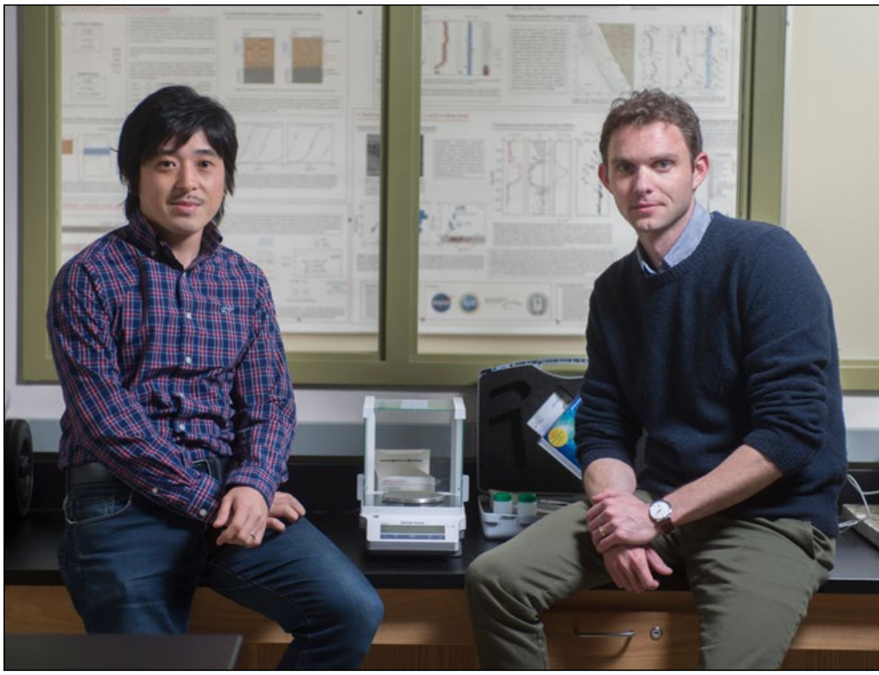
**Atmospheric Seasonality as an Exoplanet Biosignature:** A powerful way to assess exoplanets for inhabitation may be observations of their atmospheres throughout their orbits—potentially revealing life-driven changes in biosignature gases over the course of a year. In the first quantitative framework for dynamic biosignatures based on seasonal changes in the Earth's atmosphere, graduate student Stephanie Olson (UCR), postdoc Edward Schwieterman (UCR), Chris Reinhard (GT), Tim Lyons (UCR), and VPL Team Leader Victoria Meadows modeled the seasonal formation and destruction of oxygen, carbon dioxide, and methane. They also modeled fluctuations of atmospheric oxygen on a life-bearing planet with low oxygen content, like Earth billions of years ago. Ozone ($O_3$) is produced in the atmosphere through reactions involving oxygen gas ($O_2$) produced by life. On weakly oxygenated planets, ozone would be a more easily measured marker for the seasonal variability in $O_2$. Although the UV bands of ozone are saturated in the spectrum of modern Earth, the low $O_2$ concentrations of the mid-Proterozoic Earth would have been amendable for dynamic, seasonal $O_3$ signatures that changed because of differences in photosynthetic $O_2$ production with changing average solar insolation (Olson *et al*., 2018b). Based on these results, the team submitted a white paper to the National Academy of Sciences Astrobiology and Exoplanet Science Strategy (Schwieterman *et al*. 2018c) that emphasized the need to include UV capabilities to detect $O_3$ on worlds like the Proterozoic Earth where $O_2$ itself would be undetectably low in concentration.




EXPLORING EXO-SEASONS: Satellites monitor how 'greenness' changes with Earth's seasons (world map, top overlay. Credit: NASA). This study assessed the accompanying changes in atmospheric composition of gases such as $O_2$ and $CO_2$ (graph, bottom, from Olson *et al*., 2018b) as a marker for life on distant planets like Kepler 22B (NASA artist's depiction, top background. Credit: NASA / Ames / JPL-Caltech).

## New Quantitative Approaches Toward Understanding the Life History of an Inhabited Planet

Kazumi Ozaki's research with Chris Reinhard (GT) explores the mechanistic processes at play in the Earth's early atmosphere and oceans—a key step in synthesizing the geochemical observations made by other members of the Alternative Earths team. Ozaki developed CANOPS, a biogeochemical model of the C-N-P-$O_2$-S ocean cycles, and has used it to constrain global redox balance in multiple time slices of Earth's past. Working with Reinhard and colleagues at the University of Tokyo, one recent analysis of the mid-Proterozoic ocean-atmosphere system implicates severe phosphorus limitation—and a resulting sluggish biosphere—as the mechanism underlying numerous observations that atmospheric $O_2$ levels remained remarkably low for much of mid-Proterozoic time (Ozaki *et al.*, 2018a).



Kazumi Ozaki (left) and his advisor Chris Reinhard in Reinhard's office at Georgia Tech. Credit: Georgia Tech

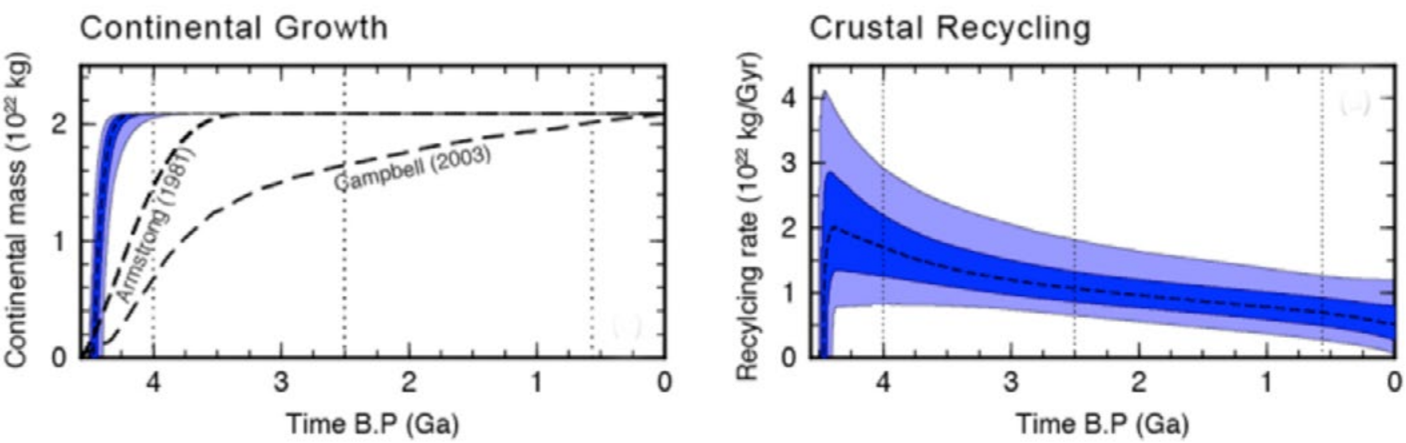
In a second effort, Ozaki and his colleagues used a coupled ecosphere-photochemistry model to explore the effects of primitive photosynthesis on Earth's early climate system (Ozaki *et al.*, 2018b). Certain microbial metabolisms produce methane, a potent greenhouse gas, but in isolation no single metabolism could have produce sufficient methane to keep the early Earth warm under a fainter sun. By including an array of microbial metabolisms within a biogeochemical cycling model more reflective of the complexity of an entire living planet, the team was able to isolate two different types of primitive bacterial photosynthetic processes that together could have produced more than enough methane to counter the famous "faint young sun paradox."

This fruitful collaboration has continued even after Ozaki began an assistant professorship at Toho University in April. Ongoing work includes modeling the impact of anoxygenic photosynthesis on planetary oxygen cycles and the long-term future of oxygen-based biosignatures on Earth. In a prime example of Alternative Earths synergy, graduate student Devon Cole, who developed chromium isotope compilations with Noah Planavsky at Yale, visited Ozaki in Japan in December to learn how to use his CANOPS model in preparation for a postdoc with Reinhard.

