

Plant Biotechnology for Biofuels



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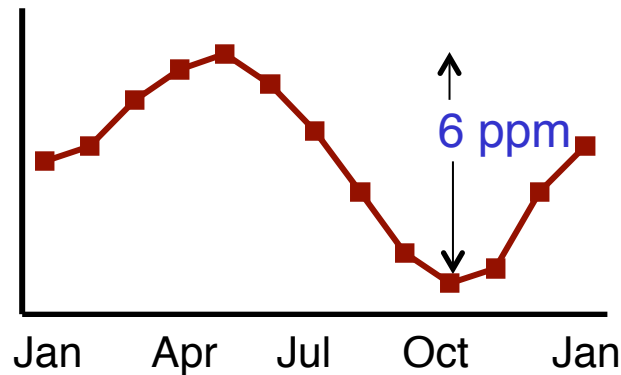
Princeton University, October 14, 2013



CO₂-concentration in the atmosphere

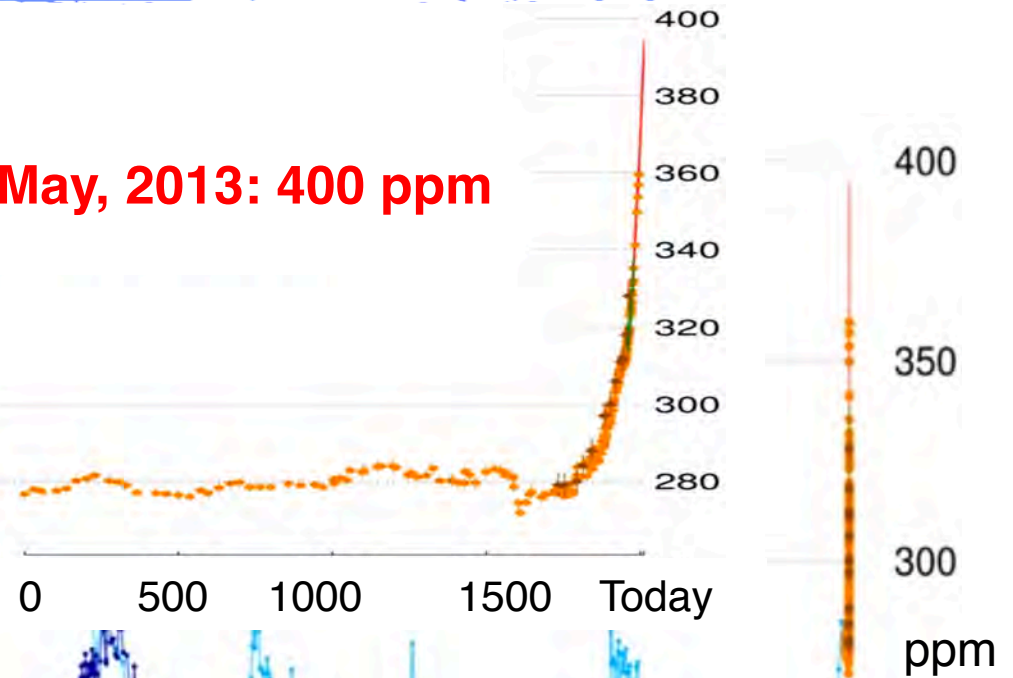
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Annual Cycle

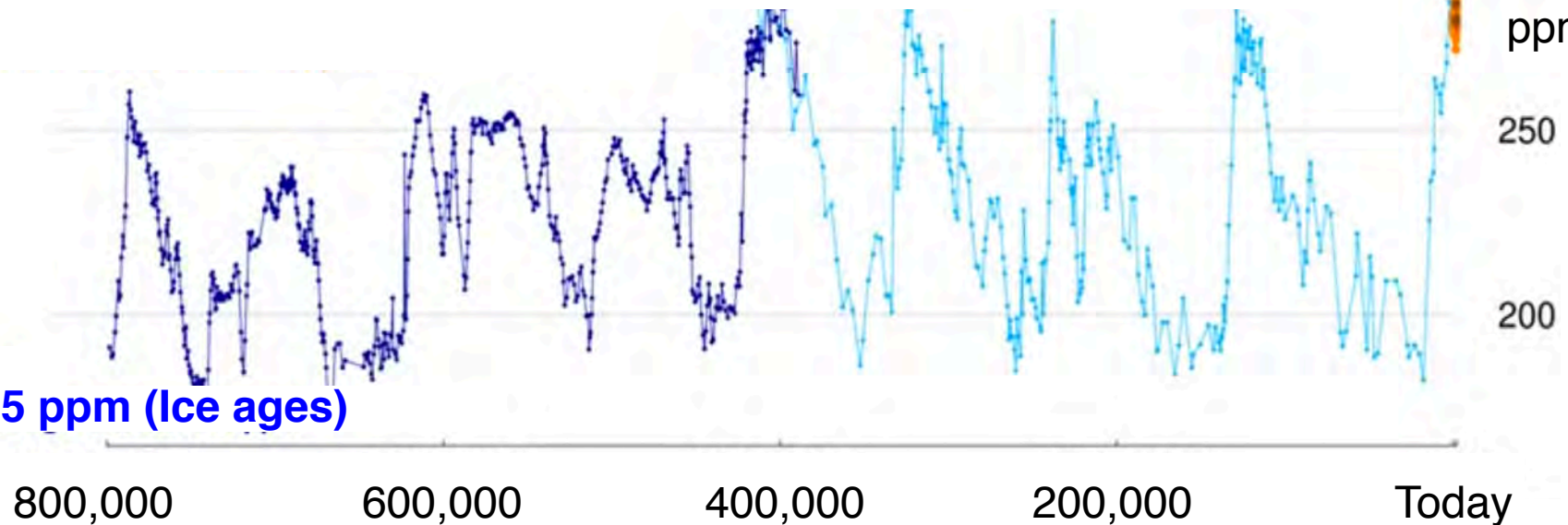


11 May, 2013: 400 ppm

280 ppm (Preindustrial)



