

Biological Systems Science Division Update

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U.S. DEPARTMENT OF
ENERGY

Office
of Science

Office of Biological
and Environmental Research

Update Outline

- Programmatic Actions – update
 - Reviews
 - Notices
 - Awards
- Significant activities
 - Workshop
 - KBase update
 - BRC status
- Science highlights

Programmatic Activities

Annual Bioenergy Research Center Reviews – Ongoing **(Reconfigured to online reviews)**

- BioEnergy Science Center (BESC) – Oct 23-24 – *completed last week*
- Great Lakes Bioenergy Research Center (GLBRC) – Nov 12-13
- Joint BioEnergy Institute (JBEI) – Dec 11-12

Upcoming Activities, Meetings and Reviews of DOE **National Laboratory programs**

- SBIR Notice – tied to Bioenergy Research Center (BRC) intellectual property
- Genomic Science Program PI Meeting (Feb 10-12, Hyatt Crystal City, VA)
- Biofuels Science Focus Area (SFA) review (immediately after PI meeting)
- Lawrence Berkeley National Laboratory (Foundational Genomics SFA)

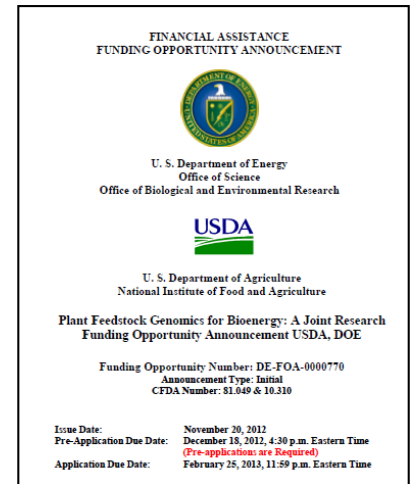
FY 2014 Notices

- Genomic Science Program
- Plant Feedstocks for Bioenergy

} **Difficult to project funding resources**

Completed Programmatic Activities Cont'd

Plant Feedstocks Genomics for Bioenergy: A Joint Research Funding Opportunity Announcement USDA, DOE (DE-FOA-0000770)



Seven New Awards

- *Functional Manipulation of Root Endophyte Populations for Feedstock Improvement*
- *Unraveling the Genetics of Two Key Biomass Traits that Differentiate Upland and Lowland Tetraploid Switchgrass Ecotypes, Colonization by Mycorrhizal Fungi and Frost Tolerance*
- *Genetic Control of Flowering in Switchgrass*
- *Pyramiding Genes and Alleles for Improving Energy Cane Biomass Yield*
- *Global Analysis of Epigenetic Regulation of Gene Expression in Response to Drought Stress in Sorghum*



- *Accelerated Development of Optimal Pine Feedstocks for Bioenergy and Renewable Chemicals Using Genome-Wide Selection*
- *Structural Polymorphisms as Causes of Heterosis in Populus*

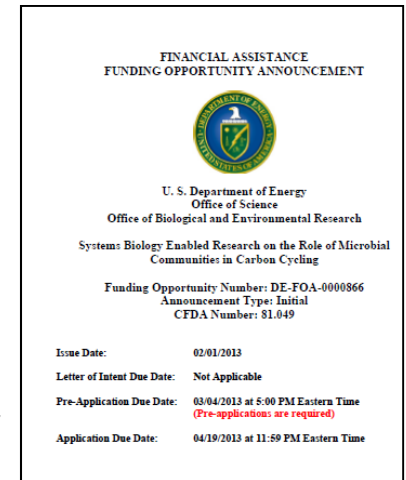
A joint DOE-USDA press release is in preparation

Completed Programmatic Activities Cont'd

Systems Biology Enabled Research on the Role of Microbial Communities in Carbon Cycling (DE-FOA-0000866)

Ten New Awards

- M. Firestone** (UC-Berk) *Mapping soil carbon from cradle to grave: drafting the molecular blueprint for C transformation from roots to stabilized soil organic C*
- V. Orphan** (Cal-Tech) *Systems level dissection of anaerobic methane cycling: quantitative measurements of single cell ecophysiology, genetic mechanisms, and microbial interactions*
- B. Hungate** (NArizSt) *Multiple element isotope probes, nano-SIMS, and the functional genomics of microbial carbon cycling in soils in response to chronic climatic change*
- J. Banfield** (UC-Berk) *Multi-'omic' analyses of the dynamics, mechanisms, and pathways for carbon turnover in grassland soil under two climate regimes*
- B. Saleska** (UAriz) *Pathways to liberation: a systems approach to understanding carbon transformations and losses from thawing permafrost regions.....*
- M. Lidstrom** (UWash) *Systems level insights into alternate methane cycling...*
- K. Hofmockel** (IowaSt) *Microbial drivers of global change at the aggregate scale: linking genomic function to carbon metabolism and warming*
- D. Buckley** (Cornell) *Cross-system analysis of carbon assimilation dynamics in soil microbial communities:...*
- J. Zhou** (U Okla) *Metagenomics-Enabled Predictive Understanding of Soil Microbial Communities*
- D. Zak** (UMich) *Atmospheric Nitrogen Deposition and Microbial Mechanisms Enhancing Soil Carbon Storage*



