New instruments and services

Árpád Somogyi
July 26, 2022
1. We provide advanced mass spectrometry-based proteomics, metabolomics, structural characterization of protein complexes, and general mass spec analytical services to OSU members, other universities, and industry.

2. We provide innovative proteomics and metabolomics data analytics and bioinformatics platforms.

3. We provide consultation with investigators on experimental design, grant/manuscript support/writing, and training users on the operation of MS.

https://www.ccic.osu.edu/MSP
Location: Biomedical Research Tower (BRT), Room 250

MAIN SERVICES

- **Proteomics**
  - Protein identification from simple and complex samples, PTMs, quantitation

- **Metabolomics**
  - Small molecule metabolites and/or lipid identifications, untargeted or targeted, multiple types of chromatography (reverse phase, HILIC, normal phase)

- **NIH P41 native MS/structural biology**
  - Structural characterization of protein:protein, protein:lipid, and protein:DNA/RNA complexes

- **MALDI tissue imaging**
  - Proteins, lipids, and small molecule metabolites

- **General mass spec analysis**
  - Multicomponent mixtures (natural organic matter), synthetic polymers and chemicals, ultrahigh resolution (1M) and mass accuracy (< 1ppm)

- **Data processing/statistical analysis/bioinformatics**
  - Data mining and visualization, single variate and multivariate statistical analysis and pathway analysis with in-house pipeline scripts using R, Python and Nextflow
**MAIN INSTRUMENTATION**

- Bruker 15T Solarix XR FT-ICR **Ultra-high Resolution** MS (S10 and IRP award)
- Bruker timsTOF Pro **ion mobility** MS/MS (NIH S10)
- Thermo LTQ Orbitrap Fusion for Proteomics (NIH S10 and IRP award)
- Thermo Quantiva Triple Quadrupole for Quant. Metabolomics (S10 and IRP award)
- Bruker amaZon ETD for MS/MS structural elucidation and nominal Mass
- Bruker UltrafleXtreme MALDI-TOF/TOF MS for imaging and large proteins (CCC IRP award)
- Thermo Orbitrap QE Plus (2) – for qualitative Metabolomics and Proteomics (2)
- Thermo LTQ Orbitrap (XL) – for nominal mass
Advantages:

- Can separate components based on mass alone
- Does not rely on LC for good separation
- Also, an ideal instrument for synthesis products!

Isotopic fine structure

http://www.matrixscience.com
Thermo Q Exactive Plus (2)

- Resolving power up to 140,000 FWHM and high mass accuracy <1ppm
- Fast scanning at 12hz and spectral multiplexing suited to UHPLC applications
- Rapid polarity switching in both MS and MS/MS modes
- In-source CID, and HCD.
- Extended mass range to 6,000m/z
- Great dynamic range (>4 orders of magnitude), along with femtogram-level sensitivity
- High spectral quality and a wide dynamic range make it possible to identify and quantify even low abundant proteins.
- Quan/qual capability make it easier to transition from proteomic discovery experiments to targeted protein quantification
Applications

- Bottom-up proteomics/untargeted metabolomics
- 1D and 2D discovery proteomics analysis
- Intact peptide and protein analysis
- Analysis of PTMs
- Targeted proteomics studies
- Quantitation-SILAC, label-free approaches
- Untargeted metabolomics
New, fast autotune improves sensitivity up to 5x for small molecule applications

Autotune technology also helps preserve fragile compounds for improved detection

New Agilent high voltage pulser and power supplies increase mass resolution performance and enhanced mass accuracy to 0.8 ppm

New ion optics elements provides greater robustness

New ion beam shaping optics provide more consistent results and longer usage time between cleanings

CID fragmentation

Applications: untargeted metabolomics
Bruker 15T FT ICR-MS

- Ultimate mass resolving power in excess of RP = 10,000,000
- Apollo II Ion Funnel Electrospray Source enabling high ion transmission efficiency over a broad mass range
- Optimized ion optics for sensitivity and mass range coverage
- Proprietary ParaCell ICR cell with patented SIDEKICK™ ion accumulation system for high detection efficiency.
- Wide range of fragmentation techniques including CID and ECD
- Dual ionization source: MALDI source with Smartbeam II laser and ESI source
Bruker 9.4 T FT-ICR Apex-Qh Tandem Mass Spectrometer

