



Ciências  
ULisboa

CQB  
Centro  
de Química  
e Bioquímica

U LISBOA

UNIVERSIDADE  
DE LISBOA

Mestrado em Química, Métodos Avançados de Análise 2017/2018

## *Bloco de Ressonância Magnética Nuclear*

Parte 1 - Novembro 2017

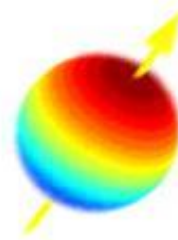
Helena Gaspar

[\*hmgaspar@ciencias.ulisboa.pt\*](mailto:hmgaspar@ciencias.ulisboa.pt)

*Gab 8.5.49, Lab 8.5.55*



# Nuclear Magnetic Resonance Spectroscopy (NMR)

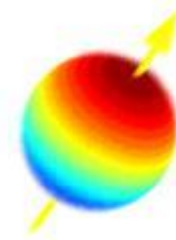


BASIC PRINCIPLES OF NMR

IDENTIFICATION OF ORGANIC COMPOUNDS

BASED ON THE INTERPRETATION OF 1D/2D SPECTRA

## BIBLIOGRAPHY



### BOOKS

- *Spectrometric Identification of Organic Compounds*  
Silverstein, Bassler, Morrill, 7<sup>th</sup> edition, 2005
- *Introduction To Spectroscopy*  
Pavia, Lampman, Kriz, 5<sup>th</sup> edition, 2008
- *Organic Structures from Spectra*  
Field, Sternhell, Kalman, 4<sup>th</sup> edition, 2007

### www (accessed November 2017)

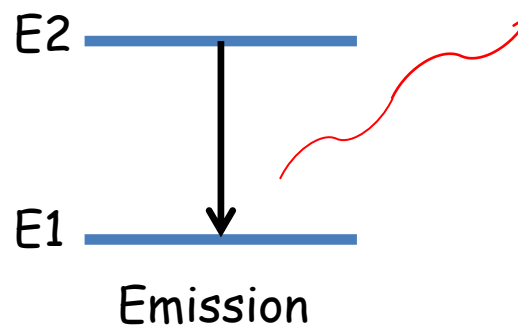
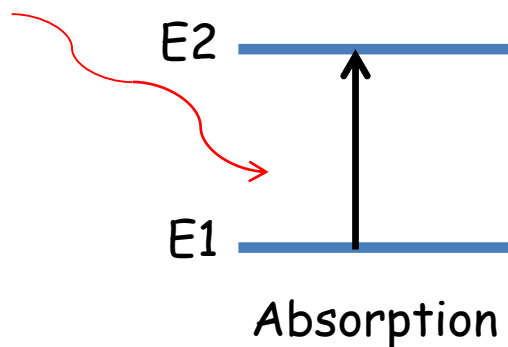
- <http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/nmr/nmr1.htm#nmr1>  
William Reusch, Michigan State University, USA
- <http://www.cis.rit.edu/htbooks/nmr/>  
Joseph P. Hornak, Rochester Institute of Technology, USA
- <http://www.chem.wisc.edu/areas/reich/chem605/index.htm>  
Hans J. Reich, University of Wisconsin, USA
- <http://chem.ch.huji.ac.il/nmr/techniques/1d/multi.html>  
Roy Hoffman, The Hebrew University of Jerusalem
- [http://chemwiki.ucdavis.edu/Organic\\_Chemistry/Organic\\_Chemistry\\_With\\_a\\_Biological\\_Emphasis/Chapter\\_\\_5%3A\\_Structure\\_Determination\\_II](http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_Biological_Emphasis/Chapter__5%3A_Structure_Determination_II)  
Tim Soderberg, University of Minnesota, USA

## SPECTROSCOPY

interaction of electromagnetic radiation with matter



## Energy states transitions



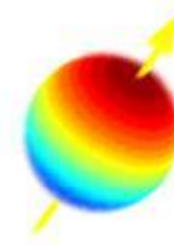
$$\Delta E = h\nu = hc/\lambda$$

$\nu$  - Radiation frequency (Hz or  $s^{-1}$ )

$h$  - Planck constant ;  $c$  - speed of light

$\lambda$  - wavelength (m)

## SPECTROSCOPY

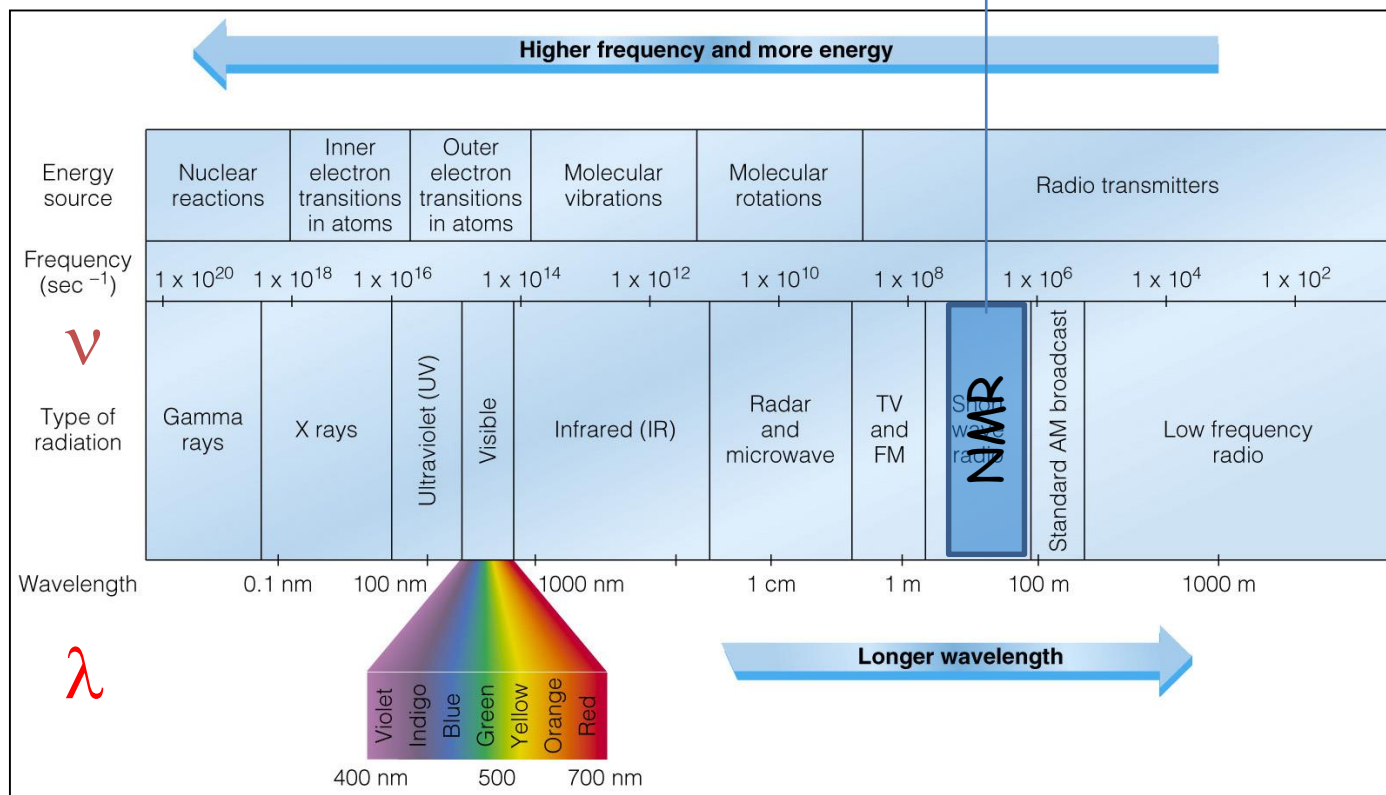


Region of Spectrum	Energy transition
X- rays	Bond Breaking
Ultraviolet/visible	Electronic
Infrared	Vibrational
Microwave	Rotational
Radiofrequencies	Nuclear spin Electronic spin

# NMR SPECTROSCOPY

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

Nuclear Spin states transitions



# Nuclear Magnetic Resonance Spectroscopy



Based on the absorption of electromagnetic radiation in the radio frequency range by certain nuclei in the molecules (spin nuclear transitions) in the presence of a strong magnetic field

# Nuclear Spin



Protons  
Neutrons



Each unpaired particule  
possesses a Spin  $1/2$



Electrons

Nuclear spin is the combination of  
neutrons spin and protons spin



$^2\text{H}$  - Deuterium


NMR active nucleus



- = one unpaired electron
- = one unpaired proton
- = one unpaired neutron

Total electronic spin  $1/2$   
Total nuclear spin  $1$

 $^1\text{H}$  -Hydrogen  
(Proton)


- = one unpaired electron
- = one unpaired proton

Total electronic spin  $1/2$   
Total nuclear spin  $1/2$



# NMR active nuclei



Must have the total nuclear spin  $I \neq 0$



- unpaired neutrons or unpaired protons
- unpaired neutrons and unpaired protons



Protons = electrons =  $Z$  atomic number

Neutrons =  $A$  mass number -  $Z$  atomic number

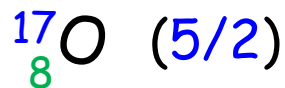
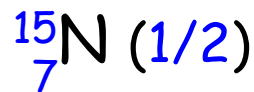
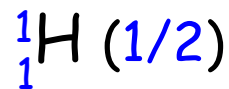
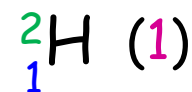
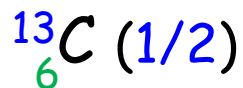
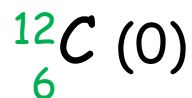


Nuclei	$Z$	Protons = $Z$	$A = P + N$	Neutrons = $A - Z$	$I$ spin
${}^1_1\text{H}$	1	1 odd	1	$1 - 1 = 0$ even	$\frac{1}{2}$
${}^2_1\text{H}$	1	1 odd	2	$2 - 1 = 1$ odd	1
${}^3_1\text{H}$	1	1 odd	3	$3 - 1 = 2$ even	$\frac{1}{2}$
${}^{12}_6\text{C}$	6	6 even	12	$12 - 6 = 6$ even	0 Inactive
${}^{13}_6\text{C}$	6	6 even	13	$13 - 6 = 7$ odd	$\frac{1}{2}$

## NMR rules



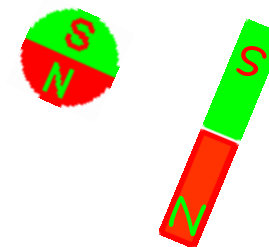
$\begin{matrix} A \\ Z \end{matrix} N$	$\begin{matrix} \text{even} \\ \text{even} \end{matrix} N$	$\begin{matrix} \text{odd} \\ \text{odd} \end{matrix} N$ $\begin{matrix} \text{odd} \\ \text{even} \end{matrix} N$	$\begin{matrix} \text{even} \\ \text{odd} \end{matrix} N$
Protons Neutrons	$\begin{matrix} \text{even} \\ \text{even} \end{matrix}$	$\begin{matrix} \text{odd/even} \\ \text{even/odd} \end{matrix}$	$\begin{matrix} \text{odd} \\ \text{odd} \end{matrix}$
I Spin	0 Inactive	$1/2, 3/2, 5/2\dots$ half-integer spin	1, 3, 5 integer spin



Nuclei  $I > 0$  have a magnetic dipole  $\mu$



spinning charge  
generates a magnetic field



Each nucleus behaves  
like a bar magnet

$$\mu = \gamma I$$

$\gamma$  gyromagnetic ratio  
(different for each nucleus since is depend of mass and charge)

Isotope	Spin (I)	$\gamma$ ( $10^7 \text{rad T}^{-1} \text{sec}^{-1}$ )
---------	----------	---

$^1\text{H}$	$1/2$	26,753
--------------	-------	--------

$^2\text{H}$	1	4,107
--------------	---	-------

$^{13}\text{C}$	$1/2$	6,728
-----------------	-------	-------

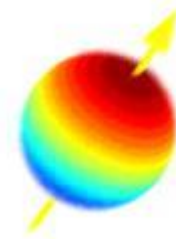
$^{17}\text{O}$	$5/2$	-3,628
-----------------	-------	--------

$^{19}\text{F}$	$1/2$	25,179
-----------------	-------	--------

$^{29}\text{Si}$	$1/2$	-5,319
------------------	-------	--------

$^{31}\text{P}$	$1/2$	10,840
-----------------	-------	--------

$$\mu = \gamma I$$



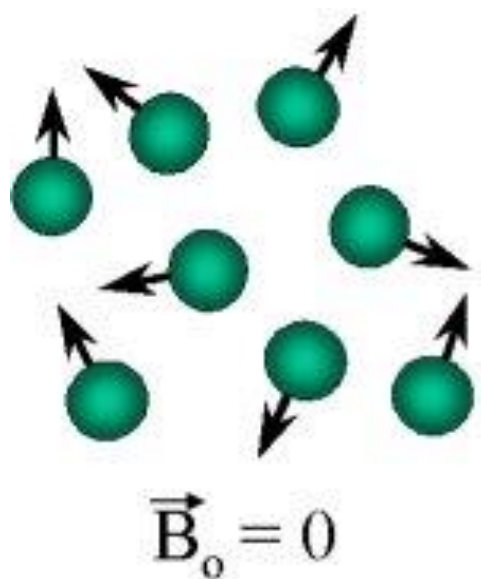
$\gamma$  gyromagnetic ratio

(different for each nucleus  
since is depend of mass and charge)

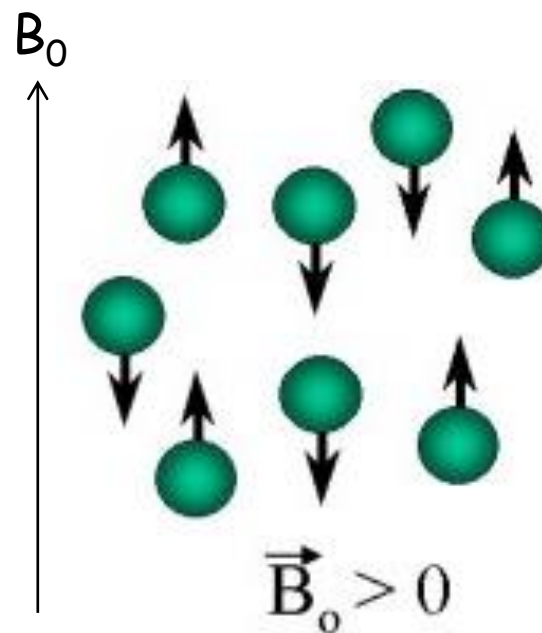
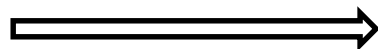
$^1\text{H}$  Proton and  $^{13}\text{C}$  carbon thirteen both with nuclear spin of  $\frac{1}{2}$

Most important nuclei in the study of organic molecules

$I = \frac{1}{2}$   $^1\text{H}$  Proton and  $^{13}\text{C}$  carbon thirteen

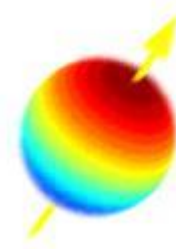
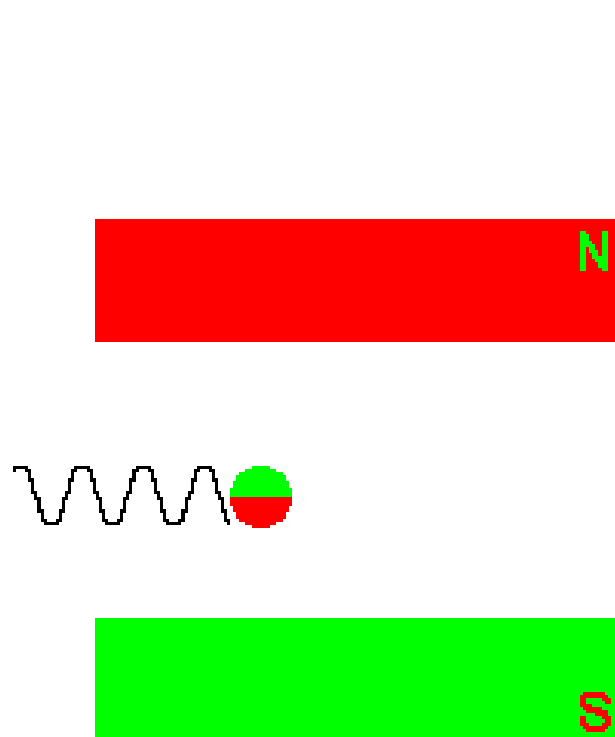
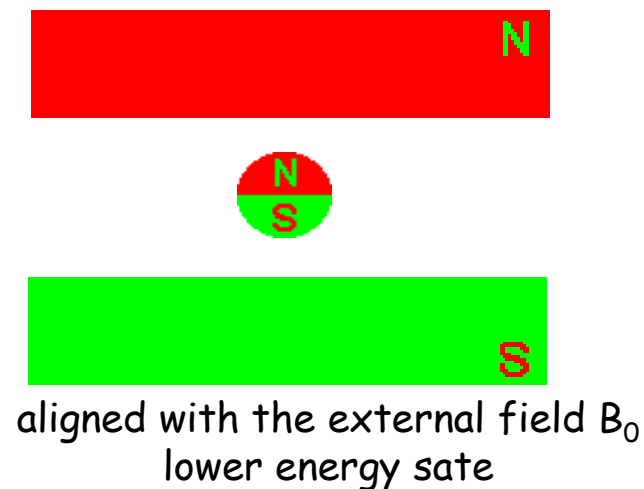
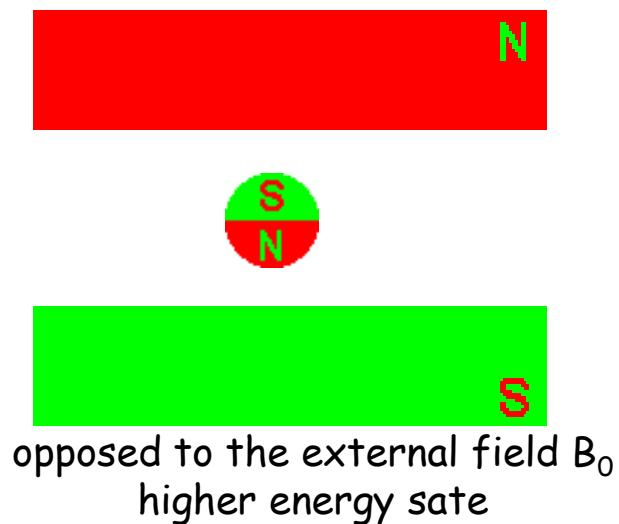


Randomly oriented

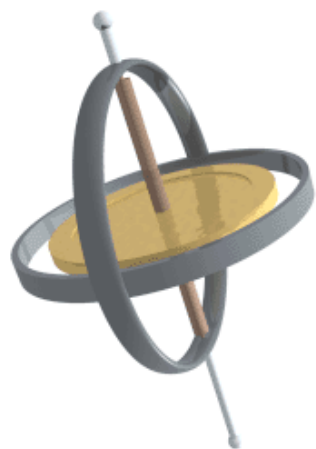


Highly oriented

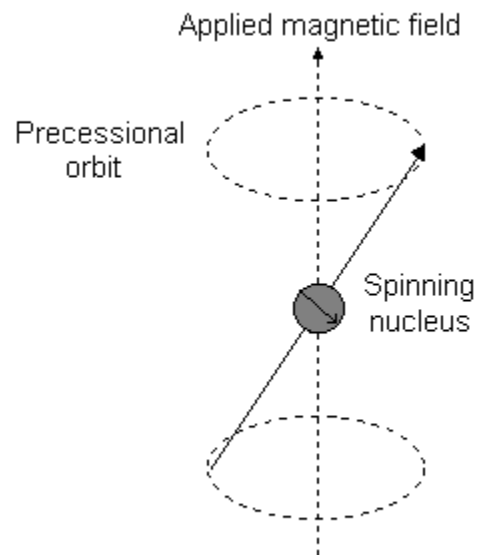
Number spin states =  $2I + 1$ ,  $I = 1/2$ , two spin states



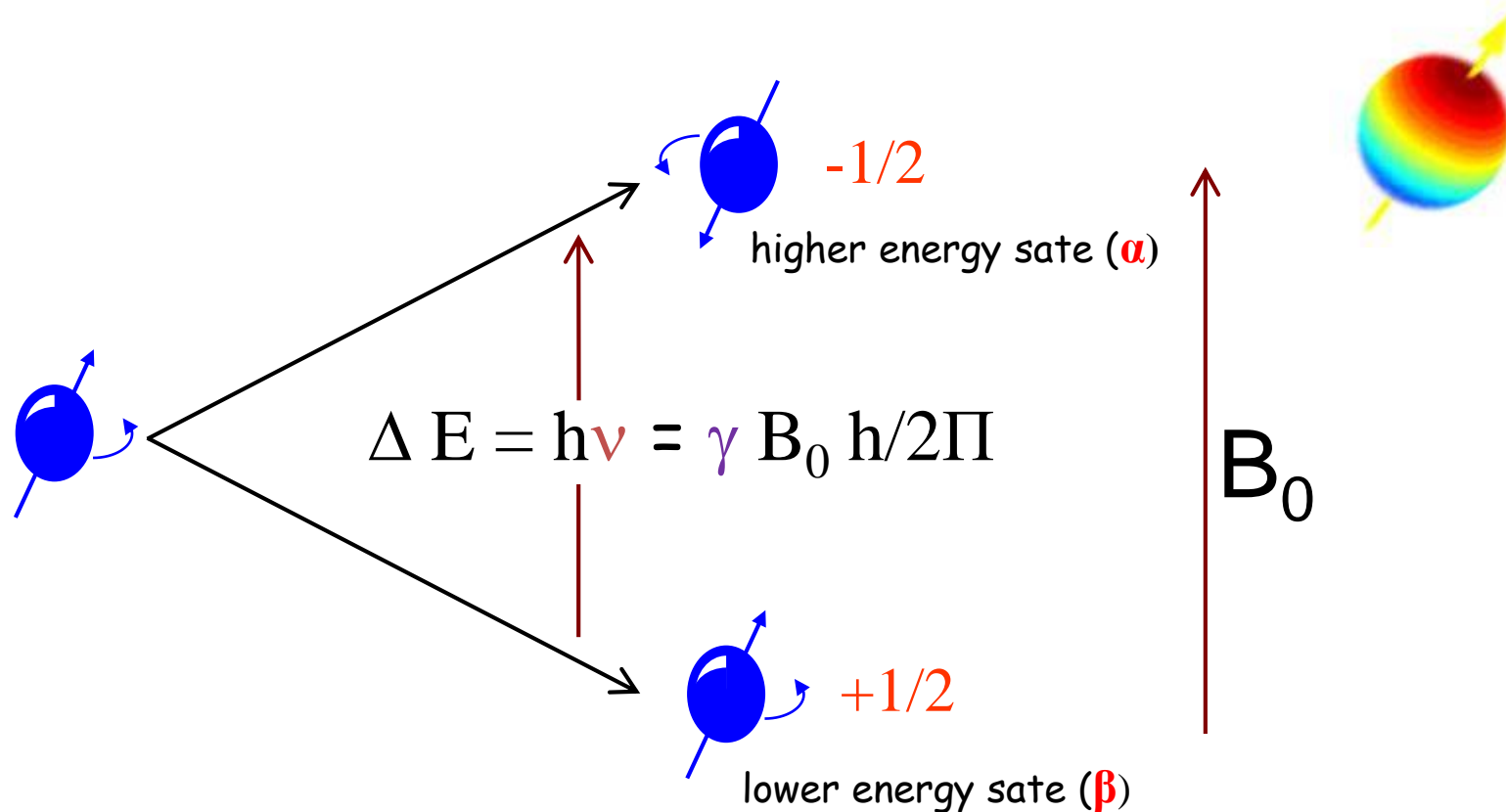




Gyroscope

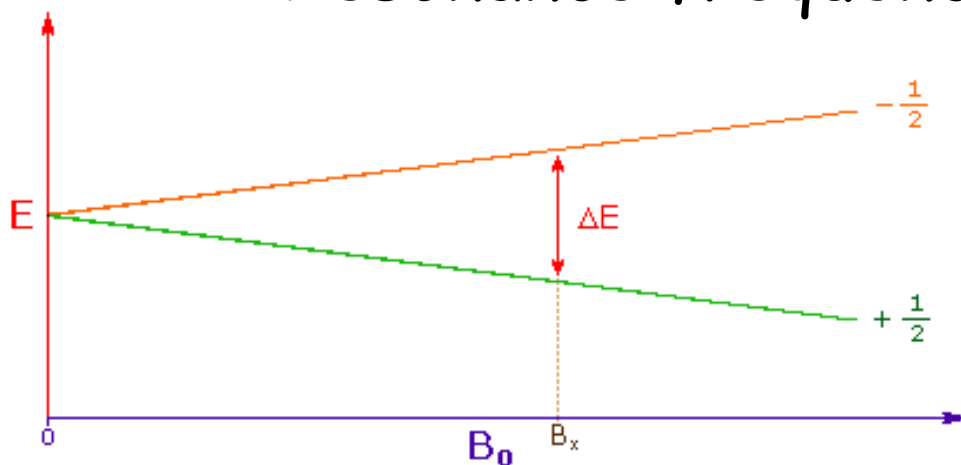
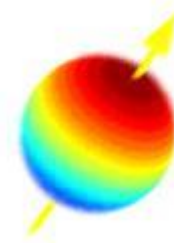


When radiation energy is absorbed by a spin  $1/2$  nucleus in a magnetic field, the angle of precession "flips" so that the magnetic moment of the nucleus opposes the applied field



For nuclei with  $I=1/2$  in the presence of an external magnetic field ( $B_0$ ), two spin states exist,  $+1/2$  and  $-1/2$

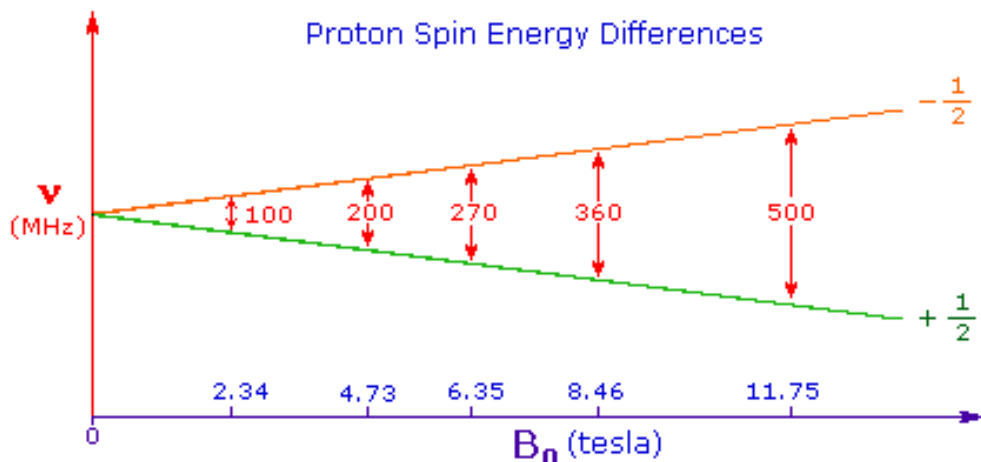
# Resonance frequency



$$\Delta E = h\nu = \gamma B_0 \frac{h}{2\pi}$$

Energy gap is proportional to the applied magnetic field ( $B_0$ )

$$h\nu = \gamma B_0 \frac{h}{2\pi}$$



$$\nu = \gamma B_0 / 2\pi$$

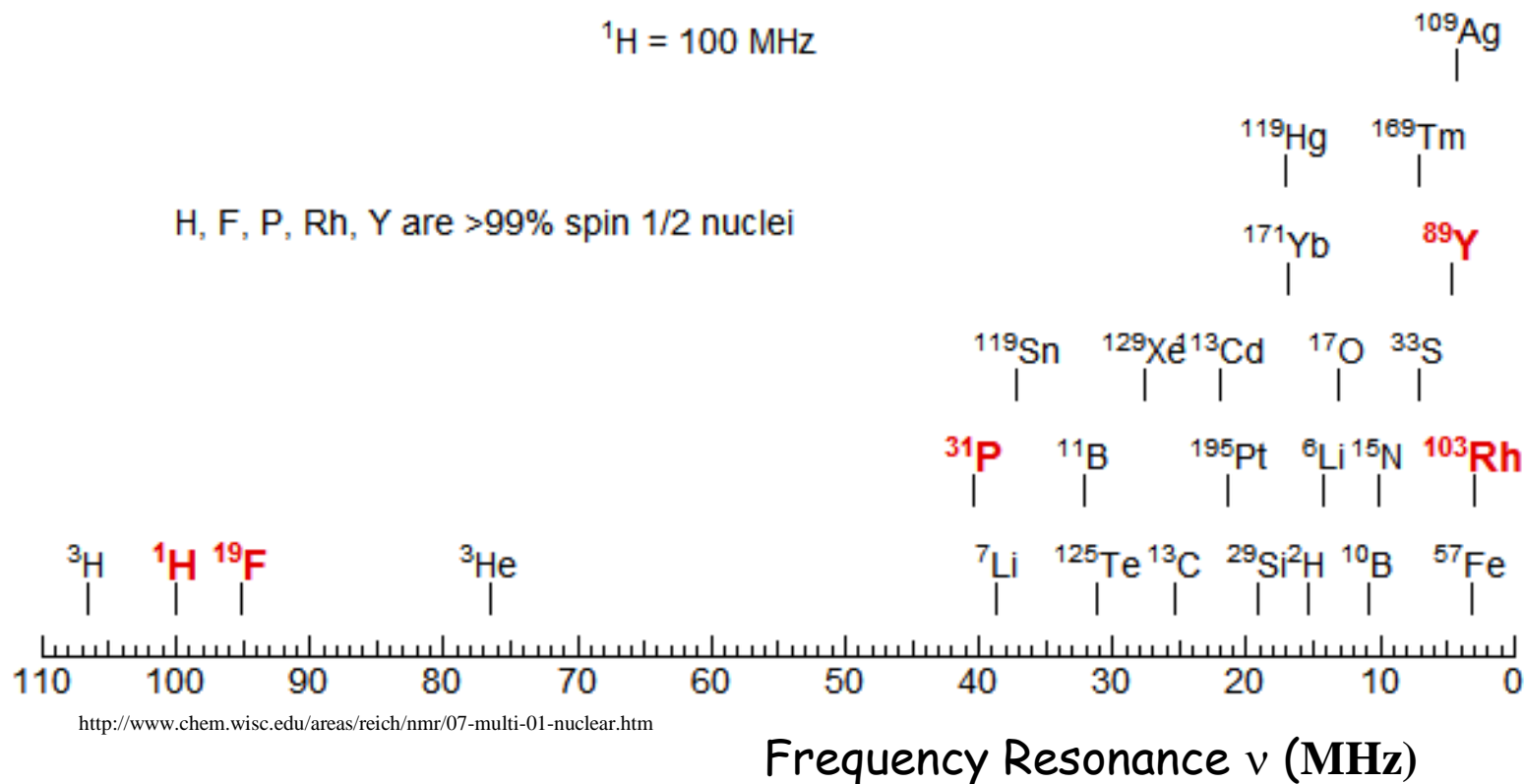
$\nu$  - Larmor frequency  
known as **resonance frequency**

Frequency Resonance  $\nu$  of  $^1\text{H}$  and  $^{13}\text{C}$  in  $\neq$  Magnetic fields

$B_0$ Tesla	$^1\text{H}$ $\nu$ (MHz)	$^{13}\text{C}$ $\nu$ (MHz)
2.1139	90	22.629
2.3488	100	25.144
4.6975	200	50.288
9.3950	400	100.577
11.744	500	125.720
21.128	900	226.296

# Resonance frequency in $B_0=3.4\text{T}$

$$\nu = \gamma B_0 / 2\pi \quad 1 \text{ Tesla} = 10^4 \text{ Gauss}$$



In an strong magnetic field of 9.4T  
(higher  $10^5$  times than the earth field)



Isotope	Spin (I)	$\gamma$ ( $10^7 \text{rad T}^{-1} \text{sec}^{-1}$ )	$\nu$ (MHz)	Natural abundance
$^1\text{H}$	1/2	26.753	400.0	99.985
$^2\text{H}$	1	4,107	61.4	0.015
$^{13}\text{C}$	1/2	6,728	100.6	1.108
$^{17}\text{O}$	5/2	-3,628	54.3	0.037
$^{19}\text{F}$	1/2	25,179	376.5	100.0
$^{29}\text{Si}$	1/2	-5,319	79.6	4.7
$^{31}\text{P}$	1/2	10,840	162.1	100.0

NMR sensitivity is dependended of  $\gamma^3$  and of Natural abundance

## NMR Sensitivity - Boltzmann Excess



The two spin states are not equally populated:  
There is a **small excess** population in the lower energy ( $\beta$ ) spin state.

$$\frac{N_{upper(\alpha)}}{N_{lower(\beta)}} = e^{-\Delta E / kT}$$

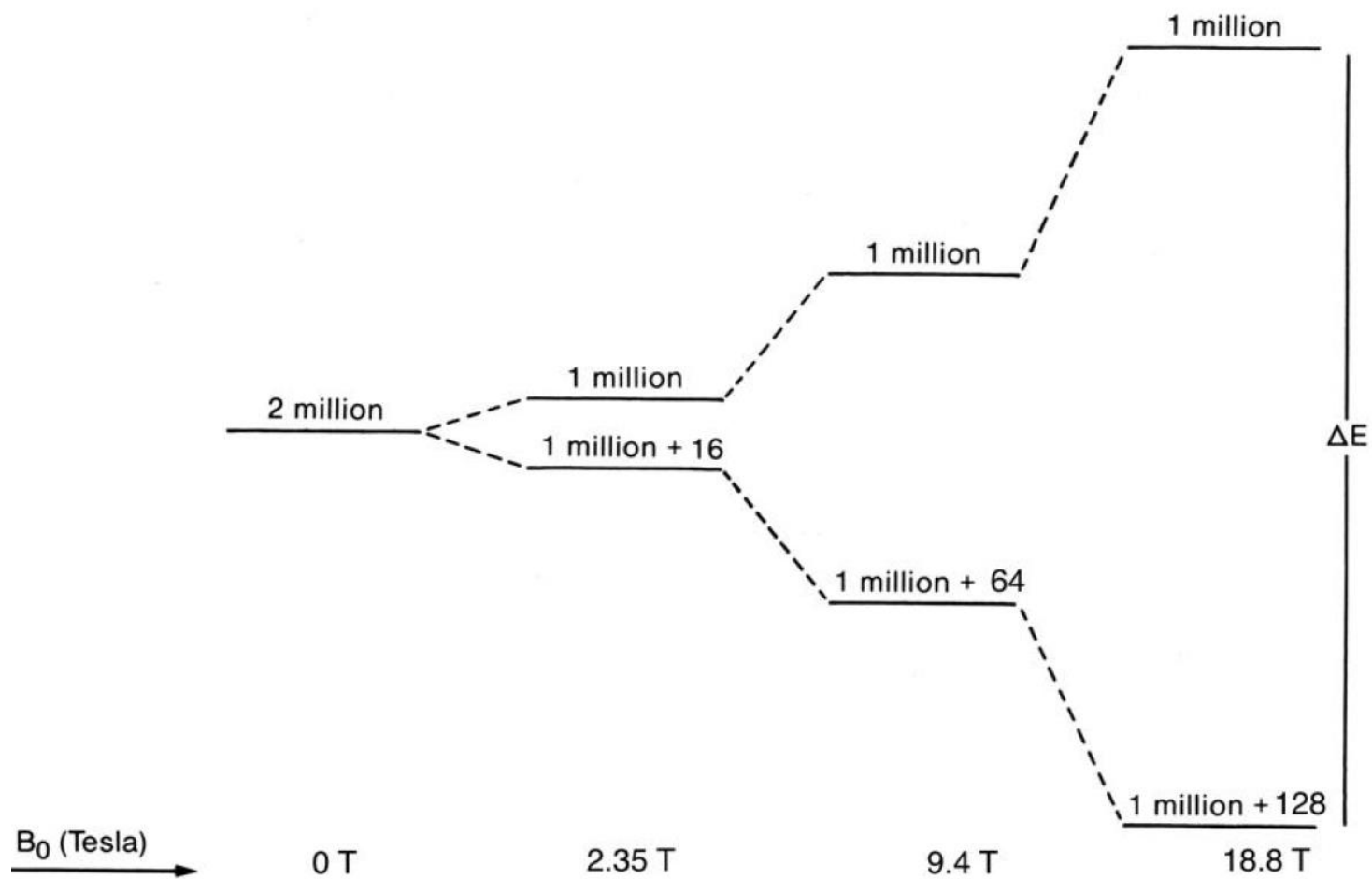
$$\Delta E = h\nu = \gamma B_0 h/2\pi$$

Resonance frequency=60 MHz ( $B_0 = 1.4$  T) @298k  $\Rightarrow$  energy gap  $\Delta E = 2.39 \times 10^{-5}$  kcal/mol

$N_\alpha / N_\beta$  (**60 MHz**) = 1000000/1000009 = 0,999991      **+9 nuclei** in lower (favored) spin state ( $\beta$ )

Resonance frequency=400 MHz ( $B_0 = 9.4$  T) @298k  $\Rightarrow$  energy gap ( $\Delta E$ ) of  $3.8 \times 10^{-5}$  kcal/mol

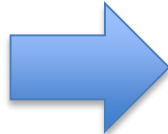
$N_\alpha / N_\beta$  (**400 MHz**) = 1000000/1000064 = 0,999936      **+64 nuclei** in lower (favored) spin state ( $\beta$ )





## NMR Sensitivity



- Signal intensity (absorption) is dependent of Boltzmann Excess:  
Typically the excess is only in  $10^6$  order (ppm) - **NMR lower sensitivity**
  
  - Highest magnetic fields ( $B_0$ )
  - Highest gyromagnetic ratio ( $\gamma$ )
  - Highest natural abundance
-  **more intense signals**

## NMR sensitivity

Sensitivity is dependended of  $\gamma^3$  and of natural abundance



### Gyromagnetic ratio

$$\gamma \text{ } ^1\text{H} (26.753) / \gamma \text{ } ^{13}\text{C} (6.73) \approx 4 \quad 4^3 = 64$$

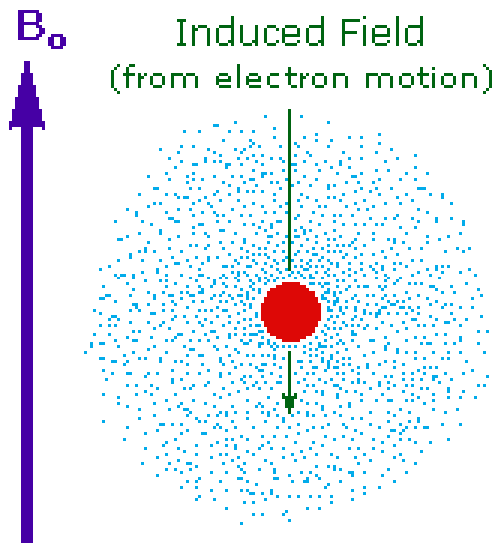
### Natural abundance

$$^1\text{H} (99.985) / ^{13}\text{C} (1.108) \approx 100$$

$$\text{Total sensitivity} = 64 \times 100$$

**$^1\text{H}$  Proton** is 6400 times more sensitive than  **$^{13}\text{C}$  carbon thirteen**

## Shielding and effective Magnetic field $B_{\text{eff}}$



$$B_{\text{eff}} = B_0 (1 - \sigma) \quad \sigma - \text{shielding}$$

$$\nu = \gamma B_{\text{eff}} / 2\pi$$

$$\nu = \gamma B_0 (1 - \sigma) / 2\pi$$

Since electrons are charged particles, they move in response to the external magnetic field ( $B_0$ ) so as to generate a secondary field that opposes the much stronger applied field. This secondary field **shields** the nucleus from the applied field, so  $B_0$  must be increased in order to achieve resonance (absorption of radiofrequency energy).

