PELATIHAN SPEKTROSKOPI NMR

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*) kompilasi dari berbagai sumber

Nuclear Magnetic Resonance (NMR)

Introduction:

Nuclear Magnetic Resonance (NMR) measures the absorption of electromagnetic radiation in the radio-frequency region (~4-1000 MHz)

- nuclei (instead of outer electrons) are involved in absorption process
- sample needs to be placed in magnetic field to cause different energy states

NMR was first experimentally observed by Bloch and Purcell in 1946 (received Nobel Prize in 1952) and quickly became commercially available and widely used.

Probe the Composition, Structure, Dynamics and Function of the Complete Range of Chemical Entities: from small organic molecules to large molecular weight polymers and proteins.

NMR is routinely and widely used as the preferred technique to rapidly elucidate the chemical structure of most organic compounds.

One of the *MOST* Routinely used Analytical Techniques
MOS **use**

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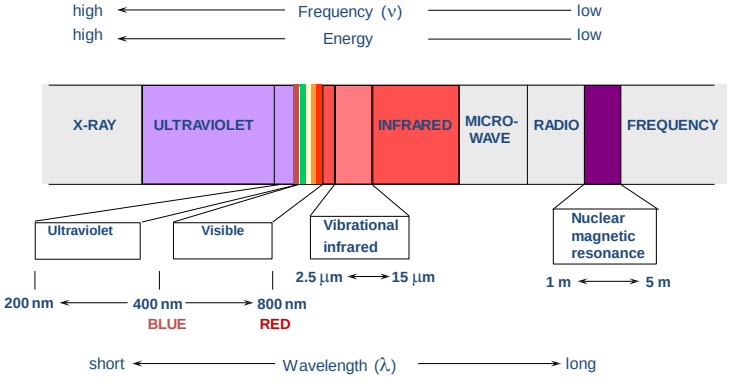
THE ELECTROMAGNETIC SPECTRUM



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Typical Applications of NMR:

1.) Structural (chemical) elucidation
- ② Natural product chemistry
- ② Synthetic organic chemistry
  - analytical tool of choice of synthetic chemists
  - used in conjunction with MS and IR

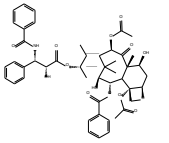
2.) Study of dynamic processes
- ② reaction kinetics
- ② study of equilibrium (chemical or structural)

3.) Structural (three-dimensional) studies
- ② Proteins, Protein-ligand complexes
- ② DNA, RNA, Protein/DNA complexes
- ② Polysaccharides

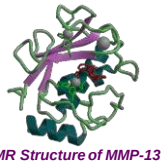
4.) Metabolomics

5.) Drug Design
- ② Structure Activity Relationships by NMR

6.) Medicine -MRI

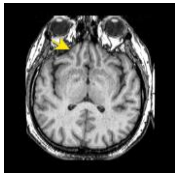


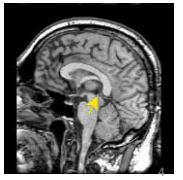
Taxol (natural product)



NMR Structure of MMP-13 complexed to a ligand

MRI images of the Human Brain





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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
1984	**Nicholson** NMR metabolomics
1985	**Wüthrich** first solution structure of a small protein (BPTI, 6511 Da) from NOE derived distance restraints
1987	3D NMR + ^{13}C, ^{15}N isotope labeling of recombinant proteins (resolution)
1990	pulsed field gradients (artifact suppression)
1996/7	**residual dipolar couplings** (RDC) from partial alignment in liquid crystalline media
	TROSY (molecular weight > 100 kDa)
2000s	**Dynamic nuclear polarisation** (DNP) to enhance NMR sensitivity

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)

NMR History

First NMR Spectra on Water

^{1}H NMR spectra of water

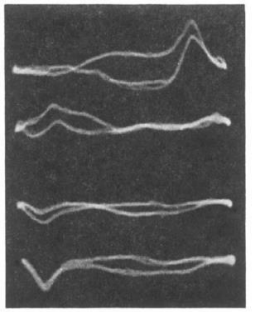


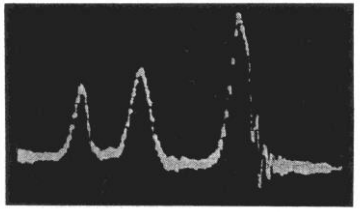
FIG. 10. Photographic record of the proton signal in water. The four traces from top to bottom correspond to the times t_1, t_2, t_3, t_4 of Fig. 9. In the text they are referred to as *a*, *b*, *c*, *d*, respectively.

Bloch, F.; Hansen, W. W.; Packard, M. The nuclear induction experiment. Physical Review (1948), 70 474-85.

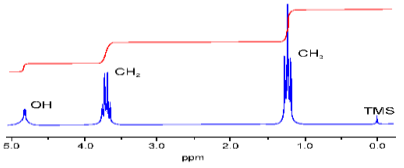
NMR History

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.

Bloch Equations

Net Magnetization (M) placed in a magnetic Field (B) will precess:

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t)$$

and relax (R)

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t) - \mathbf{R}(\mathbf{M}(t) - M_0)$$

Felix Bloch (1905-1983)

and relax individual components:

$$\frac{dM_z(t)}{dt} = \gamma[M_x(t)B_y(t) - M_y(t)B_x(t)] - \frac{M_z(t) - M_0}{T_1}$$

$$\frac{dM_x(t)}{dt} = \gamma[M_y(t)B_z(t) - M_z(t)B_y(t)] - \frac{M(t)_x}{T_2}$$

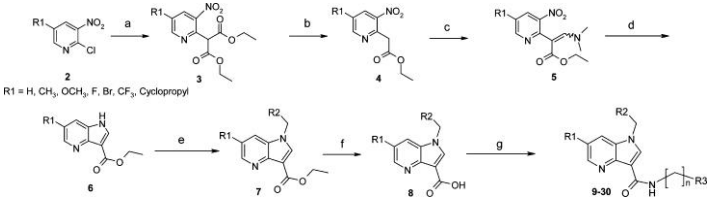
$$\frac{dM_y(t)}{dt} = \gamma[M_z(t)B_x(t) - M_x(t)B_z(t)] - \frac{M(t)_y}{T_2}$$

Course Goals

- We will **NOT** Cover a Detailed Analysis of NMR Theory
  - We will **NOT** Derive the Bloch Equations
  - We will **NOT** Discuss, in detail, a Quantum Mechanical Description of NMR
  - We will **NOT** use Product Operator Formulism to Describe NMR Experiments
- We **Will** Discuss Practical Aspects of Using an NMR
  - We **Will** Take a Conceptual Approach to Understanding NMR
  - A **Focus** of the Course will be the Application of NMR to Solving the Structure of Organic Molecules
  - We Will Use NMR to Solve the Structure of Unknowns

Course Goals

To Be Able to Go From This:



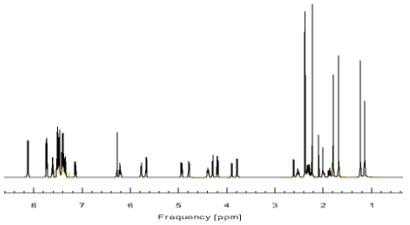
To This:

***N*-(2-Fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide (9)** MS (ES$^+$) *m/z*: 344. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.24 (s, 3H), 3.67 (d, *J* = 5.46 Hz, 1H), 3.76 (d, *J* = 5.46 Hz, 1H), 3.93 (s, 3H), 4.50 (t, *J* = 4.99 Hz, 1H), 4.66 (t, *J* = 4.99 Hz, 1H), 5.69 (s, 2H), 7.26 (dd, *J* = 8.29, 4.71 Hz, 1H), 7.94 (d, *J* = 8.52 Hz, 1H), 8.28 (s, 1H), 8.41 (s, 1H), 8.49 (d, *J* = 4.57 Hz, 1H), 8.95 (s, *J* = 5.89, 5.89 Hz, 1H). HRMS (M + H) calcd for $C_{17}H_{18}FN_5O_2$, 344.1517; found, 344.15242.

And Understand How to Read and Interpret the NMR Spectral Data

Course Goals

To Be Able to Go From This:



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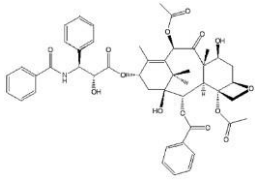
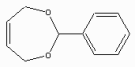
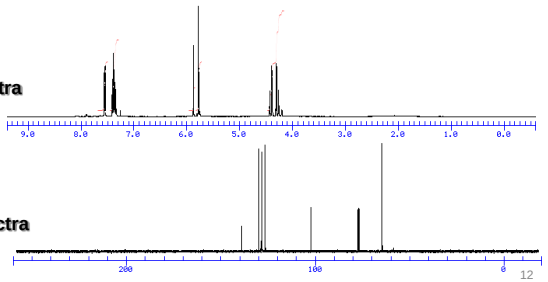


Each NMR Observable Nuclei Yields a Peak in the Spectra

"fingerprint" of the structure

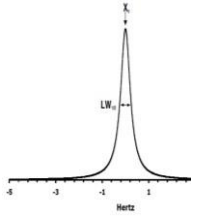
2-phenyl-1,3-dioxep-5-ene

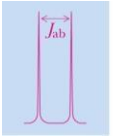


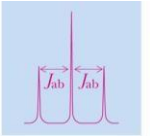


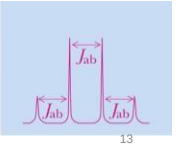
Information in a NMR Spectra

Observable	Name	Quantitative	Information
Peak position	Chemical shifts (δ)	$\delta(ppm) = \upsilon_{obs} - \upsilon_{ref}/\upsilon_{ref}$ (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	$\Delta\upsilon = 1/\pi T_2$ peak half-height	molecular motion chemical exchange uncertainty principal uncertainty in energy





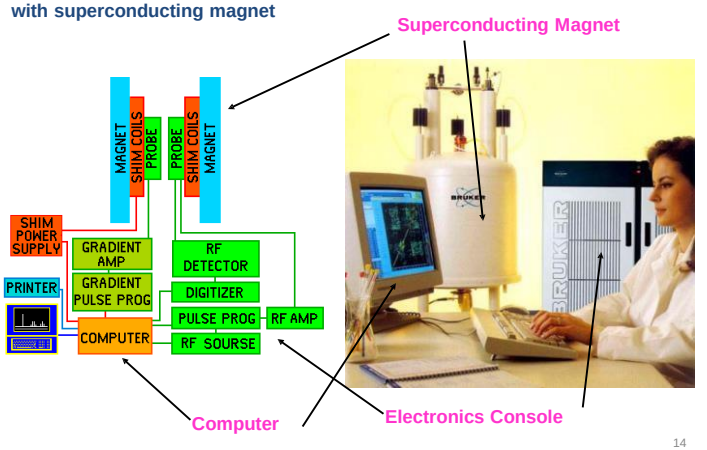




NMR Instrumentation

Modern Pulsed Fourier Transform NMR Spectrometer with superconducting magnet

Why?

