NMR-Based Metabolomics

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NMR in Metabolomics
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Basic Principles of NMR:
- Magnetization
- Chemical Shift
- J-Coupling (Peak Splits)

Modern NMR Systems:
- Magnetic Field Range
- Cryo-Magnets/Maintenance
- HR NMR Probes (conventional/cryo)
- Magic-Angle Spinning Probes (MAS)
- NMR Consoles and Sequences

^{1}H-NMR Metabolomics of Bodyfluids:
- Urine Spectra
- Blood Products
- "Exotic Fluids" (CSF, prostatic fluids, milk, saliva)

^{1}H/^{31}P-NMR Metabolomics of Tissues:
- Tissue Extracts
- MAS on Solid Tissues

1H/31P/13C-NMR on Cells:
- ^{13}C-Tracers
- Quantification of Metabolic Fluxes.

Why is NMR competitive?

- NMR is fully quantitative;
- NMR is highly reproducible;
- NMR detects all metabolites simultaneously in one snap-shot (non-selective);
- NMR allows to completely recover the samples (non-destructive);
- NMR can provide non-destructive tissue analysis;
- NMR has emerged into a high throughput analysis system with minimal sample preparation and no need for derivatization.

What are NMR limitations?

SENSITIVITY!!!!!

- NMR detects only high-abundant metabolites (micromole to millimole range);
- NMR suffers from signal overlap between individual metabolites;

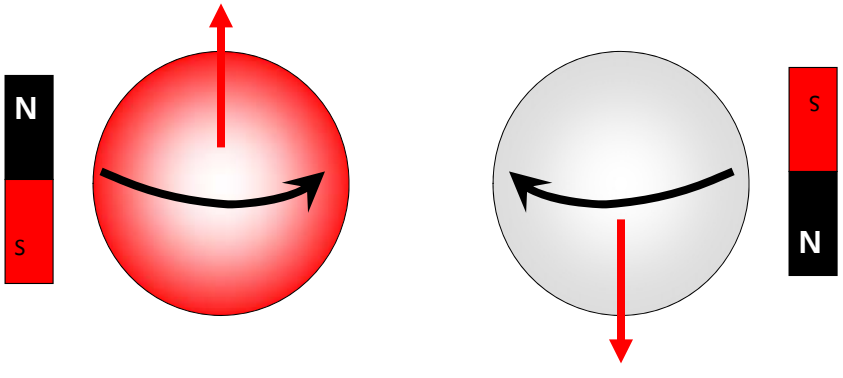
- NMR-based metabolomics (~50 metabolites identified/quantified, mM sensitivity)
- GC-MS based metabolomics (~70 metabolites identified/quantified, <mM sensitivity)
- DI-MS based metabolomics (160 metabolites identified/quantified, nM sensitivity)
- LC-MS based metabolomics (300 metabolites identified/quantified, nM sensitivity)

