National Aeronautics and Space Administration

NASA Astrobiology Institute

2019

Annual Report

2019: The NAI Concludes Operations

The NASA Astrobiology Program has announced a bold new structure to mobilize the astrobiology community towards both impacting future NASA space missions and answering the fundamental questions of; *How does life begin and evolve? Does life exist elsewhere in the Universe? How do we search for life in the Universe?* This new approach includes a system of virtual collaboration consisting of "Research Coordination Networks" (RCNs). These RCNs are designed to enable the research community to self-organize, collaborate, communicate, and network across organizational, divisional, and geographical boundaries. This new structure has replaced the NASA Astrobiology Institute (NAI) as the primary mechanism to integrate the astrobiology community, and therefore the NAI has completed its more than 20-year mission on December 31, 2019.

In collaboration with NASA HQ, the NAI was responsible for the development of eight Cooperative Agreement Notices (CAN) that solicited proposals for interdisciplinary astrobiology research concepts and projects, which resulted in the selection of 56 NAI CAN Teams that collectively included thousands of scientists, engineers, and educators, from hundreds of institutions. The NAI was also responsible for inventing and evolving NASA's very first virtual institute, which has served as a model for other virtual institutes within and outside of NASA. Through continuous and innovative efforts, the NAI pioneered the connection of people across the globe via workshops, insight courses, conferences, seminars, general meetings, field excursions, summer and winter schools, international partnerships, public outreach events, and direct personal connection and networking with colleagues around the nation and the world.

Looking forward, the NAI invites and encourages everyone seeking to engage with field of astrobiology to do so through the NASA Astrobiology Program (https://astrobiology.nasa.gov). For researchers, comprehensive information about the 2015 Astrobiology Strategy, the Research Coordination Networks, the Interdisciplinary Consortia for Astrobiology Research (ICAR) solicitations, and other funding opportunities is available under Astrobiology@NASA. Many elements of the NAI, especially those programs supporting students and early-career investigators, will continue to be accessible and well-supported through the efforts of the NASA Astrobiology Program.

We would like to thank everyone who made these two-decades of astrobiology at the NAI a period of such tremendous excitement, passion, and accomplishment: to the community of researchers for their continuous creativity and drive, to the students for their endless energy and optimism, to the public for listening to our message and recognizing its significance, to those at NASA who conceived of and supported the Institute and its mission, and finally to all those many individuals who were privileged to work at NAI Central and participate in this grand journey that is astrobiology.

In the following pages, the NAI teams demonstrate their efforts to contribute to and advance the field of astrobiology for fiscal year 2019. We thank the teams for their endeavors, and invite you to read about their accomplishments in this, the final NAI annual report.

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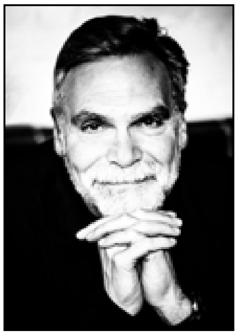

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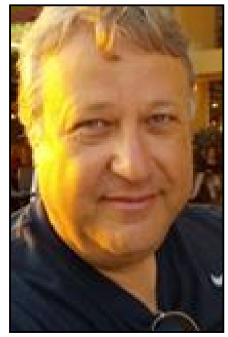

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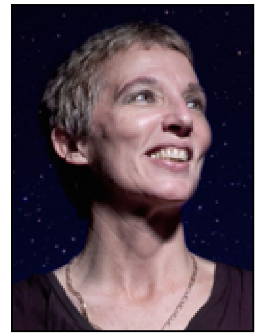

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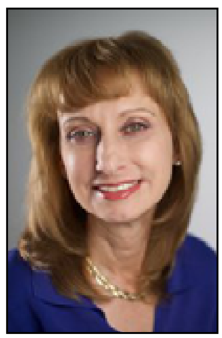

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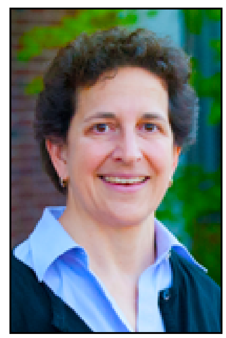

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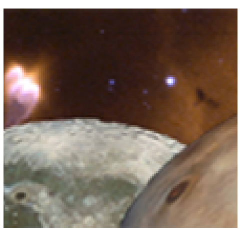
NAI 2019 Teams

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.

2019 Team Reports

The Evolution of Prebiotic Chemical Complexity
and the
Organic Inventory of Protoplanetary Disks 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 Irradiation Chemistry
- Computational Quantum Chemistry

These projects interact closely with each other so 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 benefits from inputs provided by spectral, physical, and chemical properties of molecules determined by the laboratory and computational projects, but also 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

2019 Executive Summary

During 2019 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 have developed one of the first 3-phase gas–grain chemical disk models, where gas, ice surface layers, and icy mantles of dust grains are each considered. Our work shows that the chemistry is mainly driven by photo-reactions and dust temperature gradients in the disk. The disk interior can be divided into three chemical regions: (i) a shielded, inner midplane in which complex organic molecules form, (ii) an outer midplane where hydrogenation reactions dominate and, (iii) a molecular layer below the water condensation front where photodissociation of ices significantly affects the gas-phase chemistry. This model is being used to investigate disk gas mass determinations. We find that CO conversion into CO_2 near the water ice condensation front can account for CO underabundances inferred from observational data.

Exoplanets – Our work on a two-decade-long Doppler radial velocity survey of low-mass M-type stars demonstrated that, on average, red dwarf star planetary systems contain at least three planets. The appearance of 'Oumuamua, and Borisov, the first macroscopic objects of interstellar origin detected within our Solar System, motivated us to develop dynamical and compositional models explaining their contrasting behaviors. This work placed constraints on the formation of long-period gas giant planets around Sun-like stars in the Galaxy. We showed how Spitzer's infrared orbital phase curves can be used to infer the spin-tilts of short-period extrasolar planets, and that extrasolar planets observed by the Kepler Mission are frequently forced into high-obliquity states, in which they experience enhanced interior heating as a consequence of tidal dissipation.

Laboratory Studies of Gas–Grain and Ice Irradiation Chemistry – Recent laboratory experiments of UV irradiation of astrophysical ice analogs and related computations demonstrated the formation of variants of hexamethylenetetramine (HMT), molecules that can be precursors to many species of astrobiological interest, including amino acids (Fig. 1). The Gas-Grain Laboratory constructed the ICEE (In-situ Carbon Evolution Experiments) facility. Infrared and Raman spectra can be collected from ICEE samples and ICEE has been used to investigate the chemical complexity and composition of microbial colonies in Atacama Desert salt samples as an analog to living systems on other worlds. The Raman microscope is capable of 3D mapping of the chemical composition of a microbial colony on the micron scale (Fig. 2).

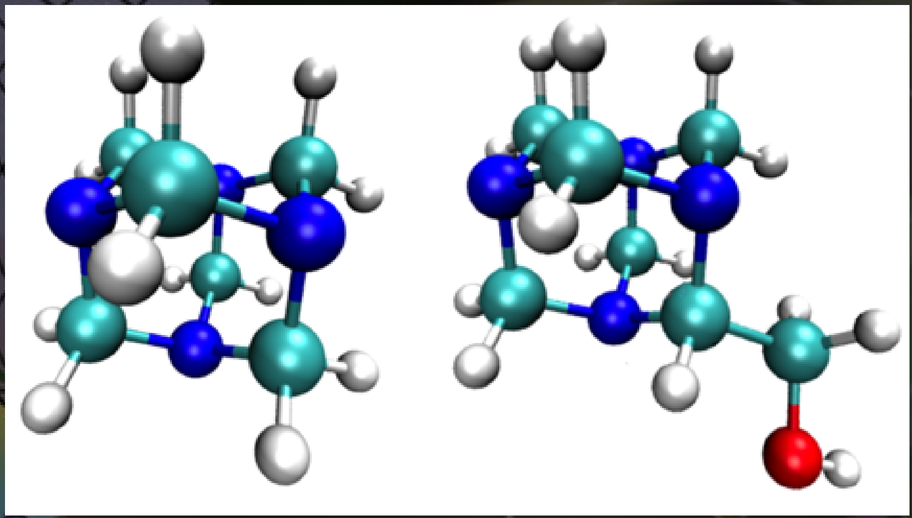


Figure 1. The structures of hexamethylenetetramine (HMT; left)) and HMT-methanol (right), a variant in which one of the peripheral H atoms on HMT is replaced with a methanol group. Laboratory and computational work show that a family of HMT variants is likely made in space and may be detectable telescopically. (From Materese *et al.* 2020)

Computational Quantum Chemistry – We performed quantum chemical calculations characterizing functionalized HMT molecules, including their vibrational and rotational data. We investigated the energetic, spectroscopic, and physical properties of N-containing cyclic molecules like isomers of pyrrole cation, azirinyl cation, and diazirene, as well as ionic and neutral growth of medium to large N-containing Polycyclic Aromatic Hydrocarbons (PAHN) molecules and their cations. Our collaboration with the Dutch Astrochemistry Network included the computation of fully anharmonic cascade emission spectra of PAHs suitable for direct comparison with astronomical data (Fig. 3). Finally, we predicted the rovibrational spectra and spectroscopic constants of small molecules for interpreting high-resolution astronomical observations.

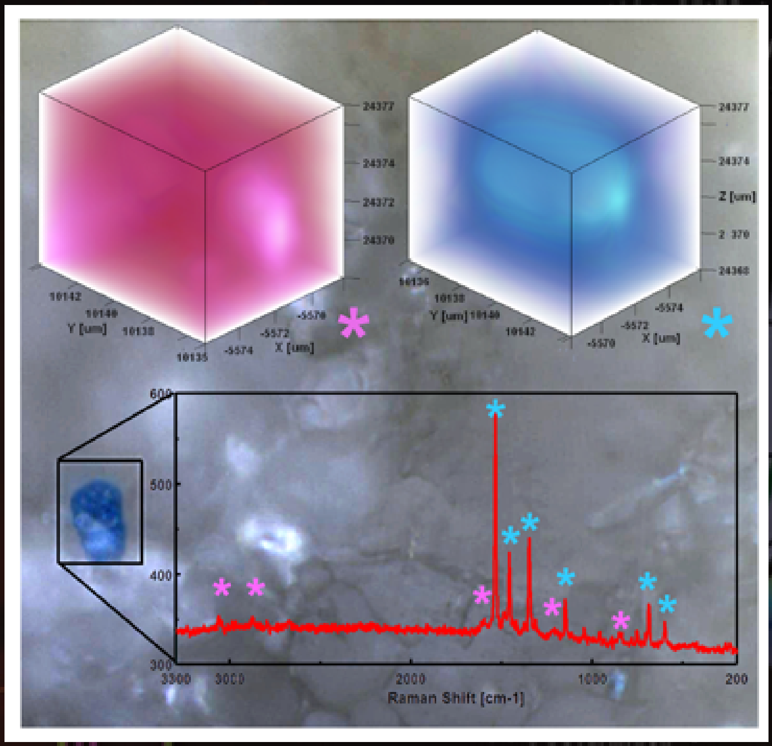


Figure 2. 3D mapping of the chemical complexity surrounding a decaying microbial colony (dark blue) in an Atacama Desert salt sample. Red asterisks denote Raman peaks attributable to aliphatic and aromatic compounds. These compounds appear to be leaking from the decaying microbial membrane. Blue asterisks denote Raman peaks associated with a porphyrin type molecular structure. This compound maps to the visible membrane of the microbial colony.

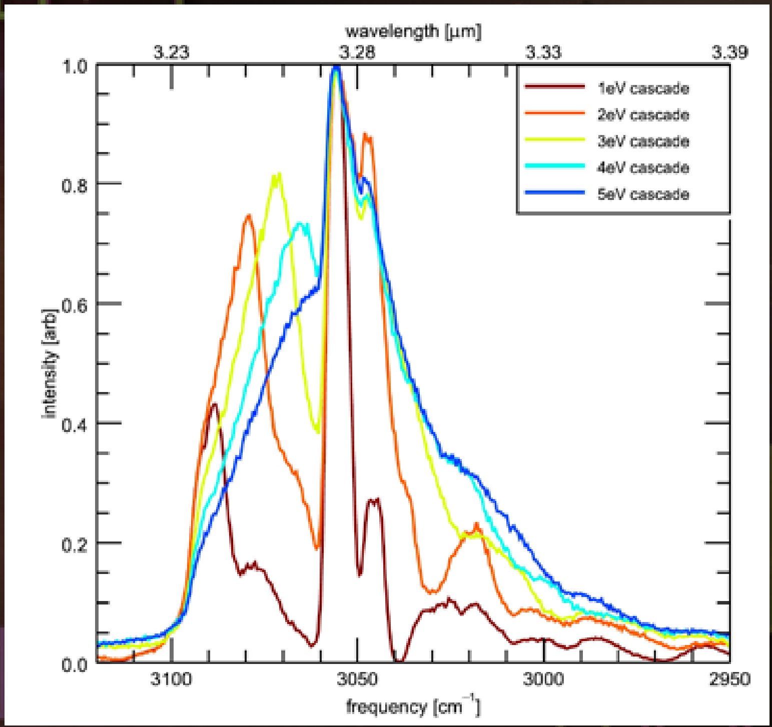


Figure 3. The fully anharmonic IR cascade spectrum of the C-H stretching region of tetracene for different starting energies. The differing starting energies result in different cascade profiles as a result of several overlapping vibrational bands in this region.

Project Reports

Modeling and Observations of Protoplanetary Disks

We have developed one of the first 3-phase gas grain chemical models as applied to disks, where gas, ice surface layers and icy mantles of dust grains are considered as being distinct. Chemistry is found to be mainly driven by photo-reactions and disk dust temperature gradients. The disk interior can be divided into three chemical regions: (i) a shielded, inner disk midplane where low FUV fluxes and warm dust (>15K) lead to the formation of complex organic molecules, (ii) an outer disk midplane where dust temperatures are low and where hydrogenation reactions dominate and, (iii) a molecular layer below the water condensation front where photodissociation of ices significantly affects the gas phase chemistry. We show that many observed gas phase species originate near the water ice condensation front. The use of the three-phase approximation over the common two-phase approximation generally leads to lower abundances for most observed molecules in disks.

This model is now being used to investigate a long-standing issue: disk gas mass determination, for which derived values differ by orders of magnitude depending on the molecular tracer used (e.g., CO isotopes, HD). We find that CO conversion to CO_2 near the water ice condensation front can account for CO underabundances typically inferred from observational data. Ongoing work on the model-predicted CO isotope emission on gas disk mass and gas/dust ratio, and comparisons to observations can successfully explain and reconcile the different data (Ruaud *et al.* 2020, in preparation, also see Fig. 4).

We have been developing a 2-D model using detailed chemical analysis to generate simpler chemical networks that focus on particular species. The stochastic evolution of dust grains as they settle, collide, fragment and coagulate and resulting changes in chemical composition are being followed via a disk evolution code to study transport of organics.

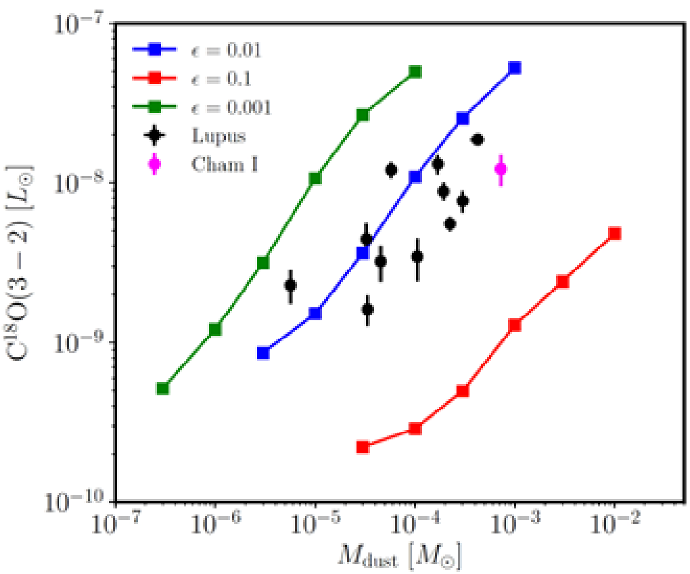


Figure. 4. $C^{18}O$ (J=3-2) emission as a function of the disk dust mass for three different dust/gas ratios. Black and magenta points show results obtained from ALMA surveys in the Lupus and Chameleon I regions. While previous studies have argued for dust/gas ratios close to 0.1 for these disks, we find that all these disks cluster close to the fiducial ISM value of 0.01. This result, if confirmed, has profound implications for our understanding of disk evolution and sequestration of chemical species on dust grains.

Exoplanet Studies

We submitted a comprehensive review of the statistics of planetary systems orbiting M-dwarf stars (Tuomi *et al.* 2019, https://arxiv.org/abs/1906.04644). Our analyses, based upon two decades worth of precise Doppler velocity data obtained by our team and its precursor teams, revealed 118 candidate planets orbiting nearby M-dwarfs and cemented the remarkable realization that low-mass planets are extremely common in orbit around low mass stars. This certified abundance of characterizable worlds has profound importance for JWST and beyond.

One of the most surprising recent astronomical stories was the appearance of *'Oumumua [Nature 552, 378–381 (2017). https://doi.org/10.1038/nature25020]*, the first macroscopic object of clearly interstellar origin to be detected within our Solar System, followed by the appearance of Borisov, the first comet of unambiguously interstellar origin to be detected. 'Oumuamua showed no sign of a dust coma, yet it displayed non-gravitational acceleration that suggested outgassing of volatile material. We developed a dynamical model that shows how sublimation jets of volatiles can simultaneously explain the observed light curve and observed non-gravitational acceleration of the body (Seligman, Laughlin & Batygin 2019). In separate work, we (Rice & Laughlin 2019) showed that the appearance of Borisov, in conjunction with gaps in protoplanetary disks seen by the ALMA array, implies that most single solar-type stars in the galaxy have planets of Neptune's mass (or larger) on distant orbits.

We also focused on the physical atmospheric structures and the global appearance of extrasolar planets. These properties can be mapped from data obtained by the Spitzer Space telescope and JWST. We published a detailed overview of how the obliquities (spin tilts) of extrasolar planets can be obtained from Spitzer light curves (Adams *et al.* 2019), and showed how secular spin-orbit resonances can explain the strange overabundances of planet pairs just wide of the 2:1 and 3:2 orbital ratios (Millholland & Laughlin 2019) (Fig. 5).

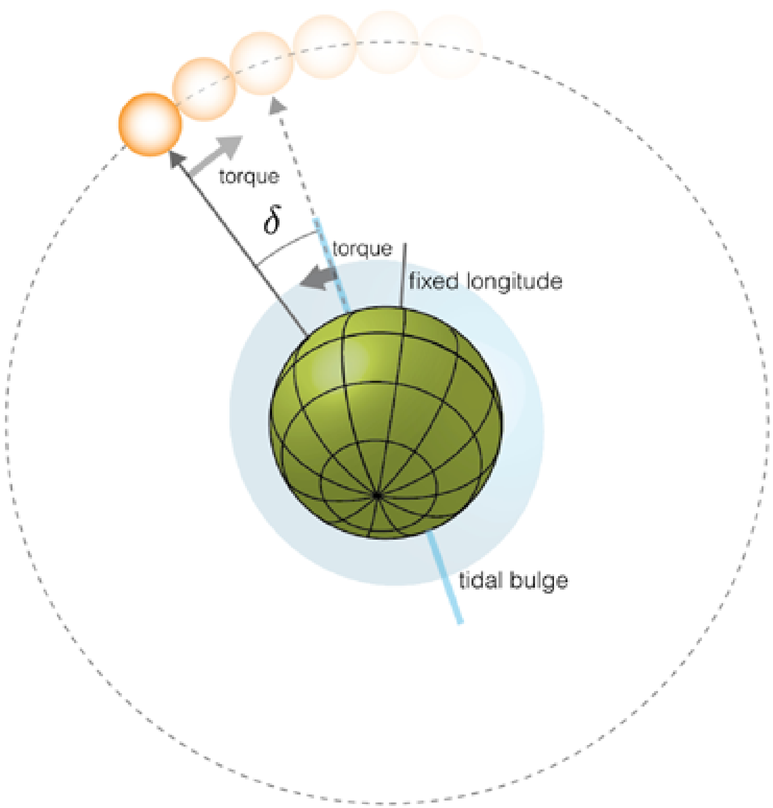
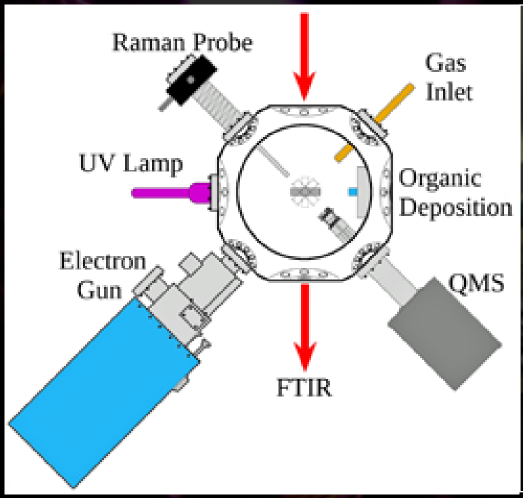


Figure 5. An exoplanet forced to maintain a large spin tilt (obliquity). As shown in the illustration, this leads to strong tides that efficiently heat the planet (much like the tidal heating of Jupiter's moon Io). Extra heat inflates the planet and provides an explanation for striking observed overabundance of exoplanet pairs just wide of the 2:1 and 3:2 period ratios.

Laboratory Studies of Gas-Grain Chemistry

In 2019, the Gas-Grain chemistry laboratory greatly expanded its capabilities with a new setup, known as ICEE (In-situ Carbon Evolution Experiments), which permits analyses of samples via IR and Raman spectroscopy and Mass spectrometry. A high vacuum chamber schematic is shown in Fig. 6 (left) identifying the *in-situ* techniques of FTIR, Raman, and Mass spectrometry and the electron gun (100 keV) and UV lamp (10.2 eV) sources. An IR transparent window in the center of the chamber is mounted to a cryo-cooler and can be rotated to face the gas and organic deposition ports, FTIR or Raman spectrometers, or the radiation sources. Samples can also be irradiated with high energy electrons and UV photons. The addition of Raman spectroscopy enhances the ability to decipher the organic chemistry occurring in water ices, as water exhibits very weak Raman modes compared to IR (Fig. 7). The addition of mass spectrometry and high energy irradiation permits us to expand on our work understanding the organic chemistry occurring in planetary systems, such as the Saturn system (Fig. 7). A Raman microscope permits detailed analysis of grain surface chemistry and enhances collaboration with the Ice Photolysis team members studying the organic residues they produce. Additionally, the Raman microscope already resulted in new collaborations with analog site astrobiology, by identifying the organic chemical signatures of microbial colonies present in analog samples. Thus, the ICEE group (formerly known as Gas-Grain Chemistry) is well poised to participate in the Astrobiology Research Coordination Networks NfoLD (Network for Life Detection), Prebiotic Chemistry and Early Earth Environments (PCE_3) and the Network for Ocean Worlds.

Ms. Julie Korsmeyer, 2017 and 2018 summer intern, spent this past summer working in the laboratory of Dr. Harold Linnartz, Leiden University and is a result of our continued international collaboration with the Dutch Astrochemistry Network (DAN).



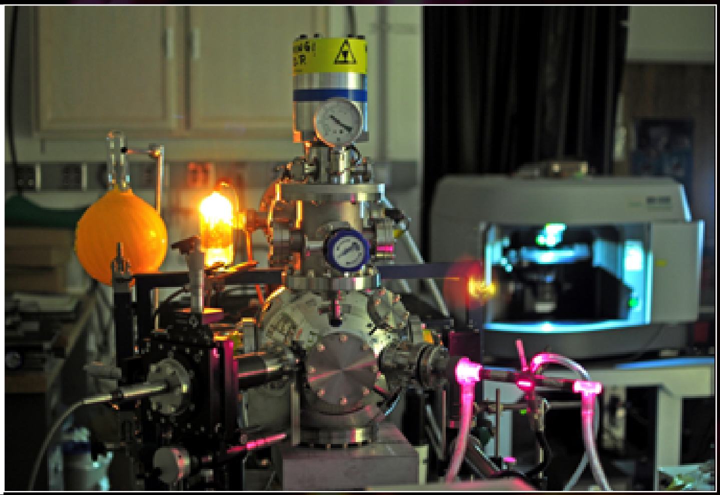
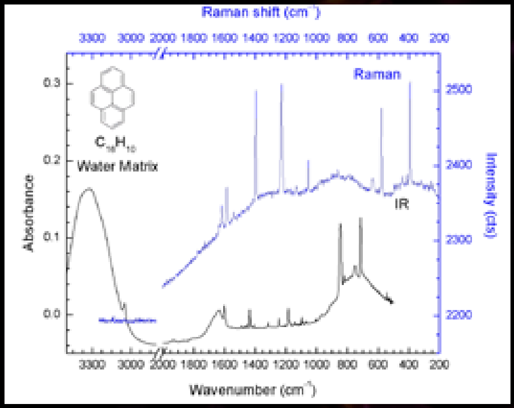


Figure 6. The new ICEE (In-situ Carbon Evolution Experiments) facility. On the left is a schematic diagram of the setup, while the Raman microscope can be seen in the background of the ICEE setup shown in the photograph on the right. Images taken from Mattioda *et al.* (2020a).



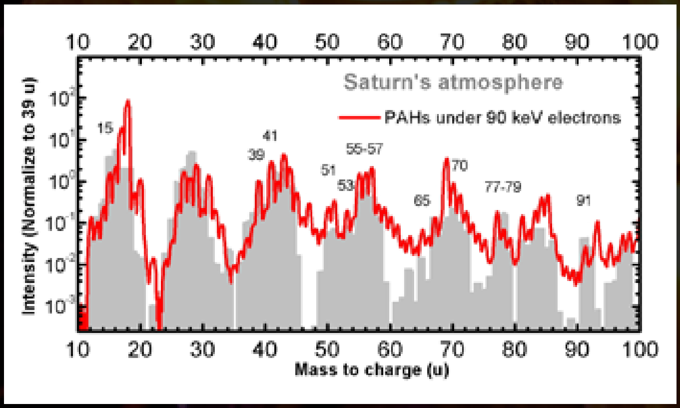


Figure 7. ICEE facility applications include understanding organic-water ice chemistry (left) and interpreting the organic chemistry of planetary systems, such as the Saturn system (right). Left -FTIR and Raman spectra of the molecule pyrene enchased in water ice (Mattioda *et al.* 2020a). Right - mass spectra of electron irradiated PAHs compared to the Ion Neutral Mass spectra of particles falling into Saturn's atmosphere from its rings (Mattioda *et al.* 2020b). Mattioda *et al.* 2020a – Ap.J. (submitted); Mattioda *et al.* 2020b A&A (submitted)

Laboratory Studies of Ice Photochemistry

The UV irradiation of ice mixtures of astrophysical interest leads to the formation of a wide variety of organic molecules, some of which are stable at room temperature and can be recovered. Among those organics, hexamethylenetetramine (HMT) is among the most abundant. Experiments conducted in previous years of this project, in which astrophysical ice analogs were UV irradiated, have led to the discovery and identification of HMT-methanol, i.e., an HMT molecule with a CH_2OH group attached to a carbon atom. This has been confirmed by additional experiments in which isotopically labeled ices (^{13}C, ^{15}N, and ^{18}O) were used. Moreover, *ab initio* computations of the infrared spectra of HMT and HMT-methanol in collaboration with the Quantum Chemistry group of our team showed strong bands associated with the high symmetry of HMT, suggesting that HMT-substituted compounds may be detectable in space. All these results were compiled in a paper that has been accepted for publication (Materese *et al.* 2020). In addition, we performed calculations of the infrared spectra for several other HMT-substituted compounds and confirmed that these molecules have infrared modes in common, making these molecules detectable in space as a family. This work has been published this year (Bera *et al.* 2019).

In addition, we have conducted two series of irradiation experiments using the NSRRC synchrotron facility in Taiwan. Samples made of organics that are found in meteorites, and mixed in relative proportions relevant to their meteoritic distribution, were irradiated with UV/EUV photons in the 4–45 eV range in order to simulate the large UV doses that grains experience in the protosolar nebula. Changes in chemical compositions have been observed, with some similarities to the insoluble organic material in meteorites. These samples are currently being analyzed with nanoSIMS (nanoscale secondary-ion mass spectrometry) to determine their isotopic composition (Fig. 8).

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Materese, C. K., Nuevo, M., Sandford, S. A., Bera, P. P., Lee, T. J. (2020). The production and potential detection of hexamethylenetetramine-methanol and other hexamethylenetetramine derivatives in space. *Astrobiology*, 20, 601-616.

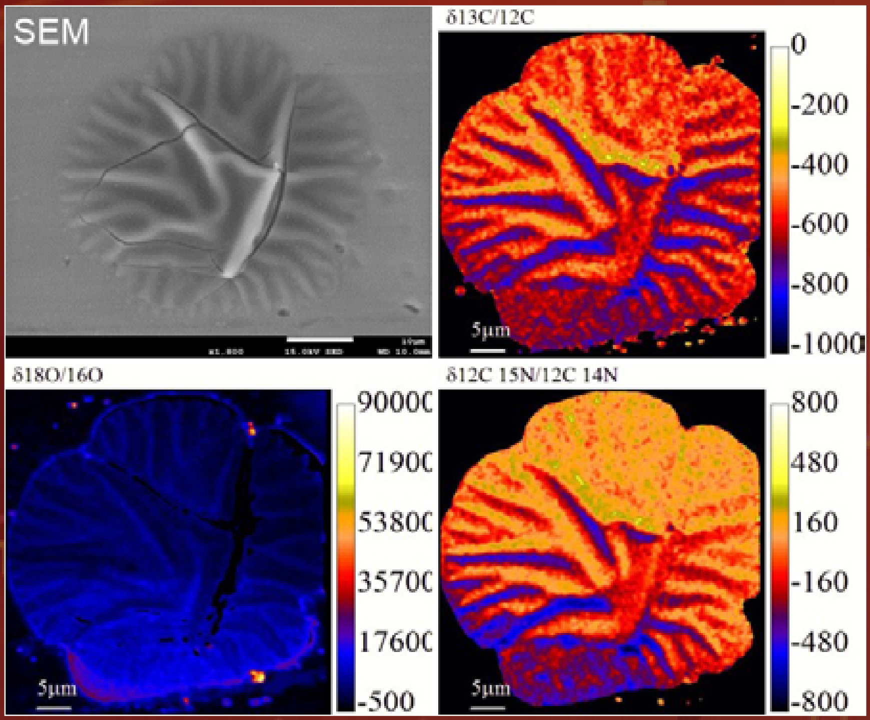


Figure 8. (Top left) Scanning electron microscopy (SEM) image of part of a residue produced from the UV irradiation of an $H_2O:CH_3OH:CO:NH_3:C_5H_4N_4$ (10:5:2:1:10^{-2}) ice at 10 K and warmed up to room temperature. (Top right) NanoSIMS image of the same residue showing the distribution in ^{13}C compared with ^{12}C in PDB (Pee Dee Belemnite). (Bottom left) NanoSIMS image of the same residue showing the distribution in ^{18}O compared with ^{16}O in SMOW (standard mean ocean water). (Bottom right) NanoSIMS image of the same residue showing the distribution in ^{15}N compared with ^{14}N in atmospheric N_2.

Computational Quantum Chemistry

Infrared spectra for HMT and HMT-methanol were computed using quantum chemistry methods and showed good agreement with observed vibrational spectra for HMT. Laboratory irradiation of astrophysical ice analogs containing H_2O, CH_3OH, CO, and NH_3 yields hexamethylenetetramine-methanol (HMT-methanol; $C_7N_4H_{14}O$). HMT-methanol may represent an abundant member of a family of functionalized HMT molecules, e.g. HMT-OH, HMT-NH_2, HMT-CH_3, HMT-CN, HMT-OCN, HMT-OCH_3 HMT-CH(OH)CHO, and HMT-NHCHO for which vibrational and rotational data have been computed. Two manuscripts have been published, in *ApJ* and *Astrobiology* (Fig. 9).

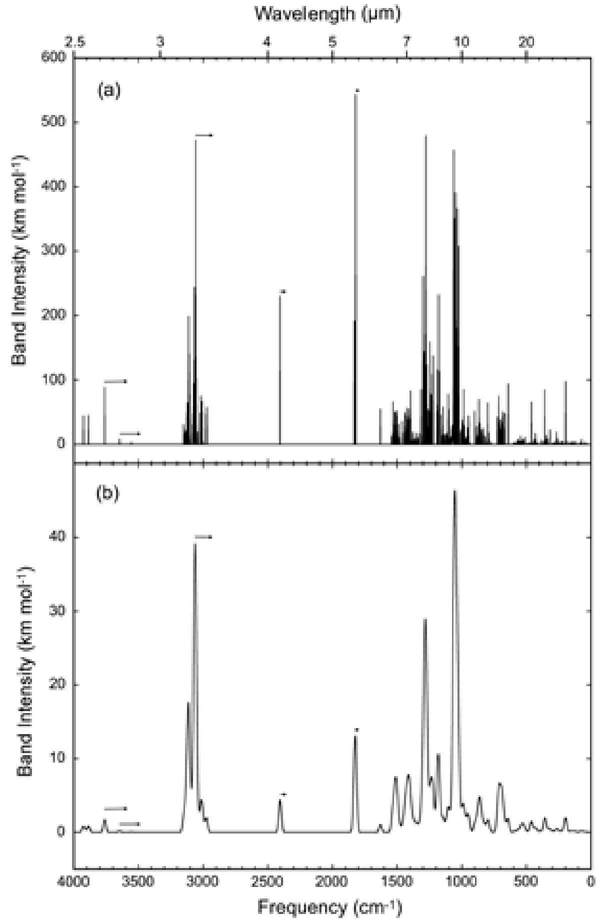


Figure 9. Composite spectra consisting of the coaddition of the computed spectra of HMT-OH, HMT-NH_2, HMT-CH_3, HMT-CN, HMT-OCN, HMT-OCH_3, HMT-CH(OH)CHO, HMT-NHCHO, HMT and HMT-CH_2OH.

Identifying nitrogenated cyclic molecules in the ISM would mark a milestone in the search for biologically relevant molecules. Continuing our previous work, we investigated the energetic, spectroscopic, and physical properties of isomers of pyrrole cation, azirinyl cation, and diazirene. One paper reported the rovibrational spectra of azirinyl cation (Fig. 10), another characterizing pyrrole cation is under review, and another (diazirene) will be submitted soon.

Ionic and neutral growth of medium to large PAH and PAHN molecules was studied using *ab initio* molecular dynamics simulations. *Ab initio* trajectory calculations performed on [*x*HCCH + *y*HCN]+ (*x*,*y*=1,2,3) clusters show interesting aspects for the growth of nitrogenated PAH (PAHN) molecules. Two manuscripts are in preparation.

Our collaboration with the Dutch Astrochemistry Network II (DAN II) was productive. We built upon last year's work by computing fully anharmonic cascade emission spectra of several PAH molecules, including their cations, which correspond to spectra actually observed by astronomers. Using these spectra, new conclusions have been made regarding the size of PAH molecules observed in the ISM, as well as the IR intensity ratio for various bands observed in the mid-IR. Two manuscripts are in preparation.

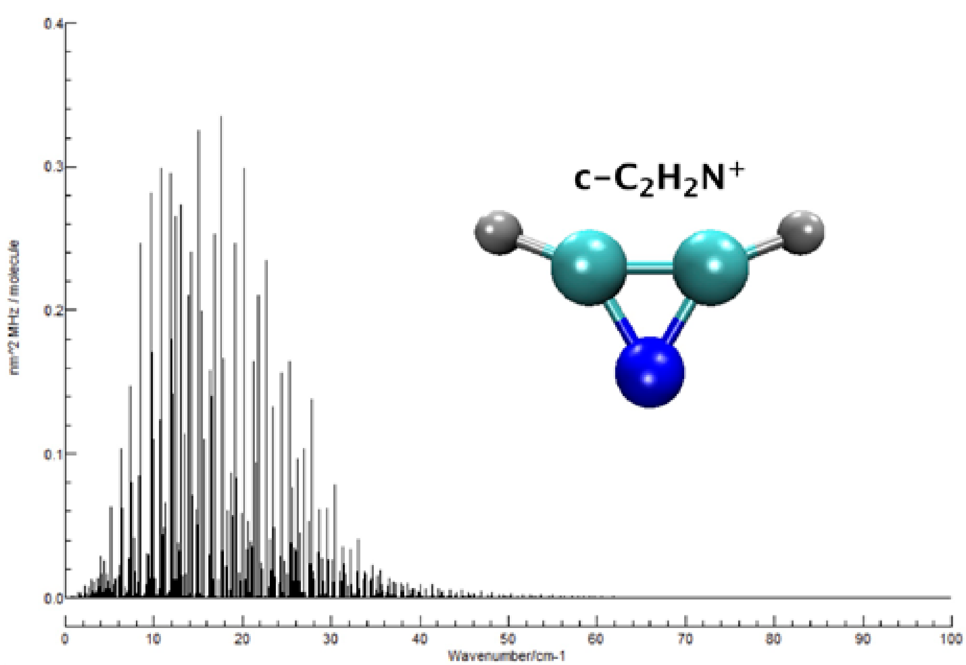


Figure 10. 50 K rotational spectrum of the azirinyl cation c-$C_2H_2N^+$.

With collaborators 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 analyzing SOFIA EXES spectra.

Collaborations

The efforts of our NAI team benefit from a number of collaborations with other researchers, some of who are also in the NAI and some of who are not.

Team members Drs. Sandford and Nuevo are working with Drs. Jamie Elsila Cook and Steve Charnley (GSFC NAI CAN 7 Team), who use GC-MS/IRMS to study some of our samples, and study isotopic fractionation in primitive solar system materials. Team member Dr. Mattioda is also collaborating with Dr. Richard Quinn's research group (SETI Institute NAI CAN 7 Team) on the OREOCube and EXOCube space-based exposure missions. These missions will measure the molecular changes in astrobiologically interesting organic molecules as they are continually exposed to radiation in space via the exposure platforms aboard the ISS (International Space Station). The changes will be measured *in situ* utilizing UV-visible and infrared spectroscopy. Additionally, this collaboration involves ESA (European Space Agency). Finally, team member Lee provides computed spectral line lists to Victoria Meadow's (VPN NAI CAN 7 team) to assist them with their exoplanet studies. This is an area in which our computational chemistry group is a recognized world leader and it is likely that we will be able to provide additional line lists in the future.

We also work with many collaborators who are outside of the NAI. For example, the organic residues made in our ice photolysis experiments are analyzed by a number of collaborators at the Carnegie Institution of Washington (Drs. Larry Nittler and Conel Alexander), the Naval Research Laboratory (Dr. Rhonda Stroud), and Academia Sinica (Drs. Der-Chuen Lee and Sung-yun Hsiao) using a variety of analytical techniques. Our work on the production of sugars and sugar-related compounds is done in close collaboration with Dr. George Cooper (Code SSX at NASA ARC). Dr. Cooper did the initial work on such compounds in meteorites, which was the initial motivation for our laboratory work. Our work on this project has resulted in several oral and poster presentations at scientific conferences during 2019 and a second paper (after the one published in *Nature Communications* in 2018) is currently in preparation.

For the past 5 years, we have also collaborated with Drs. Yu-Jung Chen [National Central University (NCU), Taoyuan] and Bing-Ming Cheng [National Synchrotron Radiation Research Center (NSRRC), Hsinchu] in Taiwan. As Dr. Bing-Ming Cheng retired at the end of 2019, this collaboration continued with Dr. Yu-Jung Wu, beamline manager of BL03 at NSRRC. Through this collaboration, we have successfully been granted several weeks of beam time on two beamlines of the NSRRC synchrotron facility, namely, BL03A, which provides photons in the ultraviolet/extreme ultra violet (UV/EUV) range (4–45 eV), and BL08B, which provides photons in the soft X-ray range (80–1200 eV). In the past year, we irradiated organic samples using BL03A (UV/EUV photons only) during two campaigns in March and October 2019. The samples irradiated during those experiments were different from those irradiated in the previous years. Indeed, previous samples were residues produced in our laboratory at NASA ARC from the UV irradiation of ices of different starting compositions, while these current samples were made from mixtures of commercial standards of organic compounds known to be present in meteorites, namely, amino acids, nucleobases, and sugar derivatives. These standards were mixed in relative proportions that reflect those observed in meteorites, and included both compounds used in modern biology and isomers and/or derivatives of these compounds that are not used in modern biology, as observed in meteorites. These samples were exposed to photons of energies 4–45 eV at NSRRC in order to verify whether such irradiation results in chemical and isotopic changes that are believed to occur in the solar nebula, and eventually lead to the formation of a material which resembles the organic materials observed in meteorites and interplanetary dust particles (IDPs). These irradiated samples are currently being analyzed with IR microscopy by team member Dr. Nuevo at NASA ARC. They will then be analyzed with Raman microscopy at NASA Ames in collaboration with team members Drs. Andrew Mattioda and Gustavo Cruz-Diaz. Finally, these samples will be analyzed with nano-SIMS by our collaborators at the Carnegie Institution of Washington and at Academia Sinica (see above). The results will be compared with those obtained in 2016 after irradiation with soft X-rays on beamline BL08B.

Dr. Mattioda is collaborating with Dr. Ana Ferreira de Barros (Professor and Researcher at the Federal Center for Technological Education – CEFET/RJ), Rio de Janeiro, Brazil, on a paper investigating the C-H stretching modes of hydrogenated and non-hydrogenated PAHs in water ice. This will be used to estimate the organic content of interstellar and planetary ices. This collaboration is in alignment with the agreements between NASA and the Brazilian Space Agency (AEB) to enhance collaborations between the agencies. This collaboration would not have been possible without funding from the NAI.

Team member Dr. Andrew Mattioda is part of a collaboration with Drs. Alfonso Davila (Code SSX at ARC), Alessandra Ricca (Code SSA at ARC), Scott Perl (JPL), Pablo Sobron (SETI), Mary Parenteau (Code SSX at ARC), Anthony Colaprete (Code SST at ARC), Christiaan Boersma (Code SSA at ARC and an NAI CAN 7 team member) and Honeybee Robotics to develop the Salt Astrobiology Geochemistry ANalyzer (SAGAN) instrument suite for the dynamic mapping of large salt deposits for the identification of samples for *in situ* analyzes or for return to Earth. Dr. Mattioda's laboratory is conducting the analysis of analog samples, identifying the chemical complexity of the organisms found within the samples.

Team member Dr. Uma Gorti is part of an international consortium on "Chemistry in Disks" (PIs, Th. Henning and A. Dutrey), and is actively involved as a theoretical/modeling expert in many large-scale programs to understand disk chemical evolution through observations and chemical modeling. These include new observations being planned for ALMA Cycle 8 and JWST Cycle 1 to investigate disk chemistry. Collaborators include researchers at Heidelberg, Bordeaux, Rochester, UA-LPL, NASA-JPL and NASA-GSFC. The NAI work has revealed the importance in understanding the composition of the solid (which form planetary objects) component in the prestellar cloud core. Ongoing collaborations include NAI Goddard Team member Steve Charnley, Earth in Other Systems (NexSS RCN) members including Ilaria Pasucci, and Neal Turner at NASA JPL (NASA XRP-related RCN). Dr. Gorti is also leading a team to investigate disk winds from young stars and their resulting evolution through a SOFIA cycle 8 pilot program.

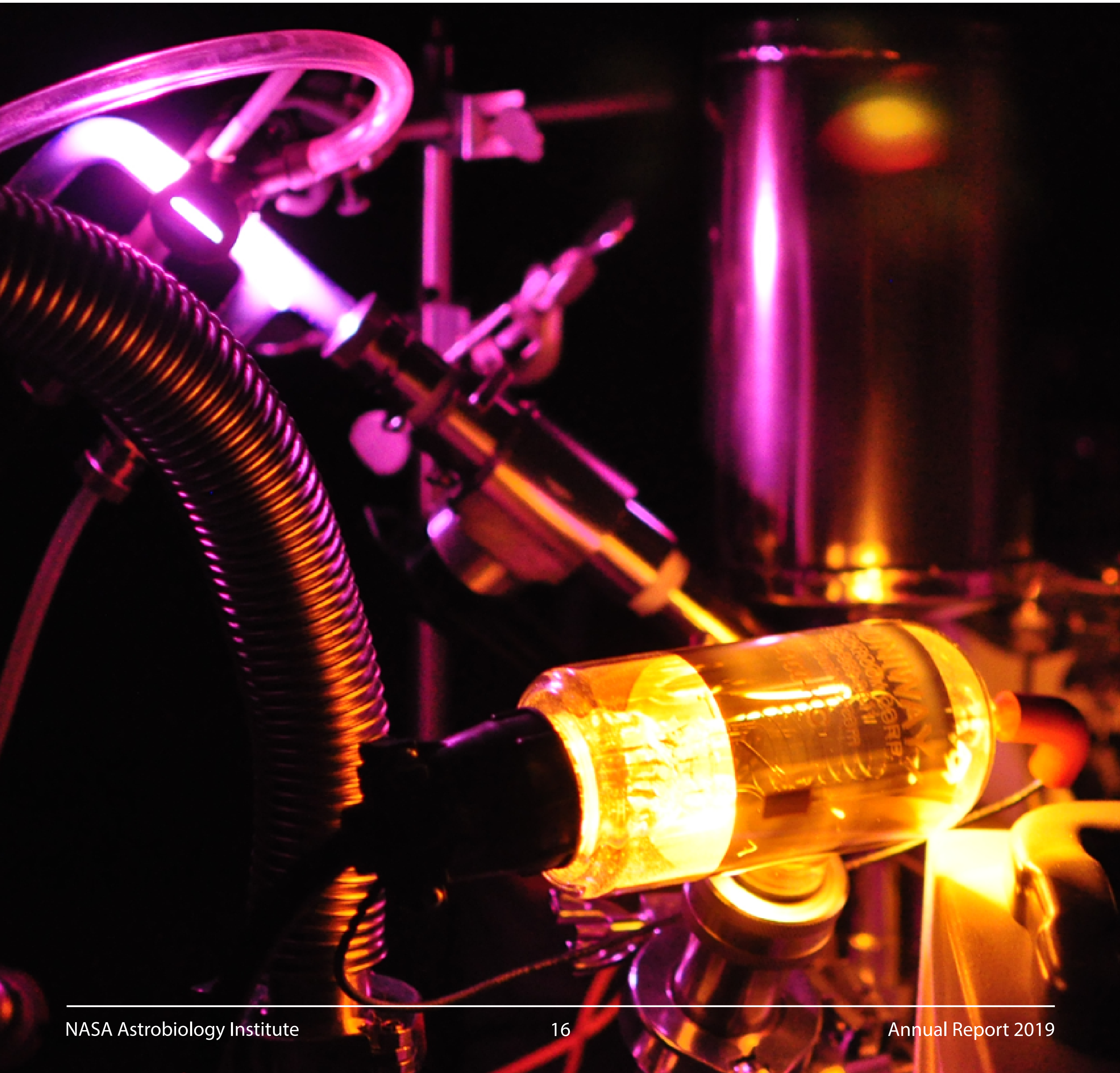
Our computational quantum chemistry effort includes a number of collaborations, including a collaboration with Dr. Xinchuan Huang from the SETI Institute, Dr. Ryan Fortenberry at the University of Mississippi, Dr. Natalia Inostroza-Pino from the Universidad Autonoma de Chile, Prof. Samy El-Shall from the Virginia Commonwealth University, Dr. Ralf Kaiser from the University of Hawaii, and Prof. Henry Schaefer at the University of Georgia.

Our largest and most visible collaboration, however, is with the DAN II, specifically with Profs. Alexander Tielens, Jos Oomens, and Wybren Jan Buma. Our Team has a Non-Reimbursable Space Act Agreement (NRSAA) with the Netherlands Organization for Scientific Research (NWO) in support of this collaboration. The primary effort associated with this collaboration is the study of the effects of anharmonicity on the vibrational spectra of PAH molecules (this effort also includes the collaboration with Dr. Xinchuan Huang from the SETI Institute). Understanding the details of anharmonicity for PAH molecules has been a major focus in the astronomy/astrochemistry community for many years, and we have developed a generalized procedure for computing the anharmonic spectra (and rovibrational spectroscopic constants) for comparison of computed spectra to experimental data, including high-resolution experiments in the C-H stretching region (the 3.2-3.4 μm region). This has been and continues to be a very productive collaboration as we extend the studies to larger and non-linear PAH molecules, as well as PAH molecules with a side methyl group and hydrogenated PAH molecules. Last year, the anharmonic spectra work on PAH molecules was extended to compute libraries of temperature dependent spectra, which were then used in a cascade emission model to compute the actual spectra of PAH molecules that astronomers observe (i.e., in emission). Over the past year, this work has been extended to compute cascade emission spectra of a whole host of PAH molecules, including some larger than we have been able to study previously. As these data are being analyzed, important new conclusions are being obtained such as the approximate size of the PAH molecules typically observed in the ISM, and the intensity ratio of various bands observed in the mid-IR. At least two papers will result from these studies and they will be written up in 2020. The importance and impact of this work for both astronomers and astrochemists was amply demonstrated this past year as Dr. Tielens' former student, Dr. Cameron Mackie (Dr. Lee was a co-advisor), was awarded the Dissertation Prize from both the Astrochemistry Subdivision of the Physical Chemistry Division of the American Chemical Society and from the Laboratory Astrophysics Division of the American Astronomical Society. Dr. Mackie is now a postdoc with team member Dr. Martin Head-Gordon, and he continues to work with Drs. Tielens and Lee on computing the cascade emission spectra of PAH molecules.

The quantum chemistry effort also included computing the rovibrational spectroscopic constants and spectra of small molecules that may appear in various astrophysical environments, with our collaborators Profs. Fortenberry (University of Mississippi) and Schaefer (University of Georgia). The collaborations with Profs. Tielens (DAN II) and Fortenberry are being extended to include very large PAH molecules, and will also include Prof. Joshua Layfield (University of St. Thomas), where we are developing new approaches that can be used to compute the anharmonic spectra of very large

PAH molecules, more consistent with those thought to exist in astrophysical environments. All of these external collaborations were made possible by the NAI funding, particularly with the DAN II as it was explicitly included in our CAN 7 proposal, for which we are grateful.

As part of our collaborations with the DAN II, Dr. Mattioda is working with Dr. Inga Los ten Kate, an Astrobiologist at the Utrecht University in The Netherlands on a NWO (Netherlands Organization for Scientific Research) funded proposal to study the crucial role mineral surfaces play in prebiotic chemistry and the subsequent origin of life. Dr. Mattioda will be hosting one of Dr. ten Kate's doctoral students in his laboratory in August 2020. The graduate student will be investigating the catalytic role of mineral surfaces on PAH chemical evolution via DRIFTS (diffuse infrared FTIR) and Raman spectroscopy. This collaboration is a direct result of NAI funding of the DRIFTS instrumentation and ongoing research in Dr. Mattioda's laboratory.



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 8 September 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 2023. 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 the 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 OREOCube and Exocube — 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.

In the News

Laughlin, G. (2019). The world that came in from the cold. *Science*, Volume 365, Issue 6460, pp. 1382-1383 (2019).

https://science.sciencemag.org/content/365/6460/1382/tab-pdf

Note: This was an illustrated Perspective opinion piece concerning the discovery of a Jupiter-like planet orbiting a low-mass red dwarf star.

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: 2019 Publications

Adams, A., Millholland, S. and Laughlin, G. (2019). Signatures of Obliquity in Thermal Phase Curves of Hot Jupiters. *The Astronomical Journal* 158(108). DOI 10.3847/1538-3881/ab2b35

Adams, A. (2019). Atmospheric Responses to Radiative Forcing: Physics and Observability of Close-in and Highly Eccentric Planets. A PhD Thesis successfully defended by Arthur Adams on 19 August 2019, Department of Astronomy, Yale University. Note: Dr. Adam's PhD Thesis was based on research he conducted with NAI Team Member Gregory Laughlin. Dr. Adams is now a post-doctoral researcher at the University of Michigan.

Agbaglo, D., Lee, T. J., Thackston, R., Fortenberry, R. C. (2019) A Small Molecule with PAH Vibrational Properties and a Detectable Rotational Spectrum: c-(C)C_3H_2, Cyclopropenylidene Carbene. *The Astrophysical Journal* 871(2): 236 (6 pp). DOI: 10.3847/1538-4357/aaf85a

Bera, P. P., Sandford, S. A., Lee, T. J. and Nuevo, M. (2019). The Calculated Infrared Spectra of Dunctionalized Hexamethylenetetramine (HMT) Molecules. *The Astrophysical Journal* 884(1): 64 (16 pp). DOI: 10.3847/1538-4357/ab3c4f

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Cruz-Diaz, G. A., Erickson, S .E., da Silveira, E. F., Ricca, A., de Barros, A. L. F., de Costa, C. A. P., Pereira, R. C. and Mattioda, A. L. (2019). PAH Products and Processing by Different Energy Sources. *The Astrophysical Journal* 882(1): 44 (16 pp). DOI: 10.3847/1538-4357/ab311f

Fortenberry, R. C., Lee, T. J. and Inostroza-Pino, N. (2019). The Possibility of :CNH_2^+ within Titan's Atmosphere: Rovibrational Analysis of :CNH_2^+ and :CCH_2. *Icarus* 321: 260–265. DOI: 10.1016/j.icarus.2018.11.026

Fortenberry, R. C. and Lee, T. J. (2019). Computational Vibrational Spectroscopy for the Detection of Molecules in Space. *Annual Reports in Computational Chemistry* 15: 173–202. DOI: 10.1016/bs.arcc.2019.08.006

Hales, A. S., Gorti, U., Carpenter, J. M., Hughes, M. and Flaherty, K. (2019). Modeling the Spatial Distribution and Origin of CO Gas in Debris Disks. *The Astrophysical Journal* 878(2): 113 (18 pp). DOI: 10.3847/1538-4357/ab211e

Materese, C. K., Nuevo, M., Sandford, S. A., Bera, P. P. and Lee, T. J. (2020). The Production and Potential Detection of Hexamethylenetetramine-methanol and other Hexamethylenetetramine Derivatives in Space. *Astrobiology* 20: 601-616.

Millholland, S., Laughlin, G. (2019). Obliquity-driven sculpting of exoplanetary systems. *Nature Astronomy* 3: 424–433. DOI 10.1038/s41550-019-0701-7

Millholland, S. and Laughlin, G. (2019). Obliquity-driven sculpting of exoplanetary systems. *Nature Astronomy* 3: 424–433. DOI: 10.1038/s41550-019-0701-7

Morgan, W. J., Fortenberry, R. C., Schaefer, H. F., Lee, T. J. (2020) Vibrational Analysis of the Ubiquitous Interstellar Molecule Cyclopropenylidene (c-C_3H_2): The Importance of Numerical Stability, *Molecular Physics*, published on line. DOI: 10.1080/00268976.2019.1589007

Morgan, W. J., Fortenberry, R. C., Schaefer, H. F. and Lee, T. J. (2019). Vibrational Analysis of the Ubiquitous Interstellar Molecule Cyclopropenylidene (c-C_3H_2): The Importance of Numerical Stability. *Molecular Physics*, published on line. DOI: 10.1080/00268976.2019.1589007

Rice, M. and Laughlin, G. (2019). Hidden Planets: Implications from 'Oumuamua and DSHARP. *The Astrophysical Journal Letters* 844(1): L22 (6 pp). DOI 10.3847/2041-8213/ab4422

Rice, M. and Laughlin, G. (2019). The Case for a Large-scale Occultation Network. *The Astronomical Journal* 158(1): 19 (18 pp). DOI 10.3847/1538-3881/ab21df

Ruaud, M. and Gorti, U. (2019). A Three-Phase Approach to Grain Surface Chemistry in Protoplanetary Disks : Gas, ice Surfaces and Ice Mantles of Dust Grains. *The Astrophysical Journal* 885(2): 146 (27 pp). DOI: 10.3847/1538-4357/ab4996

Seligman, D., Laughlin, G. and Batygin, K. (2019). On the Anomalous Acceleration of 1I/2017 U1 Oumuamua. *The Astrophysical Journal Letters* 876(2): L26 (5 pp). DOI: 10.3847/2041-8213/ab0bb5

Additional Publications:

Cruz-Diaz, G. A., Mattioda, A. L. and Ricca, A. (2020). PAH-Dust Interactions. A Laboratory Approach to Astrophysical Catalysis. *The Astrophysical Journal*, in review.

Hendrix, J., Bera, P. P., Lee, T. J. and Head-Gordon, M. (2020). The Cation, Anion, and Radical Isomers of C4H4N: Computational Characterization and Implications for Astrophysical and Planetary Environments. *The Journal of Physical Chemistry A*, in review.

Inostroza-Pino, N., Palmer, C. Z., Lee, T. J. and Fortenberry, R. C. (2020). Theoretical Rovibrational Characterization of the cis/trans-HCSH and H2CS Isomers of the Known Interstellar Molecule Thioformaldehyde. The Journal of Molecular Spectroscopy, in review.

Mattioda, A. L., Cruz-Diaz, G. A., Ging, A., Barnhardt, M., Boresma, C., Allamandola, L. J., Schneider, T., Vaughn, J. and Phillips, B. (2020). Formation of Complex Organic Molecules (COMs) from PAHs: implications for Solar System and Saturn System Organics. *The Astrophysical Journa*l. In review.

Nuevo, M., Cooper, G., Saunders, J. M., Buffo, C. E. and Sandford, S. A. (2020). Formation of Complex Organic Molecules in Astrophysical Environments: Sugars and Derivatives. *Proceedings of the IAU Symposium 350: Laboratory Astrophysics: From Observations to Interpretation*. In press.

Sandford, S. A., Nuevo, M., Bera, P. P. and Lee, T. J. (2020). Prebiotic Astrochemistry and the Formation of molecules of Astrobiological Interest in interstellar Clouds and Protostellar Disks. *Chemical Reviews*. In press, https://dx.doi.org/10.1021/acs.chemrev.9b00560.

