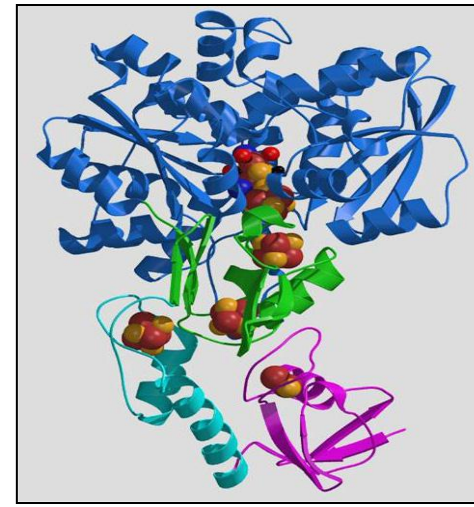
Renewable Hydrogen Production from Biological Systems

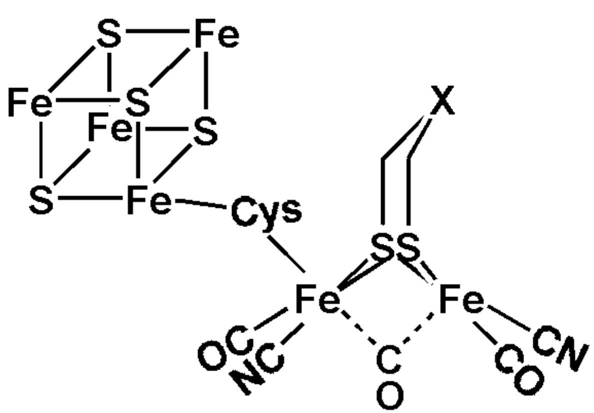


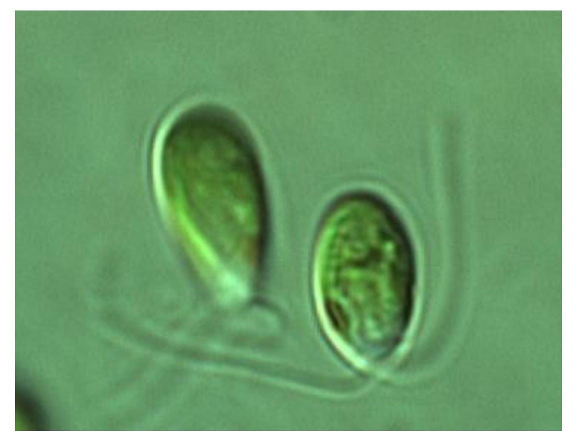
Matthew Posewitz

Colorado School of Mines

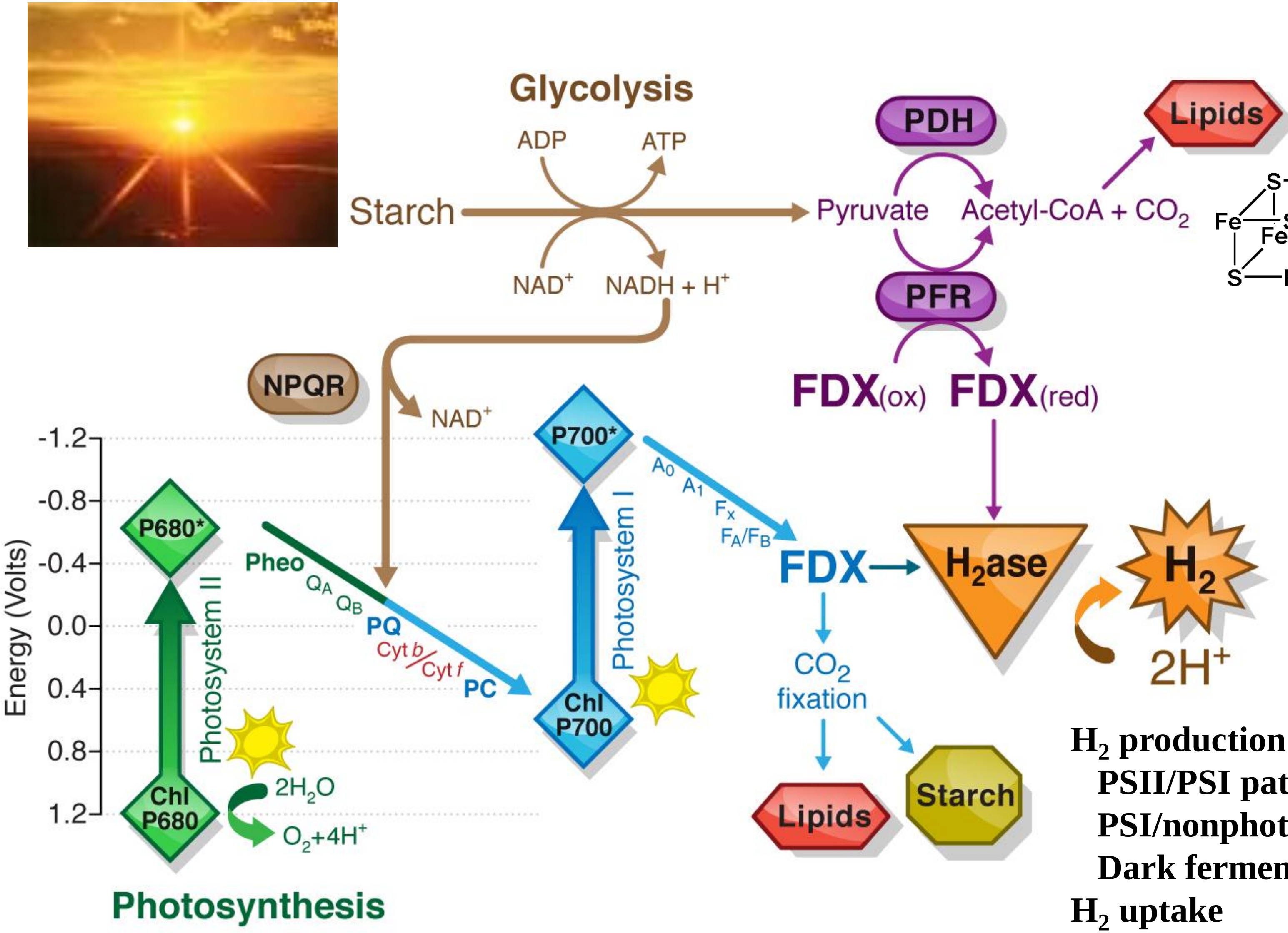
DOE Biological Hydrogen Production Workshop

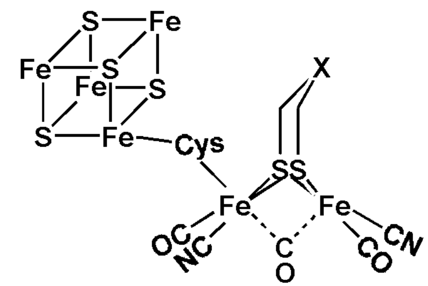
September 24th, 2013





Photosynthetic H_2 pathways





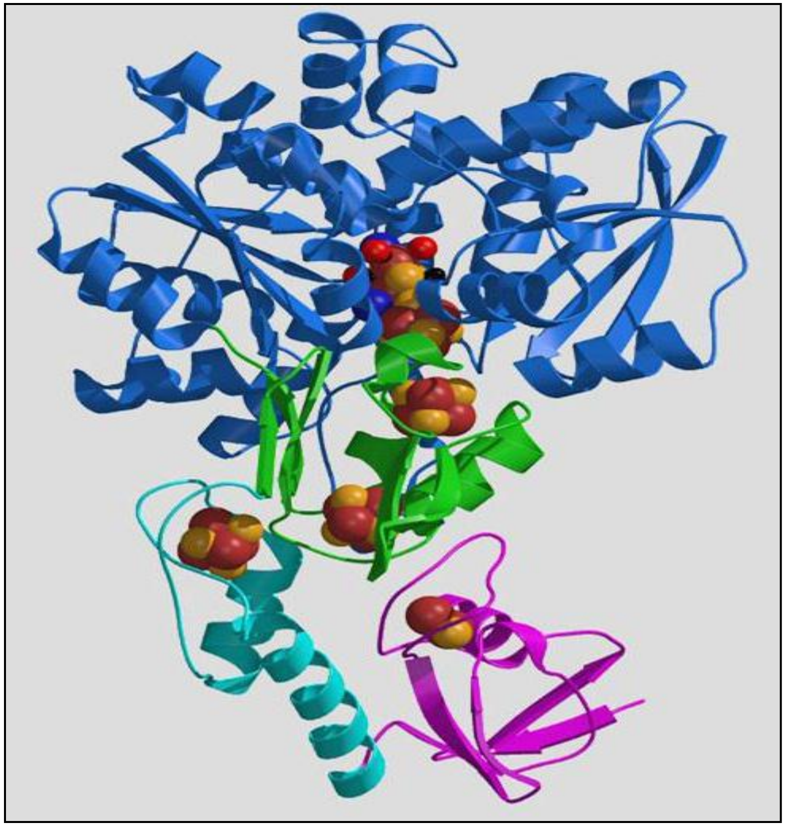
Peters JW et al. *Science* 1998

Nicolet Y et al. *Structure and Folding Des.* 1999

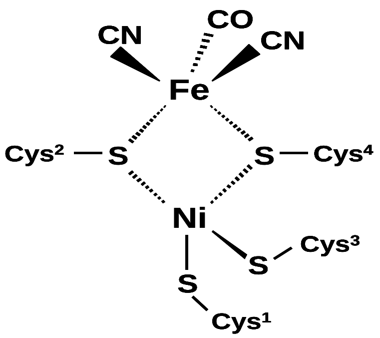
- H_2 production
  - PSII/PSI pathway
  - PSI/nonphotochemical PQ
  - Dark fermentation
- H_2 uptake
  - oxyhydrogen reaction
  - photoreduction

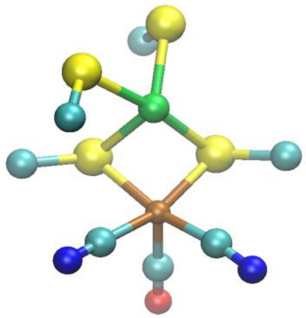
Phototroph Hydrogenases

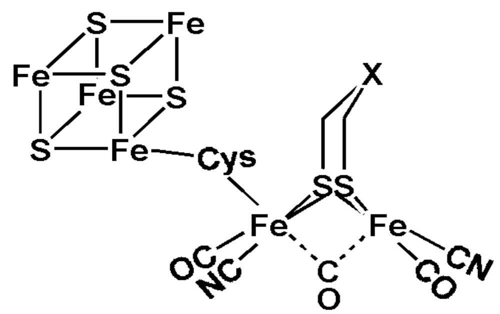
- **Cyanobacteria**
  - **Only [NiFe]-hydrogenases identified to date.**
  - **Typically dark H_2 production.**
  - **Linked to NAD(P)H.**
- **Eukaryotic Algae**
  - **Only [FeFe]-hydrogenases identified to date.**
  - **Typically two hydrogenases with only the H-cluster domain.**
  - **Linked to ferredoxin and photosynthetic electron transport.**

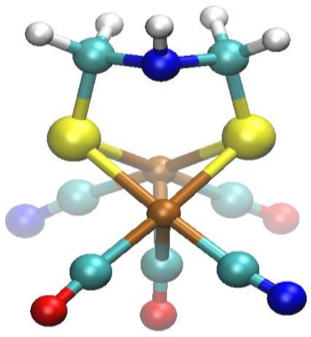


Hydrogenases







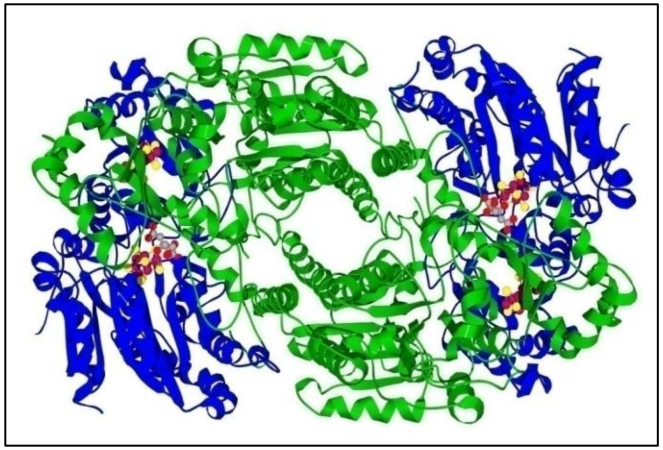


- [NiFe]-Hydrogenases
  - Reversible O_2 inhibition
  - Group 1- Uptake Enzymes Membrane bound
  - Group 2- Cyanobacterial uptake and H_2 Sensing Enzymes
    - Latter are O_2 tolerant
  - Group 3- Bidirectional
    - NAD(P)(H) linked
  - Group 4- H_2 Production Enzymes
    - Ferredoxin linked

