Biosystems Design to Enable Safe Production of Next-Generation Biofuels, Bioproducts, and Biomaterials

Summary of projects awarded in 2022 under Funding Opportunity Announcement DE-FOA-0002600

Funded Projects

Microbial Awards

- Designing Large, Genome-Wide CRISPRa/i Programs for Efficient and Effective Carbon-Conserving Bioproduction
- Integrating Cell-Free Systems and Genome Engineering to Accelerate Biosystems Design for Carbon-Negative Biomanufacturing
- Microbial Community Engineering Tools for Enhancing Polyolefin Degradation and Valorization
- Systems Engineering of *Auxenochlorella protothecoides*: From Photosynthesis to Biofuels and Bioproducts

Plant Awards

- Integrated Engineering of Whole Plant Water Use Efficiency in Sorghum and *Setaria*
- B5: Bigger Better Brassicaceae Biofuels and Bioproducts
- BioPoplar: A Tunable Chassis for Diversified Bioproduct Production

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DOE Biological and Environmental Research Program
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DOE Office of Science
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The U.S. Department of Energy (DOE) Office of Science Biological and Environmental Research (BER) program supports fundamental, interdisciplinary research to achieve a predictive systems-level understanding of Earth, environmental, and biological systems. BER's overarching goal is to support transformative science to solve critical challenges in energy security and environmental stewardship. Within BER, the Biological Systems Science Division (BSSD) aims to understand, predict, manipulate, and design biological processes that underpin innovations for bioenergy and bioproduct research and to enhance understanding of natural environmental processes relevant to DOE. BSSD supports fundamental research on the systems biology of plants and microbes through the Genomic Science program (GSP).

GSP's portfolio includes systems biology research that builds on a foundation of multiomics data and integrates multidisciplinary experimental and computational approaches. Within this framework, one GSP objective is to develop the next generation of genome-engineering technologies to unlock the potential of plants and microorganisms for advancing a sustainable and secure bioeconomy. To this end, tools are needed to enable these organisms to safely and efficiently convert renewable biomass, carbon dioxide (CO_2) captured from the atmosphere, and petroleum-derived polymers into fuels, valuable chemicals, and materials with novel properties. Iterative application and testing of these engineering technologies to design living organisms with new functional properties also lead to a deeper understanding of the fundamental principles governing those organisms. This "design, build, test, learn" (DBTL) cycle not only results in improved biosystems design, it also leads to a more comprehensive knowledge of relevant biological systems.

During the last decade, scientists made momentous advances in the fields of systems biology, synthetic biology, and artificial intelligence that have dramatically accelerated the DBTL cycle for engineering biology. More efficient approaches for genome-wide editing, analysis, and phenotyping have become available, and new computational tools and modeling algorithms can handle increasingly large datasets while continuously improving their prediction accuracy.

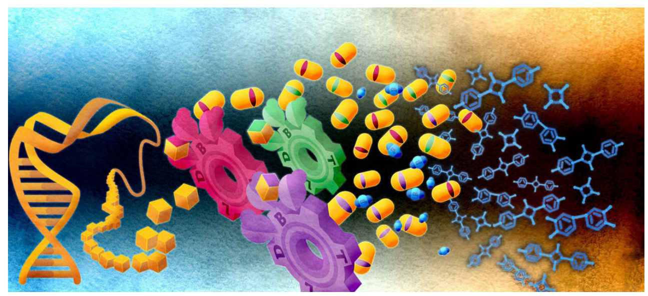
In Fiscal Year 2022, BER solicited integrative, multidisciplinary applications for highly innovative, fundamental multiomics and systems biology research and technology development for biosystems design within two research areas:

- **Microbial biosystems design for the production of biofuels, bioproducts, and biomaterials:** Interdisciplinary, fundamental systems biology research to advance the development of new genome-wide design and engineering technologies, innovative modeling, and high-throughput testing approaches for a broad range of prokaryotic and eukaryotic microbes relevant to the production of biofuels and bioproducts from biomass and synthetic polymers or as a byproduct of photosynthesis.
- **Plant biosystems design for bioenergy, bioproducts, and biomaterials:** Integrative, basic research in plant systems biology, genome-scale modeling, design, and engineering to advance the development of enhanced bioenergy crops capable of producing biofuels, bioproducts, biomaterials, and their precursors while growing in marginal environments.

