Bio-organic Chemistry

Lecture 4

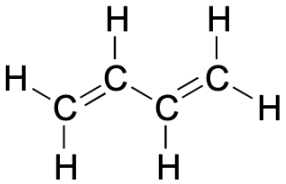
Ultraviolet and visible spectroscopy

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

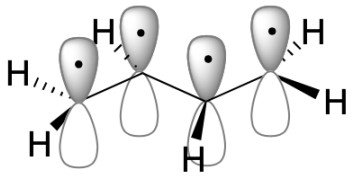
-The shorter wavelength higher energy radiation in UV (200-400 nm) and visible (400-700 nm) cause the organic molecules with conjugated π bonds to undergo **electronic transitions**

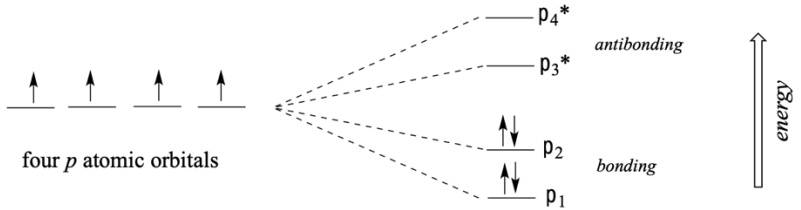
-Molecular orbital (MO) theory is useful to help understand what happens in this spectroscopy

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



1,3-butadiene





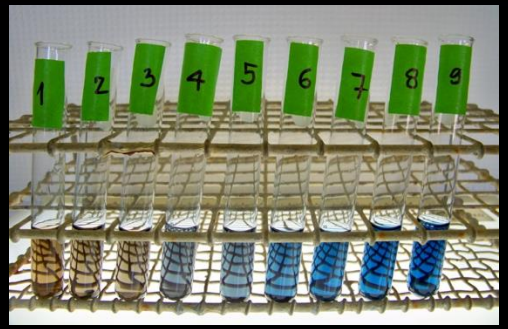
Note
p=π

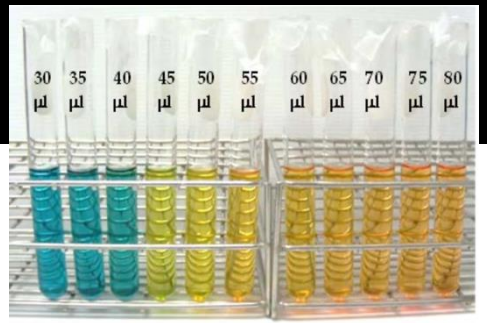
Lets get it to work

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





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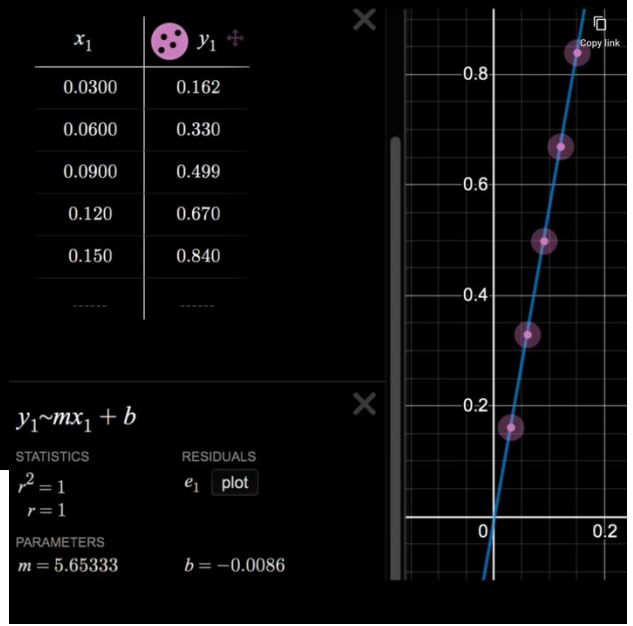
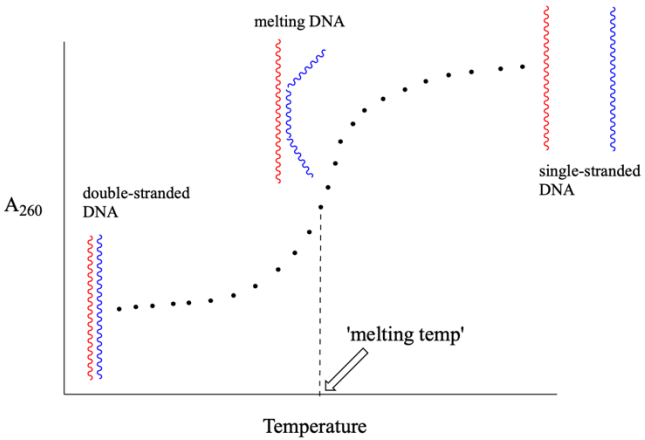
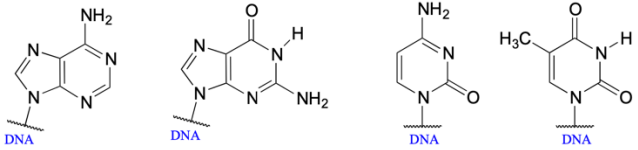


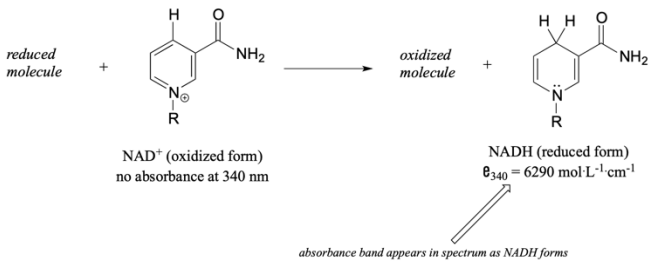
Applications of UV spectroscopy in biological chemistry

Melting of double stranded DNA

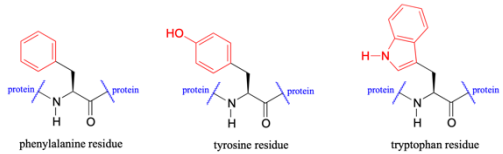




Monitoring enzyme reactions



Protein concentration



Structure Determination of Organic 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 □-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?*

Principles of nuclear magnetic resonance(NMR) spectroscopy

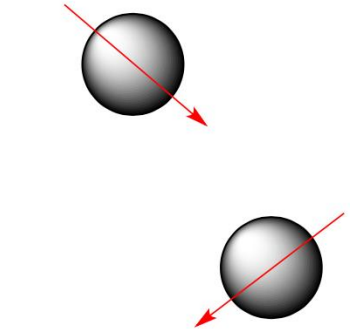
- NMR spectroscopy relies on the **magnetic properties** of specific **atomic nuclei** for molecular analysis.
- NMR provides insights into **molecular connectivity**, revealing how atoms are bonded and their arrangements, while also distinguishing between **different atomic environments** within a compound.
- Nuclei like **^{1}H, ^{13}C**, ^{19}F, and ^{31}P possess magnetic moments and are observable in NMR.
- Isotopes such as ^{12}C and ^{16}O lack magnetic moments and can't be directly detected via NMR.
- 1H-NMR and 13C-NMR are commonly used techniques, with the 1H nucleus (proton) being particularly prominent.

Magnetic nuclei	Nonmagnetic nuclei
^{1}H	^{12}C
^{2}H	^{16}O
^{13}C	^{32}S
^{14}N	
^{19}F	
^{31}P	

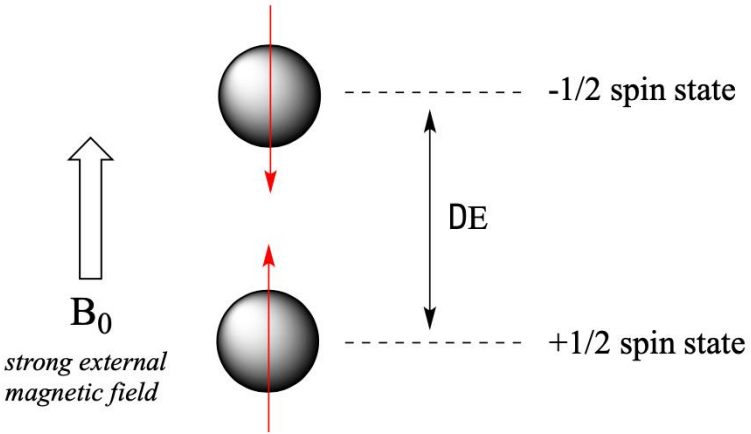
What makes NMR possible

-Samples(molecules) are subjected to magnetic fields or applied field (B_0)

-Each proton assumes one of two possible quantum spin states



no external magnetic field:
moments are randomly oriented



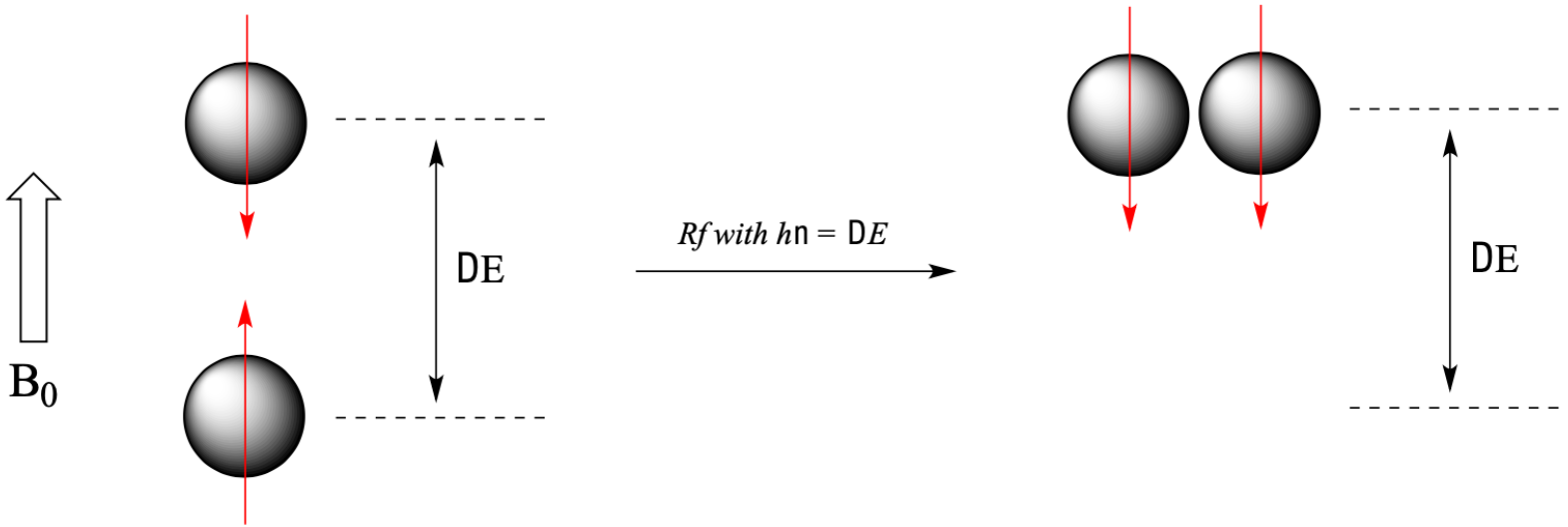
strong external magnetic field

moments align with or against external field

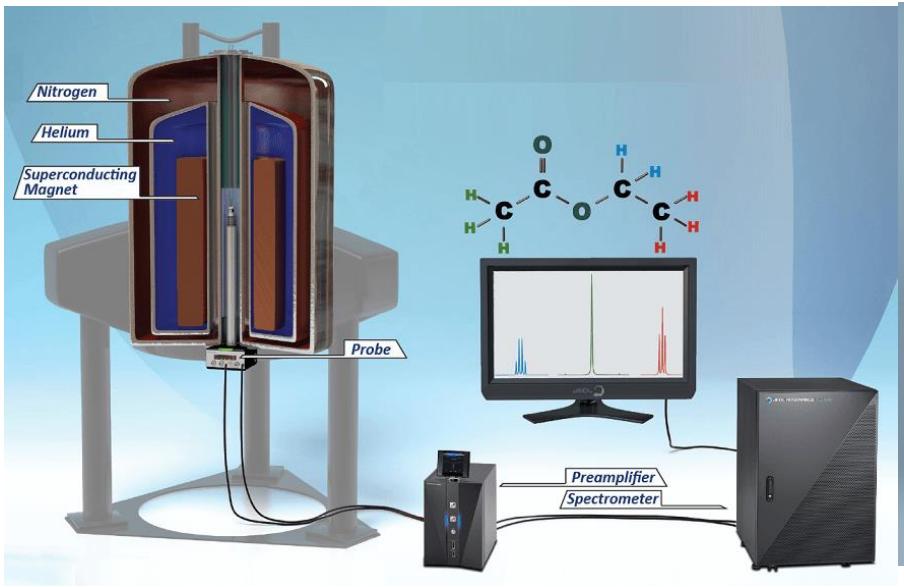
In NMR protons transition to higher energy spin states

-If the radio frequency matches the energy gap – hydrogens will flip to the higher energy state

-This is very mush similar with what you saw with IR and UV-vis spectroscopy



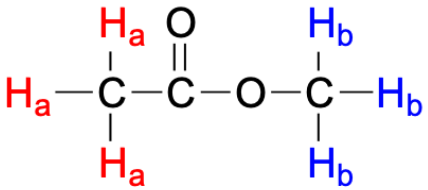
NMR instrument



Thinking about the chemical environment of protons

-Frequency of radiation absorbed by a proton during a spin transition in an NMR experiment is called its **resonance frequency**

-The principle is protons with different chemical/electronic environments will have distinct resonance frequencies

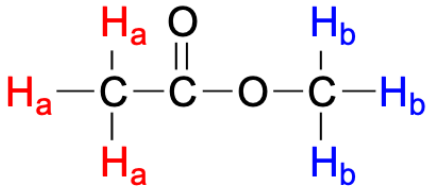


methyl acetate

- Do H_a and H_b have different chemical environments ?

