Biosystems Design to Enable Next-Generation Biofuels

Summary of projects awarded in 2012 under Funding Opportunity Announcement DE-FOA-0000640

Overview

The Genomic Science program within the Office of Biological and Environmental Research (BER) supports basic, multidisciplinary research aimed at achieving a systems-level understanding of plants, microbes, and microbial communities relevant to Department of Energy (DOE) missions in bioenergy, carbon management, and the environment. To provide the scientific foundation for a bio-economy in which new biological systems can be designed to address DOE missions, it is necessary to identify and articulate fundamental biological principles that govern biology from the molecular to the community level. These principles will allow us to predict the behavior of biological systems under changing conditions. Moreover, to be able to tailor their behavior for defined purposes, it will be necessary to re-engineer these biological systems or design new ones. Novel biosystems design tools and technologies will in turn help us better understand natural systems and their response to natural or man-made environmental inputs. Technologies developed in recent years have taken engineering of living systems to a new level, but significant advances are needed to achieve a comprehensive predictive understanding of biological systems to enable re-design of novel organisms. It is also necessary to develop computer-aided design of biological systems by leveraging other activities in the computational biosciences to predict, design, construct, and test multiscale natural and hybrid biological systems that will lead to new clean energy solutions.

In 2012, BER solicited integrated interdisciplinary applications for (1) highly innovative, fundamental genomics and systems biology research and (2) technology development for biosystems design that addresses DOE missions within two focused research areas:
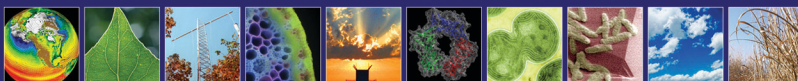
- **Microbial systems design for biofuels: from computer modeling to experimental validation.** Awardees are focusing on the design of new microbial systems, from the development of a computer model to the generation of an experimentally validated, redesigned living organism through iterative network, functional measurements, and model testing. Awardees are using integrated "omics" and computational modeling approaches and developing genome-scale engineering technologies to build innovative, exchangeable biological modules and to engineer phototrophic or fermentative microbial systems for the production of advanced biofuels from light, biomass, or other energy sources.

- **Plant systems design for bioenergy.** Awardees are focusing on the re-design of plants for efficient extraction of cellulosic sugars and their subsequent conversion to advanced biofuels. Projects feature integrative, systems biology approaches to identify regulatory and metabolic modules for re-designing energy crops. Other research involves the development of innovative technologies for large-scale genome engineering and the introduction of biological modules in plants, aiming at producing engineered bioenergy crops with increased photosynthesis capacity, biomass accumulation, and nutrient and water utilization that will allow them to grow in marginal lands.

Biosystems Design Report
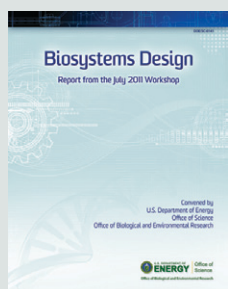
Further information on BER objectives in this area of research can be found in the DOE report *Biosystems Design: Report from the July 2011 Workshop*. The report, along with this list of funded projects, is available: genomicscience. energy.gov/biosystemsdesign/.

Office of Biological and Environmental Research

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2012 Awards

Microbial Systems Design for Biofuels

Optimizing oil production in oleaginous yeast by cell-wide measurements and genome-based models

- **Principal Investigator:** Gregory Stephanopoulos (Massachusetts Institute of Technology, Cambridge)
- **Co-Investigators:** Scott Baker (Pacific Northwest National Laboratory), Jens Nielsen (Chalmers University, Sweden), James Liao (University of California, Los Angeles)

