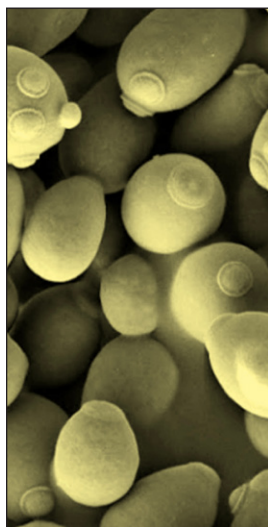


Biosystems Design to Enable Next-Generation Biofuels and Bioproducts

Summary of projects awarded in 2017 under Funding Opportunity Announcement DE-FOA-0001650

Genomic Science Program

genomicscience.energy.gov



Overview

The Genomic Science program within the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (BER) supports basic interdisciplinary research aimed at achieving a predictive, systems-level understanding of plants, microbes, and microbial communities to advance BER energy and environmental missions. Building on an ever-growing body of genomic sequence information, the program integrates large-scale systems biology (transcriptomics, proteomics, metabolomics, and other “-omics” approaches) with computational modeling to comprehensively understand the foundational principles that rule biological systems. Achieving this knowledge enables the design and engineering of DOE-relevant plants and microbes for defined purposes and reveals insights into their responses to environmental inputs. Within the Genomic Science program, Biosystems Design research has contributed to the advancement of bioengineering, often referred to as synthetic biology.

New genome-scale engineering and synthetic biology tools are opening new avenues for systems biology research. These tools not only enable the design of tailored plants and microbes by genome editing or introduction of megabase-size synthetic constructs with orthogonal pathways, they also advance fundamental understanding of biological function and inspire novel approaches for its manipulation on a large scale. Critical components of these tools are computational modeling and computer-aided design methods that integrate large

-omics datasets to solve grand challenges in biological engineering.

In fiscal year 2017, BER solicited integrative, multidisciplinary applications to conduct fundamental genomics and systems biology research and technology development to design novel biosystems within the following research areas:

- **Integrating large-scale systems biology data to model, design, and engineer microbial systems for the production of biofuels and bioproducts.** Projects should focus on the development of innovative modeling and high-throughput genome-wide design, engineering, and testing of new strains using *in vivo* or cell-free approaches for new or emerging eukaryotic and prokaryotic microbial systems that produce high levels of biofuels and bioproducts from lignocellulosic biomass or by photosynthesis.
- **Designing plant systems for bioenergy.** Projects should use multidisciplinary approaches to engineer potential bioenergy crops for sustainable production of biofuels and bioproducts from lignocellulose or oils, while growing in marginal environments. Project goals include increased abiotic stress tolerance, higher water and nutrient use efficiency, improved photosynthetic capacity, and high productivity, as well as the development of technologies for the introduction and expression of large, stable, multigene DNA constructs, aided by computational modeling and design.



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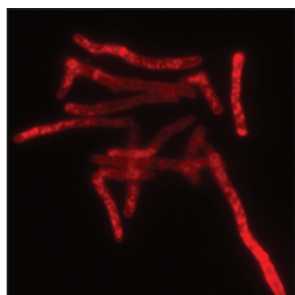
Office of
Science

Office of Biological and Environmental Research

Microbial Research Awards

Systems engineering of *Rhodococcus opacus* to enable production of drop-in fuels from lignocellulose

- **Principal Investigator:** Gautam Dantas (Washington University in St. Louis)
- **Co-Investigators:** Tae Seok Moon, Marcus Foston, Yinjie Tang, Fuzhong Zhang (Washington University in St. Louis); Hector Garcia Martin (Lawrence Berkeley National Laboratory)



Fluorescent micrograph of *Rhodococcus opacus* cells designed to convert phenolic compounds into lipids. Cells were stained with Nile Red to observe lipid bodies as bright red spots.

Building on prior funding from BER for systems biology work on *Rhodococcus opacus*, this project will apply innovative combinations of -omics and mass spectrometry data analysis, flux analysis, and machine learning to develop improved genome-scale models that will guide multiplex genome engineering of *R. opacus* strains with branched-chain fatty acid esters (BCFAE) biosynthetic capacity and improved tolerance to lignin degradation products.

The project will take advantage of this organism's capability to use both polysaccharides and lignin as carbon sources as well as its natural tolerance to phenolic compounds such as those produced by lignin breakdown.