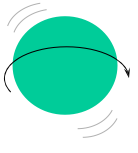


THE "RESONANCE" PHENOMENON

absorption of energy by the spinning nucleus

NUCLEAR SPIN

The nuclei of some atoms have a property called "SPIN".



These nuclei behave as if they were spinning.

….. we don't know if they actually do spin!

This is like the spin property of an electron, which can have two spins: +1/2 and -1/2 .

Each spin-active nucleus has a number of spins defined by its spin quantum number, **I**.

The spin quantum numbers of some common nuclei follow …..

Spin Quantum Numbers of Some Common Nuclei

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

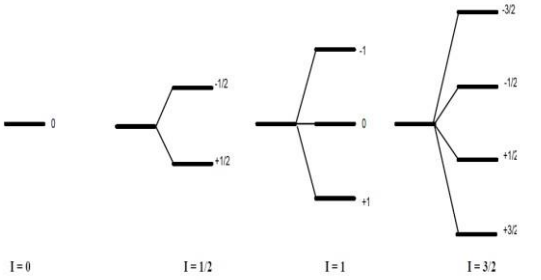
Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{16}O	^{17}O	^{19}F
Nuclear Spin Quantum No (I)	1/2	1	0	1/2	1	0	5/2	1/2
No. of Spin States	2	3	0	2	3	0	6	2

Elements with either odd mass or odd atomic number have the property of nuclear "spin".

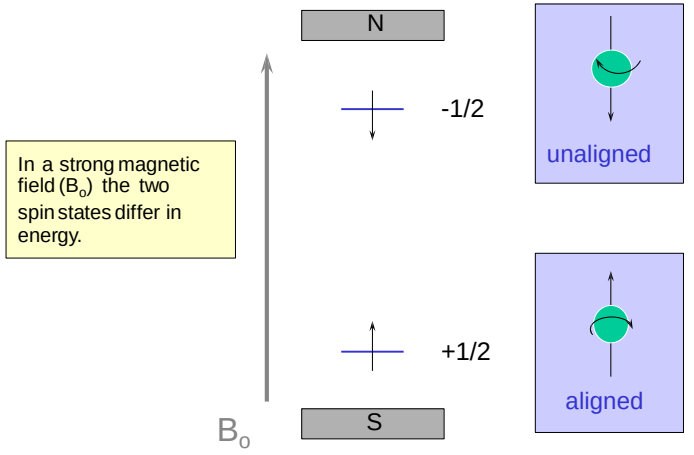
The number of spin states is $2\mathbf{I} + 1$,
where **I** is the spin quantum number.

Spin Orientation in a Magnetic Field (Energy Levels)

- The energy levels are more complicated for $I > 1/2$



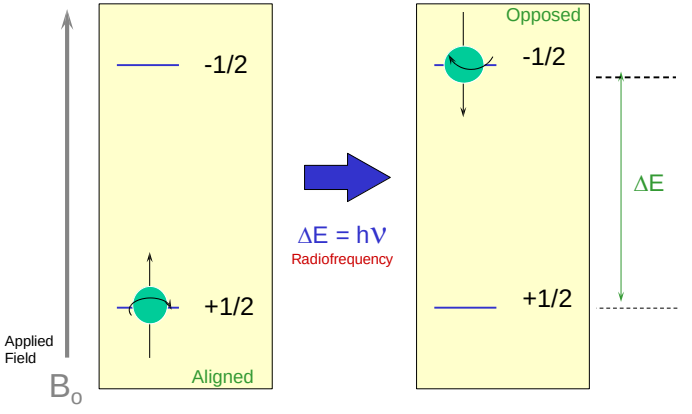
Nuclear Spin Energy Levels



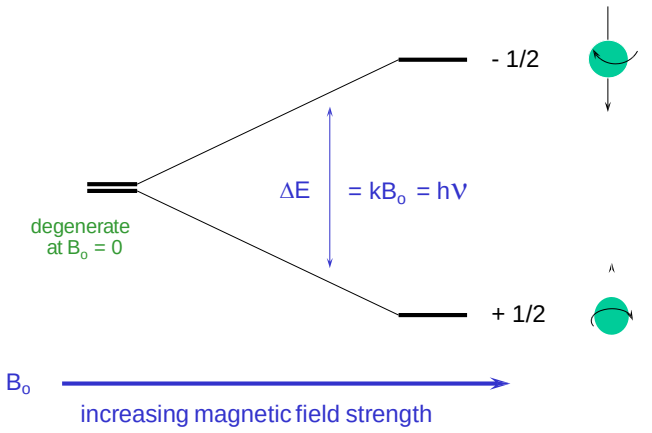
In a strong magnetic field (B_o) the two spin states differ in energy.

Absorption of Energy

quantized

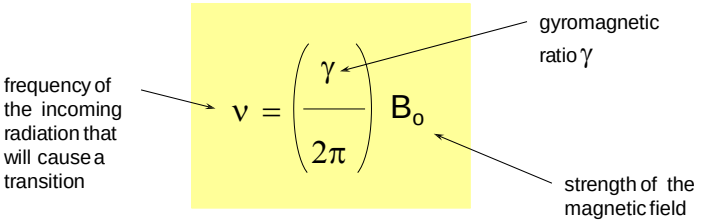


THE ENERGY SEPARATION DEPENDS ON B_o



The Larmor Equation!!!

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



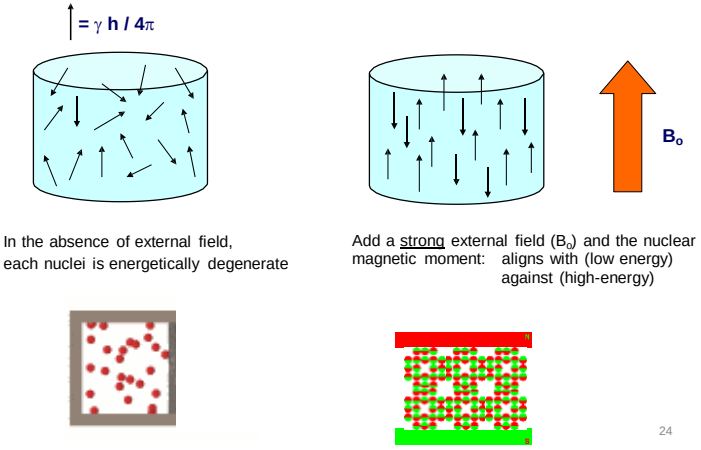
γ is a constant which is different for each atomic nucleus (H, C, N, etc)

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	
^{2}H	0.0156%	1.00	6.5	41.1
		7.05	45.8	
^{13}C	1.108%	1.00	10.7	67.28
		2.35	25.0	
		7.05	75.0	
^{19}F	100.0%	1.00	40.0	251.7

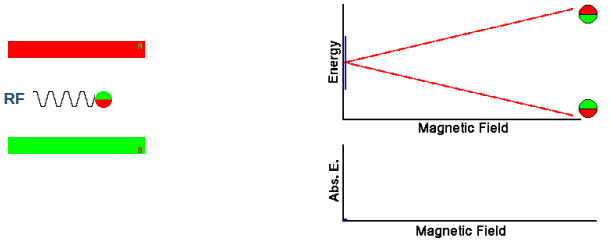
4:1

Magnetic alignment



Spins Orientation in a Magnetic Field (Energy Levels)

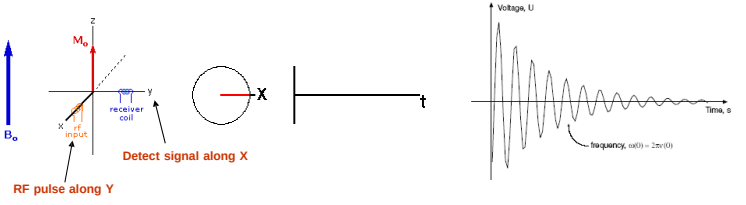
- Transition from the low energy to high energy spin state occurs through an absorption of a photon of radio-frequency (RF) energy



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

NMR Signal Detection - FID

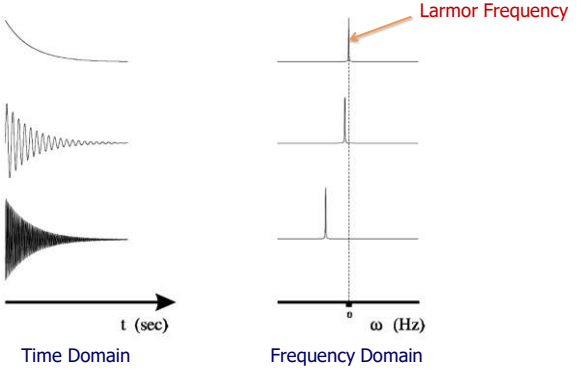
The FID reflects the change in the magnitude of M_{xy} as the signal is changing relative to the receiver along the y-axis



Again, the signal is precessing about B_o at its Larmor Frequency (ω_o).

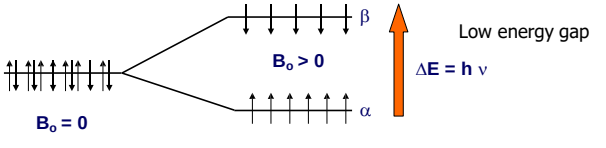
NMR Signal Detection - FID

The appearance of the FID depends on how the frequency of the signal differs from the Larmor Frequency



NMR Signal (sensitivity)

- The applied magnetic field causes an energy difference between the aligned (α) and unaligned (β) nuclei
- 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 states



- The population (N) difference can be determined from the Boltzmann distribution and the energy separation between the α and β spin states:

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

NMR Signal (sensitivity)

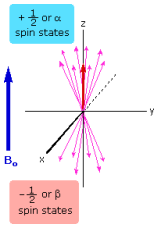
Since:

$\Delta E \equiv h\nu$

and

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

then:



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

The ΔE for ^1H at 400 MHz (B_o = 9.39 T) is 6 x 10^{-5} Kcal / mol

$N_\alpha / N_\beta = 1.000060$

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

NMR Sensitivity

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

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 ($B_o^{2/3}$, in practice, homogeneity)
5) B_1^2 exciting field strength (RF pulse)

$N_\alpha / N_\beta = e^{\Delta E / kT}$ $\Delta E = \gamma h B_o / 2\pi$

Increase energy gap -> Increase population difference -> Increase NMR signal

$\uparrow \Delta E \equiv \uparrow B_o \equiv \uparrow \gamma$

NMR Sensitivity

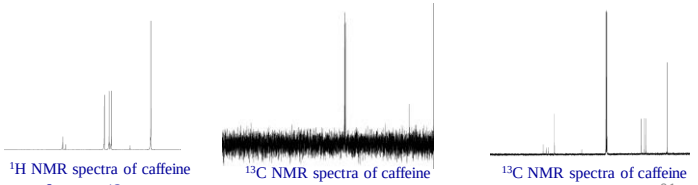
- Relative sensitivity of ^1H, ^{13}C, ^{15}N and other nuclei NMR spectra depend on
  - Gyromagnetic ratio (γ)
  - Natural abundance of the isotope

γ - Intrinsic property of nucleus can not be changed.

