



Office of Biological and Environmental Research Biological Systems Science Division Update

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October 13, 2022

Update on Programmatic Activities

Completed Reviews/Activities (since last BERAC

March

- ORNL Center for Structural Molecular Biology (CSMB)
- ORNL Biofuels SFA: Solvent Disruption of Biomass and Biomembranes

April

- LBNL Integrated Diffraction Analysis Technologies (IDAT)

May

- FOA - Biosystems Design virtual panels (plants, microbes)
- Bioenergy Research Center (BRCs) Renewal Review: In person
- FOA- Environmental Microbiome virtual panel

June

- FOA- Plant Gene Function virtual panel
- FOA- Quantum Bioimaging virtual panel
- NMDC review - virtual

July

- ORNL Plant-Microbe Interfaces (PMI) SFA: on site
- ANL Secure Biosystems Design Project Review: virtual
- LAB Bioimaging review panel: virtual

Aug

- LBNL m-CAFES SFA: virtual site visit

Upcoming Reviews/Activities

- 2023 Genomic Science PI Meeting April 17-19: Hybrid Meeting
- 2023 Bioimaging Characterization and Science PI Meeting April 17-19: Hybrid Meeting

New Biosystems Design Awards

Biosystems Design to Enable Safe Production of Next-Generation Biofuels, Bioproducts, and Biomaterials DE-FOA-0002600

7 new projects

Systems and synthetic biology research on microbial and plant systems for the production of biofuels, bioproducts, and biomaterials:

- Integrating cell-free systems and bacterial genome engineering for carbon-negative biomanufacturing.
- Engineering genome-wide, multi-gene CRISPRa/i programs for carbon-conserving bacterial bioproduction.
- Microbial community engineering for enhancing plastic waste degradation and synthesis of polymer precursors.
- Systems engineering of a green microalgae to convert atmospheric carbon dioxide into renewable fuels and bioproducts.
- Multi-pronged, integrated engineering of whole-plant water use efficiency in sorghum and the model plant *Setaria*.
- Lipid metabolic engineering in oilseed bioenergy crops for the production of chemicals, diesel, and aviation fuels.
- Precision genome and epigenome engineering of poplar for optimized plant morphology and to synthesize chemicals, fuels, and materials.



<https://www.energy.gov/articles/doe-announces-178-million-advance-bioenergy-technology>

<https://science.osti.gov/-/media/ber/pdf/Awards/Renewable-Bioenergy-and-Biomaterials-Production-FOA-2600-Award-List-2022.pdf>

New Environmental Microbiome Awards

Systems Biology Enabled Microbiome Research to Facilitate Predictions of Interactions and Behavior in the Environment (DE-FOA-0002602)

10 new projects

Projects that enhance understanding microbial interactions dynamically and predictively and facilitate realistic recapitulations of microbial ecosystems :

- Cross-kingdom interaction in response to global change, fire and drought
- Microbial interactions and predation in the context of experimental warming
- The role of volatile compounds in controlling soil microbiomes
- Climate feedbacks in methane cycling in arctic and temperate wetland environments
- The role of viruses in terrestrial carbon cycling
- Fungal-Bacterial Interactions and their impact on regulating carbon and nitrogen cycles
- The role of microbial networks in wetland carbon sequestration and the impact of the enzyme latch
- Necromass and its role in soil organic matter formation



New Plant Biology Awards

Genomics-Enabled Plant Biology for Determination of Gene Function (DE-FOA-0002601)

11 new projects

New and integrative approaches to overcome barriers in associating gene(s) to function (i.e., genotype to phenotype) in BER-relevant plant systems:

- TORC regulation and signaling networks involved in *Populus* nitrogen response
- Characterization of *Populus* ubiquitin-proteasome regulation for predictive modeling of wood formation and biomass yield
- Linking molecular control of the *Populus* winter latent state to woody biomass productivity
- Gene regulation and phenotypic plasticity in the sorghum drought response
- Genes regulating photosynthesis across the sorghum canopy for boosting biomass yields
- Integrated approach to characterize known and newly-identified nitrogen-responsive genes in sorghum
- Single-cell transcriptomics to characterize genes involved in cellular composition of biomass in *Populus* and sorghum
- Comparative genomics/AI approaches to define function of stress tolerance genes across Brassicaceae
- Identification of key genes/genomic regions in Camelina for more resilient, higher-yielding cultivars
- Biochemical/computational investigations of duckweed carbohydrate metabolic processes and glycosyltransferase function
- Experimentally validated “toolbox” of BAHD acyltransferase and acyl-CoA ligase genes of bioenergy crop plants



New Quantum Imaging and Sensing for Bioimaging Awards

Fundamental research or use-inspired technologies that exploit quantum phenomena ([DE-FOA-0002603](#))

10 New Projects

Instruments

- Entangled Raman spectroscopy on a Chip
- X-ray quantum entangled from XFEL free electron laser
- High-throughput single photon light-sheet fluorescence lifetime microscopy for 3D functional imaging
- 3 platforms IR, two-photon interference, time-resolved spectroscopy

Detectors

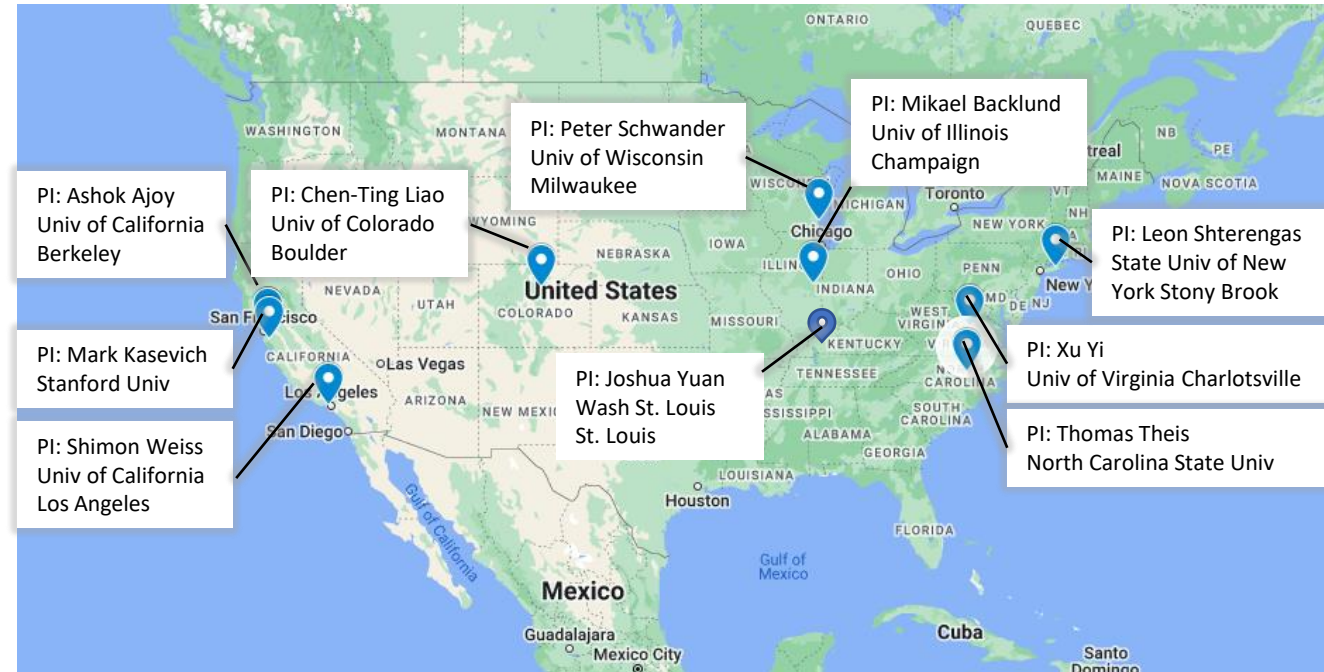
- Mid IR single photon avalanche detector (SPAD)
- Visible light SPAD detectors

