Creation of Designer Alga for Efficient and Robust Production of H_2

James W. Lee[1]*, Dong Xu[2], Barbara R. Evans[1], Laurens Mets[3], Jizhong Zhou[1], Bin Zhao[1], and Weimin Gao[1]
[1]Oak Ridge National Laboratory
[2]University of Missouri-Columbia
[3]University of Chicago

*Corresponding email: Leejw@ornl.gov, tel: 865-574-1208, fax: 865-574-1275

May 23-26, 2005

This presentation does not contain any proprietary or confidential information

Project ID #: PD17

Overview

Timeline

- Project start date: 08/2004
- Project end date 09/2008
- Percent complete 20%

Budget

- Total project funding
  - DOE share 100%
  - Contractor share
- Funding received in FY04: $100K
- Funding for FY05: $600K

Barriers

- Barriers addressed
  - J. Rate of Hydrogen Production. **The current hydrogen production rate from photosynthetic micro-organisms is far too low for commercial viability. Changes to these organisms, such as the genetic insertion of a proton channel into the thylakoid membrane, are required to overcome the restricting metabolic pathways to significantly increase the rate of hydrogen production.**

Partners

- University of Missouri-Columbia (D. Xu)
- University of Chicago (L. Mets)
- NREL (M. Ghirardi and M. Seibert) and UC Berkeley (T. Melis)

Objectives

Long-term objective:

Overcome nation's roadblocks to photosynthetic H2 production through creation of designer alga by genetic insertion of a proton channel into algal thylakoid membrane—to solve the four proton gradient-related problems in algal H2 production—to meet DOE H2 Program goal ($10/MMBtu).

FY05 objectives:

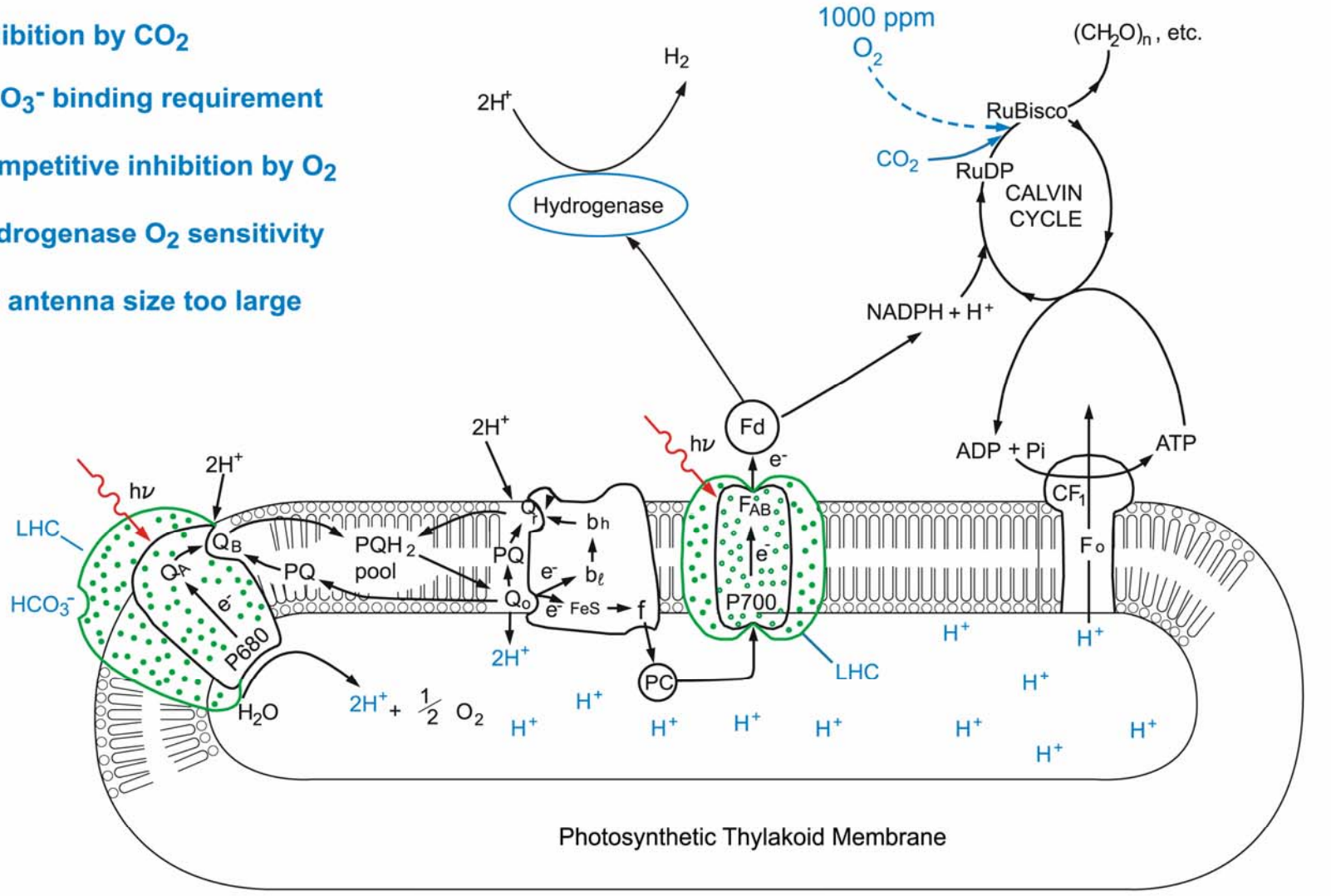
(1) Perform computer-assisted design of DNA sequence coding for a proton channel suitable for targeted insertion into algal thylakoid membrane (Task 1.4.1 described in the DOE-EERE/ORNL AOP)

(2) Synthesize the proton-channel gene linked with hydrogenase promoter and thylakoid-signal-polypeptide DNA (Task 1.4.2 described in the DOE-EERE/ORNL AOP)

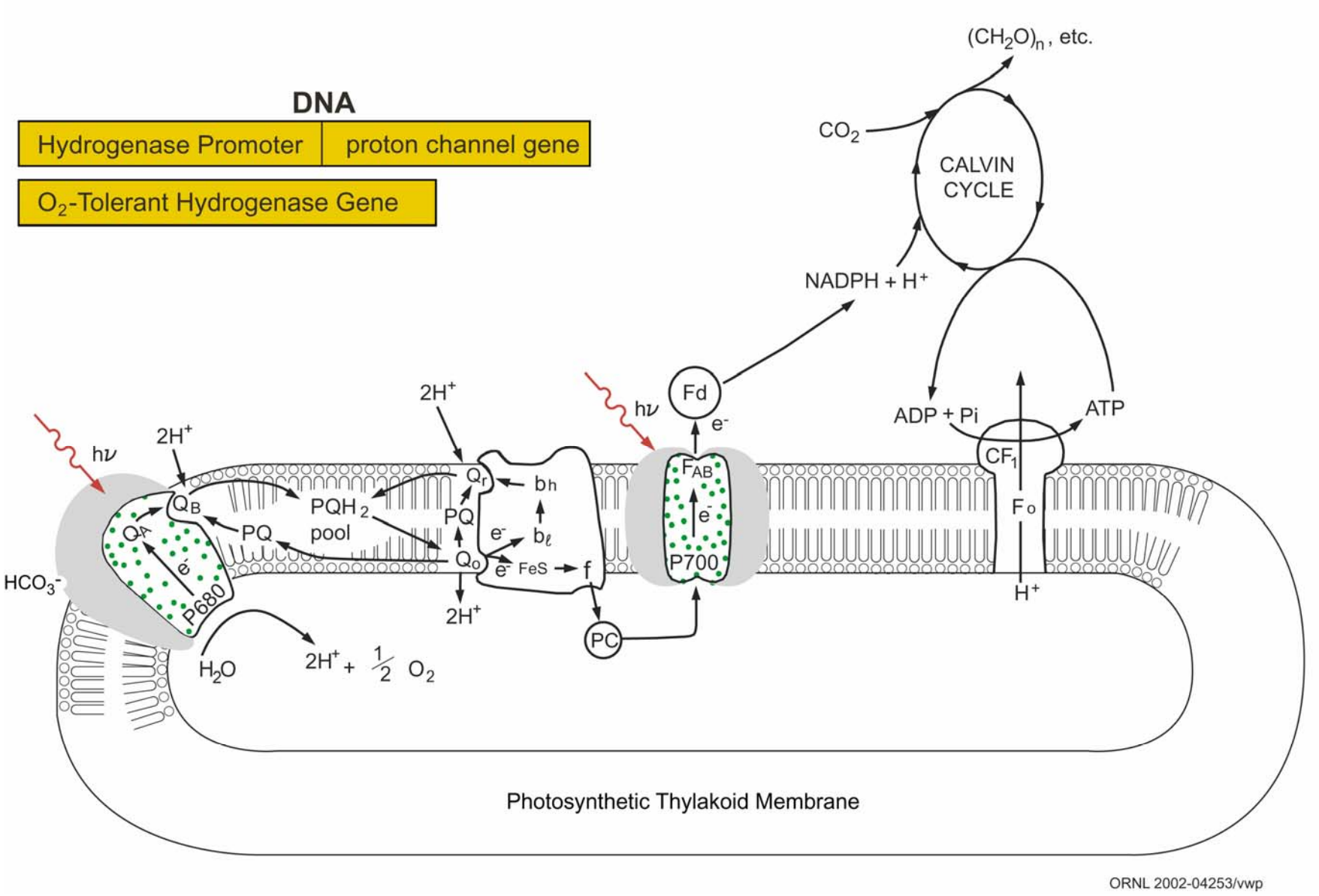
Approach:

The ORNL algal H2 project will solve the first four problems (1–4) while NREL and UC Berkeley will solve problems 5 and 6