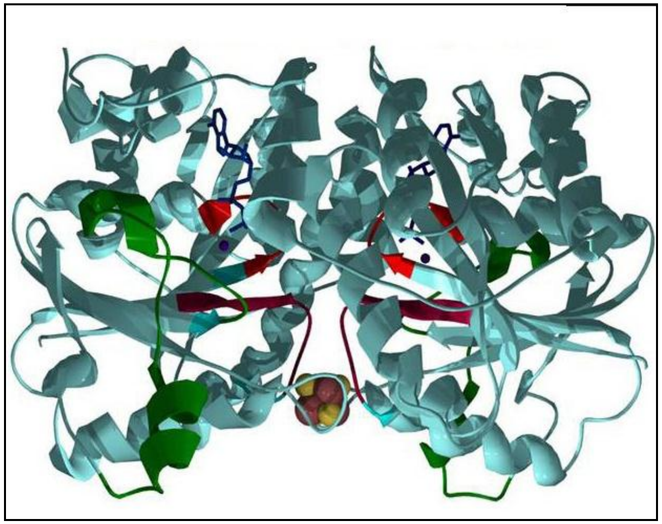
- [FeFe]-Hydrogenases
  - Algal enzymes are simplest to date
  - Often ferredoxin linked
  - Hnd multimeric enzymes are NAD(P)(H) linked
  - Differences in O_2 tolerance reported but typically irreversible inhibition
  - Capable of very high turnover $>10^3$/s

Nitrogenases

Nitrogenase MoFe Protein



Nitrogenase Fe Protein



Mo	$N_2 + 8H^+ + 9e^- = 2NH_3 + H_2$
Fe	$N_2 + 21\ H^+ + 12e^- = 2NH_3 + 7.5H_2$
V	$N_2 + 12H^+ + 12e^- = 2NH_3 + 3H_2$

~ 2 ATP/e^-

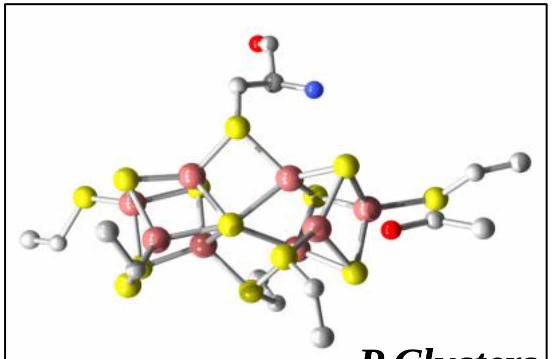
Strong thermodynamic driving force

essentially irreversible H_2 producti on

Oxygen sensitive

Heterocyst differentiation or temporal separation

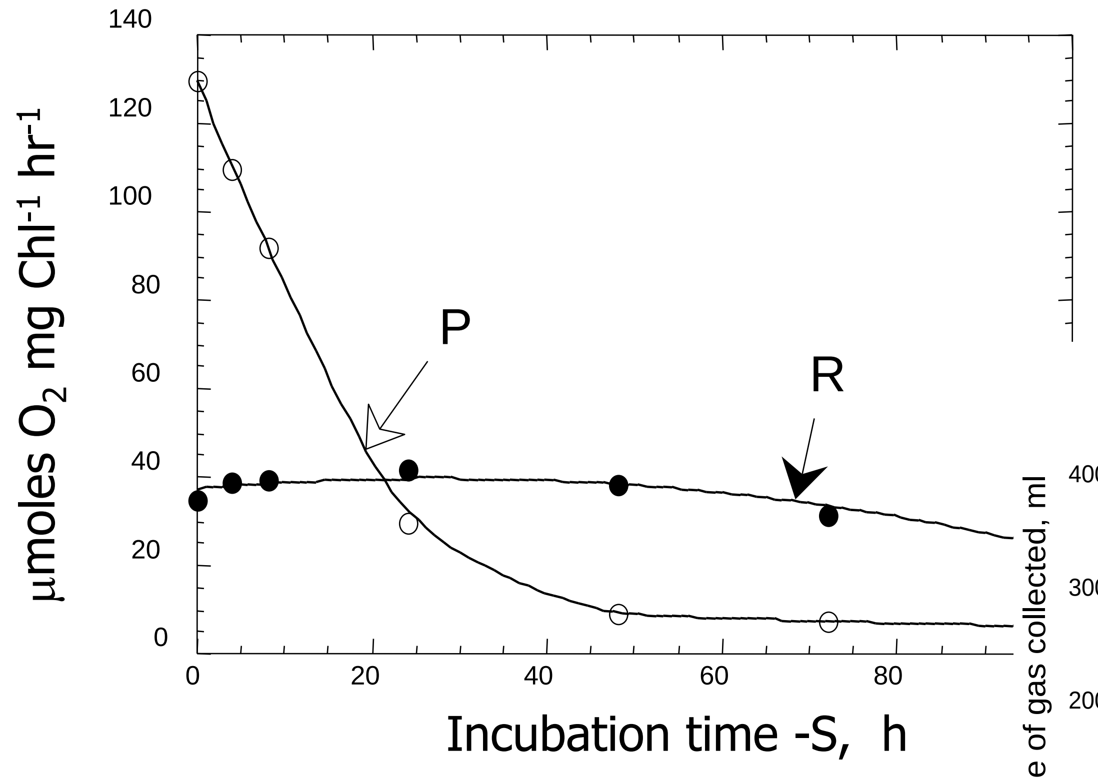
Mutants with increased H_2 production reported

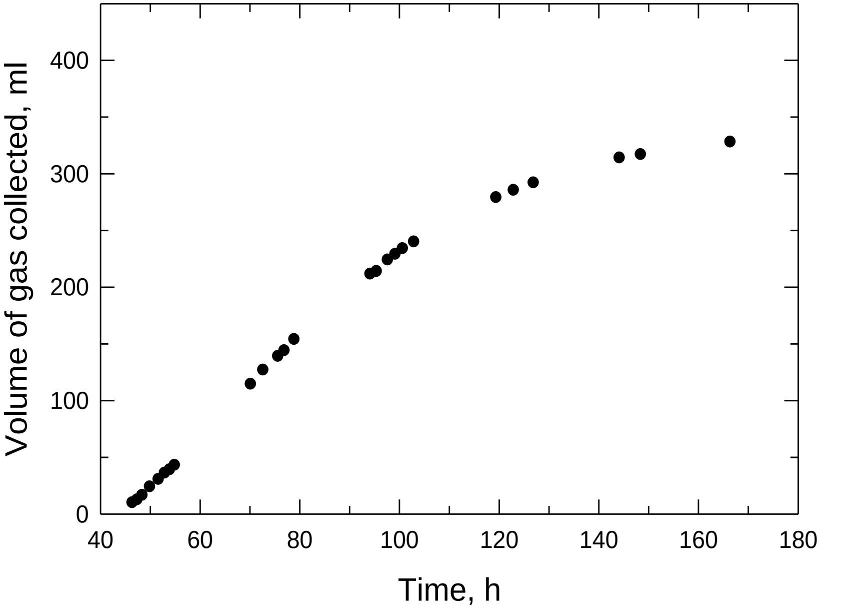


P Clusters

FeMo-cofactor

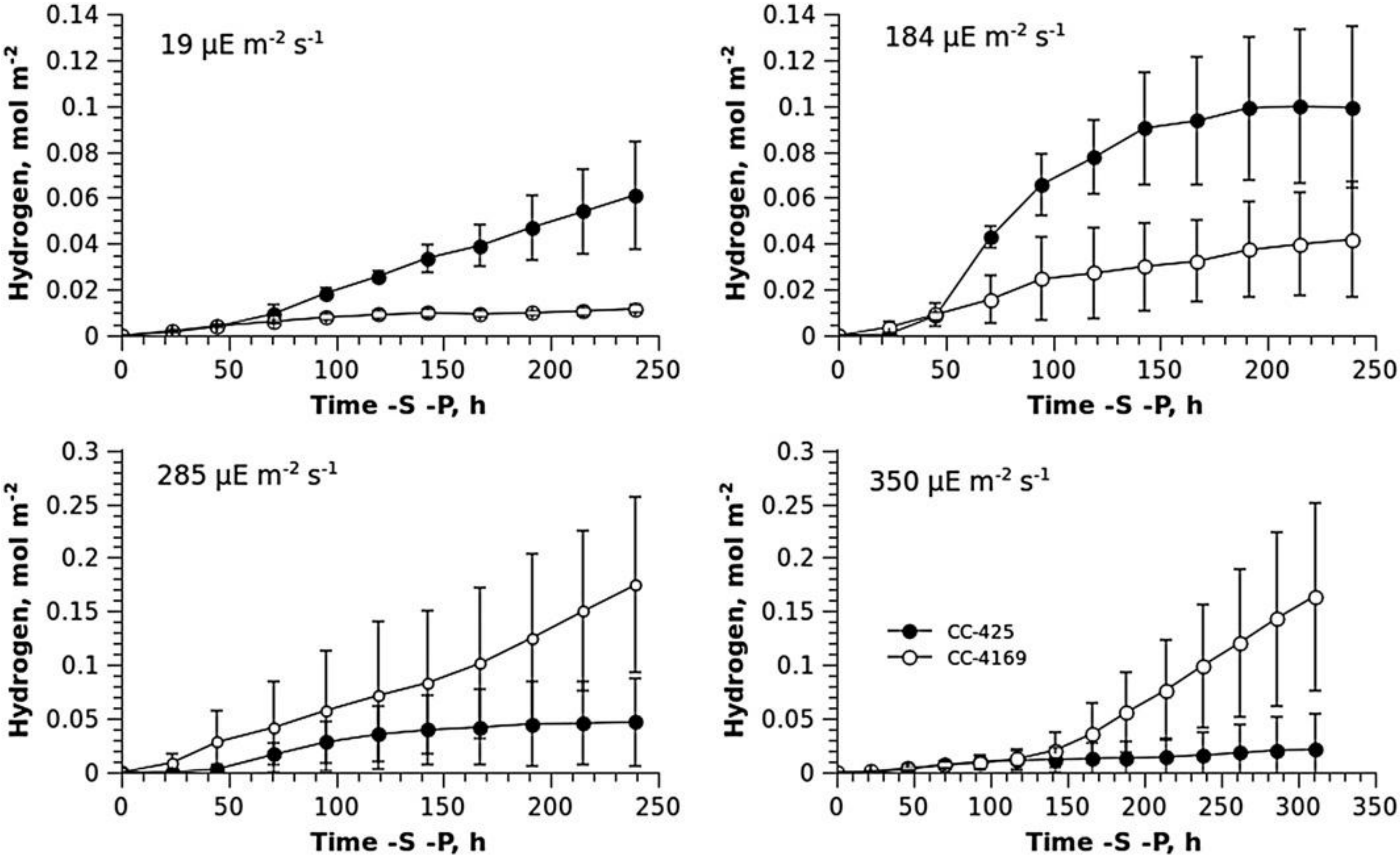
Prolonged H_2 Production in Algae Induced by Nutrient Stress





Melis et al., *Plant Phys.* 2000
Kosourov et al., 2002

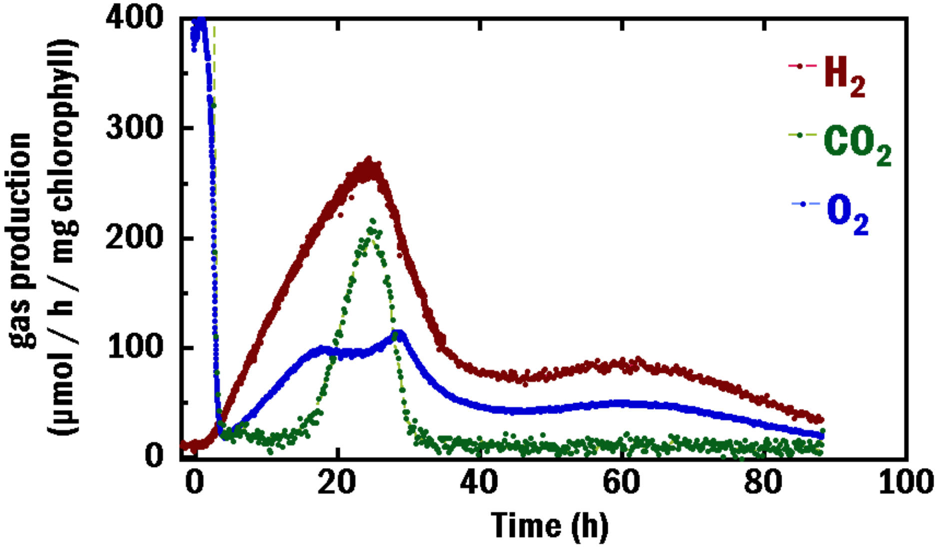
Photosynthetic antennae mutant with enhanced H_2 production



