

Joint BioEnergy Institute

Biological & Environmental Research Advisory Committee

29 November 2007

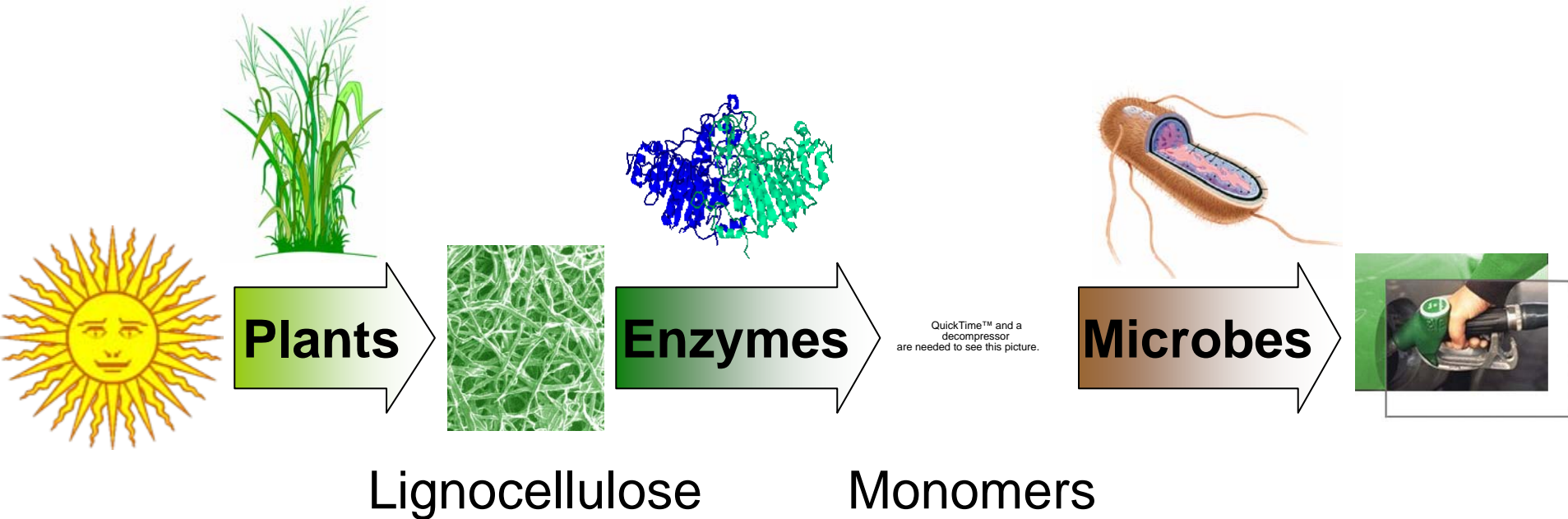
Washington, DC



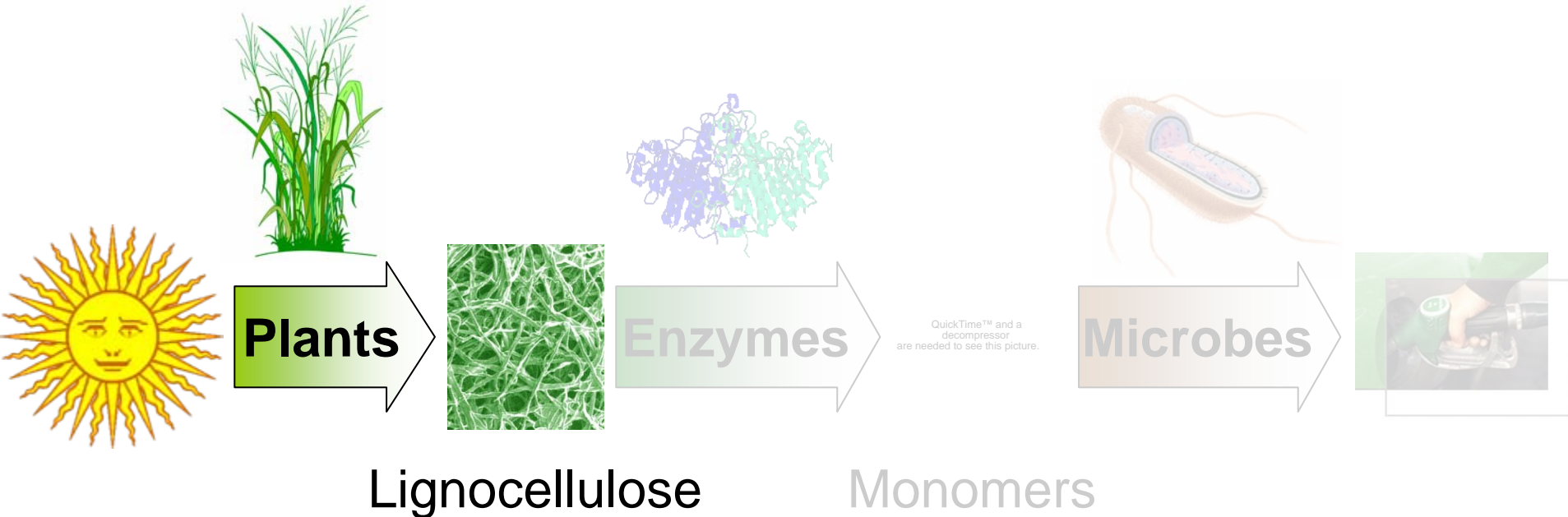
JBEI at a glance

- **Start-up company approach**
 - Highly focused research agenda
 - Single operation and facility
- **Four Science and Technology Divisions**
 - Feedstocks
 - Deconstruction
 - Fuels Synthesis
 - Cross-cutting Technologies
- **Six Partners**
 - Three DOE National Laboratories
 - Two Universities
 - One Foundation
- **Industry Partnership Program**
 - Underpin growth of the biofuels industry
 - Ensure technology transfer to biofuels industry

Lignocellulosic Biomass to Fuels



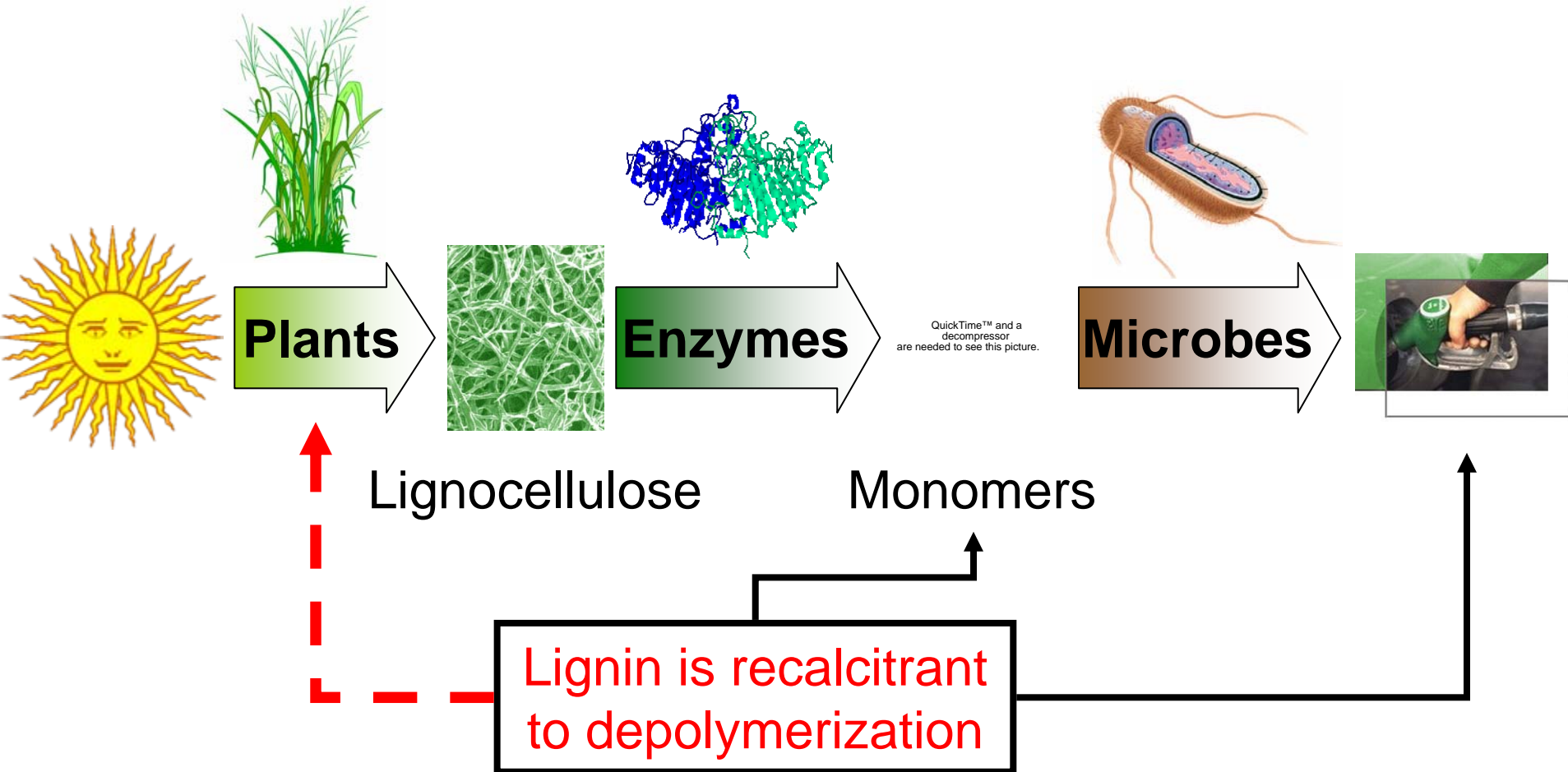
Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



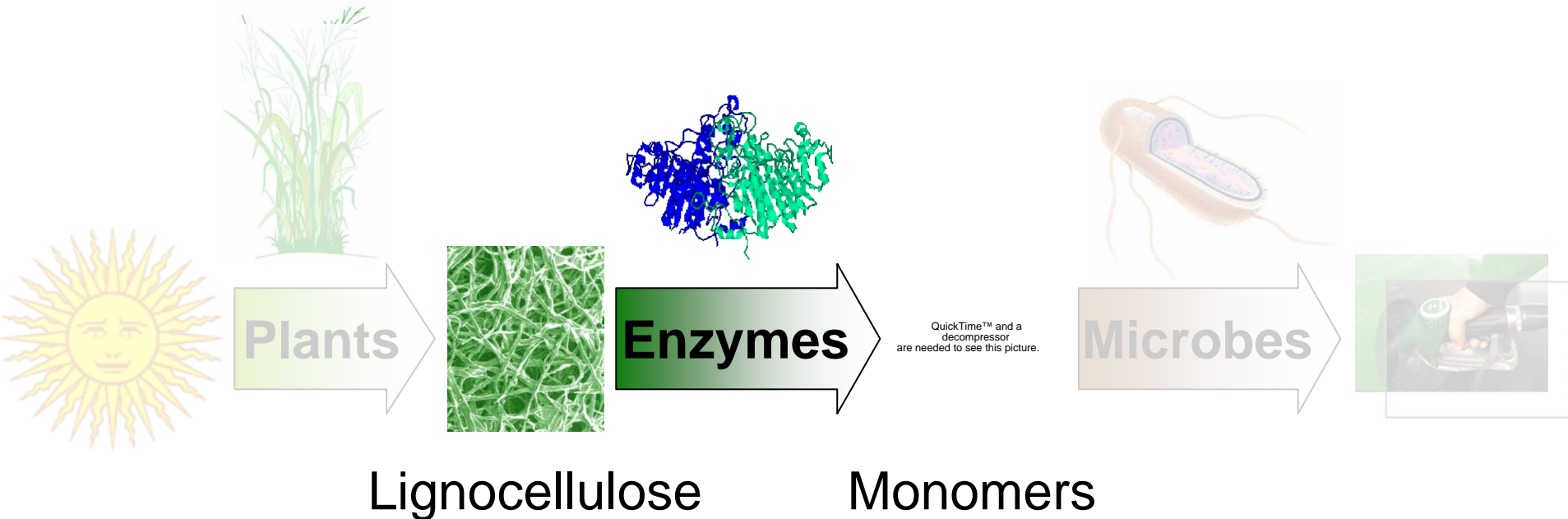
Challenges

- Cellulose and hemicellulose are occluded by lignin
- Lignin is recalcitrant to depolymerization
- Inhibitors released from biomass

Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



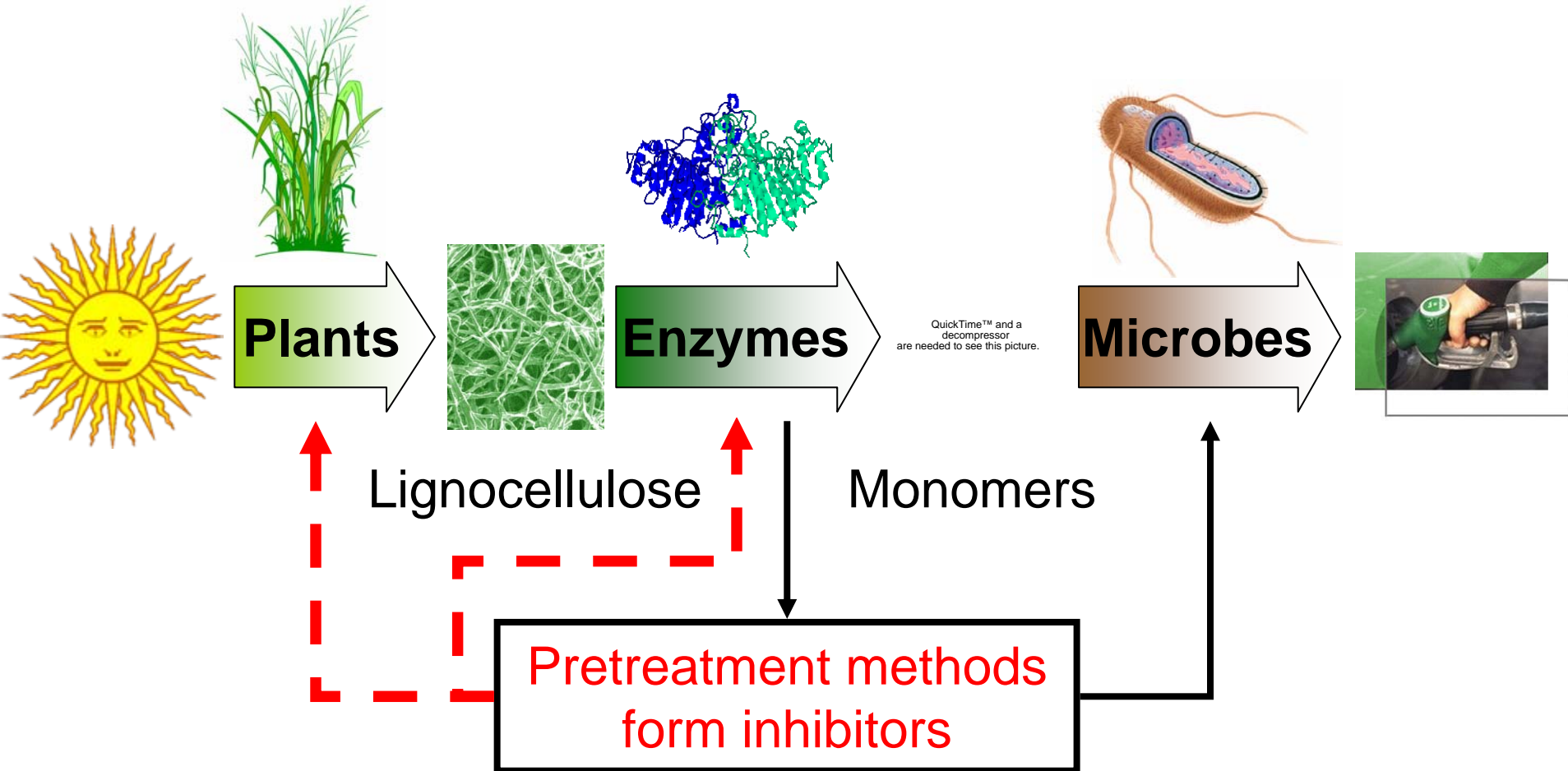
Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



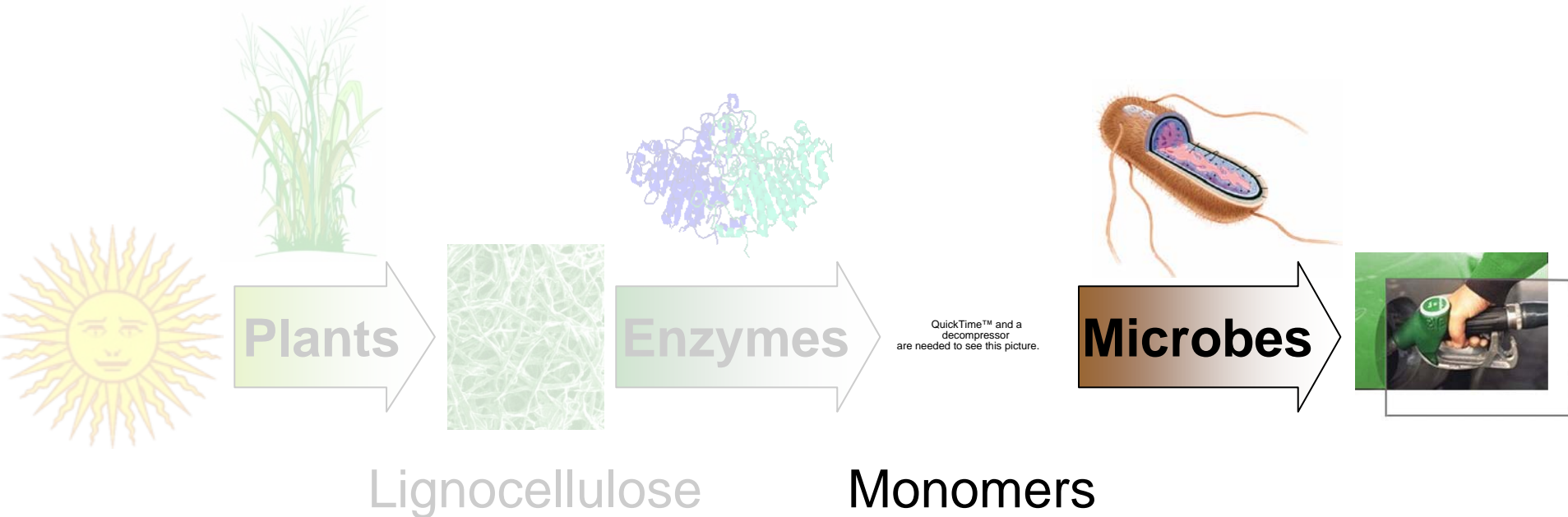
Challenges

- Lignocellulose is difficult to depolymerize
- Pretreatment methods form inhibitory by-products

Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



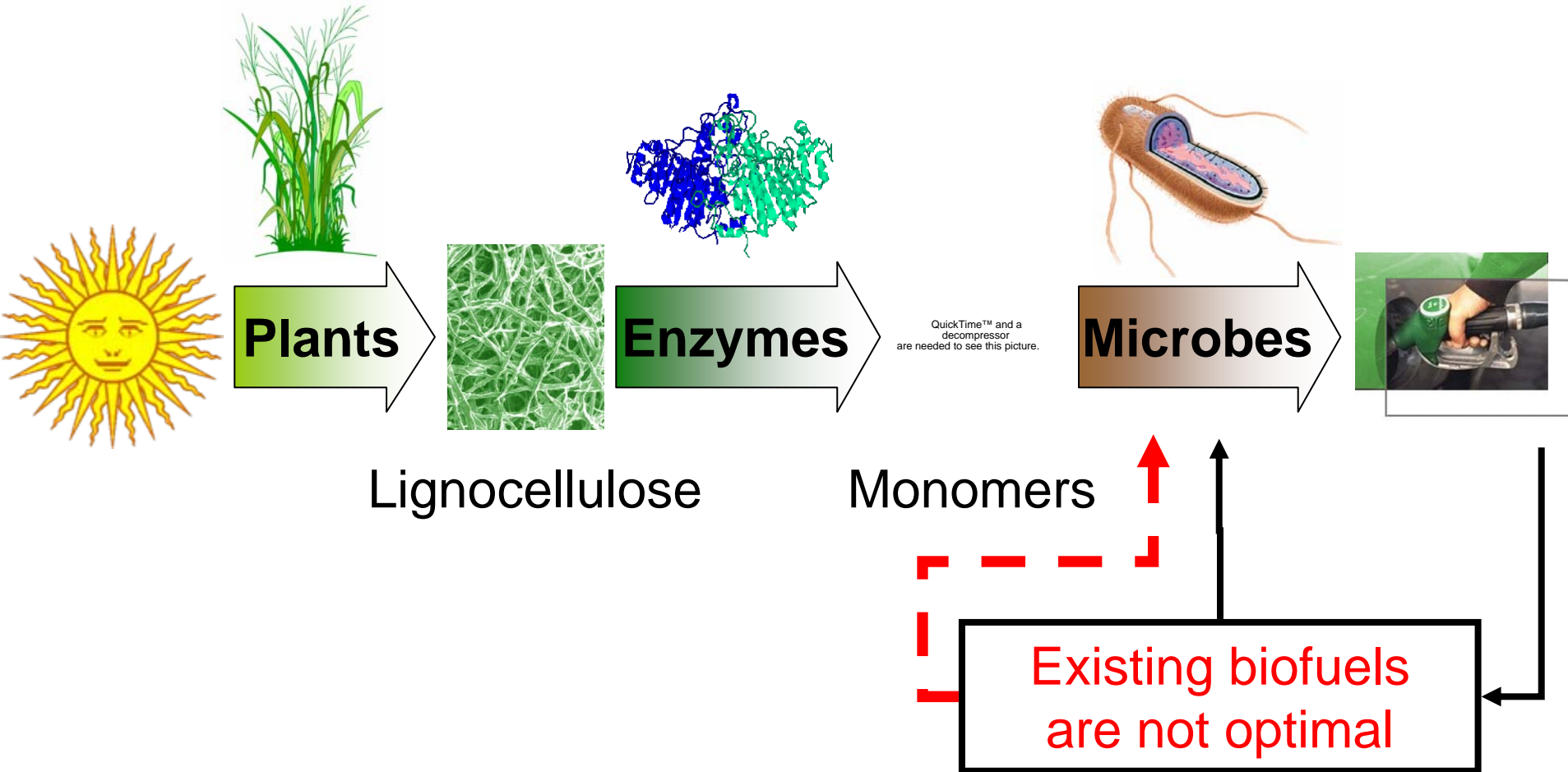
Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



