NMR-spectroscopy in solution

- an introduction

Peter Schmieder

Advanced Bioanalytics – NMR-Spectroscopy

Introductory session (11:00 – 12:30)

Basic aspects of NMR-spectroscopy

NMR parameter

Multidimensional NMR-spectroscopy

Applications of NMR-spectroscopy

Detection of protein-ligand interactions using NMR-spectroscopy

Application session (lecture and exercise, 13:30 – 15:30)

NMR-spectroscopy of proteins

Multidimensional NMR-spectroscopy with more than 2 dimensions

Sequence-specific assignment

Exercise: assignment of 9 amino acids from an SH3 domain

Basic aspects of NMR-spectroscopy

Basic aspects of NMR-spectroscopy

Nuclear Magnetic Resonance

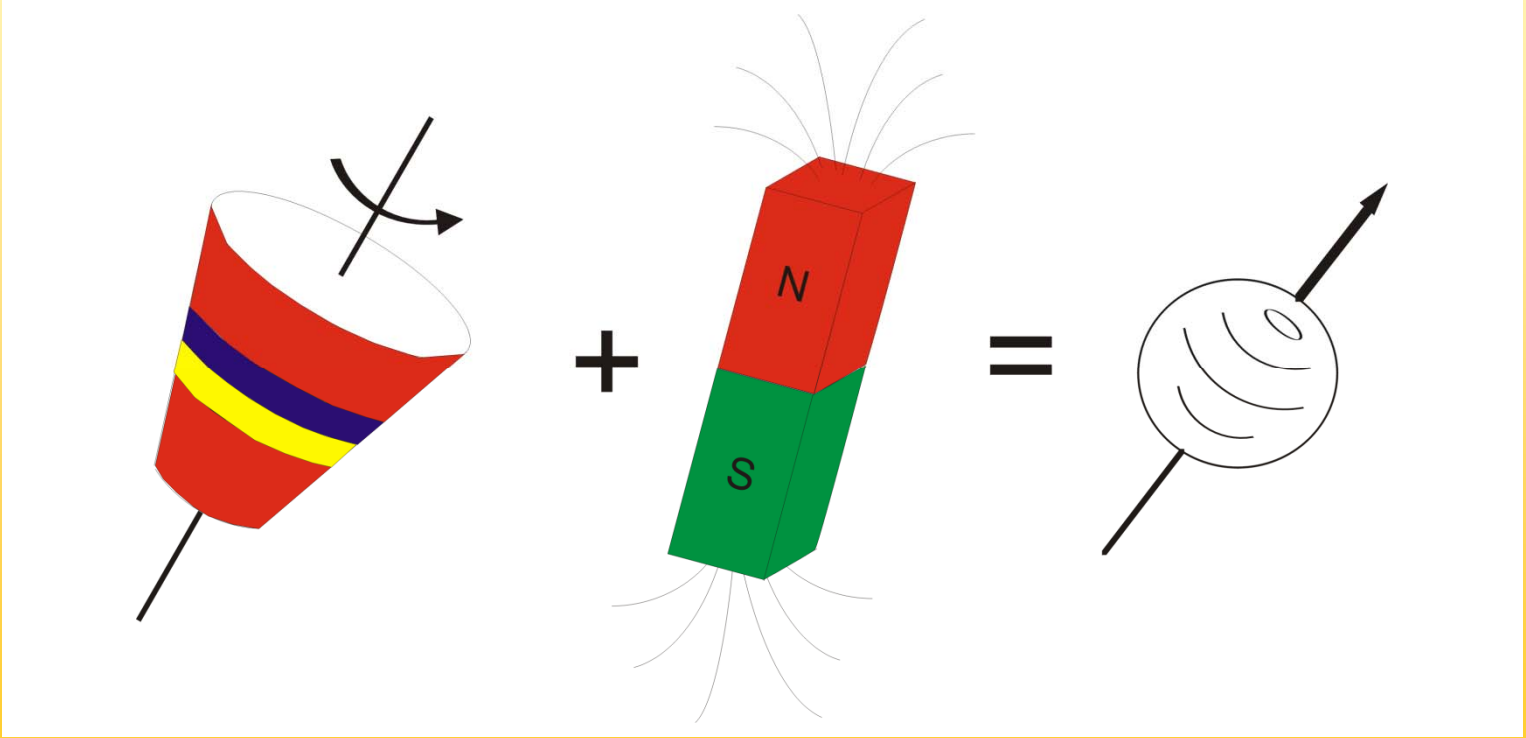
NMR-spectroscopy detects the resonance of atomic nuclei with radio waves. The effect is only readily observable in a strong magnetic field. Each nucleus is observed separately and interactions between nuclei can be observed as well.

