

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

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

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Department of Energy, Office of Biological &  
Environmental Research

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U.S. DEPARTMENT OF  
**ENERGY**

Office  
of Science

Office of Biological  
and Environmental Research

# *Programmatic Activities*

## **Completed Reviews/Activities**

- Annual review of the BRCs (Oct-Nov-Dec)
- JGI Triennial Review (Dec)
- Review of PNNL Pan-Omics Project (Feb)
- Three Panel Reviews for FY 2015 FOAs (March, May)
- Three reviews of capabilities in Structural Biology - (April and mail)
- Panel review of Bioimaging Projects at the DOE National Laboratories – (May)
- DOE National Laboratory Science Focus Area (SFA) Triennial Reviews – (Aug)
  - ANL, LANL, NREL, ORNL-Biofuels, ORNL-Foundational
- Review of the DOE Systems Biology Knowledgebase (KBase) – (Sept)

 **18 Major review activities for FY 2015!**

## **Upcoming Reviews/Activities**

- Bioenergy Research Centers annual reviews (Oct-Nov-Dec)
  - GLBRC – last week, BESC - next week, JBEI – Dec 8-11
- Annual Genomic Science PI Meeting – March 7-9, 2016
  - Sheraton Premier at Tysons Corner, VA

# Portfolio Context for the New FOAs

## **Novel in situ Imaging and Measurement Technologies for Biological Systems Science (DE-FOA-0001192)**

- Academic complement to projects ongoing at the DOE National Laboratories

## **Systems Biology Research to Advance Sustainable Bioenergy Crop Development (DE-FOA-0001207)**

- Follows a recent workshop on sustainable bioenergy research
- Builds on advances in plant and microbial research within the:
  - Genomic Science program
  - Bioenergy Research Centers (BRCs)
  - DOE National Laboratory programs (SFAs)

## **Plant Feedstock Genomics for Bioenergy: A Joint Research Funding Opportunity Announcement USDA, DOE (DE-FOA-0001249)**

- Builds on past FOAs with USDA (ten years!)
- Includes a focus on plant-plant pathogen interactions
- Complements bioenergy efforts within the BRCs

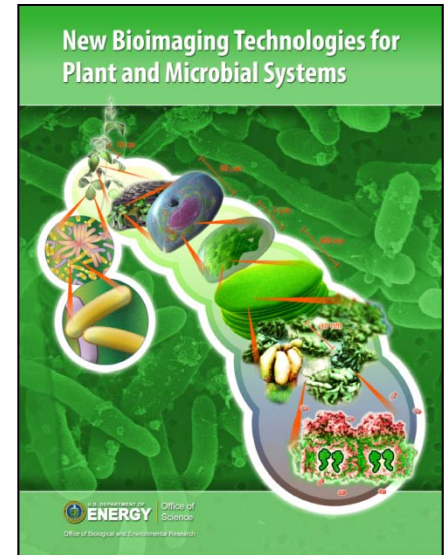


## *New Awards FY 2015*

### *Novel in situ Imaging and Measurement Technologies for Biological Systems Science FOA-0001192*

**Seven awards totaling \$15.0M (FY15-17)**

- Multi-Scale Dynamics of Water Regulation by Bacteria in Synthetic Soil Microsystems. *PI: Leslie Shor, UConn*
- Development and refinement of an in situ "molecular microscope" utilizing ultrahigh resolution mass spectrometry. *PI: Gary Stacey, Univ. Missouri*
- Integrated and dynamic multi-spectroscopic in situ imaging of plant metabolism at the level of subcellular compartments. *PI: Basil Nikolau Iowa State Univ.*
- The transparent soil microcosm: a window into the spatial distribution and dynamics of carbon utilization and microbial interspecies interactions. *PI: Elisabeth Shank, UNC*
- Development of a novel high-precision, high-resolution SIMS platform for elemental and isotopic characterization of microbial cells at a systems level. *PI: David Fike, WashU*
- Development of biosensors to measure the spatial and temporal concentration profiles of inorganic phosphate in plants during arbuscular mycorrhizal symbiosis. *PI: Wayne Versaw, Texas A&M*
- Multi-functional Plasmonics Nanoprobes for Cellular Sensing and Imaging. *PI : Tuan Vo-Dinh, Duke Univ.*

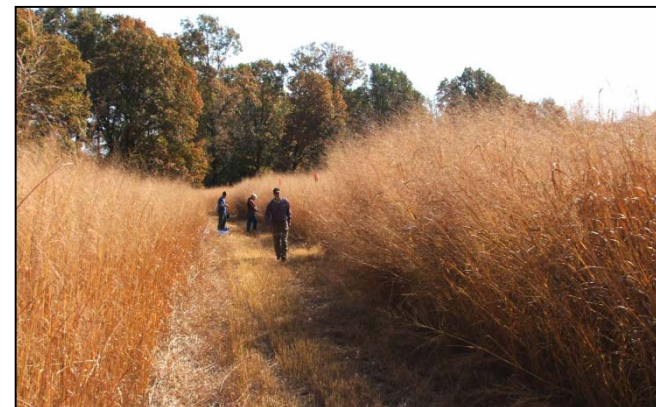


***Completes standup of a new program***

# New Awards FY 2015

## Systems Biology Research to Advance Sustainable Bioenergy Crop Development (DE-FOA-0001207)

Six awards totaling \$66.4M (FY15-19)



