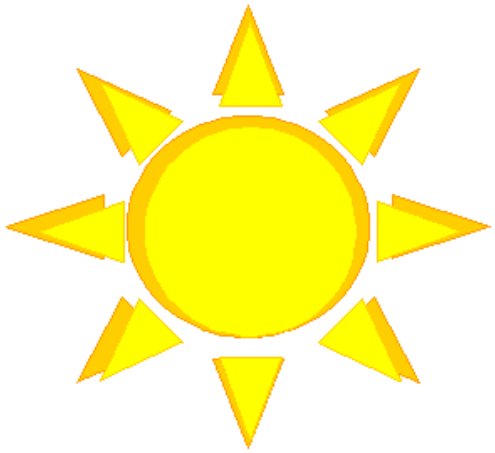
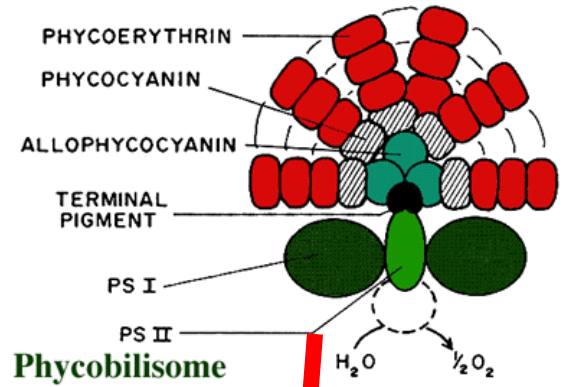
Producing Fuels the Old-Fashioned Way: Using Biology

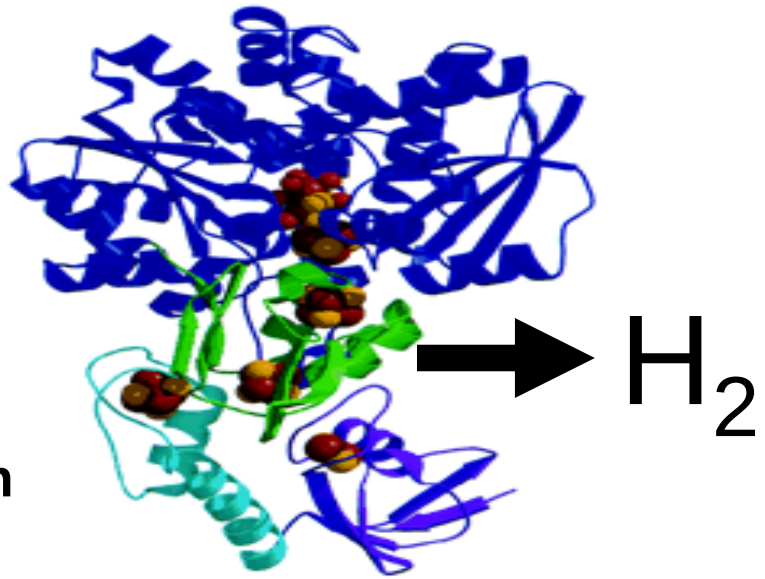




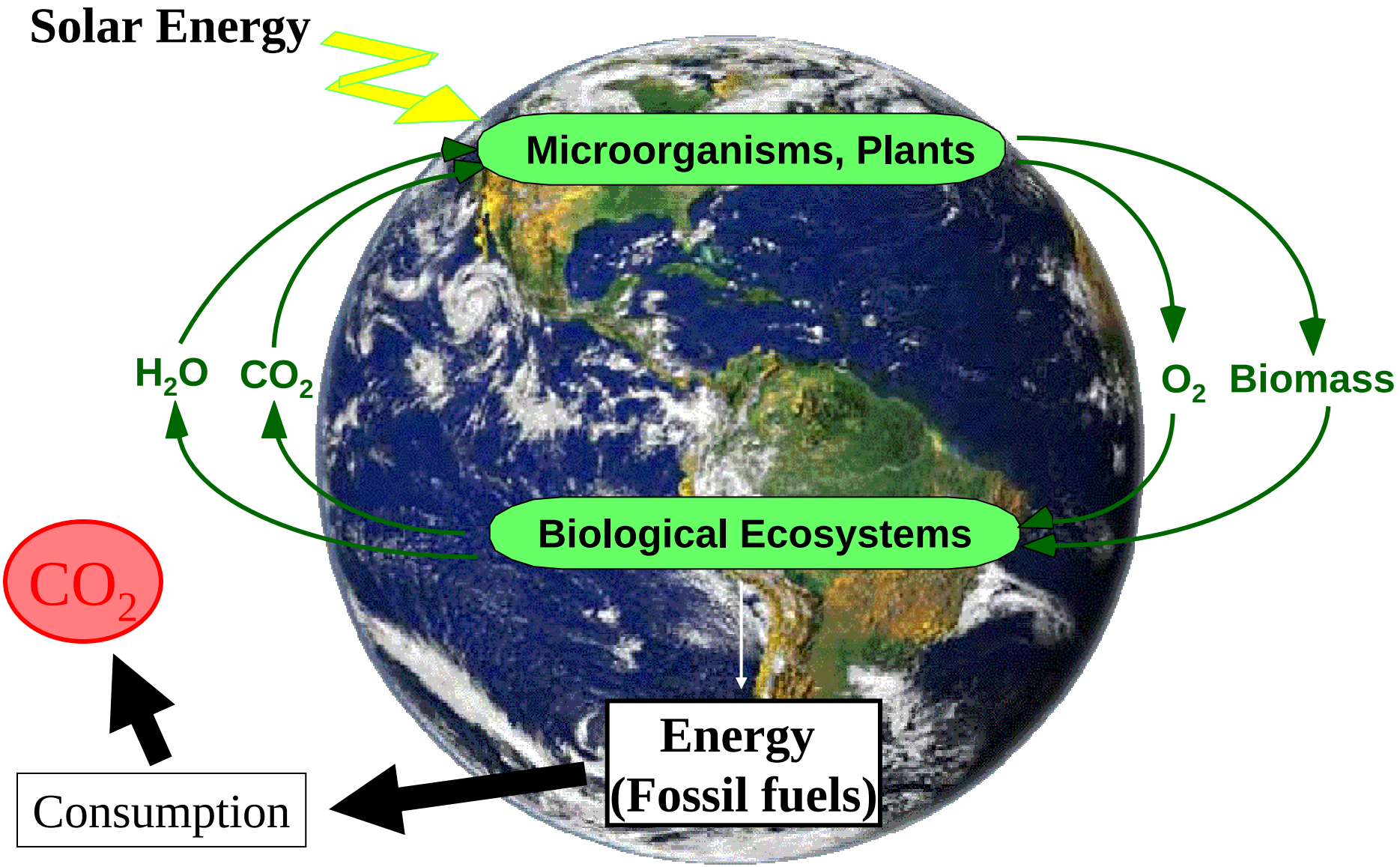
Direct Solar BioHydrogen: Part II

Jim Swartz, Dept. of Chemical Engineering
Dept. of Bioengineering
Stanford University

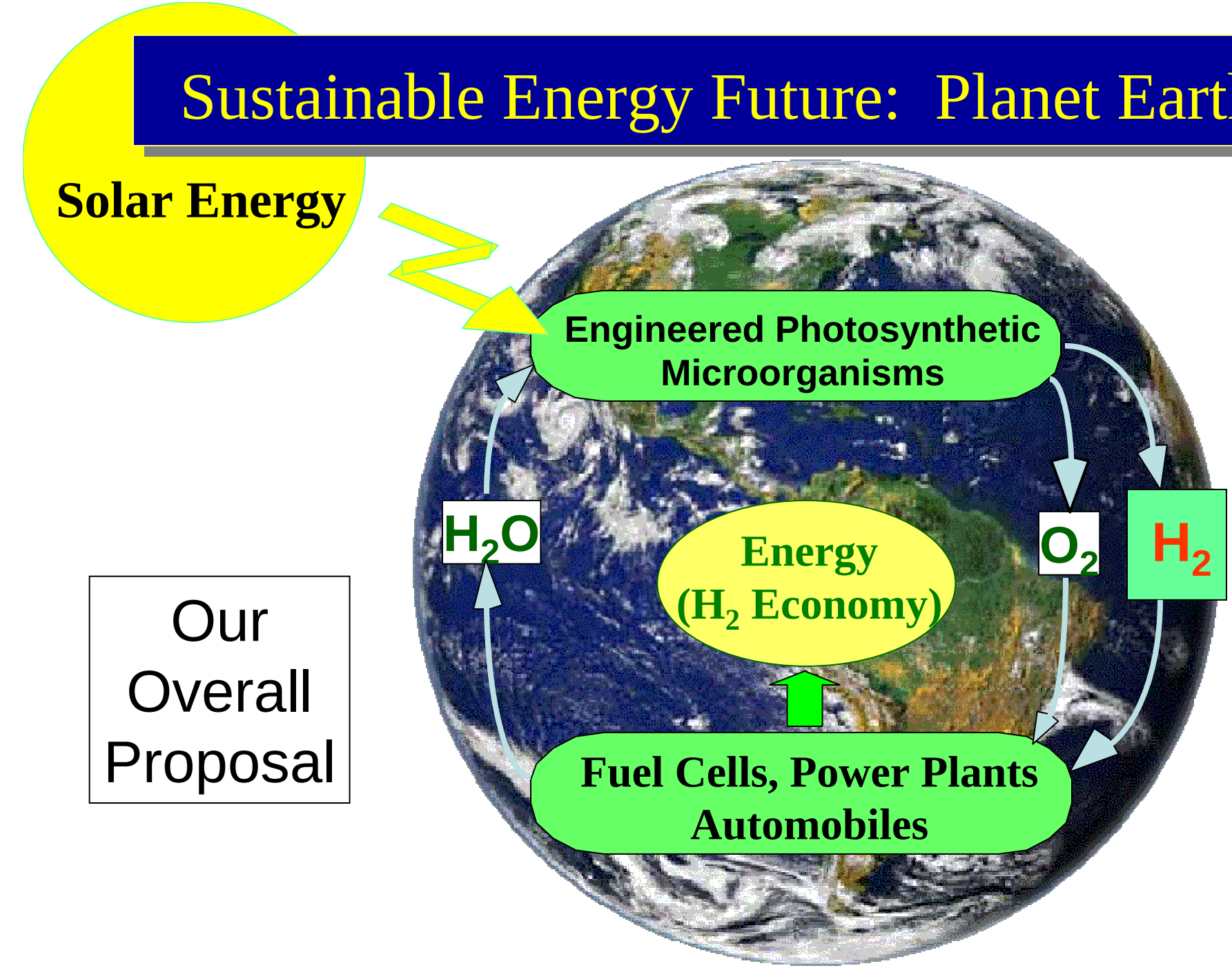
With: **Marcus Boyer, Jim Stapleton, Jon Keuchenreuther, and Phil Smith**
Dept. of Chemical Engineering



