

NMR Spectroscopy

A Brief Introduction

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Type of Spectroscopy

- IR Spectroscopy **Functional Groups**
- UV Spectroscopy **Conjugation**
- NMR Spectroscopy **Carbon-Hydrogen Framework**
- Mass Spectroscopy **Molecular Size and Formula**
- X-Ray Crystallography **Exact Structure**

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Energy Level of A Wave



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- **Nuclear magnetic resonance (NMR) obtains the structure of molecules from their unique magnetic signatures of their component atoms.**
- **NMR is the spectroscopic study of the magnetic energy levels of nuclei.**
- **NMR is a valuable tool for the study of microstructures of polymer systems, especially for proteins.**

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Why NMR Structure Determinations?

- ^{13}C NMR yields site specific information, enabling analysis of individual atoms of the molecule.
- Enables spectral characterization of samples (confirm structures, etc).
- Can be used to follow processes such as polymerization without affecting the dynamics of that process.

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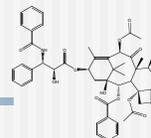
Why NMR?

- **Advantages**
 - ❖ Highly specific
 - ❖ Simple and clear interpretation of chemical structure
- **Disadvantages**
 - ❖ Low sensitivity per scan
 - ❖ Long measurement time (signal averaging)
 - ❖ Complex mixtures are difficult
 - ❖ Solubility limitations of polymers
 - ❖ Expensive equipment to operate and maintain

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Typical Applications of NMR

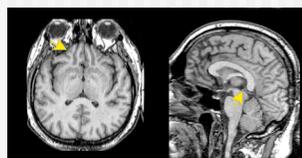
- Structural (chemical) elucidation
 - ☉ Natural product chemistry
 - ☉ Synthetic organic chemistry
 - ✱ Analytical tool of choice for synthetic chemists used in conjunction with MS and IR
- Study of dynamic processes
 - ☉ Reaction kinetics
 - ☉ Study of equilibrium (chemical or structural)
- Structural (three-dimensional) studies
 - ☉ Proteins, Protein-ligand complexes
 - ☉ DNA, RNA, Protein/DNA complexes
 - ☉ Polysaccharides
- Drug Design
 - ☉ Structure Activity Relationships by NMR
- Medicine -MRI



Taxol (natural product)



NMR Structure of MMP-13 complexed to a ligand



MRI images of the Human Brain

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How NMR Works!

- Some atomic nuclei behave as tiny bar magnets when placed in a magnetic field and align. This tiny magnets rotates around the direction of the magnet at a characteristic frequency called Larmor frequency.
- In NMR, the Larmor frequency is disturbed slightly by an surrounding electric field of functional groups, which causes a slight deviation from the Larmor frequency of the nucleus.
- This characteristic deviation (chemical shift) is on the order of ppm of the Larmor frequency and can be used to identify particular atoms and their positions.

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History of NMR

- 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)

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Nobel for Magnetic Resonance



- **Isador I. Rabi**
Nobel Prize in Physics, 1944
For his resonance method for recording the magnetic properties of atomic nuclei.
- **Felix Bloch and Edward M. Purcell, USA**
Nobel Prize in Physics, 1952
The NMR phenomenon was demonstrated for protons in 1946.

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Nobel for Magnetic Resonance



➤ **Richard Ernst, Zurich,**

Nobel Prize in Chemistry, 1991

For his fundamental contributions to NMR methodology-Nuclear Magnetic Resonance Fourier Transform Spectroscopy

<http://www.nobel.se/chemistry/laureates/1991/ernst-lecture.html>



➤ **Kurt Wüthrich**

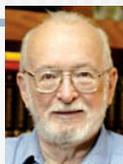
Nobel Prize in Chemistry, 2002

NMR studies of structure and function of biological macromolecules.

<http://www.nobel.se/chemistry/laureates/2002/wuthrich-lecture.html>

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Nobel for Magnetic Resonance

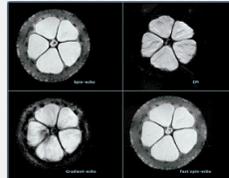


➤ **Paul C. Lauterbur (Urbana, IL) and Sir Peter Mansfield (Nottingham, UK)**

Nobel Prize in Physiology or Medicine, 2003



➤ **For their pioneering contributions which led to the application of magnetic resonance in medical imaging.**



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Nobel for Magnetic Resonance



➤ **Alexei A. Abrikosov (Argonne, IL) and Vitalij L. Ginzburg (Moscow)**
Nobel Prize in Physics, 2003



➤ **For pioneering contributions to the theory of type-II superconductors, i.e., those alloys capable of withstanding the high magnetic fields that occur in MR applications.**

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First NMR Spectrum

NMR Signal of Water

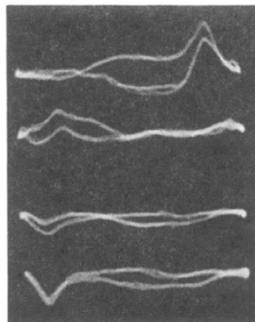


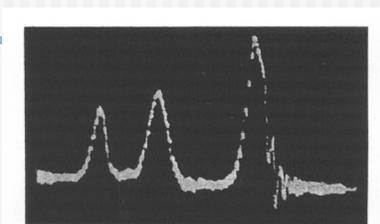
FIG. 10. Photographic record of the proton signal in water. The four traces from top to bottom correspond to the times t_1 , t_2 , t_3 , t_4 of Fig. 9. In the text they are referred to as a , b , c , d , respectively.

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

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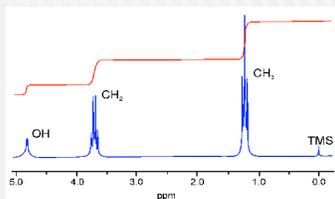
First NMR Spectrum with Chemical Shift

NMR of Ethanol



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

Comparison with modern NMR spectrum

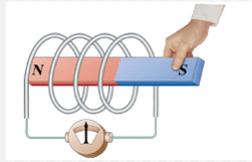


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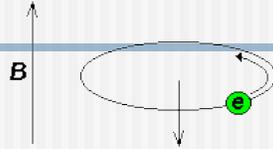
Basic Theory of NMR

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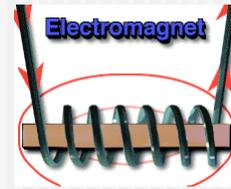
A Basic Concept in Electromagnetic Theory



A moving perpendicular external magnetic field will induce an electric current in a closed loop

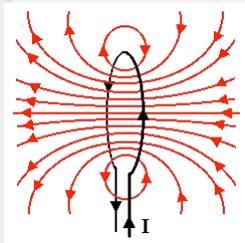


Magnetic field produced by circulating electron



An electric current in a closed loop will create a perpendicular magnetic field

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For a single loop of wire, the magnetic field, B through the center of the loop is:

$$B = \frac{\mu_0 I}{2R}$$

μ_0 – permeability of free space ($4\pi \times 10^{-7} \text{ T} \cdot \text{m} / \text{A}$)

R – radius of the wire loop

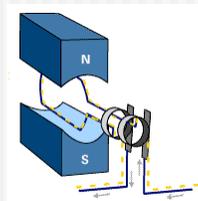
I – current

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Faraday's Law of Induction

- If the magnetic flux (F_B) through an area bounded by a closed conducting loop changes with time, a current and an emf are produced in the loop. This process is called induction.
- The induced emf is:

$$\mathcal{E} = - \frac{d\Phi_B}{dt}$$

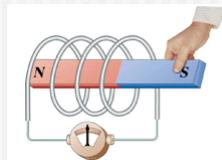


Simple AC generator

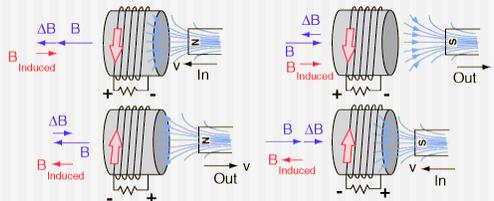
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Lenz's Law

- An induced current has a direction such that the magnetic field of the current opposes the change in the magnetic flux that produces the current.
- The induced emf has the same direction as the induced current



Direction of current follows motion of magnet



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Theory of NMR

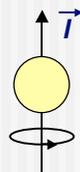
Quantum Description



➤ Nuclear Spin (just like electron spin)

- ❖ Nucleus rotates about its axis (spin)
- ❖ Nuclei with spin have angular momentum (p) or spin

1) total magnitude



$$\hbar\sqrt{I(I+1)}$$

2) quantized, spin quantum number I

3) $2I + 1$ states: $I, I-1, I-2, \dots, -I$

$$I=1/2: \quad -1/2, 1/2$$

4) identical energies in absence of external magnetic field

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NMR Periodic Table

NMR "active" Nuclear Spin (I) = $1/2$:

^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P

biological and chemical relevance

Odd atomic mass

$$I = +1/2 \text{ \& \- } 1/2$$

NMR "inactive" Nuclear Spin (I) = 0:

^{12}C , ^{16}O

Even atomic mass & number

Quadrupole Nuclei Nuclear Spin (I) $> 1/2$:

^{14}N , ^2H , ^{10}B

Even atomic mass & odd number

$$I = +1, 0 \text{ \& \- } 1$$

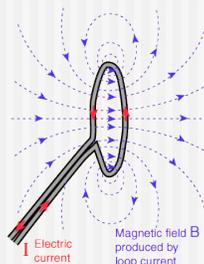
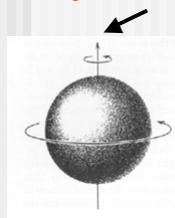
Table I.1 Nuclear properties of some of the elements

Element	Atomic mass	Spin I	Natural abundance (%)	Receptivity ($^{13}\text{C} = 1.00$)	Quadrupole moment (10^{26} m^2)	Resonant frequency (MHz) at 2.348 T
Hydrogen	1	1/2	99.985	5670	None	100.00
Deuterium	2	1	0.015	0.0082	0.287	15.35
Tritium	3	1/2	Radioactive	-	None	106.66
Helium	3	1/2	0.00014	0.0035	None	76.18
Lithium	6	1	7.42	3.58	-0.064	14.72
Lithium	7	3/2	92.58	1540	-3.7	38.87
Beryllium	9	3/2	100	78.8	5.3	15.06
Boron	10	3	19.58	22.1	7.4	10.75
Boron	11	3/2	80.42	754	4.1	32.08
Carbon	13	1/2	1.108	1.00	None	25.15
Nitrogen	14	1	99.63	5.70	1.67	7.23
Nitrogen	15	1/2	0.37	0.022	None	10.14
Oxygen	17	5/2	0.037	0.061	-2.6	13.56
Fluorine	19	1/2	100	4730	None	94.09
Neon	21	3/2	0.257	0.0036	9	7.90
Sodium	23	3/2	100	524	10	26.43
Magnesium	25	5/2	10.13	1.54	22	6.13
Aluminium	27	5/2	100	1170	14	26.08
Silicon	29	1/2	4.7	2.1	None	19.87
Phosphorus	31	1/2	100	377	None	40.48
Sulfur	33	3/2	0.76	0.098	-6.4	7.67
Chlorine	35(37)	3/2	75.53	20.2	-8.2	9.81
Potassium	39	3/2	93.1	2.69	5.5	4.67
Calcium	43	7/2	0.145	0.053	-5	6.74
Scandium	45	7/2	100	1720	-22	24.33
Titanium	49(47)	7/2	5.51	1.18	24	5.64
Vanadium	51(50)	7/2	99.76	2170	-5.2	26.35
Chromium	53	3/2	9.55	0.49	-15	5.64
Manganese	55	5/2	100	1014	40	24.84
Iron	57	1/2	2.19	0.00425	None	3.24
Cobalt	59	7/2	100	1560	42	23.73
Nickel	61	3/2	1.19	0.24	16	8.93
Copper	63(65)	3/2	69.09	368	-22	26.51
Zinc	67	5/2	4.11	0.67	15	6.25
Gallium	71(69)	3/2	39.6	322	11	30.58
Germanium	73	9/2	7.76	0.62	-17	3.48
Arsenic	75	3/2	100	144	29	17.18
Selenium	77	1/2	7.58	3.02	None	19.07
Bromine	81(79)	3/2	49.46	279	27	27.10
Krypton	83	9/2	11.55	1.24	27	3.86
Rubidium	87(85)	3/2	27.85	280	13	32.84
Strontium	87	9/2	7.02	1.08	16	4.35
Yttrium	89	1/2	100	0.676	None	4.92
Zirconium	91	5/2	11.23	6.05	-21	9.34
Niobium	93	9/2	100	2770	-32	24.55
Molybdenum	95(97)	5/2	15.72	2.92	-1.5	6.55
Technetium	99	9/2	Radioactive	-	-0.13	22.51
Ruthenium	99(101)	5/2	12.72	0.815	7.6	4.61
Rhodium	103	1/2	100	0.18	None	3.16
Palladium	105	5/2	22.23	1.43	65	4.58
Silver	109(107)	1/2	48.18	0.28	None	4.65
Cadmium	113(111)	1/2	12.26	7.69	None	22.18

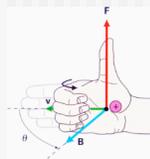
Magnetic Moment (μ)

spinning charged nucleus creates a magnetic field

Magnetic moment



Similar to magnetic field created by electric current flowing in a coil



"Right Hand Rule"

determines the direction of the magnetic field around a current-carrying wire and vice-versa

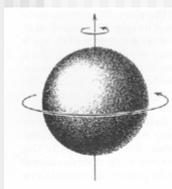
$$\vec{F} = q\vec{v} \times \vec{B}$$



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Gyromagnetic ratio (γ)

