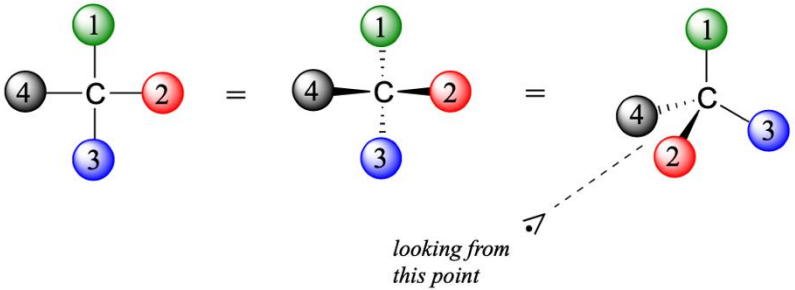
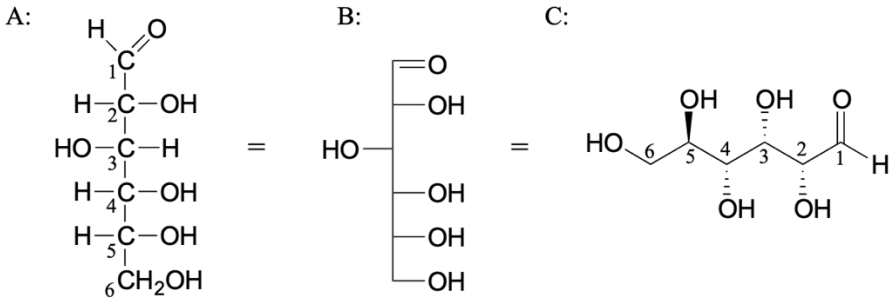
Bio-organic Chemistry

Lecture 3

Fischer and Haworth projections

-For Fischer projections the stereochemical information is conveyed by a simple rule where vertical bonds point into the plane of the page while horizontal bonds point out of the page





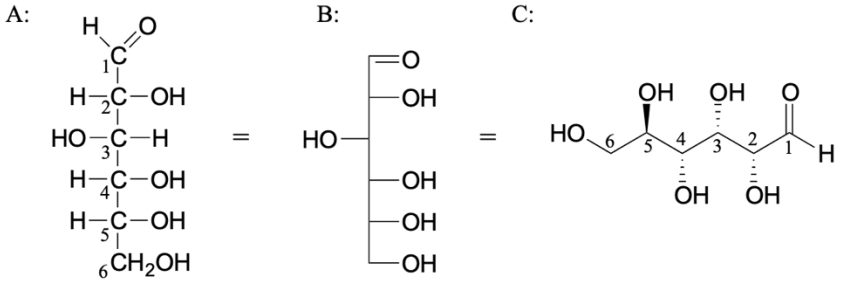
Fischer projection

Fischer projection with no carbons and hydrogens

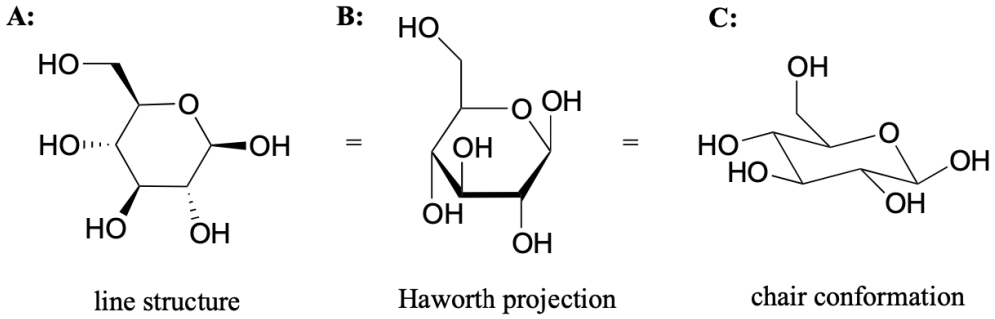
Zig-zag

Fischer and Haworth projections

Fischer projections – used for representing open-chain sugars



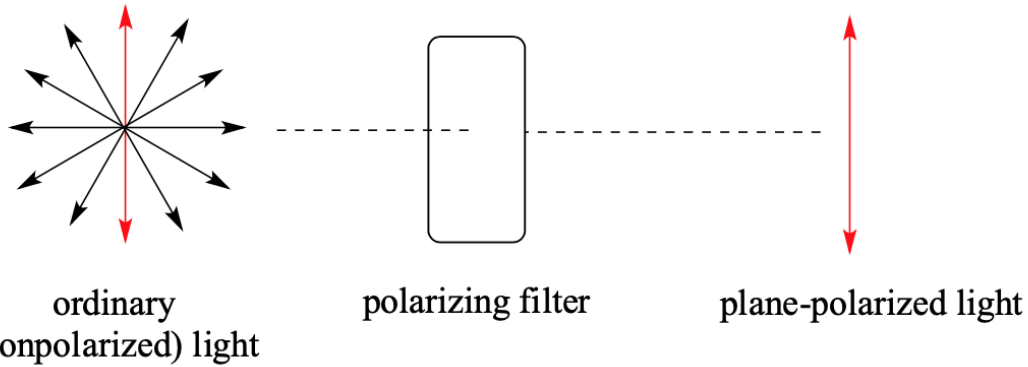
Haworth – used for representing for cyclic sugars



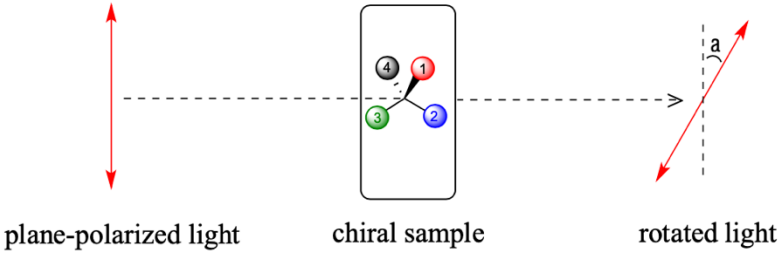
-Haworth is convenient way to show stereochemistry but not useful for conformation

Optical activity of enantiomers

-Light waves are oscillating electric and magnetic fields
-In ordinary light the oscillation is randomly oriented

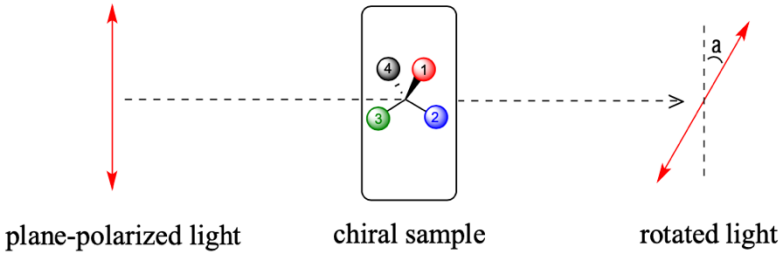


-Polarized light when passed through a sample of a chiral compound will rotate



Optical activity of enantiomers

-Polarized light when passed through a sample of a chiral compound will rotate

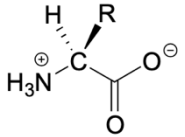


-If a compound rotates polarized light clockwise (+) direction is **dextrorotatory** if it does so in the counterclockwise (-) is **levorotatory**

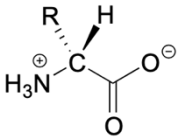
-For instance L- and D- aminoacids

-the measure specific rotations is measured as a physical property

R = *amino acid side chain*



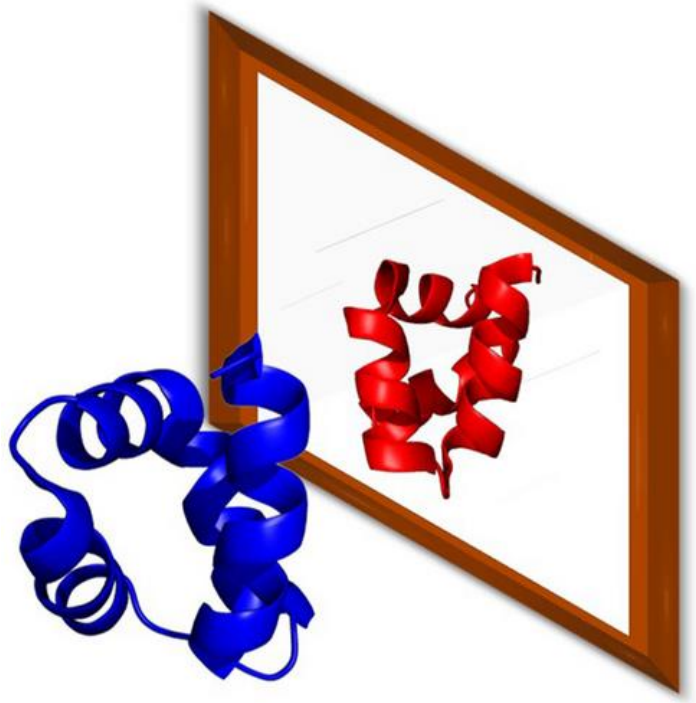
L-amino acids
(common in nature)

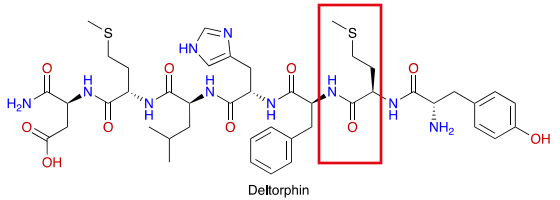


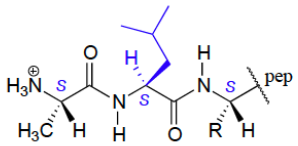
D-amino acids
(rare in nature)

There is no relationship between the chiral compound R/S designation and the direction of its specific rotation.

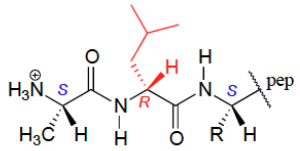
Effects on structure of an L and D protein







peptide with L-Leu at position 2



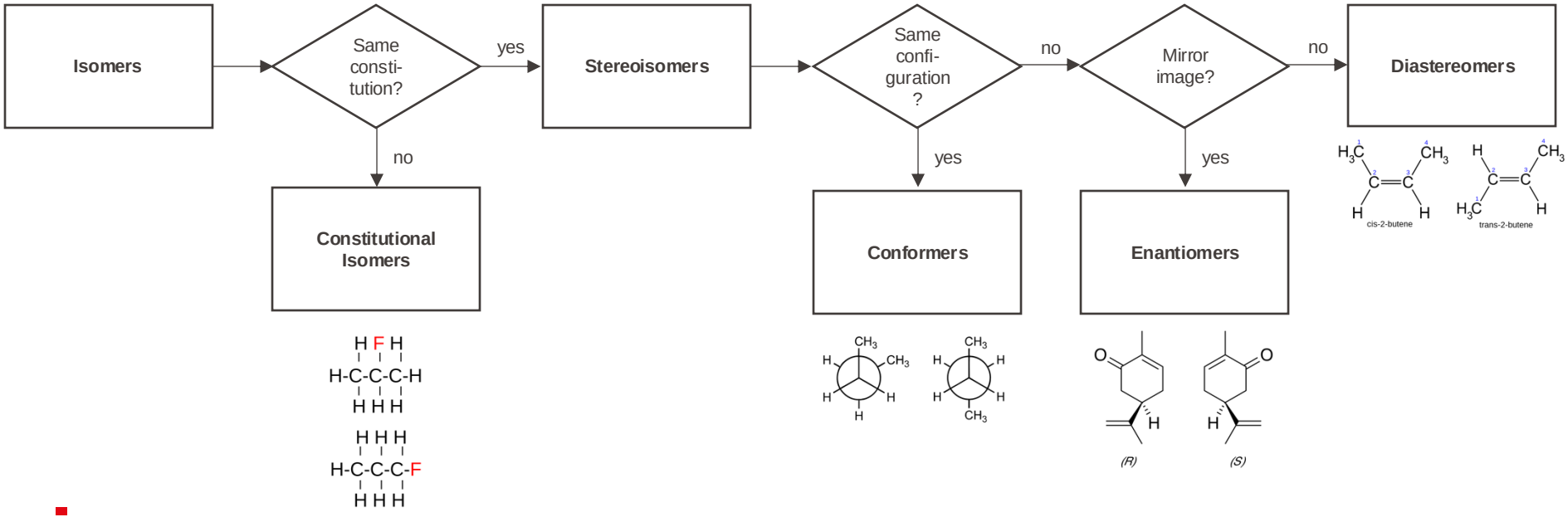
peptide with D-Leu at position 2

D-protein enantiomers can be accessed through total chemical synthesis and their preparation enables establishment of mirror-image life

Stereochemistry

Summary

Isomers = molecules with identical molecular formula but distinct arrangements of atoms in space



What do organic chemists do in the lab ?

-They use a different number of techniques to ask questions to their molecules

Mass spectrometry (MS): *What is the atomic weight of the molecule and its common fragments?*

Infrared (IR) spectroscopy: *What functional groups does the molecule contain?*

Ultraviolet-visible (UV-Vis) spectroscopy: *What is the nature of conjugated π-bonding systems in the molecule?*

Nuclear magnetic resonance spectroscopy (NMR): *What is the overall bonding framework of the molecule?*

Crystallography and X-Ray: *What is the 3D structure of a molecule?*

Mass Spectrometry

-The basic principle of Mass spectrometry is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and abundance.

-The analyte may be ionized thermally, by electric fields or by impacting energetic electrons, ions or photons.

-The ions can be single ionized ions, clusters, molecules or their fragments or associates.

-Ion separation is effected by static or dynamic electric or magnetic fields.

