Introduction to Nuclear Magnetic Resonance (NMR)

And

NMR - Metabolomics

Acknowledgment: Some slides from talks by Natalia Serkova, Wimal Pathmasiri, and from many internet sources (e.g., U of Oxford, Florida etc, journal articles)

We will briefly discuss (in qualitative terms):

What is NMR ?

Chemical Shift

J-Couplings

1D-NMR Pulse Sequences used in Metabolomics

2D-TOCSY Spectrum

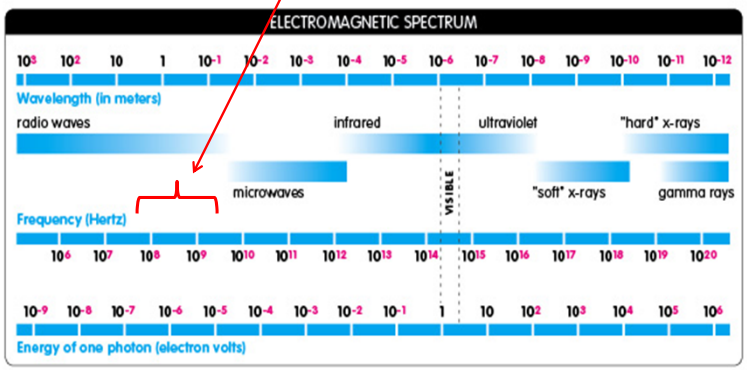
[1H-13C] HSQC spectrum.

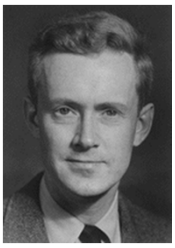
Sample preparation

Comparison of NMR and MS in Metabolomics

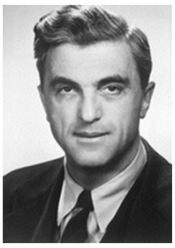
NMR Spectroscopy

Where is it?

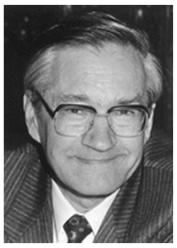




Edward M. Purcell
1912-1997

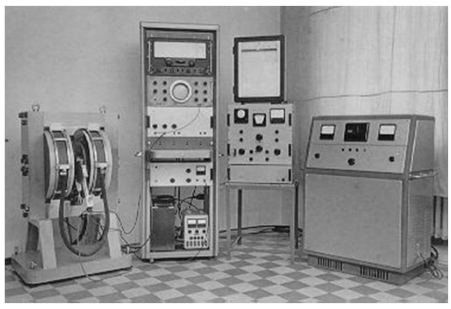


Felix Bloch
1905-1983



Richard R. Ernst
1933-

Kurt Wuthrich
1938-



The Nobel Laureates

CW NMR 40MHz

1960

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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

NMR Spectrometer

NMR Console

Computer

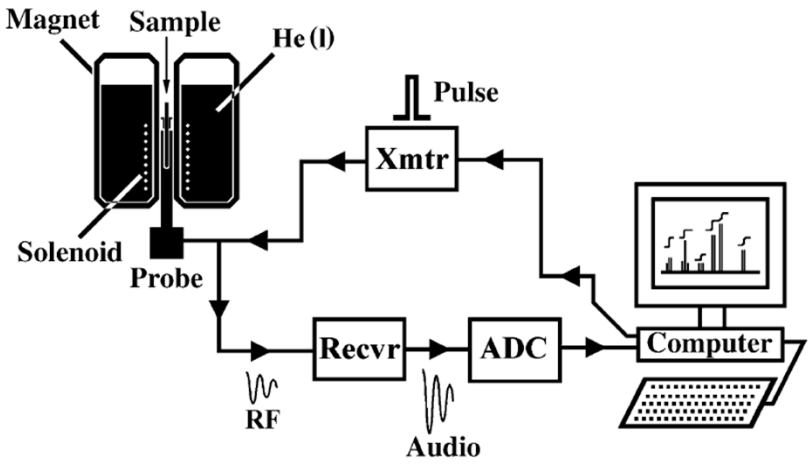
Magnet

Pre-amplifier

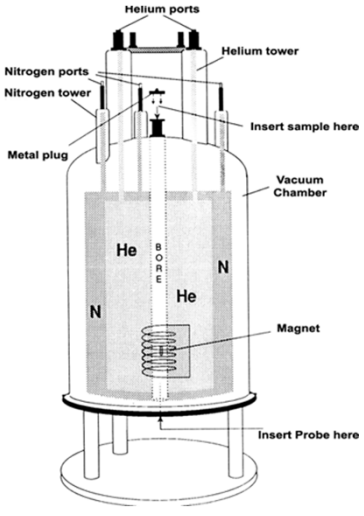
Probe and shim system

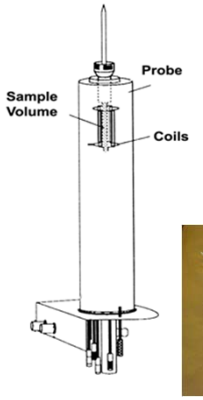
AVANCE Beginners User Guide 004 (Bruker, Germany)

NMR Experiment



NMR Magnet and the probe



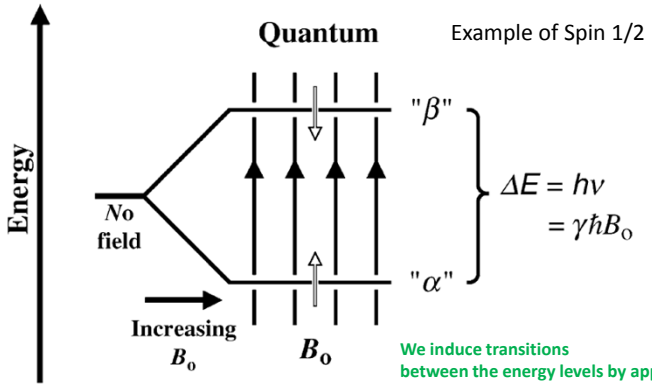


Sample is positioned on the probe using a spinner.

