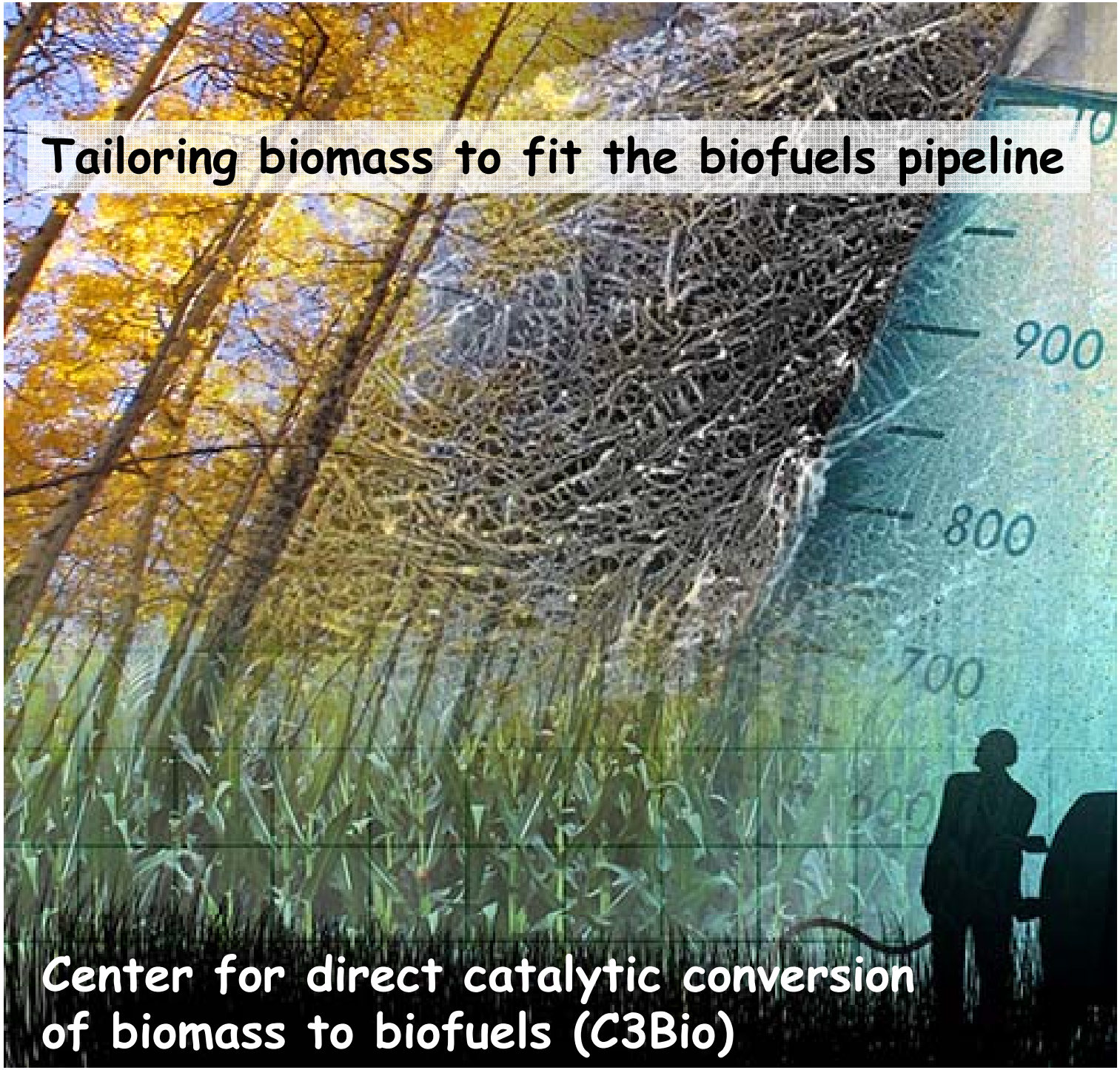
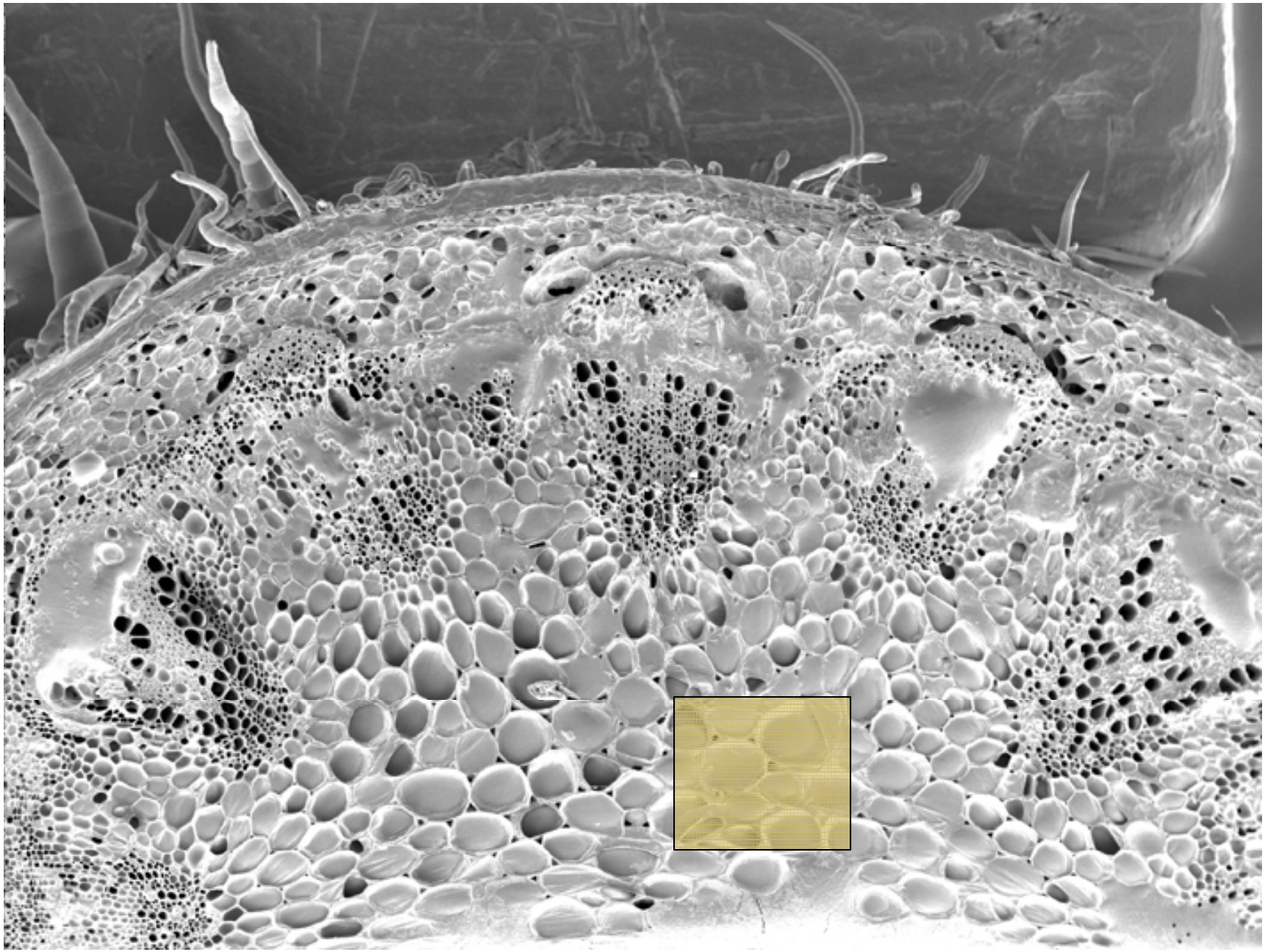
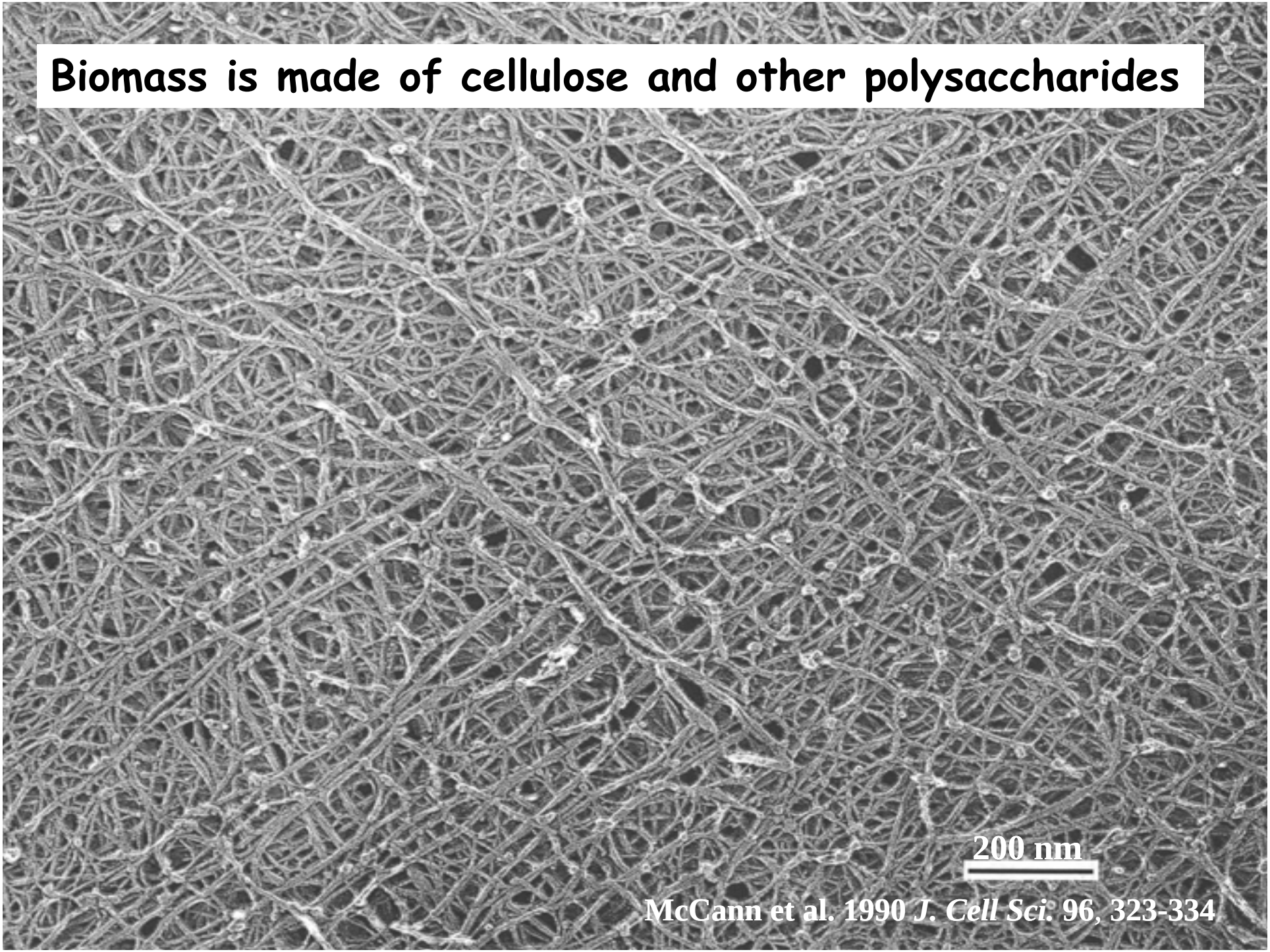

Tailoring biomass to fit the biofuels pipeline
900
800
700
Center for direct catalytic conversion
of biomass to biofuels (C3Bio)

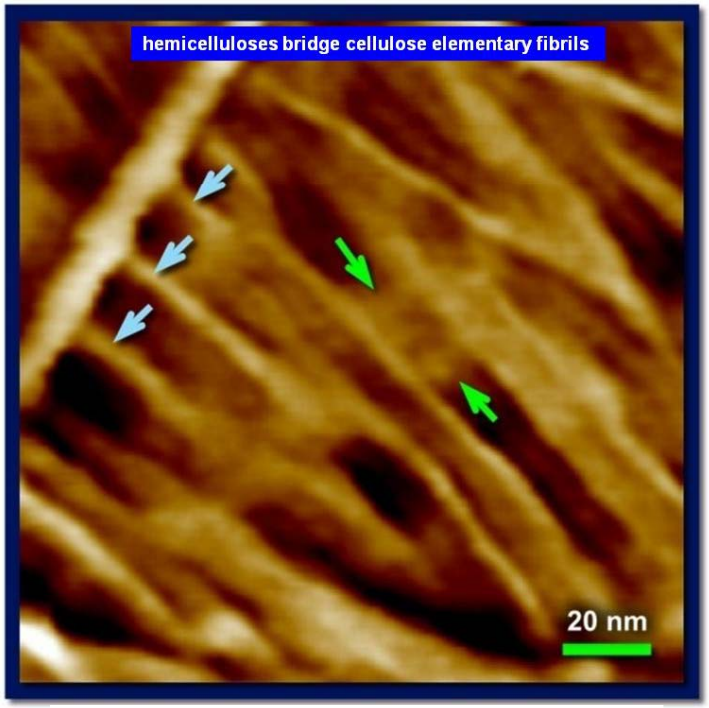


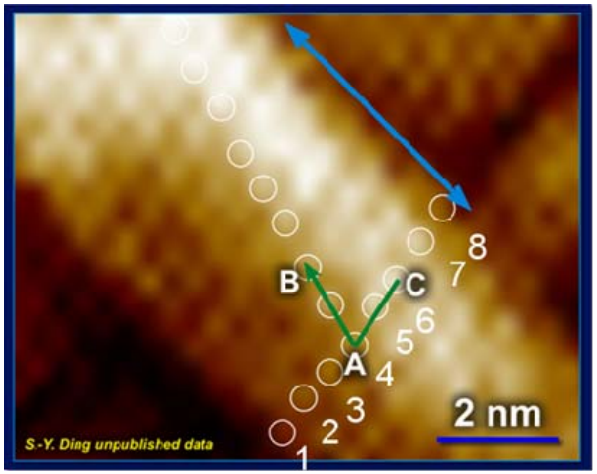
Biomass is made of cellulose and other polysaccharides

