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

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Project ID
PD039

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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

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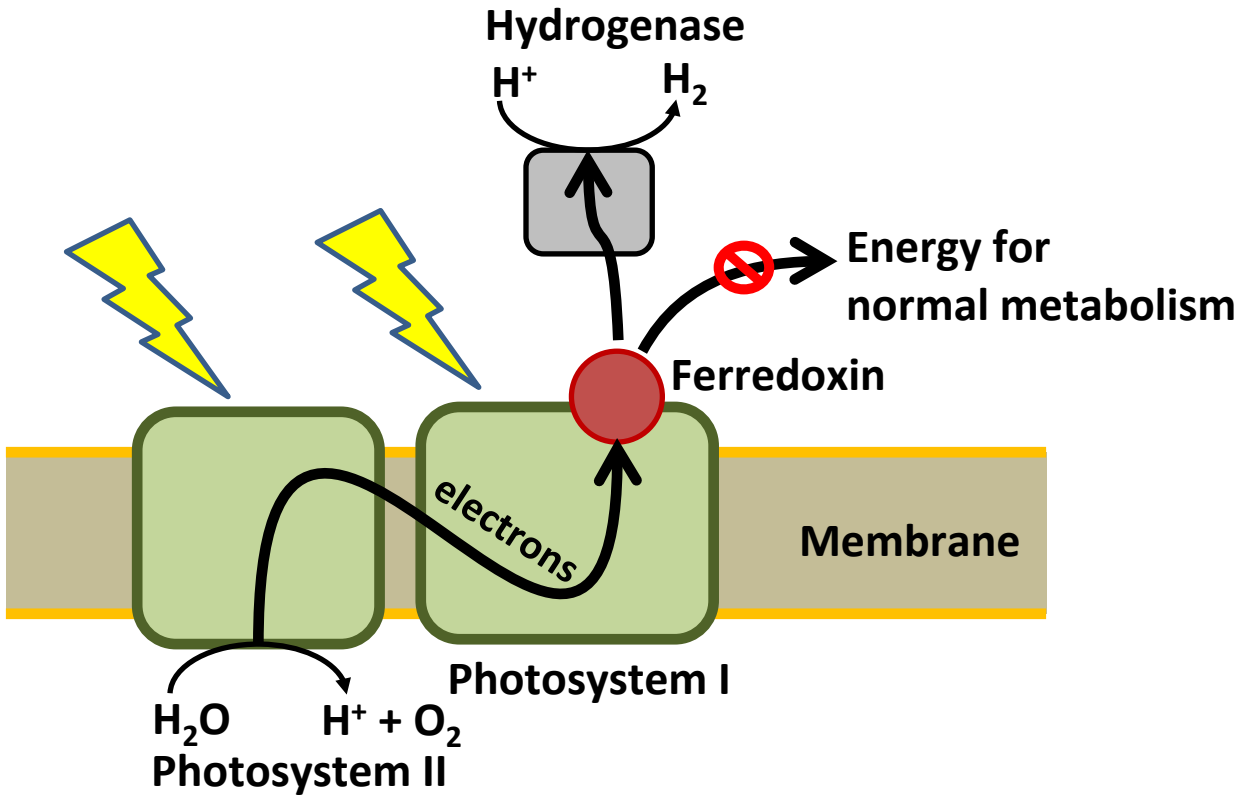
Relevance:

Develop an O_2-tolerant cyanobacterial system for continuous light-driven H_2 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

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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 *in vivo* 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 H_2 evolution assay.

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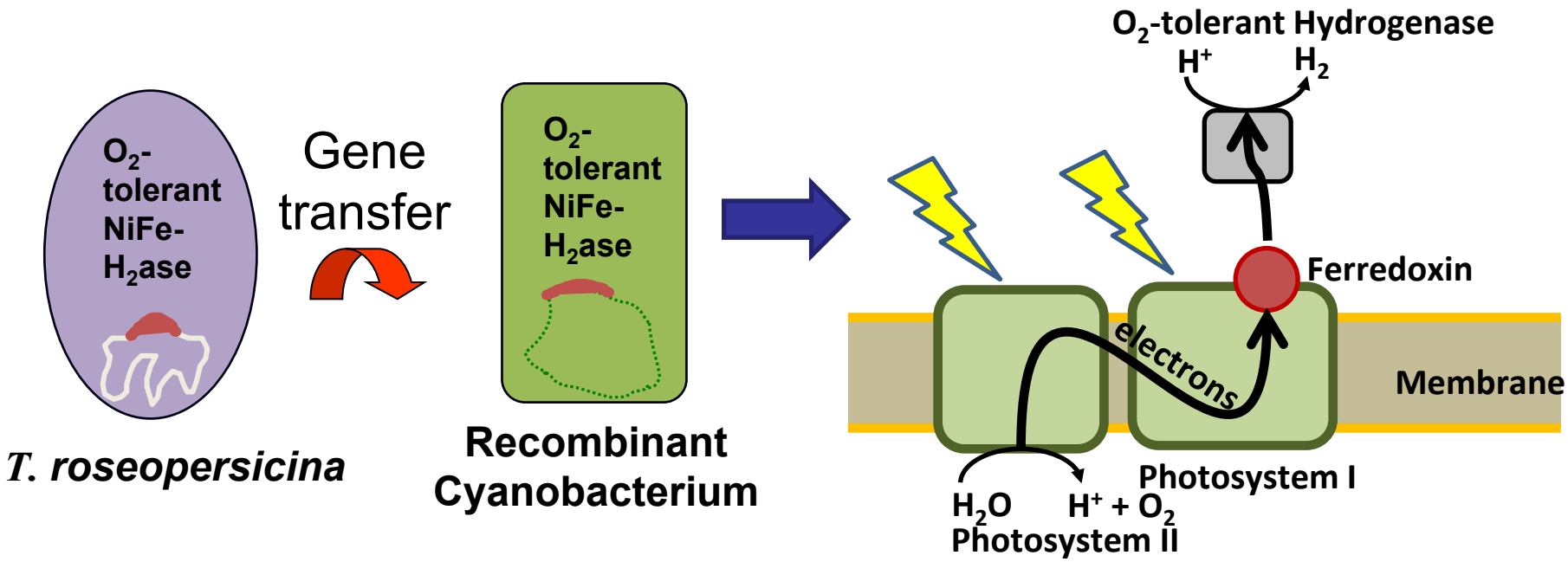
Task 1: Technical Approach

Transferring a known O_2-tolerant NiFe-hydrogenase from *T. roseopersicina* into cyanobacterium *Synechococcus sp.* PCC 7942



