Espectroscopia de Ressonância Magnética Nuclear

Relações entre Comprimento de onda e Espectroscopia

Região Espectral	Energia do fóton	Molecular Energy Changes
UV-Visível	~ 100 kcal/mol	Eletrônica
Infravermelho (IV)	~ 10 kcal/mol	Vibrações das ligações
Rádio	< 0,1 kcal/mol	Estados de spin nuclear em um campo magnético

NMR History

First NMR Spectra on Water

^{1}H NMR spectra of water

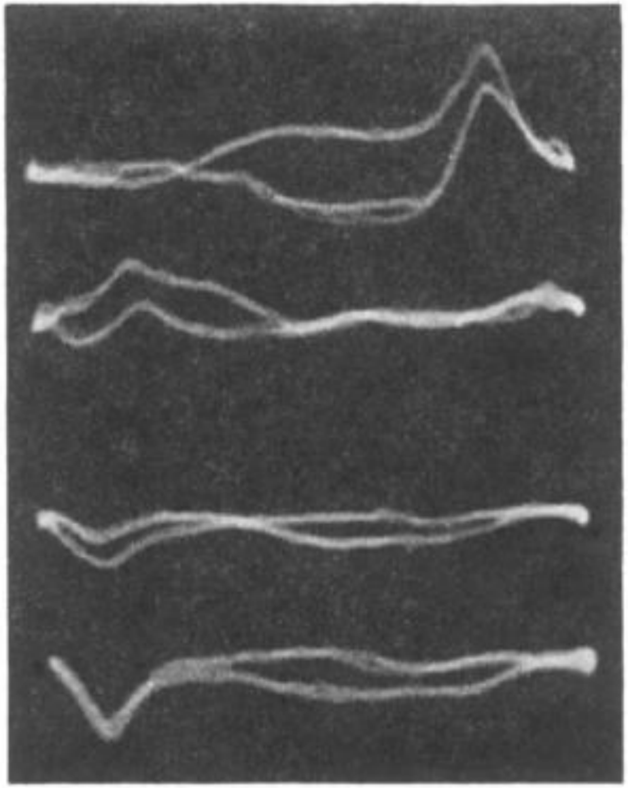


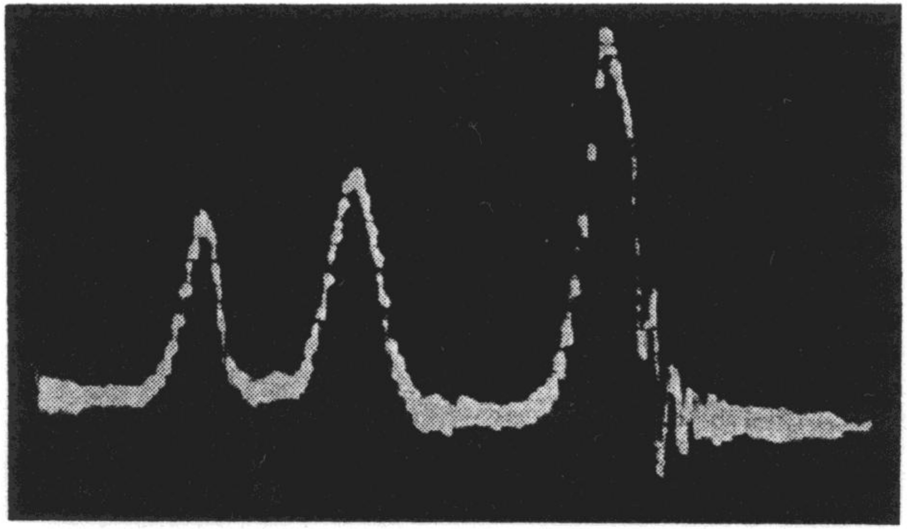
FIG. 10. Photographic record of the proton signal in water. The four traces from top to bottom correspond to the times t_1, t_2, t_3, t_4 of Fig. 9. In the text they are referred to as a, b, c, d, respectively.

Bloch, F.; Hansen, W. W.; Packard, M. **The nuclear induction experiment.** Physical Review (1946), 70 474-85.

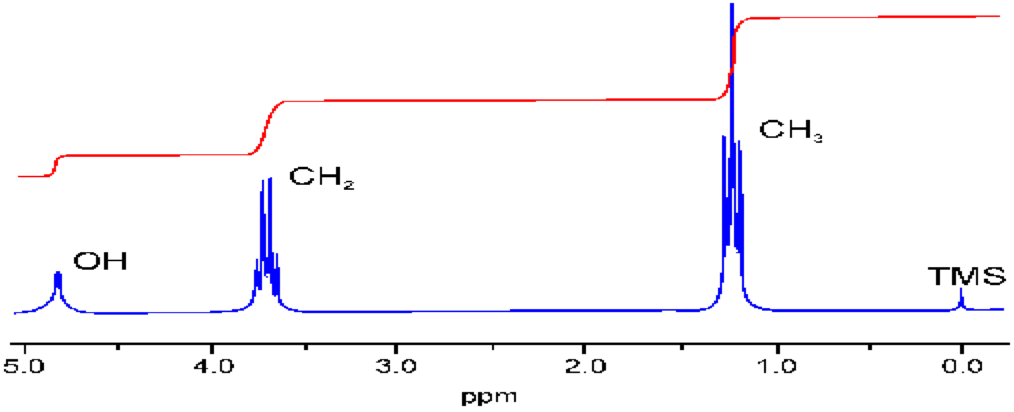
NMR History

First Observation of the Chemical Shift

^{1}H NMR spectra ethanol



Modern ethanol spectra



Arnold, J.T., S.S. Dharmatti, and M.E. Packard, J. Chem. Phys., 1951. **19**: p. 507.

NMR History

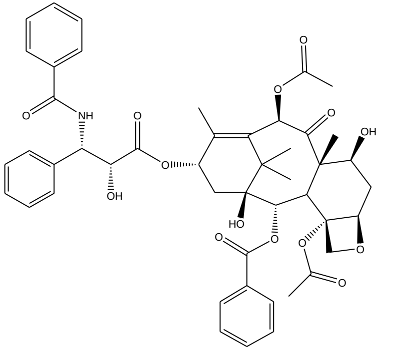
1937	**Rabi** predicts and observes nuclear magnetic resonance
1946	**Bloch, Purcell** first nuclear magnetic resonance of bulk sample
1953	**Overhauser** NOE (nuclear Overhauser effect)
1966	**Ernst, Anderson** Fourier transform NMR
1975	**Jeener, Ernst** 2D NMR
1985	**Wüthrich** first solution structure of a small protein (BPTI) from NOE derived distance restraints
1987	3D NMR + 13C, 15N isotope labeling of recombinant proteins (resolution)
1990	pulsed field gradients (artifact suppression)
1996/7	new *long range* structural parameters: - **residual dipolar couplings** from partial alignment in liquid crystalline media - projection angle restraints from **cross-correlated relaxation** TROSY (molecular weight > 100 kDa)

Nobel prizes

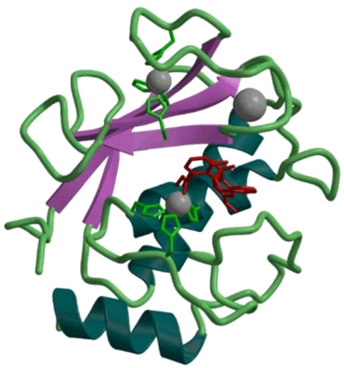
1944 *Physics* Rabi (Columbia)
1952 *Physics* Bloch (Stanford), Purcell (Harvard)
1991 *Chemistry* Ernst (ETH)
2002 *Chemistry* Wüthrich (ETH)
2003 *Medicine* Lauterbur (University of Illinois in Urbana),
Mansfield (University of Nottingham)

Typical Applications of NMR:

1.) Structural (chemical) elucidation
    - Natural product chemistry
    - Synthetic organic chemistry
        - analytical tool of choice of synthetic chemists
        - used in conjunction with MS and IR
2.) Study of dynamic processes
    - reaction kinetics
    - study of equilibrium (chemical or structural)
3.) Structural (three-dimensional) studies
    - Proteins, Protein-ligand complexes
    - DNA, RNA, Protein/DNA complexes
    - Polysaccharides
4.) Drug Design
    - Structure Activity Relationships by NMR
5) Medicine -MRI

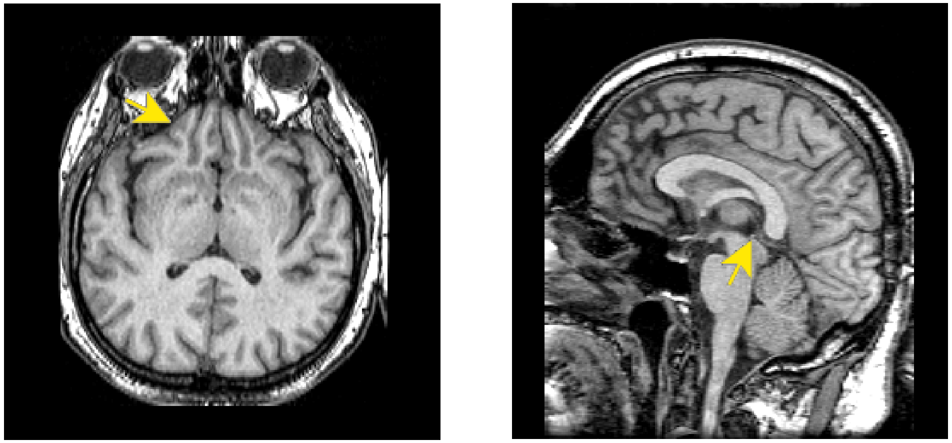


Taxol (natural product)



NMR Structure of MMP-13 complexed to a ligand

MRI images of the Human Brain



Spin dos Núcleos Atômicos

- Átomos de Spin 1/2 : número de massa é ímpar.
  exemplos: ^{1}H e ^{13}C.

- Átomos de Spin 1: número de massa é par.
  exemplos: ^{2}H e ^{14}N.

- Átomos de Spin 0: número de massa é par.
  exemplos: ^{12}C e ^{16}O.

Spin Quantum Numbers of Some Nuclei

The most abundant isotopes of C and O do not have spin.