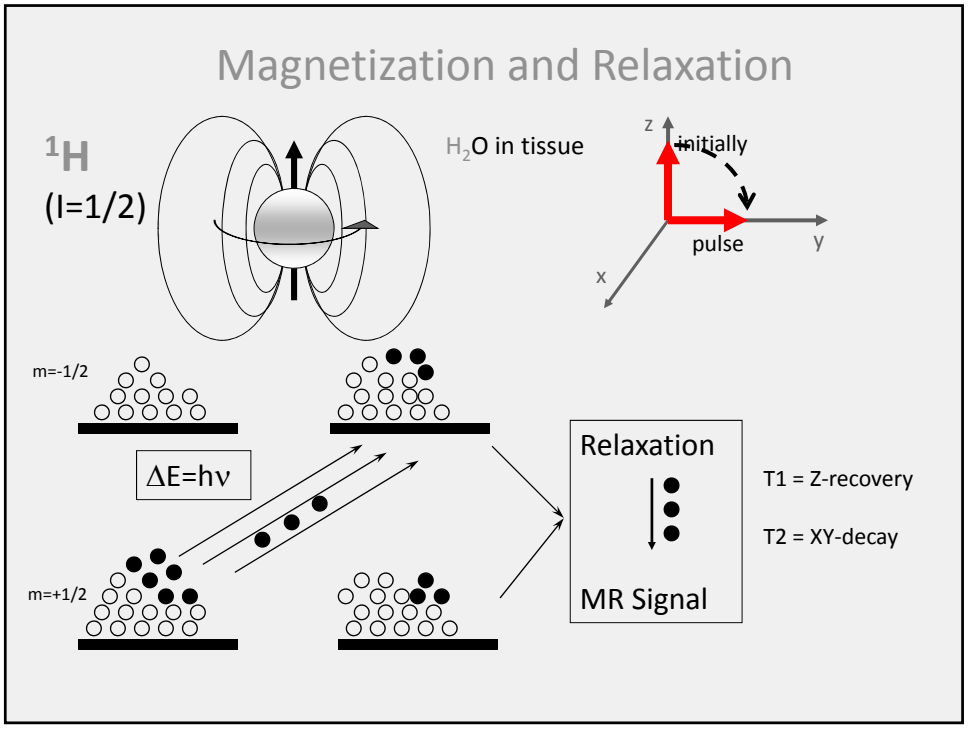
Principles of NMR

- Measures nuclear magnetism or changes in nuclear magnetism in a molecule
- NMR spectroscopy measures the absorption of light (radio waves) due to changes in nuclear spin orientation
- NMR only occurs when a sample is in a strong magnetic field
- Different nuclei absorb at different energies (frequencies)

Principles of NMR

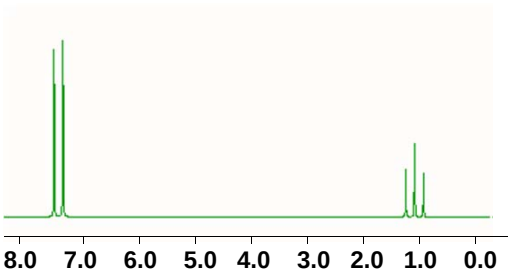
Each Spinning Proton is Like a "Mini-Magnet"





Principles of NMR

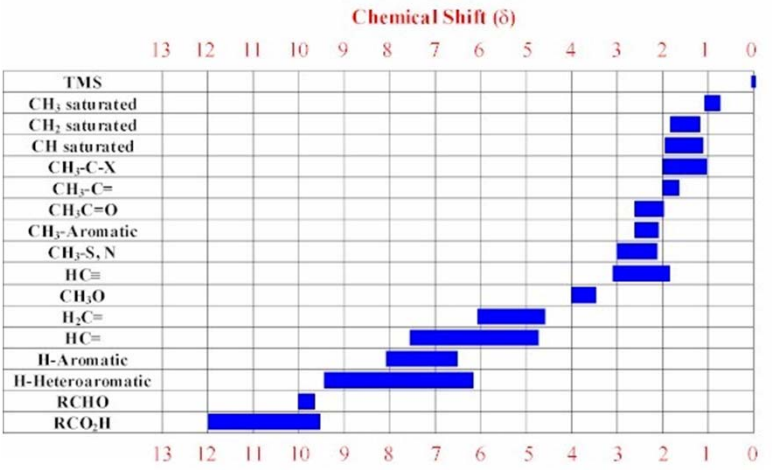
- Chemical Shifts (peaks at different frequencies or ppm values)
- Splitting Patterns (from spin coupling)
- Different Peak Intensities (# ^{1}H)



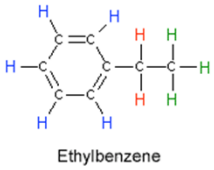
Chemical Shifts

- Key to the utility of NMR in chemistry
- Different ^{1}H in different molecules exhibit different absorption frequencies
- Each compound can be defined by a unique pattern of chemical shifts (a fingerprint)

