Office of Biological and Environmental Research Biological Systems Science Division Update

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October 22, 2021

U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

Update on Programmatic Activities

Completed Reviews/Activities

- ✓ KBase Review February 3-4
- ✓ PI Meeting End of February – *Virtual!*
- ✓ NAS - Quantum Science Concepts for Imaging March 8-10
- ✓ Early Career Applications March 30
- ✓ Review of DOE (3) Lab Microbiome SFAs May 11-13
- ✓ Bioimaging FOA Applications May 24-25
- ✓ Microbial Biofuels, Bioproducts, and Polymer Upcycling FOA Applications May 24-25
- ✓ Committee of Visitors Review July 27-29
- ✓ DOE LBNL ENIGMA SFA August 3-4
- ✓ DOE LLNL uBIOSPHERES SFA August 5
- ✓ Harvard Synbio project September 8
- ✓ KBase- SFA Partnerships September
- ✓ UCLA-DOE Institute September 21

Upcoming Reviews/Activities

- ❑ Annual BRC reviews (Nov, Dec, Jan, Feb)
- ❑ JGI Triennial Review (Dec 8-10)
- ❑ PI Meeting end of February

NEW FY 2022 Funding Opportunities (Tentative)

Genomic Science program

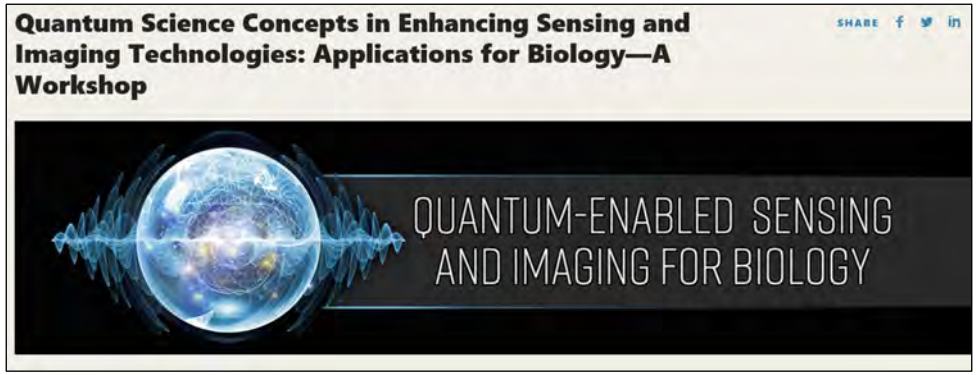
- Biosystems Design FOA
- Plant Genomics FOA
- Environmental Microbiology FOA

Biomolecular Characterization and Imaging Science

- Quantum-concepts for Imaging FOA

FOAs will post to the Grants.gov website - TBD

Workshops/Planning Activities

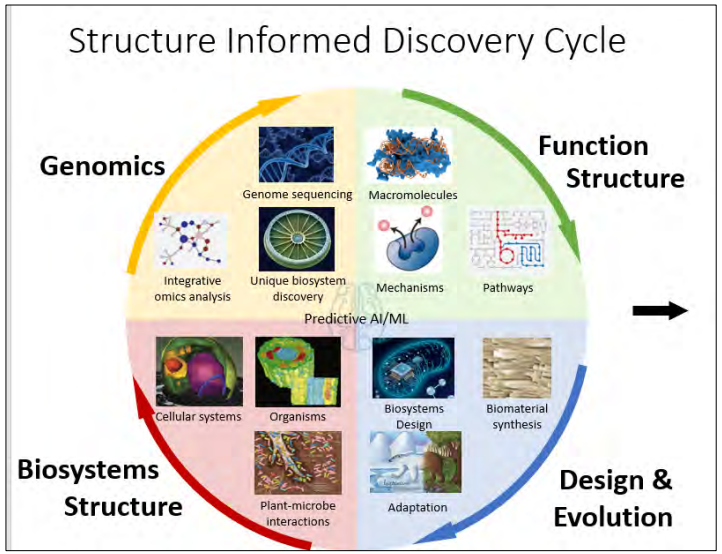


Workshop March 8-10
Proceedings now available at the NASEM website

DESIGNING THE BIOECONOMY FOR DEEP DECARBONIZATION

OPPORTUNITIES AND IMPACTS WORKSHOP | APRIL 30, 2021 11:30 AM – 3:00 PM EDT | 8:00 AM – 12:00 PM PDT

Follow-up: Incorporate automation with AI/ML techniques into experimentation and testing



New: **DOE Lab-led Workshop:**
Genomes to Structure and Function
October 27-28, 2021

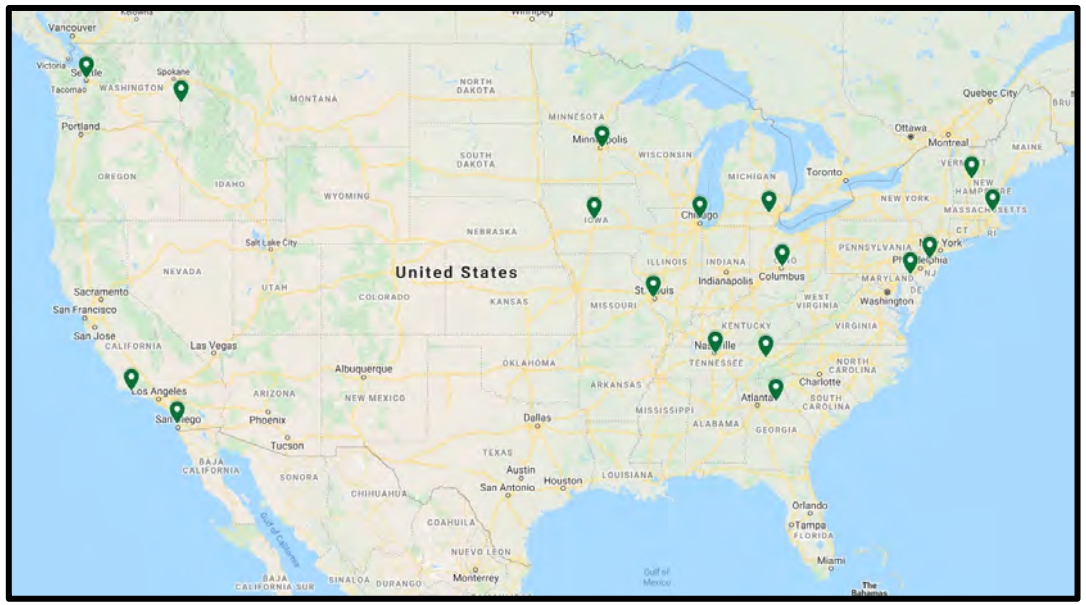
Combine unique capabilities in the BER User facilities and Structural Biology Resources

New Microbial Biofuels, Bioproducts, and Polymer Upcycling Awards

Systems Biology of Bioenergy-Relevant Microbes to Enable Production of Next-Generation Biofuels and Bioproducts (DE-FOA-0002448)

