Creation of Designer Alga for Efficient and Robust Production of H_2

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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:

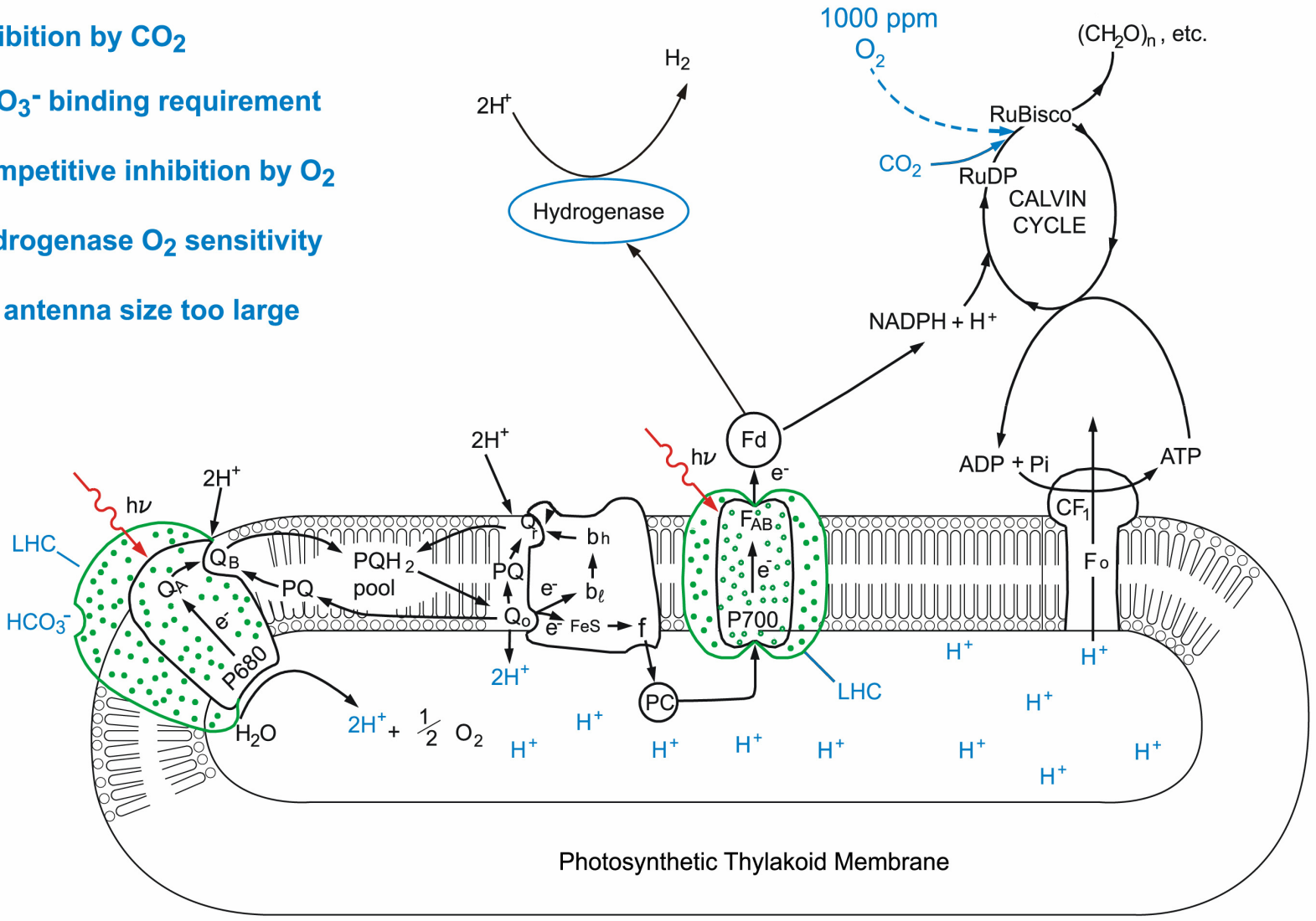
(1) Perform computer-assisted design of DNA sequence coding for a proton channel suitable for targeted insertion into algal thylakoid membrane (Task 1.4.1 described in the DOE-EERE/ORNL AOP)

