**44a A Program for the Directed Evolution of Oxygen-Tolerant Hydrogenase Enzymes for Use in Photobiological Hydrogen Generation**

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The future hydrogen economy depends on the development of technologies that can sustainably produce hydrogen in an economical and clean process. Our project aims to use microorganisms as catalysts to generate hydrogen gas from water with sunlight as the energy source. This goal is founded upon the inherent ability of photosynthetic proteins to use sunlight to mobilize electrons, coupled with the catalytic power of the enzyme hydrogenase which can combine electrons and protons to evolve hydrogen gas. The envisioned organism would be engineered to absorb a wide range of sunlight wavelengths, and would donate a large fraction of mobilized electrons to hydrogenase for hydrogen production. Given such an engineered organism, we foresee thin bioreactors covering hundreds of square miles satisfying a large portion of the country's energy needs. The first major obstacle is that hydrogenases are destroyed by oxygen. A product of photosynthesis, oxygen will be unavoidable in our system. Currently much research is being dedicated to the directed evolution of hydrogenase in an effort to obtain mutants with improved oxygen tolerance. Structural analyses of [Fe]-hydrogenases are consistent with the theory that the hydrophobic tunnel through which evolved hydrogen leaves the interior of the enzyme could be tightened to exclude oxygen and prevent it from oxidizing the complex active site. Work towards finding such a mutant has been slow because of the lack of a convenient high-throughput screen to identify oxygen-tolerant mutants. This talk will outline our plans for developing effective technology for the direct conversion of sunlight energy into hydrogen and the results of our progress to date.