

Introduction to Nuclear Magnetic Resonance

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Metabolomics

NMR Metabolomics

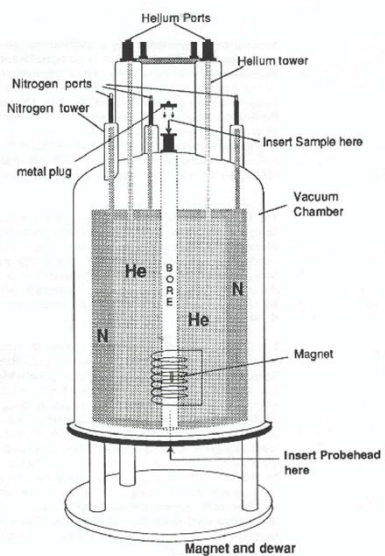
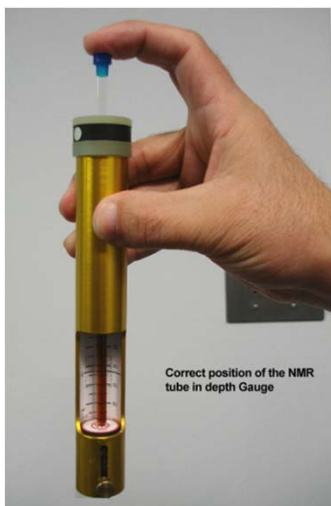
Advantages

- 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)

Innards of the Magnet



AV III HD 600

AV III HD 850

AV III HD 500



AV III HD 850

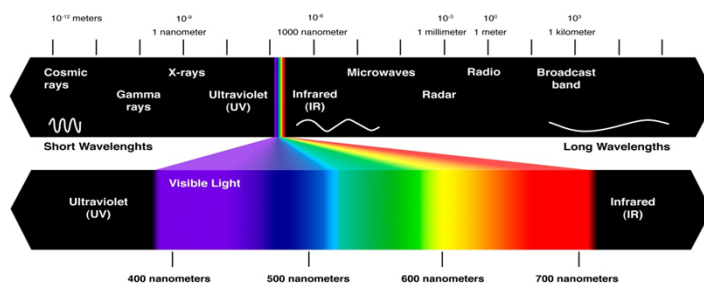


AV II 700

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

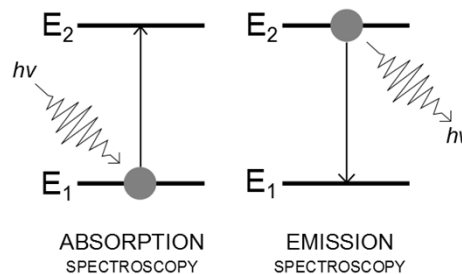
Classical Description of NMR

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



Classical Description of NMR

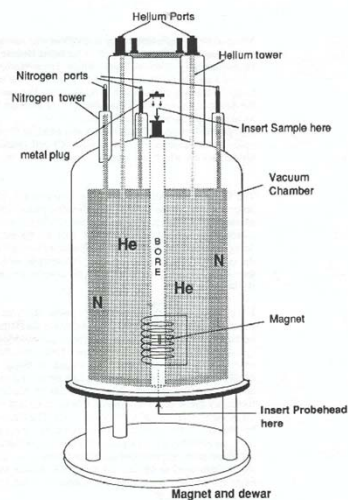
- For visible spectroscopy, an electron absorbs the energy.
- For NMR, the absorbed photon promotes a nuclear spin from its ground state to its excited state



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

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.



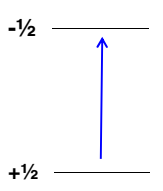
Classical Description of NMR

- 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

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

proportional to
frequency of transition

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

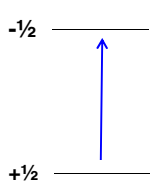
permeability of free space angular frequency

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

speed of light

Probability of spontaneous emission is virtually nonexistent

Excitation via RF Pulse



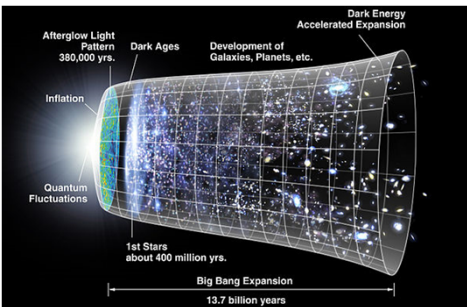
$\Delta E = h\nu$

Probability of spontaneous emission

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

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

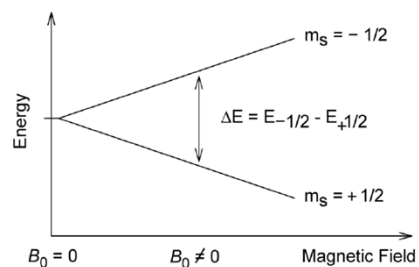
@ 500 MHz



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

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

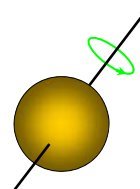


2 things are necessary

- nuclear magnetic dipole moment
- intense external magnetic field

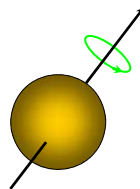
Nuclear Magnetic Dipole Moment

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



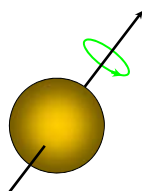
How do these states originate?

- 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

How do these states originate?



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

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

gyromagnetic ratio

Nuclear Magnetic Dipole Moment

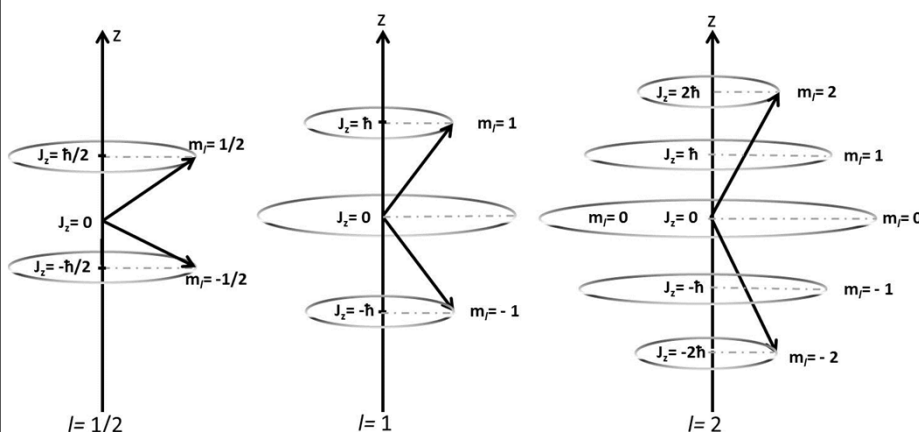
- Spin angular momentum is characterized by the nuclear spin number (I).

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 $I = \frac{1}{2}$

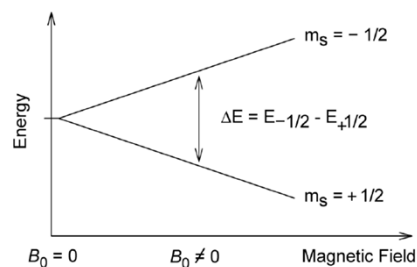
^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P

How does these states originate?



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



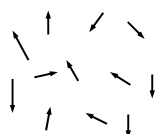
2 things are necessary

- nuclear magnetic dipole moment
- intense external magnetic field

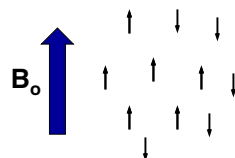
How do these states originate?

