Fluxomics

Investigating cellular metabolism on Orbitrap Excidion Pro mass spectrometer by the simultaneous use of differently labeled elemental isotope substrates

Rahul Deshpande[1], Ayush Midha[2], Claire Dauly[3], Bashar Amer[1], Isha Jain[2] and Susan S. Bird[1]
[1]Thermo Fisher Scientific, San Jose, CA, USA; [2]Gladstone Institute, UCSF, CA, USA; [3]Thermo Fisher Scientific, Bremen, DE

Abstract

Purpose:
In this study, a Thermo Scientific™ Orbitrap™ Excedion™ Pro hybrid mass spectrometer is accessed for use in stable isotope workflow.

Methods:
Pano2 cells were grown under different oxygen tensions (21% and 1.5%). Cells were unlabeled or labeled with 13C6 Glucose, 15N2 Glutamine or both 13C6 Glucose and 15N2 Glutamine. Metabolite separation was carried out on a Mixed Mode HILIC (HILIC-AEX) Column coupled to a Thermo Scientific™ Vanquish™ Horizon UHPLC system. Data acquisition was conducted on an Orbitrap Excedion Pro MS, designed for extended dynamic range, enhanced sensitivity, and minimal in-source fragmentation. Thermo Scientific™ Compound Discoverer™ 3.4 software was used for the data processing.

Result
The importance of resolution and mass accuracy is showcased for stable isotope workflows with Orbitrap Excedion Pro MS. Differences are shown in the cells grown at different oxygen conditions.

Introduction

Stable isotope labeling (SIL) in metabolomics is a notable tool to elucidate metabolic pathway associations (tracer analysis) and rate of change of metabolites (flux analysis). Analytical methods employing SIL typically focus on defined pathways of interest employing a targeted approach, yet the interconnected network of metabolic pathways may involve unappreciated pathways. Further, SIL is used to increase confidence in unknown identification and to determine true sample related mass spectral features in untargeted analysis. As the role of SIL in untargeted metabolomics and targeted analysis expands, we explore the importance of resolution and mass accuracy for these analysis.

Tissue Hypoxia

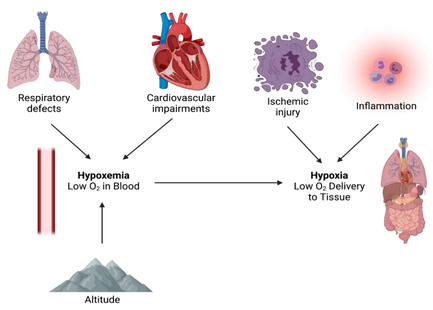

Figure 1. Tissue hypoxia refers to a condition where tissues in the body do not receive enough oxygen to function properly and is involved in various pathologies.

Materials and Method

Panc02 cells

Panc02 cells are derived from mouse pancreatic cancer cells and can be used to study the metabolic adaptability of cancer cells to hypoxia. The cell line was grown under normoxia (21% 02) and hypoxia (1.5% O2) with the following media:

1. Unlabeled Media
2. U-^{13}C Glucose
3. $^{15}N_2$ Glutamine
4. U-^{13}C Glucose and $^{15}N_2$ Glutamine

Metabolite Extraction and Data Acquisition

Metabolites were extracted from harvested cells using methanol followed by sonication. The methanol extract were dried and resuspended in 50% Acetonitrile,. Data acquisition was carried out using different mass resolution settings on the Orbitrap Excedion Pro MS.

Data analysis

Data processing was performed using Compound Discoverer 3.4 software.

Orbitrap Excedion Pro Mass Spectrometer

The new quadrupole-Orbitrap mass spectrometer.

The new system was designed to increase the coverage and sensitivity for measurements of complex samples and enable deeper profiling of metabolites with optimal relative quantitation.

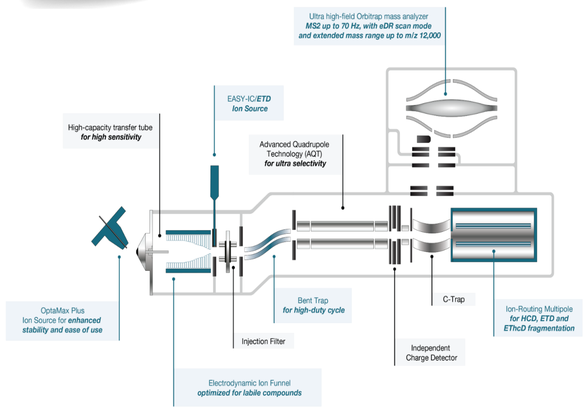

Figure 2. Orbitrap Excedion Pro MS and its schematics.