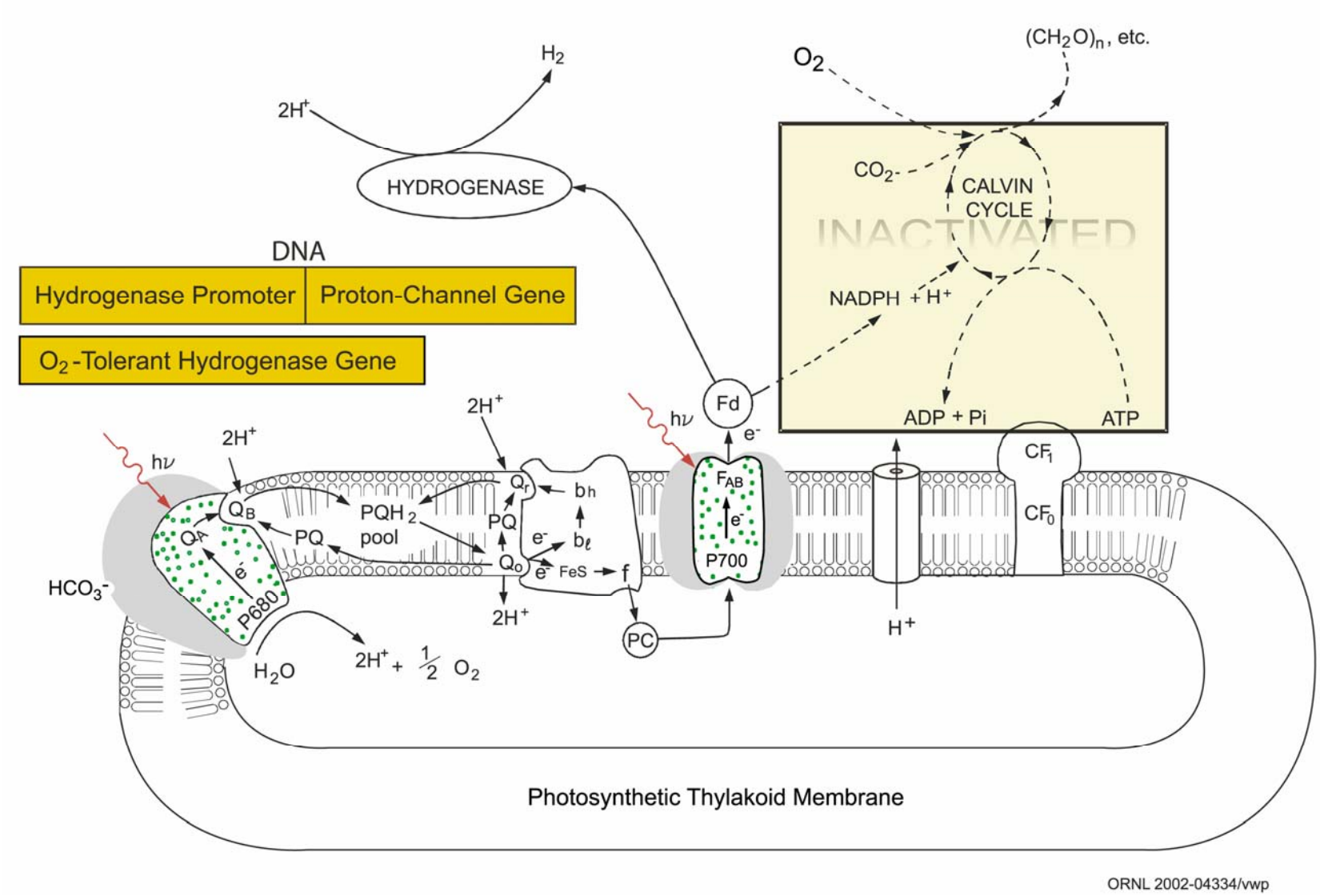
1. Proton (H^+) accumulation
2. Inhibition by CO_2
3. HCO_3^- binding requirement
4. Competitive inhibition by O_2
5. Hydrogenase O_2 sensitivity
6. RC antenna size too large



ORNL-Invented Concept: Designer Alga Performing Normal Photosynthesis under Aerobic Conditions



Solution: Designer Alga Becomes an Efficient and Robust H_2-Production System under Anaerobic Conditions



The ORNL Approach

To create switchable proton-channel designer alga through genetic insertion of proton channels into algal thylakoid membranes to simultaneously eliminate the four proton-gradient physiological problems that constitute the technical barrier "J. Rate of Hydrogen Production":

(1) Restriction of photosynthetic H2 production by accumulation of a proton gradient;

(2) Competitive inhibition of photosynthetic H2 production by CO2;

(3) Requirement of bicarbonate binding at PSII for efficient photosynthetic activity; and

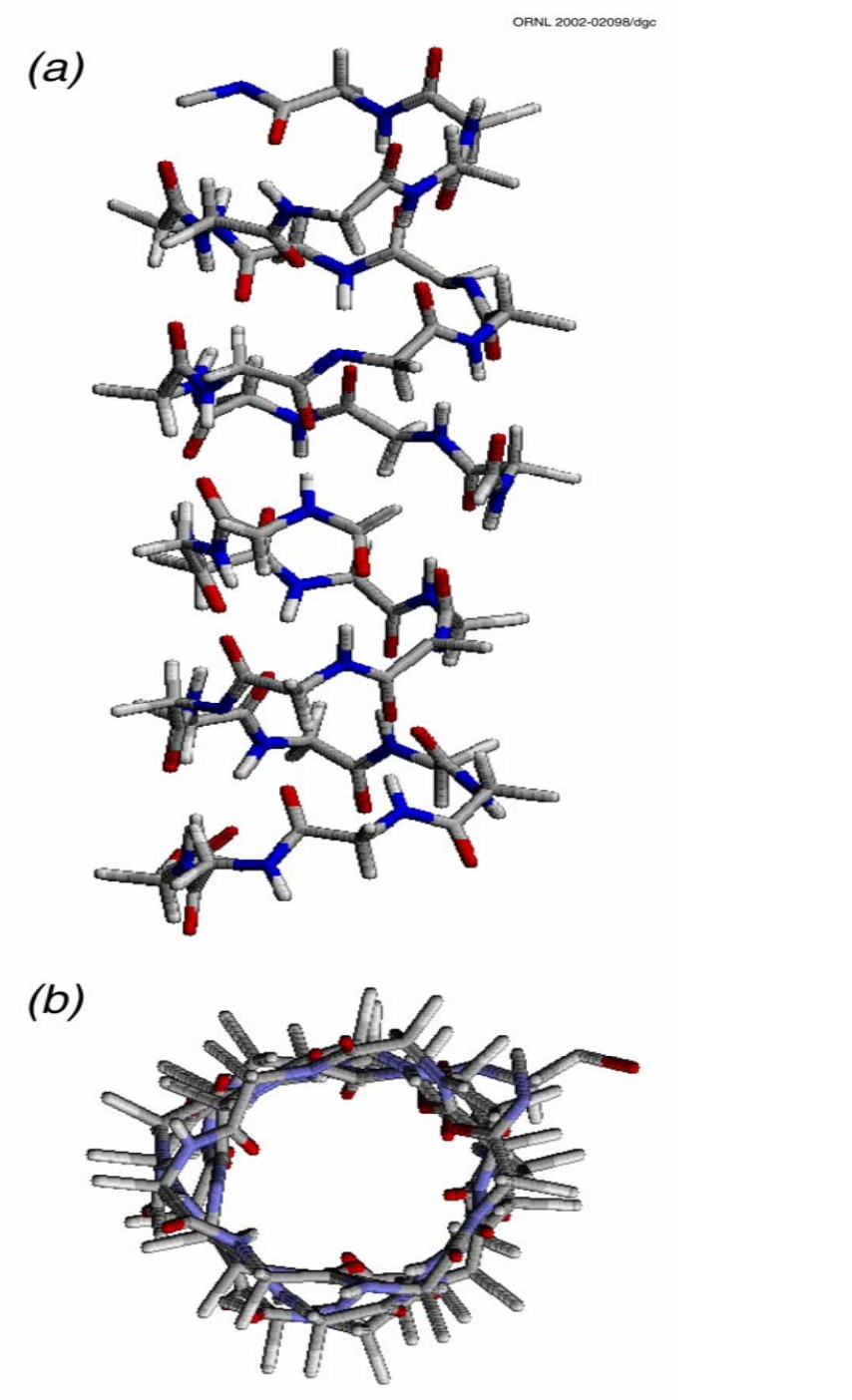
(4) Newly discovered O2 sensitivity (drainage of electrons by O2) in algal H2 production.

Technical Accomplishments/ Progress/Results

- Accomplished computer-assisted design of DNA sequences for the first set of the envisioned proton-channel genes;

- Synthesized the designed proton-channel genes linked with hydrogenase promoter and thylakoid-signal-polypeptide DNA.

A Preliminary Design of Polypeptide Proton Channel Achieved by Computer Simulations at ORNL



Accomplished: DNA Design for Synthetic Gene to Encode for a Proton Channel (gramicidin analog) in Algal Thylakoid Membrane

Design No. 1 for Expressing Gramicidin Analog

Hase promoter + *RbcS1* transit peptide + Gramicidin Analog + "natural" 3 UTR

Sequence: **570** bp

CCCACCGCTGTTTCTCCTGGATTTATGGATTTTTATACTGGCATCTTTCAAGTCACGGAA
AAAGCGCGCGCTTCCGACGAAGGTAGGGCTGCACATGGCGAGACCTGCAGCTCAGCAT-

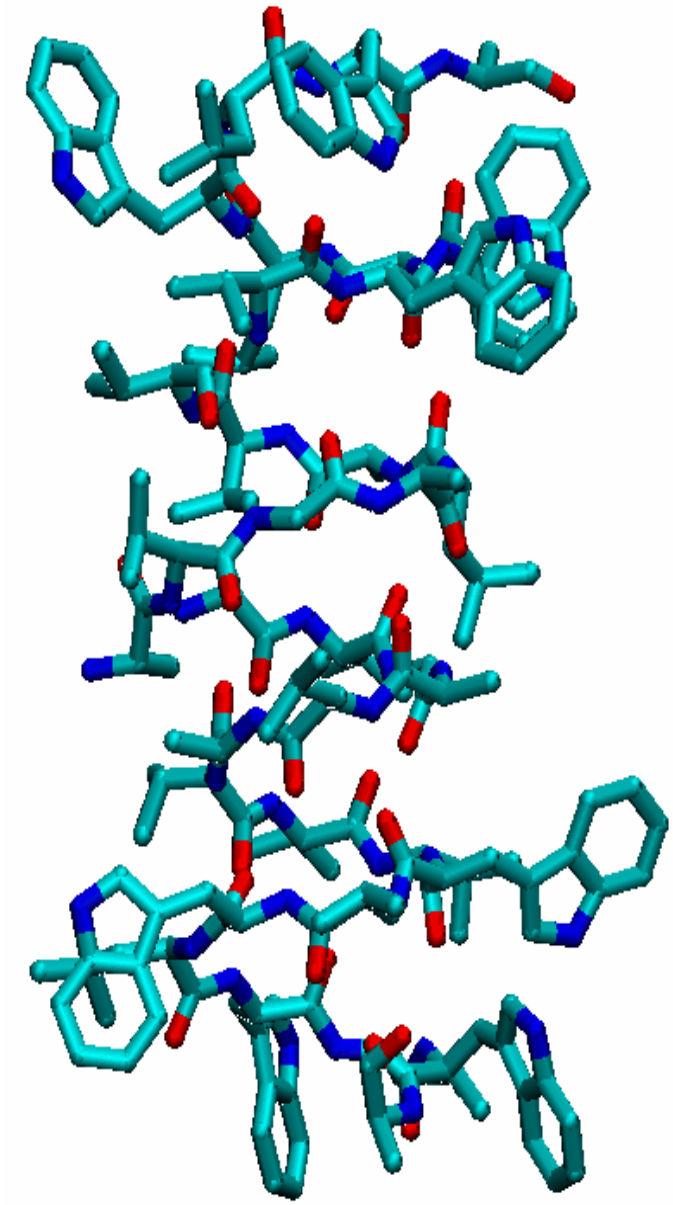
CGTTCTCATTCCGCCATTCCTACTGGCGCCTTTAAATGGCAGGACCGCATCCAAGCTTAA
ACAATCTGTTCAAATATACAAGTGC**cat**ATGGCCGCCGTCATTGCCAAGTCCTCCGTCTCC