MalDI

Dual source, MS only, no ion selection

ESI

QCID (eV) MS/MS (or SICD MS/MS)

ECD cathode
Bruker 15T FT ICR-MS applications

- High End Proteomics Studies (Top-down and Bottom-up workflows)
- PTM Investigation
- Accurate de novo sequencing
- Molecular Imaging of Tissue—Distribution of Drugs, Metabolites, and Biomarkers
- Petroleum Product Analysis
- Complex Environmental Sample Analysis
- Metabolomics Research
The longer the transient, the better the resolution!

- **C\textsubscript{9}H\textsubscript{16}N\textsubscript{5}**
  - Mass: 194.1405

- **C\textsubscript{7}H\textsubscript{12}N\textsubscript{7}**
  - Mass: 194.1154

- **C\textsubscript{11}H\textsubscript{20}N\textsubscript{3}**
  - Mass: 194.1657
Modified van Krevelen diagram for LDI ions

Red: positive LDI (FT-ICR)
Blue: negative LDI (FT-ICR)
Thermo Quantiva

- Thermo Scientific™ active ion management (AIM™) technology — electrodynamic ion funnel, ion beam guide with neutral blocker, Thermo Scientific™ HyperQuad™ quadrupole mass filter, and active collision cell — enables attogram-level sensitivity
- Ultrafast selected-reaction monitoring (SRM) of 500 SRM/s, with up to 30,000 definable SRMs, enables quantification of more compounds in less time
- Intuitive drag-and-drop method editor software with application templates simplifies method development and operation
- Applications: Targeted metabolomics
Thermo TSQ Quantiva

- Ion Max NG ion source
- High-capacity transfer tube (HCTT)
- Electrodynamic ion funnel (EDIF)
- Ion beam guide with neutral blocker
- Active collision cell with axial DC fields
- HyperQuad quadrupole mass filter
- HyperQuad quadrupole mass filter
- Dual-mode discrete-cygnalode detector
Bruker timsTOF Pro

- Ion mobility time-of-flight mass spectrometer
- Inclusion of IM permits differentiation between modified peptides of same mass-different PTM sites in the same peptide
- Increased sensitivity due to ion trapping with virtually 100% duty cycles: Allows in-depth analysis from very small sample sizes, with 50-250 ng injections routine
- High dynamic range: Allows low abundance proteins to be identified in presence of many higher abundance proteins
- High MS/MS fragmentation rate: Enables sequencing of many peptides, and hence greater number of protein identifications within shorter analysis time
The timsTOF fleX is a fully functional high speed, high sensitivity ESI instrument for all your Omics analyses with integrated MALDI source for fast MALDI Imaging
Ion mobility

The gas flow "behind" the ions and the electric potential allow for accumulation and staging of ions separated by their collisional cross sections and charges.

Selected precursors are ejected from the device by scanning (lowering) the retarding potential in the second stage.
Without ion mobility separation these overlapping precursors would give chimeric MSMS spectra
Without ion mobility separation these overlapping precursors would give chimeric MSMS spectra.

Those two peptides are co-eluted

They are 0.0009 Da apart

Distinguishing them, based on resolution only, would have required a resolving power > 900 000!

… and that alone would not have allowed to isolate them separately.

PASEF (Parallel Accumulation Serial Fragmentation)

DOES IT...

