

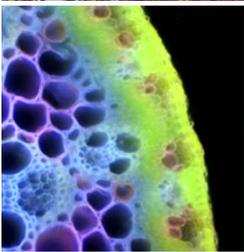
# ***Office of Biological and Environmental Research***



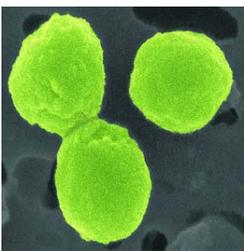
## ***Biological Systems Science Division Update***



**Todd Anderson, Ph.D.**



Director, Biological Systems Science Division,  
Department of Energy, Office of Biological &  
Environmental Research



November 2, 2017



U.S. DEPARTMENT OF  
**ENERGY**

Office  
of Science

Office of Biological  
and Environmental Research

# *Update on Programmatic Activities*

## **Completed Reviews/Activities**

- *Panel Review of Biosystems Design FOA applications (April 24-27)*
- *Panel Review of Plant Feedstocks for Bioenergy FOA applications (April 27)*
- *Committee of Visitors review of BSSD (July 11-12)*
- *Lawrence Livermore National Laboratory SFA Review (July 18)*
- *Lawrence Berkeley National Laboratory SFA Review (Aug 1-2)*
- *New DOE Laboratory SFA Review (Aug 17)*
- *DOE Systems Biology Knowledgebase review (Sept 26-27)*

## **Upcoming Reviews/Activities in FY 2018**

- *Joint Genome Institute (JGI) triennial review (Dec 6-8)*
- *Annual Genomic Sciences Program PI Meeting (Feb 26-28)*
- *Annual Bioimaging Science Program PI Meeting (Feb 28-Mar 1)*
- *Annual JGI User Meeting (Mar 12-15)*

# Strategic Planning/Activities

## Workshops

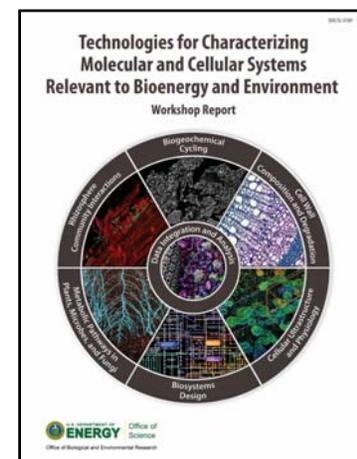
### **ASCR-BER Workshop on Exascale Computational Requirements**

- Final report completed
  - <https://science.energy.gov/~media/ascr/pdf/programdocuments/docs/2017/DOE-ExascaleReport-BER-Final.pdf>



### **Technologies for Characterizing Molecular and Cellular Systems Relevant to Bioenergy and Environment**

- Final report completed
  - <https://science.energy.gov/~media/ber/pdf/community-resources>



## National Academy Studies

### **“Developing a Research Agenda for Utilizing Gaseous Carbon Waste Streams.”**

- Board on Chemical Sciences and Technology and the Board on Energy and Environmental Systems (BER, BES, ARPA-E, FE, EERE) D. Allen, Chair
- Kickoff meeting – Oct 16<sup>th</sup>

# Potential Funding Opportunities for FY 2018

- **Plant Feedstocks Genomics for Bioenergy**
- **Systems Biology of Bioenergy-Relevant Microbes**
- **Imaging and Measurement Technologies for Biological Systems Science**

**Genomic Science Program** genomicscience.energy.gov

**Systems Biology-Enabled Research for Microbial Production of Advanced Biofuels**

Summary of Projects Awarded in 2014 Under Funding Opportunity Announcement DE-FG-0001060

The U.S. Department of Energy's (DOE) Genomic Science program, managed within the Office of Biological and Environmental Research (BER), supports fundamental research to identify the foundational principles that drive biological systems. These principles govern translation of the genetic code into integrated networks of proteins, enzymes, regulatory elements, and metabolic pools underlying the functional processes of organisms. To address DOE's mission in sustainable bioenergy development, the Genomic Science program applies "omics"-driven tools of modern systems biology to challenge associated with microbial production of advanced biofuels.

Developing an increased understanding of how biological systems function and translating that knowledge to enhance the production capabilities of microbes and plants forms the basis of DOE's mission in sustainable bioenergy. To harness the microbial world's biocatalytic processing power for advanced biofuel production, an expanded set of platform organisms is needed with appropriate metabolic capabilities and stress tolerance characteristics. The DOE BER Genomic Science program supports research aimed at improving fundamental understanding of principles that govern the functional properties of bioenergy-relevant organisms at the genome scale. This knowledge will enable development of molecular genomics approaches and computational tools for the design, construction, and validation of improved biological components and systems. This highly interdisciplinary endeavor spans multiple fields in biology, systems biology, chemical and molecular engineering, and computational biology.

Significant progress in the last decade has increased understanding of biological systems and the capabilities for manipulating them. These advances enable leaps from the traditional technological leaps in developing molecular biology tools (e.g., genomics, metabolomics, and proteomics tools) to analyze and modify the functional

properties of biological systems. Despite these advances, many fundamental gaps remain in understanding microbial metabolism and physiology related to the production of sustainable, efficient, and economically competitive biofuels derived from lignocellulosic plant biomass or from photosynthetic capture of carbon dioxide (CO<sub>2</sub>).