## Early Earth Geodynamics and Continental Evolution

Juan Rosas works with Jun Korenaga (Yale) to understand the geodynamic regime of the early Earth and the evolution of the continental crust. Two particular objectives achieved this year—to quantify crustal recycling rates during the Hadean and Archean (from Earth's accretion to 3.8 billion years ago) and to construct a new model for continental growth—resulted in a recent publication in *Earth and Planetary Science Letters* (Rosas and Korenaga, 2018). Based on the evolution of the samarium-neodymium (Sm-Nd) isotope system, this new growth model accurately reproduces a present-day recycling rate within current estimates (0 to 1022 kg Gyr-1). Likewise, the new model predicts present-day distribution of crust formation age that is remarkably similar to that inferred from the most up-to-date global compilation of zircon age data (Korenaga 2018a).

These results carry significant implications for understanding the surface environment of the early Earth. The progression of Sm-Nd isotope system throughout Earth's history is consistent with rapid continental crustal growth during the planet's first 500 million years, as well as significant crustal recycling that decreases steadily with time. Significant crustal recycling throughout the Hadean and Archean is easier to explain assuming the continuous operation of plate tectonics rather than the stagnant lid regime proposed by other researchers. Whenever it occurred, the onset of plate tectonics would have produced widespread changes in geologic cycles, including long-term climate and the global nutrient and water cycles, making it an essential factor for Earth's early habitability.




HADEAN CONTINENTS: Based on an analysis evolution of the samarium-neodymium isotope system, NPP Juan Rosas and his advisor Jun Korenaga (Yale) produced a geochemical box model that resulted in rapid continental growth (left) and significant crustal recycling (right) during the first 500 million years of Earth history. This model predicts crustal growth much earlier in Earth history than estimates from previous models (dashed lines) and suggests plate tectonics has likely been active during most of the planet's history. Credit: Rosas and Korenaga, 2018

## Visualizing Alternative Earths Through Time and Space

Edward Schwieterman leverages knowledge about Earth's atmospheric and surface evolution through geologic time to make actionable predictions about the remotely detectable signatures of terrestrial exoplanets. Working with Tim Lyons (UCR) and other members of the Alternative Earths team, his efforts this year included an examination of seasonality as a potential exoplanet biosignature—the first quantitative attempt of its kind (Olson *et al.*, 2018b). He also paired up with microbiologist Shiladitya DasSarma (University of Maryland) to expand the categories of exoplanet surface signatures expected from organisms with light-capturing pigments (DasSarama and Schwieterman, 2018).

As part of a special issue endorsed by NASA's Nexus for Exoplanet System Science published in the journal Astrobiology, Schwieterman spearheaded a robust review of potential remotely detectable biosignatures (Schwieterman *et al.*, 2018a). Lessons from early Earth are an important component of this work, which has spawned many articles and interviews in the popular press. Schwieterman contributed to a further three papers in this special issue published on diverse topics such as false positives for biological oxygen (Meadows *et al.*, 2018) and future directions for exoplanet biosignature research (Walker *et al.*, 2018). He also led the team's feedback for the 2018 National Academy of Sciences call for ""Astrobiology Science Strategy for the Search for Life in the Universe," arguing that UV characterization of exoplanet atmospheres will be necessary for minimizing $O_2$ / $O_3$ 'false negatives' and for mitigating 'false positive' $O_2$ biosignatures (Schwieterman *et al.*, 2018c).

Throughout his work, Schwieterman applies the astronomical and spectroscopic expertise he gained during his doctoral studies at the Virtual Planetary Laboratory to UCR's traditional strengths in Earth history and evolution. Continually expanding his own understanding of deep Earth history, he recently participated in the Astrobiology Grand Tour, July 2–11, 2018, which was organized by leading experts in the field of Earth's ancient environments.

FIELD ASTRONOMER: Edward Schwieterman poses by a banded iron formation at Dale's Gorge in Western Australia's Karijini State Park. Credit: E. Schwieterman

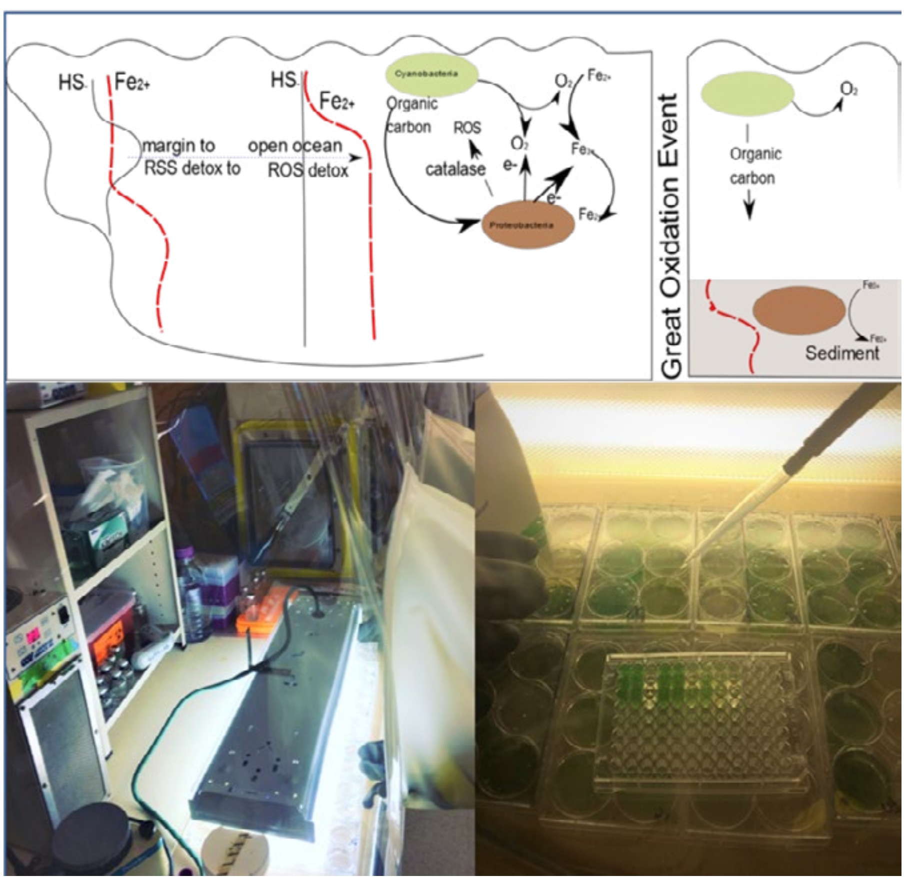
## Synthetic Microbial Communities as a Tool to Understand Metabolic Success: The Role of Microbial Interactions Leading to the Great Oxidation Event

NASA Postdoctoral Program Fellow Nadia Szeinbaum is working with Jennifer Glass and Chris Reinhard at Georgia Tech. Credit: N. Szeinbaum

Nadia Szeinbaum was awarded an NPP Fellowship in 2018 to work with Jennifer Glass and Chris Reinhard (GT). Szeinbaum is using microbial synthetic communities to study ancient microbial interactions, an experimental technique that has never before been used to test microbial metabolisms in the context of ancient ecological niches. Specifically, she is using this ecological approach to help explain the rise of oxygenic photosynthesis in ancient oceans.

Even modern oxygenic phototrophs face the unavoidable problem that photosynthesis relies on radical chemistry that produces toxic reactive oxygen species (ROS). During the Earth's transition from anoxic to oxic world, cyanobacteria specializing in anoxygenic photosynthesis could have evolved oxygenic photosynthesis, but they would have needed a way to scavenge the resulting ROS. Modern proteobacteria provide cyanobacteria with catalase to scavenge ROS. In addition, genetic analyses suggest that modern catalases in cyanobacteria appear to have been transferred from proteobacteria. Szeinbaum hypothesizes that such phototrophic-heterotrophic associations may represent a critical, long-lasting partnership that dramatically changed the planet. Using batch culture and continuous culture tests, she is reconstructing potential metabolic interactions using model bacteria to determine if ROS scavengers could have supported cyanobacteria during the rise to dominance of oxygenic photosynthesis.

