Mass Spectrometry and Proteomics

Lecture 1
30 March, 2010

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## Course Outline

<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecture 1</td>
<td>Tuesday, March 30: Mass Spectrometry Fundamentals: Instrumentation; ion optics, resolution and mass accuracy</td>
</tr>
<tr>
<td>Lecture 2</td>
<td>Wednesday, March 31: MS based methodology in system biology proteomics - sample preparation gel-based/LC methods - topdown/bottom up Microfluidics and MS</td>
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<tr>
<td>Lecture 3</td>
<td>Tuesday, April 6: Posttranslational modifications phosphorylation glycosylation/O-GlcNAc ubiquitination methylation/acetylation - histones cross-linking/other chemical biology methods.</td>
</tr>
<tr>
<td>Lecture 4</td>
<td>Friday, April 9: Infomatics for MS preprocessing - deisotoping simulated spectra/spectral libraries database search engines denovo sequencing algorithms</td>
</tr>
<tr>
<td>Lecture 5</td>
<td>Tuesday, April 13: Quantification chemical/metabolic labeling label free H/D exchange/protein turnover</td>
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What is Mass Spectrometry?

**IUPAC** Definition: The branch of science dealing with all aspects of mass spectrometers and the results obtained with these instruments.

**My** Definition: An analytical instrument that measures the mass-to-charge ratio of charged particles.

**Applications:**

1. identification
2. Quantification
3. Molecular structure
4. higher-order structure (H/D exchange, cross-link)
5. gas-phase ion chemistry
6. tissue imaging
What do we use Mass Spectrometry for in this course?

1. Protein identification, either by direct protein analysis, or by digesting the protein into smaller pieces (peptides), then identifying the peptides.
   - Complex mixture; e.g. cell organelle
   - Immunoprecipitation of protein of interest
     - ID binding partners
2. Identification of post-translational modifications: e.g. phosphorylation, acetylation.
3. Quantifying relative differences in protein/peptide levels between related samples.
4. Quantifying changes in post-translational modifications.
Outline: Lecture 1

• Mass Measurement
  – Mass definitions
  – Isotopes
  – Characteristics of a mass spectrum

• Instrumentation
  – Ion sources
  – Fragmentation methods
  – Mass analyzers
  – Ion detection methods
Isotopes and Mass Measurement
Mass Definitions

Molecular masses are measured in Daltons (Da) or mass units (u).
One Dalton = 1/12 of the mass of a $^{12}\text{C}$ atom.

**Monoisotopic mass** = sum of the exact masses of the most abundant isotope of each element present, i.e., $^1\text{H}=1.007825$, $^{12}\text{C}=12.000000$, $^{16}\text{O}=15.994915$, etc.

This is the most accurately defined molecular mass and is preferred if a measurement of it can be determined.

**Average mass** = sum of the averaged masses (“isotope abundant weighted”) of the constituent elements of a given molecule.

The result is a weighted average over all of the naturally occurring isotopes present in the compound. This is the common chemical molecular weight that is used for stoichiometric calculations (H=1.0080, C=12.011, O=15.994, etc.). The average mass cannot be determined as accurately as the monoisotopic mass because of variations in natural isotopic abundances.

**The mass to charge ratio (m/z).** A quantity formed by dividing the mass (in u) of an ion by its charge number; unit: Thomson or Th.
Isotopic Abundances of Common Elements

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotope</th>
<th>Mass</th>
<th>Natural Abundance</th>
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<tbody>
<tr>
<td>H</td>
<td>(^1\text{H})</td>
<td>1.0078</td>
<td>99.99%</td>
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<tr>
<td></td>
<td>(^2\text{H})</td>
<td>2.0141</td>
<td>0.015</td>
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<tr>
<td>C</td>
<td>(^{12}\text{C})</td>
<td>12</td>
<td>98.89</td>
</tr>
<tr>
<td></td>
<td>(^{13}\text{C})</td>
<td>13.0034</td>
<td>1.11</td>
</tr>
<tr>
<td>N</td>
<td>(^{14}\text{N})</td>
<td>14.0031</td>
<td>99.64</td>
</tr>
<tr>
<td></td>
<td>(^{15}\text{N})</td>
<td>15.0001</td>
<td>0.36</td>
</tr>
<tr>
<td>O</td>
<td>(^{16}\text{O})</td>
<td>15.9949</td>
<td>99.76</td>
</tr>
<tr>
<td></td>
<td>(^{17}\text{O})</td>
<td>16.9991</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>(^{18}\text{O})</td>
<td>17.9992</td>
<td>0.2</td>
</tr>
<tr>
<td>P</td>
<td>(^{31}\text{P})</td>
<td>30.9737</td>
<td>100</td>
</tr>
<tr>
<td>S</td>
<td>(^{32}\text{S})</td>
<td>31.9721</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>(^{33}\text{S})</td>
<td>32.9715</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>(^{34}\text{S})</td>
<td>33.9679</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>(^{36}\text{S})</td>
<td>35.9671</td>
<td>0.02</td>
</tr>
</tbody>
</table>

