International symposium on microalgal biofuels and bioproducts

(This program is tentative and subject to change)

21st November 2013

Surugadai Memorial Hall

Chuo University

3-11-5 Kandasurugadai, Chiyoda-ku, Tokyo 101-8324, Japan

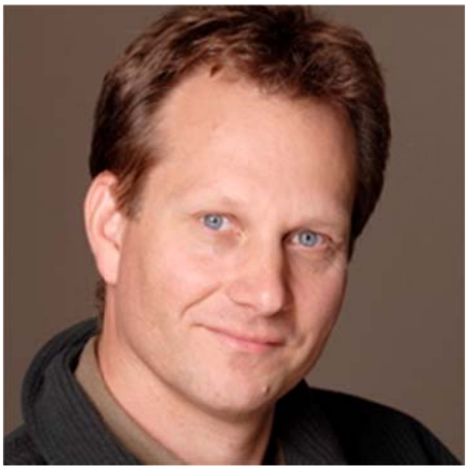
Co-hosted by
Agriculture, Forestry and Fisheries
Research Council
&
Research and Development Initiative
Chuo University

TENTATIVE PROGRAM

10:00	Welcome	Agriculture, Forestry and Fisheries Research Council
10:10	Title to be announced	
		Professor Ben Hankamer Institute for Molecular Bioscience The University of Queensland
11:00	Conversion of CO_2 to Chemicals in Cyanobacteria.	
		Professor Shota Atsumi Department of Chemistry UC Davis
11:50	Lunch	
13:00	Poster session	
15:50	Molecular breeding of green algae for biofuel production.	
		Professor Shigeaki Harayama Department of Biological Sciences Chuo University
16:10	Cost effective outdoor cultivation of green algae for biofuel production.	
		Mr. Hiroaki Fukuda General Manager of Bio R&D Department Material R&D Division, DENSO CORPORATION
16:50	Food and fuel for the 21st century - Synthetic biology in micro-algae for the production of biofuels and bio-products	
		Professor Stephen Mayfield Director, San Diego Center for Algae Division of Biological Sciences University of California, San Diego
17:40	Closing remarks	Professor Kunio Saito Director, Research and Development Initiative Chuo University
18:30	Banquet	

Profiles of invited speakers

Ben Hankamer



Professor
The Institute for Molecular Bioscience (IMB)
The University of Queensland (UQ)

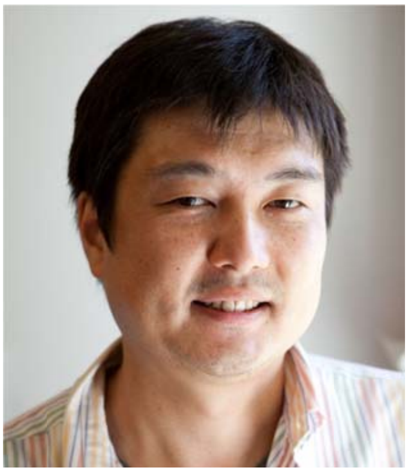
In 2002, Ben moved from Imperial College London to take up his position as a Principle Investigator at The University of Queensland's Institute for Molecular Bioscience. Ben has focused on the development of environmentally friendly high-efficiency microalgae biofuel production systems. In 2006, he established and directs the Solar Biofuels Consortium which now includes 8 international teams, ~100 researchers and ~10 industry partners.

In 2009, Ben was awarded the prestigious Eisenhower Fellowship, awarded to individuals identified as international leaders in areas of energy technology and supply. In 2013, Ben was also awarded the Discovery of Outstanding Researcher Award from the Australian Research Council.

Over the past 10 years, Ben Hankamer has focused on the development of environmentally friendly high-efficiency biofuel production systems. This area represents a rapidly expanding biotechnology. His specialisation is in the structural biology of the photosynthetic machinery, which drives the conversion of solar energy into chemical energy (fuels) and has published extensively on the water splitting Photosystem II complex, its light harvesting antenna system and V-type ATPase. Using this knowledge of the photosynthetic machinery, he embarked on the targeted engineering of the green alga *Chlamydomonas reinhardtii* for high-efficiency biofuel production. To facilitate the development of high efficiency biofuel systems, he founded the Solar Biofuels Consortium which he now directs. The consortium includes eight international teams and conducts economic analysis, bio-discovery, marine biology, structural biology, molecular biology, microbiology, genomics, metabolomics, culture optimisation and bioreactor scale up within a coordinated research program of parallel research streams. One of the biggest global challenges facing our society today is the race to discover cleaner, more affordable and sustainable energy sources. Currently, most of the world's clean energy technologies are used to produce electricity. However, 80 per cent of the global energy demand is used in the form of fuel.

