The BioEnergy Science Center
a DOE BioEnergy Research Center

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BESC Director

http://www.bioenergycenter.org/

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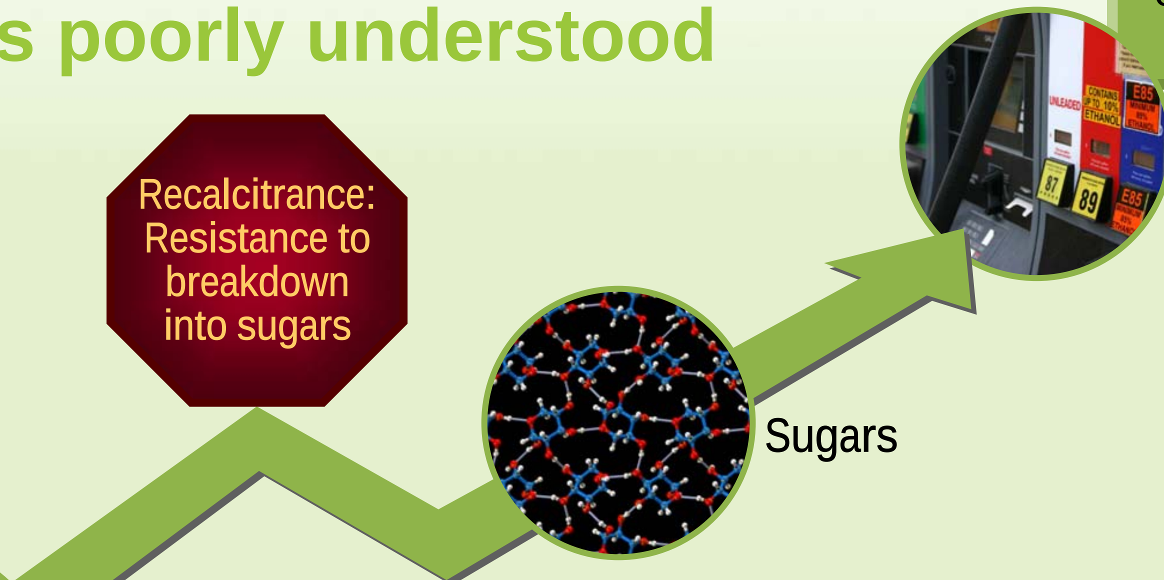
The fundamental science of biomass recalcitrance is poorly understood

Overcoming recalcitrance is the single coherent overarching theme for the BESC

- A large-scale, integrated, interdisciplinary approach is needed to overcome this problem
  - Current research efforts are limited in scope
  - BESC will launch a broad and comprehensive attack on a scale well beyond any efforts to date
- Without advances, a cellulosic biofuels industry is unlikely to emerge
- Knowledge gained will benefit other biofuels and biofeedstocks

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The BESC Team

Joint Institute for Biological Sciences (JIBS)

Alternative Fuels User Facility

Complex Carbohydrate Research Center

- Oak Ridge National Laboratory
- University of Georgia
- University of Tennessee
- National Renewable Energy Laboratory
- Georgia Tech
- Samuel Roberts Noble Foundation
- Dartmouth
- ArborGen
- Verenium
- Mascoma
- Individuals from U California-Riverside, Cornell, Washington State, U Minnesota, NCSU, Brookhaven National Laboratory, Virginia Tech

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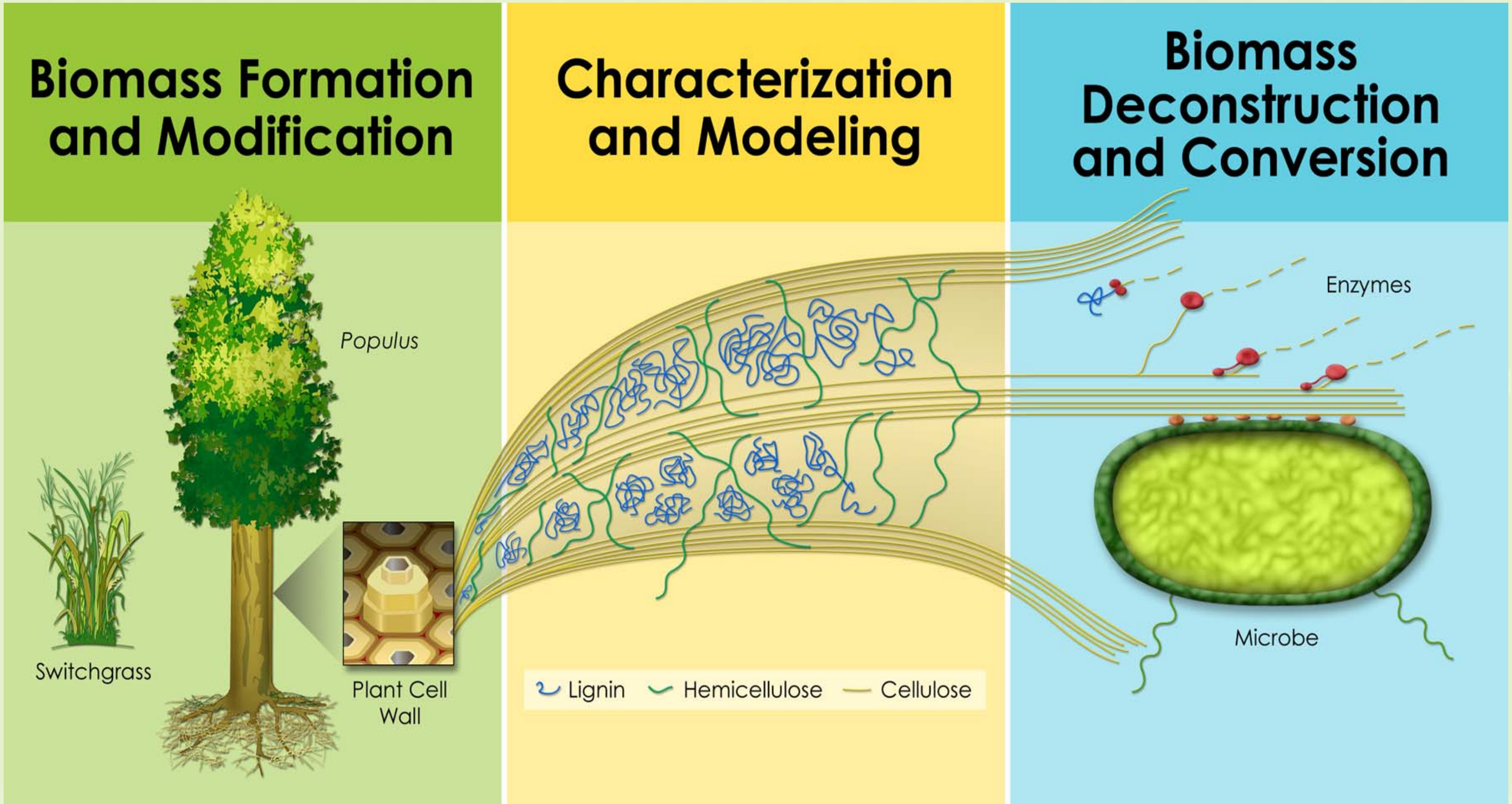
Three linked scientific focus areas will enable BESC to understand and overcome biomass recalcitrance