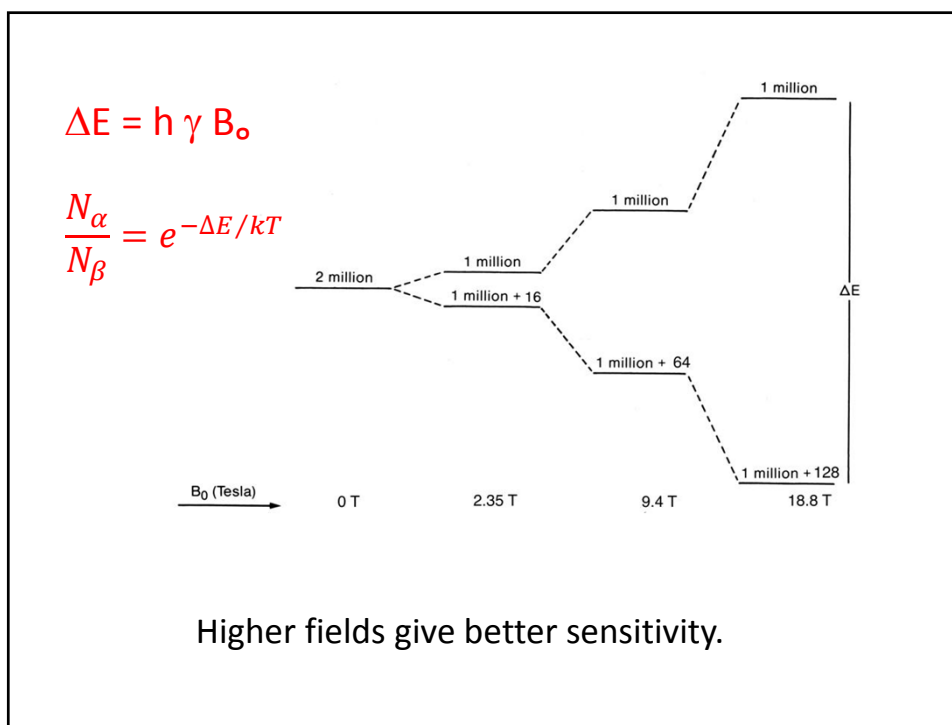
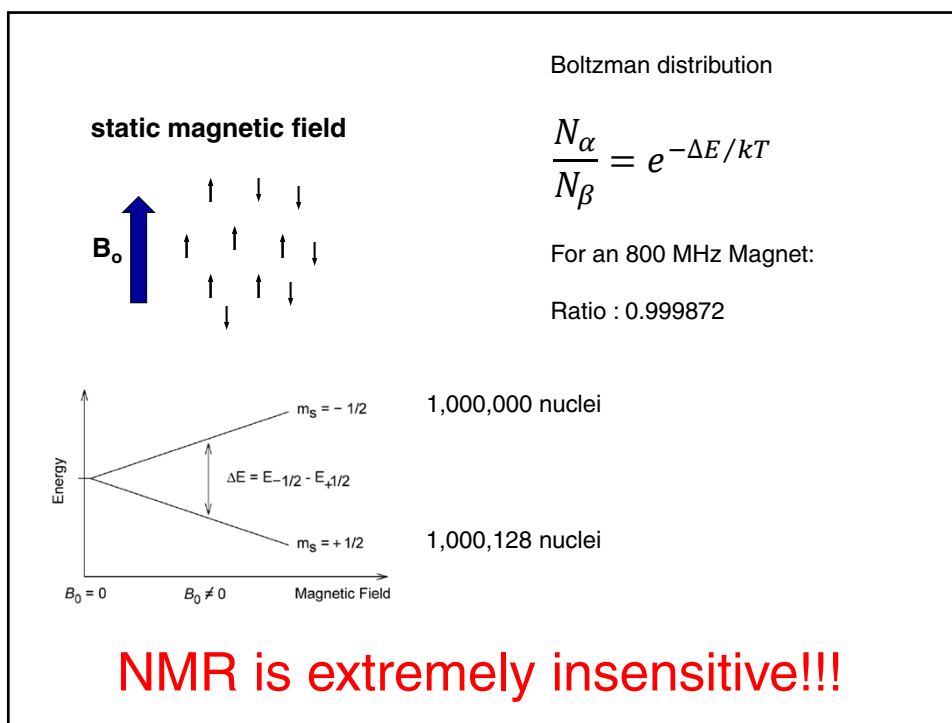
- In the absence of an external field, the quantum states corresponding to the $2I + 1$ values of m have the same energy
- In the absence of an external field, the spin angular momentum vector does not have a preferred orientation

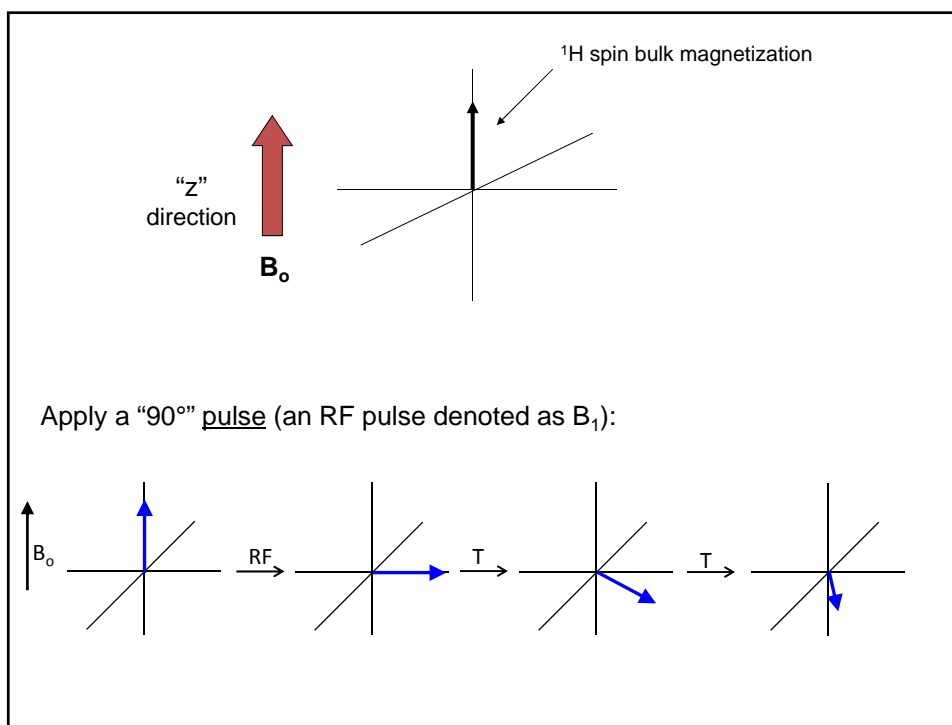
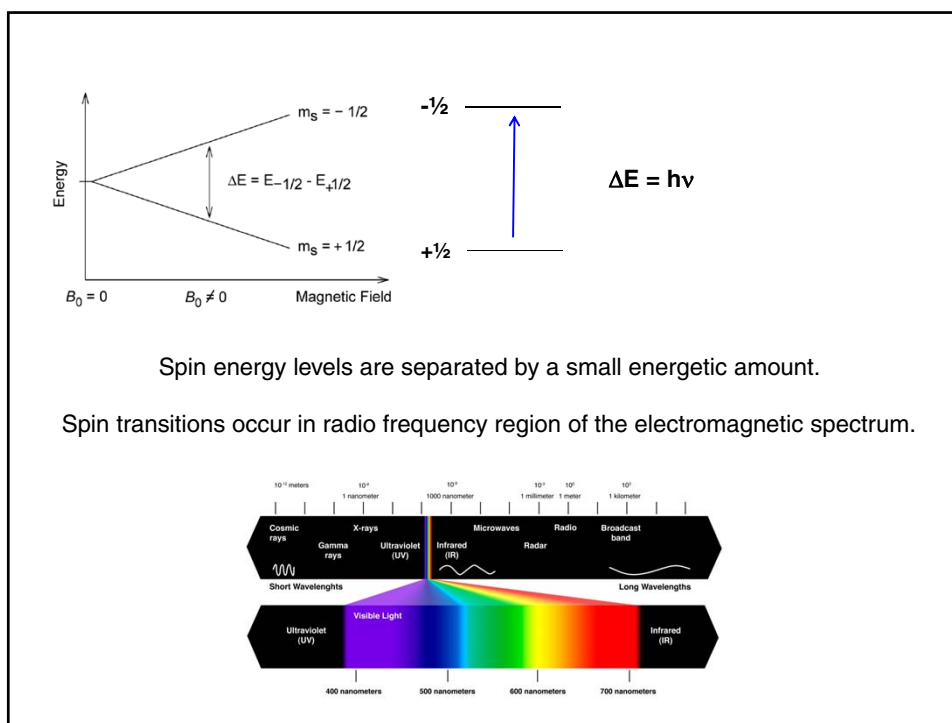
no magnetic field