Energy History: Planet Earth



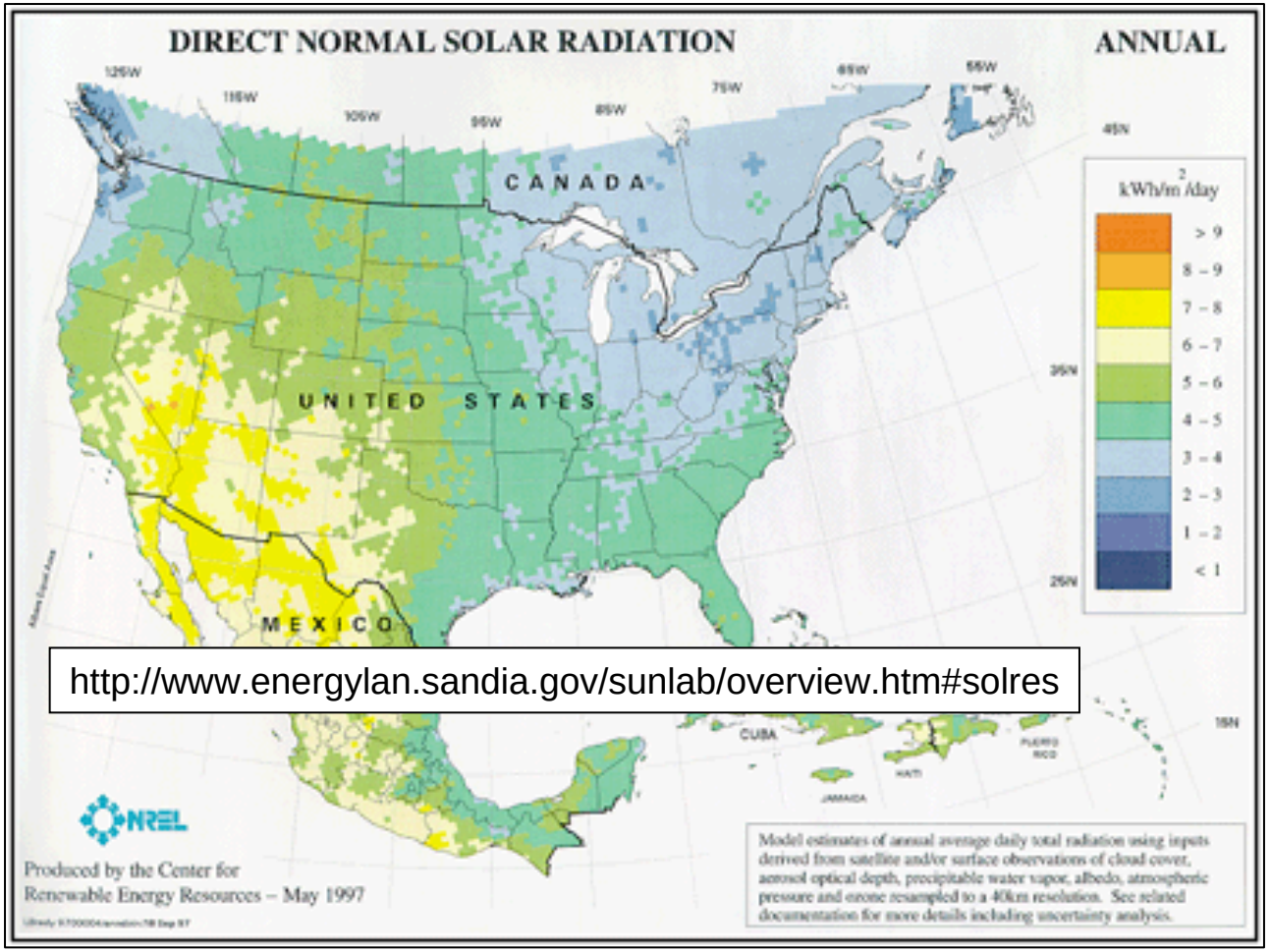
Sustainable Energy Future: Planet Earth



Our Overall Proposal

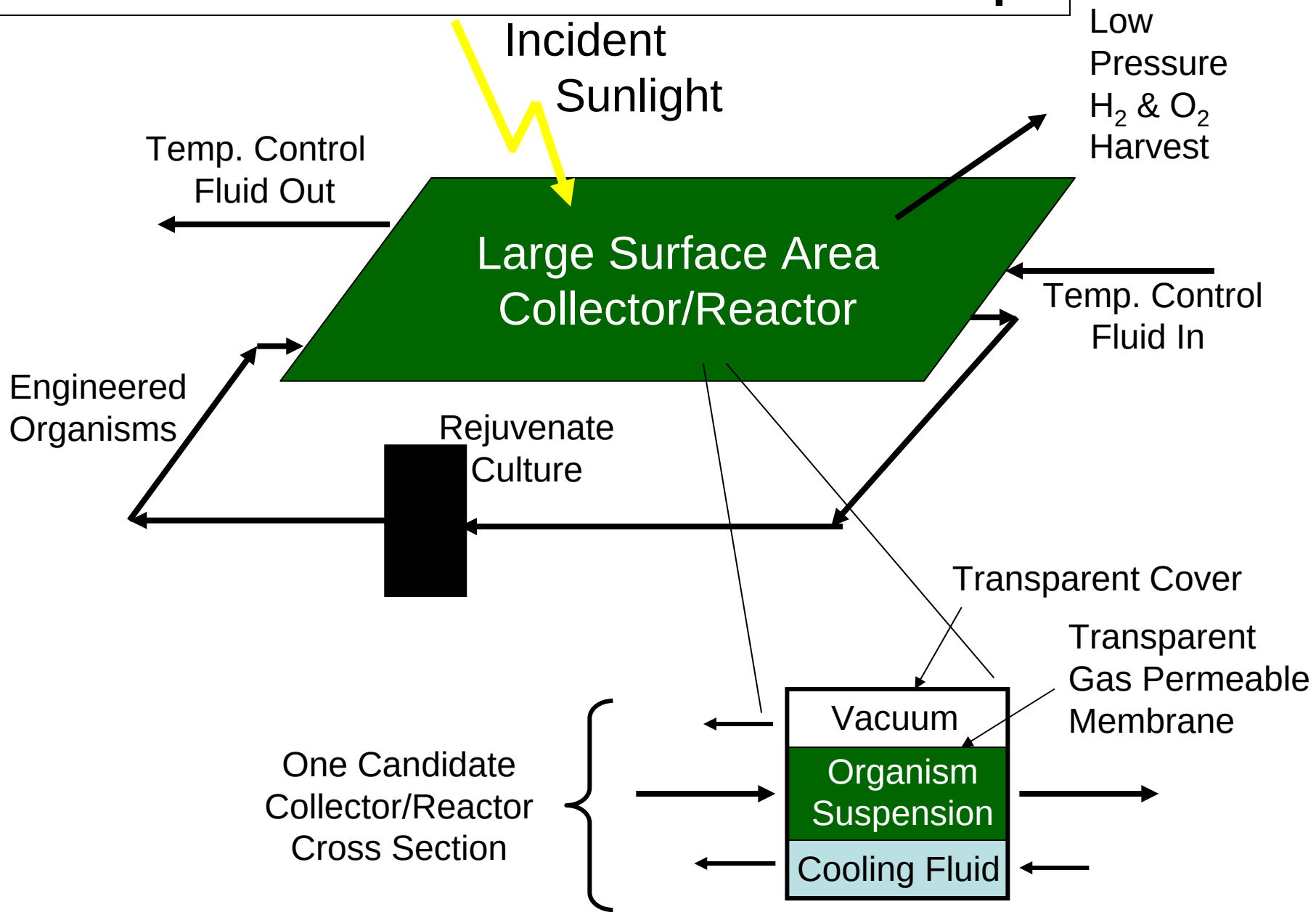
Solar Energy Could Supply **ALL** of our Energy Needs

The solar resources for generating power from concentrating solar power systems is plentiful. For instance, <u>enough electric power for the entire country could be generated by covering about 9 percent of Nevada – a plot of land 100 miles on a side*</u> – with parabolic trough systems.

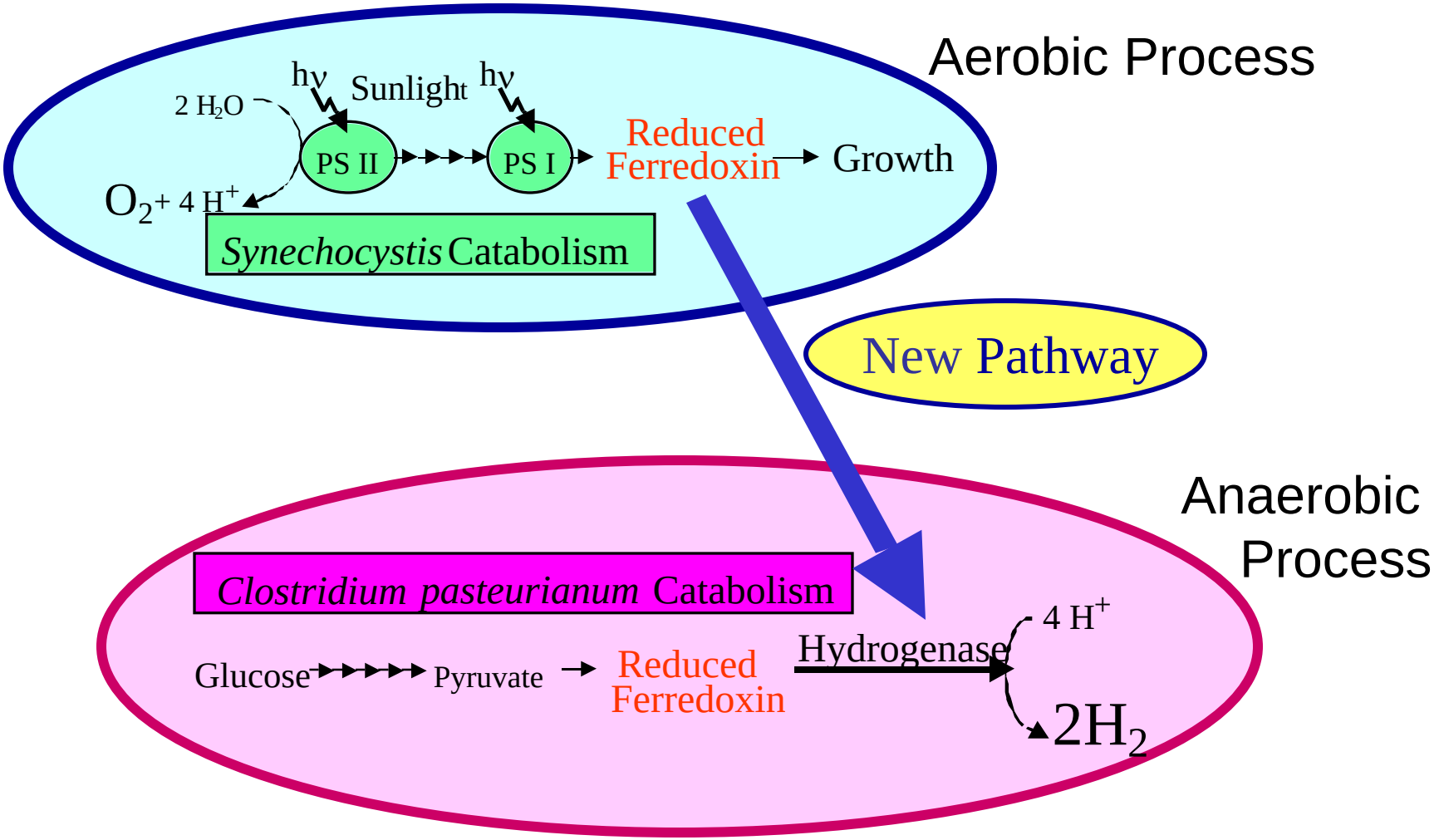


* Equivalent to 1.7% of Current Cropland

The Direct Conversion Concept



Direct PhotoBiological Hydrogen Production: Building a New Electron Pathway



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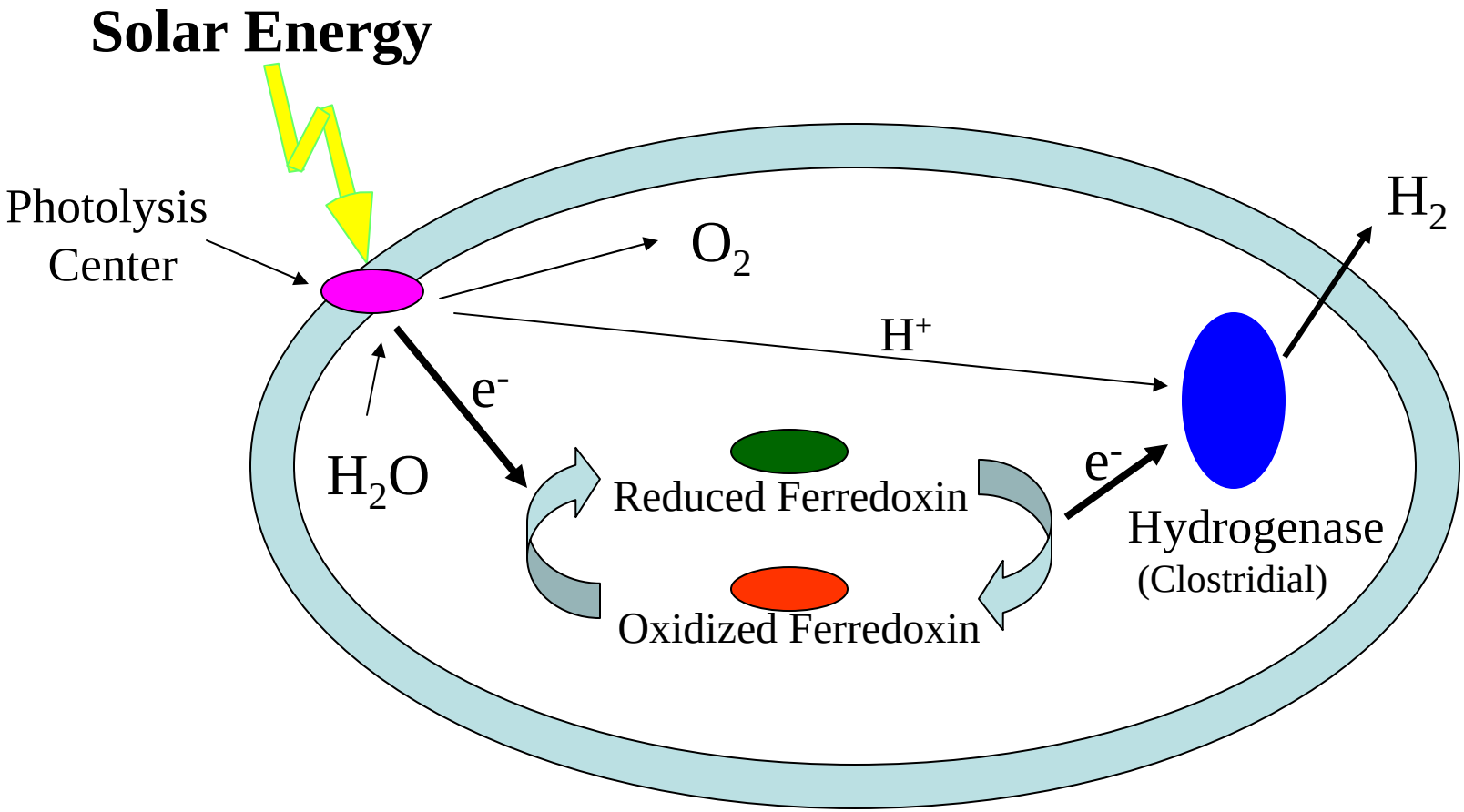
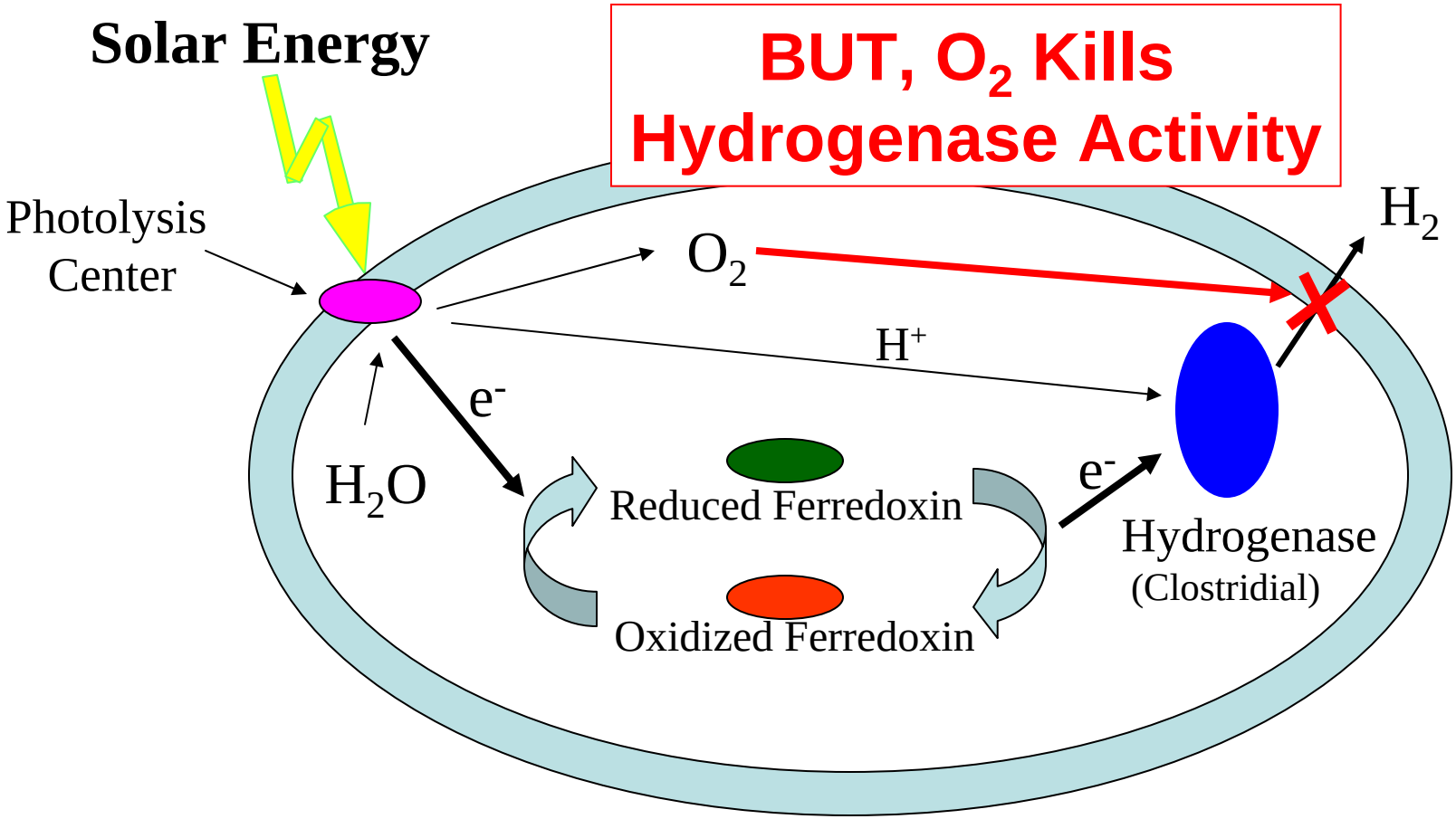