(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 2002-04335/vwp

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

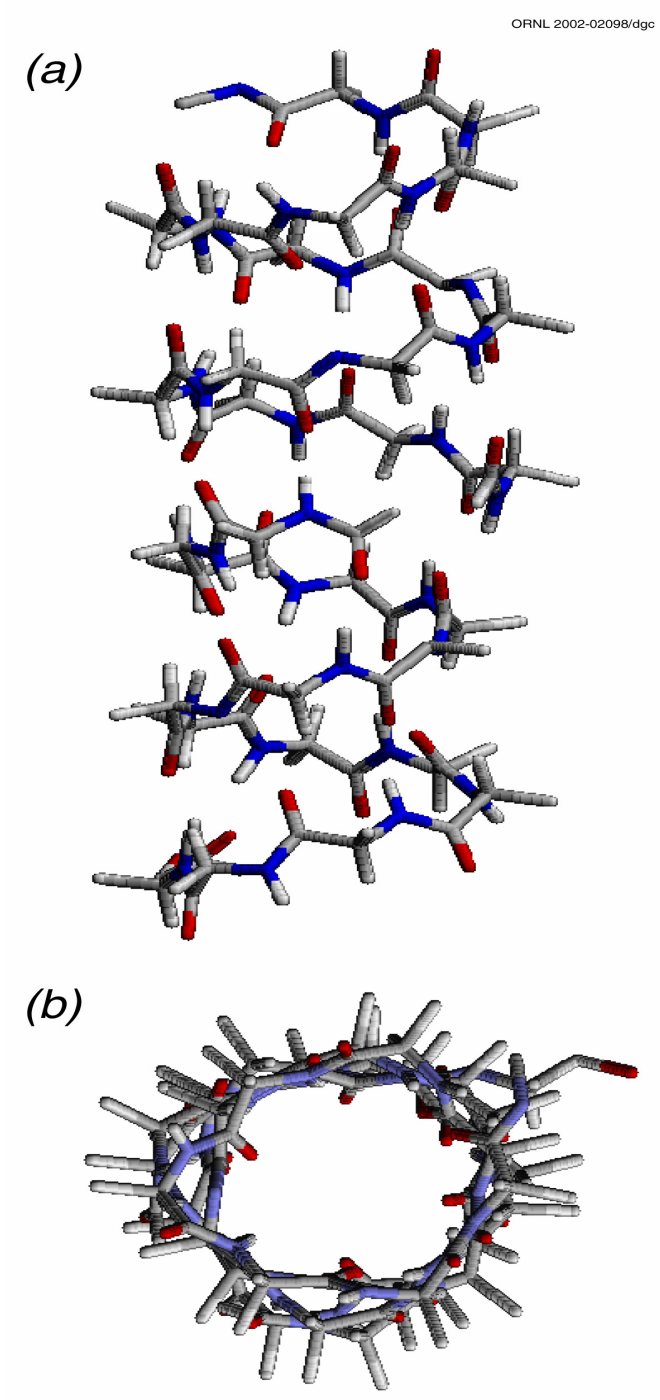
(4) Newly discovered O2 sensitivity (drainage of electrons by O2) in algal H2 production.

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-

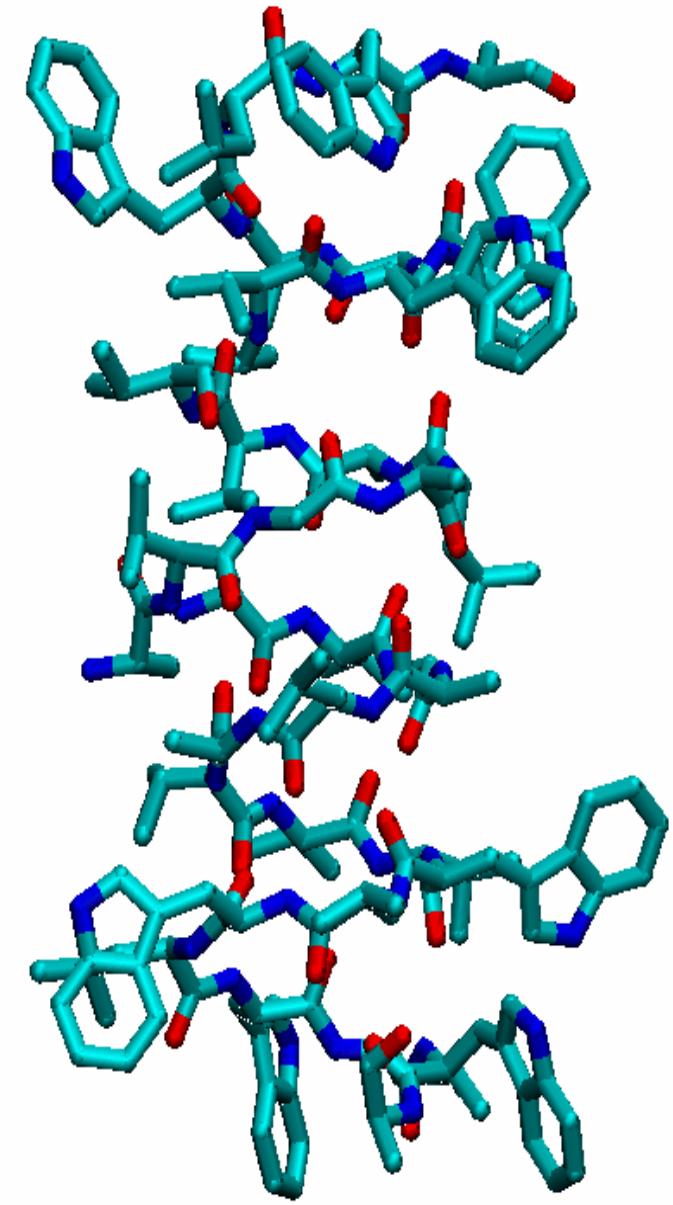
CGTTCTCATTCCGCCATTCCTACTGGCGCCTTTAAATGGCAGGACCGCATCCAAGCTTAA
ACAATCTGTTCAAATATACAAGTGC**cat**ATGGCCGCCGTCATTGCCAAGTCCTCCGTCTCC