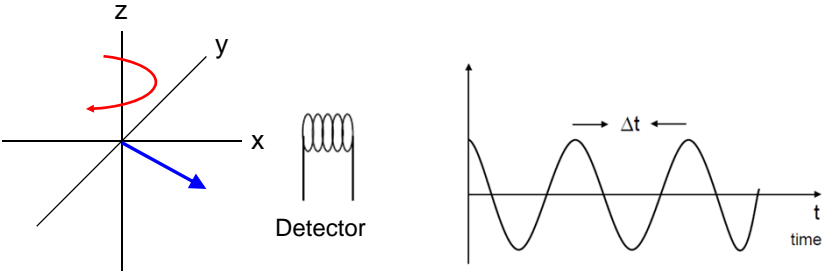


static magnetic field





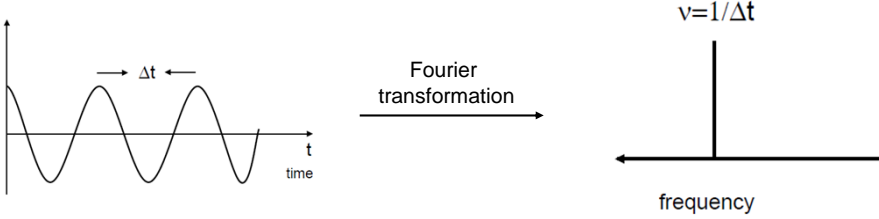




spin magnetization vector “precesses” in the “transverse plane”
(i.e. x-y plane) at a frequency

$$\omega = -B_0 \gamma$$

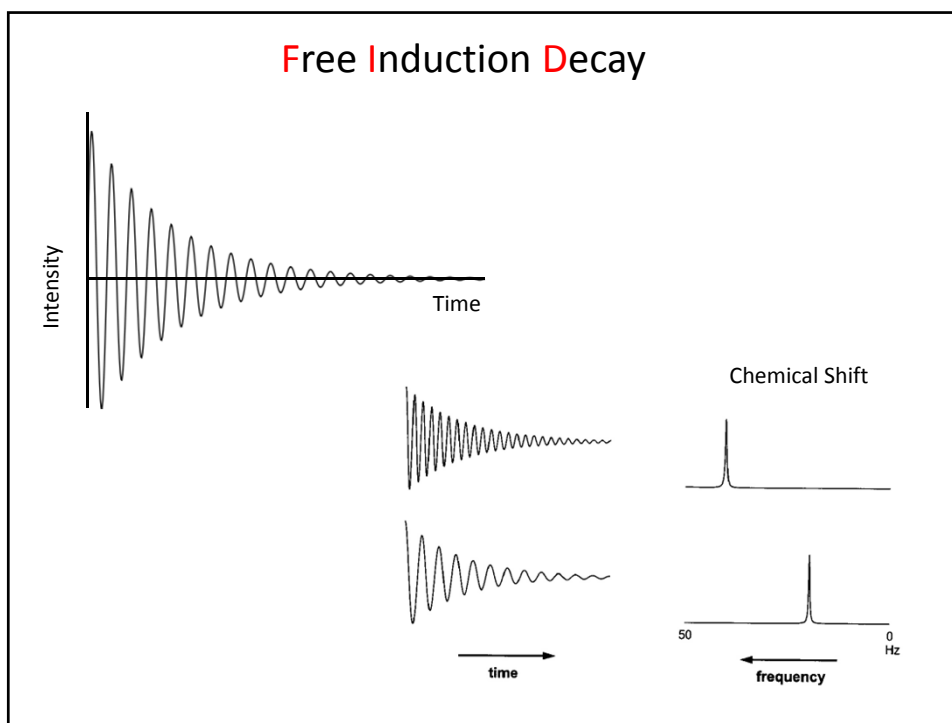
γ : gyromagnetic ratio ^1H at 11.7 Tesla is 500 MHz.



Time Domain Frequency Domain

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

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



The Chemical Shift

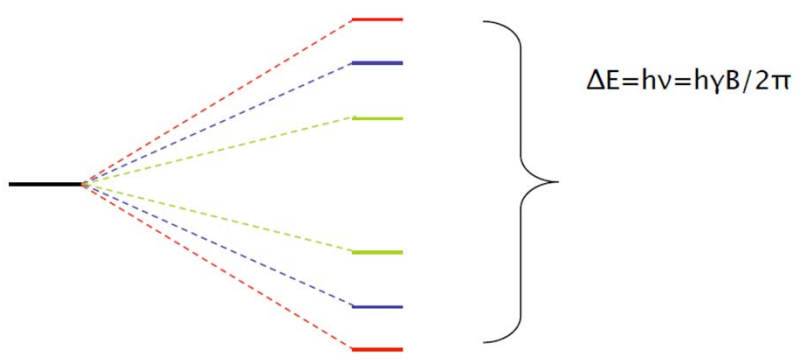
The NMR frequency ν of a nucleus in a molecule is mainly determined by its gyromagnetic ratio γ and the strength of the magnetic field \mathbf{B}

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

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**

The Chemical Shift

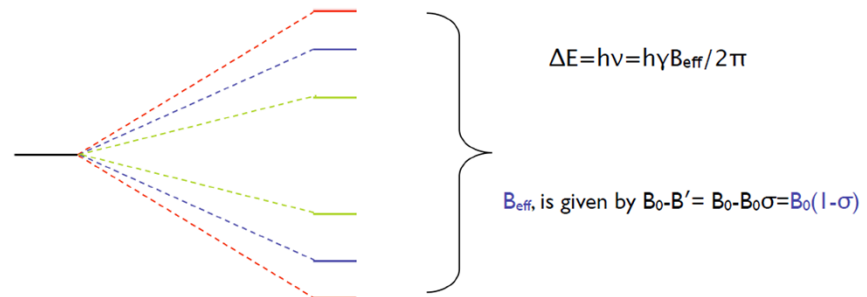


$\Delta E = h\nu = h\gamma B / 2\pi$

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!!

