

Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

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Project goals: To obtain a systems-level understanding of the biological barriers to sustained H₂ photoproduction by *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: (i) 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; and (ii) 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 2013, we used our petri plate-based H₂ detection assay to screen ~500,000 insertional mutants of *C. reinhardtii* for strains capable of high-light H₂ production. Using the assay, five such strains were isolated and verified for H₂ production under 300 μE m⁻² s⁻¹ light in comparison to negative H₂ production for the wild type strain. Characterization of H₂ production is on-going, as is identification of the mutational insertion location within the genome. We are further modifying the assay to be based on fluorescence-activated cell sorting. This will allow specific manipulation of growth conditions for single cells (e.g. light intensity and O₂ tension) as opposed to the current assay where heterogeneous conditions exist within colonies. This assay modification will also allow increased throughput in both screening rate and strain isolation. Toward this goal, we are linking the H₂-sensing *Rhodobacter* cells to the H₂-producing *Chlamydomonas* cells, either directly or through attachment to microspheres.

We had previously shown that *Chlamydomonas* FDX1 and FDX2 are capable of mediating redox reactions involving either HYDA1 or FNR, but at different rates. In an effort to better understand the differences in property and function of these proteins, we over-expressed them in *E. coli*, purified their mature versions, and used them for spectroscopic and crystallographic studies. We report the first 3D structure of a *Chlamydomonas reinhardtii* ferredoxin, FDX2, at atomic resolution of 1.18 Å, and show that its folding motif is similar to previously published plant-type FDX structures. The FDX2 crystal structure allowed us to refine the homologous FDX1 model structure and to simulate the interaction surfaces of both FDXs with the HYDA1 hydrogenase and the FNR1 protein. Moreover, based on earlier findings from the literature, we used site-directed mutagenesis to mutate two residues in FDX2 to the equivalent residues in FDX1 (M61F, -94Y and the double mutant). Our results demonstrate the mutations resulted in additively higher rates of H₂ production, with the double mutant photo-producing H₂ at 54% of the rate of the FDX1-catalyzed reaction, from 17.5% with WT FDX2. On the other hand, when NADPH photo-production was assayed using these FDX2 mutant proteins, we showed that the catalytic rates (V_{max}) for FDX1 and FDX2 were fairly similar, and neither of the mutations significantly affected the rates.

Publications:

1. Peden EA, Boehm M, Mulder DW, Davis R, Old WM, King PW, Ghirardi GL, Dubini A (2013). Identification of global ferredoxin interaction networks in *Chlamydomonas reinhardtii*. *J Biol Chem.* 288(49):35192-209.
2. Wecker MSA, and Ghirardi ML. (2014) "High-Throughput Biosensor Discriminates Between Different Algal H₂-Photoproducing Strains ". *Biotechnol. Bioeng.*, *in press*.
3. Boehm, M, Alahuhta, M, Long, H, Old, W.M, Peden, E.A, Mulder, D, Brunecky, R, Lunin, V, King, P, Ghirardi, M.L and Dubini, A. "Ferredoxin 2 of *Chlamydomonas reinhardtii*: properties, crystal structure at 1.2 Å resolution and its role in hydrogen and NADPH photo-production". *In preparation*.