Light Sources

- High-dimensional entangled anti-correlated light-pulses (HEALs) quantum-equivalent HALPs illumination

Sensors

- Voltage-sensing quantum probes lipid-coated inorganic quantum dots and lifetime-based voltage fluor. dyes
- Quantum-entangled hyperpolarized spin states, nuclear spin order in 11 parahydrogen



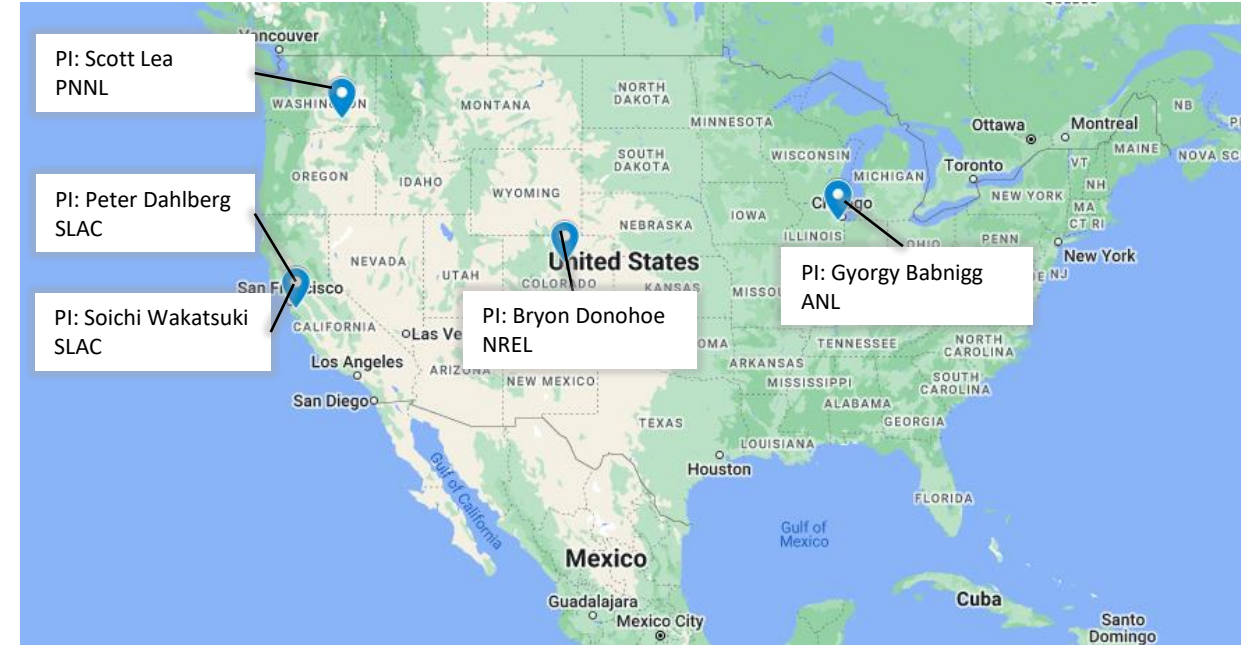
New Biomaging Projects at the DOE National Laboratories

Bioimaging Research and Approaches for Bioenergy and the Environment

Objectives

- 1) Evaluate novel, untested concepts, and theoretical models
- 2) Develop innovative prototypes of instruments and methods that include biological validation.

- Visualizing Spatial and Temporal Responses of Plant Cells to the Environment
- Developing a high-throughput functional bioimaging capability for rhizosphere interactions utilizing sensor cells, microfluidics, automation, and AI-guided analyses
- Optical and X-ray multimodal-hybrid microscope systems for live imaging of plant stress response and microbial interactions
- Novel Multimodal Chemical Nano-Imaging Approach to Visualize and Identify Small Biomolecules Exchanged in Microbial Communities
- Next-generation stimulated Raman scattering (SRS) microscopy to overcome photodamage and resolution limitations for real-time tracking of lipid/carbohydrate interfaces in plants, algae, and fungi



NEW Developing A National Virtual Biosecurity For Bioenergy Crops Center (NVBBCC)

BER-BRAVE Pilot

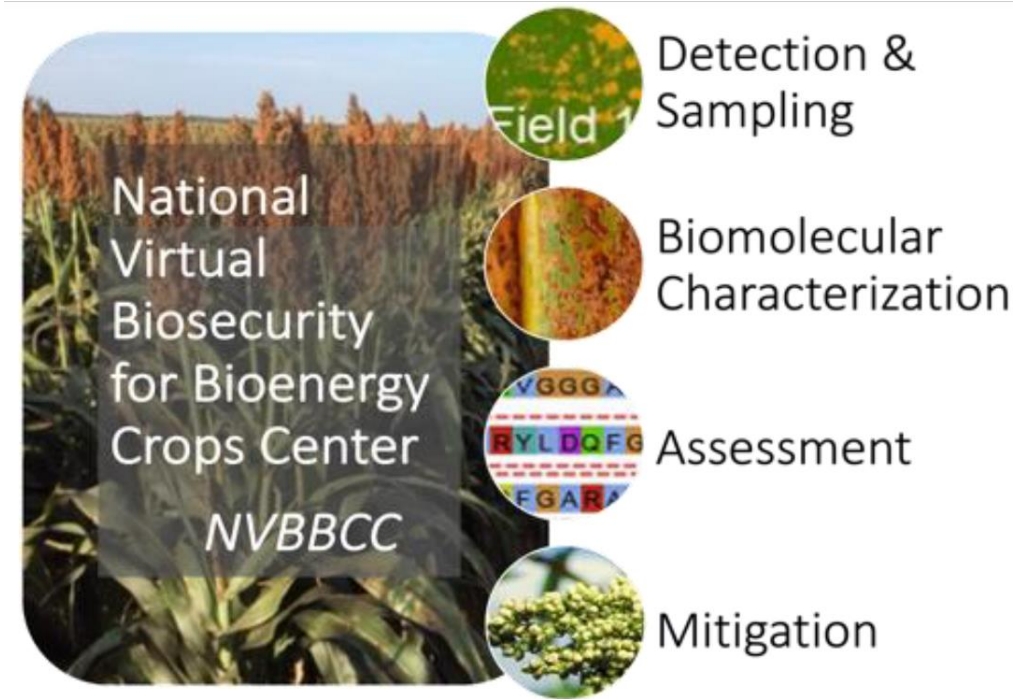


Develop a platform that supports biosecurity research of bioenergy crops but could be rapidly pivoted to a wide range of biosecurity challenges as was demonstrated by DOE's contributions to the National Virtual Biotechnology Laboratory (NVBL).

