BioEnergy Science Center:
An Integrated Strategy to Understand Biomass Recalcitrance

Presented to the
Oklahoma EPSCoR Annual State Conference

Brian Davison
Chief Scientist, Systems Biology and Biotechnology
BioEnergy Science Center

April 29, 2010

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BioEnergy Science Center

An Integrated Strategy to Understand Biomass Recalcitrance

BESC: A multi-institutional DOE-funded center

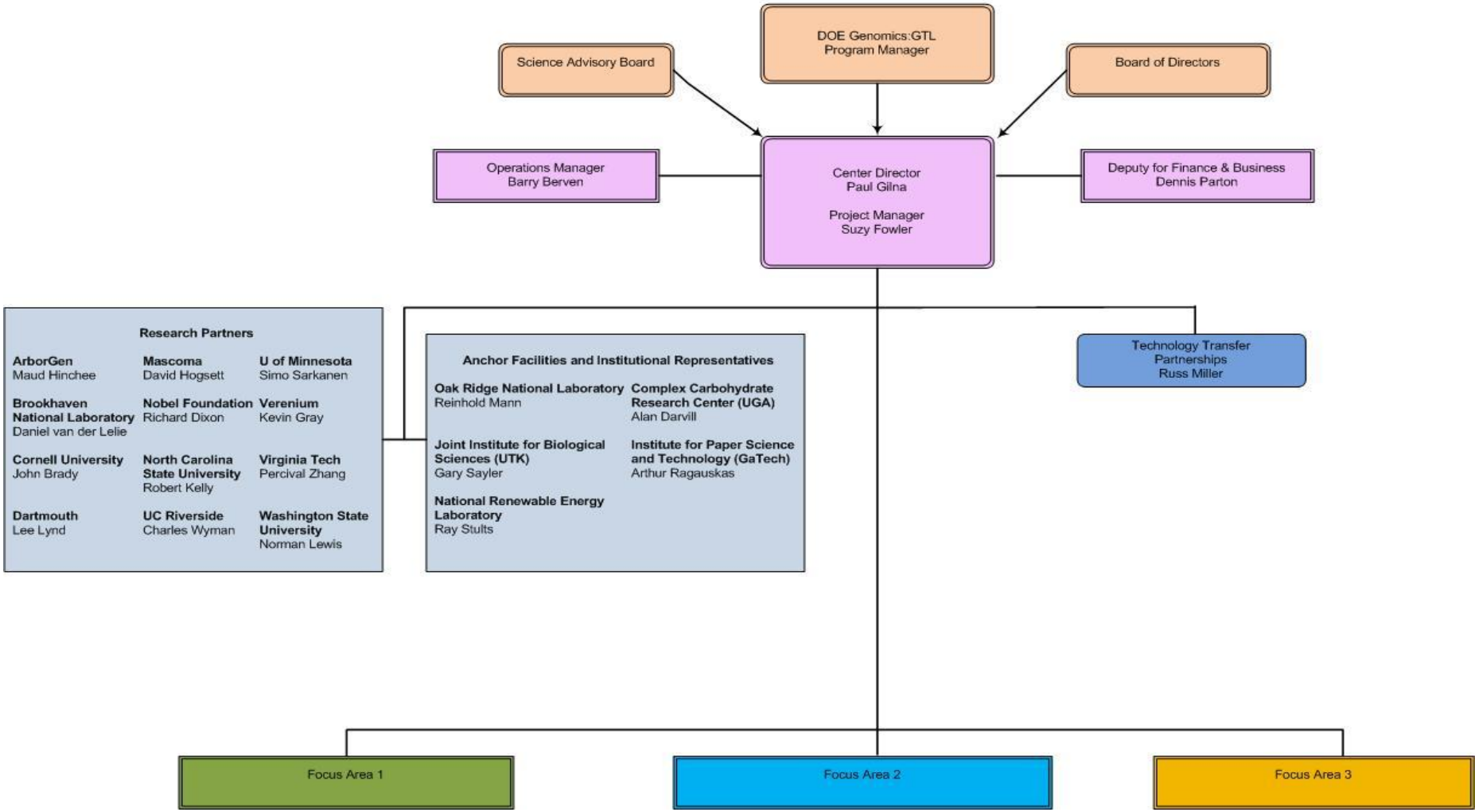
- Samuel Roberts Noble Foundation
- National Renewable Energy Laboratory
- Brookhaven National Laboratory
- University of California–Riverside
- Cornell University
- Washington State University
- University of Minnesota
- North Carolina State University
- Virginia Polytechnic Institute
- University of California–Los Angeles

- Oak Ridge National Laboratory
- University of Georgia
- University of Tennessee
- Dartmouth College
- Georgia Institute of Technology
- West Virginia University
- ArborGen, LLC
- Ceres, Incorporated
- Mascoma Corporation
- Verenium Corporation

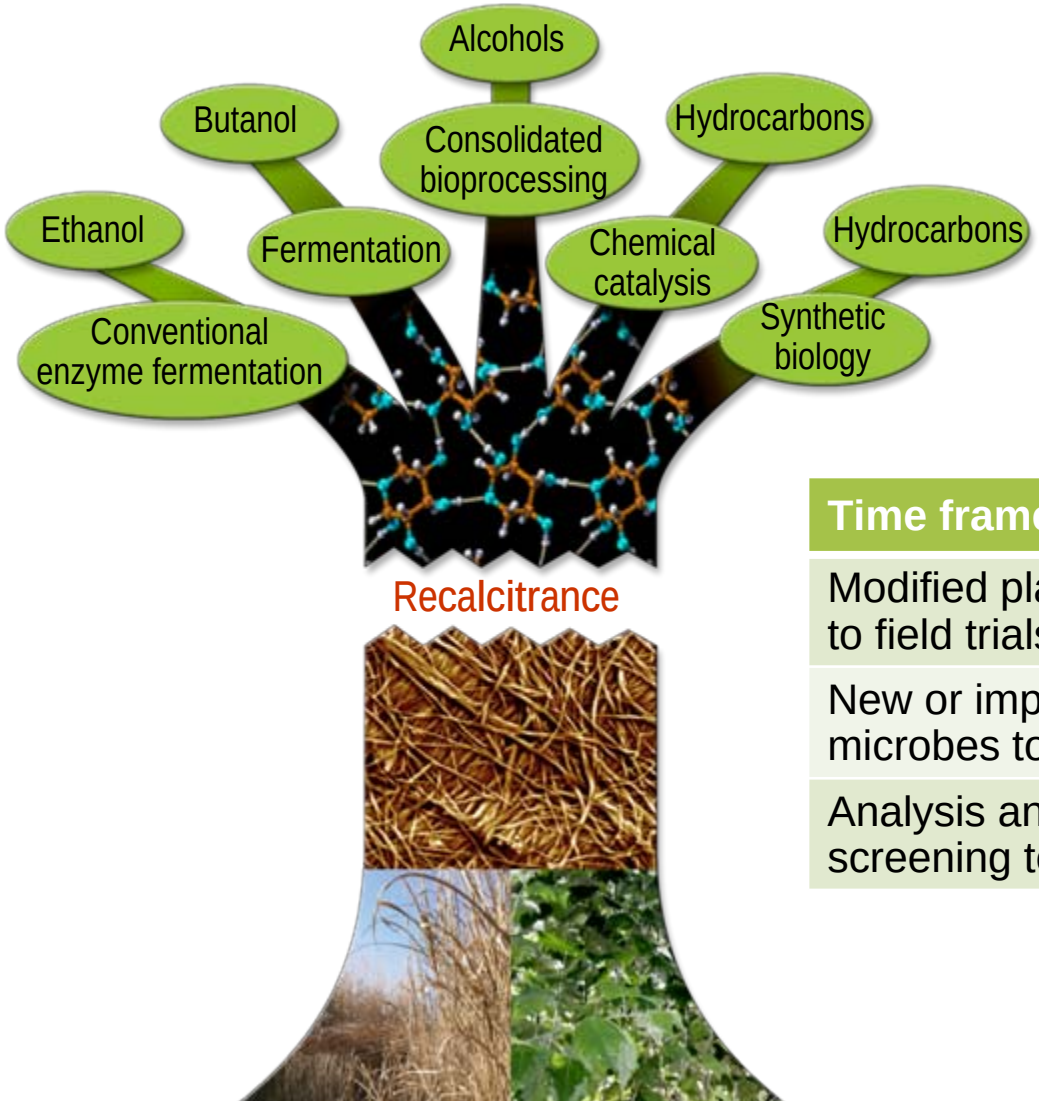
322 People in 20 Institutions

BESC is organized to lead interacting team across key areas

April 2010



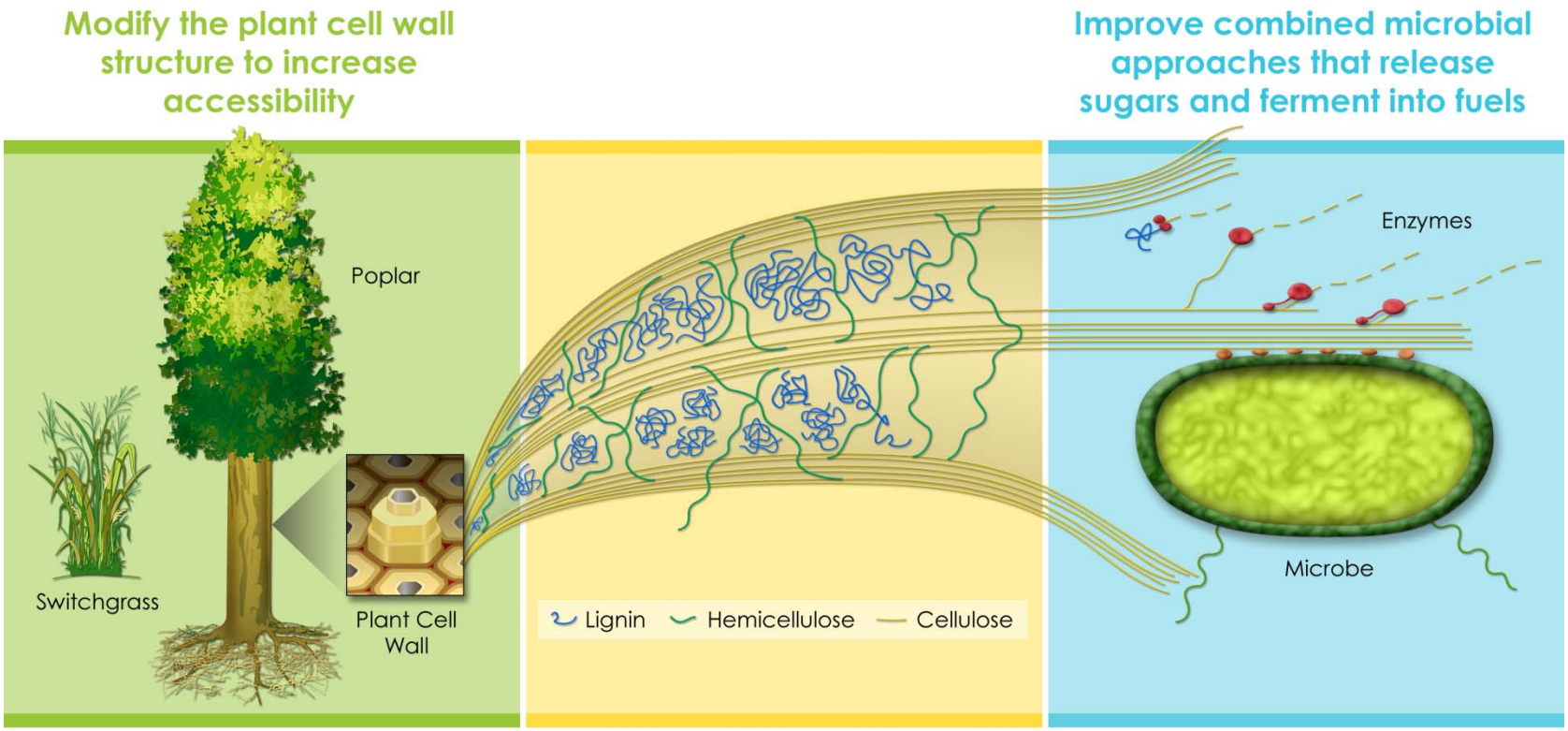
Access to the sugars in lignocellulosic biomass is the current critical barrier



