**What does a mass spectrometer do?**

1. It measures mass better than any other technique.
2. It can give information about chemical structures.

**What are mass measurements good for?**

To identify, verify, and quantitate: metabolites, recombinant proteins, proteins isolated from natural sources, oligonucleotides, drug candidates, peptides, synthetic organic chemicals, polymers
Single-protein nanomechanical mass spectrometry in real time


Nanoelectromechanical systems (NEMS) resonators can detect mass with exceptional sensitivity. Previously, mass spectra from several hundred adsorption events were assembled in NEMS-based mass spectrometry using statistical analysis. Here, we report the first realization of single-molecule NEMS-based mass spectrometry in real time. As each molecule in the sample adsorbs on the resonator, its mass and position of adsorption are determined by continuously tracking two driven vibrational modes of the device. We demonstrate the potential of multimode NEMS-based mass spectrometry by analysing IgM antibody complexes in real time. NEMS-based mass spectrometry is a unique and promising new form of mass spectrometry: it can resolve neutral species, provide a resolving power that increases markedly for very large masses, and allow the acquisition of spectra, molecule-by-molecule, in real time.
Experimental investigations on the conduction of electricity by gases led to the discovery of the electron in 1897, for which Thomson was awarded the and to the1906 Nobel Prize in Physics. Thomson J.J. On the Masses of the Ions in Gases at Low Pressures *Philosophical Magazine*, 1899, 48:295, p.547-567.

Thomson observed that the new technique could be a used profitably by chemists to analyze chemicals.
Consider a Charged Particle in an Electric Field

\[ F = qE = ma \]

\[ y = \frac{1}{2} at^2 \quad L = vt \]
Magnetic Mass Analyzer

Figure 2.39
Orientation of the magnetic force $B$ upon a moving ion

$$\vec{F}_b = qv \times \vec{B}$$

$$\frac{m}{z} = \frac{r^2 B^2}{2V}$$
Crossed Fields can be Used as a Velocity Selector

\[ v = \frac{E}{B} \]

\[ \frac{m}{z} = \frac{r^2 B^2}{2E} \]
How does a mass spectrometer work?

Sample

- **Ion source**: makes ions

- **Mass analyzer**: separates ions

- **Mass spectrum**: presents information
Mass Spectrometer Block Diagram

High Vacuum System

Inlet → Ion source → Mass Analyzer → Detector → Data System
Sample Introduction

HPLC
Flow injection
Sample plate
Ion Source

High Vacuum System

Inlet

Ion Source

Mass Analyzer

Detector

Data System

MALDI
ESI
FAB
LSIMS
EI
CI
Protonation of Peptides

First Step - Protonation of peptides

Proton affinity

N-terminus

Amide N

Amide CO

Side chain basic groups

Side chain basic groups > N-terminus > Amide CO > Amide N
Ion Sources make ions from sample molecules
(Ions are easier to detect than neutral molecules.)

Electrospray ionization:

Pressure = 1 atm
Inner tube diam. = 100 um

Sample Inlet Nozzle
(Lower Voltage)

Partial vacuum

Sample in solution
N₂ gas

N₂

High voltage applied
to metal sheath (~4 kV)

Charged droplets

MH⁺

MH₂⁺

MH₃⁺
Electrospray Ionization (ESI)

Sample solution → nebulizing gas → +HV → Mass Analyzer

1. Solvent evaporation
2. Coulombic repulsion

[M + nH]^{n+}
Electrospray Ionization (ESI) Process (Positive Mode)

Adapted from Kebarle, P and Tang, L, *Anal. Chem.*, 1993, 65(22), 972A
Electrospray Ionization (ESI)
MALDI: Matrix Assisted Laser Desorption Ionization

1. Sample is mixed with matrix (X) and dried on plate.

2. Laser flash ionizes matrix molecules.

3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$. 

