C3Bio develops transformational knowledge and technologies for the direct conversion of plant lignocellulosic biomass to advanced (drop-in) biofuels and other biobased products, currently derived from oil, by the use of new chemical catalysts and thermal treatments.

RESEARCH PLAN AND DIRECTIONS

We will maximize the energy and carbon efficiencies of advanced biofuels production by the design of both thermal and chemical conversion processes and the biomass itself. Impacts are to more than double the carbon captured into fuel molecules and expand the product range to alkanes and other energy-rich fuels.
Recent C3Bio Achievements

**Achievements**

**Duke Energy Academy**
- Plants and BioEnergy

**Spero Energy**
- Nature Publication

**Purdue Ag TEAM Award**
- Natural Gas & Biomass
  - Energy Roadmap for light – duty vehicles
  - Duke Energy Academy at Purdue
  - 2-week summer workshop for high school juniors, seniors and teachers
  - Plants and BioEnergy, Advances in Plant Biology, Vol. 4,
    - McCann, Buckeridge & Carpita (eds)
    - 3 chapters by C3Bio authors
  - Nature Publication
    - Bonawitz et al.
  - Spero Energy
    - $150K DOE Phase I SBIR grant,
    - $50K Purdue Trask Innovation Fund
    - $10K Clean Energy Student Challenge (IN)
    - $50K Regional Aviation Award (April)
  - College of Agriculture interdisciplinary award for research, teaching and outreach
  - $10K award allocated to Early Career Scientist travel grants

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Spero Energy, A C3Bio Startup Company

Scientific Achievement
Development of an integrated catalytic technology for concurrent delignification of hardwoods and selective conversion of lignin to high-value chemicals (HVCs) in a single step

Significance and Impact
- Lignin accounts for 20-30% of lignocellulosic biomass, and the only aromatic biorenewable
- Cost effective technologies for lignin conversion to methoxypropylphenols, high-value fragrance and flavor compounds
- Current methoxypropylphenols annual production volume of > 30 MM lbs and market value of $450 MM.
- Methoxypropylphenols are manufactured from petroleum feedstock and toxic chemicals via a multi-step process

Business Details
- Competitive edge identified
- Minimized pretreatment cost
- Renewable feedstock for chemicals
- Identified customer segments and lead customers
- Exploring initial R&D investment
- Awarded Phase I SBIR from DOE

Work was performed at Purdue University
Maintaining high yield in lignin-modified bioenergy plants

Scientific Achievement

We found that the dwarf phenotype of a lignin-deficient Arabidopsis mutant is dependent on the transcriptional coregulatory complex Mediator.

Significance and Impact

Cell walls of rescued med5a/b ref8 plants instead contain a novel lignin consisting almost exclusively of \( p \)-hydroxyphenyl lignin subunits, and exhibit substantially facilitated polysaccharide saccharification.

Research Details

- Guaiacyl and syringyl lignin subunits are largely dispensable for normal growth and development
- Mediator is involved in an active transcriptional process responsible for dwarfing and inhibition of lignin biosynthesis
- Signaling pathways responding to cell wall defects may be targets to include in efforts to reduce biomass recalcitrance.

Molecular mechanisms of cellulose deconstruction

Scientific Achievement
Multiple modes of imaging were used to study the behavior of maize during dilute acid and dilute acid plus iron pre-treatments.

Significance and Impact
- Iron permeated to the level of individual fibrils.
- Cellulose fibrils that were shattered or fragmented became highly susceptible to enzymatic degradation.
- Those that remained intact maintained significant recalcitrance.

Research Details
- Structural studies were carried out on milled, dried maize stover, untreated and after steam explosion pretreatment with:
  - hot water; dilute H2SO4; dilute acid plus 2 mM Fe2(SO4)3 or hot water plus 2 mM Fe2(SO4)3.

Inouye, H; Zhang, Y; Yang, L; Venugopalan, N; Fischetti, RF; Gleber, SC; Vogt, S; Fowle, W; Makowski, B; Tucker, M; Ciesielski, P; Donohoe, B; Matthews, J; Himmel, ME; and Makowski, L. MULTISCALE DECONSTRUCTION OF MOLECULAR ARCHITECTURE IN CORN STOVER, Scientific Reports 4, 3756 (2014). [10.1038/srep03756]

Work was performed at Northeastern University, NREL, ANL APS and Brookhaven NSLS.