- Overcoming this barrier will cut processing costs significantly and be used in most conversion processes
- This requires an integrated multidisciplinary approach

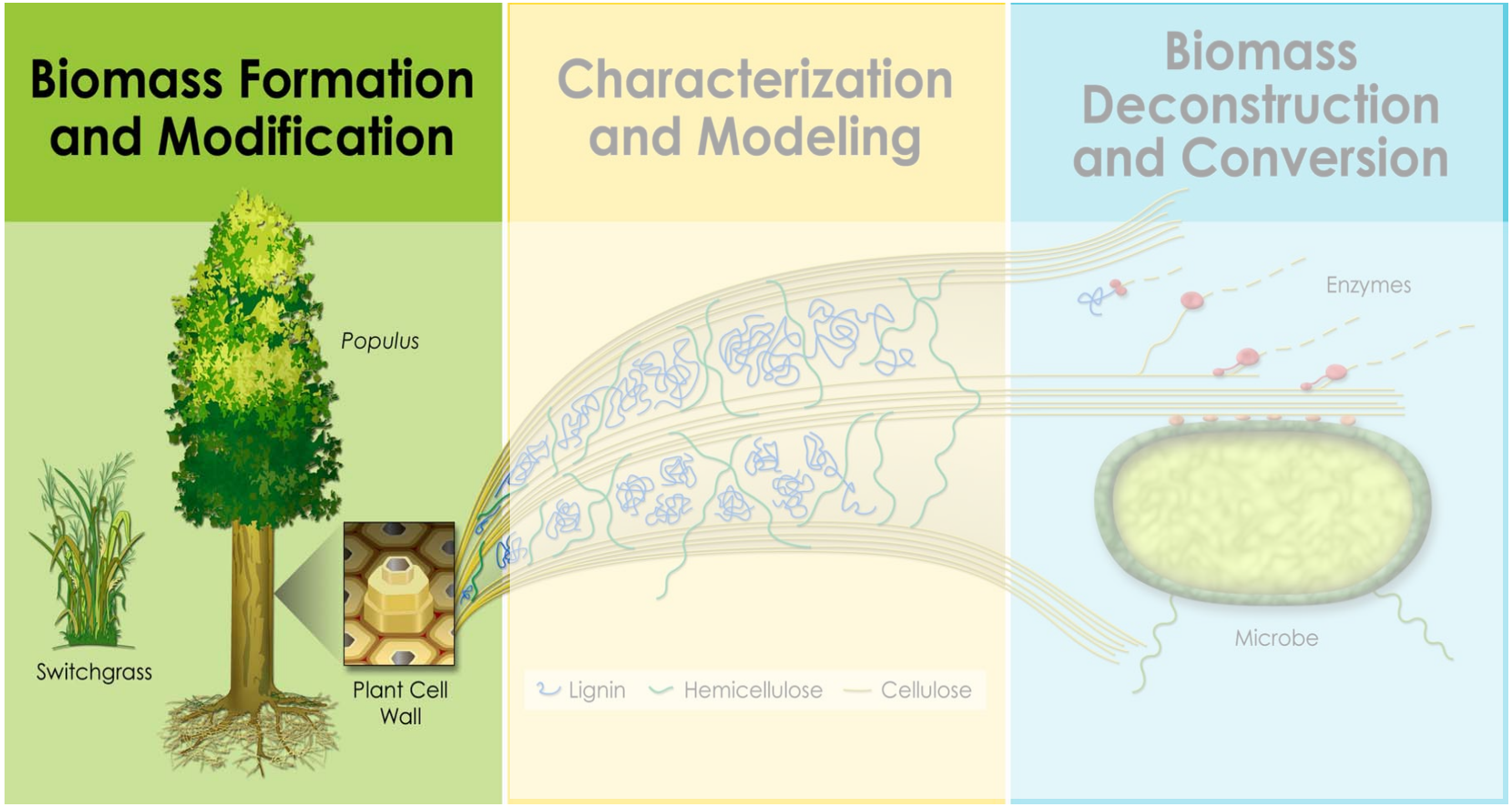
Time frame	Planned	Actual
Modified plants to field trials	Year 5	Year 4
New or improved microbes to development	Year 4–5	Year 3–4
Analysis and screening technologies	Year 3 on	Year 2 on

A two-pronged approach to increase the accessibility of biomass sugars



Both utilize rapid screening for relevant traits followed by detailed analysis of selected samples

Strategy Part 1: Identify, Understand and Manipulate the Plant Cell Wall Genes Responsible for Recalcitrance



Genetic block in lignin biosynthesis in switchgrass increases ethanol yields

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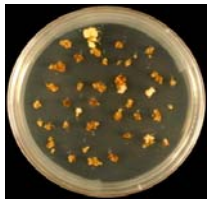
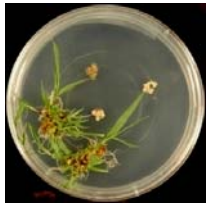
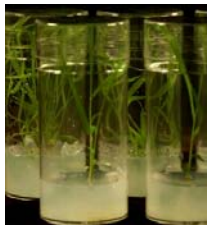
Agrobacterium-mediated transformation of switchgrass







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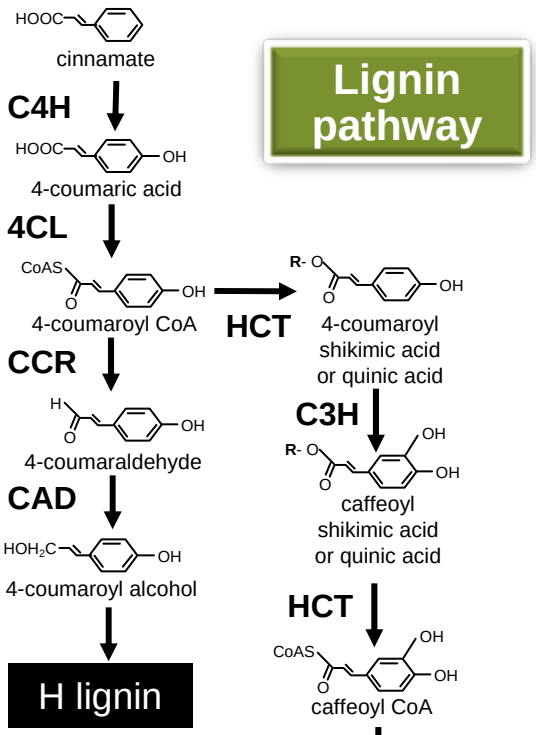
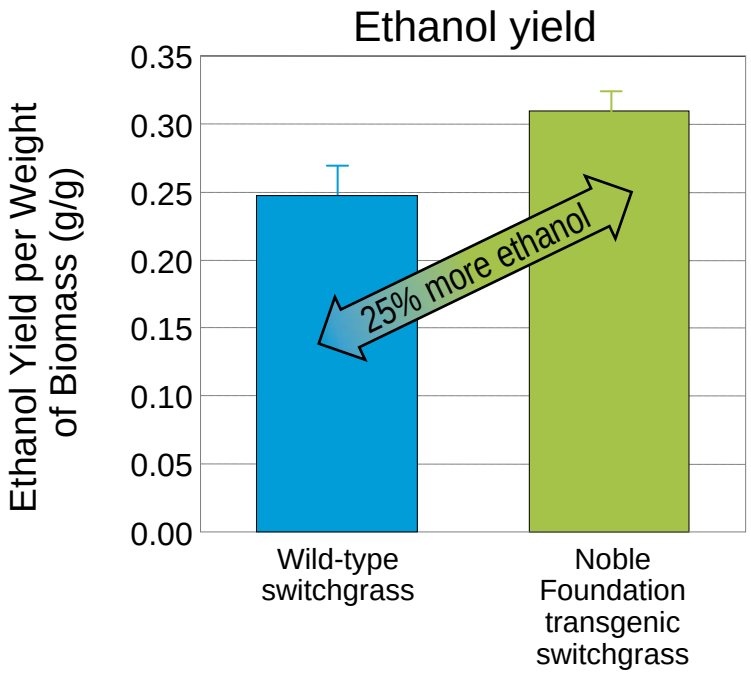
Phenylalanine → **PAL**



Lignin pathway



X. Fu and Z. Wang (Noble), J. Mielenz (ORNL), support from USDA/DOE

Mining Genetic Variation in Switchgrass

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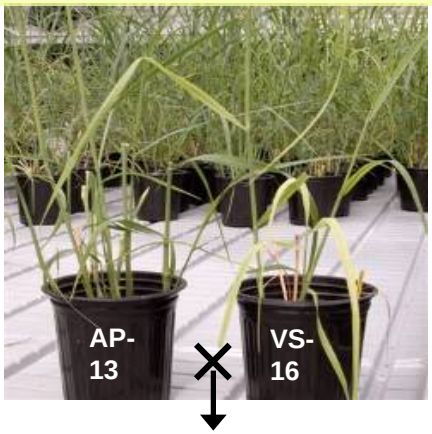
Create diverse population by cross "lowland" SG AP-13 and "upland" SG VS-16 into 385 pseudo F1 clones

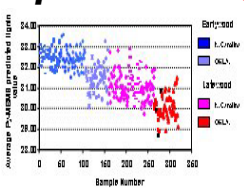


Pseudo F_1 population of 385 genotypes

Clones ready for field planting

HTS Pipeline

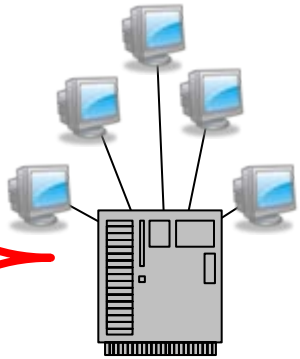
Sugar Release Assay



Analytical Pyrolysis

Create Genetic Marker Map to identify allelic variation

Identify Marker Trait Association



Cell Wall Biosynthesis Database

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Mining variation to identify key genes in biomass composition and sugar release