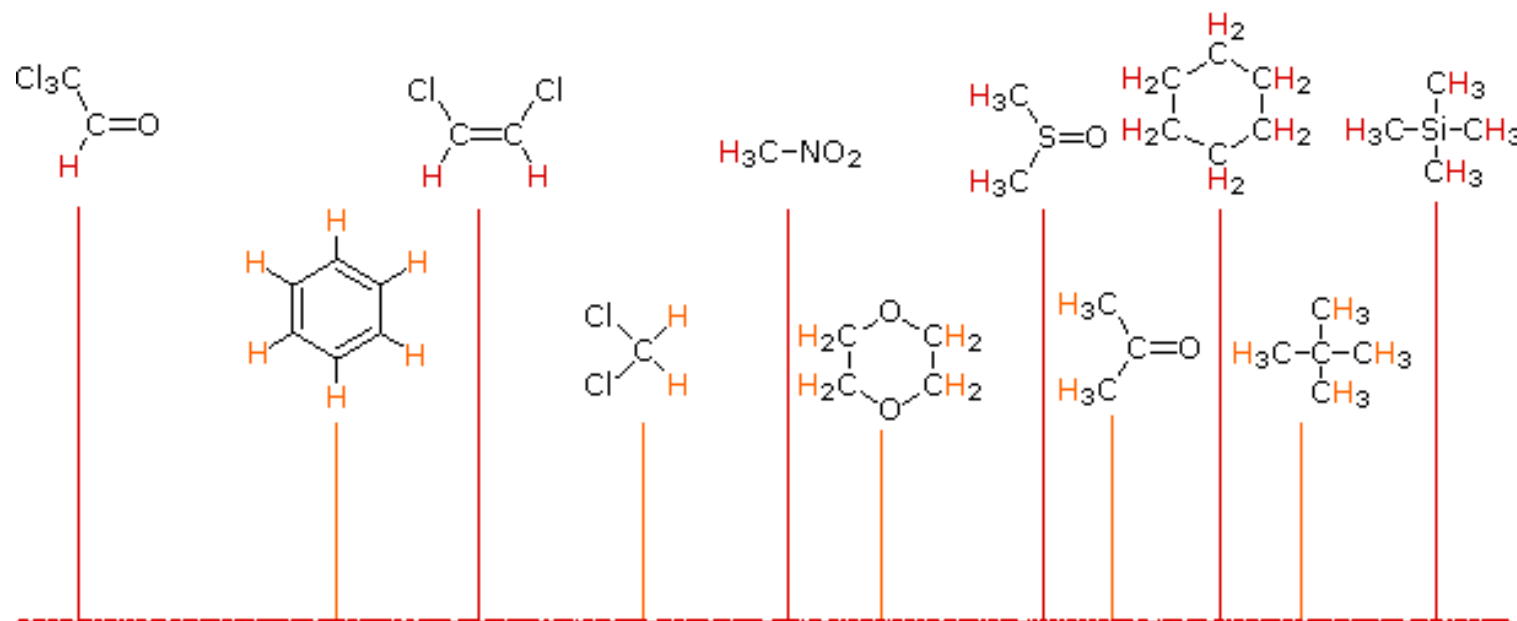
Higher shielding  $\sigma \Rightarrow B_{\text{eff}}$  lower

$$\nu = \gamma B_0 (1 - \sigma) / 2\pi$$

is necessary to increase  $B_0$  for the proton resonate at  $\nu$



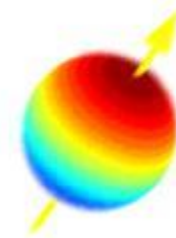
- Increasing Magnetic Field at Fixed Frequency →
- ← Increasing Frequency at Fixed Magnetic Field —
- Increased Shielding by Extranuclear electrons →



Different surrounding, different shielding, different resonance frequency

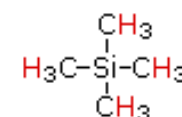
# NMR scale

$$\text{Chemical shift } \delta \text{ ppm} = \frac{\nu \text{ (Hz)} - \nu_{\text{ref}} \text{ (Hz)}}{\nu_{\text{spectrometer}} \text{ (MHz)}} = \frac{\nu \text{ (Hz)} - \nu_{\text{TMS}} \text{ (Hz)}}{\nu_{\text{spectrometer}} \text{ (MHz)}}$$

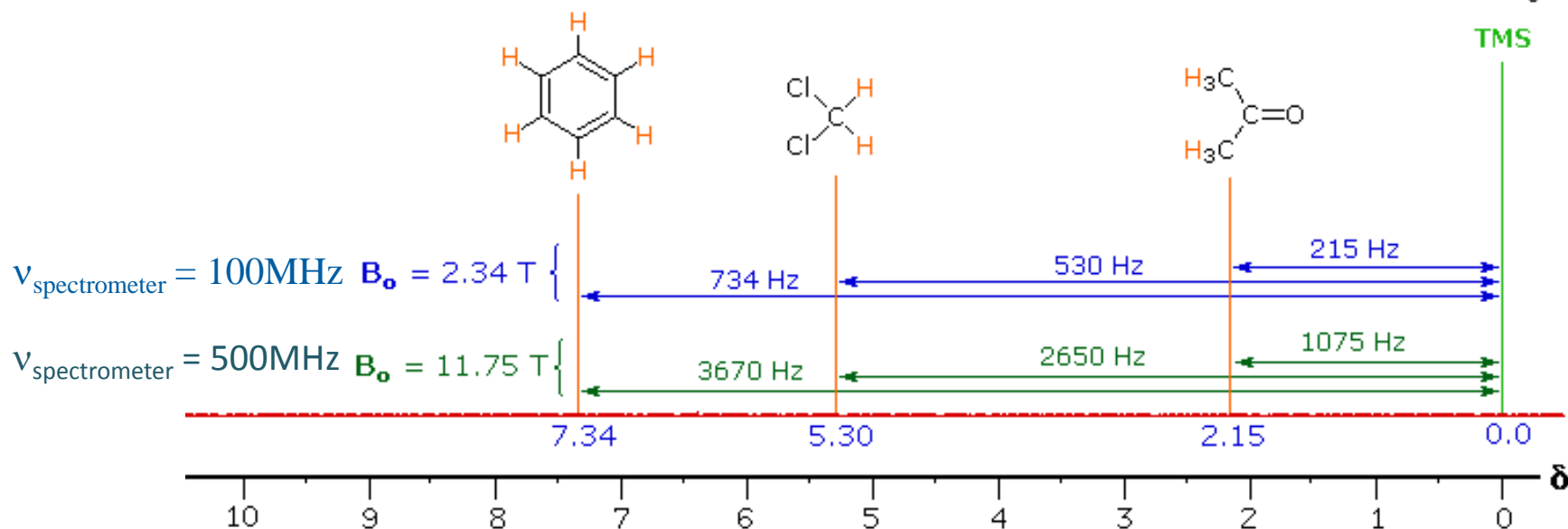


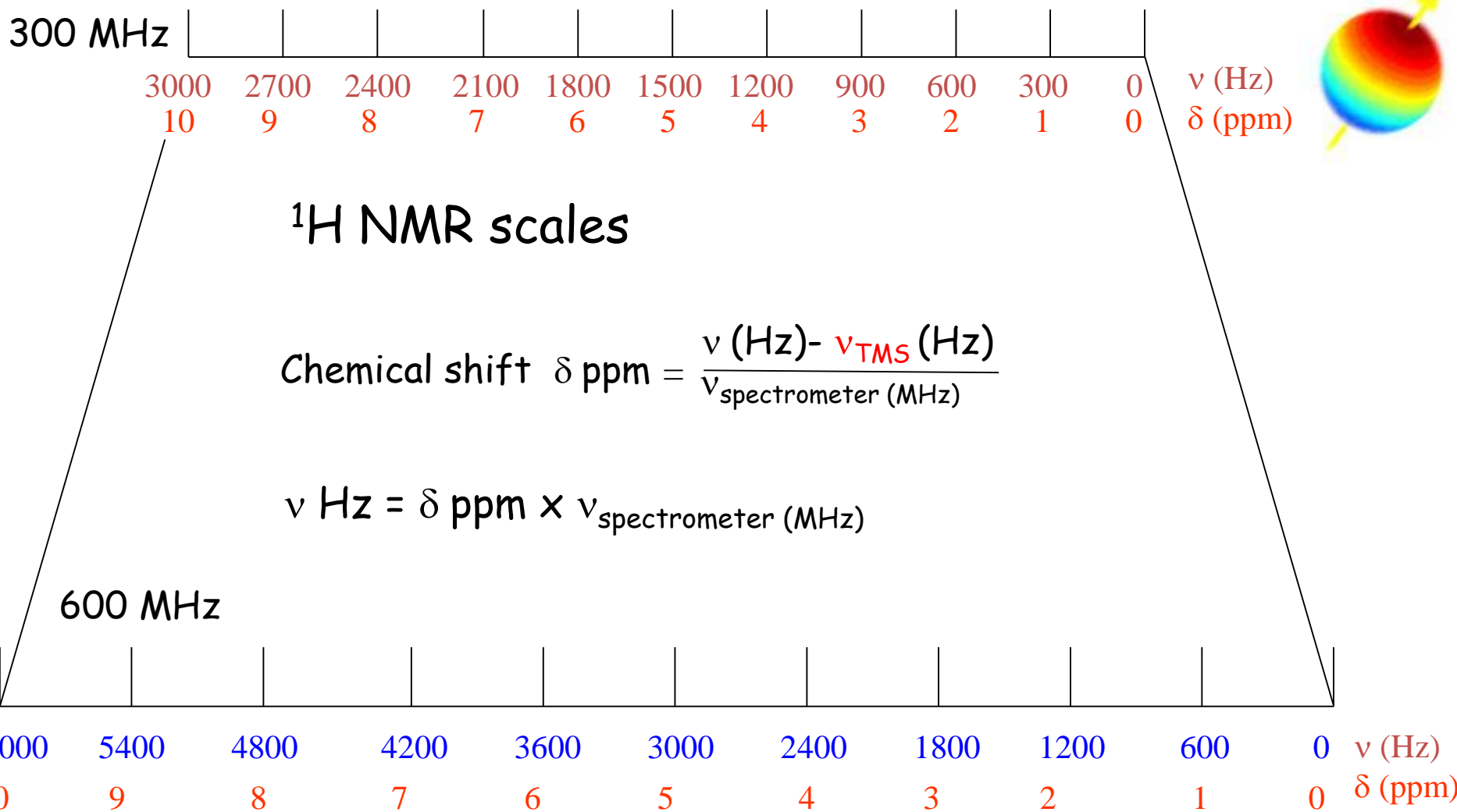
**shielding**

- Measured of the difference (shift) in Hz to a internal standard
- Most used reference tetramethylsilane - **TMS** (higher shielding, inert)
- To correct the frequency differences for the field dependence, we divide them by the spectrometer frequency

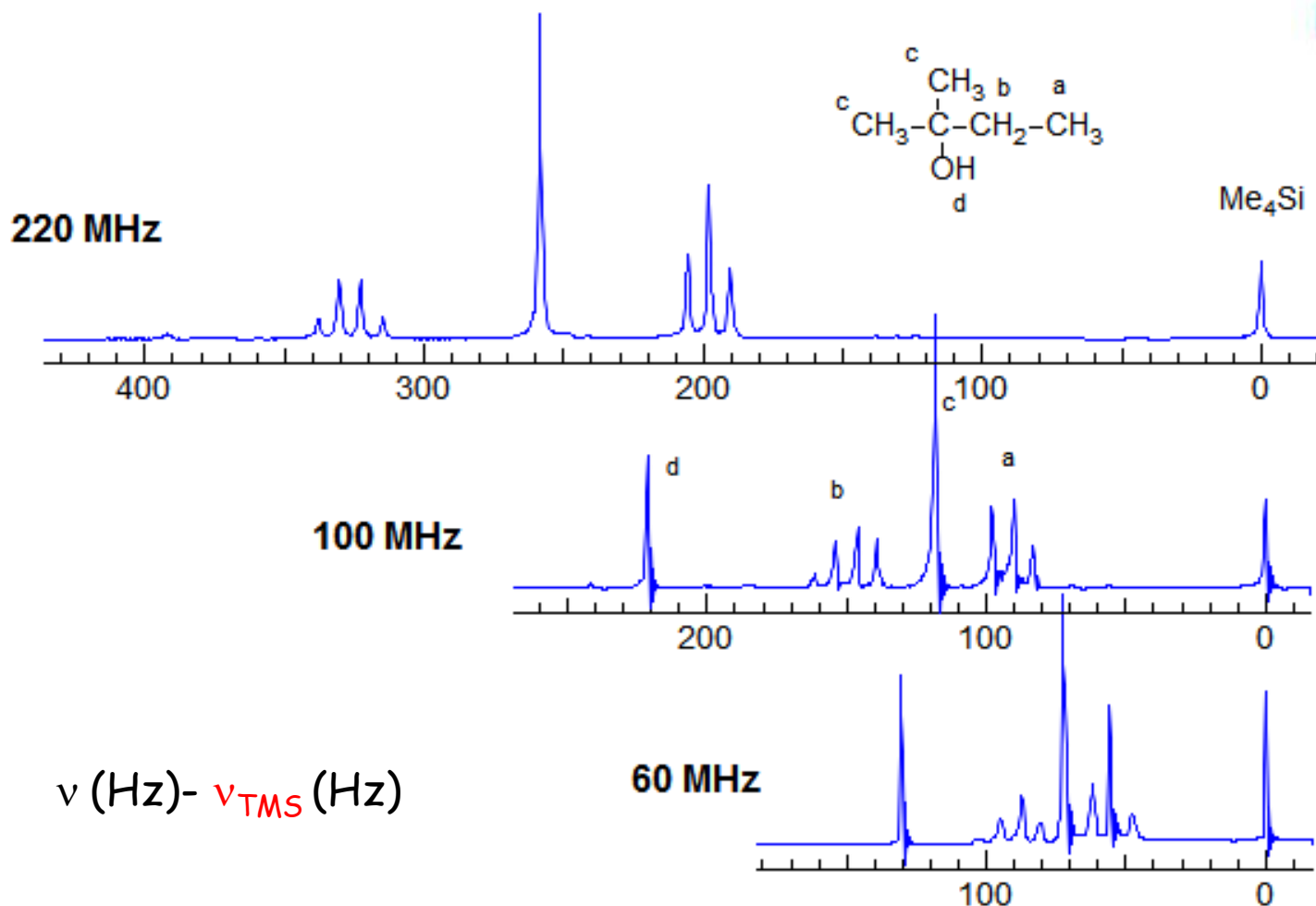


**TMS**





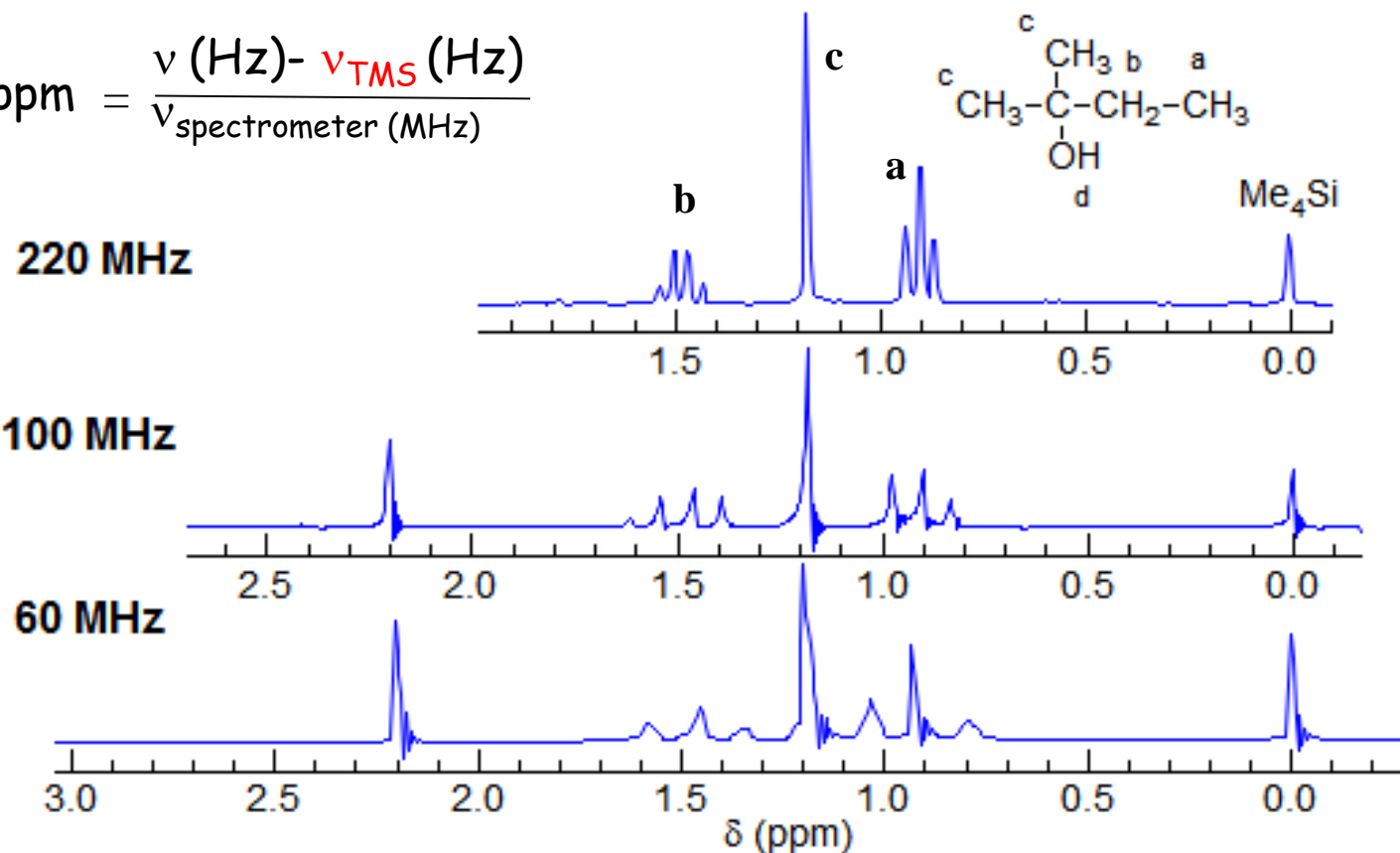
# $^1\text{H}$ NMR frequencies plotted on Hz scale



# $^1\text{H}$ NMR frequencies plotted on ppm scale



$$\delta \text{ ppm} = \frac{\nu \text{ (Hz)} - \nu_{\text{TMS}} \text{ (Hz)}}{\nu_{\text{spectrometer}} \text{ (MHz)}}$$





## NMR scales for different nuclei



**Chemical shift**  $\delta$  ppm =  $\frac{\nu \text{ (Hz)} - \nu_{\text{ref}} \text{ (Hz)}}{\nu_{\text{spectrometer}} \text{ (MHz)}} = \frac{\nu \text{ (Hz)} - \text{reference (Hz)}}{\nu_{\text{spectrometer}} \text{ (MHz)}}$

Núcleo ( $I = 1/2$ )	Natural abundance (%)	$\nu$ NMR (MHz)	range $\delta$ (ppm)
$^1\text{H}$	99.99	400.13	- 30 - (+ 20) *
$^{13}\text{C}$	1.11	100.61	- 100 - (+ 400) *
$^{19}\text{F}$	100.0	376.498	- 200 - (+ 200) #
$^{31}\text{P}$	100.0	161.976	- 170 - (+ 250) \$

Reference: \* TMS ( $\text{SiMe}_4$ ) #  $\text{CFCl}_3$ ; \$  $\text{H}_3\text{PO}_4$

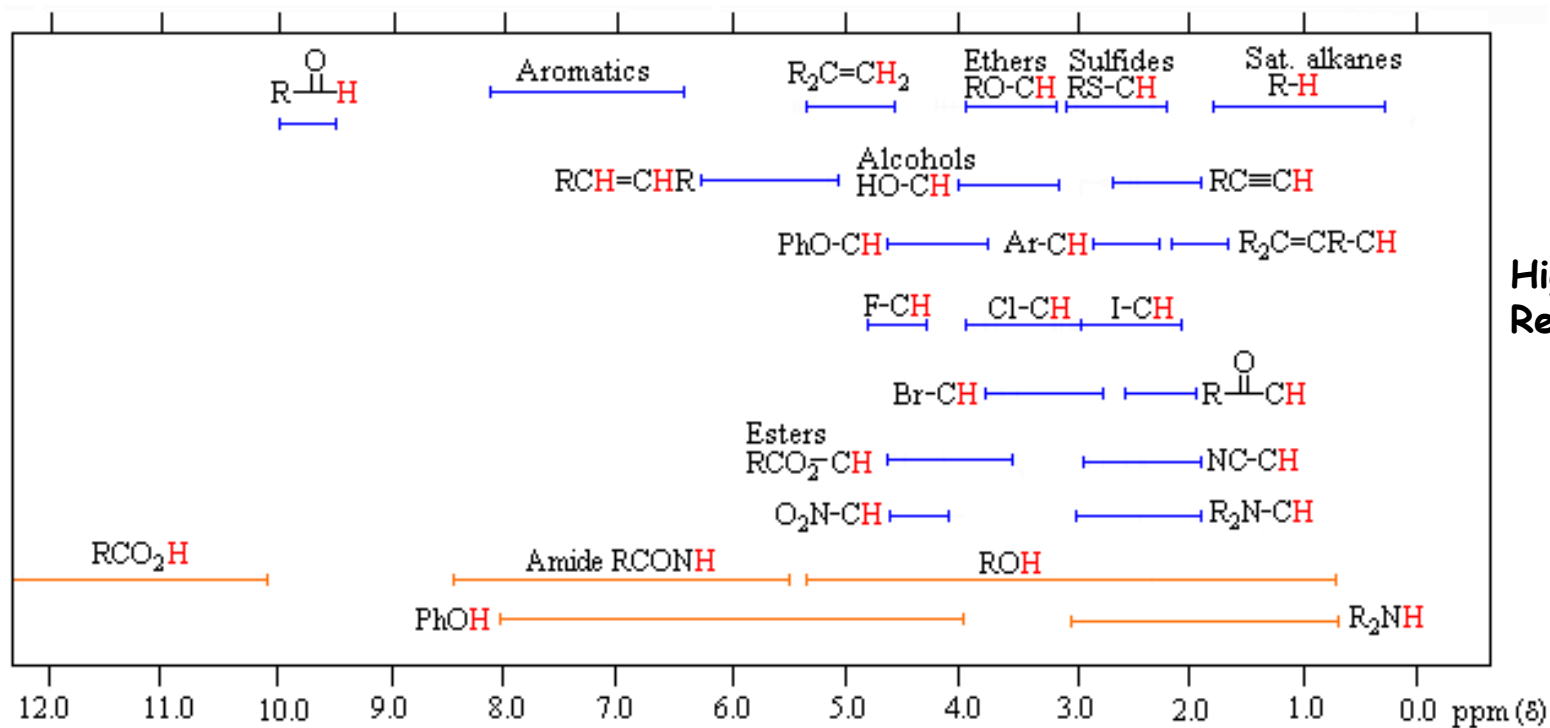
# $^1\text{H}$ NMR chemical shift depends on chemical surrounding

Structural information of molecules



## $^1\text{H}$ NMR Table - Proton chemical shift ranges

Low Field Region



High Field Region

Proton chemical shift range (20-0 ppm) reference TMS

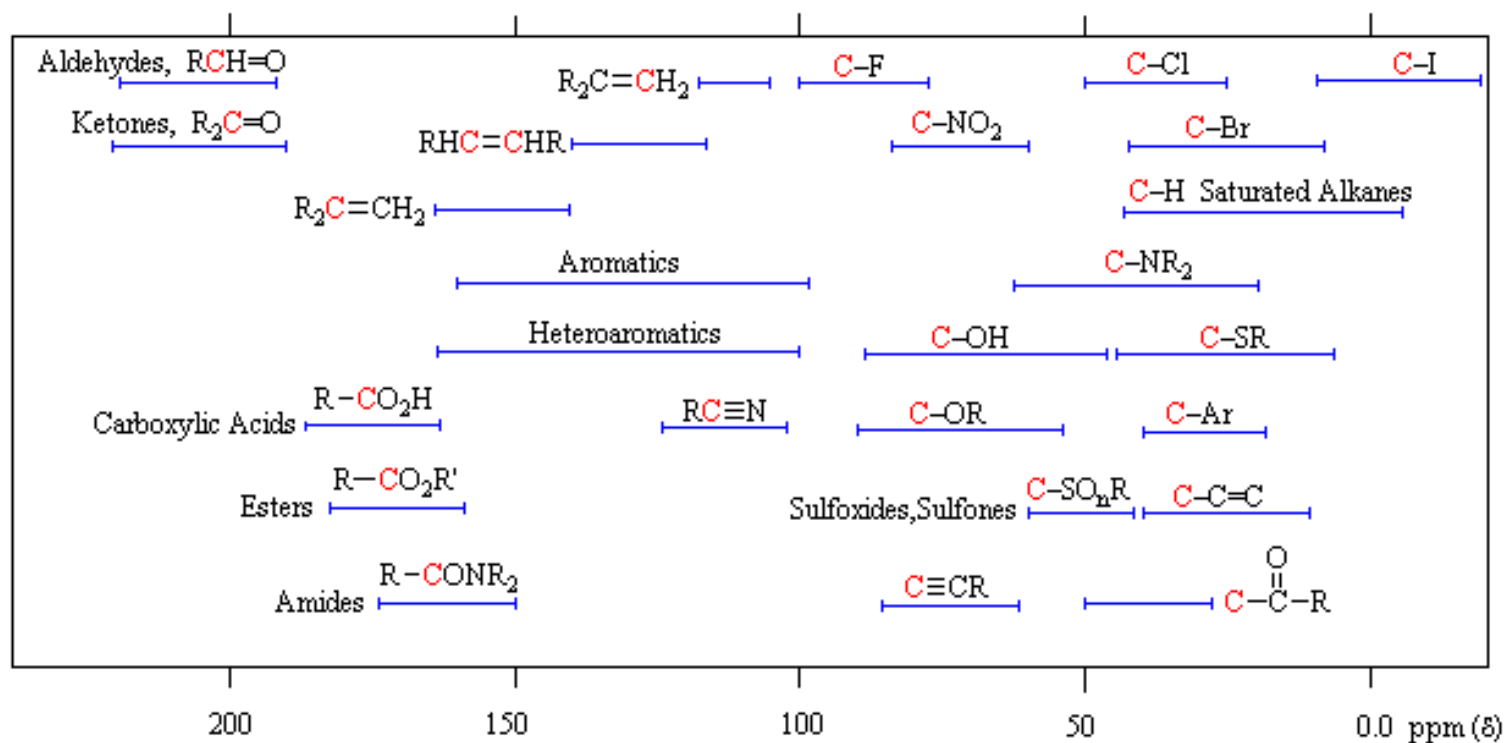
# $^{13}\text{C}$ NMR chemical shift depends on chemical surrounding

Structural information of molecules



## $^{13}\text{C}$ NMR Table - Carbon chemical shift ranges

Low Field Region



High Field Region

Carbon chemical shift range (300-0 ppm) reference TMS

Spectrometer Bruker 400  $\nu_{\text{spectrometer}} \text{}^1\text{H}$  (400.13 MHz)

$\nu_{\text{spectrometer}} \text{}^{13}\text{C}$  (100.61 MHz)



$$\nu \text{ Hz} = \delta \text{ ppm} \times \nu_{\text{spectrometer}} \text{ Nuclei (MHz)}$$

What is the resonance frequency of a **proton** with a  $\delta$  of **8 ppm**?

$$\nu \text{ Hz} = \delta \text{ ppm} \times \nu_{\text{spectrometer}} \text{}^1\text{H} \text{ (MHz)}$$

$$\nu \text{ Hz} = 8 \times 400.13 = 3201.04 \text{ Hz}$$

What is the resonance frequency of a **carbon** with a  $\delta$  of **8 ppm**?

$$\nu \text{ Hz} = \delta \text{ ppm} \times \nu_{\text{spectrometer}} \text{}^{13}\text{C} \text{ (MHz)}$$

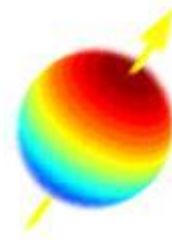
$$\nu \text{ Hz} = 8 \times 100.61 = 804.88 \text{ Hz}$$

What is the chemical shift of a **proton** with a resonance frequency of **1200 Hz** on spectrometer of **400 MHz**?

$$\nu \text{ Hz} = \delta \text{ ppm} \times \nu_{\text{spectrometer}} \text{ } ^1\text{H} \text{ (MHz)}$$

$$\delta \text{ ppm} = \nu \text{ Hz} / \nu_{\text{spectrometer}} \text{ } ^1\text{H} \text{ (MHz)}$$

$$\text{Chemical shift } \delta = 1200/400 = \mathbf{3 \text{ ppm}}$$



What is the resonance frequency of the same proton on a spectrometer of **500 MHz**?

$\delta$  ppm is the same in both spectrometers

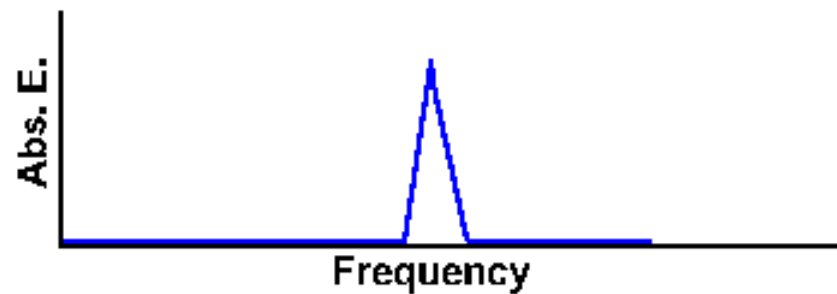
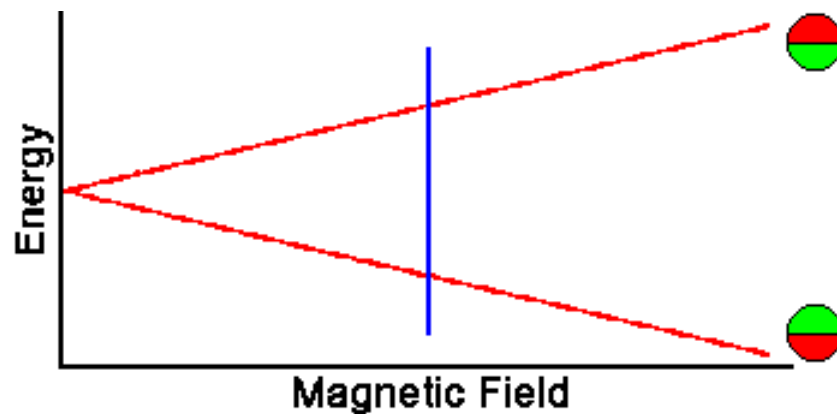
$$\nu \text{ Hz} = \mathbf{3} \times 500 = \mathbf{1500 \text{ Hz}}$$

# Continuous wave (CW)

$$\nu = \gamma B_0 (1 - \sigma) / 2\pi$$

$B_0$  constant

$\nu$  changes

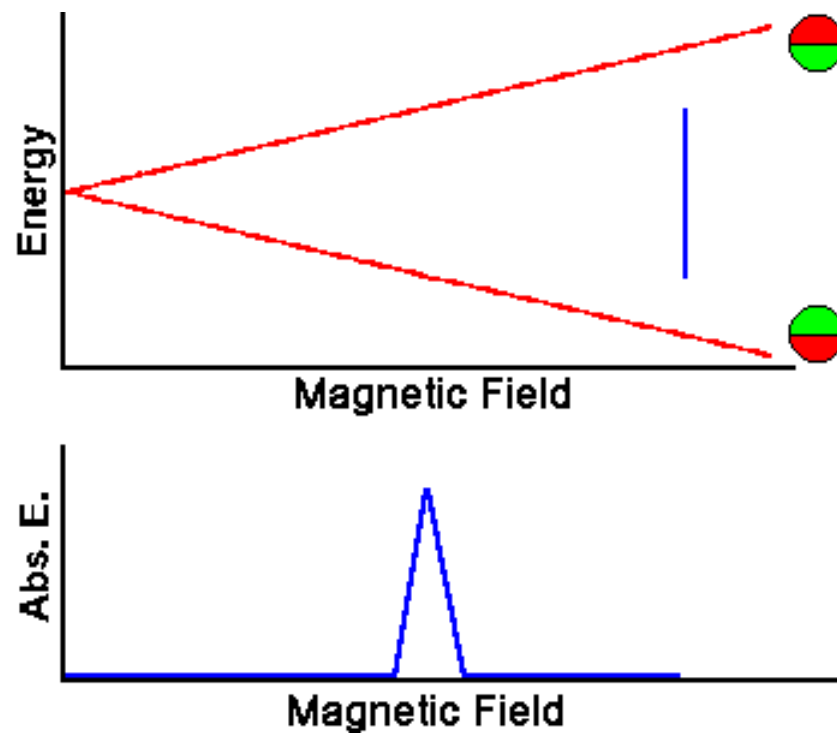


# Continuous wave (CW)

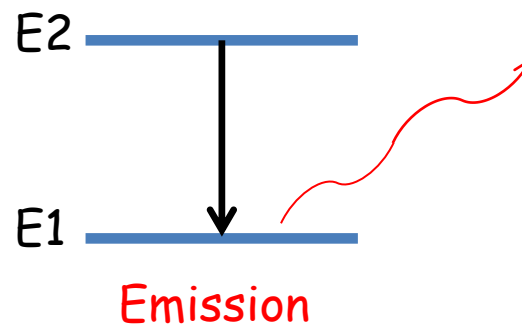
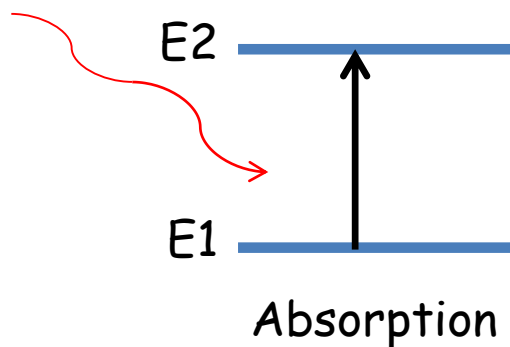
$$\nu = \gamma B_0 (1 - \sigma) / 2\pi$$

$\nu$  constant

$B_0$  changes



## Pulse NMR

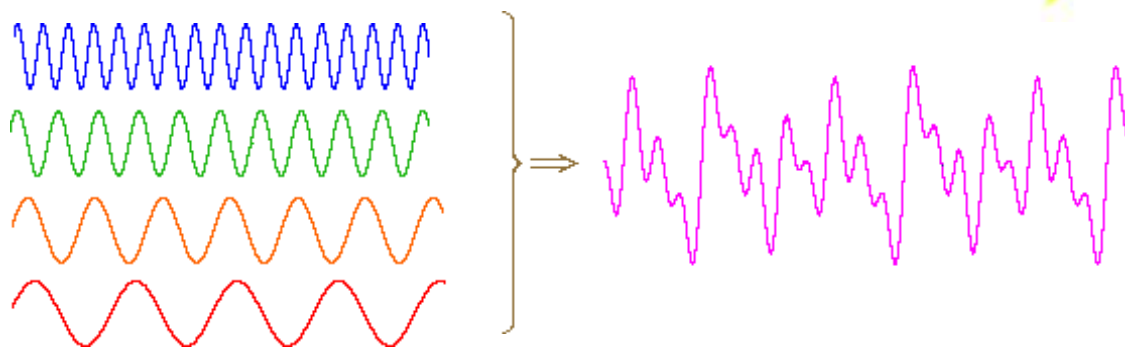
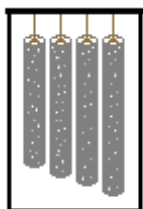


In an strong applied magnetic field a range of radiofrequencies is absorbed in order to excited all the proton nuclei in a molecule at the same time

