Programs In Alternative Energy at the University of California Berkeley

The Philomathia§ Foundation is a philanthropic organization created by the Chung family to support scholarly work that addresses some of the most pressing issues facing humanity. A generous donation by the Foundation to the University of California, Berkeley currently supports three related activities in the area of alternative energy.

The Philomathia Foundation Distinguished Chair in Alternative Energy

This chair was co-endowed by The Philomathia Foundation and the Hewlett Foundation to support a University of California Berkeley faculty member to pursue research in alternative energy.

The Philomathia Foundation Fund for Alternative Energy Research

This fund supports exploratory research projects related to the development of alternative energy. The fund is administered by a committee chaired by the Philomathia Foundation Chair in Alternative Energy.

Information concerning proposals for research support is available at the Proposals page. The next opportunity for funding will be in 2010.

The Philomathia Forum for Energy and Environment

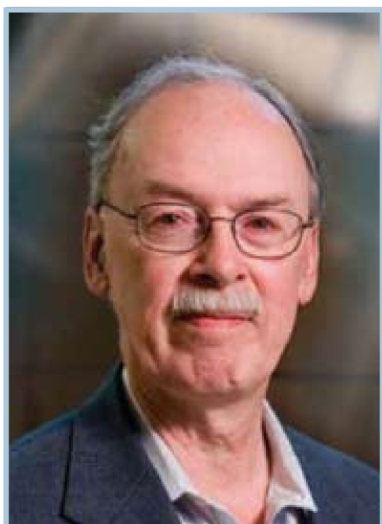
This fund provides financial support for symposia on topics related to contemporary issues concerning energy and the environment. The fund is administered by a committee chaired by the Philomathia Foundation Chair in Alternative Energy.

Information concerning proposals for research support is available at the Proposals page. The next opportunity for funding will be in 2010.

§ *Philomathia derives from the Greek words mathis (learning) and philos (friend or supporter)*

The Philomathia Foundation Chair in Alternative Energy

The first recipient of this chair was Stephen Chu who held the chair from its establishment until his departure from UC Berkeley in the fall of 2008 when he accepted the nomination to become the US Secretary of Energy. Prior to becoming U.S. Secretary of Energy, Dr. Chu served as a Professor of Physics at UC Berkeley and as the Director of the Lawrence Berkeley National Laboratory.



The current recipient of the chair is **Chris Somerville**, a professor in the **Department of Plant and Microbial Biology** and Director of the **Energy Biosciences Institute**. http://www.energybiosciencesinstitute.org/

Dr. Somerville was a professor at Stanford University and director of the Carnegie Institution for Science from 1994-2007 and a professor at Michigan State University from 1982-1993. He has published more than 200 scientific papers and patents in plant and microbial genetics, genomics, biochemistry, and biotechnology. His current research is focused on the characterization of proteins, such as cellulose synthase, implicated in plant cell wall synthesis and modification. He is a member of the US National Academy of Sciences, The Royal Society of London and the Royal Society of Canada and has received numerous scientific awards including the Gibbs and Schull awards from the American Society of Plant Biologists, the Mendel Medal from the Genetics Society, the Hopkins medal from the Biochemical Society, the Khumo Award from the Plant Molecular Biology Society and most recently the Balzan Award which he shared with Elliot Meyerowitz (Caltech) for their role in helping to develop the current paradigm in plant molecular biology.

The Philomathia Foundation Fund for Alternative Energy Research

During 2008-09 The Philomathia Fund provided financial support for the following three projects.