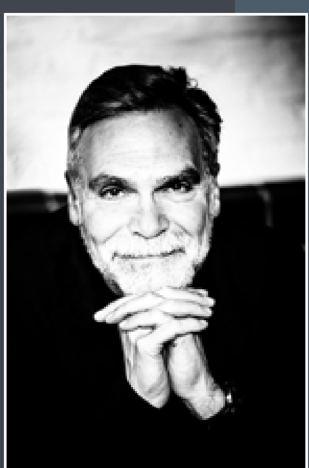
Stein, T., Bera, P. P., Lee, T. J . and Head-Gordon, M. (2020). Molecular Growth upon Ionization of Van der Waals Clusters Containing Acetylene and HCN. In preparation.

Reliving the Past:
Experimental Evolution of Major Transitions

Lead Institution:
Georgia Institute of Technology

Team Overview

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 grand challenges. Our Team is responding to these challenges by "reliving the past" using experimental evolution. This approach 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 questions, we seek to illuminate what drove the major transitions that led to evolution of complex life on our home planet:



Principal Investigator:
Frank Rosenzweig

- 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?

Team Website: https://astrobiology.nasa.gov/nai/teams/can-7/gatech/index.html

2019 Executive Summary

Major evolutionary transitions occur when simple subunits coalesce into autonomous, interdependent wholes, bringing about a quantum leap in biocomplexity. Our team integrates theory, comparative genomics and experimental evolution to tackle 5 questions related to the evolution of complex life: *How do enzymes and metabolic networks evolve? How does multicellularity evolve? How do symbioses arise? How did the eukaryotic cell come to be? And How do fundamental aspects of heredity constrain evolutionary innovation?* If life is a self-sustaining chemical system capable of Darwinian evolution, then the answers to these questions should apply wherever life arises in the Cosmos. In 2019 the Georgia Tech CAN-7 team made great strides towards answering these questions, delivered in the form of 38 peer-reviewed articles in journals such as *PNAS (USA), Cell, eLife* and *Nature* plus 4 reports on BioRxiv that are in review or revision. We disseminated our findings in 54 invited lectures at universities and scientific conferences across the globe, including a widely-acclaimed, international conference organized by our NPP Fellows on the Evolution of Complex Life (https://eclife.biosci.gatech.edu/).

We shed new light on how genes are born and integrated into existing metabolic pathways. Bioinformatic evidence suggests that pathways evolve by recruiting enzymes that have a promiscuous ability to catalyze a newly-important reaction. Shelley Copley teamed up with Vaughn Cooper to examine how promiscuous enzymes are recruited when *E. coli* is placed under strong selective pressure by deleting an essential gene. In one case, they identified mutations elsewhere in the bacterial genome that compensate for the poor function of a newly recruited enzyme (*eLife* 8:e53535). In another, they found that promiscuous activities were patched together to form an altogether new 4-step metabolic pathway *(PNAS (USA)* 113(48):24164-24173).

We succeeded in recapitulating another major transition, that of multicellularity. Matt Herron's group teamed up with PI Frank Rosenzweig and co-I Will Ratcliff to show how predation by a ciliate predator, Paramecium, selects for large size, and drives experimental evolution of multicellularity in the single-celled green alga *Chlamydomonas reinhardtii*. Remarkably, different life histories arose in different experimental populations (Figure 1).

We continue to unravel the mysteries of symbiosis, work that sheds light on eukaryogenesis, especially how nuclear and organellar genomes are reconfigured via horizontal

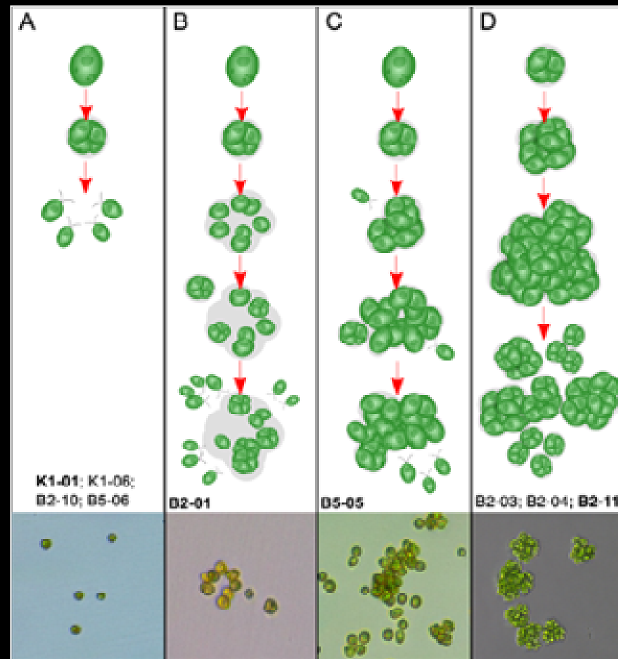


Figure 1. Depiction of *C. reinhardtii* life cycles following evolution with (B2, B5) or without (K1) predators for 50 weeks. Categories (A–D) show a variety of life cycle characteristics, from unicellular to various multicellular forms. Briefly, A shows the ancestral, wild-type life cycle; in B this is modified with cells embedded in an extracellular matrix; C is similar to B but forms much larger multicellular structures; while D shows a fully multicellular life cycle in which multicellular clusters release multicellular propagules. Evolved strains were qualitatively categorized based on growth during 72-hour time-lapse videos. Strains within each life cycle category are listed below illustrations. Representative microscopic images of each life cycle category are at the bottom (Depicted strain in boldface) (from Herron, *Scientific Reports* 9:2328, 2019). Credit: Image courtesy of Matthew Herron (*Scientific Reports* 9:2328).

Figure 2. Two mealybugs belonging to the species *Planococcus citri*, which hosts two bacterial endosymbionts, Tremblaya and Moranella. This was the study system in Bublitz *et al*. 2019. Peptidoglycan production by an insect-bacterial mosaic. *Cell* 179:703–712.

gene transfer (HGT). Working with mealybugs (Figure 2) and their bacterial endosymbionts John McCutcheon, his Caltech collaborator, Victoria Orphan, and colleagues published a landmark paper in Cell proving that a mosaic of bacterial HGTs on the insect genome, and genes retained on a bacterial endosymbiont genome work together to make a peptidoglycan layer specifically at the periphery of a particular bacterial endosymbiont (Figure 3) (*Cell* 197(3):703-712).

This shows that complex biochemical mosaics work together in examples outside of the mitochondrion and plastid, and indicates that some insect endosymbionts are proceeding down a similar path to one that the classic organelles might have taken in their transition from "endosymbiont" to "organelle."

Even if a novel trait is evolutionarily advantageous to a species, that trait may not be accessible to natural selection. Gavin Sherlock devised an elegant set of experiments using yeast showing why this sometimes occurs. His students evolved multiple barcoded yeast populations under three different growth conditions (Figure 4a), then remeasured fitness of adaptive clones isolated from each under every possible alternative, enabling them to decompose fitness into components for each part of the growth cycle (Figure 4b). Their data show that for fitness gains in fermentation and respiration, and in respiration and stationary phase, that there are no simple trade-offs but instead a Pareto front that constrains clones' ability to maximize performance in each of the phases simultaneously. (*Nature Ecology Evolution* 3(11):1539-1551).

In conclusion, whether measured as groundbreaking scholarship, public seminars, or synergistic activities, Georgia Tech's "Reliving the Past" team enjoyed a successful Year 5. As we move into our no-cost extension year we expect to leave the NASA Astrobiology Institute with a legacy of achievement in the fields of experimental and comparative genomics, a legacy that deepens our understanding of how complex life arose, and continues to arise, on Earth.

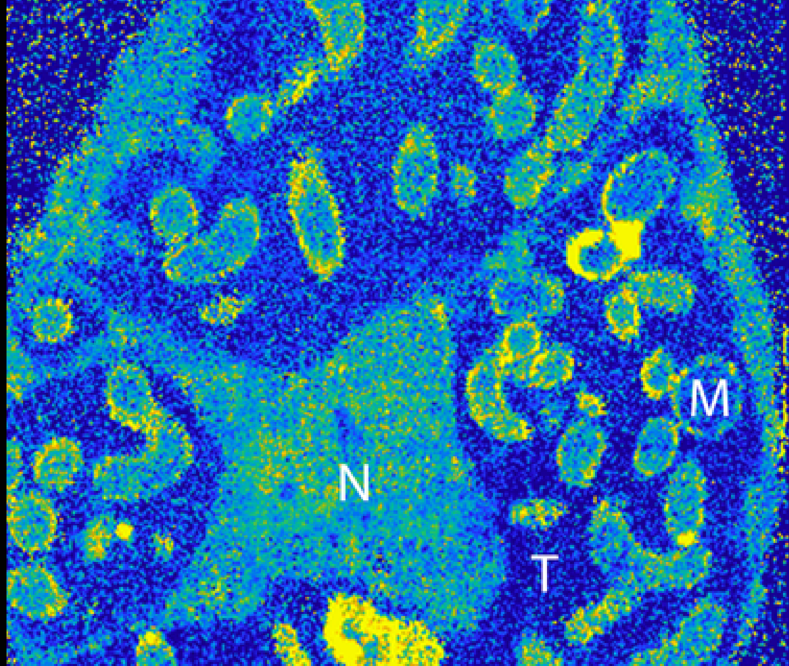


Figure 3. A nanoSIMS image of a mealybug bacteriocyte cell. Mealybugs were grown in 15N-labeled D-alanine, which is incorporated into bacterial peptidoglycan. Enrichment in 15N over 14N is shown as more yellow colors. The rings of yellow signal are the peptidoglycan layer of the bacterial endosymbiont Moranella. Insect nucleus is labeled N, the bacterium *Tremblaya* is labeled T, and a single *Moranella* cell is labeled M. Credit: Image courtesy of Victoria Orphan and John McCutcheon, published in *Cell*, 2019

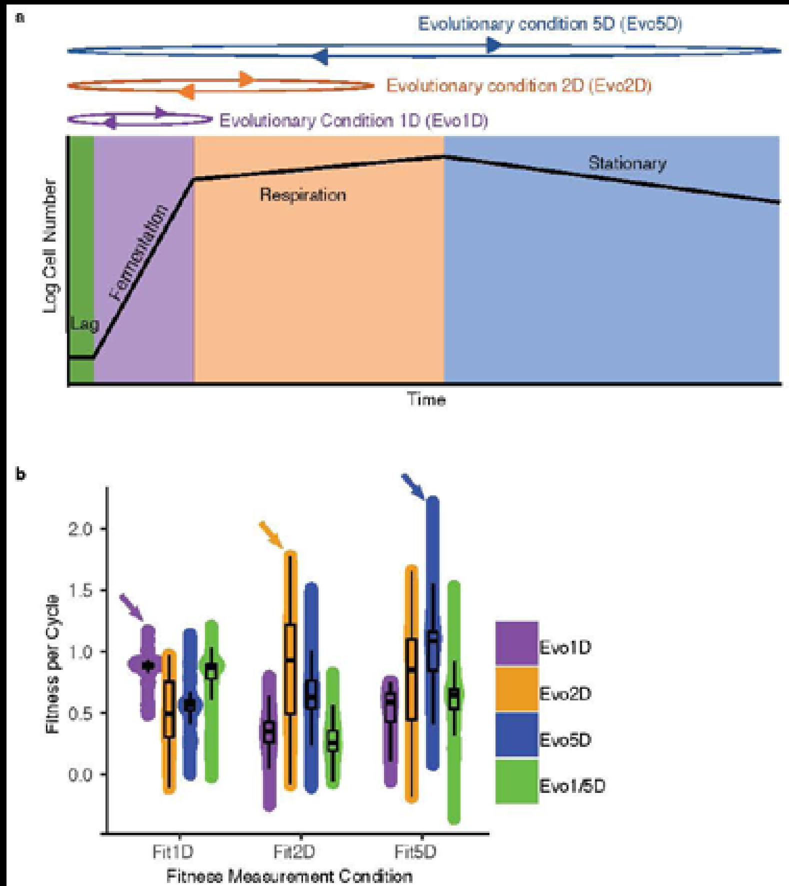


Figure 4. Experimental design and the observation of local adaptation and tradeoffs. a) Three chosen evolutionary conditions span different phases of the yeast growth cycle. Clones were also evolved in a 1-day/5-day alternating condition (Evo1/5D). b) Fitness measurements of adaptive clones, grouped by their "home" evolutionary condition, in "home" and "away" conditions. Arrows point to adaptive clones measured in their "home" condition. The lower and upper hinges of each box correspond to the first and third quartiles (the 25th and 75th percentiles). Credit: Image courtesy of Gavin Sherlock, Stanford University. Modified from *Nature Ecology and Evolution,* 2019.

Project Reports

From generalists to specialists (or not): A case study in enzyme evolution
(Shelley Copley, University of Colorado)

The Copley lab continued to investigate the role of enzyme promiscuity in the *evolution of new enzymes and metabolic pathways*. Promiscuous enzyme activities are physiologically irrelevant secondary activities that occur when non-canonical substrates bind in the highly reactive environments of enzyme active sites. Although promiscuous activities are usually inefficient, they still can accelerate rates of chemical reactions by several orders of magnitude. Thus, if circumstances change and a promiscuous activity becomes important for fitness, it can serve as an excellent starting point for evolution of an efficient new enzyme. New enzymes often evolve by amplification and divergence of genes encoding promiscuous enzymes (Figure 5). Previous experimental studies have followed the evolutionary trajectory of an amplified gene, but have not considered mutations elsewhere in the genome when fitness is limited by an evolving gene. We evolved a strain of *Escherichia coli* in which a promiscuous activity of ProA (γ-glutamyl phosphate reductase) has been recruited to replace a missing enzyme in the arginine synthesis pathway (Morgenthaler *et al.* 2019 *eLife* 9:8). The gene encoding the "weak-link" enzyme amplified in all eight populations, but mutations improving the newly needed activity occurred in only one (Figure 6). Most adaptive mutations occurred elsewhere in the genome. Some mutations increase expression of the enzyme upstream of the weak-link enzyme, pushing material through the dysfunctional metabolic pathway. Others enhance production of a co-substrate for a downstream enzyme, thereby pulling material through the pathway. Most of these latter mutations are detrimental in wild-type *E. coli,* and thus would require reversion or compensation once a sufficient new activity has evolved. *This work demonstrates that the process of evolution of a new enzyme is inextricably intertwined with mutations elsewhere in the genome that improve fitness by other mechanisms*.

The evolutionary potential of promiscuous enzymes goes beyond providing the starting point for evolution of new enzymes. Multiple promiscuous enzymes can be patched together to generate new metabolic pathways. We identified a novel pathway patched together from promiscuous enzyme activities after evolution of a strain of *E. coli* that lacked PdxB (erythronate 4-phosphate dehydrogenase), an enzyme in the pathway for

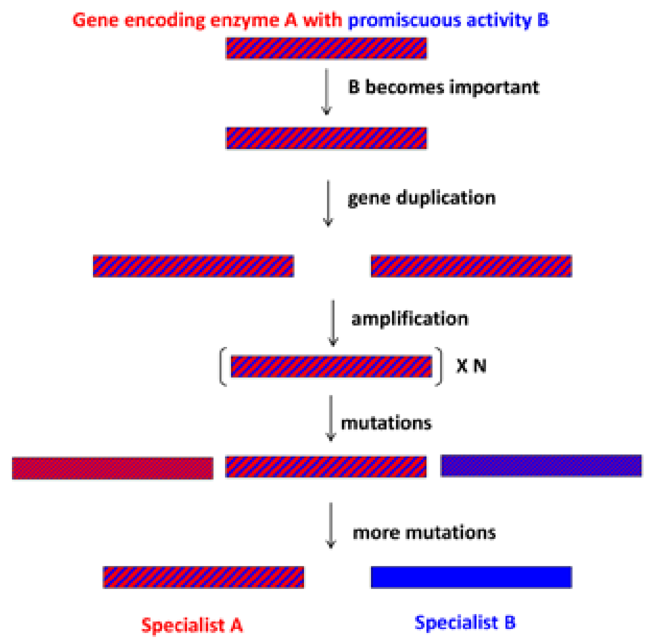


Figure 5. The Innovation-Amplification-Divergence model for evolution of new enzymes from previously irrelevant promiscuous activities (Bergthorsson *et al*. 2007 *PNAS (USA)*z104(43):17004-9). Credit: Image courtesy of Shelley Copley, University of Colorado

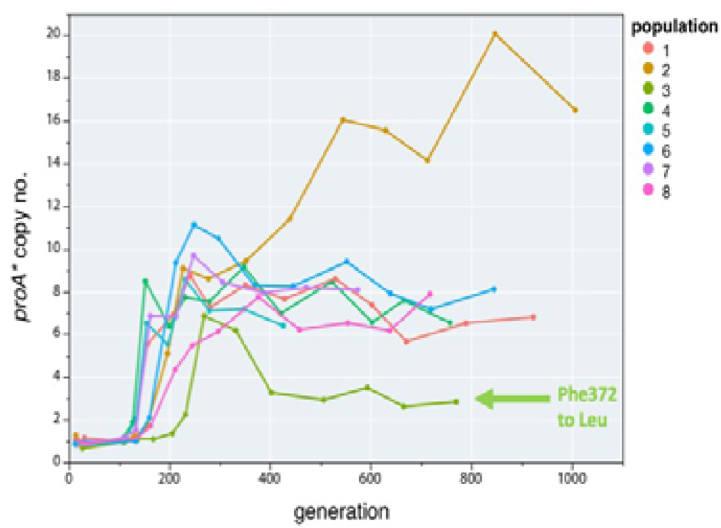


Figure 6. The gene encoding the weak-link enzyme proA* (which encodes E383A ProA, an enzyme with an inefficient ability to catalyze the missing step in arginine biosynthesis in ΔargC *E. coli*, amplifies rapidly in eight replicate lineages. However, a mutation that improves the ability of ProA* to catalyze the missing reaction occurred only in lineage 3. In all of the other lineages, fitness was improved by mutations that improved arginine synthesis by other mechanisms (Morgenthaler *et al.* 2019. *eLife* 9:8). Credit: Image courtesy of Shelley Copley, University of Colorado

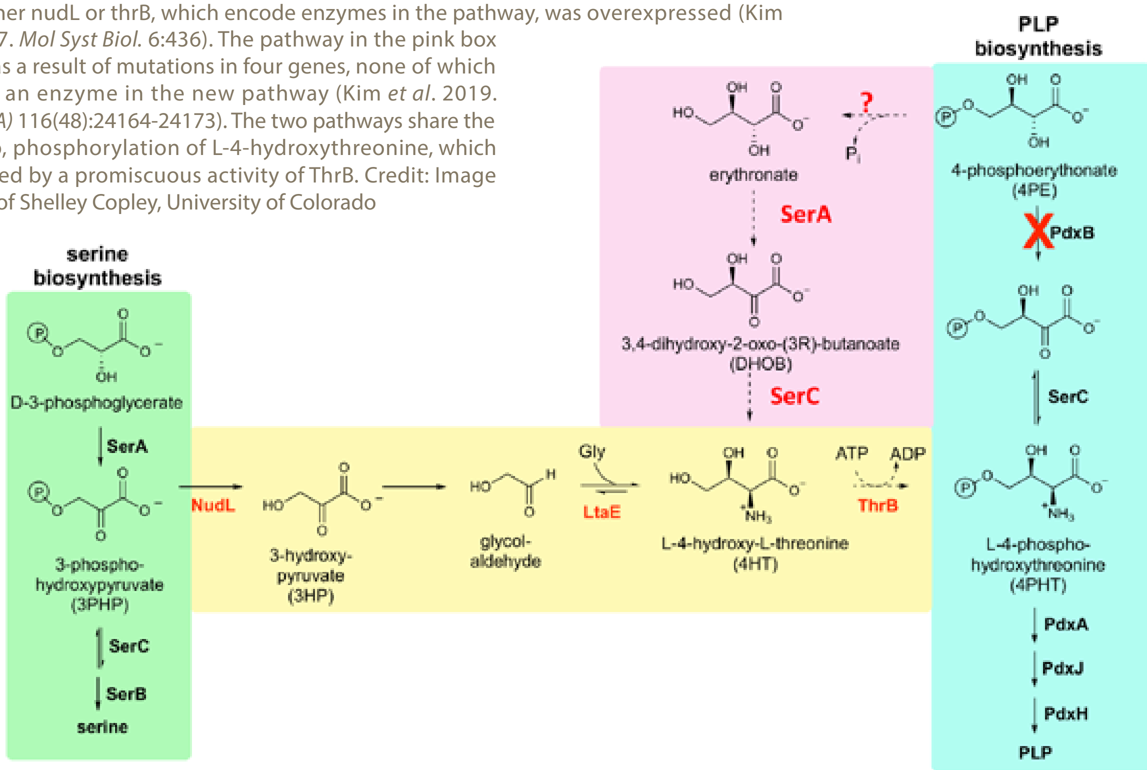


Figure 7. Two pathways that restore growth of a strain of *E. coli* that lacks PdxB in the PLP synthesis pathway. Both pathways involve promiscuous activities of enzymes that normally serve other functions. The pathway in the yellow box was previously shown to operate when either nudL or thrB, which encode enzymes in the pathway, was overexpressed (Kim *et al.* 2007. *Mol Syst Biol.* 6:436). The pathway in the pink box evolved as a result of mutations in four genes, none of which encoded an enzyme in the new pathway (Kim *et al*. 2019. *PNAS (USA)* 116(48):24164-24173). The two pathways share the final step, phosphorylation of L-4-hydroxythreonine, which is catalyzed by a promiscuous activity of ThrB. Credit: Image courtesy of Shelley Copley, University of Colorado

synthesis of the essential cofactor pyridoxal 5′-phosphate (PLP) (Kim *et al.* 2019, *PNAS* 116(48):24164-24173). Surprisingly, incubation of the Δ*pdxB* strain in medium containing glucose as a sole carbon source for 10 days resulted in visible turbidity, suggesting that PLP was being produced by some alternative pathway. Continued evolution of parallel lineages for 110-150 generations produced several strains that grow robustly in glucose (e.g. JK1). We identified a 4-step bypass pathway patched together from promiscuous enzymes that restores PLP synthesis in strain JK1 (Figure 7). None of the mutations in JK1 occurs in a gene encoding an enzyme in the new pathway. Two mutations indirectly enhance the ability of SerA (3-phosphoglycerate dehydrogenase) to perform a new function in the bypass pathway. Another disrupts a gene encoding a PLP phosphatase, thus preserving PLP levels. *These results demonstrate that a functional pathway can be patched together from promiscuous enzymes in the proteome, even without mutations in the genes encoding those enzymes.*

A new hypothesis for the role of oxygen in the evolution of organismal size
(Will Ratcliff, Georgia Institute of Technology)

Oxygen is widely thought to be a catalyst for large multicellular size. Thus, the global rise in oxygen during the Ediacaran is generally thought to be a key causal driver of large eukaryotic multicellular organisms. Despite the prevalence of this view, this hypothesis has never been directly tested, mainly because we don't have amenable model systems. A postdoc in my lab, Ozan Bozdag, modified oxygen metabolism in snowflake yeast (making them obligately aerobic, obligately fermentative, or facultatively fermentative), and performed a ~4,000 generation selection experiment favoring the evolution of increased size. Surprisingly, we found that large size evolved rapidly in either fermentative lines (those that are incapable of using oxygen) or aerobic lines cultured with an abundance of O_2, but was strongly constrained in aerobic lines given limiting O_2. Specifically, intense competition for O_2 strongly constrained the evolution of large size, because oxygen was effectively acting as a resource.

Our results (both mathematical (Figure 8) and experimental (Figure 9) demonstrate that small amounts of O_2 can constrain the evolution of large, complex multicellular organisms far more strongly than none at all, providing new insight into the lack of multicellular innovation between the great oxidation event ~2.1 GYa, and the rise of large animals in the Ediacaran ~1.5 billion years later. To investigate the role of static oxygen carriers in mitigating this trade-off, we have engineered snowflake yeast to produce sperm whale myoglobin and peanut worm myohemerythrin. This experiment is only preliminary (30 days of evolution), but so far the O_2-binding globin producing yeast have evolved to be considerably larger than the controls!

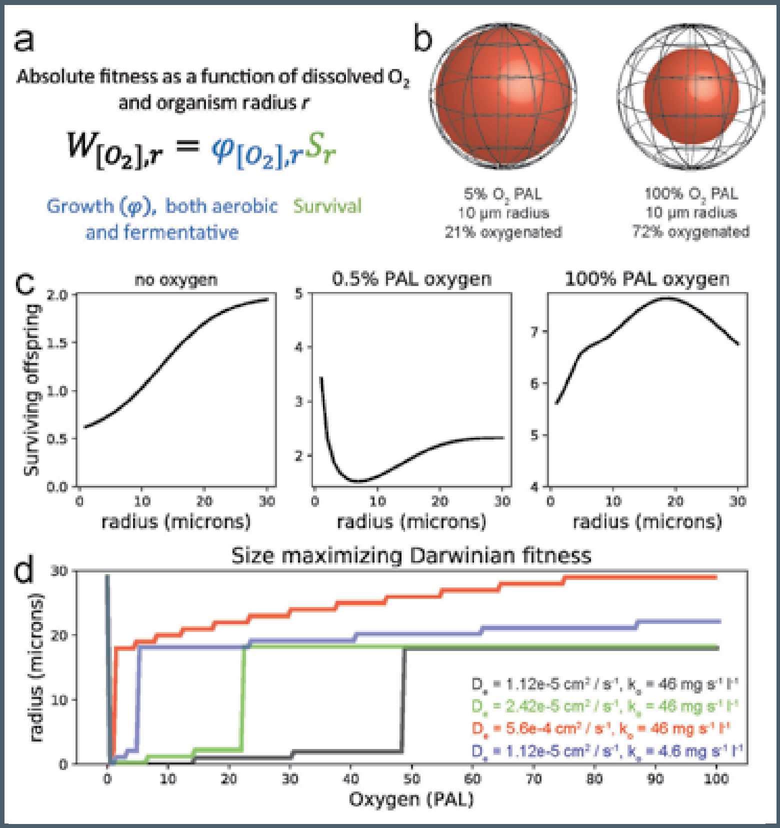


Figure 8. A model examining the evolution of organismal size across a range of oxygen availabilities. Size is bimodal: maximized when no oxygen is present or when oxygen is abundant and diffuses deeply into the organism. This is because the presence of nonfermentable carbon favors competition for oxygen. *De* refers to the diffusion rate of oxygen through the organism, while *Ko* is the volume specific consumption rate of oxygen. Credit: Image courtesy of Will Ratcliff, Georgia Institute of Technology

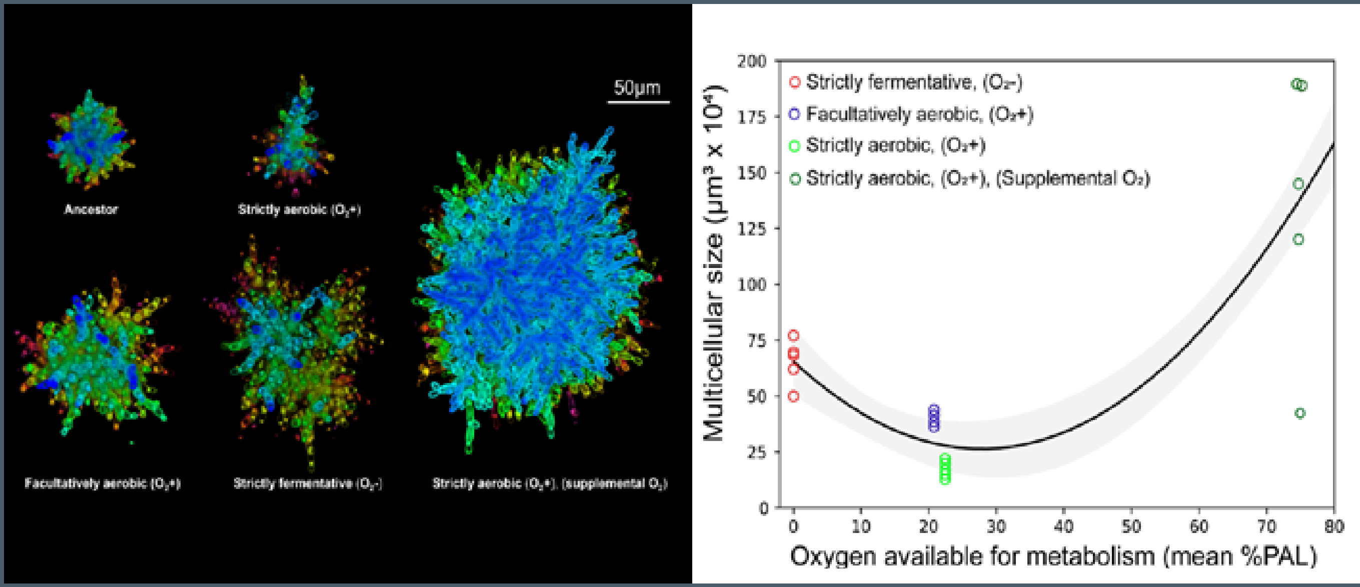


Figure 9. Direct test of our evolutionary model. Snowflake yeast evolved at intermediate oxygen availability (~20% PAL) evolved to be considerably smaller than either anaerobic or O_2-supplemented lines. This decrease in size with the transition from an anaerobic to O_2-limited environment is lends strong support to our hypothesis, that limiting oxygen constrains the evolution of multicellular size and complexity even more strongly than an anaerobic environment. Credit: Image courtesy of Will Ratcliff, Georgia Institute of Technology

Limits to Optimality in Adaptive Evolution

(Gavin Sherlock, Stanford)

NAI funding enabled four communications in 2019: two in *Nature Ecology Evolution*, one in *Nature Communications* and one in *Genome Research*. First, the Sherlock lab investigated *historical contingency*– how existing mutations constrain and/or affect future evolution. We barcoded, then further evolved, 3 adaptive lineages, each of which has a single beneficial mutation (*CYR1*, *GPB2*, or *TOR1*), affecting either the Ras/Protein Kinase A signaling pathway, or the Tor signaling pathway. 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 whole genome sequenced adaptive haploid clones from each of these evolutions and found that all three genotypes had adaptive 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 dam age from oxidative stress. Mutations in this gene have not been seen in wild-type evolved clones, suggesting this mutation is historically contingency on Ras or Tor pathway mutations. We also found that Ras pathway mutants frequently gained mutations in the Tor pathway, and that the *TOR1* mutant frequently gained Ras pathway mutations, suggesting that the beneficial effects of mutations in one of the pathways do not preclude beneficial effects in the other. Finally, we remeasured fitness for the isolated clones, and found that *TOR1* evolved mutants have greater fitness gains than seen in the Ras pathway mutants, likely due to the initial fitness of the Ras pathway mutants being higher– i.e. we are observing diminishing returns epistasis.

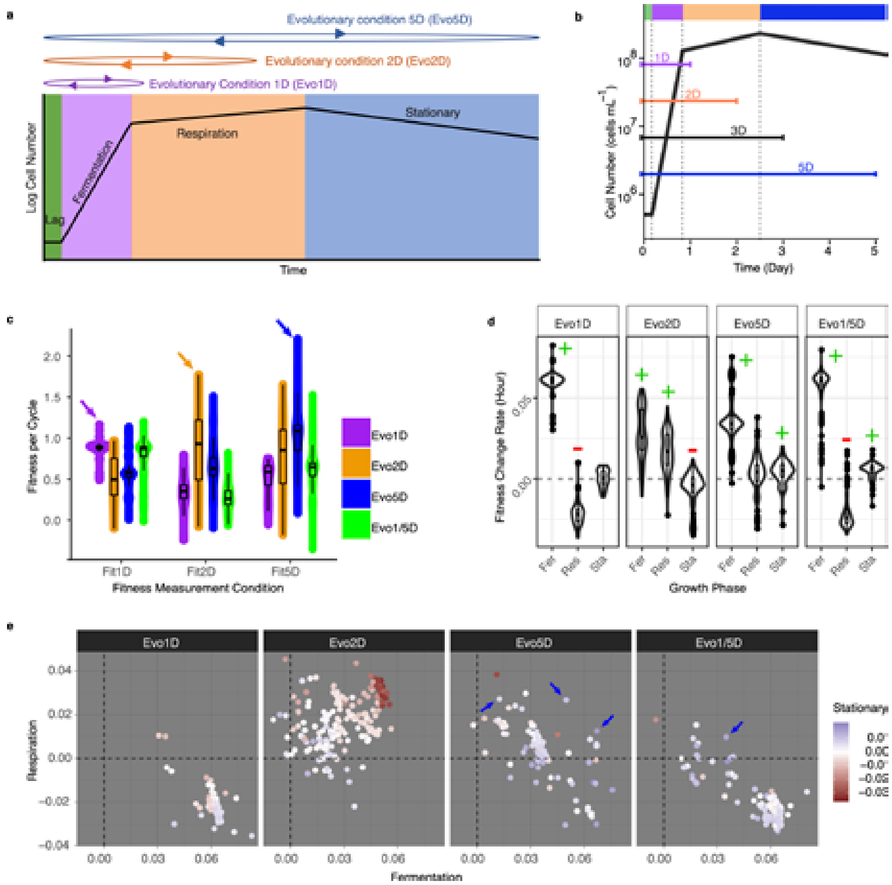


Figure 10: Experimental design and the observation of local adaptation and tradeoffs. a) Three chosen evolutionary conditions span different phases of the yeast growth cycle. Clones were also evolved in a 1-day/5-day alternating condition (Evo1/5D). b) Fitness measurement conditions designed to quantify fermentation, respiration and stationary performances (fitness change per hour) of each clone. Dashed vertical lines separate different growth phases, colored as (a). c) Fitness measurements of adaptive clones, grouped by their "home" evolutionary condition, in "home" and "away" conditions. Arrows point to adaptive clones measured in their "home" condition. The lower and upper hinges of each box correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to a value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range). The width of the violin represents the probability density of the data at different fitness values. d) Adaptive clones' fermentation, respiration and stationary performances grouped by their evolutionary condition. +/- indicates increased/decreased performance compared to the ancestor. e) Clones are separated by their evolutionary condition and colored by their stationary phase performance. Each dot represents a clone. Note that some blue colored clones from Evo5D and Evo1/5D (pointed by arrows) improve performances in all three growth phases (Li *et al.* 2020, *Nature Ecol Evoln.* 3:1539-1551). Credit: Image courtesy of Gavin Sherlock, Stanford University

Second, we have investigated *evolutionary trade-offs* – while they are believed to be pervasive, they have been challenging to demonstrate, because some adaptive individuals frequently show improved performance in multiple or even all traits tested. We evolved clones under different growth cycles, such that they would experience different amount of fermentation, respiration and/or stationary phase, then determined the performance of evolved clones in each of the phases (Figure 10). We found that while indeed, some clones improve performance in two traits simultaneously, and a few even improved performance in all three traits, there is a Pareto front, that defines an evolutionarily inaccessible space for respiration and fermentation, and for respiration and stationary phase (Figures 10 and 11). There does not appear to be a Pareto front when considering fermentation and stationary phase. We sequenced hundreds of adaptive clones and found that differing growth cycles resulted in different adaptive mutational spectra, observing mutations in several genes that we had not previously seen. One such gene, *SXM1*, has loss-of-function mutations that substantially improve performance in fermentation, but at the cost of respiration performance (Figure 12). While the exact mechanism is unknown, there is a report that Sxm1 is involved in correct localization of protein kinase A to the nucleus – thus Sxm1 loss-of-function mutations would be expected to decrease activity of the Ras/Protein kinase A pathway.

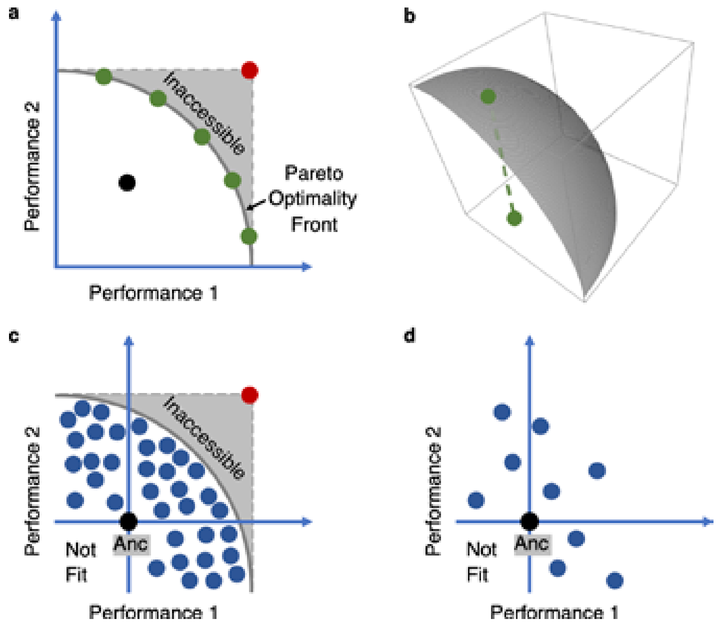


Figure 11. Evolutionary constraints in trait-performance space. a) The Pareto optimality front separates the evolutionary accessible (white space) from the inaccessible space (shaded space). The red dot represents mutants that maximize both traits simultaneously. When organisms are on the Pareto optimality front (green dots), increasing the performance for one trait decreases the performance for the other. By contrast, when organisms are behind the Pareto front (black dot), organisms can improve the performance of both traits until the front is reached. b) An organism on a three-dimensional Pareto surface (green dot) appears to be sub-optimal when it is projected onto a two-dimensional space. c-d) When the ancestor (Anc) is behind the Pareto front, many individuals occupying different parts of the trait space (c) are required to characterize the Pareto front. By contrast, too few individuals (d) are insufficient to delineate the front (Li *et al.* 2020, *Nature Ecol Evoln*. 3:1539-1551).

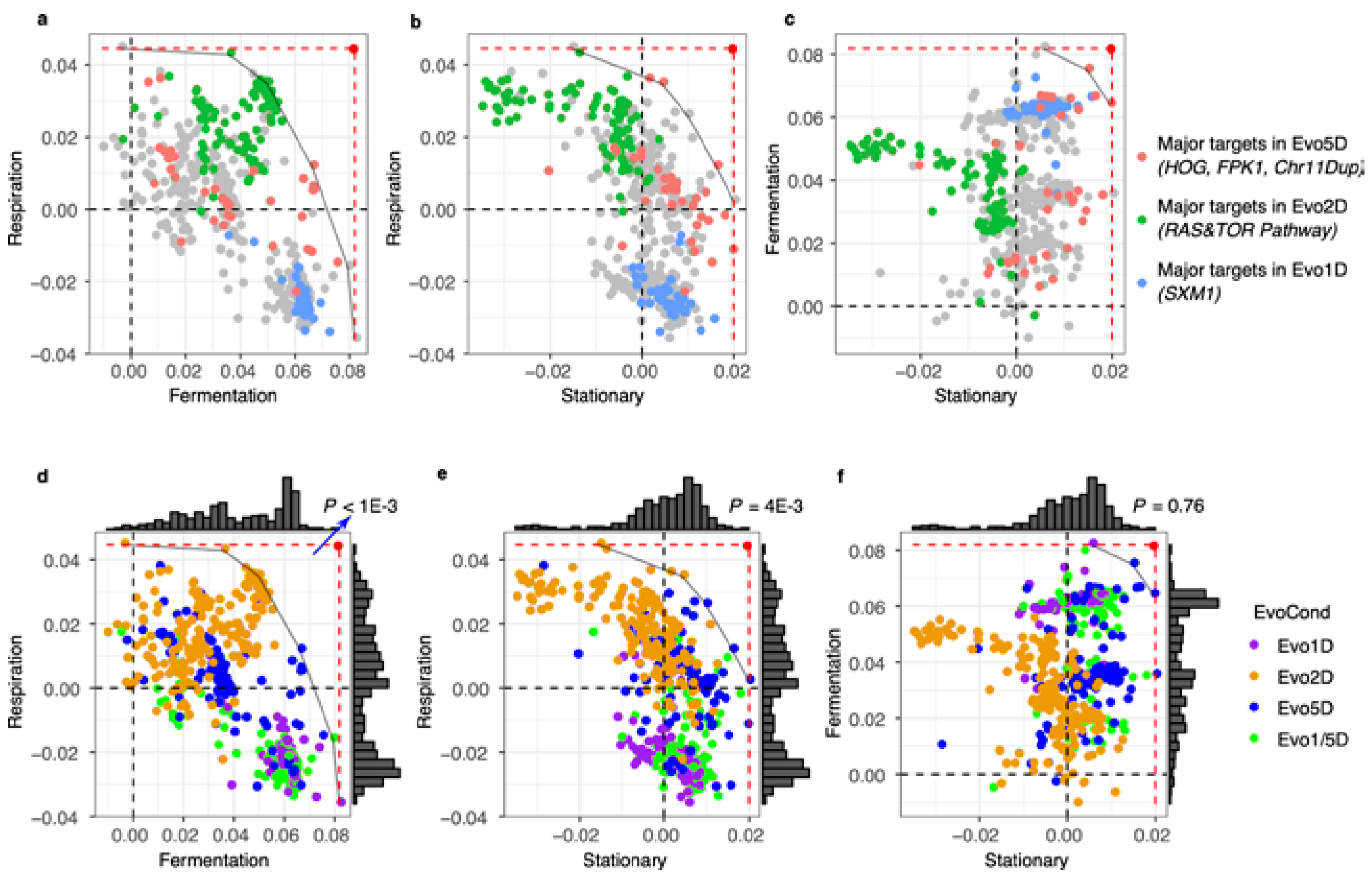


Figure 12. Mapping of the evolutionarily accessible trait space. For each pair of performances (fitness change per hour in each growth phase), adaptive clones are plotted and colored by either their molecular basis (a-c), or their evolutionary conditions (d-f). Each dot represents a clone. The large red dots represent the optimum phenotypes, achieving the upper limits (dashed lines) of each pair of performances. The grey curves, defined by the convex hull algorithm, represent putative Pareto optimality fronts. d-f, Histograms on the side represent the density distribution of each trait's performance. Based on the null distribution, the p-value of observing an empty space between the putative front and the optimal type (the large red dot) as large as observed in the real data is reported (Li *et al.* 2020, *Nature Ecol Evoln*. 3:1539-1551). Credit: Image courtesy of Gavin Sherlock

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

(Matthew Herron, Georgia Institute of Technology)

One focus of our research in 2019 has been on the question of how novel cellular traits arise. Cellular innovation is central to biological diversification and the evolution of complexity, but its mechanisms are poorly understood. Two NAI-supported projects address this issue for adaptation to novel environments. In our paper in Current Biology, we showed how the innovation of a structurally novel nitrogen-fixing cell contributed to high temperature adaptation in the cyanobacterium *Fischerella thermalis*. In addition, NAI-supported graduate student Nikea Ulrich has identified a role for horizontal gene transfer and gene duplication during the innovation of the novel light-harvesting apparatus of the cyanobacterium *Acaryochloris*. Both examples have involved the evolution of phenotypic plasticity (i.e., the environmental induction of alternative phenotypes by the same genotype). This is notable, because the role of plasticity for adaptation has been hotly debated by evolutionary biologists for decades. The second focus of our group this year has been on the evolution of the cyanobacterial endosymbiont of a group of photosynthetic algae called rhopalodian diatoms, which is in the process of becoming a nitrogen-fixing organelle. The evolution of eukaryotic organelles remains one of the great mysteries of biology, and the diatom symbiosis promises fresh insights on the early stages of this process. In 2019, we have developed genomic resources for both partners in the association. The most exciting development was our observation that the host mitochondrial genome has started to degenerate since symbiont acquisition. Strikingly, it is genes involved in translation that have been most significantly affected (ribosomal rRNAs and some tRNAs may be nonfunctional), whereas energy metabolism genes have largely not experienced accelerated evolution. Future work aims to determine the consequences of degenerative mitochondrial evolution for host function and the potential compensating mechanisms provided by the symbiont. Finally, two graduate students and an undergraduate student in the lab presented their work at AbSciCon, and one was a finalist for best poster.

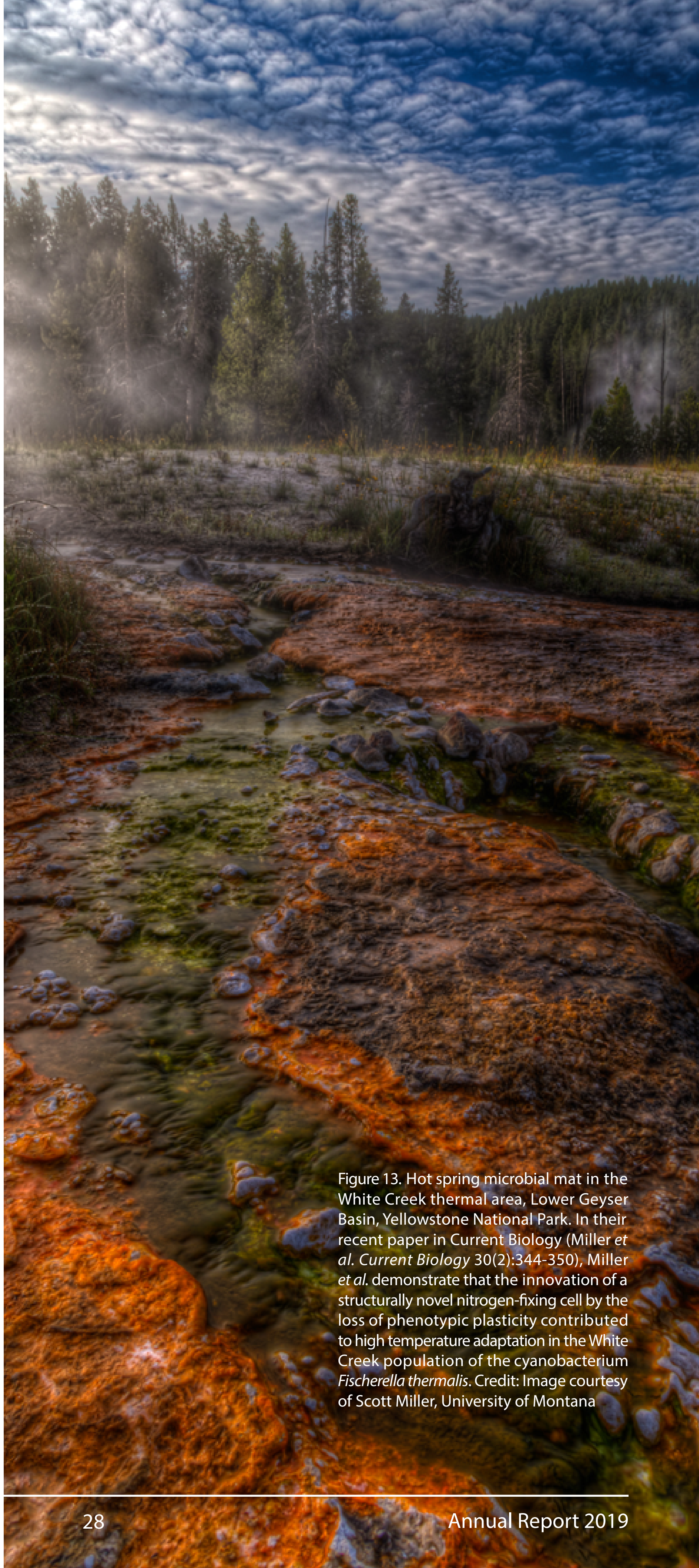


Figure 13. Hot spring microbial mat in the White Creek thermal area, Lower Geyser Basin, Yellowstone National Park. In their recent paper in Current Biology (Miller *et al. Current Biology* 30(2):344-350), Miller *et al.* demonstrate that the innovation of a structurally novel nitrogen-fixing cell by the loss of phenotypic plasticity contributed to high temperature adaptation in the White Creek population of the cyanobacterium *Fischerella thermalis*. Credit: Image courtesy of Scott Miller, University of Montana

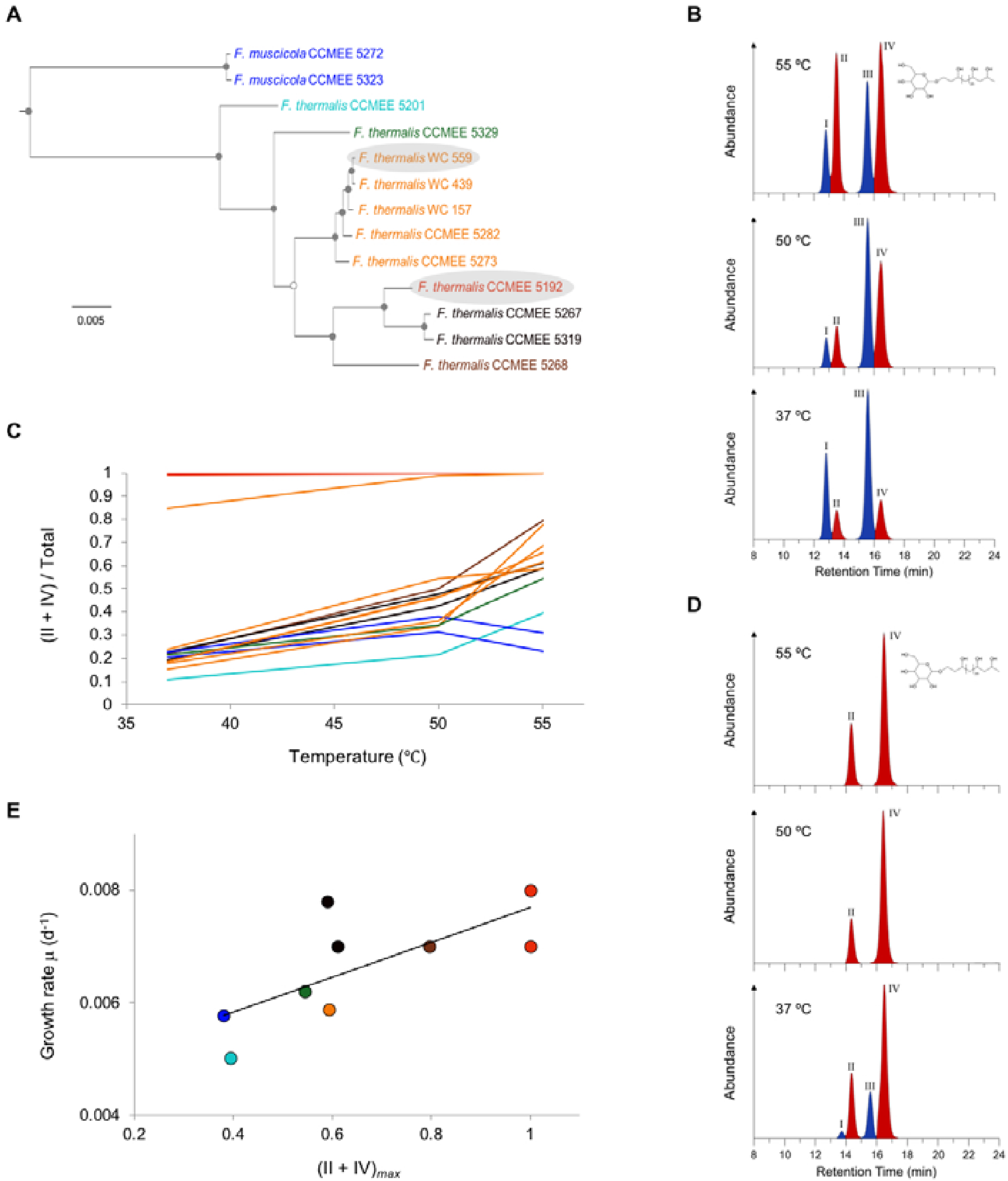


Figure 14. Plasticity of heterocyst glycolipid composition has been lost multiple times during *Fischerella* evolution. (A) Maximum likelihood phylogeny of *Fischerella* strains reconstructed from a concatenated alignment of 2,877 protein-coding genes according to a mixture model of nucleotide substitution with gamma rate heterogeneity. Strains of *F. muscicola* and the basal *F. thermalis* strain CCMEE 5201 are less thermotolerant than other representatives of *F. thermalis*, indicating that more thermotolerant *Fischerella* evolved from less thermotolerant ancestors. Shaded strains have convergently lost plasticity for heterocyst glycolipid composition (see 1C). Bootstrap values of 100% (closed circles) and > 95% (open circle) are indicated. The scale bar is in units of nucleotide substitutions per site. (B) Representative HPLC chromatograms showing the relative distribution of structural isomers of 1-(O-hexose)-3,29,31-dotriacontanetriol (HG_{32}triol; inset) at three temperatures for plastic *F. thermalis* strain WC 439. Semi-quantitative abundances are provided in Table S1. (C) The temperature dependence of the synthesis of HG_{32} triol isomers II and IV varies among *Fischerella* strains, with low-plasticity strains exhibiting constitutively high relative abundances. Color coding as in (A). (D) HPLC chromatograms showing the reduction in the temperature dependence of HG_{32} triol composition for *F. thermalis* strain WC 559. (E) *F. thermalis* strain growth rate at 50 °C versus maximum production of HG_{32} triol isomers II and IV. Color coding as in (A).

Error rate and the origin and early evolution of life: Exploring the evolutionary relationship between mutation, recombination and cooperation
(Paul Sniegowski, University of Pennsylvania;
Phil Gerrish, Georgia Institute of Technology)

NAI-supported Ben Galeota-Sprung defended his Ph.D. at Penn in December 2019. He is the third NAI-supported Ph.D. recipient from the Sniegowski lab. The first paper from Ben's thesis (Galeota-Sprung *et al.* Heredity 124(1):50-61) quantifies and partitions the contributions of lethal and non-lethal deleterious mutations to the selective cost of a mutator allele in *S. cerevisiae*. This work used a combination of experimental evolution and micromanipulation of individual yeast cells to show that the selective cost of the mutator allele is completely explained by mutational load (Figures 15 and 16). The paper was highlighted as one of the Editor's Choice papers in Heredity in 2019 (https://www.nature.com/collections/ijadaeeeaf). Further work by Galeota-Sprung (in preparation), used intensive time-series quantification of the distribution of fitness in experimental yeast populations to characterize the "relaxation" of populations toward mutation-selection balance (Figure 17).

A theoretical collaboration between Galeota-Sprung, Warren Ewens and Sniegowski investigated the effects of mutation rates and selection coefficients of deleterious mutations on the predicted functional fraction of the human genome (Galeota-Sprung *et al. Genome Biol Evol.* 2020. 12(4):273-281). This paper uses the statistics of extreme values to argue that mutational load should be calculated by comparison to the fittest individual likely to exist in the population rather than to a theoretical mutation-free individual who will never exist. The analysis indicates that the human genome—and the genomes of other creatures with very high deleterious mutation rates—can harbor a higher fraction of functional sites than had been supposed from previous load-based analyses.

Collaborative work with Philip Gerrish addressed the fundamental question of what forces favor evolution and maintenance of genetic recombination. We show that natural selection tends to favor mismatched combinations of fitness affecting alleles—i.e., combinations in which a high-fitness allele is paired with a low-fitness allele—more frequently than expected by chance. Such negative linkage disequilibrium is the broadly acknowledged population genetic precondition for recombination be favored, yet general circumstances under which it will arise have been difficult to identify. Analytical and simulation work and work with experimental *E. coli* populations in our group suggests that negative linkage disequilibrium can be a logical consequence of natural selection itself. The gist of the theory is illustrated by analogy to a "canoe race" in which predicted winning random combinations of paddlers are negatively correlated in their paddling ability, despite the intuitive prediction that combinations of two strong paddlers should win. This paper will be submitted in Spring 2020.