- related to the relative sensitive of the NMR signal
- magnetic moment (μ) is created along axis of the nuclear spin



$$\mu = \gamma p$$

$$\gamma = \frac{2\pi \mu}{h I} = \frac{\mu}{\hbar I}$$

where:

p – angular

γ – gyromagnetic ratio (different value for each type of nucleus)

magnetic moment is quantized (m)

$$m = I, I-1, I-2, \dots, -I$$

for common nuclei of interest:

$$m = +\frac{1}{2} \text{ \& \ } -\frac{1}{2}$$

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Important NMR Nuclei

	% Natural Abundance	I	M.Moment μ	γ^*	ν MHz	B_0 Telsa
^1H	99.9844	1/2	2.7927	267.53	60	1.41
^2H	0.0156	1	0.8574	41.1	60	9.23
^{12}C		0				
^{13}C	1.108	1/2	0.7022	67.28	60	5.61
^{16}O		0				
^{19}F	100.0	1/2	2.6273	251.7	60	1.50
^{31}P	100.0	1/2	1.1305	108.3	60	3.49

* Magnetogyric ratio γ unit: 10^6 radians/(Telsa*sec)

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Apply an external magnetic field

(i.e., put your sample in the magnet)

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

ω - resonance frequency
in radians per second,
also called Larmor frequency

ν - resonance frequency
in cycles per second, Hz

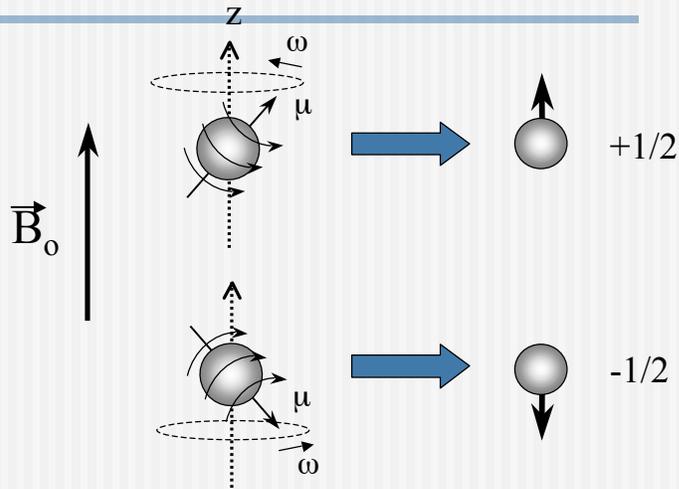
γ - gyromagnetic ratio

B_0 - external magnetic
field (the magnet)

Spin 1/2 nuclei will have two
orientations in a magnetic field
+1/2 and -1/2.

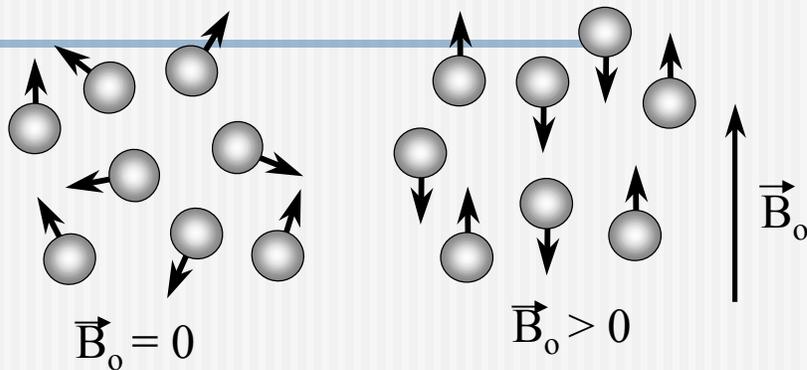
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Net magnetic moment



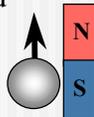
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Ensemble of Nuclear Spins



Randomly oriented

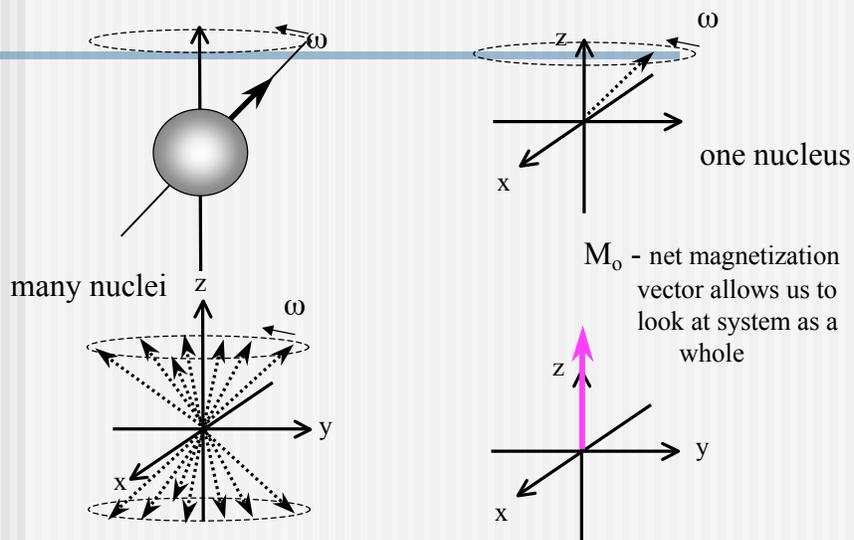
Highly oriented



Each nucleus behaves like a bar magnet.

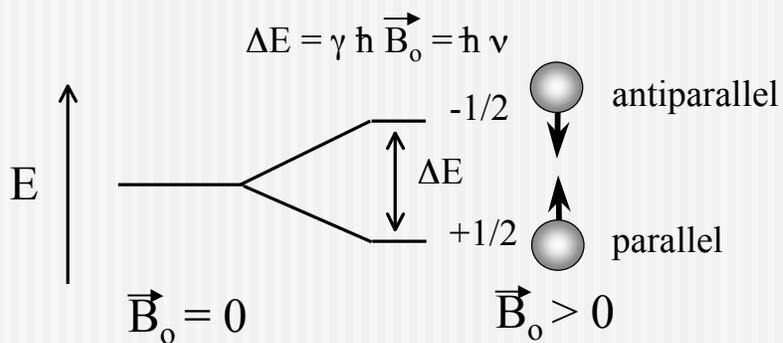
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The net magnetization vector



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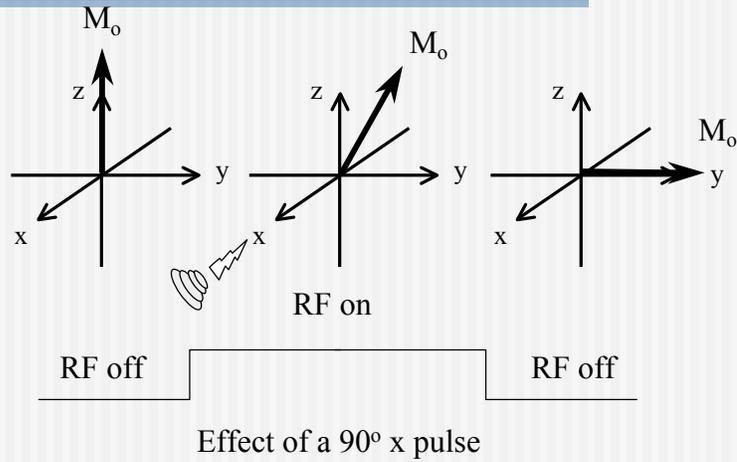
Allowed Energy States for a Spin 1/2 System



Therefore, the nuclei will absorb light with energy ΔE resulting in a change of the spin states.

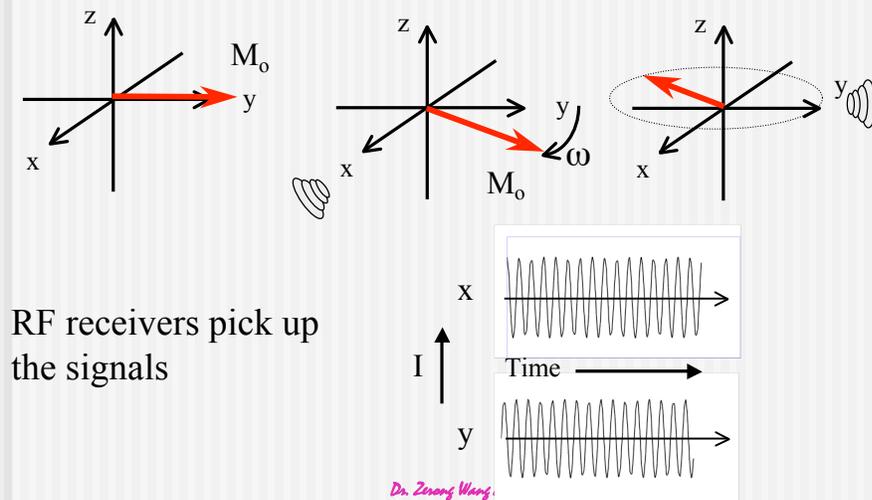
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Nuclear Spin Dynamics



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Nuclear Spin Evolution



Spin Orientation in a Magnetic Field (Energy Levels)

- Magnetic moments are no longer equivalent
- Magnetic moments are oriented in $2I+1$ directions in magnetic field

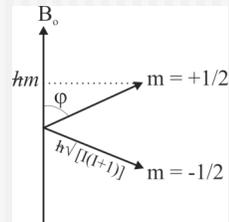
🕒 Vector length is: $\sqrt{I(I+1)}$

🕒 Angle (θ) given by: $\cos \theta = \frac{m}{\sqrt{I(I+1)}}$

🕒 Energy given by: $E = -\frac{m\mu}{I} B_0$

where,

B_0 – magnetic Field
 μ – magnetic moment
 h – Planck's constant

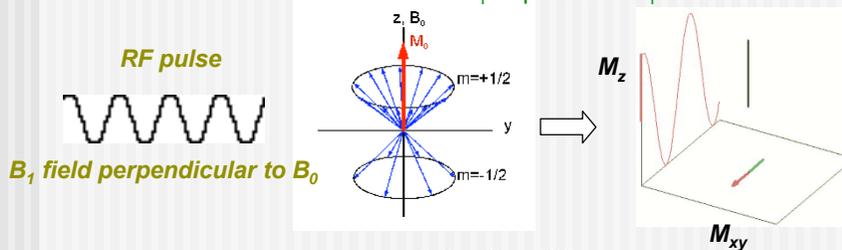


For $I = 1/2$

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Observing NMR Signal

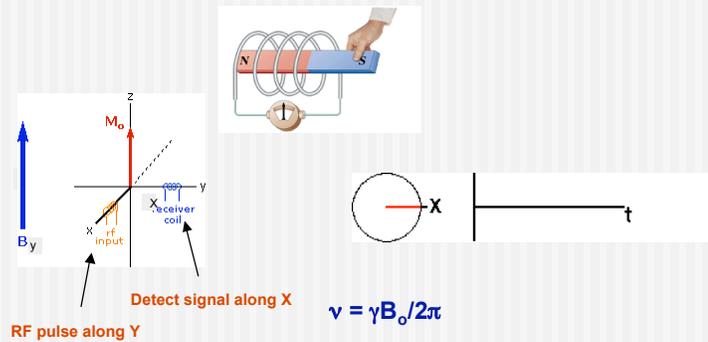
- Need to perturb system from equilibrium.
 - ✓ B_1 field (radio frequency pulse) with $\gamma B_0/2\pi$ frequency
- Net magnetization (M_0) now precesses about B_0 and B_1
 - ✓ M_x and M_y are non-zero
 - ✓ M_x and M_y rotate at Larmor frequency
- System absorbs energy with transitions between aligned and unaligned states
 - ✓ Precession about B_1 stops when B_1 is turned off



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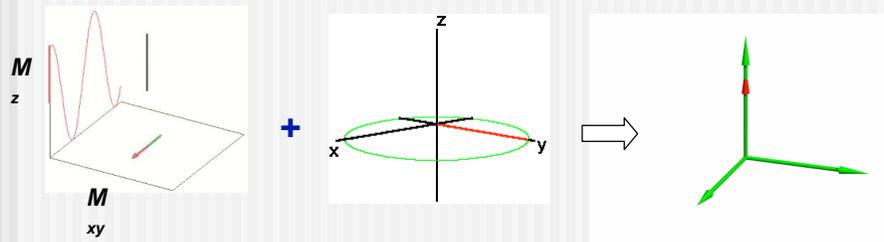
Observing NMR Signal

- **Remember:** a moving magnetic field perpendicular to a coil will induce a current in the coil.
- The induced current monitors the nuclear precession in the X,Y plane



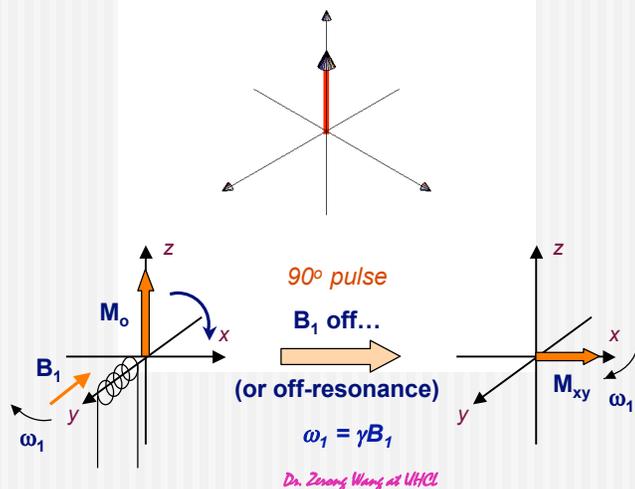
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- To simplify the vector description, the X,Y axis rotates about the Z axis at the Larmor frequency (X',Y')
- B_1 is stationary in the rotating frame



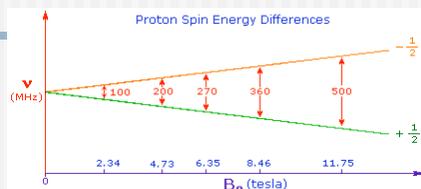
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- Applying the B_1 field for a specified duration (Pulse length or width)
- Net Magnetization precesses about B_1 a defined angle (90° , 180° , etc)



