Principles of NMR Spectroscopy

For Application to Protein Characterization

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Nuclear spin

Atomic nuclei consist of protons and neutrons (nucleons)

Protons and neutrons have spin ½

Spins tend to compensate each other but often not completely

Resulting spin quantum number of the nucleus is

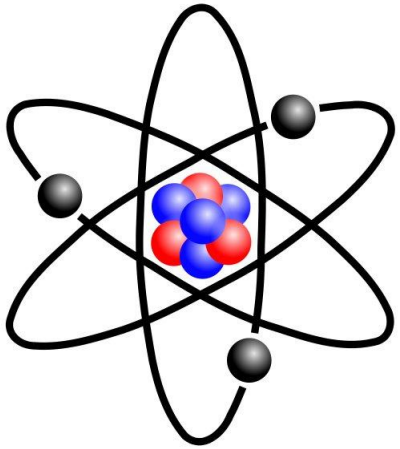
$I = k * ½$ k is integer 0, 1, 2 ....

Magnetic spin quantum number – number of possible spin states

m = -I, -I+1, -I+2 ……I-2, I-1, I

Examples

I = ½, m = -½, +½ 2 spin states example: ^{1}H
I = 1, m = - 1, 0, 1 3 spin states example: ^{2}H



Proton
Neutron

Magnetic moment

Only nuclei with non-zero spins have magnetic moments and are active in NMR

Magnetic moment

$$|\mu| = \gamma \hbar \sqrt{I(I+1)}$$

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

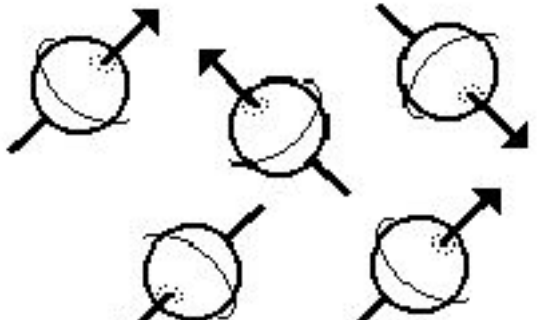
I = quantum number

m = ***I***, ***I-1***, ***I-2***… ***-I*** = allowed states

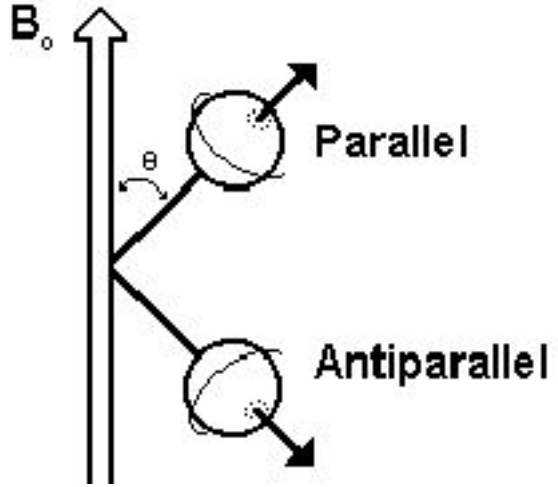
ħ=h/2π h Planck's constant γ magnetogyric ratio, specific to isotopes

Isotopes differ by the number of neutrons and have generally different spins.

In NMR, we refer to isotopes rather than elements, e.g. ^{1}H (proton) or ^{13}C instead of hydrogen and carbon