-Why ?

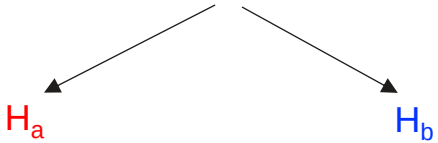
Thinking about the chemical environment of protons



methyl acetate

- H_a and H_b have different chemical environments

-The 2 sets of protons are in non identical electronic environments



Next to a carbonyl carbon Next to an oxygen

-H_a and H_b protons are equivalent to each other (**chemically equivalent**)

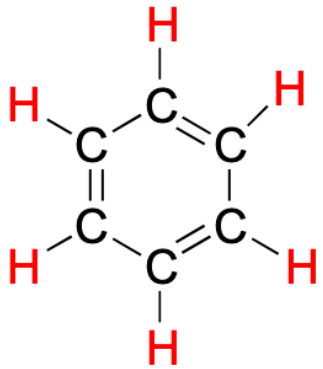
-H_a protons are not equivalent to H_b (**chemically nonequivalent**)

Different resonance frequencies

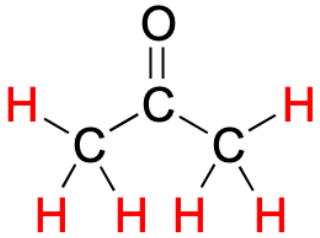
Equivalent and non-equivalent chemical environments

- Recognition of chemical equivalency and non-equivalency is critical to understand NMR

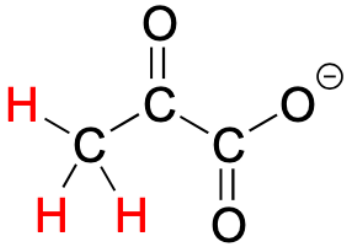
-Lets look at examples



benzene



acetone



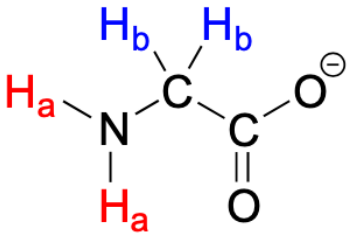
pyruvate

All protons equivalent

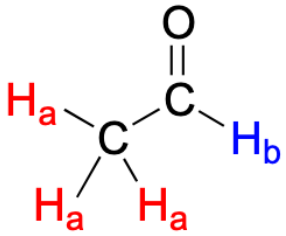
Equivalent and non-equivalent chemical environments

- Recognition of chemical equivalency and non-equivalency is critical to understand NMR

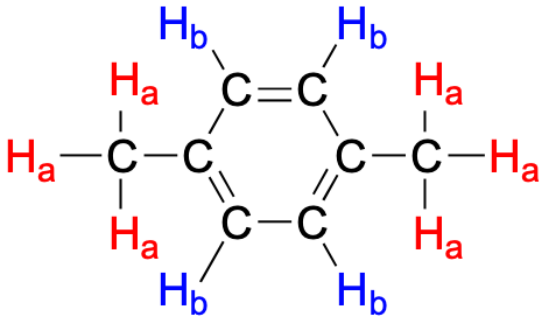
-Lets look at examples



glycine



acetaldehyde



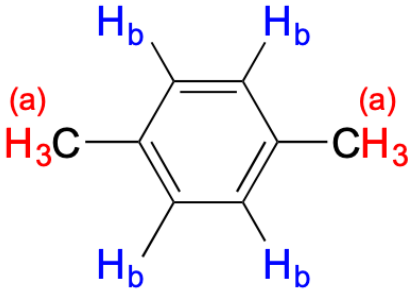
1,4-dimethylbenzene

Two sets of equivalent protons

Equivalent and non-equivalent chemical environments

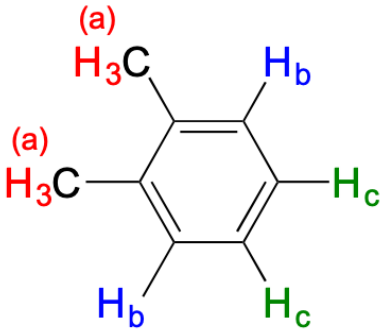
- Recognition of chemical equivalency and non-equivalency is critical to understand NMR

-Lets look at (more complicated) examples



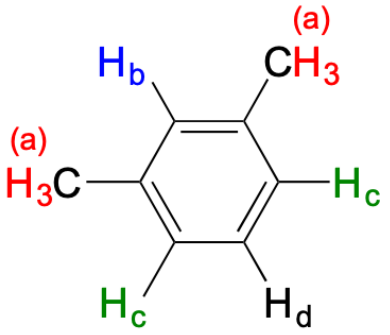
1,4-dimethylbenzene

All 4 aromatic protons equivalent



1,2-dimethylbenzene

2+2 aromatic protons equivalent
-H_b adjacent to methyl
-H_c 2 carbons away



1,3-dimethylbenzene

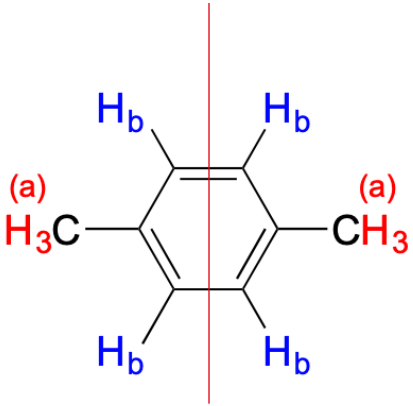
-H_b between 2 methyl groups
-H_c 1 carbon away from the methyl group
-H_d two carbons away from the methyl group

Equivalent and non-equivalent chemical environments

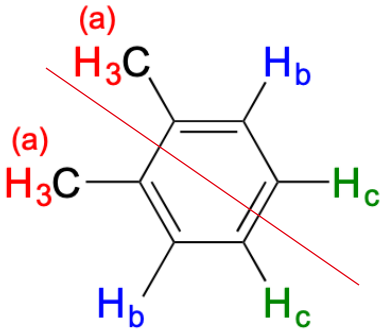
- Recognition of chemical equivalency and non-equivalency is critical to understand NMR

-Lets look at (more complicated) examples

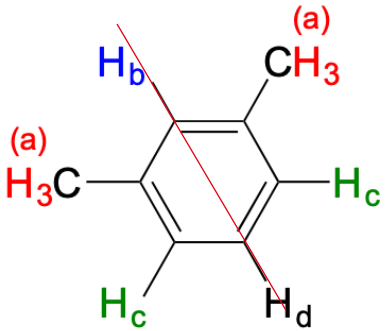
-The pattern here is the symmetry which reveals that the protons are chemically equivalent



1,4-dimethylbenzene



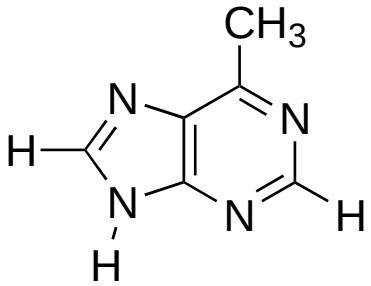
1,2-dimethylbenzene



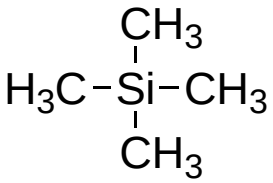
1,3-dimethylbenzene

Let's discuss

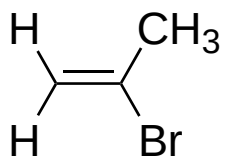
How many equivalent protons exist in the following molecules ?



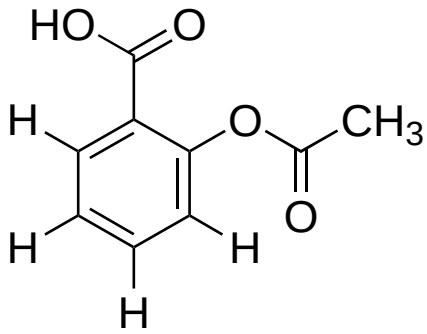
Adenosine



Tetramethylsilane (TMS)



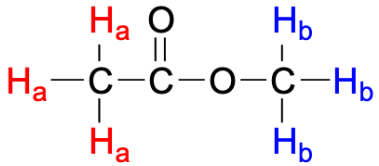
2-Bromoprop-1-ene



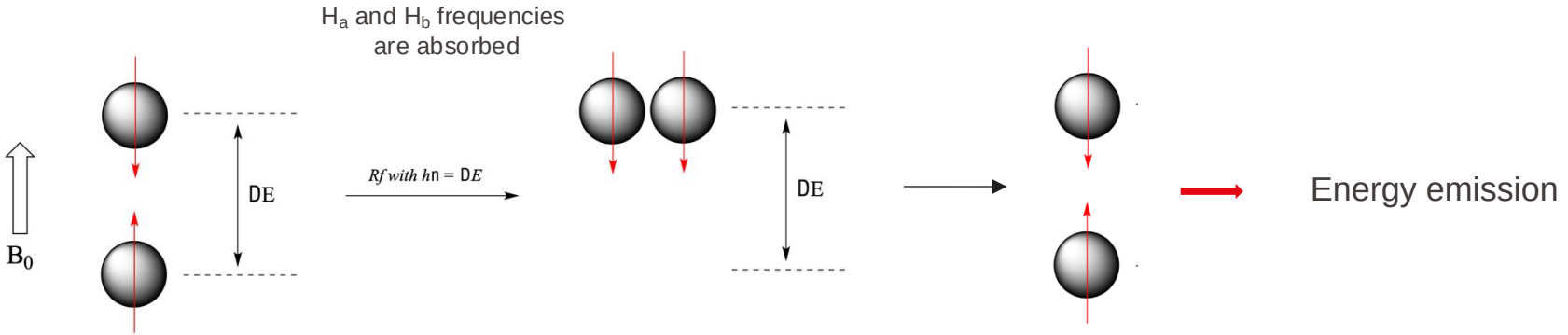
2-Acetoxybenzoic acid (Aspirin, Acetylsialic acid)

The ^{1}H-NMR experiment

-Molecules in solution are exposed to a very strong magnetic field (B_0)

