

Introduction to Nuclear Magnetic Resonance

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Metabolomics

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NMR Metabolomics

Advantages

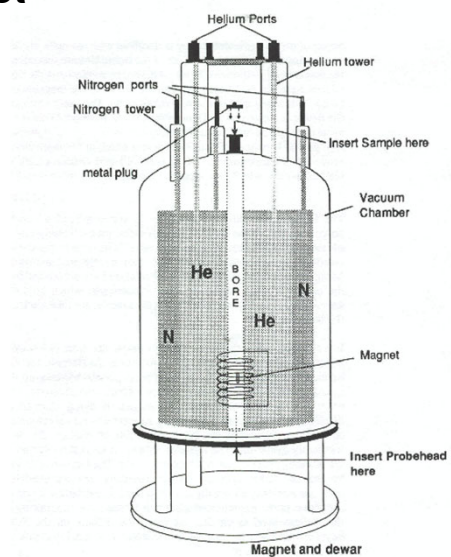
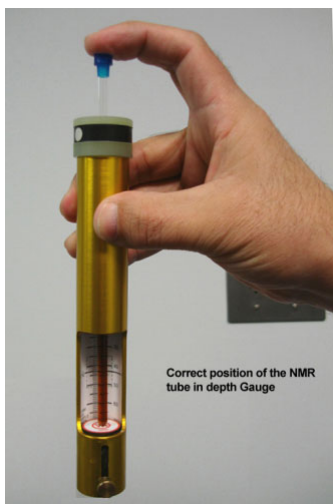
- Quantitative estimate of concentration of metabolites
- Highly Reproducible
- Detects all metabolites simultaneously
- Nondestructive. You can recover the sample completely
- Minimal sample preparation and no need for derivatization

Disadvantages:

- Sensitivity (micromole to millimole range).
- NMR spectra are complex (signals from different metabolites can overlap)

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Innards of the Magnet



3



AV III HD 600

AV III HD 850

AV III HD 500



AV III HD 850

**Central Alabama
High-Field NMR Facility
UAB**

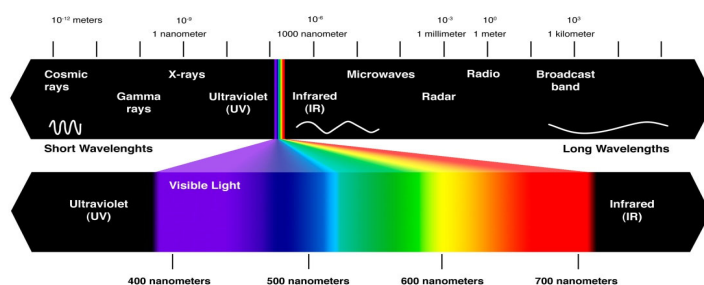


AV II 700

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Classical Description of NMR

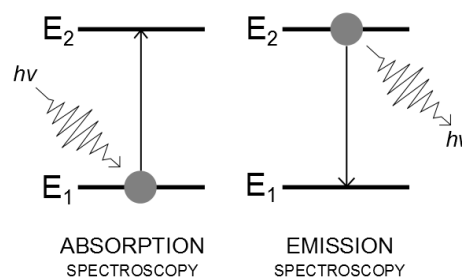
- NMR spectroscopy is similar to other forms of spectroscopy.
- A photon of light causes a transition from the ground state to the excited state



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Classical Description of NMR

- For visible spectroscopy, an electron absorbs the energy.
- For NMR, the absorbed photon promotes a nuclear spin from its ground state to its excited state

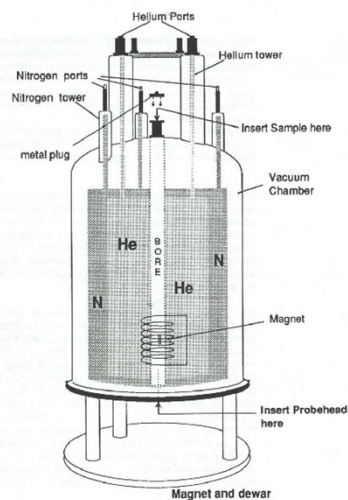


NMR differs from other types of spectroscopy in a number of ways...

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Classical Description of NMR

- Generation of the ground and excited NMR spin states requires the existence of an external magnetic field.
- This allows you to change the characteristic frequencies of transitions by simply changing the magnetic field.



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Classical Description of NMR

- The NMR excited state has a lifetime that is on order of 10^9 times longer than excited electron states.
- This lifetime follows directly from Einstein's law for spontaneous emission

lifetime of the excited state $\rightarrow \tau \propto \frac{1}{\omega^3}$ frequency of transition

proportional to \rightarrow

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Excitation via RF Pulse

$\Delta E = h\nu$

Probability of spontaneous emission

$$P = \frac{\mu_0 \gamma^2 h \omega_0^3}{12\pi^2 c^3}$$

gyromagnetic ratio Plank's constant

permeability of free space angular frequency

$$P = \frac{\mu_0 \gamma^2 h \omega_0^3}{12\pi^2 c^3}$$

speed of light

Probability of spontaneous emission is virtually nonexistent

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Excitation via RF Pulse

$\Delta E = h\nu$

Probability of spontaneous emission

$$P = \frac{\mu_0 \gamma^2 h \omega_0^3}{12\pi^2 c^3}$$

$P \approx 10^{-21} \text{ s}^{-1}$

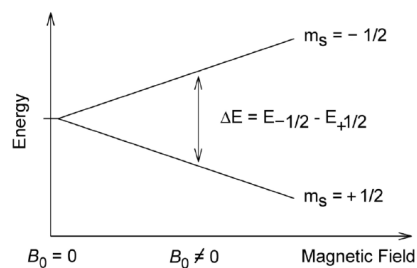
@ 500 MHz

A spontaneous emission will occur once per 316,887,646,154,127 years!!!

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How do these states originate?

- For all forms of spectroscopy, it is necessary to have two or more different states of the system that differ in energy



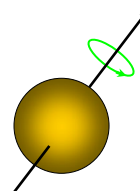
2 things are necessary

- nuclear magnetic dipole moment
- intense external magnetic field

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Nuclear Magnetic Dipole Moment

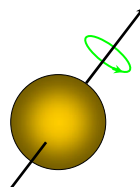
- This dipole moment arises from the spin angular momentum of the nucleus
- Spin angular momentum is a quantum mechanical property of the nucleus



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How do these states originate?

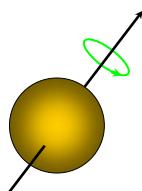
- Nuclei that have nonzero spin angular momentum also possess nuclear magnetic moments.
- The nuclear magnetic moment μ is collinear with the vector representing the nuclear spin angular momentum vector.



magnetic moment, μ , (black arrow)
perpendicular to angular spinning

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How do these states originate?



magnetic moment, μ , (black arrow)
perpendicular to angular spinning

$$\mu_z = \gamma I_z = \gamma \hbar m$$

gyromagnetic ratio

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Nuclear Magnetic Dipole Moment

- Spin angular momentum is characterized by the nuclear spin number (I).

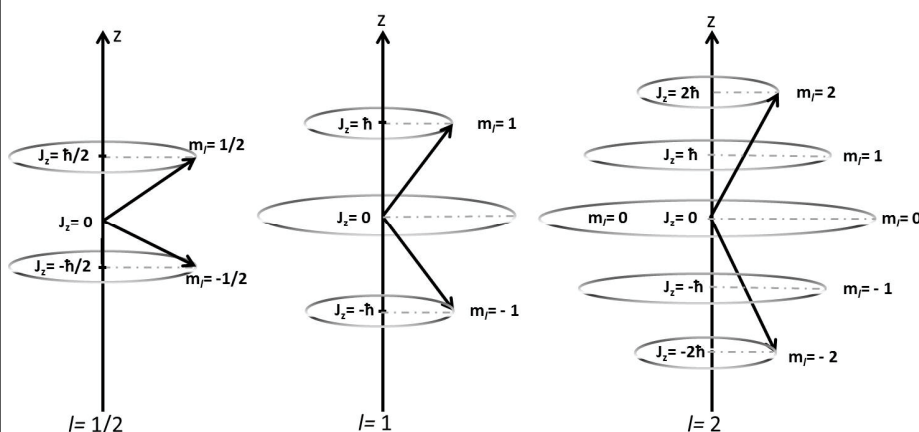
Mass Number	Number of Protons	Number of Neutrons	Spin (I)	Example
Even	Even	Even	0	^{12}C
	Odd	Odd	Integer	^2H
Odd	Even	Odd	Half-Integer	^{13}C
	Odd	Even	Half-Integer	^{15}N

For biomolecules, most important nuclei have $I = \frac{1}{2}$

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

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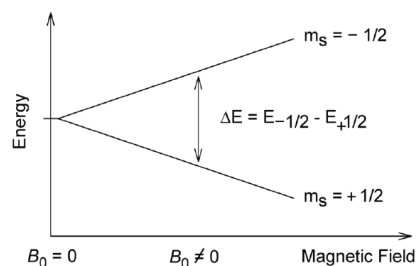
How does these states originate?



