NMR-spectroscopy of proteins in solution - an introduction

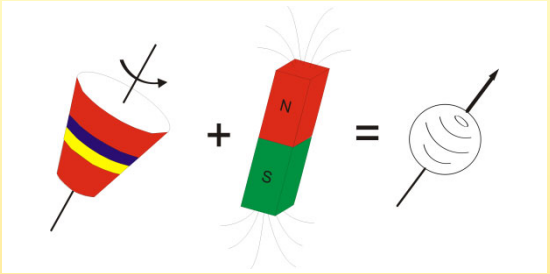
Peter Schmieder

Program

NMR-spectroscopy of proteins in solution

Part 1 (Introduction):

Basic aspects of NMR-spectroscopy

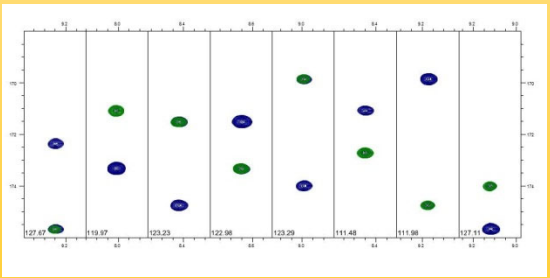


Part 2 (Demonstration):

Visit of the spectrometer

Part 3 (Exercise):

Sequence specific assignment of a protein stretch



Program

NMR-spectroscopy of proteins in solution
- an introduction

- General aspects of NMR-spectroscopy
- Basic aspects of NMR-spectroscopy
- NMR parameter
- Multidimensional NMR

NMR-spectroscopy of proteins in solution
- spectra and assignment

- NMR-spectroscopy of proteins
- Multidimensional NMR with more than two dimensions
- Sequence specific assignment of proteins using tripel-resonance-techniques

General aspects of NMR-spectroscopy

General aspects of NMR-spectroscopy

Nuclear Magnetic Resonance

NMR-spectroscopy detects the resonance of a property of the atomic nuclei (the "spin") with radio waves. The effect is only readily observable in a strong magnetic field. Each nucleus is observed separately and interactions between nuclei can be observed as well.

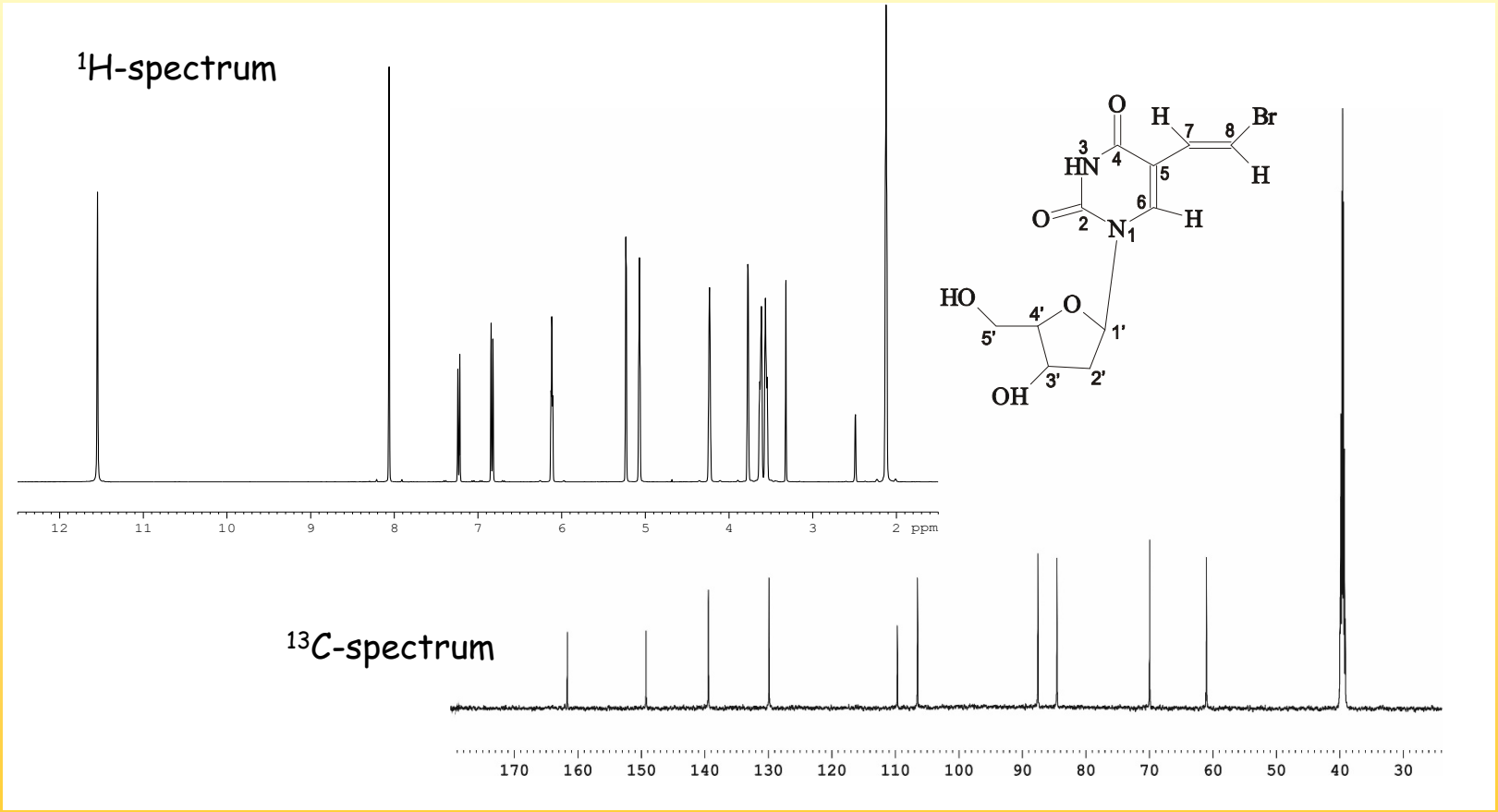
The picture of a molecule provided by NMR thus corresponds well to the view of a chemist that is seeing molecules as atoms connected by bonds.

In the areas of biochemistry and structural biology NMR yields information on structure, ligand-interaction and mobility necessarily at atomic resolution.

NMR is the only high-resolution method that can observe hydrogens !

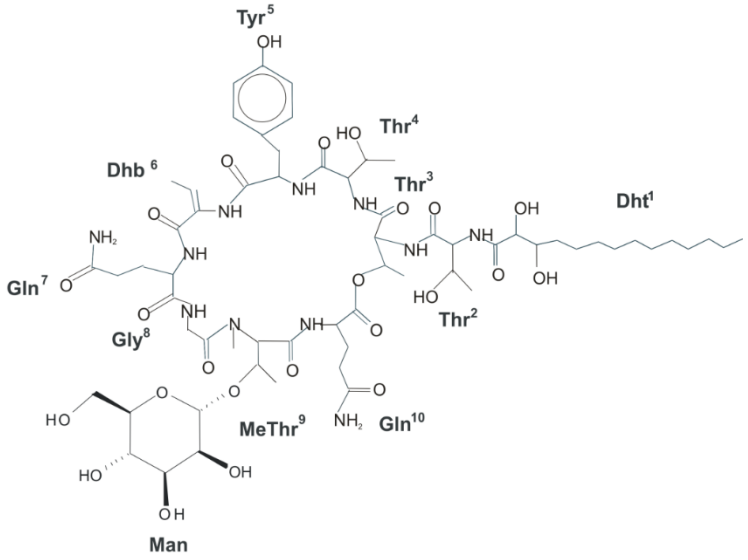
General aspects of NMR-spectroscopy

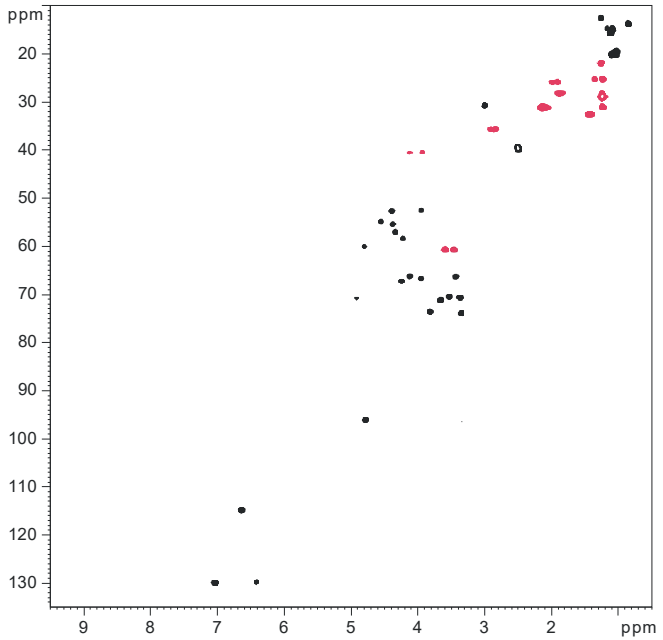
NMR as an analytic method during synthetic work



General aspects of NMR-spectroscopy

Determination of the constitution of natural products

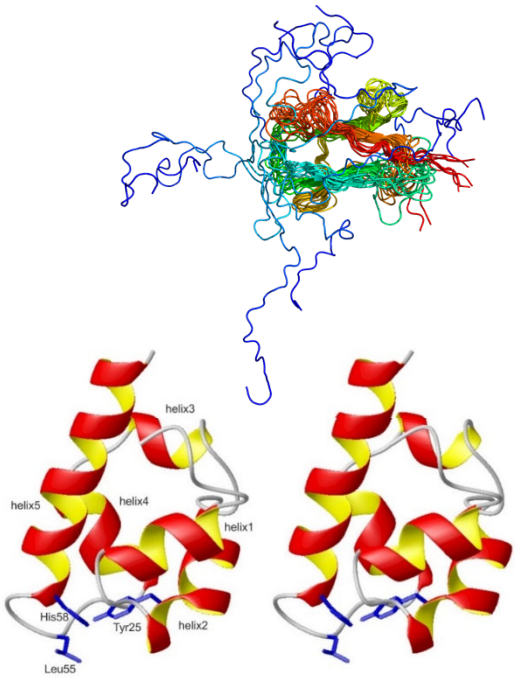


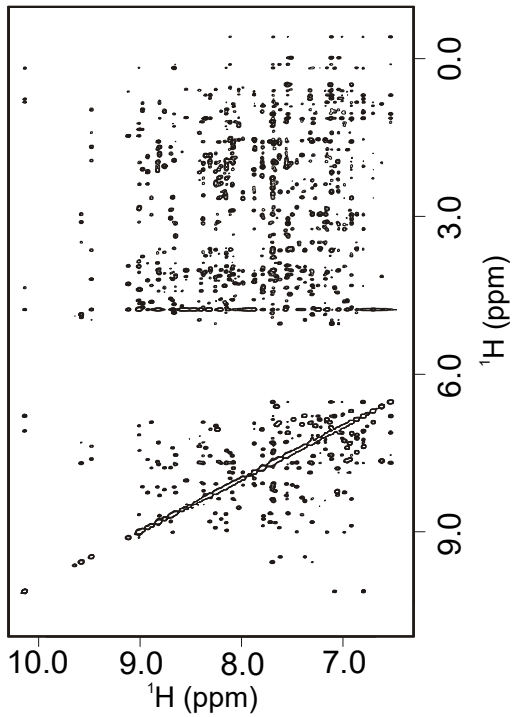


NMR is extremely powerful in determining the constitution of natural products, here 2D-NMR is indispensable.

General aspects of NMR-spectroscopy

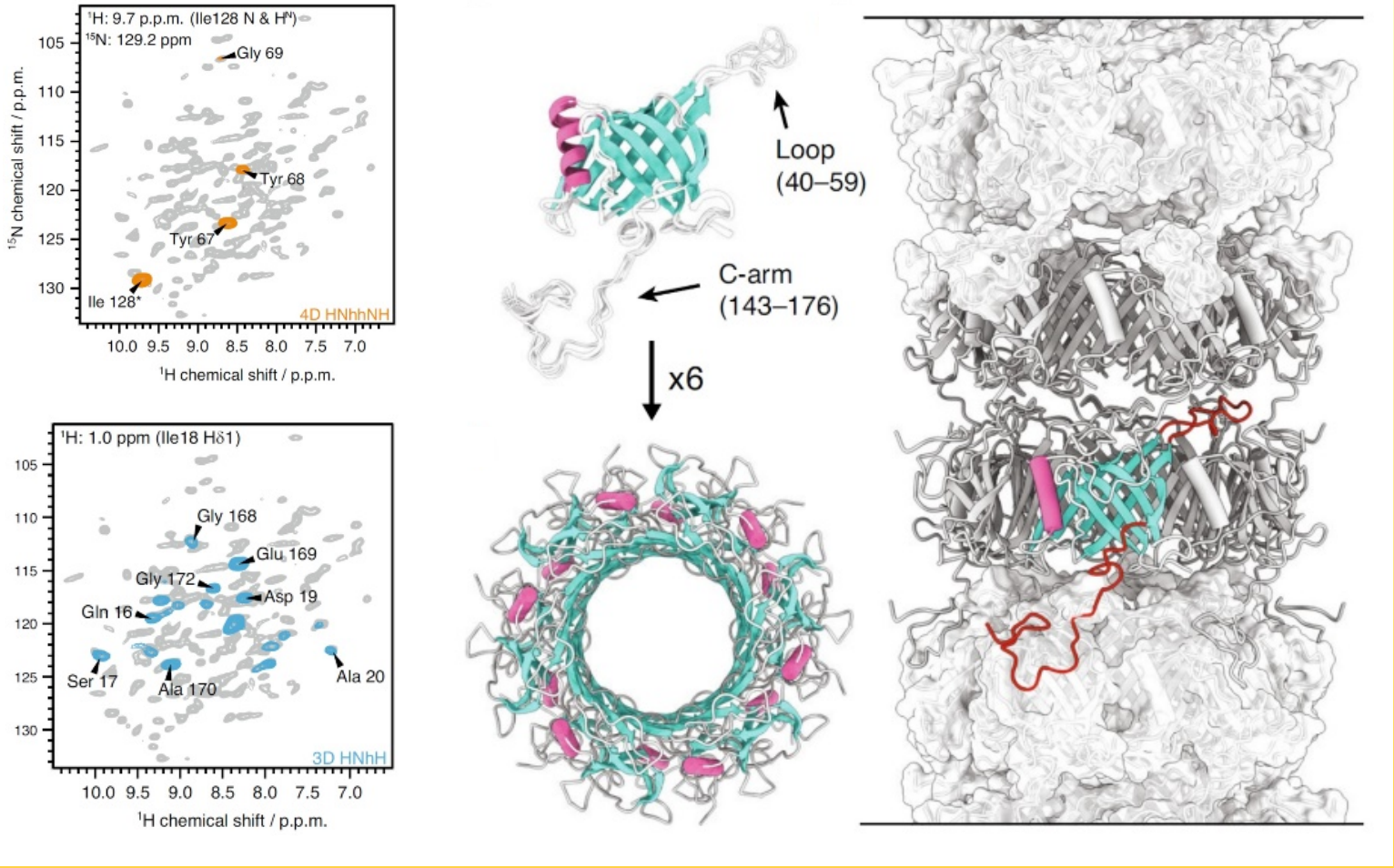
Determination of 3D structures of proteins





Using NMR 3D structures of proteins can be determined either in solution of in the solid state.

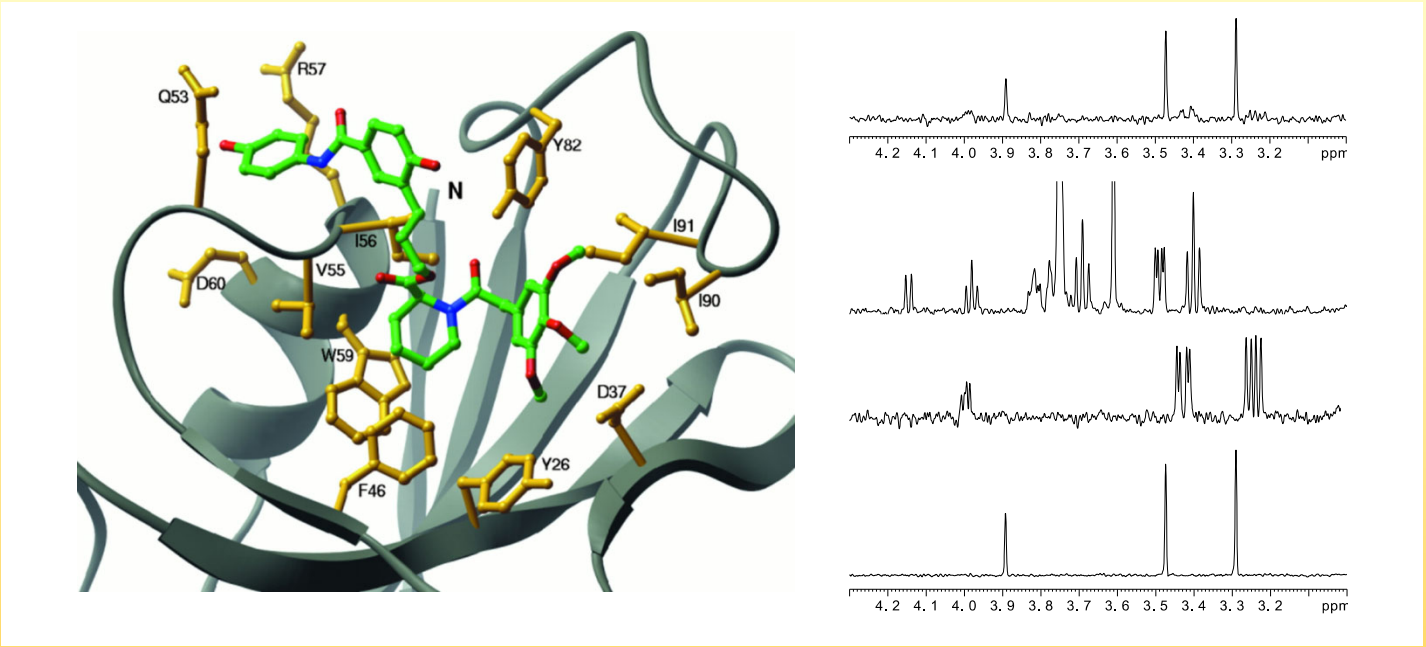
General aspects of NMR-spectroscopy



Integrated structural biology is the combination with other methods

General aspects of NMR-spectroscopy

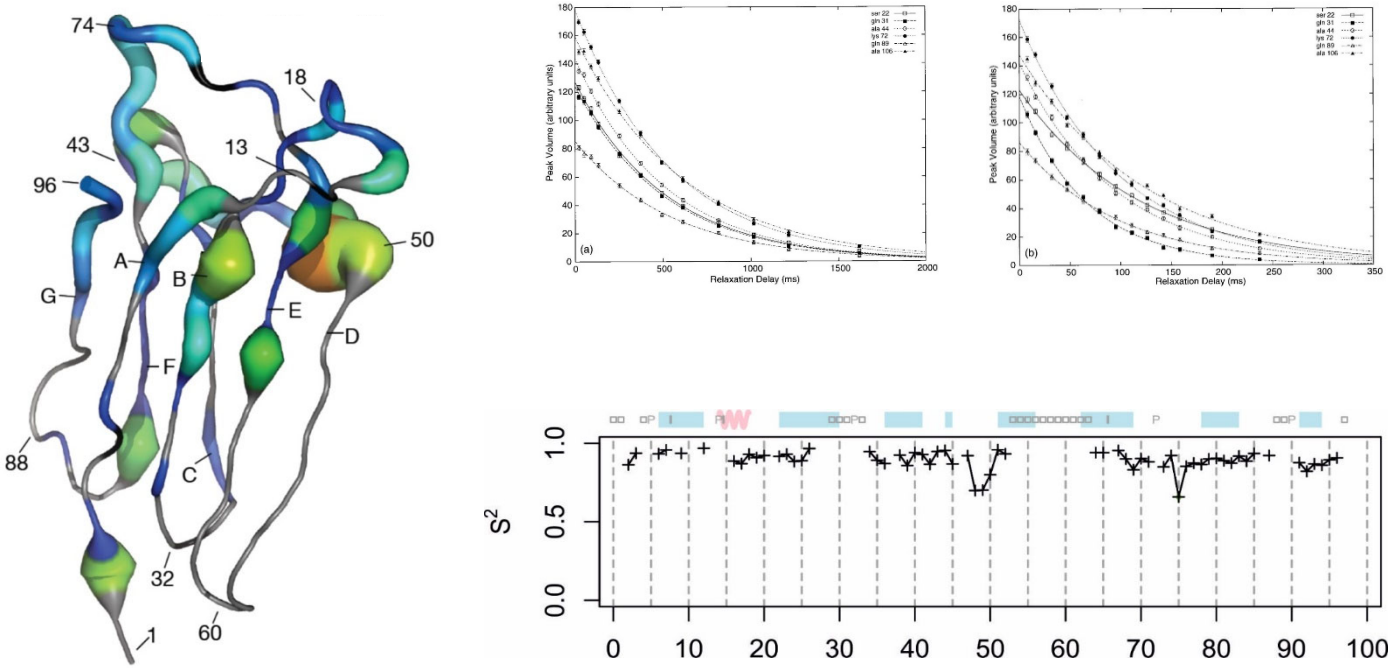
Detection of intermolecular interactions



NMR can be used for the detection of protein-ligand interaction, either for the study of one particular interaction of for the screening of libraries

