121. Characterization of Redox Signaling Pathways in Cyanobacteria during Nutrient Limitation

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Project Goals: The goal of the PNNL BSFA is to determine the natural design principles of microbial photoautotrophic systems involved in solar energy conversion to biofuels. Significant knowledge gaps exist in our understanding of regulatory events within and between metabolic subsystems that include intracellular signals to which transcriptional regulation is responding. We suggest that "redox sensing," as a means to maintain redox homeostasis in photosynthesizing cells, is likely an equally important mechanism. To investigate this hypothesis, we have developed and applied a chemical probe approach for live- cell capture, characterization, and imaging of proteins that undergo dynamic fluctuation in redox status during environmental changes. It is anticipated that the identification of redox-sensitive dithiol linkages and their modulation by thioredoxin (Trx), peroxiredoxin, and other regulators will provide key inputs for understanding the control points of flux distribution.

The primary challenge in identifying redox-regulated dithiol sensors *in vivo* is that cysteine residues are easily oxidized to dithiol linkages following cellular lysis. This eliminates the ability to experimentally measure redox regulation within native physiological settings, and therefore requires the exogenous addition of chemical or biological reductants. To overcome the dilemma lysis presents, we developed an approach for employing cell permeable probe reagents that react in live cells (*in vivo*) with thiols and permit real-time imaging and mass spectrometric characterization of probe targets. For this approach, we synthesized click-chemistry enabled chemical probes for fluorescent and mass spectrometric identification of redox regulated dithiol sensors. We have used our probes *in vivo* in both *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 5144 to identify proteins that undergo disulfide exchange in response to changes in cellular conditions. Probes were synthesized with three chemical elements: a moiety to impart cell permeability, an iodoacetamide or maleimide group for irreversibly labeling cysteines, and a reporter tag for detection and isolation of probe-labeled proteins. We exploited the multimodal bio-compatible click chemistry (CC) reaction to create "tag-free" probes for profiling proteins in living systems. Probe-labeled proteins were visualized by addition of a complementary azido-tetramethylrhodamine for fluorescent SDS-PAGE, Alexafluor-488 for confocal microscopy, or biotin-TEV-azide tag for enrichment and mass spectrometric analysis (LC-MS).

The maleimido and iodoacetamide probes were added simultaneously *in vitro* or *in vivo* to *Synechococcus* sp. PCC 7002 grown in a turbidostat under maximal growth rate conditions. Cells were also removed and placed in the dark for three hours and then labeled *in vitro* or *in vivo*. Following probe-labeling cells were lysed, and probe-labeled proteins were attached to biotin azide via CC. The probe-labeled proteins were then enriched on streptavidin, digested with trypsin, and the peptides analyzed by LC-MS. Critically, in the cells that were labeled post-lysis (*in vitro*) we found no changes in redox regulation of dithiol sensors, demonstrating that lysis rapidly oxidizes biological samples. However, when we analyzed the *in vivo* labeled cells, we identified redox-regulated proteins that were statistically different between the light and dark conditions. In a follow-up study using a carbon-limited chemostat we identified redox changes *in vivo* within 30 seconds

following the addition of CO_2. A time-course study revealed remarkable changes from 30 seconds to 60 minutes post CO_2 addition. The global proteome analysis informed us that none of the proteins abundance changed over the 60 minute time-course. Lastly, we identified the cysteine sites of probe labeling for a majority of the proteins undergoing dynamic redox fluctuations; a finding that allows for a more targeted approach in determining actual protein thiol function.

Similar approach was used to elucidate the protein redox dynamics during H_2 production by the diazotrophic *Cyanothece* sp. ATCC 5144, a cyanobacterium that can evolve H_2 using the N_2-ase pathway. Remarkably, the observed fluctuations of the protein redox status correlated with H_2 evolution dynamics. At the same time, the measured bulk ROS dissipated, thus strengthening the hypothesis that H_2 production is a mechanism, by which *Cyanothece* 51142 alleviates the detrimental effects of ROS on PSII and photosynthetic rates. Importantly, the "redox" changes cannot be measured by traditional transcriptomic or proteomic measurements because these post translational redox events occur within a time-frame that protein and mRNA content has not changed. Probe-identified proteins from both probe types map well onto multi-subunit supra- molecular complexes involving photosynthetic pathways associated with efficient collection of excitation energy (light harvesting), electron transfer reactions linked to formation of electrochemical gradients, carbon dioxide sequestration (dark reactions), and ATP synthesis. Additional redox-dependent pathways include those involving chaperone activity, transcriptional regulation, and antioxidant proteins linked to protein repair. Together, these results provide quantitative information regarding redox- dependent switches associated with photosynthetic regulation, and provide a systems biology tool capable to providing high-throughput information necessary for predictive metabolic modeling. Finally, cell permeable probe approaches represent the only existing methods for identifying and imaging live cell redox regulation, and they will be critical to informing the predictive models of metabolism needed for bioenergy applications.