Shota Atsumi

Assistant Professor
Department of Chemistry, University of California,
Davis, CA, 95616



Shota Atsumi is an Assistant Professor in the Department of Chemistry at the University of California, Davis since 2009. He received his Ph.D. from Kyoto University in 2002, where he worked with Dr. Tan Inoue. He was a postdoctoral researcher with Dr. John W. Little at the University of Arizona and with Dr. James C. Liao at the University of California, Los Angeles. He was a co-recipient of the Presidential Green Chemistry Challenge Award in 2010 awarded by the US Environmental Protection Agency. In 2012, he was awarded the prestigious Hellman Fellowship, awarded to individuals identified as promising assistant professors who show capacity for great distinction in their chosen fields of endeavor.

Shota is one of the pioneers in the study of the commercial production of 1-butanol and isobutanol from *Escherichia coli.* An increased understanding of system properties underlying cellular networks enables one to construct novel systems by assembling the components and the control systems into new combinations. His group is applying this approach to the field of metabolic engineering, which strives for the optimization of desired properties and functions, such as the production of valuable biochemicals. The production of valuable chemicals from microorganisms has the potential to solve some significant challenges, such as converting renewable feedstocks into energy-rich biofuels. He and his colleagues engineered *E. coli* to produce higher alcohols including isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-pheylethanol by taking advantage of the host's highly active amino acid biosynthetic pathway. These results have been published in *Nature*. His current research focuses on the use of synthetic biology and metabolic engineering approaches to engineer photosynthetic microorganisms to convert CO_2 to valuable chemicals using light energy. He and his colleagues engineered a model cyanobacterium, *Synechococcus elongatus* PCC 7942, to produce isobutyraldehyde and isobutanol from CO_2 published in *Nature Biotechnology* in 2009. More recently, his group developed a more efficient cyanobacterial production system published in *Proc Natl Acad Sci USA* in 2013. His group also engineered *S. elongatus* to grow without light by installing heterologous sugar transporters. The engineered strains grow continuously in light/dark conditions using saccharides such as glucose, xylose, and sucrose.

Stephen Mayfield

Director, San Diego Center for Algae Biotechnology and
John Dove Isaacs Chair of Natural Philosophy
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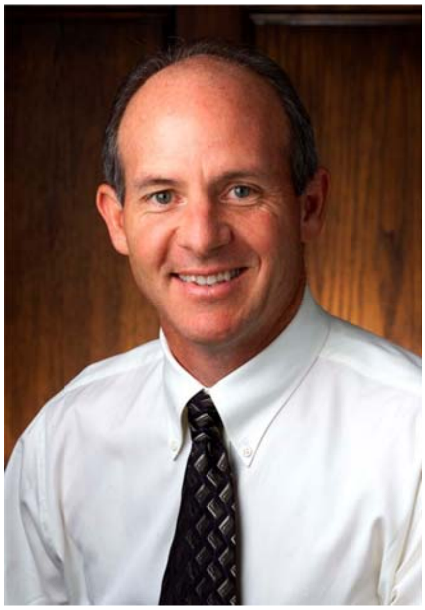

Stephen Mayfield is director of the San Diego Center for Algae Biotechnology, and a Co-director of the Food and Fuel for the 21st Century organized research unit at UC San Diego. He is also the John Doves Isaacs Chair of Natural Philosophy in the department of Biology. His research focuses on the molecular genetics of green algae, and on the production of high value recombinant proteins and biofuel molecules using algae as a production platform. Steve received BS degrees in Biochemistry and Plant Biology from Cal Poly State University in San Luis Obispo, and a PhD in Molecular Genetics from UC Berkeley. Following a post-doctoral fellowship at the University of Geneva Switzerland, he returned to California as an assistant professor at the Scripps Research Institute where he was the first person to achieve transformation of a green algae nuclear genome, work that allowed algae to become dominant organisms for the study of photosynthesis and gene function. Steve remained at Scripps for 22 years becoming the Associate Dean of Biology before joining UC San Diego in 2009. Over the last ten years work from the lab has identified mechanisms of chloroplast gene expression that has allowed for recombinant protein expression and metabolic engineering in algal chloroplast. Steve's lab was the first to show high levels of recombinant protein expression in algae, setting the stage for the use of algae as a platform for recombinant protein production, including the expression of human therapeutic proteins. These studies resulted in the founding of Rincon Pharmaceutical, company based on the low cost production of human therapeutics using eukaryotic algae as an expression platform. Recent studies from the lab have shown the potential of engineering algae for the production of superior biofuel molecules as a source of renewal energy, and Steve is a scientific founder of Sapphire Energy, the world's largest company developing biofuels in algae and photosynthetic bacteria. Steve's latest commercial undertaking is Trion Algae Innovations, a company developing high value recombinant proteins as animal and human nutraceuticals.