General aspects of NMR-spectroscopy

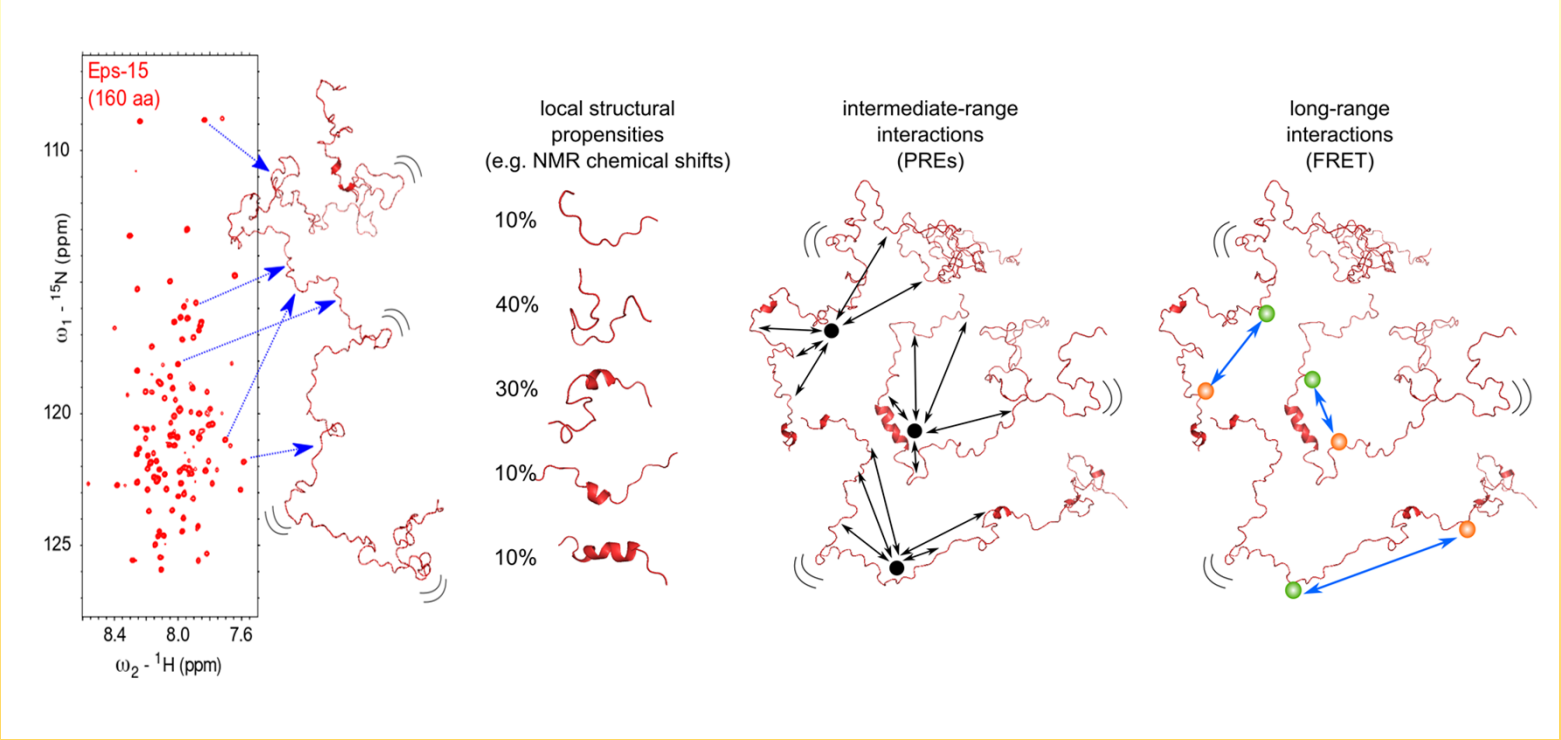
Investigation of dynamic phaenomena



Using NMR the internal dynamics of proteins (and other molecules) can be investigated

General aspects of NMR-spectroscopy

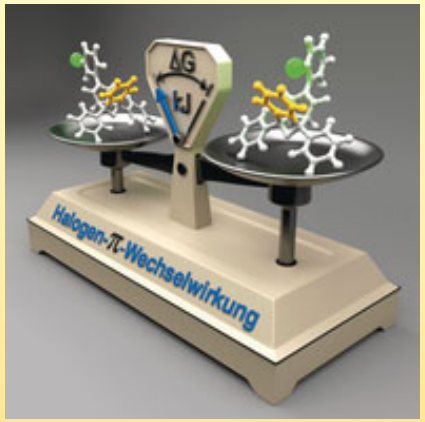
Investigation of IDPs

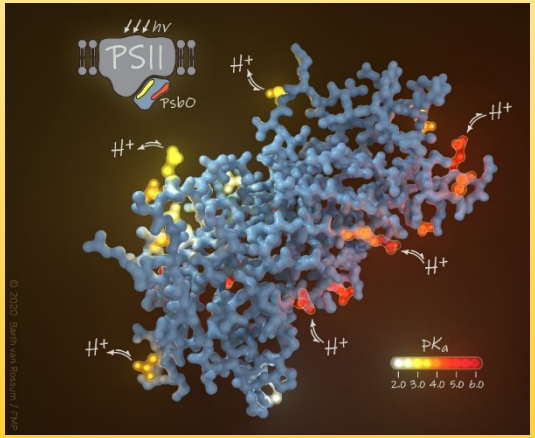


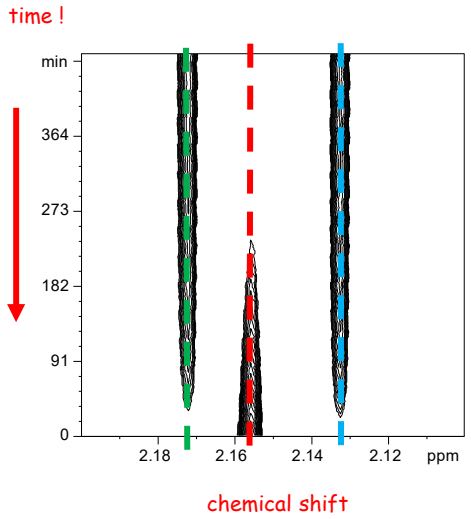
IDPs that are difficult to handle with methods for structure determination can be investigated by NMR combined with optical techniques

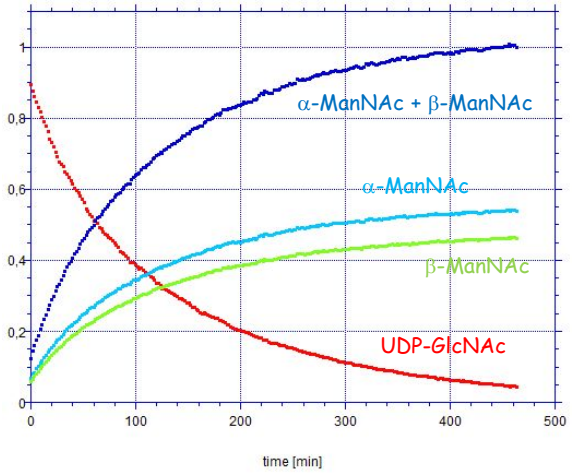
General aspects of NMR-spectroscopy

Determination of thermodynamic parameters





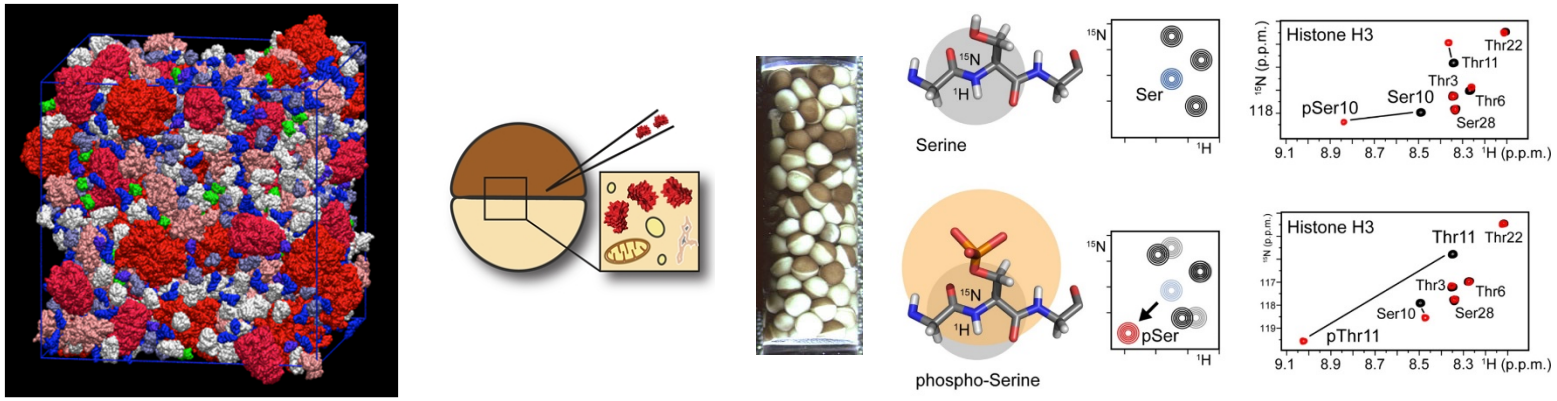




Using NMR we can follow reaction, determine equilibrium constants and pKa values

General aspects of NMR-spectroscopy

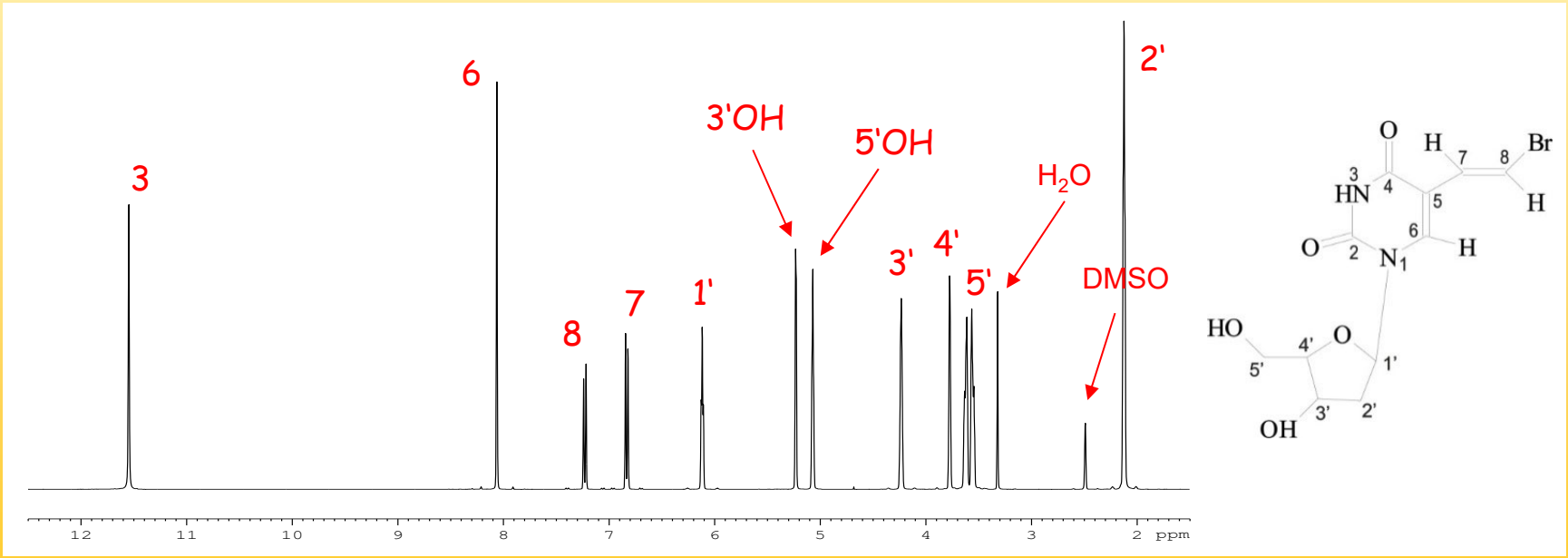
Detection of processes in living cells (in-cell-NMR)



Using in-cell-NMR-spectroscopy it is possible to follow the modifications of proteins in living cells (e.g. phosphorylation).

General aspects of NMR-spectroscopy

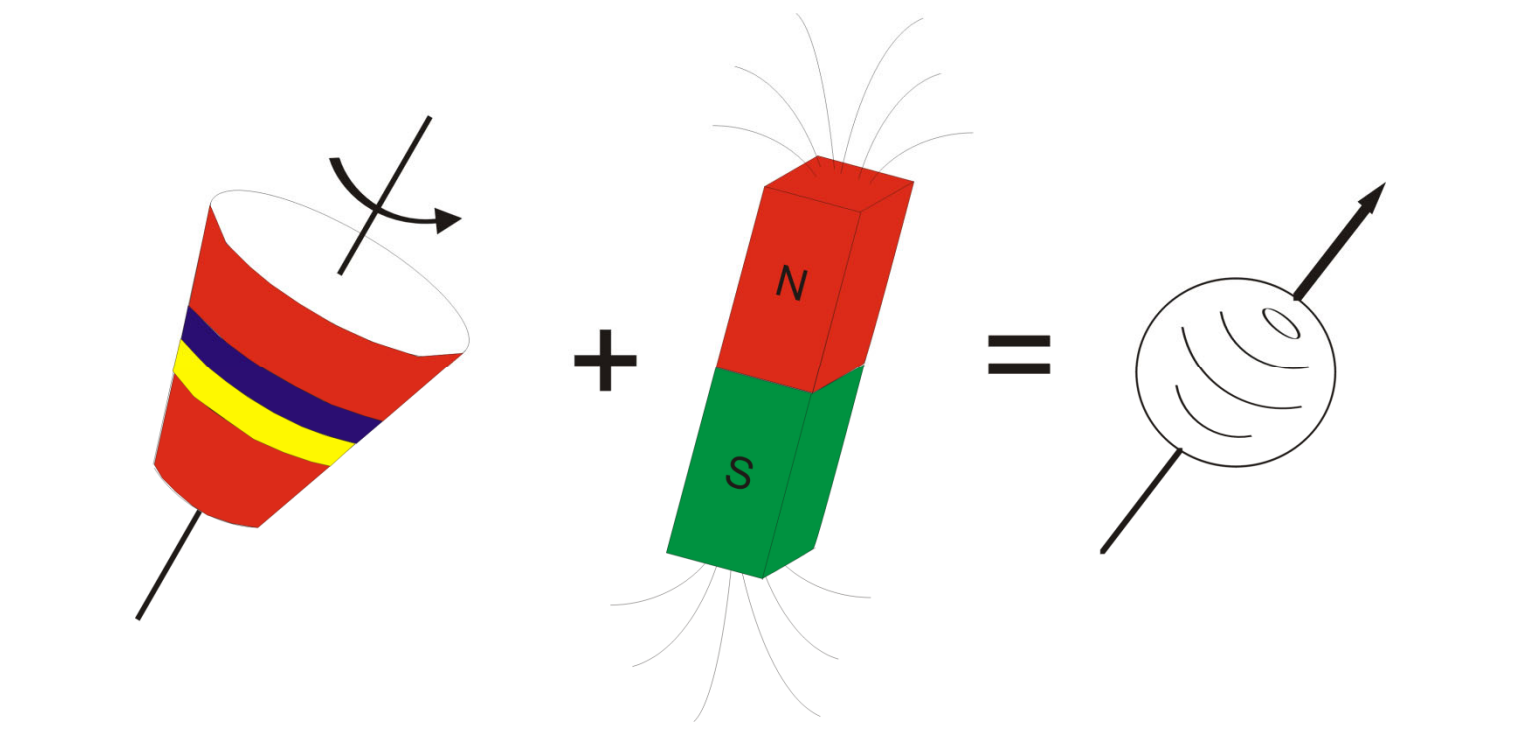
To use the information obtained at atomic resolution, we need to know which nucleus correlates with which resonance line. This essential process of determining that correlation is called „assignment".



Basic aspects of NMR-spectroscopy

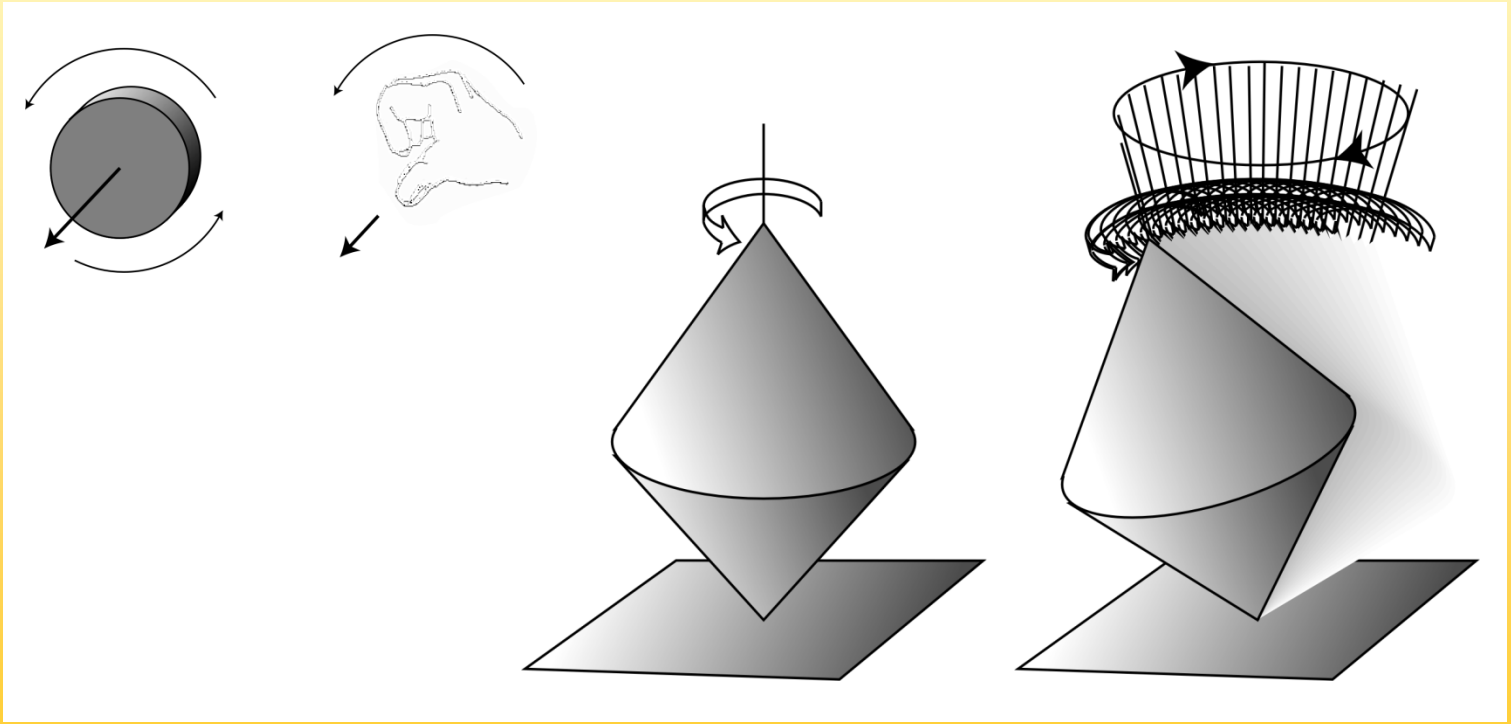
Basic aspects of NMR-spectroscopy

Prerequisite for NMR-spectroscopy is a nuclear spin that can be thought of as a mixture of a gyroscope and a little magnet



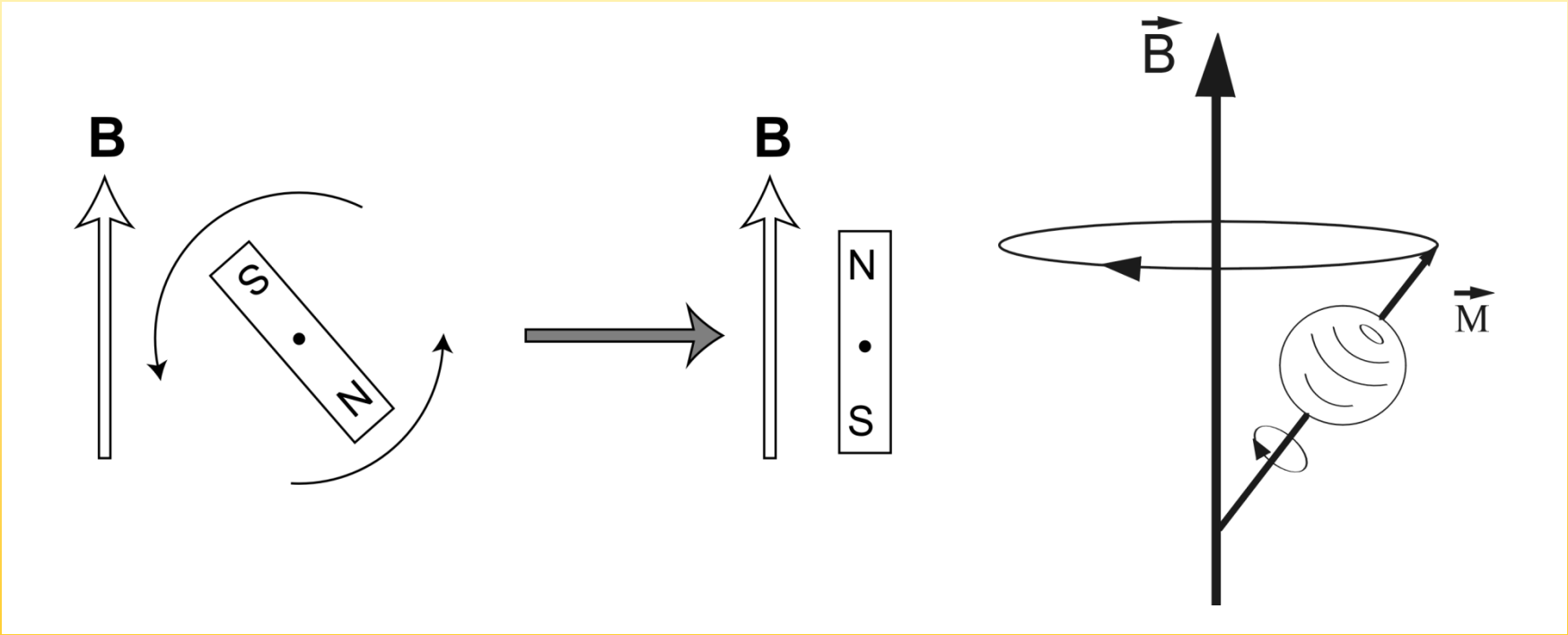
Basic aspects of NMR-spectroscopy

A gyroscope has an angular momentum that is firmly oriented in space



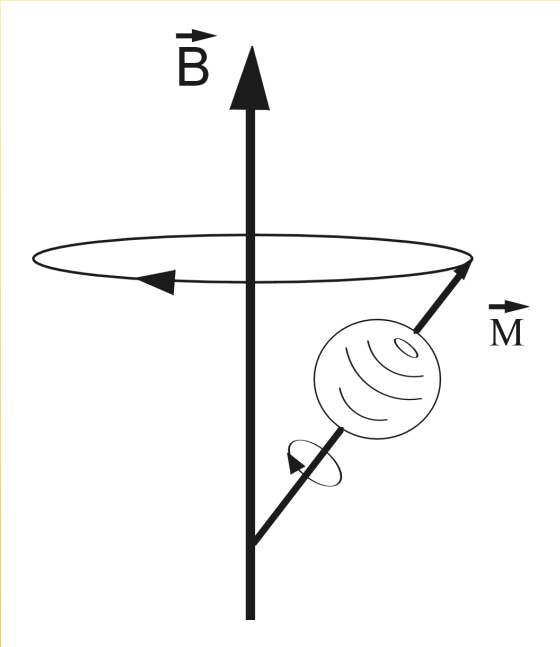
Basic aspects of NMR-spectroscopy

Orientation of the little nuclear magnet is prevented by its gyroscopic properties, the nucleus starts a precessional motion