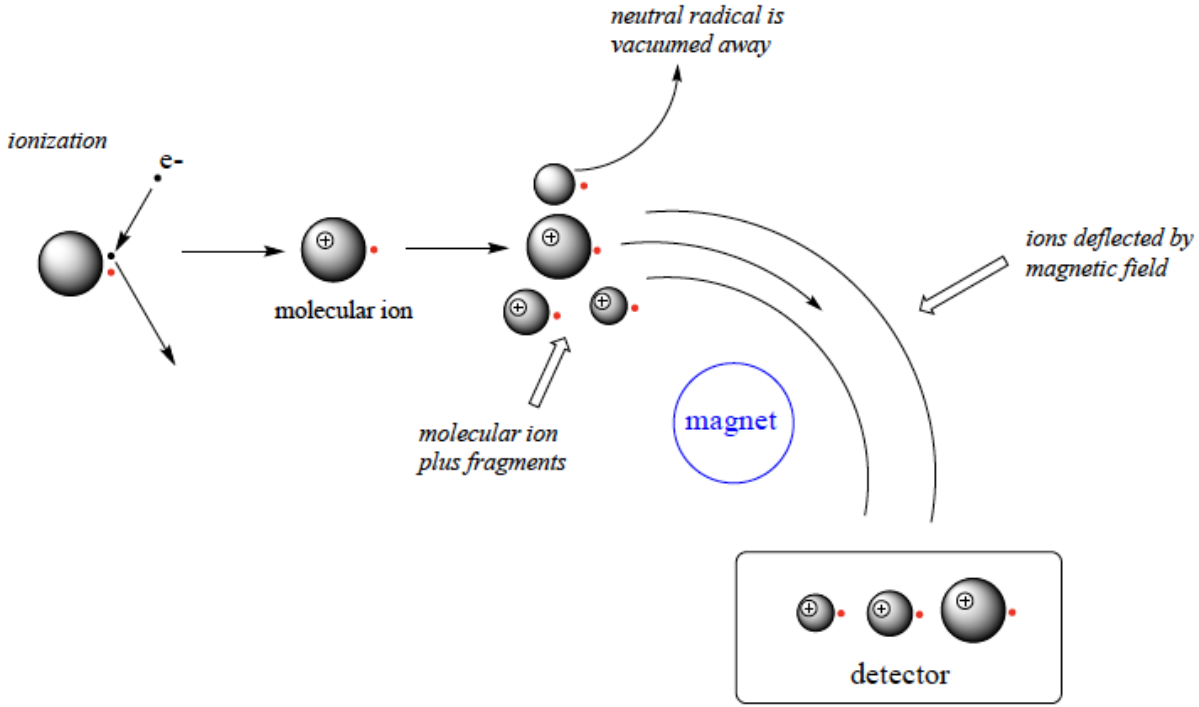
Gross 2004

Mass Spectrometry

Measure masses of individual molecules that have been converted to **ions**
Utilizes **magnetic/electric fields** to change direction/velocity

Agilent 7250 GC/Q-TOF

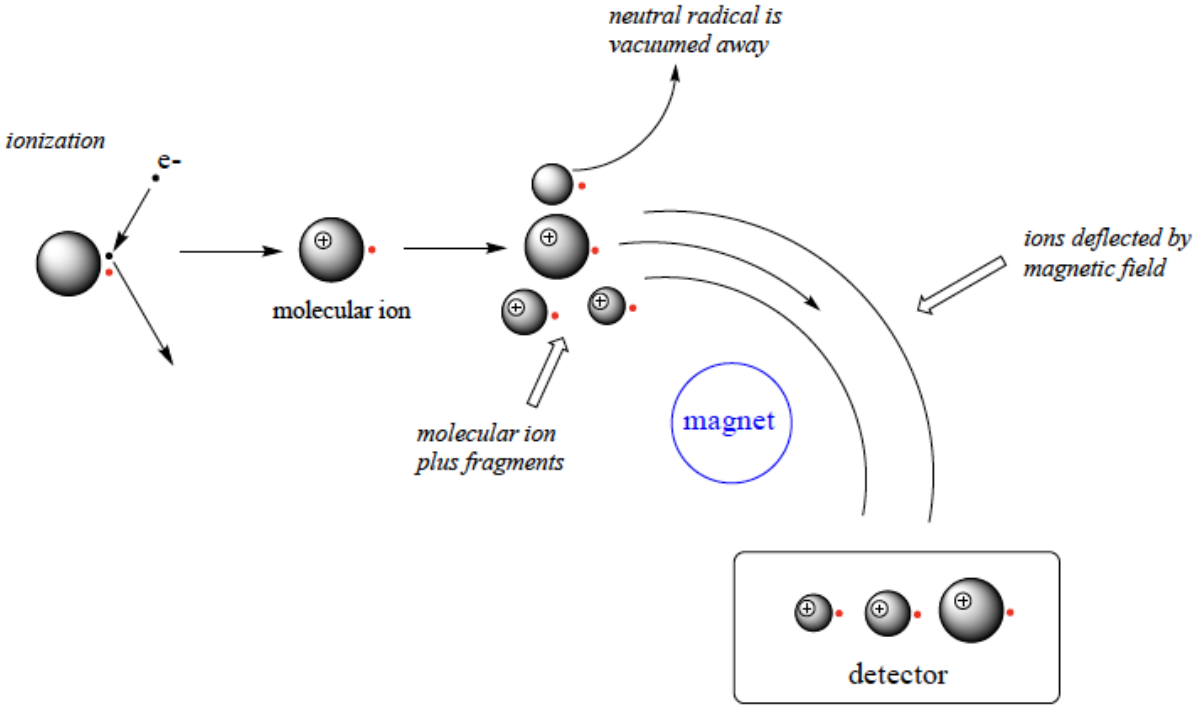
Thermo Scientific Orbitrap Exploris



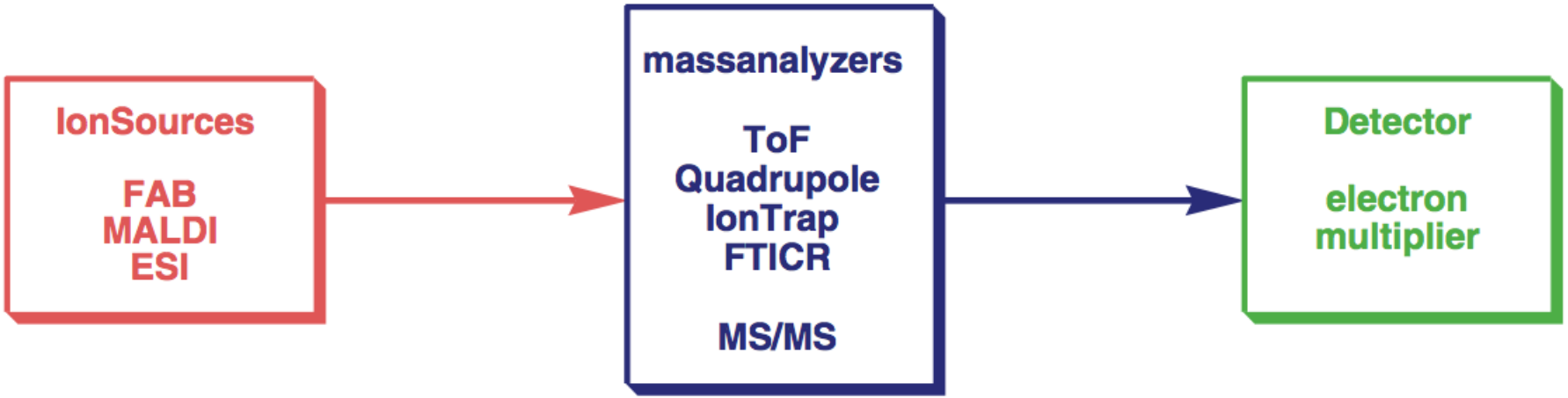
Mass Spectrometry

Measure masses of individual molecules that have been converted to **ions**
Utilizes **magnetic/electric fields** to change direction/velocity

1. Ionization of Molecules
2. Ion Separation
3. Detection



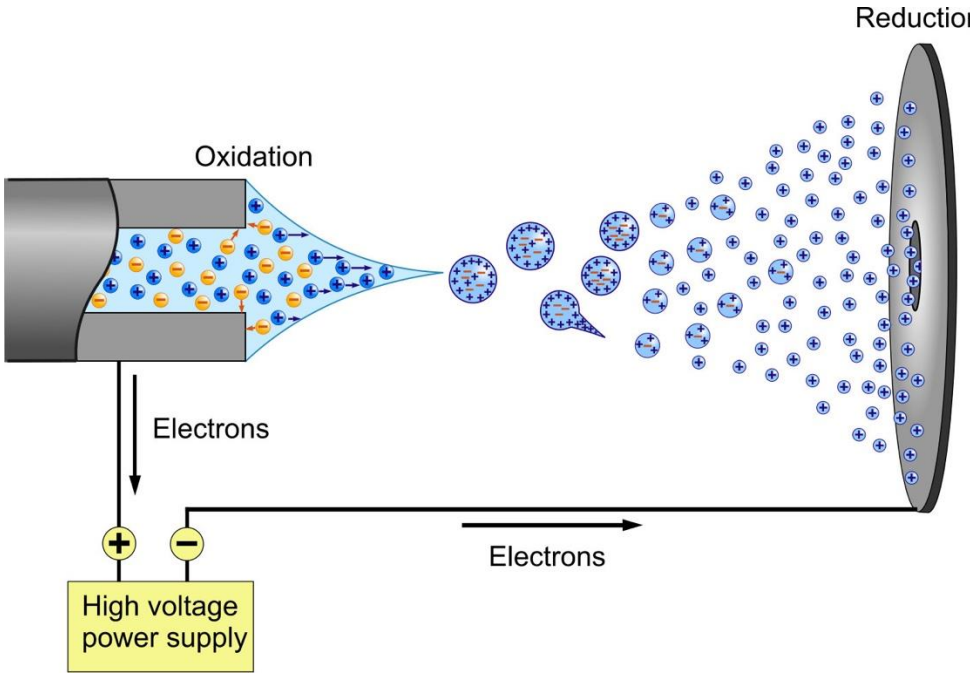
Mass Spectrometry - Instrumentation

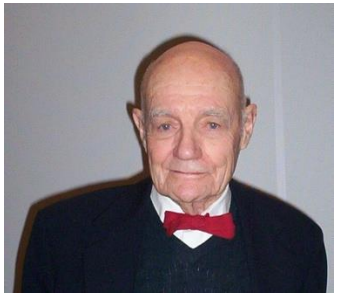


Ionization Source
-Passes molecules into the gas phase as ionic species
FAB – Fast Atom Bombardment
MALDI- Matrix assisted laser desorption and ionization
ESI – Electrospray Ionization

Ionization

There are multiple techniques for ionization of molecules.
One of the most famous: **ESI (Electron Spray Ionization)** from liquid phase



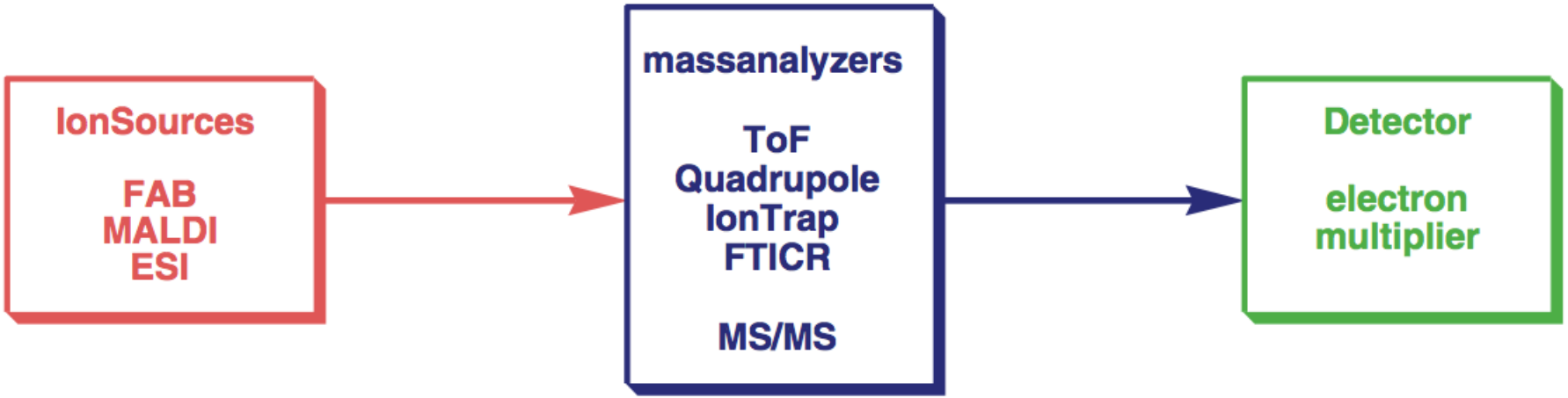


John Bennett Fenn
(Nobel Prize 2002)

1. Introduction of Sample in Liquid State
2. Creation of Charged Droplets at Capillary Tip
3. Droplet Shrinkage via Evaporation (Gas & Heat)
4. Formation of Highly Charged Tiny Droplets
5. Disintegration of Droplets due to Coulombic Charge Repulsion
6. Generation of Free Gas-Phase Ions

Source: https://flickr.com/photos/136533650@N02/21589986840

Mass Spectrometry - Instrumentation



Mass Analyzers

-Separate ions according to their mass/charge ratio(m/z)