Investigator: Krishna K. Niyogi, Department of Plant and Microbial Biology Project: Synthetic biology of photosynthesis for alternative fuel production

Natural photosynthesis is the biological process that converts solar energy into chemical energy. Elucidation of the design principles of natural photosynthetic systems is fundamentally important for understanding the biology of photosynthetic organisms and for developing biologically inspired approaches to solar energy conversion. Although decades of research have provided remarkable insights into the structure and function

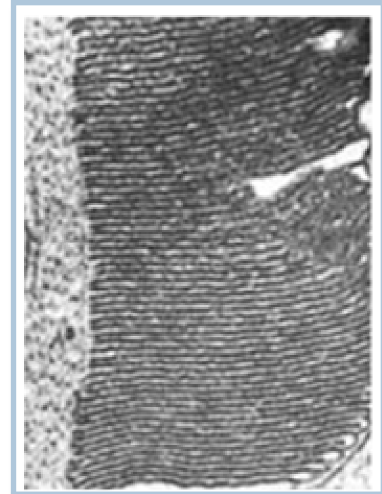
of photosynthetic complexes, a thorough understanding of how photosynthetic pigments, proteins, and cofactors are assembled into functional complexes is lacking. For example, photosystem II found in plants, algae, and cyanobacteria has the unique ability to harvest sunlight, split water, and generate high-energy electrons, yet little is known about how this remarkable complex is put together.

The goal of this project is to use synthetic biology techniques to reconstitute a functional photosystem from its component parts in order to (1) test our understanding of the system and its assembly and (2) optimize and engineer a photosystem to perform specific user-defined tasks, such as alternative fuel production. In collaboration with Chris Voigt's lab, we are starting to build a relatively simple model photosystem, the RC from a purple bacterium (Rhodobacter sphaeroides), in a heterologous host organism (Escherichia coli) using a stepwise approach, beginning with the pigments that are prerequisites for photosystem assembly and function. Thus far, we have successfully engineered E. coli to produce the necessary carotenoid hydroxyspheroidene by designing a synthetic crt operon (8 kb) that encodes the carotenoid biosynthesis enzymes of R. sphaeroides. We have also designed a set of three synthetic operons to express the more complex set of carotenoid biosynthesis enzymes from a PSIIcontaining organism, the green alga Chlamydomonas reinhardtii. These operons are currently being synthesized and tested. In the meantime, we have also made a synthetic bch operon for the first half of the bacteriochlorophyll a biosynthetic pathway from R. sphaeroides.

In the future, we would like to design a synthetic operon for the second half of the bacteriochlorophyll a biosynthetic pathway in order to produce the first green, bacteriochlorophyll-producing E. coli cells. After producing both carotenoids and bacteriochlorophyll in E. coli, we will proceed with operon construction for the structural genes that encode photosystem proteins. Successful expression of the relatively simple R. sphaeroides photosystem in E. coli will provide a proof of principle for the eventual reverse engineering of water-splitting, oxygen-evolving photosystem II, a much more complicated photosystem with enormous potential for alternative energy production.

Investigator: Jan Liphardt, Department of Physics
Project: Light-boosted fermentation in the yeast Saccharomyces cerevisiae

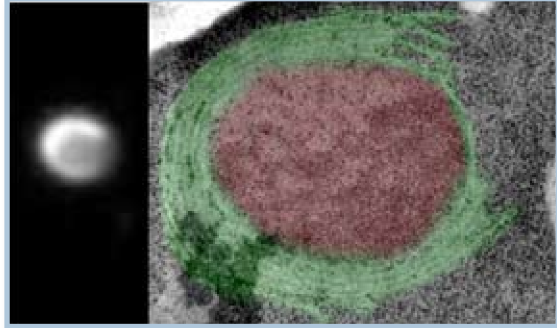
In the long term, it is possible that mankind will synthesize fuels with highly customized living cells. Unlike their natural counterparts, these cells will be constructed using synthetic biology approaches and will be fine-tuned to perform a small number of precisely defined reactions with great efficiency. Examples may include cells that convert CO_2 and sunlight into biofuels. To realize this prediction, it will be essential to discover and develop general principles that will ultimately give synthetic biologists control of cell shape and ultrastructure. Consider the photoreceptor cells in the human eye.



