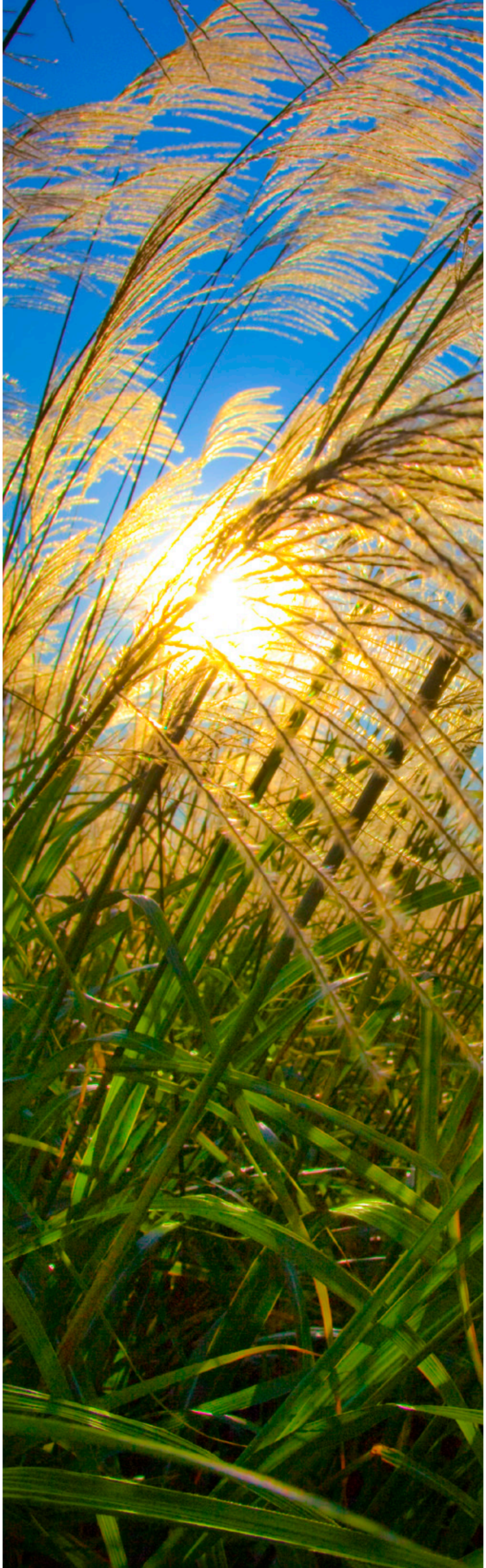
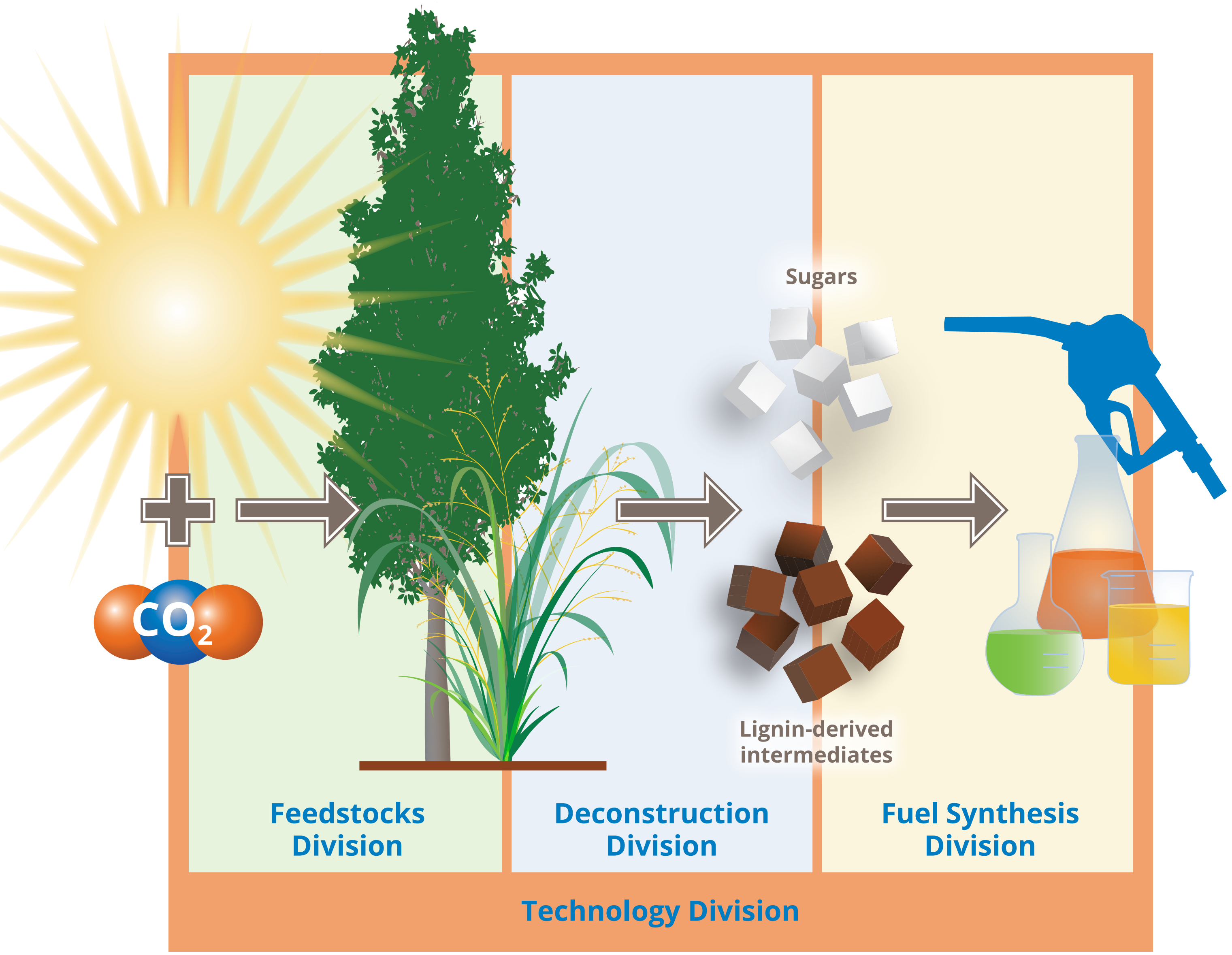