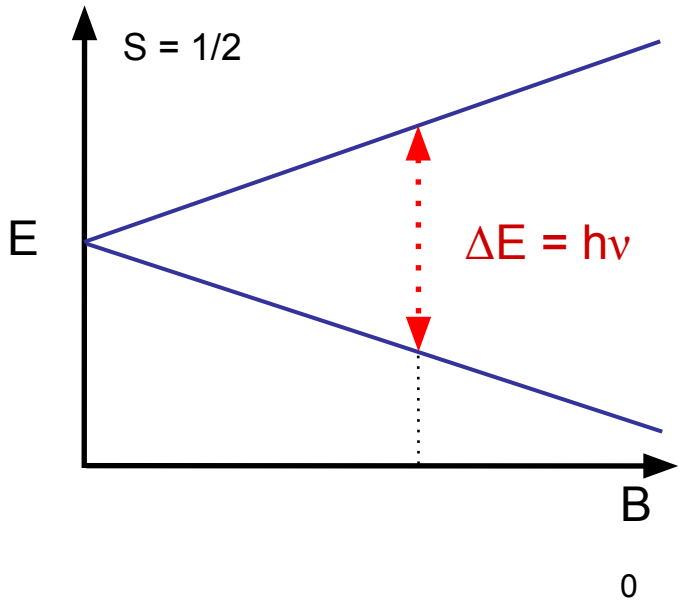


No magnetic field



In magnetic field B_0

Spins in magnetic field



$\Delta E = h\gamma B_0/2\pi$ B_0 external magnetic field

$\nu = \gamma B_0/2\pi$

$h = 6{,}626.10^{-34}$ J.s h is Planck's constant

γ Magnetogyric ratio, specific for each isotope

For ^1H $\gamma = 42\,494\,369\ s^{-1}.T^{-1}$

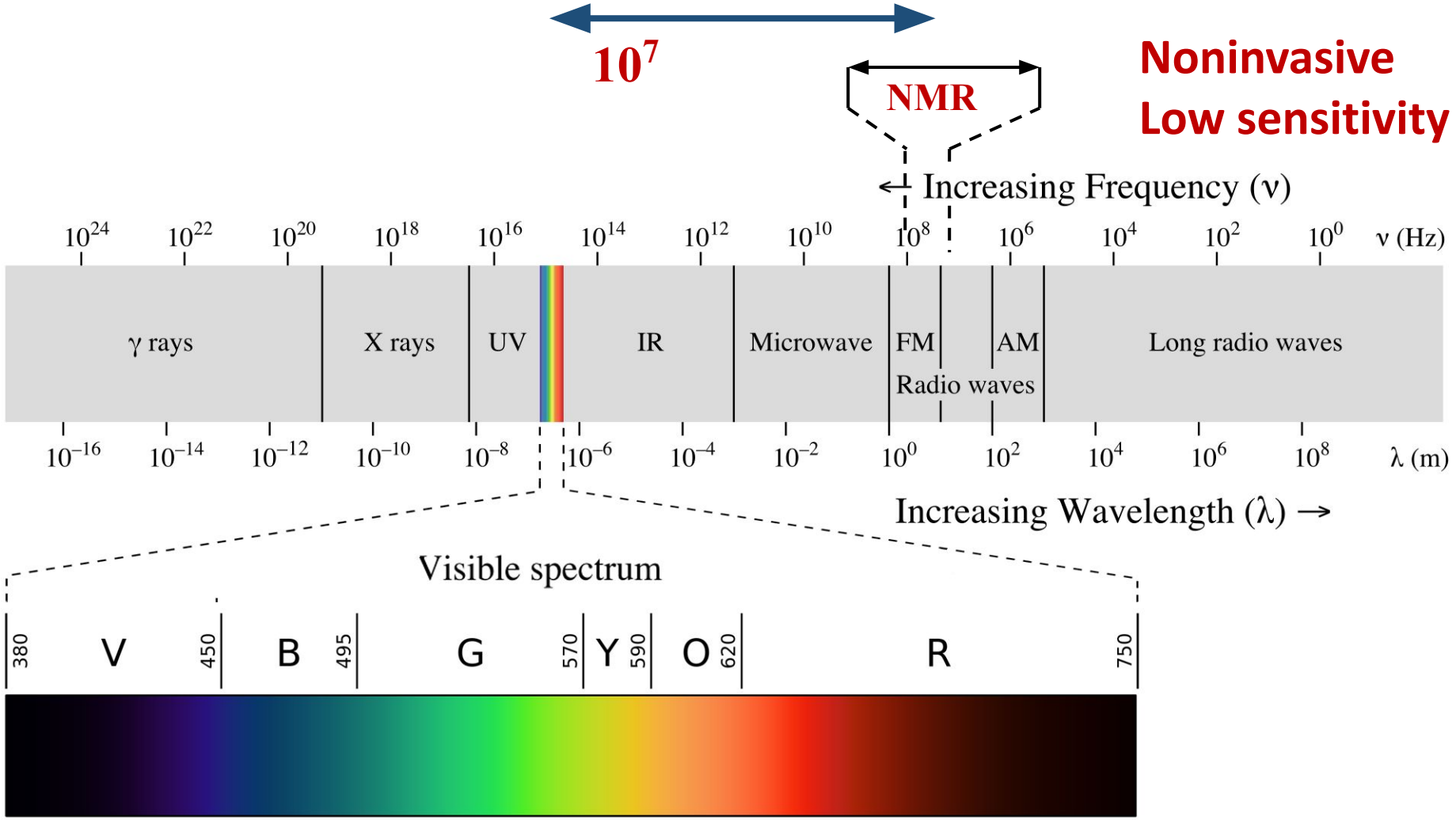
For 100 MHz resonance frequency you need magnet

$$B_0 = 100\ s^{-1} / 42.494\ s^{-1}.T^{-1} \cong 2.35\ T$$

Research grade NMR spectrometers are produced usually with ^1H resonance frequencies in multiples of 100 MHz, currently from 400 MHz up to 1,200 MHz (1.2 GHz), corresponding to magnets from 9.4 T to 28.2 T. For protein studies, high-field spectrometers with resonance frequencies 600 MHz or higher are commonly used.

On Earth, spins are always subject to a magnetic field, but the magnetic field of Earth is only about 50 µT.

NMR and electromagnetic spectrum

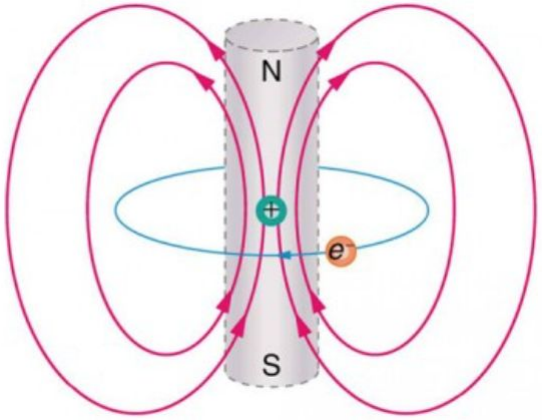


Chemical shift

Nuclei do not experience the external magnetic field B_0 only, but also the fields of other particles, especially electrons.

Net magnetic field at a nucleus

$$B = B_0 (1 - \sigma)$$ σ nuclear shielding

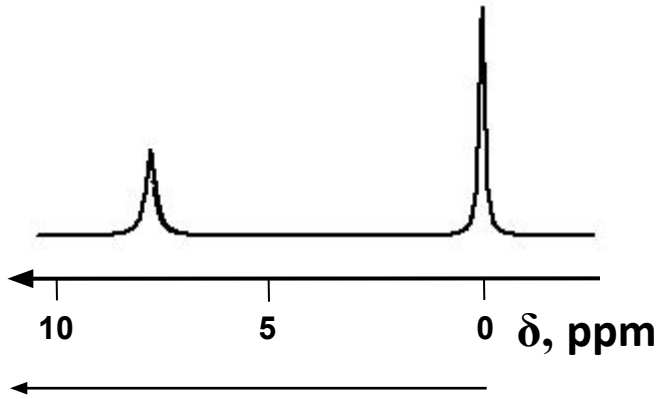


Resonance frequencies differ slightly depending on the location of the nucleus in the molecule.

Resonance frequencies are field dependent, which impractical – values are not comparable between different spectrometers.

Chemical shift – frequency difference relative to a suitable standard, expressed in ppm.

$$\delta = 10^6 (\upsilon - \upsilon_{ref}) / \upsilon_{ref}$$



For ^1H and ^{13}C, TMS (tetramethyl silane) is a common reference compound

Spin-spin interactions

Dipolar coupling

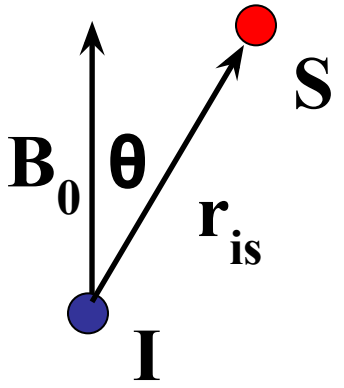
Direct interaction between nuclear spins

Depends on the orientation of the internuclear vector with respect

to external magnetic field

Important in solid-state spektra

In liquids manifests itself only through relaxation phenomena



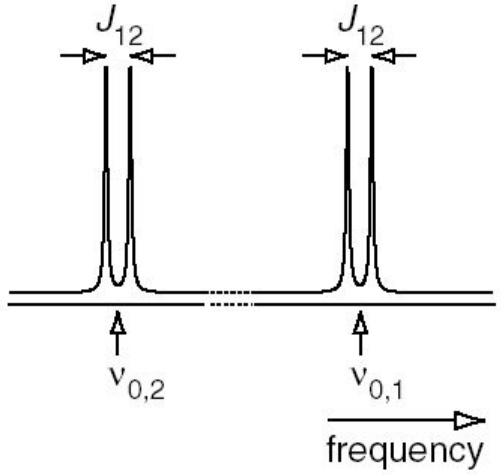
Scalar / J-coupling

Interaction of nuclei through the cloud of electrons

Does not depend on the external magnetic field

Causes splitting of signals (doublets for spins ½)

Scalar interaction constant J (in Hz, no dependence on B_0)

