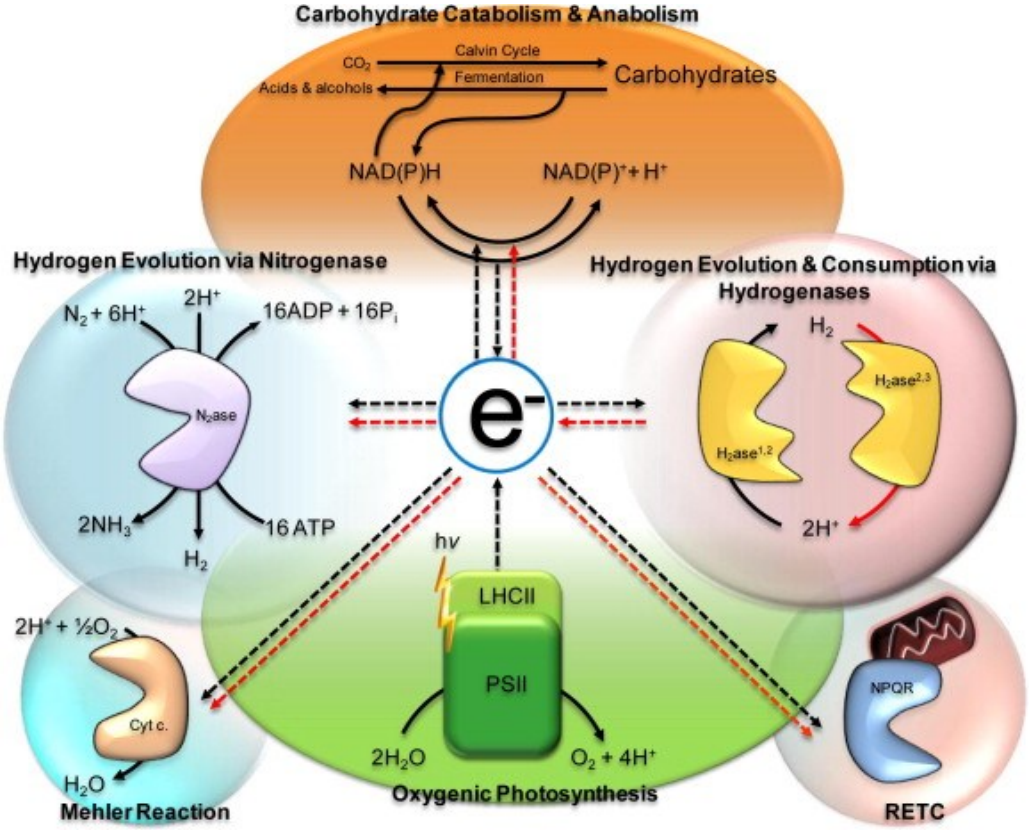
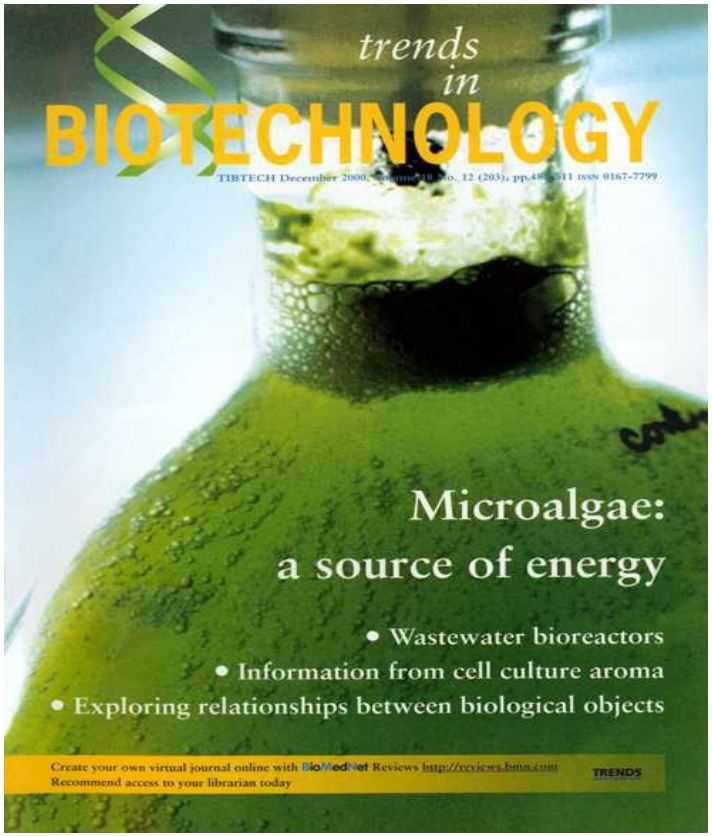
H_2 Production by Oxygenic Phototrophs