U.S. DEPARTMENT OF
ENERGY

jbei
Joint BioEnergy Institute



Mission Statement

JBEI's mission is to provide the scientific basis for converting lignocellulosic biomass to renewable, drop-in, liquid transportation fuels, as well as the production of renewable chemicals, that enable a thriving US bioeconomy.

The Joint BioEnergy Institute (JBEI) is a San Francisco Bay Area scientific partnership between Lawrence Berkeley National Laboratory (Berkeley Lab), the Sandia National Laboratories, the University of California (UC) campuses of Berkeley and Davis, the Carnegie Institution for Science, the Lawrence Livermore National Laboratory and the Pacific Northwest National Laboratory (PNNL). One of three U.S. Department of Energy (DOE) Bioenergy Research Centers, JBEI is headquartered in EmeryStation East, a state-of-the-art laboratory building in Emeryville.

The next generation of biofuels derived from lignocellulosic biomass, which includes plants, grasses, trees and agricultural residues, has the potential to reduce the U.S. current annual consumption of gasoline and diesel by about half and significantly decrease the environmental impacts of energy use. JBEI is generating new scientific insights and innovative conversion technologies that enable the production of these advanced biofuels — liquid transportation fuels derived from the solar energy stored in plant biomass. Harnessing this energy will help sustainably meet the nation's needs for liquid transportation fuels while significantly decreasing carbon emissions that contribute to global climate change. The technology transforming scientific advances taking place at JBEI are making these renewable bioenergy solutions possible. Four integrated divisions — **Feedstocks, Deconstruction, Fuels Synthesis and Technology** — perform groundbreaking fundamental research to promote these advances and build the foundation necessary for rapid commercialization.

Feedstocks Division

JBEI's research focuses on the efficient conversion of lignocellulose, the most abundant organic substance on the planet, into fuels.

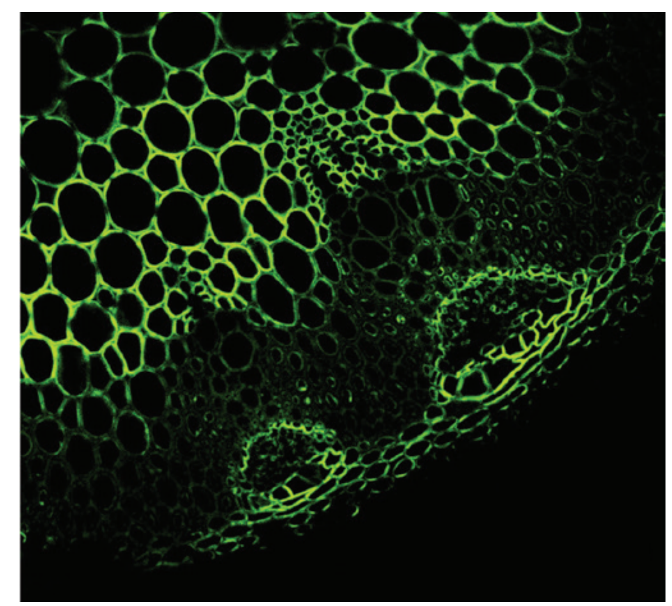
JBEI scientists are developing sustainable and high-yielding feedstocks for biofuel production.

Plant derived biomass is mainly composed of a mixture of complex sugar polymers (cellulose, hemicellulose and pectin) and lignin (robust aromatic polymer). All together these polymers form plant cell walls and the amount and/or the composition of each of them will define physical and biological properties that allow plants to develop a structural frame to collect sun light, grow in a large diversity of environments, transport water from below to above ground through their vessels, etc. Lignocellulose is highly abundant on earth and its sugar fraction (75%) could be converted into biofuels that have the same energy content as gasoline, diesels or jet-fuels and can be easily distributed through the existing pipeline and gas station infrastructure. However, lignocellulose is by nature highly resistant to being broken down into its primary constituents, sugars, a process known as "deconstruction."

