Linking Metabolic Activity, Microbial Identity, and Microscale Spatial Arrangements in Chemosynthetic Seafloor Habitats

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The characterization of potential biological activity in the deep sea and on other celestial bodies revolves around a fundamental question: how might organisms interact with their immediate surroundings to gain energy and nutrients, expel waste products, and avoid environmental hazards? Despite the fundamental importance of such spatially resolved biotic and abiotic interactions – which can enable novel metabolisms and facilitate respiration – most microbiological studies abstract organisms from their habitat or measure bulk parameters.

In this study, we present the development of a novel method combining "in place" visualization of microbial communities with measurement of metabolically active constituents. This approach exposes the abiotic and biotic controls on microbial activity in settings of water-rock interaction and provides new insight into metabolic strategies at diverse chemosynthetic ecosystems.

In field-based *in situ* incubations and lab-based mesocosms, sample material is incubated with L-homopropargylglycine (HPG), an artificial amino acid that replaces L-methionine during protein synthesis. (After incorporation, HPG can be fluorescently detected by azide-alkyne click chemistry, revealing metabolically active, protein-synthesizing cells.) In the lab, samples are fixed with paraformaldehyde and infiltrated with solidifying agents; both polyethylene glycol and quetol-651 were evaluated during method development. Samples are then sectioned; intact microbes are visualized under confocal microscopy with general DNA stains and phylogenetically resolved fluorescence *in situ* hybridization (FISH) probes, and click chemistry reactions reveal the subset of metabolically active cells. Energy-dispersive X-ray spectroscopy is coupled with X-ray diffraction analyses of subsamples. Overall, this process generates a mineralogical map of the silicate grains and carbonate rock, upon which the distribution of target taxa and active cells is overlaid. Microscale spatial relationships are maintained throughout this entire process, providing a detailed perspective of how microbial community structure, metabolic activity, mineralogy, and physicochemical parameters interact.

We deploy this approach in two astrobiologically relevant systems: silicate-rich aqueous sediments and authigenic carbonate rocks collected from anoxic horizons of methane seeps. The former demonstrates which organisms are preferentially associated with silicate minerals, providing insight into the metabolic modes supported by silicates. In seep carbonates, consortia of anaerobic methanotrophic archaea and sulfate reducing bacteria occupy micropores, while monospecific biofilms line larger pore spaces (Fig. 1). Analysis of chimney-like carbonates from the Point Dume methane seep revealed the highest rates of anaerobic methane oxidation to date, exposing new upper limits on metabolic rate in this reducing chemosynthetic environment. In both study systems, our data reveal key mineralogical and physical characteristics of a solid substrate that promote active metabolism. In the context of astrobiological investigations of other ocean worlds, this approach to viewing metabolically active microbial communities in their native physicochemical habitats will provide key constraints on mineral types, rock morphologies, fluid regimes, and redox conditions most amenable to supporting viable metabolism.

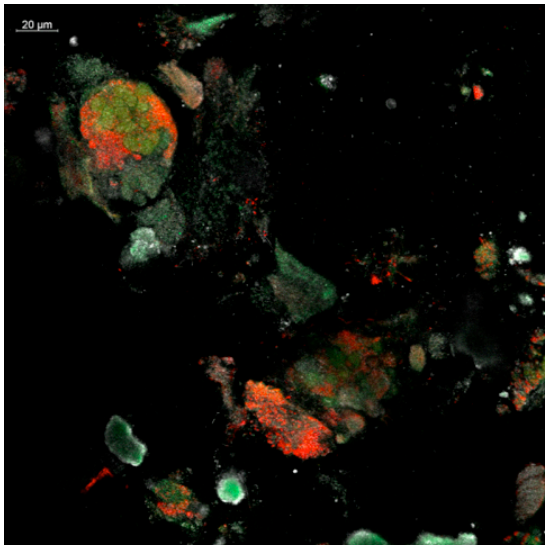

Figure 1: In-place FISH microscopy image of anaerobic methanotrophs (ANME-2a/b, red) and sulfate reducing bacteria (DSS/DSB, green); the reflected grayscale channel shows the rock surface