Abstracts of oral presentations

Conversion of CO_2 to Chemicals in Cyanobacteria

Shota Atsumi

Department of Chemistry, University of California, Davis, CA, 95616

The use of photosynthetic microorganisms as a platform for biological fuel production has gained considerable popularity as an option that would avoid global energy and environmental problems. As photosynthetic microorganisms directly fix carbon dioxide as their primary carbon source, the need for a source of fermentable sugars as a carbon feedstock for biological fuel production could be eliminated. Algae and cyanobacteria have been the primary organisms of interest for this strategy of fuel production. Both can grow much faster than plants and do not need to be grown on arable land. Furthermore, such organisms are grown in water which facilitates the use of CO_2 at higher concentrations than that of ambient air and so could potentially be fed by concentrated CO_2 emissions from waste industrial sources. The great potential of the prokaryote cyanobacteria as a biofuel production platform lies in its combination of the advantages of both algae, as a photosynthetic organism, and *E. coli*, as a relatively simple naturally transformable prokaryote. Cyanobacteria have already been engineered to produce a number of different biofuel related compounds. However, synthetic pathway construction and characterization of metabolism in cyanobacteria, is still in its infancy compared with model fermentative organisms.

We systematically developed the 2,3-butanediol (23BD) biosynthetic pathway in *Synechococcus elongatus* sp. strain PCC 7942 as a model system to establish design methods for efficient exogenous chemical production in a photosynthetic host. We identified 23BD as a target chemical with low toxicity and designed an oxygen-insensitive, cofactor-matched biosynthetic pathway coupled with irreversible enzymatic steps to create a driving force toward the target, increasing titers to 2.38 g/L, which is a significant increase for chemical production from exogenous pathways in cyanobacteria. Production of 23BD appears to redirect up to 60% of biomass toward product, this leaves 40% improvement in this system. This work demonstrates that developing strong design methods can continue to increase chemical production in cyanobacteria.

All cyanobacteria are photosynthetic organisms that utilize light energy for the reduction of carbon dioxide. Many cyanobacteria, including our model system, *S. elongatus*, have been considered obligate photoautotrophs, strictly depending upon the generation of photosynthetically derived energy for biomass production. This obligate photoautotroph is incapable of product formation in the absence of light. Thus, converting an obligate photoautotroph to a heterotroph is desirable for more efficient, economical, and controllable production systems. We determined that sugar transporter systems are the necessary genetic factors to install heterotrophy in *S. elongatus* PCC 7942. After modification, continuous growth was possible under diurnal (light/dark) conditions using saccharides such as glucose, xylose, and sucrose as both energy and carbon inputs. This modified strain showed heterotrophic growth in the dark and a 2-fold growth rate increase in the presence of light. While the universal causes of obligate photoautotrophy may be diverse, installing sugar transporters provides new insight into the mode of obligate photoautotrophy for cyanobacteria. While diurnal conditions are of keen interest for cost-effective, industrial pursuits, further work with continuously dark conditions will more fully illuminate the causes of phototrophy seen in this model cyanobacterium.

Molecular breeding of green algae for biofuel production.

Shigeaki Harayama

Department of Biological Sciences, Chuo University

Algae biofuel is considered the third generation biofuel; however, the current costs for the production of algal biofuels are not competitive with petroleum-based fuels. It is imperative to reduce the costs of cultivation, oil extraction, and conversion oil to biofuels. We are focusing on the technology development for increased productivity and stable production of triglycerides in a green alga.

