The BioEnergy Science Center – an integrated strategy to understand and overcome biomass recalcitrance

Martin Keller, Ph.D.
BESC Director

http://www.bioenergycenter.org/

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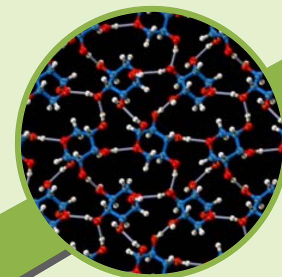
The fundamental science of biomass recalcitrance is poorly understood

Overcoming recalcitrance is the single coherent overarching theme for the BESC

Recalcitrance: Resistance to breakdown into sugars

Cellulosic biomass



Sugars

Fuel(s)

- 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)

Oak Ridge National Laboratory

UT

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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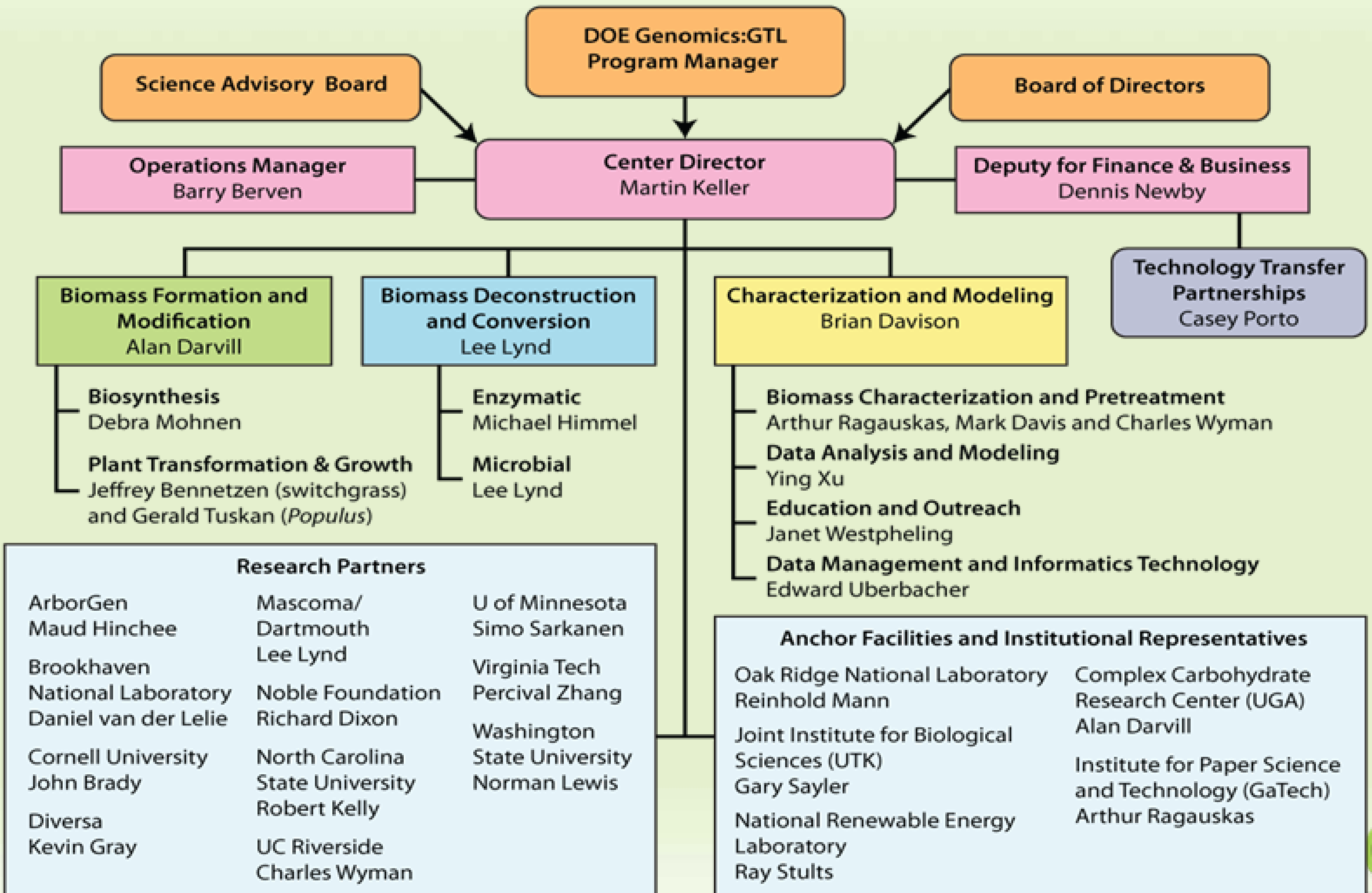
A functional management structure integrates BESC activities and objectives with clear lines of authority



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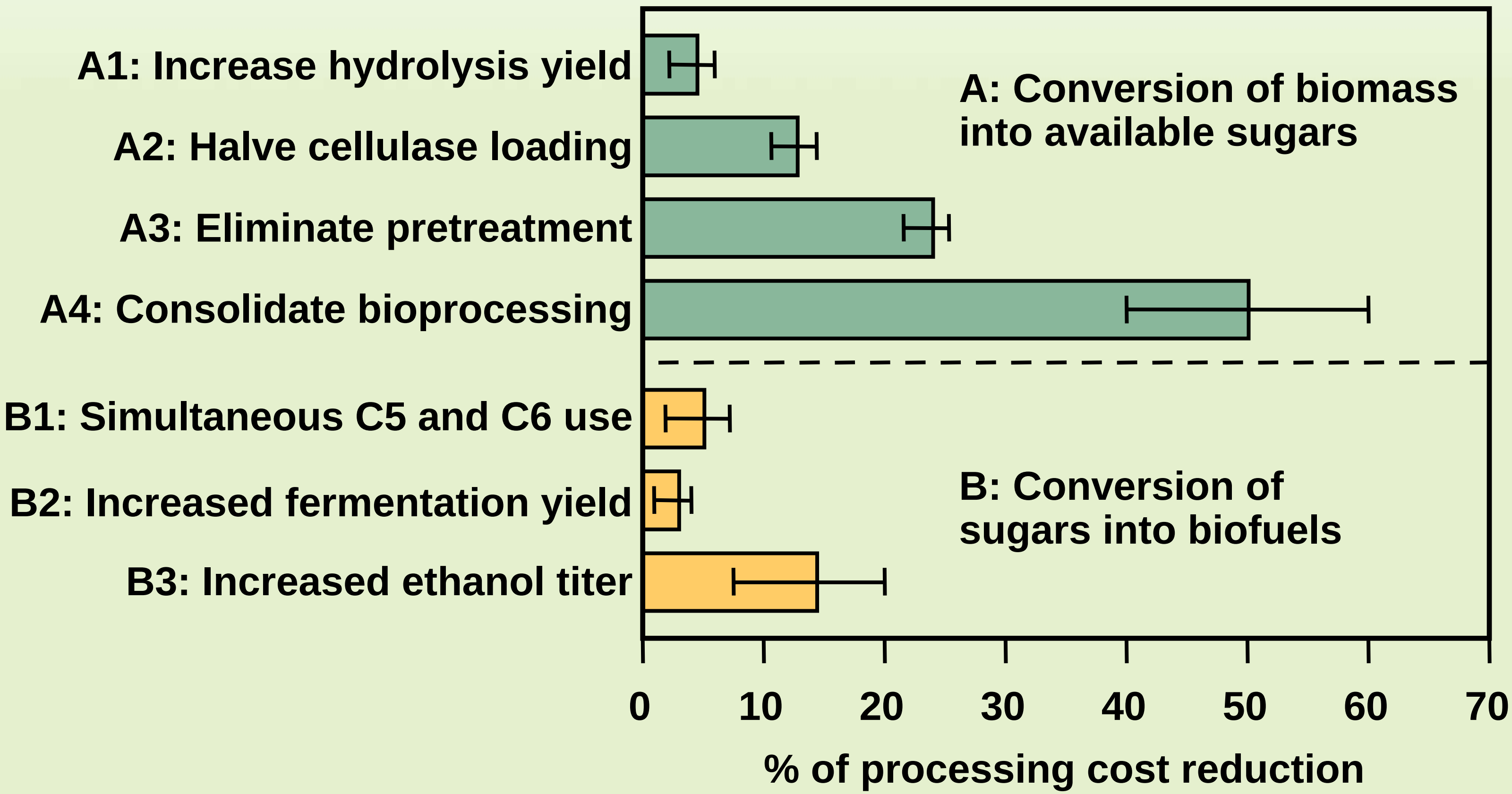
Comparative impacts of R&D on biomass processing cost



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)

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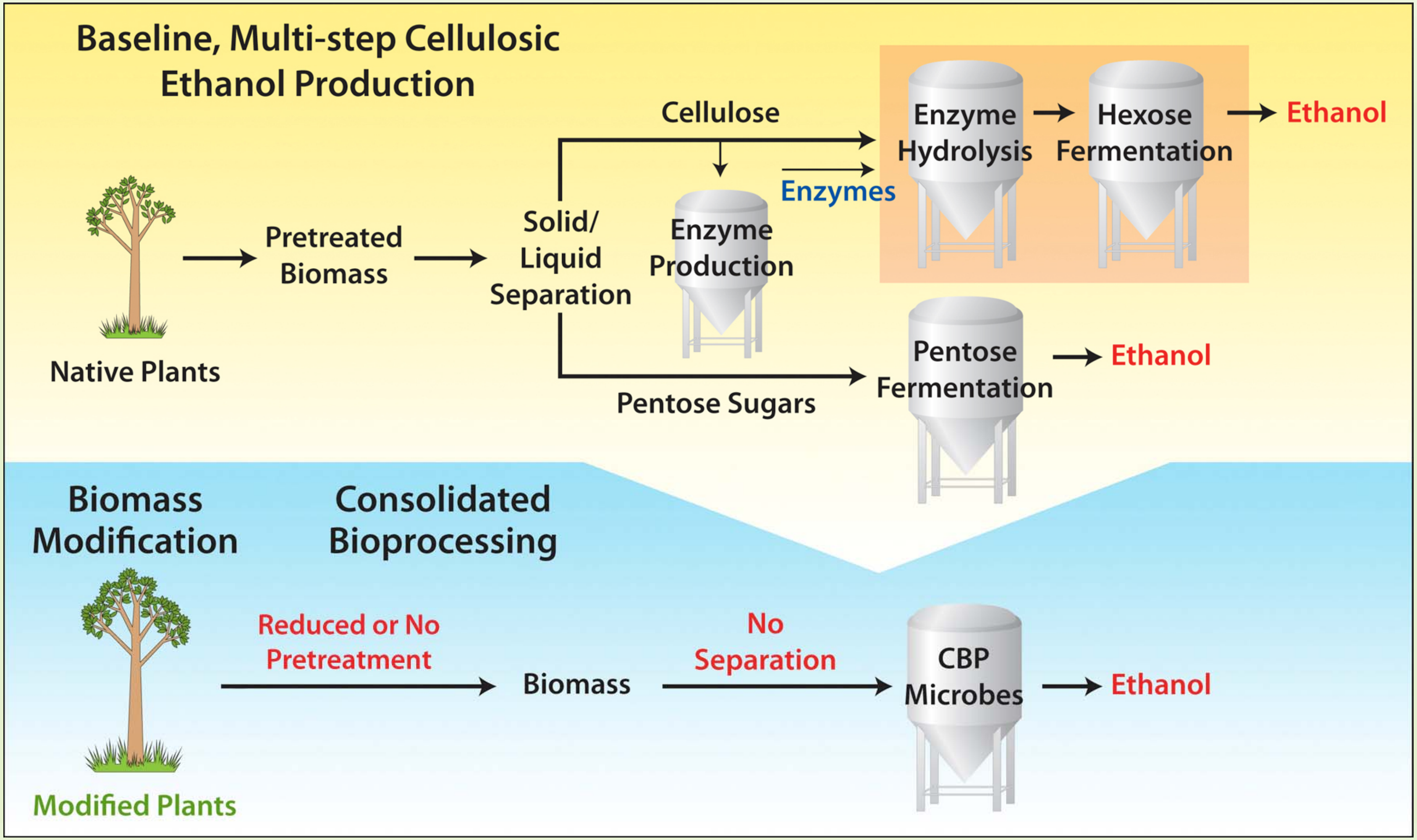
BESC will revolutionize how biomass is processed within five years