ToF– Time of Flight

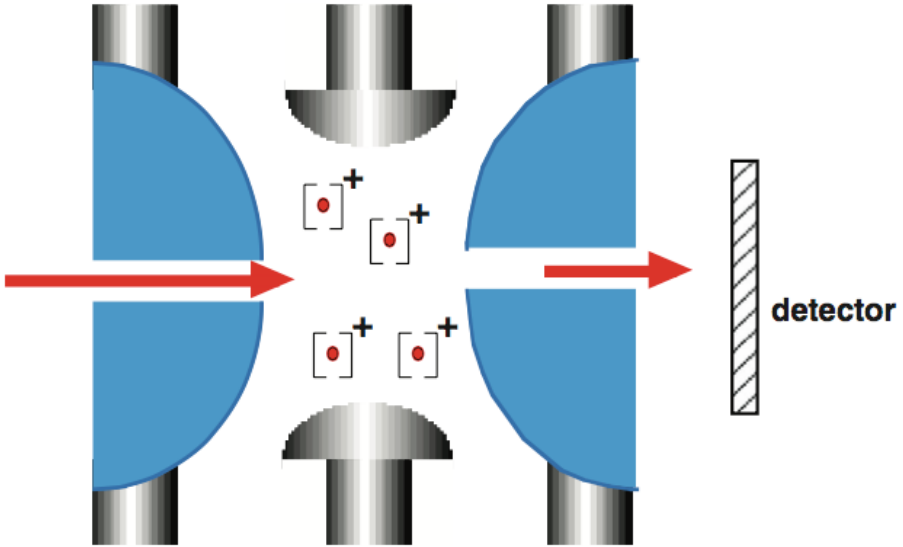
FTICR – Fourier transform ion cyclotron resonance

Ion Trap

MS/MS – tandem mass spectrometry

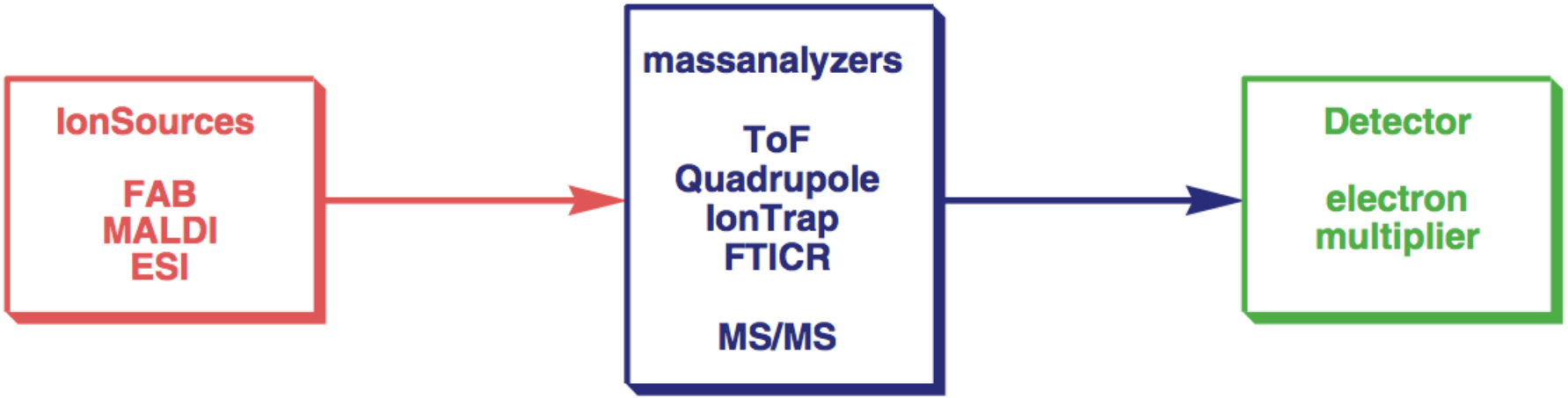
Mass Analyzers

Ion Trap & MS/MS



-The m/z of each ion is determined according to the field potential and the trajectory of the ion in the detector

Mass Spectrometry - Instrumentation

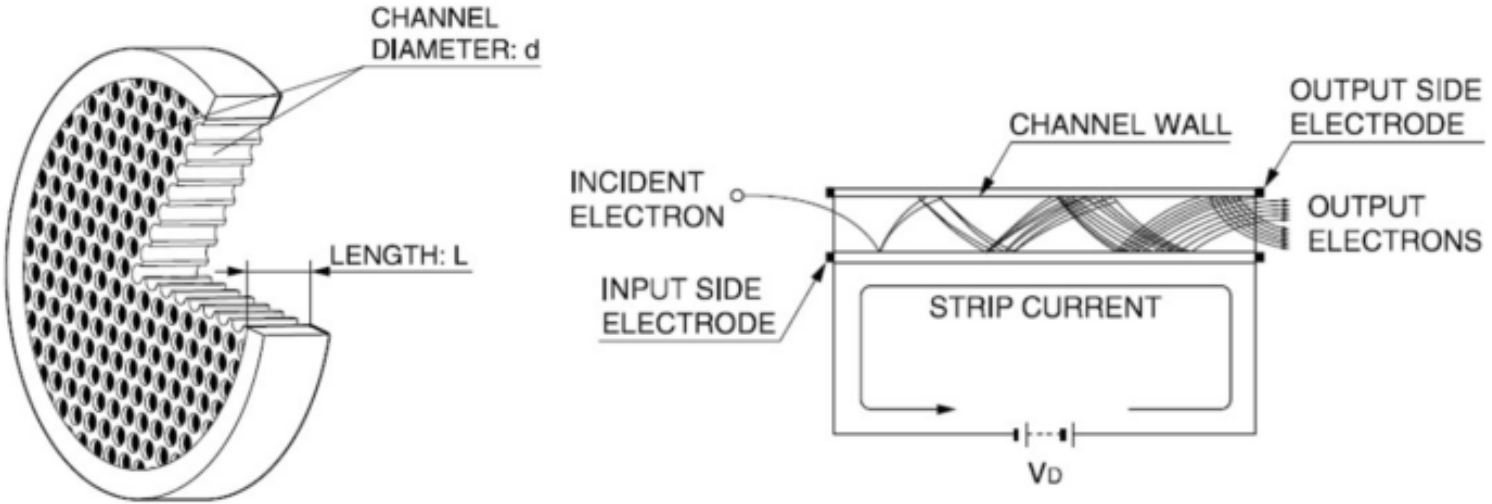


Detector
-Measures both abundance and m/z of detected ions

Detection

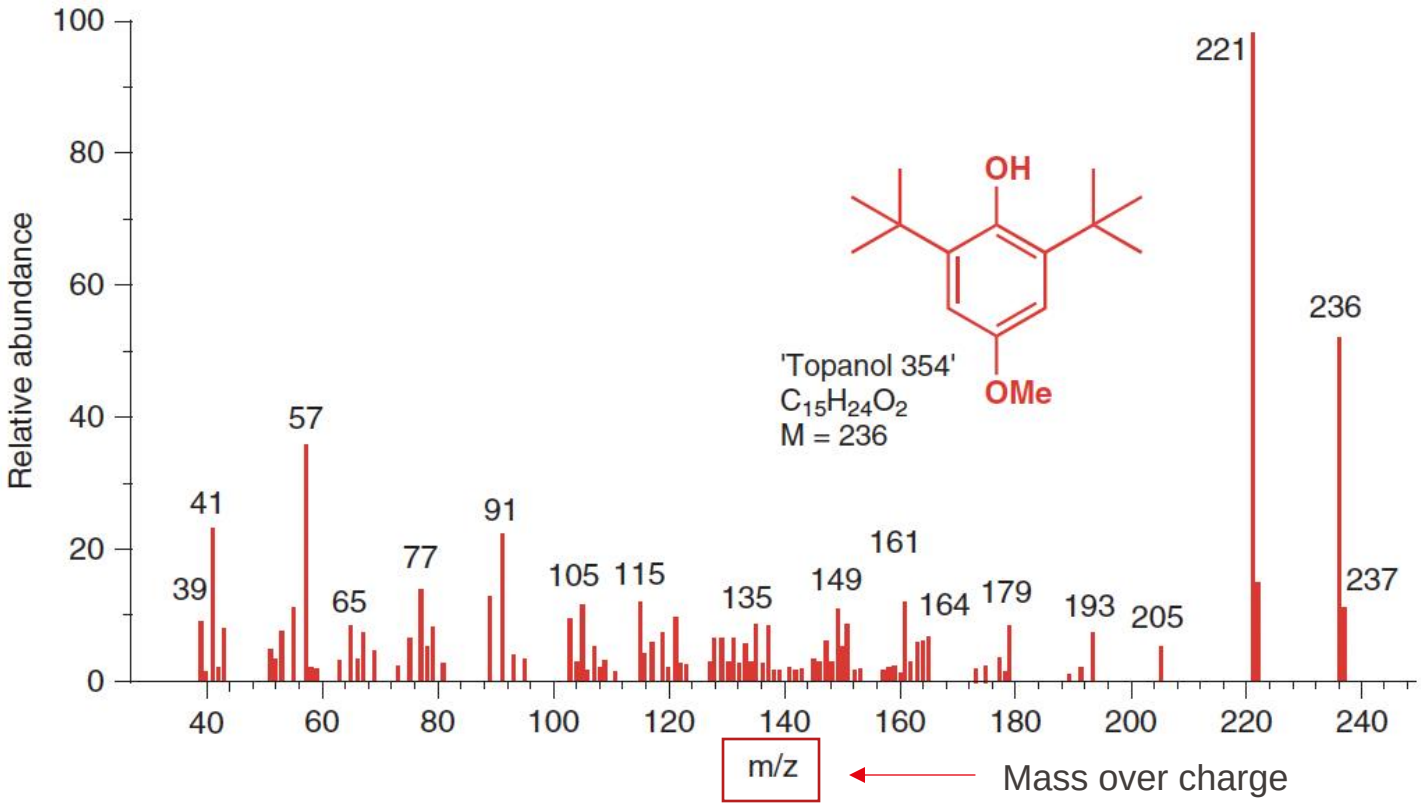
Detectors allow a mass spectrometer to generate a signal (a current) from incident ions, by generating secondary electrons which are further amplified.
-> Can be quite complex!

Example: Time of Flight - Microchannelplate detectors (MCP)



Thermal characterization of resistance and gain of microchannel plate (MCP) detectors for the JENI experiment. *CEAS Space J* **11**, 597–605 (2019)

Mass Spectrometry - An Example Spectrum



Mass and charge in mass spectrometry

“Mass over charge”: m/z is dimensionless by definition. It may be understood as the ratio of the **numerical value of ionic mass on the atomic mass scale** and the **number of elementary charges** of the respective ion.

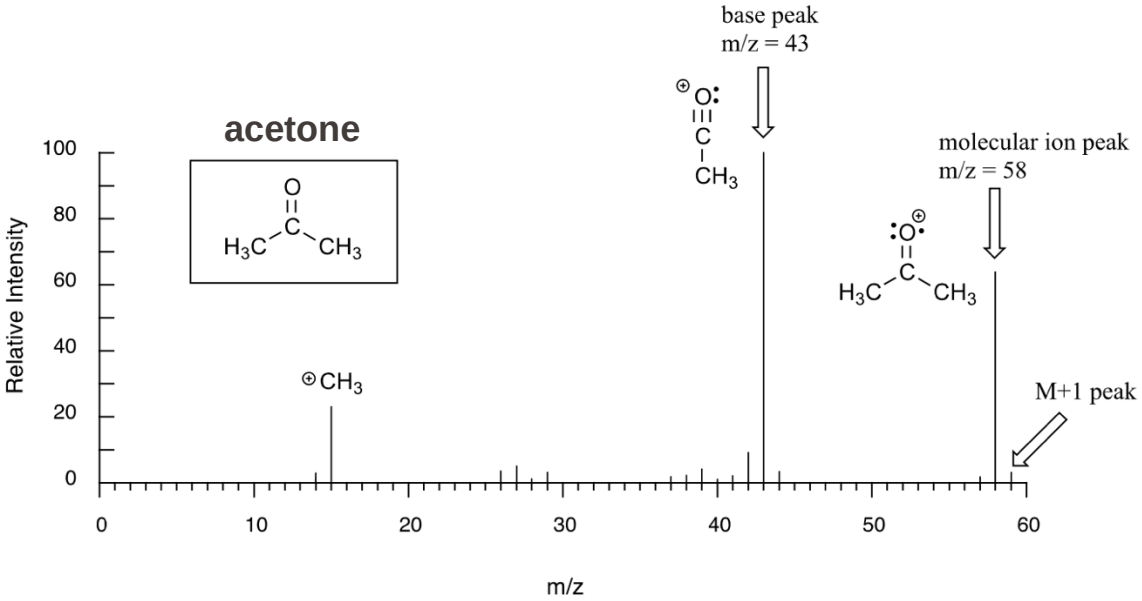
$$\text{mass-to-charge ratio} = \frac{\text{mass of cation}}{\text{charge of cation}}$$

Note: As long as only singly charged ions are observed (z = 1) the m/z scale directly reflects the atomic mass scale

$$\text{mass-to-charge ratio} = \frac{\text{mass of cation}}{+1}$$

$$= \text{mass of cation}$$

Let's take a look at an example



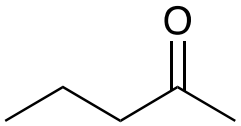
-The most abundant ion is called **base peak** (set to 100%) all other peak

- Parent peak has the full molecular weight of the compound

-Data collected is a series of m/z, each associated with their relative abundance gives a unique fingerprint of the compound

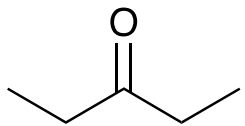
-These spectra are then used for computational searches over databases of compounds

Sometimes we are in trouble…



pentan-2-one

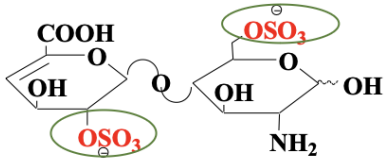
86



pentan-3-one

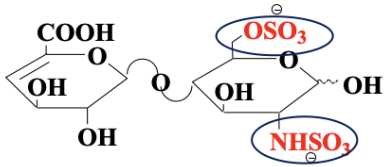
86

Same m/z!



