Comparison of mass spectrometers performances

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Mass resolution</th>
<th>Mass accuracy</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadrupole</td>
<td>$1 \times 10^3$</td>
<td>$0.1 \text{ Da}^*$</td>
<td>0.5-1.0 pmol</td>
</tr>
<tr>
<td>DE-MALDI</td>
<td>$2 \times 10^4$</td>
<td>20 ppm</td>
<td>1-10 fmol peptide</td>
</tr>
<tr>
<td>Ion trap</td>
<td>$1 \times 10^3$</td>
<td>$0.1 \text{ Da}^*$</td>
<td>1-5 pmol protein</td>
</tr>
<tr>
<td>FT-ICR</td>
<td>$1 \times 10^6$</td>
<td>&lt;1 ppm</td>
<td>20 amole</td>
</tr>
</tbody>
</table>

*depends on the mass window being used

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Mass spectra of peptides and proteins - and LC analysis of proteomes

Stephen Barnes, PhD
Overview

• A mass spectrum
• Electrospray MS
  – Analysis of intact proteins
  – Molecular weight calculations
  – Max Entropy
• Peptides
  – Purity
• Integration of MS with LC and CE
  – Multidimensional LC
• Tandem MS
  – Identifying modification sites
• MALDI spectra
  – Tryptic fingerprinting
A mass spectrum of several peptides from a tryptic digest
Isotope profile of individual peptide ion
ESI spectrum of bacterially expressed protein

Each ion is \([M+nH]^{n+}\)

For 50+ charge state of a 50 kDa protein,

\[ m/z = \frac{[50,000 + 50]}{50} = 1,001 \]
Ionizing proteins and peptides

- $^+\text{H}_3\text{NCHR}_1\text{CO}(\text{NHCHR}_n\text{CO})_n\text{NHCHR}_2\text{COOH}$ is the ion that’s found in dilute acid solution.

- If there are internal basic residues, then the ions will be of the form $[M+n\text{H}]^{n+}$, where $n = 1, 2, \text{ etc.}$

- A tryptic peptide will have a N-terminal amino group and an amino group from Arg or Lys.
  - If the peptide has a mol. wt. of 1000 Da, then the singly charged ion will have a $m/z$ of 1001, whereas the doubly charged ion has a $m/z$ of 501.
Calculation of molecular weights and ion states

- For two ions in a series for a peptide of molecular weight M, the lower m/z value (x) will be for the n+1 ion state and the larger m/z value (y) will be for the n+ ion state.
  - (1) \( \frac{M+n}{n} = y \)
  - (2) \( \frac{M+n+1}{n+1} = x \)

- Hence
  - (3) \( M+n = ny \) and \( M = ny-n \)
  - (4) \( M+n+1 = (n+1)x \) and \( M = (n+1)x-(n+1) \)

- Hence
  - \( ny-n = (n+1)x - (n+1) \)
  - \( ny-n-xn+n = x-1 \)
  - \( n(y-x) = x-1 \)
  - \( n = (x-1)/(y-x) \)

- The value of n can then be substituted in equation (1) to obtain the molecular weight of the peptide
ESI mass spectrum of ribonuclease

<table>
<thead>
<tr>
<th>Peak (m/z)</th>
<th>Intensity</th>
<th>Charge (est.)</th>
<th>Mol. Wt. (Est.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>978.00</td>
<td>7,778</td>
<td>14.00000</td>
<td>13,677.89</td>
</tr>
<tr>
<td>1,053.00</td>
<td>18,532</td>
<td>13.02656</td>
<td>13,675.90</td>
</tr>
<tr>
<td>1,141.00</td>
<td>59,087</td>
<td>11.95446</td>
<td>13,679.91</td>
</tr>
<tr>
<td>1,245.00</td>
<td>33,275</td>
<td>10.96146</td>
<td>13,683.91</td>
</tr>
<tr>
<td>1,369.00</td>
<td>32,390</td>
<td>10.03219</td>
<td>13,679.92</td>
</tr>
<tr>
<td>1,521.00</td>
<td>35,668</td>
<td>8.99995</td>
<td>13,679.93</td>
</tr>
<tr>
<td>1,711.00</td>
<td>16,624</td>
<td>7.99996</td>
<td>13,679.94</td>
</tr>
<tr>
<td>1,956.00</td>
<td>3,333</td>
<td>6.97955</td>
<td>13,684.94</td>
</tr>
</tbody>
</table>

Cumulative MW estimate = 13,680.29
SD = 2.94
Deconvolution of MS data

• When several proteins are present, then their multiply charged ion clusters overlap

• Can this be overcome - yes, use the MaxEntropy program provided by micromass
**MaxEnt** deconvolution of MWs

15,157.6 ± 0.9 Da

50,011.3 ± 18.3 Da

6xHis-tag BAT

57,195.2 ± 12.9 Da

E. coli GRoEL

**Courtesy of Mindan Sfakianos**
LC/MS of 4HNE-Modified Cytochrome C

Native Cytochrome C: 12356

Cytochrome C + One 4HNE Michael Addition (+156): 12512

Cytochrome C + One 4HNE Schiff Base (+138): 12668

Cytochrome C + Two 4HNE Michael Addition (+312): 12823

Cytochrome C + Three 4HNE Michael Addition (+467): 12823

Courtesy of Amanda Foxwell

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Use of FT-MS in ESI of proteins

- The very high resolving power of FT-MS enables a direct measure of charge state of an individual ion since each peptide or polypeptide will have several/many isotope peaks.