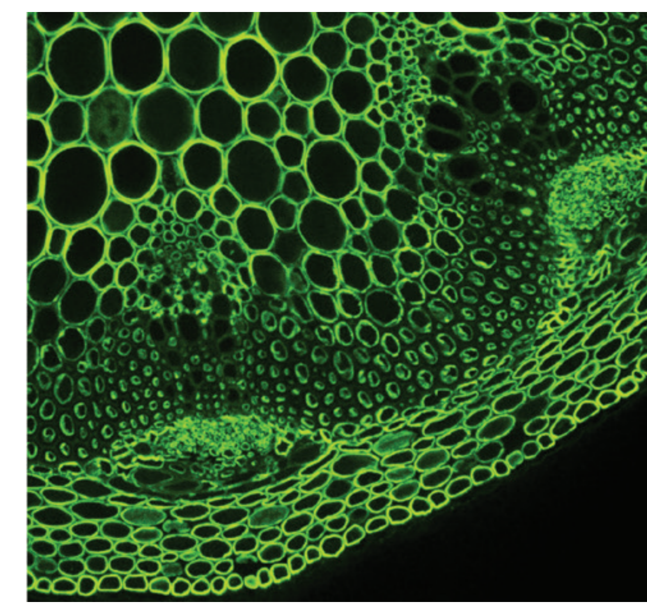
Researchers are generating basic knowledge to understand biosynthesis, regulation, structure-function and distribution of the different plant cell wall components. Together with synthetic biology this knowledge is used to develop the next generation of biofuel crops that will more easily deconstruct and yield greater quantities of fermentable sugars.

Rice is studied as a model plant for grasses such as switchgrass, and sorghum, which have great potential as energy crops. JBEI researchers also study a relative of mustard, called Arabidopsis, as a model for poplar and eucalyptus, trees that show great promises as future sugar source for the production of biofuels. These two model systems offer a great diversity of genetic tools to study cell wall biosynthesis and go from seed to mature plant in a matter of weeks, as compared to the year or more required for switchgrass, poplar and other feedstock plants.

In addition, JBEI researchers are focusing on developing novel approaches that can influence lignin content, composition and distribution with the major aim of improving cell wall susceptibility to lignolytic enzymes or other chemicals and lignin waste stream. This research effort will not only help with biomass deconstruction, but also help valorizing lignin into a valuable raw material. They are also designing and testing strategies to enrich plant biomass with hexose polymers that are non-recalcitrant to enzymatic hydrolysis. These polymers were selected because hexoses are generally more efficiently metabolized into biofuels than pentoses.