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How do these states originate?

- For all forms of spectroscopy, it is necessary to have two or more different states of the system that differ in energy



2 things are necessary

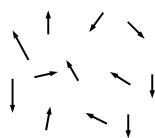
- nuclear magnetic dipole moment
- intense external magnetic field

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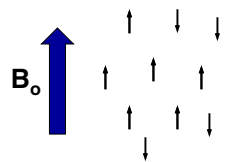
How do these states originate?

- In the absence of an external field, the quantum states corresponding to the $2I + 1$ values of m have the same energy
- In the absence of an external field, the spin angular momentum vector does not have a preferred orientation

no magnetic field



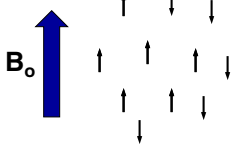
static magnetic field



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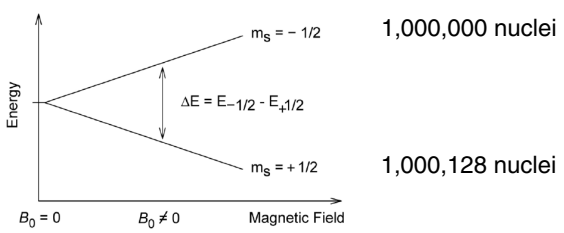
Boltzman distribution

static magnetic field



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

For an 800 MHz Magnet:
Ratio : 0.999872

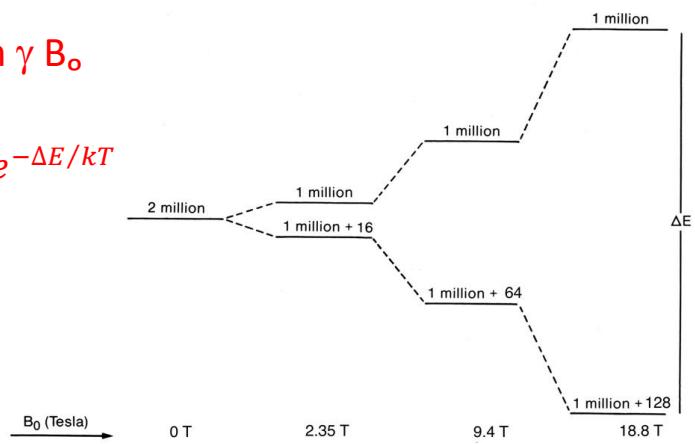


NMR is extremely insensitive!!!

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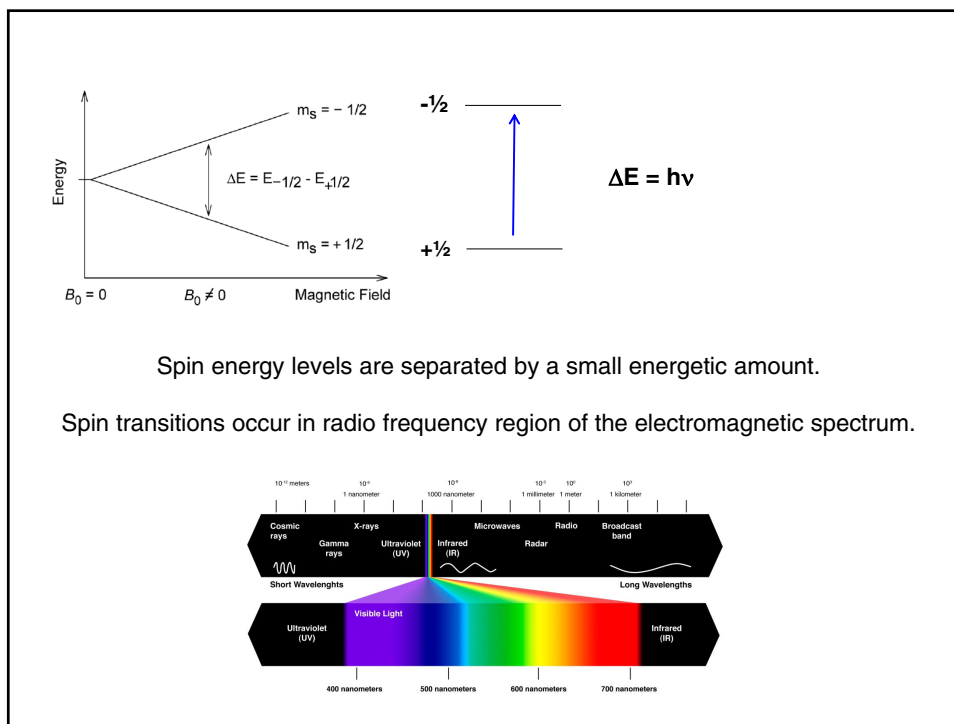
$\Delta E = h \gamma B_0$

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

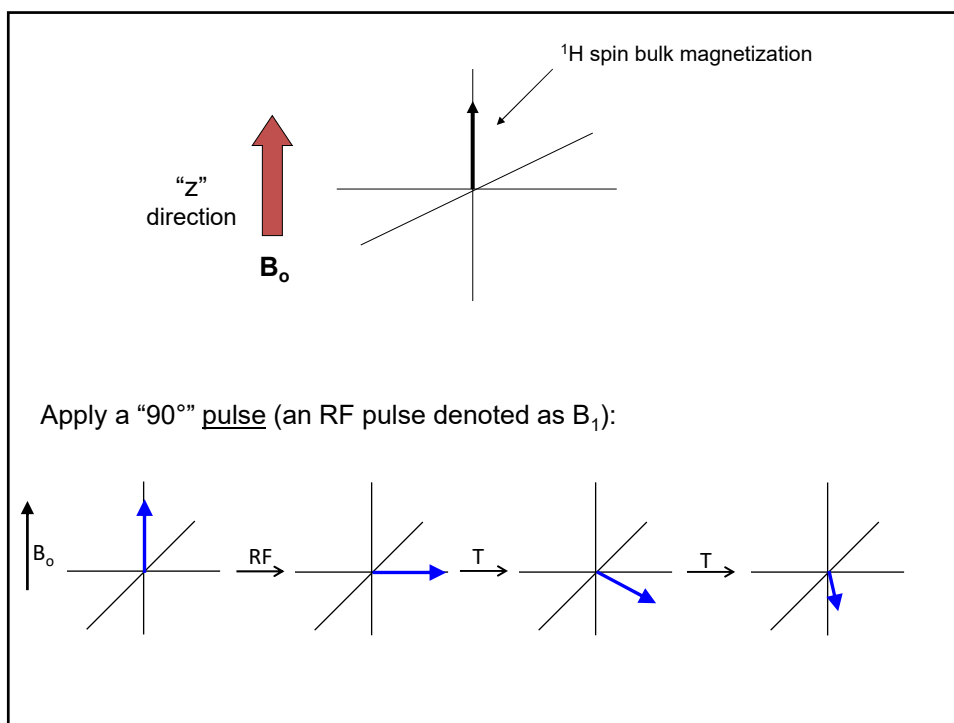


Higher fields give better sensitivity.

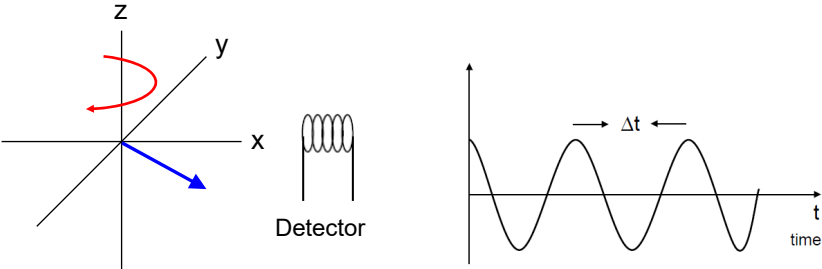
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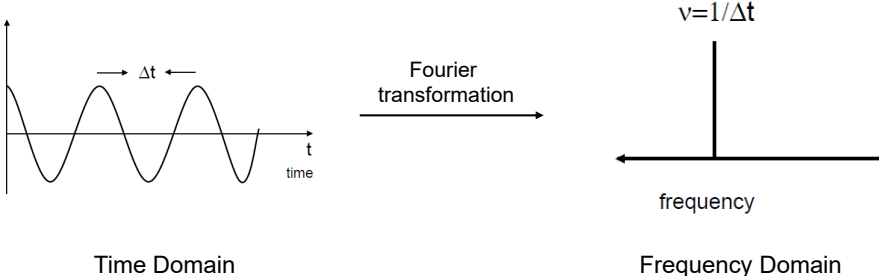


spin magnetization vector "precesses" in the "transverse plane"
(i.e. x-y plane) at a frequency

$$\omega = -B_0 \gamma$$

γ : gyromagnetic ratio ^1H at 11.7 Tesla is 500 MHz.