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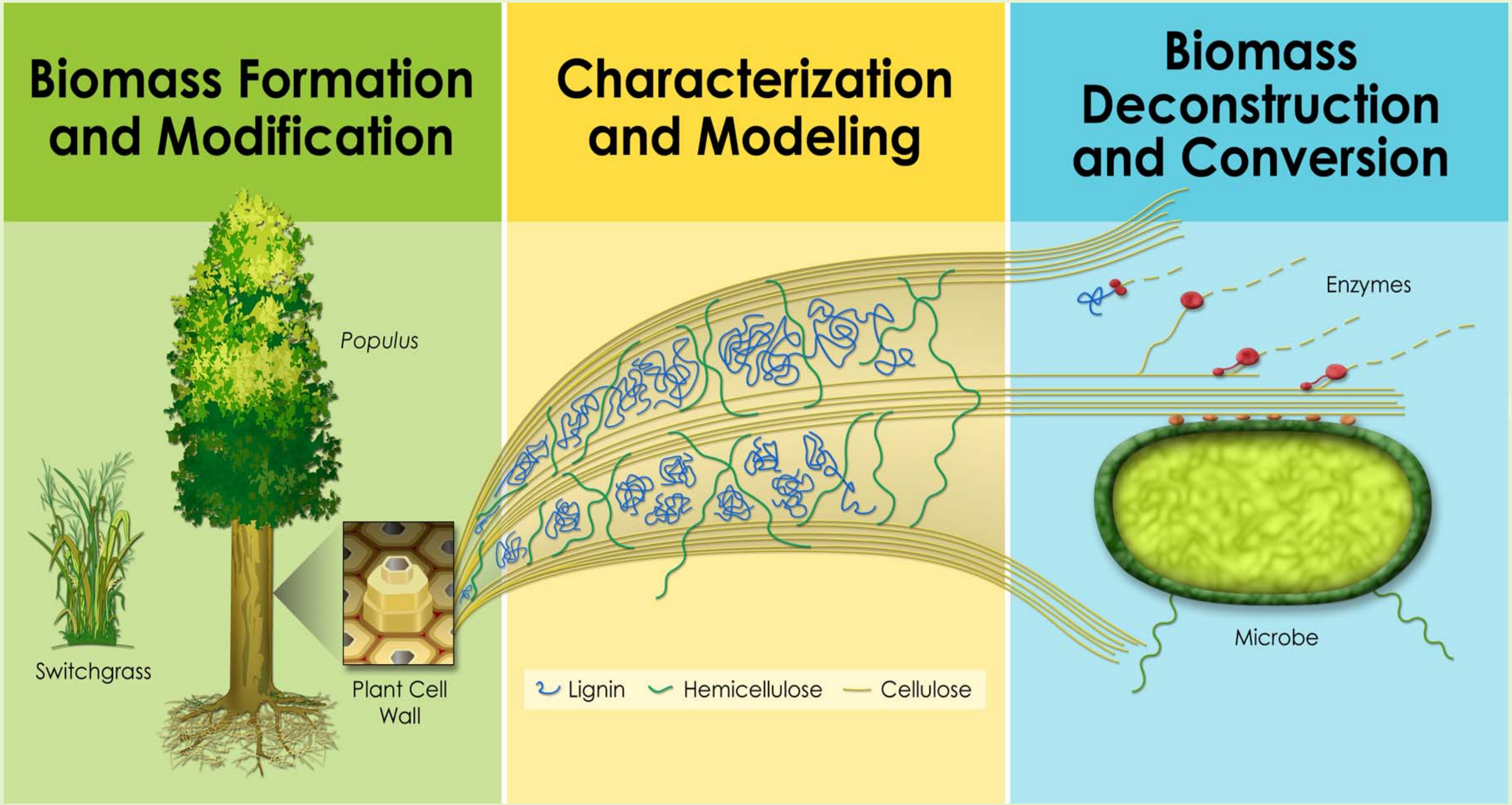
Three linked scientific focus areas will enable BESC to understand and overcome biomass recalcitrance



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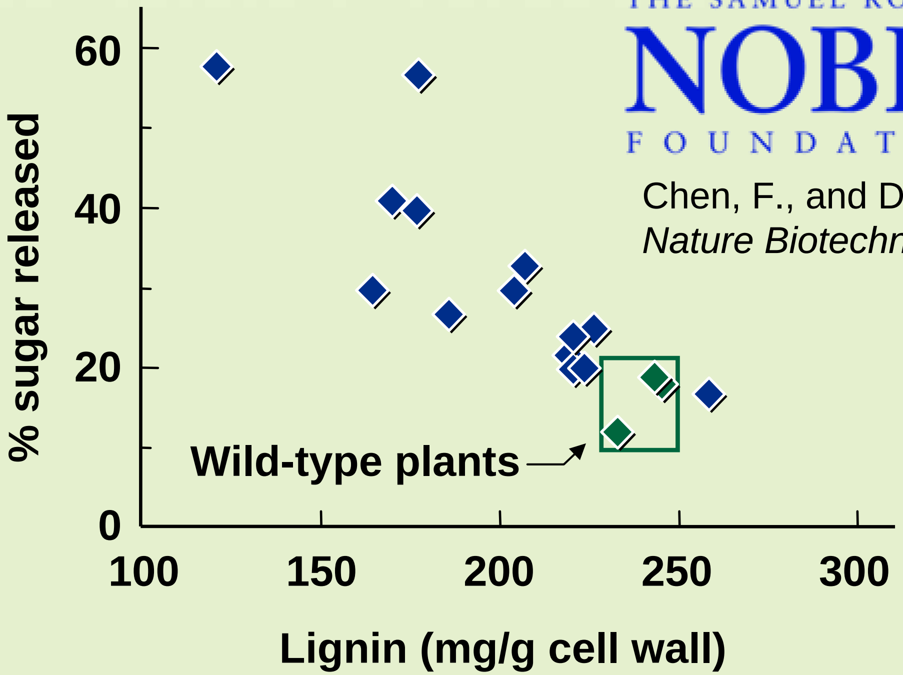
Modifying cell wall composition and structure can reduce recalcitrance



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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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The challenge: Lignocellulosic biomass is complex and heterogeneous

Most plant cell wall genes are unknown in function

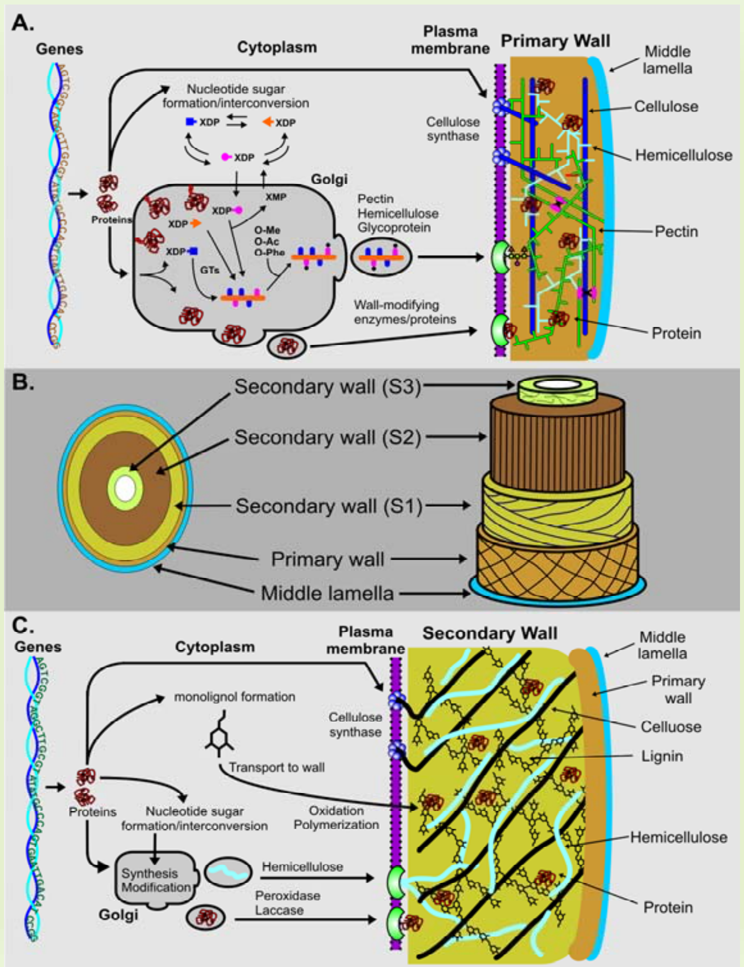

Fig. II.2. Biosynthesis of primary and secondary walls: from genes to polymers. A. Primary wall polysaccharides are synthesized at the plasmamembrane (cellulose) and in the Golgi (pectin and hemicellulose) by the action of glycosyltransferases that use nucleotide-sugar substrates. B. Some cells (e.g.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

- **Gene discovery for biomass recalcitrance**
  - **Random**
    - **Study natural variation**
    - **Study randomly modified populations**
      - **Activation tagged *Populus***
  - **Targeted**
    - **Identify candidate genes**
    - **Modify gene expression**

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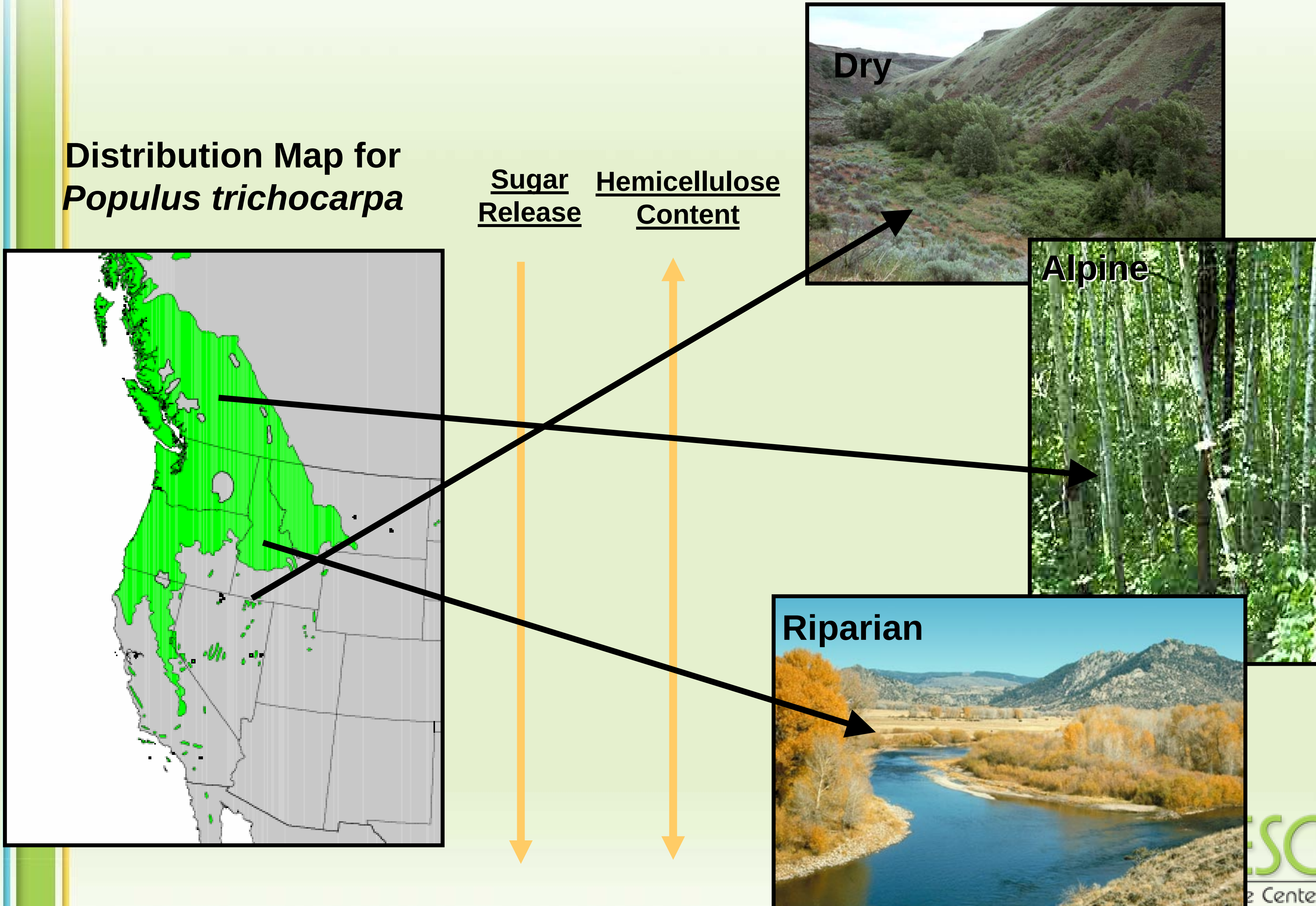
Mining adaptive allelic natural variation