Kosourov, Ghirardi and Seibert 2011

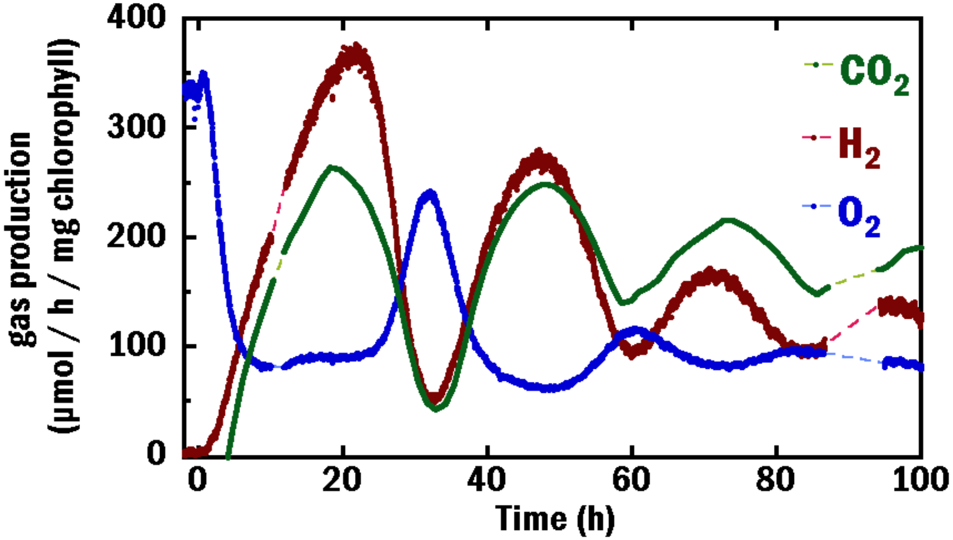
***tla1* mutant Polle and Melis (UCB)**

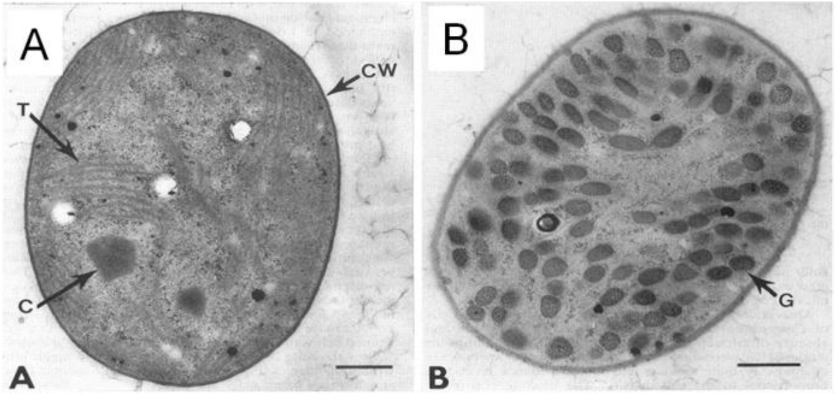
Nitrogenase Catalyzed H_2 Production in Cyanothece: Sustained Biophotolysis



- **Rates of H_2 production up to ~ 400 µmole/hr · mg chl**
- **O_2/H_2 coproduction**
- **Predominately light driven**

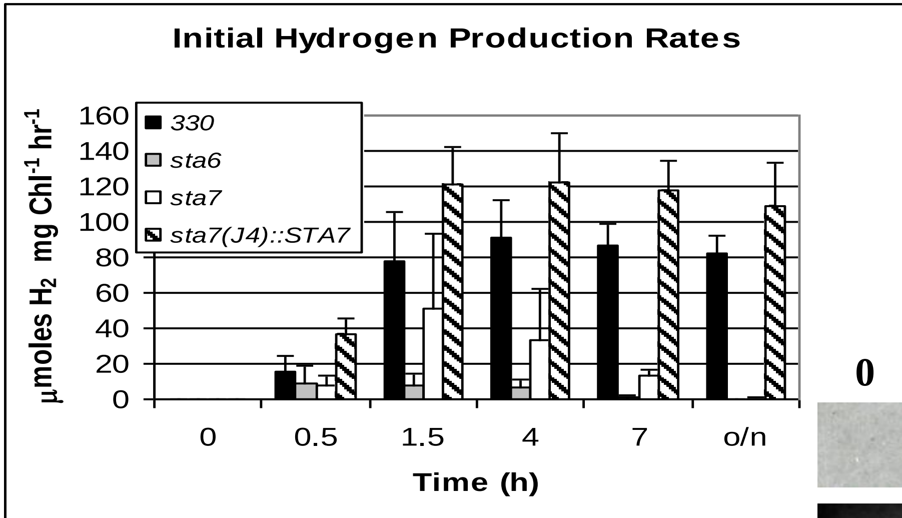
Melnicki et al., 2012, Beliaev laboratory PNNL

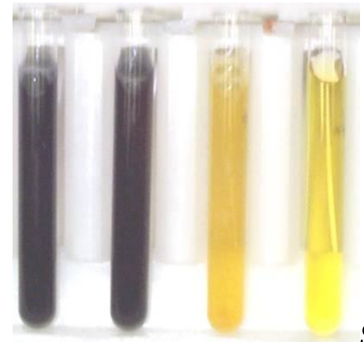




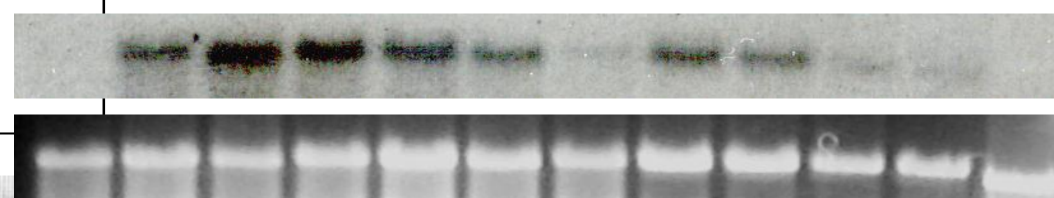
Reddy et al., 1993

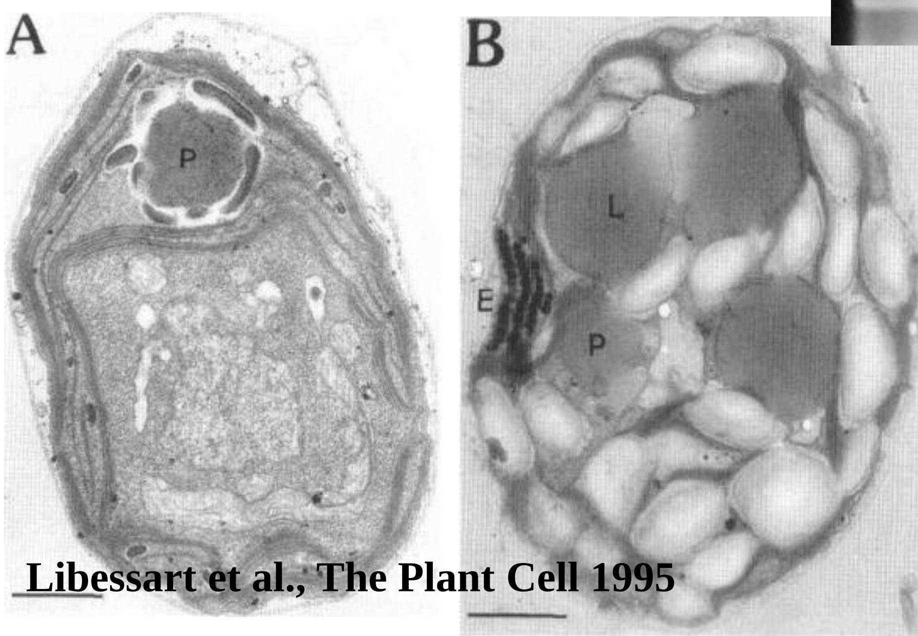
Photosynthesis and Starch- Interplay for H_2 Production



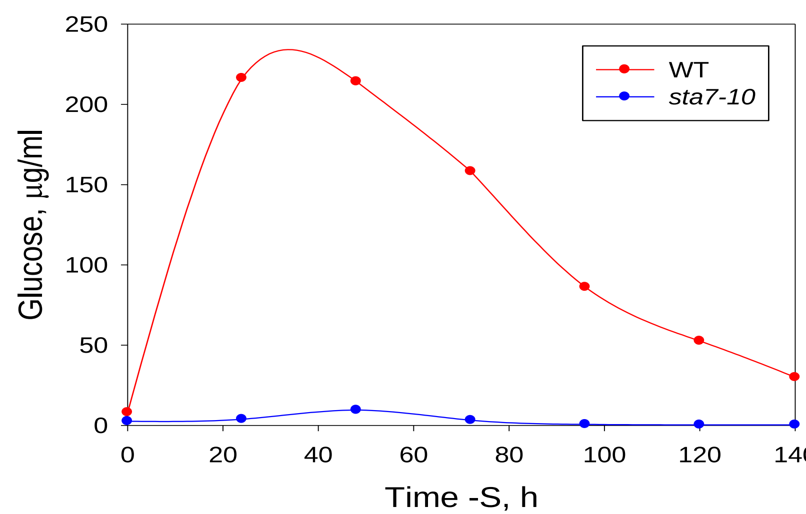


330
sta6(J5)