- **EvoNet:** A phylogenomic and systems biology approach to identify genes underlying plant survival in marginal, low-N soils. *PI: Gloria Coruzzi, New York U.*
- **Connecting Nitrogen Transformations Mediated by the Rhizosphere Microbiome to Perennial Cropping System Productivity in Marginal Lands.**  
*PI: Sarah Evans, Michigan State U.*
- **Establishment to senescence: plant-microbe and microbe-microbe interactions mediate switchgrass sustainability.** *PI: Mary Firestone, UC Berkeley.*
- **Climate adaptation and sustainability in switchgrass: exploring plant-microbe-soil interactions across continental scale environmental gradients.**  
*PI: Tom Juenger, U Texas Austin.*
- **Epigenetic Control of Drought Response in Sorghum (EPICON).**  
*PI: Peggy Lemaux, UC Berkeley.*
- **Systems Analysis of the Physiological and Molecular Mechanisms of Sorghum Nitrogen Use Efficiency, Water Use Efficiency and Interactions with the Soil Microbiome.**  
*PI: Daniel Schachtman, U. Nebraska Lincoln.*



➔ **New element in Genomic Science Program**

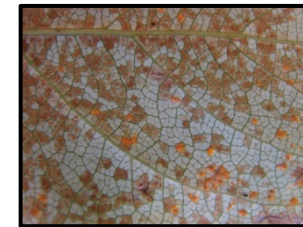
<http://genomicscience.energy.gov/>

# New Awards FY 2015

## **Plant Feedstocks Genomics for Bioenergy (DE-FOA-0001249)**

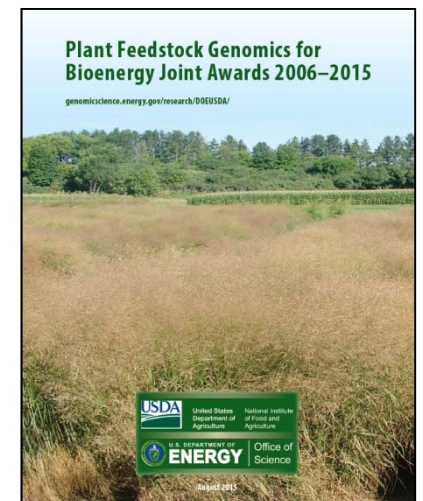
**Five awards totaling \$14.7 M (FY15-17)**

- Physiological and Molecular-Genetic Characterization of Basal Resistance in Sorghum. *PI: Peter Balint-Kurti, North Carolina St U.*
- Genomic dissection of anthracnose resistance response in sorghum [*Sorghum bicolor* (L.) Moench]. *PI: Hugo Cuevas, USDA ARS Mayaguez.*
- Characterizing the Defense Hierarchy of *Populus trichocarpa* and its Hybrids. *PI: George Newcombe, U. Idaho.*
- **Genomics-Assisted Breeding for Leaf Rust (*Melampsora*) Resistance in Shrub Willow (*Salix*) Bioenergy Crops.** *PI: Larry Smart, Cornell.*
- **Parallel analysis of *Puccinia emaculata* virulence and switchgrass resistance phenotypes.** *PI: Shavannor Smith, U Georgia.*



United States Department of Agriculture  
**National Institute of Food and Agriculture**

<http://genomicscience.energy.gov/research/DOEUSDA/2015awards.shtml>



# *Potential Upcoming Solicitations*

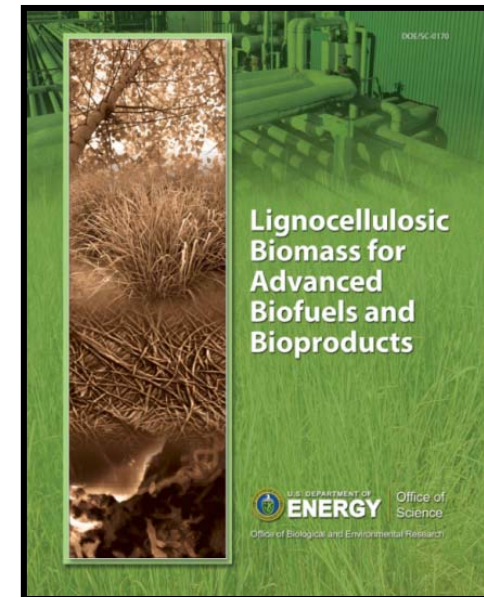
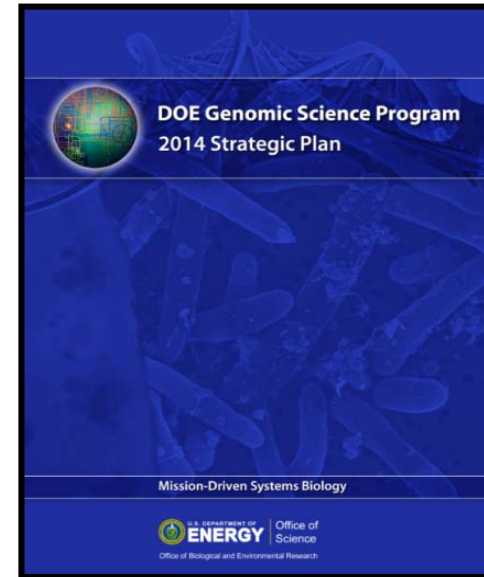
## *Genomic Science Program (FY 2016)*