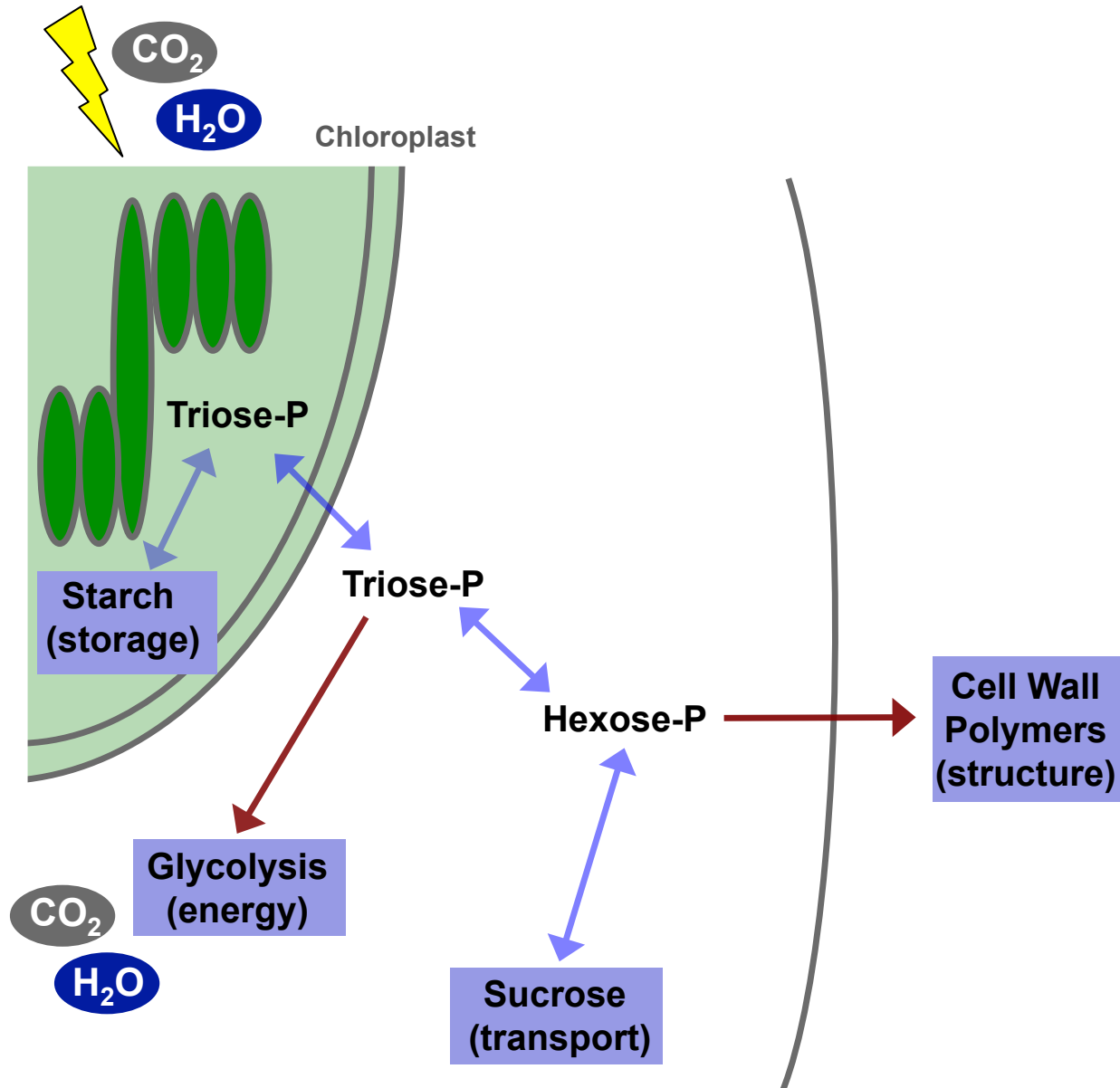
185 ppm (Ice ages)





Carbon fixation in plants

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Plant cell walls and Carbon Cycle

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Assimilated CO ₂	55 x 10⁹ t / a
Plant Biomass (land, worldwide)	170 – 200 x 10⁹ t / a
Cell walls (land, worldwide)	120 – 170 x 10⁹ t / a

Field et al., 1998; Porter and Vilar, 1997; Pauly and Keegstra 2008

Annual global production of chemicals 2010 (without pharmaceuticals)	1.2 x 10⁹ t
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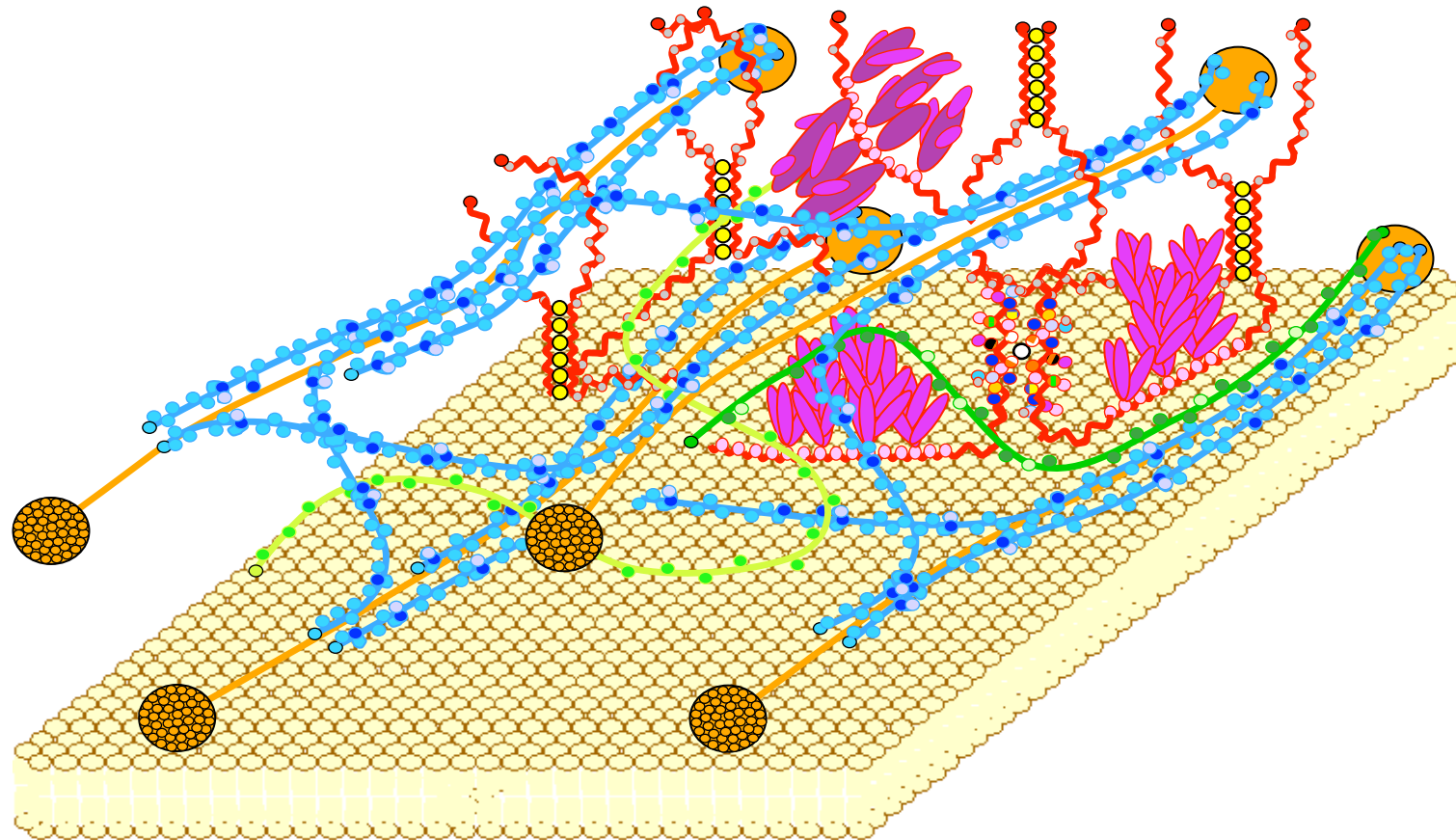
BASF report 2010 – Trends in the chemical industry