Szeinbaum further posits that a cyanobacteria-proteobacteria consortium is more likely to overcome the challenge of peroxide stress under iron-rich conditions (which dominated the early oceans) than a pure cyanobacterial culture. (Isotopes in the geochemical record suggest that during the one billion years preceding the Great Oxidation Event, oxygenic phototrophs may have inhabited euxinic coastal waters or ferruginous open oceans.) In the lab, extracellular catalase production and growth rates under an anoxic headspace will provide empirical estimates for $Fe^{2+}$ tolerance by co-occurring cyanobacteria and proteobacteria. Ultimately, these experimental systems can also be used to test hypotheses about microbial interactions on other planets.




MICROBES AND OXYGEN. Conceptual model depicting interacting microbial populations generating and scavenging oxygen and ROS in high-iron oceans may occupy separate niches on modern Earth (top). Experimental setup used to study microbial interactions of model organisms under the anoxic, iron-rich marine condition that existed on Earth prior to the Great Oxidation Event (bottom).

# Field Sites

Our ultimate goal is to model past and future atmospheres on Earth and to extrapolate those lessons learned to exoplanets. But at its core our team relies on traditional fieldwork. It takes a comprehensive deconstruction of the geologic record, as well as proxy calibration in modern environments, to deliver the data that ground-truth our models of Earth's ancient environments:

## Rio Tinto, Spain

A focus on sulfide weathering in low pH environments took UCR and ASU-based team members to this world-famous site of acid mine drainage, where the they joined a large group of students and faculty from the University of St. Andrews.


Chris Tino (UCR) uses a pH meter to track ~5-unit change in pH over a distance of less than a meter where a fresh water stream flows into the Rio Tinto (April 2018). Credit: D. Gregory

Rio Tinto cuts through the Spanish landscape like a bleeding gash. Credit: D. Gregory

## Coorong, South Australia

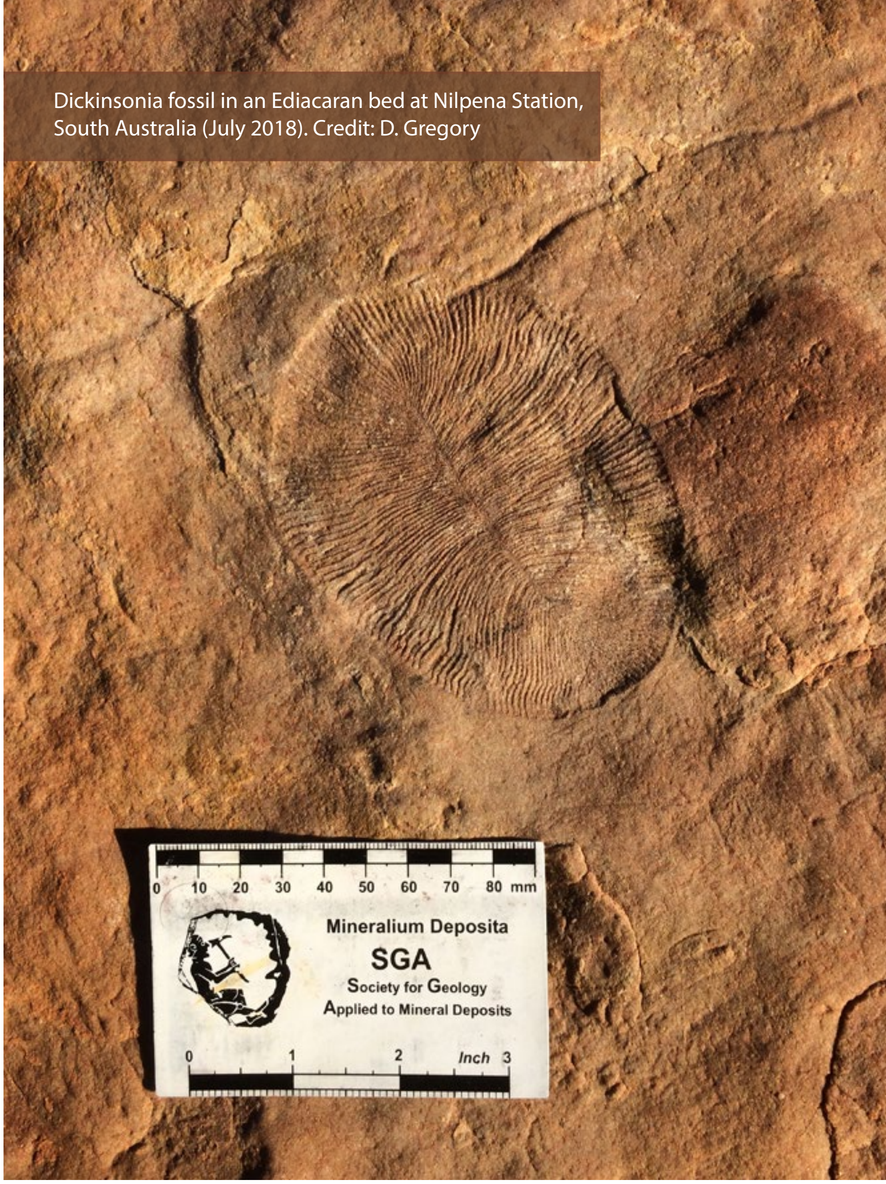
Analysis of dolomitic muds in the alkaline lakes of the Coorong coastal lagoon system complement fieldwork done at Ries crater in Germany and will improve understanding of analogous aquatic systems on ancient Mars and their potential to provide archives of the conditions for past life.


Team members pose at one of the famous alkaline lakes of the Coorong coastal lagoon system, South Australia (July 2018). From left: Daniel Gregory, Chris Tino, Jordan Todes, Eva Stüeken. Credit: T. Lyons

Tim Lyons samples dolomitic mud in one of the famous alkaline lakes of the Coorong coastal lagoon system, South Australia (July 2018). Credit: D. Gregory

**Nilpena Station (Ediacaran), Flinders Range, South Australia**

The state government of South Australia announced in early 2018 that it is investing $1.9 million to protect and preserve this world-famous Ediacaran fossil field in the Flinders Range. Mary Droser (UCR) has spearheaded fruitful discoveries on this site for the past 20 years and led an Alternative Earths team field trip to the site in July 2018.




Dickinsonia fossil in an Ediacaran bed at Nilpena Station, South Australia (July 2018). Credit: D. Gregory

**Svalbard**

Yale-based team members sampled ancient black shales on the Arctic island of Svalbard for future analysis in a variety of studies, including the reverse weathering analysis featured in our Project Reports.

Yale team members Terry Isson (seated, left) and Noah Planavsky (seated, right) led a sampling expedition to Svalbard (July 2018).

## NASA Mission Relevance

Informing future telescope design and cultivating mission involvement have been important motivations for the Alternative Earths team. Through our synergism with the Virtual Planetary Laboratory (VPL), we have extended our understanding of Earth's early atmosphere toward specific planetary biosignature detection scenarios. Specifically, Edward Schwieterman (UCR) has led the coupling of our comprehensive library of proxy-constrained estimates of ocean-atmosphere chemistry from the early Earth with the photochemistry-climate, radiative transfer models, and telescope simulators of the VPL. Schwieterman, Reinhard, Olson, and Lyons are now working together regularly to use our Earth models to inform telescope choices.

For example, our team participated in the recent solicitation of feedback for the "Astrobiology Science Strategy for the Search for Life in the Universe" and "Exoplanet Science" reports by the National Academy of Sciences (NAS). In an entirely Alternative Earths–authored submission, we argued that 'false positive' $O_2$ biosignatures can be mitigated through target selection and multi-wavelength planetary characterization (including the UV), while $O_2$/$O_3$ 'false negatives' cannot be eliminated without UV (Schweiterman *et al.,* 2018c).