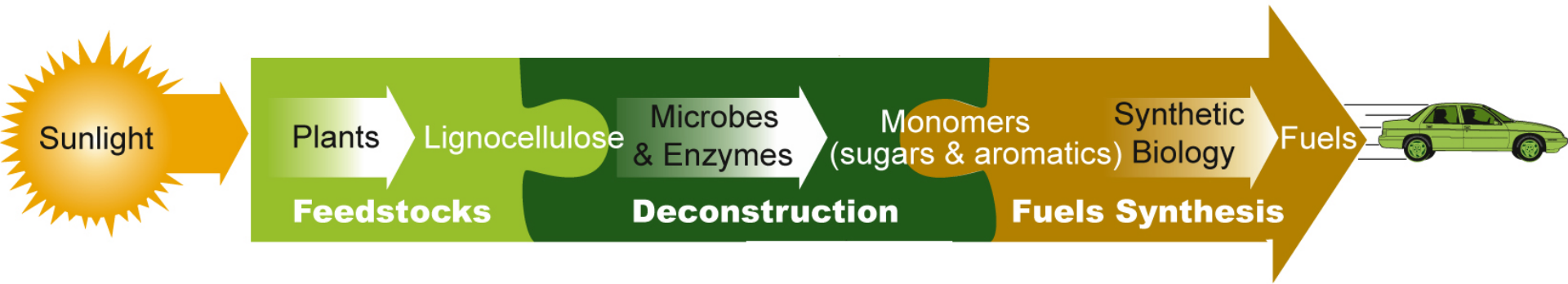
Challenges

- Existing biofuels are not optimal
- Organisms can only utilize a fraction of the monomers
- Inhibitors released from biomass limit fuels production

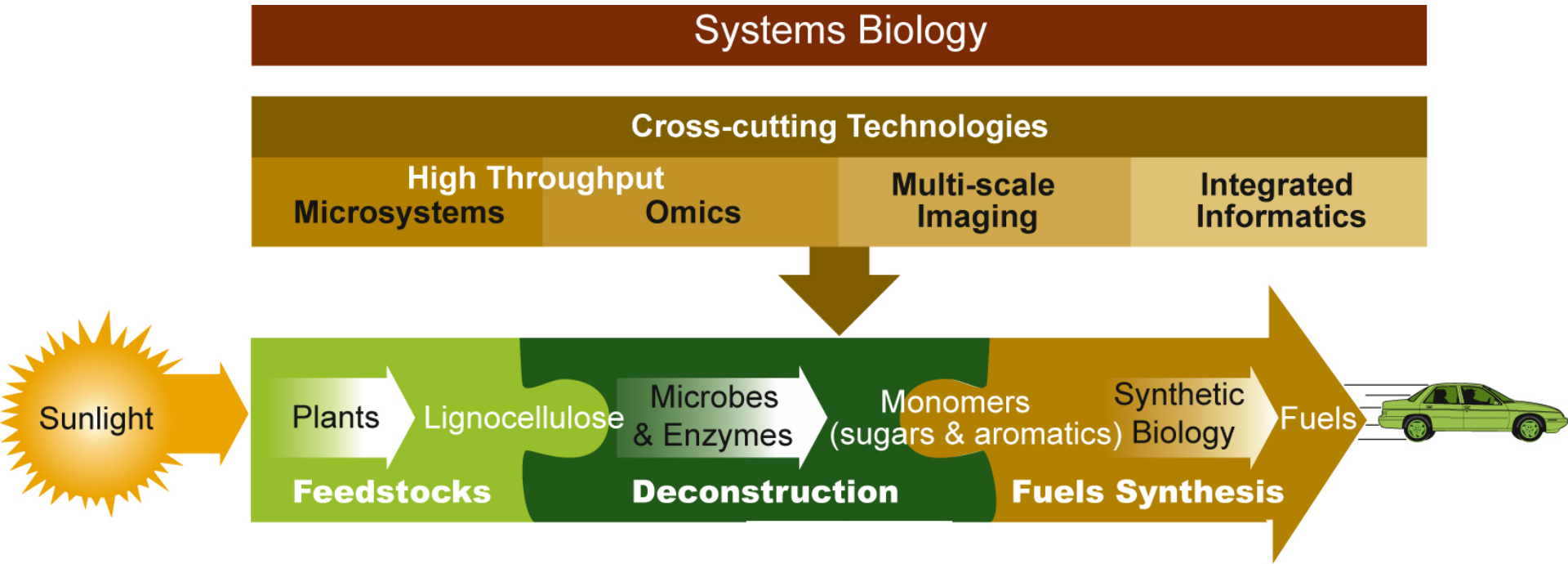
Some Key Challenges in Converting Lignocellulosic Biomass to Fuels



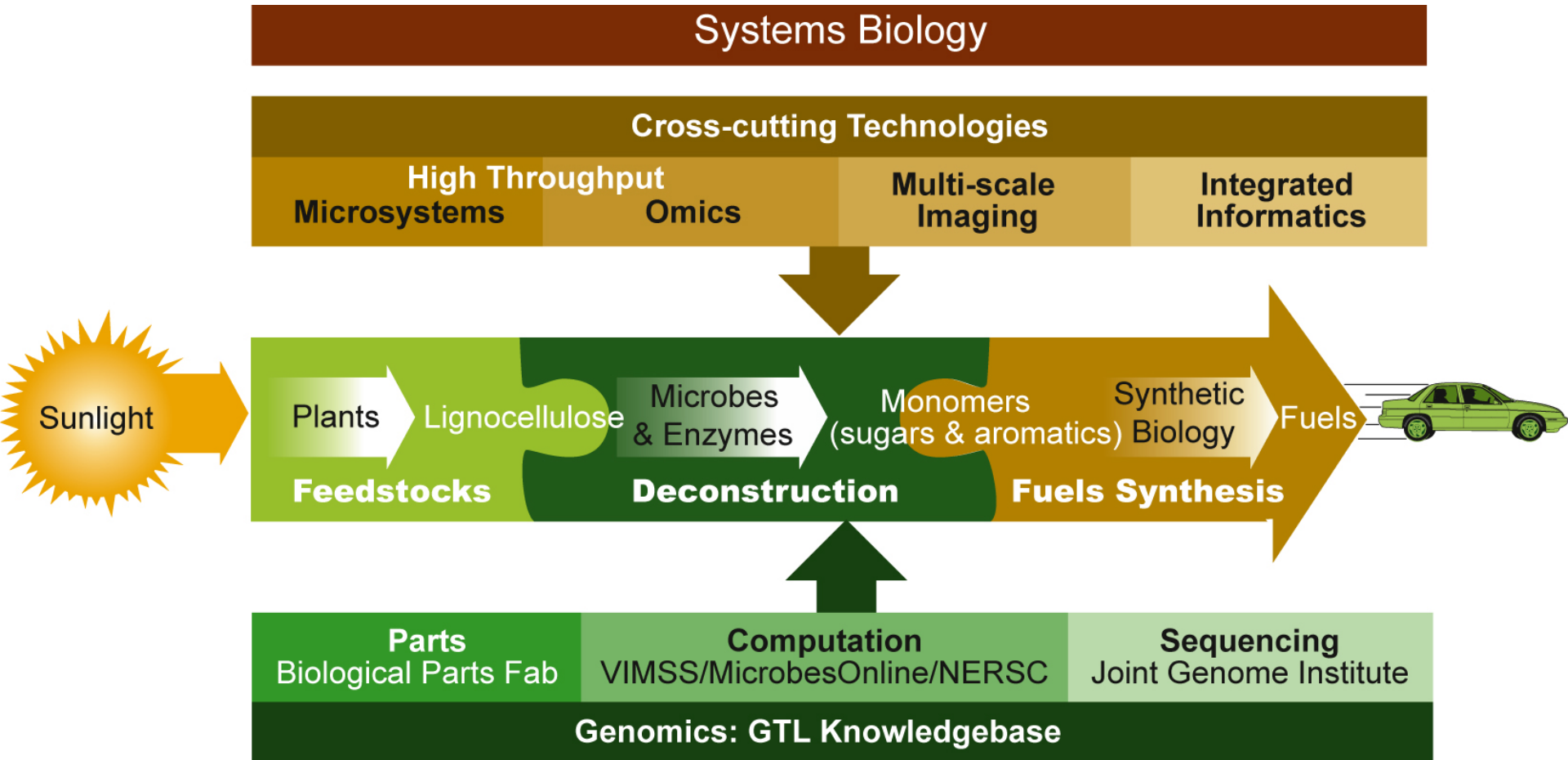
JBEI: an interlocking approach with three scientific divisions



JBEI: an interlocking approach supported by a Technologies Division



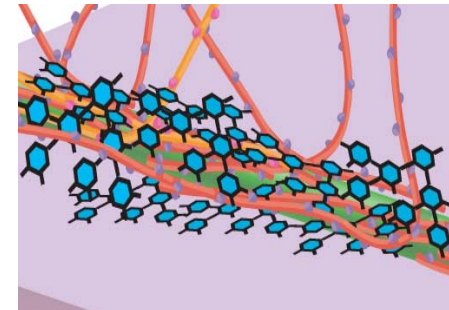
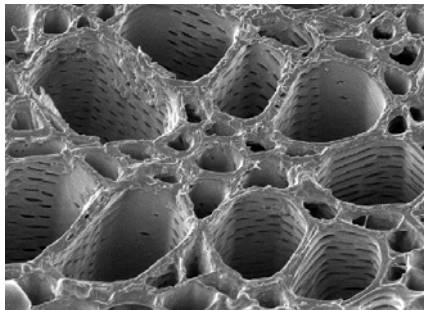
JBEI: an interlocking approach underpinned by Genomics:GTL



Feedstocks: Developing Bioenergy Crops

Challenges

- Cellulose and hemicellulose are occluded by lignin, making deconstruction difficult
- Functional groups on hemicellulose can inhibit fermentation & are not efficiently converted to fuels
- Lignin is recalcitrant to depolymerization



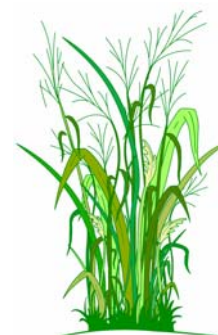
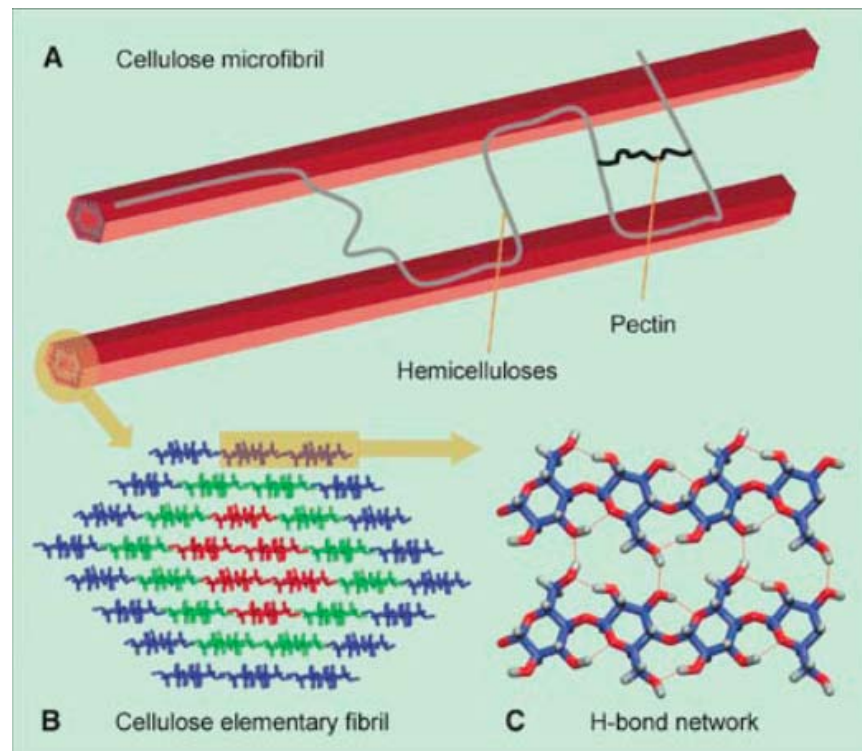
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Somerville et al. 2004, Science

