Other Methods for Generating Ions
1. MALDI  matrix assisted laser desorption ionization MS
2. Spray ionization techniques
3. Fast atom bombardment
4. Field Desorption
5. MS –MS techniques
**Matrix assisted laser desorption ionisation (MALDI)**

The sample to be analysed is dissolved in an appropriate volatile solvent, usually with a trace of trifluoroacetic acid if positive ionization is being used, and mixed with an equal volume of a solution containing a vast excess of a matrix. A range of compounds is suitable for use as matrices: sinapinic acid is a common one for protein analysis while alpha-cyano-4-hydroxycinnamic acid is often used for peptide analysis. Most commercially available MALDI mass spectrometers now have a pulsed nitrogen laser of wavelength 337 nm.
sinapinic acid

$\alpha$-cyano-4-hydroxycinnamic acid
Maldi spectrum of a peptide mixture using α-cyano-4-hydroxycinnamic acid (matrix)
<table>
<thead>
<tr>
<th>Ionization Method</th>
<th>Energy Source</th>
<th>Operating Conditions</th>
<th>Ion Formation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermospray (TS)</td>
<td>Thermal energy</td>
<td>1-10 torr, elevated temperature</td>
<td>Gas phase ion-molecule reactions</td>
<td>Temperature dependent spectra; interface for LC–MS</td>
</tr>
<tr>
<td>Electrospray (ES)</td>
<td>Electric field</td>
<td>Reduced pressure, ambient temperature</td>
<td>Ion-molecule reactions in solution, droplets</td>
<td>Forms multiply charged ions for high molecular weight determination; interface for LC–MS and capillary zone electrophoresis (CZE)–MS</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>Corona discharge</td>
<td>Atmospheric pressure, elevated</td>
<td>Gas and solution phase ion-molecule reactions</td>
<td>Multiply charged ions; interface for LC–MS and GC–MS</td>
</tr>
<tr>
<td>pressure chemical ionization (APCI)</td>
<td></td>
<td>temperature</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* There is considerable overlap in techniques and phenomena between these methods.
A schematic of an ESI source.
Electrospray
Positive ESI-MS m/z spectrum of the protein hen egg white lysozyme.
FIGURE 7.8 Appearance of the ESIMS spectrum of the hypothetical compound C₁₀₀H₂₀₀ (MW 1401.6).
$C_{100}H_{200}$

$100 \ ^{12}C = 100 \times 12 = 1200$

$200 \ ^{1}H = 200 \times 1.0782 = 202.574$

\[\text{Total} = 1402.574\]

go to parent ions
Before trying to analyze a high molecular weight compounds consider the following:

\[(^{12}\text{C} + ^{13}\text{C})^{12}\]

\[
\begin{array}{cccccc}
1 & & & & & \\
1 & 1 & & & & \\
1 & 2 & 1 & & & \\
1 & 3 & 3 & 1 & & \\
1 & 4 & 6 & 4 & 1 & \\
1 & 5 & 10 & 10 & 5 & 1 \\
1 & 6 & 15 & 20 & 15 & 6 & 1 \\
1 & 7 & 21 & 35 & 35 & & \\
1 & 8 & 28 & 56 & 56 & & \\
1 & 9 & 36 & 84 & & & \\
1 & 10 & 45 & & & & \\
1 & 11 & 55 & & & & \\
1 & 12 & 66 & & & & \\
\end{array}
\]

For each row, the number of elements is equal to the row number. The sum of each row is twice the power of 12. The coefficients in each row are the binomial coefficients for \((^{12}\text{C} + ^{13}\text{C})^{n}\).
$C_{100}H_{200}$

100 $^{12}\text{C} = 100 \times 12 = 1200$

200 $^1\text{H}$

$= 200 \times 1.00782 = 202.574$

\[\underline{1401.564}\]

$^{12}\text{C} = 0.989; \quad ^{13}\text{C} = 0.011$

The probability of all $^{12}\text{C} = 100 \times 0.989^{100} = 0.331$

The probability of finding 1 $^{13}\text{C} = 100 \times (0.989)^{99} \times (0.011) = 0.364$

The probability of finding 2 $^{13}\text{C} = 4950 \times (0.989)^{98} \times (0.011)^2 = 0.20$

go to parent ions
Selective ion monitoring

(a) Positive ion ESI-MS of chlorocarolide (10) (MW 220).