Eric L. Hegg





Bioresour. Technol. **2011**, *102*, 8589-8604

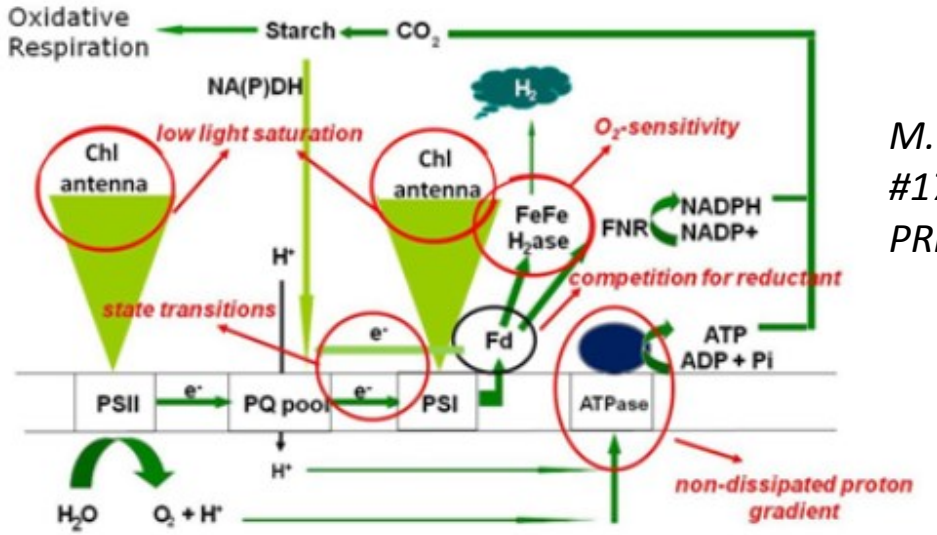
Michigan State University

Great Lakes Bioenergy Research Center

Major Challenges to H_2 Photoproduction

➢Technical Challenges

- Mixture of H_2 and O_2; H_2 separation and storage
- CO_2 addition and overall reactor design



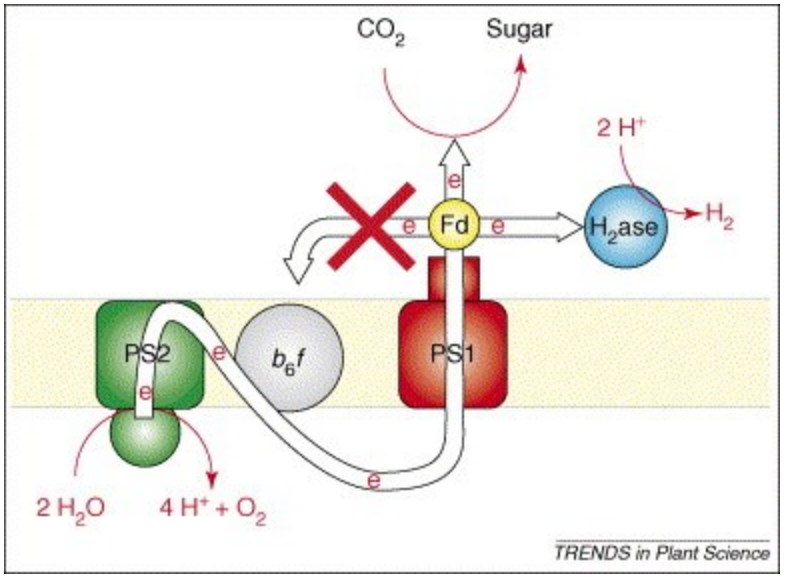
M. Ghirardi, Abstract #1751, Honolulu PRiME 2012