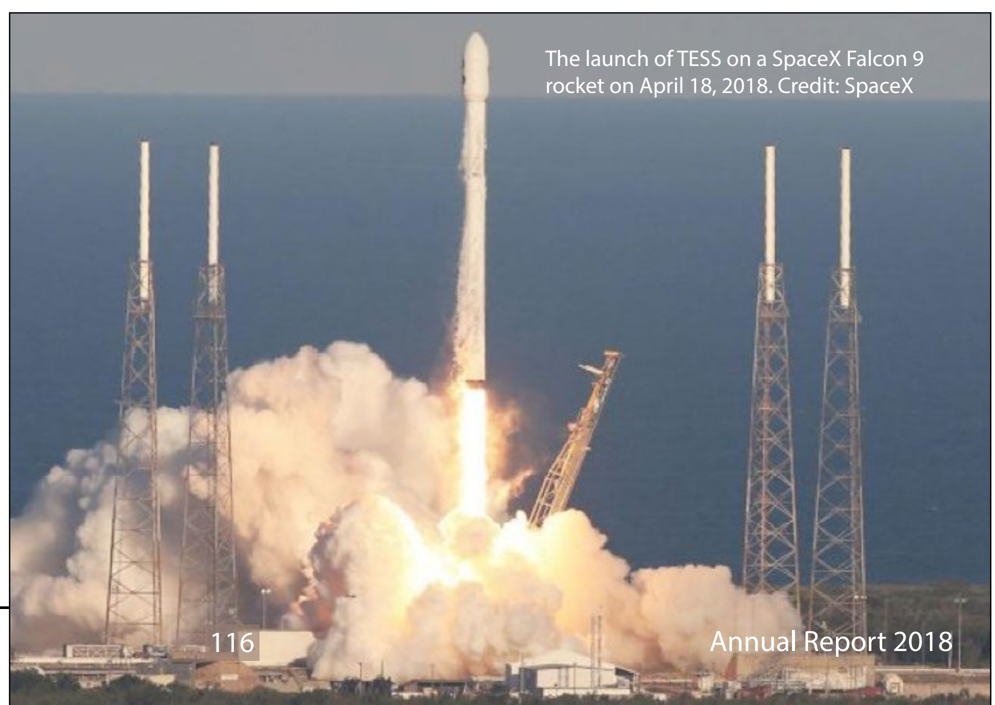
Based on this work, colleagues from JPL have reached out to our team for collaboration on a near-term, proof-of-concept mission bridging starshade technology with UV telescope capabilities. Our recent arguments for optimal ozone characterization could help justify their choice and facilitate funding. Other mission-relevant highlights of our recent work include the suggestion that seasonal compositional differences in atmospheric chemistry (e.g., $CO_2$, $CH_4$, $O_2$ and $O_3$) might be detectable biosignatures (Olson *et al.*, 2017). In the process, we make a pitch for the importance of direct imagining capabilities on future missions.

Six other responses to the 2018 NAS "Astrobiology" and "Exoplanet" solicitations included Alternative Earths authors. Collaborator Stephen Kane (UCR) argued that an improved sampling of pressure, temperature, composition, and dynamics of the Venusian atmosphere as a function of latitude would aid enormously in our ability to study exoplanetary atmospheres (Kane *et al.*, 2018). Others addressed the importance of target selection and the Earth analog in exoplanet exploration (Desch *et al.*, 2018; Barnes *et al.*, 2018); summarized the NExSS review paper efforts (Domagal-Goldman *et al.,* 2018); emphasized importance of studying Titan as an early Earth and hazy exoplanet analog (Trainer *et al.*, 2018); and explored the astrobiology of the Anthropocene (Haqq-Misra *et al.,* 2018).

With leverage stemming entirely from UCR's membership in the NASA Astrobiology Institute, Alternative Earths team leader Tim Lyons garnered college-level support to hire UC Riverside's first exoplanet specialist, with an emphasis on NASA mission involvement. Stephen Kane joined the UCR faculty in August 2017 as a Professor of Planetary Astrophysics, and the Department of Earth Sciences at UCR has since changed its name to the Department of Earth and Planetary Sciences to reflect the group's expanding focus and expertise.

Kane's research focus is the detection, characterization, and habitability of exoplanets using data from a variety of ground-based and space-based telescopes. His leadership role in a variety of current and upcoming NASA and ESA exoplanet missions makes him ideally poised to provide unique opportunities to students and postdocs alike. He is a frequent user of these space telescopes—HST, MOST, Spitzer, JWST—and is involved in the following missions:

- Kepler, Science team member and chair of the Habitable Zone Working Group
- TESS, Guest Investigator and member of the Target Selection Working Group
- CHEOPS, Collaborating Scientist
- WFIRST, Science Team member
- OST (Origins Space Telescope), Member of Exoplanets Science Working Group
- DSCOVR (climate satellite, NASA/NOAA), Collaborating Scientist


The launch of TESS on a SpaceX Falcon 9 rocket on April 18, 2018. Credit: SpaceX

# Alternative Earths: 2018 Publications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## Team Members

**Timothy Lyons**
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Mary Droser
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Marilyn Fogel
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Jennifer Glass
Daniel Gregory
Dalton Hardisty
Terry Isson
Emmanuelle Javaux
Aleisha Johnson
Stephen Kane
James Kasting
Sandra Kirtland Turner
Jun Korenaga
Bridget Lee
Cin-Ty Lee
Zijian Li
Gordon Love
Xolane Mhlanga
Marzia Miletto
Kingsley Odigie
Stephanie Olson
Jeremy Owens
Kazumi Ozaki
Noah Planavsky
Niels Peter Revsbech
Andy Ridgwell
Steve Romaniello
Juan Rosas
Edward Schwieterman
Sarah Simpson
Eva Stüeken
Nadia Szeinbaum
Yuanzhi Tang
Bradley Tebo
Mark H. Thiemens
Christopher Tino
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Pengxiao Xu
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Ming-Yu Zhao
Shiliang Zhao
Alex Zumberge

RPL

ROCK POWERED LIFE

**Lead Institution:**
**University of Colorado Boulder**

# Team Overview

**Principal Investigator:**
Alexis Templeton

The Rock-Powered Life (RPL) NASA Astrobiology Institute investigates systems on Earth and on rocky moons and planets such as Mars, Europa and Enceladus, where there is the potential to support life activity through water/rock reactions. The RPL NAI focuses on the mechanisms whereby energy may be released from the low-temperature hydration of mafic and ultramafic rocks, and the distribution, activity and biochemistry of the life forms that can harness this energy. RPL also seeks to detect the chemical and biological signatures of rock-hosted microbial activity.

In almost all of the systems we investigate, RPL focuses on understanding the geological, chemical and biological processes that control the production and consumption of $H_2$ and diverse forms of carbon. RPL efforts in 2018 continued to utilize our key field sites undergoing active serpentinization at the Atlantis Massif, the Oman ophiolite, and the California Coast Range Ophiolite Microbial Observatory (CROMO). In this annual report, we highlight advances in our understanding of how serpentinite-hosted microbial organisms metabolize or assimilate dissolved inorganic carbon, and how they produce methane, under extreme carbon and energy limitation. A combination of field-work, theoretical models, and experimental outcomes is presented in these four projects:

- Capturing new windows into the serpentinite-hosted biosphere: Oman Drilling Project 2018
- Physiological Adaptations to Serpentinization in the Samail Ophiolite, Oman
- Investigating the biological production of methane under extreme energy and carbon limitation
- Environmental assessments of habitability through cell-scale reactive transport modeling

**Team Website:** http://www.colorado.edu/lab/rockpoweredlife

# 2018 Executive Summary

In 2018, field-based activities at the California Coast Range Microbial Observatory, the Atlantis Massif, Yellowstone National Park, and the Oman ophiolite remained integral to our Rock Powered Life NAI investigations.