AVANCE Beginners User Guide 004 (Bruker, Germany)

NMR Spectroscopy

Many nuclei of atoms possess a "Nuclear Spin" that results in a "Nuclear Magnetic Moment" (similar to a bar magnet). In a Magnetic field, this nuclear spin experiences Zeeman interaction, and has well-defined quantum states.



Example of Spin 1/2

We induce transitions between the energy levels by applying RF pulses, and observe the Nuclear Magnetic Resonance signal.

Figure 1.2

AVANCE Beginners User Guide 004 (Bruker, Germany)

AT 71000 GAUSS (7.1 TELSLA)

(1T = 10,000G)

W_0(MHz)	0	30	75	121	280	300	320
Nucleus		^{15}N	^{13}C	^{31}P	^{19}F	^{1}H	^{3}H

Table 1.1 Nuclei of Major Interest to NMR Spectroscopists

Isotope	Abundance (%)	Z	Spin	μ^a	$\gamma \times 10^{-8b}$	Relative[c] sensitivity	ν_0 at 1T(MHz)	At 7.04T
^{1}H	99.9844	1	1/2	2.7927	2.6752	1.000	42.577	300
^{2}H	0.0156	1	1	0.8574	0.4107	0.00964	6.536	46
^{10}B	18.83	5	3	1.8006	0.2875	0.0199	4.575	
^{11}B	81.17	5	3/2	2.6880	0.8583	0.165	13.660	
^{13}C	1.108	6	1/2	0.7022	0.6726	0.0159	10.705	75.4
^{14}N	99.635	7	1	0.4036	0.1933	0.00101	3.076	
^{15}N	0.365	7	1/2	-0.2830	-0.2711	0.00104	4.315	30.4
^{19}F	100	9	1/2	2.6273	2.5167	0.834	40.055	282.3
^{29}Si	4.70	14	1/2	-0.5548	-0.5316	0.0785	8.460	
^{31}P	100	15	1/2	1.1305	1.0829	0.0664	17.235	121.4

a Magnetic moment in units of the nuclear magneton, $eh/(\Delta\mu M_p c)$
b Magnetogyric ratio in SI units
c For equal numbers of nuclei at constant field

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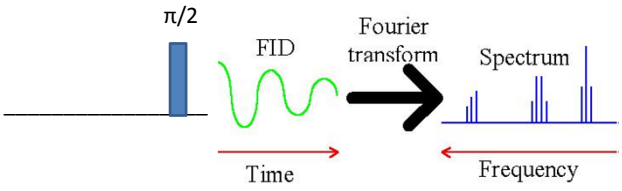
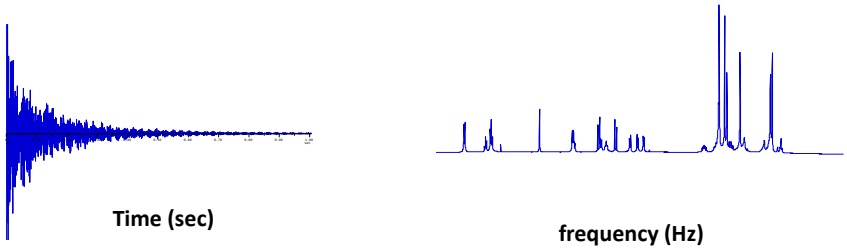
5. NMR Data Processing

- A π/2 rf pulse is applied to cause transitions. The resulting signal (called FID (free induction decay)) is then Fourier transformed to frequency domain to obtain the NMR spectrum for each different nuclei.





Time (sec) **frequency (Hz)**

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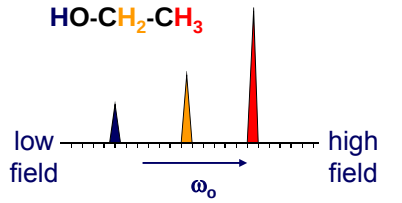

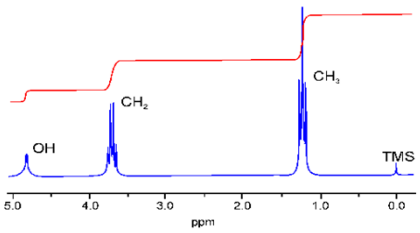

Notice that the intensity of peak is proportional to the number of H.

Note: We will discuss the fine structure in each peak in a later slide.

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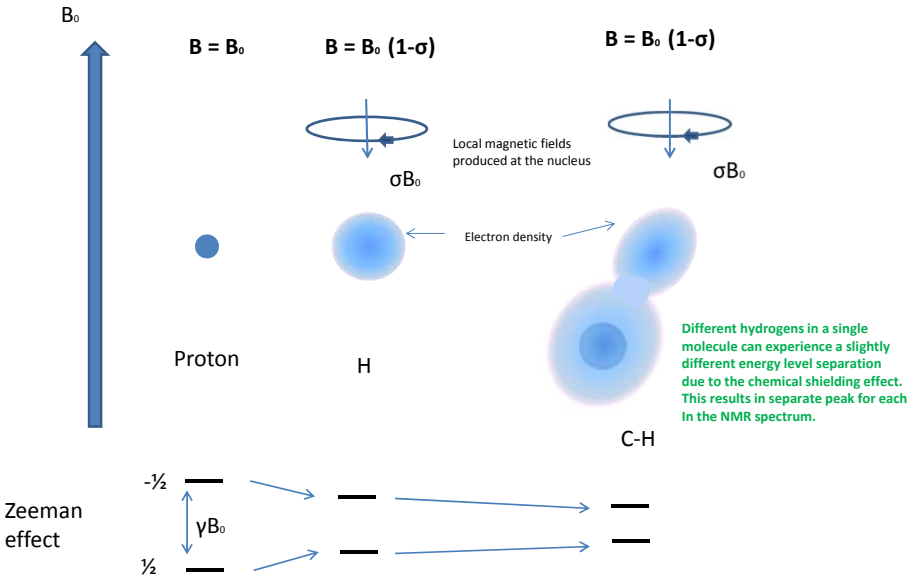
Chemical Shift

The electrons around nuclei experience local currents due to Lenz's law, and produce local magnetic fields opposite in direction to the external B_0 field.



