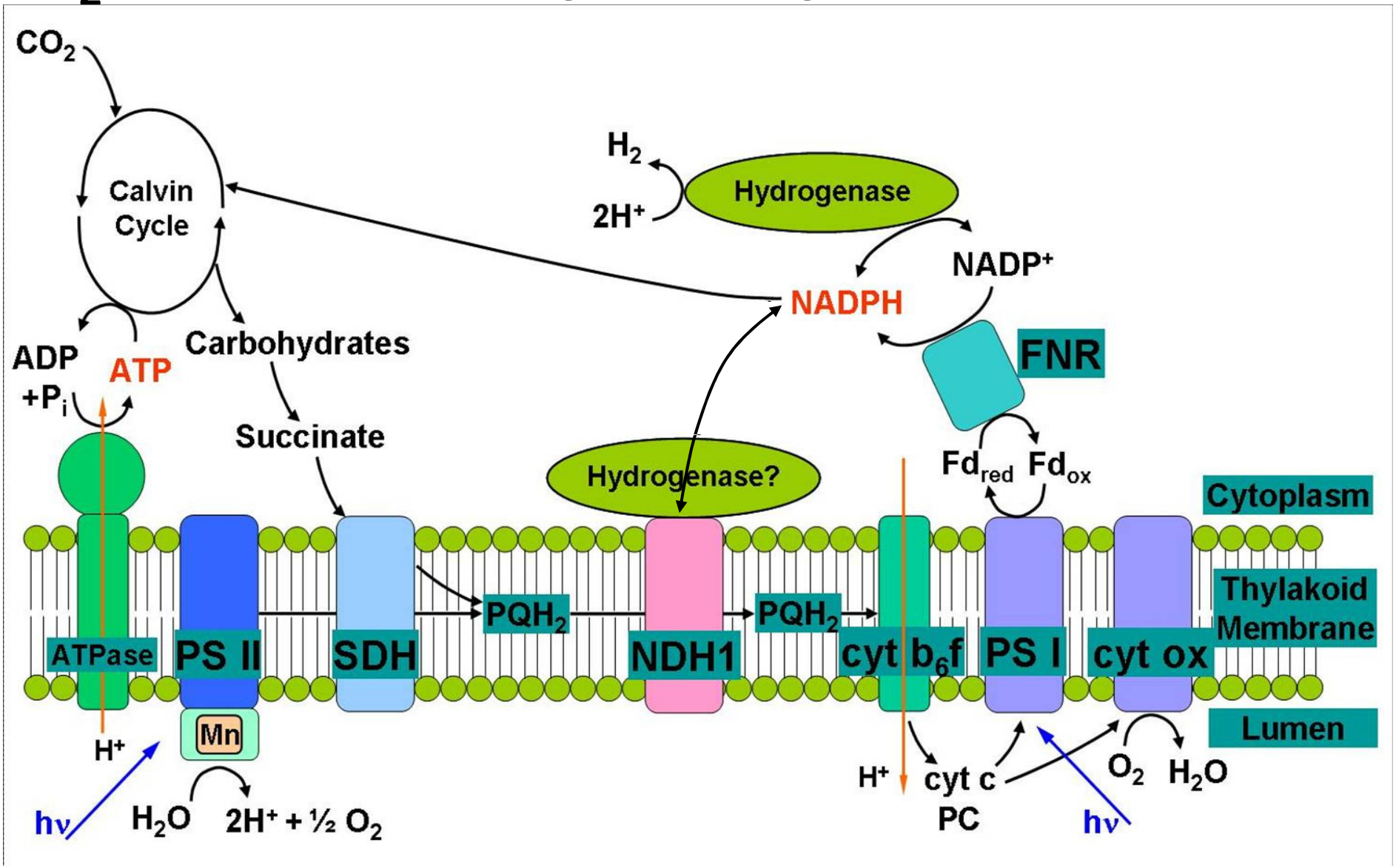
Metabolic Engineering of Light and Dark Biochemical Pathways in Wild-Type and Mutant *Synechocystis* PCC 6803 Strains for Maximal, 24-Hour Production of Hydrogen Gas

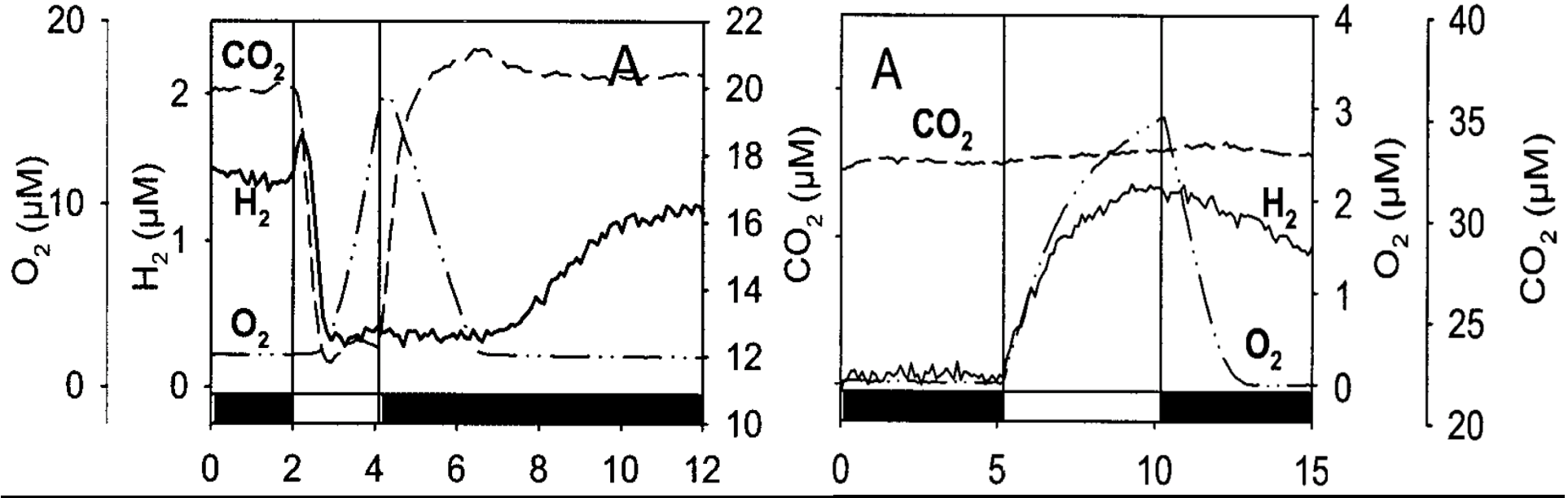
Roger L. Ely
Frank W.R. Chaplen

Oregon State University

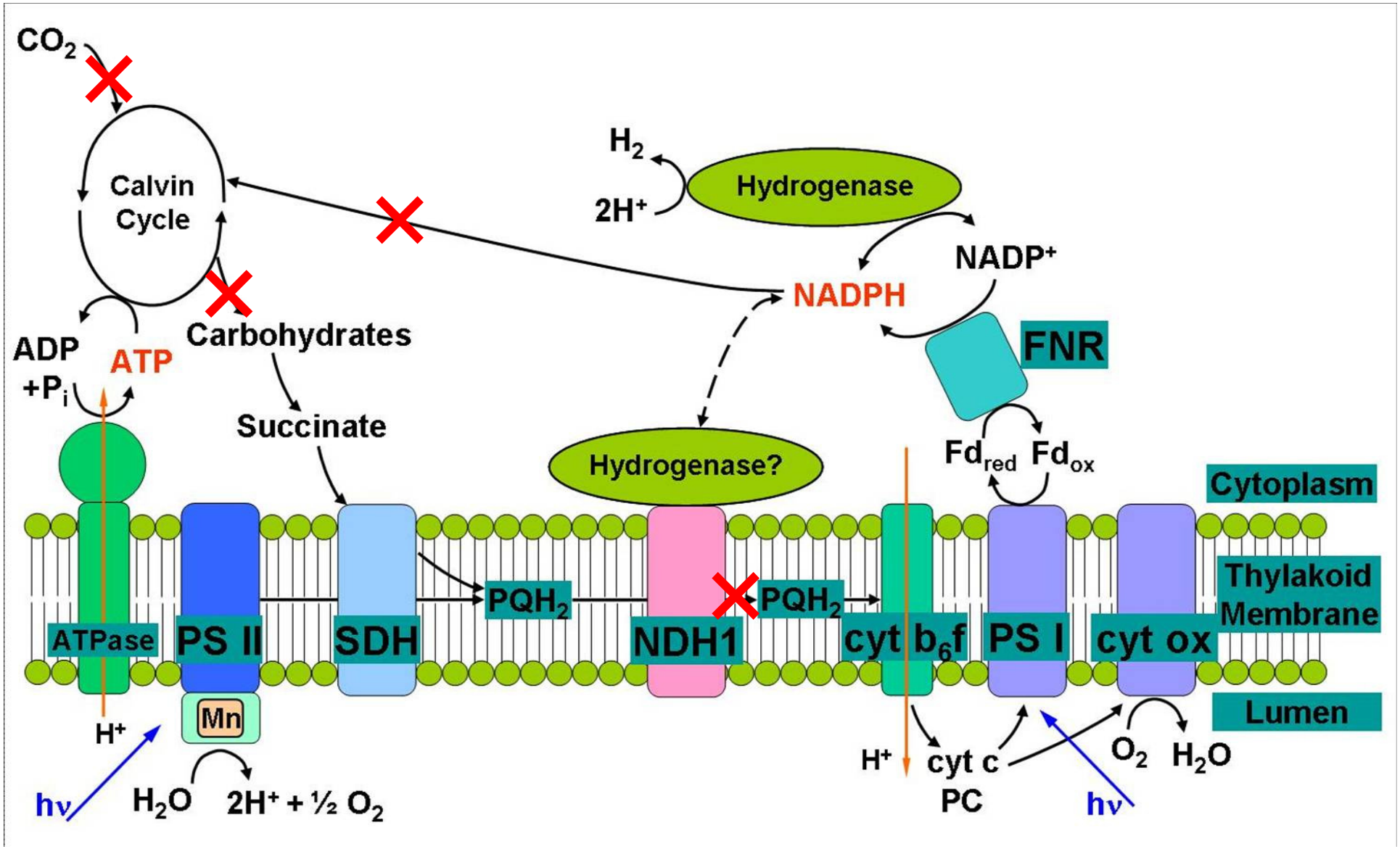
DOE-BER GTL-MEWG Annual Awardee Workshop
Feb 11, 2008