Design and engineering of synthetic control architectures

- **Principal Investigator:** Ryan Gill (University of Colorado Boulder)
- **Co-Investigators:** Christopher Voigt (Massachusetts Institute of Technology); Adam Arkin (Lawrence Berkeley National Laboratory); Carrie Eckert (National Renewable Energy Laboratory)

The strength of this project is that it will advance biological engineering to a new level of throughput and complexity. Guided by computer-aided design platforms, this research will develop new finely tuned microbial strains containing orthogonal regulatory networks. The redesigned strains will be tailored to grow on a variety of feedstocks, tolerate toxic

metabolites and other stresses, and produce valuable molecules. Building on technologies and knowledge developed with prior DOE funding from the Biosystems Design program, the project will further develop the necessary technical and computational infrastructure to achieve its goals, initially working with *Escherichia coli* and *Saccharomyces cerevisiae* and later transferring the technologies to other non-model bacteria and yeast species.

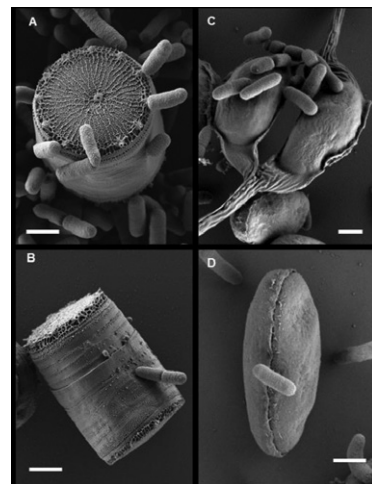
Design, synthesis, and validation: Genome-scale optimization of energy flux through compartmentalized metabolic networks in a model photosynthetic eukaryotic microbe

- **Principal Investigator:** Andrew Allen (J. Craig Venter Institute)
- **Co-Investigators:** Chris Dupont, Phil Weyman (J. Craig Venter Institute); Graham Peers, Wen Zhou (Colorado State University); Jamey Young (Vanderbilt University); Karsten Zengler, Bernhard Palsson (University of California, San Diego)

The goal of this research is to integrate genome-scale modeling efforts of the diatom *Phaeodactylum tricornutum* to

inform experimental research for the production of high-value and fuel-related metabolites. Leveraging prior work on this organism under DOE Biosystems Design funding, this project will apply genome-scale engineering to optimize carbon, energy, photosynthesis, and metabolite flux to shift the native *P. tricornutum* metabolism from carbohydrate storage into lipid and/or branched chain amino acid (BCAA)

accumulation. The team will focus on compartmentalizing relevant biosynthetic pathways on artificial chromosomes to obtain modified diatom strains with high productivity of triacylglycerols or BCAAs.



Physical association between *E. coli* and diatoms during conjugative delivery of designer diatom episomes by bacteria.

Genome-scale design and engineering of non-model yeast organisms for production of biofuels and bioproducts

- **Principal Investigator:** Huimin Zhao (University of Illinois at Urbana-Champaign)
- **Co-Investigators:** Christopher Rao (University of Illinois at Urbana-Champaign); Costas Maranas (Pennsylvania State University); Joshua Rabinowitz, Martin Wuhr (Princeton University); Yasuo Yoshikuni (Lawrence Berkeley National Laboratory)

This project will focus on *Rhodospiridium toruloides* and *Issatchenkia orientalis*, two industrial yeast species, to develop the tools needed to turn these organisms into model systems for biological design. *R. toruloides* will be engineered to produce fatty alcohols from lignocellulose-derived sugars, and the target products for *I. orientalis* will be organic acids such as muconic acid and methyl methacrylate. The project will leverage genome-scale engineering tools previously developed for *Saccharomyces cerevisiae* by the PI's laboratory that apply CRISPR-Cas methods for multi-gene disruptions (homology integrated CRISPR-Cas or HI-CRISPR) and CRISPR-based gene activation, interference, and deletion (CRISPR-AID). A highly automated robotic pipeline already operational at the University of Illinois will be used for high-throughput genetic manipulation and engineering of the two yeast species.

