Introduction to Nuclear Magnetic Resonance

William J. Placzek, Ph.D.
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 derivatiozation

Disadvantages:

Sensitivity (micromole to millimole range).

NMR spectra are complex (signals from different metabolites can overlap)

Innards of the Magnet

Correct position of the NMR tube in depth Gauge
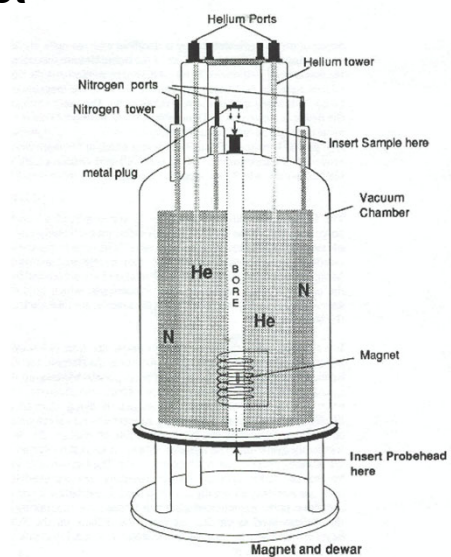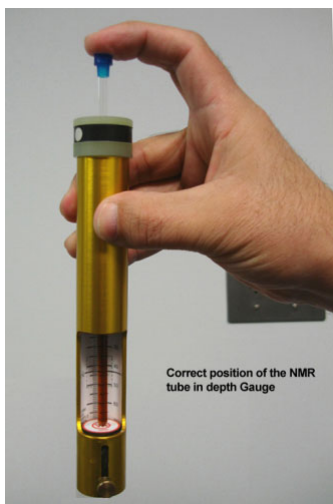
AV III HD 600

AV III HD 850

AV III HD 500

*Central Alabama High-Field NMR Facility UAB*

AV III HD 850

AV II 700

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
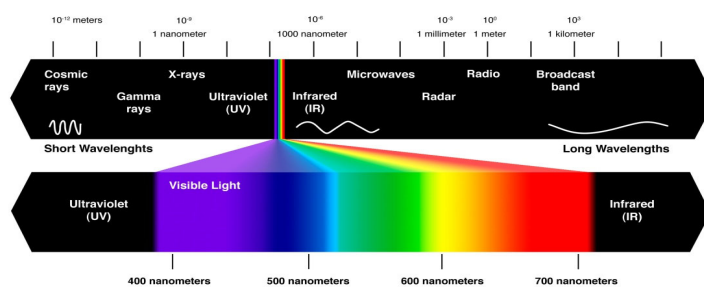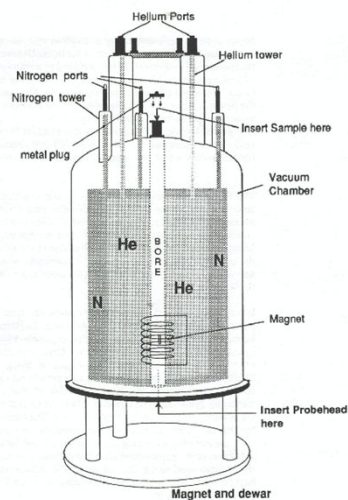

Classical Description of NMR

- 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
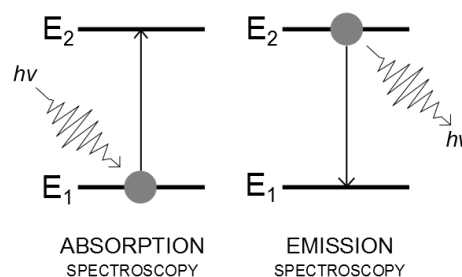
NMR differs from other types of spectroscopy in a number of ways…

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.
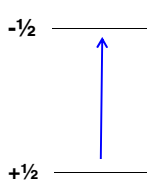
Classical Description of NMR

- 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

lifetime of the excited state

$$\tau \propto \frac{1}{\omega^3}$$

proportional to

frequency of transition

Excitation via RF Pulse

Probability of spontaneous emission

-½ ⎯⎯⎯⎯⎯

+½ ⎯⎯⎯⎯⎯

ΔE = hν

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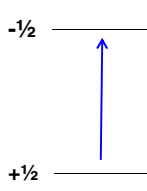
permeability of free space

gyromagnetic ratio

Plank's constant

angular frequency

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

speed of light

Probability of spontaneous emission is virtually nonexistent

Excitation via RF Pulse

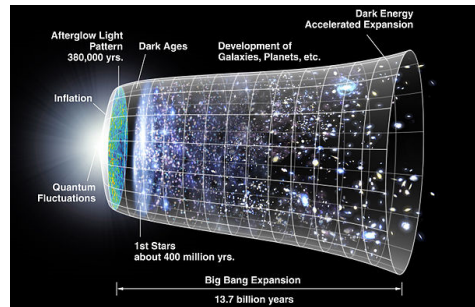Probability of spontaneous emission

-½ ⎯⎯⎯⎯⎯

+½ ⎯⎯⎯⎯⎯

ΔE = hν

$$P = \frac{\mu_0 \gamma^2 h \omega_0{}^3}{12\pi^2 c^3}$$
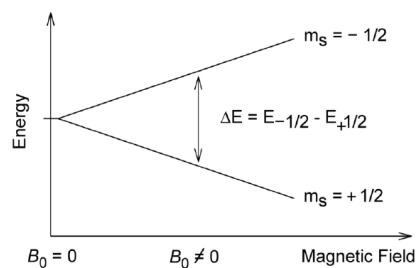
$$P \approx 10^{-21} s^{-1}$$

@ 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
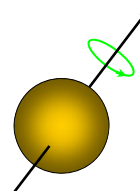

$m_S = -1/2$

Energy

$\Delta E = E_{-1/2} - E_{+1/2}$

$m_S = +1/2$

$B_0 = 0$ $B_0 \neq 0$ Magnetic Field

2 things are necessary

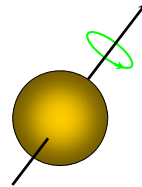1. nuclear magnetic dipole moment

2. 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?
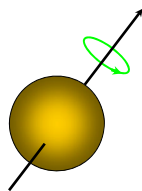
magnetic moment, μ, (black arrow)
perpendicular to angular spinning

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

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	^{2}H
Odd	Even	Odd	Half-Integer	^{13}C
	Odd	Even	Half-Integer	^{15}N

For biomolecules, most important nuclei have I = ½
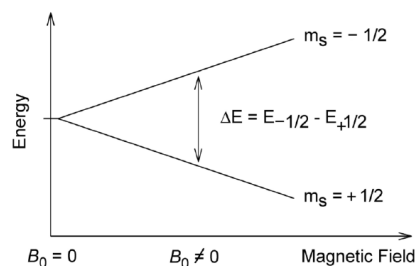
^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P

