

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

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

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Department of Energy, Office of Biological &  
Environmental Research

October 1, 2014



U.S. DEPARTMENT OF  
**ENERGY**

Office  
of Science

Office of Biological  
and Environmental Research

# *Programmatic Activities*

## **Completed/Upcoming Reviews**

- Plant Feedstocks Genomics for Bioenergy (DE-FOA-0001034) - **complete**
- Systems Biology of Bioenergy-Relevant Microbes to Enable Production of Next-Generation Biofuels (DE-FOA-0001060) - **complete**
- Committee of Visitors review (July 9-11) – **complete**  
(Briefing by Dr. Dave Stahl)
- Systems Biology Knowledgebase (KBase) (Aug 12-13) - **complete**
- Pacific Northwest National Laboratory Foundation Genomic Science SFA review (Nov. 17)
- Bioenergy Research Centers annual review
  - Great Lakes Bioenergy Research Center (GLBRC) – Oct 7-8
  - BioEnergy Science Center (BESC) – Nov 5-6
  - Joint BioEnergy Institute (JBEI) – Dec 11-12
- Joint Genome Institute (JGI) Triennial Review (Dec 8-10)

## **Completed Workshops**

- “*Research for Sustainable Bioenergy,*” – **report completed**
- “*Molecular Science Challenges Workshop*” - **report pending**  
(Briefing by Dr. Judy Wall)
- “*DOE Bioenergy Workshop*”, June 23-25, 2014 – **report pending**  
(Briefing by Dr. Kent Peters)

# Plant Feedstocks Genomics for Bioenergy (DE-FOA-0001034)

Ten Awards totaling \$12.6 M in total funds (FY14-16)

- Genetic Improvement of Bioenergy Sorghum for Compositional and Agronomic Traits
- Abiotic Stress Networks Converging on FT2 to Control Growth in *Populus*
- Exploiting Natural Diversity to Identify Alleles and Mechanisms of Cold Adaptation in Switchgrass
- A Novel Poplar Biomass Germplasm Resource for Functional Genomics and Breeding
- Genetic Dissection of AM Symbiosis to Improve the Sustainability of Feedstock Production
- Advancing Field Pennycress as a New Oilseed Biodiesel
- Biofuels in the Arid West: Germplasm Development for Sustainable Production of *Camelina* Oilseed
- The *Brachypodium* ENCODE Project - From Sequence to Function: Predicting Physiological Responses in Grasses to Facilitate Engineering of Biofuel Crops
- Genomics of Energy *Sorghum*'s Water Use Efficiency/Drought Resilience
- Quantifying Phenotypic and Genetic Diversity of *Miscanthus sacchariflorus* to Facilitate Knowledge Directed Improvement of *M. xgiganteus* (*M. sinensis* x *M. sacchariflorus*) and Sugarcane



United States Department of Agriculture  
National Institute of Food and Agriculture

USDA funded projects in red font



<http://genomicscience.energy.gov/research/DOEUSDA/2014awards.shtml>

## *Systems Biology of Bioenergy-Relevant Microbes to Enable Production of Next-Generation Biofuels (DE-FOA-0001060)*

- *Research to advance the development of promising new model organisms relevant to biofuels production.*
- *Development of novel microbial functional capabilities and biosynthetic pathways relevant to the production of advanced biofuels and the development of strategies to overcome associated metabolic challenges resulting from pathway modification*
- *Development of novel analytical technologies or high-throughput screening approaches*

### **Fourteen Awards totaling \$19.6 M in total funds (FY14-16)**

***New model organisms for bioenergy purposes:*** *Rhodococcus*, *Rhodospiridium*, fast growing cyanobacterial strains, lignocellulytic fungi, lichens, oil producing yeasts, *Bacillus* sp.

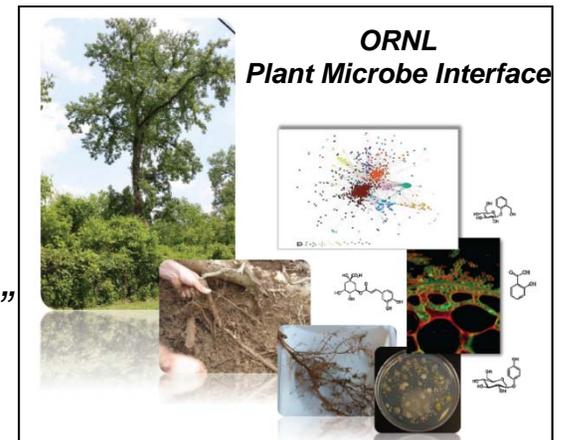
***New functional capabilities:*** novel biofuels production pathways, multi-omics approaches to understanding metabolic regulation in microbes, protein acetylation and genome regulation relevant to biofuels production.

***Novel technologies:*** cell-wide kinetic and metabolic modeling, mapping interspecies interactions and metabolic synergy.

# Portfolio Context for the New Awards

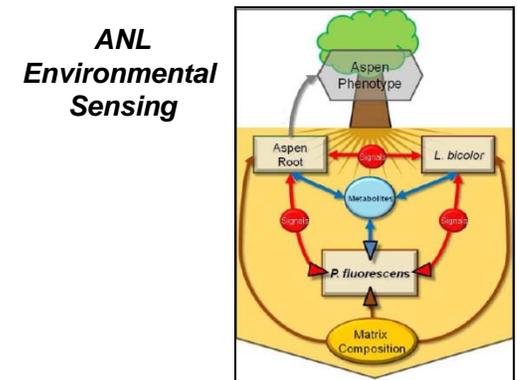
## New Plant Feedstocks Awards Complement Existing Plant and Rhizosphere Research efforts in the:

- Oak Ridge National Laboratory SFA – “Plant Microbe Interface”
- Argonne National Laboratory SFA - “Molecular Mechanisms Mediating Environmental Sensing and Response”

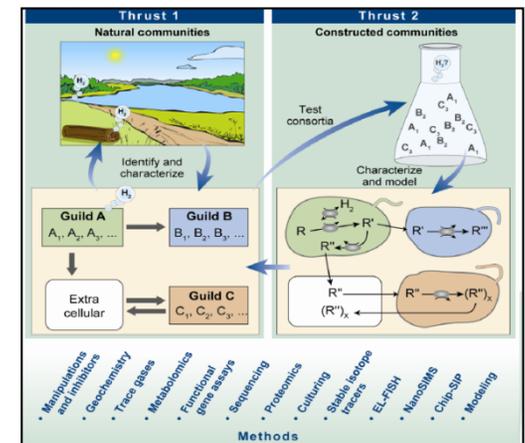


## New System Biology Research on Bioenergy-Relevant Microbes Complements Existing Efforts in the:

- Lawrence Livermore National Laboratory SFA – “A Systems Approach to Energy Flow in H<sub>2</sub>-producing Microbial Communities”
- National Renewable Energy Laboratory SFA – “...Addressing Limitations to Algal Hydrogen and Biofuels Photoproduction”
- Oak Ridge National Laboratory SFA – “Dynamic visualization of lignocellulose degradation...”



## LLNL: Energy Flow in microbial communities



In addition to existing projects and ongoing efforts in the:  
**Bioenergy Research Centers**  
**Biosystems Design projects**  
**Early Career awards**

➔ **Use the Genomic Science Program  
 PI Meeting to Encourage Collaborations**

# *New Funding Opportunity Announcements (FOAs)*



## ***Systems Biology Research to Advance Sustainable Bioenergy Crop Development (posts October 1).***

- Systems-level research to better understand the molecular and physiological mechanisms that control bioenergy crop vigor, resource use efficiency, and resilience/adaptability to abiotic stress, as well as interactions with the surrounding environment, in order to increase biomass productivity under changing and at times suboptimal conditions.
- Systems biology-enabled investigations into the role(s) of microbes and microbial communities in the complex and multi-scaled interactions of the plant-soil-environment: contribution(s) to bioenergy feedstock plant performance, adaptation, and resilience in the face of a broad range of changing environmental conditions and abiotic stressors (e.g., climate), and the impacts of introducing bioenergy cropping systems on the local ecosystem.