Topic *A: Sustainable Bioenergy*

- Development of emerging microorganisms, microbial communities, and cell free options
- Engineer and modify proteins to improve pH and temperature tolerance
- Develop and modify genetically-encoded biosensors and optogenetic circuits to control metabolic pathways and population dynamics
- Investigate use of outer membrane vesicles and bacterial microcompartments to encapsulate metabolic flux networks
- Develop and apply microbial community metabolic models for synthetic co-cultures
- Investigate impacts of engineered noise into microbial strain robustness
- Proposals include experimental and computational approaches



US map showing location of 21 projects funded by DE-FOA-0002448

Topic B: Polymer Upcycling

- Identify and design enzymes to degrade and upcycle Nylon 6, Nylon 66, polyethylene terephthalate, polyethylene, and polystyrene
- Optimization and modify both known enzymes as well as identification of new enzymes for depolymerization
- Both *in vivo* expression using microbes as well as cell-free options
- Proposals include experimental and computational-focused proposals

https://www.energy.gov/articles/doe-awards-455-million-projects-advance-biotechnology-research

21 new projects

New Bioimaging Awards

Bioimaging Research to Develop Imaging and Instrumentation Approaches DE-FOA-0002393

Developing new imaging capabilities to study plants and microorganisms to advance biofuel and bioproduct production

- This FOA encourages development of new imaging instrumentation for 3D-dynamic imaging of metabolic processes occurring in plant tissue and among rhizosphere communities
- The goal is to develop novel imaging instrumentation for dynamic in situ imaging of key metabolic processes occurring deep within the living plant and microbial systems non-destructively in real-time.
- The bioimaging research in this FOA is designed to complement the Biological Systems Science's Division's genomic science effort to understand, predict, manipulate and design metabolic processes in both plant and microbial cells for a variety of bioenergy, bioproduct and environmental purposes of interest to BER.

US map showing location of 7 projects funded by DE-FOA-0002393

https://www.energy.gov/articles/doe-awards-455-million-projects-advance-biotechnology-research

→ *7 new projects*

KBase Development and Highlights

Objective

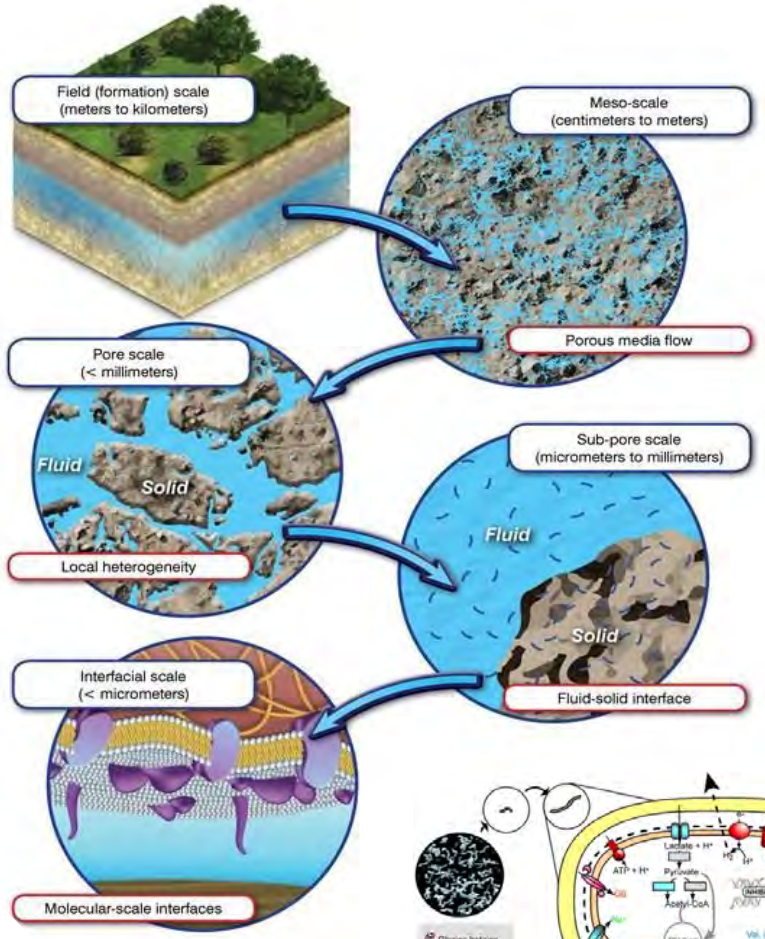
Develop the KBase platform to enable scientists to identify critical biological mechanisms and abiotic interactions in target environments, assess fitness and activity therein, and predict changes across physiology, community activity, and ecosystem services with an advanced data model and computing infrastructure, supporting over 300TB of user data and up to 850,000 CPU hours a month.

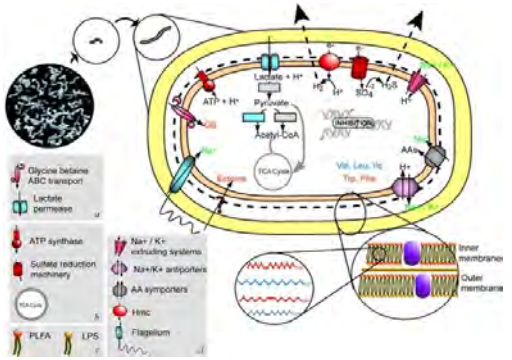
Approach

Construct a standardized data model with consistent ontology for a variety of biological data types, infer and build linkages between these data, layer and embed evidence for these associations, and then make predictions of biological and ecological behavior at multiple scales. Develop a user base for the platform through scientific engagement and collaborative development.

Results/Impacts

- Robust usage of workflows for microbial ecology, metabolic modeling, and isolate analysis across a global community of >19,000 users
- Creation of samples data type and analysis tools for amplicon and environmental chemistry data on platform
- Prototype of Knowledge Engine service for automated inference between public and user data
- 85 publications using KBase in 2021; 8 supported by DOE funding