► **Cell walls represent the dominant biological carbon sequestration system**

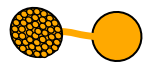


The Plant Cell Wall

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Cellulose



Hemicellulose

- xyloglucan
- galactomannan
- arabinoxylan

Homogalacturonan

- Ca²⁺-crosslinked
- non-methylesterified
- methylesterified

Pectin

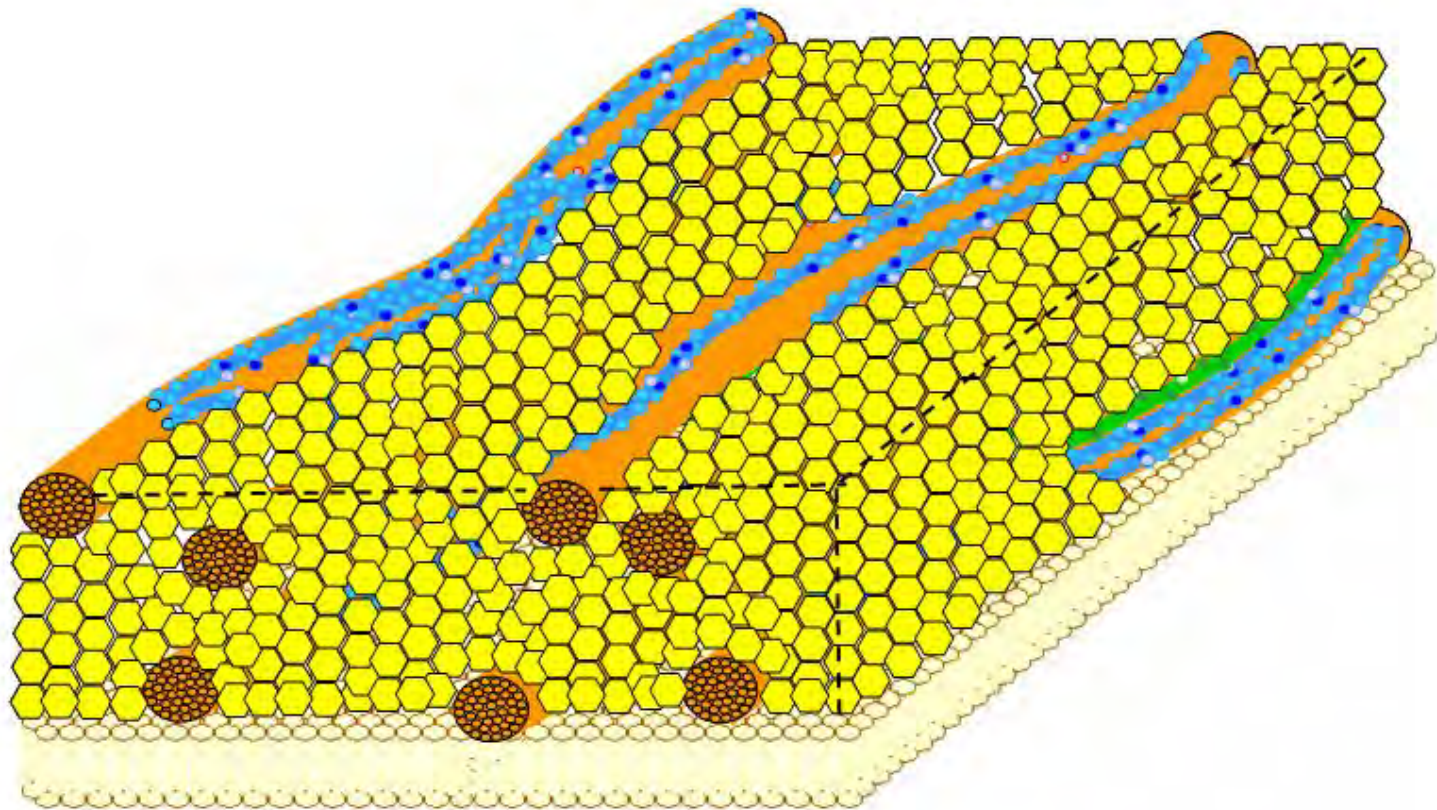
- RG I (galactan) (arabinan)
- RG II (boron-diester)

Rhamnogalacturonan

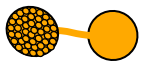


The mature Plant Cell Wall

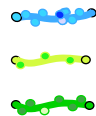
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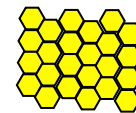
Cellulose



Hemicellulose



Polyphenols (Lignin)

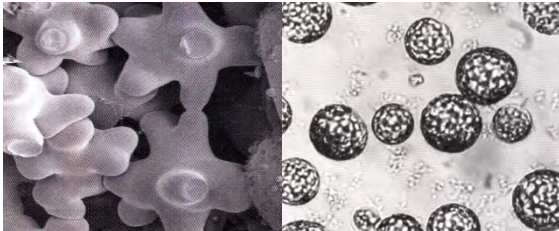




Function of plant cell walls

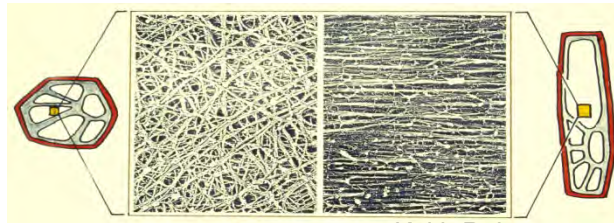
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- structural integrity of cell/ tissue/ plant
 - cell shape/ plant morphology
 - cell adhesion
 - barrier (mechanical/ path. defense)