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

^1H 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** !!



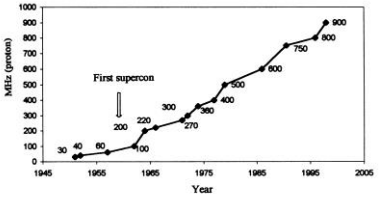
^1H NMR spectra of caffeine
8 scans ~12 secs

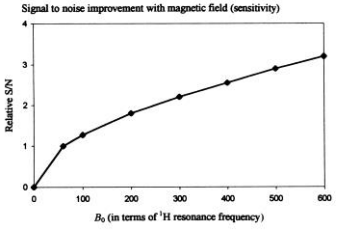
^{13}C NMR spectra of caffeine
8 scans ~12 secs

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

NMR Sensitivity

Increase in Magnet Strength is a Major Means to Increase Sensitivity





NMR Sensitivity

But at a significant cost!

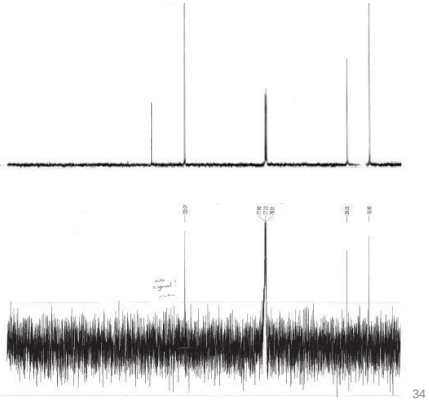
~$800,000 ~$2,000,000 ~$4,500,000

NMR Sensitivity

An Increase in concentration is a very common approach to increase sensitivity

30 mg

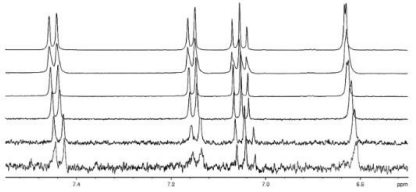
1.2 mg



http://web.uvic.ca/~pmarrs/chem363/nmr%20files/363%20nmr%20signal%20to%20noise.pdf

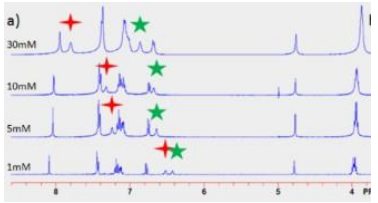
NMR Sensitivity

But, this can lead to concentration dependent changes in the NMR spectra (chemical shift & line-shape) resulting from compound property changes



Dimerization as concentration increases from 0.4 to 50 mM

Beilstein J. Org. Chem. 2010, 6, No. 3.



H-bond and multimer formation as concentration increases from 1 to 30 mM

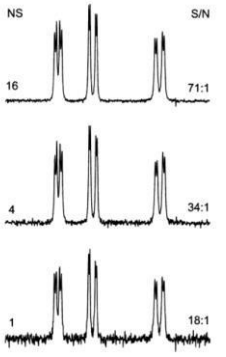
Beilstein J. Org. Chem. 2010, 6, 960–965.

NMR Sensitivity

An Increase in the number of scans (NS) or signal-averaging is the most common approach to increase sensitivity (signal-to-noise (S/N)

$S/N \approx NS^{1/2}$

NS	$NS^{1/2}$
1	1.00
8	2.83
16	4.00
80	8.94
800	28.28

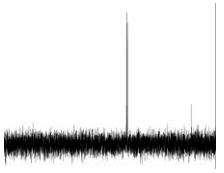


NMR Sensitivity

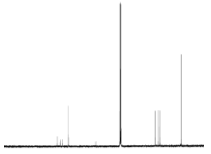
But, it takes significantly longer to acquire the spectrum as the number of scans increase:

Experimental Time = Number of Scans x Acquisition Time

8 scans ~12 secs

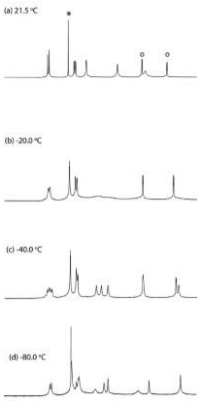


10,000 scans ~4.2 hours

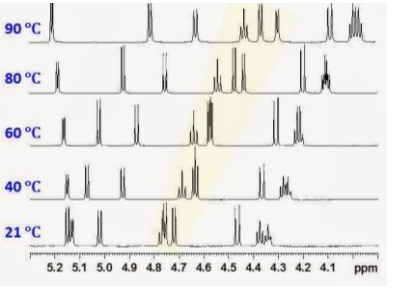


NMR Sensitivity

Lowering the temperature is usually not an effective means of increasing sensitivity because of chemical shift changes and peak broadening



Significant chemical shift changes



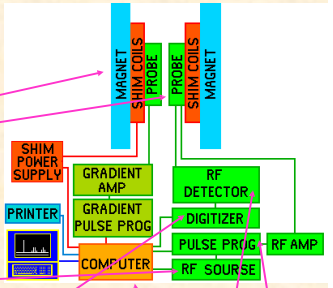
Significant peak broadening

Dalton Trans.,2014, **43**, 3601-3611

NMR Instrumentation

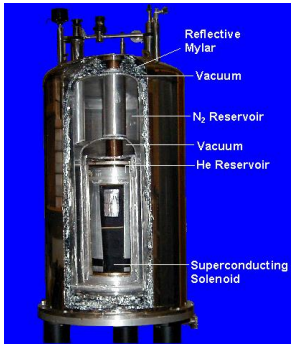
Components of a NMR spectrometer:

- An intense, homogeneous and stable magnetic field (magnet + shim)
- A "probe" which enables the coils used to excite and detect the signal to be placed close to the sample
- High-power RF transmitter/s capable of delivering short pulses (RF source + RF Amplifier)



- A sensitive receiver to amplify the NMR signals (RF Detector)
- A Digitizer to convert the NMR signals into a form which can be stored in computer memory
- A "pulse programmer" to produce precisely timed pulses and delays
- A computer to control everything and to process the data

Superconducting Magnet (SCM): A Big Stainless Steel Dewar



- use persistent superconducting magnets to generate the B_0 field;
- at low temperatures (less than 6 K, typically) the resistance goes to zero – that is the wire(eg.Nb alloy) is *superconducting*;
- To maintain the wire in its superconducting state the coil is immersed in a bath of liquid helium (4 K, expensive);
- "heat shield" kept at 77 K by contact with a bath of liquid nitrogen (cheap) to reduces the amount of liquid helium boils off;
- vacuum flask so as to further reduce the heat flow.

Advantages of SCM ?

1. Strongest Magnet; 2. Stable & homogeneous magnet field Bo;
3. Low running cost.

Sensitivity & Price

Sensitivity $\propto B_0^{3/2}$

300 MHz	1.00
400 MHz	1.54
500 MHz	2.15
600 MHz	2.83
750 MHz	3.95
800 MHz	4.35
900 MHz	5.20

NMR BUSINESS

AVERAGE SELLING PRICE

$ USD

NMR Spectrometers

300MHz	$ 200K
400MHz	$ 300K
500MHz	$ 500K
600MHz	$ 800K
Widebore solids	$1,000K
800MHz	$2,000K
900MHz	$4,500K

NMR Imaging Spectrometers

3 Tesla	$3,000K
4 Tesla	$4,000K

7 - 9.4 - 11.7 - 14 - 18.7 - 21 Tesla

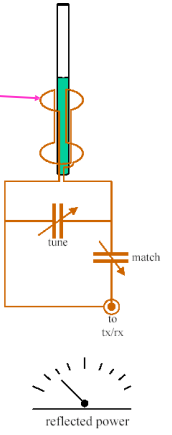
300 - 400 - 500 - 600 - 800 - 900 MHz

Shim Coils

- High resolution NMR requires linewidths of 1 Hz or less
- Magnetic field across the sample must be homogeneous so that the corresponding variation in the Larmor frequency is small
- Surround the sample with a set of *shim coils*, each of which produces a tiny magnetic field with a particular spatial profile to canceling out the small residual inhomogeneities in the main magnetic field.
- Modern spectrometers might have up to 40 different shim coils labeled according to the field profiles they generate, such as *x*, *y*, *z*, z^2, z^3, z^4, z^5, xy, xz, yz, x^2-y^2, etc…
- Shimming, the process to optimize the shims, requires skill and experience because various shims will interact with each other.

The Probe

- The key part of the probe is the small coil used to excite and detect the magnetization in radio-frequency.
- To optimize the sensitivity this coil needs to be (1) as close as possible to the sample; (2) tuned to resonant at the Larmor frequency of the nuclei being detected and (3) matched to maximize power transfer between the probe and the transmitter and receiver.
- Usually multi-coils for different nucleus: e.g. ^{1}H, ^{2}H (for locking), ^{13}C, ^{15}N, etc… with observe coil at inner-most position.

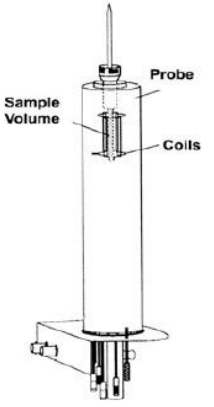


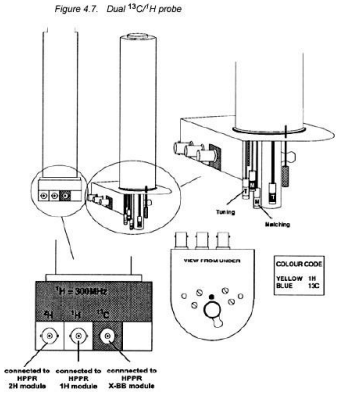
Many types: e.g. TXI, TBI, X-BB, BBI

Fixed Nucleus or Broad-Band (tunable for different nucleus)

Direct detection or Inverse detection probe

The Probe





The transmitter: Channel

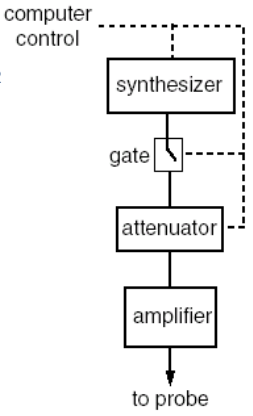
Synthesizer: RF source which produces a stable frequency which can be set precisely.