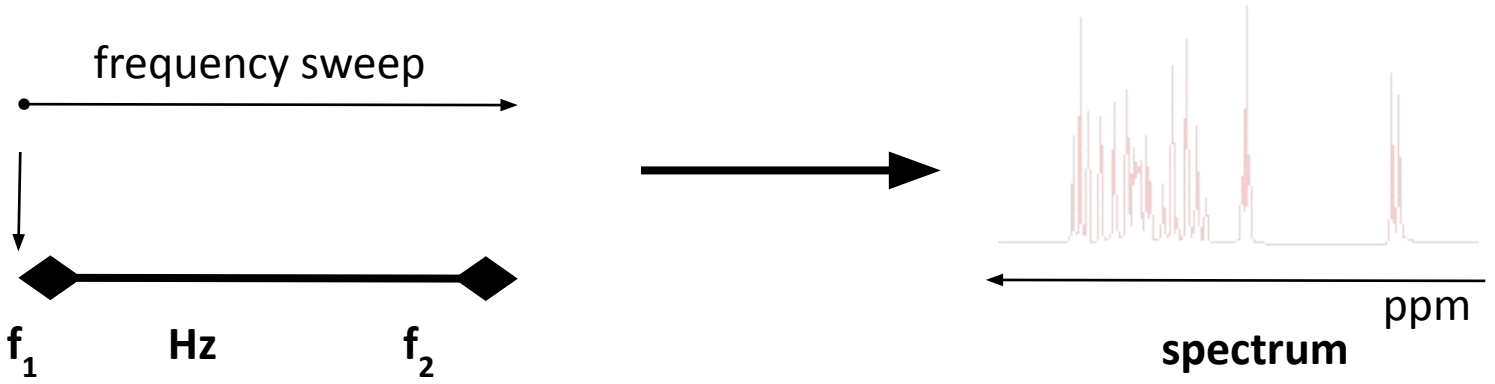


Reaching the resonance - CW

Continuous wave (CW)

Irradiation frequency is changed (swept) over the range of frequencies. When resonance occurs, the irradiation energy is absorbed which is recorded as a signal. The resulting record of intensities vs. frequency is the spectrum.

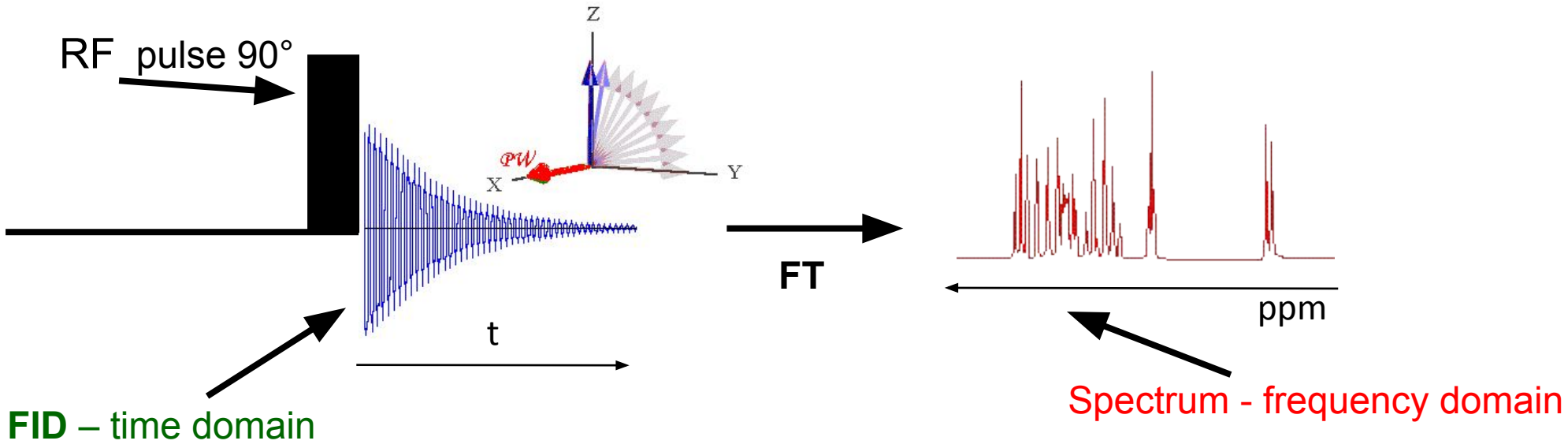
To get undistorted spectrum the process must be SLOW (minutes)



Reaching the resonance - PFT

Pulsed with Fourier Transform

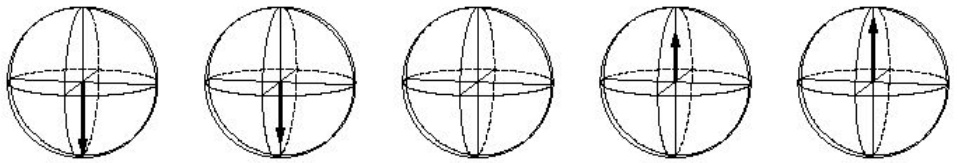
The spin system is irradiated by a single short high intensity pulse. This is equivalent to irradiating a range of frequencies. The shorter and more intense the pulse is, the broader range of frequencies is affected. After the pulse, the response of the spin system is recorded as a function of time. The record is FID (Free Induction Decay). The spectrum is produced by Fourier Transform of the FID.



Relaxation

Spin-lattice relaxation - T_1

Transferring the energy into surroundings (solvent/'lattice'). The spin system returns into equilibrium.

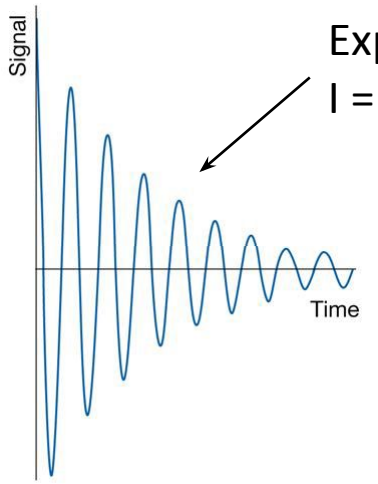


Inversion recovery

$I = I_0 [1 – 2 \exp(-t/T_1)]$

Spin-spin relaxation - T_2

The intensity of the signal in FID is dropping with time (Free Induction Decay) due to loss of coherence. The energy is still in the spin system but randomly oriented nuclear magnetic moment average to zero.



Exponential decay

$I = I_0 \exp(-t/T_2)$

Resolution

Position of signals is given by the chemical shift (relative number)

