

Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacterial System

Philip D. Weyman, Isaac T. Yonemoto, and Hamilton O. Smith,
J. Craig Venter Institute

Key Collaborator: Pin-Ching Maness, National Renewable
Energy Laboratory

Project ID
PD039

This presentation does not contain any proprietary, confidential information, or otherwise restricted information

Overview

Timeline

- Project start date: 5-01-2005
- Project end date: 1-30-2014
- Percent complete: 91%

Budget

- Total project funding
 - DOE share: \$2.019M for JCVI
 - DOE share: \$1.26M for NREL
 - JCVI cost-share: \$820K
- Funding received for FY12
 - \$200K for JCVI
- Planned funding for FY13
 - \$150K for JCVI

Barriers

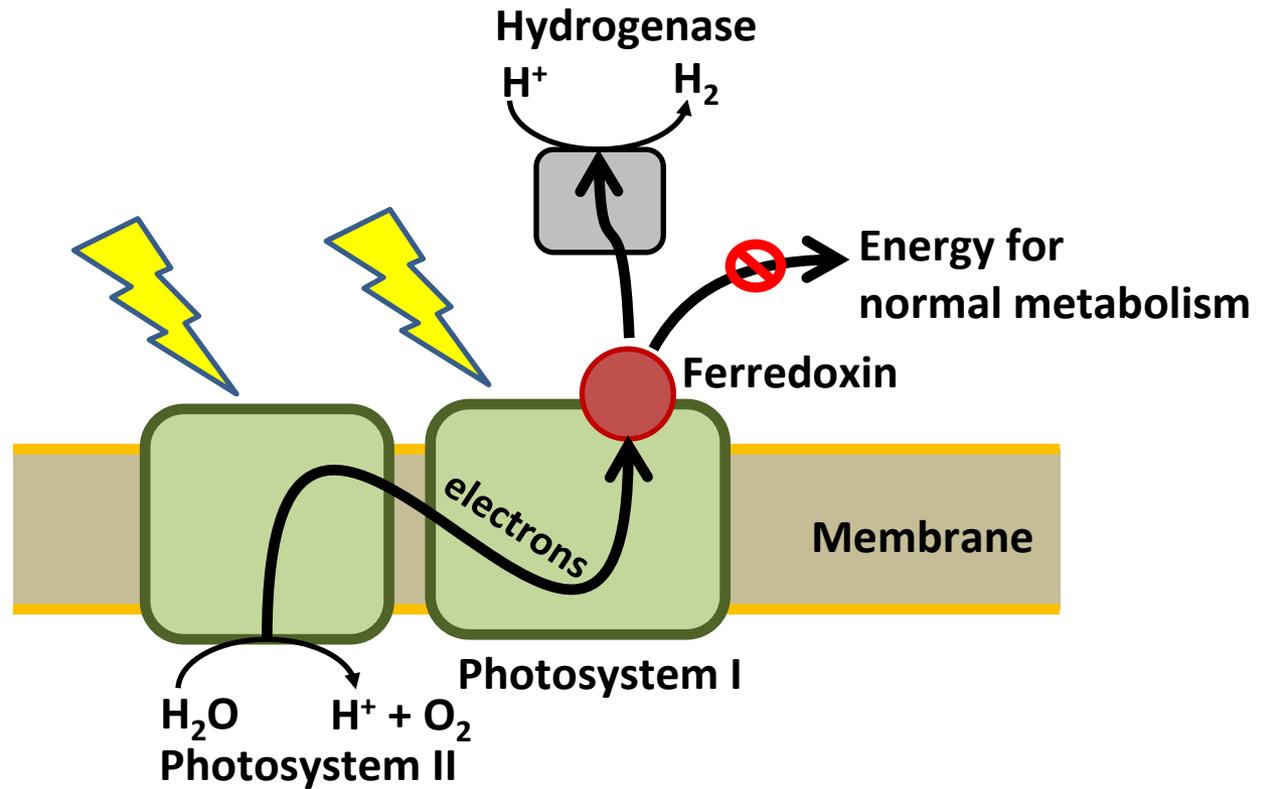
- Barriers addressed
 - Biological Hydrogen Production Barrier AP: Oxygen Accumulation

Partners

- National Renewable Energy Laboratory

Relevance:

Develop an O₂-tolerant cyanobacterial system for continuous light-driven H₂ production from water



Barrier AP: O ₂ Accumulation	2011 Status	2015 Target	2020 Target
Duration of continuous photoproduction in full sunlight	2 min	30 min	4 h

Approach: Milestones and Go/No Go

Task 1. Engineering known hydrogenases

Month/Year	Milestone	% Comp
Sept-11	Purify hydrogenase and verify functionality in O ₂	100%
Dec-10	Determine electron mediator requirement	100%
Sept-11	Verify hydrogenase activity in cyanobacteria <i>in vivo</i> and ability to make H ₂ from water	100%

Task 2. Discovery and engineering of new hydrogenases

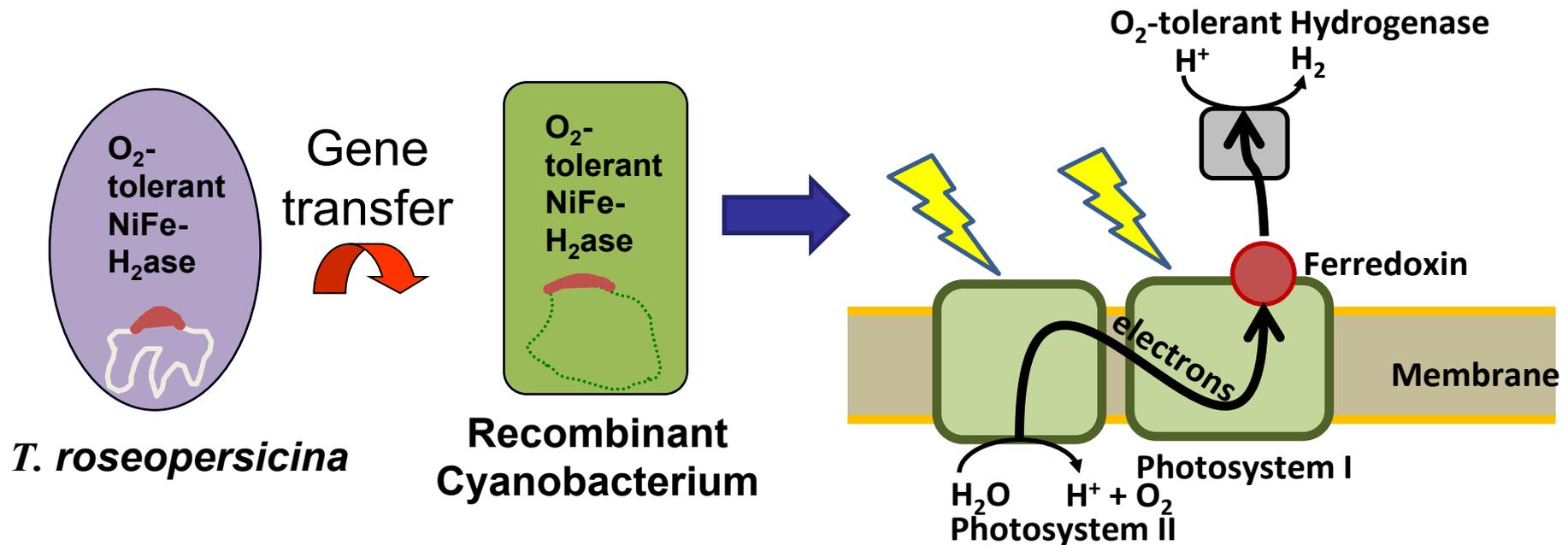
Month/Year	Milestone	% Comp
Sept-10	Identify novel hydrogenases from environment and transfer to cyanobacteria	100%
Aug-11	Construct a cyanobacterial hybrid to express an active environmental hydrogenase	100%
Apr-13	Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity.	30%
Nov-13	Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase.	20%
Jan-14	Measure light-dependent H ₂ production in modified strains.	5%

Go/No Go Decision: Due Jan-13, Achieved Nov-12

Demonstrate 5x increase hydrogenase activity from environmental H₂ase in cyanobacteria as measured by *in vitro* H₂ evolution assay.

