

Mass Spectrometry

- NOT part of electromagnetic spectrum
- What is measured are (typically) positively charged ions
 - Under certain circumstances negative ions can be detected
- Once ions are in detector, they are separated on the basis of mass/charge ratio (m/z)
- Many different ionization methods and detectors
 - Useful for different kinds of molecules
 - Vary in amount of energy
 - ↳ Varying amounts/kinds of ions
 - ↳ More or less fragments

Ionization Methods

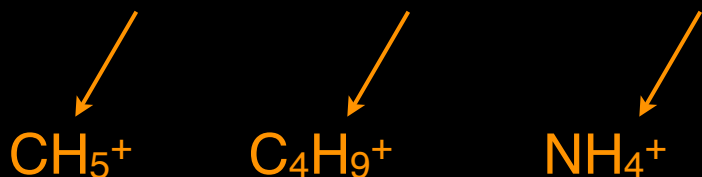
Electron impact (EI)

- Probably the most widely used
 - Vapor phase samples → from GC
- Sample bombarded with high energy electrons
 - 25-80 eV (2.4-7.6 MJ/mol, 573–1,816 kcal/mol) → 70 eV typical
- Ejects 1 electron from molecules → positively charged ion is left, odd electron ion, radical cation
- Typical ionization energies for organic molecules ~15 eV
 - ~50 eV excess energy
 - excess energy dissipated by breaking covalent bonds
 - lots of fragment ions
 - fragment pattern unique to a given molecule
 - many times molecular ion is not observed

Ionization Methods

Chemical Ionization (CI)

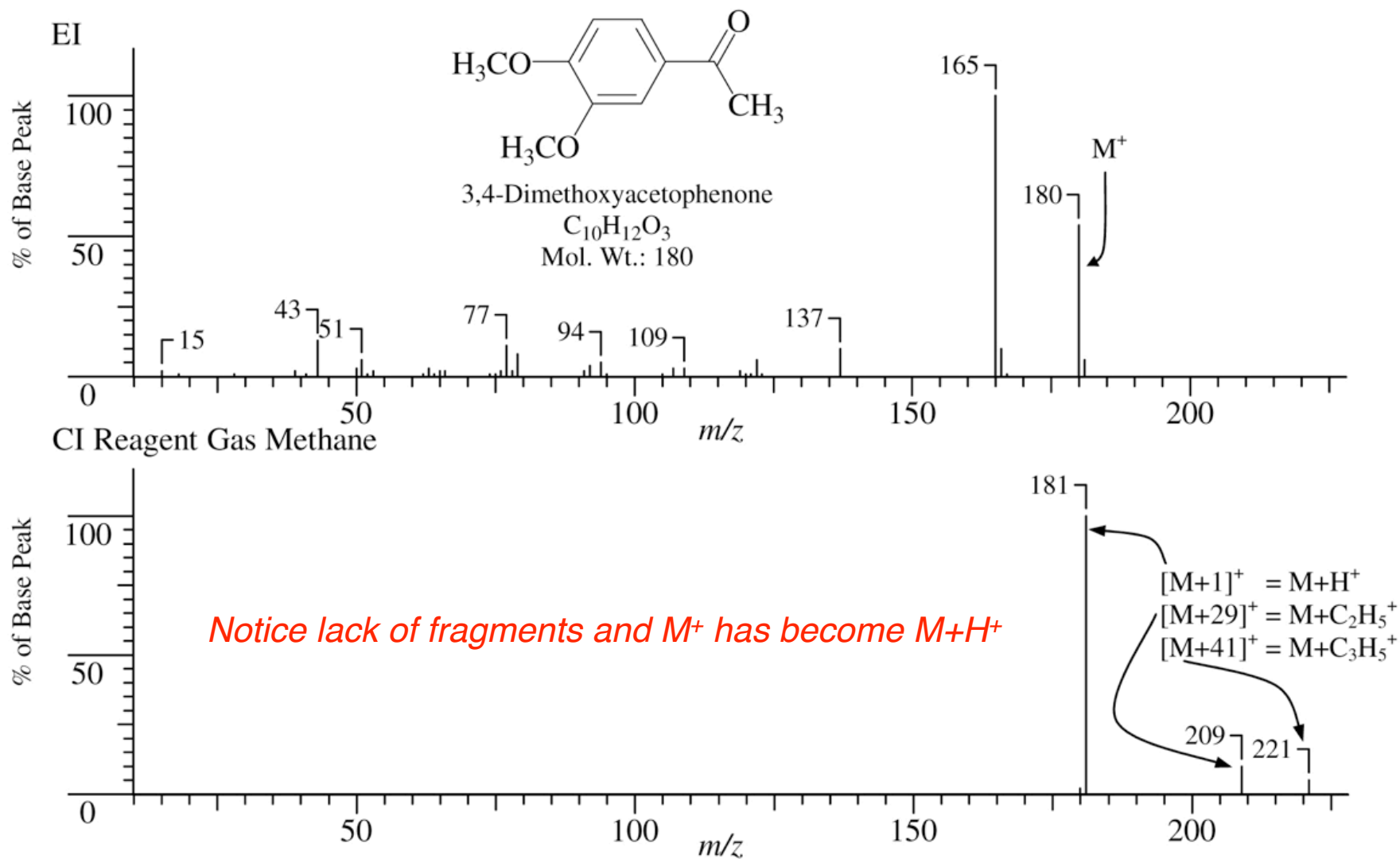
- A method for “Soft Ionization”
 - Vapor phase samples
- Sample introduced to ionized reagent gas
 - Reagent gases: Methane, Isobutane, Ammonia, others



- Collisions between sample & gas ions cause proton transfers → produces $[\text{M}+\text{H}]^+$ ions, *not* M^+ ions.
 - These are even electron ions
- Much less energy transferred $< 5 \text{ eV}$
 - less fragmentation
 - abundance of molecular ions
 - good for molecular weight determination
 - less information about structure

Mass Spectrometry

Comparison of EI and CI Spectra



Ionization Methods

Fast Atom Bombardment (FAB)