NMR Parameters

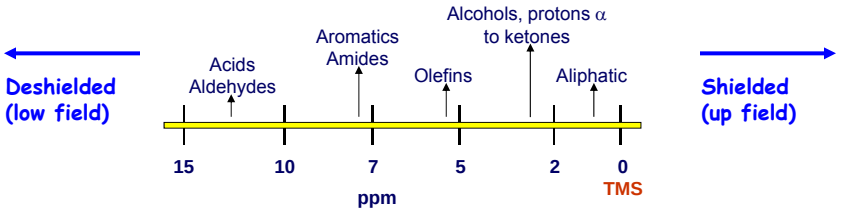
◆ Chemical Shift

- **The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol delta,**

$$\delta = (\nu - \nu_{REF}) \times 10^6 / \nu_{REF}$$

- **In NMR spectroscopy, this standard is often tetramethylsilane, $Si(CH_3)_4$, abbreviated TMS, or 2,2-dimethyl-2-silapentane-5-sulfonate, DSS (or TSP), in biomolecular NMR.**

- **The good thing is that since it is a relative scale, the δ for a sample in a 100 MHz magnet (2.35 T) is the same as that obtained in a 600 MHz magnet (14.1 T).**



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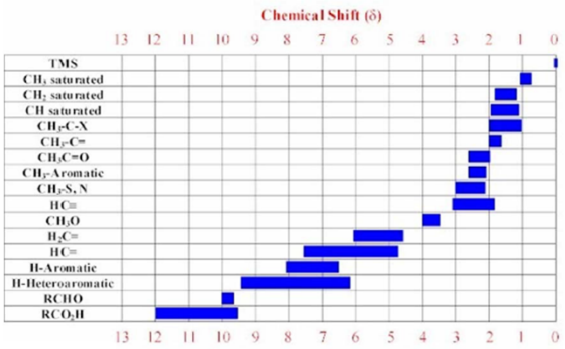
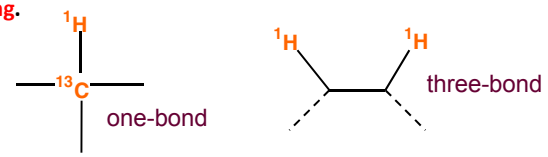
Characteristic Chemical Shifts

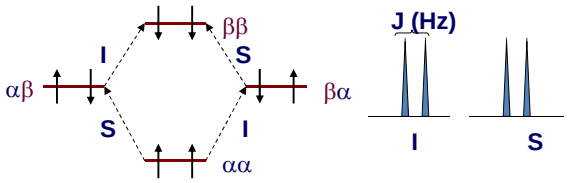


◆ J-coupling

•Nuclei which are close to one another could cause an 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.



•Each spin now seems to has two energy 'sub-levels' depending on the state of the spin it is coupled to:



The magnitude of the separation is called *coupling constant* (J) and has units of Hz.

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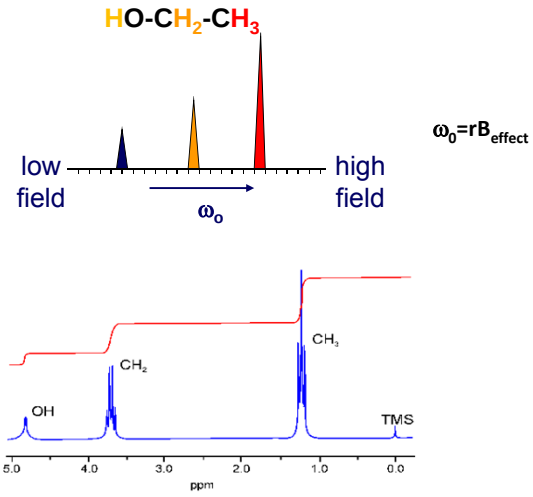

Note: the fine structure in the CH3 (triplet) and CH2 (quartet) signals is caused by the so-called J-coupling between these two sets of hydrogens. The OH Hydrogen does not show structure due to rapid exchange.

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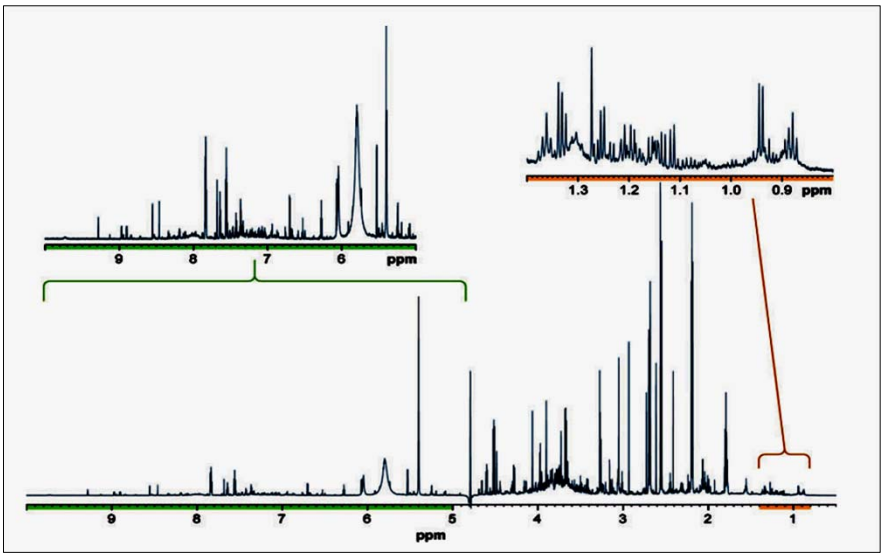
1H NMR spectra of biofluids and cell extracts can be incredibly complex !
950 MHz NMR spectrum of urine.



