DOE Genomic Science Program

# Systems Biology of Bioenergy-Relevant Microbes to Enable Production of Next-Generation Biofuels and Bioproducts

**Summary of Projects Awarded in 2018 under Funding Opportunity Announcement DE-FOA-0001865**

science.energy.gov/ber/research/bssd/genomic-science/

The U.S. Department of Energy's (DOE) Genomic Science program, managed within the Office of Biological and Environmental Research (BER), supports basic research aimed at identifying the foundational principles that drive biological systems. These principles govern the translation of the genetic code into integrated networks of proteins, enzymes, regulatory elements, and metabolite pools that are the functional processes of organisms including microbes and multispecies communities relevant to DOE missions in energy and the environment. To address the DOE mission in sustainable bioenergy development, the Genomic Science program brings to bear "omics"-driven tools of modern systems biology on the challenges associated with microbial production of advanced biofuels and bioproducts.

Developing an increased understanding of how biological systems function and translating that knowledge to enhance the production of microbial and plant capabilities form the basis of DOE's mission in sustainable bioenergy. To harness the biosynthetic processing power of the microbial world for producing advanced biofuels and bioproducts, development of an expanded set of platform organisms is needed that has appropriate metabolic capabilities and stress tolerance characteristics with a suite of modification tools. To foster this development, the DOE BER Genomic Science program supports research aimed at understanding the principles that govern the functional properties of bioenergy-relevant organisms at the genomic scale. This endeavor is highly interdisciplinary, spanning multiple fields in biology, systems biology, chemical and metabolic engineering, and computational biology.

Recent progress in understanding biological systems and the ability to manipulate them is largely due to tremendous technological advances in the development of multiomics tools, high-throughput phenotypic screening approaches, and computational modeling methods used to analyze, modify, and select specific functional properties of biological systems. There is an opportunity to potentially meet the goals of producing sustainable biofuels and bioproducts derived from lignocellulosic plant biomass or from photosynthetic capture of carbon dioxide ($CO_2$) through continued research on understanding the microbial physiology and metabolism of unique microorganisms and advancing them toward experimentally tractable organisms or systems.

This Funding Opportunity Announcement (FOA) specifically targets the production of advanced biofuels (i.e., biologically synthesized compounds with the potential to serve as energy-dense transportation fuels such as gasoline, diesel, and aviation) compatible with existing engines and fuel-distribution infrastructure and useful in producing bioproducts. The biological syntheses of advanced biofuels and bioproducts require significant advances in the basic understanding of microbial physiology and metabolism, as well as understanding the conversion of photosynthetically derived carbon compounds and how products can be shunted efficiently from central metabolism into complex products while rebalancing organismal carbon allocations and reduction-oxidation (redox) potential.

BER solicited applications for fundamental systems biology–driven basic research to enable production of advanced biofuels and bioproducts in two areas:

- **Development of emerging model microorganisms and/or microbial communities relevant to production of biofuels and/or bioproducts.** Proposed studies could include but are not limited to (1) advancing systems biology understanding and predictive modeling of specialist microbes or microbial consortia, (2) elucidating relevant regulatory and metabolic networks or environmental signal processing related to product synthesis, (3) improving fundamental understanding of integrated function and compatibility of novel enzyme systems with direct applicability to lignocellulose breakdown or advanced biofuels and/or bioproduct production, and (4) further developing genetic tools to facilitate study and manipulation of microbial species for which genomic information is available and a genetic system is at least in its initial stages of development.
- **Understanding of novel microbial functional capabilities and biosynthetic pathways relevant to the production of advanced biofuels and bioproducts.** Proposed studies could include but are not limited to (1) development of robust and efficient pathways for synthesis of advanced biofuels and bioproducts, (2) functional processes involved in deconstruction of lignocelluosic plant material, (3) elucidation and modification of phenotypes involved in tolerance to stresses relevant to biofuel and bioproduct production,

and (4) development of methods to overcome problems with recombinant expression of vital enzymes and pathways. Proposed research should include the development of strategies to identify and overcome metabolic impacts resulting from pathway modification that limit production of target molecules.