GCGTCAAGGCTGCCCCCGTGGCTGCCCCGGCTCAGGCCAACCAGGCCGTGGGCGCCCTG

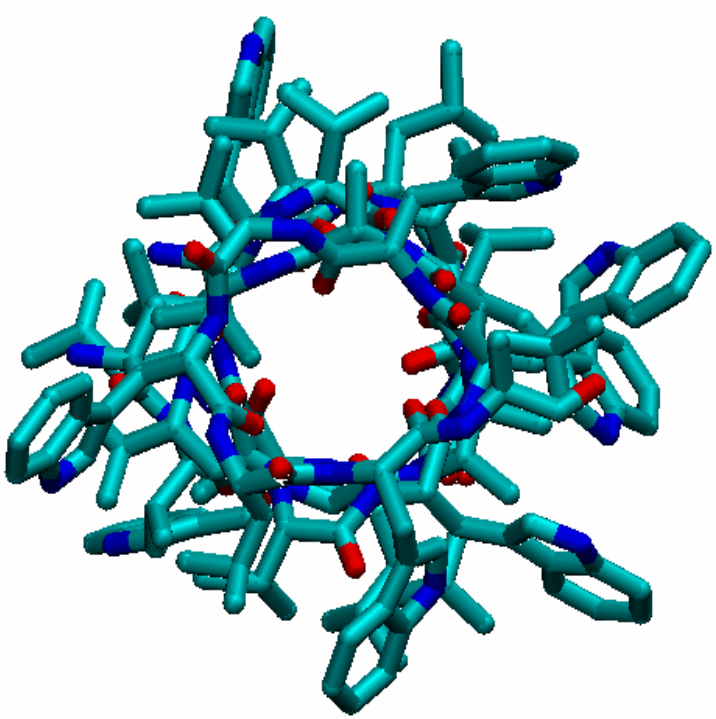
GTGGCTGTGGCTGTGGGCC*TAAG*CAGTTGACATGTTTTGG----------
ATGTAACATCCCGTGTGCA---

Our latest Design of Polypeptide Proton Channel Achieved by Computer Simulations in collaboration with Prof. D. Xu

Side View



Top View



Accomplished: DNA Sequence Design for Another Synthetic Gene to Encode for a Proton Channel (Melittin) in Algal Thylakoid Membrane

Design No. 2 for Expressing Melittin

Hase promoter + Plastocyanin transit peptide + Melittin + “natural” 3 UTR

Sequence: **603**bp

CCCACCGCTCTTTCTCCTGGATTTATGGATTTTTATACTGGCATCTTTCAAGTCACGGAA
AAAGCGCGCGCTTCCGACGAAGGTAGGGCTGCACATGGCGAGACCTGCAGCTCAGCAT-

CGTTCTCATTCCGCCATTCCTACTGGCGCCTTTAAATGGCAGGACCGCATCCAAGCTTAA
ACAATCTGTTCAAATATACAAGTGC**cat***atg*aaggctactctgcgtgcccccgcttcccgcgccagcgctgtgc-
---
gccccgtcgccagcctgaaggccgctgctcagcgcgtggcctcggtcgccggtgtgtcggttgcctctctggccctgaccctggc
tgcccacgcc*Ggca*tcggcgccgtcctg----------
aagcgccagcag*TAAG*CAGTTGACAT----------
ATGTAACATCCCGTGTGCA---

Completed the synthesizing of the first 3 designer proton-channel genes and ready for gene transformation

Reviewers' Comments

- Our reviewers clearly understood our proposed switchable-proton-channel designer alga H_2-production R&D concept. They commented that our approach is "very creative" and "addresses 4 barriers to biological production of H_2".

- They further commented, our project employs an "integrated, well thought out approach" and "could produce a significant breakthrough in biological H_2 production."

- "No cost breakdown or estimate; no attention to balance of plant or implementation"—Proof-of-principle (FCCP) experimental data demonstrated that use of this approach (genetic insertion of proton channel) could improve photobiological H_2 production rate by a factor of more than 10 times. More process economics analysis will follow if (or when) funding support allows.

- "Limited funding"—Thank the reviewers for recognizing this weakness; Hopefully the DOE H_2 Program could provide better funding support for the project.

Future Work

If the required 3.0-FTE project effort can be fully supported, we should be able to achieve the following milestones (tasks) in FY2006:

- Complete the assembly of the constructed hydrogenase promoter- thylakoid signal polypeptide-proton channel gene into a shuttle vector with a selectable marker for *Chlamydomonas reinhardtii* and *E. coli.*

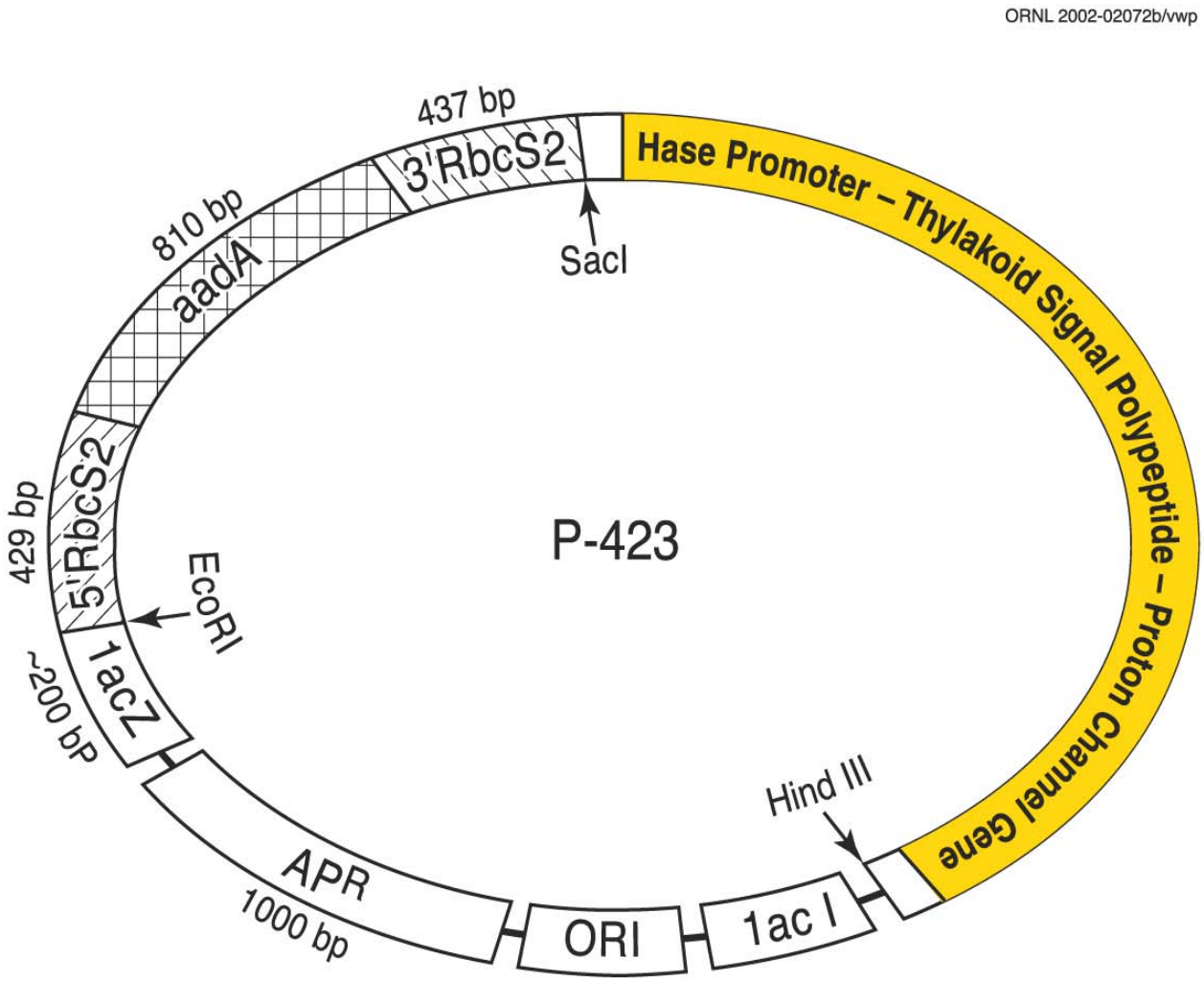
- Accomplish propagation and verification of the DNA sequence for the synthetic hydrogenase promoter-thylakoid signal polypeptide-proton channel gene.

- Achieve genetic transfer of the first hydrogenase promoter-linked polypeptide proton-channel gene (DNA) into a host *Chlamydomonas reinhardtii* strain.