Buchanan, 1999

- dynamic entity of the cell
 - cell expansion



Keith Roberts

- reposit for signaling molecules
 - pathogen attack

Redwoods:

up to 379 feet high, up to 2200 y old



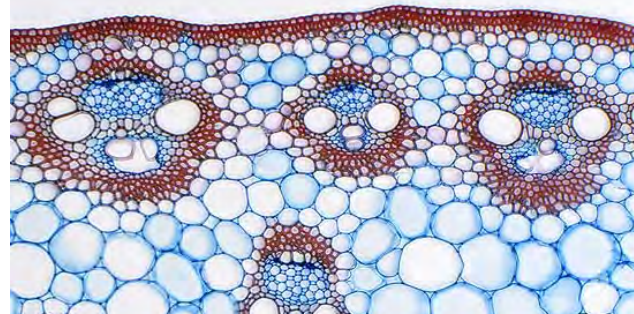


The Plant Cell Wall: Diversity

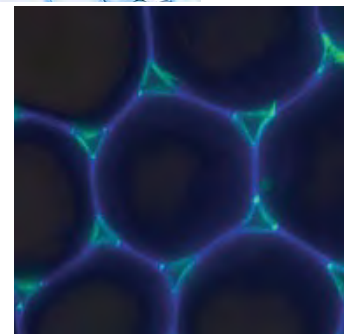
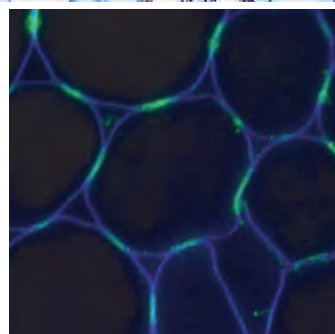
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► plant species



► plant tissue



► plant cell

Courtesy of Paul Knox, University of Leeds



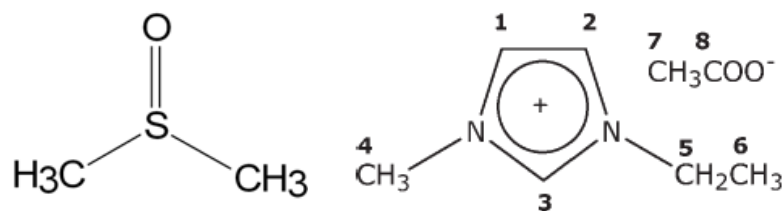
Assessment of plant cell wall structural diversity: Whole lignocellulosic 2D-NMR

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Plant tissue (Lignocellulosics) → Ball milling → Dissolution in organic solvent → 2D-NMR spectroscopy

10 mg
(dry)