Goal: Engineered *Synechocystis* Bacterium

Direct PhotoBiological Hydrogen Production: Building a New Electron Pathway



Goal: Engineered *Synechocystis* Bacterium

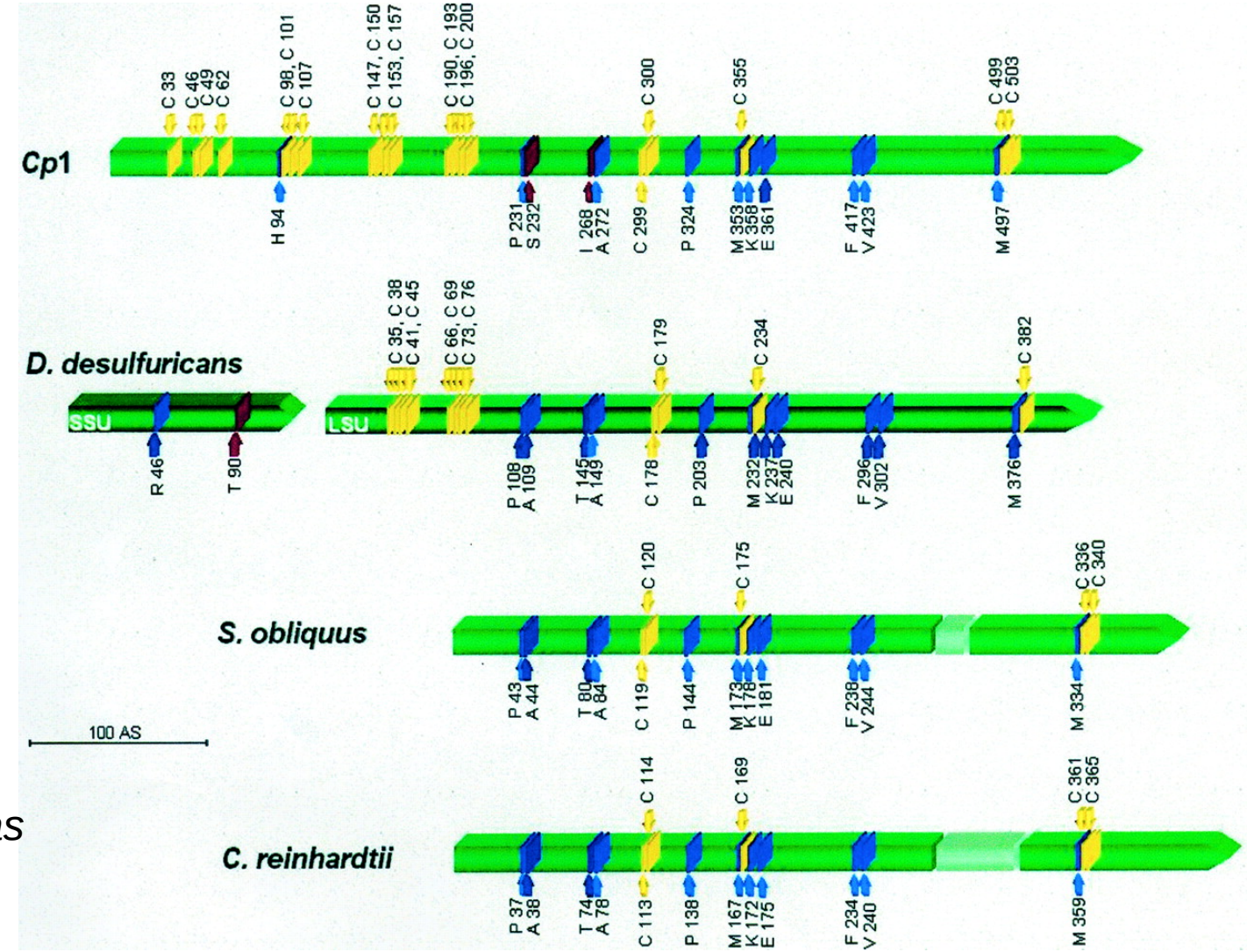
Comparison of Different Fe-Fe Hydrogenases

Clostridium pasteurianum

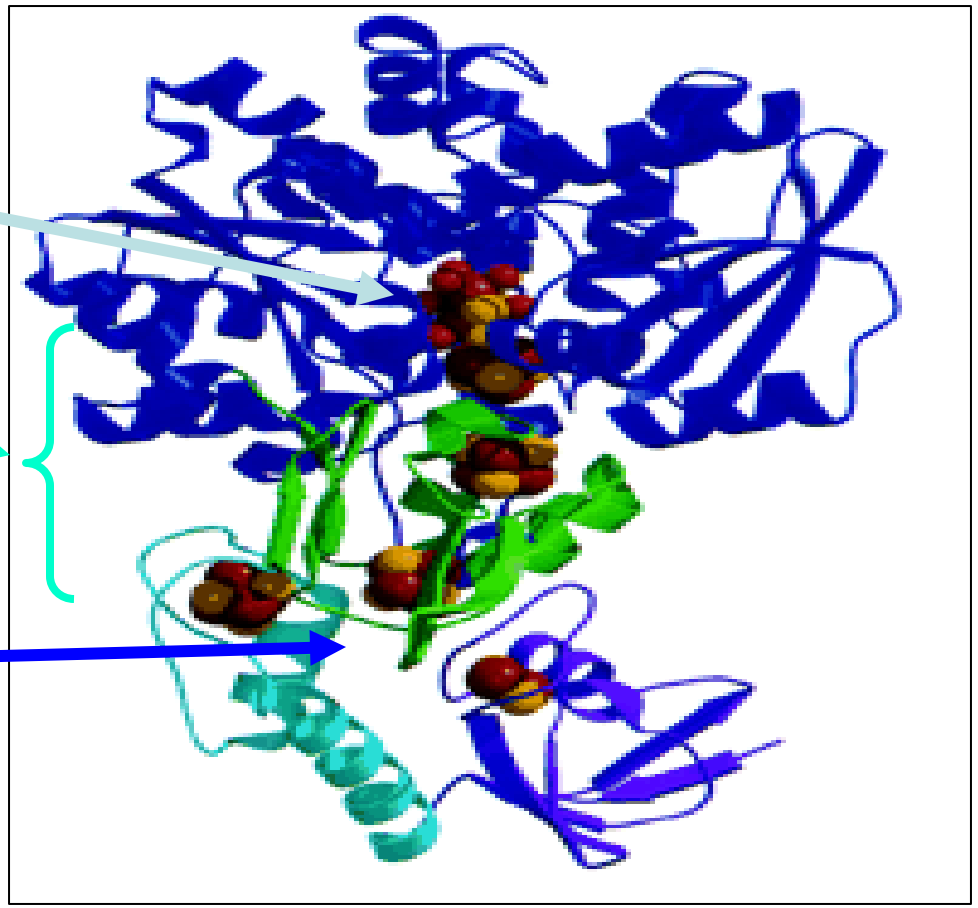
Desulfovibrio desulfuricans

Scenedesmus obliquus

Chlamydomonas reinhardtii



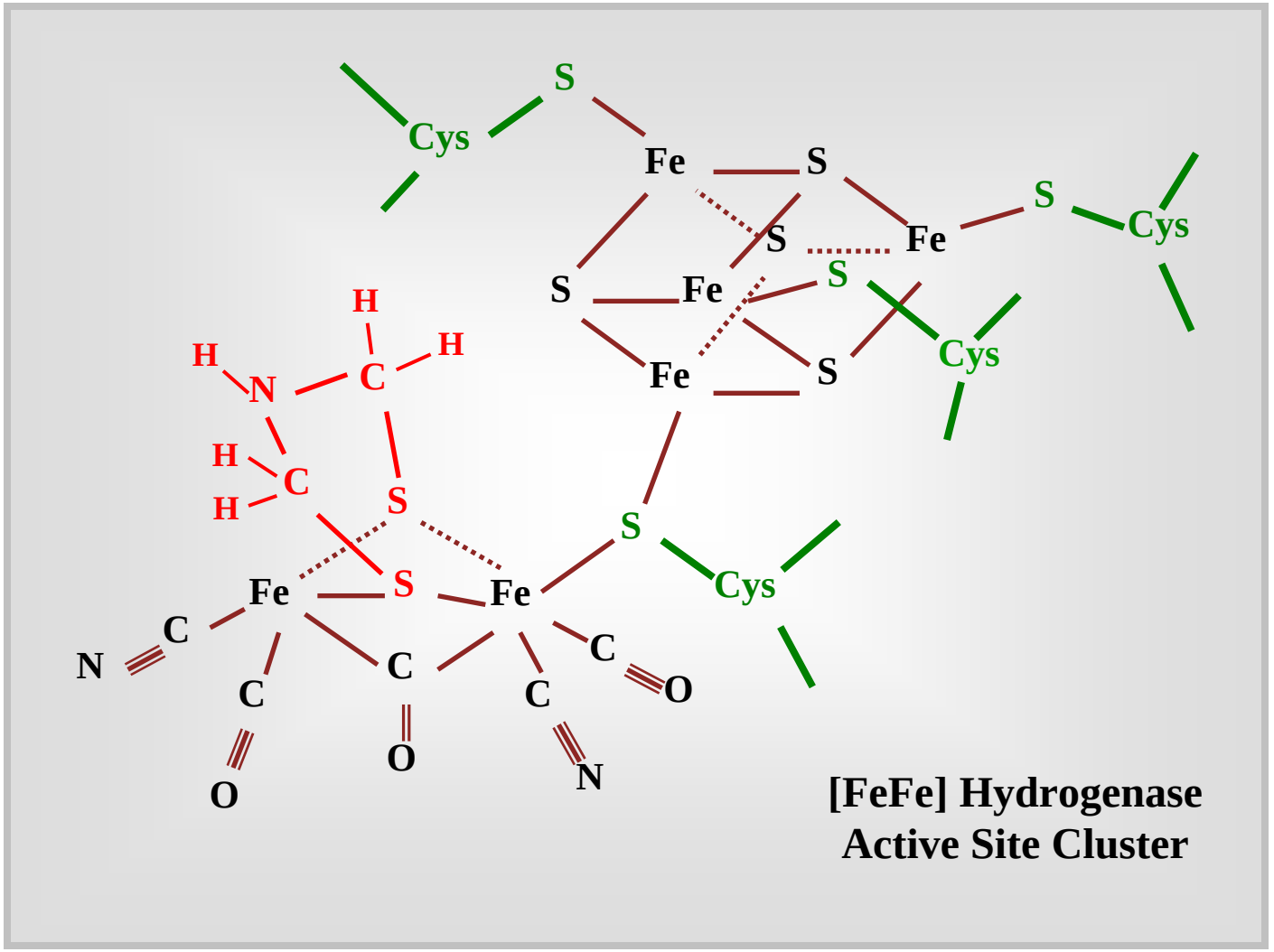
The 3-D Structure is Known for the Hydrogenase from *Clostridium pasteurianum*