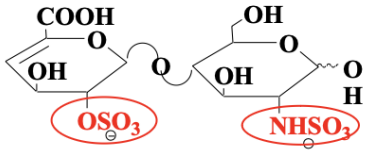
I-H

494.9988



II-S

494.9988



III-S

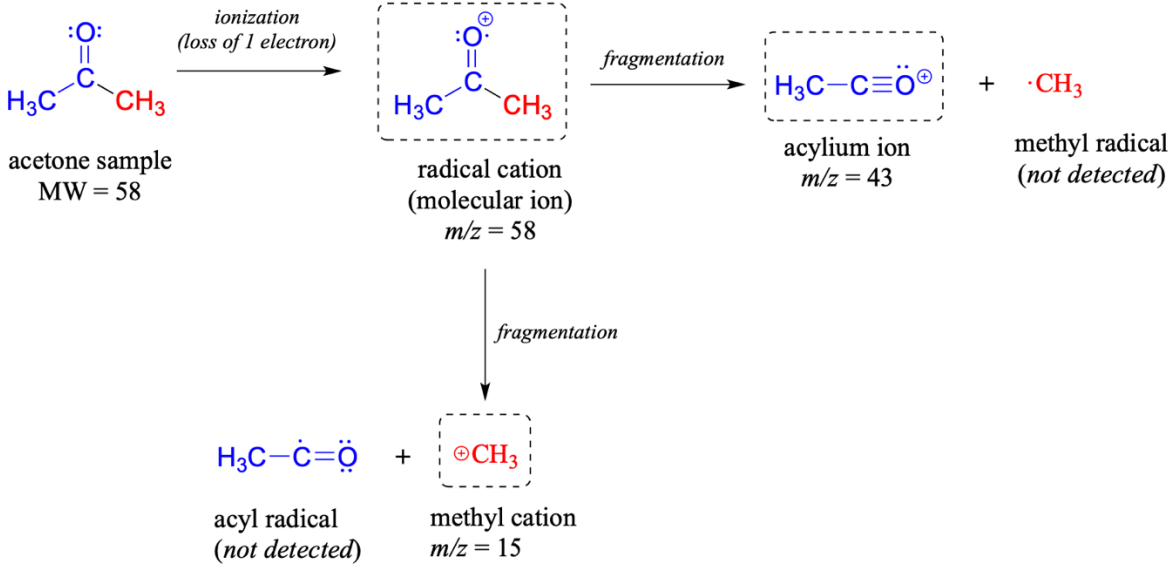
494.9988

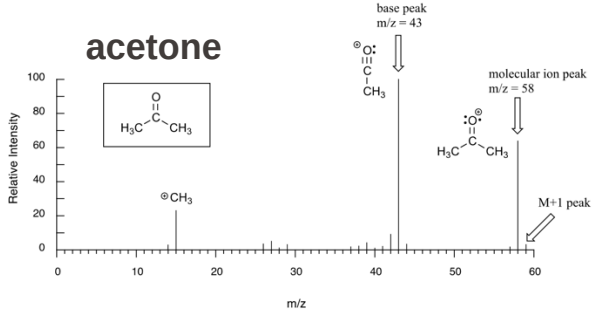
Heparin Isomers

Same m/z!

We need unique fragments!

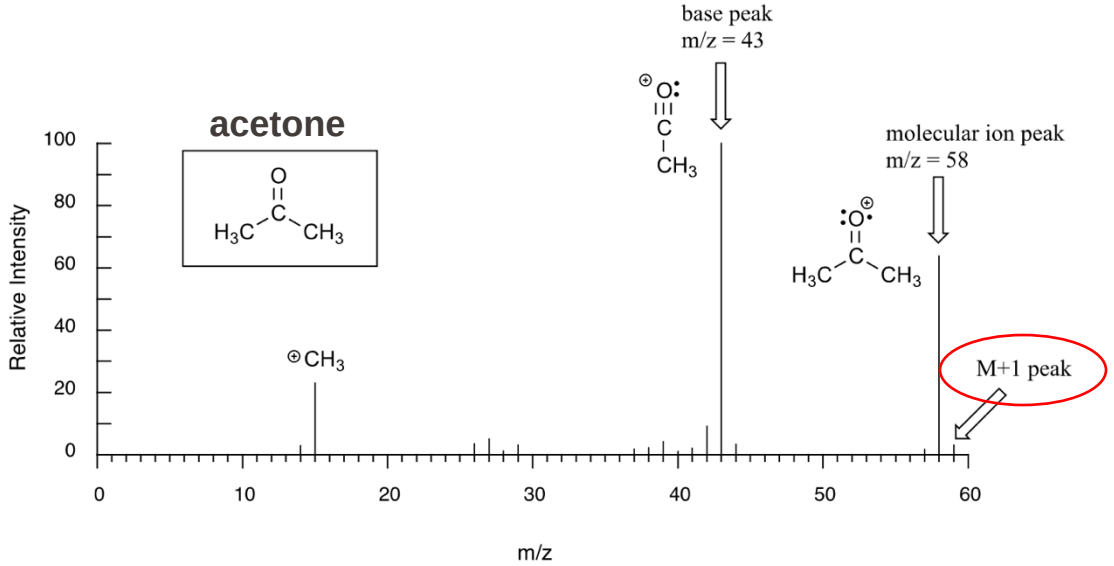
Fragmentation of molecules





-Many of the radical cations tend to be predicted in a predictable way
-With this technique radicals are not detected on positively charged species
-Stability of the fragments determines their ease of detection

The detection of isotopes in mass spectrometry



- How can there be an ion that is greater than the mass of the molecular ion ?

-About 1.1% of all carbons are ^{13}C rather than ^{12}C - so an extra neutron in the nucleus and thus heavier than ^{12}C by an extra mass unit

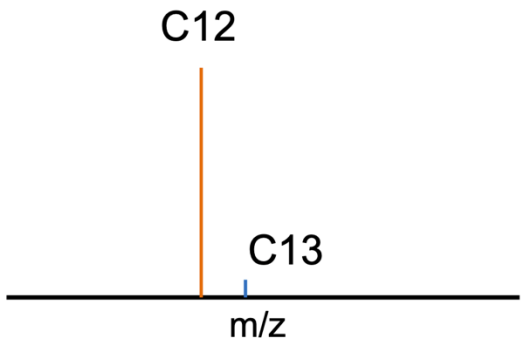
The detection of isotopes in mass spectrometry

- **Monoisotopic mass**: Mass of the most abundant isotope of a given element
- **Average mass**: a weighted average of all of the isotopes of that element, in which the mass of each isotope is multiplied by the abundance of that particular isotope
- How would a carbon atom look like in a mass spectrum? We can distinguish between isotopes!

For example: Let's consider carbon atoms

Monoisotopic mass of C: Most abundant isotope is ^{12}C, mass = 12.0000

Average mass of C: ^{12}C = 98.9%, ^{13}C = 1.1%
So average mass = (0.989 m_{C12}) + (0.011 m_{C13})



Screenshot

The detection of isotopes in mass spectrometry

Let's consider Cl_2

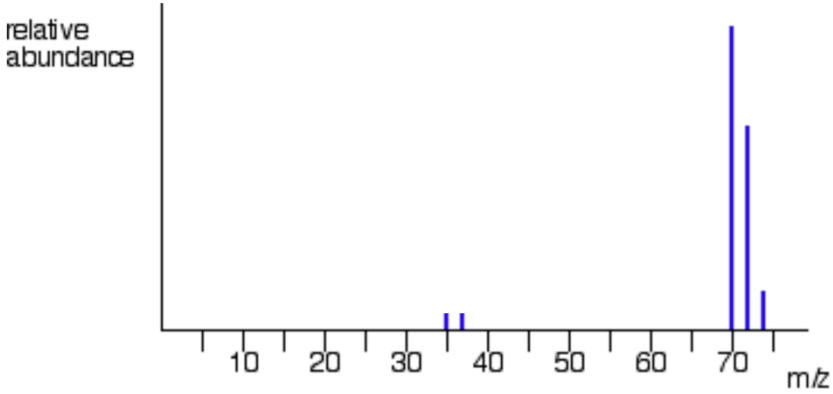
Possible cases:

^{35}Cl - ^{37}Cl

^{37}Cl - ^{35}Cl

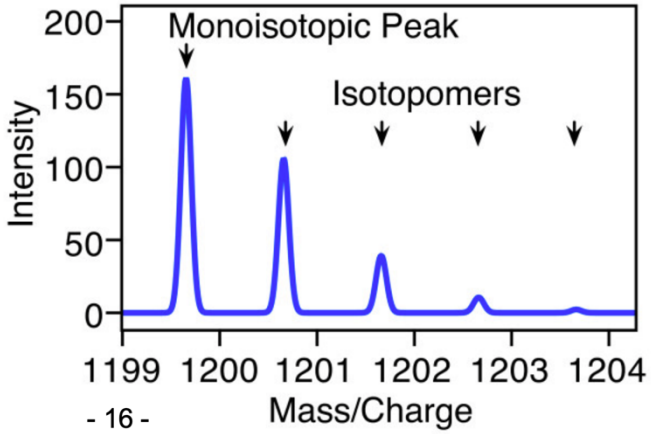
^{37}Cl - ^{37}Cl

^{35}Cl - ^{35}Cl



Small peptides

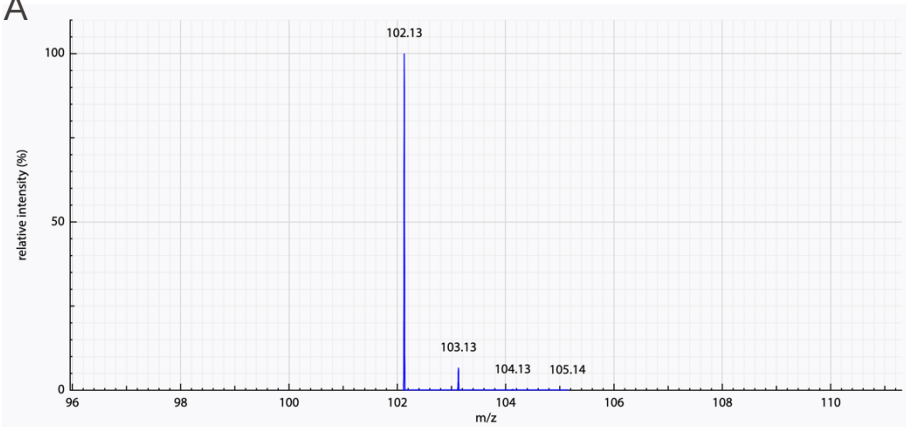
More and more peaks appearing…



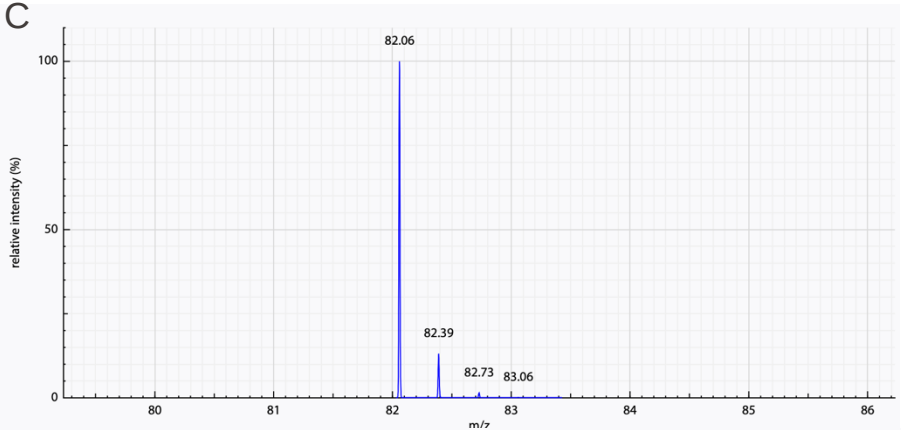
Question

By looking at the following spectra can you tell me what is the charge of the ion species ?

