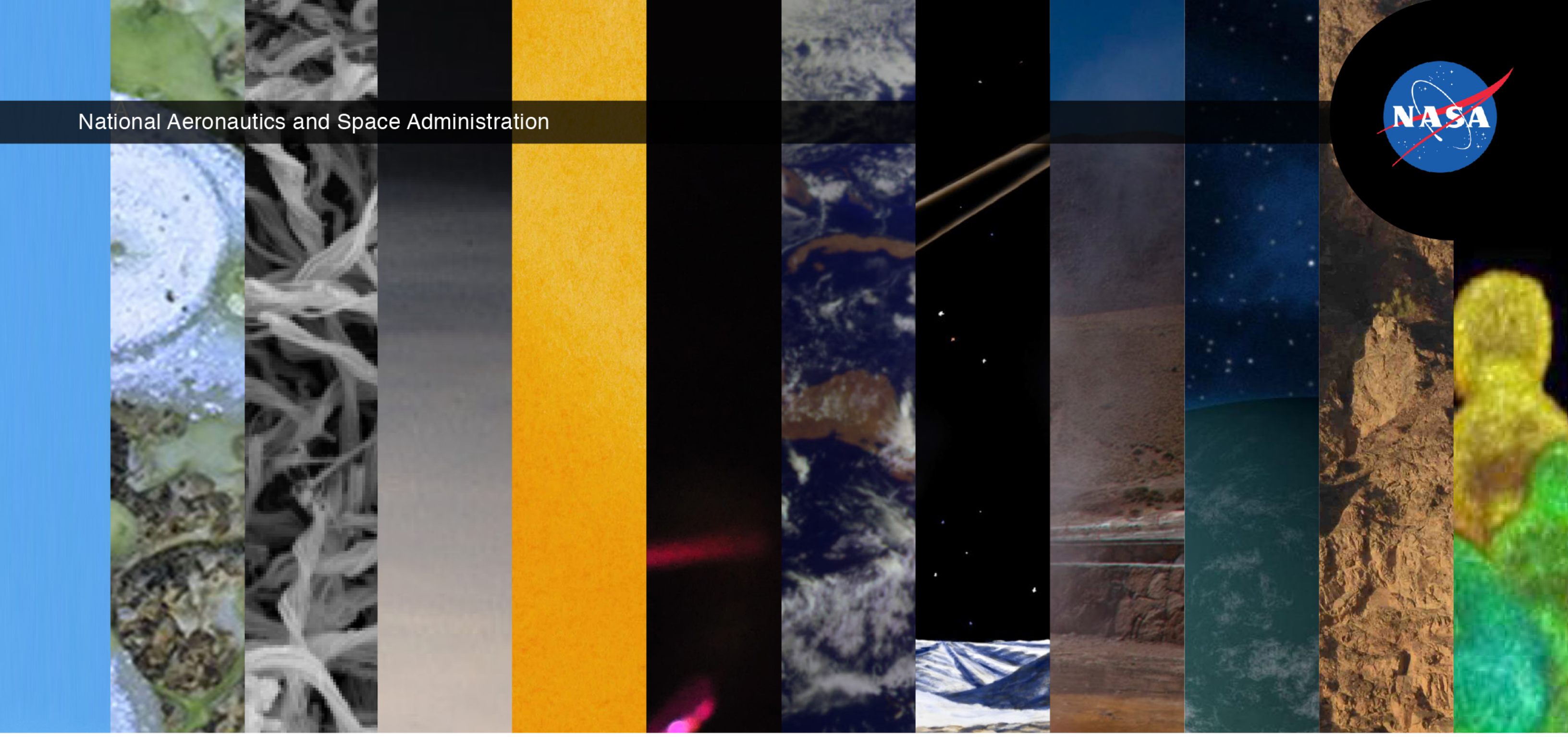
National Aeronautics and Space Administration