Hydrogenase CpI from *Clostridium pasteurianum*

$$2H^{+} + 2Fd^{red.} \rightleftharpoons H_2 + 2Fd^{oxid.}$$

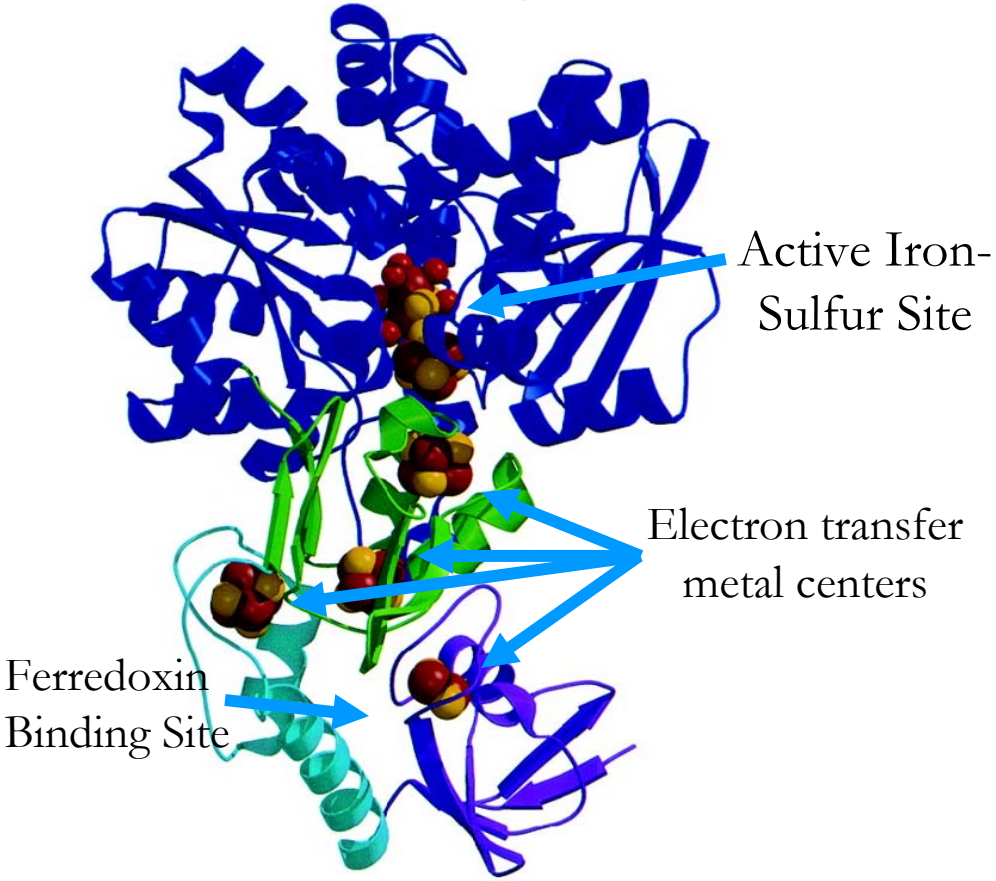
The Oxygen-Sensitive Active Site is Very Complex



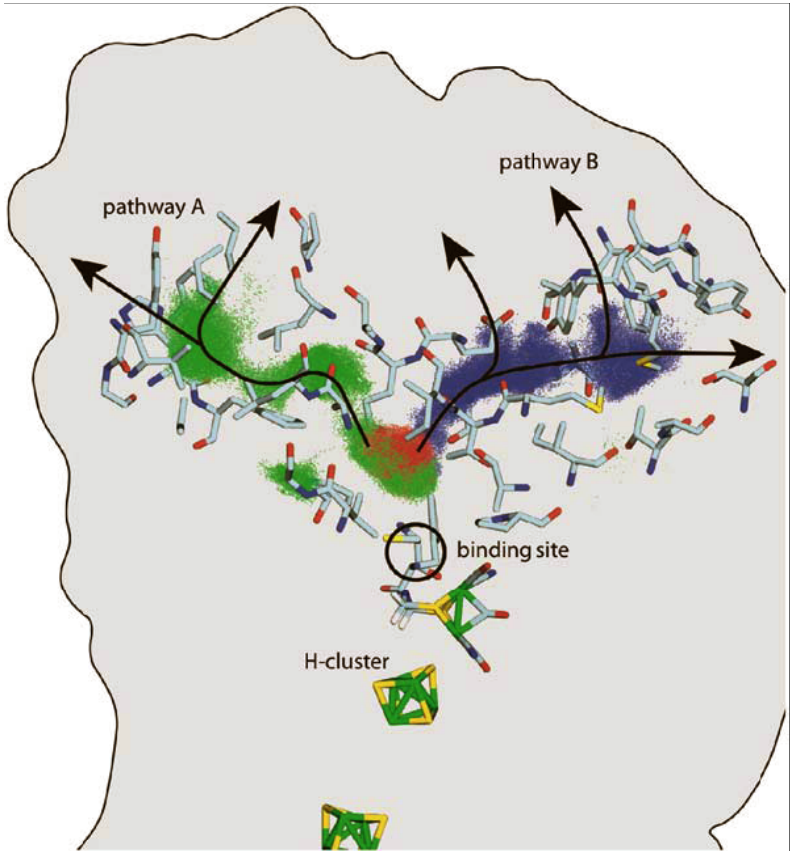
A Recent Molecular Dynamic Model Suggests Two Oxygen Channels

Hydrogenase CpI from *Clostridium pasteurianum*[1]

$2H^+ + 2Fd^{red} \leftrightarrow H_2 + 2Fd^{oxid}$



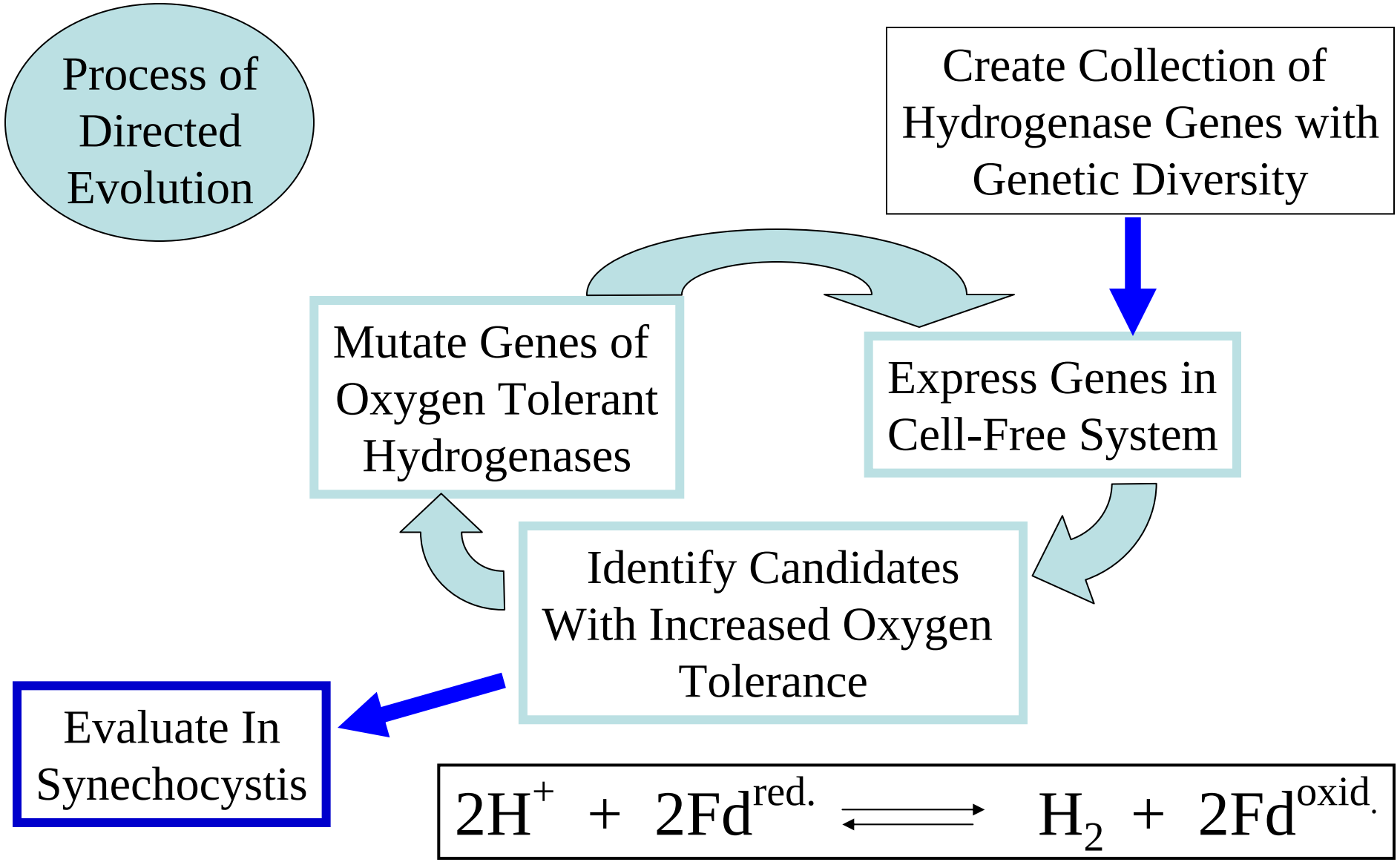
Proposed model for O_2 diffusion in CpI[2]



[1]Peters JW, Lanzilotta WN, Lemon BJ, Seefeldt LC. 1998. Science 282(5395):1853-1858.

[2]Cohen J, Kim K, King P, Seibert M, Schulten K. 2005. Structure 13(9):1321.

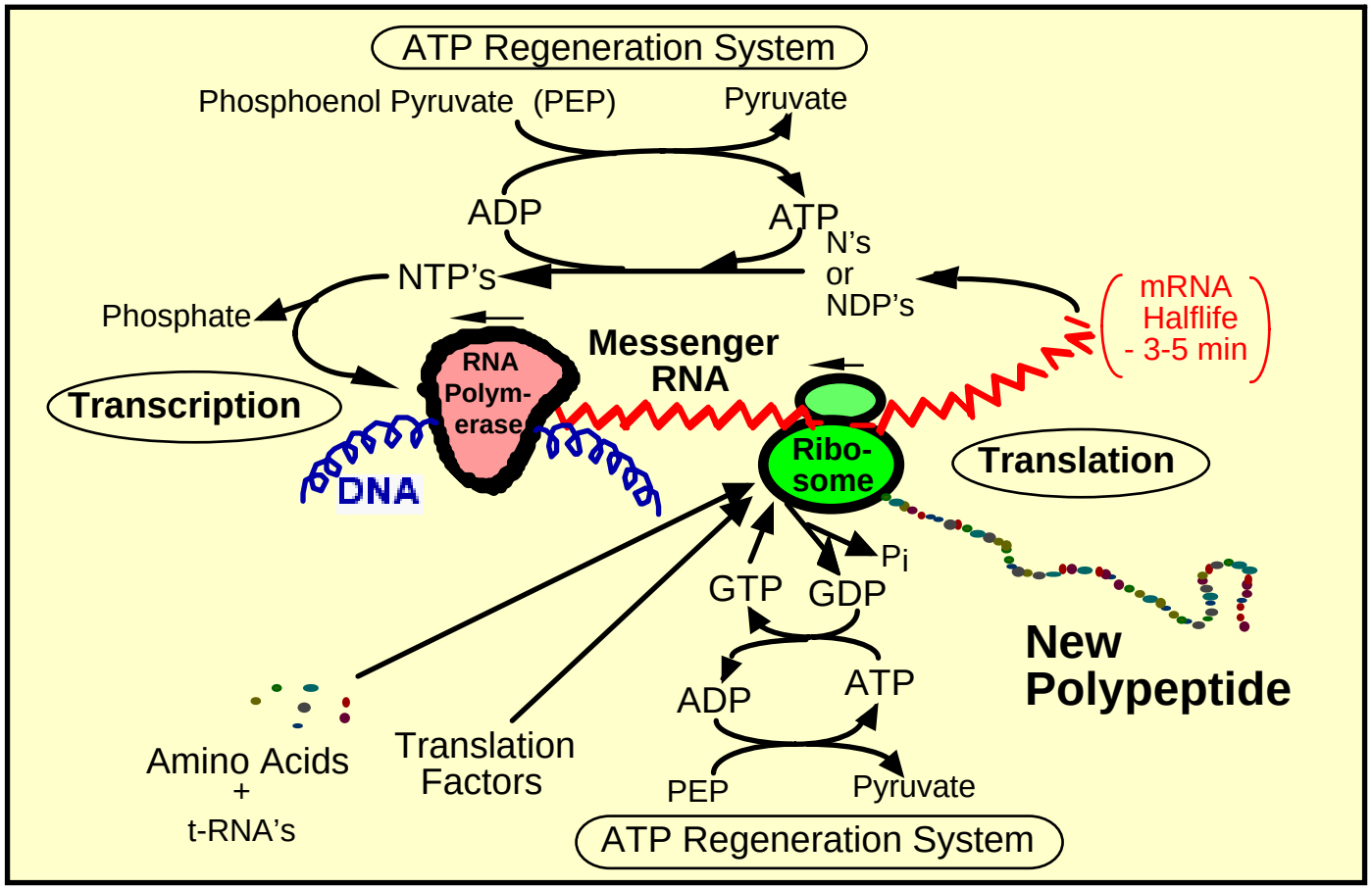
We Will Use Directed Evolution to Produce Oxygen Tolerant Hydrogenases