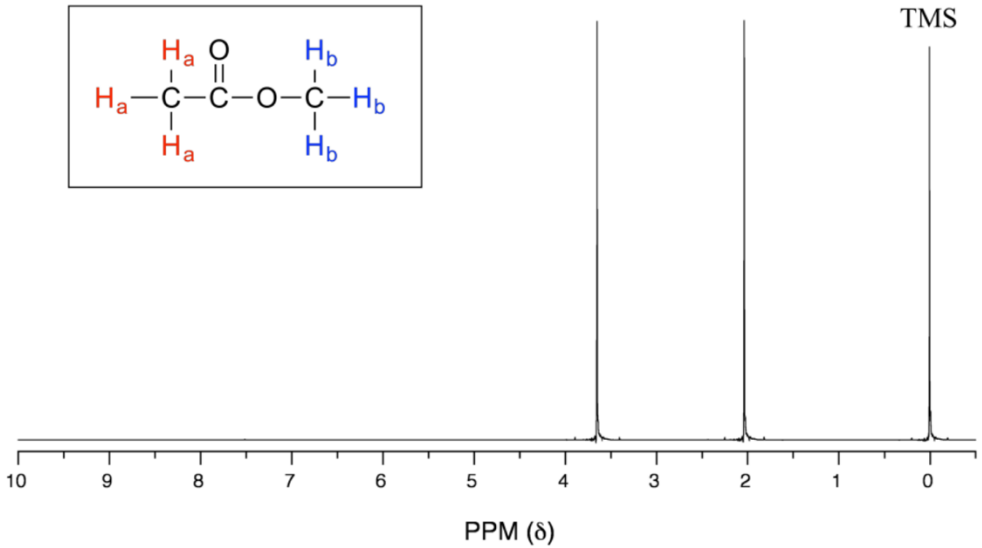


methyl acetate



The ^{1}H-NMR experiment

^{1}H-NMR spectrum of methyl acetate



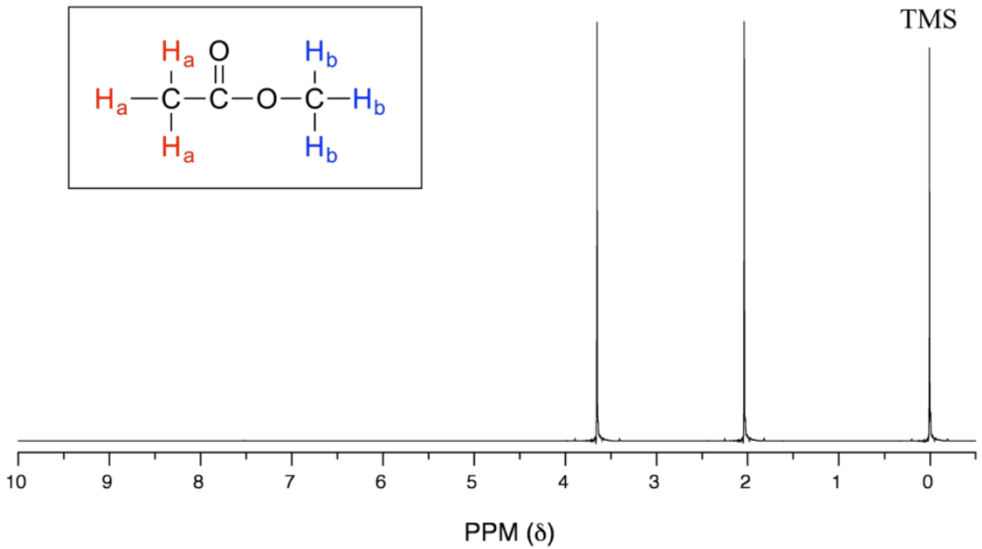
tetramethylsilane (TMS)

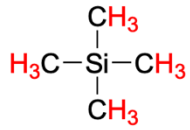
-3 absorbance peaks observed – 2 from the different protons and another one from the reference

-TMS is the reference compound to which all the other shifts are measured relative to

The ^{1}H-NMR experiment

^{1}H-NMR spectrum of methyl acetate



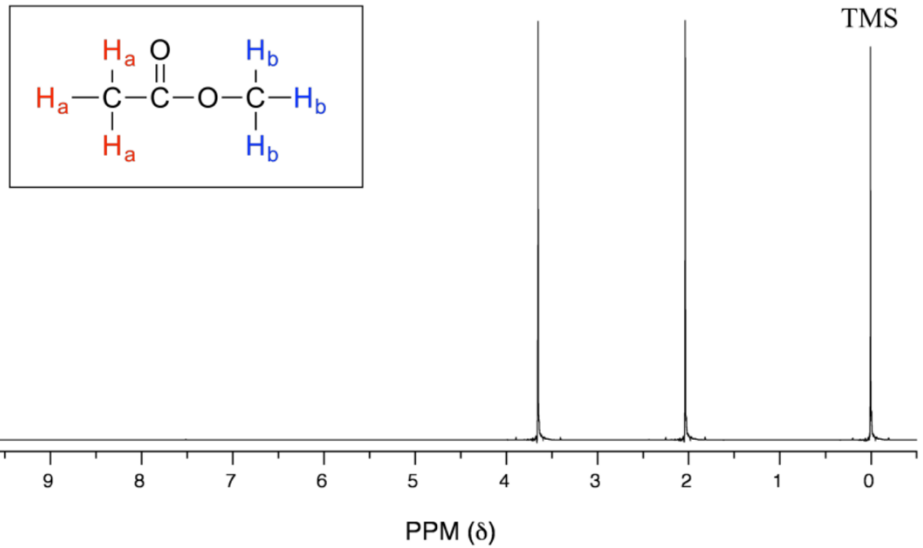


tetramethylsilane (TMS)

Focusing on the x-axis
-PPM – part per million
-The resonances of the two different types of protons have 2.0 and 3.6 parts per million higher than TMS
–This is referred as the **chemical shift (δ)**

The ^{1}H-NMR experiment

^{1}H-NMR spectrum of methyl acetate



tetramethylsilane (TMS)

Focusing on the **absence of the y-axis**

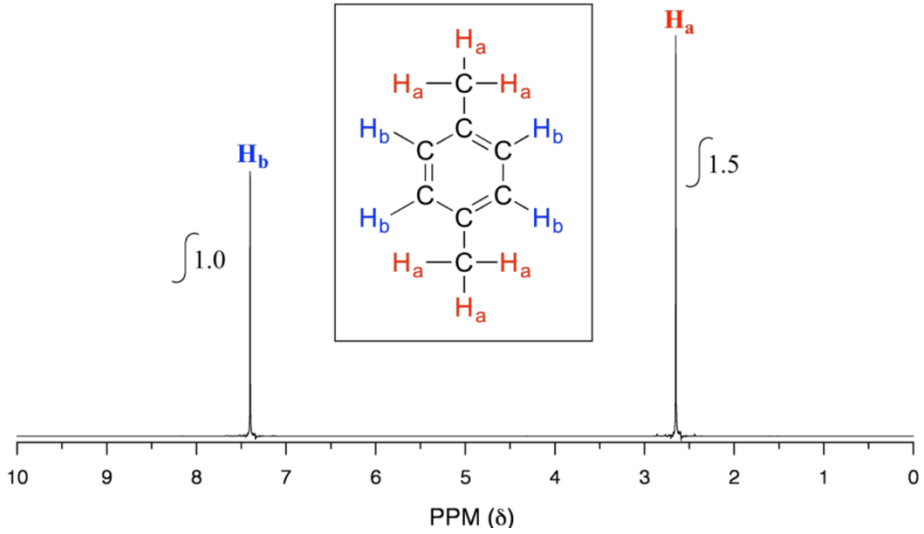
-are reported relative values and areas under the curve are integrated

-area under a signal is proportional to the number of protons to which the signal corresponds

-in this case the area under the curves are the same

The ^{1}H-NMR experiment

1,4-dimethylbenzene



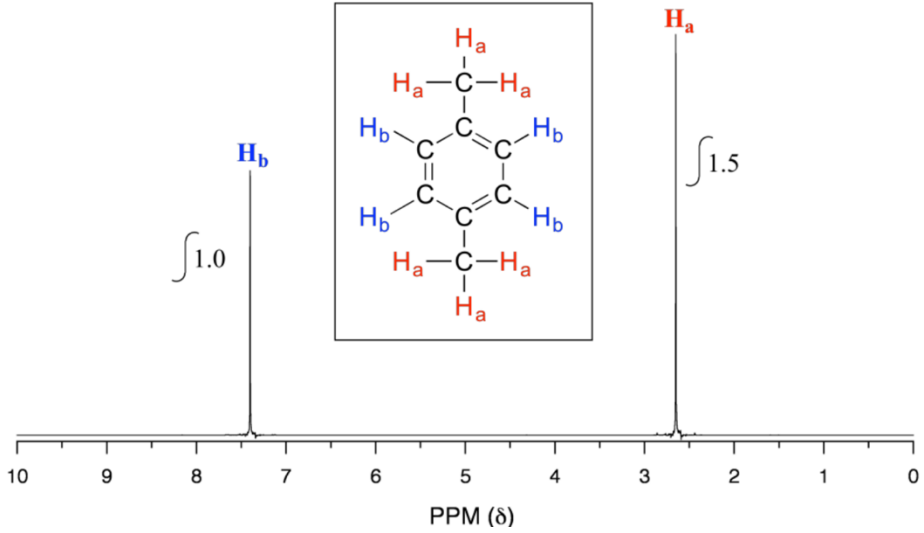
-2 sets of equivalent protons – 6 methyl protons(H_a) and 4 aromatic protons(H_b)

-Area under the peak at 2.6 ppm is 1.5 greater than that of at 7.4 ppm

Why ?

The ^{1}H-NMR experiment

1,4-dimethylbenzene



-2 sets of equivalent protons – 6 methyl protons(H_a) and 4 aromatic protons(H_b)

-Area under the peak at 2.6 ppm is 1.5 greater than that of at 7.4 ppm

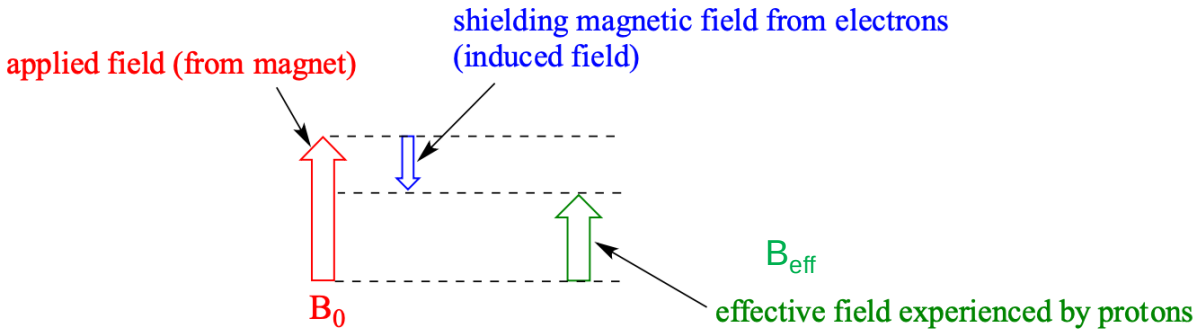
-It's the same ratio of 6/4 protons of different types

Making sense of the differences in chemical shifts

-The concept of diamagnetic shielding and deshielding

-**The chemical shift of a given proton is determined primarily by interactions with the nearby electrons**

-When electrons are subjected t an external magnetic field they form their own small induced magnetic fields in opposition to the external field



-This effect is called local diamagnetic shielding

Making sense of the differences in chemical shifts

Deshielding effect

-Electronegative atoms will pull electrons towards itself

Lower electron density → Less diamagnetic shielding → Stronger B_{eff} → Higher resonance frequency

TABLE 1.3 The Electronegativities of Selected Elements[a]

IA	IIA	IB	IIB	IIIA	IVA	VA	VIA	VIIA
H 2.1								
Li 1.0	Be 1.5			B 2.0	C 2.5	N 3.0	O 3.5	F 4.0
Na 0.9	Mg 1.2			Al 1.5	Si 1.8	P 2.1	S 2.5	Cl 3.0
K 0.8	Ca 1.0							Br 2.8
								I 2.5

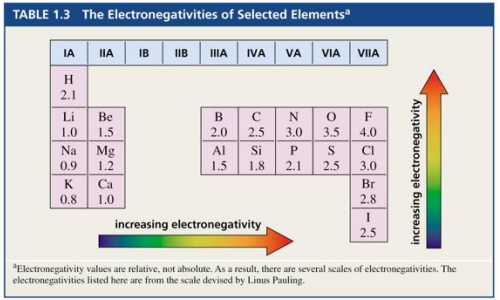
increasing electronegativity →

increasing electronegativity ↑

[a]Electronegativity values are relative, not absolute. As a result, there are several scales of electronegativities. The electronegativities listed here are from the scale devised by Linus Pauling.