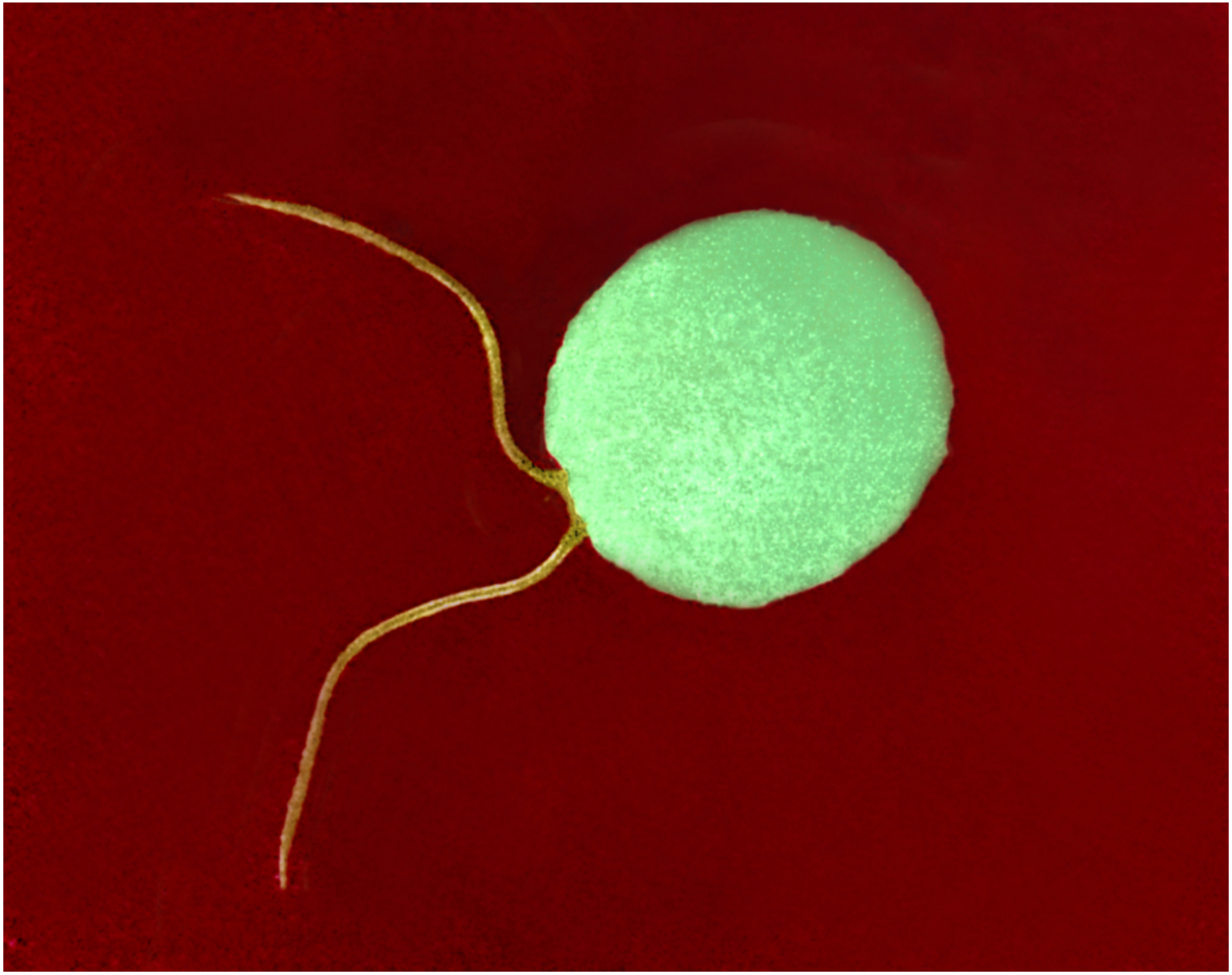
Use of Plasmid Vector for DNA Propagation and Analysis of Our Envisioned Synthetic Genes for Gene Transformation

ORNL will construct
(1st year project goal)

Commercially
availiable



We Can Deliver the Genes (DNA) into Our *Chlamydomonas* Host Cells by Use of Electroporation or Glass-Beads Method



The Transformants will Be Screened and Cultured for a Number of Assays to Test for the Predicted Features of the Designer Alga

DNA Analyzers at ORNL

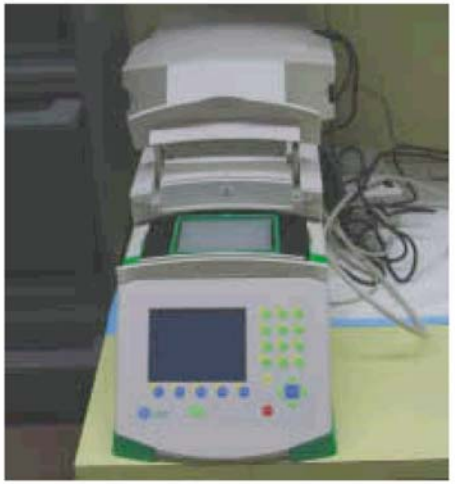
ORNL 2002-01800/vwp

ABI PRISM 3700 DNA Analyzer by Perkin-Elmer Applied Biosystems

- DNA Sequencing and Fragment Analysis
- 96 Capillary Array allows for 100s of sequences a day
- Can sequence 550 base pairs with 98.5% accuracy
- Fragment Sizing within 0.5 bases up to 500 base pairs
- Automated sample loading, electrophoresis and data analysis

HTS 7000 Plus BioAssay Reader by Perkin-Elmer

- DNA and Protein Quantitation
- Curve-fitting options provides tabluar reports of quantittive and qualitative results
- High plate reading speeds (25 seconds/96 well plate)



iCycler Thermal Cycler by Bio-Rad

- Useful for accurate real-time quantitative PCR
- Capable of rapid temperature cycling, heating at a rate of up to 3.3 °C per second and cooling at a rate of up to 2.0 °C per second
- Highly accurate and uniform temperatures
- Real time, on-line displays enable visual confirmation of amplification success

Microarray Equipment for mRNA Assays at ORNL

ORNL 2002-01799/vwp

PixSys 5500XL by Cartesian Technologies

- **High Throughput Arraying-Can prepare 48 microarrays at a time!**
- **32 or 48 ChipMaker quill pins for multiple spotting from a single sample loading**
- **Vacuum wash station for cleaning between transfers**
- **Staker and Destacker (Holds 50 Plates)**
- **Humidity chamber for maintaining humidity and reducing dust**

Scan Array 5000 by GSI Lumonics

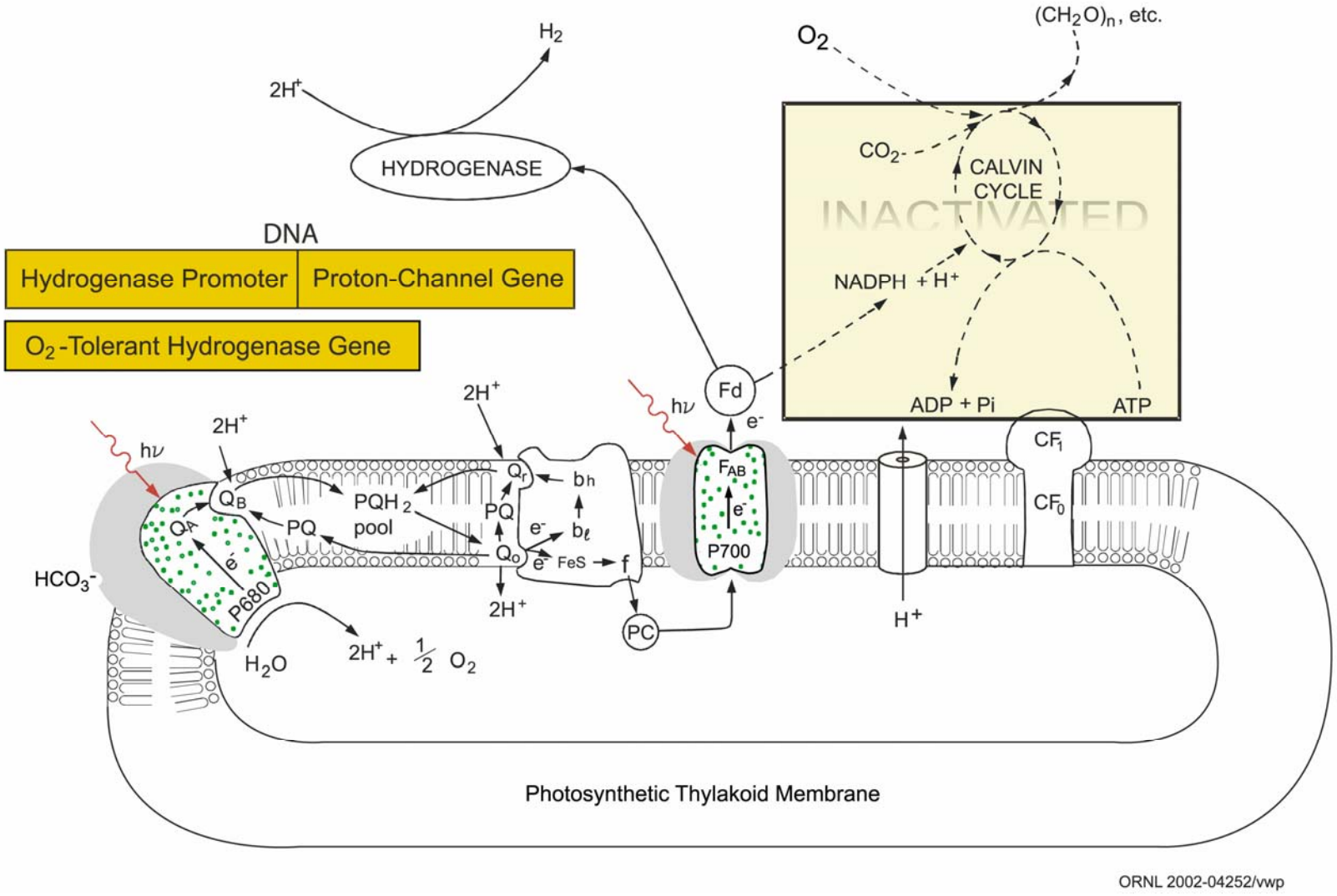
- **Compatible with many fluorescent dyes and labels**
- **(Cy2,Cy3, Cy5, FITC, TAMRA and more)**
- **Dye Choices can be combined to provide wide spectral spacing for 2, and 4 color applications**
- **Dye alternatives can provide higher sensitivity and signal variety**

GeneTAC G3 Robotic Workstation by Genomic Solutions

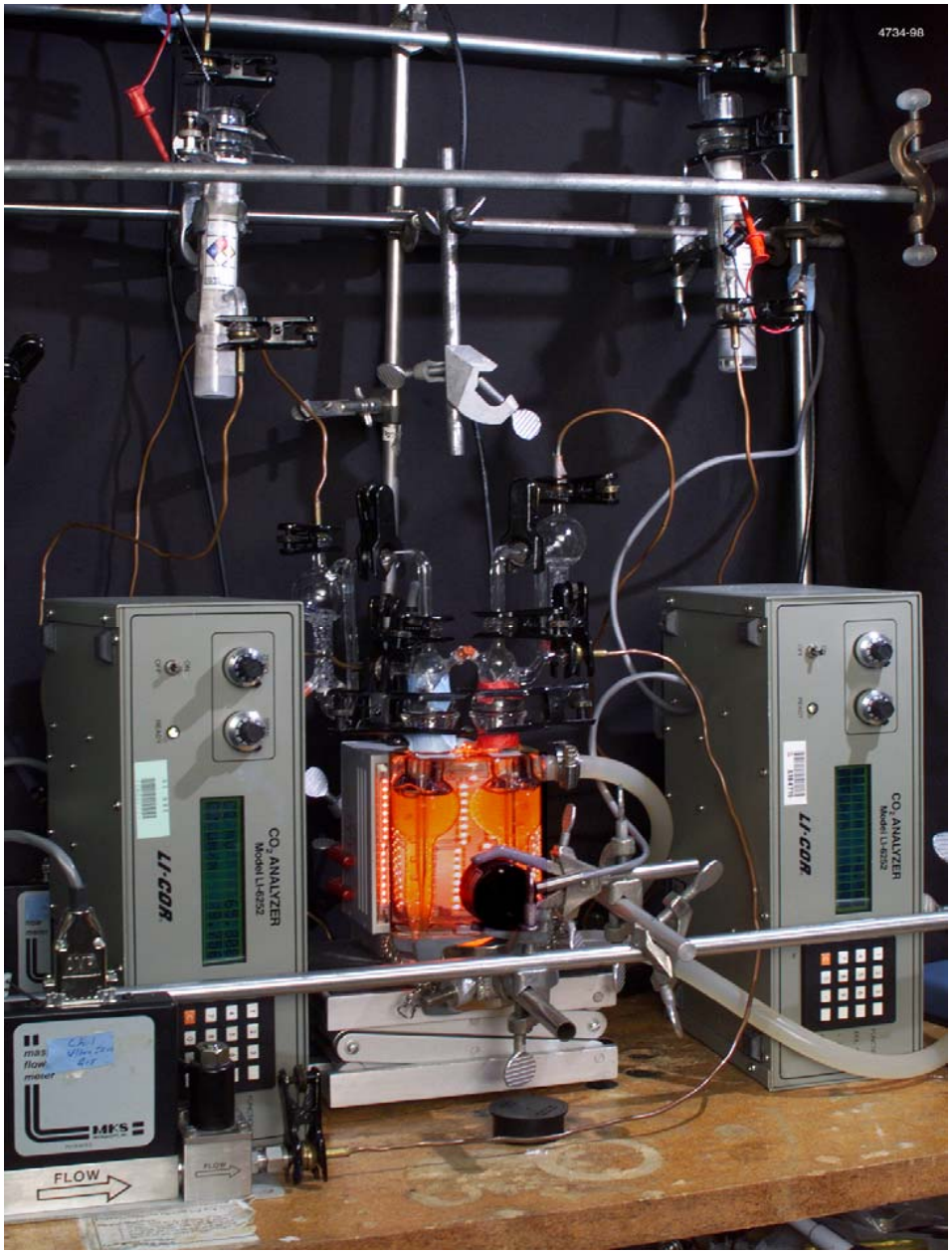
- **For microarray production, library generation, and library management**
- **Gives flexibility for printing microarrays, colony picking, macroarraying, replication, and selective re-arraying**
- **Uses "dip and print" technique so that only 1 nl of sample is used - no wasted slides**
- **Pins are made from solid titanium**

