Metabolomics measures the abundance of metabolites, however, it lacks dynamic information. Increases in metabolite abundance can result from either increased production or decreased consumption and significant changes in flux through a pathway may not result in altered metabolite abundance. Stable isotope label tracing (qualitative flux analysis) has tremendous potential to help address these situations, and allow a deeper understanding of biological systems. In qualitative flux analysis, a stable isotope tracer (typically containing 13C, 15N, or 2H) is introduced into the biological system, and results in changes in the isotope pattern (isotopologue distribution) of downstream metabolites. The approach is particularly useful in cell culture models that are amenable to the introduction of stable isotope tracers. Analysis of the resulting labeling pattern and kinetics of tracer incorporation provides insights into enzyme function, pathway dependence, and the effects of changes in gene expression or protein function.

Targeted qualitative flux analysis begins with a list of pathway metabolites, containing compound names, DB identifiers, formula and target retention times. This target list is generated using Pathways to PCDL software. Oxim Premium provides for
• high performance feature extraction
• analysis of isotope incorporation and isotopologue abundance
• natural isotope abundance correction

Omix Premium provides for
• canonical pathway diagrams from KEGG and BioCyc
• custom pathway diagrams
• flexible data visualization on pathways
• t-test and ANOVA of isotopologues

In this study, a target list of compounds (with molecular formula and expected RT) from the TCA cycle pathway was generated using Pathways to PCDL software and the Agilent METLIN Metabolite PCDL. The pathway generated list was curated in PCDL Manager to remove metabolites not detected in negative mode and to add other metabolites of interest: 2-hydroxyglutarate (2-HG), lactate, pyruvate, aspartate, glutamate, and oxidized glutathione. This simple process builds the target list for use in MassHunter Profinder.

Because of the nature of the isotope abundance relationships for label incorporated metabolites, retention times are required to constrain the search space for extracted ion chromatogram (EIC) extraction. The Batch Targeted Feature Extraction algorithm in Profinder used on unlabeled (control) samples can locate exact compound RTs for use in target list creation. These retention times are added to the target list for isotopologue feature extraction.

In the example, there are potentially four candidates at four different RT’s to be further evaluated. Next, RT and m/z tolerances are dynamically narrowed and all qualifying peaks are re-scored. In this example, the peak shown in green is reported as the isotopologue feature based on
• high coelution score
• highest number of coeluting peaks
• closest peak to target RT

There will be a natural abundance of 13C and other isotopes in unlabeled metabolites. This abundance signal will interfere with calculations of fractional labeling, etc.

The incorporation of label into 2-HG happens much faster than into TCA cycle intermediates, suggesting that the 2-HG biosynthesis proceeds directly from conversion of 2-oxoglutarate via glutamate, rather than a 4-atom incorporation from isocitrate.