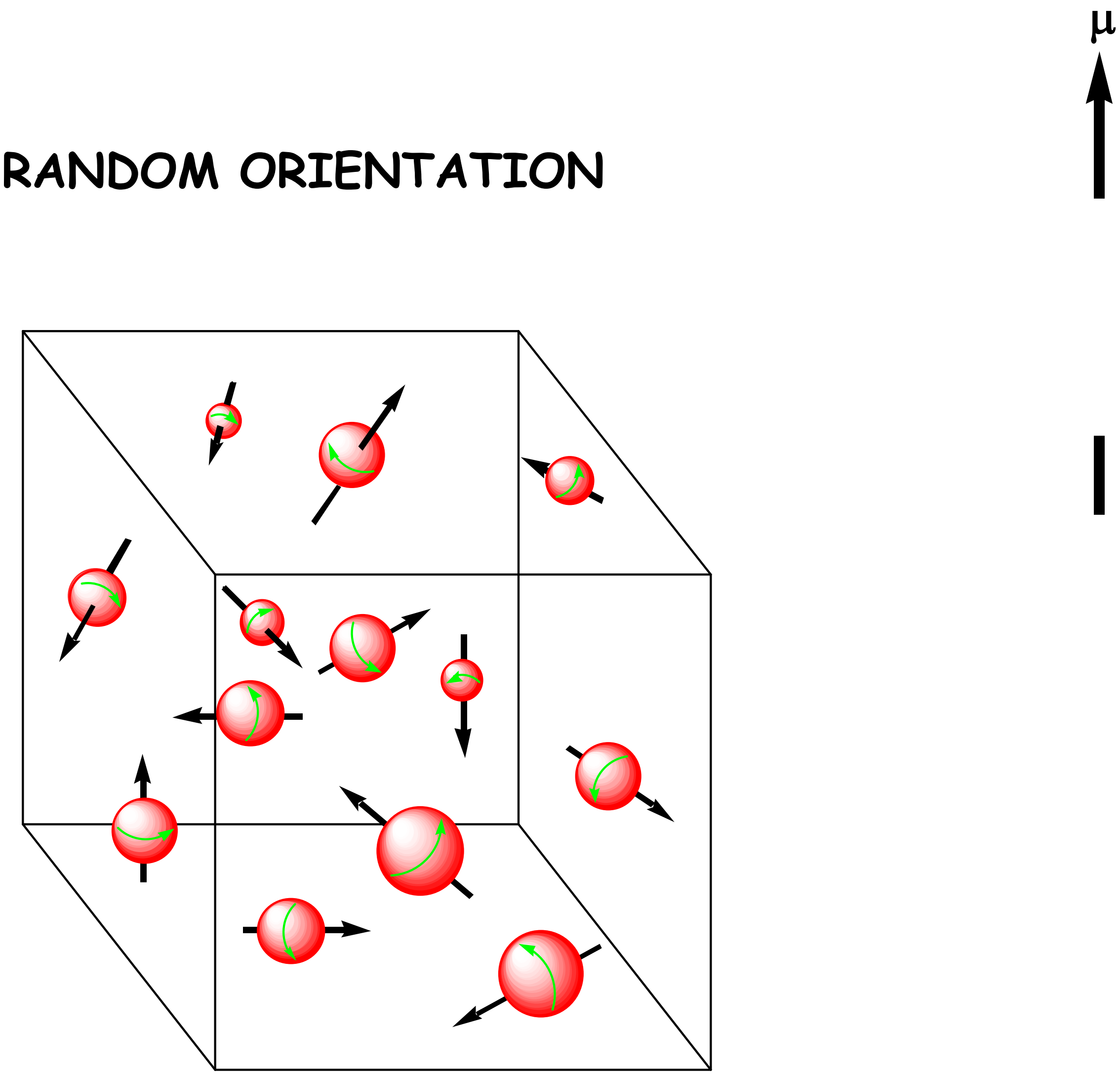
Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{16}O	^{17}O	^{19}F
Nuclear Spin Quantum No (I)	1/2	1	0	1/2	1	0	5/2	1/2
No. of Spin States	2	3	0	2	3	0	6	2

Elements with either **odd mass** or **odd atomic number** have the property of nuclear “spin”.

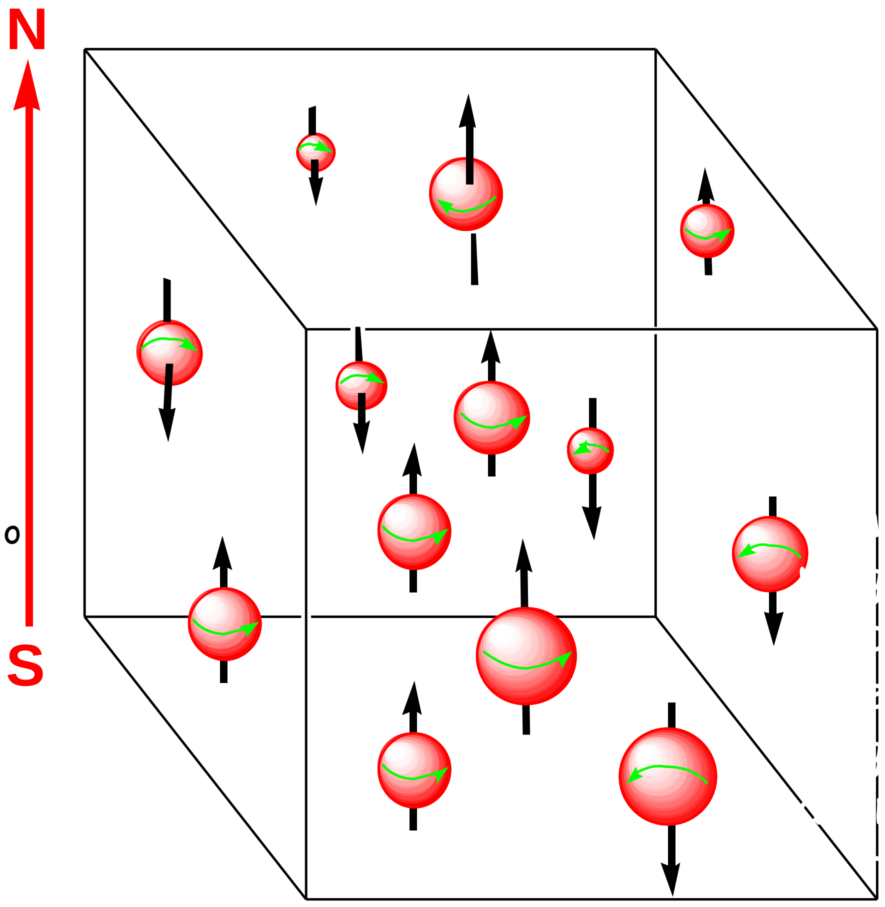
The number of spin states is $2I + 1$,

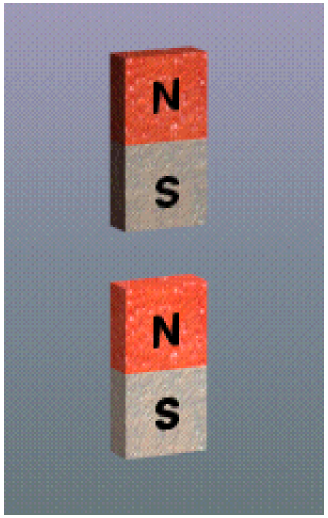
where I is the spin quantum number.

RANDOM ORIENTATION

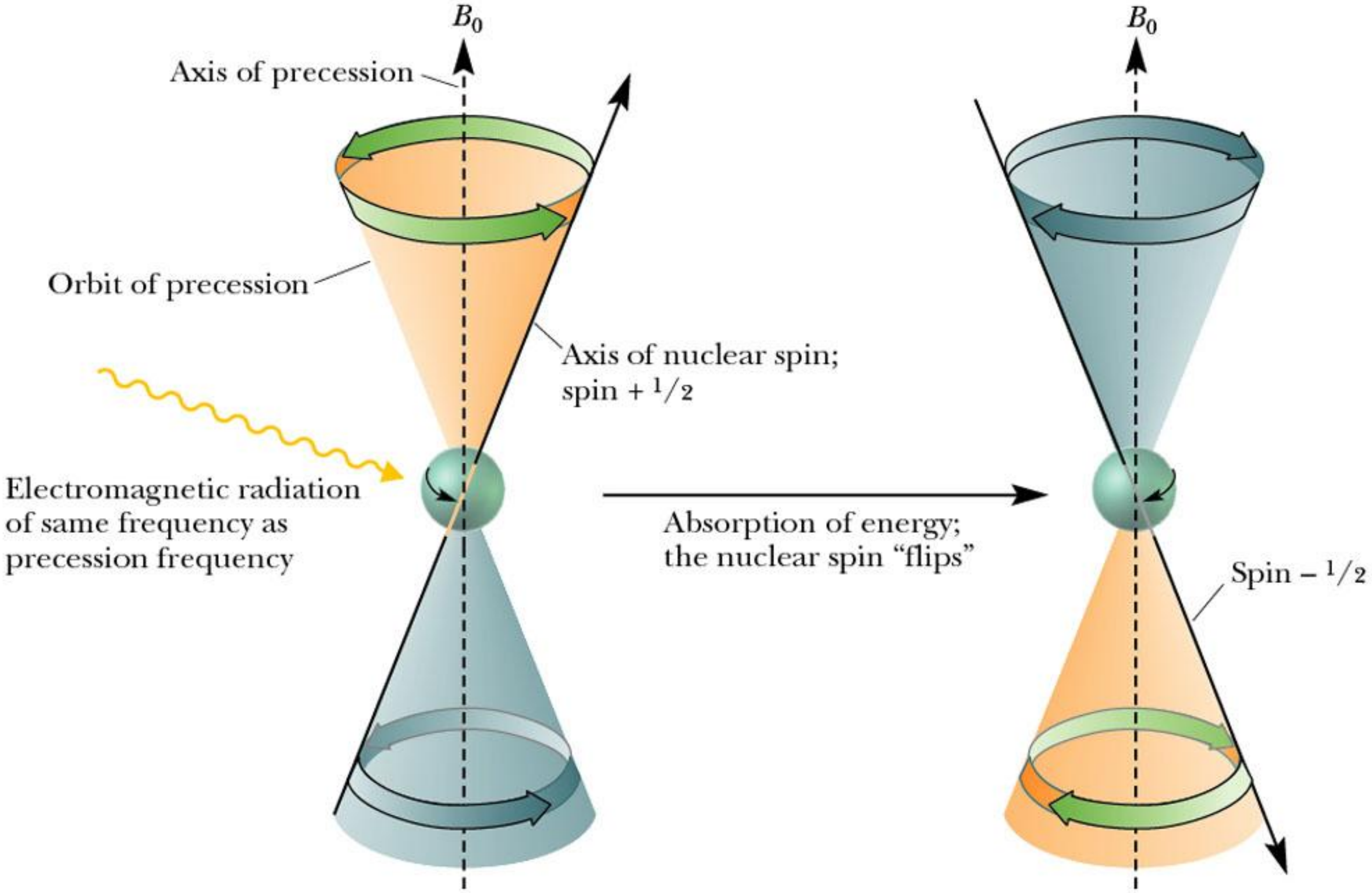


Applied
External
Magnetic
Field
of Field
Strength B_o





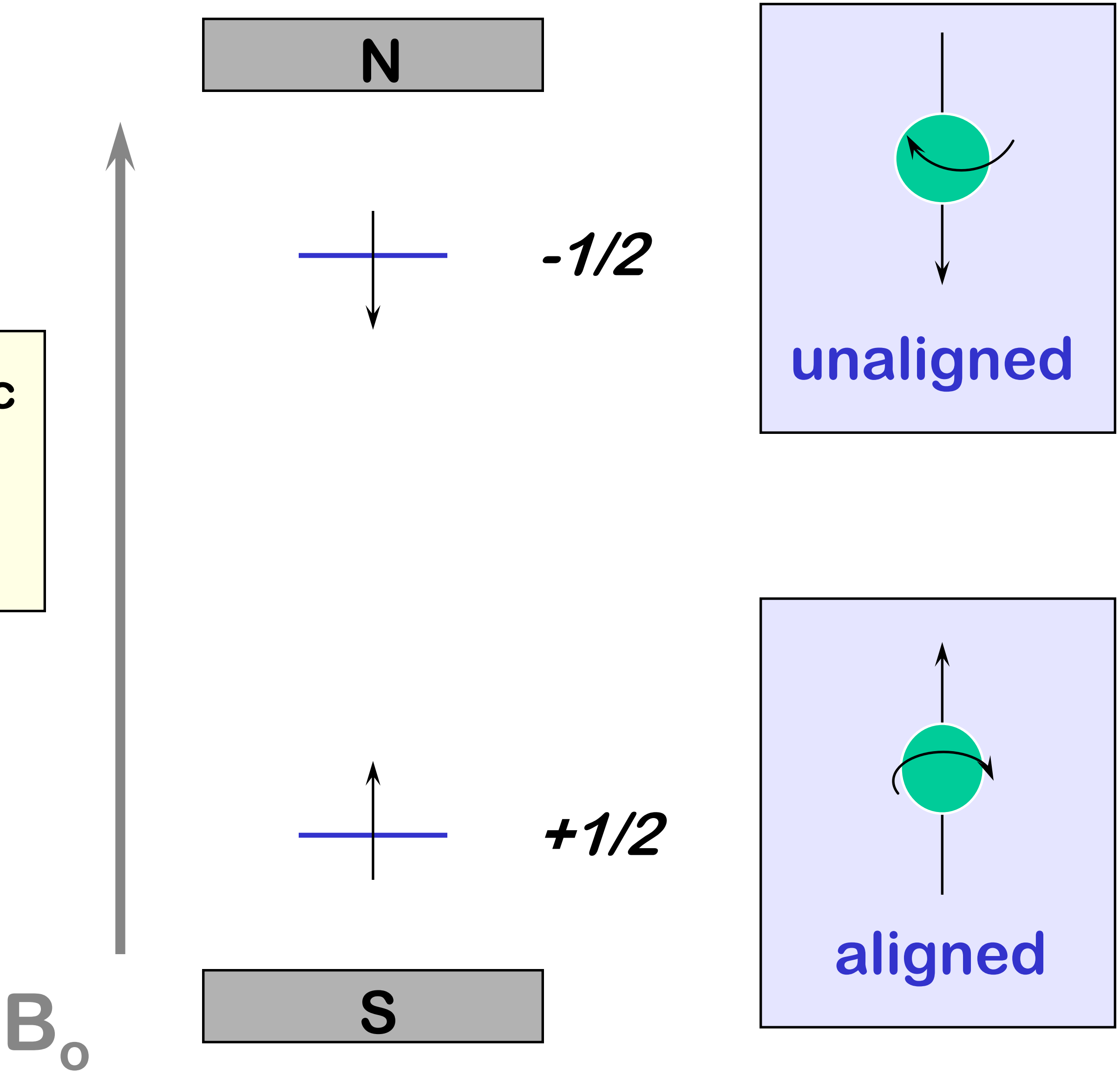
Nuclear Magnetic Resonance



Nuclear Spin Energy Levels

In a strong magnetic field (B_o) the two spin states differ in energy.