- A focus on bioenergy sorghum as a template crop

Development of:

- Detection and sampling capabilities (i.e. remote sensing techniques)
 - Connection with CABBI
- Biomolecular Characterization capabilities
- Assessment capabilities
 - Connection with ARM capabilities
- Mitigation capabilities



BNL will initiate workshops to make connections within the complex and other BRaVE efforts

Artificial Intelligence/Machine Learning (AI/ML) for BioEnergy Research (AMBER)

August 23-25, 2022 (Joint with EERE-BETO)

What are the possibilities for incorporating AI/ML techniques into Biological Research?

- What kinds of AI/ML techniques are most appropriate to genome-based basic and applied science
- What kinds of biological science/biotechnology questions could be uniquely addressed with these techniques

How could AI/ML techniques be more integral with experimentation and automation in the Laboratory?

- What would you do with an AI/ML guided, automated laboratory?
- How would that be configured for microbial and plant research?
- What science could you pursue that you can't do now?

Draft Report – Dec 2022

Genomes to Structure and Function Workshop Report



Final Draft in Review

eBERlight – an APS user program for BER community

Planned for FY24, leveraging the upgraded ANL's Advanced Photon Source

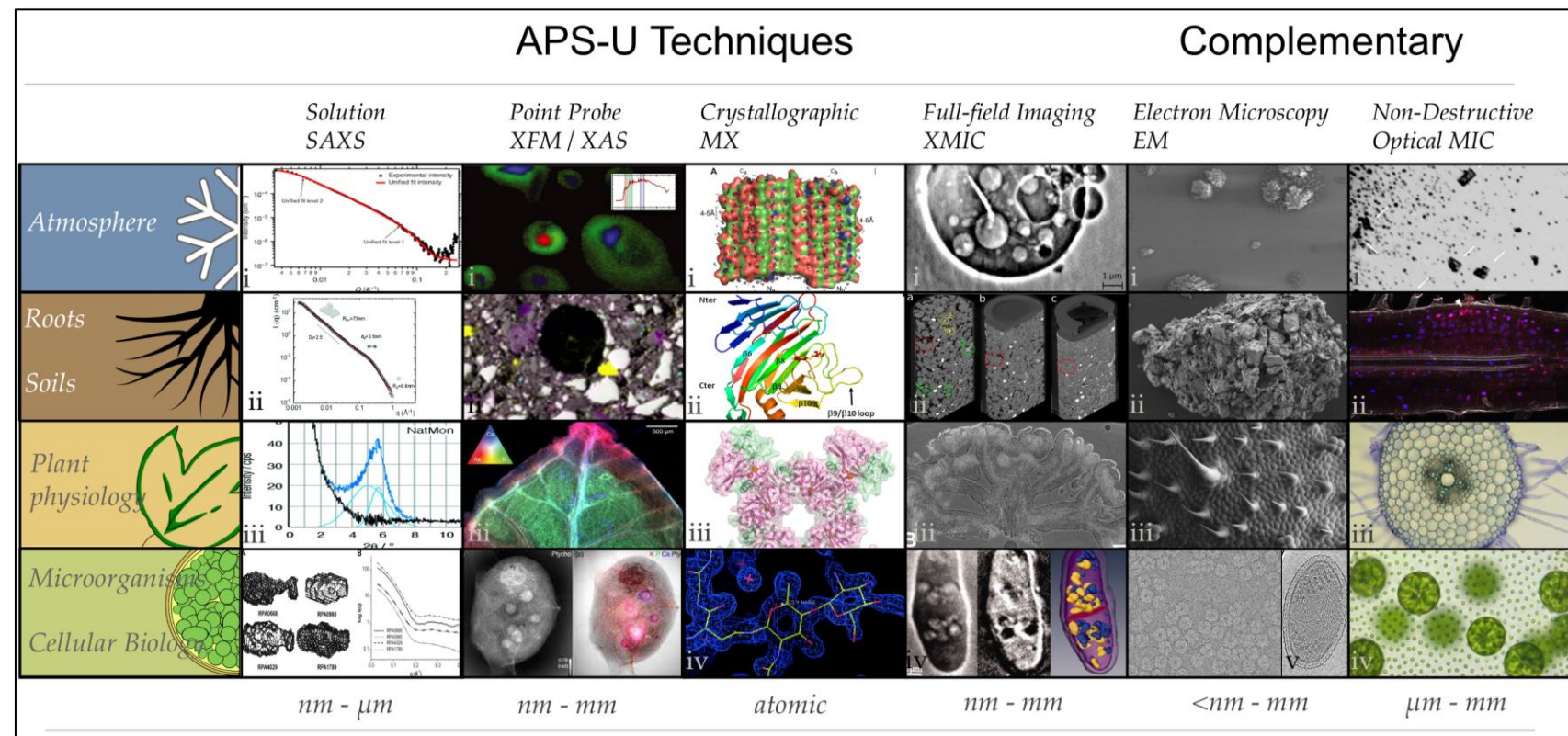


- A virtual center with single point of access for BER researchers supported by experts
- Will offer crystallography, imaging and spectroscopy, through dedicated beamtime at several beamlines

eBERlight

- A program offering multiple x-ray techniques geared for supporting BER-funded science.
- Serve as a liaison between BER researchers and the APS.
- Leverage additional ANL resources.
- Integrate with other DOE/BER facilities.

Preliminary collaborative pilot projects include:
Permafrost visualization, Soil aggregates, Grass imaging, Wood decomposition, Aerosol particles



National Microbiome Data Collaborative (NMDC)

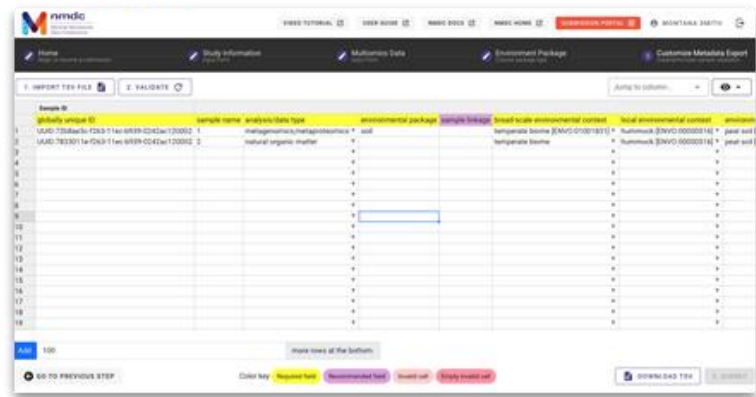
Mission: To support a FAIR microbiome data sharing network, through **infrastructure, data standards, and community building**, that addresses pressing challenges in environmental sciences