Several proposals in each Notice with linkages to KBase

Joint Call for Exploratory Collaborations

New awards in Carbon Cycling and Biofuels Research

H. Kistler, (USDA ARS) Organelles promoting high level terpenoid biosynthesis in filamentous fungi. {Biofuel Production}

P. Weyman, (JCVI) Functional genomics of moss-cyanobacteria interactions in boreal forest ecosystems {Carbon Cycling}

K. Hofmockel, (Iowa State) Development of novel approaches to target microbial drivers of C cycling in soil aggregates {Carbon Cycling}

M. Hess, (Washington State) FECB: A Functional Encyclopedia of Cyanobacteria - Building the knowledge framework for an enhanced understanding of carbon and nitrogen cycling {Carbon Cycling}

S. Harris, (University of Nebraska) Engineering morphology and secretion to enhance the productivity of fungal fermentations {Biofuel Production}

M. Firestone, (UC-Berkeley) Mapping soil carbon from cradle to grave: using comparative transcriptomics, proteomics and metabolite analysis to identify the microbial blueprint for root-enhanced decomposition of organic matter. {Carbon Cycling}

M. O'Malley, (UC-Santa Barbara) Identification and Regulation of Cellulases within Novel Anaerobic Gut Fungi {Biofuel Production}

C. Hansel, (WHOI) Genome-enabled Investigations of the Role of Secreted Proteins and Reactive Metabolites in Carbon Degradation by Pure and Mixed Ascomycete Fungal Communities {Carbon Cycling}

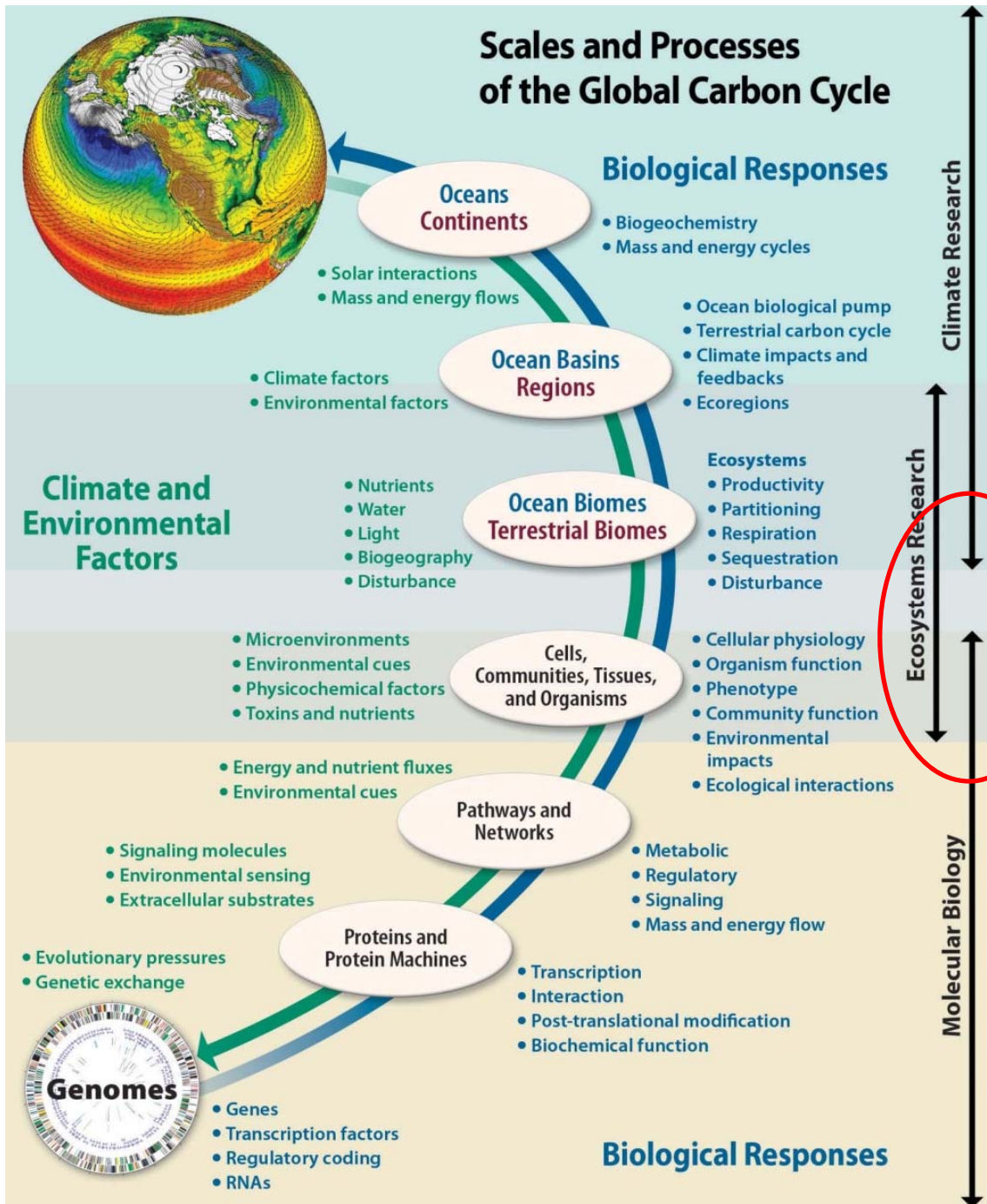


Slide from June 2013 BERAC Meeting

Resolving Biological Systems at Multiple Scales

Understanding & Model development facilitates science across larger scales relevant to BER Science

➤ Creates opportunities for interdivisional collaboration where programs meet.