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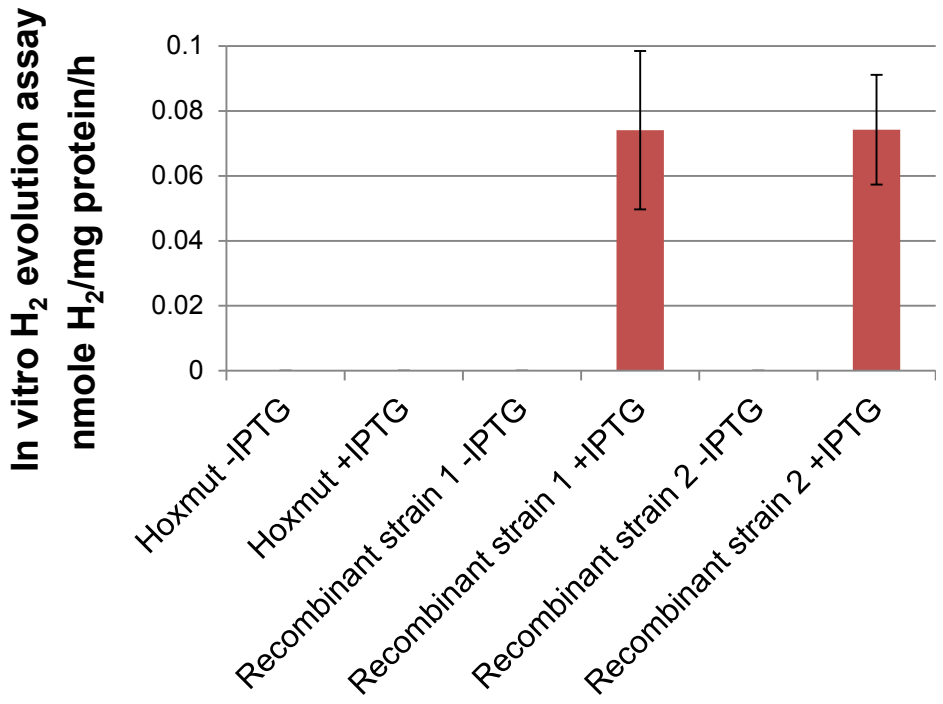
Task 1: Technical Approach

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 O_2-tolerant hydrogenases and their transfer into two model cyanobacteria.

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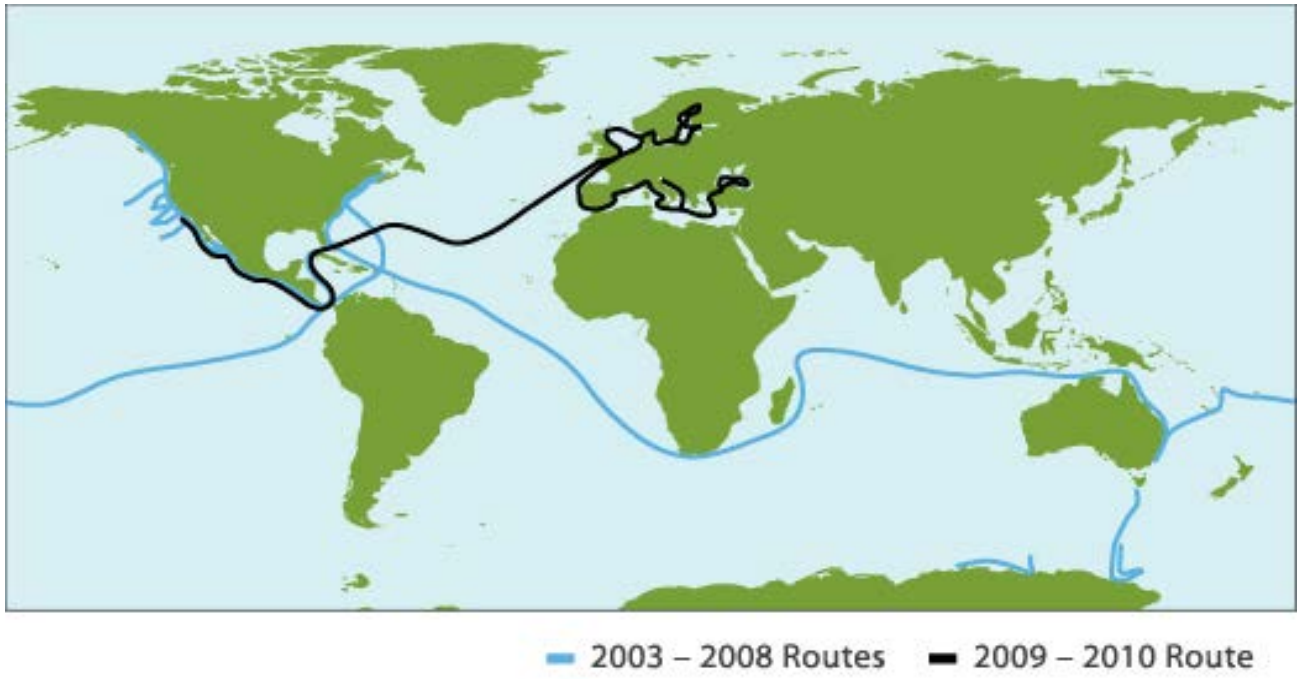
Task 2: Technical Approach

Task 2. Identifying novel O_2-tolerant hydrogenases through metagenomic analysis of marine microbes in the global ocean and transferring the hydrogenases into cyanobacteria



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 O_2-tolerant hydrogenases and their transfer into cyanobacteria.

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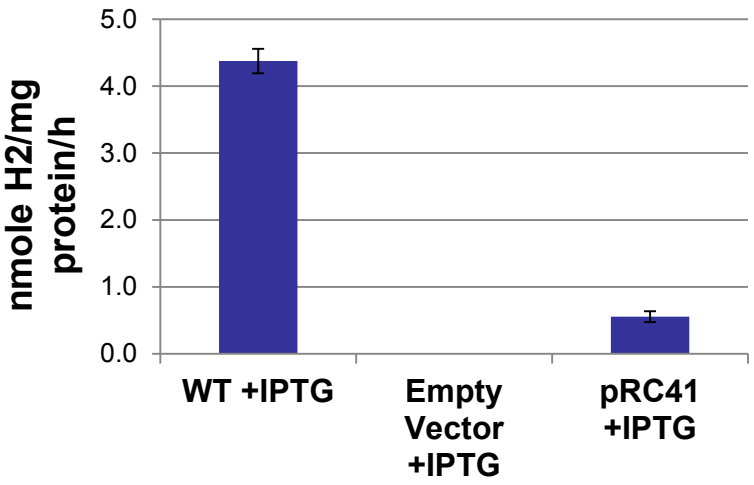
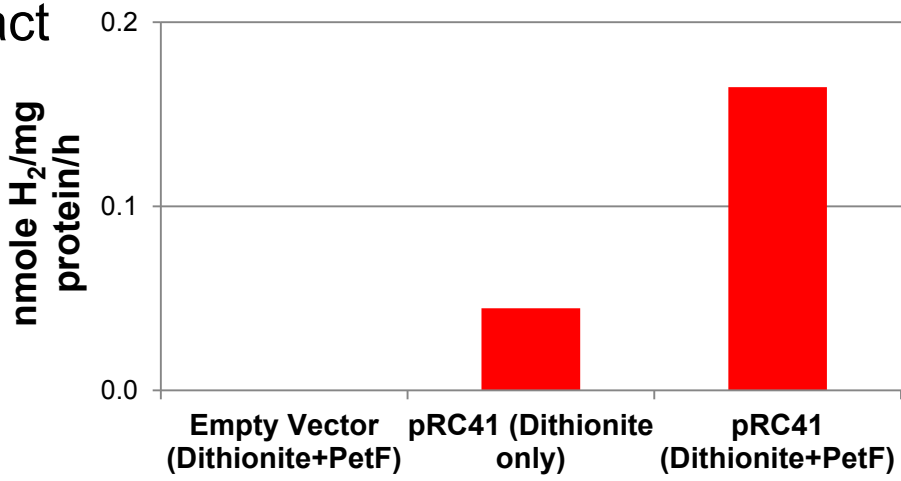
Task 2: Technical Approach

