Measurement and modeling of phenylpropanoid metabolic flux in Arabidopsis

Peng Wang[1*] (wang1155@purdue.edu), Longyun Guo,[1] Rohit Jaini,[2] Antje Klempien,[3] Rachel M. McCoy,[4] John Morgan,[1,2] Natalia Dudareva,[1] **Clint Chapple**[1]

[1] Department of Biochemistry, Purdue University, West Lafayette, IN; [2] School of Chemical Engineering, Purdue University, West Lafayette, IN; [3] Department of Agronomy, Purdue University, West Lafayette, IN; [4]Department of Horticulture, Purdue University, West Lafayette, IN

Project Goals: We propose to develop a kinetic model for the shikimate and phenylpropanoid pathways. Kinetic models provide insights into the distribution of flux control, thus permitting more intelligent, predictive and effective design of experiments to modulate fluxes towards pathway end products. For this work, we will compare flux measurements in wild-type Arabidopsis plants to plants that are mutant or down-regulated for genes of the lignin biosynthetic pathway, and, those that have been metabolically engineered to bypass the shikimate dependent branch or direct carbon away from lignin biosynthesis to the production of 2-phenylethanol. The outcomes of our proposed kinetic modeling are to identify what remains unknown about the regulation and control of metabolic fluxes to lignin, and to allow development of strategies and predictions of what targets are the most promising candidates for alteration of metabolic flux to lignin.

Lignin is a complex aromatic polymer that is deposited along with polysaccharides in the plant secondary cell wall. Lignin provides strength and hydrophobicity to plant tissues but impedes the utilization of lignocellulosic biomass for biofuel production. Lignin is derived from the phenylpropanoid pathway, the architecture of which is well understood based upon the biochemical and genetic studies conducted to date. In contrast, we lack a systematic and quantitative view of the factors that control carbon flux into and within this branched metabolic pathway in plants. To explore the control of carbon allocation for phenylalanine and lignin biosynthesis, we have developed a kinetic model of the pathway in Arabidopsis to test the regulatory role of several key enzymes. We first established an experimental system for flux analysis using excised wild-type Arabidopsis stems. We found that excised stems continue to grow and lignify during feeding and showed that distribution of PAL and 4CL activities is consistent with the pattern of lignin deposition. When ring $^{13}C_6$-labeled phenylalanine ([$^{13}C_6$]-Phe) was supplied to excised stems, corresponding isotopologues of a number of intermediates was quantified by LC/MS-MS, and incorporation of [$^{13}C_6$]-ring labeled monolignols into lignin was demonstrated by DFRC/GC/MS. Using this approach, we analyzed metabolite pool sizes and isotope abundances of the pathway intermediates in a time course from stems fed with [$^{13}C_6$]-Phe of different concentrations. The maximal activities of PAL and 4CL stayed constant during the feeding processes, while labeled lignin deposition rate increased as more labeled Phe was available. These results suggested that the availability of substrate Phe is one limiting factor for lignin flux in developing stems. In addition, we extended the feeding system to mutants

that are defective in *PAL1 PAL2* and *4CL1* to investigate the control of these enzymes on lignin flux. These measurements were all used to develop our kinetic model of the lignin biosynthetic pathway.

This research is supported by the Office of Biological and Environmental Research in the US Department of Energy.