Basic aspects of NMR-spectroscopy

The resonance frequency of the spins (here the proton spins) is determined by the strength of the magnetic field

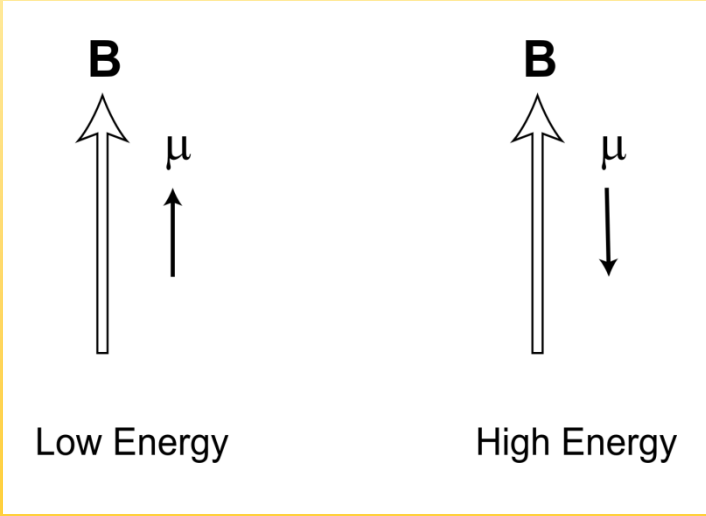


B_0 [Tesla]	ν_0 [MHz]
1.4	60
5.9	250
9.4	400
14.1	600
21.2	900

Basic aspects of NMR-spectroscopy

But we are dealing with a quantum mechanical phaenomenon, in the case that we are interested in (high resolution NMR) there are two possible orientations (α and β) for the gyroscope/magnet=spin

$$\Delta E = \hbar \gamma B_0$$



Basic aspects of NMR-spectroscopy

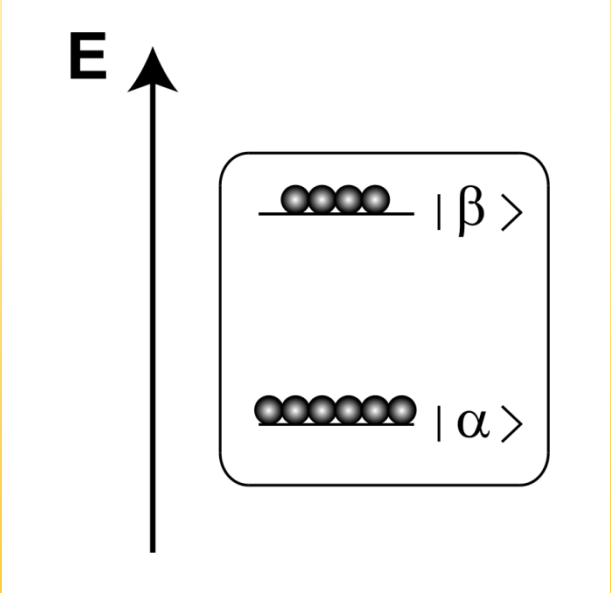
We will then have a Boltzmann-distribution

$$N_\beta/N_\alpha = \exp(-\Delta E/kT) = \exp(-\gamma\, h\, B_0 / 2\pi kT)$$

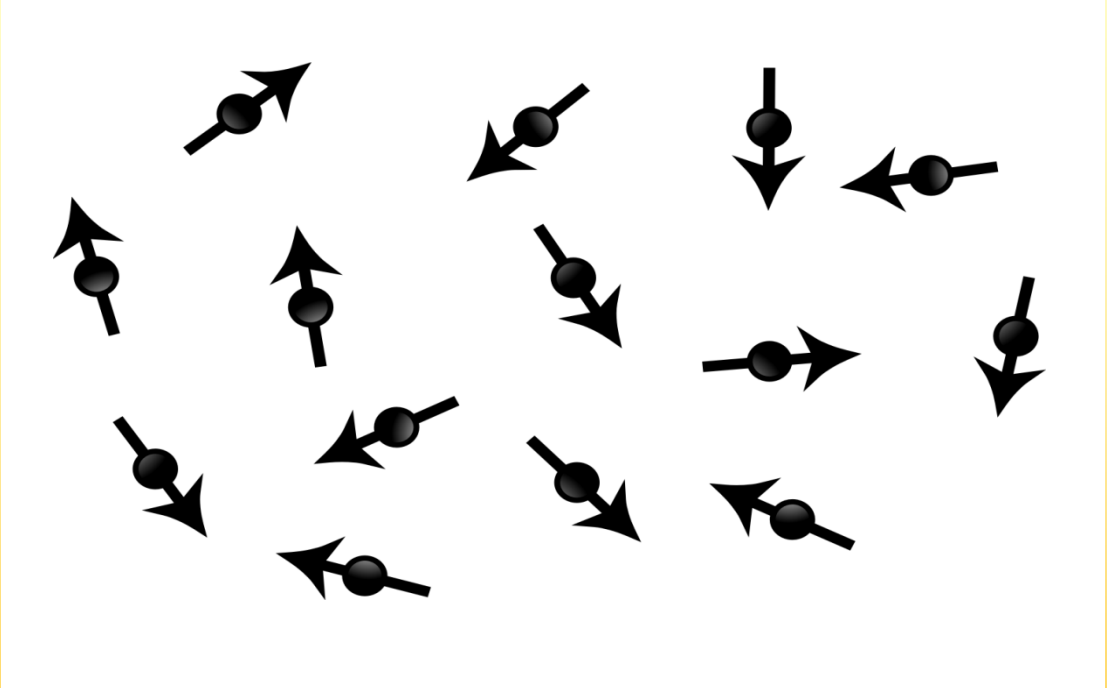
At 600 MHz frequency we get

$N_\beta/N_\alpha = 0.999904$

This extremely small difference is the reason for the low sensitivity of NMR spectroscopy

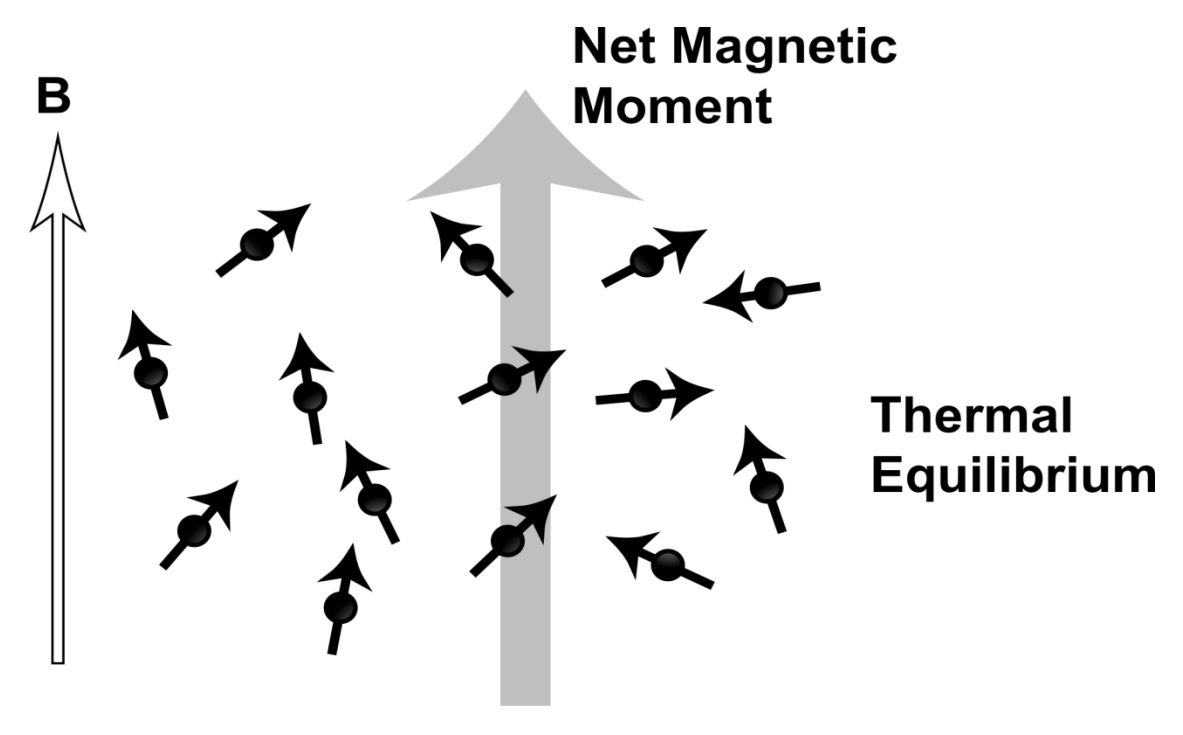


Basic aspects of NMR-spectroscopy



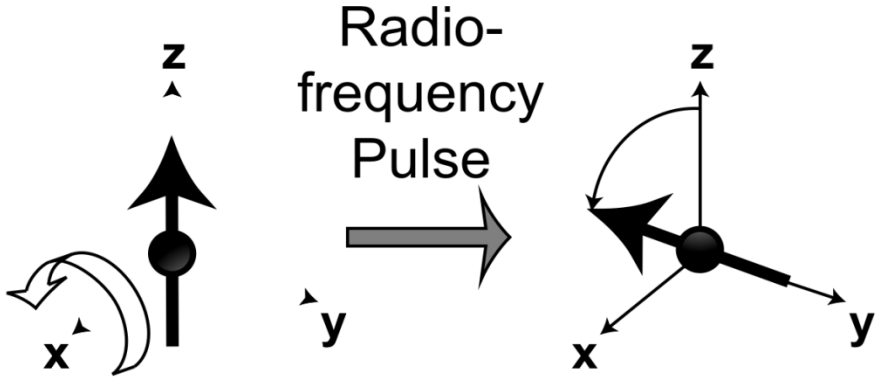
Without an external magnetic field all orientations are equal and the spins are randomly oriented

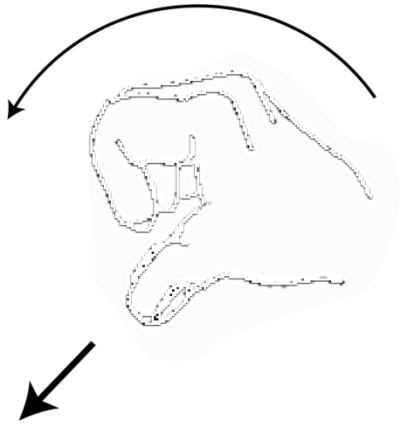
Basic aspects of NMR-spectroscopy

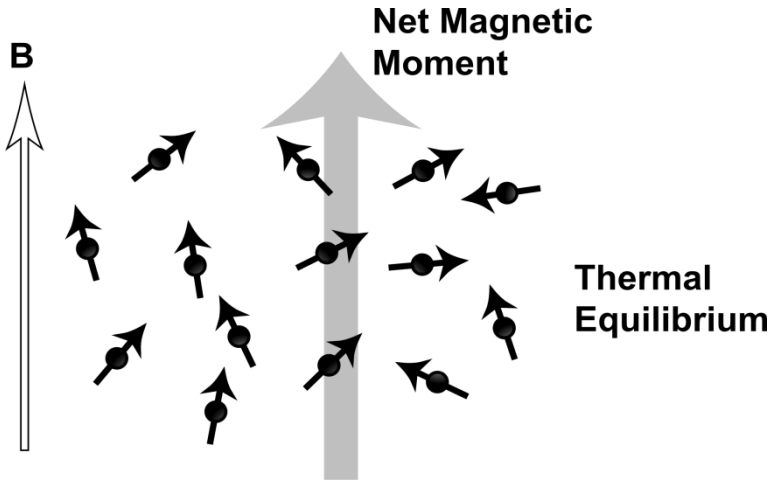


With an external magnetic field the resulting orientation yields a small magnetic moment, a small „macroscopic magnet", the axis is called the z-axis

Basic aspects of NMR-spectroscopy



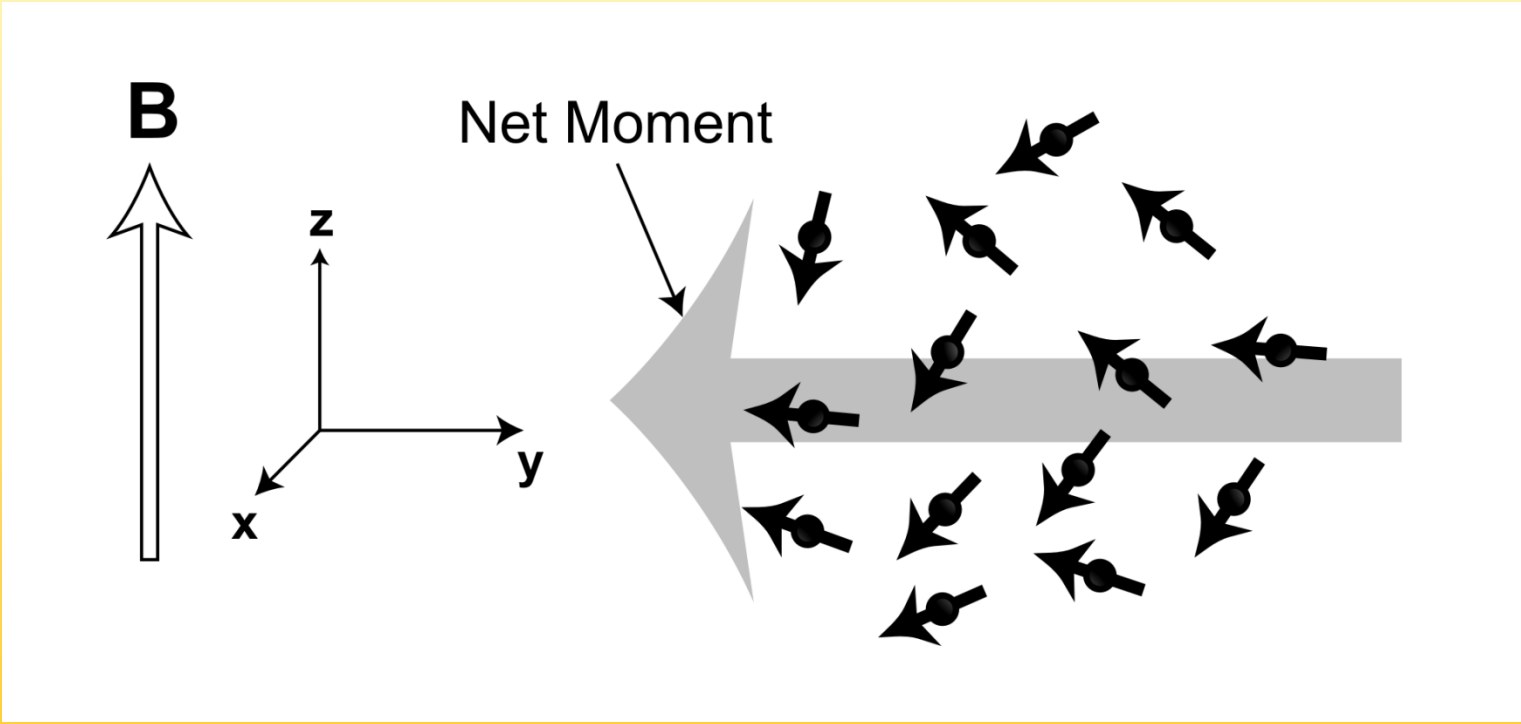




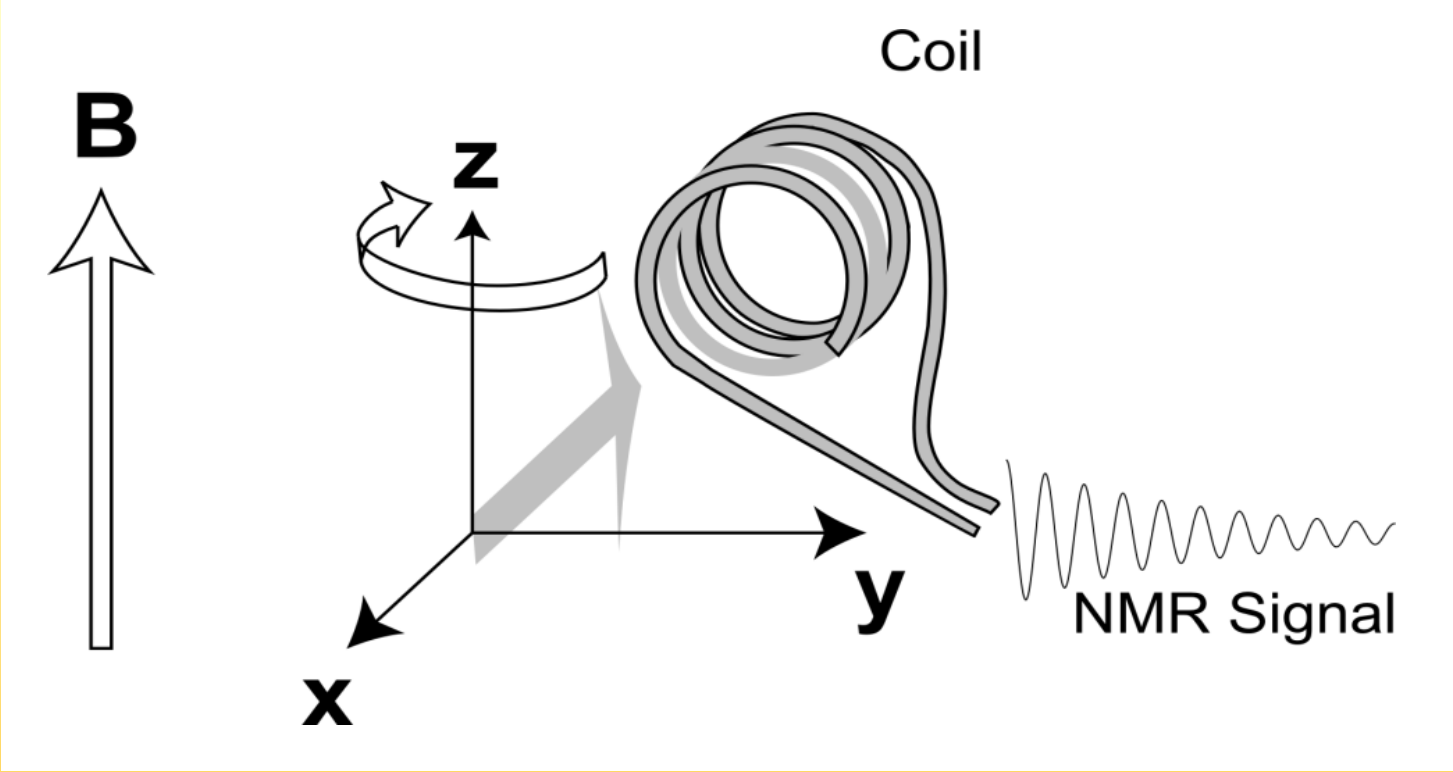
To do the experiment a radio frequency (RF) pulse turns every spin

Basic aspects of NMR-spectroscopy

which results in a rotation of the magnetic moment into the x,y-plane, no z-magnetization is left

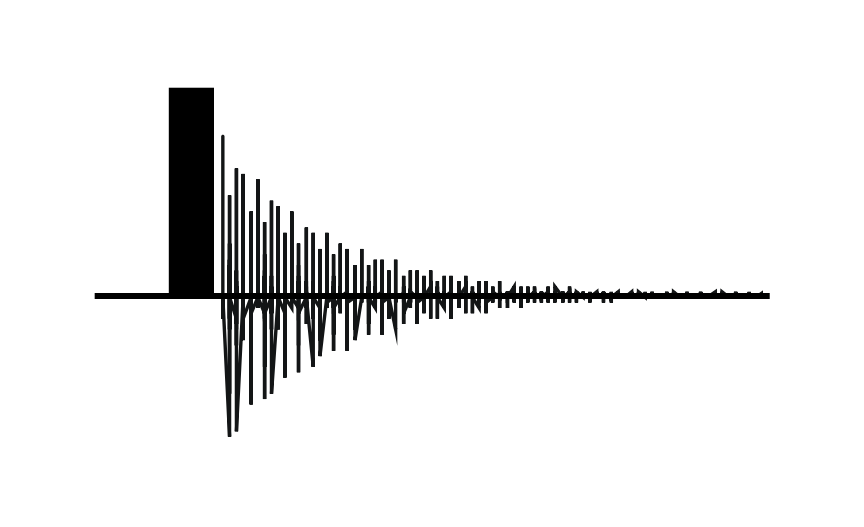


Multidimensional NMR-spectroscopy

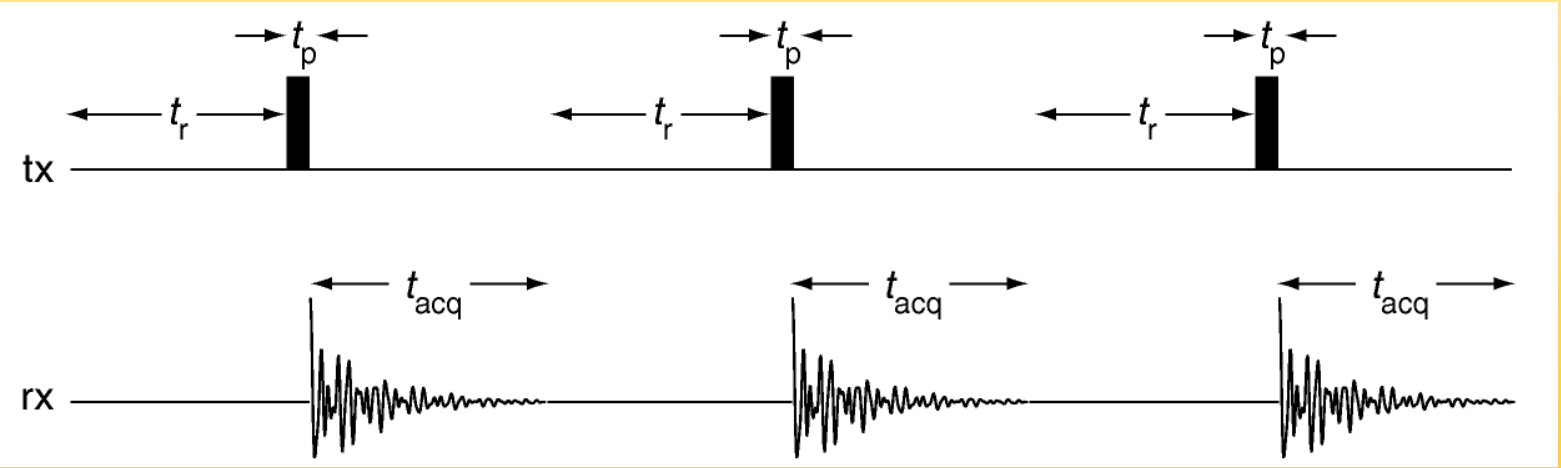


The precession that is still going on induces a current in the detection coil, the resulting signal is recorded

