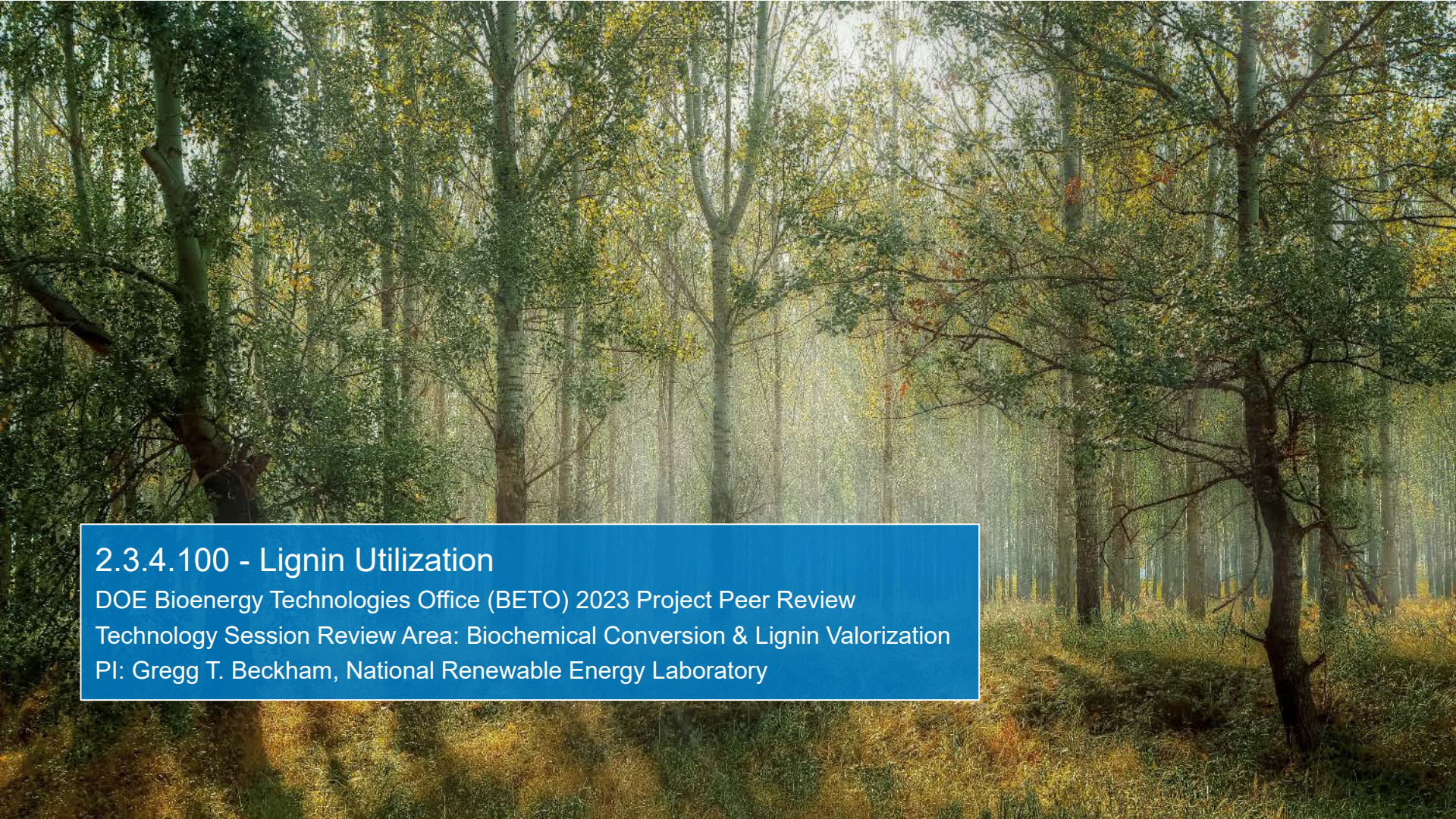


# 2.3.4.100 - Lignin Utilization

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

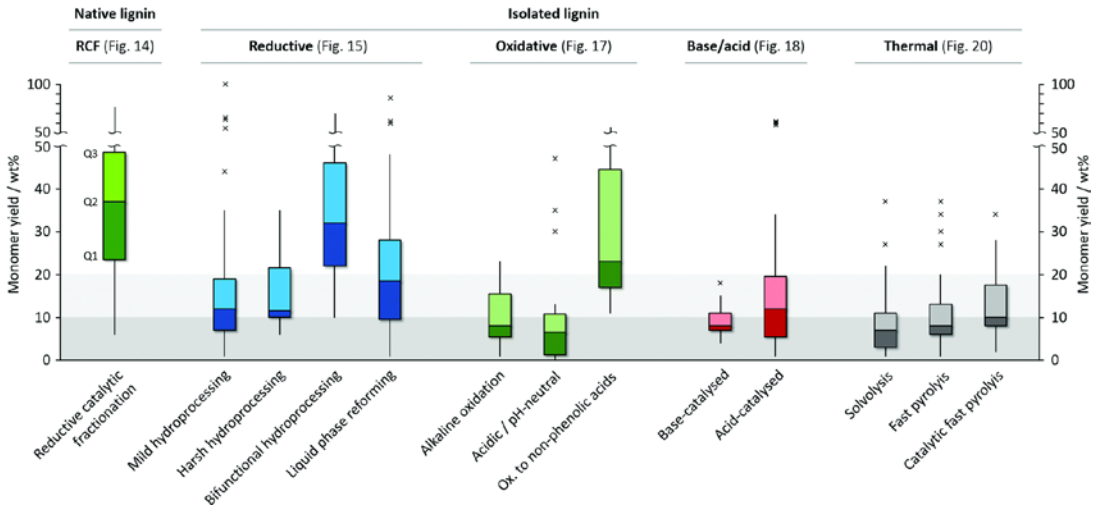
Technology Session Review Area: Biochemical Conversion & Lignin Valorization

PI: Gregg T. Beckham, National Renewable Energy Laboratory

# Project overview

**Goal**: Develop industrially-relevant, feedstock-agnostic catalytic processes to generate aromatic monomers from lignin
(and tools to characterize lignin)

- Project can enable **deconstruction-funneling paradigm**
- Deconstruction catalysis for **C–C bonds** in lignin – exceed monomer yield from C–O bond cleavage (~30-40%)
- Develop processes to provide high yields of aromatics to Biological Lignin Valorization (BLV) and Performance-Advantaged Bioproducts (PABP)
- Develop new lignin analytics and conduct model compound syntheses (down-scoped in FY23)




Schutyser *et al. Chem. Soc. Rev.* 2018

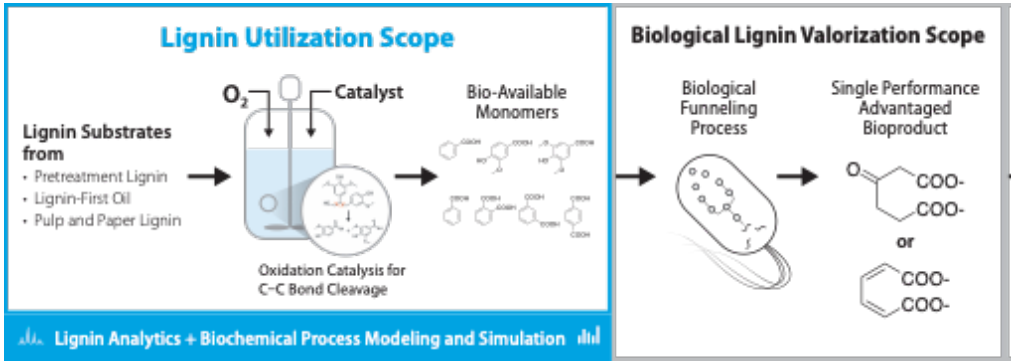
# Technical approach

## Lignin depolymerization

- Aerobic oxidation to make bio-available aromatics
- Leverage industrial oxidation processes
- Complementary to reductive methods (LigFirst/LigSAF)
- TEA and LCA with Biochem. Analysis project
- **Substrates**: hydrolysis lignin, technical lignins, RCF oil, HDO lignin
- Comprehensive **analytics** suite to characterize lignin in polymer/oligomer/monomer forms
- Work with SepCon for advanced separations
- Work with BLV and PABP to make products
- Collaborate with Shannon Stahl, UW Madison

## Lignin analytics and model compound syntheses

- LC/MS-MS; work with Biochem. Process Modeling & Simulation for data analysis, posted on GitHub
- Bespoke model compound syntheses posted on [www.protocols.io](http://www.protocols.io)



# Risks, management, and milestones

## Risks:

- **Risk**: Stabilization chemistry is too expensive
- **Mitigation**: Examine routes that use acetic acid for stabilization, alternative C–C bond cleavage routes
- **Risk**: Supply chain issues delay reactor systems
- **Mitigation**: Collaborate with groups with existing flow-based oxidation setups (UW Madison)

## Management, Communication, & DEI:

- Monthly project meetings, *ad hoc* meetings with LigFirst/BLV, open to all
- Use Dropbox for shared files
- Dedicated Project Manager
- Funding for early career staff to present and network
- Methods are open-source (protocols.io, GitHub, publications)
- Focused on physically and psychologically safe R&D environments

## Milestones:

- **FY23**: >50% yield of bio-available aromatics
- **FY24**: >80% yield of bio-available aromatics
- **FY24 Go/No-Go**: Autoxidation on phenolic lignins (TEA/LCA)
- **FY25**: ≥80% bio-available aromatics to BLV to produce a single compound with a $2/GGE and 70% reduction in GHGs relative to fossil adipic acid

