

## 155. Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

Maria L. Ghirardi 1\* ([maria.ghirardi@nrel.gov](mailto:maria.ghirardi@nrel.gov)) and Matthew Wecker2 ([matt.wecker@nrel.gov](mailto:matt.wecker@nrel.gov))

1National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401

2Genebiologics, LLC, Boulder, CO 80303

\*Presenting author

**Project Goals:** To obtain a systems-level understanding of the biological barriers that control hydrogen metabolism and prevent sustained H<sub>2</sub> photoproduction in the green alga *Chlamydomonas reinhardtii*.

Photobiological H<sub>2</sub> production from water is a clean, non-polluting and renewable technology. Although the potential light conversion efficiency to H<sub>2</sub> by biological organisms is theoretically high (about 10%), the system is currently limited by biochemical and engineering constraints. The specific objectives of this research are covered by two Tasks: (1) development, testing, validation and utilization of novel high-throughput assays to identify photosynthetic organisms with altered H<sub>2</sub>-producing activities, thus leading to the discovery of novel strategies to circumvent known biochemical limitations; and (2) deconvolution of the network of metabolic pathways centered on six ferredoxin homologs found in *Chlamydomonas*, aimed at understanding reductant flux in photobiological hydrogen production, and identifying targets for future metabolic pathway engineering strategies to reduce flux to non-productive pathways.

In Task 1, we developed, tested and validated a novel high-throughput assay to identify high H<sub>2</sub>-producing strains from an insertional mutagenesis library of *C. reinhardtii*. The assay uses the H<sub>2</sub>-sensing system of *Rhodobacter capsulatus* that responds to H<sub>2</sub>-production by algal colonies through activation of a GFP signal (Wecker et al., 2011). We validated this assay with well-characterized mutants that are either low or high H<sub>2</sub>-producers (Wecker and Ghirardi, 2014). Finally, using this H<sub>2</sub>-sensing system, we have isolated four insertional mutant strains of *C. reinhardtii* that exhibit high-light H<sub>2</sub> production and have shown that these strains show up to 100-fold increased H<sub>2</sub> production levels compared to their wild type strains when grown at elevated light levels. We are currently identifying the site of insertion and further characterizing these strains to understand which genes are responsible for these high-light H<sub>2</sub>-production phenotypes.

Concomitantly, we inserted a heterologous hydrogenase from *Clostridium acetobutylicum* into our *R. capsulatus* sensor strain. In doing so, we find that (i) hydrogen is produced by the heterologous hydrogenase; (ii) hydrogen production is detected by the H<sub>2</sub>-sensor of the organism; and, remarkably, (iii) the H<sub>2</sub> produced is derived both from fermentative and photosynthetic processes. A manuscript is in preparation. We therefore have created a novel selective means of testing and developing hydrogenases and this system is amenable to directed evolution studies. The heterologous hydrogenase shows some uptake hydrogenase activity as well, and we are currently working to understand if this uptake activity is sufficient to drive photoautotrophic (H<sub>2</sub> and CO<sub>2</sub>) or chemoautotrophic (H<sub>2</sub>, CO<sub>2</sub>, and O<sub>2</sub>) growth of the organism. If so, we may be able to use growth on H<sub>2</sub> as an additional selection tool for hydrogenase development.

Publications:

1. Wecker, M.S. and M.L. Ghirardi, High-throughput biosensor discriminates between different algal H<sub>2</sub>-photoproducing strains. *Biotechnology and Bioengineering*, 2014. 111(7): p. 1332-1340.

*This project was supported by the Office of Science (BER) under FWP #ERWER38.*