Developing a platform for multi-scale predictive biology

New KBase SFA Partnerships

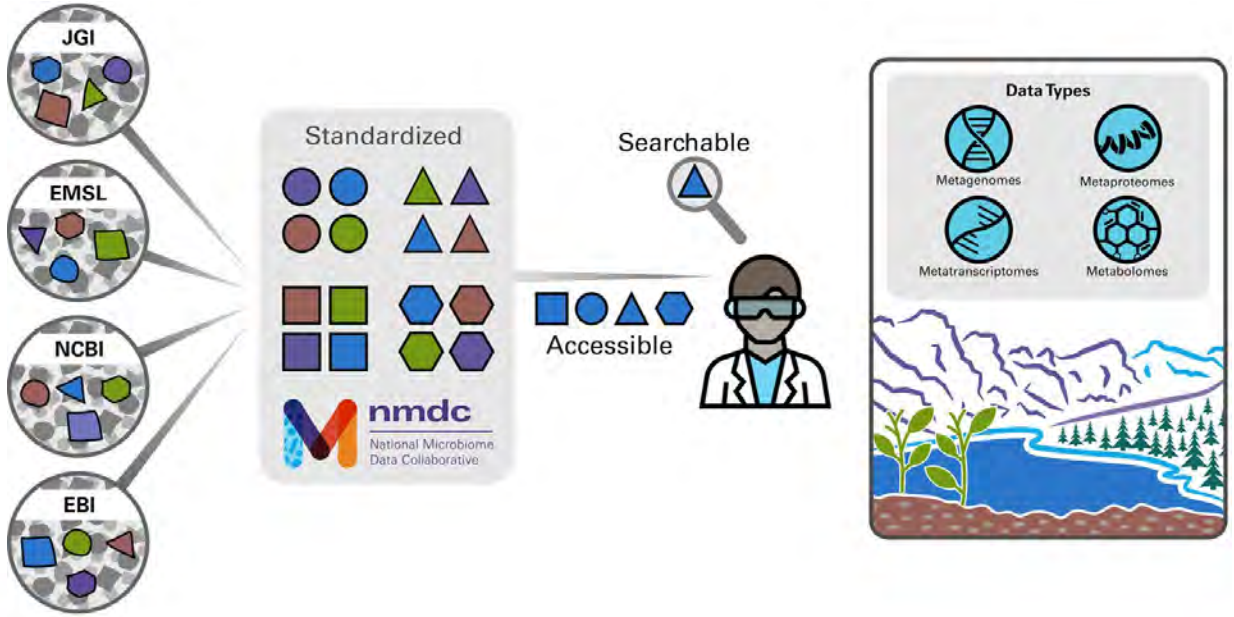
6 new projects		
LBNL	Building pipelines for long read assembly of microbial isolates and metagenomes in the DOE Systems Biology KnowledgeBase	Adams, Paul
PNNL	Omics-enabled global gapfilling (OMEGGA) for phenotype-consistent metabolic network reconstruction of microorganisms and communities	Hofmockel, Kirsten
PNNL	Improved Protein Annotation in KBase Using Machine Learning, Multi-Omics Data Integration, and Structural Prediction	Nelson, William
ORNL	Design and Omics Exploration of Synthetic Microbial Communities	Ranjan, Priya
LLNL	Probabilistic Annotation and Ensemble Metabolic Modeling in KBase	D'haeseleer, Patrik
LLNL	LLNL Soil Microbiome SFA Microbes Persist: Towards quantitative theory-based predictions of soil microbial fitness, interaction and function in KBase	Pett-Ridge, Jennifer

Towards a data science ecosystem for microbiome research

nmdc
National Microbiome Data Collaborative

Mission

Provide a gateway to FAIR multi-omics microbiome data leveraging best practices for data curation and processing



Metadata

https://github.com/microbiomedata/nmdc-metadata

Software

https://github.com/microbiomedata/WorkflowPlanning
https://hub.docker.com/u/microbiomedata

Data Portal

https://data.microbiomedata.org

Accomplishments

EMSL
JGI JOINT GENOME INSTITUTE

Coordinating standards & workflows across flagship User Facilities

SFAs

Initiating support for SFAs multi-omics data

THE NATIONAL MICROBIOME DATA COLLABORATIVE AMBASSADOR PROGRAM

Gaining strong community engagement

AMERICAN SOCIETY FOR MICROBIOLOGY

Microbiome Data Prize

Read more

nature REVIEWS MICROBIOLOGY

Wood-Charlson, E.M., *et al. Nat Rev Microbiol* **18,** 313–314 (2020). doi.org/10.1038/s41579-020-0377-0

mSystems

Vangay, P *et al.* mSystems **6**, e01194-20 (2021). doi.org/10.1128/mSystems.01194-20

JBEI
Joint BioEnergy Institute

Production Cost and Carbon Footprint of Biomass-Derived Dimethylcyclooctane as a High-Performance Jet Fuel Blendstock

Background

- Decarbonization of aviation requires energy-dense, renewable liquid fuels.
- Biomass-derived 1,4-dimethylcyclooctane (DMCO), a cyclic alkane with a volumetric net heat of combustion up to 9.2% higher than Jet A, has the potential to serve as a low-carbon, high-performance jet fuel blendstock that may enable paraffinic bio-jet fuels to operate without aromatic compounds.

Approach

- We developed detailed process configurations for DMCO production to estimate the minimum selling price and life-cycle greenhouse gas (GHG) footprint considering three different hydrogenation catalysts and two bioconversion pathways. All modeling was done in SuperPro Designer and life-cycle GHG inventory used our previously-developed BioC2G model

Outcomes and Impacts

- The platinum-based catalyst offers the lowest production cost and GHG footprint of $9.0/L-Jet-Aeq and 61.4 gCO2e/MJ, given the current state of technology.
- When the supply chain and process are optimized, hydrogenation with a Raney nickel catalyst is preferable, resulting in a $1.5/L-Jet-Aeq cost and 18.3 gCO2e/MJ GHG footprint if biomass sorghum is the feedstock.
- Because increased gravimetric energy density of jet fuels translates to reduced aircraft weight, DMCO also has the potential to improve aircraft efficiency, particularly on long-haul flights.

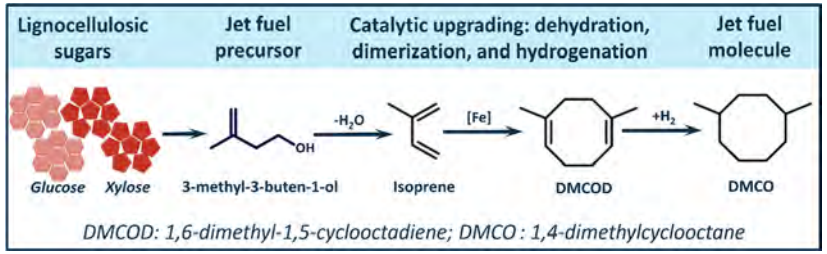


Figure 1. Overview of 1,4-dimethylcyclooctane (DMCO) synthesis processes from the biomass-derived glucose and xylose.

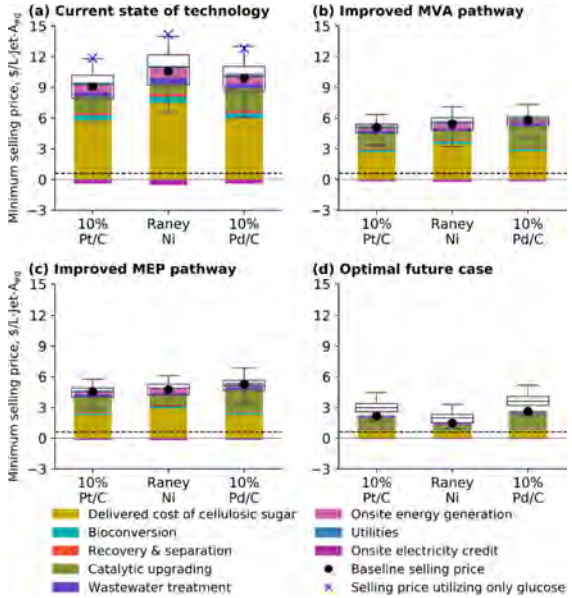


Figure 2. Minimum selling price of DMCO under different scenarios: (a) current state of technology (SOT) with the MVA pathway; (b) improved MVA pathway with 90% of the theoretical isoprenol yield; (c) improved MEP pathways with 90% of the theoretical isoprenol yield; and (d) optimal future case with the MVA pathway.