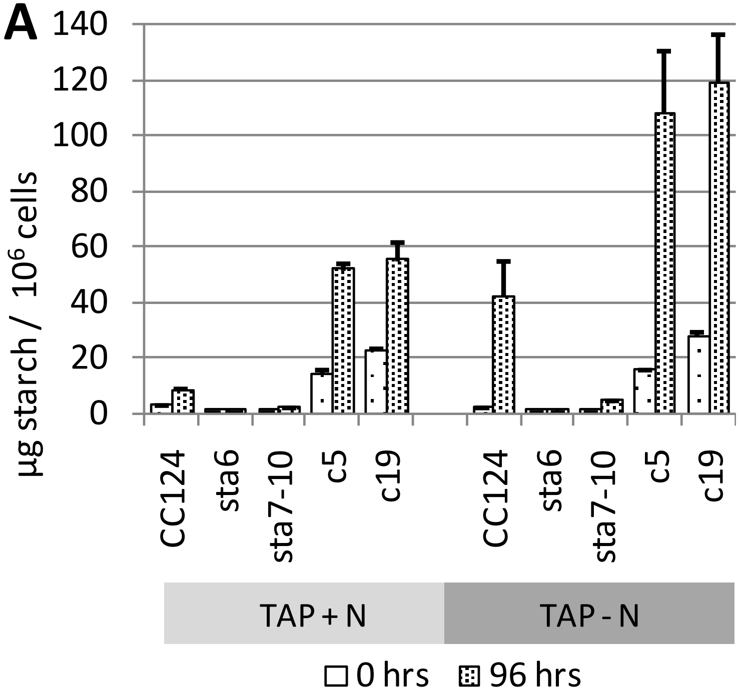


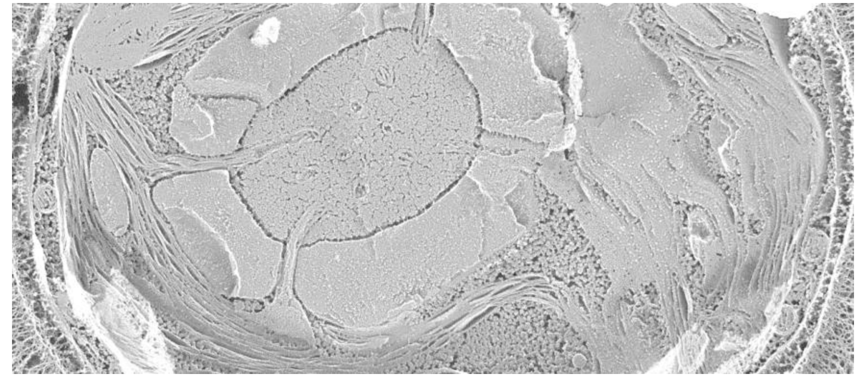


Libessart et al., The Plant Cell 1995

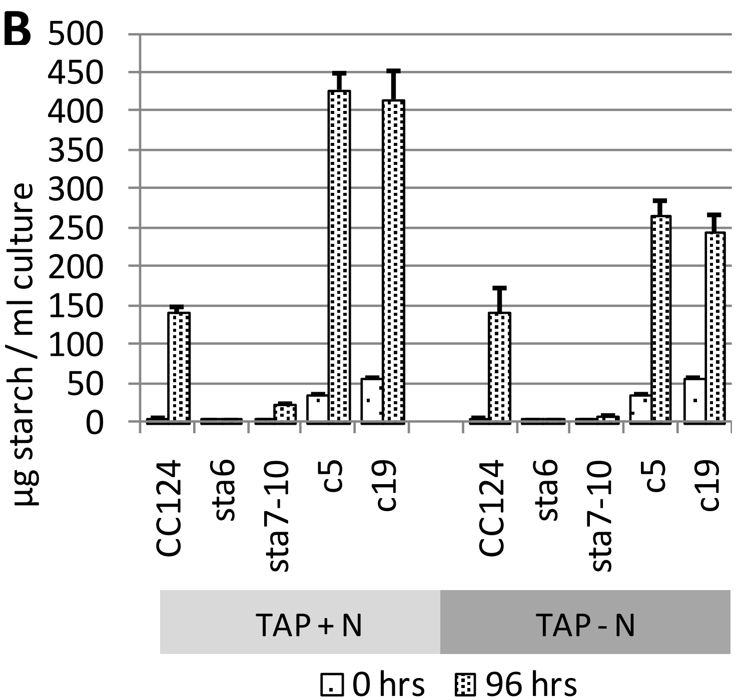


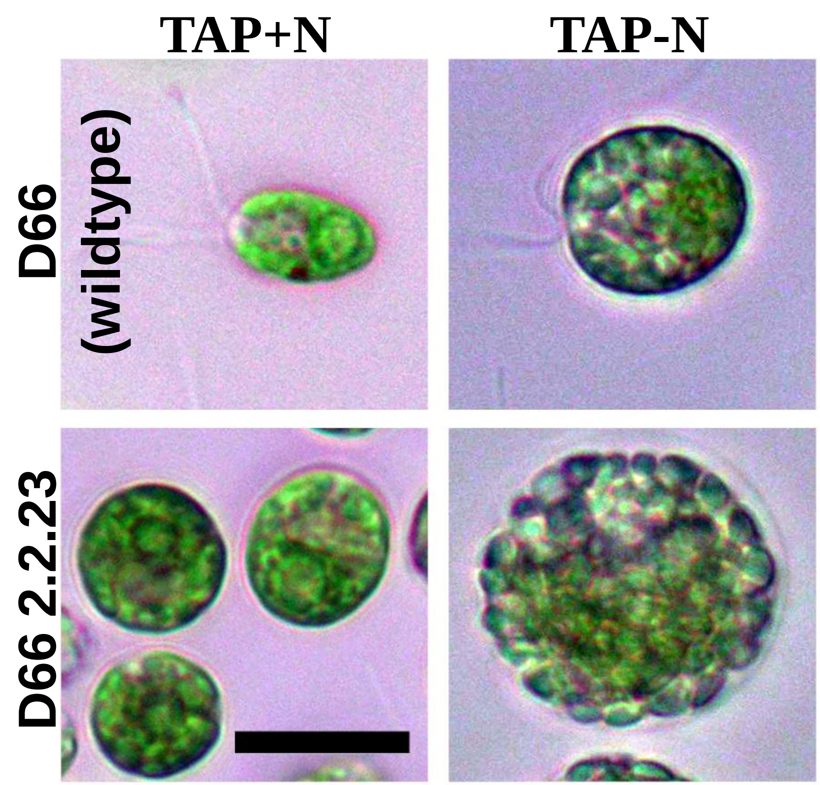
Starch Hyperaccumulation



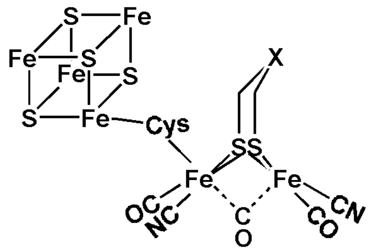


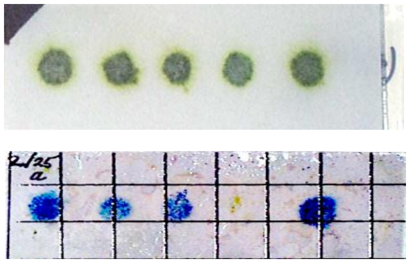
Starch sheath surrounding pyrenoid

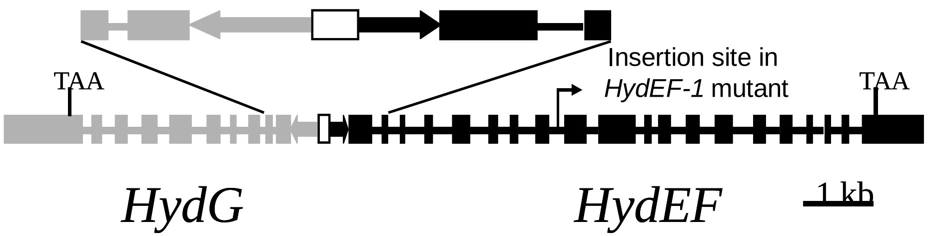


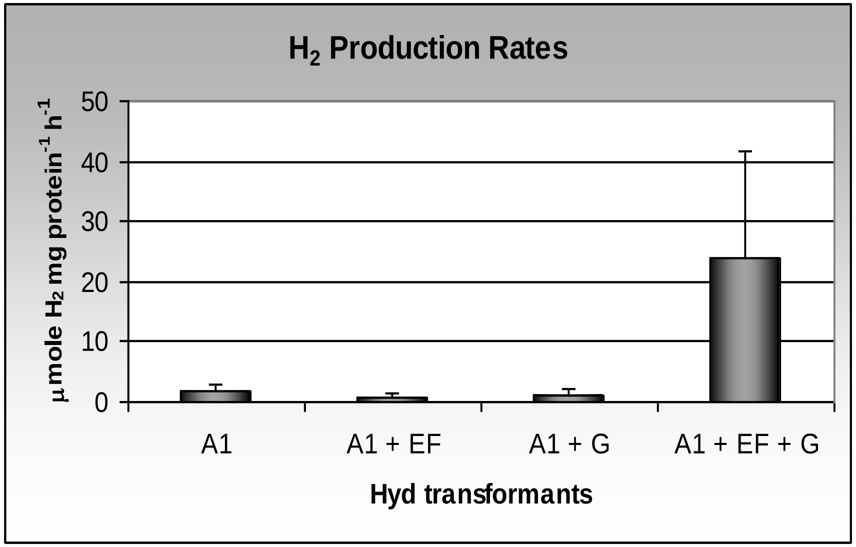


Hydrogenase Maturation Heterologous Expression and Screening in Alternate hosts

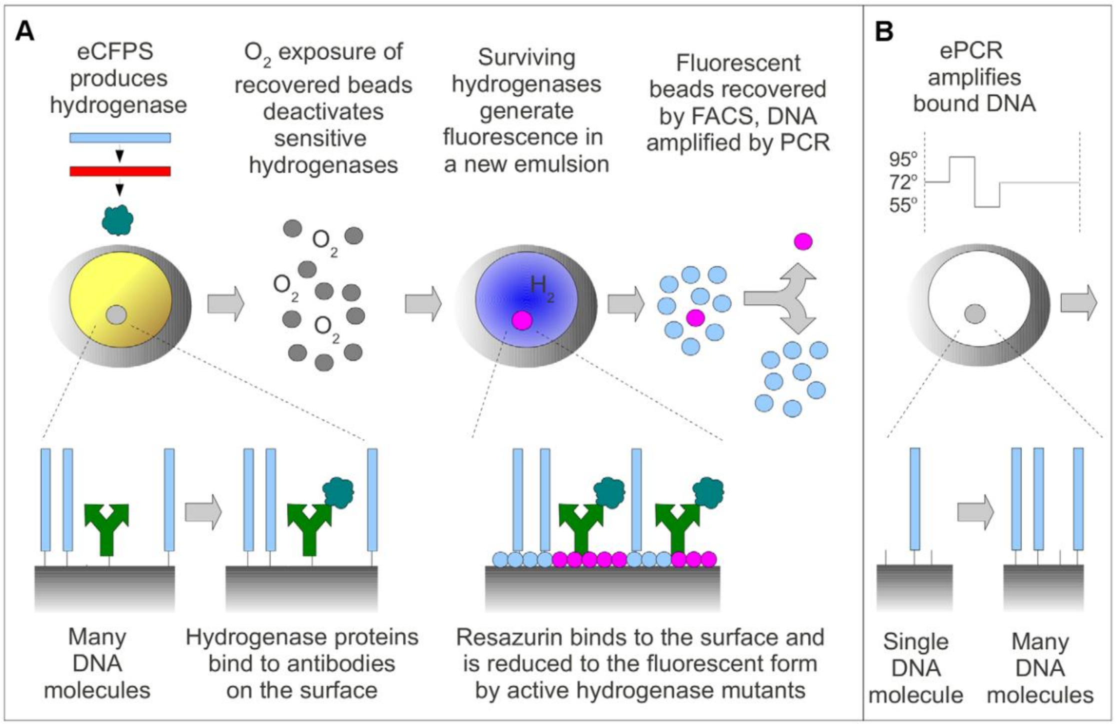




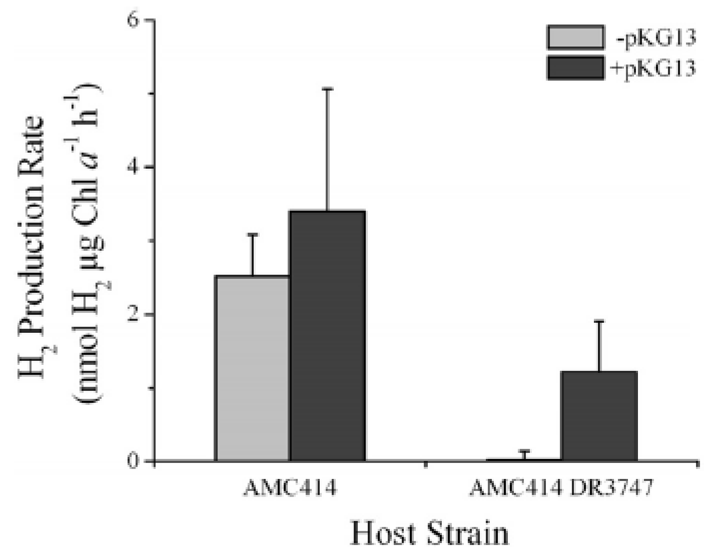




Hydrogenase Maturation Heterologous Expression and Screening in Alternate hosts

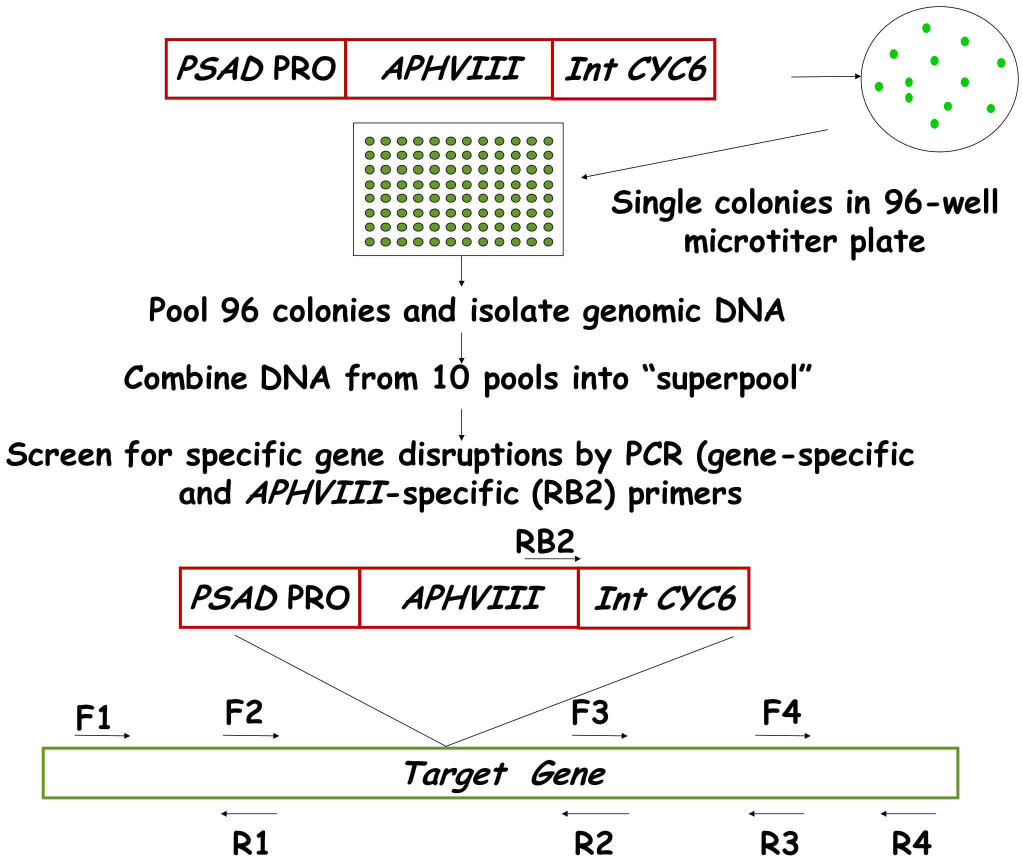


Screening mutant libraries for enhanced O_2 tolerance
Stapleton and Swartz 2010 Stanford