All the pulsed resonance frequencies emitted by the molecule are detected



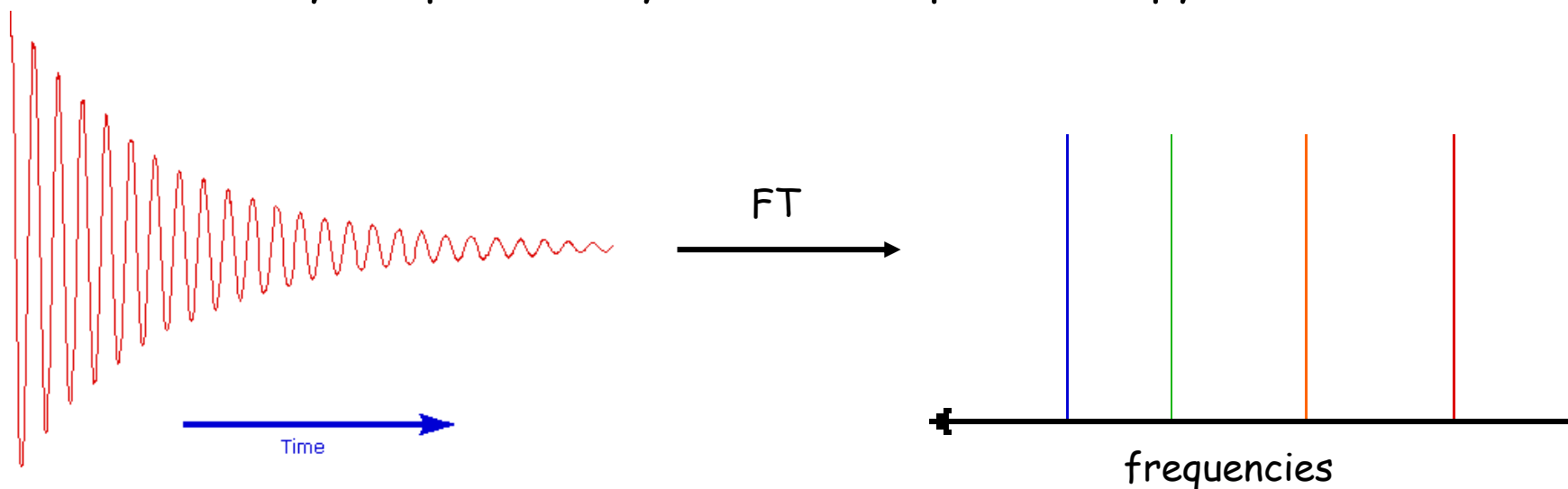
# Pulse NMR



The pulsed frequencies emitted by the molecule are added together to give a complex summation wave

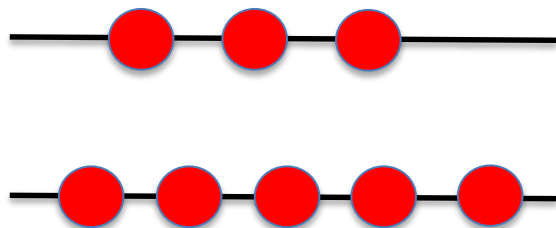
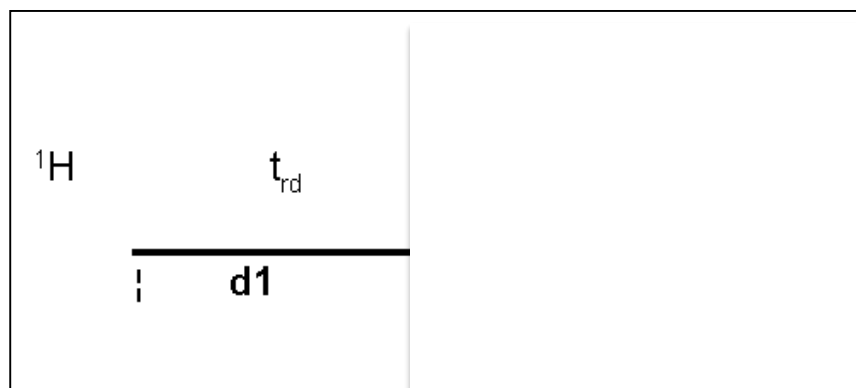
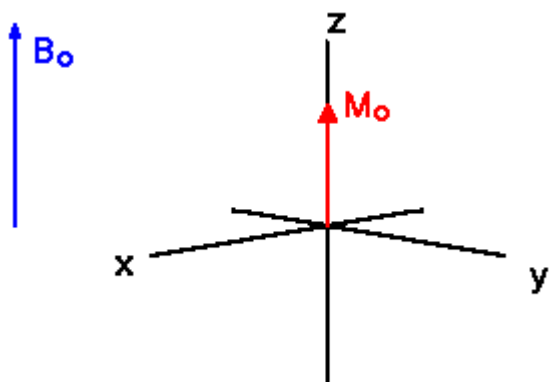
# Pulsed NMR

**Relaxation mechanism** - is a first order process, the radiofrequencies signal emitted by the sample decays exponentially- Emission spectroscopy



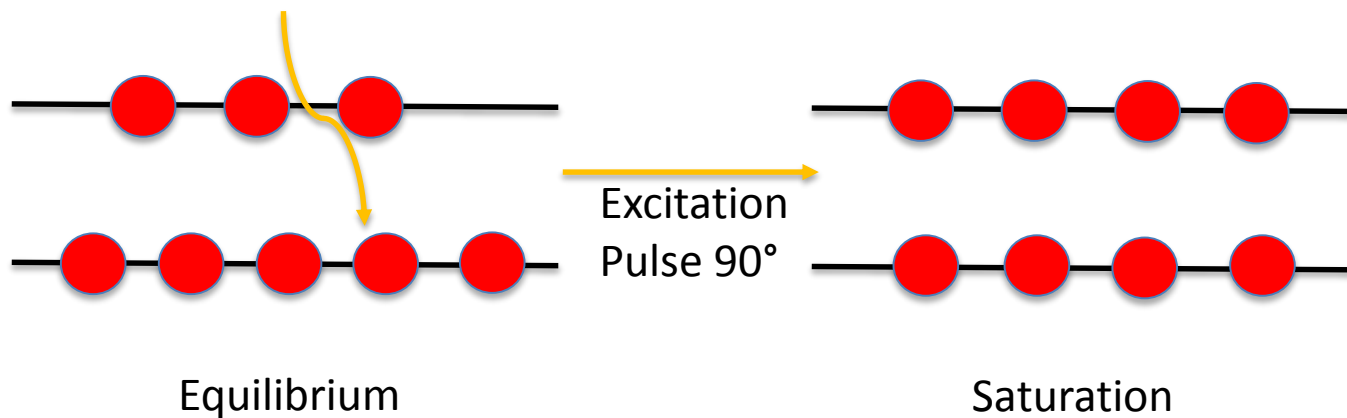
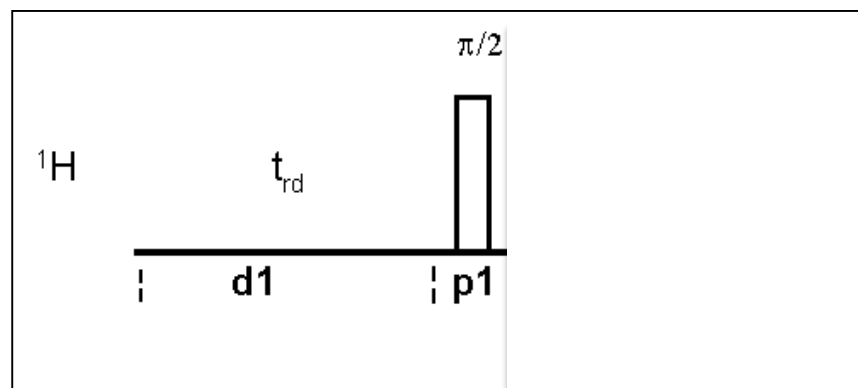
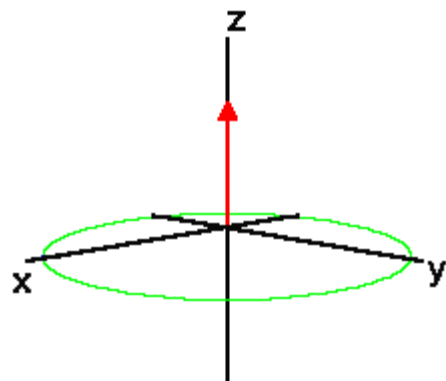
FID free induction decay signal

# Pulsed NMR

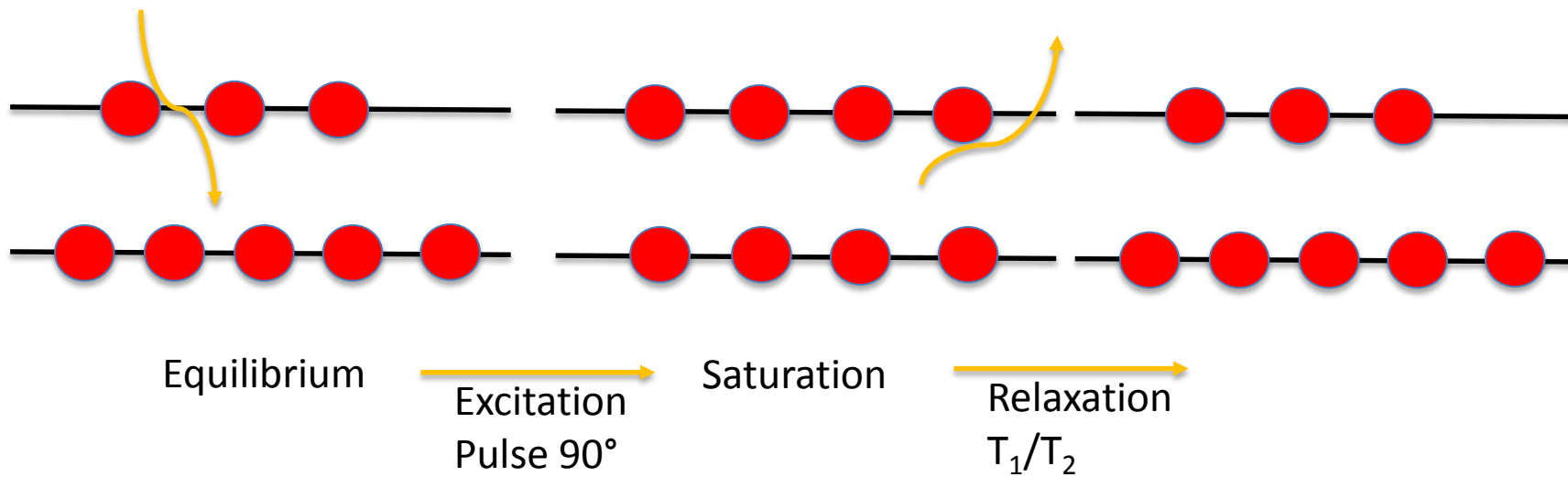
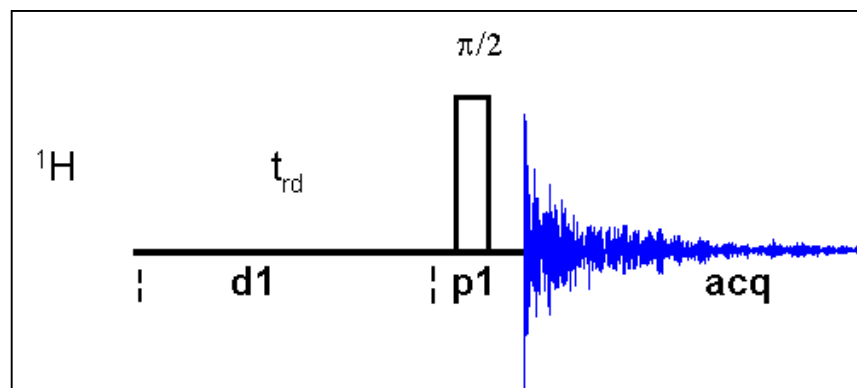
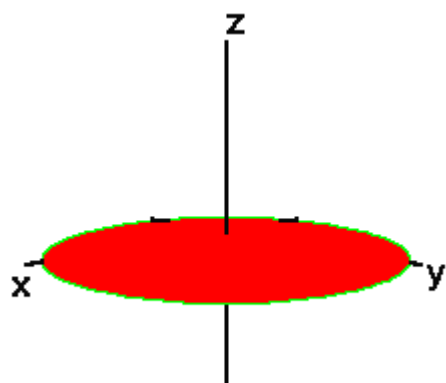


Equilibrium

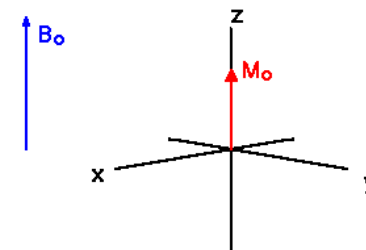
# Pulsed NMR



# Pulsed NMR

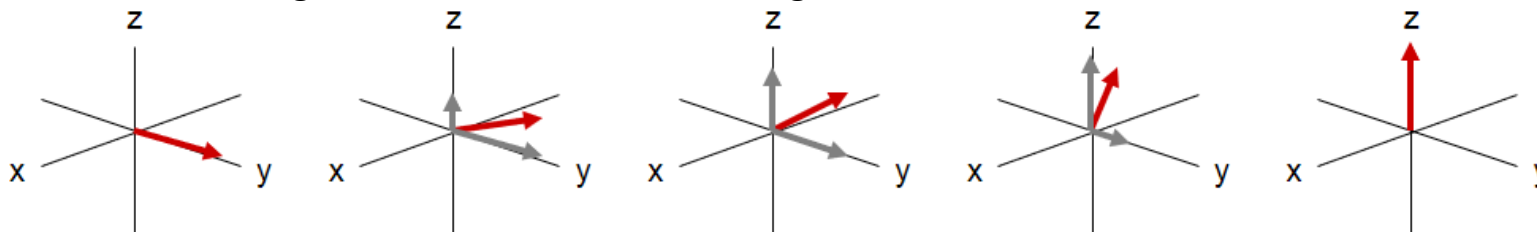


# Nuclear Relaxation



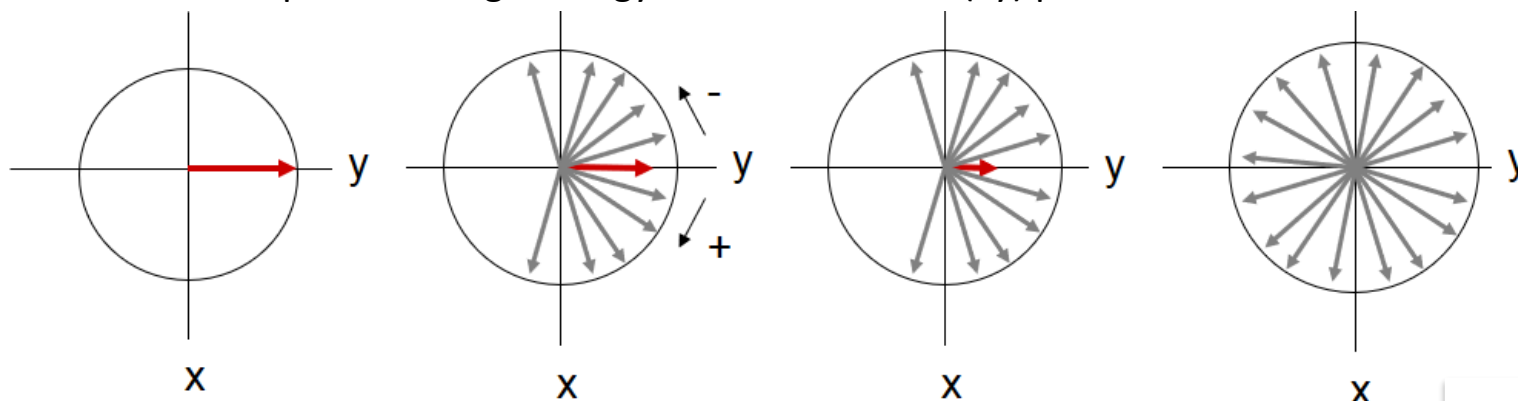
## Longitudinal Relaxation (spin-lattice) $T_1$

how fast the magnetization relaxes back along the z-axis

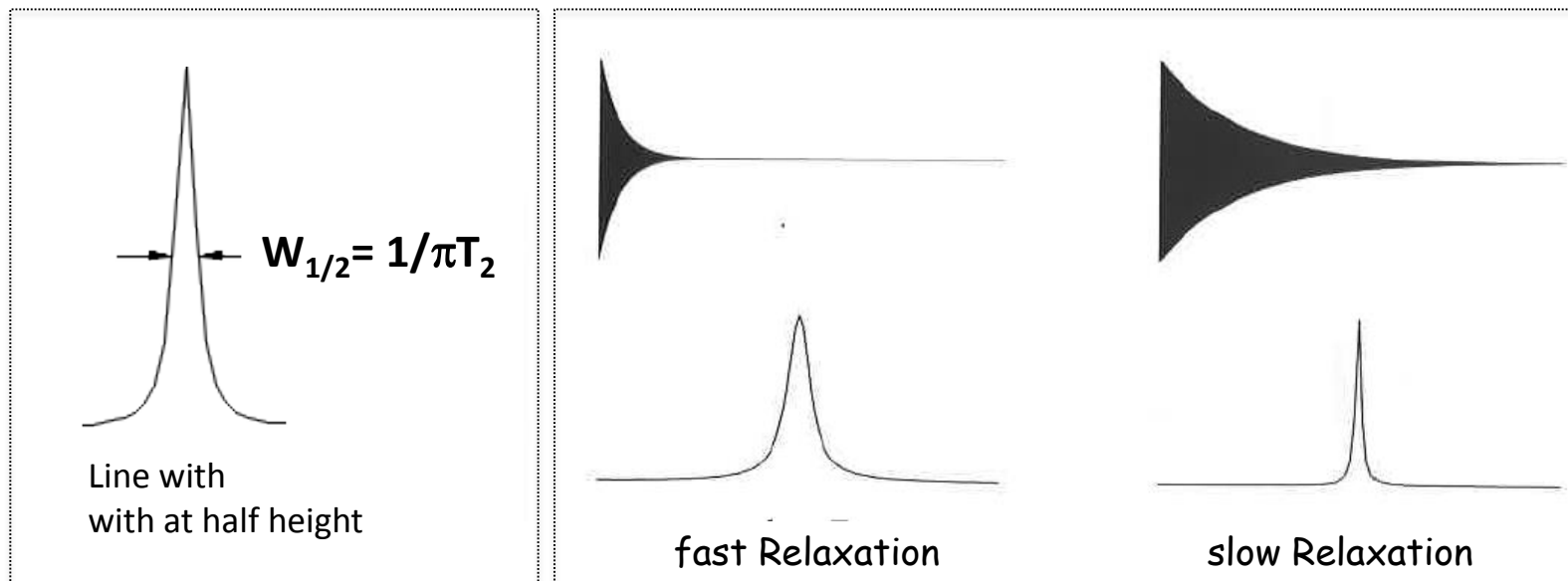


## Transversal Relaxation – $T_2$

how fast the spins exchange energy in the transverse (xy) plane



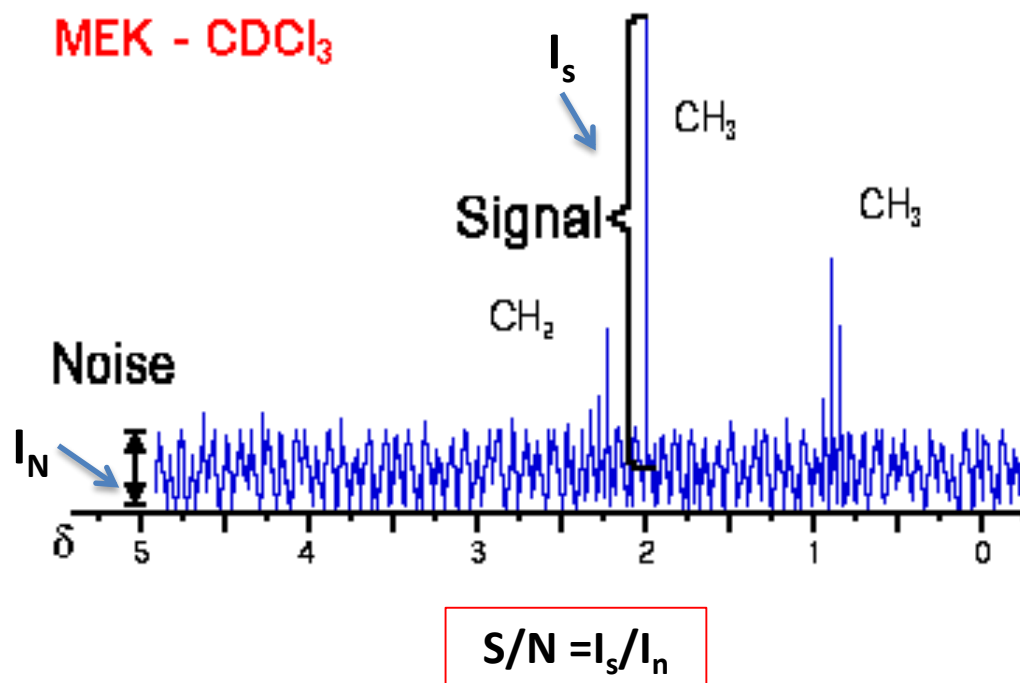
## NMR Line with



- Rapid Relaxation  $\Rightarrow$  line broad
- Slow Relaxation  $\Rightarrow$  rapid saturation  $\Rightarrow$  low intensity

## NMR Sensitivity

## Ratio Sinal/Noise





Pulse NMR increase the sensitivity (iterative process )

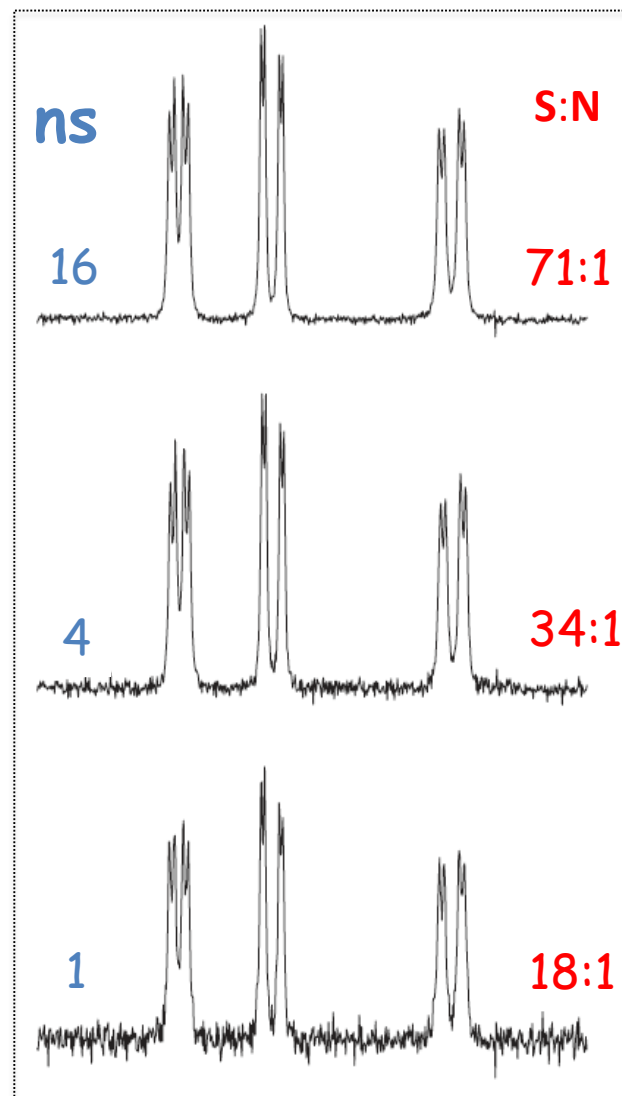
Resulting FID - cumulative FIDs

ns - numbers of scans

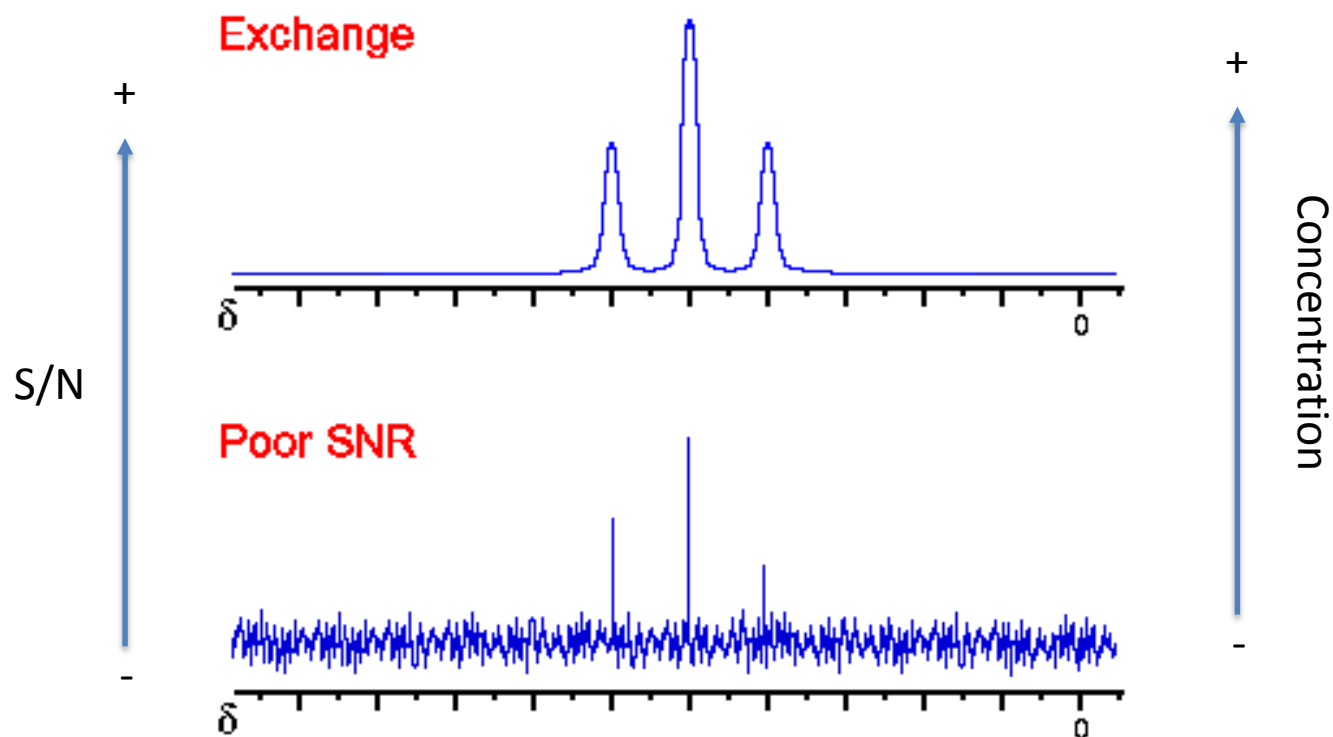
To double de sensitivity (Sinal/Noise) is necessary to acquire four-times more

$$S/N \propto ns^{1/2}$$

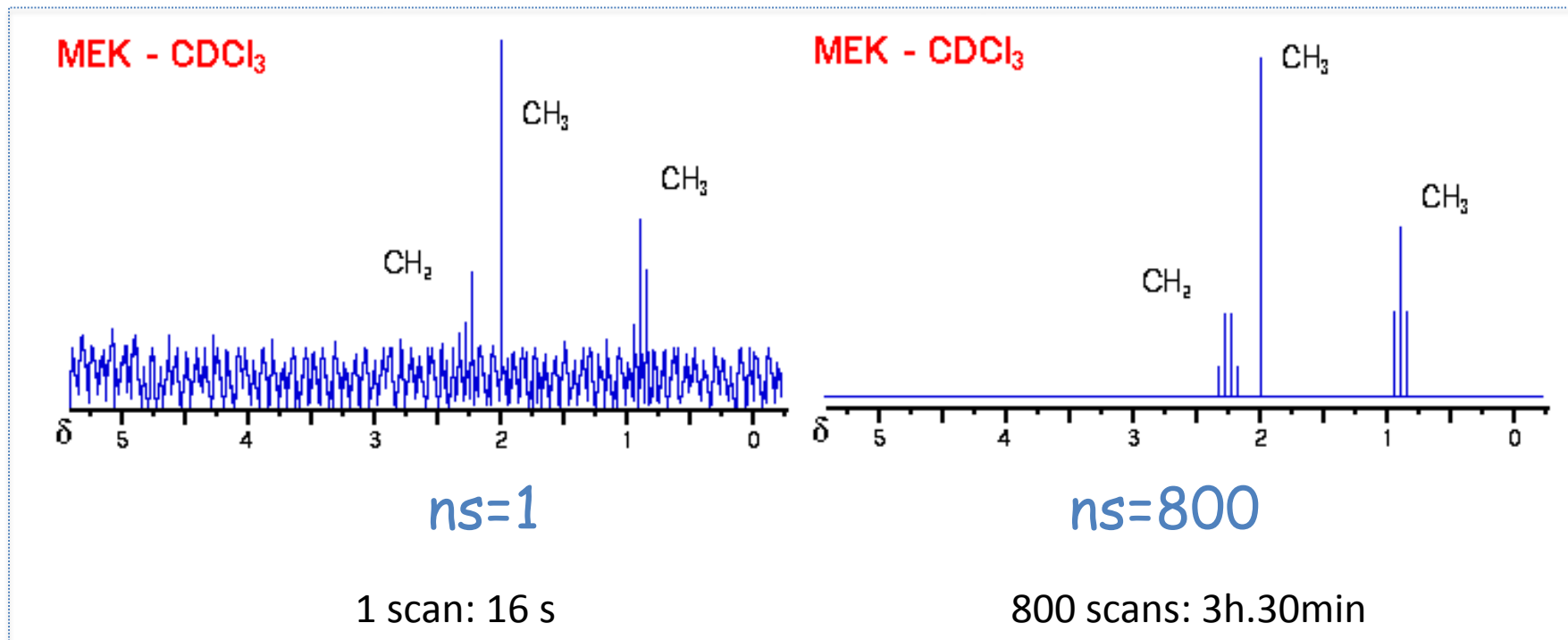
Spectrum resulting o of  $2^n$  FIDs (ns)



## Sensitivity depend on concentration

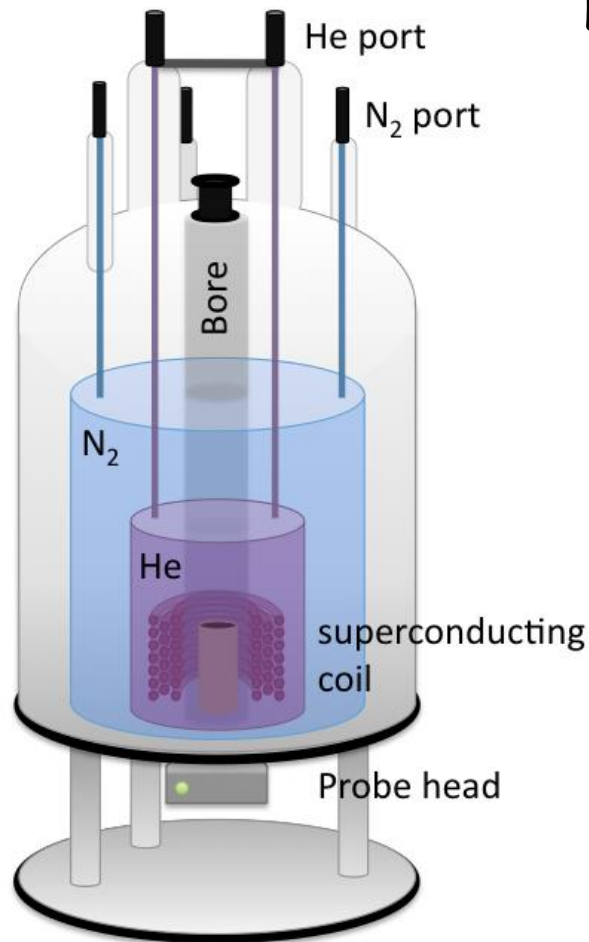


## Sensitivity depend on concentration



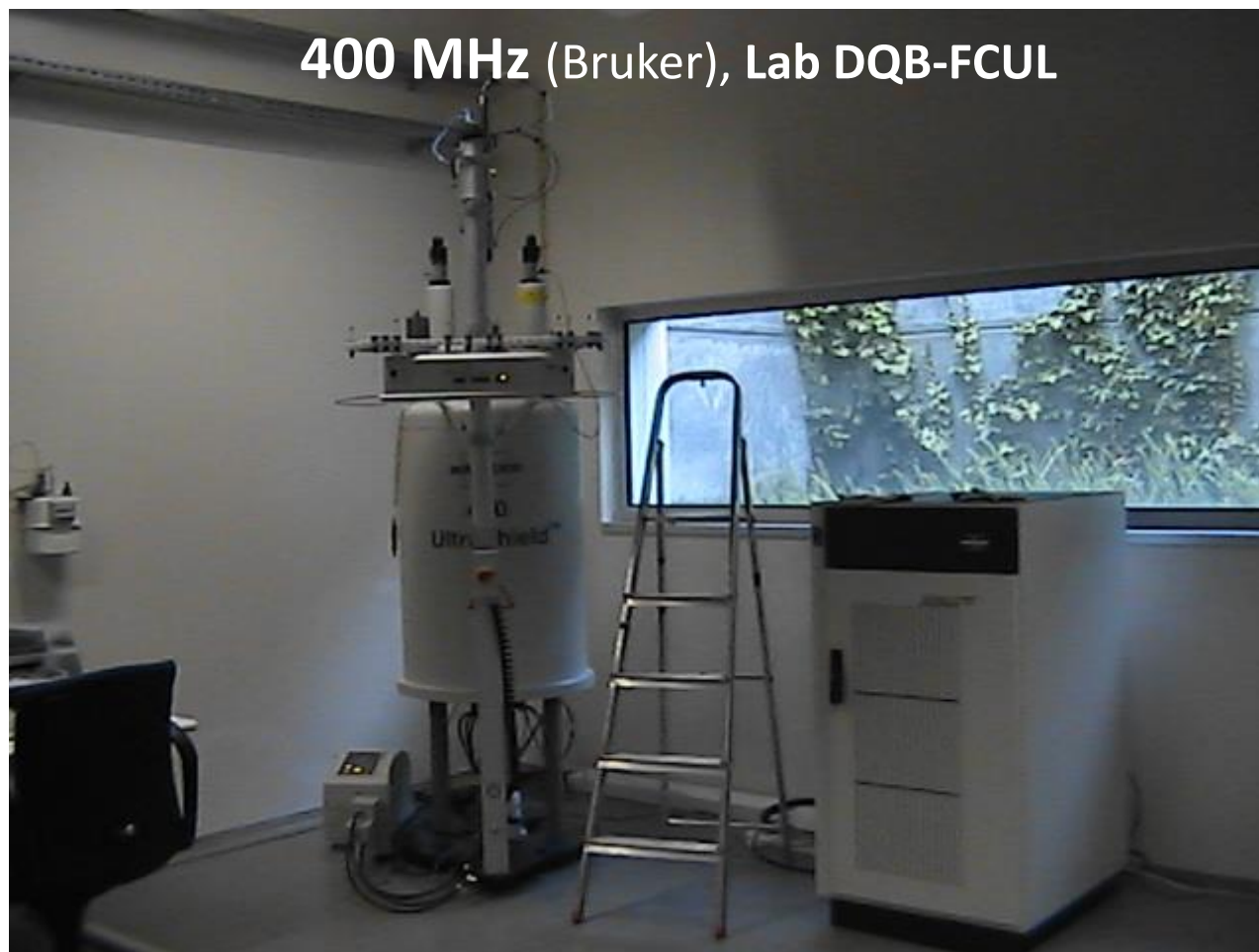
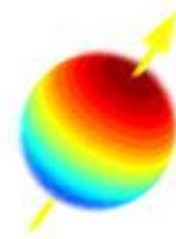
More time =&gt; more expensive!

# NMR spectrometer



- Superconducting magnet  $(\text{NbTaTi})_3\text{Sn}$ , is immersed in liquid helium (4.2K=-268.9°C).
- The helium tank is located inside a bath of liquid nitrogen to prevent the helium from evaporating.
- Inside the center bore the magnetic field is homogeneous (9.4T for a 400 MHz spectrometer).
- The probe head, contains the rf- and receiver coils, is inserted from the bottom into the homogeneous region of the magnetic field.
- The sample is inserted into the center bore and resides inside the probe head during the measurement.

<http://www.chemie.uni-hamburg.de/nmr/insensitive/tutorial/en.lproj/spectrometer.html>



# NMR in liquid solution

## Solvent

- Concentration (5 a 20 mg/400 $\mu$ L)
- Inert
- Good sample dissolution
- Without  $^1\text{H}$  signals (deuterated)
- Easy to removed (evaporated)
- **Residual signals can be used as reference**
- **Deuterium signal used for "locking"**  
(stabilise the magnetic field of the NMR magnet)

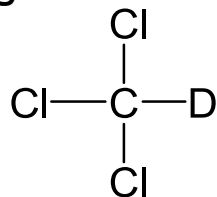


## Most common NMR Solvents

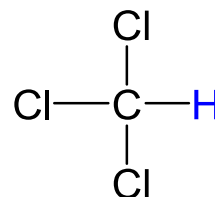
Solvent	Boling Point °C	Residual <sup>1</sup> H signal	<sup>13</sup> C signal
acetone-d <sub>6</sub>	55.5	2.05 ppm	206 & 29.8 ppm
acetonitrile-d <sub>3</sub>	80.7	1.95 ppm	118 & 1.3 ppm
benzene-d <sub>6</sub>	79.1	7.16 ppm	128 ppm
<b>chloroform-d</b>	<b>60.9</b>	<b>7.27 ppm</b>	<b>77.2 ppm</b>
cyclohexane-d <sub>12</sub>	78.0	1.38 ppm	26.4 ppm
dichloromethane-d <sub>2</sub>	40.0	5.32 ppm	53.8 ppm
dimethylsulfoxide-d <sub>6</sub>	190	2.50 ppm	39.5 ppm
nitromethane-d <sub>3</sub>	100	4.33 ppm	62.8 ppm
pyridine-d <sub>5</sub>	114	7.19, 7.55 & 8.71 ppm	150, 135.5 & 123.5 ppm
tetrahydrofuran-d <sub>8</sub>	65.0	1.73 & 3.58 ppm	67.4 & 25.2 ppm

Deuteration of solvents is not "100%"

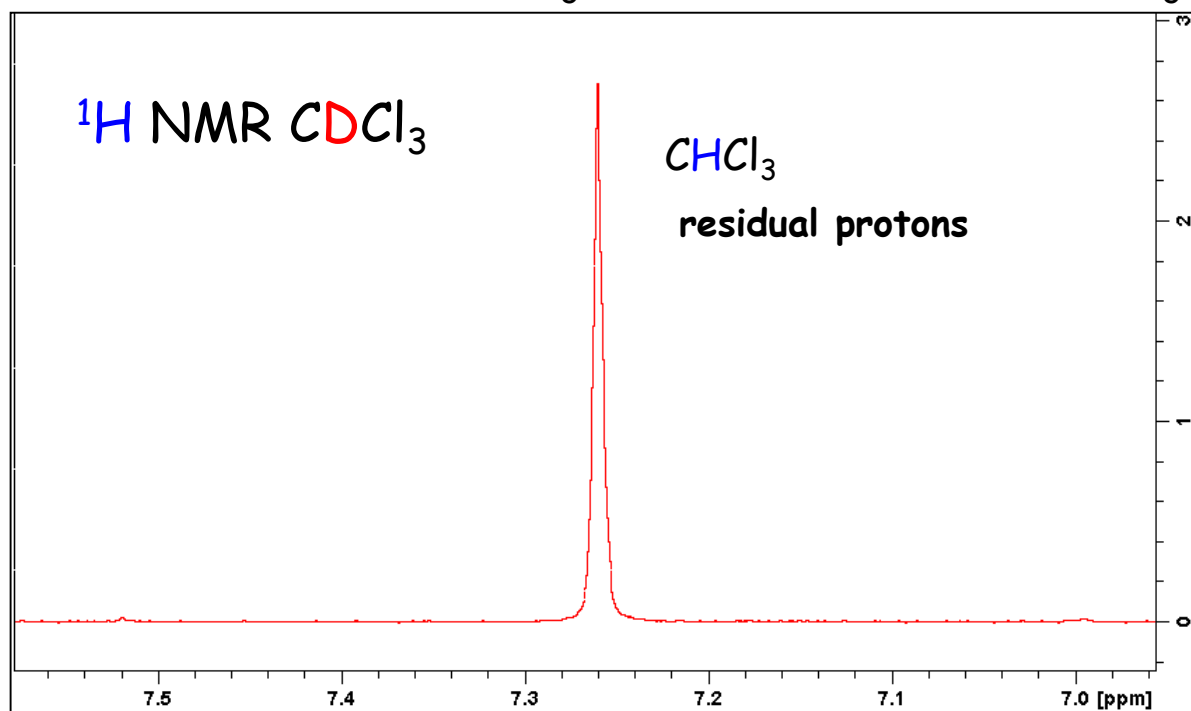
$^1\text{H}$  signals for the residual protons are observed



chloroform-d 99%  $\text{CDCl}_3$



Chloroform 1%  $\text{CHCl}_3$





## Interpretation of $^1\text{H}$ NMR spectra

- Chemical shift

(depend on chemical surrounding - shielding)

How many types of H are in the molecule

- Signal integration

(signal area is proportional to the number of proton that give rise that signal )

How many H of each type are in the molecule

- Signal multiplicity (splitting)

(splitting due to the surrounding nuclei)

What are the surrounding nucleus - connectivity in the molecule

- Line widths

## Chemical shift

- Electronic cloud "shield" nuclei from the external field causing them to absorb at a slightly higher energy (lower resonance frequency).
- Any effect on the density or spatial distribution of the electronic cloud alter the degree of shielding and consequently the value of chemical shift.