In conjunction with research addressing the two research topics outlined on p. 1, applicants could propose the development of analytical technologies facilitating characterization of relevant functional processes or high-throughput phenotyping of modified biofuel-producing strains, as long as the applications were tightly integrated with the stipulated topics.

# 2018 Awards

## Systems Biology–Based Optimization of Extremely Thermophilic Lignocellulose Conversion to Bioproducts

- **Principal Investigator:** Michael Adams (University of Georgia)
- **Collaborators:** Robert M. Kelly (North Carolina State University), Dmitry A. Rodionov (Sanford Burnham Prebys Medical Discovery Institute), and Ying Zhang (University of Rhode Island)

**Project Goal and Summary:** Use systems biology–guided approaches to develop a nonmodel, microbial metabolic engineering platform based on the most thermophilic lignocellulose-degrading organism known, *Caldicellulosiruptor bescii*, which grows optimally at 78°C. The latest metabolic reconstruction and modeling approaches will be applied to optimize biomass to product conversion using switchgrass as the model plant and acetone and 3-hydroxypropionate as products. Bioprocessing above 70°C can have important advantages over near-ambient operations. Highly genetically modified microorganisms usually have a fitness disadvantage and can be overtaken easily in culture when contaminating microbes are present. The high growth temperature of extreme thermophiles precludes growth or survival of virtually any contaminating organism or phage, thus reducing operating costs associated with reactor sterilization and maintenance of a sterile facility. In addition, at industrial scales, heat production from microbial metabolic activity vastly outweighs heat loss through bioreactor walls that would require cooling. Extreme thermophiles have other advantages—nonrefrigerated cooling water can be used if needed and heating requirements can be met with low-grade steam typically in excess capacity on plant sites. The overarching goal is to demonstrate that a nonmodel microorganism, specifically an extreme thermophile, can be a strategic metabolic engineering platform for industrial biotechnology.

## Biosensor and Optogenetics for Systems Biology of Yeast Branched-Chain Alcohol Production and Tolerance

- **Principal Investigator:** José Avalos (Princeton University)

**Project Goal and Summary:** Carry out a comprehensive systems biology study of branched-chain alcohol (BCA) production and tolerance in yeast. BCAs, including isobutanol, isopentanol, and 2-methyl-1-butanol, are some of the most promising advanced biofuels in development. These alcohols have better fuel properties than bioethanol. They have higher energy density, their refinement is less expensive and energy intensive, and they have much better compatibility with the nation's fuel use and distribution infrastructure. The project will leverage a recently developed, genetically encoded biosensor of BCA production to screen various yeast genomic libraries to measure the effects of different genetic perturbations (i.e., gene deletion, overexpression, or mutation) on BCA production or tolerance and to screen those collections using different substrates, nutritional requirements, or BCA-induced stress. In addition, by combining the biosensor with optogenetic regulation of BCA production, the team will establish a closed-loop control system for measuring transcriptomic changes under well-controlled conditions. These new insights will be used to develop improved strains for producing BCAs and to help make this very promising class of biofuels more economically competitive.

## Creating Multifunctional Synthetic Lichen Platforms for Sustainable Biosynthesis of Biofuel Precursors

- **Principal Investigator:** Michael Betenbaugh (Johns Hopkins University)
- **Collaborators:** Michael Guarnieri (National Renewable Energy Laboratory), Jon Magnuson (Pacific Northwest National Laboratory), Jamey D. Young (Vanderbilt University), and Karsten Zengler (University of California, San Diego)