How does these states originate?
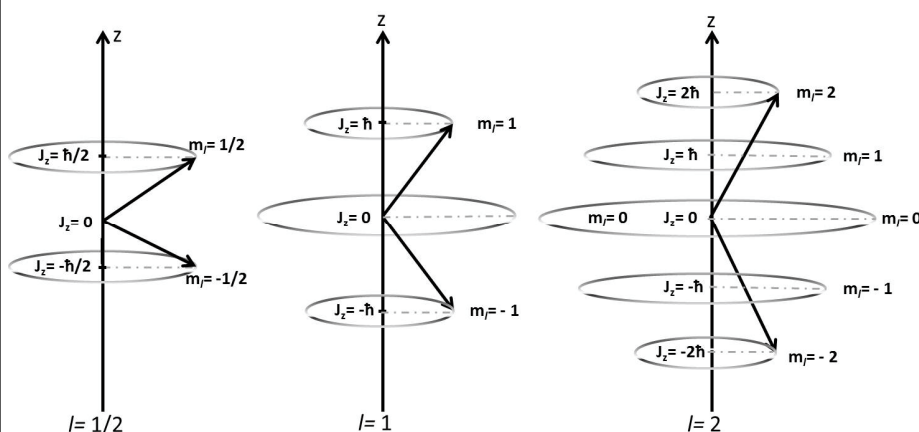
How do these states originate?

- 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
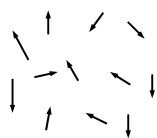
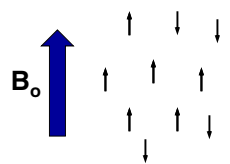1. nuclear magnetic dipole moment

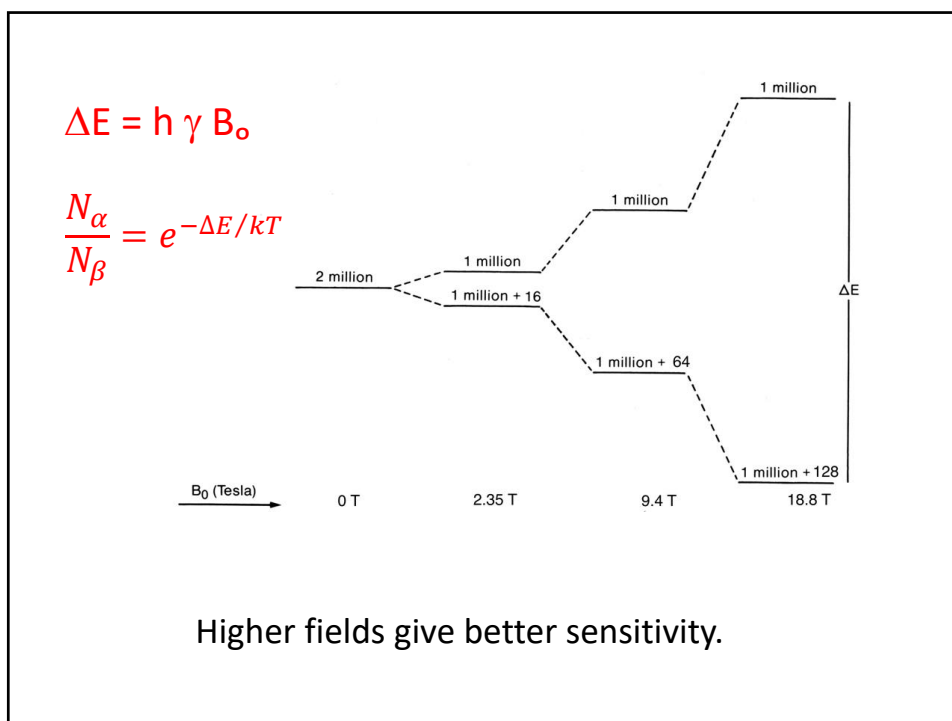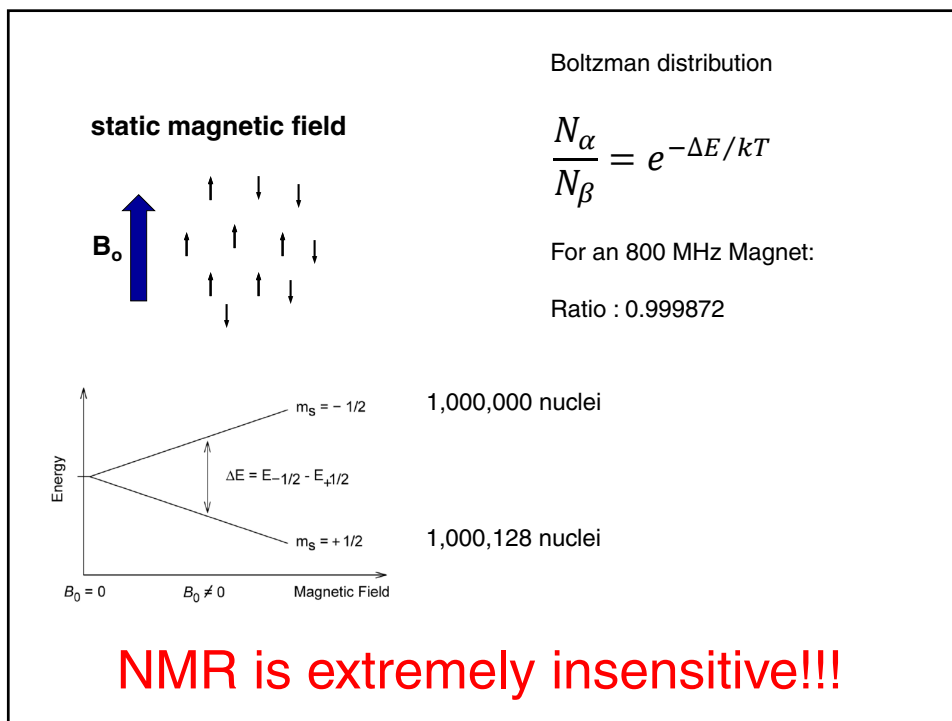2. intense external magnetic field

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**

static magnetic field

Boltzman distribution

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

For an 800 MHz Magnet:

Ratio : 0.999872

B₀

$m_s = -1/2$ 1,000,000 nuclei

$\Delta E = E_{-1/2} - E_{+1/2}$

Energy

$m_s = +1/2$ 1,000,128 nuclei

$B_0 = 0$ $B_0 \neq 0$ Magnetic Field

NMR is extremely insensitive!!!

$\Delta E = h\, \gamma\, B_o$

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

1 million

1 million

2 million   1 million

1 million + 16

ΔE

1 million + 64

B_0 (Tesla)

1 million + 128

0 T   2.35 T   9.4 T   18.8 T

Higher fields give better sensitivity.