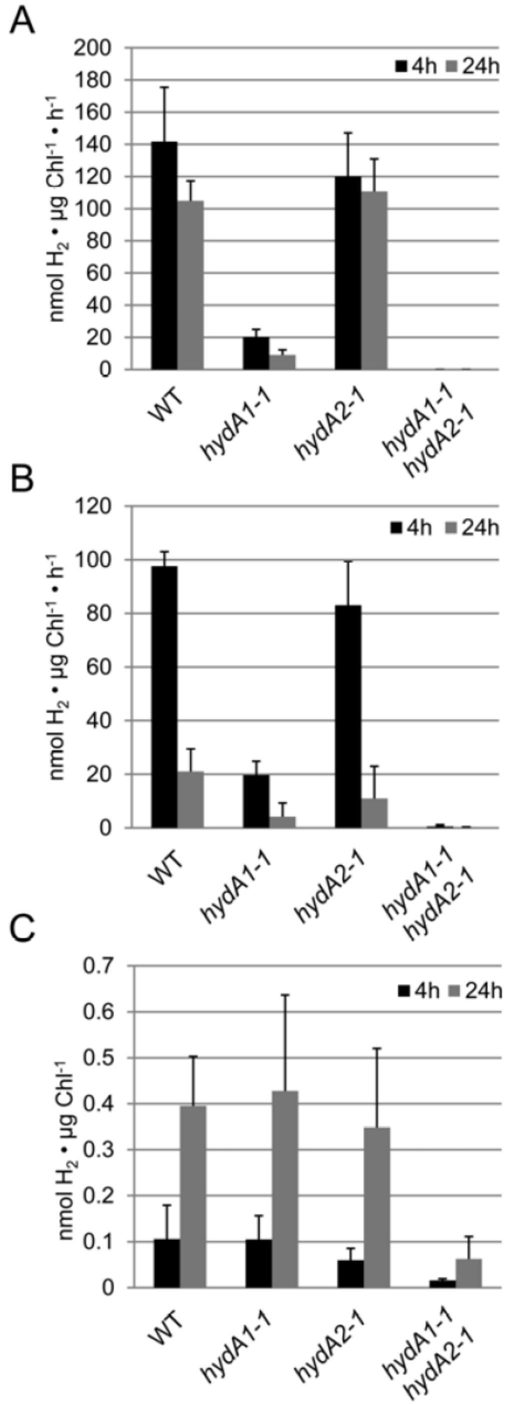
Expression of [FeFe]-hydrogenase in Anabaena heterocysts. Gartner et al. 2012, Hegg lab MSU

Reverse Genetics via PCR-Based Mutant Library Screening

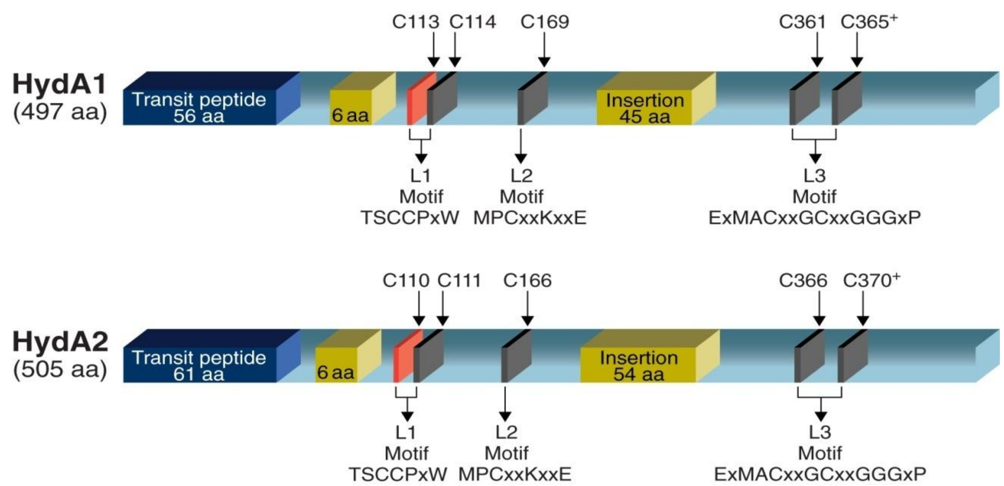


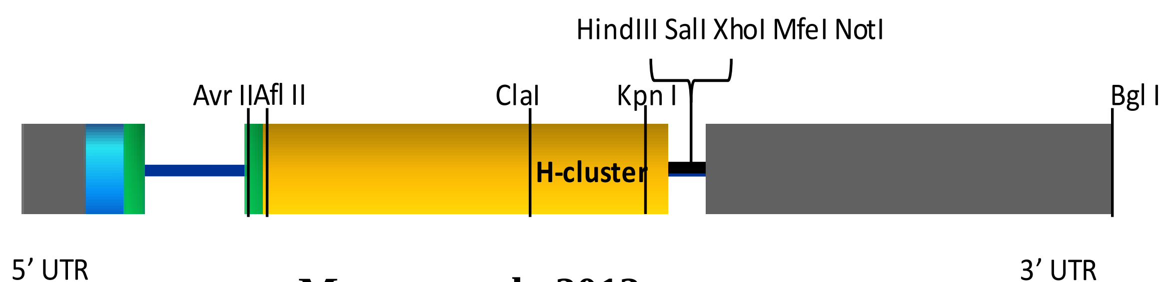
Arthur Grossman, Claudia Catalanotti, Wenqiang Yang, Venkat Subramanian, Alexandra Dubini, Mike Seibert

Chlamydomonas reinhardtii [FeFe]-hydrogenases and KO lines



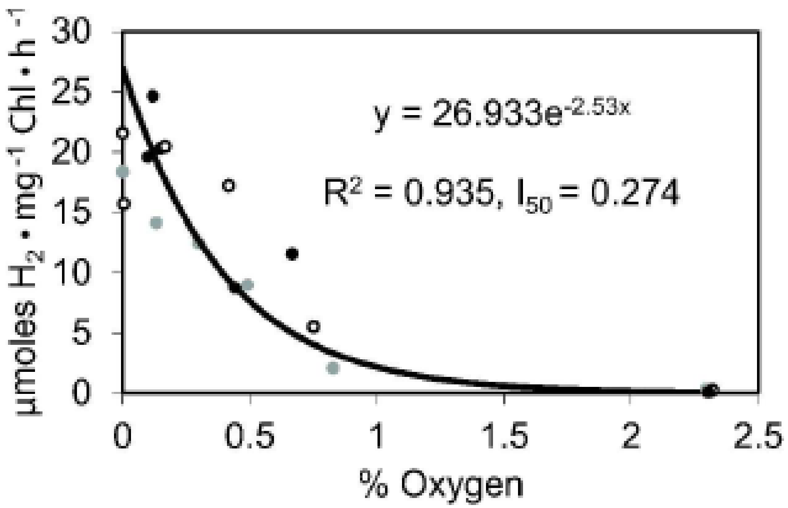
- HYDA1 is the dominant photolinked enzyme
- The two H2ases are not uniquely dedicated to photo/ferm hydrogen production
- Both enzymes can participate in photo/ferm H_2 production.
- Increases in ferm H_2 production can be achieved by increasing HYDA1 levels
- Sufficient H2ase is present in WT cells to handle all PS electron flux.