**Project Goal and Summary:** Use genetic manipulation techniques to enhance the exchange of metabolites between autotrophs and heterotrophs, creating superior synthetic lichens

able to generate useful products of interest to the energy and chemical industries. Lichens are communities of microbes that collect sunlight and carbon dioxide ($CO_2$) and apply them to power the group's activities, allowing autotrophic members to optimize photosynthesis and metabolite generation while their heterotrophic fungal partners produce biochemical compounds for the community. Additional members may provide key functions such as nitrogen fixation. While lichens can thrive in the harshest environments on Earth, they also represent a novel biotechnology platform that can transform $CO_2$ and sunlight into valuable energy-related biochemicals. Unfortunately, natural lichens have exceedingly slow growth rates, making them impractical for most industrial applications. Key metabolite excretion bottlenecks identified in cyanobacteria will be engineered to share particular metabolic intermediates with their heterotrophic partners for channeling into natural or engineered metabolic pathways, thus generating energy-related precursors of biochemicals or biofuels with high commercial value.

## High-Throughput Chemical Imaging for Optimizing Biofuel Synthesis Using Synthetic Biology

- **Principal Investigator:** Mary Dunlop (Boston University)
- **Collaborators:** Ji-Xin Cheng (Boston University) and Wilson Wong (Boston University)

**Project Goal and Summary:** Develop and use technology for directly measuring synthesis of biofuels in living cells by employing a high-throughput platform for chemical imaging of biofuel production to improve *Escherichia coli* fatty acid production. Recent advances in the fields of synthetic biology and metabolic engineering have resulted in an unprecedented ability to engineer microbial genomes and to design and build gene circuits for improving biofuel production. Stimulated Raman scattering (SRS) microscopy will be introduced as a new technology for directly measuring chemical signatures in *in vivo* samples for engineering and optimizing biofuel production strains. This technology can work on a broad range of cell types (e.g., yeast, algae, and other bacteria besides *E. coli*) and can detect *in vivo* levels of other biofuels and products (e.g., diesel and jet fuels). SRS imaging is expected to be especially valuable for assessing chemical signatures in strains where tools for genetic manipulation are limited or nonexistent.

## Dissecting the Division of Labor in Microbial Consortia for the Production of Biofuels and Chemicals

- **Principal Investigator:** Ting Lu (University of Illinois, Urbana-Champaign)
- **Collaborator:** Yong-Su Jin (University of Illinois, Urbana-Champaign)

**Project Goal and Summary:** Elucidate fundamental design principles for the division of labor (DOL) in microbial ecosystems in the context of a *Saccharomyces cerevisiae* and *Lactococcus lactis* consortium that produces 2,3-butanediol, 2-butanol, and lactic acid. Microbial metabolic engineering is an attractive strategy for clean and sustainable production of biofuels and chemicals. Over the decades, this canonical paradigm, which involves pathway construction in single strains, has led to many breakthroughs; however, this paradigm has several key limitations including inefficient and slow substrate conversion, heavy burdens in energetics and reduction-oxidation (redox) balance, and unexpected accumulation of byproducts. Synthetic microbial consortia have recently emerged as a promising solution to address these challenges by expanding the programmability and enhancing the robustness of desired functionality. The hypothesis is that the structure of the cellular interaction network in this consortium is essential to ecosystem robustness, promising to deliver a quantitative and systematic understanding of DOL in microbial ecosystems. Thus, it will advance the fundamental knowledge of microbial ecology concerning community structure and dynamics. Achieving project goals also will provide valuable insights into the design and construction of artificial microbial consortia for the synthesis of bioproducts from cellulosic biomass.