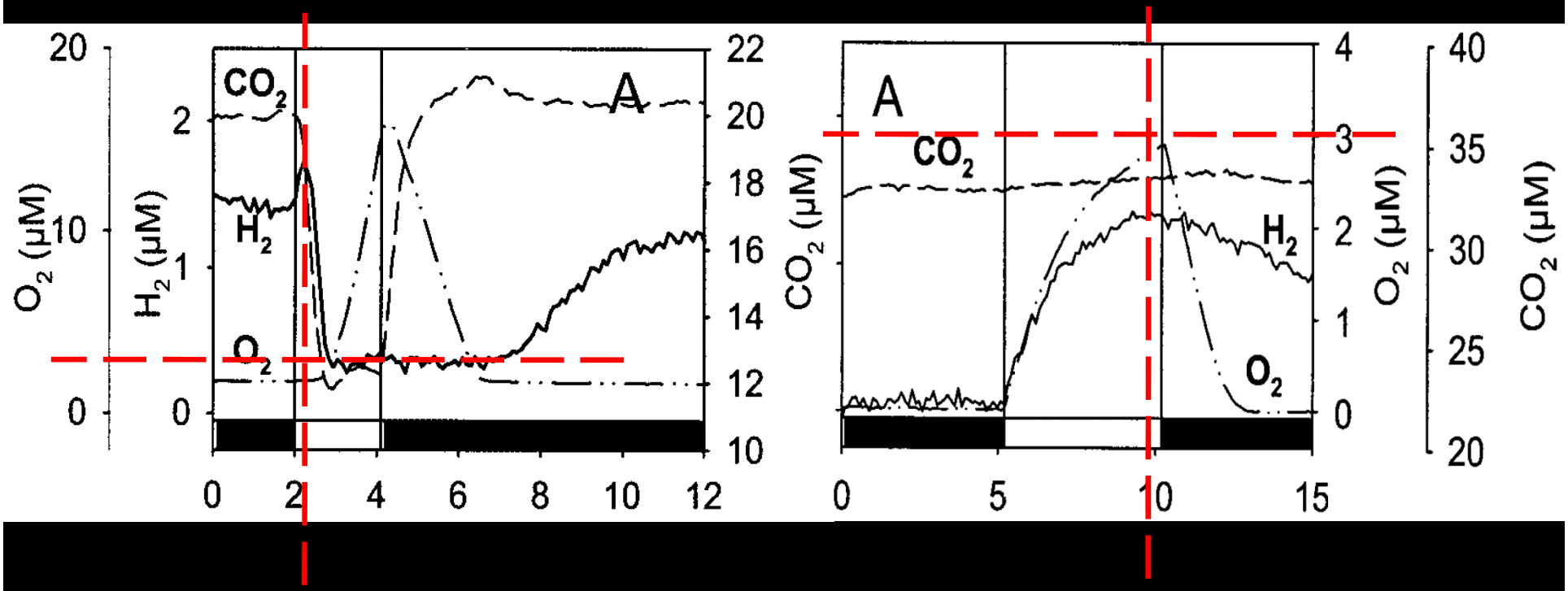
H_2 Production in *Synechocystis* PCC 6803





Source: Cournac, L., G. Guedeney, G. Peltier, and P. M. Vignais. 2004. J. Bacteriol. 186(6):1737–1746

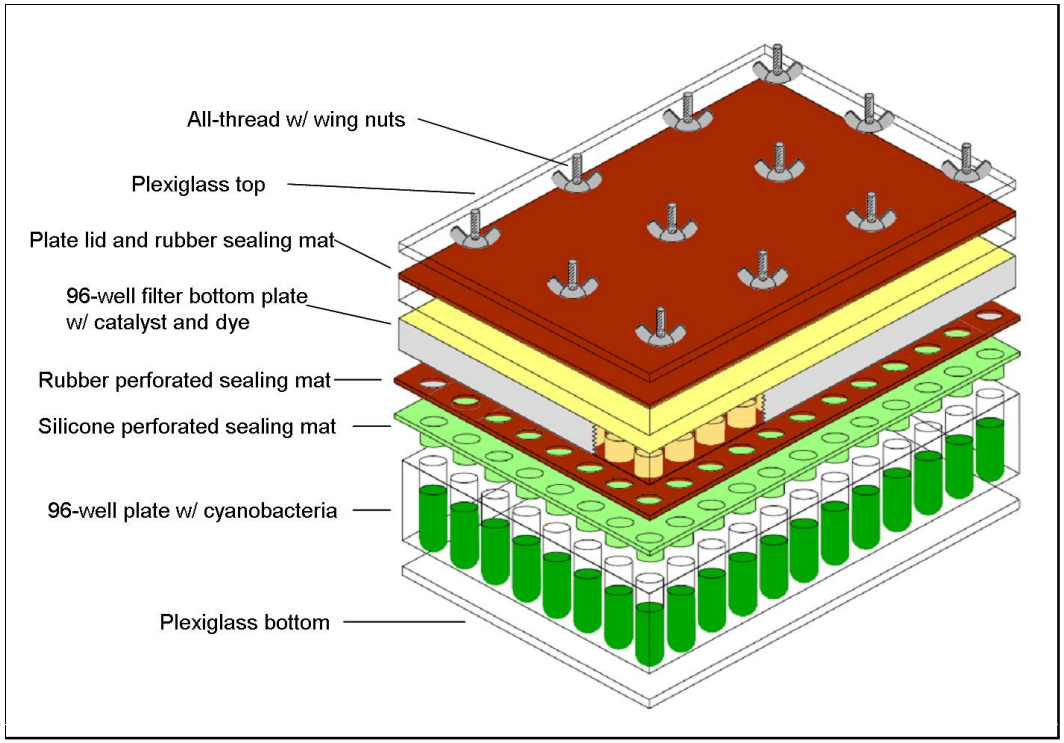

CO2
Calvin Cycle
ADP +Pi
ATP
Carbohydrates
Succinate
H2
2H+
Hydrogenase
NADP+
NADPH
FNR
Fdred
Fdox
Hydrogenase?
Cytoplasm
Thylakoid Membrane
Lumen
ATPase
PS II
SDH
PQH2
NDH1
PQH2
cyt b6f
PS I
cyt ox
Mn
H+
hν
H2O
2H+ + ½ O2
H+
cyt c
PC
hν
O2
H2O