Genome Sequence



U.S. DOE. 2008. U.S. Department of Energy, *Carbon Cycling and Biosequestration: Integrating Biology and Climate through Systems Science* Report from the March 2008 Workshop, DOE/SC-108

Research for Sustainable Bioenergy

DOE Germantown (A-410)

October 2-3, 2013

Co-Chairs

Dr. Phil Robertson (Michigan State University)

Dr. Michael Udvardi (Noble Foundation)

Dr. Jennifer Pett-Ridge (Lawrence Livermore National Laboratory)

To understand the influence of biotic, abiotic and genetic variables and combinations of variables on long-term plant feedstock performance and the delivery of ecosystem services.

Goals:

- To identify research gaps to better understand the interconnections between sustainable bioenergy feedstocks and ecosystem services such as climate mitigation, water and nutrient conservation, biodiversity amenities, and pest regulation.
- To identify paths for developing models that can accurately predict the impact of plants, microbes, and environmental attributes on the sustainability of feedstock production and ecosystem services at appropriate geographic scales.
- To identify novel ways to link genomes and ecosystems using the tools of systems biology, genomics, and ecosystem science.

Key additions to KBase's scientific functionality include:

- Metabolic and regulatory reconstructions for 5534 prokaryotic and 161 archaeal genomes
- Phylogenetic framework for analyzing annotated metagenomes with species trees for taxonomic abundance
- Genotype-to-Phenotype service that discovers genetic variations in plant populations and maps these to complex organismal traits

Data sets include: 925 data types

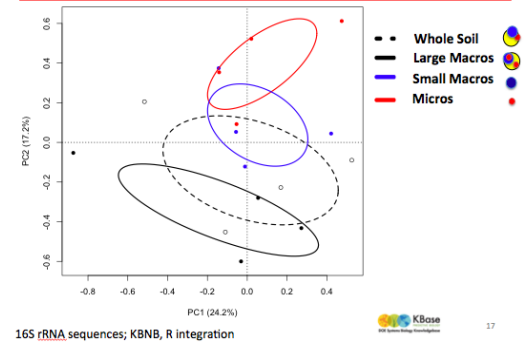
- 13085 genomes, 46,593,756 protein features
- 6202 growth curves, 521 media
- 3958 gene expression data sets, 4985 metabolic models
- 33,773 reactions, 63,479 protein-protein interactions
- 731 gene expression experiments in Arabidopsis and Poplar
- 11,000 metagenomes (>21 TBp)

Nearly 400 users registered on kbase.us

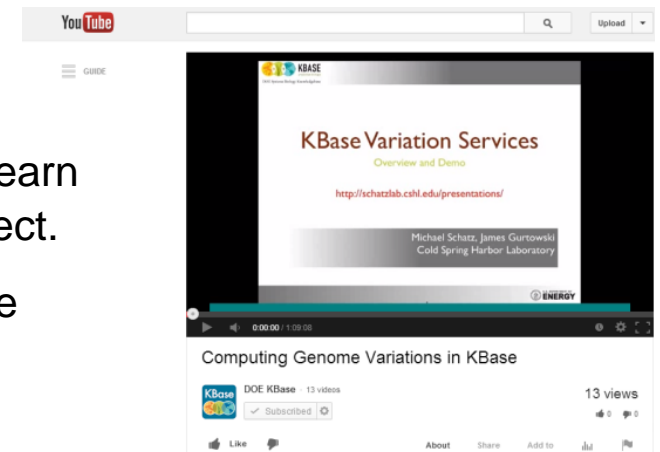
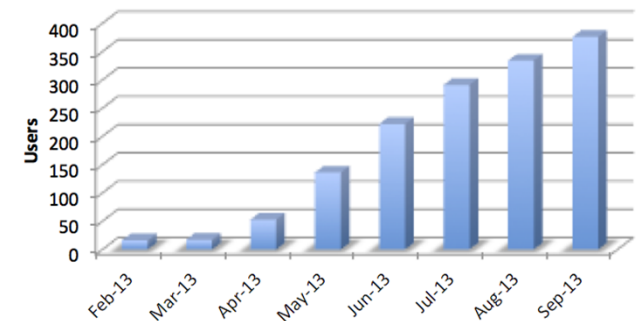
Featured webinar on [KBase YouTube Channel](#): Variation Service: Learn to identify and visualize variants in a next-gen re-sequencing project.

Outreach: In FY13 there were 67 KBase presentations, 9 KBase workshops including BRCs, and 8 KBase Developer Bootcamps.