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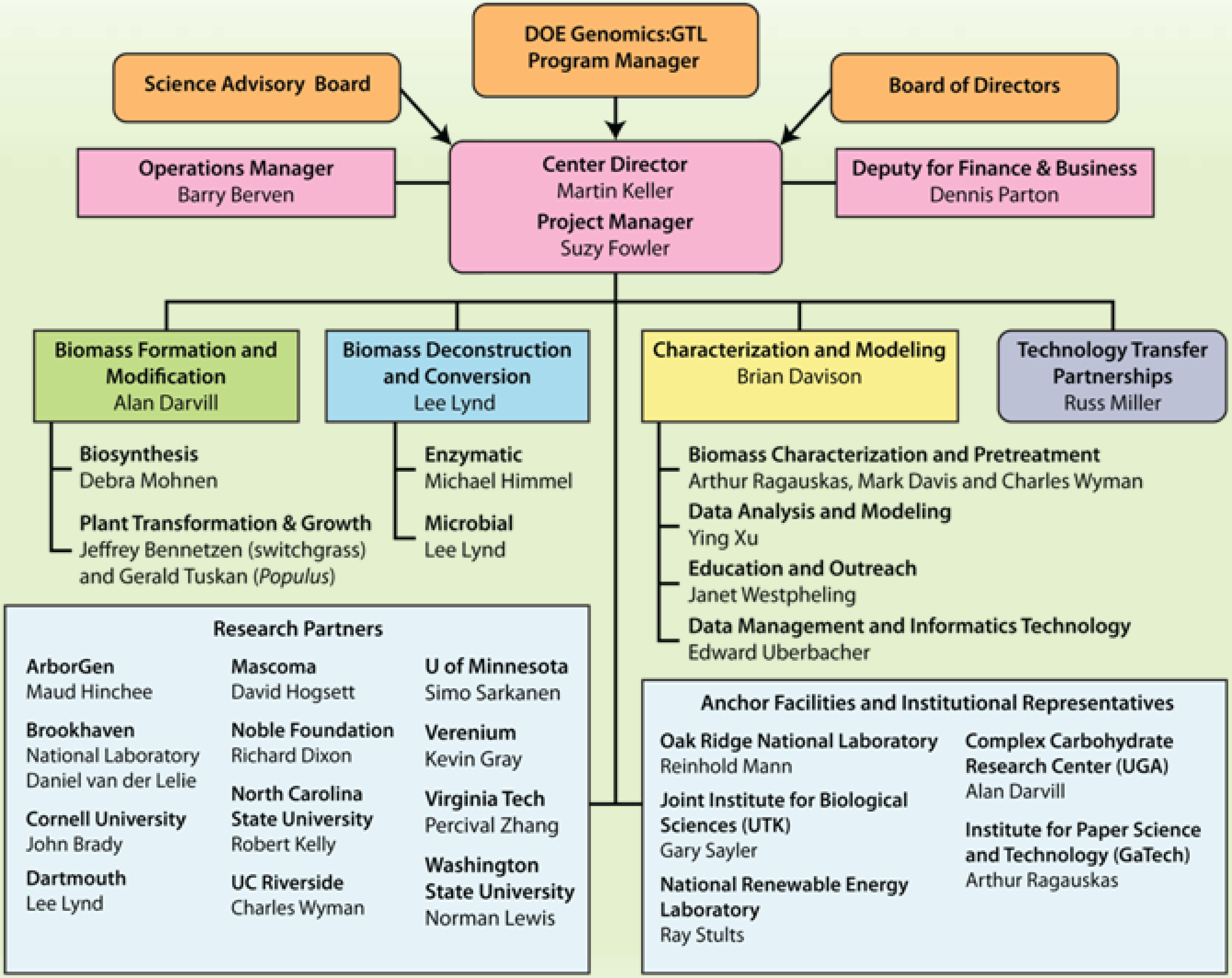
BESC is organized to provide clear operations and science accountability



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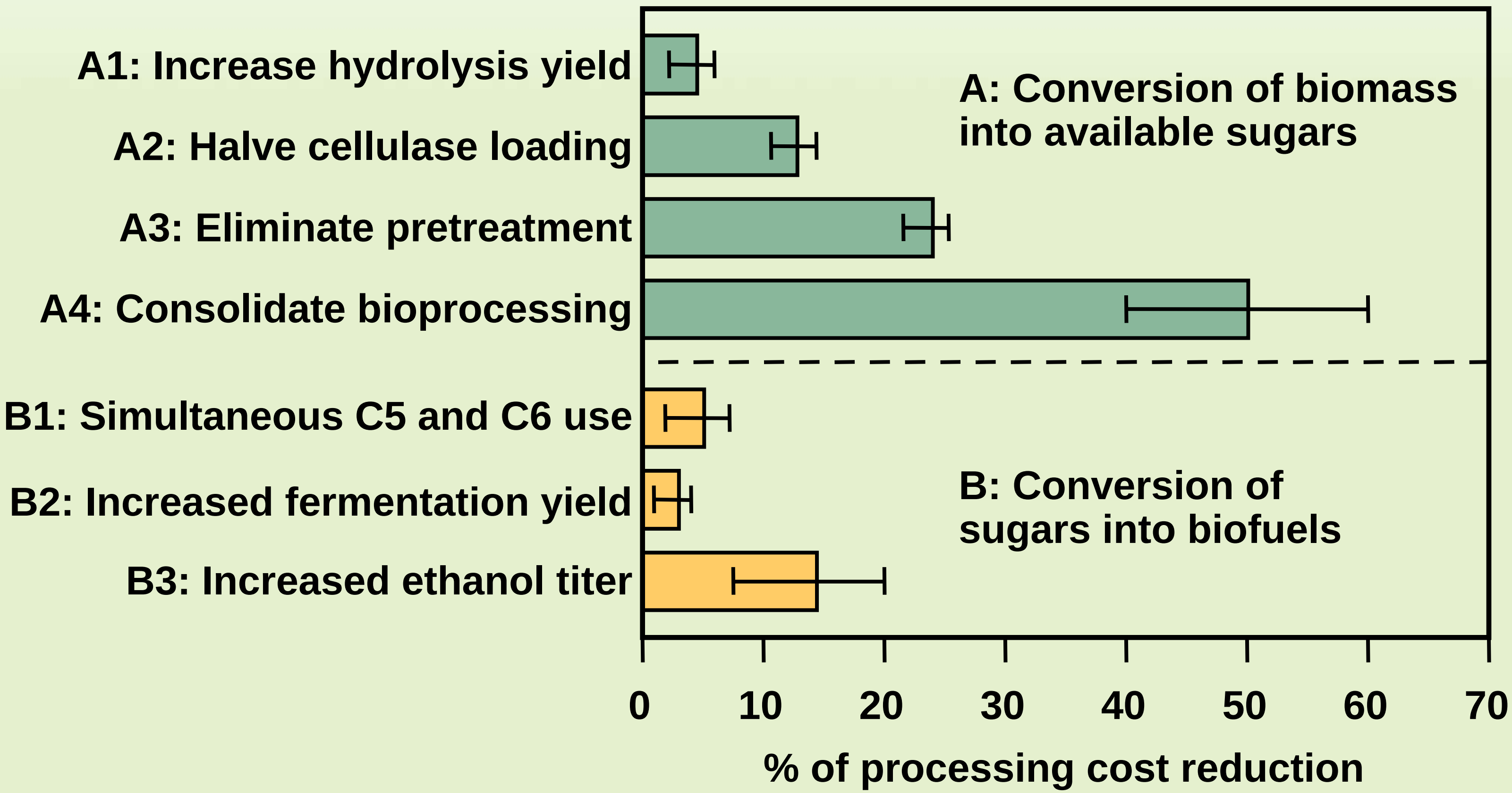
Comparative impacts of R&D on biomass processing cost



Without overcoming biomass recalcitrance (A), cellulosic biofuels will be more expensive than corn biofuels. Improved sugar conversion (B) is not enough.

Ref: Lynd, L.R., M.S. Laser, D. Bransby, B.E. Dale, B. Davison, R. Hamilton, M. Himmel, M. Keller, J.D. McMillan, J. Sheehan, C.E. Wyman, 2007. "Energy Biotechnology: Targeting a Revolution" Nature Biotechnology (in press)

BESC has well-defined objectives

Revolutionize the processing of biomass within 5 years

- **Improve overall yields**
- **Simplify operations through consolidated bioprocessing (CBP)**
- **Decrease (or eliminate) the need for costly chemical pretreatment**

Apply a systems biology approach and new higher-throughput pipelines

- **Reduce recalcitrance by targeted modification of plant cell wall composition and structures**
- **Develop and understand single microbes or microbial consortia and their enzymes to enable CBP for low-cost cellulose hydrolysis and fermentation**
- **Provide a synergistic combination of modified plants and CBP for even more cost-effective biofuel production**

