

## **Disentangling the Activity of Anaerobic Methane-Oxidizing Archaea from Their Syntrophic Sulfate-Reducing Bacterial Partner**

Hang Yu<sup>1\*</sup> (hyu@caltech.edu), Grayson L. Chadwick,<sup>1</sup> Connor Skennerton,<sup>1</sup> Silvan Scheller,<sup>1</sup> Andy O. Leu,<sup>2</sup> Gene W. Tyson,<sup>2</sup> and **Victoria J. Orphan**<sup>1</sup>

<sup>1</sup>California Institute of Technology, USA; <sup>2</sup>University of Queensland, Australia

**Project Goals: Anaerobic oxidation of methane (AOM) with sulfate is mediated by a syntrophic partnership between anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) and is a major methane sink in the global carbon cycle. Our aim is to gain a better understanding of the mechanisms of electron sharing by these syntrophs using a combination of isotope geochemistry, single-cell microscopy and meta-omics techniques. The overall energy yield of AOM with sulfate is low ( $\Delta G^{\circ} = -17$  kJ/mol) and ANME have a doubling time of 3-9 months. With an integration of these approaches, we have begun to disentangle the activity of these microbes that are symbiotic in nature and provided a fundamental understanding to the inner workings of AOM.**

A consortium of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) consumes teragrams of methane in marine ecosystems and therefore are critical players in the global carbon cycle. However, basic mechanisms of the metabolic coupling in this microbial symbiosis remains obscure. Two hypotheses exist on how electrons are transferred in anaerobic oxidation of methane (AOM) with sulfate: 1) Milucka and colleagues<sup>1</sup> propose that both methane oxidation and the initial step of sulfate reduction co-occur in ANME, and zero-valent sulfur is subsequently consumed by SRB via a disproportionation reaction into sulfate and sulfide; 2) methane oxidation in ANME and sulfate reduction in SRB occur independently and are coupled via direct interspecies electron transfer using multi-heme cytochromes or pili without a diffusible intermediate compound, such as zero-valent sulfur<sup>2,3</sup>.

We designed microcosm incubations to test these hypotheses and are refining three approaches to track activity of environmental microbes by: 1) developing stable isotope geochemistry tools to more accurately track rates of methane oxidation and sulfate reduction, 2) visualizing single-cell spatial activity patterns that link organism identity to biosynthetic activity using fluorescence *in situ* hybridization coupled to nanoscale secondary ion mass spectrometry (FISH-nanoSIMS), and 3) illuminating the pathways involved in AOM with paired metagenomics and metatranscriptomic studies that decouple this symbiosis in the laboratory and induce differential gene expression between ANME and SRB.

Zero-valent sulfur species were found to be inhibitory to AOM at concentrations higher than 0.1 mM, consistent with the idea of product inhibition. However, methane oxidation did not resume after 5 days, the time for expected consumption of sulfur as a substrate by SRB. This suggests that zero-valent sulfur prompts an unknown toxicity effect rather than being an actively exchanged metabolite in the consortia. Additionally, SRB could not be decoupled from ANME

and grown with zero-valent sulfur amendments in our long-term incubations. Instead, we found a decoupling of this syntrophy using artificial electron acceptors such as anthraquinone-2,6-disulfonate (AQDS) in which ANME were catabolically and anabolically active without sulfate or their SRB partner<sup>4</sup>. The theoretical energy yield of AOM coupled to AQDS ( $\Delta G^{\circ\prime} = -41$  kJ/mol) is higher than that coupled to sulfate ( $\Delta G^{\circ\prime} = -17$  kJ/mol). Accordingly, we found that SRB remained biosynthetically and transcriptionally inactive in the presence of both AQDS and sulfate, possibly due to a lack of methane-derived electrons that likely were shuttled to AQDS more favorably. Furthermore, all sulfate reduction genes including sulfate adenylyltransferases, adenosine-5'-phosphosulfate reductases and dissimilatory sulfite reductases were down-regulated in the metatranscriptome, indicating that pathways of methane oxidation and sulfate reduction are not linked in one organism as proposed<sup>1</sup>. Other genes up-regulated with AQDS included those involved in reverse methanogenesis and membrane electron transport. Overall, the combination of different cellular activity probes developed here provides evidence for direct interspecies electron transfer between ANME and their SRB partner as well as insight into the cellular machineries that facilitate the extracellular electron flow in AOM.

## References

1. Milucka, J. et al. Zero-valent sulphur is a key intermediate in marine methane oxidation. *Nature* 491, 541–546 (2012).
2. McGlynn, S. E., Chadwick, G. L., Kempes, C. P. & Orphan, V. J. Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526, 531–535 (2015).
3. Wegener, G., Krukenberg, V., Riedel, D., Tegetmeyer, H. E. & Boetius, A. Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature* 526, 587–590 (2015).
4. Scheller, S., Yu, H., Chadwick, G. L., McGlynn, S. E. & Orphan, V. J. Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. In press.

*This work was supported by the Department of Energy BER program (DE-SC0010574 and DE-SC0004940) and funding by the Gordon and Betty Moore Foundation through Grant GBMF3306 (to VJO).*