(b) ESI-MS reconstructed chromatogram from LC-MS analysis of a crude fermentation broth containing four diastereomers of 10 based on total ion current (below), ion current of $m/z = 222.9$ (center and $m/z = 220.9$ (top)).
3. Fast atom bombardment

High voltage applied + or -
Fast Atom Bombardment (FAB) -

**useful mass range:** up to ca 1800 amu

**sample preparation:** dissolved in a “non-volatile” matrix, glycerol or m-nitrobenzyl alcohol (NBA), concentration ca 1mg/mL; can add trifluoroacetic acid or other cation source to facilitate ion formation.

**sample introduction:** 5-10 μL of this solution on the tip of a FAB probe; sample is inserted following evacuation

**ionization:** Xenon "fast atoms"

**accurate mass determination:** PEG (polyethylene glycol), PPG (polypropylene glycol) or Ultramark (a mixture of perfluoroalkylphosphazenes) is mixed with the matrix

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**TABLE 7.3** Matrix Peaks Associated With FAB (Positive Ion)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Main Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>45, 57, 75, 93, 185, 277, 369, 461, 553</td>
</tr>
<tr>
<td>Thioglycerol</td>
<td>45, 57, 91, 109, 217, 323, 325, 429, 487, 539, 643</td>
</tr>
<tr>
<td>Magic Bullet*</td>
<td>103, 119, 135, 152, 155, 195, 279, 309, 461, 515, 613</td>
</tr>
<tr>
<td>Nitrobenzyl alcohol (NBA)</td>
<td>89, 107, 132, 154, 243, 307, 460, 613</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>89, 133, 177, 219, 459, 503, 547</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>110, 150, 194, 267, 297</td>
</tr>
</tbody>
</table>

* Mixture of dithiothreitol and dithioerythritol (5:1).
Solid sample bombarded by $^{252}$Cf source

MW 475

Sample on glycerol bombarded by Ar ions and neutrals

Sample on glycerol bombarded by Xe ions and neutrals
5. Field desorption: imposition of a high electric field gradient on a sample deposited on a special solid support
Field desorption

Sample dipped in solution and allowed to evaporate on wire
Fig. 2. (Lower) FDMS of Man$_{3}$MeMan$_{3}$-OCH$_3$: results of summation of five scans. (Upper) How the polysaccharide is assumed to fragment to give the observed ions. Only reproducible ions without isotope signals are displayed.
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8.1 Tandem mass spectrometry

Tandem mass spectrometry (MS-MS) is used to produce structural information about a compound by fragmenting specific sample ions inside the mass spectrometer and identifying the resulting fragment ions. This information can then be pieced together to generate structural information regarding the intact molecule. Tandem mass spectrometry also enables specific compounds to be detected in complex mixtures on account of their specific and characteristic fragmentation patterns.
A **tandem mass spectrometer** is a mass spectrometer that has more than one analyser, in practice usually two. The two analysers are separated by a collision cell into which an inert gas (e.g. argon, xenon) is admitted to collide with the selected sample ions and bring about their fragmentation. The analyzers can be of the same or of different types.

A single ion is focused and allowed to enter a second chamber. Collision by a neutral gas leads to fragmentation. The fragment are analysed by the second analyzer.
Product or daughter ion scanning:
the first analyser is used to select user-specified sample ions arising from
a particular component; usually the molecular-related (i.e. (M+H)+ or
(M-H)-) ions. These chosen ions pass into the collision cell, are
bombarded by the gas molecules which cause fragment ions to be
formed, and these fragment ions are analysed i.e. separated according to
their mass to charge ratios, by the second analyser. All the fragment ions
arise directly from the precursor ions specified in the experiment, and
thus produce a fingerprint pattern specific to the compound under
investigation.
There are three different types of bonds that can fragment along the amino acid backbone: the NH-CH, CH-CO, and CO-NH bonds. Each bond breakage gives rise to two species, one neutral and the other one charged, and only the charged species is monitored by the mass spectrometer.