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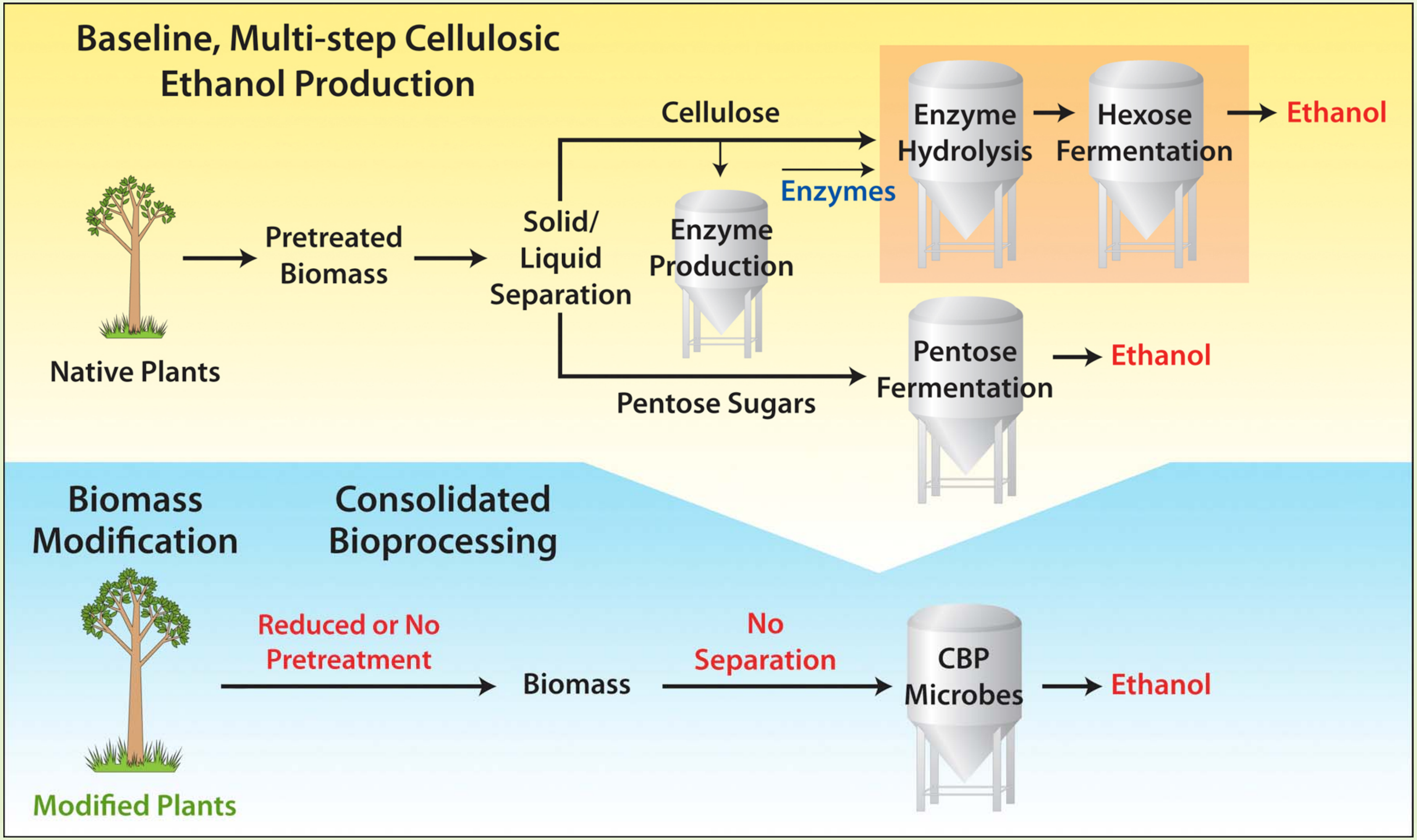
BESC will revolutionize how biomass is processed within five years



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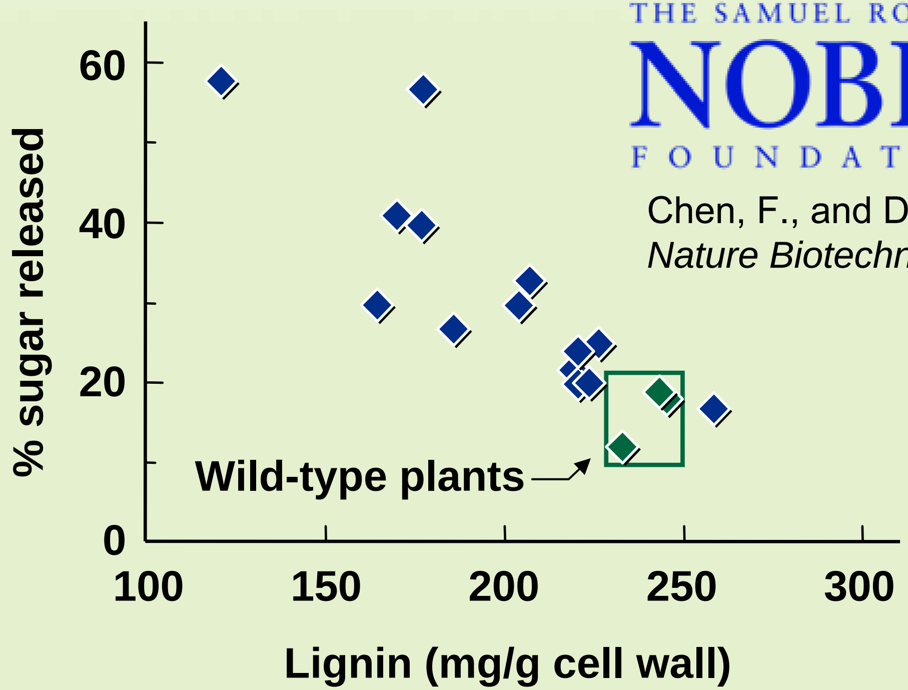
Modifying cell wall composition and structure can reduce recalcitrance



THE SAMUEL ROBERTS NOBLE FOUNDATION

Chen, F., and Dixon, R.A. (2007)
Nature Biotechnology 25, 759-761.

- **More sugar is solubilized by cellulase when the lignin content of alfalfa cell walls is reduced**
- **Strategy is feasible for *Populus* and switchgrass**

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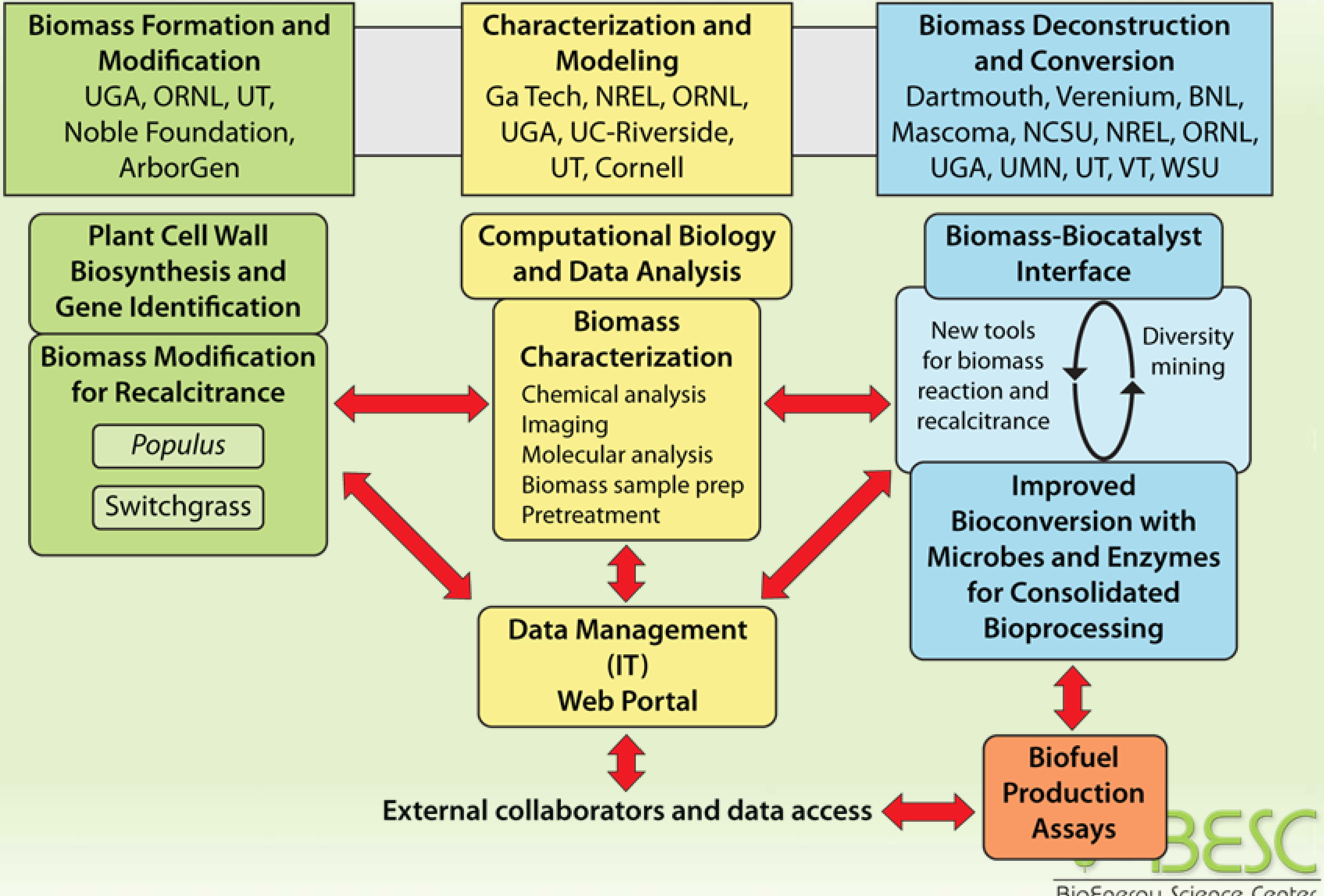
BESC – a highly integrated cutting-edge research team