- 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 O_2-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

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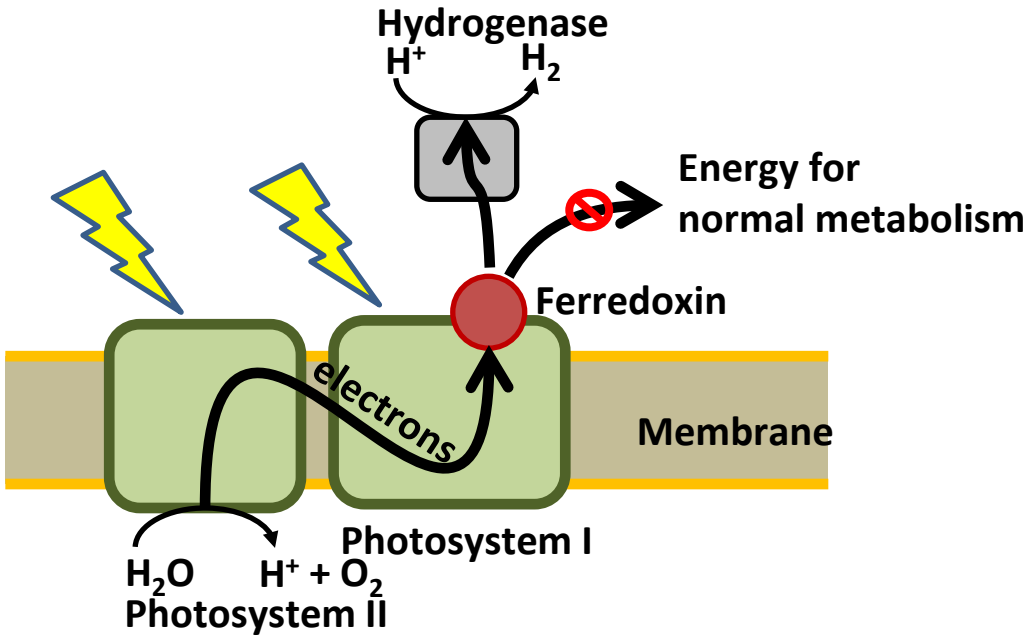
2 Current Approaches to Improve System

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



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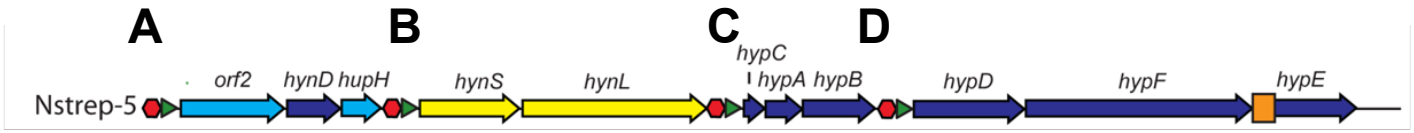
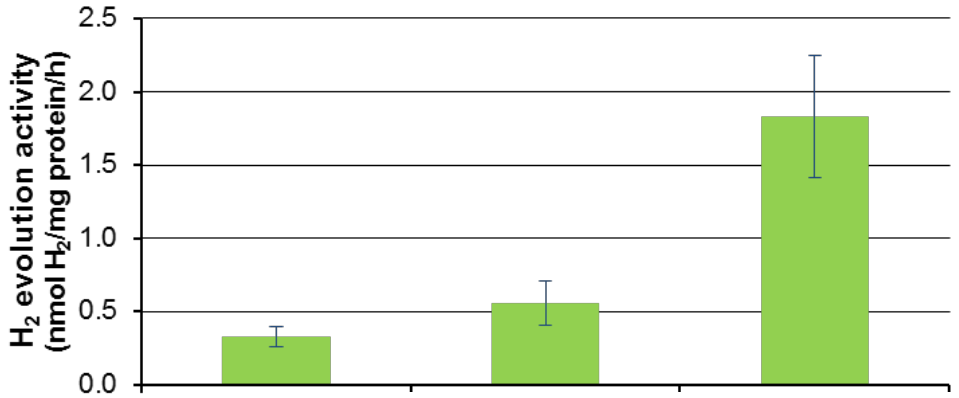
Task 2: Technical Approach