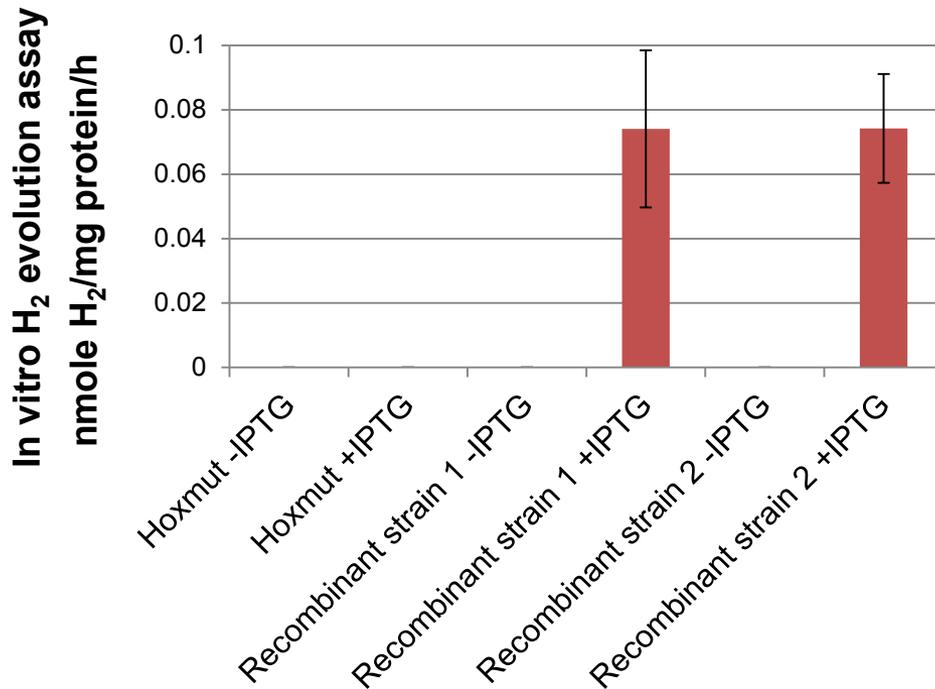
Task 1: Technical Approach

Transferring a known O₂-tolerant NiFe-hydrogenase from *T. roseopersicina* into cyanobacterium *Synechococcus* sp. PCC 7942



Task 1: Technical Approach

Review from previous AMR:



- **Completed milestone “Construct cyanobacterial hybrid to express active *Thiocapsa* hydrogenase” (8/11).**
- Effort in FY2011-13 focused on increasing expression of heterologous hydrogenase using environmentally-derived hydrogenase as model.

- JCVI approach is complementary to that of NREL in harnessing Nature’s O₂-tolerant hydrogenases and their transfer into two model cyanobacteria.

Task 2: Technical Approach

Task 2. Identifying novel O₂-tolerant hydrogenases through metagenomic analysis of marine microbes in the global ocean and transferring the hydrogenases into cyanobacteria



— 2003 – 2008 Routes — 2009 – 2010 Route

Sorcerer II Expedition: Global Ocean Sampling Project (Funded as cost share at no expense to DOE)

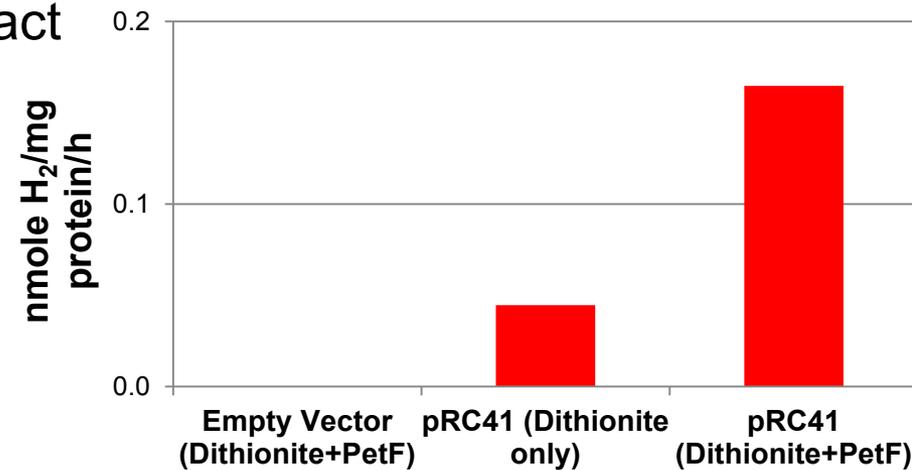
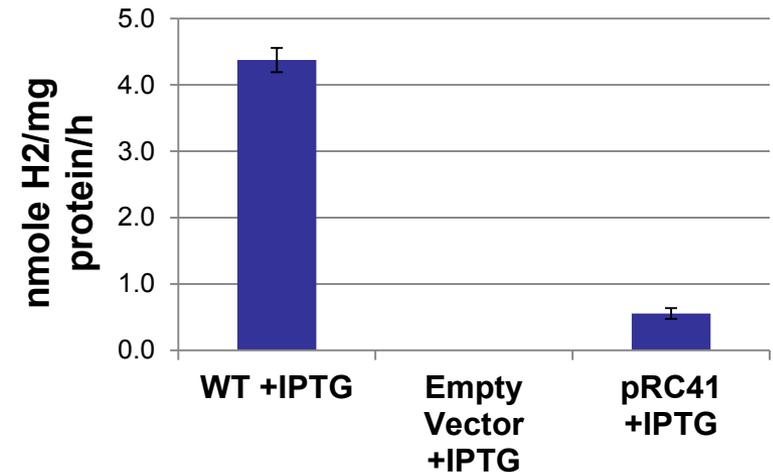
- This approach is complementary to two approaches in the Task 1 about harnessing nature's O₂-tolerant hydrogenases and their transfer into cyanobacteria.

Task 2: Technical Approach

- Previously, we reported transfer of hydrogenase from environmental DNA to *Synechococcus* PCC 7942 (Top figure, Milestone reached 4/10)
- Environmental hydrogenase is more thermostable and O₂-tolerant than *Thiocapsa* HynSL.