RF amplifier: boost this small signal to a power of 100 W or more to provide enough energy to excite the NMR active nuclei in the sample.

Attenuator: altering the RF power level in units of decibels (dB) (Bruker: 120 to –6 dB)

All under computer control

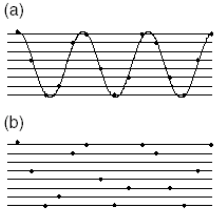
Each nucleus type required one set of transmitter channel => usually more than one channels



The receiver

The NMR signal emanating from the probe is detected by a digitizer receiver at regular time intervals (dwell time).

A device known as an *analogue to digital converter* or ADC is used to convert the NMR signal from a voltage to a binary number which can be stored in computer



memory. Dynamic range of ADC digitizer is measured by bits (e.g. 16-bit, i.e. 0 to 2^{16}-1 or 65535). Receiver Gain (rg) should be set to have the maximum signal using up all the bits in ADC.

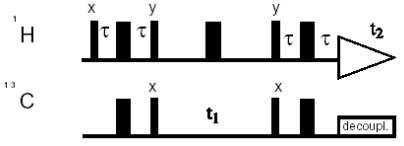
What happen maximum signal << 65535? Loss weak signals

What happen maximum signal > 65535 Clipping of FID?

Small quantization artifact noise peak appears in spectrum.

Pulse programmer

to produce precisely timed pulses and delays required by the NMR pulse experiment



Computer system

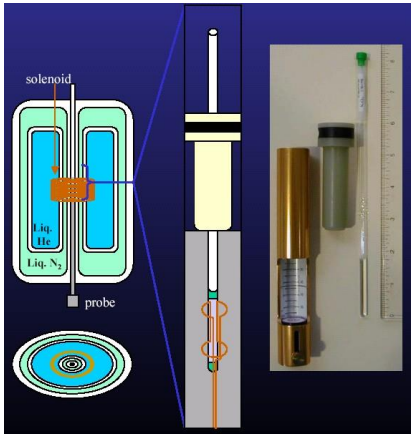
Control all electronics
Date acquisition and processing (Bruker software-XwinNMR)
Plotting Spectrum (Bruker software-Xwinplot)

Third party software may be used for processing or analysis e.g. nmrPipe, Felix, nmrView.

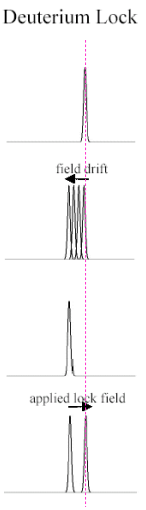
Additional Instrumentation:

Sample spinner:
Spinning equalize xy magnetic field homogeneity, i.e. better resolution

Eject/Insert system:
using air stream to eject and insert sample tube along the long bore tube



Additional Instrumentation: Locking (separate ^2H channel)



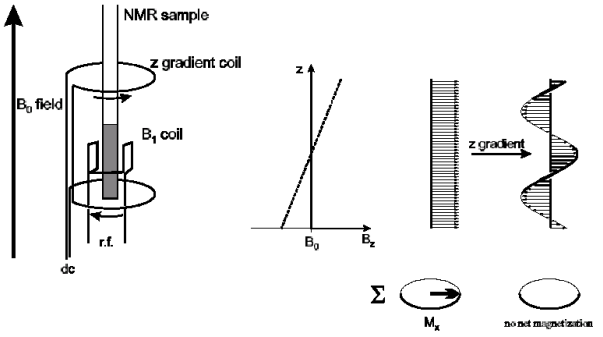
- Even in the best spectrometers the field strength varies to some extent over time
- The position of the deuterium peak is monitored
- To counteract the field drift a lock field is applied to maintain a constant deuterium resonance position

Deuteriated solvent is usually used to provide the Deuterium Lock signal e.g.

$CDCl_3$, D_2O, CD_3OD

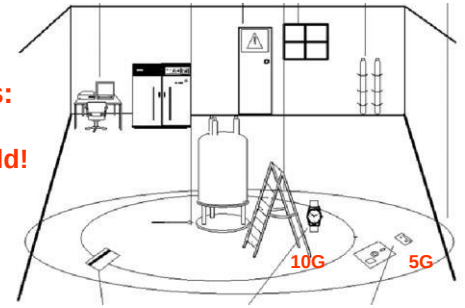
Additional Instrumentation: The pulse field gradient

Addition coils in the probe to provide a magnetic field gradient along a particular direction. Extensively used in MRI experiments to provide spatial encoding. Also useful for solvent peak suppression, artifact peaks suppression, and coherence selection in NMR experiments.



Bruker AV600 | Bruker DRX500 | Bruker AV400 | Bruker DRX300 | JEOL 270

Safety Precautions: Very Very Strong Magnetic Field!



Sample Preparation

Know as much details as possible about the sample

- stability, solubility, other properties

Solvent

- must dissolve (or be miscible with) the sample totally
- must contain deuterium atom (at least 99.5%)
- for high temperature, use CD_3SOCD_3
- for low temperature, use CD_2Cl_2, CD_3OD
- keep hygroscopic solvents in inert gas atmosphere

Reference – mandatory for Chemical Shift Referencing as per IUPAC

- tetramethylsilane (TMS) for non-aqueous medium
- 3-(trimethylsilyl)propionic acid sodium salt (TSP) for aqueous medium
- sodium 2,2-dimethyl-2-sila-pentane-5-sulfonate (DSS) for aqueous medium

Sample Preparation

Sample Tube

diameter – 3mm, 4mm, 5mm, 10mm

length – 7 inch, 8 inch

volume – 150μL to 3100μL

usage depends on probe

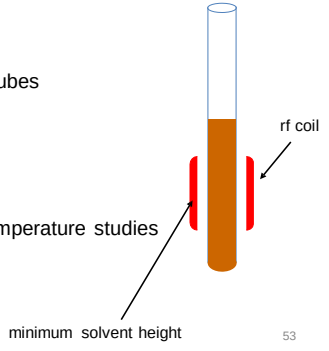
preferable to have 8 inch length tubes

Spinner Turbine

holds the sample tube intact

heat resistant variants for high temperature studies

cannot be broken



Sample Preparation

It is often realized that the samples are not prepared carefully

- place the sample in a glass vial
- add required quantity of solvent via a clean pipette or syringe
- do not use disposable syringe (peaks at ~0.1 ppm)
- ensure that the sample is fully dissolved
- place a long tailed funnel into the sample tube
- take cotton wool and place it in the funnel
- preferable to rinse cotton wool with solvent
- transfer the solution to sample tube
- degassing may be required, as per user's interest
- place the cap on the sample tube firmly

Your NMR System – Your Life

Operational Start–up Tips

- have a clear idea about experimental protocols
- go through the LOG book as soon as you take charge
- report to facility manager in case of any issues
- ensure the system is powered on
- ensure compressed air supply is on
- have an idea of the probe which is placed inside the magnet
- set the temperature regulation, say 27° C
- insert the sample into the magnet
- set the observation parameters
- tune the probe, if required

Your NMR System – Your Life

Run All Experiments under Temperature Regulation

to avoid temperature induced
chemical shift
conformational changes

to have stable Deuterium Lock

to have uniform shimming characteristics
shims under non regulated condition are impractical

to avoid sample heating
heteronuclear decoupling

Your NMR System – Your Life

Use VT regulation, even at room temperature

Large changes in temperature of the environment can affect the VT gas stream

The frequency of peaks in the spectrum and of the lock resonance (which affects all peaks) is temperature–sensitive to some extent

Shimming may also change if the probe temperature varies, which can affect the lineshape

Dan Steele, 'Indirect Detection Experiments', Page 179, Chapter 5, in 'User Guide: Liquids NMR', Manual for Varian NMR Spectrometer Systems with VNMR 6.1C Software, Varian Inc., 2002.

Observables from NMR Spectrum

Chemical Shift (δ)

Coupling Constant (J)

Chemical Shift

Chemical shift is identified with respect to a reference frequency

The reference is made where there is no distorted electron distribution

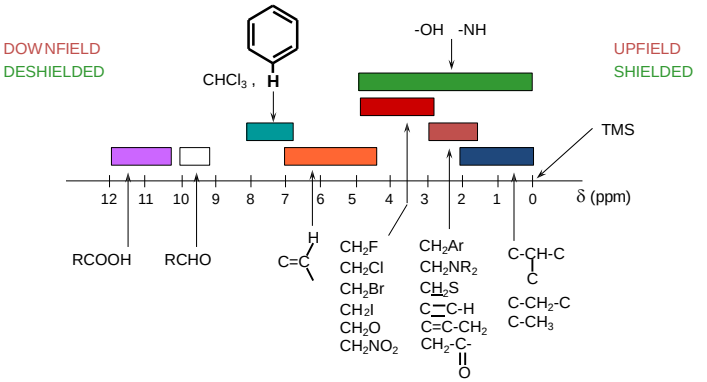
Chemical Shift

$$\delta = \{(f_s - f_r) * 10^6\} / f_i$$

f_s = Frequency of a signal
f_r = Frequency of a reference
f_i = Frequency of the spectrometer (MHz)

δ is thus expressed in 'parts per million' (ppm)

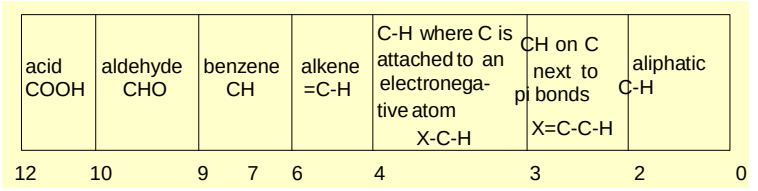
NMR Correlation Chart



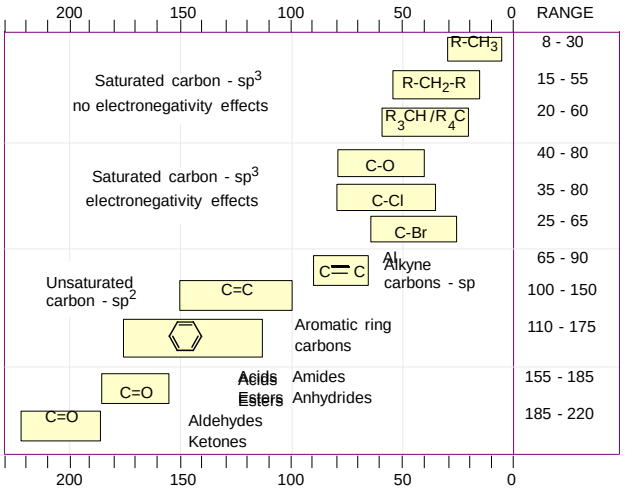
Ranges can be defined for different general types of protons.
This chart is general, the next slide is more definite.