Activation tagging to identify *Populus* genes

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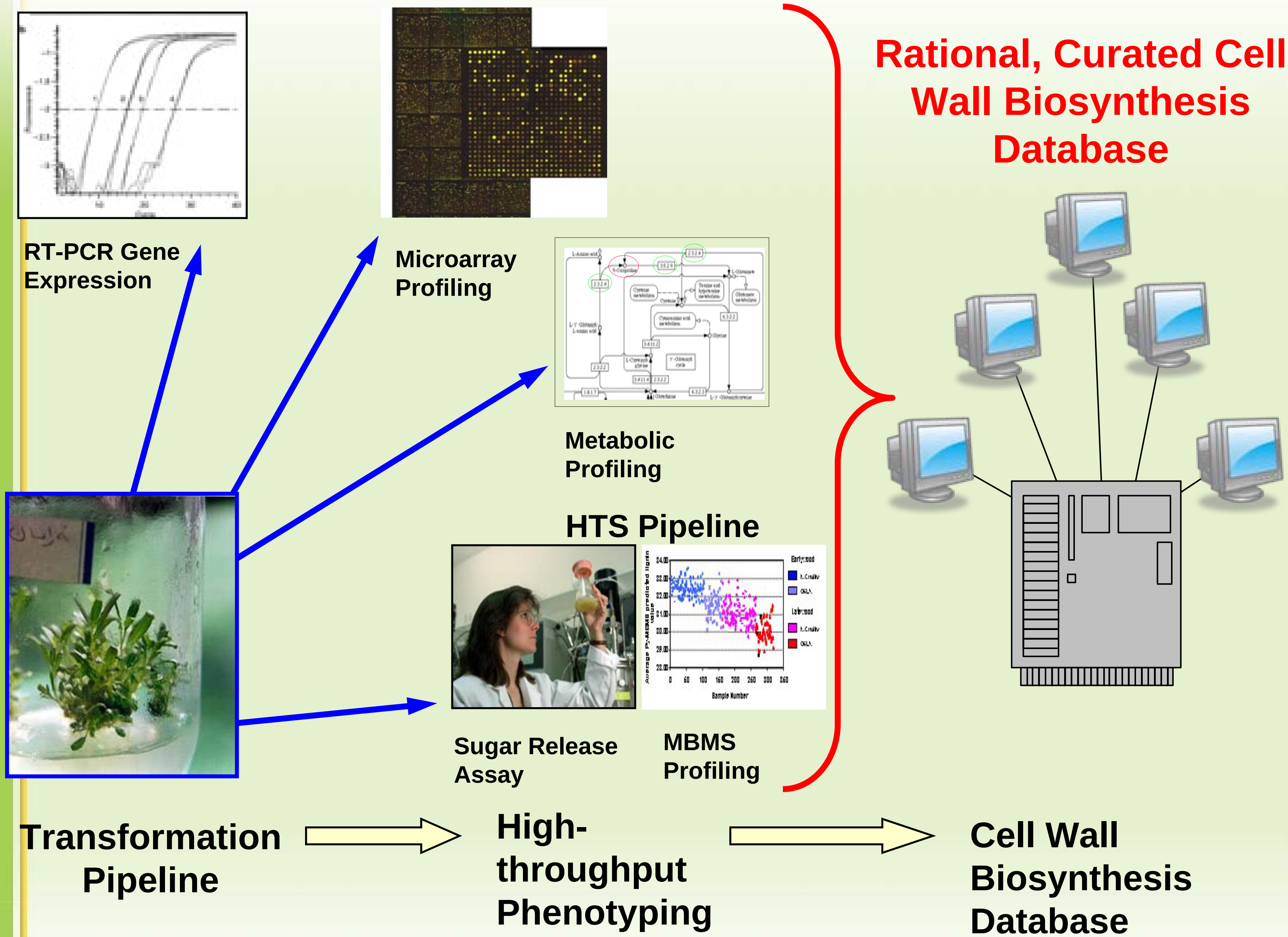
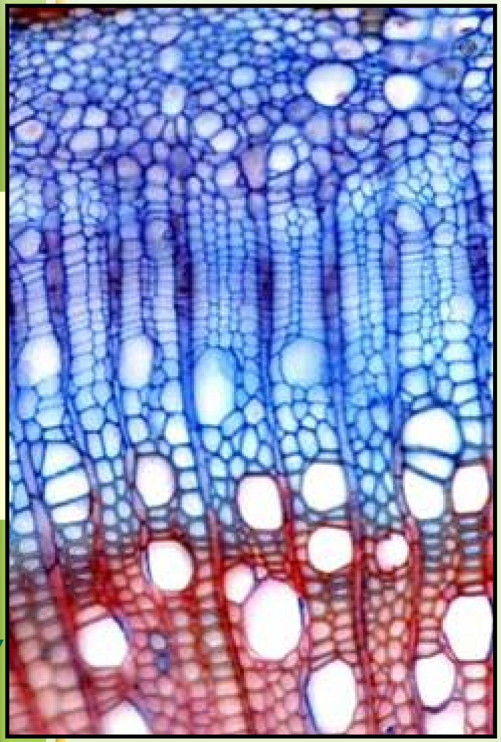
High throughput phenotyping/screening

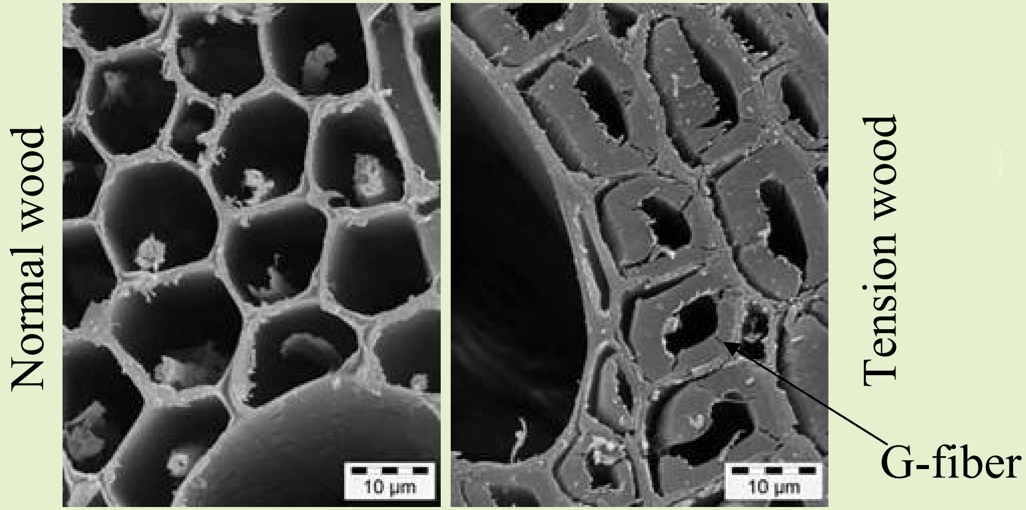


Targeted gene discovery: we are looking for cryptic wood phenotypes



Transverse section in a poplar stem (safranin-astra-blue coloration)

- Identify key-genes involved in xylem differentiation
- Focus on tension wood and G-fiber



Tension wood is produced in response to mechanical and gravitational stimuli
Tension wood is a convenient biological model
Tension wood is a major defect for wood quality in poplar

Images courtesy of Gilles Pilate, INRA-Orléans

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Current estimates of plant transformation pipeline throughput

Populus

Transformation: ~200 genes/constructs per year (over & under expression)

Activation Tagging: ~1000 genes/constructs per year

Switchgrass

Transformation: ~20 year 1, 40-60 year 2+ genes/constructs per year (under & over expression)

Screen w/ VIGS (viral induced gene silencing): 200 genes/constructs per year (under development), RNAi

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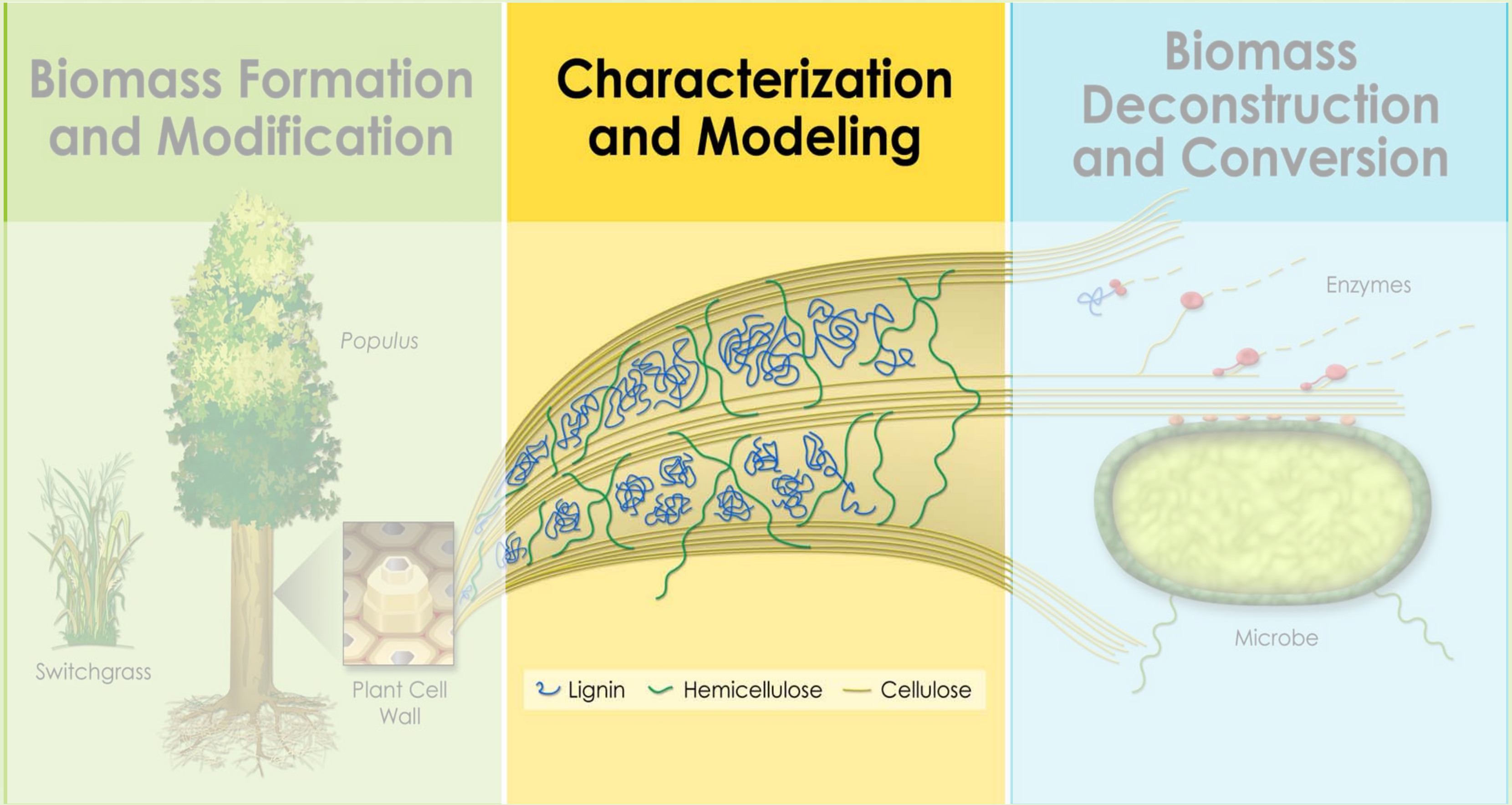
Strategy part 2: Biomass recalcitrance. Measure, understand and model