The most intensive fieldwork occurred as part of the Oman Drilling Project Phase II, with the successful recovery of 1000 meters of subsurface peridotites undergoing active hydration. More than 100 whole rock core samples were obtained to measure the abundance, activity, and biological signatures of serpentinite-hosted microbial life (see Project Report 4).

In September 2018, Co-I Brazelton and RPL graduate students Motamedi, McGonigle, and Alian participated in a new expedition to the Lost City hydrothermal field (lostcity.biology.utah.edu). The team was successful in collecting large volumes of subsurface fluids venting from Lost City chimneys using a novel hydrothermal fluid sampler (developed by Chief Scientist Susan Lang) operated by the ROV Jason. RPL team members are now sequencing metagenomes, metatranscriptomes, and single-cell genomes from the new fluid samples.

Field campaigns to the CROMO field site were completed by the Hoehler, Cardace, Schrenk, Ono and Tominaga

Figure 1. (above) RPL NAI team members in the field at the Oman Drilling Project Phase II drill site. Credit: Alexis Templeton

Figure 2. (below) Group photo of members of the RPL NAI team who attended the RPL in-person Collaboration Meeting in Boulder, Colorado in May 2018. Credit: Dan Mitchell

labs during 2018. These labs are collaborating to study microbial methanogenesis and acetogenesis using metatranscriptomic approaches. This team has also nearly completed necessary fieldwork and data integration related to the temporal characterization of the geochemistry and microbiology of the 8 groundwater wells that constitute CROMO.

Figure 3. RPL Co-I Tori Hoehler receives the H. Julian Allen award at the NASA Ames Research Center. Credit: NASA Ames

Our field-based efforts feed into experimental and computational studies. Co-I Hoehler and Sanjoy Som developed numerical models to constrain the viability of metabolisms that consume $H_2$ and organic acids as a function of the diverse environmental conditions in water-rock systems (See Project Report 4). Given an expectation that energy flux may be an important limitation on the productivity of many water rock systems, their efforts have focused on constraining the impact of multiple combined environmental controls on methanogen bioenergetics in serpentinizing systems. Members of the Templeton lab have been able to conduct methanogenesis experiments to test how the uptake of inorganic carbon at hyperalkaline pH can affect carbon isotope fractionation and rates of biological methane production (see Project Report 3). Members of the Boyd lab have conducted measurements of potential rates of biological methane formation, as well as carbon assimilation, using fluids and biomass obtained from the subsurface rock-hosted environment in Oman (see Project Report 2). The outcomes of the laboratory and field experiments are informing the next quantitative models of biological metabolism and growth under extreme carbon and energy limitation.

The RPL team not only engaged with each other in the field, but also gathered together for a full in-person team meeting at the University of Colorado Boulder in May, prior to hosting the NAI Executive Council. RPL Co-Investigators were also highly active at the Ocean Worlds 3 meeting, the Santander Astrobiology School, the Goldschmidt Geochemistry conference, the American Geophysical Union Conference, the Rocky Mountain Geobiology Symposium, and Gordon Research Conferences on "Deep Carbon" and the "Molecular Basis of C1 Metabolism". RPL Co-I Lisa Mayhew represented RPL as a member of the International MSR Objectives and Samples Team (iMOST) committee, and contributed to the identification of samples and measurements that could reveal insights about the potential for subsurface life and biosignatures on Mars. We are also proud to note that Co-I Shock received the Geochemistry Medal of the American Chemical Society, PI Alexis Templeton was recognized as a Fellow of the Mineralogical Society of America, and Co-I Tori Hoehler was the recipient of the H. Julian Allen award!

## Team Members

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Matthew Schrenk
Julio Sepulveda
Lauren Seyler
Everett Shock
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Patrick Thieringer
Christopher Thornton
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Spencer Zeigler

## Capturing New Windows Into the Serpentinite Hosted Biosphere: Oman Drilling Project 2018

In 2018, the Rock-Powered Life team was intensively involved in the Oman Drilling Project Phase II, which focused on drilling and core recovery within rocks undergoing active low-temperature serpentinization in the subsurface in the Samail ophiolite in Oman. Twelve RPL team members from the Templeton, Boyd, Spear, Cardace and Hoehler labs participated in the core handling and preservation of samples for biological analysis. Their efforts also included assembly of an on-site biological laboratory for the processing of drill core subsamples to be dedicated for geomicrobiological analyses.

The RPL team obtained excellent samples of partially-hydrated ultramafic rocks and the associated serpentinite-hosted biosphere. Whole-rock samples for biological experiments were collected every 10 meters from more than 1000 meters of core! Rocks derived from unique subsurface geochemical reaction zones, including regions of extreme changes in redox potential, pH, fluid chemistry and mineralogy were specifically targeted and subsampled for several different laboratories. For example, Eric Ellison, Spencer Zeigler, Lisa Mayhew and Alexis Templeton initiated mineralogical characterizations using a combination of x-ray diffraction and optical and spectroscopic imaging approaches. Kaitlin Rempfert is extracting and characterizing lipid biomarkers from these rocks and associated contamination controls. Clemens Glombitza has focused on measuring rates of biological sulfate reduction using a sensitive $^{35}S$ radiotracer technique, while Libby Fones and Eric Boyd started to characterize the potential rates of metabolism of inorganic C1 substrates using $^{14}C$ labeled experiments. At the same time, Emily Kraus and John Spear have initiated the molecular microbial community characterization by designing protocols to extract, amplify and sequence DNA preserved in the rock cores.

We also note that the Deep Carbon Observatory produced a film documenting field operations of the Oman Drilling Project highlighting the work of RPL PI Alexis Templeton and RPL Collaborator Peter Kelemen: https://m.youtube.com/watch?v=kLLCEPKling. In addition, Templeton and Kelemen were shadowed during this time by journalist Douglas Fox, who was commissioned to write an article about the Deep Subsurface Biosphere by the *Atlantic*. This story, called "Meet the Endoterrestrials", can be found here: https://www.theatlantic.com/science/archive/2018/10/meet-endoterrestrials/571939/

Figure 4. Oman Drilling Project Phase II drill site.


Figure 5. RPL PI Alexis Templeton finds a black "primordial ooze" seeping out of subsurface fractures in sulfurized peridotite.

Figure 6 a and b: Two examples of a partially-serpentinized rock type collected from hundreds of meters below the surface for biological analysis and experimentation.

Figure 7. RPL team members Eric Boyd and Libby Fones cleaning a core before it is logged and preserved for biological analysis.

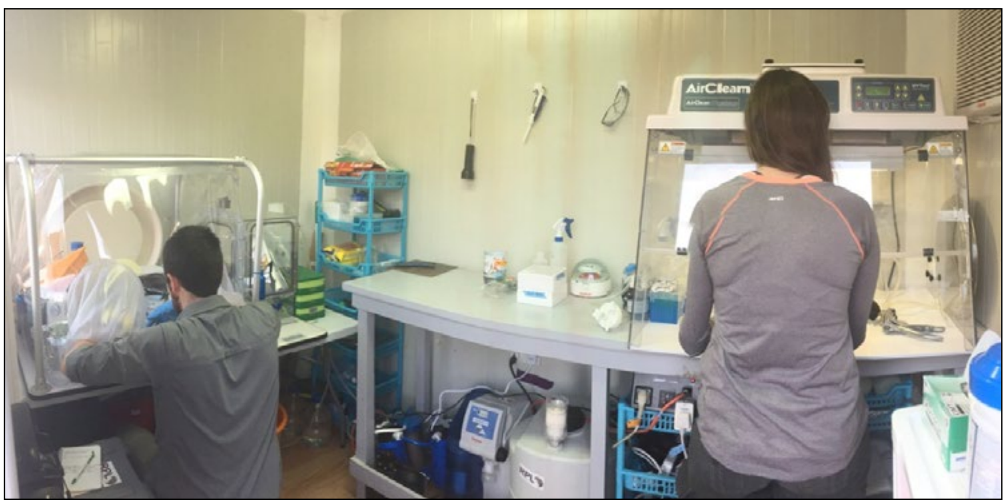

Figure 8. RPL team members Eric Ellison and Kaitlin Rempfert processing biological core samples in the on-site laboratory that they built.