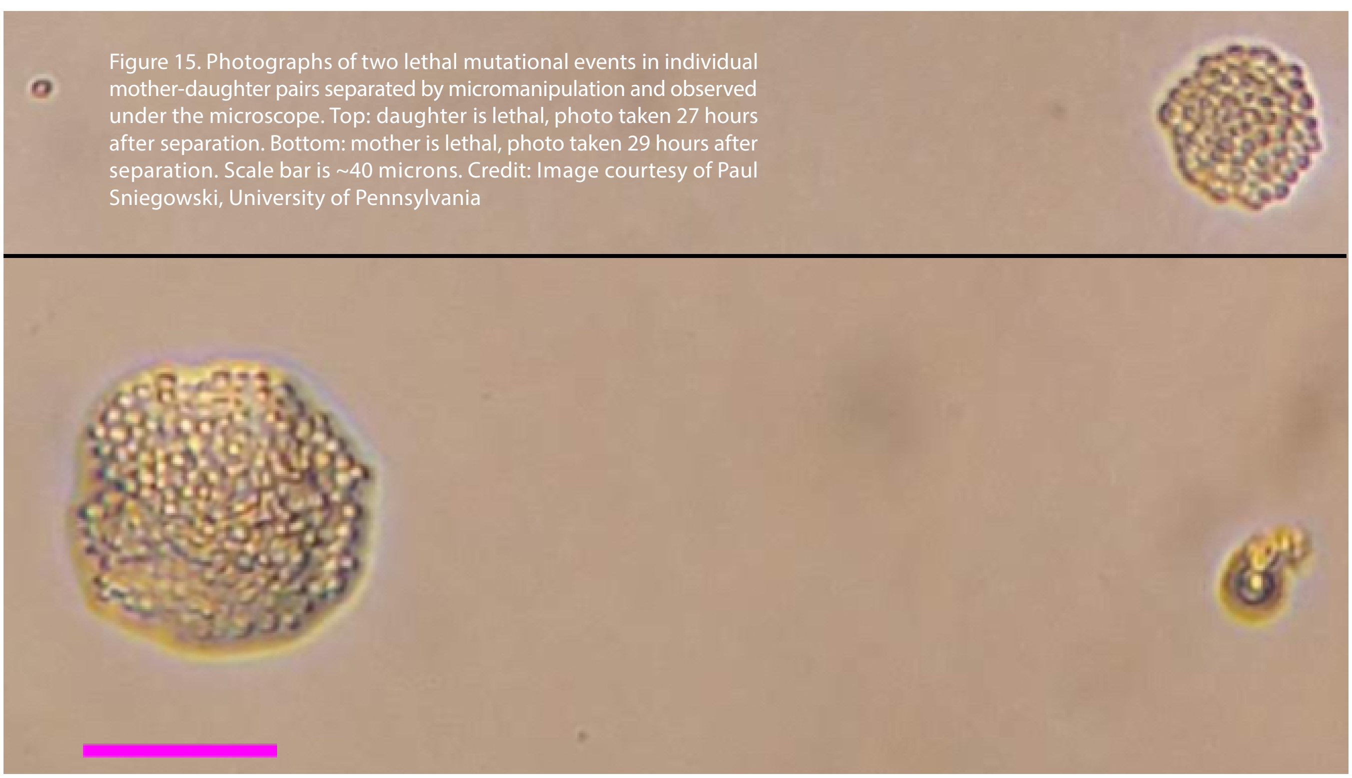


Figure 15. Photographs of two lethal mutational events in individual mother-daughter pairs separated by micromanipulation and observed under the microscope. Top: daughter is lethal, photo taken 27 hours after separation. Bottom: mother is lethal, photo taken 29 hours after separation. Scale bar is ~40 microns. Credit: Image courtesy of Paul Sniegowski, University of Pennsylvania

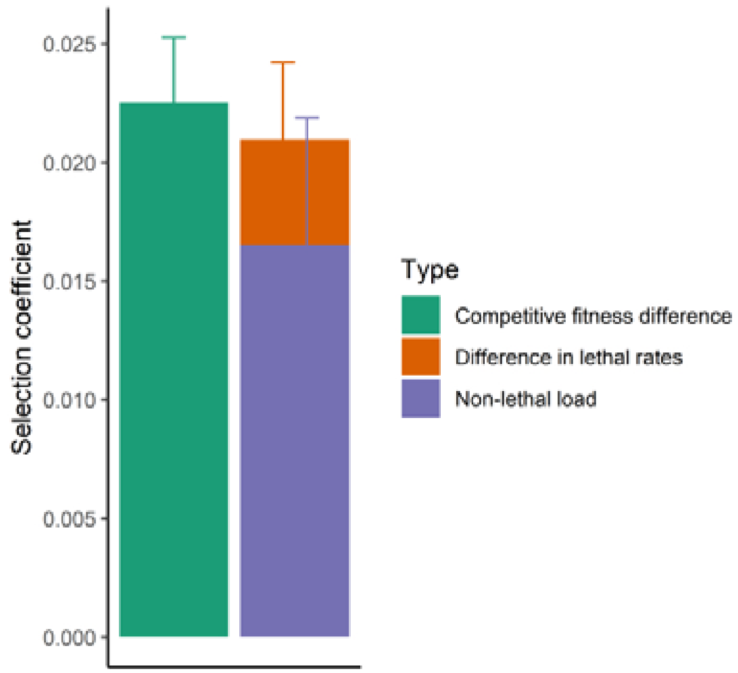


Figure 16. The sum of the nonlethal (estimated by methods illustrated in Figure 3) and lethal loads (estimated by method illustrated in Figure 1) is ~93% of the measured competitive fitness deficit of the haploid *mmr* strain relative to the wild-type strain. The difference between the two is not significant ($p > 0.2$). The difference between the competitive fitness difference and the nonlethal load alone is significant ($p < 0.02$). Credit: Image courtesy of Paul Sniegowski, Univ. of Pennsylvania

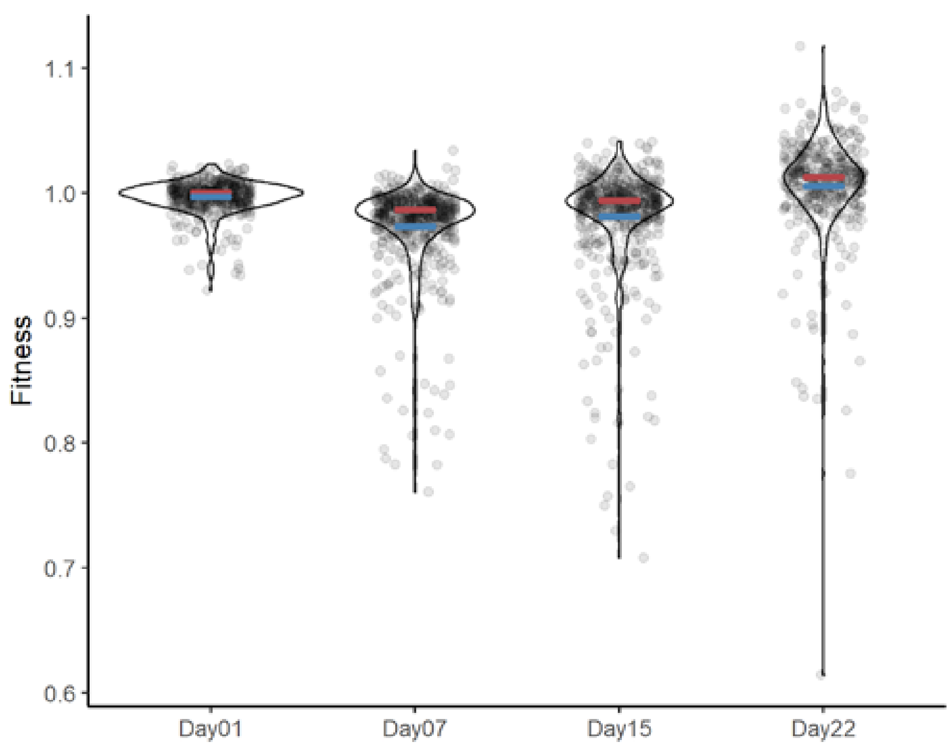


Figure 17. Time series of fitnesses in an evolving experimental population of yeast, showing the evolution of mutational load (visible as the bottom tail of fitnesses). Mean fitness is blue and mode fitness is in red. The mutational load realized at Day 7 is the difference between the mean on Day 7 and the mode on Day 1 (the mode on Day 1 being taken as the ancestral fitness). Credit: Image courtesy of Paul Sniegowski, University of Pennsylvania

NASA Postdoctoral Program Project Reports

Dr. Peter Conlin

NAI Mentor: Prof. Will Ratcliff

Project Title: Experimentally investigating the origin and consequences of fitness decoupling during the transition to multicellularity

The evolution of multicellularity is one of a small number of major transitions in evolution, events in the history of life on Earth in which previously independently replicating organisms combined to become parts of a new kind of individual. Simple multicellular groups evolve rapidly under the right selective conditions. However, simple multicellularity is typically costly in the absence of external drivers—suggesting that evolutionary transitions to multicellularity should be susceptible to reversion when environmental conditions once again favor unicellular growth. I study how nascent multicellular groups can become stabilized against reversion to unicellularity, enabling subsequent increases in complexity. My work explores this question using two approaches: experimental evolution with multicellular "snowflake" yeast and digital evolution with the Avida artificial life software platform. Preliminary data obtained by reverting multicellular 'snowflake' yeast back to unicellularity (by functional complementation with *ACE2*) suggested that the longer snowflake yeast evolve as clusters, the lower their single-celled fitness after reversion. By examining strains isolated from a long-term evolution experiment from 200, 400, and 600 transfers, we found that some strains could no longer revert to unicellularity (Figure 19a), suggesting that both the availability of potential reversion mutations and the fitness benefits of reversion are decreasing through time. We found a similar trend in the ability of digital multicellular organism to revert back to unicellularity after they evolved as multicells for prolonged periods of time (Figure 19b). I have also worked to engage with the scientific community here in Atlanta, GA. Last year, I co-organized the Evolution of Complex Life Conference, hosted at the Georgia Tech Research Institute on May 15-17, 2019. Our conference attracted the participation of 149 attendees, including 16 plenary speakers, 38 poster presenters, and 18 contributed speakers, from 28 universities. We raised a total of $27,920 to host this event.

Figure 18. Peter, happy to be hiking. Maple Pass Loop, North Cascades National Park, WA.

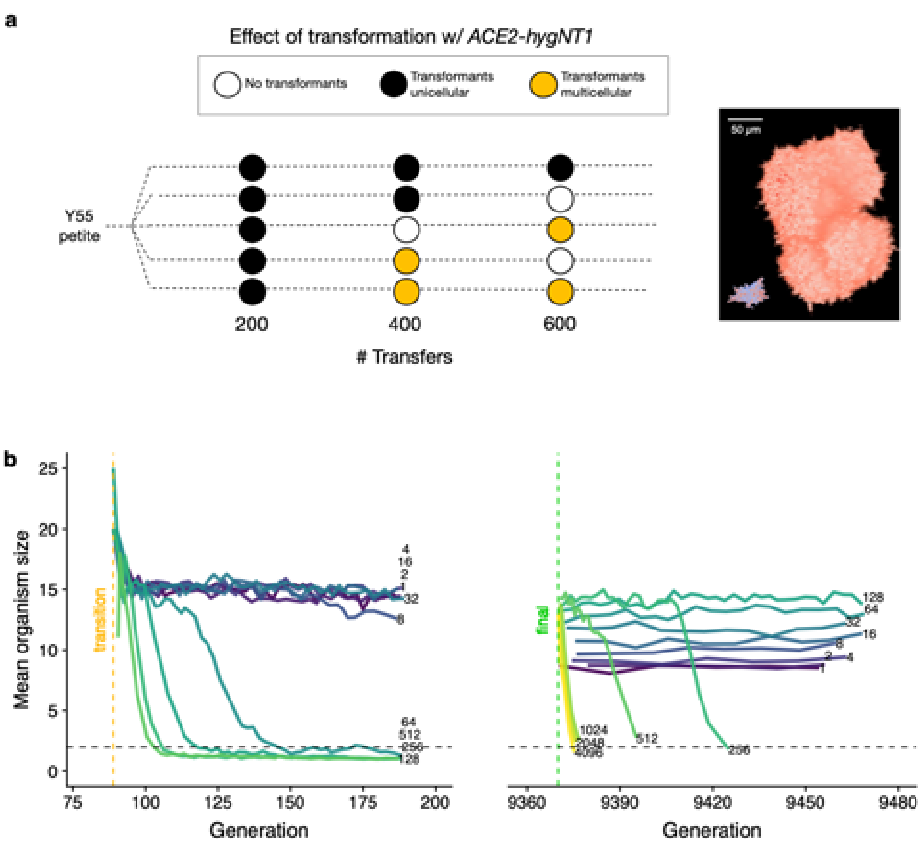


Figure 19. Changes in the probability of reversion to unicellularity. a) Snowflake yeast that evolve macroscopic size during a 600-transfer evolution experiment lose the ability to revert to unicellularity; functional complementation with the ancestral *ACE2* gene restores unicellularity in all 5 replicate lines at 200 transfers but not at transfers 400 or 600. b) Digital multicellular organisms revert to unicellularity more readily at the time of transition (when multicellularity first evolves) than at the final time point of the evolution experiment (~9000 generations later). We tested this by experimental manipulating the cost of multicellular reproduction (higher costs indicated in green and yellow). Credit: Images courtesy of Peter Conlin, Georgia Institute of Technology

Dr. Amanda Garcia

NAI Mentor: Prof. Betul Kacar,
University of Arizona
Project Title: The deep history of nitrogenases: Connecting the geochemical record of nitrogen fixation to isotopic signatures


Figure 20. Dr. Amanda Garcia

Nitrogen is an essential element for life. Throughout much of Earth's history, nitrogen has primarily been made bioavailable by the activity of nitrogenases, an early-evolved family of metalloenzymes that reduce atmospheric nitrogen to ammonia. Previous understanding of nitrogenase evolution has been limited to either phylogenetic inferences or analyses of preserved geochemical signatures, without a means to experimentally integrate both datasets. With the support of the NPP, I proposed to experimentally connect the phenotypic attributes of laboratory resurrected ancestral nitrogenases with isotopic biosignatures observed in the geochemical record. My proposal comprises four aims:

AIM 1 *Build nitrogenase phylogeny and infer ancestral protein sequences*
AIM 2 *Transform ancestral nitrogenase genes into modern bacteria*
AIM 3 *Biochemically characterize purified ancestral nitrogenases*
AIM 4 *Determine isotopic fractionation of ancestral nitrogenases*

In the past year, I have completed AIM 1 and made progress toward the completion of AIM 2. My co-authors and I have reconstructed a nitrogenase phylogeny (Fig. 21) and inferred ancestral nitrogenase protein sequences. In addition, we have analyzed both sequence features (Fig. 22) and modeled structures to infer the metal-binding properties of ancestral nitrogenases. This work has resulted in several conference abstracts as well as a manuscript published in *Geobiology*. For AIM 2, I have engineered modern bacteria, *Azotobacter vinelandii*, with recent ancestral nitrogenase genes, which preliminary data suggest are functional. I plan to complete AIMS 2 and 3 in the first six months of Year 2 of my NPP appointment, with the last six months of Year 2 dedicated to completing AIM 4. We have established a collaboration with the Seefeldt Lab (Utah State University) to purify and biochemically characterize ancestral nitrogenases *in vitro* for AIM 3. I expect the findings of AIMS 2, 3, and 4 to each result in at least one manuscript publication.

Publications

Kacar B, Garcia AK, Cavanaugh C, Why have carbon biosignatures been consistent over billions of years of biological upheaval?, *PLOS Biology*, in prep.

Garcia AK, McShea H, Kolaczkowski B, Kaçar B, Reconstructing the evolutionary history of nitrogenases: Evidence for ancestral molybdenum-cofactor utilization, *Geobiology*, https://doi.org/10.1111/gbi.12381 (2020).

Garcia AK, Kaçar B. How to resurrect ancestral proteins as proxies for ancient biogeochemistry. *Free Radical Biology and Medicine*, https://doi.org/10.1016/j.freeradbiomed.2019.03.033 (2019).

Selected oral presentations:

Garcia AK, McShea H, Kolaczkowski B, Kacar B. Reconstructing ancestral nitrogenase metalloenzymes to explore the coevolution of nitrogen fixation and marine geochemistry. *Metals in Biology Gordon Research Conference* 2020 (2020).

Garcia AK, McShea H, Kolaczkowski B, Kacar B. Reconstruction of ancestral nitrogenases: phylogenetic and structural implications for metal binding. Astrobiology Science Conference, Bellevue, WA (2019).

OoLALA Research Showcase, UWisconsin, Madison, WI (2019), invited presentation

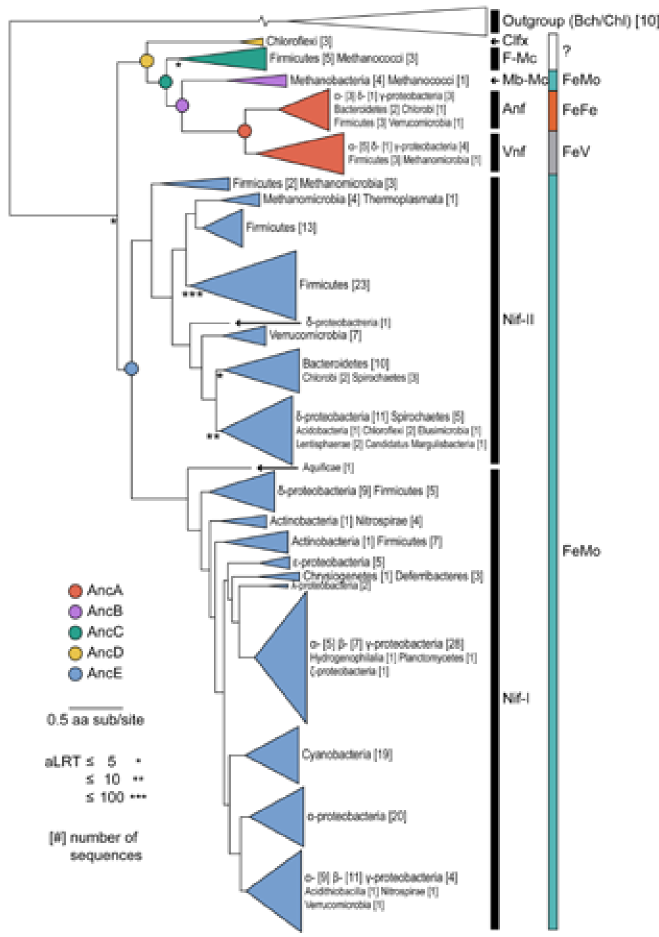
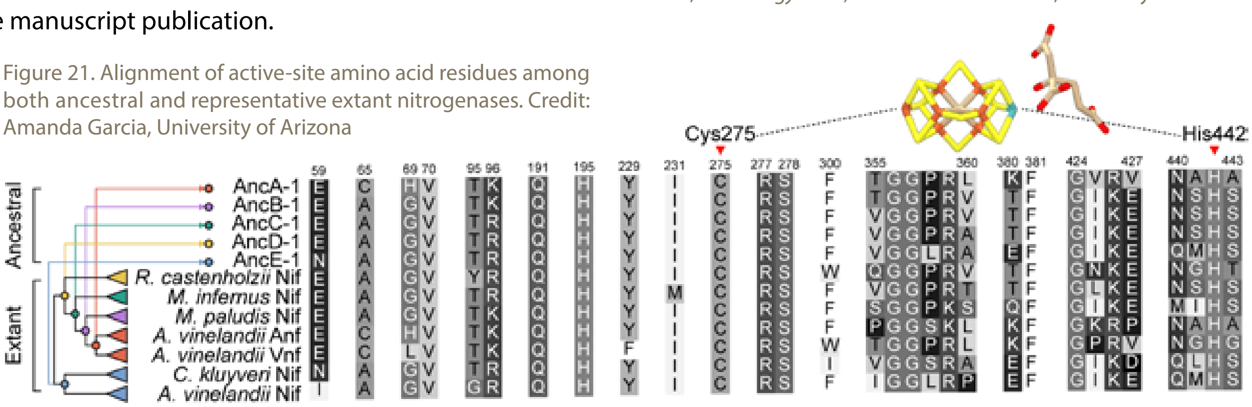


Figure 22. Maximum-likelihood phylogeny reconstructed from modern nitrogenase protein sequences. Nodes AncA-E selected for experimental ancestral sequence resurrection are highlighted (from Garcia *et al.*, *Geobiology* 2020). Credit: Amanda Garcia, University of Arizona

Figure 21. Alignment of active-site amino acid residues among both ancestral and representative extant nitrogenases. Credit: Amanda Garcia, University of Arizona



Dr. Caroline Turner
NAI Mentor: Prof. Vaughn Cooper, University of Pittsburgh,
Current appointment: Assistant Professor of Biology, Loyola University of Chicago
Project Title: Diversification and evolution of synergy in microbial biofilms

1. Published the article "Negative frequency-dependent selection maintains coexisting genotypes during fluctuating selection" in *Molecular Ecology* 29(1):138-148, https://doi.org/10.1111/mec.15307. We evolved *Burkholderia cenocepacia* under fluctuating selection for planktonic or biofilm growth and under constant selection for either planktonic or biofilm growth. We found that under fluctuating selection, two clades of bacteria arose and coexisted, with each increasing in frequency in one of the environments. We determined that this coexistence was due to negative frequency-dependent selection between the two clades. Negative frequency-dependence occurred in both environments, with the equilibrium frequency shifting in each environment. This work indicates that negative frequency dependence is a potential mechanism for the maintenance of diversity in fluctuating environments.

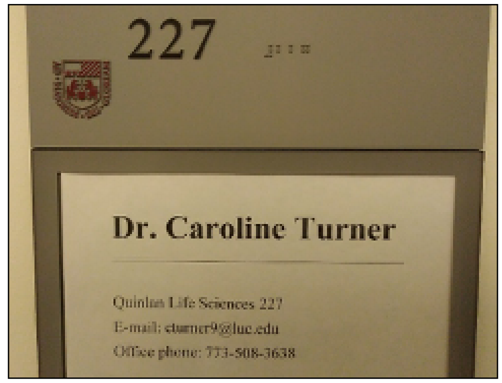


Figure 24. Nameplate on my faculty office at Loyola University Chicago. Credit: Caroline Turner, Loyola University-Chicago

2. Presented my research titled "Temporal dynamics in genetic parallelism across evolving populations" at the 2019 Microbial Population Biology Gordon Conference. This work is an ongoing collaboration with Eric Libby, Assistant Professor at Umea University and member of NASA's Laboratory for Agnostic Biosignatures.

3. I was hired as a new tenure-track faculty member in the Biology Department at Loyola University Chicago, beginning January 2020.

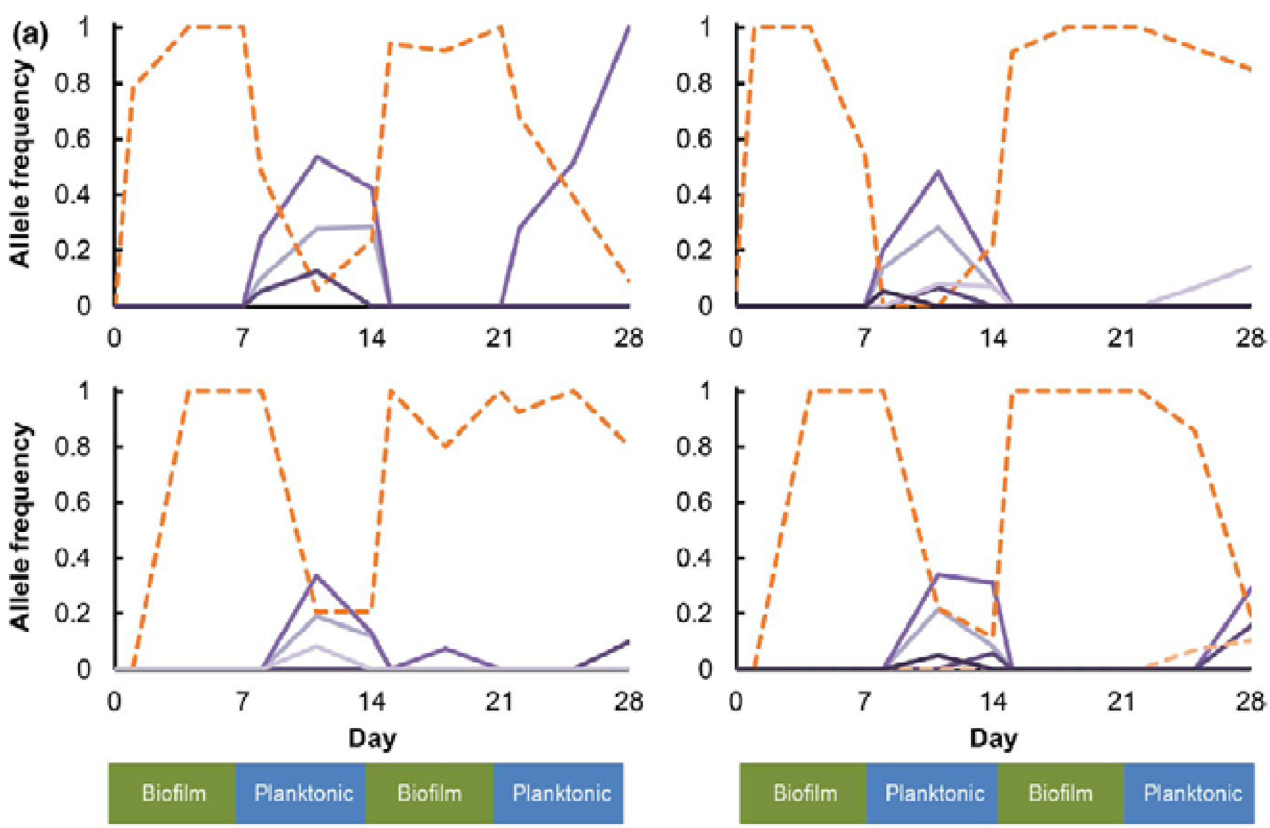


Figure 25. Evolutionary dynamics reveal the coexistence of wspA (dashed orange lines) and rpfR (solid purple lines) mutant genotypes in environments fluctuating between planktonic and biofilm selection (Turner *et al*. 2020. *Molec Ecol* 29(1):138-148). Credit: Caroline Turner, Loyola University-Chicago

Field Sites

Pilot Valley Expedition 6 - Investigating the Metabolic and Environmental Flexibility of Biological Perchlorate Reduction in a Mars analog environment.

Location: Pilot Valley Basin, Great Salt Lake Desert, Utah

The summer 2019 field study was a key part of an ongoing investigation into the relationship between naturally occurring perchlorate (NOP) and perchlorate reducing microorganisms (PRM) in the Pilot Valley Basin (Figures 18 and 19). This investigation addresses three questions: 1) What is the phylogenetic diversity of microorganisms capable of perchlorate reduction in Pilot Valley sediments; 2) what perchlorate reduction pathways exist in this environment; 3) if there is biotic perchlorate reduction ongoing in PV sediments, what ancillary metabolisms drive this process. Our overarching goal is to synthesize these data to generate a model for perchlorate-supported ecosystems on Mars. Our project is focused at the Pilot Valley Mars analog field site that Kennda Lynch established during her dissertation work. The principal summer 2019 scientific activities were: A) to perform an *in situ* gene expression experiment targeting PRM, and B) to collect sediment samples to return to the lab for PRM enrichment, isolation and characterization studies.

The personnel present during the field expedition where:

Team Leader, Kennda Lynch, Ford Foundation postdoctoral fellow, Georgia Institute Technology, Current position: Staff Scientist, Lunar Planetary Institute, Houston, TX

Professor Kevin Rey, Brigham Young University

Ms. Camille Goodale, The Evergreen State University

Ms. Cleo Abram & Film Crew, Vox Media

Please Note: This field expedition was filmed for the premiere episode of "Glad You Asked" URL of Season 1 Episode: https://youtu.be/x8fpeVICeGg

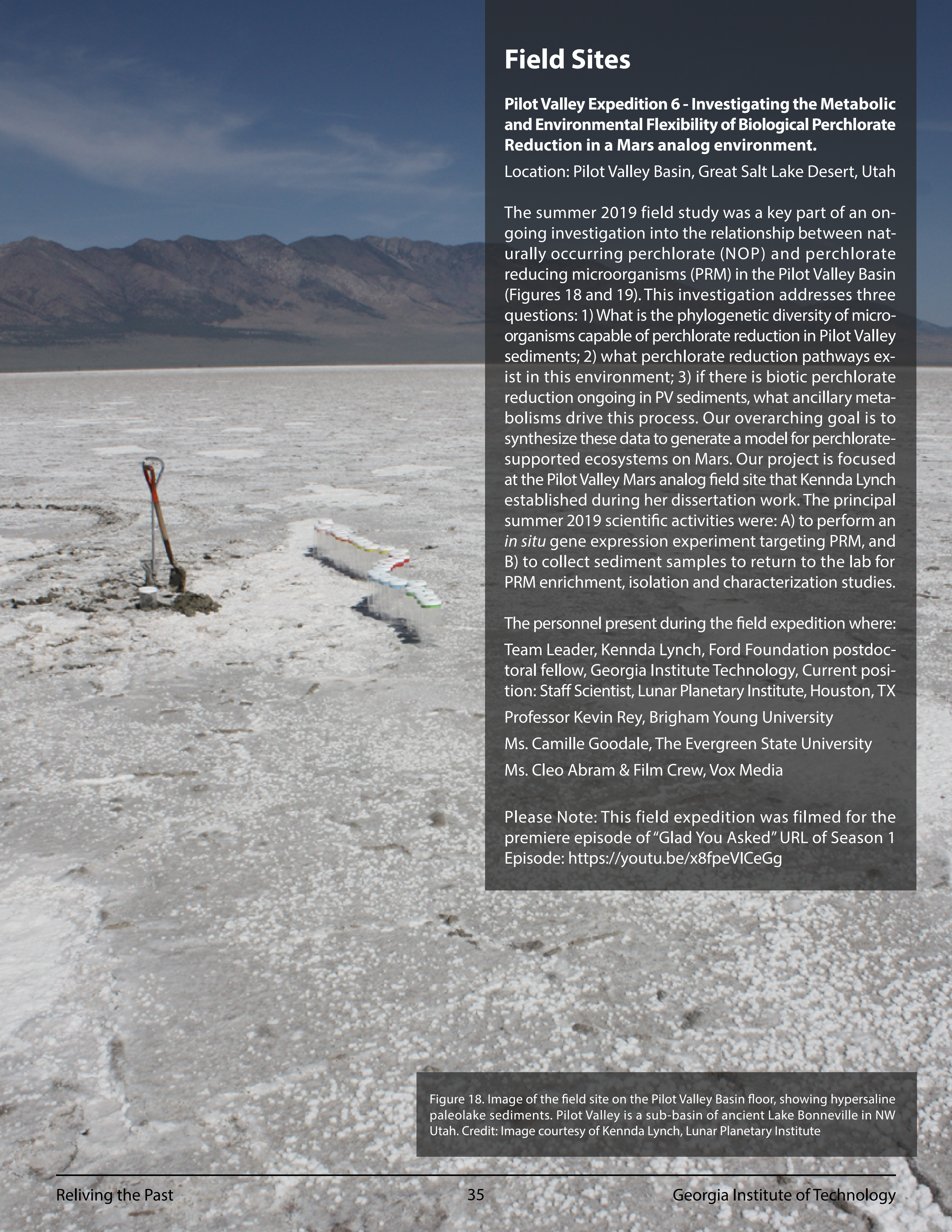


Figure 18. Image of the field site on the Pilot Valley Basin floor, showing hypersaline paleolake sediments. Pilot Valley is a sub-basin of ancient Lake Bonneville in NW Utah. Credit: Image courtesy of Kennda Lynch, Lunar Planetary Institute

Figure 19. *In situ* expression study in place on the Pilot Valley basin floor. Credit: Image courtesy of Kennda Lynch, Lunar Planetary Institute

Reliving the Past: 2019 Publications

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Garcia AK, Kaçar B. How to resurrect ancestral proteins as proxies for ancient biogeochemistry. *Free Radical Biology and Medicine,* https://doi.org/10.1016/j.freeradbiomed.2019.03.033 (2019).

Garcia, A., McShea, H., Kolaczskowski, B. and Kacar, B. (2020). Reconstructed the evolutionary history of nitrogenases: Evidence for ancestral molybdenum-cofactor utilization. *Geobiology*, Accepted DOI: 10.1101/714469*

Gloag E.S., Marshall, C.W., Snyder, D., Lewin, G.R., Harris, J.S., Santos-Lopez, A., Chaney, S.B., Whiteley, M., Cooper, V.S. and Wozniak, D.J. (2019). Pseudomonas aeruginosa interstrain dynamics and selection of hyperbiofilm mutants during a chronic infection. *mBio* 10:e01698-19. DOI: 10.1128/mBio.01698-19

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* NPP Fellow

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Dimitra Aggeli
Diane Brooks
Deanna Bublitz
Matt Campbell
Kimberly Chen
Alia Clark-Elsayed
Peter Conlin
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Jake Flood
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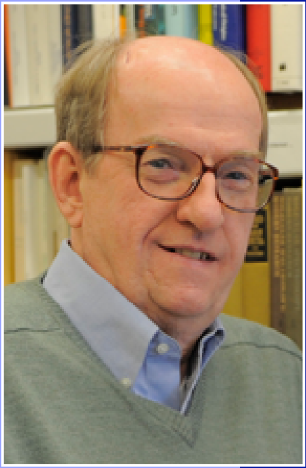
Katrina Harris
Matthew Herron
Betül Kacar
Margie Kinnersley
John Carlo Kristofich
Yuping Li
Ross Lindsey
Kennda Lynch
Chris Marshall
John McCutcheon
Scott Miller
Andrew Morgenthaler
Jennifer Pentz
William Ratcliff
Katja Schwartz
Cathryn Sephus
Gavin Sherlock
Emily Sileo
Tanya Singh
Eric Smith
Paul Sniegowski
Sophia Sukkestad
Caroline Turner
Nikea Ulrich

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

2019 Executive Summary and Historical Perspective

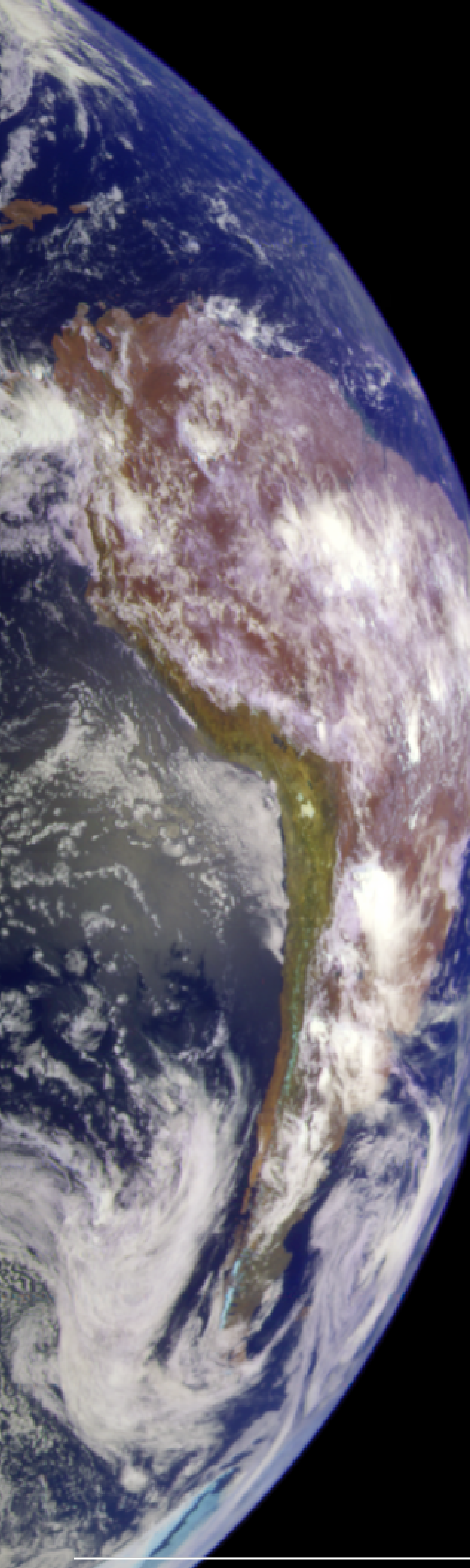
Team members pursued a vigorous and highly productive research program in all five topical areas, conducting many investigations in the laboratory and in the field (mainly astronomical).

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 in eight comets and expanded to 40 comets our taxonomy based on composition. In-depth analysis of 30 comets revealed evidence of a new class of material in the cometary nucleus with astrobiological significance (Mumma *et al.* 2019). Compared with 'normally active' comets in this group, disrupting comets within 1 au of the Sun were found to display enriched HCN and NH_3 relative to ethane, owing to the disintegration of ammoniated salts. Their delivery to barren planets and subsequent solvation by co-delivered water has profound implications for life's origins.

Three unusual cometary events occurred in 2018-19, two ecliptic comets from the Kuiper Belt underwent rare near-Earth approaches and a bright comet from the Oort cloud was discovered. 21P/ Giacobini-Zinner is the archetype for comets that show low ratios in C_2/CN at optical wavelengths, thought to be related to depletion of carbon-chain volatiles in the nucleus. Faggi *et al*. (2019) confirmed the depletion of C_2H_2 relative to HCN in 21P, consistent with this view. 46P/Wirtanen is a hyperactive comet; results for it are nearing publication. The appearance of Oort Cloud comet C/2016 R2 (PanSTARRS) enabled exploration of its unique volatile composition at 2.8 au from the Sun–dominated by hypervolatiles CO, CO_2, and N_2 with methane and water as trace species (McKay *et al.* 2019). This is the first comet of its type.

Goddard's virtual Center for Astrobiology (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 (Cordiner *et al.* 2019). This technique was also applied to the first active interstellar comet (2I/Borisov) in late 2019, demonstrating an extreme enrichment of CO in this comet relative to HCN (Cordiner *et al.* 2020).

Team scientists in the Astrobiology Analytical Laboratory (AAL) continued their ground-breaking investigations on the origin and evolution of meteoritic organic compounds, with eight papers published in 2019. Highlights include a review of chiral asymmetry as potential biosignatures in our Solar System (Glavin *et al.* 2019), and the distribution, ^{13}C-isotopic, and



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

enantiomeric compositions of monocarboxylic acids in carbonaceous chondrites (Aponte *et al.* 2019). AAL scientists have key roles in several Research Coordination Networks, including the Network For Life Detection (NFoLD) and Prebiotic Chemistry in Early Earth Environments (PCE3). Team scientists 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.

In the Cosmic Ice Laboratory, Team members 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. Seven papers were either accepted or published in 2019. Studies of the sublimation/dissociation properties of ammonium salts were re-opened to assist interpreting their role in comets.

Team members are exploring the application of novel nanopore-based detection and sequencing of informational polymers as one potential approach to life detection on future planetary missions. We investigated both the robustness of this technology to space and planetary environmental extremes (Burton *et al.* 2019, Sutton *et al*. 2019), and the optimal protocols for successful sequencing with complex samples *in situ*, starting with DNA for its accessibility and representativity for optimization.

And finally, a few notes of historical perspective. The Goddard Team was selected for membership in the NAI in 2004, and after successive selections concluded its membership in January 2020. As the 20-year existence of NASA's virtual Astrobiology Institute revolutionized the field of astrobiology, so likewise the 16-year membership of the Goddard Team enabled establishment and fruition of Goddard's virtual Center for Astrobiology (GCA). The virtual nature of both entities empowered real expansions of staff and resources far beyond the limits that organization as line management entities would have permitted. Virtuality permitted multiple line organizations to embrace the disciplines involved, and to draw upon the (independent) support offered by the Director of NAI and by the individual Team leaders (e.g., M. J. Mumma at the Goddard Center for Astrobiology), thereby avoiding concerns deriving from matrix authority and the competition for resources. At this writing, a new structure for NASA's Astrobiology Program (the Research Collaboration Networks) replaces the highly successful NAI experiment in distributed management. The challenge will be to support and enable the many institutional initiatives led by the NAI Central organization, absent the professional staff and funding resources that made them fruitful over the past 20 years. It is a recurring challenge, and new solutions will be attempted. The judgment of success or failure will be made 20 years hence. God-speed!

Team Members

Michael Mumma
Neyda Abreu
José Aponte
Geoffrey Blake
William Brinckerhoff
Steven Charnley
Martin Cordiner
Michael DiSanti
Jason Dworkin
Jamie Elsila
Sara Faggi
Frank Ferguson
Perry Gerakines
Daniel Glavin
Reggie Hudson
Natasha Johnson
Yi-Jehng Kuan
Manuela Lippi
Hannah McLain
Karen Meech
Stefanie Milam
Tom Millar
Joseph Nuth
Lucas Paganini
Eric Parker
Anthony Remijan
Mark Sutton
Geronimo Villanueva

Project Reports

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

Investigation of cometary composition is essential for the following open issues: (i) testing models of Solar System formation and evolution, (ii) assessing cometary delivery of organic compounds to the inner planets, and (iii) addressing the puzzling origin of water on Earth. The Goddard Team emphasizes the retrieval of cosmogonic indicators: isotopic ratios (isotopic enrichment is a process sensitive to the primordial conditions in which molecules formed), abundance ratios of nuclear spin isomers (which may preserve information on the molecular formation temperature), and molecular abundances (which are strongly influenced by chemical diversity and physical processes in the formative phase of the protoplanetary disk). The Team has pioneered (Mumma, Weissman, and Stern 1993) and led a taxonomic IR survey (Mumma *et al.* 2003, Bockelée-Morvan *et al.* 2004, DiSanti and Mumma, 2008) addressing these questions (Mumma and Charnley 2011), with nearly 30 comets now quantified in terms of these cosmogonic parameters – most were observed during the 16-year tenure the Team was part of NAI. By providing major increases in spectral resolving power, spectral grasp, and instrumental sensitivity, the emergence of a new class of high-resolution IR echelle spectrometers enabled successive advances during this period.

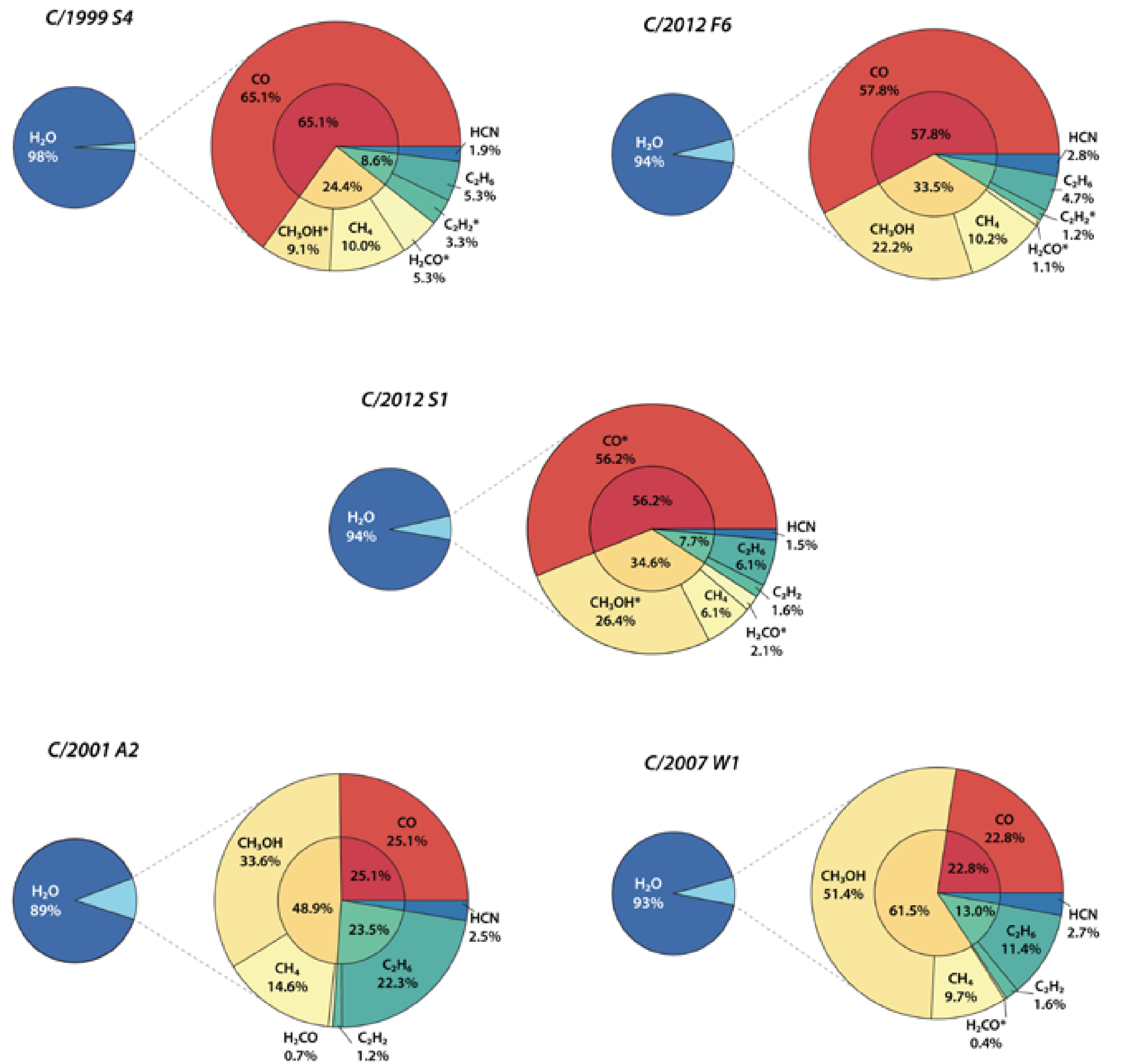


Figure 1. Double level pie charts based on updated mixing ratios in five comets, ordered with decreasing CO. For each sector the reported proportion (%) is obtained by normalizing the individual mixing ratio to the sum of all mixing ratios obtained for that particular comet. Starred labels represent 3σ upper limits. After Lippi *et al.* 2020.

With GCA collaborator Lippi and former URAA intern Camarca, co-I Villanueva and P. I. Mumma created an extensive database of high-resolution infrared spectra ($\lambda/\delta\lambda > 25{,}000$ or higher) of comets in a systematic and common fashion. So far, the archive contains data on more than 60 comets, observed since 1996. Despite the small number of observed comets, the amount of data for each target is large, making this the most rich and extensive database on comets realized until now. Some of these data have never been analyzed before, owing to the lack of specific molecular models at the time of data acquisition.

The Team's data analysis techniques and molecular models have undergone continuous changes and improvements in the 1996-2011 period, leading to unwanted systematic uncertainties in some earlier analyses. Using a robust and common set of analytical tools, we are now correcting the earlier results for unevenness related to data reductions with immature algorithms. For the initial phase of this project 20 comets were selected and re-analyzed. Lippi *et al*. (2020) report updated rotational temperatures, production rates, and mixing ratios (for H_2O, CH_4, C_2H_2, C_2H6, CO, H_2CO, CH_3OH, HCN, and NH_3) for five comets C/1999 S4 (LINEAR), C/2001 A2 (LINEAR), C/2007 W1 (Boattini), C/2012 F6 (Lemmon), and C/2012 (ISON). Changes related to the use of immature models are seen mainly in comets analyzed before 2010 (S4 and A2). Their updated compositions are compared in Fig. 1. The second paper (on 20 comets) is now being prepared for publication, and a third paper (on the full sample) is planned.

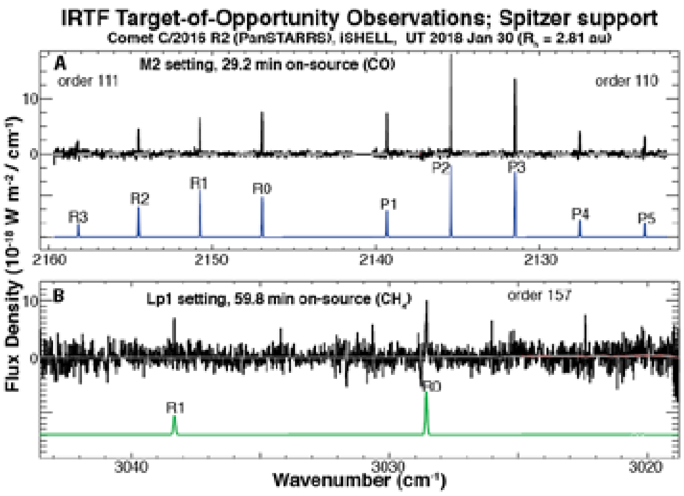
With GCA collaborators (Faggi, Lippi, Paganini) and former URAA intern Camarca), P.I. Mumma and co-I Villanueva participated in the World-wide campaigns for two comets of Kuiper Belt origin, conducting detailed studies of cosmogonic parameters (molecular abundances, isotopic ratios, and nuclear spin temperatures) encoded by biologically relevant primary volatiles. They acquired high-resolution infrared spectra of comets 21P/Giacobini-Zinner and 46P/Wirtanen and extracted chemical production rates for primary volatiles using the Goddard Team's modern quantum mechanical fluorescence models. 21P is the archetype of carbon-chain depleted comets, based on the low ratio of product species C_2 and CN seen in the coma, but their putative primary species (C_2H_2 and HCN) had not been tested in 21P until now. Faggi *et al.* (2019) extracted chemical production rates for detected water, organics and nitriles (H_2CO, CH_3OH, CH_4, C_2H_2, C_2H_6, HCN, NH_3) and verified for the first time that acetylene was depleted relative to hydrogen cyanide in this comet. Their associated campaign on 46P/Wirtanen returned a rich database that quantified the volatile fraction of this comet in detail; results were presented at several venues and are nearing submission for publication. The Team also observed other comets of Oort Cloud (C/2017 T2, C/2018 W2), Kuiper Belt (29P/SW-1, 252P/LINEAR) or interstellar origin (2I/Borisov) during this reporting period (e.g., for 252P, see Paganini *et al.* 2019).

GCA co-Investigators DiSanti and Villanueva participated in a multi-wavelength investigation of long-period (P ~ 20,000 yr) comet C/2016 R2 (Pan-STARRS), which revealed a highly unusual volatile composition. High-resolution IR (iSHELL) observations targeted primary volatiles H_2O, CO, OCS, CH_4, C_2H6, H_2CO, and CH_3OH. This resulted in secure detections of CO and CH_4 (Fig. 2, A and B), and a meaningful upper limit for C_2H_6. These observations were spurred by previously obtained optical images and spectra that revealed dominant emissions from CO^+ and N_2^+ but showed little evidence of emission from the product species normally measured in comets (e.g., CN, C_2, NH_2, and [O I]).

McKay *et al.* (2019) combined contemporaneous ground-based optical, IR, and millimeter/sub-millimeter observations together with warm Spitzer imaging, that altogether allowed quantifying the aforementioned primary volatiles plus CO_2, HCN, C_2H_2, NH_3, and N_2.

In C/2016 R2, CO was the dominant volatile, followed by CO_2 (having 18% the abundance of CO) then N_2 (5% of CO). CH_4 and H_2O were trace species in this comet, with abundance ratios of 0.59% and 0.32%, respectively relative to CO. This makes C/2016 R2 a true compositional outlier among comets (Fig. 2, C and D). The large abundances of the two most primitive molecular forms of carbon and nitrogen in the universe (CO and N_2, respectively) suggests that the region of the proto-solar disk where C/2016 R2 formed was chemically inactive and also was relatively shielded from photo-destruction. Both CO and N_2 are extremely volatile, and CH_4 and CO_2 only somewhat less so, suggesting that this comet likely formed in the farthest reaches of the early solar system. Comets of this type would deliver quite different ensembles of volatiles to barren planets.

Our study of ammoniated salts achieved new levels, with major exposure at several conferences this year. P.I. Mumma delivered an updated view of four volatiles in 30 comets, demonstrating that $NH_4^+CN^-$ (ammonium cyanide) was likely the principal origin of NH_3 and HCN in multiple comets near the Sun (Mumma *et al*. 2019). The Altwegg mass spectroscopy group presented four papers describing ROSINA/Rosetta measurements of multiple ammonium salts in comet 67P/Churyumov-Gerasimenko, including ammonium cyanide. Both teams estimate that ammonium salts represent a major fraction of refractory material in comet nuclei. Their delivery to barren planets and subsequent solvation by co-delivered water has profound implications for life's origins.



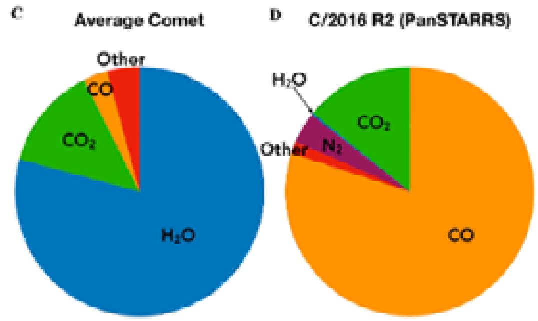


Figure 2. Detections of (A) CO and (B) CH_4 in C/2016 R2 (PanSTARRS), obtained with iSHELL, with spectral resolving power l/dl ≅ 40,000. Molecular fluorescence models (with line designations) are also shown. Panels C, D: Pie charts comparing the "average" composition measured among comets to that of C/2016 R2. This demonstrates the dominance of CO and N_2 in comet R2, unique among comets observed to date with modern capabilities. After McKay *et al*. 2019.

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Analysis of Prebiotic Organic Compounds in Astrobiologically Relevant Samples

We continued to investigate the origin and evolution of meteoritic organic compounds. We published a review article detailing the current state-of-the-art methods for analyzing soluble organic compounds in extraterrestrial samples (Fig. 3) as well as a review article detailing a set of measurement criteria that can be used to determine the origin of chiral asymmetry in future life detection missions (Glavin *et al.* 2019). We participated in research consortia analyzing recently fallen or recovered meteorites, with publications detailing results in two of these meteorites. We deepened our understanding of aldehydes and ketones in meteorites, with two papers detailing methods and findings. We published results from investigations into cyanide and its carriers in carbonaceous meteorites. We also continued a variety of collaborations, including investigations into the origin and quantitation of chiral excesses in meteorites, and we hosted two summer undergraduate students.

We supported two Research Coordination Networks: the Network for Life Detection (NFOLD) and the Prebiotic Chemistry in Early Earth Environments (PCE3). 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 new involvement in the organic analysis of samples to be returned from asteroid Ryugu by Hayabusa2. 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.

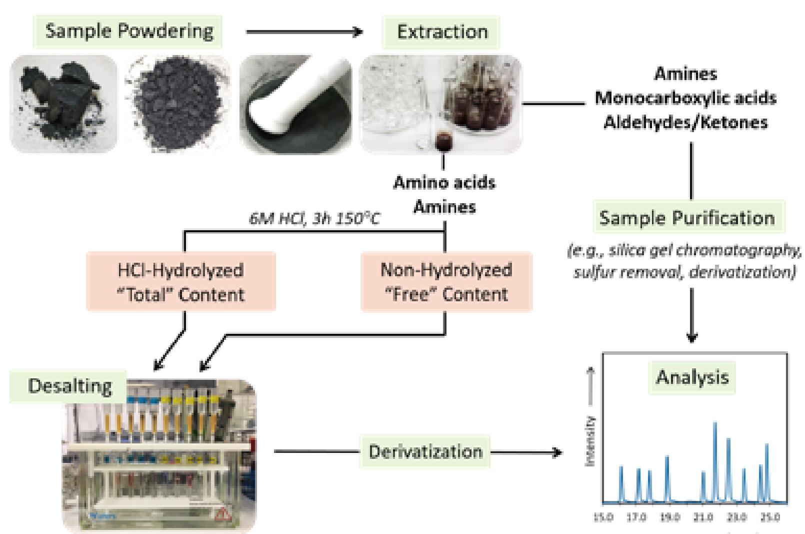

Figure 3. Our review paper detailed advanced methods for analyzing amino acid, amines, monocarboxylic acids, aldehydes, and ketones, as shown in this simplified schematic. See Simkus *et al.* 2019b.

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.

An important issue for Astrobiology is to understand how the organic chemistry in low-metallicity environments (i.e., with low abundances of elements heavier than hydrogen or helium), relevant for star formation at earlier epochs of cosmic evolution, differs from that in the Galaxy. A quantitative determination of the chemistry in high-redshift galaxies with different star formation histories, and lower abundances of the important biogenic elements (C, O, N, S, P), can shed light on the inventory of organic molecules available to their planetary systems.

The nearest laboratories for detailed studies of the formation and survival of complex organic molecules (COMs) under metal-poor conditions are the Large and Small Magellanic Clouds (LMC and SMC). Sewiło *et al.* (2019) presented a review of recent observations of a small and diverse sample of the LMC and SMC hot cores associated with COMs and made comparison to theoretical model predictions. Although a few hot cores–dense clumps of gas and dust containing young, massive protostars–were known in the LMC, unlike their Galactic counterparts, COMs had not been detected

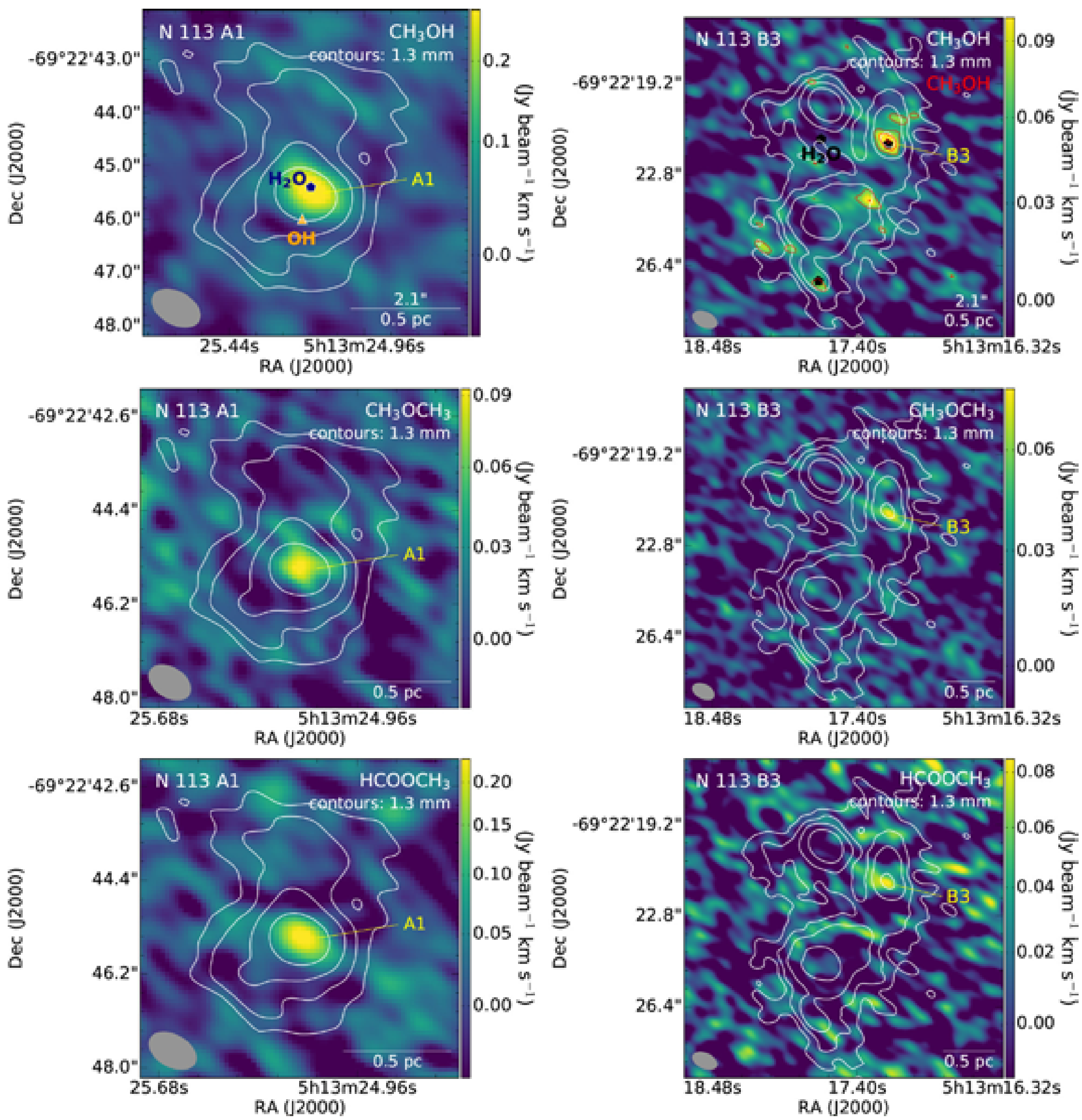

Figure 4. ALMA integrated intensity images of the A1 (left column) and B3 (right column) hot cores in the N113 star-forming region: CH_3OH (top panel) ; CH_3OCH_3 (middle panel) ; and $HCOOCH_3$ (bottom panel). Water and hydroxyl masers are indicated. The white contours correspond to the 1.3 mm continuum emission. The ALMA beam size is shown in the lower left corner of each image. From Sewiło *et al.* (2019).

in them until 2018. The Atacama Large Millimeter/submillimeter Array (ALMA) was used to detect methanol (CH_3OH), dimethyl ether (CH_3OCH_3) and methyl formate ($HCOOCH_3$) in two hot cores in the N113 star-forming region (Figure 4). Ongoing GCA work on complex chemistry in low-metallicity environments includes ALMA surveys to identify new hot cores in the LMC and SMC, deeper searches for more COMs in the N113 region, and development of new chemical models.