## ***Plant Feedstocks Genomics for Bioenergy – posts in November***

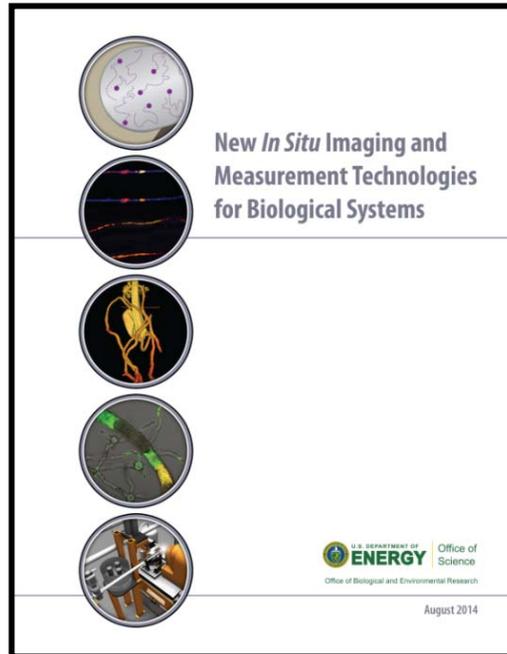
## ***Small Business Innovative Research (SBIR) – posted***

- Includes intellectual property (IP) developed by the Bioenergy Research Centers

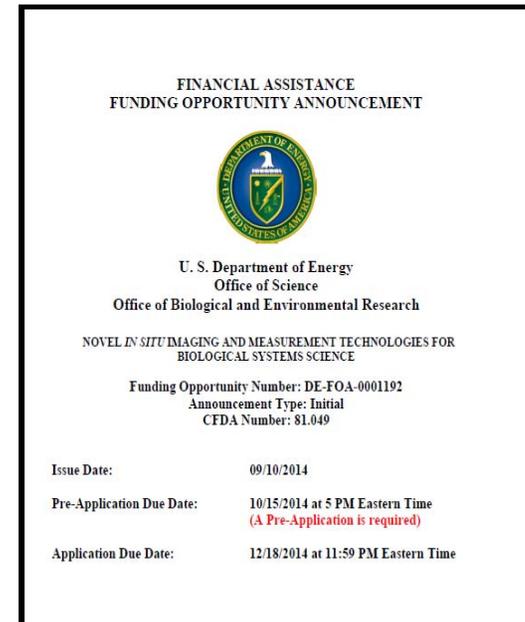
# Bioimaging Technology Development (DE-FOA-0001192)

- Understanding translation of genomic information into the mechanisms that power living cells, communities of cells, and whole organisms
- Develop new measurement and imaging technologies to visualize the spatial and temporal relationships of key metabolic processes governing phenotypic expression in plants and microbes.
- Funded five projects at the DOE National Laboratories (ANL, BNL, ORNL PNNL, SLAC)
- Issued an FOA for FY 2015 funds to the Academic community

## Brochure of Current Projects



<http://science.energy.gov/~media/ber/pdf/Brochures/imagingandmeasurement.pdf>



**Posted Sept 10**  
**Proposals due Dec 18**

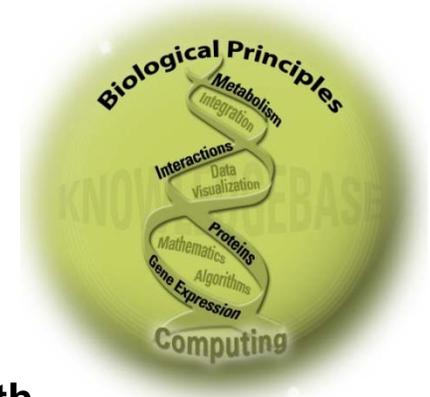
# DOE Systems Biology Knowledgebase (KBase)

## Building a Community Resource for Predictive Biology

An *open-source and open-architecture* computational environment for integrating large, diverse datasets, generated by the Genomic Sciences program and other sources, and for using this information to advance predictive understanding, manipulation, and design of biological systems.

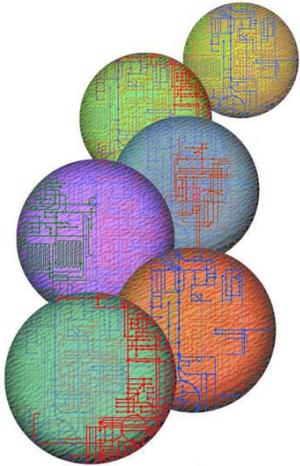
### *Reviewed this past August:*

- Support for the overall vision
- Attention to improving the access and utility of KBase
- Concerted effort to improve core capabilities in the three KBase domains (microbes, plants and microbial communities)
  - **Over 12,000 microbial genomes, 100 eukaryotic genomes, and 15,000 metagenomes**
  - **Annotated over 52 million genome features, 55,000 phylogenetic trees, and 132 million ontological terms**
  - **Generated 23,000 metabolic models with 27,000 compounds and 34,000 reactions**
  - **8,000 expression samples, 250 correlation networks, and 13 protein-protein networks including 230,000 interactions**
  - **~900 registered users from the Genomic Sciences, partnering with BRCs, JGI, and efforts at other funding agencies (e.g. iPlant)**



<http://www.kbase.us>

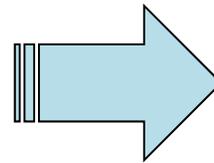
# Biological Systems Science



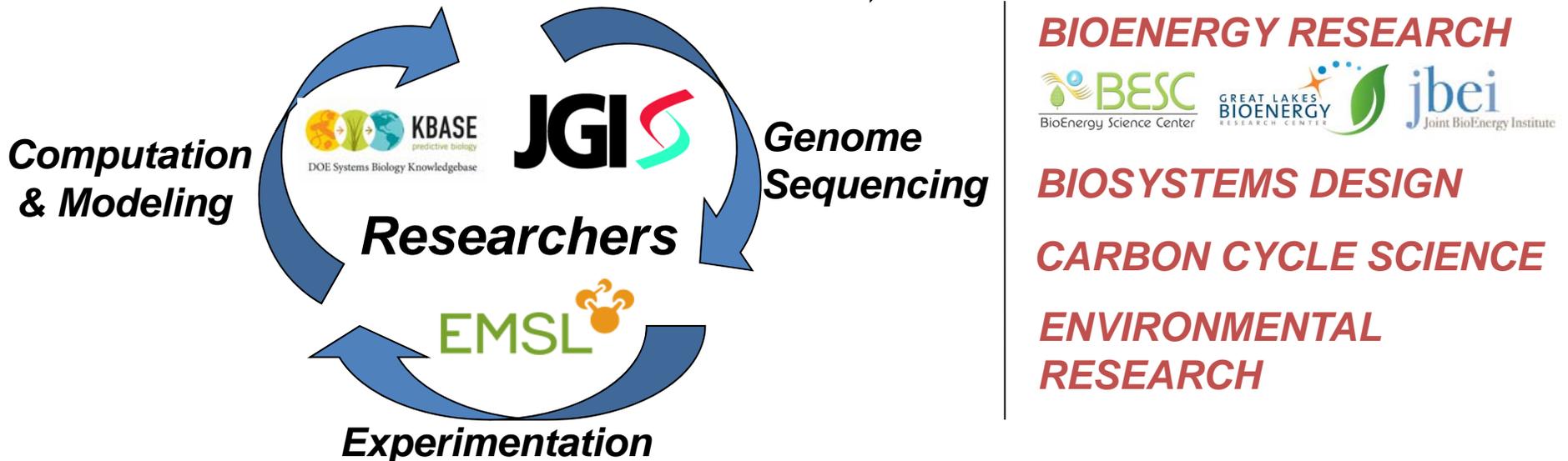
## *Enabling scientific communities*

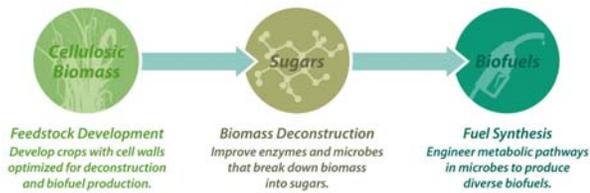
- support for individual researchers in Academia
- team-oriented research at the DOE National Laboratories
- technology development for systems biology integration
- cutting edge capabilities at the DOE User facilities
- computational infrastructure to collaboratively link researchers with each other and with facilities

