

## **Comparative genomics and functional characterization of assimilatory sulfate reduction in methanogenic and methanotrophic archaea**

Hang Yu<sup>1\*</sup> (hyu@caltech.edu), Dwi Susanti<sup>2</sup>, Shawn McGlynn<sup>3</sup>, Connor Skennerton<sup>1</sup>, Patricia Tavormina<sup>1</sup>, Biswarup Mukhopadhyay<sup>2</sup>, and **Victoria Orphan**<sup>1</sup>

<sup>1</sup>Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, 91125, USA; <sup>2</sup>Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA; <sup>3</sup>Tokyo Institute of Technology, Earth-Life Science Institute, Tokyo, 152-8550, JAPAN

**Project Goals: Biological methane production or consumption may be constrained by the availability of sulfur in the environment. Comparative genomics could be used to characterize metabolic pathways and select for key genes of interest for further experimental studies. We applied this approach in our project below to understand how methane-metabolizing archaea meet their obligate requirement for sulfur in its ecological niche.**

Methanogenic and methanotrophic archaea are critical in the global carbon cycle, and their growth is dependent on sulfur. While sulfide is the most common sulfur source, other sulfur compounds such as sulfite, thiosulfate and elemental sulfur can be present in their environments, but the capability and mechanism of assimilation are less well-understood. Here we explored new genomes of anaerobic methanotrophic archaea (ANME) by comparing to genomes of their relatives, and found a pathway for sulfate reduction that was overlooked previously. Phylogenetic analyses suggest that while intermediate sulfur species usage maybe more widespread, the ability to activate sulfate is more restricted. Multiple homologs of sulfite reductases could be found in these archaeal genomes, and we examined their transcriptional responses to different sulfur species. The result is consistent between ANME and a cultured relative in the *Methanosarcinales*, *Methanococcoides burtonii*. Further investigations on Group II Fsr, a sulfite reductase found in all methane seep environments surveyed, revealed a novel substrate of this enzyme as supported by protein homology models and heterologous expression studies. This expanded sulfur-utilizing ability may help ANME, or methane-metabolizing archaea in general, to broaden their environmental niche and thrive in conditions regardless of the oxidation state of sulfur.

*This work was supported by the Department of Energy BER program (DE-SC0016469) and funding by the Gordon and Betty Moore Foundation through Grant GBMF3306 (to VO).*