Energy

$m_S = -1/2$

$\Delta E = E_{-1/2} - E_{+1/2}$

$m_S = +1/2$

$B_0 = 0$ $B_0 \neq 0$ Magnetic Field

$-\frac{1}{2}$

$+\frac{1}{2}$

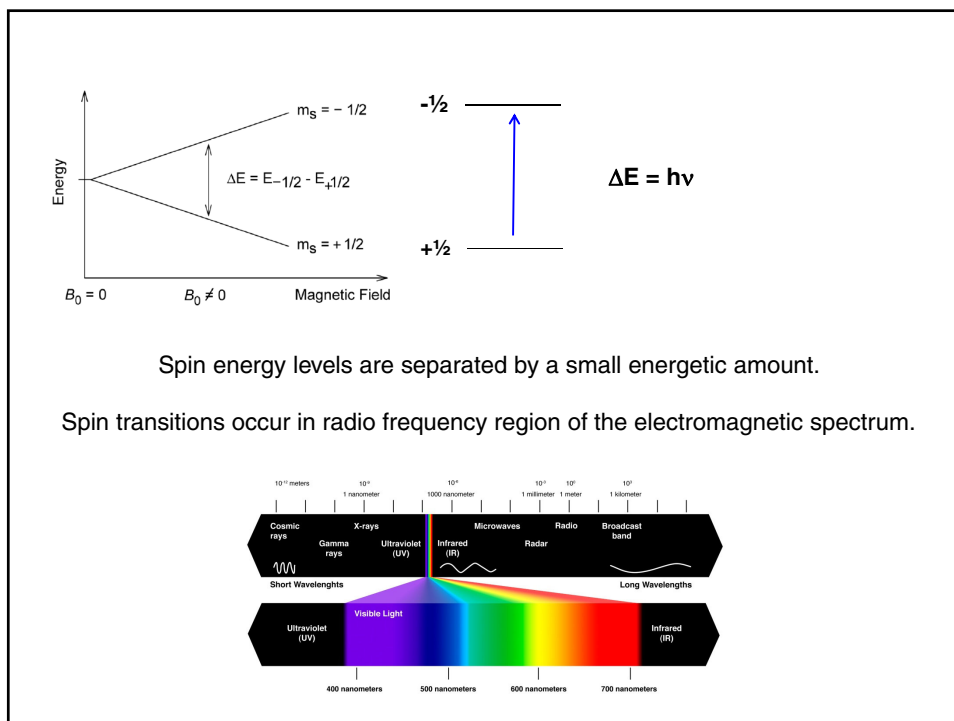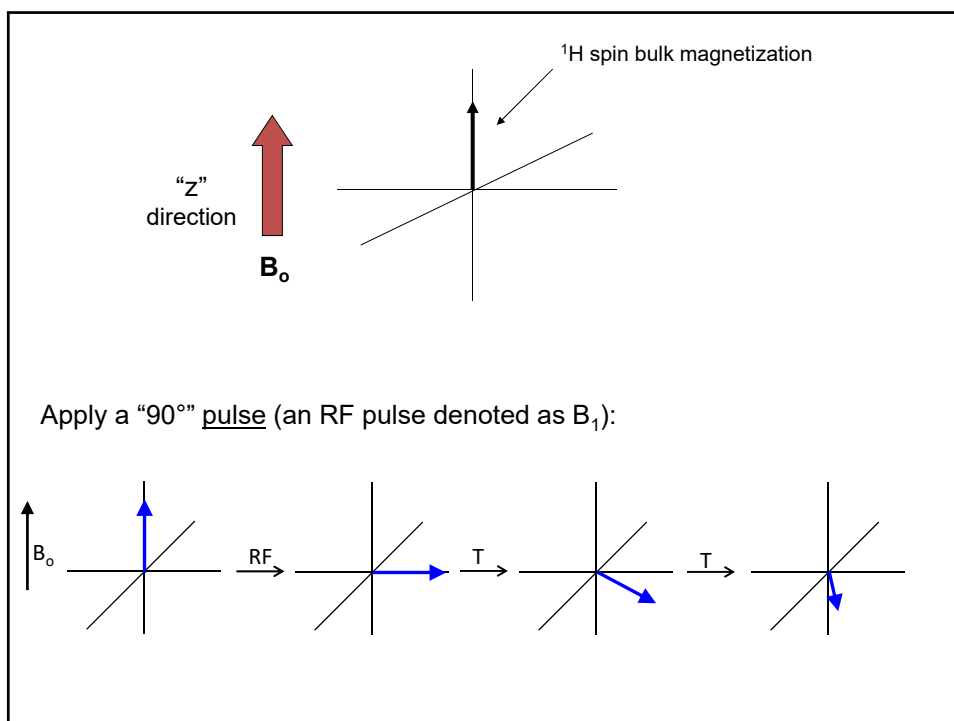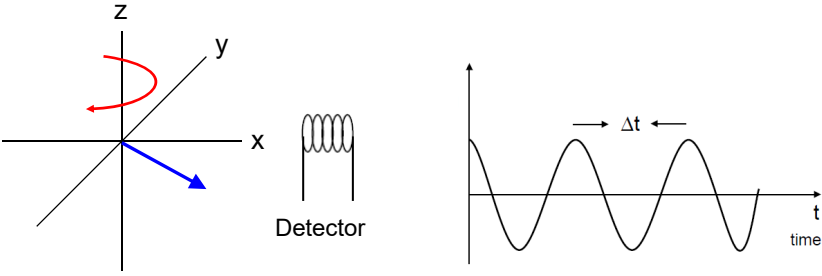$\Delta E = h\nu$

Spin energy levels are separated by a small energetic amount.

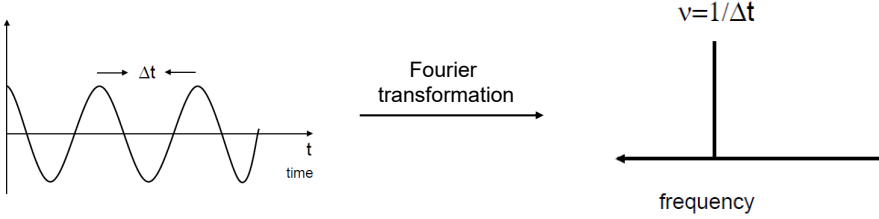Spin transitions occur in radio frequency region of the electromagnetic spectrum.

---

^{1}H spin bulk magnetization

"z" direction

B_o

Apply a "90°" pulse (an RF pulse denoted as B_1):

B_o $\xrightarrow{RF}$ $\xrightarrow{T}$ $\xrightarrow{T}$

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

$\omega = -B_o\gamma$

γ: gyromagnetic ratio

^{1}H at 11.7 Tesla is 500 MHz.