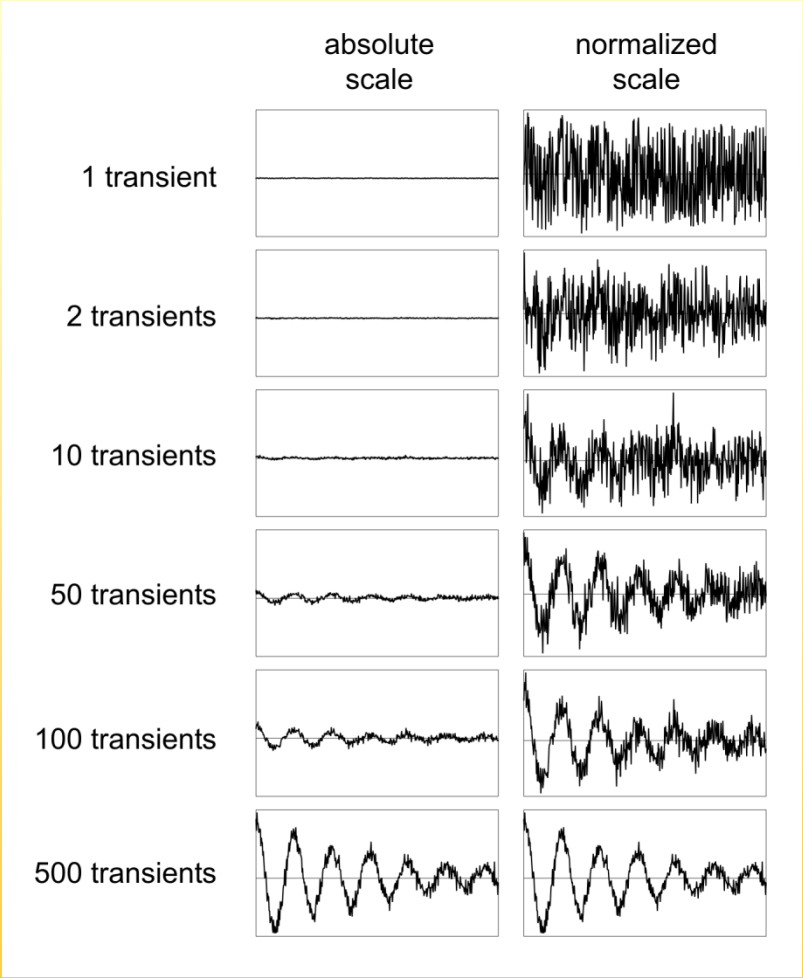
Basic aspects of NMR-spectroscopy



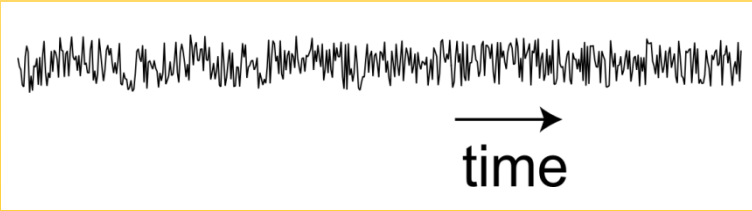
Thus the RF pulse starts the measurement which is then repeated…



NMR-parameter

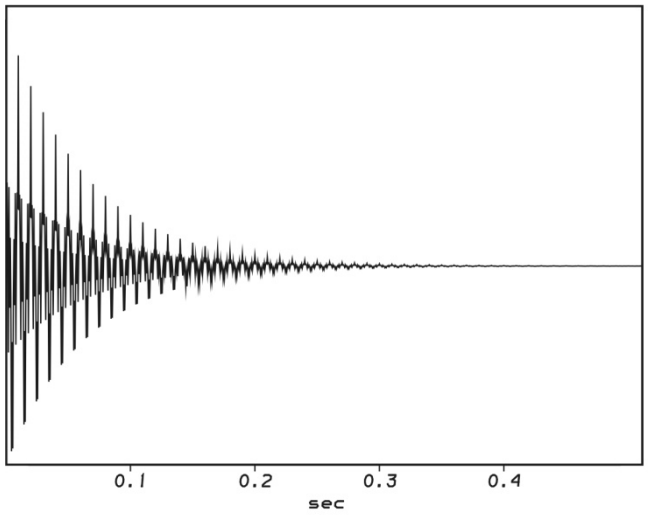


.... to get better signal-to-noise.

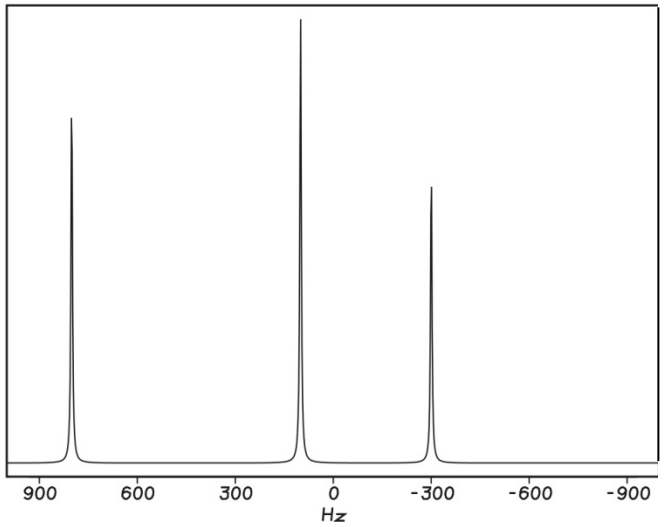


Basic aspects of NMR-spectroscopy

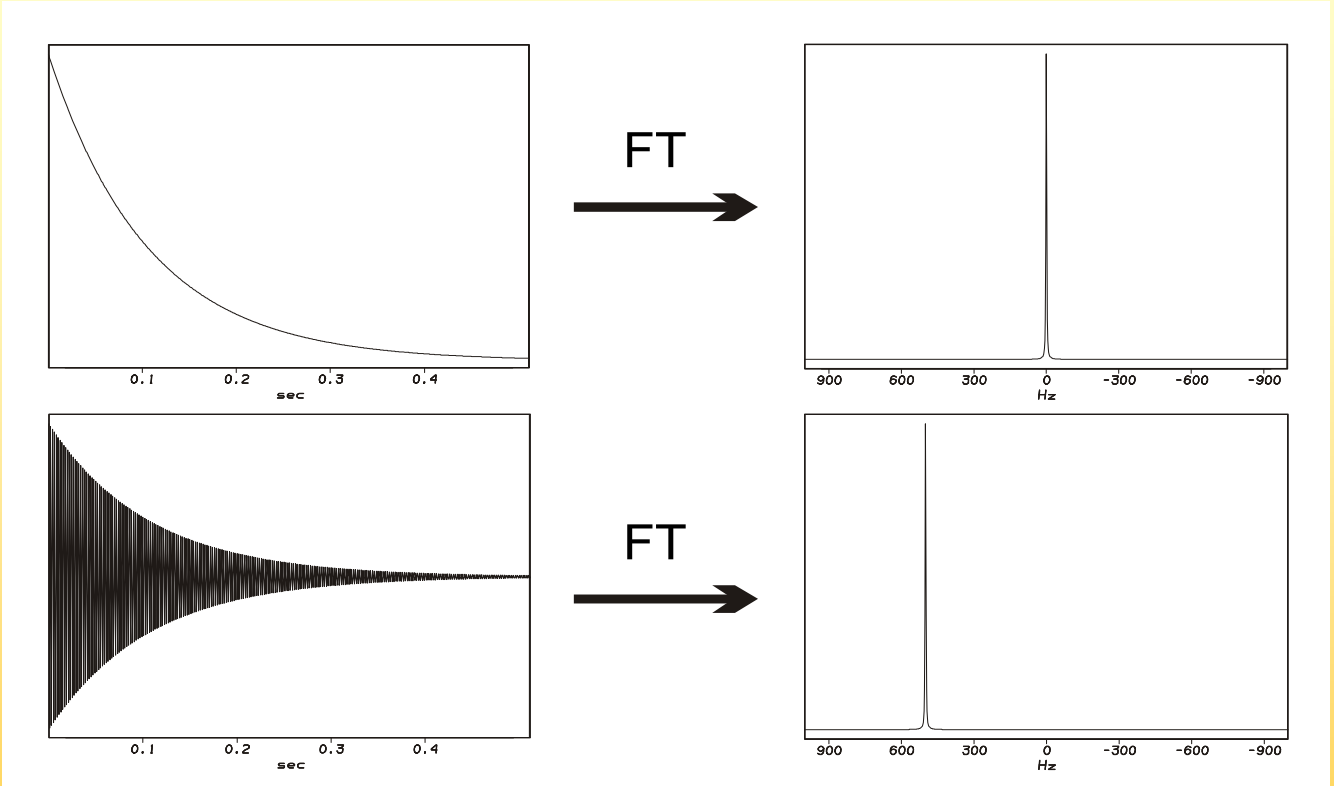
The detected time signal (the FID) is converted into a frequency spectrum by Fourier transform



FT

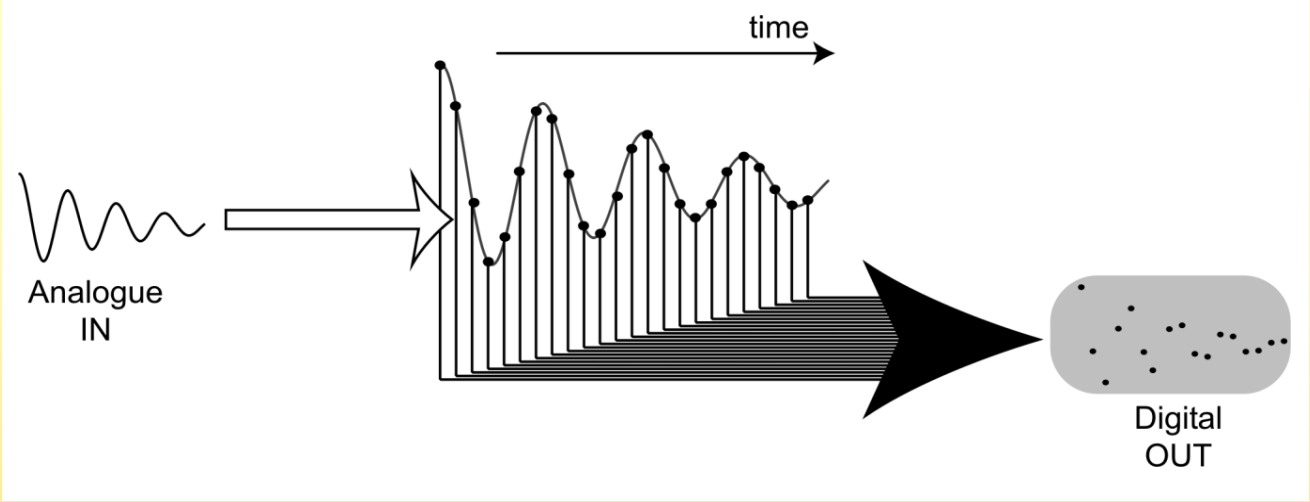


Basic aspects of NMR-spectroscopy

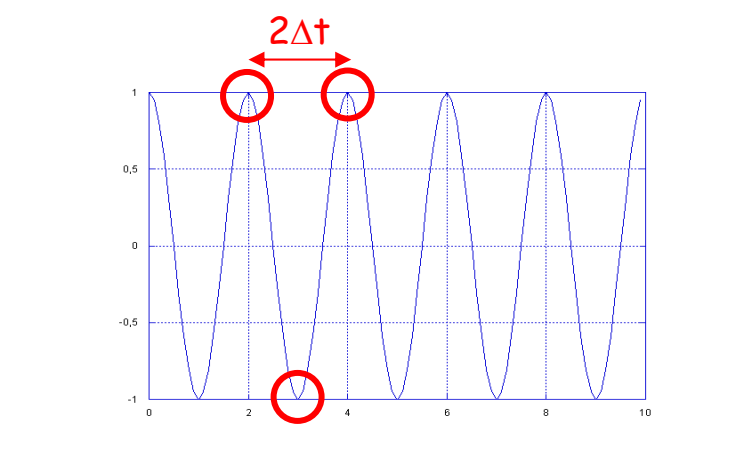


The decay determines the shape of the peak, the oscillation its position in the spectrum

Basic aspects of NMR-spectroscopy

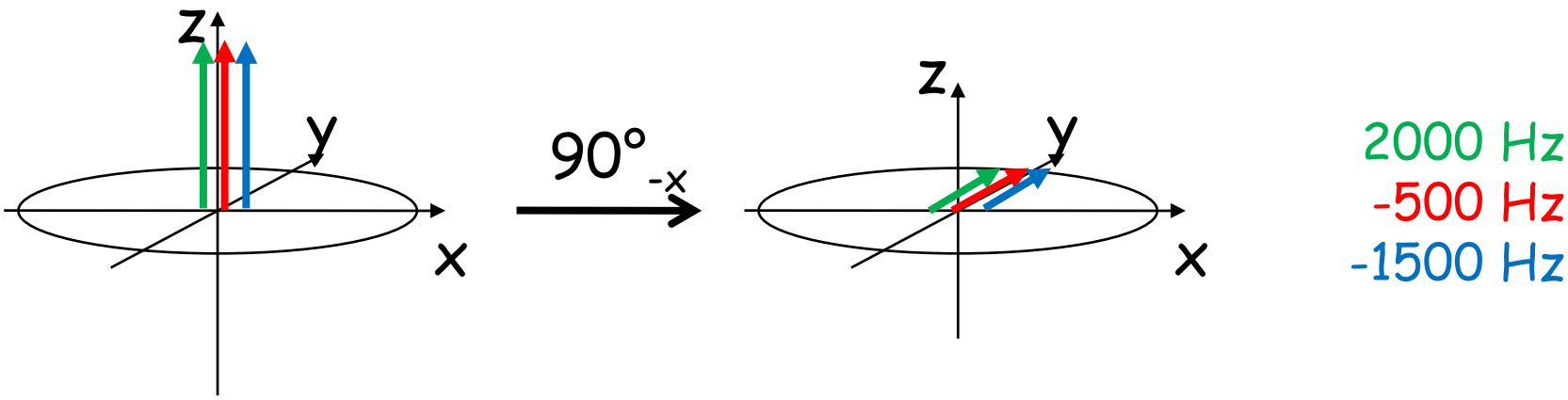


To perform the FT on a computer they need to be digitized which introduces some constraints on the experiments



Multidimensional NMR-spectroscopy

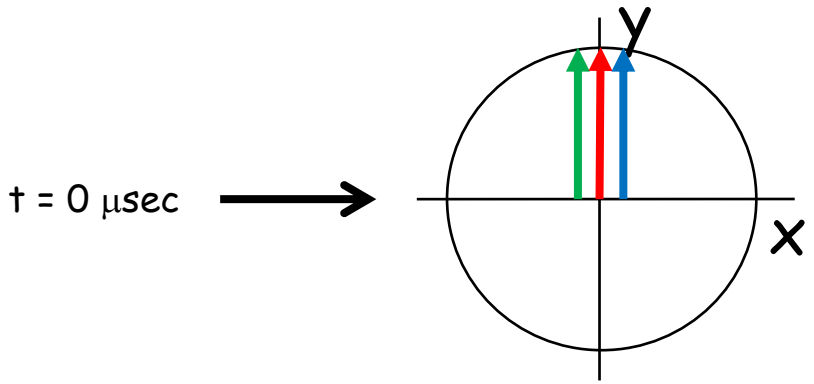
We take a closer look at that



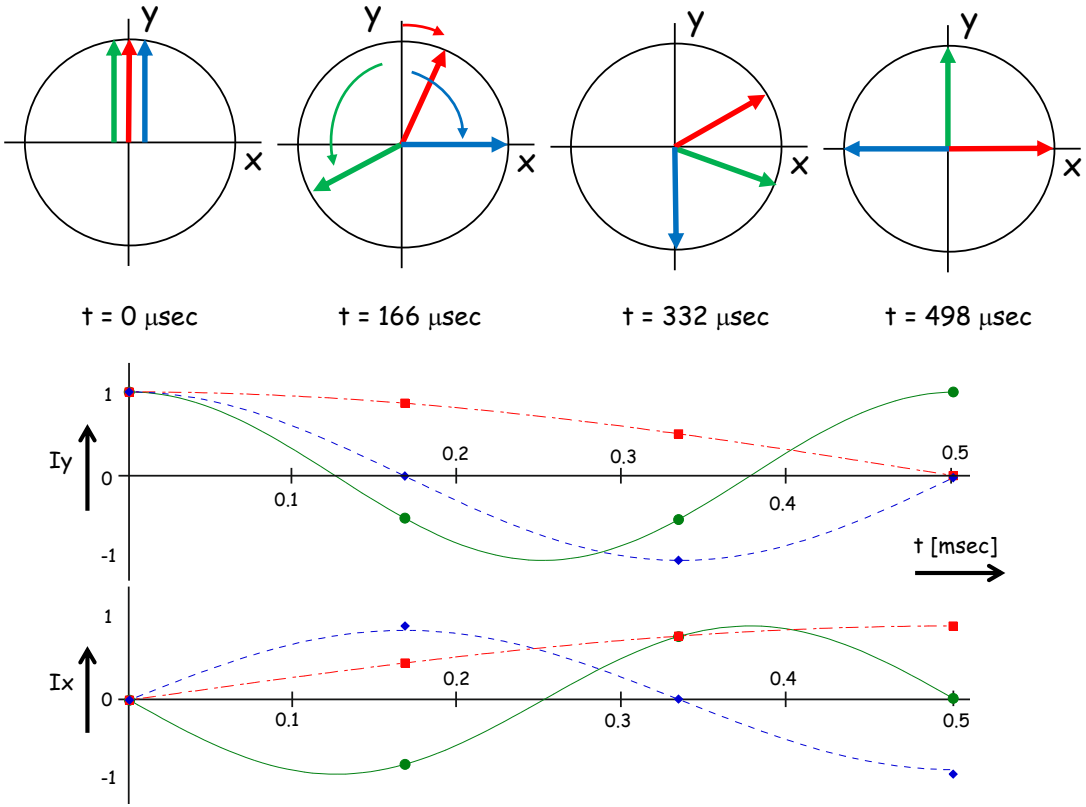
We record a spectrum using

SW = 6000 Hz (+/- 3000),

which means a Δt = 166 µsec

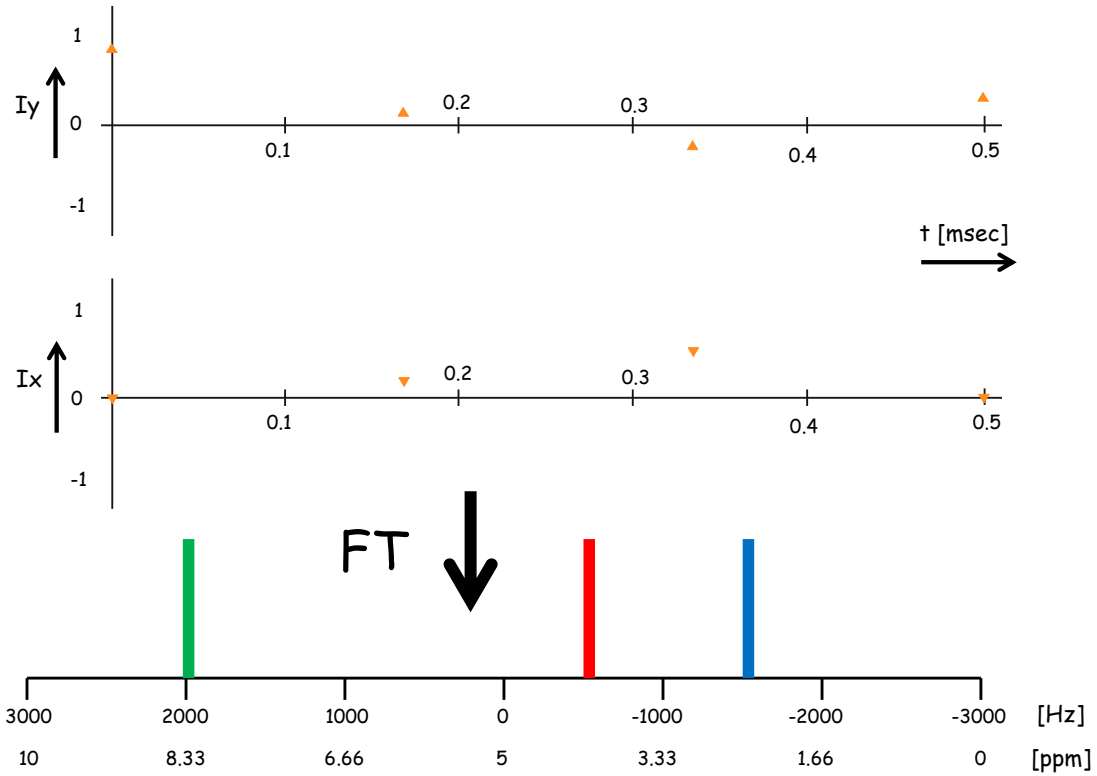


Multidimensional NMR-spectroscopy



One can see that the edges of the spectrum (+/- 3000 Hz) would be 180° apart after 166 µsec