Sample plate

Laser

$MH^+$

+/- 20 kV

Grid (0 V)
MALDI generation of ions
(Matrix-assisted laser desorption ionization)

Sample mixed with a UV-absorbing matrix and is allowed to co-crystallize on the metal target.
Matrices for MALDI analysis

Peptides/proteins
- 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid)
- α-cyano-4-hydroxycinnamic acid (CHCA)
- 2,5-dihydroxybenzoic acid (DHB)
- 2-(4-hydroxyphenylazo)-benzoic acid (HABA)

Oligonucleotides
- 2-aminobenzoic acid
- 3-hydroxypicolinic acid (3-HPA)
- 2,4,6-trihydroxyacetophenone (THAP)

The choice of matrix depends greatly on the solute to be analyzed.
Mass Analyzer

- Inlet
- Ion source
- Mass Analyzer
- Detector
- Data System

High Vacuum System

Time of flight (TOF)
Quadrupole
Ion Trap
Magnetic Sector
FT-ICR MS
Orbitrap MS
Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

- Operate under high vacuum (keeps ions from bumping into gas molecules)
- Actually measure mass-to-charge ratio of ions (m/z)

The importance of the mass-to-charge ratio is that according to classical electrodynamics two particles with the same mass-to-charge ratio move in the same path in a vacuum when subjected to the same electric and magnetic fields.

\[ F = ma \] (Newton’s second law of motion)

\[ F = q(E + v \times B) \] (Lorentz force Law)

\[ \frac{m}{q}a = E + v \times B \]

Key specifications are resolution, mass measurement accuracy, and sensitivity.
Ions are formed in pulses.

The drift region is field free.

Measures the time for ions to reach the detector.

Small ions reach the detector before large ones.
Particles on which the same amount of work was done will have the same kinetic energy.

Work is Force time distance

\[ \vec{F} = q\vec{E} \] force is proportional to charge and field strength

The distance is the same for all particles so the kinetic energy of all particles is the same and proportional to:

\[ \frac{1}{2} \frac{m}{q} v^2 \]

So a particle with twice the m/z will have a reduction in velocity of by a factor of the square root of 2. In general, the reduction in velocity is proportional to the square root of m/z.

The consequence of this is that at higher m/z the smaller the difference in arrival times.
Ions are accelerated so that they have equal kinetic energy. The ions “drift” down a 1 - 1.5 meter tube before striking a photomultiplier detector. “time of flight” (t) depends on the mass of the ion (m), where \( t = \left(\frac{m}{2eV}\right)^{1/2}D \)

\( V \) is the applied potential and \( D \) is the flight tube distance. For a given instrument, the flight time varies as the square root of the mass of the ion.
Quadrupole Mass Analyzer

Uses a combination of RF and DC voltages to operate as a mass filter.

- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.
Ions are accelerated electrically (5-15V) and passed along the long central axis of four rods arranged symmetrically. By applying combined DC and oscillating RF potentials, the ions drift along irregular flight paths along the rod axis. The DC/RF ratio is held constant and the absolute values of DC and RF are varied. Only ions with a particular $m/z$ value have stable trajectories for a given value of DC and RF.

If DC is set to 0, then all ions have stable trajectories. A scan can be accomplished over a period of 10-1000 msec.
Ion Traps

The ion trap is an energy well - ions with sufficient energy to enter the trap are retained by an energy barrier on the exit side of the trap. The advantage of the ion trap is that it accumulates selected ions prior to their analysis giving it high initial sensitivity (detection limit of approx. 20 fmol).

Ions are fragmented by collision with helium gas and their daughter ions analyzed within the trap. Selected daughter ions can undergo further fragmentation, thus allowing MS\textsuperscript{n}.

The ion trap has a high efficiency of transfer of fragment ions to the next stage of fragmentation (unlike the triple quadrupole instrument).
Detector

High Vacuum System

- Inlet
- Ion source
- Mass Analyzer
- Detector

Data System

- Microchannel Plate
- Electron Multiplier
- Mass analyzer/ion trap AC image
Principle of the (Discrete) Electron Multiplier

A series of dynodes at increasing potentials produce a cascade of electrons.

10^6 electrons out

current generated, amplified, and sent to computer.
Multichannel Plate (MCP)

- High gain
- High Temporal Resolution
- High Spatial Resolution
Detecting in the ion trap

Ion cyclotron resonance (ICR)

7 Tesla magnet, or 9.4 T or 12 T or 14.5 T
FT-Ion Cyclotron Resonance

\[ \omega = \frac{qB}{m} \]

Frequencies are converted to masses.