Fourier
transformation

$\nu = 1/\Delta t$

Time Domain

Frequency Domain

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

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

Free Induction Decay

Intensity

Time

Chemical Shift
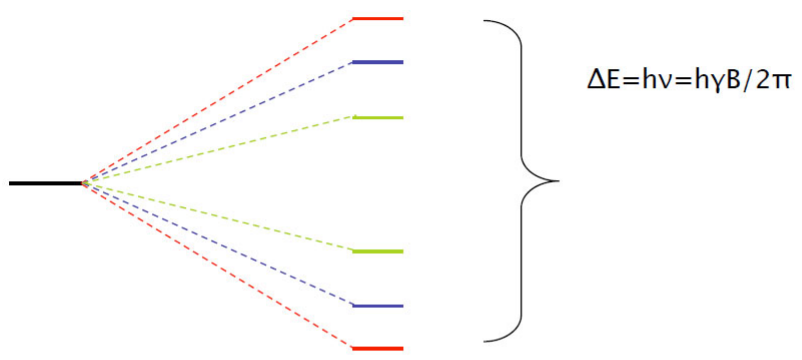
50   0
Hz
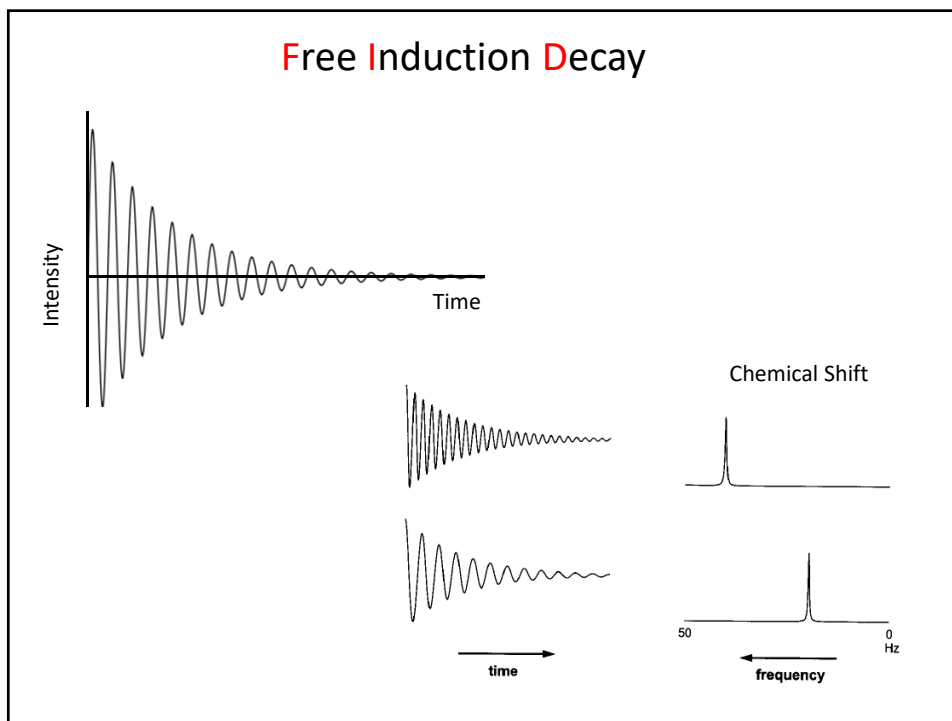
time   frequency

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 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
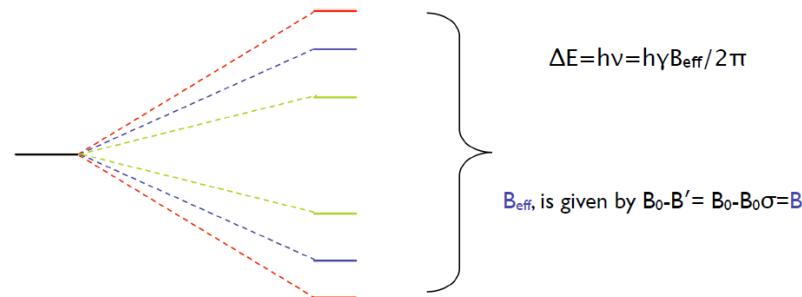
The Chemical Shift

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

Nuclei, however, in molecules are never isolated from other particles that are charg[ed]
and are in motion (electrons!).

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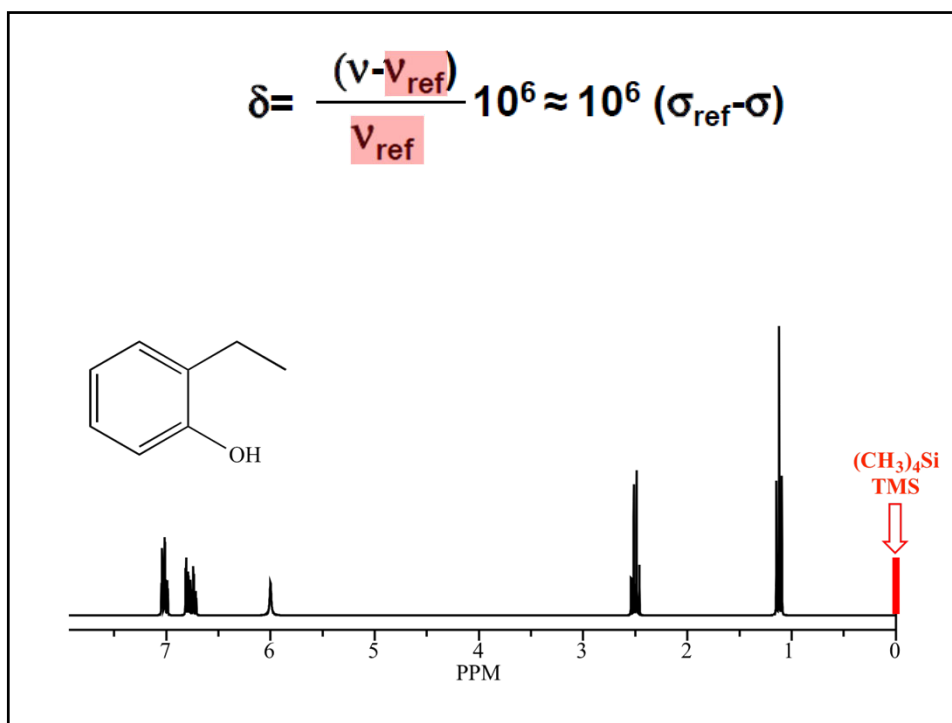external magnetic field!!