Multidimensional NMR-spectroscopy



Basic aspects of NMR-spectroscopy

Magnetic properties of some NMR nuclei

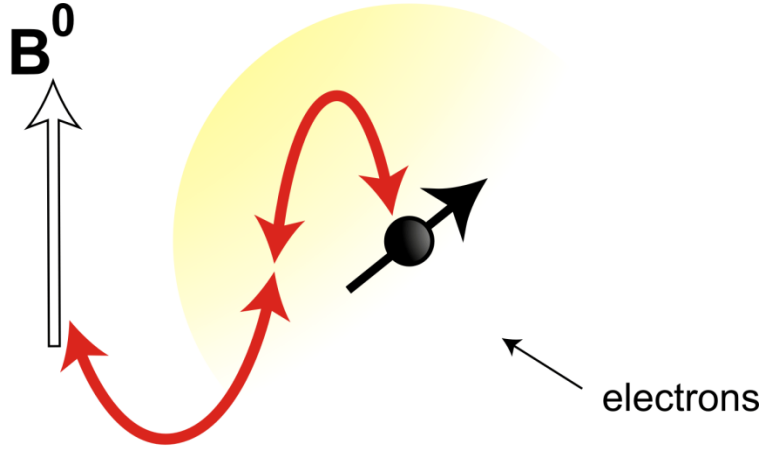
Kern	I	natürliche Häufigkeit	gyromagnetisches Verhältnis
^{1}H	1/2	99.98 %	26.75
^{12}C	0	98.89 %	0
^{13}C	1/2	1.11 %	6.73
^{14}N	1	99.63 %	1.93
^{15}N	1/2	0.37 %	-2.71
^{19}F	1/2	100 %	25.18
^{31}P	1/2	100 %	10.84
^{113}Cd	1/2	12.26 %	-5.96

NMR-Parameter

NMR-parameter

Chemical Shift

The electrons around the nucleus shield it from the external magnetic field, the more electrons there are the less field reaches the nucleus



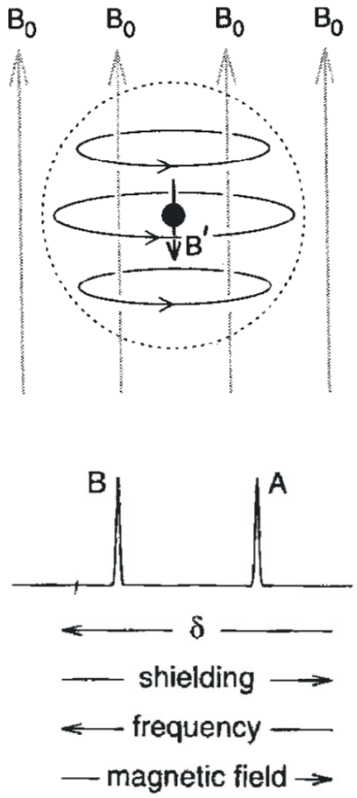
$$B_{eff} = (1 - \sigma)\, B_0$$

$$\omega = \gamma\, (1 - \sigma)\, B_0$$

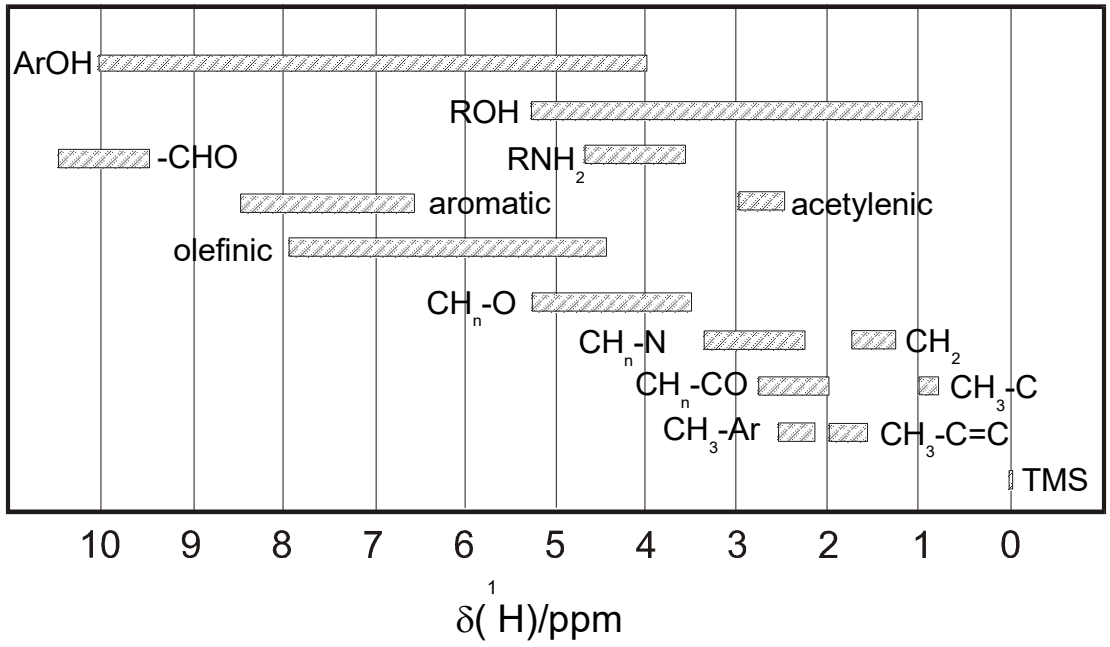
$$\delta = (\omega - \omega_{ref}) / \omega_0 \times 10^6$$

$$= (\sigma_{ref} - \sigma) \times 10^6$$

NMR-parameter



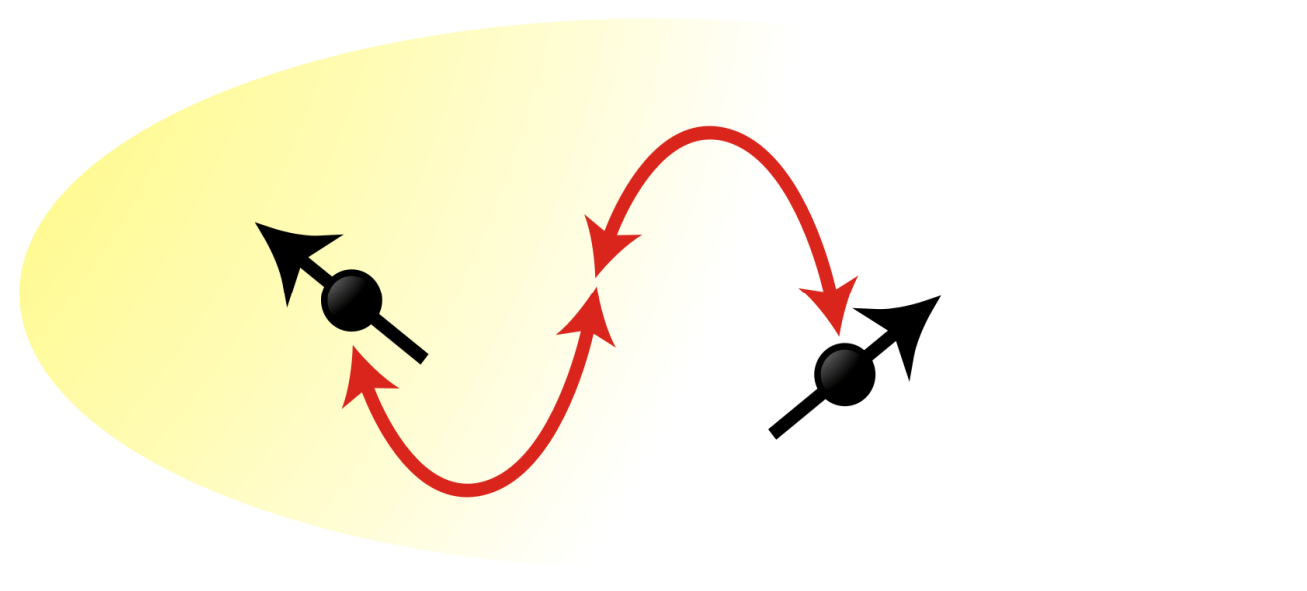
Chemical shift



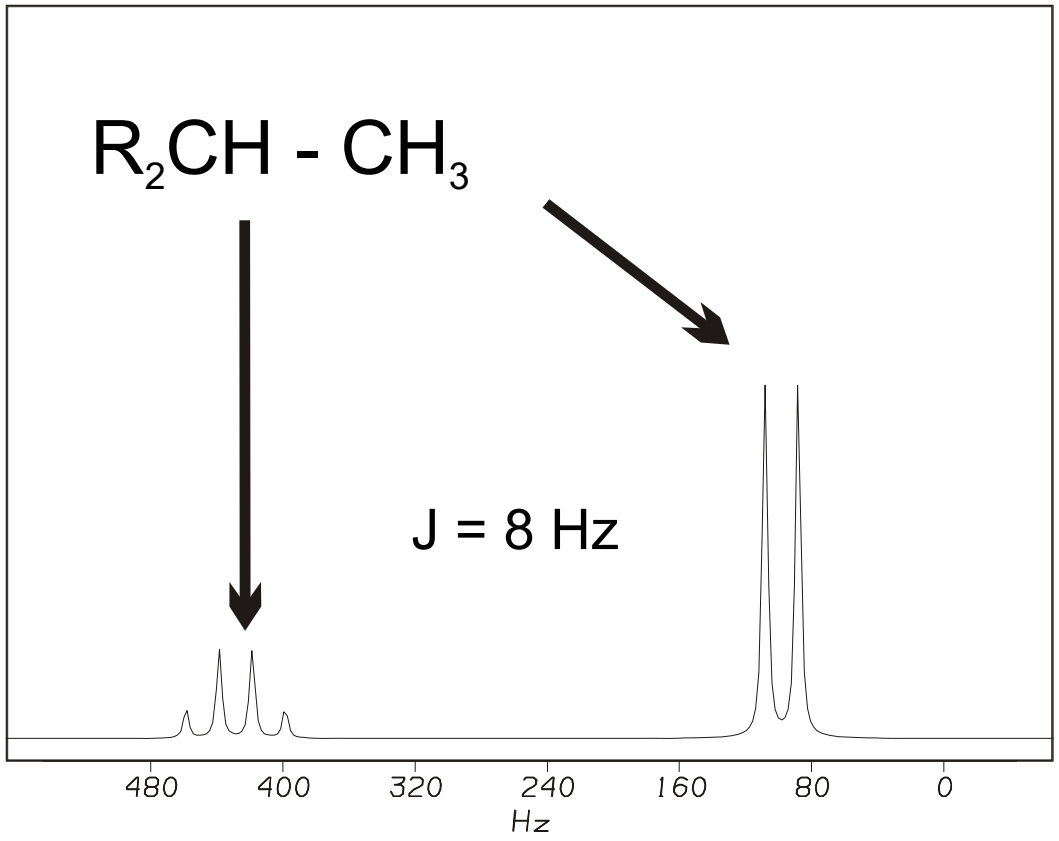
NMR-parameter

Scalar or J-coupling

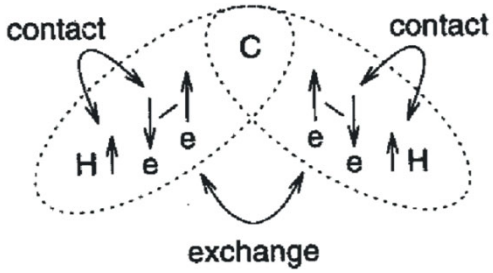
The electrons surrounding the nuclei do also establish an interaction between the nuclei



NMR-parameter

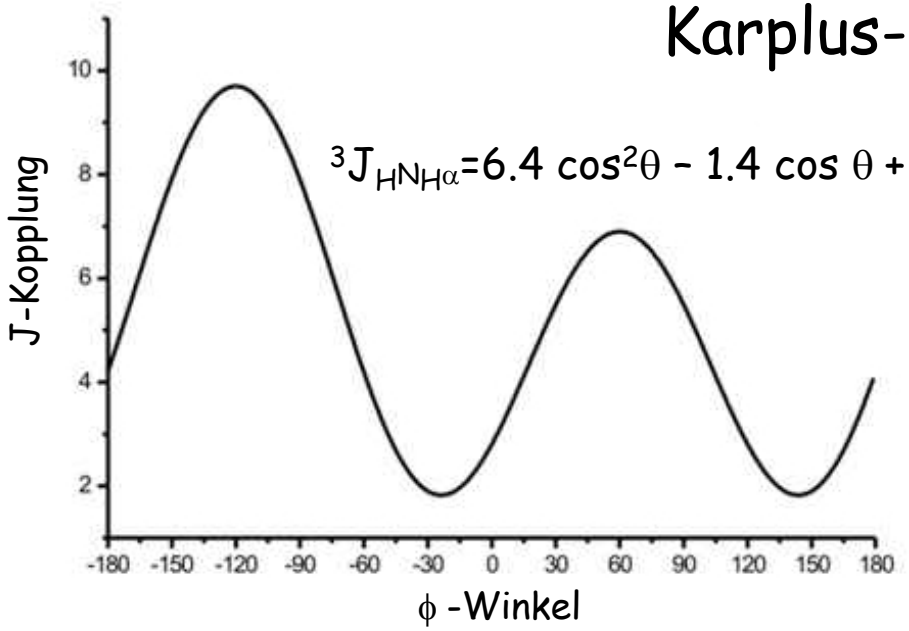


Scalar or J-coupling

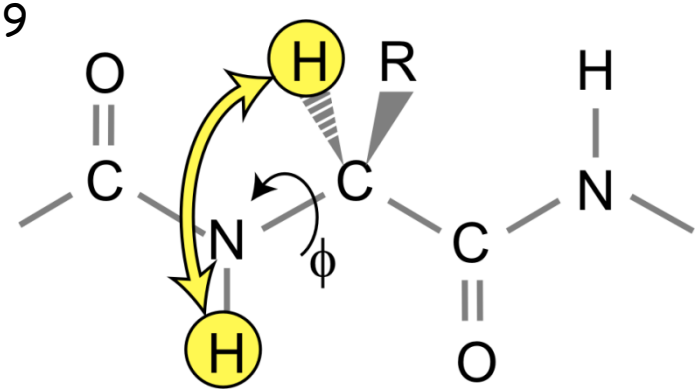


NMR-parameter

Karplus-equation



$^3J_{HNH\alpha}=6.4\ \cos^2\theta - 1.4\ \cos\theta + 1.9$

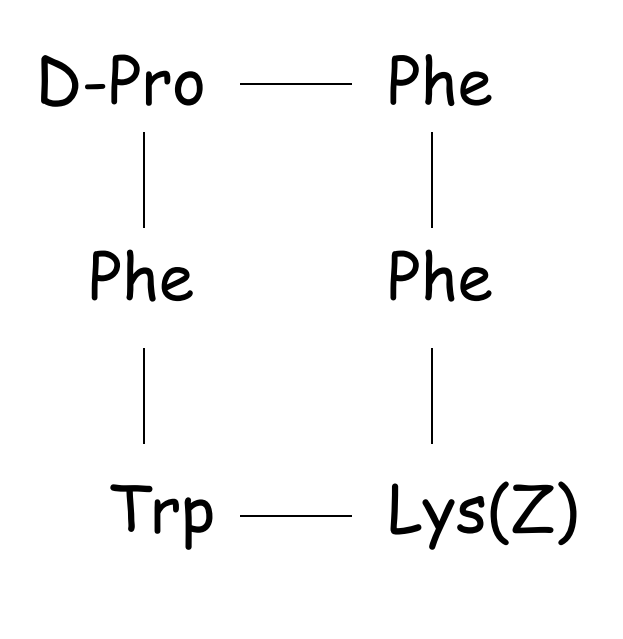


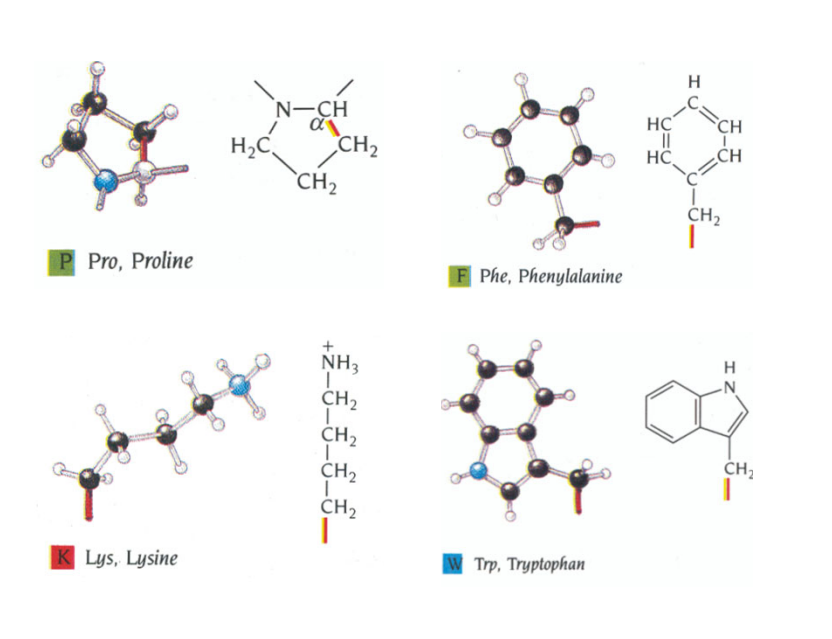
J-couplings yield structural information but are also important for the transfer of magnetization in multidimensional spectra

Multidimensional NMR-spectroscopy

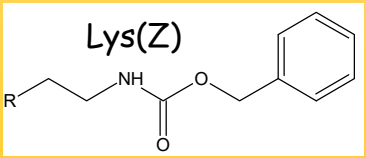
Multidimensional NMR-spectroscopy

Cyclic peptides are small peptides with usually fix conformation



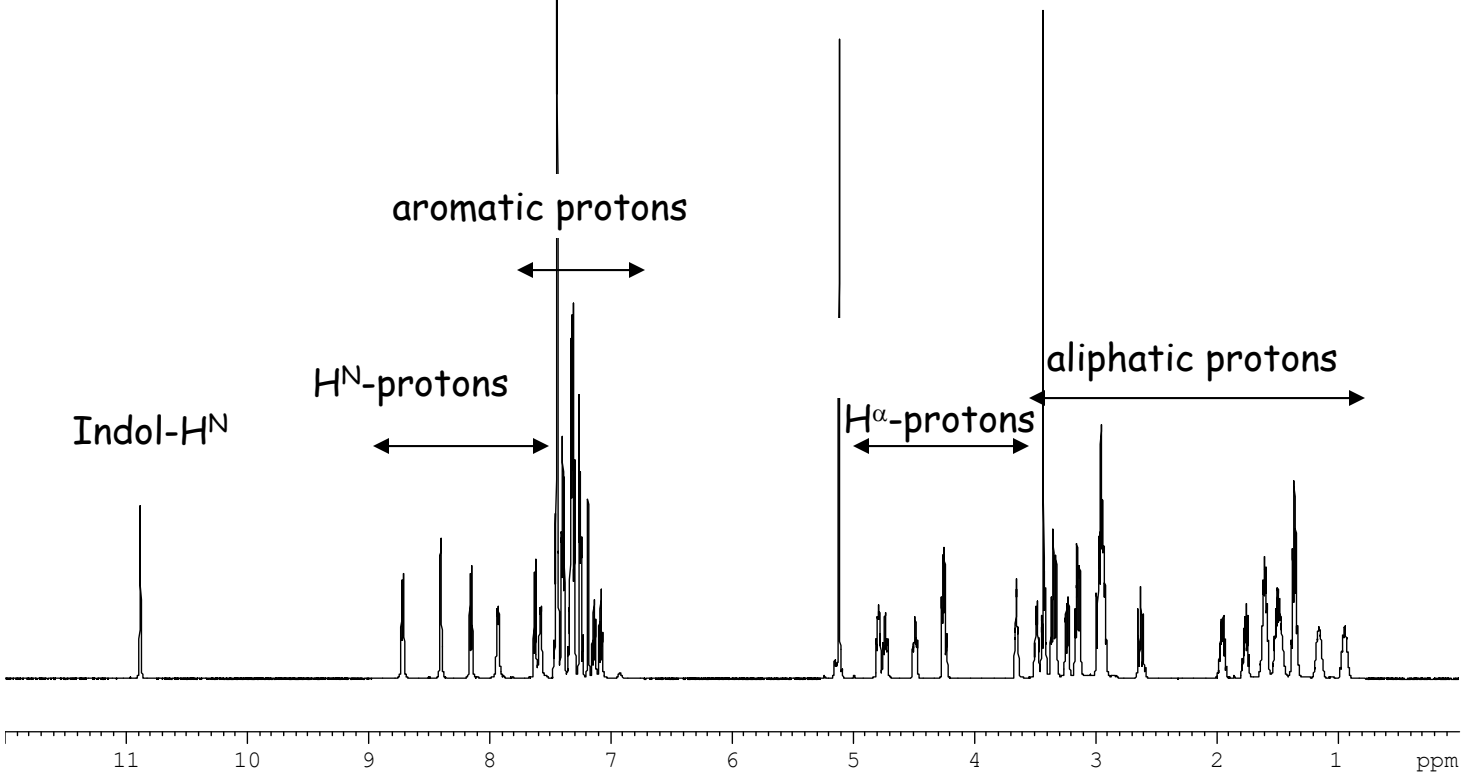


F3-008: *cyc*-(dP-F-F-K(Z)-W-F)