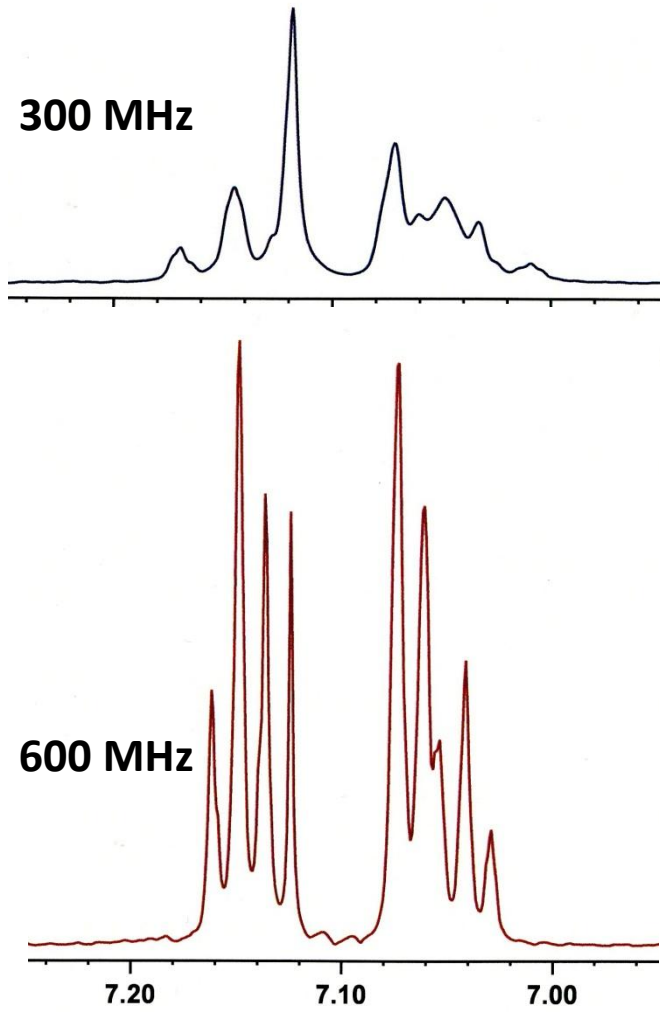
The with of 1 ppm in Hz (absolute scale) depends on magnetic field:

at 500 MHz, 1 ppm = 500 Hz,

at 1000 MHz, 1 ppm = 1,000 Hz

If the linewidth is the same, signals appear farther from each other at higher field.

Resolution (and sensitivity) increase linearly with magnetic field



Aromatic part of ethylbenzene spectrum

Sensitivity

Energy difference between spin levels are very small.

Boltzmann distribution $N_\alpha = N_\beta .\exp(-\Delta E/k_B T)$

$\Delta E = h\nu$, h Planck's constant, k_B Boltzmann constant
N_α, N_β – polulations of the spin states

For protons at 500 MHz and 303 K, the population difference between the energy levels is less than 0.01%!

We work with **a small fraction od nuclei – sensitivity** of NMR is inherently **low**

For proteins, you need about **0.5 ml of sample with 0.5 mM** concentration.

Improving sensitivity by signal accumulation – more scans

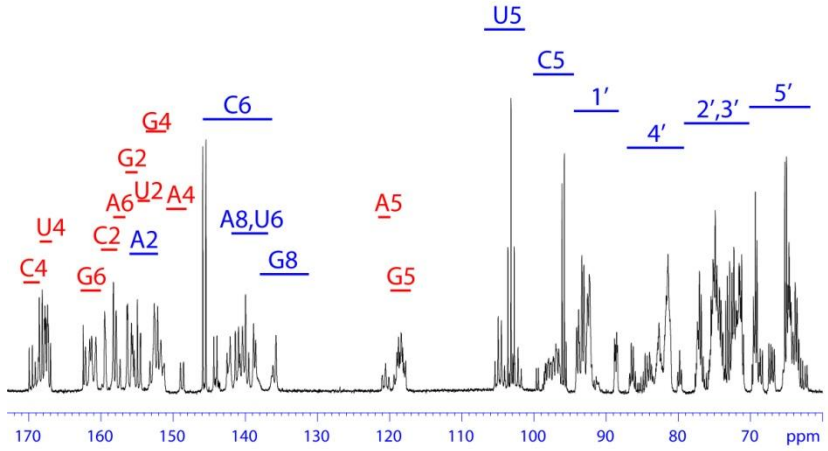
Signal-to-noise ratio increases with square root of number of scans: S/N ~ $\sqrt{n}$s

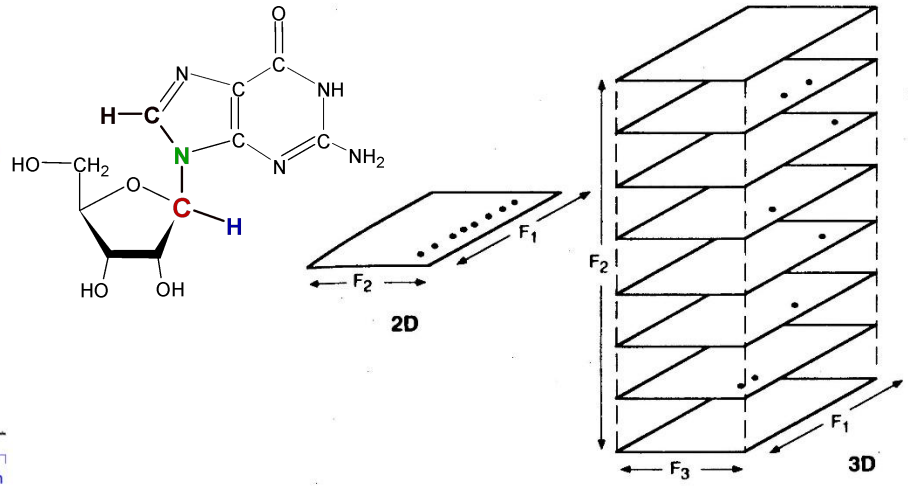
Increasing Resolution

Biopolymers: repetition of identical units (nucleotides, amino acids)