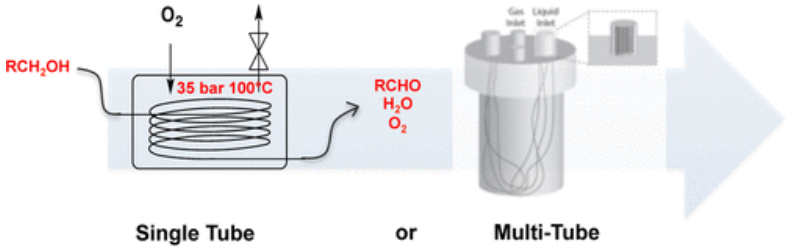
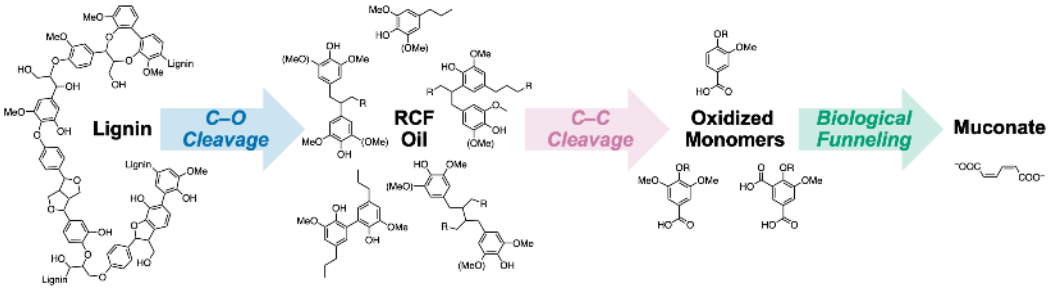


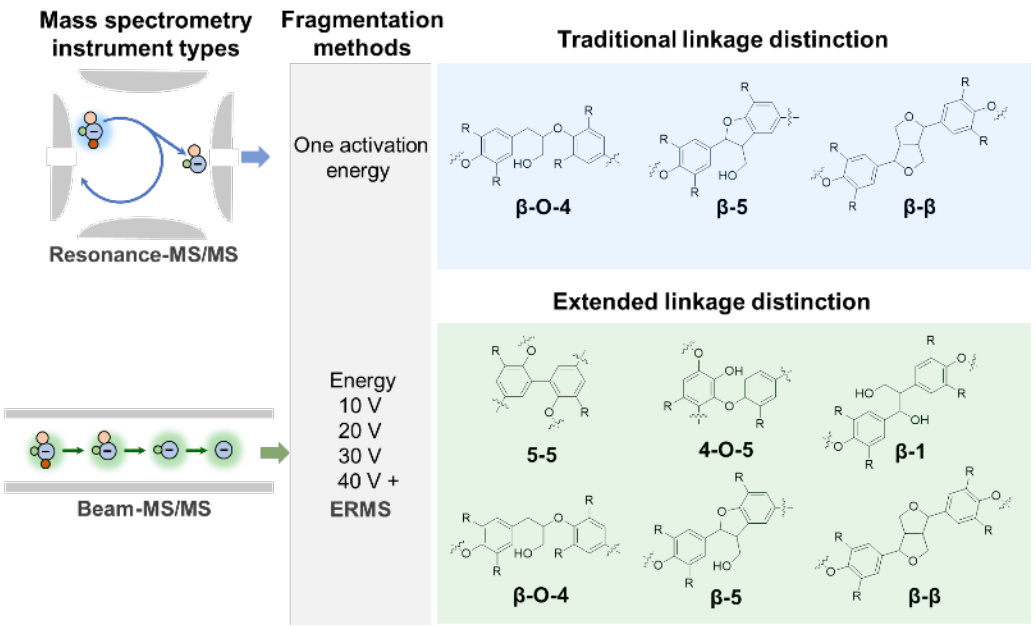

Image from Greene *et al., OPRD* 2015

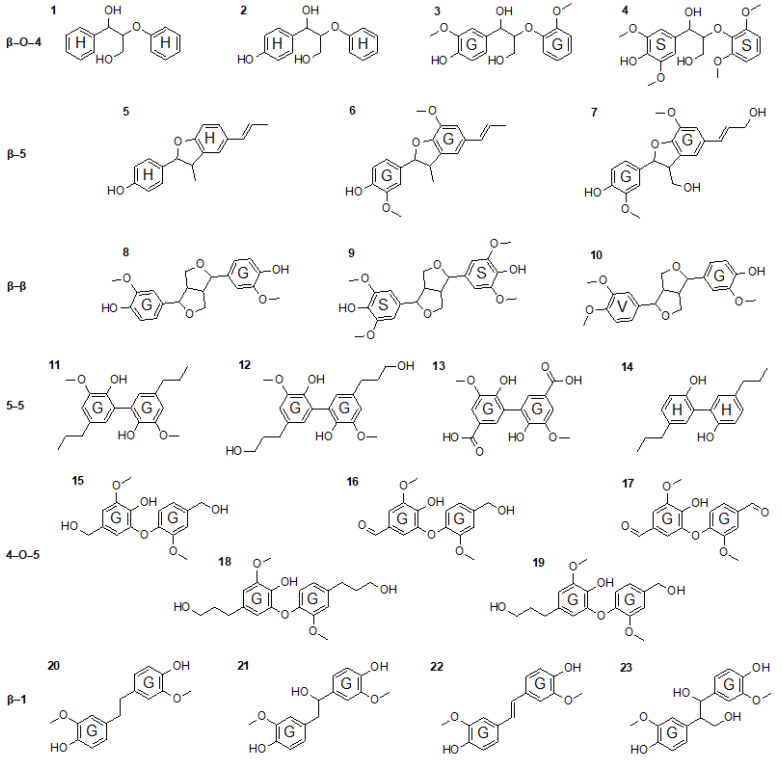
# Outline of progress and outcomes

- Lignin analytics method development for tracking lignin in the biorefinery
- Automation of lignin MS data analysis with BPMS project
- Analytics and synthesis protocols
- Catalytic autoxidation for C–C bond cleavage in lignin oils
- Catalytic autoxidation of lignin HDO substrates

# Advancing lignin analytics for linkage fingerprinting



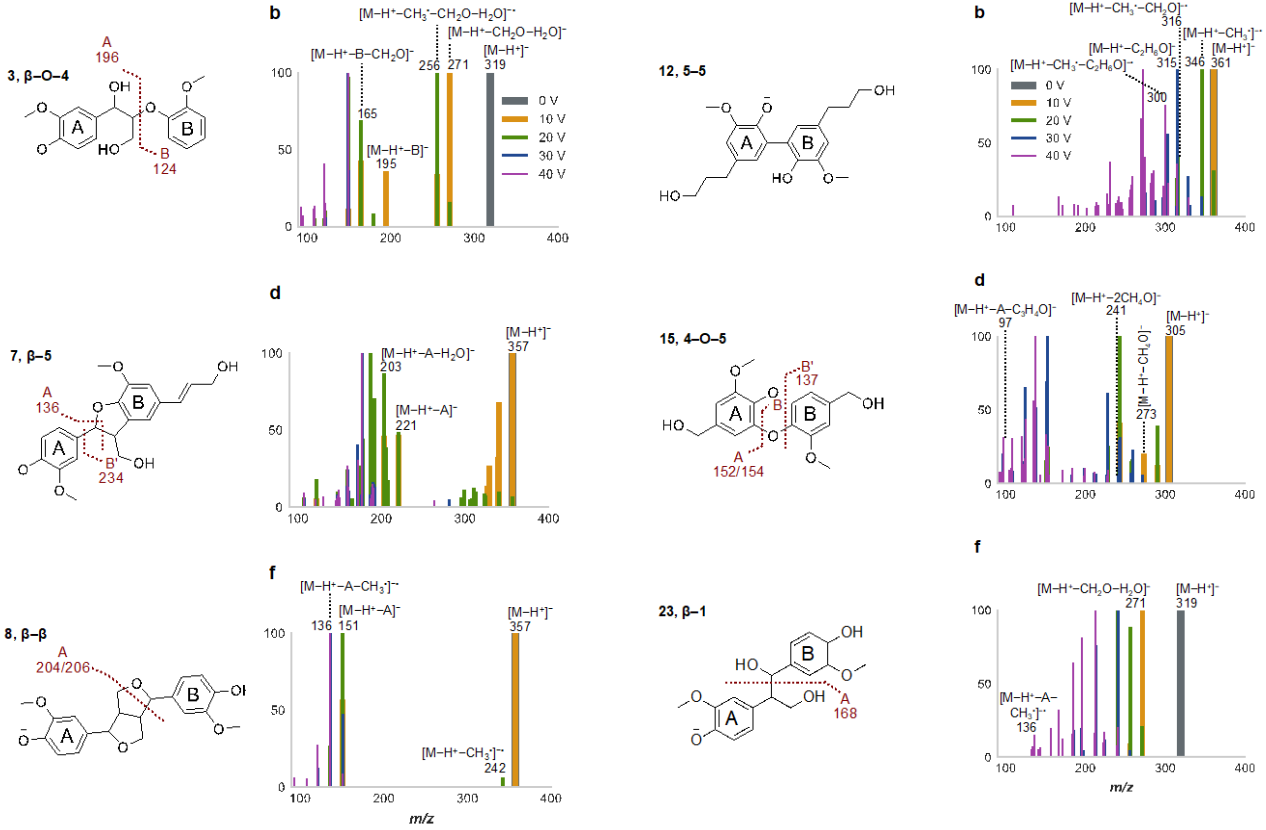




**Beam-type energy-resolved MS enables fingerprinting of 6 major lignin linkages**

- Can enable direct tracking of lignin compounds throughout a biorefinery process