There are six possible fragment ions for each amino acid residue. The most common cleavage sites are at the CO-NH bonds, which give rise to the b and/or the y" ions. The mass difference between two adjacent b ions, or y"; ions, is indicative of a particular amino acid residue.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Code</th>
<th>Formula</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>A</td>
<td>-NH.CH.(CH3).CO-</td>
<td>71.0</td>
</tr>
<tr>
<td>Arg</td>
<td>R</td>
<td>-NH.CH.[(CH2)3.NH.C(NH).NH2]CO-</td>
<td>156.1</td>
</tr>
<tr>
<td>Asn</td>
<td>N</td>
<td>-NH.CH.(CH2CONH2).CO-</td>
<td>114.0</td>
</tr>
<tr>
<td>Asp</td>
<td>D</td>
<td>-NH.CH.(CH2COOH).CO-</td>
<td>115.0</td>
</tr>
<tr>
<td>Cys</td>
<td>C</td>
<td>-NH.CH.(CH2SH).CO-</td>
<td>103.0</td>
</tr>
<tr>
<td>Gln</td>
<td>Q</td>
<td>-NH.CH.(CH2CH2CONH2).CO-</td>
<td>128.1</td>
</tr>
<tr>
<td>Glu</td>
<td>E</td>
<td>-NH.CH.(CH2CH2COOH).CO</td>
<td>-129.0</td>
</tr>
<tr>
<td>Gly</td>
<td>G</td>
<td>-NH.CH2.CO-</td>
<td>57.0</td>
</tr>
<tr>
<td>His</td>
<td>H</td>
<td>-NH.CH.(CH2C3H3N2).CO-</td>
<td>137.0</td>
</tr>
<tr>
<td>Ile</td>
<td>I</td>
<td>-NH.CH.[CH.(CH3)CH2.CH3].CO-</td>
<td>113.1</td>
</tr>
<tr>
<td>Leu</td>
<td></td>
<td>-NH.CH.[CH2CH(CH3)2].CO-</td>
<td>113.1</td>
</tr>
<tr>
<td>Lys</td>
<td>K</td>
<td>-NH.CH.[(CH2)4NH2].CO-</td>
<td>128.1</td>
</tr>
<tr>
<td>Met</td>
<td>M</td>
<td>-NH.CH.[(CH2)2.SCH3].CO-</td>
<td>131.0</td>
</tr>
<tr>
<td>Phe</td>
<td>F</td>
<td>-NH.CH.(CH2Ph).CO-</td>
<td>147.1</td>
</tr>
<tr>
<td>Pro</td>
<td>P</td>
<td>-NH.(CH2)3.CH.CO-</td>
<td>97.1</td>
</tr>
<tr>
<td>Ser</td>
<td>S</td>
<td>-NH.CH.(CH2OH).CO-</td>
<td>87.0</td>
</tr>
<tr>
<td>Thr</td>
<td>T</td>
<td>-NH.CH.[CH(OH)CH3].CO-</td>
<td>101.0</td>
</tr>
<tr>
<td>Trp</td>
<td>W</td>
<td>-NH.CH.[CH2.C8H6N].CO-</td>
<td>186.1</td>
</tr>
<tr>
<td>Tyr</td>
<td>Y</td>
<td>-NH.CH.[(CH2).C6H4.OH].CO-</td>
<td>163.1</td>
</tr>
<tr>
<td>Val</td>
<td>V</td>
<td>-NH.CH.[CH(CH3)2].CO-</td>
<td>99.1</td>
</tr>
</tbody>
</table>
b2 - b1 = amino acid
B2 - B1 = amino acid

b and y'' ions are
A cyclic peptide antibiotic, gramicidin S, was subjected to high resolution ESI mass spectrometry. The cluster at 571 amu is shown below. What is the molecular weight of gramicidin S?

```
571.36  1142.72  1714.08  2285.44
571.86  1143.72  1715.58  2287.44
572.36  1144.72  1717.08  2289.44
572.85  1145.7   1718.55  2291.4
573.36  1146.72  1720.08  2293.44
573.86  1147.72  1721.58  2295.44
```
Determining the sequence of gramicidin S by MSMS

\[ C_{60}H_{92}N_{12}O_{10} \text{ MW } 1140 \]

Amino acid analysis: Val, Orn, Leu, Phe, Pro

Val  99
Orn 114 How many degrees of unsaturation?
Leu 113 21
Phe 147
Pro  97 10 C=O; 8 Ph; pro 2:
Total = 570 570*2 1140
Ignoring N and C terminals, how many orn-val-pro isomers are there?

orn-leu-phe
orn-phe-leu
phe-orn-leu

orn-val-pro
val-pro-orn
val orn-pro

C$_{60}$H$_{92}$N$_{12}$O$_{10}$ MW 1140

val-pro-phe-leu-orn
ORN-LEU-PHE-PRO-VAL

orn-leu-phe-pro-val