DEVELOPMENT OF EFFICIENT AND ROBUST ALGAL H_2-PRODUCTION SYSTEMS

Part A:
Creation of designer alga for efficient and robust production of H_2

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Project 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).

FY04 objectives:

(1) Maintain ORNL algal H2 R&D capabilities and facilities including its valuable algal culture stocks and H2 R&D equipments;

(2) Perform computer-assisted design of DNA sequence for the envisioned proton channel (a fraction of the subtask 2.2.1 described in the ORNL AOP) as much as possible based on the extremely limited DOE EERE H2 funding support.

Budget

Total FY04 DOE Funding for the ORNL Algal H2 Project: $100K.

It did not arrive at ORNL until February 2004.

Technical Barriers and Targets

DOE Technical Barriers for Photobiological H2 Production

– **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.

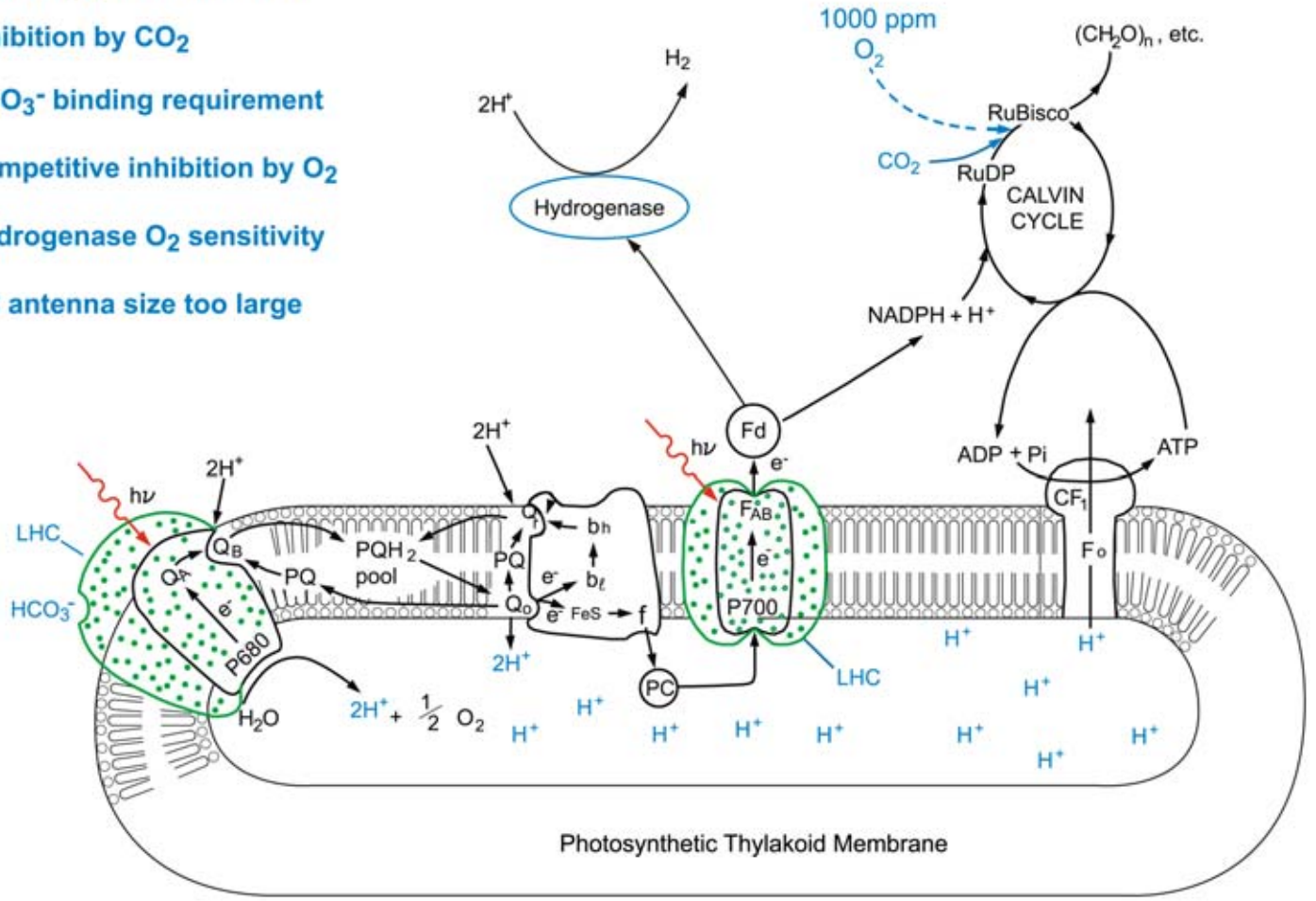
DOE Technical Target for Photobioloigcal H2 Production for 2010