Because the NMR spectra of biofluids and cell extracts can literally contain thousands of overlapping signals from several hundreds of metabolites with highly differing concentrations, one typically resorts to bioinformatic approaches :

(i) For targeted metabolomics, identify key metabolite signals using chemical shift data bases for a large library of known metabolites* (e.g., CHENOMX, Bruker-Biospin's, BMRB etc)

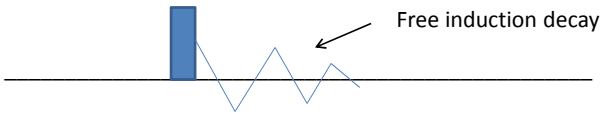
(ii) Define "bins" of spectra, measure areas, and undertake Multivariate Analyses (such as PCA, PLS-DA etc).

*** NOTE: The chemical shifts are sensitive to pH, temperature, salt and buffer conditions etc. Thus, chemical shift data bases strive to contain data collected at some standard conditions. It is absolutely critical to perform NMR Metabolomics under standard conditions identical to that in the data base being used, to successfully identify the NMR metabolites.**

Some common NMR techniques used in Metabolomics

Remember ! The standard 1D-NMR pulse sequence (a simple 90 degree pulse) will record NMR signals from **EVERY** molecule in the sample**.**

It is a truly an Equal Opportunity Detector !!!



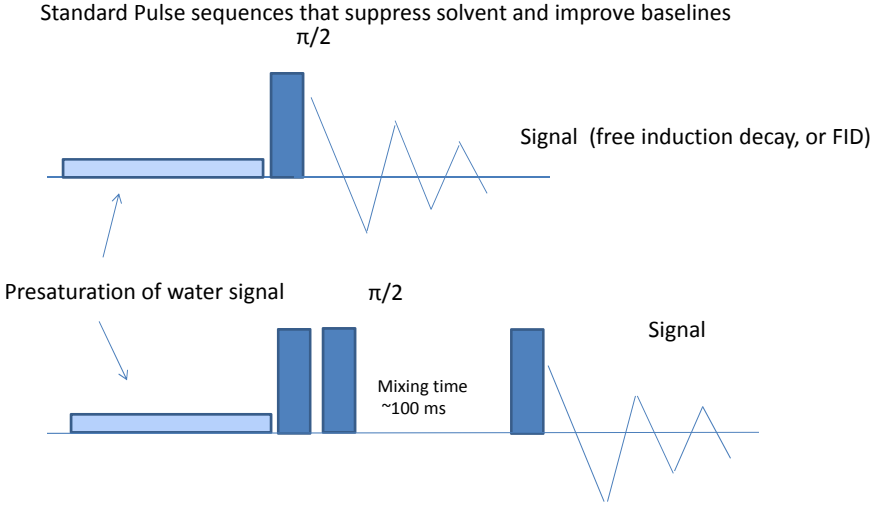
Thus, in a typical biofluid (e.g., blood serum) sample, using this pulse sequence, you will see very broad signals from proteins, lipids, as well as very sharp signals from low molecular weight metabolites. In addition, solvent peaks (H_2O) can contribute significant peaks.

Thus, one can "**edit out**" specific signals by a clever use of special NMR sequences:

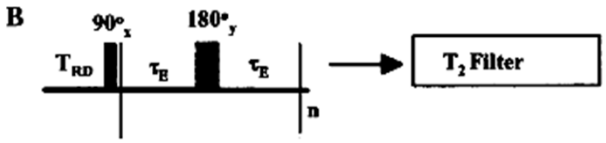
e.g., T2-filter sequence to edit out broad protein and lipid signals while retaining the sharp metabolite signals.

1D-NOESY sequence to suppress the water signal and get a relatively flatter baseline.

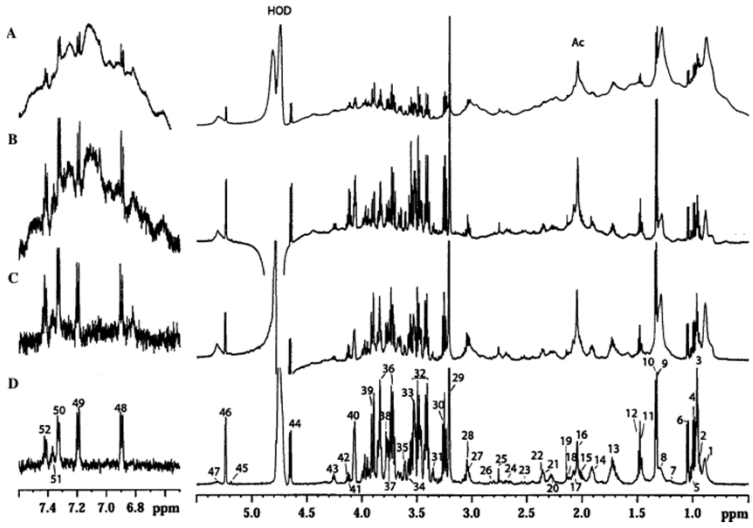
Standard Pulse sequences that suppress solvent and improve baselines



1D-NOESY Pulse sequence commonly used in NMR Metabolomics.
Improves the suppression of water, and gives flatter baselines.
Good for quantifying the metabolite concentrations.



