What is Mass Spectrometry?

- Mass spectrometry is a powerful tool in analytical/bioanalytical chemistry that provides
  - detailed structural information for a
  - wide variety of compounds (MW: 1-10^6 daltons) by using
  - a small amount of sample (ug, picomol, femtomol)
  - often and easily coupled with separation techniques (GC, HPLC) – *ideal for mixture analysis*
Sample Preparation

Sample Introduction
Direct probe/infusion
GC
HPLC

Vacuum System

Ionization Source
Electron impact (EI)
Chemical ionization (CI)
Atmospheric pressure (API)
Electrospray (ESI)
Matrix assisted laser
Desorption/ionization (MALDI)
Surface enhanced LDI (SELDI)
Fast atom bombardment (FAB)

Mass Analyzer
Electrostatic (ESA)
Magnet (B)
Time-of-flight (TOF)
Quadrupole (Q)
Ion Traps (2D & 3D IT)
Ion-Cyclotron
Resonance (ICR)
Orbitrap (OT)

Detector
Electron Multiplier
Photomultiplier
Faraday cap
Array Detectors
Multichannel plate

Computer

Rough,
Turbomolecular, and
Cryo pumps

Want to do MS or MS/MS?
Need a Mass Spectrometer
Ion movement

- Biased metal plates (electrodes, lenses) used to move ions between ion source and analyzers

- Electrodes and physical slits used to shape and restrict ion beam

- Good sensitivity is dependent on good ion transmittance efficiency in this area
Ion detection

- Ions can be detected efficiently with high amplification by accelerating them into surfaces that eject electrons.
Image Current Detectors

w/ FTICR

RF-excitation

Ion Detection Plates

w/ Orbitrap

Image current

Ubiquitin multiply charged states no selection

Ubiquitin +11 charged state Q selection
Ion energy

- Kinetic energy of ions defined by

\[ E = z e V = q V = \frac{1}{2} m v^2 \]

- \( E \) = kinetic energy
- \( m \) = mass
- \( v \) = velocity
- \( e \) = electronic charge (1.60217e-19 C)
- \( z \) = nominal charge
- \( V \) = accelerating voltage

Learning Check

Consider two electrodes,

one at 1000 V and one at ground (0 V)

\[ \begin{array}{c|c}
1000 V & 0 V \\
\end{array} \]

+ ion will travel with kinetic energy of ____________
Question: Consider an ion source block and an extraction lens. How would you bias the block and lens if you want the ions to be accelerated by

a) 8000 eV (appropriate for magnetic sector)

b) 5 eV (appropriate for entering a quadrupole)

c) 20,000 eV (appropriate for entering a TOF)

Mass Resolution

- Mass spectrometers separate ions with a defined resolution/resolving power

- Resolving power - the ability of a mass spectrometer to separate ions with different mass to charge (m/z) ratios.
Resolution defined at 10% valley

\[ R = \frac{M}{\Delta M} \]

\[ R = \frac{1000}{1} = 1000 \]

Example of ultrahigh resolution in an FTICR

J. Throck Watson “Introduction to Mass Spectrometry” p. 103
“Kaba” meteorite powder
Laser desorption/ionization (LDI)
Bruker 15 T FT-ICR

2e⁻ difference between positively and negatively charged species detected

General about mass spectrometers

• Common features
  – Accelerated charged species (ions) interact with/can be controlled by
    • Electrostatic field (ESA, OT)
    • Magnetic field (B, ICR)
    • Electromagnetic (rf) fields (Q, IT, LT)
  – Or ions just fly (TOF)
For each of the following applications, choose the most appropriate mass analyzer from the following list.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>orbitrap (OT)</td>
<td>synthetic organic chemist wants exact mass of compound</td>
</tr>
<tr>
<td>quadrupole (Q)</td>
<td>biochemist wants protein molecular weight of relatively large protein (MW 300,000)</td>
</tr>
<tr>
<td>time-of-flight (TOF)</td>
<td>EPA (Environmental Protection Agency) wants confirmation of benzene in extracts from 3000 soil samples</td>
</tr>
<tr>
<td>FTICR</td>
<td>Petroleum chemist wants to confirm the presence of 55 unique compounds at one nominal mass/charge value in a mass spectrum</td>
</tr>
</tbody>
</table>