The Chemical Shift



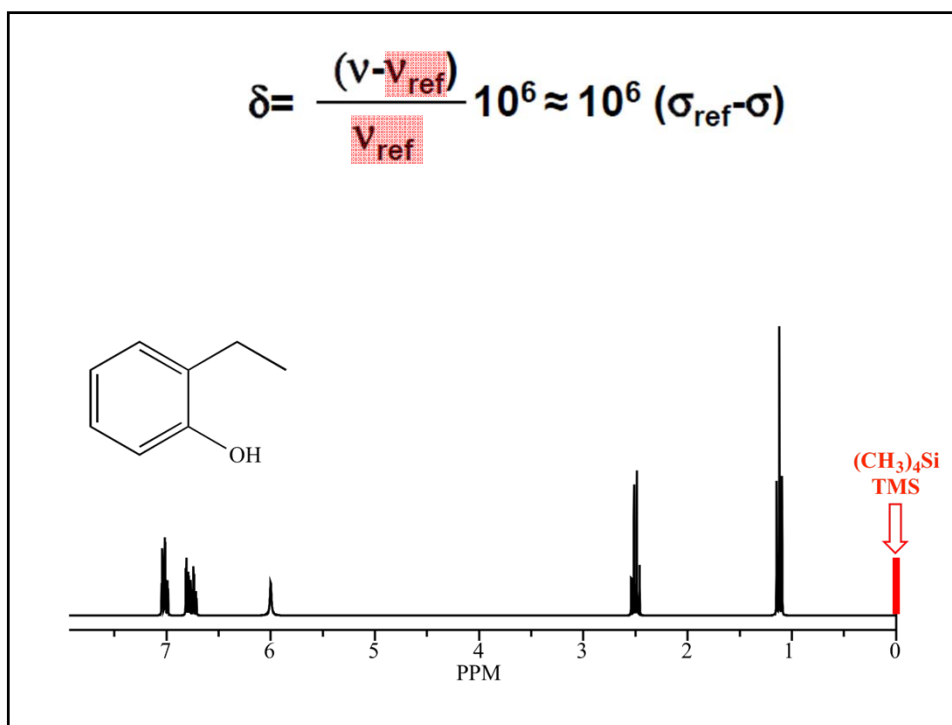
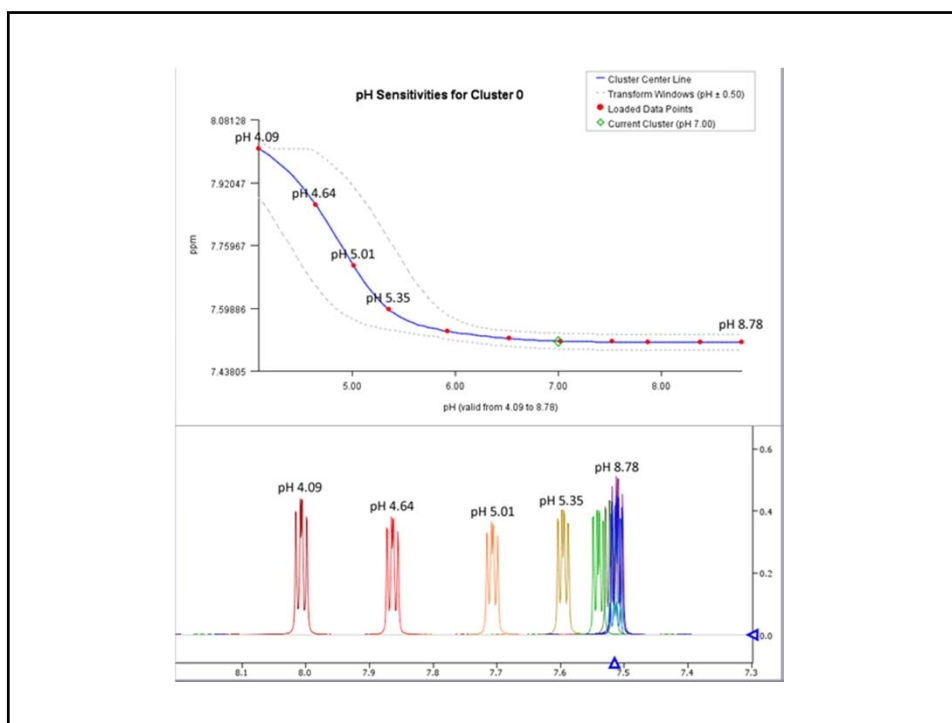
$\Delta E = h\nu = h\gamma B_{\text{eff}} / 2\pi$

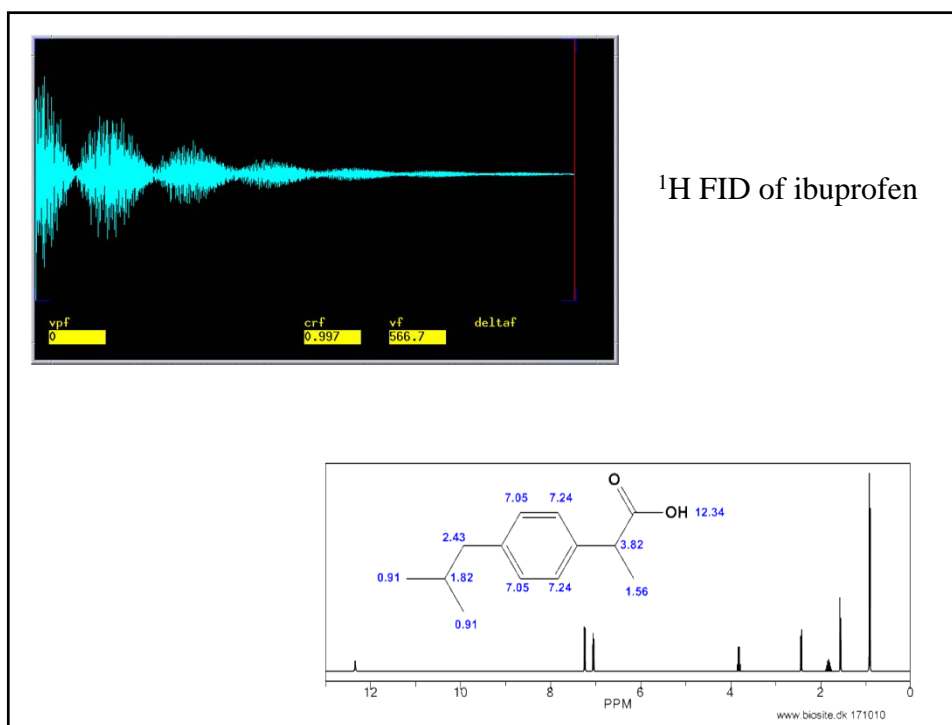
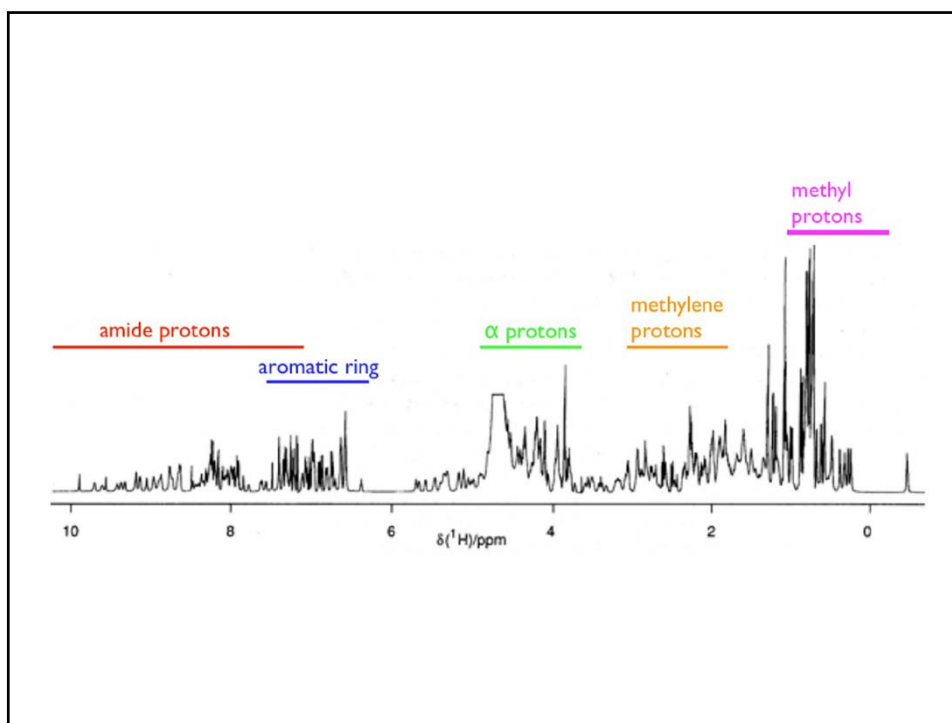
B_{eff} is given by $B_0 - B' = B_0 - B_0\sigma = B_0(1 - \sigma)$