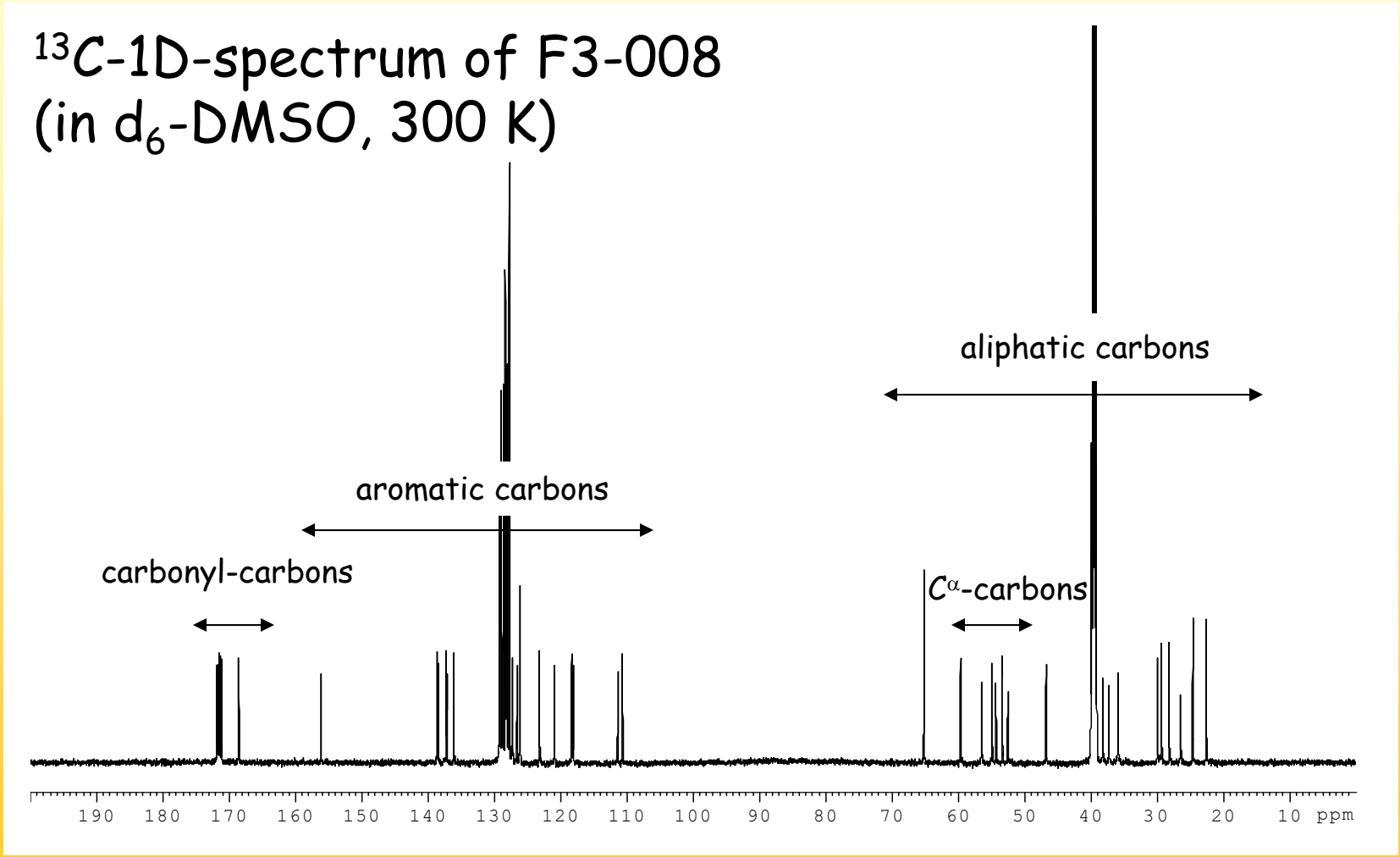


Multidimensional NMR-spectroscopy

^{1}H-1D-spectrum of F3-008 (in d_6-DMSO, 300 K)



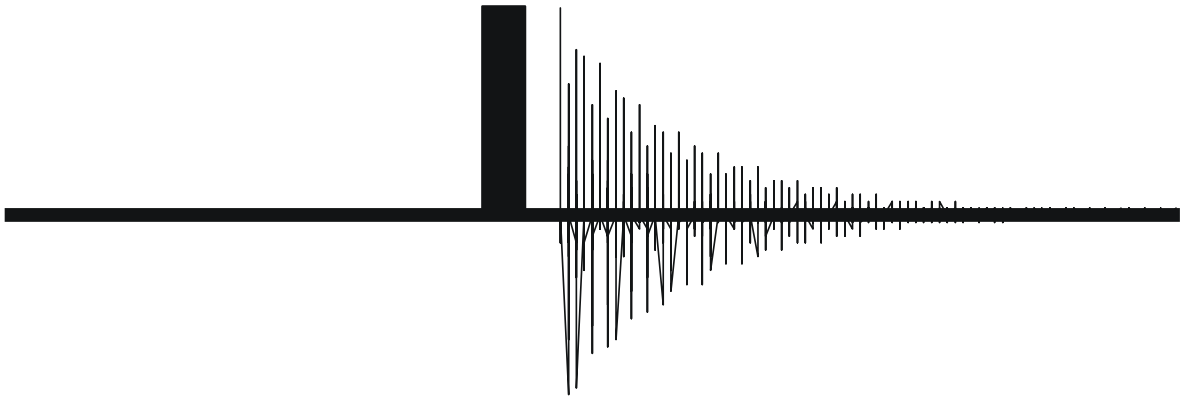
Multidimensional NMR-spectroscopy



Multidimensional NMR-spectroscopy

1D-NMR schematisch

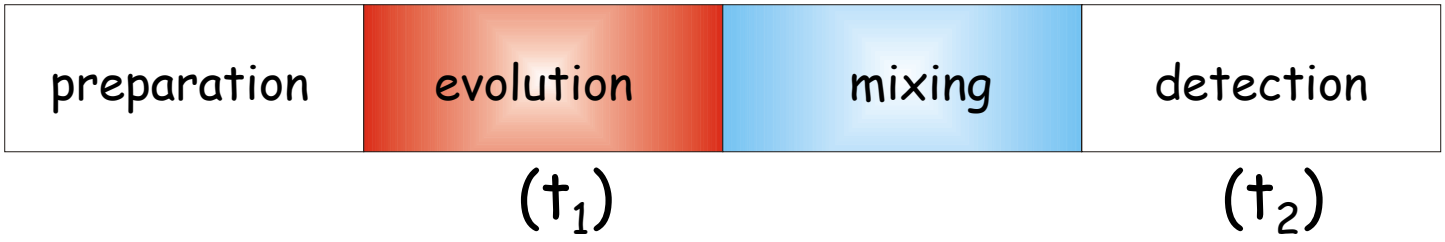
Preparation	Detektion



Multidimensional NMR-spectroscopy

2D-NMR experiments contain two new elements:

evolution time and mixing time

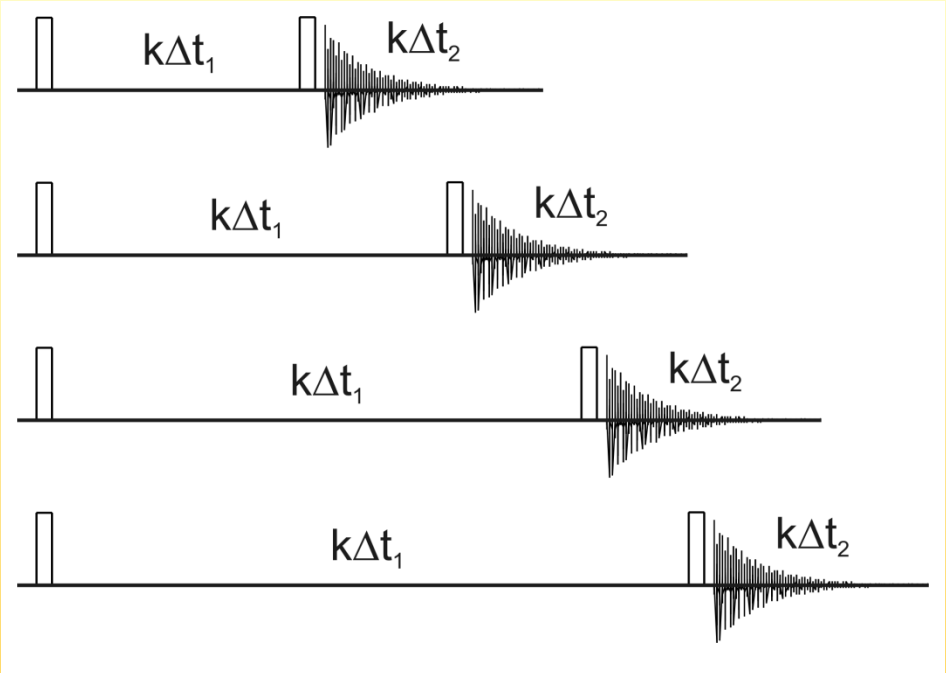


evolution time:
creation of a further frequency axis by indirect detection

mixing time:
transfer of magnetization via spin-spin interactions

Multidimensional NMR-spectroscopy

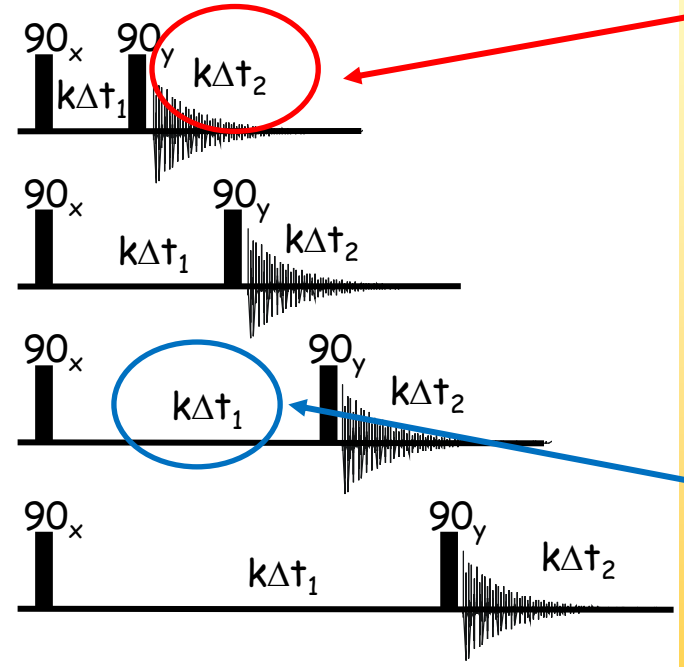
Evolution time



The indirect detection of the frequency is performed by a systematic variation of a time interval within a sequence of pulses

Multidimensional NMR-spectroscopy

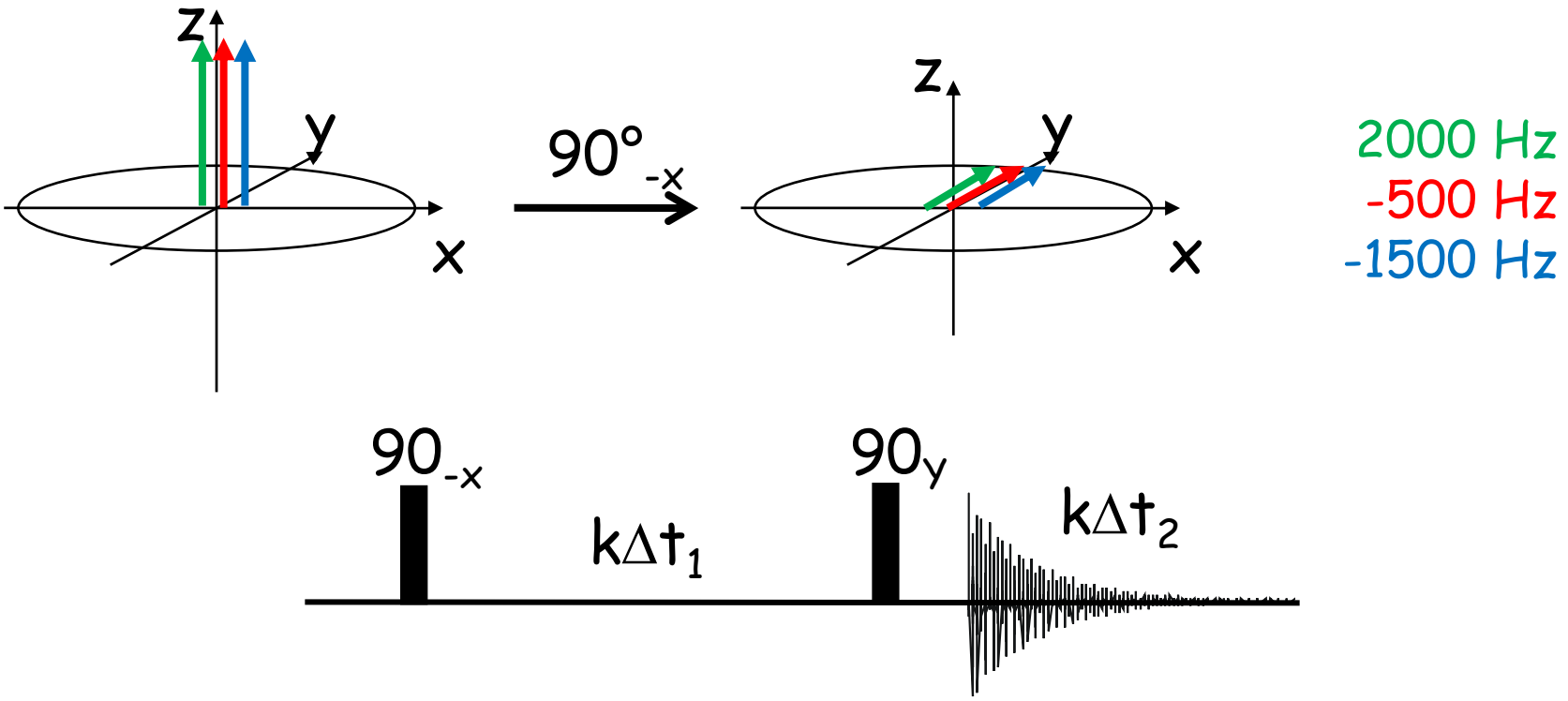
Evolution time



The recording of the FIDs we have already looked at in detail, that will be repeated for every new time point kΔt1. In the indirect dimension the data points have to be recorded at multiple integers of Δt as well

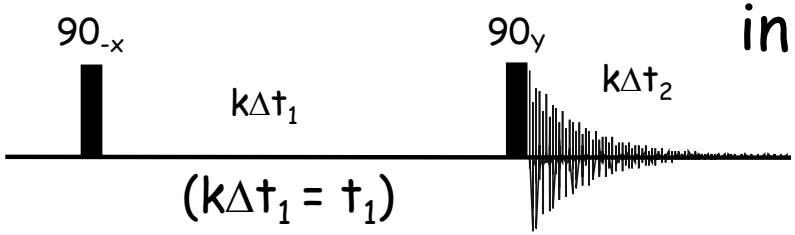
Multidimensional NMR-spectroscopy

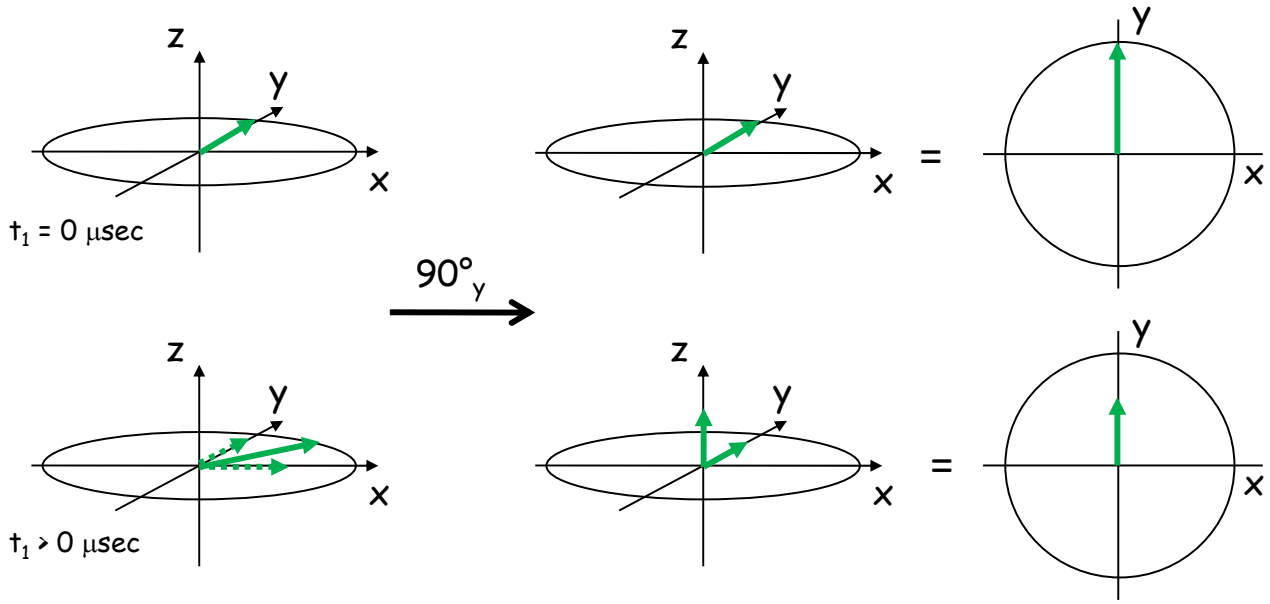
We take another closer look using the same three lines as in case of the 1D spectrum



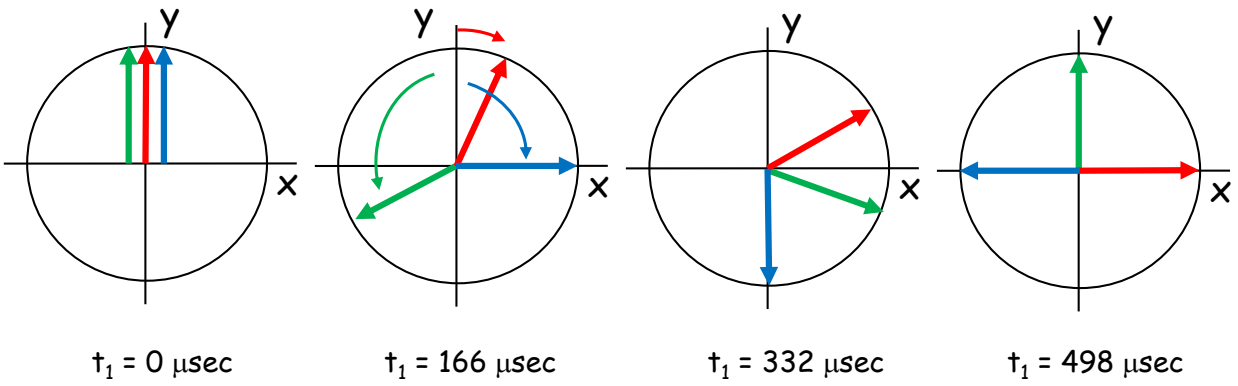
Multidimensional NMR-spectroscopy

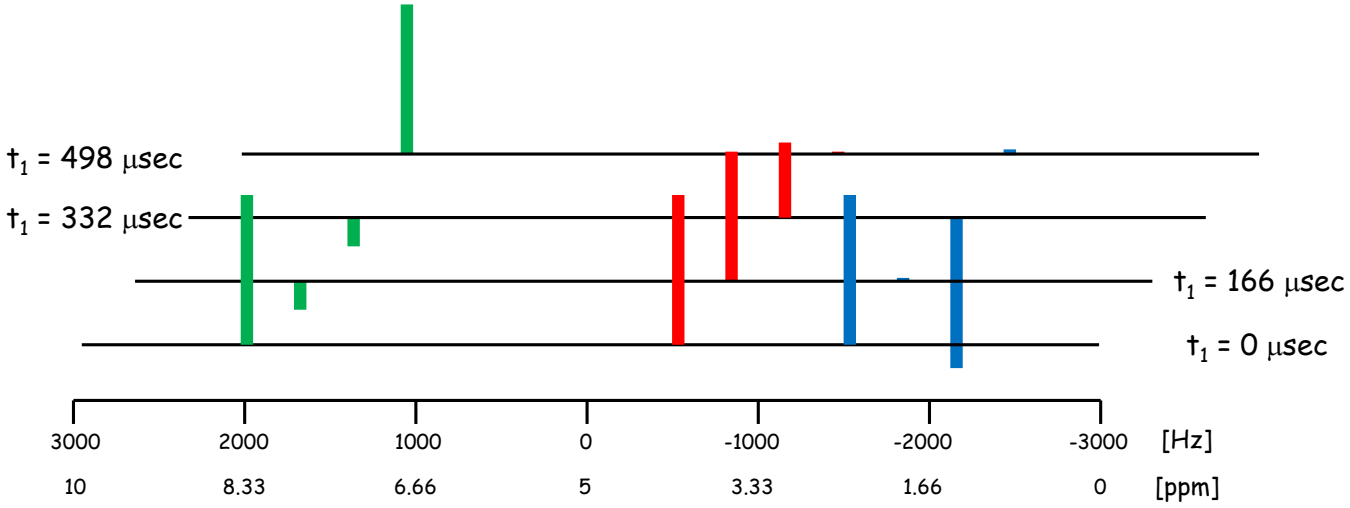
One can see that the initial intensity in t_2 depends on the frequency