## *Iterative Hypothesis Generating Engine*



## *Converging on Solutions to DOE Science Challenges*





# Bioenergy Research Centers (BRCs)

## Scientific goals (summarized) for FY 2015

### Great Lake Bioenergy Research Center (GLBRC)

- Develop a functional metagenomics description of the rhizosphere for bioenergy crops
- Modify the cell wall structure in bioenergy crops for reduced recalcitrance and sugar yield
- Engineer efficient biosynthetic pathways for NextGen biofuels

### Joint BioEnergy Institute (JBEI)

- Engineer plant cell walls with optimized matrix polysaccharide composition
- Develop and optimize new biomass pretreatment techniques based on ionic liquids
- Continue discovery and engineering of novel hydrocarbon biochemistries and biosynthesis

### BioEnergy Science Center (BESC)

- Identify key biomass structural features that increase sugar release
- Generate modified and natural variant bioenergy crops improved for conversion into biofuels
- Establish thermophilic pathways for advanced biofuel production

### *To date the BRCs have generated :*

- **602 invention disclosures and/or patent applications**
- **19 patents awarded**
- **108 licensing agreements**
- **1661 peer-reviewed publications**



## *Field Trials Demonstrate Viability of Reduced Recalcitrance Switchgrass*



### **Objective:**

Evaluate the viability of a transgenic switchgrass (*Panicum virgatum* L.) in a field setting

### **Approach:**

- Low lignin (COMT down-regulated) switchgrass was analyzed in a 2-year field trial to evaluate the viability of this engineered bioenergy crop under agriculturally relevant conditions

### **Result/Impacts:**

- Fully established harvested COMT variants produced results similar to greenhouse studies and did not appear susceptible to rust (*Puccinia emaculata*).
- Harvested plants exhibited a 34% increase in sugar yield upon deconstruction and 28% increase in ethanol yield upon conversion, relative to control plants
- The results suggests real-world improvements in a biofuel yield can be obtained with an engineered bioenergy crop

Baxter et. al. (2014) "Two-year field analysis of reduced recalcitrance transgenic switchgrass" **Plant Biotechnology J.** 12(7) 914-924 (Sept).

### **Modified plants do well in the field**

- Stable phenotypes after 4 years





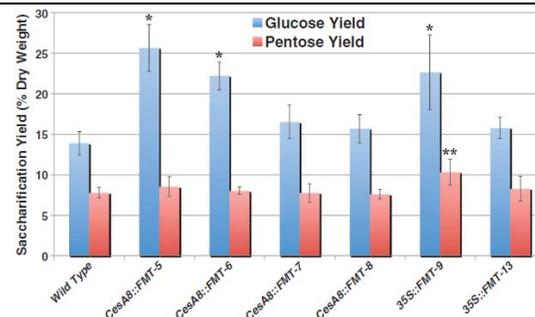
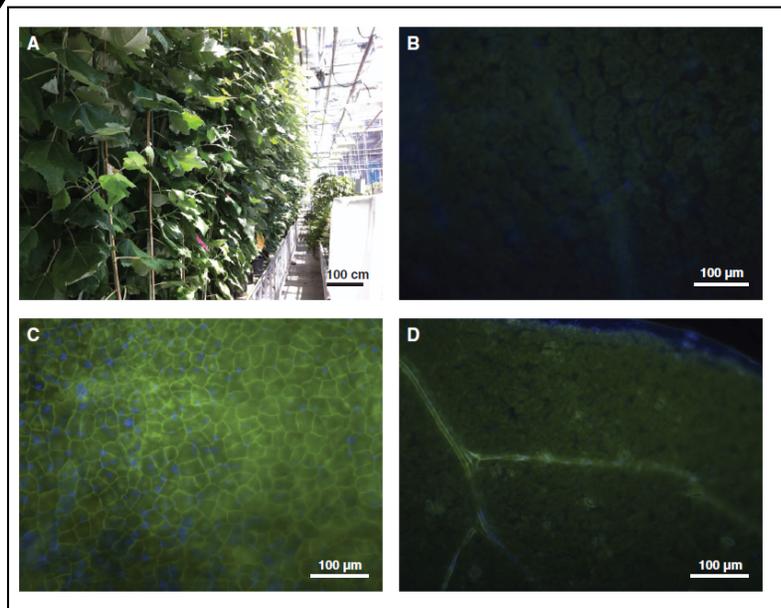
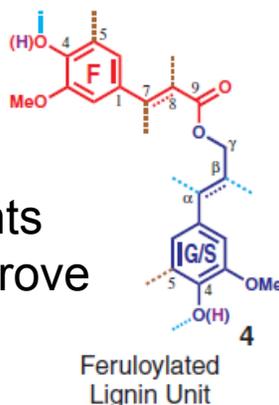
# Engineered Lignin in Poplar Improves Wood Degradability

## Objective

Engineer reduced recalcitrance into poplar cell walls

## Approach

- Design modified lignin components with labile bond structures to improve degradability
- Express enzymes *in planta* to synthesize cell walls with modified lignin components
- Demonstrate improved sugar yields in modified plants



## Results/Impact

- Engineered poplar strains grow indistinguishably from wild type
- Gene expression *in planta*, lignin component analysis and saccharification assays indicate demonstrate engineered reduced recalcitrance into poplar tissues

Wilkerson, et. al. (2014) "Monolignol Ferulate Transferase Introduces Chemically Labile Linkages into the Lignin Backbone" *Science* 4 April 2014: 90-93, doi 10.1126/science.1250161

# An Auto-Inducible Mechanism for Ionic Liquid Resistance in Microbial Biofuel Production

## Objective

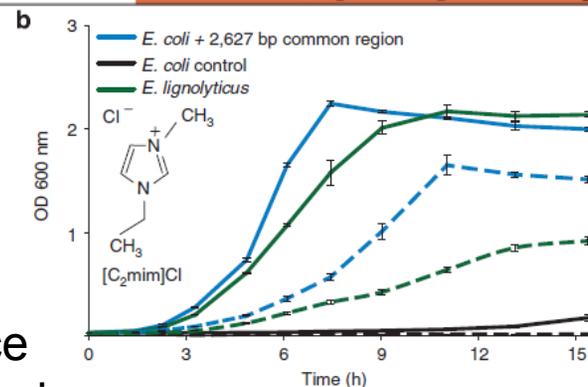
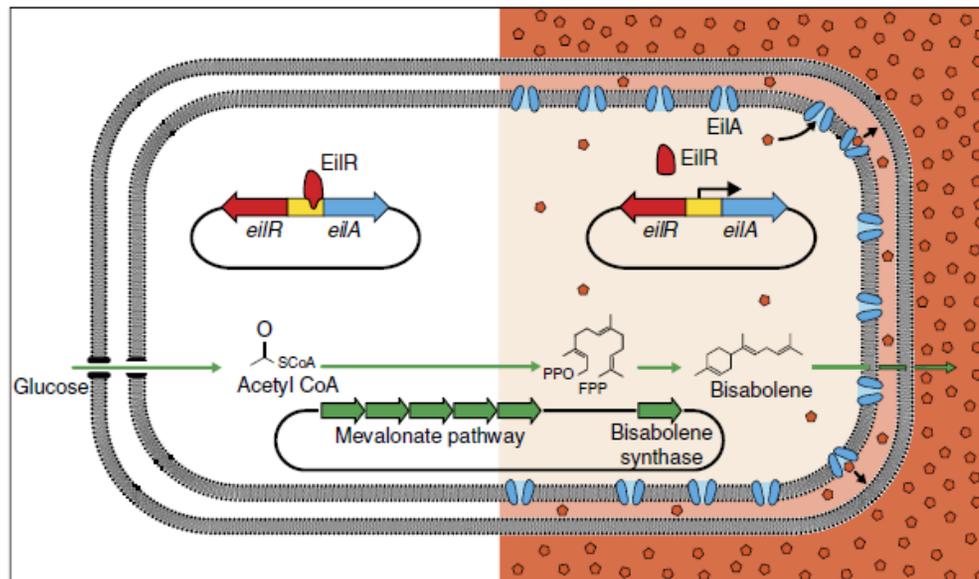
Engineer efflux pumps into fuel-synthesizing microbial hosts to increase tolerance to ionic liquids