- Soft Ionization method
- Sample is mixed with a condensed phase matrix
- Mixture is ionized with high energy (6-10 keV) Xe or Ar
 - Matrix protects sample from excess energy
- Ionization from protonation ($[M+H]^+$), cation attachment ($[M+23(Na)]^+$), or deprotonation ($[M-H]^+$).
- High resolution masses are possible
 - exact mass determination
- Can be complicated by ions from matrix

Ionization Methods

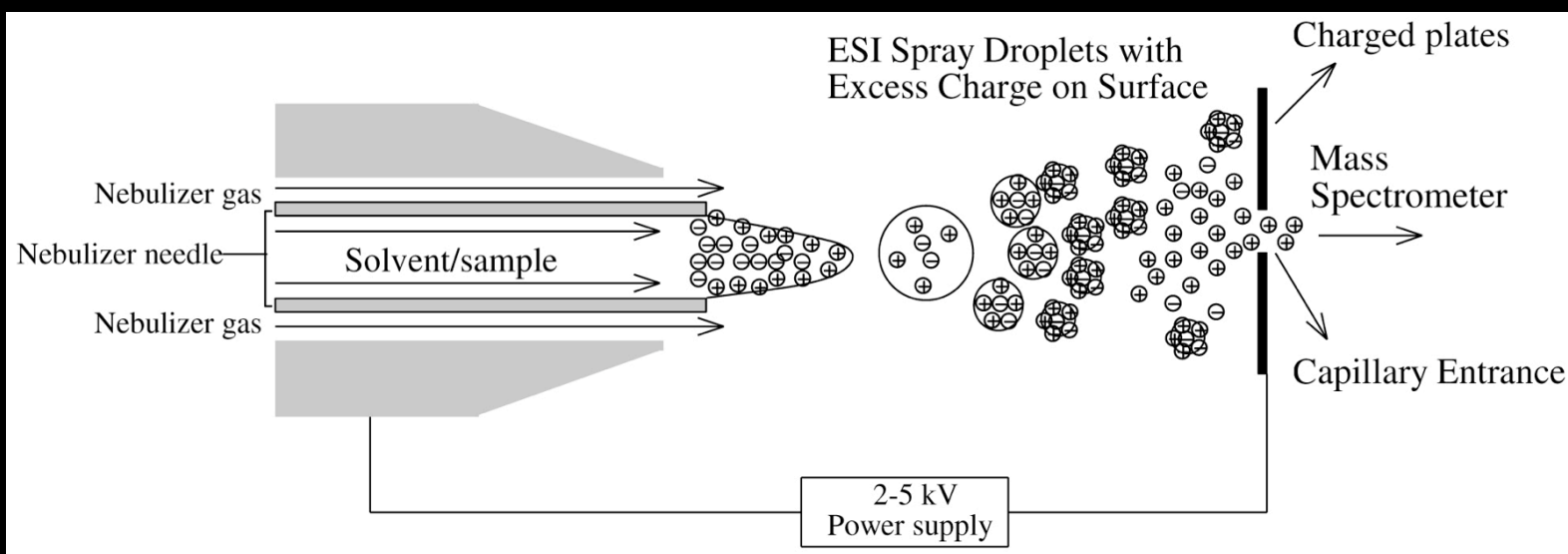
Matrix-Assisted Laser Desorption Ionization (MALDI)

- Soft Ionization method
- Sample is mixed with a condensed phase matrix
- Mixture is ionized with a laser
- Charged molecules are ejected from matrix
- Little excess energy → little fragmentation
- Good for large molecules
 - proteins
 - carbohydrates
 - polymers

Ionization Methods

Evaporative Methods

- Two methods are common: Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI)
 - Do not require vacuum pressures
- Both can be coupled to an LC system