YOU DO NOT NEED TO MEMORIZE THE PREVIOUS CHART

IT IS USUALLY SUFFICIENT TO KNOW WHAT TYPES OF HYDROGENS COME IN SELECTED AREAS OF THE NMR CHART



MOST SPECTRA CAN BE INTERPRETED WITH A KNOWLEDGE OF WHAT IS SHOWN HERE



Correlation chart for ^{13}C Chemical Shifts (ppm)

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Spin Spin Splitting

A nuclei can undergo a different NMR experience by its neighboring nuclei

The neighboring nuclei's magnetic field affects the NMR response

The effect occurs through the interaction of bonding electrons

Due to the effect, the resulting NMR response leads to changes in chemical shift

63

Spin Spin Splitting

Let us consider two protons, H_a and H_b

Corresponding energy states for these nuclei are

$H_a(\alpha)$ $H_a(\beta)$ $H_b(\alpha)$ $H_b(\beta)$

Chemical shift of H_a is defined as $\delta_a = B_0 - B_a$

B_0 static magnetic field

B_a magnetic field of H_a

The influence of H_b on H_a, that is, B_b on δ_a, can be represented by

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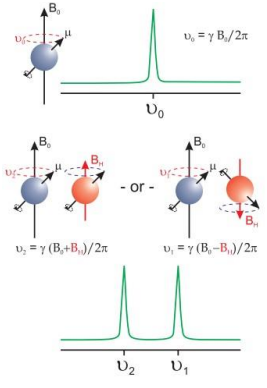
Spin Spin Splitting

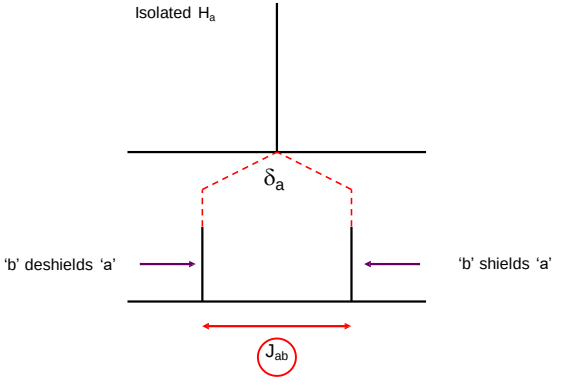


The simplistic explanation is that the neighboring spin's magnetic moment acts to either add to or subtract from the main field.

The resulting resonance frequency changes due to the change in the effective field at the nucleus.

For spin = 1/2 nuclei, there are two possible orientations for the magnetic moment and the result is two possible frequencies.

Spin Spin Splitting



Spin Spin Splitting

The Spin Spin Splitting Interaction
- Commonly known as Scalar Coupling (J)
- Measured in units of Hz

Scalar Coupling
- First Order Rules
- Second Order Rules

Facts
- First order rules are simple
- Second order rules are complicated

Spin Spin Splitting

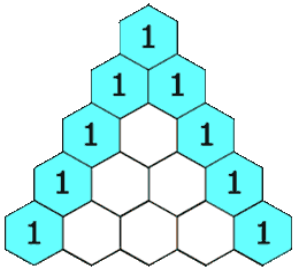
First Order Splitting

Rules

1. Equivalent protons do not interact among each other
2. The chemical shift between interacting groups is much larger than their coupling constant ($\Delta\delta >> J$)
3. Splitting Pattern
   a) $2nI + 1$, where n is the number of nearby equivalent protons and I is the spin number
   b) 2^n, where n is the number of near by non-equivalent protons

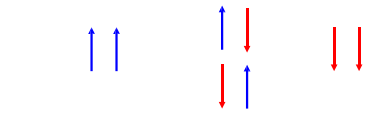
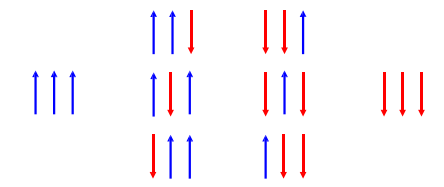
Splitting Due to Equivalent Protons

Splitting Due to Equivalent Protons ⇔ Pascal's Triangle



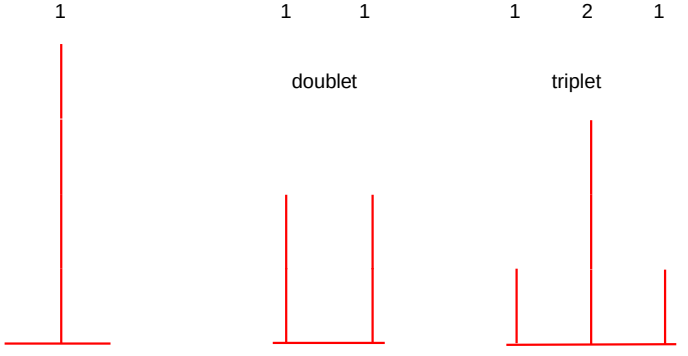
1	s
1 1	d
1 2 1	t
1 3 3 1	q
1 4 6 4 1	p

Splitting Due to Equivalent Protons

No. of Nearby Hs	Spins of Nearby Hs	Splitting
0	0	Singlet
1		Doublet
2		Triplet
3		Quartet

Splitting Due to Equivalent Protons

Intensity Pattern ⇔ Pascal's Triangle



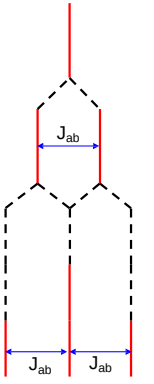
Splitting Due to Equivalent Neighbors

`2nI+1` (or) `n+1` since `I = 1/2`

n=0 : Singlet

n=1 : Doublet

n=2 : Triplet



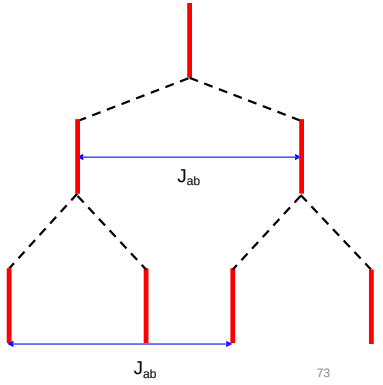
Splitting Due to Non Equivalent Neighbors

2^n

n=0 : Singlet

n=1 : Doublet

n=2 : Quartet



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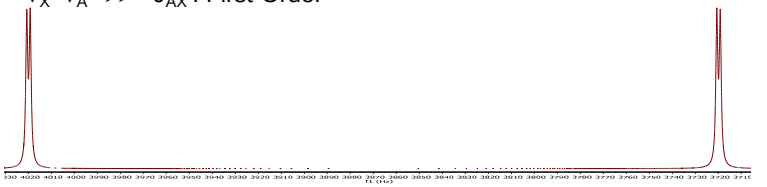
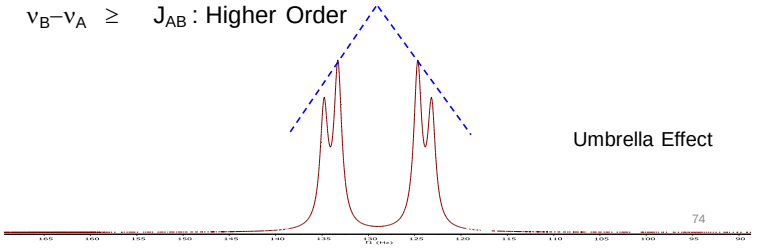
Spectra of First and Higher Order

$\nu_X - \nu_A$ >> J_{AX} : First Order



$\nu_B - \nu_A$ ≥ J_{AB} : Higher Order



Umbrella Effect

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Spin Systems & Multiplicity Pattern

Spin System	Multiplicity Pattern
AX	d, d
A_2X	t, d
A_2X_2	t, t
A_3X	q, d
A_3X_2	q, t
$A_3M_2X_2$	t, m, t

75

A Special Case of Multiplicity Pattern

$-CO-O-CH_2-CH_3$

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A Special Case of Multiplicity Pattern

Assume that the two hydrogens of $–CH_2$ are not equivalent, say, a and b

"a" of $–CH_2$ will be split by "b" resulting in a doublet and each line of the doublet will be split by $–CH_3$ resulting in a total of 8 lines

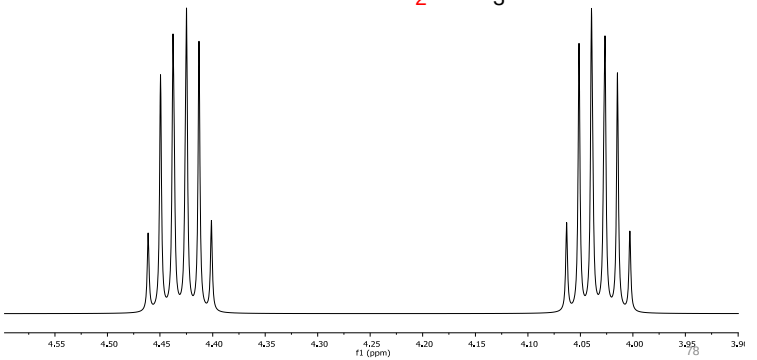
"b" of $–CH_2$ will be split by "a" resulting in a doublet and each line of the doublet will be split by $–CH_3$ resulting in a total of 8 lines

Hydrogens of $–CH_3$ will be split by "a" resulting in a doublet and each line of the doublet will be split by "b" resulting in a doublet of a doublet

A Special Case of Multiplicity Pattern

Should it not be an 8 line pattern?

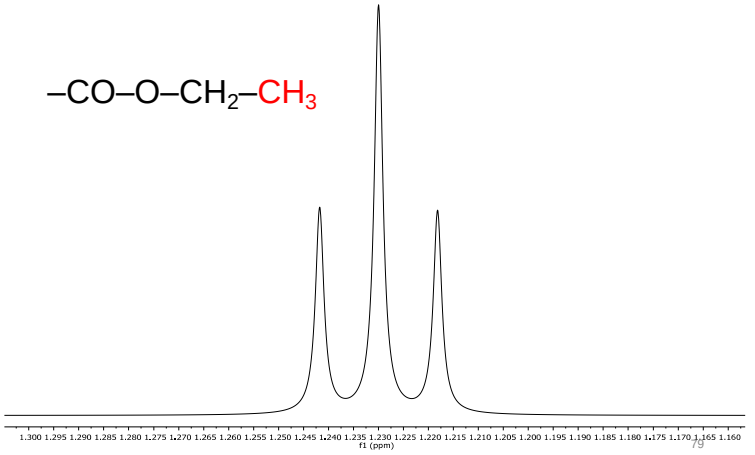
$–CO–O–CH_2–CH_3$



A Special Case of Multiplicity Pattern

Should it not be a doublet of doublet?

