304e Advances in Metabolic Flux Analysis from Stable Isotope Experiments: Theory and Applications

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My research interests focus on the development and application of novel tools for comprehensive metabolic analysis. Fluxes of molecules through metabolic pathways are a fundamental determinant of cell physiology and a necessary parameter to obtain new insights into disease mechanisms. 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. However, stable isotope techniques are still limited by the computational complexity of the underlying isotopomer networks. Repeated solution of these large-scale and highly non-linear systems is required. For realistic metabolic networks the size of the computational problem limits us to the use of ^{13}C-tracer only. To address this limitation, a new modeling strategy was 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 one can 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 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.