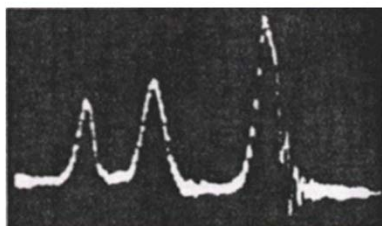
$$\nu = \frac{\gamma B_0(1 - \sigma)}{2\pi}$$

and δ is the chemical shift

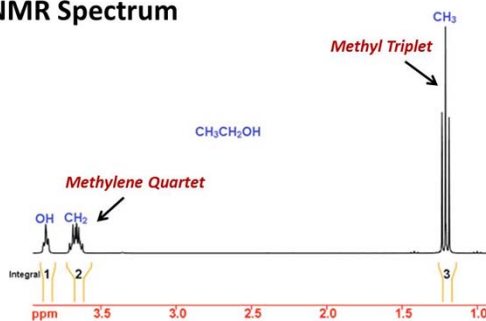
$$\delta = \frac{(\nu - \nu_{\text{ref}})}{\nu_{\text{ref}}} 10^6 \approx 10^6 (\sigma_{\text{ref}} - \sigma)$$





Ethanol ^1H NMR Spectrum

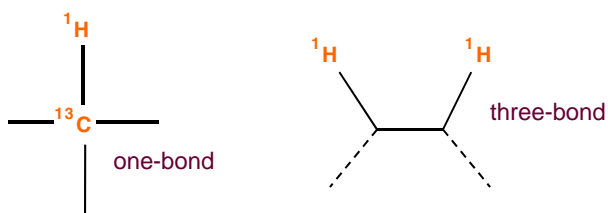
First ^1H NMR Spectrum of *ethanol*
at **30 MHz** -1951 Stanford Univ

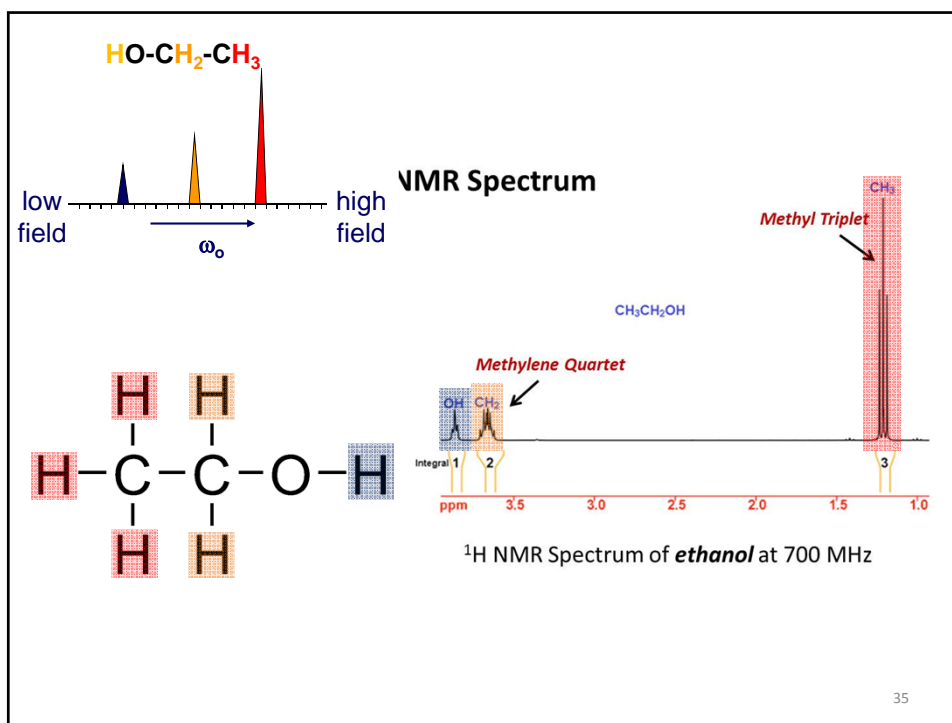


^1H NMR Spectrum of *ethanol* at 700 MHz

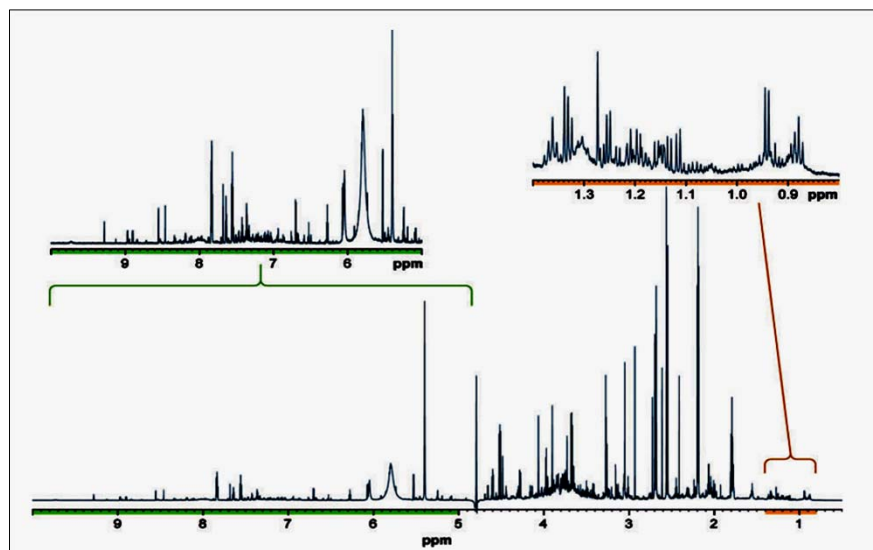
What causes the splitting of peaks?