➢Biological Challenges

- Poor efficiency of H_2 production
  - Poor heterologous expression of H_2-forming enzymes
  - Low quantum yields
  - Competition for reducing equivalents; poor electron coupling
- Sensitivity of H_2-forming enzymes to O_2

Overcoming Low Efficiency: Improving ET

- Eliminate or down-regulate pathways competing for ele
  - Production of organic acids
  - Formation of NADPH/carbon fixation

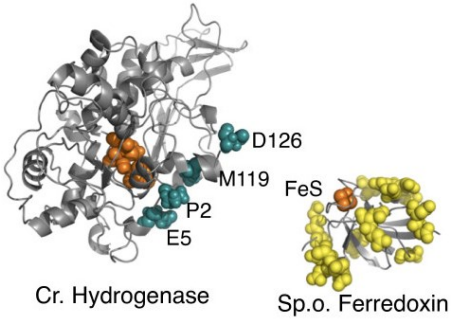


Trends Plant Sci. **2006**, *11*, 543-549

  - Strategy depends on good genetics and an understanding of the metabolic pathways
- Identify endogenous electron transfer partner
  - Which ferredoxin or cytochrome?

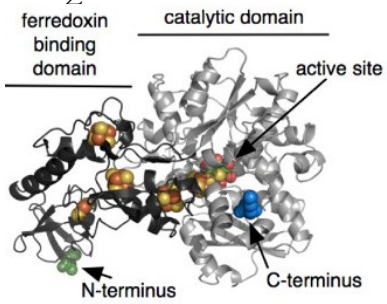
Overcoming Low Efficiency: Improving ET

- Engineer improved coupling
  - Mutate docking site for enhanced binding

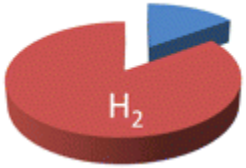


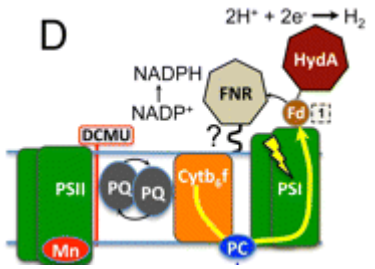
J. Biol. Eng. **2010**, *4*:3

  - Fuse H_2ase to ferredoxin



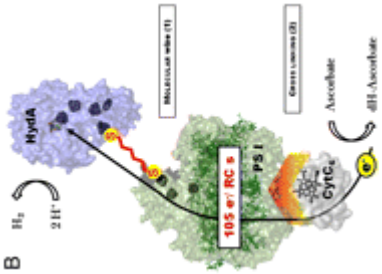
J. Biol. Eng. **2010**, *4*:3



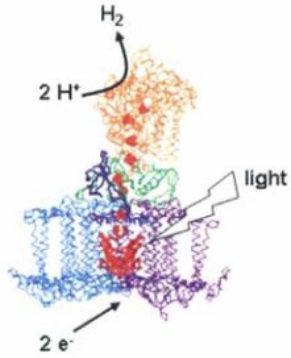


PNAS **2011**, *108*, 9396-9401

  - Fuse H_2ase directly to PS-I



PNAS **2011**, *108*, 20988-20991

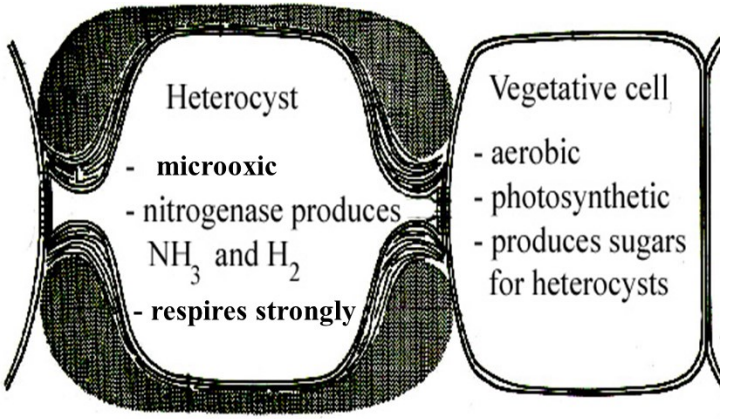