The figure on the right shows part of a photoreceptor cell. The black lines are the plasma membrane, folded back on itself numerous times. This “membrane shelving” increases the surface area of the cell membrane, and therefore, the capture cross-section for photons. Without this special shape of the cell, the human eye would be hundreds of times less sensitive to light, and we could not see at dawn or dusk. This is one of many examples where the shape or ultrastructure of a cell greatly enhances the function of an enzyme, such as the Rhodopsin protein in our photoreceptors.

We have created a strain of *S. cerevisiae* (Baker’s yeast) that features extensive internal light-collecting structures. *S.cerevisiae* does not normally harvest light energy, but it is an efficient producer of ethanol and therefore a good model system to study conversion of glucose into biofuels or biofuel precursors. To our knowledge, this is the first time that a eukaryotic cell has been re-engineered to contain a multilayer membrane structure similar to the photoreceptors in our eyes or the chloroplasts in plants. We constructed this synthetic light-harvesting structure by expressing a modified form of the Proteorhodopsin light-powered proton pump. Normally, the Proteorhodopsin enzyme pumps protons when illuminated, but it does not reconfigure the cell’s ultrastructure. However, about 10 years ago a group working on an entirely different system, cytochrome b(5), discovered that expression of GFP-tagged cytochrome b(5) lead to the formation of membrane stacks, so-called karmellae. We speculated that a GFP-tagged form of Proteorhodopsin might also exhibit this behavior. To our surprise, this prediction was correct. As can be seen in the figure, proteorhodopsin constructs containing the native bacterial localization presequence and a nonmonomeric GFP tag form semicircular membrane aggregates which can be seen both in fluorescence and electron microscopy. By influencing membrane structure and creating membrane shelves, the proteorhodopsin-GFP construct transforms certain organelles of this heterologous organism, *S. cerevisiae*, in precisely the right way to maximize its protonpumping efficiency.

Our goal in the next year is investigate the possible metabolic implications of these membrane structures. Do they increase the cell’s ability to harvest light? Our hypothesis is that the increased effective cross-section and light collection of the stacked membranes will increase the degree to which the cells can pump protons in response to illumination. Ultimately, we wish to couple these proton fluxes to the generation of cytoplasmic ATP, which is needed for enzymatic reactions such as carbon fixation and biofuel production.



Discovery of the ability of GFP-tagged Proteorhodopsin to reconfigure internal membranes, creating light collecting structures in yeast.

Proteorhodopsin (with its native localization signal and fused to a non-monomeric green-fluorescent protein) creates multi-lamellar semicircular membrane structures in yeast. Left. Fluorescence image of a single yeast cell, showing intense GFP fluorescence originating from a thick band surrounding the nucleus. Right. False-color electron micrograph of a single yeast cell showing the synthetically-encoded membrane compartment (3-5 layers thick, green) surrounding the nucleus (red).

Investigator: Jay Keasling, Department of Chemical Engineering
Project: Mechanism of fuel toxicity in fuel-producing microrganisms

One of the primary problems in producing biofuels from cellulose or starch using microorganisms is the toxicity of the fuel to the microorganism. For example, ethanol cannot be produce at high concentration in fermentation tanks because it is toxic to the yeast that produces it. Because it is produced in low oncentrations, a significant amount of energy must be expended to purify the ethanol so that it can be used as a fuel. A similar phenomenon occurs with other potential fuel molecules, including butanol, a fuel that has a higher energy content than ethanol. What's more, butanol is soluble in water only to 8%, unlike ethanol, which is completely soluble in water (e.g., up to 99.99%). If we could engineer microorganisms to survive butanol to 8%, then we would not need to distill butanol; it would come out of solution and float on top of the fermentations like oil on water.

The goal of this research project has been to determine what makes fuel alcohols (i.e., ethanol, butanol, etc.) toxic to microorganisms and to engineer tolerance to these fuels into microorganisms. We hve used state-of-the-art genomics techniques (see figure below) to measure all of the transcripts, proteins, and metabolites in the cell and determine the stress response that microorganisms undergo when exposed to alcohols.