By coincidence, the most abundant isotope of common elements has the lowest mass.
Mass spectrum of peptide with 66 C-atoms (14 amino acid residues)

EGVNDNEEGFFSAR

$^{12}\text{C}_{66}^{1}\text{H}_{95}^{14}\text{N}_{19}^{16}\text{O}_{26}$

Monoisotopic Mass
1569.66956

No $^{13}$C atom

$\Delta m \approx 1$

$^{12}\text{C}_{65}^{13}\text{C}^{1}\text{H}_{95}^{14}\text{N}_{19}^{16}\text{O}_{26}$ etc.

One $^{13}$C atom

Two $^{13}$C atoms

Average Mass
1570.5722
As the number of C-atoms in the molecule increases, the peaks due to higher mass isotopes increase in relative abundance. Data are for a series of peptides.
Protein Mass Measurement

- Protein masses are normally reported as average masses.

Effect of different resolving power on Hemoglobin beta chain peak, $\text{C}_{724}\text{H}_{1119}\text{N}_{195}\text{O}_{201}\text{S}_3$

\[
\text{MW}_{\text{Monoisotope}} = 15,857.2575 \quad \text{MW}_{\text{av}} = 15,868
\]

RP=10000 \hspace{2cm} RP=20000 \hspace{2cm} RP=30000

Monoisotopic peak is not visible!
Information from MS Resolution

Bovine Ubiquitin

\[ MW_{\text{monoisotopic}} = 8559.6158 \text{ Da} \]
Three Important Properties to Assess Performance of a Mass Spectrometer

1. **Sensitivity**
   • Minimum quantity of sample needed (always estimate how much sample you have, in femtomoles!)

2. **Mass Accuracy**
   • Needed for identifying samples by database searching or to determine elemental composition

3. **Resolving Power**
   • Determine charge state. Resolve mixtures. High resolving can also improve mass accuracy.
Peptide Mass Measurement

Monoisotopic (neutral) mass, \( M \) of peptide can be calculated from measured *monoisotopic* mass-to-charge ratio \((m/z)\) and charge state \((z)\) of protonated ion

\[
M_{\text{monoisotopic}} = (m/z)_{\text{monoisotopic}} \times z - M_{\text{proton}} \times z, \quad M_{\text{proton}} = 1.007276
\]

m/z: 785.838 785.782 785.853 785.853
M: 1569.661 1569.549 1569.720 1569.720

RP=400  
RP=800  
RP=1600  
RP=3200
Mass (Measurement) Accuracy

Mass Accuracy or Mass Measurement Error is the difference between the experimental mass \( M_{\text{exp}} \) and the theoretical value \( M_{\text{theo}} \), calculated from elemental composition.

In absolute term, \( MA = M_{\text{exp}} - M_{\text{theo}} \), in Da or milli-Da

In relative term, \( MA = \frac{M_{\text{exp}} - M_{\text{theo}}}{M_{\text{theo}}} \), unit-less (ppm for high resolution MS)

Example:

\[
\begin{align*}
M_{\text{exp}} &= 1569.684 \\
M_{\text{theo}} &= 1569.66956
\end{align*}
\]

Mass Measurement Error = 0.014Da or 9.2ppm

http://physics.nist.gov/PhysRefData/Elements/per_noframes.html
Resolving Power

• Measure of the ability to differentiate between components of similar mass.
• Two definitions:
  • Valley Definition: Neighboring peaks overlap at 10% peak apex height.
  • Full Width Half Maximum (FWHM): Width of a single peak measured at 50% peak apex. This is the most commonly used definition nowadays (because it is simpler).

\[ RP = \frac{M}{\Delta M} \]
Resolution vs Resolving Power

Resolution (Mass) – The smallest mass difference ($\Delta M$) between two equal magnitude peaks such that the valley between them is a specified fraction of the peak height.

