

4aj High-Throughput Time-Series Metabolomic Analysis of a Systematically Perturbed Plant System

Harin H. Kanani and Maria I. Klapa

Metabolomic profiling (or Metabolomics) refers to the high-throughput analysis of the metabolic state of a biological system by the simultaneous measurement of the relative concentration of small molecules in the cellular biomass. In the post-genomic era, metabolomic technologies are increasingly being used independently or in combination with gene expression profiles for environmental and genetic phenotyping, metabolic network re-construction, functional genomics, identification of disease biomarkers and screening drug targets.

The aim of this project was to overcome limitations of current metabolomic protocol using Gas Chromatography-Mass spectrometry (GC-MS) and to develop data analysis methodologies that lead to comprehensive understanding of metabolic regulation in a complex eukaryotic system using dynamic metabolomic profiles. This was done as part of a larger, NSF funded integrated metabolomic and transcriptional profiling project carried out in collaboration with The Institute of Genomic Research (TIGR). Following specific goals were pursued during the study, the results of which are presented in this poster:

Improving Metabolomic Protocol

Metabolomics protocol for analysis of polar metabolites using GC-MS was optimized. A novel data normalization and validation strategy was developed, which significantly increased accuracy and reproducibility of the acquired metabolomic measurements. This patented algorithm, addresses the major limitations faced when performing metabolomic analysis using GC-MS.

Acquiring multiple dynamic metabolomic profiles of plants

Arabidopsis thaliana liquid cultures, grown for 12 days under constant light and temperature in B5 Gamborg media were subjected to perturbations of (1) Elevated CO₂ level in their growth environment (2) Osmotic stress through addition of NaCl (3) Trehalose (sugar) signal and (4) Hormone (Ethylene) Signal (5) their growth media by replacing sucrose with glucose; applied individually or in combination. The dynamic metabolomic response of the system (for 30 hours after perturbing the system) was then obtained using the optimized metabolomic protocol.

Data analysis and modeling strategy for time series metabolomic Data

In spite of the advantages of dynamic analysis, most initial metabolomic analysis were limited to snapshot analysis, and data analysis techniques for time series metabolomic data were not available. Further, dynamic metabolomic data contain within them information about metabolic flux redistribution, thus could provide a high-throughput alternative to metabolic flux analysis in eukaryotic system. However data analysis techniques which can reconstruct metabolic flux map from dynamic metabolomic data were not available. As part of my PhD research, the large amount of experimental data obtained was used to develop metabolomic data analysis for (a) identification of individual metabolic fingerprint (b) identification of interaction between simultaneously applied perturbations (c) reconstruction of the flux map from the metabolic profiling data (d) reconstruction of the metabolic regulation network. The methodologies developed were validated in the context of the known *A. thaliana* physiology for understanding the response of the plant system to the applied environmental stresses.

Apart from generating significant information about *A. thaliana* physiology, the present study also provide an extensive example of a quantitative, high-throughput, dynamic analysis of a systematically perturbed complex biological system. In this sense, it contributes in further advancing the computational and experimental systems biology toolbox for the detailed analysis of metabolic regulation in a complex eukaryotic system.