Our Customer-Designed State-of-the-Art Photospectrometer System Can Be Used to Measure the Activity of the Envisioned Polypeptide-Proton Channels in the Designer Alga at ORNL

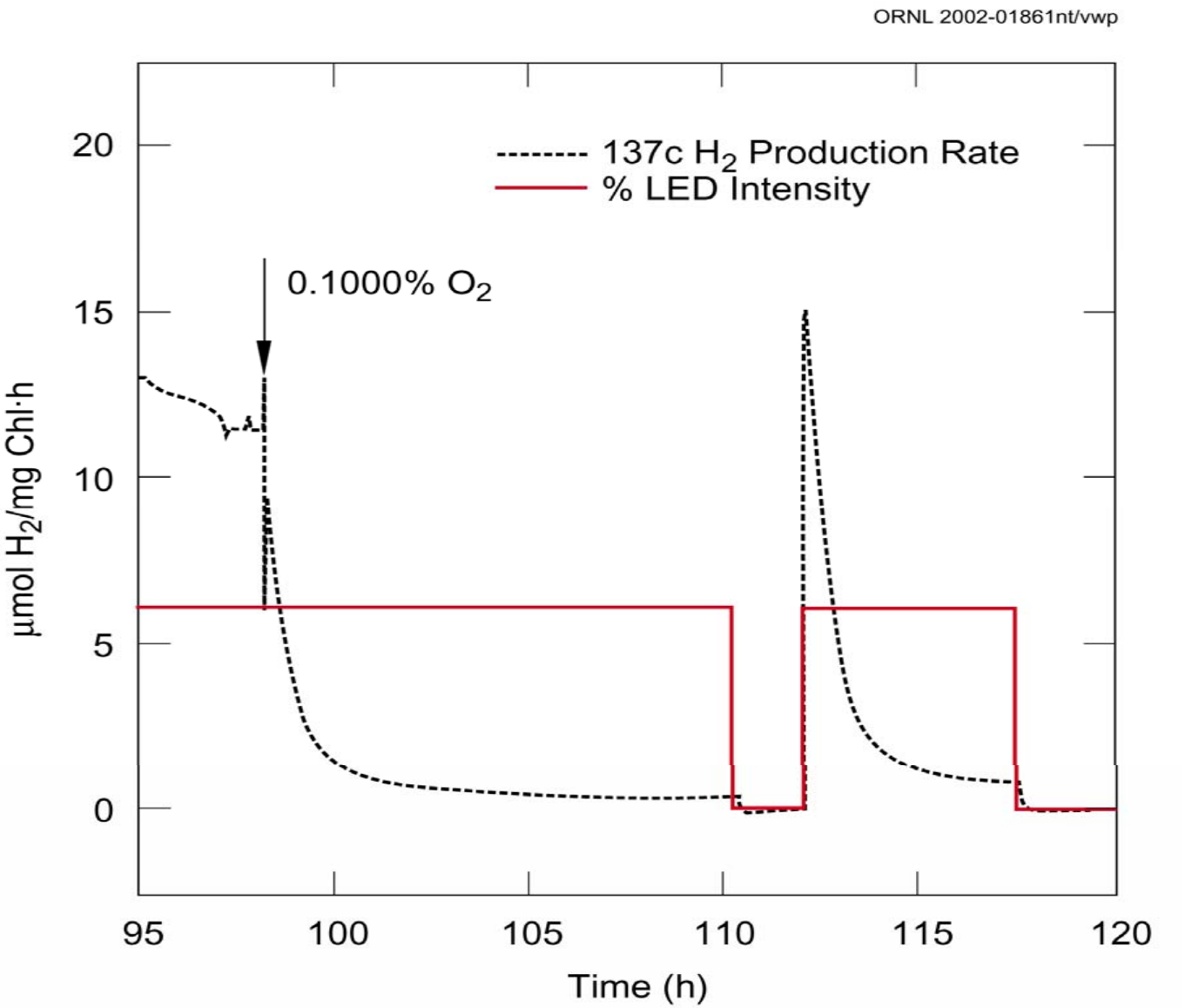
We Will Measure the Effects of the Polypeptide Proton Channels in Designer Alga through Photospectroscopic, Algal-Growth, and H_2-Production Assays



Our Dual-Reactor-Flow Detection System Can Be Used for both H_2-Production and Recyclable-Growth Assays



Property of Our Newly Discovered O_2 Sensitivity in Wild-Type (*C. reinhardtii* 137c) Algal H_2 Production Can Be Used as a Reference to Test the Designer Alga



Path Forward - Milestones

Creation of designer alga for efficient and robust production of H_2

[3.0 FTE effort by Lee, Xu, Evans, Mets, Zhou, and Zhao]

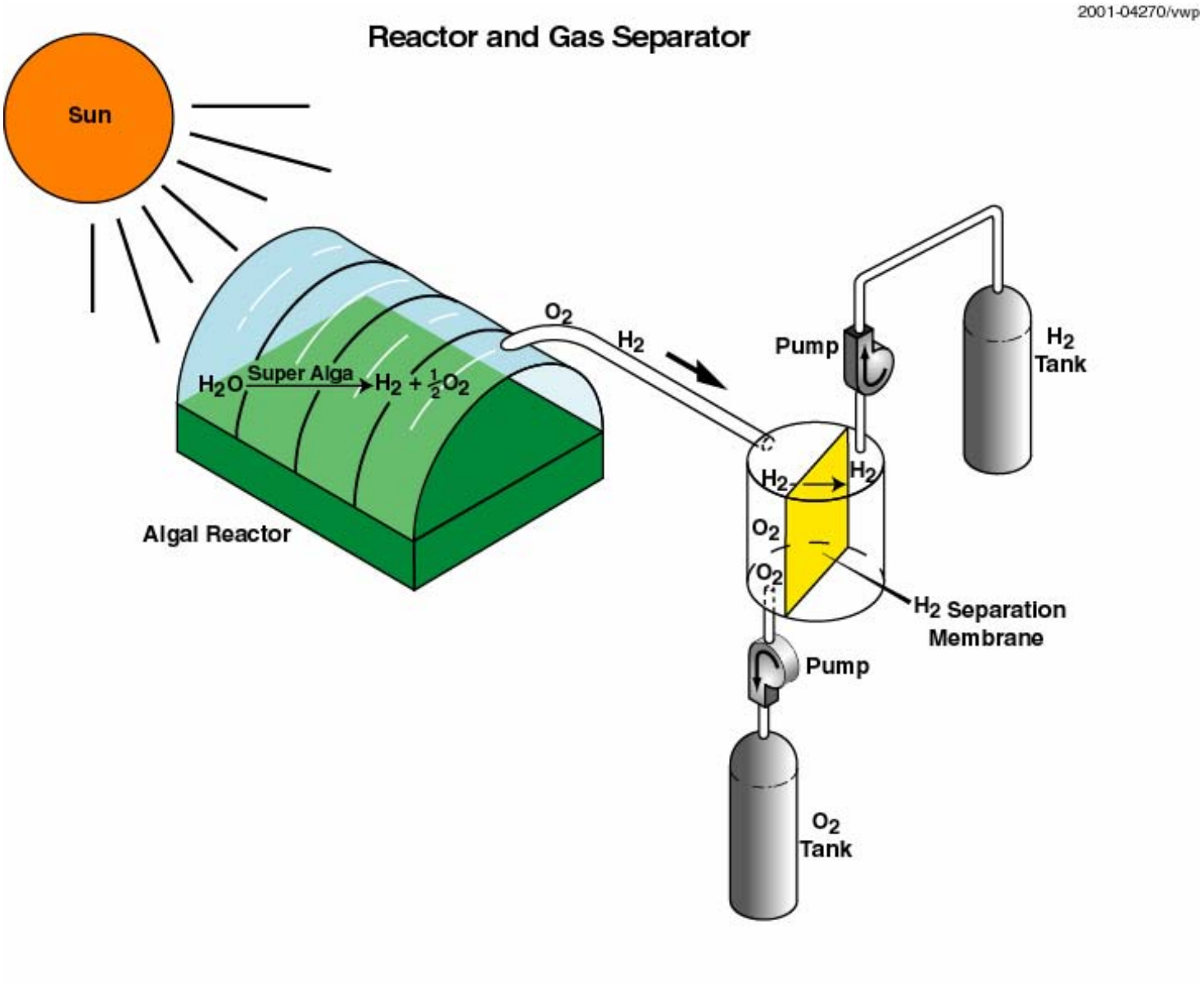
Year 1--Design and construction of DNA sequence coding for polypeptide proton channel **(accomplished for the first set of designer proton-channel genes)**

Year 2--Genetic transfer of hydrogenase promoter-linked polypeptide proton-channel DNA into DS521