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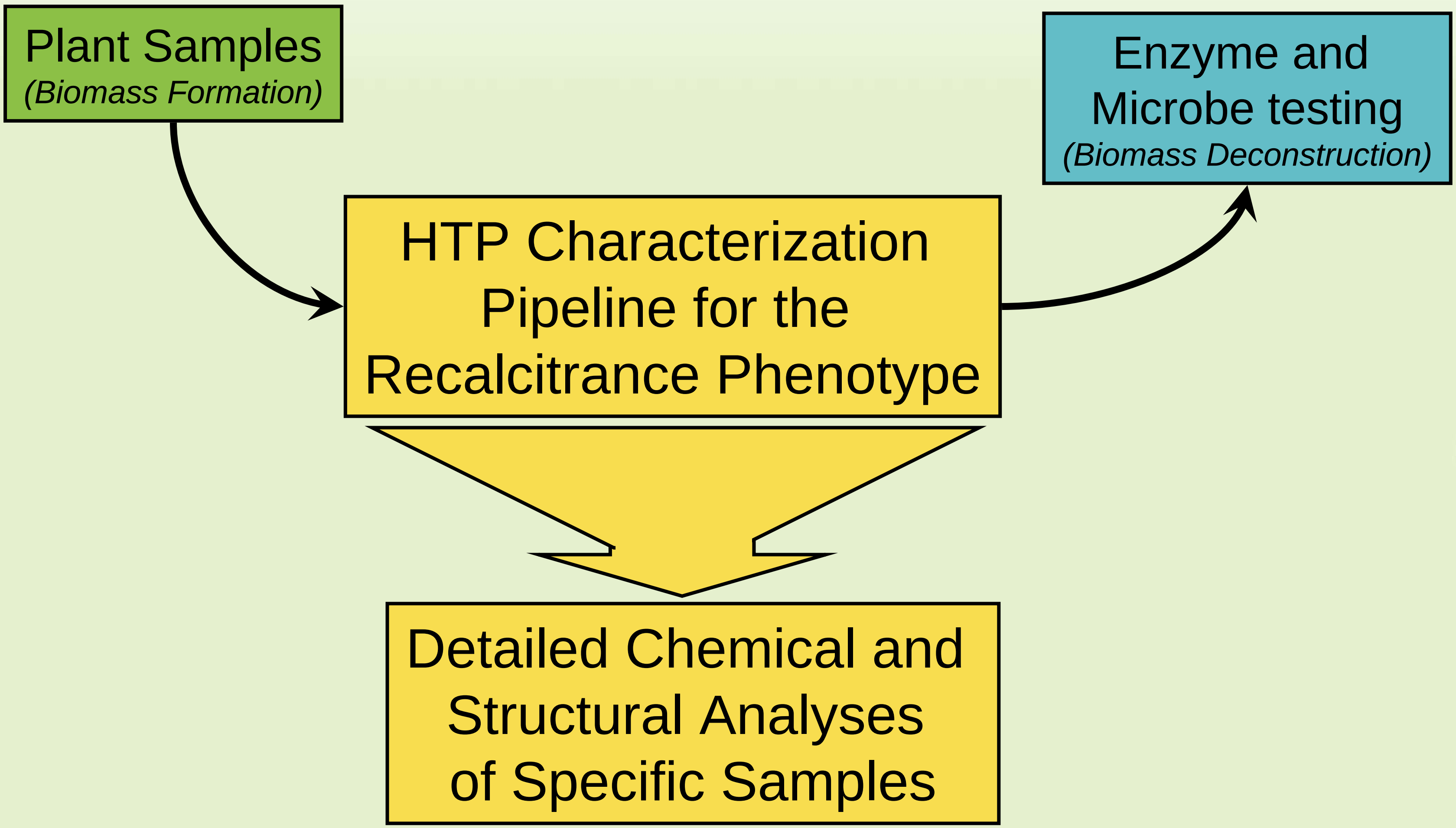
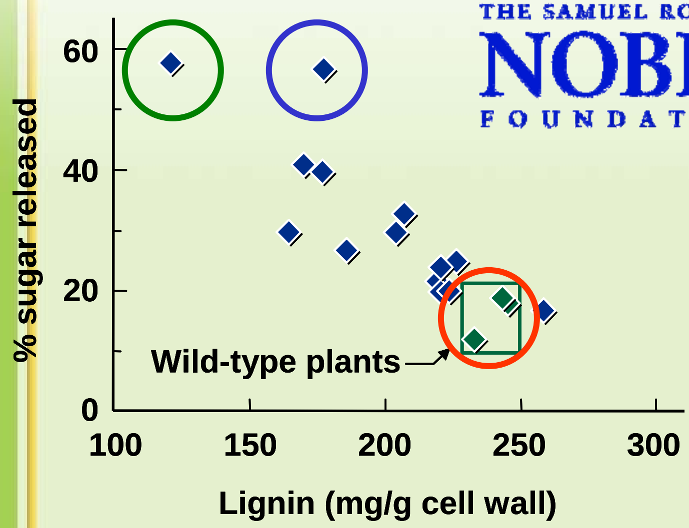
A shared sample and characterization pipeline will speed discovery and integration



Analytical pyrolysis of low lignin alfalfa

36 minutes of analysis for 6 (x3) samples



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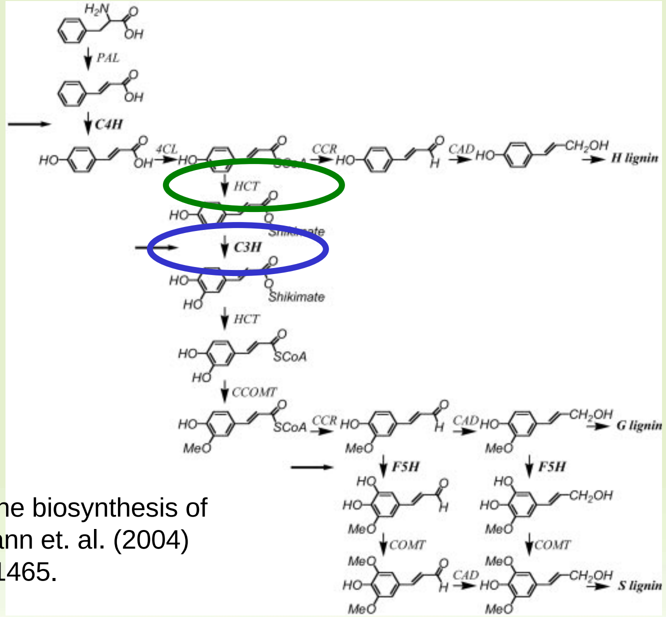
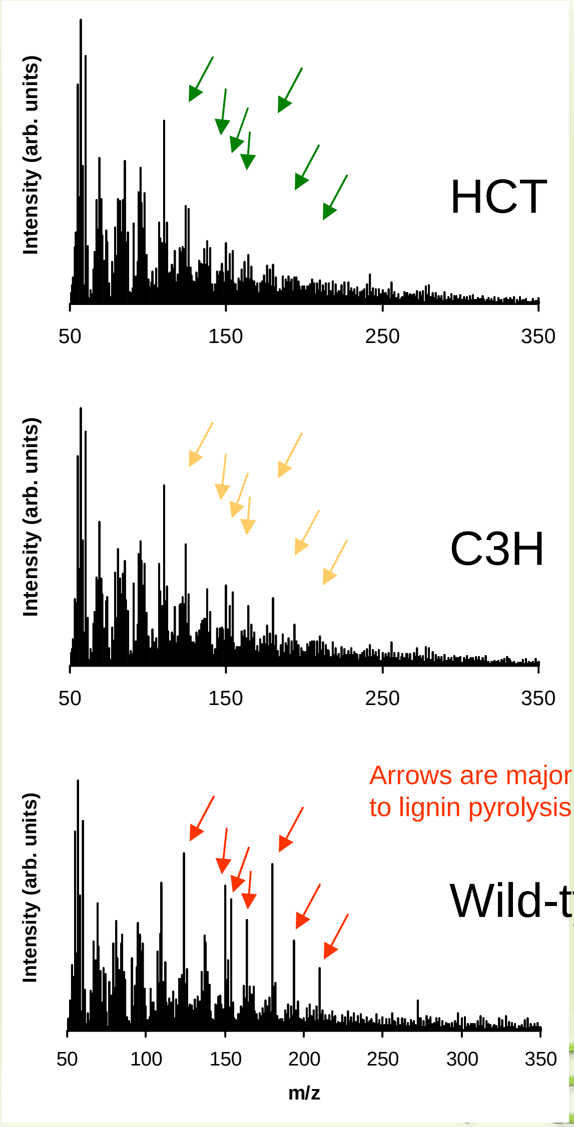


Current scheme for the biosynthesis of monolignols. (Hoffmann et. al. (2004) Plant Cell 16, 1446–1465.



Arrows are major peaks assigned to lignin pyrolysis fragments

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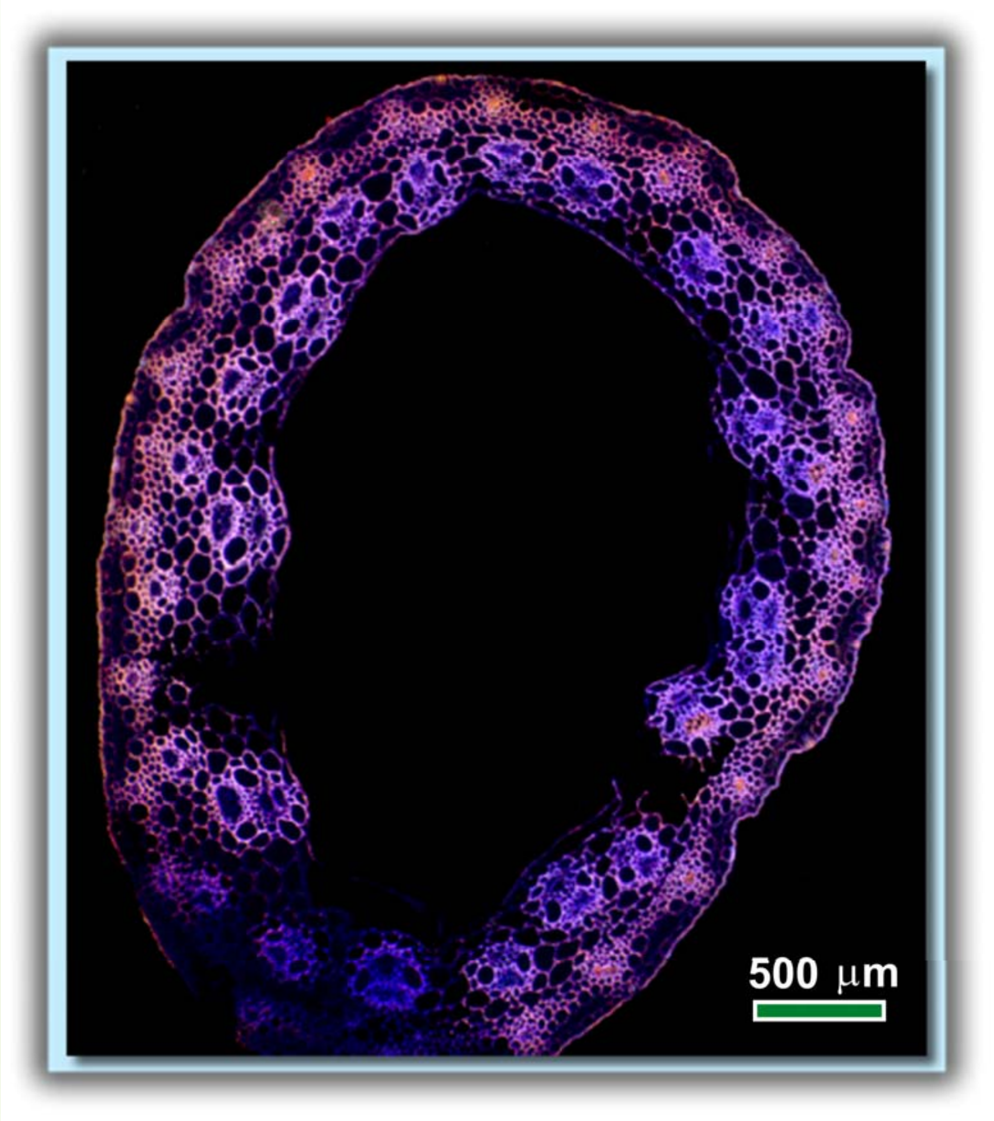
Switchgrass – Fluorescence microscopy