Higher electronic density => higher shielding => lower frequency  
=> lower chemical shift



Lower electronic density => higher deshielding => higher frequency  
=> higher chemical shift

## Chemical shift - electronegativity effect

An electronegative atom lowers the electronic density of the proton nuclei  
 ⇒ higher deshielding (lower shielding) ⇒ higher resonance frequency  
 ⇒ ⇒ higher chemical shift



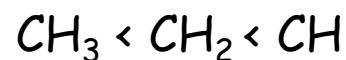
Compound CH <sub>3</sub> X	X atom	X Electronegativity	<sup>1</sup> H Chemical shift
CH <sub>3</sub> F	F	4.0	4.26 ppm
CH <sub>3</sub> OH	O	3.5	4.30 ppm
CH <sub>3</sub> Cl	Cl	3.1	3.05 ppm
CH <sub>3</sub> Br	Br	2.8	2.68 ppm
CH <sub>3</sub> I	I	2.5	2.16 ppm
CH <sub>4</sub>	H	2.1	0.23 ppm
(CH <sub>3</sub> ) <sub>4</sub> Si	Si	1.8	0 ppm


decreased shielding

Increased resonance

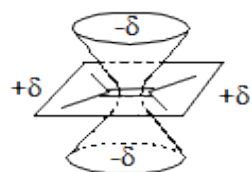
# Chemical shift

## Hybridization effect

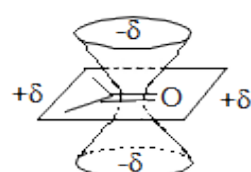
$$sp^3 < sp^2 < sp$$



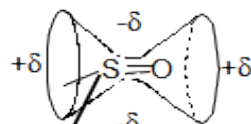
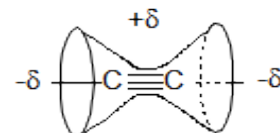
## Magnetic anisotropy



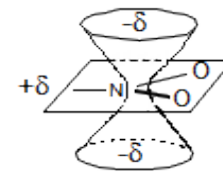
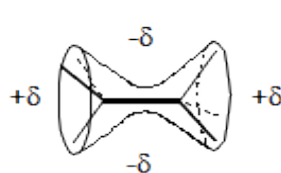
Alkene



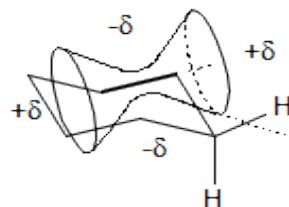
Carbonyl

Sulfoxide<sup>3</sup>

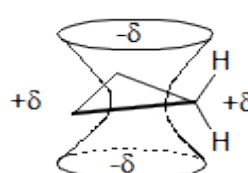
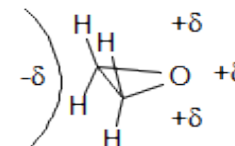
Alkyne

Nitro<sup>4</sup>

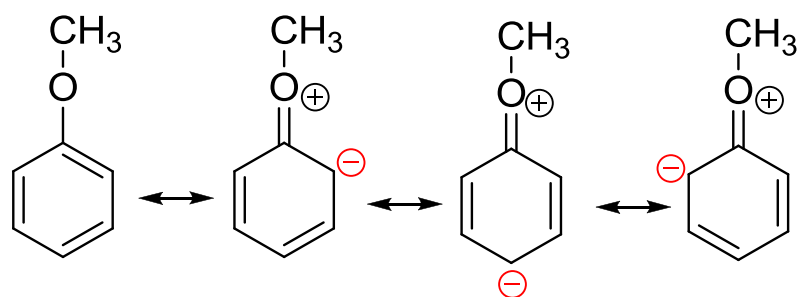
C-C Single Bond



Cyclohexane Ax-Eq

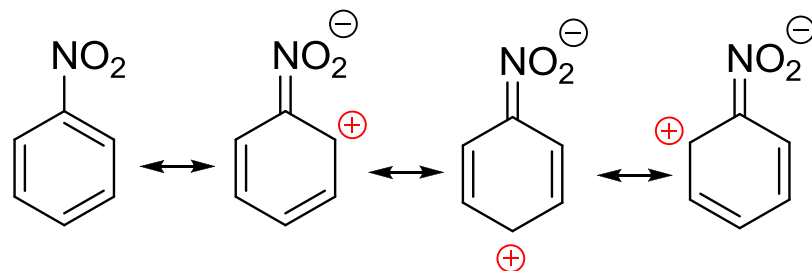
Cyclopropane<sup>2</sup>Epoxide<sup>1</sup>

# Chemical shift - mesomeric effect



shielding

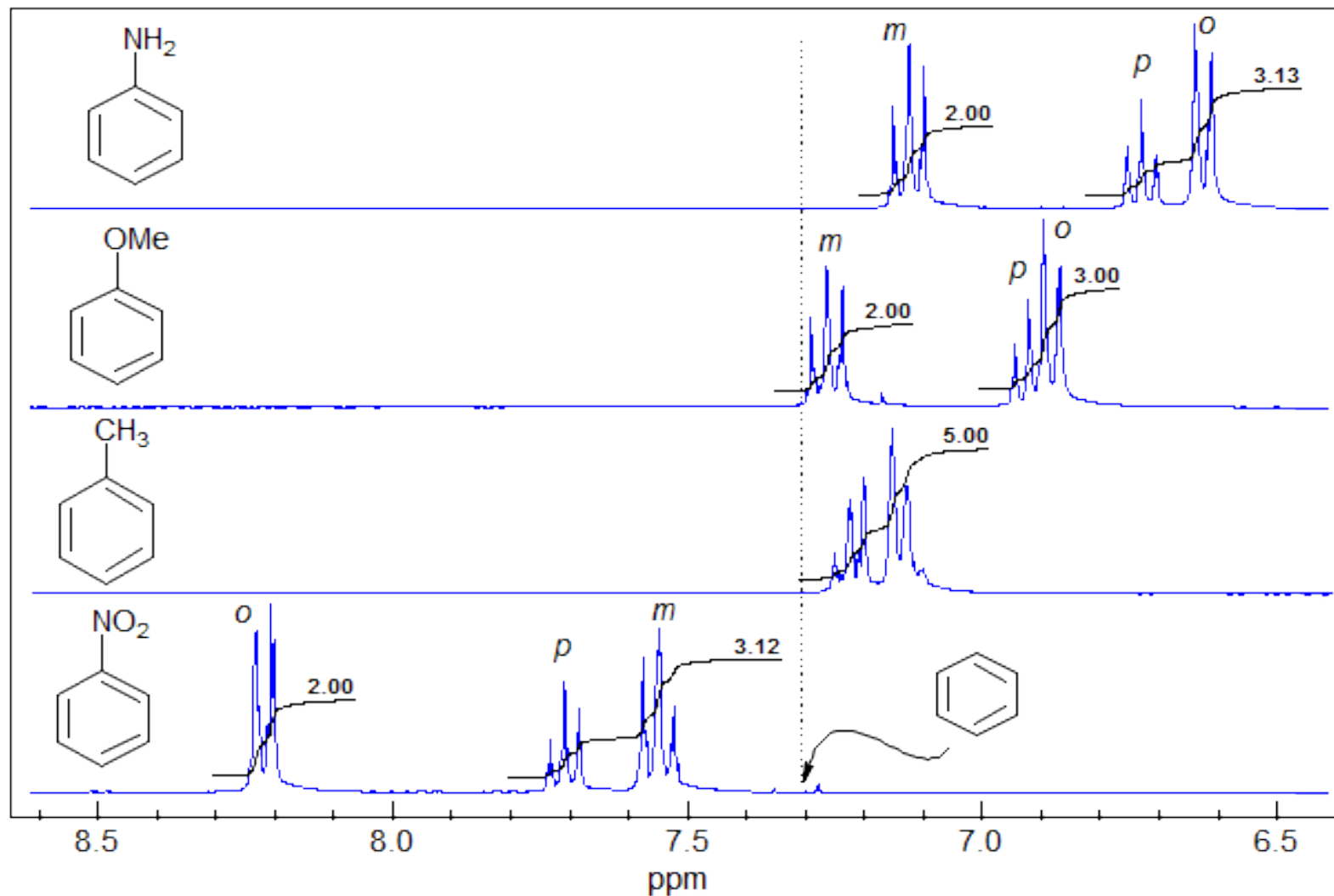
*ortho*  
*para*



deshielding

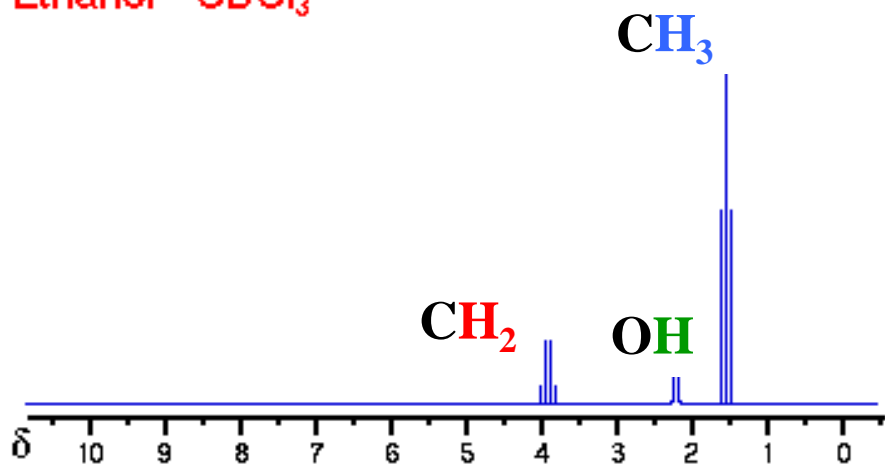
*ortho*  
*para*

## Monosubstituted Benzenes



## Chemical shift depends on the solvent

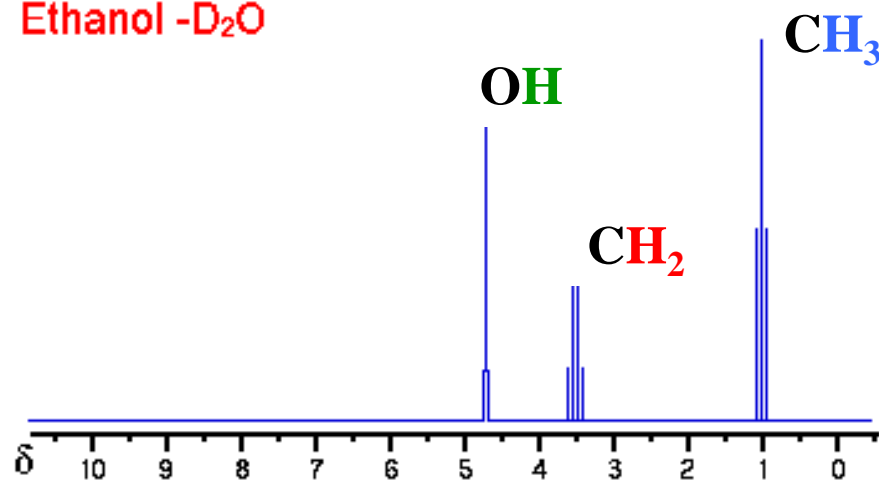
Ethanol -  $\text{CDCl}_3$



Solvation influences  
the electronic cloud density

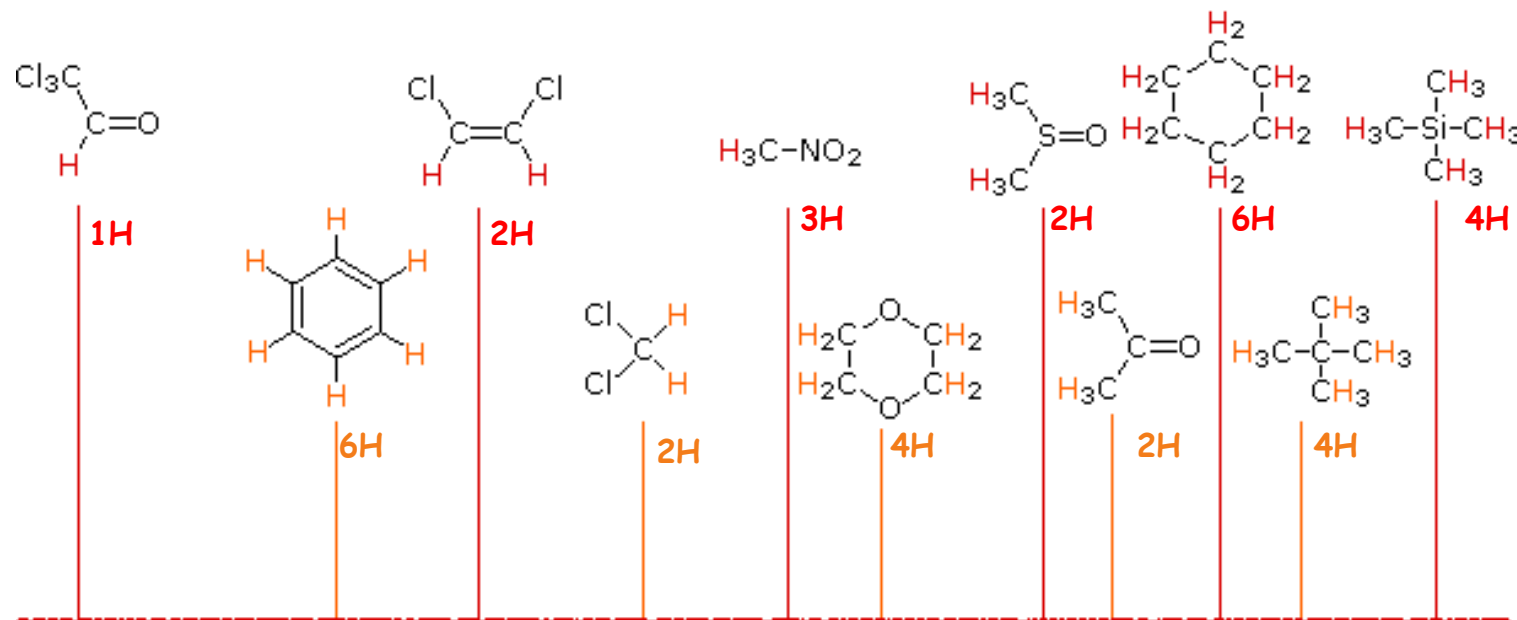
Ethanol -  $\text{D}_2\text{O}$

$\text{CH}_3\text{CH}_2\text{OH}$



Increased shielding by extranuclear electrons

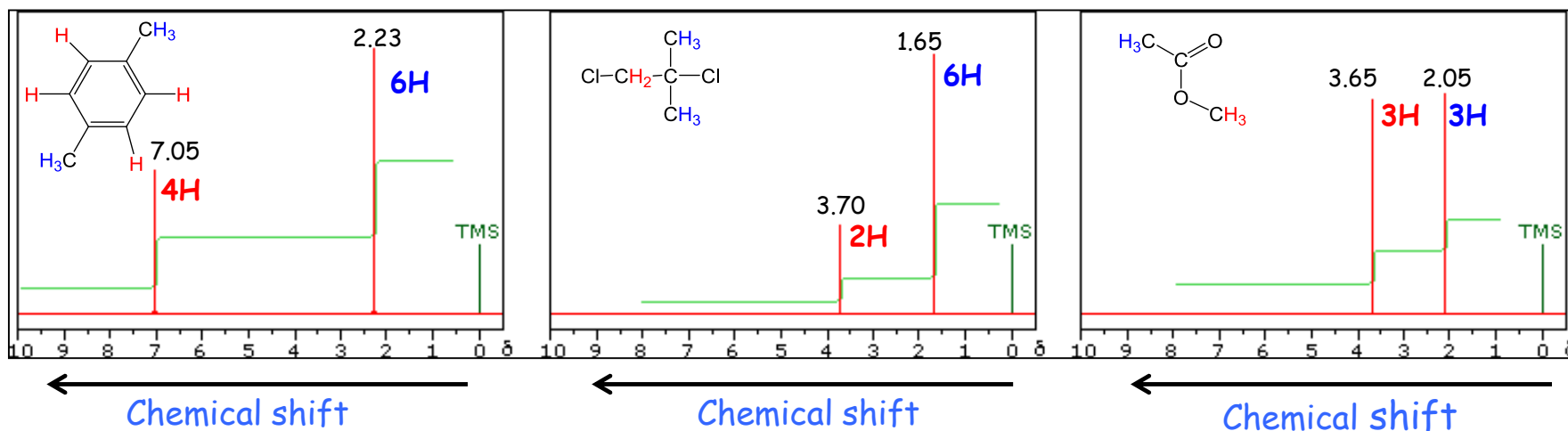
Decreased chemical shift



Different surrounding, different shielding, different resonance frequency

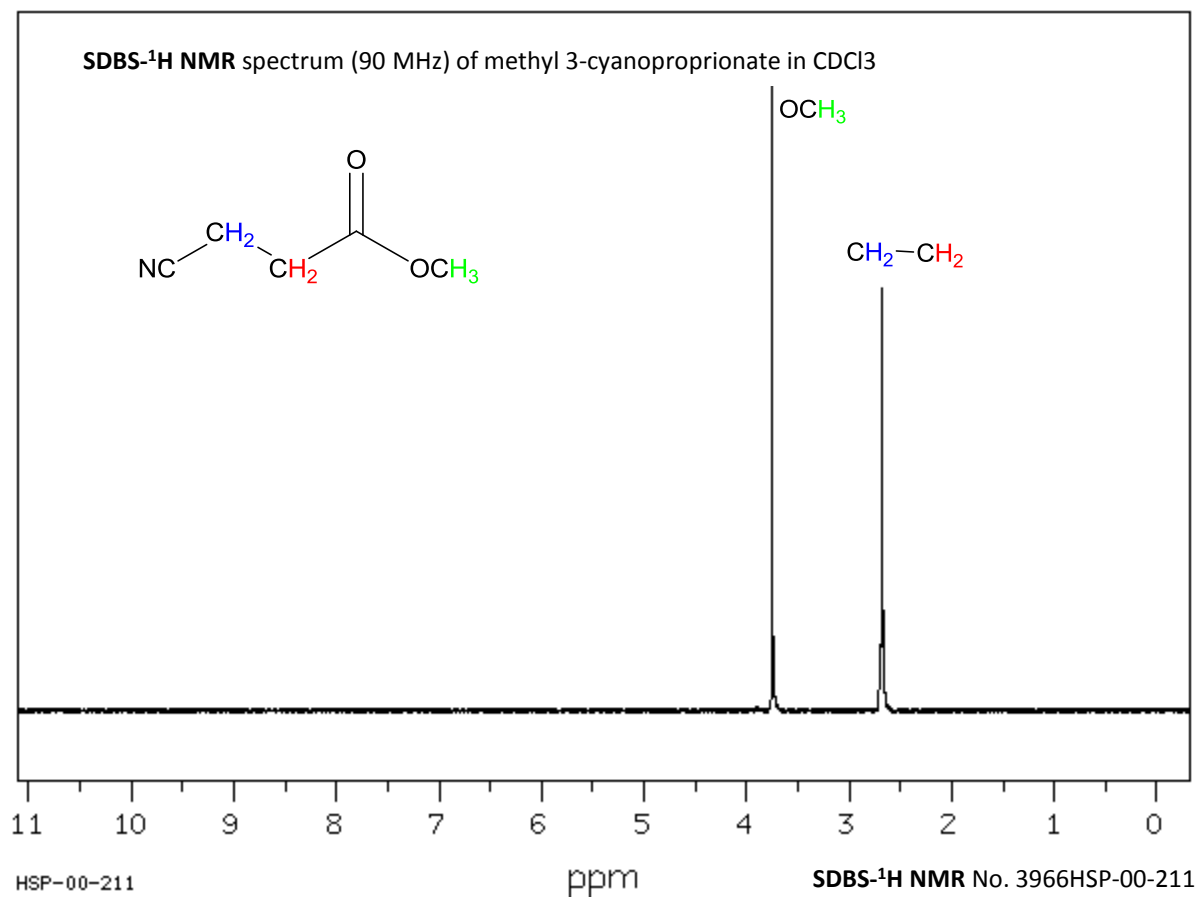
Same chemical environments => same chemical shift





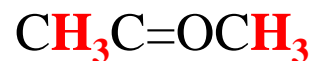
Same chemical environments => same chemical shift – **chemically equivalent**

different chemical environments => same chemical shift by coincidence!

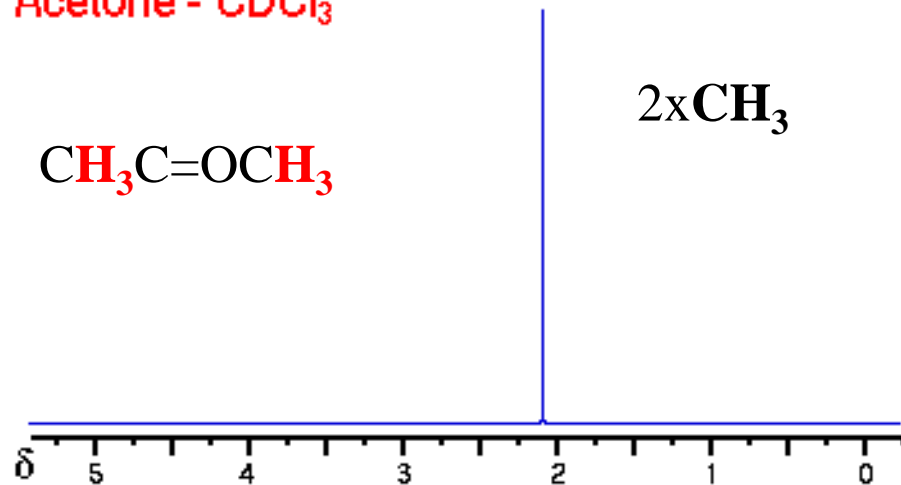


# Signal multiplicity

Acetone -  $\text{CDCl}_3$



$2\times\text{CH}_3$



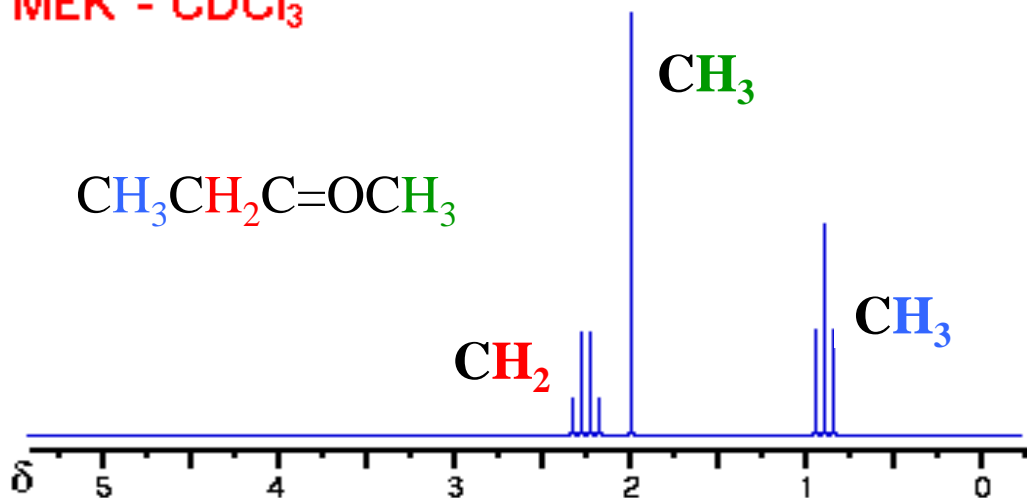
MEK -  $\text{CDCl}_3$



$\text{CH}_3$

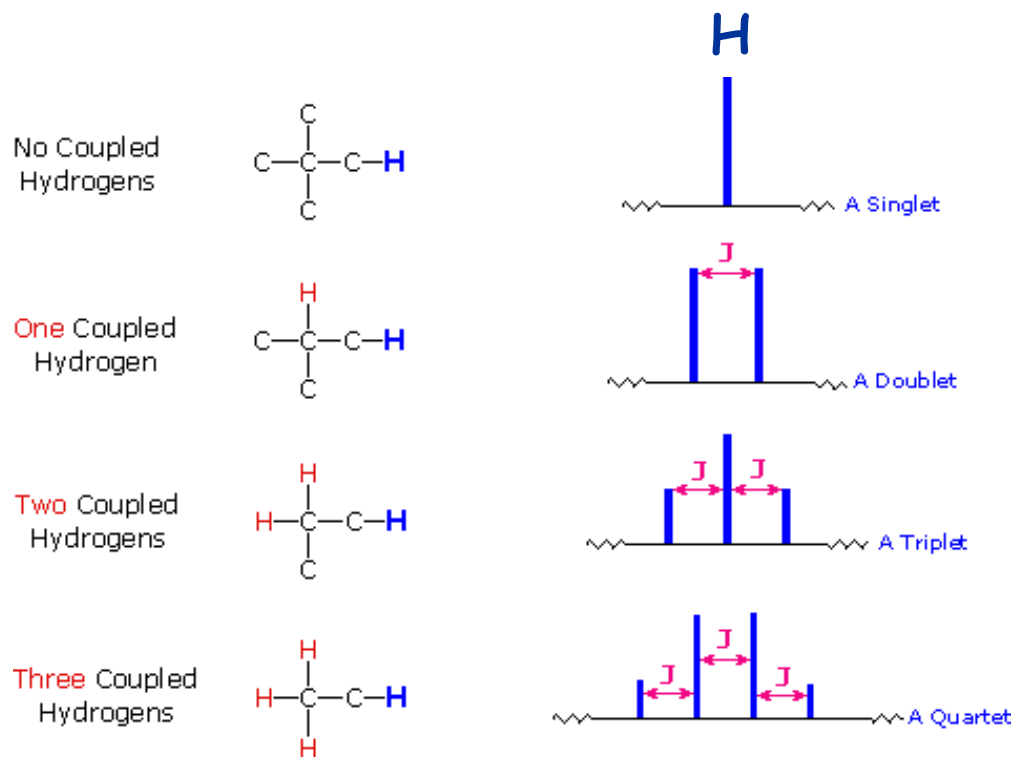
$\text{CH}_2$

$\text{CH}_3$

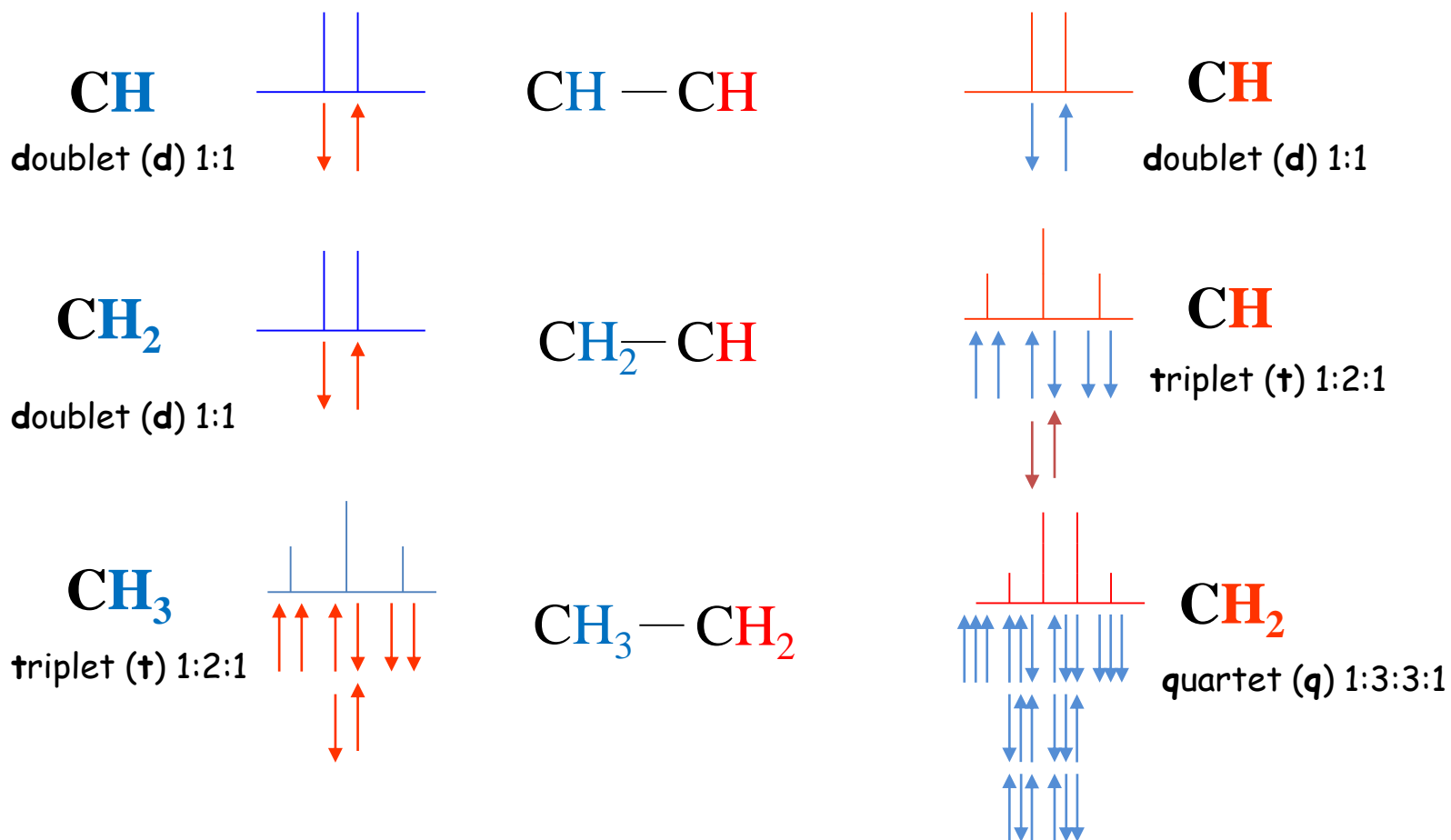


# Signal multiplicity spin-spin coupling: **equivalent protons**

spin-spin coupling - protons on adjacent carbons will interact and "split" each others signals into multiplets (through-bond interaction)








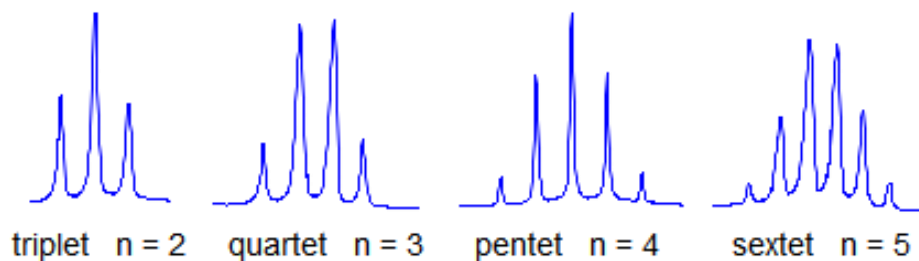
## spin-spin coupling - resonances always split each other



## spin-spin coupling: **equivalent protons**

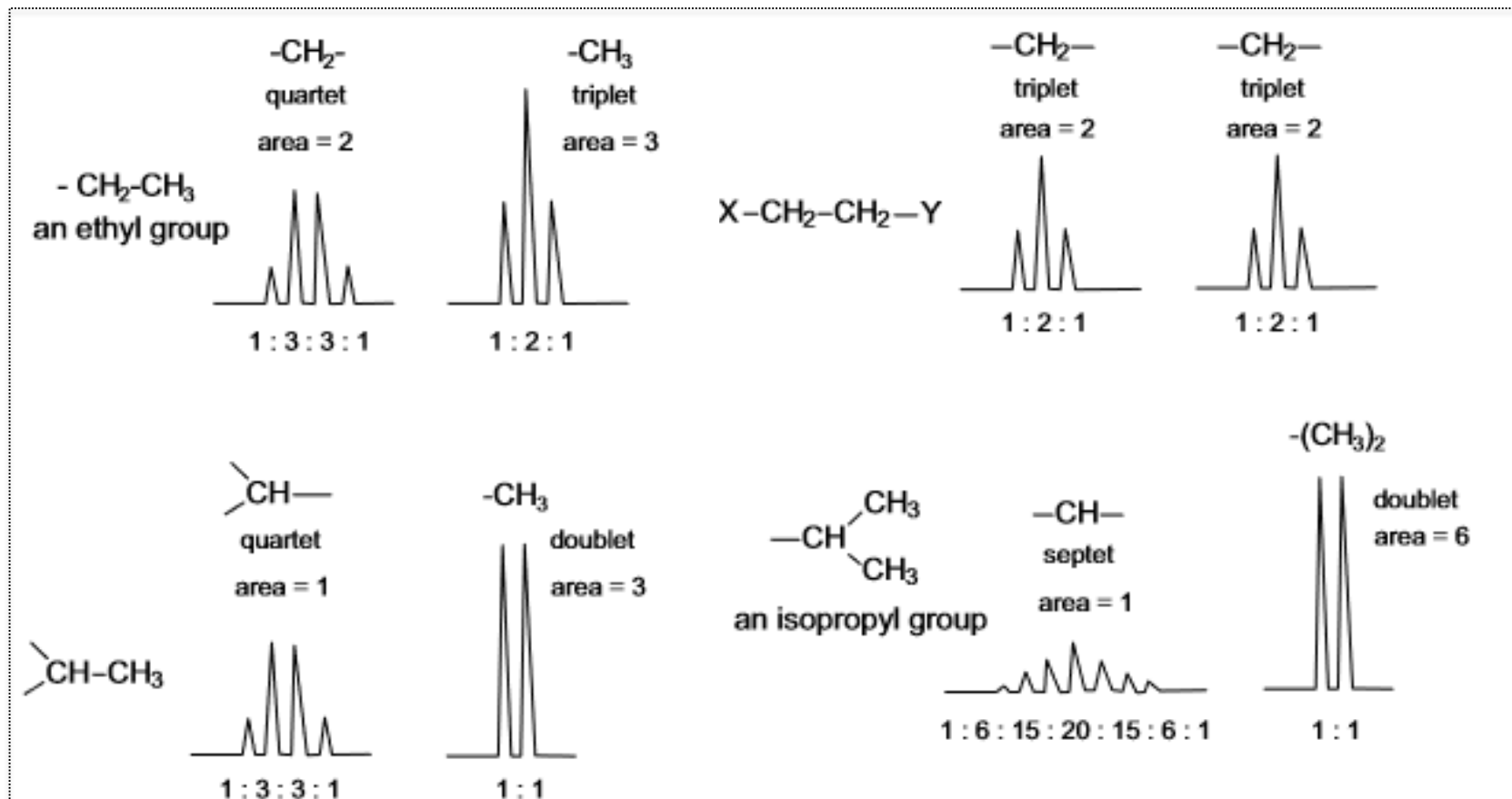
**n+1 rule** - equivalent protons that have **n** equivalent protons on the adjacent carbon will be split into **n+1 peaks**