## Approach

- Ionic liquid (IL) components can be toxic to fuel-synthesizing microorganisms
- Efflux pumps isolated from IL-tolerant *Enterobacter lignolyticus*, (rainforest isolate) show activity imidazolium-based ILs.

## Results/Impact

- Efflux pumps transferred into *E. coli* confer IL tolerance via dynamic feedback regulation of efflux gene expression
- Demonstrates a method to increase the tolerance of fuel-synthesizing microbes to biomass pretreatment conditions

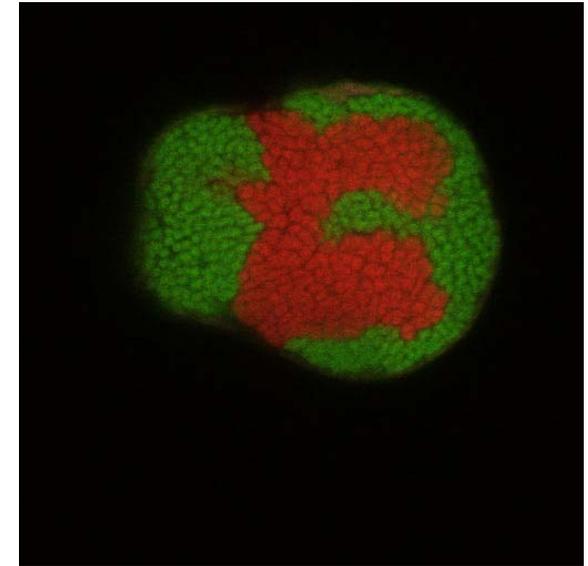


Ruegg, et. al. (2014) "An Auto-Inducible Mechanism for Ionic Liquid Resistance in Microbial Biofuel Production" *Nature Communications* 5:3490 doi: 10.1038/ncomms4490.

## ***Integration of Carbon, Sulfur, and Iron Cycling in Anaerobic Methane Oxidation***

### ***Objective:***

Examine the impacts of iron minerals on rate of anaerobic oxidation of methane (AOM) by consortia methane-oxidizing archaea and sulfate-reducing bacteria



### ***Approach:***

Track microbial community function and the fate of isotopically labeled methane ( $\text{CH}_4$ ) and sulfate ( $\text{SO}_4$ ) in sediment microcosms containing varying concentrations of the iron oxide hematite

### ***Results/Impact:***

- Hematite significantly decreases the rate  $\text{CH}_4$  release from microcosms and increased the rate of AOM.
- Isotope analysis revealed that iron oxides stimulated sulfur reduction and facilitated recycling of reduced sulfur compounds, which in turn accelerated AOM rates.
- A new biological linkage in the biogeochemical cycling of carbon, sulfur, and iron, with important implications in predicting the contribution of AOM processes to the global carbon cycle

Sivan et al. 2014. "Iron oxides stimulate sulfate-driven anaerobic methane oxidation in seeps" **PNAS** doi/10.1073/pnas.1412269111

## *Fungal and Plant Proteins Interact to Allow Beneficial Colonization in Populus*

### **Objective:**

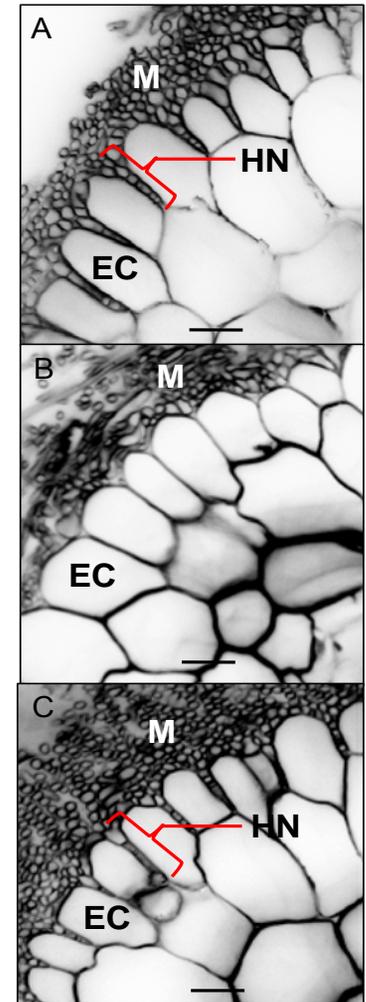
Investigate the mechanism(s) by which the *Laccaria bicolor*-encoded effector protein MiSSP7 enables establishment of fungal-plant symbiosis

### **Approach:**

Investigate the interactions between MiSSP7 and *P. trichocarpa*-encoded proteins in root cells during fungal colonization

### **Results/Impact:**

- MiSSP7 interacts with transcriptional repressor protein PtJAZ6 within *P. trichocarpa* nuclei, protecting PtJAZ6 from JA-induced degradation
- Repression of JA-induced gene transcription by MiSSP7 counters negative impacts of JA on fungal colonization of host tissues
- Mutualistic fungi, like pathogens, use effectors targeting host hormone pathways to promote fungal colonization, further delineating how beneficial and pathogenic microbes differ in the mechanisms by which they overcome plant defenses



Plett et al. 2014. "Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes". **PNAS** doi:10.1073/pnas.132267111

## ***Imaging Desert Biological Soil Crusts: PET Images of $^{11}\text{C}\text{CO}_2$ Uptake During Emergence from Dormancy***

### **Objective**

Detect microbial activity in desert crusts in response to wetting and drying as a method to measure  $\text{CO}_2$  flux in arid soils

### **Approach**

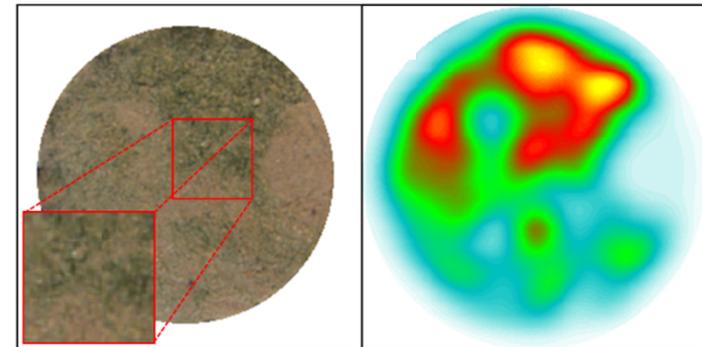
- PET imaging using  $^{11}\text{C}\text{-CO}_2$  to identify active sites of  $\text{CO}_2$  uptake in environmental samples.
- Identify sites for detailed mechanistic studies using a range of genomic and metagenomic analyses.