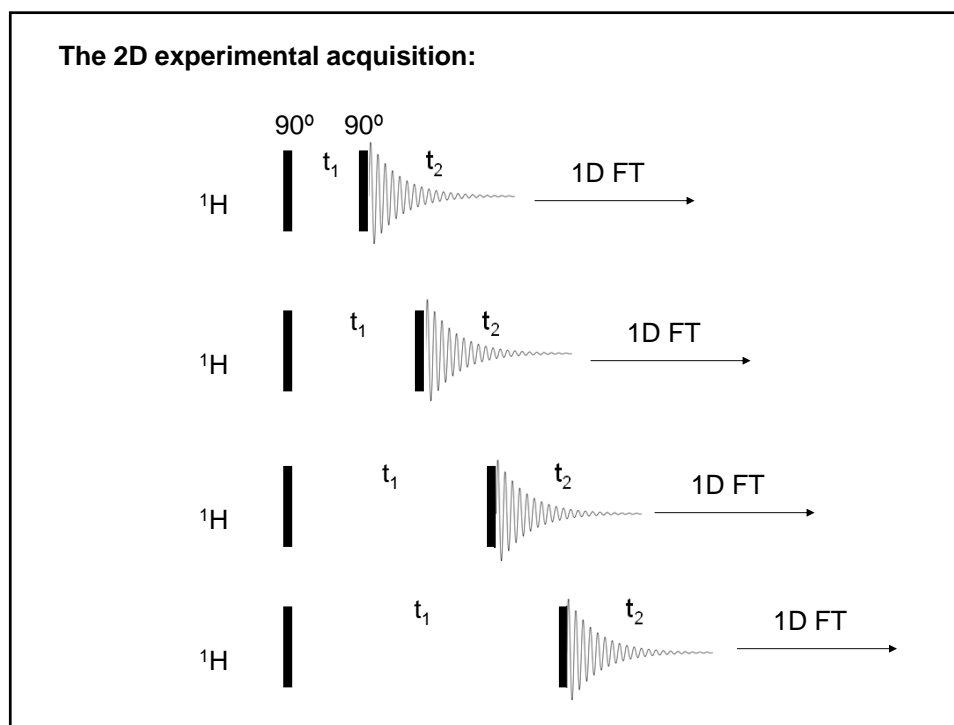
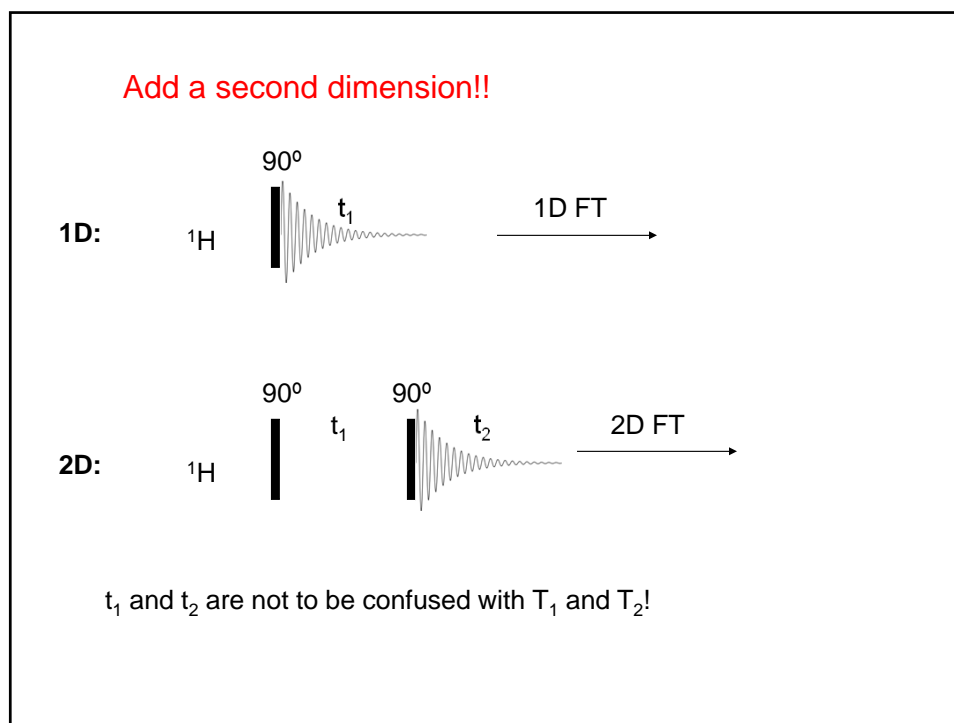
- Nuclei which are close to one another influence on each other's **effective magnetic field**.
- If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable.
- This is called **spin-spin coupling** or **J coupling**.

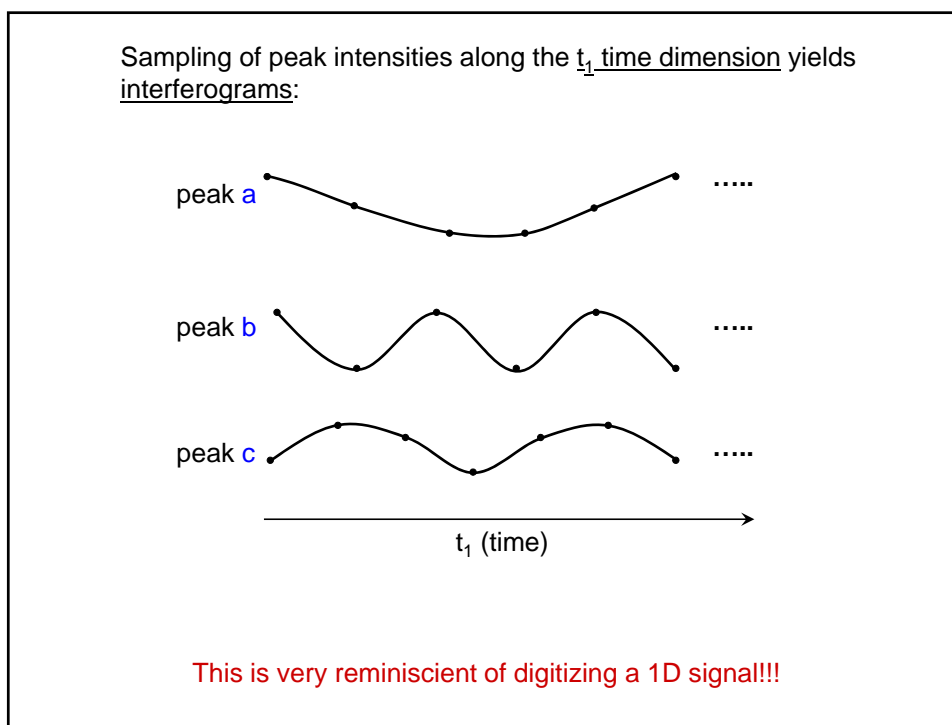
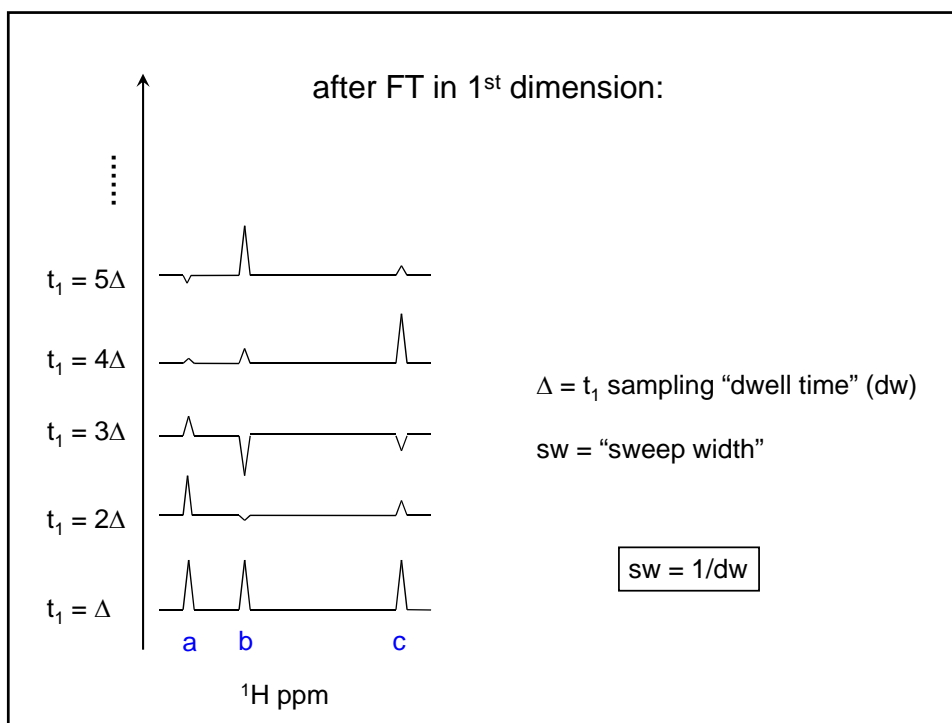




^1H NMR spectra of biofluids and cell extracts can be
incredibly complex !
950 MHz NMR spectrum of urine.