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





Trc Promoters: **A** **A+D** **A+B+C+D**

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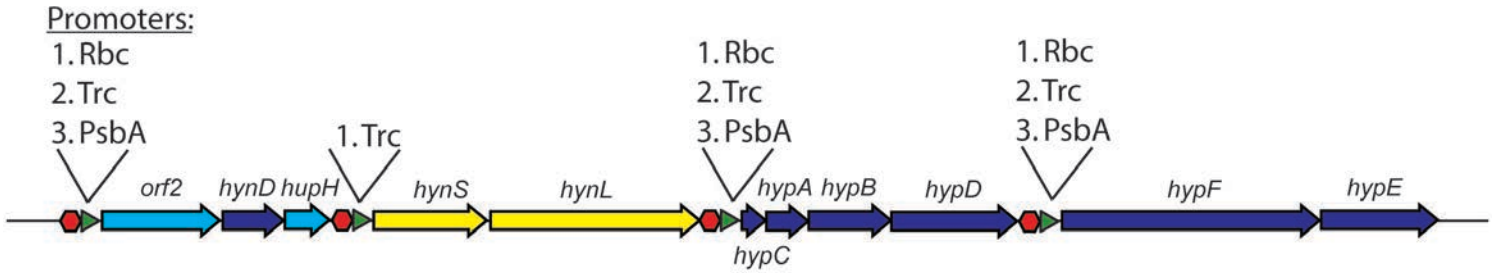
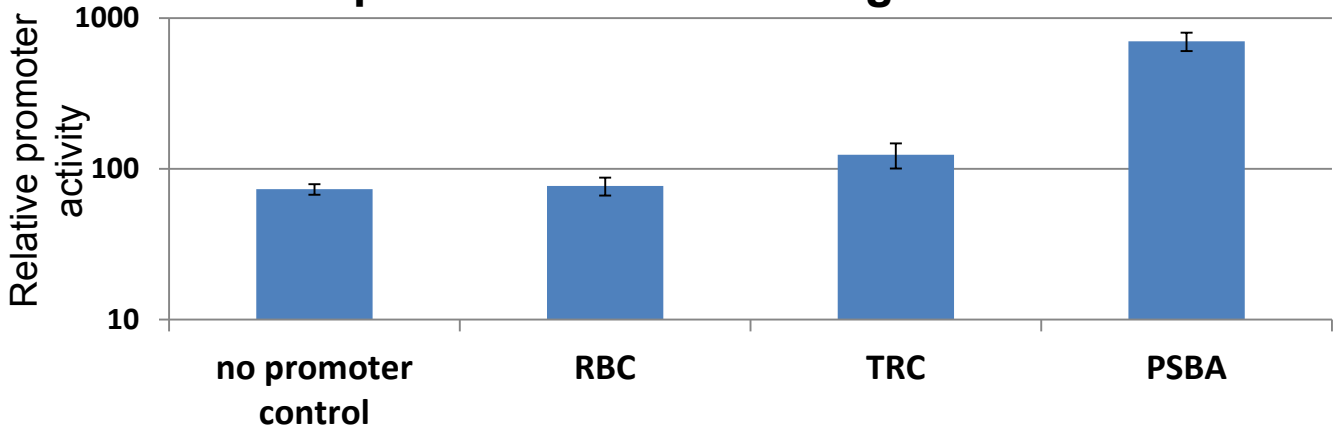
Task 2: Technical Accomplishments

Testing promoter placement, strength, and frequency

- **Plan of promoter placement = 27 different variants**



- **Relative activities of promoters we are using**



<u>On track</u> to complete Milestone "Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity."

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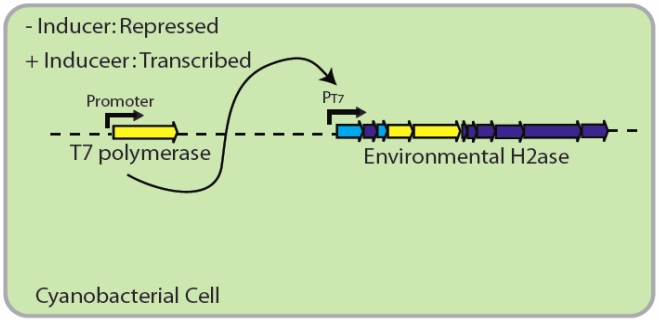
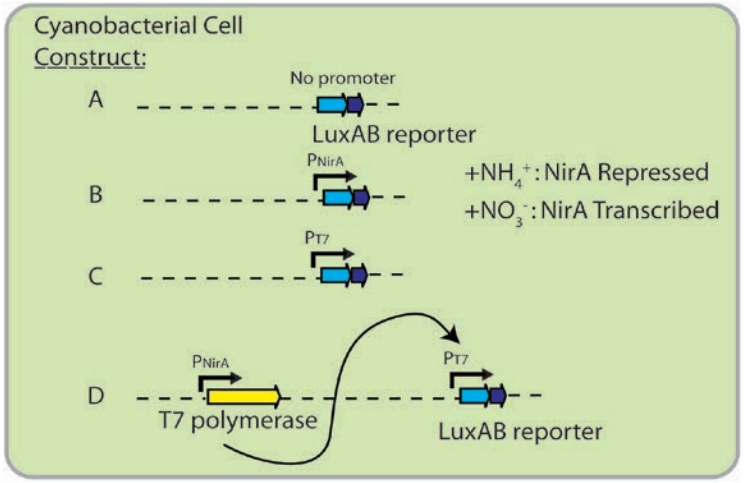
Task 2: Technical Accomplishments

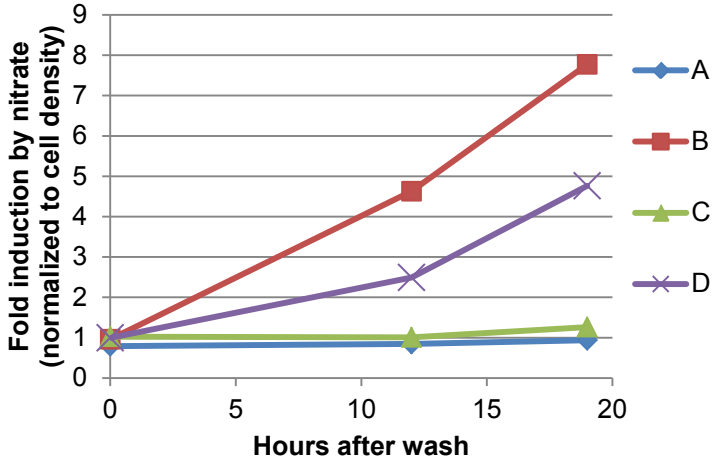
Engineer T7 polymerase strategy for hydrogenase expression.



<u>Potential Advantages of T7 polymerase:</u>

- 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).