Baral *et al.* (2021) ***ACS Sustain. Chem. Eng.***, doi: 10.1021/acssuschemeng.1c03772

Nationwide collaboration unlocks switchgrass genome

GREAT LAKES BIOENERGY RESEARCH CENTER

TEXAS
The University of Texas at Austin

Objective

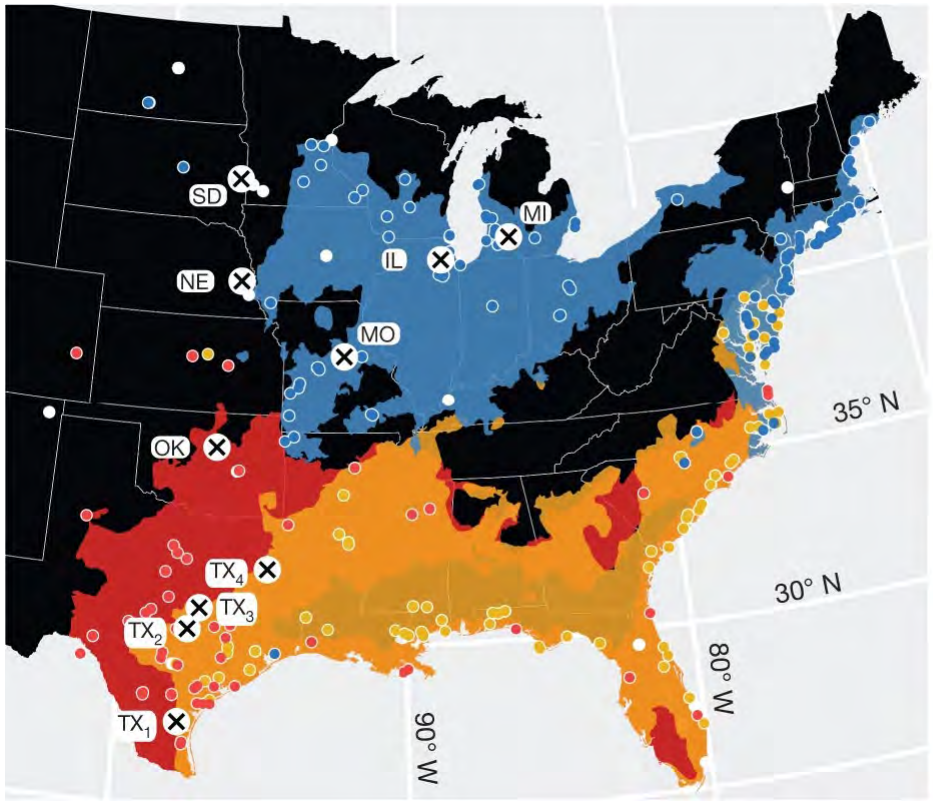
Produce a high-quality reference sequence of the complex switchgrass genome

Approach

- Ten common gardens spanning climate zones across eight states and 1,100 miles, each containing a propagated panel of 732 switchgrass genotypes.
- Sequence, assemble, and annotate the switchgrass genome.
- Analyze biomass and survival among genotypes for climate–gene–biomass associations.

Result/Impacts

- Affords the ability to assess genotype changes in expressed switchgrass phenotypes across climatic and geographic regions
- All four BRCs have expanded the network of common gardens and are exploring improvements to switchgrass through more targeted genome editing techniques to improve crop traits and customize the crop for additional end products.
- The work builds off of a BSSD Sustainability Award to (T. Juenger, UTexas) assessing switchgrass varieties across multiple field sites across the breadth of the U.S.



Geographic distribution of common gardens and plant collection locations, and spatial distribution models of each ecotype. The ecotype color legend accompanies the representative images of each ecotype to the right of the map.

Lovell, J.T., *et al.* "Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass," ***Nature*** (2021). [DOI: 10.1038/s41586-020-03127-1]

JGI JOINT GENOME INSTITUTE
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A Reference Genome for the Bioenergy Crop Switchgrass

Objective

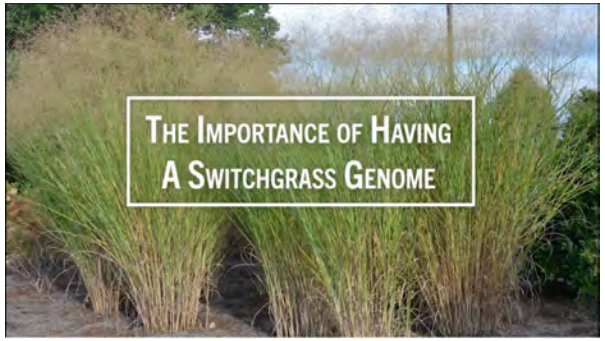
Switchgrass is a complex plant with multiple copies of its chromosomes. The Department of Energy's long-term investments in developing switchgrass as a candidate biomass feedstock crop have led to a reference sequence and several common gardens exploring the plant's diversity.

Approach

Researchers at the University of Texas at Austin led multiple institutions in collecting 700+ switchgrass plants from 25 states and establishing 10 experimental gardens across 1,100 miles. Using samples from these gardens, JGI researchers generated a high-quality reference genome.

Results/Impact

- The reference genome for switchgrass AP13 is available on the JGI's plant data portal Phytozome.
- The DOE Bioenergy Research Centers are now harnessing the switchgrass genome to explore customizing the crop for additional high-value end products.
- The switchgrass common gardens allow researchers to test associations of climate adaptations with switchgrass biology. In fall 2020, the common gardens were funded for another five years.



Podcast:
bit.ly/JGI-Switchgrass2021

Videos:
bit.ly/JGI-video-Switchgrass2021
bit.ly/JGI-Switchgrass2

JGI GENOME INSIDER

Lovell J et al. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. Nature. 2021 Jan 27. doi: 10.1038/s41586-020-03127-1.

CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Complete and Efficient Conversion of Plant Cell Wall Hemicellulose into High-Value Bioproducts by Engineered Saccharomyces cerevisiae

Background/objective

Hydrolysates from plant cell walls contain sugars but also substantial amounts of acetate, a fermentation inhibitor that hinders bioconversion of lignocellulose. Detoxifying acetate by exploiting its consumption to enhance acetyl-CoA supply in yeast could produce value-added products.