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.

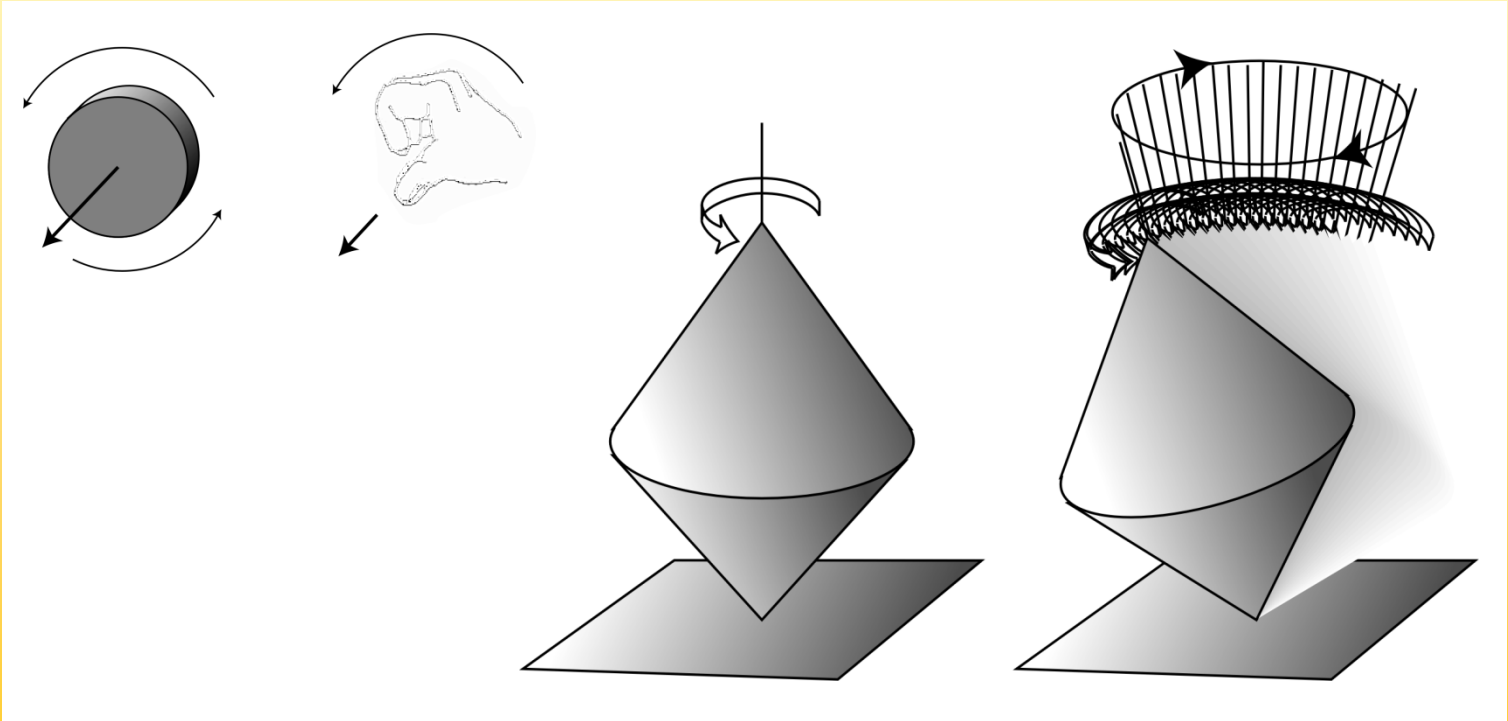
Basic aspects of NMR-spectroscopy

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



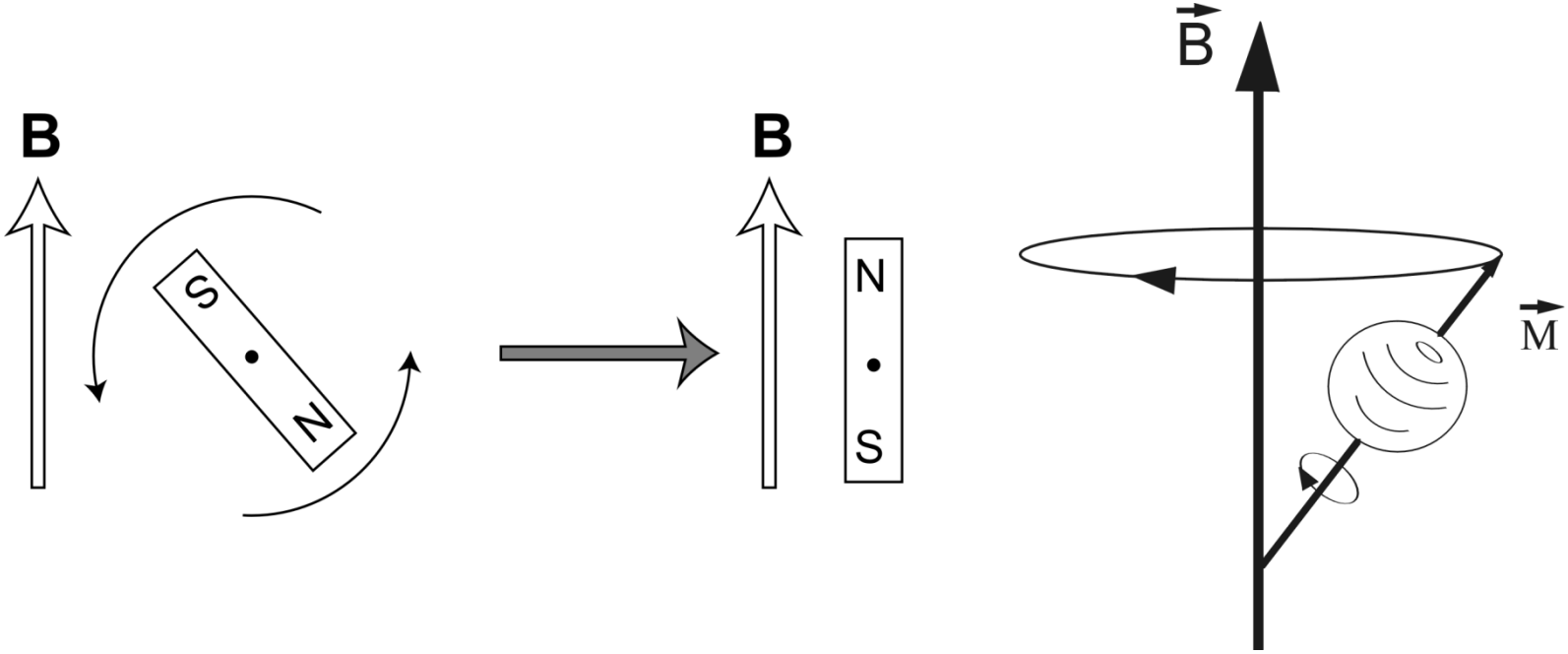
Basic aspects of NMR-spectroscopy

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