Establishing a *Clostridia* foundry for biosystems design by integrating computational modeling, systems-level analyses, and cell-free engineering technologies

- **Principal Investigator:** Michael Jewett (Northwestern University)
- **Co-Investigators:** Keith Tyo, Linda Broadbelt (Northwestern University); Michael Köpke, Sean Simpson (LanzaTech); Steven Brown, Timothy Tschaplinski, Robert Hettich, Richard Gionnone (Oak Ridge National Laboratory)

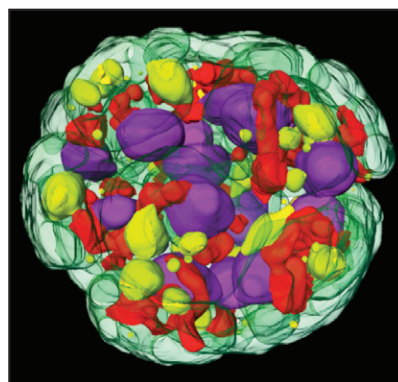
This project will develop an integrated set of technologies for high-throughput metabolic pathway design and engineering of *Clostridia*, called the Clostridia Foundry for Biosystems Design (cBioFAB), to produce fuels and chemicals from lignocellulose-derived syngas. Using multi-omics, computational modeling, genome engineering, and cell-free and *in vivo* technologies, the Foundry will focus on the production of acetone, butanol, butanediol, and mono-ethylene glycol as proof of concept. The project will leverage DOE Joint Genome Institute (JGI)

efforts to sequence the genomes of multiple *Clostridium* strains and use a computer-aided design tool called Biochemical Network Integrated Computational Explorer (BNICE) developed by team members to identify relevant metabolic genes and pathways that will be subsequently tested in cell-free systems. New CRISPR-based genome-editing techniques will be developed for *Clostridium*, and metabolic ensemble modeling will be used to predict *in vivo* pathway performance from cell-free assays.

Systems analysis and engineering of biofuel production in *Chromochloris zofingiensis*, an emerging model green alga

- **Principal Investigator:** Krishna Niyogi (University of California, Berkeley)
- **Co-Investigators:** Crysten Blaby (Brookhaven National Laboratory); Nanette Boyle (Colorado School of Mines); Mary Lipton (Pacific Northwest National Laboratory); Sabeeha Merchant (University of California, Los Angeles); Trent Northen (Lawrence Berkeley National Laboratory)

The model system on which this project will focus is the oleaginous green alga *Chromochloris zofingiensis* because it is fast-growing, can be cultured at high densities, and



Cryo-soft X-ray tomography of a reconstructed *Chromochloris zofingiensis* cell with segmented nucleus (purple), chloroplast (green), mitochondria (red), lipids (yellow), and starch granules within the chloroplast (blue).

accumulates triacylglycerols (TAG) at the highest levels observed among microalgae. *C. zofingiensis* metabolic regulation is unique because in the presence of glucose, photosynthesis is switched off by degrading the thylakoid membranes and photosynthetic machinery, while lipid production is substantially increased; the

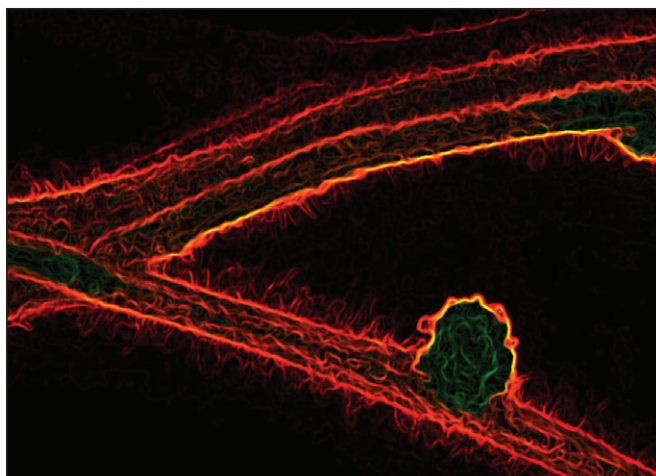
process is reversed when the glucose supply is exhausted. The project will leverage a high-quality genome assembly of the *C. zofingiensis* genome recently generated by the research team and will enhance it with multi-omics and physiological analyses to build a model to guide metabolic engineering of this alga for the production of biofuel precursors.