### **Results/Impacts:**

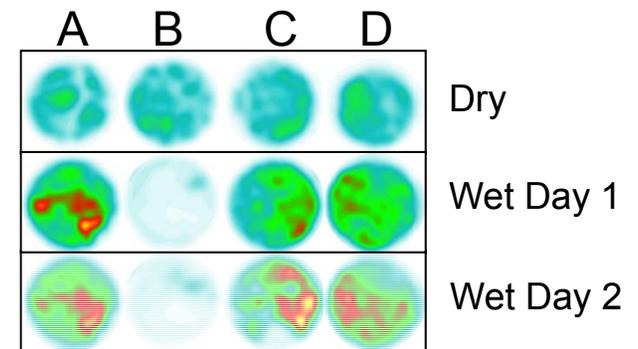
- New technique to investigate microbial activity and carbon dynamics in desert surface crusts and examine how crusts emerge from dormancy upon wetting.
- New methods to understand carbon flux in the environment.

Manuscript accepted for publication (Vandehey, et al. in Environmental Science and Technology Letters)

**Optical Image**      **PET Image**



***Intra-sample heterogeneity***



***Inter-sample heterogeneity***

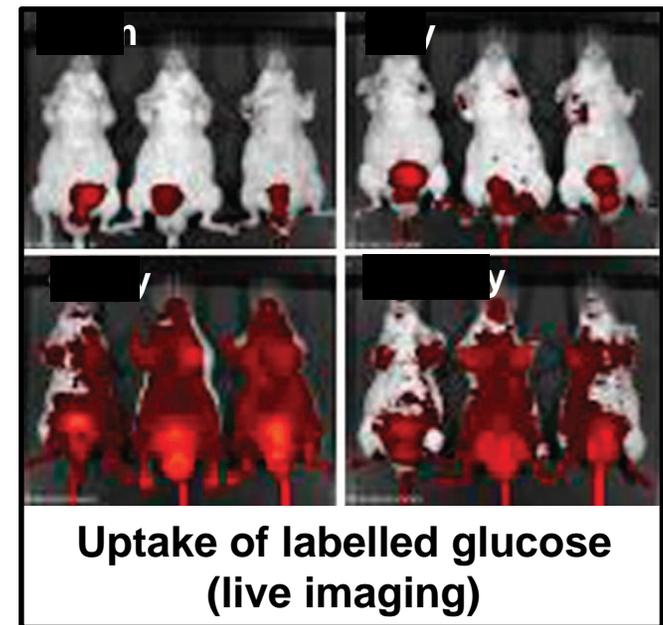
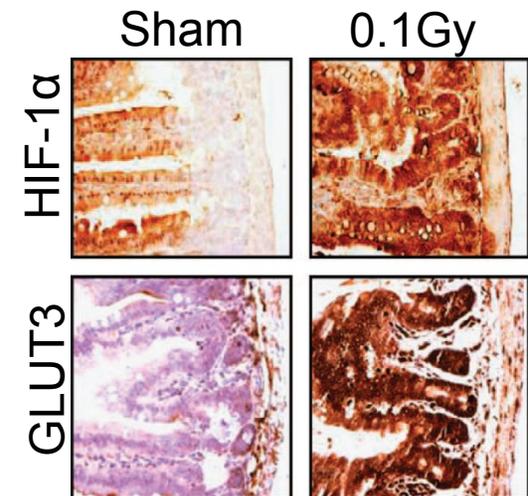
## ***Low-dose irradiation induces glucose flux and radiation resistance in vivo***

### **Objective**

Studies have shown the existence of adaptive dose–response relationships with low doses being protective and high doses causing detrimental effects. This study searches for a metabolic mechanism underlying the adaptive stress response.....

### **Results / Impact**

- Treatment of normal human cells with low-dose radiation induces a metabolic shift from oxidative phosphorylation to aerobic glycolysis, resulting in increased radiation resistance.
- Importantly, these findings are also observed systemically in mice.
- This metabolic change represents a previously unknown cellular response to low-dose radiation.



R Lall, *et al.*, Z-M Yuan, “Low-dose radiation exposure induces a HIF-1-mediated adaptive and protective metabolic response”, *Cell Death and Differentiation* 28 February 2014; doi:10.1038/cdd.2014.24

## *Celebrating 30 years of Macromolecular Crystallography at the National Synchrotron Light Source (NSLS)*

### **Objective:**

Enable access the unique capabilities of the NSLS, from 1984 to September 30, 2014.

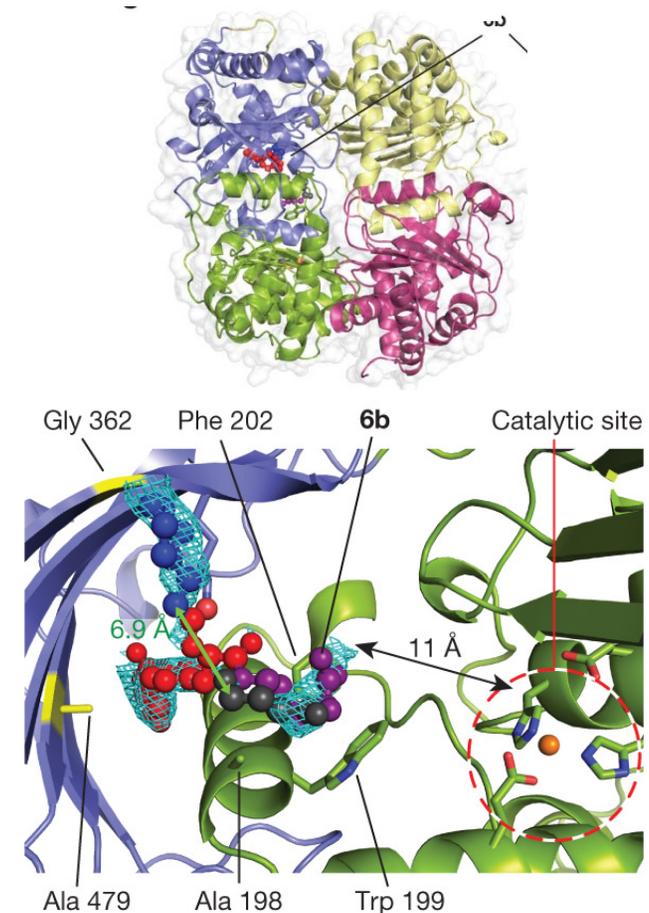
### **Approach:**

- BER and its predecessor, HER, have funded research into new technologies, design and development of beamlines, and support for a large user community for crystallography, as well as small-angle scattering and imaging, at the NSLS, the first second-generation light source in the U.S.
- New capabilities have been introduced in response to community needs, using advanced technologies developed at Brookhaven National Laboratory and other institutions.
- Jointly funded/managed effort with NIH.

### **Results/Impact:**

- The BER-funded crystallography stations have enabled nearly 5000 of the structures in the Protein Data Bank (and nearly 7300 have come from all NSLS stations)
- Forefront science (such as in this recent *Nature* paper from station X29) has been produced throughout the NSLS's 30 years.

J.P. Maianti, et al., "Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones", *Nature*, 511, 94-98 (2014). doi:10.1038/nature13297



*Above: Crystal structure of the Insulin Degrading Enzyme complex with macrocyclic inhibitor 6b  
Below: Close-up of the binding site*

# Structure of a crystal inside a living cell

## Objective:

To determine the structure of a protein as it exists in crystalline form in living cells; to demonstrate that an x-ray free-electron laser can perform such studies.