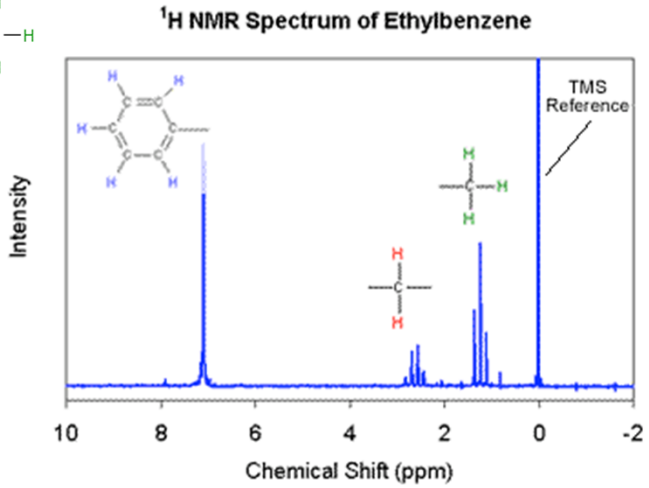
Characteristic Chemical Shifts



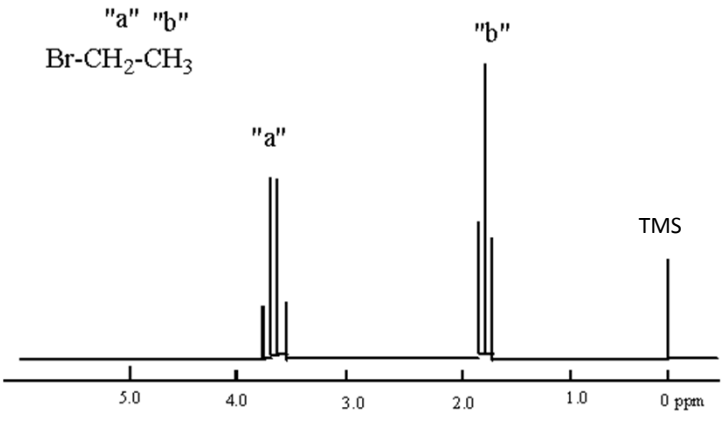
Assigning Simple NMR Spectra



Ethylbenzene



Assigning Simple NMR Spectra (J-Coupling)



Modern NMR Systems

There are two different kinds of magnets:

human or animal horizontal scanners for MRI and MRS

high-resolution vertical magnets for MRS

Human MRI/MRS scanners: 0.5 to 3.0 Tesla (clinical FDA approved)
4.0 to 7.0 Tesla (for research only: volunteers)

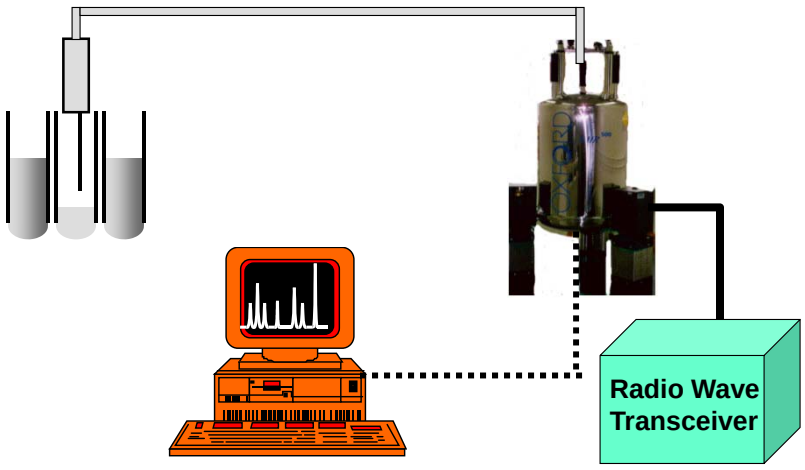
Animal scanners: 2.4 to 14.0 Tesla

High-resolution vertical magnets: 200 MHz (4.7 Tesla) to 900 MHz (**21.1 Tesla**)

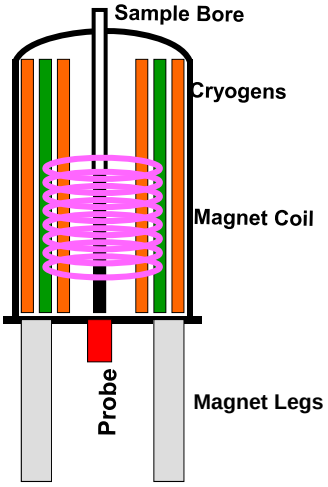
Modern NMR Systems

High-Resolution NMR Spectrometers:
300 MHz (7Tesla) – 900 MHz (21 Tesla)