Ding, et al, unpublished results

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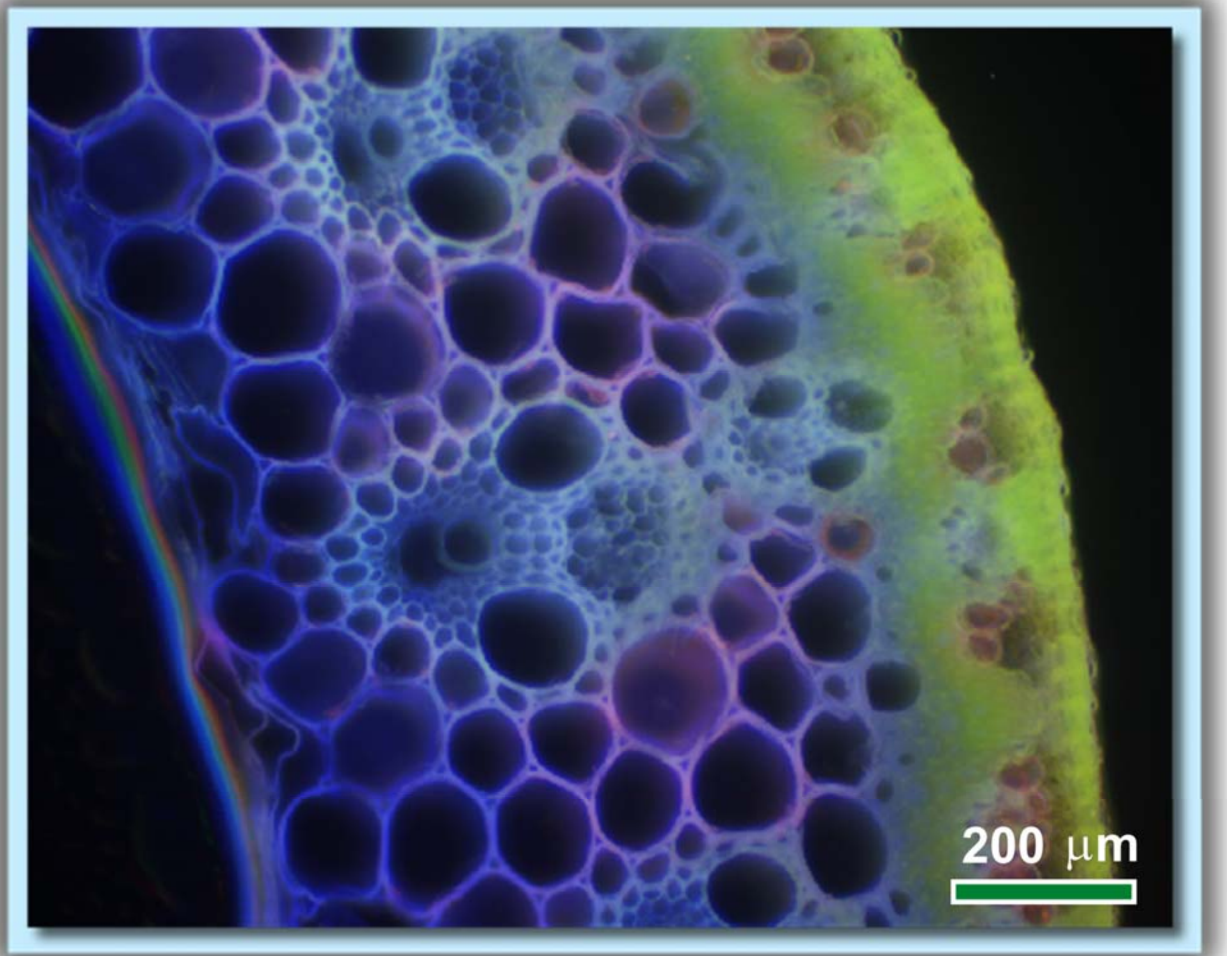
Switchgrass – fluorescence microscopy



Ding, et al, unpublished results

Fluorescence signals primarily come from chlorophyll, lignin, carotenes, and xanthophylls in plants, each with a different wavelength (color), lignin fluorescence is blue-greenish. Determine cell liginfication by using different filter sets

NREL National Renewable Energy Laboratory

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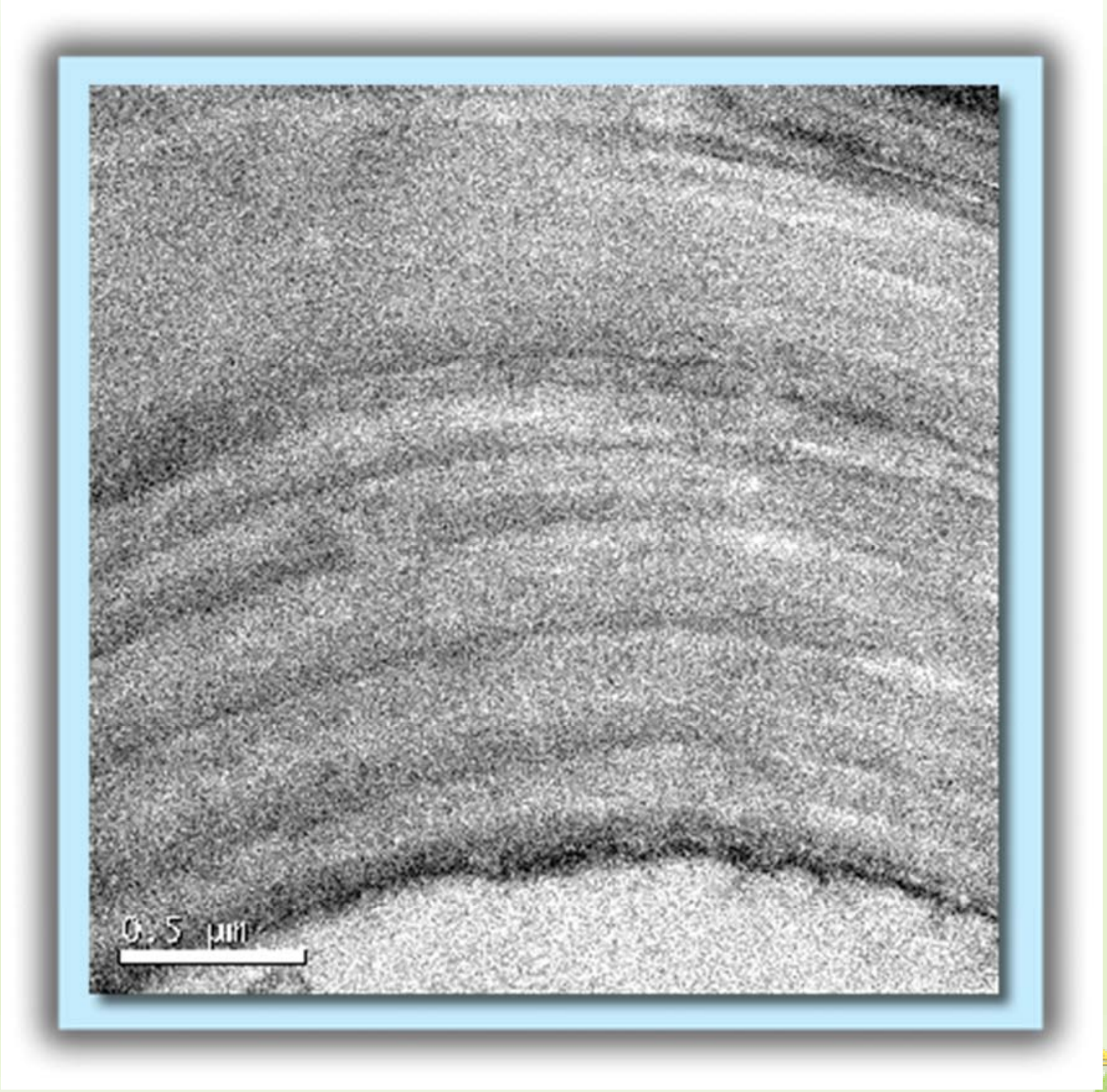
Switchgrass – Transmission electron microscopy (TEM)



Ding, et al, unpublished results

Switchgrass – Atomic force microscopy (AFM)

Ding, et al, unpublished results

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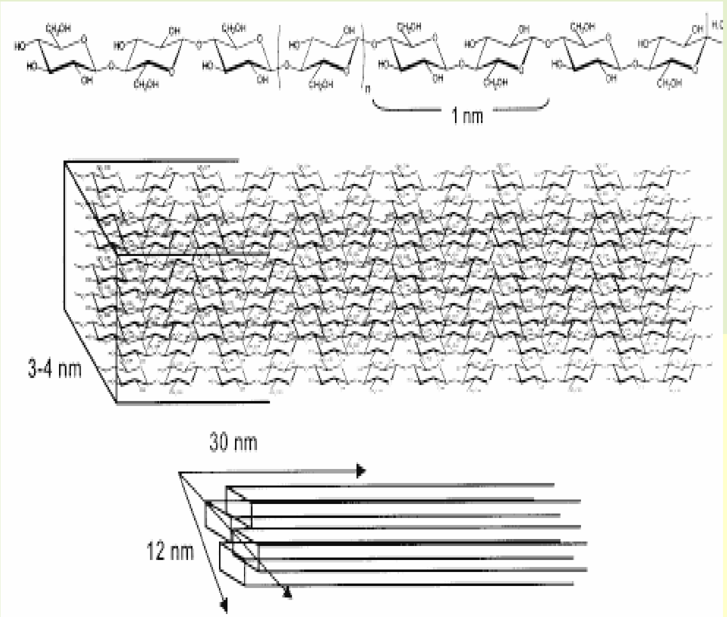
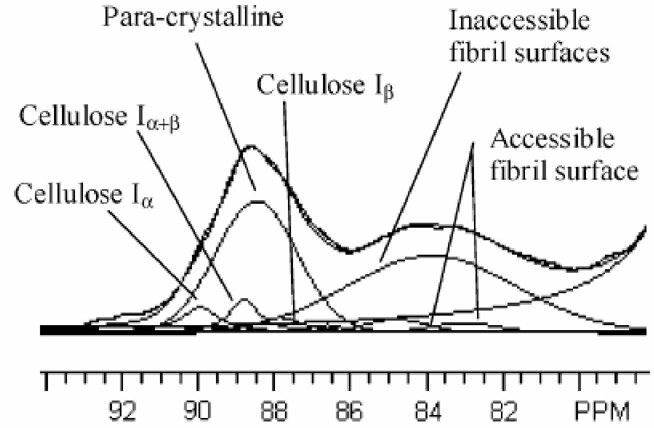
Analysis of cellulose

- **Cellulose crystalinity analysis by NMR**
  - **Critical for accessibility**
  - **Chains are parallel due to shifts in orientation of sheets**



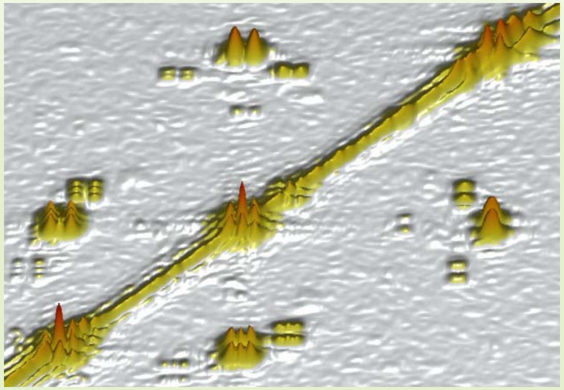


Spectral fitting for the C-4 region of CP/MAS ^{13}C NMR spectrum of the control pulp sample.

Assignments	Chemical shift (ppm)	FWHH* (Hz)	Relative Intensity, %
Cellulose I(α)	89.5	24	0.3
Cellulose I(α+β)	88.8	198	37.1
Para-crystalline cellulose	88.3	234	20.9
Cellulose I (β)	87.8	26	0.2
Accessible fibril surface	84.3	64	0.4
Inaccessible fibril surfaces and hemicellulose	83.7	467	41.2
Accessible fibril surface	83.3	0.8	-

*: FWHH is the full width at half-height.