DMSO-d₆/[Emim]OAc-d₁₄

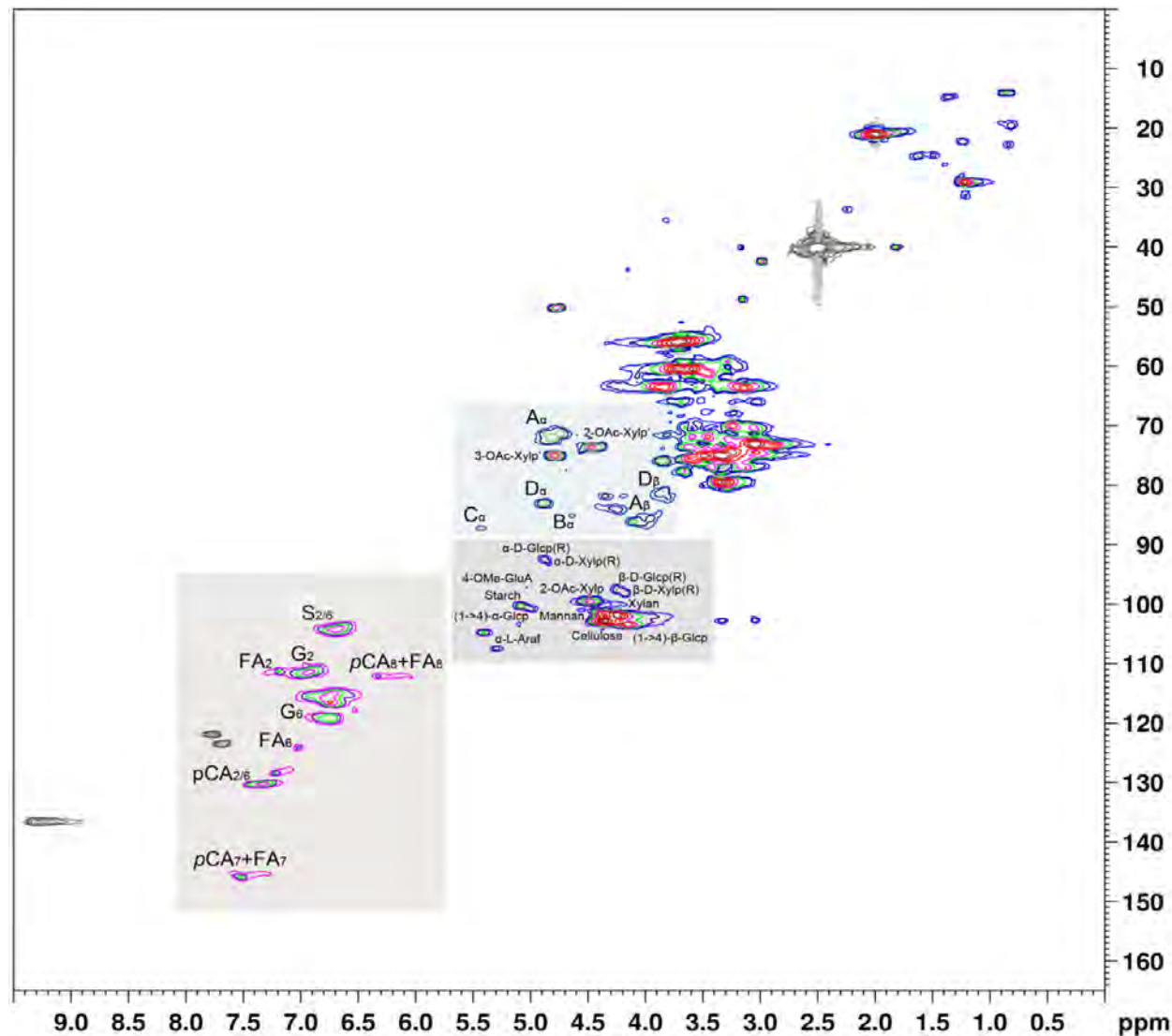
750 μl : 10 μl



Whole lignocellulosic 2D-NMR

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^1H - ^{13}C HSQC



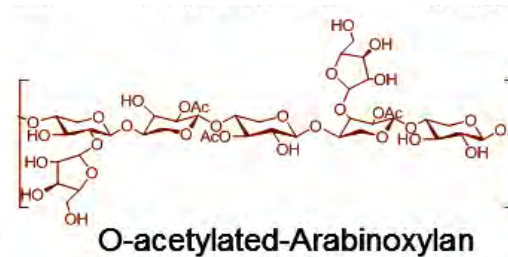
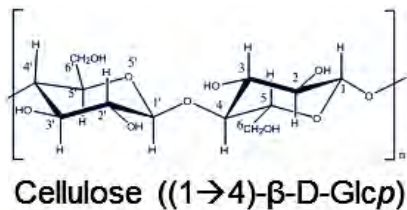
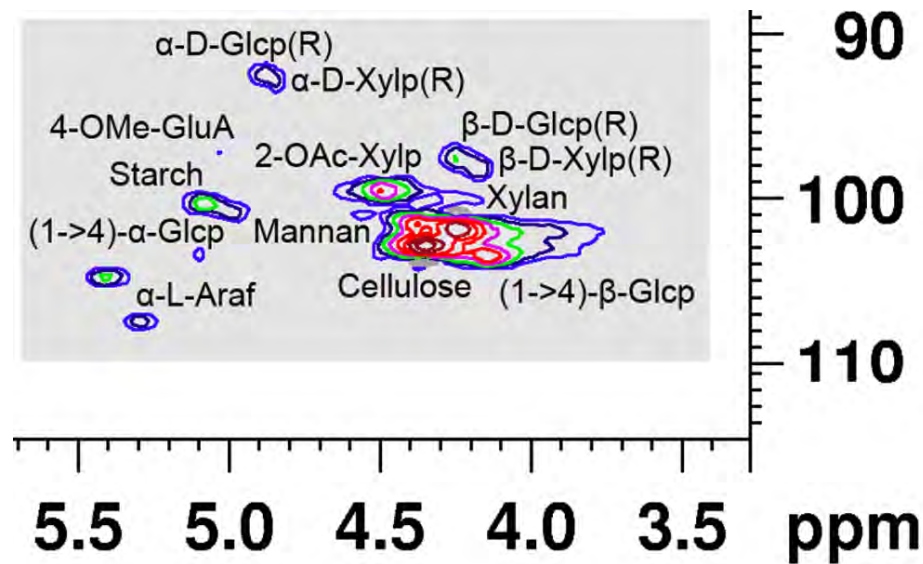
Kun Cheng



Whole lignocellulosic 2D-NMR

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Pauly

Anomeric polysaccharides

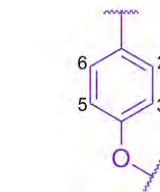
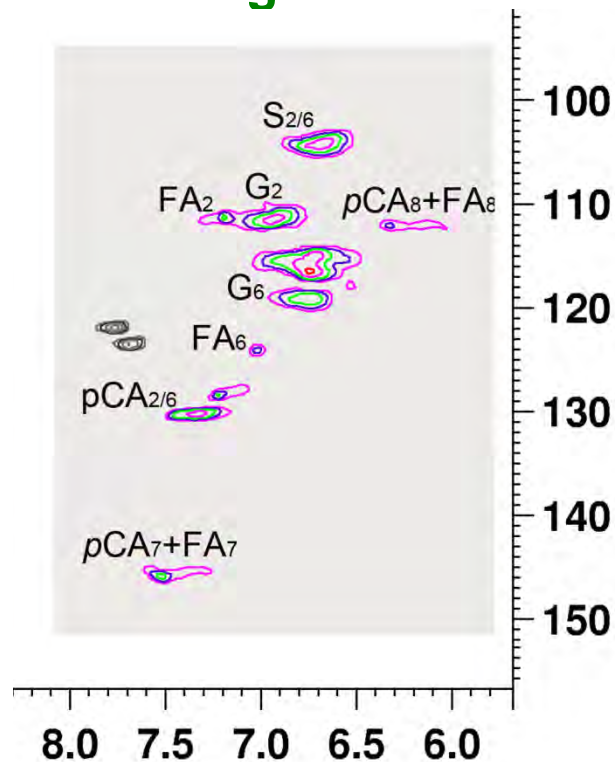




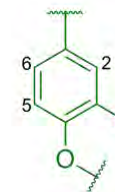
Whole lignocellulosic 2D-NMR

© Markus Pauly

Aromatic Lignin



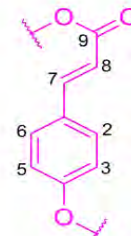
H
p-hydroxyphenyl



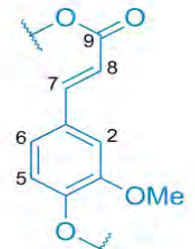
G
guaiacyl



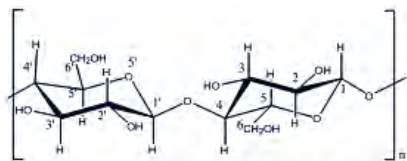
S
syringyl



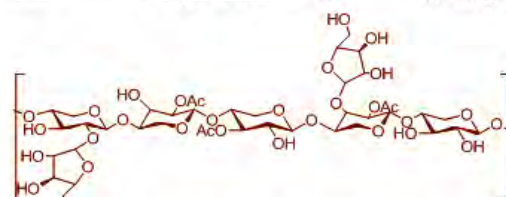
pCA
p-coumarate



FA
ferulate



Cellulose ((1→4)-β-D-Glcp)