The Chemical Shift

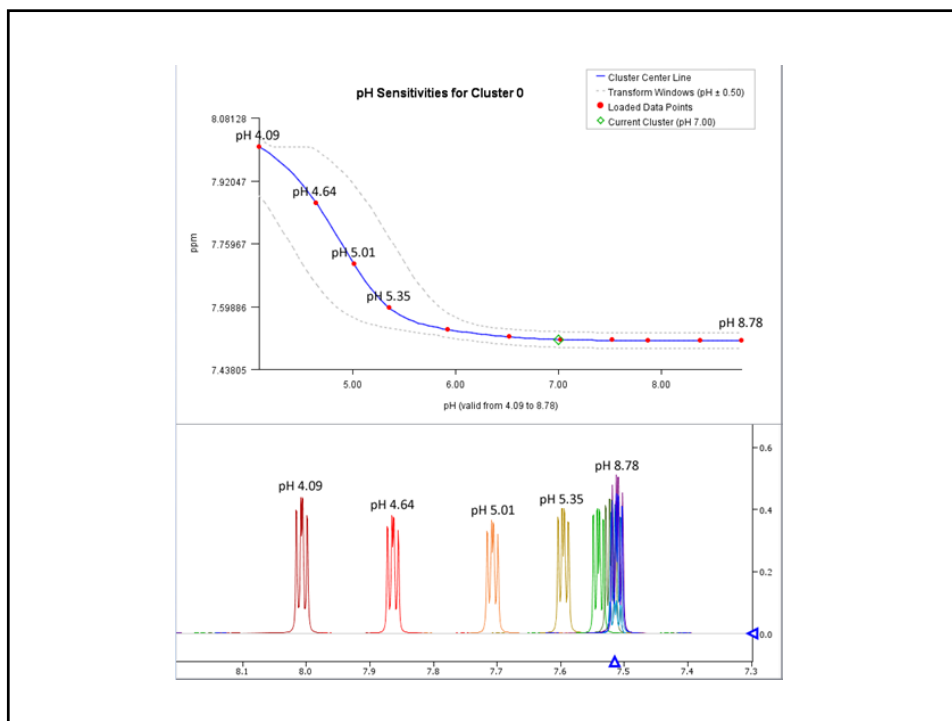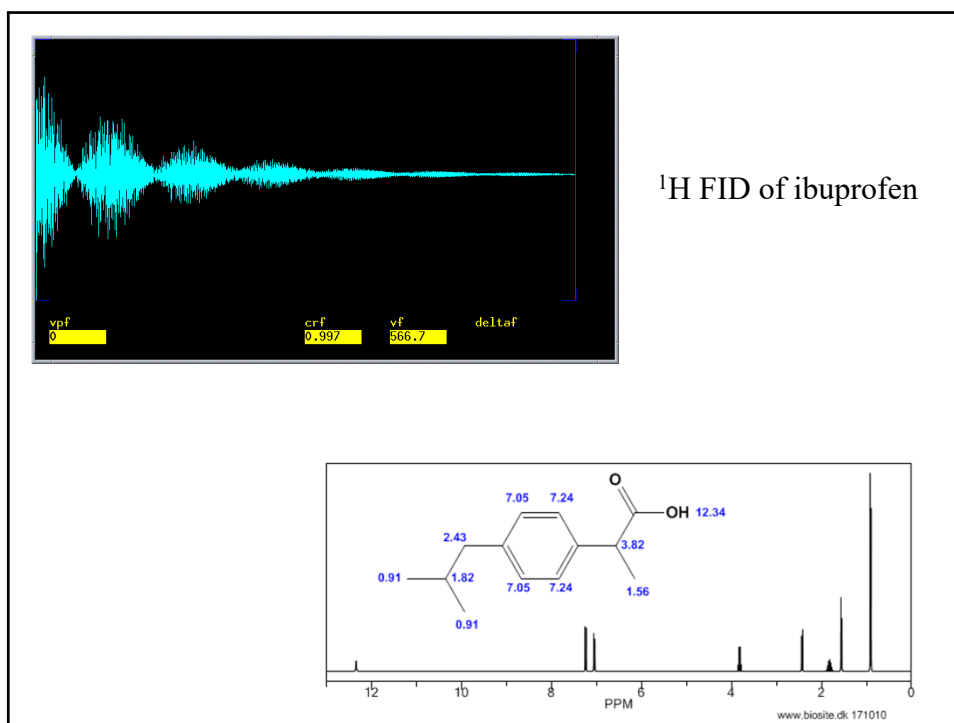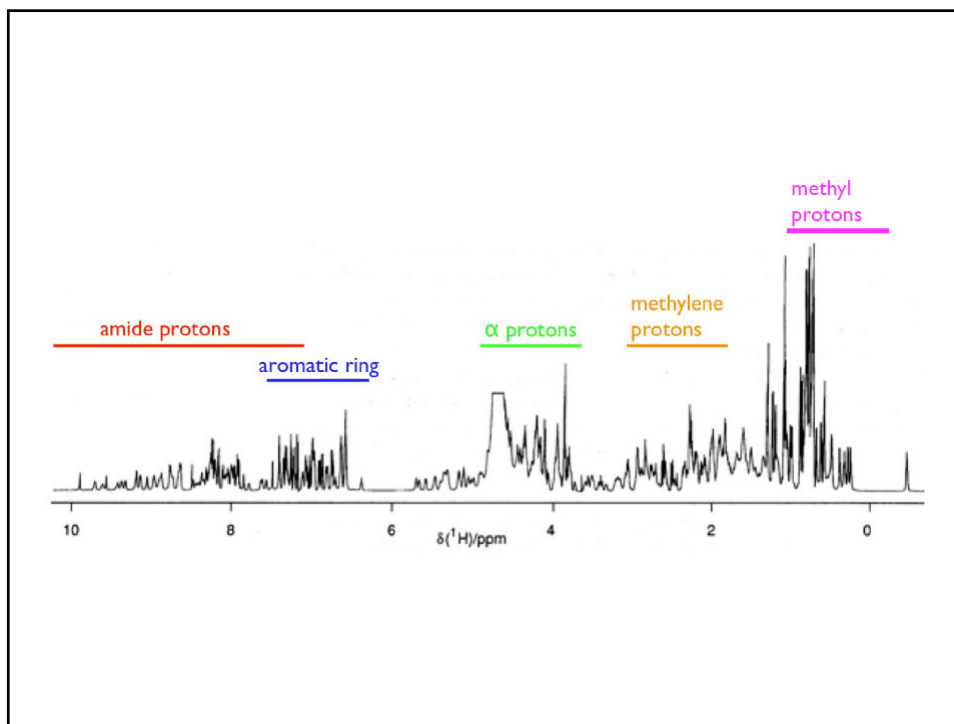$$\Delta E = h\nu = h\gamma B_{eff} / 2\pi$$

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

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

and δ is the chemical shift

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

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

¹H FID of ibuprofen

Ethanol ^{1}H NMR Spectrum
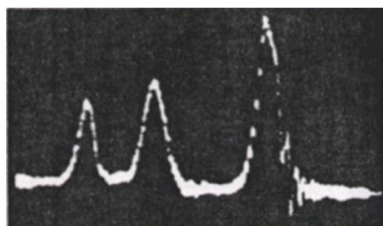
First ^{1}H NMR Spectrum of *ethanol* at **30 MHz** -1951 Stanford Univ
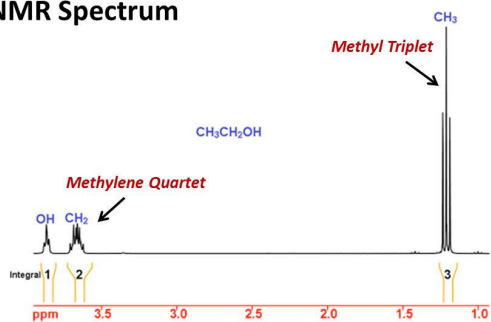
^{1}H NMR Spectrum of *ethanol* at 700 MHz