This project will take advantage of an engineered strain of the oleaginous yeast *Yarrowia lipolytica* that can produce high yields of lipids from carbohydrates and organic acids. A genome-scale dynamic metabolic modeling approach called Ensemble Modeling, aided by transcriptomics and metabolomics analysis of lipid content, will be conducted to guide further re-design and engineering of *Y. lipolytica* strains for increased lipid production. Newly optimized yeast strains showing high oil yields from sugars abundant in biomass as well as from acetate, which can be the product of inexpensive anaerobic cellulose degradation, will potentially lead to new and efficient ways for biodiesel production.
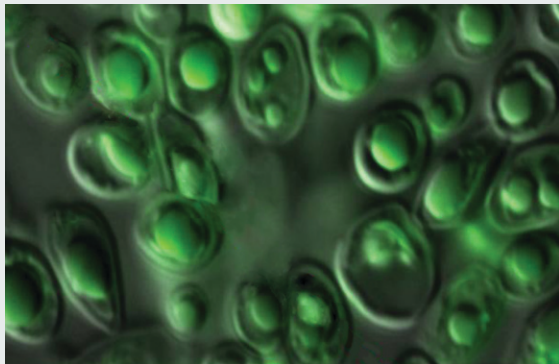
Oleaginous yeast *Yarrowia lipolytica* (Credit: Stephanopoulos Lab, Massachusetts Institute of Technology)

A platform for genome-scale design, redesign, and optimization of bacterial systems

- **Principal Investigator:** Ryan Gill (University of Colorado, Boulder)
- **Co-Investigators:** Rob Knight (University of Colorado, Boulder), Adam Arkin (Lawrence Berkeley National Laboratory), Pin-Ching Maness (National Renewable Energy Laboratory)

Building upon genome engineering technologies developed by the applicants' laboratories as well as others, the researchers will engineer *Escherichia coli* strains with enhanced recombineering capabilities, optimized for the production of ethylene and isobutanol that can be directly converted into biofuels. These strains will be used for the development of a next-generation high-throughput synthetic biology and genomic engineering technology platform for predictive design of DOE-relevant bacterial systems for biofuel production. Multiplexed mutational strategies will be developed to induce genome-wide changes not only in individual promoters but also in other regulatory features and open reading frames. Iterative cycles of metabolic modeling and high-throughput screening will allow the optimization of re-designed biofuel-producing strains.

Optimization of energy flow through synthetic metabolic modules and regulatory networks in a model photosynthetic eukaryotic microbe

- **Principal Investigator:** Andrew Allen (J. Craig Venter Institute, San Diego)
- **Co-Investigators:** Christopher Dupont (J. Craig Venter Institute, San Diego), Bernhard Palsson (University of California, San Diego), Graham Peers (Colorado State University)

This project will generate genome-scale metabolic models of the diatom *Phaeodactylum tricornutum* informed by multiple "omics" techniques, metabolic flux, protein localization, and protein interaction data. Comparative modeling analysis of diatoms and other photosynthetic organisms will be used to identify key regulatory elements targeted for reprogramming gene and metabolic networks to enhance carbon and energy flux toward the production of lipid-based biofuels. Genomic engineering technologies to introduce large extrachromosomal DNA segments into diatoms will be developed using yeast and *E. coli* intermediates to obtain new diatom strains with high oil content.

Assembling reusable genetic modules for efficient biofuel production from marine macroalgae

- **Principal Investigator:** Eric Alm (Massachusetts Institute of Technology, Cambridge)
- **Co-Investigators:** Martin Polz (Massachusetts Institute of Technology, Cambridge), Christopher Rao (University of Illinois), HuiMing Zhao (University of Illinois)

In order to take advantage of the lack of lignin and crystalline cellulose in the cell wall of brown macroalgae, this project will investigate algae-associated microbes to identify new metabolic modules capable of degrading the brown algae carbohydrates such as alginate, laminarin, and fucoidan, thus circumventing the recalcitrance problem of land plants whose lignocellulosic cell walls are very difficult to degrade. In addition to known algae-associated candidate microbes, uncultured microorganisms will be analyzed by fluorescence-based screening and single-cell genomics methods to identify new metabolic capabilities. Genetic modules comprising selected metabolic pathways will be engineered in yeast and *E. coli* using a one-step method for the design and construction of biosynthetic pathways that can be tuned with a combinatorial transcriptional engineering approach for heterologous gene expression.

Plant Systems Design for Bioenergy

Modeling and manipulating phenylpropanoid pathway flux for bioenergy

- **Principal Investigator:** Clint Chapple (Purdue University)
- **Co-Investigators:** Natalia Dudareva (Purdue University), John Morgan (Purdue University)

This project will develop advanced kinetic models for the plant shikimate and phenylpropanoid/phenylalanine pathways using *Arabidopsis* as a model system to allow predictive design of experiments to modulate fluxes toward desired products. The investigators will carry out flux measurements in plants with altered lignin and phenylalanine pathways, including plants expressing a novel hydroxylase that reroutes lignin synthesis, uncoupling it from the shikimate pool levels. As a proof of concept, they will engineer plants for the production of 2-phenylethanol, which is a promising biofuel candidate due to its high energy density, low hygroscopicity, and low volatility.