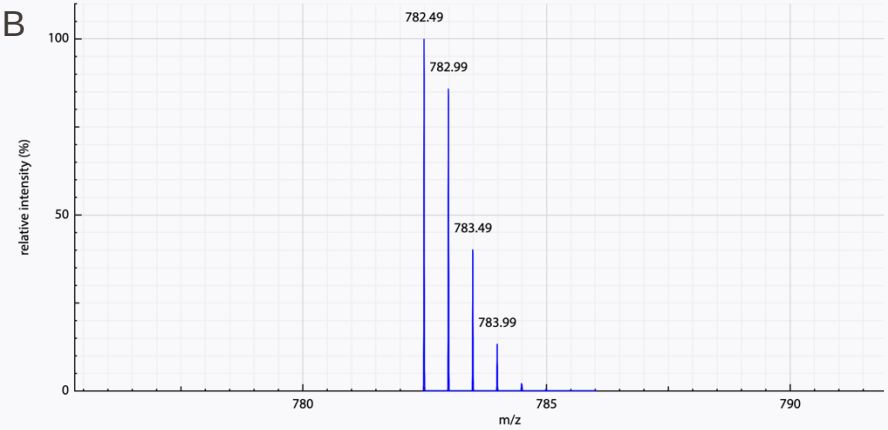
A



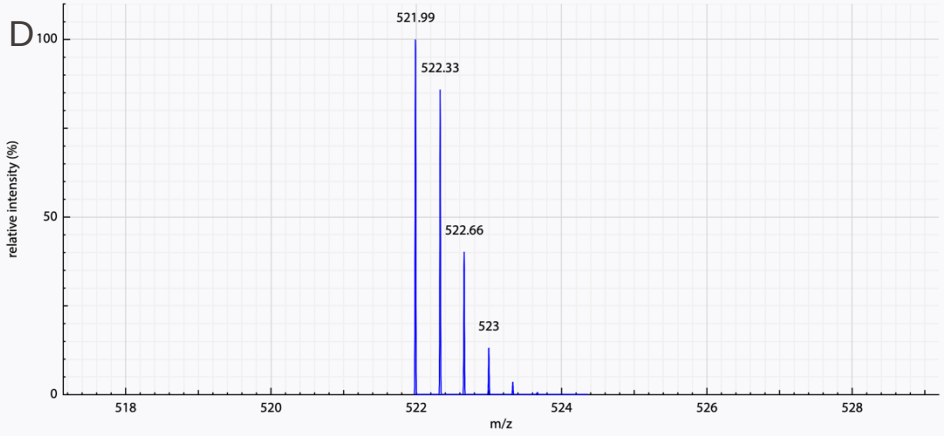
C



B

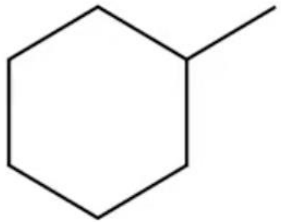


D

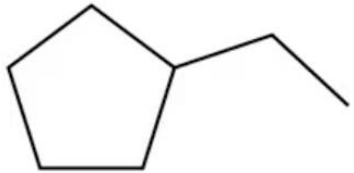


Question

A case study: Methylcyclohexane and Ethylcyclopentane



Methylcyclohexane



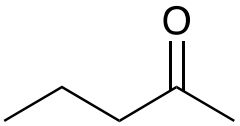
Ethylcyclopentane

Both have a mass of ~98

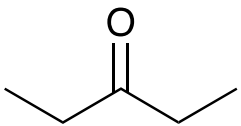
M+ = 98

Question

Back to the example brought up before: How could we distinguish the spectrum of pentan-2-one and pentan-3-one? Hint: Each of these is likely to split to produce ions with a positive charge on the CO group

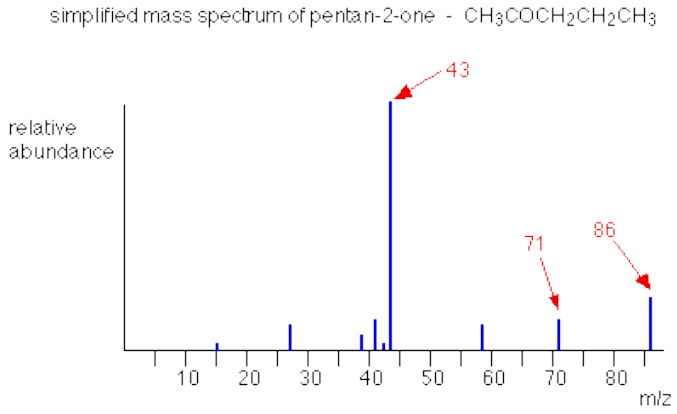


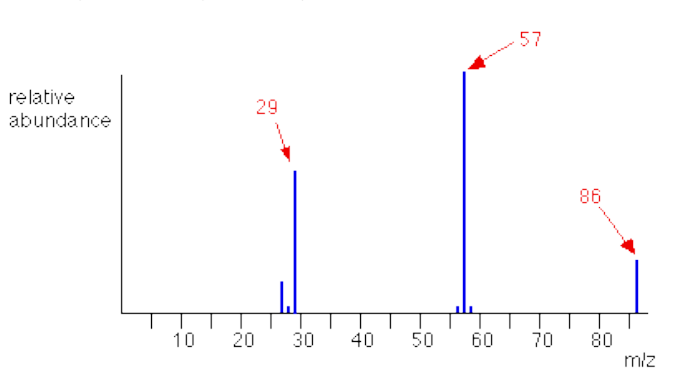
pentan-2-one



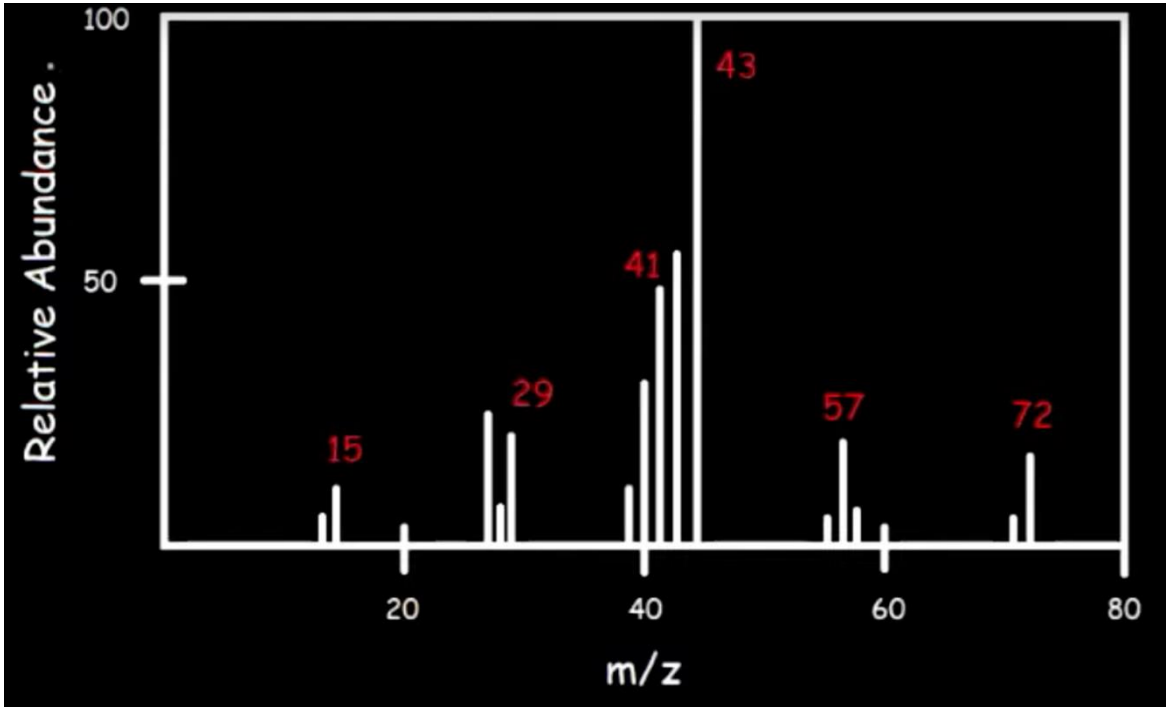
pentan-3-one

pentan-2-one:
$[CH_3CO]^+$
$[COCH_2CH_2CH_3]^+$
That would give you strong peaks at m/z = 43 and 71.
pentan-3-one:
$[CH_3CH_2CO]^+$
In that case, you would get a strong peak at 57.
The m/z = 29 peak is produced by the ethyl ion.



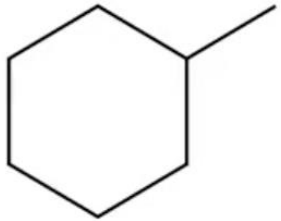


How can we read mass spectrometry data ?