O-acetylated-Arabinoxylan

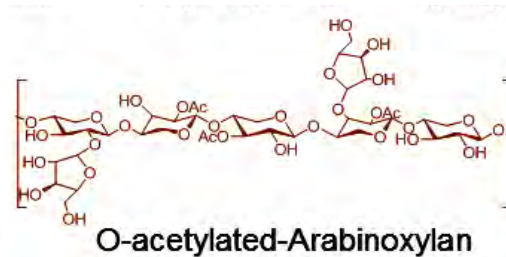
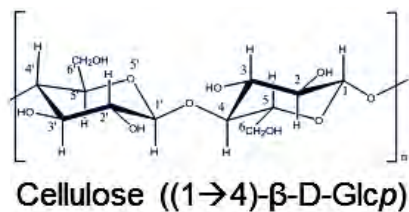
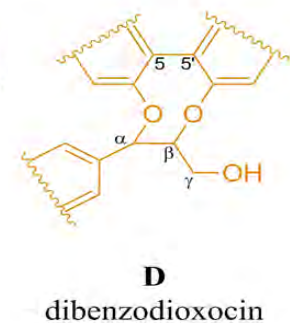
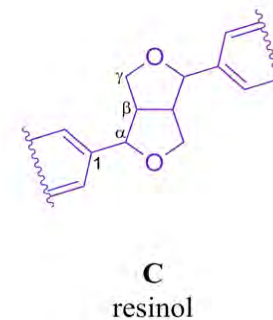
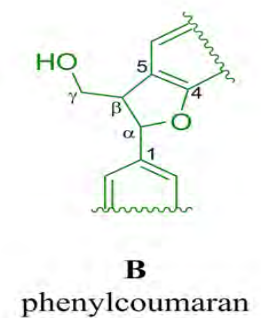
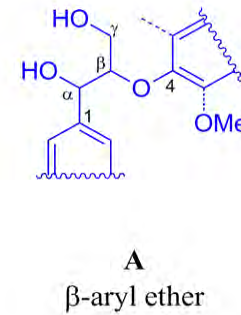
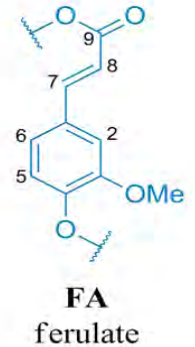
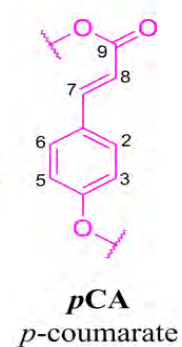
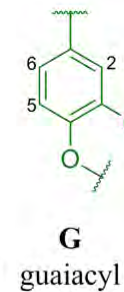
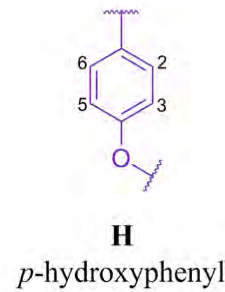
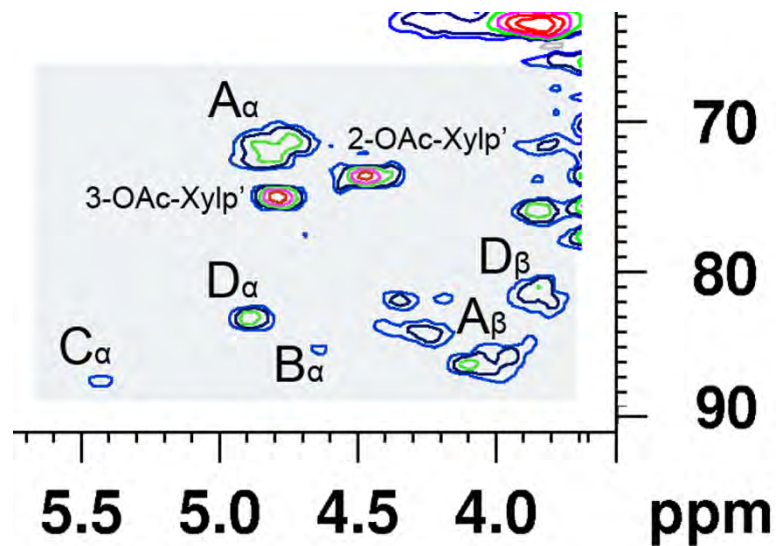




Whole lignocellulosic 2D-NMR

© Markus Pauly

Aliphatic Lignin linkages





Whole lignocellulosic 2D-NMR

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Molar percentage (Miscanthus)		
Glc	63	+/- 3
Xyl	32	+/- 1
Ara	3	+/- 0.5
Man	2	+/- 0.5
GlcA	>1	
Ac-xylan	53	+/- 2
Guaiacyl (G)	54	+/- 1
Syringyl (S)	40	+/- 1
P-hydroxyphenyl (H)	5	+/- 0.5
S/G ratio	>1	
Aryl-ether (A)	68	+/- 3
Phenylcoumaran (B)	1	
Resinol (C)	7	+/- 0.5
Dibenzodioxacin (D)	24	+/- 1



Cheng, Sorek, Zimmermann, Wemmer, Pauly (2013) Analytical chemistry



„Structural Space“ of lignocellulosics

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Plant
species

Model plant



Arabidopsis



Switchgrass



Miscanthus

Energy Grass

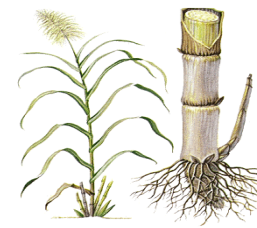
Agriculture
residues



Rice



Sorghum



Sugarcane



Maize

Forestry
resources



Pine



Maple



Willow



Poplar



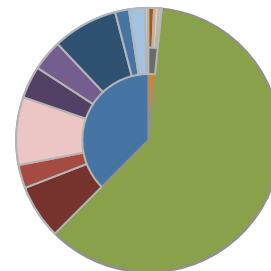
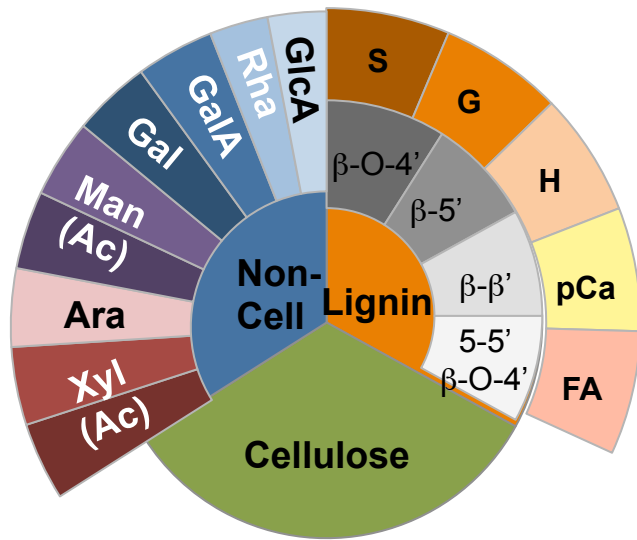
Eucalyptus