NMR Transition

Magnetic moments are oriented in one of two directions in magnetic field (for $I = 1/2$)



Difference in energy between the two states is given by

$$\Delta E = E_{-1/2} - E_{+1/2} = \gamma \left(\frac{h}{2\pi} \right) B_0$$

$$\text{If } h\nu_{\text{RF}} = \Delta E_B \rightarrow \text{NMR transition}$$

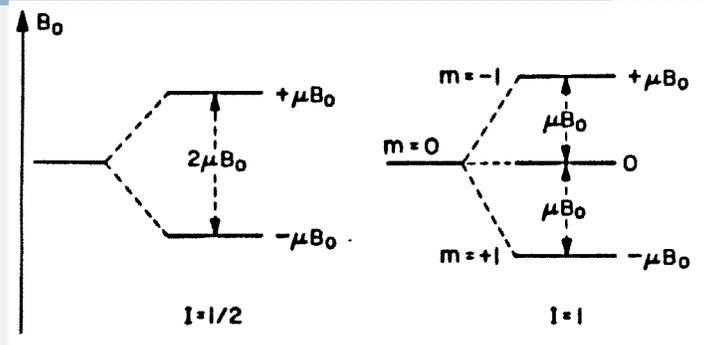
$$\text{For NMR: } B_0 = 1\text{-}20 \text{ Tesla} \quad (B_{\text{earth}} \approx 10^{-4} \text{ T})$$

$$\Delta E_{\text{NMR}} < 0.1 \text{ cal/mole} \ll kT \quad (\Delta E_{\text{IR}} \approx 1\text{-}10 \text{ kcal/mole})$$

- B_0 – external magnetic field
- h – Planck's constant
- γ – gyromagnetic ratio

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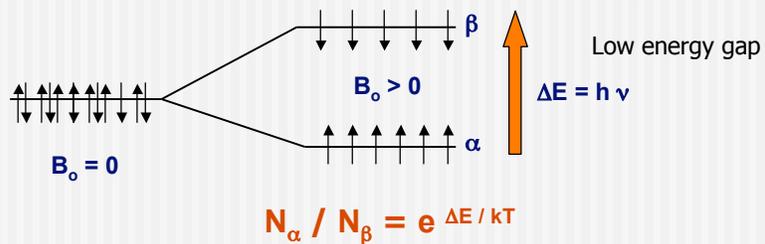
Magnetic Energy Levels for Nuclei of Spin 1/2 and 1



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NMR Signal Sensitivity

- NMR signal results from the transition of spins from the α to β state
- Strength of the signal depends on the population difference between the α and β spin
- The population (N) difference can be determined from the Boltzmann distribution and the energy separation between the α and β spin states:



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Since:

$$\Delta E = h\nu$$

and

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

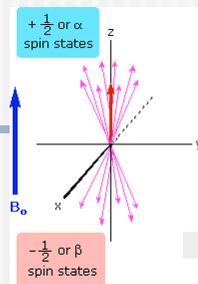
then:

$$N_\alpha / N_\beta = e^{\Delta E / kT} \implies N_\alpha / N_\beta = e^{(\gamma h B_0 / 2\pi kT)}$$

The ΔE for ^1H at 400 MHz ($B_0 = 9.39 \text{ T}$) is $6 \times 10^{-5} \text{ Kcal / mol}$

$$N_\alpha / N_\beta = 1.000060$$

Very Small !
 ~ 60 excess spins per million in lower state



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NMR Sensitivity

NMR signal (s) depends on: $s \propto \gamma^4 B_0^2 N B_1 g(\nu) / T$

- 1) Number of Nuclei (N) (limited to field homogeneity and filling factor)
- 2) Gyromagnetic ratio (in practice γ^3)
- 3) Inversely to temperature (T)
- 4) External magnetic field ($B_0^{2/3}$, in practice, homogeneity)
- 5) B_1^2 exciting field strength (RF pulse)

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$

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

Increase energy gap \rightarrow Increase population difference \rightarrow Increase NMR signal

$$\uparrow \Delta E \equiv \uparrow B_0 \equiv \uparrow \gamma$$

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NMR Signal/Noise Ratio

$$S/N = \gamma N I (I+1) [B_0/T]^{3/2} f(QV_s/b)^{1/2}$$

Where γ is the magnetogyric ratio of nucleus

B_0 external magnetic field

N number of magnetically active nuclei

T sample temperature

Q quality factor of the resonant circuit

f filling factor

b bandwidth of detector

V_s volume of sample

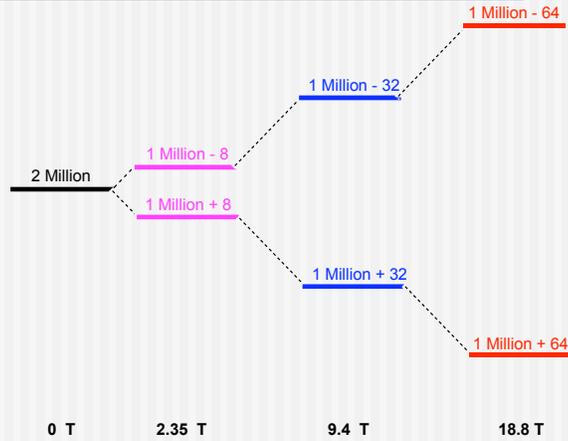
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Signal/Noise Enhancement

- **S/N ratio improves along with the increasing of the strength of magnetic field, in a relationship of:** $(B_0)^{3/2}$
- **S/N ratio increases when number of scan increases, in a relationship of:** \sqrt{N}

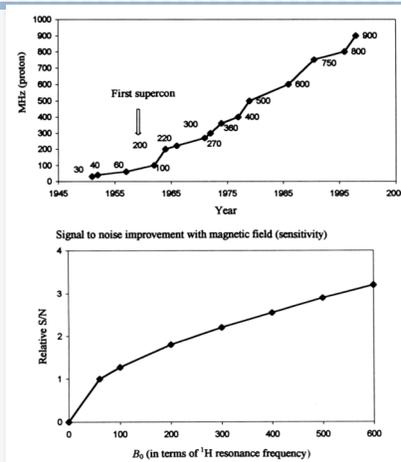
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Effect of Magnetic Field on Population Differences

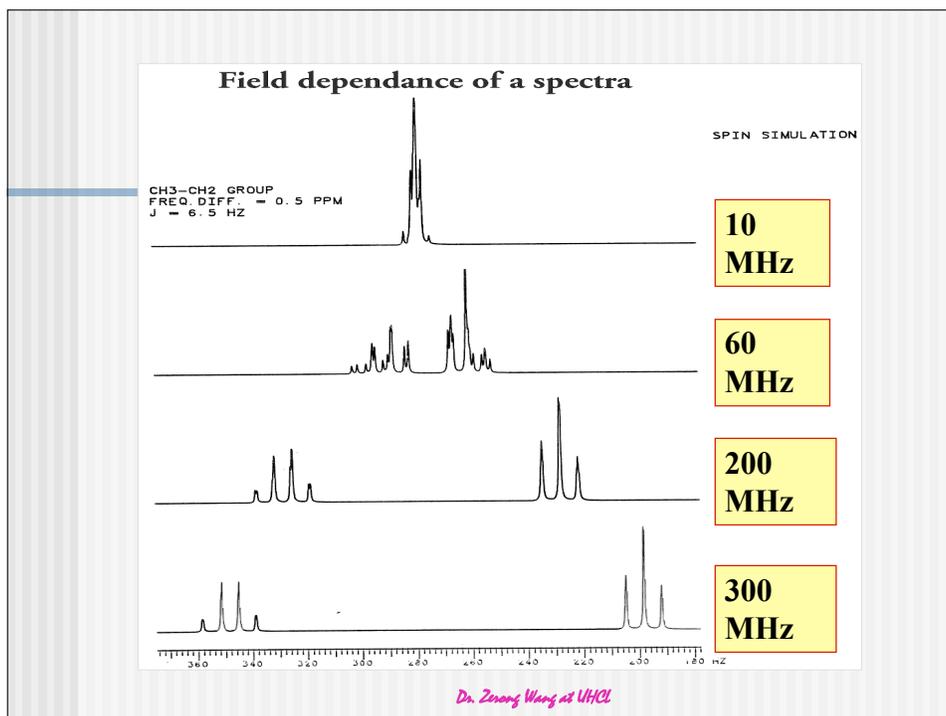


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Increase in Magnet Strength is a Major Means to Increase Sensitivity



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900 MHz NMR Spectrometer

- Analyze concentrations of 1 mM or less
- Characterize molecule with a molecular weight of 500,000

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NMR Sensitivity

- Relative sensitivity of ^1H , ^{13}C , ^{15}N and other nuclei NMR spectra depend on

- ❖ Gyromagnetic ratio (γ)
- ❖ Natural abundance of the isotope

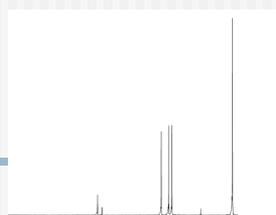
γ - Intrinsic property of nucleus can not be changed.

$$(\gamma_{\text{H}}/\gamma_{\text{C}})^3 \text{ for } ^{13}\text{C} \text{ is } 64\text{x} \quad (\gamma_{\text{H}}/\gamma_{\text{N}})^3 \text{ for } ^{15}\text{N} \text{ is } 1000\text{x}$$

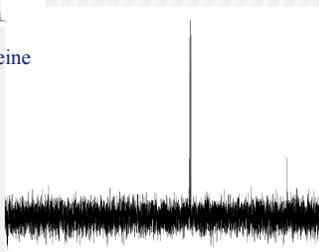
^1H is **$\sim 64\text{x}$** as sensitive as ^{13}C and **1000x** as sensitive as ^{15}N !

Consider that the natural abundance of ^{13}C is **1.1%** and ^{15}N is **0.37%**
relative sensitivity increases to **$\sim 6,400\text{x}$** and **$\sim 2.7 \times 10^5\text{x}$** !!

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^1H 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

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Increasing Magnetic Field Results in a Significant Cost!



~ \$800,000



~ \$2,000,000



~ \$4,500,000

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Varian 900 MHz NMR Instrument



Dr. Zhenqiang Wang at WHCI

Improved Sensitivity using Cryoprobes (Bruker)

- In a cryogenic probe, the pick-up coils and some electronics are cooled to ~ 30 K drastically reducing the Johnson noise, which is generated by thermal agitation of electrons in a conductor
- Increases sensitivity by a factor of 3.4-4.0
 - ❖ NMR signals are obtained by sequential averaging, this increase translates to 1/10-1/16 the number of measurements needed for averaging
 - ❖ A one hour run is reduced to 6 min
- A four-fold lower detection limit
Anal Chem, 2001, 73, A155.

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Spin Relaxation

There are two primary causes of spin relaxation:

Spin - lattice relaxation, T_1 , longitudinal relaxation

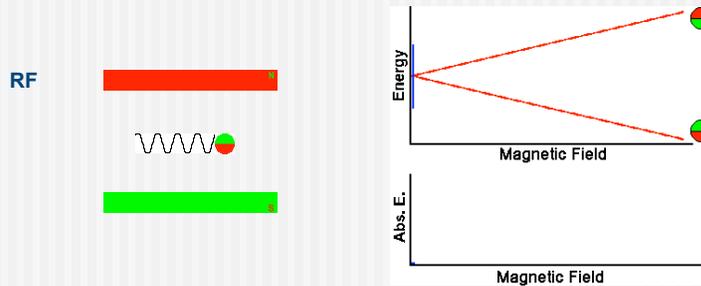


Spin - spin relaxation, T_2 , transverse relaxation.



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Transition from the low energy to high energy spin state occurs through an absorption of a photon of radio-frequency (RF) energy

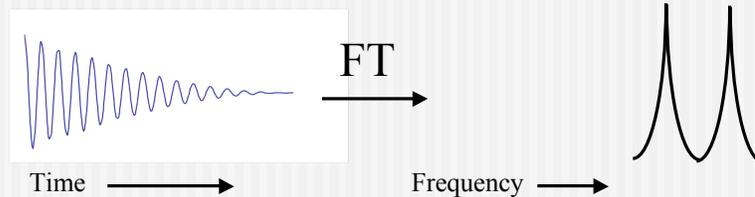


Frequency of absorption: $\nu = \gamma B_0 / 2\pi$

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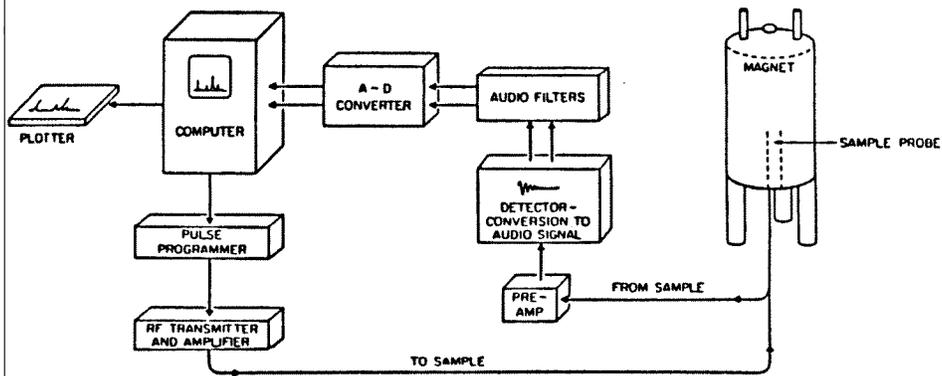
Free Induction Decay

- The signals decay away due to interactions with the surroundings.
- A free induction decay, FID, is the result.
- Fourier transformation, FT, of this time domain signal produces a frequency domain signal.



