^{13}C-based Metabolic Flux Analysis

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Abstract

The determination of fluxes is an important parameter to define the extent to which enzymes participate in metabolic networks and to simulate organism behaviour to various types of genetic and environmental perturbations. Metabolic flux analysis is the ultimate measurement of metabolic pathway activity during steady-state conditions, operating as a valuable tool in the detection of physiological alterations and to describe cell phenotypes. Since intracellular metabolic fluxes cannot be directly measured, available methodologies often estimate fluxes by applying mass balances around intracellular metabolites and from experimentally determined nutrient uptake and product secretion rates. ^{13}C-isotopic tracing is a technique that can also be used to measure fluxes. When cells are grown on a ^{13}C-labeled carbon substrate, the ^{13}C-labelling pattern in their proteinogenic amino acids can be determined through nuclear magnetic resonance or mass spectrometry (e.g.; GC-MS).

In this work we adapted a sensitive and high-throughput GC-MS method for measurement of metabolic flux distribution based on methylchlorofomate (MCF) derivatization to convert the amino acids into volatile compounds. Using the ^{13}C-labelling distribution of these compounds we determine the metabolic flux ratios in the central carbon metabolism. Great part of this work was the development of a flexible software to calculate flux ratios and estimate flux distribution in different metabolic models. Although our case studies are based on GC-MS coupled to MCF derivatization, this software is generic for different mass spectrometric methods.

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