Dong, Mayes *et al. ChemSusChem* 2022

# Energy-resolved mass spectrometry provides a unique fingerprint for all lignin linkages




**All 6 linkages provide distinct fingerprints – allows rapid identification of lignin chemistry from fragmentation pattern…**

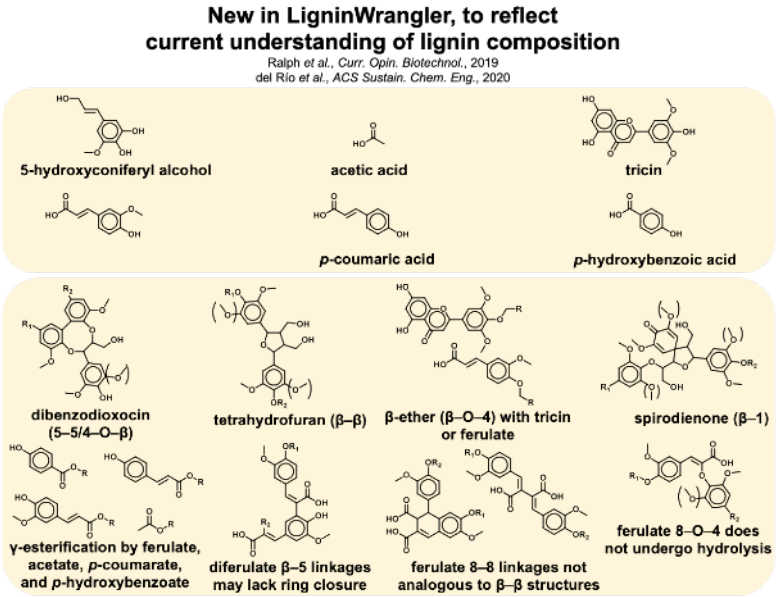
**…and the fragmentation pattern analysis can feed into automated computational analysis…**

Dong, Mayes *et al. ChemSusChem* 2022

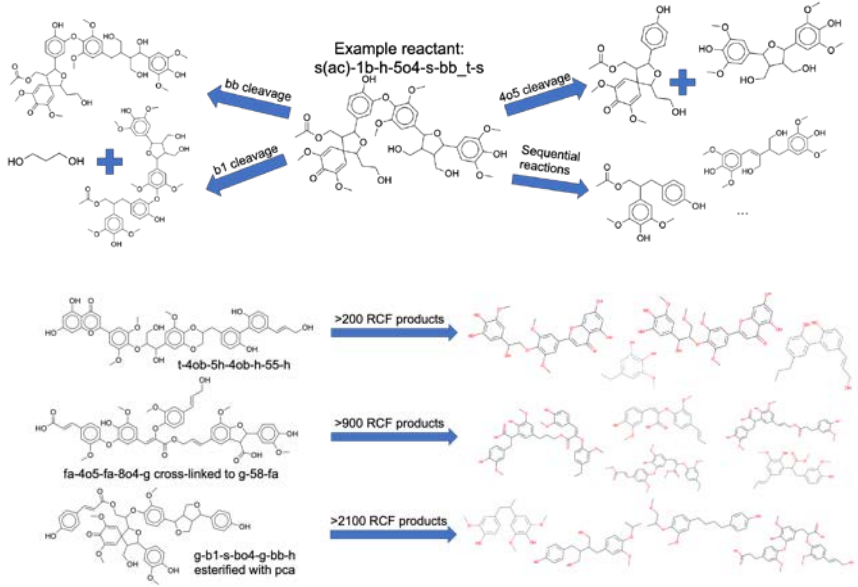
# LigninWrangler allows automated LC/MS-MS analysis of lignin

Mayes, Black *et al. in preparation*

Module 1: make_lignin




Module 2: break_lignin




**Library constructed of ~21 million lignin compounds from reductive depolymerization to date…**

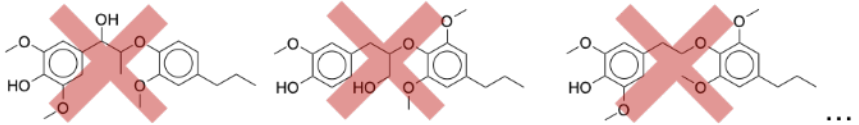
- Library contains most recent reported discoveries in lignin chemistry for maximum feedstock versatility
- Can be updated by any user and have user-fed libraries or user-fed constraints on library building

# LigninWrangler Module 3: ms2molecules to narrow compound identification

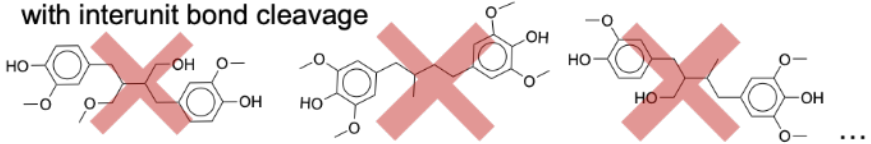
**Example:** Evaluation of high mass accuracy beam-ERMS MS/MS spectra from a RCF oil from a poplar feedstock

**Evaluation of candidate $C_{21}H_{28}O_6$ in the default library:**

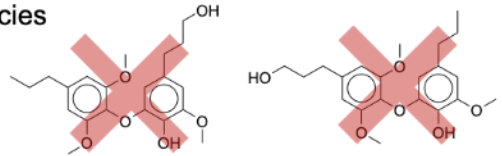
- All β–O–4 linkages ruled out due to lack of interunit cleavage at 10 V



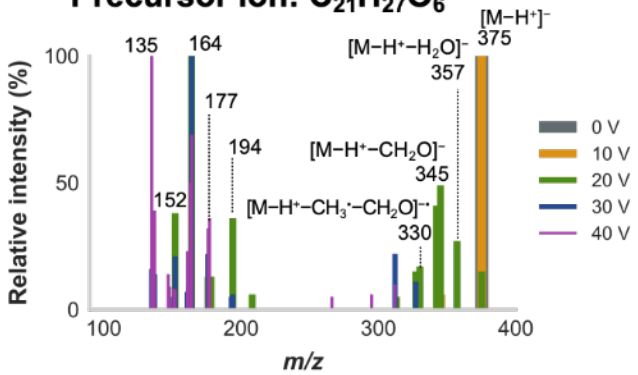
- There are no candidate 5–5 or β–1 linked species with this formula
- All β–β linked structures with this molecular formula are ring-opened, which would be expected to display far fewer product ions consistent with interunit bond cleavage



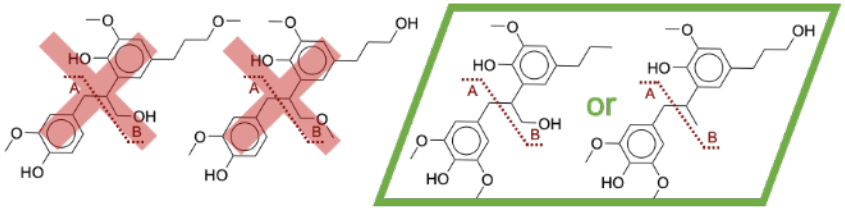
- The ~100 Da gap between lower and higher product ion *m/z* values is inconsistent with expected fragmentation of candidate 4–O–5 linked species



**Precursor ion: $C_{21}H_{27}O_6^-$**

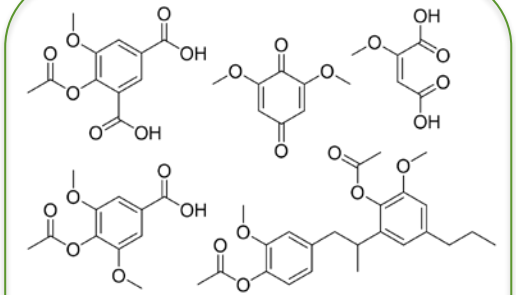



- All candidate β–5 structures are ring-opened, which, as a proposed intermediate in phenylcoumaran cleavage (Morreel *et al., Anal. Chem.*, 2010), are expected to undergo linkage cleaveage. The expected resulting fragment masses are consistent with two of the four options.