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High Resolution Pulse NMR Instrument

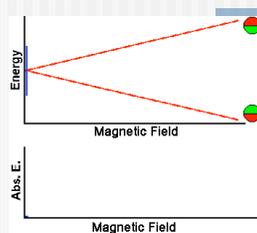


F.A. Bovey NMR Spectroscopy

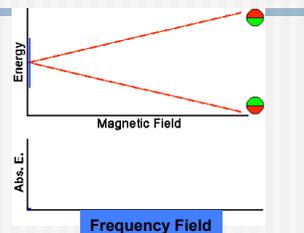
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CW NMR Experiments

The simplest NMR experiment is the continuous wave (CW) experiment. There are two ways of performing this experiment:



a constant frequency RF emission probes the energy levels while the magnetic field is varied.

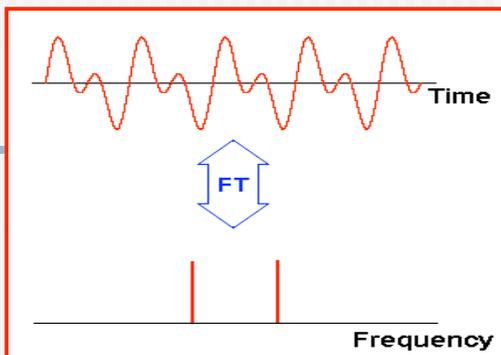


a varying frequency RF emission probes the energy levels while the magnetic field remains constant.

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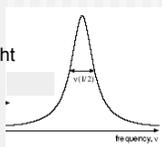
The Fourier Transform

The Fourier transform (FT) is a mathematical technique for converting time domain data to frequency domain data, and vice versa. An FT is defined by the integral :



$$f(\omega) = \int_{-\infty}^{+\infty} f(t)e^{-i\omega t} dt = \int_{-\infty}^{+\infty} f(t)[\cos(\omega t) - i \sin(\omega t)]dt$$

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<u>Observable</u>	<u>Name</u>	<u>Quantitative</u>	<u>Information</u>
Peak position	Chemical shifts (δ)	$\delta(\text{ppm}) = \nu_{\text{obs}} - \nu_{\text{ref}}/\nu_{\text{ref}}$ (Hz)	chemical (electronic) environment of nucleus
Peak Splitting	Coupling Constant (J) Hz	peak separation (intensity ratios)	neighboring nuclei (torsion angles)
Peak Intensity	Integral	unitless (ratio) relative height of integral curve	nuclear count (ratio) T_1 dependent
Peak Shape	Line width	$\Delta\nu = 1/\pi T_2$ peak half-height 	molecular motion chemical exchange uncertainty principal uncertainty in energy

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The chemical shift

The valence electrons around the nucleus are caused to *circulate* by the applied magnetic field B . This circulation, termed a local diamagnetic current, induces a *local magnetic field* δB that is oriented to *oppose* the applied field B . The net result is that the nucleus feels a *reduced magnetic field* B_{loc} ; that is, the applied field has been *shielded* by the local diamagnetic current.

σ is a dimensionless quantity called the *shielding constant* of the nucleus.

$$\delta B = -\sigma B$$
$$B_{loc} = B + \delta B = (1 - \sigma)B \quad \longrightarrow \quad \nu_L = \frac{\gamma B_{loc}}{2\pi} = (1 - \sigma) \frac{\gamma B}{2\pi}$$

Since the frequency at which resonance occurs is a direct function of the effective magnetic field B_{loc} , every nucleus that is in a distinct electronic environment will undergo resonance at a different applied frequency
scanning the frequency gives a NMR spectrum

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The δ -scale of chemical shift

The resonance frequency can be expressed in terms of *chemical shift* δ , which is related to the difference between the resonance frequency, ν , of the nucleus analyzed and that of a reference standard ν^o :

$$\delta = \frac{\nu - \nu^o}{\nu^o} \times 10^6$$

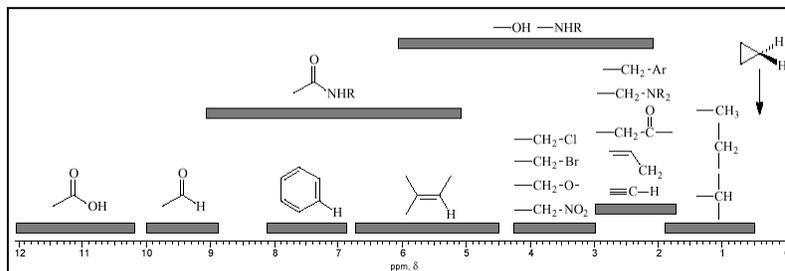
For ^1H and ^{13}C , the standard is tetramethylsilane, TMS: $\text{Si}(\text{CH}_3)_4$, for which $\delta=0$ ppm

→ With the δ -scale, shifts are *independent of the applied field*.

$$\delta = \frac{(1 - \sigma)B - (1 - \sigma^o)B}{(1 - \sigma^o)B} \times 10^6 = \frac{\sigma^o - \sigma}{1 - \sigma^o} \times 10^6 \cong (\sigma^o - \sigma) \times 10^6$$

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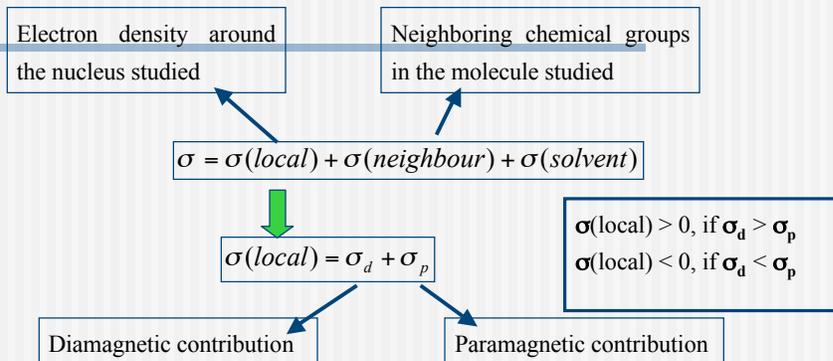
¹H NMR:



- Electronegative groups tend to move NMR signals from neighboring protons to **higher ppm values**.
- Protons on oxygen or nitrogen have **highly variable chemical shifts**.
- The π -system of alkenes and aromatic compounds strongly deshield attached protons and move them to **higher ppm values**.
- The hydrogens of carboxylic acids and aldehydes are the **most highly deshielded protons** generally encountered.

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Origin of the Shielding Constant



Note: $\sigma(\text{local}) = \sigma_d$ when the electron density is spherical or cylindrical around the nucleus. For ¹H NMR, the paramagnetic contribution is negligible.

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Diamagnetic Contribution σ_d

* B generates a circulation of charge in the ground-state electron distribution of the atom → δB is created opposite to B, which shields the nucleus and gives rise to σ_d . σ_d depends on the electron density around the protons.

Lamb formula:
$$\sigma_d = \frac{e^2 \mu_0}{3m_e} \int_0^\infty r \rho(r) dr$$

μ_0 = vacuum permeability, r = electron-nucleus distance, $\rho(r)$ is the electron density around the nucleus. Although the Lamb formula is strictly valid for spherical symmetry, when the diamagnetic contribution is dominant, it gives the right trend.

→ An electronegative atom X, directly bound to a proton (H-X) or with one carbon as intermediate (H-C-X), decreases the electron density around the proton and decreases the shielding σ_d contribution. The transition appears at high frequency, a large δ is observed.

→ As the electronegativity of the neighbor atom increases, δ increases

	CH ₃ F	CH ₃ Cl	CH ₃ Br	CH ₃ I	CH ₃ H
$\delta(\text{CH}_3)$	4.13	2.84	2.45	1.98	0.13
Electronegativity	4.0	3.0	2.8	2.5	2.1

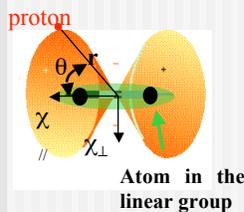
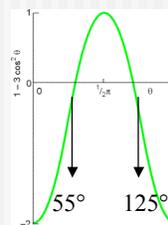
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Neighbouring Group Contribution $\sigma_{(\text{neighbour})}$

B generates current in the electron distribution of the neighboring group and gives rise to a magnetic moment M proportional to B via a constant vector χ called the magnetic susceptibility: $M = \chi B$.

For the sake of simplicity, let's consider only groups with axial symmetry (linear). The anisotropy of the group is defined with $\Delta\chi = \chi_{||} - \chi_{\perp}$. M creates an anisotropic magnetic field $B_{\text{neighbour}}$ and a shielding constant, which are function of:

- 1) The distance, r , between the proton and the group.
- 2) The anisotropy $\Delta\chi$.
- 3) The position of the proton with respect to the group, which is given by the angle θ .



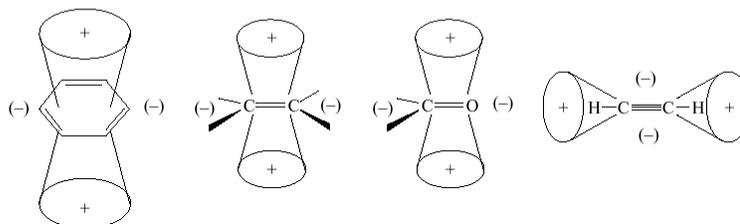
Mc Connel formula:

$$\sigma(\text{neighbour}) \propto (\chi_{||} - \chi_{\perp}) \left(\frac{1 - 3 \cos^2 \theta}{r^3} \right)$$

$\sigma(\text{neighbour})$ can be positive or negative according to $\Delta\chi$ and the position of the proton (θ). On the cone, $\delta = 0$

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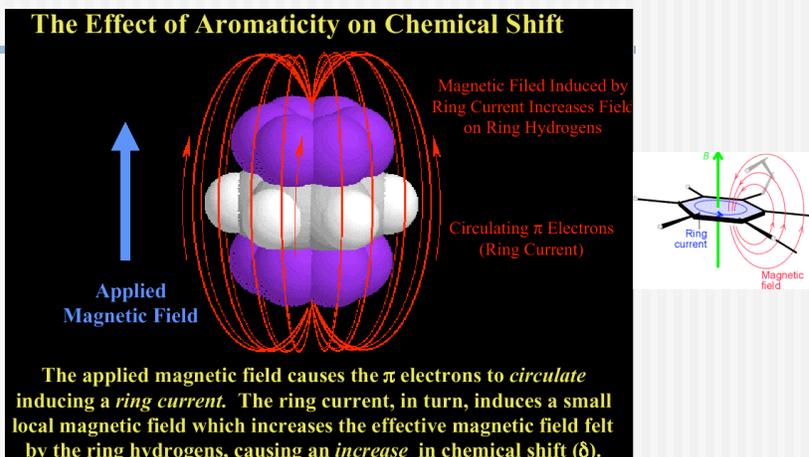
All groups in a molecule which contain π -electrons generate anisotropic secondary fields in response to an external magnetic field. The lines-of-force in these fields can either shield or deshield hydrogens, depending on the exact shape of the field and the location of the particular hydrogen nucleus. In the diagrams below, **protons within the (+) regions of space will be shielded** (smaller δ) and **protons in the (-) regions will be deshielded** (larger δ).



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Special Case of the Aromatic Compounds

The Effect of Aromaticity on Chemical Shift



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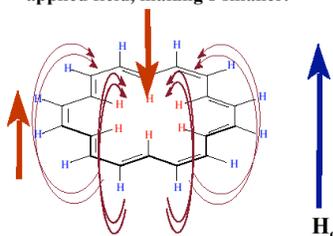
[18]Annulene

Note that [18]annulene has 12 hydrogens on the **outside** of the ring, and 6 hydrogens on the **inside**.

For the outer hydrogens, an applied magnetic field will **augment** the effective field, causing a **shift to the “aromatic” region**. The inner hydrogens, however, will experience the **opposite** effect, and should be shifted to a **smaller δ value**.

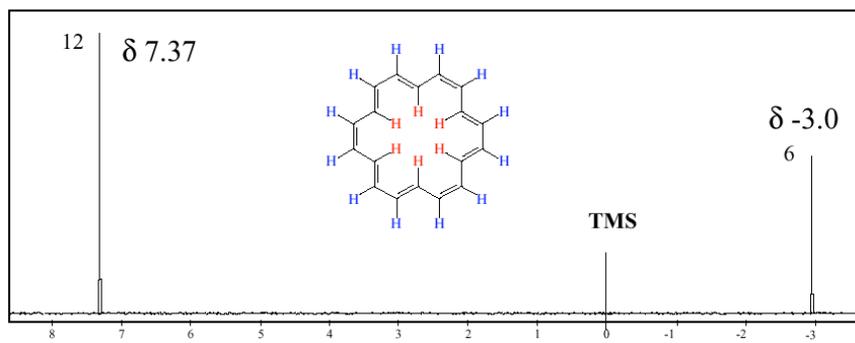
For the *inner* hydrogens, however, the induced field is **anti-parallel** to the applied field, making δ smaller.

The ring current induces a magnetic field for *outer* hydrogens which is **parallel** to the applied field, making δ larger.



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The ^1H NMR of [18]annulene shows two peaks. A **singlet at δ 7.3** (12 hydrogens) and a **singlet at δ -3.0** (6 hydrogens) consistent with the “ring current” model (**magnetic anisotropy**).