Cell-Free Protein Synthesis (CFPS) –
Can Easily Conduct Multiple Parallel Reactions

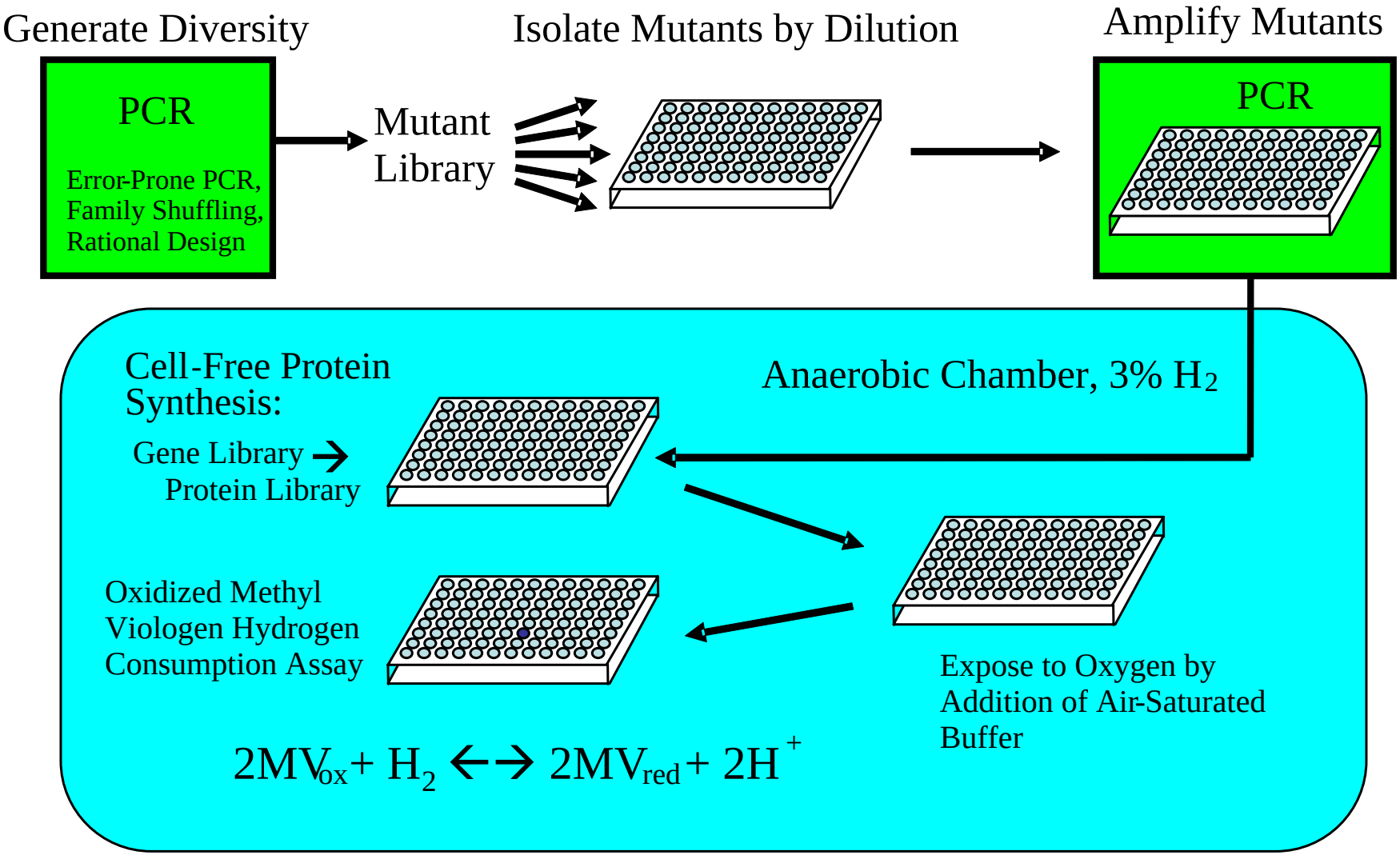
Combined Transcription/Translation: *E.coli*

1. Grow and Lyse *E.coli*
2. Prepare Extract
3. Add Substrates, Salts, and Folding Aids
4. Add Template
5. Incubate



Provides Direct Access and Control and Rapid Analysis

Screening Strategy



Jim Stapleton

Single Molecule PCR (smPCR) Allows Clonal Separation

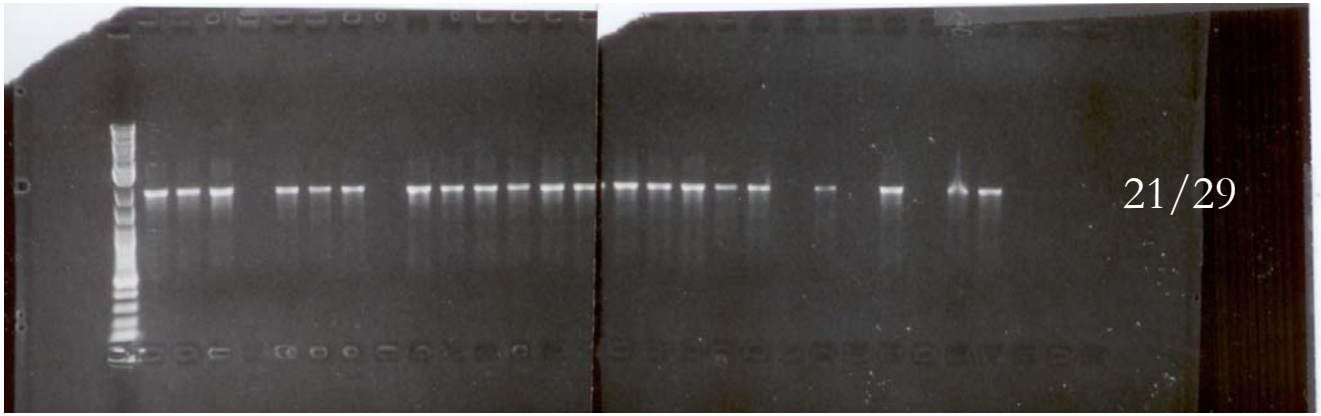
- Dilution to single molecule level is crucial
- Poisson distribution describes single molecule statistics
- For Leu66 (10^7 dilution of library):

$$P(k) = \frac{m^k e^{-m}}{k!}$$

P is the probability of getting k DNA molecules in a specified well, when m = average # DNA molecules per well

For K = 0

$$P(0) = \frac{m^0 e^{-m}}{0!} = e^{-m} \qquad m = -\ln[P(0)]$$



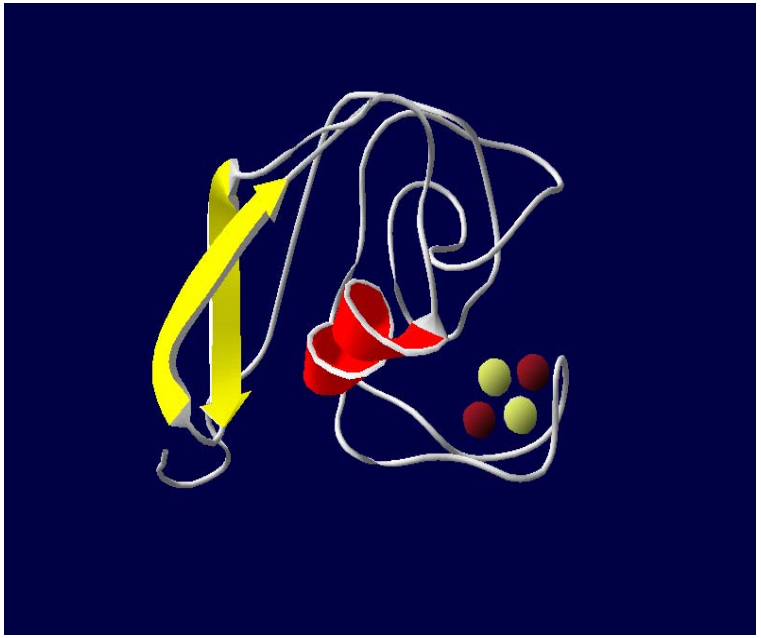
The 10^7 dilution contains 1.23 molecules/μL