Approach

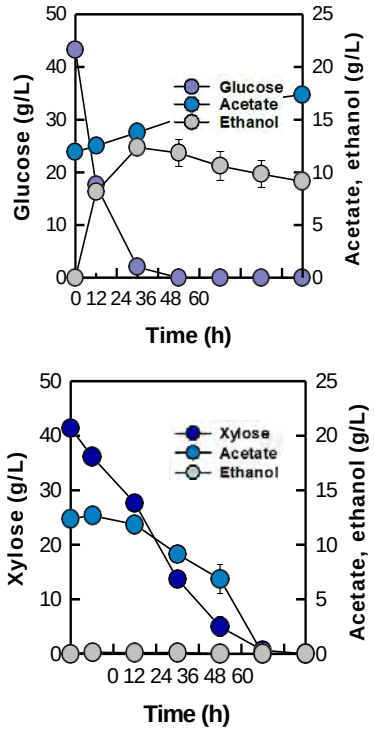
- RNA sequencing analysis revealed the underlying mechanisms of detoxification and co-consumption of acetate with xylose in yeast.
- A xylose-fermenting yeast was further engineered to produce triacetic acid lactone (TAL) and other acetyl-CoA-derived bioproducts.

Results

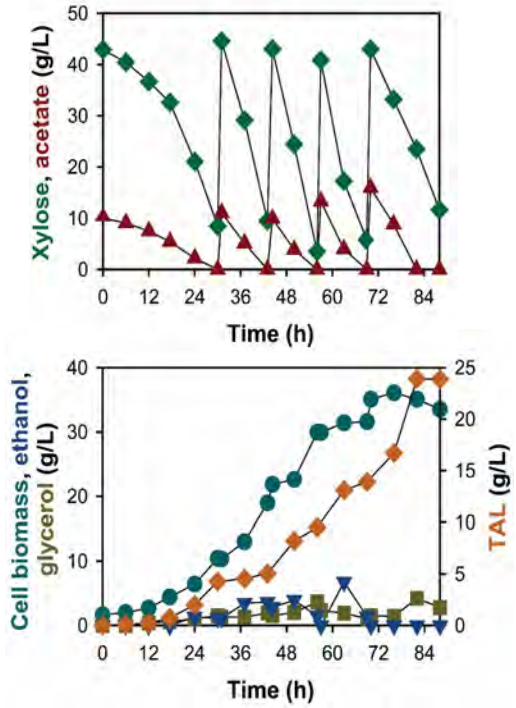
- In contrast to the hampered acetate consumption by glucose, up to 12 g/L of acetate is rapidly assimilated by engineered yeast in the xylose cultures.
- Co-feeding acetate and xylose leads to a metabolic re-configuration that boosts the synthesis of acetyl-CoA derived bioproducts, including TAL and vitamin A.
- The engineered strain produces 23.91 g/L TAL with a productivity of 0.29 g/L/h in bioreactor fermentation when co-feeding xylose and acetate. This strain also completely converts a hemicellulose hydrolysate of switchgrass into 3.55 g/L TAL.

Significance

Acetate can be rapidly co-consumed with xylose by engineered *S. cerevisiae*, which detoxifies acetate into a valuable substrate, expands the capacity of acetyl-CoA supply in *S. cerevisiae*, and enables conversion of plant cell wall hydrolysates into acetyl-CoA derived bioproducts.



Co-utilization of xylose and acetate by the xylose-fermenting yeast.



Fed-batch culture with xylose and acetate co-feeding for the production ofTAL.

Sun, L., et al., 2021. "Complete and Efficient Conversion of Plant Cell Wall Hemicellulose into High-Value Bioproducts by Engineered Yeast." ***Nature Communications*** *12, 4975. DOI: 10.1038/s41467-021-25241-y.*

THE CENTER FOR BIOENERGY INNOVATION

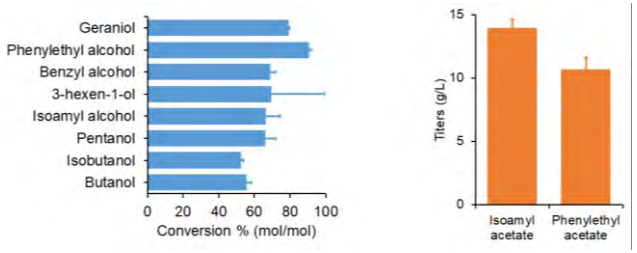
Engineering Promiscuity of Chloramphenicol Acetyltransferase for Microbial Designer Ester Biosynthesis

Background

By condensing an acyl-CoA and an alcohol, alcohol acyltransferases (AATs) can serve as an interchangeable metabolic module for microbial biosynthesis of a diverse class of ester molecules with broad applications as flavors, fragrances, solvents, and drop-in biofuels. Lack of robust and efficient AATs limits their utility with precursor pathways and microbial hosts.

Approach

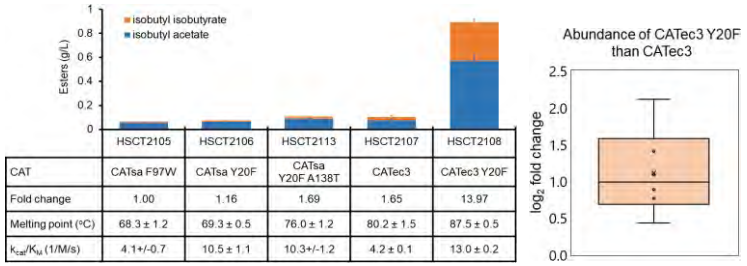
Through bioprospecting and model-guided protein engineering, we engineered substrate promiscuity of chloramphenicol acetyltransferases (CATs) to function as robust and efficient AATs compatible with at least 21 alcohols and 8 acyl-CoAs for microbial biosynthesis of linear, branched, saturated, unsaturated and/or aromatic esters in mesophiles and thermophiles.



Demonstration of efficiency and compatibility of the engineered AAT in *Escherichia coli* whole-cell biocatalyst

Outcomes and Impacts

- Developed de novo thermostable AATs that are efficient and compatible with various pathways and microbial hosts (e.g., mesophiles and thermophiles).
- Demonstrated high conversion of various alcohols and achieved about 14 g/L of isoamyl acetate with >95% (mol/mol) conversion efficiency.
- Demonstrated that CAT robustness with enhanced thermostability is critical for efficient ester production in thermophiles by maintaining high level of intracellular CAT abundance.
- Engineered *C. thermocellum* to produce up to 1 g/L of isobutyl esters from cellulose.



	HSCT2105	HSCT2106	HSCT2113	HSCT2107	HSCT2108
CAT	CATsa F97W	CATsa Y20F	CATsa Y20F A138T	CATec3	CATec3 Y20F
Fold change	1.00	1.16	1.69	1.65	13.97
Melting point (°C)	68.3 ± 1.2	69.3 ± 0.5	76.0 ± 1.2	80.2 ± 1.5	87.5 ± 0.5
k_{cat}/K_M (1/M/s)	4.1+/-0.7	10.5 ± 1.1	10.3+/-1.2	4.2 ± 0.1	13.0 ± 0.2

Impact of enzyme thermostability in ester production by *C. thermocellum* fermenting cellulose

Significance

- This work not only presents a robust, efficient, and highly compatible AAT platform for designer bioester production, but also elucidates the impact of enzyme thermostability on engineering heterologous pathways in thermophiles.