GCGTCAAGGCTGCCCCCGTGGCTGCCCCGGCTCAGGCCAACCAGGCCGTGGGCGCCCTG

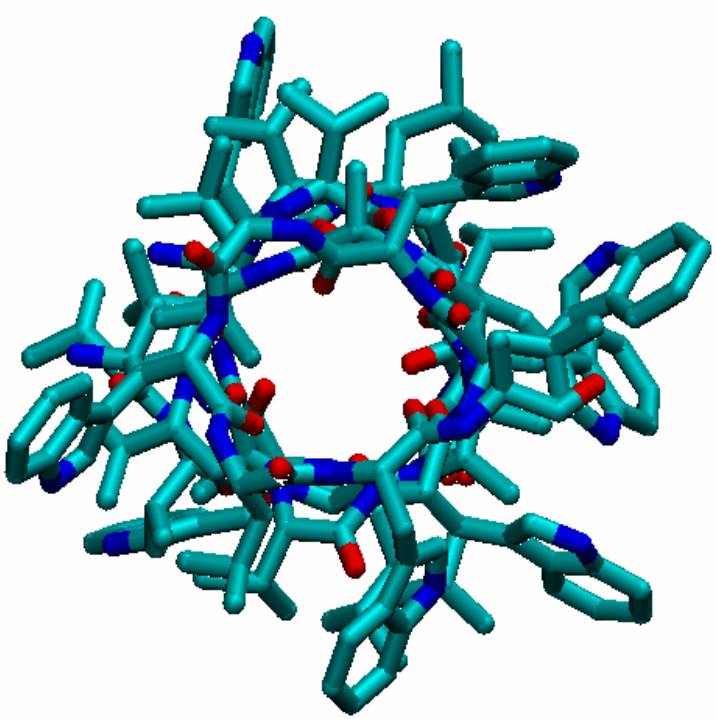
GTGGCTGTGGCTGTGGGCC*TAAG*CAGTTGACATGTTTTGG----------
ATGTAACATCCCGTGTGCA---

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-
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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.

Publications and Presentations

Lee, James W. "Genomic Biotechnology: Creation of Designer Alga for Enhanced H2 Production from Water", presented at the 26th Biotechnology Symposium for Fuels and Chemicals, May 9-13, 2004, Chattanoonga, TN.

Lee, J. W. (2004) Method for creating efficient and robust photosynthetic H2-production systems, U.S. Patent Application pending.

Lee, J. W. (2005) Switchable photosystem II designer alga for photobiological H_2-production, U.S. Patent Application pending.

Lee, James W., Dong Xu, Laurie Mets, Barbra Evans, and Jizhong Zhou. "Creation of designer alga for efficient ad robust production of H_2," presented at the DOE Hydrogen, Fuel Cells & Infrastructure Technologies Program Merit Review meeting, May 24-27, 2004, Philadelphia, Pennsylvania.

Lee, James W. and Elias Greenbaum (2003). "A new oxygen sensitivity and its potential application in photosynthetic H_2 production," *Applied Biochemistry and Biotechnology*, Vol. 105-108, pg 303-313.

Lee, James W., Laurie Mets, Dong Xu, Barbra Evans, and Jizhong Zhou. "Development of efficient ad robust algal hydrogen production systems," presented at the DOE Hydrogen, Fuel Cells & Infrastructure Technologies Program Merit Review meeting, May 19-22, 2003, Berkeley, California.

Lee, James w. "Overcoming nation's roadblocks to photosynthetic H_2 production," presented at the 14th Annual U.S. Hydrogen Conference and Hydrogen Expo USA, March 4-6, 2003, Washington, DC.

Hydrogen Safety

The most significant hydrogen hazard associated with this project is: the follow-on tests of the envisioned switchable proton-channel designer alga for photosynthetic production of H_2 and O_2 from water, which will be performed likely after FY06 when the designer alga is created.

Hydrogen Safety

Our approach to deal with this hazard is:

- **Project has undergone "Integrated Safety Management Pre-Planning and Work Control" (Research Hazard Analysis and Control)**
- **Experienced Subject Matter Experts are required for all Work Control for Hydrogen R&D including**
  - **Fire Protection Engineering**
  - **Certified Safety and Industrial Hygiene expertise**
- **Periodic safety reviews of installed systems**
- **Typical controls include:**
  - **Systems design to prevent air-hydrogen mixtures in the flammable-explosive range**
  - **Minimization of available potential energy**
  - **Use of robust, enclosed systems and gas cabinets, inert gas purging**