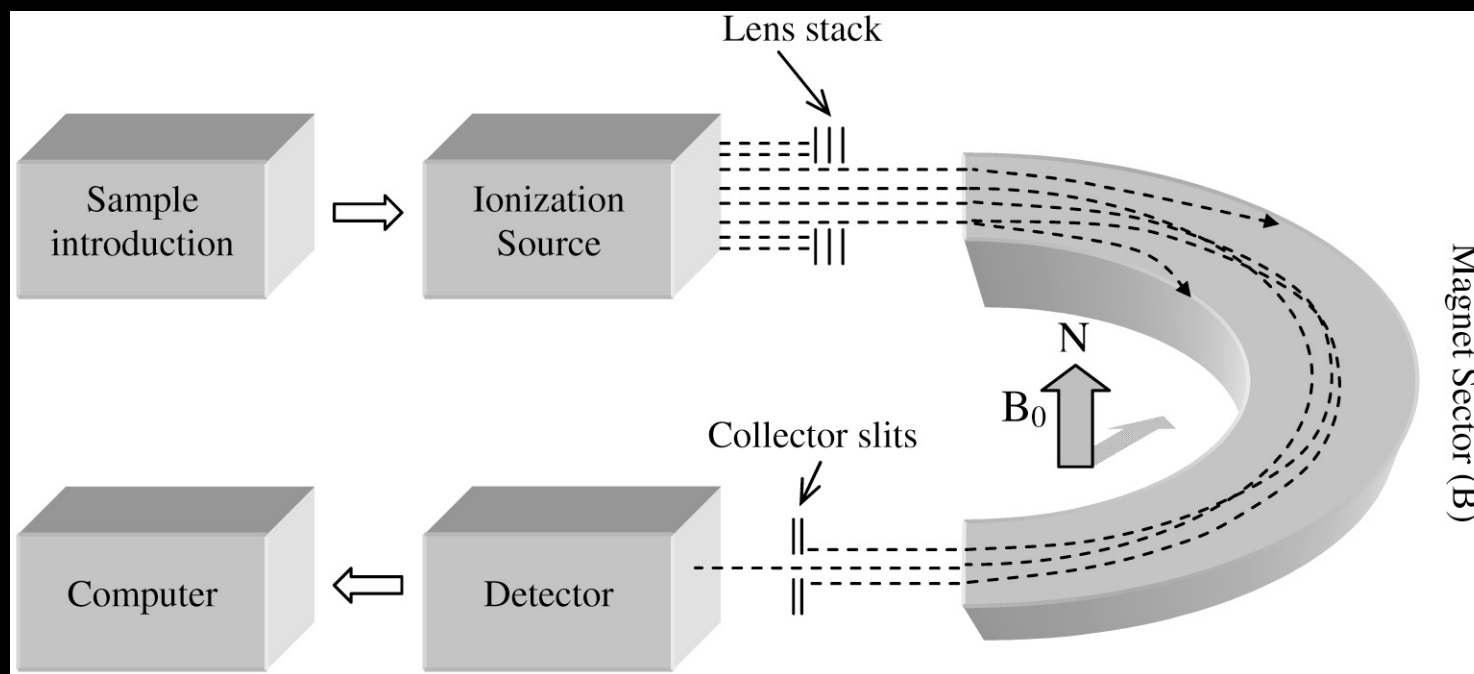


- Multiply charged ions are possible and can be useful for large molecules (e.g. proteins).
 - $m/z = 100,000 \text{ Da}/40 \text{ charges} \Rightarrow 2,500 \text{ } m/z$

Detectors

Magnetic Sector

- Magnetic field is used to deflect ions around curved path
 - Magnetic field is scanned to bring ions into focus

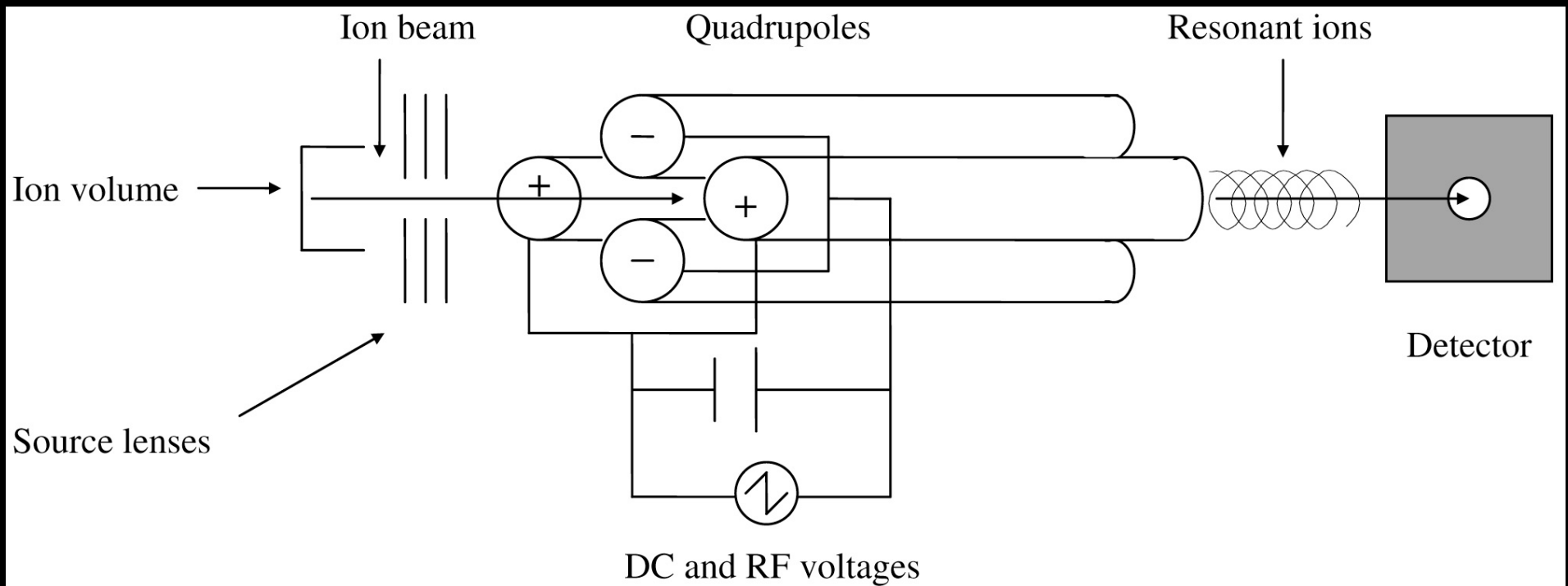


- Ions with correct m/z pass through the detector
 - If too heavy or too light do not make it through

Detectors

Quadrupole

- Ions fly through a tunnel of four charged rods
 - Voltage of the rods is changed in order to focus ions



- Ions with correct m/z are able to fly through to detector
 - If too heavy or too light deflected into rods

Detectors

Time-of-Flight

- Ions accelerated through potential and allowed to drift down a tube to the detector
 - all ions have the same energy