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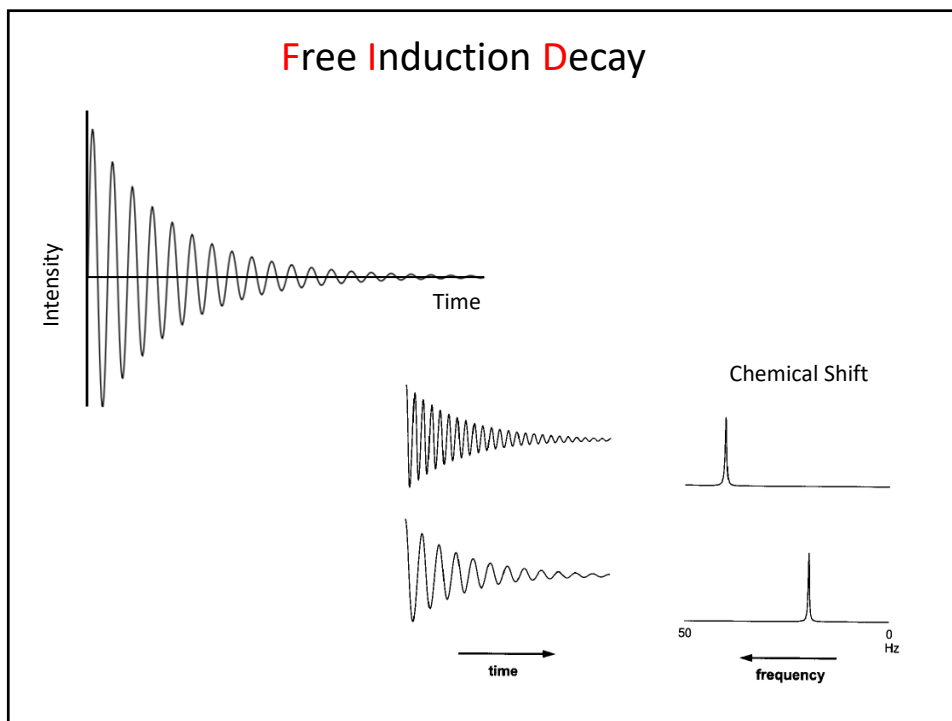


Time Domain Frequency Domain

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \tilde{F}(\omega) e^{j\omega t} d\omega$$

$$\tilde{F}(\omega) = \int_{-\infty}^{\infty} f(t) e^{-j\omega t} dt$$

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The Chemical Shift

The NMR frequency ν of a nucleus in a molecule is mainly determined by its gyromagnetic ratio γ and the strength of the magnetic field \mathbf{B}

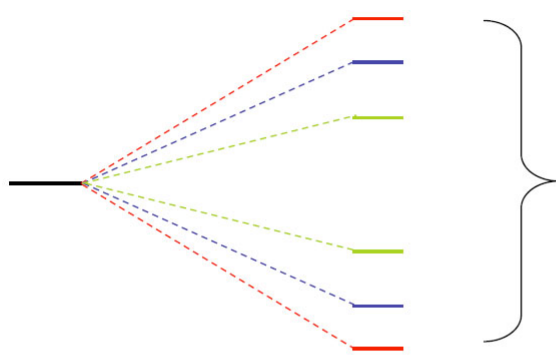
$$\nu = \frac{\gamma B}{2\pi}$$

The exact value of ν depends, however, on the position of the nucleus in the molecule or more precisely on the local electron distribution

this effect is called the **chemical shift**

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The Chemical Shift



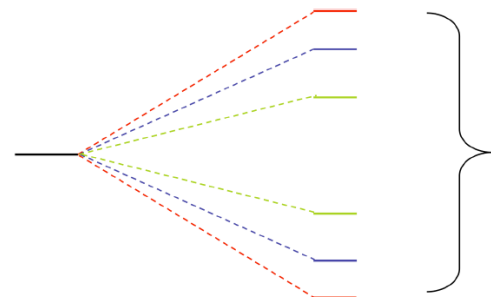
$\Delta E = h\nu = h\gamma B / 2\pi$

Nuclei, however, in molecules are never isolated from other particles that are charged and are in motion (electrons!).

Thus, the field actually felt by a nucleus is slightly different from that of the applied external magnetic field!!

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The Chemical Shift



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

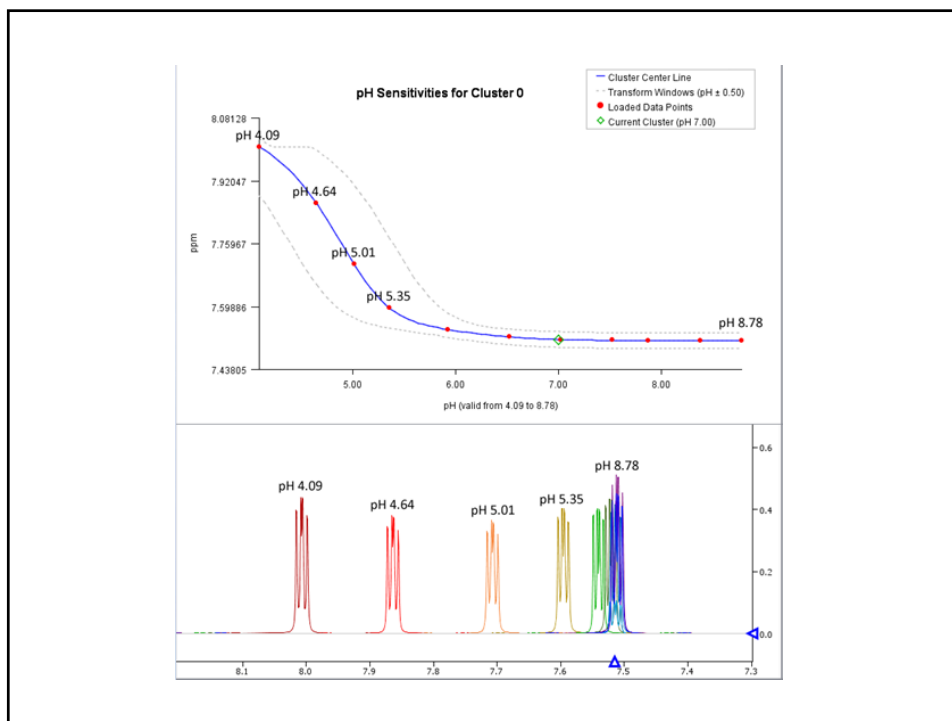
B_{eff} is given by $B_0 - B' = B_0 - B_0\sigma = B_0(1 - \sigma)$

$$\nu = \frac{\gamma B_0(1 - \sigma)}{2\pi}$$

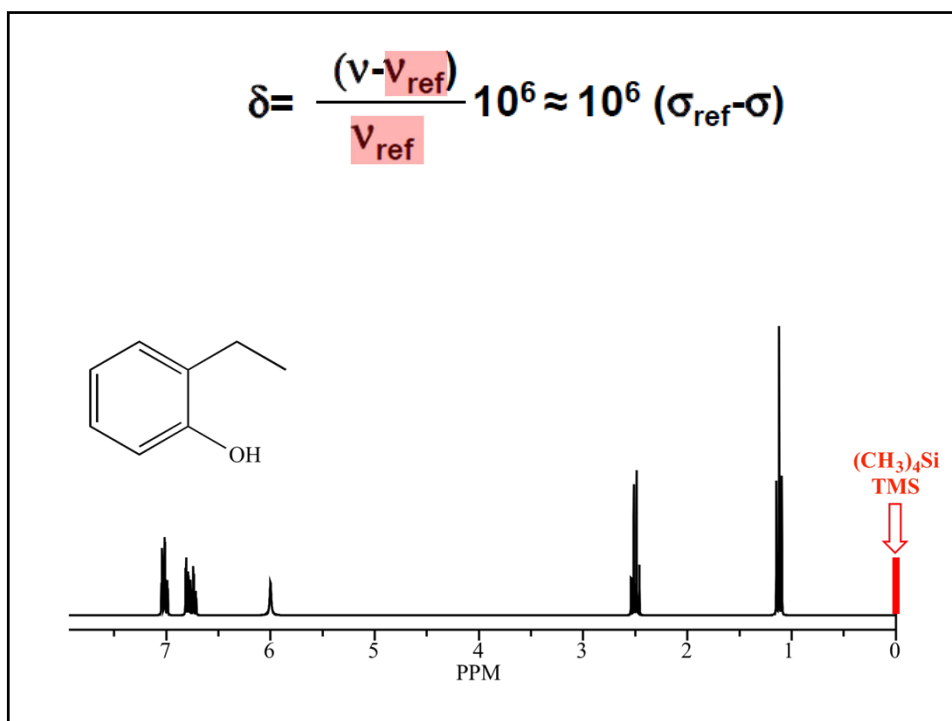
and δ is the chemical shift

$$\delta = \frac{(\nu - \nu_{\text{ref}})}{\nu_{\text{ref}}} 10^6 \approx 10^6 (\sigma_{\text{ref}} - \sigma)$$