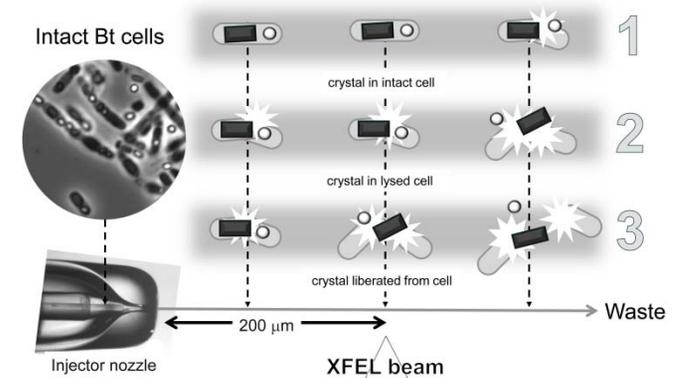
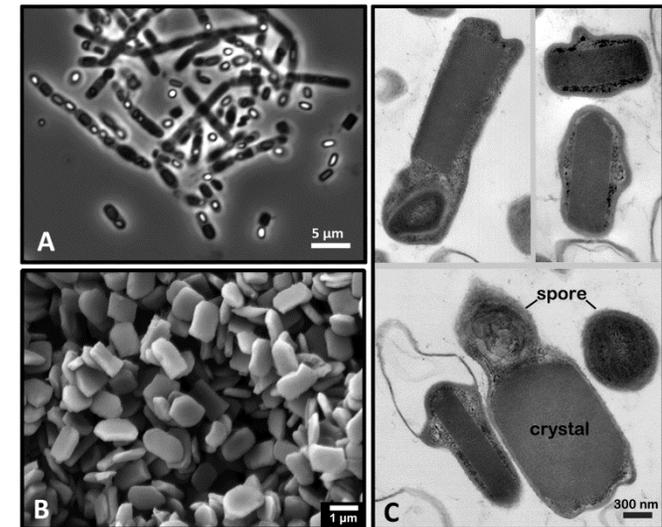
## Approach:

- Select a well-characterized microbial species that is known to have a protein stored within the cell in crystalline form: *Bacillus thuringiensis* (Bt), which stores its toxins in this way.
- Develop a way to introduce living Bt cells into the beam of the CXI instrument of the Linac Coherent Light Source, which can obtain diffraction data on extremely small crystals.
- Compare the in vivo structures obtained for the toxin Cry3A with those determined using conventional in-vitro crystals.

## Results/Impact:

A crystal structure with resolution to 2.9 Å was obtained from the experiments. It is consistent with the conventional structure of Cry3A. This demonstrates possibilities for studying crystals and other ordered structures in their native environments in living cells.

M.R. Sawaya, et al., "Protein crystal structure obtained at 2.9 Å resolution from injecting Bacterial cells into an X-ray free-electron laser beam", *Proceedings of the National Academy of Sciences (USA)*, 111, 12769-74 (2014). doi:10.1073/pnas.1413456111



Above: samples used for the studies;

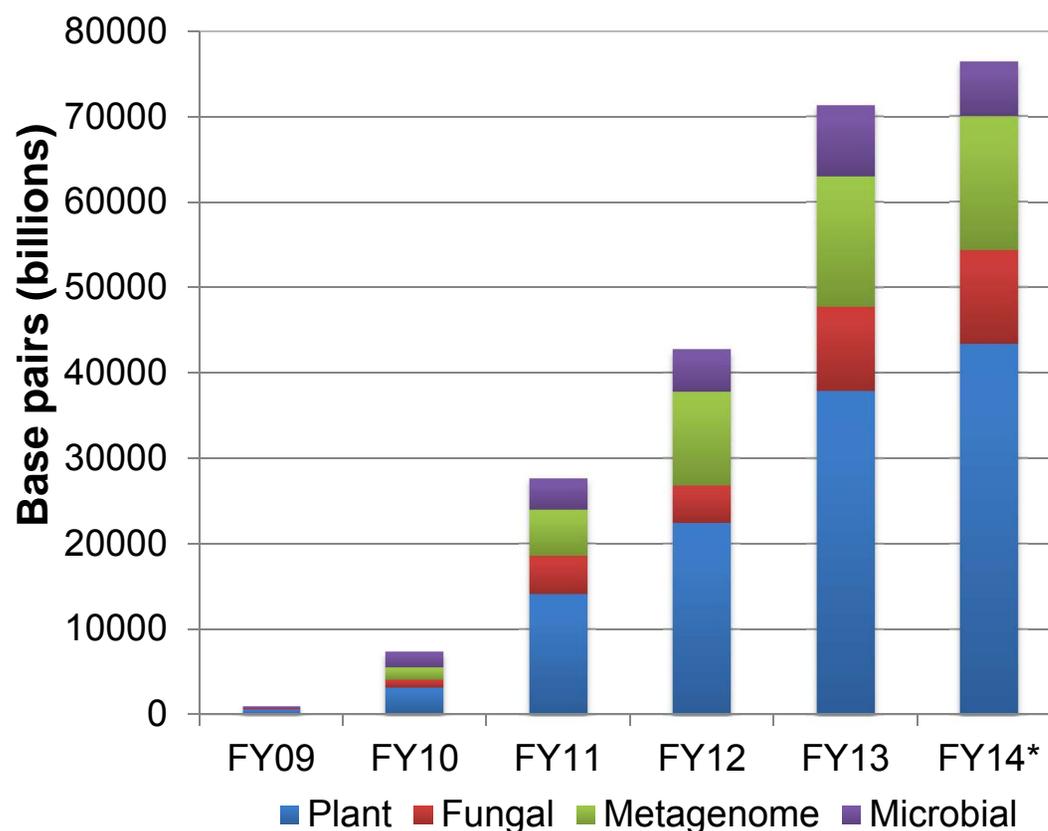
Below: Three ways in which the XFEL beam might interact with Bt cells



# DOE Joint Genome Institute

*Meeting the DNA sequencing needs of the bioenergy, carbon cycle, and subsurface science communities*

- Complex plant, rhizosphere and microbial community sequencing
- New strategies for interpreting complex genomes through new high-throughput functional assays, DNA synthesis and manipulation techniques and, genome analysis tools in association with KBase.
- Supporting biosystems design efforts for biofuels and environmental process research.
- Metagenome (genomes from multiple organisms) sequencing and analysis from environmental samples and single cell sequencing for hard-to-culture microorganisms from understudied environments relevant to DOE .
- Joint projects with EMSL