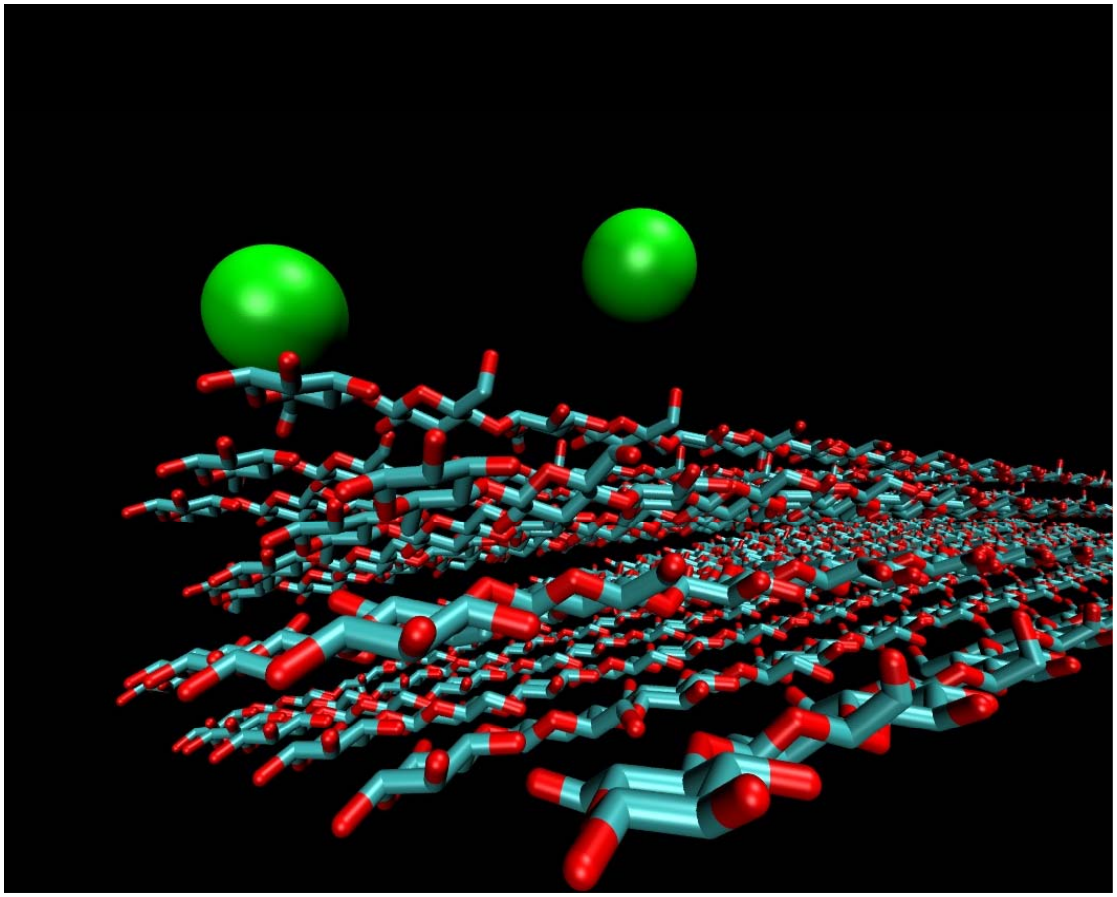


McCann et al. 1990 *J. Cell Sci.* 96, 323-334

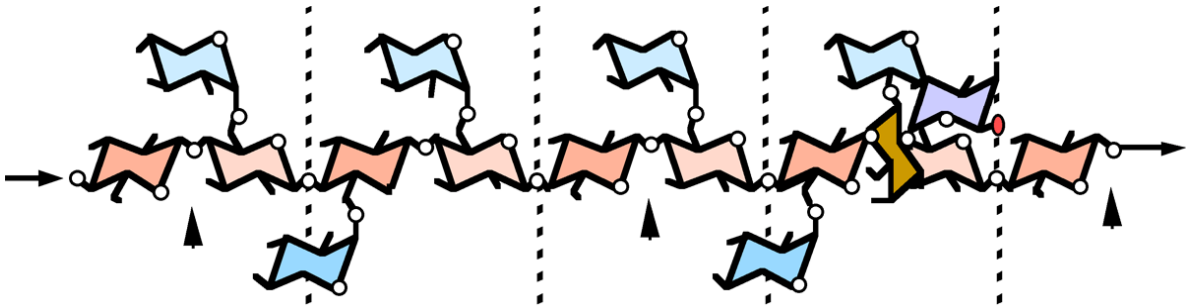
Imaging and modeling of cellulose microfibrils