### Environmental Assessments of Habitability Through Cell-Scale Reactive Transport Modeling

The potential for subsurface serpentinizing systems to host methanogens is related to the availability of hydrogen and carbon. The availability of $CO_2$, which is the form of carbon that can readily diffuse across biological membranes, is strongly pH dependent. Thus we are conducting numerical simulations on the energy available for methanogens, that accurately quantify the impact of the carbon-poor, alkaline conditions that characterize such fluids on the energy available for methanogens.

To place this study in a broader ecological context, we are documenting the differing carbon availability across not only a range of serpentinizing systems, but also other traditional methanogenic environments such as sediments, oil reservoirs, and animal rumens. The concentration of $CO_2$ in serpentinized fluids is many orders of magnitude lower than in traditional methanogenic environments (Figure 8, Hoehler *et al.*, in prep). Thus, alternative mechanisms of carbon acquisition by methanogens must be necessary for them to overcome energetic limits associated with serpentinization environments, despite the elevated $H_2$ that is common to those systems.

The model of methanogen biochemistry and bioenergetics incorporates a realistic treatment of $CO_2$ uptake (enzyme kinetics) and cross-membrane carbon transport (Hoehler *et al.*, in prep). The simulations are performed at the cellular scale to quantify the changes in energy produced by the consumption of the limiting substrate (either $CO_2$ or $H_2$) across the suite of environments described above. In a parallel effort, the cell-scale simulation is coupled to the equilibrium geochemistry code EQ3/6, in order to predict how variations in host rock type, water-rock ratio, and reaction conditions, which impact equilibrium fluid chemistry, will affect methanogen bioenergetics (Figure 9, Som *et al.*, in prep).

Finally, we are planning strategies to ground truth both simulations against actual biological distribution and activity, collected across a range of subsurface conditions (namely, rock and water composition), from the active serpentinization field site in Oman.

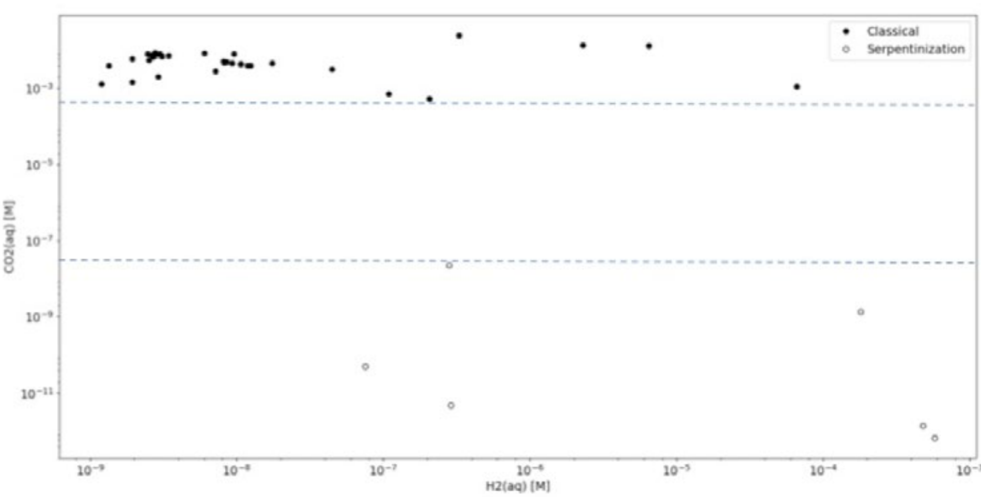


Figure 9. Differences in $CO_2$ concentration in fluids of serpentinizing systems (open circles) vs. traditional methanogenic environments, including sediments, oil reservoirs, and rumen fluids. >10,000-fold lower concentrations in serpentinizing environments would yield order-of-magnitude lower methanogenic rates and/or require alternative strategies for C acquisition.

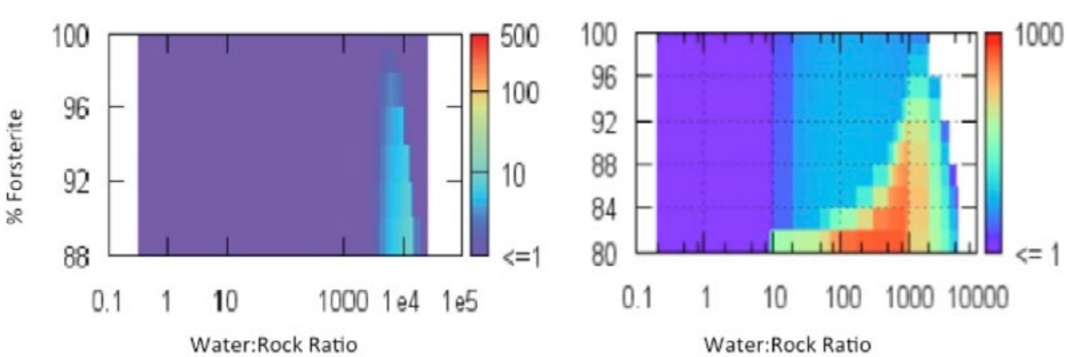


Figure 10. Heat map of 'habitability index' (defined as cell-specific metabolic power generation divided by cell-specific energy demand), plotted as a function of water: ratio and olivine Mg content (as % Forsterite). Left panel: fresh water reacting with high Ca rocks (18% clinopyroxene). Right panel: seawater reacting with low Ca rocks (0.2% clinopyroxene). For a given W:R and forsterite content, the habitability index may vary by >100x between these conditions.

## Investigating the Biological Production of Methane Under Extreme Energy and Carbon Limitation

Methane is a molecule that can serve as a critical energy source, as well as a potential signature of life activity on Earth and other planetary bodies in our solar system.

However, it is often challenging to determine the biological or abiogenic pathway through which large sources of subsurface methane have been formed. Debates about the origin of subsurface methane hinge strongly on our understanding of the conditions under which microbial organisms can produce methane and the isotopic signatures of the methane generated. Therefore, in a series of experiments, RPL team members Hannah Miller, Nabil Chaudhry and Alexis Templeton, and core collaborators Sebastian Kopf and Mark Conrad, investigated biological methane production under conditions that simulate "serpentinizing systems" (Miller *et al.*, 2018).

We isolated a methanogen that functions in pH 11 fluids in its native subsurface environment in Oman and tested its ability to form methane from two critical types of inorganic carbon sources ($NaHCO_3$ and $CaCO_3$ minerals). As such, we were able to probe critical controls on alkaline methanogenesis. We conducted experimental measurements of the rate of $CH_4$ generation, and tracked the $\delta^{13}C$ and $\delta D$ of $CH_4$ produced by *Methanobacterium sp.* as it varied as a function of the carbon source and availability, which are strongly affected by pH. Incredibly large carbon isotopic shift effects were observed across the environmentally relevant range of pH and Gibbs Free energy availability that we explored. We also produced a predictive isotope flux model that can be tested under numerous scenarios where microbial organisms may be expected to reduce $CO_2$ to $CH_4$ through hydrogenotrophic methanogenesis, which is one of the most widespread and primordial metabolisms known.

This work highlights the potentially important role that carbonate minerals might play in sustaining biological methane production in hyperalkaline systems and serpentinites. This novel insight is important in focusing our search for rock-hosted life. These studies also highlight how challenging it will be to use stable isotopic composition of methane to discriminate between biological and abiotic methane formed at slow rates in carbon limited systems.