2022 Microbial Awards

Designing Large, Genome-Wide CRISPRa/i Programs for Efficient and Effective Carbon-Conserving Bioproduction

- **Principal Investigator:** James Carothers (University of Washington)
- **Co-Investigators:** Jesse Zalatan, Herbert Sauro, Georg Seelig (University of Washington); Anna Kuchina (Institute for Systems Biology); Pamela Peralta-Yahya (Georgia Institute of Technology); Alexander Beliaev, Jeremy Zucker, Nathalie Munoz, Joshua Elmore (Pacific Northwest National Laboratory); Héctor García Martin, Yasuo Yoshikuni (Lawrence Berkeley National Laboratory)



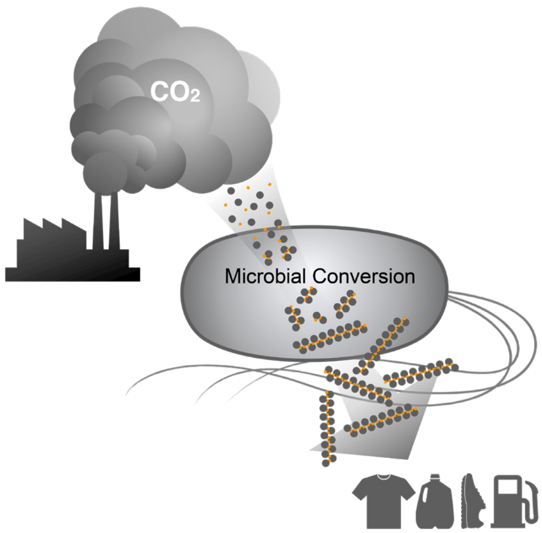
A University of Washington-led team of synthetic biologists is engineering microbial genomes to transform carbon dioxide into high-value chemicals. [Courtesy University of Washington]

This project will develop bacterial CRISPR/Cas-based genome-editing technologies, including the challenging CRISPR-activation, to enable editing 25 or more gene targets at a time as well as rewiring dynamic gene regulatory networks. The team will use machine learning and systems biology approaches such as high-throughput, single-cell transcriptomics to assess the potential design space for increasing carbon efficiency of central metabolism. These technologies and approaches will be integrated to engineer heterotrophic and phototrophic bacteria for increased carbon conservation and sequestration. Demonstrating the application of the engineering technologies to gram-positive, gram-negative, and purple nonsulfur bacteria will facilitate the portability of the methods to diverse bacterial species, advancing toward species-agnostic genome engineering. Up to a third of the carbon metabolized through microbial glycolysis is released as CO_2 waste, thus this project's goal of redesigning central metabolism to redirect carbon flux from CO_2 to valuable products will increase carbon conservation in bioproduction processes.

Integrating Cell-Free Systems and Genome Engineering to Accelerate Biosystems Design for Carbon-Negative Biomanufacturing

- **Principal Investigator:** Michael Jewett (Northwestern University)
- **Co-Investigators:** Ashty Karim (Northwestern University); Michael Köpke, Ching Leang (LanzaTech); Farren Isaacs (Yale University); Christopher Johnson, Gregg Beckham (National Renewable Energy Laboratory)

Focusing on the anaerobic acetogen *Clostridium autoethanogenum* and the aerobic hydrogenotrophic *Cupriavidus necator*, this project will develop bacterial



Schematic representation of the project's approach to fix atmospheric carbon dioxide for carbon-negative biomanufacturing using redesigned and optimized microbial systems. [Courtesy National Renewable Energy Laboratory]

bioprocessing approaches to produce valuable industrial products while sequestering CO_2 from the atmosphere. The team will develop synthetic biology tools, including CRISPR-associated transposases, multiplexed recombineering, biosensors, machine learning–guided enzyme engineering, computer-aided design software, and genome-reduction approaches, along with cell-free systems for optimized bioproduction. Superspecialist, genome-reduced strains will be intrinsically biocontained because they cannot survive in natural environments. These bacterial and cell-free systems will be testbeds for carbon capture and production of solvents, jet and diesel fuels, and plastic precursors enabling secure carbon-negative biomanufacturing.