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.

one-bond

three-bond

NMR Spectrum

HO-CH₂-CH₃

low field — high field

ω₀

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

35
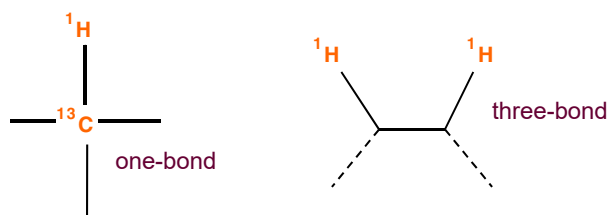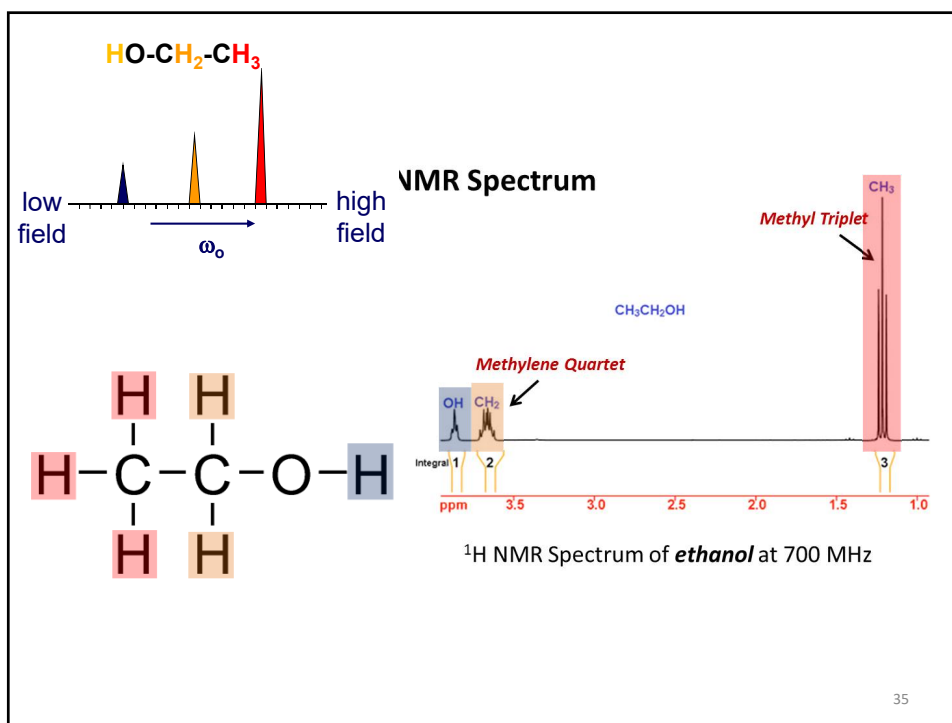
1H NMR spectra of biofluids and cell extracts can be incredibly complex !
950 MHz NMR spectrum of urine.

Add a second dimension!!

1D: ^{1}H — 90° — t_1 — 1D FT →

2D: ^{1}H — 90° — t_1 — 90° — t_2 — 2D FT →

t_1 and t_2 are not to be confused with T_1 and T_2!

The 2D experimental acquisition:

^{1}H — 90° — t_1 — 90° — t_2 — 1D FT →

^{1}H — t_1 — t_2 — 1D FT →

^{1}H — t_1 — t_2 — 1D FT →

^{1}H — t_1 — t_2 — 1D FT →

after FT in 1st dimension:

$t_1 = 5\Delta$

$t_1 = 4\Delta$

$t_1 = 3\Delta$

$t_1 = 2\Delta$

$t_1 = \Delta$

a b   c

^1H ppm

$\Delta = t_1$ sampling "dwell time" (dw)

sw = "sweep width"

$$sw = 1/dw$$

Sampling of peak intensities along the t_1 time dimension yields interferograms:

peak a

peak b

peak c

t_1 (time)

This is very reminiscent of digitizing a 1D signal!!!

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

2-dimensional ^{1}H NMR
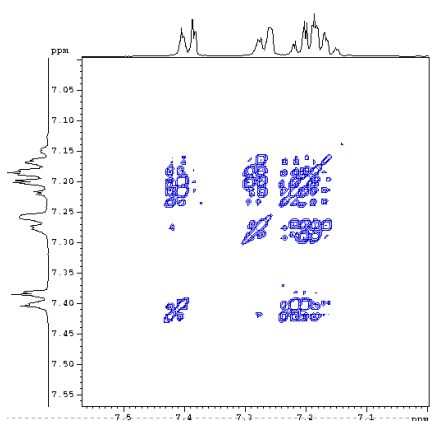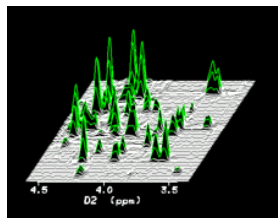
⭐ **Major Advance #1 in NMR methodology: the 2nd dimension**

2D HSQC (Heteronuclear Single Quantum Coherence)

"magnetization pathway"

HSQC pulse sequence:

^{1}H

^{15}N

t_1

t_2
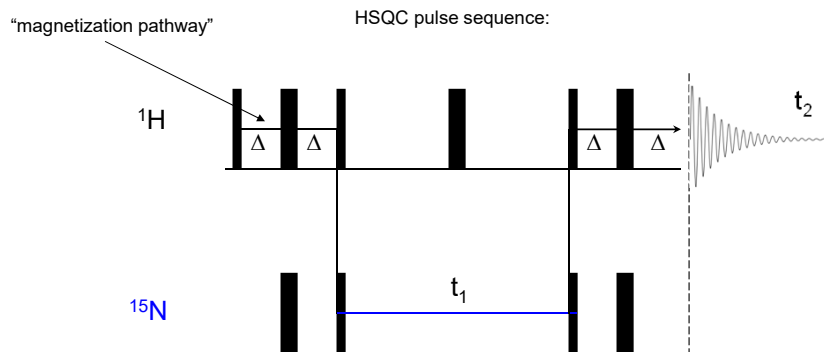
- "indirect" heteronuclear (^{15}N) detection without sensitivity reduction from the low γ.