Data from Phil Smith

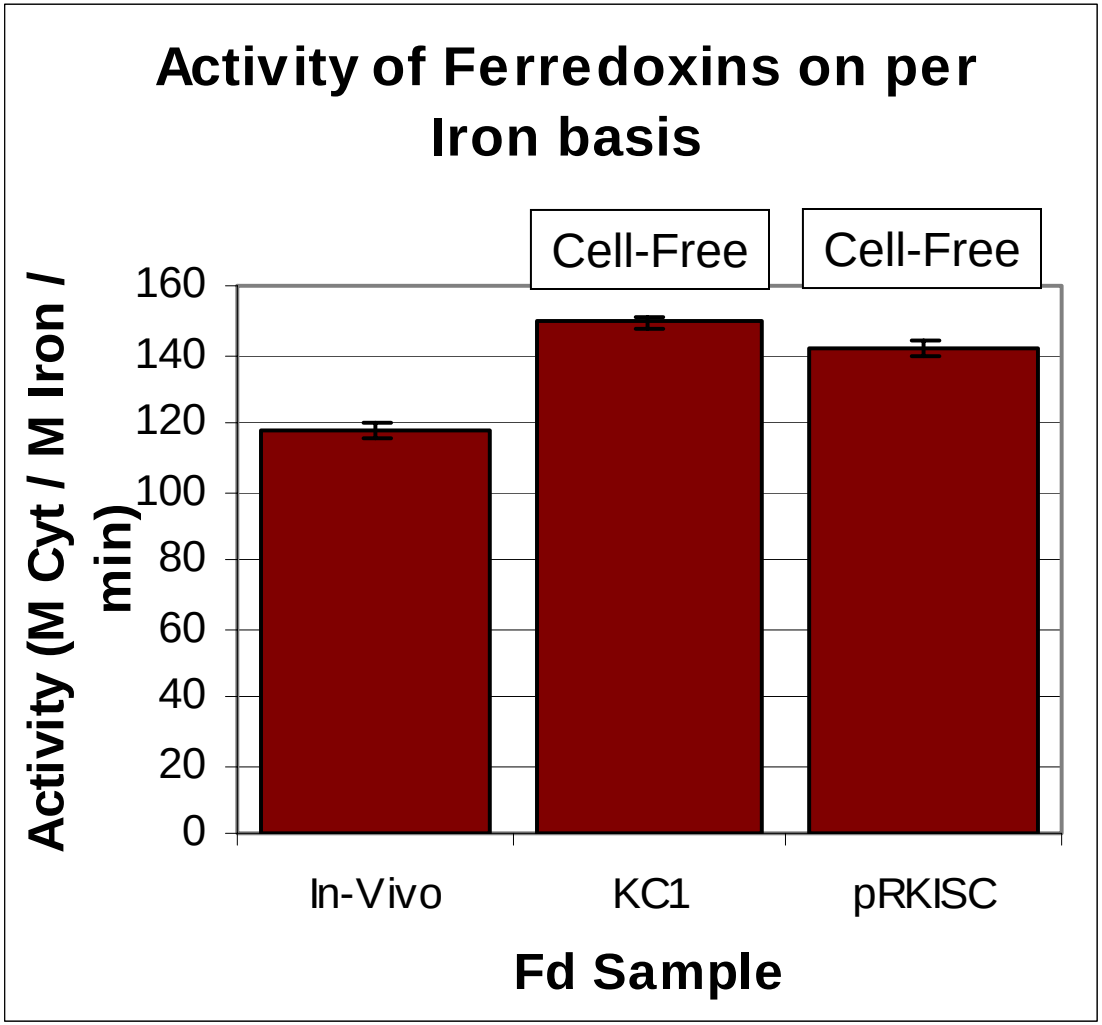
Methodology developed by Jim Stapleton

We Began with Ferredoxin. It also Needs an Fe-S Center

Cell-Free Produced Ferredoxins are Fully Active

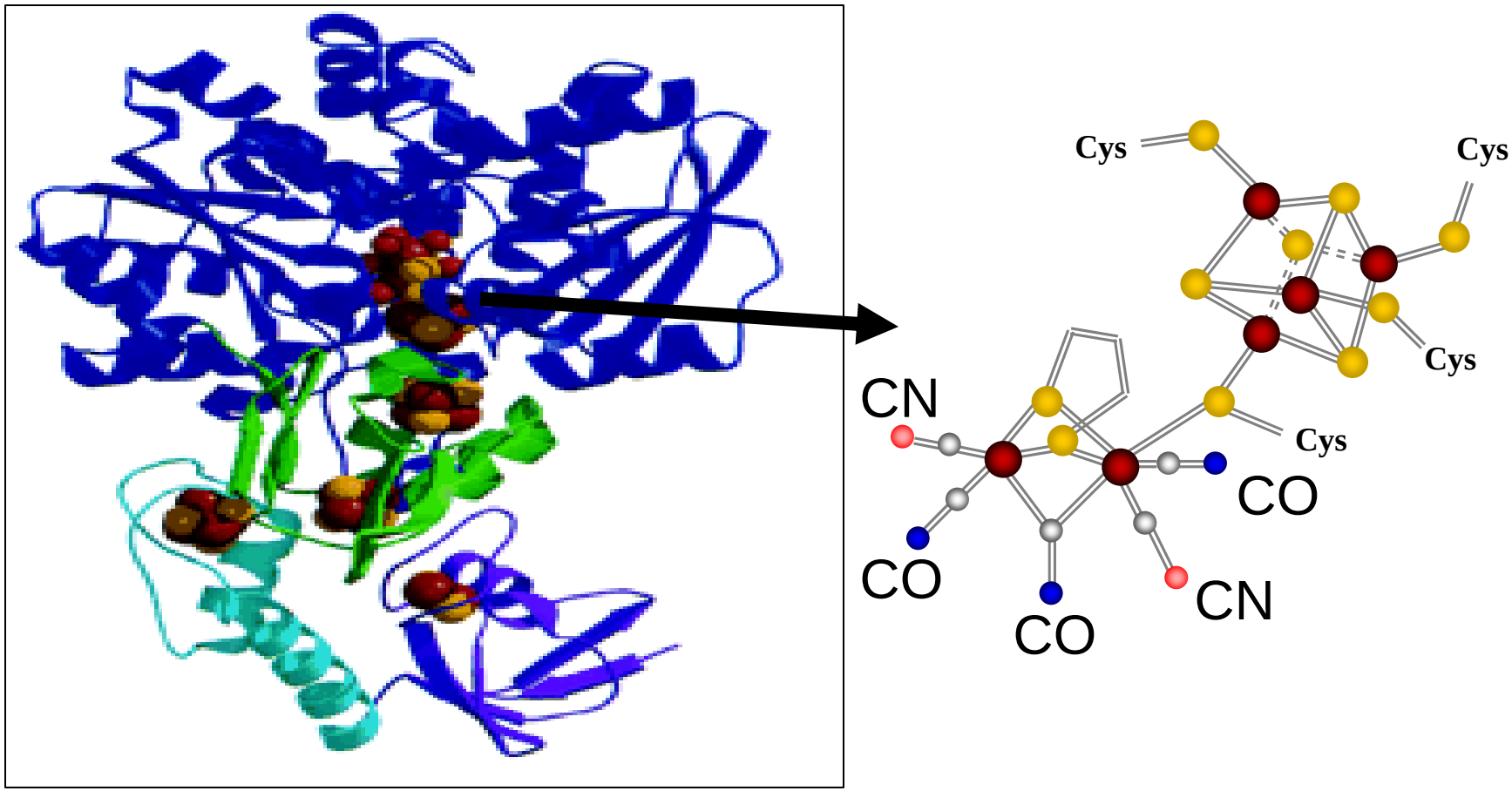


Synechocystis Ferredoxin



Marcus Boyer

The Active Site of Fe-Fe Hydrogenases is Complicated: Stabilized by Cysteines, Carbon Monoxide, and Cyanide



Discovery of Two Novel Radical *S*-Adenosylmethionine Proteins Required for the Assembly of an Active [Fe] Hydrogenase*

Received for publication, March 23, 2004, and in revised form, April 13, 2004
Published, JBC Papers in Press, April 13, 2004, DOI 10.1074/jbc.M403206200

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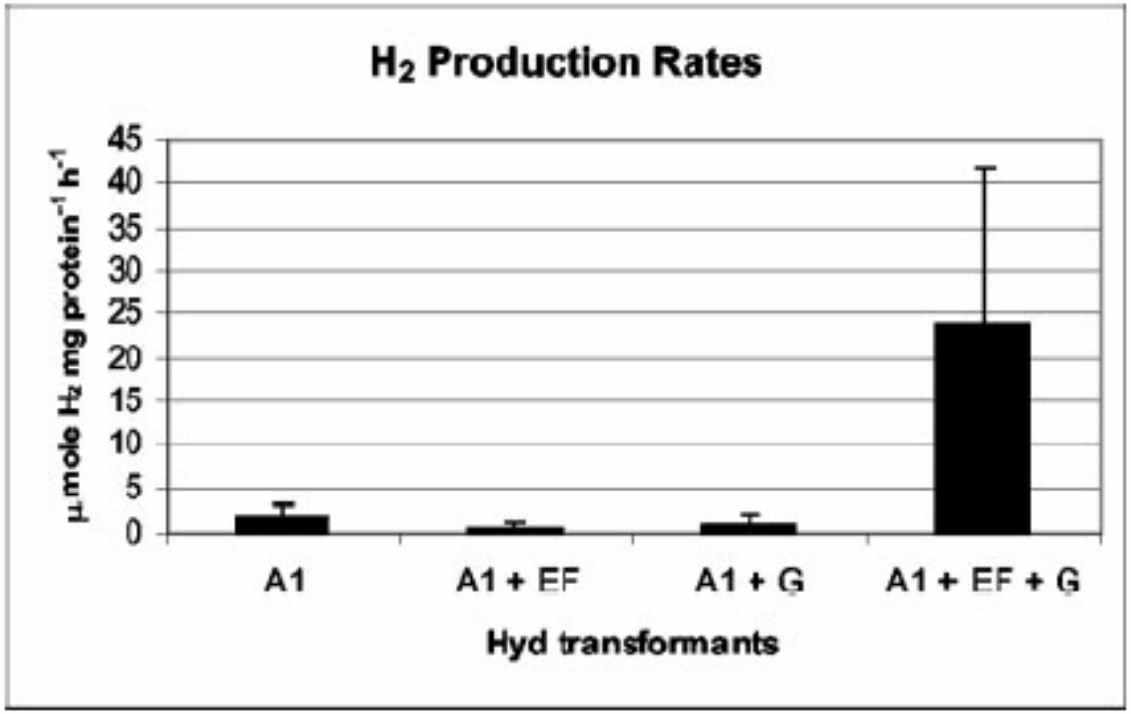
From the ‡National Renewable Energy Laboratory and the §Department of Environmental Science and Engineering, Colorado School of Mines, Golden, Colorado 80401

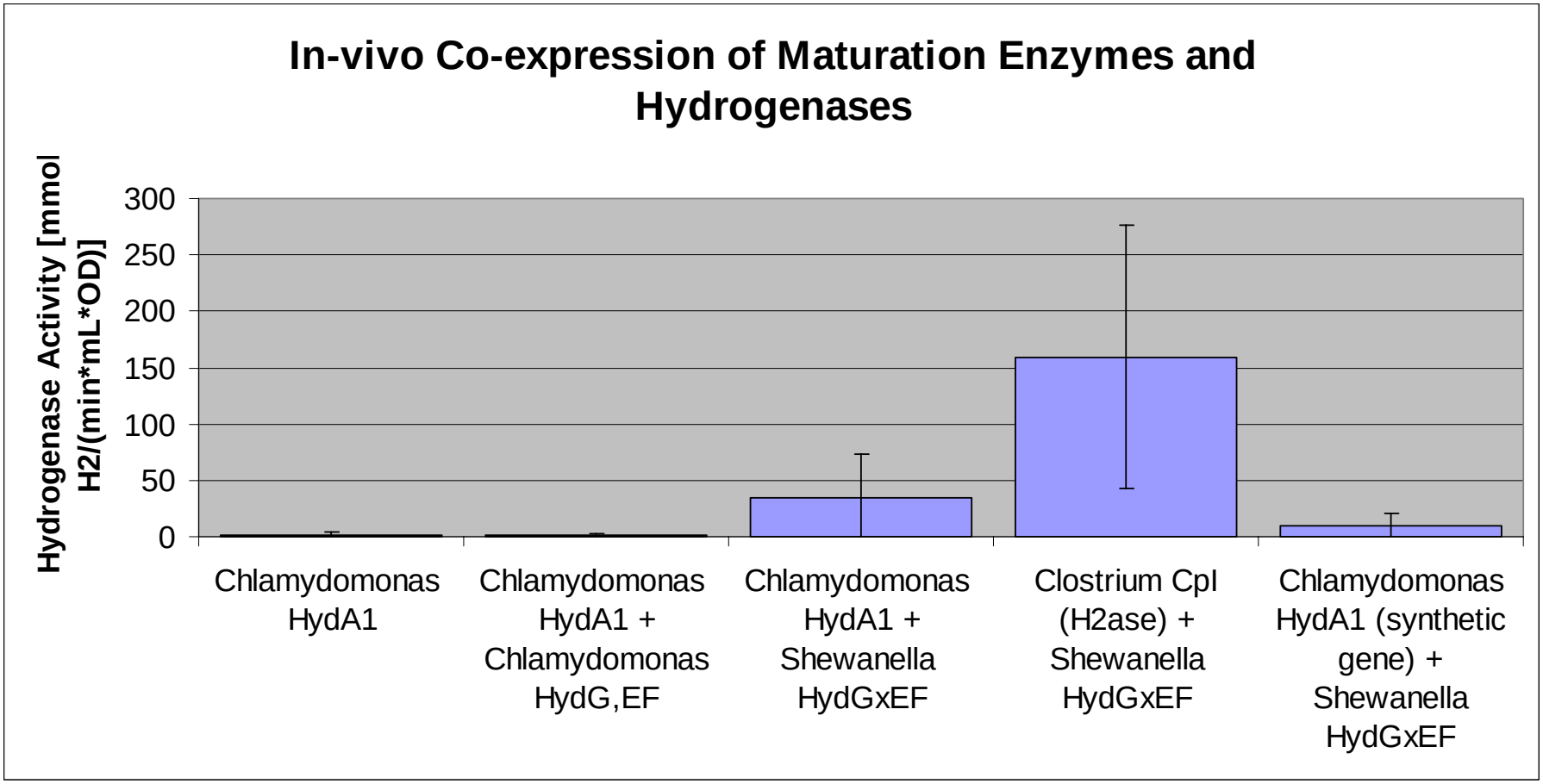
FIG. 5. **Hydrogen production rates from purified HydA1 heterologously expressed in *E. coli* either alone or co-expressed with the indicated Hyd proteins.**

Hydrogen production was measured using the methyl viologen-based assay. The data shown represent the average of four independent experiments; average deviations from the mean are shown.



*Genes Taken from *Chlamydomonas reinhardtii*

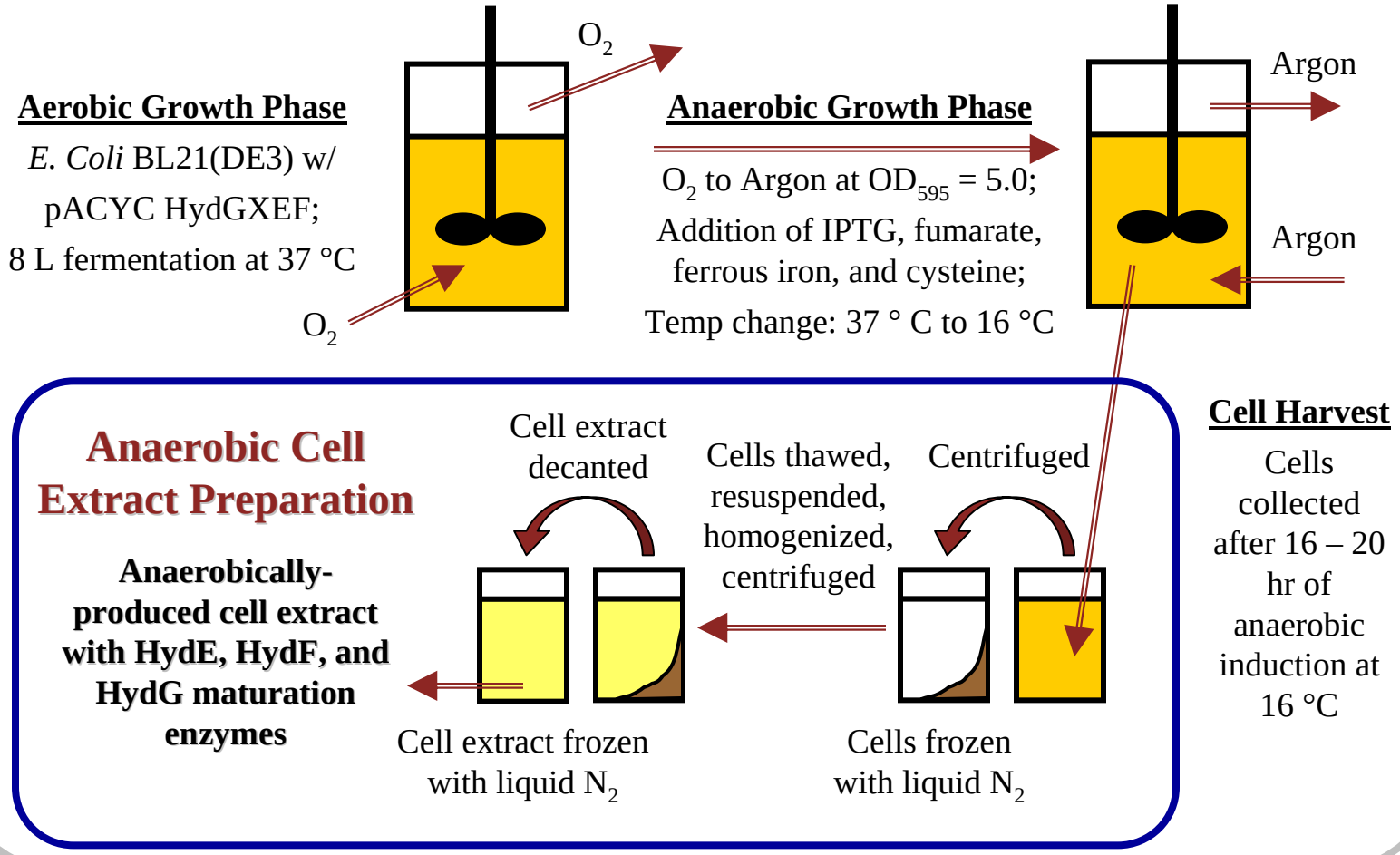
Hydrogenase Expression in *E.coli* Identified Much Better Helper Proteins