The detection of COMs in the Magellanic Clouds has important implications for Astrobiology. As the metallicity of the Magellanic Clouds is similar to that of galaxies at high redshift, these indicate that a similar prebiotic chemistry leading to the emergence of life, as it happened on Earth, is possible in low-metallicity systems in the earlier epochs of the Universe.

At a high level, GCA supports co-Investigator Prof. Geoffrey A. Blake (CalTech) to work with team members and via additional collaborations to examine the major speciation of the key volatile elements C, N and O along the pathway from the dense interstellar medium to protostars and protoplanetary disks to planetesimals and ultimately planets such as the Earth. In this reporting period, Prof. Blake participated in deep surveys of the water and key nitrogen tracer compounds in the inner regions of protoplanetary disks using the MICHELLE and TEXES mid-infrared spectrographs (Pontoppidan *et al.* 2019, Salyk *et al.* 2019). Beyond these individual tracers and disk studies, the papers lay out the near-term future with improved ground-based instruments, such as NIRSPEC-2 and CRIRES+, and especially the space borne capabilities provided by JWST. The latter in particular will enable the first robust detections of isotopically labeled water (and CO) in disks, and thus can provide exacting tests of the proposed self-shielding hypothesis for the generation of the widely observed mass independent oxygen isotopic signatures in early solar system materials.

With Blake, GCA associate and CalTech graduate student Ms. Olivia Wilkins is taking an isotopic approach to the now more complex organic chemistry that operates throughout the star- and planet-formation process. Her efforts are guided by the amazing results from the GCA's Astrobiology Analytical Lab (see Analysis of Prebiotic Organic Compounds in Astrobiologically Relevant Samples, in this report). Ms. Wilkins is using ALMA observations to examine the carbon, oxygen, and hydrogen isotopic signatures in key O-bearing (acetaldehyde, methanol, methyl formate) and N-bearing (cyanoacetylene, methyl and ethyl cyanide) organics that are likely feedstocks for alteration in parent bodies. Her first steps are in Giant Molecular Cloud complexes such as the Orion Nebula. These are extremely large and complex data sets, even by ALMA standards, and the key last pieces of main array and Atacama Compact Array (ACA) data were only recently acquired at the end of Cycle 6 and have just passed pipeline inspection. Thus, only recently has this project moved to a sufficiently advanced state to enable data analysis and interpretation (in total there are three ALMA cycles in the data sets, spread over four years). The data clearly reveal the presence of the sought isotopologues at high S/N.

Ms. Wilkins is also carrying out laboratory analysis of the isotopically substituted COMs she is studying with ALMA both to better characterize their pure rotational spectra and to determine whether high precision microwave and THz spectroscopy offers a non-destructive route to the site-specific isotopic signatures in astrobiologically relevant samples (in both meteorites and in possible samples returned by missions to the asteroid belt or to short period comets). Our hope is that this seed effort, supported by the NAI through GCA, will lead to method and instrument development support from other NASA R&A programs.

These studies of COMs are coupled with studies of complex organic molecules in comets at radio wavelengths led by Team collaborator Martin Cordiner and co-Investigator Stefanie Milam. A new NASA postdoctoral fellow, Nathan Roth, has started at GSFC (September 2019) working on the analysis of ALMA data obtained for comets – specifically he has begun the full analysis of ER61. We triggered two Target-of-Opportunity (TOO) proposals on the first active interstellar comet (2I/Borisov) (one at the end of ALMA cycle 6 and a second during perihelion in cycle 7). The analysis of these data is nearing completion and compelling results will be submitted imminently. The team also vigorously addresses all suitable sub-mm single dish facility proposal calls with either TOO proposals or targeted ones. These are supportive of our ALMA data and are also important stand-alone proposals – especially for targets that get too close to the sun for other observatories. During this reporting year, we published the first detection of isotopic HCN in a comet (C/2012 S1 ISON) using a novel autocorrelation approach to improve the sensitivity of ALMA for molecular line emissions (Cordiner *et al.* 2019). We now routinely use this approach for other targets, most recently for comet 2I/Borisov.

Investigations of Cosmic Ice Analogues and Processes

In the Cosmic Ice Laboratory, co-Investigators Gerakines and Hudson 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. Seven papers were either accepted or published in 2019.

Four papers focused on the physical and spectroscopic properties needed to quantify abundances of organic molecules in interstellar ices and laboratory ice analogs. One paper includes newly measured physical properties needed to determine abundances of thirty-one compounds spanning seven classes of organic molecules (Hudson *et al.* 2020). The three other papers were on similar measurements and predictions for cyclic organics (Figures 5, 6), an organic carbonate, and an organic ester. Hudson and Gerakines (2019a) described how IR observations achievable with JWST will be able to search for solid propynal (Figure 7). Hudson and Ferrante (2020) identified a 40-year-old 80% error in the IR band strengths of the acetaldehyde molecule, an amino acid precursor through the Strecker synthesis, and report the first IR spectrum of one crystalline form of the solid compound. Hudson and Gerakines (2019b) described and illustrated methods for identifying reaction products in astronomical ice analogs, using an N_2-rich ice as an example.

We also continued to educate and motivate the next generation of astrobiologists. Co-Investigator Gerakines again taught an on-line astrobiology course for about 100 students at the University of Alabama at Birmingham, and both he and co-Investigator Hudson gave seminar presentations to undergraduates. The work of our most-recent undergraduate astrobiology student, Ms. Ella Mullikin (Wellesley College) was published in this reporting year (Hudson and Mullikin 2019, see Figure 8). Also in 2019, Dr. Yukiko Yarnall joined our group through a NASA Postdoctoral Fellowship and is now working on salts and salt precursors relevant to cometary chemistry.

Dr. Chris Materese, who joined us as a new civil servant in 2018, led and completed our study of the survival to ionizing radiation of the nucleobase thymine this past year, and a manuscript on this work has been submitted for publication in Astrobiology; co-Is Hudson and Gerakines are co-authors.

In the Cosmic Dust Laboratory, co-Investigators Johnson and Nuth continued studies on 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. 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. We also found that refractory carbonaceous deposits can catalyze additional surface reactions. These types of investigations can be wide ranging and we have looked into the 'dusting' or sequestering of iron into the hydrocarbons and the possible formation of iron carbides using Fe-based substrates that appear to occur over a much wider temperature range than expected ($575K < T < 1200K$). 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. In this reporting period we primarily emphasized collaborations and distributions of dust analogs and reacted samples to colleagues (Nuth *et al*., 2019). For example, one of these collaborations is preparing a foundation for possible microgravity experiments to investigate eutectic condensation that may have implications for circumstellar outflows (Rietmeijer *et al*., 2019).

Cosmic Ice Lab members

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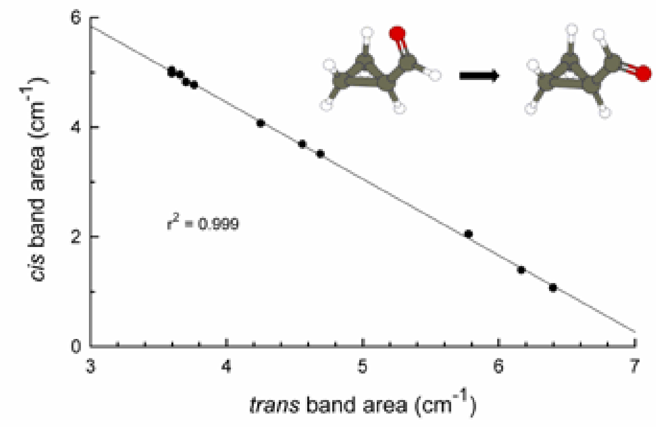

Figure 5. Conversion of cis-cyclopropanecarboxaldehyde into the *trans* isomer as an amorphous sample was warmed from 14 to 80 K. After Hudson and Coleman 2019a.

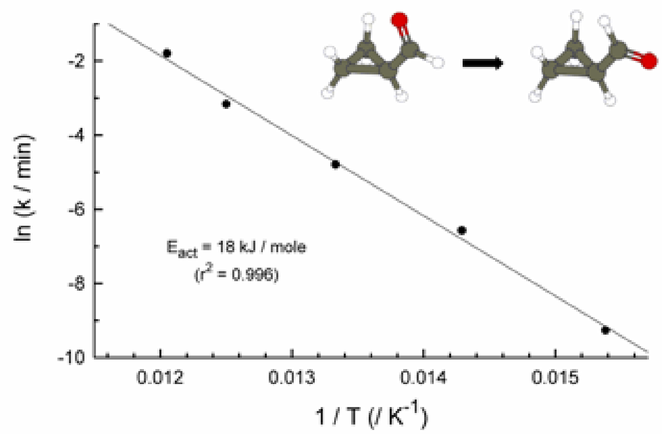

Figure 6. Rate constants and activation energy for the conversion of cis-cyclopropanecarboxaldehyde into the trans isomer. After Hudson and Coleman 2019b.

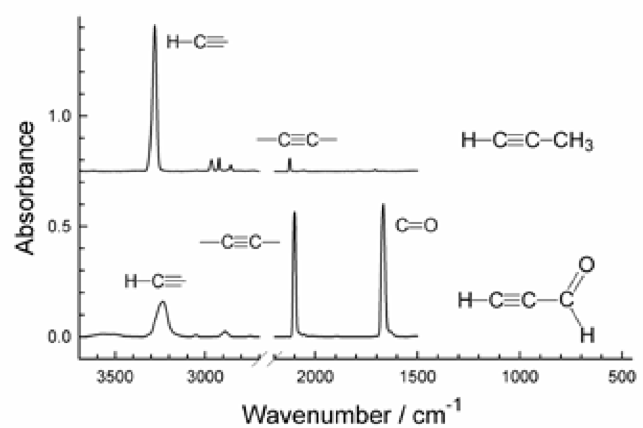

Figure 7. IR spectra of amorphous propyne (top) and amorphous propynal (bottom), each ice made, and its spectrum recorded, at 10 K. Note the dramatic difference in intensities of the C≅C peaks in a region covered by JWST. After Hudson and Gerakines 2019a.

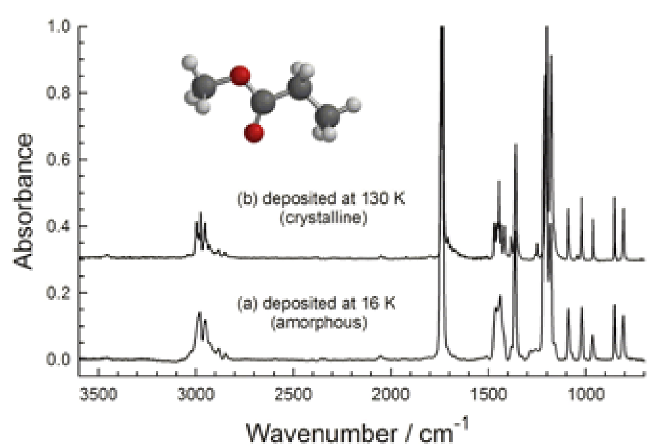

Figure 8. Mid-infrared survey spectra of methyl propionate, a candidate interstellar molecule. The ice thicknesses were about (a) 2.6 and (b) 1.2 µm. The upper spectrum has been offset vertically for clarity. After Hudson and Mullikin 2019.

Analytical Protocols in Sequencing Technology

We have continued to develop the application of novel nanopore-based detection and sequencing of informational polymers as one potential approach to life detection on future planetary missions. Such polymers, of which RNA and DNA represent individual types, may arguably provide a universal molecular modality that enables information storage, processing, and replication: by definition an agnostic biosignature not dependent on any particular, earth-centric collection of monomers. Recently GCA research has 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*, starting with DNA for its accessibility and representativity for optimization.

Following the work by former URAA intern and science associate Mark Sutton and team on radiation impacts on nanopore detection (Sutton *et al.* 2019; Burton *et al.* 2019), during this year URAA intern Margaret Weng and co-workers extended research to include impacts of salt concentrations as would be expected in samples on Mars, Europa, and Enceladus. Using recent models of the MinION™ device from Oxford Nanopore, Inc., Weng *et al.* tested sequencing metrics in analog planetary sample environments incorporating heavy salts such as epsomite and perchlorate at various concentrations. It was found that higher salinity was required for both of these salts, compared to the simpler KCl, before significant degradation in read counts or quality was detected. Furthermore, the impact of perchlorate in reducing sequencing effectiveness was greater (harsher) than that of epsomite, at all salinity levels (Figure 9). Notably, however, adequate sequencing was achieved even at relatively high salt concentrations of 3.5 wt% in both cases. These preliminary results were recently presented at the Fall Meeting of the American Geophysical Union (Weng *et al.* 2019). A model in which charge effects of dissolved salts can directly impede the translocation of DNA through the pores, at various levels, is under active continued research.

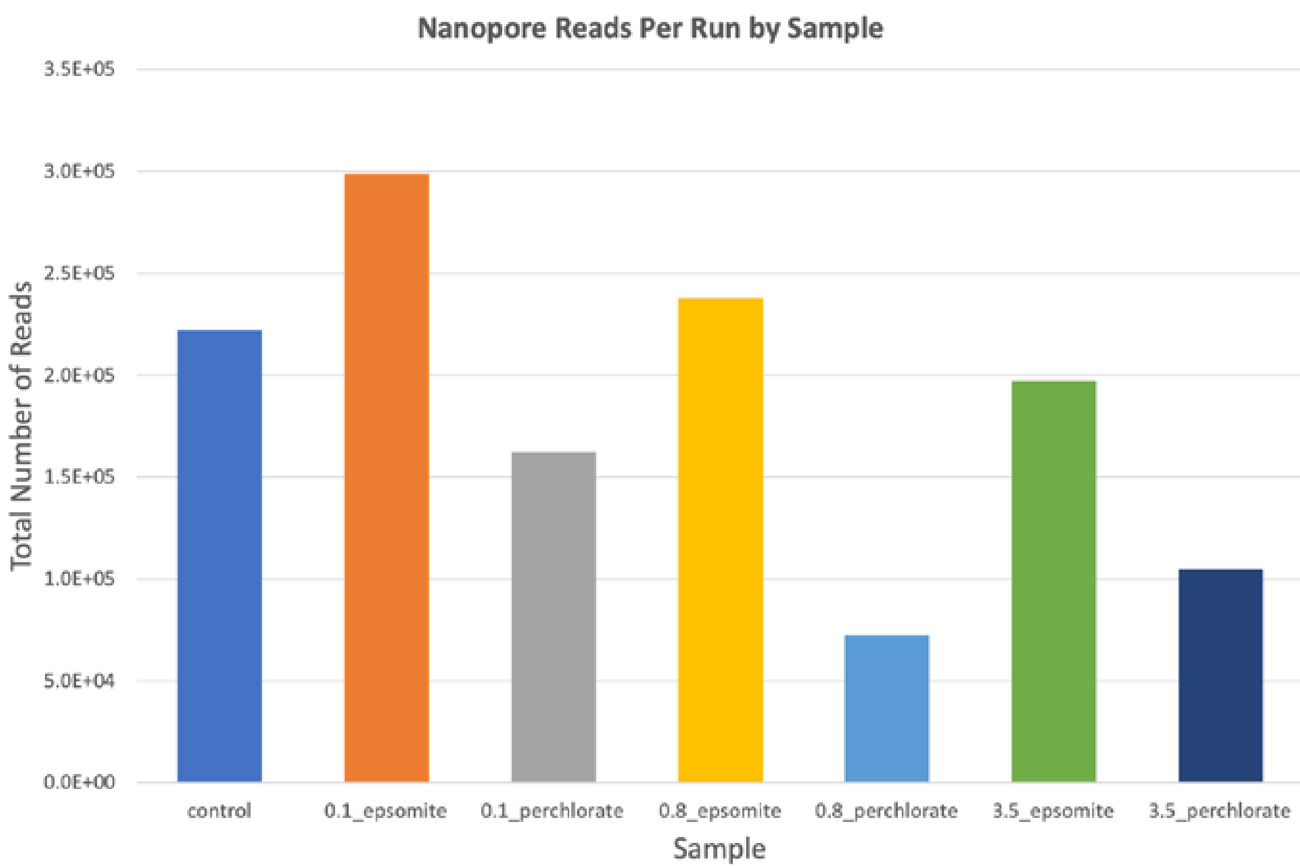

Figure 9. Absolute read count of Lambda DNA standard in nuclease-free water brine with "Europa analog" $MgSO_4 \cdot 7H_2O$ (epsomite) and "Mars analog" $Ca(ClO_4)_2 \cdot 5H_2O$ (calcium perchlorate) salts at weight percentages indicated by the axis labels. After Weng *et al.* 2019.

Collaborations

We collaborated with Professor Matthew Powner for the synthesis of enantiopure aldehyde standards that may be the chemical precursors of amino acids. The analysis of those synthesized aldehydes was key to understanding the limitations of a newly developed method for the analysis of meteoritic aldehydes, work that was published in ACS Earth and Space Chemistry.

We have an ongoing collaboration with Professor Ram Krishnamurthy (The Scripps Research Institute) pertaining to the investigation of polypeptide synthesis from exposure of amino acids to a plausible prebiotic phosphorylating agent. A manuscript detailing the findings of this study is in preparation for submission.

We continue to collaborate with Professor Donna Blackmond (The Scripps Research Institute), focusing on the development of new analytical tools to detect and quantify polypeptide chiral excesses brought about by amino acid polymerization reactions performed under possible early Earth conditions.

A new collaboration with Professor Irene Chen (University of California, Santa Barbara) has emerged, focusing on the analysis of amino acid enantiomeric abundances in laboratory experimental samples produced in her laboratory. The collaboration has implications for chemical evolution and the origin of life on Earth, and is facilitated via the Simons Collaboration on the Origin of Life.

We have a new collaboration with Queenie Chan (Open University, UK) focused on the amino acid analysis of asteroid Itokawa particles returned from the JAXA Hayabusa mission. A manuscript detailing our findings is in preparation for submission.

We continue to collaborate with Professor Hiroshi Naraoka in Japan (Kyushu University) to investigate hydroxy amino acids in meteorites and hosted one of his students in 2019.

We completed participation in three large research consortia investigating the Diepenveen, Saricicek, and LA 2018 meteorites, with publications resulting from the first two groups and the third in preparation.

We began a collaboration with Philippe Schmitt-Kopplin (Helmholtz Zentrum München) into the organic content of meteorite NWA 11118.

We established a new collaboration with Yoshi Furukawa (Tohoku University, Japan) to investigate the distribution and carbon isotopic composition of sugars in carbonaceous meteorites. A manuscript which included the first confirmation of extraterrestrial ribose in a meteorite was published in the Proceedings of the National Academy of Sciences USA.

We collaborated with Uma Gorti (SETI Institute/NASA Ames) on disk chemistry modeling.

Flight Mission Involvement

OSIRIS-REx
Team Member(s): Jason Dworkin, Daniel Glavin, Jamie Elsila, José Aponte (and also Joseph Nuth from the GCA team but not the AAL)
How are they involved: Project Scientist – Dworkin; Co-Investigator – Glavin; Collaborator – Elsila; Collaborator – Aponte; Deputy Project Scientist – Nuth

Mars Science Laboratory (MSL)/Sample Analysis at Mars instrument
Team Member(s): Daniel Glavin, Jason Dworkin
How are they involved: Co-Investigator – Glavin; Collaborator – Dworkin

ExoMars/Mars Organic Molecular Analyzer (MOMA) instrument
Team Member(s): Jason Dworkin, Daniel Glavin
How are they involved: Co-Investigator – Dworkin; Co-Investigator – Glavin

Hayabusa2 (JAXA)
Team Member(s): Jason Dworkin, Jose Aponte, Jamie Elsila, Daniel Glavin, Hannah McLain, Eric Parker
How are they involved: All are members of the international soluble organic analysis team; Dworkin is International Deputy Lead for the Soluble Organics for the team.

Mars Sample Return Capture, Contain, and Return System (CCRS)
Team Members(s): Daniel Glavin, Jason Dworkin
How are they involved: Sample Integrity Lead – Glavin; Deputy Sample Integrity Lead – Dworkin

CAESAR Phase A Study
Team Member(s): Daniel Glavin, Jason Dworkin (and also Michael Mumma, Stefanie Milam, and Perry Gerakines from the GCA team but not the AAL)
How are they involved: Project Scientist – Glavin; Co-Investigator – Mumma, Dworkin; Deputy Project Scientist – Gerakines, Milam

Europa Lander Ice Desiccation Study
Team Member(s): Perry Gerakines (Lead), Daniel Glavin and Jason Dworkin are Co-Investigators
How are they involved: Support laboratory testing to determine the ice sublimation rate as a function of temperature under Europa surface conditions using a suite of Europa near surface ice analogs.

James Webb Space Telescope
Team Member(s): Stefanie Milam
How are they involved: JWST Deputy Project Scientist for Planetary Science

Origins Space Telescope
Team Member(s): Stefanie Milam
How are they involved: Member of the Science and Technology Design Team

WFIRST
Team Member(s): Stefanie Milam
How are they involved: Solar System Lead

Origin and Evolution of Organics and Water in Planetary Systems: 2019 Publications

Aponte, JC., Whitaker, D., Powner, M.W., Elsila, J.E. and Dworkin, J.P. (2019). Analyses of Aliphatic Aldehydes and Ketones in Carbonaceous Chondrites. *ACS Earth and Space Chemistry*, 3 463-472 DOI: 10.1021/acsearthspacechem.9b00006

Aponte, J.C., Woodward, H.K., Abreu, N.M., Elsila, J.E. and Dworkin, J.P. (2019). Molecular distribution, 13C-isotope, and enantiomeric compositions of carbonaceous chondrite monocarboxylic acids. *Meteoritics and Planetary Science* 54 (2): 415-430. DOI: 10.1111/maps.13216

Burton, A.S., Sutton, M.A., Zaikova, E., Sutton, R.E., Brinckerhoff, W.B., Bevilacqua, J.G., Weng, M.M., Mumma, M.J. and Johnson, S.S. (2019). Radiation Stability Evaluation of Protein-Based Nanopores for Mars and Europa Missions. Proc. 2019 Astrobiology Science Conf., Seattle, WA. Presentation 142-181. https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20190002053.pdf

Cordiner, M.A., Palmer, M.Y., de Val-Borro, M., Charnley, S.B., Paganini, L., Villanueva, G., Bockelée-Morvan, D., Biver, N., Remijan, A.J., Kuan, Y.-J., Milam, S.N., Crovisier, J., Lis, D.C. and Mumma, M.J., (2019). ALMA Autocorrelation Spectroscopy of Comets: The HCN/H13CN ratio in C/2012 S1 (ISON). *Astrophys. J. Letters* 870:L26 (6pp). DOI: 10.3847/2041-8213/aafb05

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Hudson, R.L. and Coleman, F.M. (2019b). Infrared Intensities and Molar Refraction of Amorphous Dimethyl Carbonate - Comparisons to Four Interstellar Molecules. *Physical Chemistry Chemical Physics*, 11284-11289. DOI: 10.1039/c9cp01709g

Hudson, R.L. and Ferrante, R.F. (2020). Quantifying Acetaldehyde in Astronomical Ices and Laboratory Analogues: IR Spectra, Intensities, ^{13}C Shifts, and Radiation Chemistry. *Monthly Notices of the Royal Astronomical Society*, in press. DOI: 10.1093/mnras/stz3323

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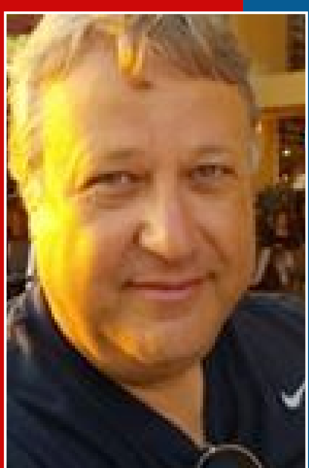
Icy Worlds: Astrobiology at the Water-Rock Interface and Beyond

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 JPL Icy Worlds NAI team. 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

2019 Executive Summary

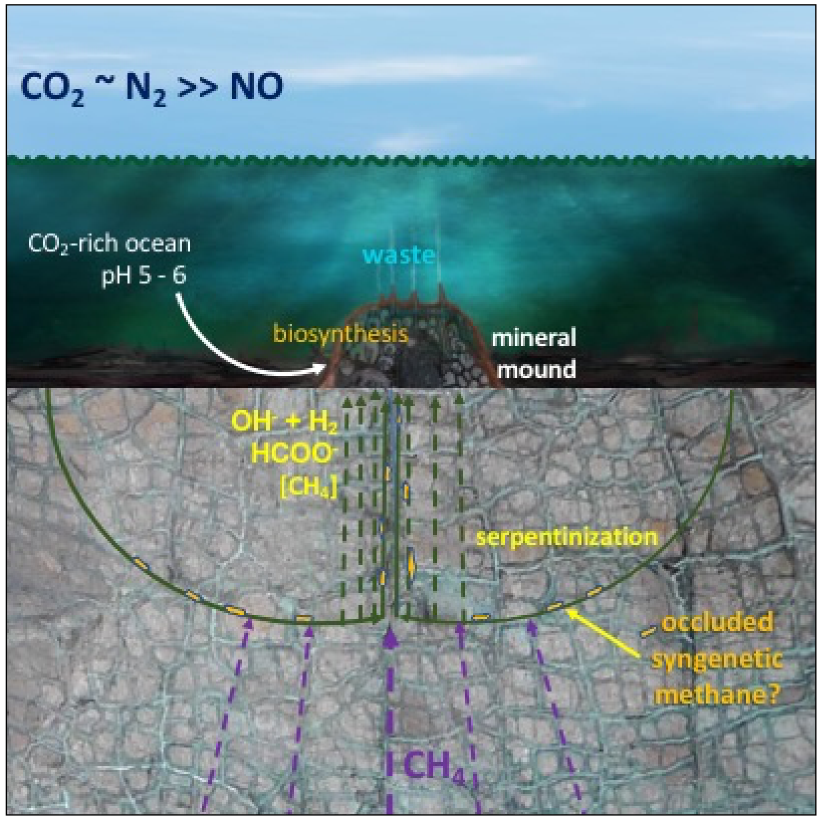


Figure 1. Schematic representation of alkaline hydrothermal vent model for the emergence of life on the early Earth.

The INV 1 team continued to work on developing the Alkaline Vent Theory (AVT) and carrying out investigations relevant to the origin of life. Through a series of experiments, using the JPL hydrothermal flow reactor, they simulated the geochemical conditions that likely resulted from the interaction of alkaline fluids generated in open system convection in ultramafic Hadean crust and carbonic ocean at and just below the ocean floor (Fig. 1). Solid materials and the derived solutions showed some evidence of serpentinization after subjecting the ultramafic ocean crust simulants to alternating hydrothermal and ocean fluids under hydrothermal pressures (100 bar), temperatures (120°C), and pH conditions similar to those occurring at the best-known modern analogue, the Lost City hydrothermal field. Oscillations in the concentrations of dissolved magnesium, calcium, and iron (in the alkaline solutions) correlated very well with alternations in the flow of ocean and alkaline hydrothermal simulants and were consistent with partial serpentinization. High calcium concentrations, independent of the fluid source, and effluents with up to 9 mmol/kg of dissolved sulfide, demonstrated the propensity of these alkaline systems to offer bisulfide to the growing mounds. The detection of mackinawite in altered samples further supports the notion that it would have been present in ancient alkaline hydrothermal systems. Depleted Mg levels in ultramafic compositions as compared to unaltered (pre-experiment) materials was consistent with the observed dissolution of silica from komatiite and precipitation in solid samples where the pH's of the solutions were below those controlling silica solubility.

INV 2 team members have conducted experiments and shown the reductive amination of pyruvate to alanine, and glyoxylate to glycine, by mixed-valence iron minerals that would have been present in early Earth or ocean world hydrothermal systems. They demonstrated the importance of redox and pH gradients to the synthesis of alanine and lactate from pyruvate, showing that both alkaline pH and an intermediate iron redox state in the mineral are required for formation of the amino acids.

In characterizing these prebiotic reaction networks, INV 2 team has also observed very interesting results in redox and pH gradients that build upon our initial study. Generally, more reduced iron minerals lead to increased yield of alpha-hydroxy acids (αHA) over amino acids. However, the pH has a pronounced effect on product distribution at a given Fe(II)/Fe(III) ratio in the mineral. At alkaline pH for both pyruvate and glyoxylate, αHA's were dominant when the minerals were highly reduced and amino acids were dominant when the minerals were more oxidized. In contrast, for glyoxylate reactions at neutral pH, amino acids were dominant with reduced minerals and αHA's were dominant with more oxidized minerals (pyruvate did not form any amino acid at neutral pH). At any particular gradient condition, pyruvate and glyoxylate did not give identical relative yields of their corresponding αHA and amino acid, so it is likely that other carboxylic acid precursors in these gradients also would act distinctly (Fig. 2). Our results show that a complex prebiotic and proto-metabolic network can emerge in the overlapping geochemical gradients of redox and pH that would have been present in alkaline hydrothermal vent systems on early Earth and perhaps ocean worlds. Though this reaction starts with only two simple precursors, a variety of organic distribution patterns were produced depending on the position of the reaction in the overlapping geochemical gradients. It is likely that the geochemical gradients that affect the initial distribution of amino acid and αHA monomers will provide an additional chemical pressure to shape the composition and function of any oligomers/polymers formed from that seeding reservoir. We are now currently testing these gradient reactions incorporating the effects of sulfide plus other nitrogen sources, and other mineral catalysts that might be present in vents.

INV 3 members investigated how, where, and for how long might disequilibria exist in icy worlds, and what that may imply in terms of habitability. In 2019, INV 3 team members continued to synthesize laboratory investigations of the properties of icy world oceans and applied these new data to understanding their possible thermal histories and transport properties. INV 3 Lead, Steve Vance, working with INV 2 team members, investigated the possible formation of inverted ice chimneys,

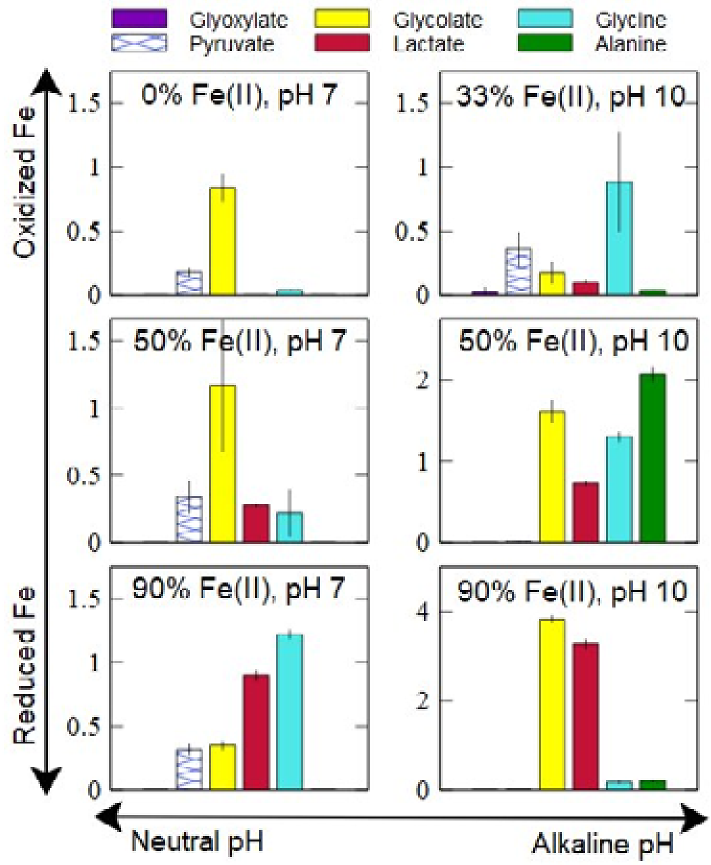


Figure 2. Organic distribution signatures in redox and pH gradients. The Y-axis is the area of the methyl peaks relative to DSS, not accounting for the number of protons integrated over.

or brinicles, at the ice-ocean interface on Europa and Enceladus. To assess the possible lifetimes of such features and thus their potential as long-term drivers of chemical gradients that might be useful for life, the work includes estimates of the possible amounts of salt trapped in the ice, a parameter that might be assessed by the planned Europa Clipper and JUICE missions to Jupiter's icy ocean moons. Our INV 3 Co-I at the University of Washington (UW), Olivier Bollengier, studied liquid water and came up with the most precise equation of state yet for the world's most studied liquid. This new data set, which makes use of the local basis function approach, developed by Co-I J. Michael Brown of UW, is the necessary starting point for describing the aqueous solution chemistry of the different possible compositions of icy world oceans. The complete data set for the aqueous Na-Mg-Ca-Cl-SO_4 system, also obtained under Bollengier's direction, is being developed in the final year of the project. Meanwhile, UW postdoc Baptiste Journaux has continued his laboratory studies of high-pressure ices in the presence of aqueous solutions.

Investigation 4 (INV 4) researchers concentrated on investigating possible links between species observed on the surface of Europa and the characteristics of the underlying ocean. They innovatively combined a recent body of work studying the chemistry of frozen putative Europa ocean brines with new experiments to develop predictions of the sequence by which the hydrated minerals form when a four-ionic component ocean (Na^+, Cl^-, Mg^{2+}, and SO_4^{2-}) freezes as a function of relative ionic concentrations and pH. This in turn provides a means to begin linking observed surface chemistry and the chemical environment of the subsurface ocean as well as insight into endogenic versus exogenic origin of detected species.

Team Members

Isik Kanik
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Jun Kimura
Victoria Meadows
Robert Pappalardo
Michael Russell
Takazo Shibuya
Christophe Sotin
Ken Takai
Steve Vance
David VanderVelde

INV 1: Energy Production at Water-Rock Interfaces

During this past year the focus of the Emergence-of-Life group progressed from concerns of theory and the constraints ordered through initial conditions, as investigated through the origin-of-life experimentation (e.g., White *et al.*, 2020 in press), to the kind of mechanisms likely required for the conversions of those disequilibria as identified by research produced over the previous three grant years (eight in total including CAN 5).

The environmental thermodynamic disequilibrium permitting life to exist is a redox tension. Electron transfer from reducing environmental compounds (substrates) to oxidizing ones collapses this electrochemical disequilibrium but while doing so generates chemical mass-action disequilibria in the form of high ATP/ADP or PPi/Pi ratios, life's immediate source of "free energy". Green rust (GR, or fougerite) appears to offer such redox potential. GR1 harbours mono- and divalent anions with spherical (e.g. Cl^-) or 'flat' (e.g. carbonate) shapes, while GR2 contains molecules featuring tetrahedral geometry (for example, SO_4^{2-} and PO_4^{3-}). The astonishing structural flexibility of GR is on a par with its unparalleled redox versatility and, we have argued, that the mineral had the physical and chemical flexibility to act as a complex of compartments to account for the earliest steps toward life (Figure 3; Duval *et al.*, 2019). How these steps may have been taken from the base at white rust (WR) is illustrated in Figure 4.

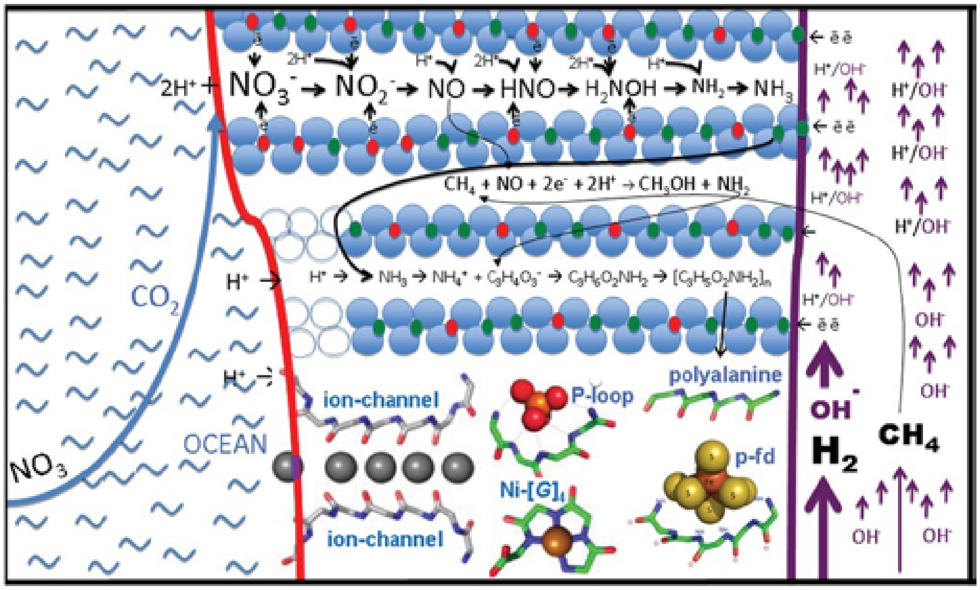

Figure 3. Sketch of a fougerite nano- to micro-crystallite situated to hold disequilibria between the chemistries of an alkaline and highly reduced hydrothermal solution against the acidulous carbonic ocean bearing the high potential electron acceptor, nitrate. Fougerite, a redox and physically flexible hydrous mineral, is shown here as a putative combined proto-enzyme (nano-engine) with the capacities of (i) nitrate reductase, (ii) methane monooxygenase (iii) aminase, and (iv) peptidase. Amyloid peptide comprising a mixture of apolar α-sheets and polar β-sheets is extruded from the hydrous interlayers to form (i) a protoferredoxin (p-fd), (ii) a P-loop (P-l), (iii) Ni-tetra-glycine residue nest (Ni-[G]4), (iv) ion channels (ion-channel), and ultimately (v) aggregating to form a dielectric and osmotic barrier both encapsulating and embedding fougerite nanocrystals (Duval *et al.*, 2019).

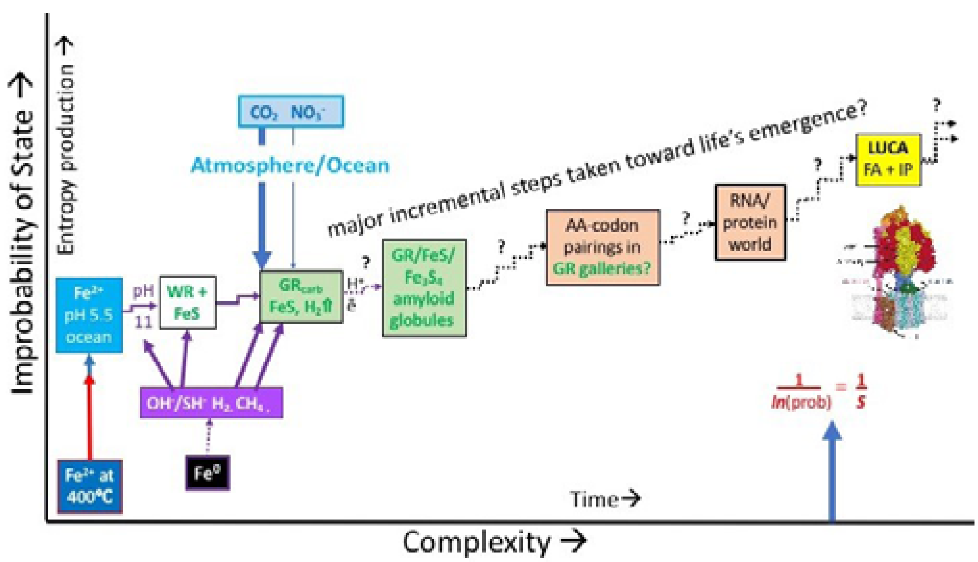

Figure 4. A recursive staircase model revised from Branscomb and Russell (2017) comprises a set of discreet evolutionary transitions of increasing organization to address the processes we consider most likely to have been involved at life's emergence. Though there will have been an untold number of steps taken to the last universal common ancestor a limited number of these are isolated here. The theoretical ground floor to this abridged staircase is the precipitation of white rust and cubic iron mono-sulfides at an alkaline hydrothermal vent on the floor of the Priscoan Ocean ~4.4 billion years ago. The top floor of the staircase is the LUCA "penthouse" suspended from the phylogenetic tree. On the 'perception' of any disequilibria, the universe appears to 'discover' mechanisms – entropy generators – to bring about more relaxed outcomes resulting paradoxically in increasing complexity of the generators themselves – this on the way to making the universe more uniform. The myriad steps taken to life must have been long drawn out (at the nano-scale), each step facilitated by a decrease in internal entropy of the evolving open (proto)-metabolizing system.

INV 2: From Geochemistry to Biochemistry

INV 2 work focused on several topics including prebiotic organic chemistry in hydrothermal systems, green rust chemistry with nitrogen, and phosphorus prebiotic chemistry.

At the Oak Crest Institute of Science (Oak Crest), the current grant year was dedicated to expanded synthesis and analysis of green rust (GR), a reactive and prebiotically relevant iron oxyhydroxide that would have been present on early Earth and may be present on the seafloors of ocean worlds. We have developed methods for the synthesis of pure GR1 and GR2 minerals using rigorous pH control and anaerobic techniques. We have also debunked literature methods that incorrectly claim to afford GR1(F^-) and GR2(CO_3^{2-}). Our minerals have been characterized spectroscopically, including anaerobic XRD and Mössbauer spectroscopy. We also have developed novel methods for analyzing these samples anaerobically by electron microscopy (SEM and TEM) as well as atomic force microscopy (Fig. 5-6). A manuscript about green rust synthesis methods is in preparation.

NV 2 also studied the reduction of nitrate/nitrite mixtures by green rust (producing nitrogen compounds including ammonia, which can react with organics in hydrothermal vent systems). This work is generating new results about the types of reduced N compounds that can be produced by green rust, and under what conditions organic reactions may be facilitated; a manuscript is in prep. This year also, Oak Crest has developed a series of analytical methods that will continue to benefit the Icy Worlds team and future collaborations, including: GC-MS method for the analysis of carbonyl compounds (Bock *et al*. 2017), methods for the analysis of reactive nitrogen species (Key *et al.* 2011), and non targeted LC-QTOF-MS for the analysis of complex mixtures (Barge *et al*. 2019). In addition, we have developed a headspace GC-MS method to measure gas-phase products, such as observed in the GR-mediated reduction of nitrate-nitrite mixtures (Fig. 7).

INV 2 researchers investigated the organic reactions that occur when simple organic acids (e.g. pyruvate and glyoxylate) are reacted with nitrogen species and redox-active iron minerals including green rust. These studies have experimentally shown the reductive amination of pyruvate to alanine, and glyoxylate to glycine, by mixed-valence iron minerals that would have been present in early Earth or ocean world hydrothermal systems. Our first published study (Barge *et al.* 2019) demonstrated the importance of redox and pH gradients to the synthesis of alanine and lactate from pyruvate, showing that both alkaline pH and an intermediate iron redox state in the mineral are required for formation of the amino acid. However, in this study we

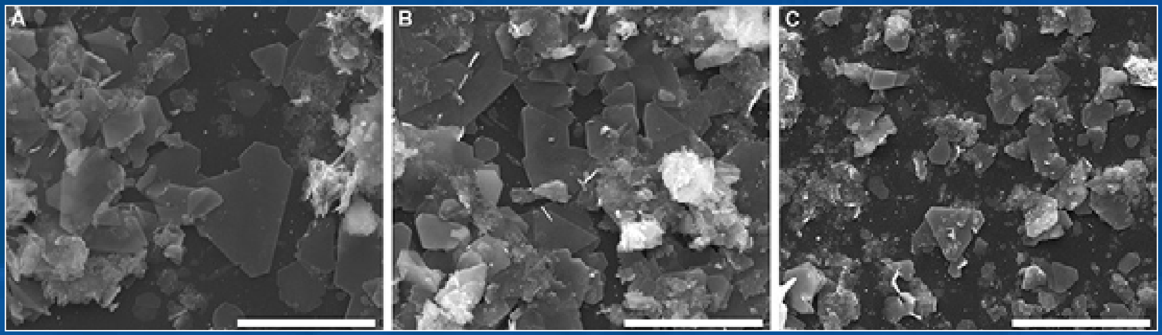

Figure 5. Anaerobic SEM analysis of GR1(Cl-) reveals regular plate structures. (A-B) scale bar, 10 μm; (C) scale bar, 20 μm.

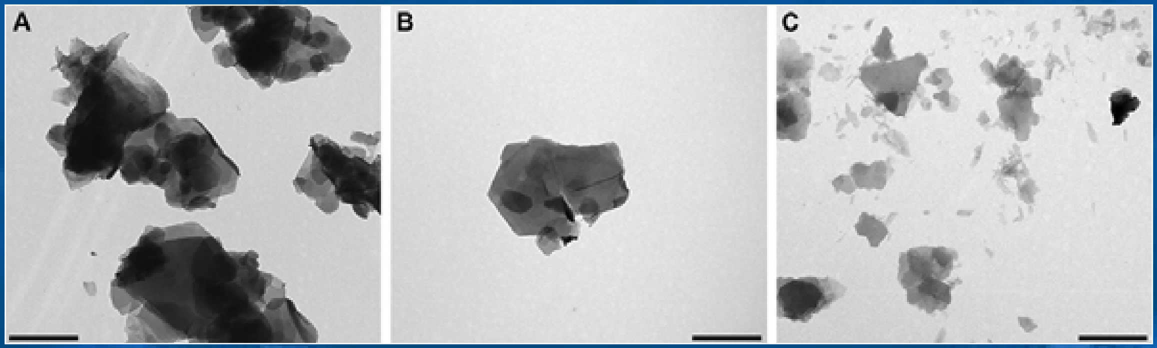

Figure 6. Structures in agreement with SEM data; scale bar, 0.5 μm.

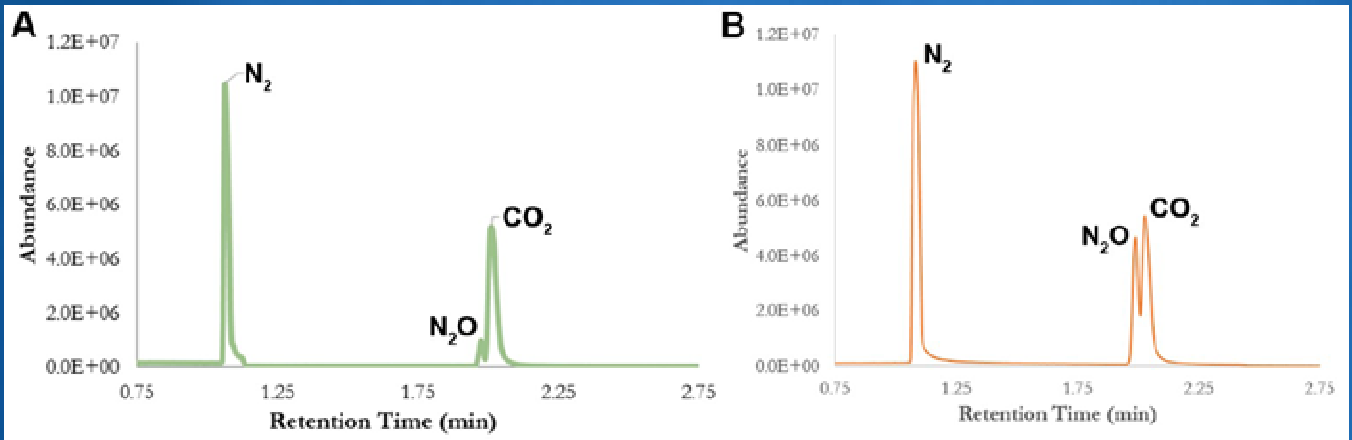

Figure 7. GC-MS Chromatograms of headspace samples from GR reactions with nitrate-nitrite mixtures show that oxyanion reduction produces differing amounts of nitrous oxide (N_2O). (A) GR2(SO_4^{2-}); (B) GR1(Cl^-).

used a very high concentration of ammonia (to be consistent with previous studies in this area) that is unrealistic for hydrothermal vent or seafloor settings. In our subsequent study (manuscript submitted) we investigated a combined network of pyruvate and glyoxylate, which is relevant to a variety of prebiotic metabolic cycles and products, in a broader range of gradients. These gradients included ammonia concentration to determine the minimum [NH_4] that would still enable reductive amination. Our lowest ammonia concentrations tested (5-10 mM, compared to 375 mM in the first study) still enabled reductive amination of glyoxylate to glycine, both with and without the presence of an iron hydroxide mineral. This indicates that amino acid formation as shown in (Barge *et al.* 2019) is likely to still proceed even at relatively low ammonia concentrations that are realistic for geological systems (Figure 8).

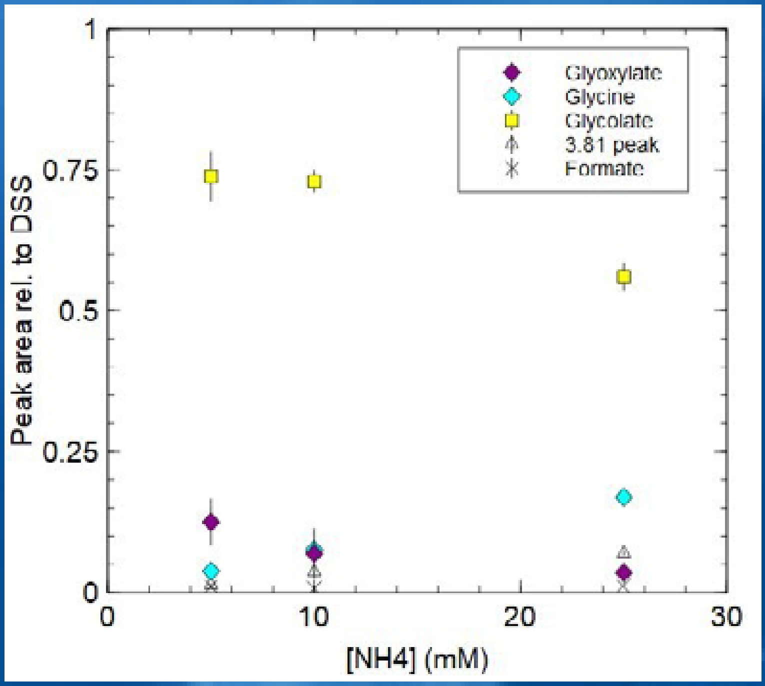

Figure 8. Reductive amination of glyoxylate with iron hydroxide minerals showing amino acid formation even at 5 mM ammonia.

INV3: Characterization of Ocean Worlds and Implications for Habitability

The accumulated UW mineral physics work highlighted in the Executive Summary has been synthesized into an open source computer framework (Journaux *et al*. 2019), referred to as SeaFreeze and available on github (github.com/Bjournaux/SeaFreeze), that allows the rapid computation of Gibbs energy and associated properties using the local basis function method. Journaux gave a well-received talk at the 2019 Fall AGU meeting in San Francisco. Team member Steve Vance has incorporated SeaFreeze data into the PlanetProfile program, also available on github (github.com/svance/planetprofile). Using PlanetProfile, Vance and JPL postdoc Mohit Melwani Daswani worked with University of Nantes researchers Gabriel Tobie and Gael Choblet to investigate the ability of the planned JUICE mission to use gravity data to reveal the sulfur composition of Ganymede's metallic iron core (Vance *et al.* 2019a).

Melwani Daswani's work has invigorated the study of the rocky lithospheres and iron cores of icy ocean worlds by infusing more sophisticated geochemical modeling, allowing us to consider what the elemental makeup of these bodies reveals about their formation, thermal history, and the resulting redox states and compositions of their oceans.

As a bridge between INV 1-2 and INV 3, the team published a paper in Astrobiology about the formation and energetic potential of ice stalactites formed from brine channels on Earth, and possibly in icy ocean worlds Europa and Enceladus (Vance *et al.* 2019b).

Meanwhile, the INV 3 team continues to apply the new fundamental data to model planetary processes linked to the search for life. Associate Simon Stähler led a paper (Stähler *et al*. 2019) investigating the seismic signals of wind-driven waves on Titan's seas that may be detectable near Titan's equator where the Dragonfly mission in slated to land in the 2030's. Caltech intern Ana Lobo, working with team members Vance and Thompson, presented results of models for the lateral transport of materials in the Enceladus ocean arising from melting and freezing at the ice-ocean interface (Lobo *et al.* 2019). This work will lead to predictions for the global redistribution of potential nutrients in the ocean. We will also evaluate whether the ocean composition inferred from Cassini spacecraft samples of the south polar plume may be skewed by dilution of melt at the ice-ocean. Vance, Bills, and Styczinski are using PlanetProfile's chemical models for oceans to investigate the use of magnetic induction measurements to evaluate the ocean compositions and thus their redox states.

INV 4: Observable Chemical Signatures on Icy Worlds

INV 4's researchers concentrated on investigating possible links between species observed on the surface of Europa and the characteristics of the underlying ocean. In order to better understand the composition of icy minerals that may form from freezing of brines on Europa's surface, we have conducted a number of experimental investigations that have been reported in the peer reviewed literature. During the period covered by this report, INV 4 has focused on a study that combines chemical divide modelling with Raman and X-ray diffraction experiments to examine the freezing of a four ionic component (Na, Mg, SO_4, Cl) solution. We have quantitatively identified the minerals that are formed upon freezing as a function of relative ionic concentration and freezing rate and assessed the effectiveness of using the chemical divide model to infer the ocean's chemical composition. As mineral formation largely depends on freezing conditions, this study can provide guidance as to whether future missions should focus on direct analysis of plume materials, or should look to explore other terrains such as diapirs in order to gain the most insights into the ocean.

Two methods have been utilized:

Chemical Divide Modeling: This approach predicts the sequence of precipitation of salts based on their solubility and relative ionic concentrations in solution. In the case of putative Europan brines with pH ≤ 8.4 containing the four ionic components Na, Mg, Cl and SO_4, chemical divide modeling for thermodynamic freezing yields the flowchart in Johnson *et al.* (2019)

Experimental Techniques: We examine a greatly simplified brine system where the concentrations of Na and Cl are exactly twice those of Mg and SO_4, thus only mirabilite ($Na_2SO_4 \cdot 10H_2O$) and $MgCl_2 \cdot 12H_2O$ are expected as products. We studied two freezing scenarios (flash vs slow freezing) as well as two concentration regimes (molar vs tenths of molar) using complementary Raman (surface) and cryogenic X-ray diffraction (XRD, bulk) techniques. The latter is a fairly unique facility for investigating planetary ices that has recently been developed at JPL.

In all cases, the XRD experiments detected only the two products as predicted by the chemical divide model. The Raman results are more complex, where the two expected salts were detected in only 2 scenarios. In the case where the molar brines were slowly frozen, epsomite and hydrohalite also formed along with mirabilite and $MgCl_2$•$12H_2O$. In addition, flash freezing of the diluted brine often produces water ice together with $MgCl_2$ glass, greatly hindering detection. These results hold important implications for both instrument as well as landing site selection for a potential Europa lander. Specifically, the Raman payload needs to be accompanied by a complimentary instrument that can detect salts in low-concentration brines, especially for the plumes where brines are likely to be flash frozen. In addition, terrains such as diapirs where brines are more likely to freeze slowly and closer to thermodynamic equilibrium should be considered for exploration in order to gather more comprehensive chemical information from the subsurface ocean.

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Collaborations, Extended Scientific Directions, Flight Mission Involvement

INV 2 team members have been very active in events, conferences, and outreach. Six student researchers presented at AbSciCon; and we hired a new Icy Worlds postdoc, Dr. Jessica Weber (Ph.D. in Chemistry from MIT). Barge, along with student researcher Erika Flores and postdoc Jessica Weber, conducted a research visit to ELSI this fall to start new collaborations for origin of life research (Barge and Weber gave seminars, and Weber and Flores led an origin of life discussion meeting about early Earth anoxic environments). Barge and Flores also visited University of Nevada, Las Vegas to start new collaborations with the Hausrath group regarding prebiotic chemistry driven by iron rich clays. Barge visited Dakota State University to participate in a Women in STEM workshop; was an invited guest speaker at the NYC Intrepid Museum about Europa (World Water Day); and gave seminars / lectures at various universities including UNLV and the University of Southern California. Following up on research established under a 2018 NAI Early Career collaboration grant that started a collaboration between the JPL Icy Worlds team and Penn State (Keating group), a NASA FINESST fellowship was awarded to graduate student Saehyun Choi, who will continue to collaborate with us for her thesis research (studying how co-acervates incorporate into simulated hydrothermal chimneys).

The INV 3 team is contributing to the development of robotic exploration missions. Vance continued his work facilitating the Habitability Working Group. Team member Mathieu Choukroun took over Vance's role as Investigation Scientist for the Clipper's Mass Spectrometer for Planetary EXploration (MASPEX). INV 3 Postdoctoral Fellow Melwani Daswani joined the Europa Clipper science team as an affiliate working with Dr. Steve Vance. Melwani Daswani and Vance participated in work supporting the Europa Clipper MASPEX instrument, which was presented at the Fall AGU Meeting (Glein *et al*. 2019). Also joining the Clipper team as an affiliate was UW Seattle graduate student Marshall Styczinski, who is working with Vance and team member Bruce Bills on the magnetic properties of icy ocean worlds. Vance is leading an effort to evaluate the optimal set of geophysical investigations of Enceladus that would provide needed habitability context for any future mission with the primary goal of searching for life (Vance *et al.* 2019d). Vance also contributed to seismic noise estimates for the Dragonfly mission to Titan (Panning *et al.* 2019).

The team is also involved in planetary science leadership internationally. Vance was recently elected as the incoming President of the Planetary Science section of the Asia Oceania Geosciences Society (AOGS). Vance participated in the convening of a special session at the AGU Fall Meeting entitled "Ice and Ocean Worlds: Geology, Oceanography, Chemistry, Habitability". Vance gave an invited talk at the AGU meeting on his Icy Worlds work entitled, "Hydrothermal Activity in the Solar System's Ocean Worlds" (Vance 2019). The associated paper is in press (Vance and Melwani Daswani 2019).

