

Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

Task 1

Maria L. Ghirardi ^{1*} (maria.ghirardi@nrel.gov) and Matthew Wecker ²
(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 H₂ photoproduction in the green alga *Chlamydomonas reinhardtii*.

Photobiological H₂ production from water is a clean, non-polluting and renewable technology. Although the potential light conversion efficiency to H₂ 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 H₂-producing activities, thus leading to the discovery of novel strategies to circumvent known biochemical limitations (this task is now completed); 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 completing Task 1, we developed a novel high-throughput assay that allows the screening of up to 500K algal colonies per minute for H₂-production characteristics. This assay is now in use by eight different labs; it is notable for its ease of use as a bench-top screen for H₂-producing organisms whose H₂ production systems are essentially O₂-intolerant. The assay uses the H₂-sensing system of *Rhodobacter capsulatus* and couples the sensor to activation of a GFP signal (Wecker et al., 2011). We validated this assay with well-characterized mutants that are either low or high H₂-producers (Wecker and Ghirardi, 2014). We then used the assay on a *C. reinhardtii* insertional mutagenesis library and isolated four strains of *C. reinhardtii* capable of high-light H₂ production. These mutants showed up to 180-fold greater levels of H₂ production at elevated light levels in comparison to the wild type strain. The greatest high-light H₂-production increase is putatively the result of a mutation in a phosphatase gene. We are complementing this mutation to determine if the mutant with a replacement gene regains its original phenotype.

We have also created a model system for developing photosynthetic H₂ production. We inserted a heterologous hydrogenase from *Clostridium acetobutylicum* into our *R. capsulatus* sensor strain. The imported enzyme produces H₂, and the H₂ produced in turn causes the organism to fluoresce. Moreover, H₂ production can be repeatedly induced by dark cycles followed by illumination. This

system therefore represents a novel means of testing, developing and selecting for *in vivo* hydrogenase activity, for photosynthetic H₂ production, and for metabolic support of H₂ production with real time output of hydrogen production reported on a cellular level. A manuscript is near completion.

Publications:

1. Wecker, M.S. and M.L. Ghirardi, *High-throughput biosensor discriminates between different algal H₂-photoproducing strains*. *Biotechnology and Bioengineering*, 2014. 111(7): p. 1332-1340.
- 2.
3. Wecker, M.S. and M.L. Ghirardi, *Expression and Photosynthetic Activity of a Clostridial [FeFe] Hydrogenase in the Bacterium Rhodobacter capsulatus* (In Preparation)

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