In Vivo MRI/MRS Scanner:
1.5 Tesla (80 MHz) – 7 Tesla (300 MHz)
4.7 Tesla (200 MHz) – 14 Tesla (600 MHz)



Modern NMR Systems



Modern NMR Systems

Both NMR approaches, MRI and MRS, involve the collection and display of signals as a function of frequency,

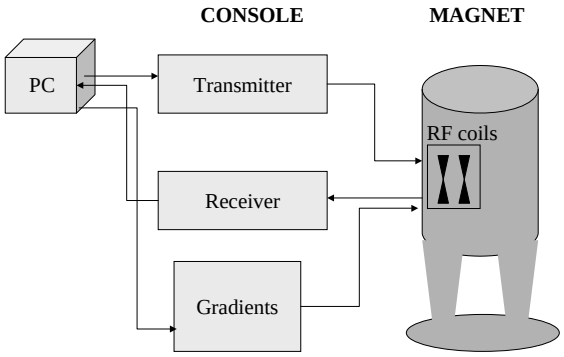
the main technical difference being that in spectroscopy the frequency encodes for chemical compounds,

whereas in imaging the use of gradients causes the frequency to encode for special pictures.

The integral of an NMR signal is strictly linearly proportional to the amount of atoms in the probe volume.

The signals are a measure of molar ratios of molecules, independently of the molecular weight.

Modern NMR Systems



the magnet and shim coils
the gradient system
the transmitter
the radiofrequency coils
the receiver
the computer

Modern NMR Systems

Both the magnetic field and the RF frequency is slowly varied from one sample to another –
we need to perform **"shim"-**procedure (magnetic field homogeneity)

and "**match-tune"-**procedure (RF-frequency adjustment).

However, magnets cannot provide and keep the required homogenous field stability and need additional control mechanism –

a lock signal.

The lock signal usually is the deuterium (2D) from solvent solutions.

Modern NMR Systems

CONSOLE

Transmitter.
The transmitter generates RF pulses of the appropriate frequency, power, shape, and timing.

The transmitter consists of a frequency source and amplifiers to boost the RF signals to the desire level.

Receiver.

NMR receivers consist of amplifier stages to make the RF signal from the sample large enough, followed by a detector which removes the RF carrier so that only the signal envelope, which is the desire information, remains.

Modern NMR Systems

Gradients.

Small linear distortions to B0, known as gradient fields or gradients, are required to localize the tissue imaging.

Max. gradient strength: 20-25 mT/m.

RF coils.

The RF coils are used for transmitting the B1 field into the region of interest, for transmitting the RF pulse and for detecting the resulting signal.

Modern NMR Systems

MAGNETS

From historical point of view, the NMR evolution counts on three different types of the magnets:

Resistive magnets (in 1950s to 1980s): electromagnets generating very low magnetic fields: FORTGET IT!

Permanent magnets (in 1950s): ferromagnetic materials creating very low magnetic fields: FORGET IT!

Superconducting magnets: current magnets with the field strength up to 21 Tesla

Modern NMR Systems

Superconductors are materials such as niobium-titanium alloys that lose all their electrical resistance at very low temperatures.

To achieve a sufficiently low temperature, the conductors need to be enclosed in Dewar vessel of liquid helium.

The boil-off of liquid helium is minimized by means of
-surrounding vacuums:
-radiation shields
-liquid nitrogen chambers

and this complete cooling system forms a major component of the magnet design.

Modern NMR Systems

The NbTi coil magnet is operated at 4.2 ° K.

Liquid Helium: 4.2°K

Liquid Nitrogen: 52°K

Liquid nitrogen has to be refilled every week.

Liquid helium has to be refilled every three to six months.