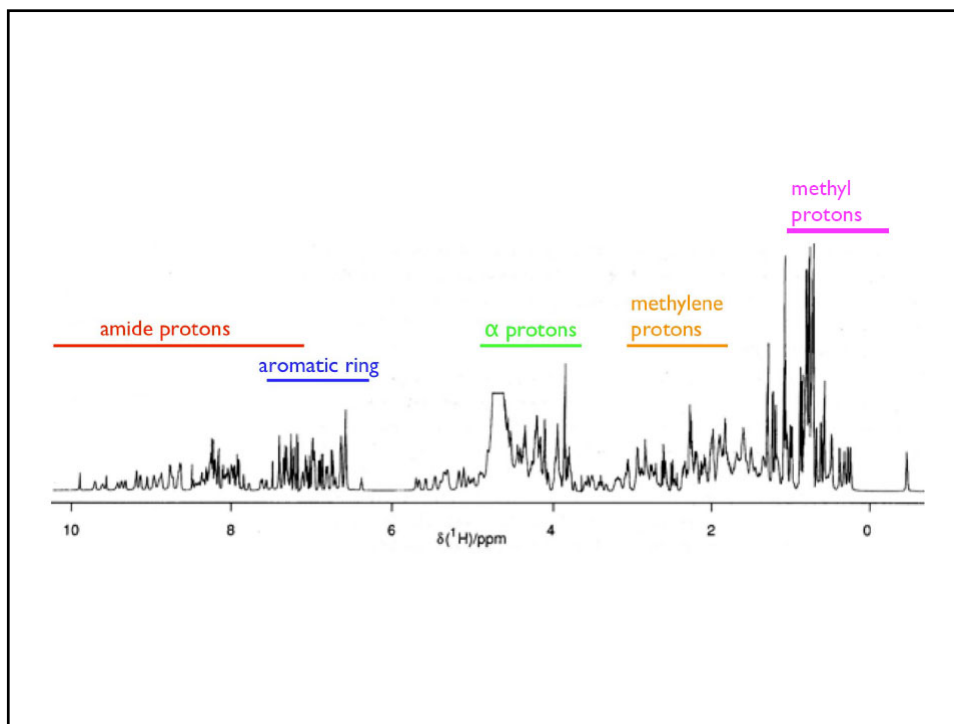
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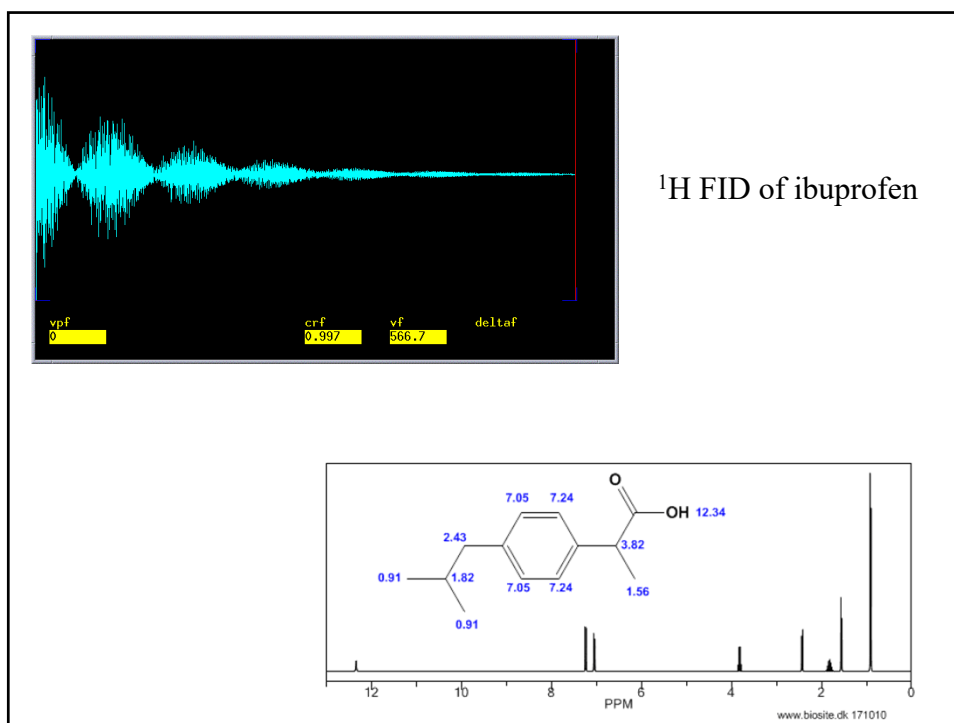
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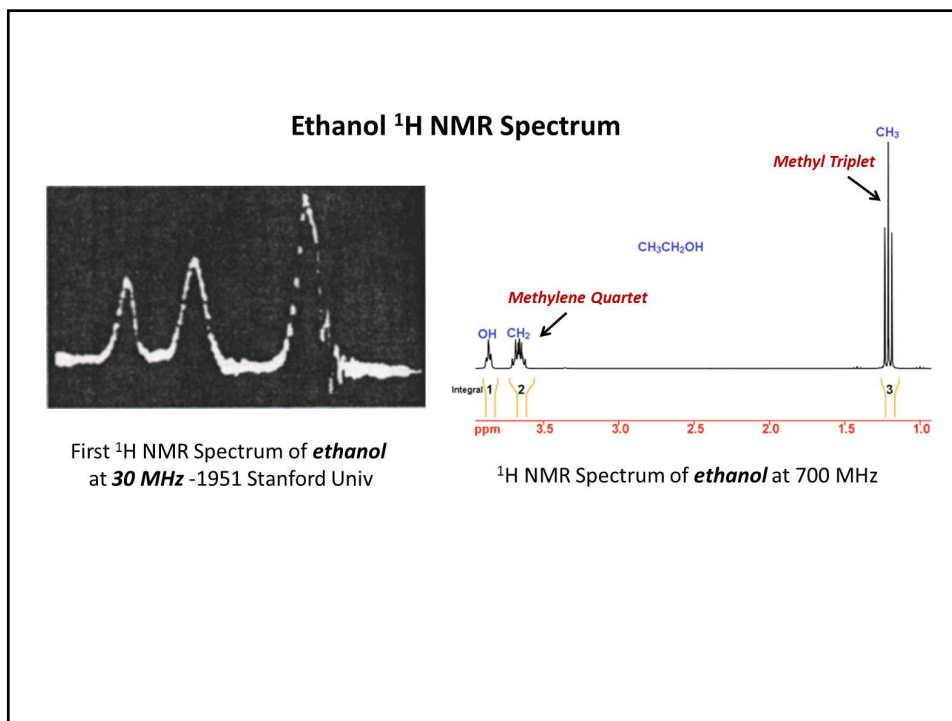
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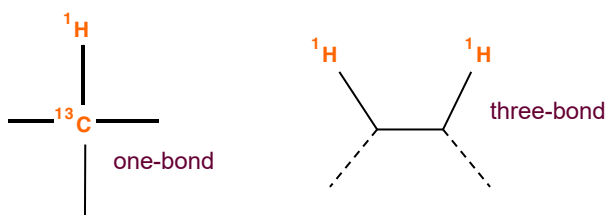
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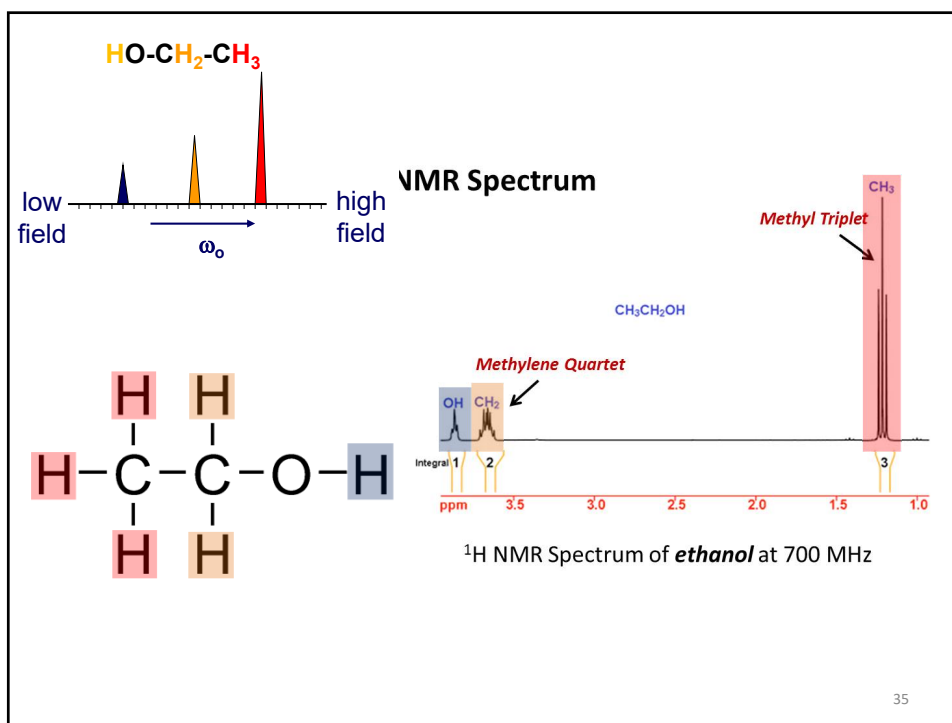
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What causes the splitting of peaks?