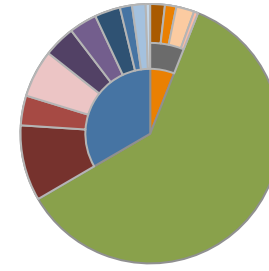


Lignocellulosic structural diversity

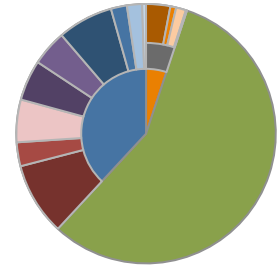
© Markus Pauly



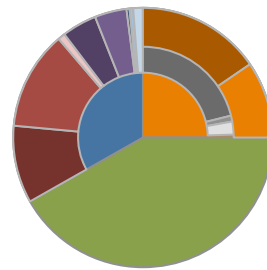
Agave americana



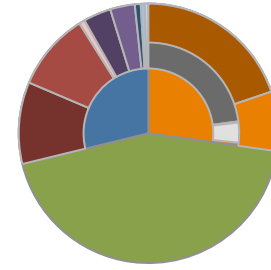
Agave fourcroydes



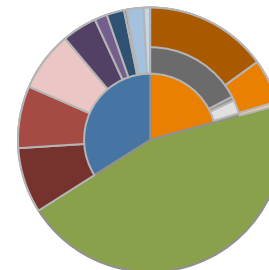
Agave tequilina



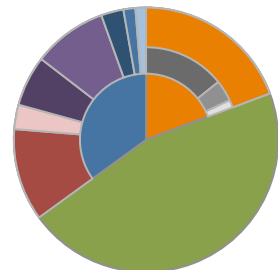
Poplar wood



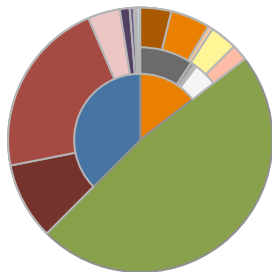
Willow wood



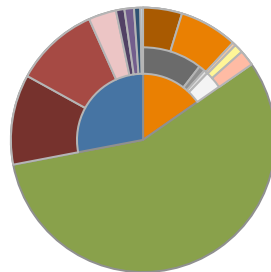
Eucalyptus wood



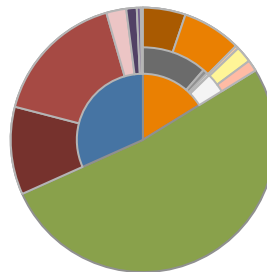
Pine wood



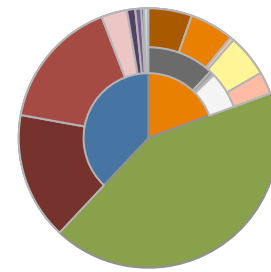
Sugarcane bagasse



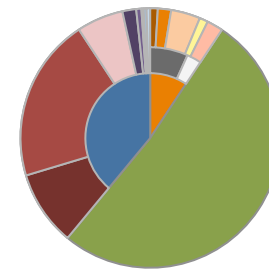
Cordgrass straw



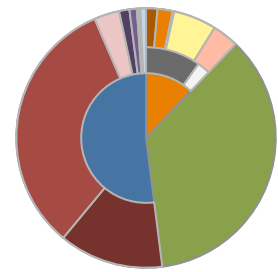
Miscanthus straw



Sorghum



Maize leaf

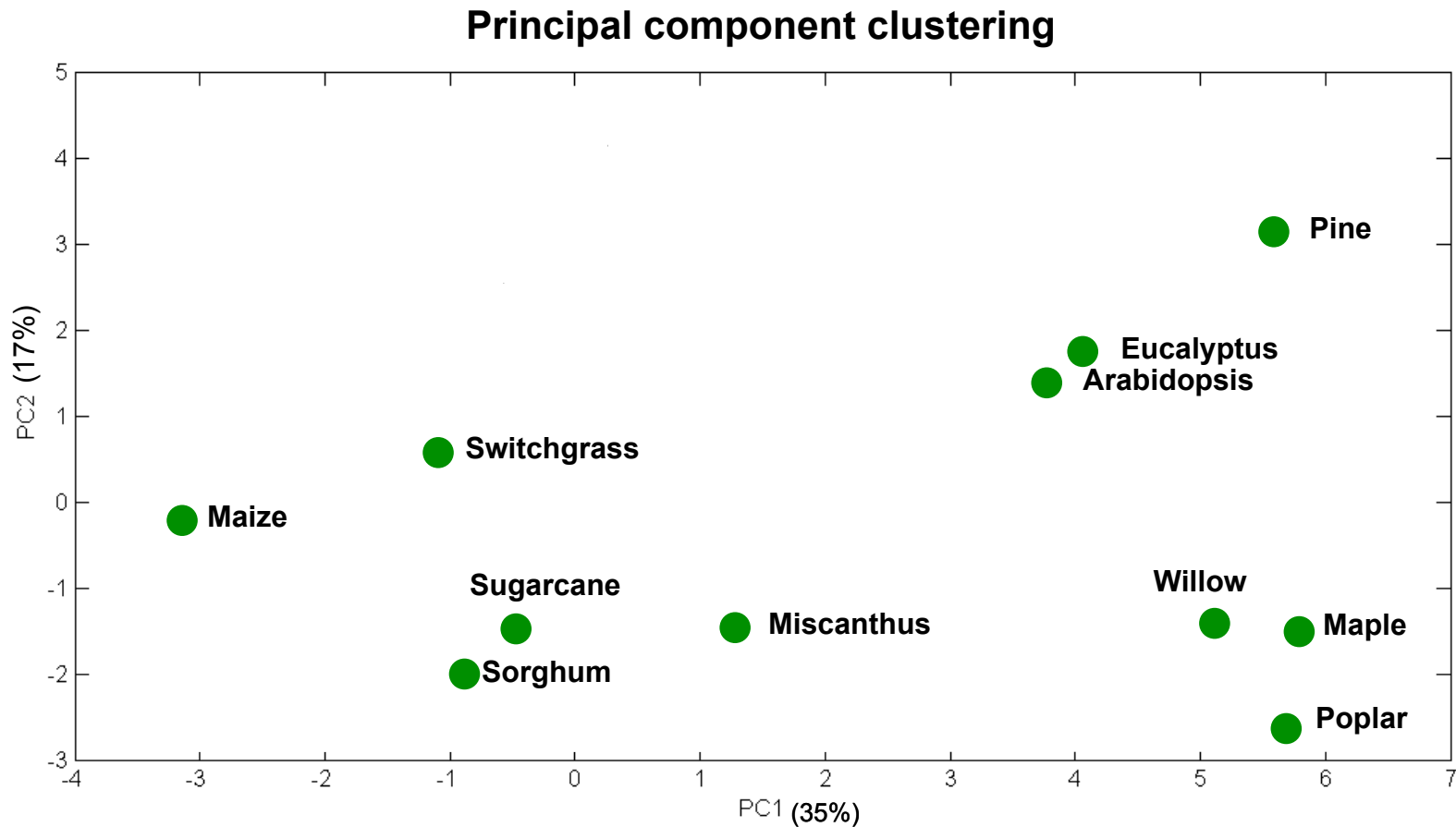


Maize cob



„Structural Space“ of lignocellulosics

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Plant
species

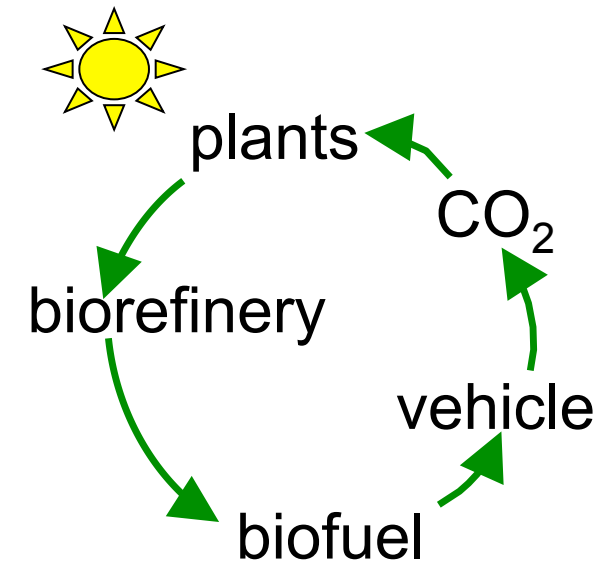
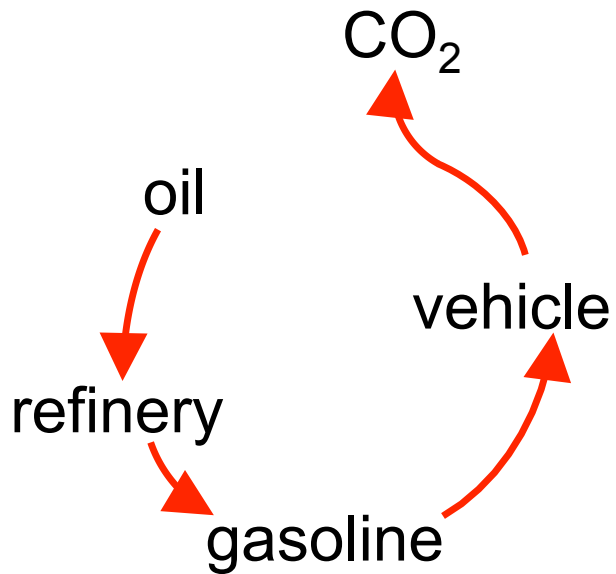
► Grasses ≠ Wood (Hardwood ≠ Softwood)





