

# <u>The complex polyploid genome</u> <u>architecture of sugarcane</u>

#### **Background/Objective**

- Sugarcane, the world's most harvested crop by tonnage is currently responsible for 80% of sugar production worldwide.
- Modern sugarcane hybrids are the last remaining major crop without a reference-quality genome due to its complexity.

#### Approach

We generated a polyploid reference genome for R570, a typical modern cultivar derived from interspecific hybridization between *Saccharum officinarum* and *Saccharum spontaneum*.

#### Results

Our 8.7 billion base assembly contains a complete representation of unique DNA sequences across the approximately 12 chromosome copies in this polyploid genome.

# Significance/Impacts

This polyploid genome assembly and molecular targets for biotechnology will help accelerate molecular and transgenic breeding.

Healey A., et. al. Nature. doi: 10.1038/s41586-024-07231-4



Field-grown cultivar R570 sugarcane



Chromosome preparation of R570 (red probe = *S. spontaneum*)



Figure: (Left) An image of field-grown R570 (approximately 4 m in height), (right) Chromosome preparation of R570 after in situ hybridization



# An engineered laccase from Fomitiporia

mediterranea accelerates lignocellulose degradation

## **Background/Objective**

- Laccases catalyze lignin depolymerization and accelerate glucose release.
- We hypothesized that addition of a carbohydrate-binding module (CBM) to a laccase would bring the enzyme closer to lignocellulose and enhance biomass degradation and glucose yields.

#### Approach

A laccase from *Fomitiporia mediterranea* (Fom\_lac) and a variant engineered to have a CBM (Fom\_CBM) were studied for their ability to catalyze cleavage of  $\beta$ -O-4' ether and C–C bonds in lignin and to enhance glucose yields from untreated and cholinium lysinate ([Ch][Lys]) pretreated pine.

### Results

- Fom\_lac and Fom\_CBM catalyzed  $\beta$ -O-4'ether and C–C bond breaking, with higher activity under acidic conditions (pH < 6).
- Fom\_CBM to mixtures of cellulases and hemicellulases improved sugar yields by 140% on untreated pine and 32% on [Ch][Lys] pretreated pine when compared to the inclusion of Fom\_lac to the same mixtures.

### Significance/Impacts

Fom\_lac engineered to have a CBM accelerated biomass degradation at reduced enzymes loading, a potential cost saving in the biomass conversion process.

Pham L.T.M., et. al. Biomolecules. doi: 10.3390/biom14030324

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Figure 1. Proposed mechanism of lignin-catalyzed Fom\_lac in the presence/absence of CBM. (A) Long-distance electron transfer through an aqueous solution is inefficient from lignin to mediator. (B) CBM brings Fom\_lac in close contact with lignin and shortens the electron transfer pathway between mediator and lignin.



# Edible mycelium bioengineered for enhanced nutritional value and sensory appeal using a modular synthetic biology toolkit

#### **Background/Objective**

Filamentous fungi have the potential to power the bioeconomy, including producing sustainable food for a growing population

#### Approach

We developed a modular synthetic biology toolkit for an edible and industrial workhorse fungus, *Aspergillus oryzae* 

#### **Results**

Our toolkit enables precise, efficient genome integration and expression of genes in *A. oryzae* for metabolic engineering. We used it to engineer the nutritional value and sensory appeal of the edible mycelium for food.

#### Significance/Impacts

Our study generates new genetic engineering tools for filamentous fungi while also demonstrating their promise for food



Figure 1. Example of engineered edible *A. oryzae* mycelium using synthetic biology tools. We engineered heme production in the fungus, creating a meat substitute that looks and cooks like red meat, much like the plant-based commercially available IMPOSSIBLE meat.

Maini Rekdal V., et. al. Nature communications. doi: 10.1038/s41467-024-46314-8





# <u>Inhibition of Asphaltene Aggregation using Deep Eutectic</u> <u>Solvents: COSMO-RS Calculations and Experimental Validation</u>

# Background/Objective

- Asphaltene precipitation/deposition adversely affects various oil and gas processes
- Deep eutectic solvents (DESs) have recently gained interest as inhibitors of asphaltene aggregation

#### Approach

Derived the mechanism of inhibition of petroleum asphaltene aggregation by a novel screening of DESs using a combined computational and experimental approach.

### Results

It was found that DESs containing thymol were the most promising of the 153 DES combinations screened.

# Significance/Impacts

The present approach opens pathways to rationally design and understand the impact of structural variation of DESs based on their interactions with asphaltene. This study also provides new insights into potential routes for lignin solvation and conversion.

Kumar N., et. al. Journal of Molecular Liquids. doi: 10.1016/j.molliq.2024.124471





Figure 1. Solubility of asphaltene in different DESs at 298.15 K and ambient pressure. Error bars represent the standard deviation calculated from three independent experiments (p = 0.05). Significant differences between groups (p < 0.05) are indicated with lowercase letters.



# <u>A guayule C-repeat binding factor is highly activated in</u> <u>guayule under freezing temperature and enhances freezing</u> <u>tolerance when expressed in Arabidopsis thaliana</u>

#### Background/Objective

- We had previously identified a master regulator, PaCBF4, of rubber biosynthesis and cold response in guayule
- The project aimed to validate the function of PaCBF4 in a model plant prior to investigating its potential in crop improvement

#### **Approach**

The PaCBF4 transcription factor was expressed in Arabidopsis and the tolerance to cold and freezing investigated

#### Results

The transgenic plants exhibited very high tolerance to freezing. The plants showed normal vegetative growth.