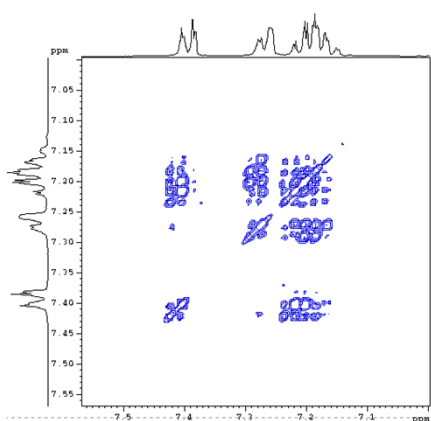
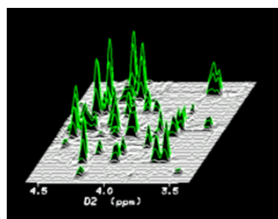






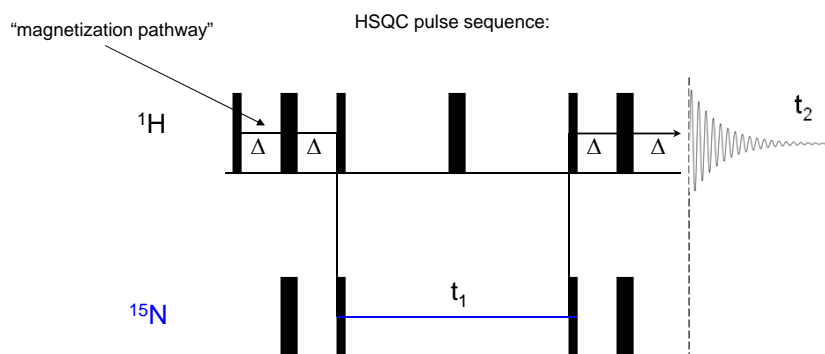
Upon Fourier transformation in both dimensions,
spectral peaks with 2-dimensional character are obtained.

2-dimensional ^1H NMR

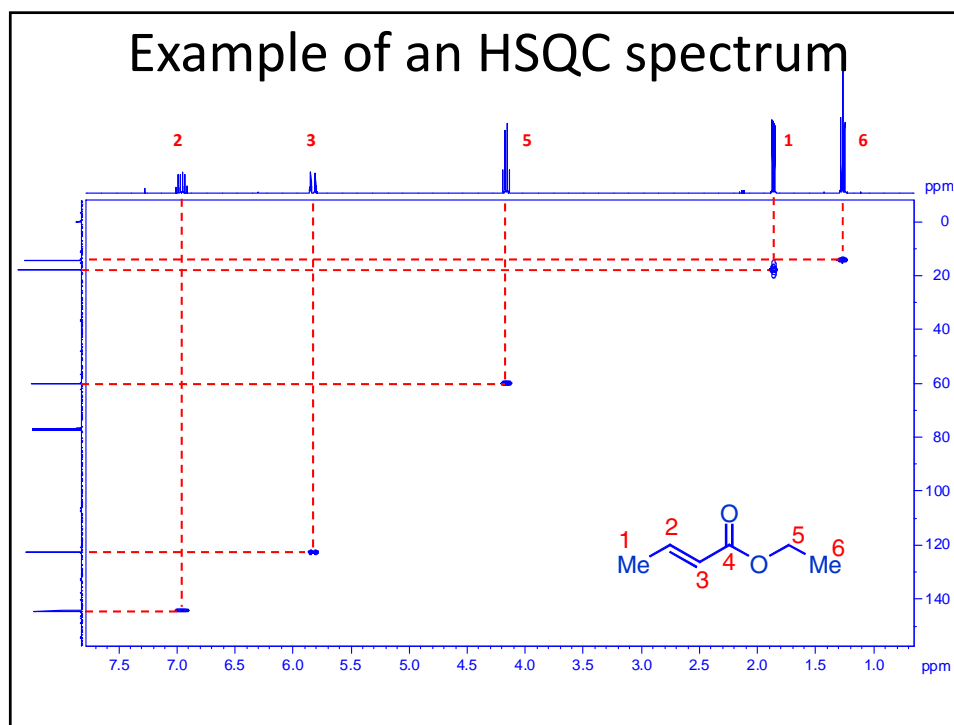
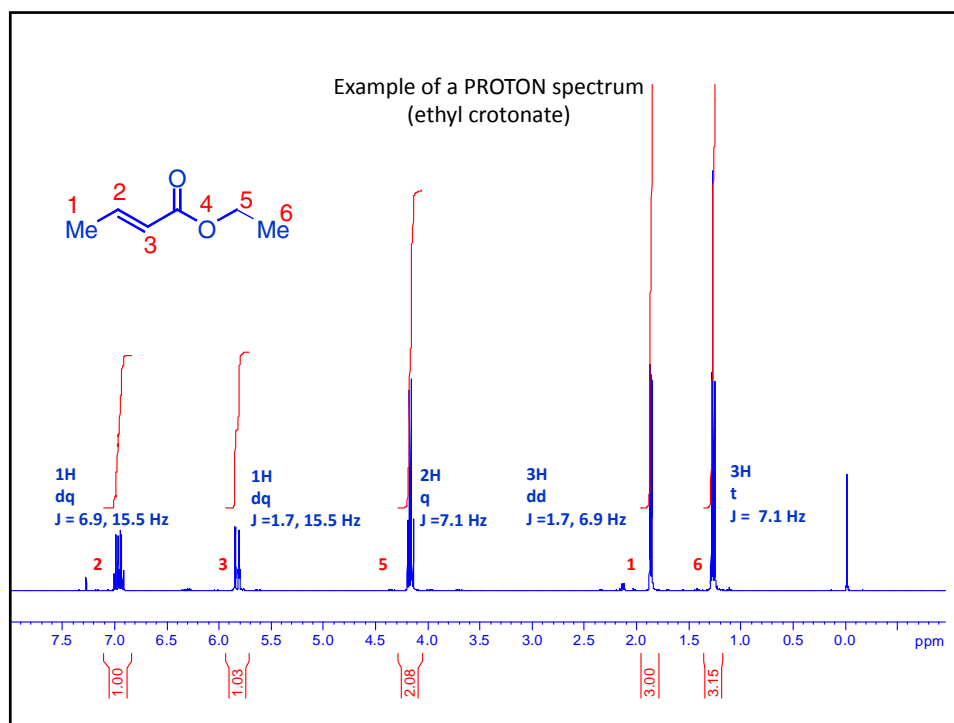


★ Major Advance #1 in NMR methodology: the 2nd dimension

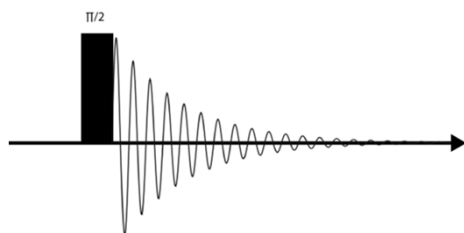
2D HSQC (Heteronuclear Single Quantum Coherence)



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



One more thing to think about...