-Pulling electron density away deshields protons making them more exposed to B_0

Making sense of the differences in chemical shifts

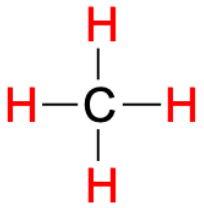


Deshielding effect

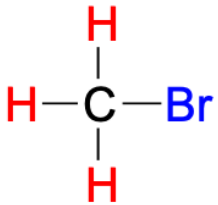
-Electronegative atoms will pull electrons towards itself

-Different halogens have different effects of the chemical shifts

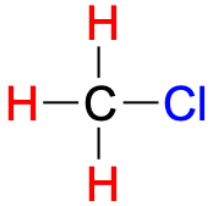
-As the electronegativity of the substituent increases so does the extent of the dishielding and of the chemical shift



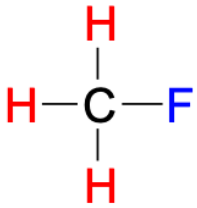
0.23 ppm



2.68 ppm



3.05 ppm

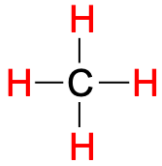


4.26 ppm

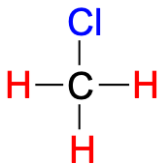
-One can predict the trend of the perturbation

Making sense of the differences in chemical shifts

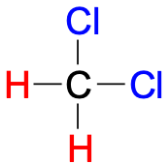
-Chemical shift of trichloromethane is higher than that of cloromethane



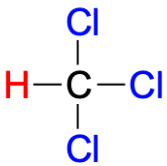
0.23 ppm



3.05 ppm

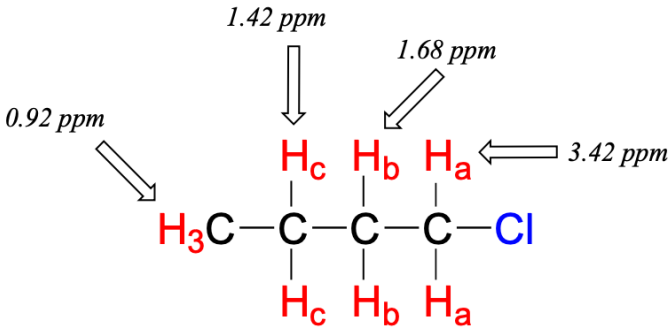


5.30 ppm



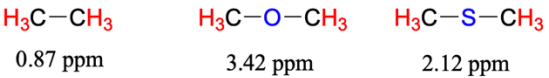
7.27 ppm

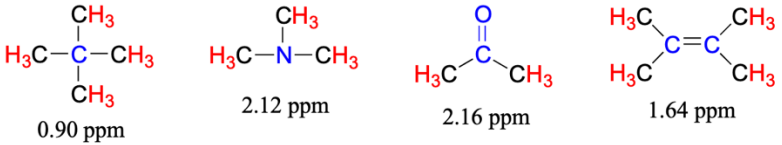
-The deshielding effect of an electronegative substituent diminishes sharply with the distance



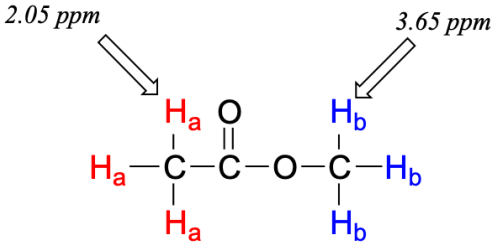
Making sense of the differences in chemical shifts

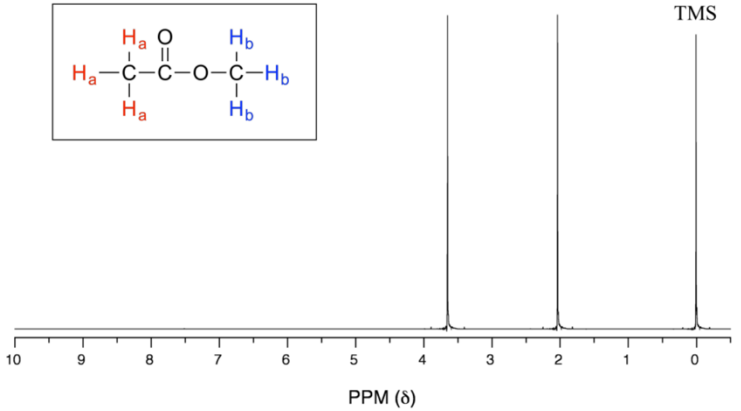
-Oxigens, sulfurs , sp2-hybridized carbons other electronegative atoms also shift the the signals of nearby protons





-Going back to our initial methyl acetate

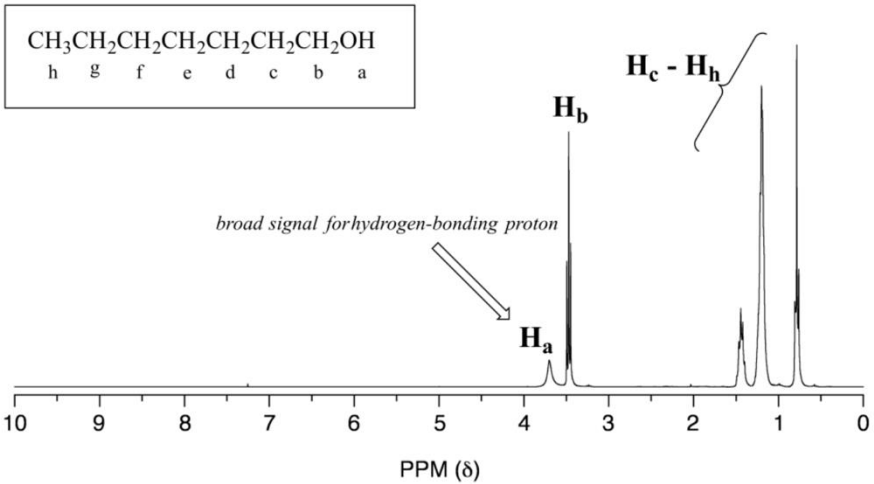




Making sense of the differences in chemical shifts

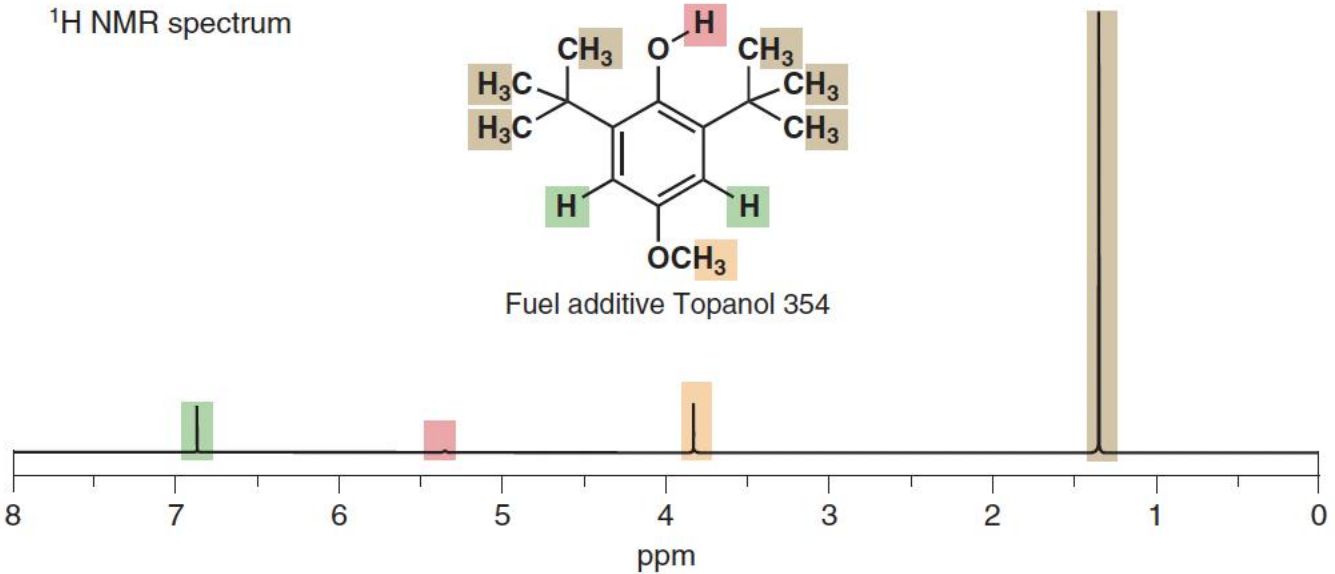
-Hydrogen bonded protons – have an impact on the electron density around the proton

-just like in other spectroscopies you can use reference values to guide yourself



type of proton	chemical shift range (ppm)
bonded to sp^3 carbon	0.5 - 4
bonded to N (amine)	1 - 3
bonded to O (alcohol)	1 - 5
alkene/ vinylic	3.5 - 6.5
terminal alkyne	2 - 3
bonded to N (amide)	5 - 9
aromatic	6 - 9
aldehyde	9.5 - 10
carboxylic acid	10 - 13

^{1}H-NMR – Chemical Shift



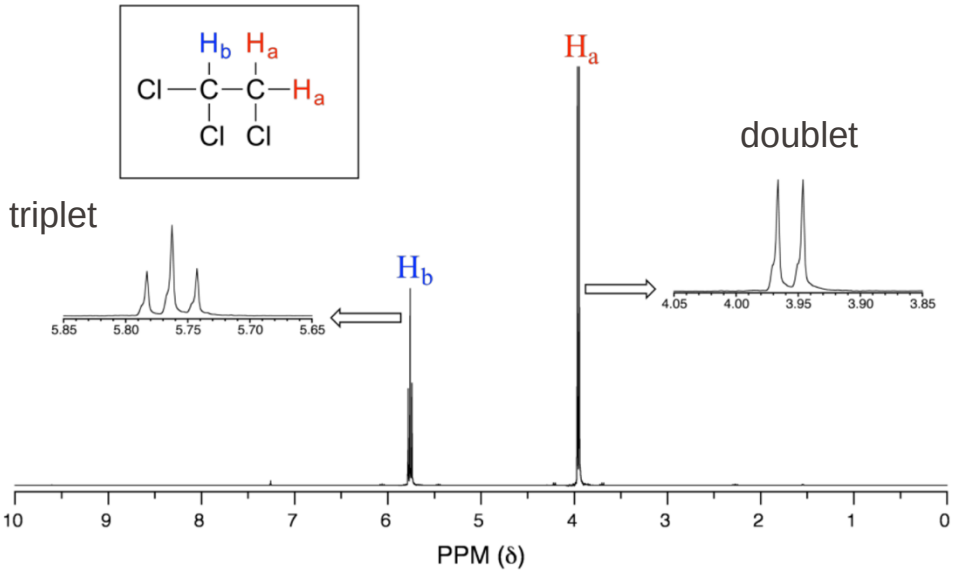
Region Division: 1H NMR spectrum regions
Right-hand Region (0 to 5 ppm): Saturated carbon-bonded hydrogen atoms, more shielded.
Left-hand Region (5 to 10 ppm): Hydrogen atoms bonded to unsaturated carbon atoms (e.g., alkenes, arenes, or carbonyl groups), less shielded.
Electron-Withdrawing Effect: Nearby oxygen atoms shift signals towards the left end of each region.

Spin-spin coupling

-Most of the organic molecules contain proton signals that are split into two or more sub-peaks

-Yet another source of useful information about sample molecules

1,1,2-tricloroethane



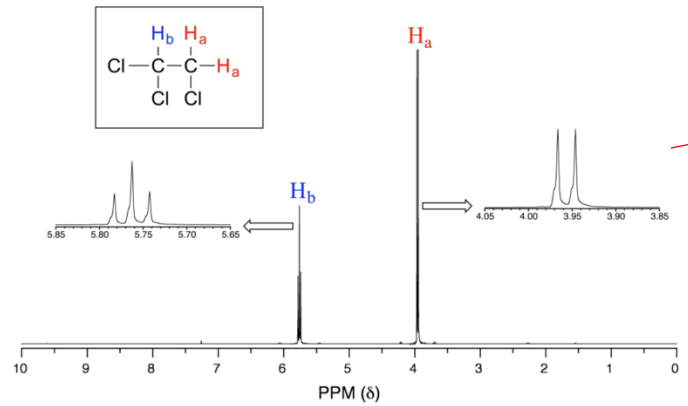
-area of the mid peak

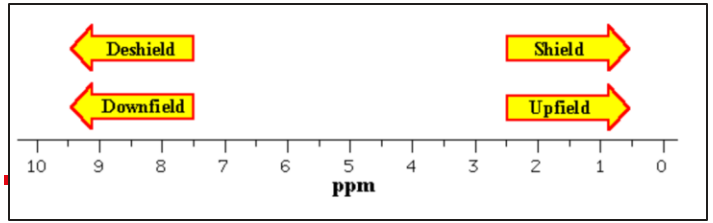
Equal height and area

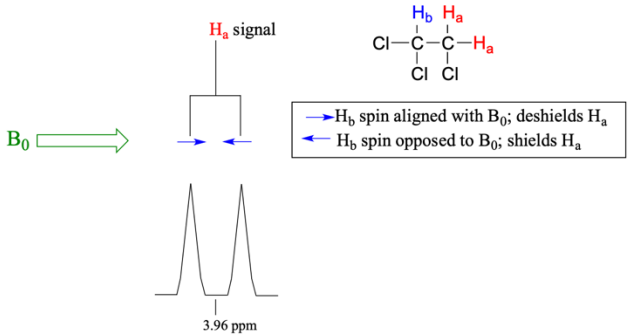
Spin-spin coupling

-Phenomenon that is the source of signal splitting
-Describes the magnetic interaction between neighboring non-equivalent NMR-active nuclei

1,1,2-tricloroethane







-H_a is influenced by the presence of H_b

-Magnetic moment of Hb is aligned in 50% of the molecules and opposed in the rest

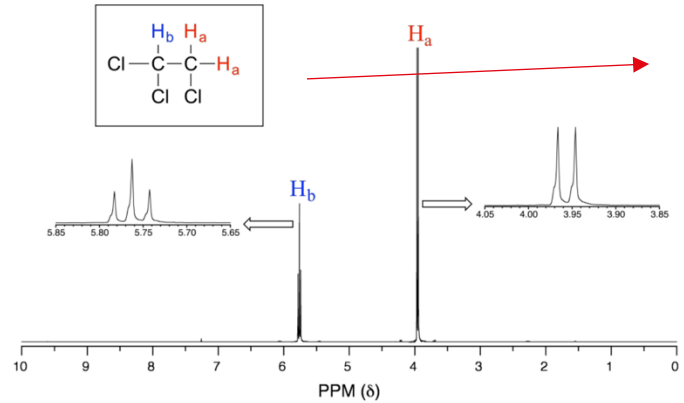
-Half of the molecules the magnetic field is shielded(shifts upfield)