– Absorbed light energy to hydrogen efficiency 5%
– Durability 1500 hours
– Cost $30/kg H2

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.

Project Safety

- 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

Project Timeline

Creation of designer alga for efficient and robust production of H_2

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

If DOE can provide funding support of this 3.0 FTE effort, the project objective can be achieved within 4 years with the following milestones

Year 1—Complete the Design and construction of DNA sequence coding for polypeptide proton channel

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

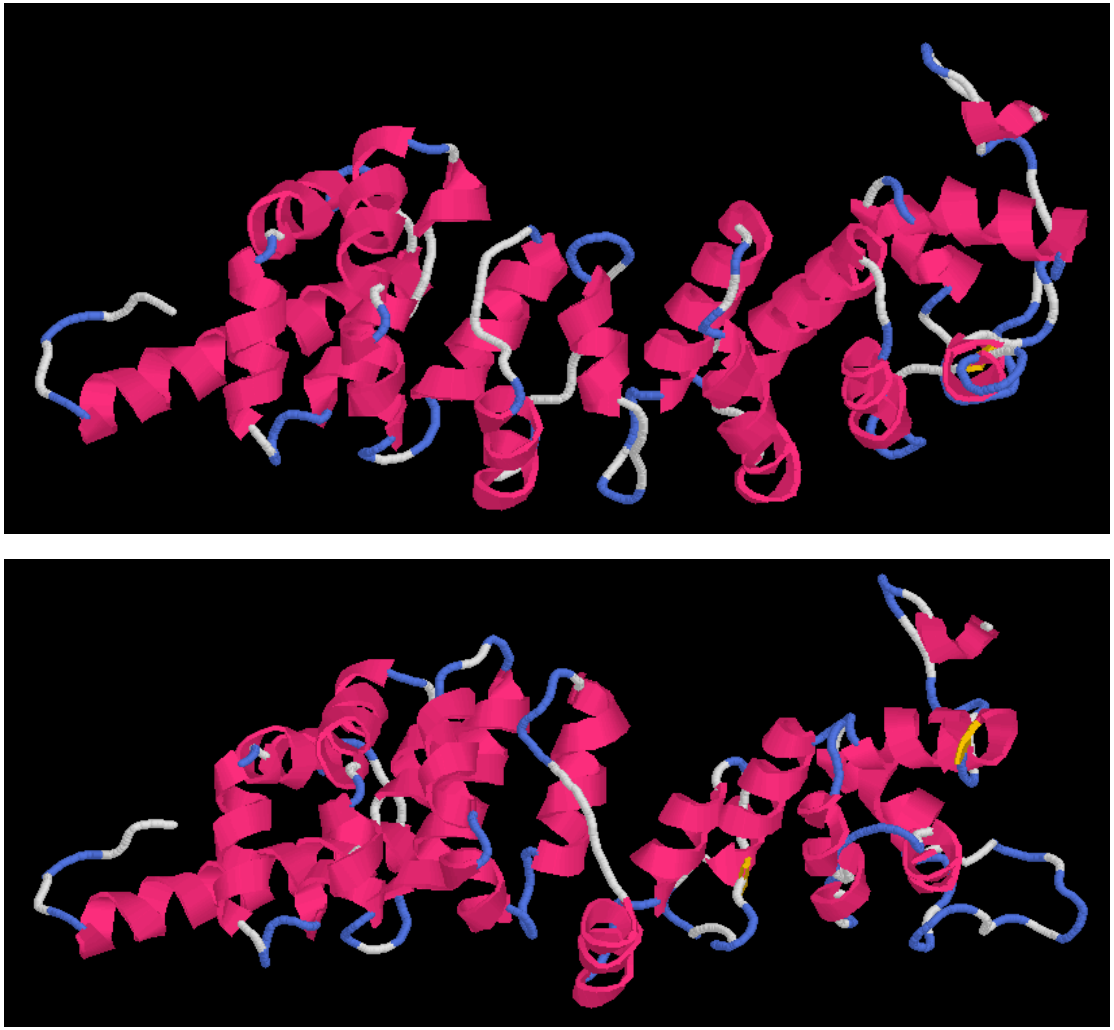
Year 4--Demonstration of efficient and robust production of H_2 in designer alga (ready for next phase: scale up and commercialization)

