Narrative Progress Report:
1. PRF: 57209-DNI2; Institutional Account: 268887
2. Project Title: *The inner lives of Archaea*: the hydrogen isotopic composition of Archaeal lipids as a proxy of metabolic state.
3. Leavitt, William (Dartmouth College)
4. Collaborator: Pearson, A. (Harvard University)
5. Reporting Period: 01 July 2017 to 31 August 2018

Summary and Impact

Archaea are ubiquitous microbes in Earth's ocean sediments, extreme environments and oil/gas reservoirs. Prominent members, including methanogens, anaerobic methanotrophs, aerobic ammonium oxidizers, as well as the more well-studied thermoacidophiles. Different archaea perform critical biogeochemical reactions with global economic consequences. *Sulfolobus* strains are essential for high-temperature sulfur cycling in hydrothermal vents, hot springs and hydrocarbon reservoirs. Critically, the lipids from thermoacidophilic archaea are frequently used to understand environmental conditions, which are heretofore poorly calibrated due to our severely limited knowledge of how archaeal physiology is recorded in lipids – the result of few lab calibrations. Here we are working to develop a stable isotope-based organic geochemical tool to disentangle different archaeal metabolic phenotypes. It was recently shown that H-isotopic compositions in bacterial lipids (δD_{lipid}) are significantly offset from ambient water, where δD_{lipid} values vary strongly in response to changing environment. Because archaea in general tend to occupy systems characterized by extremes, including energy limitation, we anticipate that archaeal δD_{lipid} values also are particularly sensitive to variations in energy flux. In this study we are cultivating model archaeal thermoacidophiles in batch and continuous cultures under different electron donor supply regimes in order to quantify the response of δD_{lipid} to 'energy rich' versus 'energy poor' conditions. This approach will lend insight into the physiological response of archaea to energy stress and will directly impact our understanding of archaeal lipids, perhaps recalibrating them as *in situ* proxies for energy stress. The results from this work will fundamentally improve our knowledge of archaeal metabolic plasticity, and can be extended to archaea critical in to oil and gas-production, and reservoir preservations.

We are well on our way toward the successful completion of this project. In year one of the two year ACS-PRF-DNI proposal we have (i) constructed and calibrated three parallel chemostatic bioreactors (Figure 1), (ii) successfully completed three parallelized growth rate experiments with the model thermoacidophilic archaeon, *Sulfolobus acidocaldarius* and analyzed the GDGT abundances. (iii) In separate batch experiments we have already characterized the response of archaeal lipid cyclization in response to pH, temperature, and oxygen availability (Figure 2), and (iv) we have also quantified the response of *Acidianus sp.* DS80, an exceptionally challenging to grow chemolithoautotrophy, on three different redox coupled (Figure 3).

In the upcoming year we will complete rate experiments under electron donor limitation, and move on to oxygen limitation experiments. We will then move to quantifying the H-isotopic compositions of individual GDGT lipids and that of the water. The extraction HPLC preparation and TC/EA and HTC-GC IRMS and methodologies have primarily been worked out on *Sulfolobus sulfotaricus* GDGT's generated in our lab in the last year, with sample presently being prepared in the Pearson lab at Harvard, for H-isotopic analysis by colleague Kopf at CU Boulder.

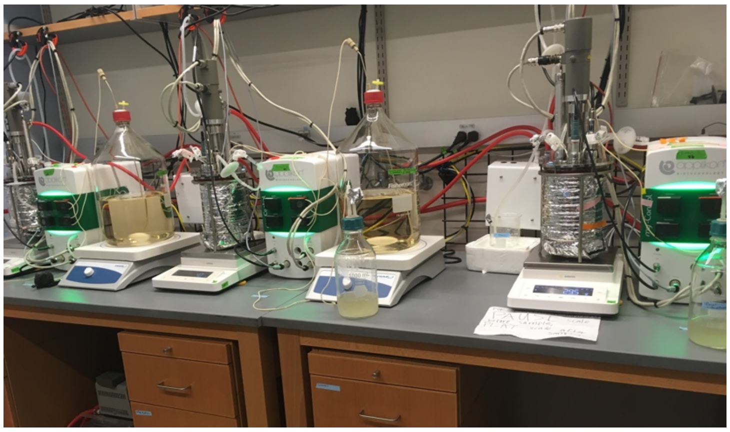


Figure 1. Leavitt lab Applikon chemostats. Operating in 3*x* parallel at 70°C and pH 2.5 with *Sulfolobus acidocaldarius*. Operated by graduate student A. Zhou.