The 2014 Funding Opportunity Announcement described herein specifically targets production of advanced biofuels, which in the context refers to biologically synthesized compounds with the potential to serve as energy-dense transportation fuels (e.g., diesel, gasoline, and aviation fuels) compatible with existing engines and fuel distribution infrastructure. Advanced biofuel production requires significant progress in the basic understanding of microbial metabolism and the conversion of photosynthetically derived carbon compounds (either via direct photosynthesis or acquired via breakdown of lignocellulosic plant biomass).

Another goal is to determine how products can be efficiently obtained from central metabolism into complex products with associated inhibition of organismal carbon allocation and yield potential.

BER solicited applications for systems biology-driven basic research in three areas of development focused on enabling advanced biofuel production:

- **Examining new model organisms relevant to bio-fuels production.** Proposed studies could include but are not limited to: (1) advancing systems biology understanding and predictive modeling of special- or microbes or microbial consortia; (2) elucidating relevant regulatory and metabolic networks involved in product synthesis or environmental signal processing; (3) improving fundamental understanding of integrative function and compatibility of novel enzyme systems with direct applicability to specialized biomass or advanced biofuels production; and (4) developing genetic tools to facilitate study and manipulation of genetically diverse native species.

**Office of Biological and Environmental Research**

**ENERGY** Office of Science

**Plant Feedstock Genomics for Bioenergy Joint Awards 2006–2016**

genomicscience.energy.gov/research/DOEUSDA/

**USDA** United States Department of Agriculture

**U.S. DEPARTMENT OF ENERGY** Office of Science

June 2016

**New Bioimaging Technologies for Plant and Microbial Systems**

**ENERGY** Office of Science

Office of Biological and Environmental Research

# *New Bioenergy Research Centers*

## **Great Lakes Bioenergy Research Center (GLBRC)**

**University of Wisconsin-Madison** – *Develop the science and technological advances to underpin a sustainable lignocellulosic bioindustry (PI: T. Donohue, UWisc)*



## **Center for Bioenergy Innovation (CBI)**

**Oak Ridge National Laboratory** – *Accelerate the domestication of bioenergy crops and targeted consolidated bioprocessing innovations to improve cost efficiencies within the bioenergy supply chain (CEO: G. Tuskan, ORNL)*



## **Joint BioEnergy Institute (JBEI)**

**Lawrence Berkeley National Laboratory** – *Broaden and maximize production of economically viable fuels and chemicals from plant biomass to enable biorefinery development (CEO: Jay Keasling, LBNL)*



## **Center for Advanced Bioenergy and Bioproducts Innovation (CABBI)**

**University of Illinois at Urbana-Champaign** – *Enable the direct production of drop-in fuels and chemicals in plants as sustainable biofactories for a range of bioproducts (Director: E. DeLucia, UIUC)*



# The Bioenergy Research Centers Address Key Science Themes



Sustainability	Feedstock Development	Deconstruction	Conversion
<i>Long-term studies of producing bioenergy crops on marginal land</i>	<i>Designing improved dedicated bioenergy crops</i>	<i>Renewable biomass deconstruction and separation</i>	<i>Novel biomass conversion microbes</i>
<i>Water and nutrient use in dedicated bioenergy crops</i>	<i>Multi-omics tools for development of high-yield bioenergy crops</i>	<i>Integrated and consolidated thermophilic bioprocessing</i>	<i>Drop-in biofuels and bioproducts from biomass and lignin residues</i>
<i>Studies of environmental resilience of engineered bioenergy crops</i>	<i>Plants engineered for atom-economical conversion into biofuels and bioproducts</i>	<i>Feedstock-agnostic biomass deconstruction with renewable ionic liquids</i>	<i>High throughput synthetic biology tools and hosts for scalable, atom economical, biofuel &amp; bioproducts</i>
<i>Integrated economic and environmental analyses for biomass supply</i>	<i>“Plants as Factories” concept for biofuels and bioproducts</i>		<i>Automated biofoundry concept for fuels and bioproducts</i>