T2-edited 1D-NMR sequence to remove broad signals from proteins and lipids
And retain signals from only low-molecular weight metabolites

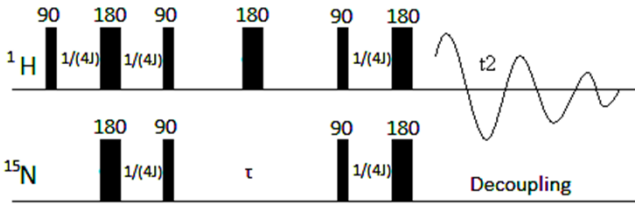


1D-1H NMR spectra of a human blood plasma sample, (A) NOESYPR1D with water presaturation; (B) T1-edited NMR spectrum plotted inverted (with 180° phase shift); τ1=0.265 s; (C) T1ρ-edited NMR spectrum; spin-locking time, 120 ms; spin-locking power, 5 kHz; (D) T2-edited NMR spectroscopy.

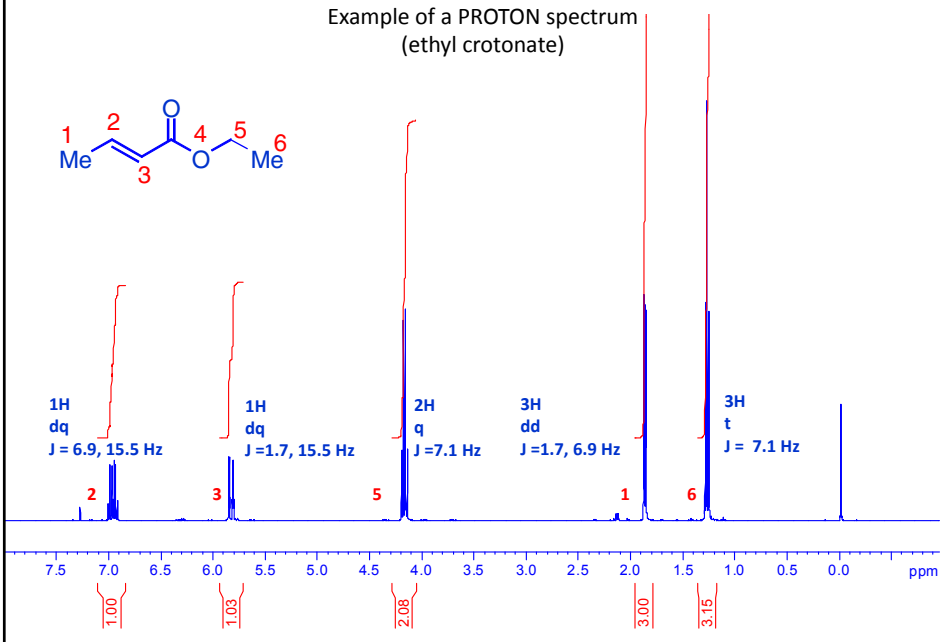
Tang et al., Anal Biochem., 325:260-272 (2004)