*Systems Biology Research to Understand Carbon Cycling in the Environment*

*Plant Feedstock Genomics for Bioenergy:  
A Joint Research Funding Opportunity  
Announcement USDA, DOE*

## *On the Horizon: New Bioenergy Research Efforts*

- *Guided by a recent Bioenergy Workshop*
- *Next steps in basic bioenergy research*
- *Expanded focus on biofuels and bioproducts from biomass*



# Strategic Planning

## BSSD Strategic Plan:

- **Foster interdisciplinary research**
- **Develop high throughput enabling technologies**
- **Integrate high performance computing into genomic science**
- **Foster collaboration among DOE user facilities**

**Structure the portfolio to accelerate the understanding of Biology for DOE missions in bioenergy and the environment**





## Early Career Highlights

### ***Publication in Nature Communication***



***Trent Northen  
Staff Scientist  
Life Sciences Division  
Lawrence Berkeley National Laboratory***

### ***Desert soil crusts bacteria show specific preferences in organic compound consumption***

Baran R, Brodie E, Mayberry-Lewis J, Hummel E, Nunes Da Rocha U, Chakraborty R, Bowen B, Karaoz U, Cadillo-Quiroz H, Garcia-Pichel F, & Northen T (2015) ***Nature Communications*** doi:10.1038/ncomms9289

### ***Early Career Scientist Named One of MIT's Innovators Under 35***



***Michelle O'Malley,  
Assistant Professor  
University of California,  
Santa Barbara***

MICHELLE O'MALLEY, 33  
PIONEER

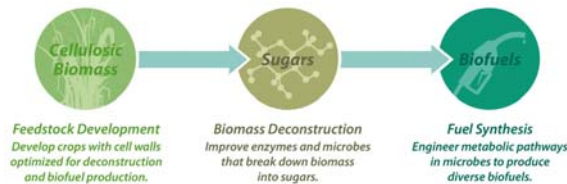
### 35 Innovators Under 35 2015

**Understanding a tricky kind of single-cell creature could help reduce the cost of biofuels.**

**C**hemical engineer Michelle O'Malley is trying to figure out how an understudied type of microbe could be harnessed to make better biofuels or pharmaceuticals. O'Malley works with anaerobic microbes – organisms that can't live in the presence of oxygen, making them extremely difficult to cultivate. In fact, her lab at the **University of California, Santa Barbara** is the only one in the United States that is able to study the behavior of anaerobic microbial communities.

Why go to all the trouble? Because these organisms are more efficient than aerobic ones at chewing up plant material and secreting something else, like a biofuel. They also create fewer unintended by-products, which are costly to deal with.

- Introduction
- Inventors
- Entrepreneurs
- Visionaries
- Humanitarians
- Pioneers
- Past Winners



# Bioenergy Research Centers (BRCs)

## Scientific goals (summarized) for FY 2016

### Great Lake Bioenergy Research Center (GLBRC)

- Evaluate transgenics for biosynthesis genes, transcription factors and, promoters for increased hemicelluloses in stem parenchyma tissues
- Design optimal 'drop-in' fuel plant oils and direct production to vegetative tissues
- Evaluate optimal enzyme mixtures for recalcitrant species

### Joint BioEnergy Institute (JBEI)

- Optimize pathways for new lignin traits using synthetic biology tools from Fuels Synthesis Division
- Demonstrate conversion of lignin-derived aromatics to methyl ketones
- CRISPR-based chromosomal integration and systematic optimization of isoprenoid pathway in *E. coli*

### BioEnergy Science Center (BESC)

- Incorporate omics data into genome-scale metabolic network to identify reaction bottlenecks and improve strain design of *C. thermocellum* for enhanced biofuels production
- Identify correlations between the chemical properties and altered recalcitrance in TOP Lines using advanced characterization methods
- Improve the *C. thermocellum* cellulosome and determine the synergy of *C. thermocellum* cellulosomes with heterologous cellulolytic enzymes

#### To date the BRCs have generated :

- **660 invention disclosures and/or patent applications**
- **24 patents awarded**
- **105 licensing agreements**
- **1799 peer-reviewed publications**