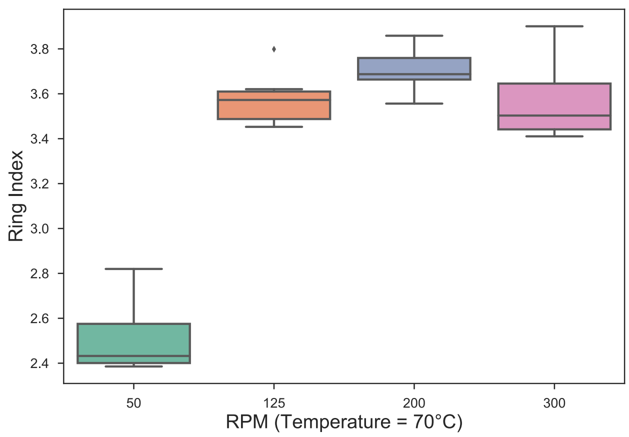
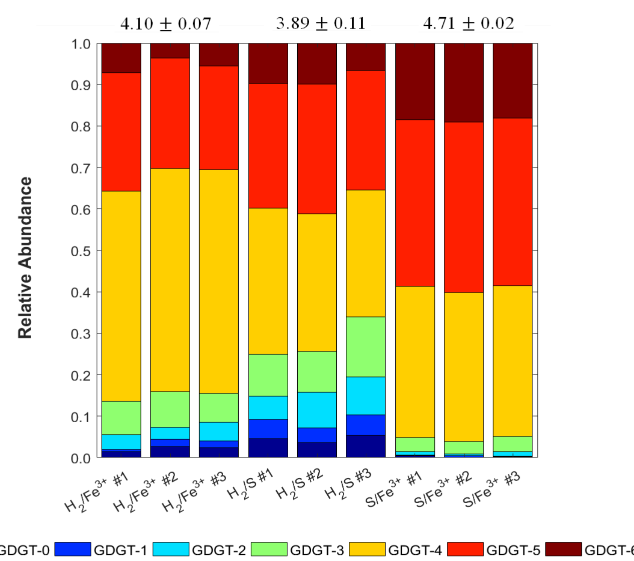


Figure 2. The relative abundance of different cyclopentyl rings in the GDGT lipids of *Sulfolobus acidocaldarius* grown at different temperatures as a function of shaking speed, which is a simplified proxy for oxygen availability. Experiments by undergraduate A. Cobban.

Figure 3. Lipid profiles show that **ring index varies with metabolic substrate independent of temperature**. Notably, it is lowest when DS80 is utilizing its preferred redox couple, H_2/S.



Career Impact: This project represents the first major award to Leavitt (PI) and has enabled the hiring of Zhou (masters student) and an undergraduate (Cobban). As such, this project is supporting three early career scientists at different stages, and intermittently, a postdoctoral fellow (Y. Weber). This project will allow for this first publications from the Leavitt lab, a planned submission to the journal Organic Geochemistry later in 2018, and has already supported A. Zhou in her first presentation at the international Goldschmidt Conference in August of 2018.

Published Abstracts

Zhou[#], A, M Amenabar, Y Weber, FJ Elling, A Pearson, **WD Leavitt** . Archaeal GDGT profiles as recorders of free energy availability. 2018, 28th V.M. Goldschmidt Conference, Boston, MA, USA.

Personnel

Leavitt (PI): 0.5 summer month in 2017 and 0.5 summer month in 2018 (rounded)
Cobban (undergraduate student): $2,445. December 2017
Zhou: (graduate student): 9 months, (December 2017 to August 2018).