Strategies for Nontargeted Metabolite Profiling using UHPLC/MS in an Open Access Core Laboratory

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Brief history of the MSU facility

- Founded in 1968 by Chuck Sweeley
- NIH Regional Resource for 28 years until 1997
- Converted to open access facility in 2005
- Now consists of 12 mass spectrometers, 3 staff, 1 grad student assistant
Research Interests in Nontargeted Metabolome Analysis at MSU

- College of Agriculture
- Veterinary School
- Two medical schools
- Two DOE bioenergy centers
Organization of Mass Spectrometers

**Nontargeted Profiling**
- GC/quad 1: central metabolites (silylated)
- GC/quad 2: fatty acids (FAMEs, butylamides)
  - GC/quad 3: volatiles
  - GC/quad 4: carbohydrates
- GC/TOF: accurate mass measurements

- QTof 1: specialized metabolites, lipids, carbohydrates, lignin (no ion-pairing)†
  - QTof 2: central metabolites (with or without TBA ion pairing)**
  - MALDI-TOF: metabolite imaging

**Targeted Profiling**
- QQQ 1: amino acids/vitamins*
- QQQ 2: hormones/nucleotides**
- QQQ3: oxylipins/pheromones
- QQQ4: anything else

~ 130 trained users from ~50 PI labs
User fees range from $13-$47/hr
Training in nontargeted metabolomics

• How to not damage the instrument
• Experiment design
• Sample processing
• Analytical method selection
• Data analysis
Nontargeted specialized metabolomics for discovery of plant gene functions

- Breed introgression lines: plants with short inserts of genomics DNA from another genotype
- Rapid screening of metabolic phenotypes

http://zamir.sgn.cornell.edu/Qtl/il_story.htm
Rapid UHPLC-MS profiling of plant specialized metabolites

Schilmiller AL; Shi F; Jones AD; Last RL (2010)
Plant J. 61: 579-90.
Data-independent collision-induced dissociation
Mining for metabolites using fragment ion masses

Virtually all GSH conjugates yield a fragment ion of \( m/z \) 306.08 in negative mode
Stable isotope dynamics in specialized metabolism

Introduce $^{13}$C-labeled precursors to whole plant metabolism

Tomato seedlings $\rightarrow$ $^{13}$CO$_2$ Labeling (pulse/chase) $\rightarrow$ Leaf Dip Extraction $\rightarrow$ LC-MS analysis $\rightarrow$ $^{13}$C enrichment

Acylsugar S4:17 $\quad C_{29}H_{48}O_{15}$ $[\text{M+HCOO}]^-$

Unlabeled control 0.15 mol% $^{13}$C  Labeled sample 8.62 mol% $^{13}$C
Multiplexing CID aids measurement of labeling of substructures with minimal overlap

- Large numbers of heavy isotopes may lead to overlapping features
- Energy-resolved CID helps resolve fragments

Sensitive targeted profiling using TOF-MRM
Simultaneous nontargeted and sensitive targeted profiling using TOF-MRM

- TOF-MRM mode rivals limits of detection of high-end triple quadrupoles
- Targeted and nontargeted profiling performed in a single analysis

5 µL of 10 pM solution injected (50 amol)
Summary

• Data-independent multiplexing of CID yields extensive information in nontargeted profiling

• User training is key to method performance and minimizing operating costs
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