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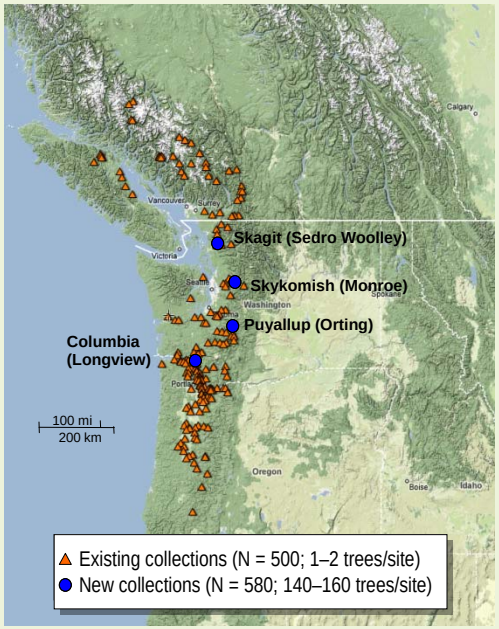
Collected ~1300 samples for *Populus* association and activation-tag study

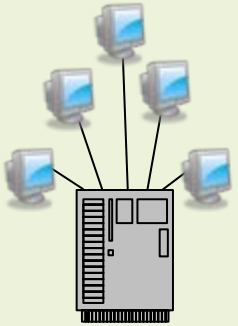


High-throughput screening pipeline

- Create genetic marker map to identify allelic variation
- Identify marker trait association

Sugar release assay

Cell wall biosynthesis database



Establish common gardens for association and activation-tag populations with thousands of plants

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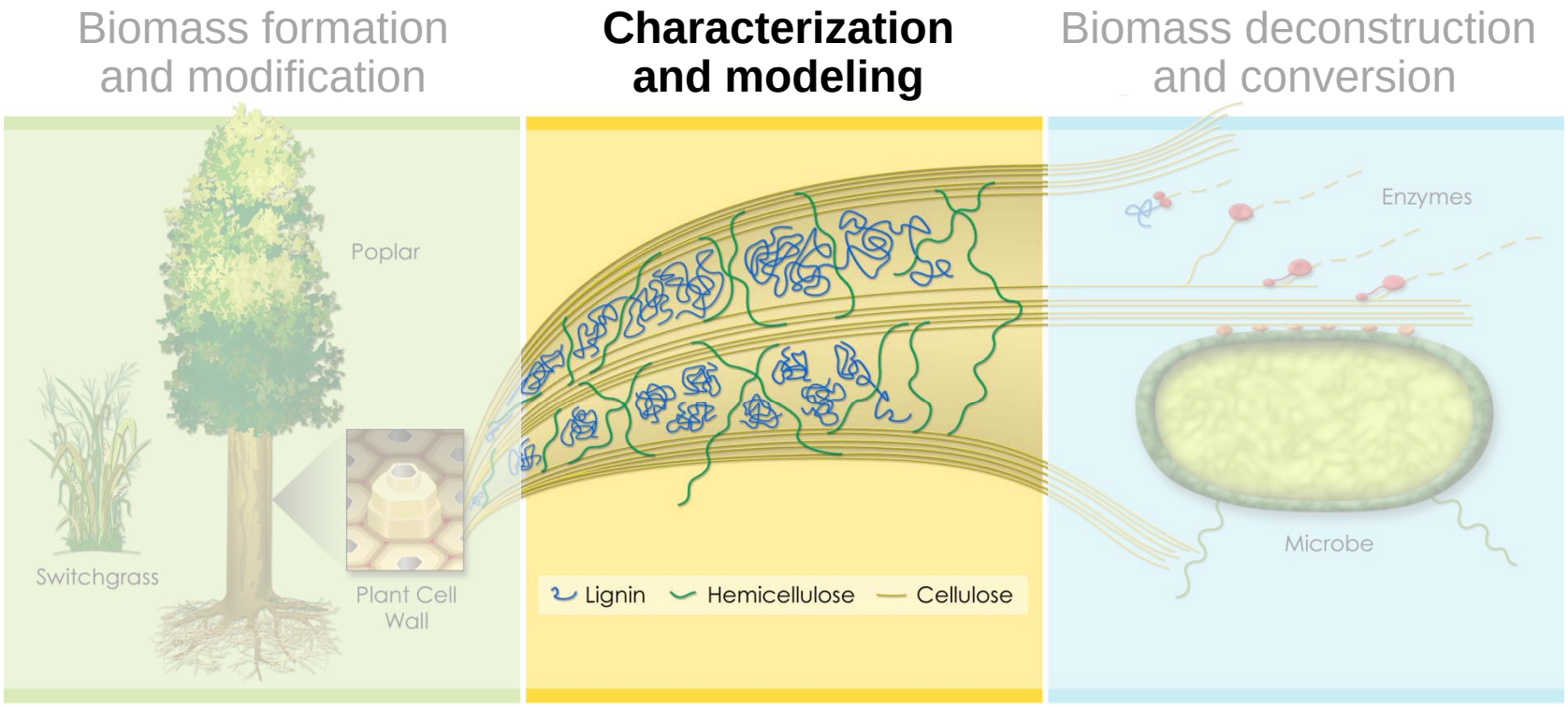
Strategy, part 2:
Measure, understand, and model biomass recalcitrance



High-throughput characterization pipeline for the recalcitrance phenotype

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Screening thousands of samples

Composition analytical pyrolysis, IR, confirmed by wet chemistry

Pre-treatment new method with dilute acid and steam

Enzyme digestibility sugar release with enzyme cocktail

Detailed chemical and structural analyses of specific samples

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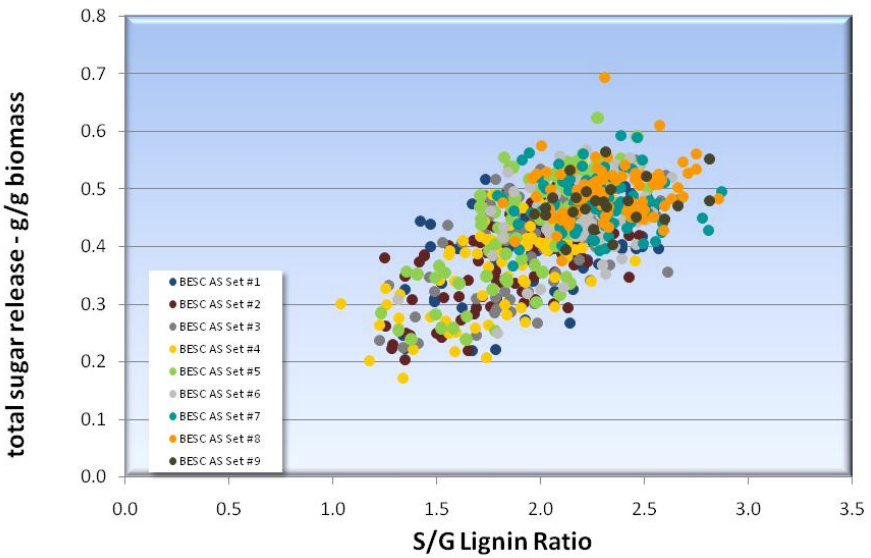
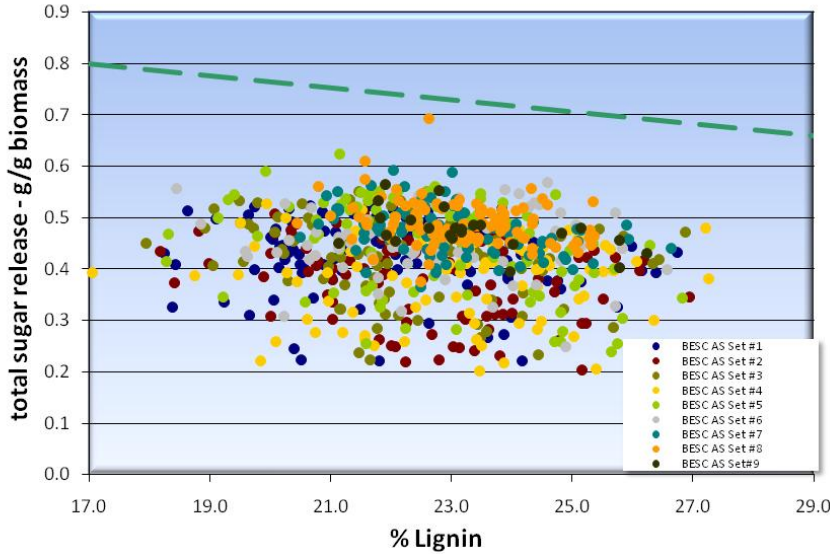
High-throughput screening to analyze natural *Populus* trees

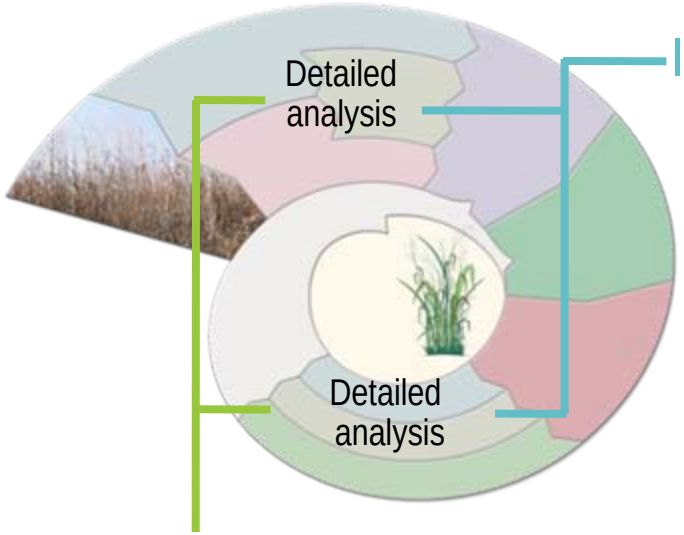
- Screening of 1200 natural *Populus* trees
- Hot water as pretreatment only
- Sugar release varies from 25% to >90% of theoretical value





Environmental vs genetic?

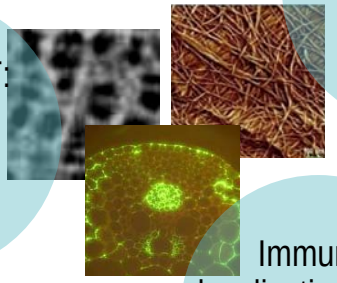
Detailed analysis of specific samples inform cell-wall chemistry and structure



AFM of switchgrass showing cellulose microfibrils

NREL

Bio-ultraCAT: 3-D density of *Populus* cell walls

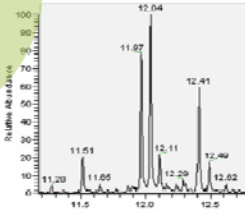


OAK RIDGE National Laboratory

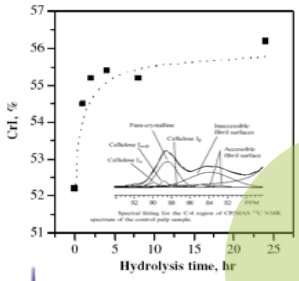
Immuno-localization using wall antibodies on switchgrass