$$zeV = mv^2/2$$

$$v = (2zeV/m)^{1/2}$$

*different masses have
different velocities*

$$t = (L^2m/2zeV)^{1/2}$$

V = potential v = velocity
L = length of tube t = time
m = mass

*time of flight
varies with mass*

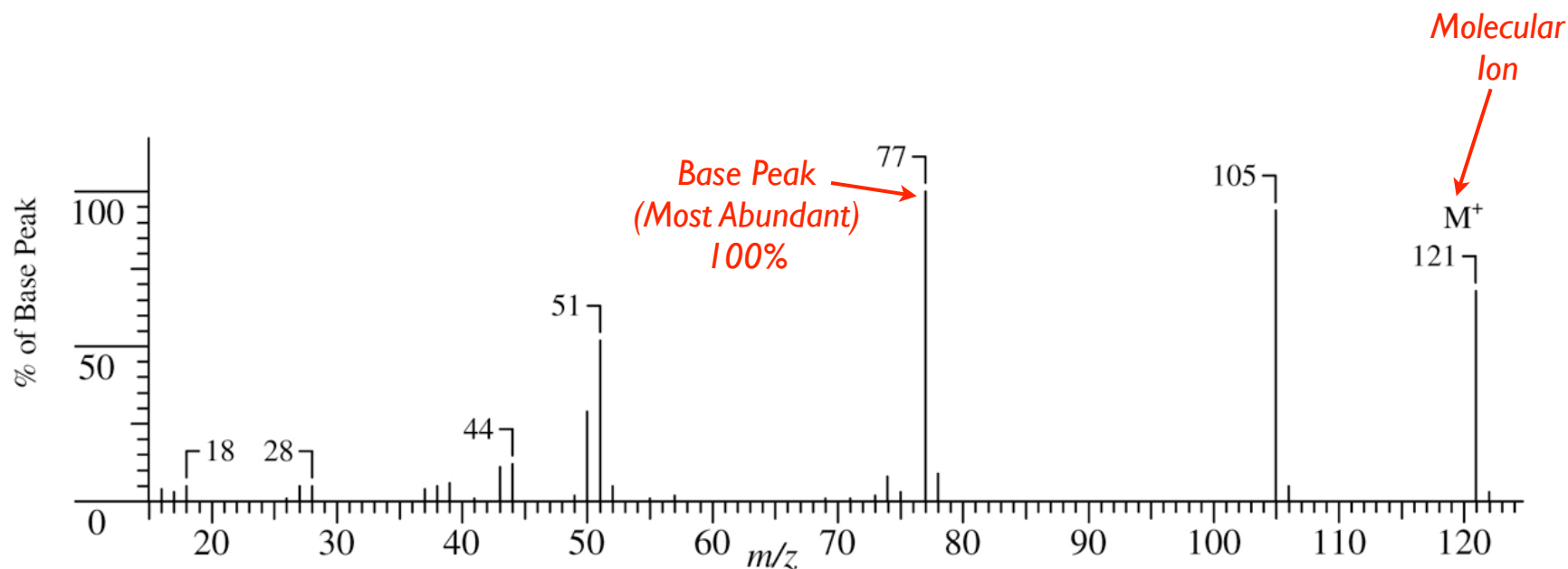
*As mass ↑
time ↑*

- Unlimited mass range
 - Often combined with MALDI

Mass Spectrometry

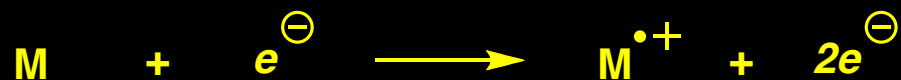
What does the spectrum look like?