Technical Accomplishments/Progress

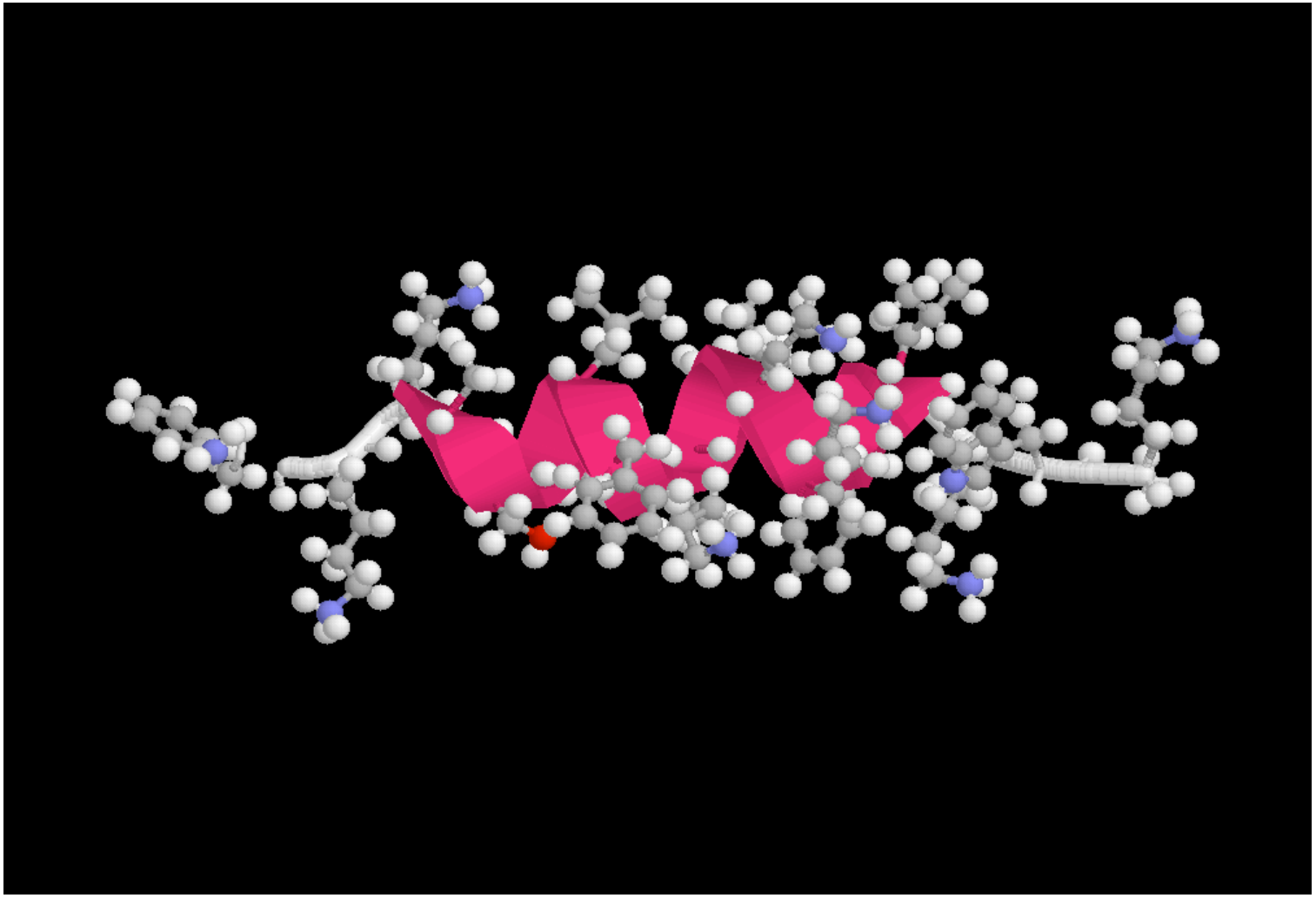
- Maintained valuable ORNL algal culture stocks, instruments, and R&D capabilities