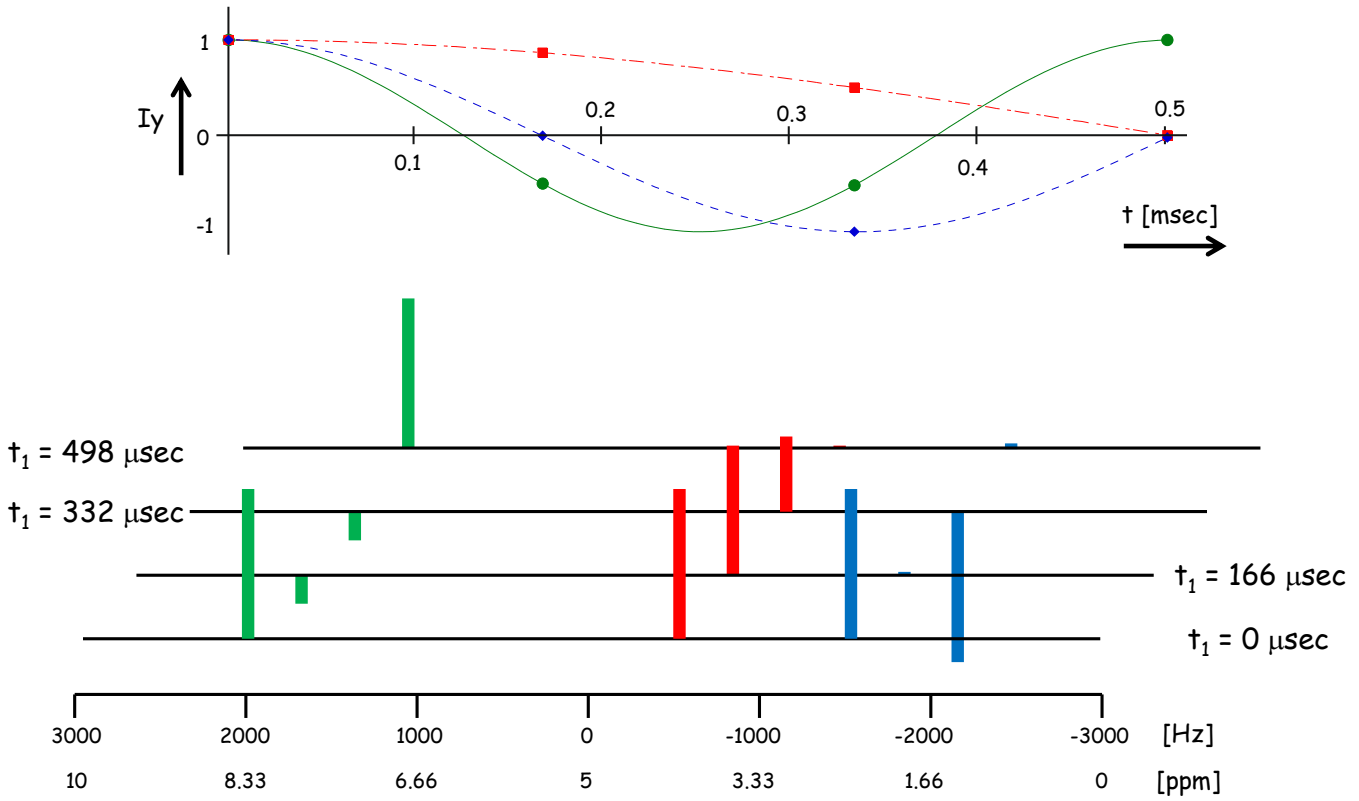
Multidimensional NMR-spectroscopy





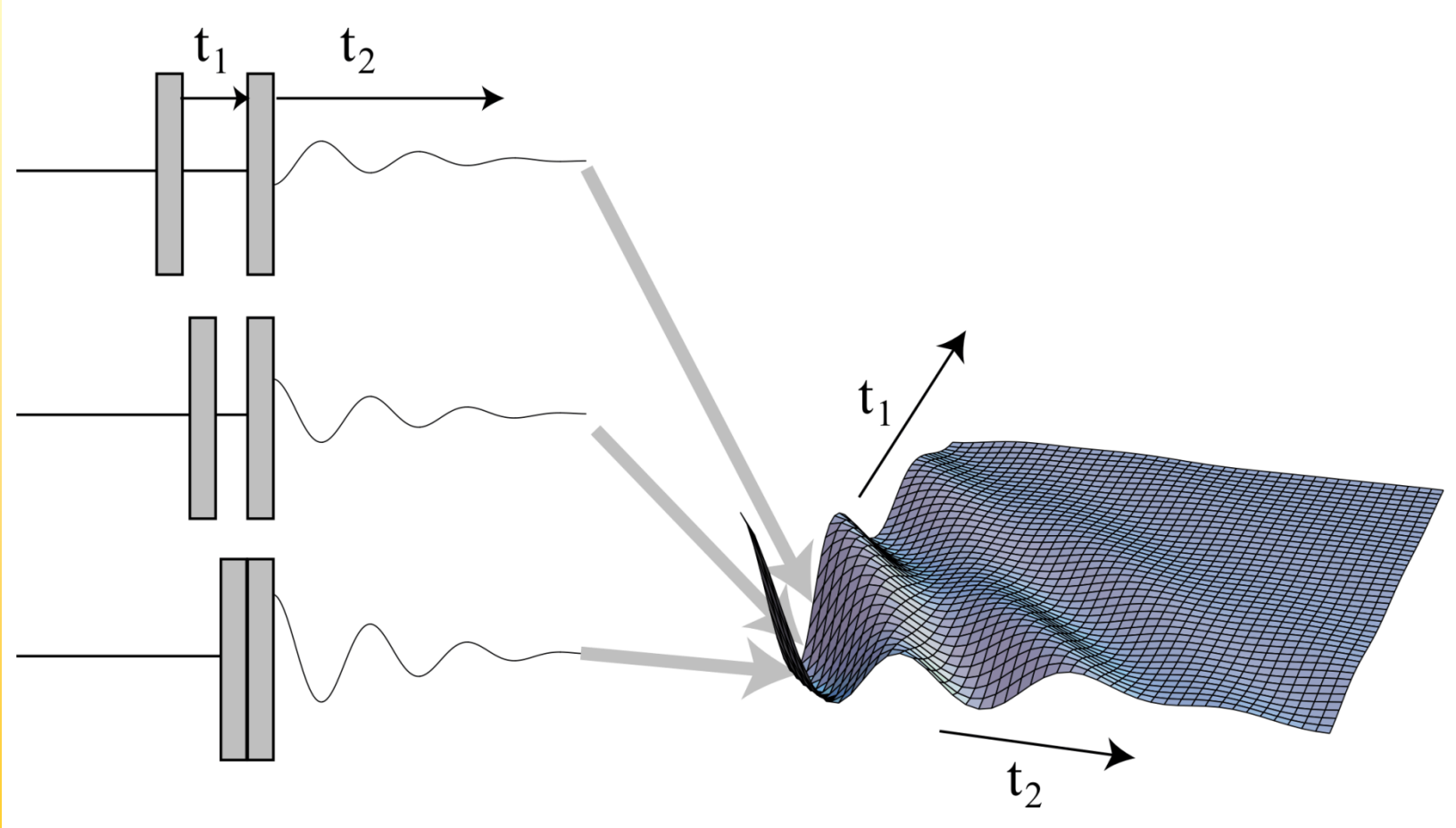
Multidimensional NMR-spectroscopy

We get a similar picture along the time in the indirect dimension as we did in the direct one.



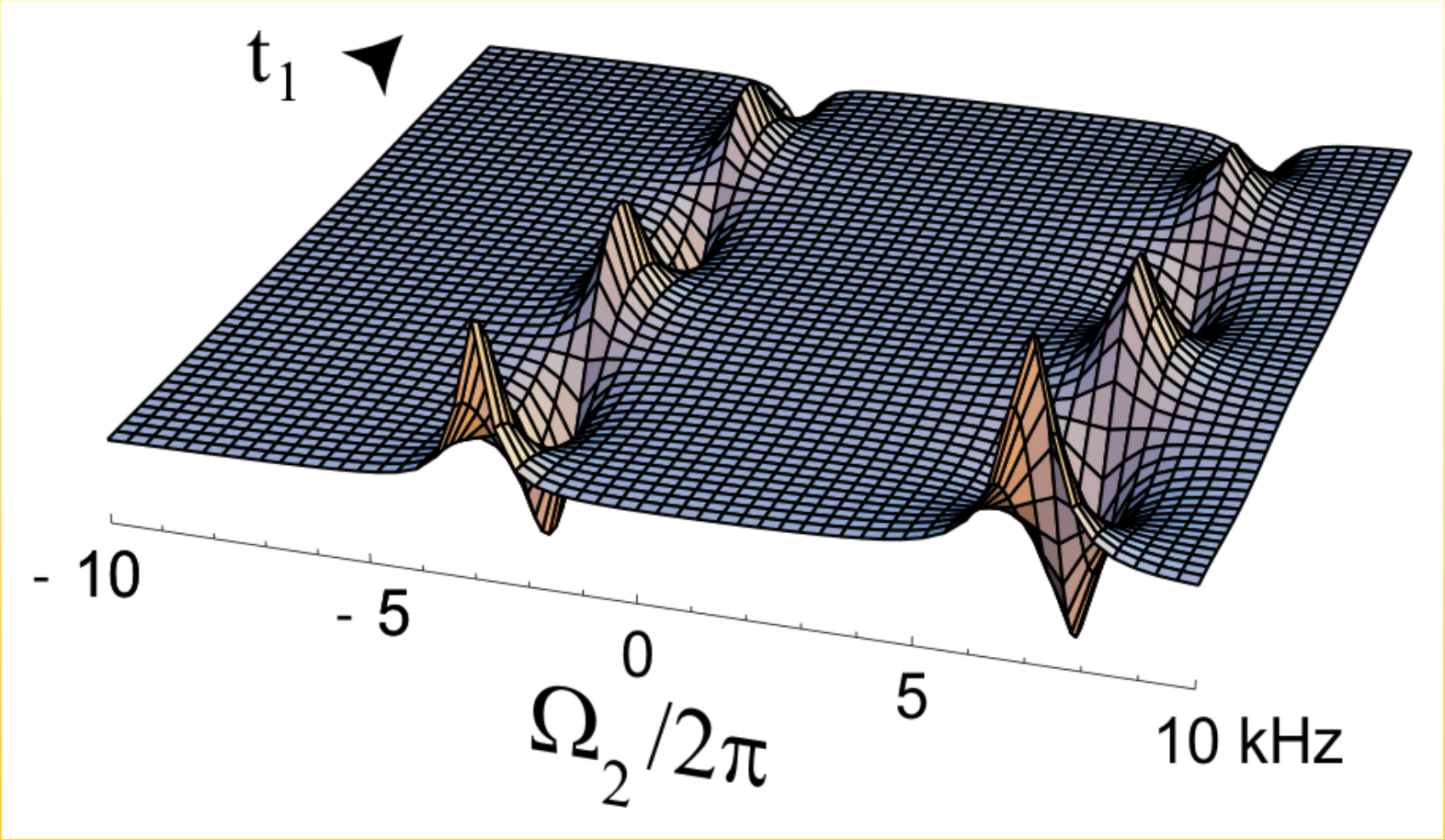
Multidimensional NMR-spectroscopy

We obtain a two-dimensional FID



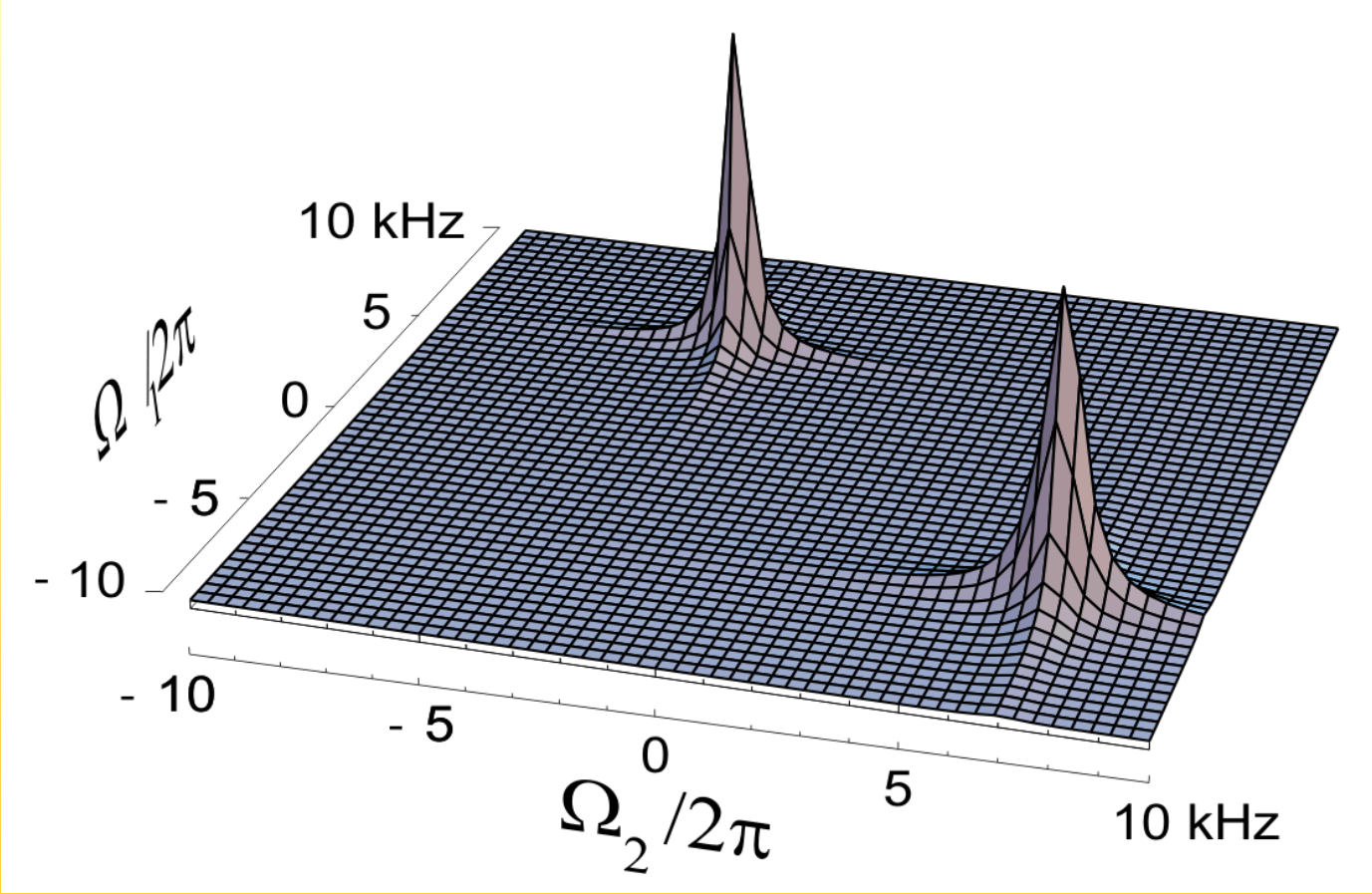
Multidimensional NMR-spectroscopy

a first FT results in an „interferogram“

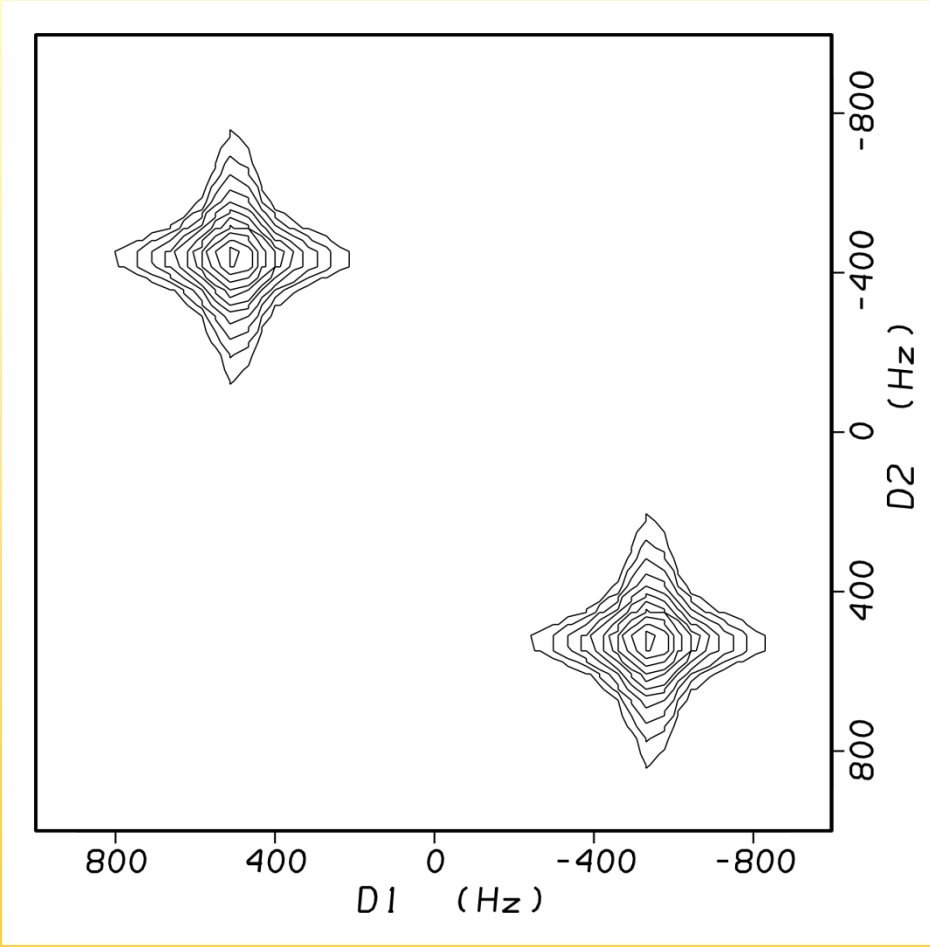


Multidimensional NMR-spectroscopy

a second FT yields the two-dimensional spectrum

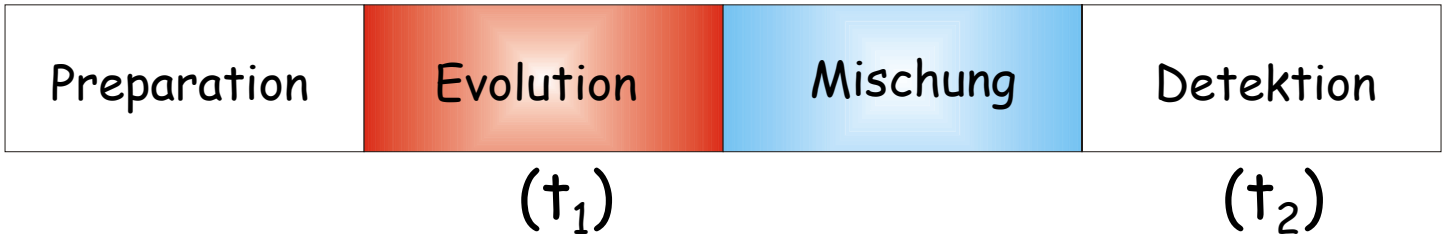


Multidimensional NMR-spectroscopy



To analyze the spectra they are viewed as contour-plots, in which intensities are display as contour levels

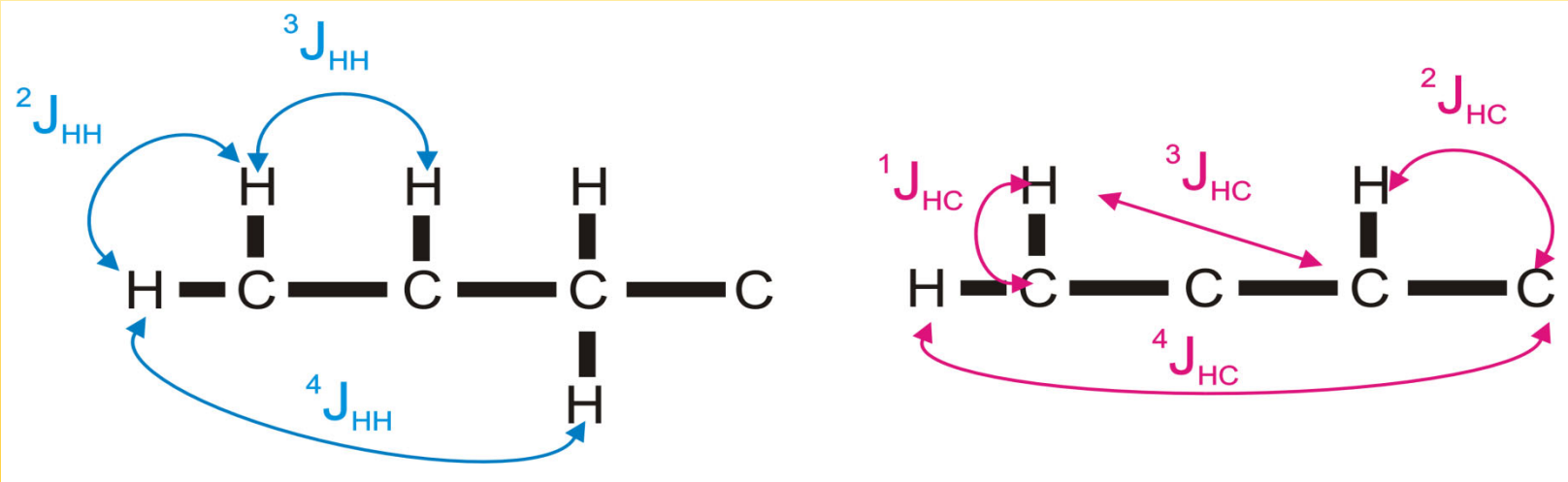
Multidimensional NMR-spectroscopy



If there was just evolution and detection we would detect the same frequency in both time domains and not gain anything. Therefore the mixing time is of major importance, since it enables the transfer of magnetization from one nucleus to the next.

Multidimensional NMR-spectroscopy

This transfer can take place via several mechanisms, the one used most often for multidimensional NMR and assignment experiments a scalar coupling (J-couplings).