Most work in this class will be based on EI



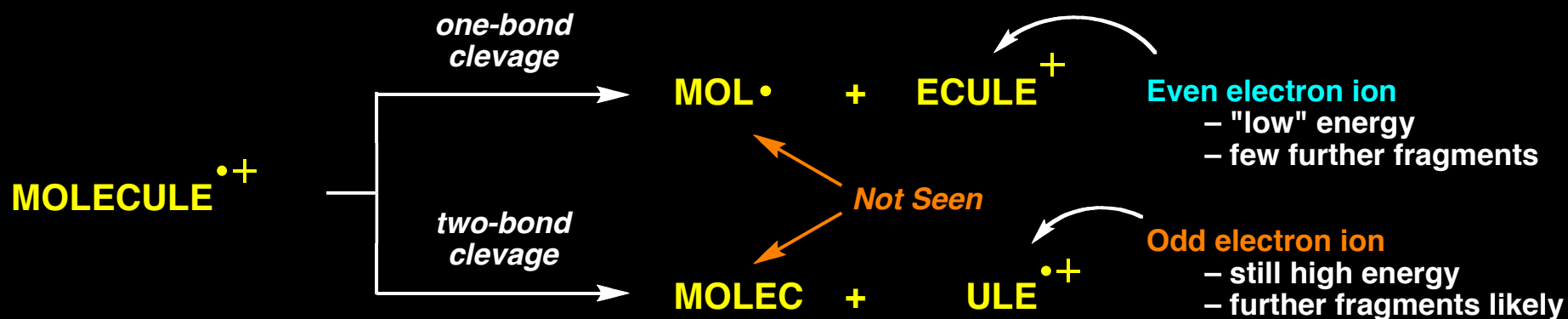
Mass Spectrometry

Basics of ionization and fragmentation



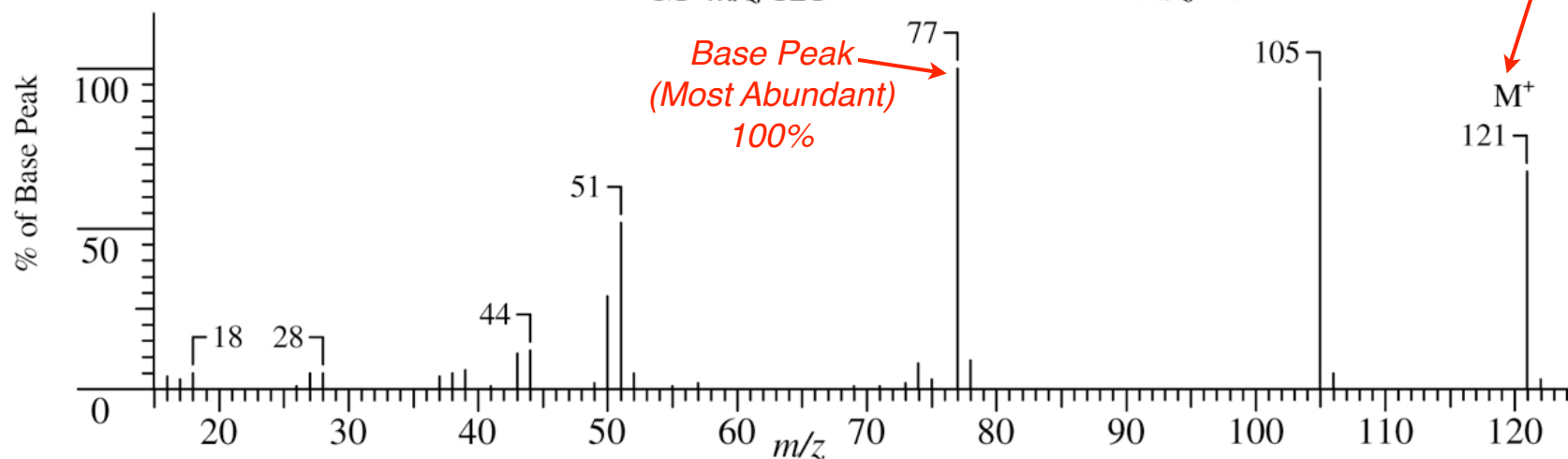
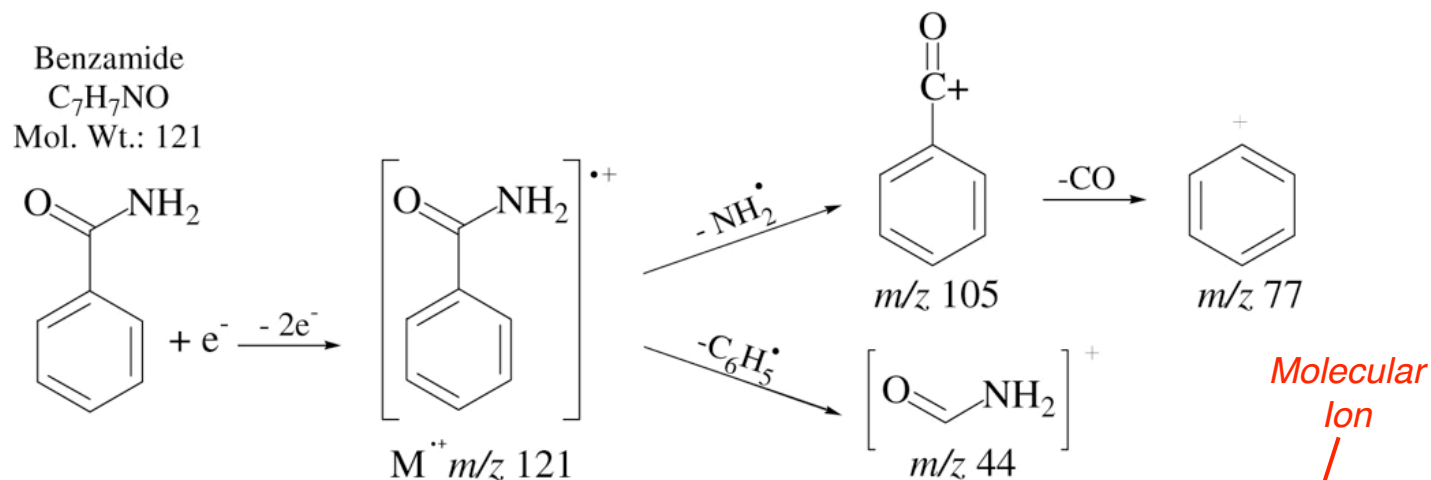
Molecular ion

- odd electron ion
- gives exact mass
- high energy, releases energy by bond cleavage



Mass Spectrometry

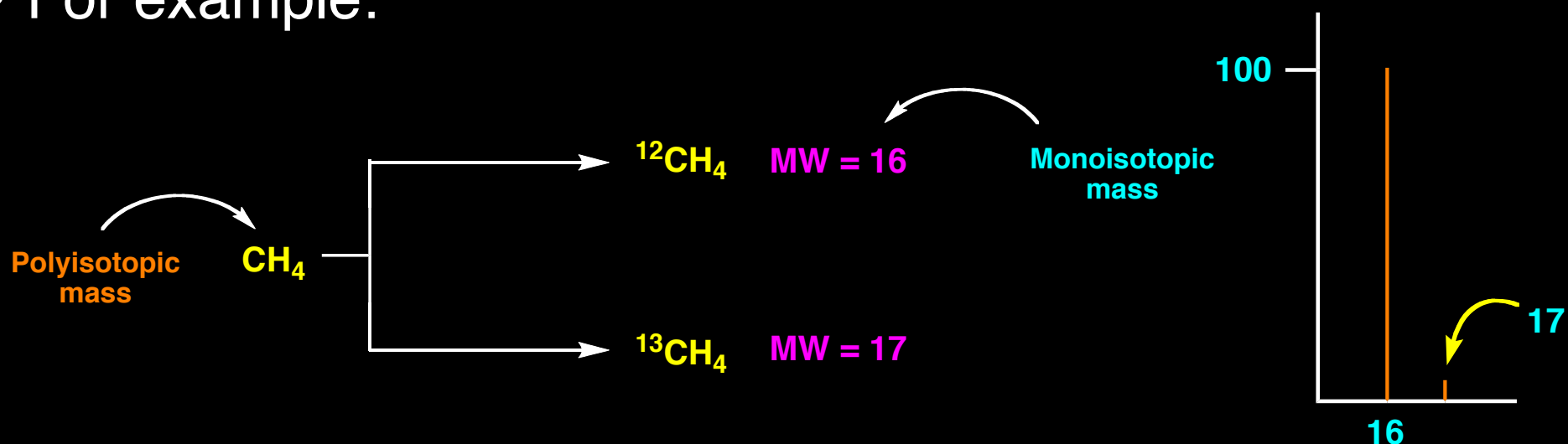
What does the spectrum look like?



Mass Spectrometry

Effects of Isotope Differences

- *Keep in Mind* $m/z = \underline{\text{mass}}$ over charge
 - this means isotopes have different m/z values and show up as different peaks
- Samples are actually a mixture of isotopic species
- To get formulas/structures must use monoisotopic masses, *not* those from periodic table which are weighted averages of all isotopes
- For example:



Mass Spectrometry

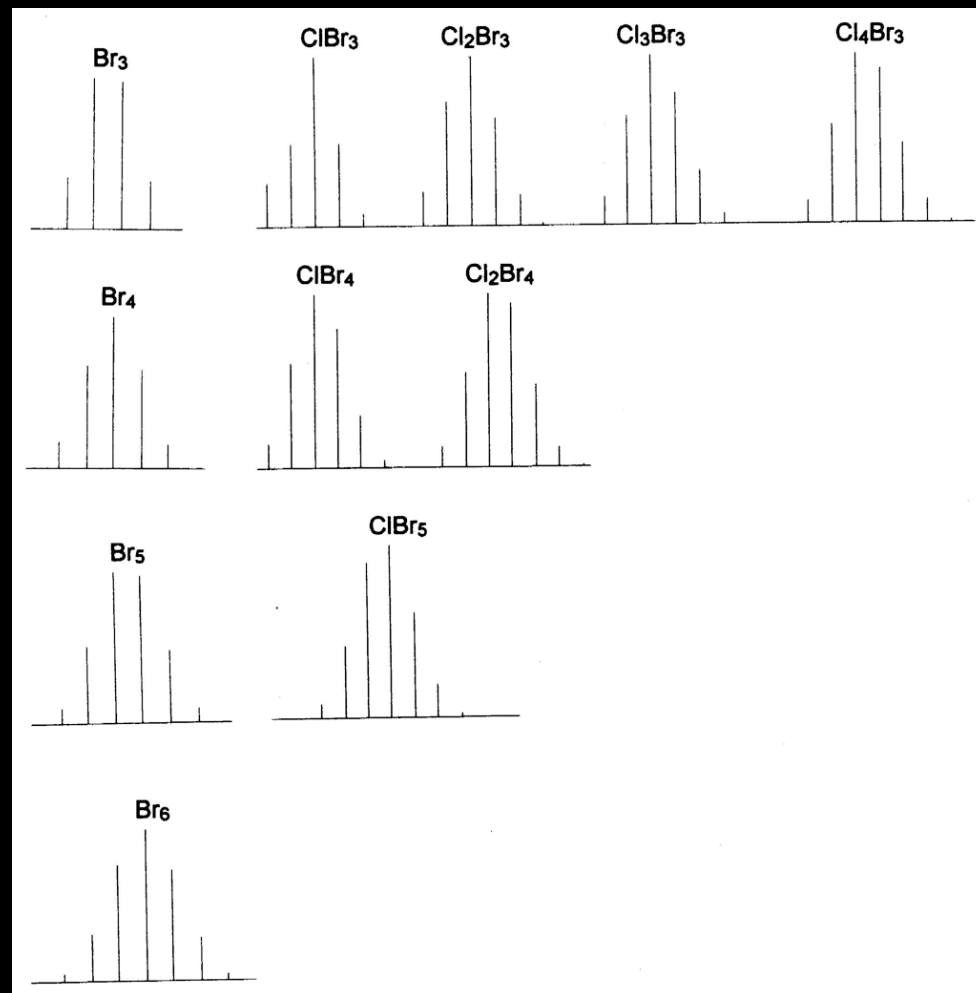
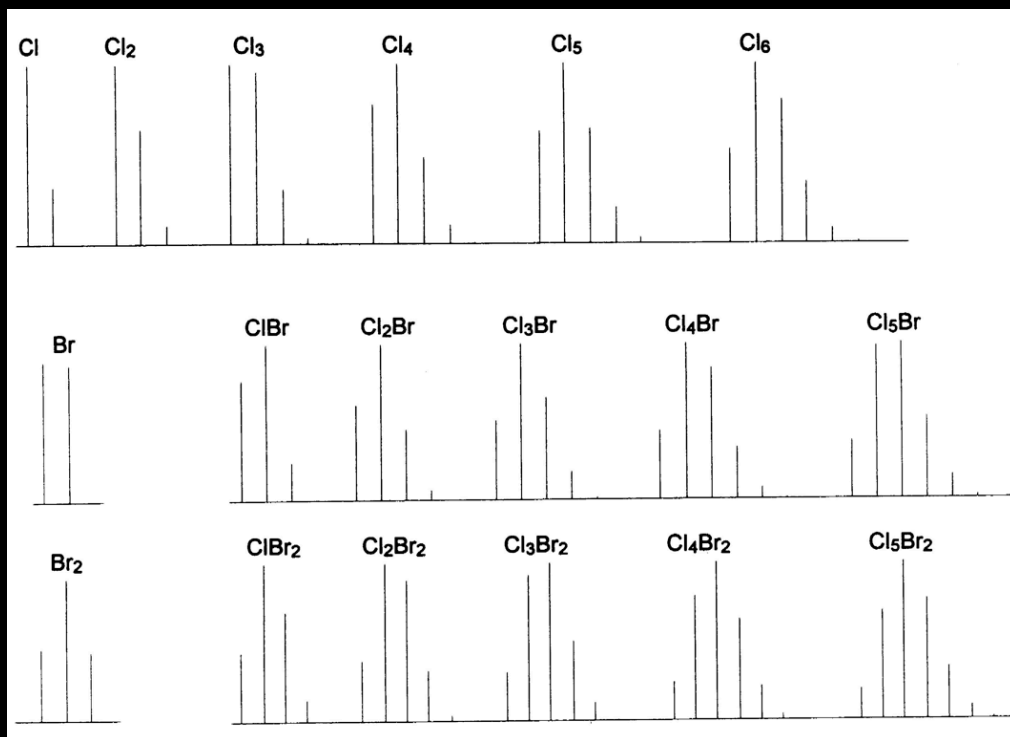
Relative Abundance for Common Elements

^1H	100	^2H	0.016	—	—
^{12}C	100	^{13}C	1.11	—	—
^{16}O	100	^{17}O	0.04	^{18}O	0.02
^{14}N	100	^{15}N	0.38	—	—
^{28}Si	100	^{29}Si	5.1	^{30}Si	3.35
^{31}P	100	—	—	—	—
^{32}S	100	—	—	^{34}S	4.4
^{19}F	100	—	—	—	—
^{35}Cl	100	—	—	^{37}Cl	32.5
^{79}Br	100	—	—	^{81}Br	98.0
^{127}I	100	—	—	—	—

Distinct isotope patterns

Mass Spectrometry

Isotope Patterns for Multiple Halogens



Mass Spectrometry

A “More Complicated” Molecule



	<u>m/z</u>	<u>Rel. Ab.</u>
$^{12}\text{C} \rightarrow$	12	8.7
$^{16}\text{O} \rightarrow$	16	9.6
$\text{M}^{2+} \rightarrow$	22	1.9
$^{12}\text{C}^{16}\text{O} \rightarrow$	28	9.8
$^{12}\text{C}^{17}\text{O} + ^{13}\text{C}^{16}\text{O} \rightarrow$	29	0.13
$^{12}\text{C}^{18}\text{O} \rightarrow$	30	0.02
$\text{M}^+ \rightarrow$	44	100 $\leftarrow ^{12}\text{C}^{16}\text{O}_2$
	45	1.2 $\leftarrow ^{13}\text{C}^{16}\text{O}_2 + ^{12}\text{C}^{16}\text{O}^{17}\text{O}$

Why don't we see other combinations?
-low probability (“non-existent”)

Determination of Formula

What mass to use?