Desirable mass analyzers characteristics
Desirable mass analyzers characteristics

1) They should sort ions by $m/z$

2) They should have good transmission (improves sensitivity)

3) They should have appropriate resolution (helps selectivity)

4) They should have appropriate upper m/z limit

5) They should be compatible with source output (pulsed or continuous)

Mass spectrometers record $m/z$ values

$m/z$ values are determined by actually measuring different physical parameters

<table>
<thead>
<tr>
<th>Type of analyzer</th>
<th>Physical parameter used as basis for separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>electric sector</td>
<td>kinetic energy/z</td>
</tr>
<tr>
<td>magnetic sector</td>
<td>momentum/z</td>
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<tr>
<td>quadrupole, ion trap</td>
<td>$m/z$</td>
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<tr>
<td>time-of-flight</td>
<td>flight time</td>
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<tr>
<td>FT-ion cyclotron resonance</td>
<td>$m/z$ (resonance frequencies)</td>
</tr>
</tbody>
</table>
Types of Mass Analyzers

- Magnetic (B) and/or Electrostatic (E) (HISTORIC/OLDEST)
- Time-of-flight (TOF)
- Quadrupole (Q)
- Quadrupole Ion Trap (IT)
- Linear Ion Trap (LT)
- Orbitrap
- Fourier Transform-Ion Cyclotron Resonance (ICR)

Performance Advantages / Disadvantages / $$$

TOF

Quadrupole

Linear Ion Trap

Orbitrap

FTICR
Notice: accelerating voltages vary with analyzer (has consequences for MS/MS)

- High voltage (keV energy range)
  - magnet (B)
  - electrostatic (E)
  - time-of-flight (TOF)

- Low voltage (eV energy range)
  - quadrupole (Q)
  - ion trap (IT, LT)
  - ion cyclotron resonance (ICR)
  - orbitrap

MS and MS/MS revisited
Simulation of Two-Step Process

Current popular MS/MS arrangements

Tandem in Space
  QqQ
  Q Trap
  Q TOF
  TOF TOF
  LTQ-Orbi (QExactive)

Tandem in Time
  Ion Trap (2 or 3 D)
  FT-ICR (FT)
Decision factors when choosing a mass spectrometer

- Speed
- Resolution
- Sensitivity
- Dynamic range
- Cost

Disclaimers:

We are not intentionally favoring any single manufacturer. We are more familiar with the operation of instruments we use.

Some of our slides involve simplifications. Listeners could likely find an exception for almost any statement we make.
**Time of Flight**

\[ KE = zeV = \frac{1}{2}mv^2 \]

\[ v = \frac{D}{t} \]

\[ \frac{1}{2}m(D/t)^2 = zeV \]

\[ t = \left( \frac{m}{2zeV} \right)^{1/2} D \]

- **m** = mass
- **V** = velocity
- **D** = distance of flight
- **t** = time of flight
- **KE** = kinetic energy
- **e** = charge
How does the ion generation step in TOF influence \( m/z \) analysis?

Consider MALDI

Analyte ion may have (1) Kinetic Energy distribution or (2) Spatial distribution.

How will KE spread influence the spectrum?

Ions of same \( m/z \)

Has slightly greater KE

Effect is broad peaks

c/o Cotter
How will KE spread influence the spectrum?

Solution to peak broadening caused by kinetic energy spread:

Reflectron (ion mirror)

Series of ring electrodes, typically with linear voltage gradient

Time-of-Flight Reflectron

- Increased resolution by compensating for KE spread from the source

http://www.jic.bbsrc.ac.uk/services/proteomics/tof.htm
KE = \frac{1}{2} mv^2
We talked about how to deal with kinetic energy spread.