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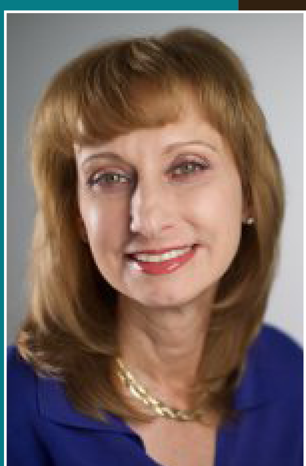
Habitability of Hydrocarbon Worlds: Titan and Beyond

Lead Institution:
NASA Jet Propulsion Laboratory

Team Overview



Principal Investigator:
Rosaly Lopes

Our team explores the potential biochemical pathways for organic materials extending from the atmosphere of Titan down to the potentially habitable ocean and for any extant chemical biosignatures to ascend from the ocean to the surface and atmosphere (Fig. 1). 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.

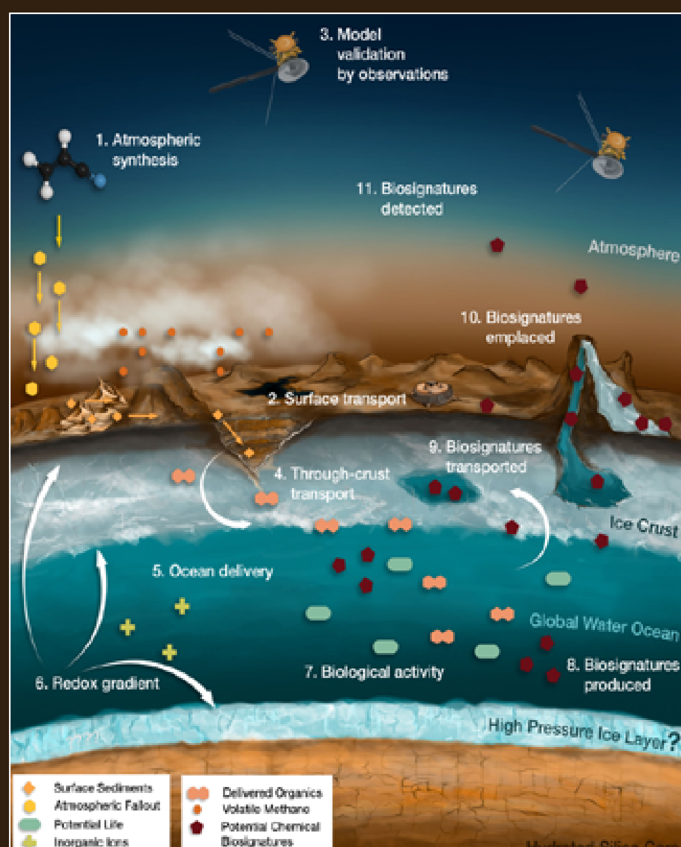


Figure 1. Titan is an Ocean World rich in organics. We will explore its potential biochemical pathways for organic materials to go from the atmosphere down to the potentially habitable ocean and for any extant chemical biosignatures to go up from the ocean to the surface and atmosphere. We will investigate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

Team Website: https://astrobiology.nasa.gov/nai/teams/can-8/jpl-titan/index.htmlcan-7/gatech/index.html

2019 Executive Summary

We have made significant progress on photochemical and dynamical modeling of Titan's atmosphere. On the modeling side, coupling two atmospheric models that cover different altitudes in Titan's atmosphere allowed integration of the entire atmosphere of Titan. On the observational side, analysis of ALMA data resulted in the first observation of the CH_3D molecule at sub-millimeter wavelengths (Thelen *et al.*, 2019). Analysis of NASA IRTF data resulted in the first detection of propadiene (CH_2CCH_2) in Titan's atmosphere (Lombardo *et al*., 2019a), a paper that was highlighted in AAS Nova. Spatial and seasonal changes in Titan's gases from the final years of the Cassini mission were the subject of several papers, using data from ALMA (Cordiner *et al.*, 2019) and CIRS (Teanby *et al.*, 2019, Lombardo *et al*., 2019b).

To understand how materials falling from the atmosphere are transported across the surface, we are developing a landscape evolution model, which involved re-writing and optimizing the DELIM code that is used for Mars. Titan's surface is more complex. We have added a new module to describe groundwater flow, and a dissolution subroutine. The flow routing modeling is novel and has broad applications beyond Titan. The new DELIM model is now the only such landscape evolution model that tackles both dissolution and mechanical erosion simultaneously.

We have published the first global geomorphologic map of Titan (Lopes *et al.*, 2019), which will serve as a constraint for the landscape evolution model by showing how sedimentary and depositional materials are distributed over the surface. In a complementary study, we have obtained an updated estimate of the amount of organic materials on Titan, which is important as a constraint on the amount of chemical energy and building blocks available for potential life.

To investigate the molecular pathways from surface to subsurface ocean, we have performed a series of numerical simulations on the effect of a clathrate layer capping Titan's icy crust on the convection pattern in the stagnant lid regime (Kalousova and Sotin, 2019). In the investigation of habitats resulting from molecular transport, we have modeled the accretion of Titan to understand the effects of thermal evolution on the rocky interior, and to constrain the composition of volatiles exsolved from the interior

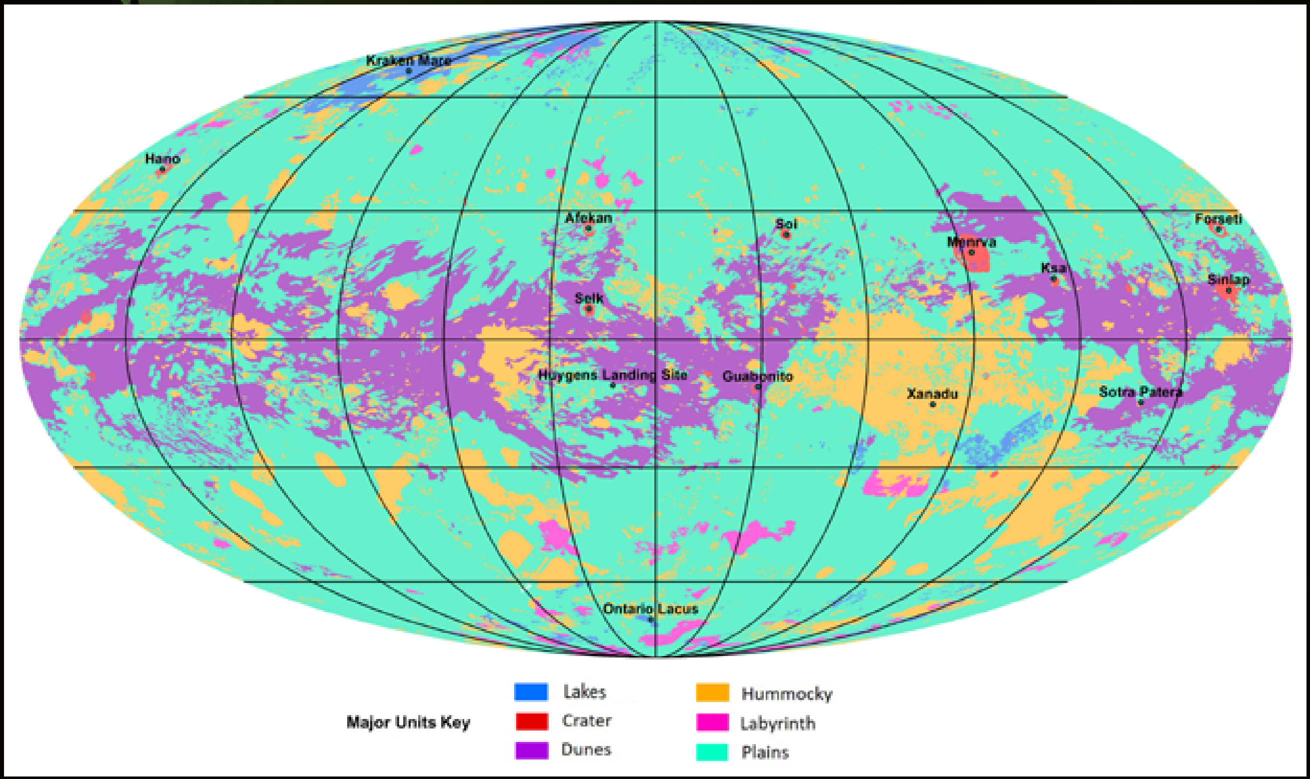


Figure 2. The first global geologic map of Saturn's largest moon, Titan, is based on radar and visible and infrared images from NASA's Cassini mission. Black lines mark 30 degrees of latitude and longitude. Map is in Mollweide projection, centered on 0 degrees latitude, 180 degrees longitude. Map scale is 1:20,000,000. The map is labeled with several of the named surface features. Also located is the landing site of the European Space Agency's (ESA) Huygens Probe. The map legend colors represent the broad types of geologic units found on Titan: plains (broad, relatively flat regions), labyrinth (uplifted regions often containing fluvial channels), hummocky (hilly, with some mountains), dunes (mostly linear dunes, produced by winds in Titan's atmosphere), craters (formed by impacts) and lakes (regions now or previously filled with liquid methane or ethane). Modified from Lopes *et al.* (2019). NASA PIA23174.

and that may have migrated vertically to build up the ocean early in Titan's history (Neri *et al*. 2019). We have also published results of modeling water-hydrocarbon mixtures using the CRYOCHEM code, which now successfully allows chemical modeling of both the hydrocarbon-rich condensed fluid phases and the water-rich condensed fluid phases (and vapor phases, too) simultaneously (Tan *et al*. 2019).

Preliminary results for our investigation of ocean habitats led to new insights into the origin of methane and nitrogen (N_2) on Titan by modeling D/H exchange between organics and water, as well as high pressure C-N-O-H fluid speciation in Titan's rocky core (Miller *et al*., 2019a; 2019b; 2019c). Results suggest an important role for organic compounds in the geochemical evolution of Titan's core, which may feed into the habitability of Titan's ocean.

Our team has submitted several science nuggets to the NAI and organized sessions at meetings including AbSciCon, AOGS, and AGU.

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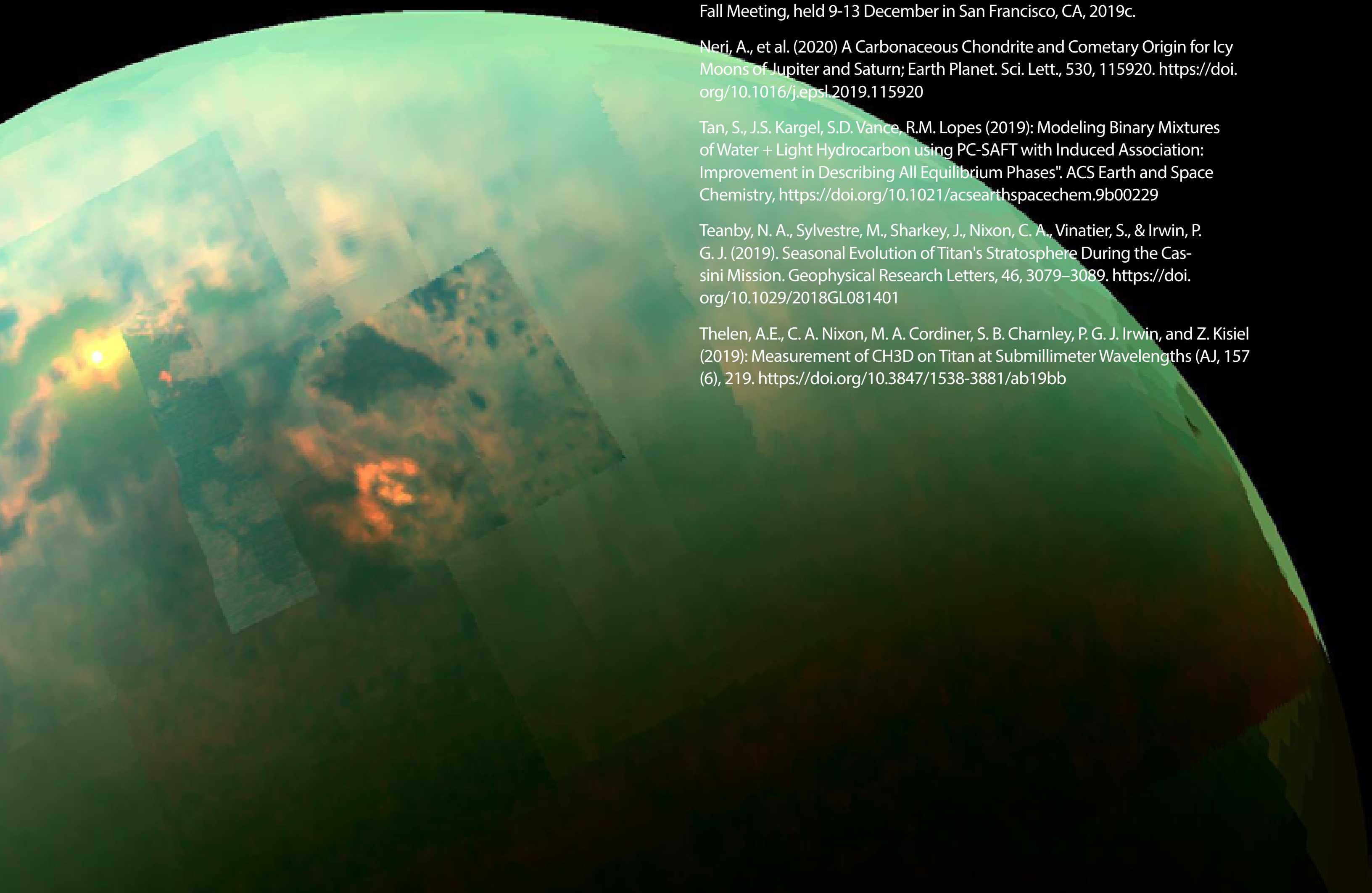

Objective 1: Transfer of Organics from Atmosphere to Ocean (Lead: Steve Vance)

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)

<u>Photochemical modeling of Titan's atmosphere and dynamical modeling of Titan's Atmosphere:</u> As Titan general circulation models (TGCMs) cannot span the full atmosphere from surface to the latitudes required for our project, our goal in this project is to model Titan's atmosphere from 0 to ~1350 km by combining two atmospheric models: TitanWRF GCM, which covers the lower atmosphere (0 to ~400km), and the TGCM thermospheric model, which covers the thermosphere (~600-1350km). For this work, the TGCM's bottom boundary was lowered dynamically to 380km (~1Pa) to overlap with TitanWRF, although new physical processes operating within the ~380-600km region were not included. Output from the 1Pa level of TitanWRF every 10 Titan minutes (i.e. 144 times per Titan sol) - specifically, the (i) zonal (west to east) wind, (ii) meridional (south to north) wind, (iii) vertical wind, and (iv) altitude of that pressure level - was then used to force the lower boundary of the TGCM.

Coupling the models in this way enables us to capture the effects of wave forcing on the upper atmosphere of Titan. Significant wave activity exists in Titan's stratosphere (e.g. Newman *et al.*, 2011) with wave numbers of ~1-3 and periods of a few Titan hours. As waves propagate upward toward regions of lower atmospheric density, they grow in amplitude to conserve energy and ultimately break, dumping angular momentum, which can strongly affect the upper atmosphere circulation. Hence waves in TitanWRF can have a large impact on the TGCM.

Initially, the TGCM was forced using TitanWRF output from a simulation at planetocentric solar longitude Ls=0° (northern spring equinox) with no surface topography or methane cycle. These results showed (a) a large impact on temperatures and winds (Figure 3) compared to a TGCM simulation with only solar forcing, and (b) hugely increased variability in thermospheric temperature and air density, which better matches Cassini INMS observations (Figure 4). TitanWRF output for Ls=90, 180, and 270° was then used to force the TGCM at northern summer solstice, fall equinox, and winter solstice, respectively. Following this, full 3-D dynamical output from TitanWRF (from 0 to ~400km) and the TitanWRF-forced TGCM runs (~400-1350km) was provided to the team at Caltech for combination into a time-varying, 3D prediction of the atmospheric state from 0 to ~1350km, for each of the four seasons.

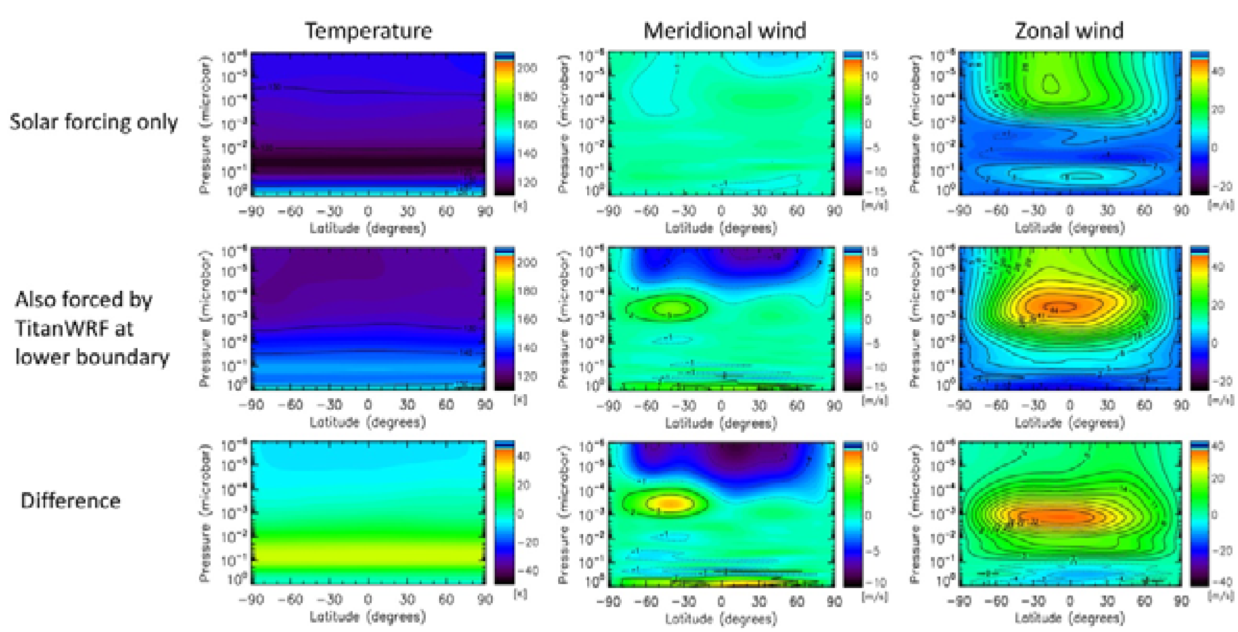

Figure 2. Impact on TGCM temperatures and winds of lower boundary TitanWRF forcing at equinox.

The next step was to convert this coupled model output into a 2D (latitude-altitude) transport circulation and parameterization of horizontal mixing, as needed by the 2D chemical-transport model (KINETICS). This process revealed mismatches between the lower and upper atmospheric predictions at the 1 Pa level, suggesting that the TGCM had not been forced correctly by the TitanWRF output. We have found that the error involved output from TitanWRF being applied to latitudes in the opposite hemisphere, due to the models' latitude grids being reversed. The coupled model is now being re-run with the correct forcing for all seasons.

In parallel, TitanWRF has now been run with a surface topography map and an active methane cycle for several Titan years, which produces different circulations and different results at the 1 Pa level used to force the TGCM. Hence, the output from these more realistic simulations is now also available to force the TGCM's lower boundary. Future work will include raising TitanWRF's upper boundary to ~600km to fully capture haze processes. This will involve the addition of appropriate physics (e.g. UV heating and molecular diffusion) for the ~400-600km region, and will enable more realistic coupling between the models (now at ~600km).

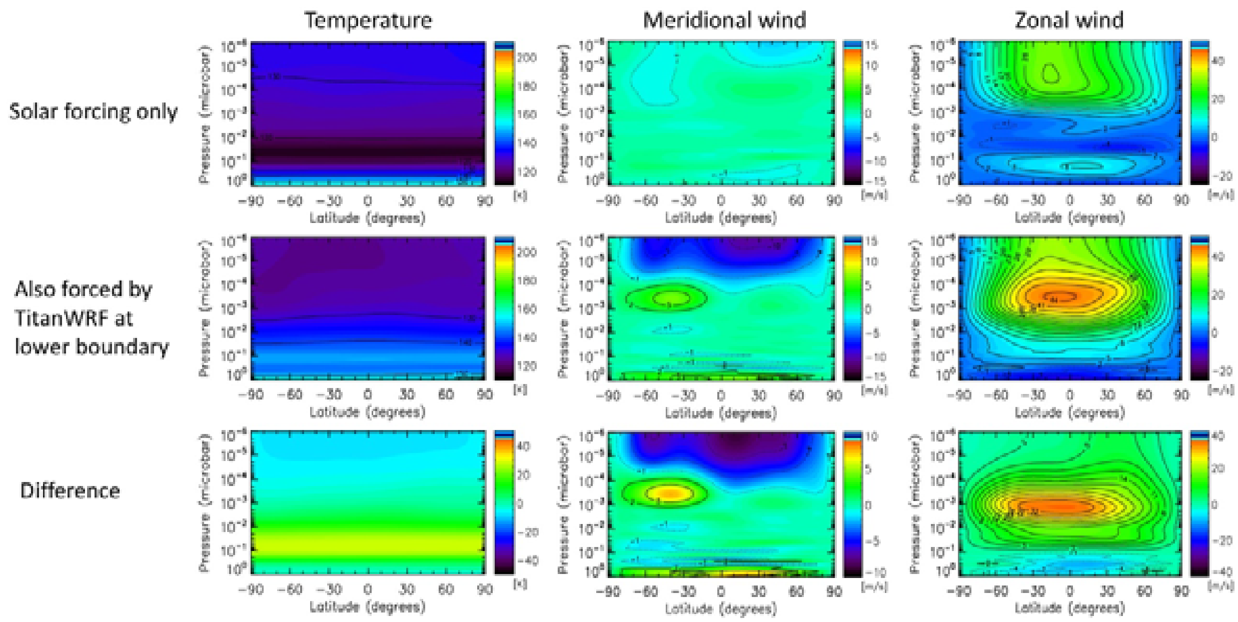

Figure 3. Impact on TGCM temperatures and winds of lower boundary TitanWRF forcing at equinox.

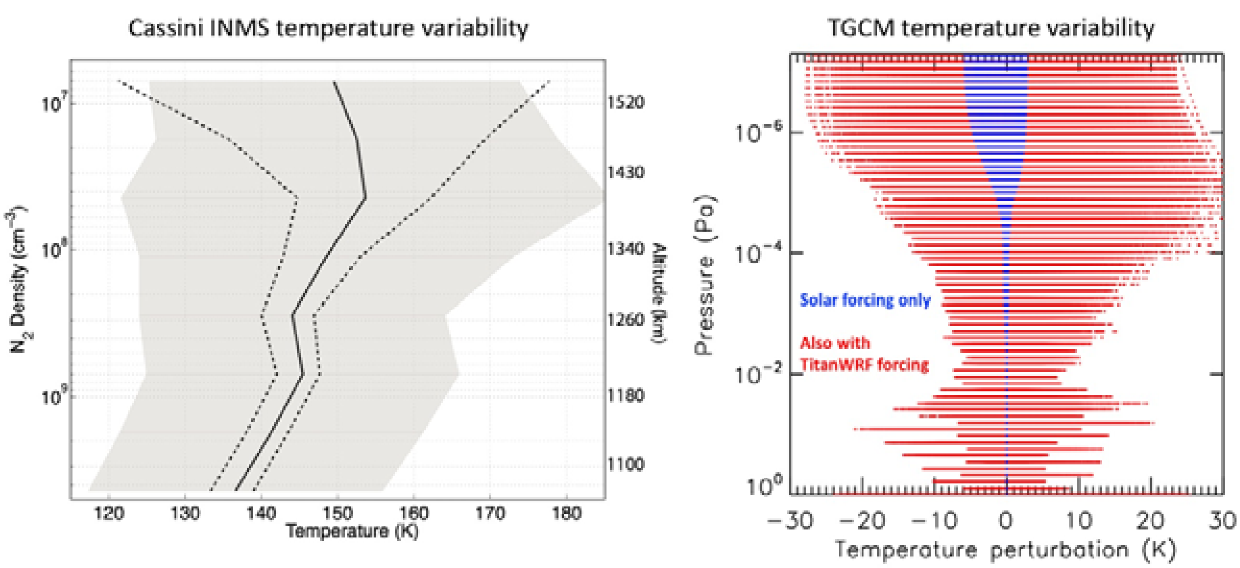

Figure 4. (left) Temperature variability measured by Cassini INMS over one flyby. (right) Temperature variability simulated for the same season and same range of times and locations by the TGCM with (blue) only solar forcing and (red) the addition of TitanWRF forcing at the lower boundary of the TGCM.

Observational Measurements of Titan's Atmosphere: Analysis of ALMA data continued, including observations made by us in 2016 and 2017, and additional archive datasets. The latter resulted in the first observation of the CH_3D molecule at sub-millimeter wavelengths (Thelen *et al*. 2019, Fig. 5). Although CH_3D has been observed on Titan previously in the infrared, this finding will allow us to map any temporal changes in the stratospheric distribution of methane using ALMA, which currently provides the best spatial resolution for measuring molecular abundances, providing potential insights into dynamics and mixing that will help to further constrain photochemical models.

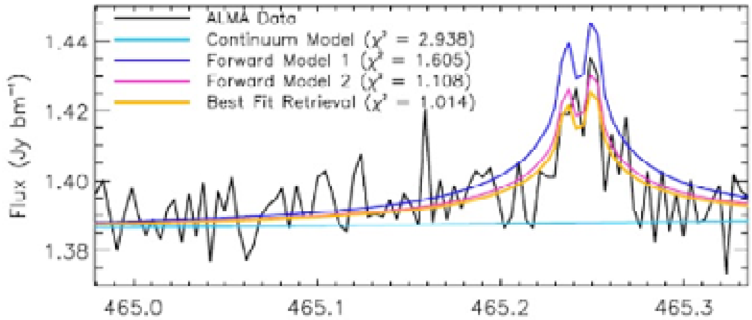

Figure 5. First detection of CH_3D on Titan at sub-millimeter wavelengths (Thelen *et al.* 2019).

Spatial and seasonal changes in Titan's gases from the final years of the Cassini mission were the subject of several papers, using data from ALMA (Cordiner *et al.*, 2019) and CIRS (Teanby *et al.*, 2019, Lombardo *et al.*, 2019a). Our ALMA data reveals differences in the distribution of gases, for example between HCN and HNC (Fig. 6), with HNC being concentrated at higher altitudes, and also showing a longitudinal asymmetry, which may be evidence for a very short lifetime (less than one Titan day).

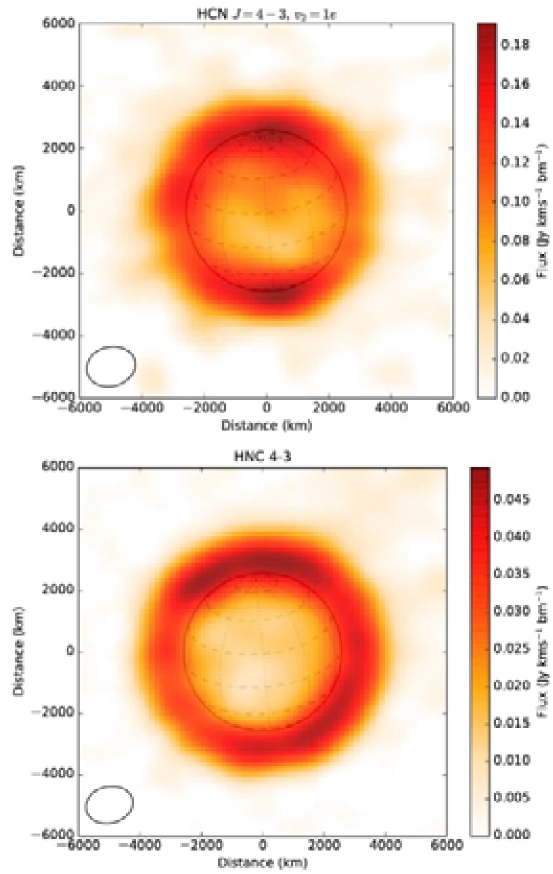

Figure 6. Flux maps of nitriles in Titan's atmosphere measured by ALMA in 2017 (Cordiner *et al*. 2019). Top: HCN. Bottom: HNC. These maps show substantial differences in distributions, with HNC emission originating at higher altitudes, and also displaying a surprising longitudinal asymmetry.

We made the first detection of the propadiene molecule (CH_2CCH_2) using data recorded at the NASA IRFT in 2017 with the high-resolution infrared TEXES instrument (Lombardo *et al*., 2019). This molecule has not previously been observed in space, and the ratio between propadiene and its isomer, propyne (CH_3C_2H) may have important implications for the abundance of atomic hydrogen in Titan's atmosphere. We were also successful in winning new observation time on ALMA and SOFIA, and look forward to analyzing and publishing more results in coming years.

Investigation 1.2: Molecular Transport across Titan's Surface (Lead: Alex Hayes):

To identify locations of astrobiological interest, we need to understand the production, transport, and modification of organic materials across Titan's surface. 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. 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.

Landscape evolution model: The landscape model we are developing utilizes numerous modules that are individually responsible for modeling the physical/chemical weathering of rocks into a transportable regolith, mass wasting by non-linear creep, fluvial channel erosion, and fluvial transport and deposition.

While these modules have been successfully applied to understand erosion and deposition on the Martian surface many times, and recently across the solar system, they are not sufficient to capture the full complexity of Titan's surface. Specifically, there is ample evidence for at least a polar groundwater water aquifer, while most interestingly, there may be both dissolvable and non-dissolvable bedrock in close proximity on the surface. This necessitated the development of two additional modules to capture the effects of groundwater fluid and solute transport and dissolution erosion.

Some extra work was needed, as we could not simply add modules to the existing DELIM model without complications. For instance, there is a vast gap in temporal and spatial scales over which these processes act, where the timescale of groundwater flow is many orders-of-magnitude larger than the time it takes to dissolve a given volume of bedrock. Further, for the Titan environment nearly all the parameters that go into these calculations are unknown or poorly constrained. While some tasks of this NAI project will better measure properties of some materials we think are on Titan, the reality is that Titan's surface will be more complex.

To tackle these many issues requires many simulations that sweep across broad parameter spaces. The previous framework of the DELIM model, though more than sufficient to model processes on Mars, was not capable of performing such massive calculations over reasonable timescales, necessitating a re-write and optimization of the underlying source code. Over this past year, we have performed this complete rewrite and modernization of the code. This involved many additions to DELIM, such as a new parallelization of how material is routed across the surface, a new module to describe groundwater flow, and the addition of a dissolution routine.

The flow routing routine is novel and has broad applications beyond Titan, as it allows efficient parallelization of the model not previously available. The latter two of these were equally significant additions, as the new DELIM model is now the only such landscape evolution model that tackles both dissolution and mechanical erosion simultaneously. Therefore, we now have the robust model we need for applications to Titan.

<u>Global geological map of the surface</u>: We have published the first global geomorphologic map of Titan (Lopes *et al*., 2019, see Fig. 2), which will serve as a constraint for the landscape evolution model by showing how sedimentary and depositional materials are distributed over the surface. The map used data from Cassini RADAR and VIMS and shows significant latitudinal dependence of the major geomorphologic units. Equatorial regions are dominated by vast dune fields and the mid-latitudes are dominated by plains, while the lakes and labyrinth units are found primarily in the polar regions. This is possibly related to more humid conditions in the polar regions. The hummocky unit, interpreted as exposed crustal materials, is seen at all latitudes, but primarily in the equatorial Xanadu region, for reasons yet unknown. In terms of composition, the Cassini emissivity data are consistent with organic materials forming the plains, dunes, lakes, and labyrinth units, and a higher abundance of water-ice materials in the crater and hummocky units. Relative ages and distribution of the major units imply that Titan's old, icy crust (hummocky materials) has been covered by sedimentary materials like the dunes and plains, particularly at lower latitudes, with the exception of Xanadu region. In the polar regions, where cumulative rainfall outpaces infiltration/evaporation of liquids, lakes are abundant. Labyrinth terrains, which are older than plains and mostly located at higher latitudes, may have begun as uplifted or otherwise elevated terrains, predominantly of organic deposits, that later became heavily incised and dissolved by rainfall, like karstic formations on Earth.

<u>Updated estimate of organic materials</u>: Obtaining an updated estimate of the amount of organic materials on Titan is important as a constraint on the amount of chemical energy and building blocks available for potential life. In addition, by backward calculating using the production rates of photochemical models we can constrain the degassing history of Titan and obtain an integrated time of organic photochemical production based on observed deposits. This will allow us to determine if Titan's methane outgassing and photochemical production has been present for a long period of time, or is a relatively recent phenomenon.

Previous estimates by Lorenz *et al*. (2008) only considered the liquid lakes and dune materials based on partial coverage of Titan's surface as the Cassini mission was still in progress. Recent mapping and more detailed investigations of labyrinth terrains (Lopes *et al*., 2019; Malaska *et al*., submitted) with the completion of the Cassini mission and the final (although partial) coverage of Titan's surface, we carried out an estimation of Titan's organic deposits; this was presented as a table in Malaska *et al*., submitted. Our estimates show that Titan's largest repository of organics are the dunes, although the undifferentiated plains are also significant. Our work shows that the plateaus in the labyrinth terrains are a significant repository of Titan's organics due to their spatial extent and volume. The labyrinth terrains account for between 14-35% of Titan's organics, although these terrains only account for roughly 2% of Titan's surface area. The liquid hydrocarbon lakes account for a smaller fraction of organics compared to the other terrains; these account for between 6-16% of the total organics and are mostly methane, rather than higher order photochemical products such as ethane, propane or higher dissolved hydrocarbons.

Overall, we find that Titan has from 11.5 x 10^5 km^3 - 4.9 x 10^5 km^3 organics at the surface. When divided by the total surface area (8.3 x 107 km^2), this is the equivalent of a global layer 13.8 m – 5.9 m deep. This value includes both solids (dominant) and liquid (minor) photochemical products. Further refinements are in progress, particularly in the extensive polar scalloped terrain units as well as other terrain units that may have less organic overburden. These estimates suggest, when analyzed against the photochemical layers, that 1 Gyr of atmospheric photochemical production can deliver this amount of solid organics to the surface. Interestingly, that amount of photochemical production should have created a large amount of liquid hydrocarbons such as ethane and propane. The observed dearth of these compared to predicted rates suggests that either 1) the photochemical ethane: solid branching ratio is incorrect or 2) there is a large unseen reservoir of ethane sequestered in the subsurface. Furthermore, if ethane is sequestered in the subsurface, other organic materials, particularly those that could dissolve in ethane or propane, may also have been transported and sequestered in the surface. This provides the exciting possibility that higher organics have moved from the atmosphere, to the surface, and down into the icy crust, at least the shallow subsurface. Thus, this work also provides some inputs into Investigation 1.3 for transport deeper into the subsurface and ocean.

Investigation 1.3: Molecular pathways: Surface to ocean (Lead: Christophe Sotin)

We have investigated the effect of a clathrate layer capping Titan's icy crust. Clathrates have been shown to form quickly in Titan's surface conditions from the reaction of liquid ethane with water ice (Vu *et al*., 2020). Because clathrates have a thermal conductivity an order of magnitude lower than pure water ice, they act as an insulator. We have performed a series of numerical simulations investigating the effect of this layer on the convection pattern in the stagnant lid regime. First, the thickness of the stagnant lid, and therefore the thickness of the lithosphere is dramatically reduced by the presence of a clathrate layer. In the example shown in Figure 7,

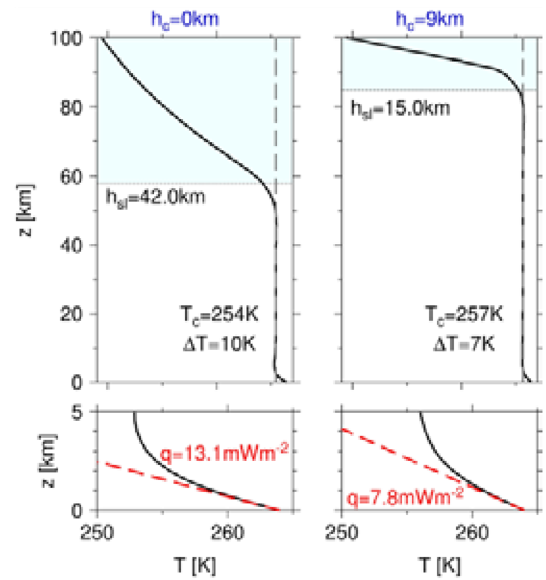

Figure 7. The presence of a clathrate cap dramatically reduces the thickness of the stagnant lid from 42 km (no clathrate, upper left panel) to 15 km (9 km thick clathrate cap, upper right panel). It also reduces the amount of heat that can be transferred by convection by 40% (compare the two lower panels), such increasing the lifetime of an ocean on Titan.

the stagnant lid thickness is 42- and 15-km without and with a 9-km clathrate layer, respectively. Second, the amount of heat that can be transferred by convection is reduced by about 50% from 13.1 mW/m^2 down to 7.8 mW/m^2. These results have profound implications for modeling Titan's evolution. The results have been presented at the DPS-EPSC meeting (Kalousova and Sotin, 2019) and a paper will be submitted soon to GRL. The results above will be used for task 1.3.1 (fracture depth and porosity) which will drive the flux of organics that can be transferred to the convective region.

Investigation 1.4: Habitats resulting from molecular transport (Lead: Steve Vance)

Progress towards understanding the effects of thermal evolution on the rocky interior of Titan has enabled us to constrain the composition of volatiles exsolved from the interior and that may have migrated vertically to build up the ocean early on in Titan's history. Vance and Melwani Daswani performed accretion models of Titan with their code AccretR (https://github.com/mmelwani/AccretR) to resolve the possible bulk composition of the body. They found that the bulk composition of Titan is a mixture of CI chondrite and cometary material, in line with Néri *et al.* (2020). They then used Gibbs energy minimization code Perple X (Connolly, 2009) to calculate phase equilibria in the interior of Titan, and have discovered that during thermal excursions caused by radiogenic heating and tidal dissipation, the amount (Figure 8) and composition of exsolved volatiles (CH_4,

H_2O, H_2, CO_2, Figure 9) varied markedly. Specifically, they found that hydrocarbons and organics (e.g., carboxylic acids) were probably exsolved from the rocky interior at low temperatures, signifying that they may have been released from the interior to build up the ocean early in Titan's history (Melwani Daswani & Vance, 2019b, 2019a). At moderate to high temperatures, water was released from the dehydration and dehydroxylation of phyllosilicates, and at 400 – 600 °C, carbonates were destabilized, releasing CO_2 into the fluid phase. Thus, the ocean's chemistry is heavily influenced by the thermal evolution of the interior. Thermal evolution pathways are thus distinguishable from each other if we are able to resolve the ocean's composition directly or indirectly in future observations. Additionally, the extraction of volatiles affects the properties of the solid interior, such as the density (Fig. 10). Some results will also feed into Objective 4, since some of the fluids exsolved from the interior at moderately high temperature were hydrogen rich.

While results have been able to constrain the composition of the volatiles exsolved from the rocky interior as a result of thermal evolution, the absolute amount of volatiles that migrate from the interior into the ocean remains poorly constrained. In future work, we will explore the parameter space of retained versus extracted volatiles. Thermodynamic data for volatile-mineral equilibria at >6 GPa remains inadequate. We have extrapolated available data from the Deep Earth Water model (Huang & Sverjensky, 2019) to simulate conditions at the higher pressures of Titan's interior, but the reasonableness of this approach can only be tested with further thermodynamic data derived from experimental studies.

<u>Modeling of water-hydrocarbon mixtures using CRYOCHEM:</u> Work lead by Co-I S. Tan (Tan *et al*., 2019) using the CRYOCHEM code now successfully allows chemical modeling of both the hydrocarbon-rich condensed fluid phases and the water-rich condensed fluid phases (and vapor phases, too) simultaneously. Previously, for accurate modeling, it was only possible to model one or the other, either methane-rich or water-rich. This is a significant result, as one model is now able to do both at the same time, and the applications can be broadened to a wider range of pressure-temperature conditions, and to various planetary bodies. For Titan, it allows us to model the ocean (water phase with dissolved hydrocarbons) and equilibrium condensed fluid and vapor hydrocarbon phases (that contain dissolved water and water vapor).

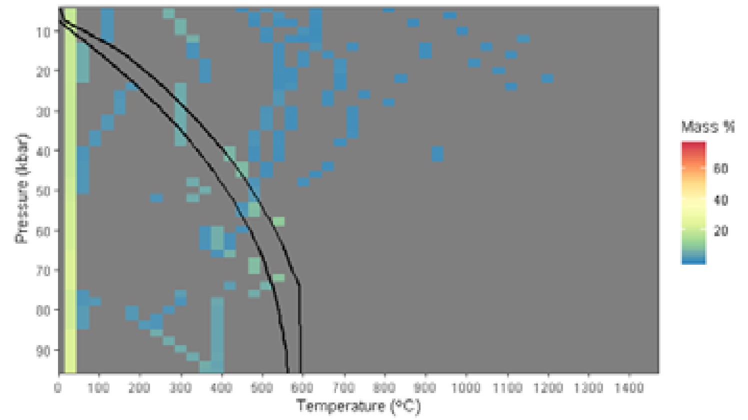

Figure 8. Mass % of fluids extracted from different depths and temperatures within the interior. Black curves are possible geotherms within Titan from Vance *et al.* (2018).

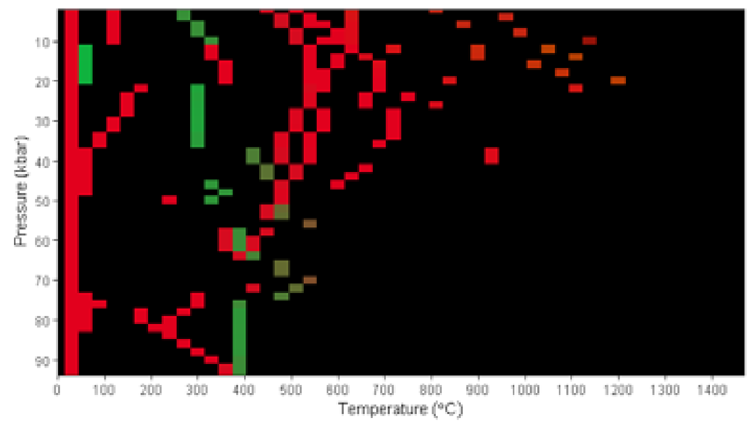

Figure 9. Color composite summarizing the composition of fluids exsolved (cf. masses of fluid exsolved in Fig. 1). Red = oxygen-rich, green = carbon-rich, blue = hydrogen-rich. The oxygen-rich cells are mainly H_2O and CO_2. The carbon-rich cells are mainly methane and other hydrocarbons.

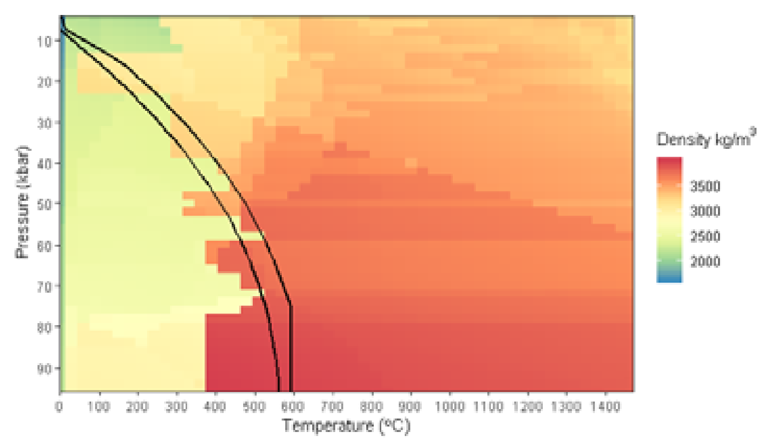

Figure 10. The extraction of volatiles changes the physical properties of the rocky interior of Titan, such as the density, shown here. Black curves are possible geotherms for modern Titan from Vance *et al.* (2018).

Ocean Conditions and Habitability (Lead: Rob Hodyss)

Investigation 2.1: Ocean Habitats (Lead: Chris Glein)

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.

A review article (Glein and Zolotov, 2020, in press) covers several ocean worlds, with a major emphasis on Titan. It discusses the methane-based meteorological cycle including organic-liquid hydrocarbon interactions at the surface, as well as recent insights into the origin of Titan's enigmatic atmosphere. The intent of writing this article was to increase awareness of the science that has been accomplished at Titan, and to broaden interest in Titan from affiliate scientific communities. Glein also addressed the latter objective by giving invited talks featuring Titan at the Goldschmidt Conference (Glein, 2019a), and at the concluding meeting of the decade-long Deep Carbon Observatory program (Glein, 2019b). In a second task, Glein worked with Kelly Miller (Southwest Research Institute) to gain new insights into the origin of methane and nitrogen (N_2) on Titan by modeling D/H exchange between organics and water, as well as high pressure C-N-O-H fluid speciation in Titan's rocky core. Preliminary results from these projects were presented by Miller at major conferences (Miller *et al*., 2019a; 2019b; 2019c). They suggest an important role for organic compounds in the geochemical evolution of Titan's core, which may feed into the habitability of Titan's ocean. Finally, it was recognized that this theme of organic thermogenesis initially developed for Titan could have broader applications in the outer solar system, creating an opportunity to pursue the "beyond" part of this NAI project. Dr.

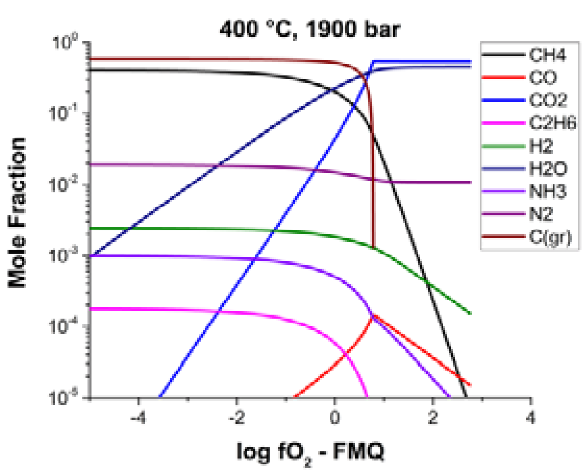

Figure 11. Speciation of cometary CHON matter mixed with CI chondritic rock and heated inside Pluto. These results suggest a large graphitic component accompanying thermogenic CH_4 and N_2. FMQ refers to the fayalite-magnetite-quartz buffer of oxygen fugacity (i.e., the oxidation state).

Glein has been collaborating with William McKinnon (Washington University in St. Louis) to explore the potential for accreted organics to generate the observed methane and nitrogen ices on Pluto (McKinnon *et al*., 2019). Figure 11 shows an example of how reducing conditions can favor significant production of both methane and nitrogen. This type of analysis represents a key initial step in constraining the contributions of hot volatiles to the potential habitability of oceans in organic-rich worlds.

Investigation 2.2: Ocean Organic Alteration (Lead: Rob Hodyss)

Investigation 2.2 concerns how organic molecules are altered in the subsurface ocean. While we expect the primary form of chemical alteration in the ocean to be hydrolysis, chemical alteration may also occur as organics are carried through the subsurface crust, presumably as solutes in cryogenic hydrocarbon fluids. Their solubility in these fluids will therefore be a critical parameter for both the quantity and identity of the organics that reach the subsurface ocean.

In order to determine the solubilities of relevant organics in cryogenic hydrocarbons under the temperature and pressure condition of Titan's crust, CoIs Hodyss and Malaska have partnered with collaborators at the University of Western Australia in Perth. Professor Eric May and Dr. Arman Siahvashi have developed several tools, initially designed for use by the petroleum industry, to understand solubility in cryogenic hydrocarbons. The first, the Joint Expert Speciation System (JESS), is an online hydrocarbon solubility database that draws from around 100 literature references [http://jess.murdoch.edu.au/cryobase.shtml]. The second, ThermoFAST, is a new software tool for prediction of solid formation and solubility in liquid hydrocarbons [https://www.fsr.ecm.uwa.edu.au/thermofast-full/]. Both JESS and ThermoFAST are freely available online. As well, Prof. May's laboratories include a number of unique experimental systems that can be used for the determination of solubilities under Titan subsurface conditions. CoIs Hodyss and Malaska have been working with Prof. May and Dr. Siahvashi to identify Titan-relevant organics to be studied with these systems (acetonitrile and acetylene are the first two candidates), and to utilize the existing databases and software to help constrain Titan lake and subsurface chemistry.

This work also relates to Investigation 1.2, Molecular transport across Titan's surface. Investigation 1.2 deals with the transport and modification of organics materials as they move across Titan's surface. Specifically, in Task 1.2.2, the solubilities of various organics in cryogenic liquid hydrocarbons representative of Titan's seas lakes are used in surface transport models and in theoretical calculations of lake composition. The JESS database, ThermoFAST, and the experimental work being conducted at the University of Western Australia will directly contribute to this task's goals.

Additionally, work performed by collaborator Morgan Cable and CoI Robert Hodyss elucidated the formation of a co-crystal between acetylene and butane (Cable *et al.*, 2019). Figure 12 shows an image of the co-crystals obtained in the laboratory. Modeling suggests that these two organic molecules may be the main components of evaporite deposits in Titan's dry lake beds. This new organic mineral could play important roles in landscape evolution on Titan. Understanding Titan's surface composition and the processes that shape the landscape are an important goal of Objective 1. This work was featured in an article in Chemical and Engineering News (https://cen.acs.org/physical-chemistry/astrochemistry/Newly-found-organic-mineral-influence/98/i3).

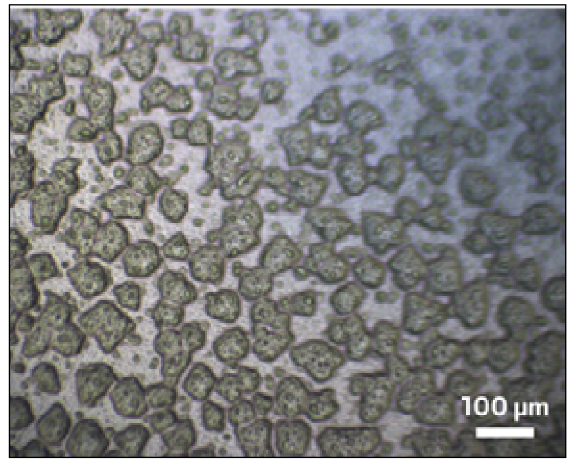

Figure 12. Image of the co-crystals between acetylene and butane obtained in the laboratory (Cable *et al.*, 2019).

Oceanic Biosignatures

The Goal of Objective 3 is to determine what biosignatures might be produced if Titan's subsurface is inhabited. In Investigation 3.1 (Oceanic Biotic Survivability and Growth, Lead: D'Arcy Meyer-Dombard) we will determine if microorganisms can survive and grow in Titan's subsurface habitable zones. In Investigation 3.2 (Oceanic Biosignatures, Lead: Fabien Kenig) we investigate what biosignatures could be produced in Titan's subsurface habitable zones. We will use the experiments conducted in Investigation 3.1 to determine the isotopic and molecular biomarkers that result from the enrichment cultures, and understand the biochemical basis of their production.

Investigation 3.1: Oceanic Biotic Survivability and Growth (Lead: D'Arcy Meyer-Dombard)

The investigation began with identifying potential piezophiles, hyperpiezophiles, psychrophiles, and halophiles that can be used in our 'Adaptive Laboratory Evolution' (ALE) protocol. In short, 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 known 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 started by purchasing strains of Bacteria and Archaea that are available in culture collections of the American Type Culture Collection (ATCC), or the German collection Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Currently, we are cultivating the strains in the table below.

Organism	T range	P range	Notes
Shewanella oneidensis	optimum 30 C	up to 1.5 GPa	used previously in ALE work
Shewanella frigidimarina	<0 to 27C	unknown	up to 9% NaCl
Colwellia psycrerythraea	0 to 18C	unknown	other strains are pieozophiles
Pyrococcus 'Pikanate 5017'	80-108C	20-120 MPa	non-methanogen Archaeon
Halorubrum lacusprofundi	-1 to 40C	unknown	Antarctic halophile;1.5-4.5M NaCl

We are also obtaining strains from private laboratory collections, and using samples obtained from relevant Earth analog environments to enrich our diversity of extremophiles. For example, we are currently working to isolate Bacteria with high pH tolerance which may also tolerate high concentrations of ammonia salts, as predicted to be present in the ice of Titan. This isolation effort is underway, and proceeding as planned.

Plans for moving forward in the short term [January-April 2020] include beginning the low temperature and high salt ALE training on the strains in the table above. We are ready to begin this work. We are also preparing extractions of DNA and cellular membrane materials [lipids] for analysis. For strains that lack recently sequenced genomes, we will use the extracted DNA to obtain genome sequencing.

Investigation 3.2: Oceanic Biosignatures
(Lead: Fabien Kenig)

Investigation 3.2 has passed through the development stage since the last update. This investigation involves building and testing the very high pressure culturing chamber [VHPCC] that will be needed to achieve growth of organisms at the pressures relevant to Titan's subsurface. A specific requirement of the VHPCC for this project is a large sample volume (tens of mL, equivalent to several test tubes), unusual for high-pressure experiments but required to produce sufficient biomass to carry out our intended analyses. We have recruited a promising postdoctoral researcher, Olivier Bollengier, who is an expert in high pressure experiments, to help with this investigation as well as Investigation 3.1. Dr. Bollengier began at the University of Illinois at Chicago (UIC) in September, 2019. In the time he has been at UIC, the design of the VHPCC system has been completed and the order submitted to Harwood Engineering. This initial order includes two pressure vessels to begin simultaneous experiments. The initial design of the sample holder inside the pressure vessels has been revisited to accommodate for specifics of high-pressure experiments (variation of volume and sealing of the samples). The stainless steel sampling and media reinjection solutions offered by Harwood Engineering are currently on hold as we are looking for affordable titanium-based alternatives more compatible with the Titan chemistry that will be explored later in the project. As of January 15th, the safety pressurization tests carried out by Harwood Engineering have been completed for the whole system but the pressure vessels; we are expecting a delivery on February 3rd. To save on costs and give our team greater flexibility in the use of multiple pressure vessels, Dr. Bollengier will handle the final assembly of the VHPCC upon delivery and will lead the testing of the instrument prior to beginning our high pressure culturing experimentation. In the interim, he has been learning the needed laboratory techniques for culturing extremophiles. We have also begun addressing the lipid biomarker portion of Investigation 3.2 – all strains will be subjected to lipid analysis via GC-MS.

To date, we have involved two graduate students [Judy Malas, Michael Tanzillo], and two undergraduate students [Gracie Fischer, Sarah Khoury] in the above work. Both graduate students will work on this project as part of their thesis work. Ph.D. candidate Judy Malas is also forging relationships with UIC researchers to extend our proposed work into the area of proteomics using mass spectrometry. These analyses will characterize and quantify the proteins produced at Titan conditions, providing additional context for biomarkers that may be found on Titan. Both "bottom-up" and "top-down" proteomic analyses are being planned, and each method provides a unique dataset. Bottom up refers to analyses conducted on the proteins after being digested into peptides, which will elucidate whether the amino acid residues change after the high-pressure treatment. Top-down analyses on the intact proteins will provide information on changes in tertiary and quaternary folding patterns of the proteins after high pressure adaption. The proteomes can then be compared with the genomes and transcriptomes of the organisms after they have adapted to high pressures to better understand how changes in the genome (if observed) affect changes in the proteome.

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)

This task focusses on development of quantitative models of the mechanisms of transport of biosignature-hosting materials from the ocean–ice shell interface to the surface. In the past year, progress has been made investigating convective transport through the deep ice shell (Co-I Sotin, collaborator Kalousova). Preliminary findings suggest that the presence of a layer of methane clathrate, which is highly insulating, in the shallow portion of the ice shell, allows for convective upwelling to approach much closer to the surface than when the clathrate layer is absent. Such convective transport has the potential to entrain liquid bodies from the ocean–ice interface, or develop partial melts during ascent, and deliver them close to the surface, where other mechanisms operating in the shallow crust might facilitate transport to the surface. (See also complementary work in Investigation 1.3.)

To that end, other work in the past year has included modeling of the tectonic response to Titan's diurnally-varying tidal stress field (graduate student Burkhard, Co-I Fagents). This approach combines the computation of tidal stresses in the ice shell from tidal potential theory, with the calculation of Coulomb failure conditions that allow strike-slip faulting. Early results from this work (Burkhard *et al.*, 2019, 2020; Figure 13) suggest that, in certain locations, the near-surface crust is capable of undergoing shear failure over a substantial portion of Titan's orbital cycle, provided that pore fluids (e.g., methane) are present in the subsurface. Investigations of several localities exhibiting hummocky or labyrinth terrains, indicate that such locations might experience failure conditions that might provide pathways for ascent of fluids in the near-surface ice shell.