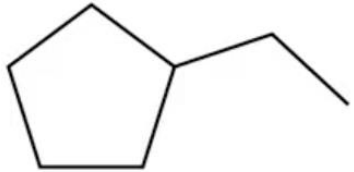


Question

A case study: Methylcyclohexane and Ethylcyclopentane



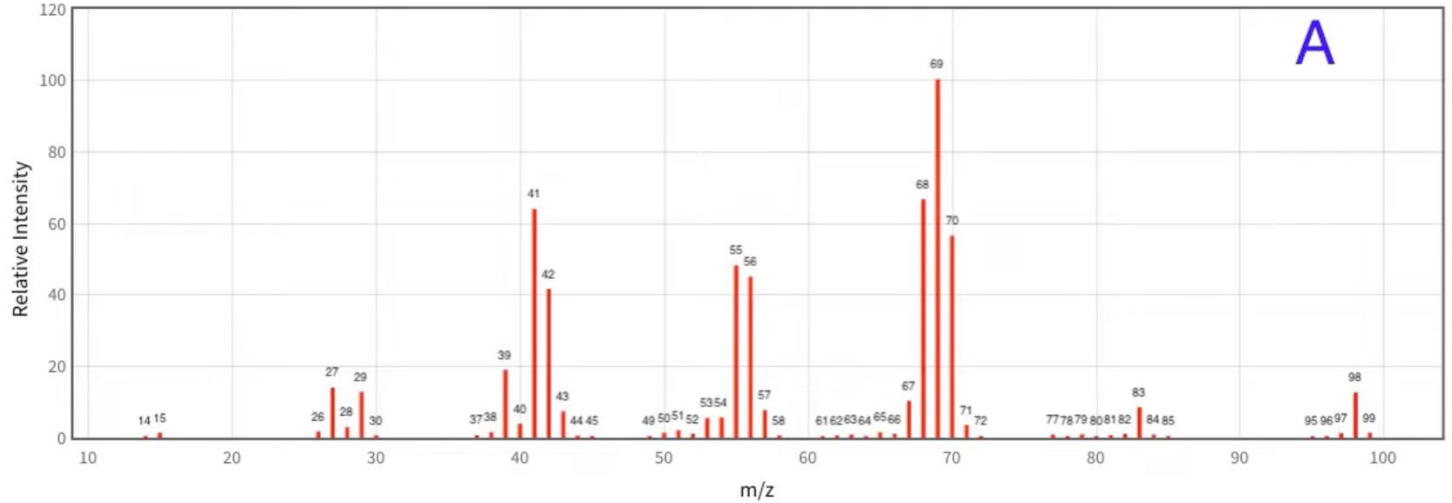
Methylcyclohexane

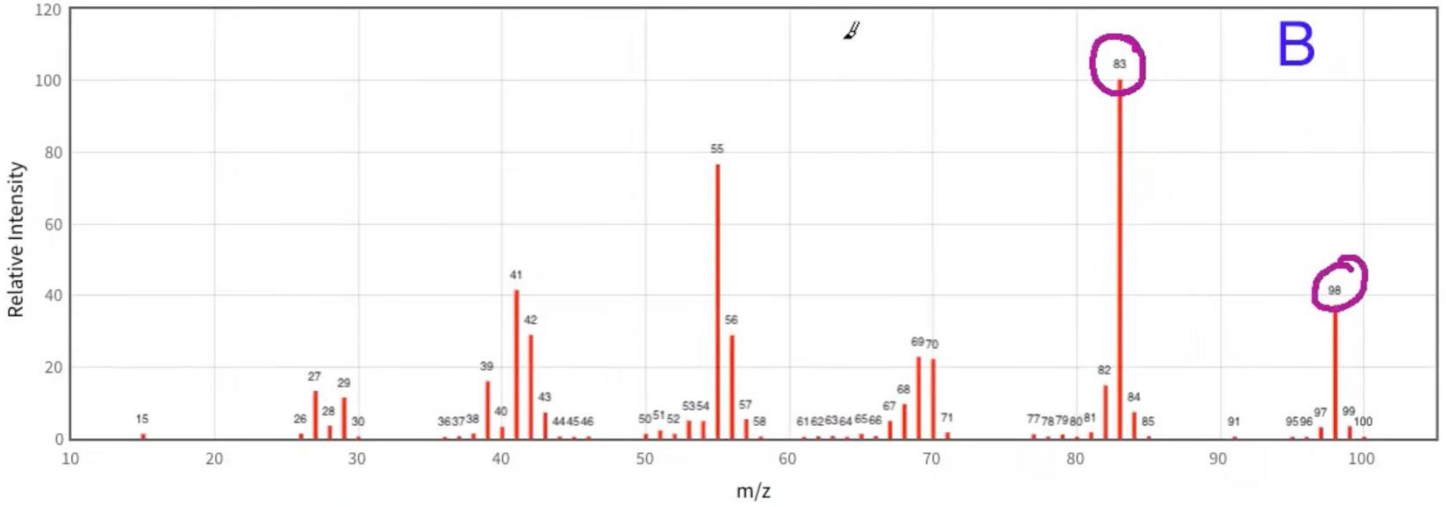


Ethylcyclopentane

Both have a mass of ~98

M+ = 98

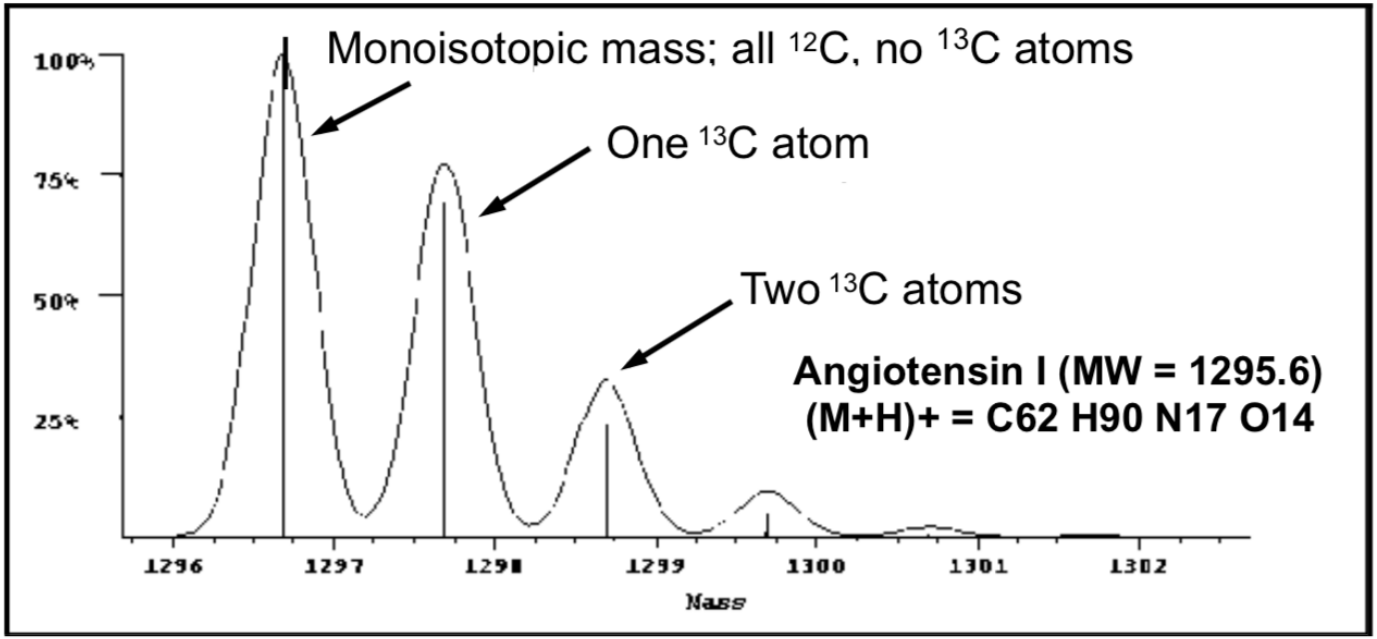

A
Relative Intensity
m/z


B
Relative Intensity
m/z

Common Ion Fragment	m/z
CH_3	15
OH	17
CN	26
CO	28
CH_3CH_2 and CHO (Aldehyde)	29
OCH_3	31
Cl	35, 37
$CH_3CH_2CH_2$ and CH_3CO (Methyl ketone)	43
COOH (Carboxylic Acid)	45
C_4H_9 (Butyl) and C_2H_5CO (Ethyl ketone)	57
CH_3OCO (Methyl Ester)	59
C_5H_{10} (Cyclopentane)	70
C_5H_{11} (Pentyl)	71

Mass spectrometry in peptides and proteins

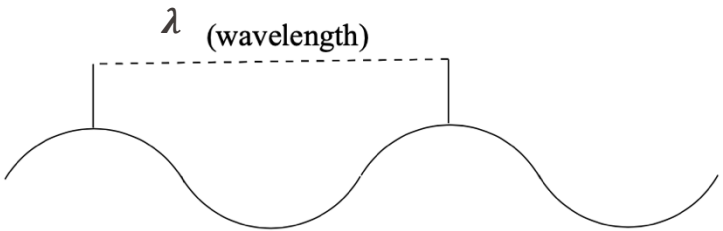
-spectrum of a peptide



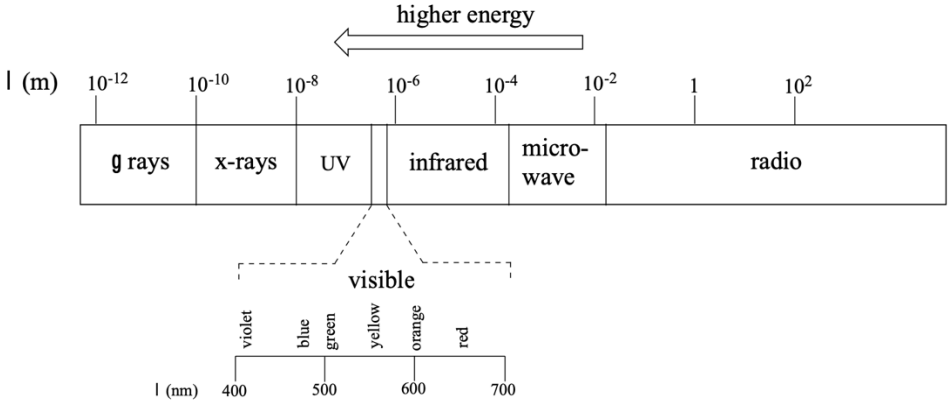
- As the number of atoms of any given element increases, the percentage of the population of molecules having one or more atoms of a heavier isotope of this element also increases
- The most significant contributors to the isotopic peak pattern for peptides is the 13C isotope of carbon (1.1%) and 15N peak of nitrogen (0.36%)

Basics in molecular spectroscopy

-Electromagnetic radiation is defined by its wavelength – distance between one wave crest to the next



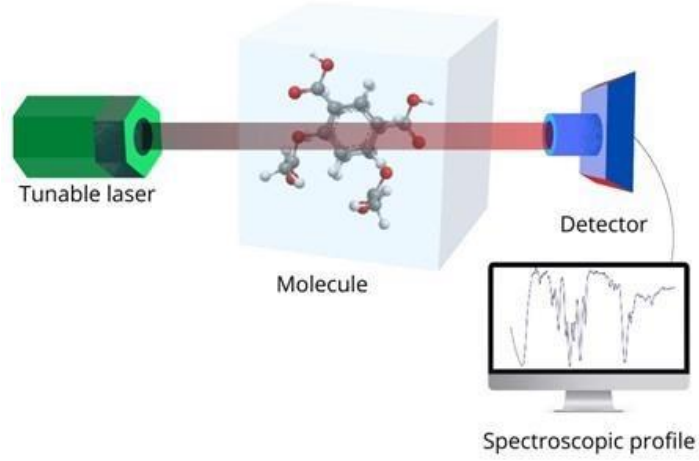
Electromagnetic spectrum

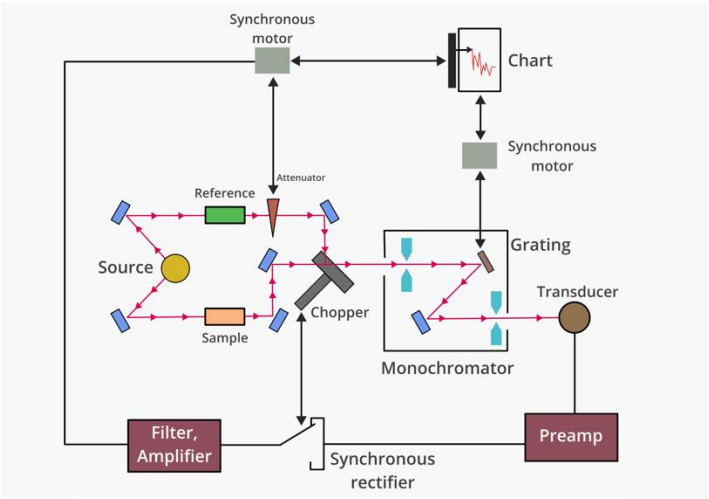


λ – wavelength (distance)
υ – frequency (s^{-1})
c – speed of light constant (3.0×10^8 ms^{-1})

Basics in molecular spectroscopy

- Setup of spectroscopy instrument

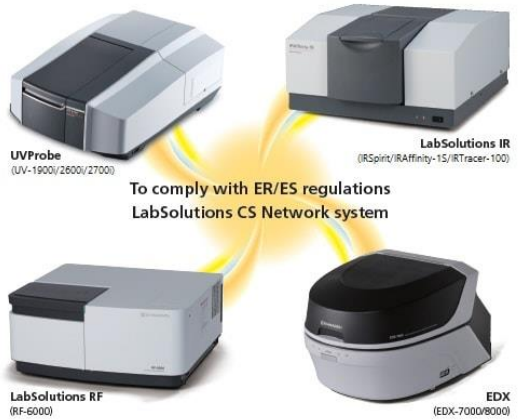




-Sample molecules absorb energy from some of the wavelengths

-Molecules go from ground state -> excited state

-Detector records which wavelengths were absorbed and to what extent



Infrared spectroscopy

-Covalent bonds in organic molecules are not rigid sticks

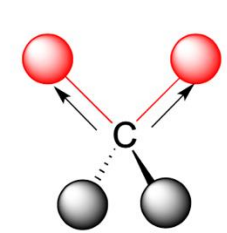
Behave more like springs

-Bonds have vibrational modes (10^{13} to 10^{14}) vibrations per second – infrared region of the electromagnetic spectrum

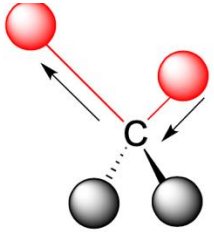
-Upon absorption of electromagnetic radiation matching a vibrational mode the molecule jumps to a higher vibrational state

-The amplitude of the vibration changes but the frequency remains the same (example in the next slide)

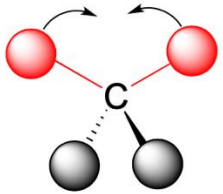
Bond vibrational modes (examples)



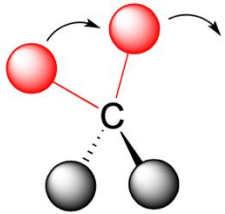
symmetric stretching



asymmetric stretching

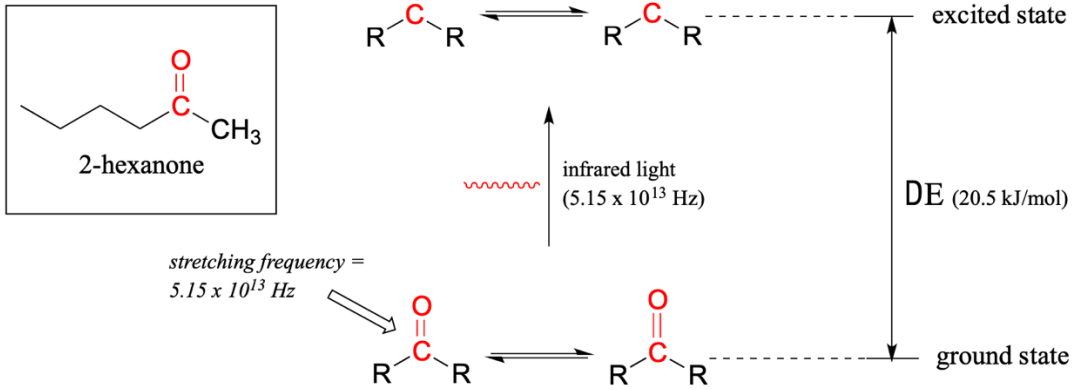


scissoring



rocking

Infrared spectroscopy



Speed of light

$$\lambda \upsilon = c$$

Wavelength Frequency

$$E = hc/\lambda = h\nu$$

Energy Planck constant

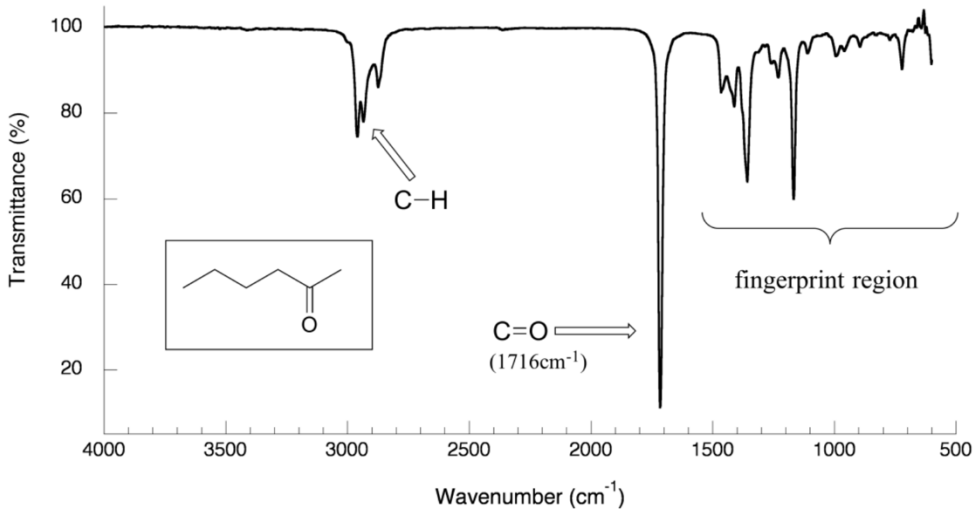
-Take a look at the carbonyl bond

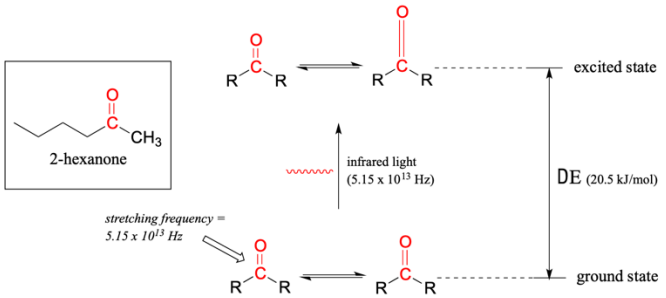
-If ketone is irradiated with infrared red if absorbs light of the stretching frequency 5.15×10^{13} Hz

-This light using the upper right equations corresponds to a wavelength of $\lambda = 5.83 \times 10^{-6}$ m and E = 20.5 kJ/mol