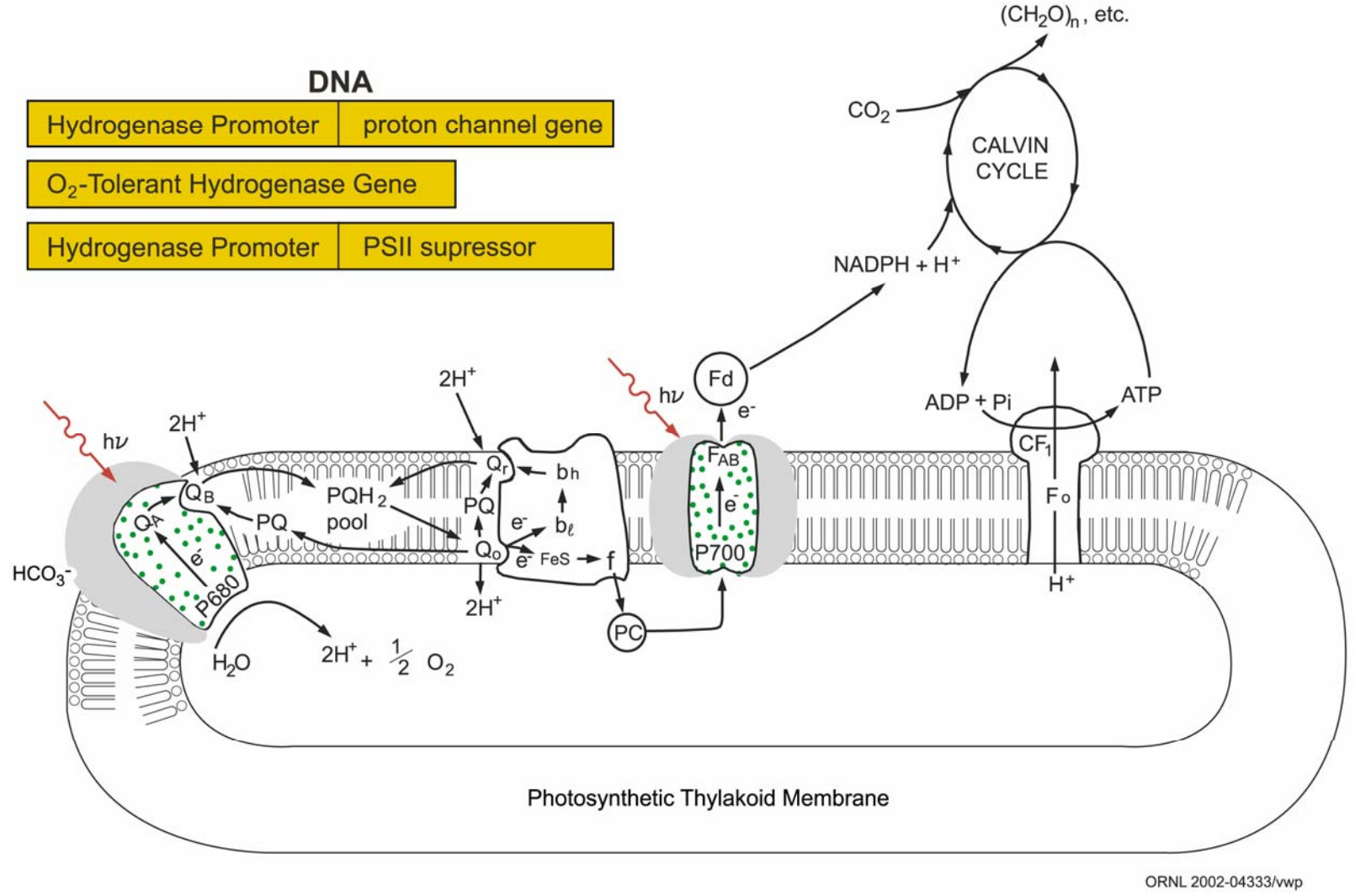
Year 3--Characterization and optimization of the polypeptide proton-channel gene expression

Year 4--Demonstration of efficient and robust production of H_2 in designer alga (ready for next phase: scale up and commercialization)

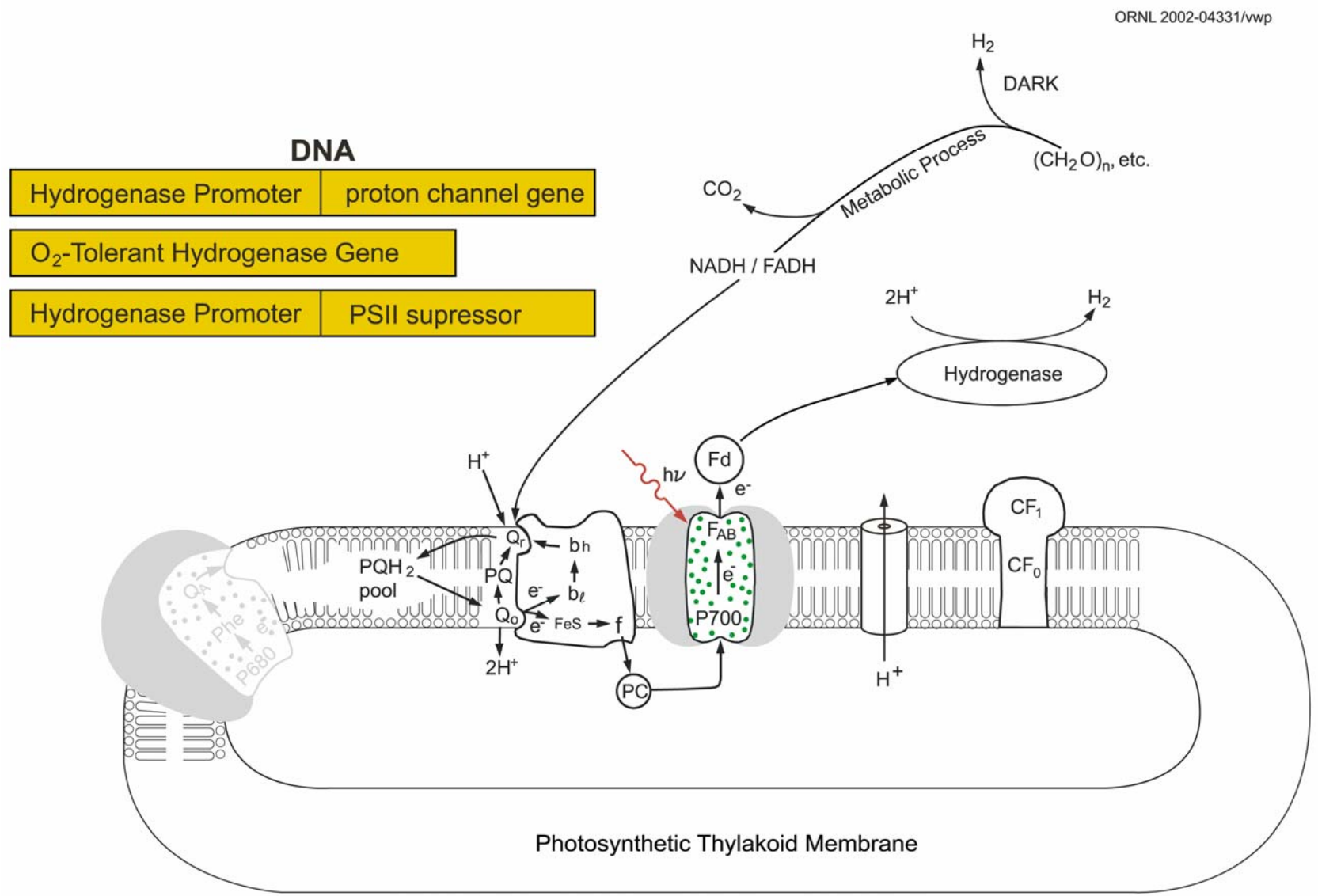
Our Envision (in Part B) How the Designer Alga will Be Used for Clean H_2 Production



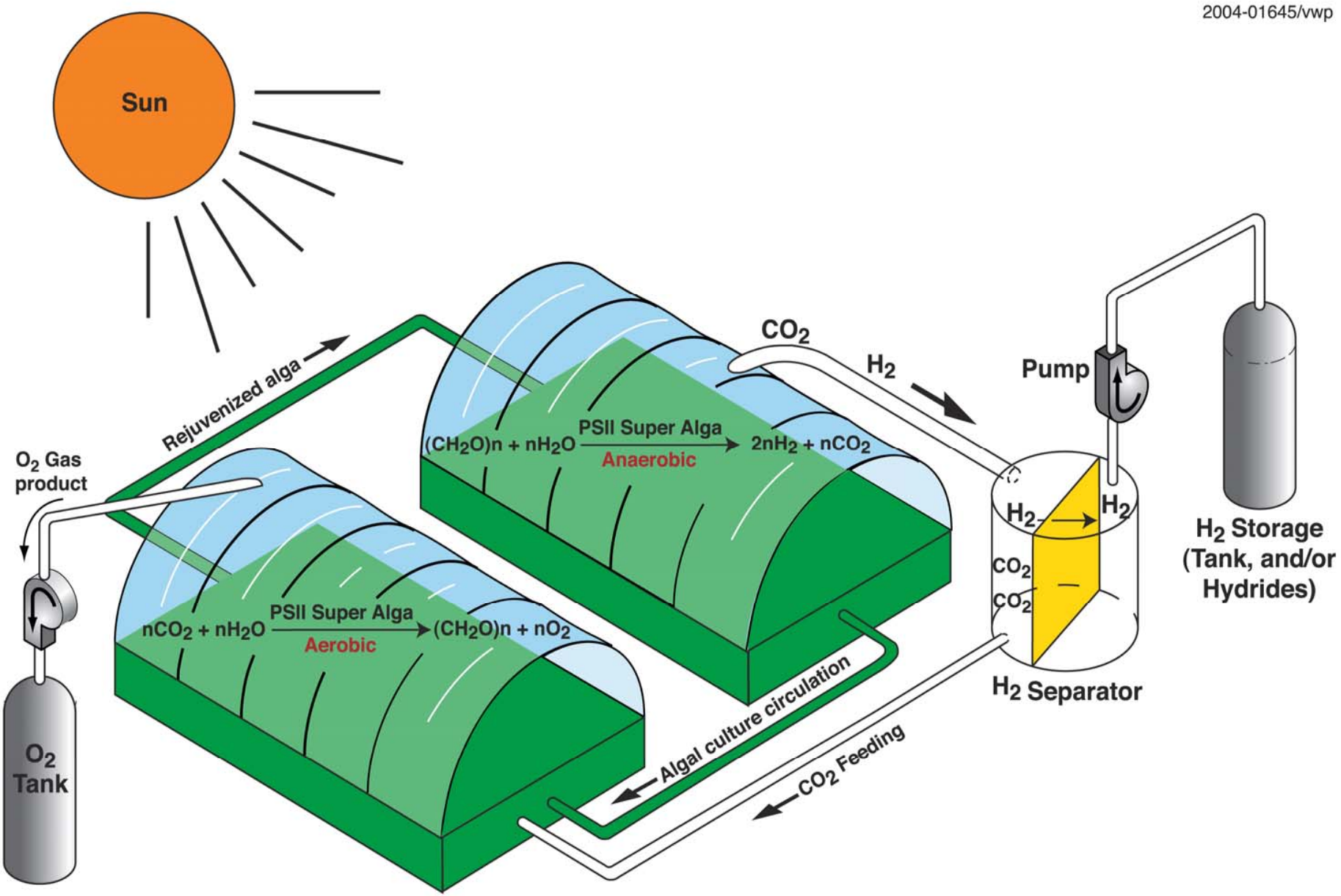
Designer Alga Upgraded with a Hydrogenase Promoter-Linked PSII Inhibitor (Suppressor)



