4cr Comprehensive Analysis of Metabolic Pathways through the Use of Multiple Isotopic Tracers

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Fluxes of molecules through metabolic pathways are a fundamental determinant of cell physiology and a necessary parameter to obtain new insights into cellular and disease mechanisms. The tools for determination of metabolic flux are fundamentally different from the tools for measuring static information such as concentration profiles or transcript levels. Currently the most powerful method for metabolic flux determination in complex biological systems is based on the use of stable isotopes. Metabolic conversion of isotopically labeled substrates generates molecules with distinct labeling patterns (i.e. isotopomers) that can be detected by mass spectrometry and NMR. The isotopic enrichments of metabolites in a metabolic system are strongly dependent on the relative fluxes values. Different flux patterns result in significant tracer redistribution and yield different label distributions. We determine metabolic fluxes by solving comprehensive mathematical models that describe the relationship between metabolite labeling patterns and fluxes.

My research is primarily focused on the development and subsequent utilization of new tools and strategies for metabolic flux analysis. Most importantly, a new modeling strategy has been developed allowing us to investigate the full complexity of metabolic systems by probing them simultaneously with multiple isotopic tracers. In addition, rigorous statistical tools were developed to gain insight into the statistical significance of flux estimation results. We have shown how reliable flux confidence intervals can be determined from these highly non-linear systems, and how to quantify the relative importance of various measurements. All these methods were implemented into the software platform Metran (MEtabolic TRancer ANalysis) that allows rational design and comprehensive analysis of isotopic tracer experiments. I have successfully applied this tool to study a number of prokaryotic and eukaryotic systems.

First, the pathway of gluconeogenesis was investigated in cultured primary hepatocytes. Through the combined use of multiple isotopic tracers, both ^{2}H- and ^{13}C-labeled tracers, and GC/MS analysis of glucose labeling we determined all fluxes in the upper gluconeogenic pathway including the rates of all reversible reactions. In a separate study, the complete central carbon metabolism of *E. coli* was investigated using a mixture of ^{13}C-labeled glucose tracers. Here, we quantified the fluxes from a fed-batch fermentation experiment. In two additional collaborative projects the metabolism of glutamine in brown fat cells, and histidine metabolism in yeast were successfully measured.