-IUPAC definition

For most people in the field, mass resolution and mass resolving power are used interchangeably.
Charge State Determination

**High Resolution**
- isotope peaks resolved

1. counting isotope peaks in ONE m/z unit
2. if the measured spacing of neighboring isotopes is $\Delta(m/z)$, 
   \[ z = 1 / \Delta(m/z) \] or more accurately 
   \[ z = 1.00235 / \Delta(m/z) \]
   1.00235 is the average isotope spacing

**Low Resolution**
- isotope peaks are not resolved

Use neighboring charge states $(m/z)_1$ [higher charge]
and $(m/z)_2$ [lower charge, higher m/z]

Solve the following linear equations
   for $z$ (for $(m/z)_1$) and $M$ (neutral mass)

\[
(m/z)_1 X z - z = M \\
(m/z)_2 X (z-1) - (z-1) = M
\]
Electrospray Mass Spectrum of Bovine Ubiquitin

\[ M_{\text{theo}} = 8559.6112 \]
\[ M_{\text{exp}} = 8559.603 \]

\[ Z = +10 \]
Instrumentation
Mass Spectrometer Schematic

Inlet systems:
- HPLC
- Simple vacuum lock

Ion sources:
- Electrospray (ESI)
- MALDI

Mass analyzers:
- Time-of-flight (TOF)
- Quadrupole
- Ion trap
- FT-ICR
- Orbitrap
Ion sources

MALDI & ESI
Matrix-Assisted Laser Desorption/Ionization (MALDI)

- Analyte is dissolved in solution with excess matrix (>10^4).
- Sample/matrix mixture is dried on a target and placed in the MS vacuum.

Requirements for a satisfactory matrix:

- It must co-crystallize with typical analyte molecules
- It must absorb radiation at the wavelength of the laser (usually 337 nm)
- To transfer protons to the analyte it should be acidic

Typical successful matrices for UV MALDI are aromatic carboxylic acids.

- Sinapinic acid
- α-cyano-4-hydroxycinnamic acid (CHCA)
- 2,5-dihydroxybenzoic acid (DHB)
1. Laser pulse produces matrix neutrals, + and - ions, and sample neutrals: \( M \rightarrow M^* , MH^+, (M-H)^- \) (M= Matrix)

2. Some analyte molecules are ionized by gas-phase proton transfer:

\[
MH^+ + A \rightarrow AH^+ + M \quad \text{(A=Analyte)}
\]

\[
(M-H)^- + A \rightarrow (A-H)^- + M
\]
MALDI Mass Spectrum of Protein Tryptic Digest
Electrospray Ionization

Sample in solution flows into capillary tube

Tube held at +1-5 kV

Nitrogen flowing in outer tube aids nebulization

$\rightarrow M \rightarrow \cdots \rightarrow MH_{n}^{n+}$
Electrospray Ion Formation

Droplets formed in electric field have excess positive ions.
Evaporation of neutrals concentrates charge.
Droplets break into smaller droplets.
Eventually one molecule + $n$ protons is left.
Electrospray ionization
Nanospray

ESI: 1-100µL/min flow

Online analysis

~ 20 µm tip ID

Interface with nanoLC

Flow rate: ~300nL/min

Offline analysis (static infusion)

~ 2 µm tip ID

Flow rate: ~40nL/min

Requires pure sample free from salt
## Ionization Methods for Biomolecule Analysis

<table>
<thead>
<tr>
<th>Electrospray</th>
<th>MALDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Online LC/MS possible</td>
<td>• Very long sample lifetime; repeated measurements possible</td>
</tr>
<tr>
<td>• Poor for mixtures without LC</td>
<td>• Good for mixtures</td>
</tr>
<tr>
<td>• Quantitation possible</td>
<td>• Salt tolerant</td>
</tr>
<tr>
<td>• Good for MW &lt;600</td>
<td>• Matrix peaks can interfere at MW &lt;600</td>
</tr>
<tr>
<td>• Generate highly charged ions</td>
<td>Generate ions with few charges</td>
</tr>
</tbody>
</table>
Mass analyzers

TOF
Quadrupole
Ion Trap
FTICR
Orbitrap
Ion Optics

A device for manipulating ion beams. A mass spectrometer consists of many *ion optical* components.