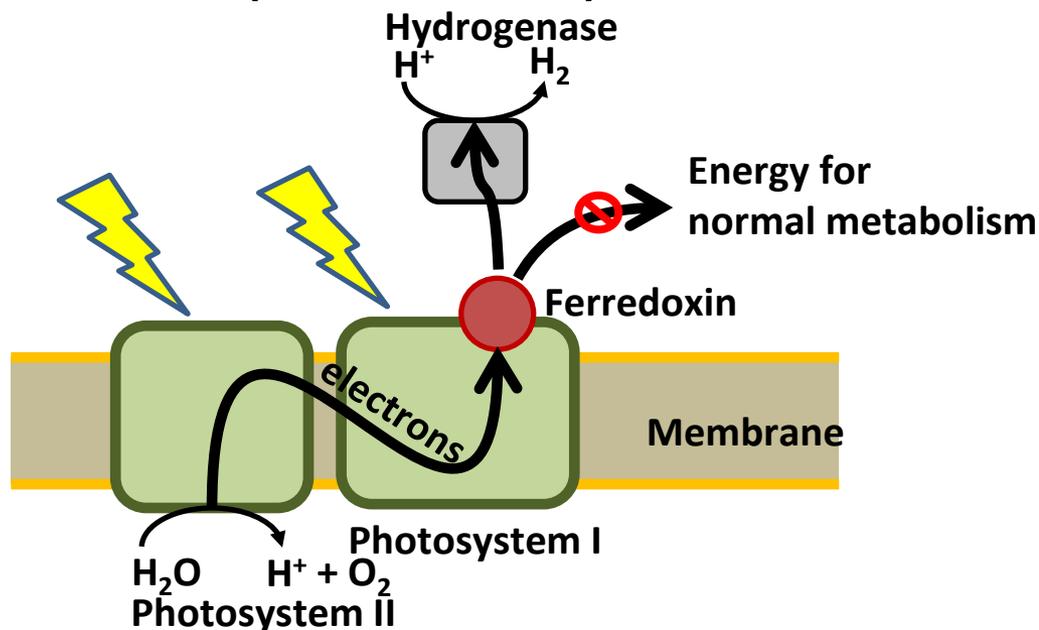
- Cyanobacterial ferredoxin (PetF*) can act as an electron mediator to the environmental hydrogenase in *E. coli* crude extract (Bottom figure, Milestone “Determine Electron Mediator Requirement” reached 12/10)

* PetF cloned and purified by NREL and provided to JCVI through our collaboration



2 Current Approaches to Improve System

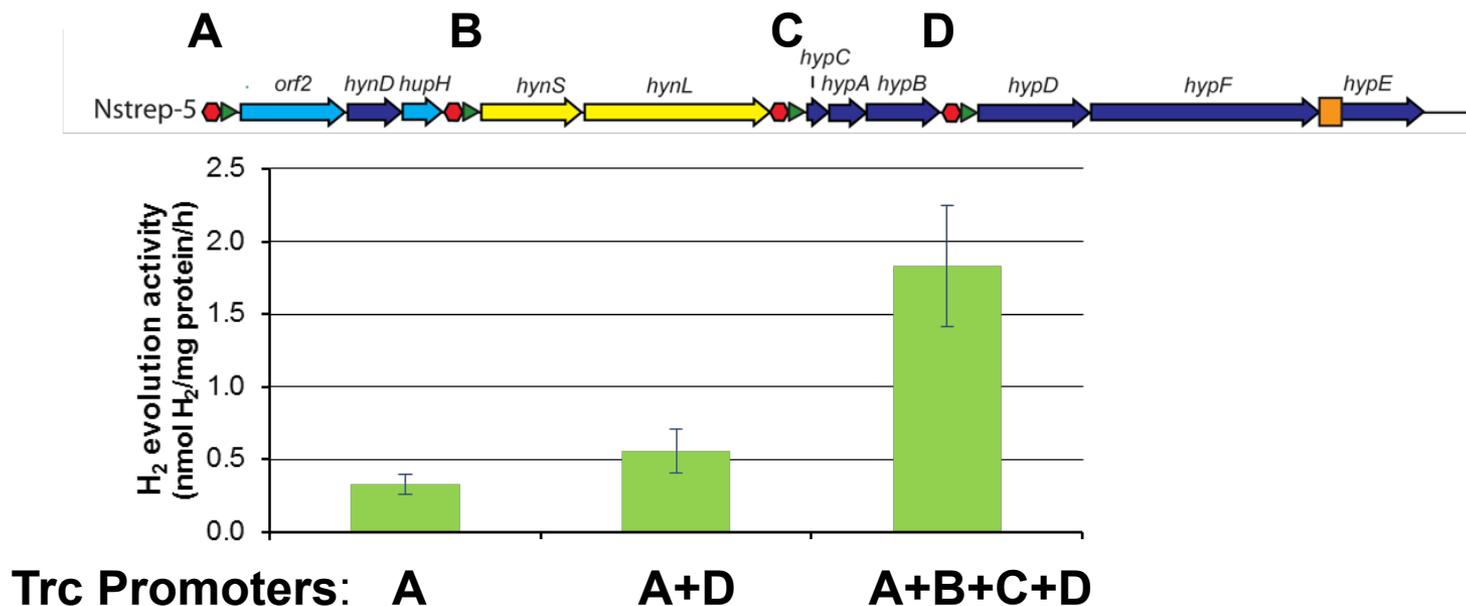
1. Improve expression and activity of hydrogenase in cyanobacteria (Task 2.4)
2. Improve hydrogenase-ferredoxin interaction (Task 2.5)



Task 2: Technical Approach

Review from last year's AMR:

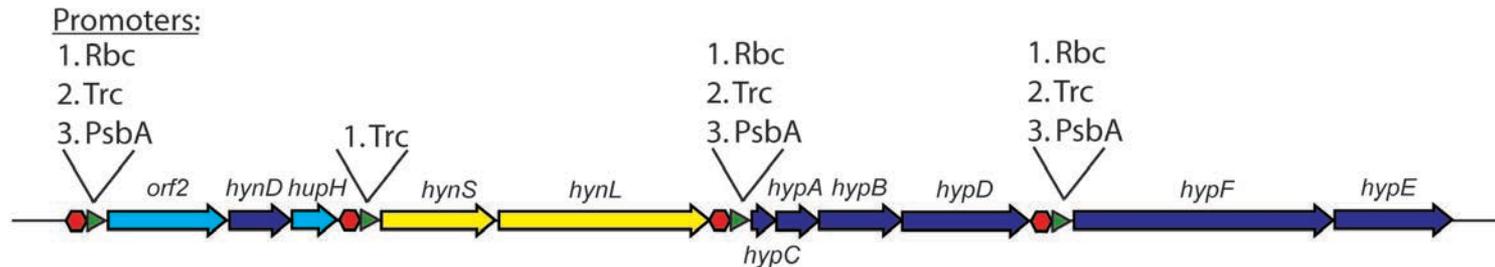
- More Trc promoters increases hydrogenase expression
- Ratios of maturation proteins may be important for maturation efficiency
 - Promoters at A+D led to more “mature” HynL but no increase in activity