$–CO–O–CH_2–CH_3$



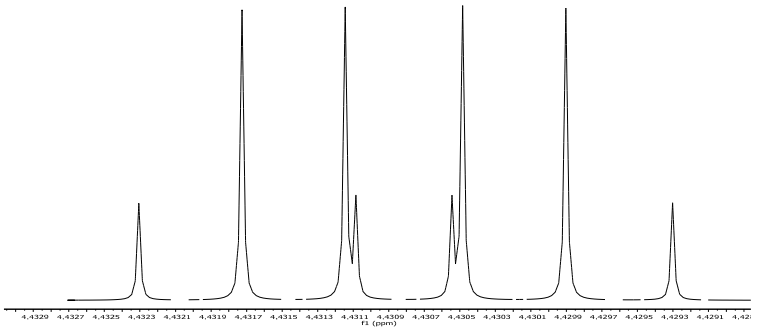
A Special Case of Multiplicity Pattern

Pseudotriplet Ψt

A triplet-like splitting pattern caused by the identical coupling of the resonance of the observed spin to two other spins not related to each other by symmetry

A Special Case of Multiplicity Pattern

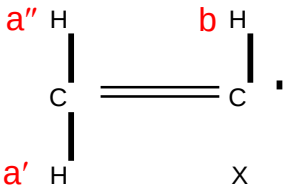
One can see 8 lines if the field strength is enormously high



The above result is from a 10 GHz NMR system (!!)

Of course, it is a simulated spectrum

Facts about Scalar Coupling Constant (J)



a″ = a′ : a → b 3 bonds : J_{ab} (vicinal)

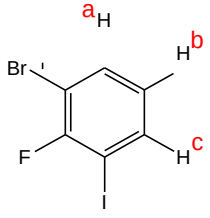
a″ ≠ a′ : a″ → b 3 bonds : $J_{a''b}$ (vicinal)

a″ ≠ a′ : a′ → b 3 bonds : $J_{a'b}$ (vicinal)

a″ ≠ a′ : a″ → a′ 2 bonds : $J_{a''a'}$ (geminal)

3 bonds : J_{vic} : J_{cis} = 8 to 12 Hz J_{trans} = 14 to 18 Hz

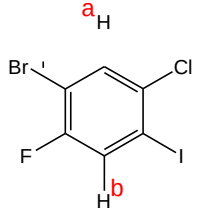
2 bonds : J_{gem} : J_{gem} = 2 to 4 Hz



a → b 3 bonds : J_{ab} (ortho) : 6 – 8 Hz

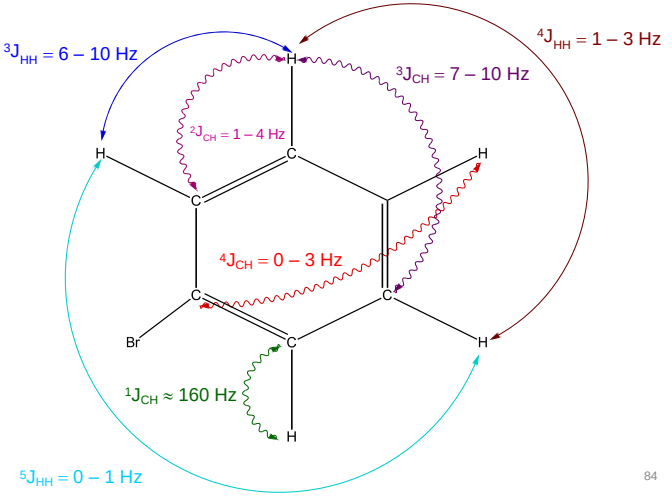
b → c 3 bonds : J_{bc} (ortho) : 6 – 8 Hz

a → c 4 bonds : J_{ac} (meta) : 2 – 3 Hz



a → b 5 bonds : J_{ab} (para) : 0 – 1 Hz

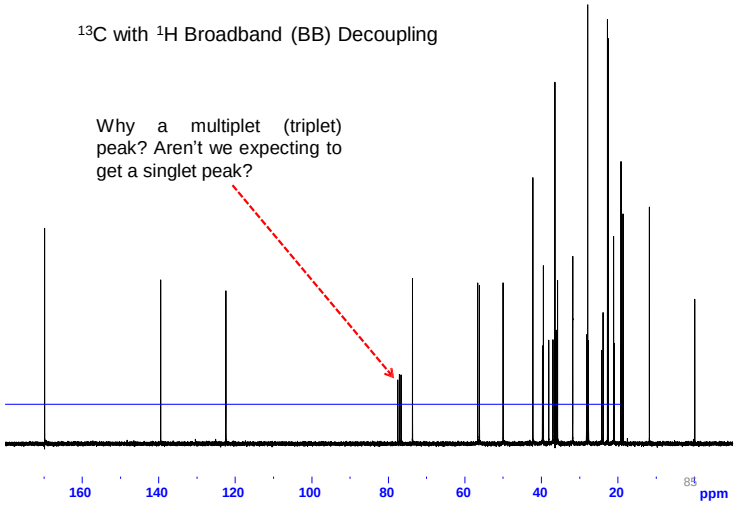
Note: It is very difficult to observe para coupling as it usually appears as a broadened peak



^{13}C NMR

^{13}C with ^{1}H Broadband (BB) Decoupling

Why a multiplet (triplet) peak? Aren't we expecting to get a singlet peak?



^{13}C NMR

Multiplet Peaks in ^{13}C NMR

The presence of multiplet (no singlet) peaks in ^{13}C NMR is due to the presence of a deuterated solvent

Typically, ^{13}C NMR is recorded with ^{1}H BB Decoupling only

Since Deuterium (^{2}H) is not decoupled, the interaction between ^{13}C and ^{2}H leads to multiplet peaks

^{2}H has a I value of 1

Examples

Solvent	n	Pattern { (2nI+1) }
$CDCl_3$	1	3
CD_2Cl_2	2	5
CD_3SOCD_3	3	7

^{13}C NMR

Facts

Peaks in ^{13}C NMR arise due to FOUR types of Carbons

CH_3	Primary	(C_p)	Methyl
CH_2	Secondary	(C_s)	Methylene
CH	Tertiary	(C_t)	Methine
C	Quaternary	(C_q)	(No ^{1}H attached)

Number of peaks in ^{13}C NMR ≡ Number of types of Carbons

^{13}C NMR spectrum is not integrated

^{13}C NMR spectrum contains singlet peaks
Due to "Broadband Decoupling" of ^{1}H

Sculpting 13C

Though a ^{13}C spectrum contains Singlet Peaks, the complexity of molecule makes the analysis difficult if not impossible

Sculpting ^{13}C spectrum to discriminate C_p, C_s, C_t and C_q

"Distortionless Enhancement by Polarization Transfer (DEPT)"

DEPT

Performed as DEPT45, DEPT90 and DEPT135
DEPT135 discriminates Carbons by way of amplitude of the peaks

C_p, C_t : Positive peaks
C_s : Negative peaks
C_q : Not Present

Process is also known as "Spectral Editing"

Sculpting 13C – DEPT

The signal intensity in a DEPT experiment is expressed as

$$I = n * \sin\theta * \cos^{n-1}\theta$$

where θ is the final proton pulse angle of DEPT experiment and n is the number of hydrogens attached to carbon

Type	Nature of Peak Intensity		
	$\theta = 45°$	$\theta = 90°$	$\theta = 135°$
C	**0**	**0**	**0**
CH	+	+	+
CH_2	+	**0**	–
CH_3	+	**0**	+

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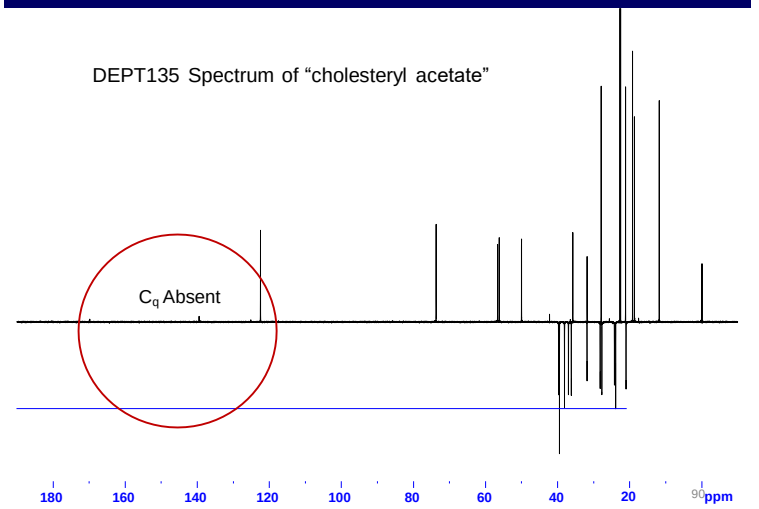
Sculpting ^{13}C



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Spectral Editing with DEPT

Extract Precise Information about types of CH

(1). DEPT90
Gives information about CH

(2). Subtract DEPT45 and DEPT135
Gives information about CH_2

(3). Subtract DEPT45 and DEPT135
Gives information about CH and CH_3

(4). Subtract (1) and (4)
Gives information about CH_3

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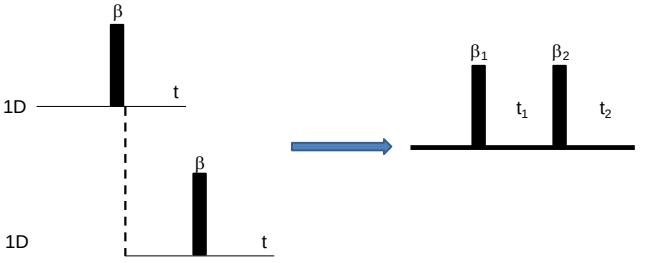
Two Dimensional (2D) NMR

Schematic (Pulse Sequence) Diagram of 2D NMR



2D – Concatenation of 1D experiments wherein the responses from nuclei are spread across two dimensions

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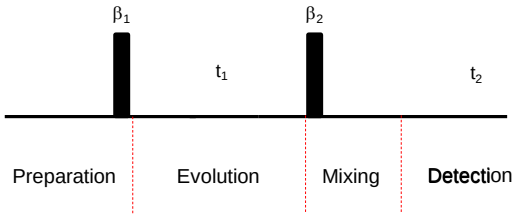
Two Dimensional (2D) NMR

Four Stages of a 2D Experiment



Evolution and Detection are time based events

Two Dimensional Fourier Transform

(t_1, t_2) → (f_1, f_2)