## Using Gene Editing and an Accumulated Bioproduct as a Reporter for Genotypic and Phenotypic Heterogeneity in Growth-versus-Production for *Methylobacterium extorquens* Conversion of Aromatics to Butanol

- **Principal Investigator:** Christopher Marx (University of Idaho)
- **Collaborators:** Ankur B. Dalia (Indiana University), Sergey Stolyar (University of Idaho), and Andreas E. Vasdekis (University of Idaho)

**Project Goal and Summary:** Develop *Methylobacterium extorquens* as a catalyst to convert methoxylated aromatics from lignin hydrolysate into a model bioproduct, 1-butanol, and develop a novel approach that combines the advantages of gene editing, deep-sequencing, and analysis of phenotypic heterogeneity for both growth and production. Lignin-derived compounds from plant biomass are among those most recalcitrant for microbial conversion. Hydrolysates contain a wide variety of aromatic molecules, and a particular issue with these molecules is that many of them are methoxylated; these methoxy groups are released as formaldehyde during degradation, possibly overloading the detoxification ability of standard heterotrophs. Methylotrophic bacteria, on the other hand, not only rapidly generate internal formaldehyde from oxidation of single-carbon compounds, like methanol, but also can oxidize it fast enough to prevent toxicity. In an earlier DOE project, the team also discovered that some *Methylobacterium* strains grow exceptionally well on aromatics and do not release formaldehyde into the medium

from the methoxy groups present, unlike classic systems for aromatic degradation (e.g., *Pseudomonas putida*). The project has since demonstrated that the pathways for methoxylated aromatic use can be introduced into the emerging model organism, *M. extorquens*, and enable it to grow on aromatics. These conceptual advances could broadly revolutionize work in DOE-relevant biosystems design.

## Systems Analysis of a Fast-Growing $N_2$-Fixing Cyanobacterium for Production of Advanced Biofuels and Nitrogen-Containing Petrochemical Replacement Compounds

- **Principal Investigator:** Himadri Pakrasi (Washington University in St. Louis)
- **Collaborators:** Maciek R. Antoniewicz (University of Delaware) and Costas Maranas (Pennsylvania State University)

**Project Goal and Summary:** Use an integrated systems biology approach to develop the filamentous cyanobacterium *Anabaena* sp. PCC 33047 as a model fast-growing, photosynthetic, diazotrophic production platform. Cyanobacteria are photosynthetic prokaryotes with significant potential as cell factories for sustainable production of biofuels and chemicals by directly using energy from sunlight and carbon dioxide ($CO_2$). One key issue with the current cyanobacterial production strains concerns the growth rates of these microbes. Compared to other oxygenic photosynthetic organisms such as plants and eukaryotic algae, many cyanobacterial strains have superior growth rates. However, they grow significantly slower than heterotrophic microbes such as *Eshcherichia coli* and yeast, which are commonly used in biofuels research. The team's recent discovery of the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973 demonstrates that there are strains available whose production potential far exceeds that of current model systems. Notably, most cyanobacterial production systems require the input of fixed nitrogen, which has been reported as one of the highest operational costs for biofuel production. This taxing requirement can be largely eliminated through the use of ($N_2$)-fixing cyanobacteria. The team has identified *Anabaena* 33047, which has a remarkable 3.8-hour doubling time under nitrogen $N_2$-fixing conditions. Because nitrogen demand is a major cost for photosynthetic bioproduction, the use of this fast-growing diazotrophic strain should significantly improve the cost outlook of target bioproducts. The team will pursue a systems approach to develop *Anabaena* 33047 as a versatile photosynthetic $CO_2$-fixing and $N_2$-fixing production platform for use by the bioenergy research community during the coming era.

## Syntrophic Co-Cultures of *Clostridium* Organisms to Produce Higher Alcohols and Other C6-C8 Metabolites

- **Principal Investigator:** Eleftherios Papoutsakis (University of Delaware)
- **Collaborators:** Maciek R. Antoniewicz (University of Delaware) and Costas Maranas (Pennsylvania State University)