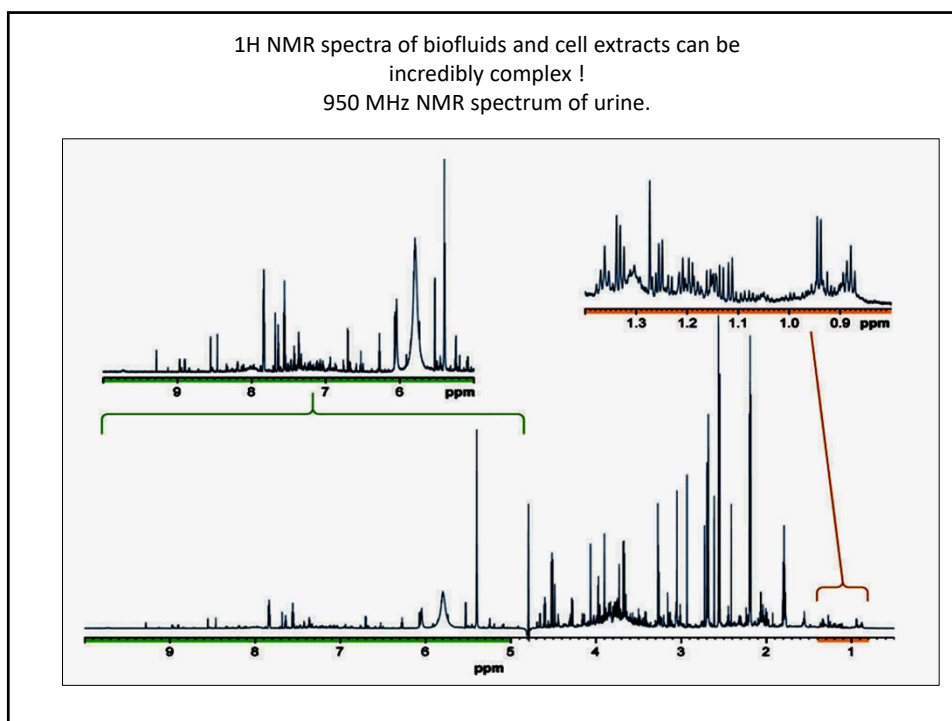
- Nuclei which are close to one another influence on each other's **effective magnetic field**.
- If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable.
- This is called **spin-spin coupling** or **J coupling**.