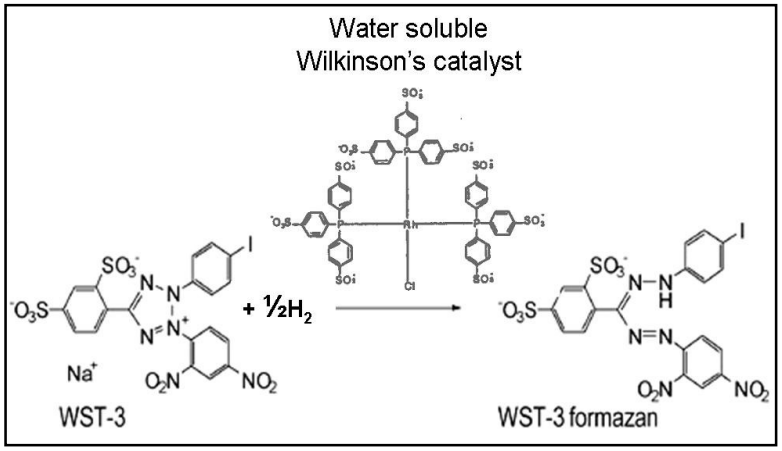


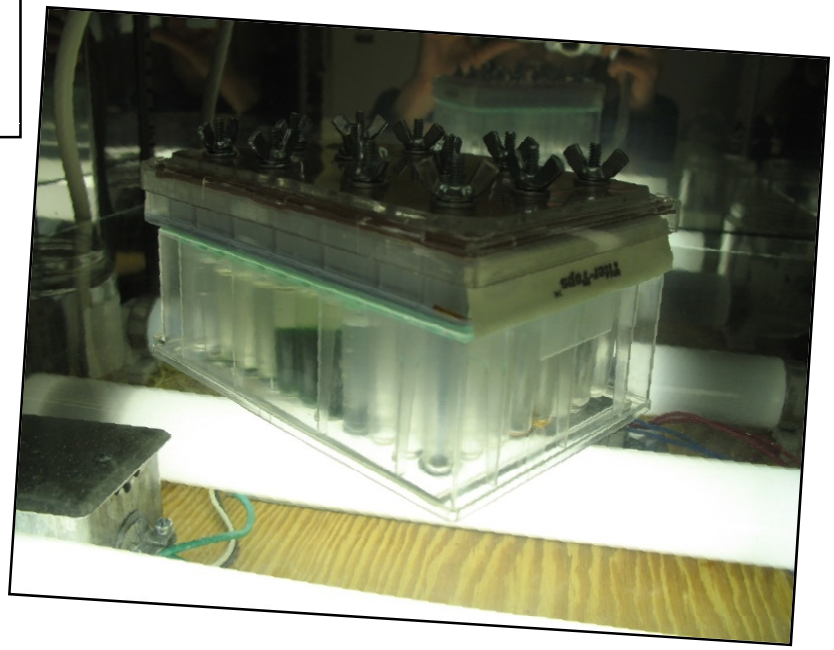
Source: Cournac, L., G. Guedeney, G. Peltier, and P. M. Vignais. 2004. J. Bacteriol. 186(6):1737–1746

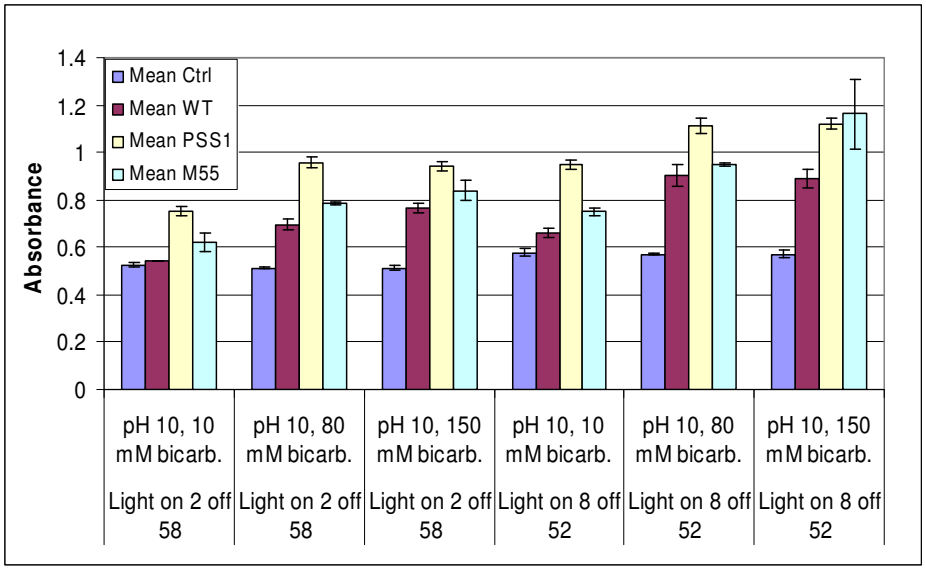
Approach

- **Reductant Supply**
  - Initial Screening
  - H_2 Production Profiling
  - Metabolic Flux Analyses
  - Specific Inhibitors
- **Oxygen Sensitivity**
  - Rational Enzyme Modifications
  - Directed Evolution

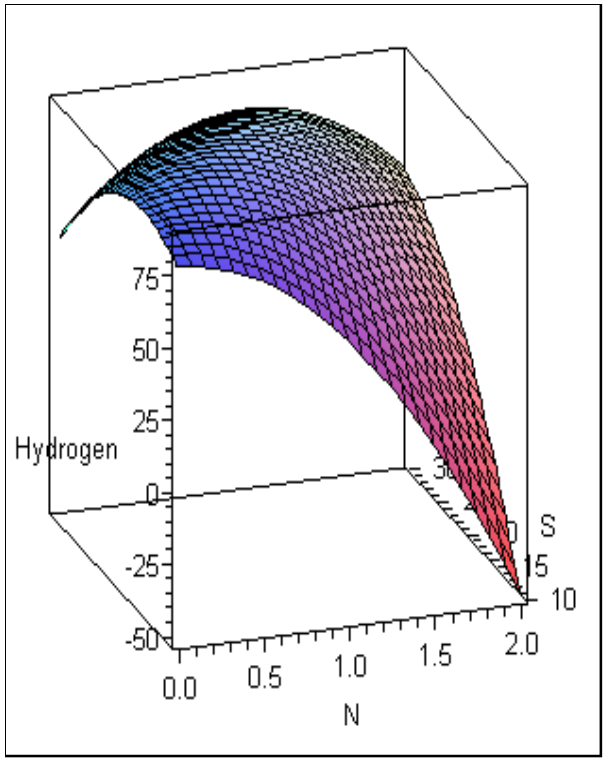

All-thread w/ wing nuts
Plexiglass top
Plate lid and rubber sealing mat
96-well filter bottom plate w/ catalyst and dye
Rubber perforated sealing mat
Silicone perforated sealing mat
96-well plate w/ cyanobacteria
Plexiglass bottom

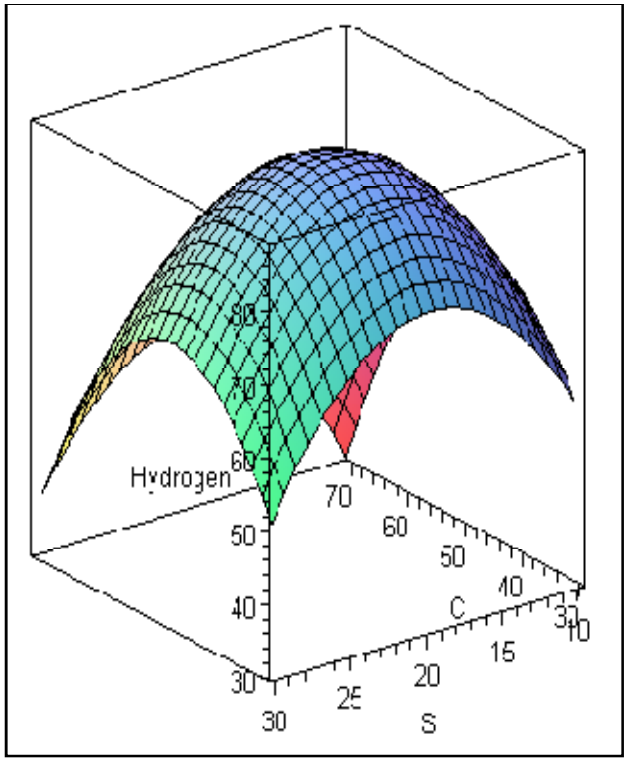

Water soluble
Wilkinson's catalyst
+ ½H2
WST-3
WST-3 formazan

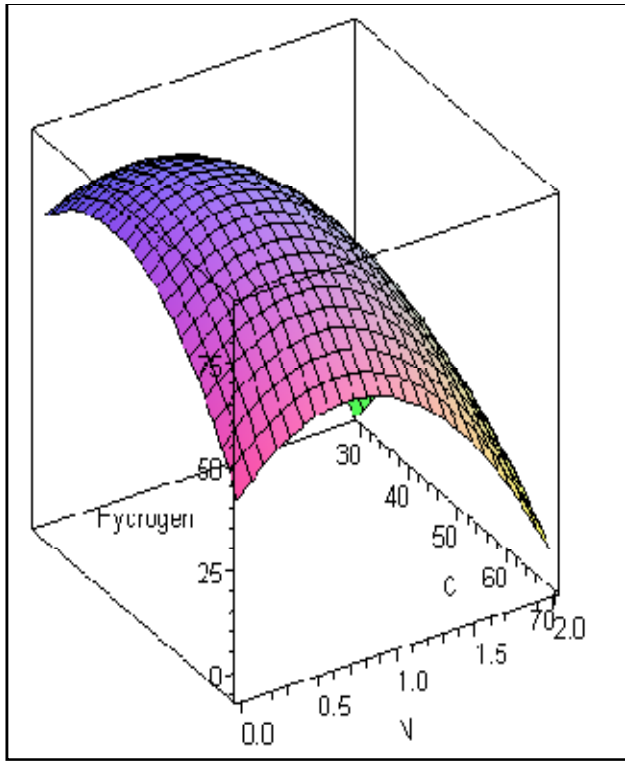



Absorbance
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mean Ctrl
Mean WT
Mean PSS1
Mean M55
pH 10, 10 mM bicarb.
pH 10, 80 mM bicarb.
pH 10, 150 mM bicarb.
pH 10, 10 mM bicarb.
pH 10, 80 mM bicarb.
pH 10, 150 mM bicarb.
Light on 2 off 58
Light on 2 off 58
Light on 2 off 58
Light on 8 off 52
Light on 8 off 52
Light on 8 off 52