"The number of spin states is 2I + 1"



N

-1/2

unaligned

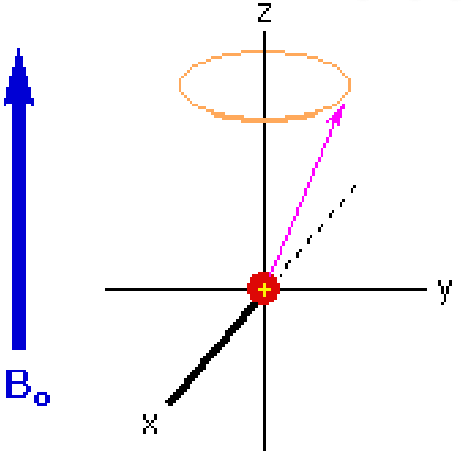
+1/2

aligned

S

B_o

The Larmor Equation!!!



$\Delta E = kB_o = h\nu$ can be transformed into

$$N = \left(\frac{\gamma}{2\pi}\right) B_o$$

gyromagnetic ratio γ

frequency of the incoming radiation that will cause a transition

strength of the magnetic field

γ is a constant which is different for each atomic nucleus (H, C, N, etc)

IRRADIATION FREQUENCY VS FIELD STRENGTH

ENERGY OF A PHOTON

$E = h\nu$

SPIN STATE ENRGY DIFFERENCE

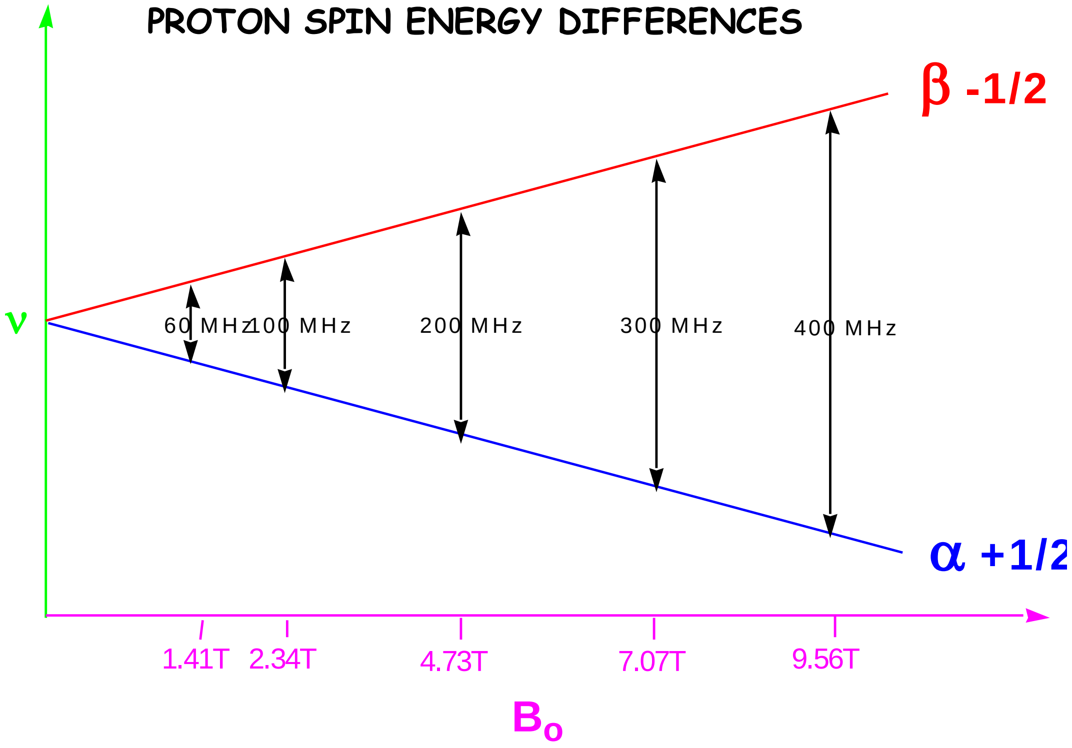
$\Delta E = \gamma h B_o / 2\pi$

WHEN $E = \Delta E$, SPIN FLIP OCCURS

$h\nu = \gamma h B_o / 2\pi$

THE NECESSARY FREQUENCY IS:

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



NMR Sensitivity

But at a significant cost!

~$800,000

~$2,00,000

~$4,500,000

NMR Sensitivity

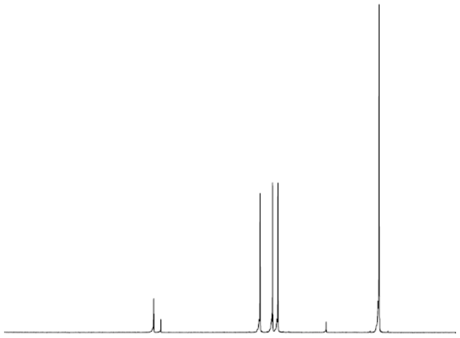
- Relative sensitivity of ^{1}H, ^{13}C, ^{15}N and other nuclei NMR spectra depend on
  - Gyromagnetic ratio (γ)
  - Natural abundance of the isotope

γ - Intrinsic property of nucleus can not be changed.

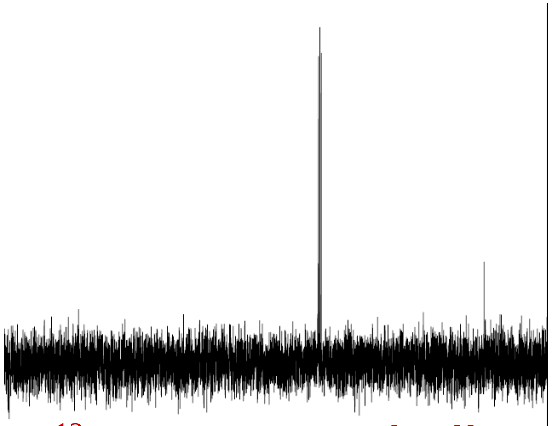
$(\gamma_H/\gamma_C)^3$ for ^{13}C is 64x $(\gamma_H/\gamma_N)^3$ for ^{15}N is 1000x

^{1}H is **~ 64x** as sensitive as ^{13}C and **1000x** as sensitive as ^{15}N !

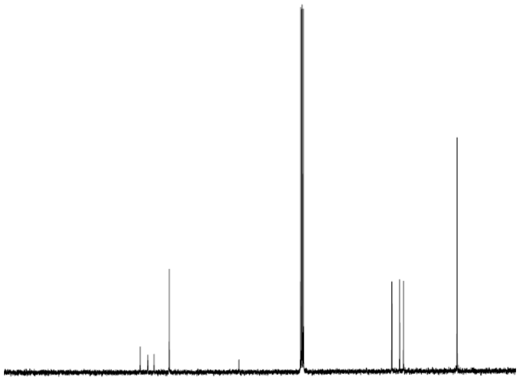
Consider that the natural abundance of ^{13}C is **1.1%** and ^{15}N is **0.37%** relative sensitivity increases to **~6,400x** and **~2.7x10^{5}**x !!



^{1}H NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
10,000 scans ~4.2 hours

Resonance Frequencies of Selected Nuclei

Isotope	Abundance	B_o (Tesla)	Frequency(MHz)	γ(radians/Tesla)
^{1}H	99.98%	1.00	42.6	267.53
		1.41	60.0	
		2.35	100.0	
		7.05	300.0	
^{13}C	1.108%	1.00	10.7	67.28
		2.35	25.0	
		7.05	75.0	

Ressonância Magnética Nuclear (RMN)
Nuclear Magnetic Resonance (NMR)

- **Ressonância** – fótons de ondas de rádio podem apresentar a diferença de energia exata entre os estados de spin + ½ and – ½ resultando na absorção de fótons enquanto os prótons têm seus estados de spin alterados.

- **Magnética** – um campo magnético forte causa uma pequena diferença de energia entre os estados de spin + ½ e – ½.

- **Nuclear** – nuclídeos de spin ½ (e.g. prótons) comportam-se como pequenas barras magnéticas.

The NMR Experiment

- The sample, dissolved in a suitable NMR solvent (e.g. $CDCl_3$ or CCl_4) is placed in the strong magnetic field of the NMR.

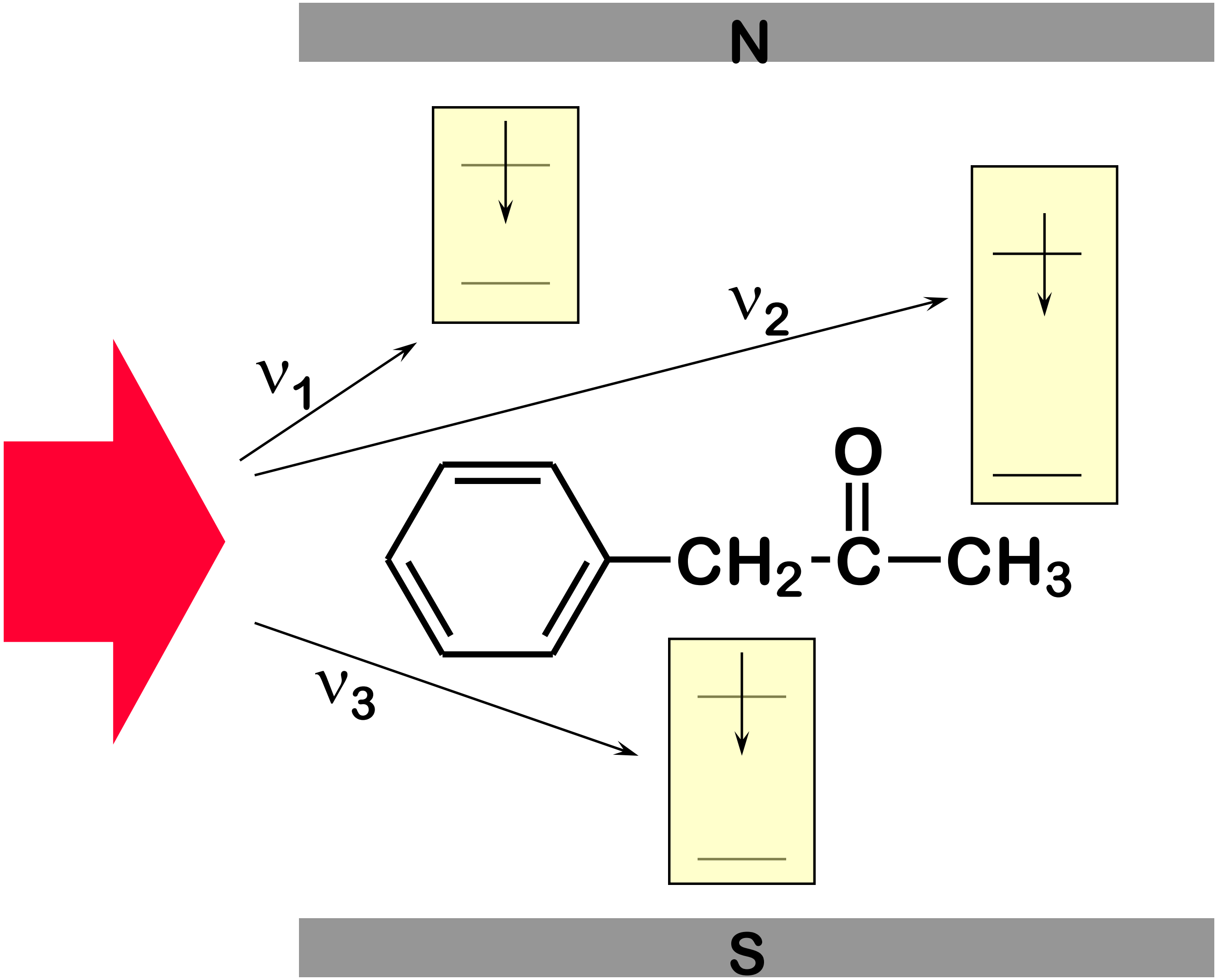
- The sample is bombarded with a series of radio frequency (Rf) pulses and absorption of the radio waves is monitored.