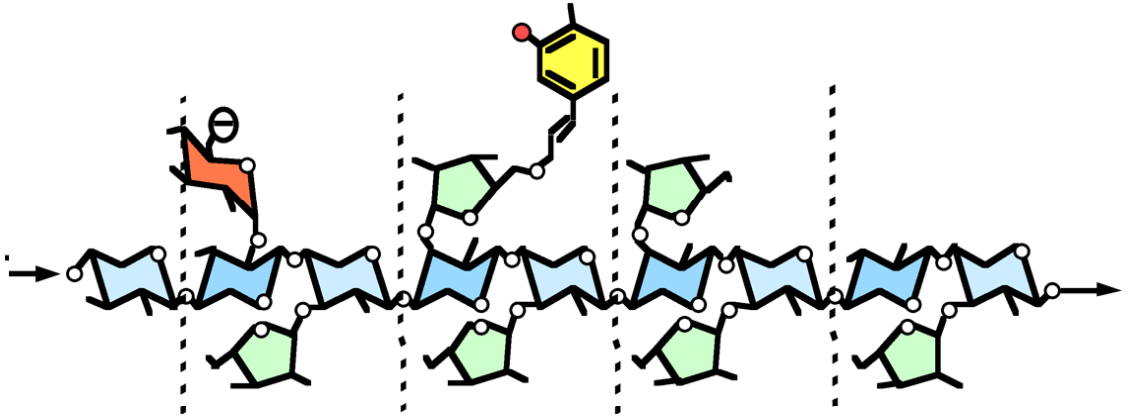


Biomass of different species has different hemicelluloses

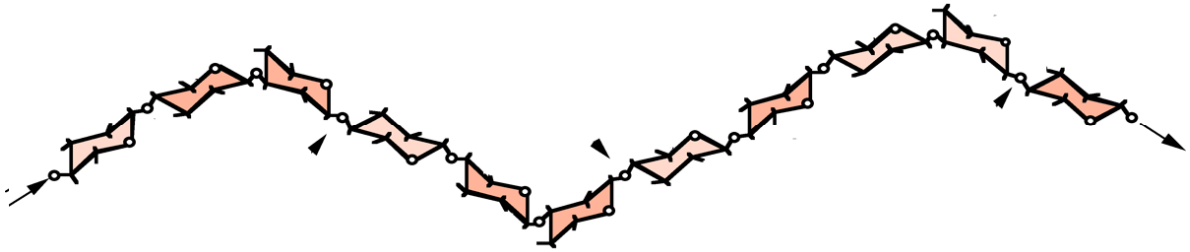


Xyloglucan is the major cross-linking glycan of the Type I wall

Cross-linking glycans of the Type II walls of grasses

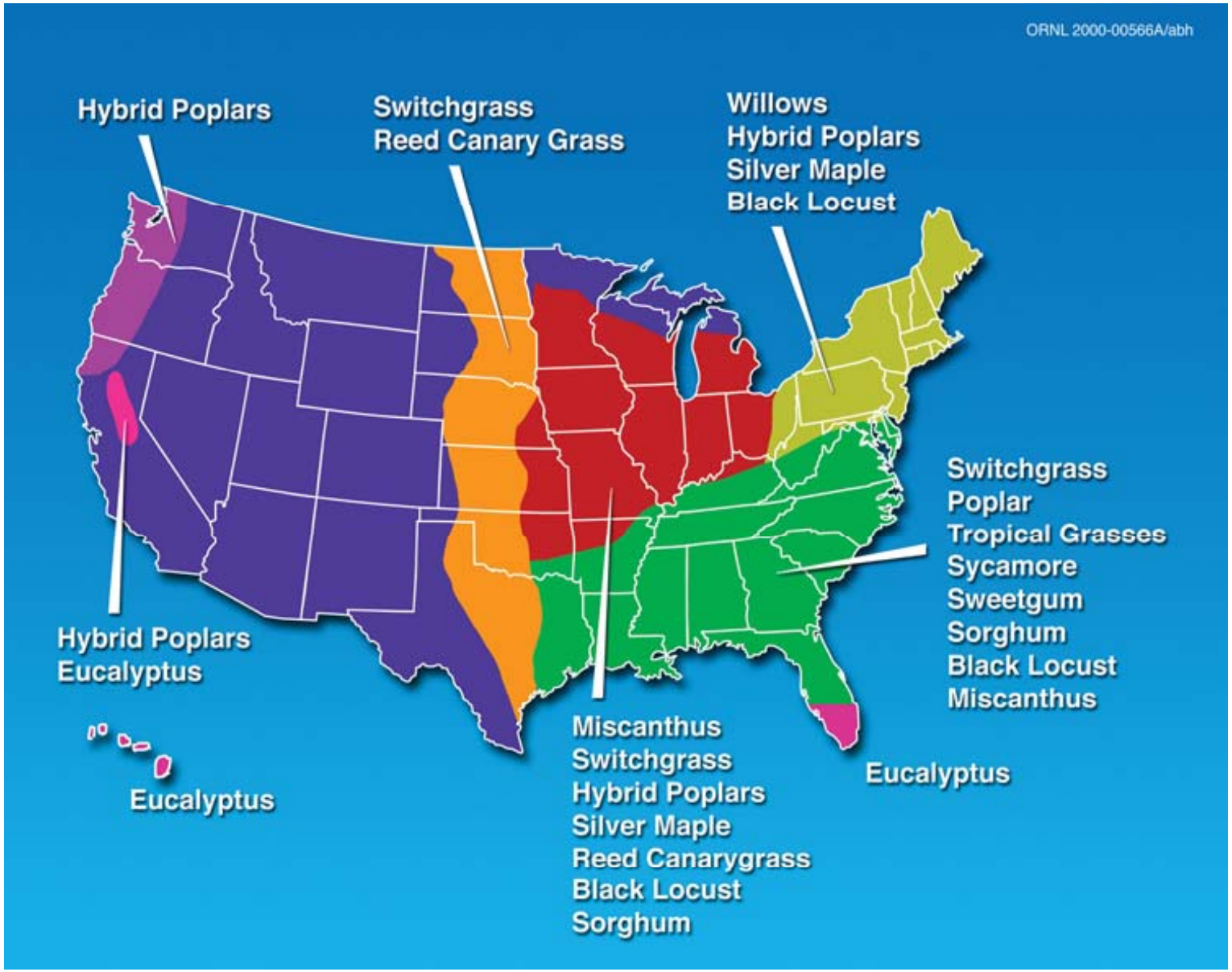


Feruloylated glucuronoarabinoxylan

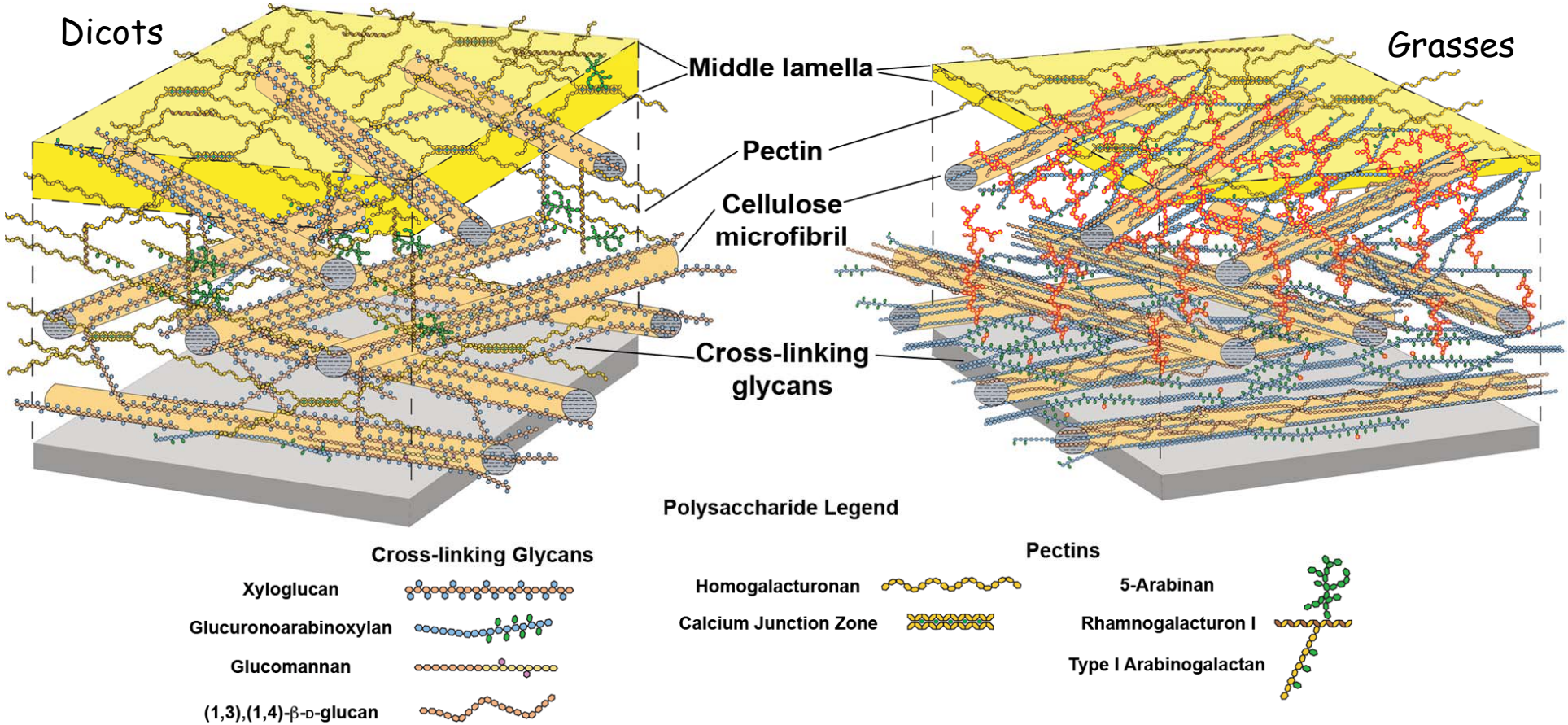


Mixed-linkage (1,3),(1,4)-β-D-glucan

Different biomass feedstocks will be developed regionally



Two cell wall models are needed for growing cells



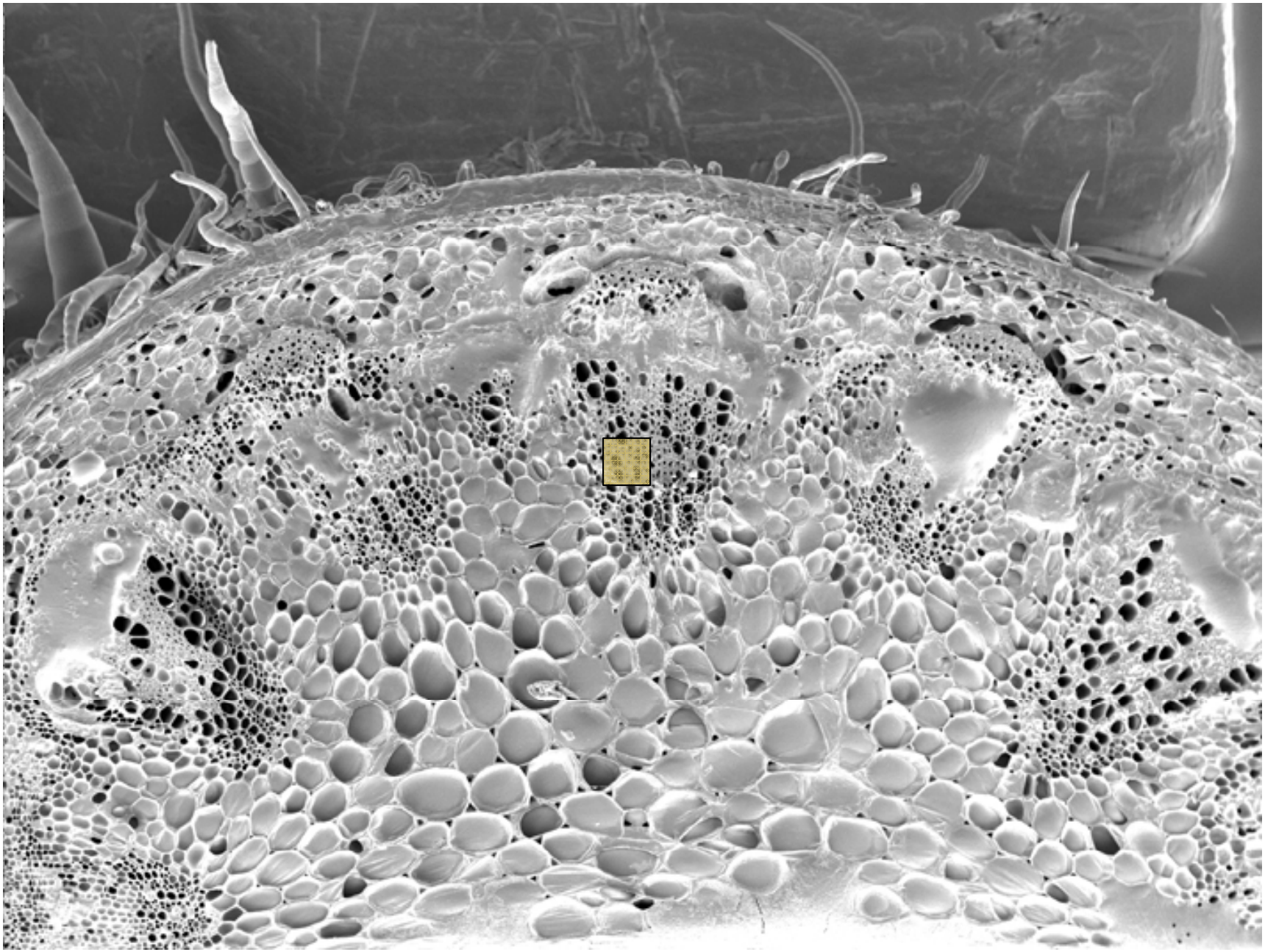
Arabidopsis, Poplar

Poplar, Willow, Silver maple,
Eucalyptus, Sweet Gum, Black locust

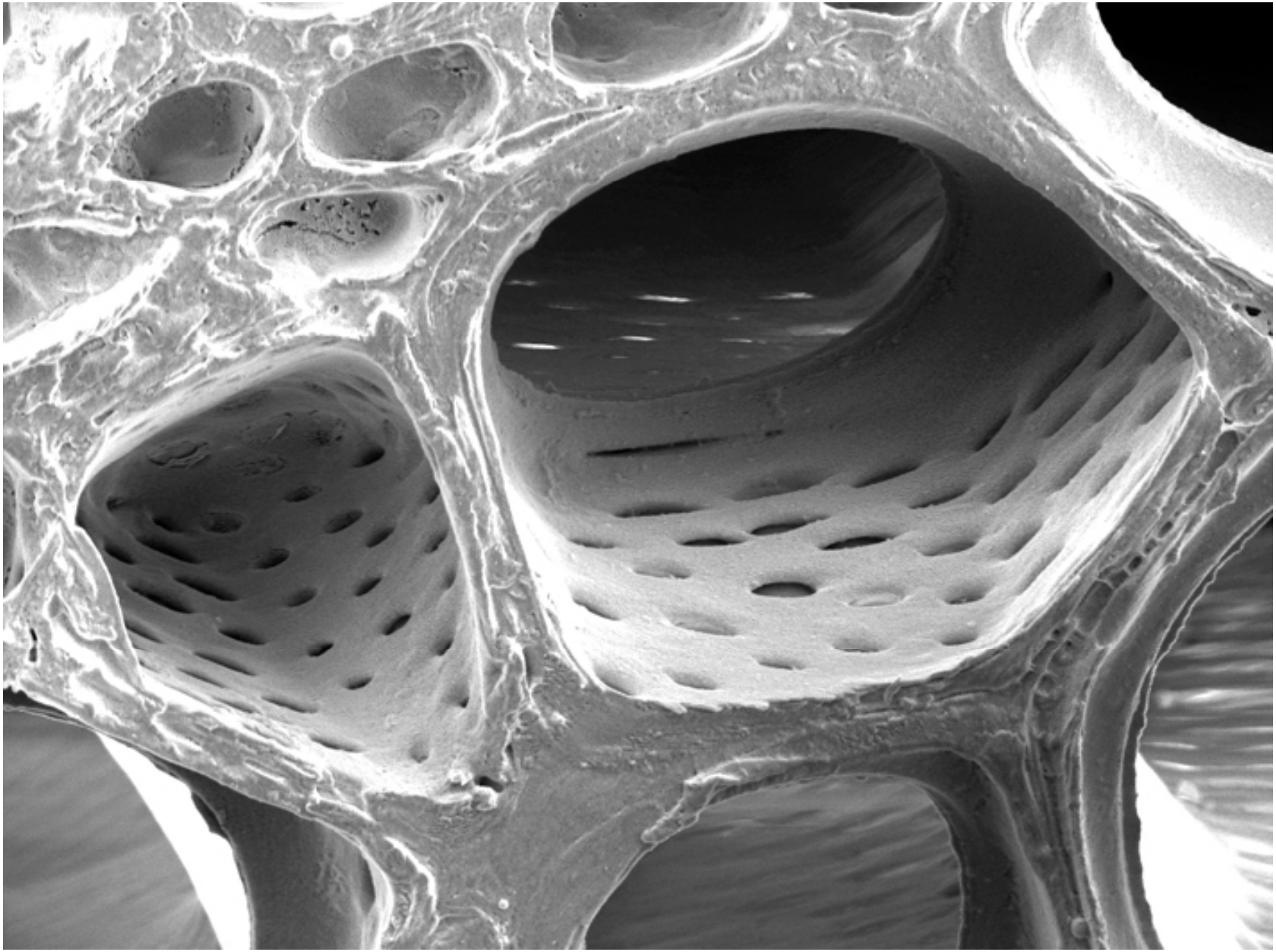
Maize, Sorghum, Rice

Switchgrass, Sorghum, Miscanthus,
Sugarcane, Big Bluestem, Reed canarygrass,
Tropical grasses

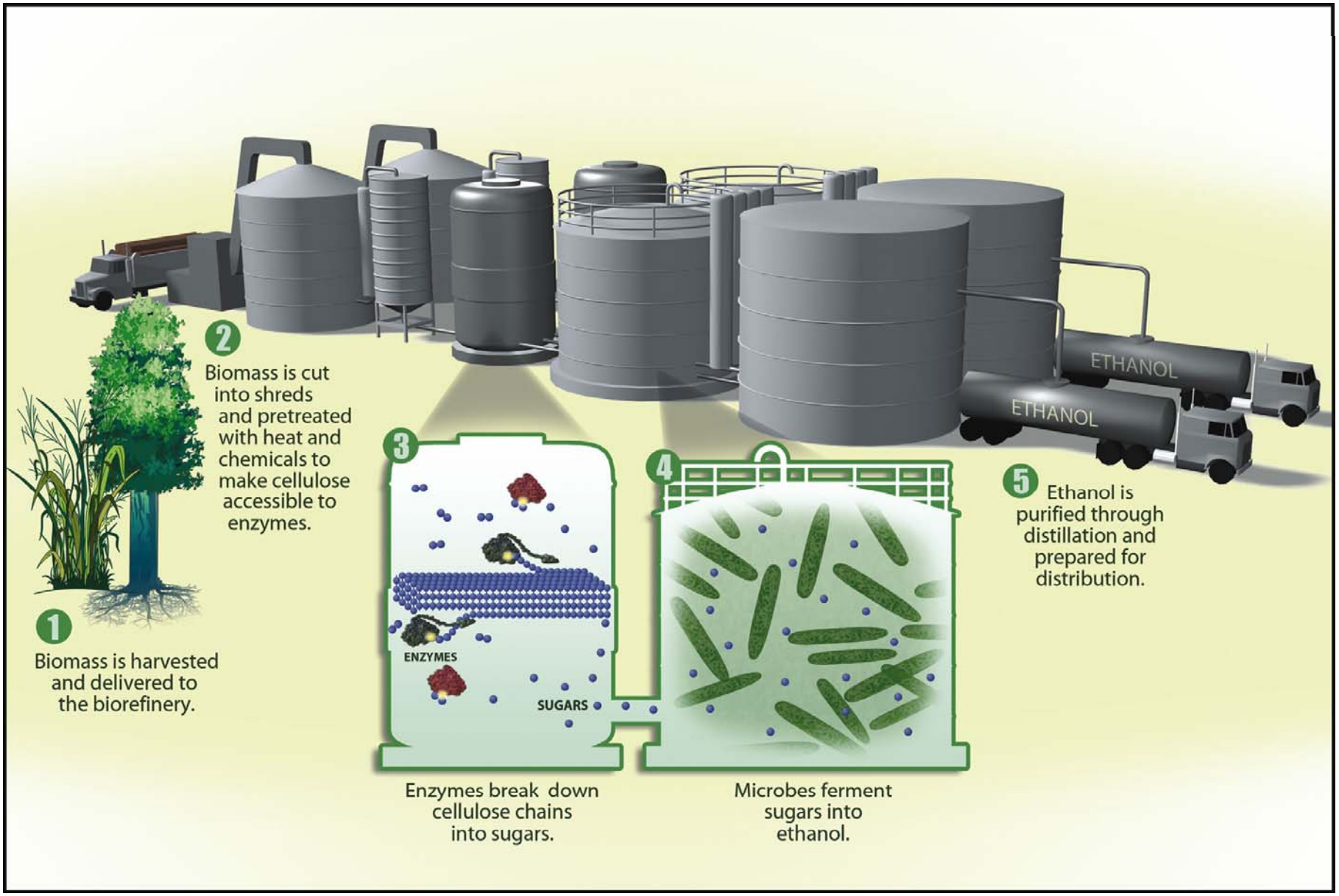
Carpita and McCann, 2008; after McCann and Roberts, 1991



Lignified secondary walls surround some specialized cell types but contribute greatly to the biomass

