Kinetic modeling of the phenylpropanoid pathway in Arabidopsis

Longyun Guo[1]* (guo165@purdue.edu), Peng Wang[1], Rohit Jaini[2], Natalia Dudareva[1], Clint Chapple[1] and **John Morgan**[1, 2]

[1]Department of Biochemistry, Purdue University, West Lafayette, IN; [2]School of Chemical Engineering, Purdue University, West Lafayette, IN-47907

Project Goals: This project aims to rationally manipulate lignin metabolism resulting in reduced costs for biofuel production. There are 11 enzyme families involved in lignin monomer biosynthesis from phenylalanine, which makes impractical a comprehensive experimental approach to search for the optimal combinations of genetic targets for pathway manipulation. We are developing a kinetic model for lignin biosynthesis in wild type Arabidopsis, and validate with time course intracellular metabolite measurements in various mutant lines. Such a kinetic model will serve as the basis for reliable and rigorous *in silico* analysis of genetic targets to obtain desired pathway features. Meanwhile a competing pathway towards the biofuel 2-phenylethanol has also been engineered into Arabidopsis to redirect carbon flux away from lignin, and we have included this route into our kinetic model.

Lignin is the second most abundant polymer in the plant cell wall, which is essential for normal growth because of its cross-linking property and hydrophobicity. On the other hand, lignin impedes the efficient breakdown of lignocellulosic biomass for industrial applications. Precursors of lignin are synthesized from phenylpropanoid metabolism in plants, which makes the genetic engineering of this pathway a promising strategy to manipulate lignin content and composition for improved biofuel yield. Although individual enzymatic steps in phenylpropanoid metabolism have been well characterized, a systematic scheme connecting all the steps into a single model is lacking. Kinetic modeling combined with *in vivo* time-course metabolite profiling provides a mechanistic and biologically relevant way to understand the plant metabolism, from which reliable predictions can be made to guide metabolic engineering design. We selected Arabidopsis primary stem as the experimental system for modeling lignin formation. In order to obtain *in vivo* measurements for model training, excised 5-week-old stems were fed with different concentrations of $^{13}C_6$-ring labeled phenylalanine, and both the amount and isotopic enrichment of downstream intermediates were quantified with LC-MS/MS at multiple time points after feeding. Maximal activities of phenylalanine ammonia lyase (PAL) and 4-coumarate: CoA ligase (4CL) were determined along the same feeding period, and averaged lignin deposition rate was estimated from total lignin content over development. A kinetic model for general phenylpropanoid metabolism in Arabidopsis was then constructed, with the parameters identified through fitting the model's outputs with training datasets, and validated with another dataset from an independent experiment. The current model is able to capture pathway dynamics over a wide range of feeding treatments, and can be used to explore *in vivo* metabolic behaviors under different conditions.

This research is supported by the award DE-SC0008628 from the Office of Biological and Environmental Research in the US Department of Energy.