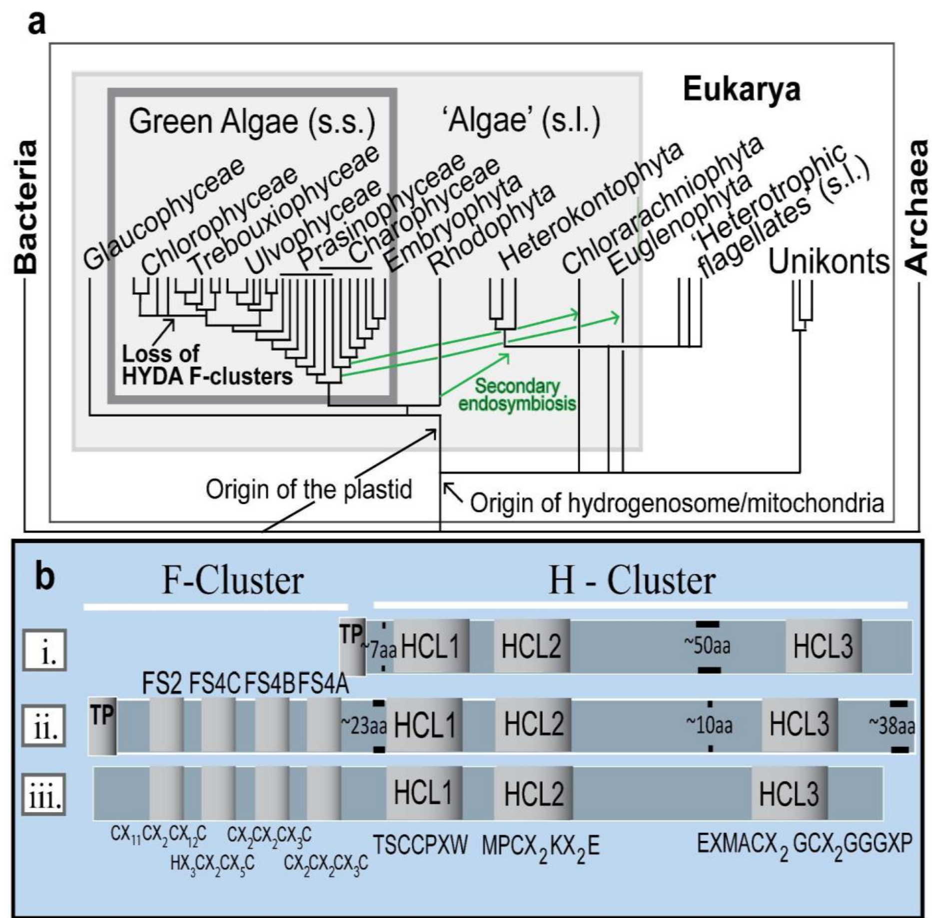


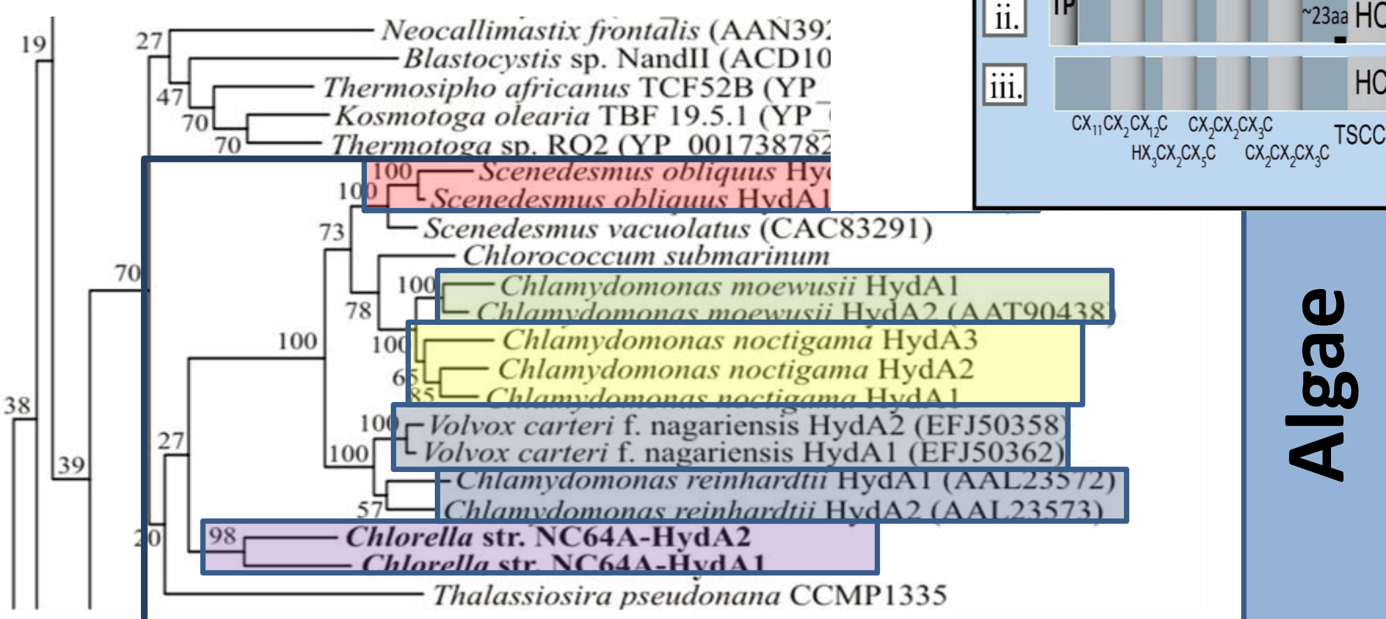
Meuser et al., 2012

HYDA paralogs are reciprocally monophyletic



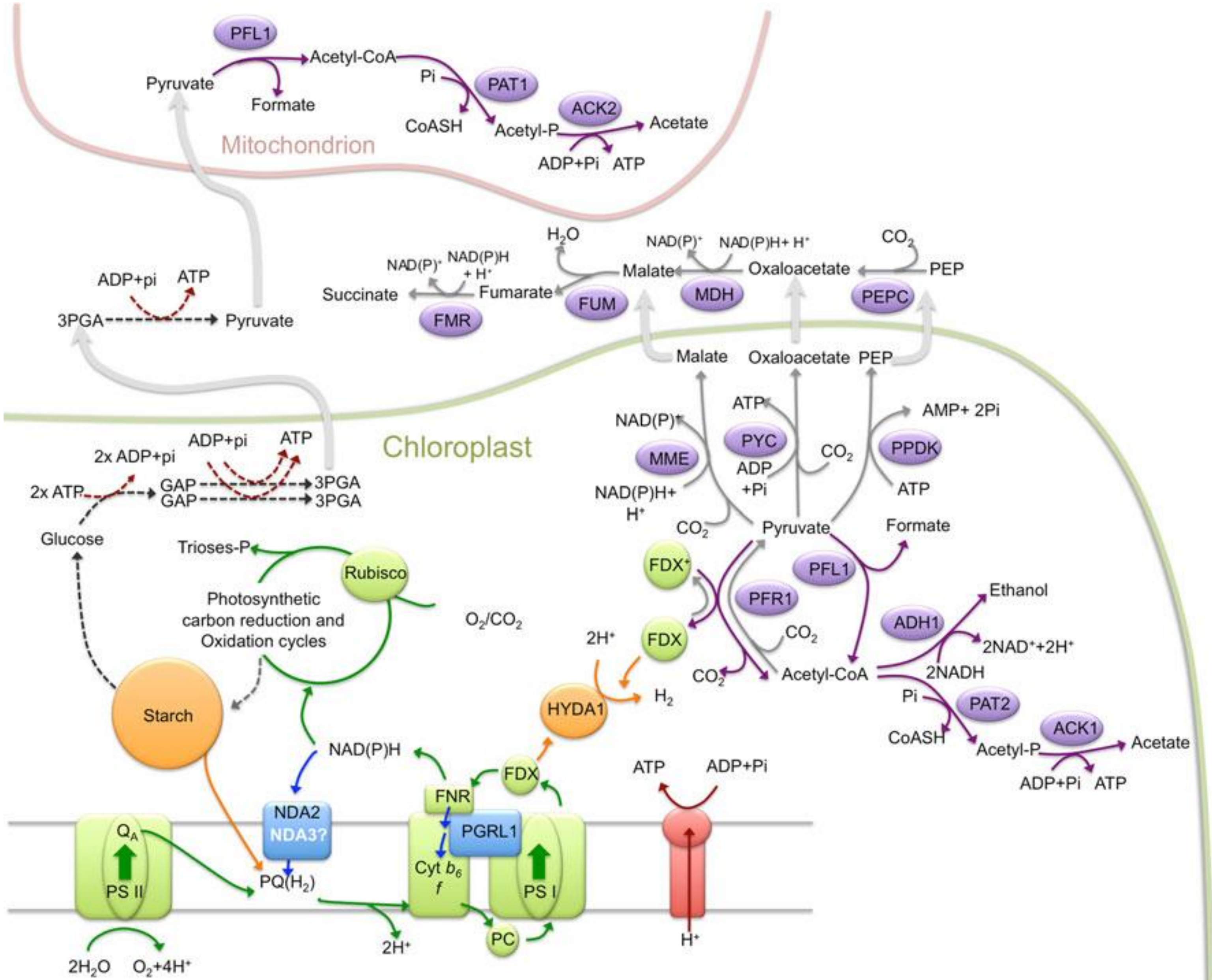
hydrogenase oxygen sensitivity





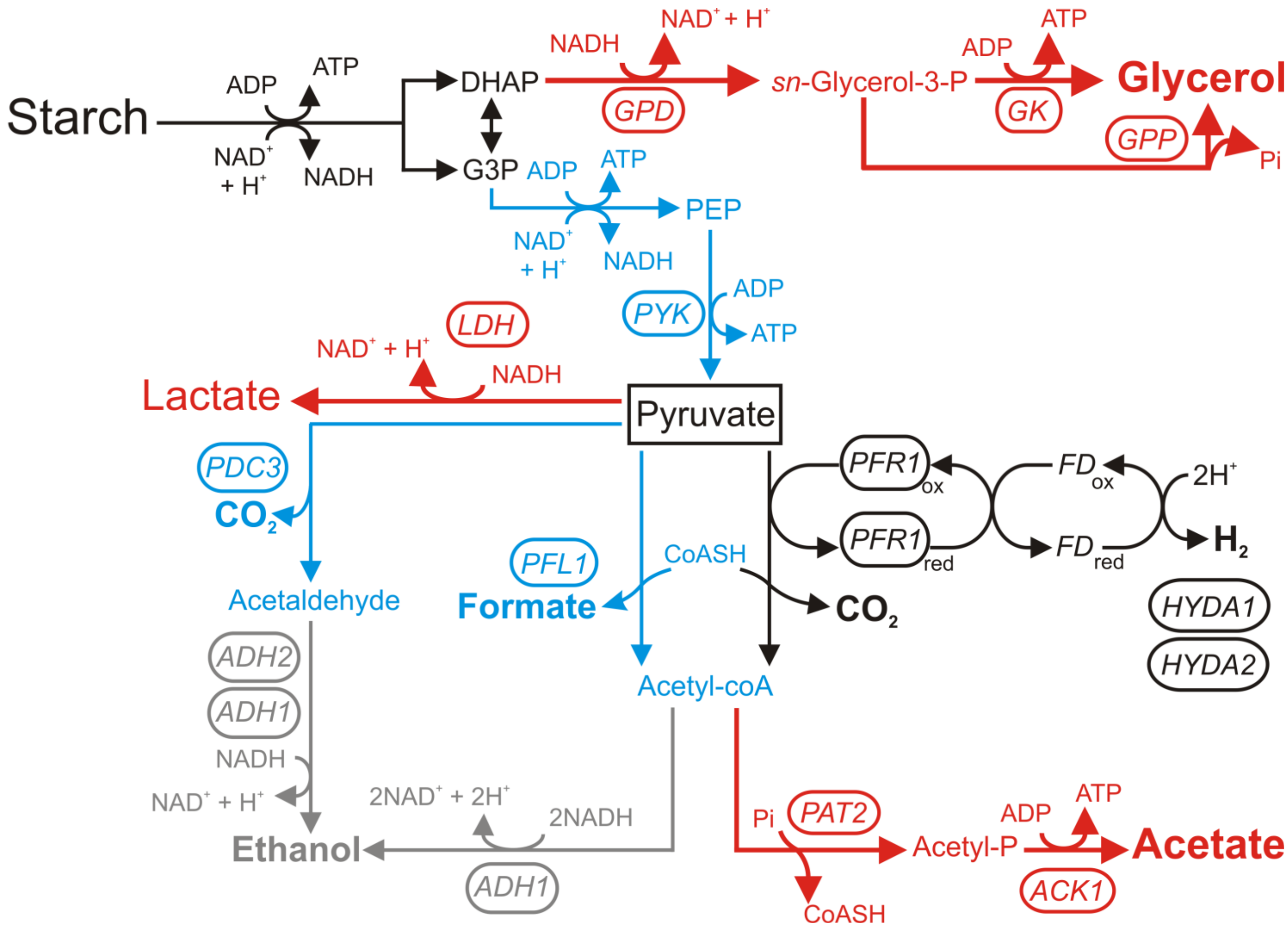
Meuser et al., 2011 *Planta*

Docking Hydrogenase into Cellular Metabolism



Terashima et al. 2011

Fermentation to H_2: PFL1 and ADH1 Deletions in Chlamydomonas



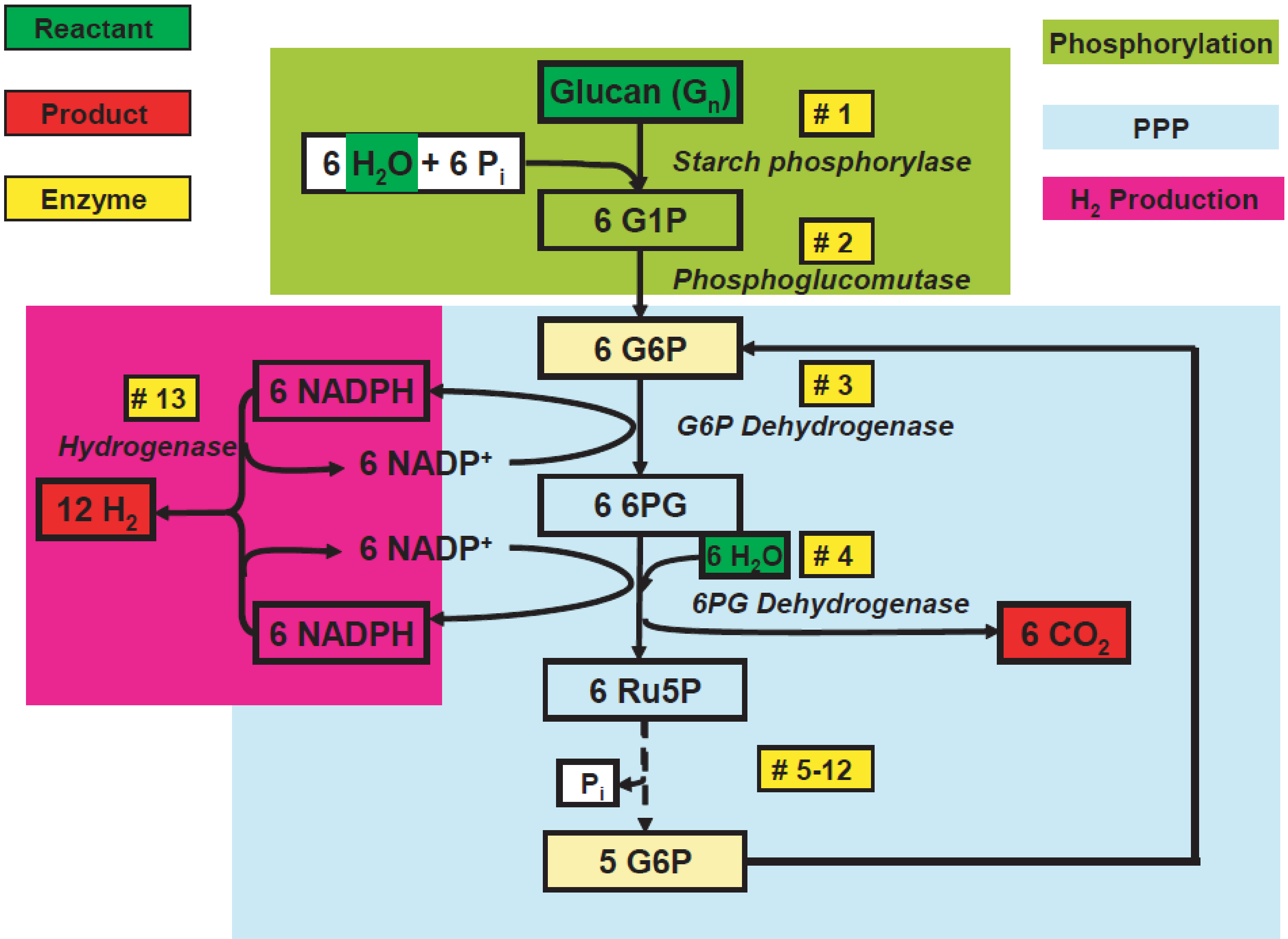
Hypotheses:

1) PFL competes with PFR and H_2 production
2) ADH1 competes with H2ase for reductant at the level of NADH

Elimination of either or both should stimulate H_2 production

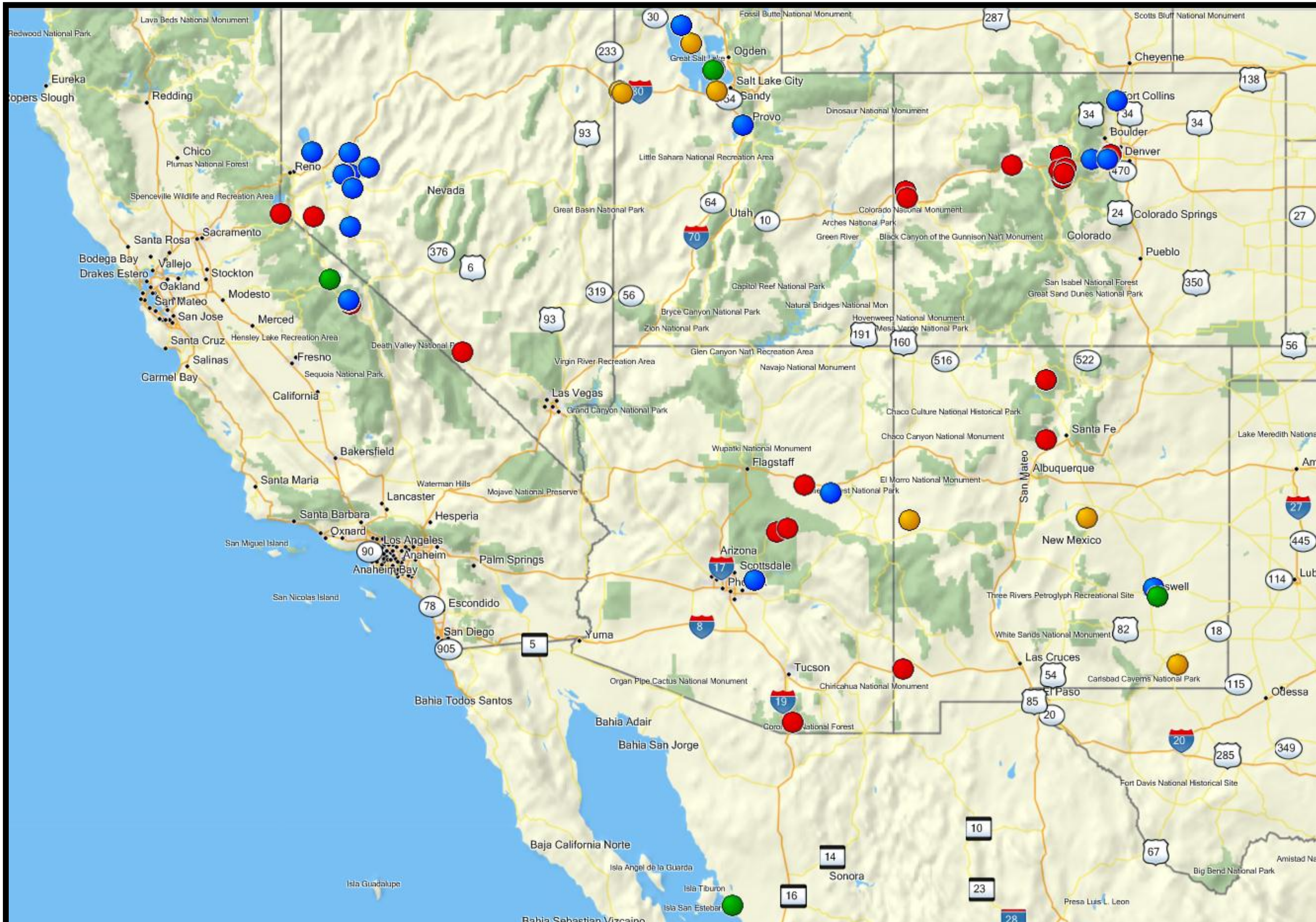
Reality: neither the single mutants nor the double mutant increase fermentative H_2.

Hydrogen From Starch Using *in vitro* Pentose Phosphate Pathway or Acetate Microbial Fuel Cells

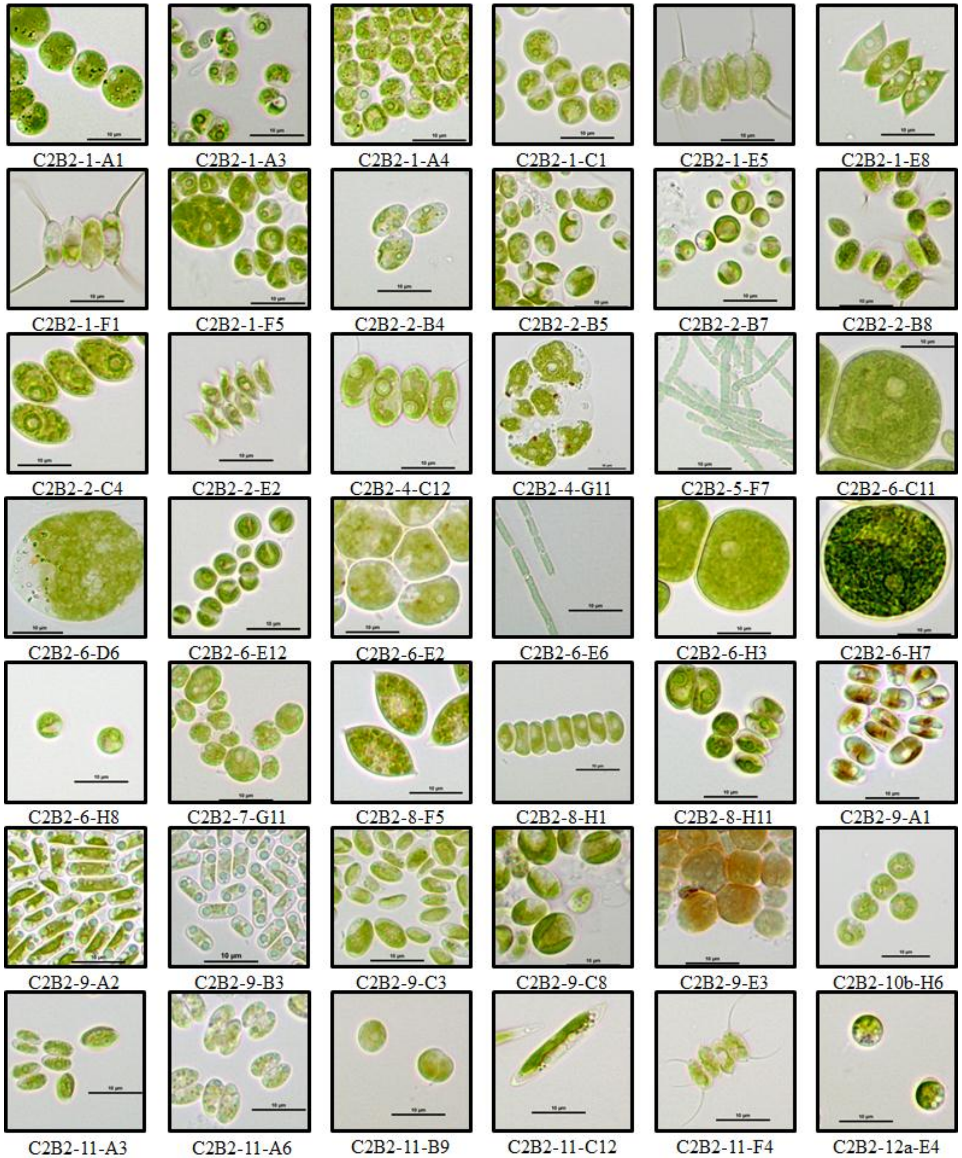


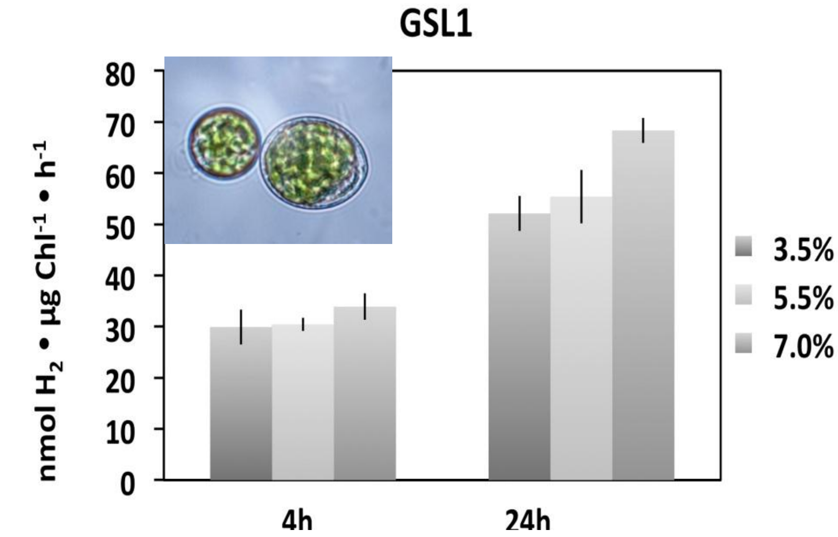
Zhang et al., 2007 PLoSOne

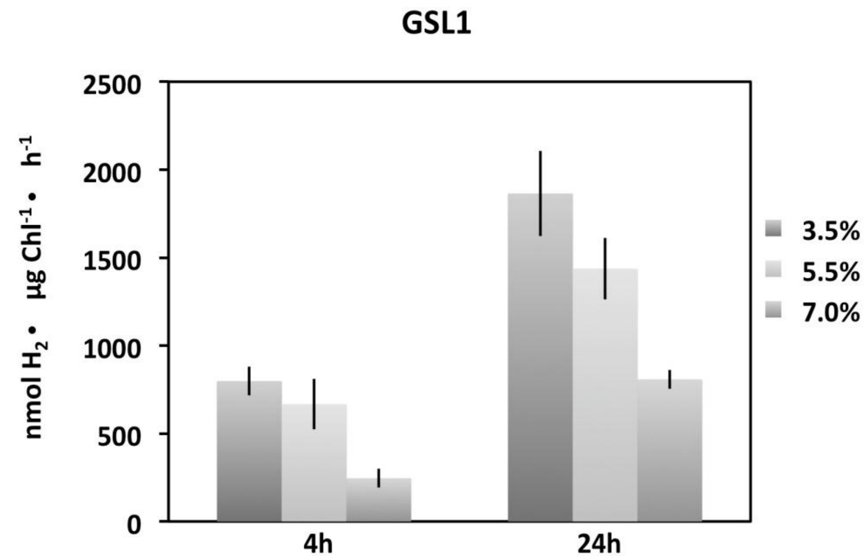
Prospecting for New Enzymes and Organisms