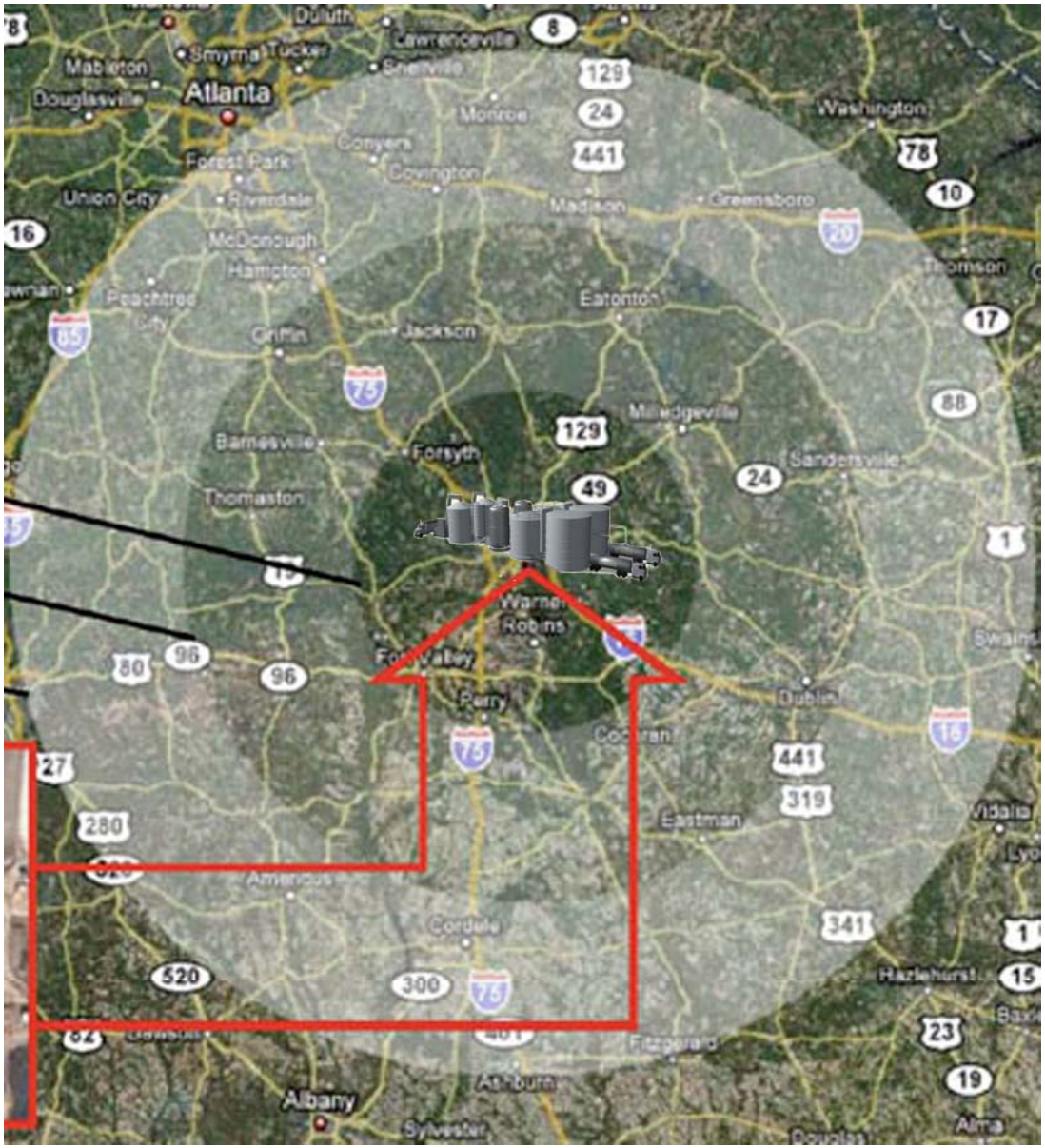


Biological conversion route for biomass to biofuel

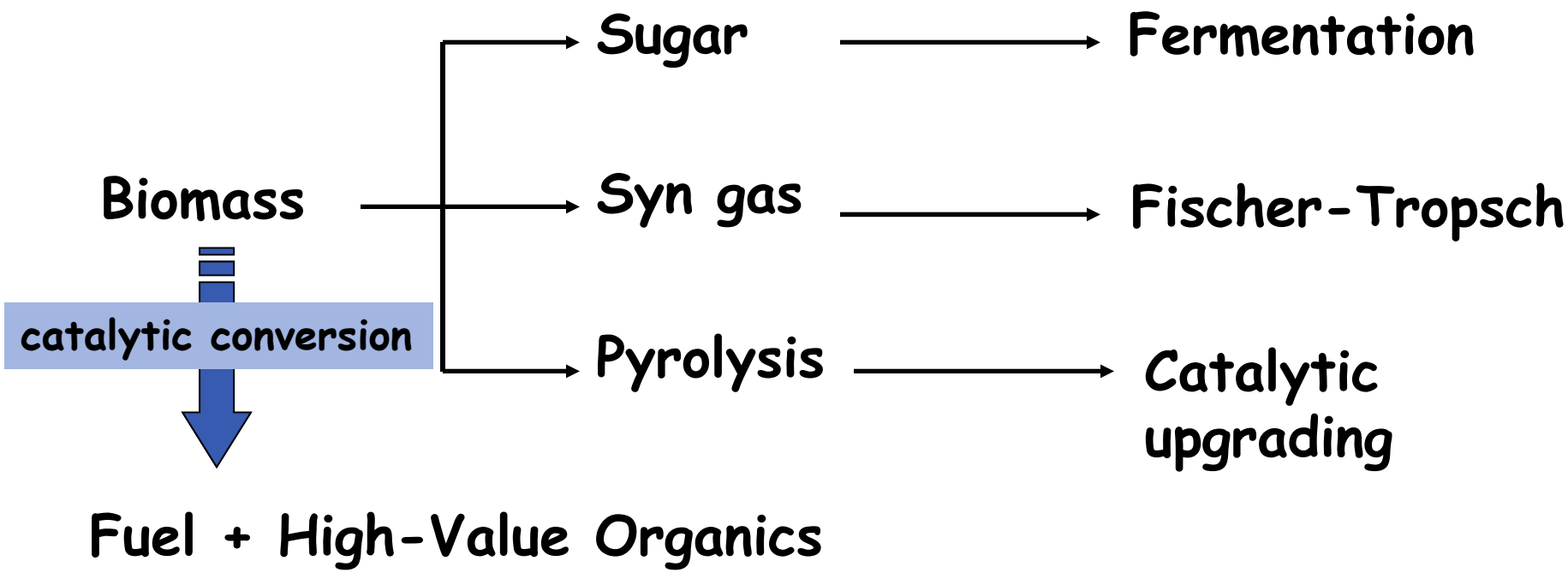


DOE-GTL *Research Roadmap to Breaking the Biological Barriers to Cellulosic Ethanol* 2005

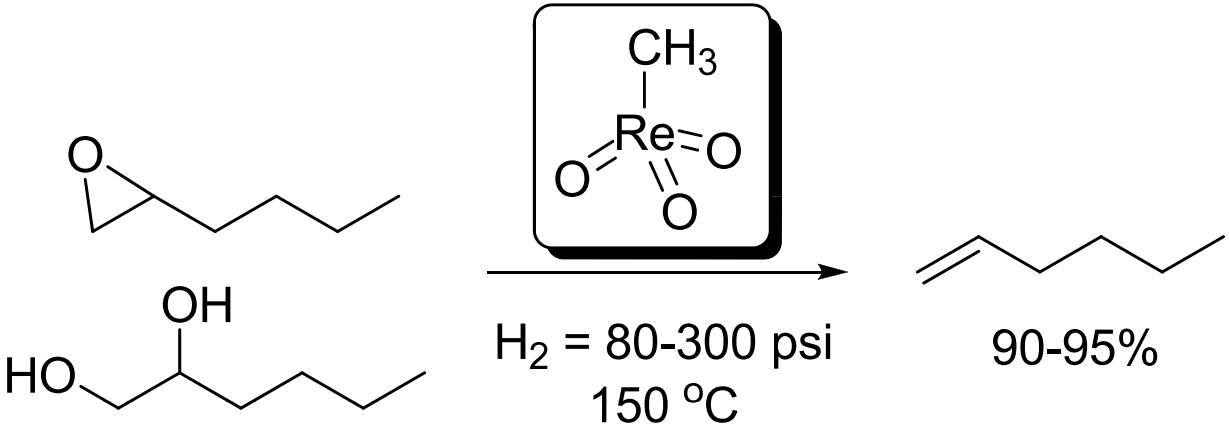
Transport costs will limit where biomass feedstocks are grown



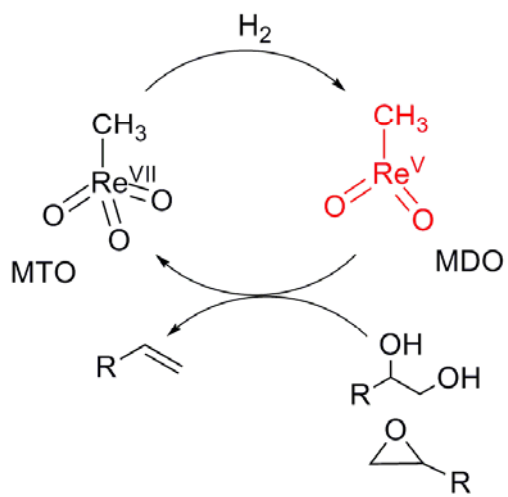
Conversion technologies for next-generation biofuels

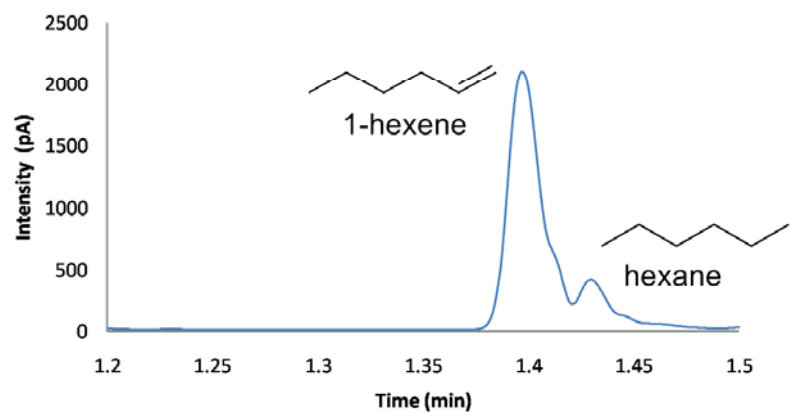


Converting sugars to alkenes: diol-to-alkene reaction



Mechanism

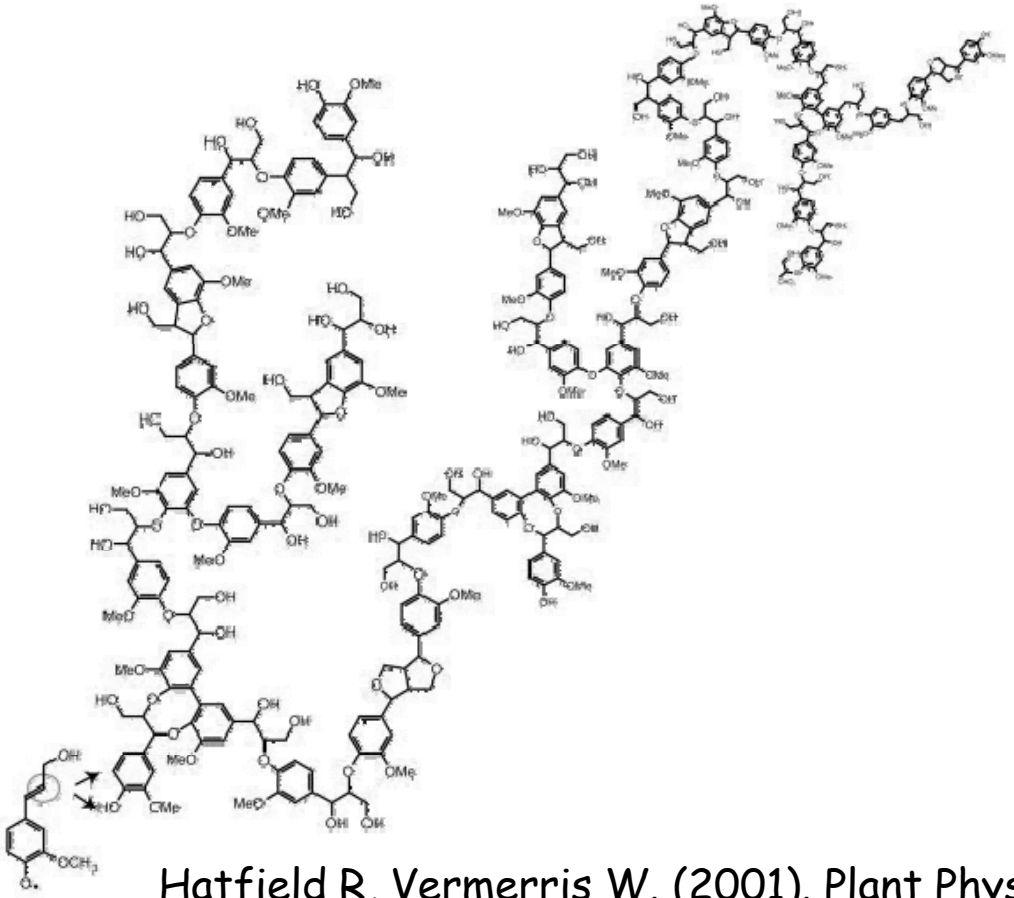




J. E. Ziegler, M. J. Zdilla, A. J. Evans, and M. M. Abu-Omar, "H_2-Driven Deoxygenation of Epoxides and Diols to Alkenes Catalyzed by Methyltrioxorhenium" *Inorg. Chem.* **2009**, *48*

