The Biodegradation of Lignin: harnessing the power of enzymes

Amanda C. Kohler[1,2*] (ACKohler@lbl.gov), Jijiao Zeng[1,2], Michael S. Kent[1,2], Jose Henrique Pereira[1,3], Paul D. Adams[1,3,4], Blake A. Simmons[1,2], and **Kenneth L. Sale**[1,2]

[1]Joint BioEnergy Institute, Emeryville, CA, USA; [2]Sandia National Laboratories, Livermore, CA, USA; [3]Lawerence Berkeley National Laboratory, Berkeley, CA, USA; [4]Department of Bioengineering, University of California Berkeley, Berkeley, CA, USA

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Project Goals: Our objective is to characterize and optimize key lignin degrading enzymes for the development of multi-component enzyme mixtures aimed at producing defined, utilizable end products. Enzymes of particular interest for this research include fungal lignin-acting peroxidases as well as bacterial lignin metabolic enzymes. Thus, two goals of this work are to engineer a versatile peroxidase with enhanced temperature and pH stability, and to functionally and structurally characterize a key *Sphingobium* sp. SYK-6 metabolic enzyme, *O*-demethylase LigM.

Lignin is a key structural component of plant cell walls and one of the most abundant natural polymers on earth. A complex network of aromatic subunits, lignin serves as a rich and renewable source of valuable aromatic compounds, which can be used as precursors for the synthesis of pharmaceuticals, plastics, fuels, and other organic products. However, due to the recalcitrance of lignin, current cellulosic ethanol production methods fail to constructively extract lignin-derived compounds from plant biomass, thus leaving this important commodity untapped. Biodegradation of lignin using lignin-degrading enzymes offers a sustainable method of increasing cellulose availability and releasing low molecular weight aromatic molecules, thus increasing the profitability of biofuel production. We are studying lignin-degrading enzymes, and synergies among them, from a variety of fungi and bacteria that degrade and metabolize lignin. A combination of biochemical analyses, structural biology, and protein engineering techniques are presently being employed to characterize and optimize native enzymes along this pathway. The work presented here focuses on a fungal versatile peroxidase from *Pleurotus ostreatus*, which is influential in the first steps of lignin depolymerization, and on an *O*-demethylase, LigM from the lignin metabolizing bacteria *Sphingobium* sp. SYK-6, involved in late stage lignin degradation – the opening of lignin-derived aromatic rings. LigM is predicted to have a novel fold, as it shares minimal sequence homology with proteins of known structure. Thus, we are characterizing LigM using X-ray crystallography and HPLC-based kinetic assays and currently have LigM protein crystals that diffract to 1.75Å.

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