- The data is collected and manipulated on a computer to obtain an NMR spectrum.

PULSED EXCITATION

BROADBAND RF PULSE

contains a range of frequencies $(\nu_1 \ldots\ldots \nu_n)$



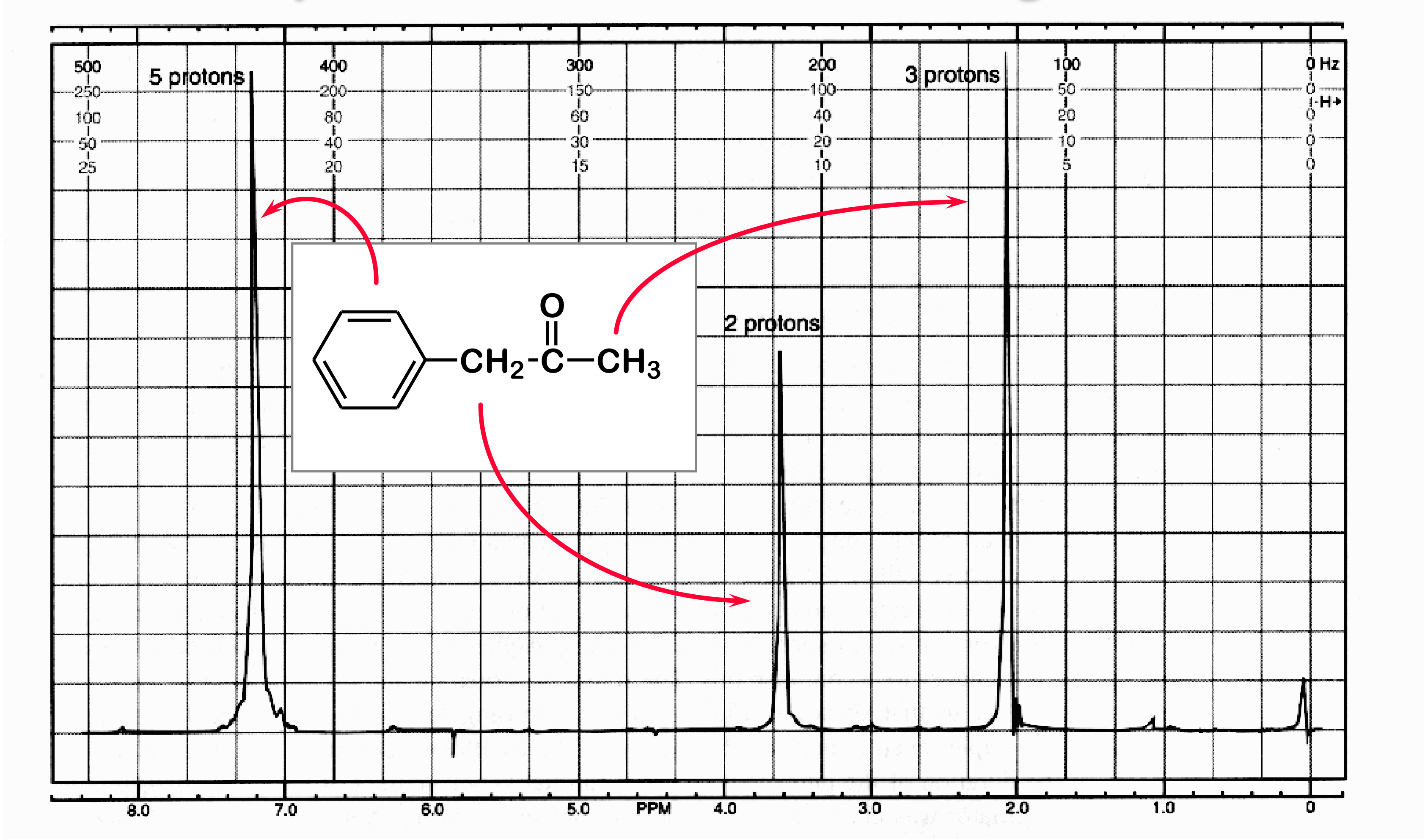
All types of hydrogen are excited simultaneously with the single RF pulse.

Some Generalizations

- Tetramethylsilane (TMS) is the reference.
- Chemical shift in Hz from TMS vary according to frequency of spectrometer!
- Delta values (δ) are independent of frequency of spectrometer (ppm).

Espectro de RMN de Hidrogênio
(Proton NMR Spectrum)

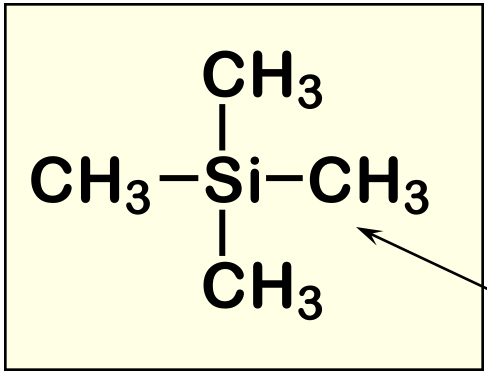
NMR Spectrum of Phenylacetone



EACH DIFFERENT TYPE OF PROTON COMES AT A DIFFERENT PLACE - YOU CAN TELL HOW MANY DIFFERENT TYPES OF PROTONS THERE ARE BY INTEGRATION.

PEAKS ARE MEASURED RELATIVE TO TMS

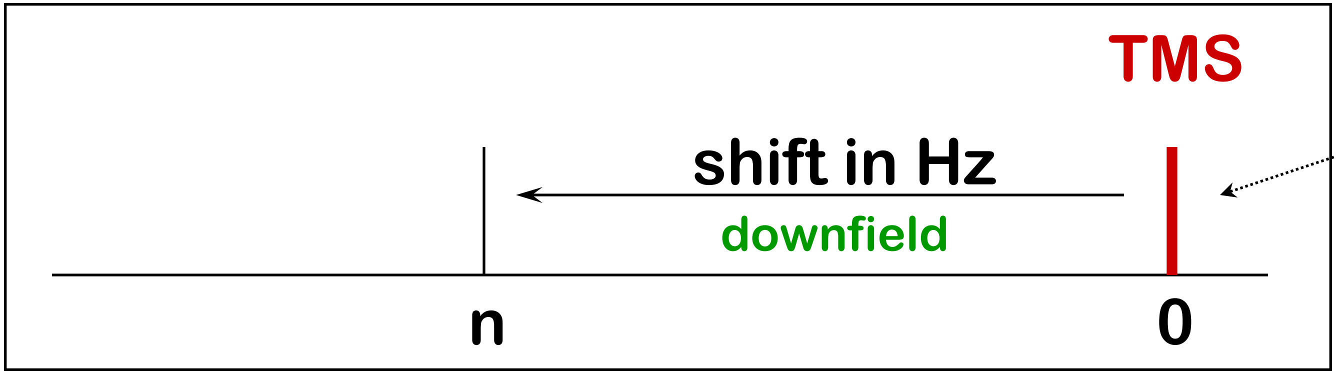
Rather than measure the exact resonance position of a peak, we measure how far downfield it is shifted from TMS.



$$\begin{array}{c} \mathrm{CH_3} \\ | \\ \mathrm{CH_3{-}Si{-}CH_3} \\ | \\ \mathrm{CH_3} \end{array}$$

reference compound
tetramethylsilane
"TMS"

Highly shielded protons appear way upfield.

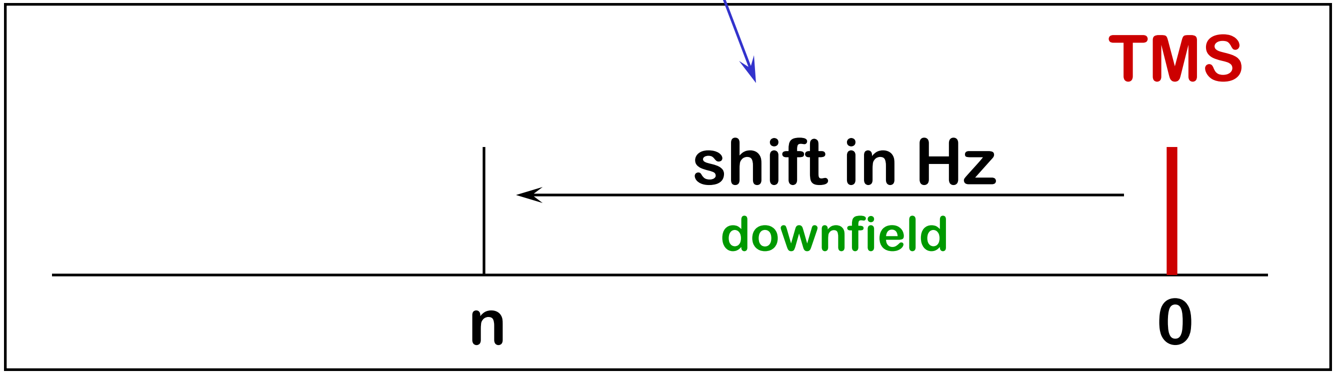


Chemists originally thought no other compound would come at a higher field than TMS.

HIGHER FREQUENCIES GIVE LARGER SHIFTS

The shift observed for a given proton in Hz also depends on the frequency of the instrument used.

Higher frequencies = larger shifts in Hz.



TMS

shift in Hz

downfield

n

0

THE CHEMICAL SHIFT

The shifts from TMS in Hz are bigger in higher field instruments (300 MHz, 500 MHz) than they are in the lower field instruments (100 MHz, 60 MHz).

We can adjust the shift to a field-independent value, the “chemical shift” in the following way:

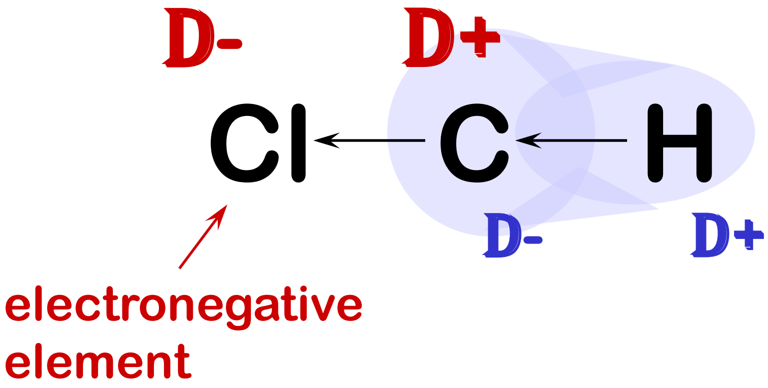
parts per million

$$\text{chemical shift} = \delta = \frac{\text{shift in Hz}}{\text{spectrometer frequency in MHz}} = \text{ppm}$$

This division gives a number independent of the instrument used.