Marcus Boyer, Chia-Wei Wang. Jackie Ng

Producing Active Cell Extract Is a Complicated Procedure

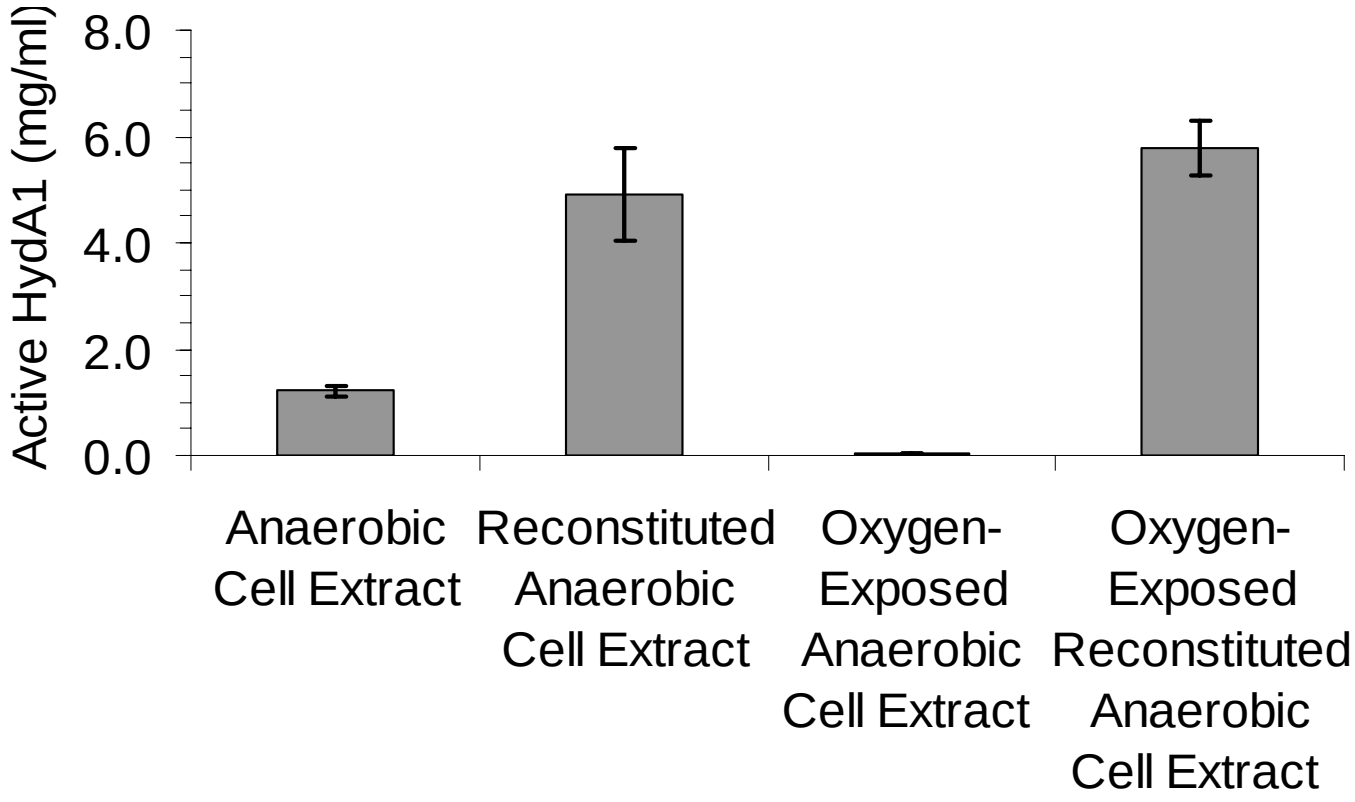
Anaerobic Production of Cell Extract for Cell-Free Synthesis of Active [FeFe] Hydrogenase



Marcus Boyer, Jim Stapleton, Jonny Kuchenreuther, Chia Wei Wang

Activity of Cell Extracts Can be Significantly Increased by “Reconstitution”

The Helper Proteins HydE, HydG, and HydF are all 4Fe-4S Proteins.*

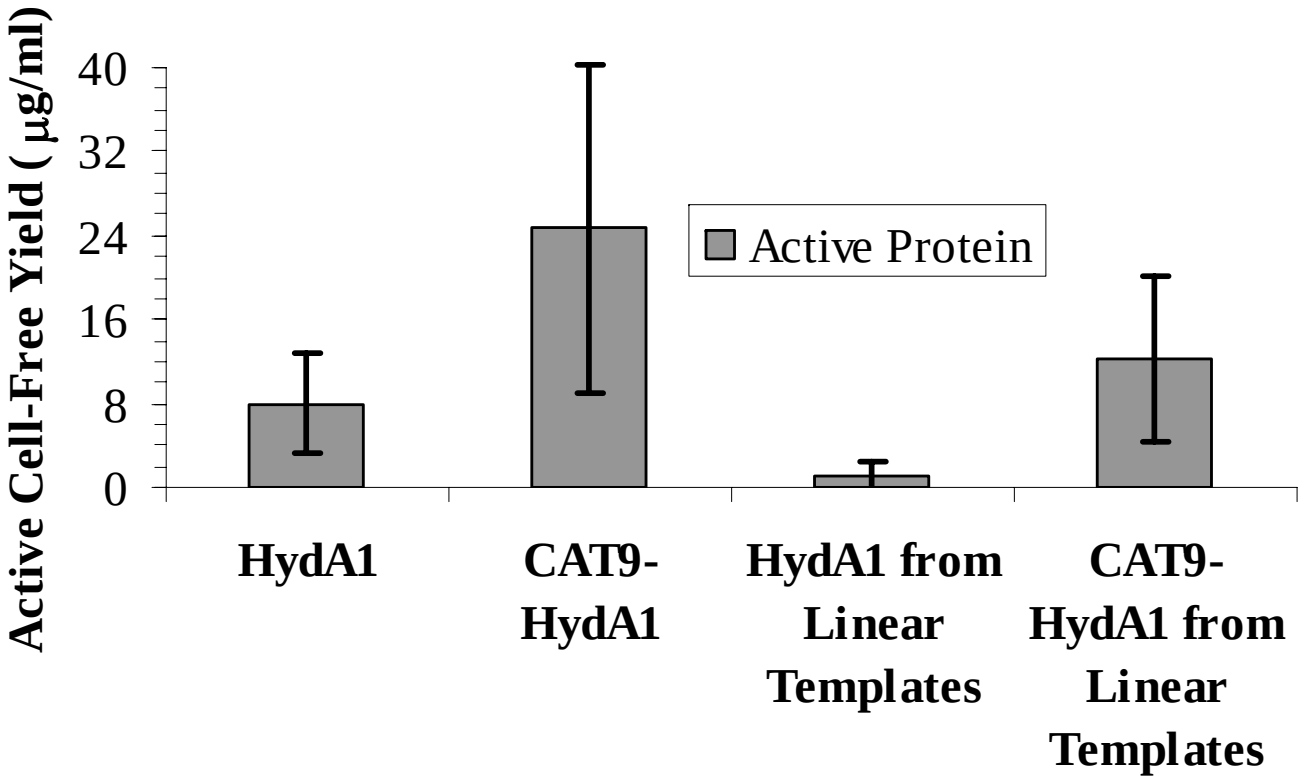


“Reconstitution” is Effected by Incubation of Cell Extract with:
1mM $Fe(NH_4)_2(SO_4)_2$, 1mM Na_2S, and 1 mM Dithiothreitol (DTT)

Jonny Kuchenreuther and Marcus Boyer

*Brazzolotto, …, Marc Fontecave, J. Biol. Chem. 281:769, 2006

Improved Translational Initiation Gives Higher Active Yields



Jonny Kuchenreuther

New Insights: The Devil is in the Details

We must better understand:

The Function and the Activation of the Helper Proteins
as well as any other Requirements for Hydrogenase Activation:

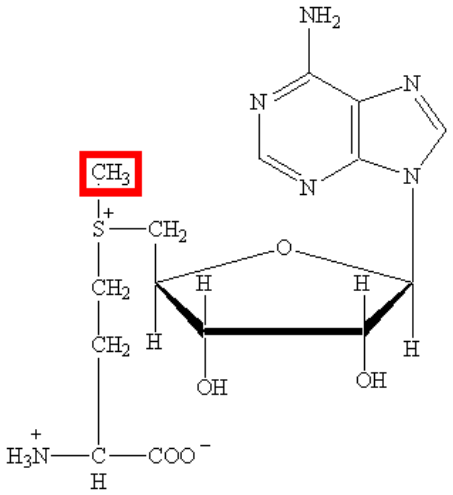
A) Required for Effective Screening of Hydrogenase Mutants

B) Required for Activation of Hydrogenase when it is Expressed in Photosynthetic Organism.

Fontecave's work suggests requirements for:
Guanosine Tri-Phosphate (GTP)

and S-Adenosyl Methionine (SAM)

What do GTP and SAM do?
What is the Origin of the Active Site Atoms?



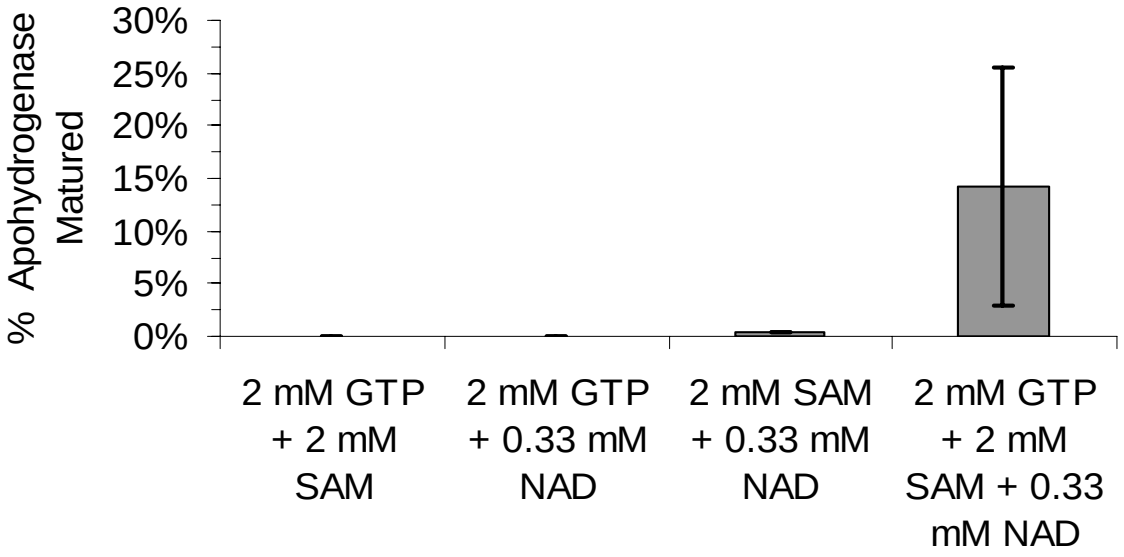
SAM

Post-Synthesis Activation (Maturation) of ApoHydrogenase Enables Detailed Studies of Maturation Requirements

A. Hydrogenase polypeptide is produced **without** helper proteins and under **aerobic** conditions. Small molecules are removed.