We study on the green alga, *Pseudococcomyxa ellipsoidea* that accumulates 30% (w/w) or more of triglycerides in lipid bodies inside cells upon nitrogen starvation. We mutagenized the strain by N-methyl-N'-nitro-N-nitrozoguanidine, and isolated many mutants of different phenotypes, including low-chlorophyll mutants, oil-accumulating mutants, fragile-cell-wall mutants, high-light tolerant mutants, dark-metabolism-deficient mutants, uracil-requiring mutants, nitrate-reductase deficient mutants, etc. We determined the genomic sequences of these mutants, and compared with that of the wild-type strain.

One of the low-chlorophyll mutants named strain 5P was defective both in chlorophyllide *a* monooxygenase and in one of chlorophyll a/b binding proteins. The biomass productivity of strain 5P was approximately 30% higher than that of wild-type strain when light intensity was high (300 µmol m^{-2} sec^{-1}) and the depth of the culture vessel was 10 cm or deeper. We further mutagenized strain 5P, and isolated mutants capable of accumulating triglycerides at high concentrations (>50%).

In parallel, we are developing recombinant DNA techniques to accelerate the breeding speed of this alga. We established genetic transformation methods with particle bombardment, and constructed several cloning vectors for gene expression.

Cost effective outdoor cultivation of green algae for biofuel production

Hiroaki Fukuda
General Manager of Bio R&D Department
Material R&D Division, DENSO CORPORATION

Fossil fuels are widely accepted as unsustainable energy source due to depleting oil reserves and causing global warming. Thus, interest in biofuels is increasing across the world. However, biofuels of the first generation are produced from crops, and it happened to compete with food, resulting in a rise in food prices. Currently, microalgae are recognized as a resource for the third generation biofuels. The main advantages of the microalgae are that they have a higher photon conversion efficiency (higher biomass yield) compared with terrestrial plants, and that they do not compete with food crops.

Since July 2008, DENSO has been involved in an algal research of cultivating *Pseudochoricystis* in open-type ponds. *Pseudochoricystis* is a tentative name and it may belong to the *Pseudococcomyx*a genus. We constructed pilot-scale raceway ponds in our Zenmyo factory on June 2010. As *Pseudochoricystis* can grow under acidic conditions, it was possible to cultivate this alga for several weeks in the raceway ponds without contamination of other algal species. In these cultures, flue gas from a cogeneration power system in the Zenmyo factory was used as the CO_2 source, while the effluent from an activated sludge wastewater treatment plant in the factory was used as water resource.

In the MAFF project which started on July 2010, DENSO is engaging in the development of new culture processes to lower power consumption and reduce operational cost. We are developing an automated cultivation system and water recycling technologies. In addition, we recently succeeded in developing a system to predict the triacylglycerol productivity in raceway-pond cultures.

In this presentation, I would like to introduce some of our efforts descried above.

Food and Fuel for the 21st Century - Synthetic biology in micro-algae for the production of biofuels and bio-products

Stephen P. Mayfield
Director, San Diego Center for Algae Biotechnology and Professor, Division of Biological Sciences, University of California, San Diego

Fuel, food, and all biological products are all different forms of chemical energy, and as such are closely related. All of these products are ultimately derived from photosynthesis, the process by which sunlight energy is converted to chemical energy. Over the last 100 years we have exploited cheap fossil fuels to drive unprecedented economic and agricultural growth, but in so doing we have released sequestered CO2 into the atmosphere, which is now beginning to impact our climate. In addition, fossil fuel reserves are finite, and we are now starting to see the initial signs of depletion of these reserves, including the rising cost of fuel and food. Together these factors have provided the impetus behind the development of new renewable energy sources that can supplant fossil fuels while greatly reducing carbon emissions into the atmosphere. Eukaryotic algae offer tremendous potential for the large scale production of biofuels and bio-products as algae require only sunlight as an energy source and sequester CO2 during the production of biomass, and algae can be much more efficient then terrestrial plants in fixing CO2 and producing biomass. Using “designed for purpose” photosynthetic organisms we have the opportunity to develop production platforms for fuel and food that have unmatched efficiencies and productivities. We are developing the genetic and synthetic biology tools to enable the production of designer algae as a bio-fuels and bio-products platform. The challenges, potential, and some early successes of synthetic biology in algae for the production of high value products will be discussed.

Abstracts of poster presentations