Multidimensional NMR-spectroscopy

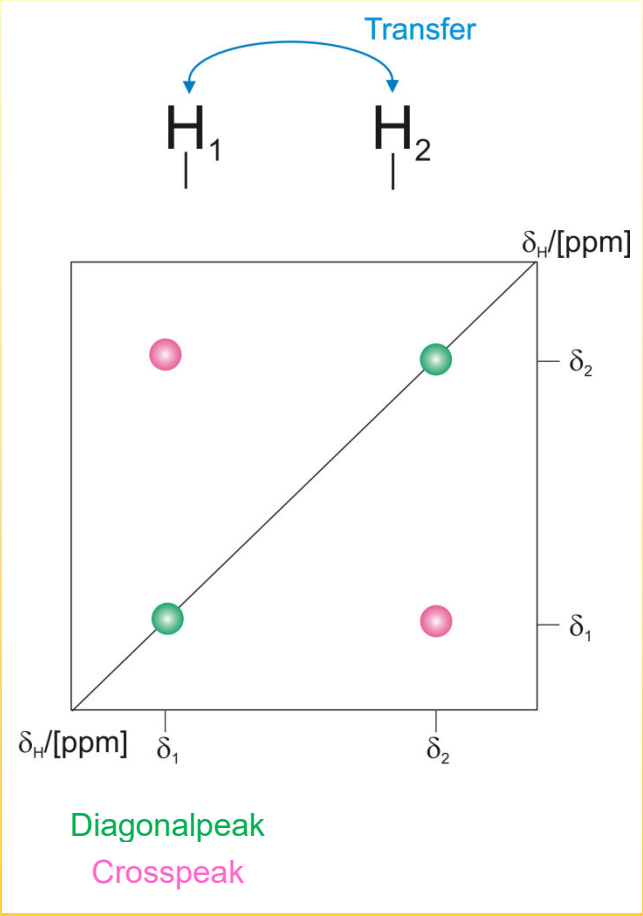
homonuclear spectra

Here the transfer of magnetization takes place between nuclei of the same type. Both frequency axes then show the same type of chemical shift.
If there is a transfer this results in two different chemical shifts in both dimensions:

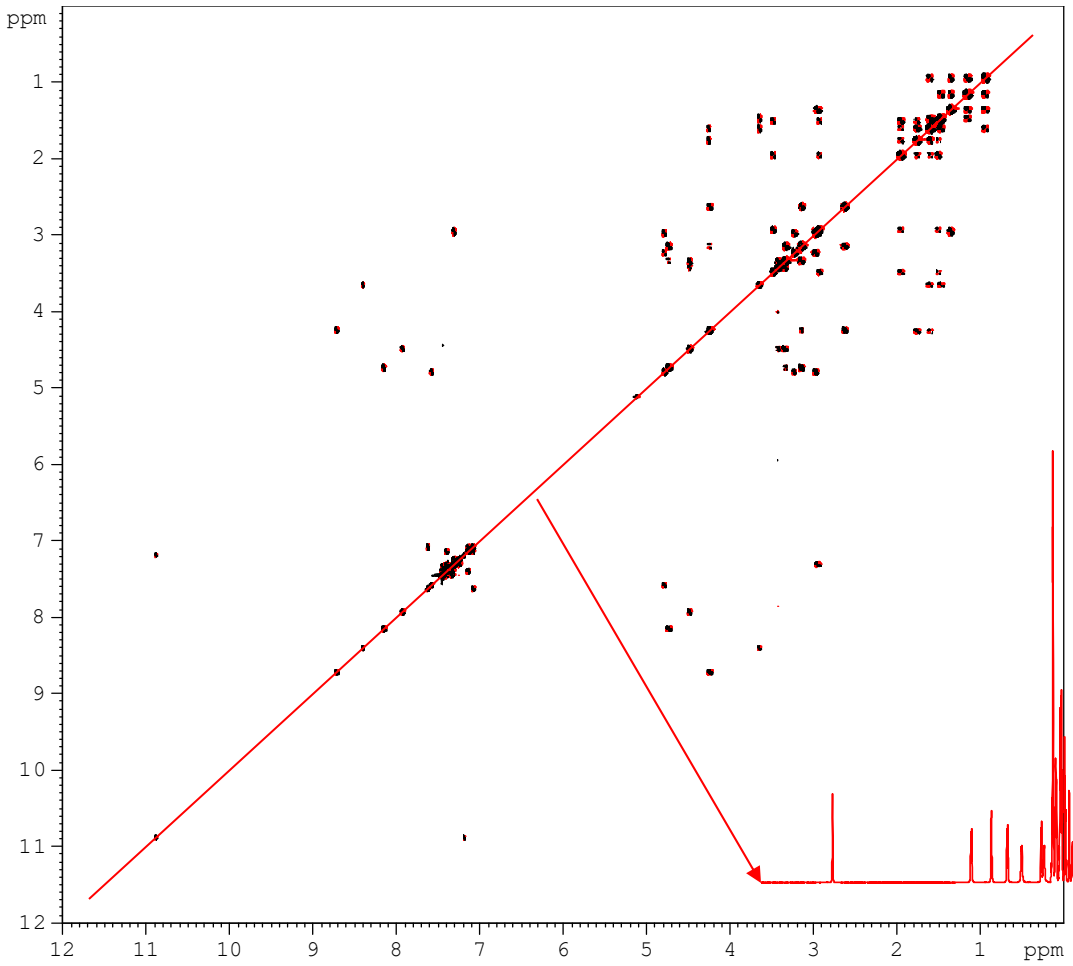
Crosspeak

If there is no transfer the chemical shift in both dimensions is identical:

Diagonalpeak



Multidimensional NMR-spectroscopy

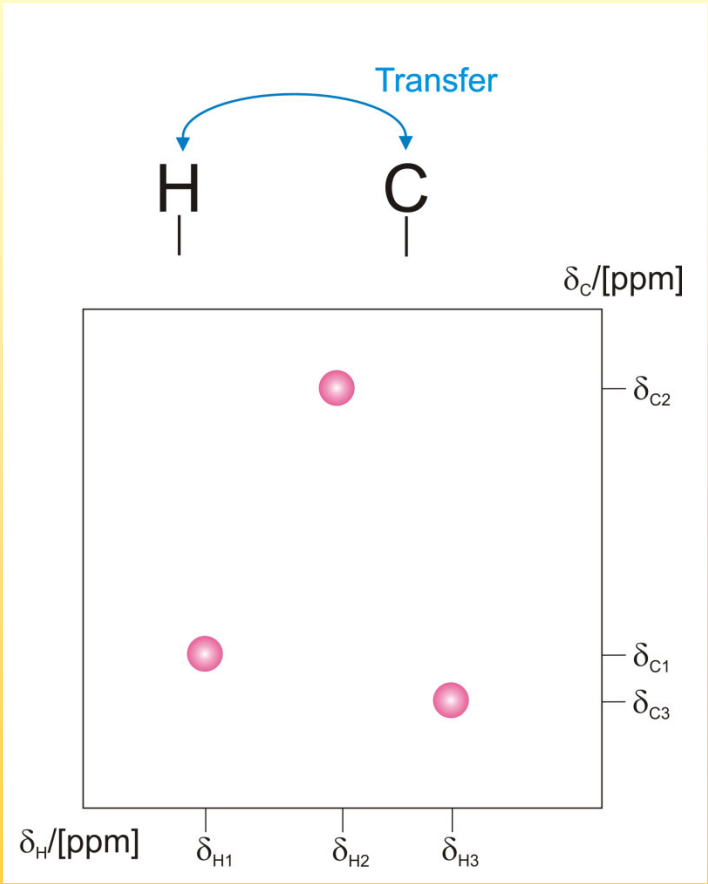


COSY of F3-008, correlations via J-coupling (max. 3 bonds)

Multidimensional NMR-spectroscopy

heteronuclear spectra

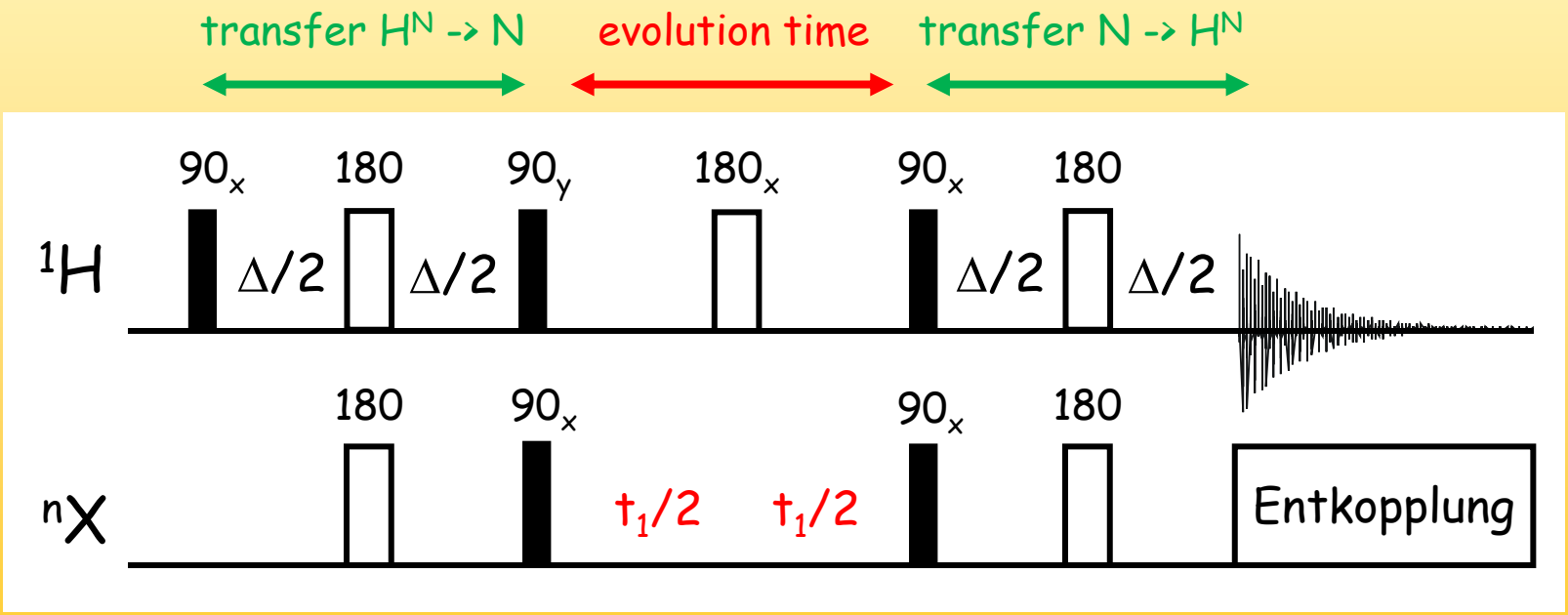
Here the transfer takes place between different types of nuclei und thus both axes exhibit different chemical shifts. If there is no transfer then there will be no peak, but if there is, the peak appears at the intersection of the chemical shifts of the two nuclei involved.



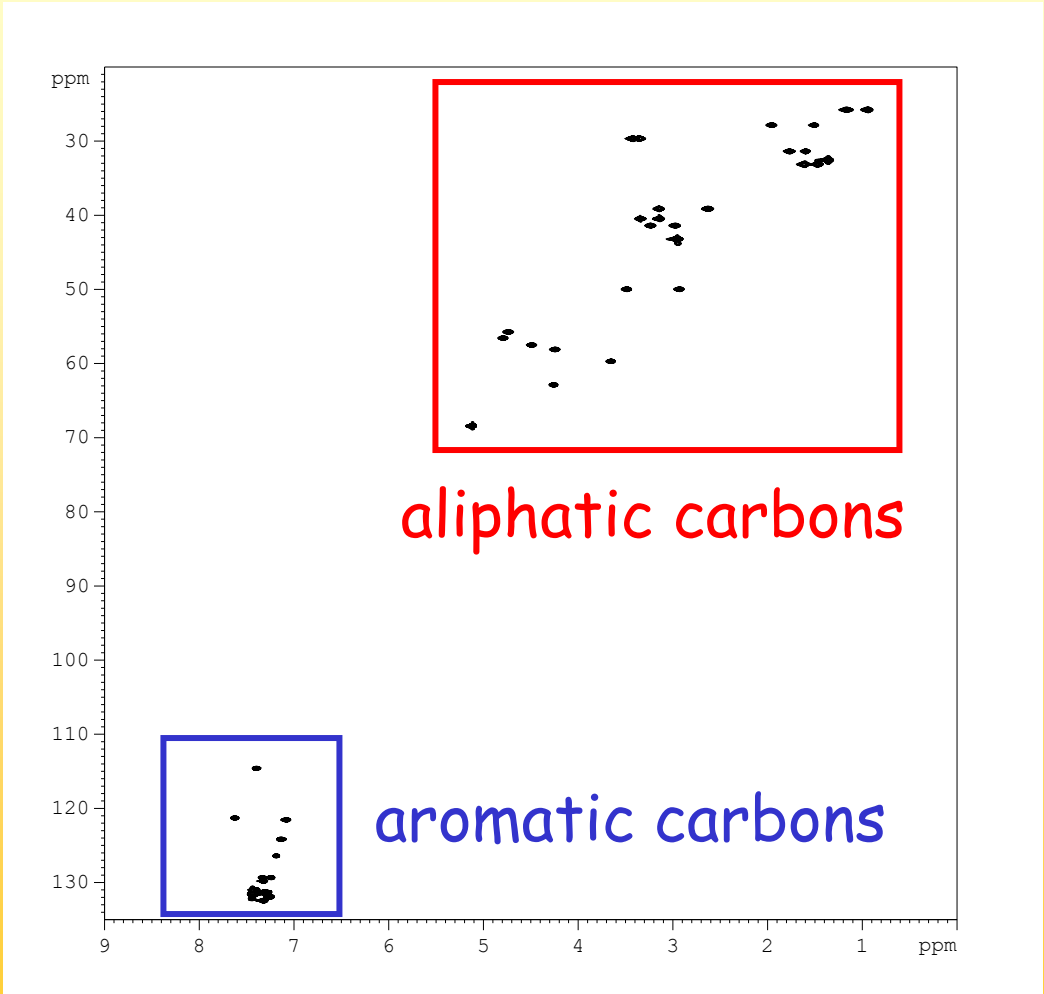
Multidimensional NMR-spectroscopy

Pulse sequence of the HSQC

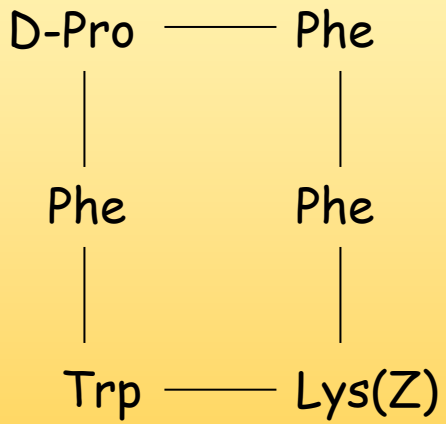
To create more complex spectra the number of pulses in the experiment increases, there order and timing matters but can be controlled very precisely by the hardware.



Multidimensional NMR-spectroscopy

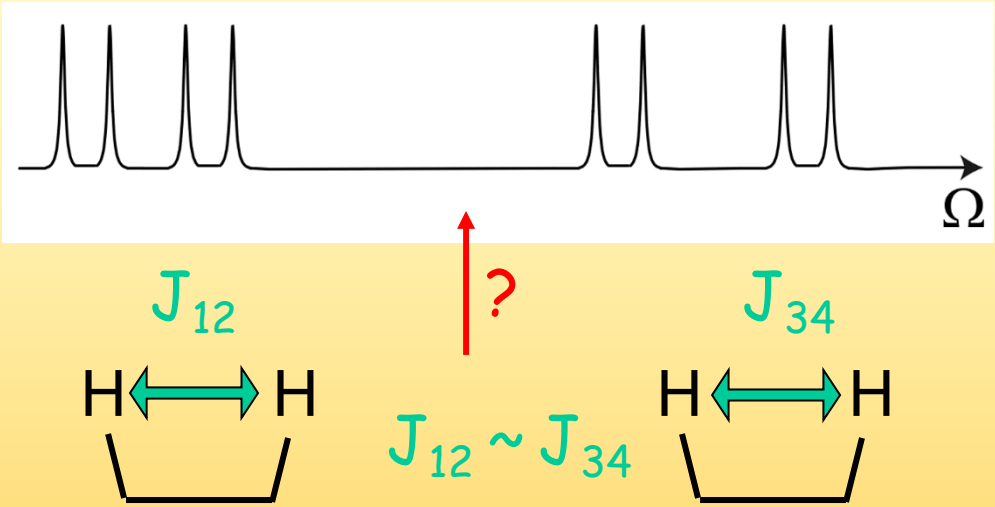


^{13}C-HSQC
of F3-008



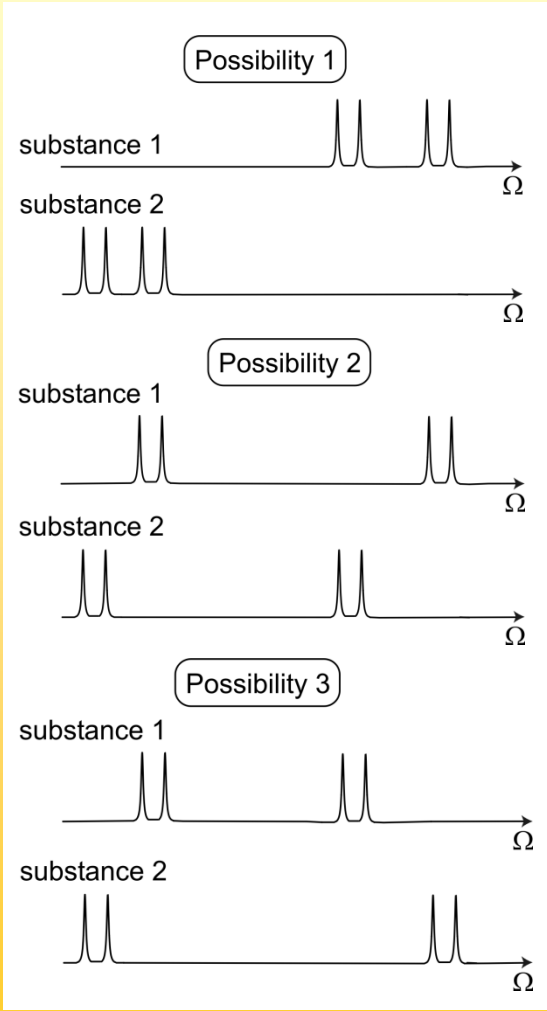
Multidimensional NMR-spectroscopy

A simple example:



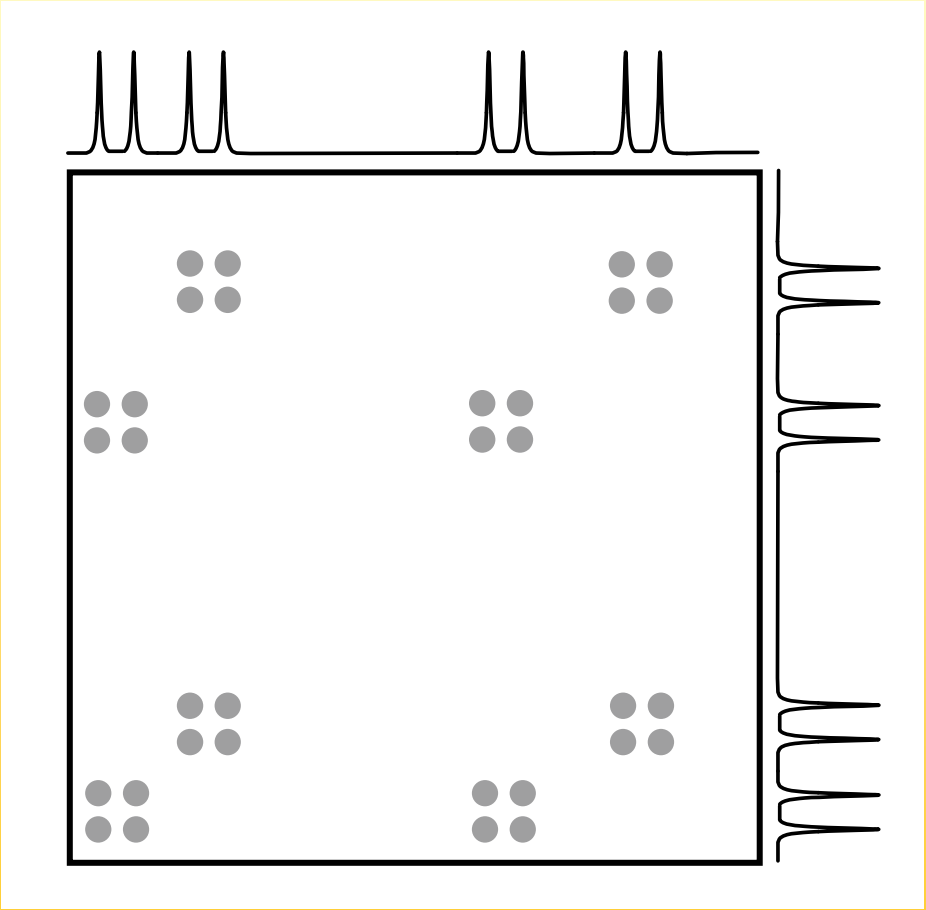
Two Tyrosine aromatic rings:

We can exclude possibility 1 using some experience but an assignment using 1D is not possible...



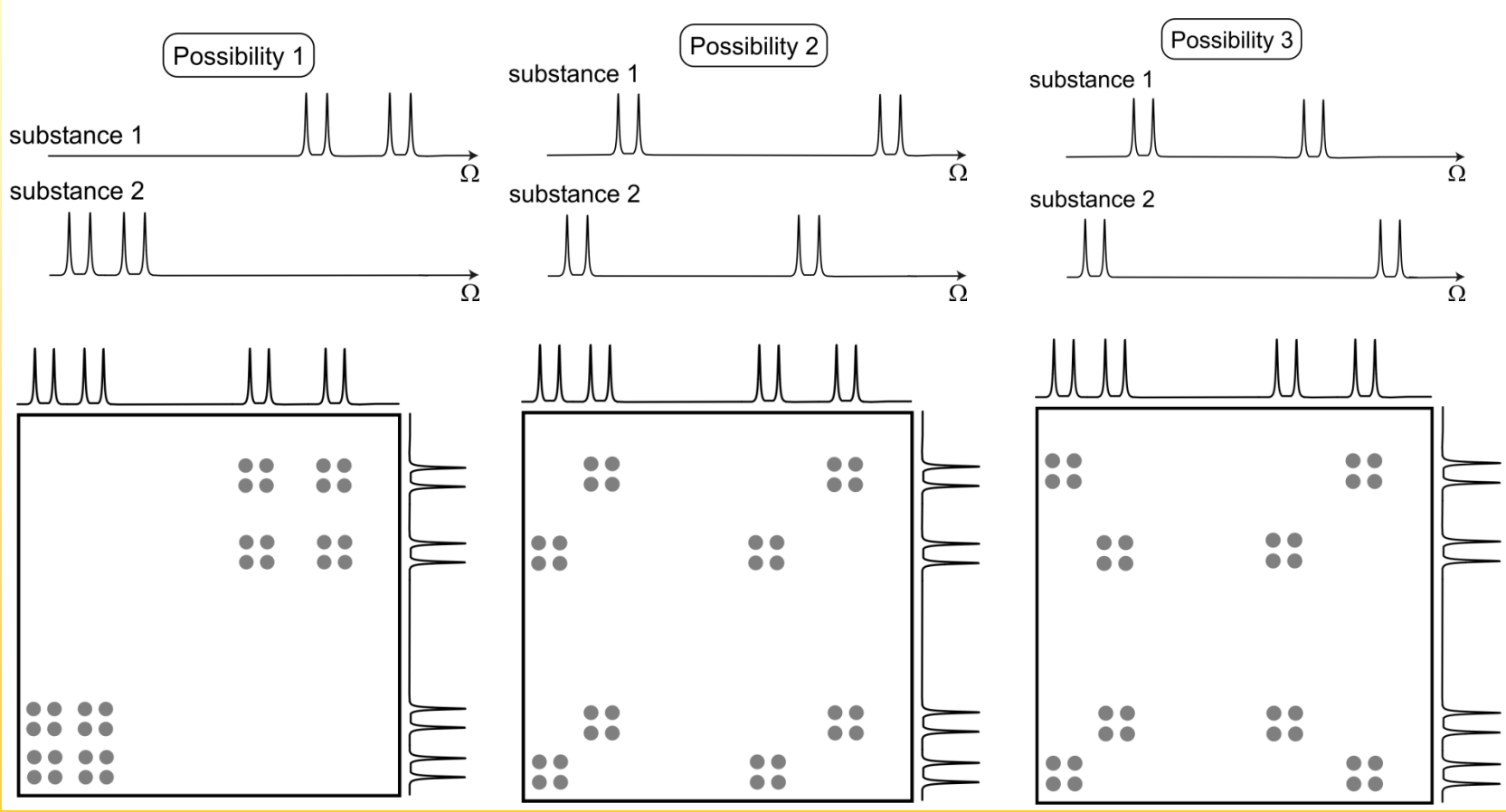
Multidimensional NMR-spectroscopy

....but if we record a simple 2D...



Multidimensional NMR-spectroscopy

....it is easy !



That's it

https://www.schmieder-nmr.de/teaching/praktikum.htm