Poster # 23

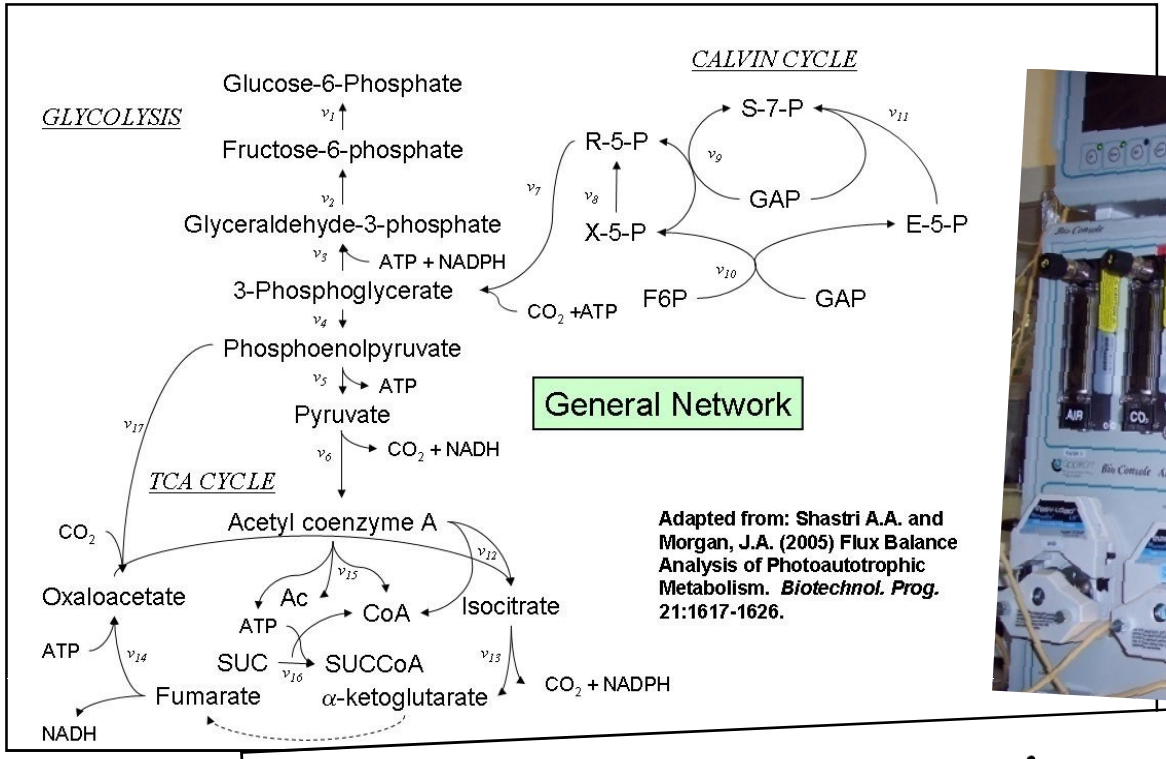




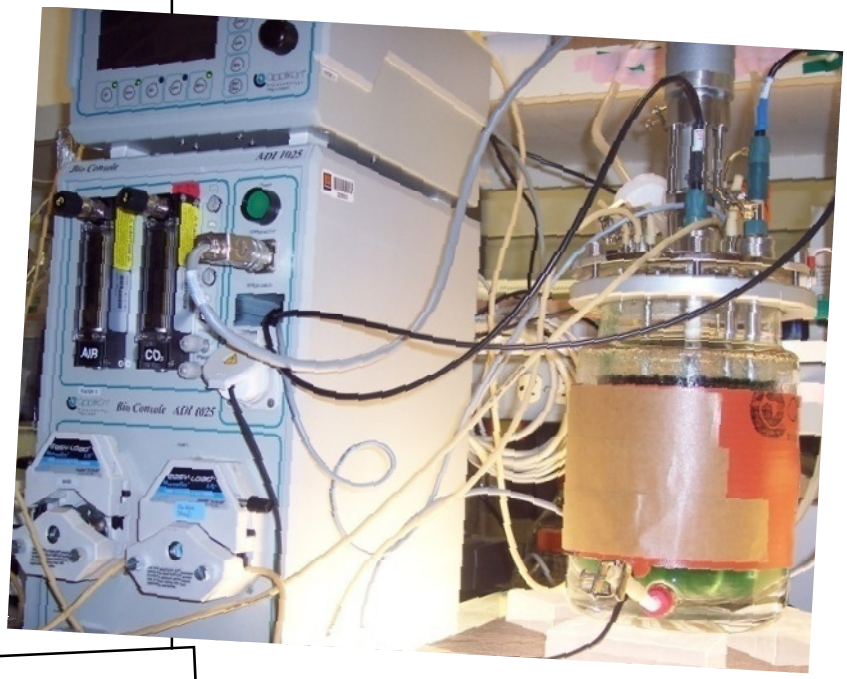


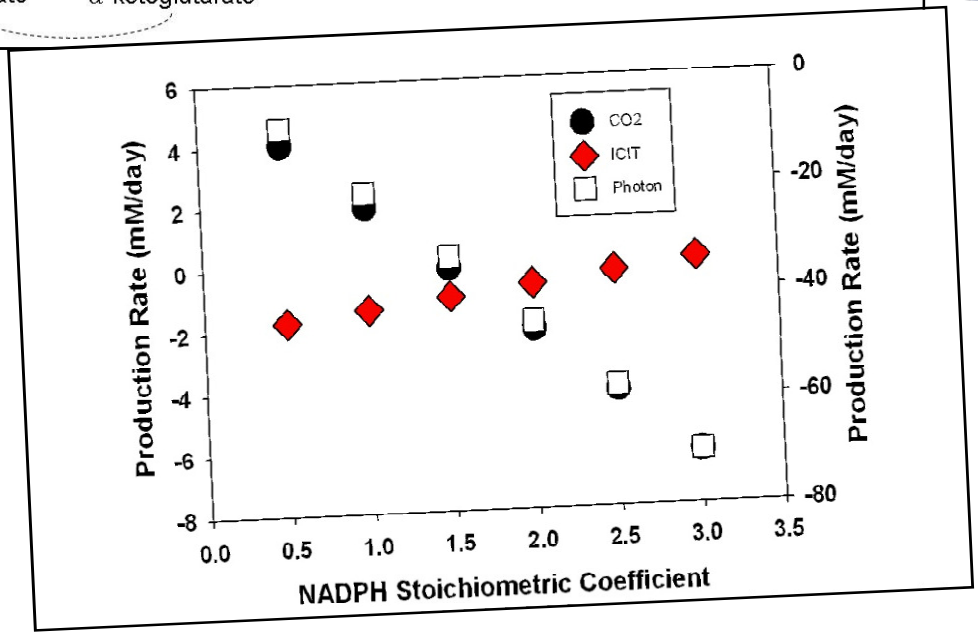
	H_2 accumulation in headspace (μM)	
	14 hours	40 hours
Predicted maximum	85 (23)	210 (54)
S-deprived cultures	0 (0)	0.16 (0.3)