Example of a PROTON spectrum
(ethyl crotonate)

Example of an HSQC spectrum

One more thing to think about…

π/2
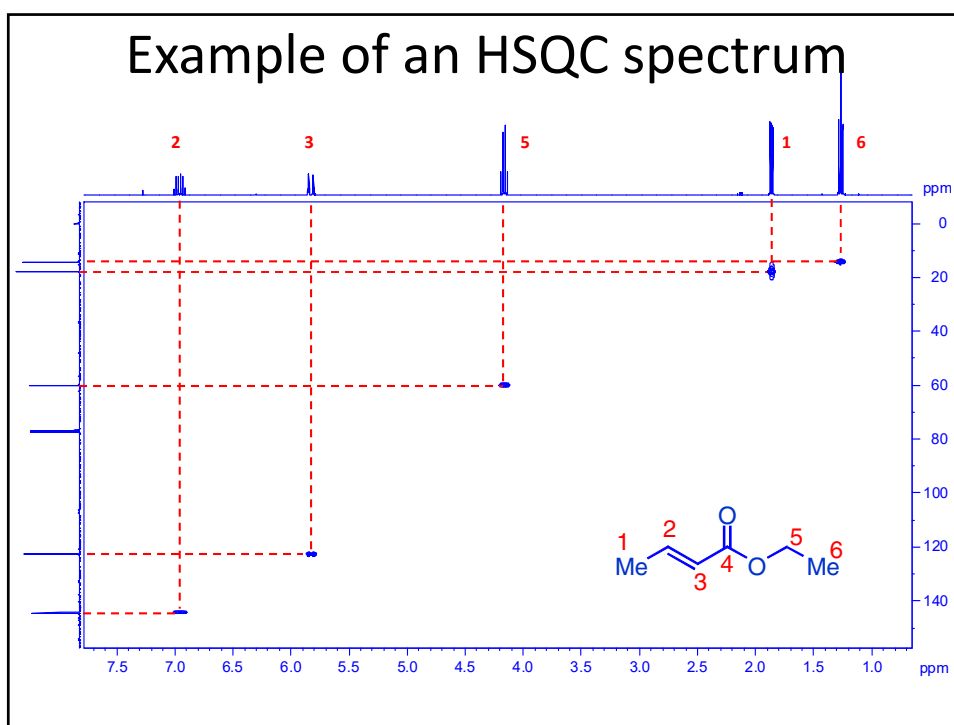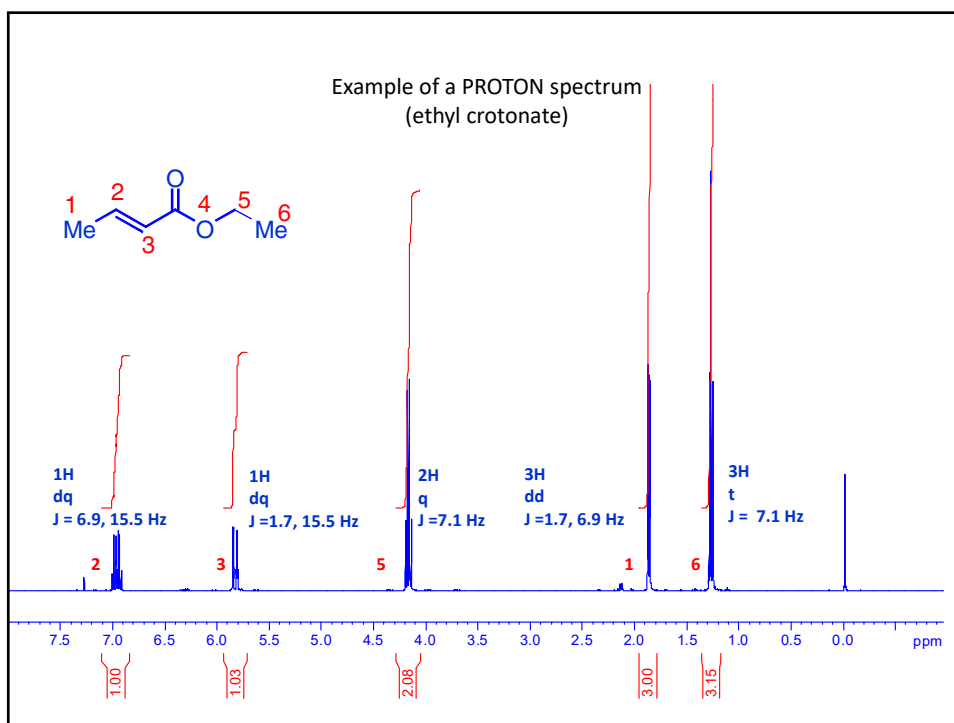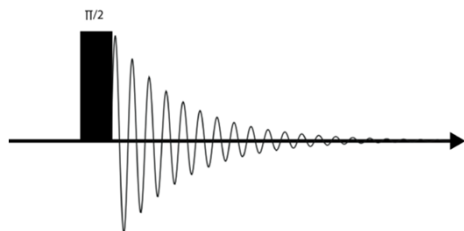
Target Molecule: ≈ μm – mm