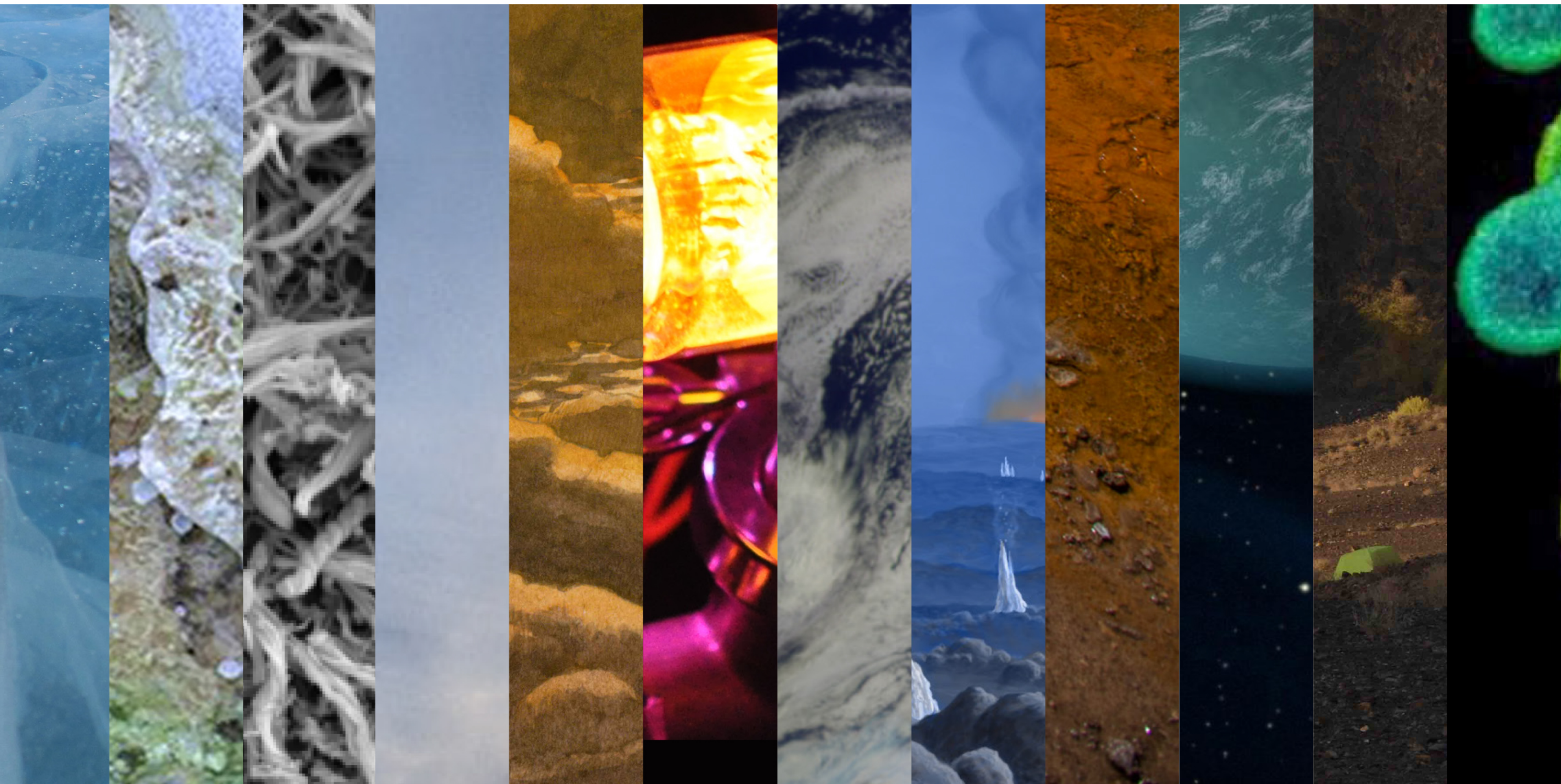


NASA ASTROBIOLOGY INSTITUTE

2017 Annual Science Report

Rock Powered Life

University of Colorado Boulder



RPL
ROCK POWERED LIFE

Lead Institution:
University of Colorado Boulder

Principal Investigator:
Alexis Templeton

Team Overview

The goal of the Rock Powered Life Team is to reveal how energy flows from the lithosphere to the biosphere. Rocks store chemical energy that can power living systems when released through extensive interaction with water. However, there is a need to mechanistically define how, when, and where geological systems can directly sustain biological activity, particularly within the vast realm of environmental conditions where rocks and water react across the shallow subsurface of rocky planets and moons.

RPL identifies controls upon the production and consumption of energy sources such as hydrogen, methane and carbon monoxide in "Serpentinizing Systems", which are environments where ultramafic rocks react with water. Establishing how chemical and biological processes are coupled together during Serpentinization will have important implications for developing strategies for detecting extraterrestrial life beyond Earth, such as in the shallow crust of Mars and icy satellites (Europa, Enceladus), and improving hypotheses concerning primordial ecosystems on Earth.

RPL efforts in 2017 led to significant accomplishments highlighted within four main project areas:

- How geochemistry supports chemosynthetic life at high temperatures in hot springs
- Advances in characterizing physics and chemistry of serpentinizing systems
- Experimental production of hydrogen at low temperatures
- Geochemical disequilibria within serpentinizing systems

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

2017 Executive Summary

In 2017, the 12 Rock-Powered Life investigators, and their lab members, have been identifying habitable environments and in-situ microbial activity within rock-hosted ecosystems. Notable accomplishments include new insights into the metabolic activity, genes and evolutionary history of subsurface chemosynthetic life in Yellowstone National Park, and advances in characterizing the geochemistry and geophysical properties of systems undergoing active water/rock interaction that are all highlighted in our Project reports. RPL NAI team members also played an integral role in hosting and presenting at a NASA Workshop Without Walls: Serpentinizing Systems Science.