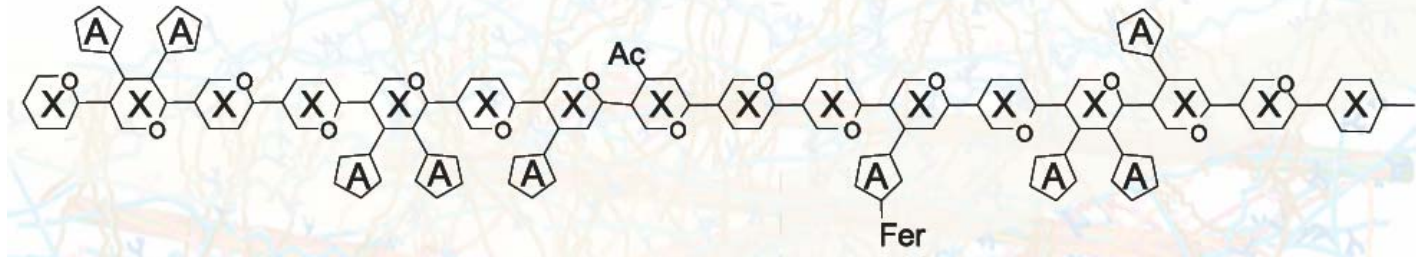
Feedstocks: Developing Bioenergy Crops

Approach

- Understand & modify polysaccharide biosynthesis
 - Focus on hemicellulose
- Reduce feruloylation by engineering alternative pathway
- Modify lignin to aid deconstruction.
 - Introduction of cleavable linkages
- Switchgrass, rice and Arabidopsis as model plants
 - Switchgrass sequencing



Example: reduce the complexity of cell wall building blocks or change their composition



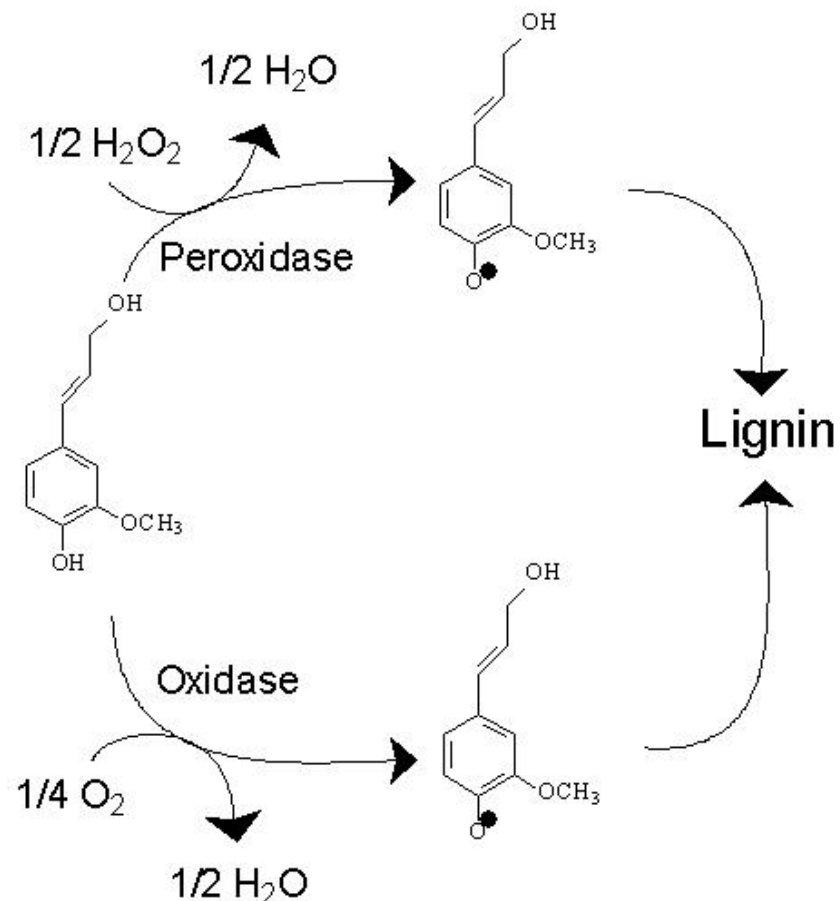
Arabinoxylan, a type of xylan found in grasses

- Xylans contain acetate esters and grass xylans contain additional ferulic acid esters
- Acetate esters are problematic for deconstruction and subsequent fuels fermentations
- Ferulate & diferulate esters are crosslinked with lignin. This results in grass cell walls being difficult to enzymatically digest

Objective:
change composition & crosslinking

Example: change the monomers in lignin

- Systematic analysis of (lignin) cell wall oxidases
- Develop replacement strategies for lignin
- Apply advanced imaging technology to determine structure of plant cell walls



Objective:
change the monomer composition of lignin

Feedstocks: developing bioenergy crops

Deliverables

- Improved understanding of all cell wall synthesizing and modifying enzymes in rice and *Arabidopsis*
- Transgenic plants with optimized cell wall composition for deconstruction
- Translate genetic developments from model plant systems to proposed bioenergy crops



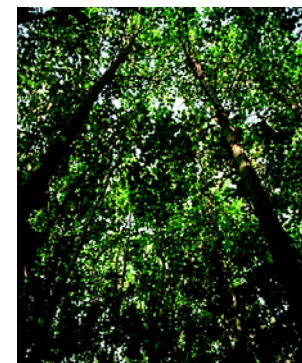
Rice



Switchgrass



Arabidopsis

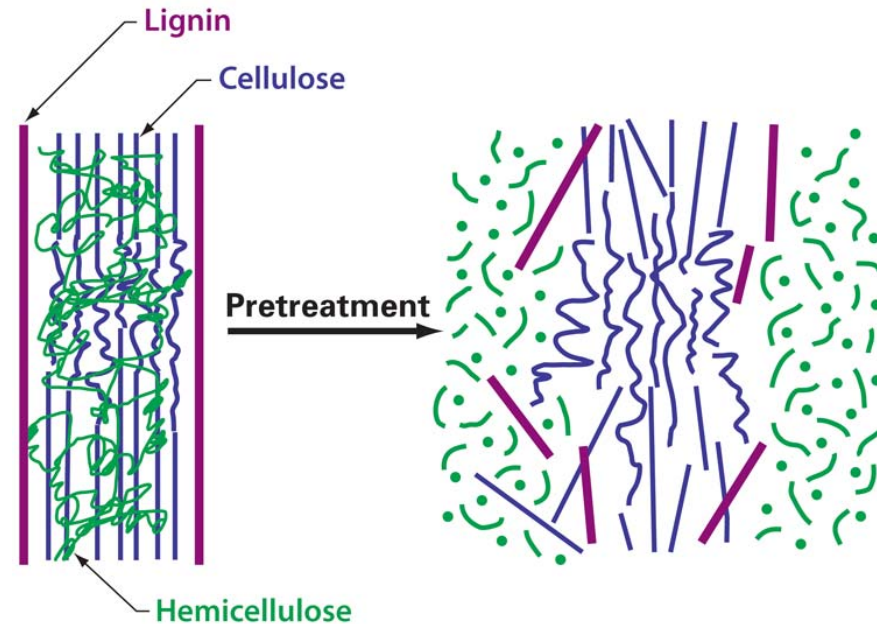


Poplar

Deconstruction: providing a source of fermentable sugars

Challenges

- Lignocellulose is difficult to process due to:
 - low accessibility of crystalline cellulose fibers
 - presence of lignin “seal” & hemicellulose cross-links
 - small pore sizes in lignocellulose



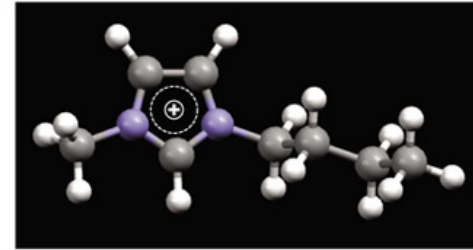
- Acid pretreatment methods result in the formation of by-products that are inhibitory to subsequent biofuels fermentation and result in a loss of sugars

Deconstruction: providing a source of fermentable sugars

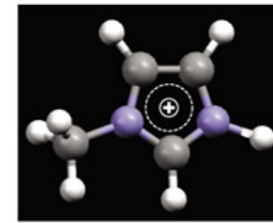
Approach

- Understand the chemical and structural changes resulting from current and new biomass pretreatment approaches
- Understand the fundamental interactions that govern lignocellulolytic enzymes
- Explore new microbial environments and employ **directed evolution** to produce more active and stable lignocellulolytic enzymes

a

[bmim]⁺

b

[hmim]⁺

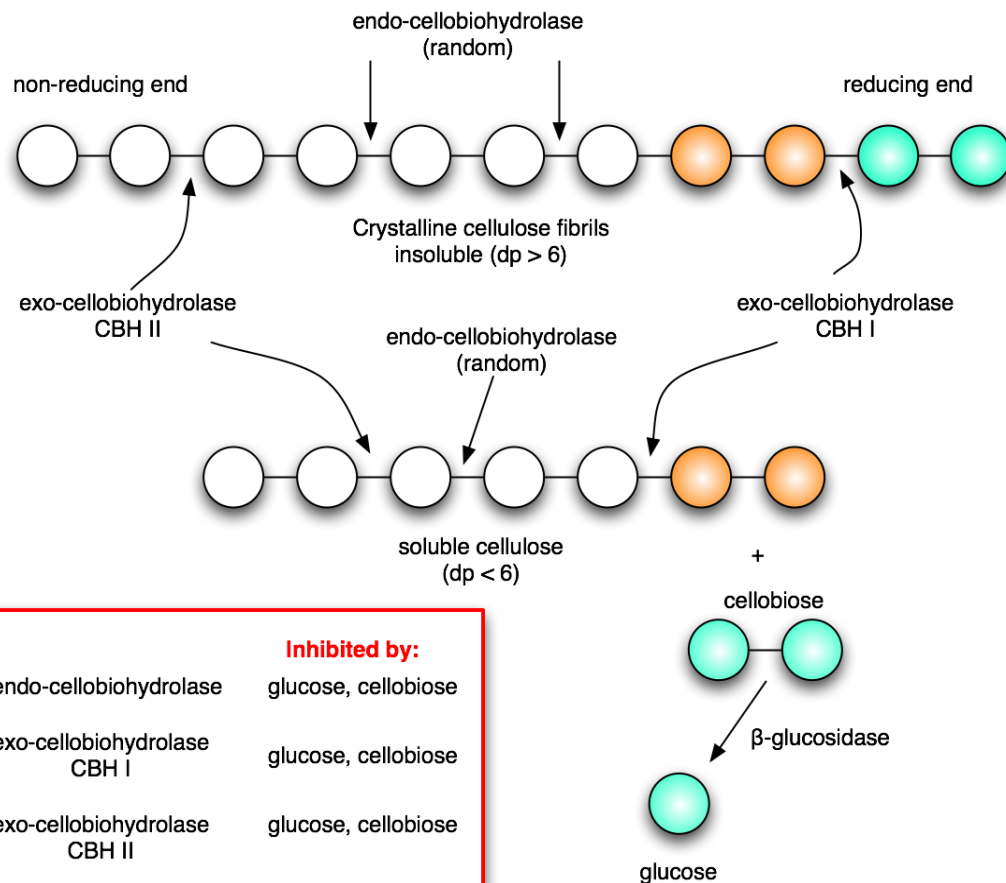
Ionic liquids as cellulose solvents



Rain forest floor

Example: Improving enzyme performance

- Enzyme-substrate, enzyme complexes, and enzyme-product interactions are key components of enzyme performance
- We will delineate the enzymatic mechanisms involved in lignocellulose depolymerization
- Utilize directed evolution to optimize enzymes to improve performance characteristics and lower cost