We hypothesize that delivery of materials from the ocean–ice interface to the surface involves a combination of deeper, convective processes and shallow, brittle processes (fracturing). Examination of the variability of the brittle–ductile transition depth (BDT; post-doc Schurmeier) allows constraints to be placed on where brittle and ductile processes operate, and will allow coupling of results of convection modeling with inferences from brittle processes. Figure 14 shows the influence on BDT of the presence of methane clathrates in the ice shell, compared to BDT for pure ice. In agreement with the convection modeling of Co-I Sotin, the presence of clathrates is predicted to substantially influence

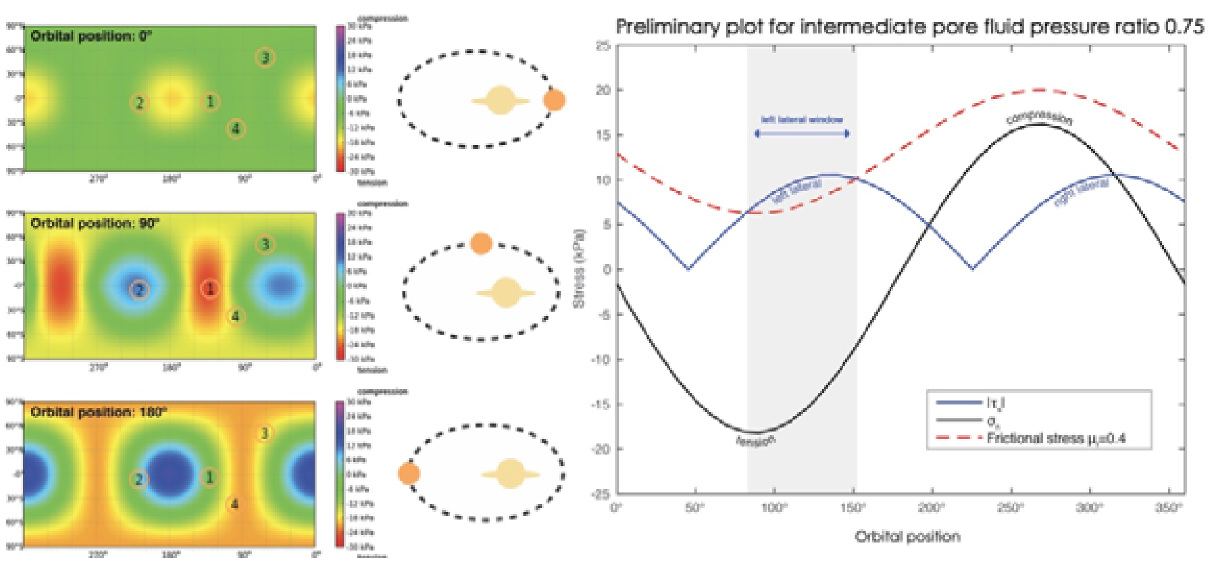

Figure 13. Left panel shows variation of tidal stress field in the ice shell during Titan's orbital cycle. Warm colors indicate tension, cool colors indicate compression. Right panel shows the normal stresses (black curve) and shear stresses acting on a fault plane (blue curve), as well as the friction stress (red dashed curve) for a coefficient of friction of 0.4 and a pore fluid pressure ratio of 0.75. The gray shaded box shows a window of left-lateral slip under tensional conditions where the Coulomb failure criterion is met (i.e., where the red dashed friction stress curve drops below the blue shear stress curve between ~90˚ and 150˚ orbital position). The plot represents location 1 on the stress map, Mithrim Montes in the Xanadu region, where parallel ridges are observed at a spacing of 25 km with an ESE orientation.

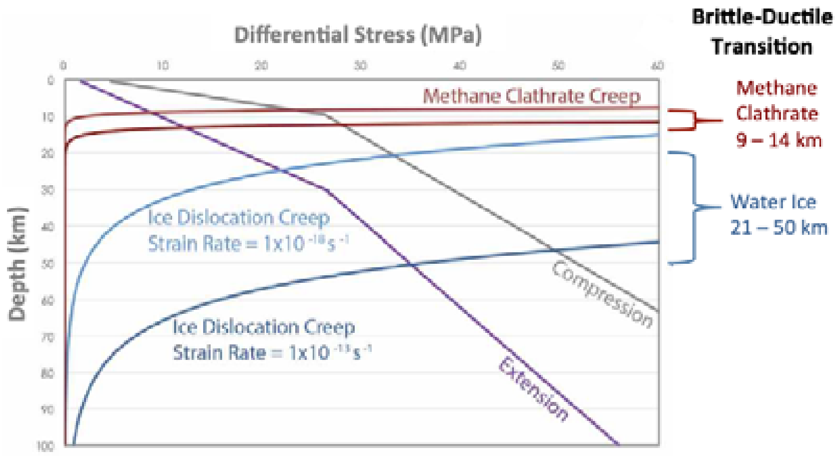

Figure 14. Titan's lithospheric strength envelope calculated for methane clathrate (red curves) and pure water ice (blue curves). The brittle–ductile transition occurs where the ductile creep curves cross the brittle failure curves (gray = compression, purple = extension), and is seen to be substantially shallower when the outer ice shell consists of methane clathrate.

where brittle vs. ductile transport processes can operate in Titan's ice shell.

In the upcoming year, we will continue exploring the parameter space in the convection, shear failure, and BDT modeling. In addition, we will examine the formation, evolution, and consequences of liquid bodies in the near-surface crust (Co-I Fagents, graduate student Brouwer, post-doc Schurmeier), whether entrained in convective upwellings from depth or produced *in situ* by heating of impurity-rich ice by thermal upwelling. Volumes fluxes, residence times, and pressure- and temperature-pathways of ascending materials will be passed to Investigations 4.2 and 4.3 to investigate molecular alteration and potential habitable environments en route to the surface from the ocean.

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Collaborations

Since the start of the work, we have added 19 Collaborators who were not in the original proposal, including 7 postdocs. We have also collaborated with 7 students. This proposal has catalyzed people interested in Titan and ocean worlds in general. Below is a list of our added Collaborators and students, and the people and Objective they are working with.

New Collaborators:

Klara Kalousova (Charles Univ., Prague) working with C. Sotin

Jonathan Lunine (Cornell) working with R. Lopes and others

Sam Birch (postdoc, MIT) working with A. Hayes

Martin Cordiner (GSFC) working with C. Nixon

Baptiste Journaux (U of Washington) working with J. Michael Brown

Alexander Thelen (NMSU/GSFC/CUA) working with C. Nixon

Orkan Umurhan (SETI) working with A. Hayes

Alvaro P. Crosta (Campinas Univ., Brazil) working with R. Lopes and M. Malaska

Mohit M. Daswani (postdoc, JPL) working with S. Vance

Olivier Bollengier (postdoc, UIC) working with D. Meyer-Dombard

Nick Lombardo (postdoc, Yale) working with C. Nixon

Morgan Cable (JPL) working with R. Hodyss

Olivier Bollanger (postdoc, U. Illinois) working with F. Kenig

Benoit Seignovert (postdoc, JPL) working with C. Sotin

Kelly Miller (SWRI) working with C. Glein

Ingo Mueller-Wodarg (Imperial College, UK) working with C. Newman

Laureen Schurmeier (postdoc, U of Hawaii) working with S. Fagents

Eric May (Univ. of Western Australia) working with R. Hodyss, M. Malaska

Arman Siahvashi (Univ. of Western Australia) working with R. Hodyss, M. Malaska

Elizabeth Silber (Brown University) working with R. Lopes and others

Graduate Students:

Marika Leitner (Cornell University) working with J. Lunine

Siteng Fan (Caltech) working with Y. Yung

Judy Malas (U. Illinois) working with D'Arcy Meyer-Dombard

Michael Tanzillo (U. Illinois) working with D'Arcy Meyer-Dombard

A. Schoenfeld (UCLA) working with R. Lopes and M. Malaska

Liliane Burkhard (U of Hawaii) working with S. Fagents

Gwen Brouwer (U of Hawaii) working with S. Fagents

Extended Scientific Directions

We had not considered the role of impact cratering in enabling material from Titan's surface to reach the ocean and, in 2018-19, started a collaboration with Prof. Alvaro Crosta from the State University of Campinas in Brazil. Prof Crosta is an expert on impact craters and a member of the Brazilian National Academy of Sciences. Prof Crosta spent a sabbatical at JPL in 2018-19, collaborating with the PI, Rosaly Lopes. Prof Crosta is working with Dr. Elizabeth Silber on modeling the formation of Titan's largest impact crater, Menrva and implications for habitability. Our preliminary runs produced an impact crater consistent with the dimensions of Menrva. Higher resolution simulations will be needed to better evaluate the final crater depth and compare to the observed values. The 75 km and 100 km thick ice shell breaks up during the crater collapse, establishing a pathway to the ocean. The 125 km and 150 km thick ice shells remain intact; however, there is significant amount of deformation and heating. The work was presented at LPSC and a manuscript is in preparation.

Flight Mission Involvement

The PI and several team members have had long-standing collaborations on Titan work with the PI and several Co-Is on Dragonfly. One new Collaborator on the NAI, Dr Morgan Cable of JPL, is a Co-I on Dragonfly. We plan to have a closer collaboration with Dragonfly team members and believe that our results will help Dragonfly with planning and interpretation of future results.

Europa Clipper
Team Member(s): Steve Vance
How are they involved: science team member

JUICE
Team Member(s): Rosaly Lopes (unfunded)
How are they involved: Co-I on JANUS camera

Mission Name: Mars 2020
Team Member(s): Rob Hodyss
How are they involved: science team member (PIXL)

Cassini (in close out phase)
Team Members: Rosaly Lopes, Mike Malaska, Alex Hayes
How are they involved: RADAR Associate Team members; Lopes also Investigation Scientist

Dragonfly
Team Members: Morgan Cable (Collaborator on NAI)
How are they involved: Dr Cable is a Co-I on Dragonfly

In the News

The Lopes *et al.* (2019) *Nature Astronomy* publication with the first geomorphologic map of Titan had very wide publicity worldwide. This paper was covered by more than 70 science and news outlets including *Nature*, *Scientific American*, the *New York Times* and CNN. The Titan map image released in NASA's planetary photojournal was picked up by the site Redbubble and is now available on 72 of their products.

Team Members

Rosaly Lopes
Jesse Beauchamp
Samuel Birch
Mark Boryta
J. Michael Brown
Martin Cordiner
Alvaro Penteado Crosta
Mohit Daswani
Sarah Fagents
Siteng Fan
Christopher Glein
Alexander Hayes
Robert Hodyss
Patrick Irwin
Paul Johnson
Baptiste Journaux
Klara Kalousova
Isik Kanik
Jeffrey Kargel
Fabien Kenig
Marika Leitner
Jonathan Lunine
Michael Malaska
D'Arcy Meyer-Dombard
Claire Newman
Conor Nixon
Ashley Schoenfeld
Florian Schwandner
Anezina Solomonidou
Christophe Sotin
Sugata Tan
Nicholas Teanby
Alexis Templeton
Alexander Thelen
Orkan Umurhan
Steve Vance
Véronique Vuitton
Catherine Walker
Karen Willacy
Yuk Yung

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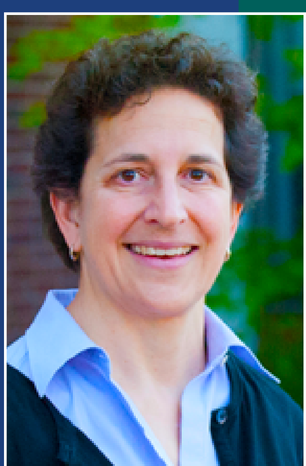
Astrobiology
Center for Isotopologue Research

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 is comprised of world-leading experts in isotopic studies and analysis who seek to advance the analytical and computational capacity to include organic isotopologues. These are molecules that differ by the position of rare isotopes within the structure. Our broader aim is to develop understanding of the biological and non-biological drivers for isotope patterns within molecules and to apply this rich resource of new information to potential biosignatures relevant to solar system and planetary exploration, and for studies of NASA-relevant Earth-life dynamics, both past and present. The team is comprised of researchers at Penn State, Caltech, University of Texas at El Paso, Lehigh University, NASA Goddard, UC Riverside, and the Earth-Life Science Institute (ELSI) at Tokyo Tech. This report highlights the following themes in isotopologue research:

ACIR Research Themes:

- Analytical Capacity
- Predictive Models
- Earth Fluids
- Organics, Ice, and Minerals

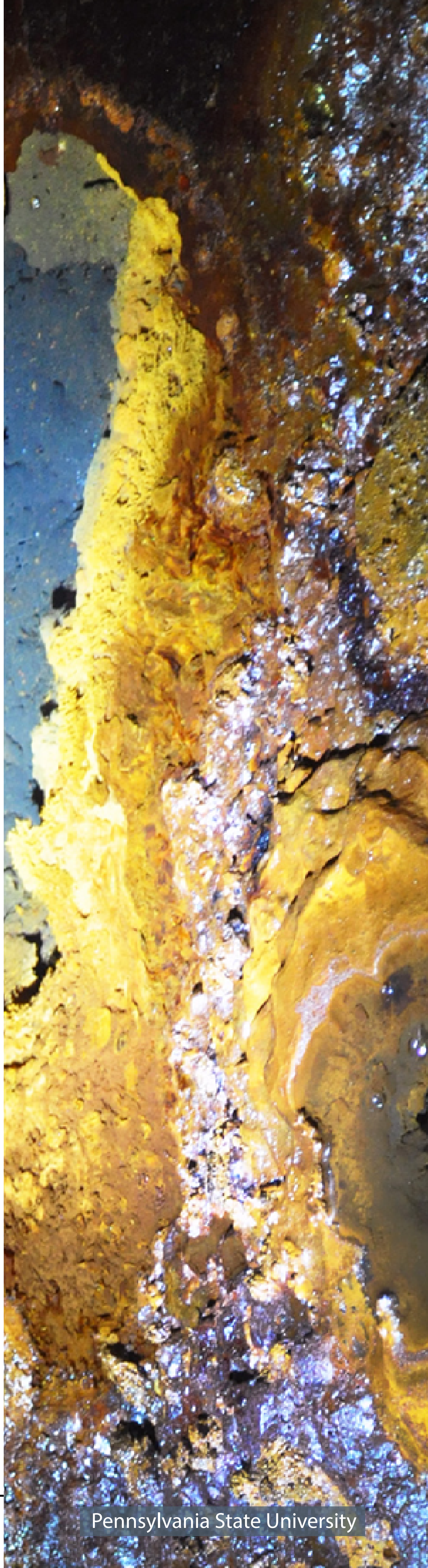
Team Website: https://astrobiology.nasa.gov/nai/teams/can-8/psu/index.html

2019 Executive Summary

The Astrobiology Center for Isotopologue Research (ACIR) is comprised of world-leading experts in isotopic studies and analysis. ACIR members seek to advance the analytical and computational capacity to include organic isotopologues, which are molecules that differ by the position of rare isotopes within the structure. Our broader aim is to develop understanding of the biological and non-biological drivers for isotope patterns within molecules and to apply this rich resource of new information to potential biosignatures relevant to solar system and planetary exploration, and for studies of NASA-relevant Earth-life dynamics, both past and present.

ACIR seeks to build the community of scientists working with isotopologues. Our community-building activities focused both inwardly, as we build our team, and externally, as we engage within the larger scientific community. Academic nodes of the ACIR team attracted new students, postdocs and staff to a diversity of research projects. A central priority of ACIR is to exchange scholars, methods, standards, and samples. In 2019, we made significant strides to have students and postdocs visit different institutions to learn new methods, including extended visits by PSU student Allie Fox to Nantes, and PI Freeman as a visiting scholar at Caltech. ACIR collaborators using computational models have actively engaged through virtual collaboration tools. Many of the exchanges and research connections were fostered by ACIR-sponsored gatherings at professional meetings and at our all-hands meeting at Penn State in the spring. Freeman co-led a session at AGU on multiple isotopes in molecules, which featured presentations by ACIR members as well as a panel discussion including ACIR investigators. The community-building event was capped by a dinner that ACIR co-sponsored for participants, including over 30 isotope scientists and students, who have interests in molecular isotopes and isotopologues. Finally, Alexander Sessions, with help from Wilkes, organized and ran the summer International Geobiology Course, a 5-week training course for graduate students and postdocs. Work with the course included organic stable isotope measurements, and discussion of position-specific isotope measurements.

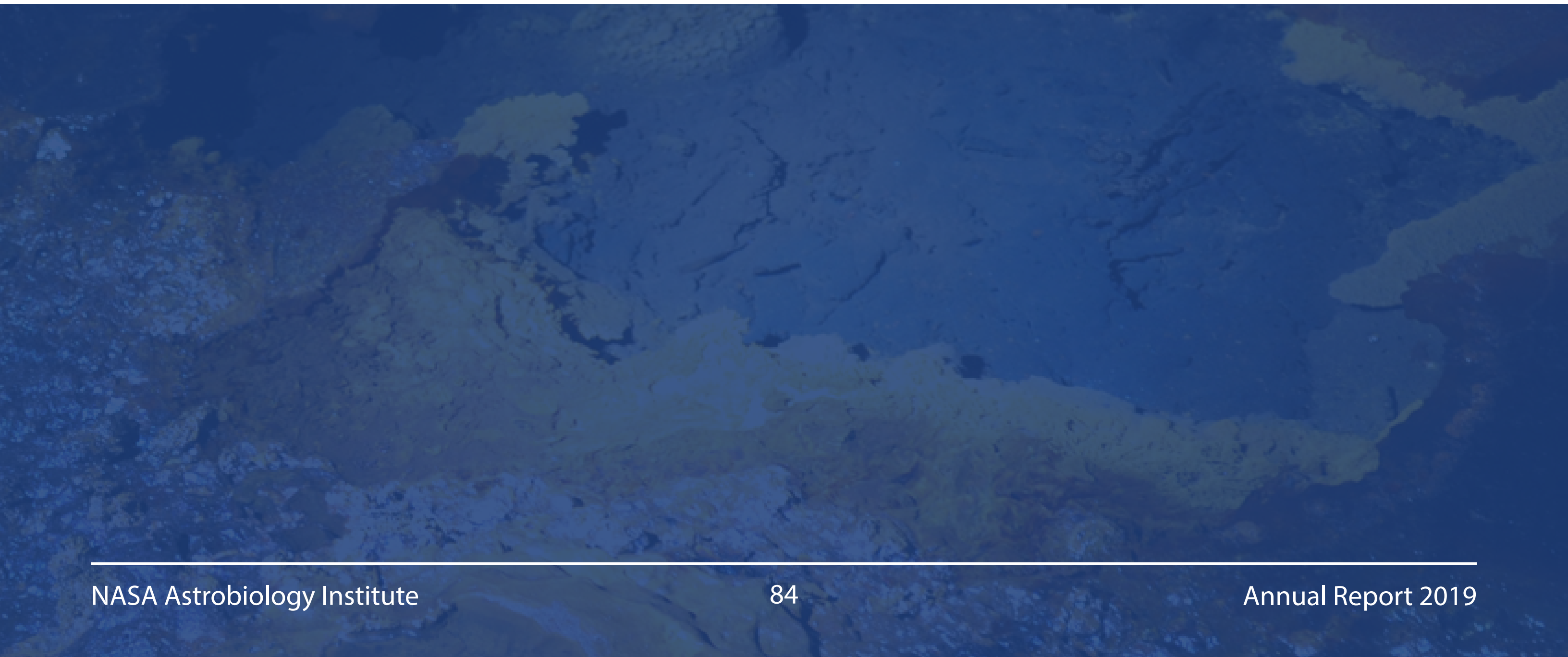
Scientific advances in 2019 built analytical and computational capabilities. As indicated in Table 1 below, ACIR members significantly expanded the diversity of analytical methods, and target molecules for analysis. These efforts were accompanied by acquisition of new instrumentation to build capacity for studies at more institutions and by more researchers. Significantly, the Tokyo team (led by Yoshida, Gilbert, and postdoc Maxime Julien) contributed a full suite of alanine standards, which are now being used for the first ACIR round-robin comparison. Researchers using density functional theory (DFT) models made advances on equilibrium calculations for position-specific isotopes in a suite of hydrocarbons and amino acids, and during sorption processes (Kubicki, Boettger, Watts, Fox).



Expanding application studies include the first data on the influence of photorespiration on isotope patterns within serine (Wilkes, Sessions), the alteration of organics in a high-radiation environment (Fox, Eigenbrode, Freeman), the first whole-molecular isotope studies of Ediacaran steranes (Pehr, Love, Baczynski, Freeman), isotopologue studies of alanine in the Murchison meteorite (Chimiak, Sessions, Eiler), and isotope studies of deep Earth fluids (McDermott, Dowd, Keele).

Table 1. Summary of compounds currently being investigated by the ACIR team for isotopologue analyses. Investigations have included analyses of ^{13}C, ^{15}N, ^{34}S, and ^{2}H.

Compound	Method	Team (PI)	Intended Application
Methionine	LC orbitrap	Caltech (JE, AS)	"M+1" ion method
Alanine	IRMS	Tokyo (NY, AG)	Standards
Alanine	Orbitrap (LC, GC)	Caltech& PSU (JE, KF)	Standards (planned for 2020)
Alanine	NMR	Nantes (GR)	Standards; Sorption studies
Serine, methionine	GC Orbitrap, IRMS	Caltech (AS)	Standards; Arabidopsis C-fixation
Serine	NMR	Nantes (GR, KH)	Sorption studies
Glycine	NMR	Nantes (GR, KH)	Sorption studies
Leucine	NMR	Nantes (GR, KH)	Sorption studies
Serine	NMR	Nantes (GR, KH)	Sorption studies
Phenylalanine	NMR	Nantes (GR, KH)	Sorption studies
Uridine, thymidine, adenine	LC Orbitrap	PSU (CH, KF)	Method development
Hexane and isoprene	Prep and isolation	Caltech (JE)	Method development
High mass (>500 u)	FT-ICR	Caltech (JE)	Method development
Inorganic anions	LC Orbitrap	Caltech (JE, AS)	Method development
Methylamines	Prep and isolation	Lehigh (JM)	Method development
Organic acids	Prep and isolation	Lehigh (JM)	Method development



Project Reports

Analytical Capacity

NAI funding in 2019 made possible widely expanding development of organic isotopologue analyses by the ACIR team (see Table 1 in the executive summary). Notably, progress was fostered for amino acids (and other electrospray ionization amenable compounds) using the "M+1" ion method developed by Neubauer, Eiler and Sessions (Neubauer *et al.*, 2018; *Int. J. Mass Spec.* 434, 276-286). This method also led to measurement of isotopes carried by inorganic anions in aqueous solution (Neubauer *et al.*, *Int. J. Mass Spec.*, in press). Progress with GC-Orbitrap includes new methods for serine and methionine, now used by the Sessions group to investigate intramolecular isotope patterns tied to photorespiration during carbon fixation.

Isotopologue standards are critical to progress with all analysis approaches, and a major goal this year has been new standards for the ACIR community and beyond. Team members at Tokyo Tech (Yoshida, Gilbert, Maxime Julien) contributed a full suite of alanine standards, which have been distributed for round-robin analytical comparison. Sessions' team has produced a serine standard as part of their method development efforts.

Continued pioneering isotopologue method exploration by the Eiler group included new studies using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). Expanding studies have also fostered new compound targets, including nucleotides (House), hexane and other hydrocarbons (Eiler), and methyamines and organic acids (McDermott).

The team has increased our capacity for isotopologue studies. Notably, new LC-MS at Caltech and PSU will foster molecular isolation and characterization. Penn State has acquired a new GC-Orbitrap, and members of the team have been exploring isotopologue studies for nucleosides with help from Caj Neubauer and two different LC-Orbitrap instruments on the PSU campus. Finally, the new isotope mass spectrometry labs funded by Penn State, leveraged in direct result of NAI funding, were completed late in 2019, with new and existing instruments being set up in the expansive facility.

Predictive Models

The results from density functional theory (DFT) computational methods powerfully aid understanding of experimental data. We applied DFT with the goal of distinguishing among position-specific equilibrium fractionation factors for H isotopes at primary, secondary, tertiary, alkene (sp^2), aromatic, and methyl C sites. We found distinct equilibrium fractionation factors for each type of substitution and this work will culminate in a manuscript from this collaboration between the groups of Freeman and Kubicki.

The DFT-calculated equilibrium ^{13}C NMR chemical shifts agreed well with infrared frequencies and corresponding data for guaiacol, ethanol, acetic acid, ethene, propane, butane, and isobutane. We next intend to relate the equilibrium DFT results to experimental data for similar compounds that are not at equilibrium, as is likely the case for organics in planetary environments regardless of their synthetic origin. This work involves a collaboration with the teams of Kubicki, Remaud and Yoshida groups in ACIR.

ACIR postdoc Jason Boettger modeled all 20 proteinogenic amino acids at different pH/protonation states and determined that equilibrium ^{13}C composition mostly varied with oxidation state and compared well with whole-molecule data in natural samples. The pH-dependent $\delta^{15}N$ equilibrium of amino acid groups may explain natural variability of amino acid $\delta^{15}N$ values. New modeling efforts focus on the effects of aqueous solvation and bond torsion conformational changes. Boettger has assisted graduate student Allison Fox using molecular models to study isotope effects on amino acid sorption to ice and clay surfaces.

Model combinations that most accurately predict equilibrium isotopic fractionation in gases are less computationally expensive due to a previously unrecognized error cancellation. Current work addresses anharmonicity and isotopic clumping in aqueous sulfate, isotopic effects of vanillin solvation and gaseous molecule solvation, central metabolites and organic acids, surface sorption of hydrocarbons, and application of models to enzymes.

Organics in Earth Fluids

Under the leadership of McDermott, the Lehigh team has been working on the investigation of saline brines and anoxic gases collected from 2.7 Ga banded iron formation bedrock within the Soudan Iron Mine. The goal is to understand how microorganisms establish and enhance habitability deep within continental fractured-rock systems. Highly saline fluids flow into the mine through boreholes accompanied by effervescent anoxic gases. Initial n-alkane abundance ratios, CH_4 $d^{13}C$ values (determined by PhD student William Dowd and presented at the Goldschmidt Conference in Barcelona Spain), combined with new $d^{13}C$ data for C1-C4 n-alkanes may reveal biotic and abiotic controls on total dissolved inorganic carbon species. Ongoing work is focused on the solid phase extraction of methylamine compounds and carboxylic acids as a target for position-specific isotope analysis, with the goal to elucidate their origins and any active processes that impact their cycling through a deep biosphere ecosystem.

Upcoming field efforts at the mine will focus on the drilling of a new ~100 m deep borehole. The Lehigh team welcomed a new Director of Instrumentation, Dr. Tesia Chciuk, in August and a new PhD student, Christopher Keele, in September. A funded research cruise to 9N East Pacific Rise planned for April 2021 (ROV Jason) will provide an additional opportunity to collect samples for organic isotope analysis in a deep-sea hydrothermal system.

Organics, Ice, and Minerals

Organic molecules could provide some of the best evidence for the presence of life on early Earth and potentially evidence of life beyond Earth. Planetary missions, such as to Mars, are often equipped to detect small organic molecules, although they cannot be definitively tied to life. Organics on Mars experience a harsh radiation environment as, unlike Earth, Mars lacks a magnetic field and is exposed to high radiation that can penetrate below the surface and alter or destroy organic material. A collaboration between the PSU (Freeman and student Allie Fox) and NASA Goddard (Eigenbrode) ACIR team members, sought to determine the potential alterations of complex organic molecules in different mineral matrices that had been previously exposed to high-energy radiation. We found that complex molecules had broken down into smaller organic acids and that similar products were produced with different initial organic sources and a variety of rock types. This work suggests radiation exposure can limit preservation of unaltered small organics, and thus future missions should target samples that have been protected from extensive radiation exposure.

In a collaboration between PSU (Freeman, Fox), UTEP (Kubicki, Boettger) and Nantes (Remaud), we have investigated the influence of surface sorption on organic and isotope fractionation for hydrocarbons and amino acids. Fox has used DRIFT spectroscopy to document bond vibrational energy shifts during sorption for comparison with DFT computational models. In addition, she has modeled amino acid sorption to water ice, and has spent several months using the NMR facility at Nantes to provide new data for comparison. While the isotope fractionation is modest, this work does point to the importance of site polarity and dispersive forces on the sorption surface.

Field Sites

Soudan Iron Mine
Minnesota, USA

McDermott led studies of saline fluids from the iron-ore mine. The mine first opened in the 19th century, but it is no longer a working mine. A new scientific borehole is planned for 2020 studies.

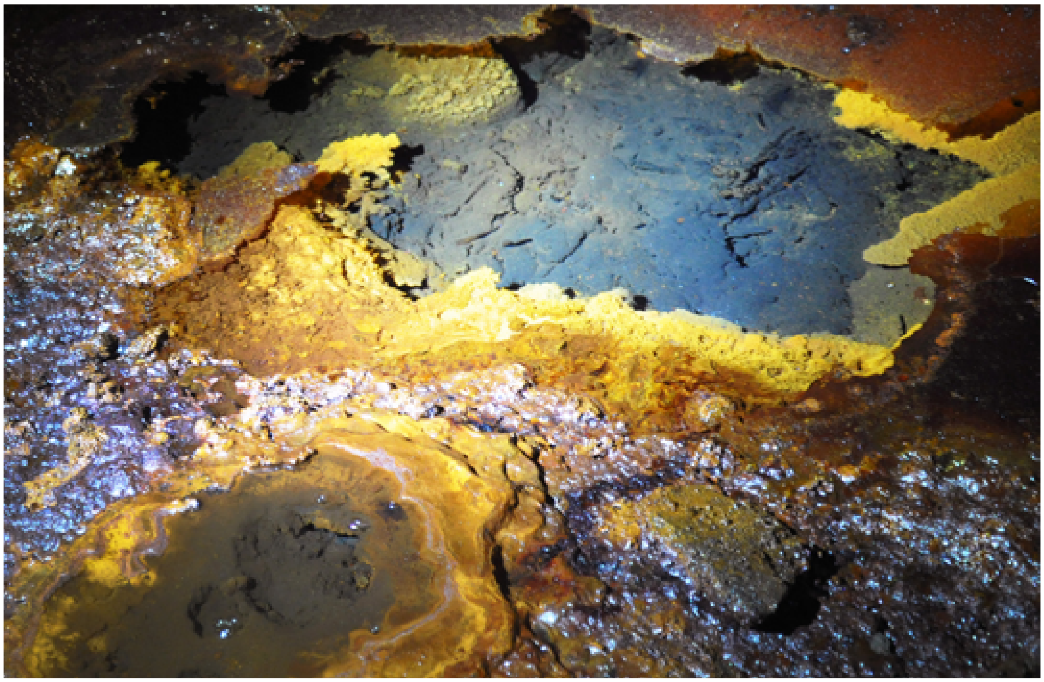


Figure 1. Highly saline brines outflow from boreholes drilled through the Neoarchean (~2.7 Ga) Wawa-Abitibi Subprovince of the Canadian Shield. Iron redox reactions form the basis for many microbial metabolic strategies, both in the oxic-anoxic interface at the fluid outflow point and at depth in the anoxic boreholes. (Photo credit: Jill McDermott)

Figure 2. Lehigh team members Jill McDermott and William Dowd collect fluid and gas samples that are free from ambient air contamination by using custom-built silicone and steel packer systems. (Photo credit: Brandy Toner)

Figure 3. William Dowd excavates a borehole opening in the mine floor. (Photo credit: Jill McDermott)

The Origins of Molecules in Diverse Space and Planetary Environments and Their Intramolecular Isotope Signatures: 2019 Publications

Fox, A.C., Eigenbrode, J.E. and Freeman, K.H. (2019). Radiolysis of macromolecular organic material in Mars-relevant mineral matrices. *JGR Planets* 124: 3257-3266. DOI: 10.1029/2019JE006072

Boettger, J.D. and Kubicki, J.D. (2019). Evaluating Computational Chemistry Methods for Isotopic Fractionation between CO_2(g) and H_2O(g). *Journal of Chemical Information and Modeling* 59(11), 4663-4677. DOI: 10.1021/acs.jcim.9b00392

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Heike Betz
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Andrew Hyde
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Christopher Keele
James Kubicki
Max Lloyd
Gordon Love
Jill McDermott
Alexandra Phillips
Gerald Remaud
Alexander Sessions
Heath Watts
Gabriella Weiss
Elise Wilkes
Fenfang Wu
Hui Yang
Naohiro Yoshida
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e·nig·ma

Evolution of Nanomachines In Geospheres and Microbial Ancestors

ENIGMA: Evolution of Nanomachines in Geospheres and Microbial Ancestors

Lead Institution:
Rutgers University

RUTGERS

Principal Investigator:
Paul Falkowski

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.

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/

2019 Executive Summary

The ENIGMA team significantly increased its strength; the team now has 47 members, including faculty, post-doctoral fellows, graduate and undergraduate students. Several students have applied for a graduate degree to study astrobiology at Rutgers. The post-docs are being cross mentored in the three themes (Fig. 1). The entire ENIGMA team met in May 2019 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. Together with our colleagues at Rice University, we showed that an artificial symmetrical ferredoxin actually can function in vivo (Fig. 2). The work was published in *PNAS* (Mutter, 2019). Simultaneously, we have developed novel peptides that can bind iron sulfur clusters and are catalytic. Structural analyses of the metallopeptides are being conducted with our colleagues at UCSD and in Israel. 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 *Escherishia 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 two primordial metal-protein folds that likely served as building blocks for complex electron transport pathways critical for the evolution of 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 sequence variation in naïve protein libraries. We have demonstrated simple protein folds are capable of iron-

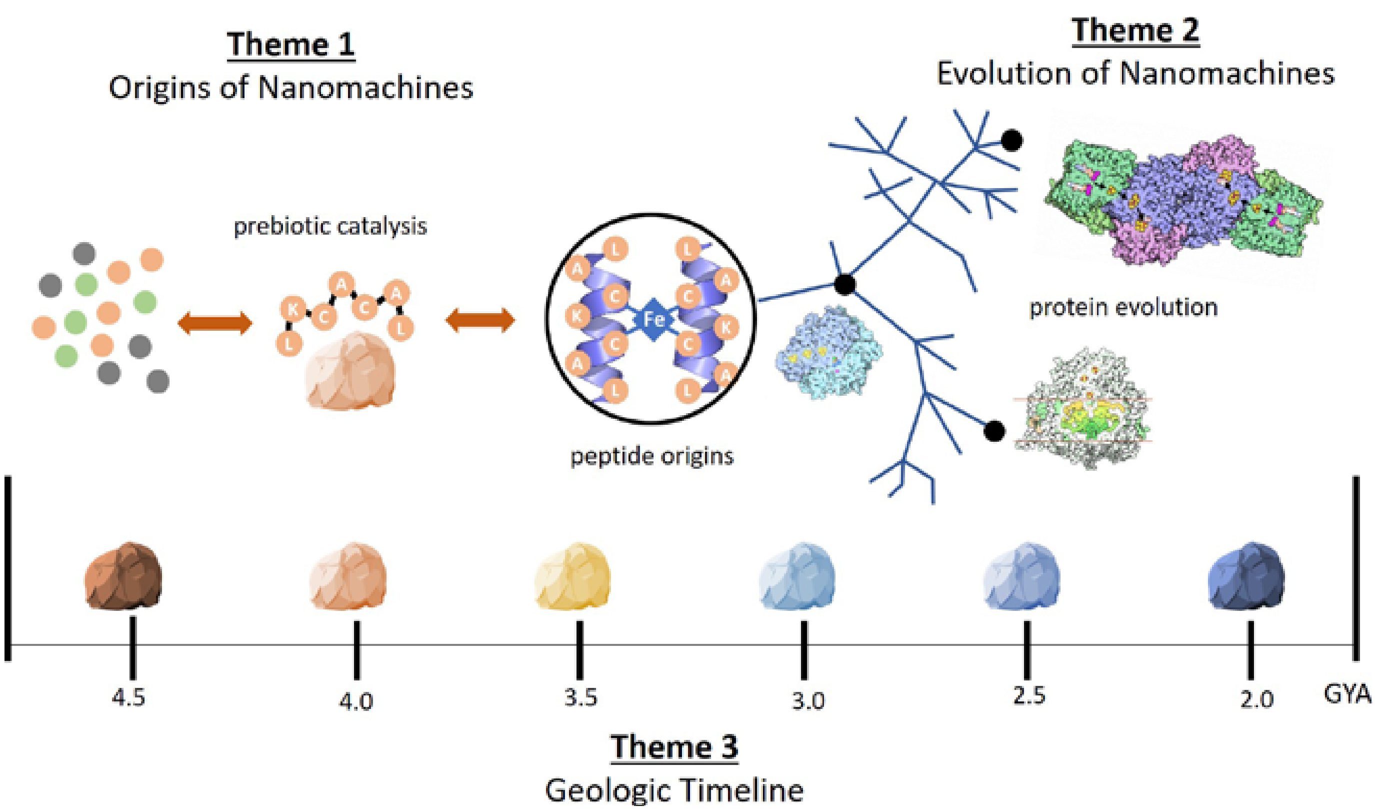


Figure 1. ENIGMA: Evolution of Nanomachines In Geospheres and Microbial Ancestors. This NAI team will explore the catalysis of electron transfer reactions by prebiotic peptides to microbial ancestral enzymes to modern nanomachines, integrated over four and a half billion years of Earth's changing geosphere. 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

sulfur cluster binding, suggesting that the evolution of metallopeptides/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 from sequence. Working with NPP fellow Adrienne Hoarfrost and post-doc Ariel Apetekmann, they are building a deep learning architecture that captures the functionally relevant features of microbial communities.

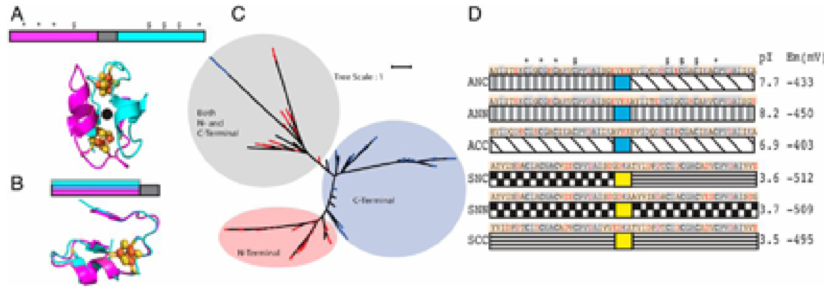


Figure 2. Structural symmetry and consensus designs. (A) Color blocked diagram of bacterial ferredoxin's asymmetric sequence accompanied by a cartoon representation of PDB ID 1FDN with axis of symmetry highlighted by a dot, N- and C-terminal colored magenta and cyan, respectively. (B) Structural alignment of N and C structures showing structural symmetry of the parent protein. (C) Unrooted maximum-likelihood phylogenetic tree of N-terminal motif (red) and C-terminal motif (blue) of ferredoxin. Bootstrap value of each node is >54. (D) Color block diagrams of asymmetric vs. symmetric designs with consensus sequence aligned above each diagram with negative and positive residues colored red and blue, respectively, along wvith highlighting for symmetric core residues (gray) and variable outer-shell residues (orange), * and $ indicate cluster binding. Credit: Andrew Mutter.

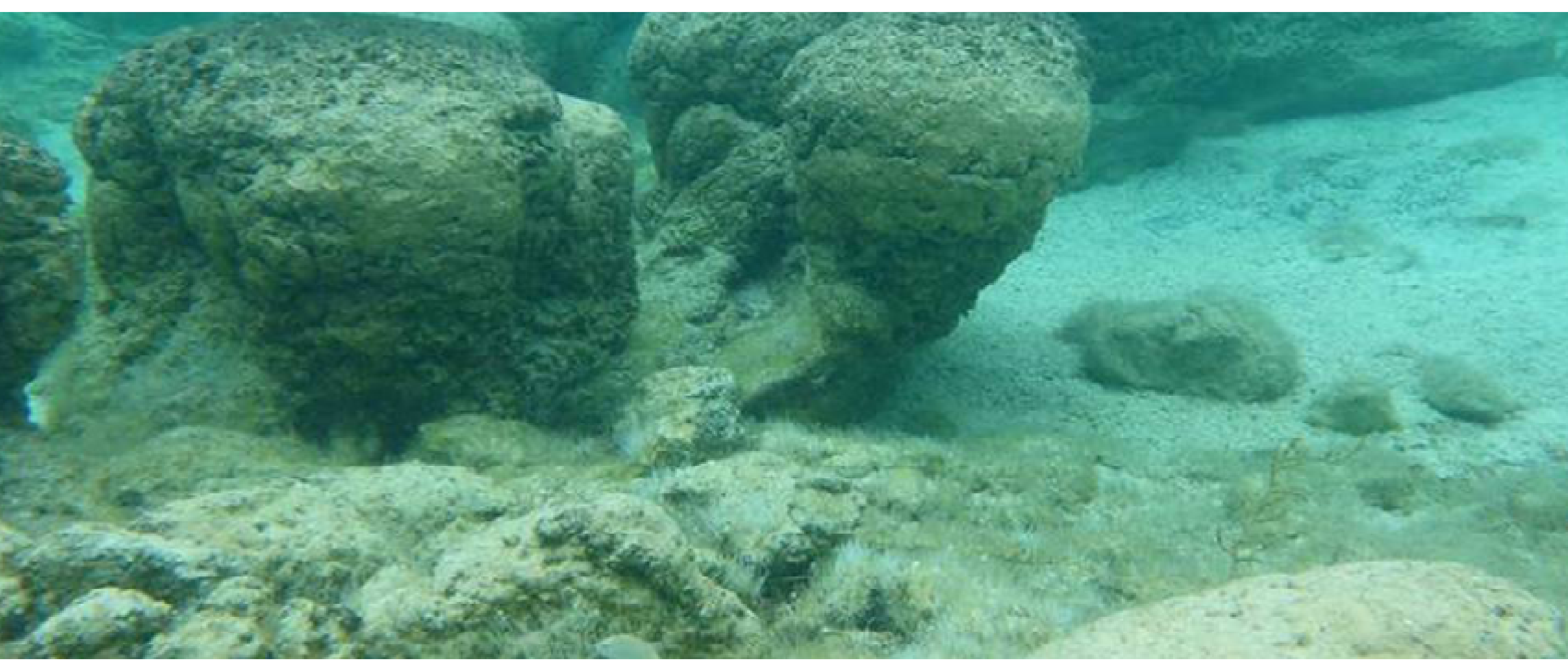
To consider the question of remote protein similarity from the sequence perspective, Diego Ferreiro (University of Buenos Aires) and his group developed an algorithm to search for amino acid patterns in metalloprotein sequences. The results further guide our exploration of functional evolution of proteins.

Akif Tezcan's group at UCSD is 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 (Rutgers), and serves in molecular self-organization and the assembly of proteins that contain transition metals.

Figure 3. Extant stromatolites in Shark Bay (Western Australia). Credit: Shaunna Morrison



Project Reports

Synthesis of Nanomachines in the Origin of Life

The Vikas Nanda and Paul Falkowski (Rutgers) groups continue to make significant contributions to our understanding of putative early proteins, their evolution and function in energy conversion and catalysis. Simple models of ferredoxin, proposed to be among the earliest electron carriers in metabolism, were shown to have robust redox behavior, and in work with the ENIGMA team at Rice University led by Jon Silberg, we showed these simple proteins could function inside cells, setting a high bar in the field for characterizing electron transfer of model proteins (Fig. 4). This work was published in *PNAS* (Mutter 2019).

Even simpler ferredoxin models have been constructed with similar structural and redox behavior to extant proteins, indicating that we are likely retracing the steps of ferredoxin evolution back to its earlies stages (Raanan 2020). We have also developed novel peptides that bind iron-sulfur complexes that are redox active and can assemble inside cells, suggesting alternate pathways for redox protein evolution that perhaps were not explored on Earth. With the ENIGMA teams at UCSD (Akif Tezcan) and at MIGAL, Israel (Dror Noy), we are solving the high resolution structure of this alternative ferredoxin. One paper is currently in review with several more submissions in 2020.

Using both bioinformatic and computational chemistry approaches, our team has recently created very short peptides that are capable of acting as hydrogenases. These are being developed as model systems for studying hydrogen-driven metabolism at a prebiotic or early-life stage.

In cross-theme collaborations, we are working with the Yana Bromberg (Rutgers, Theme 2) and Diego Ferreiro (University of Buenos Aires) groups to combine protein structure with sequence to better define the minimal peptides capable of metal coordination and catalysis that could have existed at the root of metabolism. With Shaunna Morrison (Carnegie Institution) and Nathan Yee (Rutgers, Theme 3), we are comparing protein and mineral crystallographic databases to uncover parallel chemistries that might have constrained and coupled biochemical and geochemical evolution throughout Earth history.

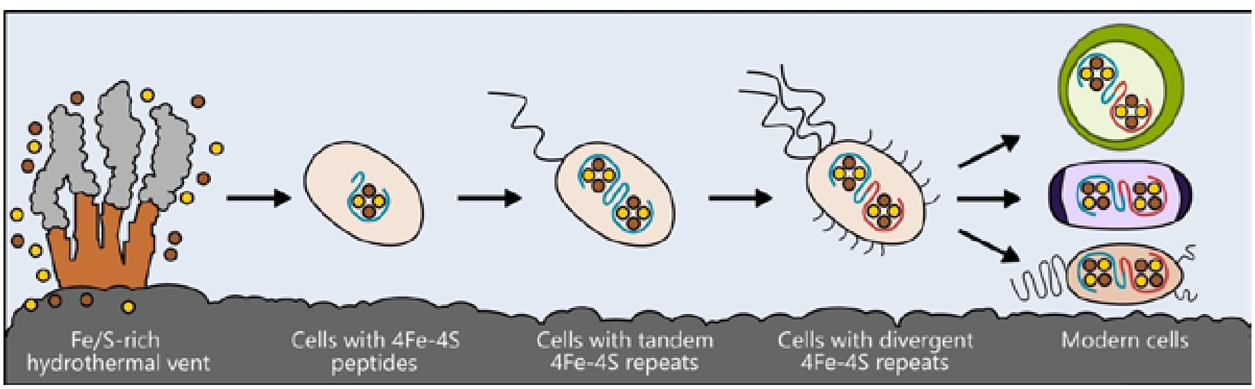


Figure 4. Life may have arisen near hydrothermal vents rich in iron and sulfur. The earliest cells incorporated these elements into small peptides, which became the first and simplest ferredoxins -- proteins that shuttle electrons within the cell, to support metabolism. As cells evolved, ferredoxins mutated into more complex forms. The ferredoxins in modern bacteria, plant and animal cells are all derived from that simple ancestor. Credit: Ian Campbell, Rice University and Mutter *et. al.* (2019) *PNAS*.

Increasing Complexity of Nanomachines in Microbial Ancestors

Yana Bromberg's (Rutgers) group developed a novel method for annotating protein functional, and particularly allosteric, sites from sequence. Working with post-doctoral fellow Maximillian Miller (Rutgers), they showed that variation in the protein sequence is constrained by the specifics of the affected amino acids (Fig. 5). Their method categorizes protein positions based on their expected range of variant impacts: Neutral (weak/no effects), Rheostat (function-tuning positions), or Toggle (on/off switches). They found that position types do not correlate strongly with familiar protein features such as conservation or protein disorder. Within the metal-binding spheres (15A from ligand center in protein 3D structure), fewer than a fifth of the residues were Neutral, while they made up half of the amino acids overall. As protein evolution likely progresses via accumulation of mild-to-moderate effect variants, an in-depth understanding of sequence position specifics is fundamental for both tracing evolutionary history and for synthetic protein design. For instance, conservation of Rheostat designations in protein evolutionary history will guide future design and synthesis of relevant metal-binding structural motifs.

To further guide the exploration of biotic metal binding, ENIGMA post-doctoral fellow Ariel Aptekmann (Rutgers) worked in collaboration with Diego Ferreiro (University of Buenos Aires) to develop an algorithm for metal binding prediction. The method (manuscript in preparation) achieves 95% accuracy in identifying sequences of metal binding proteins and 75% (Ni) to 95% (Fe) accuracy in identifying ion-specific proteins. The method does not use homology and can thus be used for the annotation of previously unseen and fragmented sequences.

ENIGMA NASA Astrobiology post-doctoral fellow Adrienne Hoarfrost's (Rutgers) work to develop a nucleotide-based language model (LM) of metagenomes has also reached a critical stage of development. The deep learning models using this LM are currently able to predict reading frames in metagenomic reads with 95% accuracy and encoded enzymatic functions with 88% accuracy. Both Adrienne's and Ariel's work will be used to further guide exploration of the microbiomes specific to geologically characterized sites.

Furthermore, Julian Esselborn (a new ENIGMA/UCSD post-doc) in Akif Tezcan's group (University of California, San Diego), is getting very close to solving the crystal structure of a 4Fe-4S protein, *de novo* designed by the Nanda group (Rutgers,Theme 1). Completing this project will get some exciting results and publications.

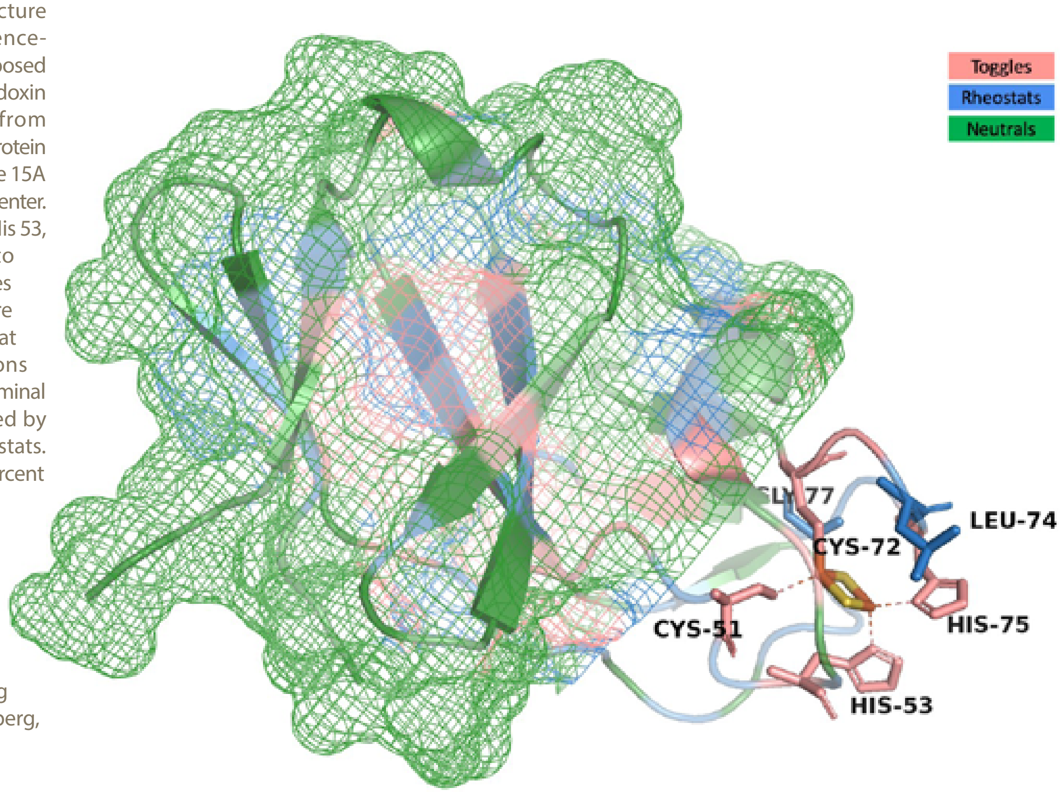


Figure 5. Ferrodoxin protein structure highlighted according to sequence-driven funTRP predictions. The exposed cartoon representation of the Ferredoxin of carbazole 1,9a-dioxygenase from *Nocardioides aromaticivorans* IC177 protein corresponds to the atoms within the 15A sphere around the ligand [2Fe-2S] center. Predicted Toggles (pink) at Cys 51, His 53, Cys 72, and His 75 correspond to Rieske cluster-coordinating residues and additional hydrogen bonds are produced by the Rheostats (blue) at Leu 74 and Gly 77. Other functions of the protein, i.e. binding of the terminal oxygenase, are likely highlighted by the remaining Toggles and Rheostats. Note that while more than sixty percent of the protein outside the 15A sphere is highlighted in green (Neutral, i.e. non-functional, residues; 34 Neutrals, 13 Rheostats, 7 Toggles), only about a third is Neutral within the sphere (18 Neutrals, 18 Rheostats, 14 Toggles) indicating its importance. Credit: Yana Bromberg, Max Miller.

Co-evolution of Nanomachines and the Geosphere

Robert Hazen (Carnegie Institution) developed a new evolutionary system for mineral classification. The new system of mineralogy is based on the co-evolution of the geosphere and biosphere over geologic time. It employs "natural kind clustering" of Earth and planetary materials to reflect different paragenetic origins of minerals in the context of planetary evolution. Diamond is used as an example for this new approach. In the context of cosmic evolution, the mineral species "diamond" can be described as five different natural kinds of minerals, each with a distinct set of properties, mode of origin, and age range of formation (Fig. 6). The theoretical framework and application of this evolutionary system of mineralogy are reported in Hazen (2019) and Hazen & Morrison (2020).

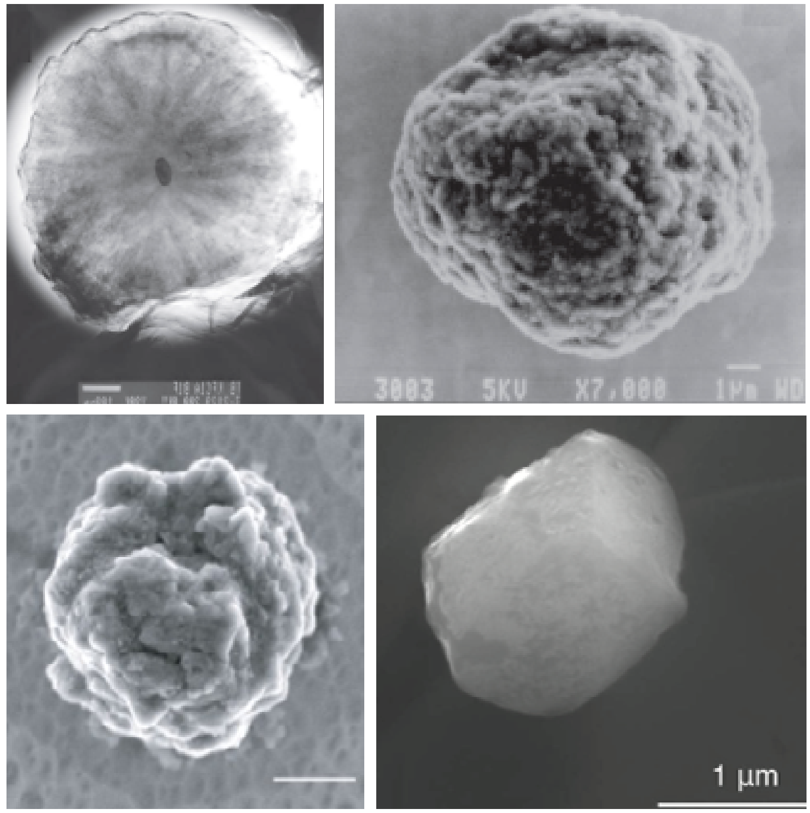


Figure 6. Electron microscope images of stellar minerals. (A) Cross-section of a 1 µm diameter "onion" AGB graphite with central khamrabaevite (TiC) inclusion (courtesy of M. Bernatowicz); (B) 13 µm diameter "cauliflower" SN-II graphite grain—a composite of smaller crystallites (courtesy of S. Amari); (C) 3 µm presolar SiC grain (courtesy of L. Nittler); (D) 1.4 µm diameter euhedral AGB corundum (Al_2O_3) crystal, sample QUE060 (Takigawa *et al.* 2018; licensed under CC BY 3.0).

Robert Hazen (Carnegie Institution) published a new book entitled "Symphony in C: Carbon and the Evolution of (Almost) Everything". Hazen reveals that carbon's grand symphony began with a frenzied prelude shortly after the dawn of creation, bringing new attention to the tiny number of Big Bang–created carbon atoms that often get overlooked. In minutes, violently colliding protons and neutrons improbably formed the first carbon atoms, which can still be found within our bodies. His book then unfolds in four movements, building momentum as he explores carbon as the element of Earth, Air, Fire, and Water. Aimed at a general audience, Hazen explains how carbon holds the answers to some of humanity's biggest questions: Where did Earth come from? What will ultimately become of it—and of us? This must-read is a Science News Favorite Book of 2019.

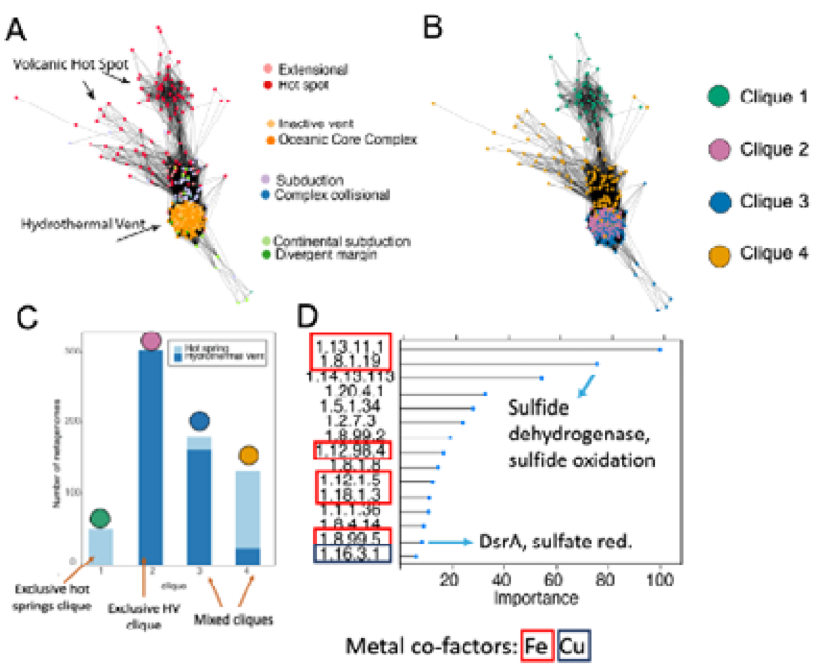


Figure 7. (A) Community network of oxidoreductase composition. Node represent metagenomic samples and edges represent Spearman correlation of a suite of 286 oxidoreductase enzymes within each metagenome. Colors are rendered based upon tectonic setting or hydrothermal vent type. (B) Same community network as in (A), but colors are rendered based upon clique or cluster defined by Louvain modularity. (C) Clique composition with respect to hot spring or hydrothermal vent. (D) Results of random forest, showing rankings of oxidoreductase importance in clustering model. Enzymes beginning with 1.8 indicate those that act on sulfur species.