## *BESC partner Mascoma launches C5 FUEL™ a yeast engineered for improved cellulosic biofuel production*

### **Objective**

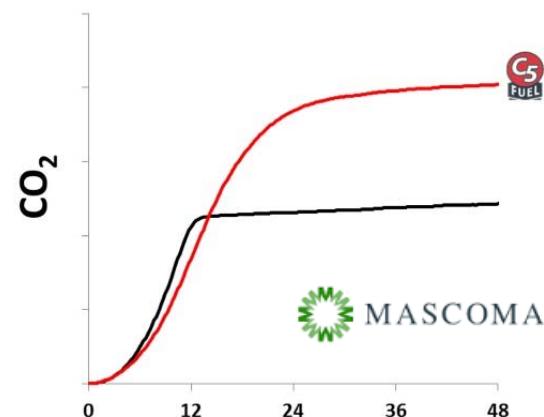
Develop new methods to utilize hemicellulose and xylose leftover from cellulosic feedstock processing methods.

### **Approach**

- C5 FUEL™ was engineered by Mascoma scientists in collaboration with BESC to utilize xylose as well as glucose.
- The new yeast strain simultaneously yields 97 percent conversion of xylose – the C5 sugar – and glucose and it accomplishes this feat in less than 48 hours
- C5 FUEL™ was launched at the Fuel Ethanol Workshop in June 2015

### **Results/Impact**

- The launch of a commercially available product designed for producers in the cellulosic biofuels market is an important milestone for BESC and the BRC program.
- Combining the expertise at Mascoma and BESC in engineering microbes to release and convert sugars from lignocellulosic biomass greatly accelerated the translation of basic research outcomes to a commercial product.



Kevin Wenger of Mascoma, a subsidiary of Lallemand, LLC and Paul Gilna of BESC



## Field production, purification, and analysis of high-oleic acetyl-triacylglycerols (TAG) from transgenic *Camelina sativa*

### Objective

Evaluate acetyl-TAG production and field performance in engineered *Camelina sativa* expressing *Euonymus alatus* diacylglycerol acetyl transferase (EaDAcT).

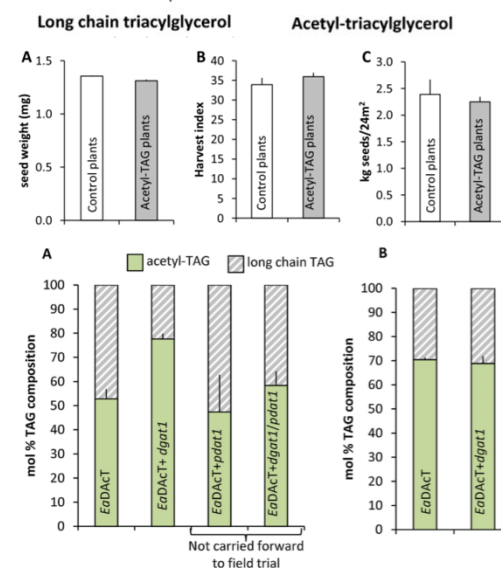
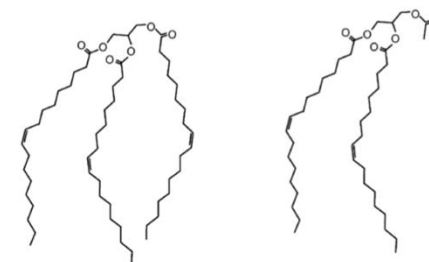
### Approach

- A high-oleic line of *Camelina* was engineered to produce high levels of acetyl-TAG by expressing EaDAcT and down-regulating two competing metabolic pathways.
- Seeds from transformed *Camelina* lines were bulked and used for field trials (0.7 ha total) designed to evaluate growth, yield, and acetyl-TAG production.
- Seed oil was analyzed for composition and physical properties

### Result/Impacts

- Engineering of *Camelina* had little or no impact on seed weight, harvest index, seed yield and oil content but had a significant increase in acetyl-TAG oil.
- Achieved reductions in desirable physical properties, such as crystallization temp., viscosity, and caloric content, of oil from engineered plants.
- Direct production of high-oleic acetyl-TAG in crops could offer an alternative, lower-cost supply of acetyl-TAG for biodegradable lubricants, hydraulic fluids, and drop-in diesel fuels.