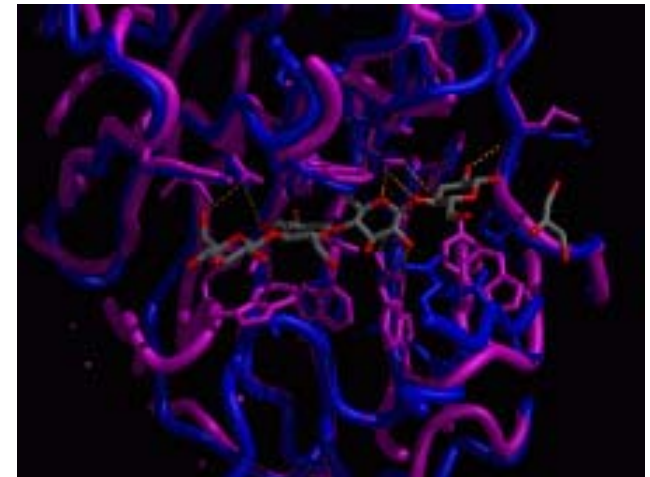
	Inhibited by:
endo-cellobiohydrolase	glucose, cellobiose
exo-cellobiohydrolase CBH I	glucose, cellobiose
exo-cellobiohydrolase CBH II	glucose, cellobiose
β -glucosidase	glucose

Objective:
optimize enzyme structure and function

Deconstruction: providing a source of fermentable sugars

Deliverables

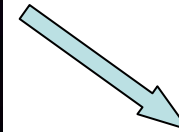
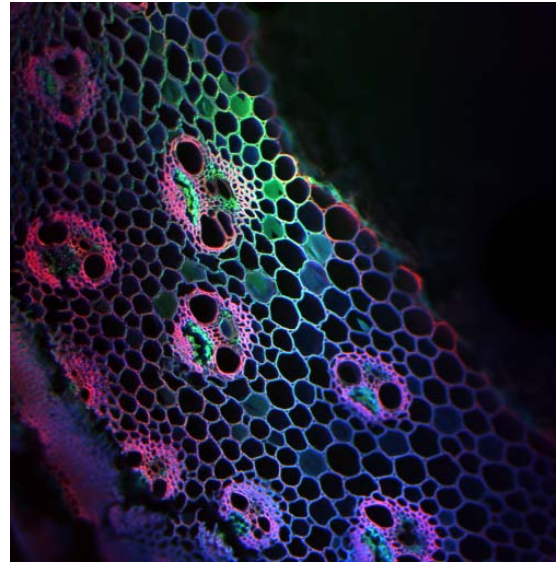
- Optimal pretreatment methods for target biomass feedstocks
- Improved lignocellulolytic enzymes with enhanced activity and stability
- Understanding of how microbial communities degrade lignocellulose
- Cost-effective pretreatment & enzymatic depolymerization methods with minimal by-products and inhibitor formation



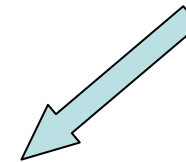
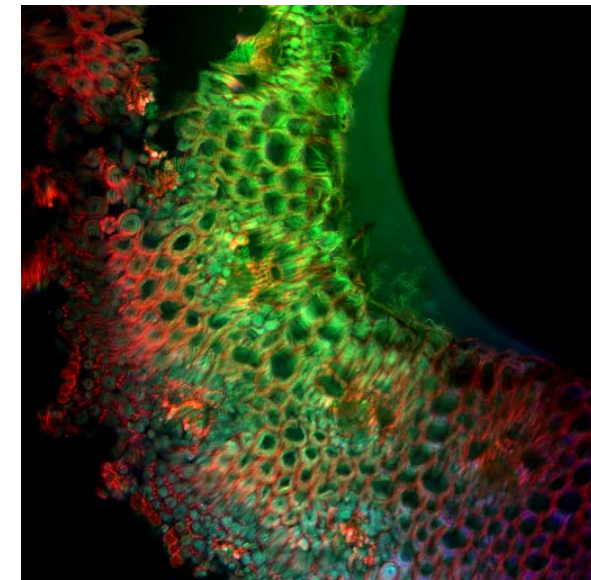
Recent Results: Ionic Liquid Pretreatment Studies of Switchgrass

- Raw switchgrass samples were processed to isolate different parts of the plant
- Intact bulk samples were then exposed to 1-ethyl-3-methylimidazolium (acetate salts) at 120°C
- Biomass deconstruction tracked as a function of temperature and time
- Confocal images taken with dual wavelength excitation (405 and 543 nm)

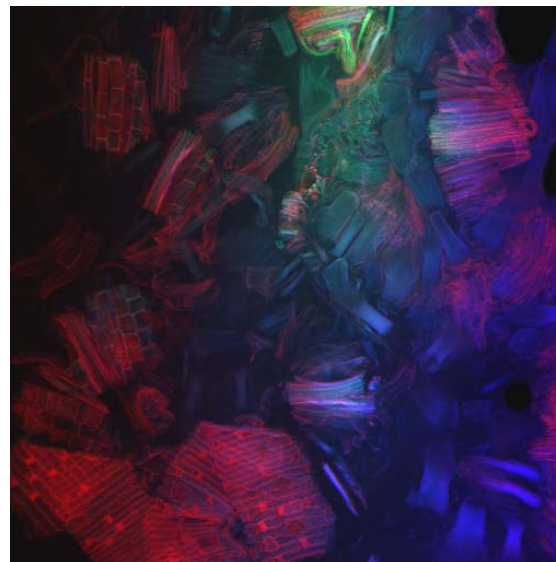
Initial



$T = 120^{\circ}\text{C}$, $t = 20$ mins



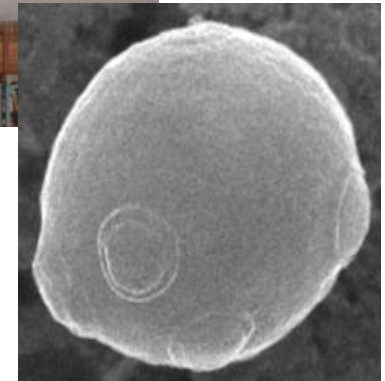
$T = 120^{\circ}\text{C}$, $t = 50$ mins



Biofuels Synthesis: ethanol and next generation biofuels

Challenges

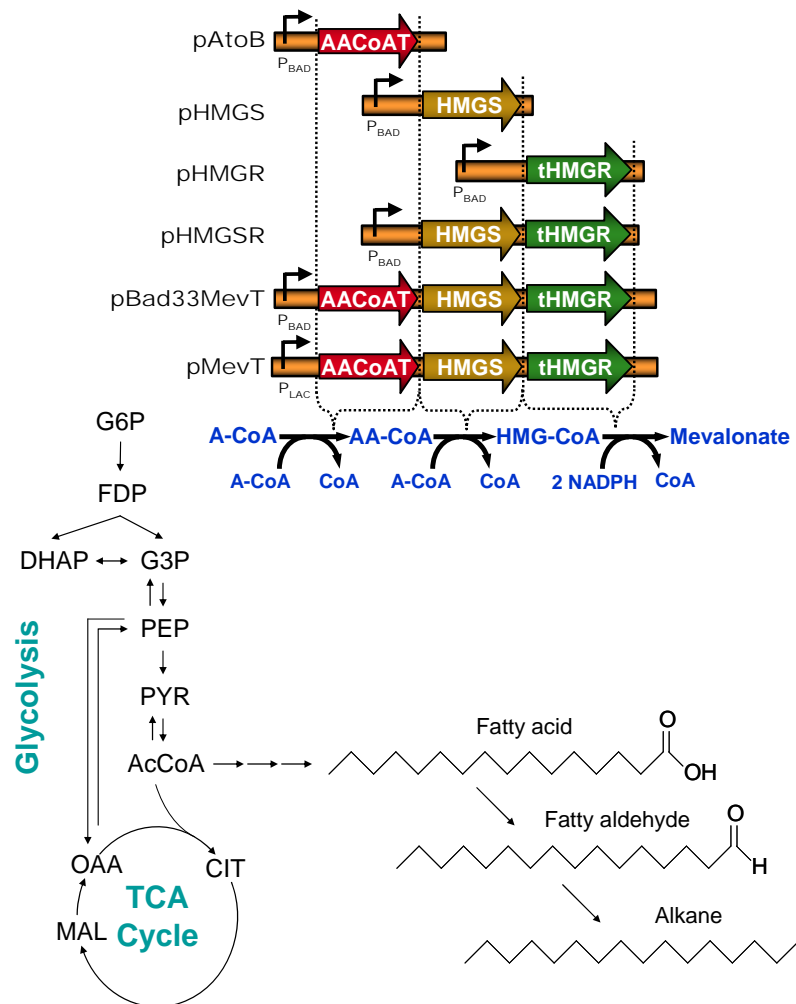
- Existing biofuels
 - do not have the full fuel value of gasoline
 - require energy-intensive purification processes
 - are toxic at high concentrations
 - cannot be transported using traditional means
- Microorganisms convert only a limited number of precursors to fuels.
- Inhibitors resulting from the pre-treatment process prevent growth and biofuel production