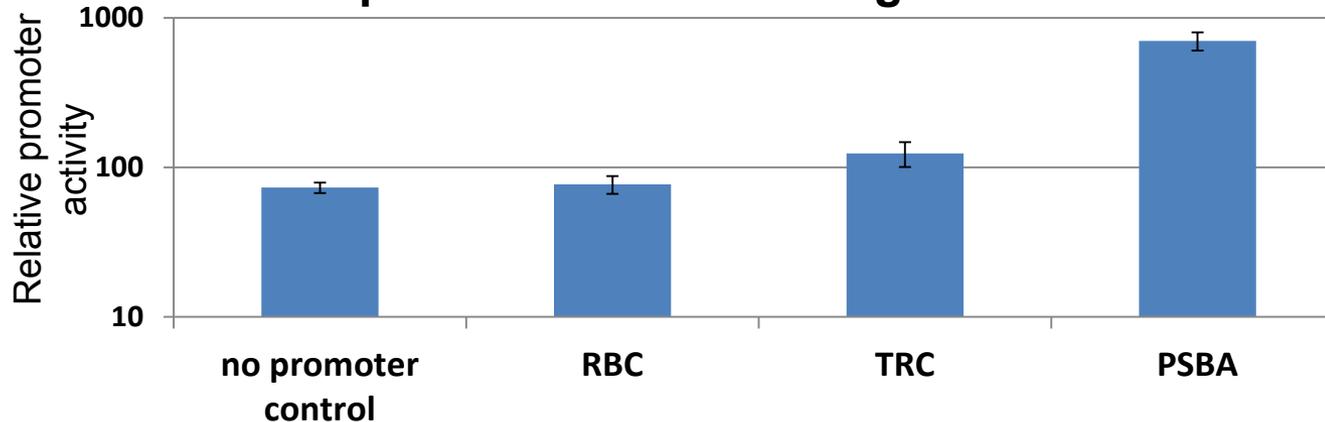
Task 2: Technical Accomplishments

Testing promoter placement, strength, and frequency

- Plan of promoter placement = 27 different variants



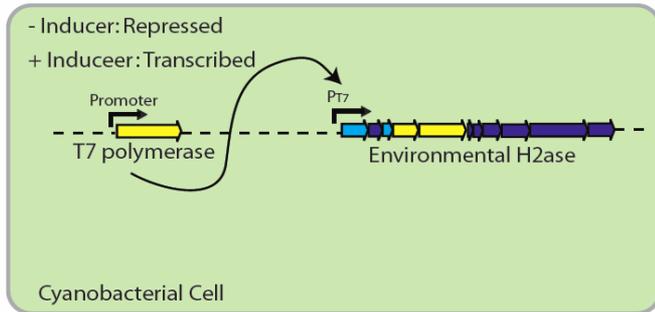
- Relative activities of promoters we are using



On track to complete Milestone “Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity.”

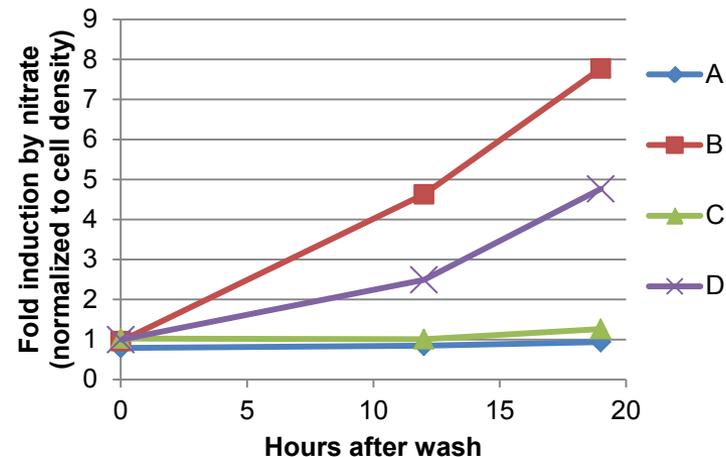
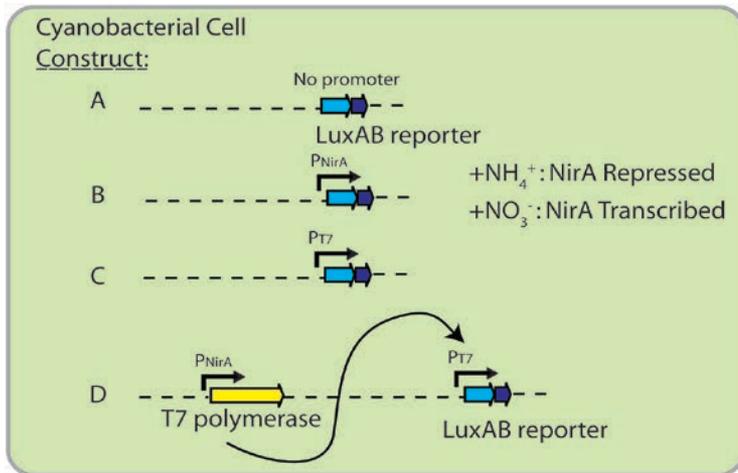
Task 2: Technical Accomplishments

Engineer T7 polymerase strategy for hydrogenase expression.



Potential Advantages of T7 polymerase:

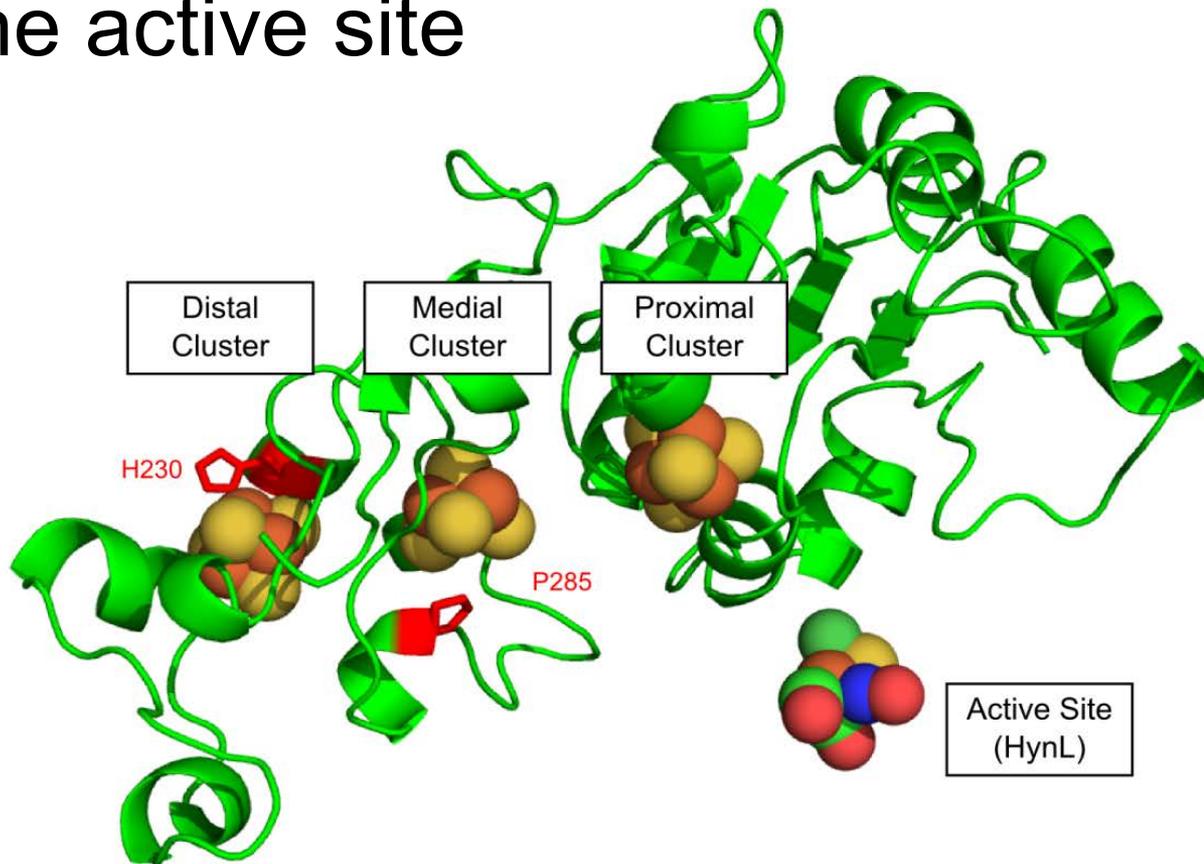
- Tighter control of transcription
- Lower frequency of early termination, may function better with long transcripts.