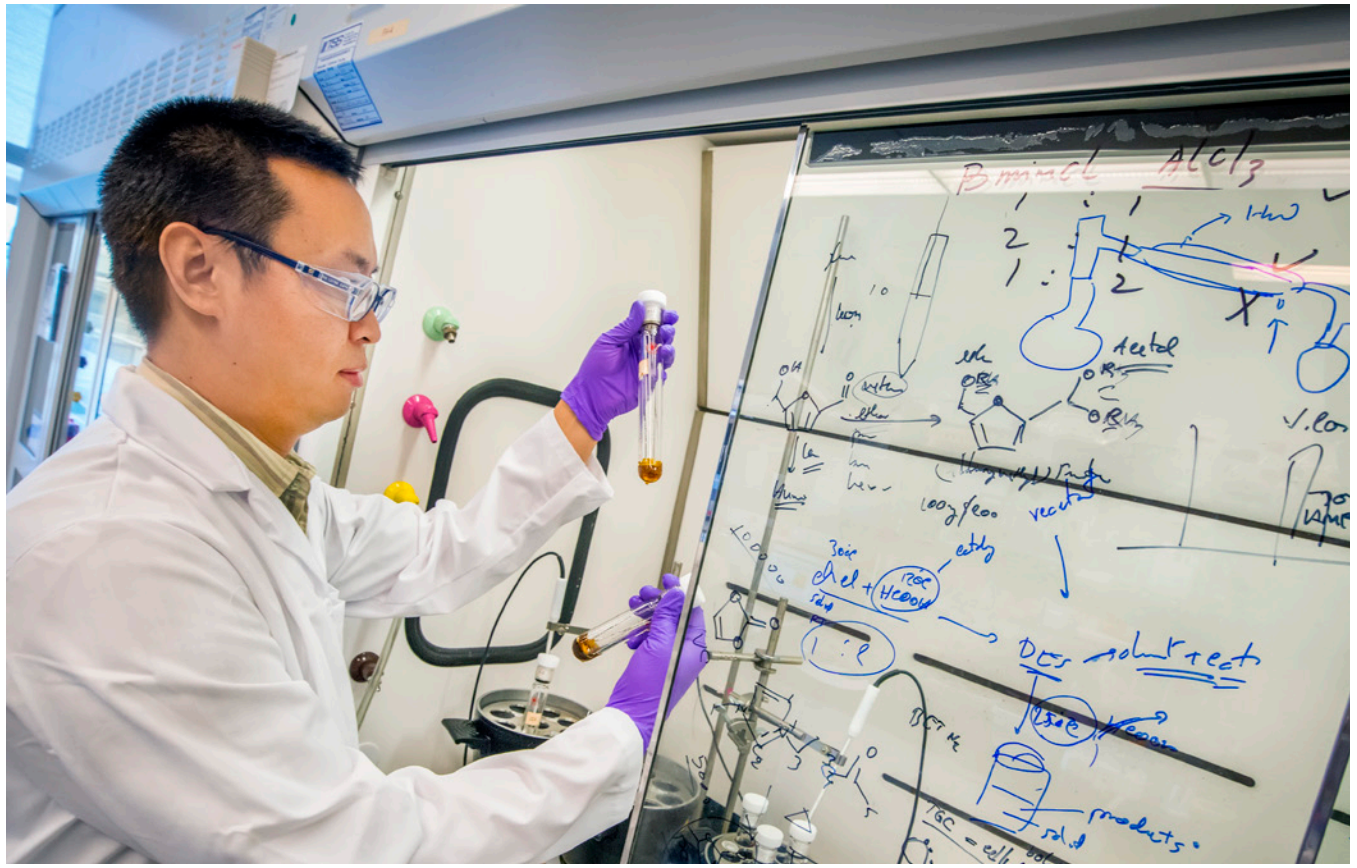


Control stem



Galactan enriched stem

Biomass enrichment with hexose-polymers. Several approaches were developed by the Feedstocks Division to enrich plant cell walls with hexose rich polymers. This image depicts an example in which 2 genes (UGE2 and GalS1) were stacked and co-expressed to boost the carbon flux through the galactan biosynthesis pathway. This engineering resulted in an enrichment of plant cell wall with galactan as shown by immuno-detection using the LM5 antibody since engineered stem (right image) exhibits a stronger signal in the than control stem (left image).



Deconstruction Division

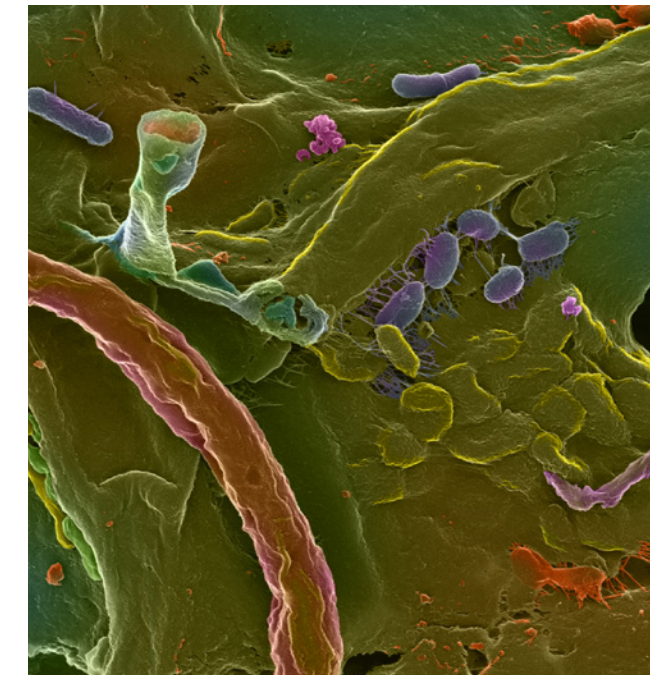
Unlike the simple starch-based sugars in corn and other grains, the complex polysaccharides in the plant cell walls of lignocellulosic biomass are locked within a tough material called lignin.

JBEI scientists are developing new and improved ways to generate targeted intermediates from biomass — a process called "deconstruction".

The deconstruction process normally requires two steps: pretreatment, followed by enzymatic hydrolysis. The development of novel ionic liquids, molten salts that are liquid at room temperature, as a novel means of pretreating biomass is one of JBEI's focuses. Deconstruction and feedstock researchers are collaborating to investigate the effects of ionic liquids on wild type and engineered biomass. Deconstruction researchers are also collaborating with fuels synthesis researchers to investigate the fractionation of lignocellulose into targeted output streams suitable for bioconversion into biofuels and renewable chemicals through the discovery and development of novel process technologies. This includes ionic liquids that can be produced from renewable feedstocks that lower costs of the overall biorefinery process.