Expression of the Designed Genes for the PSII Inhibitor and the Polypeptide-Proton Channel under Anaerobic Conditions



Envisioned Follow-on Bioreactor Development Project to Apply the Switchable PSII Designer Alga for Clean H_2 Production



Designer Alga H_2-Production Technology with 0.7% U.S. Land Could Provide H_2 Energy ($30x10^{15}$ Btu) for All U.S. Cars

U.S. Total Land	U.S. Cropland	U.S. CRP (Set-aside land)	To produce $30x10^{15}$ Btu of H_2 from H_2O by the Algal Technology
2,300 MM Acres	377 MM Acres	32.7 MM Acres	13.3 MM Acres
100%	16%	1.4%	0.6%

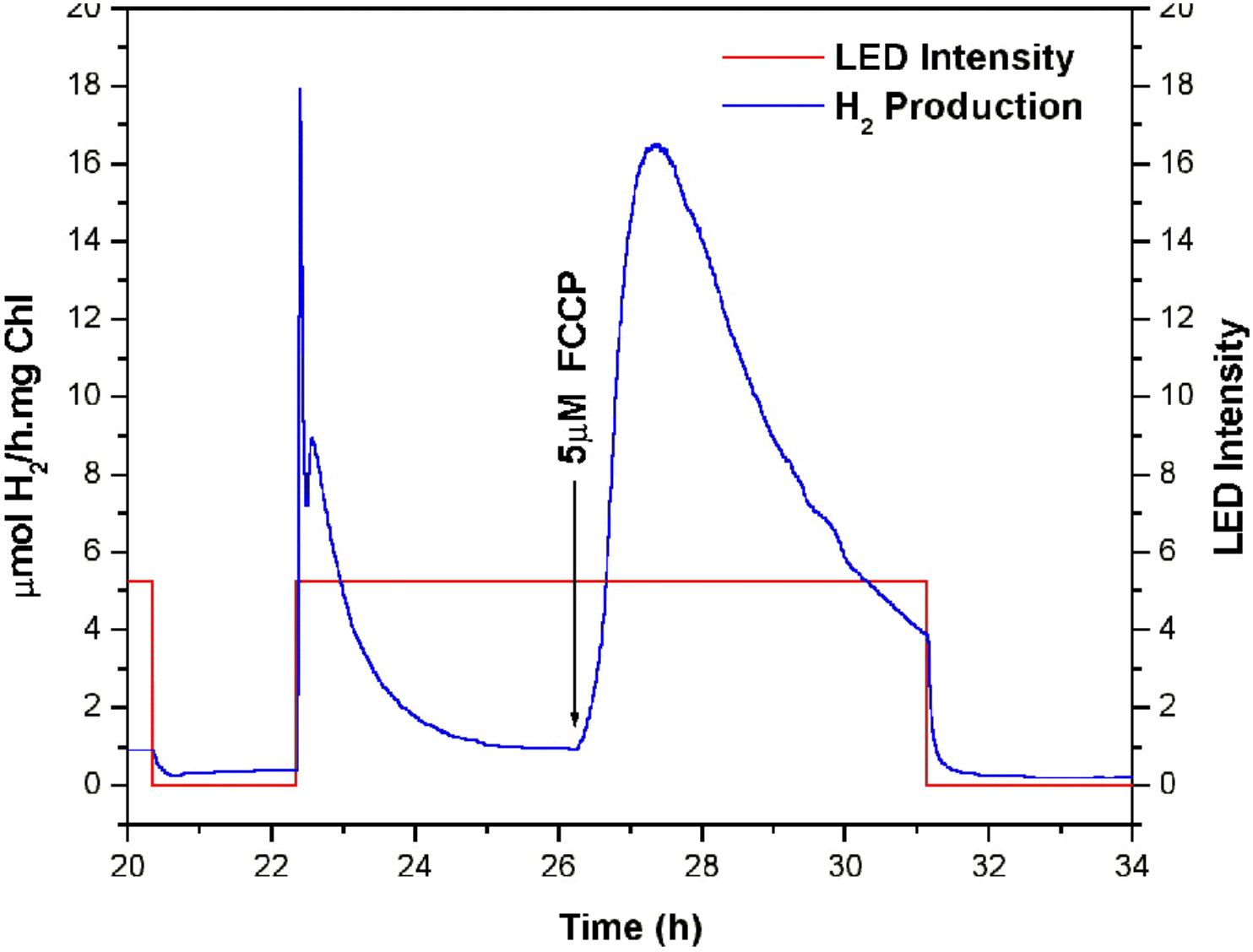
Calculated by Mark Downing and James Lee, using 1997 USDA NASS data and assuming 10% solar energy conversion efficiency for the Designer Alga H_2-production process

Designer Alga H_2-Production Technology Could Be an Attractive New Energy Business

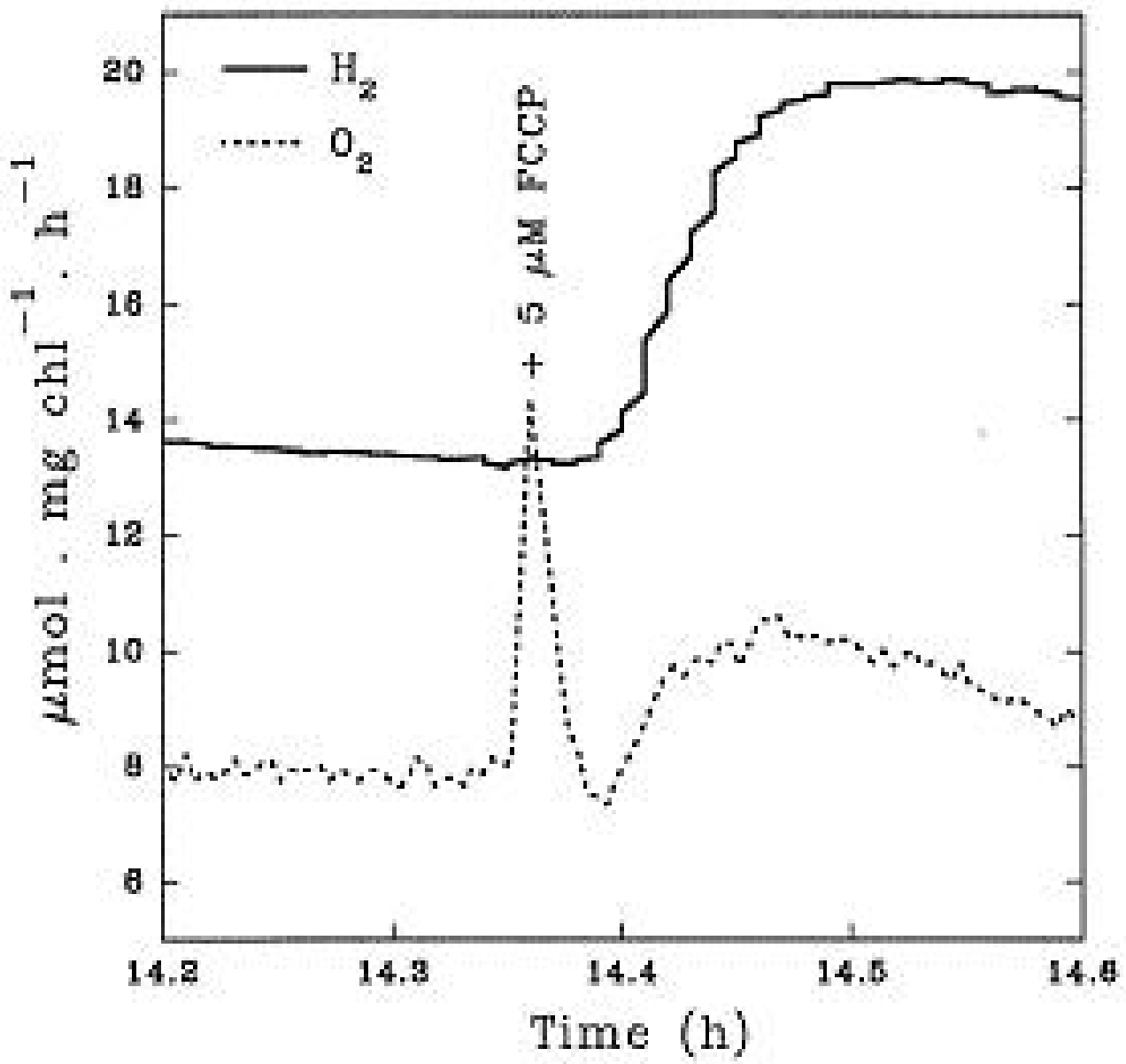
Designer-alga H_2 productivity	H_2 energy value produced	H_2 cash value at production site	Number of cars could be supported
21,519 Kg H_2/acre.year	**2,419 MM Btu/acre.year**	**$18,622/acre.year**	**140 cars/acre.year**

Calculated by Dick Ziegler and James Lee, assuming the value of H_2 at production site will be $1.00 per 115,400 Btu (equivalent to 1 gal of gasoline) and 10% solar energy conversion efficiency for the designer alga H_2-production process