[1] Seo, H., et al. ***Metab Eng***, accepted. (2021) doi.org/10.1016/j.ymben.2021.04.005

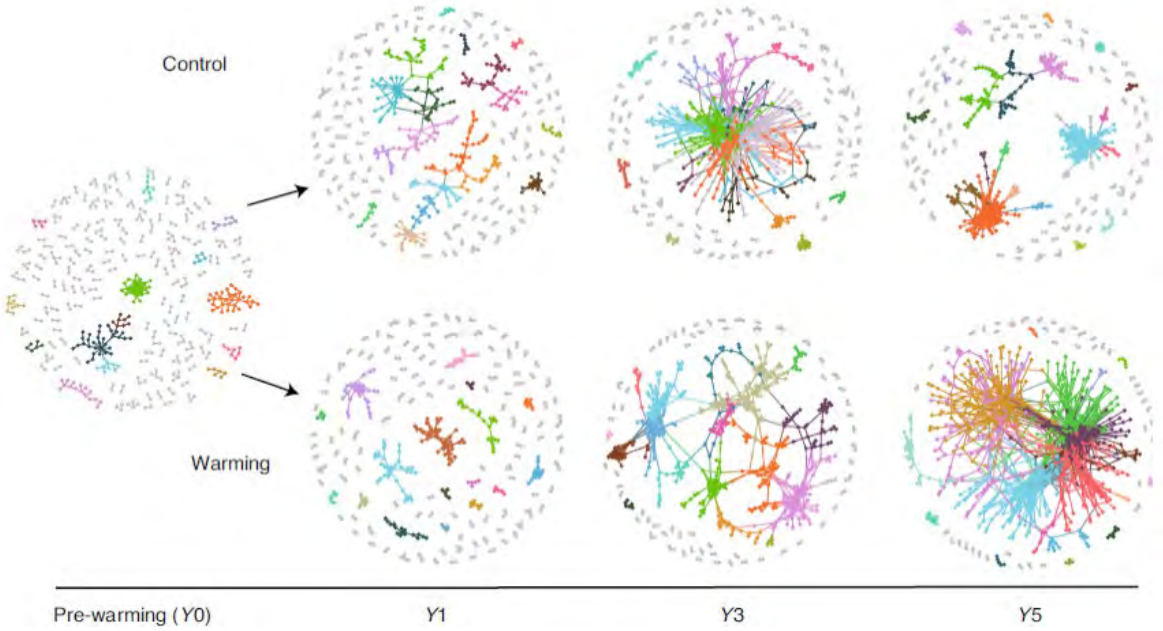
Soil Warming Leads to Greater Microbial Network Complexity and Community Stability

Approach

- Leverage long-term *in situ* prairie soil warming experiment
- Sampled soils for six years to conduct molecular community analysis and monitored physical/chemical parameters.
- Conduct statistical and network analysis to determine patterns of network connectivity and stability with respect to warming.

Results/Impacts

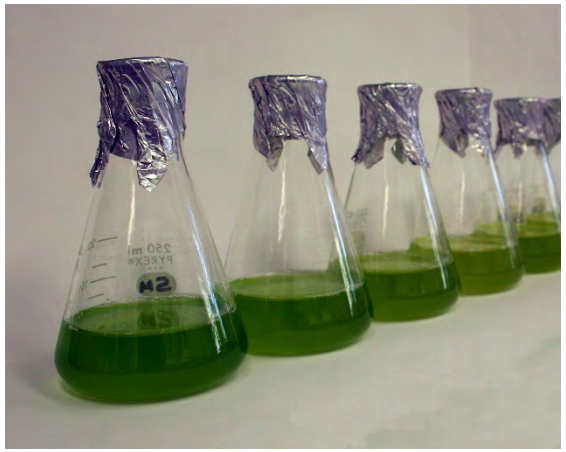
- Community networks were significantly altered by warming as compared to un-warmed prairie soils.
- Warming significantly increased network complexity, size, connectivity, clustering, relative modularity, and the number of keystone species.



***Nature Climate Change* (2021)**. https://doi.org/10.1038/s41558-021-00989-9

- Greater network complexity was associated with greater community stability, which may impact ecosystem behavior across scales.
- Greater community stability might accelerate microbially driven processes, such as the loss of organic matter from soils, and suggests that microbial processes become less vulnerable to perturbations in a warmer world.

Polycistronic Gene Expression Is Widespread in Green Algae

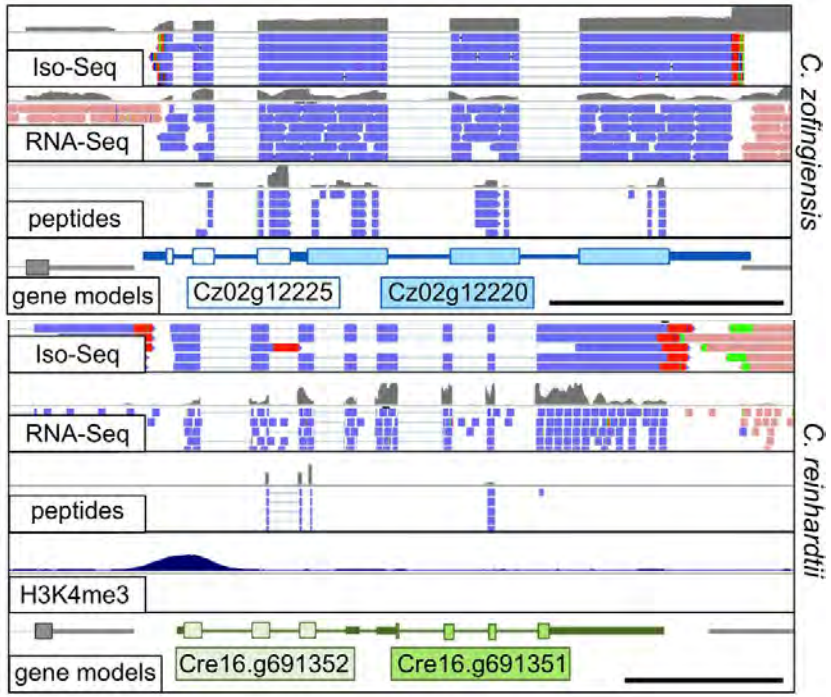


Objective

Improve our understanding of gene organization and transcriptional regulation in green algae

Approach

Multiple genome-wide gene expression analyses, including full-length transcriptomics (Iso-Seq), chromatin immunoprecipitation, polyadenylation mapping, co-expression analysis, and proteomics were used to determine the presence and expression of polycistronic transcripts in *Chlamydomonas* and *Chromochloris*. The discovery was validated by genome-wide comparison with five other algae and by expressing synthetic polycistronic gene pairs.



