156. Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

Maria L. Ghirardi (maria.ghirardi@nrel.gov) and Alexandra Dubini* (Alexandra.dubini@nrel.gov)

National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401
*Presenting author

Project Goals: To obtain a systems-level understanding of the biological barriers that control hydrogen metabolism and prevent sustained H_2 photoproduction in the green alga *Chlamydomonas reinhardtii*. Photobiological H_2 production from water is a clean, non-polluting and renewable technology. Although the potential light conversion efficiency to H_2 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_2-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 ferrredoxin 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.

Our previous work in Task 2 demonstrated that both FDX1 and FDX2 interact with FNR and HYDA1 and are able to reduce NADP+ and protons in vitro, although with different kinetic properties. We are currently assessing whether the differences are due to their dissimilar redox potentials or to altered binding to their interacting partners. In an effort to continue characterizing FDX2 function, we generated and successfully identified a FDX2 knock out strain. Our data show that the FDX2 mutant strain evolves around 15% less hydrogen than the wild-type, confirming that it does have a role, although secondary to FDX1 in hydrogen metabolism. On the other hand, preliminary results suggest that this minor ferredoxin is probably more closely associated with cyclic electron flux (CEF), diverting photosynthetic reductant away from hydrogen production. FDX2 was shown to interact with PGRL1, an essential component of the PSI supercomplex that meditates CEF in Chlamydomonas. Interestingly, Western blot analysis indicates also that the levels of other minor FDXs and hydrogenases in the FDX2 knock-out mutant vary, depending on the conditions tested, providing an additional clue that FDX2 is an important player on cell adaptation and electron flux modulation. Future work will include metabolomics and fluxomics analysis to understand the specific vs. complementary roles of the different FDXs in algal metabolism under stress conditions.

Publications:

1. Boehm, M, Alahuhta, M, Long, H, Old, WM, Peden, EA, Mulder, D, Brunecky, R, Lunin, V, King, P, Ghirardi, ML and Dubini, A. Two amino acid residues contribute to functional differences between the *Chlamydomonas reinhardtii* root-type Ferredoxin 2 and the leaf-type Ferredoxin 1. *In preparation.*

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