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NMR Spectroscopy

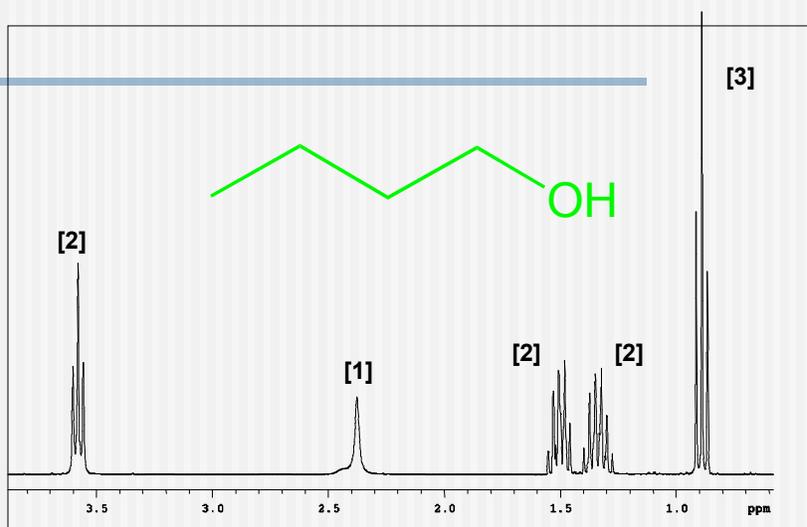
Main Features ---- Integration

The area under the NMR resonance is proportional to the number of hydrogen which that resonance represents. In this way, by measuring or integrating the different NMR resonances, information regarding the relative numbers of chemically distinct hydrogens can be found.

Integration only gives information on the relative number of hydrogens different, not the absolute number.

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^1H NMR Spectrum of 1-Butanol



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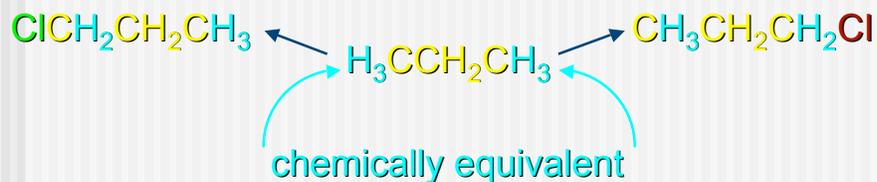
Number of Signals

- Protons that have different chemical shifts are chemically nonequivalent
- Exist in different molecular environment

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Chemically Equivalent Protons

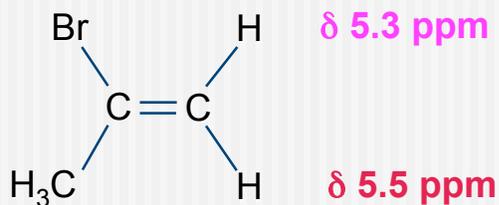
- are in identical environments
- have same chemical shift
- replacement test: replacement by some arbitrary "test group" generates same compound



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Diastereotopic Protons

- Replacement by some arbitrary test group generates diastereomers
- Diastereotopic protons can have different chemical shifts

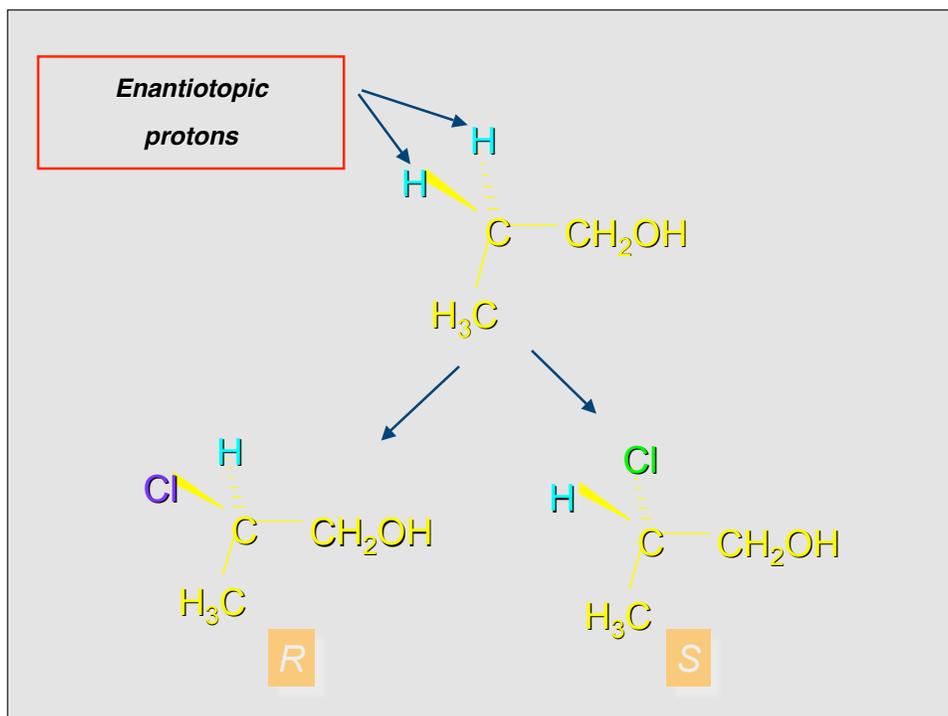


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Enantiotopic Protons

- are in mirror-image environments
- replacement by some arbitrary test group generates enantiomers
- enantiotopic protons have the same chemical shift

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Spin-Spin Coupling

- Nuclei with the same chemical environment or the same chemical shift are called **equivalent**. Nuclei with different environments or having different chemical shifts are nonequivalent.
- Nuclei which are close to one another affect each other's effective magnetic field. This effect is observable for nonequivalent nuclei at a distance of three or less bond lengths from each other.
- The effect is called spin-spin coupling or J coupling. The size of J is given in Hz unit, and is therefore dependent on the strength of the applied magnetic field.

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Spin-Spin Coupling

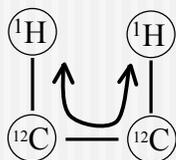
- For two nuclei, A and B, apart from one another by three bonds in a molecule, there are a total of four possible configurations for the two nuclei in a magnetic field. The vertical lines in this diagram represent the allowed transitions between energy levels.
- An allowed NMR transition is one where the spin of one nucleus changes from spin up to spin down or vice versa. Absorptions of energy where two or more nuclei change spin at the same time are not allowed.

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Scalar J Coupling

Electrons have a magnetic moment and are spin 1/2 particles.

J coupling is facilitated by the electrons in the bonds separating the two nuclei. This through-bond interaction results in splitting of the nuclei into $2I + 1$ states. Thus, for a spin 1/2 nucleus the NMR lines are split into $2(1/2) + 1 = 2$ states.



$$\text{Multiplet} = 2nI + 1$$

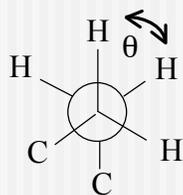
n - number of identical adjacent nuclei

I - spin quantum number

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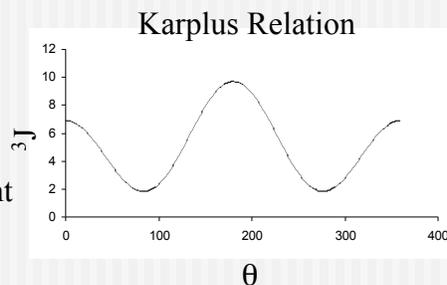
Scalar J Coupling

The magnitude of the J coupling is dictated by the torsion angle between the two coupling nuclei according to the Karplus equation.



A, B and C on the substituent electronegativity.

$$J = A + B \cos(\theta) + C \cos^2(\theta)$$
$$A = 1.9, B = -1.4, X = 6.4$$



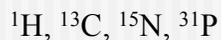
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Torsion Angles

Coupling constants can be measured from NMR data.

Therefore, from this experimental data we can use the Karplus relation to determine the torsion angles, θ .

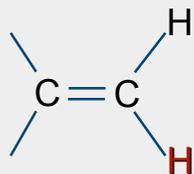
Coupling constants can be measured between most spin 1/2 nuclei of biological importance,



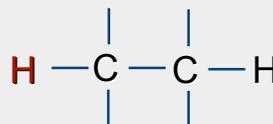
The most significant limitation is usually sensitivity, S/N.

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Two-bond and Three-bond Coupling

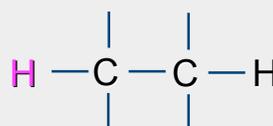
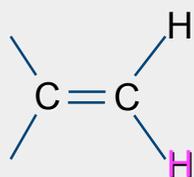


protons separated by
two bonds
(geminal relationship)
Large Coupling
10-15 Hz



protons separated by
three bonds
(vicinal relationship)
Smaller Coupling
about 7 Hz

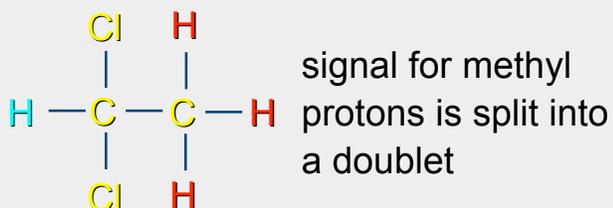
Two-bond and Three-bond Coupling



**in order to observe splitting, protons cannot
have same chemical shift**

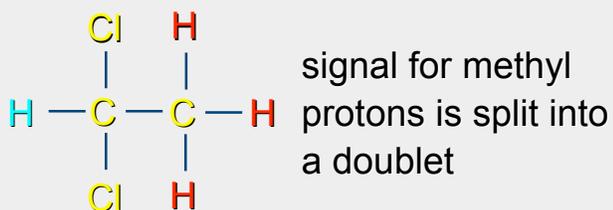
**coupling constant (2J or 3J) is independent
of field strength**

*Why do the methyl protons of
1,1-dichloroethane appear as a doublet?*



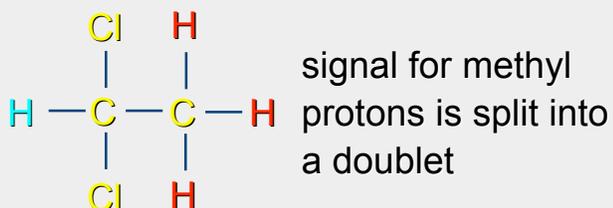
To explain the splitting of the **protons** at C-2, we first focus on the two possible spin orientations of the **proton** at C-1

*Why do the methyl protons of
1,1-dichloroethane appear as a doublet?*



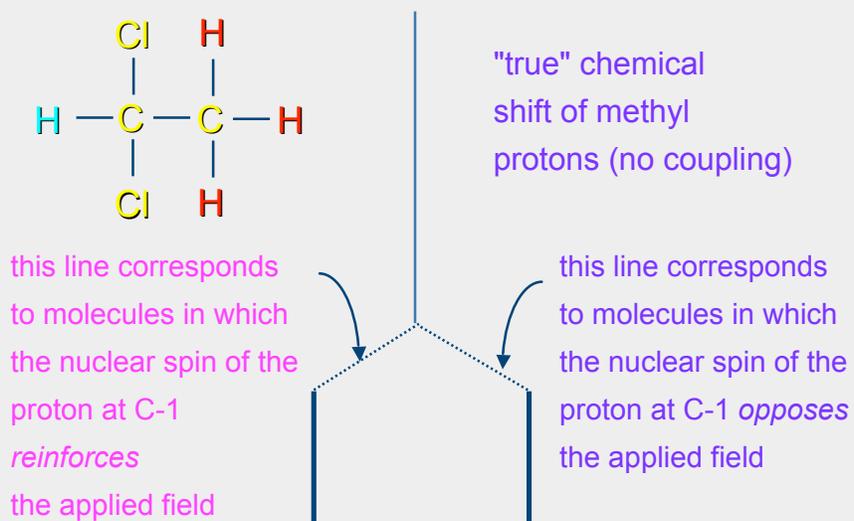
There are two orientations of the nuclear spin for the proton at C-1. One orientation shields the protons at C-2; the other deshields the C-2 protons.

Why do the methyl protons of
1,1-dichloroethane appear as a doublet?



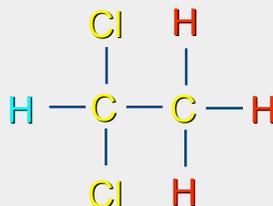
The protons at C-2 "feel" the effect of both the applied magnetic field and the local field resulting from the spin of the C-1 proton.

Why do the methyl protons of
1,1-dichloroethane appear as a doublet?



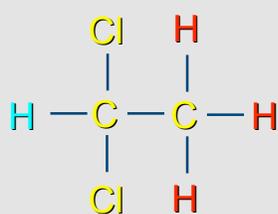
Why does the methine proton of
1,1-dichloroethane appear as a quartet?

signal for methine
proton is split into a
quartet

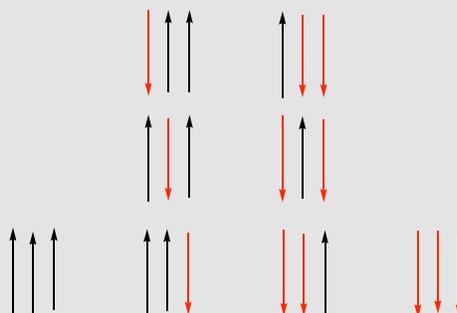


The proton at C-1 "feels" the effect of the applied magnetic field and the local fields resulting from the spin states of the three methyl protons. The possible combinations are shown on the next slide.

Why does the methine proton of
1,1-dichloroethane appear as a quartet?



There are eight combinations of nuclear spins for the three methyl protons. These 8 combinations split the signal into a 1:3:3:1 quartet.



The splitting rule for ^1H NMR A Much Simpler Rule

For simple cases, the multiplicity of a signal for a particular proton is equal to the number of equivalent vicinal protons + 1.

