Engineering a Cytochrome P450 System for Oxidative Demethylation of Lignin-Related Aromatics

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The Center for Bioenergy Innovation (CBI) is a multidisciplinary center with the vision of *accelerating domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations across the bioenergy supply chain.* The CBI Lignin Valorization Team aims to integrate lignin refining, lignin depolymerization, microbial conversion of lignin-related compounds, and production of new materials from lignin polymers to enable a holistic biorefinery paradigm. The microbial conversion aspect of this effort requires new and improved biological platforms, including bacteria designed to simultaneously catabolize a variety of lignin-related compounds and convert them to valuable products – a process known as "biological funneling".

Abstract: Biological funneling of lignin-related aromatic compounds is a promising approach for valorizing its catalytic depolymerization products. Industrial processes for aromatic bioconversion will require efficient enzymes for key reactions, including demethylation of *O*-methoxy-aryl groups, an essential and often rate-limiting step. The GcoAB cytochrome P450 system comprises a coupled monoxygenase (GcoA) and reductase (GcoB) that catalyzes oxidative demethylation of the *O*-methoxy-aryl group in guaiacol, which serves as the base unit for G lignin. We employed structure-guided protein engineering and detailed biochemical assays to identify mutants of the GcoA monooxygenase that catalyze *O*-demethylation of syringol (the base unit of S lignin) as well as the aromatic aldehydes *o*- and *p*-vanillin. One variant, GcoA-T296S, was utilized for *in vivo* demethylation of *p*-vanillin in *Pseudomonas putida*, an industrially relevant bacterial host. We are also combining structure-guided design with high throughput enzyme evolution screens to identify variants of GcoA that can accept vanillate, a carboxylic acid, as a substrate. This will lay the foundation for a larger effort to compare cytochromes P450 with other enzymatic paradigms for aromatic *O*-demethylation and thereby establish the most efficient strategy for biological funneling.

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