Bacterial Community Composition



Growing User Base





The DOE Bioenergy Research Centers

FY 2014 Milestones

BioEnergy Science Center (Oak Ridge National Lab)

- Validate key genes in target consolidated bioprocessing microbes and enzymes and initiate modifications to achieve improved conversion and product synthesis
- Complete sampling of poplar and switchgrass common gardens
- Initiate testing of potential thermophilic microbes in biomass conversion

Great Lakes Bioenergy Research Center (University of Wisconsin, Michigan State University)

- Begin studies of cropping systems and land use scenarios on marginal lands
- Begin translation of fundamental research on grasses to dedicated bioenergy crops
- Increase emphasis on NextGen fuels
- Evaluate lignin valorization

Joint BioEnergy Institute (Lawrence Berkeley National Lab)

- Validate function of genes for biomass traits in model plants and in biofuels production processes
- Develop tools that enable enzyme cocktail optimization for biomass deconstruction
- Engineer central metabolism in target microbial hosts to facilitate fuels synthesis

New gene discovery clarifies lignin biosynthesis pathway

Objective:

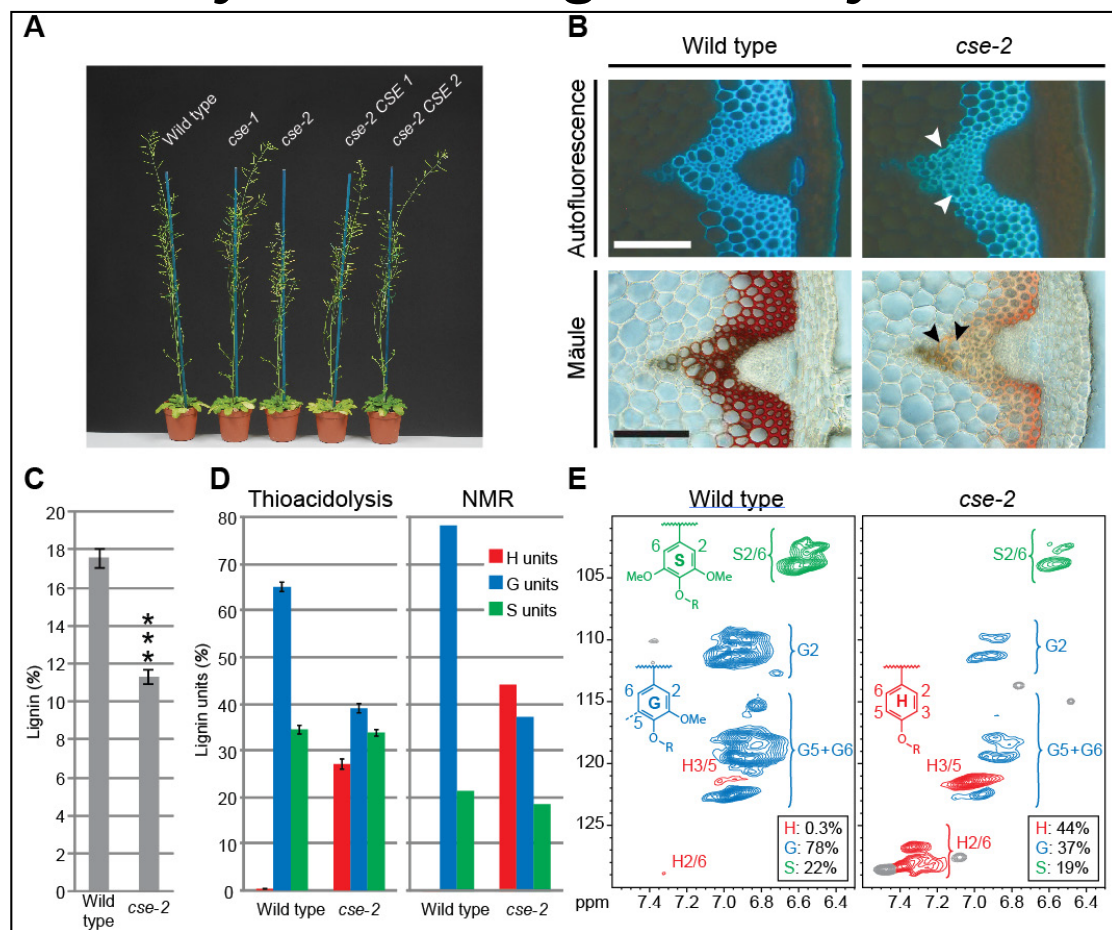
Identify the enzymatic steps in lignin biosynthesis and clarify the pathway(s).

Approach:

Use publically available gene expression databases for genes of unknown function that co-express with known lignin biosynthesis genes

Results/Impact:

- Identified caffeoyl shikimate esterase (CSE)
- Mutants in CSE had reduced lignin with altered component structure
- Knockout mutant had 78% saccharification compared to 18% in wild-type
- New methods to intelligently alter lignin composition



Vanholme, *et al.*, "Caffeoyl Shikimate Esterase (CSE) is an Enzyme in the Lignin Biosynthetic Pathway", **Science** (2013) 341,1103-6. doi: 10.1126/science.1241602

Pectin and Xylan Structures are Covalently Interconnected via Arabinogalactan Proteins

Objective:

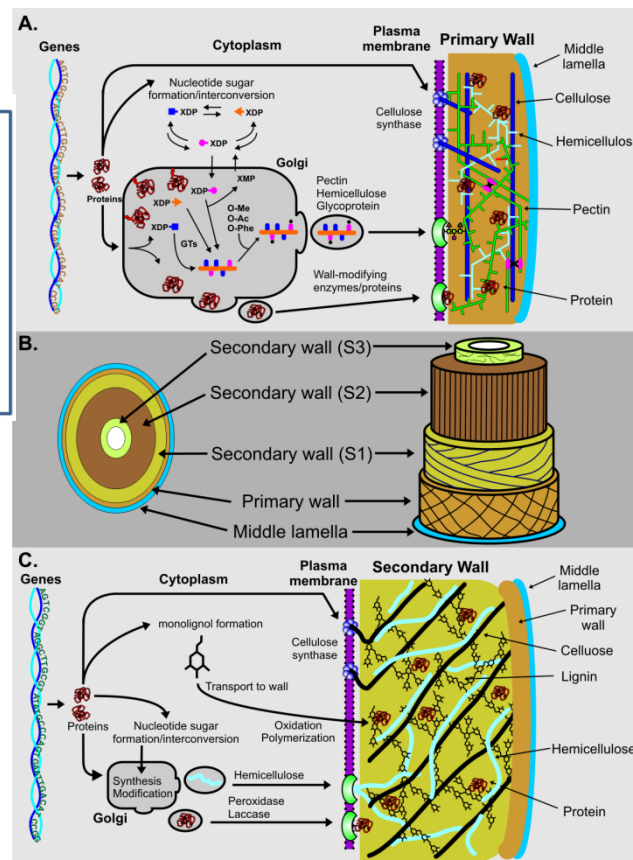
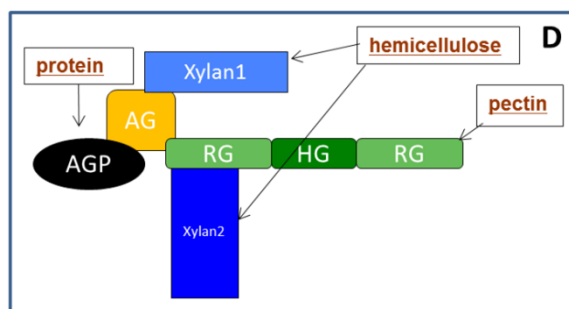
- Clarify the covalent linkages among pectin, xylans and cell wall proteins
- Current models suggest these structures are not covalently linked

Approach:

- Isolate and characterize APAP1 and its two glycoforms using proteomics, carbohydrate linkage and structural analysis

Results/Impact:

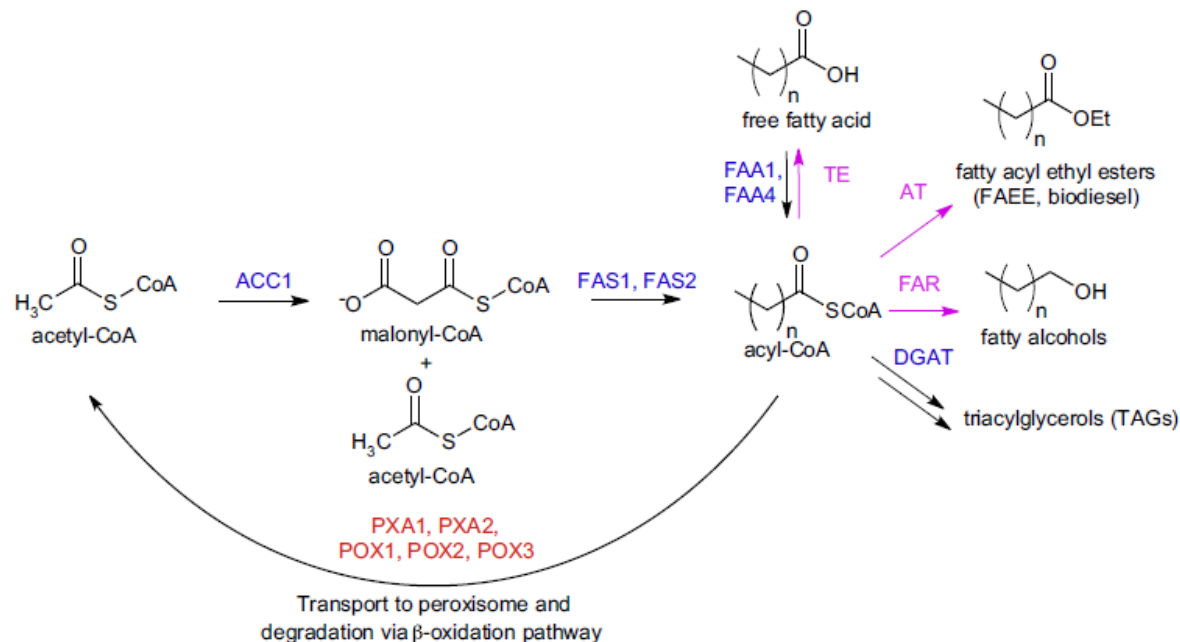
- The discovery of APAP1 provides evidence contrary to prevailing cell wall models that depict independent protein, pectin and hemicellulose polysaccharide networks
- Pectin, a primary cell wall component, has now been shown to be important in secondary cell wall structure, and thus recalcitrance
- The existence of APAP1 in plant walls has significant consequences for understanding of plant wall architecture and function, and potentially for engineering plant cell walls for improved agronomic and renewable biomaterial uses



Genetically Engineering Yeast to Produce Higher Yields of Advanced Biofuels

Objective:

- Genetically engineer yeast to produce advanced biofuels and overcome feedback inhibition of fatty acid production



Approach:

- Overexpress fatty acid production machinery by replacing native promoters with strong constitutive promoters
- Re-route fatty acyl-CoA away from triacylglycerols and breakdown products to form biofuel-like products with use of an appropriate tailoring enzyme (pink)

Results/Impact:

- Platform strain developed for fatty acid overproduction
- Tailoring provided highest recorded titers of advanced biofuels in yeast: 400 mg/L free fatty acids; 100 mg/L of fatty alcohols; 5 mg/L of fatty acid ethyl esters

Runguphan, W., Keasling, J.D., Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid-derived biofuels and chemicals. **Metabol. Eng.** (2013)

A method to fine-tune transcription and translation control in bacteria

Objective:

Accurately predict expression levels of multiple genes introduced in engineered bacteria.