-Most frequencies pass right through and do not get absorbed

Infrared spectroscopy



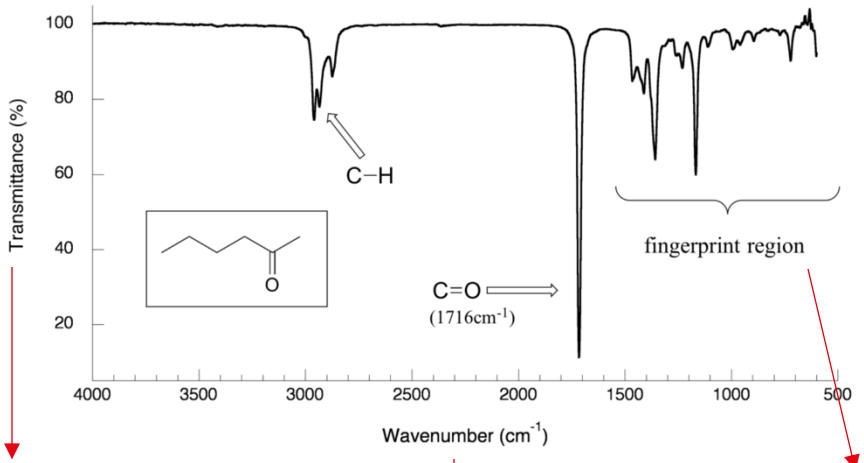


-The vibrations for 2-hexanone are not limited to the simple stretching of the carbonyl bond. The various carbon-carbon and carbon-hydrogen bonds also stretch and bend in different frequencies

-Not all bonds are infrared active – in general the greater is the polarity of the bond the stronger is the IR absorption

-For example – carbonyls absorb very strongly; alkynes – are **infrared inactive** as they are less polar

Infrared spectroscopy

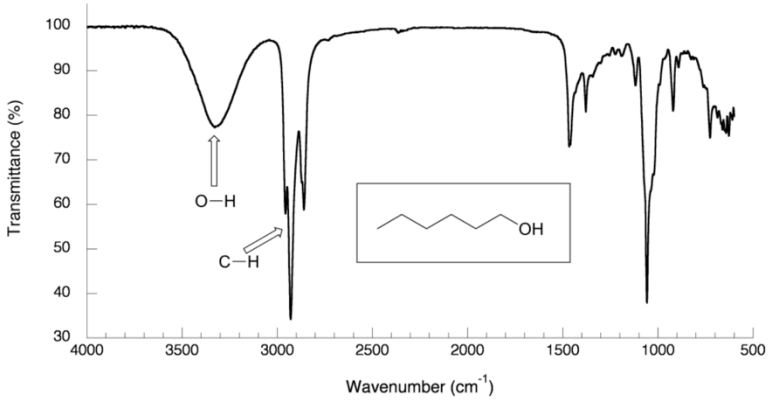


How strongly does light get absorbed at each frequency

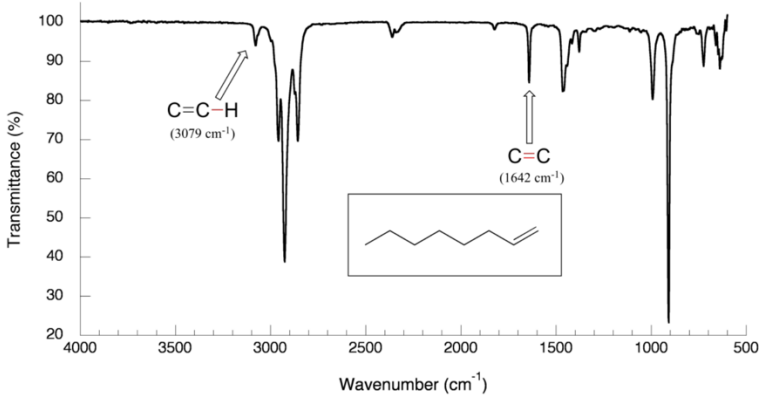
Region that display patterns unique to every molecule

$$\text{wavenumber (in cm}^{-1}) = 1/100\lambda$$

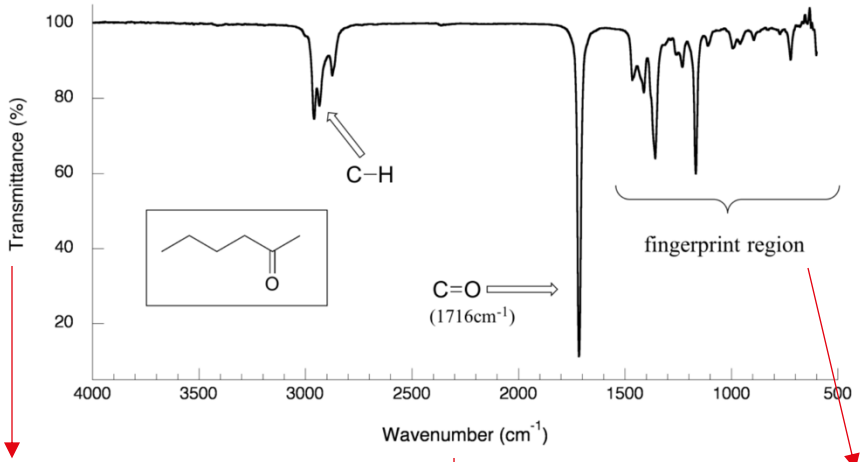
1-hexanol



1-octene



Infrared spectroscopy



How strongly does light get absorbed at each frequency

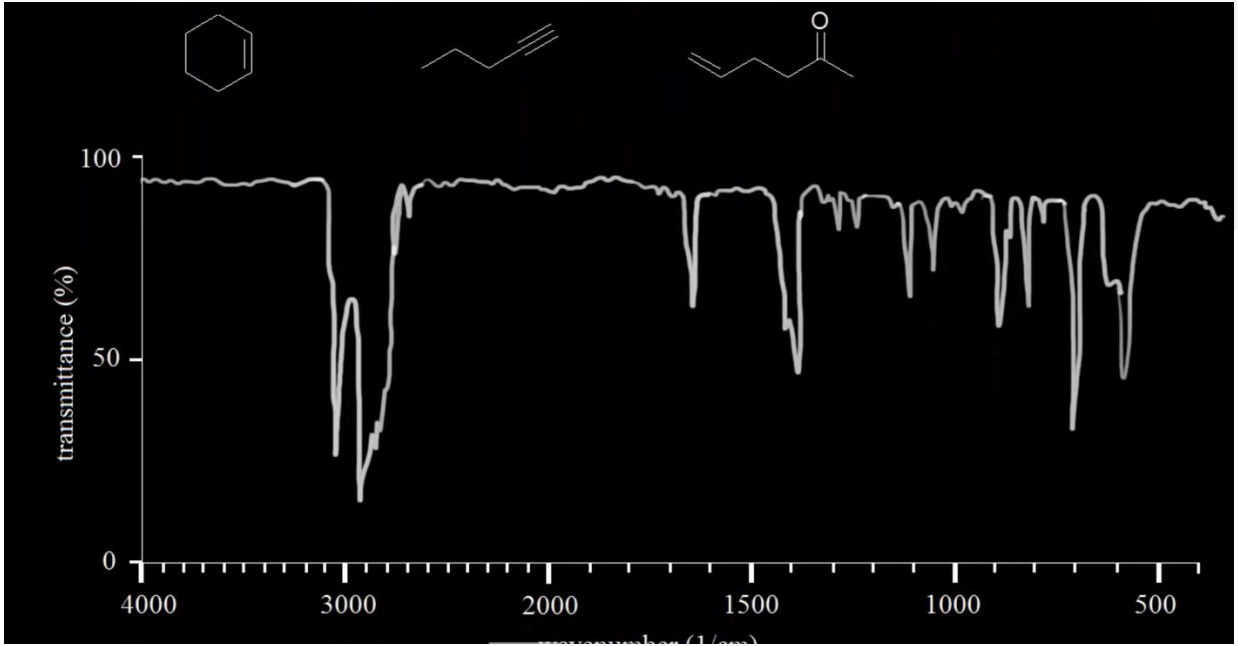
Region that display patterns unique to every molecule

wavenumber (in cm^{-1}) = $1/100\lambda$

Characteristic IR absorbances

Functional group	Characteristic IR absorbance(s) (cm^{-1})	Source of signal
carbonyl	1650-1750 (strong)	C=O stretching
alcohol	3200 - 3600 (broad)	O-H stretching
carboxylic acid	1700-1725 (strong)	C=O stretching
	2500-3000 (broad)	O-H stretching
alkene	1620 - 1680 (weak)	C=C stretching
	3020 - 3080	vinylic C-H stretching
alkyne	1620 - 1680 (weak)	triple bond stretching
	3250-3350	terminal C-H stretching

Which molecule do we have ?



Bond	Wavenumber (cm^{-1})
O–H	3600-3400
N–H	3400-3200
C–H	3080-2760
C≡N	2260-2215
C≡C	2150-2100
C=O	1815-1650
C=C	1660-1600
C–O	1200-1050

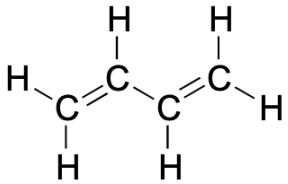
Ultraviolet and visible spectroscopy

- Ultraviolet and visible (UV-Vis) spectroscopy provides information about aromatic and other conjugated π systems

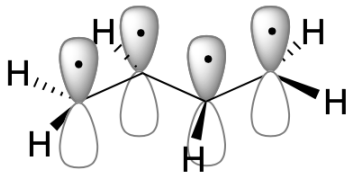
-The shorter wavelength higher energy radiation in UV (200-400 nm) and visible (400-700 nm) cause the organic molecules with conjugated π bonds to undergo **electronic transitions**

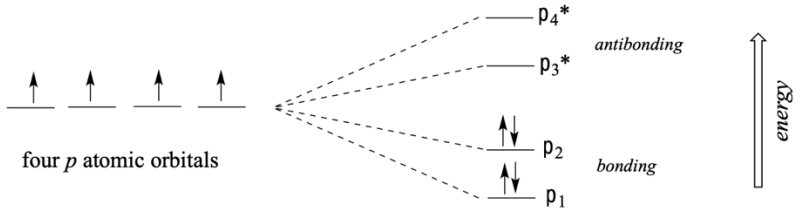
-Molecular orbital (MO) theory is useful to help understand what happens in this spectroscopy

-**Chromophore** – any molecule that has the property of absorbing light in the ultraviolet or visible region of the spectra



1,3-butadiene

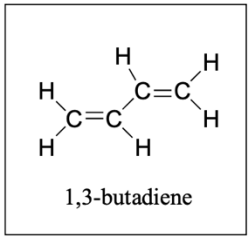


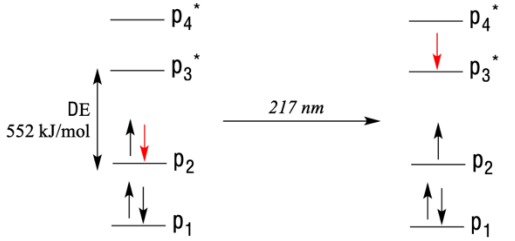


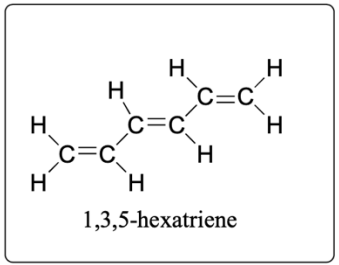
four p molecular orbitals

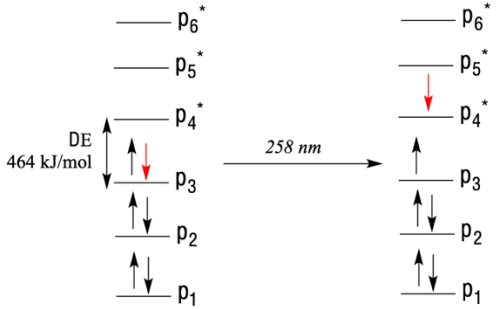
Note
p=π

Ultraviolet and visible spectroscopy







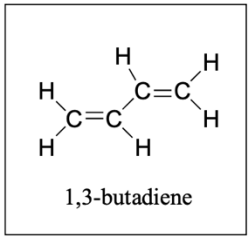


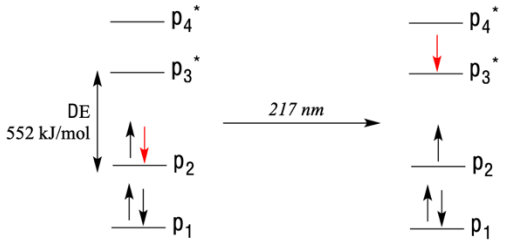
Note
p=π

-This spectroscopy takes advantage of π -> π* transition that occur between the **highest occupied molecular orbital (HOMO)** and the **lowest unoccupied molecular orbital (LUMO)**

- The gap between these two molecular orbitals is often called **HOMO-LUMO energy gap**

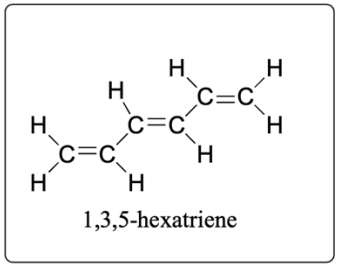
Ultraviolet and visible spectroscopy

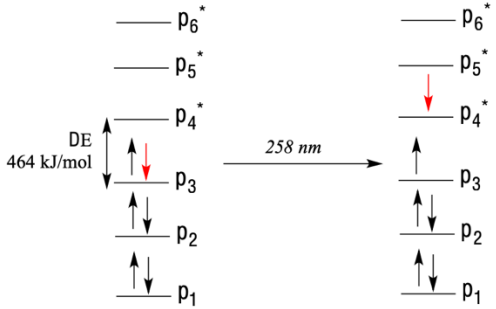




As conjugated π systems become more extended,

a) the HOMO-LUMO gap shrinks, and
b) the wavelength of absorbed light becomes longer.