Inouye, H; Zhang, Y; Yang, L; Venugopalan, N; Fischetti, RF; Gleber, SC; Vogt, S; Fowle, W; Makowski, B; Tucker, M; Ciesielski, P; Donohoe, B; Matthews, J; Himmel, ME; and Makowski, L. MULTISCALE DECONSTRUCTION OF MOLECULAR ARCHITECTURE IN CORN STOVER, Scientific Reports 4, 3756 (2014). [10.1038/srep03756]

Work was performed at Northeastern University, NREL, ANL APS and Brookhaven NSLS.
The Structure of the Catalytic Domain of a Plant Cellulose Synthase and Its Assembly into Dimers

**Scientific Achievement**
The catalytic domains of plant cellulose synthases (CesAs) dimerize in vitro, and may form the scaffolding units of construction of large synthase complexes.

**Significance and Impact**
In contrast to structure modeling predictions, solution x-ray scattering studies demonstrate that recombinant CesA proteins form a two-domain, elongated structure, with the smaller domain coupling two monomers into a dimer. The arrangement of the catalytic domain within the CesA monomer and dimer provides a foundation for constructing structural models of the synthase complex and defining the relationship between the rosette structure and the cellulose microfibrils they synthesize.

**Research Details**
The 57 kDa catalytic domain of a rice CesA dimerizes when DTT is removed or when the protein is concentrated. Docking experiments showed that this domain, homologous to bacterial cellulose synthase, can only occupy the central region of the solution structure, with plant-specific sequences that are not present in bacterial synthases flanking it. The best fit model places the Class-Specific Region (CSR) in the small domain involved in dimerization. Dimerization of CesA proteins provides a simple hypothesis for how their respective Zn-finger domains in the N-terminus may couple dimers into the larger complex.


Work was performed at Purdue University, the Brookhaven National Lab, and NREL
**Scientific Achievement**

Furions are synthesized from biomass-derived furfural and 2-methylfurfural in high yields in green solvents using N-heterocyclic carbene organocatalysts. The resulting furion molecules are hydrodeoxygenated over heterogeneous Pd/Zeolite-β catalyst to C10-C12 hydrocarbons in high yields (76%).

**Significance and Impact**

- Use of organocatalysis in 100% atom-economic reaction in green solvents.
- Building C10, C11, and C12 molecules from biomass-derived dehydration products.
- Selective HDO with high selectivity for C-O over cracking, no C-C cleavage.
- Producing drop in hydrocarbons C10-C12.

**Research Details**

- Investigated more than 12 organocatalysts and multiple solvent systems.
- Pd/C or Ru/C produces oxygenated mixtures instead of complete HDO.
- Combination of HDO metal on acidic support results in ring opening followed by HDO.

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Work was performed at Purdue University
Oxygen removal from intact biomass to produce liquid fuel range hydrocarbons via fast-hydropyrolysis and vapor-phase catalytic hydrodeoxygenation

**Scientific Achievement**
Production of hydrocarbons directly from biomass and cellulose using fast-hydropyrolysis (FHP) combined with downstream vapor phase catalytic hydrodeoxygenation (HDO)

**Significance and Impact**
- Carbon yield of 54% C$_1$-C$_{8+}$ Hydrocarbons from Intact Poplar Biomass and yield of 73% C$_1$-C$_{8+}$ from cellulose
- Lowering temperature between fast-hydropyrolysis and catalytic HDO improves carbon recovery
  - This has implications for approaches that utilize catalytic pyrolysis
- Development of a novel, selective bimetallic Pt-Mo catalyst for complete removal of oxygen from the entire product slate of FHP reactor
- A simple two step process for the conversion of the entire biomass species

**Research Details**
Initial FHP + HDO were performed at 27 bar total pressure using 25 bar P$_{H_2}$ and balance N$_2$. Research in progress should lead to lower char formation. Furthermore, char combustion and/or gasification can be used synergistically to improve overall process carbon and energy efficiency beyond the results of the lab-scale reactor reported here.

Vinod Kumar Venkatakishnan, W. Nicholas Delgass, Fabio H. Ribeiro and Rakesh Agrawal. Oxygen removal from intact biomass to produce liquid fuel range hydrocarbons via fast-hydropyrolysis and vapor-phase catalytic hydrodeoxygenation Green Chem., 2015, DOI: 10.1039/C4GC01746C  *Work was performed at Purdue University*