Transitioning from Pilot to Production
DOE Review: June 29 & 30, 2022



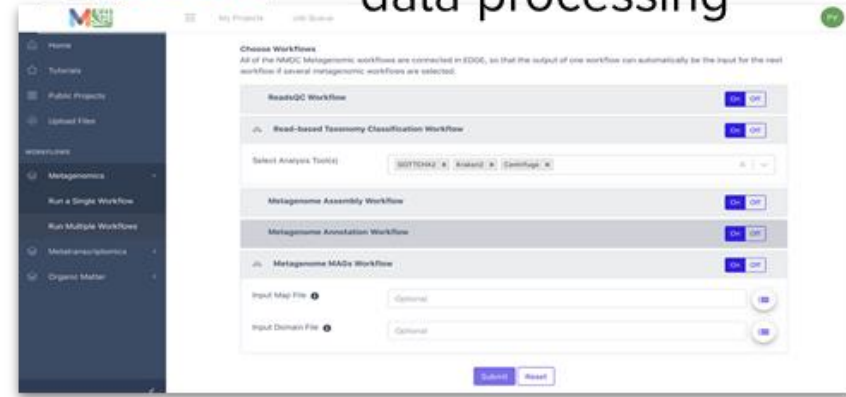
NMDC Submission Portal

Lower barriers to collect study and biosample data



NMDC EDGE

Streamline multi-omics data processing



NMDC Data Portal

Access and discovery of multi-omics data

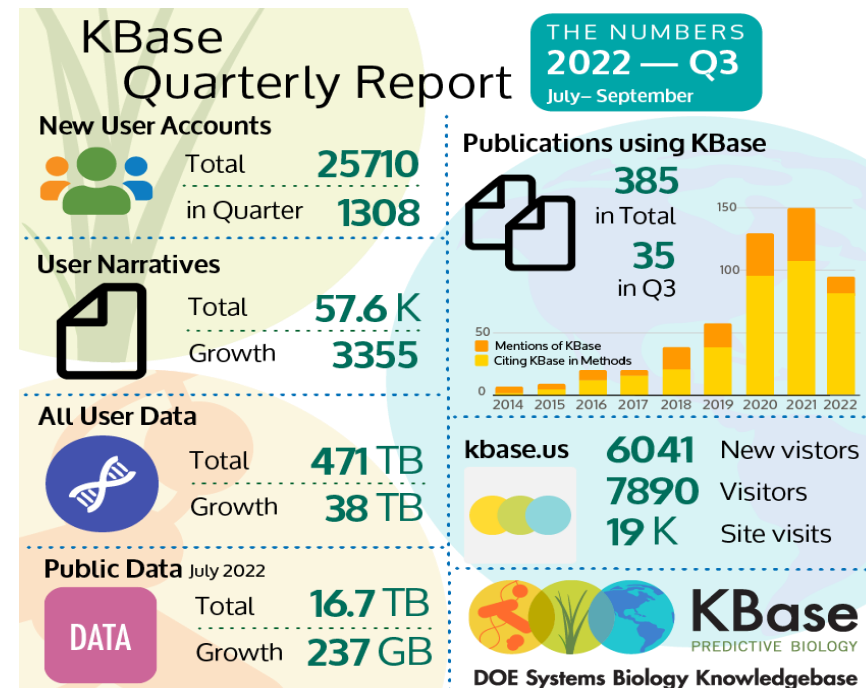


DOE Systems Biology Knowledgebase

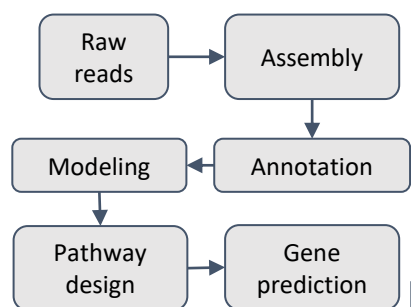


Latest Developments in KBase

- Working with ESS-DIVE, NMDC, & JGI to design cross platform data citation service
- Sequence mapping service between KBase and IMG - users are shown sequence matches via the IMG platform that link directly to KBase data landing page.
- Bulk upload released enabling users to easily upload large numbers of files.
- New tools in KBase to support integration with PDB, including direct queries from KBase genomic datatypes with import, visualization, and citation of PDB / AlphaFold DB structures.

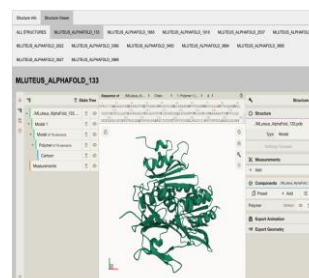


Connect KBase Genomics Workflows to PDB



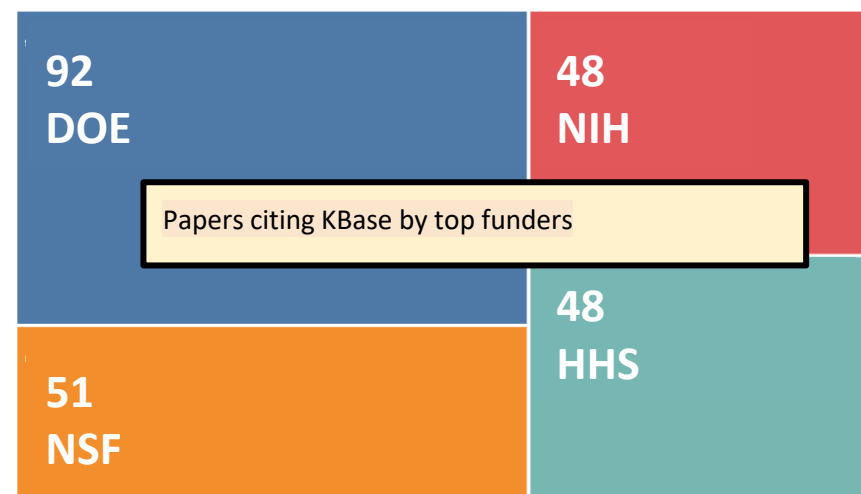
Protein structures successfully uploaded

Structure	Genome	Feature	Model and chain matching	Sequence identity
NLUTERIS_ALPHA_FOLD_133	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_133	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_180	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_180	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_1919	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_1919	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_2637	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_2637	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_2777	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_2777	Model 1 Chain A	93.02%
NLUTERIS_ALPHA_FOLD_2922	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_2922	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_366	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_366	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_360	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_360	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_364	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_364	Model 1 Chain A	100.0%
NLUTERIS_ALPHA_FOLD_365	Multikv_JCOC_4960	Multikv_mssource_PAST_CDS_365	Model 1 Chain A	100.0%



Import structures from PDB/AlphaFold

Visualize/analyze structures



Modified Poplar with Altered Lignin Composition is More Valuable and Easier to Break Down

Background/Objective

- Harness the flexibility of the lignin biosynthesis pathway to engineer bioenergy crops that are more valuable and less expensive to break down.