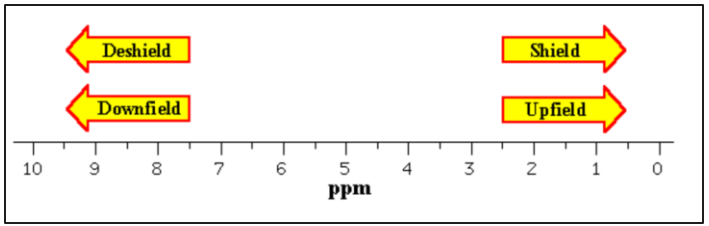
- In the other half is deshielded (shifts downfield)

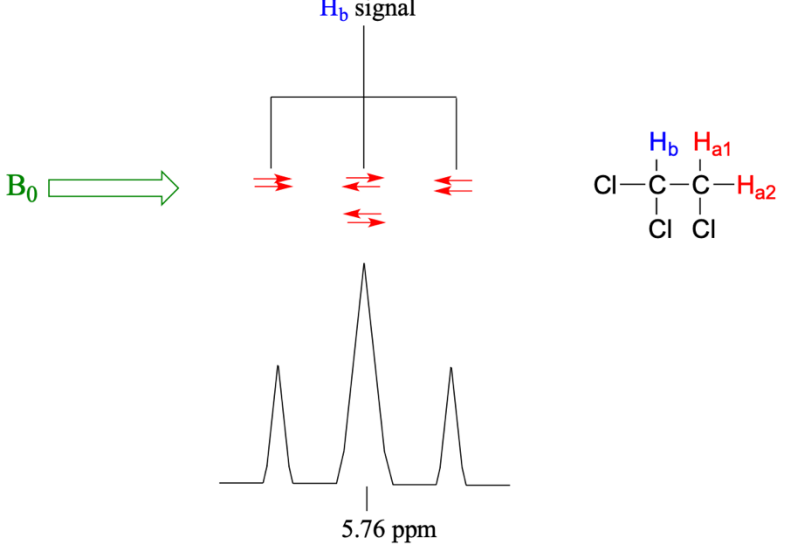
Spin-spin coupling

-Let's think about the H_b signal

1,1,2-tricloroethane







-H_b is influenced by the fields of both Ha protons:

-H_{a1} and H_{a2} are aligned with B0 shifting signal downfield

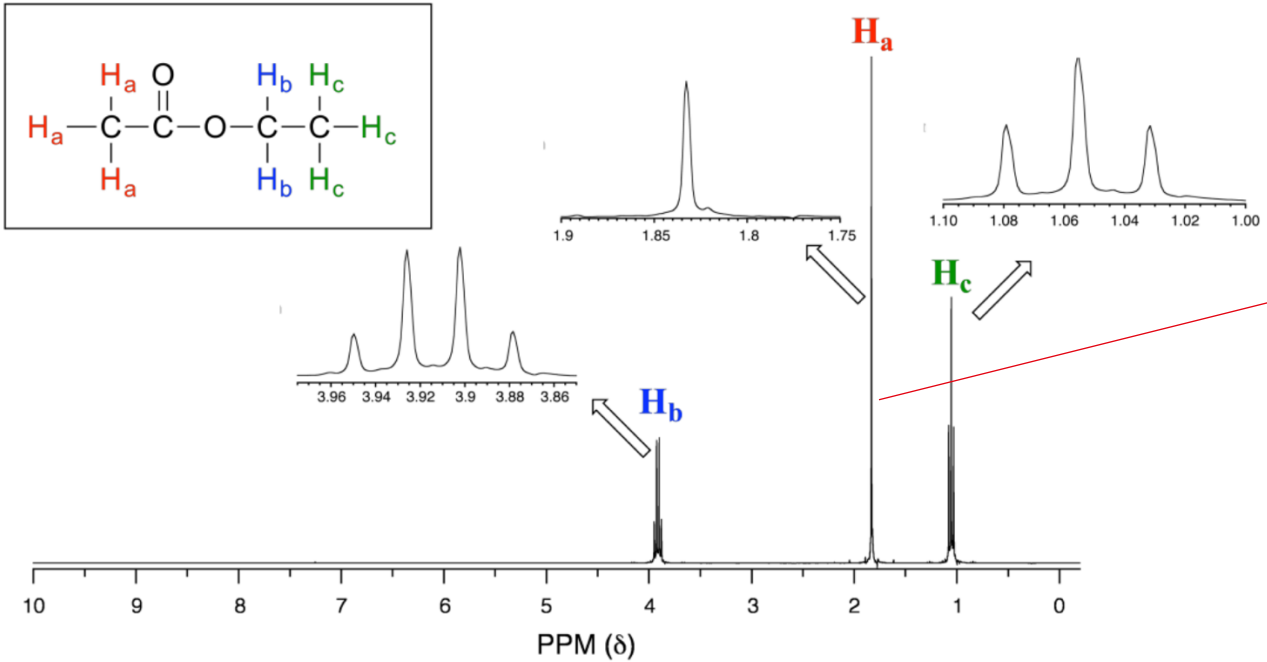
-H_{a1} and H_{a2} are opposed to B0 shifting signal upfield

-H_{a1} aligned and H_{a2} opposed – canceling effect

-H_{a1} opposed and H_{a2} aligned – canceling effect

Spin-spin coupling

-Looking ethyl acetate

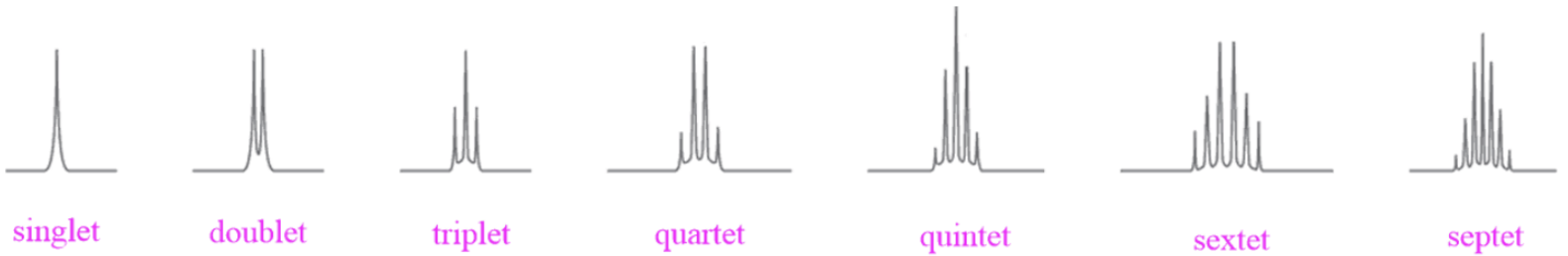


Notice that H_a has no splitting

There are no adjacent protons in the molecule

Spin-spin coupling- N+1 rule

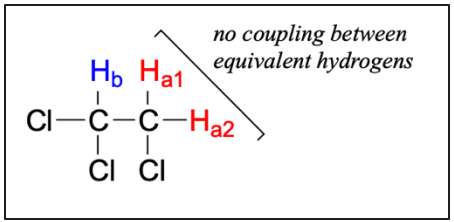
-A few things to remember



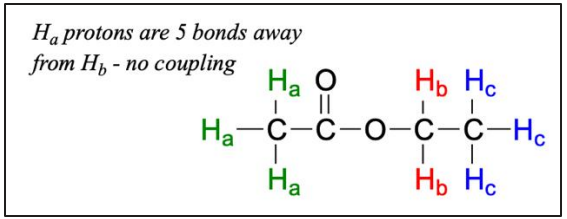
Multiplicity Determination:

- **N+1 Rule:** Number of peaks = N+1 (where N is the number of distinguishable neighbouring protons).
- For example:
  - One neighboring proton results in a doublet (1+1).
  - Two adjacent protons yield a triplet (2+1).