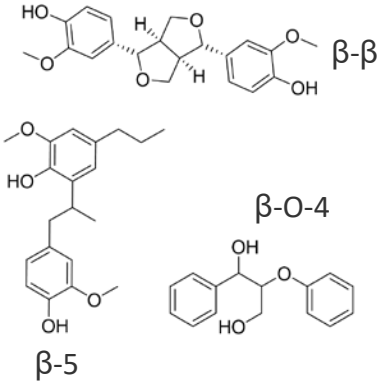

**Allows for rapid, automated identification of compound chemistry in a high-throughput manner**

# Protocols for synthesis and analytics



Exemplary compounds for analytical quantitation

Exemplary model compounds for synthesis



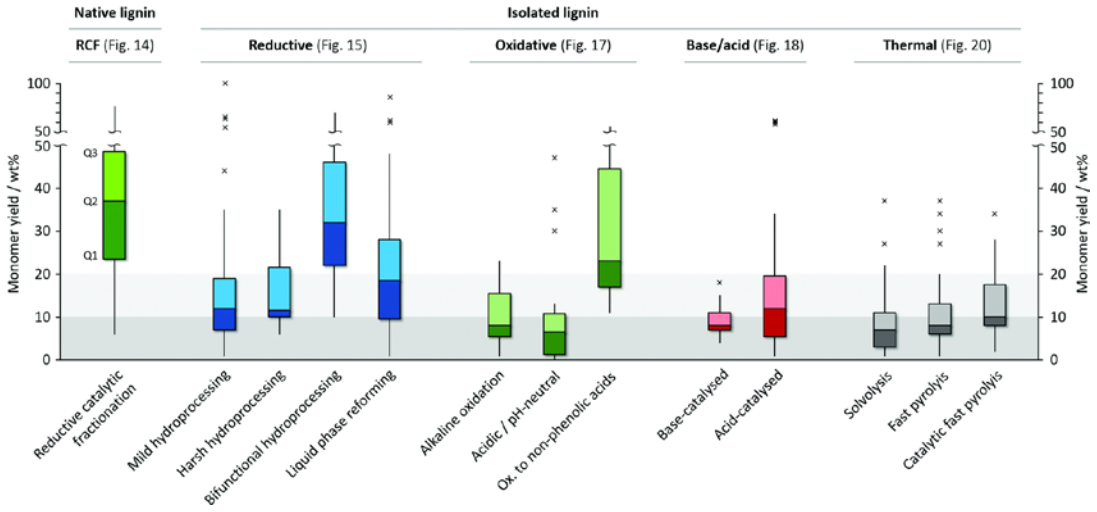

protocols.io

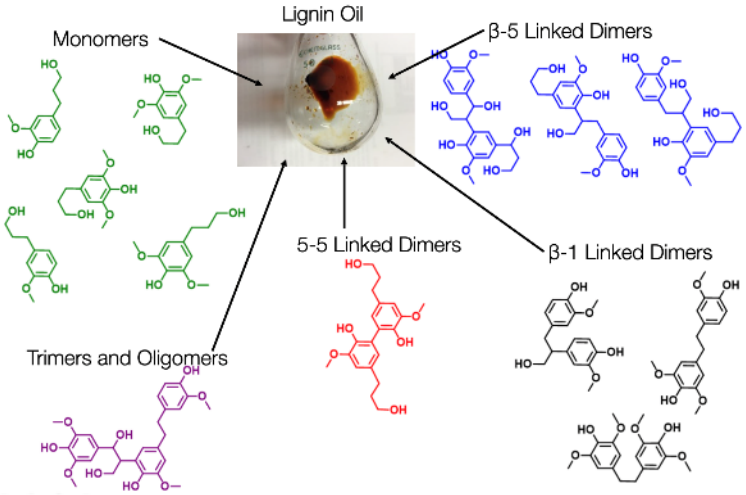
- Lignin Reductive Catalytic Fractionation (RCF) Acetylated Monomers Analysis by Gas Chromatography Flame Ionization Detection (GC-FID)
- Acetylated Products from Lignin Autoxidation by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS)
- Lignin Reductive Catalytic Fractionation (RCF) Methylated Oxidation Products by Ultra High Performance Liquid Chromatography (UHPLC)
- Lignin Reductive Catalytic Fractionation (RCF) Monomers Analysis by Gas Chromatography Flame Ionization Detection (GC-FID)
- Synthetic Procedure of 2-(1-(4-hydroxy-3-methoxyphenyl)propan-2-yl)-6-methoxy-4-propylphenol
- Synthetic Procedure of Pinoresinol
- Synthetic Procedure of 2-Phenoxy-1-phenylethanol (PE)

**protocols.io allows for an open-source collection of step-wise protocols with aim towards reproducibility**

- Published multiple analytics and model compound syntheses online for the community to use

# Carbon–carbon bond cleavage in lignin is a major technical hurdle



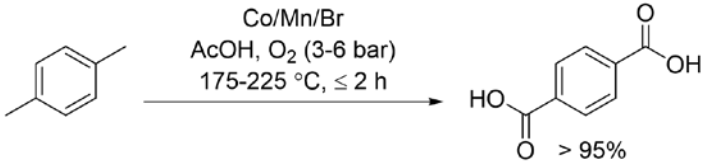

**Lignin depolymerization yields from most optimal methods usually only ~30-40%**

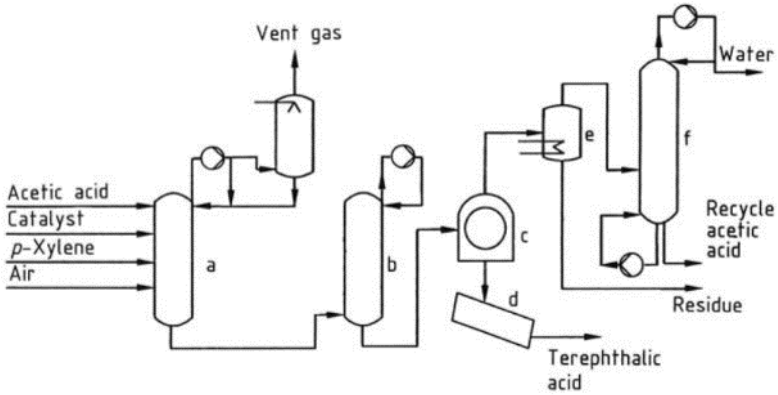
- Refractory carbon–carbon bonds in lignin inherently limit aromatic monomer yields

Schutyser *et al. Chem. Soc. Rev.* 2018

# Adapting industrial autoxidation catalysis for C–C bond cleavage in lignin

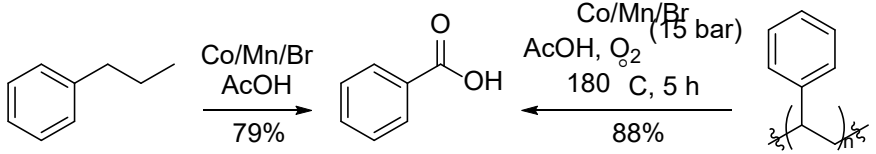
## Amoco process:



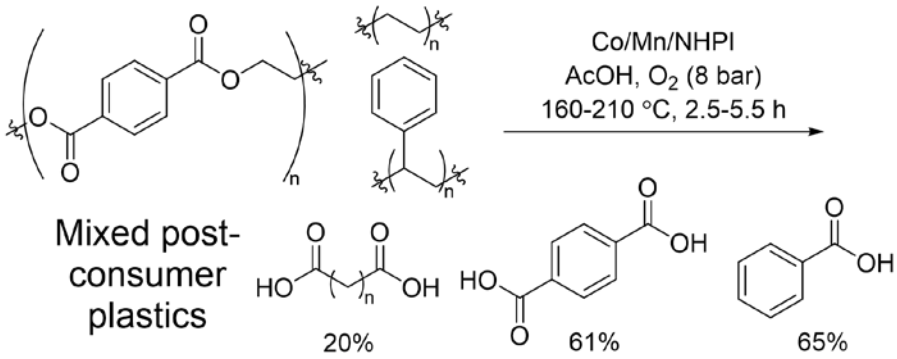




Sheehan, *Ullmann's Encyclopedia*, 2000

## Previous studies of C–C bond cleavage:




Partenheimer, *Catal. Today*, 2000; Partenheimer, *Catal. Today* 2003

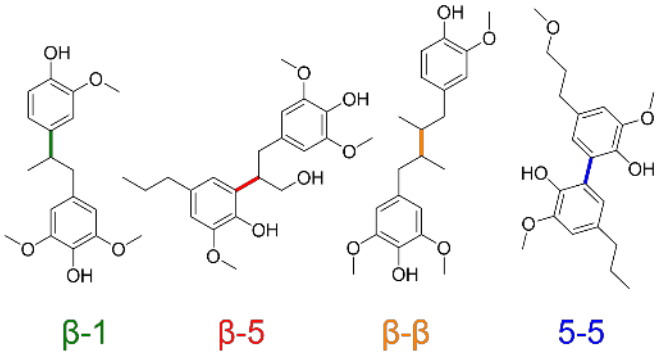



Sullivan *et al. Science*, 2022

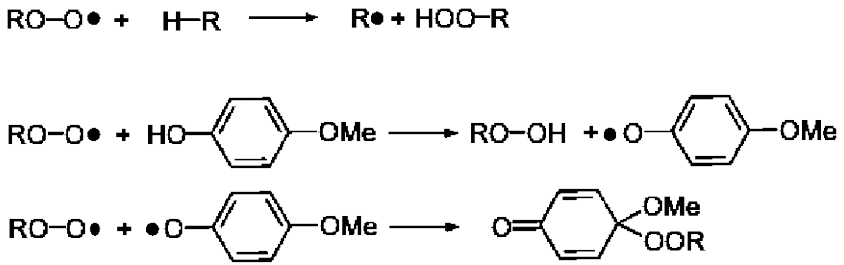
## Co/Mn/Br oxidation with $O_2$ in acetic acid is effective for C–C bond cleavage in hydrocarbon substrates

- Substantial industrial precedent for this chemistry in terephthalic acid manufacturing