Approach

- Transform a hybrid poplar with 3-dehydroshikimate dehydratase gene (*QsuB*), which has shown the potential to divert carbon away from the lignin biosynthesis pathway and toward soluble dihydroxybenzoate (DHB), a platform chemical and precursor to high-value products like plastics.

Results

- Transgenic poplar wood had up to 33% less lignin, and an improved saccharification, releasing up to 40% more glucose.
- Poplars also revealed novel incorporation of DHB into the backbone of lignin, introducing weaker bonds, or ‘zips’, to the polymers.

Significance/Impacts

- This work demonstrates how engineering bioenergy crops can not only improve the efficiency of industrial biomass deconstruction by adding cleavable ‘zip-lignin’ but also increase the value of the biomass, as soluble DHB could be a valuable coproduct in future biorefineries.



Genetically engineered poplars showed changes in lignin composition for easier deconstruction and greater sugar yield. Image courtesy of Faride Unda.

Unda, F., Mottiar, Y., Mahon, E.L., Karlen, S.D., Kim, K.H., Loqué, D., Eudes, A., Ralph, J. and Mansfield, S.D., “A new approach to zip-lignin: 3,4-dihydroxybenzoate is compatible with lignification.” *New Phytologist*, 235: 234-246 (2022) [DOI: 10.1111/nph.18136]

Metabolic Engineering of Oleaginous Yeast *Rhodotorula toruloides* for Overproduction of Triacetic Acid Lactone

Objective

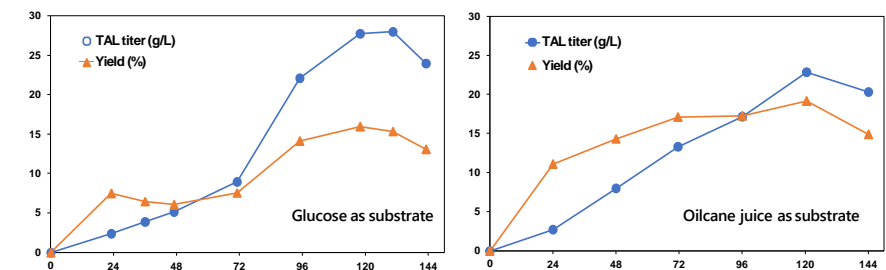
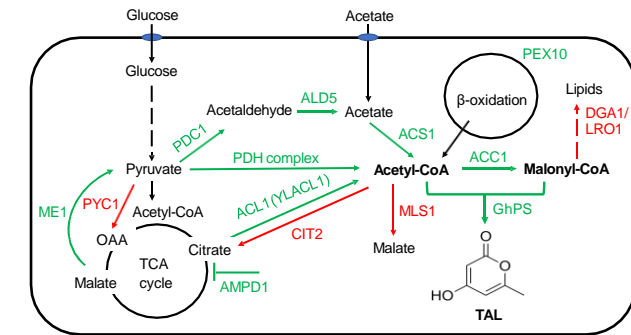
Rhodotorula toruloides is a promising production host to synthesize chemicals from biomass. The organism produces high levels of acetyl-CoA during lignocellulosic breakdown, a precursor to valuable chemicals such as triacetic acid lactone (TAL) which can be converted to a variety of valuable intermediates and end products, such as phloroglucinol, acetylacetone, and sorbic acid.

Approach

- Screened several sources of the 2-pyrone synthase (2PS) gene, and functionally expressed the gene from *Gerbera hybrida* in *R. toruloides* to produce TAL.
- Systematically evaluated various metabolic targets to increase acetyl-CoA and malonyl-CoA levels, which increase TAL production.
- Performed fed-batch bioreactor fermentation using glucose or oilcane juice as substrates and evaluated TAL production.

Results

- *R. toruloides* produced 2 g/L TAL in a culture tube.
- Sequential overexpression of key pathway metabolic targets increased TAL by significantly
- *R. toruloides* engineered with overexpressed enzymes produced 28 g/L or 23 g/L TAL in glucose or oilcane juice medium using fed-batch fermentation, respectively.



Metabolic engineering strategies and fed-batch bioreactor fermentation of *R. toruloides* for TAL production

Cao, M., Tran, V., Qin, J., Olson, A., Mishra, S., Schultz, J.C., Huang, C., Xie, D., Zhao, H. June 14, 2022. "Metabolic Engineering of Oleaginous Yeast *Rhodotorula toruloides* for Overproduction of Triacetic Acid Lactone." *Biotechnology and Bioengineering* 2022:1-12. DOI: 10.1002/bit.28159.

Comparing *in planta* Accumulation with Microbial Routes to Set Targets for a Cost-competitive Bioeconomy

Objective

- Plants and microbes can make some of the same bioproducts, yet there is no systematic way to determine which route is preferable.
- This study aimed to establish break-even yield and accumulation rates.

Approach

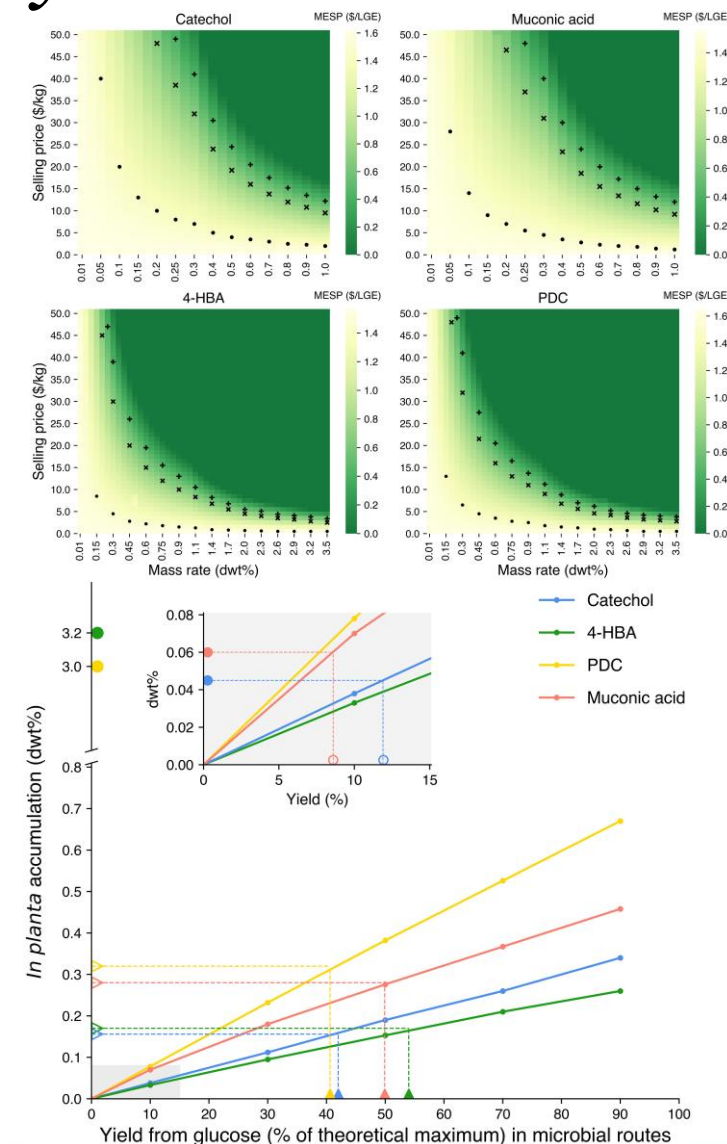
The study uses techno-economic analysis to compare *in planta* and microbial production routes for catechol, muconic acid, 4-HBA, and PDC in biorefineries.