The challenges (part 1): Lignocellulosic biomass is complex and heterogeneous

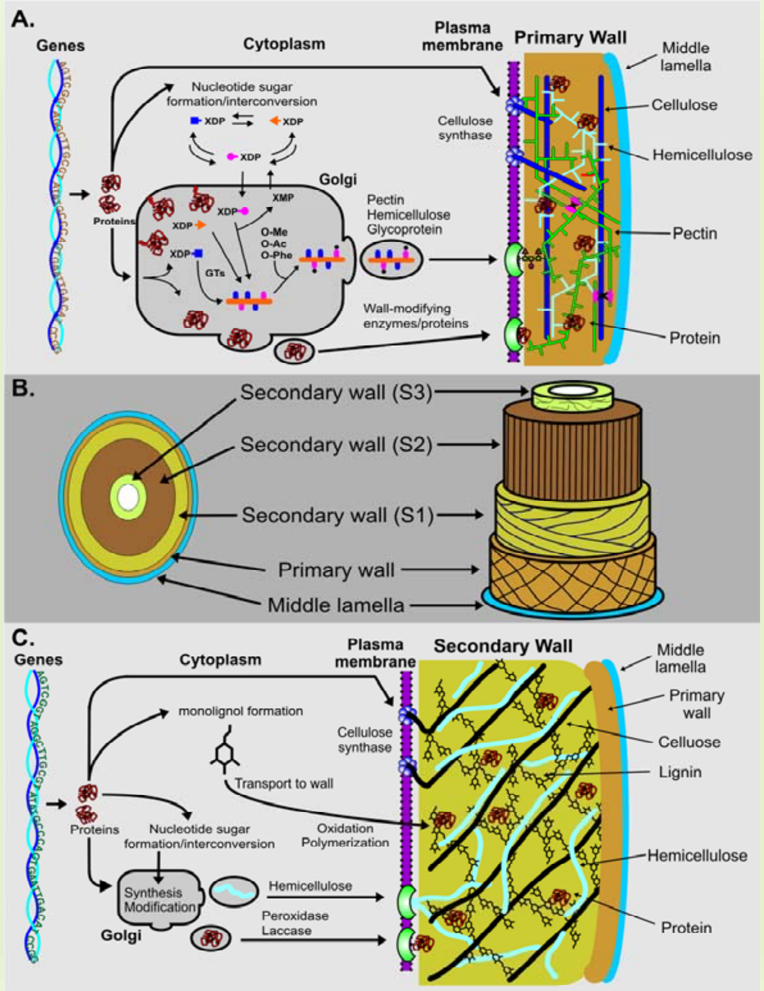


Fig. II.2. Biosynthesis of primary and secondary walls: from genes to polymers. A. Primary wall polysaccharides are synthesized at the plasmamembrane (cellulose) and in the Golgi (pectin and hemicellulose) by the action of glycosyltransferases that use nucleotide-sugar substrates. B. Some cells (e.g. xylem) form secondary walls internal to the primary wall. C. Secondary wall synthesis includes cellulose, hemicellulose and lignin deposition.

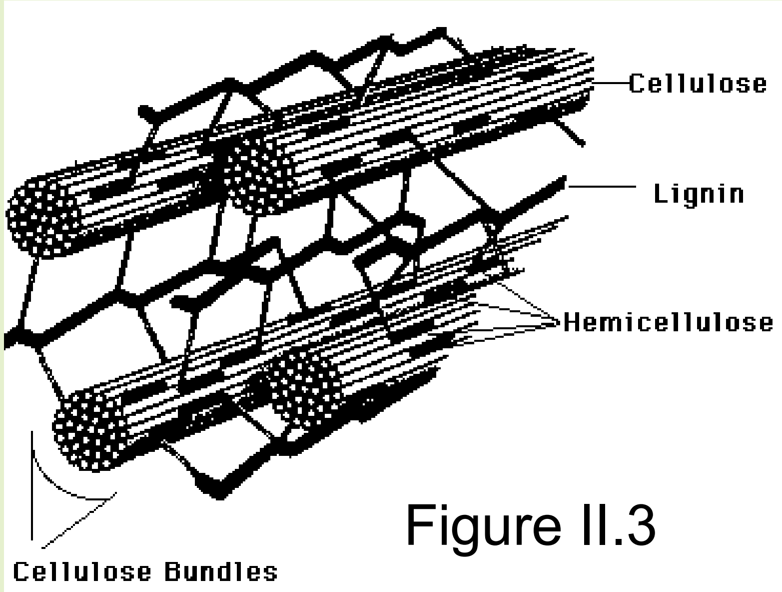


Figure II.3

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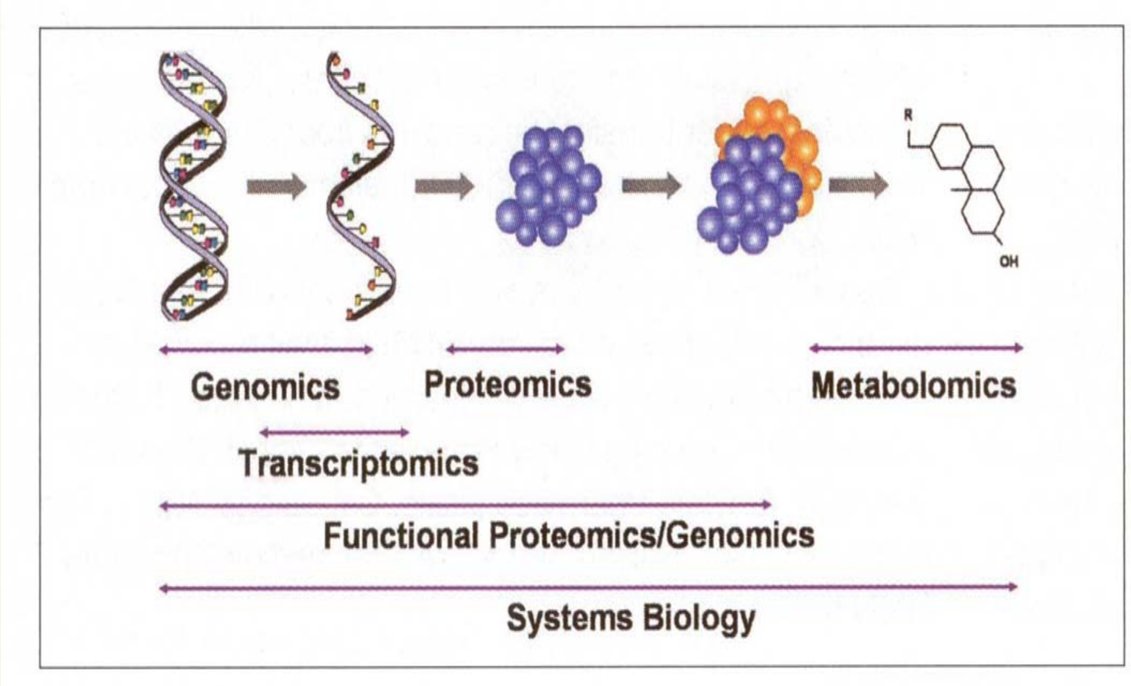
“Omic” capabilities for systems biology

- Genomics
- Transcriptomics
- Proteomics
- Interactomics
- Metabolomics



- Together these can provide a deeper picture of how a microbe or plant is functioning
- This can help identify where improvements need to be made

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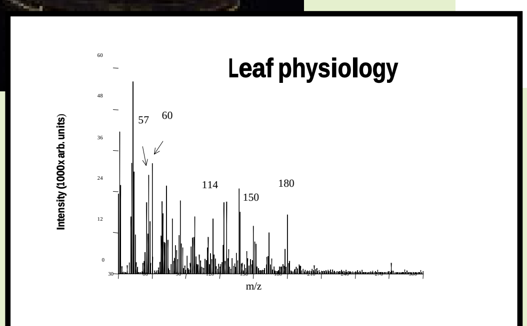
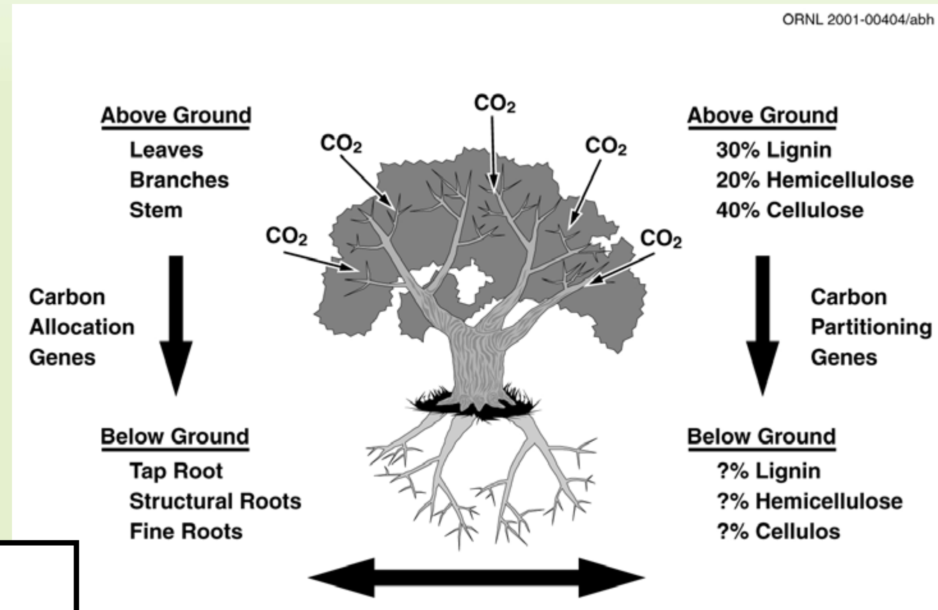
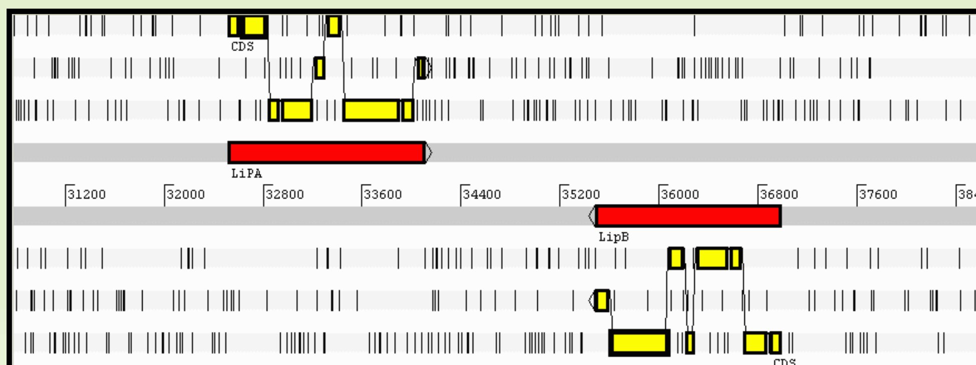
Woody plant genomics – *designing crops for energy & C sequestration*