Poster # 26

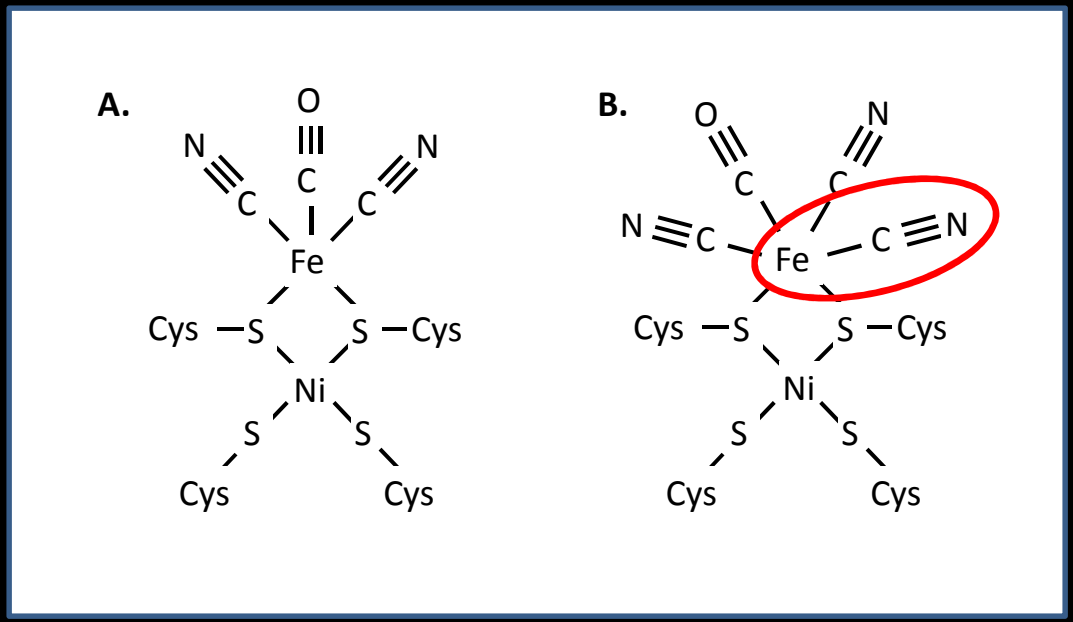


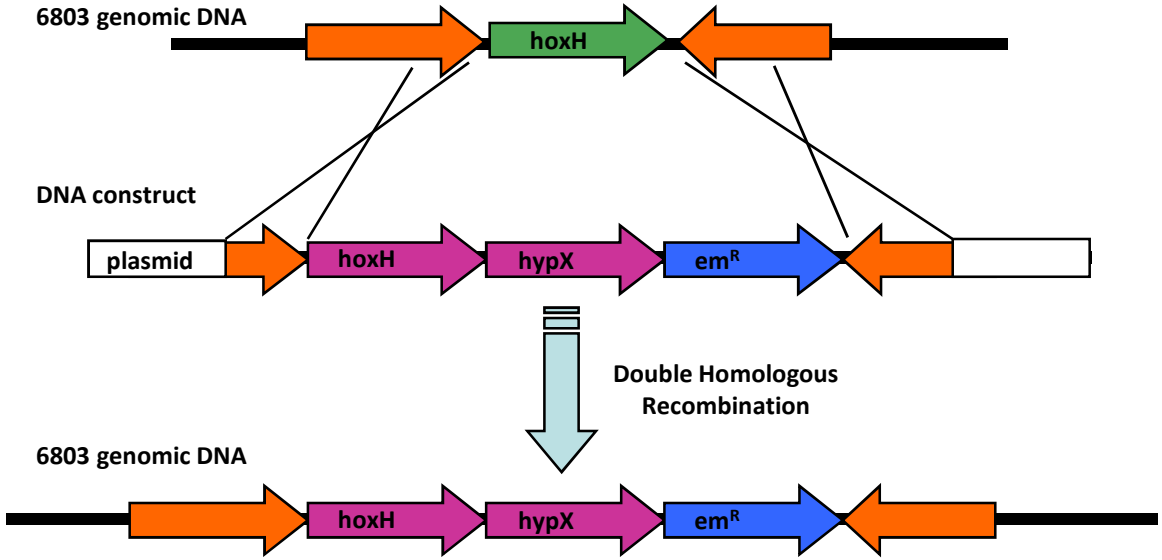
Adapted from: Shastri A.A. and Morgan, J.A. (2005) Flux Balance Analysis of Photoautotrophic Metabolism. *Biotechnol. Prog.* 21:1617-1626.