A systems-level analysis of drought and density response in the model C4 grass *Setaria viridis*

- **Principal Investigator:** Tom Brutnell (Donald Danforth Plant Science Center, St. Louis)
- **Co-Investigators:** Ivan Baxter (USDA Agricultural Research Service; Donald Danforth Plant Science Center, St. Louis); Asaph Cousins (Washington State University); Jose Dinneny, Sue Rhee (Carnegie Institution for Science, Stanford); Andrew Leakey (University of Illinois); Todd Mockler (Donald Danforth Plant Science Center); Daniel Voytas (University of Minnesota)

This multidisciplinary research team will address two important challenges to developing high-yield bioenergy crops that can grow in arid, marginal lands to avoid land competition with food crops. The project will start by conducting an extensive quantitative trait loci (QTL) study of drought tolerance and planting density in the model C4 grass *Setaria viridis*, which is a close relative of biomass crops such as switchgrass, sugarcane, sorghum, and *Miscanthus*. Extensive molecular and physiological studies will be carried out in selected lines to build integrated metabolic and genetic networks; candidate genes will then be analyzed in the context of those network models. New transformation technologies will be tailored for *Setaria* biodesign, and methods for monitoring flow of transgenes will be developed as part of this project.

Engineering CAM photosynthetic machinery into bioenergy crops for biofuels production in marginal environments

- **Principal Investigator:** John Cushman (University of Nevada, Reno)
- **Co-Investigators:** James Hartwell (University of Liverpool); Anne Borland, Jin-Gui Chen, Madhavi Martin, Timothy Tschaplinski, Gerald Tuskan, David Weston, Xiaohan Yang (Oak Ridge National Laboratory); Karen Schlauch (University of Nevada, Reno)

Crassulacean Acid Metabolism (CAM) is a photosynthetic CO_2 fixation mechanism common in plants that grow in hot and arid environments to maximize water

use efficiency. This project will attempt to engineer a functional CAM pathway in poplar and *Arabidopsis*. The introduction of this metabolic machinery into a bioenergy crop such as poplar will increase its water use efficiency allowing cultivation in marginal lands for biofuel production. Genomics and systems biology analyses of monocotyledonous and dicotyledonous CAM species will identify the necessary carbon assimilation and stomatal control modules for engineering C3 plants such as poplar, as well as the model plant *Arabidopsis*, to acquire CAM metabolism.

The weed *Setaria viridis*, or green bristlegrass, a relative of *Setaria italica* (foxtail millet) and an emerging model system for bioenergy crops. (Credit: Daniel Waxler, Donald Danforth Plant Science Center)

Expanding the breeder's toolbox for perennial grasses

- **Principal Investigator:** Eduardo Blumwald (University of California, Davis)
- **Co-Investigators:** John Vogel, Christian Tobias, Roger Thilmony (USDA Agricultural Research Service)

The goal of this project is to engineer the bioenergy crop switchgrass to facilitate molecular breeding approaches for genetic improvement, overcoming major problems posed by its tetraploid nature and the lack of inbred lines. Investigators will engineer new tools to dramatically advance breeding in switchgrass by developing homozygous plants through the generation of double haploids. To do this, the researchers will create a mutant in a centromere-specific histone that produces haploid plants, which can be diploidized upon crossing to wild type individuals. This project will also use a perennial *Brachypodium sylvaticum* model system to understand and improve drought tolerance and nutrient use efficiency and to develop new tools for further genomic engineering of perennial grasses. Translation of these technologies to switchgrass will include the development of transgenic systems to minimize gene flow.

Contact and Websites

Websites

DOE Office of Science
- science.energy.gov

DOE Office of Biological and Environmental Research
- science.energy.gov/ber/

BER Genomic Science program
- genomicscience.energy.gov

Biosystems Design Report
- genomicscience.energy.gov/biosystemsdesign/