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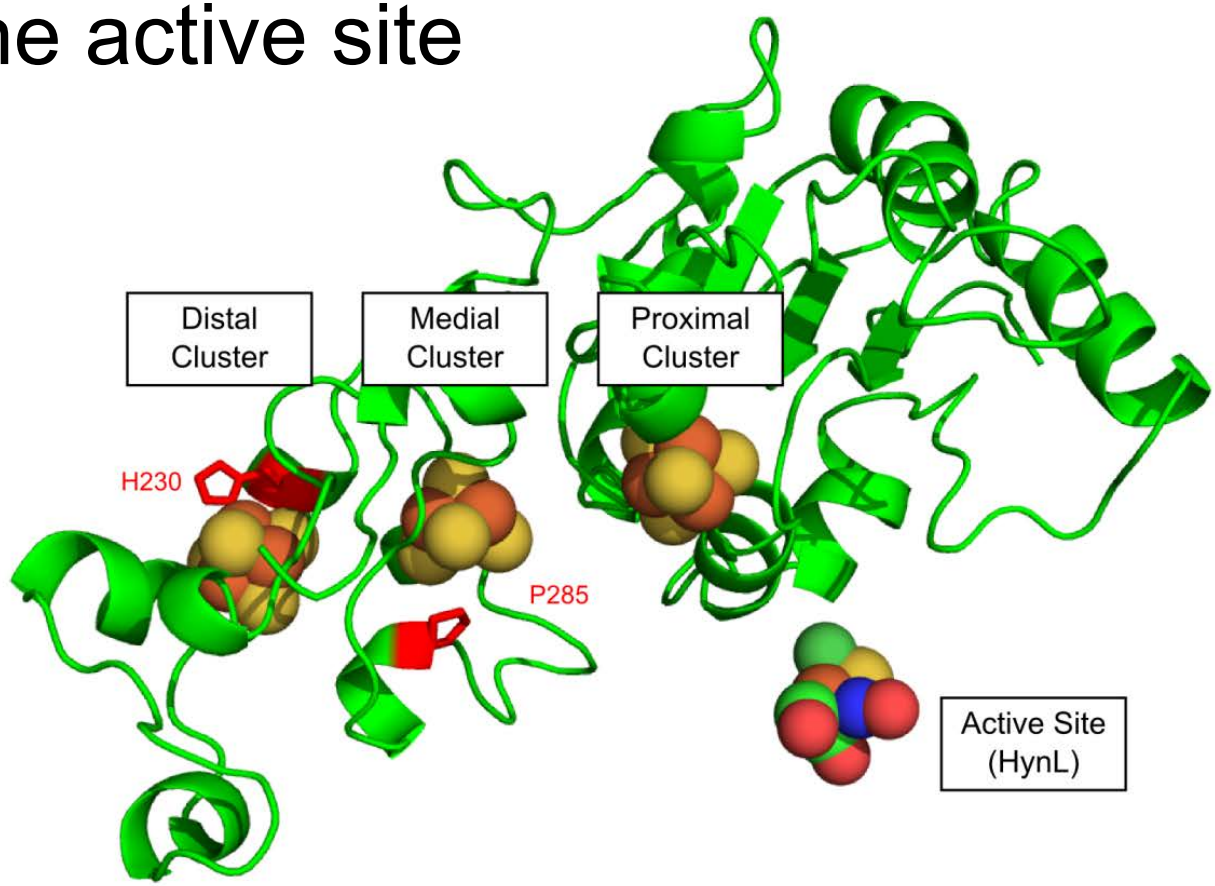
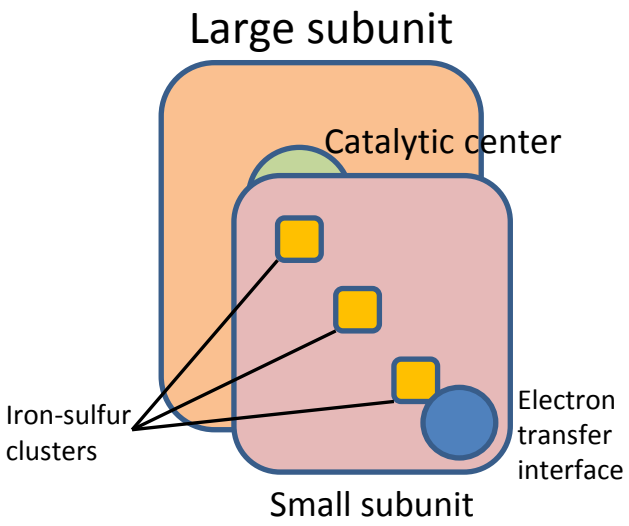
Task 2: Technical Approach

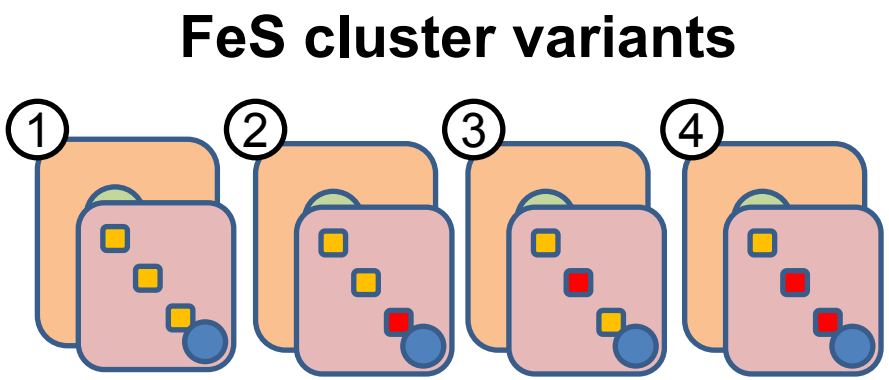
- FeS clusters form a molecular wire to/from the active site



Task 2: Technical Approach

Point mutants alter electrochemistry of the hydrogenase small subunit "molecular wire"





Tests the potential to further modify the environmental hydrogenase to favor H_2 production *in vivo* using PetF as an electron mediator.

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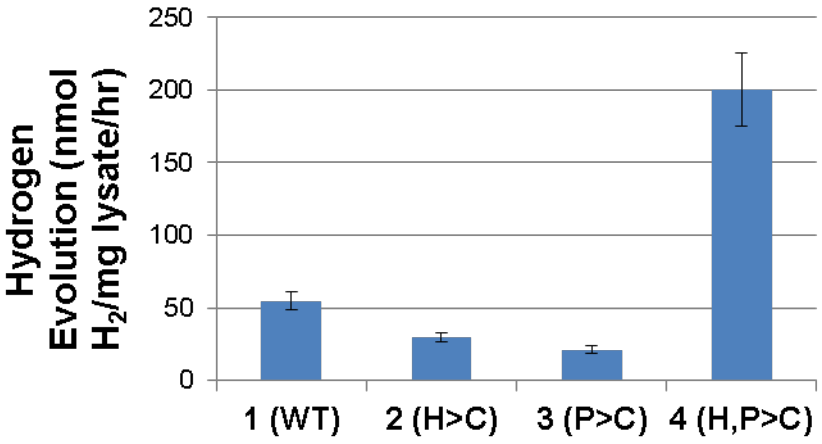
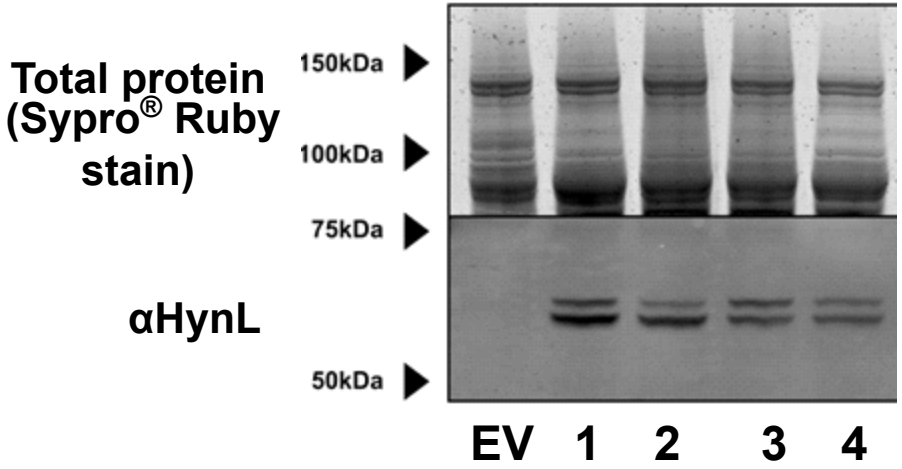
Task 2: Technical Approach