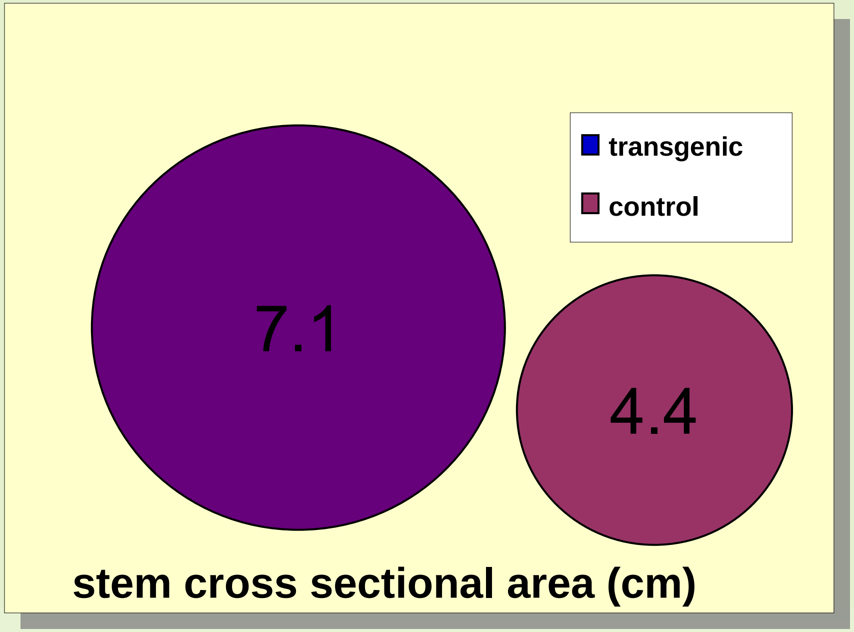


High-throughput Phenotying

Populus - early results from genome sequence availability (Jerry Tuskan, ORNL)

90-day-old *Populus* cuttings

Using Poplar tree genome, the expression of one gene (IAA16.3) was altered. This resulted in enhanced radial growth of IAA16.3 transgenics vs. controls



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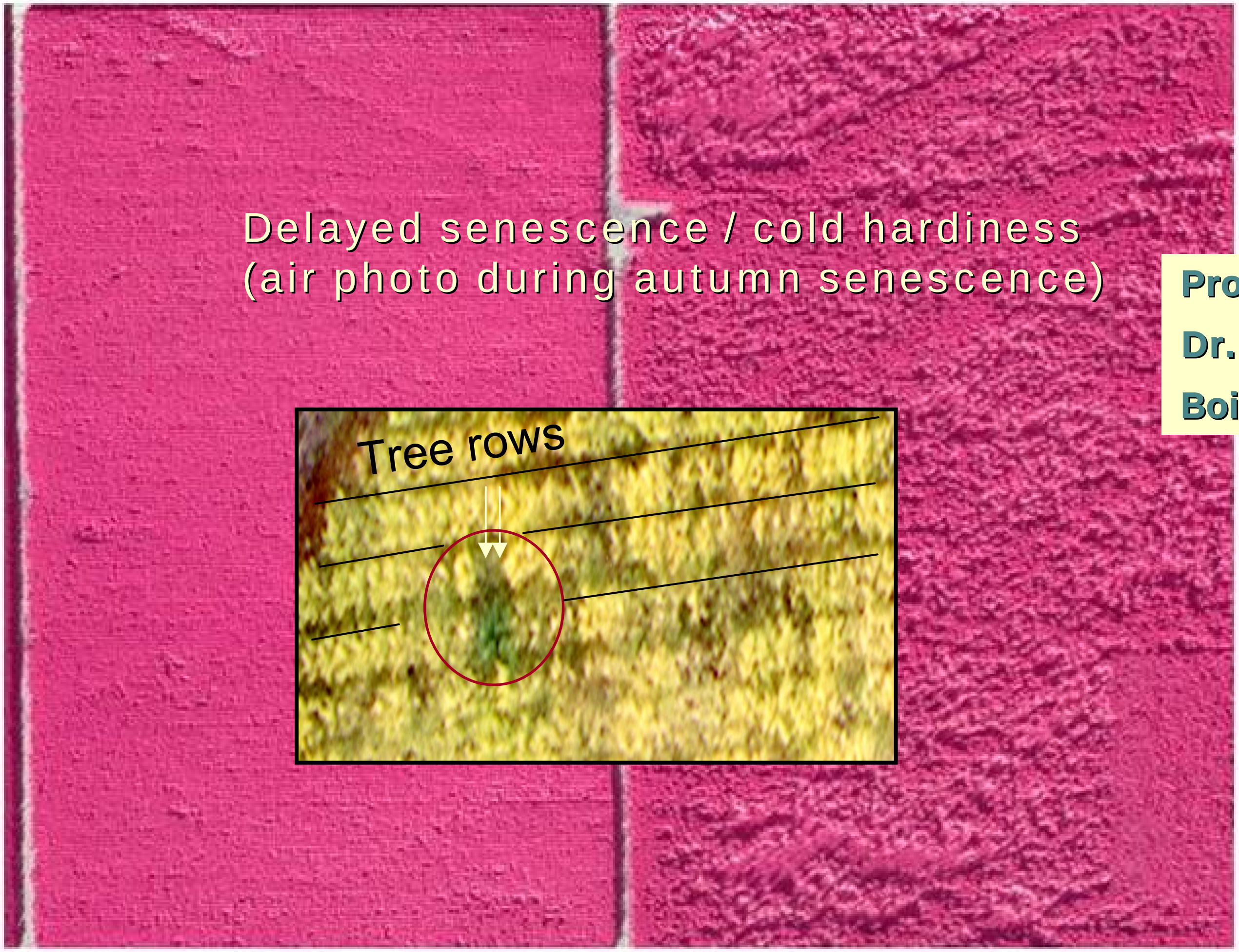
Activation tagging to identify Poplar genes: Some mutants can be detected using infrared aerial photography



Provided by:

Dr. Peggy Payne,

Boise Cascade

Populus trichocarpa x deltoides clone block

P. trichocarpa x nigra clone block

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Others are evident at the whole-tree level