Liu J, et. al. Field Production, Purification, and Analysis of High-Oleic Acetyl-Triacylglycerols from Transgenic *Camelina sativa*. **Industrial Crops and Products**. 65 (2015) 259-268



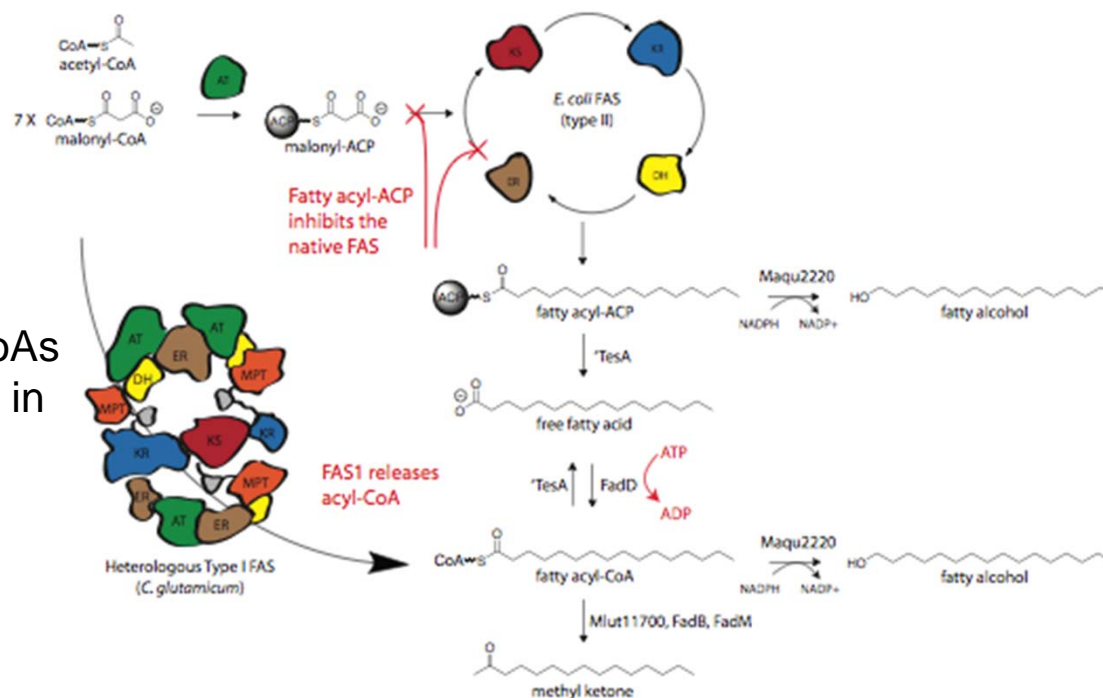
# Development of an orthogonal fatty acid biosynthesis system in *E. coli* for oleochemical production

## Objective

Modify native fatty acid synthase (FAS) in *E. coli* to increase production.

## Approach

- Actinobacterial possess type I FAS enzymes that directly produce acyl-CoAs and are not transcriptionally regulated in engineered *E. coli*.
- Cloned and expressed type I FAS enzymes from actinobacteria and investigated their capacity for oleochemical production



## Results/Impact

- We demonstrated the *in vivo* activity of several type I FASs and used *C. glutamicum* FAS to produce fatty alcohols and methyl ketones.
- This is the first example of a heterologous FAS pathway in *E. coli*, and will be further optimized to improve renewable production of oleochemicals.

Haushalter, R. W. *et al.* (2015). "Development of an orthogonal fatty acid biosynthesis system in *E. coli* for oleochemical production". *Metab Eng*, DOI: 10.1016/j.ymben.2015.04.003

## *A new method to track combinatorial mutations in engineered cell populations*

### **Objective**

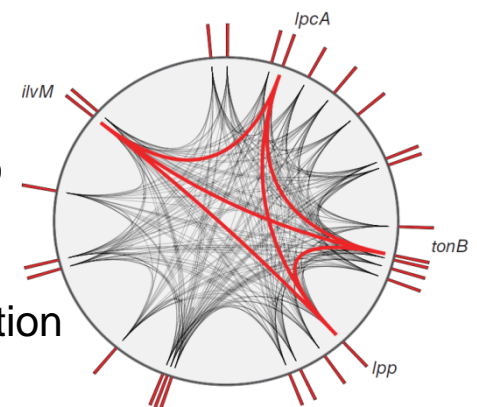
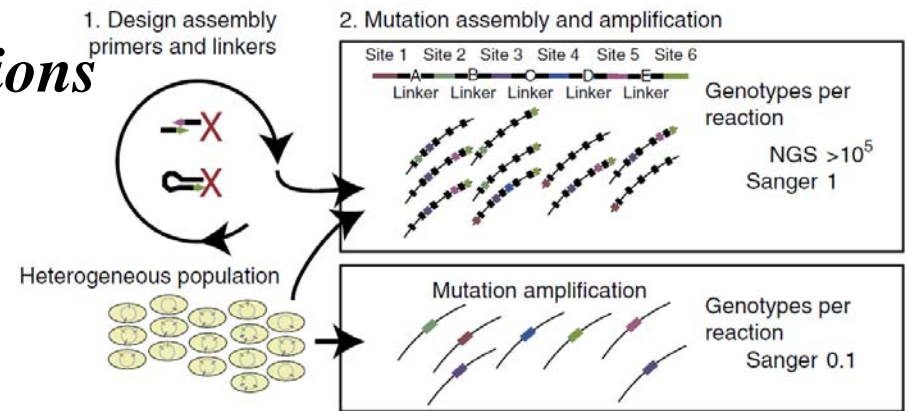
Design a method to map combinations of genetic mutations in individual cells within engineered cellular populations.