How do we deal with ions formed at different locations in the source (spatial distribution)?
Low mass (below 40 k Da)
High resolution

Continuous vs. Delayed Extraction

- **Continuous TOF**
  - Resolution = 700 (FWHM)

- **Delayed Extraction**
  - Resolution = 6000 (FWHM)
Recent TOF designs: improved resolution, better sensitivity and mass accuracy (Ultraflex III MALDI TOF-TOF)

Resolution: 25,000
S/N: 46

**Typical TOF Specs**

- **m/z Range:** unlimited u
- **Resolution:** ~20,000 (Reflectron)
- **Mass Accuracy:** ~ 3-10 ppm (300-4,000 u)
- **Scan Speed:** $10^6$ u/s
- **Vacuum:** $10^{-7}$ Torr
- **Quantification:** low - medium
- **Positive and Negative Ions**
- **Variations:**
  - Linear, Reflectron,
- **Tandem:** w/ quads, TOFs and/or sectors
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MALDI TOF-TOF fragmentation spectrum of a sodiated polymer

\[
\begin{array}{c}
\text{3-OEB, } m=1-3 \ (164, 44) \\
\text{Copolymer of 2-hydroxybenzoic acid and ethylene carbonate}
\end{array}
\]
## Advantages and Disadvantages

<table>
<thead>
<tr>
<th>Mass Spectrometer</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>TOF-TOF</td>
<td>high resolution, high m/z fragment ions</td>
<td>Large size</td>
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<td>keV CID</td>
<td>Not ideal for continuous ionization source</td>
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<td>easier de novo peptide sequencing</td>
<td>$$$</td>
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<td>$d_n$ and $w_n$ to distinguish Ile/Leu</td>
<td>MALDI source</td>
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### Diagrams

- **TOF**
- **Quadrupole**
- **Linear Ion Trap**
- **Orbitrap**
- **FTICR**
Quadrupole (Q)

- four parallel rods or poles
- fixed DC and alternating RF voltages

- only particular $m/z$ will be focused on the detector, all the other ions will be deflected into the rods
- scan by varying the amplitude of the voltages – (AC/DC constant).
Quadrupole Field Animation

http://www.kettering.edu/~drussell/Demos/MembraneCircle/Circle.html
Ion Motion in Quadrupoles
- a qualitative understanding

- + DC, ions focused to center
- - DC, ions defocused
- +DC w/ rf, light ions respond to rf, eliminated (high mass filter)
- -DC w/ rf, light ions respond to rf, focused to center (low mass filter)
Quadrupole animation

http://www.youtube.com/watch?v=pjCun7QF19U

Initial kinetic energy affects ion motion in quad
Figure 9. The \( a-q \) stability diagram: a) The shaded area represents those areas in \( a-q \) space which correspond to stable solutions of Mathieu's differential equation. B) The one amu bandpass mass filter: Notice that only ions of \( m/e \ m+1 \) fall within the stability diagram.


Quadrupole Typical Specs

- **\( m/z \) Range:** 2-4000 u
- **Resolution:** Unit
- **Mass Accuracy:** ca +/- 0.1 u
- **Scan Speed:** 4000 u/s
- **Vacuum:** \( 10^{-4} – 10^{-5} \) Torr
- **Low Voltages:**
  - RF \(~6000 – 10000\) V
  - DC \(~500V-840V\)
  - Source near ground
- **Quantification:** good choice
- **Positive and Negative Ions**
- **Variations:** SingleQ, TripleQ, Hybrids
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What MS/MS instruments can be produced from Q?

What MS/MS instruments can be produced from Q and TOF?
Triple Quadrupole
- since 1970’s and still going strong!