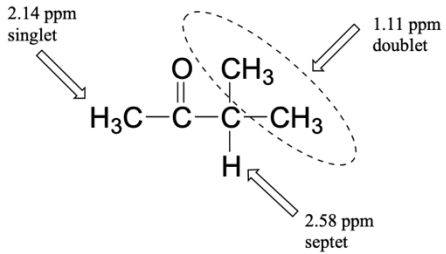
Equivalent protons don't couple

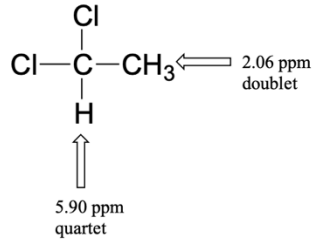


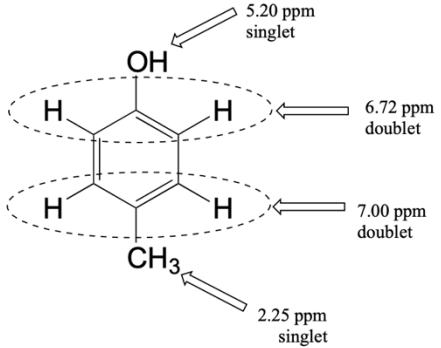
Equivalent protons don't couple

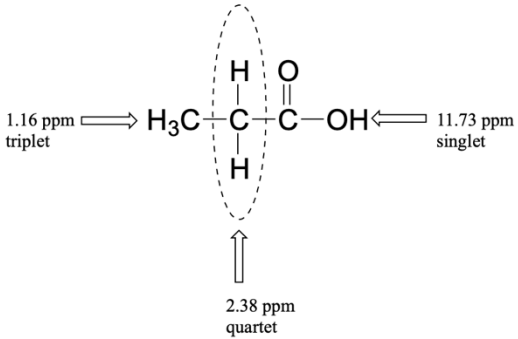


Splitting pattern in simple molecules

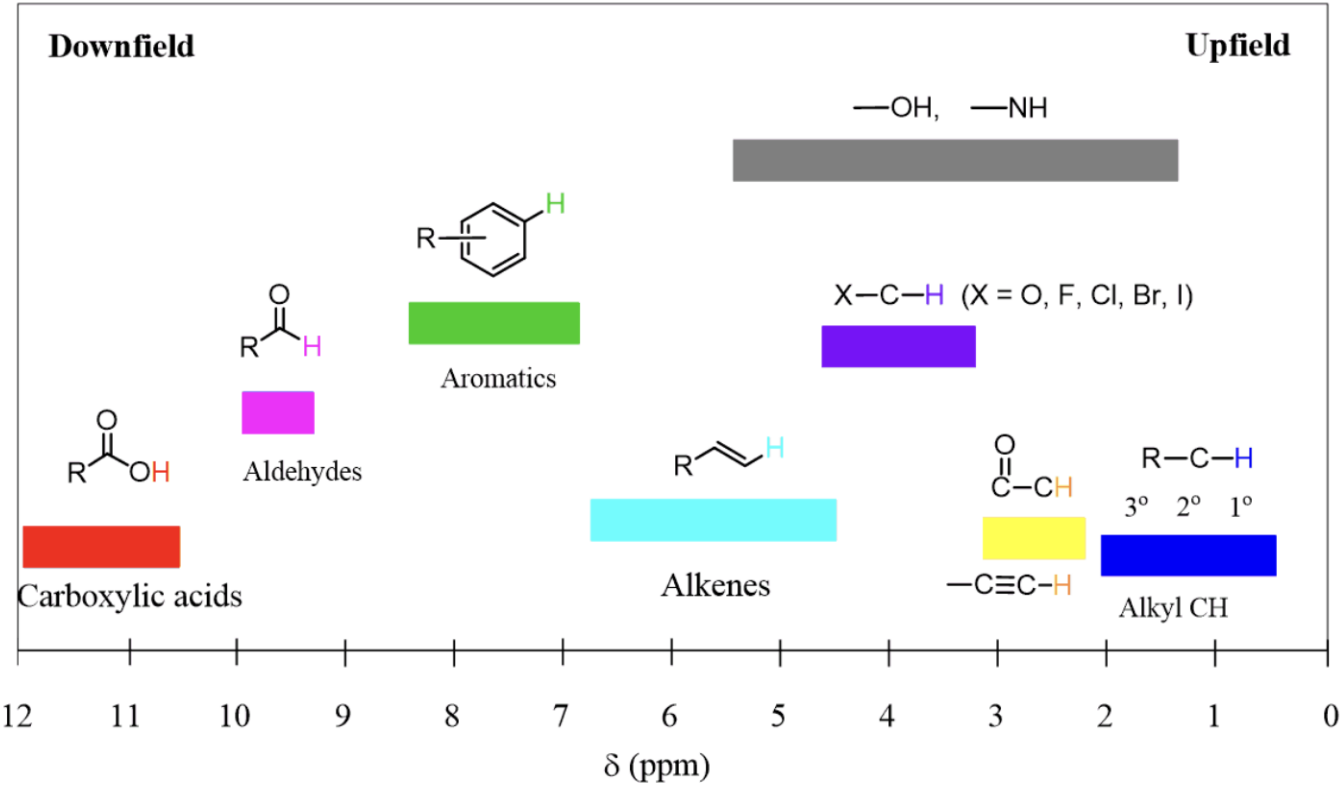




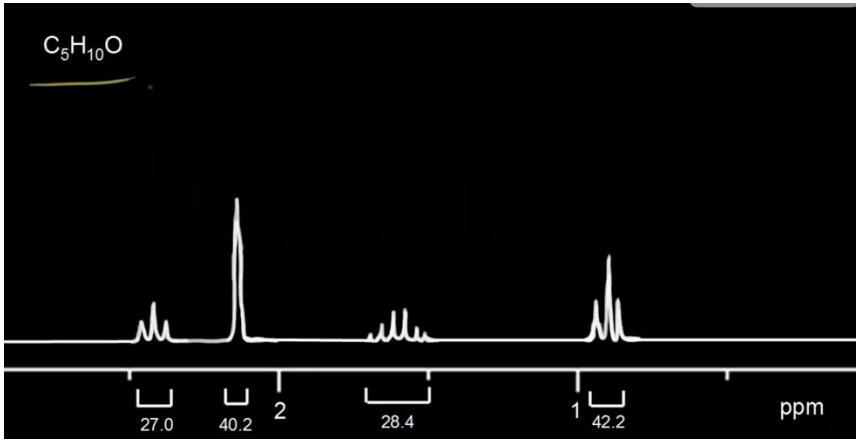


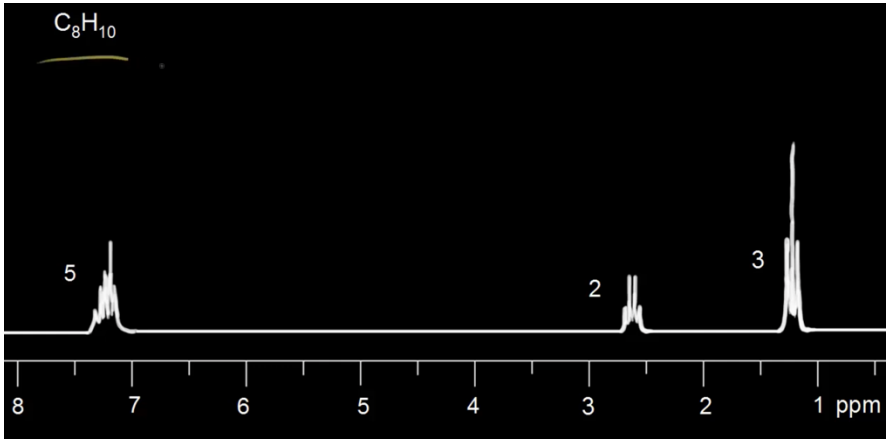


^{1}H-NMR – Chemical Shift



Let's solve problems with this





Solving unknown structures

Step 2 – Calculating Index of Hydrogen Deficiency

-Will tell us the how many hydrogen bonds an/or ring structures our molecule has
Simple idea – presence of a double bond or a ring means that two fewer hydrogen atoms can be part of the compound

Calculating Index of Hydrogen Deficiency:

$$IHD = \frac{(2n+2) - A}{2}$$

where:

n = number of carbon atoms
A = (number of hydrogen atoms) + (number of halogen atoms) - (number of nitrogen atoms) - (net charge)

Examples:

C_6H_{14} -> IHD = 0 -> no double bonds or rings
C_6H_{12} -> IHD = 1 -> one double bond or ring like cyclohexane or 2-hexene
C_6H_6 -> IHD = 4 -> three double bonds and one ring benzene

- For our molecule C_4H_9Cl -> IHD = 0 -> no double bonds or rings

^{13}C-NMR spectroscopy

- Remember ^{12}C does not have a nuclear magnetic moment and is not NMR active

-However ^{13}C is NMR active

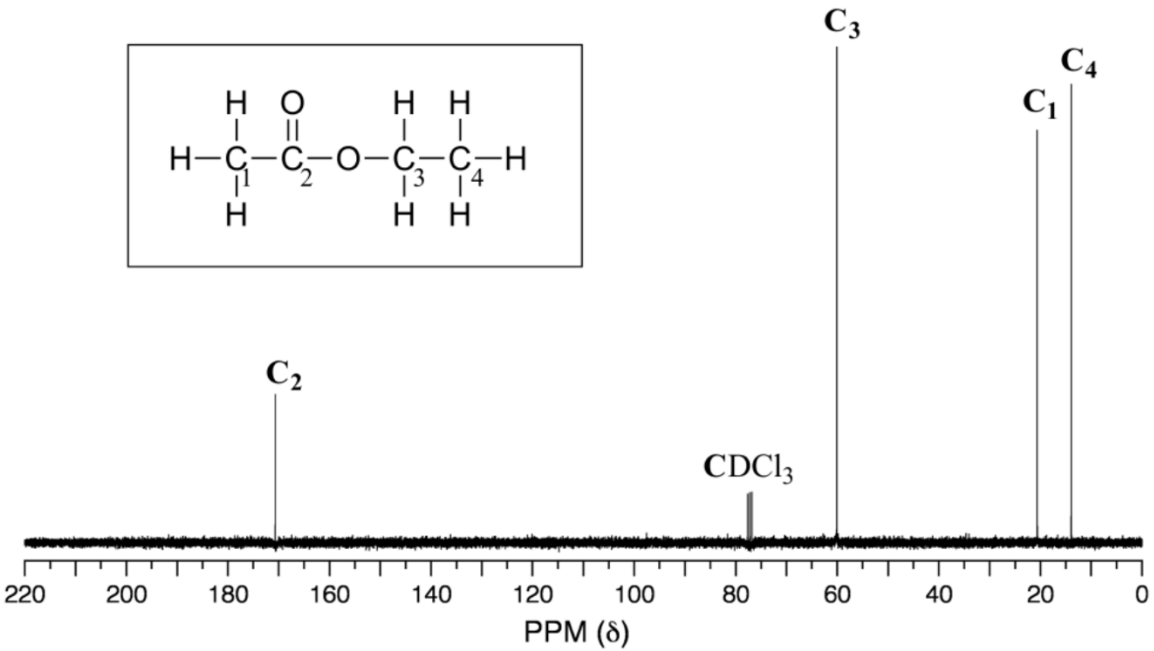
-Many things are common between ^{13}C and ^{1}H NMR spectroscopy but many aren't:
- -NMR signals much weaker
- -More sample required
- -More complex experiments
- -Area under the peak is not as informative as for ^{1}H

-Generally ^{13}C don't couple and to avoid complexity in the interpretation of the data ^{1}H-^{13}C coupling is turned off

-The peak you see labeled as $CDCl_3$ is deuterated chloroform included in the solvent

^{13}C-NMR spectroscopy

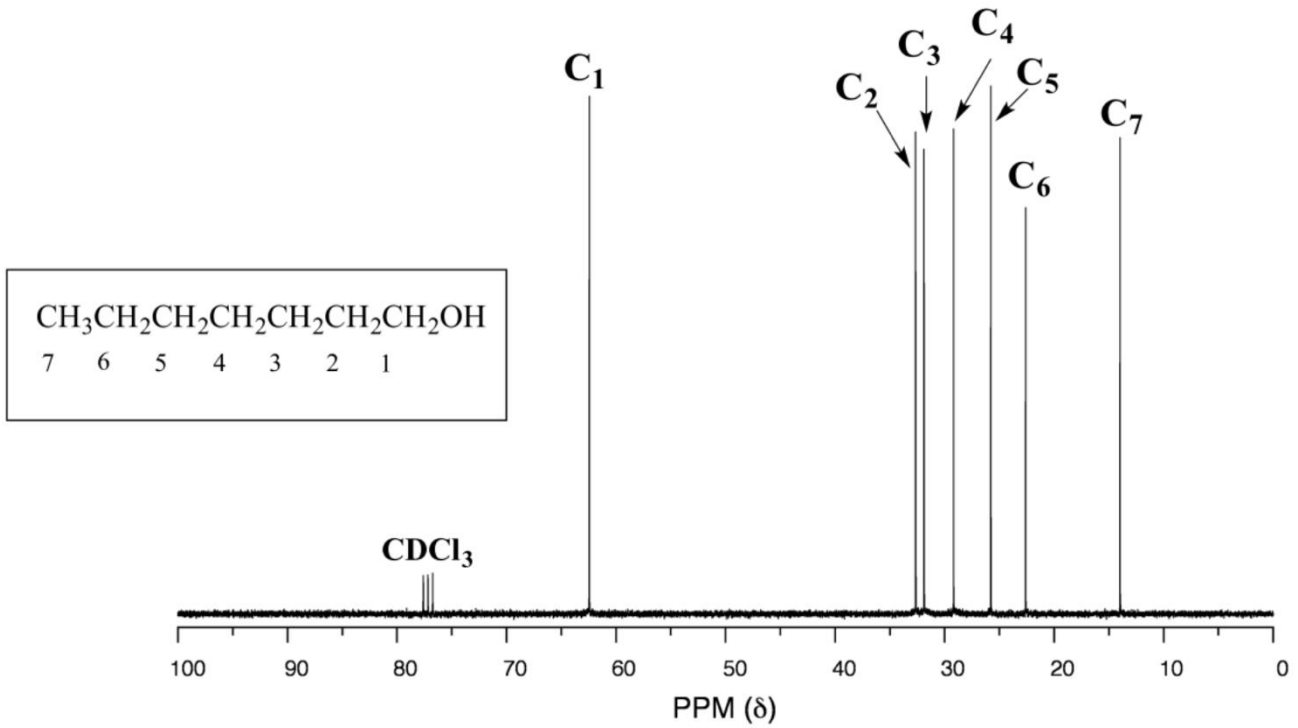
-let's look an example



-Carbon peaks almost never overlap making the interpretation of the data easier

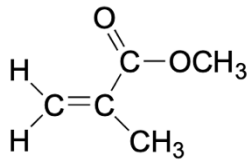
^{13}C-NMR spectroscopy

-let's look an example

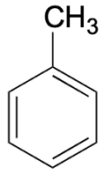


Let's discuss

Which spectrum belongs to which molecule ?

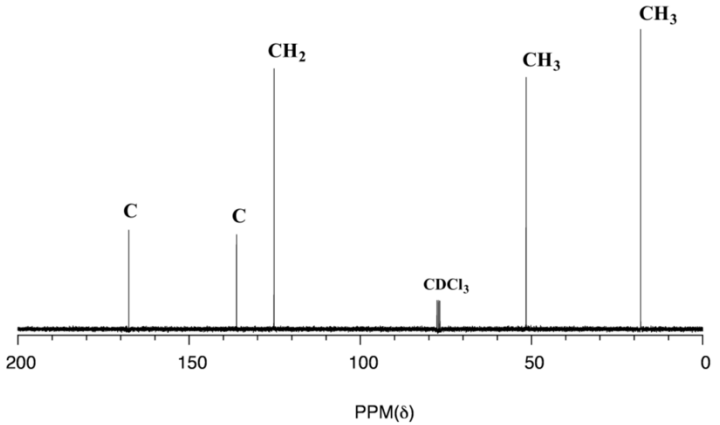


methyl methacrylate

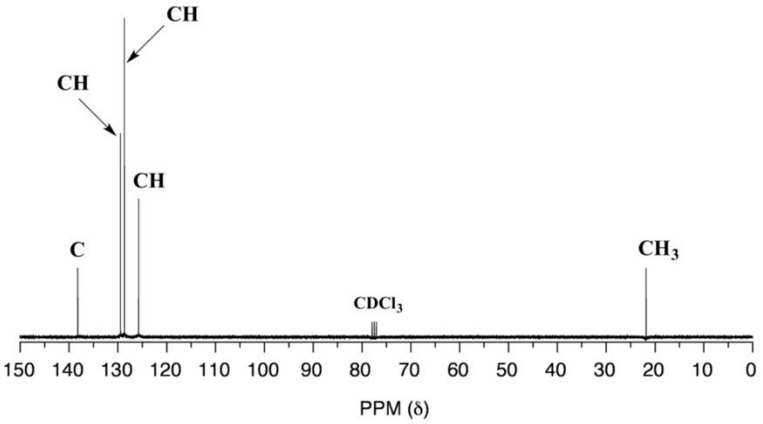


toluene

Spectrum A:

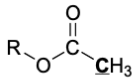
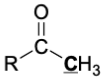
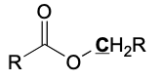
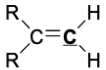
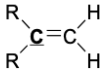


Spetrum B:



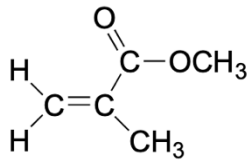
Let's discuss

If I would give you this information would it become easier ?