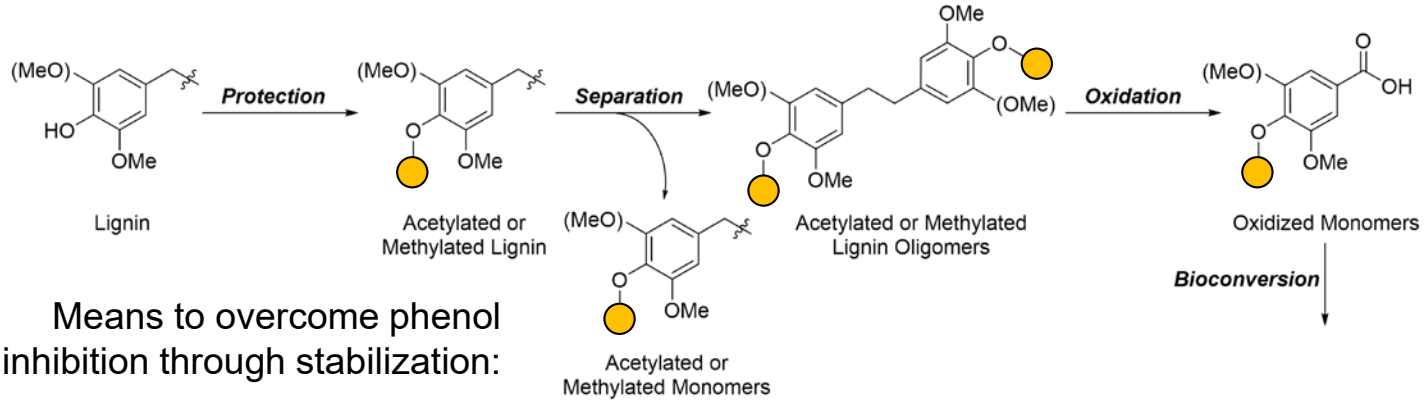
# Phenols are inhibitory to autoxidation




Exemplary C–C bonds in lignin that catalytic approaches must cleave

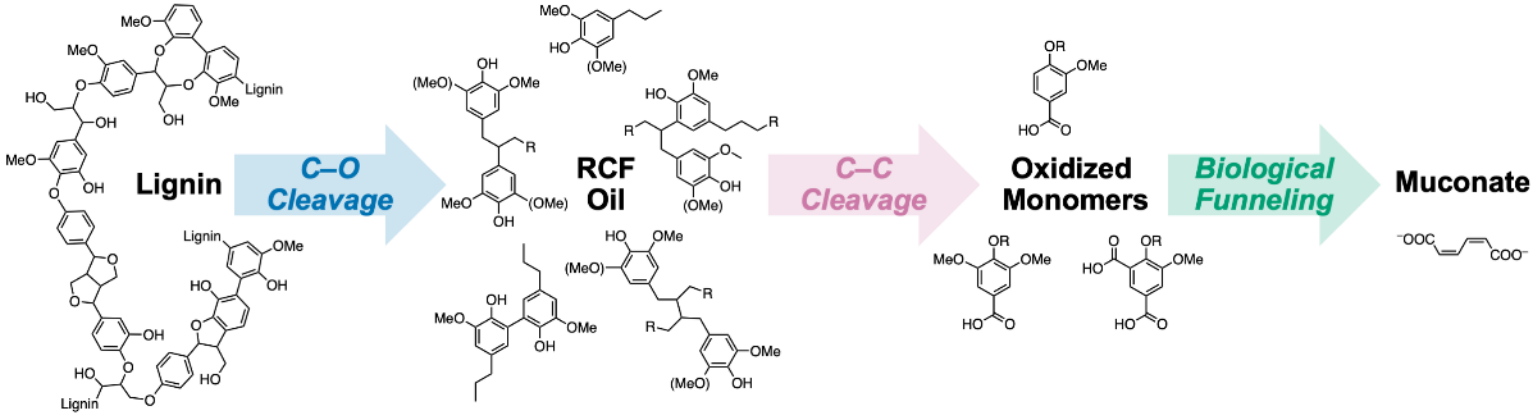


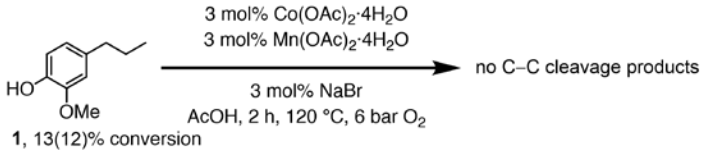

Lucarini & Pedulli, *Chem. Soc. Rev.* 2010

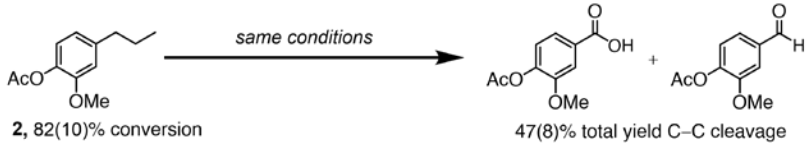



Means to overcome phenol inhibition through stabilization:

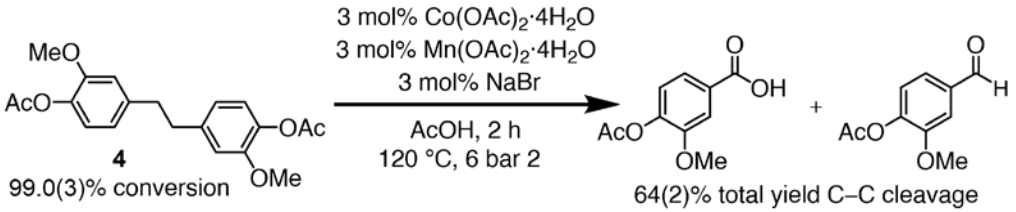
# Acetyl stabilization of phenols leads to effective C–C bond cleavage



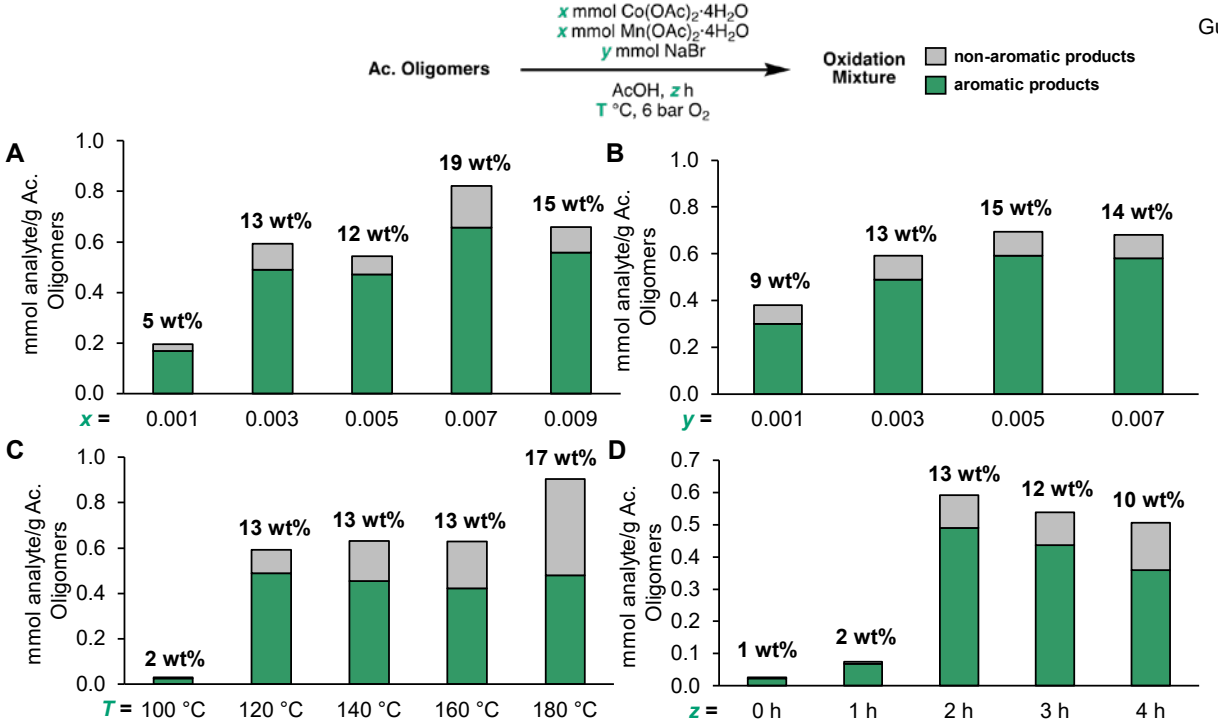

Catalytic strategy is effective for C–C bond cleavage in model compounds




Control reactions illustrate role of stabilization

# Screening reaction conditions with C–C-linked dimers and oligomers

Gu, Palumbo, Bleem *et al. in review*




**C–C-linked dimers and oligomers converted to aromatic monomers at mild conditions**

- Yields are based on total lignin and higher yields will be accessible in flow

# Proof-of-concept autoxidation catalysis on C–C-linked dimers and oligomers

Optimal conditions on poplar lignin oil with monomers removed

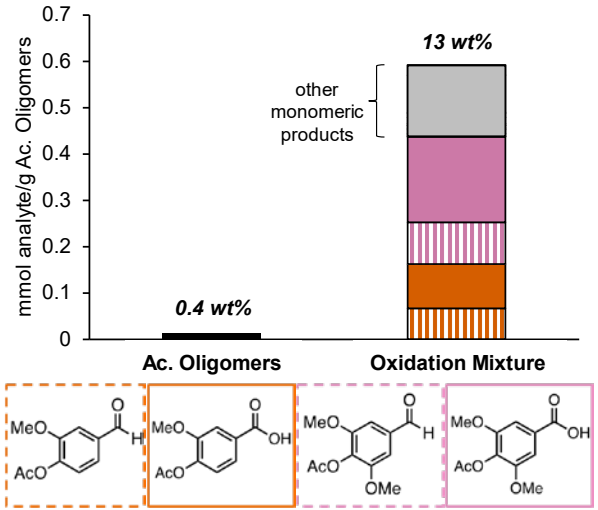
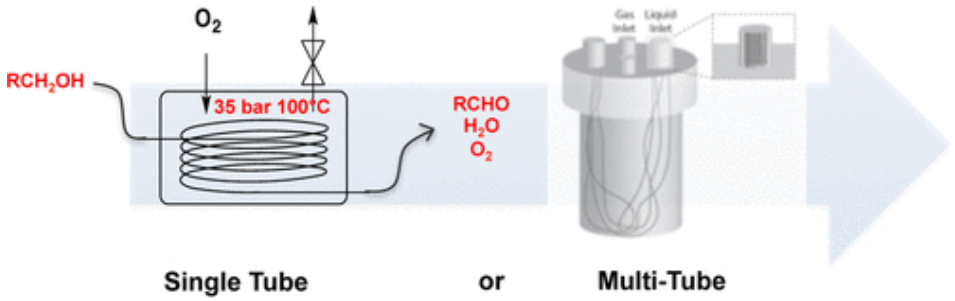