High resolution is needed

High magnetic field

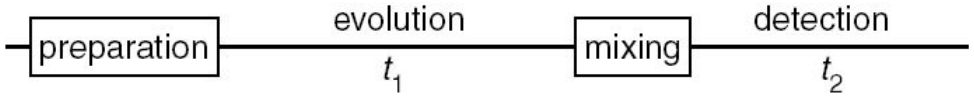




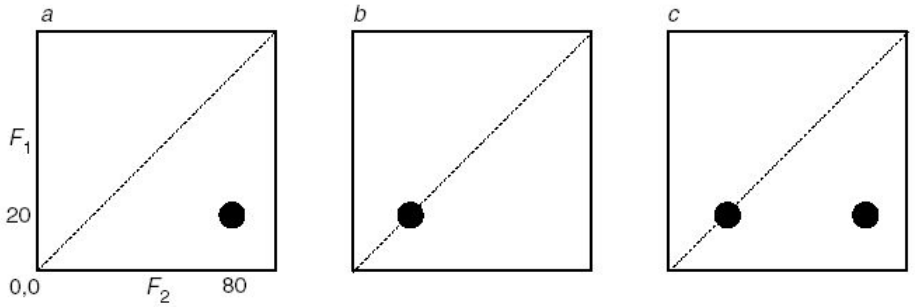
Increasing number of dimensions

Introducing Higher Dimensions

General scheme

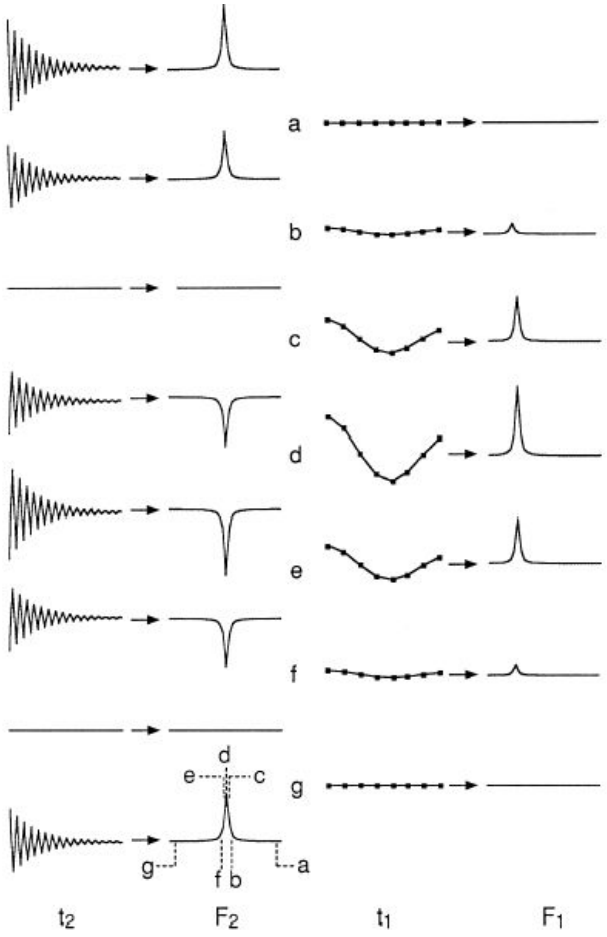


Peak types