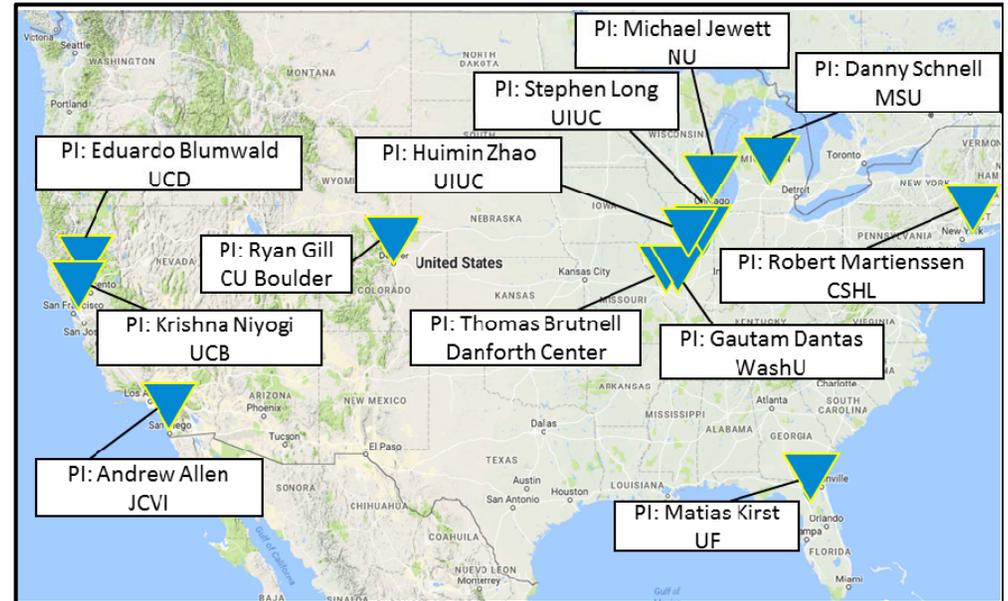


# New Biosystems Design Awards

Biosystems Design to Enable Next-Generation Biofuels and Bioproducts (DE-FOA-0001650)

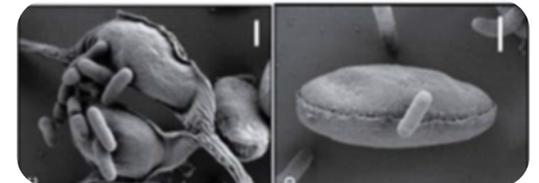
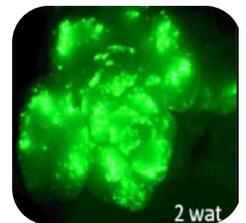
## Expands BER's Plant Biosystems Design Portfolio:

- Transitioning from model plants such as *Arabidopsis* and *Setaria* to *Camelina* and sorghum
- New efforts in energy cane and an aquatic monocot (duckweed)
- Targeting nitrogen fixation via engineering symbiosis
- Increased water use and photosynthetic efficiency
- Engineering oil production in stems and leaves



## ...And Microbial Biosystems Design Portfolio:

- New non-model yeasts that produce oils and organic acids
- Expanding genome-scale design and engineering to diatoms and green algae
- Developing *in vivo* and cell-free microbial bioprocessing systems
- Computer-aided design and high throughput re-combineering in model and non-model microorganisms



➡ **12 new projects (\$21M/yr over 5 yrs)**

# ***New Plant Feedstock Genomics Awards***

## ***Plant Feedstock Genomics for Bioenergy: A Joint Research Funding Opportunity Announcement USDA, DOE (DE-FOA-0001688)***

**J. LeBoldus** (Oregon St.) *Towards durable resistance to Septoria stem canker and leaf spot: A molecular understanding of resistance*

**R. Bart** (Danforth Center) *Optimizing tradeoffs implicit during bioenergy crop improvement: Understanding the effect of altered cell wall and sugar content on sorghum-associated pathogenic bacteria*

**E. Eisenstein** (Univ. Maryland) *Elucidating Mechanisms of Rust Pathogenesis for Engineering Resistance in Poplar*

**D. Lowry** (Michigan St.) *Identification of Adaptive Fungal Pathogen Resistance Loci in Switchgrass*

**J. Sedbrook** (Illinois St.) *Advancing field pennycress as a new oilseed biofuels feedstock that does not require new land commitments*