**Project Goal and Summary:** Advance the systems biology understanding and predictive modeling of synthetic and syntrophic *Clostridium* microbial consortia, focusing on elucidation of metabolic networks and environmental signals in the consortia. Microbial communities are ubiquitous in nature and have a wide range of applications, including production of biofuels and chemicals. It is now well appreciated that the capabilities of multimicroorganism systems cannot be predicted by the sum of their parts. Rather, synergistic interactions at different levels often result in better overall performance of these systems. The emerging field of co-culture synthetic biology promises the assembly of different metabolic capabilities into functional systems, where the diversity of metabolic pathways and the ability of microorganisms to exchange metabolites and larger molecules dramatically expand the possible metabolic space. *Clostridium* organisms are uniquely capable of using a large variety of biomass-derived carbohydrates, and some of them can also fix carbon dioxide ($CO_2$) autotrophically, thus enabling maximal use of substrate carbon. These organisms possess diverse biosynthetic capabilities for producing a broad spectrum of metabolites, which, together with their derivatives, could serve as commodity chemicals, biofuels, and biofuel precursors. Significantly, syntrophic *Clostridia* consortia can fix extensive $CO_2$ amounts, thus achieving product yields that cannot be achieved by monocultures. The ultimate goal then is to use the knowledge developed from these systems as a basis for future developments of syntrophic systems to produce a broad spectrum of metabolites via modular syntrophic co-cultures, involving engineered and nonengineered microorganisms from various genera in addition to the *Clostridium* organisms.

## Rapid Development of Acetogenic *Clostridia* Using Highly Multiplexed Genome Engineering for Control of C1 Bioconversion

- **Principal Investigator:** Howard Salis (Pennsylvania State University)
- **Collaborators:** Steve Brown (LanzaTech) and Michael Koepke (LanzaTech)

**Project Goal and Summary:** Develop the commercially scalable emerging model organism, *Clostridium autoethanogenum*, which converts a single-carbon (C1) feedstock (e.g., carbon dioxide and carbon monoxide from waste gas emissions) into a short-chain fatty acid, 3-hydroxyproprionic acid. This

3-hydroxypropionic acid is an ideal biorenewable precursor to industrially important polymers such as acrylates. The team will apply several systems and synthetic biology technologies, coupling together algorithmic design approaches, highly multiplexed genome-scale engineering techniques, and omics measurements, to exert complete control over the metabolism of *C. autoethanogenum*. First, the team will employ an integrated computational, experimental approach to engineer optimized biosynthesis pathways for 3-hydroxypropionic acid in *C. autoethanogenum*. Second, to redirect metabolic flows toward 3- hydroxypropionic acid production, the team will develop and demonstrate a very highly multiplexed version of CRISPR (i.e., clusters of regularly interspaced short palindromic repeats) that uses highly nonrepetitive genetic parts to up-regulate or down-regulate up to 20 targeted genes simultaneously. Third, the team will perform technoeconomic assessments of C1 bioconversion to 3-hydroxypropionic acid and couple those assessments to algorithm-designed genetic modifications, determining genotype-phenotype-cost relationships across several metrics. This project will result in a commercially scalable emerging model organism capable of producing 3-hydroxypropionic acid at economically competitive, high productivities from low-cost C1 feedstock.

## Gene Regulatory Networks Enabling Fungi to Selectively Extract Sugars from Lignocellulose

- **Principal Investigator:** Jonathan Schilling (University of Minnesota)
- **Collaborators:** Igor Grigoriev (Lawrence Berkeley National Laboratory), David Hibbett (Clark University), Young-Mo Kim (Pacific Northwest National Laboratory), and Claudia Schmidt-Dannert (University of Minnesota)

**Project Goal and Summary:** Identify and characterize brown rot–specific gene regulation patterns to address key knowledge gaps, enabling *in vivo* manipulations such as CRISPR/Cas9 and metabolomics to map metabolite expression feedback over time to produce an integrated regulatory model for brown rot fungi. However, understanding of fungal brown rot metabolism is limited. Fungi dominate the biological decomposition of wood and other lignocellulosic plant tissues in nature through a range of pathways for unlocking the sugars embedded in lignin, offering a proven model for the sustainable production of energy from biomass. Modern approaches to bioenergy production aim to depolymerize polysaccharides to release fermentable sugars (saccharification), saving lignin as a coproduct that is a good fit for the carbohydrate-selective pathways of brown rot fungi. This project will enable omics-driven tools for organisms highly relevant to bioenergy, with broader scientific impacts in the fields of ecology, evolution, and biogeochemistry.