- Performed bioinformatics analyses on natural ionophores: the brown adipose tissue uncoupling proteins (UCP-1 and UCP-2) and melittin for design of the envisioned proton channel to enhance algal H2 production

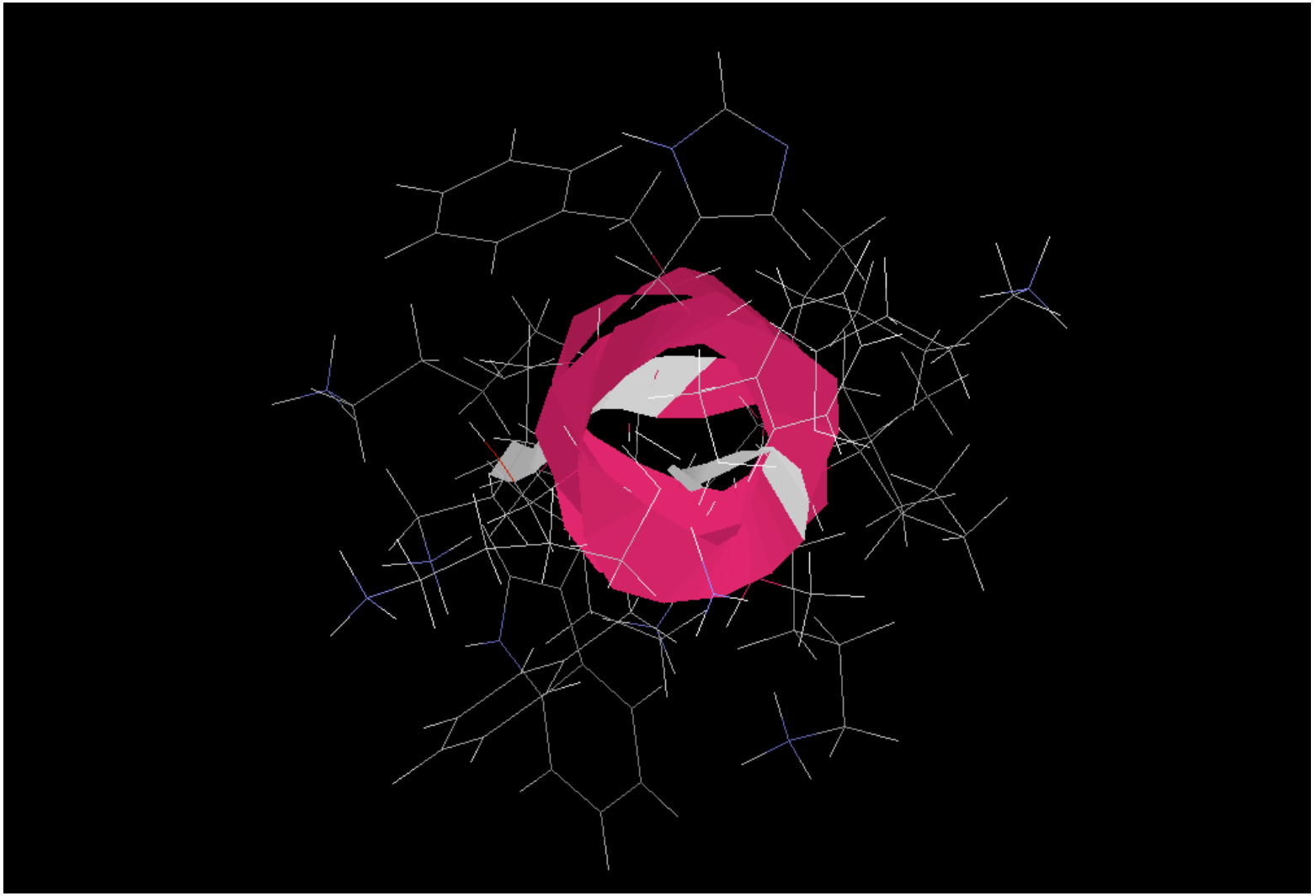
Performed Computational Analysis on Uncoupling Proteins UCP-1 (top) and UCP-2 (bottom) Using the PROSECT Software at ORNL