Plant Research Awards

Phylogenomic discovery and engineering of nitrogen fixation in the bioenergy woody crop poplar

- **Principal Investigator:** Matias Kirst (University of Florida)
- **Co-Investigators:** Sushmita Roy, Jean-Michel Ané (University of Wisconsin, Madison); Robert Guralnick, Pamela Soltis, Douglas Soltis (University of Florida)

To identify the plant genes involved in the development of symbiotic nodules containing nitrogen-fixing microbes (diazotrophs), this project will analyze the evolution of those symbioses. Prior studies by members of the research team showed that microbe-mediated nitrogen fixation was originated and lost multiple times during the evolution of the nodulating plant clade. Therefore, investigators intend to identify the genetic machinery used by nodulating plants and introduce it into non-nodulating bioenergy crops such as poplar, thereby reducing this crop's need of nitrogen fertilization. To achieve this ambitious goal, the team will conduct extensive phylogenetic analysis of over 20,000 species using available genomic sequence data as well as samples from multiple plant species, including preserved museum specimens from around the world. Selected engineered poplar lines will be tested for their ability to establish symbiotic relationships with diazotrophs, as well as for their biomass productivity and composition.



Transgenic roots of the model legume *Medicago truncatula* expressing DsRed, with nodules formed by its symbiont (*Sinorhizobium meliloti*).

SyPro poplar: Improving poplar biomass production under abiotic stress conditions: an integrated omics, bioinformatics, synthetic biology, and genetic engineering approach

- **Principal Investigator:** Eduardo Blumwald (University of California, Davis)
- **Co-Investigators:** Amir Ahkami (Pacific Northwest National Laboratory); C. Neal Stewart (University of Tennessee); Stephen DiFazio (West Virginia University)

To engineer bioenergy crops that can grow in dry, marginal lands, this project will introduce into poplar trees tolerance to different environmental stresses such as water deficit, high soil salinity, and heat. As plants are often exposed to combinations of these different types of abiotic stress, poplar lines will be engineered to express multiple stress-related genes, each one controlled by a synthetic promoter that is only induced under specific stress conditions and within the tissues where its function is needed, improving biomass production under unfavorable conditions while avoiding negative effects of unwanted transgene expression. The project will focus on a poplar hybrid clone for which a genome sequence is available and will design tissue- and stress-specific synthetic promoters using plant promoter databases and computational tools to identify cis-regulatory elements.

Using systems approaches to improve photosynthesis and water use efficiency in sorghum

- **Principal Investigator:** Thomas Brutnell (Donald Danforth Plant Science Center)
- **Co-Investigators:** Ivan Baxter (U.S. Department of Agriculture and Donald Danforth Plant Science Center); Todd Mockler, Hector Quemada (Donald Danforth Plant Science Center); Asaph Cousins (Washington State University); Jose Dinneny, Sue Rhee (Carnegie Institution for Science); Albert Kausch (University of Rhode Island); Andrew Leakey (University of Illinois, Urbana-Champaign); Daniel Voytas (University of Minnesota)

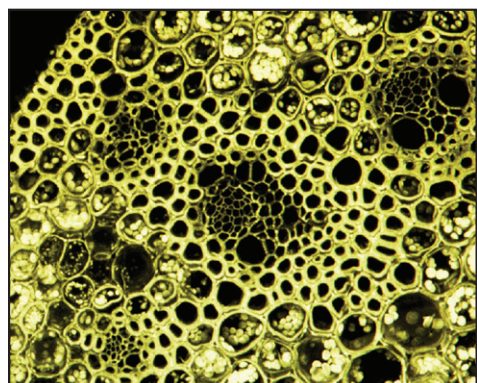
Building on prior work on the model grass *Setaria*, funded by DOE as part of the Biosystems Design program, this project aims at increasing water use and photosynthetic efficiencies in the bioenergy crop sorghum. Leveraging the resources previously developed by the research team for *Setaria*, investigators will accelerate the generation of engineered high-biomass sorghum lines

that grow in marginal lands using innovative genome-editing tools, genome-scale modeling, -omics approaches, genome-wide association studies, and high-throughput phenotyping in both laboratory and field settings. The work will exploit the efficient transformation and screening pipelines available for *Setaria* to facilitate gene discovery and pathway design that can be transferred to sorghum. Because biomass productivity strongly depends on photosynthesis and water availability, the engineering work will focus on three main topics: enhanced carbon assimilation, increased root water uptake, and reduced water loss through stomata.