Lignin is an opportunity as well as a challenge

- UV resistance
- structural support
- water transport

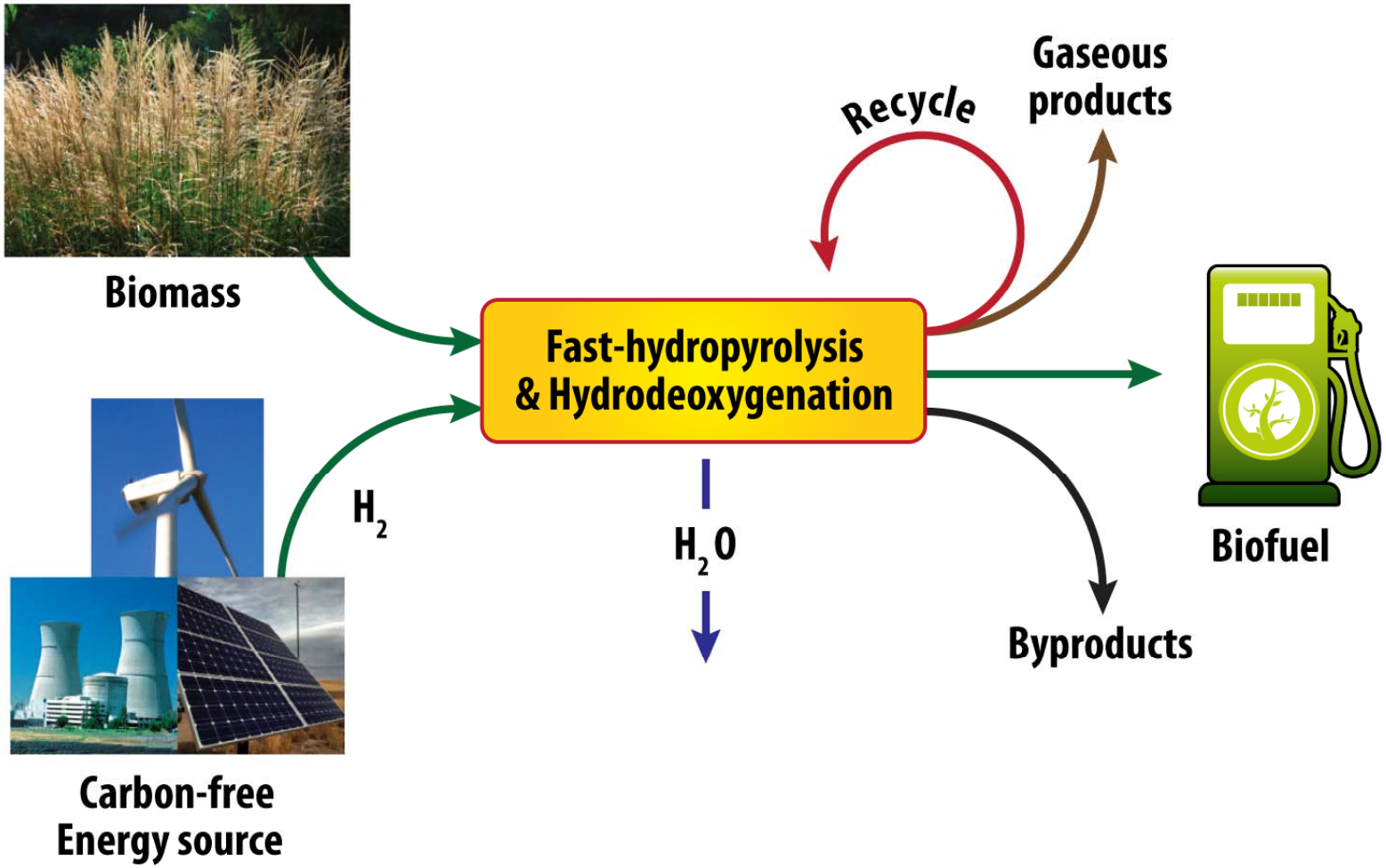


Hatfield R, Vermerris W. (2001). Plant Physiol. 126: 1351-1357

Desired characteristics of a winning conversion process

- the process is independent of the feedstock species
- it will maximize the carbon and energy conversion efficiency (amount of product recovered per unit biomass)
- desired co-product streams can be recovered
- can be scaled to smaller, even mobile, biorefineries

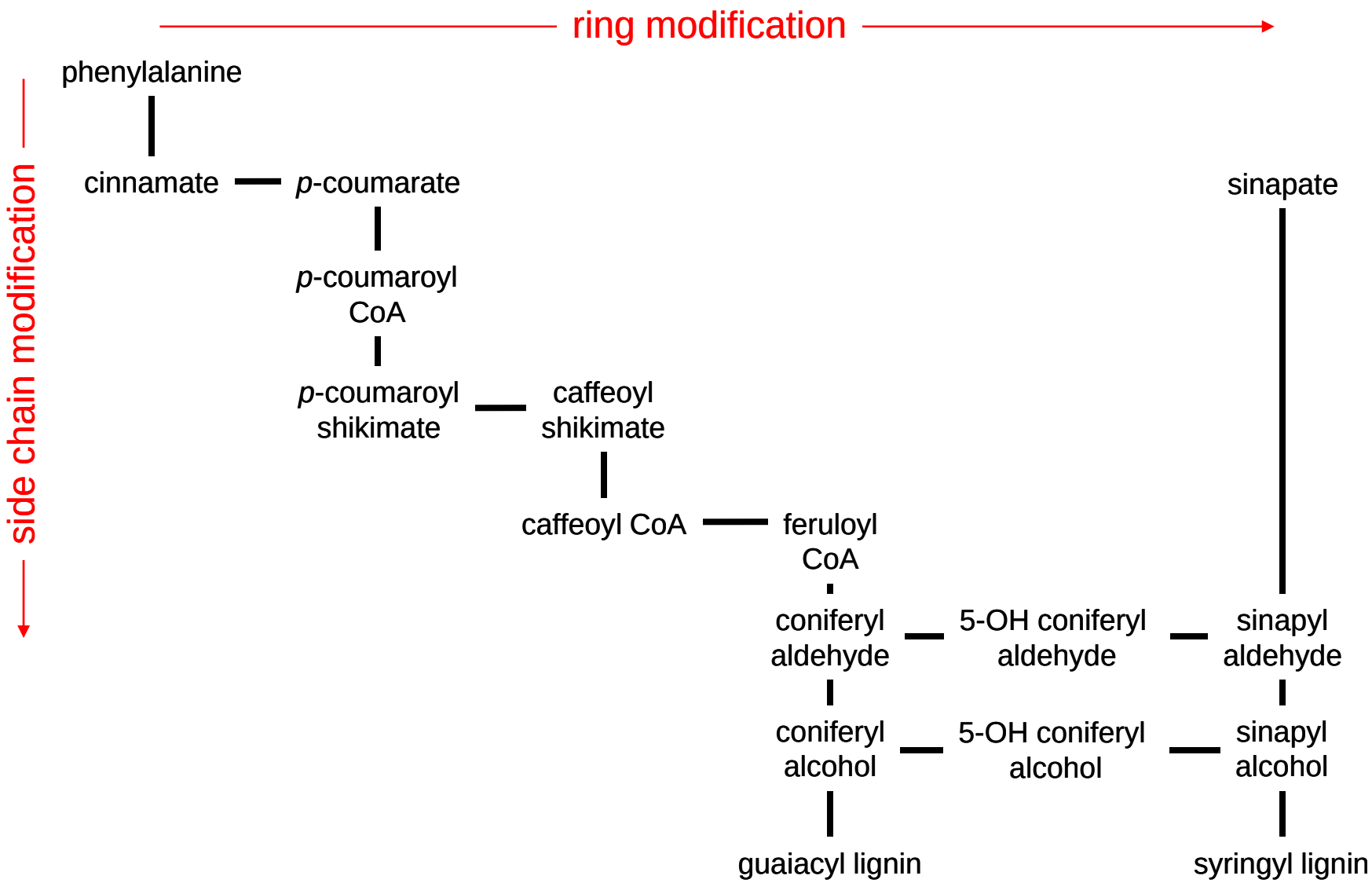
High-temperature treatments may produce a bio-crude oil for biorefinery fractionation



R. Agrawal and N. Singh, AIChE Journal, **55**, 1898 (2009)

What can plant biologists do to help optimize carbon and energy conversion efficiencies?

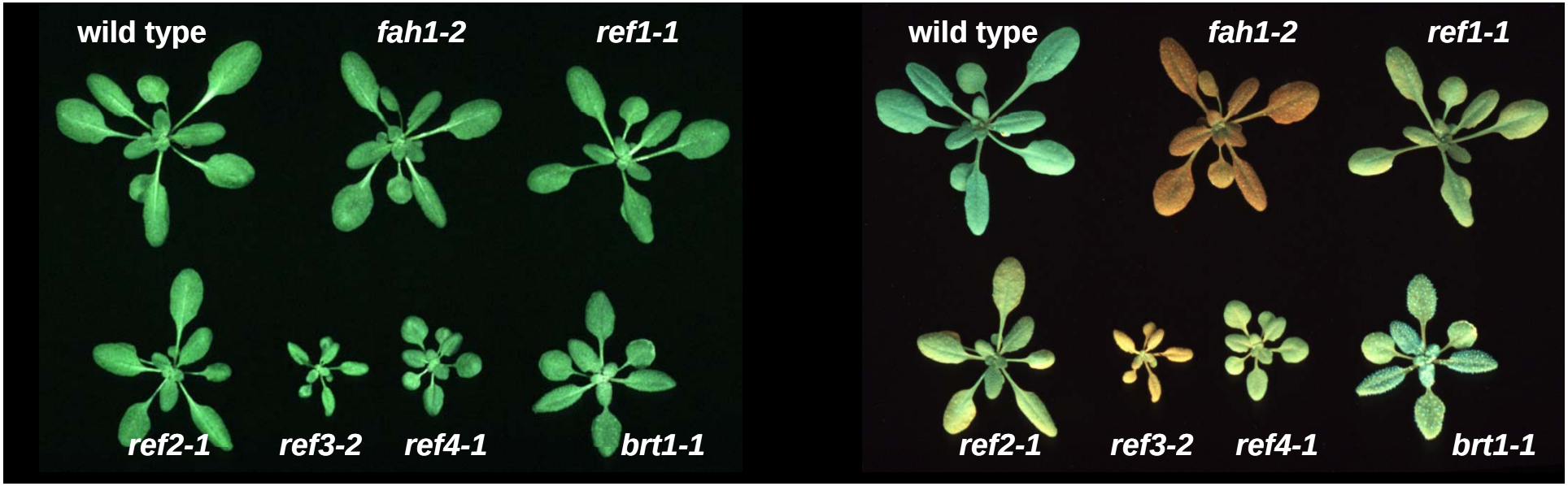
The lignin biosynthetic pathway



Clint Chapple, Purdue University

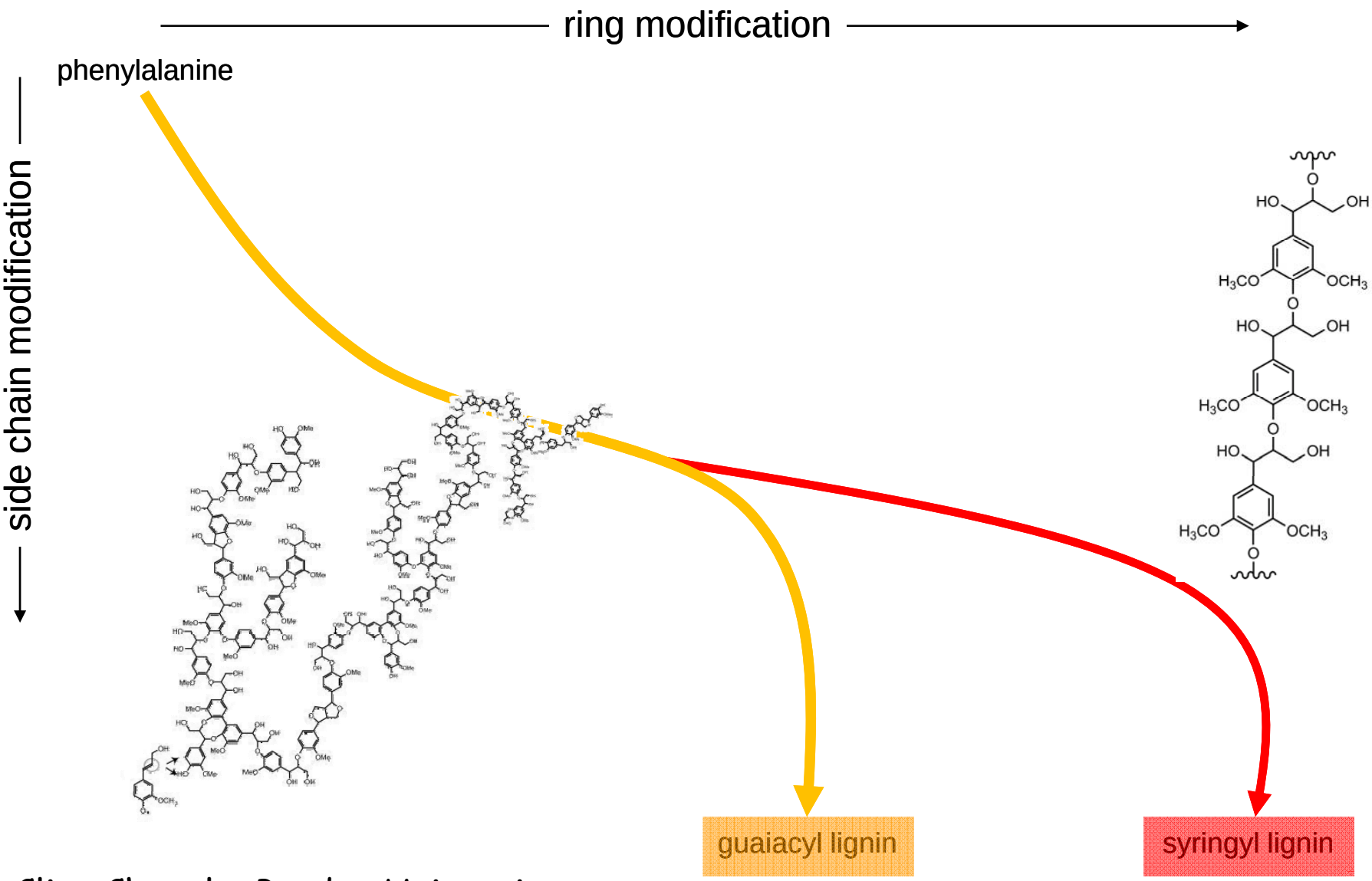
Phenotypes of lignin mutants under UV light

VISIBLE UV



Clint Chapple, Purdue University

The lignin biosynthetic pathway



Clint Chapple, Purdue University

Maize *brown midrib* mutants: naturally occurring mutants with altered lignin-carbohydrate interactions