Ions of different mass have different cyclotron frequencies.
Block Diagram of Triple Quad
Thermo-Finnigan LTQ-FT

**Linear Ion Trap MS**
- MS, MS/MS and MS\(^n\) Analysis
- AGC Control
- Secondary Electron Multiplier Detector

**FTICR MS**
- Ion Image Current Detector
- Accurate Mass
- High Resolution

**7 T Actively Shielded Superconductive Magnet**

**Triple-Ported Turbopump**
- 40 L/sec
- 15 L/sec
- 300 L/sec
- 400 L/sec
- 210 L/sec
- 210 L/sec
Quadrupole – Time of Flight (Q-ToF)
Mass analyzers

**Ion traps** – most popular mass analyzers in peptide MS
- 3D – traditional trap
- Linear ion trap – high ion capacity

  relatively low resolution
  mediocre mass accuracy
  low mass limit (~1/3 of precursor m/z)

  Compatible with CID (most recently also ETD (electron transfer diss.))

**Triple quadrupoles** – not very common in peptide MS

  relatively low resolution
  mediocre mass accuracy
  very good for quantitation (multiple reaction monitoring)
Mass analyzers

QqTOF – hybrid quadrupole – TOF instrument - common in peptide MS
  high mass resolution
  good mass accuracy (< 5 ppm)

  Compatible with CID

Cyclotron instruments – popular in peptide MS
  FT-ICR
  Orbitrap

  very high mass resolution
  excellent mass accuracy (<1 ppm)

Tandem TOF-TOF – not quite common in peptide MS

  high mass resolution
  very good mass accuracy (<5 ppm)
  disconnect between LC and MS analysis allows targeted reanalysis
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Applications</th>
<th>Resolution</th>
<th>Mass accuracy</th>
<th>Sensitivity</th>
<th>Dynamic range</th>
<th>Scan rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIT (LTQ)</td>
<td>Bottom-up protein identification in high-complexity, high-throughput analysis, LC-MS&lt;sup&gt;n&lt;/sup&gt; capabilities</td>
<td>2000</td>
<td>100 ppm</td>
<td>Femtomole</td>
<td>1e4</td>
<td>Fast</td>
</tr>
<tr>
<td>TQ (TSQ)</td>
<td>Bottom-up peptide and protein quantification; medium complexity samples, peptide and protein quantification (SRM, MRM, precursor, product, neutral fragment monitoring)</td>
<td>2000</td>
<td>100 ppm</td>
<td>Attomole</td>
<td>1e6</td>
<td>Moderate</td>
</tr>
<tr>
<td>LTQ-Orbitrap</td>
<td>Protein identification, quantification, PTM identification</td>
<td>100,000</td>
<td>2 ppm</td>
<td>Femtomole</td>
<td>1e4</td>
<td>Moderate</td>
</tr>
<tr>
<td>LTQ-FTICR, Q-FTICR</td>
<td>Protein identification, quantification, PTM identification, top-down protein identification</td>
<td>500,000</td>
<td>&lt;2 ppm</td>
<td>Femtomole</td>
<td>1e4</td>
<td>Slow, slow</td>
</tr>
<tr>
<td>Q-TOF, IT-TOF</td>
<td>Bottom-up, top-down protein identification, PTM identification</td>
<td>10,000</td>
<td>2–5 ppm</td>
<td>Attomole</td>
<td>1e6</td>
<td>Moderate, fast</td>
</tr>
<tr>
<td>Q-LIT</td>
<td>Bottom-up peptide and protein quantification; medium complexity samples, peptide and protein quantification (SRM, MRM, precursor, product, neutral fragment monitoring)</td>
<td>2,000</td>
<td>100 ppm</td>
<td>Attomole</td>
<td>1e6</td>
<td>Moderate, fast</td>
</tr>
</tbody>
</table>
Ion Mobility Mass Spec (IMS)

- Provides information about the size and shape of a molecule in the gas phase

- Imagine putting a size exclusion column in front of the mass spectrometer
Ion Mobility Mass Spec (IMS)

Provides information about the
Size and shape of a molecule
In the gas phase
Data System

High Vacuum System

Inlet  --  Ion source  --  Mass Analyzer  --  Detector  --  Data System

PC
The mass spectrum shows the results

MALDI TOF spectrum of IgG

Relative Abundance

Mass (m/z)

MH+

(M+2H)^2+

(M+3H)^3+
Assigning numerical value to the intrinsic property of “mass” is based on using carbon-12, $^{12}\text{C}$, as a reference point.