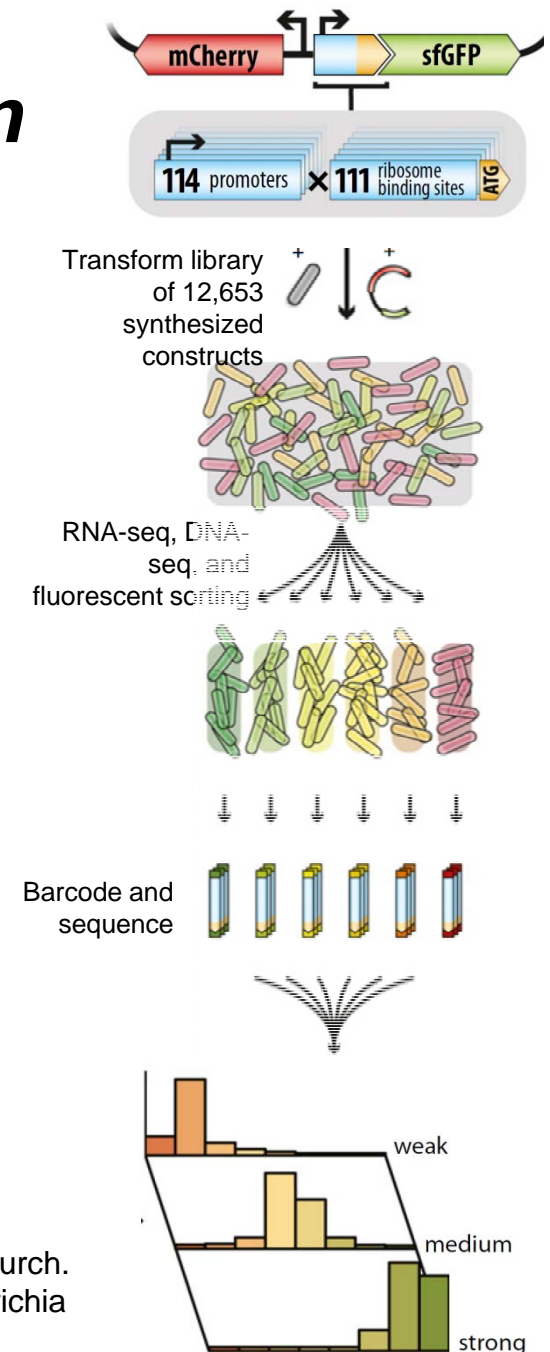
Approach:

Researchers synthesized thousands of combinations of promoters and ribosome binding sites and measured DNA, RNA, and protein levels using next-generation sequencing and fluorescent cell sorting.

Results/Impact:

Most transgenes behaved as predicted but a significant fraction did not. This facile method to measure gene expression controlled by thousands of regulatory sequences will allow precise genome engineering of bacteria for multiple purposes such as the efficient production of biofuels.

S. Kosuri, D. B. Goodman, G. Cambray, V. K. Mutalik, Y. Gao, A. P. Arkin, Drew Endy, G. M. Church. 2013. "Composability of regulatory sequences controlling transcription and translation in *Escherichia coli*" *Proceedings of the Natl. Acad. Sci.* 110: 14024–14029. doi/10.1073/pnas.1301301110



Systemic Acquired Resistance: Carbon-11 azelaic acid for mechanistic studies on plant immunity

Objective:

Use C-8, C-9 and C-10 dicarboxylic acids labeled with ^{11}C and ^{14}C to test the hypothesis that azelaic acid is mobile in plants.

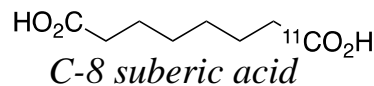
Approach:

$[^{11}\text{C}]$ azelaic acid was applied to a tobacco leaf to examine its short term movement (50 Min) and $[^{14}\text{C}]$ azelaic acid was applied to *Arabidopsis* for 24hr distribution

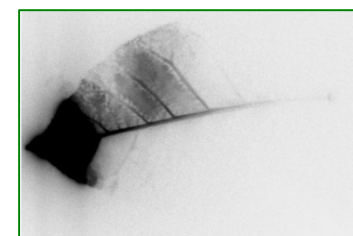
Results/Impact:

Understanding the chemical mechanisms mediating plant defense is important in developing robust plants for agriculture and biofuel production.

Carbon-11 Labeled Tracers



In vivo imaging with $[^{11}\text{C}]$ azelaic acid show movement in the leaf in 50 min



Imaging with $[^{14}\text{C}]$ azelaic acid shows movement through the whole plant in 24 hrs

Collaborative studies with Kachroo et al., (2013) using $[^{14}\text{C}]$ azelaic acid from BNL identified an intricate regulatory feedback loop regulating SAR.