Mass Resolution

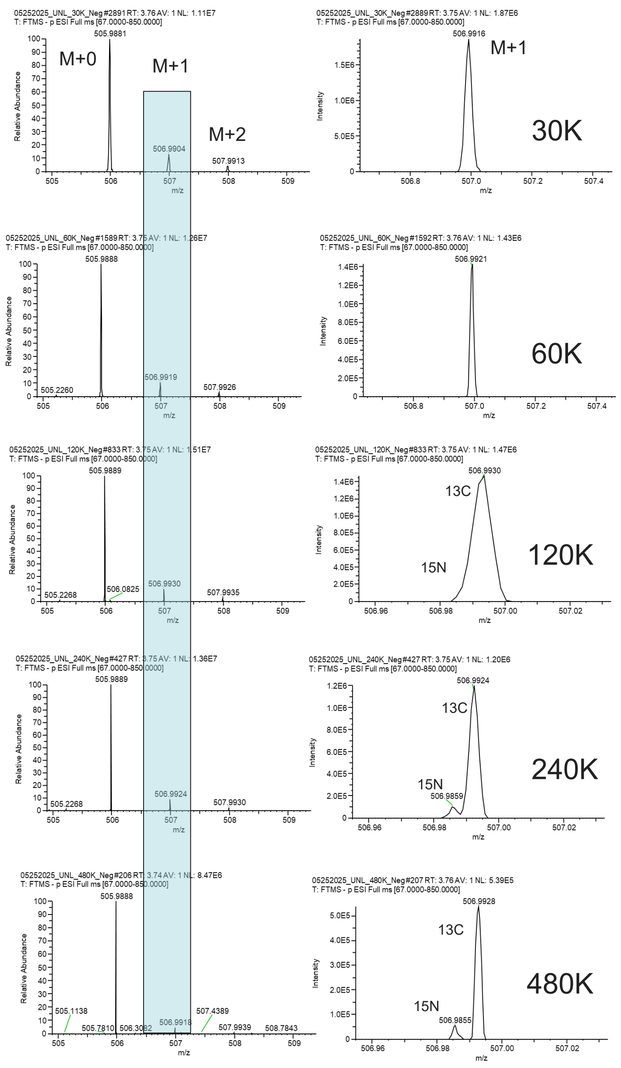

Figure 3. Detection of isotopologue pattern and fine isotope structure is obtained from use of high resolution. Here Adenosine triphosphate (ATP) which is an important energy metabolite is shown. It has a molecular formula of C10H16N5O13P3. Resolution of 240K or more is required for the determination of fine isotopic pattern which can be achieved on Orbitrap Excedion Pro MS.