**L. Comai** (UC Davis) *Discovery and characterization of dosage-dependent disease resistance loci in biomass poplar*

 ***6 new projects (\$7M over 3 yrs; 2 recommended for funding by **USDA**)***

# ***New Science Focus Area (SFA) Projects at the DOE Labs***

## **Soil Microbiome Research**

### ***Phenotypic Response of the Soil Microbiome to Environmental Perturbations***

**Pacific Northwest National Laboratory (PNNL) (PI: Janet Jansson) \$2.5M/yr**

### ***Bacterial:Fungal Interactions and Their Role in Soil Functioning***

**Los Alamos National Laboratory (LANL) (PI: Patrick Chain) \$2.5M/yr**

### ***Microbes Persist: Systems Biology of the Soil Microbiome***

**Lawrence Livermore National Laboratory (LLNL) (PI: Jennifer Pett-Ridge) \$2.5M/yr**

## **Two New Projects (time limited)**

### ***Quantitative Plant Science Initiative (QPSI)***

**Brookhaven National Laboratory (BNL) (PI: Crysten Blaby-Haas) \$1M**

### ***m-CAFÉs: Microbial Community Analysis and Functional Evaluation in Soils***

**Lawrence Berkeley National Laboratory (LBNL) (PI: Louise Glass) \$1.6M/yr 2 yrs**

# Engineering high-level production of fatty alcohols from lignocellulosic feedstocks

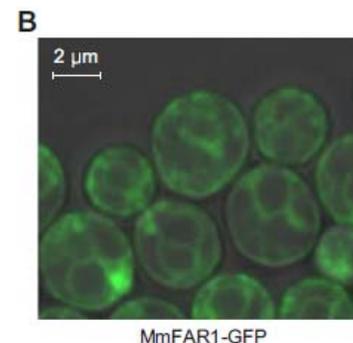
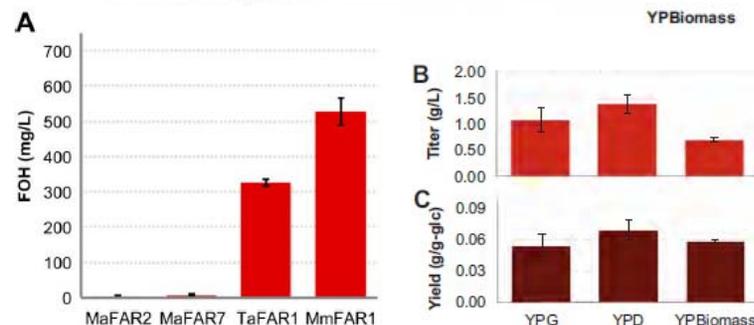
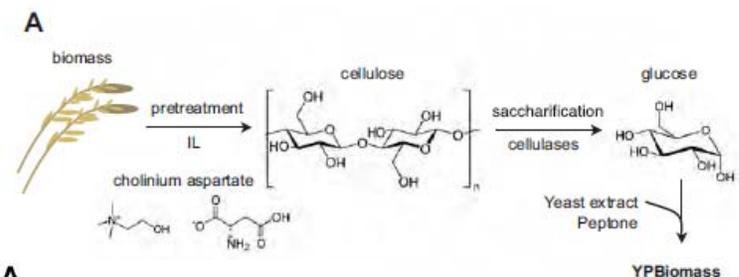
**Objective** Improve titer and yield of fatty acid-derived products in *Saccharomyces cerevisiae*

## Approach

- Proteomic/metabolic flux analysis to identify targets to improve fatty alcohol titer.
- Compared 4 fatty acid reductases finding *Mus musculus* FAR best, localized to ER in yeast
- Tested 24 gene edits, stacked best 6 into strain

## Results/Impacts

- Final strain produced 1.2 g/L fatty alcohols in shake flasks, and 6.0 g/L in fed-batch fermentation
- Corresponds to ~ 20% of the maximum theoretical yield from glucose, the highest titers and yields reported to date in *S. cerevisiae*
  - Previously yields *S. cerevisiae* remained under 2% of maximum theoretical yield from glucose
- Represents progress towards efficient and renewable microbial production of fatty acid-derived products



D'Espaux *et al.*, "Engineering high-level production of fatty alcohols by *Saccharomyces cerevisiae* from lignocellulosic feedstocks" (2017), *Metabolic Engineering* DOI: <https://doi.org/10.1016/j.ymben.2017.06.004>

## Field-grown transgenic switchgrass has no effect on soil chemistry, microbiology or carbon storage potential

**Objective** Understand agronomic impacts of growing transgenic switchgrass

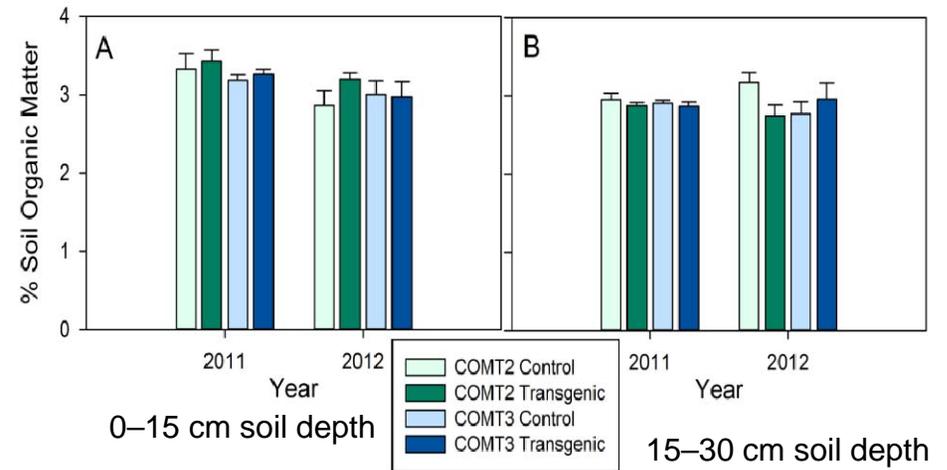
### Approach

Over two seasons of growth in the field, BESC COMT-regulated switchgrass showed:

- No impact on soil pH or total concentrations of 19 elements
- No impact on microbiome (mainly bacterial) diversity, richness, or community composition
- No impact on soil carbon storage capacity or in switchgrass-contributed carbon

### Results/Impact

Lignin modification in switchgrass through manipulation of COMT expression does not have an adverse effect on soils in terms of total elemental composition, bacterial community structure and diversity, and capacity for carbon storage.



Mean soil organic matter during first 2 growing seasons in soils below transgenic and control switchgrass plants

DeBruyn *et al.*, “Field grown transgenic switchgrass (*Panicum virgatum* L.) with altered lignin does not affect soil chemistry, microbiology and carbon storage potential” (2017) **Global Change Biology** DOI:10.1111/gcbb.12407



## *Increasing the revenue from lignocellulosic biomass: Maximizing feedstock utilization*

**Objective** Determine the yield of high-value products from the primary components of lignocellulosic biomass to assess the economic viability of an integrated biorefinery.