Building on success in systems design of high-yielding, low-input energy canes for marginal lands

- **Principal Investigator:** Stephen Long (University of Illinois, Urbana-Champaign)
- **Co-Investigators:** Don Ort, Vijay Singh, Li-Qing Chen (University of Illinois, Urbana-Champaign); John Shanklin (Brookhaven National Laboratory); Fredy Altpeter (University of Florida); Brian Baldwin (Mississippi State University)

This project will pursue an innovative approach to engineer oil accumulation in vegetative tissues of bioenergy crops. Leveraging previous efforts toward



Section of a *Miscanthus* stem under a confocal microscope showing oil accumulation within parenchymatic cells.

sugar cane, CRISPR/Cas9-based genome-editing technologies previously developed in sugar cane will likely be transferable to these new plant systems along with novel genome-engineering methods. The ultimate goal of the project is to reach a TAG accumulation of up to 20% of dry stem weight, which represents a productivity increase of more than one order of magnitude

producing oil in sugar cane leaf tissue, the researchers will attempt to redirect and increase TAG accumulation in the stems of energy cane and *Miscanthus*. As these species are close relatives of

relative to oilseed crops. Computational modeling will be used to design improved photosynthetic capacity that they expect to reach 50%, along with increased nitrogen and water use efficiency, to account for the required redirection of carbon flow from lignocellulosic biomass to oil in the redesigned plant lines.

Biological design of *Lemnaceae* aquatic plants for biodiesel production

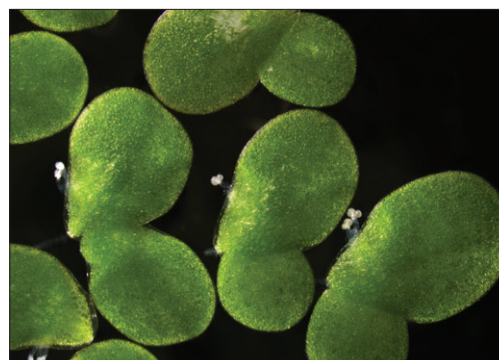
- **Principal Investigator:** Robert Martienssen (Cold Spring Harbor Laboratory)
- **Co-Investigators:** James Birchler (University of Missouri); Eric Lam (Rutgers University); Jorg Schwender, John Shanklin (Brookhaven National Laboratory)

The goal of this project is to divert carbon flow from starch accumulation to oil production in resting fronds of *Lemnaceae* (duckweed) species, thereby redesigning existing

commercial strains towards biofuel production.

Lemnaceae's rapid growth, high proportion of photosynthetic tissue due to its

lack of lignified supportive tissue, and its ability to grow in marginal wastewater environments are among the advantages of these small aquatic plants. An available genome sequence and other -omics resources will be leveraged, along with other products from prior DOE funding (ARPA-E) to significantly increase oil content in *Lemnaceae*. Metabolic pathways will be re-engineered to maximize TAG yields while preserving rapid biomass accumulation, and tools for genetic manipulation, metabolic flux models, and a catalog of key transcription factors and promoters will be developed to elevate this emerging plant model system to a potential bioenergy crop.



Duckweed fronds (photosynthetic part of the plant body) and flowers (white protrusions).

A systems approach to increasing carbon flux to seed oil for biofuels and bioproducts production in *Camelina sativa*

- **Principal Investigator:** Danny Schnell (Michigan State University)
- **Co-Investigators:** Erich Grotewold, Yair Shachar-Hill (Michigan State University); Heike Sederoff (North Carolina State University); Kristi Snell (Yield10 Bioscience)

This project will establish *Camelina sativa* as a commercially viable, dedicated biofuel and bioproduct feedstock. Its low agronomic input requirements, natural tolerance to different stresses, available molecular

and genomics tools and resources, and suitability of its oil for transportation as liquid fuel blends are some of *Camelina*'s advantages over other oilseed crops. However, oil yield from *Camelina* seeds is relatively low. To improve oil production, an integrative approach including metabolic flux modeling, proteomics, regulatory network analysis, and multi-gene stacking and genome-editing techniques will be employed to optimize photosynthetic carbon fixation in leaves, its transport to seeds, and the allocation of fixed carbon to TAG. With this approach, the team aims at increasing oil production to 300% per hectare, thus greatly enhancing the feasibility of developing *Camelina* as a cost-competitive bioenergy crop.

Contact and Websites

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Websites

Biosystems Design report

- genomicscience.energy.gov/biosystemsdesign/

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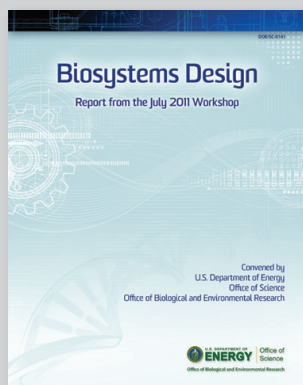
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Biosystems Design Report

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

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November 2017