Our first approach has been to grow the yeast Saccharomyces cerevisiae and the bacterium Escherichia coli (E. coli) in the presence of various concentrations of fuel alcohols, namely butanol. We have examined response of these microorganisms to the presence of added alcohols using DNA arrays, proteomics, and metabolomics and then analyzed the results using various computer algorithms. We find that the cells induce a number of stress responses that help them cope with high concentrations of fuels. Indeed, as the concentration of fuel increases in the medium, the cells grow more and more

poorly and induce more and more stress responses. However, at high concentrations of fuels, the cells cannot induce stress responses that will allow them to grow; so the cells do not grow. Nonetheless, the information we have gathered using the various functional genomics techniques will allow us to engineer yeast and E. coli to better withstand high concentrations of fuels. This work will include expressing pumps to transport the fuel out of the cell as it is made so that the cells can withstand the fuel they are producing.

Another, and potentially easier, approach is to evolve yeast and E. coli to withstand higher and higher concentrations of alcohol added to the growth medium. To accomplish this goal, we are generating mutants of E. coli and yeast that can cope with high concentrations of alcohol in the growth medium and then fusing them together to form mutant cells (super bugs) that can withstand very high concentrations of alcohol. To do the fusion of cells, we remove the outer cell wall of these cells, which allows the cells to fuse their membranes and mix the contents of the two cells in a single cell. When the cells are fused, their chromosomes recombine into a single chromosome that contains genes from each of the individual cells that were fused together. If we apply a method to select for fused cells that have a desire trait, then we can often generate the desired cells. For example, we can select for mutant cells that grow on higher and higher concentrations of alcohol and then fuse the mutant cells together to get cells that grow on even higher concentrations of alcohol. We have spent the past year optimizing the protocols for removing cell walls and then regenerating the cells once they are fused. Now, we are in a position to being fusing mutant cells together to generate “super bugs” capable of withstanding very high concentrations of fuels. If funded, we plan to continue this work over the next year. We will continue the work to analyze the stress responses organisms subjected to fuels, and we will continue our work to generate mutant cells that can withstand higher and higher concentrations of fuels. We will also use the functional genomics techniques described above to analyze these mutant cells to determine why they are more resistant to fuels than the natural microorganisms. In the future, these organisms will be engineered to produce the fuels themselves.

The Philomathia Forum for Energy and Environment

The first symposium to be supported by the Philomathia Forum will be a meeting on carbon capture and sequestration that will be held at Beijing University in the fall of 2009. The symposium is currently being organized by Don DePaulo, Berend Smit, Hui-Hai Liu (UC Berkeley), Dongxiao Zhang (Beijing University), Lynn Orr (Stanford University) and colleagues.

Joint US-China Workshop on Carbon Capture and Sequestration

Donald DePaolo, Berend Smit, and Hui-Hai Liu* Earth Sciences and Materials Science Divisions (MSD, ESD) of LBNL Earth and Planetary Sciences and Chemistry Departments, U.C. Berkeley

Background and Objectives:

Anthropogenic emissions of carbon dioxide (CO2) into the atmosphere are recognized to have a significant effect on global warming, one of the most important problems facing society. Due to its rapid economic development and energy demands, China is currently the largest CO2 emitter in the world. Carbon capture and sequestration (CCS) is widely regarded as an essential approach to reduce CO2 emissions.

Primary Technical Areas

The workshop will focus on basic and applied research and technology development in the following areas:

- Overview of the current status of Chinese and American CCS development;
- Key Chinese and international collaborative CCS projects;
- Fundamental processes and advanced technologies for CCS;
- Risk assessment and regulation development for CCS.

Venue/Location

The symposium will be held on the campus of Beijing University. Approximately 250 people are expected to attend.

Time (tentative)

November, 2009.

Proposal Submission Page
for The Philomathia Foundation Fund
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