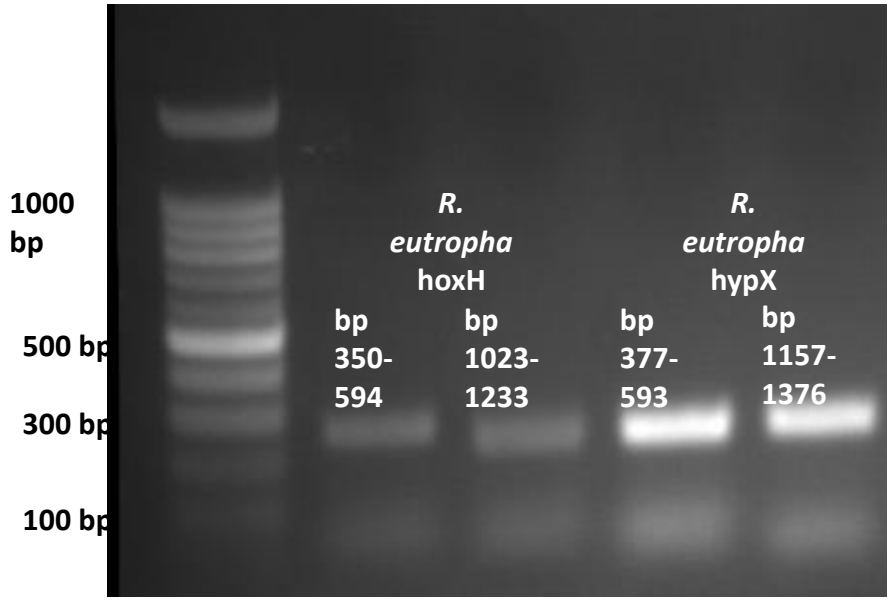


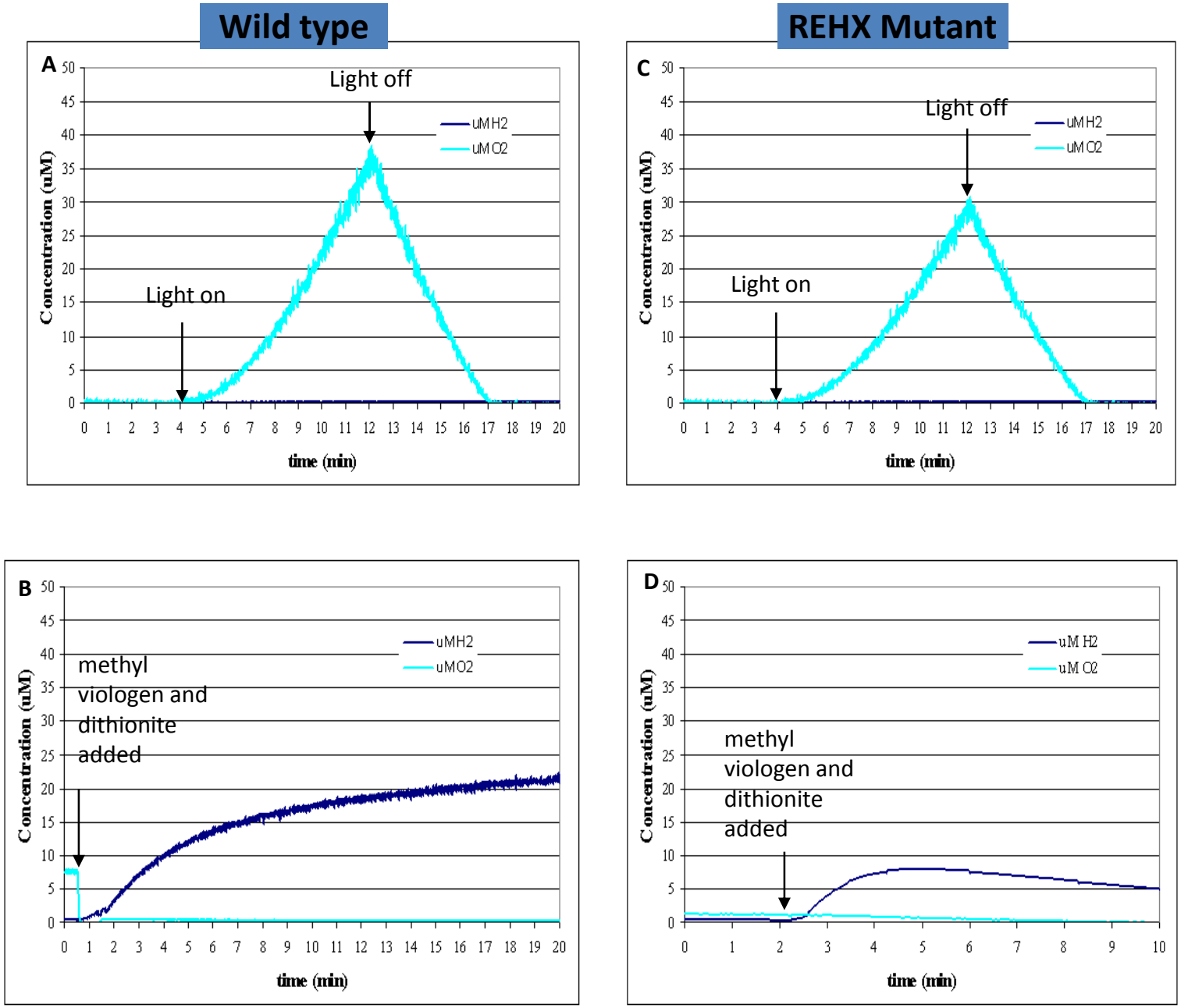


Poster # 25

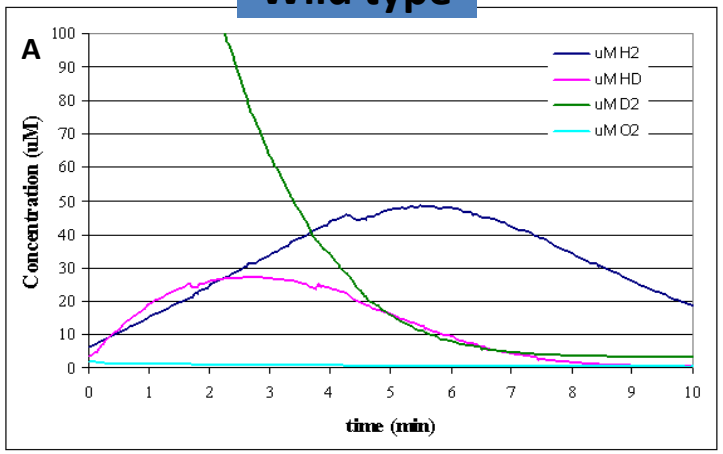

A.
O
N
N
C
C
C
Fe
Cys
S
S
Cys
Ni
S
S
Cys
Cys
B.
O
N
C
C
N
C
Fe
C
N
Cys
S
S
Cys
Ni
S
S
Cys
Cys


6803 genomic DNA
hoxH
DNA construct
plasmid
hoxH
hypX
emR
Double Homologous
Recombination
6803 genomic DNA
hoxH
hypX
emR

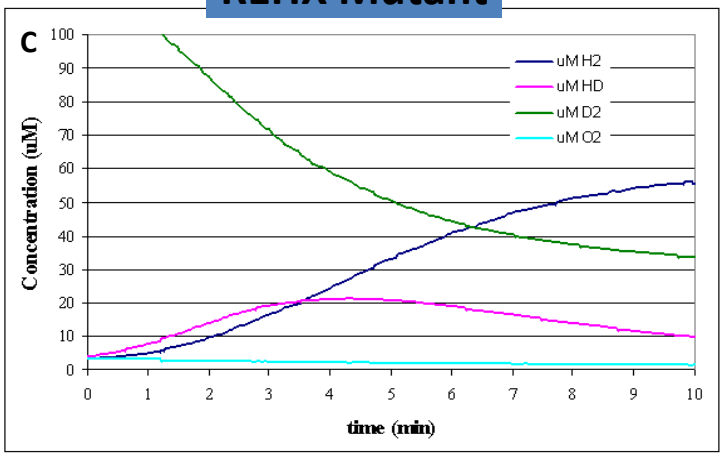

1000
bp
500 bp
300 bp
100 bp
R.
eutropha
hoxH
bp
350-
594
bp
1023-
1233
R.
eutropha
hypX
bp
377-
593
bp
1157-
1376

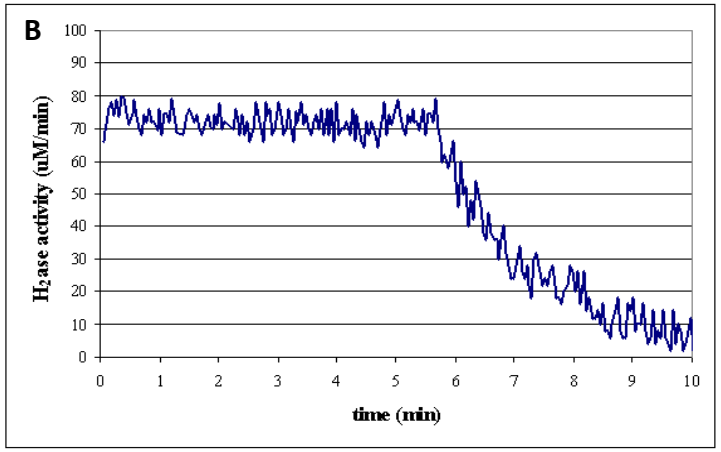

Wild type
REHX Mutant
A
C
B
D
Light off
Light on
uM H2
uM O2
uMH2
uMO2
methyl viologen and dithionite added
Concentration (uM)
time (min)