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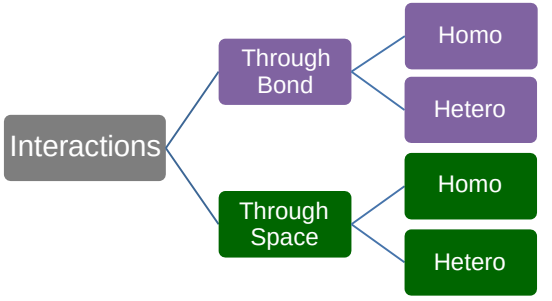
Two Dimensional (2D) NMR

Classification of 2D NMR

Homonuclear
Identical nuclear species ($^{1}H–^{1}H$, $^{13}C–^{13}C$, $^{19}F–^{19}F$)

Heteronuclear
Different nuclear species ($^{1}H–^{13}C$, $^{1}H–^{15}N$, $^{1}H–^{31}P$, $^{19}F–^{13}C$)



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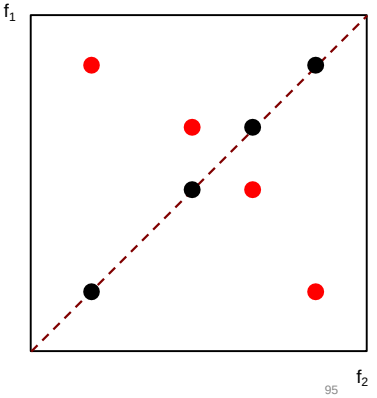
Two Dimensional (2D) NMR

Homonuclear 2D
$f_1 = f_2$

Represented by

Diagonal peaks ●
Cross (Off Diagonal) peaks ●



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Two Dimensional (2D) NMR

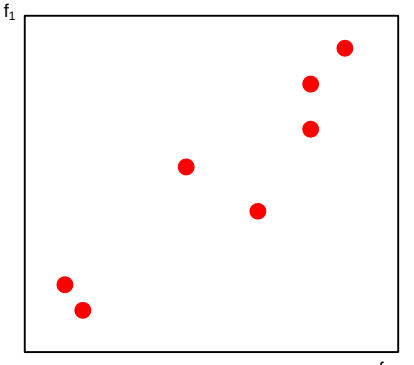
Heteronuclear 2D
$f_1 \neq f_2$

Represented by

Cross peaks ONLY

There are

No Diagonal Peaks



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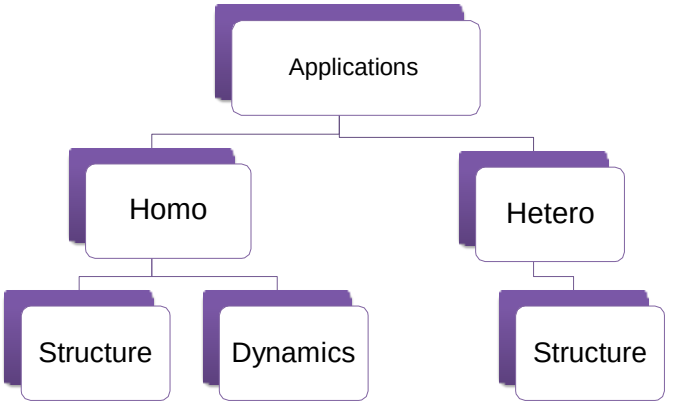
Two Dimensional (2D) NMR



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Two Dimensional (2D) NMR

Acronym	Full Name
COSY	COrrelated SpectroscopY
DQFCOSY	Double Quantum Filtered COrrelated SpectroscopY
TOCSY	TOtal Correlated SpectroscopY
NOESY	Nuclear Overhauser Effect SpectroscopY
ROESY	Rotating frame Overhauser Effect SpectroscopY
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation

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Two Dimensional (2D) NMR

Name	**Type**	**Information**	**Correlation**
COSY	Homo	Through Bond	2 and 3 bonds
DQFCOSY	Homo	Through Bond	2 and 3 bonds
TOCSY	Homo	Through Bond	2, 3, 4 and 5 Bonds
NOESY	Homo	Through Space	Internuclear Distance
ROESY	Homo	Through Space	Internuclear Distance
HSQC	Hetero	Through Bond	1 Bond
HMBC	Hetero	Through Bond	2, 3, and 4 Bonds

Almost 95% of 2D NMR methods are studied among I=1/2 nuclei

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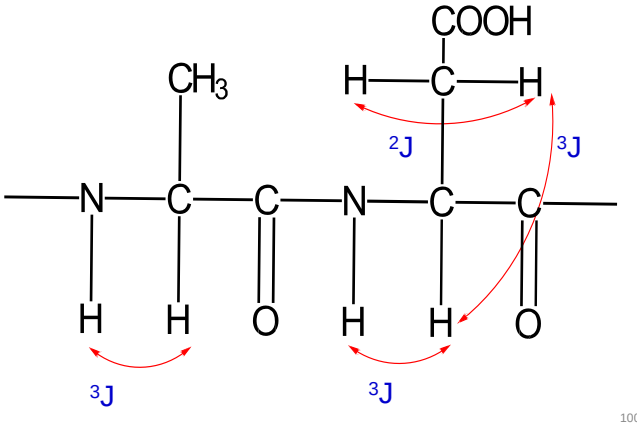
Exploitation of Scalar Coupling in NMR



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Exploitation of Scalar Coupling in NMR

Coupled protons exhibit two bond (^2J) and/or three bond (^3J) couplings

^2J and ^3J couplings reveal sequential connectivity among protons

COSY experiment exploits ^2J and ^3J couplings

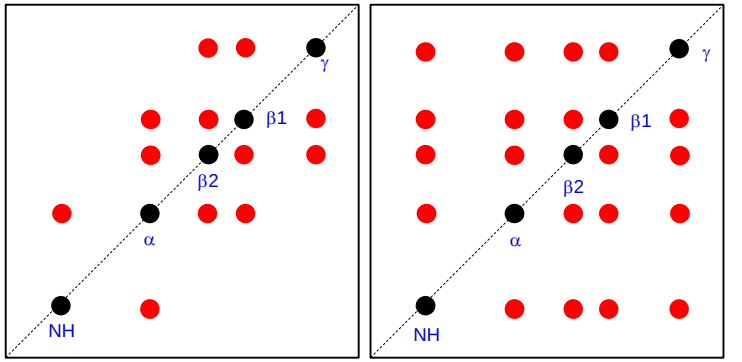
In order to identify a group or set of protons, known as spin system, TOCSY experiment is used

TOCSY produces peaks beyond ^2J and ^3J by way of a relay transfer

For example, consider the amino acid residue, Arginine. While COSY produces to ^{N}H–$^{\alpha}H$ peak, TOCSY produces ^{N}H–$^{\alpha}H$, ^{N}H–$^{\beta 1}H$, ^{N}H–$^{\beta 2}H$ and ^{N}H–$^{\gamma}H$ peaks

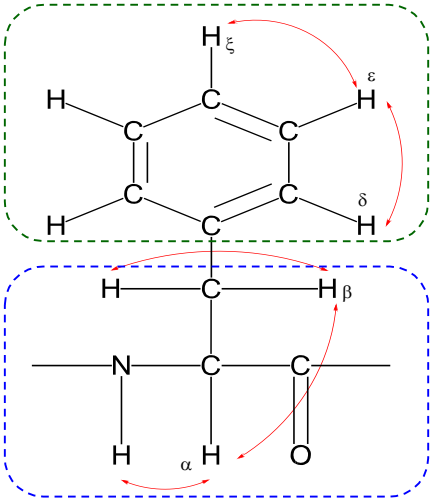
The relay transfer is controlled by an experimental parameter known as “mixing period” which yields a sinusoidal behavior of the transfer

Exploitation of Scalar Coupling in NMR



COSY TOCSY

Spin System Identification by TOCSY



Spin System 2

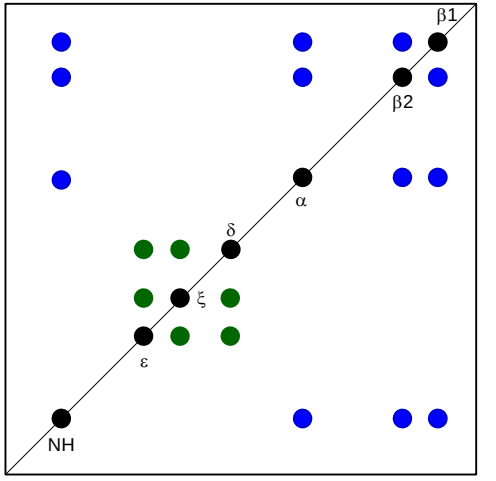
Side Chain

Spin System 1

Backbone

Phenyl alanine

Spin System Identification by TOCSY

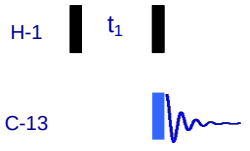


Spin System 1 Backbone

Spin System 2 Sidechain

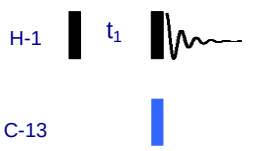
Phenyl alanine

Heteronuclear (HX) 2D NMR



Carbon Detection

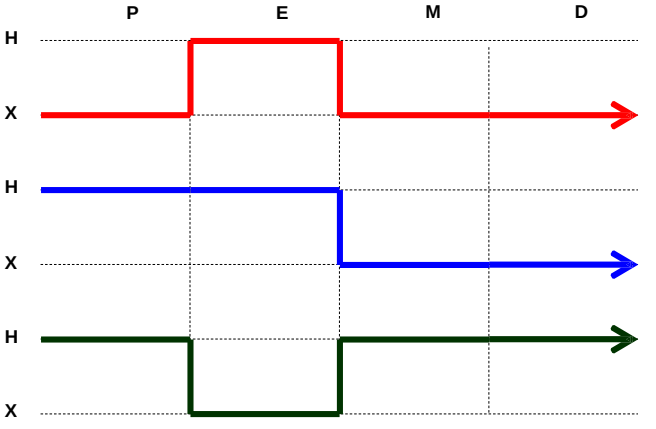
2D ^{13}C-^{1}H COSY
(HETCOR)



Proton Detection

2D ^{1}H-^{13}C COSY
(HSQC or HMQC)

Detection in HX 2D NMR – H or X ?



Detection in HX 2D NMR – H or X ?

