TWO-DAY COURSE

15 Untargeted Metabolomics: From Basic Methods to Advanced Workflows and Isotope Labeling

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It has become relatively routine to acquire mass spectrometry-based metabolomic data, either in one's own laboratory or using one of the many service facilities around the world. Despite this progress, however, interpreting metabolomic results continues to be a major challenge for many researchers that severely limits potential applications of the technology. The overarching objective of the two-day metabolomics short course is to guide students to overcome this critical interpretation barrier.

Although the challenge of interpreting metabolomic data may seem to be purely informatic in nature, we will discuss how the problem fundamentally starts with poorly designed experiments that adversely affect data quality and unnecessarily complicate results. Opportunities to optimize metabolomic workflows with respect to extraction, chromatography, mass spectrometry, and informatics will be reviewed in great depth and some of the latest technologies in the field introduced. The latter ranges from plug-and-play vendor platforms to R scripts for SWATH-like data acquisition. Focus will be placed on understanding the underlying principles of techniques, rather than simply detailing step-by-step protocols. Untargeted metabolomic datasets that have been thoroughly annotated will be used for instructional illustration of successes and failures of various approaches.

Lastly, we will cover how interpretation of untargeted metabolomic data can be enhanced by complementary analyses such as NMR and Seahorse assays. Particular consideration will be dedicated to the role of stable isotopes in metabolomics. Isotope-based approaches for metabolite identification, quantitation, unbiased fate tracking, and metabolic flux analysis will be reviewed. Experimental considerations ranging from choice of tracer to labeling time and software will be discussed.

Learning objectives:

• Develop an understanding of how to compare performance of untargeted metabolomic methods (e.g., extraction, chromatography, mass spectrometer, etc.). This may be useful to set up a new metabolomics lab or to optimize an existing metabolomic platform.

• Understand challenges that complicate metabolite identification. Develop a working knowledge of strategies to improve success. This may include modifying experimental workflows and/or implementing advanced software algorithms.

• Appreciate the basic principles underlying various state-of-the-art metabolomic workflows and informatic pipelines. This knowledge will enhance data interpretation.

• Gain an understanding of the metabolic insights that NMR, Seahorse, and stable isotope tracing can provide, either when used independently or as a complement to untargeted metabolomics.

Prerequisite: working knowledge of mass spectrometry

Basic course outline:

Overview of untargeted metabolomics
- What are realistic goals?
- How many metabolites are profiled?
- How many unknowns are in a dataset?

Establishing an experimental workflow
- Integral steps
- How do you optimize each?
- Best extraction, separation, and MS approaches

Principles of data processing
- Basic steps and goals
- Theory behind algorithms
- Overview of available software and databases
- Strategies for data reduction
- Translating results to biochemical insight

Barriers to metabolite ID
- Why can't we ID everything?
- Poor assumptions and failures
- Approaches to improve metabolite ID

Advanced metabolomic workflows
- Data-dependent vs independent analysis
- Autonomous metabolomics
- SWATH-like experiments
- Barcoding

Complementary techniques
- Isotope-assisted metabolomics and flux analysis
- NMR-based metabolomics
- Seahorse (respirometry)

Applications