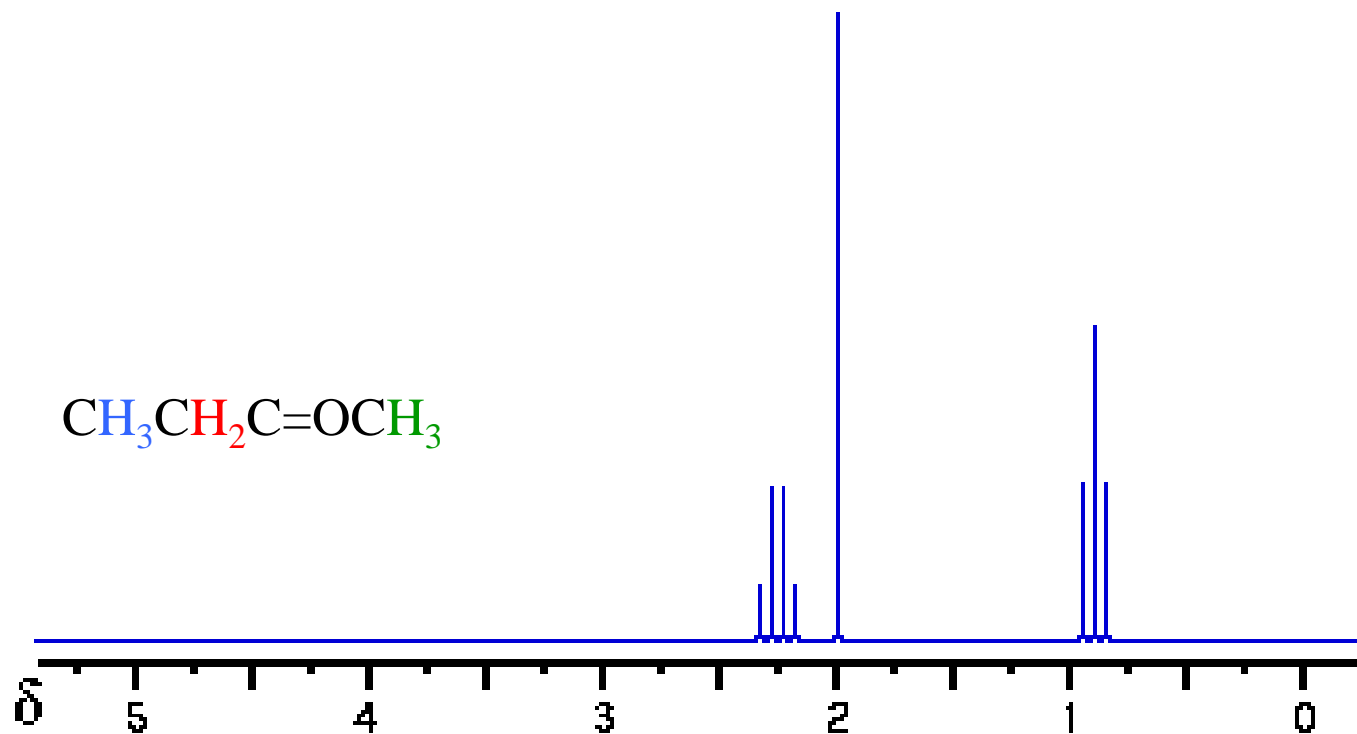
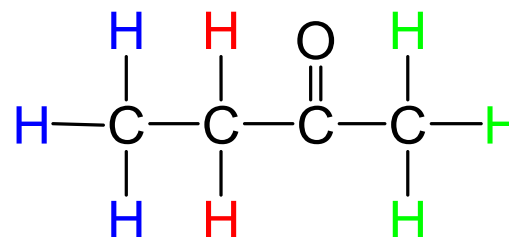
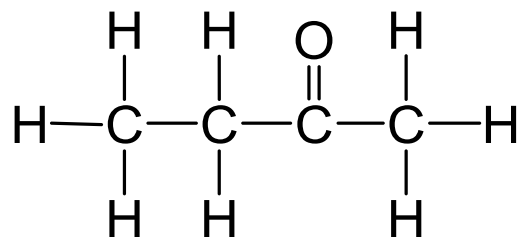
n	n + 1	pattern intensities	Multiplet	Symbol
0	1		singlet	s
1	2		doublet	d
2	3		triplet	t
3	4		quartet	q
4	5		pentet	m
>4	>5	-	multiplet	m



**multiplicity - the number of peaks and the pattern intensities**

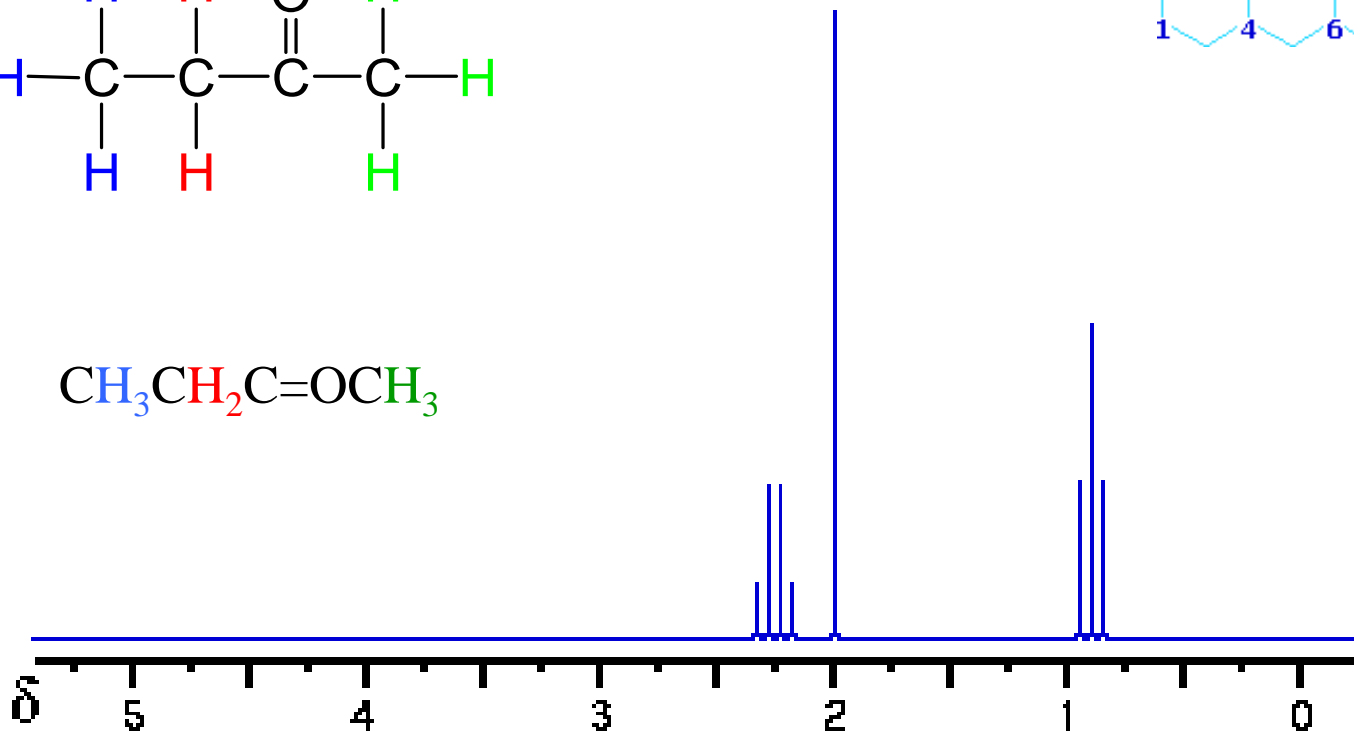
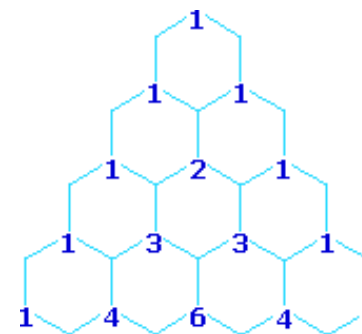
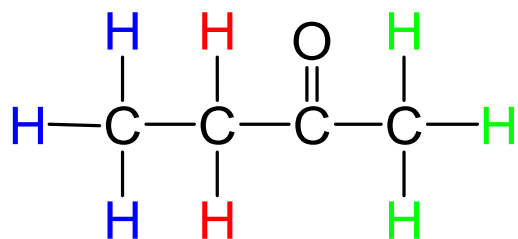
## Examples of multiplet Patterns







Group	n	n + 1	Multiplet	Symbol	Intensities
-------	---	-------	-----------	--------	-------------

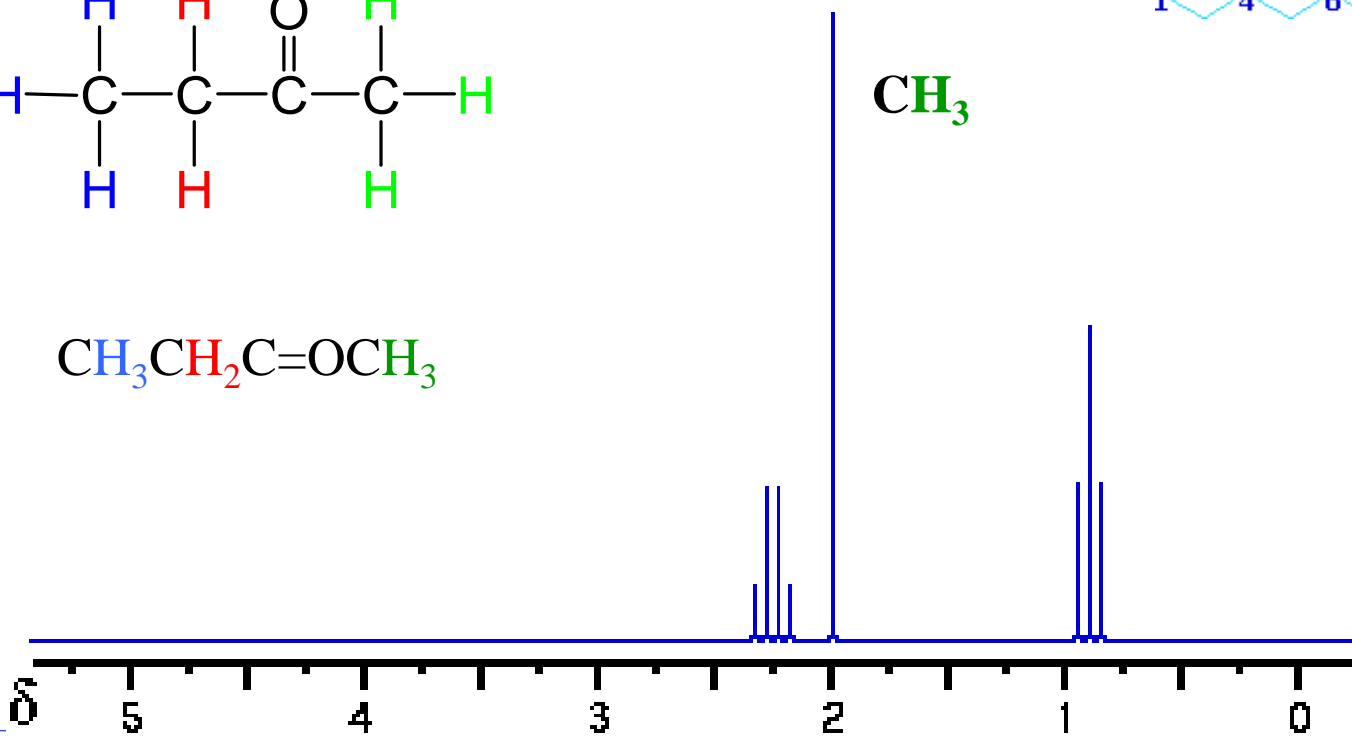
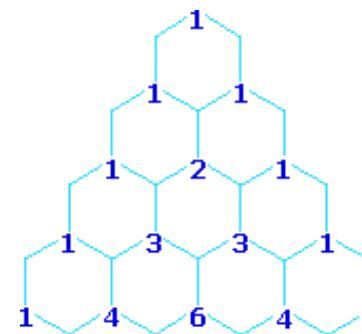
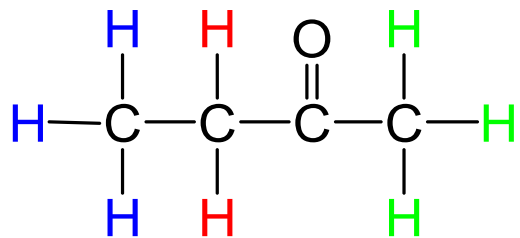
CH<sub>3</sub>CH<sub>2</sub>CH<sub>3</sub>

Group	n	n + 1	Multiplet	Symbol	Intensities
-------	---	-------	-----------	--------	-------------

CH <sub>3</sub>	0	1	singlet	s	1
-----------------	---	---	---------	---	---

CH <sub>2</sub>	1	2	doublet	d	1:1
-----------------	---	---	---------	---	-----

CH <sub>3</sub>	2	3	triplet	t	1:2:1
-----------------	---	---	---------	---	-------

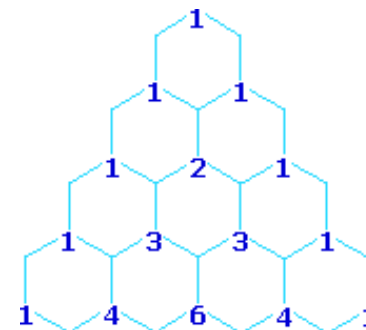
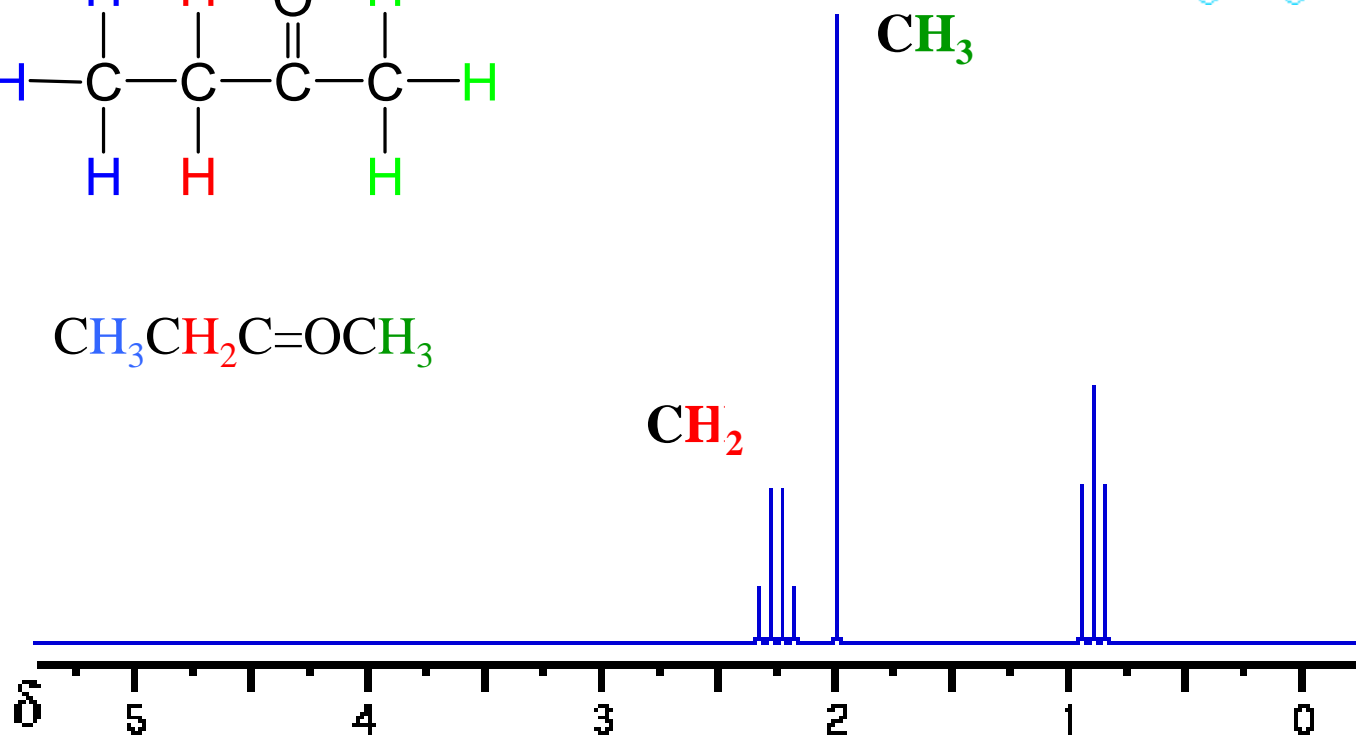
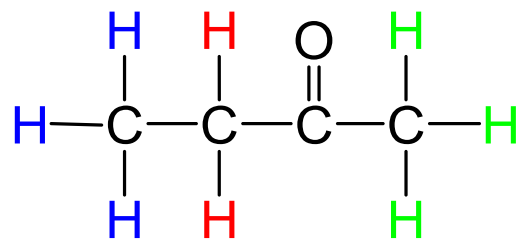


Group	n	n + 1	Multiplet	Symbol	Intensities
-------	---	-------	-----------	--------	-------------

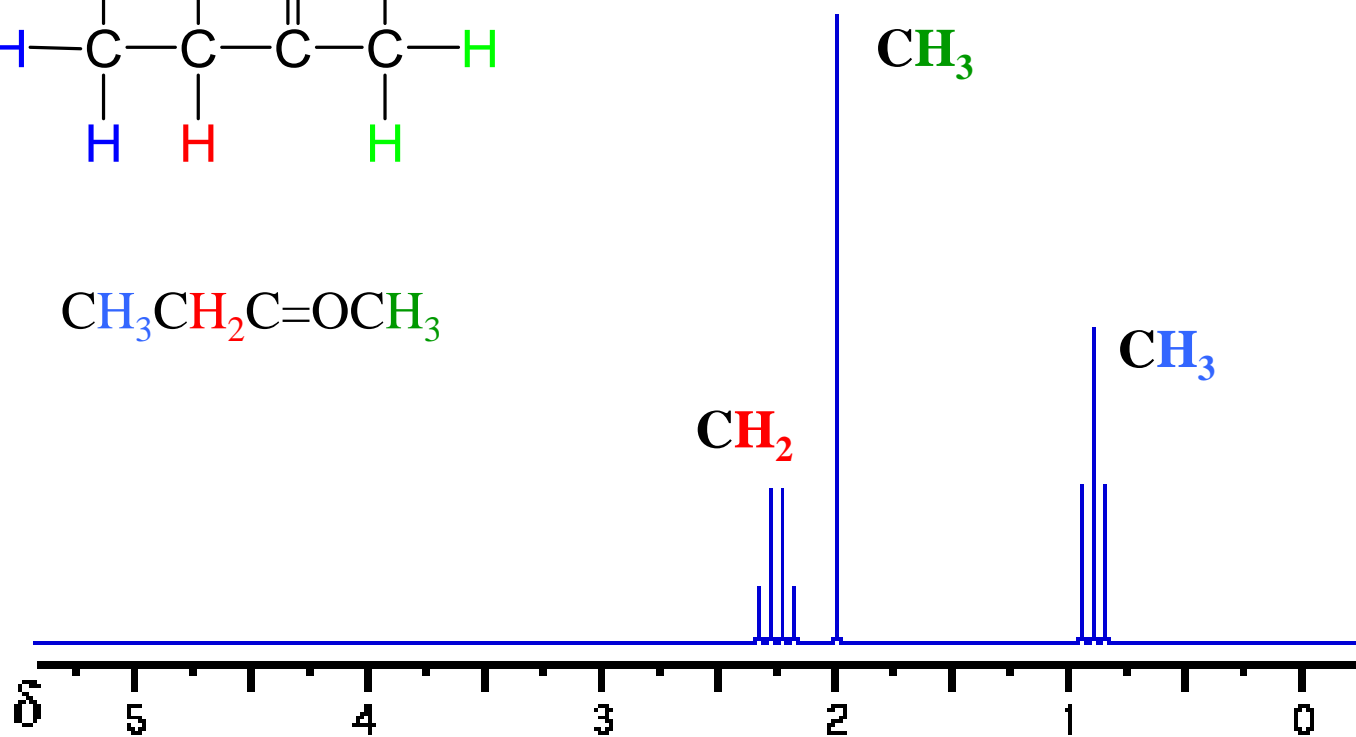
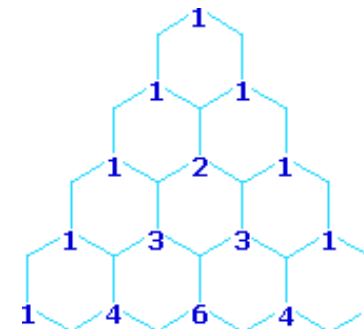
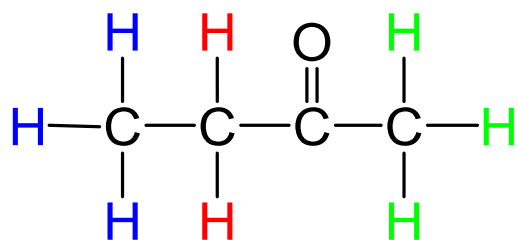
CH <sub>3</sub>	0	1	singlet	s	1
-----------------	---	---	---------	---	---

CH <sub>2</sub>	3	4	quartet	q	1:3:3:1
-----------------	---	---	---------	---	---------

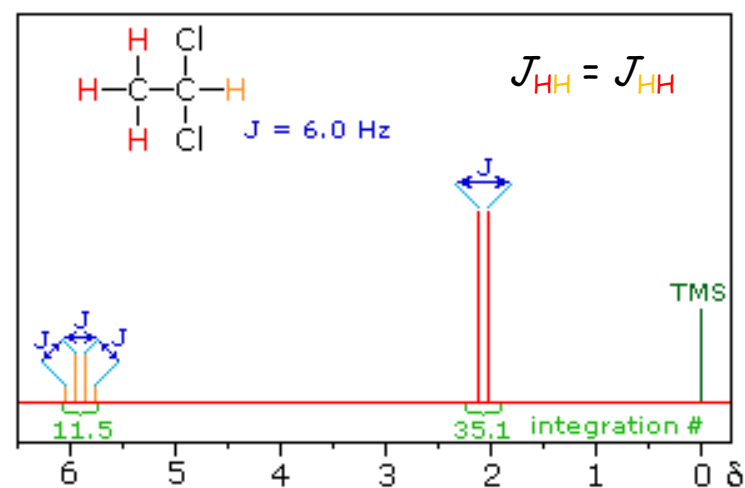
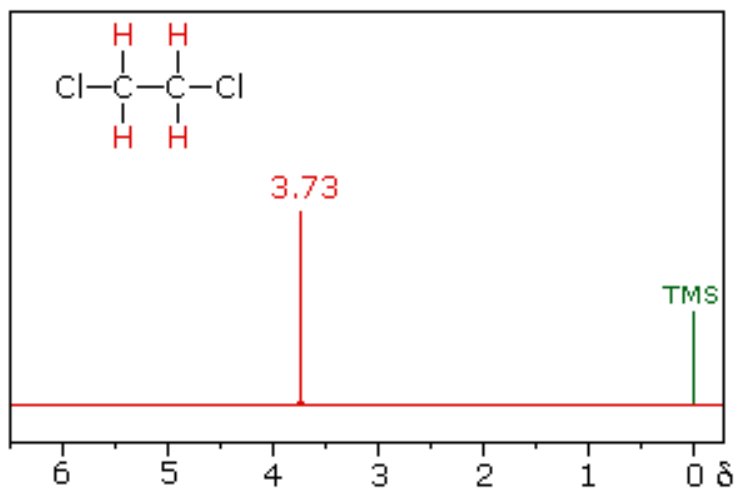
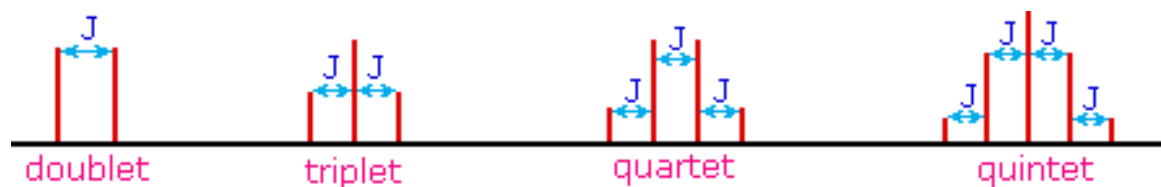
CH <sub>3</sub>					
-----------------	--	--	--	--	--



Group	n	n + 1	Multiplet	Symbol	Intensities
CH <sub>3</sub>	0	1	singlet	s	1
CH <sub>2</sub>	3	4	quartet	q	1:3:3:1
CH <sub>3</sub>	2	3	triplet	t	1:2:1

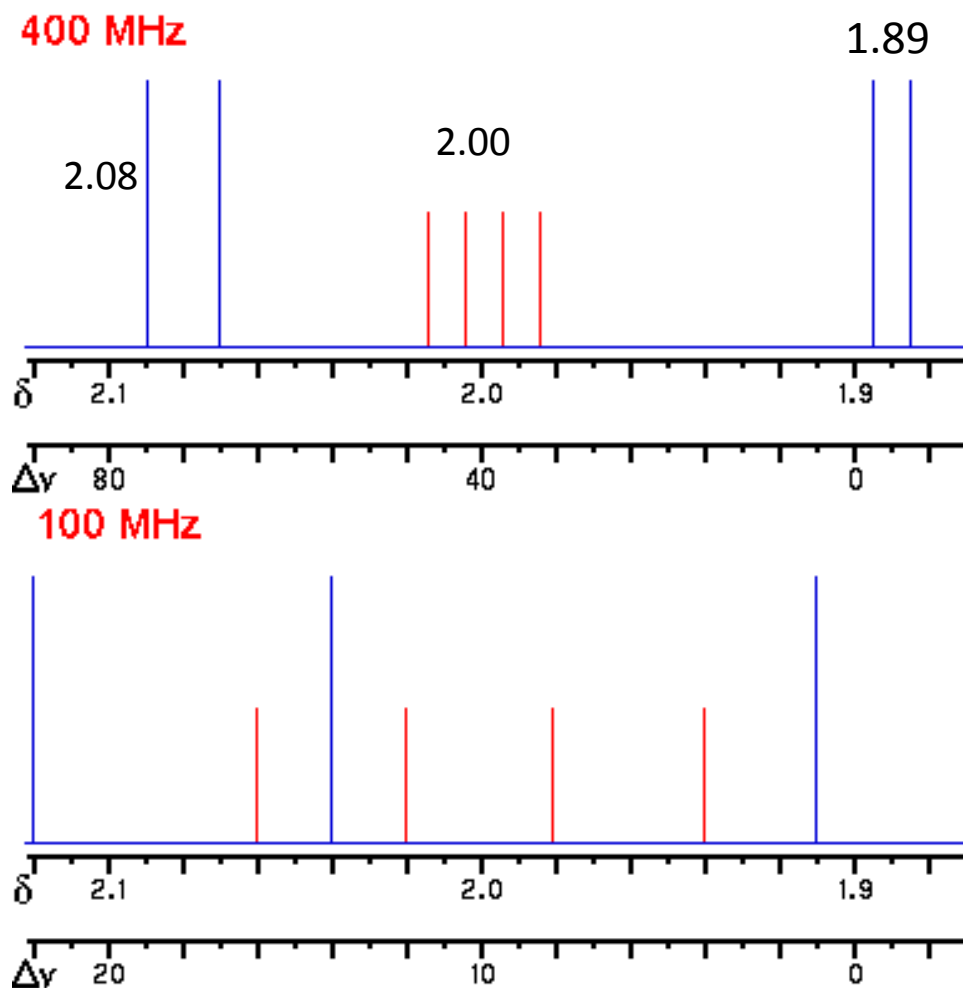


## Coupling constant $J$

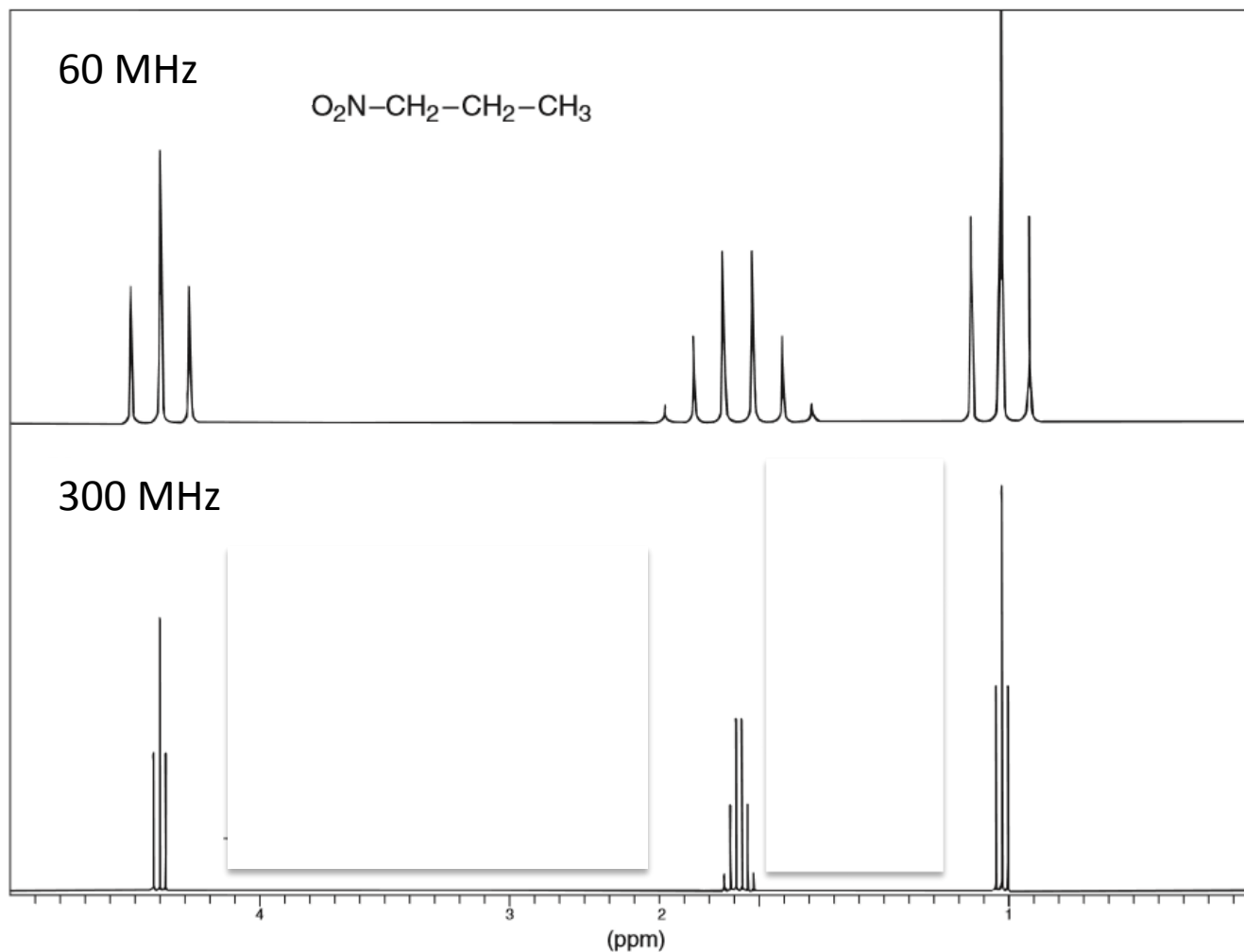


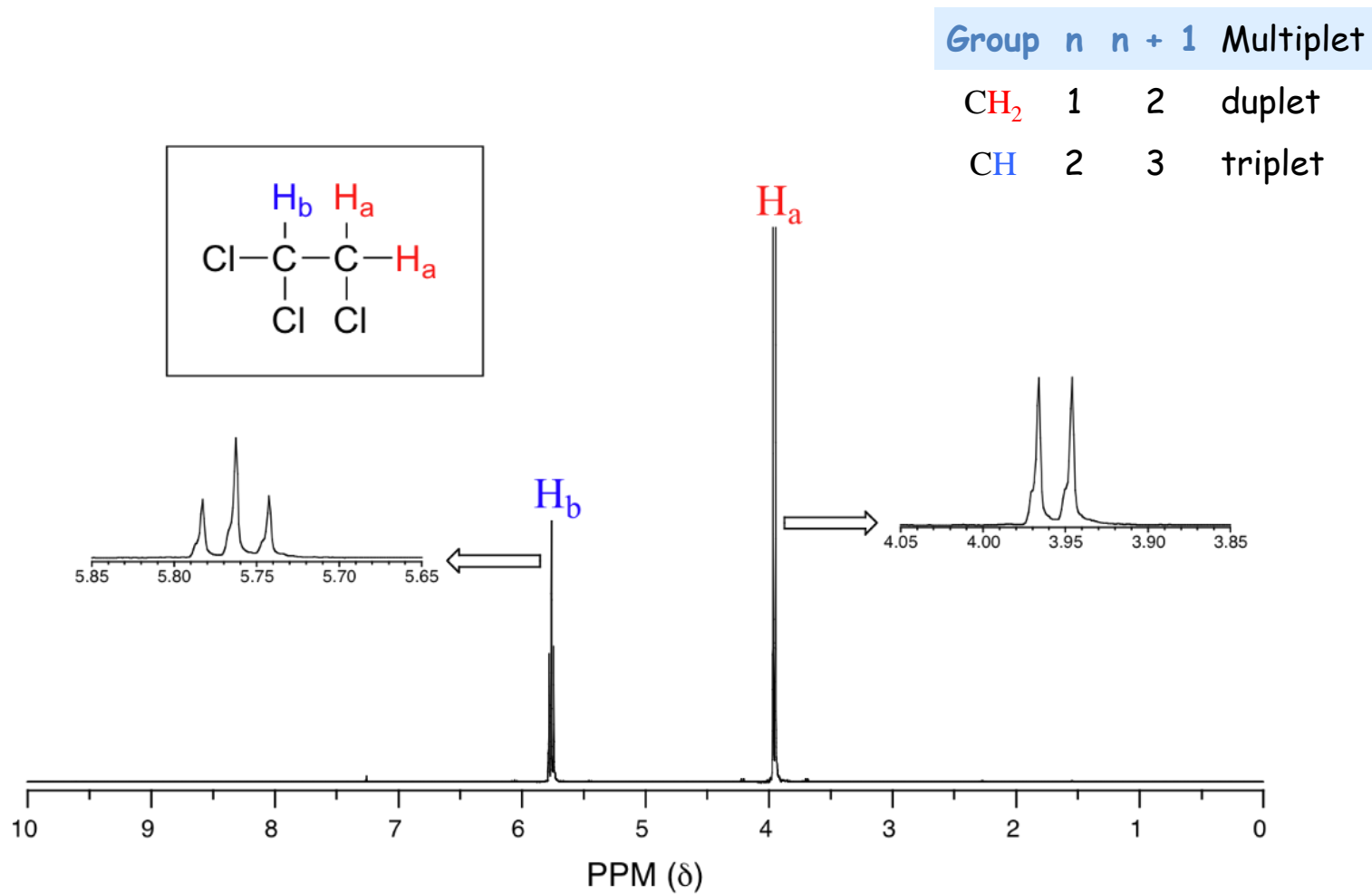
The  **$J$  coupling** (always reported in Hz)  
 $J$  is **constant** is mutual (eg:  $J_{HH} = J_{HH}$ )