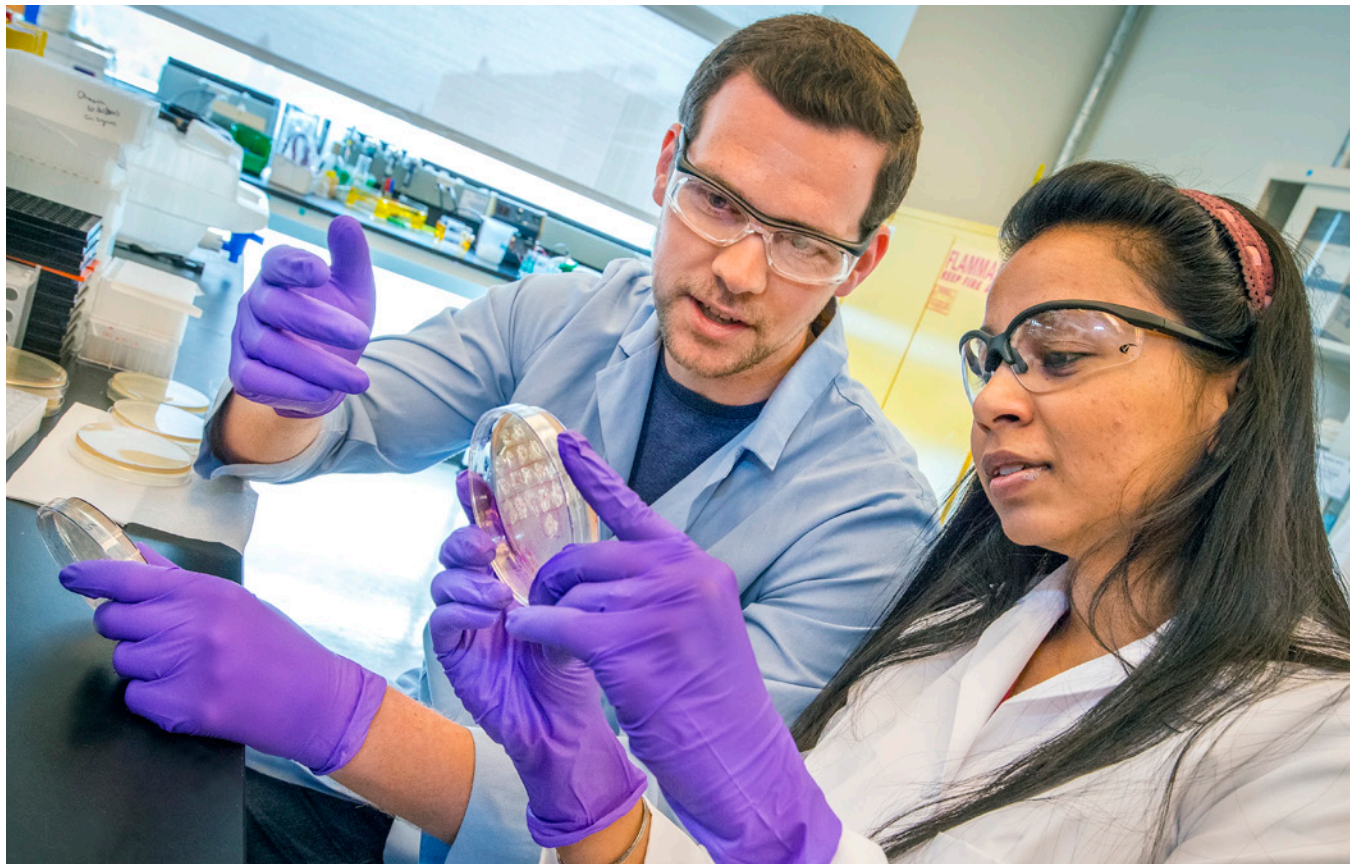
Deconstruction researchers are also exploring known ecosystems, such as rain forest floors, marshes, and composts, for new enzymes that are capable of efficiently hydrolyzing both the sugar and lignin components of plant cell walls in the presence of ionic liquids. The enzymes required to convert polysaccharides into fermentable sugars represent a major portion of the current cost of producing advanced biofuels. A key to lowering production costs is to reduce the quantity of enzyme used. Researchers are also developing enzymes that can break down lignin into targeted products that simultaneously enable more efficient production of fermentable sugars by disrupting the lignin-carbohydrate complex and provide a unique opportunity to valorize lignin into advanced biofuels and renewable chemicals. Cost-effective enzyme mixtures are being developed by analyzing both protein structure and genetic sequences of enzymes to better understand the properties that will allow enzymes to tolerate potential industrial conditions, such as extremes of temperature, pH and the presence of ionic liquids. JBEI researchers, in collaboration with the DOE funded Joint Genome Institute and the Environmental Molecular Sciences Laboratory, are studying microbial communities, through a targeted combination of genomics, proteomics, and transcriptomics to identify promising new enzymes.

After effective new enzymes are generated, they will be studied at the molecular level to gain a better fundamental understanding of the different biological mechanisms used by nature to deconstruct lignocellulose. Deconstruction researchers are using high-throughput enzyme screening methods, developed in collaboration with JBEI's Technology Division, to characterize the activities of enzymes and enzyme mixtures identified through genomics and proteomics.



This helium ion micrograph illustrates how communities of fungi and bacteria cooperate to deconstruct biomass. Researchers at JBEI exploit these natural strategies, utilizing bacterial and fungal enzymes to liberate sugars from biomass for conversion into biofuels and bioproducts. *Image: Bruce Arey, Sue Karagiosis, Scott Baker and Nathan Johnson from EMSL, PNNL*

High-throughput assays are run under a variety of conditions and on many pretreated biomass samples to develop a database of activity and performance. The knowledge gained from characterizing large numbers of enzymes is used to engineer properties of interest through a combination of directed and random mutations to the gene encoding the protein. Researchers are also developing highly efficient methods to express these recombinant proteins using industrially relevant filamentous fungi to enable screening of these enzymes at biorefinery-relevant process conditions, such as high solids loading, and to provide new insights into the genetic motifs that can hinder recombinant protein expression in non-native hosts.

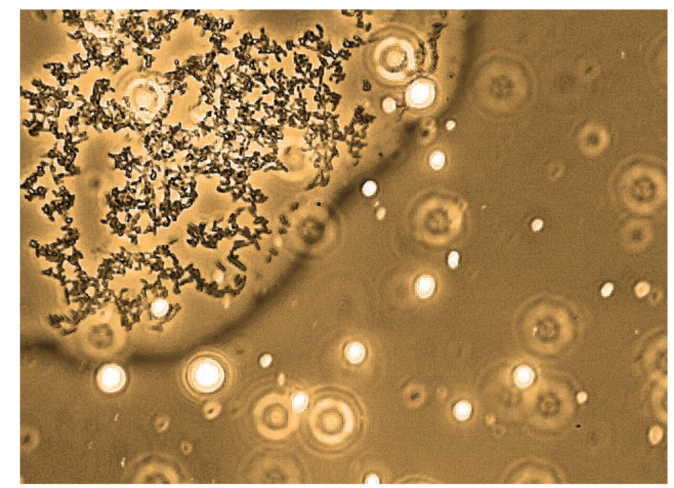


Fuels Synthesis Division

JBEI's Fuels Synthesis Division develops tools and methods to facilitate the engineering of microorganisms to efficiently convert sugars derived from cellulosic biomass into advanced biofuels tailored to match properties of petroleum-based fuels enabling their use in existing engines.