### **Approach**

- Fractionate biomass into its primary components (cellulose, hemicellulose, lignin) using gamma-valerolactone (GVL).
- Determine experimental yield of higher value products, develop biorefinery process model, and perform technoeconomic evaluation for cost comparisons.



### **Result/Impacts**

- Obtained up to 80% of deconstructed biomass as high-value products (high-purity cellulose, furfural from hemicellulose, and carbon foam from lignin).
- Economically competitive with calculated revenues of ~\$500 per ton of dry biomass.
- Technology can be applied to production of advanced biofuels or chemicals that is cost-competitive with a current petroleum refinery.

Alonso *et al.* "Increasing the revenue from lignocellulosic biomass: Maximizing feedstock utilization." (2017) **Science Advances** DOI: 10.1126/sciadv.1603301

## *A new high-throughput genome editing technique to map phenotype to genotype*

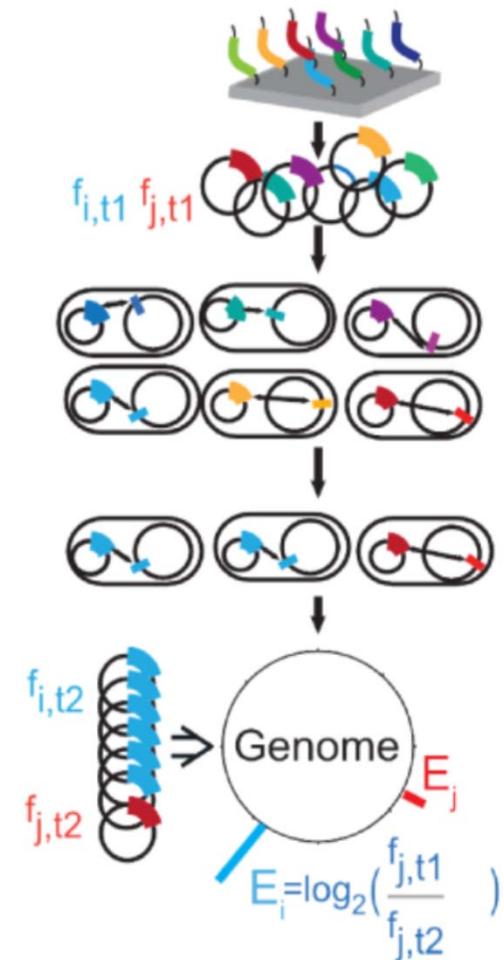
**Objective** Design approaches for high-throughput, genome-wide targeted mutagenesis in bacteria that simultaneously assigns mutation effects to the responsible genes

### **Approach**

The CREATE (CRISPR-enabled trackable genome engineering) method combines CRISPR–Cas9’s editing efficiency with bar-coding to mutate thousands of specific genome locations and track the mutated loci to determine their effect on physiology.

### **Result/Impacts**

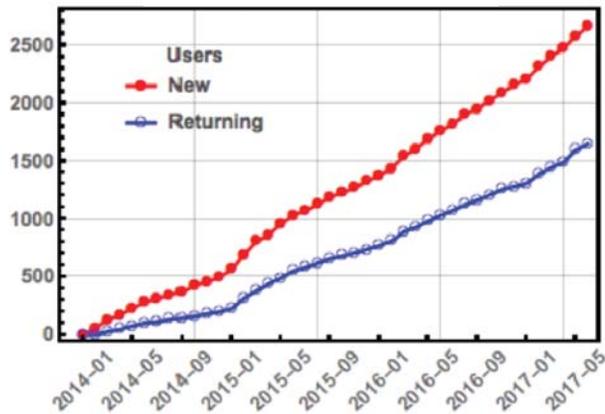
- Allows introduction of mutations throughout a bacterial genome in parallel, and associate each mutation (or combination) with the resulting phenotype at single-nucleotide resolution.
- Tested with a library of 50,000 CREATE cassettes targeting multiple genes in *E. coli* under heat stress and identified hundreds of known and new heat stress-related genes with 70% editing efficiency
- Allows high-throughput engineering of new metabolic pathways for production of biofuels and bioproducts