RPL investigators conducted intensive groundwork related to our core field investigations – the California Coast Range Microbial Observatory, the Atlantis Massif, and the Oman ophiolite – that are integral to all of our NAI activities.

In January 2017, nine members of RPL (representatives of Templeton, Boyd, Spear, and Schrenk labs) conducted a field campaign to sample deep subsurface hyperalkaline fluids in the Samail Ophiolite. The goal was to capture biomass and dissolved gases from highly reduced fluids to probe how ongoing low-temperature serpentinization shapes the distribution and activity of life within subsurface peridotite. PI Templeton and graduate students Rempfert and Nothaft characterized the geochemistry of dissolved gases and redox-active carbon, nitrogen, sulfur and iron species. Co-I Spear and graduate student Kraus conducted molecular microbial community characterization of DNA extracted from fluid and rock samples. Co-I Boyd and graduate student Fones began to characterize the abundance of cells in the fluids, and used radiotracer techniques to determine the maximum potential rates of carbon substrate assimilation and transformation. In parallel, NPP Postdoc Glombitza utilized 35S-labelled rate assays to calculate in-situ microbial turnover rates of sulfate that were lower than observed in

Fig. 1. RPL NAI team members at CROMO redeploying a pressure-temperature datalogger string into the CSW well to measure hydrogeological properties of the serpentinite subsurface.Credit: Matt Schrenk

Fig. 2. Nine members of the RPL NAI, representing four different labs, conduct fieldwork in Oman in 2017. Credit: Alexis Templeton

any other habitat yet probed on Earth (approximately a few fmol to a few pmol per mL fluid per day).

RPL investigators significantly progressed in their comprehensive geophysical, geochemical, hydrological and microbiological characterization of the California Coast Range Ophiolite Microbial Observatory. Co-I Tominaga and graduate student Ortiz characterized changes in the subsurface magnetic and electrical properties associated with groundwater flow during on-going serpentinization processes. Co-I Schrenk and graduate student Williams are completing a time-series analysis of fluid chemistry and microbiology that has been ongoing since 2011, conducting statistical analyses of the data to decipher both seasonal and episodic effects upon the predominant microbial populations. Graduate student Sabuda, working with co-I Hoehler and RPL researcher Kubo, measured geochemical profiles related to methane oxidation and used this as a basis to set up stable isotope tracer experiments. Post-doc Seyler complemented this work with analysis of dissolved organic matter in fluids from CROMO using FT-ICR-MS, GC/MS, and LC/MS/MS. This metabolic output data is being integrated with existing metagenomic data to gain a sense of the function of the carbon cycle at CROMO.

Lastly, Co-I Brazelton and graduate student Motamedi made critical progress developing a powerful method for the extraction and purification of DNA from extremely low-biomass rock samples, using serpentinized rocks collected from the Atlantis Massif during IODP Expedition 357. These advances set the stage for how RPL will comprehensively conduct a molecular-level investigation into the subsurface biosphere across our suite of serpentinite-hosted ecosystems.

Fig. 3. RPL graduate student Shahrzad Motamedi extracts DNA from low biomass serpentinites from the Atlantis Massif in the Brazelton Lab at the University of Utah. Credit: William Brazelton

Team Members

Alexis Templeton
Eric Boyd
Grayson Boyer
William Brazelton
Laura Bueter
Peter Canovas
Dawn Cardace
Nabil Chaudhry
Carol Cleland
Dan Colman
Julie Cosmidis
Vince Debes
Eric Ellison
Elizabeth (Libby) Fones
Clemens Glombitza
Tori Hoehler
Alta Howells
Brian Hynek
Abigail Johnson
Jena Johnson
Peter Kelemen
Sebastian Kopf
Emily Kraus
Mike Kubo
Graham Lau
James Leong
Melody Lindsay
Juerg Matter
Lisa Mayhew
Thomas McCollom
Julia McGonigle
Hannah Miller
Shahrzad Motamedi
Daniel Nothaft
Juan Carlos Obeso
Shuhei Ono
Estefania Ortiz
Kaitlin Rempfert
Jeemin Rhim
Kirtland Robinson
Mary Sabuda
Michelle Scherer
Matthew Schrenk
Julio Sepulveda
Lauren Seyler
Everett Shock
Sanjoy Som
Alexander Sousa
John Spear
Christopher Thornton
Masako Tominaga
Chris Trivedi
Katrina Twing
Noah Vento
Lindsay Williams
Kristin Woycheese

Project Reports

Advances in Characterizing the Chemistry of Serpentinizing Systems

In 2017 the RPL team advanced our ability to characterize critical components of subsurface mineralogy and gas chemistry in serpentinizing field sites towards an effort to more accurately assess the habitability of the deep rock-hosted biosphere.