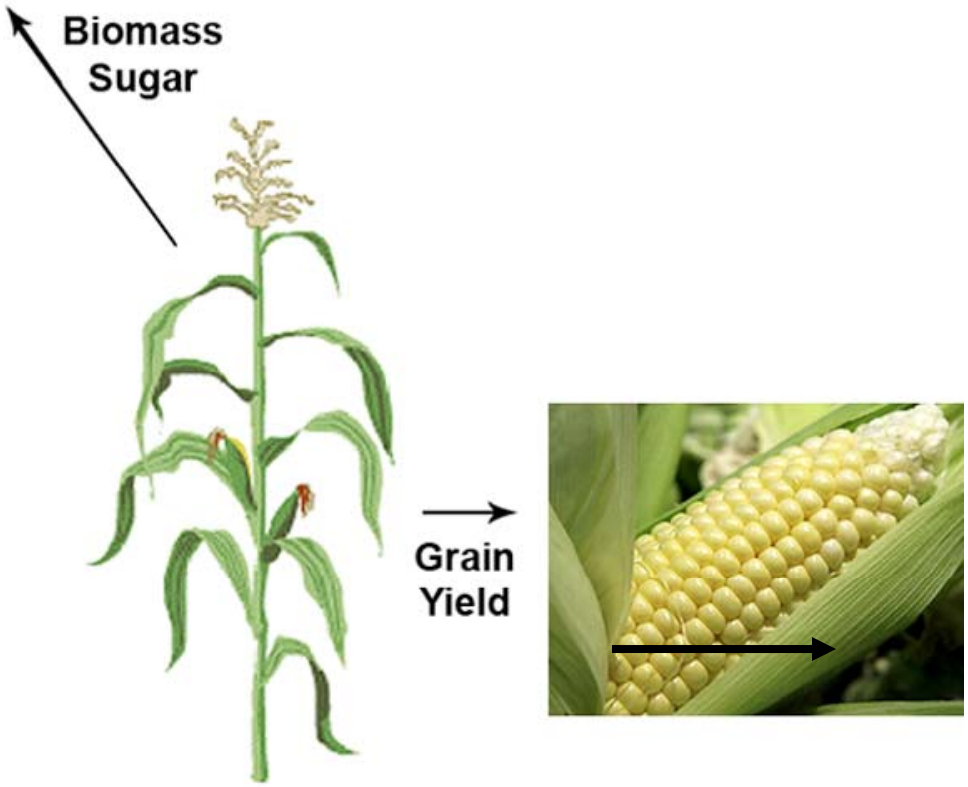
Wilfred Vermerris

The genetic diversity of all humans is less than 0.1%

We vary from chimps by 1.23-1.34%

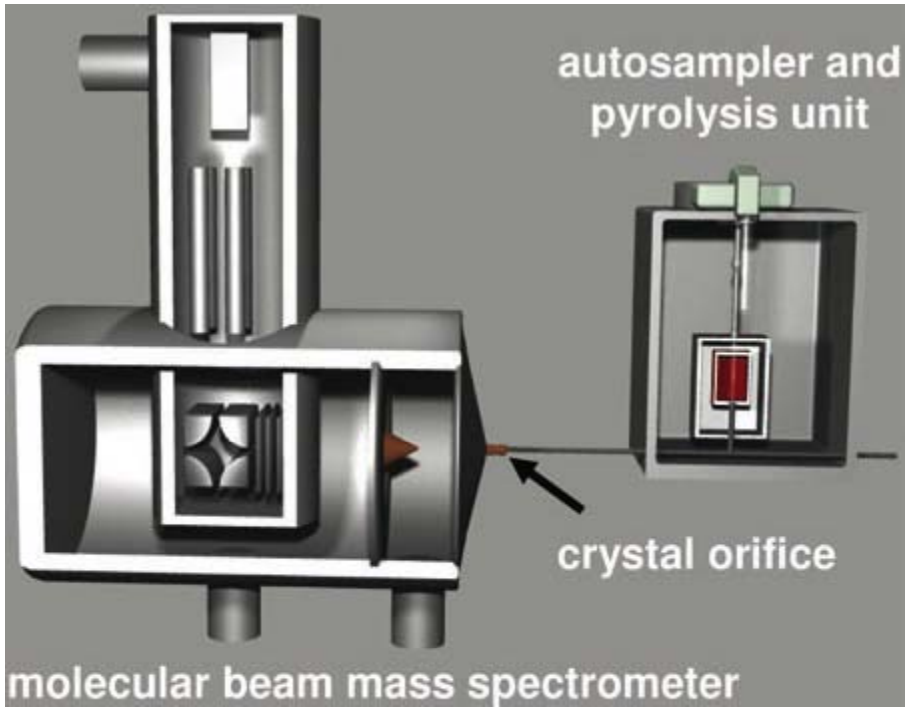
The genetic diversity of maize is estimated at nearly 1.9%

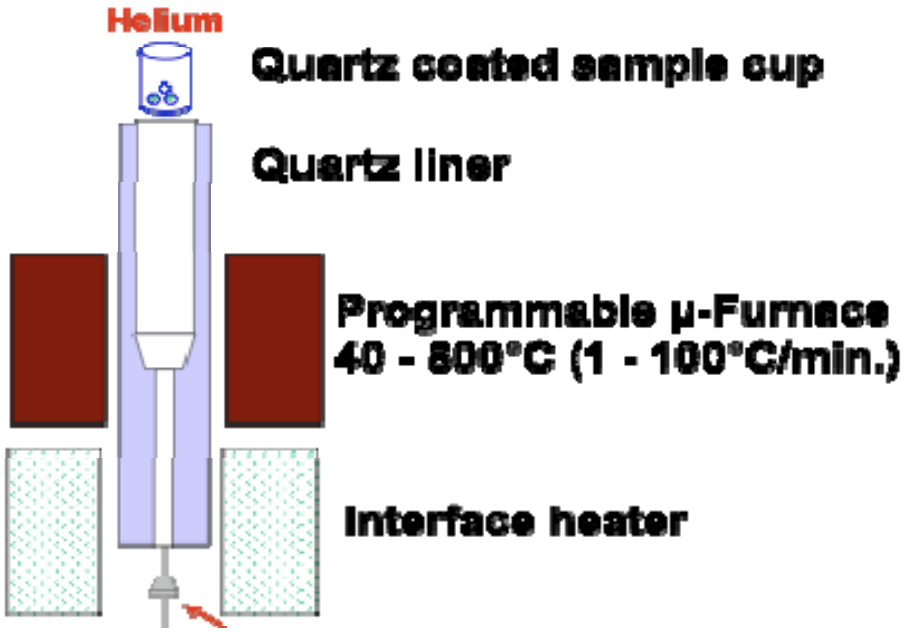
Maize genetic diversity permits selection of several desirable traits; focus on the grain has increased yields almost 9-fold since the advent of hybrid corn



Maize has quality traits for advances in not only grain yield but also biomass accumulation and sugar accumulation

Pyrolysis molecular-beam mass spectrometry (Py-MBMS) as a research tool to screen for biomass variants impacting hydropyrolysis behavior

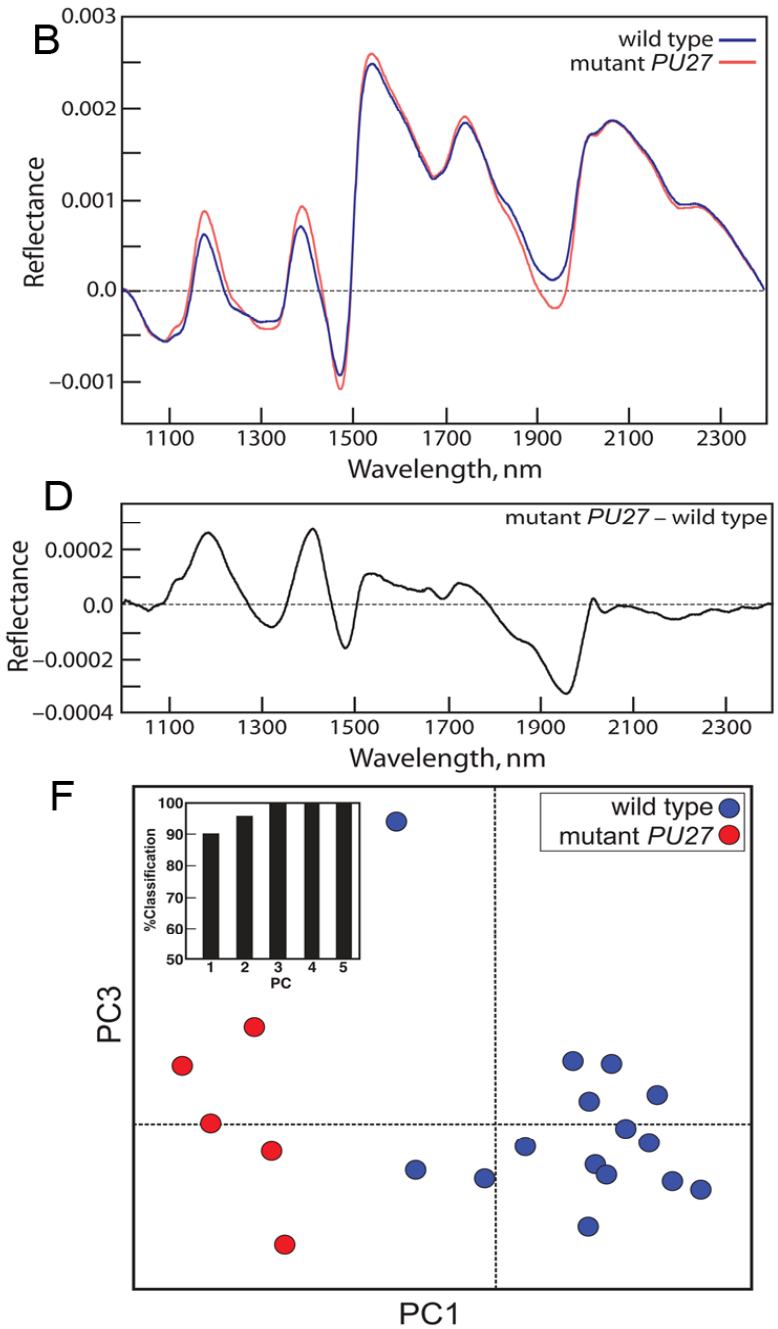


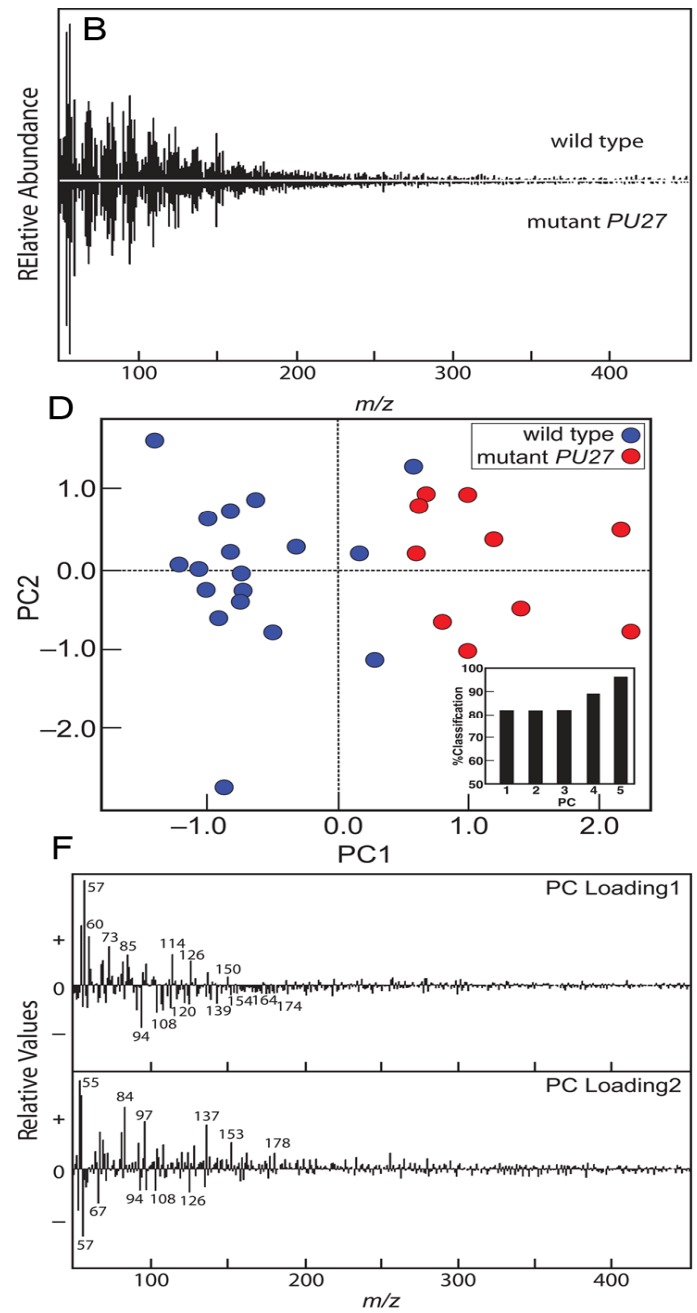


Frontier Labs drop-tube micropyrolyzer, in use at NREL in the Py-MBMS setup

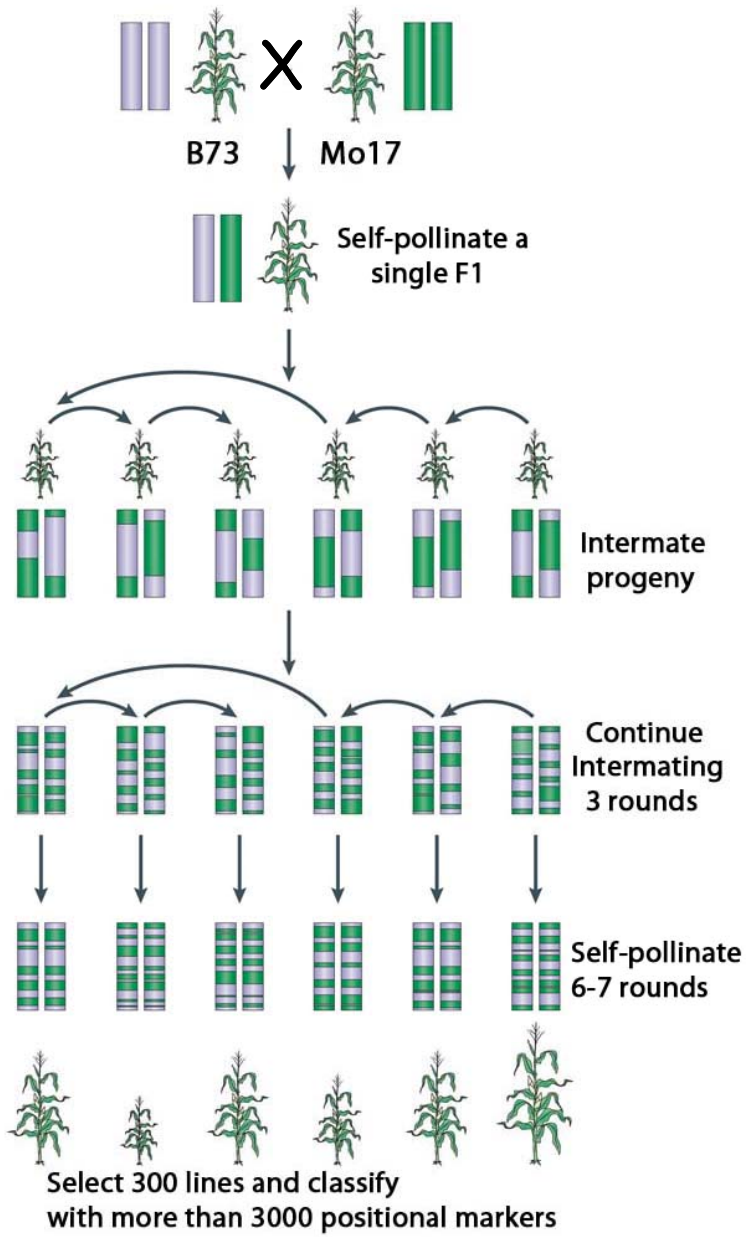
Jonathan R. Mielenz (ed.), Biofuels: Methods and Protocols, Methods in Molecular Biology, vol. 581, Chapter 12, Springer