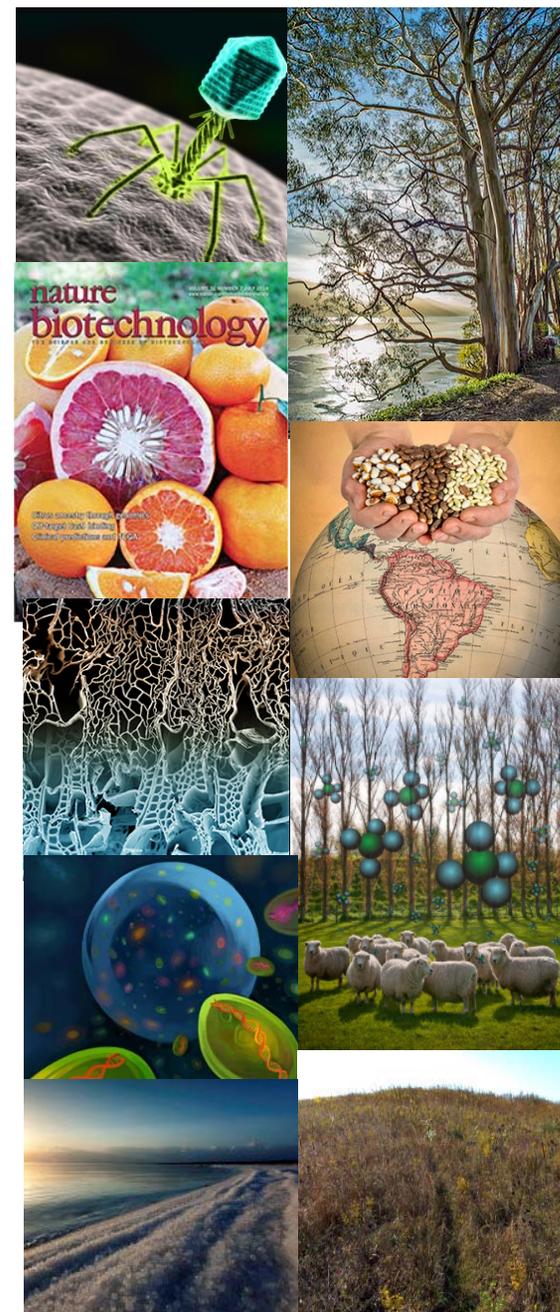
*\*through FY14-Q3*

## Selected Publications

- Ivanova, NN, et. al., (2014) [Stop codon reassignments in the wild](#) *Science*, 344: 909-913 doi:10.1126/science.1250691
- Myburg, AA, et. al., (2014) [The genome of \*Eucalyptus grandis\*](#) *Nature* 510:356-362 doi:10.1038/nature13308
- Wu, GA, et. al., (2014) [Sequencing of diverse mandarin, pummelo and orange genomes reveals complex history of admixture during citrus domestication](#) *Nature Biotechnology* 32:656–662 doi:10.1038/nbt.2906
- Schmutz, J., et. al., (2014) [A reference genome for common bean and genome-wide analysis of dual domestications](#) *Nature Genetics* 46: 707–713 doi:10.1038/ng.3008
- Riley, R., et. al., (2014) [Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi.](#), *Proc. Nat. Acad. Sci.* 111(27):9923-8. doi: 10.1073/pnas.1400592111.
- Shi, W., et. al., (2014) [Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome](#) *Genome Research* 24: 1517-1525 doi:10.1101/gr.168245.113
- Kis-Papo, T., et. al., (2014) [Genomic adaptations of the halophilic Dead Sea filamentous fungus \*Eurotium rubrum\*](#), *Nature Communications* 5:37-45 doi:10.1038/ncomms4745
- *Kashtan, N., et. al., (2014) [Single-Cell Genomics Reveals Hundreds of Coexisting Subpopulations in Wild \*Prochlorococcus\*](#), *Science* 344:416-420 DOI: 10.1126/science.1248575*
- Howe, AC., et. al., (2014) [Tackling soil diversity with the assembly of large, complex metagenomes](#) *Proc. Nat. Acad. Sci.* 111: 4904-4909 doi: 10.1073/pnas.1402564111

**67 publications since the last BERAC meeting**

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





# *Latest Opportunities*

## ***The Community Science Program 2015:***

- **Functional Genomics and Microbiomes of DOE JGI Flagship Plants**
- **Functional diversity of microbes**
- **Microbial emission and capture of greenhouse gases in terrestrial systems**
- **Discovery and expression of natural product pathways relevant to energy-related and environmental processes.**

***LOIs: 85***

***Full Proposals: 76***

***Selected Projects: 32***

## ***The JGI-EMSL Collaborative Science Initiative:***

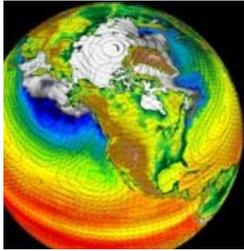
- **Biogeochemistry**
- **Carbon Cycling**
- **Biofuels Production**

***LOIs: 37***

***Full Proposals: 31***

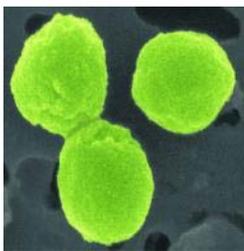
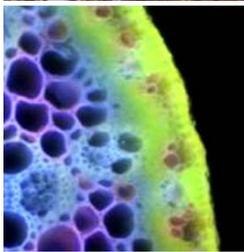
***Selected Projects: 12***

[http://www.jgi.doe.gov/CSP/user\\_guide/index.html](http://www.jgi.doe.gov/CSP/user_guide/index.html)



**Systems science to meet DOE mission needs in bioenergy, climate and the environment.**

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



**Thank you!**

<http://genomicscience.energy.gov>



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and Environmental Research

# Additional Science Highlights

## *Analysis of 33 Basidiomycete Fungi Blurs “White Rot” and “Brown Rot” Distinction*

### **Objective:**

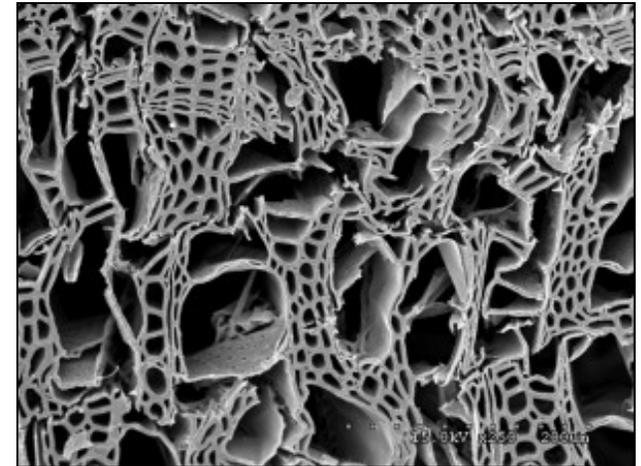
Characterize the diversity of fungal wood-degrading enzymes and pathways

### **Approach:**

DOE JGI researchers analyzed 33 basidiomycete fungal genomes, 22 of which are known wood decayers, four recently sequenced by the DOE JGI.

### **Results/impact:**

- The distinction between classic “white rot” fungi (able to break down cellulose, hemicellulose, and lignin) and “brown rot” fungi (able to break down cellulose, hemicellulose, but not lignin) is no longer clear-cut.
- Fungal genetics and biochemistry that are focused on wood degradation are more complex than previously thought, suggesting that additional options for exploring wood-degrading enzymes and pathways exist.



The white rot fungus  
*Botryobasidium botryosum*

Riley, R., et. al. (2014) Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi **PNAS** 111(27):9923-9928.

## *Measuring Nitrogen Fixation in Conifer Trees*

### **Objective:**

Endophytic N<sub>2</sub> fixation in the needles of pine trees has been hypothesized by UC Merced / LBNL researchers to be a potential source of the missing N budget in sub-alpine ecosystems.

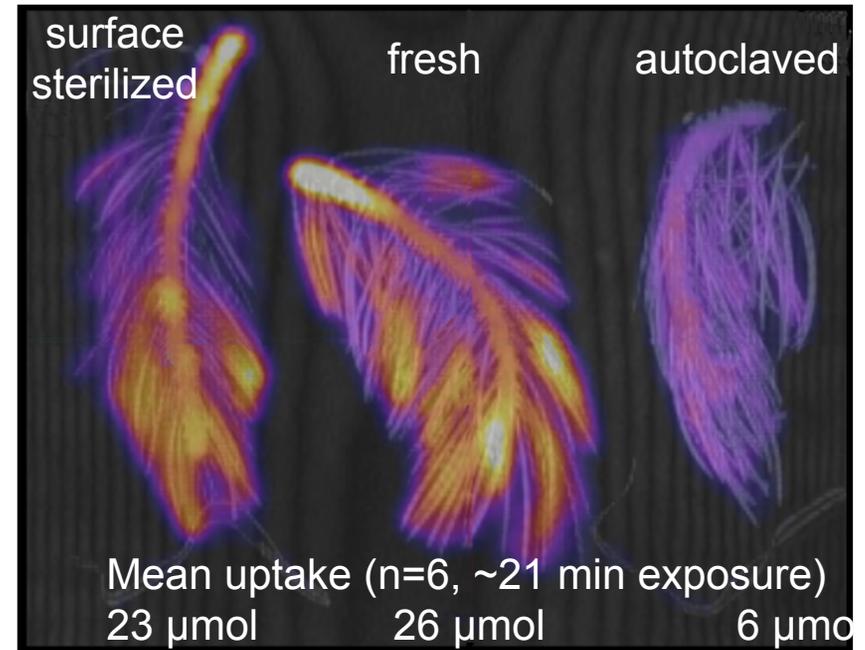
### **Approach:**

N<sub>2</sub> uptake was visualized and quantified in pine needles using <sup>13</sup>N<sub>2</sub> radiotracer imaging.