Co-I Mayhew, early career scientist Ellison and PI Templeton worked to optimize synchrotron-radiation based spectroscopic techniques that utilize Fe K-edge spectra as a measure of the oxidation state of Fe in mineral phases that are produced during the hydration of ultramafic rocks (Mayhew et al., 2018; Ellison et al., in preparation). When integrated with microscale mineral identification analyses, partially altered Oman serpentinites were found to possess Fe(II)-bearing relict primary phases and secondary serpentine, likely Fe(II)-bearing, intergrown with Fe-bearing brucite. Highly altered Oman serpentinites were dominated by more Fe(III)-rich serpentine and carbonate (Mayhew et al., 2018). Oxidation associated with the transformation of partially to highly altered rocks may have produced H_2 that could have acted as an electron donor for microbial communities.

Natural serpentinized samples sometimes appear to be rich in super-paramagnetic sized (diameter <50 nm) magnetites, yet magnetic analyses usually only account for hydrogen production from 'normal' sized magnetite. Members of the Tominaga lab are using samples from CROMO to test whether super-paramagnetic magnetites should be considered in estimations of hydrogen production and predictions of biomass hosted by in situ serpentinization processes (Noah et al., 2017). Frequency-dependent susceptibil-

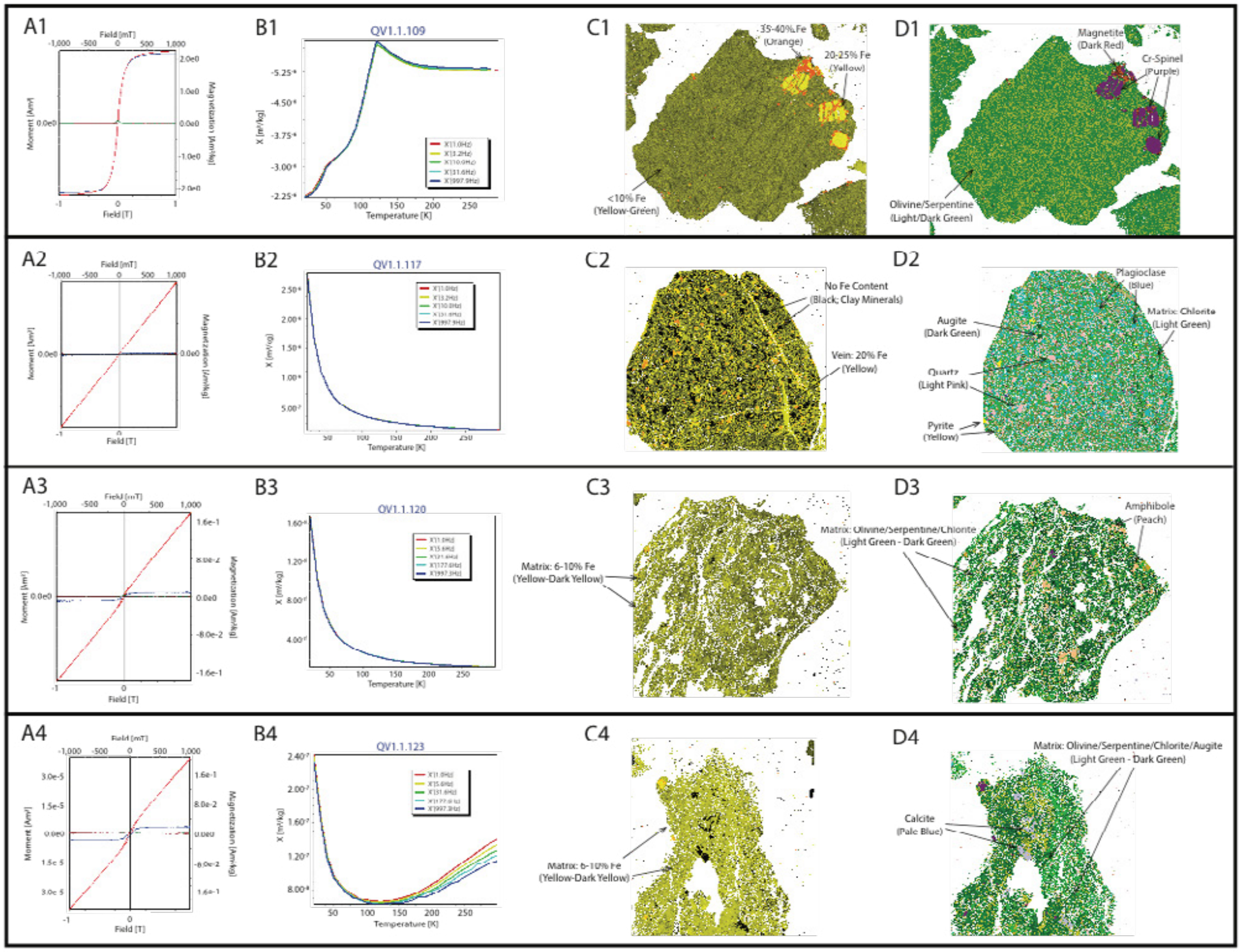


Fig. 4. Magnetic measurements and microscopy analyses of QV1.1 samples: (A1-A4) Hysteresis curves of samples displaying ferromagnetic (QV1.1.109), paramagnetic (QV1.1.117), and superparamagnetic (QV1.1.120 and QV1.1.123) magnetic behavior; (B1-B4) Frequency-dependent susceptibility curves; (C1-C4) Fe-Maps; (D1-D4) Phase maps. Credit: Noah Vento