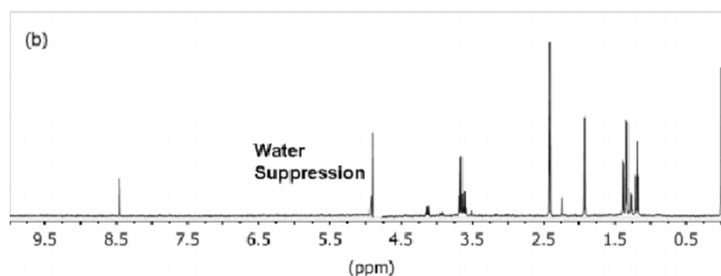
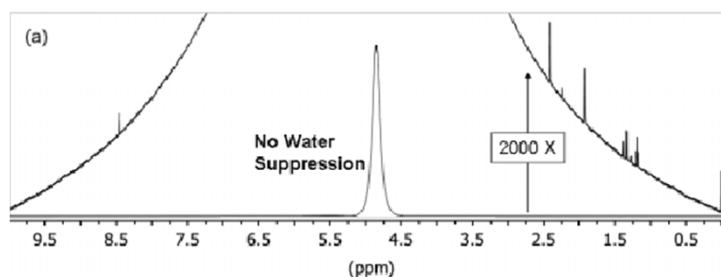
Target Molecule: $\approx \mu\text{m} - \text{mm}$

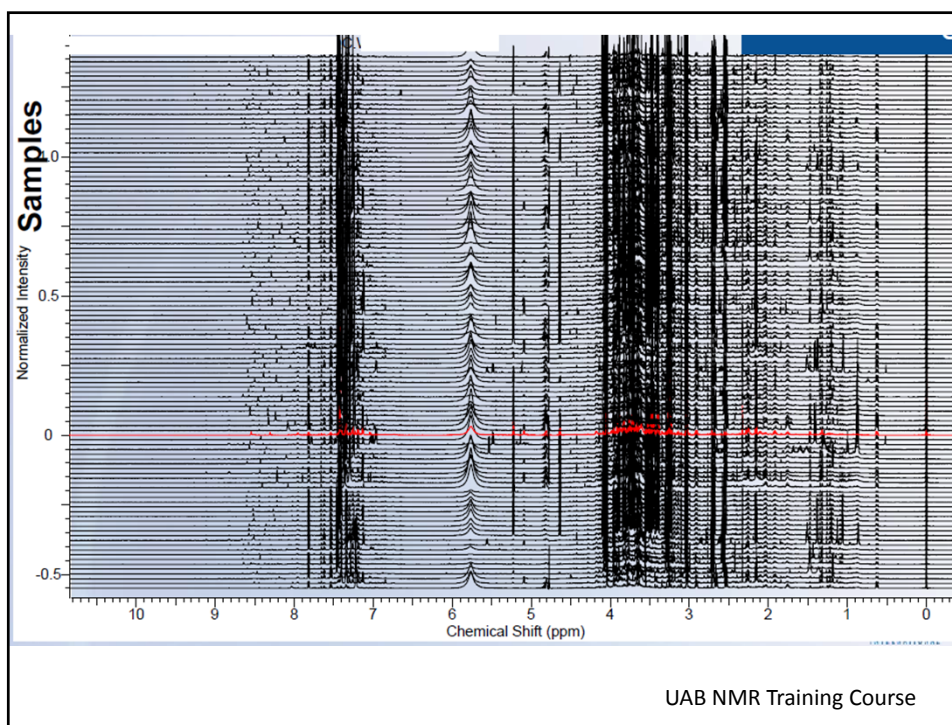
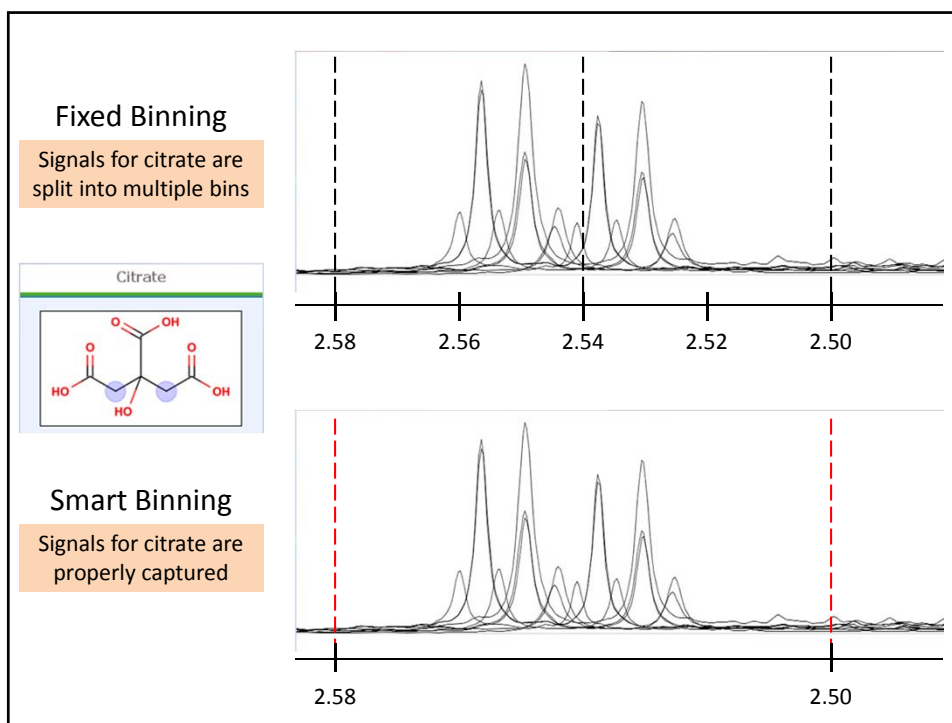
Solvent (H_2O): 55.5 M

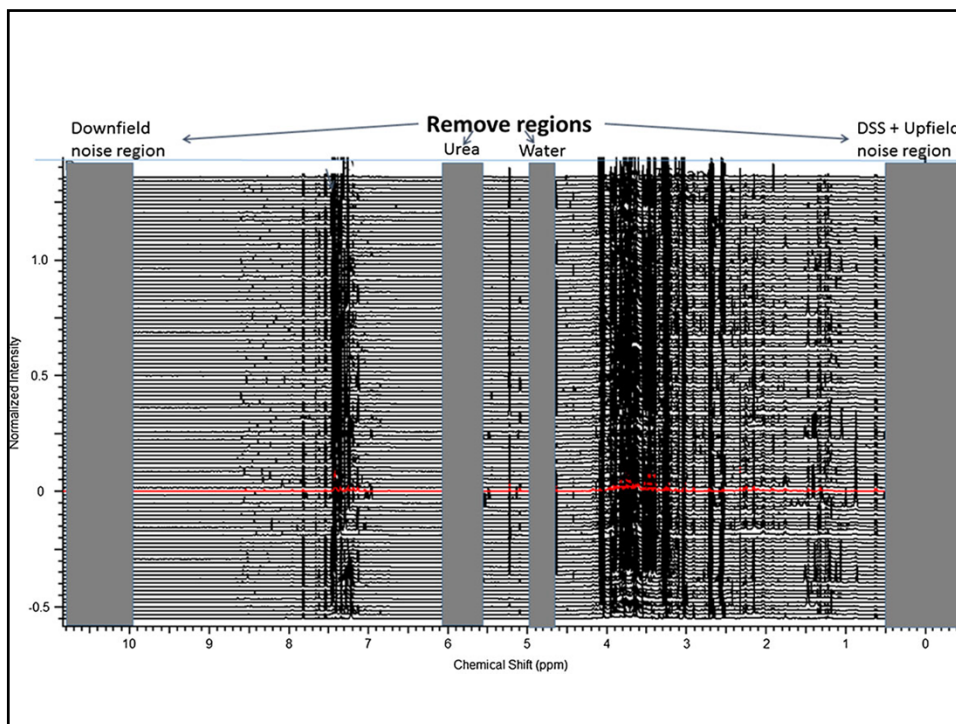
- The standard 1D-NMR pulse sequence will record NMR signals from **EVERY** molecule in the sample.
- Thus, in a typical biofluid (e.g., blood serum) sample, using this pulse sequence, you will see very broad signals from proteins, lipids, as well as very sharp signals from low molecular weight metabolites.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

It is a truly an Equal Opportunity Detector !!!

One more thing to think about...







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
E0309	Cases	4.11E-03	0.00E+00	2.23E-02	7.54E-03	3.06E-03	3.34E+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	1.62E-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	4.78E-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