Biofuels Synthesis: ethanol and next generation biofuels

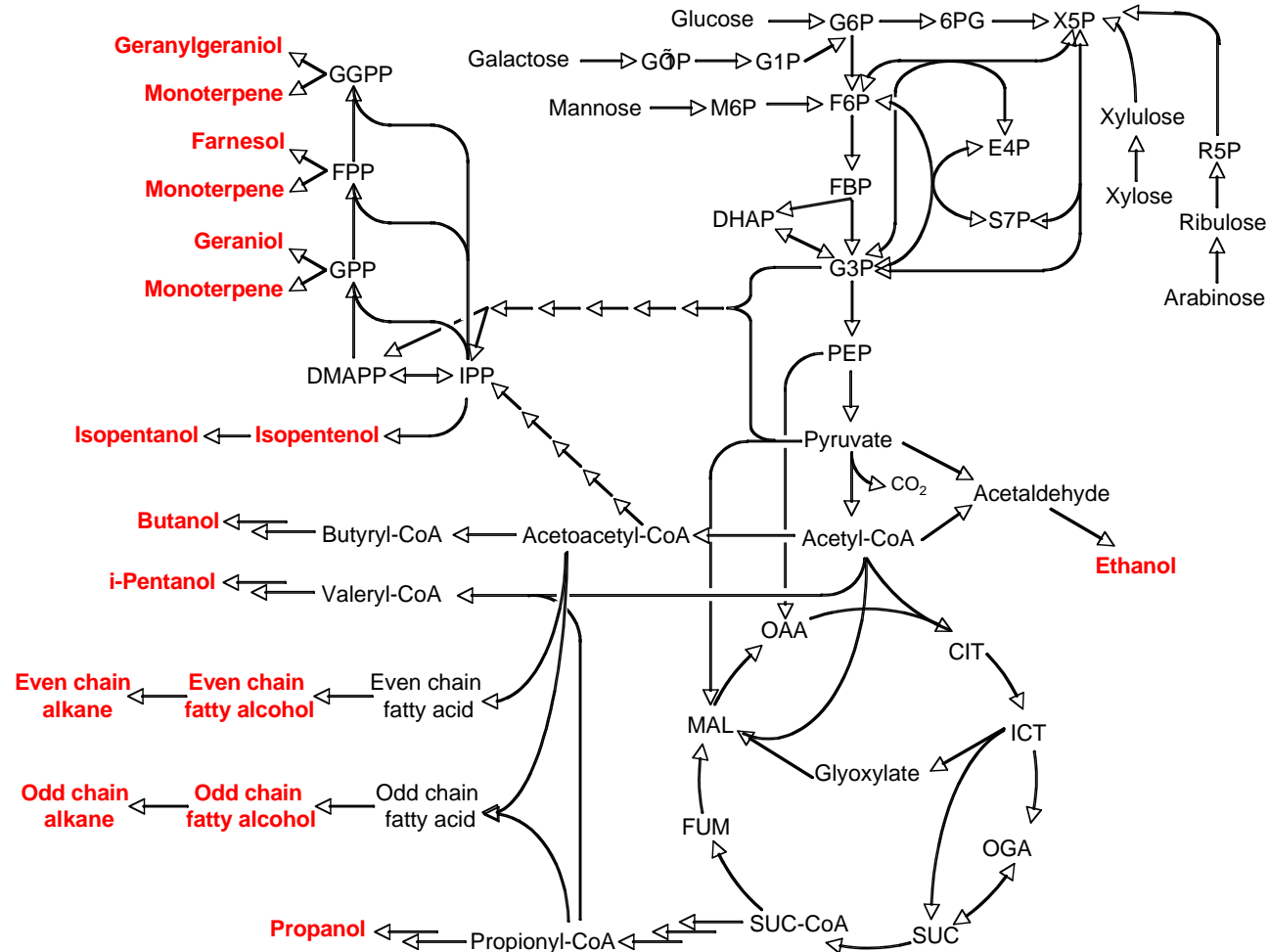
Approach

- Develop pathways for production of future biofuels
- Understand mechanisms of fuel toxicity and stress response
- Engineer organisms to produce & withstand high concentrations of biofuels
- Engineer organisms for consolidated bioprocessing (cellulase production with simultaneous fermentation of sugars to biofuels)



- Large number of potential fuel molecules can be produced from central metabolic intermediates.
 - Alkanes
 - Alcohols
 - Esters
- Need to construct precursor biosynthetic pathways.
- Understand their impact on cell physiology.

Example: Production of next-generation biofuels



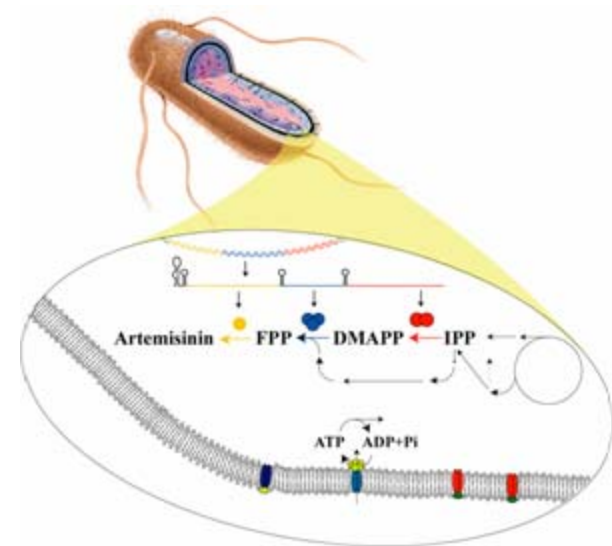
Objective:
Engineer pathways for fuels synthesis



Biofuels Synthesis: ethanol and next generation biofuels

Deliverables

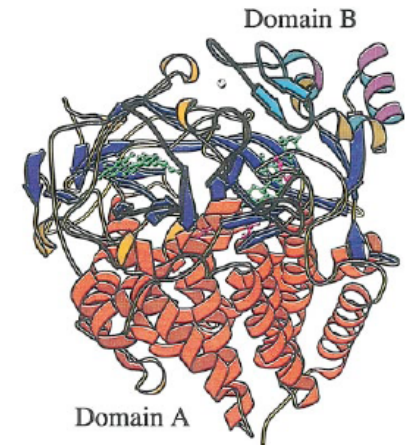
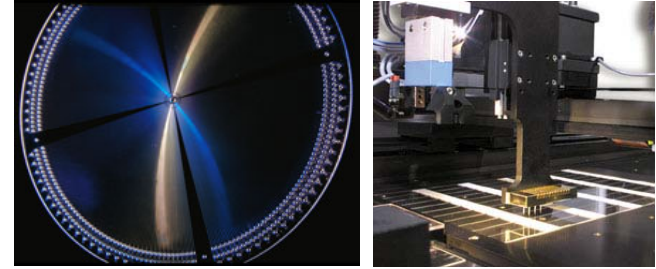
- Organisms engineered to produce and withstand high concentrations of biofuels
- Organisms resistant to by-products formed during deconstruction
- Sequence and regulatory information for metabolic pathways producing biofuels
- Models of metabolic pathways for fuel synthesis and their mode of regulation



Technology: new tools for biofuels research

Challenges

- Few tools available for bioenergy/biomass research
- New high throughput biochemical and omics approaches needed for all aspects of bioenergy research
- Advanced imaging techniques can be leveraged to characterize biomass and biomass deconstruction processes

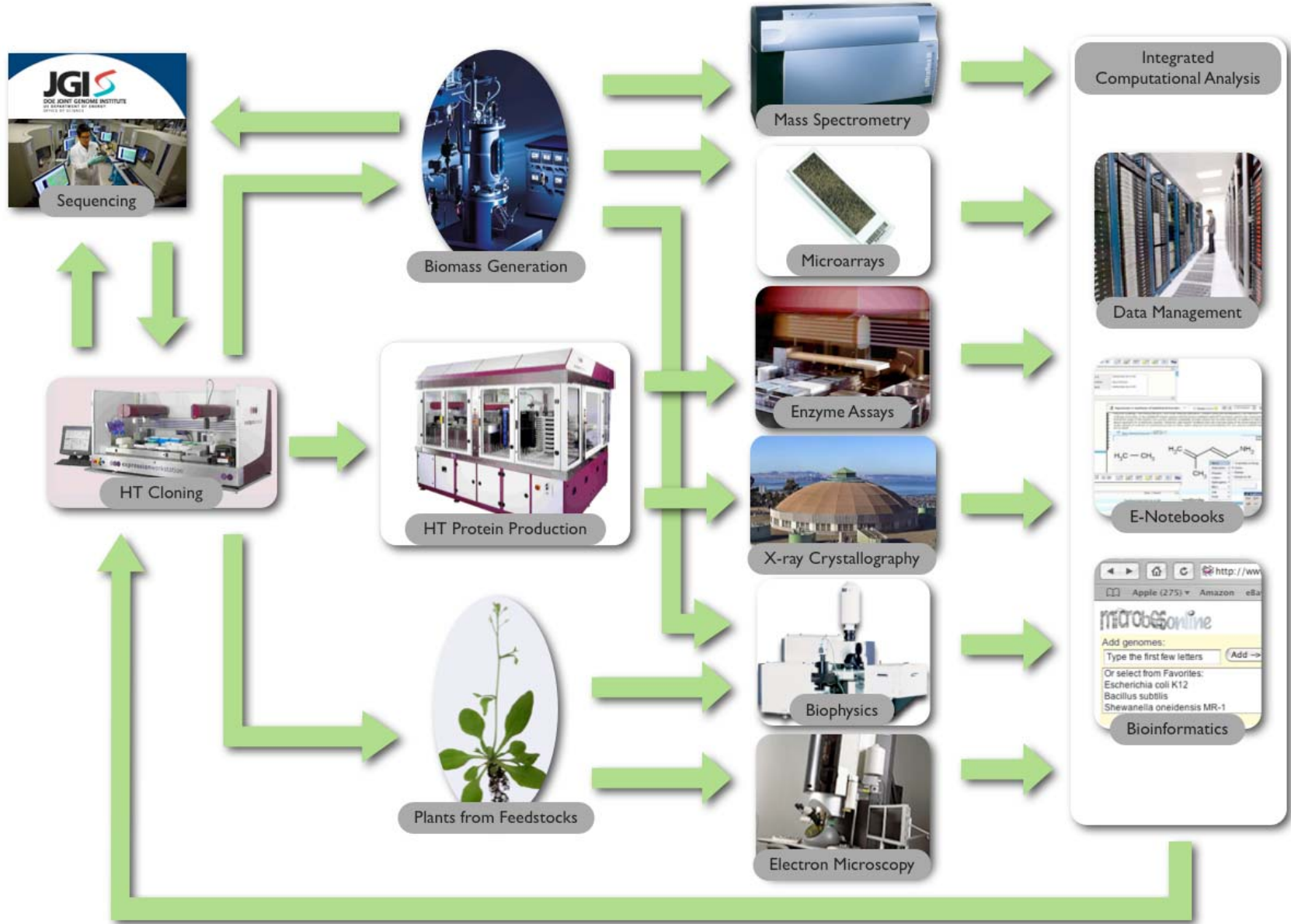


Technology: new tools for biofuels research

Approach

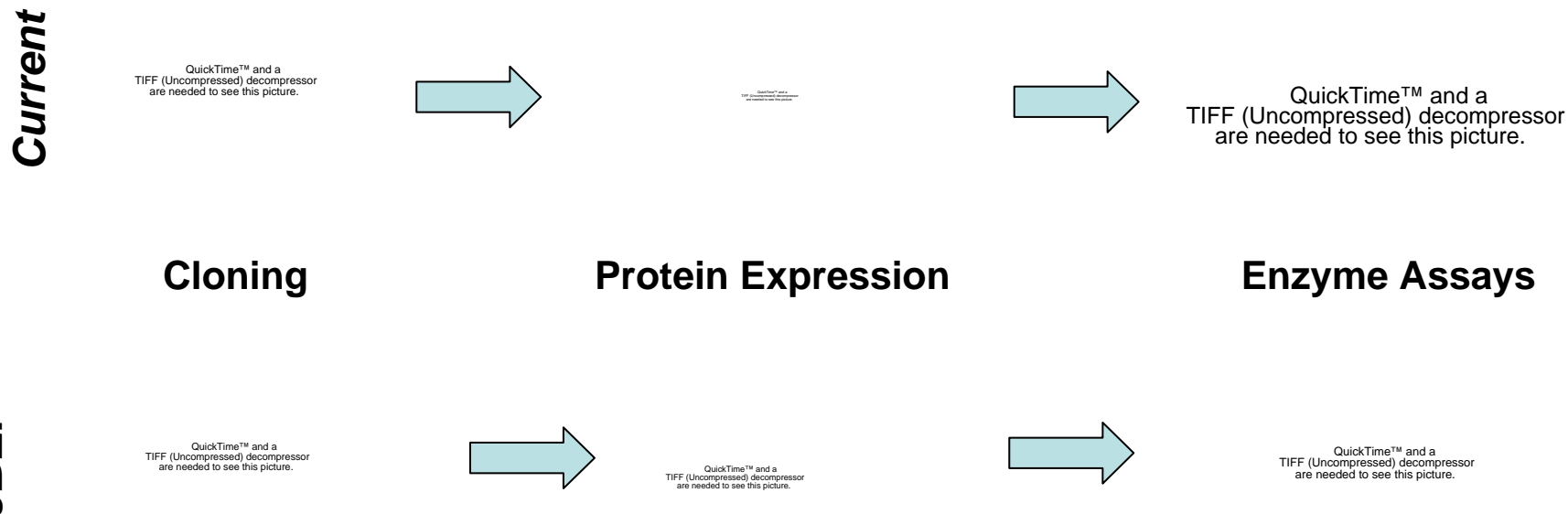
- Provide technologies for scientific discovery
- Implement high-throughput off-the-shelf systems
- Automate, parallelize and miniaturize throughput-limiting procedures
- Develop new technologies for enzyme characterization