## Novel Microbial Routes to Synthesize Industrially Significant Precursor Compounds

- **Principal Investigator:** F. Robert Tabita (Ohio State University)
- **Collaborators:** William R. Cannon (Pacific Northwest National Laboratory) and Kelly C. Wrighton (Colorado State University)

**Project Goal and Summary:** Maximize the potential of microorganisms to convert lignocellulose-derived compounds and carbon dioxide ($CO_2$) to important synthetic precursor compounds such as ethylene and propylene, the most widely employed organic compounds in industry. With these compounds being used for the synthesis of several multibillion-dollar products, there is an increasing demand for bioproducts and biofuels from plentiful starting materials such as lignocellulose and $CO_2$ feedstocks. In this project, a combination of systems biology and bioinformatics approaches, along with a unique toolbox of analytical, omics, molecular, and biochemical approaches, will be applied to meet project goals. Current chemical processes for precursor synthesis require huge amounts of energy derived from fossil fuels, but recently discovered, efficient anaerobic ethylene synthetic processes offer the potential to significantly impact biological ethylene (and propylene) formation, tenable with the plentiful starting materials of lignocellulose and/or $CO_2$ feedstocks. Overall, this project aims to develop an industrially compatible process to synthesize ethylene in high yields using microbial systems.

## Understanding and Harnessing the Robustness of Undomesticated *Yarrowia lipolytica* Strains for Biosynthesis of Designer Bioesters

- **Principal Investigator:** Cong Trinh (University of Tennessee, Knoxville)
- **Collaborators:** Bruce J. Dien (U.S. Department of Agriculture's Agricultural Research Service, Peoria, Illinois), Richard Giannone (Oak Ridge National Laboratory), and Patricia Slininger (U.S. Department of Agriculture's Agricultural Research Service, Peoria, Illinois)

**Project Goal and Summary:** Harness the potential of robust undomesticated *Yarrowia lipolytica* isolates to produce designer bioesters from undetoxified biomass hydrolysates. These isolates will be derived from genetic and phenotypic screening approaches using a rigorous microbe selection platform. Genomic and molecular characterization will be leveraged to elucidate and characterize the underlying mechanisms of how these new strains yield desirable bioesters and other bioproducts. Specifically, the team will detail how these *Y. lipolytica* (1) tolerate and effectively assimilate inhibitory biomass hydrolysates for superior lipid accumulation under hypoxic compared

with oxygen-sufficient conditions; (2) tolerate organic solvents that are required to produce biofuels and bioproducts in a two-phase fermentation system; and (3) endogenously degrade lipids to produce targeted esters with potential use as fuels, solvents, flavors, and fragrances. Project results will provide the needed tools to allow *in situ* production and integrated recovery of custom esters, as well as the insight necessary for engineering *Yarrowia* strains to produce a wide variety of biofuels and bioproducts from lignocellulosic biomass.

## Employing Bacterial Microcompartments to Create Privileged Redox Pools for Biofuel Production

- **Principal Investigator:** Danielle Tullman-Ercek (Northwestern University)
- **Collaborators:** Niall M. Mangan (Northwestern University) and Keith Edward Jaggard Tyo (Northwestern University)

