AFOSR MURI Update June 2005-Jan 2006:

Renewable Bio-solar Hydrogen Production from Robust Oxygenic Phototrophs

**BioSolarH\(_2\) \rightarrow Team**

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Goal 1: High Throughput Screening for BioSolar H₂ Production

- BioSolarH₂ →

Goal: screen libraries of phototrophs in search of most active H₂ producers using multiple sensors for H₂, photosynthetic capacity and O₂ consumption

Colonies on agar plates

Chemochromic H₂ sensor

Kinetic Fluorescence CCD Camera

- PS I
- 2H⁺ + 2e⁻ → H₂

- PS II
- 2H₂0 → 4H⁺ + O₂

- H₂ase

Payoff to Air Force
- development of robust screening protocol to identify unique H₂ producing organisms
- development of clean renewable hydrogen fuel
Goal 2: Design/Fabricate Powerful Instrumentation

Current Tools for:
- high throughput screening of gaseous H\textsubscript{2} production
- Light induced electron transfer rates in/out of PSII: \( \text{H}_2\text{O} \rightarrow \text{O}_2, \text{Q}_A^{-} \rightarrow \text{Q}_A \)
- Intracellular redox status PSII: PQ/PQH\textsubscript{2}
- Nanomolar sensitivity for dissolved O\textsubscript{2} and H\textsubscript{2} concentrations

- Colonies on agar
- Fast repetition rate fluorimeter
- PSII Quantum Efficiency
- Dissolved H\textsubscript{2} & O\textsubscript{2}
- LED + Clark cells
- NREL H\textsubscript{2} gas sensor
Goal 3: Elucidate Pathways for e⁻ & H⁺ fluxes in Microalgae: Light-Induced H₂ Production in *Chlamydomonas reinhardtii*

**Mechanistic Pathway in WT strain cc124**
- Two pools of photo-electron acceptors in PSI identified as precursors to photo-H₂
- Established conditions for absence of competing pathways to photo-H₂

**Genetically Engineered Strains**
- Expression of a single set of H₂ase assembly genes has been demonstrated to be sufficient to assemble a diverse set of [FeFe]-hydrogenase structural enzymes from foreign *HydA* genes taken from clostridial bacterial strains.
Goal 3: Elucidate Pathways for e⁻ & H⁺ Fluxes in Cyanobacteria:

Dark Fermentative H₂ production by *Arthrospira maxima*

- Robust photoautotrophic growth over long periods
- High carbonate-requiring hypersaline alkalophile
- Optimal temporal separation of H₂ and O₂
- High rates of fermentative H₂ production optimized by environment conditions (T, pH) and micro-nutrient optimization (Ni, Fe, trace metals): 5.5 ml H₂ (liter culture)⁻¹ hour⁻¹

Two stage indirect pathway to generate H₂ via an O₂-tolerant NiFe-H₂ase:
1) classical photosynthetic generation of glycogen,
2) fermentation of glycogen
Goal 4: Gene shuffling to rapidly increase diversity of [FeFe]-hydrogenases

**Goal:** Use recombinant DNA technology to rapidly generate large libraries of novel [FeFe]-hydrogenases. Select robust enzymes for application in BioSolar H₂-production applications.

**Payoff to Air Force**
- Rapid generation of millions of novel [FeFe]-hydrogenases in less than a week
- Efficiently generates enzyme libraries to be screened for more robust properties
  - Established biotechnology for improving enzyme function

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**Examples of shuffled products with activity**

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FeFe-hydrogenase catalytic H-cluster