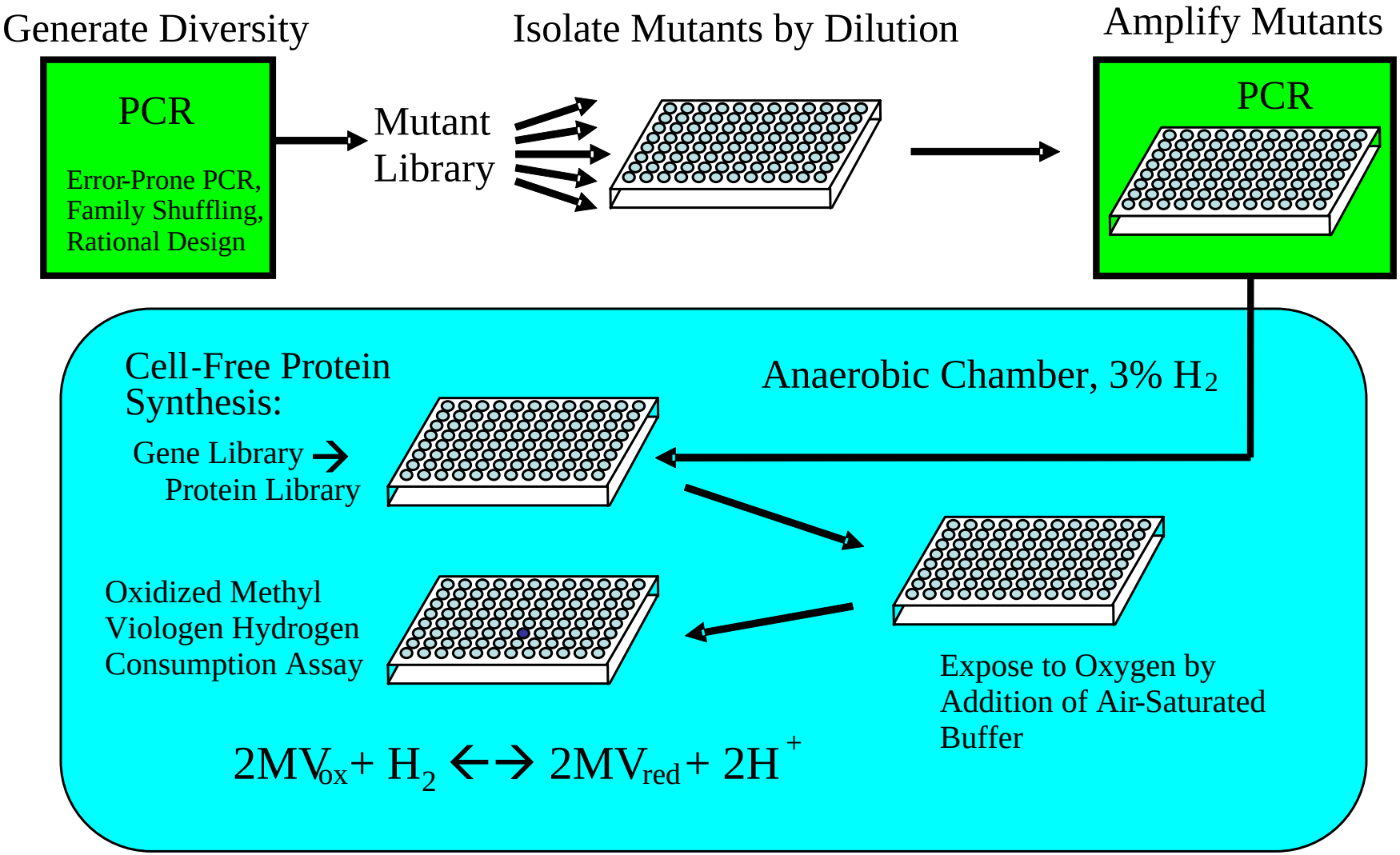
B. Cell Extract with Helper Proteins is Reconstituted under **anaerobic** conditions. Small molecules are removed.

C. Under **anaerobic** conditions, the preparations are mixed and candidate small molecules are added.



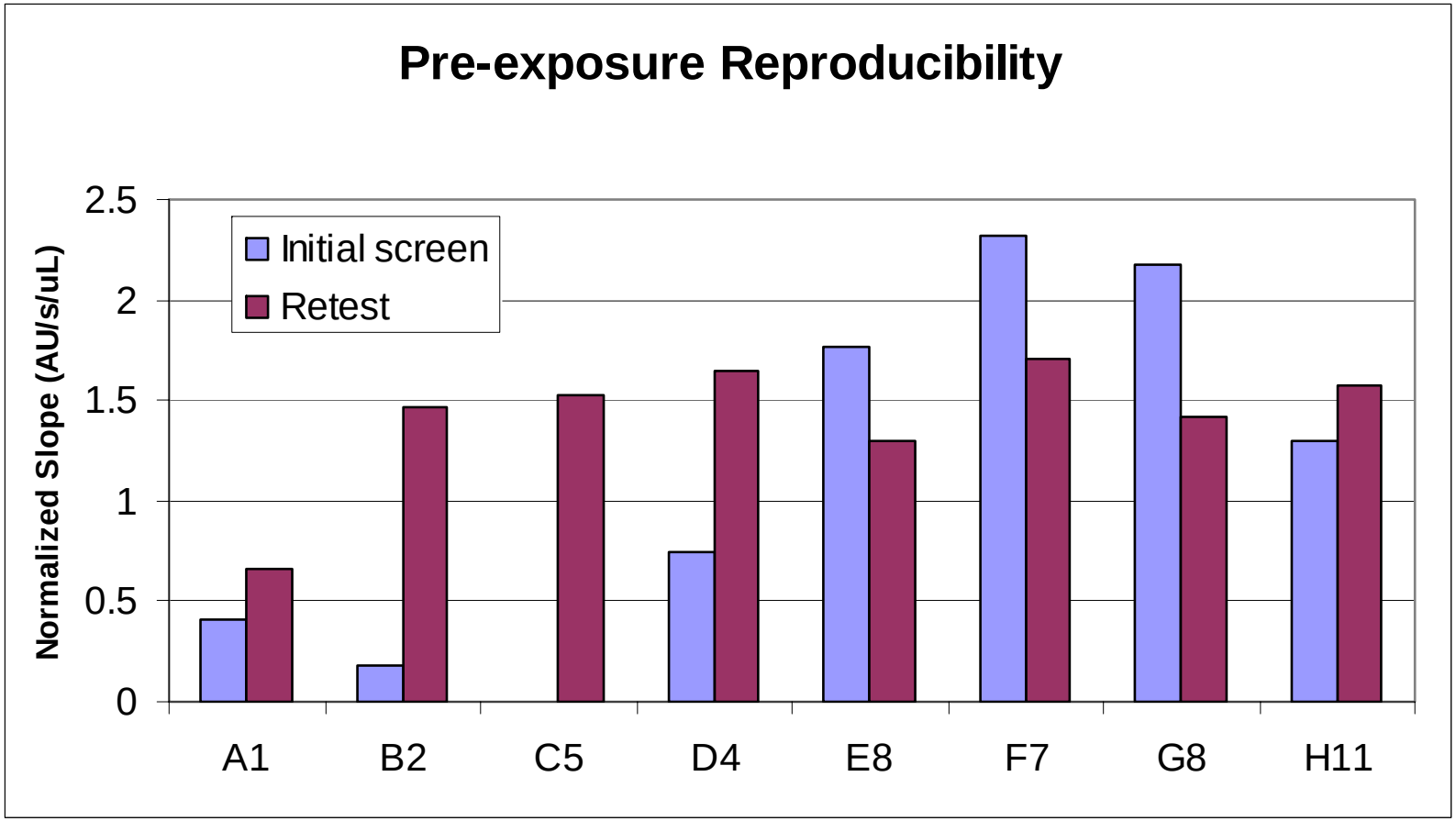
Marcus Boyer and Jonny Kuchenreuther

Screening Strategy



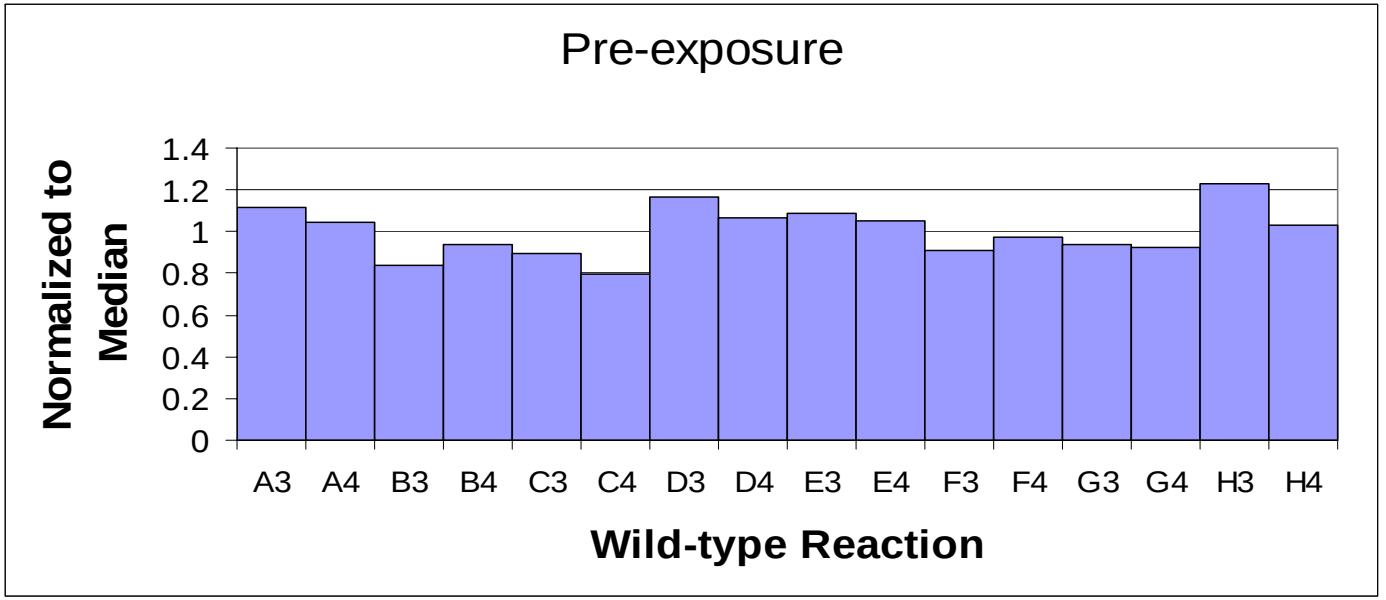
Jim Stapleton

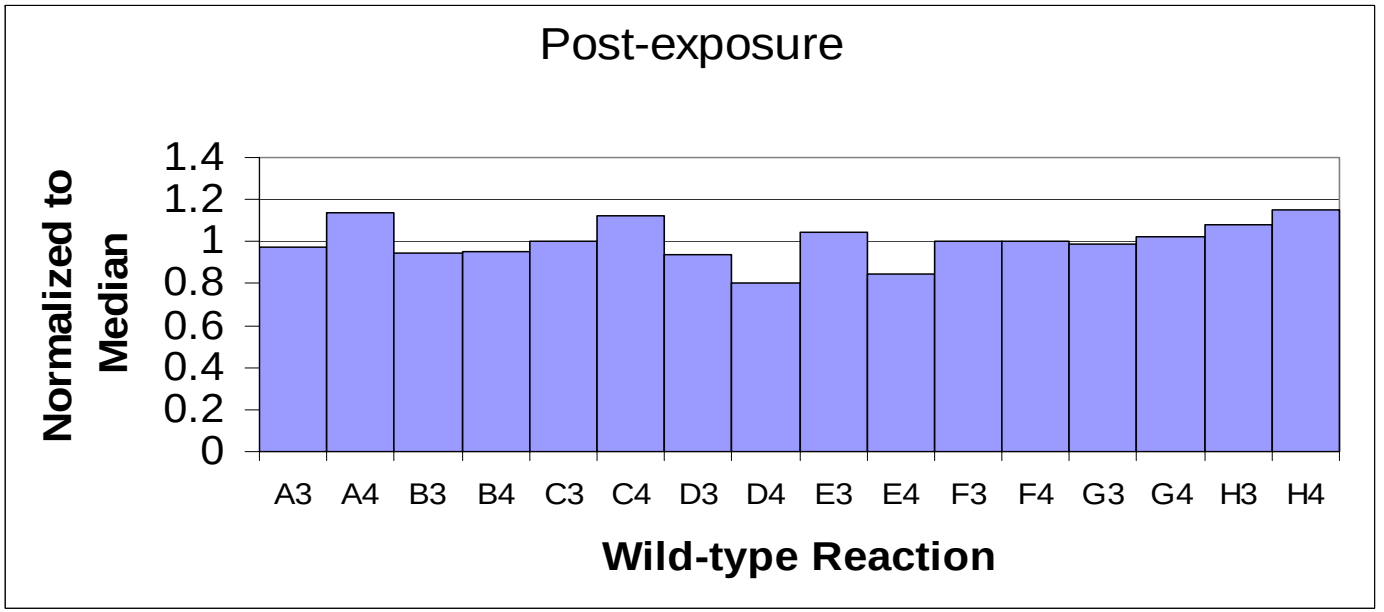
Initial Screening Assays Were Highly Variable



For Unmutated Hydrogenase

We are Working to Make the Screen Performance More Reproducible





Jim Stapleton,

Sean Kendall,

Phil Smith

Conclusions

- Solar BioHydrogen Appears to be Technically and Economically Feasible
- BUT, We must first Evolve an Oxygen Tolerant Hydrogenase
- We can express Fe-Fe Hydrogenases With Cell-Free Technology Using Activated Cell Extracts
- We can accomplish Single-Molecule PCR for Clonal Separation
- GTP, SAM, and NAD are Required for H_2ase Activation
- We Have Gained Significant Knowledge To Help Us Express The Hydrogenases in Photosynthetic Organisms
- The Search for Oxygen Tolerant Hydrogenases is Underway

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- Stanford School of Engineering
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- Stanford Global Climate and Energy
  Program (GCEP) for Major Funding