Microbial Community Engineering Tools for Enhancing Polyolefin Degradation and Valorization

- **Principal Investigator:** Mark Blenner (University of Delaware)
- **Co-Investigators:** LaShanda Korley, Kevin Solomon (University of Delaware); Yinjie Tang (Washington University, St. Louis); Carrie Eckert, Adam Guss, William Alexander (Oak Ridge National Laboratory)

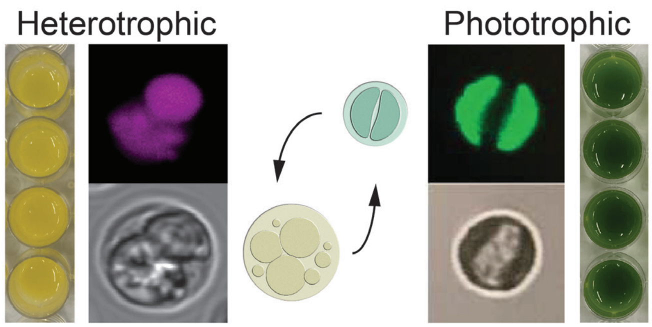
This project will address the global plastic waste crisis and reduce the climate impacts of plastic production by focusing on the yellow mealworm gut microbiome. This microbiome has a natural capacity to degrade a broad range of plastics without chemical pretreatment, which is enhanced by co-feeding with a mixture of plastics and organic supplements. The research will elucidate the metabolic and genetic basis of the observed co-feeding effect, and new genome-engineering techniques will be developed for diverse members of this interkingdom community. The project's objectives include the improvement of heterologous gene expression and transformation methods for nonmodel bacteria and fungi based on restriction-modification and epigenetic mechanisms. These technologies will be used to redesign metabolic pathways of plastic-degrading microbial strains or communities to enable the degradation of polyethylene and polystyrene, as well as the synthesis of polyester precursors, advancing toward a circular bioeconomy.

Feed additives can greatly accelerate the rate of plastic consumption by mealworms and alter the composition of the gut microbiome responsible for plastic depolymerization. [Courtesy University of Delaware]

Systems Engineering of *Auxenochlorella protothecoides*: From Photosynthesis to Biofuels and Bioproducts

- **Principal Investigator:** Sabeeha Merchant (University of California, Berkeley)
- **Co-Investigators:** Melissa Roth, Krishna Niyogi, Jeffrey Moseley (University of California, Berkeley); Nanette Boyle (Colorado School of Mines); Mary Lipton (Pacific Northwest National Laboratory); Crysten Blaby, Trent Northen, Setsuko Wakao (Lawrence Berkeley National Laboratory)

Leveraging the potential of microalgae to capture and convert atmospheric CO_2 into renewable fuels and bioproducts, this project will study and engineer the oleaginous green microalga *Auxenochlorella protothecoides*, which is already used in industrial settings. Taking



Center: Schematic of *Auxenochlorella protothecoides* reversibly cycling between photoautotrophic growth and heterotrophic lipid synthesis. Green fluorescence corresponds to chlorophyll, purple fluorescence corresponds to lipids, black/white corresponds to differential interference contrast microscopy images of the cells above. **Left:** Cell culture growing in heterotrophic mode (yellow indicates high lipid concentration). **Right:** Cell culture growing in photoautotrophic mode (green indicates presence of chlorophyll). [Courtesy Lawrence Berkeley National Laboratory and University of California, Berkeley]

advantage of existing genetic tools and genomic resources for this organism, this project will expand the synthetic biology toolkit for *A. protothecoides* and use it to increase photosynthetic efficiency while producing high titers of cyclopropane fatty acids of tailored lengths that can be used as precursors for jet fuels, minimizing downstream chemical processing requirements. Innovative engineering approaches will be used to improve photosynthesis by increasing ribulose 1,5-bisphosphate regeneration in the Calvin-Benson cycle, enhancing CO_2 capture capacity and augmenting tolerance to light and copper-deficiency stresses in this microalga. The organism's small streamlined genome and the lack of conserved components of known algal carbon-concentrating mechanisms will allow the planned multiomics and systems biology analyses to potentially discover new targets for photosynthetic improvement.