### **Approach**

Using mathematical modeling, highly specific primers are designed to bind targeted mutation sites spread around the genome in an engineered cell population. The primers contain linkers to allow subsequent concatenation of the amplification products into a single DNA molecule. By performing the amplification and assembly of the DNA products in emulsion-PCR format, each cell in the population is confined to a single droplet, where 6 to 8 target sites are assembled into a >1 kb DNA fragment and analyzed by next generation sequencing to identify which cells contain mutations in one or more target sites.

### **Results/Impact**

- With this new method combinations of mutations that confer tolerance to cellulose hydrolysate or isobutanol were readily identified.
- The technique also works in eukaryotic cells.
- The approach will accelerate the engineering of microbes for the production of biofuels.



Ramsey Zeitoun, Andrew Garst, George Degen, Gur Pines, Thomas Mansell, Tirzah Glebes, Nanette Boyle & Ryan Gill. Multiplexed tracking of combinatorial genomic mutations in engineered cell populations. *Nature Biotechnology* (2015) doi:10.1038/nbt.3177

## *A novel technology to engineer microalgae with biofuel production potential*

### **Objective**

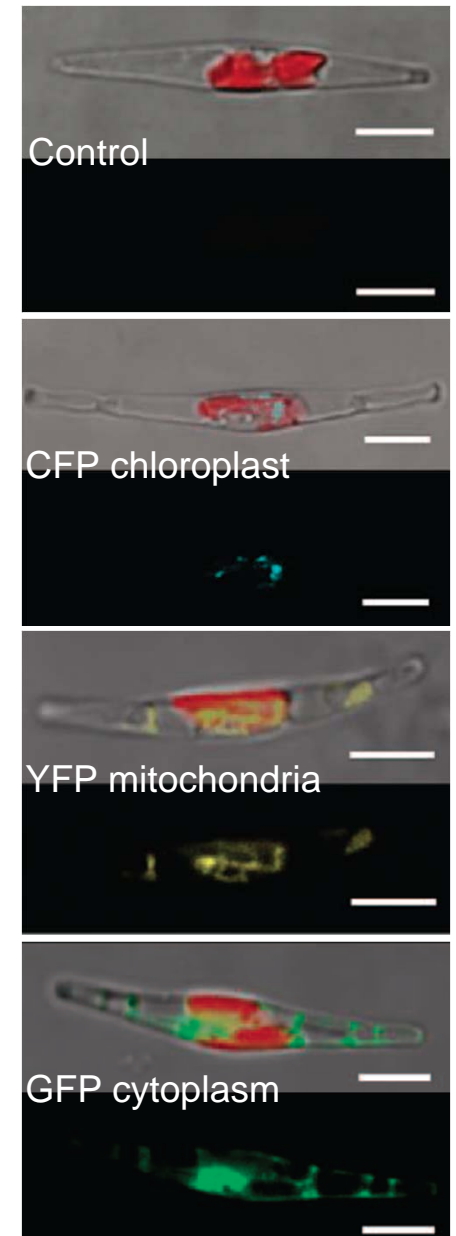
Develop an efficient and simple transformation technique for diatoms.

### **Approach**

Using a DNA sequence from yeast that supports episomal replication in two diatom species and the *E. coli* DNA sequence needed to start conjugative DNA transference, researchers at the J.C. Venter Institute designed a vector that can be used to clone large fragments of heterologous DNA and efficiently introduce it in diatoms without the need of plasmid purification.

### **Results/Impact**

- The new vector could be efficiently introduced in both *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* and reporter genes were expressed and maintained in a stable fashion for at least two months.
- A 49 kb fragment of cyanobacterial DNA was successfully introduced in diatoms.
- The method will advance engineering of diatoms for the production of biofuels and valuable chemicals.



## ***Direct Electron Transfer via Nanowires in Anaerobic Oxidation of Methane (AOM) Consortia***

### **AOM Consortia Activity Measurement**

#### **Objective**

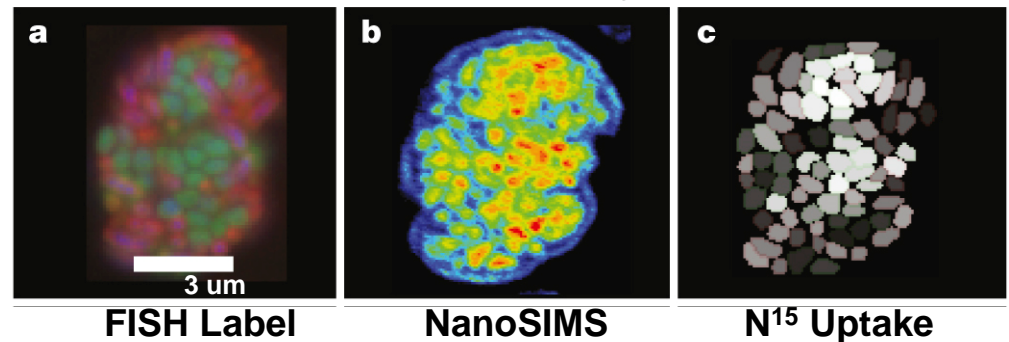
Examine metabolic properties and energy transfer mechanisms of anaerobic oxidation of methane (AOM) symbiotic consortia