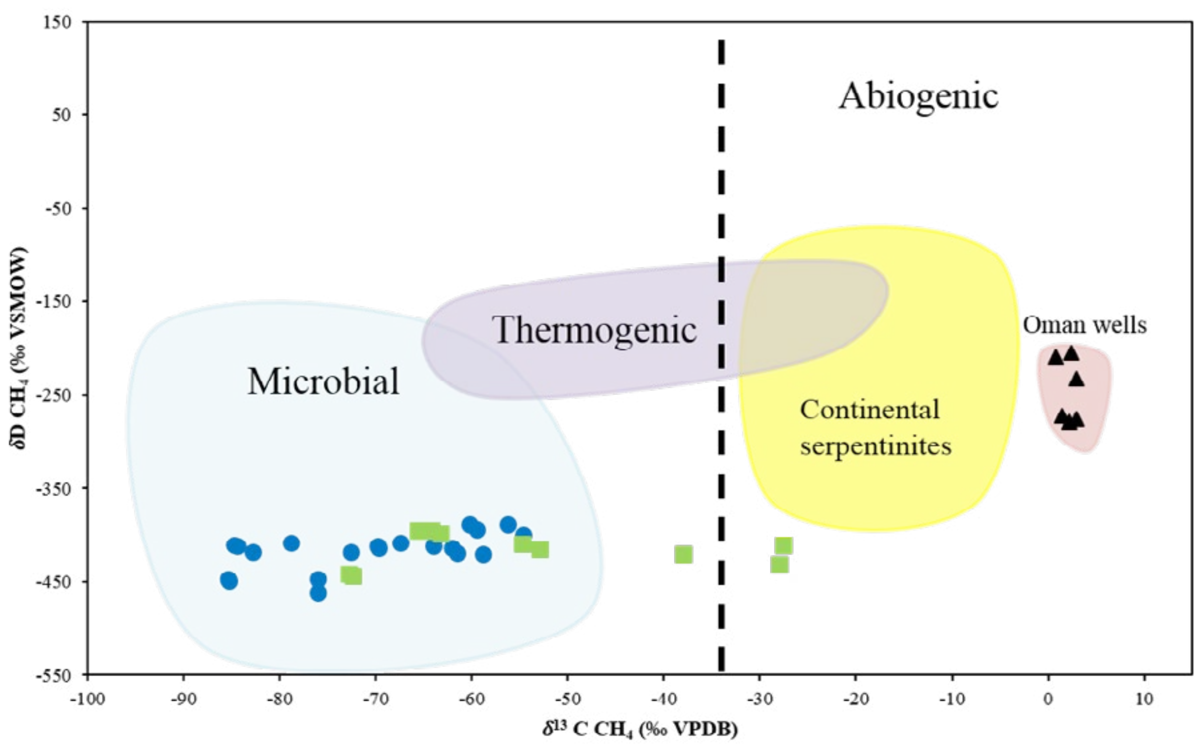


Figure 11. Carbon and hydrogen stable isotopic compositions of methane produced by *Methanobacterium sp.* (blue circles - $HCO_3$, green squares - $CaCO_3$) can vary enormously as a function of carbon availability at hyperalkaline pH, giving rise to methane that is easily recognized as "microbial" as well as methane that would typically be considered "abiogenic" when detected in serpentinite rocks (see Miller *et al.* 2018).

## Physiological Adaptations to Serpentinization in the Samail Ophiolite, Oman

The serpentinization reaction may have provided substrates that fueled early life's carbon and energy metabolisms. Multiple constraints are placed on microbial life with increasing water-rock reaction progress, which generates fluids characterized by high pH, highly reducing conditions, and limited DIC (dissolved inorganic carbon). To better understand how microbial life withstands these extreme conditions, we collected subsurface water samples spanning a pH and redox gradient from the actively serpentinizing Samail Ophiolite. In work recently accepted to the ISME Journal (Fones *et al.*, accepted), we enumerated planktonic microbial cells and subjected them to metagenomic and physiological analyses. We found that the abundance of planktonic cells and their activities generally declined with increasing pH. However, microcosm assays revealed that microorganisms sampled from waters of progressively higher pH were increasingly utilizing select single-carbon substrates for biomass synthesis rather than energy generation. This may represent an adaptation to minimize energetic and physiologic stresses imposed by serpentinizing conditions. Consistent with this hypothesis, community functional potentials inferred from metagenomes were found to group by fluid type, and estimated genome sizes and average oxidation states of carbon in inferred proteomes were lower in hyperalkaline waters than in alkaline waters. Our data collectively suggest that microorganisms inhabiting serpentinized waters exhibit unique adaptations that allow for their persistence under serpentinizing conditions that may be reminiscent of those present on early Earth.


Figure 12. Injection of well waters into pre-sterilized anoxic vials for microcosm assays that were used to determine potential rates of microbial utilization of select single-carbon substrates. Photo credit: John Spear

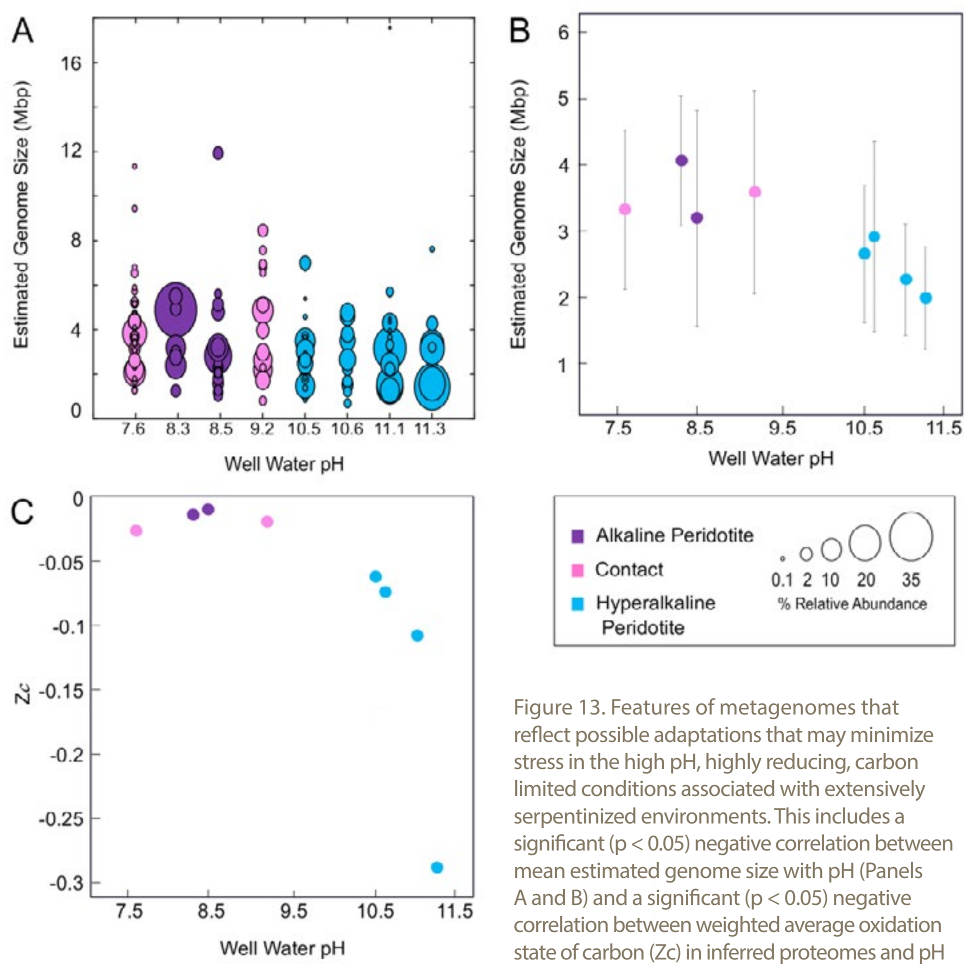


Figure 13. Features of metagenomes that reflect possible adaptations that may minimize stress in the high pH, highly reducing, carbon limited conditions associated with extensively serpentinized environments. This includes a significant ($p < 0.05$) negative correlation between mean estimated genome size with pH (Panels A and B) and a significant ($p < 0.05$) negative correlation between weighted average oxidation state of carbon (Zc) in inferred proteomes and pH (Panel C). (Fones *et al.*, accepted ISME Journal)