In late 2018, postdoc Joy Buongiorno (Carnegie Institution) was awarded the NASA Astrobiology Institute Early Career Collaboration Award to spend several months at the beginning 2019 training at the University of Naples, Italy with co-advisor Dr. Donato Giovannelli. As part of this time abroad, she participated in a field campaign in Argentina where she sampled hot springs across the Andean volcanic arc. These samples will provide paired microbiological and geochemical/geological data crucial for the Theme 3 goal of describing evidence for biosphere and geosphere co-evolution. This data will eventually be added to the existing database of manually curated co-located biological and geological data that Buongiorno has constructed during the past year. Currently, this database has approx. 750 metagenomic samples from hot springs and hydrothermal vents with paired geochemical/mineralogical data and continues to grow. Preliminary multivariate analysis suggest that microbial community oxidoreductase composition is distinct between marine hydrothermal vent settings compared to volcanic hot spring settings (Fig. 7). These results have been presented by Buongiorno at the 2019 Goldschmidt meeting in Barcelona, the 2019 Deep Carbon Observatory meeting in DC, the 2020 American Society of Microbiology DC Branch meeting, and the Mid-Atlantic Geobiology meeting in Philadelphia.

ENIGMA Education and Outreach

I. Community and K-12 Education/Outreach

Science Communication training for the Next Generation of Scientists: Janice McDonnell and Alesha Vega (Rutgers) conducted preparation sessions/meetings with members of the ENIGMA team to help improve science communication and collaborations with urban youth. Participants had 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 Events and Short Term Exploratory Program (STEP) for urban youth: In April 2019, Janice McDonnell and Alesha Vega (Rutgers), offered two family science programs centered on the complex topic of Astrobiology in local New Brunswick schools for K-8 students and their families (Fig. 8 & 9). These first interactive K-8 Family Science Nights entitled "Exploring Life on Other Planets", were interdisciplinary focused. Bi-lingual Spanish student volunteers were provided to make sure all family members were included in the discussion and activities. Dinner was also provided to make sure there were not barriers for families to be able to attend. Close to 200 were in attendance at each Family Science Night.

In collaboration with two local K-8 schools, (Greater New Brunswick Charter and McKinley Community School), the New Brunswick Public School System and the Supervisor of Science in K-12 New Brunswick Public Schools, McDonnell and Vega implemented two six-week Short Term Exploratory Program (STEP) clubs for urban youth during Fall/Winter 2019 (Fig. 10). We utilized effective practices from the NASA Astrobiology Afterschool guide, 4-H STEP Club activities which emphasize STEM learning and building leadership skills through a learn by doing approach, as well as employing activities from current research of ENIGMA Scientists. Each school had consistently 25-30 students in attendance during the course of the 6-week program.

There was significant press coverage on both the ENIGMA Family Science Nights and the after school programs.

Next Steps: We have begun developing the script and conceptual goals with Tilapia Films to create the ENIGMA science videos. They will be added to the successful Tools-of-Science series (toolsofscience.org) focused on ENIGMA content. The video will be designed to illustrate the non-linear nature of the scientific process and the creative vision and concepts behind ENIGMA's cutting-edge scientific research. Social Media outlets have been created to share these videos widely.

Figure 8 and 9. Astrobiology Family Science Night at Greater Brunswick Charter School. Credit Alesha Vega.

Figure 10. ENIGMA after school program at McKinley Community School. Credit Nick Romanenko.

II. Undergraduate Education

Nathan Yee (Rutgers, Theme 3) and the ENIGMA team members created and taught a new Astrobiology course in 2019. The inaugural offering was extremely popular and highly rated by students in the teaching evaluations. The course will be taught annually in Fall semester. Yee is currently developing an Astrobiology minor that will be offered at Rutgers University starting Fall 2020.

Linking Life and Earth with Deep Transfer Learning:
ENIGMA NASA Astrobiology post-doctoral fellow Adrienne Hoarfrost has been working with Yana Bromberg (Rutgers, Theme 2) on this project. It aims to build a deep learning architecture that captures the functionally relevant features of microbial communities. Leveraging these features will facilitate modeling the complex relationship between microbiome functions and their geochemical and mineralogical context.

A deep learning architecture was developed using the AWD-LSTM model (Merity *et al.* 2017) and trained to predict a masked nucleotide from its surrounding sequence context. This model accounts not only for the co-occurrence of nucleotides in the sequence, but also the order in which they appear. Note that the ability to correctly predict the nucleotide is not an end goal of the project, but rather an evaluation of the model's ability to parse complex sequence patterns from DNA reads. In a test run, a model was trained for one epoch using a truncated training set of 25 million short sequences from 23,458 genomes, obtaining 39% accuracy. After hyperparameter tuning is complete, this model will be trained further on all sequences from the representative genomes, as well as from metagenomes sourced from diverse environmental biomes.

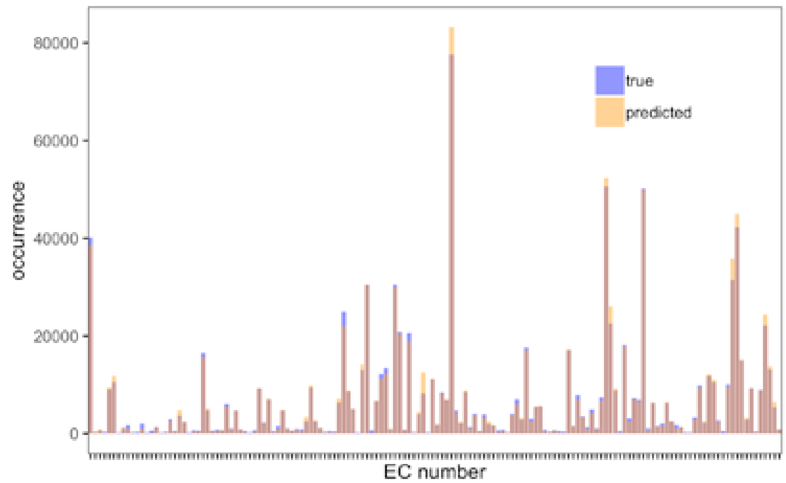


Figure 11. The distribution of true and predicted functional annotations of sequences. Each bar represents an EC number to the third place, e.g. 1.1.1. Overlap in distributions appears in pink. 88% accurate prediction results in tight overlap.

Fine tuning the pretrained model to predict the functional annotation of individual reads attains 88% accuracy to the third EC number (Fig. 11). This performance holds for reads derived from oxidoreductases (Fig. 12) and can also accurately predict the metal-binding protein preference (Fig. 13). This suggests that the architecture is indeed learning functionally-relevant patterns from sequence information. A publication describing this approach is in preparation. The code used to implement and train the model will be made available as a software package along with the pretrained models resulting from this study.

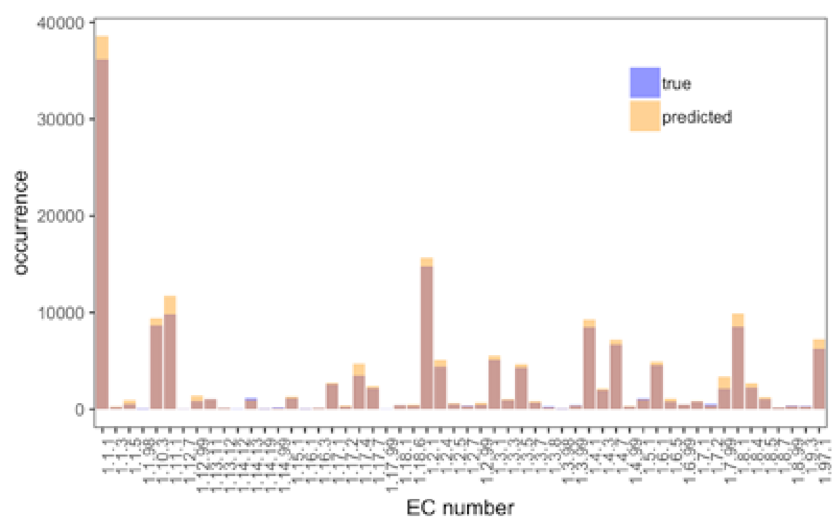


Figure 12. The distribution of true and predicted functional annotations of sequences derived from oxidoreductases to the third EC number. Overlap in distribution is indicated in pink.

Future efforts will focus on fine-tuning the pretrained models to predict the geochemical and mineralogical conditions associated with specific microbial communities. These models will be used to identify sequence motifs most tightly linked with geochemical and mineralogical conditions, which characterized Earth conditions through deep time.

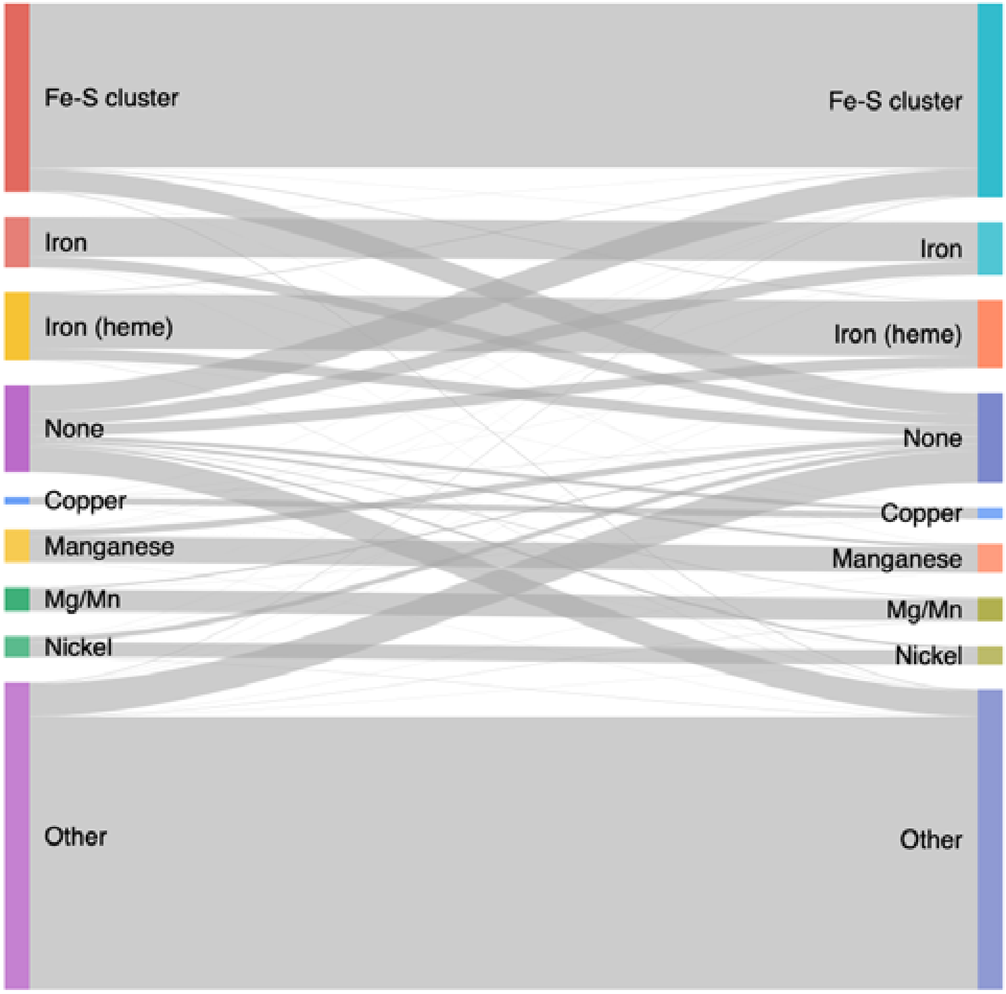


Figure 13. Flow Sankey diagram showing consistency of labeling between predicted (left) and true (right) labels. Where a metal is predicted to be bound, the metal is generally correctly predicted; incorrect predictions occur when the model predicts a metal is bound where it is not, and vice versa.

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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 Mars environment that may have once either supported life as we know it, or sustained pre-biological processes leading to an origin of life, Mars 2020 is 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, our Team's research focus on these three themes:

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

Team Website: https://astrobiology.nasa.gov/nai/teams/can-7/seti/index.html

2019 Executive Summary

The continued collaboration between our team's main research areas allowed us to merge and focus our results and findings from previous years around the central theme of ***Astrobiology Research Applied to Missions***, which is the primary goal of our NAI team. In 2019, using this theme as a guide, we designed and implemented new field-exploration methods and strategies focused on biosignature identification and detection from orbit the ground.

Major progress and many achievements have been made in the following areas:

1) ***In situ technologies and exploration strategies***, focused on astrobiology science readiness of instruments including: definition, testing, and validation of operational concepts for in-situ characterization of biomarkers and associated geochemistry and mineralogy.

2) ***Identification and collection high-priority field samples,*** for further lab investigation as precursor to sample caching on Mars by Mars 2020 and sample return to Earth.

3) ***Developing recommendations for upcoming and future astrobiology missions***. Our team has been also especially active in advocating for an increased role of the Astrobiology community in a new era of planetary exploration.

One of the key achievements was the identification, development, and implementation of methods, processes, and tools that provide a guideline to integrate scales of exploration from orbit to the ground, and connect megascale, to mesoscale and microscale. Through field deployment at Salar de Pajonales, Chile, our work has established a pathway to link planetary habitability (using current mission data) to microbial ecology (past and present biosignature detection at local to microscale). The evaluation of the nature, spatial scales, and spectral resolution that are needed to support upcoming missions was accomplished using drones, visible, multi- and hyperspectral imagery. Current orbital mission data can allow scientists to reach a "threshold of attention," in other words, the identification of potential habitable environments. Our field work established that a "threshold of detection"

Salar de Pajonales, Chilean Altiplano. Credit: Nathalie A. Cabrol, SETI Institute NAI team.

for habitat potential could be achieved in the field at a resolution of ~ 4cm/pixel during simulated descent imaging. The factors that make up microbial habitats at Salar de Pajonales, and their structure, were clearly visible at a resolution of ~ 2mm/pixel, which we defined as the “threshold of identification."

Our significant 2019 science and technology pay-offs include: The identification and implementation in the field of a pathway to link planetary habitability to microbial life; the development and optimization of strategies to maximize science return of *in situ* astrobiological missions and surveys, sampling, and analysis, as well as, the advancement of technologies for astrobiology exploration of Mars and Oceans Worlds through the development of enabling methods to explore terrestrial analogs.

Figure 1. SETI NAI team members in the Chilean Altiplano at Salar de Pajonales
Credit: Victor Robles, Campoalto and SETI Institute

Figure 2. View of the night sky at Salar de Pajonales in the Chilean Altiplano during the 2019 expedition.
Credit: Victor Robles, Campoalto and SETI Institute

What to Search For?

In 2019, our team developed and optimized strategies to maximize science return of *in situ* astrobiological missions and surveys, sampling, and analysis. Our research informs survey, coring, and caching operations for the Mars 2020 missions; We continued to contribute to the development of databases and libraries for upcoming Mars missions, with support to the lipid standard database for the ExoMars RLS instrument (Co-I Pablo Sobron in collaboration with Mary Beth Wilhems, NASA Ames), and the Mars 2020 Raman spectral library through the analysis of samples in the lab and in the field (Co-I Pablo Sobron); the deployment of a prototype UV Fluorescence Imager (Fig. 3) to understand best practice for data acquisition by an analog instrument onboard Mars 2020 (Coll. Evan Eshelman). Finally, a deep drill and a coring system relevant to ExoMars and Mars 2020, respectively (Co-I Zacny) were used to extract subsurface materials at Salar de Pajonales during the 2019 field expedition. Overall, in addition to populating spectral libraries for Mars 2020 and ExoMars, we also contributed equipment and facilities to the Earth, planetary, and astrobiology communities through the delivery of sample and data libraries, and the production of both drill and coring kits that can be used by astrobiology project teams across NASA programs.

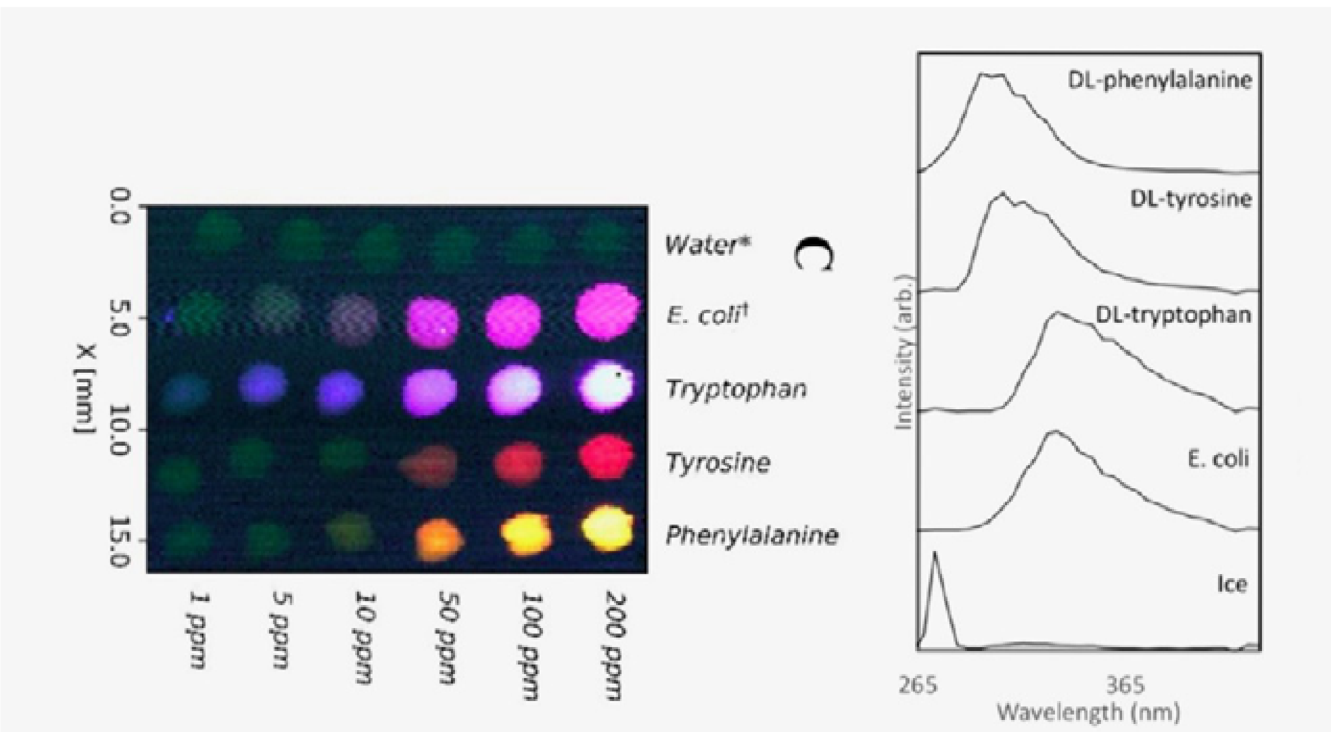

Figure 3. Prototype UV Fluorescence Imager. While spectra indicate the presence of organics, the brightness, color, and size of the spots (to the left) indicate the types and abundance present. Credit: SETI Institute

How to Search?

Our contributions to missions continued through the advancement of tools, data products, and operational concepts and was supported by a thorough statistical survey of microbial habitats at Salar de Pajonales, Chile with the goal to understand and characterize the distribution and abundance of microbial life. Representative units were documented and sampled (Co-Is Warren-Rhodes, Hinman, Pwl; Grad Students McInley and Phillips; Coll. Tebes, Atkinson), and samples brought back to the lab for correlative analysis including LChips, DNA sequencing, thin section preparation, petrography, SEM, IR, Raman (field and lab), VNIR (lab) NIR (field) (Co-Is Farmer, Cady, Hinman, Bishop, Gulick, Sarrazin, Sobron, Summers). Critically, this field survey showed that while microbial colonization (Fig. 4) is patchy at microscale, distribution patterns are repeatable at various scales throughout the landscapes, demonstrating a "fractal" nature of polyextreme microbial habitats, with local (m-μm) factors driving habitat location and distribution. Correlation of datasets resulted in the development of a microbial "heat map" that showed hotspot distribution and abundance over the landscape. This finding offers promising research avenues and the development of probability maps combining multi-source, and multi-scale and resolution data to search for biosignatures during missions. The fractal nature of microbial habitat distribution may also lead to the development of automated biosignature detection algorithms that will integrate landscape knowledge on their own "on the go" through data accumulated during rover traverses and orbiter and drone flyovers.

Figure 4. Pigmented algae under the salt crust of Salar de Pajonales. They were found at the new exploration site in the active part of the Salar. The crust is about 2 mm thick with water within a few centimeters of the surface. Credit: Rebecca McDonald, the SETI Institute.

Where to Search?

A team goal this year was the development of methods enabling orbit-to-ground reconstruction using analog data to what is produced by Mars orbiters. This was achieved through drone imagery, and the production of hyper-resolution mosaics from our analog sites with the development of intelligent Digital Surface Mosaics (DSM). These DSM were produced using deep learning for image analysis and automated terrain mapping (Co-I Wettergreen, Grad Student Candela). This technique allowed us to bridge resolution gaps and also provided key information on the structure and composition of the landscape through intelligent/adaptive image segmentation, with mapping improving with each additional data acquisition. Hyper-resolution mosaic imaging also provided an additional next step to link aerial imagery to the ground imaging (rover view, Fig. 5). This critical step allowed the generation of 3D views of potential microbial habitats from aerial imaging, and is a promising tool to preselect sampling targets before landing and/or during traverses performed by collaborative robotic missions using, for instance, rovers and instrumented drones.

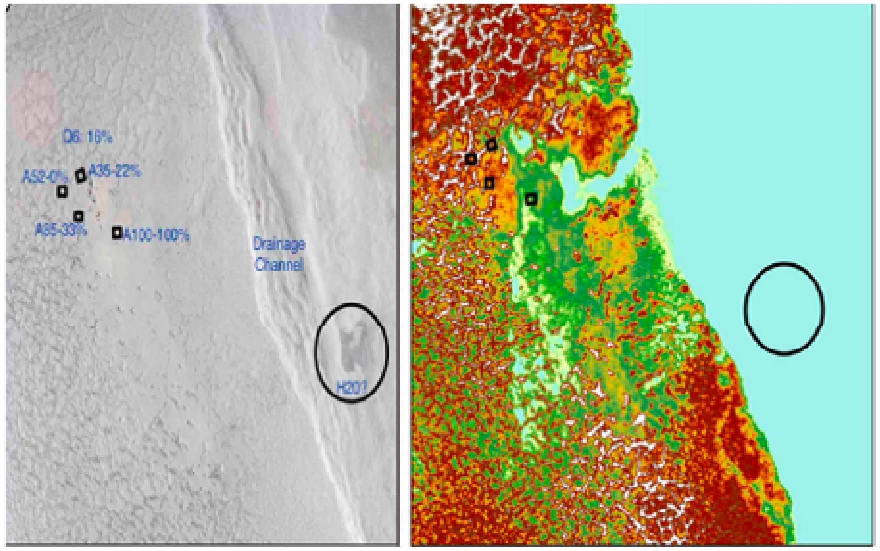

Figure 5. Digital Surface Mosaics provide a first level segmentation/organization of the landscape, pointing to potential microbial habitats.

One of the most promising demonstrations of integration between orbit to ground information at scales and resolution that matter for microbial habitats was made with our hyperspectral imager (VNIR: 400-1000 nm) SWIR (2500 nm), which was available to our team through the selection of a NASA Planetary Major Equipment grant awarded to Co-I Jeffrey Moersch. The use of principal component-stretched false subframe analysis of landforms at Salar de Pajonales (Fig. 6) showed subtle spectral differences between bio-rich and bio-poor surfaces in gypsum domes that were confirmed through ecological survey (Co-Is Warren-Rhodes, Hinman, Wettergreen, Tebes; Grad Student Phillips, Coll. Atkinson and Rhodes).

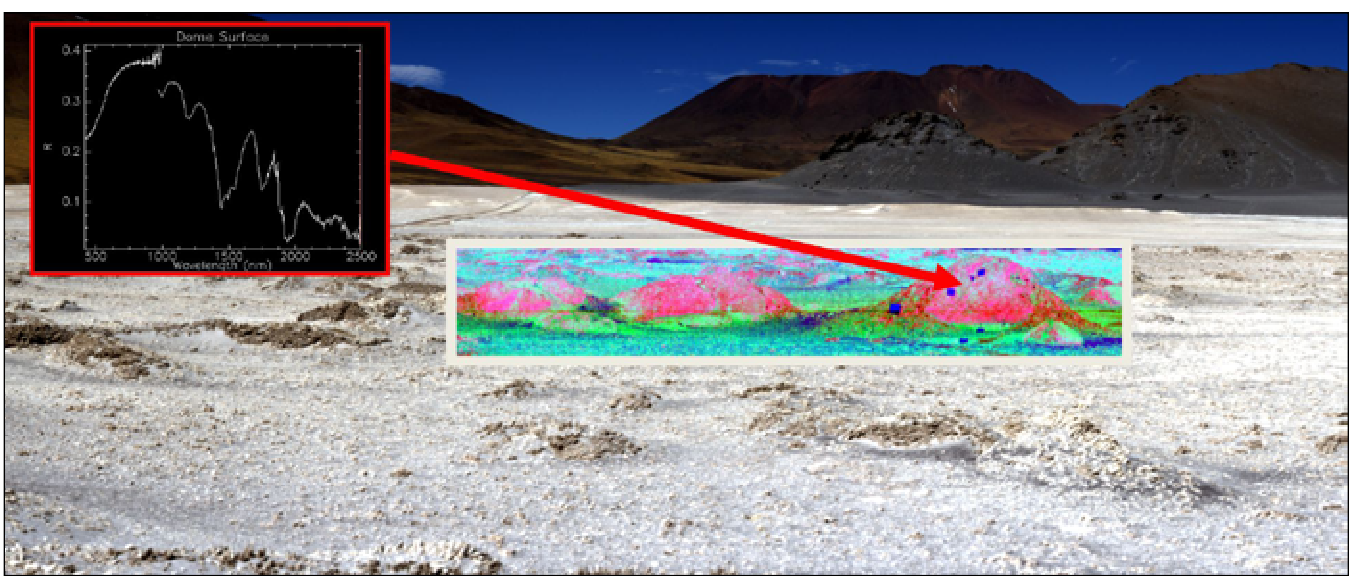

Figure 6. Principal component-stretched false subframe analysis of landforms at Salar de Pajonales showed subtle spectral differences between bio-rich and bio-poor surfaces in gypsum domes. Credit: SETI Institute

NPP Project Report: Exploring hydrated minerals and cryosalts on Mars by bridging laboratory analogs with Martian orbit data

The overall aim of this project is to identify hydrated minerals and cryosalts on the near surface of Mars in order to reveal the history of water activity and potential water resources for future human exploration. NASA Postdoctoral Program (NPP) researcher Dr. Merve Yesilbas has completed several low temperature spectroscopy experiments on mineral-salt mixtures that illustrate phase changes among these materials over the -50 to 0 °C temperature range. This study provided data to help understand the interactions of these minerals with water and brine solutions using the mid-IR (4000-600 cm^{-1}) spectral region to probe the activity of water both at elevated temperatures (up to 900°C) and under cold temperatures (down to -90°C) and to help develop a VNIR spectral database for martian orbital analyses. Spectral changes are observed for these mineral-salt mixtures as the temperature was increased from -90°C to -50°C that are consistent with a mixed frozen-liquid brine system forming from the frozen permafrost samples. Continued warming of the samples to -30°C revealed a disappearance of the ice features. These results indicate that frozen subsurface soil on Mars could include a liquid brine component at -50 to -30°C if Cl salts are present. This implies that near surface liquid brine reservoirs could even be present on Mars today, thus greatly expanding the realm of potentially habitable sites on that planet.

Field Sites

2019 SETI Institute NAI Team Field Expedition

Salar de Pajonales, Second Region (Antofagasta), Chile
25o08’40’S/68o49’12’W

Cryptic niches such as salt deposits are some of the last microbial refuges in many of the world’s deserts, including the Atacama—one of Earth’s oldest and driest deserts and a Mars analog for its hyperaridity, severe diurnal temperature fluctuations and extremely high ultraviolet radiation. A common geomorphological and hydrological feature of the Atacama Desert and the altiplano (high-altitude plateau) in Northern Chile is their extensive evaporite deposits, known as salars, or intra-continental evaporitic basins, which exhibit negative water balance. Both active and fossilized salars exist, and serve as key biological repositories for macro and micro-fauna and cryptic microbial life, such as photosynthetic endolithic communities. Typical of these basins, Salar de Pajonales (SP, 3517 m) is located at 3,537 m elevation in the Chilean altiplano (Figs. 5-8). An ancient lakebed covering an area of 104 km^2, Pajonales still has some active lagoons (~1.4 km^2). Low precipitation (80-150 mm/yr) and high evaporation potential at the site (1,350 mm/yr) are typical of the dry conditions of the Atacama and aliplanic region. While the average temperature is 5°C, lower values recorded can reached -20°C. At Pajonales, our team investigated a fossilized dome field characterized by a gypsum-dominated salt landscape with four main geomorphic/ecological habitat units: (i) networks of polygonal ridges (m to 100s of m); (ii) domes (comprising radial selenite crystals, cm to tens of m); (iii) polygonal patterned ground (mm to 100s of m) and (iv) detrital/aeolian cover (over gypsum salar surface) —analogs for the evaporitic basins, dome-like features and desiccation polygonal structures at the Noachian/Hesperian transition on Mars.

Figure 7. 2019 SETI Institute NAI Team Field Site. Salar de Pajonales, Second Region (Antofagasta), Chile. Credit: SETI Institute

Figure 8. 2019 SETI Institute NAI Team Field Site. Salar de Pajonales, Second Region (Antofagasta), Chile. Credit: SETI Institute

Figure 9. Drone view of the Salar de Pajonales in the Chilean altiplano. The scene was captured during data acquisition for integration mapping. Credit: SETI Institute NAI team

Figure 10. Fossil spring on the paleo lake bed at Salar de Pajonales, Chile. Credit: Rebecca McDonald, SETI Institute

Changing Planetary Environments and the Fingerprints of Life: 2019 Publications

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*Not acknowledged: News and View article stemming from NAI work.

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ALTERNATIVE EARTHS

Lead Institution:
University of California, Riverside

Team Overview

Principal Investigator:
Timothy Lyons

A single question 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 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:

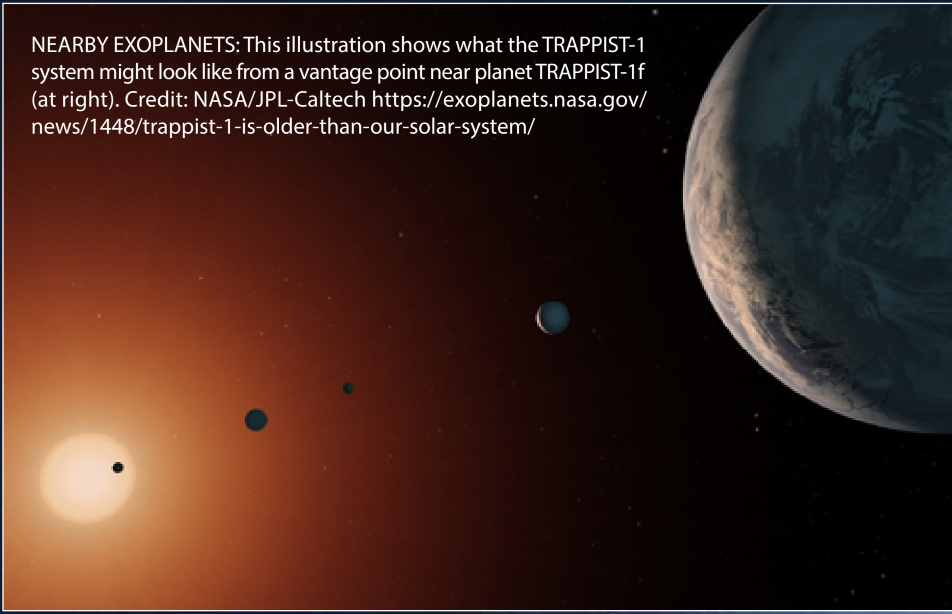
- Composition of the oceans and atmosphere: proxy development
- Gas fluxes and ecological impacts: 3D Earth system models
- Stability and remote detectability of biosignature gases: 1D photochemical and radiative transfer models that define synthetic spectra for evaluation using telescope simulators

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

2019 Executive Summary

The Alternative Earths Team is focused on reconstructing how Earth's atmosphere has changed through time and the factors driving this evolution. The ultimate goal is a more robust framework for the next stages of exoplanet exploration. We focused on two main areas—(1) remotely detectable biosignatures and (2) greenhouse gases that controlled planetary habitability. However, as our team reported this year, we have provided new insights into environmental and biotic co-evolution. This progress was highlighted, foremost this year, in new work that explored the physiological impacts of high and CO and CO_2 as related to searches for biological complexity beyond our solar system—including intelligent life or technosignatures. Specifically, we found that the habitable zone for complex life is highly restricted relative to the zone defined by the potential for liquid water due to toxic buildup of one or both of these gases. Conversely, high levels of carbon monoxide alone could actually signal a robust microbial biosphere on certain exoplanets.

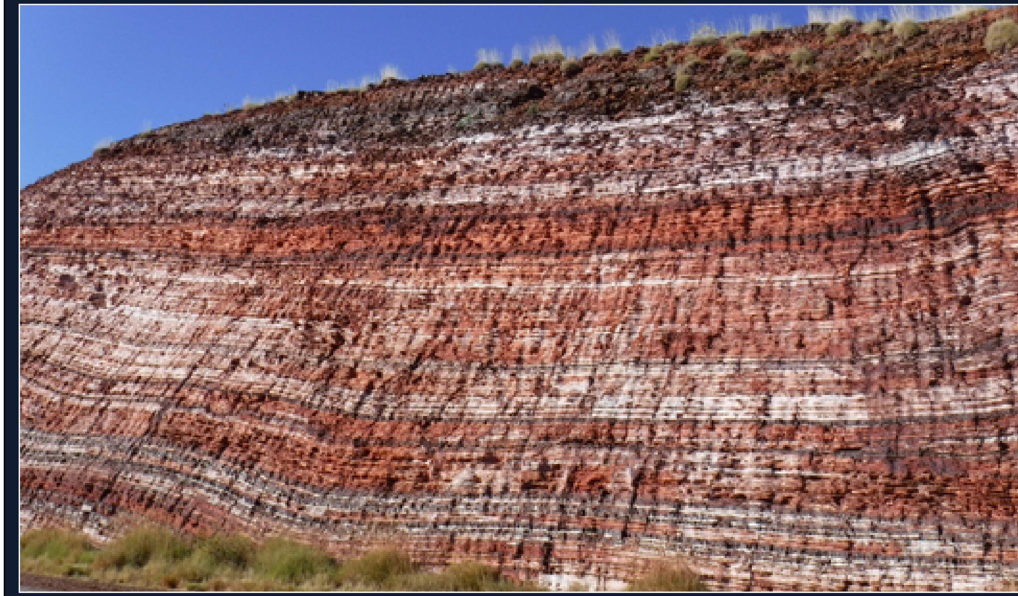
Using Earth's past to guide exoplanet exploration requires a careful vertical integration of research efforts, anchored by the core strengths of our team: development of geochemical proxies that reveal the composition of the ancient oceans and atmosphere. When applied carefully these geochemical proxies allow us to test predictions from global biogeochemical models. We view this two-pronged approach as the most robust means of moving forward in our understanding of how life can transform a terrestrial planet. Several geochemical systems continue to provide new evidence for very low pO_2 for the majority of Earth history. The idea that planets can stabilize at relatively low levels of atmospheric oxygen (< 1% modern) has obvious implications for the tools we will design to look for and interpret oxygen on exoplanets. For example, our new chromium isotope data from paleosols add to a growing body of work suggesting a widespread, permanent, stepwise increase in baseline atmospheric pO_2 to >1% of present atmospheric level during the Neoproterozoic


NEARBY EXOPLANETS: This illustration shows what the TRAPPIST-1 system might look like from a vantage point near planet TRAPPIST-1f (at right). Credit: NASA/JPL-Caltech https://exoplanets.nasa.gov/news/1448/trappist-1-is-older-than-our-solar-system/

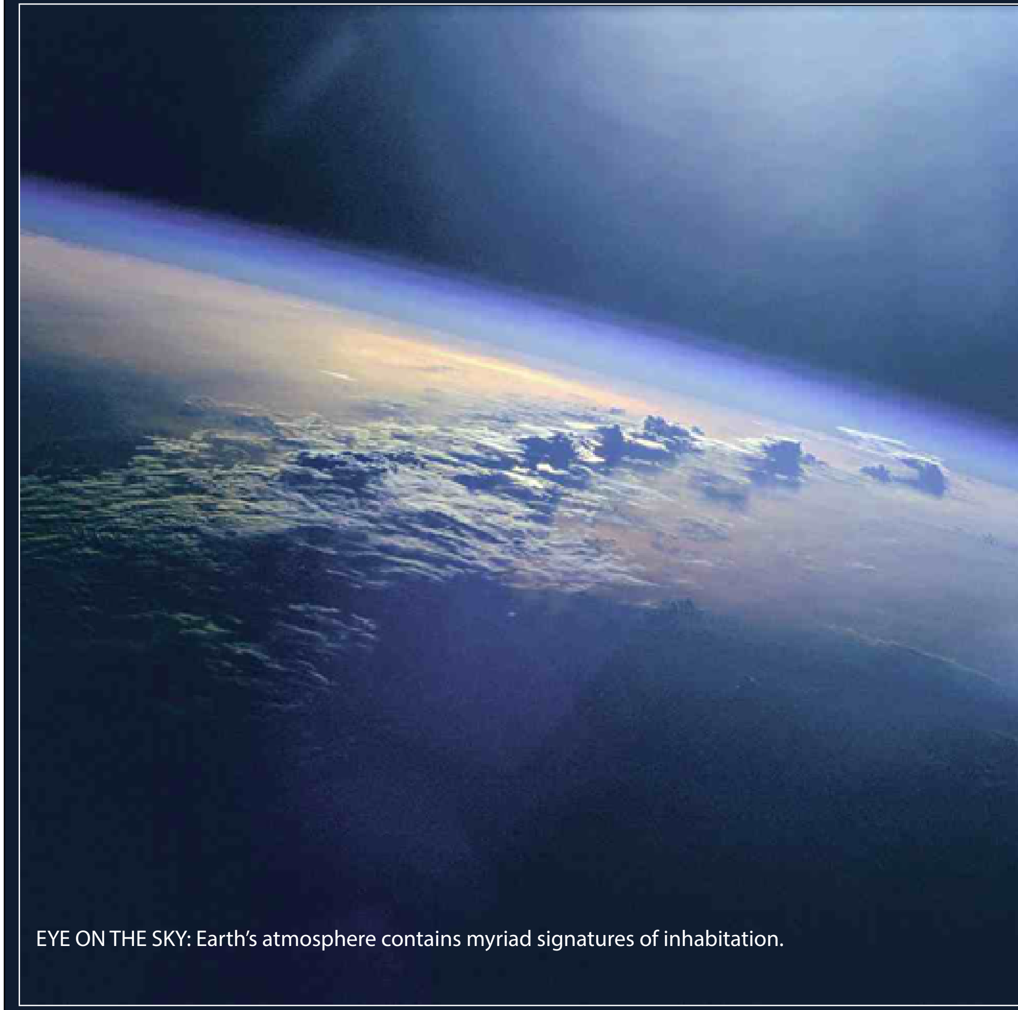

SAANICH INLET. This modern anoxic fjord in Canada is one place the Alternative Earths team samples water and mud for redox proxy development and calibration.

(1,000 to 541 million years ago). Our latest synthesis of experimental microbiology, genomic analyses and global biogeochemical models offers a look at how and why atmospheric pO_2 remained low during the Proterozoic Eon. Another key focus of our team's mission is making sense of what controls greenhouse gas composition of Earth-like planets. This year we continued to provide support for the idea that reverse weathering—clay formation in the oceans—is a major control on climate, challenging traditional views of the controls on carbon dioxide levels.

Collaboration and creative leadership by early career scientists are hallmarks of our team's success. Graduate students first-authored some of the team's most important results this year, and NASA Postdoctoral Program fellows James Eguchi (UCR), Edward Schwieterman (UCR), Nadia Szeinbaum (GT), and Sebastiaan van de Velde (UCR) brought new areas of modeling and experimentation to the mix. 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 and participation, public talks, and honors.



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.



EYE ON THE SKY: Earth's atmosphere contains myriad signatures of inhabitation.

Project Reports

Composition of the Oceans and Atmosphere

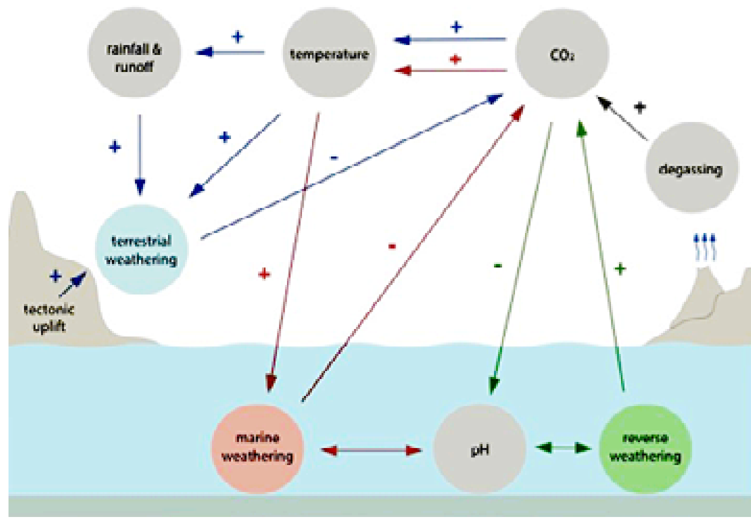
Paleosols, the remnants of ancient soils, are ideal archives for tracking the history of atmospheric oxygen (O_2) levels because chemical weathering in the terrestrial environment occurs in contact with the atmosphere. We produced new stable chromium isotope data from multiple paleosols, which offered snapshots of Earth surface conditions over the past three billion years (Colwyn *et al.*, 2019). Paleosols through the majority of Earth's history had significantly different geochemical signatures than soils that formed over the past 500 million years. Specifically, prior to roughly 500 million years ago metals (Cr, Mn, Fe) in the soil environment where not quantitatively oxidized—pointing to very low atmospheric oxygen levels. These data add to a growing body of work suggesting a widespread, permanent, stepwise increase in baseline atmospheric pO_2 to >1% of the present atmospheric level during the Neoproterozoic (1,000 to 541 million years ago). Solid evidence that Earth-like planets can stabilize at such low atmospheric oxygen levels gives us a new target for oxygen and ozone detection limits with next generation telescopes that will be involved in searching for remotely detectable biosignatures on exoplanets.

ANCIENT SOIL: Outcrop of 500-million-year-old paleosol in Francois Mountains, MO, showing granite corestone. CREDIT: R. Gaines

Gas Fluxes and Ecological Impacts

Our group has provided evidence from geochemical proxies that the Earth, and by inference Earth-like planets in general, can stabilize at very low levels of atmospheric oxygen. This hypothesis is a strong deviation from the traditional view of Earth's evolution. Therefore, an obvious question is what factors could have allowed for this fundamentally different Earth state. In a new model, we argued that ecosystem system structure in a largely anoxic world would have stabilized the planet in a very low oxygen state (Ozaki *et al.*, 2019). The emergence of oxygenic photosynthesis created a new niche with dramatic potential to transform energy flow through Earth's biosphere. However, the extent of oxygenic photosynthesis in the oceans would have been limited by the availability of nutrients. With a synthesis of experimental microbiology, genomic analyses, and Earth system modeling, we argued that a simple biological mechanism—competition for light and nutrients between oxygenic and anoxygenic photosynthesizers—prevented atmospheric oxygenation. We presented a robust quantitative framework that provides a simple solution for how—in the face sustained photosynthetic O_2 production—Earth's atmosphere would have remained in a low atmospheric pO_2 during the Proterozoic Eon (Ozaki e*t al*., 2019).

The existence of stabilizing feedbacks on Earth is generally thought to be necessary for the persistence of liquid water and life. Earth's atmospheric composition must have adjusted to the gradual increase in solar luminosity, resulting in persistently habitable surface temperatures. Further, with limited exceptions, the Earth system recovered rapidly from dramatic climatic perturbation. Carbon dioxide (CO_2) regulation via negative feedbacks within the coupled global carbon-silica cycles are classically viewed as the main processes giving rise to climate stability on Earth. We recently provided a new view on the long-term global carbon cycle budget and how controls on the carbon cycle and related impacts on Earth's climate system have changed over time. We specifically provided new evidence—by exploring a solution that allows for mass balance with the global carbon cycle—pointing to marine processes as important components of the silicate weathering feedback. These processes likely played a more important role in pCO_2 regulation than traditionally imagined. Foremost, we provided new evidence suggesting that higher dissolved silicon (Si), magnesium (Mg) and iron (Fe) in the oceans for the majority of Earth's history would have fostered more extensive reverse weathering (Isson *et al.*, 2019). This work highlights the need for better reconstructions of the history of marine dissolved silica, better characterizations of authigenic clay assemblages captured in Earth's sedimentary record and improvements in our understanding of the kinetics of reverse weathering.



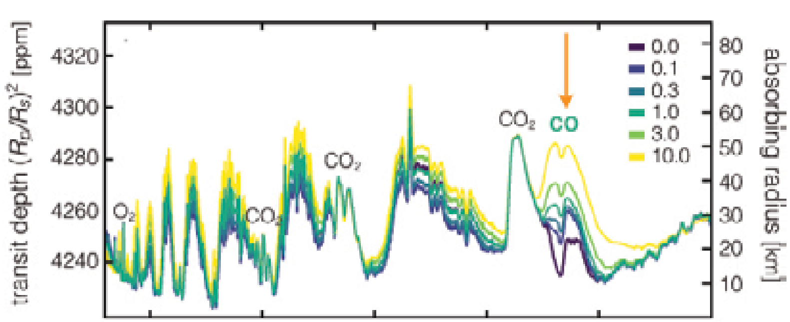
REVERSE WEATHERING: Schematic of the processes that give rise to Earth's thermostat.

Stability and Remote Detectability of Biosignature Gases

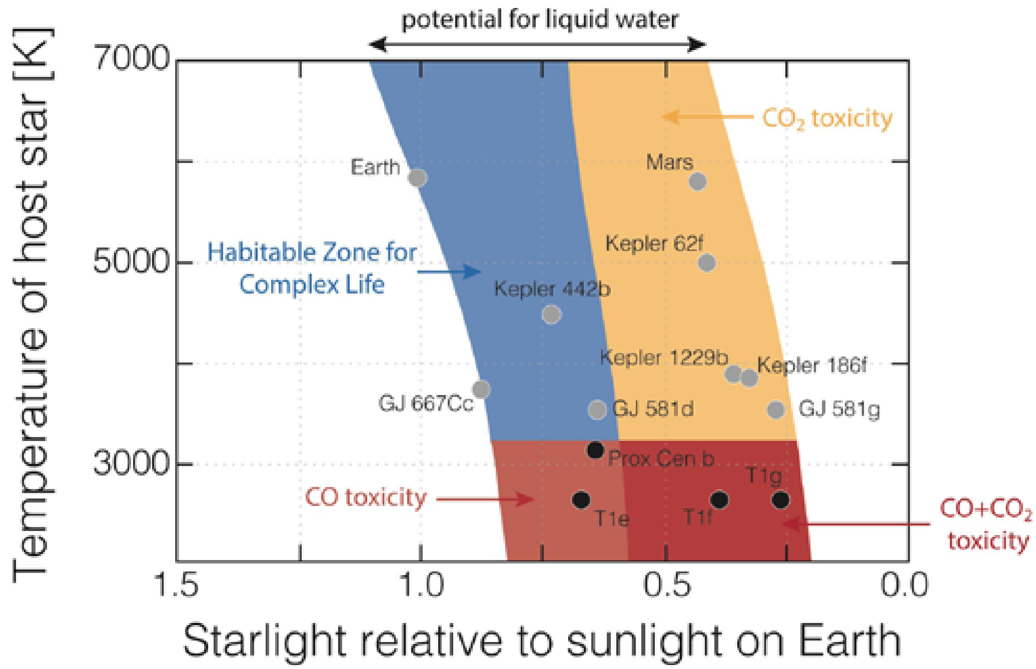
Toxic gases may preclude macroscopic animal life on many distant worlds. For some exoplanets, however, remotely detectable carbon monoxide may actually be diagnostic of a robust microbial biosphere. Certain atmospheric gases such as carbon monoxide (CO) have been proposed as 'antibiosignatures'— evidence that a planet is not inhabited—if remotely detectable at sufficient abundance. We used 1-D ecosphere-atmosphere and photochemical models to quantify the extent to which CO could exist in the atmospheres of living planets. Reducing biospheres around sun-like stars, like the Archean Earth of three billion years ago, can maintain CO levels of ~100 parts per million (ppm)—orders of magnitude greater than the parts-per-billion traces of CO in the atmosphere of modern Earth (Schwieterman *et al*., 2019a).

Photochemistry around M-dwarf stars like Proxima Centauri is considerably more favorable for buildup of CO, with plausible concentrations for inhabited, oxygen-rich planets extending from hundreds of ppm to several percent. Transit spectroscopy of rocky exoplanets with the James Webb Space Telescope could detect CO that is compatible with—or even diagnostic of—the presence of life. Validating CO as an anti-biosignature rather than a product of life will require a comprehensive planetary assessment.

Beyond the search for microbial biospheres, atmospheric photochemistry and stellar environment are critically important for constraining a planet's potential to support complex life. The concept of the habitable zone—the range of distances from a host star where liquid water could exist on a planet's surface—is based on the minimum requirements for a simple microbial biosphere. We were the first to consider the roles of CO_2 and carbon monoxide (CO) in limiting the planetary environments suitable for animal-like life (Schwieterman *et al.*, 2019b). Using a suite of models for atmospheric climate and photochemistry, we compared predicted CO_2 and CO levels to known toxicity limits to quantitatively describe a 'Habitable Zone for Complex Life.' This zone is less than half the size of the conventional habitable zone for sun-like stars and may be non-existent for the coolest M dwarf stars.



CARBON MONOXIDE is a prominent feature in simulated transmission spectra for oxygen-rich, modern Earth–like atmospheres in the habitable zone of an M-dwarf star like Proxima Centauri. Colors correspond to the magnitude of the assumed surface molecular CO flux scaled to that of the modern Earth (e.g., modern = 1.0). Unlabeled features are due to CH_4. CREDIT: From Schwieterman *et al.* (2019a)



THE HABITABLE ZONE FOR COMPLEX LIFE (blue) is highly restricted relative to the zone defined by the potential for liquid water, due to toxic buildup of CO2 (yellow), CO (red), or both (orange). This safe zone excludes many potentially water-bearing exoplanets, including Proxima Centauri b and TRAPPIST-1e, f, and g (black dots). CREDIT: From Schwieterman *et al*. (2019b)

THREE PLANETS orbiting TRAPPIST-1 fall within that star's habitable zone. CREDIT: R. Hurt/NASA/JPL-Caltech

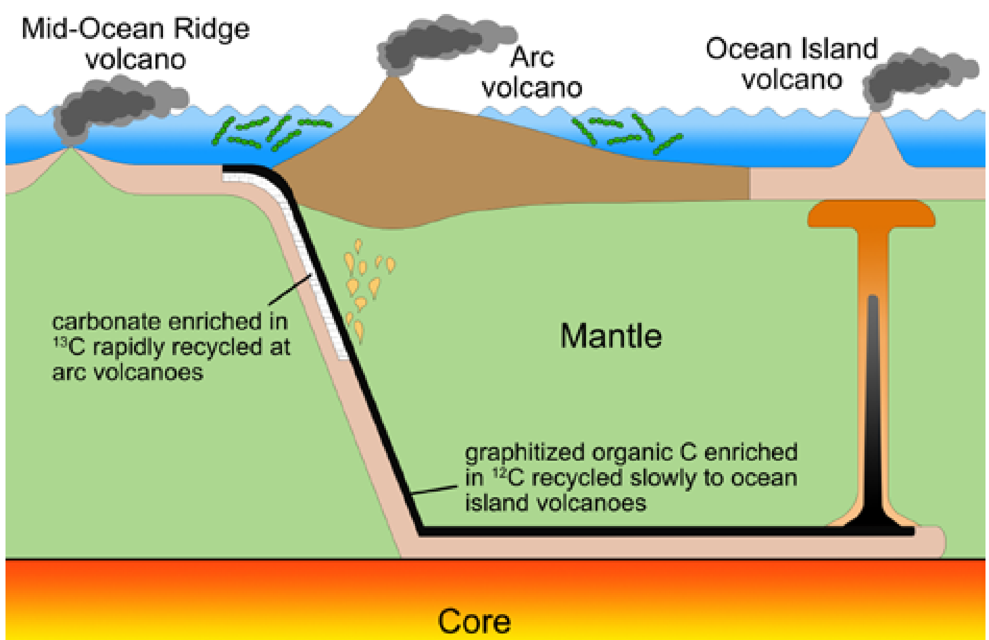
NPP Project Reports

Evolution of Atmospheric CO_2 and O_2 Driven by Planetary Tectonics

James Eguchi joined the Alternative Earths team in 2019 and is based at UCR under the supervision of Timothy Lyons. Eguchi studies how processes occurring in the mantle play a role in controlling the cycling of carbon and other volatiles between planetary interiors and surfaces. He is interested in identifying how igneous and metamorphic processes occurring in the mantle can affect the evolution of important atmospheric gases such as CO_2 and O_2. Specifically, his project proposes a new mechanism for driving large carbon isotope excursions.

Eguchi recently lead authored a paper published in Nature Geoscience (DOI: 10.1038/s41561-019-0492-6) that proposed the temporal association of the Great Oxidation Event and a large positive carbon isotope excursion known as the Lomagundi Event is due primarily to Earth's interior processes. The hypothesis is that enhanced volcanic emissions of CO_2 led to enhanced continental weathering, which in turn stimulated elevated levels of carbonate and organic carbon burial, leading to the accumulation of O_2 in the atmosphere. He proposed that different residence times of carbonate versus organic carbon in the mantle drove the associated rise in carbon isotopes of marine carbonates.

The hypothesis described above predicts that different CO_2 emissions at different volcanic settings have different carbon isotope signatures. Measuring carbon isotopes of different volcanic settings is a part of Eguchi's ongoing research as part of the Alternative Earths Team. Additionally, there are other times in Earth's history where the geologic record provides evidence of large oxidation events with large carbon isotope excursion, and Eguchi is interested in investigating how solid Earth processes may have played a part in the associations.

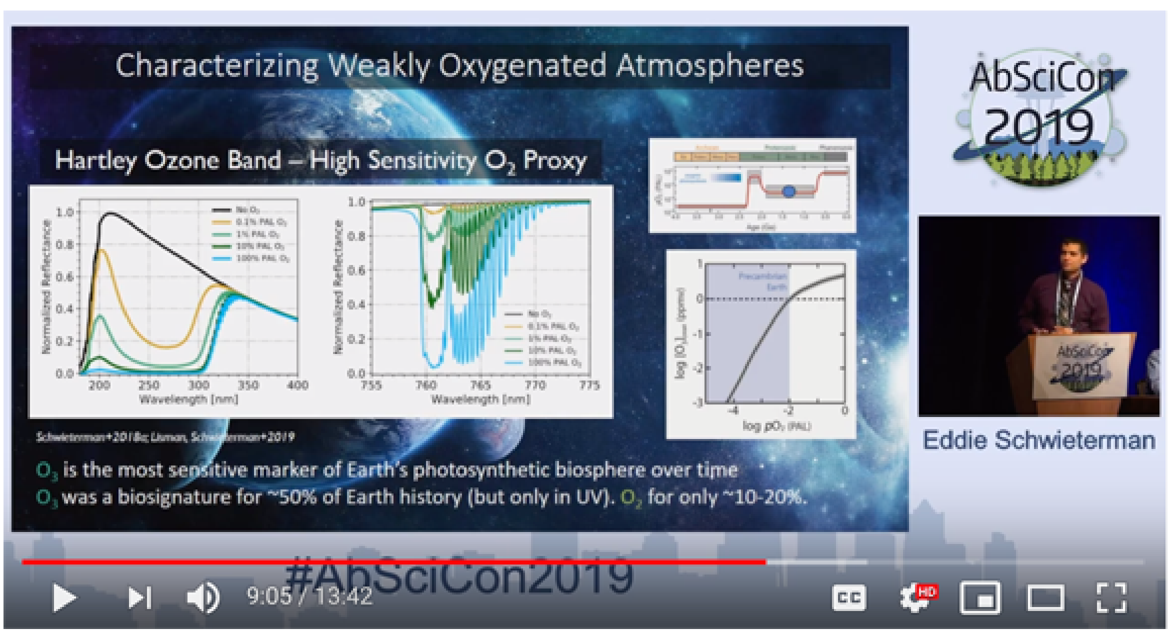


Enhanced volcanism increases delivery of weathering products and nutrients to the ocean leading to increased biological production of photosynthetic organisms. This causes increased deposition of carbonates and organic carbon on the seafloor, allowing oxygen to accumulate in the atmosphere. Carbonates and organic carbon then get subducted into Earth's mantle, and due to different chemical behaviors carbonates are rapidly recycled back to Earth's surface at arc volcanoes, while organic carbon is recycled more slowly at ocean island volcanoes.

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 habitability and remotely detectable signatures of terrestrial exoplanets. 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. His efforts this year included an examination of the common presumption that carbon monoxide is a so-called "anti-biosignature." Using photochemical and spectral models, he and the team illustrated several cases where spectroscopically detectable CO on a terrestrial planet would be compatible with a robust biosphere (Schwieterman *et al.*, 2019a). Schwieterman also led a project that compared known CO_2 tolerances for metazoans with the CO_2 concentrations needed to maintain clement surface temperatures at various locations through the habitable zone. The team found that the toxic impact of very high CO_2 may limit Earth-like complex biospheres to a small portion of the conventional habitable zone (Schwieterman *et al.*, 2019b). Both of these papers were featured in UCR press releases, science nuggets on the NASA Astrobiology Institute website, and several media reports.