Result/Impacts

- Polycistronic transcription was found in seven diverse chlorophyte species, indicating that this phenomenon has been conserved in algae for over 700 million years.
- Synthetic polycistronic transcripts containing markers or reporters were successfully expressed in vivo.
- The relative expression of each gene in a polycistronic transcript can be tuned, demonstrating its applicability for synthetic biology.

Gallaher, et al. ***Proc. Natl. Acad. Sci.*** 118: e2017714118 (2021)

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Green Algae Reveal One mRNA Encodes Many Proteins

Objective

In green algae, researchers found multiple instances of two or more proteins translated from a single messenger RNA (mRNA). This suggests two or more genes are being encoded on a mRNA (polycistronic). The idea counters the long-held belief that in eukaryotes, a single gene makes messenger RNA, which encodes a single protein.

Approach

A team led by Sabeeha Merchant of the University of California (UC) Berkeley and including researchers at UCLA and Brookhaven National Laboratory discovered this previously unknown similarity between algae and bacteria. Their work was enabled through the JGI-EMSL collaborative science FICUS initiative.

In a JGI-produced video, researchers Sean Gallaher, Sabeeha Merchant and Crysten Blaby-Haas describe the significance of a recent finding that, in two species of green algae, multiple proteins are translated from a single mRNA molecule.

bit.ly/JGI-Unexpected-Algae

Results/Impact

- Researchers found polycistronic gene expression is common in the green algae *Chlamydomonas reinhardtii* and *Chromochloris zofingiensis.*
- This ability to encode multiple genes in a single mRNA could improve processes for engineering algae to produce biofuels and other bioproducts.
- The team's findings also contribute to larger questions focused on the role of conserved plant genes in photosynthesis, which could help improve the growth of sustainable bioenergy crops, especially under stressful conditions.

Gallaher SD et al. Widespread polycistronic gene expression in green algae. *PNAS*. 2021 Feb 16;118(7):e2017714118. doi: 10.1073/pnas.2017714118.

ENIGMA ECOSYSTEMS & NETWORKS INTEGRATED WITH GENES & MOLECULAR ASSEMBLIES

Systematic Discovery of Pseudomonad Genetic Factors Involved in Sensitivity to Tailocins

Objective

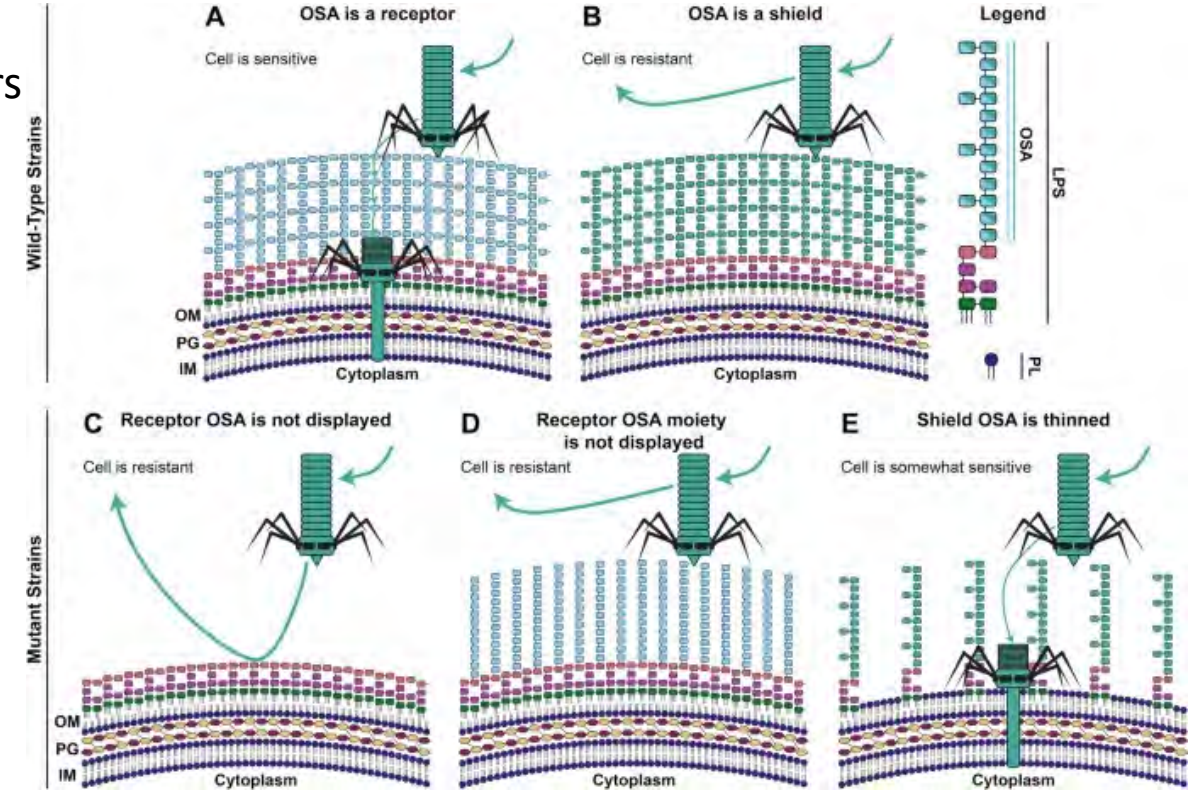
- Investigate tailocin (phage-tail-like bacteriocins) sensitivity factors
- Identify mechanisms behind resistance to self-intoxication

Approach

- Identify tailocin gene clusters in *Pseudomonas* genomes and characterize induced particles
- RB-TnSeq libraries to identify mutants with altered fitness in the presence of tailocins; specifically assess O-specific antigen (OSA) composition as well as phospholipid transport.
- Profile tailocin sensitivity in 130 sequenced *Pseudomonas* isolates in reference to OSA composition.

Results/Impacts

- First systematic effort to identify genetic factors involved in sensitivity to tailocins
- Identified genes related to tailocin infectivity in LPS core and the OSA biosynthetic gene clusters



Carim. et al. Systematic discovery of pseudomonad genetic factors involved in sensitivity to tailocins. **ISME J 15, 2289–2305 (2021).** https://doi.org/10.1038/s41396-021-00921-1

- Genes encoding outer membrane lipid asymmetry and LPS transport involved in sensitivity to tailocins
- Strains with the same overall OSA cluster typically display the same tailocin sensitivity pattern, but gene content can not completely explain sensitivity.