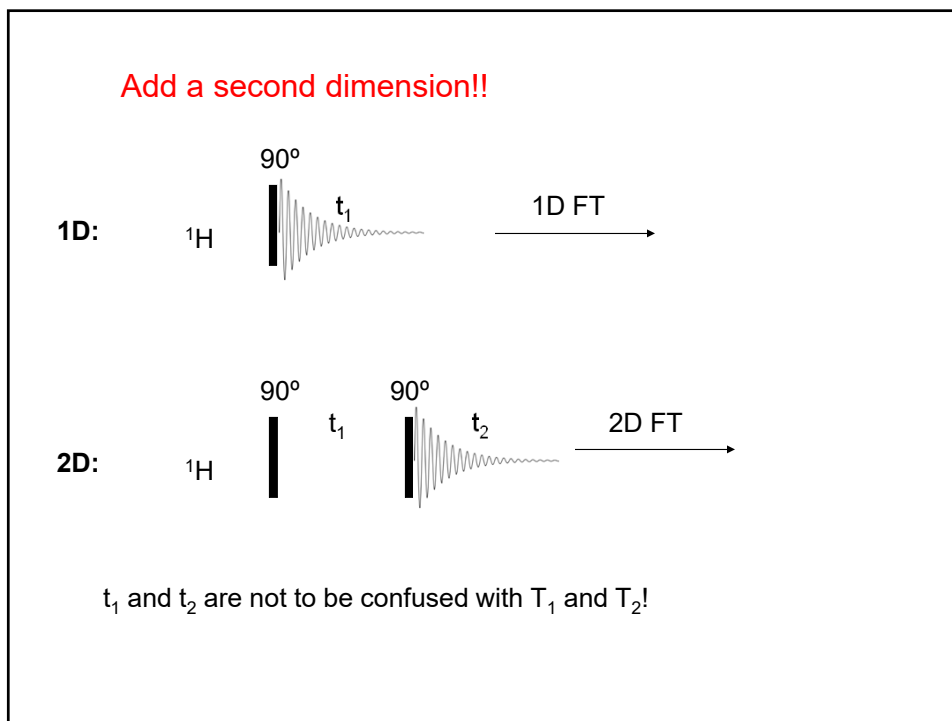
34



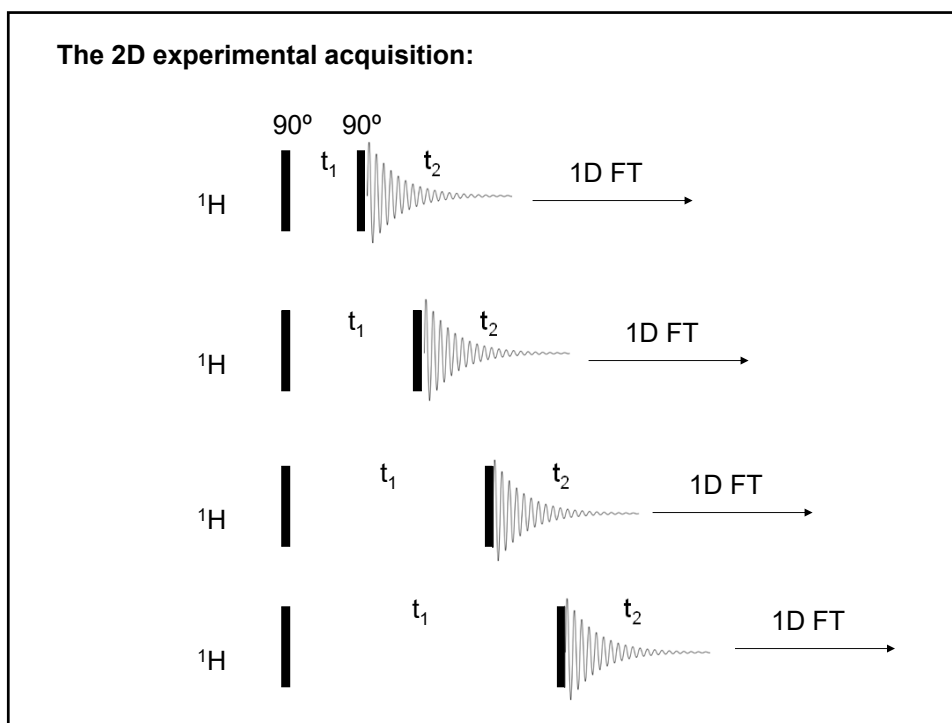
35



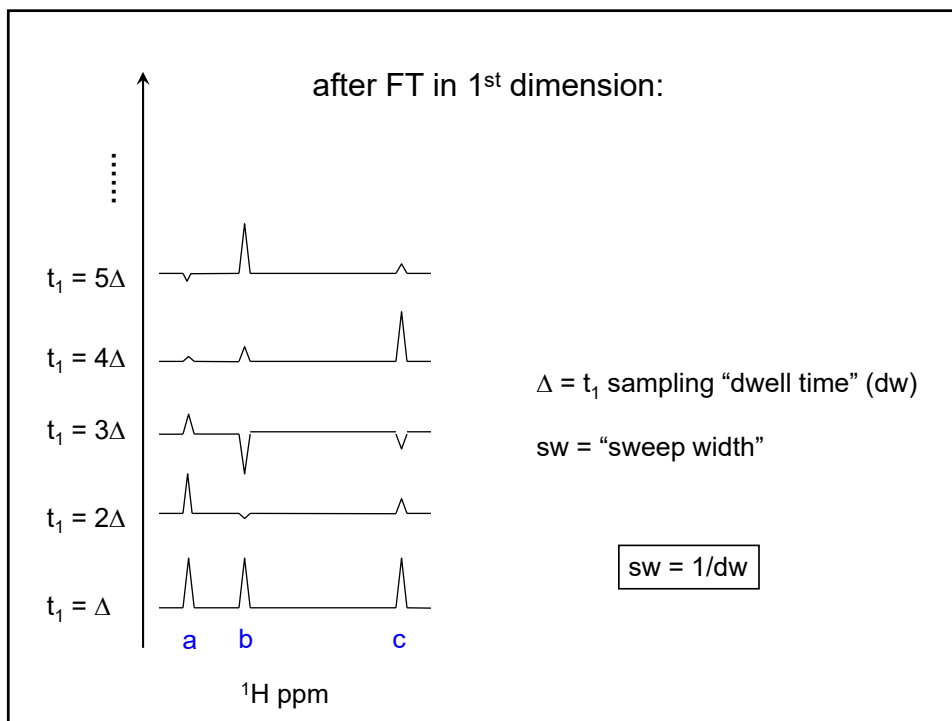
36



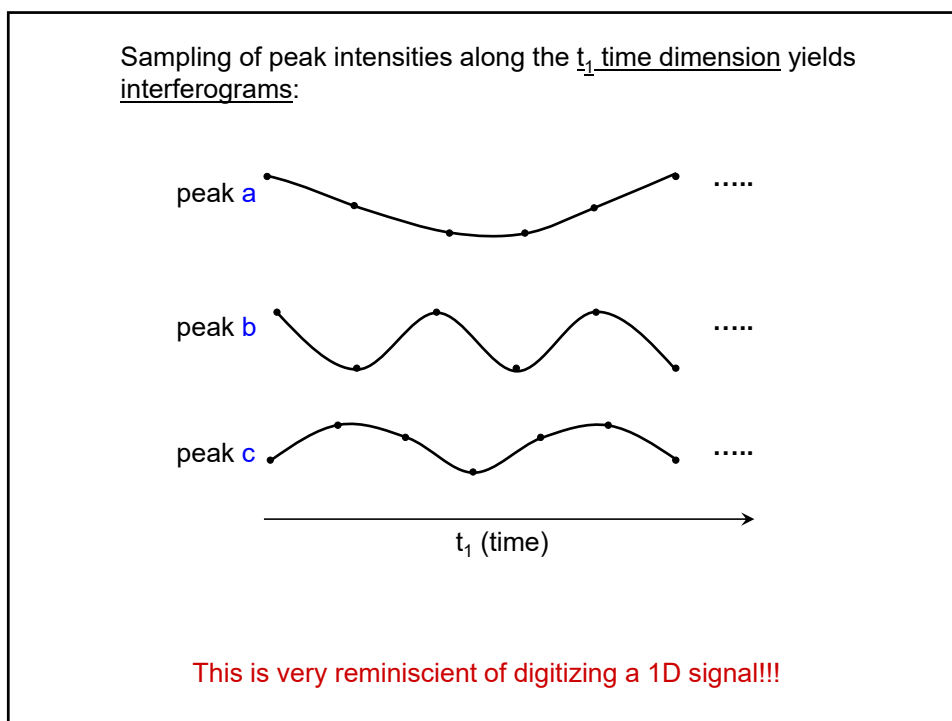
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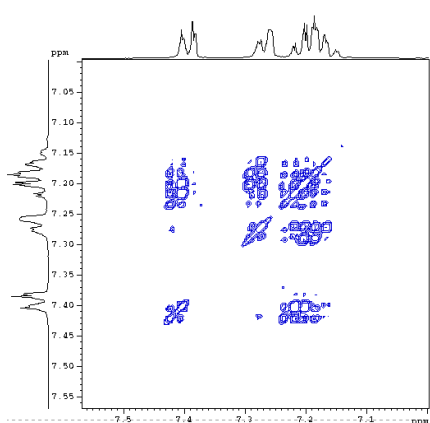
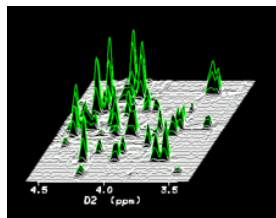
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Upon Fourier transformation in both dimensions,
spectral peaks with 2-dimensional character are obtained.

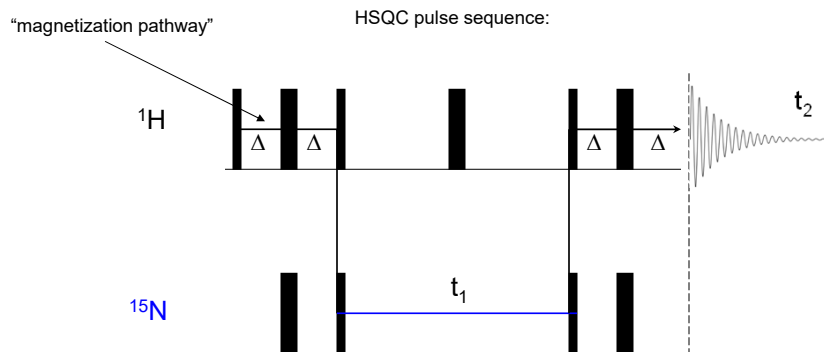
2-dimensional ^1H NMR



★ Major Advance #1 in NMR methodology: the 2nd dimension

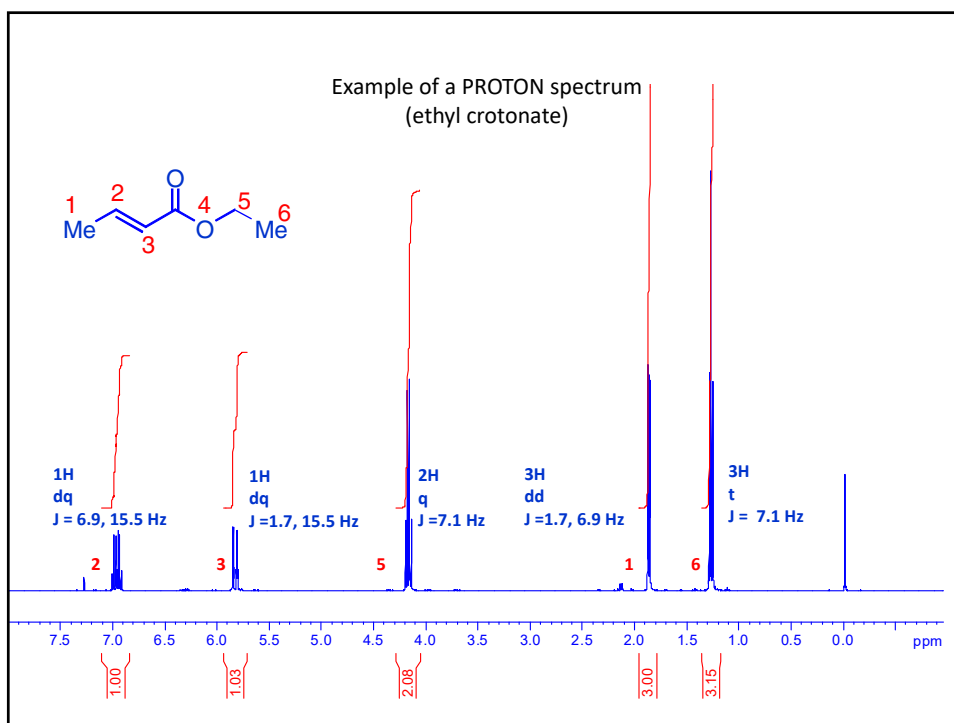
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2D HSQC (Heteronuclear Single Quantum Coherence)