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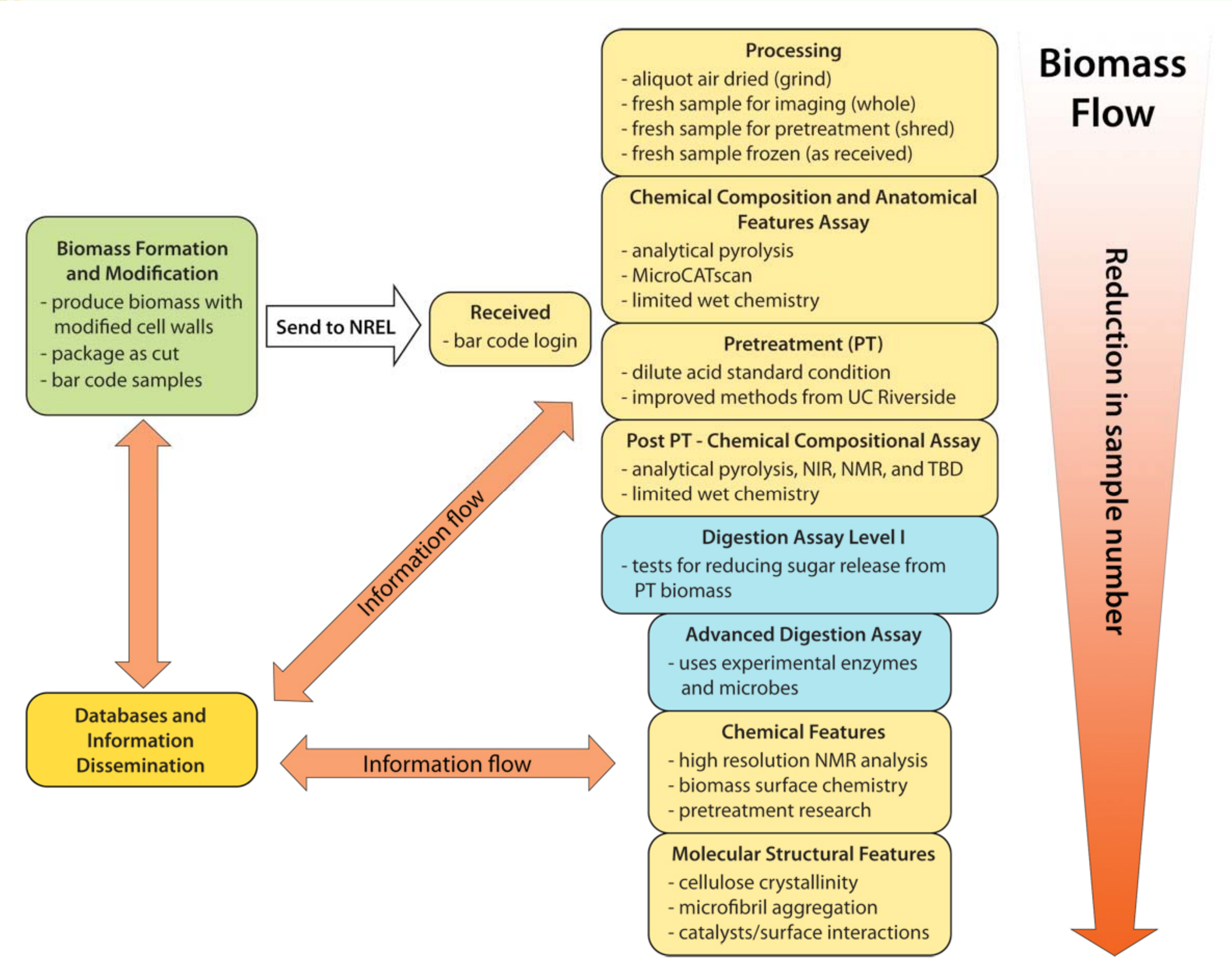
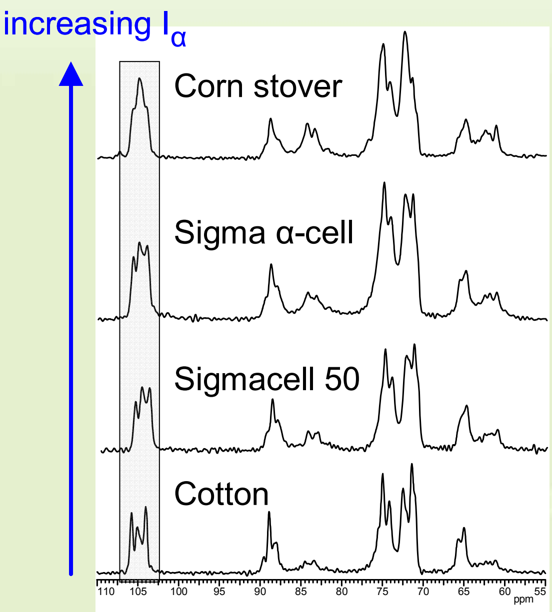
Schematic of sample flow in BESC



Identify structural and chemical features of recalcitrance

- High throughput analysis
  - Compositional analysis of 1000's of samples
    - NREL pbMS is being adapted to *Populus* and switchgrass
  - Pretreatment screens
  - Biomass enzyme digestibility (recalcitrance phenotoype)
- High resolution analysis of plant cell walls
  - Monoclonal antibodies
  - AFM mapping of surface chemistry
  - Imaging MS
  - MicroCat for plant anatomy
- Biomass surface chemistry
- Baseline samples of biomass provided across BESC for methods testing, control, and shared insights
- Pretreatment insights to understanding recalcitrance



Comparison of [13]C CPMAS NMR spectra of different celluloses show increasing crystalinity (Iα).

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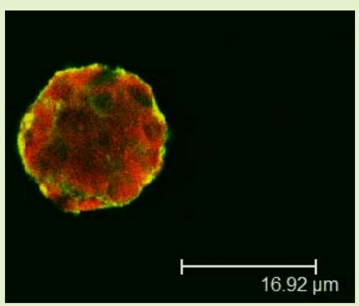
New combined imaging tools to elucidate cell wall formation

- **utilizing polysaccharide antibodies to visualize layers in the cell wall**

Green fluorescence from positive antibody reaction

Red autofluorescence from Chloroplasts (intracellular)



Positive signal in cell walls of 2-day old spheroplasts (*Populus* protoplasts growing in wall formation culture condition) obtained using CCRC monoclonal antibodies *.

*Antibody mix composition: CCRC-M1, M13, M38, M48, M56. This mixture of antibodies targets wall polysaccharides such as pectin (Rhamnogalacturonan I) and hemicelluose (Xyloglucan).

<u>Kalluri, Hahn: unpublished results</u>

In the near term, the study will involve the use of ~140 antibodies to track spatiotemporal developments during wall formation around a single cell.

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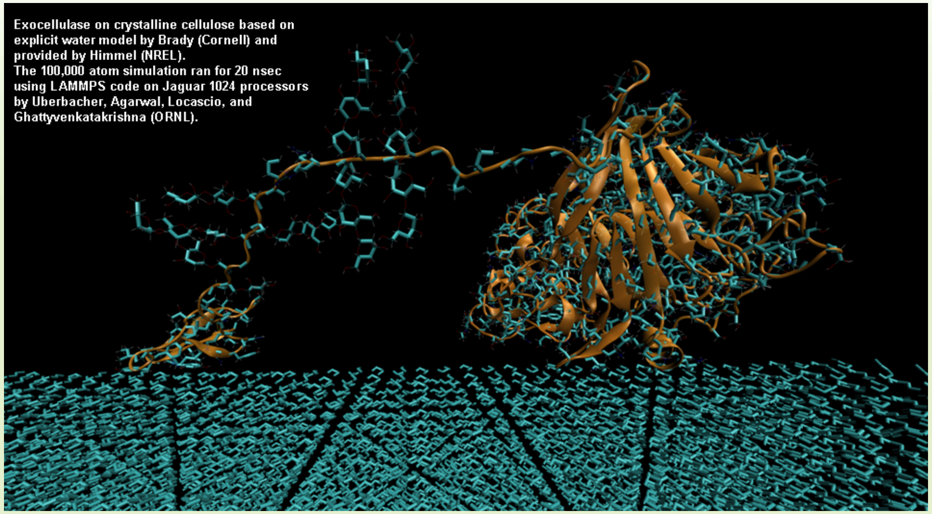
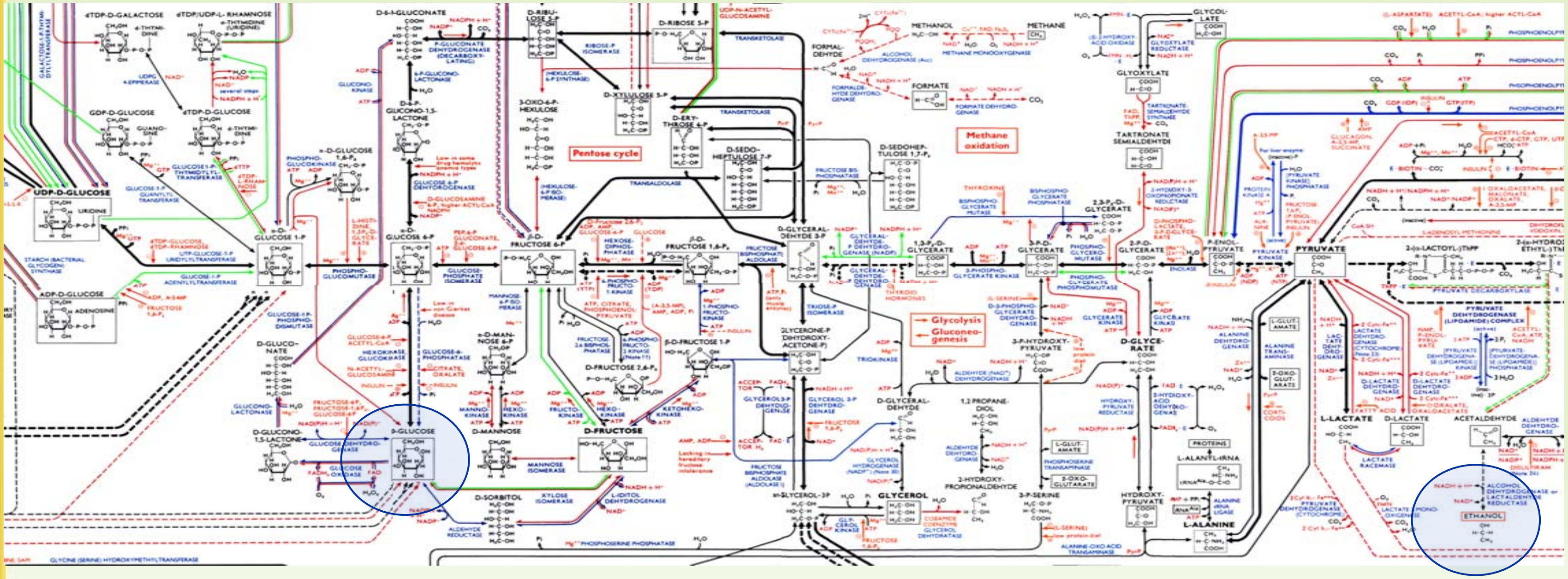
The challenge (part 2): Lignocellulosic biomass is difficult to breakdown and ferment