PRINCETON UNIVERSITY PennState UIC

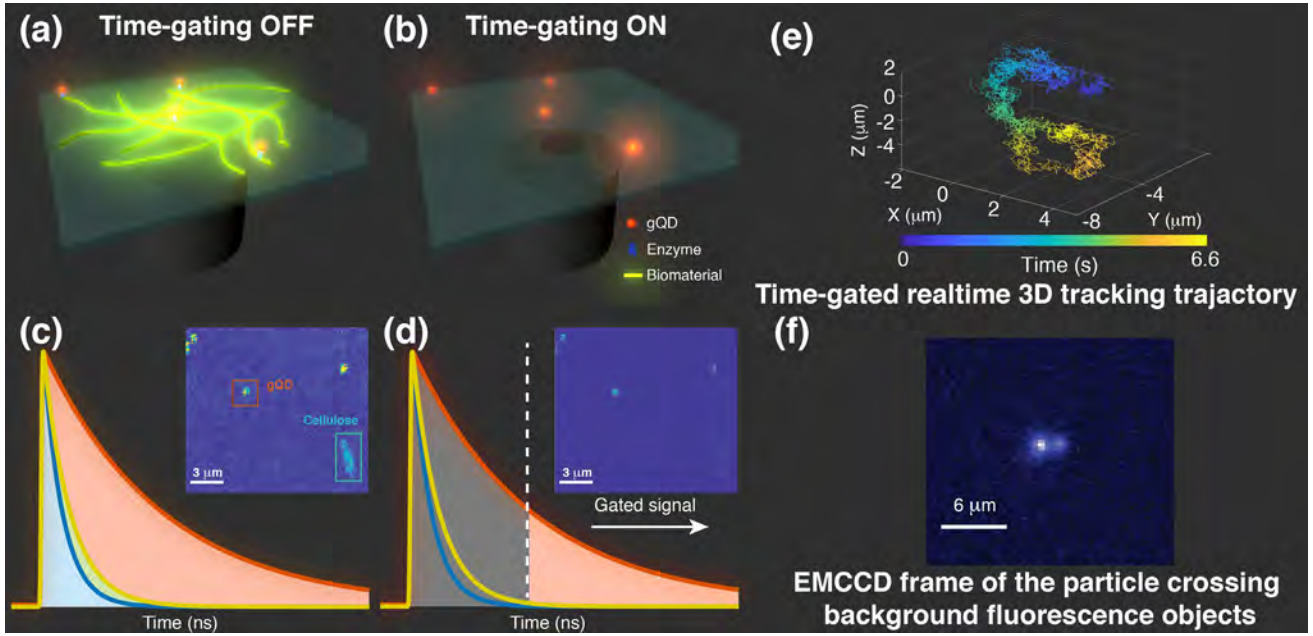
Lifetime-gated real-time 3D single-particle tracking

Objective

To understand dynamics in complex systems by tracking, in real-time and 3D, nano-scale particles moving in noisy environments.

Approach

A time-gating module of a real-time 3D single-particle tracking microscope analyzes the arrival times of individual photons as they arrive at detectors. This enables the rejection of photons from the noisy background environments and thus uses only photons from the probe for robust fast 3D tracking.



Results/Impact

- Emission lifetime gating was integrated into 3D single-particle experiments and was shown to reject early arriving photons from noisy backgrounds.
- Two-photon excitation was used for tracking to further increase penetration depth for imaging in complex 3D environments and to reject background light by decreasing the out-of-focus excitation.
- Using newly designed gQDs, time gating allows up to 200-fold increase in signal-to-background ratio, allowing tracking of particles moving with a diffusion coefficient of 3.3 um^2s^{-1} which is 33 times faster than had been previously possible.
- This will be useful for overcoming the high background emission from cellulose fibers, or plant cells in general, when monitoring plant metabolic processes, rhizosphere bacterial dynamics, and the cellulase-cellulose degradation mechanism.

Zhao et al. (2021) "Leveraging lifetime information to perform real-time 3D single-particle tracking in noisy environments", ***J. Chem. Phys.***, in press.

Methanobactin Complexation of Group 12 Transition Metals

Objective

Methanobactins are post-translationally modified peptides secreted by methanotrophic bacteria to facilitate Cu acquisition. Methanobactins can also bind other trace metals; the structure of these metallic complexes has implications for the transport, bioavailability and toxicity of these trace metals in the environment. This study sought to understand the structure and geometry of methanobactin binding to Zn, Cd and Hg.

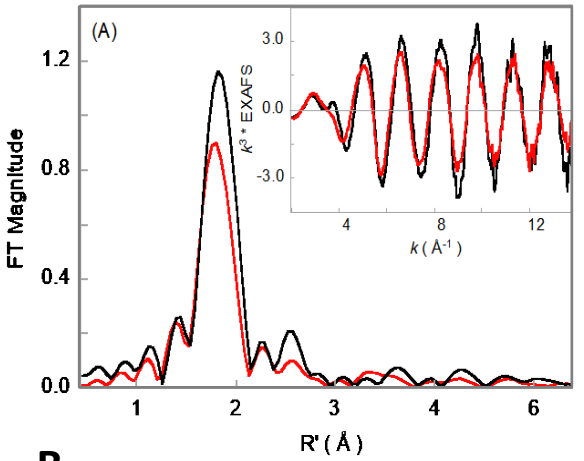
Approach

- Methanobactin was isolated from *Methylocystis* sp. (SB2), purified, and mixed with 1 mM metal salt solutions. Various ratios of methanobactin:metal and aging experiments (for time resolved data) were studied.
- Hg L_3-edge EXAFS in combination with UV-vis and fluorescence spectroscopy and TD-DFT calculations, provides a comprehensive overview of the resulting peptide-metal interactions.

Results/Impact

- Hg L_3-edge EXAFS spectroscopy and TD-DFT calculations revealed a linear 2- coordinate Hg-S site. Upon increasing the ratio of methanobactin:Hg, a mixture of 2- and 3-coordinate complexes is formed, while TD-DFT calculations predict a tetrahedral geometry for Zn and Cd.
- Absorption and fluorescence spectroscopies indicate the formation of dimeric complexes that become monomers when methanobactin:metal is equivalent.
- These results lay the foundation for future work exploring the impact of Cu binding metallophores on the speciation and biogeochemical cycling of transition metals, including highly toxic methylmercury.

A



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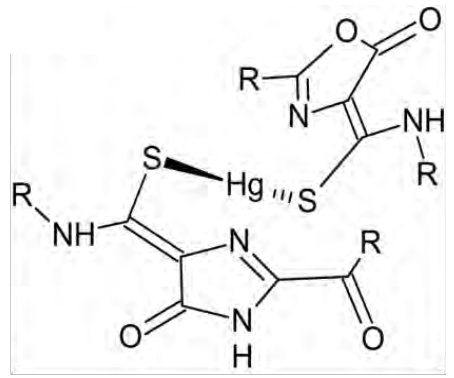

Eckert, P., Johs, A., Semrau, J.D., DiSpirito, A.A., Richardson, J., Sarangi, R., Herndon, E., Gu, B., and Pierce, E.M., **J. *Inorg. Biochem***. doi./10.1016/j.jinorgbio.2021.111496

Thank you

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