The first step in producing a fungible biofuel is to identify potential molecules for candidate fuels. Microorganisms are then engineered to produce the most promising molecules from cellulosic biomass and the resulting biofuel is then tested in engines.

Fuels synthesis researchers have analyzed the properties of several candidate biofuels and engineered metabolic pathways to optimize their production in microbial hosts. These metabolic pathways were created by assembling genes from plants, bacteria, archaea, and animals; and placed into two robust, industrial microorganisms, Saccharomyces cerevisiae (yeast) and Escherichia coli *(E. coli)*. Additionally, new metabolic pathways have been discovered and this knowledge is being used to produce novel biofuel molecules. JBEI has developed a unique and chemically diverse portfolio of biofuels including diesel replacements, bisabolane and fatty acid-derived methyl ketones; a gasoline replacement, isopentenol; and jet fuel precursors, α-pinene and limonene.

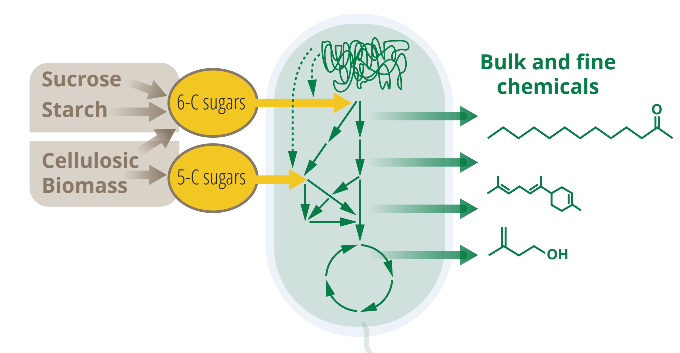


Once *E. coli* have secreted oil, they sequester themselves from the droplets as shown by this optical image, thereby facilitating oil recovery. *Image: Eric Steen, JBEI*

Cost is an important factor in consumer adoption of biofuels over the petroleum-based fuels that currently dominate the market. Economics require that advanced biofuel-producing microorganisms efficiently transform all varieties of sugar generated from cellulosic biomass into fuels, and be capable of producing fuel by the most cost-efficient means. To identify bottlenecks in metabolic pathways and improve yields of fuels, researchers developed computational metabolic models to trace how carbon is routed from sugars, such as glucose, to the final biofuel product.

Some advanced fuels and components of the biomass hydrolysates inhibit the growth of biofuel-producing microorganisms. To address this challenge, JBEI researchers engineered tolerance mechanisms into biofuel-producing microorganisms to increase tolerance to these products, such as efflux pumps to transport the fuel molecules out of the cell and to exclude biomass hydrolysate inhibitors from the cell. In addition, they have gained a better understanding of the growth inhibition associated with some of the biofuels and biomass hydrolysate and are using this information to engineer biofuel producers that are more optimized for fuel production conditions. Sensor-regulator systems have been engineered that sense biomass inhibitors, end-products or toxic intermediates in biofuel pathways and regulate these pathways to improve fuel yields; and regulators have been developed that alter the expression of large numbers of genes involved in central carbon metabolism.

Finally, the speed, cost, and reliability of genetic engineering have been a significant deterrent in the development of microorganisms that can produce biofuels and other chemicals. To reduce the cost and time involved and increase the reliability of genetic modifications, software and robotic methods have been developed to rapidly and in a high-throughput manner assemble DNA that encodes the fuel production pathways, together with tolerance mechanisms and optimal regulatory systems and genome wide edits, to develop the next generation of microbial platforms for biofuels and chemicals production.



Microbes can be optimized and engineered to efficiently convert sugars from renewable sources to many fuels and chemicals.

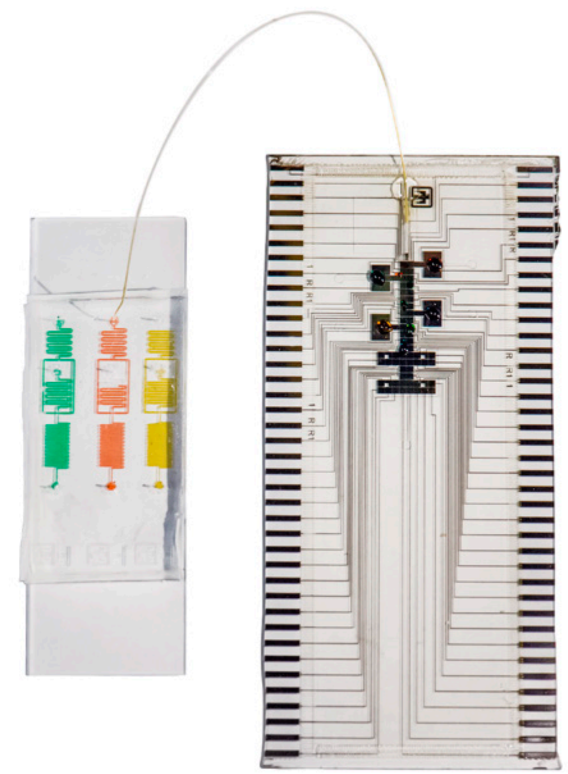
Technology Division

JBEI researchers in the Technology Division create and apply advanced technologies to enable the development of biofuels.