H_2 production in GSL halophilic Algae

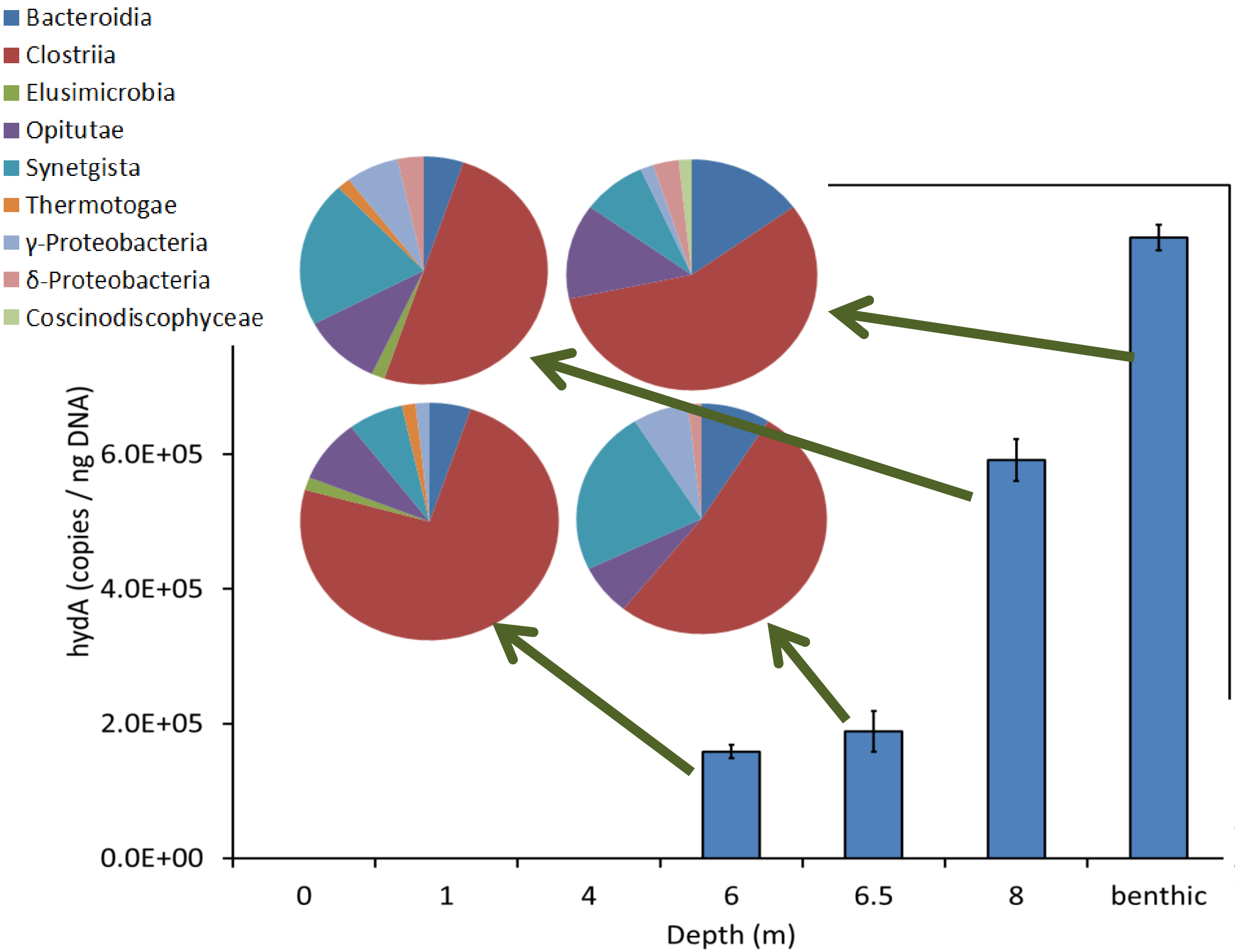


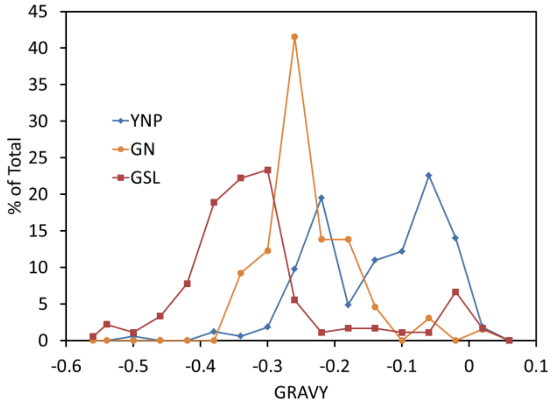




H_2 production in high salt

Hydrogenase Diversity in Naure: *hydA* along Great Salt Lake Vertical Gradient

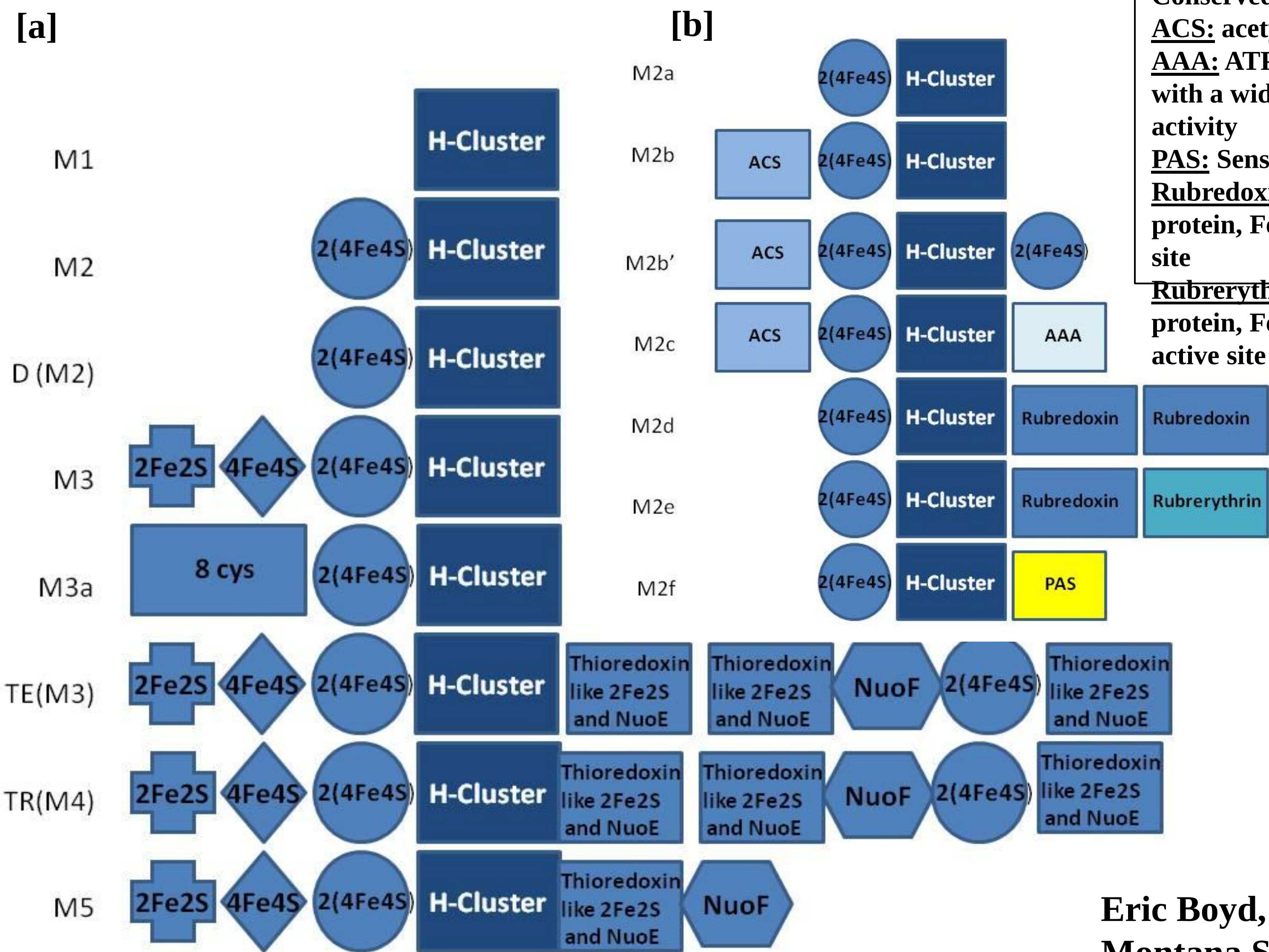




Eric Boyd, John Peters Montana State University

Jon Meuser, CSM

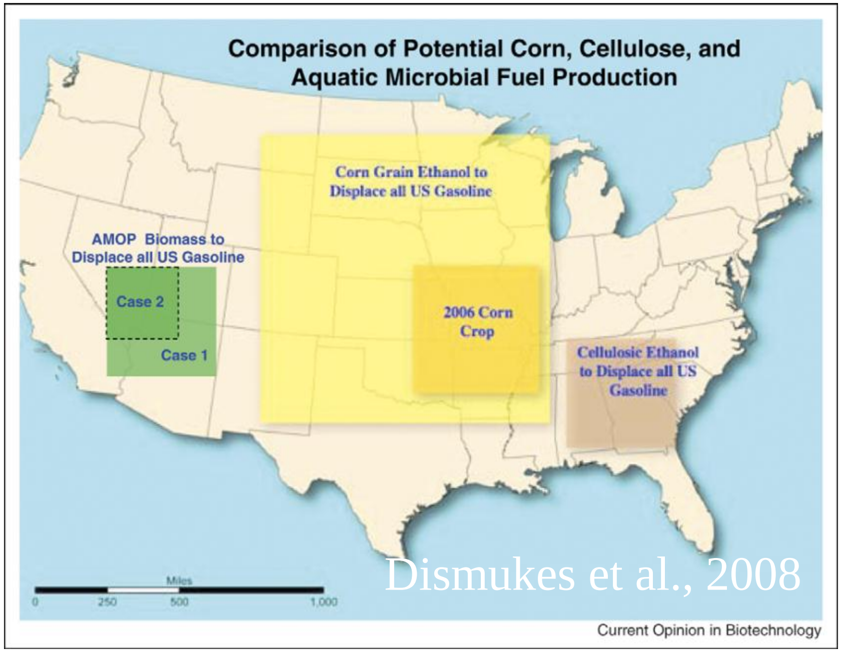
Inferred HydA Structural Variation



Conserved Accessory Domains:
ACS: acetyl-CoA synthase
AAA: ATPase associated with a with a wide variety of cellular activity
PAS: Sensory domain
Rubredoxin: Iron containing protein, FeS_4 domain at the active site
Rubrerythrin: Iron containing protein, FeS_4 diiron domain at the active site

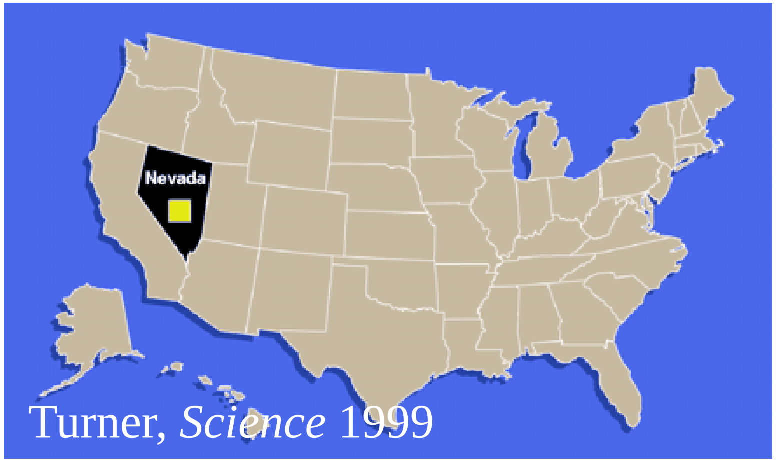
Eric Boyd, John Peters
Montana State University
Updated from Meyer, 2007

Area Required for US Transportation Needs 10% Solar-to-Fuel Conversion Efficiency



1000 µmoles PAR $m^{-2}s^{-1}$
12 hours light/day
15768 moles of PS photons/yr
7884 moles of reductant
3942 moles H_2/yr
1 kg H_2 = 495 moles of H_2 = 1 gallon of gasoline
~12100 L H_2 gas
~ 8 kg H_2/yr ·m^2

Current efficiencies are considerably less than 1% and short term. Among most productive systems are heterocyst based nitrogenase systems in cyanobacteria and nutrient deprived eukaryotic algae



Recent Advances Accelerating Progress

- Enzyme structure/function/maturation
- Genetic/metabolic tools
- Synthetic biology
- Metabolic engineering
- Generation of key mutants

10,000 square miles (100 x 100 miles)