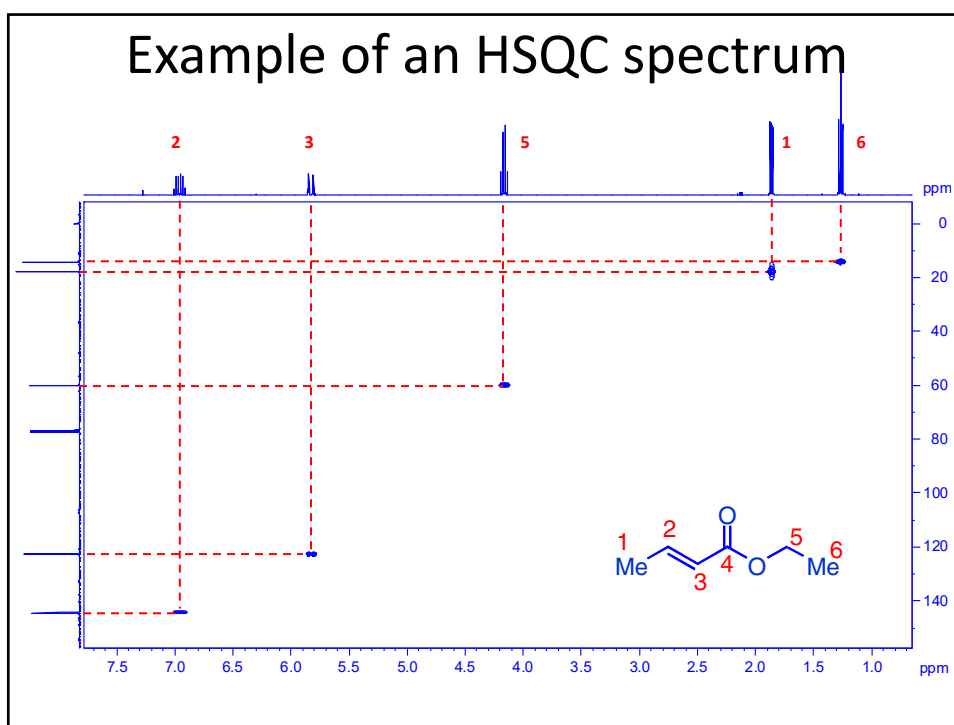


- "indirect" heteronuclear (^{15}N) detection without sensitivity reduction from the low γ .

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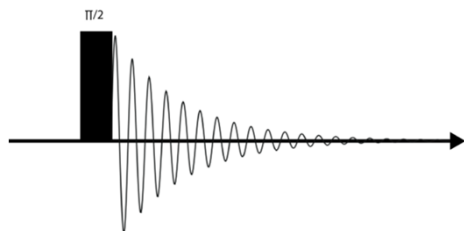


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One more thing to think about...



Target Molecule: $\approx \mu\text{m} - \text{mm}$

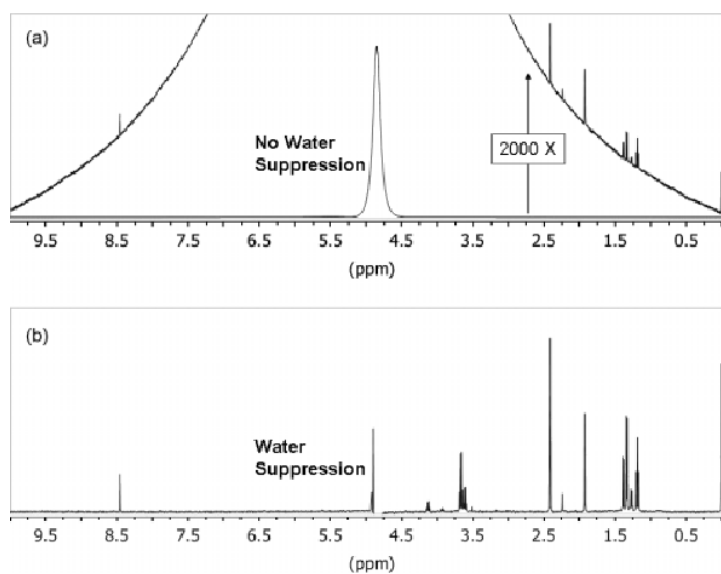
Solvent (H_2O): 55.5 M

- The standard 1D-NMR pulse sequence will record NMR signals from **EVERY** molecule in the sample.
- Thus, in a typical biofluid (e.g., blood serum) sample, using this pulse sequence, you will see very broad signals from proteins, lipids, as well as very sharp signals from low molecular weight metabolites.
- In addition, solvent peaks (H_2O) can contribute significant peaks.

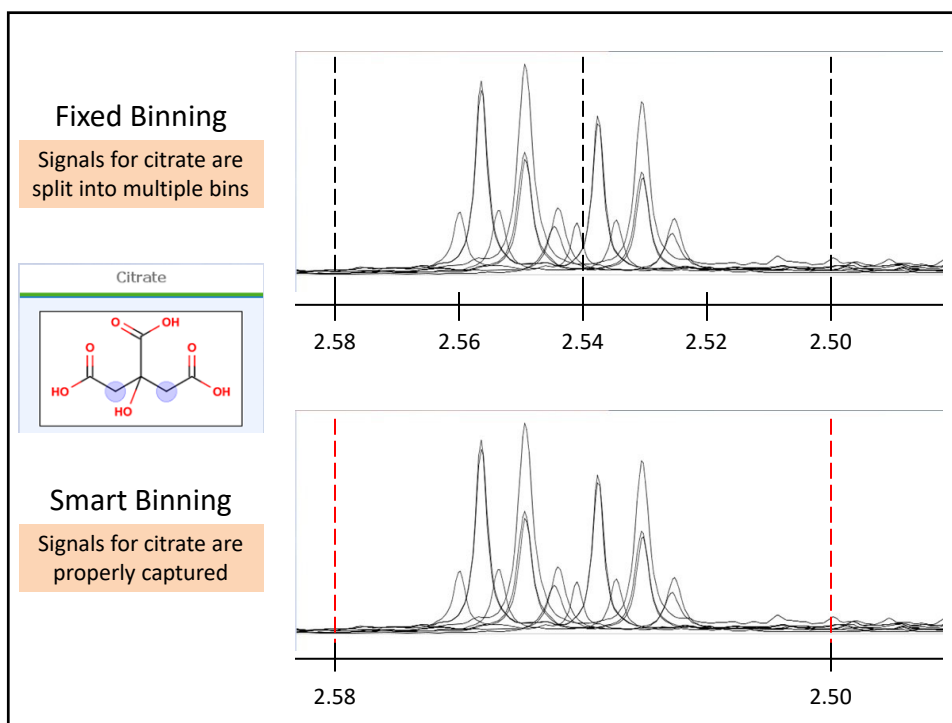
It is a truly an Equal Opportunity Detector !!!

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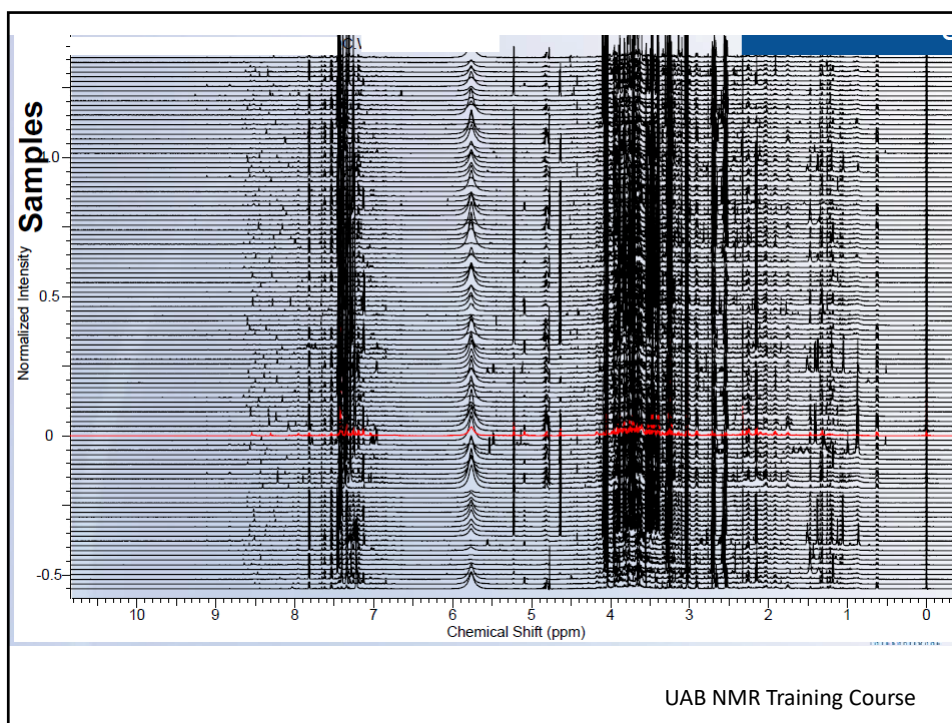
One more thing to think about...



