**Project Goals**: The LLNL Biofuel SFA investigates systems biology of complex microbial communities relevant to bioenergy production. To understand nutrient cycling and potential biofuel production in complex microbial communities we employ an integrated analysis of energy flow using multi-scale approaches including biogeochemical, stable isotope probing, metagenomic/transcriptomic, proteomic/metabolomic and computational analyses. Our ultimate goal is the development of multi-scale models that can predict ecological and biochemical relationships within multi-trophic microbial systems.

Cyanobacterial carbon excretion is crucial to carbon cycling in many microbial communities, but the nature and bioavailability of the carbon excreted is dependent on its physiological function, which is often unknown. Hypersaline laminated photosynthetic mats are an excellent model system for the study of carbon flow in a complex community because they are sustained primarily by photosynthesis in a relatively small-scale closed system. These mats have a large reservoir of carbon in the extracellular matrix, but how cyanobacterial matrix production is regulated and who consumes it is poorly understood. To better understand cyanobacterial carbon excretion, we examined the macromolecular composition of the extracellular matrix of microbial mats from Elkhorn Slough in Monterey Bay, CA USA. In collaboration with PNNL’s Pan-Omics group we characterized the mat exoproteome and discovered predominantly cyanobacterial proteins, many with predicted roles in breakdown of organic matter, and detected enzymatic activities which indicate capacity for cyanobacterial degradation of matrix organic matter. To further explore the regulation of these breakdown abilities, we characterized a biofilm-forming cyanobacterial isolate from Elkhorn Slough, ESFC-1, that has a similar extracellular composition to that of our field mats, providing us with a model culture. Using this culture, we identified exoproteins that change in abundance over a diel cycle, and exoproteins that were dark stress induced, suggesting light-dependent regulation of...

**Figure 1**: Cyanobacterial trichomes incubated with $^{13}$C-labeled EPS become enriched in $^{13}$C. NanoSIMS analysis of ESFC-1 trichomes incubated with $^{13}$C EPS. (A) Points represent $^{13}$C APE (atom percent excess, % over background) values for cyanobacterial trichomes from three biological replicates (1,2 or 3) at 2 time points following addition of $^{13}$C label. “Killed” represents killed control cells that were fixed before incubation. (B) Scanning electron microscopy image of representative ESFC-1 trichomes after 12 hours incubation with $^{13}$C EPS. (C) $^{12}$C $^{14}$N NanoSIMS image of same trichomes and (D) $^{13}$C APE NanoSIMS image.
matrix material breakdown. We then used high resolution imaging mass-spectrometry (NanoSIMS) to characterize EPS-carbon re-uptake in ESFC-

Our results demonstrated light-dependent, rapid uptake of EPS-associated carbon by ESFC-1 in culture. Based on these findings, we propose that mat Cyanobacteria store and recycle their organic carbon from the mat extracellular matrix. Cyanobacteria are such a large percentage of the biomass in the upper phototrophic layer of the microbial mats, that their re-uptake of organic carbon has the potential to re-define carbon availability and turnover in these systems.

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