Image from Greene *et al., OPRD* 2015



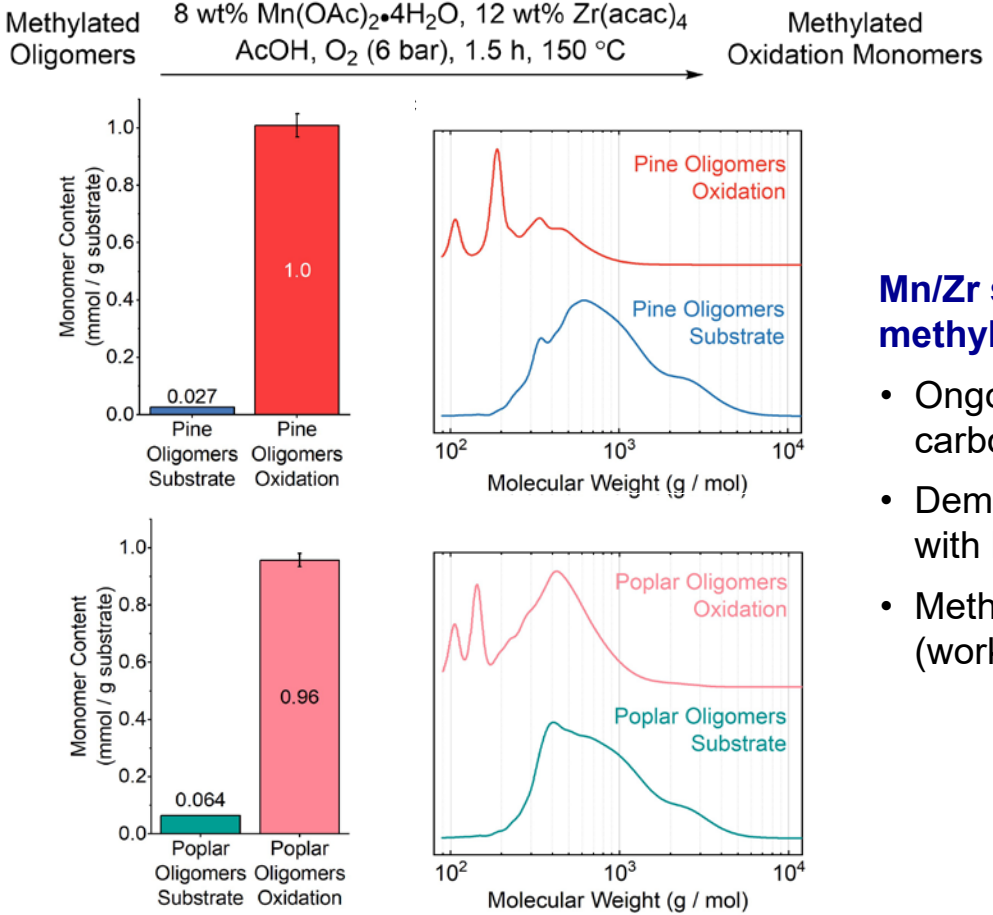

Collaborating with Shannon Stahl, UW Madison on transition to flow

**C–C-linked dimers and oligomers converted to aromatic monomers at mild conditions**

- Flow chemistry is being developed now to improve selectivity to and yield of aromatic products
- Ongoing work to simplify stabilization reaction, including with acetic acid only
- Compounds are bio-available (see BLV presentation)

Gu, Palumbo, Bleem *et al. in review*

# Methylation of phenols is another effective stabilization strategy

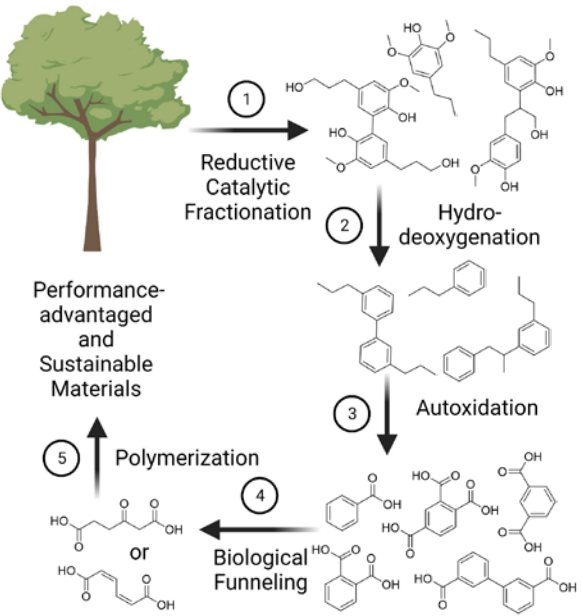


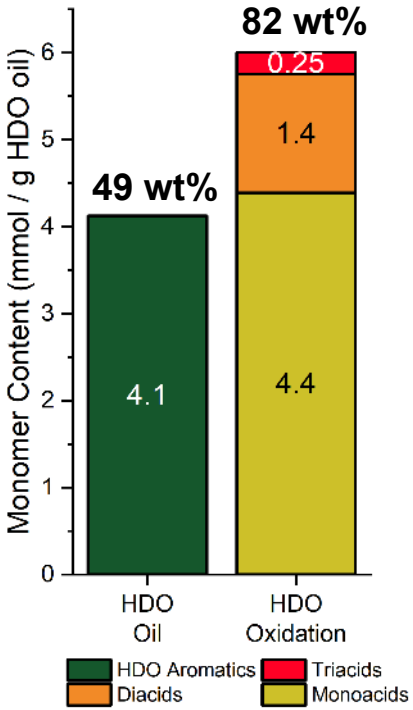

**Mn/Zr system shown to be effective with methylated lignin**

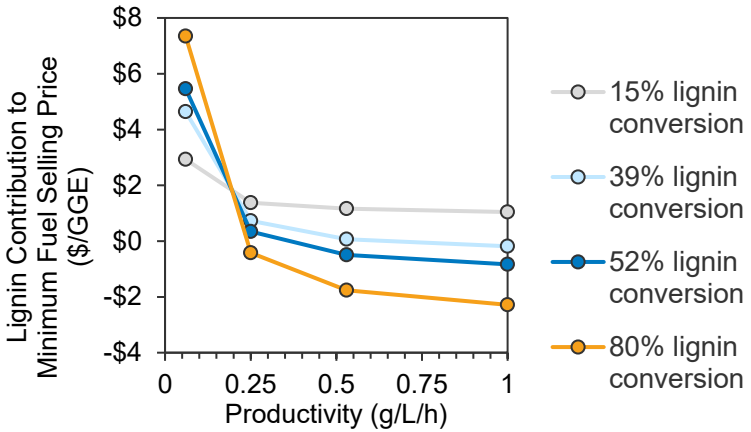
- Ongoing work to determine if dimethyl carbonate can be used for methylation
- Demonstrated this chemistry to be effective with both pine and poplar RCF oil
- Methylated products are also bio-available (work with BLV)

Palumbo, Gu, Bleem *et al. in review*

# Oligomers from lignin hydrodeoxygenation result in high product yields



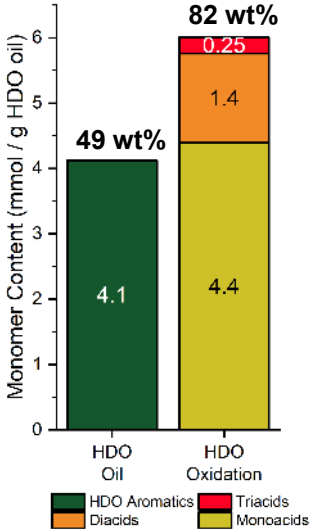

**These yields can enable a >$2/gge contribution to biorefinery economics with biological funneling (BLV)**

Palumbo, Werner, Webber *et al.* ongoing

# Impact

**Scientific:**

- First catalytic strategy for lignin depolymerization to aromatics to definitively cleave C–C bonds in lignin
- **Breaks the "monomer yield ceiling" set by C–O bond cleavage alone**
- **Enables "single valuable product" lignin valorization strategy**
- Publications and patent applications for lignin autoxidation will be online in 2023, with TEA and LCA ongoing
- Lignin analytical tools for high-throughput, automated lignin analysis