$J$  is **constant** at different external magnetic field strength

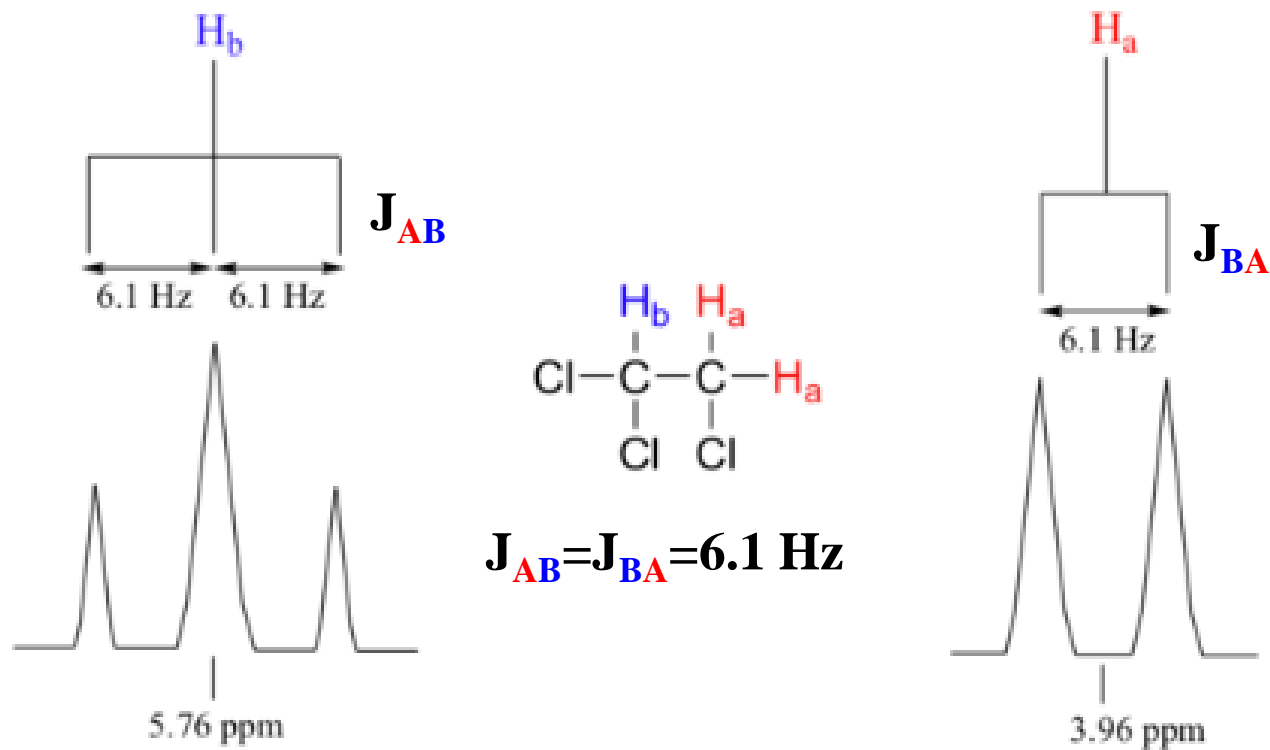


$J$  is **constant** at different external magnetic field strength

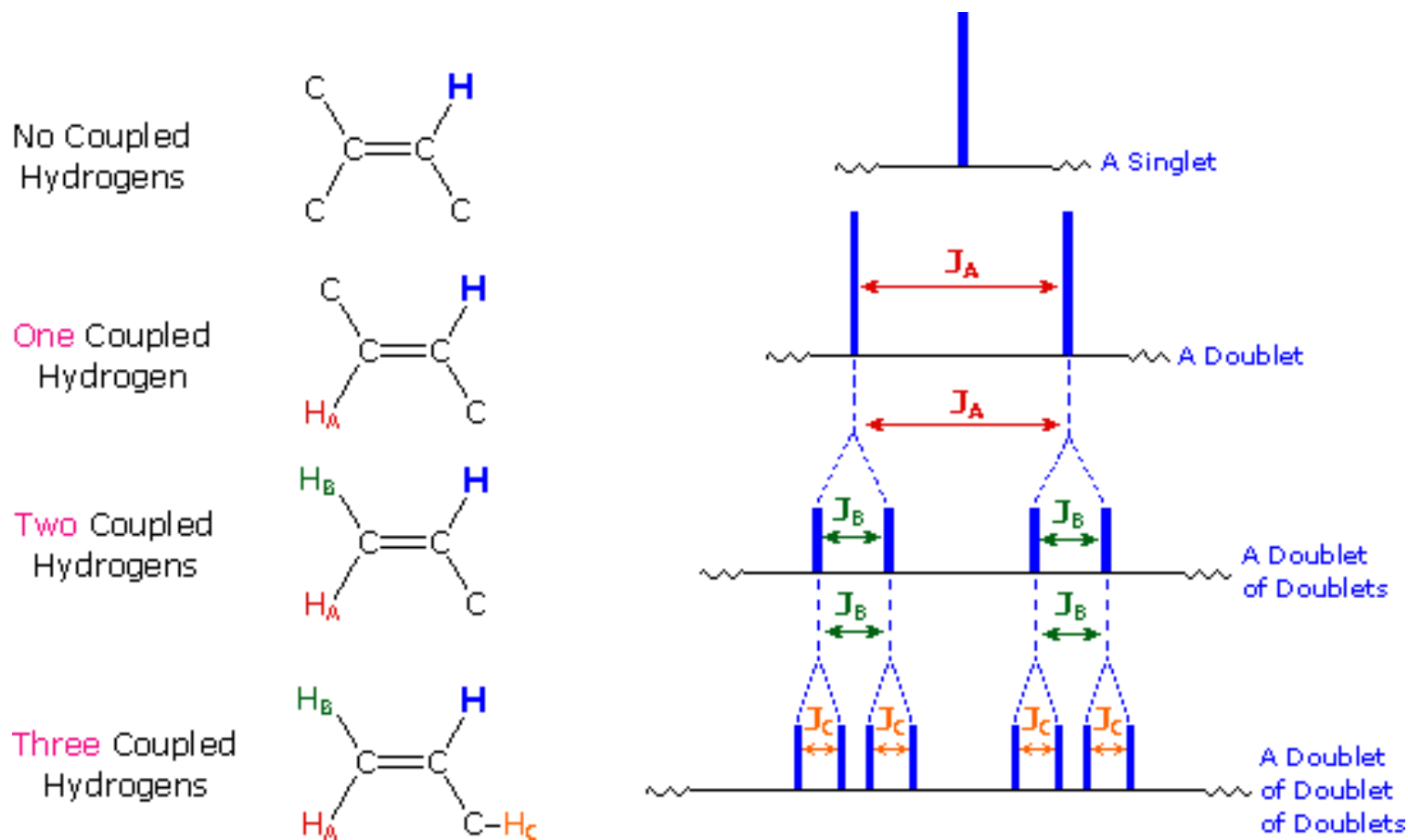






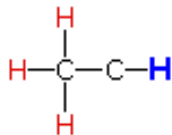


## spin-spin coupling: non equivalent protons will couple independently

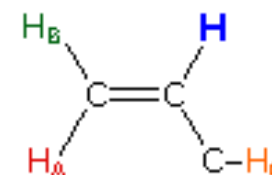




Three Coupled  
Hydrogens

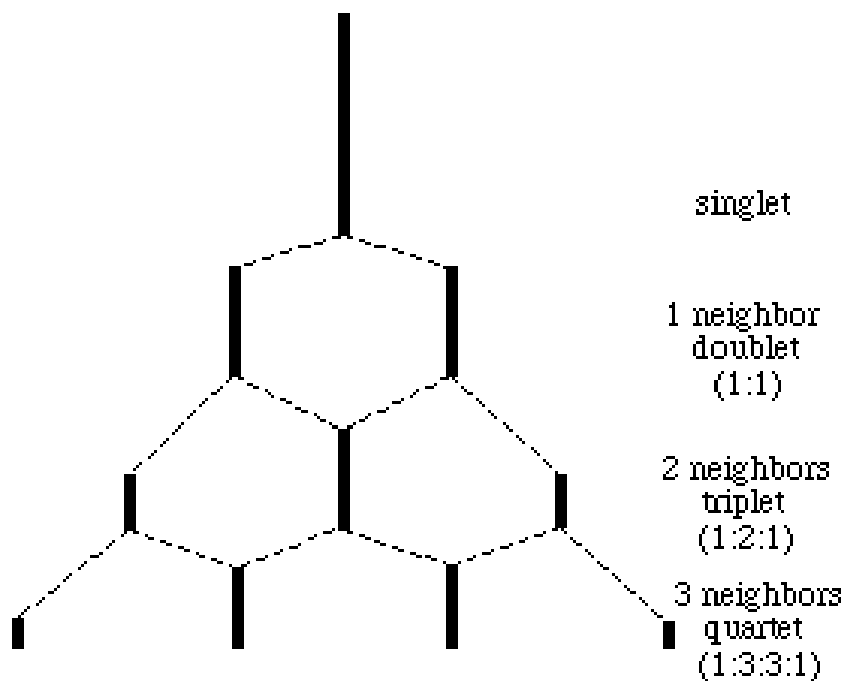


Three Coupled  
Hydrogens



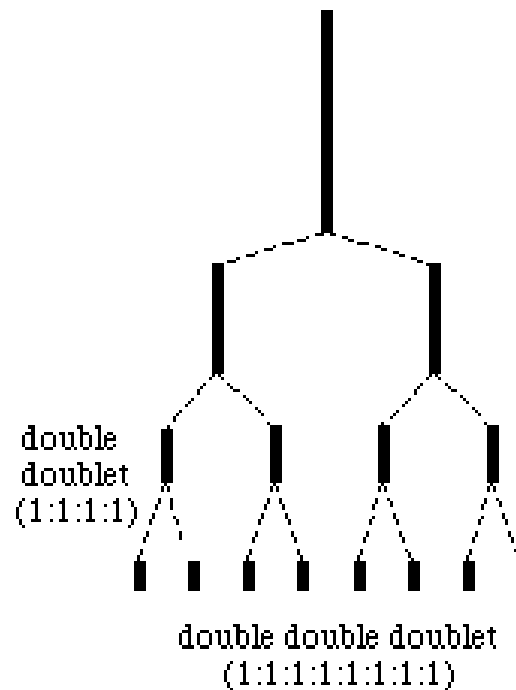
## Splitting Patterns

### Equivalent Splitting



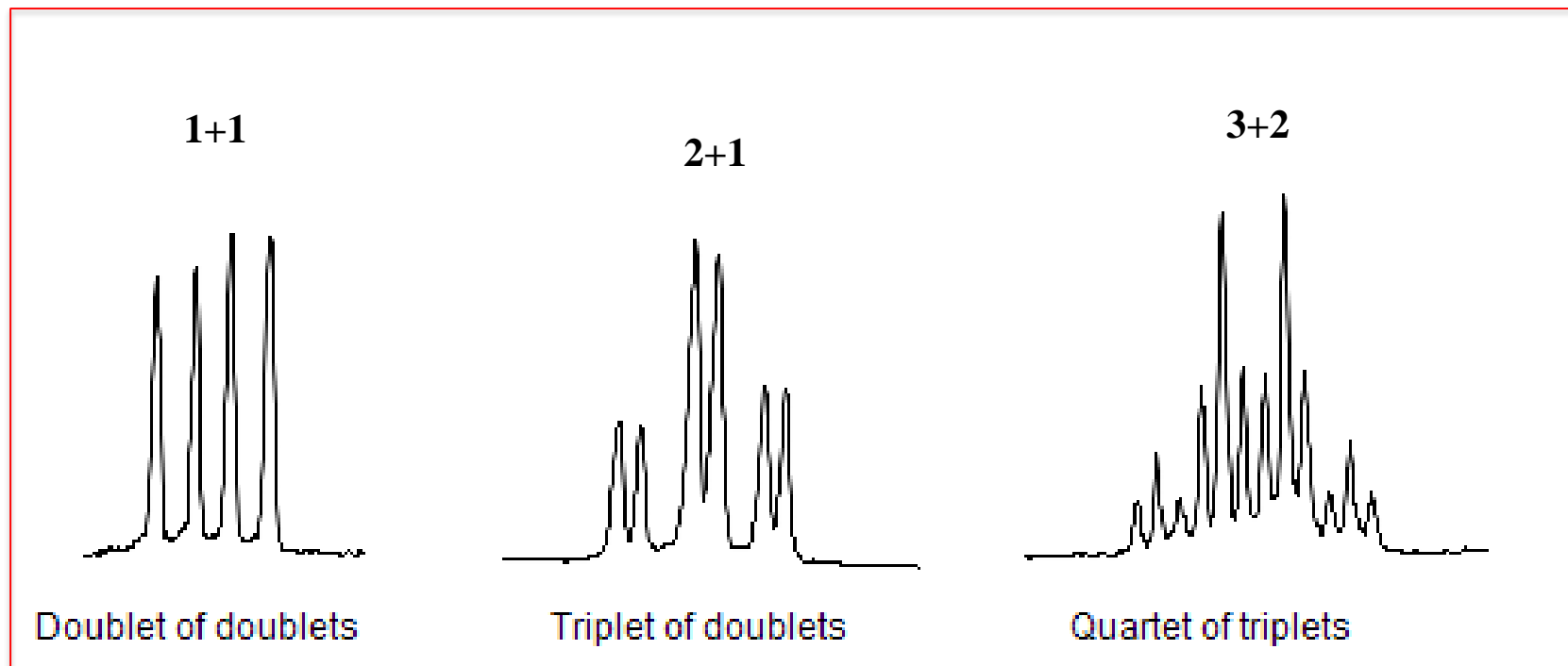
**(N+1 peaks)**

### Nonequivalent Splitting



**(2<sup>N</sup> peaks)**

<http://www2.ups.edu/faculty/hanson/Spectroscopy/NMR/coupling.htm>



Doublet of doublets

Triplet of doublets

Quartet of triplets

**dd**

**td** not a dt

**qt** not a tq

$J$  triplet  $>$   $J$  doublet

$J$  quartet  $>$   $J$  triplet

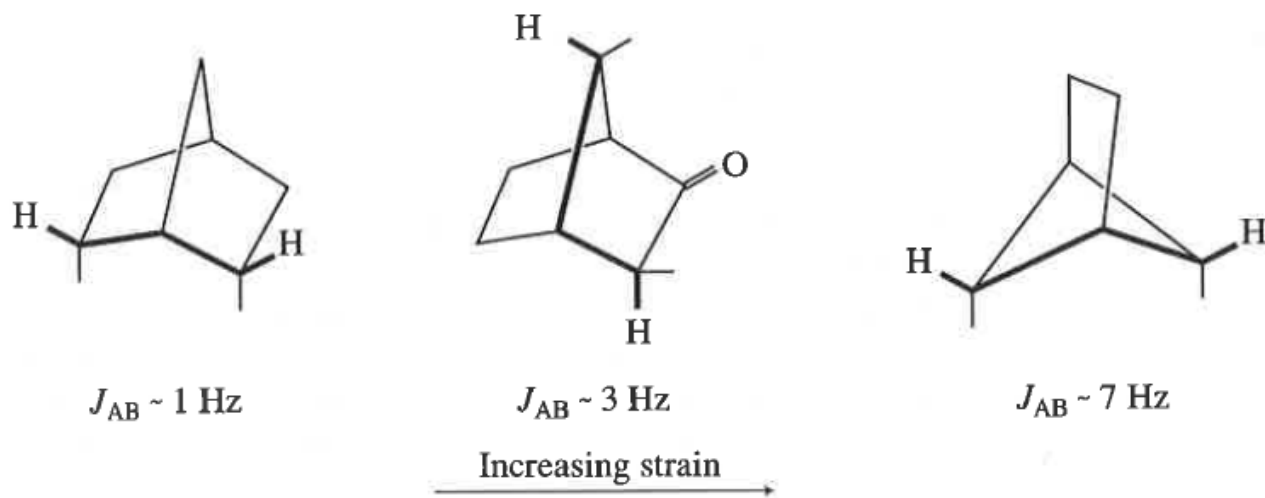
## Typical coupling $J$

Structural Type	$J$ (Hz)
	0 (unless in a rigid ideal orientation)
	6 to 8 $^3J$
	5 to 7 $^3J$
	2 to 12 $^3J$ (depends on dihedral angle and the nature of X and Y)
	0.5 to 3 $^3J$
	12 to 15 $^2J$ (must be diastereotopic)

## $^nJ$ (through $n$ bonds)

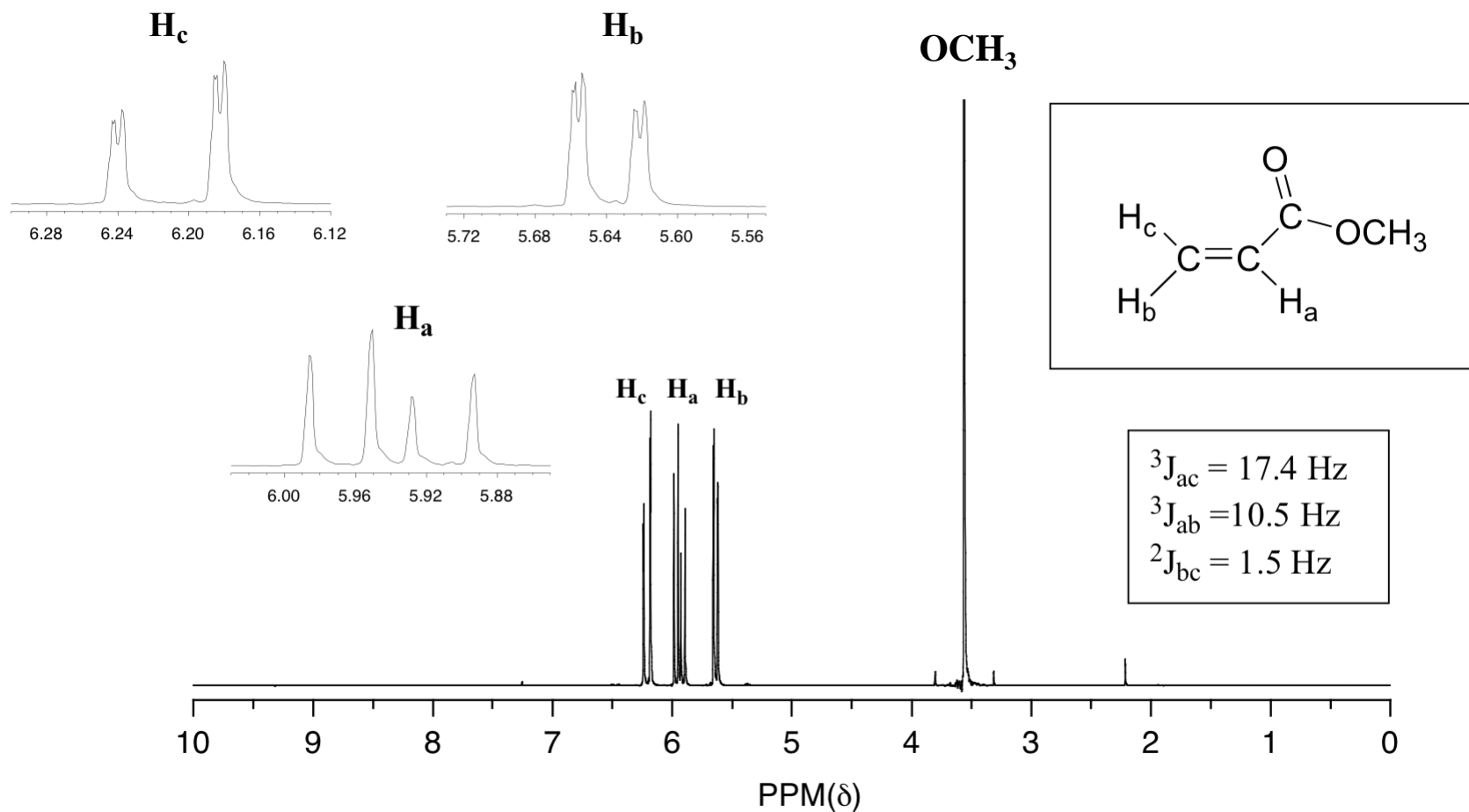
Structural Type	$J$ (Hz)
	12 to 18 $^3J$
	7 to 12 $^3J$
	0.5 to 3 $^3J$
	3 to 11 $^3J$ (depends on dihedral angle)
	2 to 3 $^4J$
	o 6 to 9 $^3J$ m 1 to 3 $^4J$ p 0 to 1 $^5J$

#### $^4J$ homoallylic coupling - W



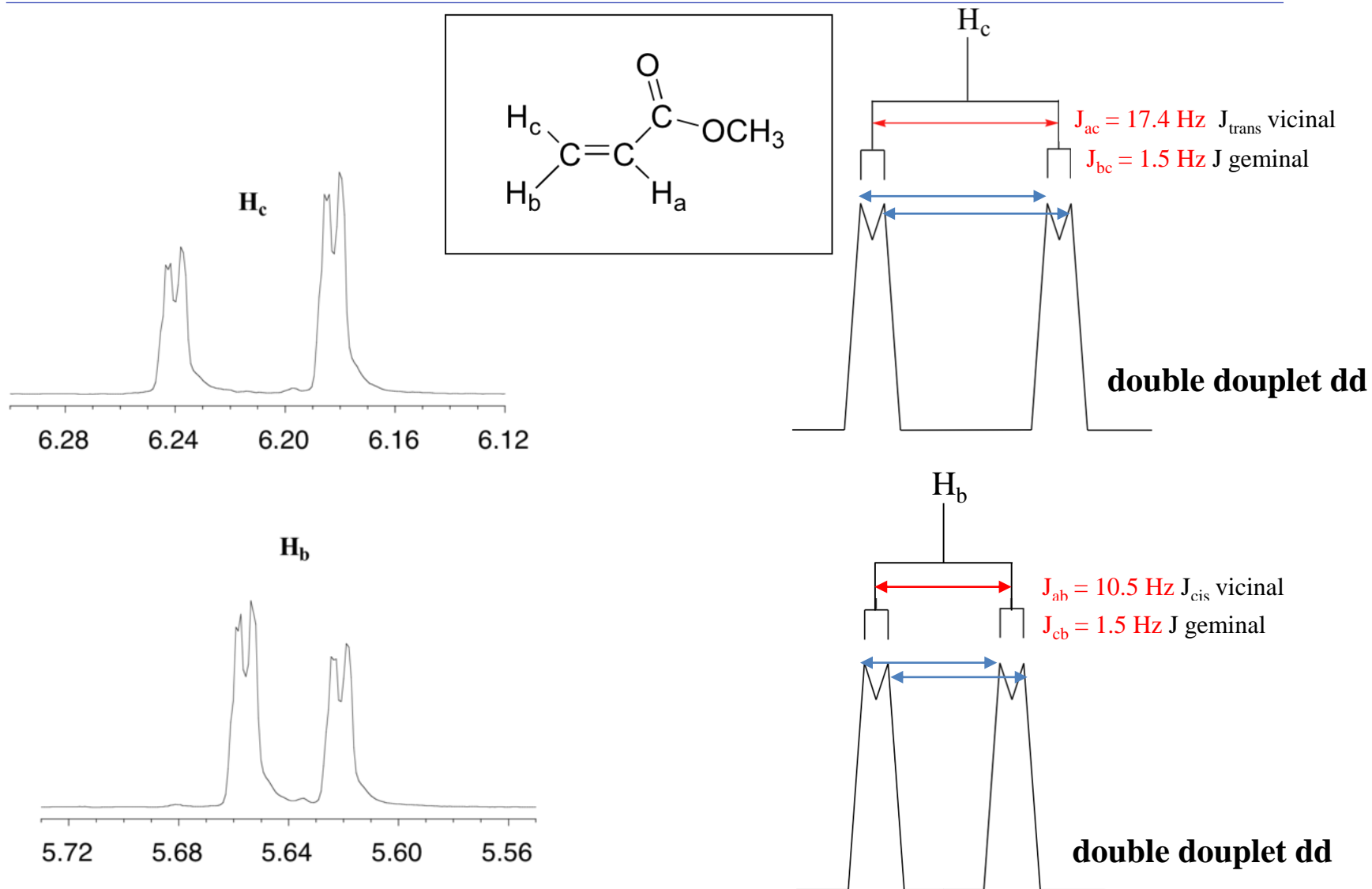
# Summary $^1\text{H}$ - $^1\text{H}$ spin-spin coupling

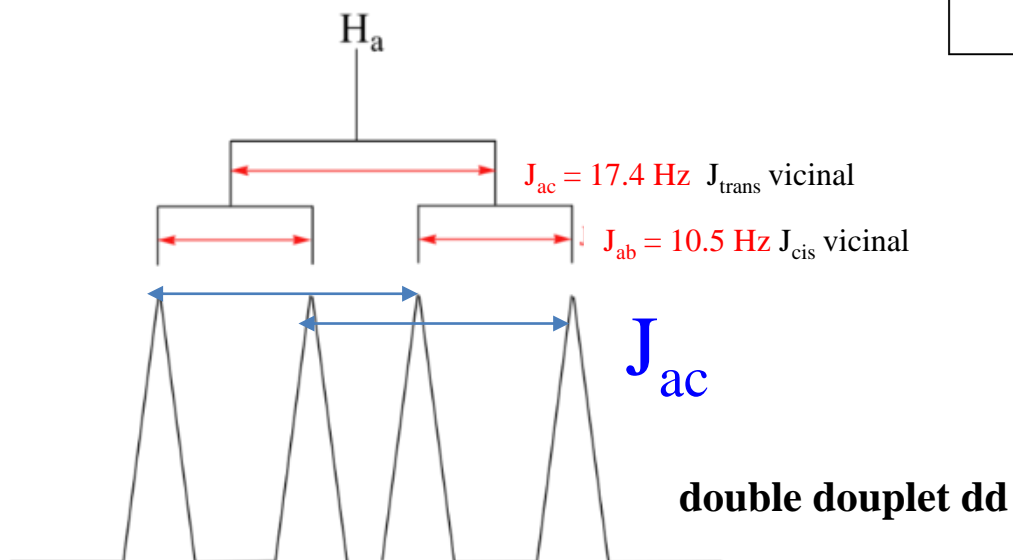
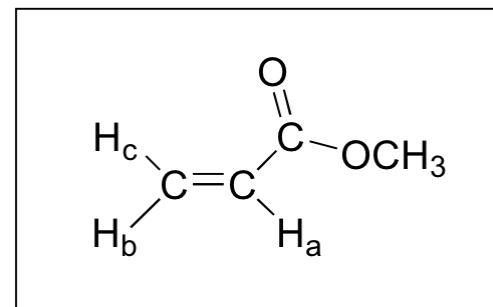
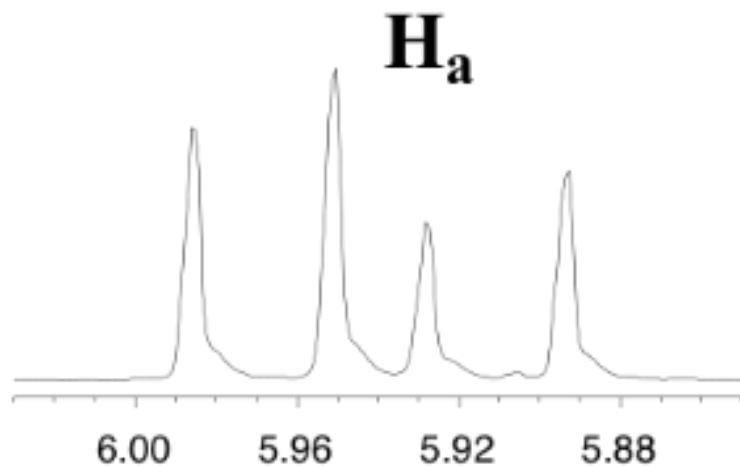
- Equivalent protons do not exhibit spin-spin coupling to each other
- Equivalent protons ( $n$ ) will split the resonance signal of other protons into  $n+1$  peaks (multiplicity) with a coupling constant  $\mathcal{J}$
- Protons that are coupled each other have the same  $\mathcal{J}$
- Non equivalent ( $n$ ) protons will split the resonance signal of other protons independently into  $2^n$  peaks (complex multiplicity) with  $n$  different  $\mathcal{J}$
- Spin-spin coupling is normally observed between nuclei that are 1, 2 and 3 bonds away. But in certain situations can be 4 or even 5!

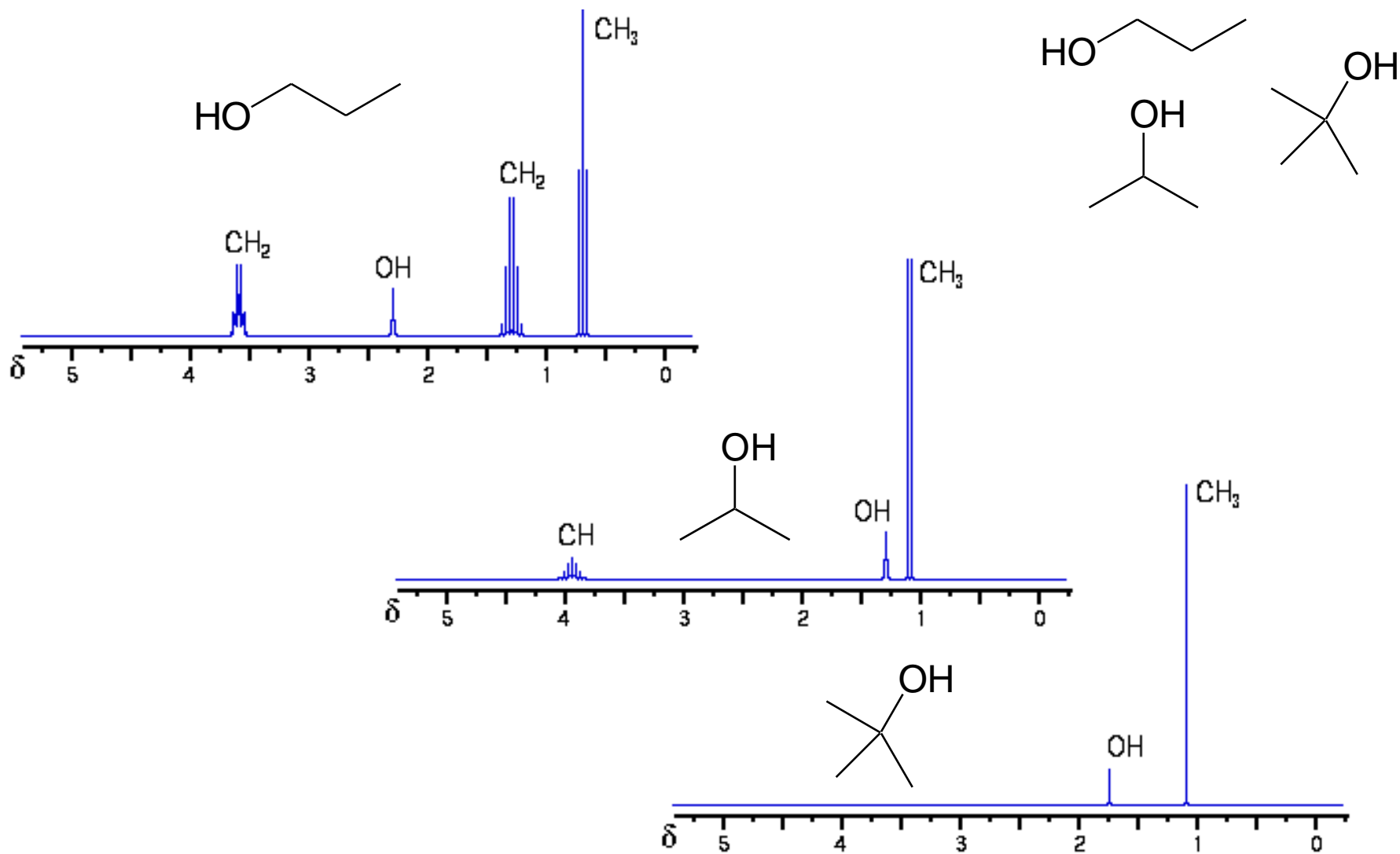


[http://chemwiki.ucdavis.edu/Organic\\_Chemistry/Organic\\_Chemistry\\_With\\_a\\_Biological\\_Emphasis/Chapter\\_5%3A\\_Structure\\_Determination\\_II/Section\\_5.5%3A\\_Spin-spin\\_coupling](http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_Biological_Emphasis/Chapter_5%3A_Structure_Determination_II/Section_5.5%3A_Spin-spin_coupling)



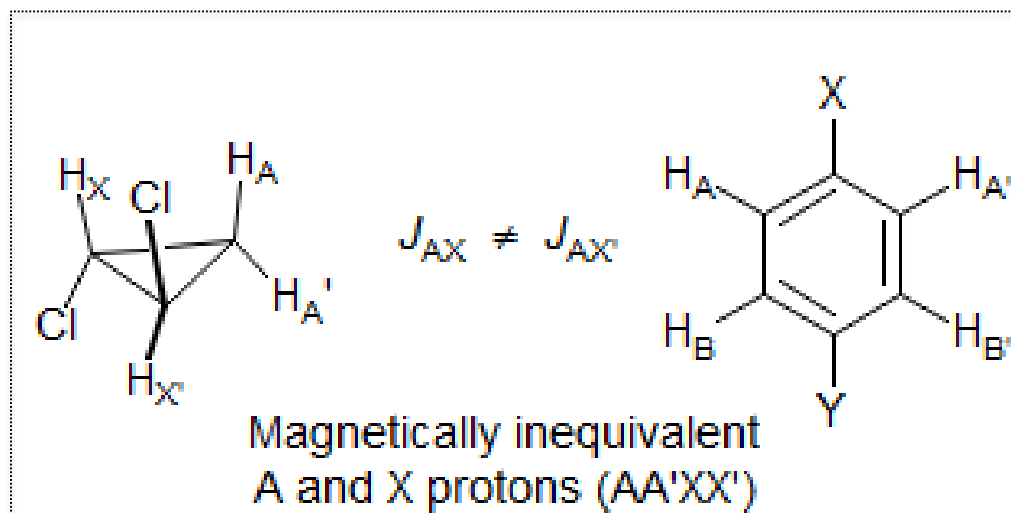
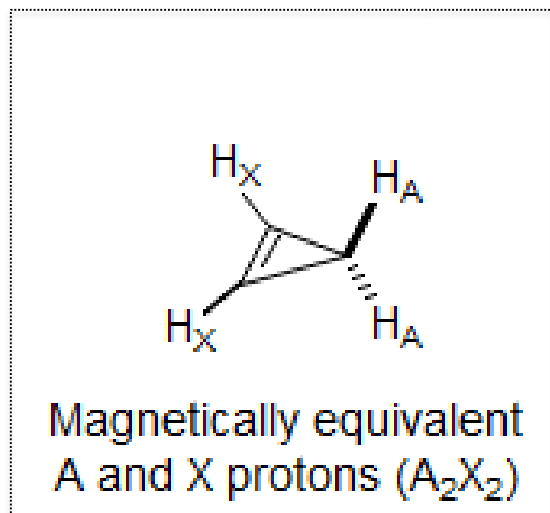


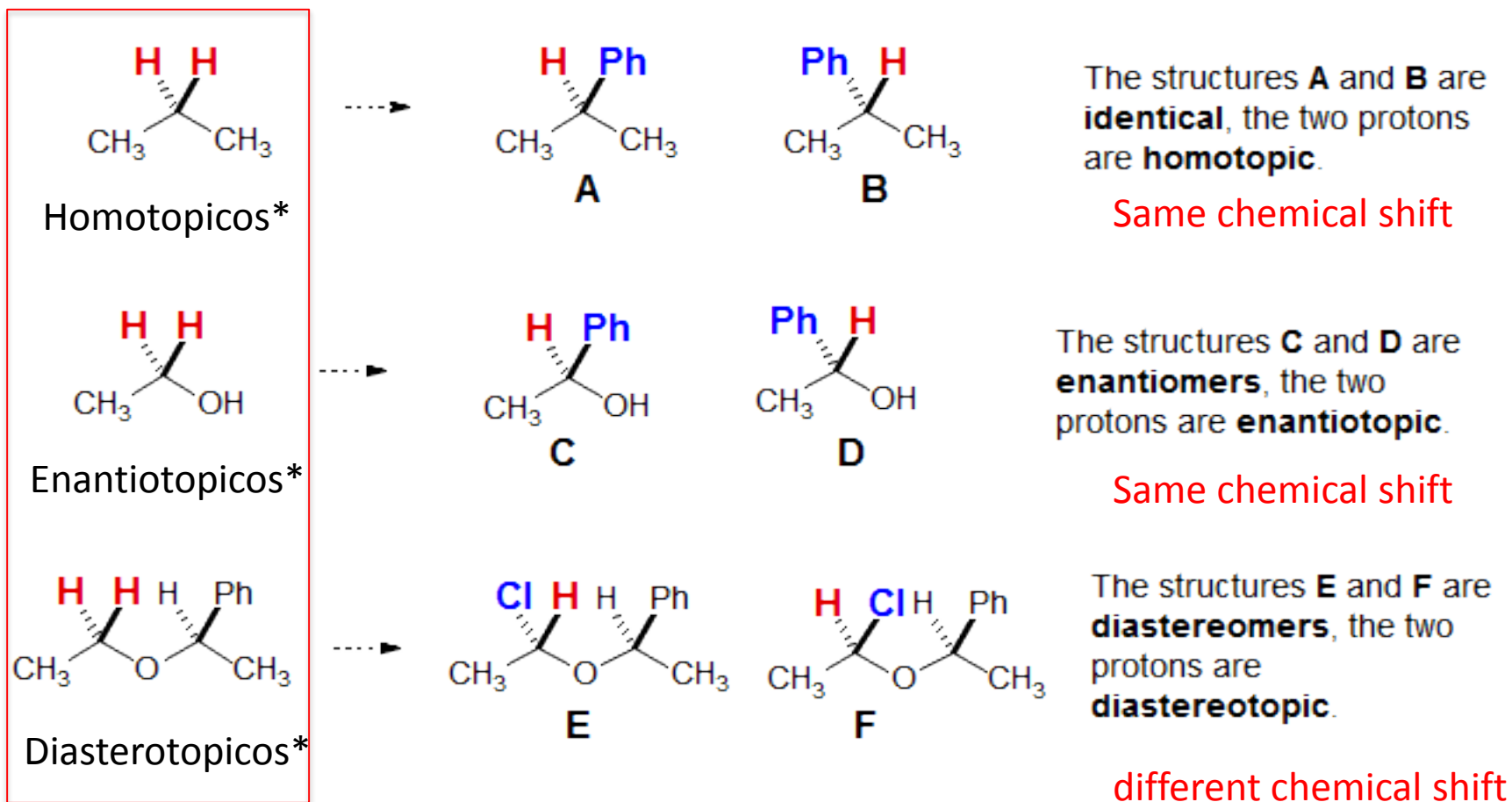




## Magnetic equivalence/Chemical equivalence

A group of protons is **magnetically equivalent** when they have the same chemical shift (**chemical equivalence**) and **identical spin-spin coupling** (same  $J$ ) to other magnetic nuclei in the molecule.





