Hyper-accumulated Mn, co-localized in *Chlamydomonas reinhardtii* acidocalcisomes with Ca and P, can be mobilized in Mn-deficient situations and protects against oxidative stress

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Project Goals: The LLNL Biofuels SFA is developing advanced methods to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere, we can advance the efficiency and reliability of algal biofuel production. Our research includes studies of probiotic traits of phycosphere-associated bacteria, systems biology studies of model algae, and genome-enabled metabolic modeling to promote bioenergy production and healthy co-cultures. Our overall goal is to develop the comprehensive understanding of complex microbial communities needed to advance the use of biological properties for practical energy production.

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Acidocalcisomes are 100-200nm diameter electron-dense organelles, rich in calcium and polyphosphate. They have emerged as an important intracellular compartment for handling metal homeostasis in eukaryotes, both in the context of nutrition and toxicity. Using a reference organism, *Chlamydomonas reinhardtii,* for which we have genome-level information on the occurrence and expression of metal transporters and homeostasis factors, we are using state of the art imaging methodologies to visualize the function of and interactions between individual pathways for Mn, Fe, Cu and Zn utilization, and Ag, Hg, Cd sequestration. Because metals occur at parts per million and lower levels in some cells, they can be difficult to image by standard methods. Here we use high spatial resolution secondary ion mass spectrometry with the LLNL Cameca NanoSIMS 50 to image metals in embedded and sectioned cells. The NanoSIMS data are correlated with other methods to provide a more complete understanding of metal metabolism in these microalgae.

In this study we examined manganese (Mn) metabolism in microalgae. Mn is an essential element for most forms of life. In photosynthetic organisms like *Chlamydomonas reinhardtii*, a eukaryotic alga, photosystem II and Mn-superoxide dismutases are major sites of Mn utilization. Mn^{2+} is assimilated via divalent cation transporters of the NRAMP family or via phosphate

transporters as a counterion with inorganic phosphate. In contrast to Fe and Cu assimilation, Mn can be hyper-accumulated in Chlamydomonas cells in proportion to extracellular Mn supply. Hyper-accumulation is independent of phototrophic vs. heterotrophic physiology. While hyper-accumulation does require aerobic conditions, X-ray absorption near edge structure (XANES) suggests predominantly Mn^{2+} species. We used multiple imaging approaches to localize intracellular Mn, including X-ray fluorescence microscopy, transmission electron microscopy energy dispersive X-ray spectroscopy (TEM-EDS) and NanoSIMS, which indicate that the primary foci of intracellular Mn accumulation are co-localized with Ca and P, suggesting this accumulation is within acidocalcisomes, which are known to be acidic vacuoles containing polyphosphate. Mutants in components of the VTC polyphosphate polymerase which have reduced intracellular total P and dramatically lower Ca content, cannot hyper-accumulate Mn. Growth on low inorganic phosphate, but not low Ca, recapitulates this phenotype, suggesting that Mn hyper-accumulation requires an interaction with phosphate/polyphosphate. Electron nuclear double resonance (ENDOR) spectroscopy confirms association of Mn ions with inorganic phosphate and phytate, but in cells which hyper-accumulated Mn, the bulk of the Mn ions are associated with other species. Hyper-accumulated Mn is bio-available because it can be mobilized in situations of Mn-deficiency for synthesis of Mn-SOD, consistent with a role of the acidocalcisome in metal homeostasis.

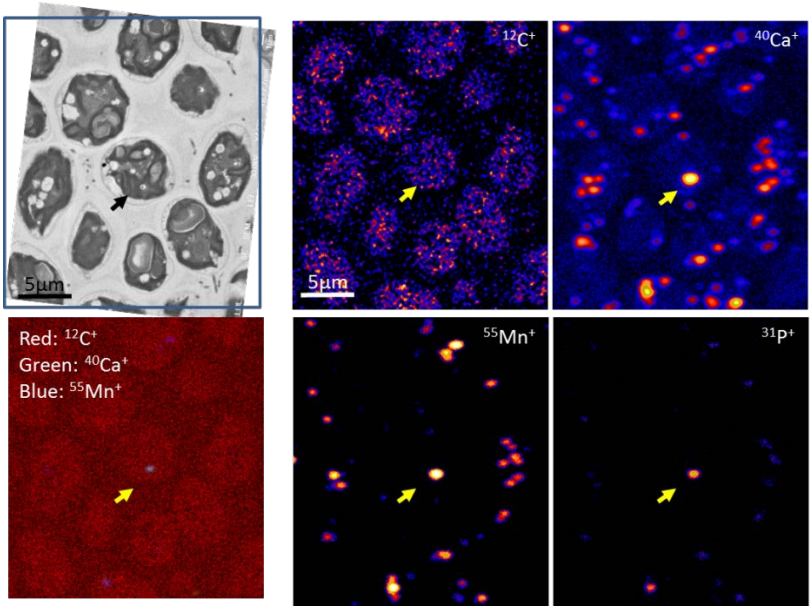

Fig. 1. Correlated STEM and NanoSIMS ion images. The location of the cells can be visualized in the C and Ca ion images. The color addition image (lower left) combines the NanoSIMS C^+, Ca^+ and Mn^+ images. The arrows indicate the same location in all images. Inspection shows that Mn foci tend to correlate with Ca and P foci. Count rates for P are relatively low because of low P^+ yield.

This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52- 07NA27344 and supported by the Genome Sciences Program of the Office of Biological and Environmental Research under the LLNL Biofuels SFA, FWP SCW1039. Use of the Advanced Photon Source at Argonne National Laboratory was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract number: DE-AC02-06CH11357. BMH and TLS acknowledge support by the NIH (GM 111097 and DK068139).