Photochem. Photobiol. **2006**, *82*, 676-682

  - Localize to a synthetic protein scaffold

Overcoming O_2 Sensitivity

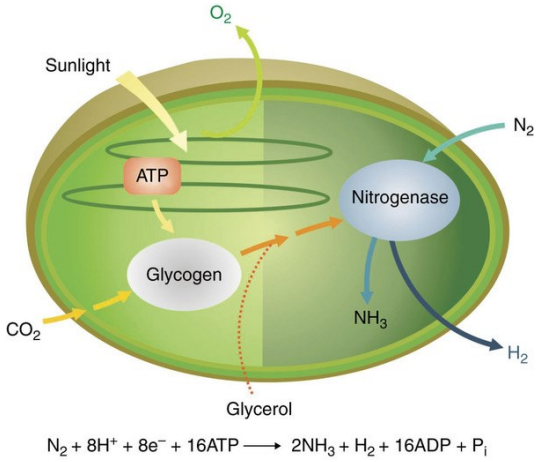
- Utilize non-oxygenic photosynthesis
  - Purple bacteria (*Rhodobacter sphaeroides*)
  - Selective light
  - Sulfur-deprivation
- Engineer enzyme to be less O_2 sensitive
  - Inhibit diffusion of O_2
  - Alter redox potentials
- Separate H_2 and O_2 biosynthesis
  - Temporal separation (e.g. H_2 produced from fermentation)
  - Spatial separation
    - Heterocyst forming bacteria
      - Expression of [FeFe]-H_2ase in *Anabaena* sp. PCC 7120
      - Mutations can increase heterocyst frequency
    - Other compartments? Carboxysomes?



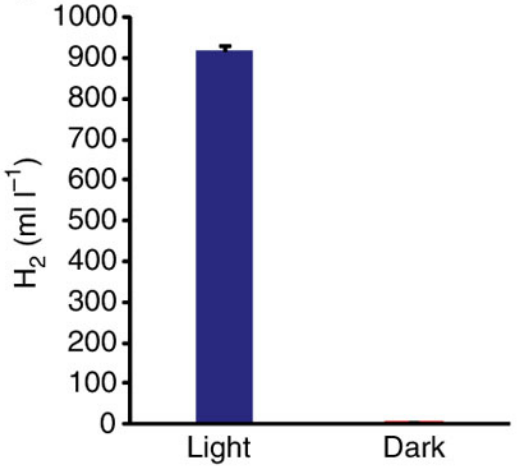
Wolk et al.

Identifying New Organisms

- *Cyanothece* sp. ATCC 51142
  - H_2 from nitrogenase
  - 465 µmol H_2 per mg chl per hr
  - Simultaneous light-driven H_2 and O_2 production
    - Over 100 hrs in presence of CO_2



Nat. Commun. **2010**, *1*:139



Nat. Commun. **2010**, *1*:139

- *Volvox carteri*
  - Multicellular green alga with differentiated cells
    - *C. reinhardtii* is most well-characterized relative
  - First multicellular eukaryote discovered to have H_2 metabolism