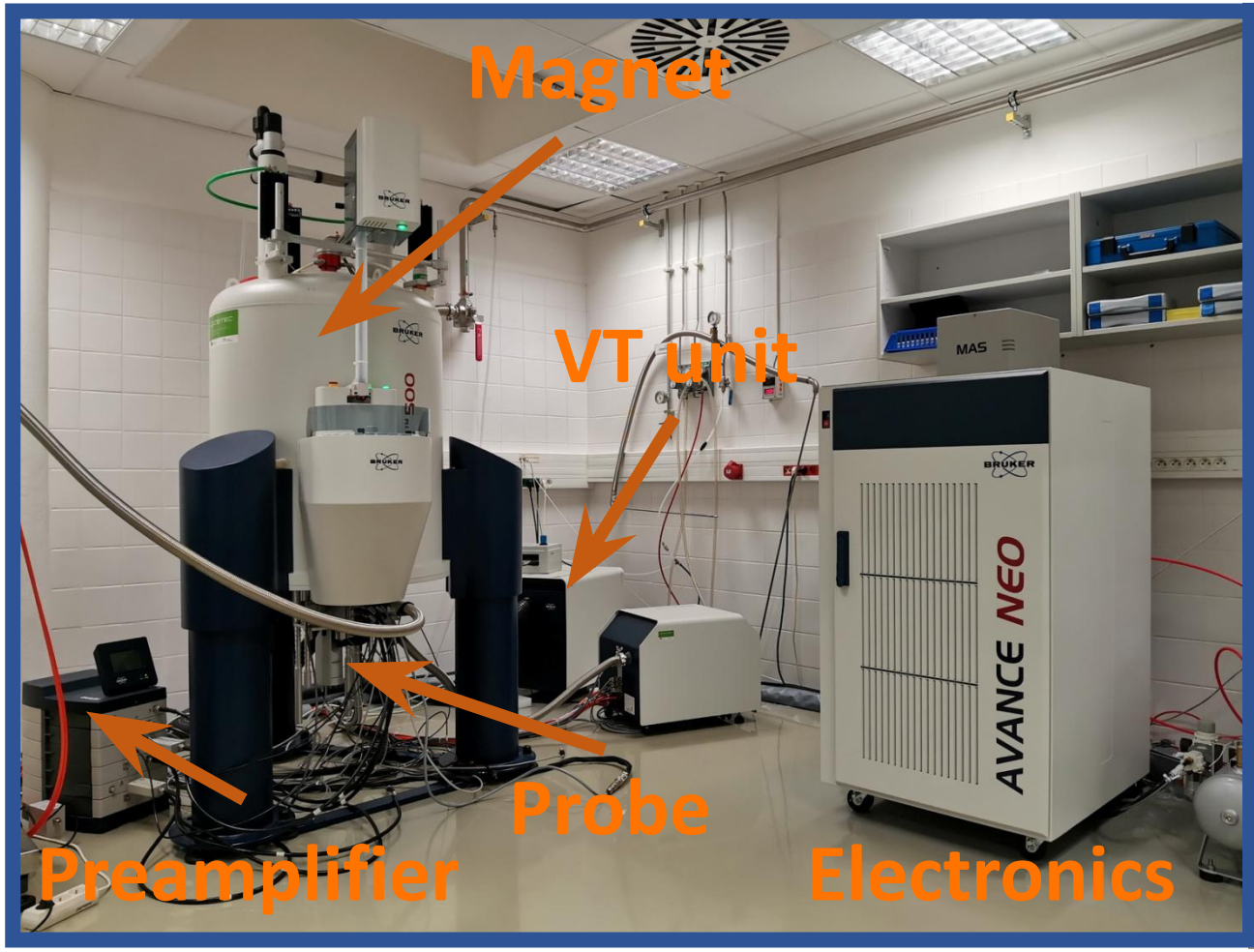
crosspeak diagonal peak

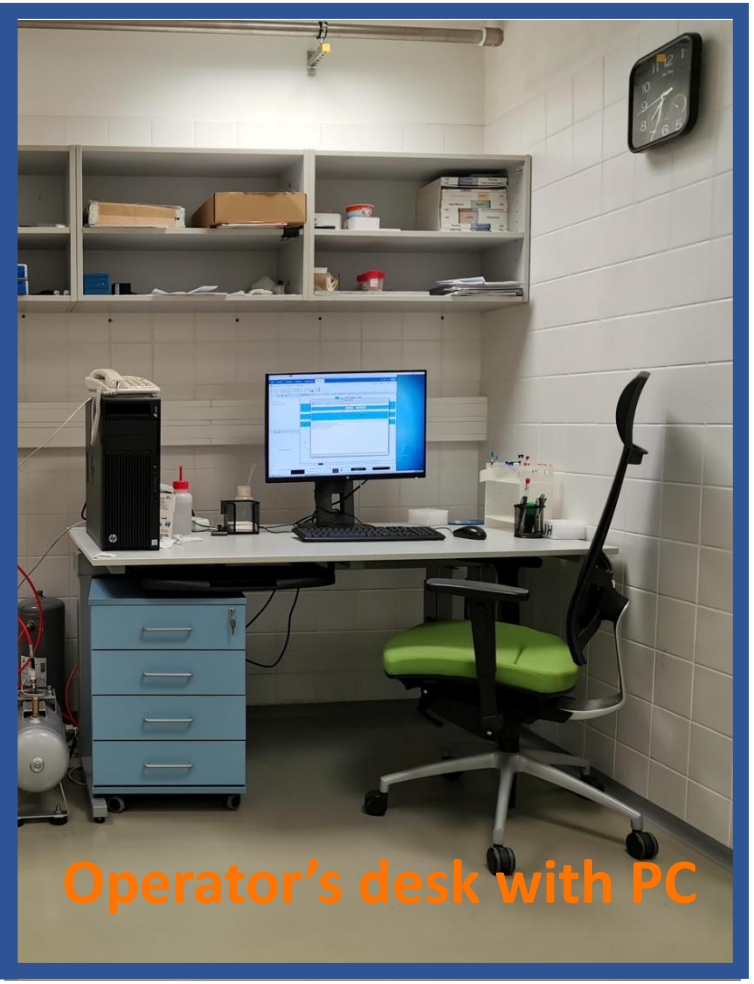
Modulation



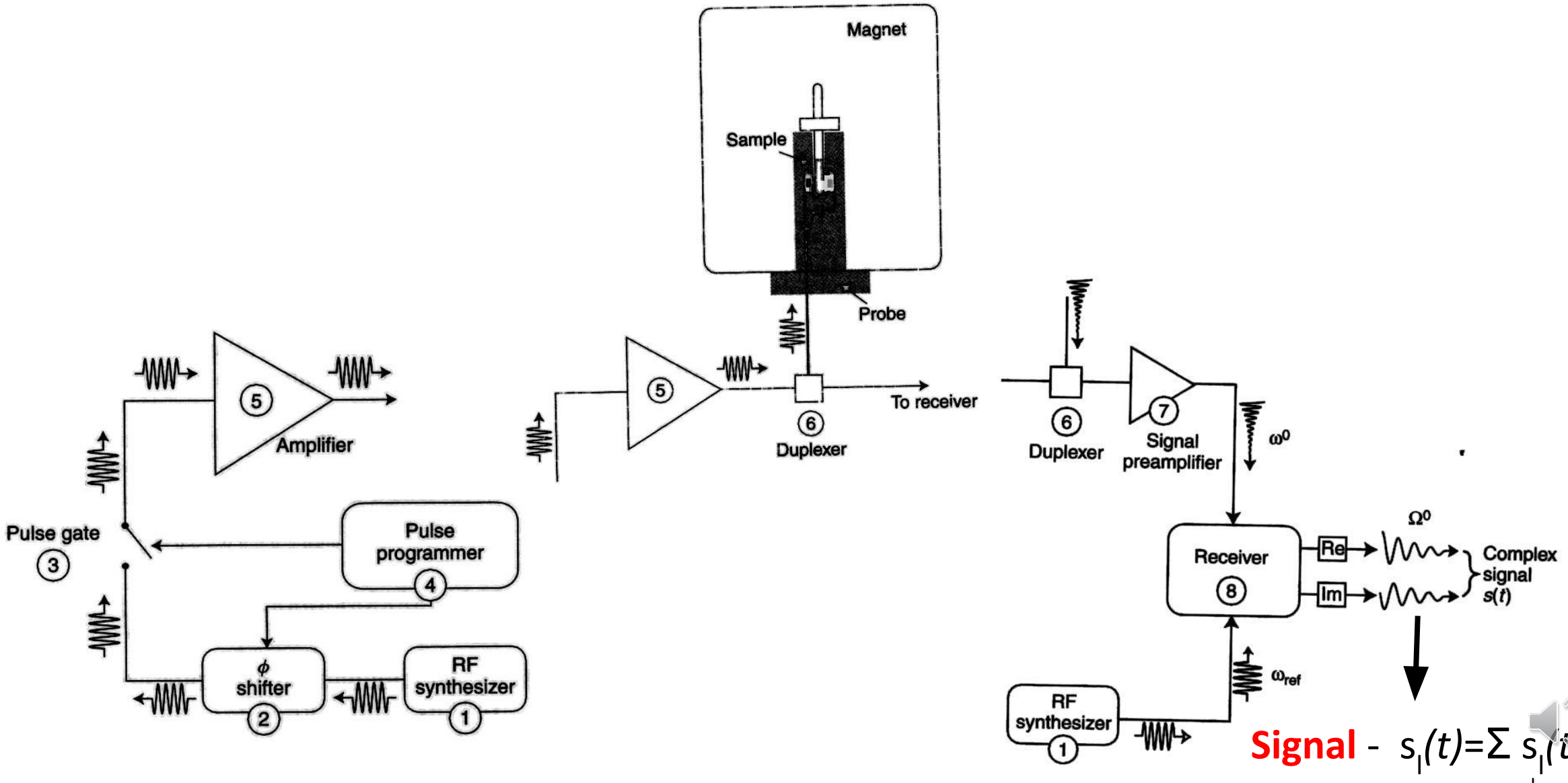
NMR Spectrometer

Major parts



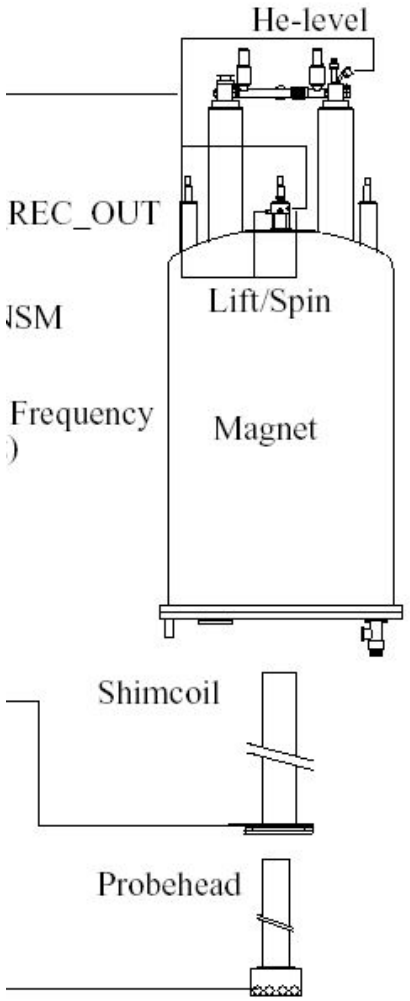


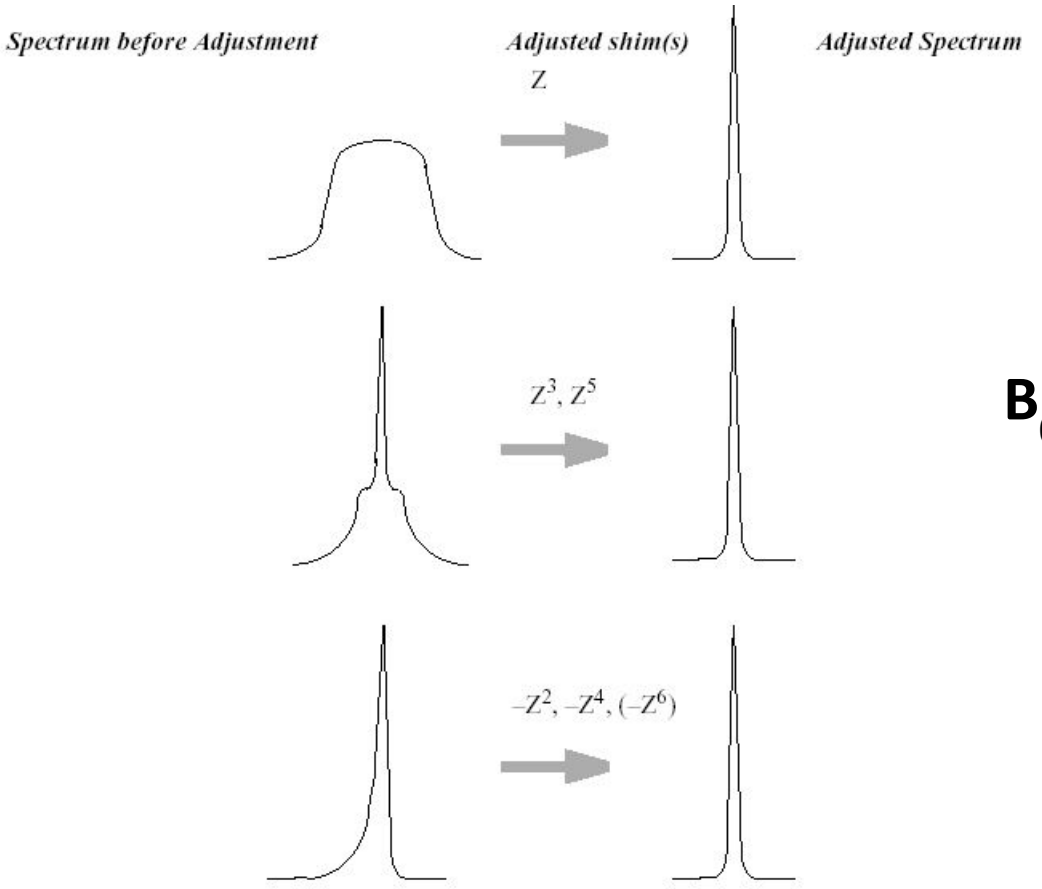