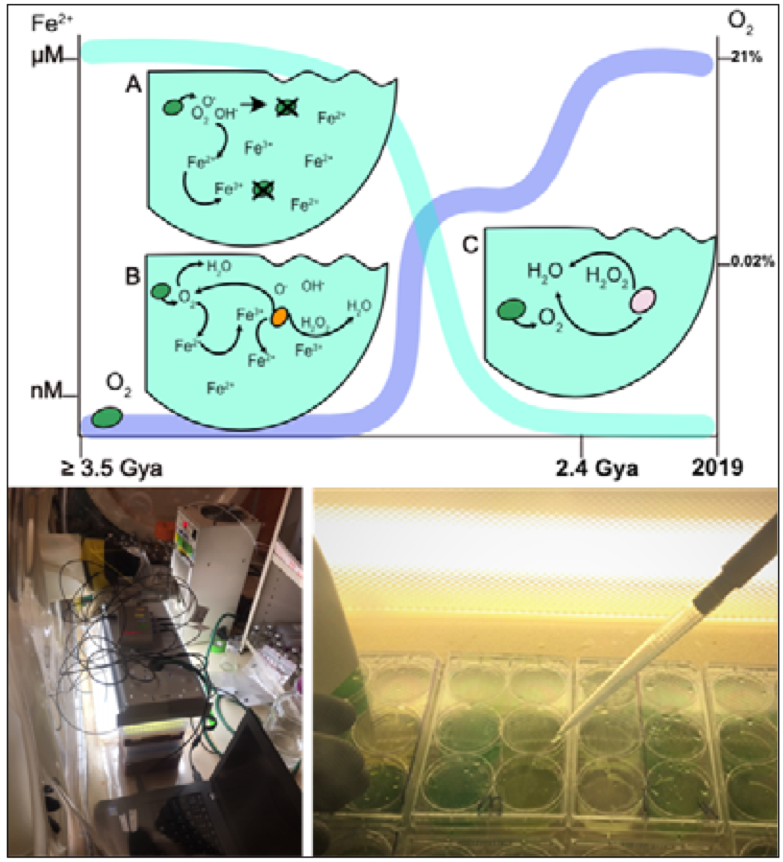
In response to a 2019 call for the Decadal Survey on Astronomy and Astrophysics (Astro2020), Schwieterman contributed to several white papers: the remote detectability of Earth's biosphere through time and implications for future missions (Reinhard *et al.*, 2019), a Probe Class space mission to detect ozone on exoplanets (Lisman *et al.*, 2019), the importance of thermal emission in studying exoplanets (Line *et al.*, 2019), and a method for using statistical analysis for testing the habitable zone concept (Checlair *et al.*, 2019). Schwieterman also contributed his Earth modeling expertise to a project focused on constraining the contribution of Earthshine to the radiation environment in otherwise permanently shadowed lunar crater regions (Glenar *et al.*, 2019).



Schwieterman presents an invited talk on "Exoplanet Biosignatures" during the Frontiers in Biosignatures Plenary Session at the Astrobiology Science Conference on June 24, 2019, in Bellevue, Wash.

The Role of Microbial Interactions Leading to the Great Oxidation Event

Working with Jennifer Glass and Chris Reinhard (GT), Nadia Szeinbaum uses microbial synthetic communities to study ancient microbial interactions 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 rich in iron. Modern oxygenic phototrophs face the unavoidable problem that photosynthesis produces toxic reactive oxygen species (ROS). During Earth's transition from an anoxic to an oxic world, cyanobacteria would have needed a way to scavenge the resulting ROS exacerbated from Fenton chemistry by higher Fe^{2+} concentrations. Modern proteobacteria provide cyanobacteria with catalase to scavenge ROS. Szeinbaum hypothesizes that phototrophic-heterotrophic associations like this one may represent critical partnerships for the ecological success of phototrophs.



Experimental approach to study microbial interactions relevant to pre-GOE environments. Top panel, conceptual model depicting A) Fenton-driven toxicity from oxygen production by cyanobacteria and B) Co-existing microbial populations cycling oxygen, ROS, and iron, under pre-GOE conditions (anoxic, iron-rich); C) modern heterotrophs scavenge ROS increasing the fitness of cyanobateria in open oceans. Models based on Morris *et. al* (2011), Swanner *et al.* (2015), and Szeinbaum *et al.* (2017) [1-3] . Bottom panel, experimental setup used to study microbial interactions of model organisms under simulated pre-GOE conditions.

To better understand the role of the microbial ecology of the Great Oxidation Event, she is reconstructing potential metabolic interactions using model iron-reducing proteobacteria as ROS scavengers with model oxygenic phototrophs. Szeinbaum analyzed the phylogenetic relationships between different types of catalases in metal-reducing bacteria. Notably, periplasmic catalases are present in only a handful of Cyanobacteria and are candidate enzymes to scavenge extracellular ROS. Phylogenies of these genes suggest that Gammaproteobacteria may have transferred a periplasmic catalase to Cyanobacteria, supporting a close association.

In the lab, Szeinbaum tested how cyanobacteria grow in the presence of different strains under iron-poor and iron-rich conditions compared to a pure cyanobacterial culture. The strains that are able to tolerate higher hydrogen peroxide concentrations increase cyanobacterial fitness allowing cyanobacteria to grow at higher iron concentrations that are otherwise prohibitive. Ultimately, these experimental systems can also be used to test hypotheses about microbial interactions on other planets. Szeinbaum presented these results at AbSciCon 2019, where she also co-chaired the first "Astroecology" session, dedicated to the study of microbial interactions on Earth and beyond.

Ocean Redox, Nutrient Availability, and the Evolution of Earth's Biosphere

Sebastiaan van de Velde works with Andy Ridgwell (UCR) to address the constraints on biological evolution in an ocean that was fundamentally different than today. Their approach is the development of a unique representation of the coupled iron-sulfur-phosphorus cycle in a 3D, ocean-based, Earth System model (cGEnIE) that is able to spatially resolve the redox structure and nutrient availability of the ancient ocean. This model will be able to quantitatively test many of the hypotheses generated by the Alternative Earths Team.

Eukaryotic life evolved in a shallow oxygenated surface layer overlying an ocean that was largely anoxic below. In the absence of oxygen, the ocean interior should have been either iron-rich ('ferruginous'), sulfide-rich ('euxinic'), or a spatially segregated mix of the two, an inference supported by the geologic record, much of which has been generated by other members of the Alternative Earths Team. These contrasting redox states can exert profound and very different controls on nutrient concentrations and cycling. Increases in ocean nutrient concentrations have likely been an important factor during major ecological transitions, such as the Cryogenian rise of algae or the Cambrian rise of animals, suggesting that changing ocean redox played an important role in constraining the evolution of eukaryotic life.

Although the key environmental controls on life—oxygen and nutrient availability—are inextricably intertwined, their dynamical relationship in the global ocean, much less the feedback with marine ecosystems, remains largely unexplored. By developing a mechanistic and spatially explicit model of ocean redox and nutrient cycling, van de Velde expects to elucidate the feedbacks between algal productivity, ocean redox, and large-scale nutrient limitation. This work will represent an important step toward understanding the co-evolution of life and its environment, applicable not just to early Earth, but to any planet where marine life shares similar metabolisms and elemental requirements.

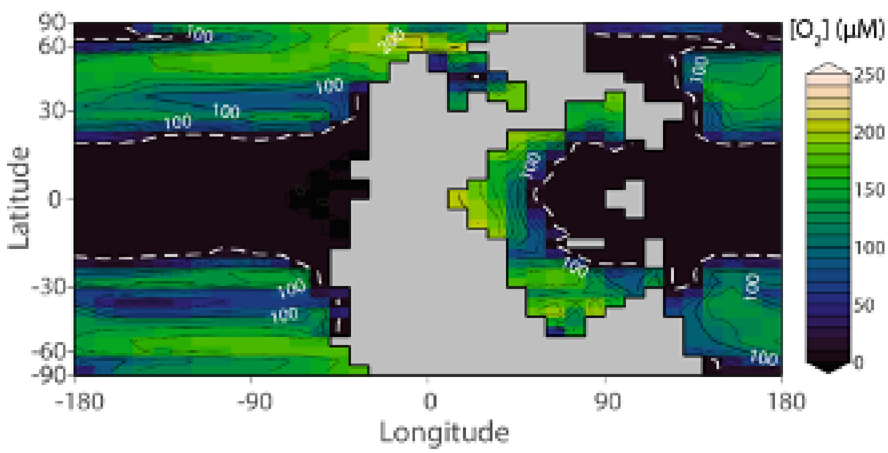


Illustration of the spatial (and paleo) capabilities and grid resolution of the cGEnIE Earth system model. Shown is the distribution of sub-surface anoxia (<10 µM O2, highlighted as a white dashed line) and hence projected pattern of unfavourable benthic habitat, in a shallow biological pump and enhanced ocean phosphate, late Permian model experiment.

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:

Exuma Cays, The Bahamas

Co-I Noah Planavsky (Yale) did fieldwork to determine how modern carbonates trap metal isotope signatures in the world's largest shallow water nonskeletal carbonate factory today—the Bahamas.

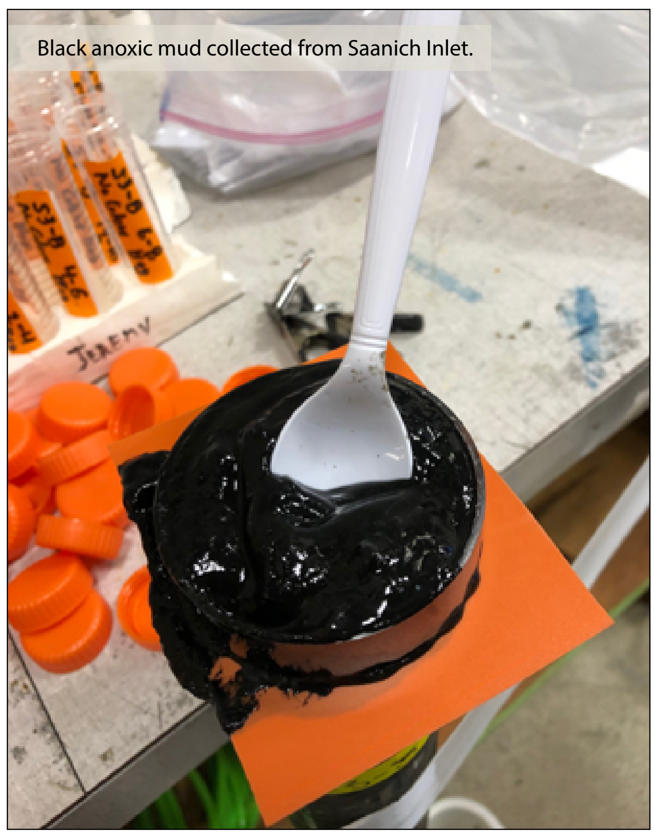
Exuma Cays, The Bahamas. CREDIT: N. Planavsky

Saanich Inlet, Canada

PI Tim Lyons, graduate student Maria Figueroa (UCR) and international collaborator Dan Gregory (Univ. of Toronto, pictured below) traveled to Saanich Inlet, a modern anoxic fjord in Canada, to collect mud and water samples for redox proxy development and calibration via an array of elements, including sulfur and trace metals in pyrite.

Research vessel prepares for sampling tour of Saanich Inlet. Photo credits: T. Lyons

Dan Gregory uses a glove bag to prepare mud samples for analysis.



Black anoxic mud collected from Saanich Inlet.

In the News

Exoplanet Biosignatures: A Review of Remotely Detectable Signs of Life was cited the first week of January as one five "Top-Read Articles of 2018" in *Astrobiology*. https://arxiv.org/abs/1705.05791.

The week of August 21, the journal *Astrobiology* advertised Exoplanet Biosignatures: A Review of Remotely Detectable Signs of Life (Schwieterman *et al*., 2018) as being in the top 1% of its academic field based on number of citations.

Eddie Schwieterman (UCR) consulted on graphics in the March 19 issue of ***National Geographic***: "Life probably exists beyond Earth. So how do we find it?"

Stephen Kane (UCR) co-authored an article in the February 2019 issue of ***Scientific American***: "How Visiting Venus Will Help Us Find Life on Distant Planets"

Space.com wrote a story on our paper, Rethinking CO antibiosignatures in the search for life beyond the solar system in *The Astrophysical Journal*. https://www.space.com/carbon-monoxide-indicator-alien-life.html

Marc Kaufman's Many Worlds blog was one of the best among a broad array of scientific and popular press coverage of our team's Moment of Science for May. Kaufman's take is that "Exoplanets With Complex Life May Be Very Rare, Even in Their "Habitable Zones."

Outreach & Education

Are We Alone' Astrobiology Lecture Series at UCR Palm Desert Center (Winter-Spring 2019) —The UCR team hosted the third annual 'Are We Alone' science lecture series at the UCR Palm Desert Center.

- **March 5:** FILM SCREENING – "The Farthest" (Voyager documentary)
- **March 12**: HABITABILITY OF EARLY EARTH – Tim Lyons, UC Riverside
- **April 2:** HABITABILITY OF EARLY VENUS – Stephen Kane, UC Riverside
- **April 16**: HABITABILITY OF EARLY MARS – Michael Tuite, NASA JPL

Innovate Riverside (May 2019) — The UCR Alternative Earths Team took a lead role in organizing a community fair for the Riverside STEM Academy, a public middle and high school serving grades 5-12. More than 1,000 people passed through the event held in downtown Riverside.

Cosmic Thursday. May 23, 2019 — "The Planet We Could Not Imagine," a free, public lecture at UCR, given by Stephen Kane (UCR)

Riverside Parks and Recreation STEAM Camp. June 26, 2019 — Hour-long lesson on "How Starlight Helps Scientists Discover Planets Outside Our Solar System."

Institutional PI Noah Planavsky (Yale) gave a public lecture on the 'team' for the Geological Society of Australia Selwyn Public Lecture with title: Alternative Earths: Explaining Persistent Inhabitation on a Dynamic Early Earth.

Institutional PI Chris Reinhard (GT) was recently interviewed for the ScienceMatters podcast that the Georgia Tech College of Sciences puts together.

Long Night of Arts & Innovation, Riverside, CA, October 11 — UCR-based team members presented our "Sensing the Universe" outreach program at this large, public event in downtown Riverside, held one day every other year from 5-11pm

UCR Astronomy Club, April 25, 2019, Are we alone? How Earth's past guides the search for alien life

UCR public lecture, July 11, 2019, Apollo 11 Celebration

Sagan Workshop, Pasadena, July 15, 2019, Rise of oxygen on Earth and detectable biosignatures.

Awards & Honors

Jennifer Glass (GT) was promoted to associate professor with tenure.

Stephanie Olson, a former Lyons (UCR) graduate student, who is now completing a postdoc at University of Chicago, has accepted a faculty position at Purdue University.

NPP Fellow Edward Schwieterman (UCR) has been offered a tenure-track faculty position in the UCR Department of Earth and Planetary Sciences.

The UCR Department of Earth Sciences formally changed its name to the Department of Earth and Planetary Sciences to reflect the department's growing focus on astrobiology.

Co-I Ariel Anbar (ASU) was presented the 2019 Samuel Epstein Innovation Award at the Goldschmidt Conference in August. Tim Lyons (UCR) gave the public citation, which emphasized Ariel's career of leadership in studies of Earth's redox evolution using consistently novel geochemical and isotopic approaches.

PI Tim Lyons (UCR) was named a Highly Cited Researcher for 2019 (for the third consecutive year) based on multiple papers that rank in the top 1% by citations for field and year in the Web of Science.

Co-I Noah Planavsky (Yale) was awarded the George Pemberton Medal by the Geobiology Society for outstanding contribution to the field from an early career researcher.

Alternative Earths: 2019 Publications

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RPL
ROCK POWERED LIFE

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Team Overview

The Rock-Powered Life (RPL) NASA Astrobiology Institute team 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 team 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 2019 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 provide updates in three areas of activity. **Project Report 1** describes advances in our understanding of how serpentinite-hosted groundwater chemistry shapes microbial community dynamics. In **Project Report 2**, we provide an update on the RPL team's intensive efforts to successfully extract and characterize DNA and lipid membranes from serpentinite rocks hosting active and fossil microbial activity. In **Project Report 3**, we highlight some of the most recent RPL perspectives on the potential for life in serpentinites on Earth derived from a series of 2020 papers that synthesize findings and hypotheses presented at a Royal Society of London Discussion Meeting held in 2018.

- **Project Report 1:** Biogeochemistry in serpentinization influenced groundwater at the California Coast Range Microbial Observatory and its relationship to microbial community dynamics and bioenergetics
- **Project Report 2:** Detecting and identifying microbial life inhabiting subsurface serpentinite rocks at the Atlantis Massif and Oman
- **Project Report 3:** Rock Powered Life – synthesizing how geologic systems fuel biologic processes

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

2019 Executive Summary

In 2019, the Rock-Powered Life team addressed scientific questions focused on habitable environments and *in situ* microbial activity that can be detected within rock-hosted ecosystems.

At the California Coast Range Microbial Observatory (CROMO), the field team completed an observational time series designed to track the changes in aqueous chemistry and microbial community composition and dynamics in well fluids following the drilling of the CROMO wells in 2011 (**See Project Report 1**). The data set captures the effects of drilling-induced perturbations upon microbial activity and community structure, and provided the chemical and interpretive context to support publications focused on sulfur cycling (Sabuda *et al*., 2020) and carbon uptake (Seyler *et al*., 2020).

Several RPL members focused on the analysis of subsurface serpentinite rocks obtained in 2018 during the Oman Drilling Project. Rigorous DNA and lipid extraction and characterization protocols were developed and applied to the processing of ~25 subsurface serpentinite cores (**See Project Report 2**). RPL also started to decipher the complex mineralogy of the highly serpentinized rocks recovered from drilling in the active serpentinization zone. Templeton lab members identified highly reduced metallic iron-nickel alloys, as well as highly oxidized ferric iron bearing phases, such as hydroandradite garnet, as unexpectedly abundant phases playing a role in buffering the subsurface redox conditions and availability of H_2. Eric Ellison, co-I Lisa Mayhew and PI Alexis Templeton also completed developing integrated Raman microspectroscopy analyses for mineral identification and synchrotron-based x-ray absorption microspectroscopy to map the Fe-oxidation state of the distinct mineral phases in serpentinized samples by quantifying $Fe(III)/Fe_{total}$ at the microscale using the Fe pre-edge (Ellison *et al.*, in press). This approach is now being widely used on Oman and Atlantis Massif samples in order to characterize which reactions during low-serpentinization give rise to habitable conditions.

The RPL team intensively reconstructed the metabolism and activity of *Acetothermia* and *Methanobacterium* from metagenomic and metatranscriptomic data derived from deep fracture fluids in the Oman ophiolite (Fones *et al*., in review; Coleman *et al*. in prep; Howells *et al.* in prep; Kraus *et al.* in review). Efforts in the Boyd, Spear,

RPL field work in Oman 2019 was also conducted on a small scale for the targeted pumping of waters from deep boreholes. Graduate student Dan Nothaft (Templeton lab) engaged in efforts to use packer systems to collect fluids from isolated depth intervals that intersect distinct redox and pH conditions in the subsurface.
Photo credit: Sherry Paukert

Templeton, Shock and Hoehler labs focused on identifying metabolic adaptations that allow populations to overcome the stress imposed by hyperalkaline pH and the resultant effects that this has on inorganic carbon limitation.

For our senior personnel, Rock-Powered Life team congratulates co-investigator Professor Everett Shock, ASU, for receiving the Geochemistry Medal of the American Chemical Society. RPL also congratulates early-career co-investigator Dr. Lisa Mayhew for being selected as a Participating Scientist on the Mars2020 Sample Return team. The RPL team was also pleased to again host the Astrobiology Graduate Conference, a key early-career development event this time led by Julia McGonigle at the University of Utah in July 2019.

Co-I Ono's lab continued to develop methane isotopologue geothermometry to distinguish methane generated by microbes from abiotic processes. Graduate students Cumming and Beaudry applied the technique to methane sampled from sites of serpentinization and volcanic fumaroles and geothermal wells in Iceland (Cumming *et al.*, 2019). RPL Graduate Student Jeemin Rhim (Ono Lab) is shown sampling gases from a steam vent from Kerlingarfjöll in central Iceland. Photo credit: Shuhei Ono

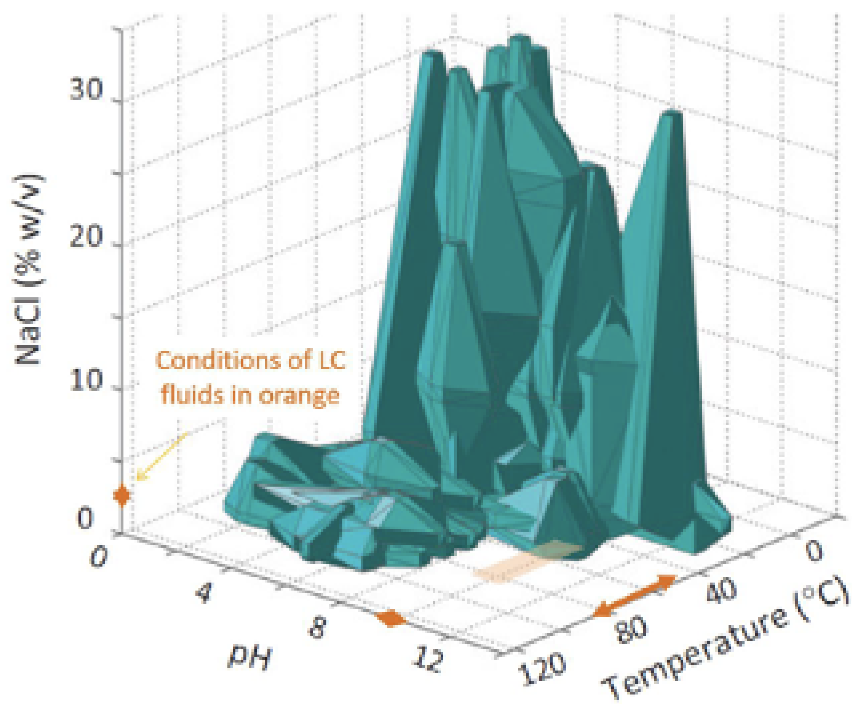
AbGradCon 2019 was hosted by RPL graduate students at the University of Utah. The meeting engaged 75 participants from 47 institutions and 7 different countries (Australia, Brazil, Canada, France, Israel, Japan, and the US). Photo credit: Julia McGonigle

Project Reports

Rock Powered Life – deciphering how geologic systems fuel biologic processes

An overarching goal of the Rock-Powered Life team is to identify how the mechanisms of low temperature water/rock reactions control the distribution, activity, and biochemistry of life in rock hosted systems undergoing serpentinization. In January 2020 a thematic issue 'Serpentinite in the Earth System', published in the Philosophical Transactions of the Royal Society A, included five research papers authored by RPL team members. These papers reflect perspectives shared at an international discussion meeting hosted by the Royal Society of London in 2018. Templeton & Ellison (2020) explore the role of Fe-bearing brucite in H_2 production at low temperature. They find that Fe-brucite reactivity may be a key control on the timing and extent of H_2 generation and thereby also influence the potential for serpentinizing systems to support microbial life. They hypothesize that locations in which ferroan brucite has been formed and then consumed may have high biosignature preservation potential. McCollom *et al.* (2020) address the effect of pH on reaction pathways and rates during serpentinization finding that highly alkaline conditions may favor H_2 production and thus increased biological activity. Mayhew and Ellison (2020) conduct a quantitative meta-analysis of Fe chemical and redox data to investigate the relationship between iron, serpentine, and water/rock reaction conditions across a variety of previously studied systems. They find a general lack of coupled chemical and redox data and suggest that standardizing such measurements will aid in unraveling the complex relationships between reactants, products, and conditions. Boyd *et al*. (2020) relate the conditions that characterize serpentinizing systems, e.g. high pH, high [H_2] and the correspondingly low redox potential to the biochemistry and evolution of early autotrophic metabolisms. They suggest that the hyperalkaline, H_2-rich conditions of serpentinizing systems would have permitted the use of low-potential ferredoxins as primordial electron carriers, without requiring the complex molecular machinery required for electron bifurcation strategies utilized by extant H_2-dependent autotrophic life. Lang and Brazelton (2020) also consider system conditions and their relationship to the activity of life in the rock-hosted Lost City hydrothermal system. They conclude that life in such systems is likely not limited by a single limiting factor but instead by a combination of factors that may include the amount of bioavailable CO_2 and/or N coupled with the stress of high temperatures and hyperalkaline conditions. They suggest that clarifying how these factors interact and influence one another will be key to assessing the potential for such environments to host life on Earth and other planets.

Figure 1. Lang and Brazelton (2020) show the known boundaries for life based on prokaryote cultures in green, adapted from Harrison *et al.*, 2013. The pH and temperature conditions for Lost City fluids are highlighted in orange.



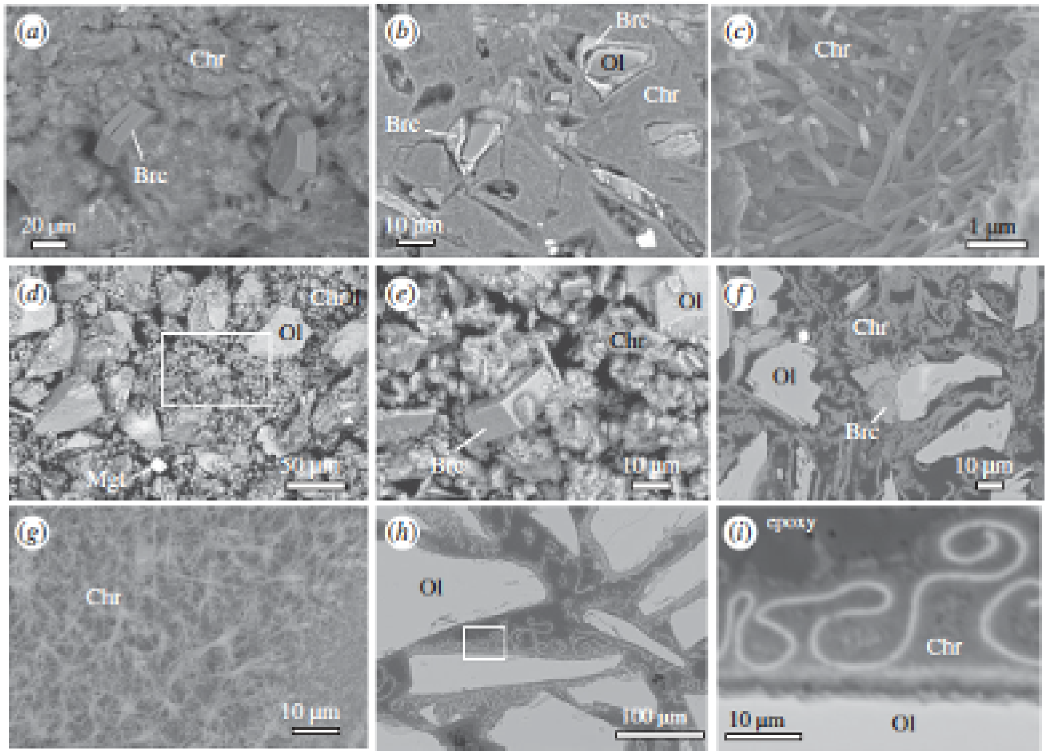


Figure 2. Chrysotile (chr) is the main serpentine phase identified in all water/rock experimental conditions though the morphology varies as does the presence of other secondary phases, including brucite (brc) and magnetite (mgt) (McCollom *et al*., 2020). Relict primary olivine (ol) is present in many of the experimental conditions.

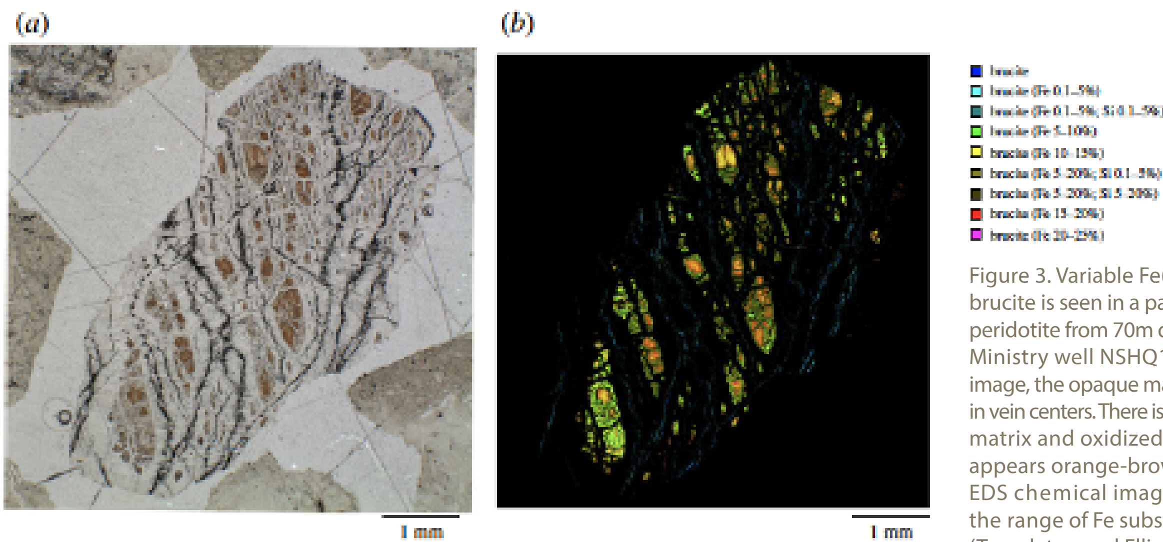


Figure 3. Variable Fe(II) substitution into brucite is seen in a partially serpentinized peridotite from 70m depth in Oman Water Ministry well NSHQ14. (a) In the optical image, the opaque magnetite grains occur in vein centers. There is a colorless serpentine matrix and oxidized Fe-bearing brucite appears orange-brown. (b) Quantitative EDS chemical imaging by Zeiss shows the range of Fe substitution into brucite. (Templeton and Ellison, 2020).

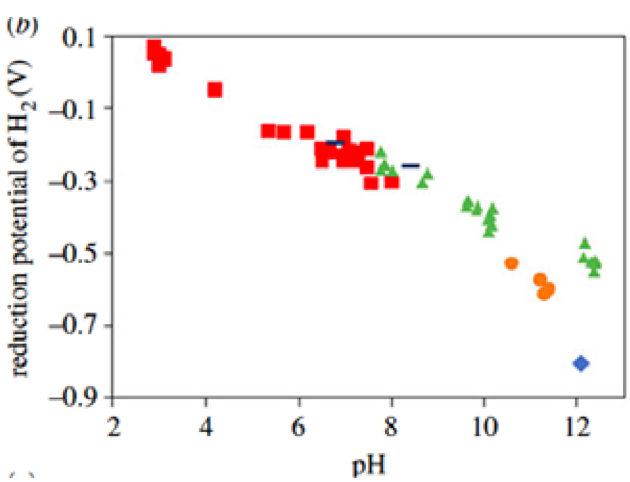


Figure 4. The reduction potential of H2 in early Earth analogue natural environmental samples decreases with increasing pH. Data from: hot springs environments = red squares; subglacial environments = black lines; CROMO = green triangles; Oman ophiolite = orange circles; Lost City = blue diamonds. (Boyd *et al*., 2020; Figure 2b).

Biogeochemistry in serpentinization influenced groundwater and its relationship to microbial community dynamics and bioenergetics

The Rock-Powered Life team has utilized the Coast Range Ophiolite Microbial Observatory (CROMO) as a key field-site for unraveling the coupled hydrology, geochemistry and microbiology of serpentinizing systems. Such integrated studies inform our understanding of how low temperature water-rock reactions impact microbial processes, an overarching theme of the Rock Powered Life team. Studies by the CROMO RPL team (Co-Is Cardace, Hoehler, McCollom, Schrenk) have focused upon using the strong hydrological and microbiological framework (Cardace *et al*. 2013; Crespo-Medina *et al*., 2014; Twing *et al*., 2017; Ortiz *et al*., 2018) to hone in on specific processes influencing biogeochemistry at the site. For example, groundwater time-series data demonstrates elevated hydrogen concentrations in the groundwater due to drilling-induced perturbations followed by a transition to highly reducing conditions that favored acetogenesis (Putnam *et al*., in preparation). Analysis of CROMO groundwater, and integration of hydrodynamic, metagenomic, and geochemical data over the past seven years has shown the segregation of microbial communities across different niches, such as surface regimes influenced by recent mixing between meteoric water and serpentinization-derived fluids, low connectivity isolated aquifers at hyperalkaline pH, and deeper regimes influenced by mixing of serpentinization-derived fluids with paleoseawater.

Most recently, the Schrenk lab investigated the impact of stored seawater, a remnant of submarine alteration prior to the formation of ophiolites, to stimulate biological activity such as microbial sulfur cycling in serpentinites (Fig. 1, 2). Geochemical and metagenomic data of biomass pumped from wells was integrated together, revealing the role of cryptic cycling of intermediate sulfur oxidation states in driving microbial activity in the deepest, most saline water at CROMO (Fig. 2; Sabuda *et al*., 2020).

Parallel investigations into the organic geochemistry in CROMO fluids and its relationship to water-rock reactions, diagenetic processes, absorption to minerals, and microbial metabolism have shown that C-1 compounds, including methane and formate, are important carbon sources for sustaining microbial metabolism in hyperalkaline well waters (Fig. 3; Seyler *et al*., 2020). This work builds upon previous isotopologue analyses that found a mixed biogenic/thermogenic origin for methane at CROMO (Wang, *et al*., 2015), and helps to identify potential pathways for methane formation and consumption.

Co-I Tominaga and undergraduate student Paiden Pruett (Texas A&M) have been establishing a new geophysical approach to map out the phase and extent of *in situ* chemical processes, such as serpentinization and carbonation of mantle peridotite. A particularly novel aspect of their approach to studying the CROMO serpentinite weathering process is using rock magnetic signals as a proxy for tracking changes in Fe-bearing mineralogy and ground truthing this with QEMScan and tomography data.

The Cardace lab has employed spectroscopic approaches (FTIR, Raman) to study the character of carbon-containing compounds in solid matrices at CROMO, and their relationship to mineralogy (Fig. 4). Cardace and Johnson (in prep) use FTIR investigations of carbonate caps associated with serpentinites to show that in carbonate matrices, dispersed organics are detectable when present at high concentration. Cardace and Sousa (in prep) use

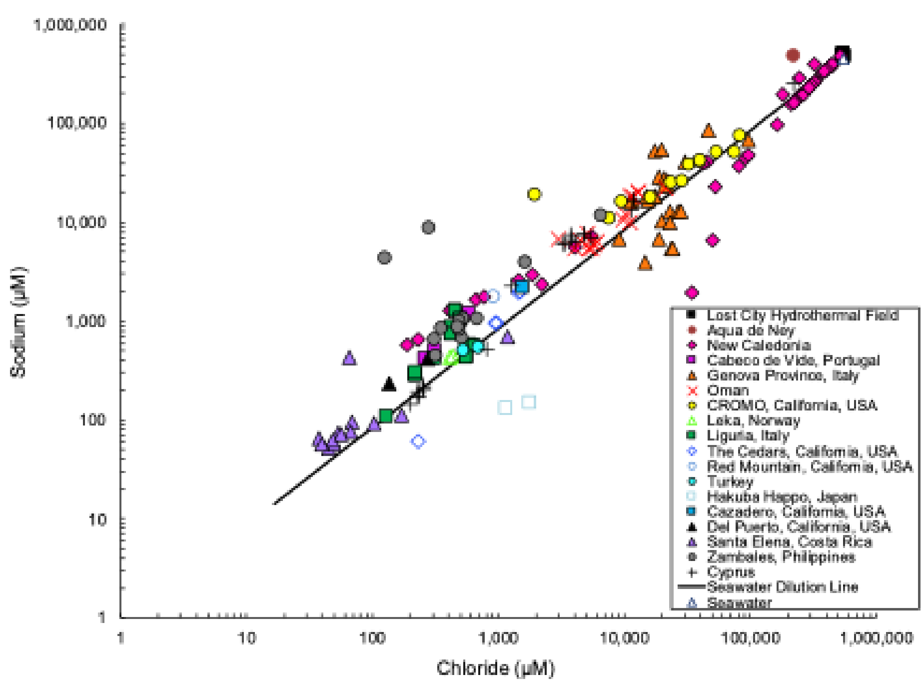


Figure 5. Sodium and chloride values (□M) are plotted for published subaerial serpentinizing systems worldwide, with a black seawater dilution line plotted for reference. CROMO wells plot as yellow circles. This data compilation show that many continental serpentinization-influenced springs and groundwater are moderately saline which has implications for both the chemistry of water-rock reactions and the bioenergetics of the microbial life they sustain. (From Sabuda *et al*., 2020).

FTIR and Raman to detect organic films with a serpentinite background; robust spectral data allowed discrete accounting of methyl groups in live and sterile organic-containing solutions. Hart and Cardace (in prep) use these results, combined with data from other serpentinites, to develop bioenergetic models that incorporate the potential of solid mineral phases to sustain life on Earth and elsewhere. They propose that as serpentinization proceeds, the oxidation of methane, as well as Fe and S in pyrite grains, are the most energy-yielding reactions. Aqueous Fe cycling also appears feasible and should be more intensively investigated.

Finally, the CROMO team is collectively using the multi-year geochemical and genomic datasets to develop a comprehensive microbiological and bioenergetic framework for understanding subsurface life in serpentinization-influenced groundwater. This work takes into account interactions between fluids and minerals, hydrologic data, and microbial physiology to develop a holistic model incorporating these diverse processes. The range of conditions encapsulated by the CROMO site reveals the bioenergetic potential and nutrient resources available to serpentinite-hosted microbial communities, and consequently their impact upon serpentinite biogeochemistry (Cardace *et al.*, in prep.).

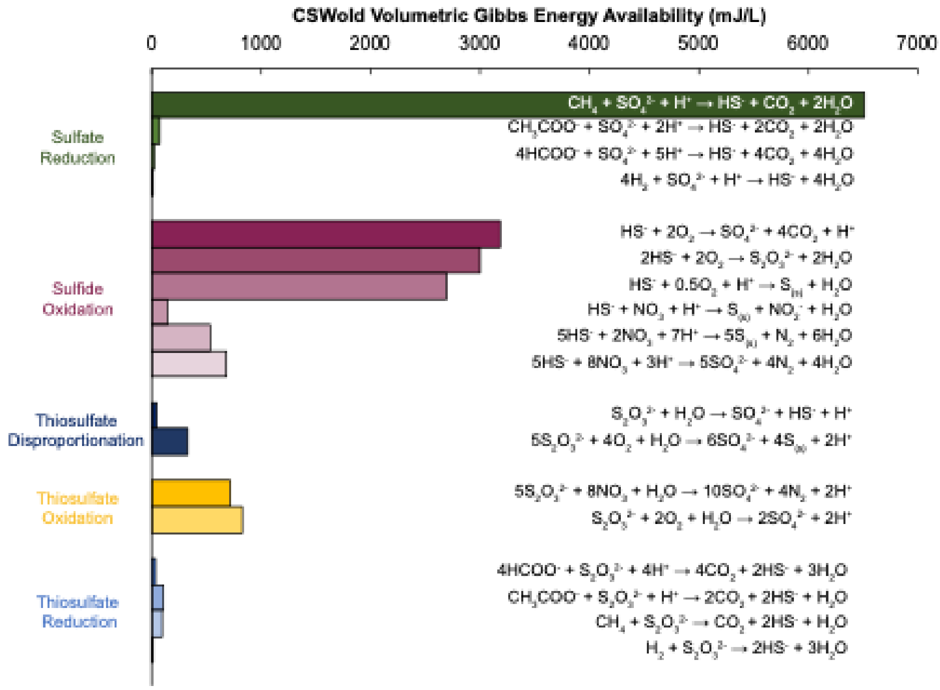


Figure 6. MilliJoules of energy available per liter of fluid (energy density) for a suite of sulfur reactions calculated using measured aqueous geochemistry data for specific sulfur reactions within the deepest, most saline well at CROMO, CSWold. These calculations show the substantial bioenergetic potential available in saline wells when products of serpentinization (e.g. H_2, CH_4, formate) are mixed with seawater fluid compositions. (From Sabuda *et al.*, 2020)

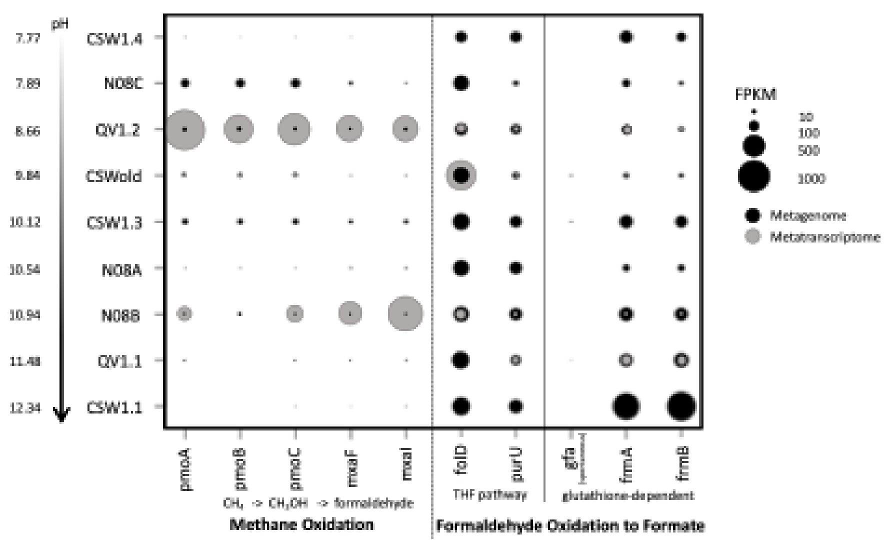


Figure 7. Fragments per kilobase of sequences per million mapped reads (FPKM) of genes in the methane oxidation, tetrahydrofolate (THF), and glutathione-dependent formaldehyde oxidation pathways. Metagenomes are represented in black; metatranscriptomes are represented in gray. These data show the relative absence of known methanotrophy genes in high pH wells, while genes involved in formaldehyde oxidation and assimilation are present and expressed. These data suggest that low temperature geochemical reactions may lead to the formation of metastable C-1 compounds that may sustain microbial metabolism in the CROMO wells. (From Seyler *et al.* 2020).

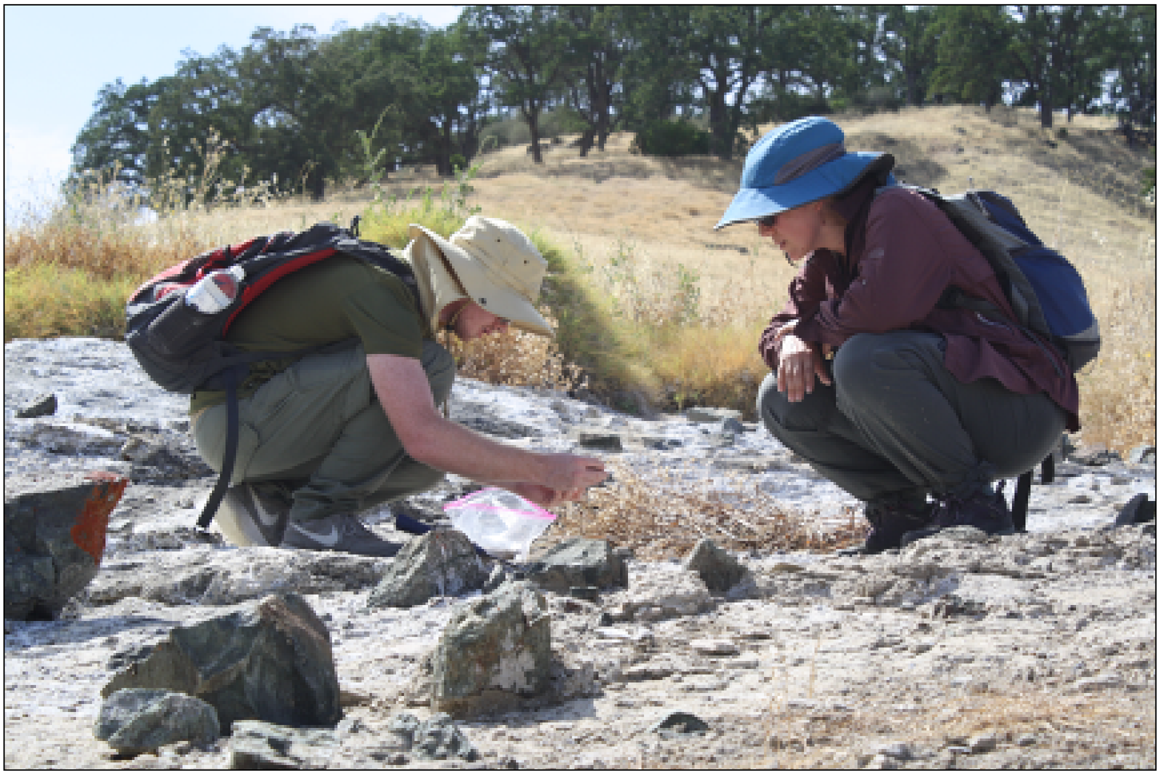


Figure 8. Undergraduate researcher Holden Faraday collects travertine samples (overlaying weathered serpentine-chlorite-smectite slope materials) with Dawn Cardace in the vicinity of CROMO wells to examine the co-occurring carbonate minerals and green, organic-rich layers near sample surfaces. Faraday is applying Raman spectroscopy to powdered and polished subsamples of these travertines to characterize background inorganic and organic spectral components, and is beginning (January 2020) with biofilm formation studies using these specimens.

Detecting and identifying microbial life inhabiting subsurface serpentinite rocks

The generation of H_2, reduced carbon compounds and other chemical products of water-rock reactions can sustain a subsurface microbiome within serpentinites. Given the potential ubiquity of serpentinization reactions on other rocky bodies in the solar system and across Earth's history, detecting and characterizing serpentinite-hosted life is a high priority target for astrobiological investigations. To date, microbiological studies of serpentinization zones have relied on biomass recovered from reacted fluids, due in part to the technical and physical challenges in obtaining and extracting biomass from rock samples. To distinguish endemic microbial taxa hosted in serpentinite subsurface rocks from aquifer microorganisms and other potential contaminants, biomass and DNA must be extracted from serpentinite rock samples and control samples. The methodology and results of this project have implications for life detection experiments, including sample return missions, and provide the first window into the diversity of microbial communities inhabiting subseafloor serpentinites.

International Ocean Discovery Program (IODP) Expedition 357 to the Atlantis Massif (Mid-Atlantic Ridge 30°N) collected cores of altered oceanic crust, including serpentinites, from seventeen drilled holes at nine sites. A major component of the project was the minimization and detection of contamination into the extremely low-biomass samples. To extract DNA, rock core samples were processed in collaboration with the Kochi Core Center (JAMSTEC) in Japan, using a sterile rock saw to remove exterior surfaces of samples, leaving only pristine interiors for subsequent analyses. Many samples of seawater, drilling lubricants, and laboratory air were included in our DNA sequencing study and used to identify and remove contaminant DNA sequences from the final results.

This study provides the first sequences of environmental DNA from subseafloor serpentinites, enabled by the high recovery of rock cores by IODP Expedition 357. DNA was extracted from at least one rock core sample from each site. The extremely low biomass of the serpentinites as well as the presence of minerals such as phyllosilicates, that act as inhibitors to DNA extraction, purification and sequencing, caused multiple challenges necessitating the development of a novel DNA extraction and purification protocol. Use of chemical solutions that block inhibitors, in addition to SCODA (synchronous coefficient of drag attenuation) technology for purification and concentration of DNA from these extremely low-biomass samples, helped us overcome those challenges. The taxonomic compositions of serpentinite samples were highly variable (Figure 1). No two samples were alike, and no clear correlations with subseafloor depth or mineralogy were apparent. Our results do highlight a few candidate residents of the shallow serpentinite subsurface, including uncultured representatives of the *Thermoplasmata, Acidobacteria*, *Acidimicrobia*, and *Chloroflexi*. These microbes were detected previously in similar environments such as deep-sea sediments in the Okinawa Trough, marine sediments and altered rocks of the Mariana subduction zone, where serpentinization can also occur.

The Rock-Powered Life team is also investigating the microbial communities inhabiting the continental serpentinite subsurface in the Samail ophiolite of Oman. As part of the Oman Drilling Project Phase II, twelve members of RPL collected rock core segments for biological studies from three boreholes drilled 300 to 400 meters into the ophiolite. These boreholes (BA1B, BA3A, BA4A) vary in mineralogy and aqueous geochemical parameters (e.g. pH/Eh as a function of depth). A major goal is to identify members of the microbial communities within these subsurface regimes through culture-independent high-throughout DNA sequencing studies and intact polar lipid biomarker analysis. This will elucidate how life is distributed in this continental serpentinized environment and what adaptations may be used to meet the challenges of energy and nutrient limitation and hyperalkaline pH. Additionally, the methodologies developed may be useful for future studies of rock-hosted ecosystems.

To extract DNA a rigorous workflow had to be established to address the challenges of abundant contaminants and low biomass samples which required large volumes of rock. Exterior surfaces of the core were removed with a modified rock saw to reduce contamination. Core exteriors and interiors were processed and extracted separately along with a broad suite of contamination controls from the field drilling sites and laboratory. The workflow minimized binding of the DNA phosphate backbone to positively-charged mineral surfaces using a modified phenol-chloroform-isoamyl alcohol extraction method. A portion of the small subunit ribosomal RNA (SSU rRNA) gene was then targeted for amplification and sequencing.

Initial results from borehole BA1B show low numbers of sequences recovered from most, but not all, cores. The most abundant taxa look similar across all control and

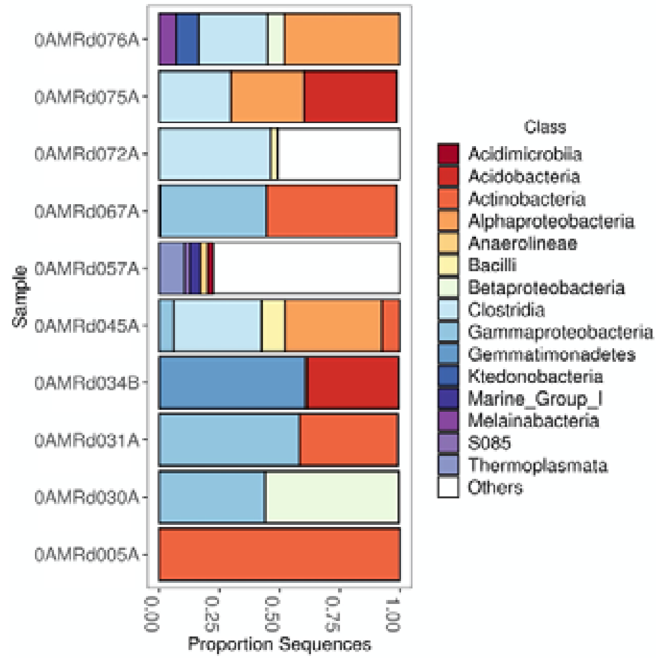


Figure 9. Taxonomic summary of AM Expedition 357 serpentinite samples after contaminant removal. The listed taxa showed the top 50 microbial classes that are the most abundant ones among all of the serpentinite samples. Sample 0AMRd057 has the highest number of sequences. It also has the highest taxonomic diversity. Some of the abundant taxa, Thermoplasmata, Anaerolineae and Acidobacteria, were previously found in deep-sea and marine sediments with similar environmental conditions to the marine subsurface.

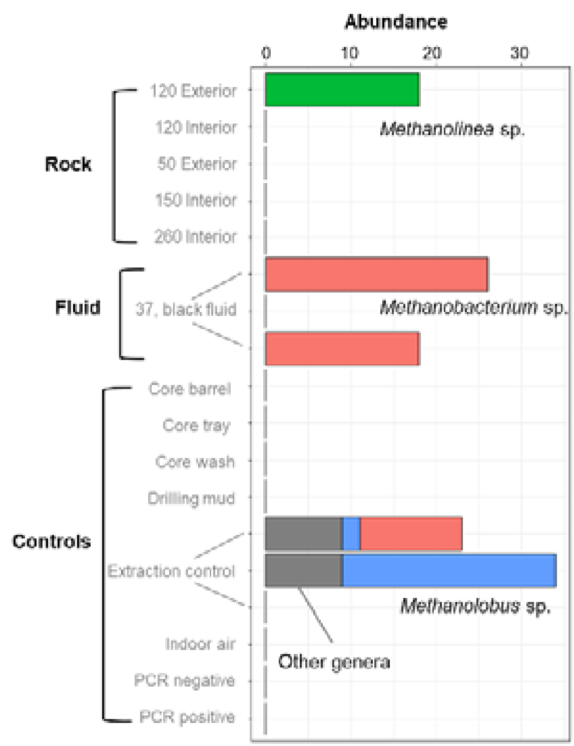


Figure 10. An example of differences in presence/absence of Archeal strains between core, fluid, and control samples. Methanogens such as Methanobacterium sp. have been found in aquifer waters of the Samail ophiolite previously.

rock samples; belonging to the *Proteobacteria*, *Firmicutes* and *Actinobacteria* phyla. Generally, contamination controls group by location (field drilling site versus laboratory) and rock core samples cluster with either of the two groupings. However, contaminating sequences were expected to be a large part of the data set and further examination of the rare sequence variants shows promise. Many of the lower abundance sequences are identified as taxa that have been previously detected by sequencing the ophiolite aquifer fluids (e.g. *Meiothermus sp*., *Methanobacterium sp*., members of Acetothermia, Patescibacteria, and Dependentiae phyla). Of these taxa, some show differences in presence/absence between the rock and all control samples, possibly indicating that these sequences are specific to the rock-hosted environment (Figure 10). This work is in the preliminary stages and analysis of initial samples from BA1B is ongoing while rock and control samples from BA3A and BA4A are currently being sequenced.

As with the extracted DNA, establishing the syngeneity of lipid biomarkers in deep serpentinite core has proven to be analytically challenging. Surface contamination is removed by paring off the exterior centimeter of the core using a combusted, solvent-cleaned blade [French *et al*., 2015]. Only the interior subcore sample is utilized for biomarker analysis and a combusted brick blank is processed in tandem with the interior of the core to quantify any contamination introduced during sawing and subsequent crushing, milling, and lipid extraction. To deal with the low predicted biomass of serpentinite core, we extract from 50 g of interior rock core (or brick blank) powder using a modified Bligh and Dyer extraction [Wormer *et al*., 2015]. We use 10% of the total lipid extract to identify intact polar lipid biomarkers using hydrophilic interaction liquid chromatography coupled to electrospray ionization high resolution mass spectrometry (HILIC-ESI-HRMS) using a completely untargeted approach. To be able to recognize when organic contaminants may have penetrated into the interior of the serpentinite core, we have characterized a deep library of field contamination controls. HILIC-ESI-HRMS signatures of total lipid extracts from serpentinite core samples are dominated by organic contamination introduced both in the field and during laboratory processing. However, in some of the rock-derived lipid extracts, we are able to detect compounds with peak intensities many orders of magnitude higher than the contaminating signal (Figure 3). In borehole BA1B, we have tentatively identified betaine lipids that have previously been identified in Oman fluids and have been described to be produced by bacteria under phosphate limitation [Rempfert *et al*., in prep; Bosak *et al*., 2016; Sebastian *et al*., 2016]. This indicates the presence of very likely endogenous rock-hosted lipid signatures and illustrates great potential to successfully apply these methods to the rest of the rock core samples.

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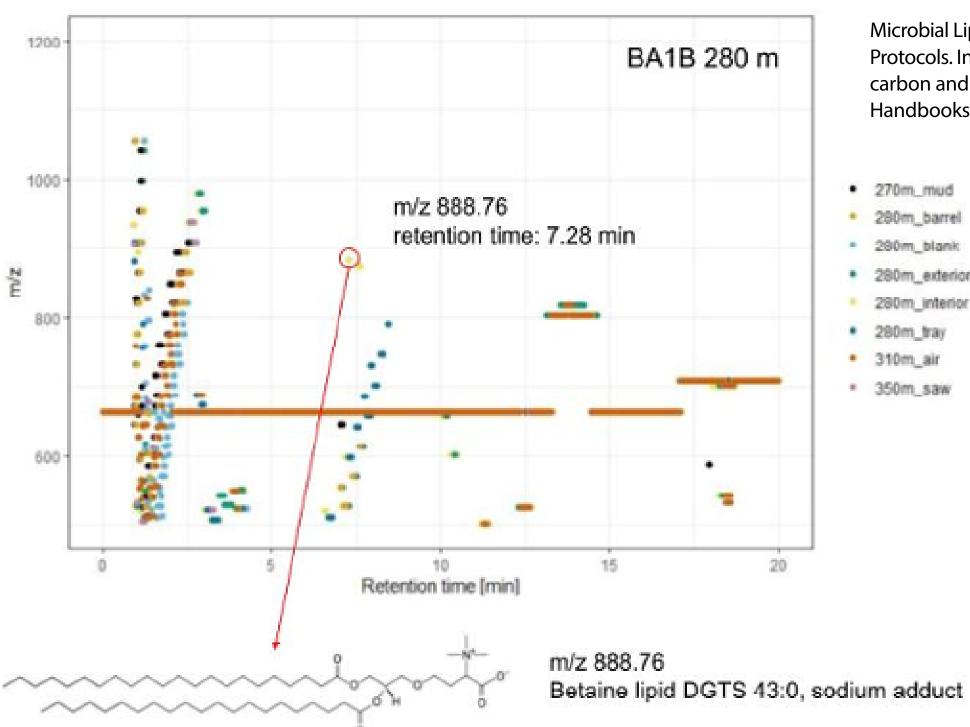

Figure 11. Plotted mass to charge value vs. retention time of analytes with peaks at 75% base peak intensity or greater for a serpentinite core sample retrieved from hole BA1B at 280 m depth and associated contamination controls and blank. Note, clusters of compounds only detected in the sample (exterior and interior) can be identified. The peak intensity for the IPL with a MS1 m/z 881.76 and retention time of 436 seconds is six orders of magnitude higher in the interior sample than the associated contamination controls. This IPL is tentatively identified as a betaine lipid, which has previously been identified in Oman serpentinized fluids [Rempfert *et al*., in prep] and has been described to be produced by bacteria under phosphate-limiting conditions [Bosak *et al*., 2016; Sebastian *et al*., 2016].

Rock Powered Life: 2019 Publications

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Lauren Seyler
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Alexander Sousa
John Spear
Patrick Thieringer
Christopher Thornton
Masako Tominaga
Chris Trivedi
Katrina Twing
Noah Vento
Lindsay Williams
Kristin Woycheese
Spencer Zeigler

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