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





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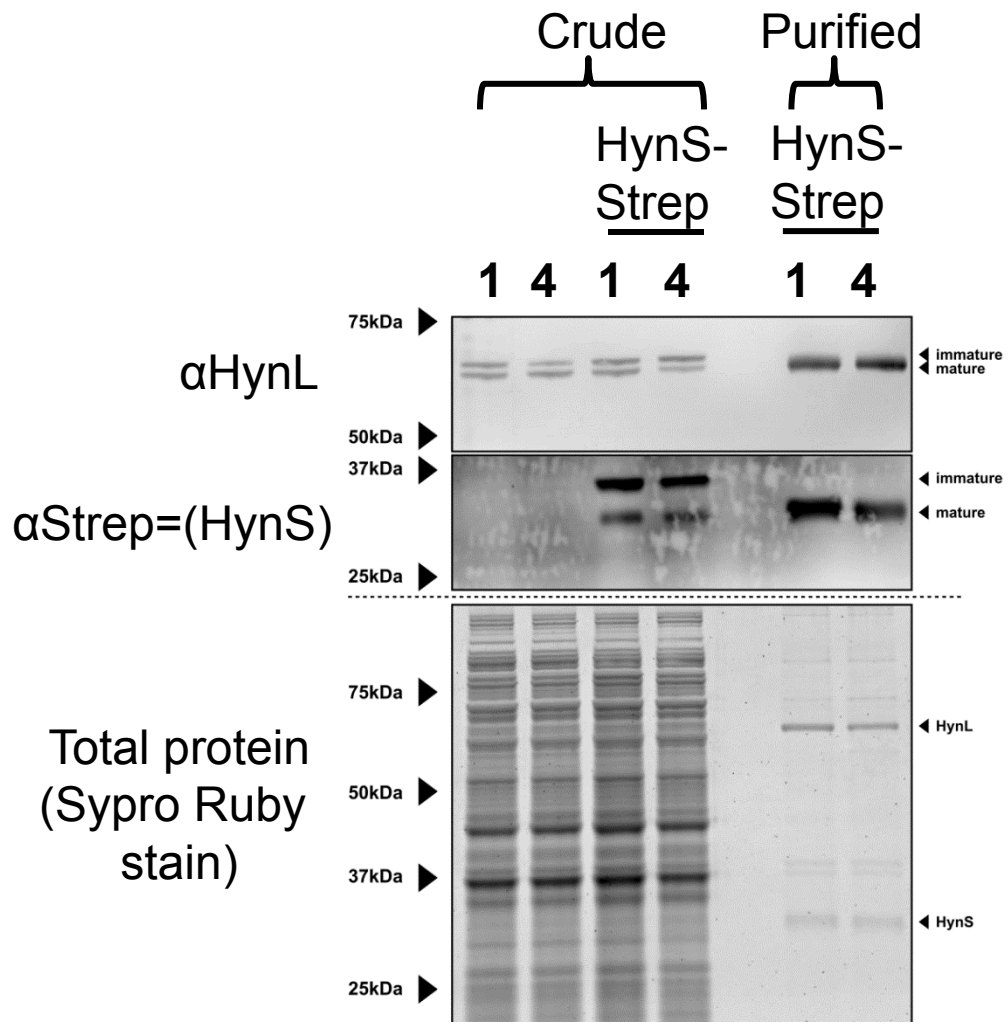
Task 2: Technical Accomplishments



Increased H_2 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).

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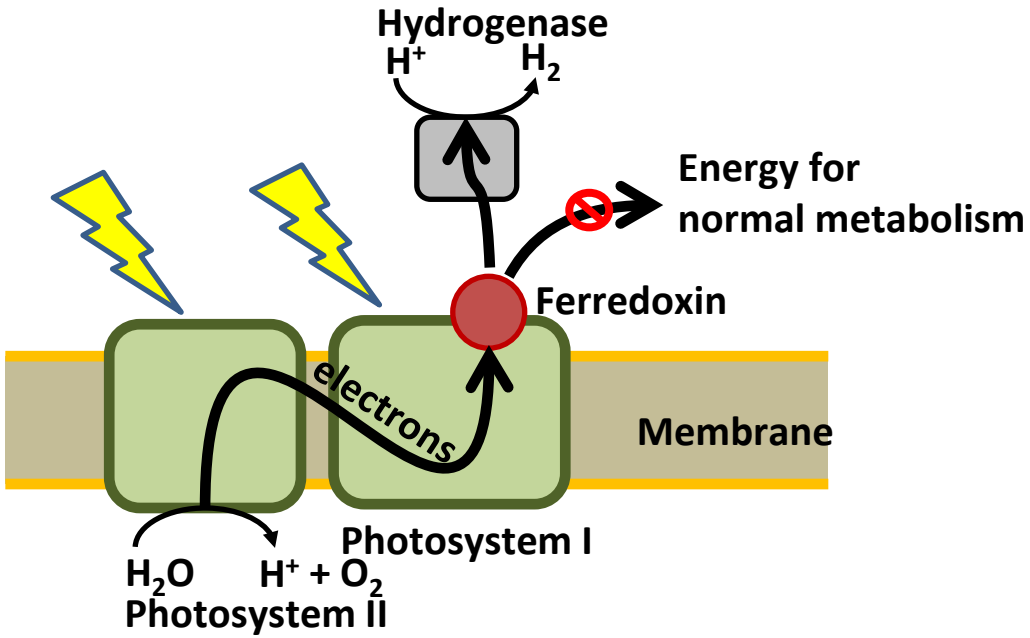
2 Current Approaches to Improve System

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



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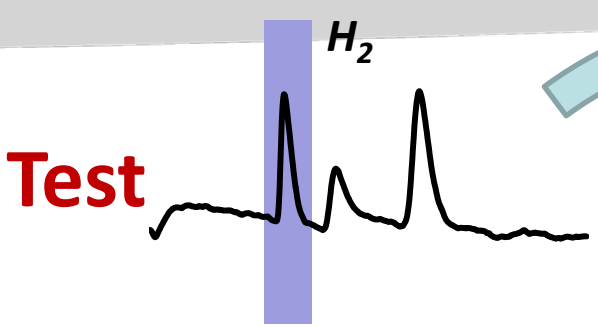
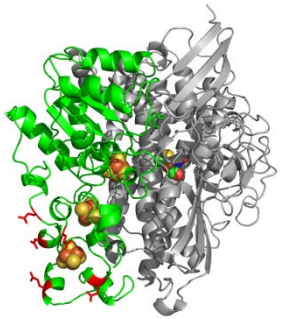
Engineering cycle for improving H2ase activity