NMR Spectrometer - Scheme

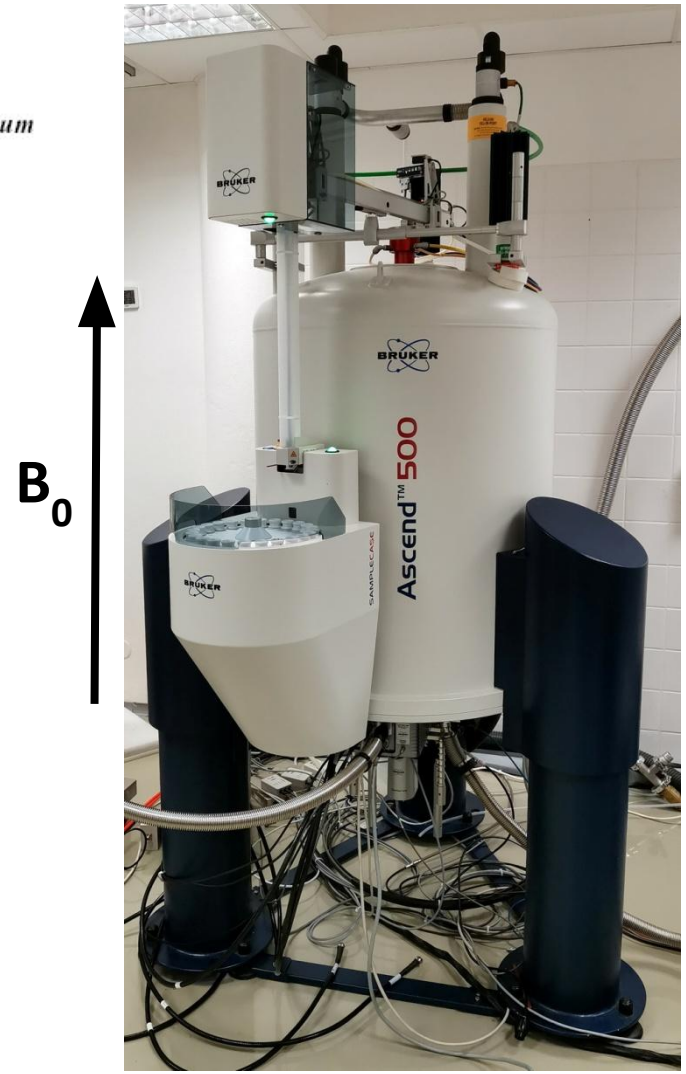


Signal - $s_l(t)=\Sigma\, s_l(t)$

The Magnet







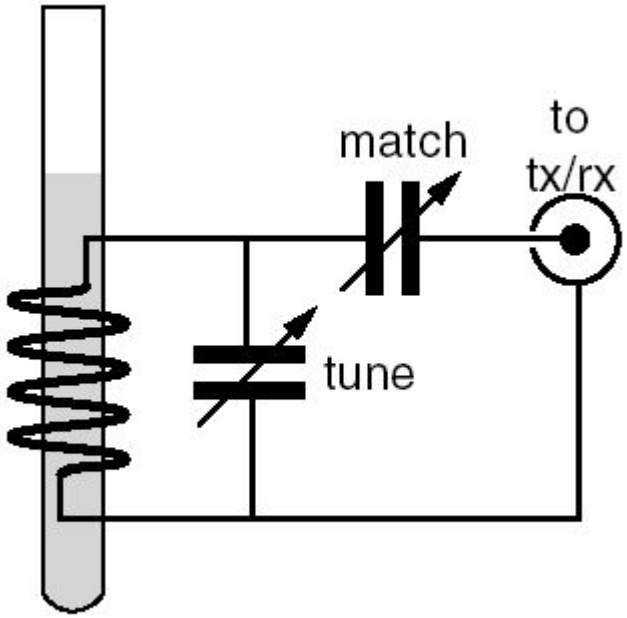
Adjusting the magnetic field homogeneity

The Probe

Houses the sample

Changes electric current into magnetic field and back.