The University of Georgia

Mass spectrometry for key metabolites



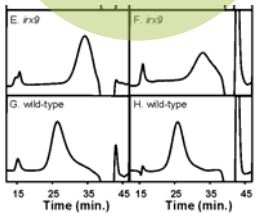
OAK RIDGE National Laboratory



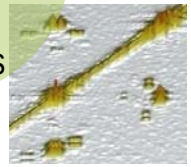
Georgia Tech

NMR for cellulose crystallinity

Fractionation and chromatography



2D ^{1}H-NMR sees altered bonds in polysaccharides and lignin in biomass



The University of Georgia

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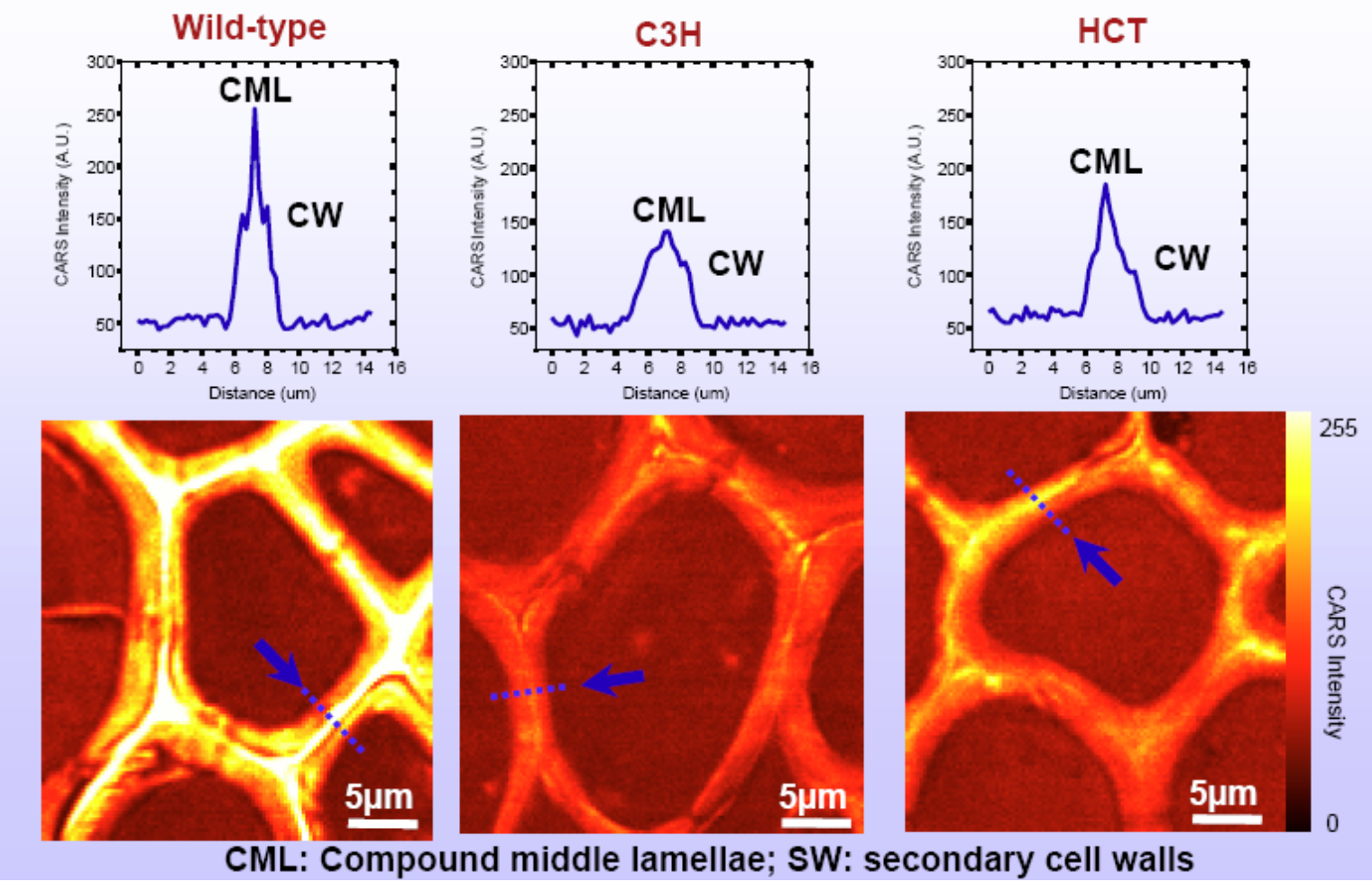
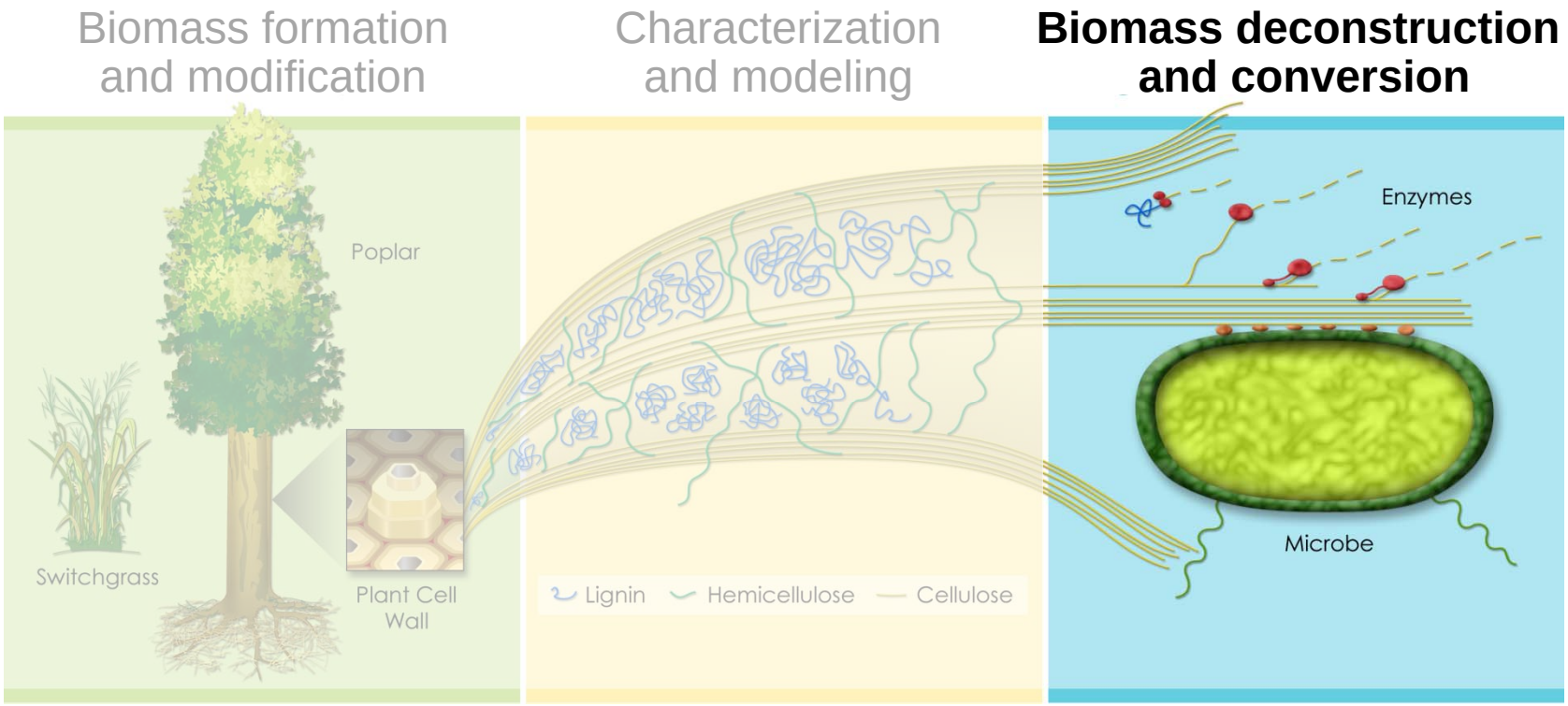
CARS (Coherent Anti-Stokes Raman Scattering) Imaging of Lignin in Interfascicular Fiber Cell Walls in Alfalfa

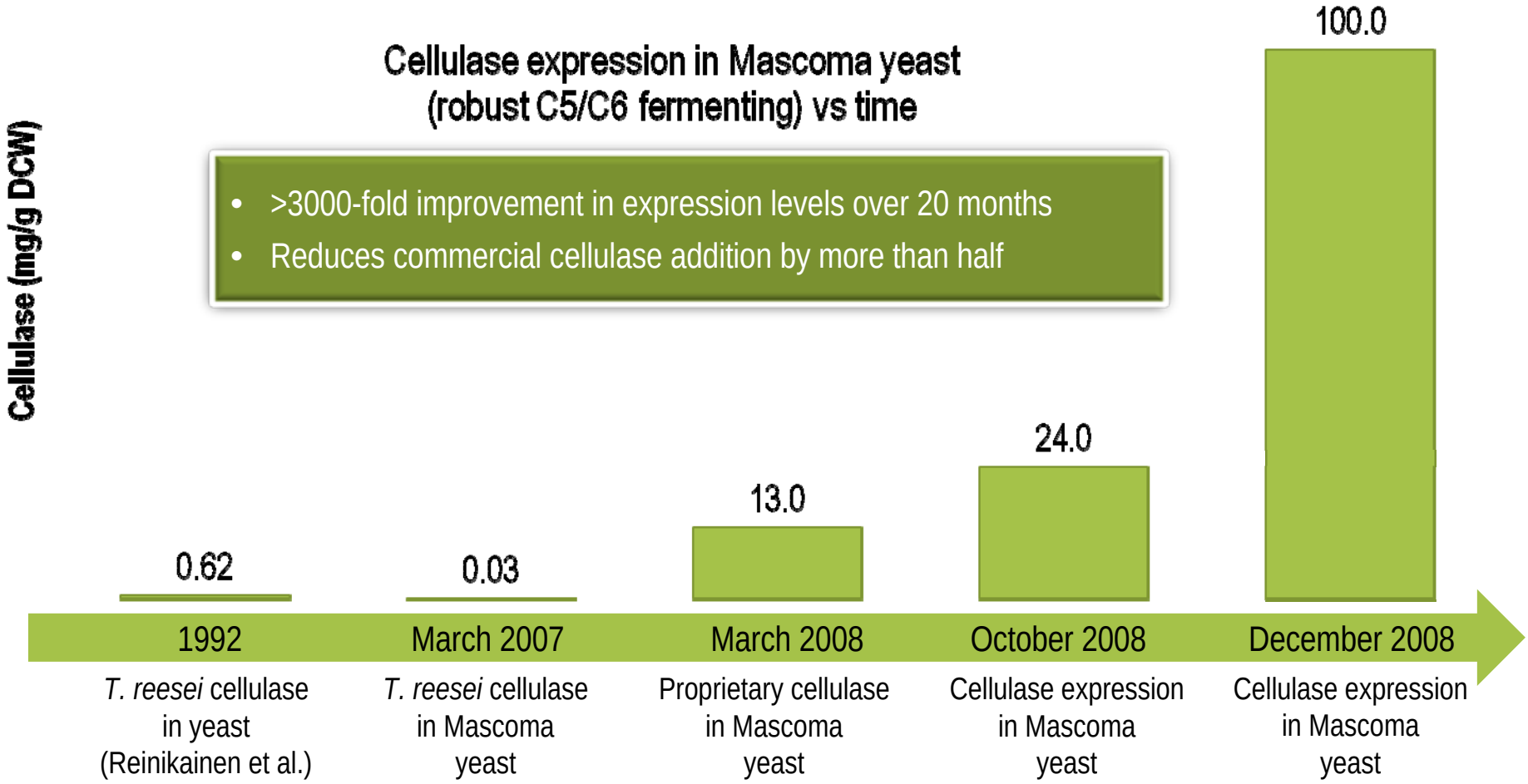


S-Y Ding (NREL) and X. S. Xie (Harvard)
tools under BER imaging grant; sample analysis under BESC, 2010
Mutant alfalfa from Noble Foundation