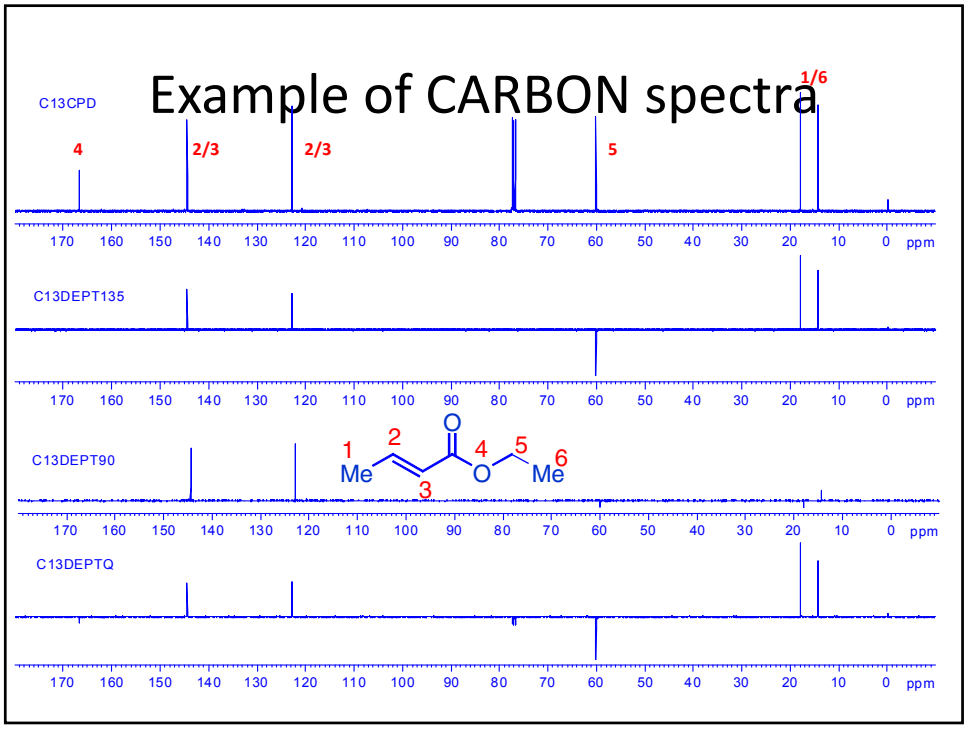
Introduction to HSQC

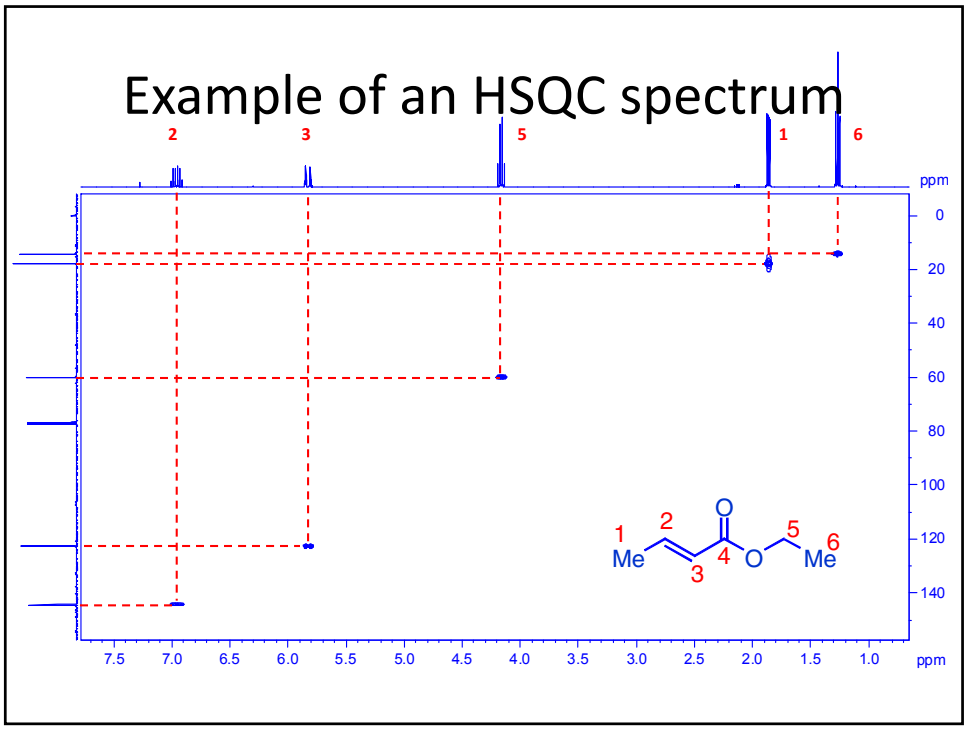
It is technique that "correlates" the heteronucleus (e.g., 13C or 15N to its directly bonded hydrogen.
Thus, it is extremely useful in making assignments of metabolite signals.



Example of a PROTON spectrum
(ethyl crotonate)



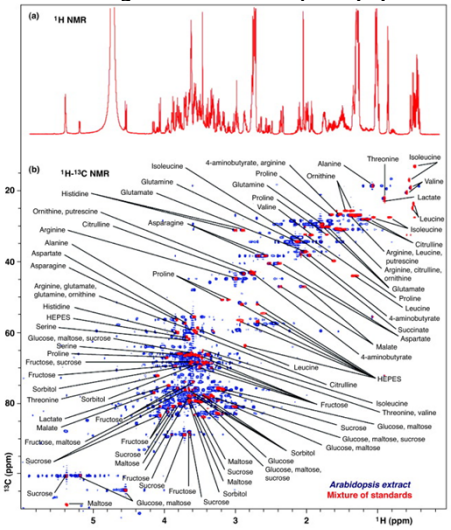

Example of CARBON spectra
C13CPD
4
2/3
2/3
5
1/6
C13DEPT135
C13DEPT90
C13DEPTQ
Me
O
Me
1
2
3
4
5
6
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


Example of an HSQC spectrum
2
3
5
1
6
ppm
0
20
40
60
80
100
120
140
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
Me
O
Me
1
2
3
4
5
6

Metabolic Profiling Methods

Main Analytical Techniques

Nuclear Magnetic Resonance (NMR) Spectroscopy



HSQC used to select for protons directly bonded to ^{13}C.

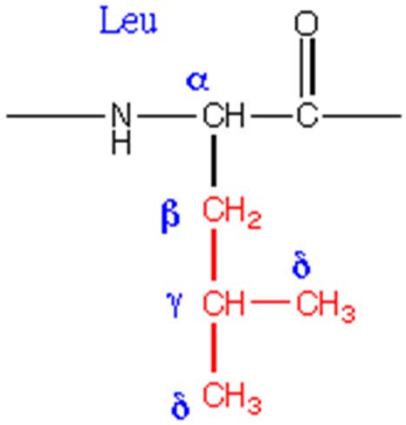
Use of HSQC spectroscopy for analysis of common metabolites. In 1D spectra, overlapped signals hamper identification of individual metabolites, whereas in 2D correlation, spots are easily visible.

(**a**) 1D ^{1}H NMR spectrum of an equimolar mixture of the 26 standards.

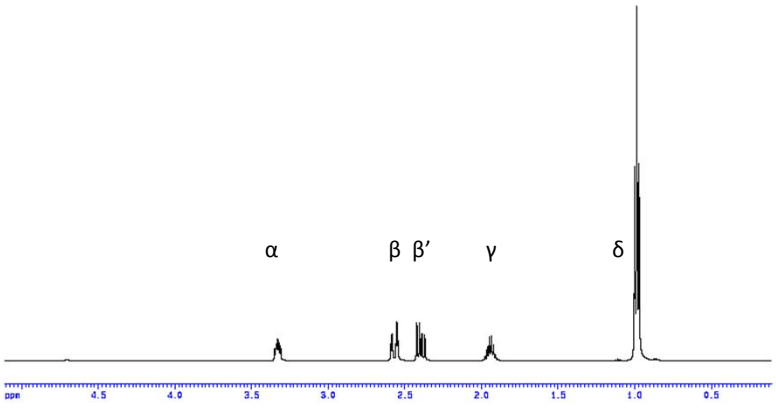
(**b**) 2D ^{1}H–^{13}C HSQC NMR spectra of the same synthetic mixture (red) overlaid onto a spectrum of aqueous whole-plant extract from *Arabidopsis* (blue).