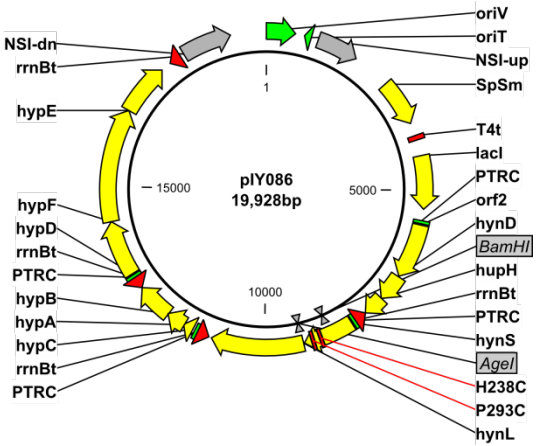
Test



Design

E. coli
Frontline work

Synechococcus elongatus
Best candidates



Build

Fast

Slow

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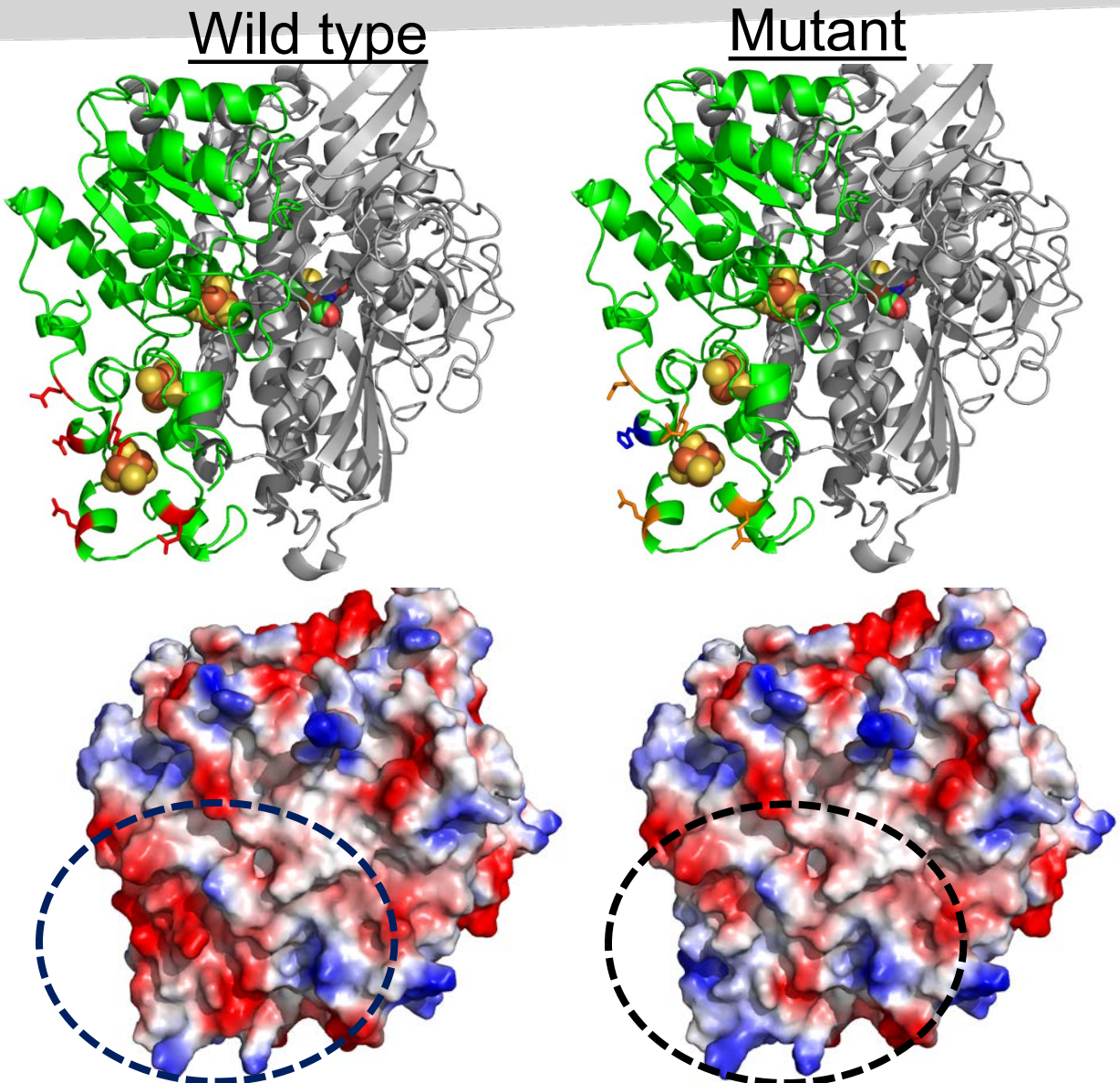
Task 2: Technical Accomplishments



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).