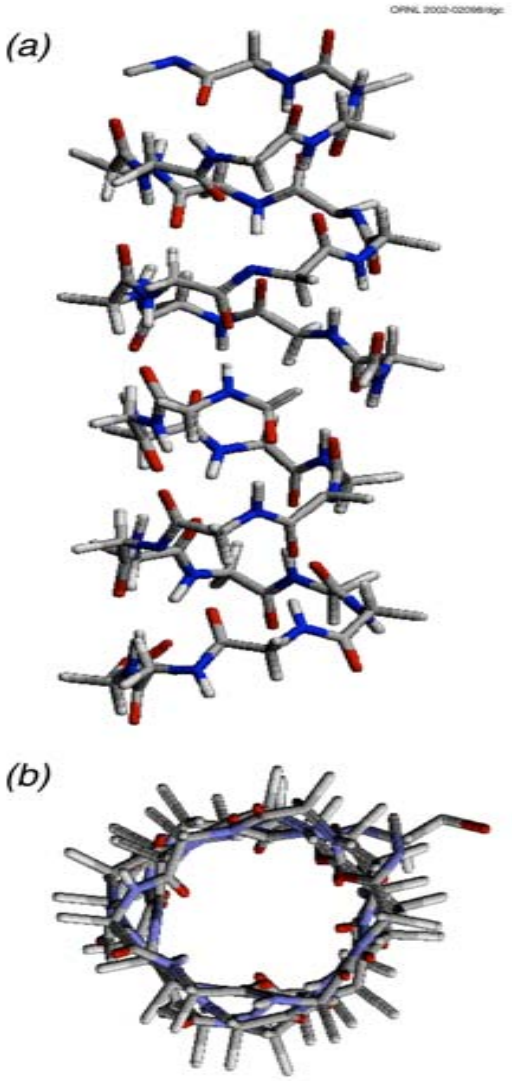
Bioinformatics Analysis of Melittin Using the PROSECT Software



Top View of Melittin Structure Showing Its Channel Pore Size



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



Interactions and Collaborations

We have been working with all the relevant DOE Photobiological H2 Program teams and collaborators including the National Renewable Energy Laboratory (M. Ghirardi and M. Seibert), University of California-Berkeley (T. Melis), University of Chicago (L. Mets), and University of Missouri-Columbia (D. Xu).

Reviewers' Comments

- Our reviewers clearly understood our proposed switchable-proton-channel designer alga H2-production R&D concept. The reviewers' comments are very helpful. They commented that our "approach is novel, sound and even exciting." They also recommended that we should also use natural ionophores, such as the "brown adipose tissue peptide (uncoupling protein)" and perhaps "melittin" in addition to our "synthesis" approach.

- We have now followed reviewers' recommendation and analyzed the structures and DNA sequences of both the brown adipose tissue uncoupling protein and melittin. We are now applying these natural ionophores for our design of the envisioned proton channel to enhance algal H2 production. The proposed project work is entirely achievable.

- Currently, the single limiting factor in this ORNL project is the slow and inadequate DOE/EERE photobiological H2 funding support.

Future Work

The small FY04 funding support will be used up before June 2004. Significantly improvement on DOE/EERE Photobiological H2 Program funding support is needed_hopfully this could happen in the coming FY05 budget. If the required 3.0-FTE project effort can then be fully supported, we should be able to achieve the following milestones in FY2005:

- Complete the computer-assisted design of DNA sequence coding for a proton channel suitable for target insertion into thylakoid membrane;
- Synthesis of the proton-channel gene linked with hydrogenase promoter and thylakoid-signal polypeptide DNA;
- 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;*
- Propagation and verification of the DNA sequence for the synthetic hydrogenase promoter- thylakoid signal polypeptide-proton channel gene_get ready for gene transformation in FY06.