Results

Mass accumulation ranging from 0.1 to 0.3 dry weight % can achieve costs comparable to microbial routes operating at 40 to 55% of maximum theoretical yields from glucose.

Significance/Impacts

The study provides generalizable insights into breakeven points between plants and microbes, assuming residual plant biomass can be converted to biofuel. Both plants and microbes have an important role in producing bioproducts.



Yang, M., Liu, D., Baral, N.R., Lin, C.Y., Simmons, B.A., Gladden, J.M., Eudes, A. and Scown, C.D., 2022. Comparing *in planta* accumulation with microbial routes to set targets for a cost-competitive bioeconomy. *Proceedings of the National Academy of Sciences*, 119(30), p.e2122309119.

Background

- There are three switchgrass ecotypes: coastal, lowland and upland.
- Segregation distortion, which is a genetic deviation from expected Mendelian ratios, is a driving force of evolution that impacts the genetic compatibility between ecotypes (i.e., ability to inter-breed).
- Wide crosses between ecotypes can benefit switchgrass breeding efforts to improve growth and yield in unfavorable environmental conditions by selecting for desired genes and traits.

Approach

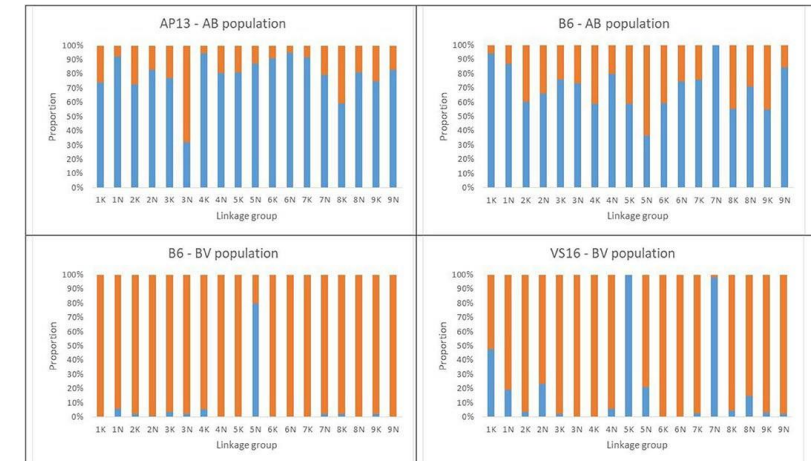
- Two inter-specific ecotypic switchgrass populations were generated: Lowland (AP13) x Coastal (B6) and Coastal (B6) x Upland (VS16) as AB and BV, respectively.
- The extent of genetic variance and patterns of segregation distortion were mapped for the two populations, AB (285 progeny) and BV (227 progeny) derived from these crosses.
- Biomass yield was analyzed across two years, and quantitative trait loci (QTL) mapping was performed to link genotypic and phenotypic information.

Results

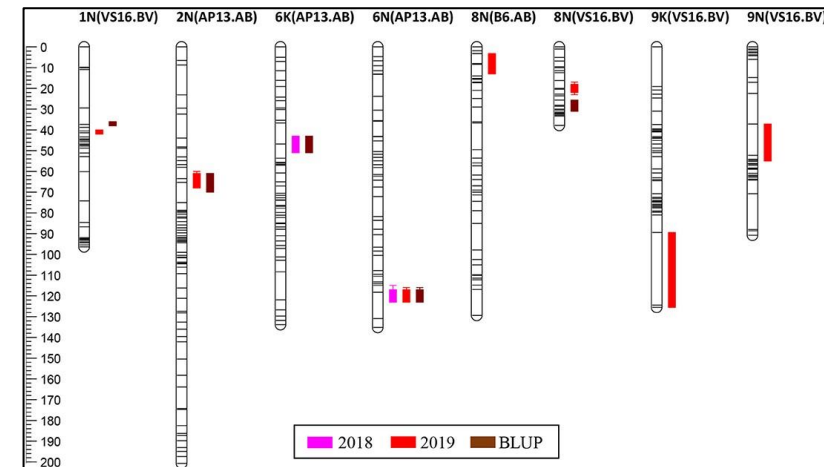
- More segregation distortion of alleles was seen in the AB population, and likely a result from zygotic or post-zygotic selection for increased levels of heterozygosity.
- The results suggest lower genetic compatibility between the lowland AP13 and the coastal B6 ecotypes
- Four biomass yield QTLs were mapped in the AB population and six QTLs in the BV population

Significance

- Strong evidence for biomass yield QTLs indicate that breeding can improve switchgrass, especially across compatible inter-ecotypes. A challenge to improved traits is segregation distortion leading to unexpected biases in what traits become inherited.



Proportion of distorted (blue bars) and Mendelian markers (orange bars) across LGs assessed by the Chi-square (χ^2) values for the goodness-of-fit test to 1:1 expected segregation ratios ($p < 0.05$).



QTL positions on the genetic map. QTL are positioned at the right side of each LG; solid bars and whiskers on one or both ends represent coverage at LOD drop intervals of 1.0 and 2.0, respectively. QTL were mapped using LS means for each year and the BLUP value.

Razar, RM et al. (2022) *Frontiers in Plant Science* 13:739133, <https://doi.org/10.3389/fpls.2022.739133>

RuBisCO Evolution Enabled by Structural Plasticity

Objective

Better understand the phylogenetic distribution of the oligomeric states of form II RuBisCO, found in bacteria and certain photosynthetic microbes.