Carbon type	Chemical shift (ppm)
RCH_3	13 - 16
RCH_2R	16 - 25
R_3CH	25 - 35
	18 - 22
	28 - 32
RCH_2NHR	35 - 45
RCH_2OH	50 - 65
$R-C\equiv C-R$	65 - 70
$ROCH_2R$	50 - 75
	50 - 75
	115 - 120
	125 - 140
aromatic carbon	125 - 150
 (carboxylic acid derivatives)	165 - 185
	190 - 200
	200 - 220

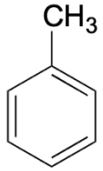
Let's discuss

Which spectrum belongs to which molecule ?



methyl methacrylate

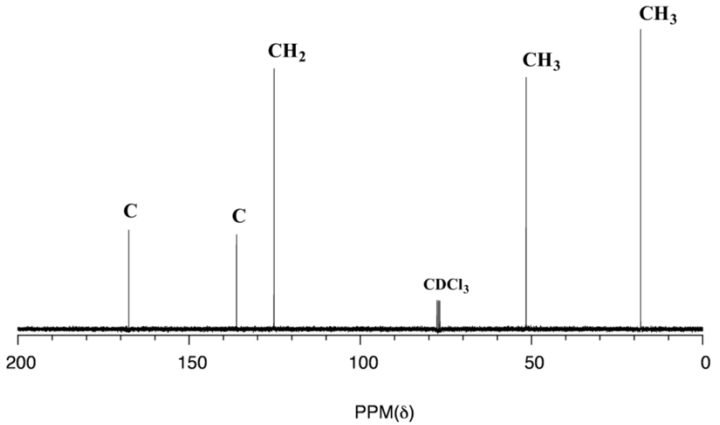
a



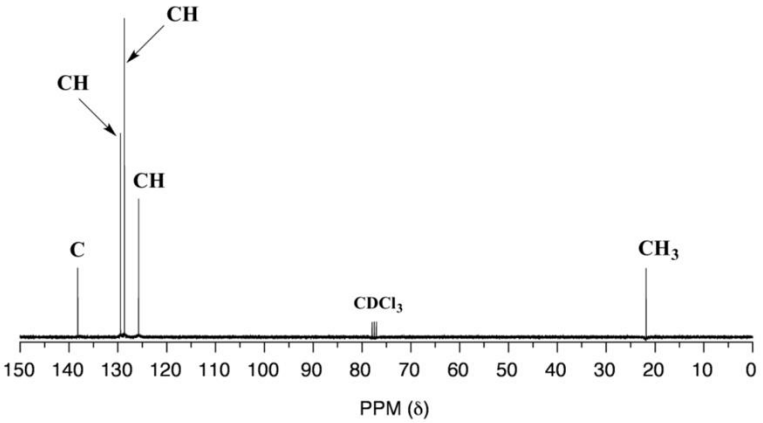
toluene

b

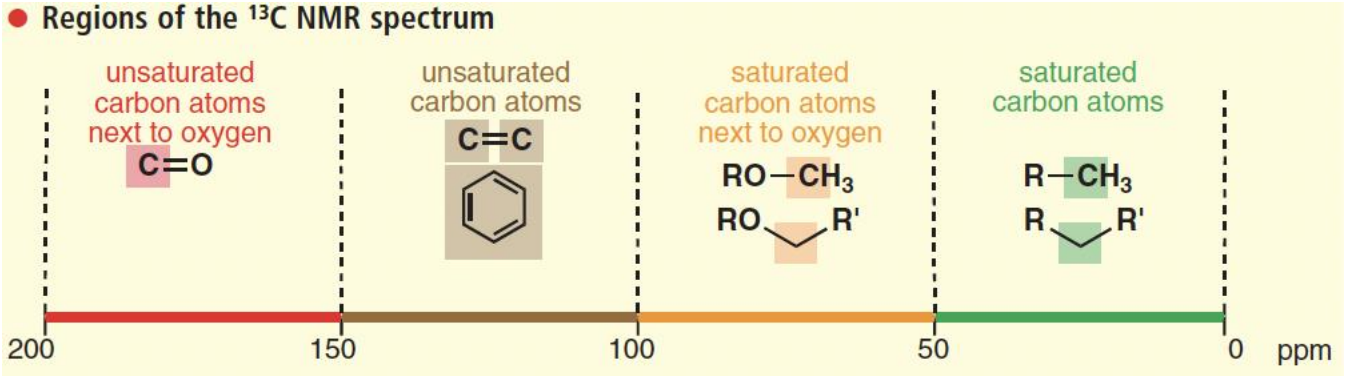
Spectrum A:

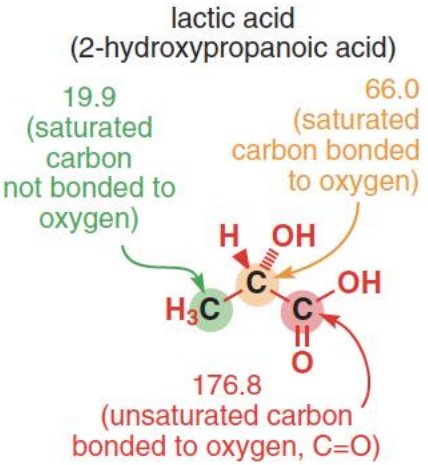


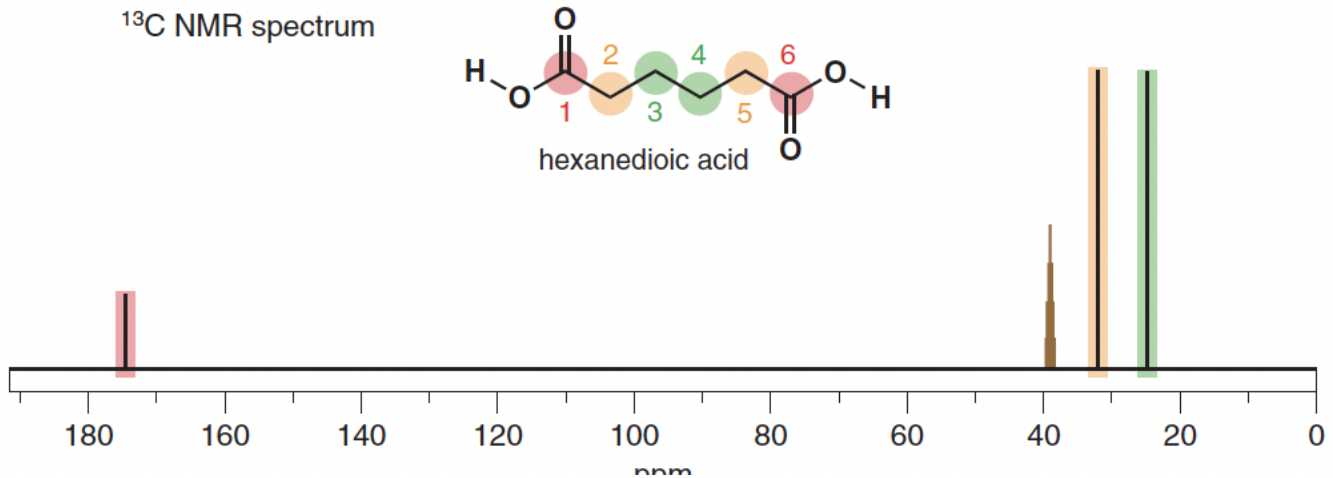
Spetrum B:



^{13}C-NMR


Regions of the ¹³C NMR spectrum
unsaturated carbon atoms next to oxygen
C=O
unsaturated carbon atoms
C=C
saturated carbon atoms next to oxygen
RO—CH3
RO
R'
saturated carbon atoms
R—CH3
R
R'
200
150
100
50
0
ppm


lactic acid
(2-hydroxypropanoic acid)
19.9
(saturated carbon not bonded to oxygen)
66.0
(saturated carbon bonded to oxygen)
H
OH
C
OH
H3C
C
O
176.8
(unsaturated carbon bonded to oxygen, C=O)


¹³C NMR spectrum
O
H
O
1
2
3
4
5
6
O
H
O
hexanedioic acid
180
160
140
120
100
80
60
40
20
0
ppm

Solving unknown structures

- you are given a vial of an unknown sample – you are the first one to analyze it !!!!!

Step 1 – Use MS and combustion analysis to determine the molecular formula

Combustion analysis – will tell us the mass % of each element; the mass of O gest combusted

working example -> 52% of C; 38.3% Cl; 9.7% H

Next – we need to know its molecular mass - How to do it ?

Molecular ion peak in mass spec tells us that the molar mass 92 g/mole

Moles of C -> 0.52* 92g = 47.8 g -> 1 mole of compound X has 4 moles of carbon
Analogously -> 9 moles of H and 1 Cl

The formula is C_4H_9Cl

Solving unknown structures

Step 2 – Calculating Index of Hydrogen Deficiency

-Will tell us the how many hydrogen bonds an/or ring structures our molecule has
Simple idea – presence of a double bond or a ring means that two fewer hydrogen atoms can be part of the compound

Calculating Index of Hydrogen Deficiency:

$$IHD = \frac{(2n+2) - A}{2}$$

where:

n = number of carbon atoms
A = (number of hydrogen atoms) + (number of halogen atoms) - (number of nitrogen atoms) - (net charge)

Examples:
C_6H_{14} -> IHD = 0 -> no double bonds or rings
C_6H_{12} -> IHD = 1 -> one double bond or ring like cyclohexane or 2-hexene
C_6H_6 -> IHD = 4 -> three double bonds and one ring benzene

- For our molecule C_4H_9Cl -> IHD = 0 -> no double bonds or rings

Solving unknown structures

Step 3 – Use available spectroscopy data to identify discrete parts of the structure

-In this case we will just use NMR data (C_4H_9Cl)

^{1}H-NMR

δ (ppm)	splitting	integration
3.38	d	2
1.95	m	1
1.01	d	6

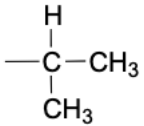
^{13}C-NMR

52.49 (CH_2)
31.06 (CH)
20.08 (CH_3)

-only 3 signals in each NMR spectrum -> tell us that two carbons are chemically equivalent

-1.01 ppm in the proton spectrum corresponds to six protons strongly suggests that the molecule has two equivalent methyl (CH_3) groups

-Since this signal is a doublet there must be a CH carbon bound to each of these two methyl groups



Solving unknown structures

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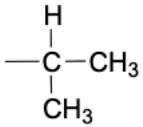
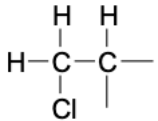


- 1H-NMR signal at 3.38 ppm must be for protons bound to the carbon which is bound to the chlorine (electromagnetic atom which dishields)

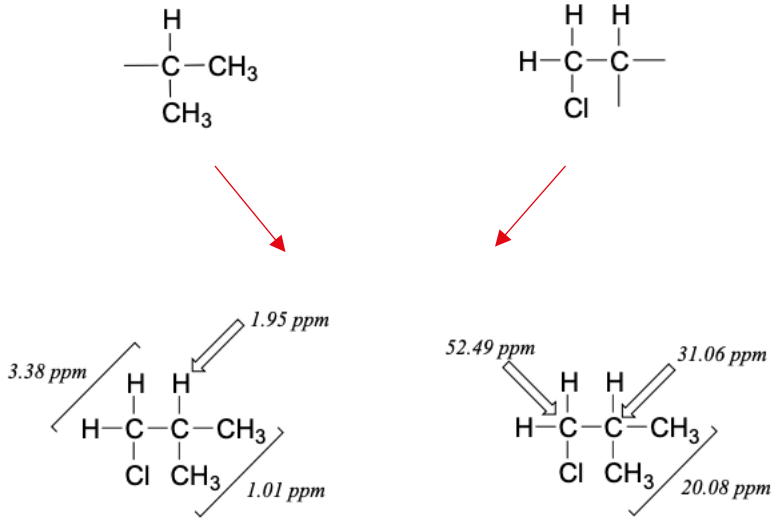
-The signal is for 2 protons and is a doublet meaning there is a single non-equivalent proton on an adjacent carbon



Solving unknown structures

Step 4 – Put the pieces of the puzzle together

-In this case we will just use NMR data (C_4H_9Cl)



^{1}H-NMR assignments

^{13}C-NMR assignments

Key concepts

-Identify groups of chemically equivalent protons and carbons in a structure

- Understand how to look at an NMR spectrum including meaning of the ppm label and chemical shift

-Predict trends in chemical shift

-Understand how to use proton peak integration values to determine how many protons a peak is worth