\*in Portuguese

## Coupled Spin Systems - Pople Nomenclature

A, B, C, D – nucleus strongly couple ( $\Delta\nu / J < 10$ ) (consecutive letters)

A, X, M – nucleus weakly couple (break in the alphabet)

$A_2, X_2$  – subscripts give the number of nucleus magnetically equivalent

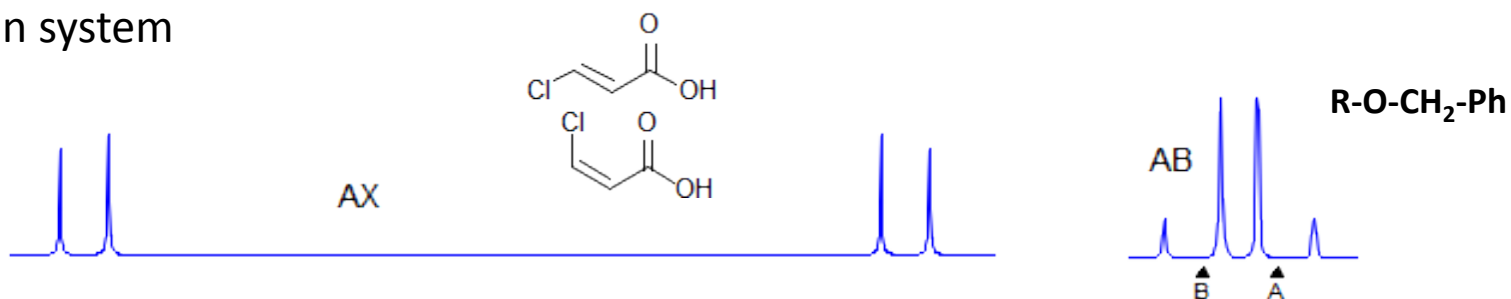
$AA', XX'$  – prime denote nucleus chemically equivalent but magnetically nonequivalent

$\Delta\nu / J$  (all in Hz) is  $> 5$  first order

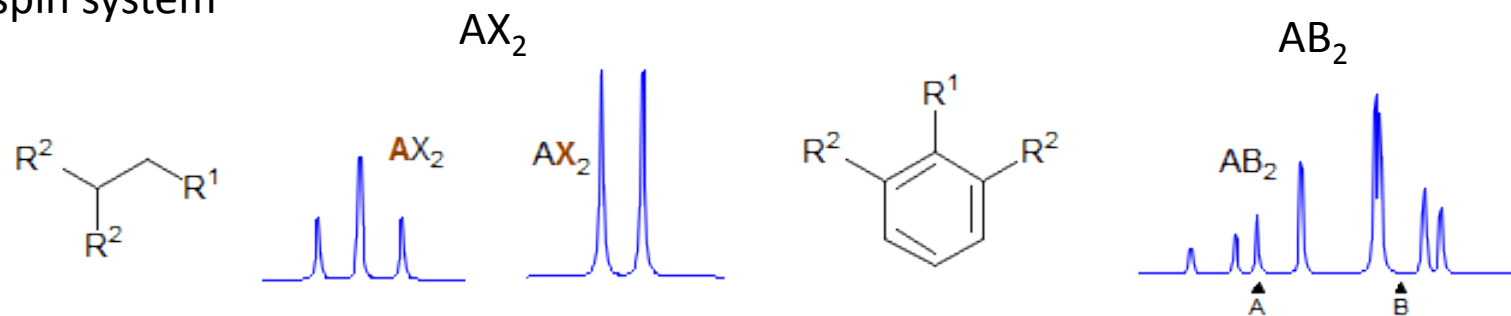
$\Delta\nu / J$  (all in Hz) is  $< 5$  then **second order**

$\Delta\nu / J < 1$  cannot assume first order behavior

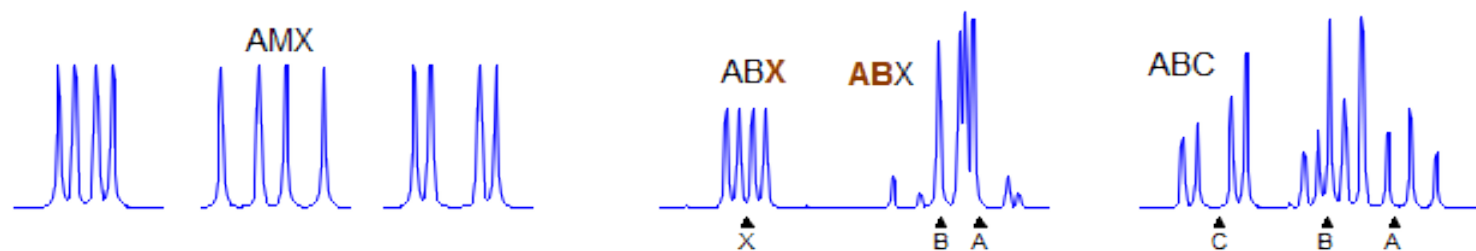
## 2-spin system



## 3-spin system

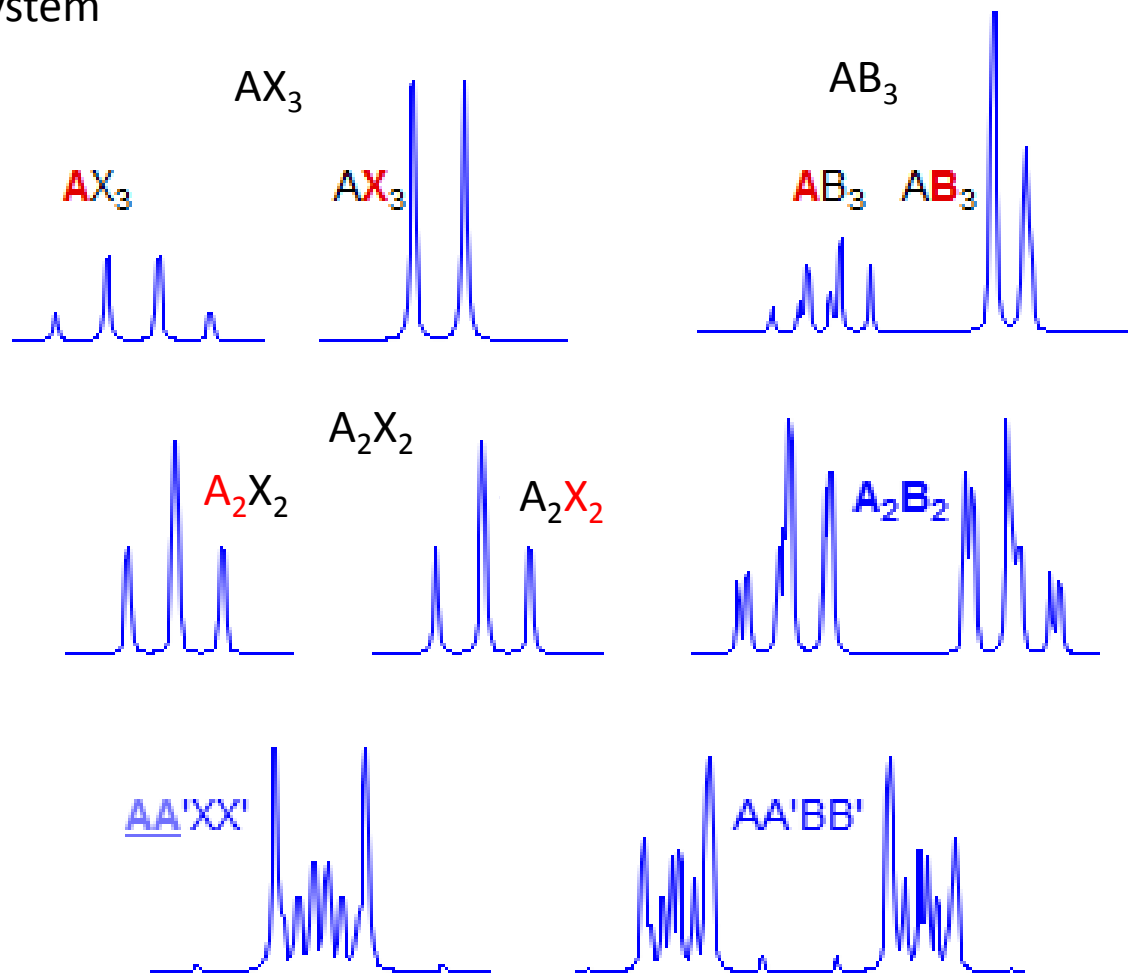


## 3-spin system



<https://www.chem.wisc.edu/areas/reich/chem605/index.htm>

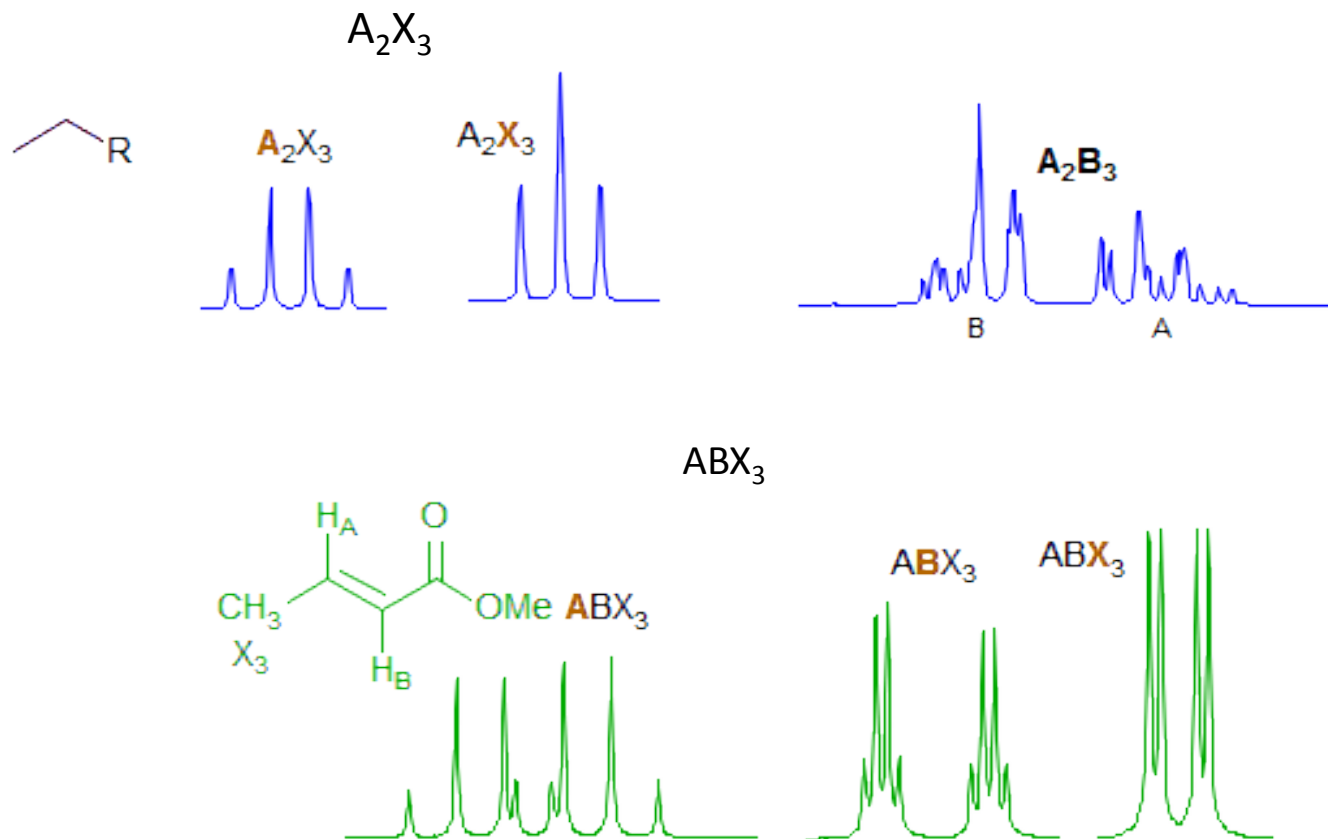
## 4-spin system



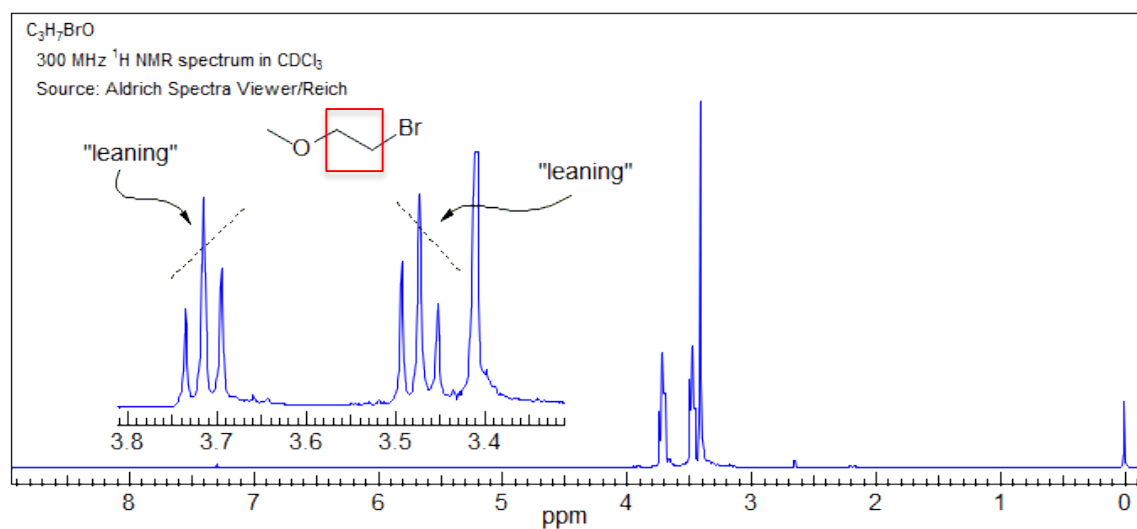
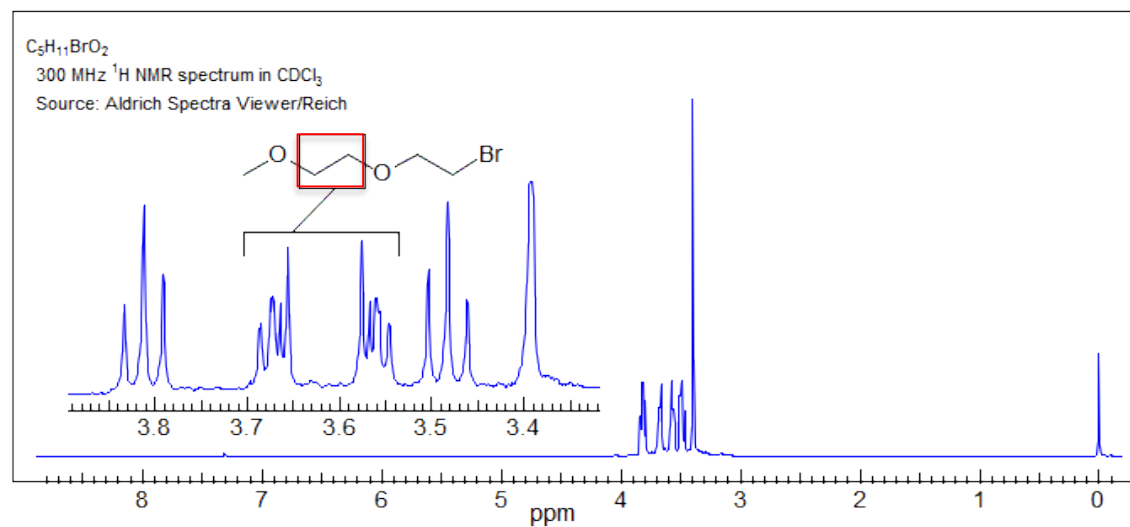
<https://www.chem.wisc.edu/areas/reich/chem605/index.htm>



## 5-spin system



<https://www.chem.wisc.edu/areas/reich/chem605/index.htm>

$A_2X_2$ 

 $A_2B_2$ 


$2D/\Delta\nu$  is the error in the measured chemical shift  $\nu_{AB}$ .

AB Quartet with increasing  $\nu_{AB}$   
Constant  $J_{AB} = 10$  Hz  
all printed at **same Hz scale**

AB Quartet with  $J_{AB} = 10$  Hz and  $\nu_{AB} = 0.1$  ppm  
at various spectrometer frequencies  
all printed at **same ppm scale**

$\Delta\nu/J$      $2D/\Delta\nu$

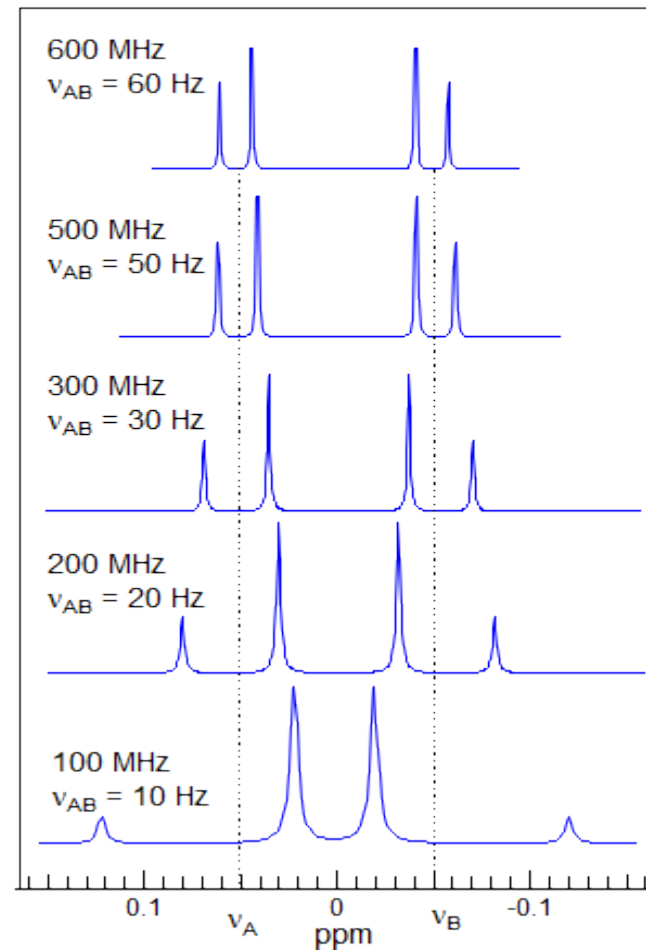
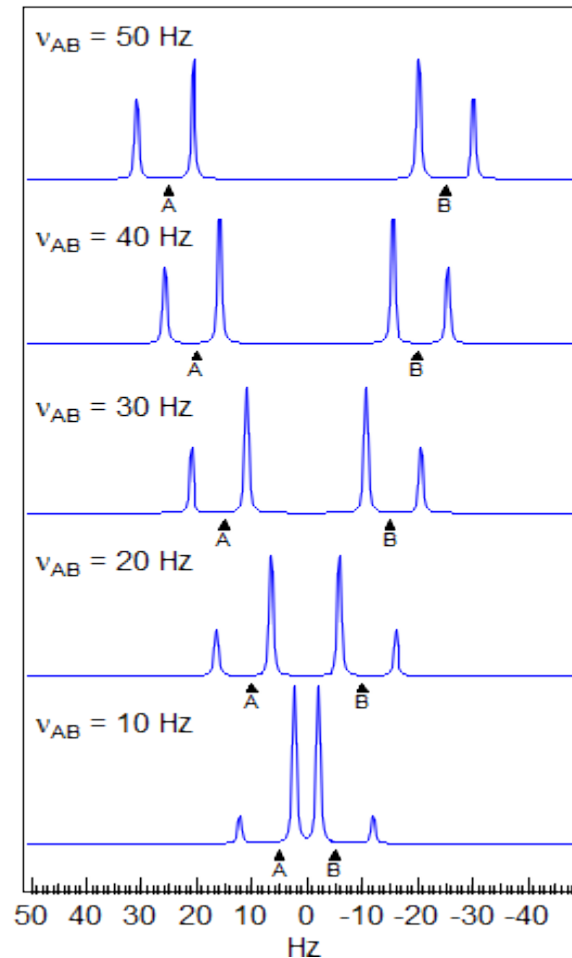
5    1.02

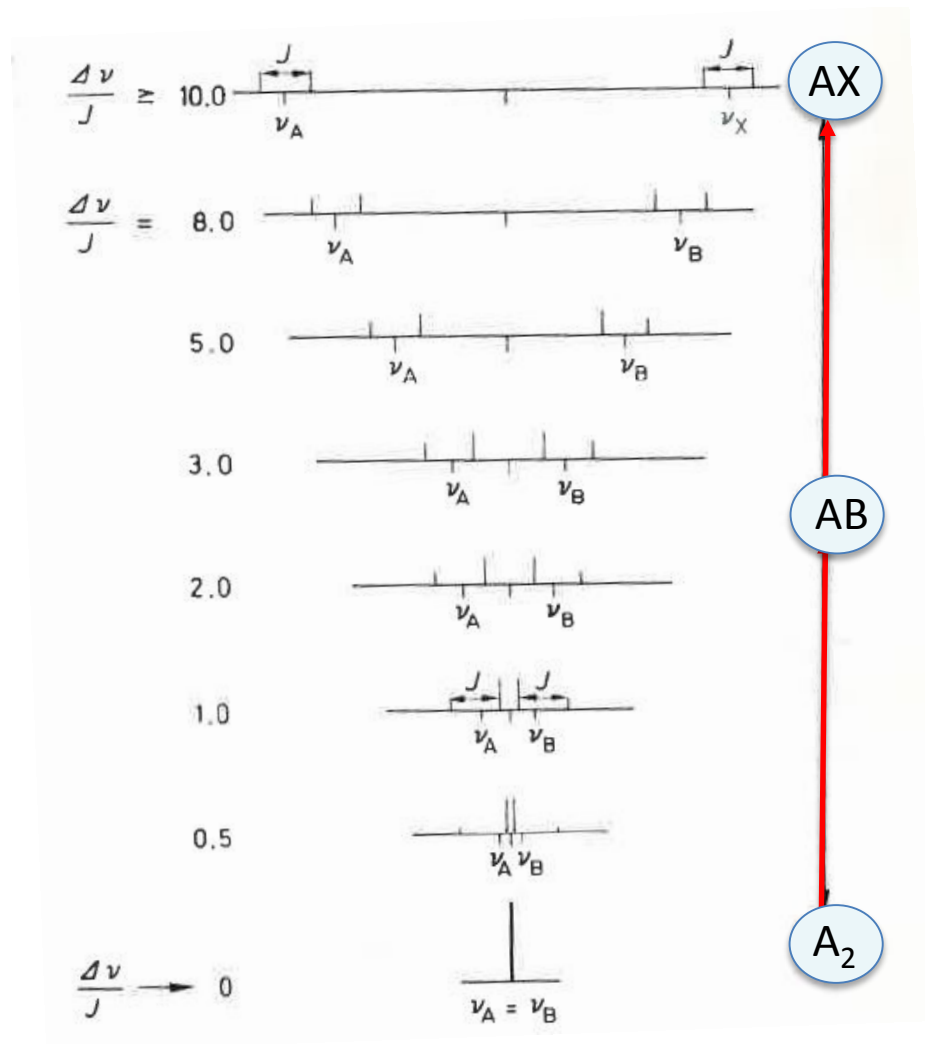
4    1.03

3    1.05

2    1.12

1    1.41





## How to Analyse an AB quartet

Treat the pattern as first order for AB quartets with a large  $\nu_{AB} / J_{AB} > 4$

For low  $\nu_{AB} / J_{AB} < 4$   
Second order analyse

1. Determine the four line positions in Hz, and measure  $J_{AB}$

$$|J_{AB}| = (\nu_1 - \nu_2) = (\nu_3 - \nu_4) = 10 \text{ Hz}$$

2. Calculate the center position (in Hz):

$$\nu_{\text{center}} = \frac{1}{2} (\nu_2 + \nu_3) = 2024.1$$

3. Calculate  $\nu_{AB}$ .

$$\Delta\nu_{AB} = \sqrt{(\nu_1 - \nu_4)(\nu_2 - \nu_3)} = 9.94 \text{ Hz}$$

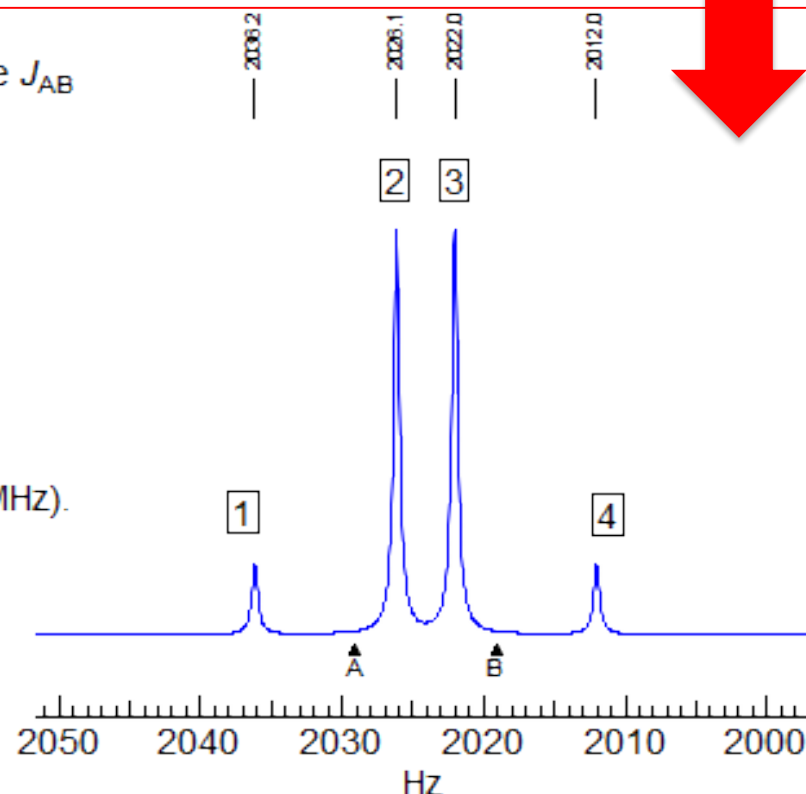
4. Calculate  $\nu_A$  and  $\nu_B$  (spectrometer frequency: 300 MHz).

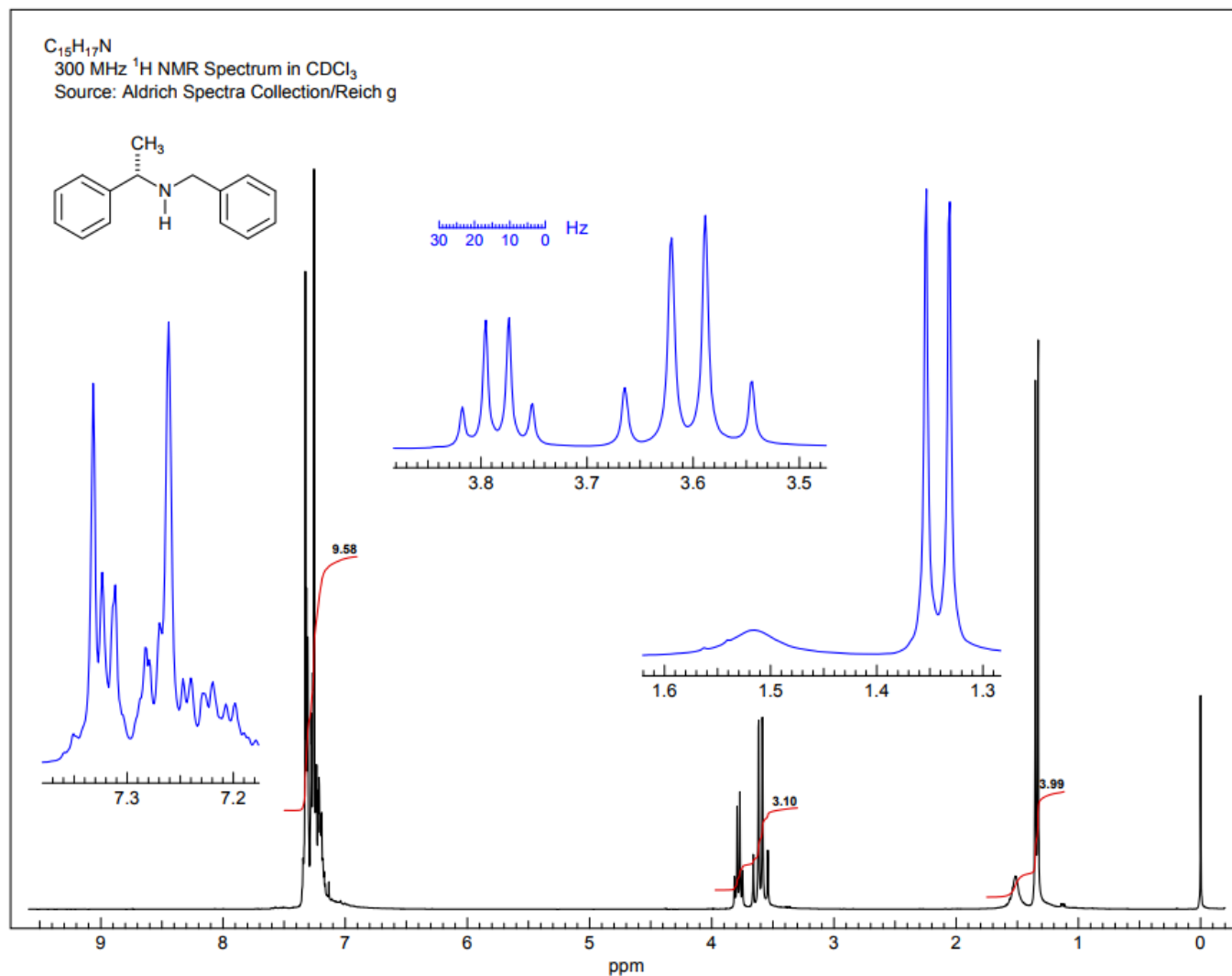
$$\nu_A = \nu_{\text{center}} + \frac{1}{2} \Delta\nu_{AB} = 2029.1 \text{ Hz}$$

$$\nu_B = \nu_{\text{center}} - \frac{1}{2} \Delta\nu_{AB} = 2019.1 \text{ Hz}$$

$$\delta_A = \nu_A / \text{MHz} = 6.76$$

$$\delta_B = \nu_B / \text{MHz} = 6.73$$

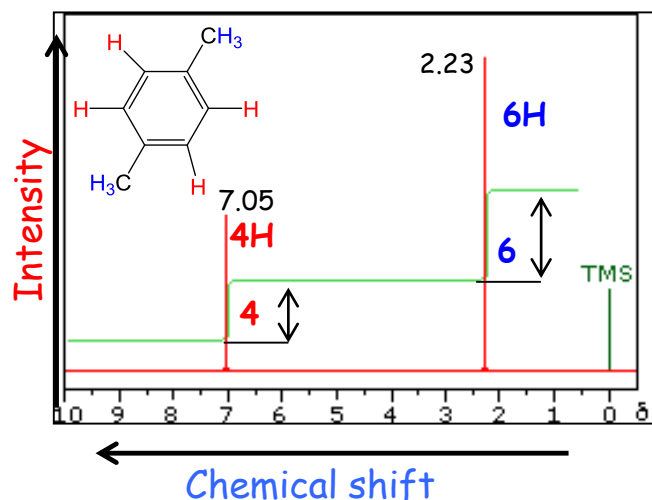




<https://www.chem.wisc.edu/areas/reich/chem605/index.htm>

## Signal integration

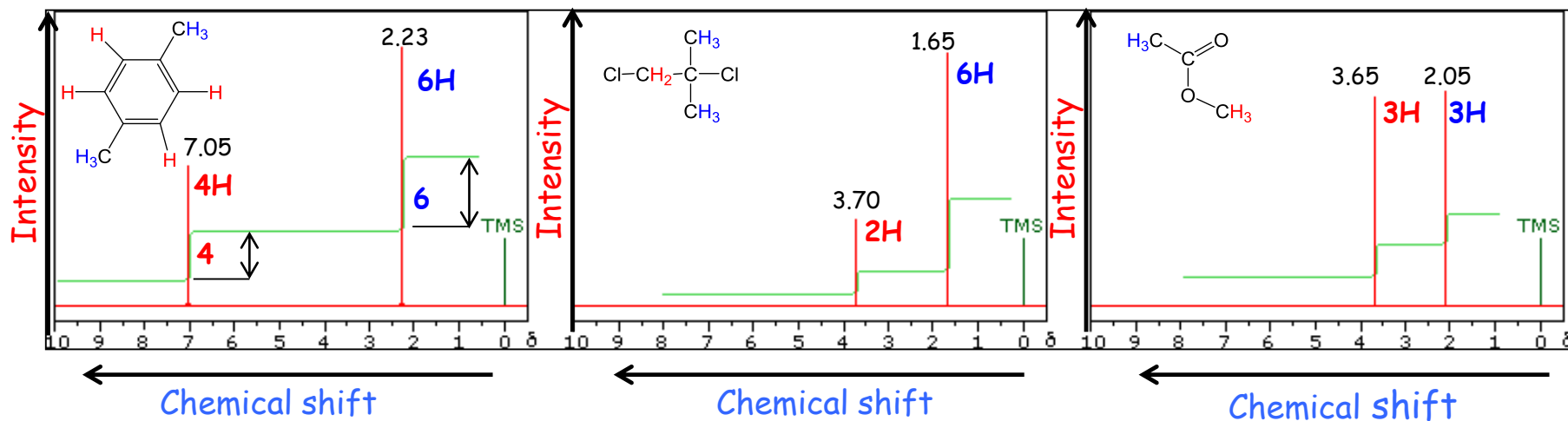
The signal intensity is directly proportional to the number of nuclei causing the signal (all absorption coefficients for a given nucleus are identical).



The rule is that peak area (*integral*) is proportional to the number of a given type of spins in the molecule and in the sample (concentration).

The *integral* is superimposed over the spectrum as a "stair-step" line. The height of each step is proportional to the area.

# Signal integration



The **relative area** under the resonances:

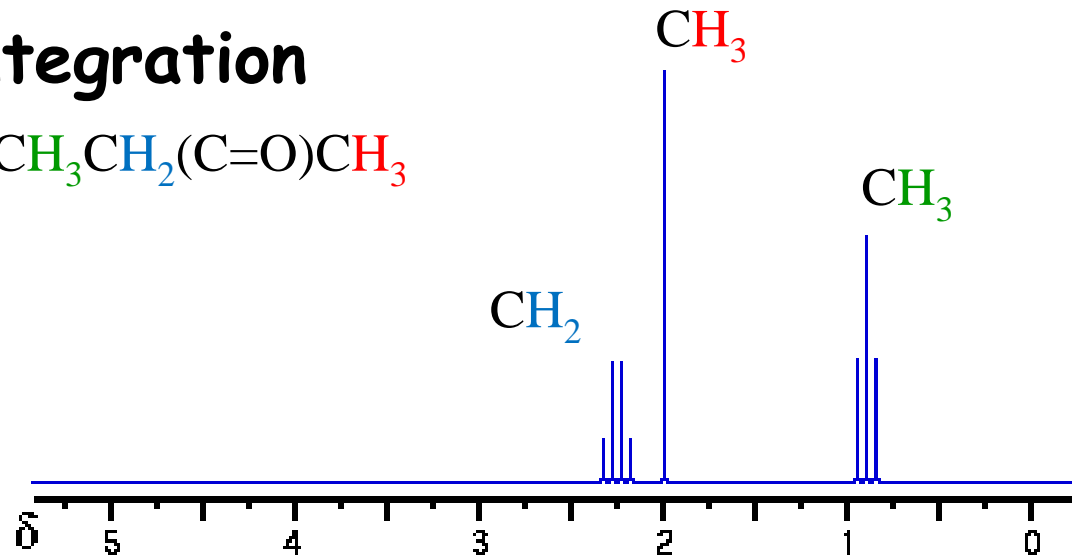
7.05 and 2.23 ppm is 4:6  $\Rightarrow$  **2:3**

3.70 and 1.65 ppm is 2:6  $\Rightarrow$  **1:3**

3.65 and 2.05 ppm is 3:3  $\Rightarrow$  **1:1**



# Signal integration



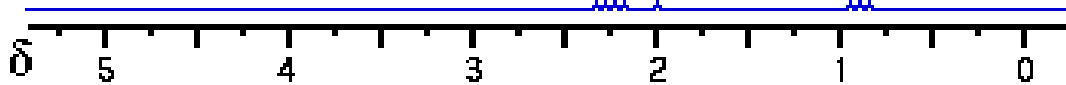
26:39:39

26/26:39/26:39/26

1:1,5:1,5

$\text{CH}_3$  39  
 26  
 $\text{CH}_3$  39

2:3:3

 $\text{CH}_2:\text{CH}_3:\text{CH}_3$ 


## Relative quantification by $^1\text{H}$ NMR

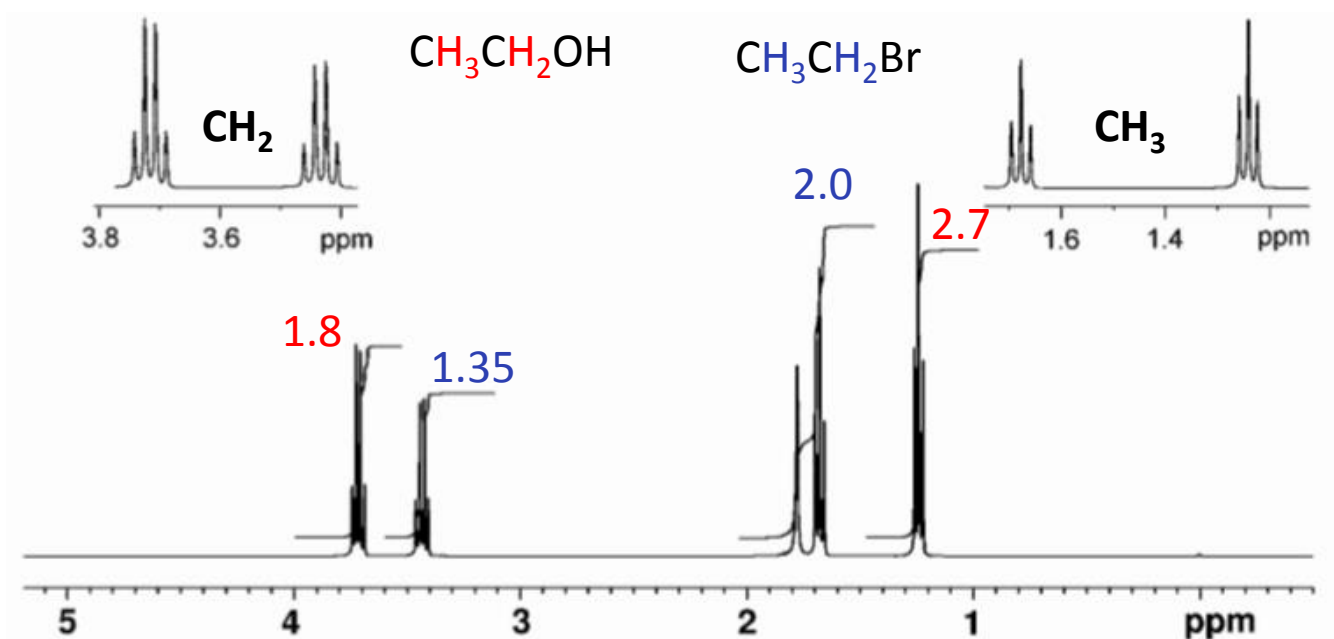
The integration (signal area) is proportional to the number of moles of protons that originates resonance peak

$$\frac{I_{\text{compound 1}}}{I_{\text{compound 2}}} = \frac{n_{\text{compound 1}}}{n_{\text{compound 2}}} \times \frac{n_{\text{compound 1}}^{\text{H}}}{n_{\text{compound 2}}^{\text{H}}}$$

$$n = m \text{ (g)}/M \text{ (mol/g)}$$

$$\frac{m_1}{m_2} = \frac{I_1}{I_2} \times \frac{n_2^{\text{H}}}{n_1^{\text{H}}} \times \frac{M_2}{M_1}$$

Determine the %molar of each compound in the mixture



$$1,8/2 = 0.9 \text{ mol ethanol}$$

$$1,35/2 = 0.68 \text{ mol bromoethane}$$

$$\% \text{ etanol} = 0.9/(0.9+0.68) \times 100 = 57\%$$

CH<sub>3</sub>

$$2.7/3 = 0.9 \text{ mol ethanol}$$

$$2.0/3 = 0.68 \text{ mol bromoethane}$$

$$\% \text{ Bromoethane} = 0.68/(0.9+0.68) \times 100 = 43\%$$

## Exchangeable protons

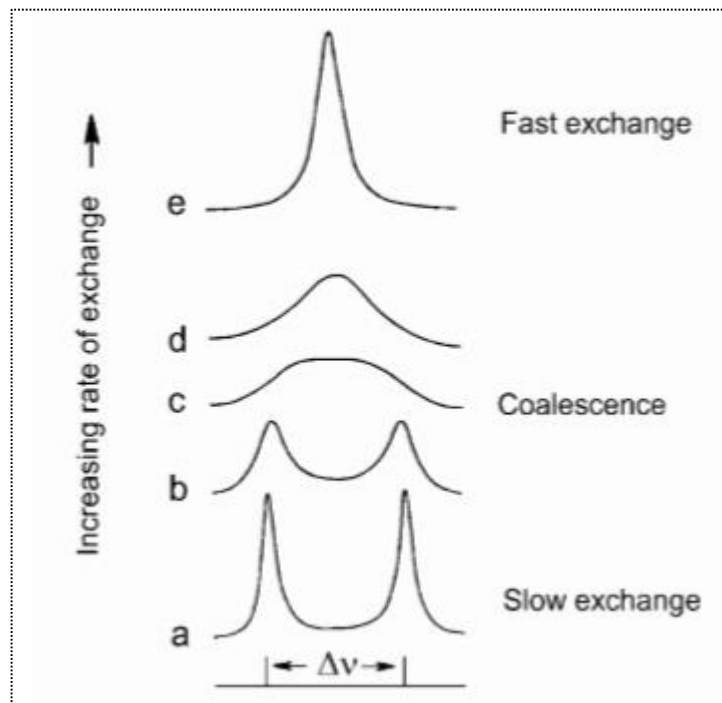
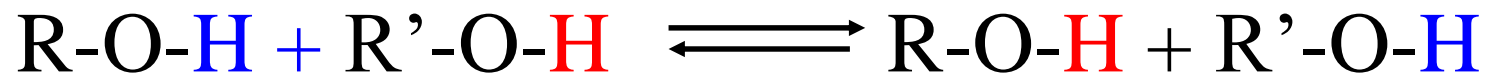
Alcohols (R-OH), amines (R-NH-), carboxylic acids (RCOOH), thiols (R-SH) and amides (R-CO-NH-) are labile protons