2022 Plant Awards

Integrated Engineering of Whole Plant Water Use Efficiency in Sorghum and *Setaria*

- **Principal Investigator:** Ivan Baxter (Donald Danforth Plant Science Center)
- **Co-Investigators:** Todd Mockler (Donald Danforth Plant Science Center); Asaph Cousins (Washington State University); Jennifer Brophy, José Dinneny (Stanford University); Sue Rhee (Carnegie Institution for Science); Albert Kausch (University of Rhode Island); Daniel Voytas (University of Minnesota); Andrew Leakey (University of Illinois)

This project will address the challenge of improving water use efficiency (WUE) while increasing biomass in sorghum to advance its potential as a bioenergy crop. The team will accomplish this by enhancing photosynthetic capacity, optimizing water evaporation and CO_2 conductance through stomata and the cell wall, and increasing water acquisition through the root system. To achieve these goals, new virus-induced germline gene editing in Cas9 will be deployed in transgenic plants to increase transformation efficiency and throughput in sorghum. Systems biology approaches, multiomics data, and multiscale modeling methods will be leveraged to identify candidate genes to engineer synthetic regulatory circuits. These engineering approaches will be tested and tuned in the fast-growing model C4 grass *Setaria*, leveraging high-throughput phenotyping, imaging, and robotics capabilities. These approaches will enable rapid experimental iterations leading to faster and substantial WUE improvements in bioenergy sorghum.

B5: Bigger Better Brassicaceae Biofuels and Bioproducts

- **Principal Investigator:** Edgar Cahoon (University of Nebraska)
- **Co-Investigators:** Douglas Allen (U.S. Department of Agriculture, Agricultural Research Service, and Donald Danforth Plant Science Center); Malia Gehan (Donald Danforth Plant Science Center); Philip Bates (Washington State University); Timothy Durrett, Ruth Welti (Kansas State University); Jerome Fox (University of Colorado, Boulder); Trupti Joshi, Jay Thelen, Dong Xu (University of Missouri); Chaofu Lu (Montana State University); Michael Smanski (University of Minnesota)

Due to their high productivity under water and nutrient limitations, the Brassicaceae *Camelina* and pennycress have high potential as bioenergy crops. This project will engineer fatty acid and triacylglycerol (TAG) metabolism

Field testing of *Camelina* biodesigned to produce oils with medium-chain fatty acids for sustainable aviation fuel synthesis optimization. Plots are from the 2020 growing season at the Eastern Nebraska Research, Extension, and Education Center. [Courtesy University of Nebraska]

in these oilseeds to produce industrial chemicals and diesel and aviation fuels, which cannot be replaced by electric power. Using computational model–guided genome editing and comprehensive biochemical and multiomic analyses, the research will focus on understanding and controlling plastid oil metabolism, particularly acetyl-CoA carboxylase and fatty acid synthase activities, to engineer more homogeneous fatty acids with shorter carbon chains and less polyunsaturation, which will increase their industrial value.

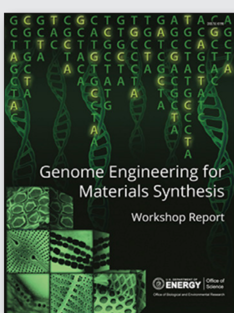
BioPoplar: A Tunable Chassis for Diversified Bioproduct Production

- **Principal Investigator:** C. Robin Buell (University of Georgia)
- **Co-Investigators:** Wayne Parrott, Robert Schmitz, Chung-Jui Tsai, Breeanna Urbanowicz (University of Georgia); Patrick Shih (University of California, Berkeley); Christopher Dardick (U.S. Department of Agriculture, Agricultural Research Service)

Using precision-genome and epigenome-engineering approaches, this project will design new poplar varieties to enable the use of poplar as a multipurpose crop for the production of valuable chemicals and materials in addition to biofuels. To achieve this goal, a poplar gene expression atlas at single-cell resolution will be developed. The resulting high-resolution gene regulatory networks will be leveraged to engineer tree architecture, leaf morphology, and leaf hair density to optimize crops for desired growth and harvesting characteristics. Metabolism and cell wall composition will be further engineered for customized production of chemicals, fuels, and materials while also increasing photosynthesis through enhanced light capture. This research will generate chemotypes and morphotypes of poplar that can be used as chassis for tailored biodesign.

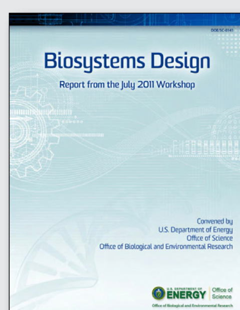
More Information

BER objectives in this program element can be found in the following DOE reports. Both reports, along with lists of previously funded projects, are available at genomicscience.energy.gov/biosystems-design/.



Genome Engineering for Materials Synthesis

Report from the October 2018 Workshop



Biosystems Design

Report from the July 2011 Workshop

May 2023