Thrust Area 5: Carbon Capture & Nuclear

Biocatalytic Lignin Modification for Carbon Sequestration

PI: Jon Stewart
Students: Bradford Sullivan (postdoctoral fellow), Filip Boratynski (postdoctoral fellow)

Description: After cellulose, lignin is the second most abundant forma of carbon in plants. Lignin's complex structure makes it difficult to use this material in value-added products, and ahte vast majority of lignin is currently burned to provide energy for factory operations. While burning plant derived lignin does not add to global greenhouse gas levels, having options to remove lignin from the global carbon cycle would lead to diminished atmospheric CO2 levels. This could be accomplished by chemically altering lignin's structure to facilitate long-term terrestrial sequestration or using it in value-added products that would not be discarded immediately. We will use Nature's catalysts (enzymes) to tailor the chemical structure of lignin for both deep-well injection (by using lignin derivatives as drilling "muds") and for materials that can be used in building, packaging, and other manufactured products.)

Budget: $200,000
Universities: UF

Progress Summary

Bradford Sullivan joined this project as a postdoctoral fellow in February 2010 with extensive experience in both organic synthesis and in dioxygenase enzymes. To the best of our knowledge, no one has applied dioxygenases to lignin and/or lignin model compounds. Enzymes such as toluene dioxygenase offer the possibility of converting this renewable feedstock into valuable building blocks. In preliminary studies, Brad has applied toluene dioxygenase to model compounds derivable from lignin to create small molecule mediators required by laccases for lignin breakdown. Some reaction was observed. We are also setting up a collaboration between our lab and those of Steven Sherman and Charles Turick (Savannah River National Laboratory), who have developed a simple method for lignin extraction from a variety of soft materials such as switchgrass as well as woody tissues. This will provide us with the material for exploring ionic liquids and deep eutectic solvents for laccase-catalyzed lignin conversions. Filip Boratynski joined the project in September 2011 with a background in biocatalysis. He will be focusing on experiments using the lignin samples provided by our collaborators at Savannah River.

Funds leveraged/new partnerships created:

New collaborations		
Steven Sherman, Charles Turick (Savannah River National Laboratory)	Steve and Chuck have agreed to supply us with lignin samples prepared in their lab using a newly-developed extraction method. This product stream will be employed for enzyme-catalyzed reactions in our lab using safe, non-volatile solvents (ionic liquids and deep eutectic solvents)	No external funding yet for this work

Proposals						
Title	Agency	Reference Number	PI, Co-investigators and collaborators	Funding requested	Project time frame (1 year, 2 years, etc.)	Date submitted
Adapting Kernel Metabolism to Enhance Cereal Yield Under Adverse Conditions	USDA	2011-67003-30215	L. Curtis Hannah (P.I.), Tracy Hennen-Bierwagen (co-P.I.), Karen Koch (co-P.I.), Don McCarty (co-P.I.), Alan Meyers (co-P.I.), Mark Settles (co-P.I.), Jon Stewart (co-P.I.), William Tracy (co-P.I.)	$5M	5 years	June 2010
Improving Alkene Reductases for Applications in Asymmetric Synthesis	NSF	NSF 10-1	Jon Stewart (P.I.)	**497,851**	3 years	December 2010

Grants Awarded					
Title	Agency	Reference Number	PI, Co-investigators and collaborators	Period of Performance	Funding awarded
Adapting Kernel Metabolism to Enhance Cereal Yield Under Adverse Conditions	USDA	2011-67003-30215	L. Curtis Hannah (P.I.), Tracy Hennen-Bierwagen (co-P.I.), Karen Koch (co-P.I.), Don McCarty (co-P.I.), Alan Meyers (co-P.I.), Mark Settles (co-P.I.), Jon Stewart (co-P.I.), William Tracy (co-P.I.)	$5M	5 years
Improving Alkene Reductases for Applications in Asymmetric Synthesis	NSF	CHE-0615776	Jon Stewart (P.I.)	**497,851**	3 years

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Lignin makes up approximately 20% of the carbon fixed by plants [1] and must be separated from the cellulosic fraction in a number of processes including pulping and bioethanol production [2]. Traditional Kraft pulping chemically derivatizes lignin with sulfonic acid moieties, allowing it to be soluble under basic conditions, but also imparting a strong odor that makes it difficult to employ the lignin for any purpose other than combustion [3]. Steam treatment is typically employed in cellulosic ethanol processes, and this operation yields a lignin stream better suited to value-added uses [4]. It should be noted that sugarcane bagasse has a lower density of ortho-substitution, increasing its ability to be derivatized [5].