A particular proton in a given molecule will always come at the same chemical shift (constant value).

DESHIELDING BY AN ELECTRONEGATIVE ELEMENT



Chlorine “deshields” the proton, that is, it takes valence electron density away from carbon, which in turn takes more density from hydrogen <u>deshielding</u> the proton.

NMR CHART

“deshielded“
protons appear
at low field

highly shielded
protons appear
at high field

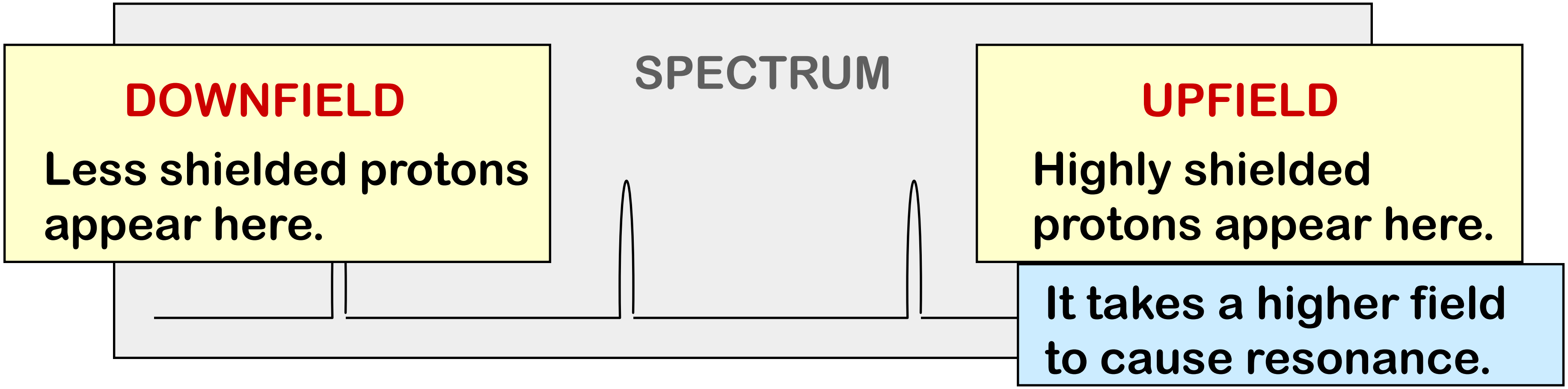
deshielding moves proton
resonance to lower field

PROTONS DIFFER IN THEIR SHIELDING

All different types of protons in a molecule have a different amounts of shielding.

They all respond differently to the applied magnetic field and appear at different places in the spectrum.

This is why an NMR spectrum contains useful information (different types of protons appear in predictable places).



SPECTRUM

DOWNFIELD

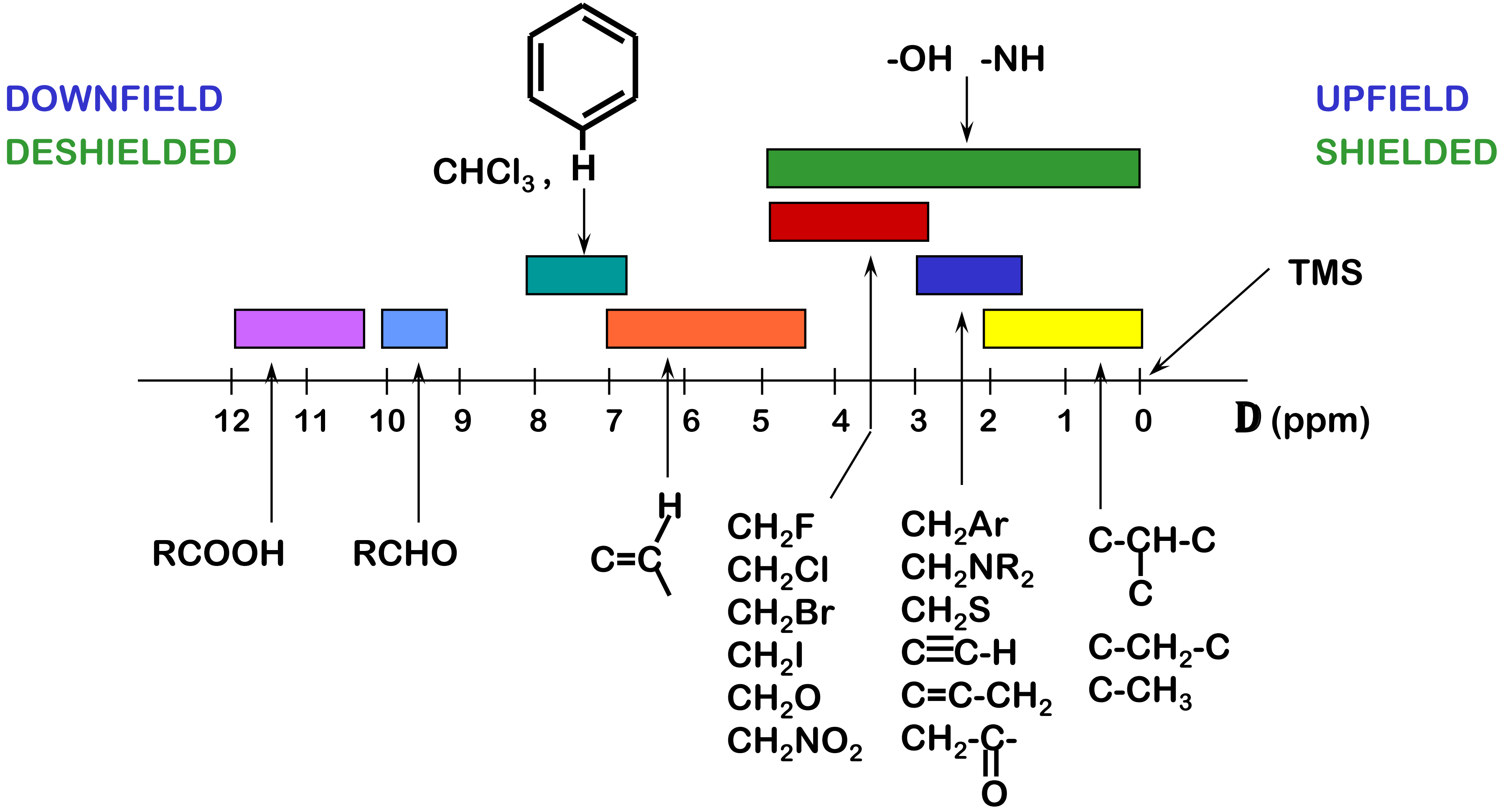
Less shielded protons appear here.

UPFIELD

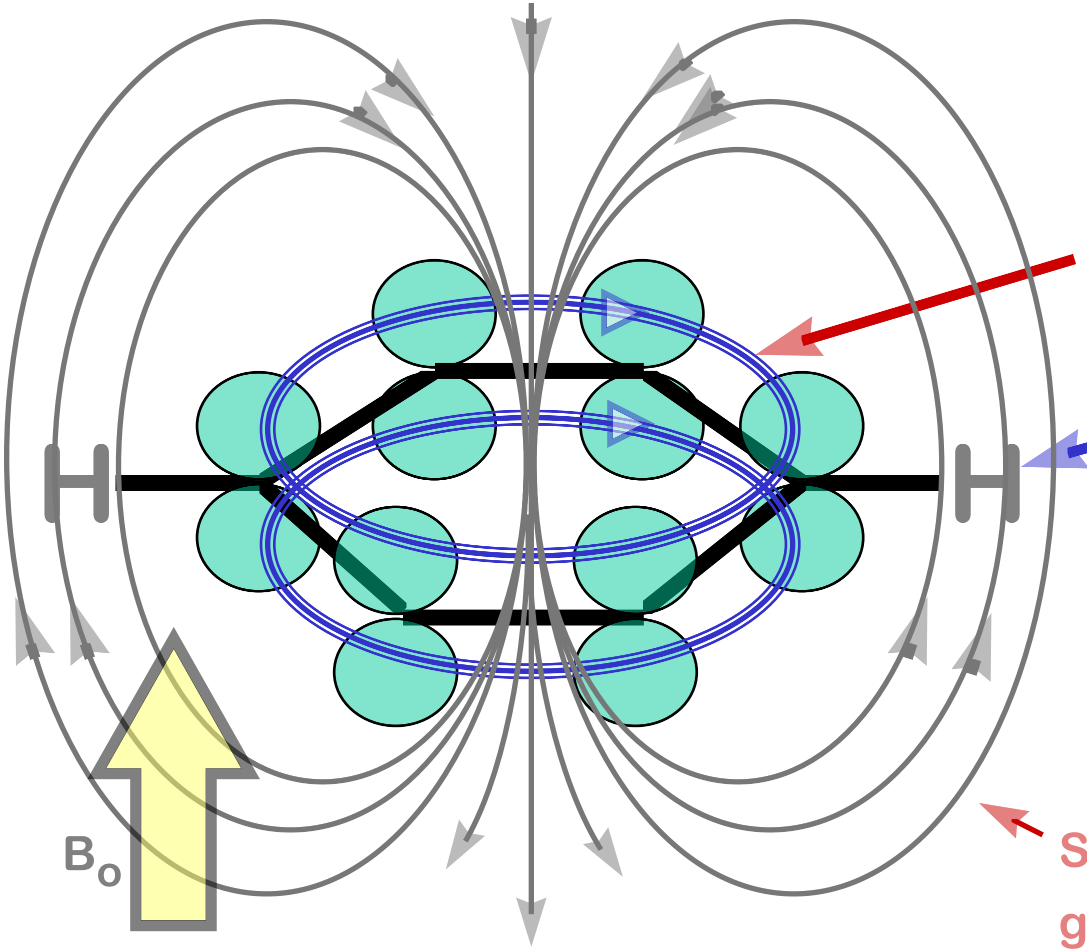
Highly shielded protons appear here.

It takes a higher field to cause resonance.