Mass Accuracy

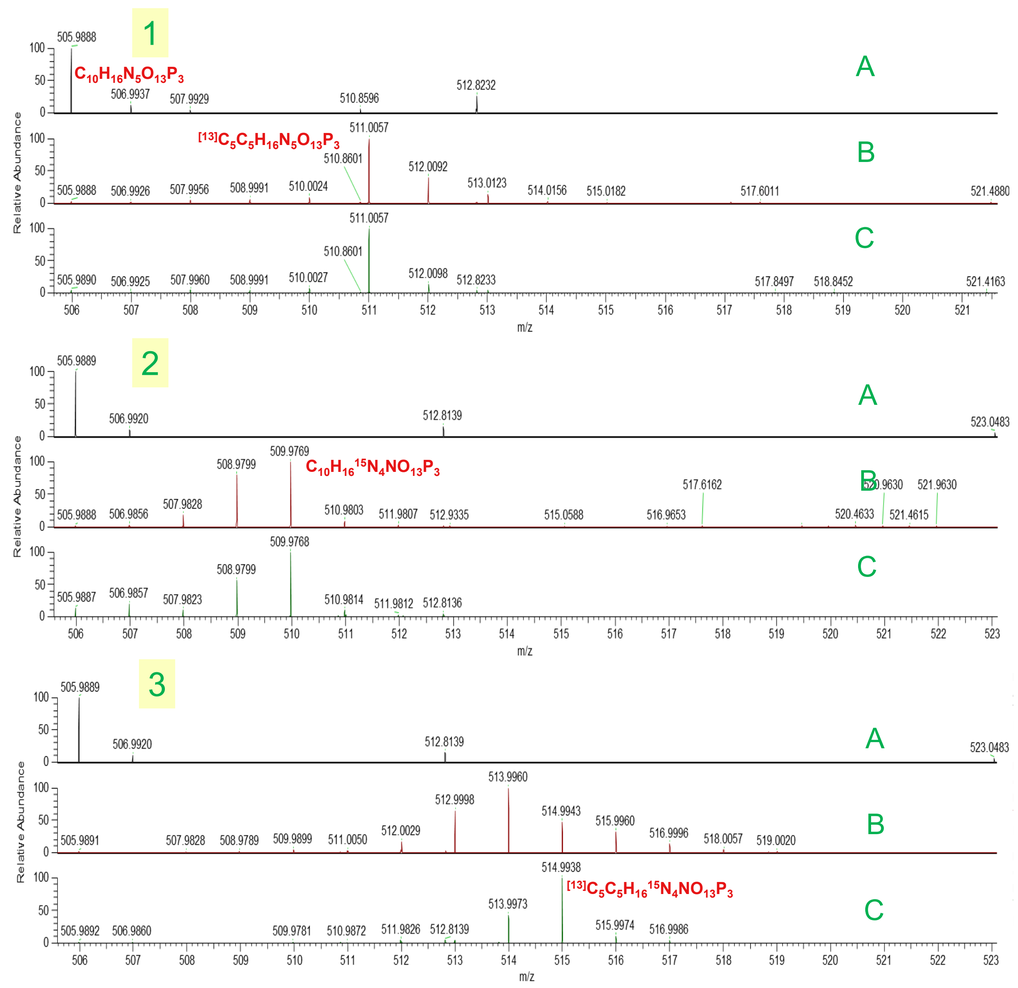

Figure 4. High Mass Accuracy is a prerequisite for accurate determination of isotopologues. Here ATP molecule labeled differentially with 13C and 15N is shown The high mass accuracy (<1ppm) of the instrument can predict the molecular formula of he isotopologues with high precision. 1. Samples labeled with 13C glucose. 2. Samples labeled with 15N glutamine. 3. Samples labeled with both 13C glucose and 15N glutamine. A i: Unlabeled sample. B: Normoxia (21% O2) C: Hypoxia (1.5% O2)

Compound Discoverer 3.4 Software

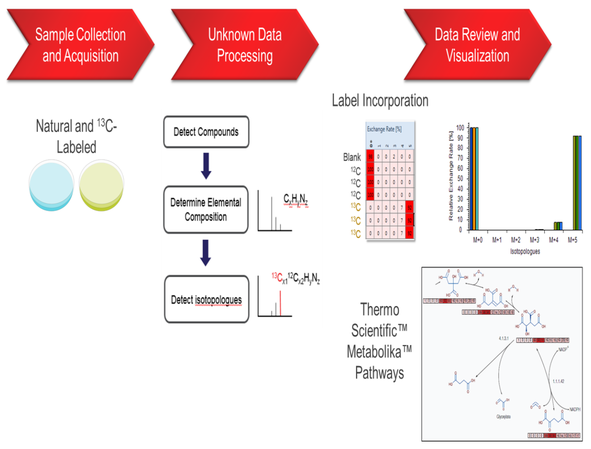

Figure 5. Workflow for SIL in Compound Discoverer 3.4 software

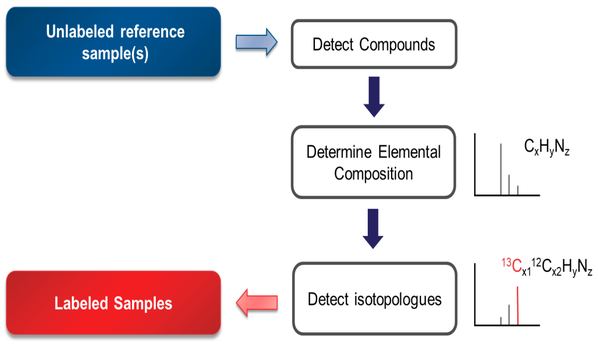

Figure 6. Compound Discoverer 3.4 software automatically detects isotopologues based on compound formulae obtained in reference (unlabeled sample) file

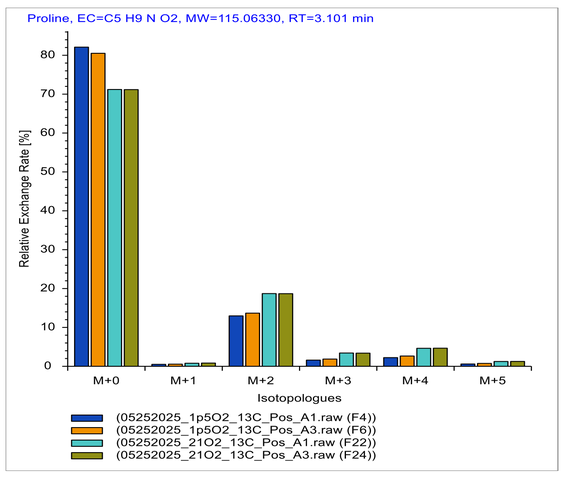

Figure 7. Compound Discoverer 3.4 software plots the 13C relative abundances of isotopologues of detected compounds. Here we see a difference in the labeling of proline in normoxia vs hypoxia., with the proline in hypoxia incorporating less 13C compared to normal conditions.

Conclusion

We have successfully showed the utility of Orbitrap Excedion Pro MS with its high resolution and accurate mass for stable isotope applications using Compound Discoverer 3.4 software. We applied this to study hypoxia in pancreatic cancer cell lines.

Trademarks/licensing

Learn more at thermofisher.com/orbitrapexcedionpro