NIR and PyMBMS identify maize mutants with altered CHO-lignin interactions

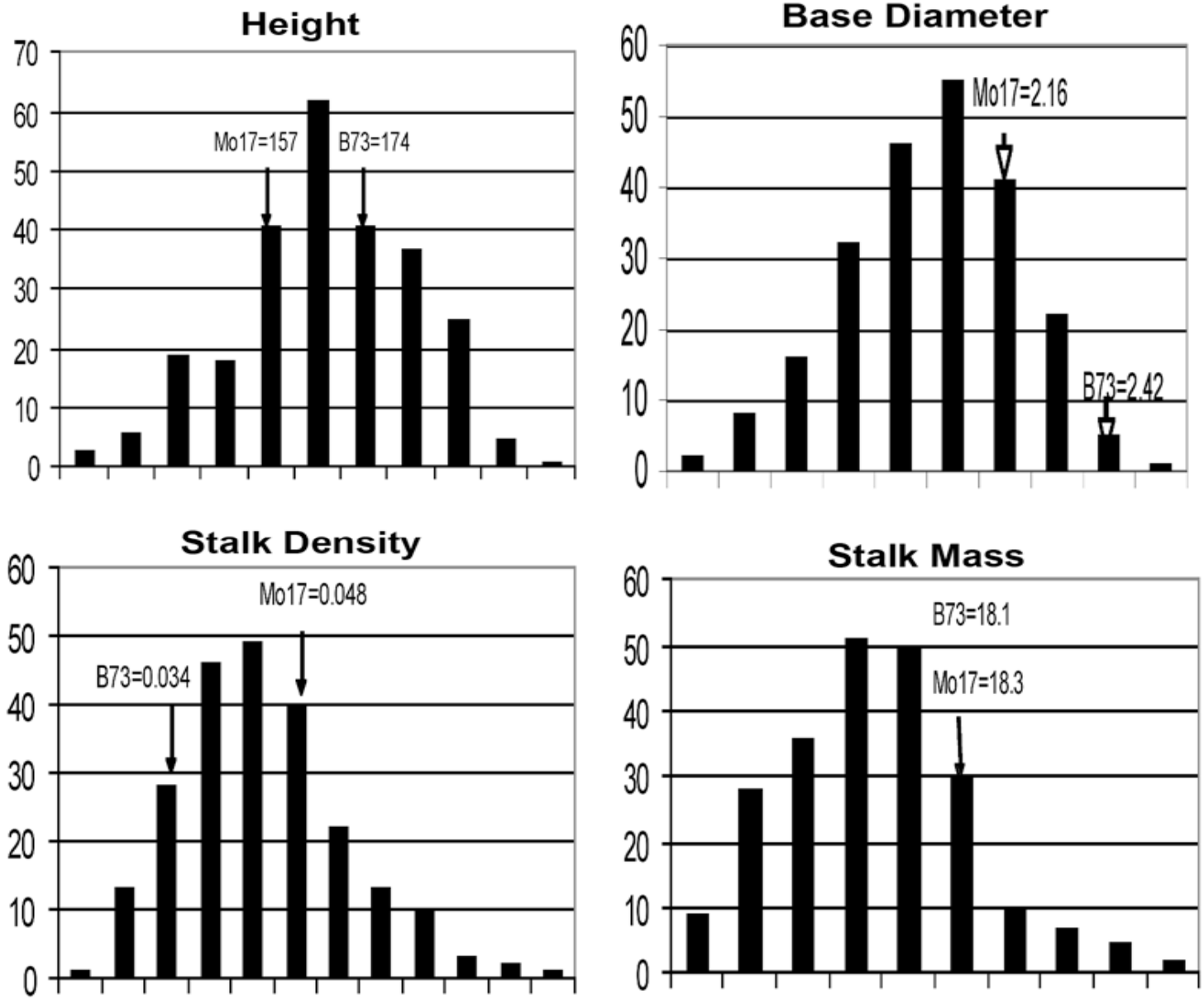




Structured populations allow us to correlate small chromosomal regions with desirable characteristics



The Intermated B73 x Mo17 (IBM) lines represent a valuable test resource for enhanced biomass characteristics



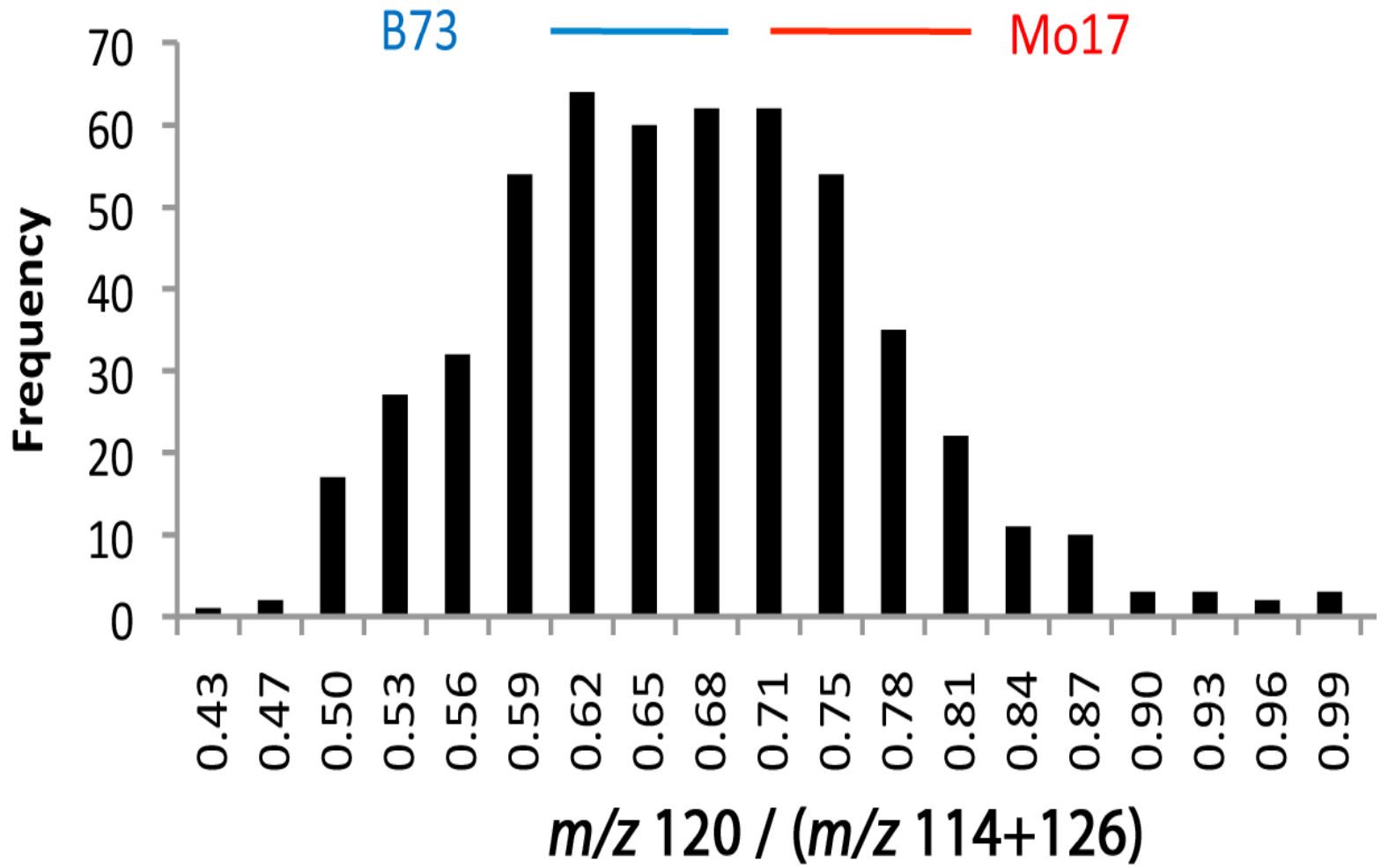
Origins of the *m/z* fragments detected by PyMBMS

m/z	
55	polysaccharide
73	polysaccharide
85	polysaccharide
94	phenol
98	polysaccharide
109	guaiacol [M-15]$^+$ (Guaiacyl Lignin) (G)
114	pentose
117	indole or phenylacetonitrile (protein)
120	4-vinylphenol [M-44]$^+$ (p-Coumaric Acid) (pCA)
123	4-methyl guaiacol [M-15]$^+$ (G)
124	guaiacol (G)
126	hexose
144	hexose
137	coniferyl alcohol [M-43]$^+$ (G)
138	4-methyl guaiacol (G)
139	2,6-dimethoxyphenol [M-15]$^+$ (Syringyl Lignin)(S)
147	*p*CA [M-17]$^+$

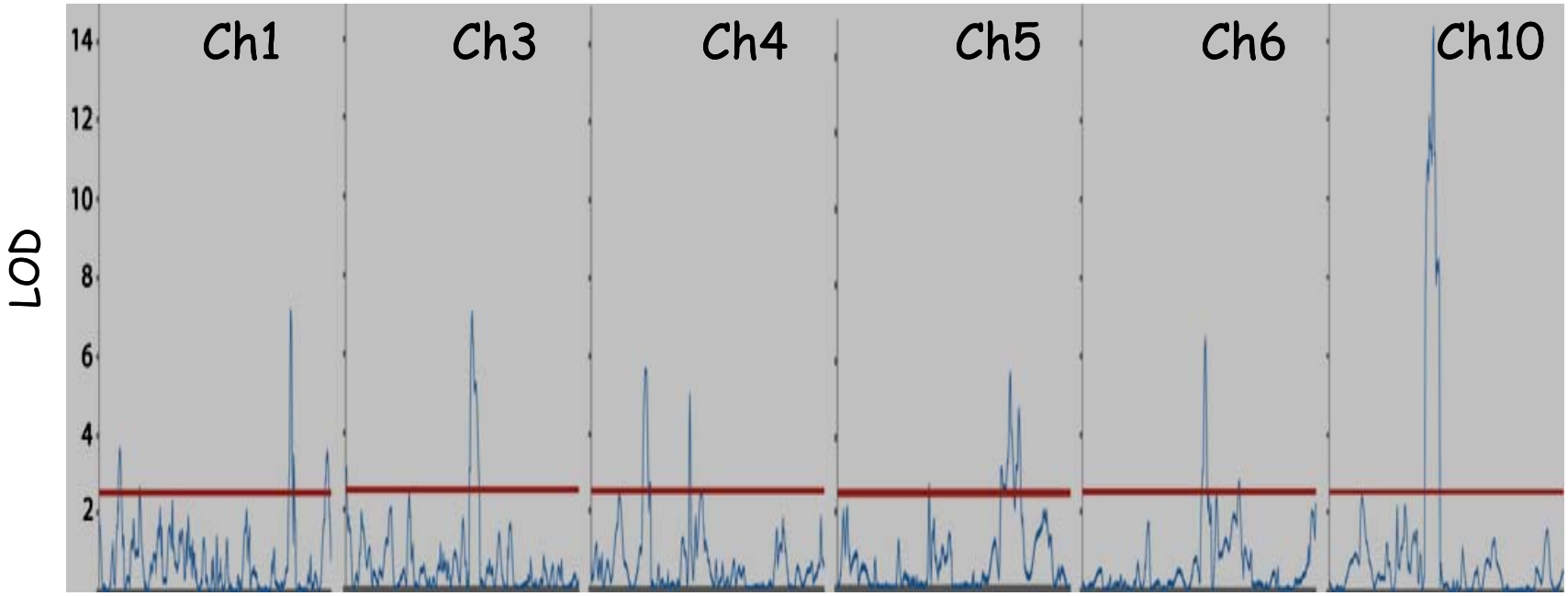
m/z	
149	(iso)eugenol [M-15]$^+$ (G)
150	4-vinylguaiacol (Ferulic Acid)(FA)
151	vanillin (G) [M-1]$^+$
152	vanillin (G)
153	2,6-dimethoxy-4-methylvinylphenol [M-15]$^+$ (S)
154	2,6-dimethoxyphenol (S)
164	*p*CA; (iso)eugenol (G)
	sinapaldehyde [M-43]$^+$ (S); 2,6-dimethoxy-4-
165	vinylphenol [M-15]$^+$ (S)
167	sinapyl alcohol [M-43]$^+$ (S); syringyl acetone (S)
168	2,6-dimethoxy-4-methylvinylphenol (S)
178	coniferaldehyde (G)
	coniferyl alcohol (G); 2,6-dimethoxy-4-vinylphenol
180	(S)
194	FA; 2,6 dimethoxypropenylphenol (S)
208	sinapaldehyde (S)
210	sinapyl alcohol (S)