NMR Correlation Chart



Ring Current in Benzene



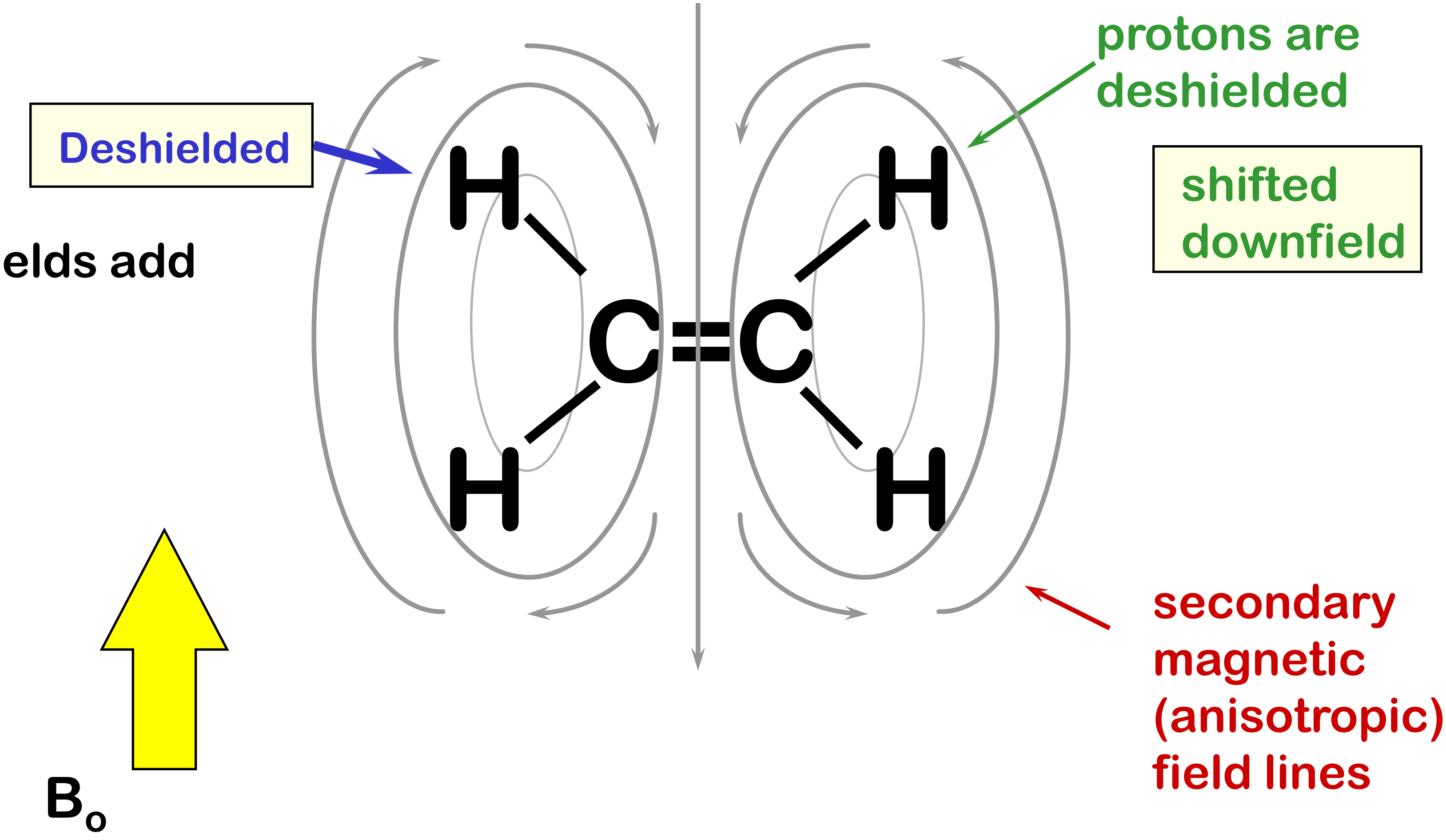
Circulating π electrons

Deshielded

fields add together

Secondary magnetic field generated by circulating π electrons deshields aromatic protons

ANISOTROPIC FIELD IN AN ALKENE



Deshielded

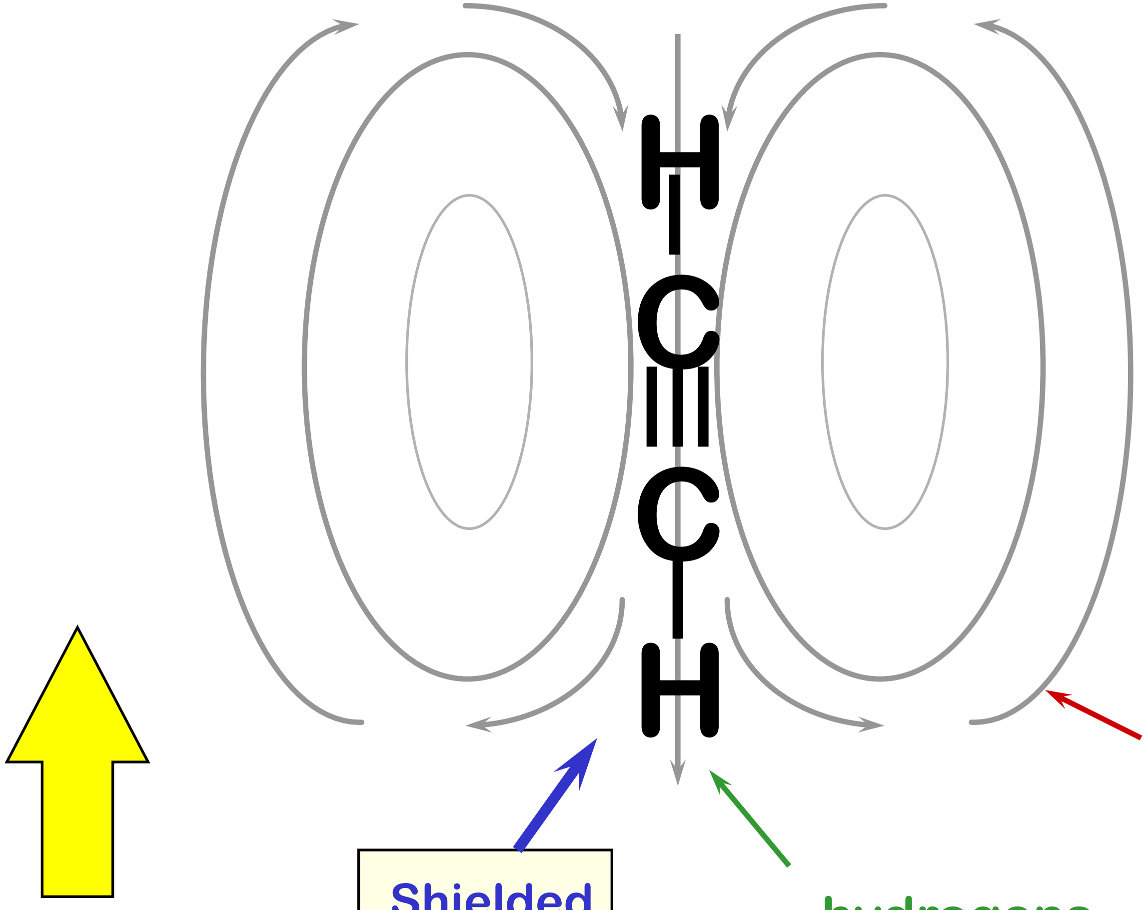
fields add

protons are deshielded

shifted downfield

secondary magnetic (anisotropic) field lines

ANISOTROPIC FIELD FOR AN ALKYNE



B_o

Shielded

fields subtract

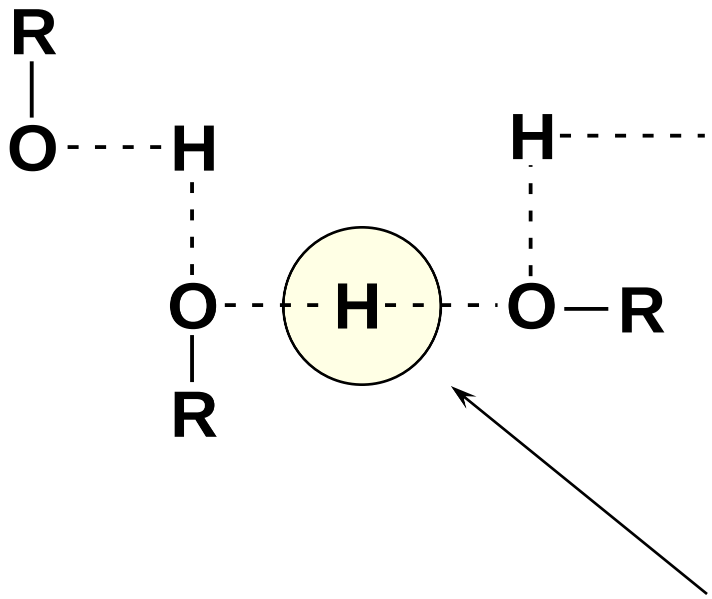
hydrogens are shielded

secondary magnetic (anisotropic) field

Generalizations

- electrons shield nucleus
- electronegativity: withdraws electrons to deshield nucleus
- downfield (deshielding) = left side of spectrum
- upfield (shielding) = right side of spectrum
- delta values increase from right to left!

HYDROGEN BONDING DESHIELDS PROTONS

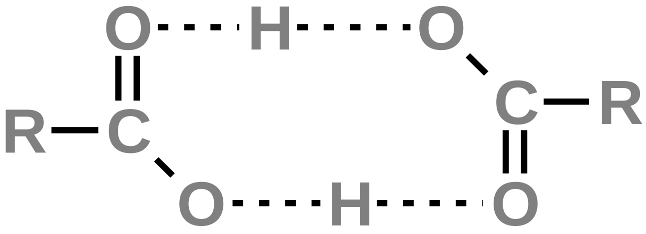


The chemical shift depends on how much hydrogen bonding is taking place.

Alcohols vary in chemical shift from 0.5 ppm (free OH) to about 5.0 ppm (lots of H bonding).

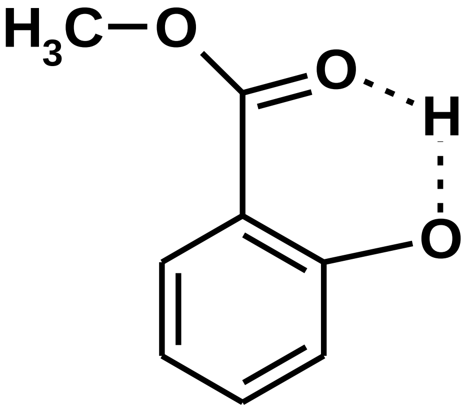
Hydrogen bonding lengthens the O-H bond and reduces the valence electron density around the proton
- it is deshielded and shifted downfield in the NMR spectrum.

SOME MORE EXTREME EXAMPLES



Carboxylic acids have strong hydrogen bonding - they form dimers.

With carboxylic acids the O-H absorptions are found between 10 and 12 ppm very far downfield.



In methyl salicylate, which has strong internal hydrogen bonding, the NMR absorption for O-H is at about 14 ppm, way, way downfield.

Notice that a 6-membered ring is formed.

NMR: Some Specific Functional Group Characteristics

- O-H and N-H will often show broad peaks with no resolved splitting, and the chemical shift can vary greatly.

- Aldehyde C-H is strongly deshielded.
  (δ = 9-10 ppm)

- Carboxylic Acid O-H is very strongly deshielded. (δ = 10-12 ppm)

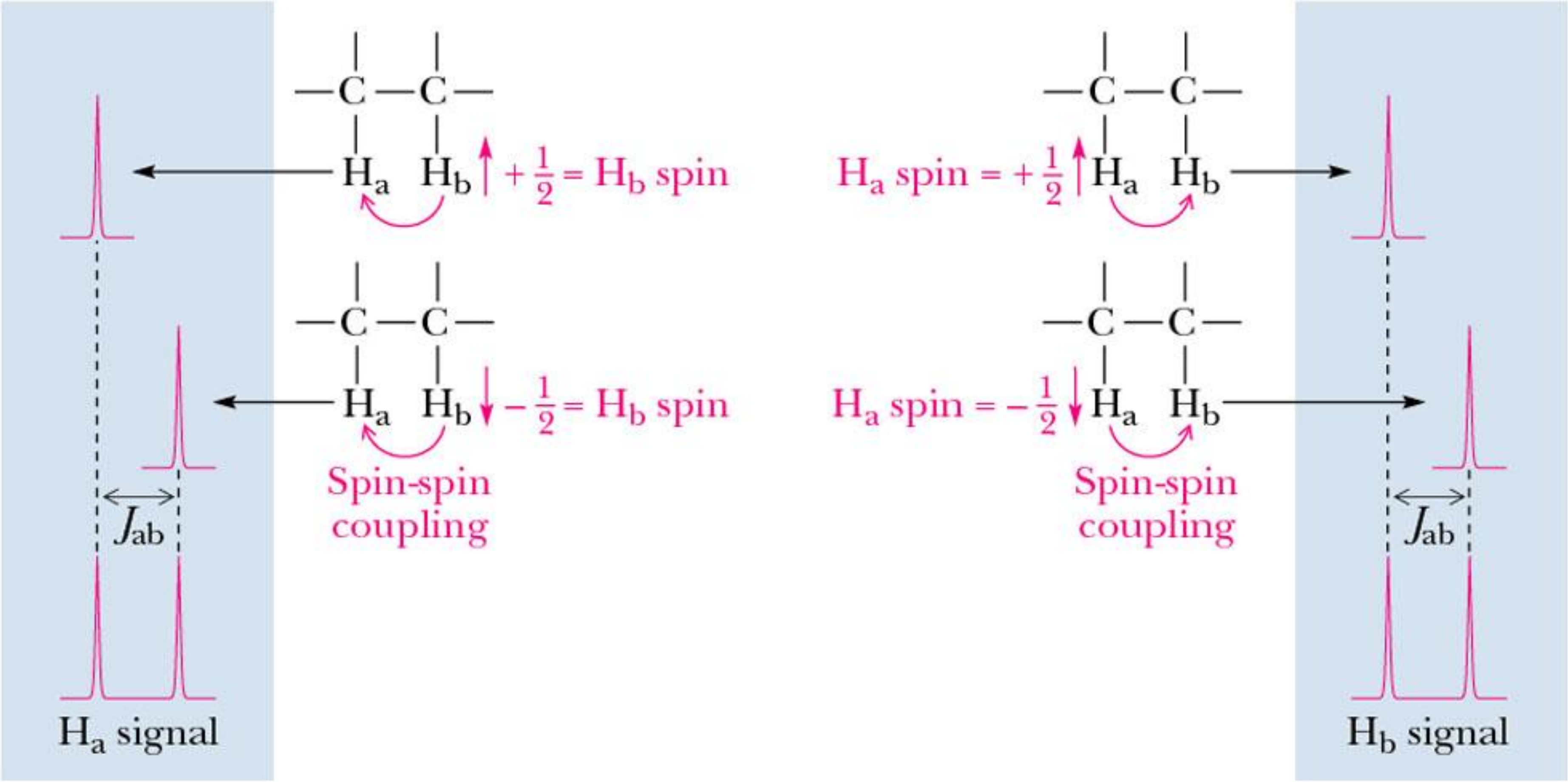
Spin-Spin Splitting

- Non-equivalent hydrogens will almost always have different chemical shifts.