**Industrial:**

- Companies actively funding our lignin analytics capabilities and/or using our new methods in their labs
- Energy I-Corps team will be proposed around lignin autoxidation in early FY24
- Can enable **≥80% yield to a single product from lignin** and also be **complementary to LigSAF efforts** (light and heavy ends)

Honeywell UOP

ExxonMobil

Borregaard

SUSTAINABLE FIBER TECHNOLOGIES

**Overall:** Catalytic C–C bond cleavage can enable substantially higher-yielding lignin valorization processes

# Summary

## Overview

- Aim to develop viable processes to increase the theoretical monomer yield from lignin alongside robust analytics and accessible compound syntheses

## Approach

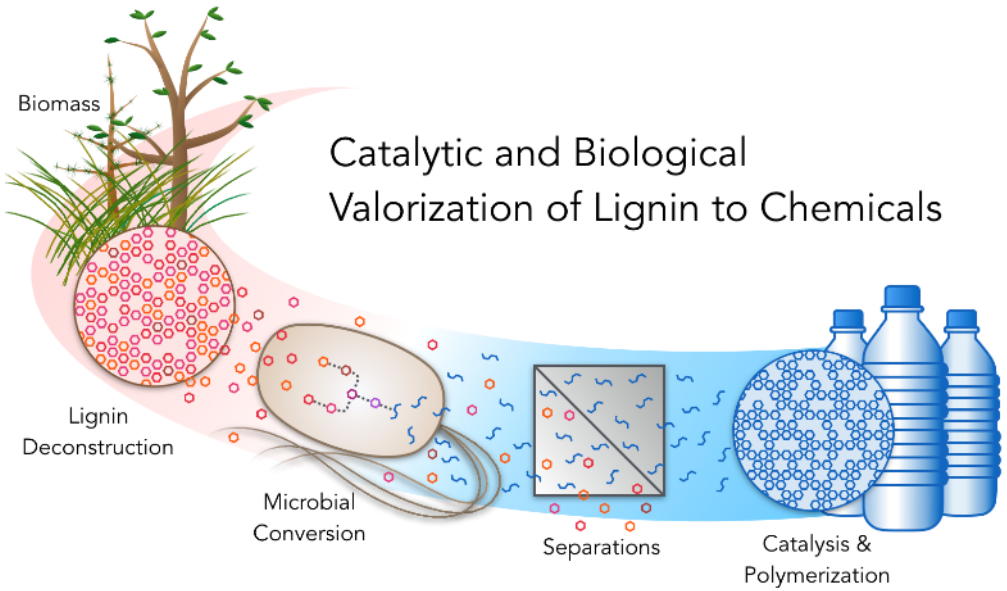
- Oxidation catalysis for C–C bond cleavage to make water-soluble, bio-available monomers

## Progress and outcomes

- Developed an automated MS analysis pipeline for rapid lignin compound identification
- Demonstrated a robust method for C–C bond cleavage leveraging an industrial process from multiple lignin substrates

## Collaborations and impact

- Work with all BETO-relevant lignin projects and with multiple industry partners




Beckham *et al., Curr. Opin. Biotech.* 2016

# Quad chart overview

## Timeline

- Active Project Duration: 10/1/2022 – 9/30/2025
- Total Project Duration: 10/1/2016 – 9/30/2025

| | FY22 funding | Total Award |
|---|---|---|
| **DOE Funding** | $1,450,000 (10/01/2021–9/30/2022) | $625,000 – FY23 |

## Project Partners

**BETO projects**: Lignin-First Biorefinery Development, Biological Lignin Valorization, Separations Consortium, Biochemical Platform Analysis

**Nat'l labs**: Oak Ridge National Laboratory

**University collaborators**: Massachusetts Institute of Technology, University of Wisconsin Madison

## Project Goal

Develop industrially-relevant processes and tools for lignin valorization

## End of Project Milestone

Deliver concentrated, lignin monomer-rich streams that contain 80% bio-available aromatic compounds to BLV, which can enable muconic or β-ketoadipic acid production at industrially relevant bioprocess parameters. Demonstrate a $2/GGE and 70% reduction in GHG emissions relative to fossil-based adipic acid production.

## Funding Mechanism

Bioenergy Technologies Office FY23 AOP Lab Call (DE-LC-000L015) – 2022

TRL at Project Start: 2

TRL at Project End: 4

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BIOENERGY TECHNOLOGIES OFFICE

# Q&A

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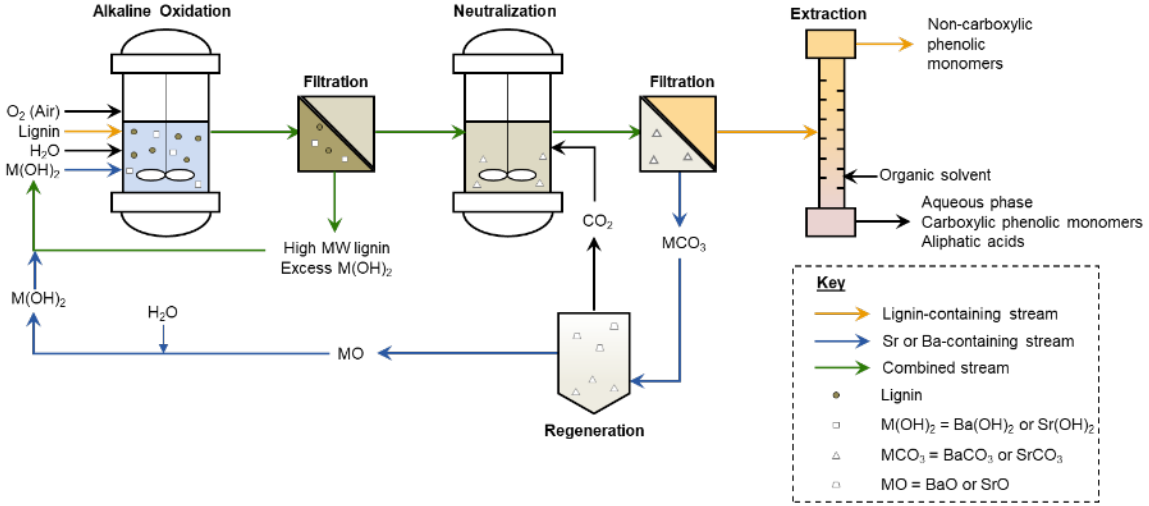
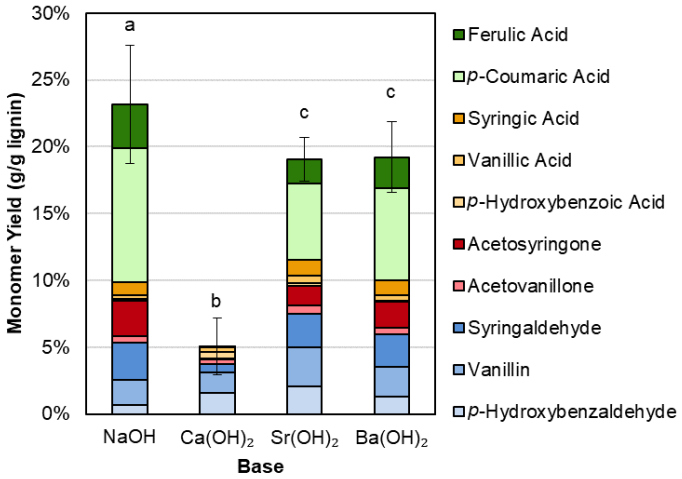
# Additional Slides

# Recoverable bases for base-catalyzed depolymerization of lignin

Kruger *et al. Green Chem.* 2022




**NaOH use is a major contributor to environmental impacts of some lignin processes**

- Sr(OH)2 is a recoverable base that shows similar yields of monomeric aromatics to NaOH
- TEA and LCA both indicate promise in using recoverable bases to replace NaOH

# Responses to previous reviewers' comments

- More details on the composition of the oxidized lignin mixtures will be helpful, but the literature suggests that complex mixtures of products will result.
  - We agree that yield measurements and mass balances are critical. However, as the reviewers know, these measurements take a substantial amount of time to do rigorously for lignin. In terms of products, we fully agree that lignin oxidation products will be heterogeneous—this is why the Lignin Utilization project is working very closely with the Biological Lignin Valorization project to use microbes downstream of oxidative depolymerization to be able to produce high yield of single lignin-derived products.

# Publications, patents, and presentations

**Publications**

In preparation or review

Chad T. Palumbo, Allison Z. Werner, Matthew S. Webber, David G. Brandner, Jeremy R. Bussard, Shannon S. Stahl, Yuriy Román-Leshkov, Gregg T. Beckham, High atom economy conversion of lignin into aromatic monomers through carbon–carbon bond cleavage, manuscript in preparation.

Chad T. Palumbo, Nina X. Gu, Alissa Bleem, Kevin P. Sullivan, Mikhail O. Konev, Rui Katahira, Lisa Stanley, Kelsey J. Ramirez, Stefan J. Haugen, Morgan A. Ingraham, Caroline R. Amendola, Shannon S. Stahl, Gregg T. Beckham, Catalytic carbon–carbon bond cleavage in lignin via manganese–zirconium-mediated autoxidation, manuscript in preparation.

Nina X. Gu, Chad T. Palumbo, Alissa C. Bleem, Kevin P. Sullivan, Stefan J. Haugen, Sean P. Woodworth, Kelsey J. Ramirez, Lisa Stanley, Rui Katahira, Shannon S. Stahl, Gregg T. Beckham, Autoxidation catalysis for carbon–carbon bond cleavage in lignin, in review.