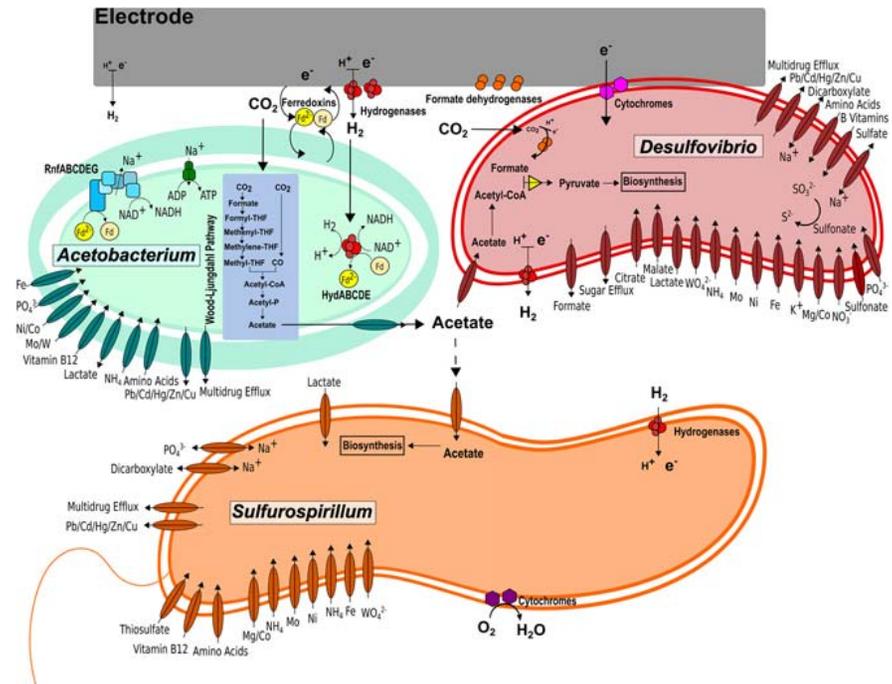
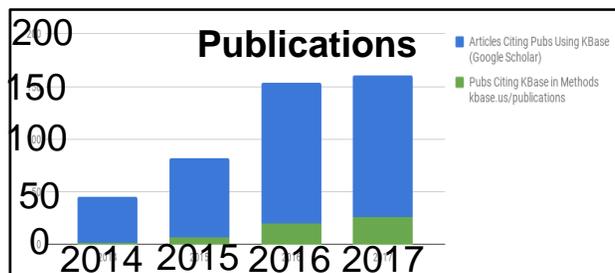
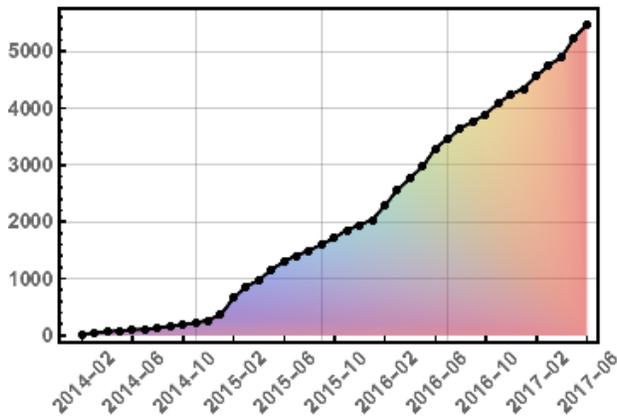
Garst *et al* “Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering.” **Nature Biotechnology** (2017) [DOI: 10.1038/nbt.3718]

# Metabolic Reconstruction and Modeling Microbial Electrosynthesis

### Cumulative Users



### Cumulative Narratives



- Modeled production of volatile fatty acids in a microbial electrosynthesis cell (MEC)
- Hypothesis testing enabled via metabolic pathway analysis and metatranscriptomics to deduce dominant pathways and dependencies
- Approach stored in a KBase Narrative for independent replication of results

Marshal C. et. al. **Scientific Reports 7:** 8391 (2017),  
 doi:10.1038/s41598-017-08877-z



# Instrument development: multimodal chemical and topographic imaging of biological systems

**Objective** Development of multimodal micro-nano-spectroscopic and topographic imaging for biological systems

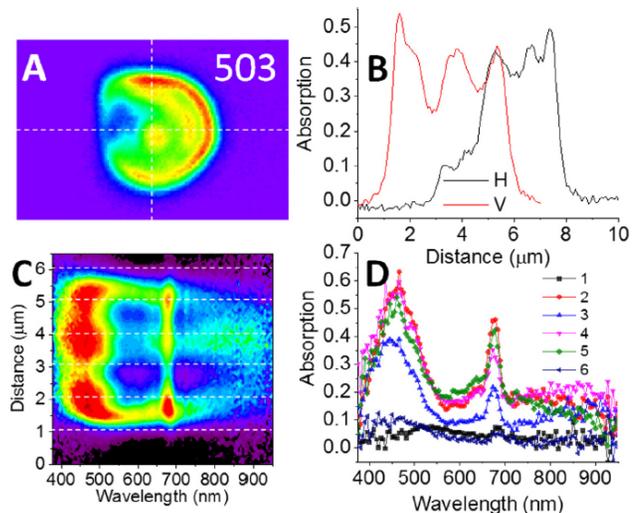
## Approach

- Integrated a hyperspectral optical imager with a custom-built atomic force microscopy-based tip-enhanced Raman microscope to enable:
  - hyperspectral fluorescence,
  - optical absorption,
  - dark-field scattering,
  - Raman scattering, and
  - topographic imaging
 of model biological systems on a single platform.



## Results/Impact

- Ability to image and identify pigments in lipid monolayers and within a single live cell in solution using hyperspectral optical absorption microscopy.
- Paves the way for multimodal hyperspectral imaging of organisms and processes relevant to bioenergy and environmental research.