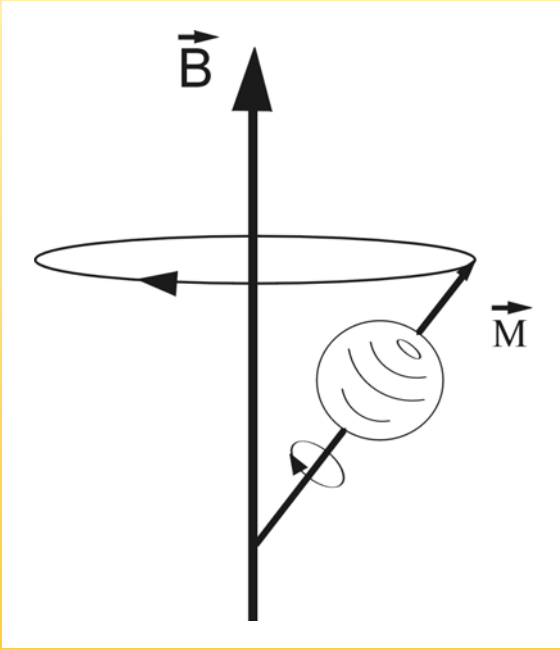
Basic aspects of NMR-spectroscopy

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

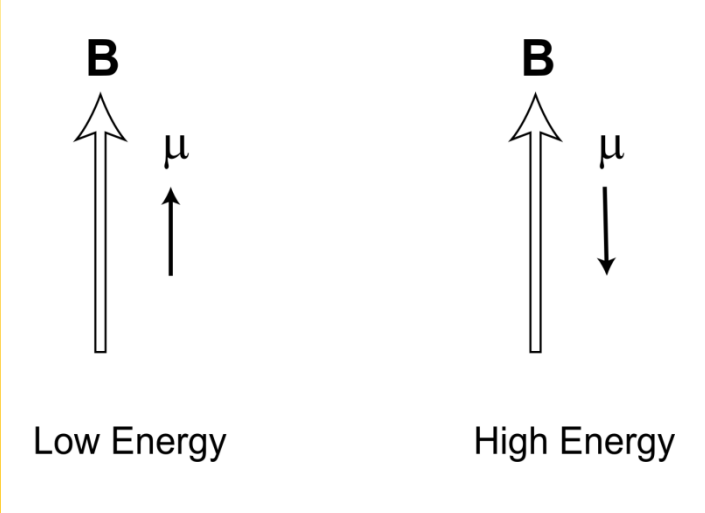


B_0 [Tesla]	ν_0 [MHz]
1.4	60
5.9	250
9.4	400
14.1	600
21.2	900

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