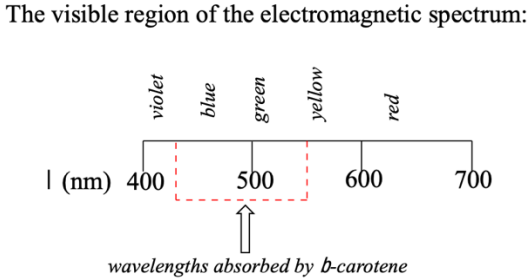


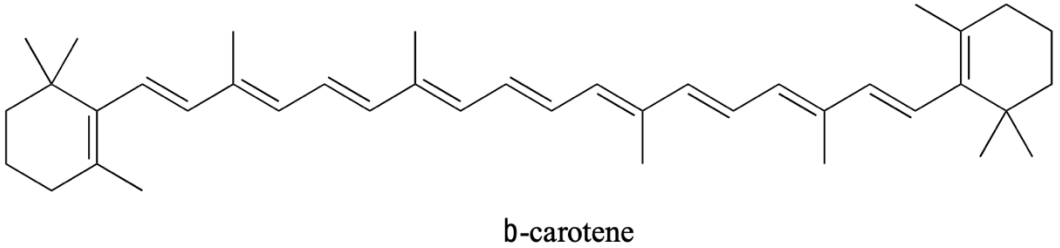


Note
p=π

When conjugated π systems become very large

-In extended conjugated π systems the π -> π^* energy gap becomes so small that absorption occurs in the visible rather than the UV region of the electromagnetic spectrum



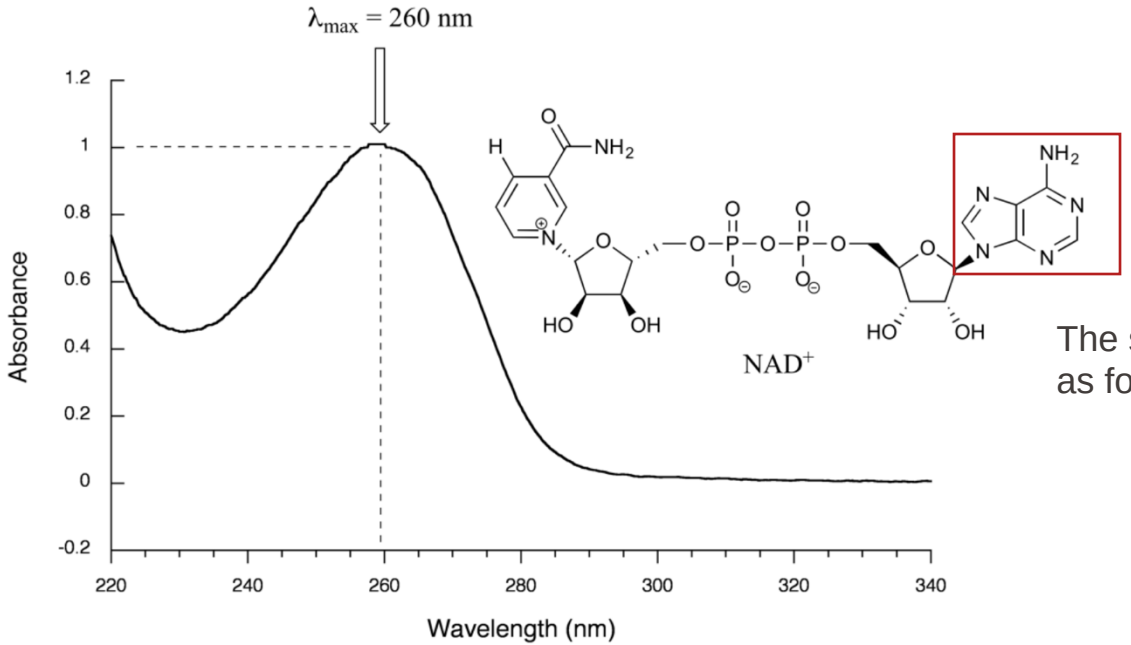


-β-carotene is an 11 conjugated double bond system and absorbs between 420-550 nm , with λ_{max} = 470 nm spanning the blue/green wavelengths

- Blue/green wavelengths are absorbed and the red and yellow region passes through as such it appears to our eyes as orange

Looking at UV-vis spectra

-Setup is similar to IR spectrometry – radiation directed to a sample and a detector recording which wavelengths were absorbed



Nicotinamide adenine nucleotide

Adenine absorbs at 260 nm

The spectrometer computes absorbance as follows:

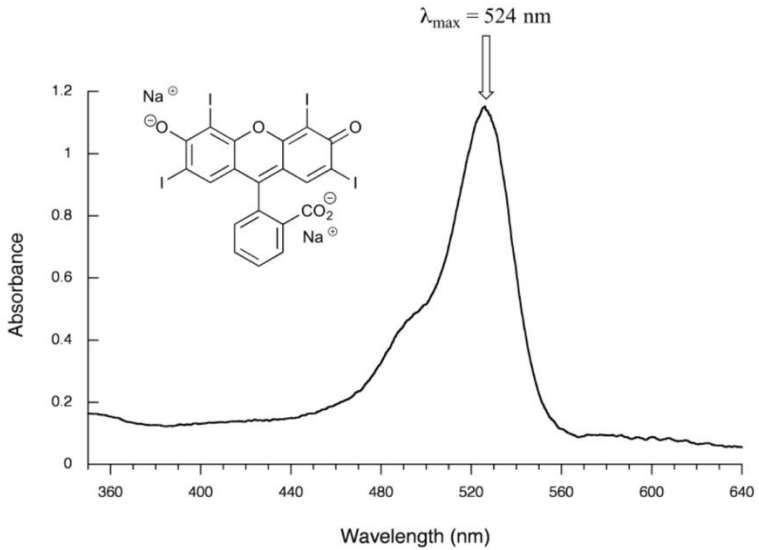
$$A = \log (I_0/I)$$

I_0 - Intensity of light with no sample
I- Intensity of light after the sample

Looking at UV-vis spectra

-A few things to look for on UV-vis spectrum:
λ_{max} – wavelength at maximal absorbance
Absorbance (A) at λ_{max} which depends on the concentration of the sample

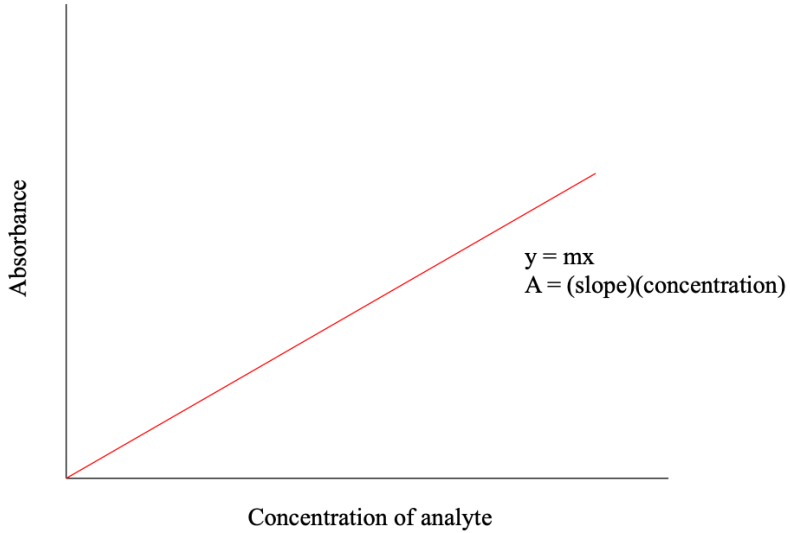
Food coloring example Red #3:



- extended system of conjugated π bonds causes the molecules to absorb in the visible

- λ_{max} = 524 nm which is blue/green range meaning reds pass through so solution is red

Applications of UV spectroscopy in biological chemistry



Beer-Lambert law - is certain ranges the absorbance of UV-active compound varies in linear fashion with its concentration

$$\varepsilon = A/cl$$

ε – characteristic value of the compounds ($L \cdot mol^{-1} \cdot cm^{-1}$)
A – absorbance
c – concentration (mol/L)
l- path length (cm)

Lets get it to work

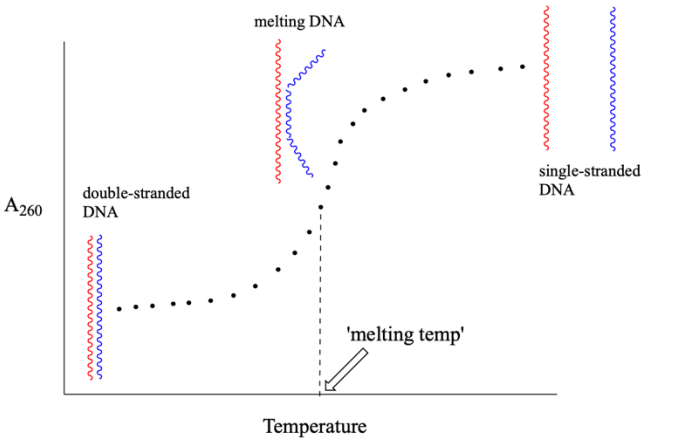
A solution of $KMnO_4$ has an absorbance of 0.539 when measured at 540 nm in a 1.0-cm cell. What is the concentration of the $KMnO_4$? Prior to determining the absorbance for the unknown solution, the following calibration data were collected for the spectrophotometer.

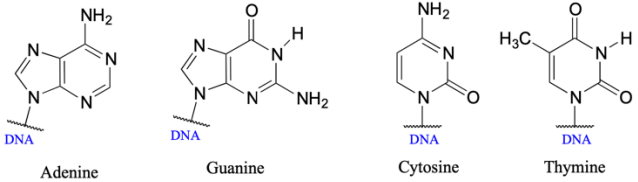
Concentration of $KMnO_4$ (M)	Absorbance
0.0300	0.162
0.0600	0.330
0.0900	0.499
0.120	0.670
0.150	0.840

From Kotz, Treichel, and Townsend Chemistry and Chemical Reactivity

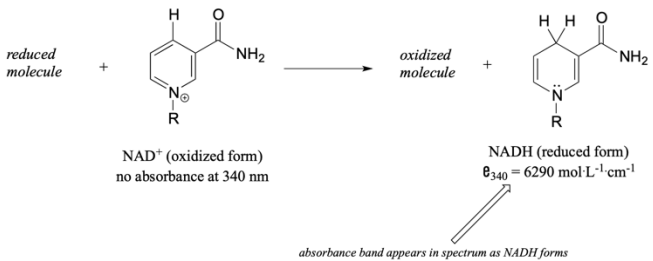
Applications of UV spectroscopy in biological chemistry

Melting of double stranded DNA

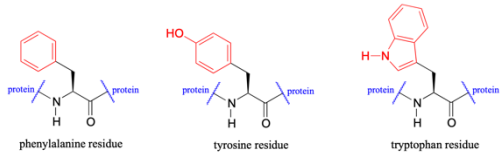




Monitoring enzyme reactions



Protein concentration



Take home

-Fundamentals of an MS experiment

-Recognize molecular ion peaks

-Spectrometry basics

-IR spectroscopy basics including the concept of vibrational transition

-To identify IR-active and inactive functional groups and which will lead to more intense absorbance

-Based on an IR spectrum predict the presence of the functional groups

-Understand the basic idea of π->π^* transitions

-Recognize a cromophore

-Use the Beer-Lamber law for simple calculations

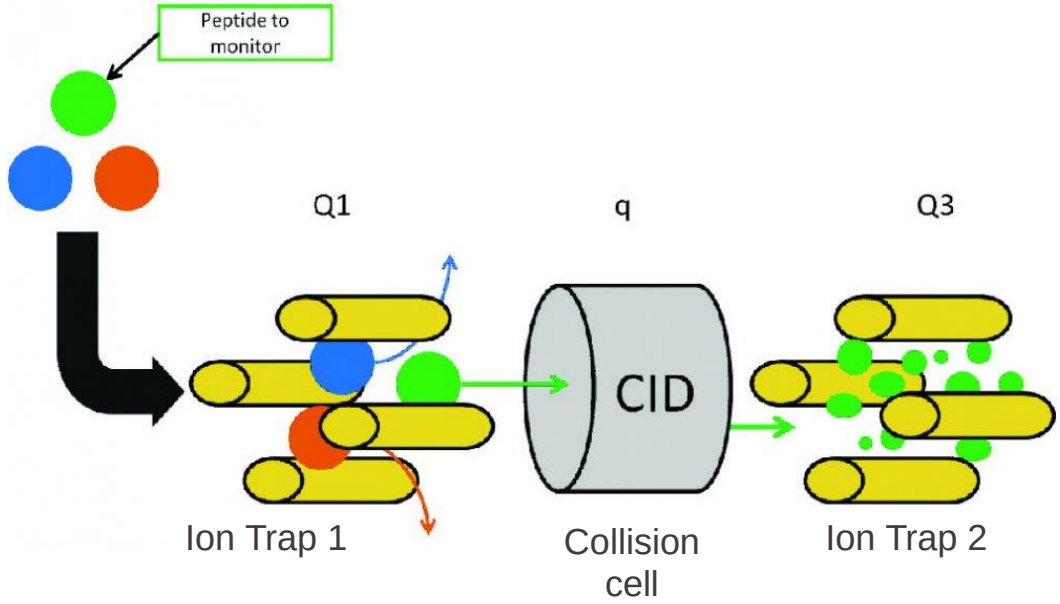
Next lecture

Structure determination NMR (Chapter 5)

Questions ?

Mass Analyzers

Ion Trap & **MS/MS**



MS/MS

-**Mass selection** of a user-defined precursor parent ions

-**Fragmentation** of the parent ion to form product daughter ions

-**Mass analysis** of the product ions

The Protein-Sequencing Problem

Same Mass, Different Sequences
Proteins with identical mass may have diverse amino acid sequences, posing a challenge in identification

Solution: Digestion into smaller fragments leads to distinguishable units that can later be aligned to a database