ity magnetic measurements were conducted at the Institute of Rock Magnetism (IRM) (Fig. 4). Multiscale EDS phase mapping, Back Scattered Electron scanning, FIB-nanotomography and STEM-EELS were employed to identify and quantify the superparamagnetic minerals that contribute to the measured magnetic susceptibility signals (Fig. 1).

The hydrogen produced from serpentinization can often drive carbon reduction reactions, resulting in the production of reduced-rich molecules such as methane. Members of the Ono lab are constraining the extent of abiotic methane production during serpentinization from measurement of methane isotopologue compositions in samples from ophiolites in California, Oman, Turkey, Canada and the Philippines, as well as several sites on the seafloor along the Mid-Atlantic ridge (Fig. 5) (Wang et al., 2018). Multiple sources of methane were identified. Terrestrial sites appear to share clumped isotope characters with microbial methane or thermogenic methane. In contrast, methane from seafloor hot springs yield high temperature signals (250 to 400°C), suggestive of a deep origin. A model that supports the occurrence of methane-rich aqueous fluids in the oceanic lithosphere, where methane is formed during serpentinization at temperatures between 250 and 400°C was proposed. This study implies a strong kinetic barrier for abiotic methane generation at temperature below 250°C during serpentinization (Wang et al., 2018).

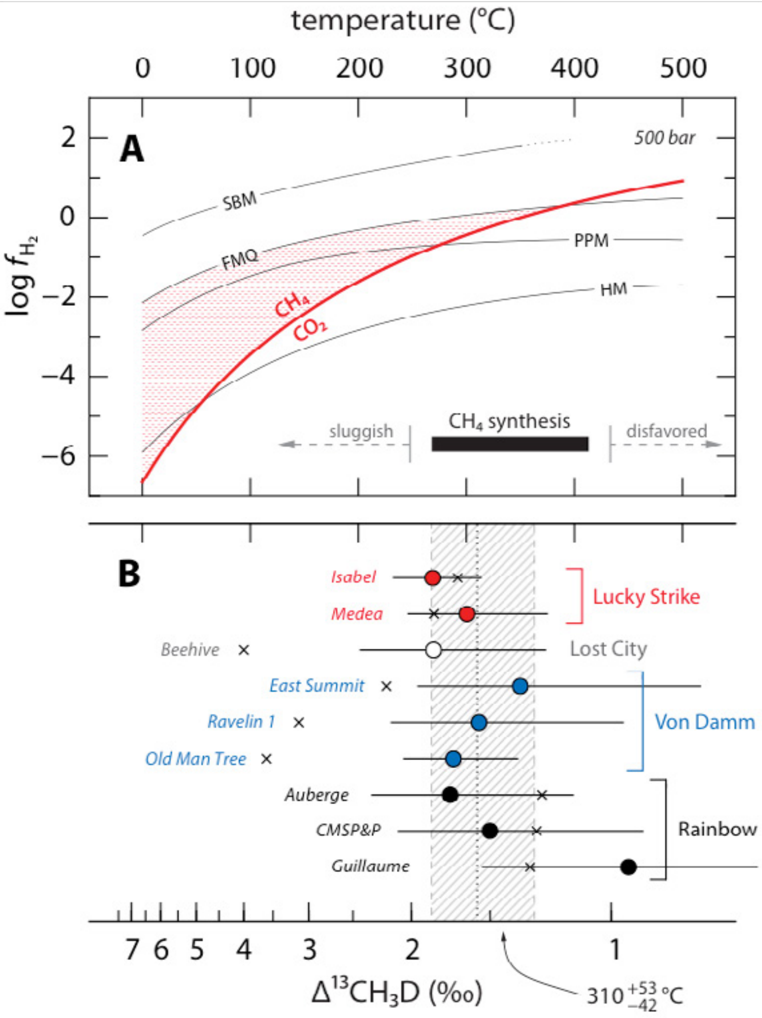


Fig. 5. Constraints on abiotic methane formation and stability from thermodynamics and clumped isotopologue data. (A) fugacity of H_2 as a function of temperature for different redox buffers: hematite-magnetite (HM), pyrite-pyrrhotite-magnetite (PPM), fayalite-magnetite-quartz (FMQ), and serpentine-magnetite-brucite (SBM) (from Shock, 1992; Sleep et al., 2004). The SBM buffer is truncated above 400C where serpentinization is unlikely to occur. Red shaded area represents the intersection of regions corresponding to geologically-relevant H_2 fugacity and where CH4 is thermodynamically stable relative to CO_2. (B) Clumped isotopologue temperatures of methane from seafloor vent fields. From Wang et al., 2018. Credit: Wang