Strategy, part 3:
Identify, understand, and manipulate "biological catalysts" to overcome recalcitrance

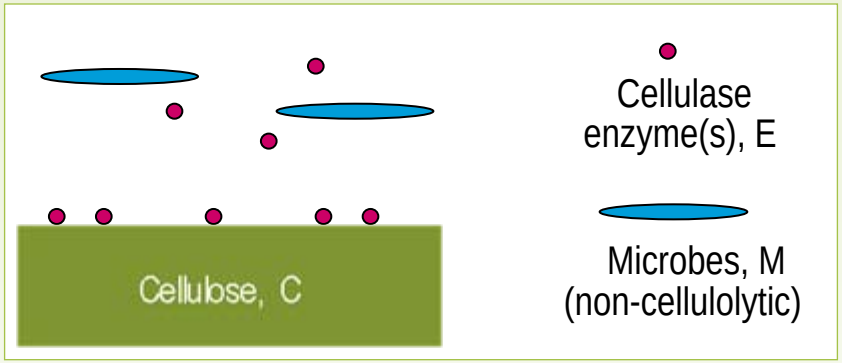


CBP organism development yeast

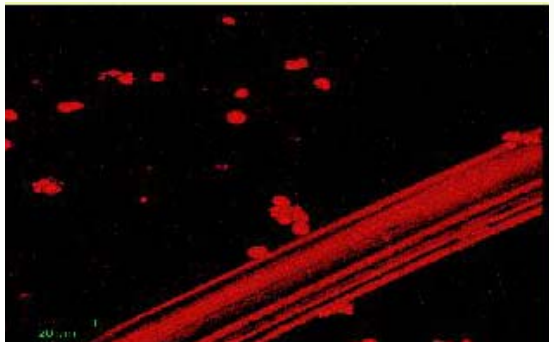


Enzymatic and microbial hydrolysis
A fundamentally different relationship between microbes and cellulose

Enzymatic hydrolysis (classical approach)

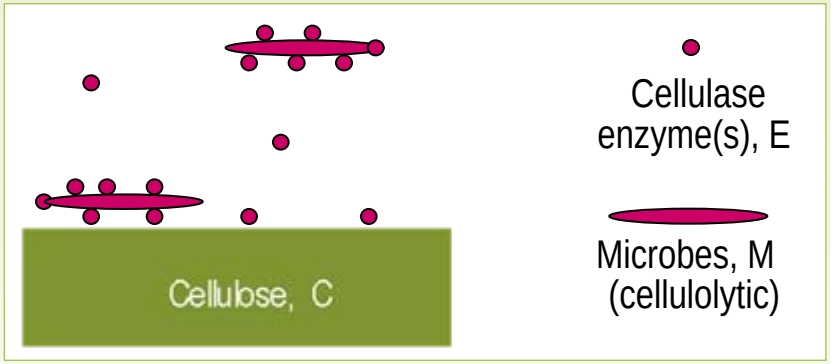


- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound and free
- Cells may or may not be present

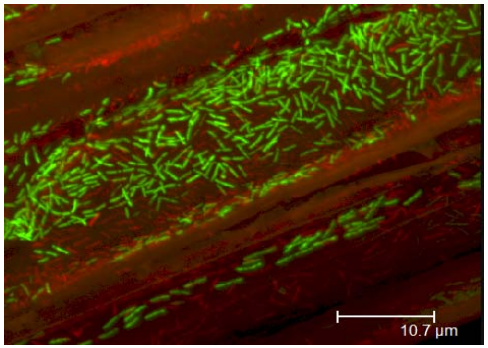


Yeast, enzymes with biomass (Dumitrache and Wolfaardt)

Microbial hydrolysis (CBP)



- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound and free
- Cells both bound and free



C. thermocellum on poplar (Morrell-Falvey and Raman, ORNL)

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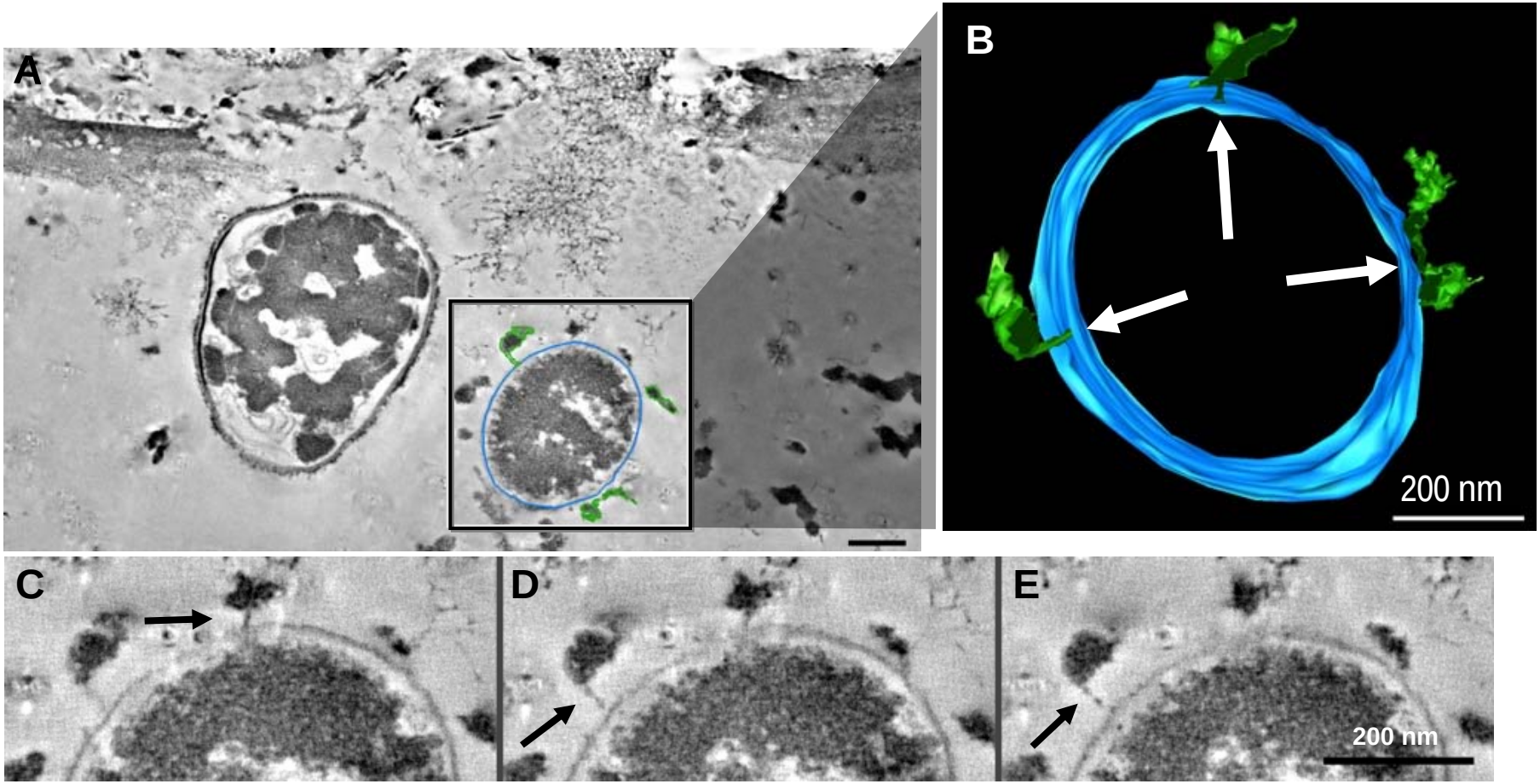
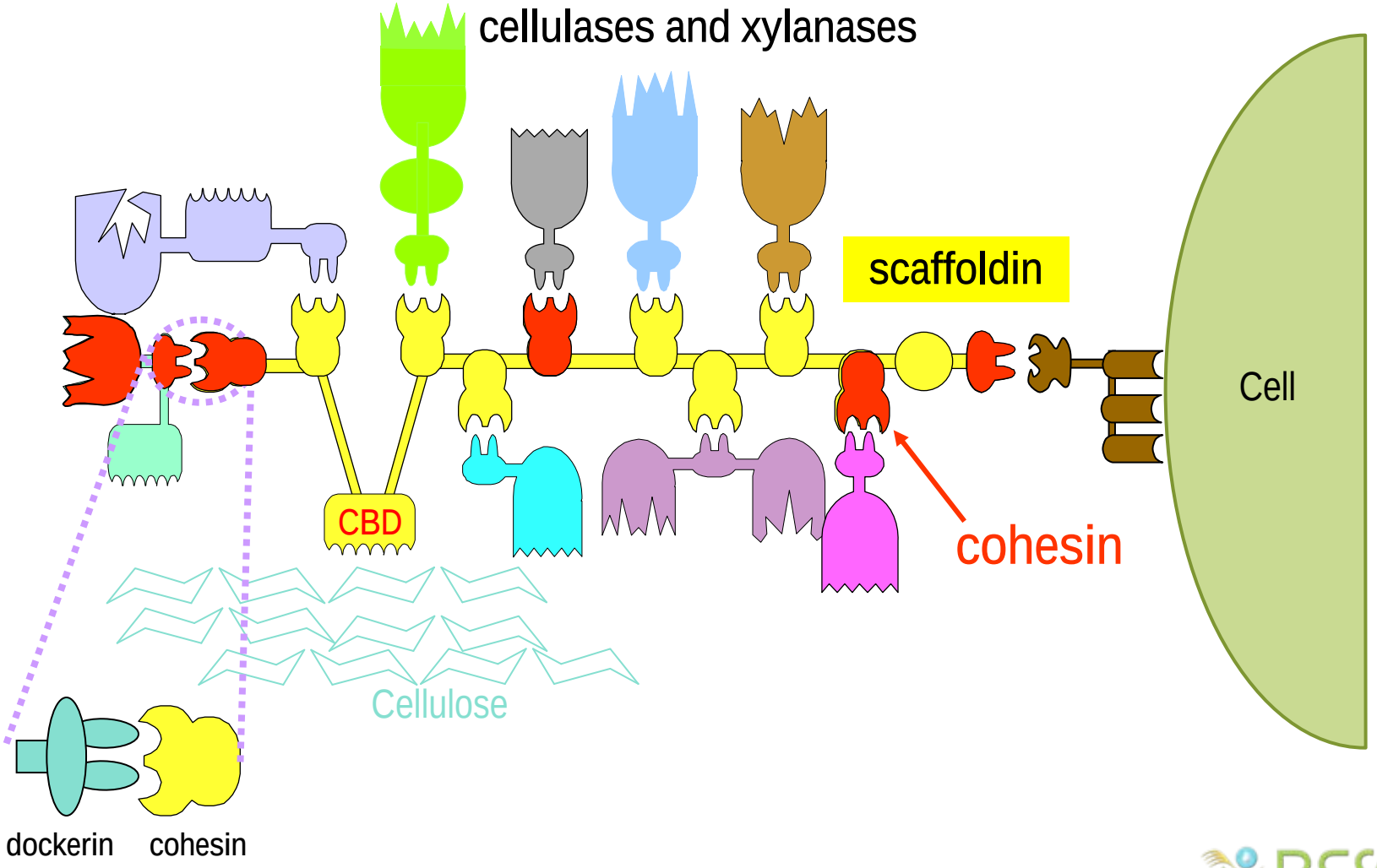
3D electron tomography of *C. cellulolyticum*