**Project Goal and Summary:** Deploy compartmentalization as a strategy to overcome a critical roadblock in biosynthesis—the requirement for reduction-oxidation (redox) cofactor recycling. Metabolic engineering holds great promise for creating efficient, competitive routes for the production of biofuels and biochemicals without harsh chemicals and hazardous byproducts. Successes in biochemical production include the use of bacteria for producing Dupont's Sorona fibers from 1,3-propanediol from glucose and the use of yeast for the manufacture of the antimalarial drug artemisinin. However, roadblocks to biosynthesis prevent many biochemicals from being produced biologically, given current technology. Nature uses compartmentalization (e.g., organelles in eukaryotes and bacterial microcompartments in prokaryotes) to solve issues such as intermediate leakage, toxicity, and byproduct formation. In traditional systems, redox cofactors are lost to cellular growth and maintenance needs. Compartmentalizing redox cofactors with the biochemical synthesis enzymes is anticipated to increase thermodynamic efficiency and prevent the loss of valuable intermediates and cofactors. If successful, this strategy would be the first direct demonstration of this feature of a bacterial microcompartment and would provide a tool for improving metabolic pathway performance for all enzymes with redox or other cofactors. In addition, this work would reveal insights into the native function of these structures, while also providing a detailed method for selecting and improving biochemical pathway performance. Ultimately, research results will lead to the cost-efficient production of chemicals currently derived from petroleum.

## Biosynthesis of Bioprivileged, Linear Molecules via Novel Carboligase Reactions

- **Principal Investigator:** Keith Edward Jaggard Tyo (Northwestern University)
- **Collaborators:** Linda J. Broadbelt (Northwestern University), Neil L. Kelleher (Northwestern University), and Paul Martin Thomas (Northwestern University)

**Project Goal and Summary:** Characterize and engineer carbon-carbon (C-C) bond-forming enzymes to enable novel biosynthetic pathways to a variety of long-chain and di-functional fatty acids. More broadly, this project will harness enzyme substrate promiscuity to access a broad range of products not found in nature, while mitigating toxic or undesirable side reactions, potentially unlocking facile synthesis of new classes of molecules to enable biomanufacturing. Its significance lies in two areas—enabling synthesis of new molecules difficult to obtain by petrochemical routes and modeling the genome-scale consequences of enzyme promiscuity. First, many useful fuel and chemical molecules, like heptanoic and suberic acid, are difficult to produce by oil refining and petrochemistry. Functionalizing the ends of alkanes is particularly difficult because interior carbons are more reactive. Biosynthesis of terminally functionalized molecules mediated by enzymatic coupling reactions would allow production of valuable biochemicals, making scale-up more feasible and lower risk. Secondly, the team will develop and use tools to predict enzyme promiscuity and mitigate the negative consequences associated with unwanted side reactions. This research and accompanying tool development will help identify deleterious promiscuous reactions and pave the way for other metabolic engineers to readily avoid toxicity and productivity loss due to unwanted side reactions.

## Harnessing Methanotroph-Photoautotroph Interactions for Biogas Conversion to Fuels and Chemicals Using Binary Consortia

- **Principal Investigator:** Jin Wang (Auburn University)
- **Collaborators:** Alexander S. Beliaev (Pacific Northwest National Laboratory), Q. Peter He (Auburn University), and Marina G. Kalyuzhnaya (San Diego State University)

**Project Goal and Summary:** Use a model co-culture of photoautotroph-methanotroph *Synechococcus* sp. PCC 7002 and *Methylomicrobium alcaliphilum* 20ZR for developing experimental and computational tools to gain a qualitative and quantitative understanding of the interactions and dynamics of the co-culture at both systems and molecular levels. Biogas from conversion of organic waste streams has immense potential for use as a feedstock to produce high-density fuels and commodity chemicals. However, the use of biogas represents

a significant challenge due to its low pressure and the presence of contaminants such as hydrogen sulfide, ammonia, and volatile organic carbon compounds. Tapping into this immense potential requires effective biotechnologies that co-utilize both carbon dioxide and methane. Fundamental understanding of the photoautotroph-methanotroph interaction will lay the foundation for designing and optimizing synthetic binary consortia to produce fuels and chemicals from biogas. Knowledge gained from this project may be generally applicable to other cross-feeding binary consortia, and the tools developed can be adapted to study the interactions and dynamics of other multi-organism platforms.