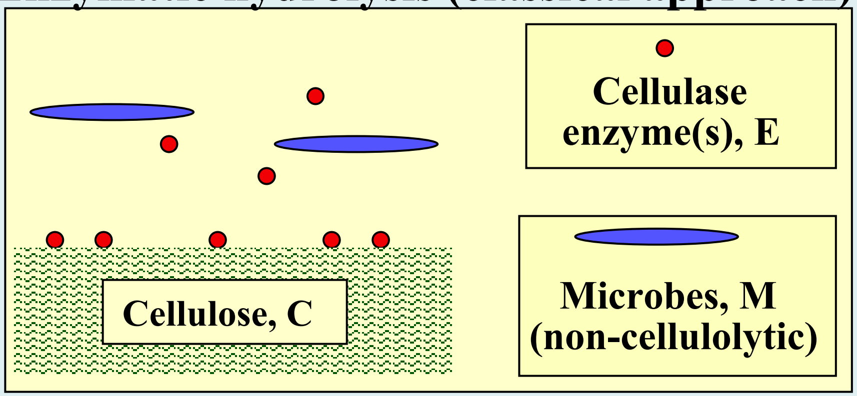


GLUCOSE

ETHANOL

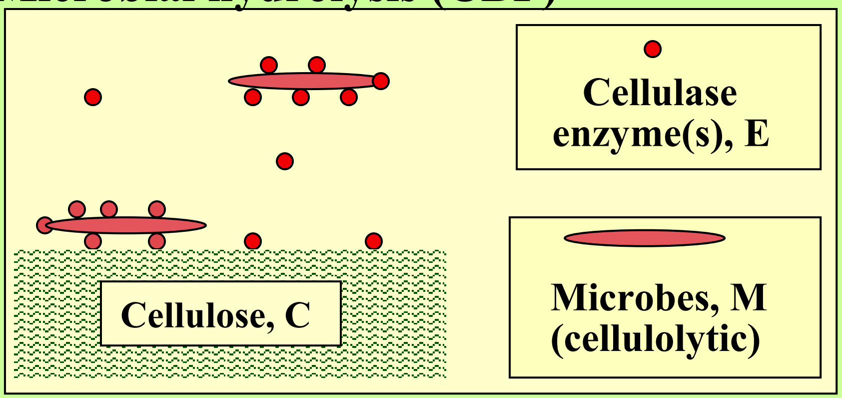
Microbial hydrolysis and enzymatic hydrolysis: A fundamentally different relationship between microbes and cellulose

Enzymatic hydrolysis (classical approach)

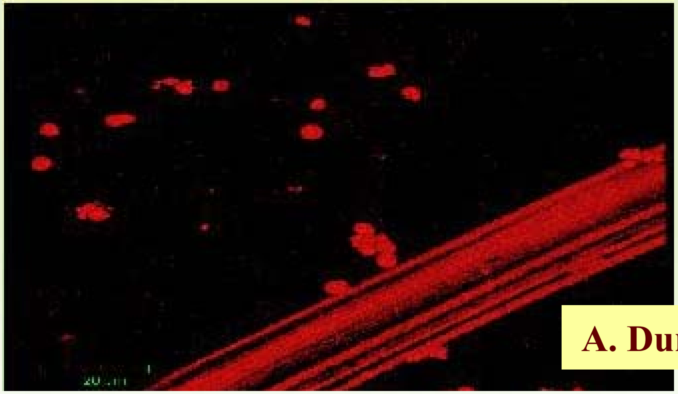


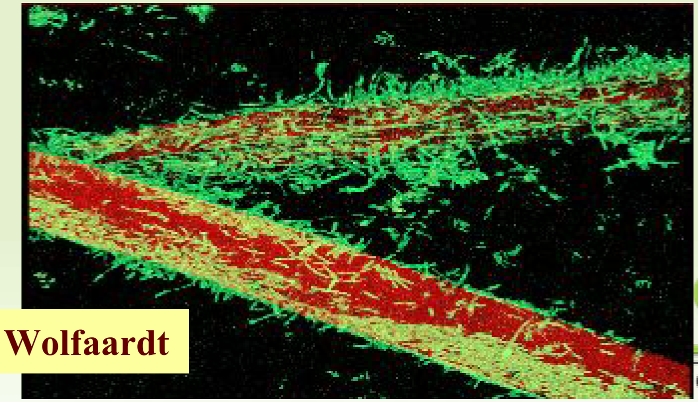
- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound & free
- Cells may or may not be present

Microbial hydrolysis (CBP)



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

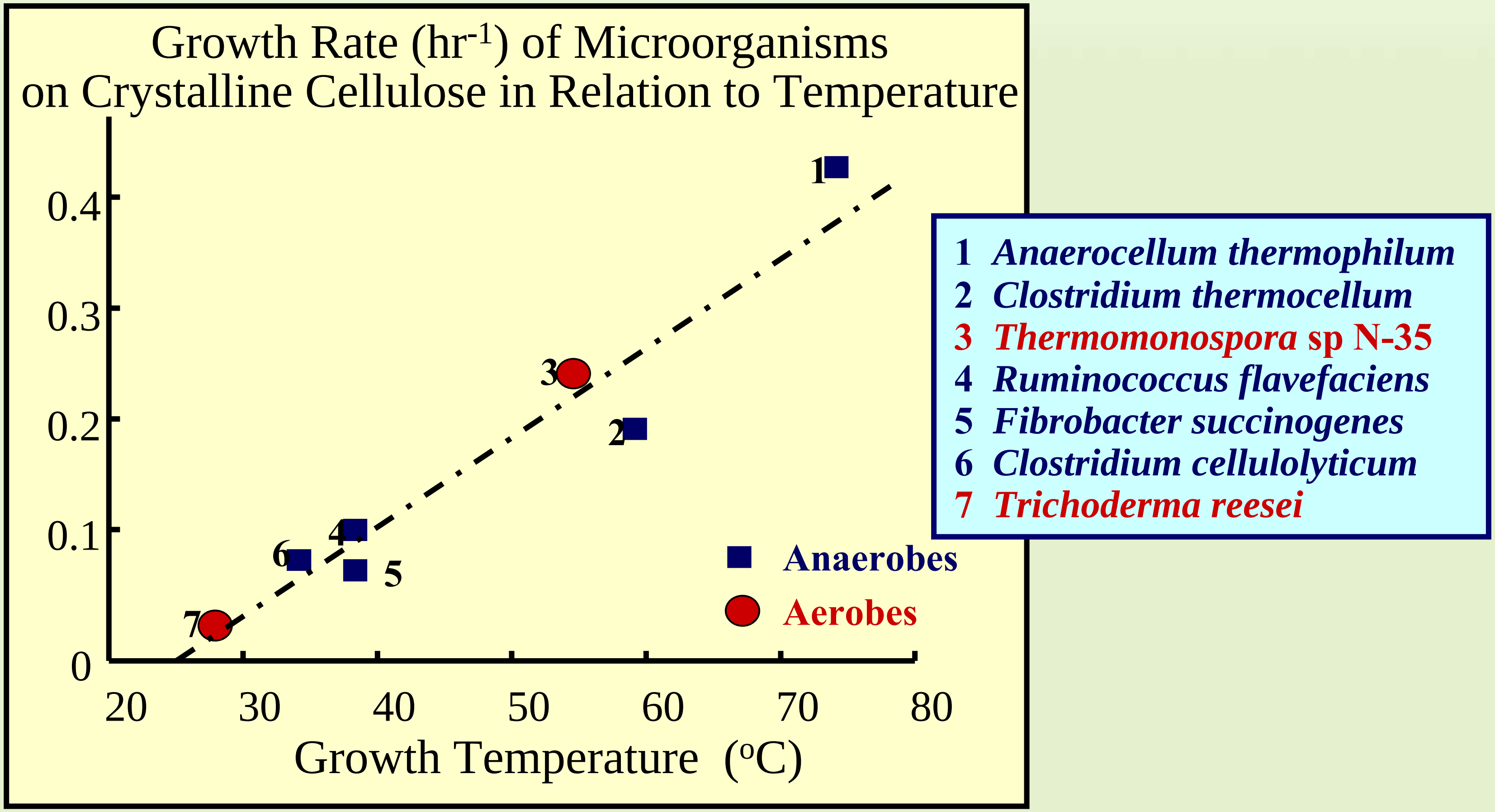




A. Dumitrache & G. Wolfaardt

Search for new biocatalysts

- **Hypothesis: will higher temperature microbes be more effective?**



The growth of microbes on cellulose increases linearly with temperature. Lynd, et al., *Microb. Molec. Biol. Rev.* 66: 506 (2002).