One unit of mass is defined as a Dalton (Da).

One Dalton is defined as $1/12$ the mass of a single carbon-12 atom.

Thus, one $^{12}\text{C}$ atom has a mass of 12.0000 Da.
Isotopes

Most elements have more than one stable isotope. For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

Why do we care?

Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.
**Stable isotopes of most abundant elements of peptides**

<table>
<thead>
<tr>
<th>Element</th>
<th>Mass</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.0078</td>
<td>99.985%</td>
</tr>
<tr>
<td></td>
<td>2.0141</td>
<td>0.015</td>
</tr>
<tr>
<td>C</td>
<td>12.0000</td>
<td>98.89</td>
</tr>
<tr>
<td></td>
<td>13.0034</td>
<td>1.11</td>
</tr>
<tr>
<td>N</td>
<td>14.0031</td>
<td>99.64</td>
</tr>
<tr>
<td></td>
<td>15.0001</td>
<td>0.36</td>
</tr>
<tr>
<td>O</td>
<td>15.9949</td>
<td>99.76</td>
</tr>
<tr>
<td></td>
<td>16.9991</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>17.9992</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Calculating Molecular Weight

**Monoisotopic mass (exact mass)** Mass of an ion calculated using the exact mass of the most abundant isotope.

**Average mass** Mass of an ion calculated using average atomic weight of each element.

**Nominal mass** Mass of an ion calculated using the integer mass of the most abundant isotope.

---

**Table 3.2. An illustration of the differences between monoisotopic, average, and nominal mass for some elements, a lipid, a sugar, and a peptide.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Formula</th>
<th>Average Mass (Da)</th>
<th>Monoisotopic Mass (Da)</th>
<th>Nominal Mass (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>H</td>
<td>1.0080</td>
<td>1.0078</td>
<td>1</td>
</tr>
<tr>
<td>Carbon</td>
<td>C</td>
<td>12.0112</td>
<td>12.0000</td>
<td>12</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N</td>
<td>14.0067</td>
<td>14.0031</td>
<td>14</td>
</tr>
<tr>
<td>Oxygen</td>
<td>O</td>
<td>15.9994</td>
<td>15.9949</td>
<td>16</td>
</tr>
<tr>
<td>Sulfur</td>
<td>S</td>
<td>32.0600</td>
<td>31.9721</td>
<td>32</td>
</tr>
<tr>
<td>A lipid</td>
<td>( \text{C}<em>{16}\text{H}</em>{35}\text{N}_1\text{O}_1 )</td>
<td>281.4858</td>
<td>281.2718</td>
<td>281</td>
</tr>
<tr>
<td>A sugar</td>
<td>( \text{C}<em>{59}\text{H}</em>{116}\text{N}<em>4\text{O}</em>{14} )</td>
<td>1071.5833</td>
<td>1070.8844</td>
<td>1070</td>
</tr>
<tr>
<td>A peptide</td>
<td>( \text{C}<em>{161}\text{H}</em>{258}\text{N}<em>2\text{O}</em>{24} )</td>
<td>2193.3288</td>
<td>2191.9704</td>
<td>2190</td>
</tr>
</tbody>
</table>
Isotopic Distribution of Carbon

Fig. 3.2. Calculated isotopic patterns for carbon. Note the steadily expanding width of the pattern as \( X+2, X+3, X+4, \ldots \) become visible. At about \( C_{90} \) the \( X+1 \) peak reaches the same intensity as the \( X \) peak. At higher carbon number it becomes the base peak of the pattern.