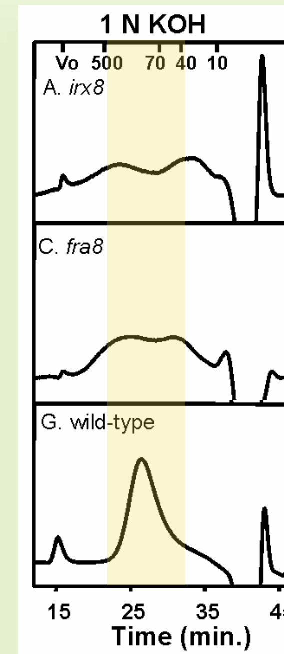
Polysaccharide analysis will distinguish genetically diverse biomass

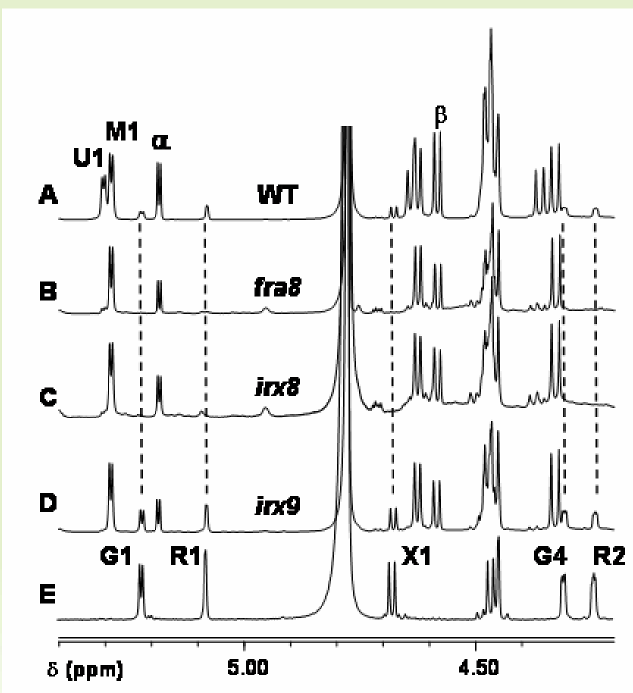


2D ^{1}H-NMR sees altered bonds in polysaccharides and lignin in biomass

Size-exclusion chromatography shows mutants can lose the ability to control the chain length of glucuronoxylan

Arabidopsis *irx* and *fra* mutants in secondary walls





^{1}H NMR spectra shows changes in glycosyl residues

Glucuronoxylans isolated from Arabidopsis *irx* and *fra* mutants are deficient in the unique sequence of glycosyl residues at the reducing end

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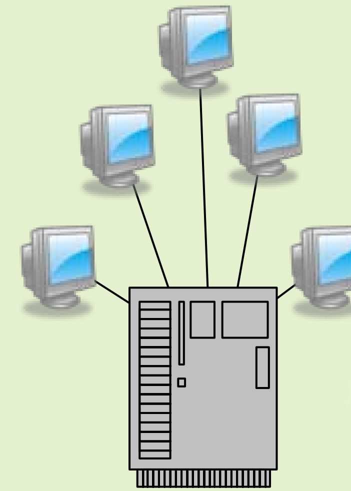
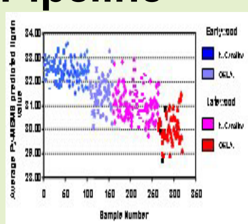
What genes influence recalcitrance in what way?

Transformation Pipeline → **High-throughput Phenotyping** → **Cell Wall Biosynthesis Database**



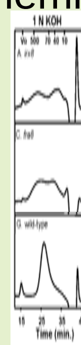
HTS Pipeline



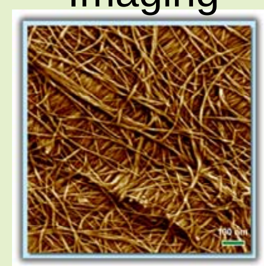
Sugar Release Assay

MBMS Profiling

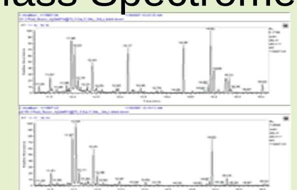
Chemistry



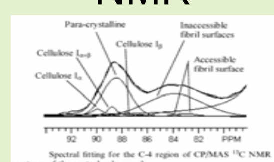
Imaging



Mass Spectrometry

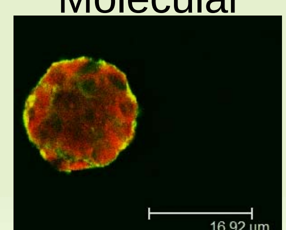


NMR



Spectral fitting for the C-4 region of CP/MAS ^{13}C NMR spectrum of the control pulp sample.

Molecular



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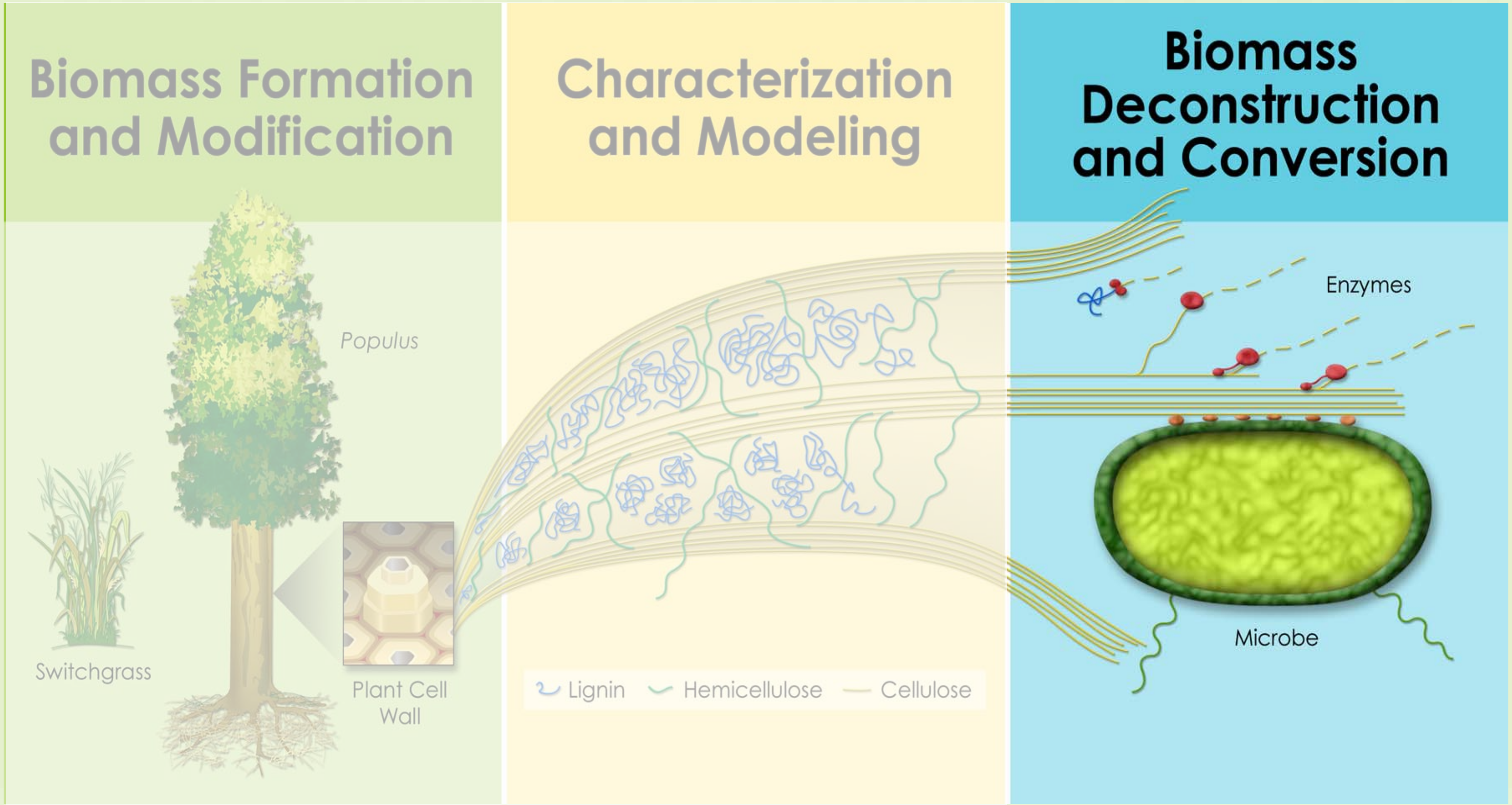
Strategy part 3: Identify, understand and manipulate "biological catalysts" to overcome recalcitrance



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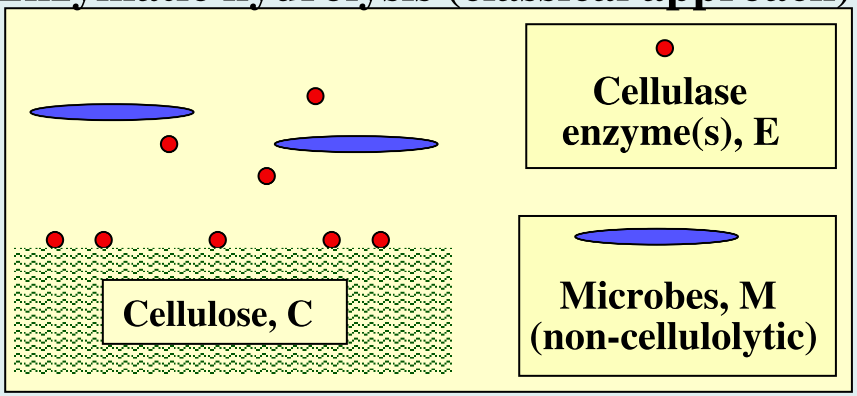
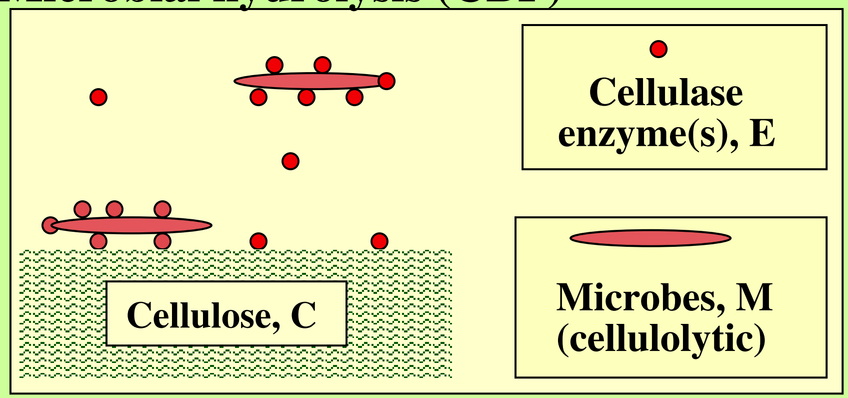
Microbial hydrolysis and enzymatic hydrolysis: A fundamentally different relationship between microbes and cellulose

Enzymatic hydrolysis (classical approach)

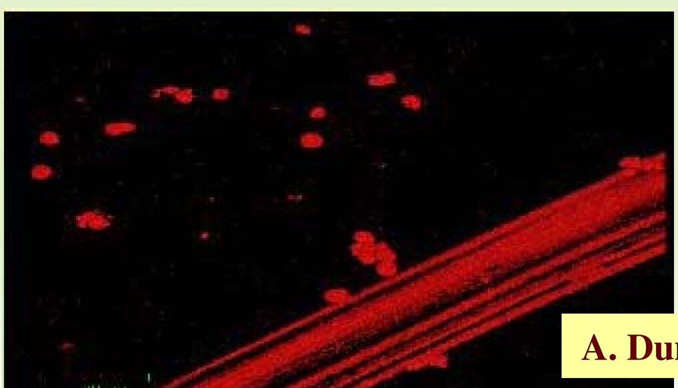


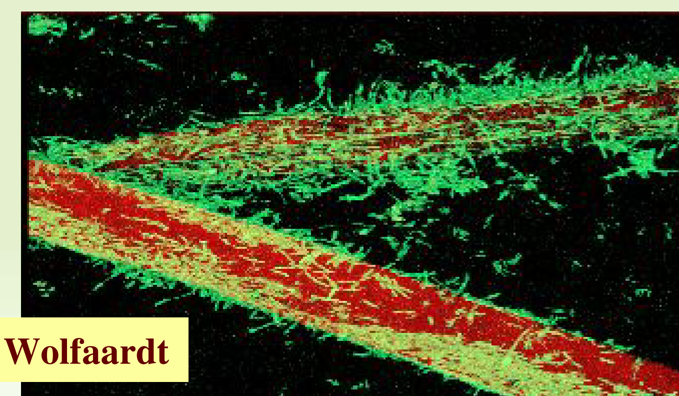
- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound & free
- Cells may or may not be present