PMID: 21435731

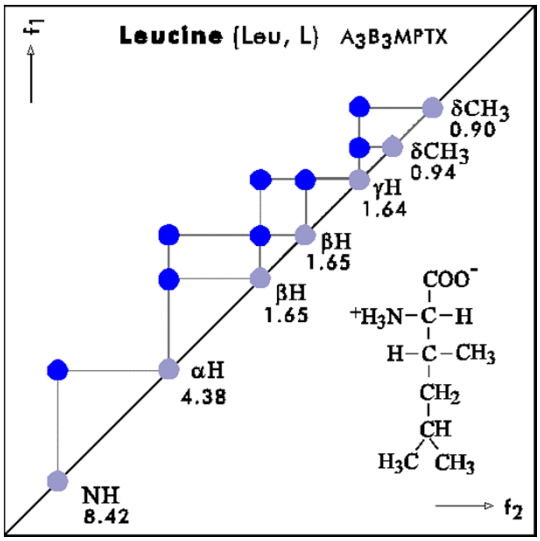
2D-TOCSY



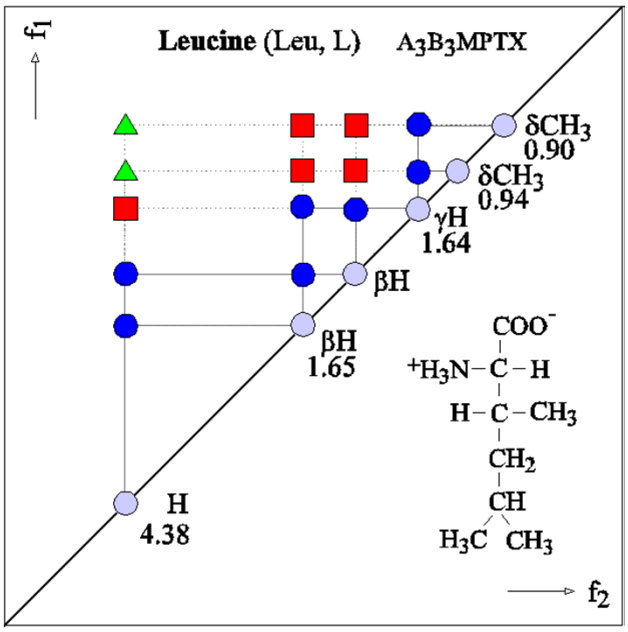
There are <u>through-bond 1H-1H couplings</u> that are finite over 2 and 3 bonds, and vanish rapidly after that. They cause multiplet structure.
These are the basis of the COSY and TOCSY experiments.



1H 1D-NMR spectrum of Leucine/D2O, showing splittings from J-couplings

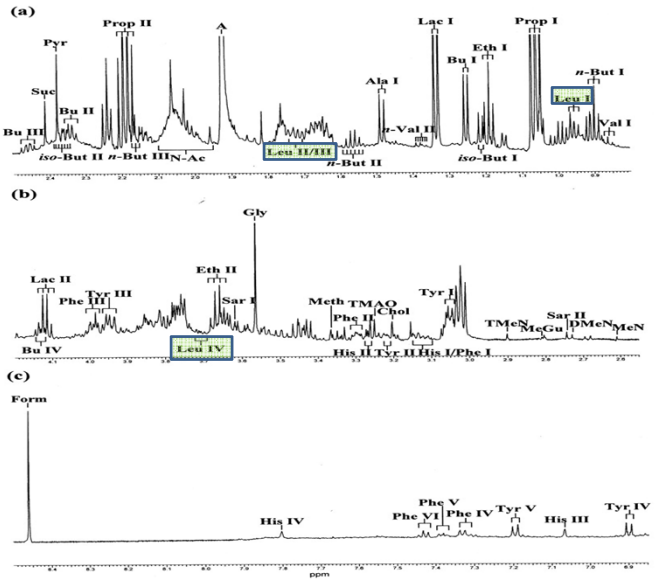


2D-Chemical Shift Correlation Spectroscopy (2D-COSY)



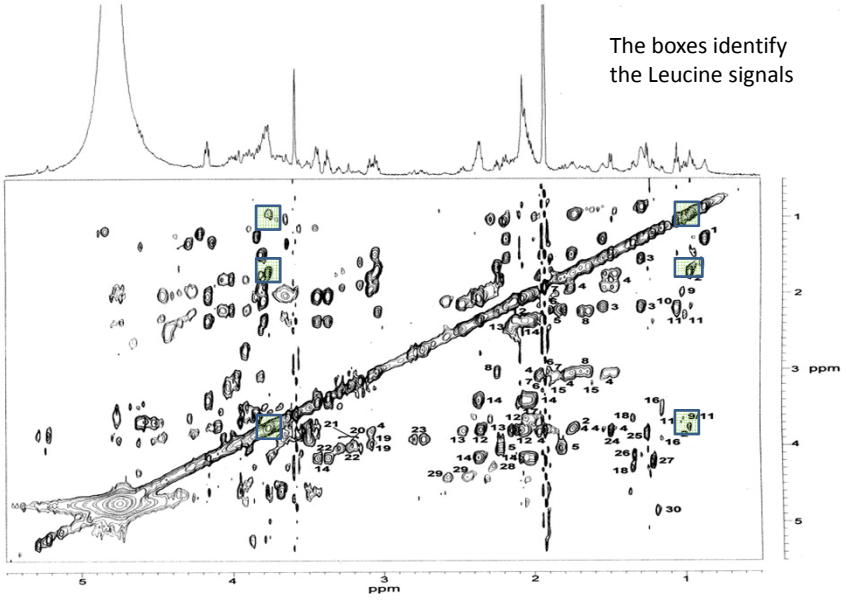
2D-Total Correlation Spectroscopy (2D-TOCSY)

Figure 1. Single-pulse 1D 1H NMR spectrum of a human salivary supernatant specimen.



