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## Background

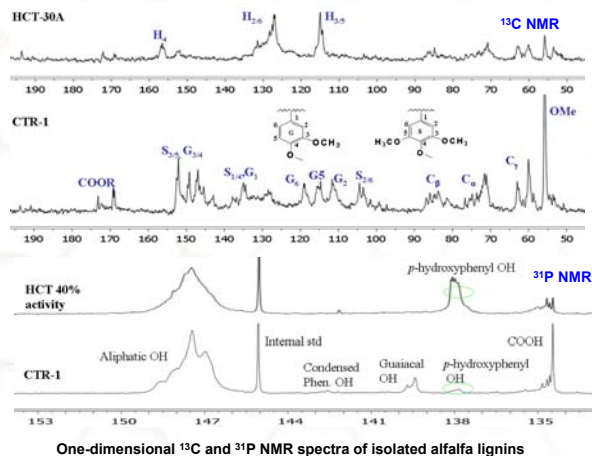
- Sustainable supplies of renewable, carbon-neutral energy need viable, cost-saving biological energy production from plant biomass.
- Processing improvements are the best option to lower the high estimated cost of biofuels from lignocellulosics.
- BESC aims to develop improved plant materials with low recalcitrance and consolidated bioprocessing methods that facilitate cost-effective conversion of biomass to fermentable sugars.
- Analytical characterization can provide detailed knowledge of:
  - The physical and chemical properties of biomass contributing to biomass recalcitrance.
  - Fundamental understanding of the relationship between plant polysaccharides, lignin and how these biopolymers are integrated in the plant cell wall.
  - How biomass properties change during pretreatment and how such changes affect biomass deconstruction by enzyme/microorganisms

## Lignin characterization through NMR spectroscopy

- Baseline and transgenic alfalfa: C3H and HCT gene down-regulation
- Ball-mill lignin isolation
- One-dimensional <sup>1</sup>H and <sup>13</sup>C NMR
- One-dimensional <sup>31</sup>P NMR
- Two-dimensional (<sup>13</sup>C-<sup>1</sup>H) heteronuclear correlation spectra

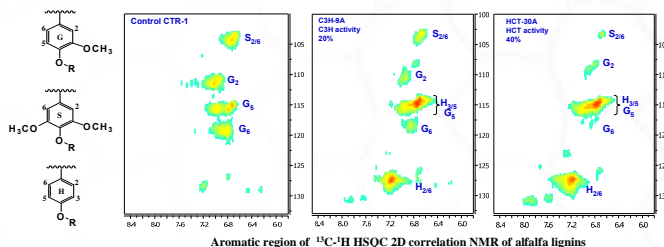


## One-dimensional NMR analysis

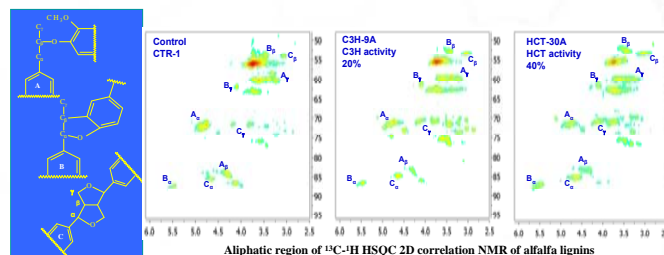


One-dimensional <sup>13</sup>C and <sup>31</sup>P NMR spectra of isolated alfalfa lignins

## <sup>13</sup>C-<sup>1</sup>H HSQC 2D correlation NMR



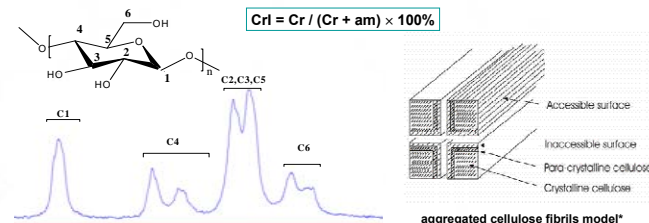
Aromatic region of <sup>13</sup>C-<sup>1</sup>H HSQC 2D correlation NMR of alfalfa lignins



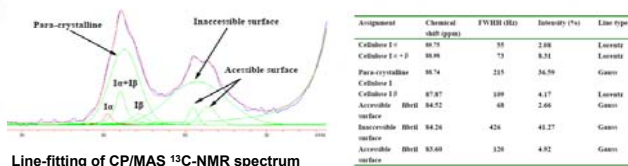
Aliphatic region of <sup>13</sup>C-<sup>1</sup>H HSQC 2D correlation NMR of alfalfa lignins

## Solid-state CPMAS <sup>13</sup>C NMR analysis

- The alfalfa samples were first holopulped to removed lignin
- Holopulp samples were then treated with 2.5 M HCl to remove hemicelluloses.
- Samples were packed and spun at 8 kHz for CPMAS NMR



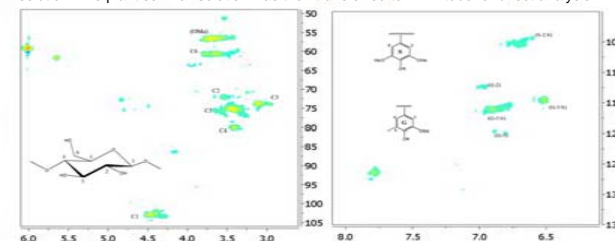
CPMAS <sup>13</sup>C NMR spectrum of alfalfa



Line-fitting of CP/MAS <sup>13</sup>C-NMR spectrum

## Perdeuterated ionic liquid for direct NMR analysis of plant cell walls

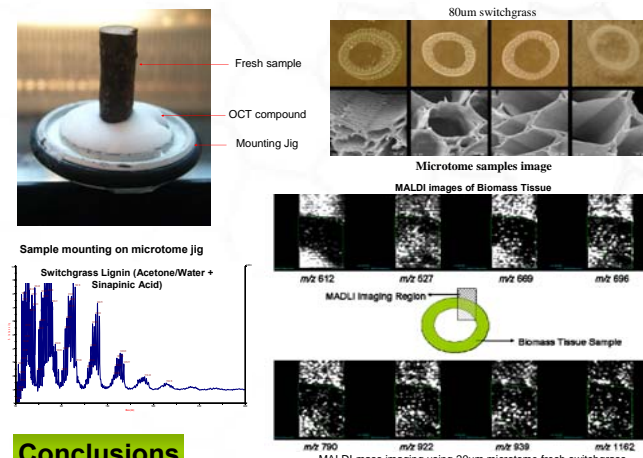
- Plant cell wall samples were added into perdeuterated pyridinium ionic liquid-dx /DMSO-d6 solution. The mixture was stirred vigorously at 70 °C for 1-4 h to form a homogeneous solution. The plant cell wall solution was then transferred to NMR tube for direct analysis.



2D HSQC of poplar in 1:2 [Cmpy]Cl-d7/DMSO-d6

## Microtome sample: MALDI-mass image analysis

- Spatial analysis of biomass chemical constituents across the plant cell wall for native and deconstructed biomass.
- The fresh samples are attached on the mounting head and sectioned into 20-80 um slices in a cryostat (-20°C)
- Extractive free, lignin free and hemicellulose free samples are prepared
- Microtome samples are analyzed using MALDI-mass image analysis.



## Conclusions

- Transgenic alfalfa showed significant changes of lignin structure as revealed by NMR
- Reduced recalcitrance appeared to be not related to the crystallinity of cellulose
- Ionic liquids provided great potential for better NMR characterization of nonderivatized plant cell wall structures

## Acknowledgements

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