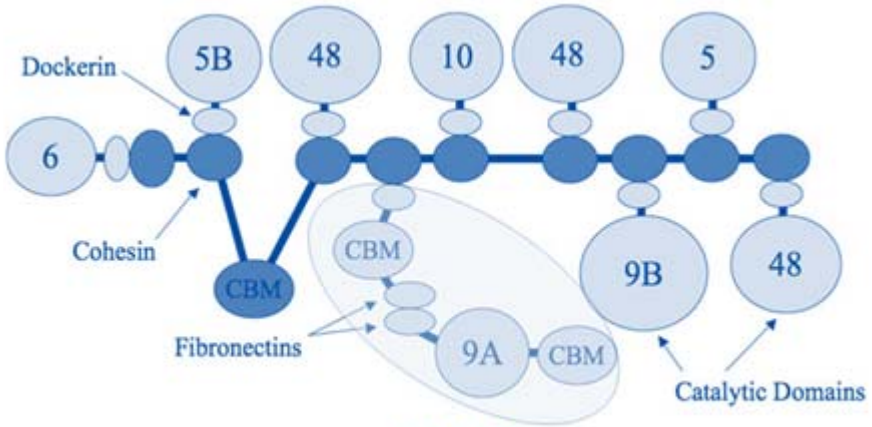
Tomogram slices and surface rendered segmentation of bacterial cells and tethered cellulosomes. C–E: Serial slices taken every ~8 nm through tethered cellulosomes. These tethers are seen at one end of most polycellulosmes found near the bacterial cell surface and are ~5 nm in diameter and up to 50 nm in length.

Cellulosome of *C. thermocellum*



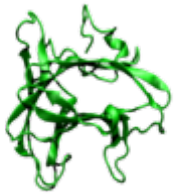
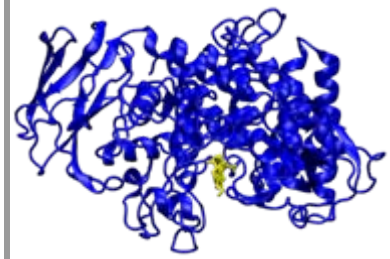
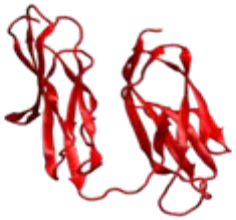
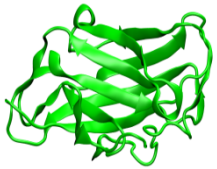
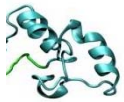
Understanding cellulosome structure/function

- Assembled 7 domain enzymes
- Ready for computer simulations

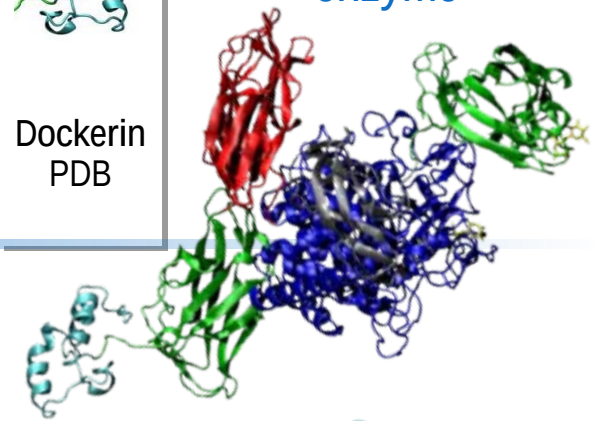


CbhA

C. thermocellum CbhA

CBM4	IG GH9	$FN3_1$ $FN3_2$	CBM3b	D
				
CBM4 NREL 2009	Ig-GH9 PDB	$Fn3_1$–$Fn3_2$ NREL 2009	CBM3b Bayer et al., 2009 (unpublished)	Dockerin PDB

CbhA is a critical enzyme



New structures solved at NREL

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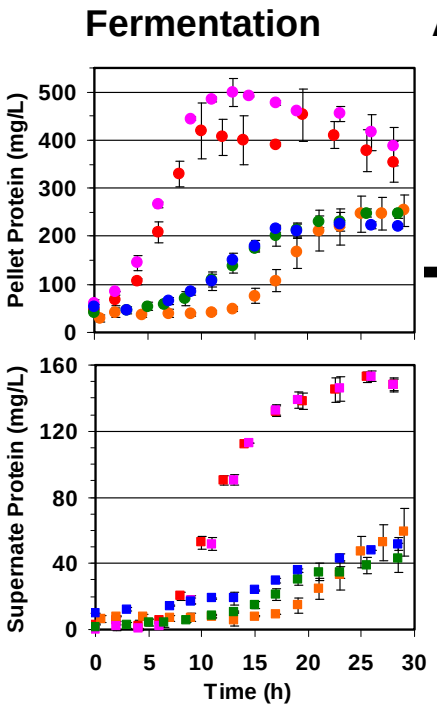
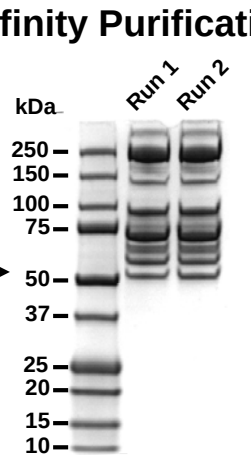
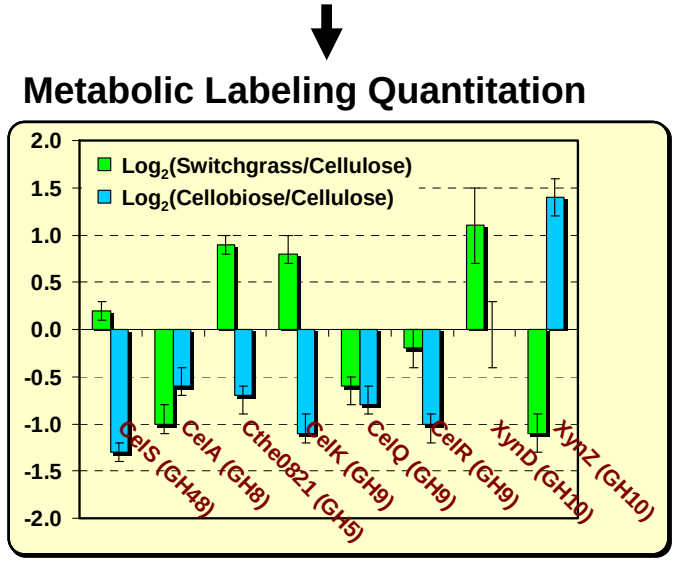
Cellulosome Changes in *C. thermocellum* on Different Biomass Substrates





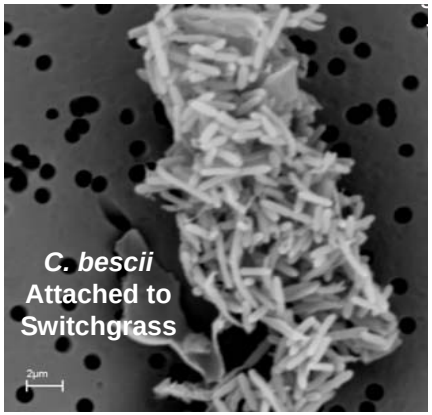


- Pretreated Switchgrass
- Cellobiose
- Amorphous Cellulose
- Avicel - ^{14}N
- Avicel - ^{15}N
- Avicel-Pectin
- Avicel-Xylan
- Avicel-Pectin-Xylan

- ***C. thermocellum*** **alters its cellulosome catalytic composition depending upon the growth substrate**
- **We identified and experimentally verified 16 "new" cellulosome components**
- **Insights aid in constructing designer cellulosomes with tailored enzyme composition for industrial ethanol production**

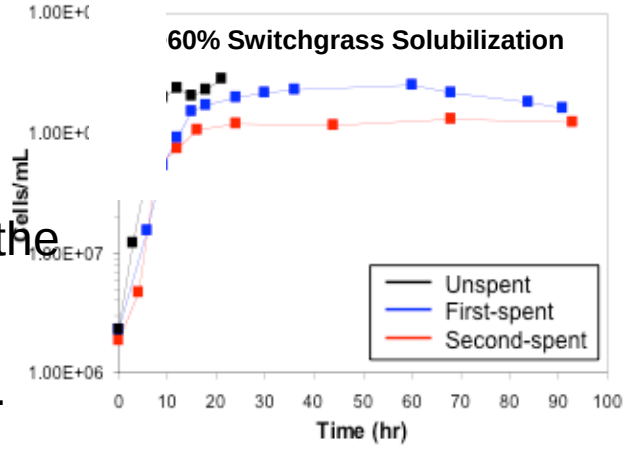
Citation: "Raman B, *et al*. (2009) Impact of Pretreated Switchgrass and Biomass Carbohydrates on *Clostridium thermocellum* ATCC 27405 Cellulosome Composition: A Quantitative Proteomic Analysis. PLoS ONE 4(4): e5271. doi:10.1371/journal.pone.0005271"

Thermophilic Cellulose-Degrading Microbe Degrades Plant Biomass Without Pretreatment



Caldicellulosiruptor bescii (formerly *Anaerocellum thermophilum*)

- The most thermophilic cellulolytic organism known (Tmax 90°C)
- Grows on unprocessed plant biomass (switchgrass, poplar and peanut shells) and spent insoluble material (after primary growth)
  - >60% switchgrass biomass solubilized by three successive cultures
  - glucose:xylose:lignin ratio comparable in unspent and spent biomass
- Genome contains > 100 CBH-metabolizing (CAZy) genes, many in clusters
  - candidate genes to mediate cell-biomass adhesion identified
- Growth on cellulose and switchgrass accomplished at the 600-liter scale for
  - characterization of extracellular proteins (> 8 grams)
  - transcriptomics and proteomics analyses underway of C. bescii grown on cellulose, xylan, switchgrass and poplar
- Significance: conversion of direct unprocessed biomass to biofuel may be possible



Source: Adams (UGa): Yang (2009), Kataeva (2009) Yang (2010)

Biodiversity Access for New Biocatalysts

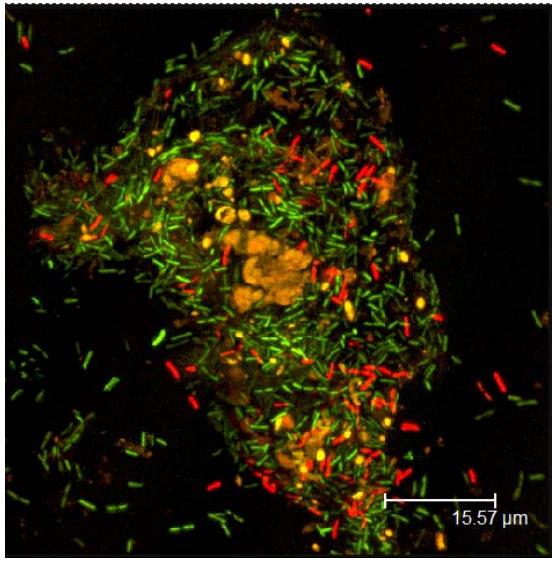
- State-of-the-art cultivation techniques to isolate novel high-temperature microbes with powerful lignocellulolytic enzymes
  - Collect samples from thermal biotopes
  - Establish primary enrichment cultures at relevant temperatures and conditions