Experimentally-exploring the production of hydrogen at low temperatures and alkaline pH

Hydrogen is a critical electron donor source that may sustain life activity in rock-hosted systems. The RPL team utilizes laboratory experiments to evaluate the rates and pathways whereby reduced compounds such as hydrogen are produced by the reaction of water with ultramafic rocks and minerals within the temperatures considered habitable for life. In particular, we are simulating the hydration of peridotites under the cooler and more alkaline conditions that persist across large volumes of the Earth's shallow subsurface, as well as on other planetary bodies such as Mars, Europa and Enceladus, in order to identify the potential rates and extent of hydrogen generation that can sustain microbial activity.

In recent work, Miller et al. (2017) tested the H_2 generating capabilities of naturally occurring, partially-serpentinized peridotites in contact with fluids at 100°C. The highest observed rates of low-temperature hydrogen generation were measured, in addition to the notable formation of small molecular weight reduced carbon substrates such as formate and acetate.

In this study, RPL investigators Miller, Mayhew, Ellison, Kelemen, Kubo, and Templeton also successfully identified the key mineralogical changes and Fe-oxidation reactions giving rise to hydrogen production through the integration of a variety of spectroscopic and analytical techniques. This work highlighted that partially-serpentinized rocks bearing reactive minerals such as Fe(II)-rich brucite have the potential to generate significant amounts of hydrogen and organic acids, which is critical for generating habitable conditions.

McCollom et al. (in prep) further expanded our ability to predict environmental conditions that may enhance serpentinization reaction rates. They have demonstrated that the rates of hydrogen generation can be significantly increased 4-10 fold by increasing pH, and they suggest that the commonly observed transitions to strongly alkaline conditions in low-temperature serpentinizing systems likely contributes to high hydrogen concentrations.

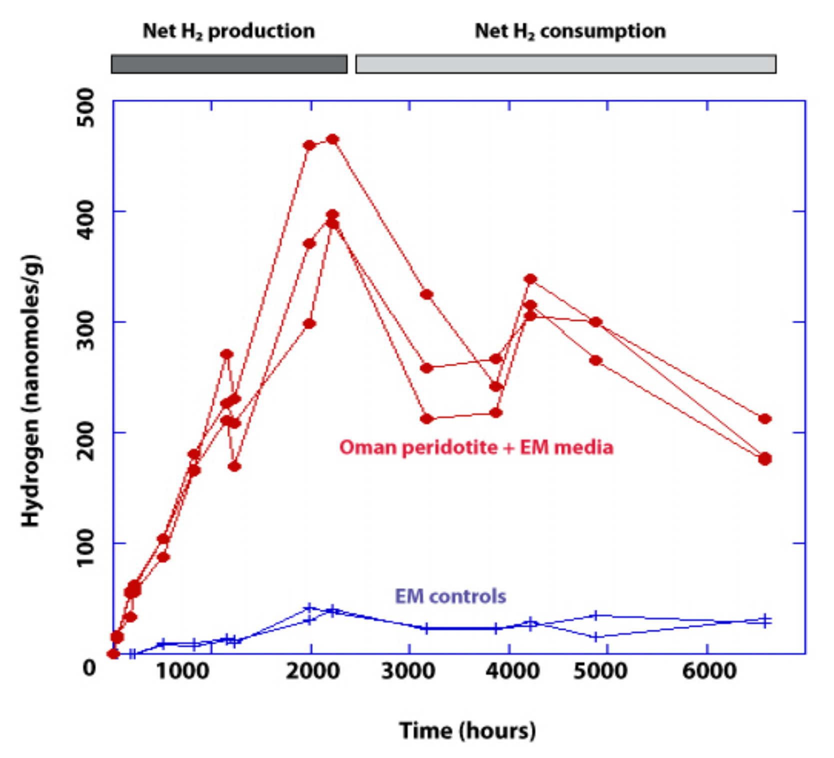


Fig. 6. Hydrogen production at 100°C from reaction of partially serpentinized Oman dunite with seawater EM media (red lines) conducted in triplicate. Controls (vials filled with fluids, no minerals, purged with N_2:CO_2) are shown with blue lines. Net H_2 concentrations increase for approximately 2000 hours and then decrease. See Miller et al., 2017 for full paper and datasets.