– For low resolution, round to nearest amu

$$^{16}\text{O} = 15.9949 \rightarrow 16$$

$$^{13}\text{C} = 13.00336 \rightarrow 13$$

– For high resolution, round to nearest 0.0001 amu

$$^1\text{H} = 1.00783 \rightarrow 1.0078$$

$$^{13}\text{C} = 13.00336 \rightarrow 13.0034$$

With high resolution masses you can differentiate molecules with the same nominal mass

Example: For MW = 28

Exact mass – within 10 ppm

#.XXXX

Difference of 2 = 1 ppm ↑

$\text{C}\equiv\text{O}$	$\text{N}\equiv\text{N}$	$\text{H}_2\text{C}=\text{CH}_2$
12.0000	14.0031	12.0000
+ 15.9949	+ 14.0031	+ 4.0312
<hr/>	<hr/>	<hr/>
27.9949	28.0062	28.0312

← All can be distinguished!!

see
handout

Determination of Formula

The Rule of 13

Assumes C_nH_n and amu equivalent (13 for $n=1$) is present in all molecular fragment ions

Step 1: Divide M^+ mass by 13, this gives n

Step 2: Any remainder represents count of additional H's

example 1: for $M^+ = 78$

$$78 \div 13 = 6 \rightarrow n = 6 \rightarrow C_6H_6$$

example 2: for $M^+ = 92$

$$92 \div 13 = 7.077 \rightarrow n = 7$$

$$7 \times 13 = 91 \rightarrow 1 \text{ extra H is present}$$

$$\text{Formula is } C_7H_{7+1} = C_7H_8$$

Determination of Formula

The Rule of 13

example 3: for $M^+ = 161$

$$161 \div 13 = 12.385 \rightarrow n = 12$$

$$12 \times 13 = 156 \rightarrow 5 \text{ extra H's are present}$$

$$\text{Formula is } C_{12}H_{12+5} = C_{12}H_{17}$$

What about heteroatoms?

Step 1: First derive formula as above

Step 2: Next, modify using C_nH_m equivalents

<u>Element</u>	<u>C_nH_m Equiv</u>	<u>Element</u>	<u>C_nH_m Equiv</u>
$^1H_{12}$	C	^{31}P	C_2H_7
^{16}O	CH_4	^{32}S	C_2H_8
^{14}N	CH_2	$^{16}O^{32}S$	C_4
$^{16}O^{14}N$	C_2H_6	^{35}Cl	C_2H_{11}
^{19}F	CH_7	^{79}Br	C_6H_7
^{28}Si	C_2H_4	^{127}I	$C_{10}H_7$

Determination of Formula

The Rule of 13

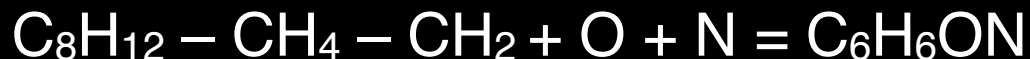
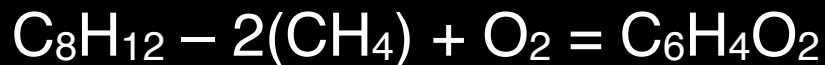
example 4: for $M^+ = 108$

$$108 \div 13 = 8.308 \rightarrow n = 8$$

$8 \times 13 = 104 \rightarrow 4$ extra H's are present

Formula is $C_8H_{8+4} = C_8H_{12}$

Possible candidates with heteroatoms



MS will only give you molecular weight data. Must use other spectral techniques to glean information about the presence of heteroatoms.

Determination of Formula

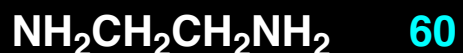
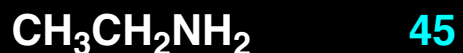
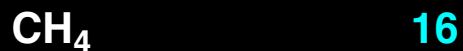
The Nitrogen Rule

- A molecule with even numbered MW must contain either no N or even number of N
- A molecule with odd numbered MW must contain an odd number of N
- Holds for all compounds with C, N, O, S, X, P, B, Si, As, & alkaline earths
- Corollary: for fragmentation at a single bond
 - even # M^+ will give odd # fragment ion
 - odd # M^+ will give even # fragment ion
 - fragment ion must contain all N if any

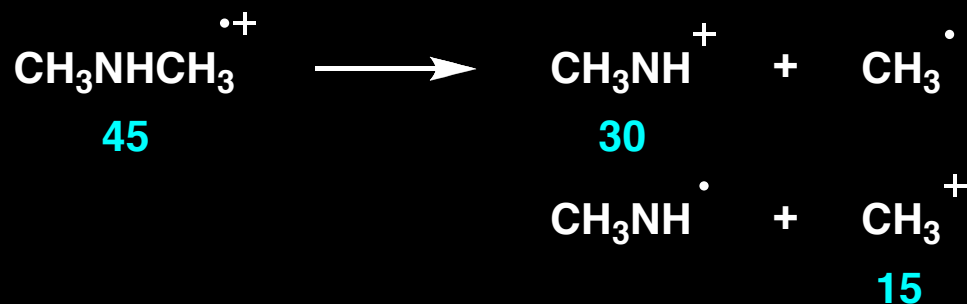
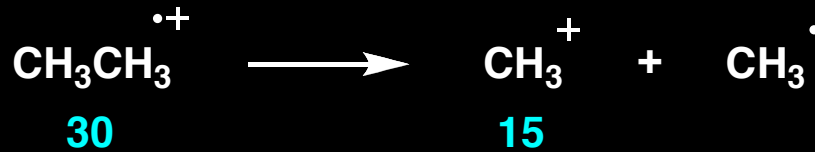
Determination of Formula

The Nitrogen Rule

Examples:

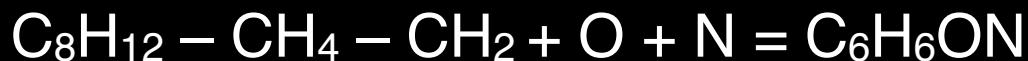


Fragment Ions:



Back to example of MW = 108...