#### **Approach**

Used a combination of microscopy, stable isotope labeling, nanoSIMS analysis, and computational modeling to examine metabolic activity of individual cells and predict the energy transfer mechanism from methane oxidizing archaea to sulfate-reducing bacteria

#### **Results/Impact**

- NanoSIMS analysis showed that metabolically active cells are found throughout the consortia, and partner microbes do not need to be closely associated with each other
- Genes encoding a multi-haem complex electron transfer system were identified in the AOM archaea genome, and respiratory chain components were observed in the extracellular space between partner cells using an active staining approach
- Advances understanding of the specific mechanism driving a globally significant carbon cycle process







# Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists

## Objective

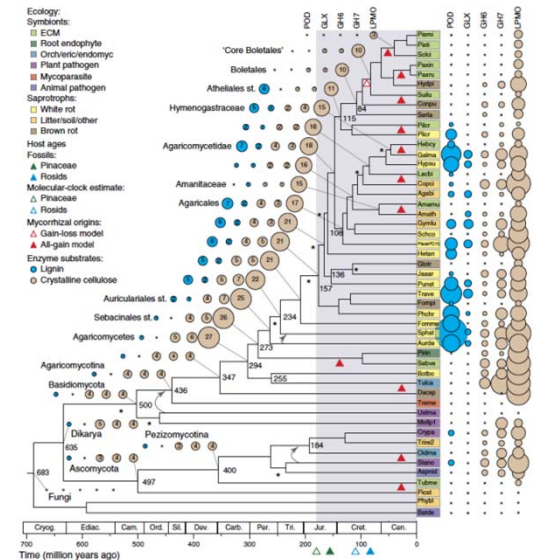
Improve the understanding of ectomycorrhizal fungi to resolve mechanisms of nutrient cycling, lignocellulose degradation and plant growth promotion.

## Approach

- Phylogenomic and comparative genomic analyses of 13 new and 31 other sequenced fungal genomes were performed to elucidate the genetic bases of mycorrhizal lifestyle evolution.
- ECM fungi have a reduced complement of genes encoding plant cell-wall degrading enzymes, yet retain a distinct suite of these enzymes indicating that they possess diverse capabilities to decompose lignocellulose.
- Symbiosis development between ECM fungi and host-plant development and immunity requires lineage-specific genes encoding small secreted effector proteins (SSEPs). Of induced genes, 7 to 38% are orphan genes, including genes that code for mycorrhiza-induced small secreted proteins.

## Results/Impact

- Convergent evolution of mycorrhizal habit in fungi occurred via the repeated evolution of a “symbiosis toolkit”, with reduced numbers of plant cell-wall degrading enzymes and lineage-specific suites of mycorrhiza-induced genes.



Kohler *et al.* 2015, Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. **Nature Genetics**. Doi: 10.1038/ng.3223

# Exploring the repeat protein universe through computational protein design

## Objective

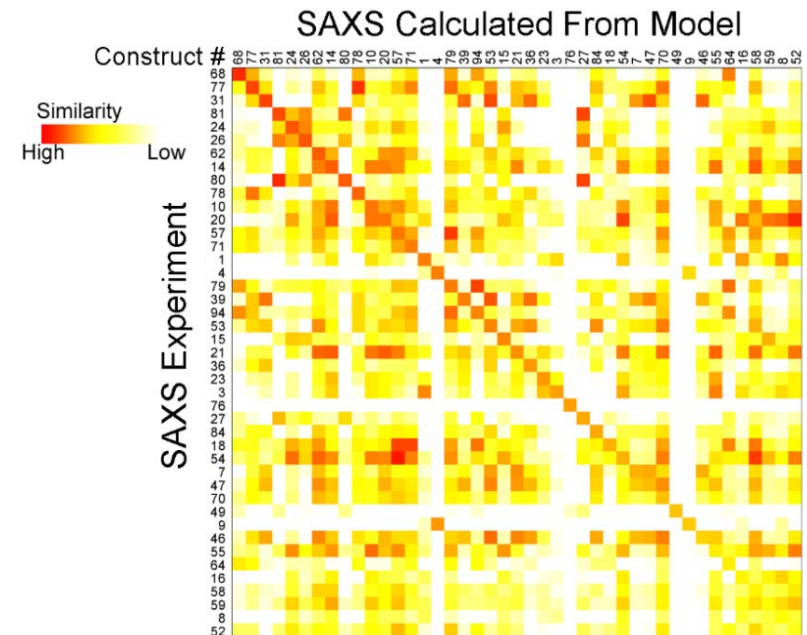
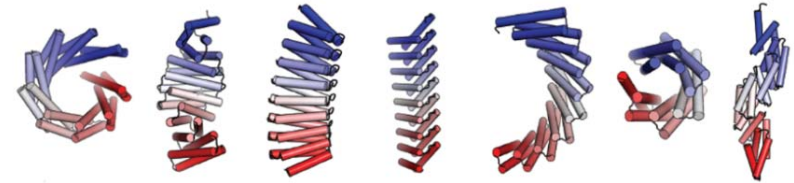
Understand the range of shapes possible for a tandem repeat protein