Xu Du, Mikhail O. Konev, Kevin P. Sullivan, Rui Katahira, Chad T. Palumbo, Nina X. Gu, Sean P. Woodworth, David G. Brandner, Shannon S. Stahl, Gregg T. Beckham, Tandem acid-promoted and aerobic cleavage of C(OH)–C bonds with a polyoxometalate catalyst, in review.

Accepted

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# Publications, patents, and presentations

2022

Jacob S. Kruger, Reagan J. Dreiling, Daniel G. Wilcox, Arik J. Ringsby, Katherine L. Noon, Camille K. Amador, David G. Brandner, Kelsey J. Ramirez, Stefan J. Haugen, Bruno C. Klein, Ryan Davis, Rebecca J. Hanes, Renee M. Happs, Nicholas S. Cleveland, Earl D. Christensen, Joel Miscall, Gregg T. Beckham, Lignin alkaline oxidation using reversibly-soluble bases, *Green Chem*. (2022), 24, 8733-8741.

Jun Hee Jang, David G. Brandner, Reagan J. Dreiling, Arik J. Ringsby, Jeremy R. Bussard, Lisa M. Stanley, Renee M. Happs, Anjaneya S. Kovvali, Joshua I. Cutler, Tom Renders, James R. Bieleberg, Yuriy Román-Leshkov, Gregg T. Beckham, Multi-pass flow-through reductive catalytic fractionation, *Joule* (2022) 6, 1859-1875.

Patrick O. Saboe, Emily G. Tomashek, Hanna R. Monroe, Stefan J. Haugen, Ryan L. Prestangen, Nick S. Cleveland, Renee M. Happs, Joel Miscall, Kelsey J. Ramirez, Rui Katahira, Eric C. D. Tan, Jipeng Yan, Ning Sun, Gregg T. Beckham, Eric M. Karp, Recovery of low molecular weight compounds from alkaline pretreated lignin via membrane separations, *Green Chem.* (2022) 24, 3152-3166.

Hang Dao Thi, Korneel Van Aelst, Sander Van den Bosch, Rui Katahira, Gregg T. Beckham, Bert F. Sels, Kevin M. Van Geem, Identification and quantification of lignin monomers and oligomers from reductive catalytic fractionation of pine wood with GC×GC-FID/MS, *Green Chem.* (2022) 24, 191-206.

2021

Mahdi M. Abu-Omar, Katalin Barta, Gregg T. Beckham, Jeremy S. Luterbacher, John Ralph, Roberto Rinaldi, Yuriy Román-Leshkov, Joseph S.M. Samec, Bert F. Sels, and Feng Wang, Guidelines for performing lignin-first biorefining, *Energy Env. Sci.* (2021), 14, 262-292.

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Gerald N. Presley, Allison Z. Werner, David C. Garcia, Stefan J. Haugen, Caroline B. Hoyt, Rui Katahira, Kelsey J. Ramirez, Richard J. Giannone, Gregg T. Beckham, and Joshua K. Michener, Pathway discovery and engineering for cleavage of a ß-1 lignin-derived biaryl compound, *Metabolic Eng*. (2021), 65, 1-10.

Laura Berstis, Thomas Elder, Richard A. Dixon, Michael F. Crowley, Gregg T. Beckham, Coupling of flavonoid nucleation sites with monolignols studied by density functional theory, *ACS SusChemEng (*2021) 9, 4, 1518–1528.

Sandra Notonier, Allison Z. Werner, Eugene Kuatsjah, Linda Dumalo, Paul E. Abraham, E. Anne Hatmaker, Caroline B. Hoyt, Antonella Amore, Kelsey J. Ramirez, Sean P. Woodworth, Dawn M. Klingeman, Richard J. Giannone, Adam M. Guss, Robert L. Hettich, Lindsay D. Eltis, Christopher W. Johnson, and Gregg T. Beckham, Metabolism of syringyl lignin-derived compounds in *Pseudomonas putida* enables convergent production of 2-pyrone-4,6-dicarboxylic acid, *Metabolic Eng*. (2021) 65,111-122.

2020

Morgan M. Fetherolf, David J. Levy-Booth, Laura E Navas, Jie Liu, Jason C Grigg, Andrew Wilson, Rui Katahira, Gregg T. Beckham, William M. Mohn, Lindsay D. Eltis, Characterization of alkylguaiacol-degrading cytochromes P450 for the biocatalytic valorization of lignin, *PNAS* (2020), 117, 25771-25778.

Thomas Elder, José Carlos del Río, John Ralph, Jorge Rencoret, Hoon Kim, Gregg T. Beckham, Michael Crowley, Coupling and reactions of lignols and new lignin monomers: A density functional theory study, *ACS Sus. Chem. Eng.* (2020), 8, 11033-11045.

# Publications, patents, and presentations

Nicholas E. Thornburg, M. Brennan Pecha, David G. Brandner, Michelle L. Reed, Josh V. Vermaas, William E. Michener, Rui Katahira, Todd B. Vinzant, Thomas D. Foust, Bryon S. Donohoe, Yuriy Román-Leshkov, Peter N. Ciesielski,* Gregg T. Beckham*, Mesoscale reaction-diffusion phenomena governing lignin-first biomass fractionation, *ChemSusChem* (2020), 13, 4495-4509.

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Josh V. Vermaas, Michael F. Crowley, Gregg T. Beckham, A quantitative molecular atlas for interactions between lignin and cellulose, *ACS SusChemEng*. (2019) 7, 19570-19583.

Josh V. Vermaas, Richard A. Dixon, Fang Chen, Shawn D. Mansfield, Wout Boerjan, John Ralph, Michael F. Crowley, Gregg T. Beckham, Passive membrane transport of lignin-related compounds, *PNAS* (2019), 116, 23117-23123.

Josh V. Vermaas, Loukas Petridis, Michael F. Crowley, Gregg T. Beckham, Systematic parameterization of lignin for the CHARMM force field, *Green Chem*. (2019) 21, 109-122.

Michael J. Orella, Terry Z. H. Gani, Josh V. Vermaas, Michael L. Stone, Eric M. Anderson, Gregg T. Beckham, Fikile R. Brushett, Yuriy Román-Leshkov, LIGNIN-KMC: A toolkit for simulating lignin biosynthesis, *ACS Sus. Chem. Eng*. (2019) 7, 18313-18322.

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Terry Z. H. Gani, Michael J. Orella, Eric M. Anderson, Michael L. Stone, Fikile R. Brushett, Gregg T. Beckham and Yuriy Román-Leshkov Computational evidence for kinetically controlled radical coupling in lignin polymerization, *ACS Sust. Chem. Eng.* (2019) 7, 13270-13277.

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Andrea Corona, Mary J. Biddy, Derek R. Vardon, Morten Birkved, Michael Hauschild, and Gregg T. Beckham, Life cycle assessment of adipic acid production from lignin, *Green Chem*. (2018) 20, 3857-3866.

Wouter Schutyser, Jacob S. Kruger, Allison M. Robinson, Rui Katahira, David G. Brandner, Nicholas S. Cleveland, Ashutosh Mittal, Darren J. Peterson, Richard Meilan, Yuriy Román-Leshkov, and Gregg T. Beckham, Revisiting alkaline aerobic lignin oxidation, *Green Chem*. (2018) 20, 3828-3844.

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# Publications, patents, and presentations

**Patents (issued)**

| | |
|---|---|
| ROI-20-129 | Polyoxometalate Catalyzed Reductive Catalytic Fractionation (RCF) Oil and Kraft Lignin Depolymerization |
| ROI-20-84 | Method for Quantifying Inorganic Carbon in Solution |
| ROI-21-40 | Process for converting CO2 into Conductive Carbons via Flash Joule Heating |

**Patents (provisional)**

| | |
|---|---|
| ROI-18-88 | Heterogeneous Catalytic Oxidation of Lignin Carbon-Carbon Linkages |

# Publications, patents, and presentations

**Presentations (2021 – 2022)**

Using catalysis as a discovery tool to develop better poplar feedstocks and find new lignin building blocks, Plant Biochemistry Symposium in honor of Richard Dixon, UNT, October 2022

Advances in lignin and plastics conversion, VITO, September 2022

Recent efforts in NMR spectroscopy, high-throughput screening, mass spectrometry, and computational tools for lignin characterization, Lignin Gordon Research Conference, August 2022

Catalytic Depolymerization of Lignin Oligomers in Reductive Catalytic Fractionation Oil through C–C Bond Cleavage. LignoBiotech2022, August 2022

Recent adventures in lignin valorization, Ligno COST Workshop, June 2022

Biological conversion of lignin and plastics-related substrates (via webinar), CIB-CSIC, May 2022

Lignin valorization through integrated process modeling, chemical catalysis, material science, metabolic engineering, and separations research, Wallenberg Wood Science Center (via webinar), June 2021

# Publications, patents, and presentations

Catalysis for valorization of lignin and plastics, Great Plains Catalysis Society (via webinar), June 2021

The critical role of economic and environmental analysis to guide research in lignin valorization and plastics upcycling, Keynote Invited Lecture, ACS Green Chemistry and Engineering (via webinar), June 2021

Recent progress in performance-advantaged bioproducts and plastics upcycling, Arizona State University (via webinar), April 2021

Recent adventures in biomass conversion and plastics upcycling, Rutgers University (via webinar), April 2021