- The distance in Da between successive isotope peaks of a multiply charged ion is the reciprocal of the number of charges.
Bovine Serum Albumin (66 kDa)
4.7 T Actively Shielded Magnet

ESI: BSA

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Bruker Daltonics
Guarantees of purity based on observation of “a single peak by reversed-phase HPLC” and by “it gave the correct sequence when analyzed by Edman degradation” are hollow. The lower spectrum was of a “pure” HPLC peak. The method of purification was amended and the upper spectrum was obtained.
LC-MS of peptide mixtures

Analytical reverse phase column
75 μm i.d. x 15 cm

Flow rate 200 nl/min

Acetonitrile gradient

Collision gas

Q1
Q2

Electrostatic reflector

TOF detector

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Tandem mass spectrometry on a triple quadrupole instrument

• **Daughter ion spectra**
  – The molecular ion is selected in Q1, collided with Ar gas in Q2, and “daughters” analyzed in Q3

• **Parent ion spectra**
  – All molecular ions allowed into Q1, collided in Q2, and a selected daughter ion measured in Q3

• **Multiple reaction ion monitoring (MRM)**
  – A single molecular ion selected in Q1, collided in Q2 and a selected daughter ion measured in Q3. Up to 8 pairs of parent/daughter ions
MUDPIT - MUlti-Dimensional Protein Identification Technology

- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

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John Yates
Pros/Cons of laying down LC or EC separations on matrix plate

- Allows off-line analysis both in real time and then in a retrospective mode
- MALDI-TOF analysis is very fast
- Can do TOF-TOF MS-MS analysis
- BUT what happens chemically on the acidic environment on the surface of the plate during storage
- Also, can the laser beam cause chemical changes?
Example of MS-MS to detect a modification

- Cytochrome c, a mitochondrial enzyme was reacted with 4-hydroxynonenal, an aldehyde formed by oxidation of long chain, unsaturated fatty acids
- Site of attachment believed to be on lysine groups (to form a Schiff’s base)
- However, increase in MW consistent with Michael addition
- Protein hydrolyzed with trypsin
Qtof Tryptic Digest of Control Peptide

100

% 102 159 256 370 483 620 677 790 937 994 1150
T G P N L H G L F G R

Y₇ - 799.462

Y₆ - 686.357

Y₅ - 549.308

B₆ - 620.306

Y₈ - 913.470

Y₉ - 1010.536

Y₁₀ - 1067.535

0

100 200 300 400 500 600 700 800 900 1000 1100 1200

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Amanda Foxwell
Conclusions of experiment

• A peptide was identified that increased its molecular weight by 156 Da

• Tandem MS revealed that the 4-HNE was attached to His-33 to form a Michael adduct
MALDI-TOF MS

- Whole proteins
- Tryptic fingerprinting
- MS-MS
MALDI spectra usually contain only the molecular ion \([\text{M}+\text{H}]^+\). This is an advantage since it maximizes the signal, but is a disadvantage in that it gives no clues as to structure.
Protein analysis by MALDI 2003

MALDI plate

Desalting Ziptip

Eppendorf tube

Incubate overnight

destain

trypsin 1:20

Speed-Vac

Water Bath 37°C

Counts

Mass (m/z)

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MASCOT Search

1016.55
1034.54
1181.59
1461.75
1443.76
1687.98
1950.80
2368.13
2813.32

Trypsin Digest of porin-P1;
Voltage-dependent anion-selective channel
Connecting CE and LC to MALDI analysis

Creates 20 mm wide tracks that can be scanned by MALDI laser for MS analysis

Parallel capture of effluents of 8 nanoLC separations on Mylar - can be scanned simultaneously by fast laser
Post-source decay experiments in a TOF-mass spectrometer

Daughter fragment ions formed in the drift region are separated by the reflector. Suitable resolution only occurs over a limited range of m/z values. This can be overcome by recording individual spectra over a wide range of voltage settings (10-12) for the reflector. Alternatively, a curved applied voltage can be used to obtain the daughter ion spectrum in a single experiment.

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TOF-TOF - high speed MSMS
Decomposition of -NO$_2$ group in MALDI-TOF MS

A

BSA

YLYEIAR

B

BSA + TNM

NO$_2$

C

BSA + TNM + Dith

NH$_2$