They pioneered nanoscale mass spectrometry approaches that enable JBEI researchers to rapidly discover, design and characterize lignocellulose degrading enzymes.

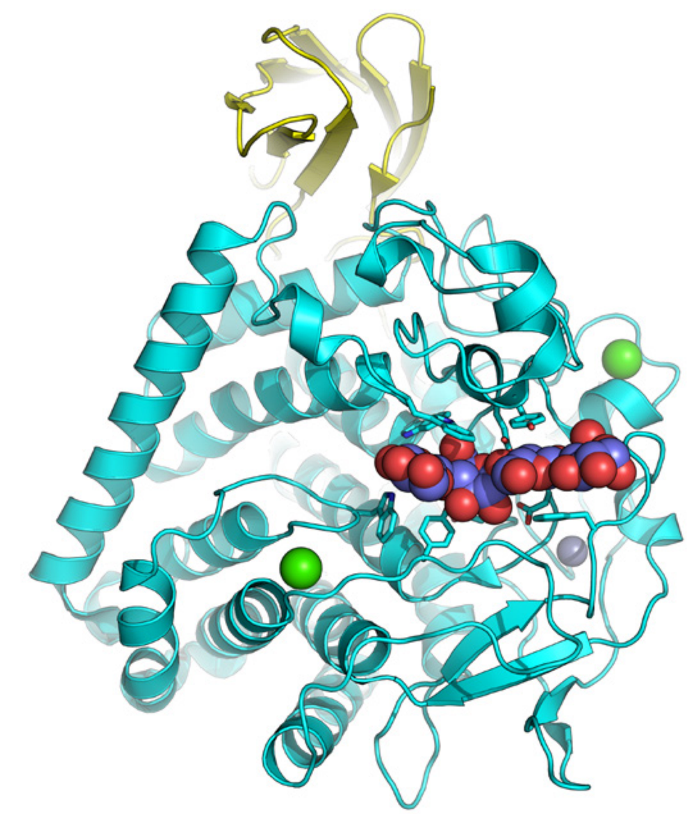
The recent coupling of these technologies with DNA synthesis and cell-free protein expression enabled characterization of thousands of enzyme treatment conditions for efficiently making biomass fermentable sugars. Microfluidic approaches are being developed that enable assembly of metabolic pathways to convert the resulting sugars into target biofuels.

State of the art methods are being developed to provide protein identification and quantification for JBEI research programs. This work includes identification of novel biomass-degrading enzymes for the Deconstruction Division, identification and quantification of organelle proteomes for the Feedstocks Division, and quantification of key proteins in engineered microbes for the Fuels Synthesis Division. Research also focuses on developing methods that increase sample throughput to speed iterations of the metabolic engineering cycle (design, build, test, learn) and fulfill JBEI's mission.



A droplet-to-digital microfluidic device for analyzing the effects of ionic liquid on single cells. *Image: Steve Shih, JBEI*

Technology researchers are developing and applying methods for characterizing plant biomass and biofuels-relevant enzymes across a broad range of resolutions. Electron microscopy methods are being used to visualize plant cell walls to develop 3-dimensional models of native and mutant plants to be used by the Feedstocks Division to help predict the impact of genetic mutations or natural variants. Lignocellulosic degrading enzymes are being characterized at atomic resolution using X-ray crystallography, and combined with functional studies by the Deconstruction Division to help design better saccharification processes. Enzymes involved in the synthesis of the plant cell wall are being studied in collaboration with the Feedstocks Division, and enzymes used for the microbial production of fuels are being characterized in collaboration with the Fuels Synthesis Division.



The X-ray crystal structure of the cellulose degrading enzyme Cel9A from the thermoacidophilic microbe Alicyclobacillus acidocaldarius, with substrate bound in the active site. *Image: Paul Adams, JBEI*