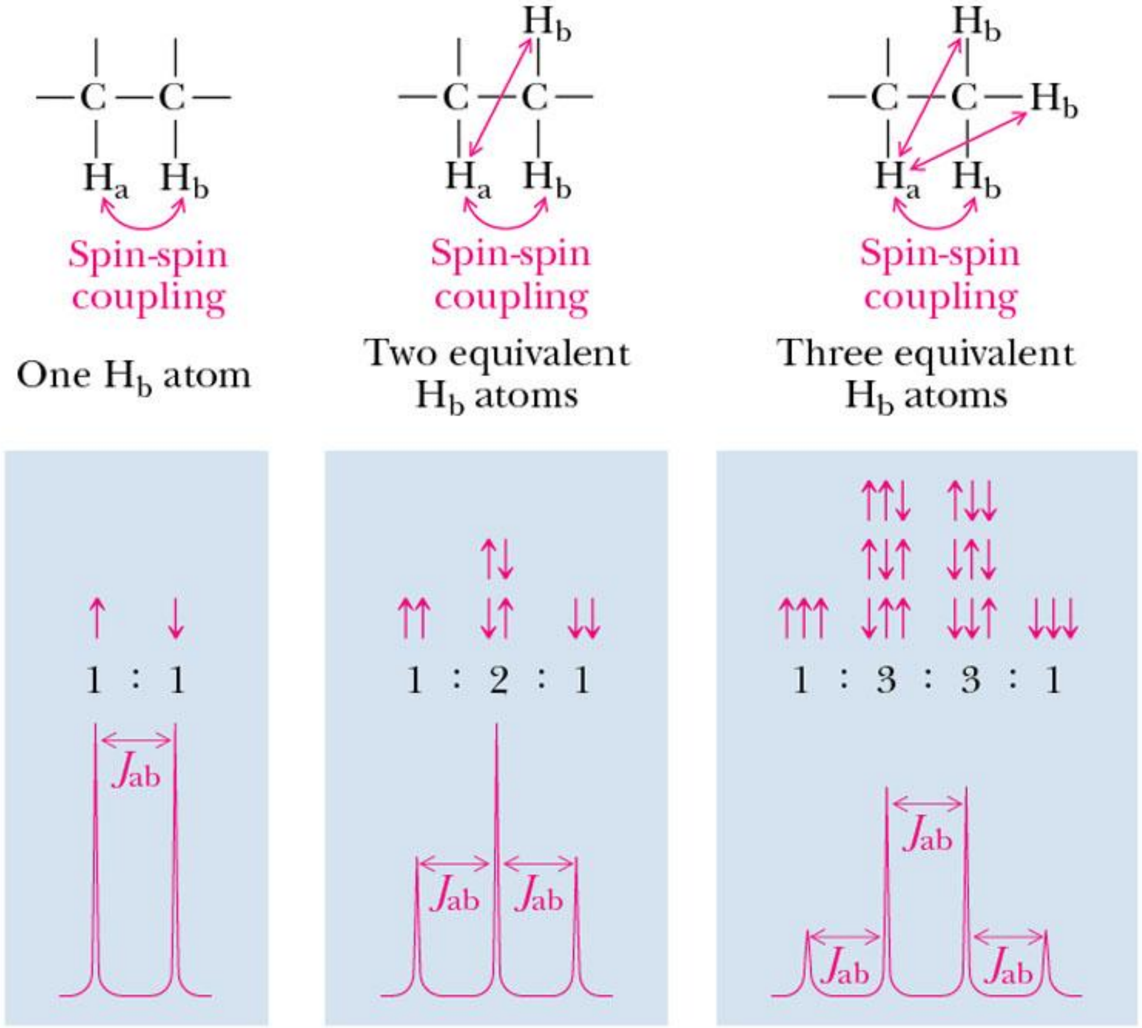
- When non-equivalent hydrogens are on adjacent carbon atoms spin-spin splitting will occur due to the hydrogens on one carbon feeling the magnetic field from hydrogens on the adjacent carbon.

- The magnitude of the splitting between two hydrogens (measured in Hz) is the coupling constant, *J*.

Origins of Signal Splitting



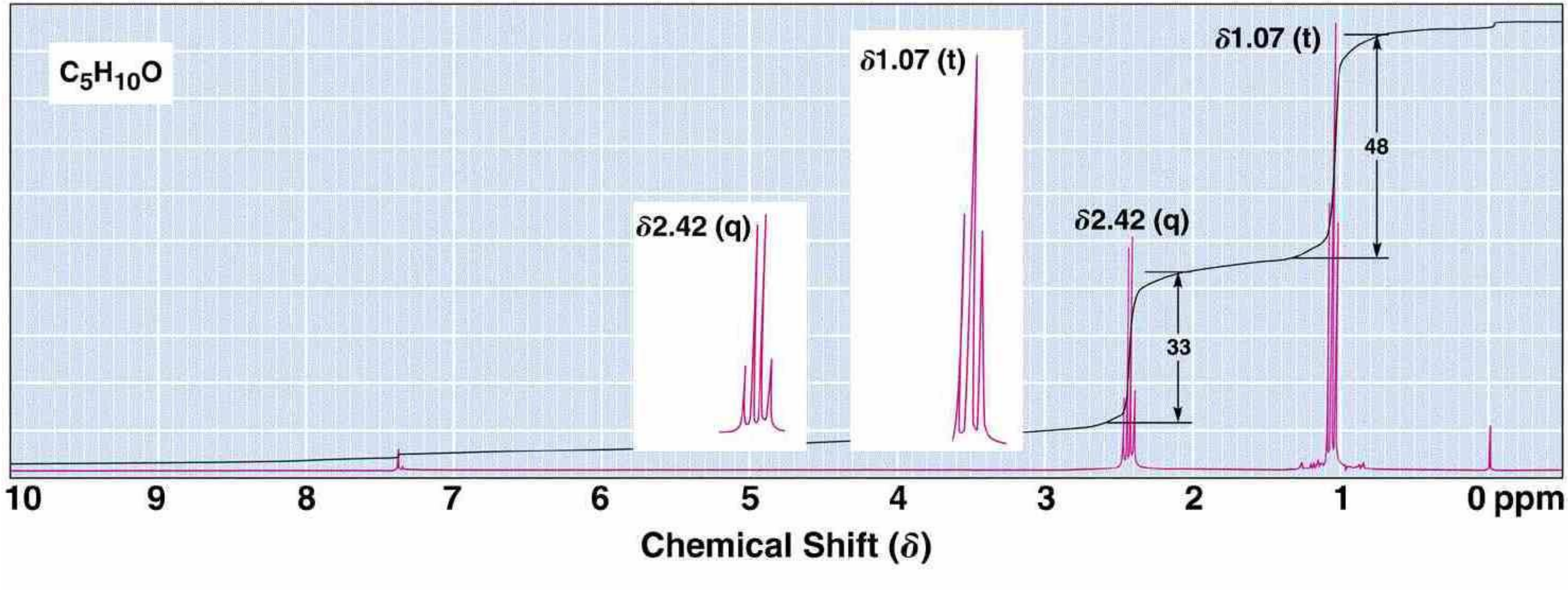
Origins of Signal Splitting



Observed splitting in signal of H_a

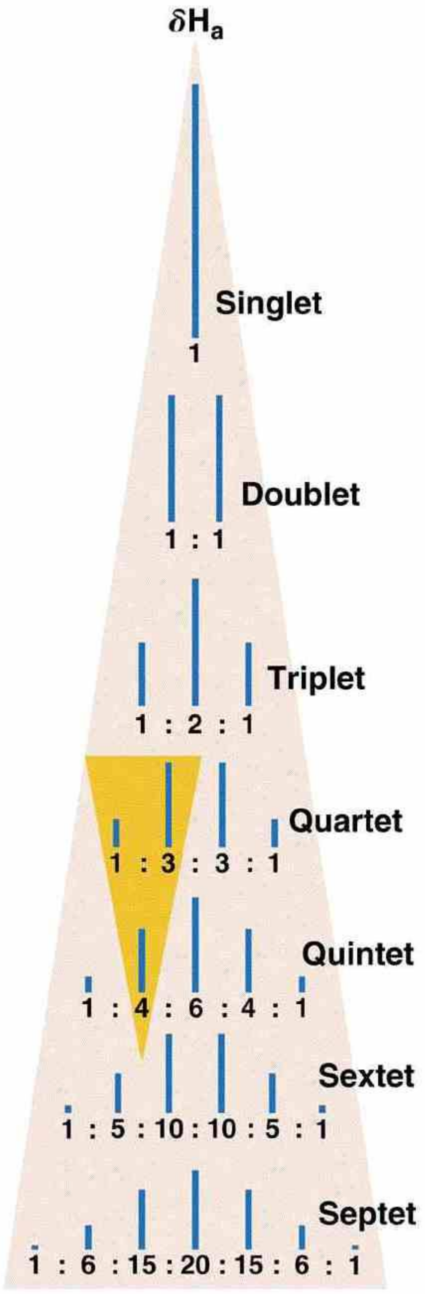
Origins of Signal Splitting

- because splitting patterns from spectra taken at 300 MHz and higher are often difficult to see, it is common to retrace certain signals in expanded form
- ^{1}H-NMR spectrum of 3-pentanone; scale expansion shows the triplet quartet pattern more clearly



Signal Splitting

- Pascal's Triangle
  - as illustrated by the highlighted entries, each entry is the sum of the values immediately above it to the left and the right



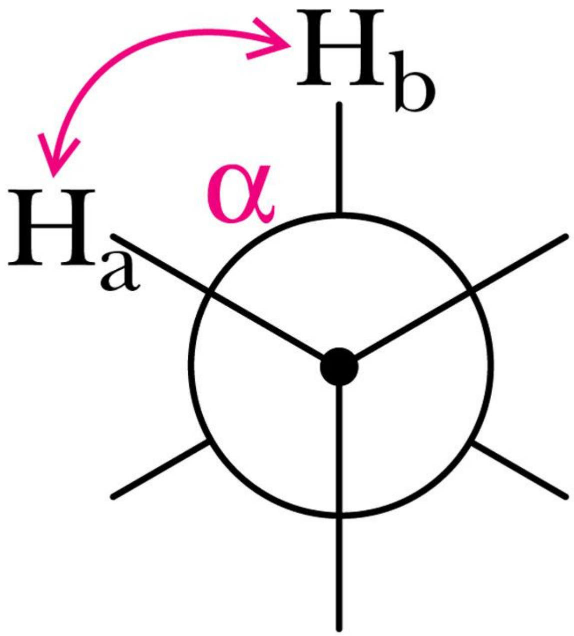
The n + 1 Rule

If Ha is a set of equivalent hydrogens and Hx is an adjacent set of equivalent hydrogens which are not equivalent to Ha:

- **The NMR signal of Ha will be split into n+1 peaks by Hx (where n = number of hydrogens in the Hx set).**
- **The NMR signal of Hx will be split into n+1 peaks by Ha (where n = number of hydrogens in the Ha set).**

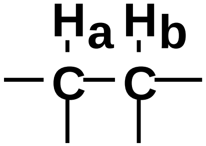
Coupling Constants

- an important factor in vicinal coupling is the angle α between the C-H sigma bonds and whether or not it is fixed
- coupling is a maximum when α is 0° and 180°; it is a minimum when α is 90°

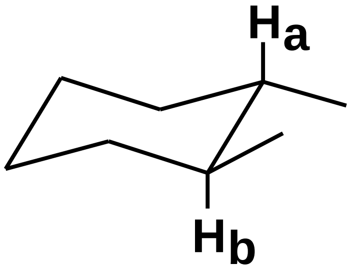


Coupling Constants

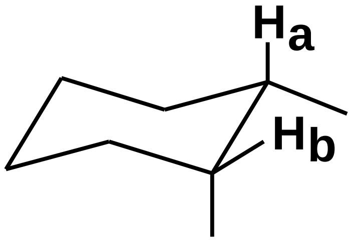
- Coupling constant (J): the distance between peaks in a split signal, expressed in hertz
  - the value is a quantitative measure of the magnetic interaction of nuclei whose spins are coupled



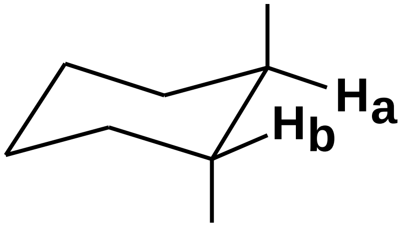
6-8 Hz



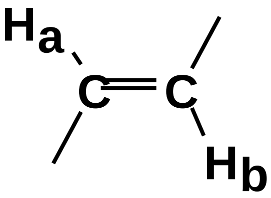
8-14 Hz



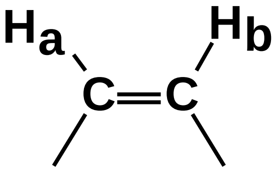
0-5 Hz



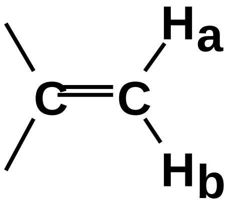
0-5 Hz



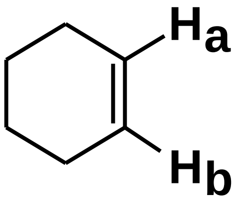
11-18 Hz



5-10 Hz



0-5 Hz



8-11 Hz

13.8 Integration

INTEGRATION OF A PEAK

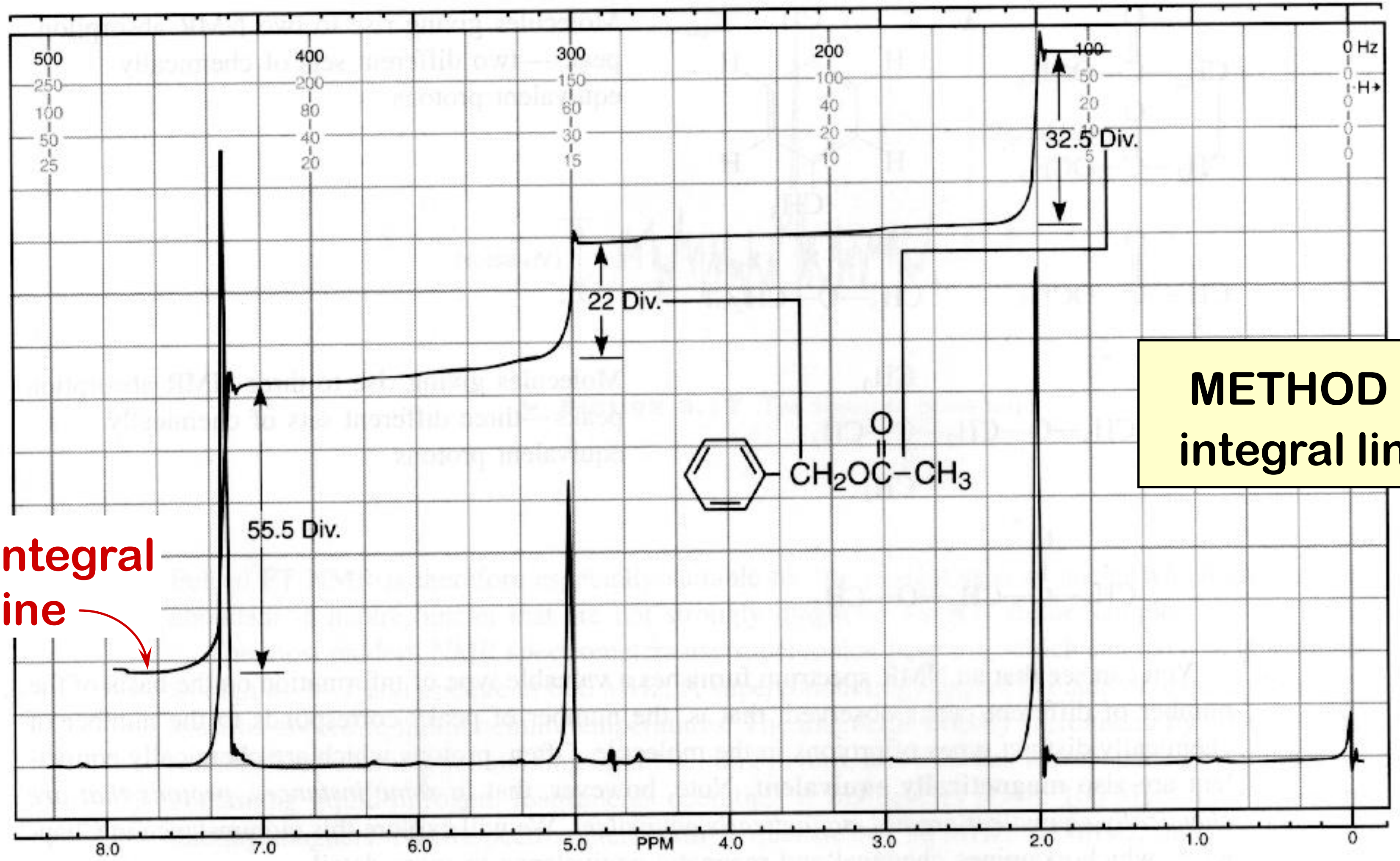
Not only does each different type of hydrogen give a distinct peak in the NMR spectrum, but we can also tell the relative numbers of each type of hydrogen by a process called integration.

Integration = determination of the area under a peak

The area under a peak is proportional to the number of protons that generate the peak.

Benzyl Acetate

The integral line rises an amount proportional to the number of H in each peak



integral line

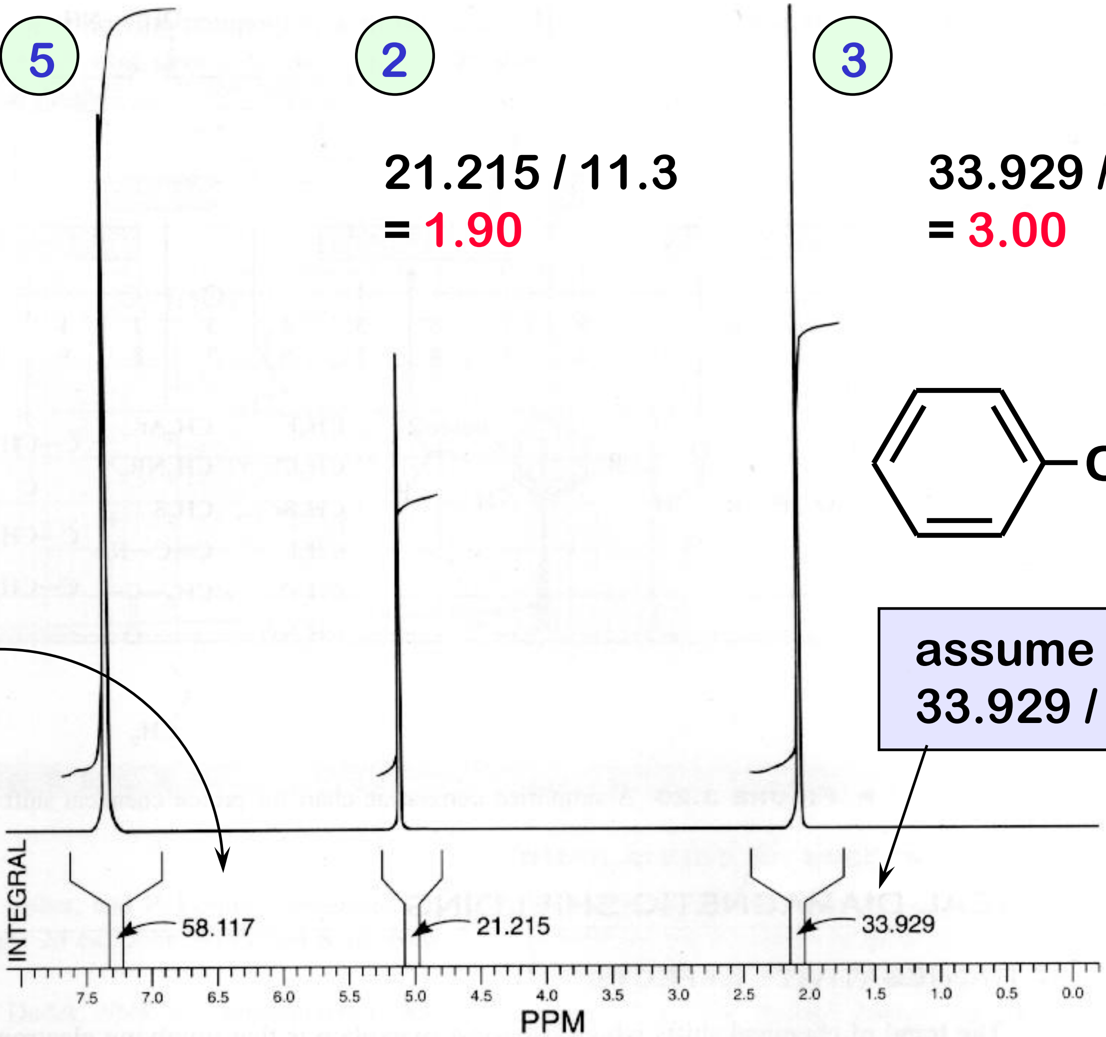
METHOD 1
integral line

55 : 22 : 33 = 5 : 2 : 3

simplest ratio of the heights

Benzyl Acetate (FT-NMR)

Actually :

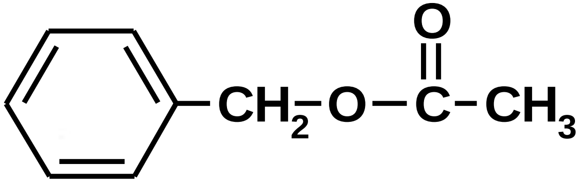


⑤ ② ③

$58.117 / 11.3 = 5.14$

$21.215 / 11.3 = 1.90$

$33.929 / 11.3 = 3.00$



METHOD 2

digital integration

assume CH_3

$33.929 / 3 = 11.3$ per H

Integrals are good to about 10% accuracy.

Modern instruments report the integral as a number.