Constructing the Nitrogen Flux Maps (NFMs) of Plants

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Website: https://nfluxmap.github.io/

Project Goals: To construct plant N flux maps (NFMs) from plant genomes and to determine both biochemical and system-level functionality of plant N metabolic network.

Abstract: Nitrogen (N) is an essential element of organic molecules, such as amino acids and proteins, but is often limited in plants. Thus, N use efficiency (NUE) directly impacts overall yield and performance of bioenergy and agricultural crops. Improved NUE can also reduce the use of N fertilizers and environmental issues caused by N eutrophication. Despite the critical roles N play in both plant productivity and environmental health, unlike extensively-studied carbon (C) flux map of plant metabolism, little is known about how assimilated N flows through the metabolic network, namely the "**N flux map (NFM)**". **Aminotransferases (ATs)** play pivotal roles in interconnecting different branches of N metabolic pathways, but the multi-substrate specificities of ATs remain largely uncharacterized due to their poor sequence-function relationships and tedious aminotransferase activity assays[1-3]. The main objectives of this project are **to construct plant NFMs from plant genomes and to determine system-level functionality of AT enzymes and plant N metabolic network**. The obtained NFMs will provide a novel framework to advance basic understanding of plant N metabolism and facilitate rational engineering of plants having high productivity with limited N input.

To address this grand challenge, our project makes use of rapidly growing numbers of plant genomes, high-throughput functional characterization platforms, and computational modeling to deduce both biochemical and systems level functionality of ATs and NFMs. Over the past year we have made progress on each aim. In **Aim 1** to construct the framework of NFMs, we generated initial NFMs, the N atomic map of C3 plants, based on models that include primary as well as genome-scale metabolic pathways (i.e. AraCore and PlantSEED models, respectively)[4,5], which will be further expanded to include more N compound-containing metabolic pathways. In **Aim 2**, we have successfully set up high-throughput AT enzyme assay platforms using nanostructure-initiator mass spectrometry (NIMS)[6-8], and have characterized broad substrate specificity of tyrosine and tryptophan ATs from Arabidopsis. The detailed kinetic analyses of these enzymes further confirmed the findings from the NIMS assay. Additional ATs are currently being generated by various means—wheat germ *in vitro*[8,9], *E. coli*, and *Nicotiana benthamiana* expression systems—to screen their substrate specificities. These biochemical data will be used to further

refine the NFMs and also mapped onto AT phylogeny and sequence similarity network to infer AT functions from other species. In **Aim 3** for validating the NFM and using it in reaction flux estimation, ^{15}N-labeled precursor feeding has been set up using Arabidopsis hydroponic growth system, and the time-dependent incorporation of ^{15}N labeling was observed in major N-containing compounds (e.g. amino acids) were obtained. After further optimization (e.g. to determine metabolic steady state), the kinetic ^{15}N labeling data will be integrated into the N atomic map (from Aim 1) and be used to determine which reactions carry flux in specific conditions by using non-stationary metabolic flux analysis (MFA) at a genome-scale level.

The resulting NFMs will serve as a novel framework to i) elucidate how N flows through the plant metabolic network in a quantitative manner, ii) simulate how plant metabolism responds to different N availability at a systems level, and iii) identify potential targets for improving N use efficiency. We will also establish **open source public databases and pipelines** in DOE Systems Biology Knowledgebase (KBase) for other researchers to be able to predict AT functions and construct NFMs from any given plant genomes.

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