### **Results/Impact:**

- Significant <sup>13</sup>N<sub>2</sub> uptake by limber pine twigs was observed, supporting previous observations of endophytic diazotrophs in these conifer needles.
- This work reveals a previously unknown nitrogen source in sub-alpine systems important for understanding the biogeochemical cycling of N in the environment.

### **Image of Radiotracer (<sup>13</sup>N) Uptake in Limber Pine Twigs**



The ability to fix nitrogen was determined by exposing limber pine twigs to <sup>13</sup>N-labeled N<sub>2</sub> for ~21 minutes, flushing with unlabeled N<sub>2</sub>, then imaging the <sup>13</sup>N distribution. This figure shows rapid uptake of N<sub>2</sub> in the fresh twigs, with ~10% in the woody stems and 90% in the needles. Similar uptake in fresh and surface sterilized samples indicated that the uptake is not due to surface microbes. We see low uptake in autoclaved samples, indicating that the uptake is not due to diffusion into the twig (manuscript in preparation).

# Direct conversion of plant biomass to ethanol by engineered *Caldicellulosiruptor bescii*

## Objective:

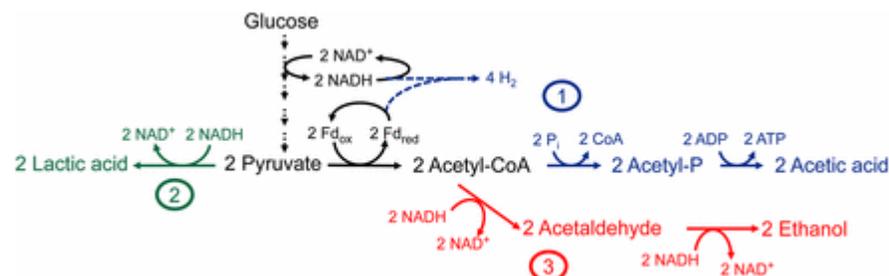
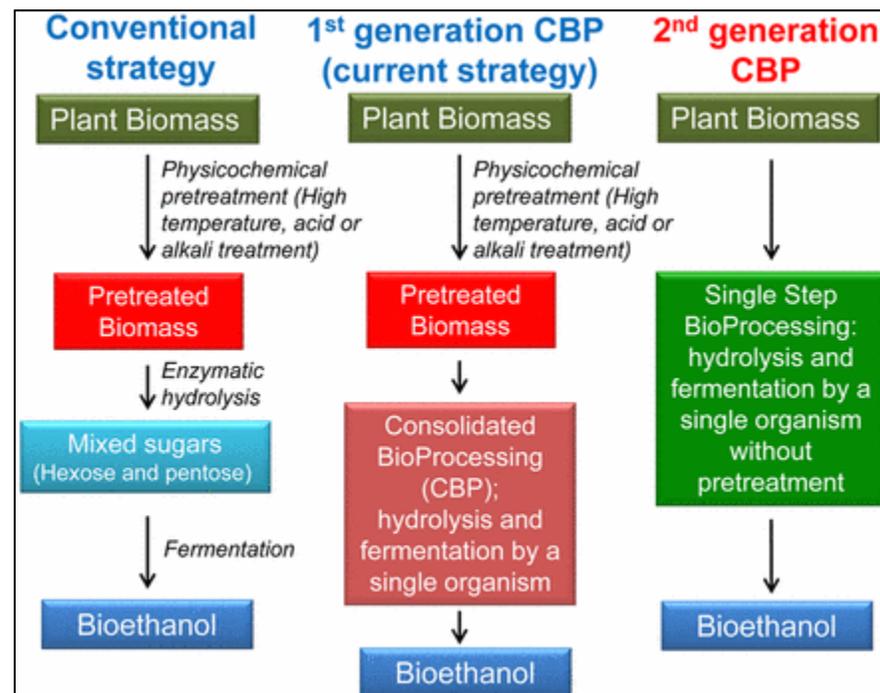
Combine cellulolytic activity and ethanol production in one bioprocessing step

## Approach:

- Use bioinformatics analysis and metabolic engineering techniques to modify cellulolytic *C. bescii* with ethanol-producing genes from *C. thermocellum*

## Result/Impacts:

- Modified *C. bescii* able to degrade unpretreated cellulose and ferment the resulting sugars to ethanol
- Approach demonstrates a 2<sup>nd</sup> Generation CBP process for bioethanol production



Chung et. al. (2014) "Direct conversion of plant biomass to ethanol by engineered *Caldicellulosiruptor bescii*"  
**PNAS** 111(24): 8931-8936 (Jun).

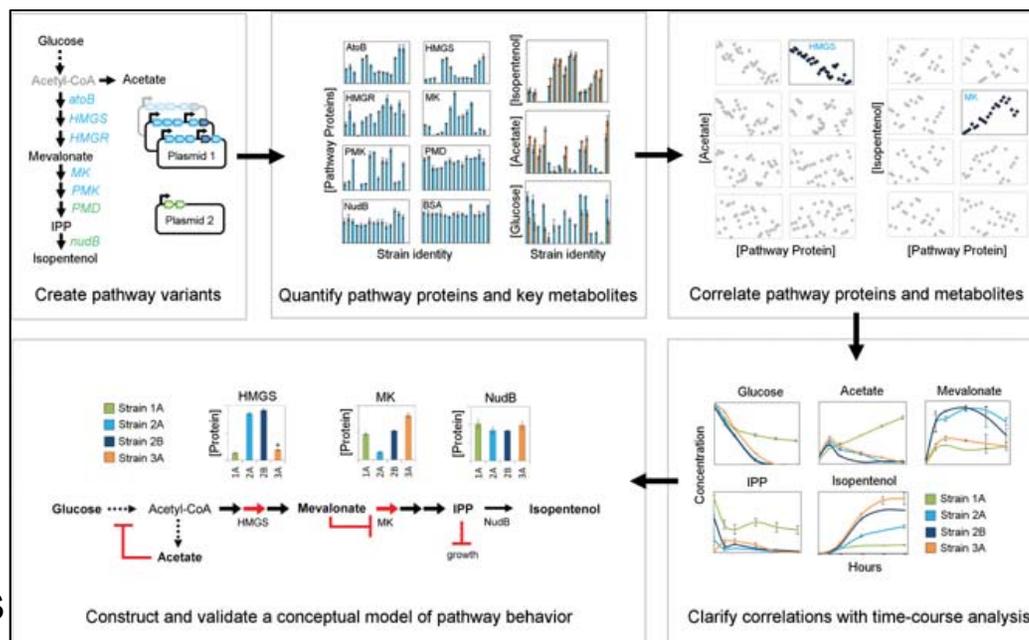
# Engineered approaches to isopentenol production in microbial hosts

## Objective

Optimize engineered isopentenol production in a microbial host

## Approach

- Isopentenol can be produced from the mevalonate pathway in *E. coli* but titers are low.
- Combine classic molecular biology with high-throughput proteomics/metabolomics analysis and computational methods to optimize isopentenol production



## Results/Impact

- Pathway optimization increased isopentenol titers 5-fold to 1.5g/L, 45% of theoretical
- The approach demonstrates a pipeline approach to pathway optimization for metabolic engineering in biofuels production in particular.

George, et. al. (2014), "Correlation analysis of targeted proteins and metabolites to assess and engineer microbial isopentenol production". *Biotechnol. Bioeng.*, 111: 1648–1658. doi: 10.1002/bit.25226