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Biodiversity access

- **Sampling trip to Yellowstone in October**
- **Enrichments are growing at different temperatures**

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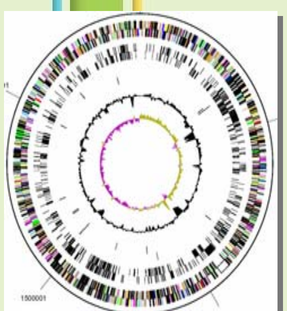
"Omics" technologies applied to help design better CBP microbes

Biochemical pathway

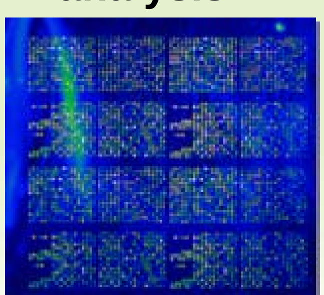
Cellulose

Ethanol
& Byproducts

Genome annotation gives possible genes, proteins, etc.



Transcriptome - Gene expression measured by microarray analysis



Proteome- Protein expression measured by mass spectrometry

Metabolome - Cellulose hydrolysis fragments and fermentation products measured by mass spectrometry

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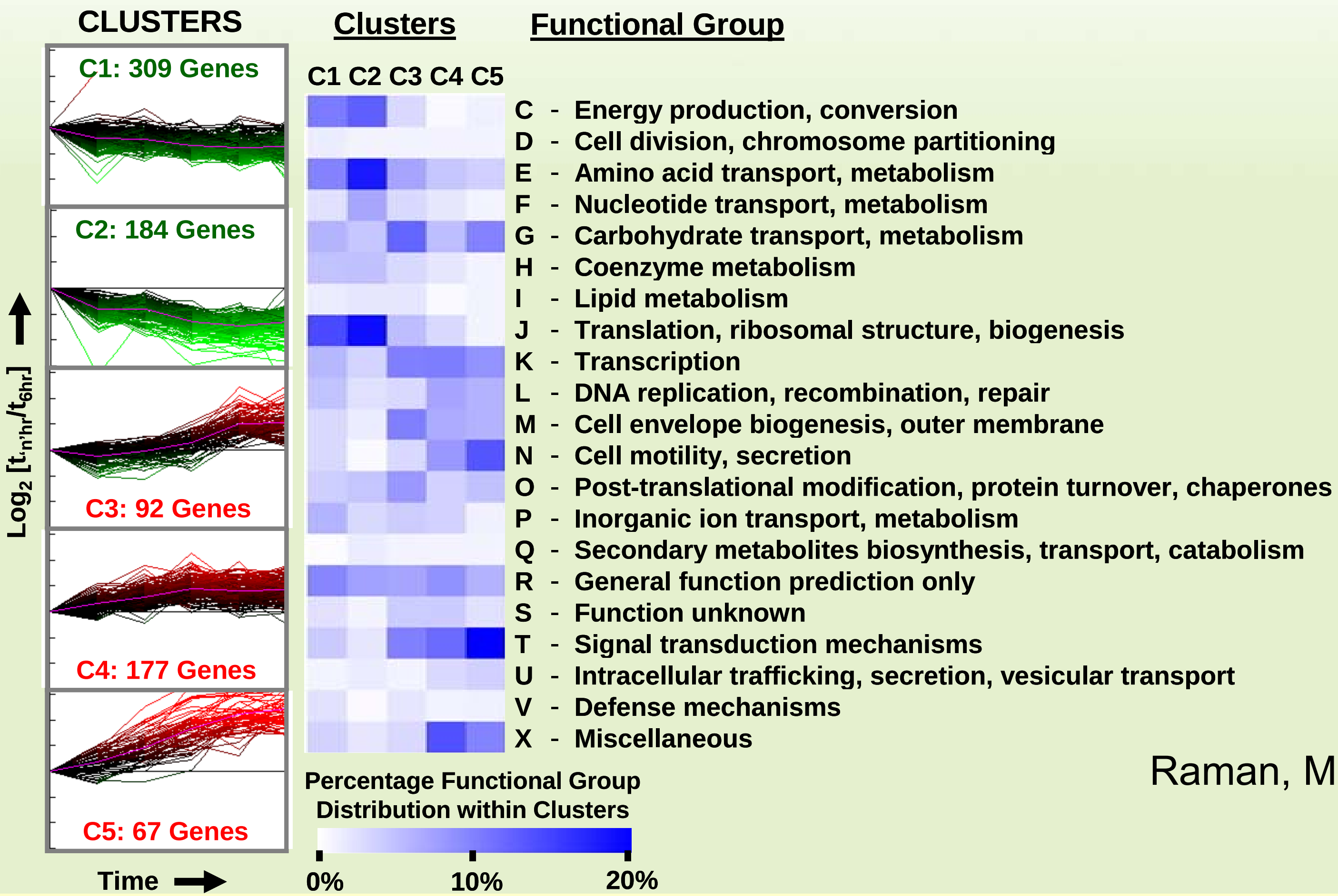
Gene expression changes during *C. thermocellum* fermentation

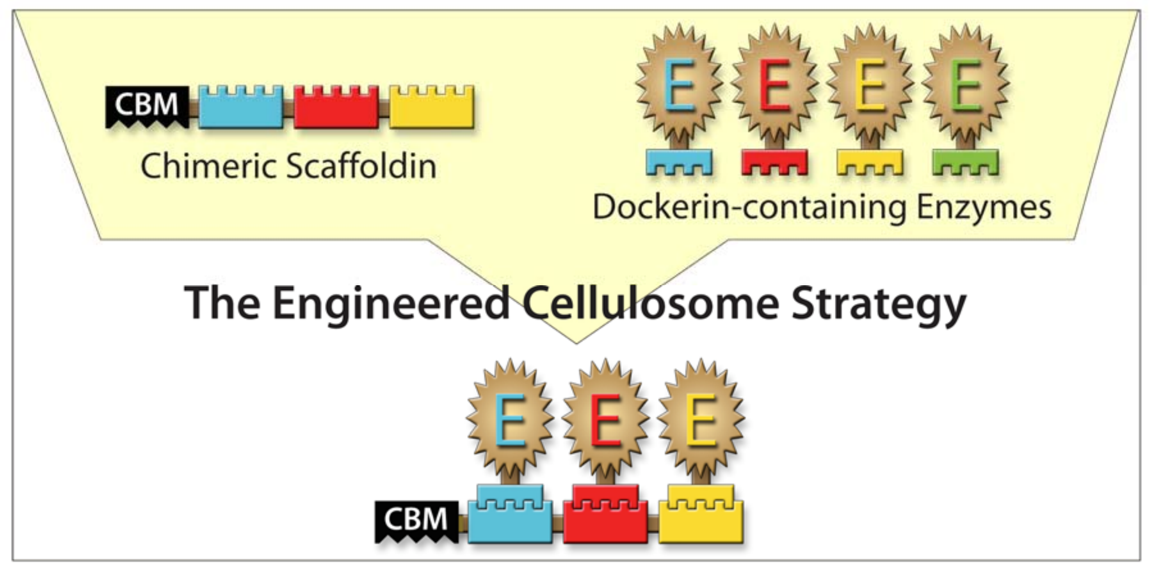


Raman, Mielenz 2007

During cellulose fermentation, the capacity of *C. thermocellum* to sense and respond to its environment increases and cells become more motile over time; however the metabolic capacity decreases progressively with time during batch growth.

Goal: Understanding leading to an improved cellulosome

- **A deep proteome analysis of the cellulosome of C. thermocellum identified more than 20 ‘new’ cellulosomal components**



C. thermocellum image courtesy of Bayer and Lamed, The Weizmann Institute of Science.

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Thank you

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