Evans and Milne (1987) *Energy Fuels* **1**, 123-137
Tuskan et al. (1999) *Appl. Biochem. Biotech.* **77-79**, 55-65

Transgressive segregation gives over 2-fold differences in *p*-coumaric acid

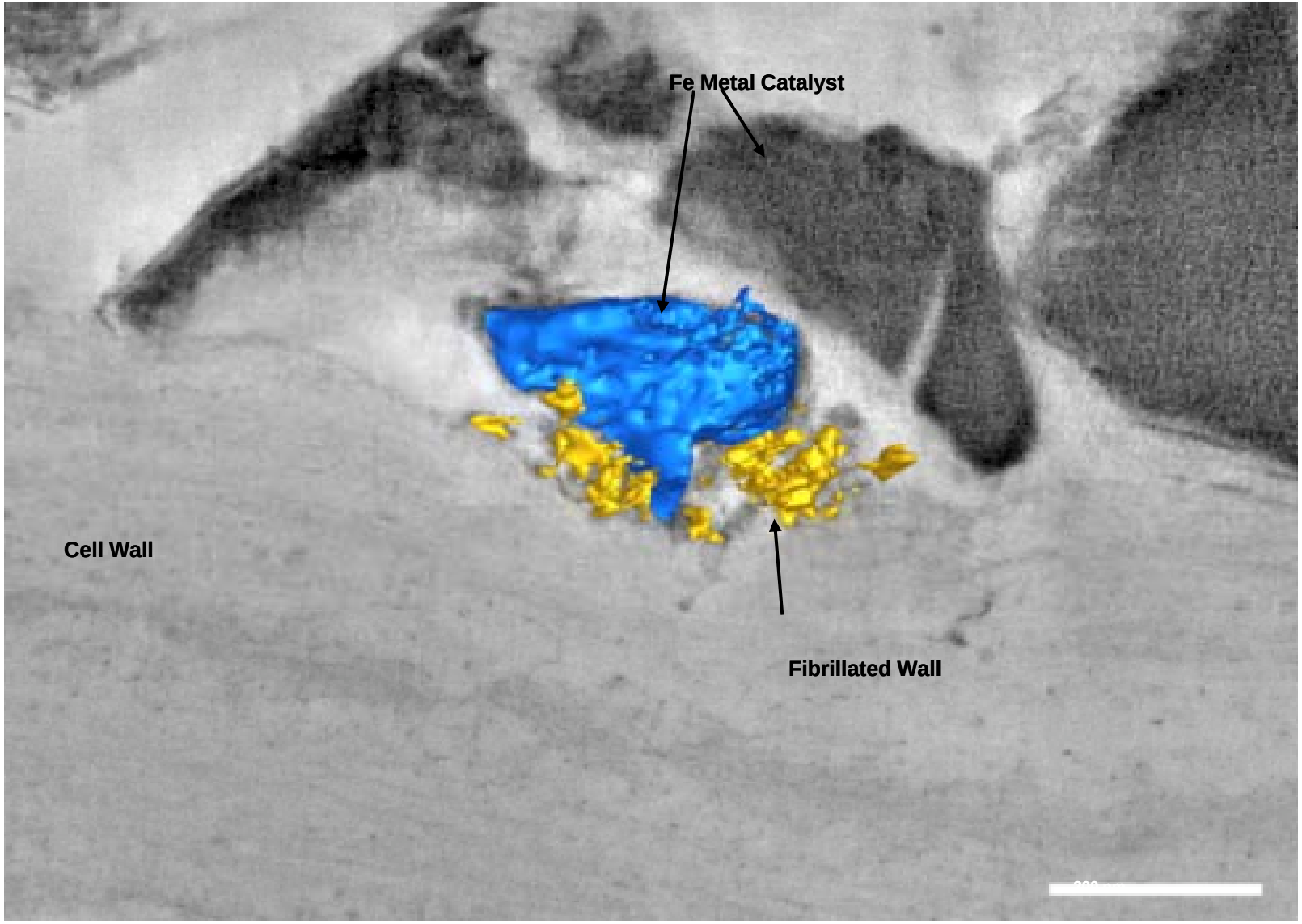


The ratio of *m/z* 120/*m/z* 114 + 126 identifies locations on chromosomes where there are genes controlling *p*-coumarate abundance

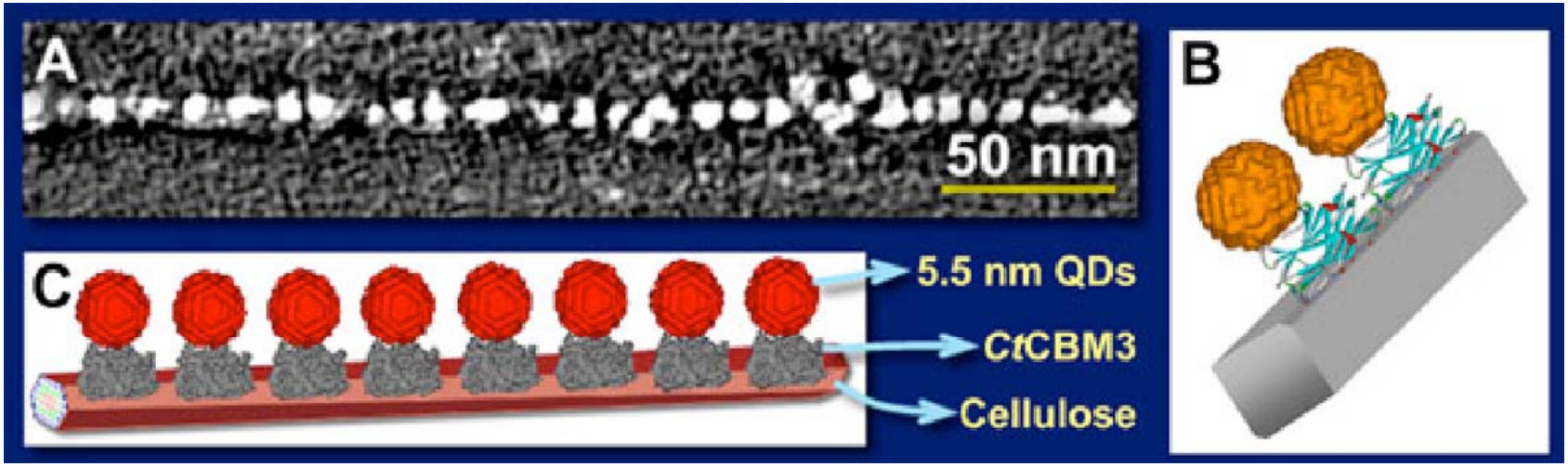


GRMZM2G104302_P01 – cytochrome P450
GRMZM2G032853_P01 – Lactate/malate or Glucose/ribitol dehydrogenase
GRMZM2G057294_P01 – Lactate/malate or Glucose/ribitol dehydrogenase
GRMZM2G090946_P01 – Plant ascorbate oxidase
GRMZM2G076936_P01 – cytochrome P450
GRMZM2G117786_P01 – Lipid binding domain with dehydrogenase active site

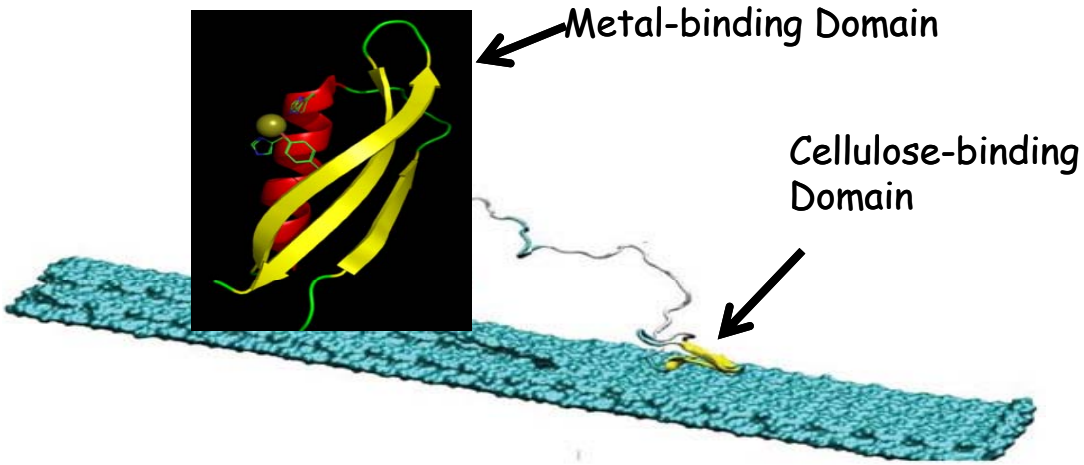
A metal catalyst contacts a very limited surface of cell wall. Can we deliver metal catalysts throughout the volume of the cell wall and target them to specific molecules?

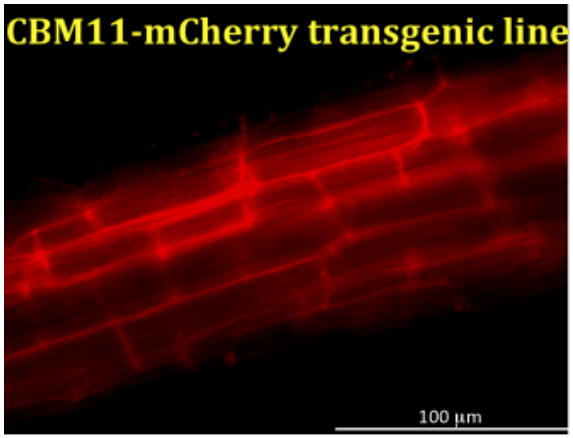


Tailored biomass – introducing catalysts and catalytic sites as plants grow



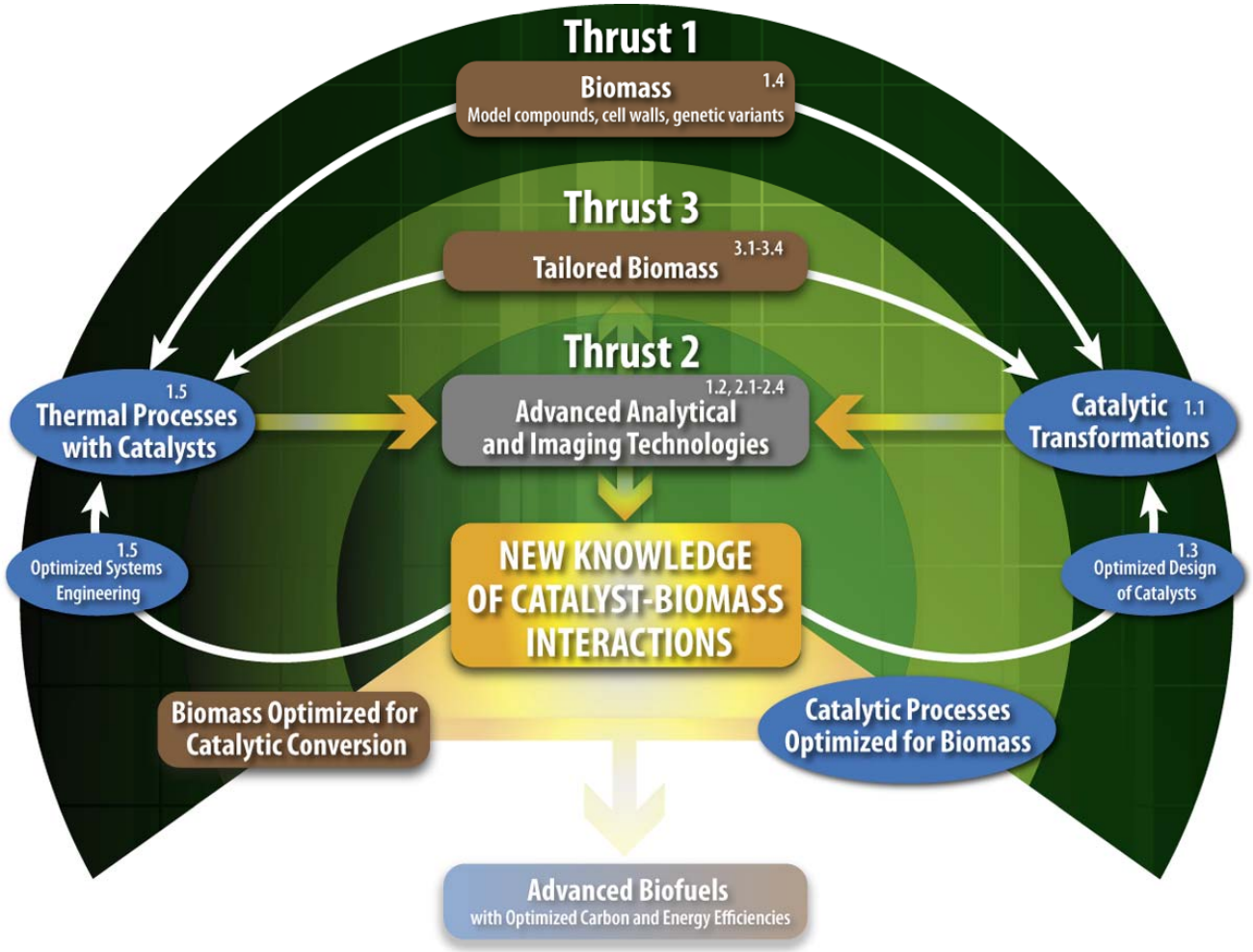
Quantum dots are specifically labeled on the planar face of crystalline cellulose directed by carbohydrate-binding module (CBM).





What can plant biologists do to help optimize conversion?

- Modulate biomass composition by mutation or by genetic engineering
- Exploit natural genetic diversity
- Build novel genetic circuits in synthetic biology approaches



Purdue University: M. McCann (Director), M. Abu-Omar (Associate Director), R. Agrawal, N. Carpita, C. Chapple, K. Clase, N. Delgass, H. Kenttämaa, N. Mosier, F. Ribeiro, G. Simpson, C. Staiger, D. Szymanski, K. Thomson

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Argonne National Laboratory: L. Makowski, J. Lal

University of Tennessee: J. Bozell, C. Barnes, A. Buchan

National Science Foundation
EFRI-HyBi Program

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