Technology: new tools for biofuels research



Example: Automation of Limiting Processes

- Cloning, protein expression and enzyme assays will be rate limiting without high-throughput technologies.
- JBEI will implement HT cloning and expression technologies and develop new microfluidic tools for HT enzyme assays



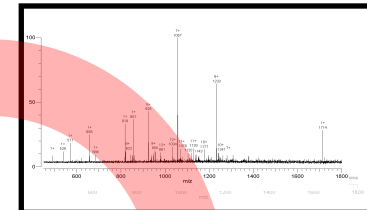
Technology: new tools for biofuels research

Deliverables

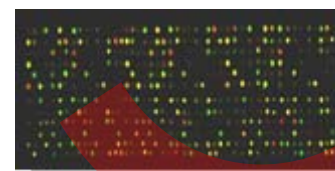
- High-throughput microfluidics platforms for large scale analysis of plant and microbial enzyme activities
- Ligno- and glyco-arrays for rapid screening of enzymatic function
- `Omics pipelines for systems biology
- Integrated data capture, analysis and dissemination
- Parts, devices, chassis for synthetic biology



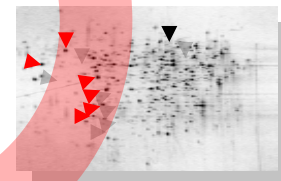
Comparative Genomics



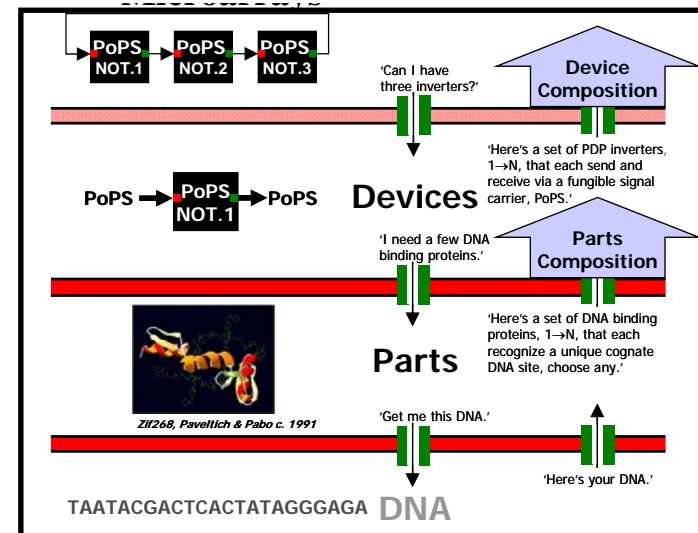
Metabolomics



DNA Microarrays

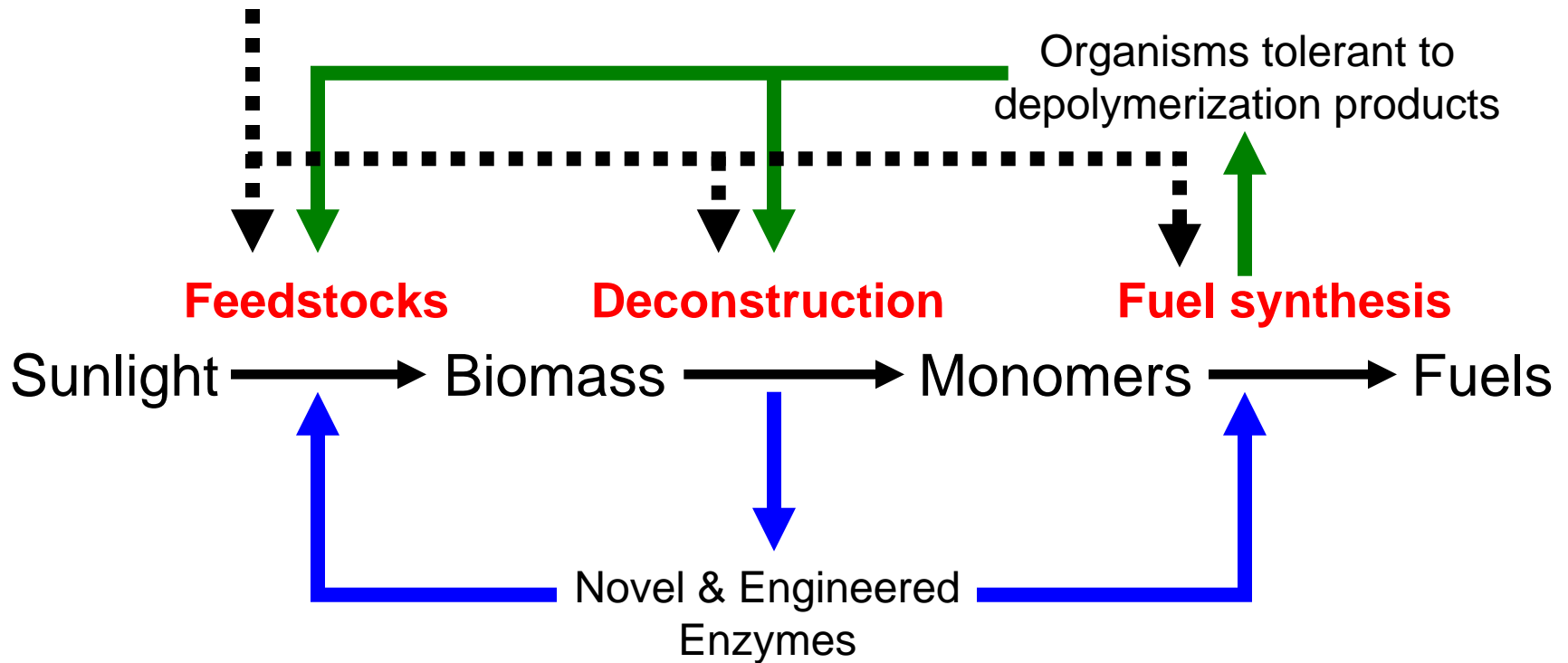


Proteomics



Interdependent research

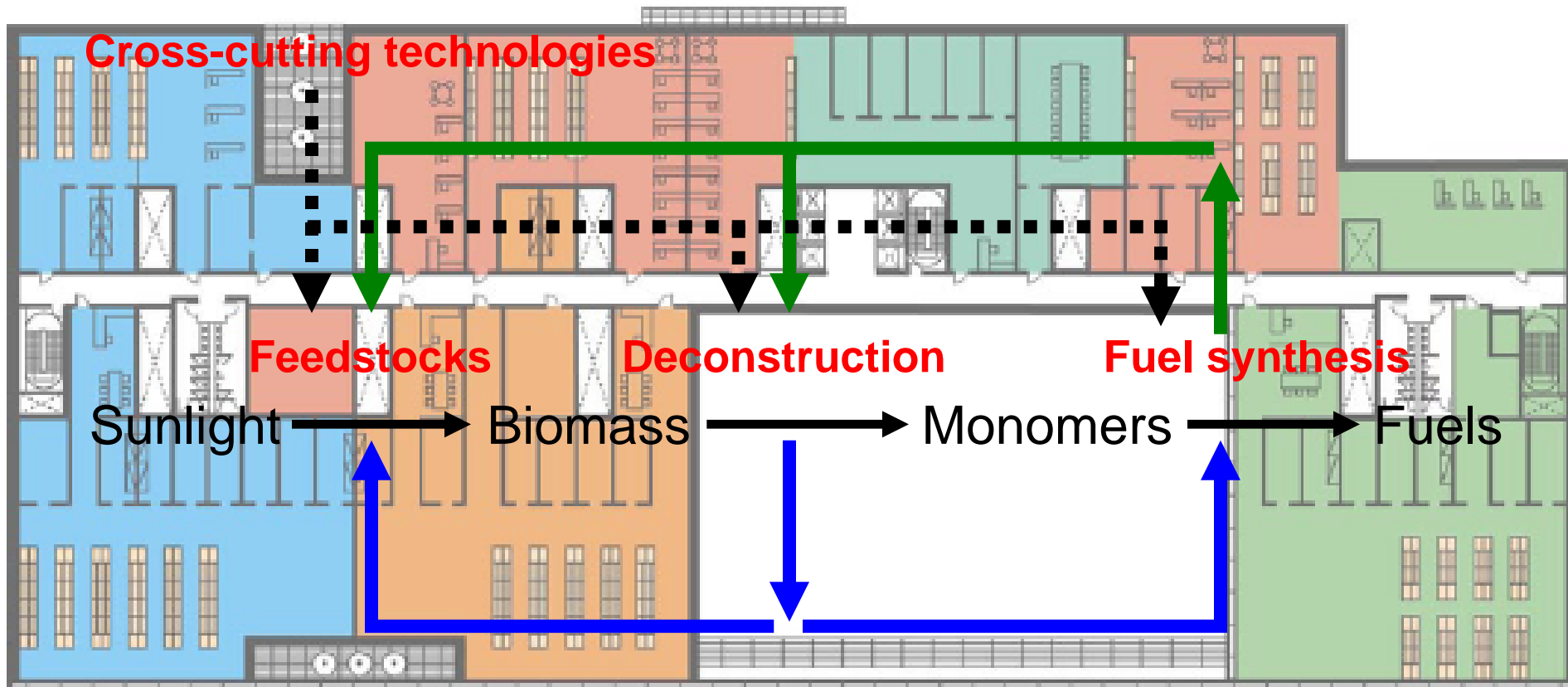
Cross-cutting technologies



- Cross-cutting technologies will aid the research in multiple divisions
- Research will be interdependent with discoveries in one area influencing the research in the other areas.