Progress toward Milestone “Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity.” (4/13).

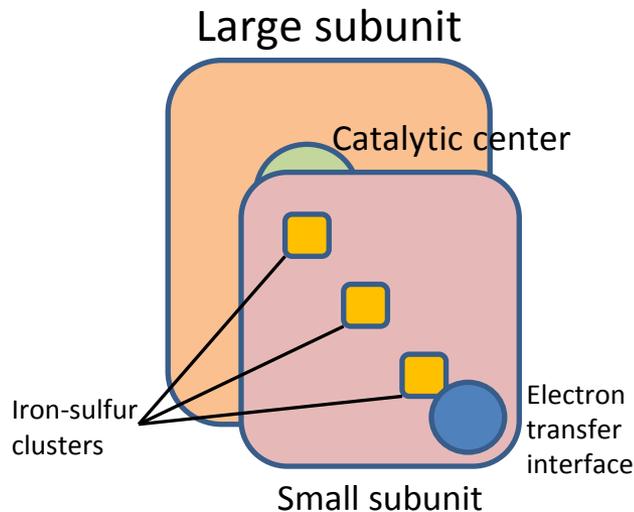
Task 2: Technical Approach

- FeS clusters form a molecular wire to/from the active site

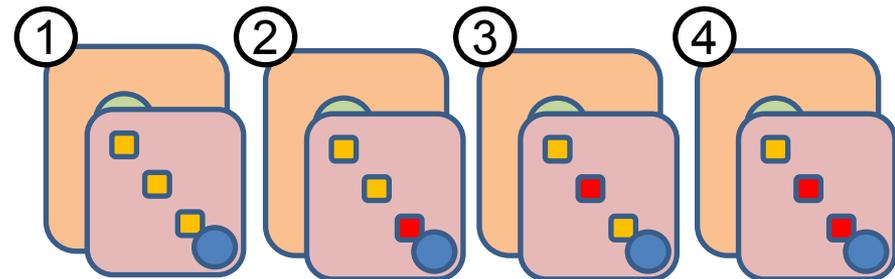


Task 2: Technical Approach

Point mutants alter electrochemistry of the hydrogenase small subunit “molecular wire”



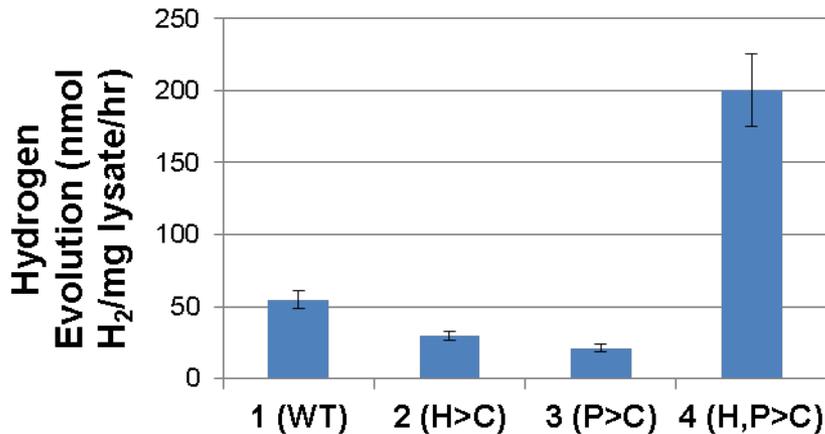
FeS cluster variants



Tests the potential to further modify the environmental hydrogenase to favor H₂ production *in vivo* using PetF as an electron mediator.

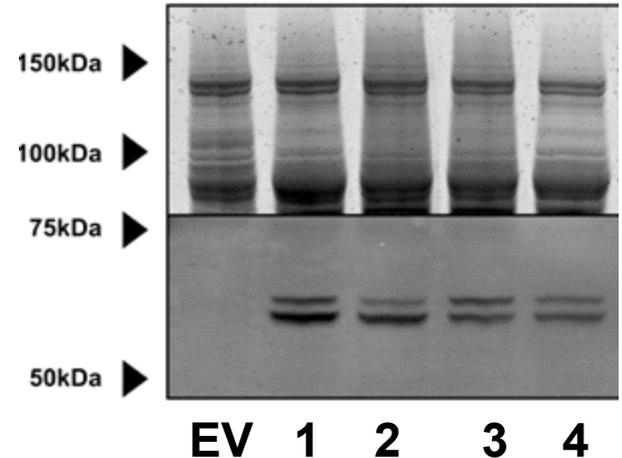
Task 2: Technical Approach

Review from last year's AMR: Double-substituted HynS yields increased evolution activity relative to uptake



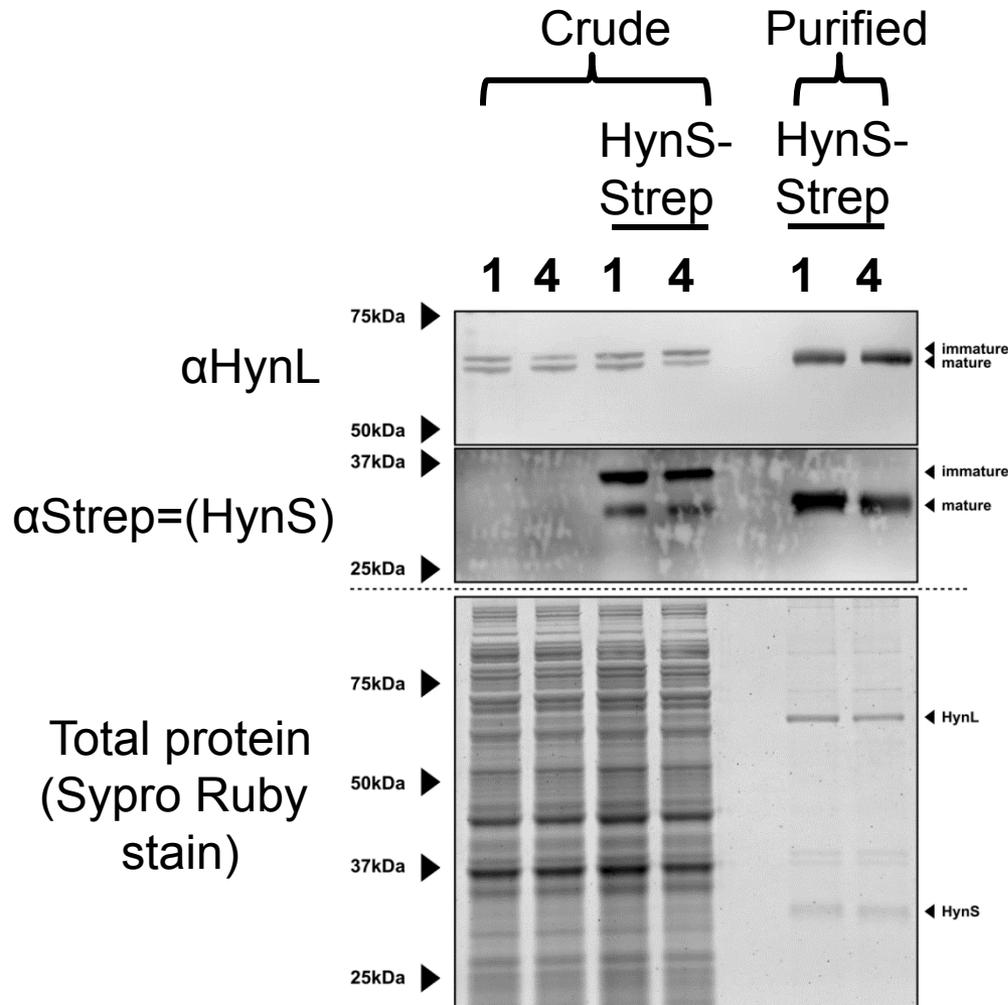
Total protein
(Sypro[®] Ruby
stain)