Microbial hydrolysis (CBP)



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

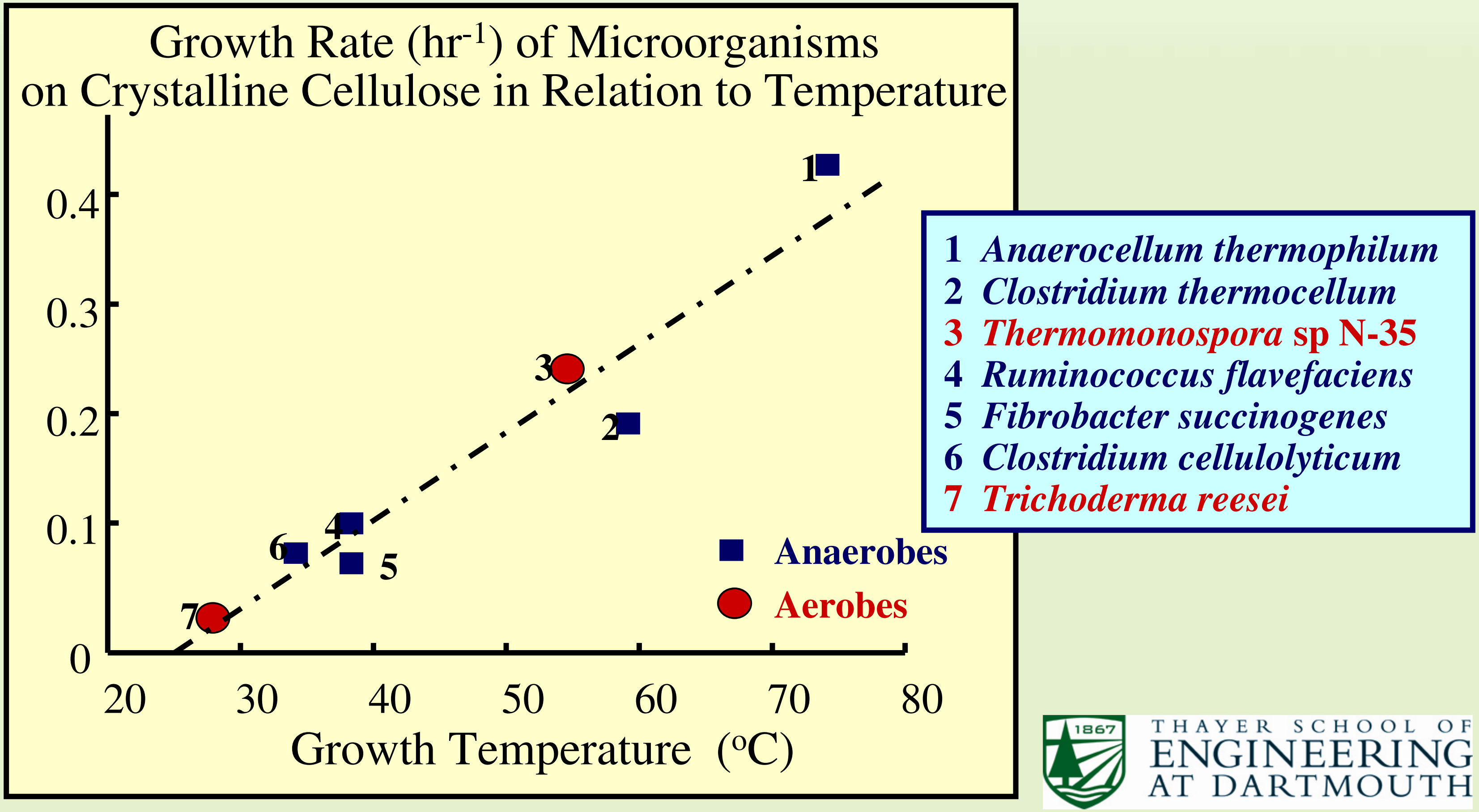




A. Dumitrache & G. Wolfaardt

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).

THAYER SCHOOL OF ENGINEERING AT DARTMOUTH

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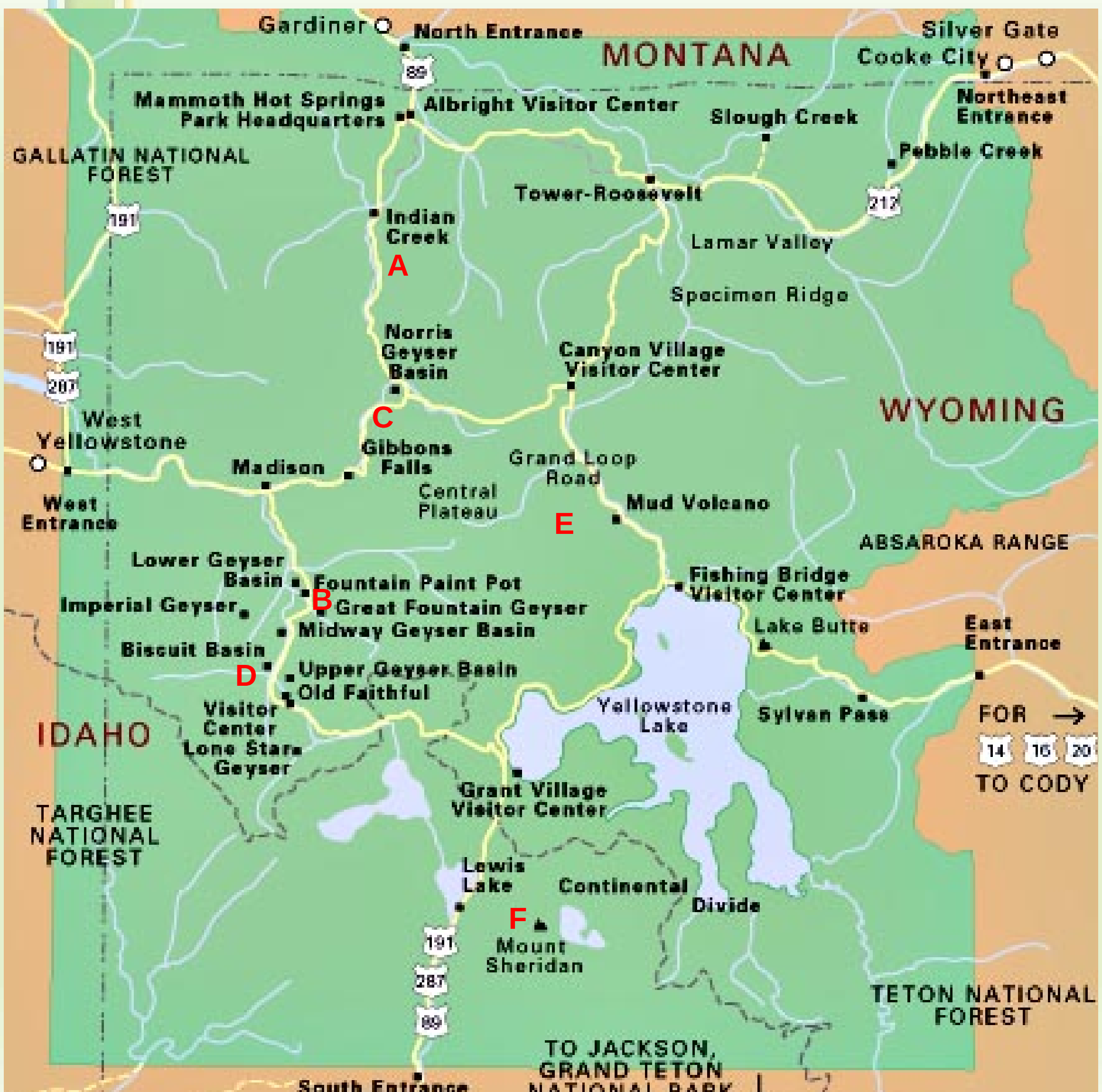
Yellowstone Sample Collection 2007



A – Amphitheater

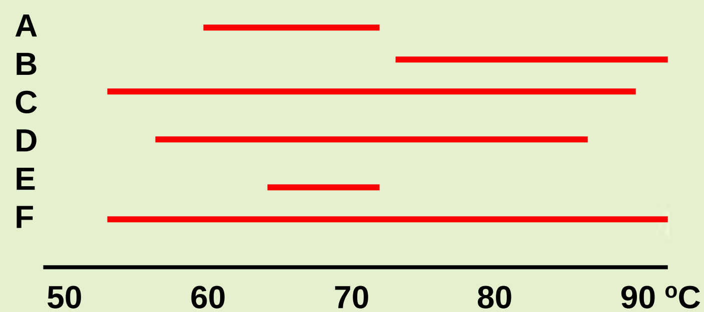
B – Fire Hole Spring, Octopus, White Creek Area, Five Sisters

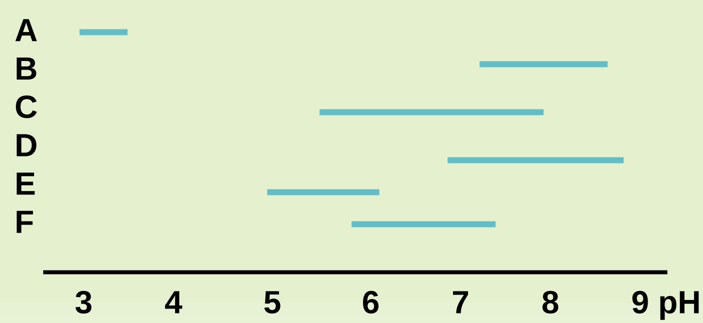
C – Artist Paintpots

D – Black Sands Basin

E – Obsidian Pool

F – Witch Creek, Heart Lake trail





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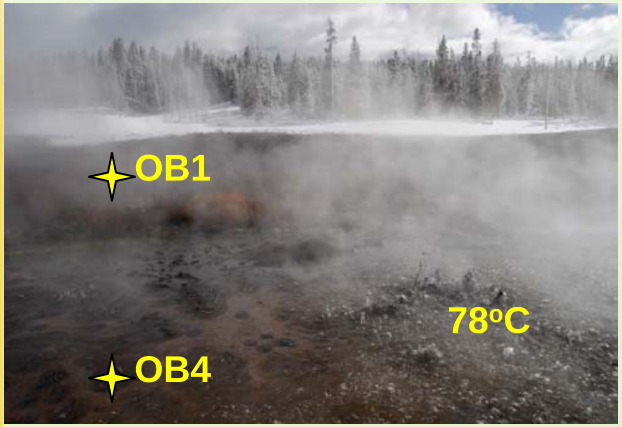
Thermophilic cellulose degrading bacteria from YNP

Enrichments (6) => gDNA isolation => 16S rRNA cloning => RCA TempliPhi => sequencing

Obsidian Pool



<u>OB1</u>: 66°C, pH5, Water, sediment, grass
<u>Enrichment (3)</u>: 60°C, poplar or switch grass
<u>Clones</u>: *Caldicellulosiruptor* sp., *Thermoanaerobacter* sp.

<u>OB4</u>: 71°C, pH5, Water, wood
<u>Enrichment (1)</u>: 80°C, poplar
<u>Clones</u>: Uncultured *Dictyoglomus* sp.

