Title: Development and usage of atomic mapping for estimation of nitrogen fluxes in plant metabolic networks

Authors: Sebastian Huß,[1,2] Presenting Author* (shuss@uni-potsdam.de), Rika Judd,[3] Kaan Koper,[3] Hiroshi A. Maeda,[3] and **Zoran Nikoloski**,[1,2]

Institutions: [1]Bioinformatics, Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany; [2]Systems Biology and Mathematical Modeling, Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany; and [3]Department of Botany, University of Wisconsin-Madison, Madison, WI, USA

Website URL: 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 limiting for important traits, like biomass, growth, and yield. In addition, the supply of N in the soil is often limited, and the distribution of N throughout plant metabolic pathways is elusive. Understanding the impact that N has on the overall (re)distribution of cellular resources can provide insights useful for improving N use efficiency of model plants and crops such as determining rate limiting pathways that hinder N flow and therefore plant growth. One way towards achieving this goal is to assemble the **N flux map (NFM)**, embedded in large-scale models of plant (Arnold & Nikoloski 2014, Küken & Nikoloski 2019), and use it to estimate reaction fluxes by integration of isotope labeling patterns under different N availabilities. However, the available metabolic network and data resources in plants cannot address this challenge since they are focused on simulation and integration of carbon labeling patterns.

Estimation of intracellular fluxes based on isotope labeling patterns of metabolites in a metabolic network relies on approaches from metabolic flux analysis (MFA) (Wiechert, 2001, Basler, Fernie & Nikoloski 2018). Application of MFA requires a stoichiometric model with **atomic mappings** that are currently not available for most large-scale metabolic network models, particularly in plants. Here we established an automated workflow to apply reliable automated atom mapping (AAM) approaches, such as Reaction Decoder Toolkit (RDT) (Rahman, et al. 2016), on large-scale metabolic models of *Arabidopsis thaliana* to facilitate estimation of fluxes By using atom maps from the MetaCyc database, that cannot be readily used in flux estimation, we show that the atom maps created by RDT are accurate. The established workflow is made available at https://github.com/sebahu/UniversalRDT/ and the resulting atomic mappings will be integrated in KBase.

We further demonstrate the utility of our automated workflow by simulating ^{15}N-isotope enrichment and identifying metabolites which show enrichment patterns that are informative

regarding one or several fluxes and thus need to be measured in ^{15}N-MFA studies of *A. thaliana*. To experimentally examine isotope enrichment throughout plant metabolism, 21-day-old *A. thaliana* Col-0 plants were fed with ^{15}N-labeled nitrate and ammonia over a course of 3 days using a hydroponics (Conn et al. 2013) and the resulting ^{15}N incorporation was analyzed by both GC-MS and LC-MS/MS. Starting with amino acids, we demonstrate ^{15}N label incorporation over 24 hours for the labeled amino acids detected (e.g., phenylalanine, alanine) while some other compounds (e.g. serine, methionine) showed much slower labeling kinetics. These ^{15}N kinetic labeling data for amino acids, as well as other N containing compounds, will be incorporated into the developed atomic mappings to determine reaction fluxes of N in the large-scale models.

In addition to increasing plant biomass to address high demands for important crops, characterizing N flow and building the NFM will also allow the development of metabolic engineering of N pathways towards increase the production of compounds of interest and crop growth. The developed atom mapping of Arabidopsis metabolic network will be further expanded by incorporating additional connections of N flow based on novel aminotransferase (AT) activities identified by high-throughput AT substrate screening within this same project (as presented in a separate poster, Koper *et al.*). Beyond *A. thaliana*, the proposed workflow that we developed so far in *A. thaliana* will be expanded to other plant species, such as sorghum, for which metabolic models have been assembled and will facilitate MFA at the levels of large-scale metabolic networks. The comparison of NFMs from the model dicot and bioenergy monocot plants (*A. thaliana* vs. sorghum) will allow us to identify conserved and unique features of N metabolic network among different plants.

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