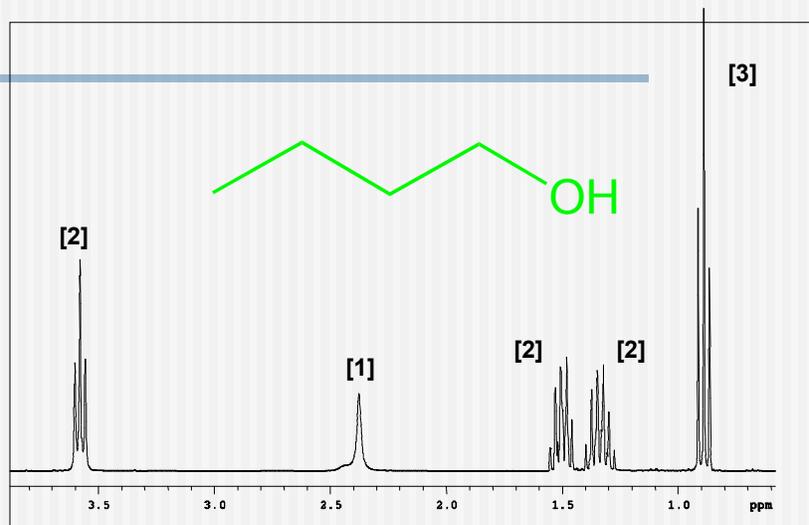
This is called the N+1 Rule: The number of proton neighbors plus itself gives the splitting number.

Pascal's Triangle --- "True" For NMR

Splitting Patterns of Common Multiplets

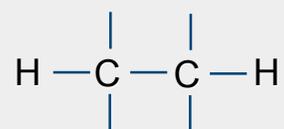
Number of equivalent protons to which H is coupled	Appearance of multiplet	Intensities of lines in multiplet
1	Doublet	1:1
2	Triplet	1:2:1
3	Quartet	1:3:3:1
4	Pentet	1:4:6:4:1
5	Sextet	1:5:10:10:5:1
6	Septet	1:6:15:20:15:6:1

^1H NMR Spectrum of 1-Butanol



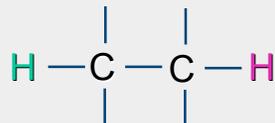
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Pairs of Doublets



Consider coupling between two vicinal protons.

If the protons have different chemical shifts,
each will split the signal of the other into a
doublet.



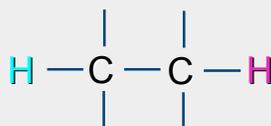
Let $\Delta\nu$ be the difference in chemical shift in Hz between the two hydrogens.

Let J be the coupling constant between them in Hz.



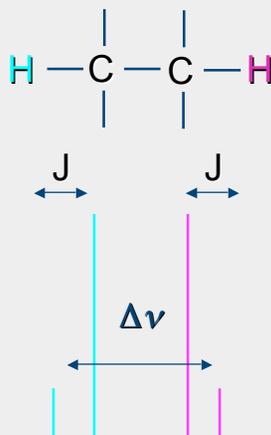
When $\Delta\nu$ is much larger than J the signal for each proton is a doublet, the doublet is symmetrical, and the spin system is called AX.

AM



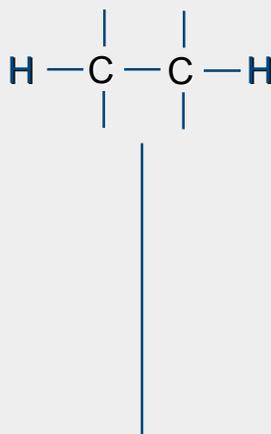
As $\Delta\nu/J$ decreases the signal for each proton remains a doublet, but becomes skewed. The outer lines decrease while the inner lines increase, causing the doublets to "lean" toward each other.

AB



When $\Delta\nu$ and J are similar, the spin system is called **AB**. Skewing is quite pronounced. It is easy to mistake an AB system of two doublets for a quartet.

A₂



When $\Delta\nu = 0$, the two protons have the same chemical shift and don't split each other. A single line is observed. The two doublets have collapsed to a singlet.

^{13}C NMR

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More Complicated or Simpler Than ^1H Spectra?

Heteronuclear coupling: between different nuclei

^1H - ^{13}C , ^1H - ^{31}P , ^{13}C - ^{19}F , etc.

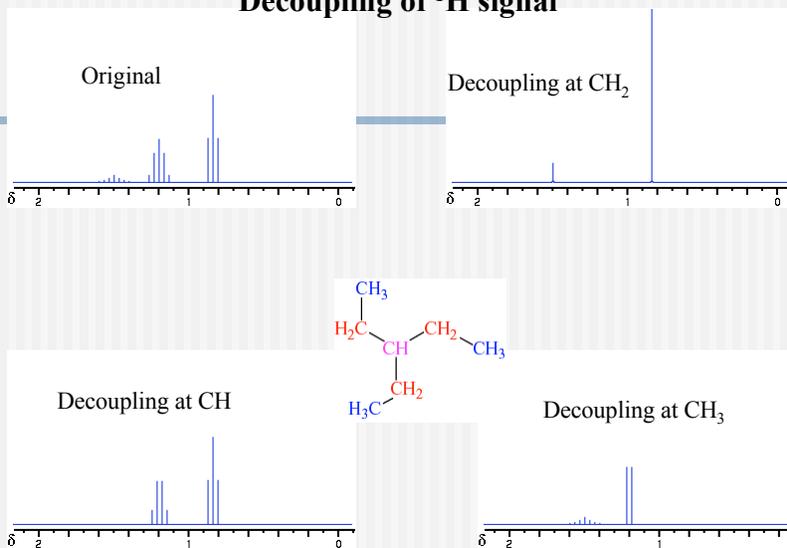
$^1J_{\text{CH}} \sim 100$ to 250 Hz

$^1J_{\text{CC}} = 550$ s(i)s(j), e.g., $\text{CH}_2=\text{C}=\text{CH}_2$, $J = 98.7$ ($1/2 * 1/3 = 1/6$)

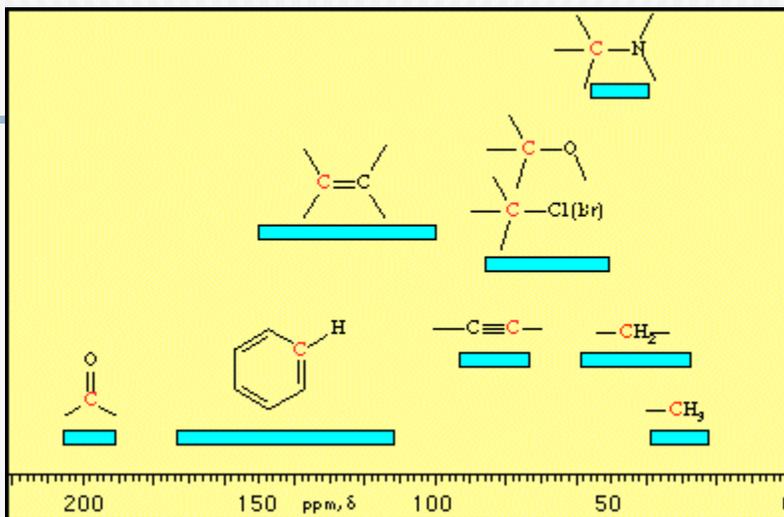
^1H -coupled ^{13}C spectra often have overlapping resonances, so ^{13}C spectra usually acquired in ^1H -mode

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Decoupling of ^1H signal

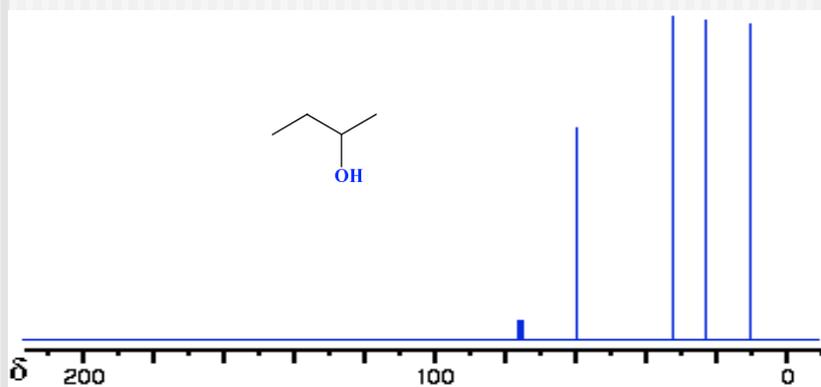


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Decoupling of ^1H on ^{13}C spectrum



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^{13}C DEPT NMR

The DEPT (distortionless enhanced polarization transfer) experiment is a useful 1D NMR experiment that provides information on the number of protons attached to the various ^{13}C resonances in a carbon NMR spectrum. In the DEPT experiment we use two rf transmitters, one to apply pulses to the ^1H spins and one to apply pulses to the ^{13}C spins. We acquire the ^{13}C signal during the FID time period. During the ^{13}C acquisition, the ^1H transmitter is used for broad-band (BB) decoupling to remove the splitting of ^{13}C signals by attached protons. For the proton channel, it is important to note that we are using the transmitter that is normally used to do decoupling and not the one that is used for routine proton acquisitions.

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A DEPT spectrum actually consists of several spectra, with the final data presentation depicting one spectrum for each type of carbon atom. Thus, CH, CH₂ and CH₃ carbons are each printed out on separate spectra, together with a ¹³C spectrum where all carbon types are shown. Each carbon type is thus identified unambiguously.

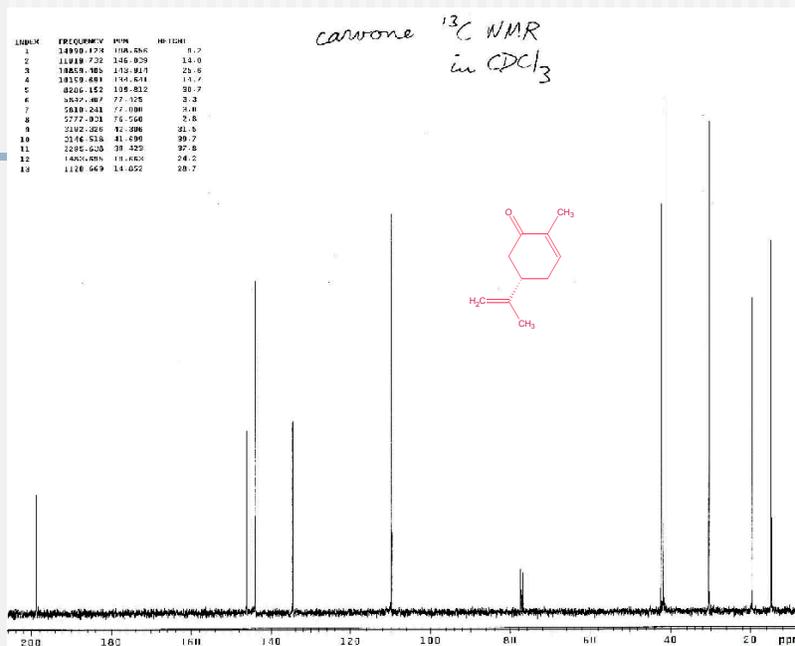
Results of the DEPT experiments (quaternary carbons are null in all spectra):

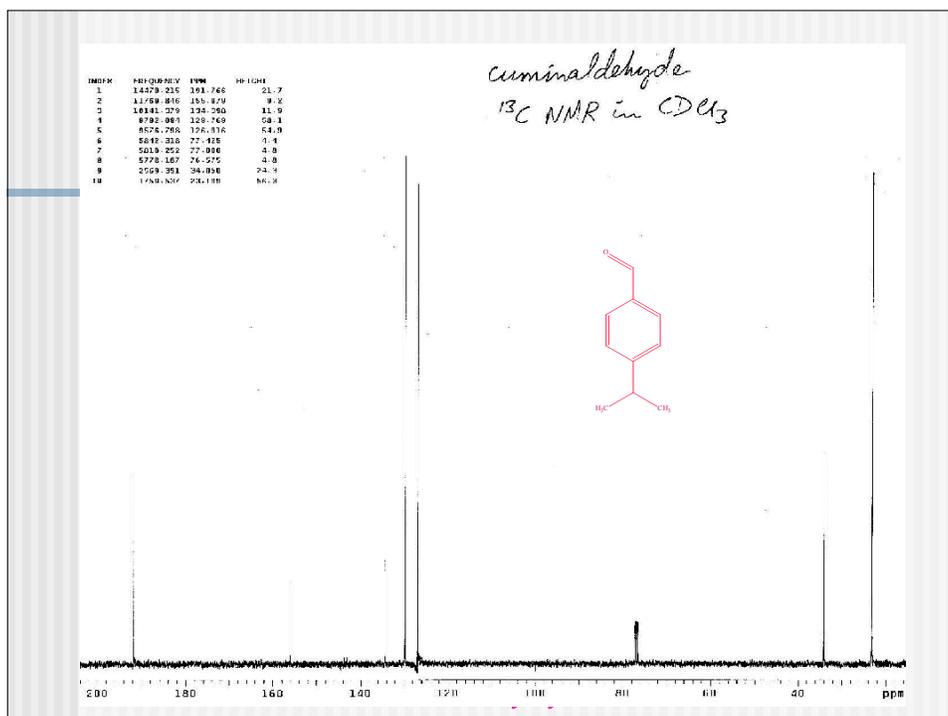
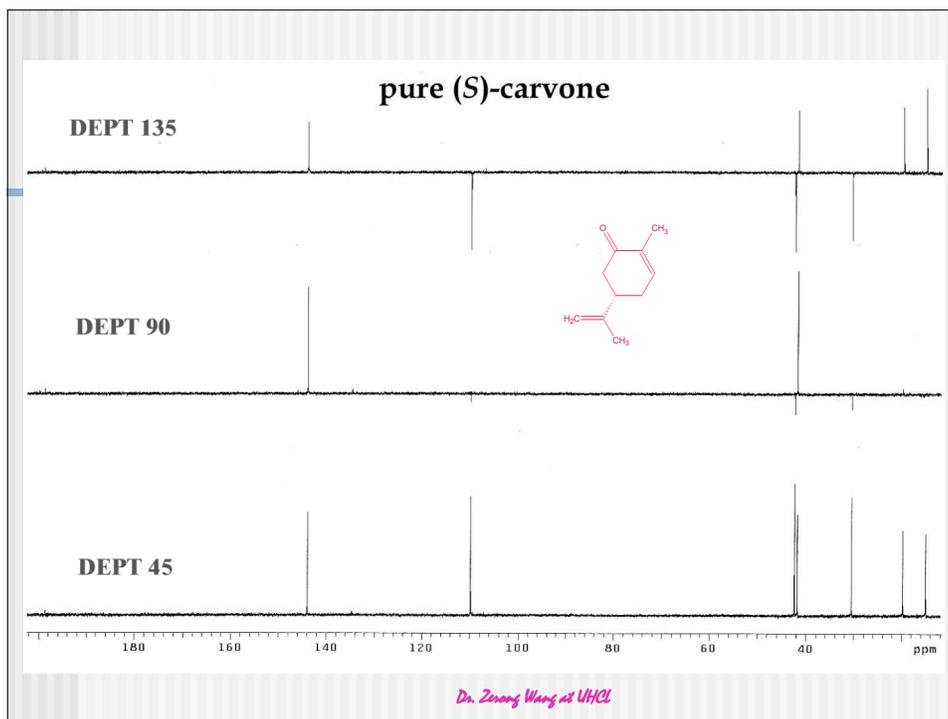
DEPT 45: CH, CH₂, CH₃ positive.