@ > 100 Hz MS/MS

While preserving sensitivity!
Bruker timsTOF Pro: Iosbaric peptides
TIMS separation of co-eluted isobaric HEK Phosphopeptides

- Similar sequences
- Several phosphorylation sites on the sequence
- Probable isoforms
New services

- Proteomics Quantitation-label free, TMT (Tandem Mass Tag), SILAC (Stable Isotope Labeling Amino Acid in Cell culture)

- MADLI Tissue Imaging

- Top-down Proteomics
## ORBITRAP EXPLORIS 480 MASS SPECTROMETER

### Making Genius Simpler

- **480,000 Resolution** to resolve spectra interference and enable data certainty
- **Maximized proteome** coverage and quantitation with FAIMS Pro interface
- **Novel scan modes** for higher throughput without compromising sensitivity, precision and accuracy
- **Robust and reliable design** for maximum uptime
- **Application modes** with best-practice default parameters and drag-n-drop methods templates for portability from system to system
- **Next generation user interface** common with TSQ Triple Quad MS and Orbitrap Tribrid MS systems

### Specifications

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Mass range</td>
<td>40 - 6000 m/z (8000 m/z optional)</td>
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<tr>
<td>Quad isolation</td>
<td>down to 0.4 u</td>
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<tr>
<td>Scan rate MS²</td>
<td>up to 40 Hz</td>
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<td>Max resolution</td>
<td>480k at m/z 200</td>
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<td>Dynamic range</td>
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<td>Mass Accuracy</td>
<td>3 ppm RMS ext., 1 ppm RMS int.</td>
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<td>Dissociation</td>
<td>Higher energy Collisional Dissociation (HCD)</td>
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<td>Analyzer</td>
<td>Quadrupole, Orbitrap</td>
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<tr>
<td>Compact</td>
<td>530 x 760 x 700 mm (w,d,h)</td>
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<tr>
<td>Options</td>
<td>BioPharma , FAIMS Pro</td>
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</table>
Proteome Discoverer 3.0

TMT SAMPLES C,D,E – DATA PROCESSING

• SEQUEST HT + INFERYS Rescoring
• Database: Homo sapiens (Swissprot canonical - retrieved 03/02/2022) –20,306 sequences
• Enzyme: Trypsin (full specificity), 2 missed cleavages
• Mass tolerances: 10 ppm precursor/0.02 Da fragment ion
• Variable M oxidation
• Static C carbamidomethylation, Static TMTpro (N-term), Static TMTpro (K)
• 1% FDR filtering

TMT Quantification
• Protein ratios based on protein abundances
• P-value calculations: t-test (background based)
• Normalization based on total peptide amount
• Scaled to Pooled1-(3x) channels
• Co-isolation threshold: 50%
MOUSE LIVER—TIC NULL1-4 AND OE5-8

Null 1-4

OE 5-8
# All Mouse Liver IDs

## Proteins

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<th>SEQUEST</th>
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<th>SEQ + INF + CHIM</th>
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<th>SEQ + INF</th>
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6001 proteins identified across entire dataset!
A tandem mass tag (TMT) is a chemical label that facilitates sample multiplexing in mass spectrometry (MS)-based quantification and identification of biological macromolecules such as proteins, peptides and nucleic acids.

Stable isotope labeling by amino acids in cell culture (SILAC) is a simple, robust, yet powerful approach in mass spectrometry (MS)-based quantitative proteomics. SILAC labels cellular proteomes through normal metabolic processes, incorporating non-radioactive, stable isotope-containing amino acids in newly synthesized proteins.
Top Down vs Bottom up

Top-down proteomics

1. Protein extraction
2. Separation by gel or LC
3. MS
4. Protein MS
5. MS/MS
6. Data Analysis
7. Protein ID Quantitation
8. PTM mapping

Bottom-up proteomics

1. Protein extraction
2. In-gel or in-solution digestion
3. Peptide MS
4. MS Separation by LC
5. MS/MS
6. Data Analysis
7. Protein ID Quantitation
8. PTM mapping
Protein Identification by Top Down MS/MS

Parents ions (750 - 770, 15+)

754.7814 +1Ac + 2Me
758.0864 + 4Ac + 5Ac
+ 2Ac + 2Me

Histone H4

ECD MS/MS

m/z
Protein Identification by Top Down MS/MS

Known Modification

Modification Detected

$500 on Fusion or ICR

Slide courtesy of Liwen Zhang