-Interpret splitting in NMR spectrum

-Understand ^{13}C NMR spectra

-Match structures to ^{1}H and ^{13}C spectra

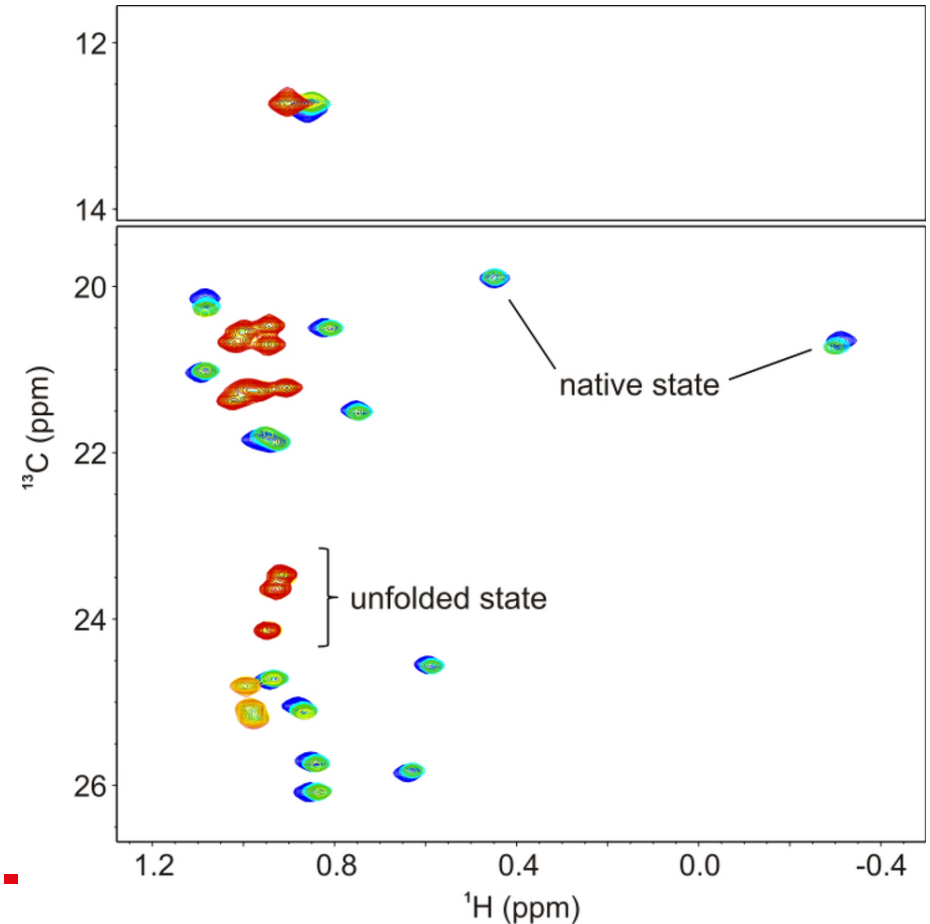
-Capable of solving an unknown structure from multiple experimental data sources

Questions ?

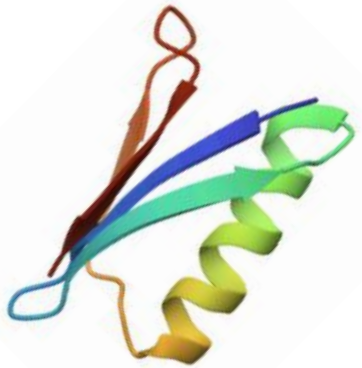
Next lecture

Organic Reactivity (Chapter 6)

Monitoring protein unfolding by NMR



Methyl region of 2D $^{1}H^{13}C$ HMQC spectra of 900 µM GB1 in 20 mM HEPES, pH 7.0 at 300 K and urea concentrations of 0 M (blue), 2.9 M (cyan), 4.05 M (green), 5.4 M (yellow), and 6.52 M (red)

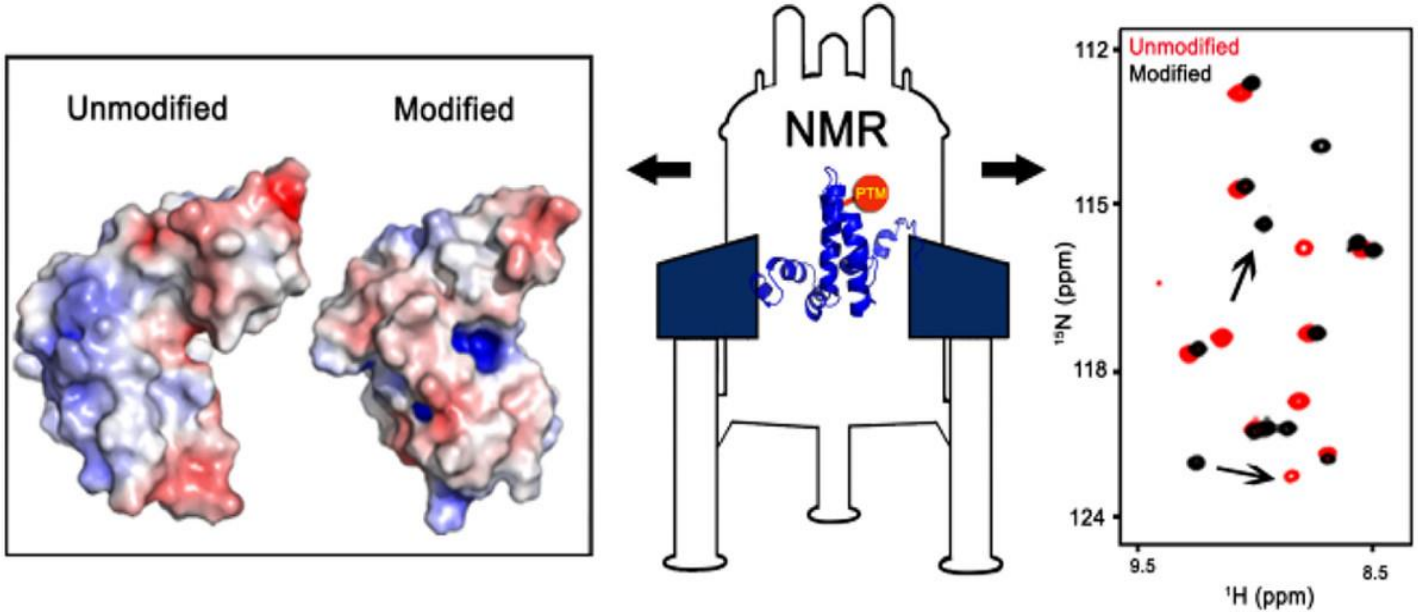


Dreydoppel, M., Balbach, J. & Weininger, U. Monitoring protein unfolding transitions by NMR-spectroscopy. *J Biomol NMR* **76**, 3–15 (2022). https://doi.org/10.1007/s10858-021-00389-3

Detection of Protein-Modifications using NMR

Effect of PTM on protein conformation

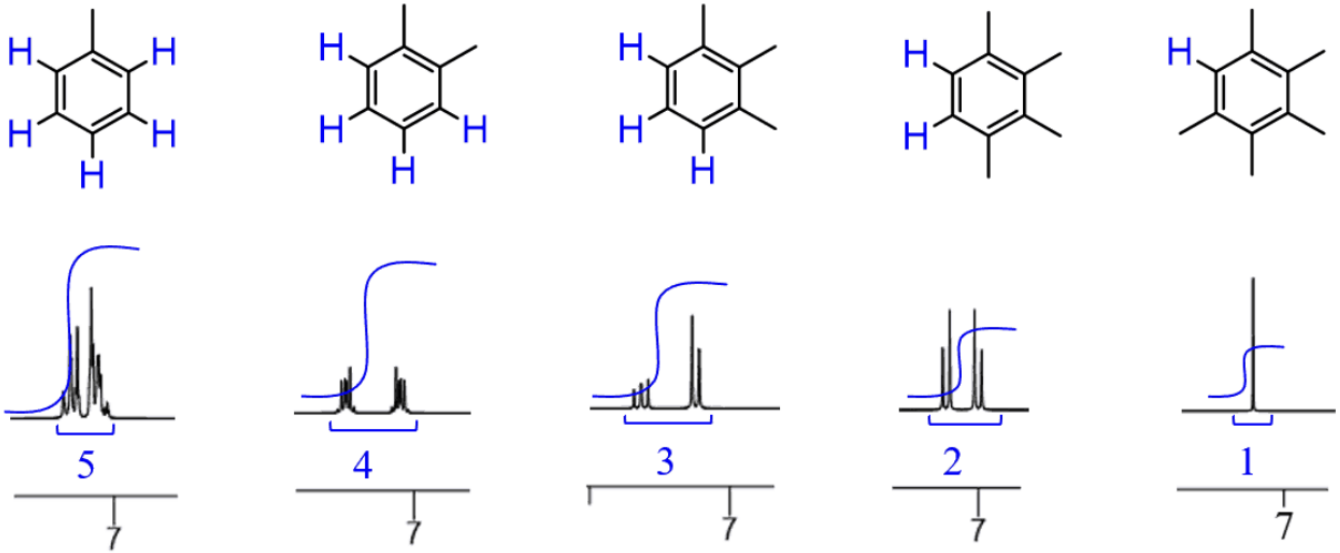
Site-specific detection of PTM



Using NMR, specific PTMs such as phosphorylation, acetylation, and glycosylation are detected by observing alterations in protein structure at the atomic level. By **comparing NMR spectra of modified and unmodified proteins**, distinct shifts or changes in peaks signify the presence and location of PTMs.

Kumar *et al. Biochemistry* 2020 *59* (1), 57-73

^{1}H-NMR – Number of Protons

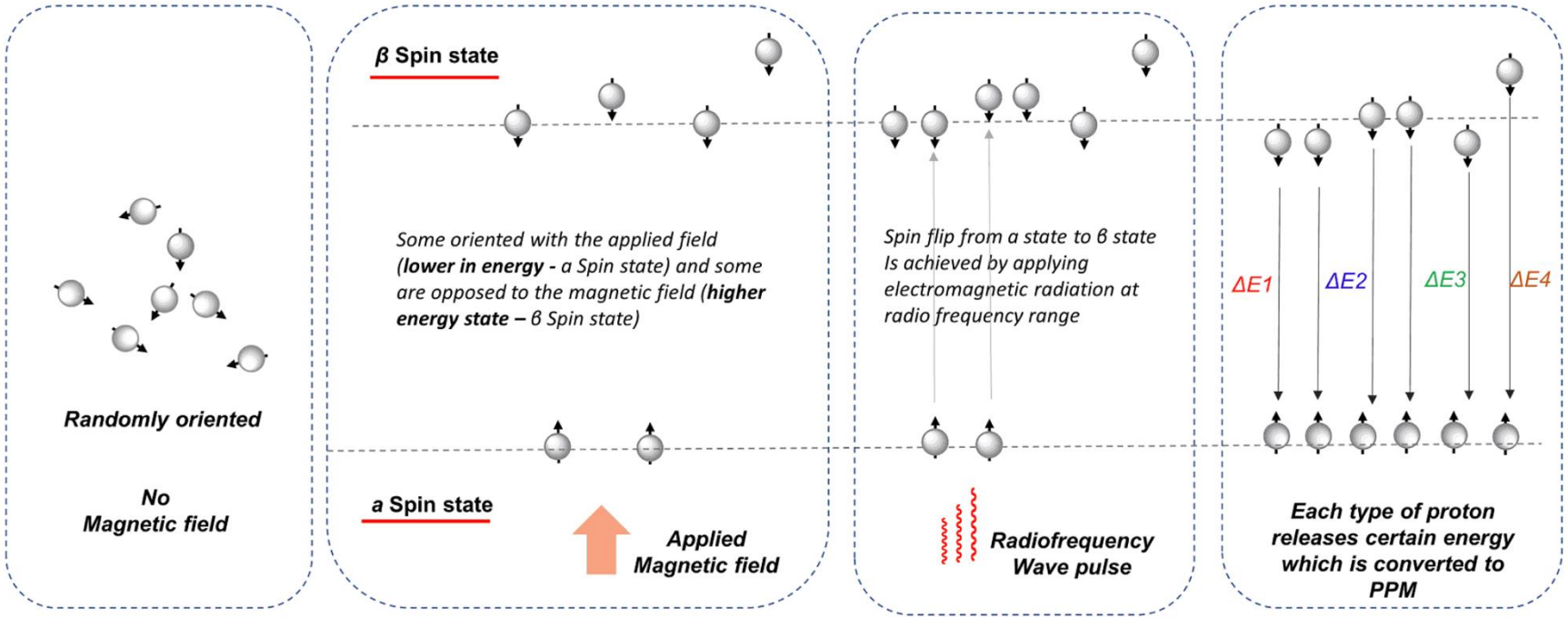


The number of protons is represented beneath the integral sign, and the integral's height correlates with the proton count.

Integral Significance:

- **Relative Proton Number:** Number of protons indicated beneath the integral sign.
- **Proportional Height:** Integral height reflects the relative abundance of protons.

Nuclear magnetic resonance spectroscopy



The energy gap between the α and β states is slightly different for each type of proton depending on their environment (neighboring atoms)