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…

Fixed Binning

Signals for citrate are split into multiple bins

Citrate

Smart Binning

Signals for citrate are properly captured

UAB NMR Training Course

Remove regions

Downfield noise region   Urea   Water   DSS + Upfield noise region

Metadata   **Normalized binned data**
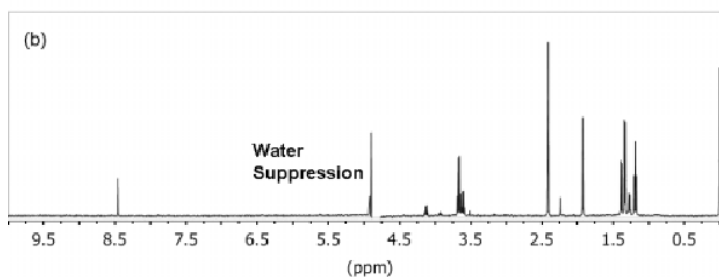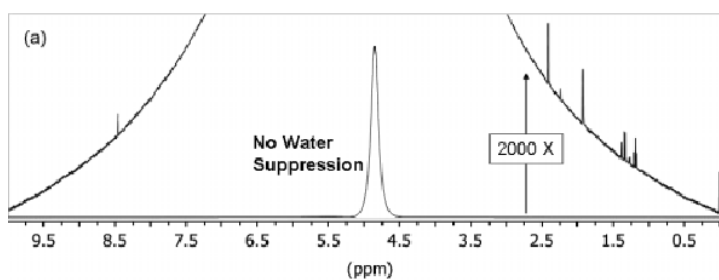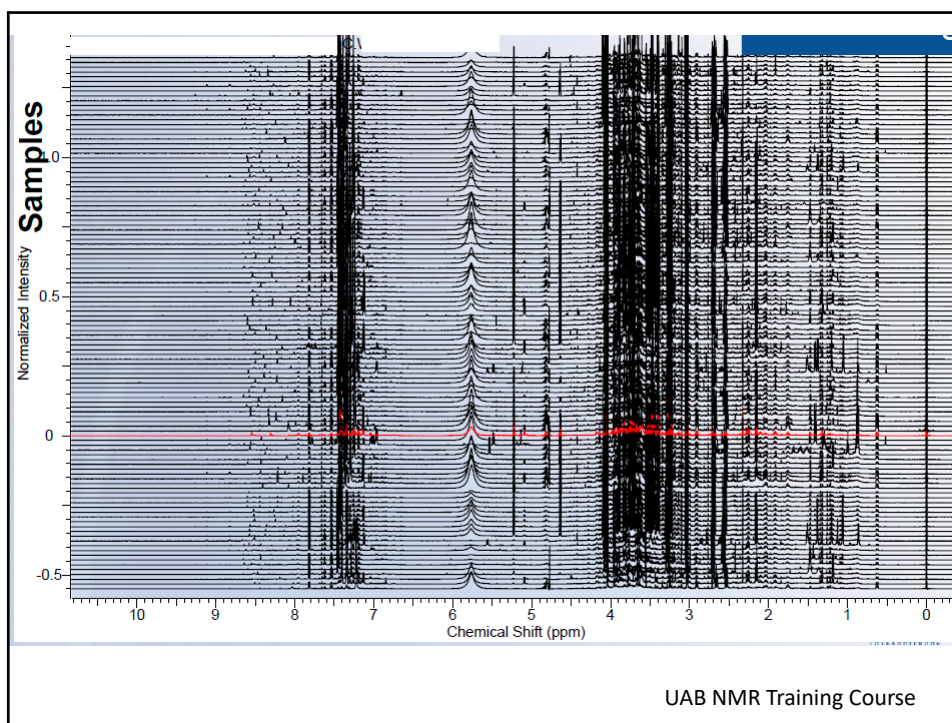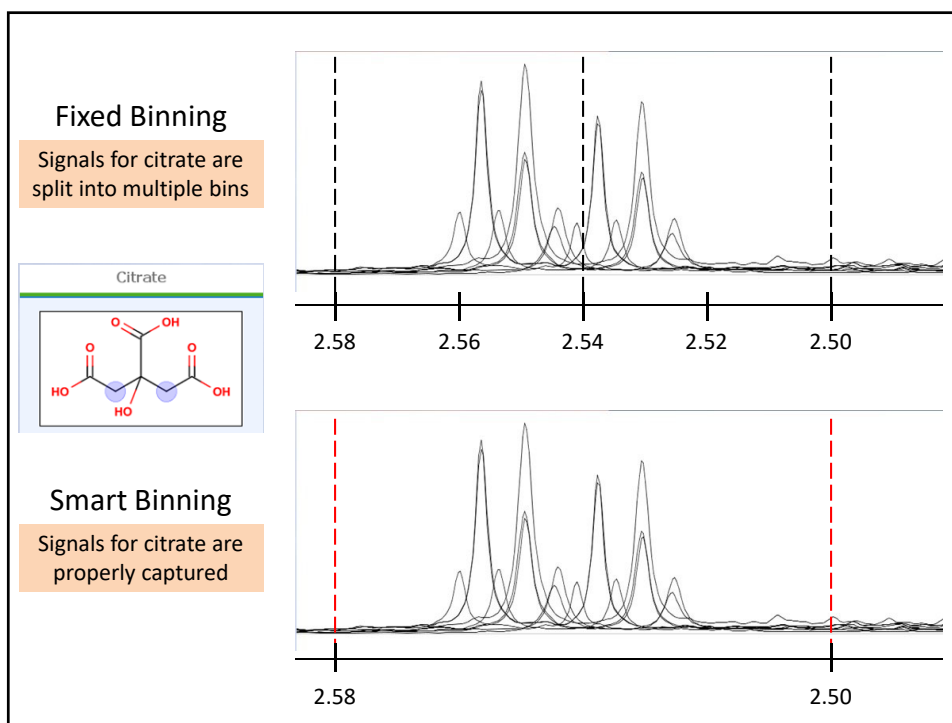
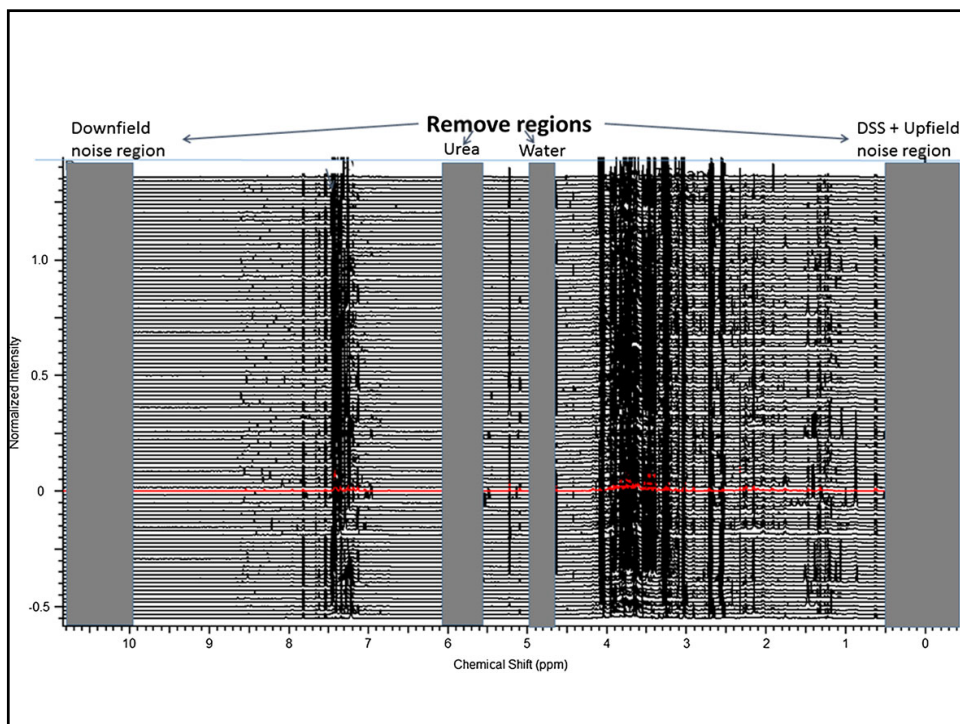| Sample ID	Disease Group	[0.40 .. 0.46]	[0.46 .. 0.52]	[0.52 .. 0.54]	[0.54 .. 0.57]	[0.57 .. 0.60]	[0.60 .. 0.66]	[0.66 .. 0.68]	[0.68 .. 0.71]	[0.71 .. 0.75]
C0559	Cases	7.60E-05	0.00E+00	7.32E-02	8.48E-02	3.20E-02	1.84E+00	1.31E-01	3.60E-01	3.67E-01
C0629	Cases	0.00E+00	1.78E-02	0.00E+00	2.18E-02	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
E0425	Cases	1.25E-03	0.00E+00					0.00E+00	1.08E-02	2.30E-02
E0909	Cases	4.11E-03	0.00E+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