Attractive Features:

• Source near ground and operates at relatively high pressure
  Couples well to source and to chromatography
• Multiple scan modes easy to implement

Triple Quadrupole (QQQ)
c/o Thermo Finnigan Corp.
c/o Agilent Corp.
Quadrupole Time of Flight (Q-TOF)

The Q-ToF® combines a quadrupole neo-tube [Q1], a hexapole collision cell, and an octof mass analyzer [Q2] to deliver high performance MSxMS.

http://www.waters.com/WatersDivision/waters_website/products/micromass/ms_top.asp (outdated)

Low-energy (eV) Collisions with Gas
QTOF with Ion Mobility

Ion Mobility

http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml

Disadvantages of MS

- Expensive
- Large/heavy
- No direct molecular shape information
- Requires vacuum
Ion Mobility

Ion transit through bath gas in presence of applied electric field

- **Analytical** (examples on following slides)
  - Airport security (explosives)
  - Industrial processes
  - Military uses including chemical warfare detection
  - Drugs of abuse

- **Biological** (usually coupled with m/z measurement)
  - Protein/peptide shape
Ion Mobility Process – Injection into Drift Tube


Ion Mobility Process – Separation

Ion Mobility Process – Detection

![Ion Mobility Process Diagram]

Mobility Spectrum

![Mobility Spectrum Diagram]

Principles of Mobility Separation

\[ \frac{L}{t} = v = KE \]

- K – mobility, cm²/V-s
- K reported as reduced mobility, Ko
- K related to collisional cross section, Ω
- Low E/N only

Indicative of size and shape – compare to computationally calculated cross sections


QTOF with Ion Mobility
Selection of \([M+2H]^{+2}\) at \(m/z\) 246.1

Transfer CID of \([M+2H]^{+2}\) at \(m/z\) 246.1
Ion mobility tutorial

https://zenodo.org/record/3268737#.XR_wCXDKi70

Differential Mobility Analyzers (DMA)
J. Fernandez de la Mora (< 1998)
Aerosol sciences

Travelling wave IMS (TWIMS)
K. Giles
Waters SYNAPT (2006)
Structural biology

Commercial DTIMS
PNNL designs (R.D. Smith)
Agilent 6560 IMS-Q-TOF

Trapped ion mobility spectrometry (TIMS)
M. Park, M. Rigdeway, F. Fernandez-Lima
Bruker timsTOF Pro (nano-LC ion mobility Q-TOF)
What is small, inexpensive and still gives great MSMS ????

**Quadrupole Ion Traps**

*Miniature IT*: Cooks, R. G. and coworkers


Ion path in a trap
RF ION TRAP ELECTRODE STRUCTURES

LCQ-Type 3D Quadrupole Trap

LTQ-Type (2D) Linear Quadrupole Trap

2D vs. 3D Ion Traps
Linear Trap Demo

Overall Performance Gains

<table>
<thead>
<tr>
<th></th>
<th>LTQ</th>
<th>3D Traps</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapping efficiency</td>
<td>~ 55-70%</td>
<td>~5%</td>
<td>~11-14x</td>
</tr>
<tr>
<td>Detection efficiency</td>
<td>~ 50-100%</td>
<td>~50%</td>
<td>~1-2x</td>
</tr>
<tr>
<td>Trapping capacity</td>
<td>~ 20,000 ions</td>
<td>~500 ions</td>
<td>~40x</td>
</tr>
</tbody>
</table>
Motivating Factors Realized

• Increased Trapping Efficiency
• Increased Trapping Capacity

Which means....

• Increased Sensitivity
• Increased Inherent Dynamic Range
  • Increased S/N for full Scan MS
  • Practical MS^n
• Faster Scan Times - no μscans (only one)
How are 3-D traps and linear ion traps used in MS/MS?

Stand alone
  3-D traps
  LTQ
Front or back end of tandem-in-space
  LT-TOF
  LT-FT, LT-Orbitrap,
  QTrap

Activation:

  CID
  Low-energy (eV) Collisions with Gas

  IRMPD
  (Infrared Multiphoton Dissociation)

  ETD
  (electron transfer dissociation)
### General Conclusions

- Many instruments are complementary
- More Activation methods, the better
- Assess your needs
- Assess your budget
- @ Research level, may want to build your own