Experiment	Preparation	Detection	ED Ratio	Relative Sensitivity	
				^{1}H–^{13}C	^{1}H–^{15}N
RED	X	X	$Z_X\, Z_X^{3/2}$	1	1
BLUE	H	X	$Z_H\, Z_X^{3/2}$	4	9.9
GREEN	H	H	$Z_H\, Z_{H3/2}$	31.6	306

RED Reference

BLUE HETCOR

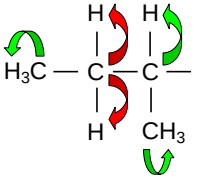
GREEN HSQC

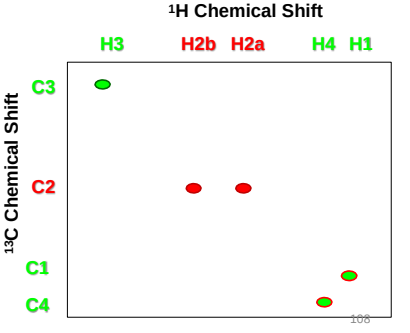
$$Z_H = 4 * Z_X$$

Heteronuclear Single Quantum Coherence

HSQC

Establishes connectivity between H and X via $^{1}J_{XH}$

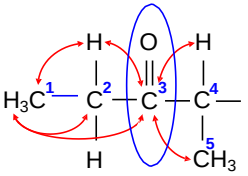


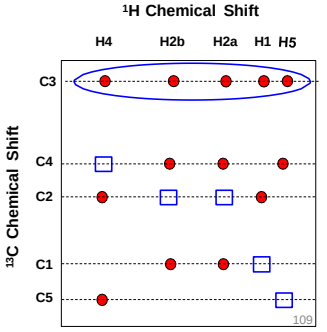


Heteronuclear Multiple Bond Correlation

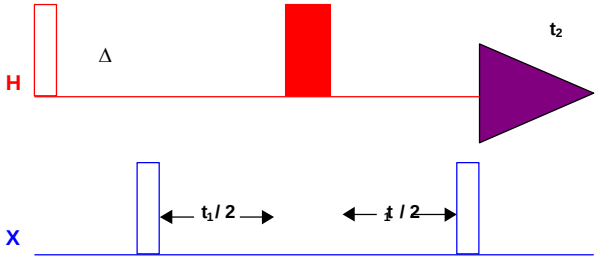
HMBC

Establishes connectivity between H and X via $^{n}J_{XH}$





HMBC Experiment



$\Delta = 1/(2 * {}^{n}J_{XH})$

^{n}J Values

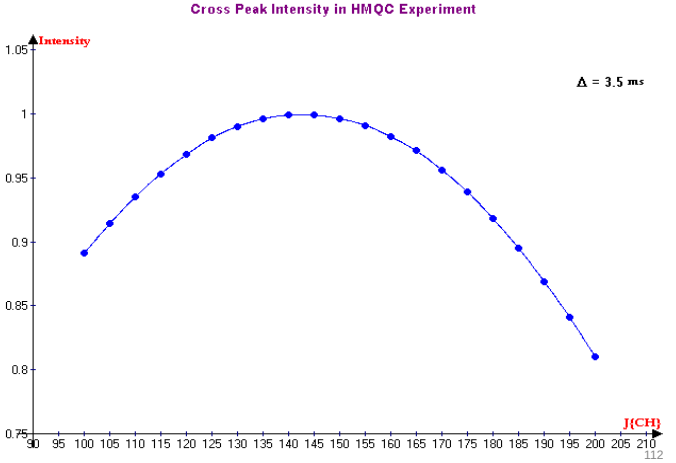
System	Value	Type
H-C-C	~ 5	^{2}J
H-C=C	~ 10	^{2}J
H-C≈C	40–60	^{2}J
H-C(=O)-C	20–25	^{2}J
H-C-C-C	~ 5	^{3}J
H-C=C-C	~ 15	^{3}J
H-C≈C-C	~ 5	^{4}J
H-C=C-C=C	~ 1	^{4}J
H-C-C-C-C	~ 1	

Facts

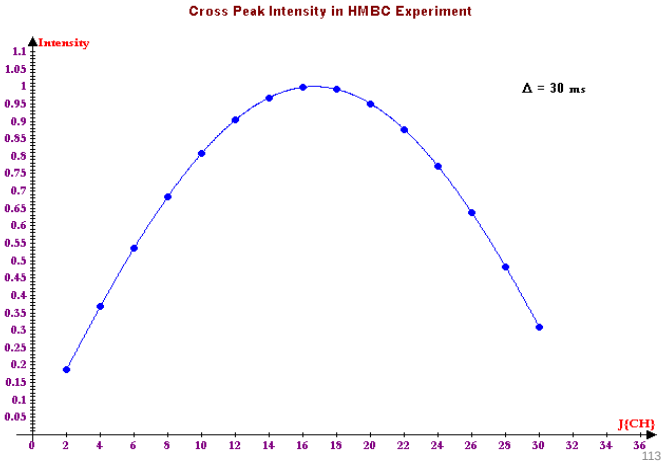
Typically Optimized for ^{2}J or ^{3}J

Discrimination of ^{n}J Ruled Out

HMBC – Variation in Δ



HMBC – Variation in Δ



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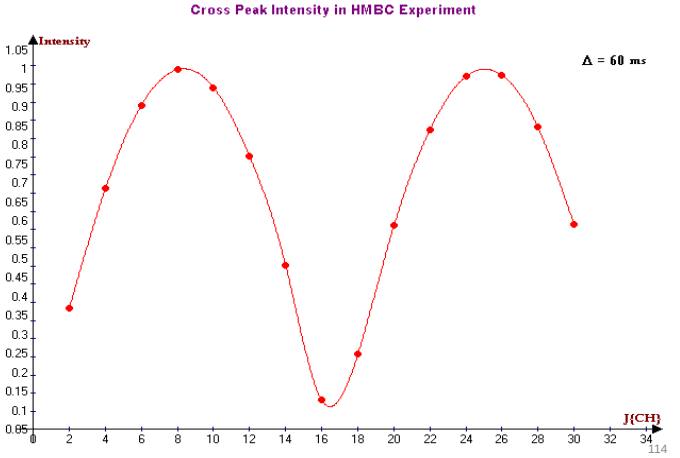
HMBC – Variation in Δ



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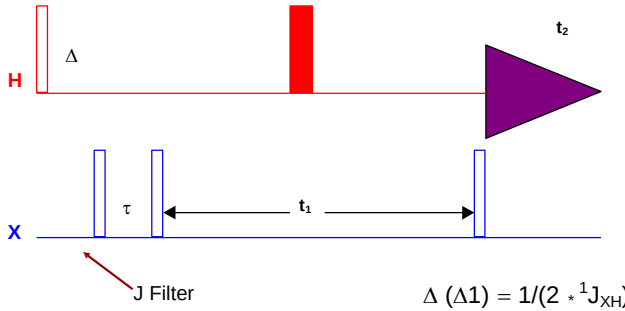
HMBC – ^{1}J Filter

Suppression of $^1J_{CH}$ Contribution – J Filter



Δ (Δ1) = 1/(2 * $^1J_{XH}$)

τ (Δ2) = 1/(2 * $^nJ_{XH}$)

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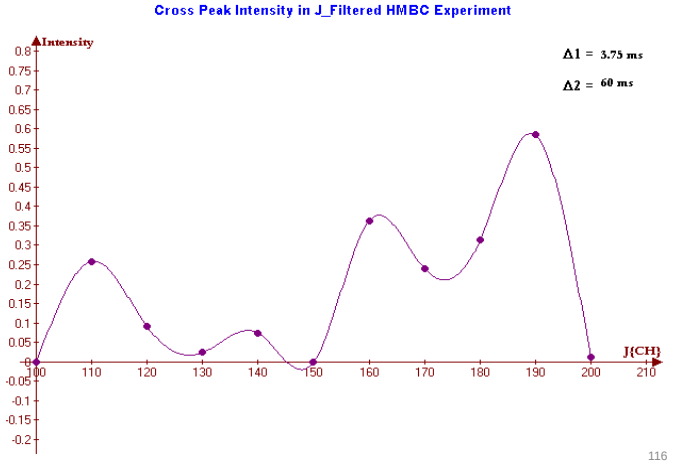
HMBC – ^{1}J Filter



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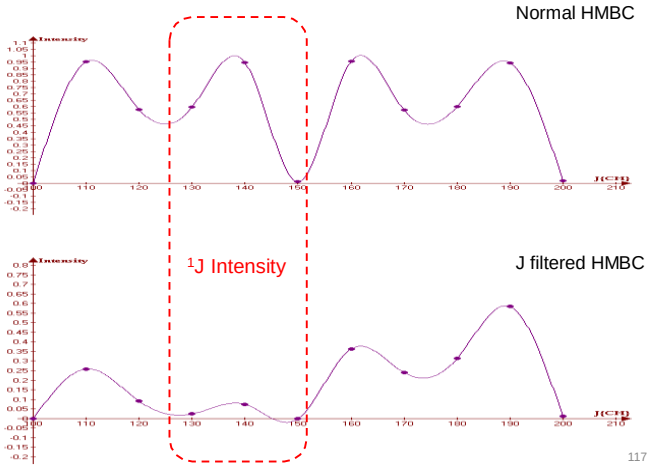
HMBC – With and Without ^1J Filter



Experiments & Key Parameters

Experiment	Delay	Scans	Increments	Mixing	swH1	swC13	Data Points	Total Time
gCOSY	1.0 sec	2	256		10 ppm		1024	12 min
gDQCOSY	1.0 sec	2	256 * 2		10 ppm		1024	25 min
NOESY	1.5 sec	16	256 * 2	500 msec	10 ppm		1024	311 min
TOCSY	1.0 sec	2	256 * 2	80 msec	10 ppm		1024	25 min
gHSQC	1.0 sec	2	256 * 2		10 ppm	170 ppm	1024	25 min
gHMBC	1.0 sec	16	512		10 ppm	240 ppm	1024	178 min
CIGAR	1.0 sec	16	512		10 ppm	240 ppm	1024	187 min
NOESY1D	1.5 sec	512		900 msec	10 ppm		12000	38 min
TOCSY1D	1.0 sec	16		100 msec	10 ppm		11000	2 min

pw90 for H1 **9.5 microsec**
pw90 for C13 **11.5 microsec**

What Could Have Possibly Gone Wrong ?!

Assuming that hardware is perfect

Pulse-width	(Observe [1H], Decouple [X])
Offset	(Transmitter, Decoupler)
Decoupling	(X nucleus)
Gradients	(Strength, Ratio)
J Values	(one bond, multiple bond)
Processing	(Window Functions)

What Could Have Possibly Gone Wrong ?!

Proper use of J_{AB} values

Homonuclear – TOCSY

Mixing Time = $1/(2J_{HH})$

Heteronuclear – HSQC and HMBC

Delay = $1/(2 * {}^1J_{HX})$ and $1/(2 * {}^nJ_{HX})$

$^1J_{HC}$ values

~125 Hz for sp^3
~167 Hz for sp^2
~250 Hz for sp

Do remember a molecule may contain a mix of sp^3, sp^2 and sp hybridizations