- Broadened peaks (line widths enhance)
- Chemical shifts are critical dependent  
(solvent, concentration and temperature)

“ Labile protons do not have characteristic chemical shift ranges”

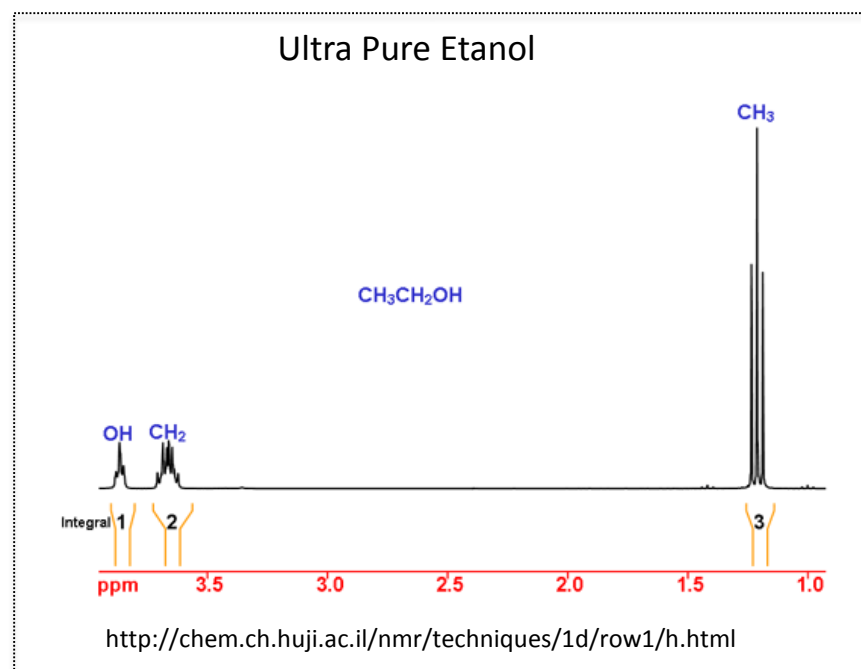
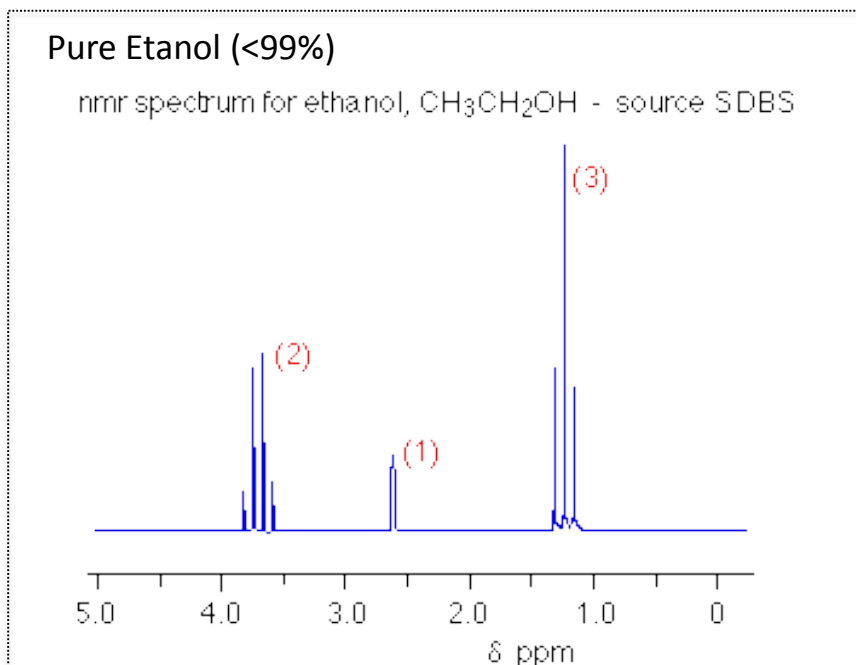
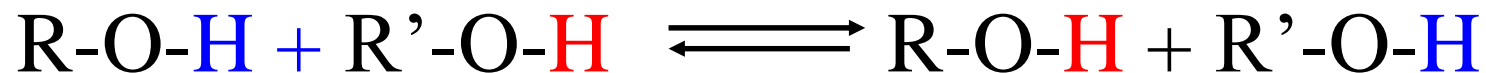
<https://www.chem.wisc.edu/areas/reich/nmr/05-hmr-02-delta.htm>

## Exchangeable protons



Intermolecular interchange  
(broadened the peaks, lost of multiplicity)

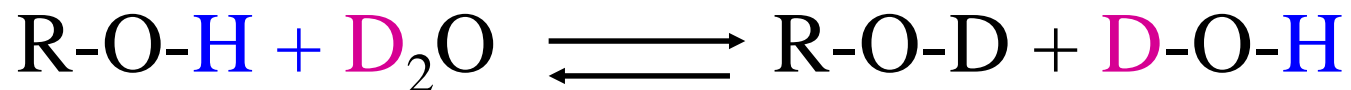
## Exchangeable protons



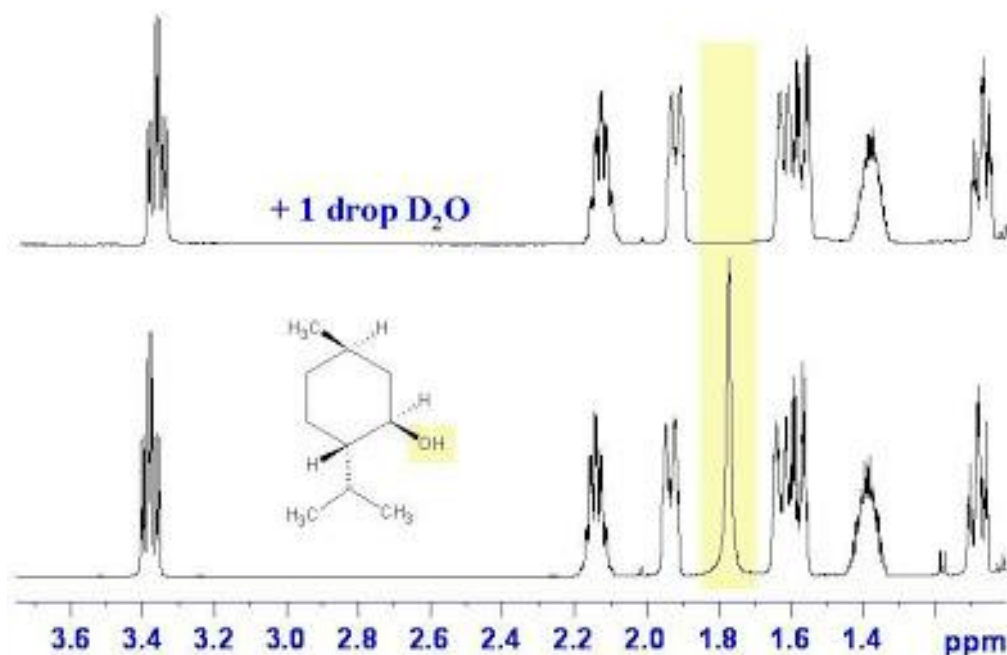
Water promotes a faster intramolecular interchange – lost of multiplicity

[http://www.facstaff.bucknell.edu/drovnyak/Ethanol-Water\\_exp.pdf](http://www.facstaff.bucknell.edu/drovnyak/Ethanol-Water_exp.pdf)

## Exchangeable protons



Partial  $^1\text{H}$  NMR Spectrum of Menthol in  $\text{CDCl}_3$



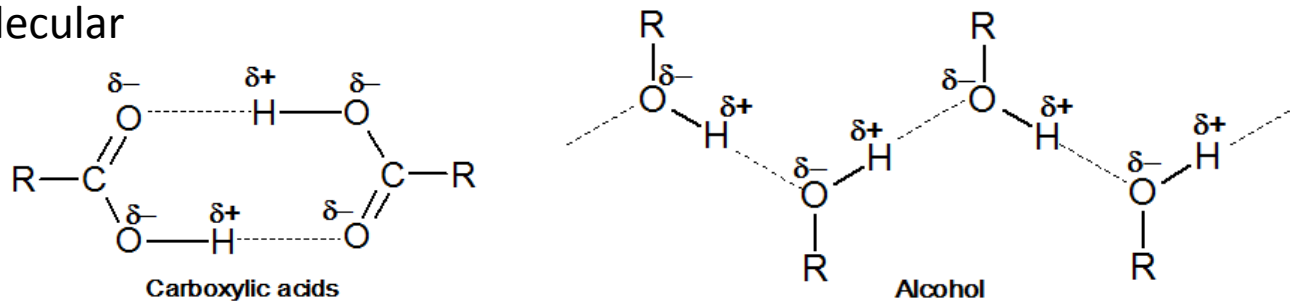
To identify labile protons

OH peak disappears  
after  $\text{D}_2\text{O}$  shake

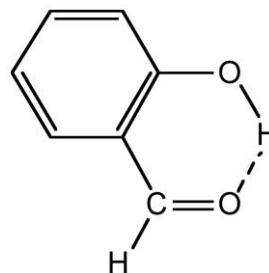
# Exchangeable protons

Hydrogen bonding shifts the resonance signal of a proton to lower field

intermolecular



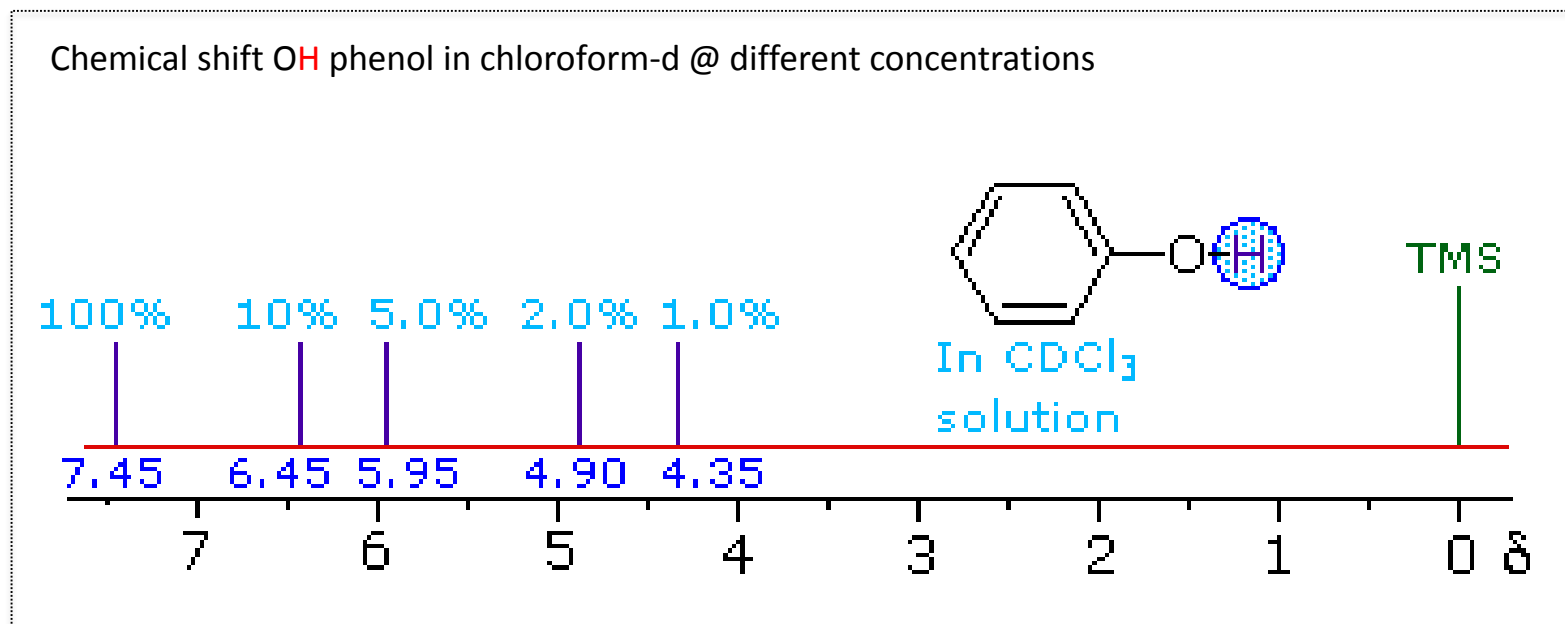
intramolecular



High concentration => more hydrogen bonding => high chemical shift

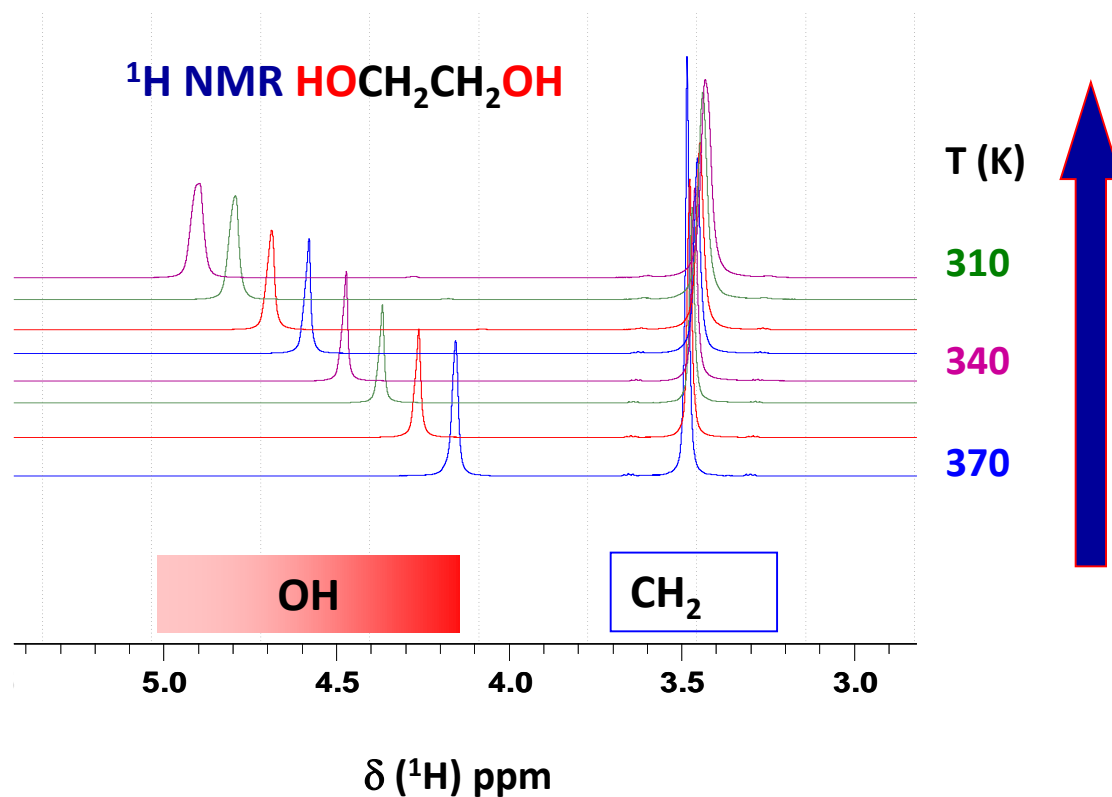


Hydrogen bonding shifts the resonance signal of a proton to lower field



High concentration  $\Rightarrow$  more hydrogen bonding  $\Rightarrow$  high chemical shift

# Exchangeable protons



High Temperature => less hydrogen bonding => lower chemical shift

## Heteronuclear coupling

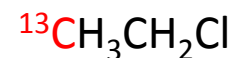
Spin-spin coupling takes place between all NMR active nuclei, not just between protons.

### Coupling of $^1\text{H}$ to $^{13}\text{C}$

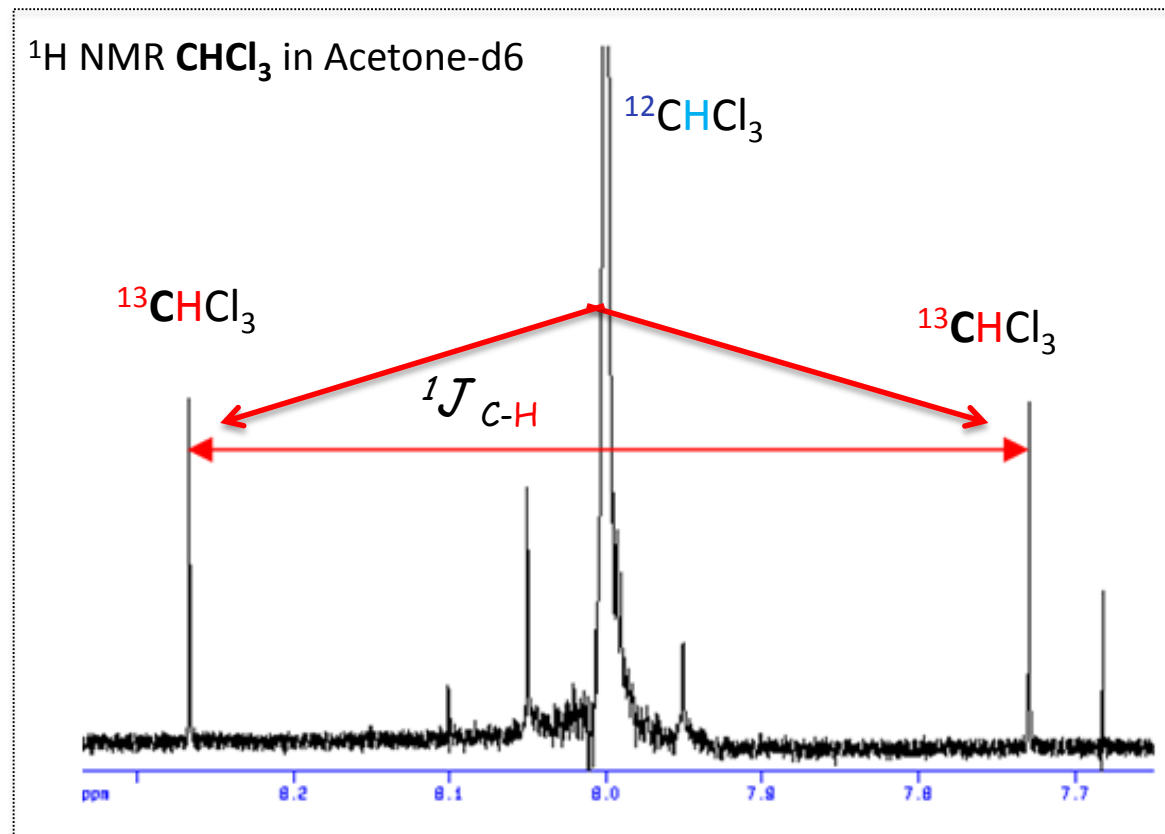
$^{13}\text{C}$  has a natural abundance just over 1% and the major isotope ( $^{12}\text{C}$ ) is not NMR active so very little of the proton signal is coupled. The coupled signal appears as small satellite signals either side of the main uncoupled signal.

$^1J_{\text{C-H}}$  coupling constants = 115-250 Hz (usually 125 to 160 Hz)

**Isotopomers** - compounds having different isotopes of the same element or having the same isotope in a different position



## $^{13}\text{C}$ Satellites

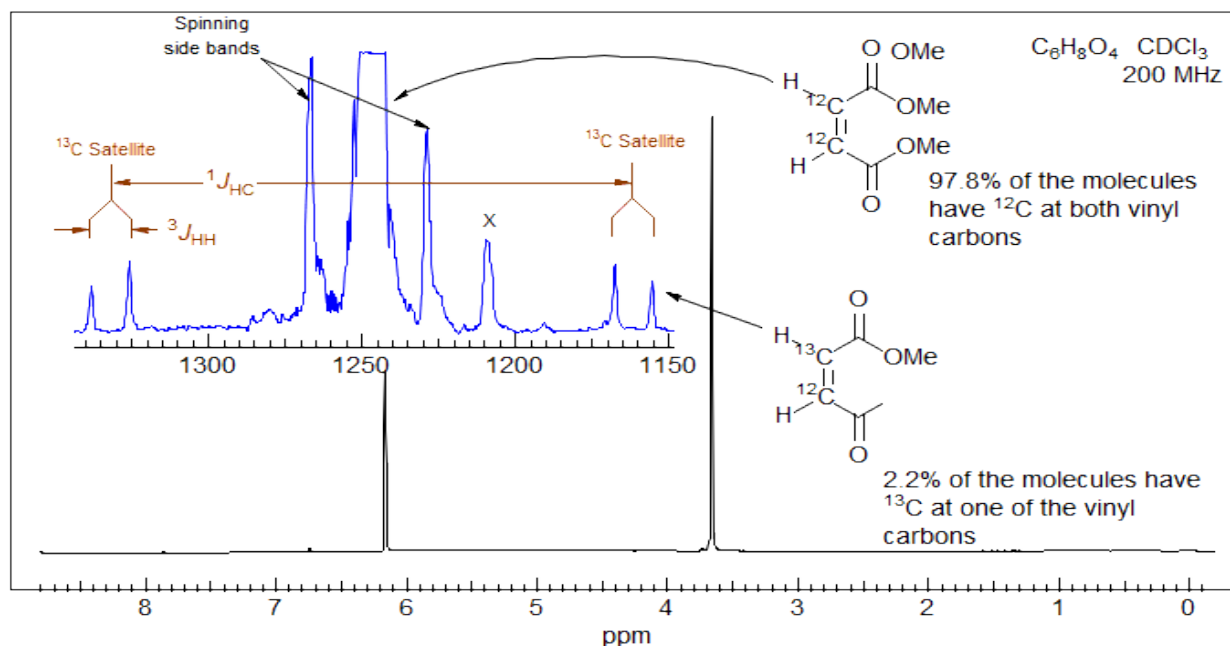


Isotopomer of the main peak	$^{13}\text{CHCl}_3$	d
main peak	$^{12}\text{CHCl}_3$	s

## $^{13}\text{C}$ Satellites

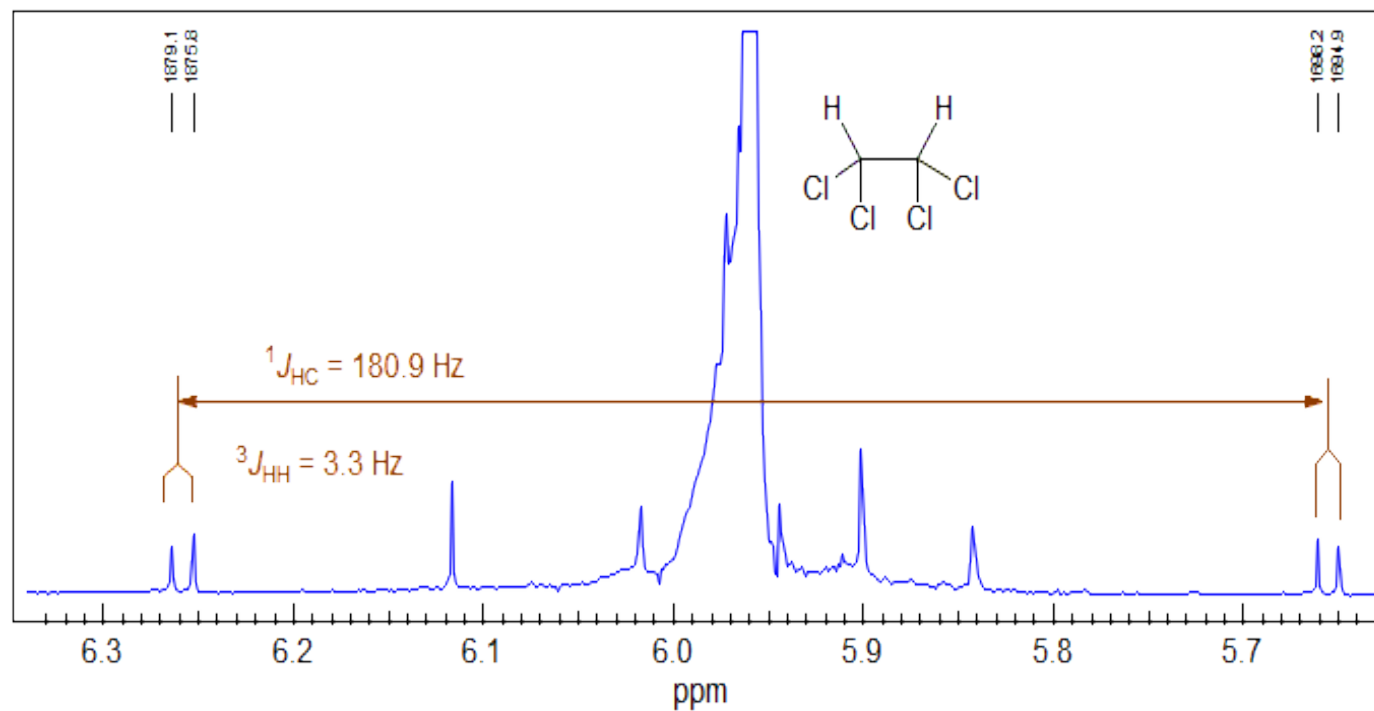
Vicinal couplings between homotopic or enantiotopic protons  $^3J_{\text{HH}}$  can often be obtained by analysis of the  $^{13}\text{C}$  satellites.

$A_2$  spin system of the  $^{12}\text{C}$  isotopomer has become an ABX pattern in the mono- $^{13}\text{C}$  labelled compound, where X is the  $^{13}\text{C}$  nucleus, and A and B are the two vinyl protons, one on  $^{13}\text{C}$  and the other on  $^{12}\text{C}$ .



<https://www.chem.wisc.edu/areas/reich/nmr/05-hmr-03-jcoupl.htm>

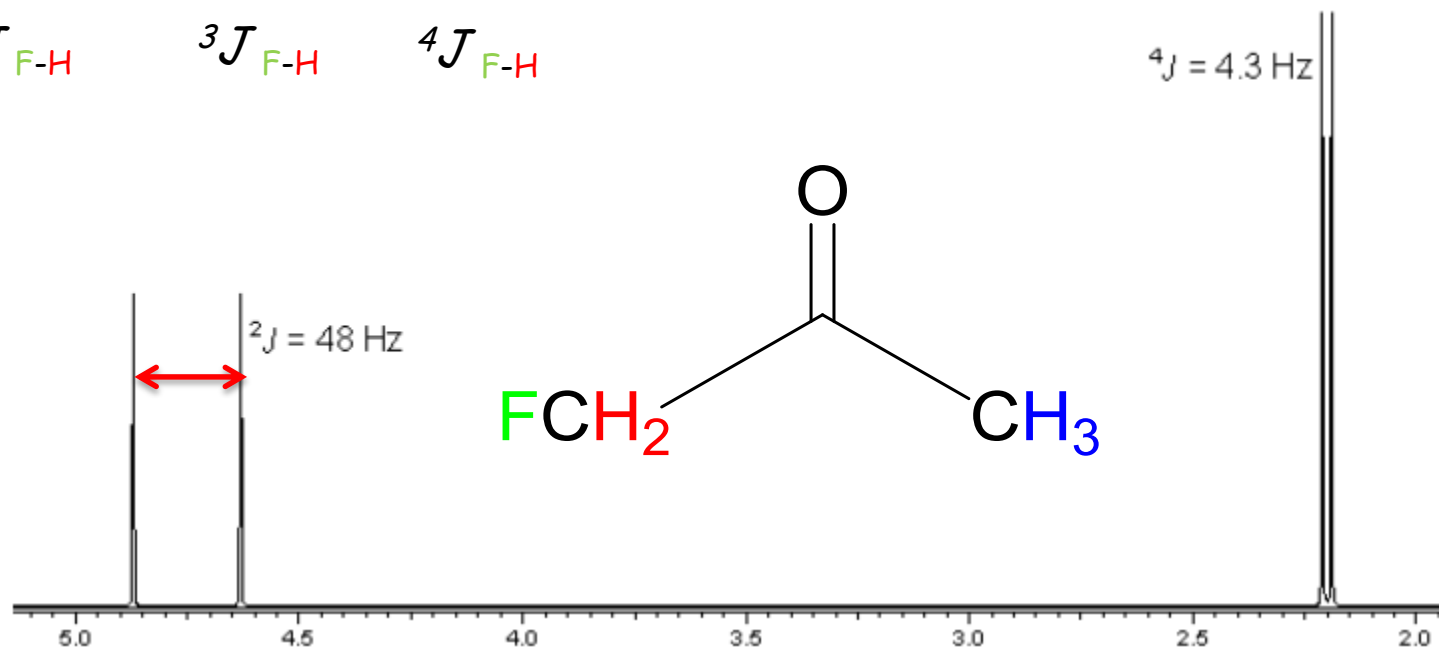
## $^{13}\text{C}$ Satellites



## Coupling of $^1\text{H}$ to $^{19}\text{F}$

Natural abundance 100%  $I=1/2$

$^2J_{\text{F-H}}$      $^3J_{\text{F-H}}$      $^4J_{\text{F-H}}$



## Coupling of $^1\text{H}$ to D

Number spin states =  $2I + 1$

$I=1$  (2H deuterium D), 3 spin states

Almost all deuterated solvents are not 100%

Signal Multiplicity =  $2nI + 1$

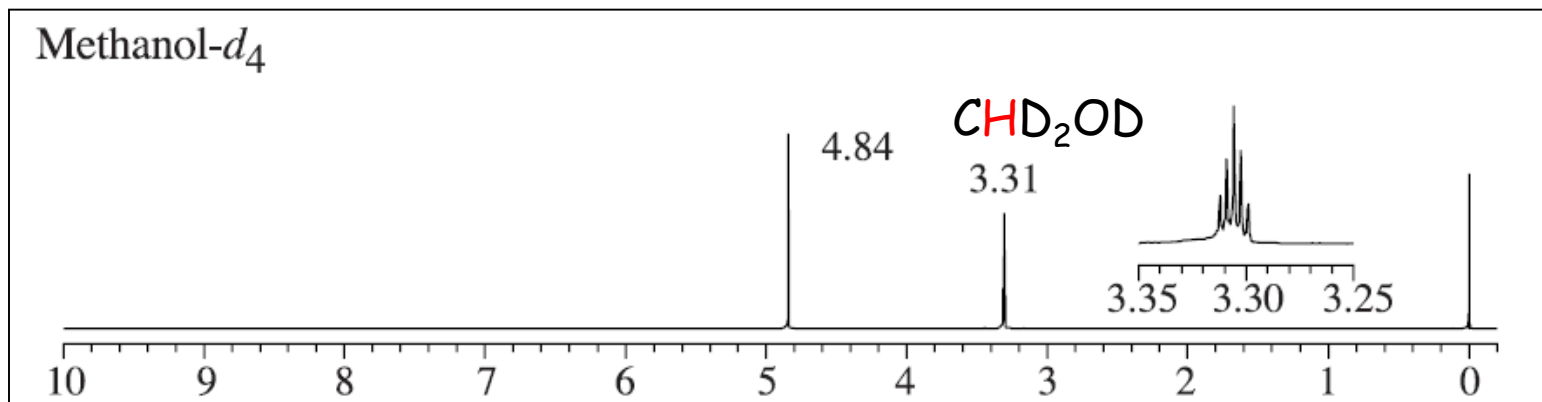
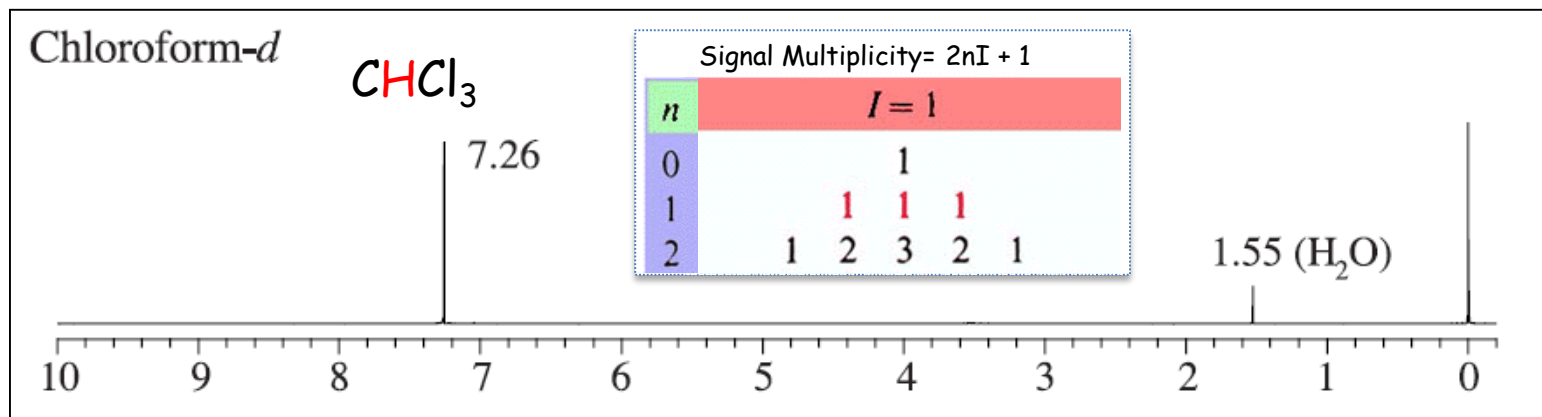
$n$	$I = 1$							
0	1							
1	1 1 1							
2	1	2	3	2	1			
3	1	3	6	7	6	3	1	
4	4	10	16	19	16	10	4	1

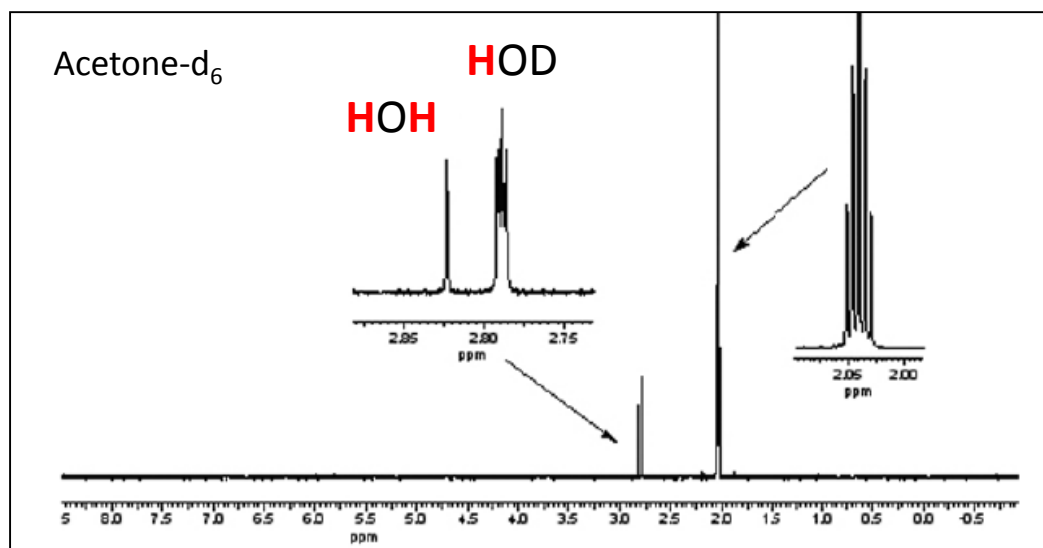
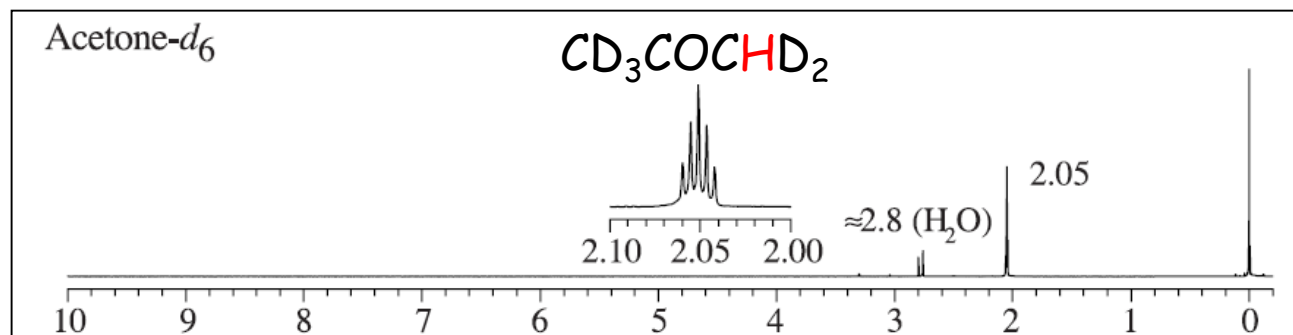
## Multiplicity of residual protonated impurity

Solvent	Impurity	Signal	Multiplicity
Chloroform- $d_1$	$\text{CHCl}_3$	7.26	$n=0$ singlet
Acetone- $d_6$	$\text{CD}_3\text{COCHD}_2$	2.05	$n=2$ pentet
DMSO- $d_6$	$\text{CD}_3\text{SOCHD}_2$	2.50	$n=2$ pentet
Methanol- $d_4$	$\text{CHD}_2\text{OD}$	3.33	$n=2$ pentet

<https://webspectra.chem.ucla.edu/NotesOnSolvents.html>







Signal Multiplicity=  $2nI + 1$

$n$	$I = 1$
0	1
1	1 1 1
2	1 2 3 2 1

**HOH** peak appears as a singlet at 2.82 ppm; **HOD** peak appears as a triplet at 2.78 ppm  
 Peak splitting is due to coupling from D nucleus with a coupling constant  $^2J_{HD} = 2.07$  Hz

[https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General\\_Information/double\\_water\\_peaks.pdf](https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General_Information/double_water_peaks.pdf)

# Interpretation of $^1\text{H}$ NMR spectra

## Chemical shift

(depends on the chemical surrounding)



How many types of H are in the molecule

It's diagnostic of the chemical environment (shielding/deshielding)

## Signal integration

(signal area is proportional to the number of protons that give rise that signal )

How many H of each type are in the molecule

## Signal multiplicity (splitting)

(splitting due to the surrounding nuclei)

What are the surrounding nucleus - connectivity in the molecule

Line widths Allows to identify Labile proton

# $^1\text{H}$ NMR codeine ( $\text{CDCl}_3$ , 400 MHz)