Einzel lens modeled with SIMION ion optics simulation program (computing electric and magnetic fields and ion trajectories)

http://simion.com/
Time-of-Flight (TOF) Mass Analyzer

- Ions formed in pulses.
- Measures time for ions to reach the detector.

\[ \frac{m}{z} = \frac{2t^2V}{L^2} \quad \text{or} \quad t \propto \sqrt{\frac{m}{z}} \]
Linear and Reflector TOF Analyzers

Reflector compensates for initial variation in kinetic energy, improving resolving power and mass accuracy.
Quadrupole Mass Analyzer/Filter

Uses a combination of RF and DC voltages to operate as a mass filter.
• Mass analyzer.
• Mass selection device
• Ion transport device (RF-only collision cell).

Mass scan and stability diagram
Quadrupole Ion Trap

- Uses a combination of DC and RF fields to trap ions
- Ions are sequentially ejected by scanning the RF voltage

Linear Trap

- Essentially a quadrupole with end-caps
- Advantage: Larger ion storage capacity, leading to better dynamic range
Electron Multiplier

Multi-Channel Plate (MCP)

From Detector Technolgy: http://www.detechinc.com/
Fourier Transform Ion Cyclotron Resonance (FT-ICR)

- Ions trapped and measured in ultrahigh vacuum inside a superconducting magnet.

\[ \omega \propto \frac{1}{m/z} \]
Fourier Transform Ion Detection

Differential Amplifier

FT

Image Current

Bovine Ubiquitin

Calibration

Frequency (kHz)

m/z

Time (ms)

100 150 200 250

1072 1071

1071 1072

12+ 11+ 10+ 9+ 8+ 7+

600 1000 1400 1800

A.G. Marshall
Orbitrap

TOF
- Simultaneous excitation

FTICR
- Confined ion trajectory
- Image current detection
- Fourier transform data conversion

Unique to Orbitrap
- 3D electric field trapping
- No need for magnet
- Easy access
- Final detection device
Image Current Detection in Orbitrap
## Comparison of Analyzer Types

<table>
<thead>
<tr>
<th></th>
<th>Ion Trap/Quadrupole</th>
<th>TOF</th>
<th>OrbiTrap</th>
<th>FT-ICR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>+++</td>
<td>++* to +++</td>
<td>++*</td>
<td>+*</td>
</tr>
<tr>
<td>Mass Accuracy</td>
<td>+**</td>
<td>++</td>
<td>+++</td>
<td>+++**</td>
</tr>
<tr>
<td>Resolving Power</td>
<td>+**</td>
<td>++</td>
<td>+++</td>
<td>+++**</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>+ to +++**</td>
<td>++</td>
<td>+++</td>
<td>+++**</td>
</tr>
<tr>
<td>Upper m/z</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Sensitivity lowered due to losing ions on way to analyzer, rather than inherent sensitivity.
**Can be improved by scanning narrower mass range or slower.
Hybrid/Tandem Instruments

• Combine (1) ion selection, (2) ion dissociation, and (3) mass analyzer devices

  • Quadrupoles and ion traps good for selective isolation of precursor ions and for fragmentation (required for MSMS - Topic of Lecture 2)

  • Reflectron TOF, FT-ICR, and OrbiTrap have higher mass accuracy and resolving power (high mass accuracy is good for identification – Lecture 4)
Ion Isolation

• Quadupole
  Continuous ion beam

• Quadrupole ion trap
  Pulsed-mode operation; space charge issue

• SWIFT in FTICR
  Ultrahigh selectivity; only works well in ICR traps

• TOF
  Only implemented on TOF/TOF
Ion Dissociation

• Collision Induced Dissociation (CID or Collision Activated Dissociation (CAD))
  ion traps: off-resonance excitation
  rf-only multi-poles: higher kinetic energy (HCD) and cascaded CID
  TOF/TOF: single collision

• Electron capture dissociation (ECD) and Electron transfer dissociation (ETD)
  ECD: FTICR, reagent: electron
  ETD: ion traps, reagent: free radical anion

Other important factors to consider: how product ions are collected and detected
Multi-Reaction Monitoring (MRM)

A

phosphopeptide  fragmentation  loss of phosphate

\[ M^{2+} = 1092.0 \]

\[ M^{2+} = 1043.0 \]