Formula of $\text{C}_6\text{H}_6\text{ON}$ *must* be a fragment ion, *not* M^+



However... $\text{C}_8\text{H}_{12} - 2(\text{CH}_2) + \text{N}_2 = \text{C}_6\text{H}_8\text{N}_2$ is possible M^+

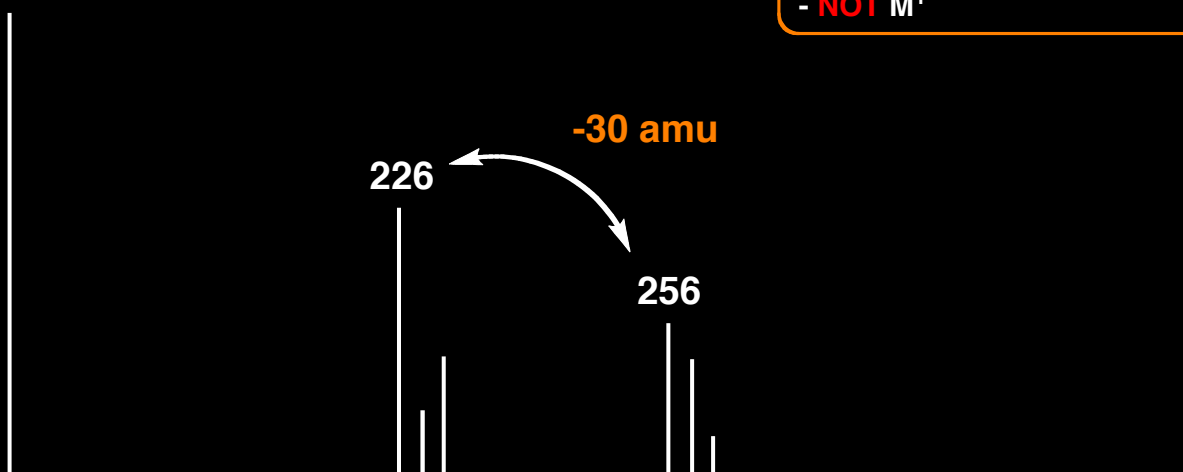
Determination of Formula

How do we know if we have the molecular ion or a fragment ion?

1. Must obey Nitrogen Rule
2. Must generate lower mass ions by logical neutral losses

Unlikely losses

4 – 14
21 – 25
33, 37, 38



- ok mass loss
- even # "M⁺", even # fragment
- **NOT** M⁺

Determination of Formula

How do we know if we have the molecular ion or a fragment ion?

1. Must obey Nitrogen Rule
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Unlikely losses

4 – 14
21 – 25
33, 37, 38



- ok mass loss
- even # "M⁺", odd # fragment
- ✓ M⁺

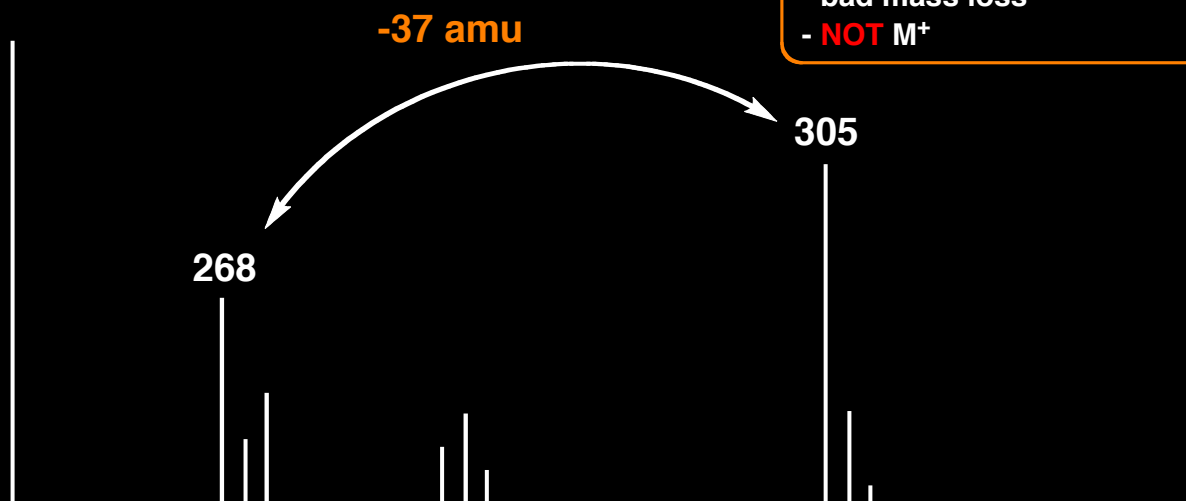
Determination of Formula

How do we know if we have the molecular ion or a fragment ion?

1. Must obey Nitrogen Rule
2. Must generate lower mass ions by logical neutral losses

Unlikely losses

4 – 14
21 – 25
33, 37, 38



- odd # "M⁺", even # fragment
- bad mass loss
- **NOT** M⁺

Determination of Formula

How do we know if we have the molecular ion or a fragment ion?

Stability plays a factor in whether or not we see molecular ion.

Decreasing ability to give prominent M^+ :

aromatics > conjugated alkenes > cyclic compounds >
organic sulfides > alkanes > mercaptans

Decreasing ability to give recognizable M^+ :

ketones > amines > esters > ethers >
carboxylic acids ~ aldehydes ~ amides ~ halides

M^+ is frequently not detectable from:

aliphatic alcohols, nitrites, nitrates, nitro compounds,
nitriles, highly branched compounds