α HynL



Task 2: Technical Accomplishments

Increased H₂ evolution in construct 4 is also observed in purified protein

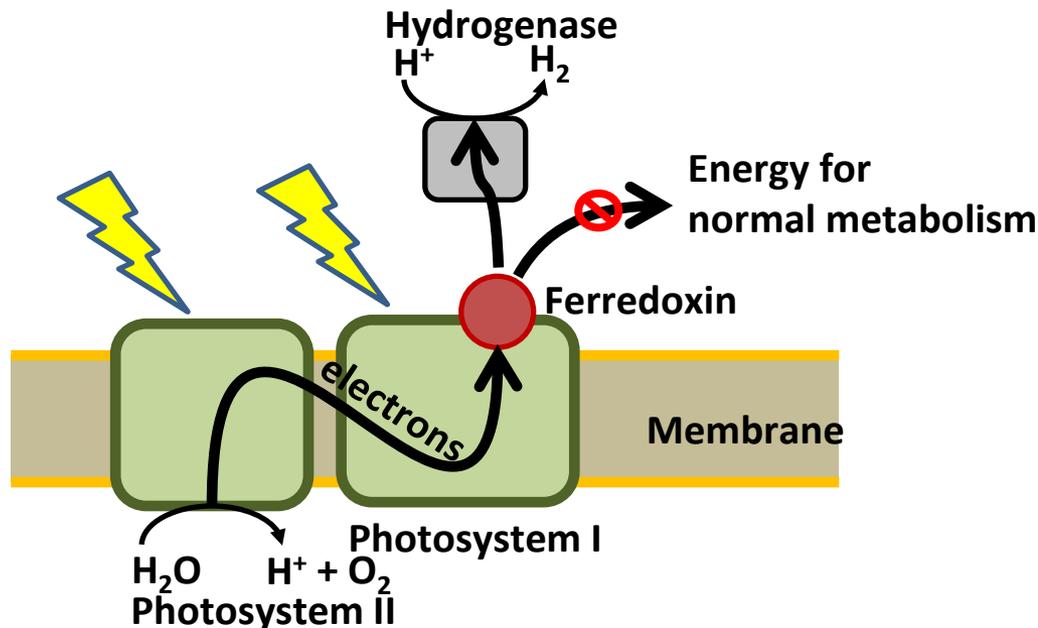


	Specific activity (nmole H ₂ produced/mg protein/h)	Fold purification
Construct 1 (crude)	369	
Construct 1 (purified)	41,450	112
Construct 4 (crude)	765	
Construct 4 (purified)	134,214	176

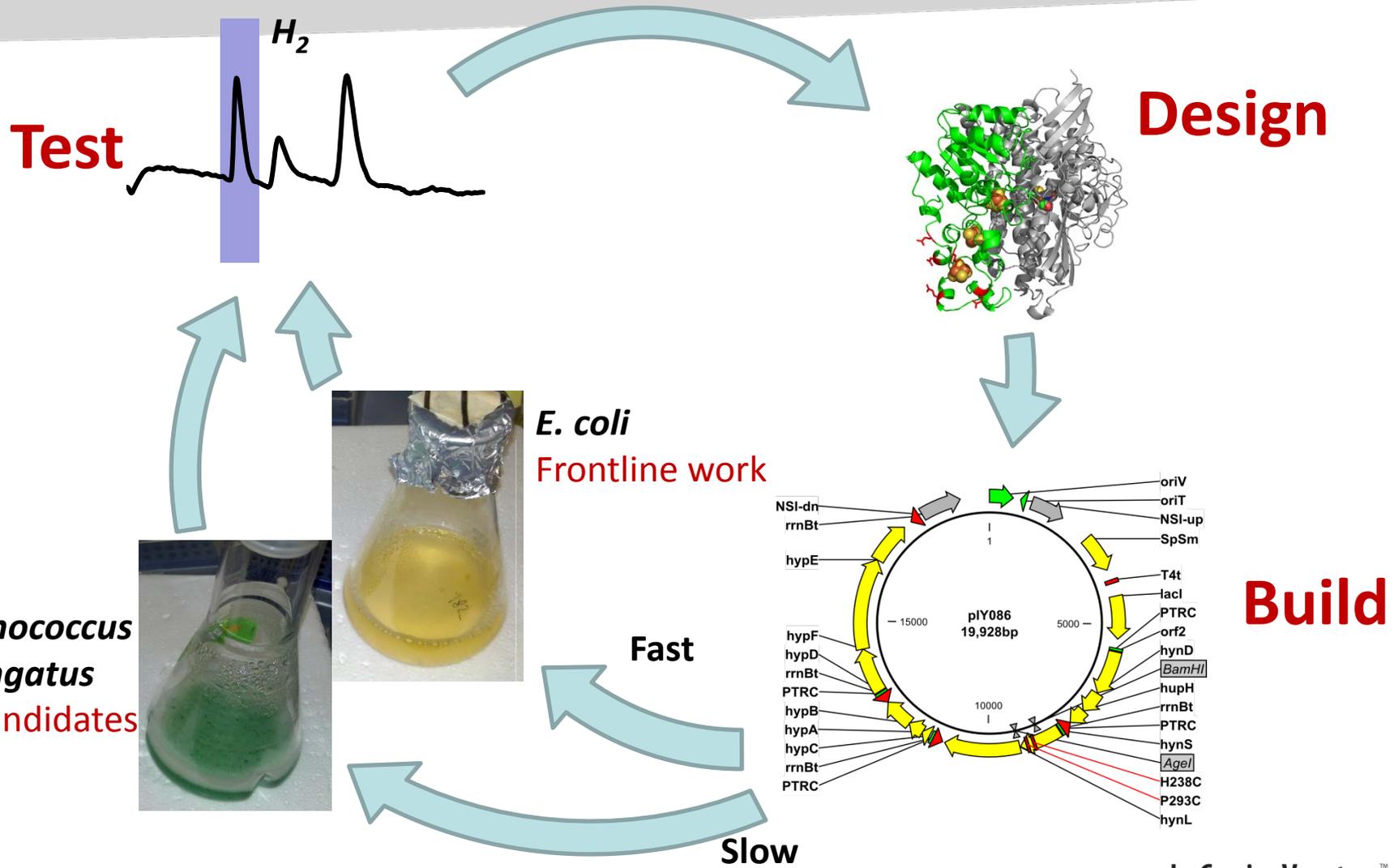
Completed Milestone 2.5.1 “Develop an affinity-tagged purification system for HynSL” (08/12).