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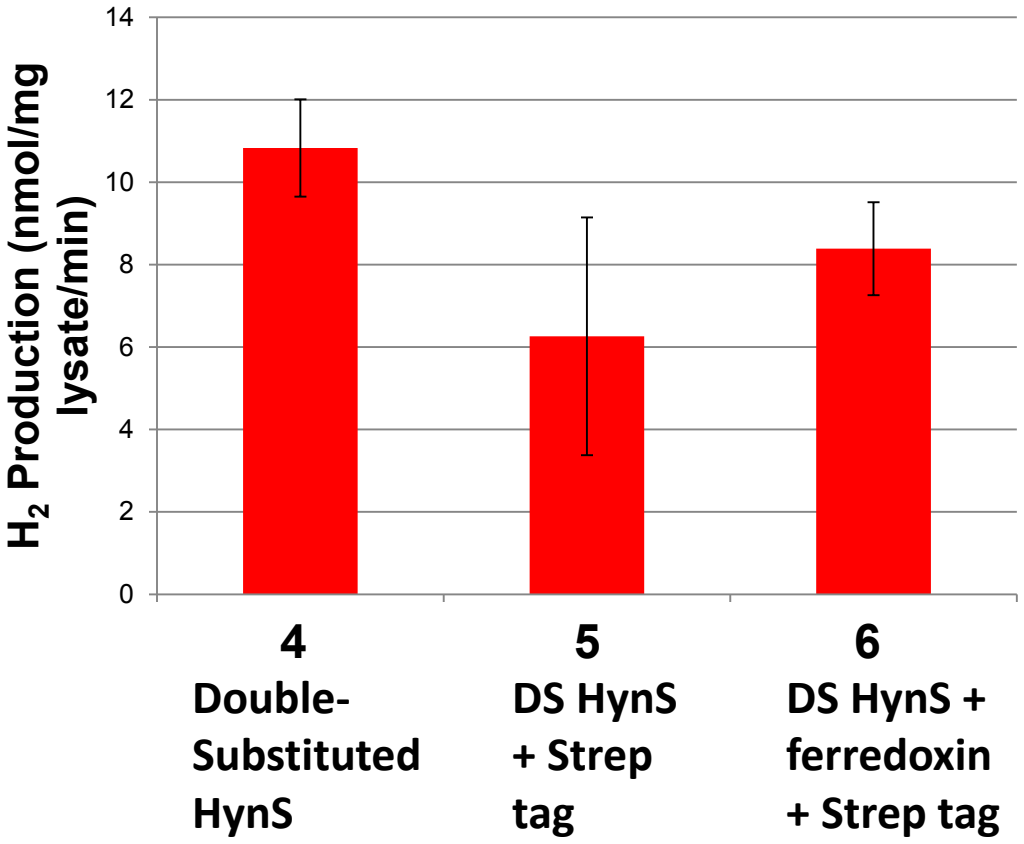
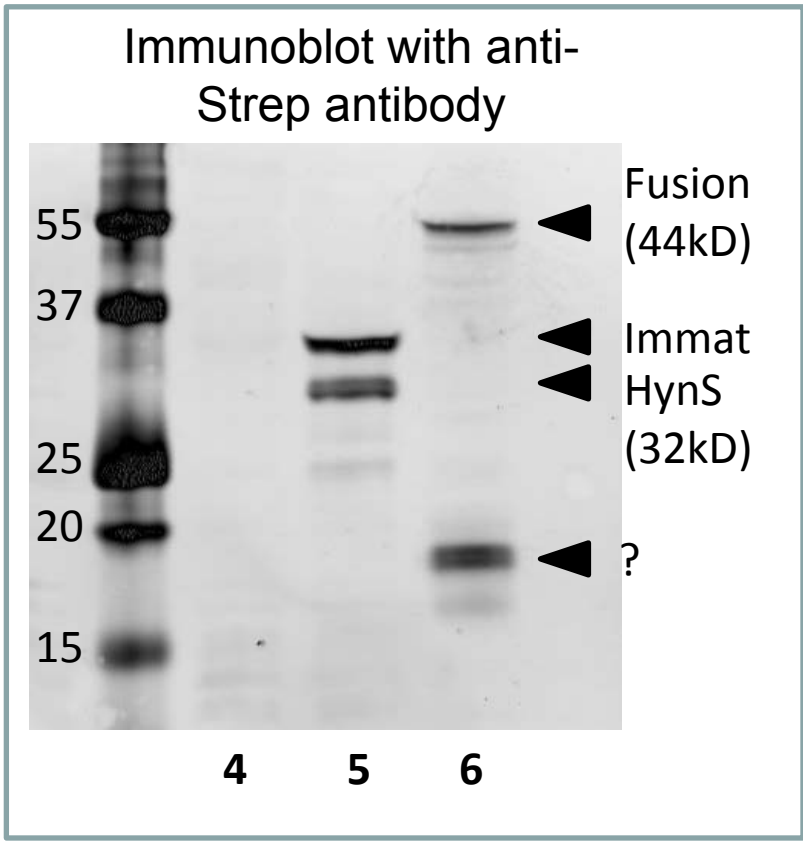
Task 2: Technical Accomplishments

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).

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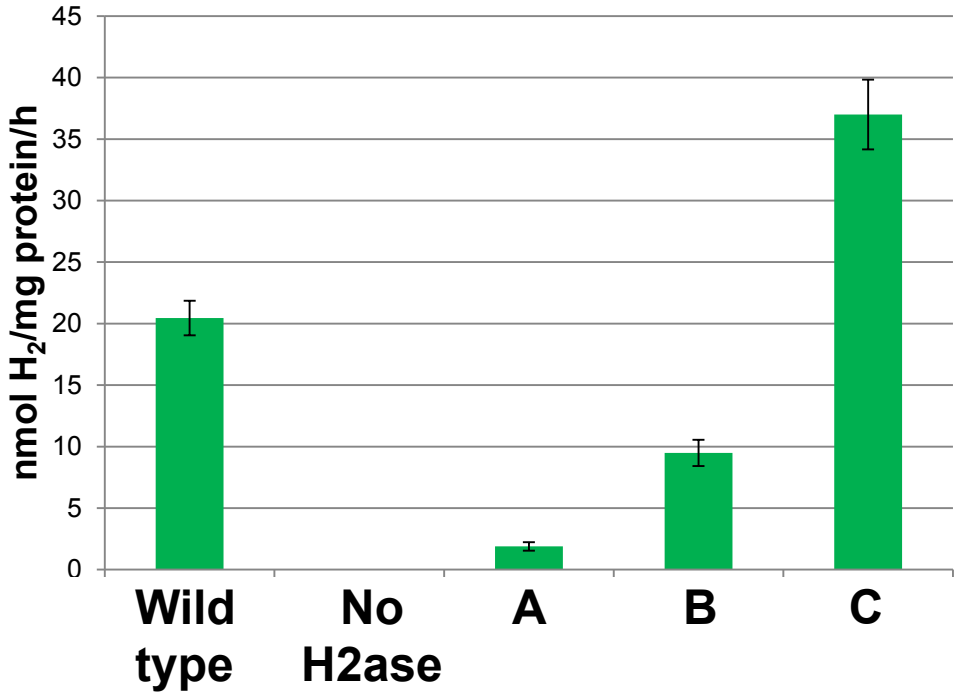
Task 2: Technical Accomplishments

Activity improvements can be combined



Key:

A = Envi H2ase

B = A + extra promoters

C = B + Double substitution HynS

Exceded Go-No Go Criteria "Demonstrate 5x increase hydrogenase activity from environmental H2ase in cyanobacteria as measured by in vitro H_2 evolution assay." **(11/12)**

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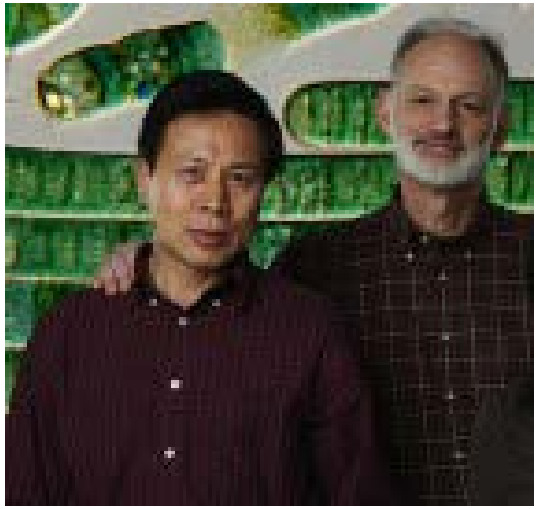
Collaborations

- 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 O_2-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 H_2 evolution activity
- Developed strategies for increasing hydrogenase-ferredoxin interaction
  - Constructed a ferredoxin-hydrogenase fusion protein that maintains activity.