Rock Powered Life in Yellowstone National Park

Significant advances were made in understanding how geochemistry supports chemosynthetic life at high temperatures in Yellowstone National Park hot springs through collaborative efforts by RPL researchers Boyd, Shock, Spear and their research teams. Three high profile research products were generated in 2017. New data on the distribution of genes in subsurface versus surface microbiomes indicate subsurface populations are adapted to the use of lithogenic substrates (Colman, et al., 2017a). This data was put into the context of the past 25 years of deep hydrothermal biosphere research in a Perspective paper for PNAS (Colman, et al., 2017a). In a second study, we investigated how a metabolically flexible, chemoautotrophic microorganism isolated from a hot spring in YNP chooses among available geologically derived substrates (Amenabar et al., 2017). We found that the energy required for electron transfer dictates the choice of substrate, not the energy supplied from the substrate. This novel insight indicates that thermodynamic calculations of energy supplies cannot alone accurately predict the distribution of microorganisms in natural environments. Instead, models must also take into account the biomass yield of strains with diverse metabolic strategies (Amenabar et al., 2017). A third study focused on understanding the distribution and diversity of thermoacidophilic Archaea among YNP hot springs, their potential relationship to the formation of acidic hot springs, and the evolutionary history of these archaeal lineages (Colman et al., 2017b). Similarities in protein-coding genes between independent lineages of archaeal thermoacidophiles suggest that horizontal gene transfer between lineages occurred during convergent evolution. Oxygen is generally required to both produce and inhabit acidic environments making it likely that these thermoacidophiles evolved after the rise of oxygenic photosynthesis (Colman et al., 2017b).

RPL also examines how electron acceptor availability has influenced the diversification of microbial life. In the first of two papers focused on a chemosynthetic thermophile, we show that electron acceptor availability alters carbon and energy metabolism in an isolated strain (Amenabar et al., in press). We also examine mechanisms that allow cells to access insoluble minerals to support growth (Amenabar et al., in reviewb). A third study used the natural environments of a paired set of hot springs in YNP that have differing availabilities of oxidants due to differences in subsurface processes that supply hydrothermal fluids to these springs. Using a combination of geochemical, molecular, microcosm, and culture-based analyses, we showed that the availability of oxidants in these two springs varied, which in turn impacted the abundance and composition of hydrogen-oxidizing autotrophic microbial populations (Lindsay et al. 2018).

Fig. 7. Suspended elemental sulfur and silicate nanoparticles together diffract light to yield this aquamarine colored hot spring in Yellowstone. Not surprisingly, this spring is dominated by sulfur dependent chemosynthetic microbial communities. Credit: Eric Boyd

Geochemical Disequilibria Within Serpentinizing Systems

Several RPL team members are investigating how water-rock-organic reactions confer habitability during serpentinization. In 2017, Co-I Shock and RPL-affiliated graduate students Robinson, Howells, Leong and Canovas completed thousands of forward reaction-path models to develop quantitative predictions regarding the geochemical evolution of surface waters as they move through the subsurface within ultramafic aquifers. These efforts reveal thermodynamic drivers of the carbon cycle during serpentinization, and the influence of chemical energy supplies on structuring microbiomes and the distributions of metabolic strategies as functions of reaction progress.

These projects build on recently published results from serpentinizing systems in Oman that quantify the affinities of key redox reactions, such as hydrogen oxidation, aerobic methane oxidation and methanogenesis, that are most closely tied to known isolates, microbial strategies, and gene distributions (Canovas et al., 2017).

In one almost completed project, Co-I Shock and recent PhD graduate Robinson focused on the central role of carbon chemistry in many processes during low temperature serpentinization, including the precipitation of carbonate minerals as fluids become more alkaline, maintaining biomolecules, and powering biological metabolisms via redox reactions. They used geochemical models to predict when transformations between inorganic and organic carbon should occur during low temperature serpentinization. The production of H_2 in the presence of inorganic carbon during serpentinization theoretically sets up an energetic drive for the formation of organic compounds, although carbonate reduction reactions can often be kinetically hindered at low-temperatures. Thus Robinson and Shock have been assessing when and where there is a metabolic potential for the formation of formate, acetate and methane in the subsurface, and examining why many fluids remain out of equilibrium with respect to the carbonate and organic species. Identifying formation conditions and equilibration pathways for organic compounds will help to target the location of active processes and associated microbial communities.

Photo credit: Tori Hoehler

Geophysical Remote Sensing of Water/Rock Interactions

Geophysical remote sensing has emerged as a powerful approach to characterize in situ water-rock interaction processes in time and space. Members of the Tominaga Lab conducted 2D Electrical Resistivity Tomography (ERT) surveys to investigate in situ geological and hydrogeological architecture within the tectonic mélange portion of CROMO during wet and dry seasons where water/rock interaction processes are thought to facilitate a subsurface biosphere (Ortiz et al., 2017). Changes in in situ hydrological properties, i.e., the lateral and vertical distribution of conductive zones and their temporal behavior that are dependent upon seasonal hydrology were documented. A hydrogeological architecture model, in which the *in situ* formation is composed of four distinct aquifer systems: serpentinite aquifer without seasonal dependency (shallow system), fractured well-cemented serpentine confining beds with seasonal dependency (intermediate system), serpentinite aquifer with seasonal dependency (deep system), and the ultramafic basement that acts as a quasi-aquiclude (below the deep system) (Figure Ortiz2) was proposed. The stunning contrast between the seasonality in the surface water availability and groundwater storativity in the formation allows us to locate zones where serpentinite weathering and possibly deeper serpentinization processes might have taken place primarily based on the lithological composition and the distribution of the conductive formation and highlights the link between these processes and the source of water in space and time.