## Approach

- Computationally engineered 100s of helix-turn-helix protein folds with unique topologies
- Experimentally validated computational models with SAXS at SIBYLS station at the Advanced Light Source

## Results/Impacts

- Identified novel repeat proteins with precisely specified geometries
- Demonstrated that “existing repeat proteins occupy only a small fraction of the possible repeat protein sequence and structure space”
- And “that it is possible to design novel repeat proteins with precisely specified geometries, opening up a wide array of new possibilities for biomolecular engineering.”

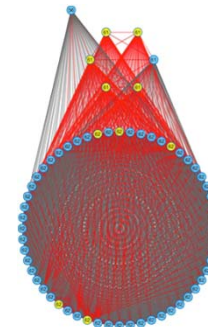
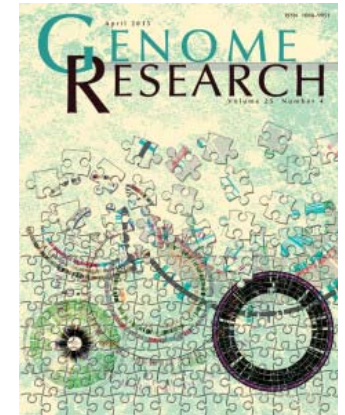
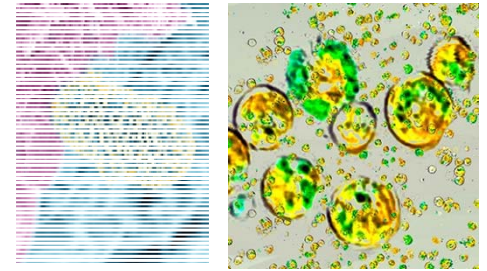


Upper diagrams: Some different topologies for a protein sequence containing 18 helices as predicted by Rosetta  
Lower diagram: Diagonal compares SAXS experimental data and models: red indicates high and white indicates low similarity

\*TJ Brunette, Fabio Parmeggiani, Po-Ssu Huang, Gira Bhabha, Damian C. Ekiert, Susan E. Tsutakawa, Greg L. Hura, John A. Tainer, and David Baker (2015) Exploring the repeat protein universe through computational protein design. *Nature* in press.

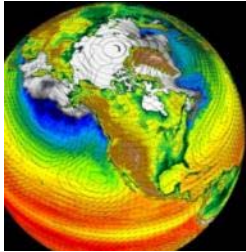
## Selected Publications

- Ngan, CY, et. al., (2015) Lineage-specific chromatin signatures reveal a regulator of lipid metabolism in microalgae *Nature Plants*, 1 (15158) doi:10.1038/nplants.2015.107
- Lebeis, SL, et. al., (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa *Science*, 349 (6250): 860-864 DOI: 10.1126/science.aaa8764
- Varghese, NJ, et. al., (2015) Microbial species delineation using whole genome sequences *Nucleic Acids Research*, 43 (14): 6761-6771 doi: 10.1093/nar/gkv657
- Nobu, MK, et. al., (2015) Phylogeny and physiology of candidate phylum 'Atribacteria' (OP9/JS1) inferred from cultivation-independent genomics *The ISME Journal*, doi: 10.1038/ismej.2015.97
- Tennesen, K, et. al., (2015) ProDeGe: a computational protocol for fully automated decontamination of genomes *The ISME Journal*, doi: 10.1038/ismej.2015.100
- Sharon, I, et. al., (2015) Accurate, multi-kb reads resolve complex populations and detect rare microorganisms *Genome Research*, 25: 534-543 doi:10.1101/gr.183012.114
- Hultman, J, et. al., (2015) Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes *Nature*, 521, 208–212 doi:10.1038/nature14238
- Kohler, A, et. al., (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists *Nature Genetics*, 47, 410–415 doi:10.1038/ng.3223



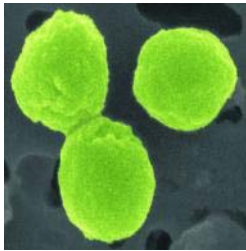
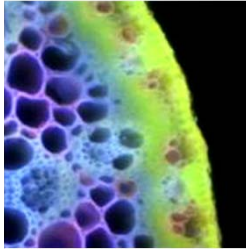
**181 publications in FY 2015; 127 since the last BERAC meeting**

JGI Publication Highlights: <http://www.jgi.doe.gov/News/pubs.html>



# Systems science to meet DOE mission needs in bioenergy and the environment.

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



# Thank you!



<http://genomicscience.energy.gov/index.shtml>



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