Artists Paintpots

<u>AR4</u>: 54°C, pH7.5, Water, wood, sediment
<u>Enrichment (1)</u>: 60°C, switch grass
<u>Clones</u>: *Caldicellulosiruptor* sp., uncultured *Nitrospira* sp., *Thermobacteroides acetoethylicus*, uncultured *Thermodesulfovibrio* sp., *Thermoanaerobacter ethanolicus*

- **43 unique OTUs out of 57 clones.**
- **Same OTUs were found in different enrichments for the same sample.**
- **No clones from different samples fell into same OTU.**

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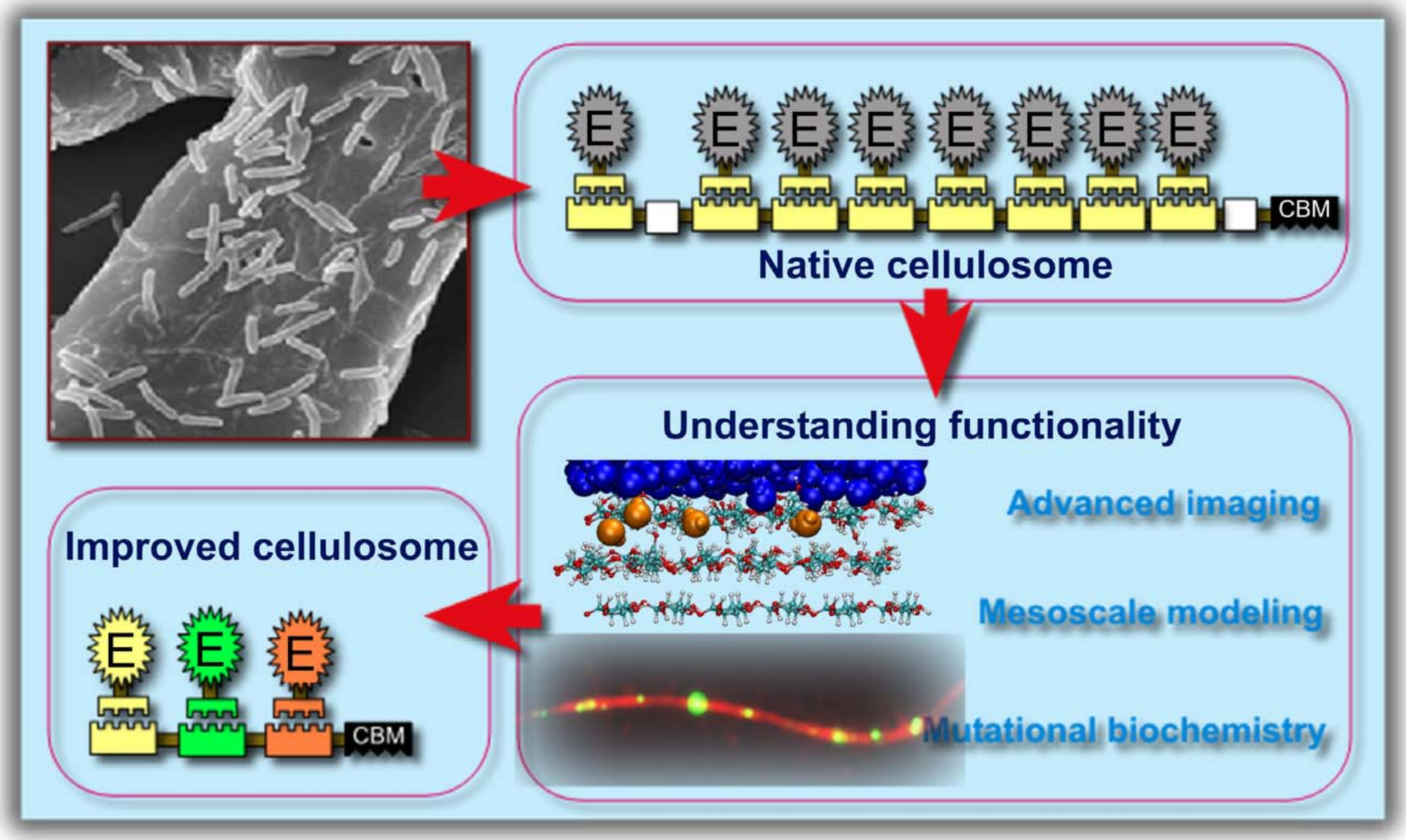
Cellulosome understanding and improvement



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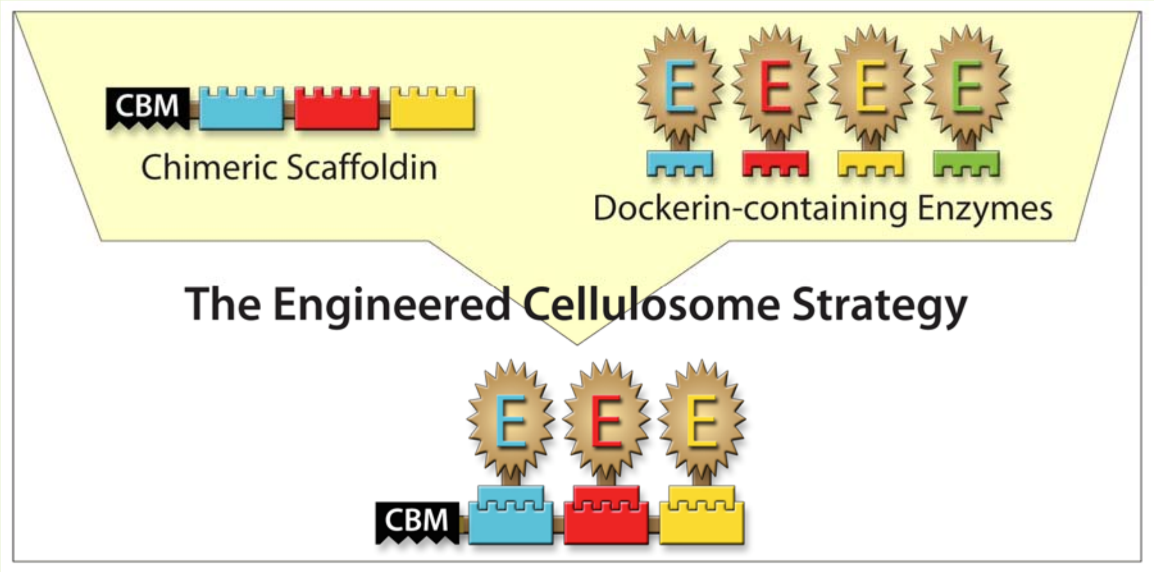
Cellulosome understanding and improvement

- *C. thermocellum* cellulosome proteomics analysis: identified more than 20 'new' cellulosomal components.

- Assignment of these components to specific growth substrates is ongoing



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

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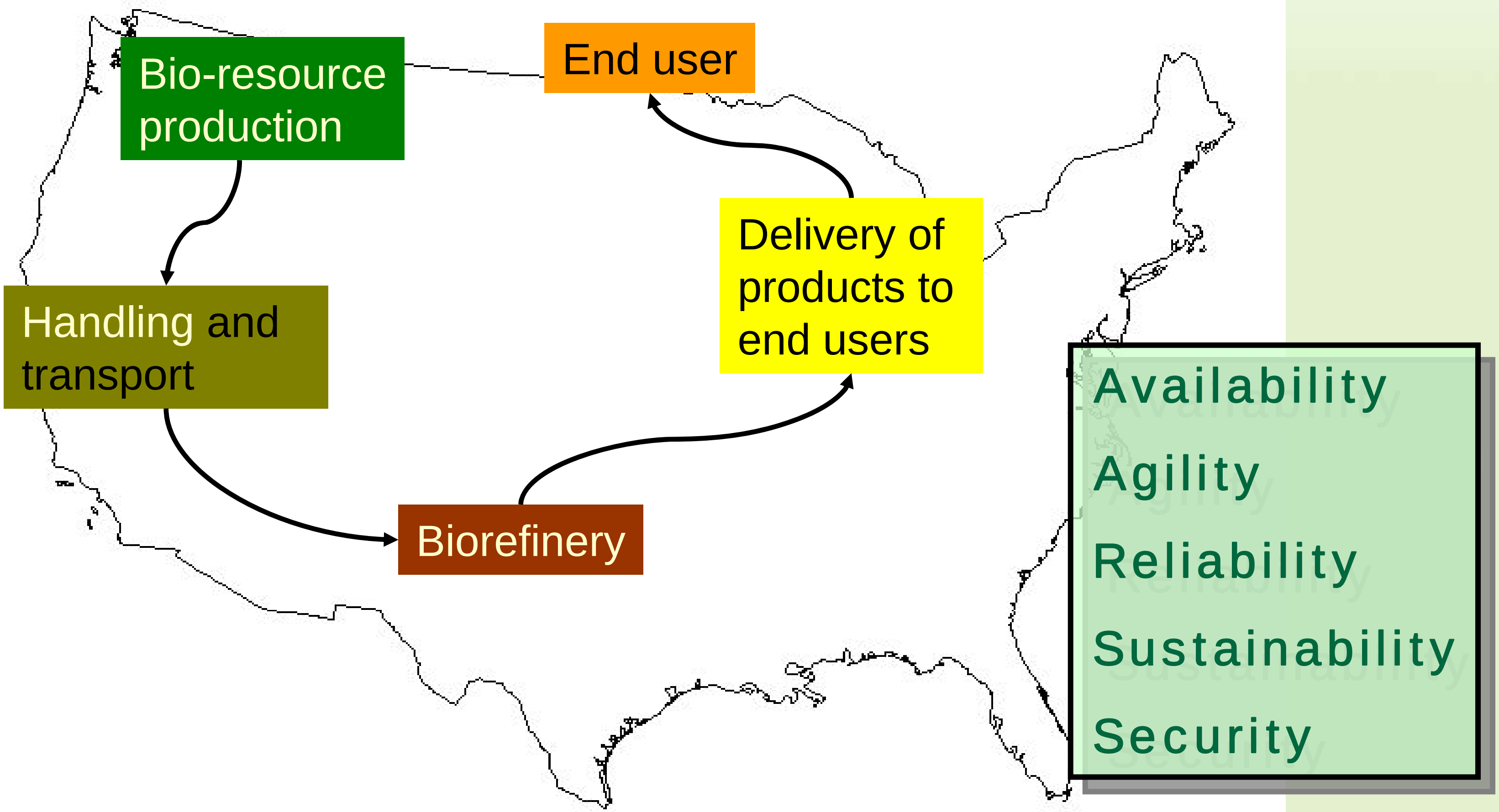
Bioenergy supply chain



Bioenergy is analogous to other existing energy networks

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The bioenergy grid will need to successfully address 5 key requirements

- ***Availability***
  - **Bioenergy needs to meet customer demand when and where its demanded**
- ***Reliability***
  - **The country must be able to count on adequate feedstock production, feedstock conversion and storage and distribution of the bioenergy products… and the quality of those products**
- ***Security***
  - **Bioenergy production and product distribution must be safe from deliberate acts to disrupt them**
- ***Agility***
  - **Bioenergy industry must be able to absorb periodic natural and technological perturbations to the system (e.g. regional drought or breakdowns in the transport system)**
- ***Sustainability***
  - **The bioenergy supply chains must be sustainable – environmentally, economically and socially**

Bioenergy supply chain

- To be successful, the physical and socioeconomic components and their interconnections of the supply-chain must be able to sustain the reliable generation and delivery of energy to the nation
- Will need to be integrated with the existing energy grids and transportation networks
- Will have strong interactions with other Critical Infrastructures
  - Agriculture & Forestry
  - Electric grid & oil network
  - Transportation
  - Water
  - Banking and Finance (Commerce)

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For a successful bioenergy supply chain we will need to address bioenergy's special issues

- **Biomass production**
  - **Geographically dispersed**
  - **Many viable production and generation pathways**
  - **Influenced by Ag/Forest/environmental policy**
  - **Rapidly changing through (bio)engineering**
    - Higher production rates
    - Adaptive to newer production environments
  - **Strongly influenced by external forcings**
    - Climate change and weather (extremes)
- **Bioenergy generation and storage & transportation**
  - **Many conversion technologies & forms of bioenergy**
  - **Evolving conversion technologies**
  - **Nascent supply logistics** (including feedstock storage)
  - **Progressively improving efficiency**
- **Environmental impacts**
  - **Water quality, biodiversity, soil productivity and air quality issues**
- **Societal adaptability and implications**

  Driven by security and climate change considerations

  Risk of undermining food security

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Looking to the future and thinking about bioenergy infrastructure

- **The US will need an organization or structured approach of some kind to continuously improve the reliability and security of the bioenergy resource, refinery, delivery, and end-use assets in North America**

- **Some sort of centralized function (private or government) will eventually be needed to**
  - develop and enforce bioenergy reliability standards
  - monitor the performance of the bioenergy supply chain
  - assess future resource adequacy
  - forecast future biofuel supplies
  - audit resource, refinery, delivery, and other asset owners for preparedness
  - and educate and train industry personnel ranging from the growers of energy crops to bulk end-users

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It is time to start developing

- A national decision support framework
  - Underpinned by comprehensive data and modeling resources
  - Providing analytical and visualization targeted to multiple stakeholder for efficient planning, developing and managing of the US Bioenergy supply chain
    - Federal Gov't
    - State & local Gov't
    - The private components of the supply chain
      - Farmers
      - Transporters
      - Equipment manufacturers
      - Builders of thermo and bio conversion facilities
      - End use manufacturers (e.g. engine manufacturers)
  - Integrating key findings from research supported by multiple agencies

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Thank you

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