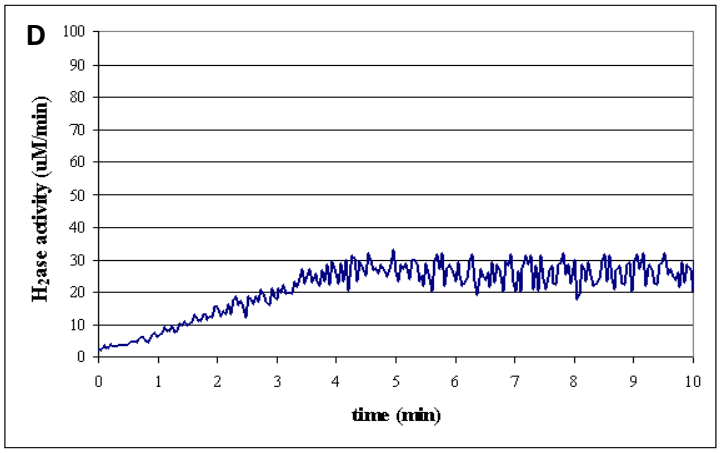
Wild type



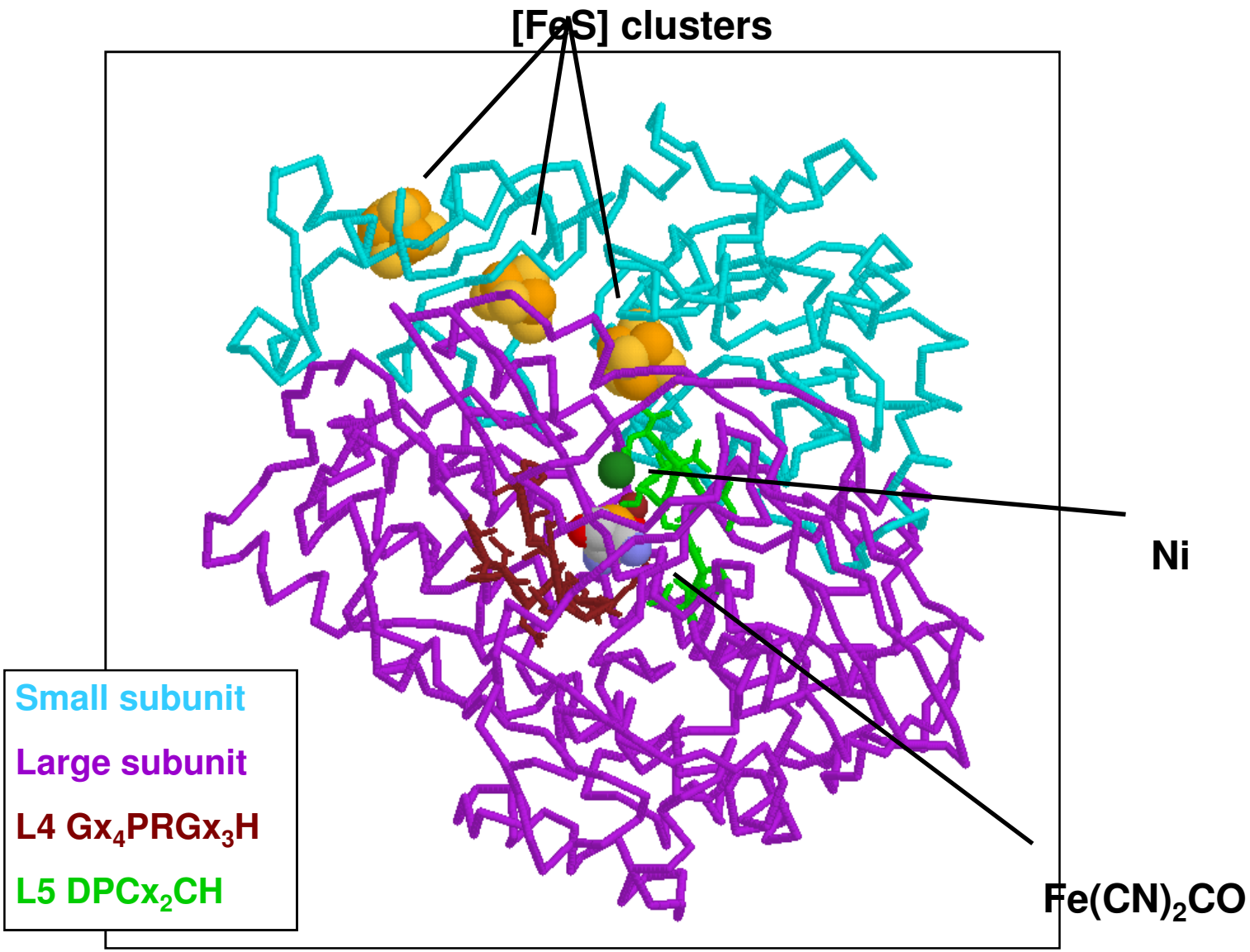
REHX Mutant

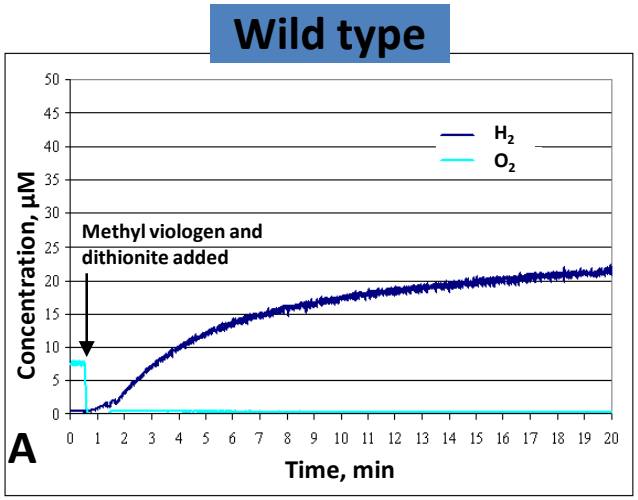


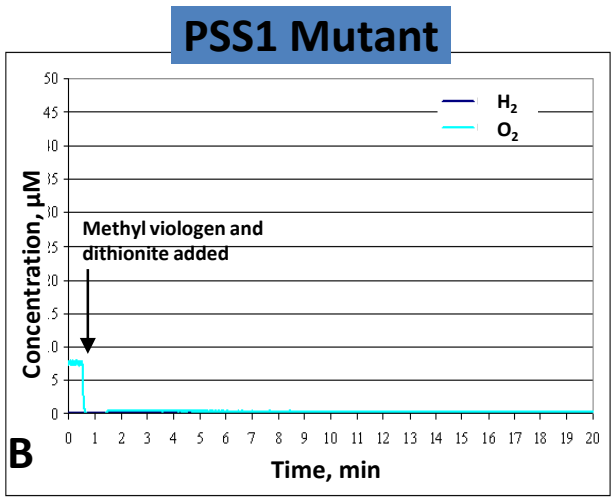


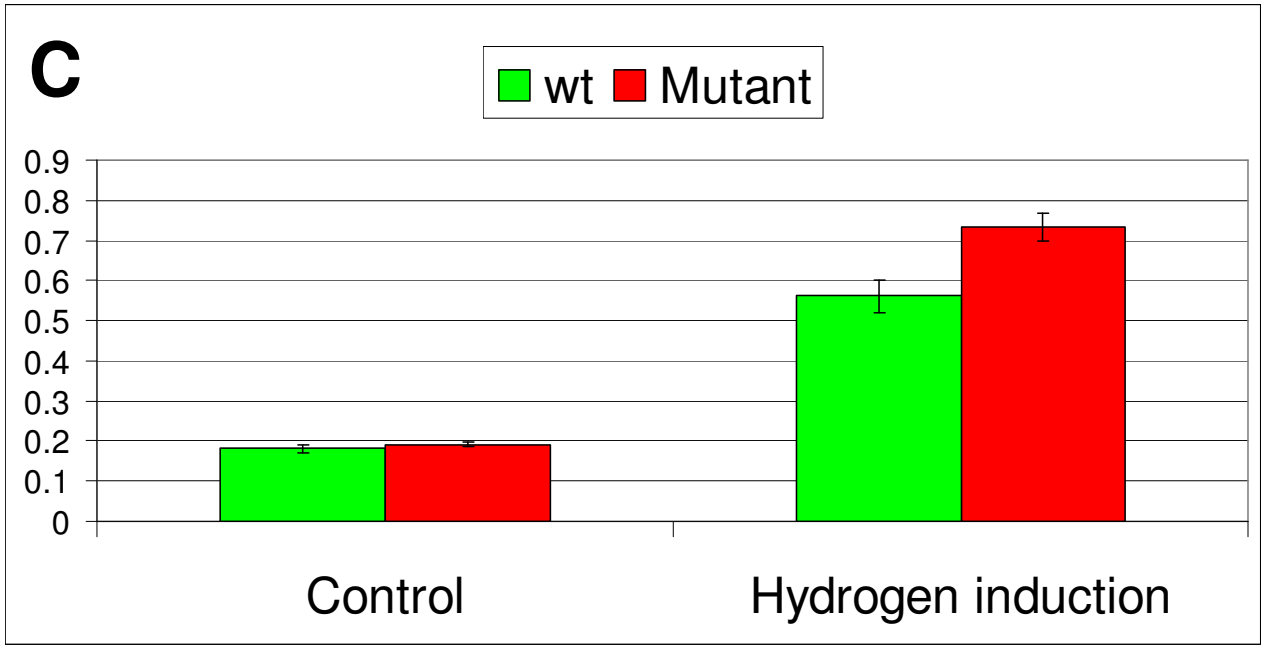


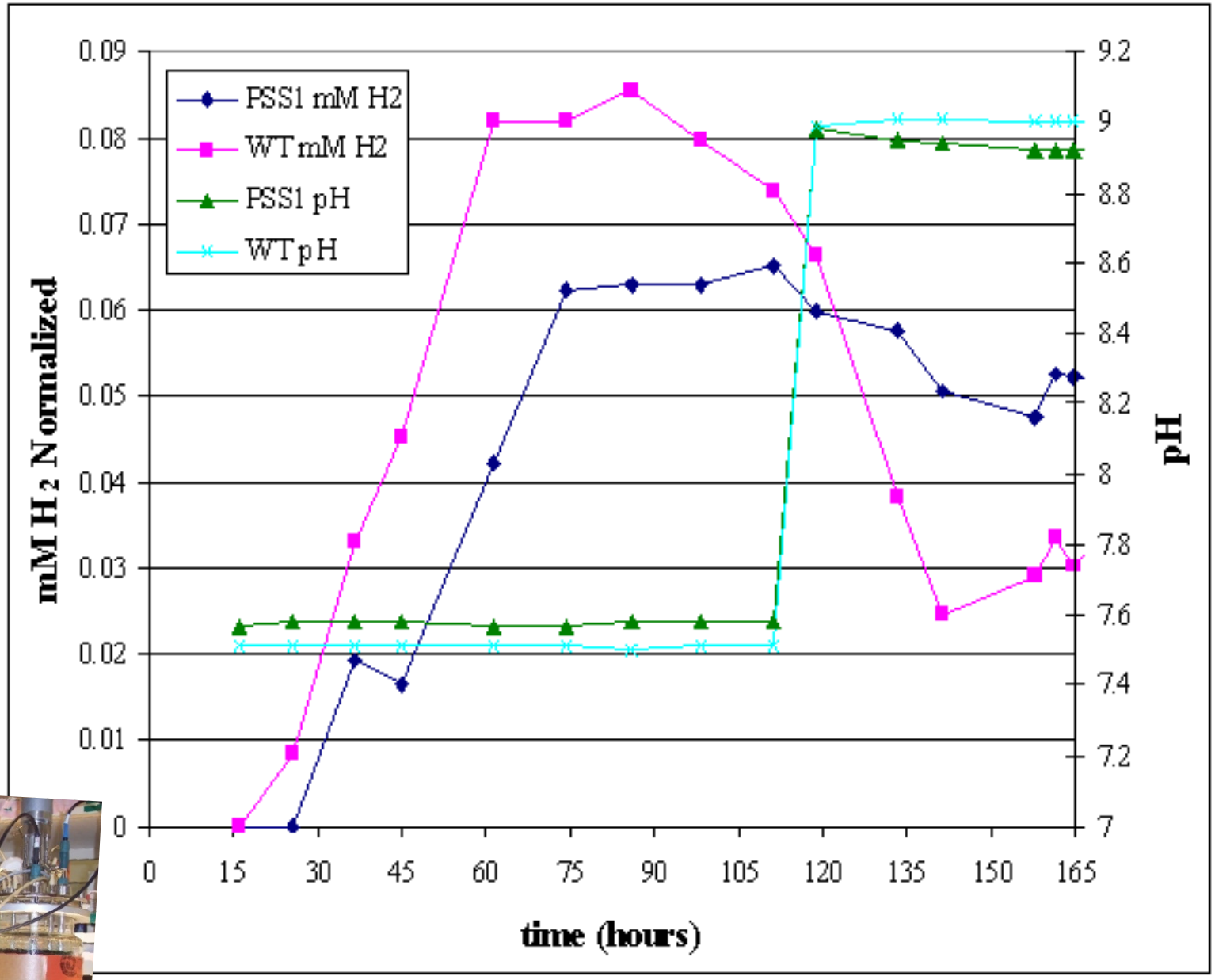
Poster # 27

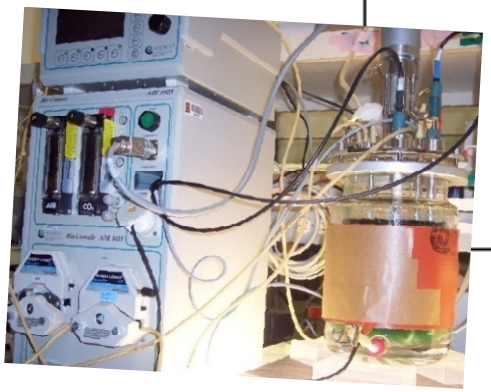

[FeS] clusters
Ni
Fe(CN)$_2$CO
Small subunit
Large subunit
L4 Gx$_4$PRGx$_3$H
L5 DPCx$_2$CH