Magnet quenching:

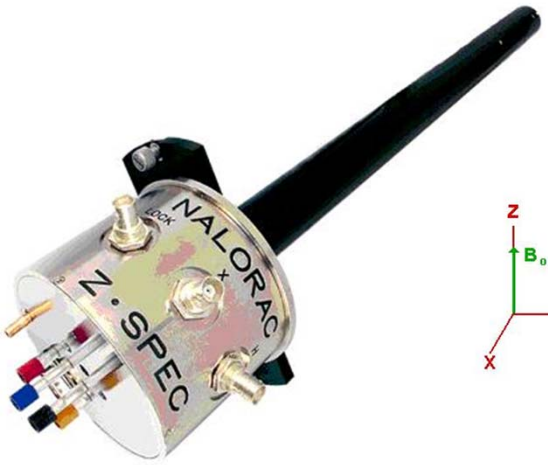
Loose of helium

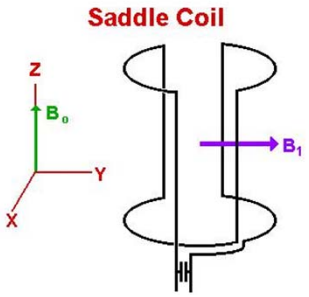
Loose of nitrogen

Loose of vacuum

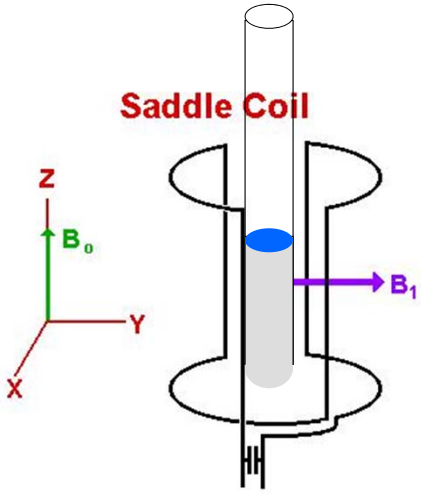
And the magnet becomes resistant.

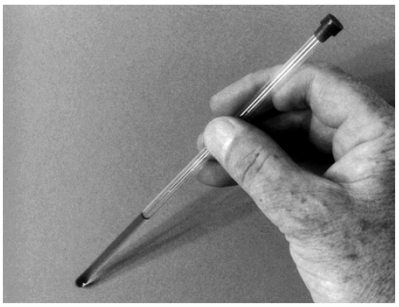
Modern NMR Systems
A HR Liquid State NMR Probe



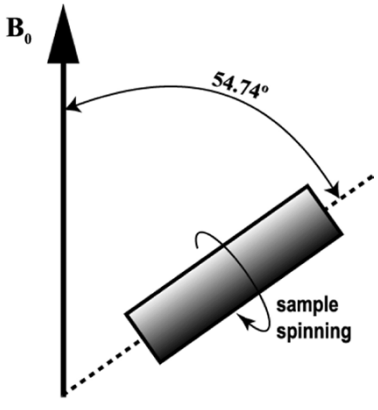


NMR Sample & Probe Coil





Modern NMR Systems
A HR Solid State NMR Probe
(Magic Angle Spinning, MAS)



NMR Safety

- Any metal near the magnet should be non-magnetic.
- Metal items such as chairs, gas tanks, scissors, stethoscopes, and even pens may just FLY to the magnet,
- causing not only magnet defects,
- but also POSSIBLE PERSONAL INJURY.

Remember:
your watches,
your credit,
ID,
Cell phones/beepers
and telephone cards have magnetic strips on them.

They will be destroyed near the magnet.

There is no dangerous to your health or magnet wellbeing of that, but you better leave your watches and cards outside of 5 Gauss* line.

NMR Safety

PERSONS WITH SURGICAL IMPLANTS or METAL FRAGMENTS in their bodies SHOULD NOT ENTER THE NMR ROOM.

PERSONS WITH PACEMAKERS ARE NOT ALLOWED to be present in the NMR room.

PERSONS WITH FERROMAGNETIC INTRACRANIAL ANEURYSM CLIPS ARE NOT ALLOWED to enter the NMR room.