Table 3.2. Calculated isotopic distributions for carbon based on the \(^{13}\text{C} \) content according to IUPAC. © IUPAC 1998. [4]

<table>
<thead>
<tr>
<th>Number of carbons</th>
<th>( X+1 )</th>
<th>( X+2 )</th>
<th>( X+3 )</th>
<th>( X+4 )</th>
<th>( X+5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.3</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.3</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.5</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.6</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.7</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9.7</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.8</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>13.0</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>16.1</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>21.6</td>
<td>2.2</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>27.0</td>
<td>3.5</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>32.3</td>
<td>5.0</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>43.2</td>
<td>9.0</td>
<td>1.3</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>54.1</td>
<td>14.5</td>
<td>2.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>60</td>
<td>65.0</td>
<td>20.6</td>
<td>4.2</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>90</td>
<td>97.2</td>
<td>46.8</td>
<td>14.9</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>120</td>
<td>100.0</td>
<td>64.4</td>
<td>27.3</td>
<td>8.6</td>
<td>2.2</td>
</tr>
</tbody>
</table>

\(^{a}\) The \( X \) peak has an abundance of 77.0 \% in that case.
Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)

“Monoisotopic mass”

1981.84

No \(^{13}\)C atoms (all \(^{12}\)C)

1982.84

One \(^{13}\)C atom

1983.84

Two \(^{13}\)C atoms
Isotope pattern for a larger peptide (207 C-atoms)
Insulin has 257 C-atoms. Above this mass, the monoisotopic peak is too small to be very useful, and the average mass is usually used.
When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.
Average mass

When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the isotope peaks in the cluster, which is the same as the average or chemical mass.
What if the resolution is not so good?

At lower resolution, the mass measured is the average mass.
Peptide mixture: [Val\textsuperscript{5}]-Angiotensin II
Lys-des-Arg\textsuperscript{9}-Bradykinin
Sequence: DRVYVHPF
KRPPGFSPF
Formula: C\textsubscript{49}H\textsubscript{69}N\textsubscript{13}O\textsubscript{12}
C\textsubscript{50}H\textsubscript{73}N\textsubscript{13}O\textsubscript{11}
Exact mass: [M+2H]\textsuperscript{2+} = 516.76671
[M+2H]\textsuperscript{2+} = 516.78490
\(\Delta m\) (mmu): 18.2 mmu

RP = 18,000

RP = 56,700
How is mass resolution calculated?

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

FWHM = \Delta M
Mass measurement accuracy depends on resolution

High resolution means better mass accuracy
Mass Resolution = \( \frac{m}{\Delta m} \)

FT-ICR MS

\[
\text{RP} = \frac{491.2594}{0.0055} \text{ amu} = 89,319
\]

LTQ MS

\[
\text{RP} = \frac{491.45}{0.69} \text{ amu} = 712
\]
How Do We Determine Charge State?

\[ \Delta = 0.33 \]
How Do We Determine Charge State of a Protein?

\[ \text{m/z} = \frac{(\text{MW} + n\text{H}^+)}{n} \]
Mass Spectrum of Denatured Intact Protein

Mix 406 (7.446) Cm (399:413)

TOF MS ES+ 1.06e3
Quadrupole-Time of Flight (Q-ToF)

Sample

ULTRA STABLE QUADRUPOLE MASS FILTER

HIGH EFFICIENCY COLLISION CELL

ACCELERATOR

Sample

Effective Flight Path = 2.5 m

Ion Mirror (reflector)
Fragmentation of Peptides

\[ \text{b2-b1 = amino acid} \]
\[ \text{B2 - B1 = amino acid} \]

\[ \text{b and y'' ions are} \]
Ion Activation – Inducing fragmentation

CID – collision induced dissociation – collision with inert gas molecule
most common mode of ion fragmentation

IRMPD – infrared multiphoton dissociation – irradiation of ions with IR laser
one of major modes of ion fragmentation in FT-ICR

ECD – electron capture dissociation – irradiation of ions with electrons
one of major modes of ion fragmentation in FT-ICR

SID – surface induced dissociation – colliding ions with surface
similar to CID, narrow internal energy distribution
requires specialized instrumentation
• Provides information about the size and shape of a molecule in the gas phase

• Imagine putting a size exclusion column in front of the mass spectrometer
Ion Mobility Mass Spec (IMS)

Provides information about the Size and shape of a molecule In the gas phase