2 Current Approaches to Improve System

1. Improve expression and activity of hydrogenase in cyanobacteria (Task 2.4)
2. Improve hydrogenase-ferredoxin interaction (Task 2.5)

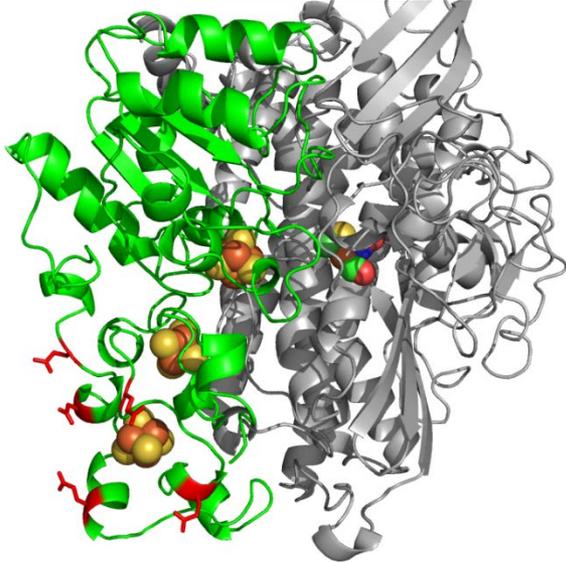


Engineering cycle for improving H2ase activity

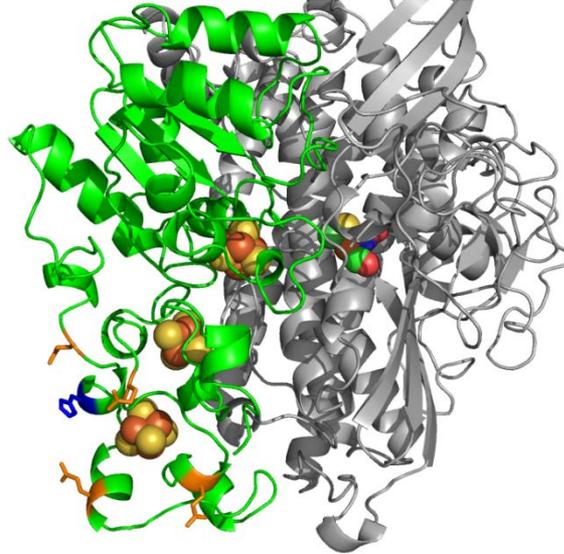


Task 2: Technical Accomplishments

Wild type



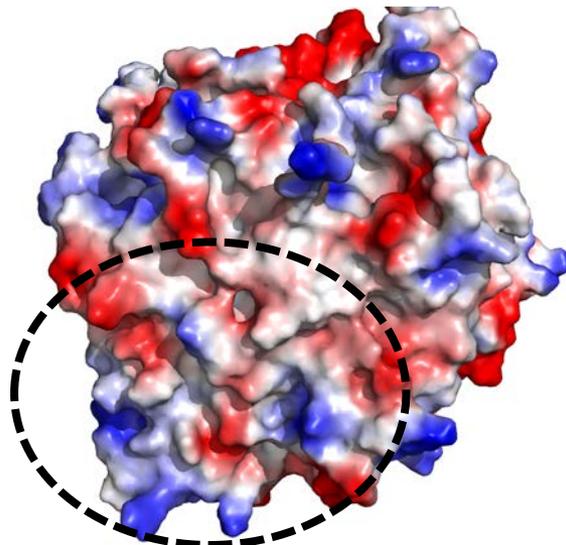
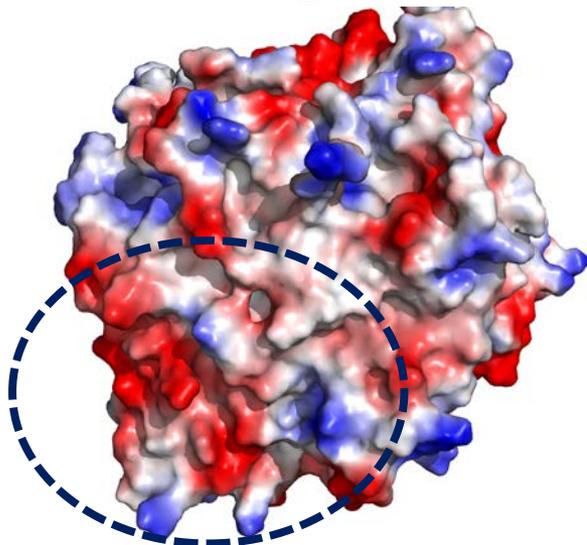
Mutant



HynS mutants with altered ferredoxin binding sites retain activity

Key:

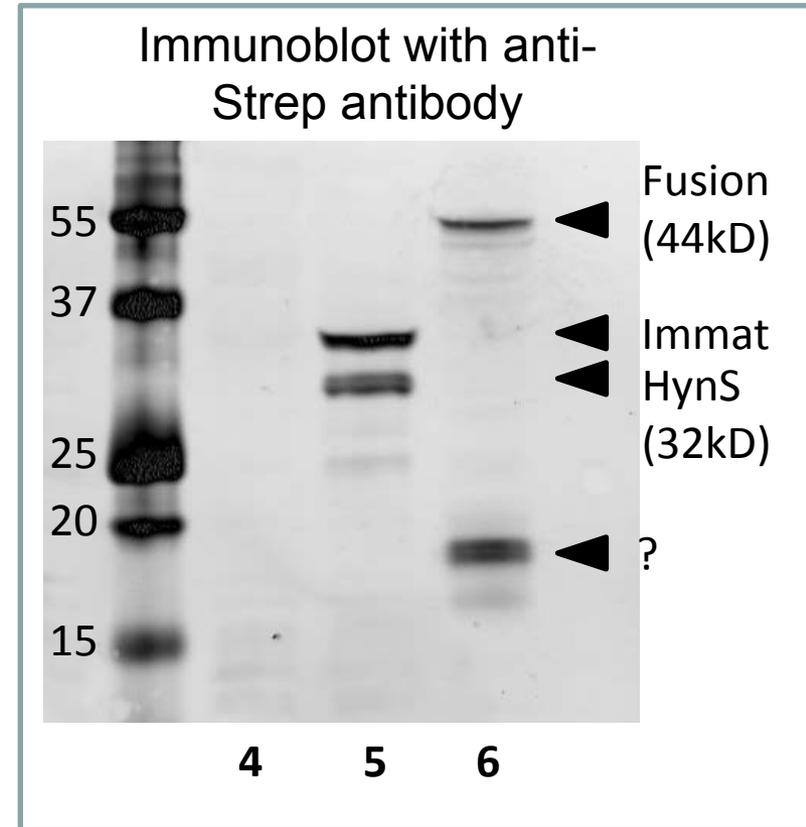
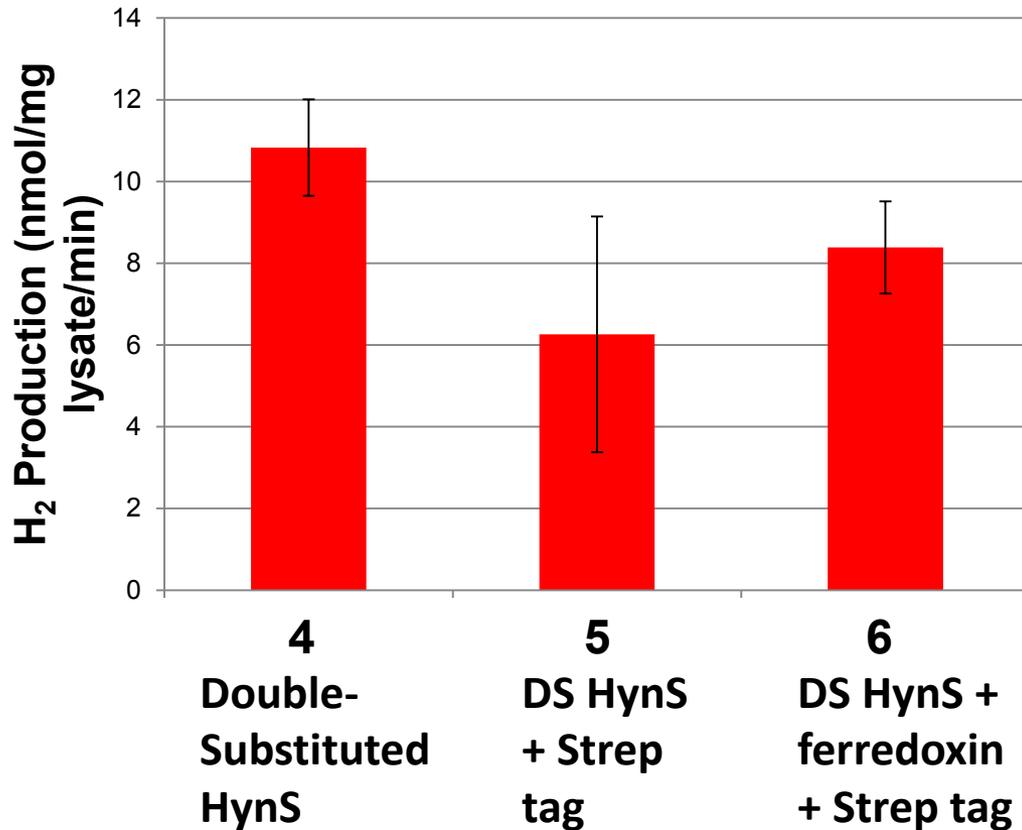
Red = negative charge
Blue = positive charge



On track to complete Milestone “Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase.” (11/13).

Task 2: Technical Accomplishments

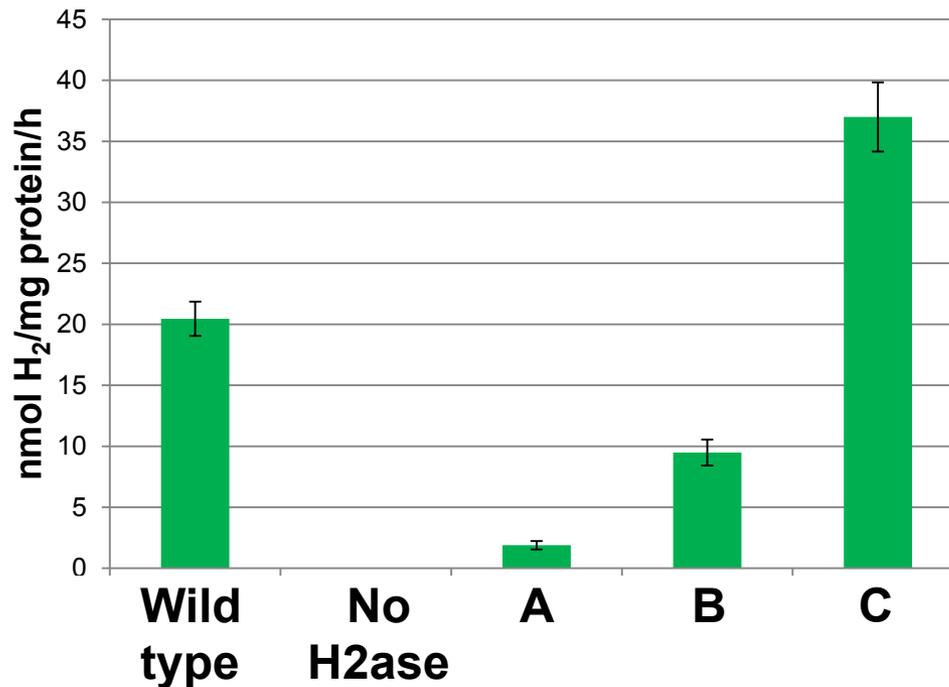
Ferredoxin-fusion H2ases are active when expressed in *E. coli*



On track to complete Milestone 2.5.3 “Construct an Fd-HynS fusion protein” (11/13).

Task 2: Technical Accomplishments

Activity improvements can be combined



Key:

A = Envi H2ase

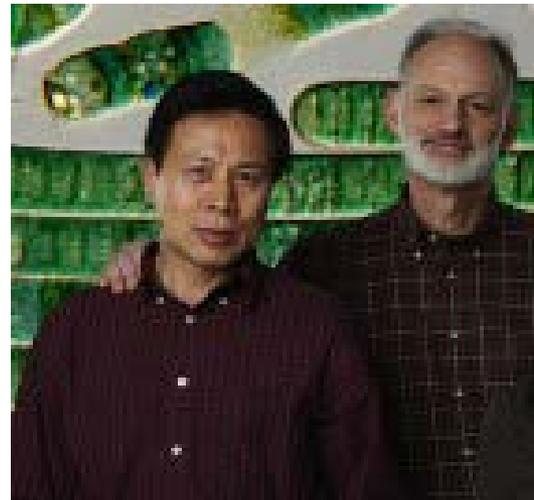
B = A + extra promoters

C = B + Double substitution HynS

Exceeded Go-No Go Criteria “Demonstrate 5x increase hydrogenase activity from environmental H2ase in cyanobacteria as measured by in vitro H₂ evolution assay.” (11/12)

Collaborations

- NREL – Dr. Pin-Ching Maness
 - Expressing environmentally-derived hydrogenase in her *Synechocystis* sp. PCC 6803 system
 - Purified cyanobacterial ferredoxin
- Vanderbilt University – Dr. Carl H. Johnson and Dr. Yao Xu
 - Using circadian rhythm modification to enhance expression of O₂-tolerant hydrogenases in cyanobacteria
 - Manuscript in preparation



Proposed Future Work

FY2013

- Continue optimization of promoter strength to achieve maximum expression of active hydrogenase.
- Continue to modify small subunit to increase ferredoxin binding.

FY2014

- Combine all positive modifications into a single cyanobacterial strain and test for hydrogen production from light and water.

Summary

- Developed strategies for increasing expression and activity of the environmentally-derived hydrogenase in cyanobacteria
 - Changed the frequency and strength of promoters.
 - Tested a novel T7 polymerase strategy for expression of hydrogenase.
 - Altered the FeS cluster ligation to increase H₂ evolution activity
- Developed strategies for increasing hydrogenase-ferredoxin interaction
 - Constructed a ferredoxin-hydrogenase fusion protein that maintains activity.