Novikova et al. (2017) "Multimodal Hyperspectral Optical Microscopy" *Chemical Physics* S0301-0104(17)30502-5 DOI:10.1016/j.chemphys.2017.08.011

## Neutrons reveal the *in vivo* organization of bacterial membranes and indicate lipid rafts

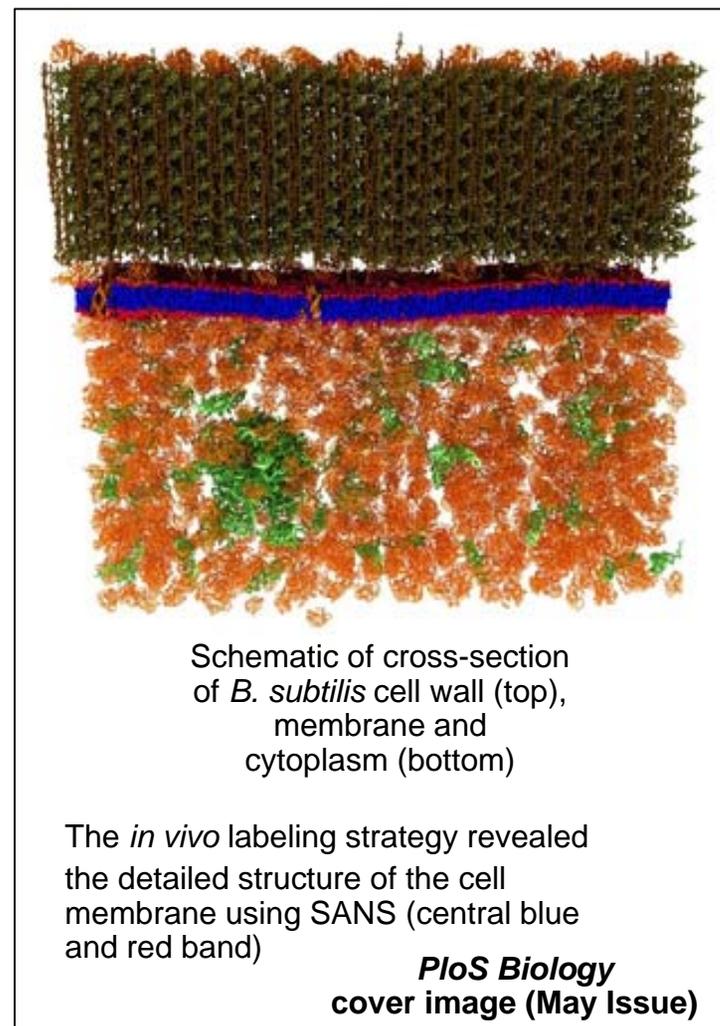
**Objective** Resolve membrane organization in living organisms has been a grand challenge due to the inherent nanoscopic size of the system

### Approach

- Construction of *Bacillus subtilis* genetic knockouts deficient in fatty acid synthesis to enable manipulation of the isotopic composition (H/D) of its membrane
- First observation of the *in vivo* membrane structure with a hydrophobic thickness of  $24.3 \pm 0.9$  Å.
- The observed lipid segregation in the membrane is consistent with nanoscopic lipid assemblies, implying that lipid domains are an integral feature of biological membranes

### Results/Impact

- First observation of lipid domains *in vivo* (~40 nm in size), consistent with presence of lipid rafts.
- New approach, contrast matching *in vivo*, for systematic investigations of cell membrane structure



Nickels *et al.*, "The *in vivo* structure of biological membranes and evidence for lipid domains" (2017) ***PLoS Biology*** DOI:<https://doi.org/10.1371/journal.pbio.2002214>

## *A Parts List for Fungal Cellulosomes Revealed by Comparative Genomics*

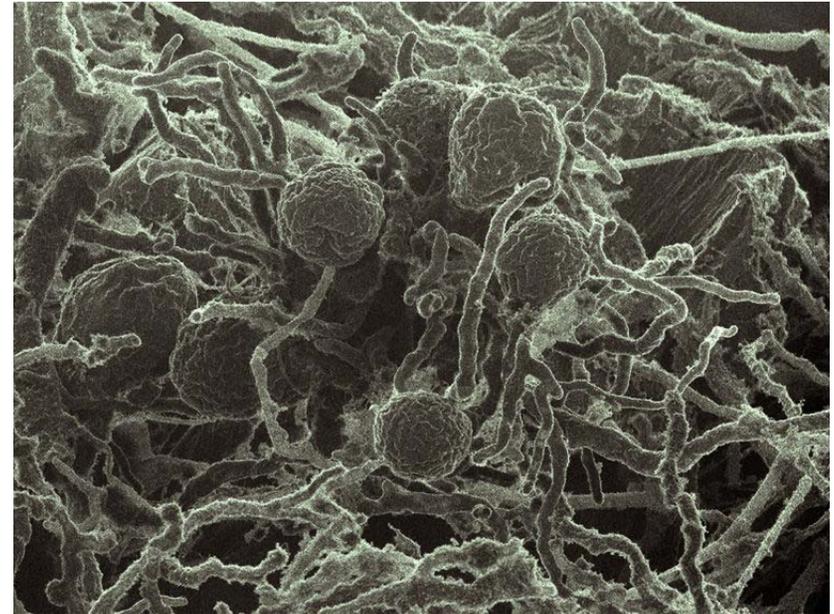
**Objective** Describe the comprehensive set of proteins that play a role in fungal cellulosome assembly