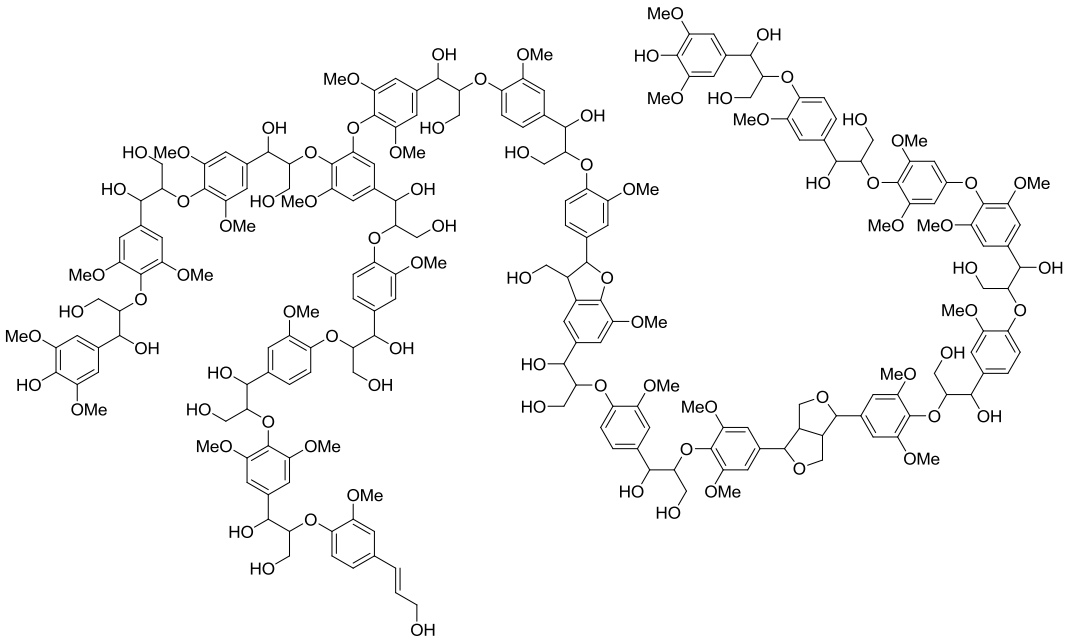
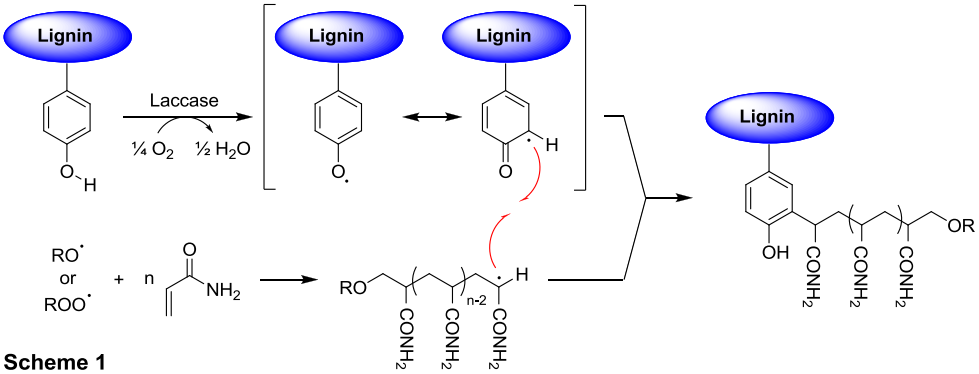


Figure 1: Typical lignin structure (re-drawn from http://www.dfrc.ars.usda.gov/ligninmodels.html).

Laccases are the best-known enzymes that accept lignin as a substrate. These multi-copper proteins are produced by a wide variety of species and play important roles in lignin degradation by white- and brown-rot fungi [6]. These enzymes mediate the four-electron reduction of O2 using lignin as the ultimate electron source. Because lignin can be highly crosslinked and interior portions are difficult to access by large proteins, laccases are paired with small molecule, diffusible electron carriers (mediators) [7]. Depending on reaction conditions, laccase / mediator systems can cross-link lignin internally, covalently add small molecules to lignin or degrade the lignin substrate [7-9]. We will focus on laccase-mediated molecular additions since these conversions can alter lignin properties in useful ways. To facilitate re-using laccases, these enzymes have been immobilized on a variety of solid supports (for a summary, see [9]).

Mai and co-workers have shown that polyacrylamide can be grafted onto lignin by simultaneous treatment with laccase and a small-molecule peroxide [10, 11]. The reaction is believed to proceed by by laccase-mediated radical formation within lignin. These radicals couple with radicals found at growing ends of polyacrylamide chains (Scheme 1). The resulting co-polymer had solubility properties suitable for use as deep-well drilling fluid. In addition to polymer grafting, laccase also cross-links the lignin into

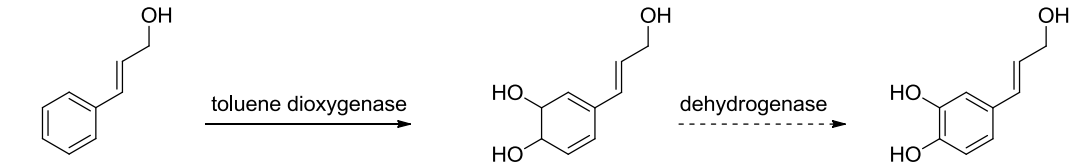
higher molecular weight assemblies, increasing its mechanical strength. These results suggest that other living radical polymerizations might also be amenable to lignin attachment.



Scheme 1

Non-covalent polymer blends represent an important means of using lignin for value-added products. Unfortunately, native lignin interacts poorly with existing materials. In an effort to solve this problem, Thielemans and Wool acylated kraft lignin in an effort to identify a derivative that dissolved in styrene [12]. While successful in this regard, only a limited number of acyl chains were examined in this study. This approach also depended on chemically synthesizing activated acyl derivatives (anhydrides, acyl chlorides). By contrast, lipases can utilize carboxylic acids directly and tolerate a wide variety of functional groups.

We are investigating the first step in the conversion of aromatic substrates into catechols by using cinnamyl alcohol as a model compound for lignin-derived materials. It appears that bacterial toluene dioxygenase does indeed accept cinnamyl alcohol and convert it to the corresponding diol. The next steps will be to combine this enzyme system with a dehydrogenase to yield the catechol in situ and to probe the possibility of oxidizing more complex lignin-derived materials. This can be combined with the polymer grafting approach described above to lower the costs of these materials even further.



cinnamyl alcohol 1,2-dihydrodiol catechol

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