B

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Q1</th>
<th>Q3</th>
</tr>
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<tbody>
<tr>
<td>NROVFTTK</td>
<td>537.3</td>
<td>466.3</td>
</tr>
<tr>
<td>MRMDAWVT</td>
<td>545.2</td>
<td>496.2</td>
</tr>
<tr>
<td>NFIAVSAANR</td>
<td>571.8</td>
<td>522.8</td>
</tr>
<tr>
<td>SEPISPPRDR</td>
<td>568.3</td>
<td></td>
</tr>
<tr>
<td>KNFIAVSAANR</td>
<td>617.3</td>
<td></td>
</tr>
<tr>
<td>TNSDIVEALNK</td>
<td>358.8</td>
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<tr>
<td>ISSSGALDDDK</td>
<td>642.3</td>
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<tr>
<td>IQITRIMDER</td>
<td>651.8</td>
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<tr>
<td>NGYASTDMK</td>
<td>677.8</td>
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<td>LFQYASTDMK</td>
<td>699.8</td>
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<tr>
<td>TNSDIVEALNK</td>
<td>706.3</td>
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</tr>
<tr>
<td>TNSDIVEALNKK</td>
<td>709.4</td>
<td></td>
</tr>
</tbody>
</table>

sequence

MRM transitions

mass spec detection
Multi-Reaction Monitoring (MRM)

AB SCIEX QTRAP® 5500 System

AcQuRate™ Pulse Counting Detector

Linear Accelerator™ Trap

Q1 Curved LINAC® Collision Cell

QJet® 2 Ion Guide

Q0 High-Pressure Cell
MALDI-TOF/TOF (4700 Proteomics Analyzer)

- High performance TOF analysis for MS1 and MS2 give high resolving power and good mass accuracy.
- High accelerating voltage allows high energy CID, giving a wider range of fragment ions and facilitating side-chain cleavages that distinguish isomeric amino acids Ile and Leu.

Hybrid Instrument: QqTOF Mass Spectrometer (QSTAR)

Linear Ion Trap – FT-ICR (LTQ-FT)

Linear Ion Trap (LTQ)

Ion Cyclotron
Data Dependent Acquisition

• *Data Dependent Scans*

  MSMS based on intensity ranking of precursor ions

• *Dynamic Exclusion*

  Precursor m/z of previous MSMS are memorized and no MSMS done on them during a defined time period

• *Automatic Gain Control (AGC, unique to ion trap)*

  Control how many ions are scanned – to achieve signal/noise ratio and to minimize space charge effect
Scan Sequence of LTQFT

Time

FT

LT

Pre-scan  Inject  MS/MS #1  MS/MS #2  MS/MS #3

Snap Shot @ 25k RP  Continue FT Acquisition at 100k RP

"Free" (in parallel) MS/MS Data

Thermo Application Note: 30046
Linear Ion Trap - Orbitrap - ETD
Newest Velos - Orbitrap - ETD

Dual Cell Linear Ion Trap
  HP cell: ion accumulation and dissociation
  LP cell: fast detection
  (ASMS2008WPAA039)

S-lens or ion funnel
  (MassSpectromRev2010v29p294)
Key Milestones Leading to LTQ Orbitrap ETD

- **Penning Trap**
  - Magnetic trapping
  - Dehmelt, 1959

- **FTICR**
  - Marshall/Comisarow, 1974

- **Magnetic trapping**
  - FT/IMAGE current

- **ECD**
  - Zubarev/McLafferty, 1998

- **ETD**
  - Syka/Hunt, 2004

- **Kingdon Trap**
  - Electrostatic trapping
  - Kingdon, 1923

- **Orbitrap**
  - Makarov, 1999

- **TOFMS**
  - Stephens, 1952

- **z-instability scan**
  - Stafford, 1985

- **Paul Trap**
  - rf trapping
  - Paul, 1953

- **Simultaneous excitation**

- **LTQ**
  - Schwartz, 2002

- **C-trap**
  - Horning, 2005

**Historical Concept**

**Realized as**

**Mass spectrometer**

Mass spectrometry history: http://massspec.scripps.edu/mshistory/mshistory.php
Linear quadrupole ion trap (LTQ) video clip
Mass Spectrometry Online Resources

NIH NCRR Mass Spectrometry Facility, UCSF

http://ms-facility.ucsf.edu/

American Society for Mass Spectrometry (ASMS)

http://www.asms.org

Molecular & Cellular Proteomics

http://www.mcponline.org