Alternative to fossil fuels: Plant biomass

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- ▶ **Plants capture energy from the sun**
- ▶ **Plants convert energy into durable chemicals**
- ▶ **Plants sequester CO₂ from the atmosphere**



Lignocellulosics as renewable resource

© Markus
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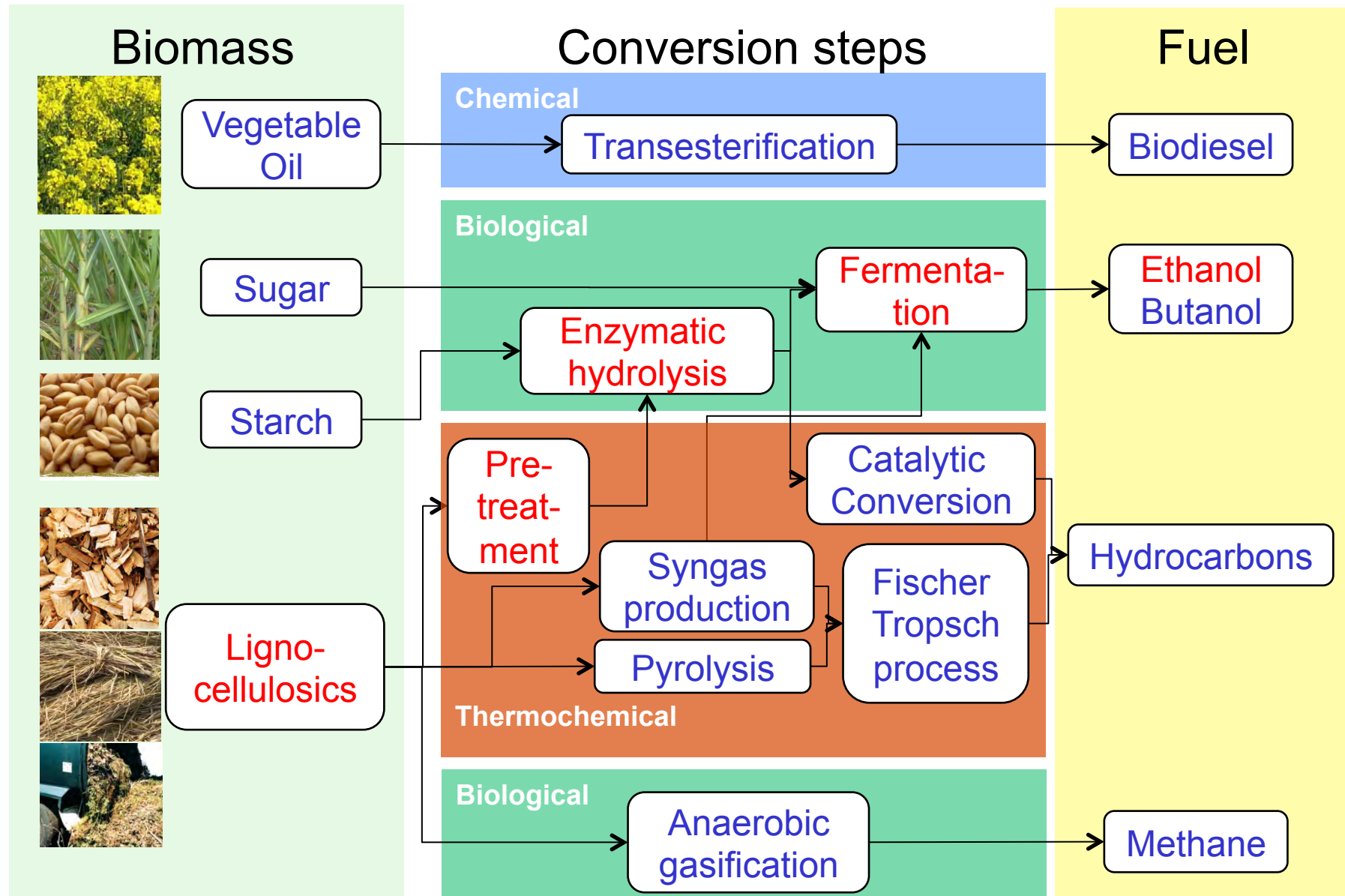


- ▶ Cell walls (lignocellulosics) are non-food components
- ▶ Lignocellulosics are highly abundant
 - Dominant biological carbon sequestration system
- ▶ The conversion of lignocellulosics is energy and cost-intensive



Biomass to Biofuels

© Markus Pauly





Challenges: Plant biology – 2nd Gen Biofuels

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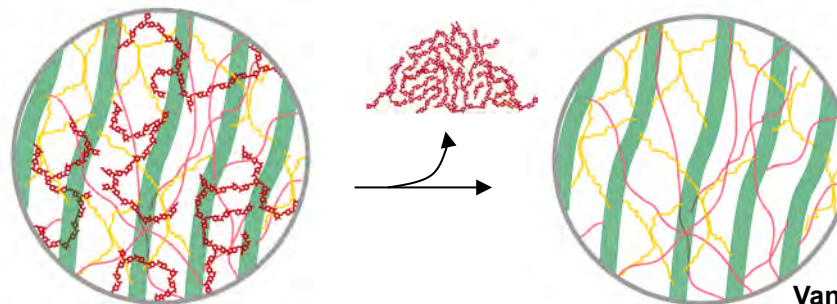
► Increase plant biomass/ ha

- Choice of crops
- Increase resource efficiency (water, fertilizer)
- Delayed flowering (energy maize: www.kws.com)



► Increase the yield of fermentable sugars

- Create a more open wall architecture (reduce, alter lignin)
- Reduce process inhibitors
- Increase cellulose (amorphous), hemicelluloses (hexoses)



Van Holme, 2007

Acknowledgements

Pauly lab



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Amancio Souza
Nasim Mansoori
Alex Schultink
Julia Schulbert
Dan Naylor

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Patrick Brown,
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ISU transformation
facility