A single JBEI facility will foster research interactions

Integrated operation ensures effectiveness, cost-efficiency, and unity



JBEI Facility: EmeryStation East



61,000 rentable square feet on-floor

~43,000 assignable square feet

- Environmentally friendly building
- Access to adjacent 80 seat conference center
- Shuttle services and 90 parking spaces



Accelerated start-up: Research at Partner Facilities

FY07 research starts at partner institutions

- LBNL, UCB
 - Berkeley West Biocenter
 - dedicated 12,000 sf lab, office space
- Sandia National Laboratories, California
- Lawrence Livermore National Lab
- Carnegie Institute of Science
- UC Davis



Carnegie Institute



National Combustion Research
Facility



Berkeley West Biocenter



Center for Accelerator Mass
Spectrometry

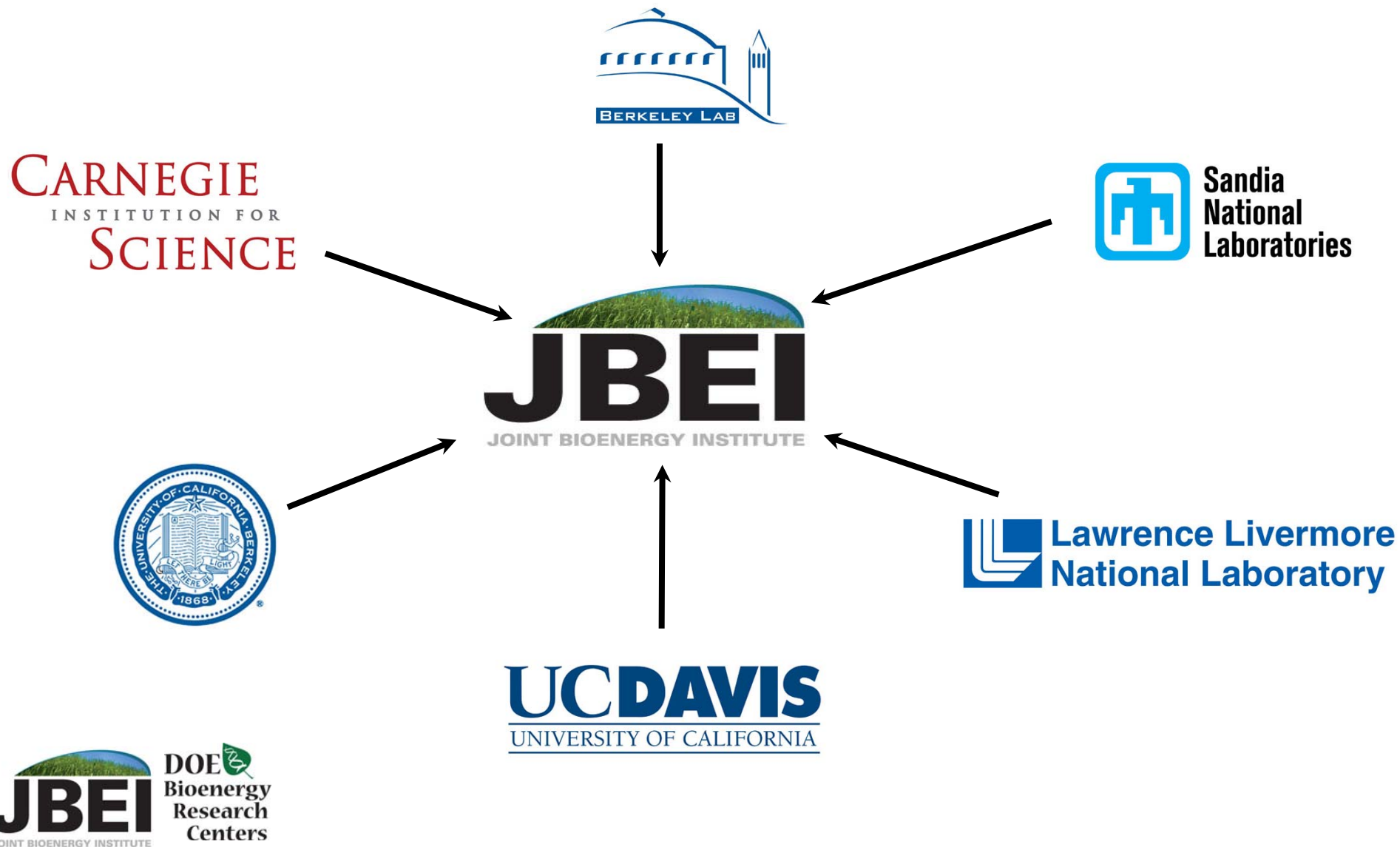


UC Davis
Genome Center
& Plant Genomic Program



Advanced
Light Source

A single location



JBEI leverages key capabilities of partner institutions

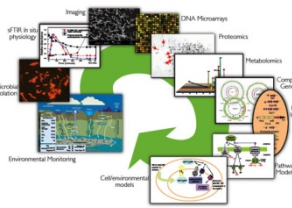
Systems & Synthetic Biology

institutions

Plant Research



Synthetic Biology Engin. Res. Center (SynBERC)

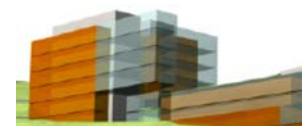


DOE: Genomics VIMSS ESPP



Joint Genome Institute

Energy Research



Helios



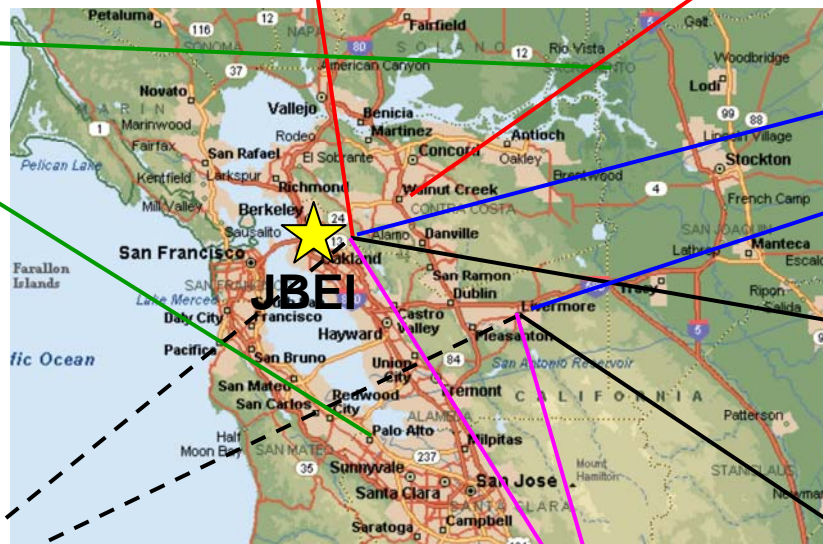
National Combustion Research Facility



UC Davis Genome Center & Plant Genomic Program



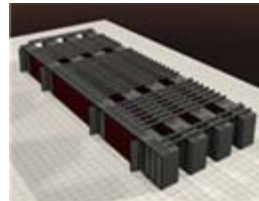
Carnegie Institute of Stanford



Computation



National Energy Res. Supercomputing Center



Red Storm Supercomputer

Imaging



UC Berkeley Imaging



National Center for Electron Microscopy

Nanoscience



Molecular Foundry



Center for Integrated Nanoscience



Center for Accelerator Mass Spectrometry

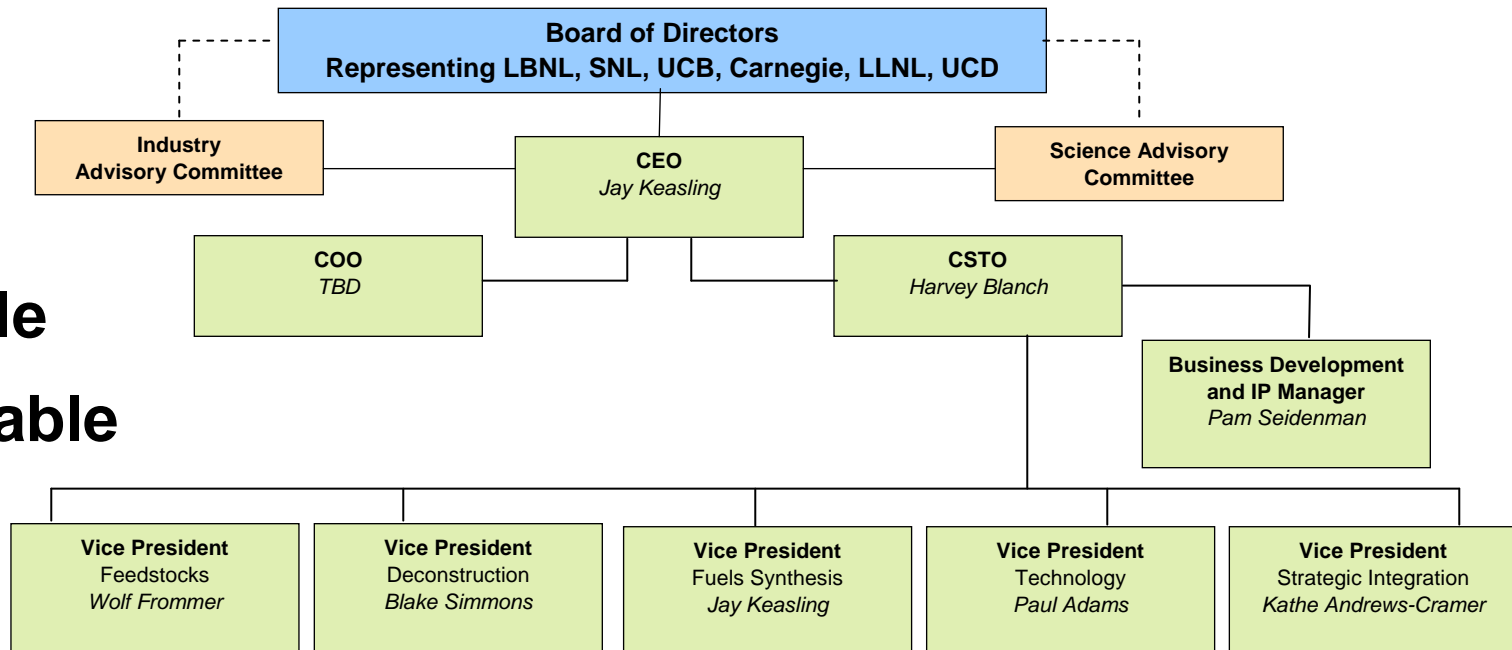


Advanced Light Source

JBEI Organization

Research organization designed to be decisive and nimble, modeled on **technology “start-up”**

- **Bold**
- **Flexible**
- **Adaptable**



Dedicated management for science, technical integration, and scalability

JBEI leverages the Bay Area biotech and high tech industry



Vibrant industries grow around intellectual centers

- Silicon Valley and Biotech Industry around UCB, UCSF, Stanford
- Bay Area and CA becoming centers for renewable energy

Benefits of JBEI location in Bay Area and CA

- Intellectual environment
- Recruiting
- Commercialization

Interactions with industry

Equipment makers/suppliers

Bioinformatics companies

Cross-cutting Technologies

**High Throughput
Microsystems**

Omics

**Multi-scale
Imaging**

**Integrated
Informatics**

Sunlight

Plants

Lignocellulose

Microbes
& Enzymes

Monomers
(sugars & aromatics)

Synthetic
Biology

Fuels

Feedstocks

Deconstruction

Fuels Synthesis

**Crop genetics
companies**

**Biofuels
companies**

**Automobile
companies**

**Biomass
suppliers**

Commercializing JBEI's Products

JBEI's Technology Transfer Program will:

- Efficiently commercialize innovative biofuels technologies
- Promote dialogue among researchers, industry, and VCs
- Provide opportunities for industry to collaborate with JBEI
- Complement and further JBEI's biofuels research

Mechanisms:

- Industry Advisory Committee
 - Companies from key sectors: feedstocks, enzymes, fuels production, biotechnology, genetics, chemistry
 - Provide feedback on JBEI research from an industry perspective
- Central management of IP and industry interface
- Industry Partnership Program
- Central data repository of all JBEI IP and IP agreements
- Industrial scientist sabbaticals at JBEI

Impacts

- Elucidate & modify plant cell wall structure and synthesis
- Efficient, cost-effective routes for deconstruction of lignocellulose
- Engineered organisms for scalable production of ethanol and next generation biofuels
- Enabling and integrating technologies for bioenergy research
- Integrated science & technology to transform the U.S. biofuels industry

Accelerated start-up: research and operations personnel