### Development of Emerging Model Microorganisms: *Megasphaera elsdenii* for Biomass and Organic Acid Upgrading to Fuels and Chemicals

- **Principal Investigator:** Janet Westpheling (University of Georgia)
- **Collaborator:** Adam Guss (Oak Ridge National Laboratory)

**Project Goal and Summary:** Develop *Megasphaera elsdenii* as a platform for the conversion of lignocellulosic biomass sugars and organic acids into hexanol and other valuable chemicals. The native ability to condense acetyl–coenzyme A (CoA) groups to efficiently generate C4 to C8 compounds makes *M. elsdenii* a compelling platform for producing fuels and chemicals from lactate and plant carbohydrates. Engineering *M. elsdenii* to efficiently produce next-generation, drop-in lignocellulosic fuels such as hexanol at high yield and titer could provide an efficient bioengineering platform. Initially, lignocellulosic sugars will be converted to hexanol and related products; however, because *M. elsdenii* ferments lactate to organic acids, this project also will lay the foundation for more advanced processing options such as a co-culture or sequential fermentation in which one organism converts sugars to lactate and an engineered *M. elsdenii* converts the lactate to a higher-value product.

### Establishing the Thermotolerant Yeast *Kluyveromyces marxianus* as a Host for Biobased Fuels and Chemicals Production

- **Principal Investigator:** Ian Wheeldon (University of California, Riverside)
- **Collaborators:** Nancy Da Silva (University of California, Irvine) and Stefano Lonardi (University of California, Riverside)

**Project Goal and Summary:** Develop the thermotolerant yeast *Kluyveromyces marxianus* as a platform host for industrial bioprocessing. A critical area of the U.S. industrial biotechnology sector is the conversion of biomass and other renewable feedstocks to fuels and chemicals. Robust microorganisms that are genetically accessible can grow rapidly at high temperature and low pH, and those that can effectively assimilate a wide range of different sugars, such as *K. marxianus*, are needed to sustain technological and economic growth. New genome-wide CRISPR-based tools for genome editing, genetic screening, and rapid strain development will be developed and applied in systems biology studies to understand industrially desirable phenotypes and for metabolic engineering. A key aim will be to enhance acetyl–coenzyme A (CoA) production, a central precursor in the synthesis of many fuels and chemicals. Anticipated outcomes include rapid engineering of *K. marxianus* strains that produce biofuels and chemicals at high titer, rate, and yield, leading to a new, robust platform for low-cost bioprocessing.

### Rapid Flux Phenotyping to Accelerate Metabolic Engineering of Cyanobacteria

- **Principal Investigator:** Jamey D. Young (Vanderbilt University)
- **Collaborators:** Carl H. Johnson (Vanderbilt University), John A. McLean (Vanderbilt University), and Brian Pfleger (University of Wisconsin, Madison)

**Project Goal and Summary:** Develop technologies to optimize cyanobacteria and other microbes for producing renewable chemicals at commercially feasible rates and yields by establishing a rapid flux phenotyping platform that can be applied to accelerate metabolic engineering of cyanobacterial hosts. The ability to quantify flux alterations in response to targeted genetic manipulations is a key requirement for rational metabolic engineering, but the time needed to complete a comprehensive $^{13}C$ flux analysis can far exceed the time needed to introduce new genetic modifications to a recombinant host. Matching the throughput of $^{13}C$ flux phenotyping to the rate of strain generation will provide the foundation for a rational "design-build-test-learn" metabolic engineering cycle. Project findings are expected to be generalizable to a diverse range of biochemical products derived from major metabolic hubs, enabling a systematic strategy for metabolic engineering of cyanobacteria and other microbes.

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