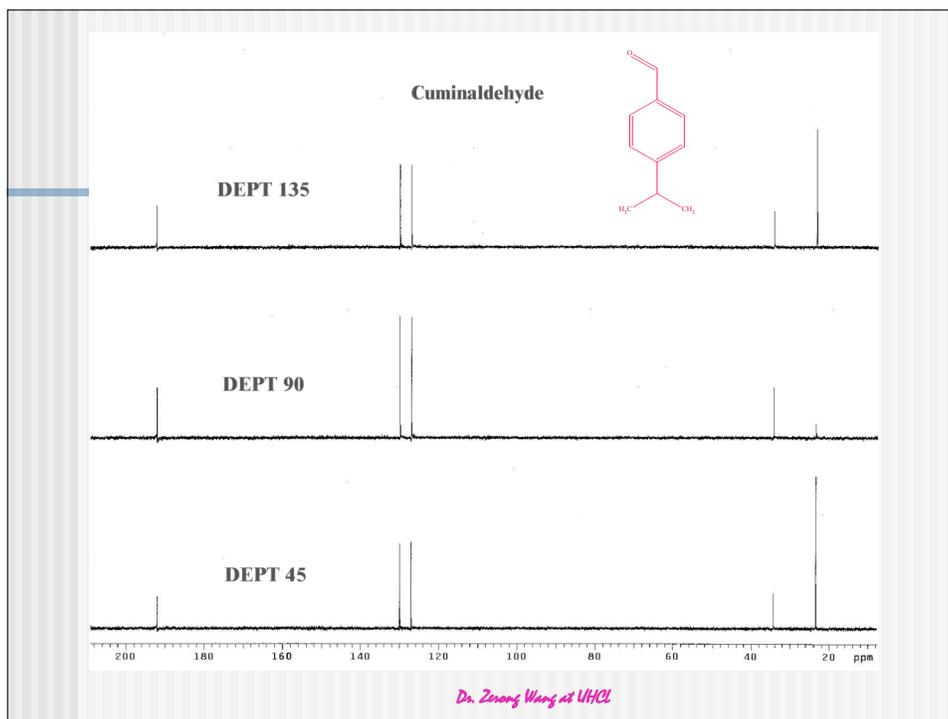
DEPT 90: CH positive, CH₂, CH₃ null (often a small residual signal is seen).

DEPT 135: CH, CH₃ positive, CH₂ negative.

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Prediction of ^1H NMR Signal

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Shoolery chemical shift rules for ^1H

• As we have seen, most of the different effects on ^1H chemical shifts have been tabulated in one way or another.

• Furthermore, we also saw that most of the effects are *additive*, meaning that if we can estimate the different effects on the chemical shift of a certain ^1H from different groups and bonds, we can in principle estimate its chemical shift by adding all the effects together.

• There are several empirical rules, derived mostly by *Shoolery* in the late 50s/early 60s.

• In order to use them, we first have to identify the type of proton we have, such as aliphatic CH_3 , CH_2 , CH , olefinic CH_2 or CH , aromatic, a or b to a ketone or alcohol, belonging to an a,b-unsaturated system, etc. They will have a base value.

• Then we look up the contributions from different groups attached to carbons in the surrounding of our system, and add them up to obtain the estimated chemical shift.

• We'll analyze several cases to see how they work...

$$\delta H = \delta H_{\text{base}} + \sum \text{contributions}$$

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Shoolery rules (continued)

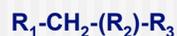
• *Aliphatic compounds*. There are two approaches to the calculation of additive effects on the ^1H chemical shifts.

• The first one is very simple. We just use two 'skeletons' with two base values, $\text{R}_1\text{-CH}_2\text{-R}_2$ or $\text{R}_1\text{-CH-(R}_2\text{)-R}_3$, and add the effects from the R_1 , R_2 , or R_3 groups:

• So CH_2Br_2 would be $d = 1.25 + 1.9 + 1.9 = 5.05$ ppm, which compares pretty well with the experimental value of 4.94 ppm.



$$\delta = 1.25 + \text{R}_1 + \text{R}_2$$



$$\delta = 1.50 + \text{R}_1 + \text{R}_2 + \text{R}_3$$

Substituent	δ
Alkyl	0.0
-C=C-	0.8
-C \equiv C-	0.9
-C ₆ H ₅	1.3
-CO-R	1.3
-OH	1.7
-O-R	1.5
-O-CO-R	2.7
-NH ₂	1.0
-Br	1.9
-Cl	2.0

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Shoolery rules (...)

The second method is pretty more general. We start with methane (d of 0.23 ppm), and then we add substituent effects directly.

- Now, if instead of a substituent we have another carbon chain, we have to consider how many carbons it has, and each carbon will have an increment we need to add:
- Furthermore, if the carbons of these chains are substituted, we have to add increments according to their position in the carbon chain.
- It is a lot more complicated, but as we see, more general (and some say more accurate).

$$\delta = 0.23 + \sum S(\delta)$$

$\frac{0.248}{\text{---C}_2}$

$\frac{0.244}{\text{---C}_2\text{---C}_3}$

$\frac{0.147}{\text{---C}_2 \begin{matrix} \diagup \text{C}_3 \\ \diagdown \text{C}_3 \end{matrix}}$

$\frac{0.006}{\text{---C}_2 \begin{matrix} \diagup \text{C}_3 \\ \diagdown \text{C}_3 \\ \text{---C}_3 \end{matrix}}$

CH ₃ -	0.47
C ₆ H ₅ -	1.85
RO-	2.36
RC(=O)O-	3.13

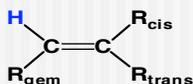
	C ₁	C ₂	C ₃
HO-	2.47	0.048	0.235
Br-	1.995	0.363	0.023
CH ₃ O-	-	-0.374	-
-O-CO-CR ₃	2.931	0.041	-0.086

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Shoolery rules (...)

- **Olefines.** For alkenes we change the tables for the base values, but we also have to consider the stereochemistry of the substituent (*cis*, *trans*, or *gem*):

So for cinnamic acid (*trans* Ph-CH^a=CH^b-COOH), we get that $\delta_{H^a} = 5.25 + 1.38 + 0 + 0.98 = 7.61$, and $\delta_{H^b} = 5.25 + 0.80 + 0 + 0.36 = 6.41$, pretty close to the reported values of 7.82 and 6.47 ppm.



$$\delta = 5.25 + R_{\text{gem}} + R_{\text{trans}} + R_{\text{cis}}$$

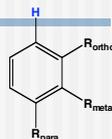
Substituent	δ_{gem}	δ_{cis}	δ_{trans}
H-	0.0	0.0	0.0
Alkyl-	0.45	-0.22	-0.28
-OR	1.21	-0.60	-1.00
-COOH	0.80	0.98	0.32
-Ar	1.38	0.36	-0.07
-C=C-	1.24	0.02	-0.05
-OH	1.22	-1.07	-1.21
-Cl	1.08	-0.40	-1.02

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Schoolery rules (...)

- Aromatics.** Finally, the Schoolery rules allow us to calculate the approximate chemical shifts in aromatic compounds. Again, we have a different base value of 7.26 (benzene...).

$$\delta = 7.26 + R_{\text{ortho}} + R_{\text{meta}} + R_{\text{para}}$$



Substituent	δ_{ortho}	δ_{meta}	δ_{para}
H-	0.0	0.0	0.0
CH ₃ -	-0.18	-0.10	-0.20
-NO ₂	0.95	0.26	0.38
-COOH	0.85	0.18	0.25
-OCH ₃	1.38	0.36	-0.07
-Cl	1.24	0.02	-0.05
-F	1.22	-1.07	-1.21
-CONH ₂	1.38	0.36	-0.07
-CH=CH ₂	1.24	0.02	-0.05
-SO ₃ H	1.22	-1.07	-1.21

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Schoolery rules (...)

- For *p*-Xylene:

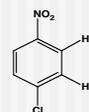
$$\delta H_a = 7.26 - 0.18 - 0.10 = 6.98 \text{ (6.97)}$$

$$\delta H_b = \delta H_a$$

- For 1-Chloro-4-nitrobenzene

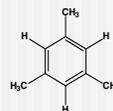
$$\delta H_a = 7.26 + 0.95 - 0.02 = 8.19 \text{ (8.17)}$$

$$\delta H_b = 7.26 + 0.03 + 0.26 = 7.55 \text{ (7.52)}$$



- For mesitylene

$$\delta H = 7.26 - 2 * 0.18 - 0.20 = 6.70 \text{ (6.78)}$$

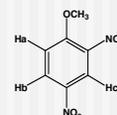


- For 2,4-dinitro-1-methoxybenzene

$$\delta H_a = 7.26 - 0.48 + 2 * 0.26 = 7.30 \text{ (7.28)}$$

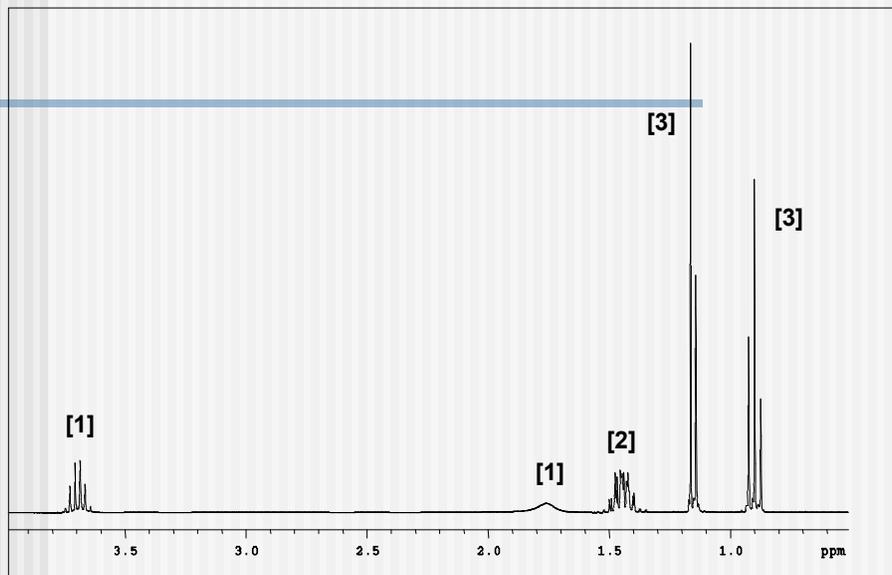
$$\delta H_b = 7.26 + 0.95 + 0.38 - 0.09 = 8.50 \text{ (8.47)}$$

$$\delta H_c = 7.26 + 2 * 0.95 - 0.09 = 9.07 \text{ (8.72)}$$



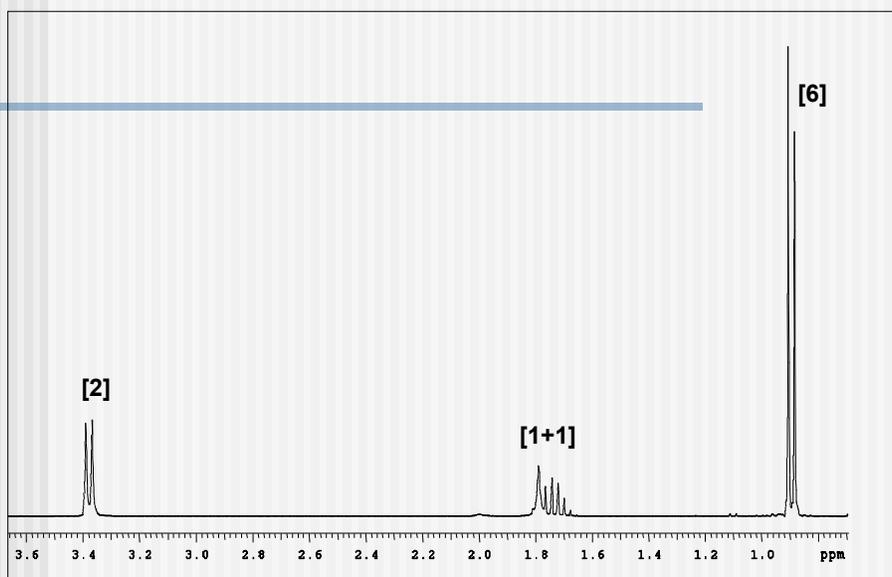
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NMR Results: 2-Butanol