Must be tuned for best sensitivity



Electronics

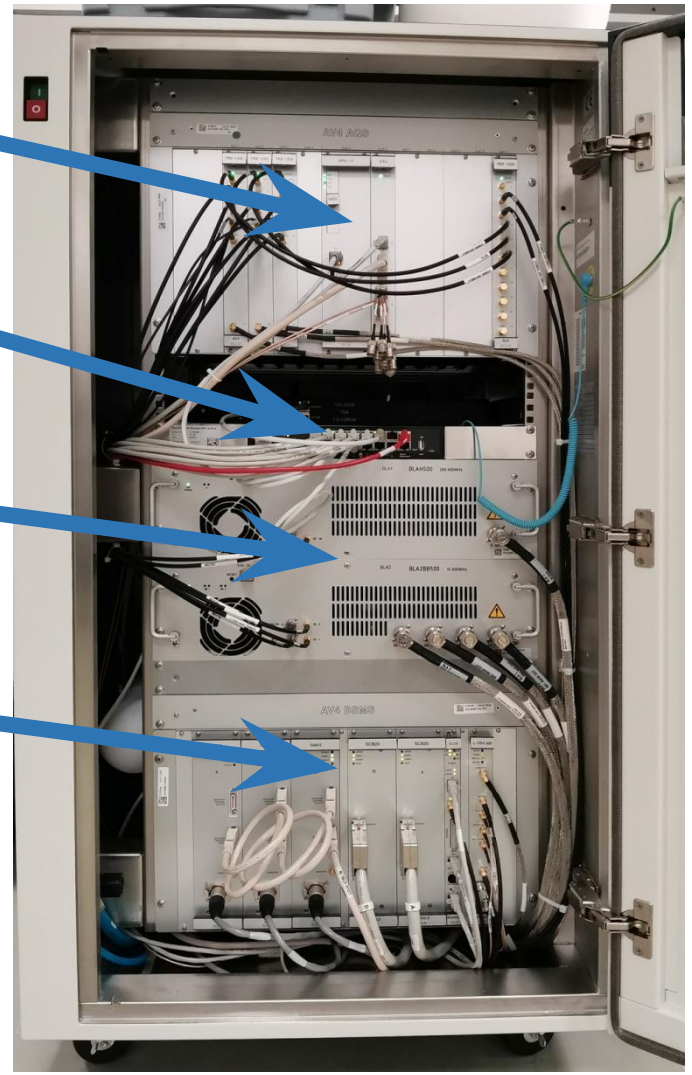
Frequency synthesis & signal processing

Computer (real-time control)

Power amplifiers

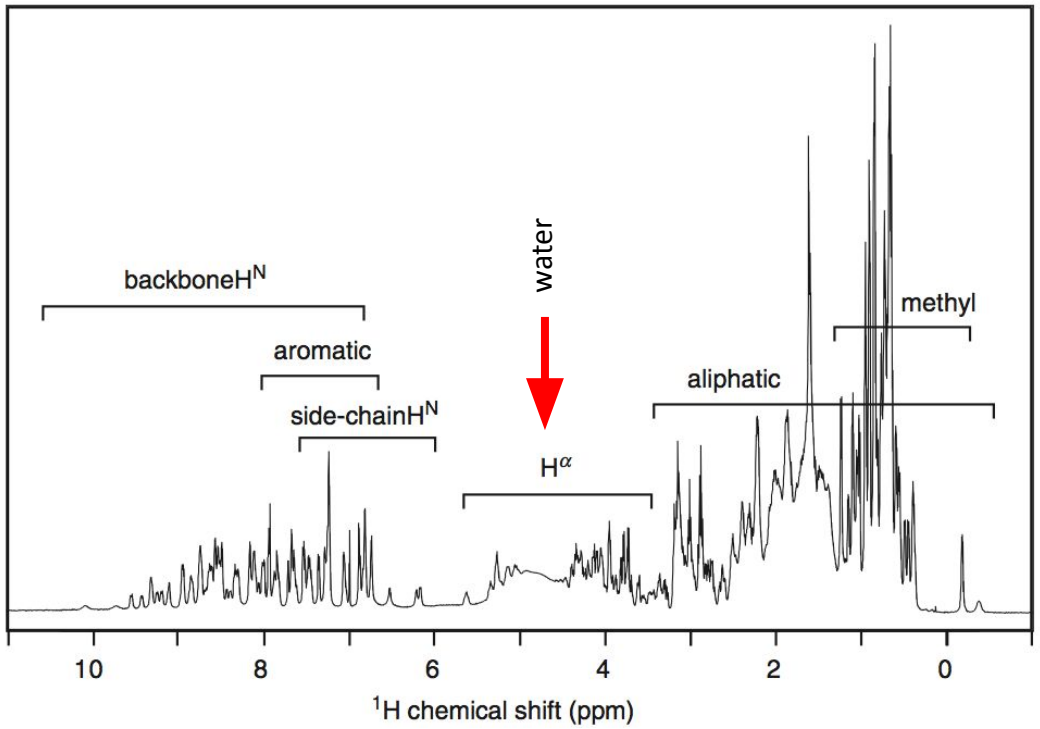
Magnet control

- sample insert & eject
- shimming
- temperature control



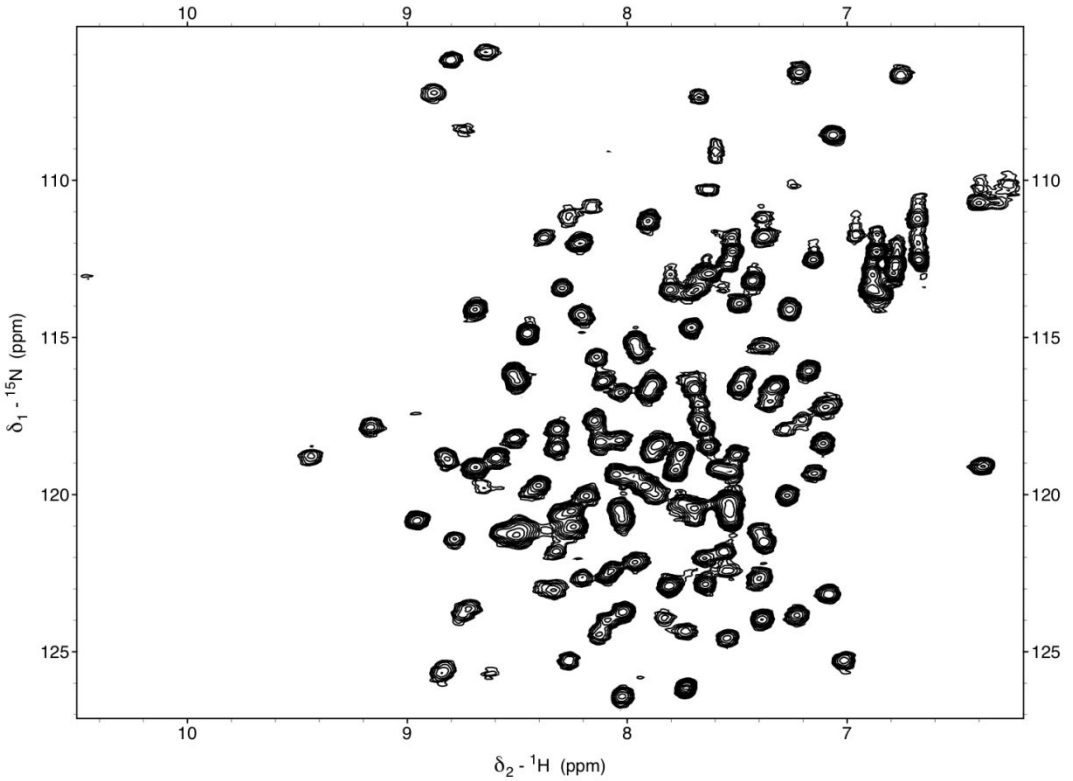
Examples

1D proton spectrum of a protein



^{1}H 1D, Cavanagh et al., Protein NMR Spectroscopy, 2007

^{1}H-^{15}N HSQC of a well folded protein at 293 K with approximately 155 amino acids, 600MHz



The End

Thank you for your attention

Questions, comments?

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