

## 23. Systems-level dissection of anaerobic methane cycling: single cell ecophysiology, genetic mechanisms, and microbial interactions

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**Project Goals:** Energy and resource limitation typify much of microbial existence, and proliferation in such regimes often requires the interaction of multiple species, each catalyzing unique sets of reactions relevant to the community at large. Multispecies microbial consortia which carry out the anaerobic oxidation of methane are premier examples of growth permitting metabolic coupling, yet very little is known about the nature or controls of these globally relevant associations. By applying a diversity of 'omics and advanced microscopy techniques complimented by geochemical measurements, we are developing a unique systems-level perspective of methane-based microbial interactions. This coordinated research effort will yield fundamental insight into the associations driving anaerobic methane oxidation, and provides direction for the investigation of structured microbiological communities at-large, be they antagonistic, competitive, mutualistic, or engineered.

We are investigating nutrient utilization in multispecies consortia at multiple levels using community targeted 'omics based approaches in tandem with targeted microscale analysis of syntrophic microbial consortia at the level of individual cells using epifluorescence microscopy (FISH), stable isotope labeling and nanoSIMS. Metaproteomic investigations of sediment-hosted anaerobic methane oxidizing consortia have identified a potential tungsten-containing formylmethanofuran dehydrogenase- the first evidence of a tungstoenzyme expressed by psychrophilic methanotrophic archaea<sup>1</sup>. Analysis of <sup>15</sup>N enriched proteins from these methanotrophic consortia recovered from stable isotope tracer experiments is yielding insight into the pool of actively synthesized proteins by these slow growing anaerobic microorganisms. High resolution fluorescence and single cell stable isotope analysis of cells within consortia provide evidence that aggregate structure and distance between microbial partners are major contributors to the distribution of single cell metabolic activity within consortia mediating the anaerobic oxidation of methane. Between individual cells, anabolic activity rates are strongly correlated between neighboring members within a population, as well as between symbiotic partners. Surprisingly, the average anabolic activity of an individual aggregate does not appear to be a function of spatial structure (i.e. layered or well mixed), suggesting that environmentally derived resources are differentially partitioned within co-associated populations based on the geometry of cell- cell interactions. Using modeling approaches, we are now investigating the metabolic consequences of variable types of physiological interaction, and thereby confirm or eliminate particular nutrient and energy exchange regimes as candidates for producing our empirical observations.

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<sup>1</sup>Glass, J. et al (2013). Geochemical, metagenomic and metaproteomic insights into trace metal utilization by methane-oxidizing microbial consortia in sulphidic marine sediments. *Environ. Microbiol.* DOI: 10.1111/1462-2920.12314