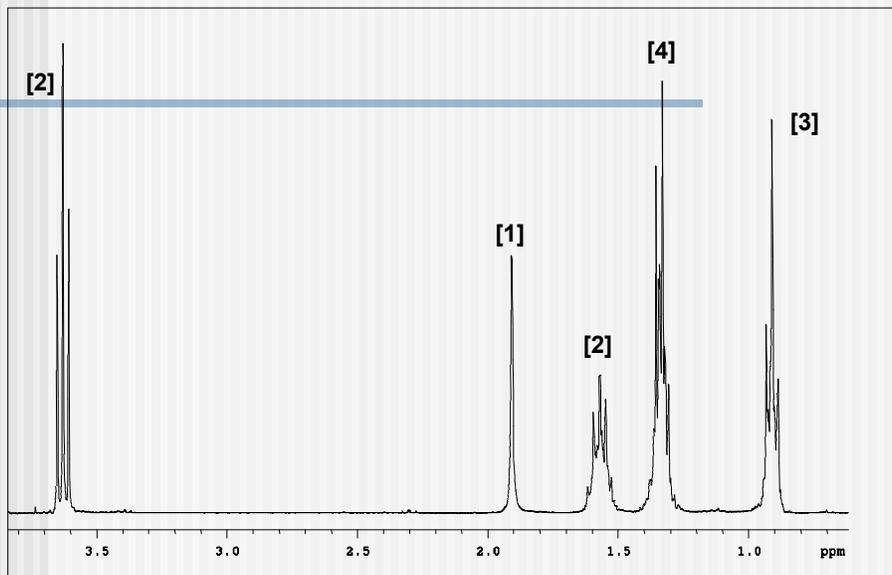
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NMR Results: 2-Methyl-1-propanol (Isobutanol)



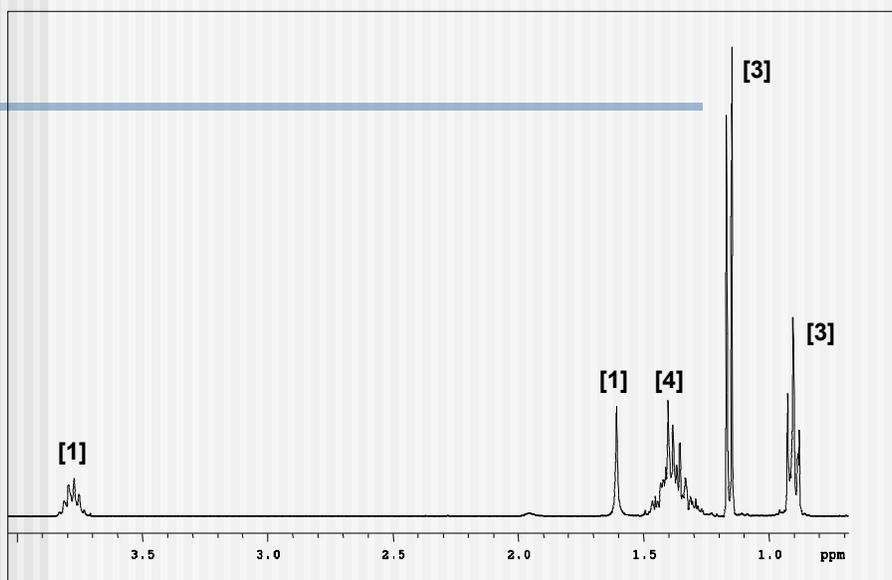
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NMR Results: 1-Pentanol



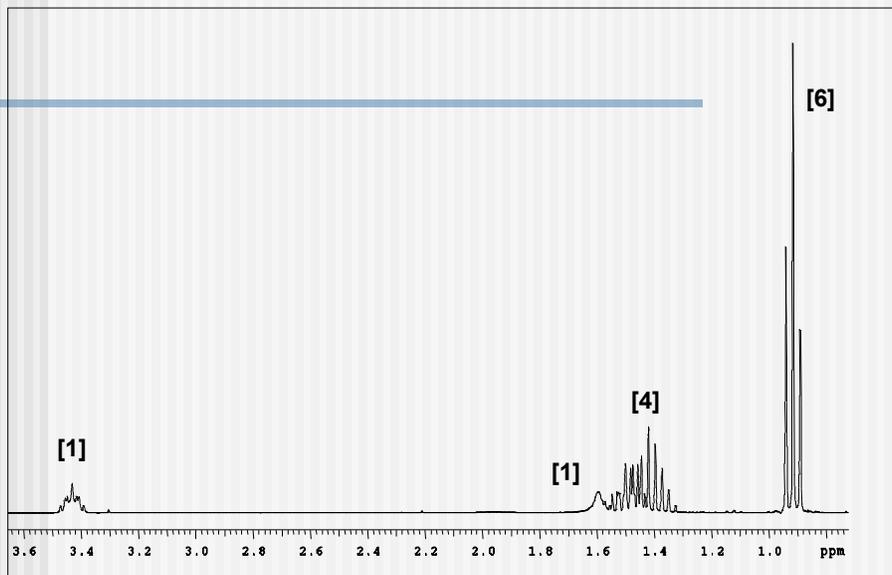
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NMR Results: 2-Pentanol



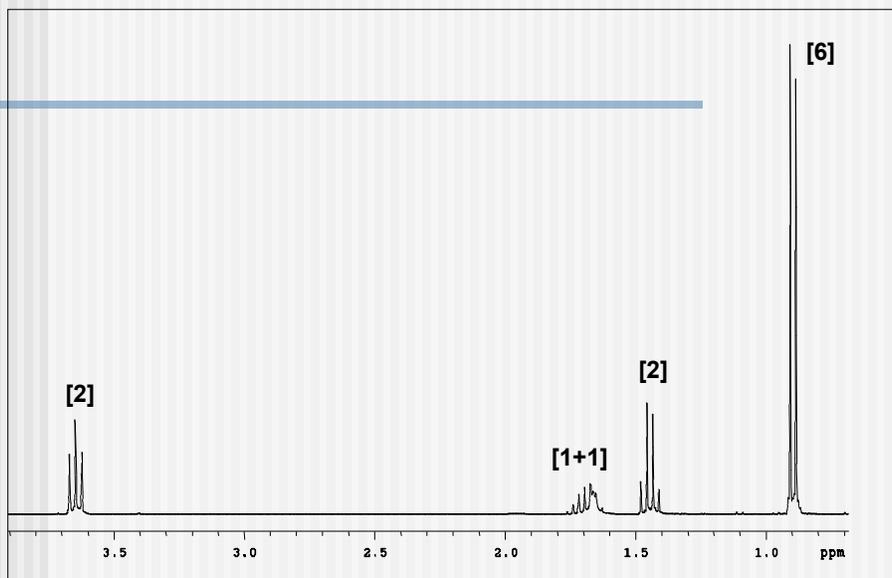
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NMR Results: 3-Pentanol



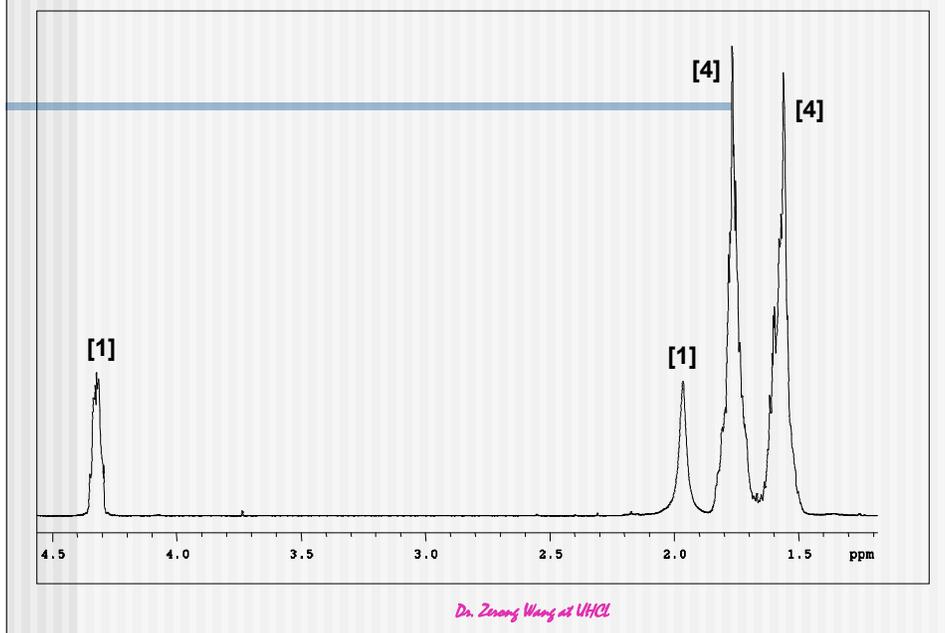
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NMR Results: 3-Methyl-1-butanol (Isopentanol)



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NMR Results: Cyclopentanol

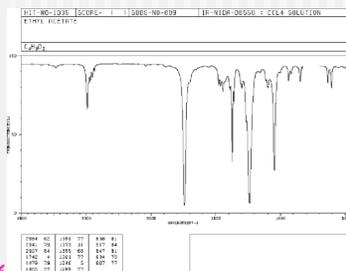


One chemical with molecular formula as $C_4H_8O_2$, its 1H NMR, ^{13}C NMR, IR and MS spectra are given below, Please identify its structure.

Assign.	Shift(ppm)	ppm	Int.
		171.08	527
A	4.119	60.44	1000
B	2.038	21.00	571
C	1.260	14.28	857

$J(A,C)=7.1\text{HZ.}$

14.0	1	42.0	4
15.0	6	43.0	100
18.0	2	44.0	2
26.0	1	45.0	14
27.0	6	61.0	14
28.0	2	70.0	9
29.0	13	73.0	4
31.0	1	88.0	5



Another Example

Molecule Formula: $C_{10}H_{11}NO_3$

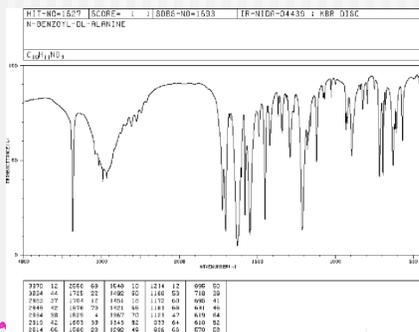
Assign. Shift(ppm)

A	12.
B	8.69
C	7.925
D	7.59 to 7.39
E	4.488
F	1.443

ppm Assign.

174.12	1
166.11	2
133.91	3
131.23	4
128.13	5
127.35	6
48.11	7
16.84	8

45.0	1	104.0	0
50.0	3	105.0	100
51.0	13	106.0	8
52.0	1	148.0	33
71.0	1	149.0	10
74.0	1	175.0	1
76.0	2	193.0	4
77.0	38		



Dr. Zou

Structure Determination by NMR

Biological molecules such as proteins and nucleic acids can be large and complex. They can easily exceed 2000 atoms. Knowing their structure is critical in understanding the relationship between structure and function.

X-ray crystallography is an excellent method to determine detailed 3D structures of even some of the largest biological molecules. However, it has some significant difficulties. Getting crystals and is the structure biologically relevant.

NMR can be used to determine 3D structure and dynamics in solution! It's limitation is molecular size. However, this is changing.

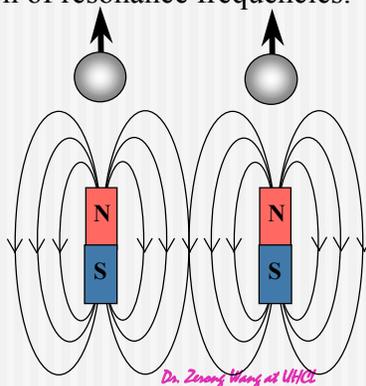
A good online book about basic NMR is at
<http://www.cis.rit.edu/htbooks/nmr/>

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Nuclear Overhauser Effect

Caused by dipolar coupling between nuclei.

The local field at one nucleus is affected by the presence of another nucleus. The result is a mutual modulation of resonance frequencies.

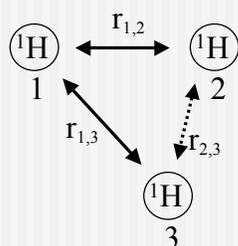


Nuclear Overhauser Effect

The intensity of the interaction is a function of the distance between the nuclei according to the following equation.

$$I = A (1/r^6)$$

I - intensity
A - scaling constant
r - internuclear distance



Arrows denote cross relaxation pathways
 $r_{1,2}$ - distance between protons 1 and 2
 $r_{2,3}$ - distance between protons 2 and 3

The NOE provides a link between an experimentally measurable quantity, I, and internuclear distance.

NOE is only observed up to $\sim 5 \text{ \AA}$.

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Biomolecular NMR Experiments

➤ J Correlated Based Experiments

- ⊕ COSY - Correlated Spectroscopy
- ⊕ 2QF-COSY - Double Quantum Filtered Spectroscopy
- ⊕ HETCOR - Heteronuclear Correlated Spectroscopy
- ⊕ E.COSY - Exclusive COSY
- ⊕ HOHAHA - Homonuclear Hartmann Hahn (TOCSY)

➤ Nuclear Overhauser Based Experiments

- ⊕ NOESY - Nuclear Overhauser Effect Spectroscopy
- ⊕ ROESY - Rotating Frame Overhauser Effect Spectroscopy

➤ Three Dimensional Experiments Use a Combination

- ⊕ NOESY - TOCSY
- ⊕ NOESY - NOESY

Dr. Zhenqiang Wang at WHCI

Sample Factors Influencing NMR Sensitivity

- **System should have short longitudinal T_1 to allow maximum number of scans**
- **Exhibit longest possible transverse T_2 to yield sharpest lines**
- **Be decoupled from other magnetically active centers so as to yield single line**
- **Be stable on NMR time scale**
- **Transfer of magnetization from adjacent scalar coupled nuclei to spin site under observation**
- **Isotopic enrichment of observed nucleus**

Dr. Zhenqiang Wang at WHCI