### **Approach**

- Combined next-generation sequencing with functional proteomics to analyze three anaerobic fungi strains for proteins critical to fungal cellulosome assembly

### **Results/Impacts**

- Found ~312 non-catalytic dockerin domain-containing proteins per strain, mostly CAZymes, w/ 95 fungal scaffoldins identified across four genera that bind to NCDDs
- Uncovered a new family of genes that likely serve as scaffoldins in the cellulosomes of anaerobic fungi
- Fungal cellulosomes have little genetic similarity to bacterial analogues, indicating that they evolved independently. However, several catalytic domains are shared via horizontal gene transfer with gut bacteria
- This finding of convergent evolution suggests that co-locating biomass enzymes at cell surface is inherently advantageous for efficient biomass deconstruction



O'Malley, "A Parts List for Fungal Cellulosomes Revealed by Comparative Genomics." *Nature Microbiology* (2017). DOI 10.1038/nmicrobiol.2017.87

## *Engineering a More Efficient System for Harnessing Carbon Dioxide*

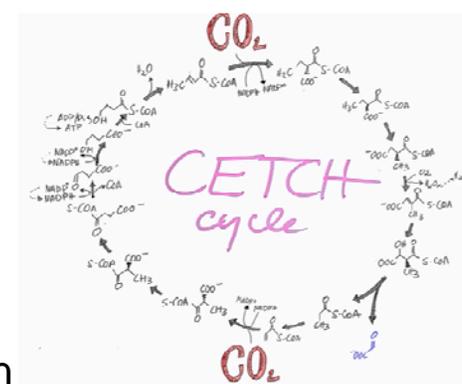
**Objective** To reverse-engineer a biosynthetic pathway for more effective carbon fixation.

### **Approach**

- Conceived several theoretical CO<sub>2</sub> fixation routes that (i) start with a carboxylase reaction, (ii) regenerate the carboxylation substrate to allow for continuous cycling, and (iii) feature a dedicated output reaction to channel the fixed carbon into a product

### **Results/Impact**

- In the end, through sequencing and synthesis, 17 different enzymes from 9 different organisms across the three kingdoms of life were incorporated.
- These parts were combined to achieve a proof of principle CO<sub>2</sub> fixation pathway performance that exceeds what can be found in nature
- Potential: Synthetic CO<sub>2</sub>-fixation cycles can be introduced into organisms to bolster natural photosynthesis or, in combination with photovoltaics, lead the way to artificial photosynthesis



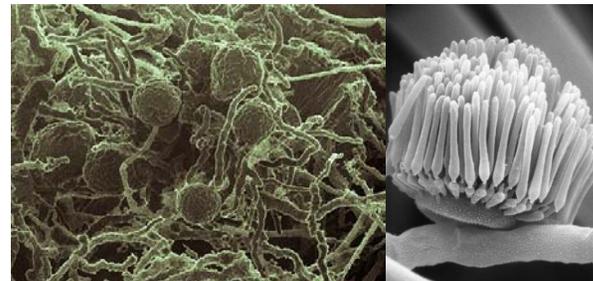
Schwander *et. al.* "A synthetic pathway for the fixation of carbon dioxide in vitro" **Science** 354. 6314 (2016). [DOI: 10.1126/science.aah5237]

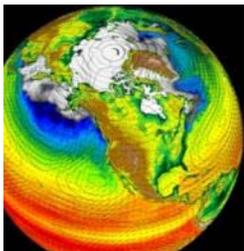
**Selected publications Q3-Q4 2017:**

- *Virophage Database Doubled with Discovery in Freshwater Lakes Datasets, **Nature Communications***
- *Liverwort Genome Analysis of Sheds Light on How Plants Thrive on Land, **Cell***
- *Benchmarking Computational Methods for Metagenomes, **Nature Methods***
- *Defining Standards for Genomes from Uncultivated Microorganisms, **Nature Biotechnology***
- *1,003 Reference Bacterial & Archaeal Genomes Released, **Nature Biotechnology***
- *Fungal Enzymes Team Up to More Efficiently Break Down Cellulose, **Nature Microbiology***
- *New Major Gene Expression Regulator in Fungi Found, **Nature Genetics***

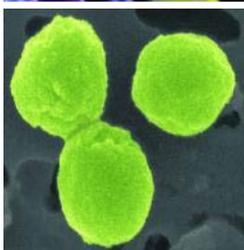
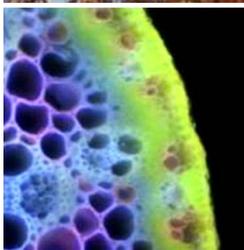
**66 papers published in FY2017 Q3/Q4**

JGI news releases & highlights:  
<http://jgi.doe.gov/news-publications/>  
<http://jgi.doe.gov/category/science-highlights/>





<http://science.energy.gov/ber>



# Thank you!

<http://genomicscience.energy.gov>



U.S. DEPARTMENT OF  
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Office of Biological  
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Integrative Genomics Building (10-30-17)

