

Biohydrogen Generation

Investigators: James Swartz, Professor, Chemical Engineering, Alfred Spormann, Associate Professor, Civil and Environmental Engineering; Chia-Wei Wang and Wing-On (Jacky) Ng, Post-doctoral Researchers; Marcus Boyer and Jim Stapleton, Graduate Students

Sponsor: Global Climate and Energy Project

Objective: The long term goal for this project is to develop efficient and economical technology for the biological conversion of solar energy into molecular hydrogen. The first portion of the project seeks to develop an organism/bioreactor system employing a genetically engineered organism that is effective in the direct conversion of sunlight to hydrogen. The organism will use a shuttle protein, ferredoxin, to transfer electrons from the reaction of water photolysis to the hydrogenase enzyme.

Background: Figure 1 below shows that there is a simple and short pathway for water photolysis in a biological organism that may therefore deliver an attractive conversion efficiency. The photosystem of a bacterium such as *Synechocystis* captures sunlight and splits water to generate molecular oxygen, protons, and mobilized electrons. These electrons are transferred to an electron carrying protein, ferredoxin. By introducing into the cyanobacterium a new hydrogenase enzyme, the enzyme can accept the electrons from ferredoxin and combine them with the protons to make molecular hydrogen. However, the first and major problem is that hydrogenase enzymes are inactivated by molecular oxygen. Thus, the initial focus of this part of the project is to establish protein evolution methods capable of evolving a highly active hydrogenase (such as the one from *Clostridium pasteurianum*) to be insensitive to inactivation by molecular oxygen.

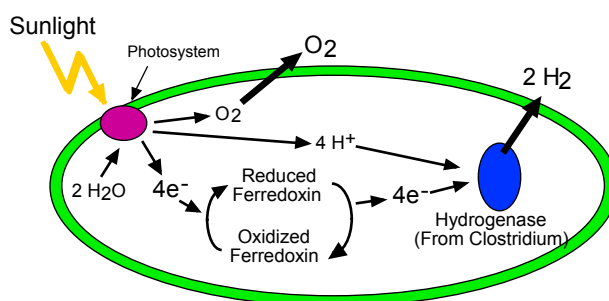


Figure 1: Proposed Engineered *Synechocystis* Bacterium

Approach: Based on the 3-D structure and the molecular properties of the Fe-S hydrogenase (CpI) from *Clostridium pasteurianum*, this enzyme will be modified to become oxygen tolerant. It is expected that it will be possible to engineer an altered enzyme structure so that molecular oxygen is sterically excluded from the Fe-S cluster at the active site but that hydrogen can still diffuse away. The predictive capability for protein folding does not allow one to *a priori* select the amino acid changes that would provide such a change. Instead it is now a well established strategy to pursue a protocol called “protein evolution”.

For protein evolution to be successful, one must generate genetic diversity around the initial DNA sequence that encodes for expression of the protein. This part is reasonably straightforward. The more difficult challenge is establishing methods for searching through tens of thousands of candidate proteins to find the few that have the new property of oxygen tolerance. If one can develop this capability, then it is possible to iteratively search for enzymes with increasing oxygen tolerance. It is anticipated that this search can be conducted relatively quickly and effectively at the facilities at Stanford.

The major enabling capability is cell-free protein synthesis. Using this approach, a number of hydrogenase candidates can potentially be synthesized in each well of 96-well microtiter plates. Procedures will also be established to allow processing of many plates per day. Each candidate protein will have an extension that will absorb onto the wall of the microtiter plate well. When the reaction is completed, the well will be washed clean of the reaction solution, but the product hydrogenase will be retained. Then the extent of reaction will be monitored using reduced ferredoxin as the source of electrons, and the reaction will be conducted under controlled partial pressures of oxygen.

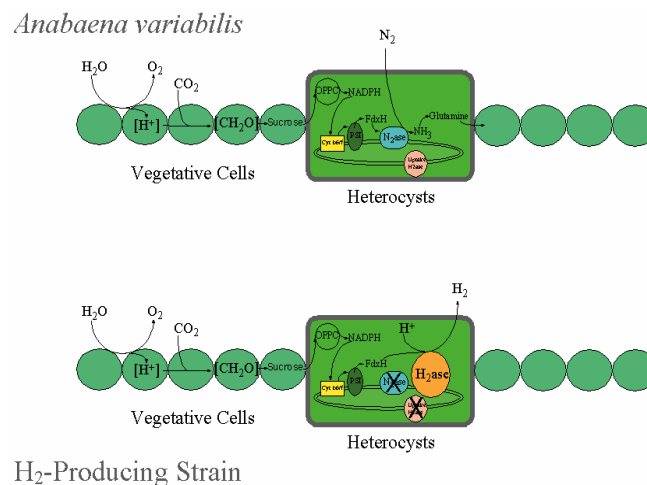


Figure 2: Photosynthesis and nitrogen fixation in filamentous cyanobacteria (top panel). Proposed scheme for a hydrogen-producing strain (lower panel).

Since the engineering of an oxygen tolerant hydrogenase is a significant experimental challenge, an alternative approach is also being explored, in which photosynthetic hydrogen production is directed to an anoxic compartment (heterocysts) found in filamentous cyanobacteria (Figure 2). In filamentous cyanobacteria, photolytic water cleavage is separated spatially from oxygen-sensitive nitrogen fixation. Light-dependent oxygen release proceeds only in vegetative cells, whereas nitrogen fixation is restricted to the anoxic heterocysts (Figure 2, upper panel). Within heterocysts, oxygenic photosynthesis is suppressed. This, together with other oxygen-scavenging mechanisms, provides an anoxic environment for the proper functioning of nitrogenase. The remaining vegetative cells (Figure 2, upper panel) can perform oxygenic photosynthesis, storing light energy into fixed carbon such as sucrose. The sucrose then can be transported into the heterocysts to fuel nitrogen fixation. The lower panel in Figure 2 depicts the

research objective: an organism that would use the sucrose to fuel hydrogen production instead of nitrogen fixation.

Contacts:

Alfred M. Spormann, spormann@stanford.edu

James R. Swartz, swartz@chemeng.stanford.edu