Technology Commercialization at JBEI

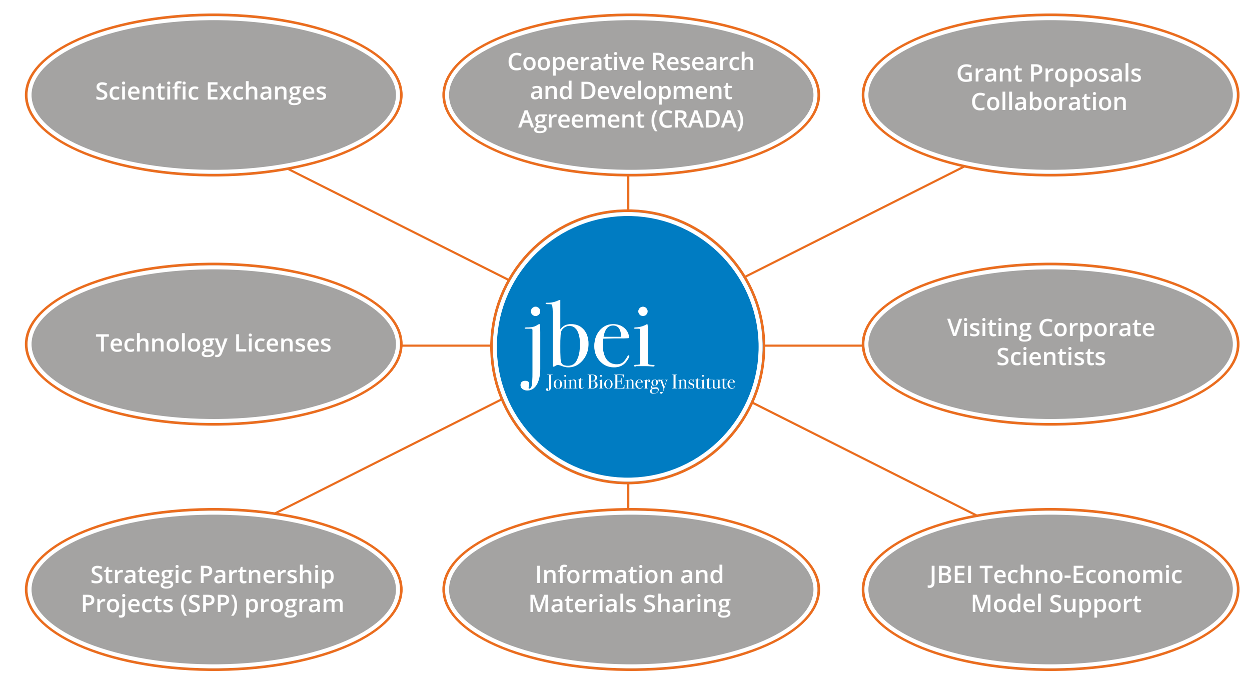
JBEI is unique in combining leading researchers from seven partner institutions with corporate collaborators on one floor of one state-of-the-art building in highly integrated, cross-disciplinary, big-team, basic science for challenging commercial applications.

World-leading scientists from multiple research programs are interspersed and work side-by-side in JBEI's environment of open scientific exchange, with multiple research programs coordinated both by top scientific management as well as through daily interactions at the bench. JBEI's exceptionally productive research culture leads US research institutions in inventions per research dollar, creating high-impact discoveries and innovations in bio-based products to benefit the public, environment, and industry.

JBEI's Industry Partnership Program facilitates commercialization of JBEI's scientific advances and technical inventions through interactive collaborative research with company partners, funded research, intellectual property licenses, scientific exchanges and visiting corporate scientists, access to biological material, and career advancement of JBEI Post-Doctoral students to industry.

JBEI's Industry Advisory Committee provides strategic guidance and industry perspective on JBEI research programs, including representatives from leading companies in the agricultural, biotechnology, transportation, chemical, materials and energy business sectors.

Industry Partnership Opportunities at JBEI



JBEI Industry Interactions

JBEI’s Education and Outreach Program

As part of its mission, JBEI is committed to preparing the next generation of scientists, promoting the benefits of biofuels and expanding interest in science.

JBEI’s education and outreach programs include curriculum development, K-12 bioenergy educational programs, internships, scientific academies, seminars, and collaborations with academic and industry-based science institutions.

JBEI’s highly successful internship program, iCLEM (Introductory College Level Experience In Microbiology), was developed in partnership with UC Berkeley. iCLEM is an eight-week paid summer biotech internship program for high-potential, low income high school sophomores and juniors. The program broadens students’ understanding of biotechnology and career opportunities in the field. iCLEM has seen 97% of its alums attend college, with nearly 80% of those majoring in STEM (science, technology, engineering and mathematics) fields. JBEI also partners with the Biotech Partners program to provide underserved youth with research experiences to increase participation in higher education and access to fulfilling science careers.

UC Berkeley’s Lawrence Hall of Science (LHS) and JBEI are partnering to develop a new biofuel education program targeting 4th – 7th grade students. A leader in K-12 STEM education, LHS engages 150,000 students at schools, hosts 200,000 student, teacher and public visitors at its public science center each year and reaches 20% of the nation’s K-12 students with its curriculum. JBEI’s partnership in the future will include the development of biofuel-centered curriculum supporting the Next Generation Science Standards, as well as biofuel-centered public outreach activities.

JBEI provides research internships for undergraduates and graduates students. Mentored by JBEI scientists, interns work in the lab on projects related to JBEI’s research program. Both US and international students are welcome to apply for internships.

Ongoing education on the state-of-the-art is key to JBEI’s discovery and innovation efforts, and to the growth of the cellulosic biofuels industry. Beyond recruiting top talent, JBEI keeps researchers abreast of advancements in the fields of biofuel discovery and industry’s current challenges and opportunities through continuing education and training. JBEI contributes to national and international scientific meetings, provides in-house seminars and tutorials, and collaborates with academic and industry partners across the world.

Informing policymakers, industry members and the public debate is critical to the nation’s effort to successfully deploy and adopt advanced biofuels. By promoting and sharing research breakthroughs and associated benefits in the public forum and through media outlets, JBEI contributes to disseminating broadly the key message that advanced biofuels are a cleaner, greener and renewable form of bioenergy that contribute to boosting the nation’s economy, safeguarding the environment and supporting national security.

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