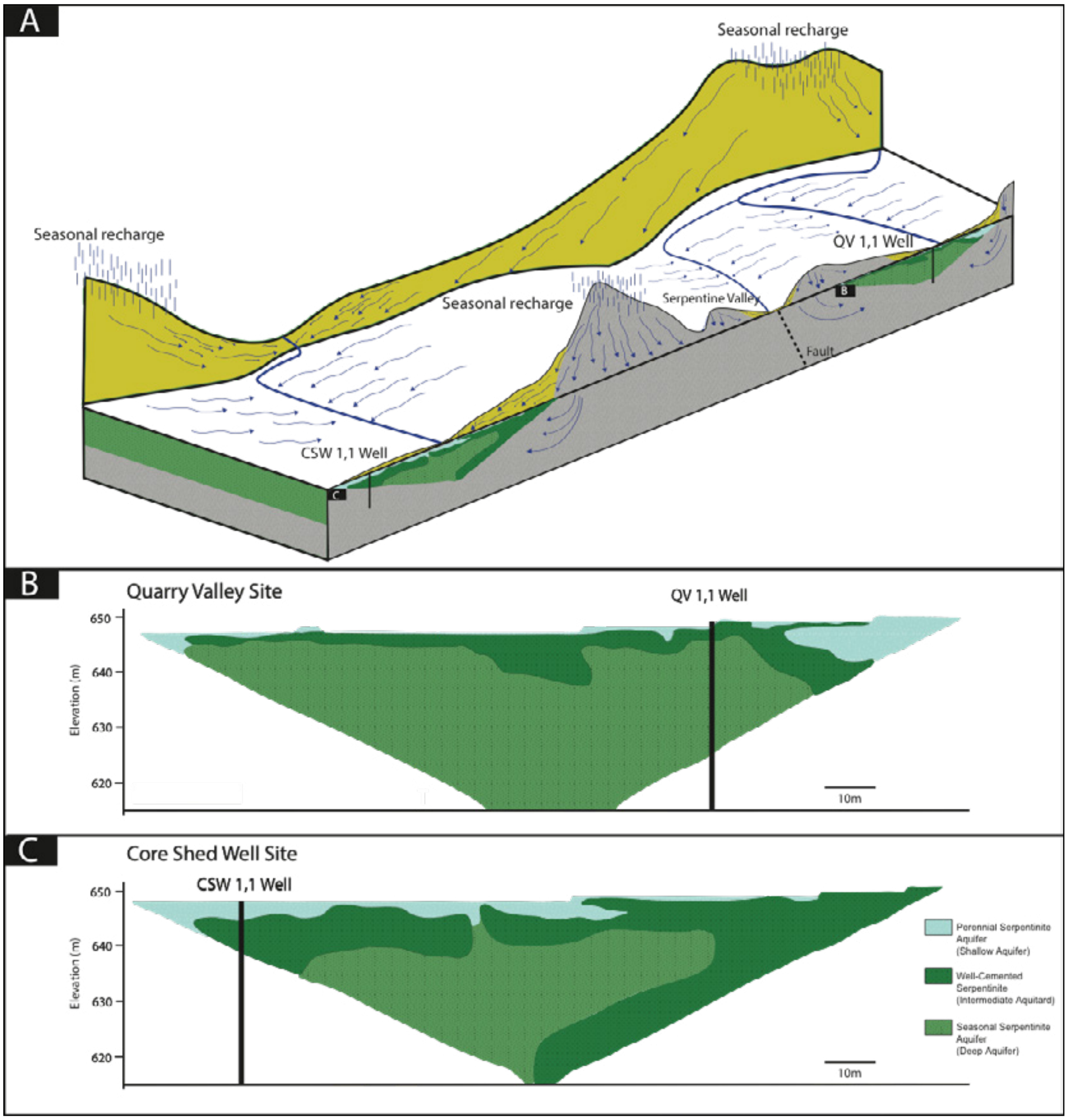


Fig. 8. (A) CROMO Hydrogeological architecture model, (B) local hydrogeological architecture model of QV site and, (C) local hydrogeological architecture model of CSW site. Model based on core-log ERT integration. Credit: Ortiz et al., 2018

Rock Powered Life: 2017 Publications

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