## Microbial Sulfate Reduction in Serpentinizing Rocks

Within the framework of NASA Astrobiology Institute Rock Powered Life (NAI RPL), we investigated the potential of serpentinizing mantle rocks to support microorganisms that utilize abiotically generated hydrogen together with sulfate for metabolic energy conversion (sulfate reduction). We studied two locations where actively serpentinizing mantle rocks are accessible and which collectively provide a range of environmental conditions such as *in situ* fluid temperature, pH, redox potential and chemical composition (i.e. concentration of sulfate, hydrogen and methane), namely the Coast Range Ophiolite (California, US) and the Semail Ophiolite (Oman). In 2017, formation fluids of both locations were collected from a total of 18 previously drilled wells. In 2018, we participated in the OmanDP effort and recovered rock cores from three sites in the Semail Ophiolite. The preserved fluid and rock samples were incubated with a radioactively labeled sulfate tracer to determine inherent microbial turnover rates (sulfate reduction rates). We found that serpentinizing mantle rocks host active microbial sulfate reducers. Compared to known highly active sulfate-reducing sediments, the *in situ* activities in the mantle rocks were low, in the range of a few fmol to a few pmol of sulfate turned over per $cm^3$ of fluid or rock (e.g. Fig. 16 from OmanDP site BA1B). The low activities, which could only be approximately stimulated by amendment with excess electron donor ($H_2$, $CH_4$ or organic acids), suggest that the microorganisms employ a slow metabolism which is most likely not limited by the availability of the generated hydrogen or methane. We suggest that in this particular environment, other factors, such as carbon and nutrient availability, limit the microbial activity. An ultimate limit seemed to occur where the pH of the fluids was increased to hyperalkaline conditions (> pH 10.5 - 11) and sulfate concentration was concomitantly lowest (few tens of micromolar).

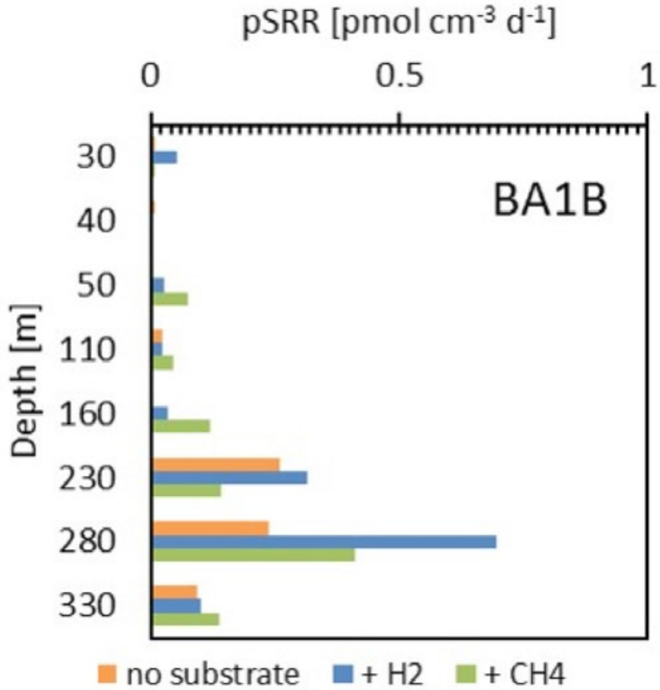


Figure 14. Potential sulfate reduction rates measured in the rock cores retrieved in the Semail Ophiolite, Oman, from hole BA1B. Colors of the bars relate to incubations with and without amendment of excess electron donor (i.e. hydrogen and methane)

# Field Work

The majority of work being conducted by RPL scientists is based upon field observations and samples obtained from sites such as Yellowstone National Park, the California Coast Range Microbial Observatory, the Oman ophiolite, the Atlantis Massif along the mid-Atlantic ridge, and Edgar Mine, Colorado. In 2018, 12 members of RPL participated in the Oman Drilling Project (from the Templeton, Boyd, Spear, Cardace, Hoehler labs; see Project Report #1). In addition, numerous RPL field trips to the CROMO site were conducted by members of the Schrenk, Tominaga, Hoehler and Cardace labs in order to complete their time-series studies of serpentinization-influenced groundwater metabolomics and geochemistry. In 2018, members of the Brazelton and Schrenk labs participated in a new expedition to the Lost City hydrothermal field. The scientific party included 10 undergraduate and graduate students who had never been at sea before and who worked very hard, especially during the 5.5 sleepless days the team was able to spend on site due to weather constraints. They are now conducting sequencing of metagenomes, metatranscriptomes, and single-cell genomes with the new fluid samples and will coordinate the multidisciplinary datasets that will be generated by the other expedition participants. In addition, the Boyd, Spear and Shock labs have conducted multiple years of hydrothermal investigations characterizing dissolved gas, organic acid, and aqueous geochemistry of a chemically diverse set of springs in Yellowstone National Park. In 2018, the Shock lab focused on the exploration of previously unsampled systems, and initiated sampling for genomes and trace elements chemistry in order to trace microbial cycling of $H_2$.


Figure 15. Group photograph of the Return to Lost City research expedition team.

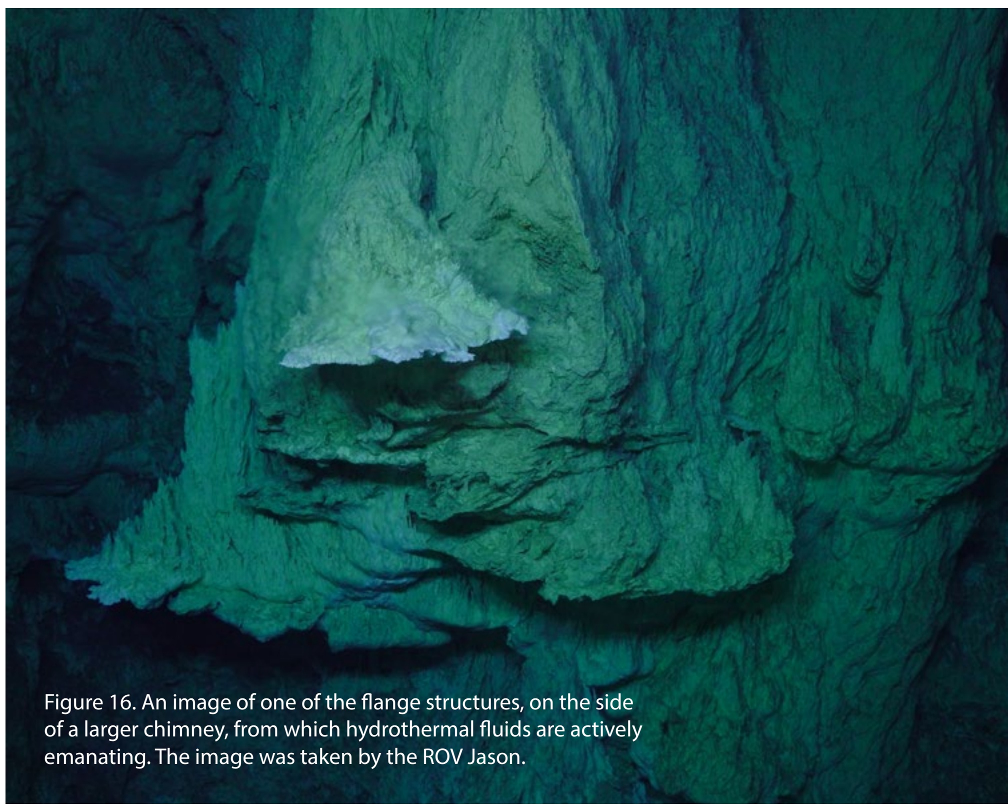

Figure 16. An image of one of the flange structures, on the side of a larger chimney, from which hydrothermal fluids are actively emanating. The image was taken by the ROV Jason.

## Rock Powered Life: 2018 Publications


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