Yu, et. al. (2013) A feedback regulatory loop between G3P and lipid transfer protein DIR1 and AZI1 mediates azelaic-acid-induced systemic immunity. *Cell Rep.* 3(4):1266-78. Best M, et. al. (2012) Rapid radiosynthesis of $[^{11}\text{C}]$ and $[^{14}\text{C}]$ azelaic, suberic, and sebaccic acids for in vivo mechanistic studies of systemic acquired resistance in plants, *Journal of Labelled Compounds and Radiopharmaceuticals* 55:39-43;

X-ray spectroscopy shows details of solution structure**Objective:**

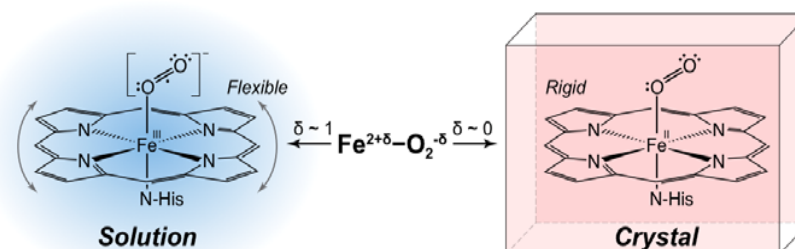
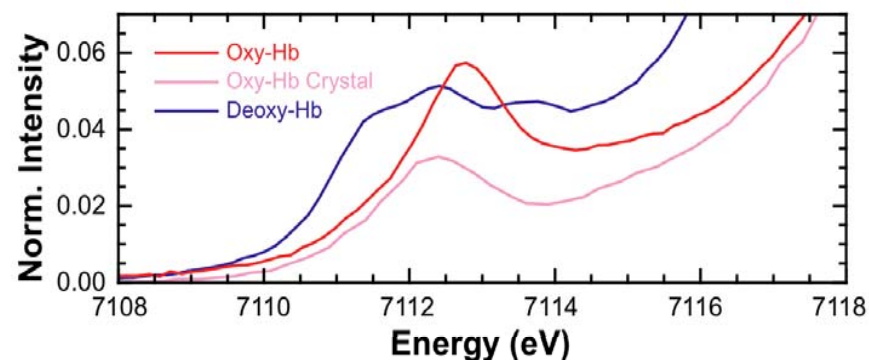
To identify critical differences between solution and crystal electronic structures of a metallo-protein that help explain its behavior in vivo.

Approach:

Use iron K-edge X-ray absorption spectroscopy (XAS) at beamline 9-3 at the Stanford Synchrotron Radiation Lightsource (SSRL) in combination with density function theory calculations to show that oxyhemoglobin is in a ferric-superoxide form in solution and a ferrous-dioxygen form in crystals.

Results/Impact:

Through this research, XAS is shown to provide valuable information for understanding the behavior of the active site of metalloproteins. Many systems being studied in BER's biological research involve such proteins and this new capability will help enable progress in areas from biosystem design to biogeochemistry to carbon cycling.



Top: The Fe pre-edge XAS for solution and crystalline oxyhemoglobin and deoxyhemoglobin.
Bottom: Schematic showing the electronic change in crystal and solution forms of oxyhemoglobin.

Collaboration of SSRL, Stanford University, Reed College and Laboratori Nazionali di Frascati: S.A. Wilson, *et al.*, (2013) "X-ray absorption spectroscopic investigation of the electronic structure differences in solution and crystalline oxyhemoglobin", **PNAS** (USA). DOI: 10.1073/pnas.1315734110

DOE-JGI: High resolution structures of two cyanobacterial carboxysome proteins starting from their synthesis

Objective:

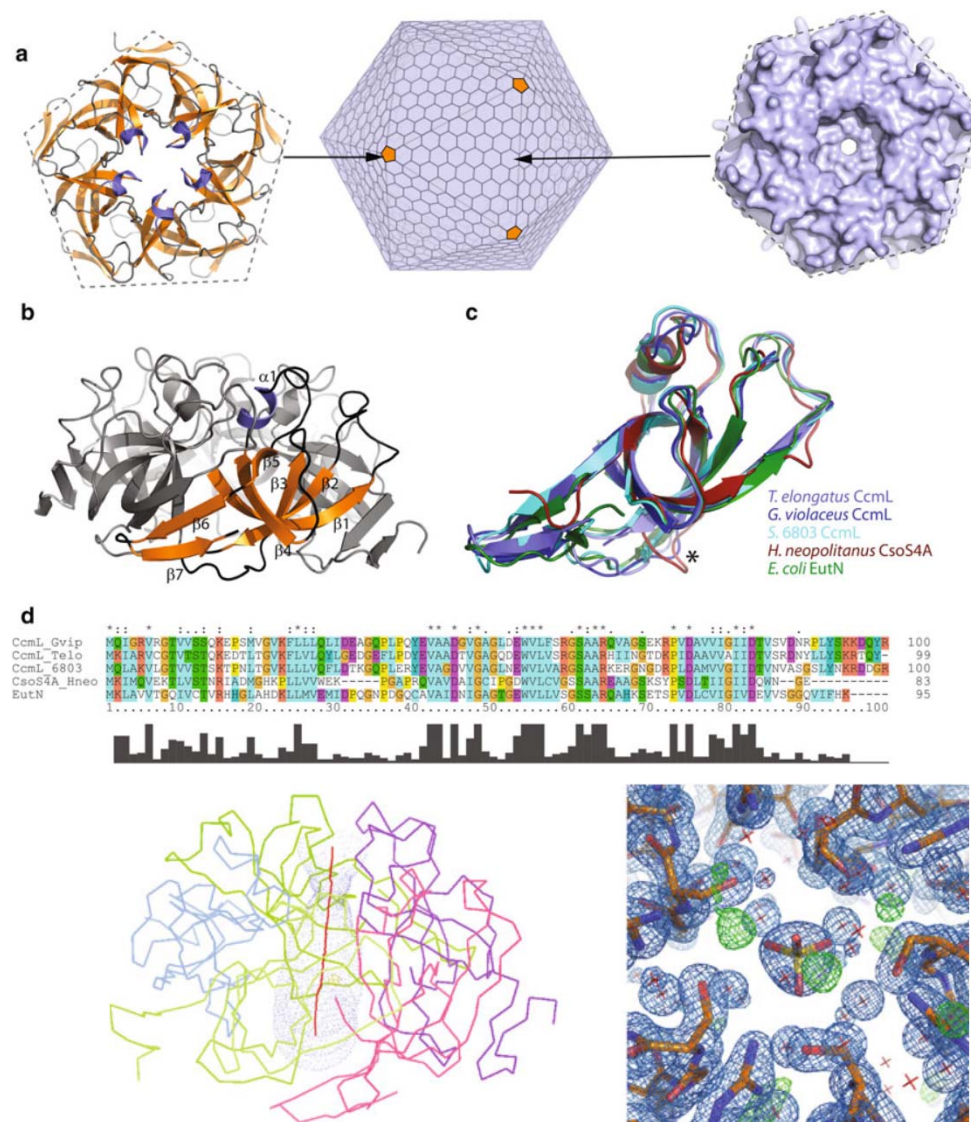
Characterization of carboxysome proteins from cyanobacteria for detailed structural and functional characterization.

Approach:

JGI researchers synthesized bacterial genes from *G. violaceus* and *T. elongatus* carboxysomes enabling the expression, crystallization and determination of crystal structures at Angstrom-scale resolution.

Results/Impact:

Demonstration of the use of gene synthesis for structural characterization not only of the native protein but also of deliberately “designed” alterations, vastly enhancing functional analyses.



Sutter, M., et.al.(2013) Two new high-resolution crystal structures of carboxysome pentamer proteins reveal high structural conservation of CcmL orthologs among distantly related cyanobacterial species. *Photosynthesis Res.* DOI 10.1007/s11120-013-9909-z



Selected JGI Publications

Environmental Metagenomics

- Bendall ML et al. [Exploring the roles of DNA methylation in the metal-reducing bacterium *Shewanella oneidensis* MR-1](#). *J Bacteriol.* 2013 Aug 30. [Epub ahead of print]
- Beyersmann P et al. [Genome sequence of *Phaeobacter caeruleus* type strain \(DSM 24564^T\), a surface-associated member of the marine *Roseobacter* clade](#). *Stand Genomic Sci.* 2013;8(3). doi:10.4056/sigs.3927623. Epub 2013 Jul 30.
- Binder M et al. [Phylogenetic and Phylogenomic overview of the Polyporales](#). *Mycologia.* 2013 Aug 11. [Epub ahead of print]
- Castelle CJ et al. [Extraordinary phylogenetic diversity and metabolic versatility in aquifer sediment](#). *Nat Commun.* 2013 Aug 27;4:2120. doi: 10.1038/ncomms3120.
- D'haeseleer P et al. [Proteogenomic analysis of a thermophilic bacterial consortium adapted to deconstruct switchgrass](#). *PLoS One.* 2013 Jul 19;8(7):e68465. doi: 10.1371/journal.pone.0068465. Print 2013 Jul 22
- Everroad RC et al. [Draft Genome Sequence of an Oscillatorian Cyanobacterium, Strain ESFC-1](#). *Genome Announc.* 2013 Aug 1;1(4). doi:pii: e00527-13. 10.1128/genomeA.00527-13.
- Haudry A et al. [An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions](#). *Nature.* 2013 Aug;45(8):891-8. doi: 10.1038/ng.2684. Epub 2013 Jun 30.

Asian Longhorn beetle



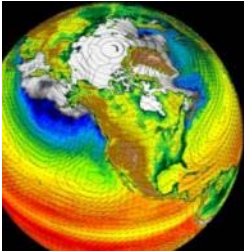
Physcomitrella



“Dark Matter” global sampling sites

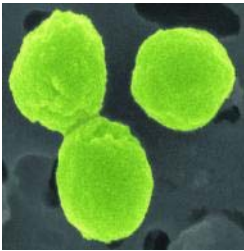
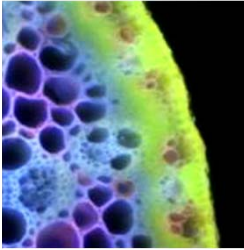
31 publications since the last BERAC meeting

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



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

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



Thank you!

<http://genomicscience.energy.gov>



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