Sampling at Yellowstone National Park, October 2007 and July 2008

High-throughput Isolation of Cellulolytic Extreme Thermophiles Using Flow Cytometry

U.S. DEPARTMENT OF ENERGY

Complex Enrichment

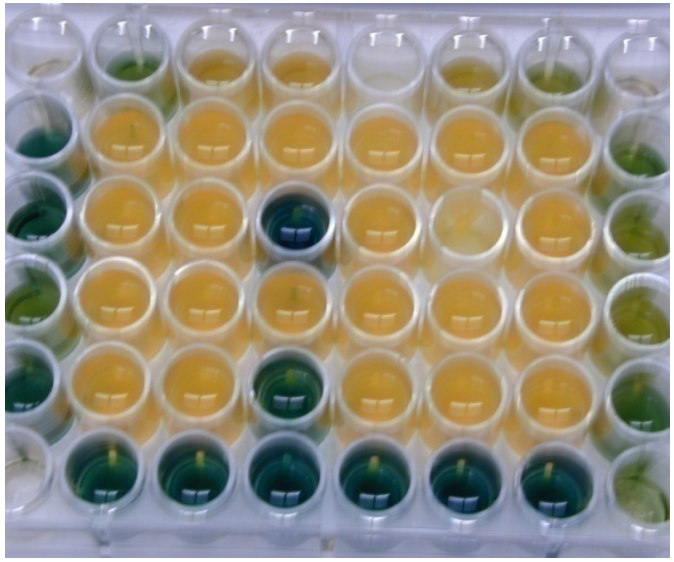


1. Establish primary enrichments from environmental samples on biomass.
2. Screen for growth and hydrolysis of pretreated biomass.

Single Cell Isolation

3. A single cell is deposited by Flow Cytometry in a culture well containing pretreated biomass.
4. Multi-well plates are incubated at 70-80^{o} C in the absence of oxygen.

High-throughput Screening



5. Plates are screened for growth and biomass hydrolysis.
6. High-throughput screening allows thousands of isolates to be evaluated with natural substrates.

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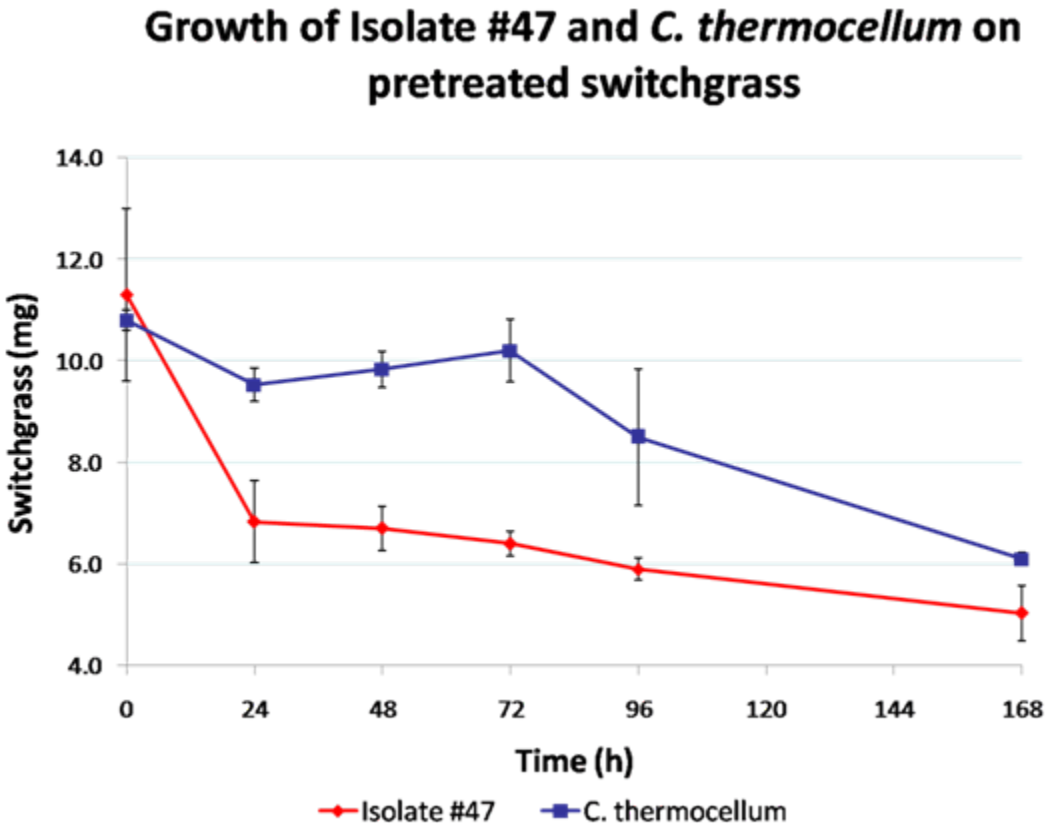
New Isolates Show Enhanced Biomass Hydrolysis Rates

Isolate #47

Control



Preliminary results show visual disappearance of pretreated switchgrass solids during growth at 78˚C relative to a benchmark organism

Elkins et al. - ORNL

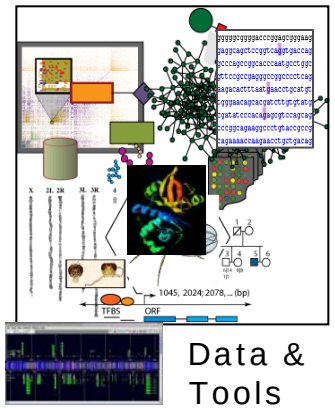
OB#47 submitted as Caldicellulosiruptor sp.

BioEnergy Science Center

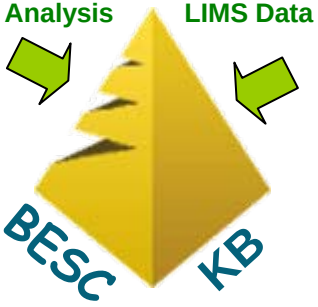
BESC Knowledge Base

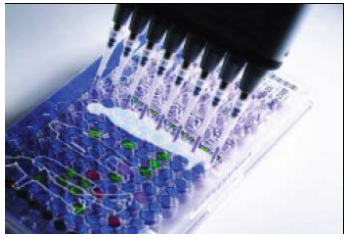
KB Mission

- Analyze and present integrated data and results in the context of cell and organism systems biology