# Significance/Impacts

PaCBF4 has a potential to improve cold tolerance in different crops. In guayule it is also likely to improve rubber production without the requirement for chilling.

Chen G.Q., et. al. Industr Crops and Products. 10.1016/j.indcrop.2024.118303





Figure 1: Guayule (*Parthenium argentatum*) is a woody shrub native to southwestern USA. It produces high quality rubber in response to chilling. The residue after rubber extraction can be used for lignocellulosic biofuel production. Photo source: USDA



Figure 2: Wild-type (WT) and two transgenic lines overexpressing PaCBF4 were exposed to  $-5^{\circ}$ C for 24 h and then returned to 24°C for 5 recovery days. Survival rate, scored as the percentage of plants showing healthy leaves after 5 days recovery from the freezing treatment (solid bar). Non-freezing controls were grown under 24°C (open bar). Number in parenthesis indicate survival rate of 0% for the WT. Data are mean ± SD of three independent experiments. Each treatment had 64 individuals.



# **ENABLED PUBLICATIONS**





# <u>teemi: An open-source literate programming approach for</u> <u>iterative design-build-test-learn cycles in bioengineering</u>

#### Background/Objective

Integral to synthetic biology is the aspiration to efficiently find, access, interoperate, and reuse high-quality data on genotype-phenotype relationships of native and engineered biosystems under FAIR principles, and from this facilitate forward-engineering strategies.

#### Approach

To enable user-friendly simulation, organization, and guidance for the engineering of biosystems, we developed an open-source python-based computer-aided design and analysis platform operating under a literate programming user-interface hosted on Github, called teemi.

#### Results

Natural language protocols team design Literate protocols open source life science test build

Figure 1. Conversion of natural language lab protocols for iterative design-build-test-learn cycles to literate protocols using teemi. Natural language protocols (left—blue) comprehensible to humans are converted into computer code (right—yellow) that can be understood by both computers and humans. In teemi, each procedure in natural language protocols is connected with names of python modules in literate protocols, thus lowering the programming entry level needed for adopting teemi.

We apply teemi for i) designing and simulating bioengineering, ii) integrating and analyzing multivariate datasets, and iii) machine-learning for predictive engineering of metabolic pathway designs for production of a key precursor to medicinal alkaloids in yeast.

# Significance/Impacts

The platform is called teemi and is fully compliant with FAIR principles, and it is is publicly available at PyPi and GitHub.

Petersen S., et. al. PLoS Comp Bio. doi: 10.1371/journal. pcbi.1011929





# Engineered Saccharomyces cerevisiae as a **Biosynthetic Platform of Nucleotide Sugars**

#### Background/Objective

Glycosylation is a ubiquitous modification found in proteins, lipids, and natural products, and the presence of sugars on these molecules can drastically change their bioactivity and stability

#### Approach

We engineered *Saccharomyces cerevisiae* to heterologously express nucleotide sugar synthases to access a wide variety of uridine diphosphate (UDP)-sugars from simple starting materials.

#### Results

We observed strong inhibition of UDP-D-Glc 6-dehydrogenase (UGD) by the downstream product UDP-D-Xyl, which we circumvented using an induction system (Tet-On) to delay the production of UDP-D-Xyl to maintain the upstream UDP-sugar pool.

#### Significance/Impacts

These engineered yeast strains are a robust platform to (i) functionally characterize sugar synthases in vivo, (ii) biosynthesize a diverse selection of UDP-sugars, (iii) examine the regulation of intracellular UDP-sugar interconversions, and (iv) produce glycosylated secondary metabolites and proteins.

Crowe, S. et. al. ACS Synth. Bio. doi: 10.1021/acssynbio.3c00666





Figure 1. Uridine diphosphate sugar biosynthetic pathways engineered into Saccharomyces cerevisiae



# Land Resources for Wind Energy Development <u>Requires Regionalized Characterizations</u>

#### Background/Objective

Machine learning and high-resolution geospatial modeling capabilities developed at JBEI were leveraged to explore the land footprint of wind energy in the U.S., including wind farms located on agricultural lands

#### Approach

Developed a method that combines machine learning-based imagery analysis and geographic information systems and examined the land area of 318 wind farms (15,871 turbines) in the U.S. portion of the Western Interconnection

### Results

Prior land use and human modification impact the land-use efficiency of wind projects. The developed estimation method enables more granular estimates of wind energy potential on different land cover types, including cropland.

### Significance/Impacts

Decarbonization in the U.S. will have a substantial land footprint and understanding the potential for dual land uses, and the resulting outputs is critical to developing national strategies for renewable energy generation and the bioeconomy.

Tao Dai, et. al. Environmental Science & Technology. doi: 10.1021/acs.est.3c07908





Figure 1: Three types of wind farms based on the level of human modification in the project area. (b) Distribution of capacity-based LUE. Mixed Projects are a mixed of new projects and old projects in the same area. Actively-Used Land represents a scenario with only actively used class being occupied and all regenerating classes are reclaimed. Initial Land Use is a scenario where all the initial disturbances are considered. Reference Year considers land use in the reference year of this study (2018). (c) Spatial distribution of LUE in the Western Interconnection.