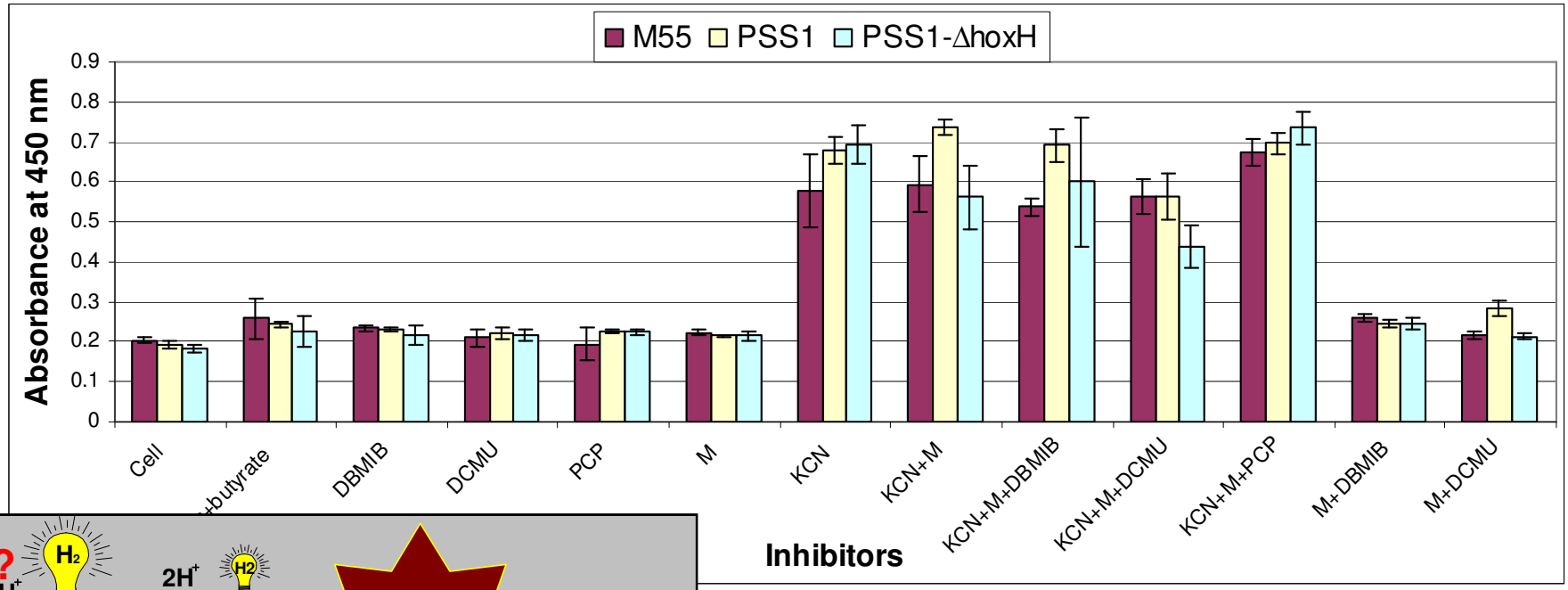

Wild type
A
Concentration, µM
Time, min
Methyl viologen and dithionite added
H_2
O_2

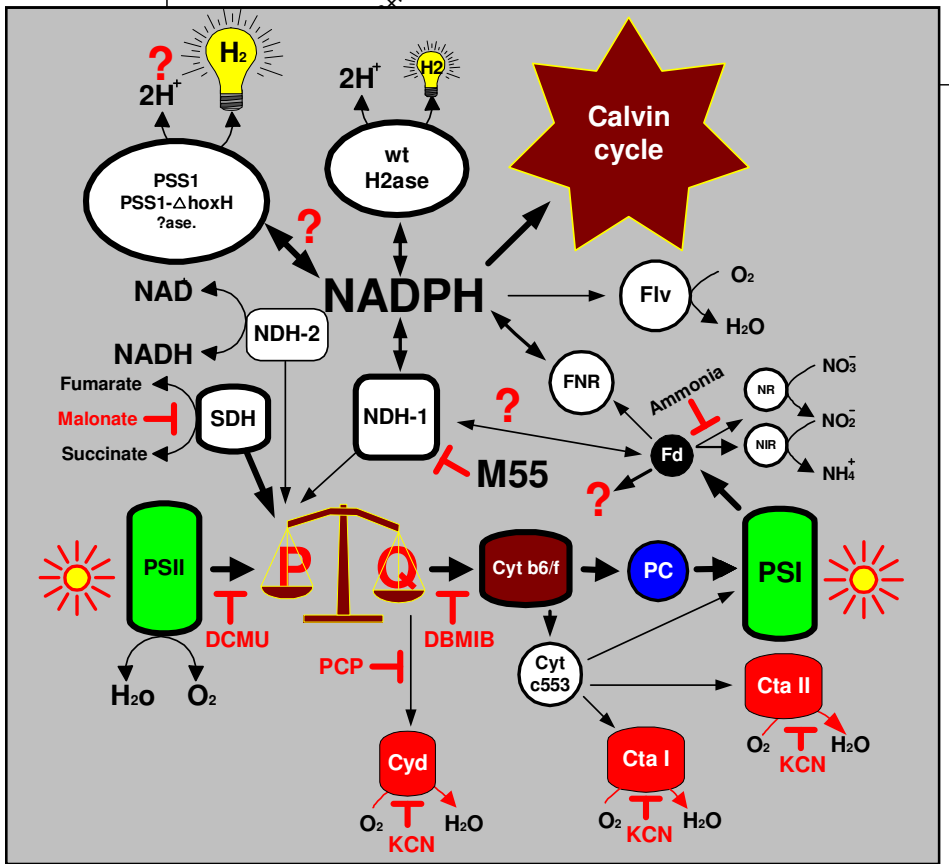

PSS1 Mutant
B
Concentration, µM
Time, min
Methyl viologen and dithionite added
H_2
O_2


C
wt
Mutant
Control
Hydrogen induction


PSS1 mM H2
WT mM H2
PSS1 pH
WT pH
mM H 2 Normalized
pH
time (hours)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
9.2
9
8.8
8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
0
15
30
45
60
75
90
105
120
135
150
165




M55
PSS1
PSS1-ΔhoxH
Absorbance at 450 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Cell
+butyrate
DBMIB
DCMU
PCP
M
KCN
KCN+M
KCN+M+DBMIB
KCN+M+DCMU
KCN+M+PCP
M+DBMIB
M+DCMU
Inhibitors


H2
2H+
H2
2H+
PSS1
PSS1-ΔhoxH
?ase.
wt
H2ase
Calvin
cycle
NAD
NADH
NDH-2
NADPH
Flv
O2
H2O
Fumarate
Malonate
Succinate
SDH
NDH-1
FNR
Ammonia
NR
NIR
NO3-
NO2-
NH4+
Fd
M55
PSII
P
Q
Cyt b6/f
PC
PSI
DCMU
DBMIB
PCP
H2O
O2
Cyt
c553
Cta II
Cyd
Cta I
O2
KCN
H2O

Poster # 24

Recap

- High-Throughput Screening Assay
- Optimization
- Metabolic Flux Analysis
- REHX Mutant
- MV-DT Assay and Performance of Mutants

Acknowledgments

DOE-BER:
John Houghton
Sharlene Weatherwax
David Thomassen

NSF-MEWG:
Fred Heineken

Post-doctoral Associate:
Hatem Mohamed

PhD Students:
Paul Schrader
Liz Burrows

This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-05ER64130.

THANK YOU!