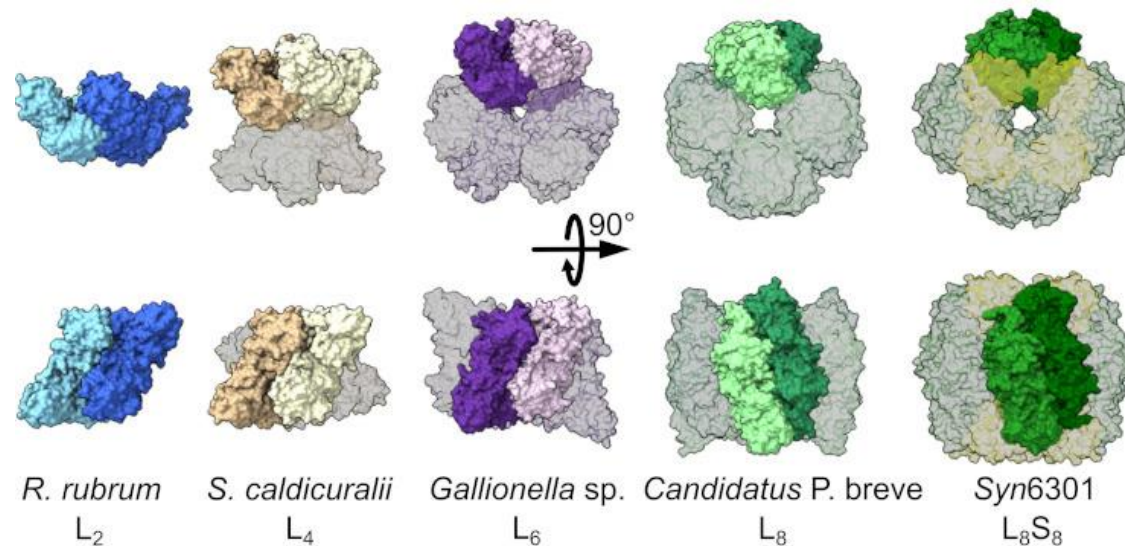
Approach

Structurally characterized 28 candidates across the phylogeny using macromolecular X-ray crystallography (MX) and small-angle X-ray scattering (SAXS), which can reveal natural oligomerization states. Ancestral sequence reconstruction guided the selection of evolutionary representatives from the phylogenetic tree.

Results/Impacts

- While Form I RuBisCO found in plants is “structurally entrenched” in an octomeric state, Form II (microbial) has been observed mostly as hexamers, but also as dimers and a tetramer.
- Ancestral sequence reconstruction enabled by combining the structural data with the gene sequences supported the evolutionary structural plasticity evidenced by the variety of Form II oligomers.
- Quaternary structure plasticity may tune enzyme kinetics which could contribute to selective evolutionary pressure and also inform enzyme biodesign.

A comparison of RuBisCO assemblies from different microbial species illustrates the enzyme’s wide diversity of oligomeric states



Liu, A.K., et al. 2022. Structural plasticity enables evolution and innovation of RuBisCO assemblies. *Science Advances*, 8 (34). [DOI: 10.1126/sciadv.adc9440]

New Approach Produces 90-Fold Increase in Known Viral Taxa

Objective

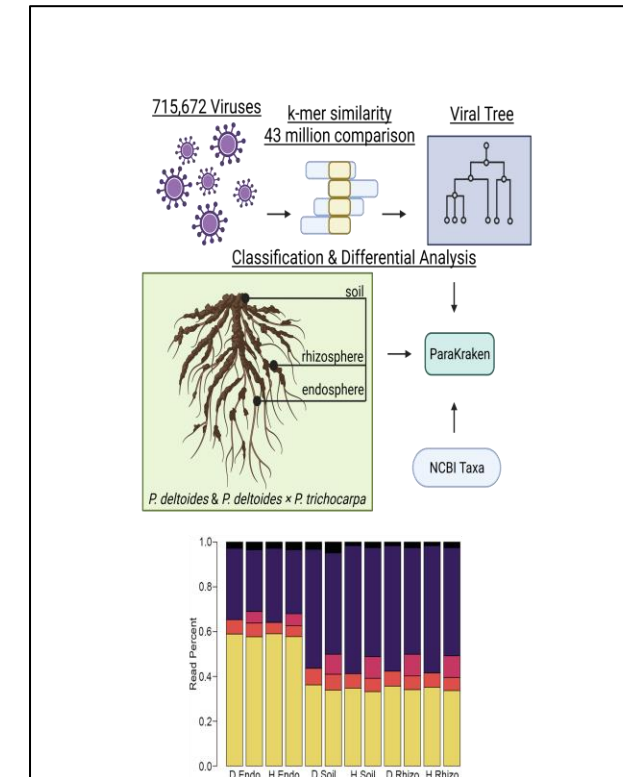
- Remedy viral taxa underrepresentation for taxonomic classification through large-scale comparison of viral genomes.

Approach

- Novel dynamic programming algorithm for viral classification tree of 723,672 viral genomes without a taxonomy.
- Improved classification of endosphere, rhizosphere, and soil metatranscriptome samples showing differential viral taxa. Highlighting putative compartment and genotypic effects,
- Viral taxa comprise a non-trivial component (average 15%) of the metatranscriptome.

Result/Impacts

- This approach allows for the classification of viruses without requiring a taxonomy and results in an increase in the number of potential viral taxa by 90-fold. Better classification of viruses helps in identifying associations between viruses and their hosts as well as viruses and other microbiome members.



Garcia, BJ, et al. *Comp Struc Biotech J*, doi: 10.1016/j.csbj.2021.10.029

Cross-kingdom expression of biosynthetic gene clusters



Cover of Cell!

Objective

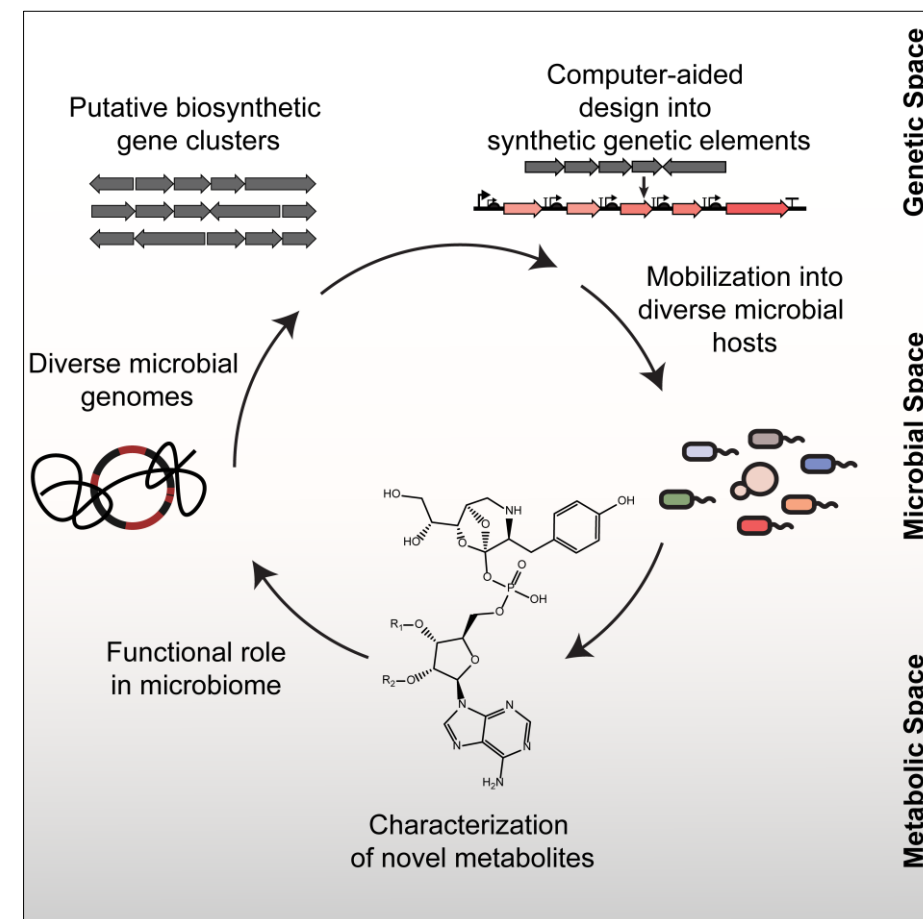
Develop a computational and experimental strategy to redesign biosynthetic gene clusters (BGCs), determine their biosynthetic products, and express them in diverse hosts.