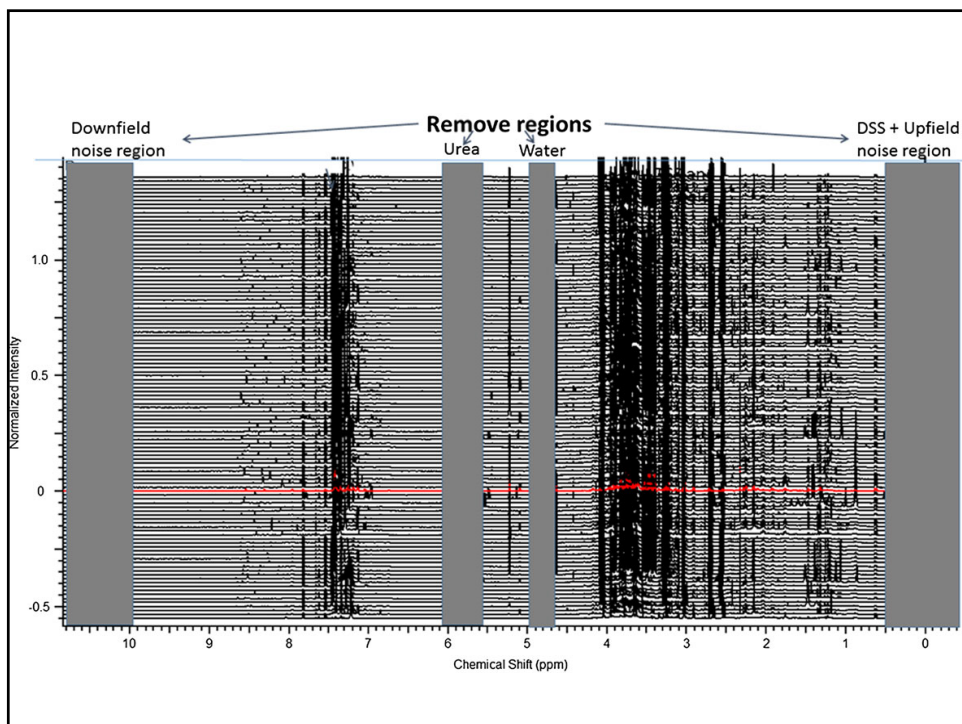
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Sample ID	Disease Group	[0.40 .. 0.46]	[0.46 .. 0.52]	[0.52 .. 0.54]	[0.54 .. 0.57]	[0.57 .. 0.60]	[0.60 .. 0.66]	[0.66 .. 0.68]	[0.68 .. 0.71]	[0.71 .. 0.75]
C0559	Cases	7.60E-05	0.00E+00	7.32E-02	8.48E-02	3.20E-02	1.84E+00	1.31E-01	3.60E-01	3.67E-01
C0629	Cases	0.00E+00	1.78E-02	0.00E+00	2.18E-02	0.00E+00	1.08E+01	0.00E+00	0.00E+00	3.02E-02
C0640	Cases	3.44E-04	0.00E+00	1.83E-03	1.86E-04	0.00E+00	4.51E+00	0.00E+00	0.00E+00	0.00E+00
C0835	Cases	6.41E-04	0.00E+00	6.44E-03	0.00E+00	3.96E-03	3.28E+00	0.00E+00	5.12E-03	1.75E-02
D0613	Cases	6.63E-03	0.00E+00	0.00E+00	1.06E-02	0.00E+00	5.79E+00	0.00E+00	6.36E-02	3.02E-01
D0762	Cases	0.00E+00	0.00E+00	1.79E-02	1.98E-02	0.00E+00	9.37E+00	0.00E+00	0.00E+00	1.74E-02
D1113	Cases	3.14E-03	2.42E-03	8.02E-02	1.04E-01	5.32E-03	3.74E+00	0.00E+00	2.02E-02	1.84E-01
D1158	Cases	0.00E+00	3.71E-03	2.35E-02	4.83E-02	0.00E+00	5.02E+00	0.00E+00	1.91E-02	0.00E+00
D2090	Cases	0.00E+00	0.00E+00	2.45E-03	9.98E-04	0.00E+00	5.76E+00	0.00E+00	1.24E-02	1.04E-02
E0004	Cases	1.72E-03	0.00E+00	6.85E-02	3.05E-02	0.00E+00	1.47E+00	6.90E-02	3.61E-01	4.08E-01
E0195	Cases	0.00E+00	1.69E-03	5.57E-02	6.29E-02	0.00E+00	2.77E+00	1.34E-01	2.04E-01	4.56E-01
E0225	Cases	1.25E-03	0.00E+00	4.40E-03	1.69E-02	0.00E+00	9.17E+00	0.00E+00	1.08E-02	2.30E-02
E0339	Cases	4.11E-03	0.00E+00	2.33E-02	1.68E-02	3.09E-02	3.98E+00	0.00E+00	3.28E-02	9.09E-01
E0487	Cases	1.72E-03	0.00E+00	0.00E+00	1.00E-02	0.00E+00	4.00E+00	0.00E+00	1.36E-02	0.00E+00
F0036	Cases	1.66E-02	0.00E+00	0.00E+00	2.06E-02	0.00E+00	1.22E+01	1.04E-02	0.00E+00	5.97E-01
F0108	Cases	0.00E+00	2.31E-03	6.30E-03	1.11E-02	0.00E+00	7.17E+00	0.00E+00	1.65E-02	2.21E-01
A0233	Control	0.00E+00	1.86E-02	0.00E+00	1.82E-02	0.00E+00	1.61E+01	0.00E+00	2.91E-03	0.00E+00
A0490	Control	0.00E+00	0.00E+00	2.99E-03	3.60E-02	0.00E+00	2.97E+00	0.00E+00	4.00E-02	5.46E-01
A2003	Control	0.00E+00	0.00E+00	3.45E-02	2.20E-02	0.00E+00	1.80E+00	0.00E+00	0.00E+00	0.00E+00
C0586	Control	0.00E+00	1.69E-02	0.00E+00	6.64E-03	0.00E+00	1.92E+01	0.00E+00	6.51E-02	0.00E+00
C2177	Control	0.00E+00	0.00E+00	3.02E-02	3.59E-02	0.00E+00	2.35E+00	0.00E+00	3.19E-02	1.49E-01
D0177	Control	9.21E-03	0.00E+00	1.69E-02	1.47E-02	0.00E+00	2.43E+00	0.00E+00	4.46E-02	0.00E+00
D0729	Control	0.00E+00	1.88E-03	5.58E-02	7.87E-02	2.92E-02	3.16E+00	6.59E-02	2.80E-01	4.30E-01
D0909	Control	0.00E+00	1.08E-03	0.00E+00	5.69E-03	0.00E+00	2.49E+00	0.00E+00	1.01E-02	1.87E-01
D0945	Control	0.00E+00	4.79E-04	7.00E-03	0.00E+00	4.19E-03	3.99E+00	0.00E+00	1.11E-03	3.96E-02
D1174	Control	0.00E+00	9.33E-04	0.00E+00	3.43E-03	1.30E-02	7.21E+00	6.53E-03	0.00E+00	1.66E-02
D2054	Control	1.55E-03	0.00E+00	0.00E+00	1.22E-02	0.00E+00	2.07E+00	0.00E+00	1.28E-02	3.90E-01
D2062	Control	2.39E-05	0.00E+00	6.04E-02	2.99E-02	0.00E+00	4.94E+00	0.00E+00	9.95E-03	0.00E+00
D2079	Control	2.73E-02	0.00E+00	1.81E-03	1.17E-02	0.00E+00	3.38E+01	7.87E-02	0.00E+00	5.91E+00

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