Data & Tools



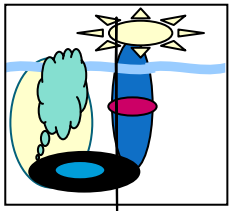


Experiments

Experiment Design

Researcher

Discovery

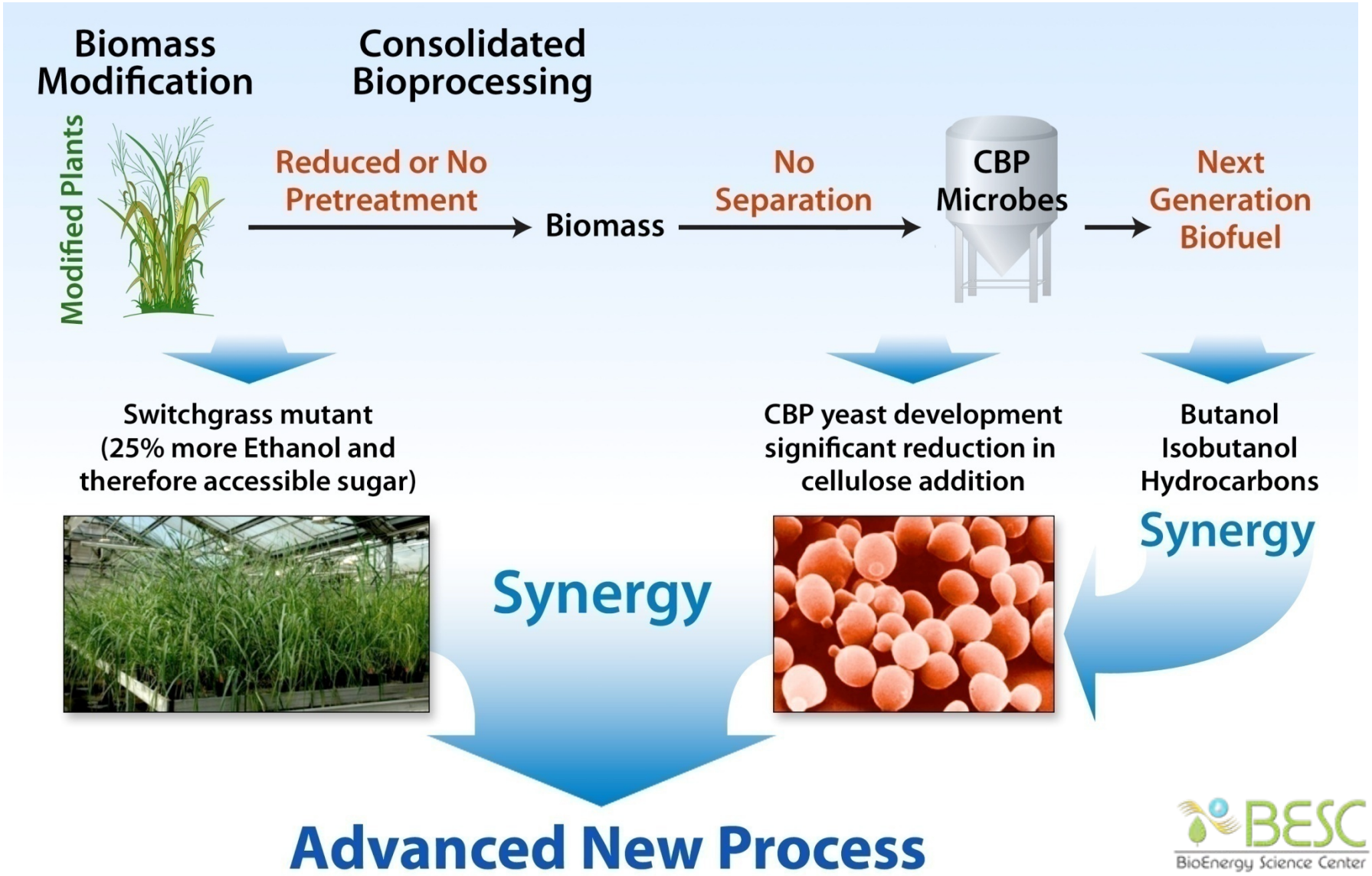


Hypothesis

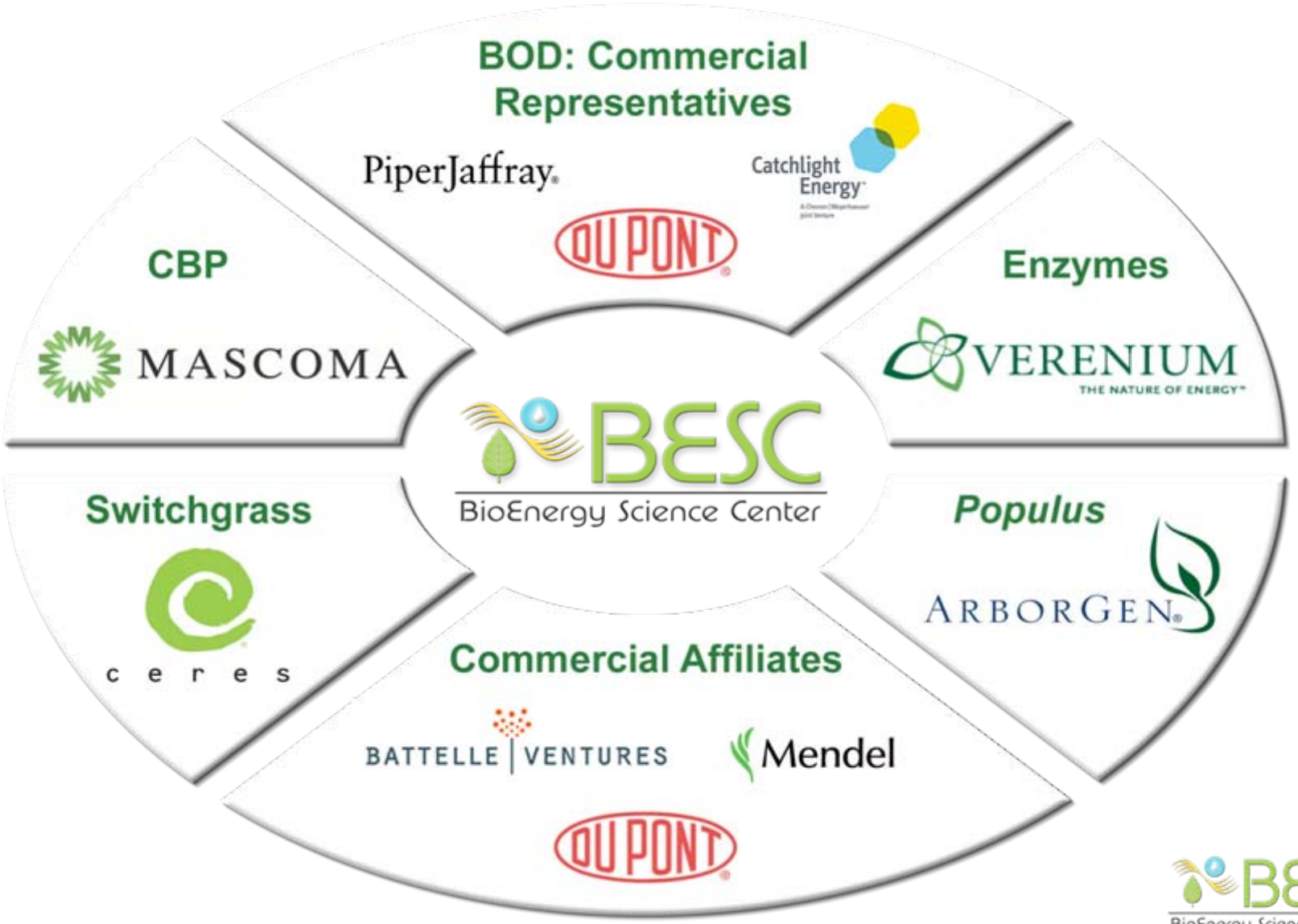
Methodology

- **BESC Knowledge discovery and data mining environment for *design* of experiments**
- ***Interpretation of BESC experimental* data thru integration with community / reference data such as genomes, pathways, networks, ontologies**
- Integrate diverse data types across BESC **data to provide new insights in to cell wall recalcitrance, etc.**
- **Provides systems level representations, knowledge, data, and information on key plants, microbes, and molecules**
- **Serves as a biological discovery / data mining platform for larger community**

BESC will revolutionize how biomass is processed and converted

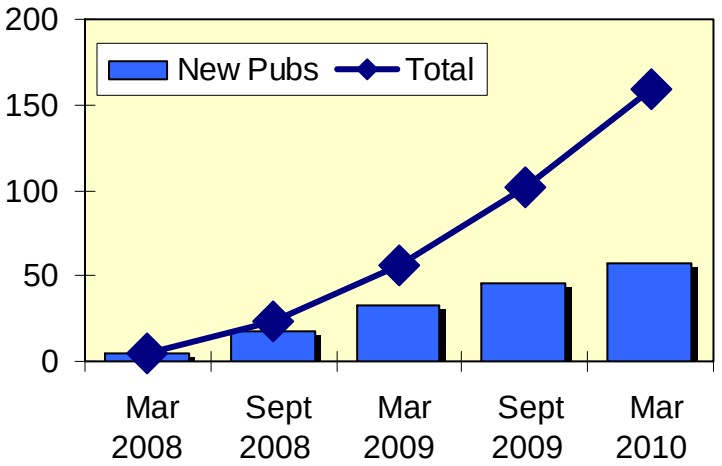


Industrial partners facilitate strategic commercialization



Translating discoveries to the scientific community

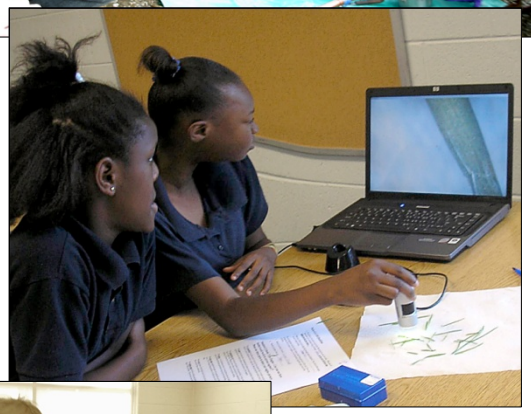
- 159 scientific publications
  - 33% of publications include external collaborators at non-BESC Institutions
- BESC publications have already been cited 262 times in peer-reviewed journals
- Several publications in top-tier journals
  - *Nature Biotechnology*, 2008, Lynd *et al*., How biotech can transform biofuels
  - *PNAS*, 2008, Shaw *et al*., Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield
  - *Nature Nanotechnology*, 2010, Tetard *et al*., New modes of subsurface atomic force microscopy through nanomechanical coupling



- 17 inventions disclosed (under evaluation by BESC Commercialization Council)

Influencing next generation of scientists

- National Geographic, The Jason Project, filmed and generated an educational module on bioenergy with BESC researchers
  - This module is available from www.jason.org
- Created an interactive biofuels outreach lesson for students in Grades 3-8
  - Piloted more than 220 lessons which reached over 6,000 students
  - Partnered with the Creative Discovery Museum
  - Available on www.bioenergycenter.org
- Piloted ten Biofuels Family Science Nights with an average attendance of 250 people each



BESC model for multidisciplinary and integrated research program

- It is possible to be highly integrated without being co-located
- A strong management team with centralized funding source is needed
- Allowing scientists to participate from home institutions enables rapid assembly of a team with a much higher proportion of the most accomplished experts
- Capital costs are lowered by utilizing existing infrastructure/equipment available at partner facilities
- A coherent vision, goal, and defined milestones are critical

Thank you

SCIENCE RETREAT DECEMBER 2008

SCIENCE RETREAT JUNE 2009

DOE Bioenergy Research Centers

DOE GENOMICS:GTL SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT

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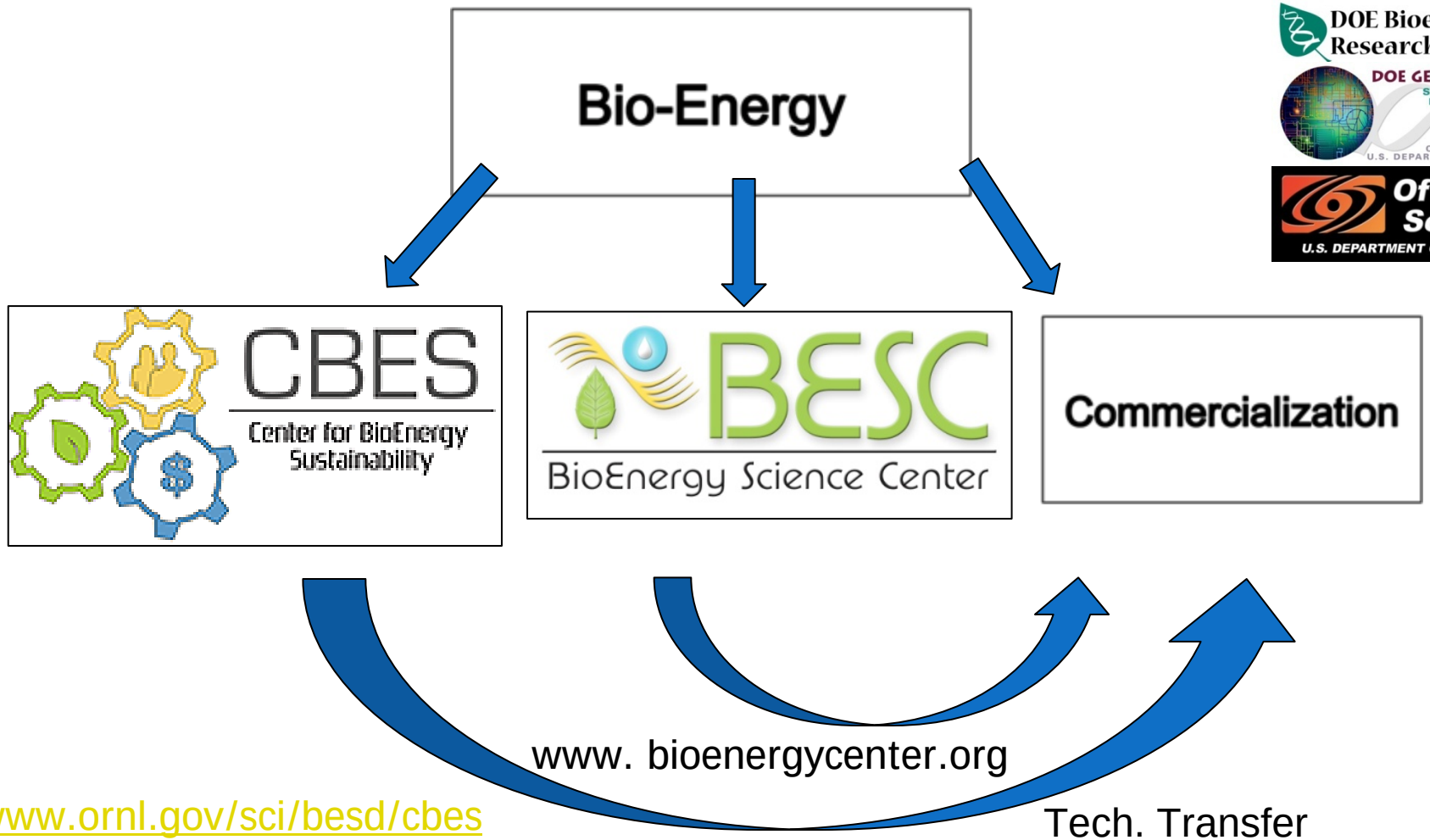
BESC is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science

BESC BioEnergy Science Center

Bio-Energy and Bioproducts at ORNL



www.ornl.gov/sci/besd/cbes

Tech. Transfer
Nat'l Transport. Res. Center
Bioproducts, etc.

Acknowledgements: slides from BESC, Robin Graham, Mark Downing, L. Russo (USDOE-OBP), etc.