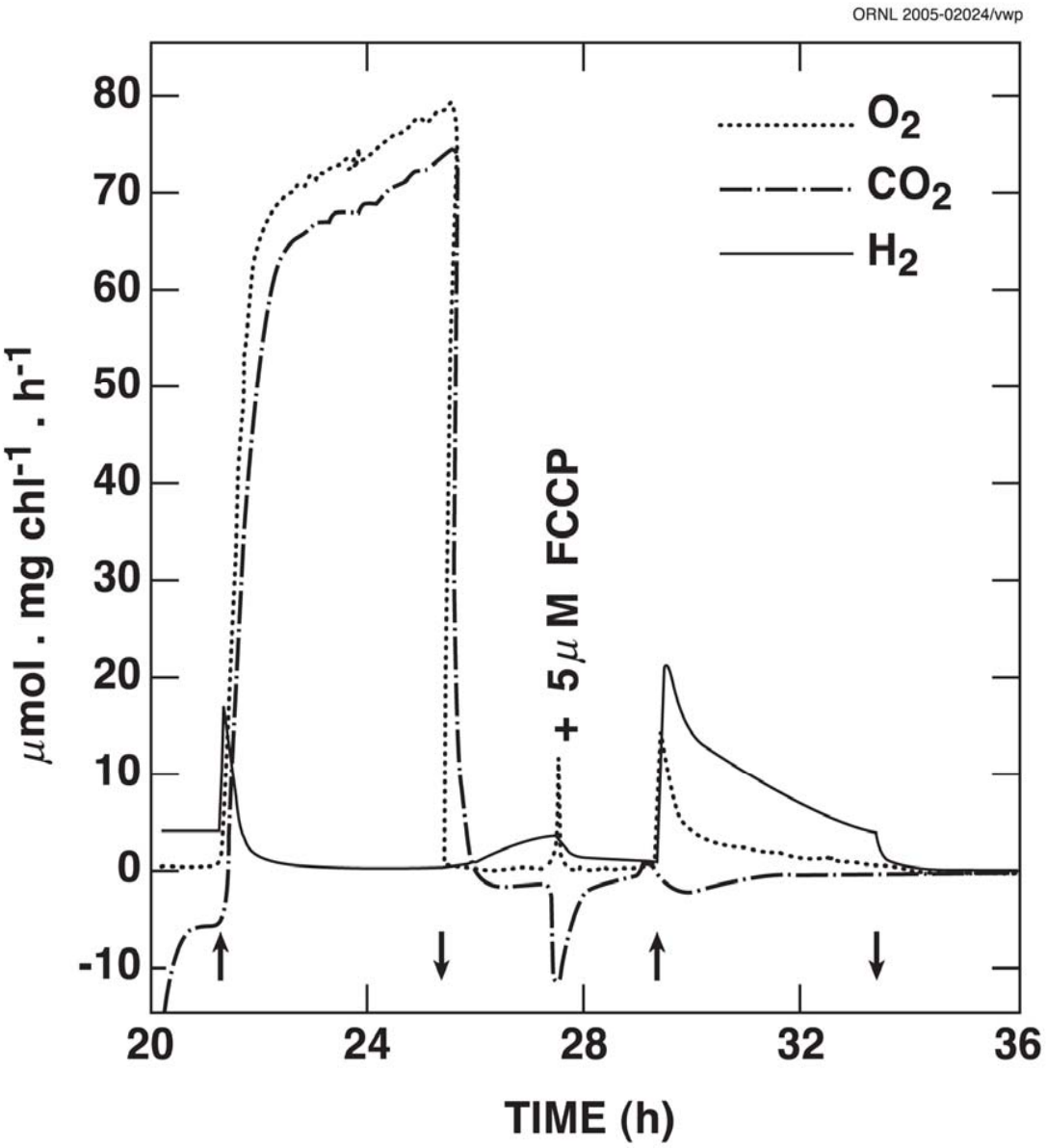
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 Production with 1000 ppm O_2



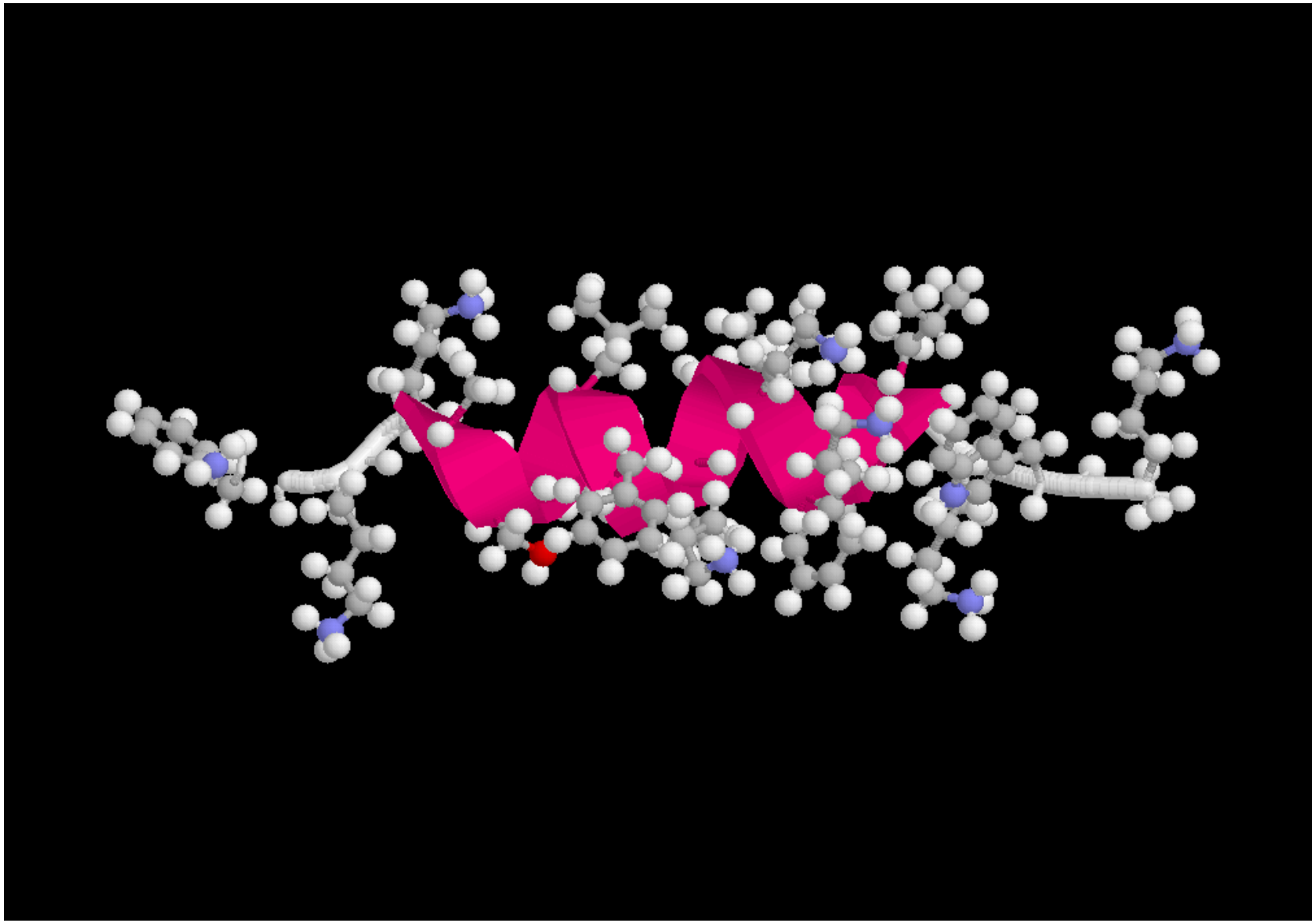
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 and O_2 Production under Helium Atmosphere



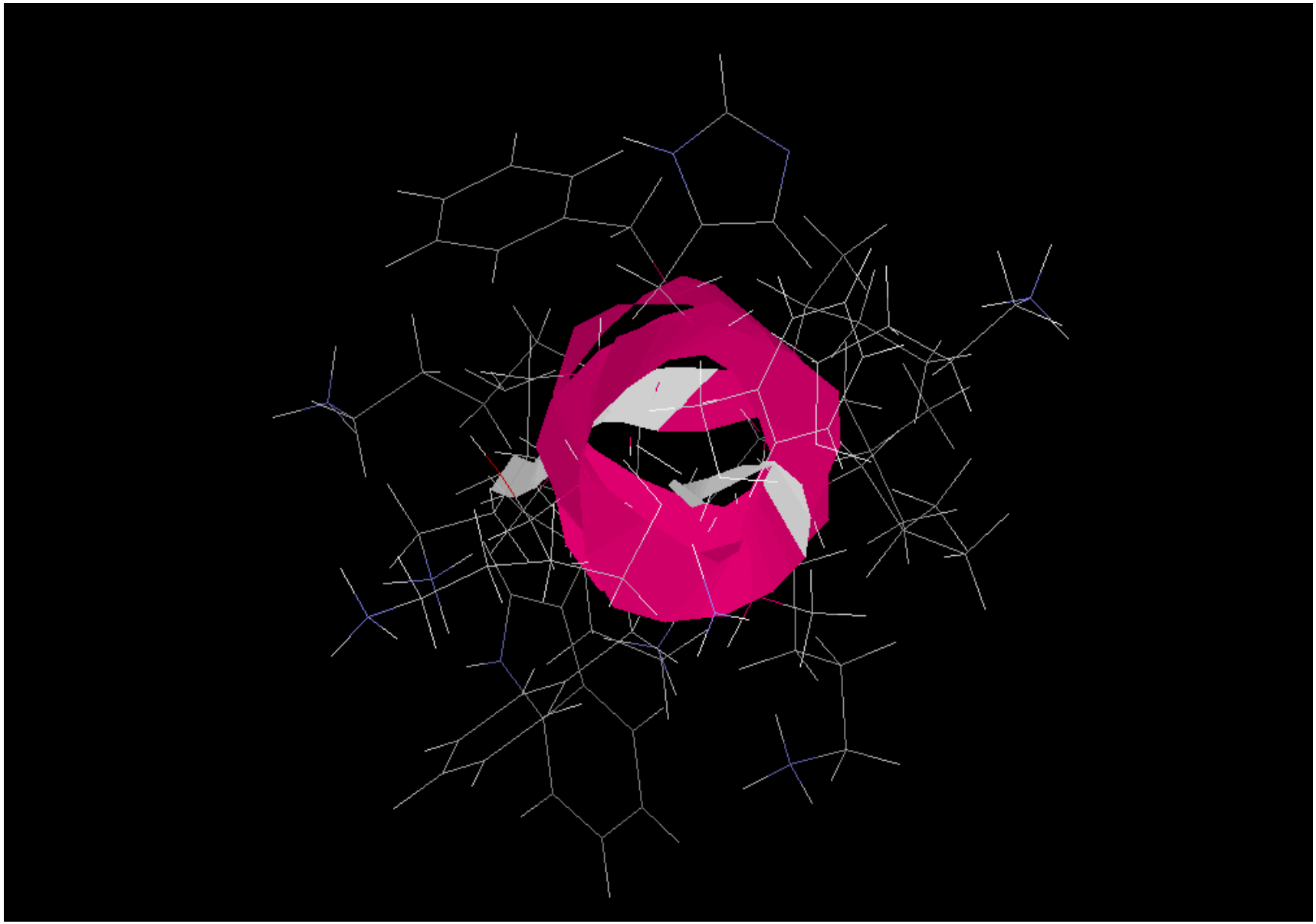
Proof of Principle Demonstrated by Proton Uncoupler FCCP Experiments in Wild-Type Algal H_2 and O_2 Production with 700 ppm CO_2 in Helium



Bioinformatics Analysis of Melittin Using the PROSECT Software



Top View of Melittin Structure Showing Its Channel Pore Size



Preliminary Results:
The Transformants will Be Screened and Cultured for a Number of Assays to Test for the Predicted Features of the Designer Alga