Approach

A computer-aided design of synthetic gene elements (CAD-SGE) strategy was developed to redesign BGCs with hybrid eukaryotic/prokaryotic regulatory elements and optimized coding sequences. A combination of conjugation transposable elements facilitates the introduction and stabilization of heterologous sequences. The redesigned BGCs can be expressed in diverse yeast and bacterial hosts.

Result/Impacts

- Redesigned multigene pathways can be mobilized and stably expressed in multiple hosts across kingdoms.
- The computational/experimental strategy allows to validate biosynthetic capacity of predicted BGCs and to determine their functional role in the microbiome.
- A wealth of untapped biosynthetic potential from microbes and microbial communities can be harnessed to engineer the production of new bioproducts.



Patel et al., *Cell* (2022) 185,1487–1505

BNL NSLS II Ghost Imaging with X-rays 101

Ghost Image is generated from quantum-entangled x-rays that did not pass through the sample

Objective

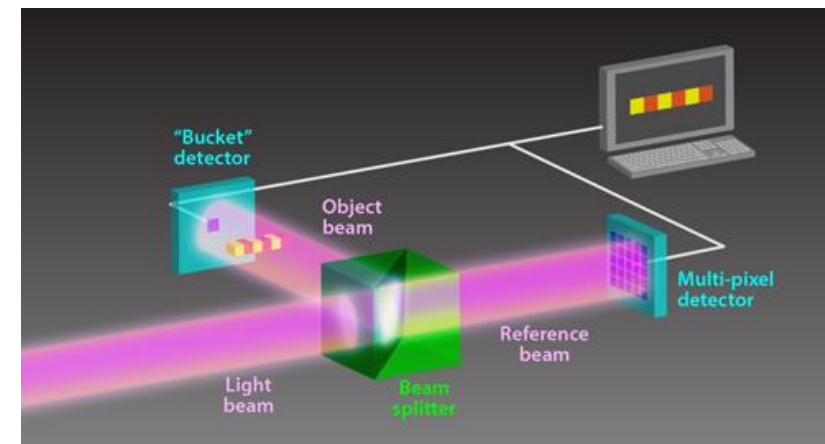
- Reduce required X-ray dose 10-fold compared to a Direct Image

Approach

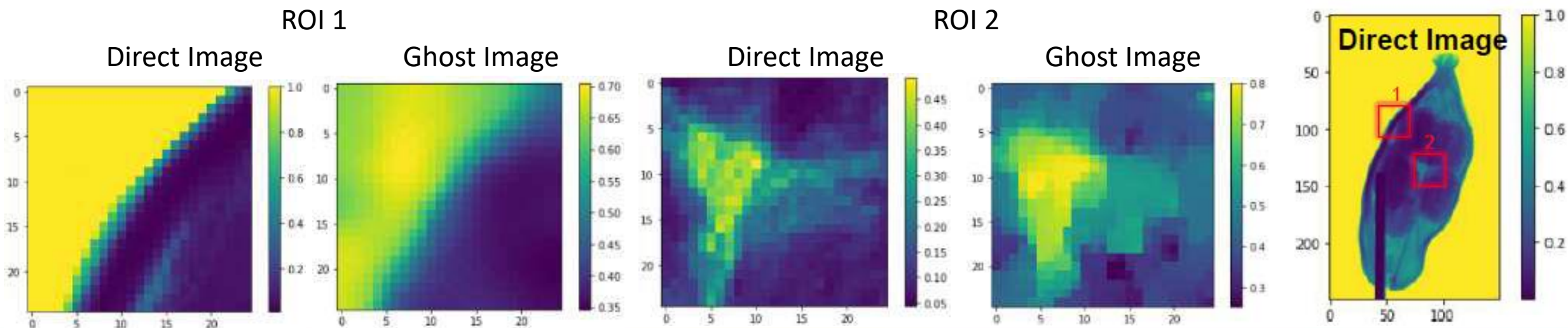
- Thermal source of X-rays to develop physics of bioimaging
- Future use of Synchrotron X-ray source

Result/Impacts

- Successful deployment of type-2 ghost imaging for near-field imaging
- First report of successful imaging of interior structure using x-ray Ghost Imaging on a cardamom seed!
- Future goals: faster data acquisition and more complex data analysis, better signal/noise, resolution.



Saldin Ghost Imaging with X Rays, APS Physics (2016)



118 JGI Publications April 1, 2022 - September 28, 2022

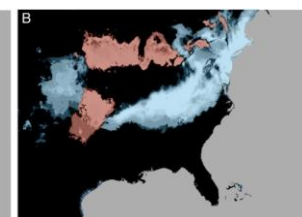
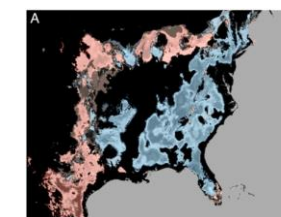
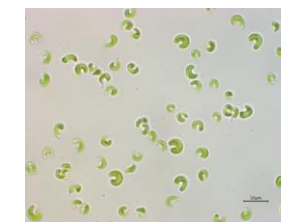
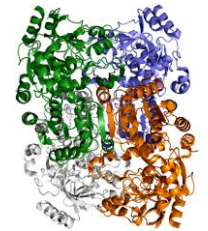
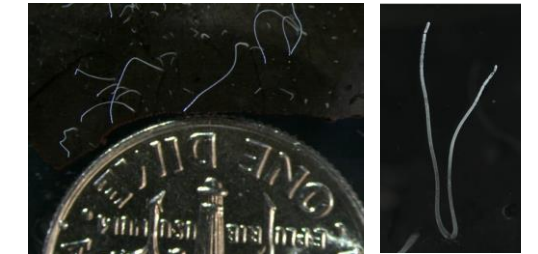
Selected Highlights:

- A centimeter-long bacterium with DNA contained in metabolically active, membrane-bound organelles. **Science**
- Intersubunit Coupling Enables Fast CO₂-Fixation by Reductive Carboxylases. **ACS Central Science**
- A generalist–specialist trade-off between switchgrass cytotypes impacts climate adaptation and geographic range. **PNAS**
- Identification of beneficial and detrimental bacteria impacting sorghum responses to drought using multi-scale and multi-system microbiome comparisons. **ISME Journal**
- Dynamic genome evolution in a model fern. **Nature Plants**
- Wildfire-dependent changes in soil microbiome diversity and function. **Nature Microbiology**
- Multi-omics profiling of the cold tolerant *Monoraphidium minutum* 26B-AM in response to abiotic stress. **Algal Research**
- Expansion of the global RNA virome reveals diverse clades of bacteriophages. **Cell**

JGI news releases & highlights:

<http://jgi.doe.gov/news-publications/>

<http://jgi.doe.gov/category/science-highlights/>





Thank you

<https://science.osti.gov/ber>

<https://www.energy.gov/science/ber/biological-and-environmental-research>