Silwood C et al. J DENT RES 2002;81:422-427

Figure 2. Partial 1H-1H TOCSY NMR spectrum of a human salivary supernatant specimen.



Silwood C et al. J DENT RES 2002;81:422-427

Clin Cancer Res January 15, 2009 vol. 15 no. 2 431-440

Clinical Applications of Metabolomics in Oncology: A Review

Table 1.

Biofluid and sample preparation requirements

Biofluid	Required sampling handling	
Urine	Add deuterated phosphate buffer to 0.2-0.4 mL urine	
Blood/plasma /serum	For 0.5 mL of heparinized blood product	
		—Add deuterium oxide (to lock)
		—Add acetonitrile (for protein precipitation)
		—Add methanol/chloroform extraction (for lipid extraction)
CSF	Add deuterium oxide to 0.5 mL of CSF	
EPS	Add deuterium oxide to 0.03-0.10 mL of EPS	
Bile	Add deuterium methanol to 0.5 mL of bile	
BALF	Add deuterium oxide to 0.5 mL of BALF	
Tissue	—Add 0.01 mL of deuterium oxide to 3-10 g of tissue in MAS rotor	
		—Add perchloric acid extraction on 20-200 g frozen tissue
		—Add methanol/chloroform extraction to 20-200 g frozen tissue

• NOTE: Adapted from ref. 13.

Abbreviations: CSF, cerebrospinal fluid; EPS, expressed prostatic secretions; BALF, bronchoalveolar lavage fluid; MAS, magic angle spinning.

Note: It is typical to add some deuterated solvent (e.g., 5% D2O) to the solution for Field-frequency lock, to compensate for the slow field drift of the magnet

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)

Lindon & Nicholson, Ann Rev of Analytical Chem., 1:45-69 (2008).

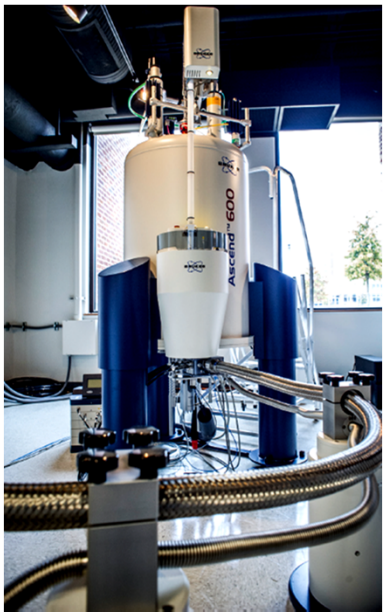
Table 1 The relative strengths and weaknesses of nuclear magnetic resonance and mass spectrometry for metabolic profiling[a]

	NMR	MS
Detection limits	Low-micromolar at typical observation frequencies (600 MHz), but nanomolar using cryoprobes	Picomolar with standard techniques, but can be much lower with special techniques
Universality of metabolite detection	If metabolite contains hydrogens it will be detected, assuming the concentration is sufficient or protein binding does not cause marked line broadening	Usually needs a more targeted approach. There can be problems with poor chromatographic separation; with the loss of metabolites in void volumes; with ion suppression (but this is reduced when using UPLC); lack of ionization; ability to run both +ve and -ve ion detection gives extra information
Sample handling	Whole sample analyzed in one measurement	Different LC packings and conditions for different classes of metabolite; usually samples have to be extracted into a suitable solvent; samples have to be aliquoted but some recent studies have avoided the need for chromatography
Amount of sample used	Typically 200–400 μL, but much less for microcoil probes, down to 5–10 μL	Low μL range
Sample recovery	Technique is nondestructive	Technique is destructive but only small amounts used
Analytical reproducibility	Very high	Fair
Sample prepreparation	Minimal: addition of buffer, D_2O and chemical shift reference (not always required)	Can be substantial; often needs different LC columns and protein precipitation
Ease of molecular identification	High, both from databases of authentic material and by self-consistent analysis of 1D and 2D spectra	Difficult, often only the molecular ion is available; this needs extra experiments, such as routine tandem MS; GC-MS is generally better with accurate retention times and comprehensive databases of spectra
Time to collect basic data	5 min for 1D ^{1}H NMR	10 min for UPLC-MS run
Quantitation	1–5%	5% intraday and interday is now common with or without prior chromatography
Robustness of instruments	High	Low
Molecular dynamics information	Yes, from T1, T2 relaxation time and diffusion coefficient measurements	No
Analysis of tissue samples	Yes, using MAS NMR	No
Availability of databases	Not yet comprehensive but increasing; several are available freely on the web; some commercial products also exist	Comprehensive databases for electron impact MS allow spectral comparisons; For electrospray ionization, as is usual in LC-MS, only mass values can be compared

[a]Abbreviations: GC, gas chromatography; LC, liquid chromatography; MAS, magic angle spinning; MS, mass spectrometry; NMR, nuclear magnetic resonance; UPLC, ultraperformance liquid chromatography.

Bruker-Biospin Avance III HD
600 MHz NMR Spectrometer with
TCI-CryoProbe and Sample Case

Central Alabama High-Field NMR Facility



Bruker-Biospin Avance III 600 MHz NMR system with
TCI CryoProbe and Sample Case