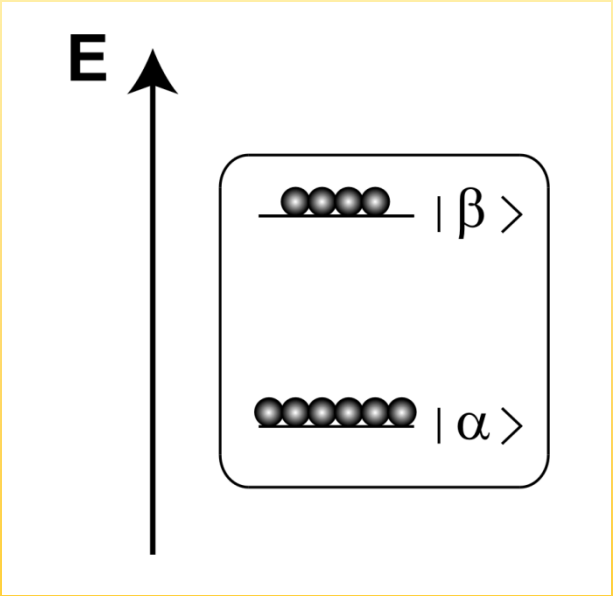
We will then have a Boltzmann-distribution

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

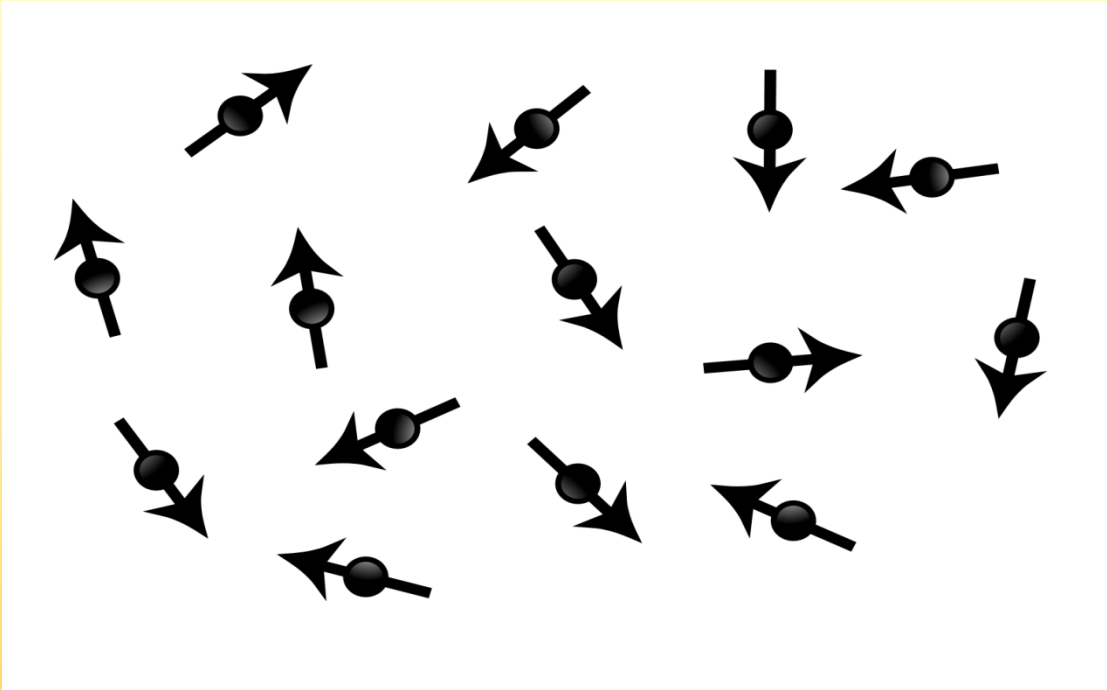
At 600 MHz frequency we get

$N_\beta/N_\alpha = 0.999904$

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

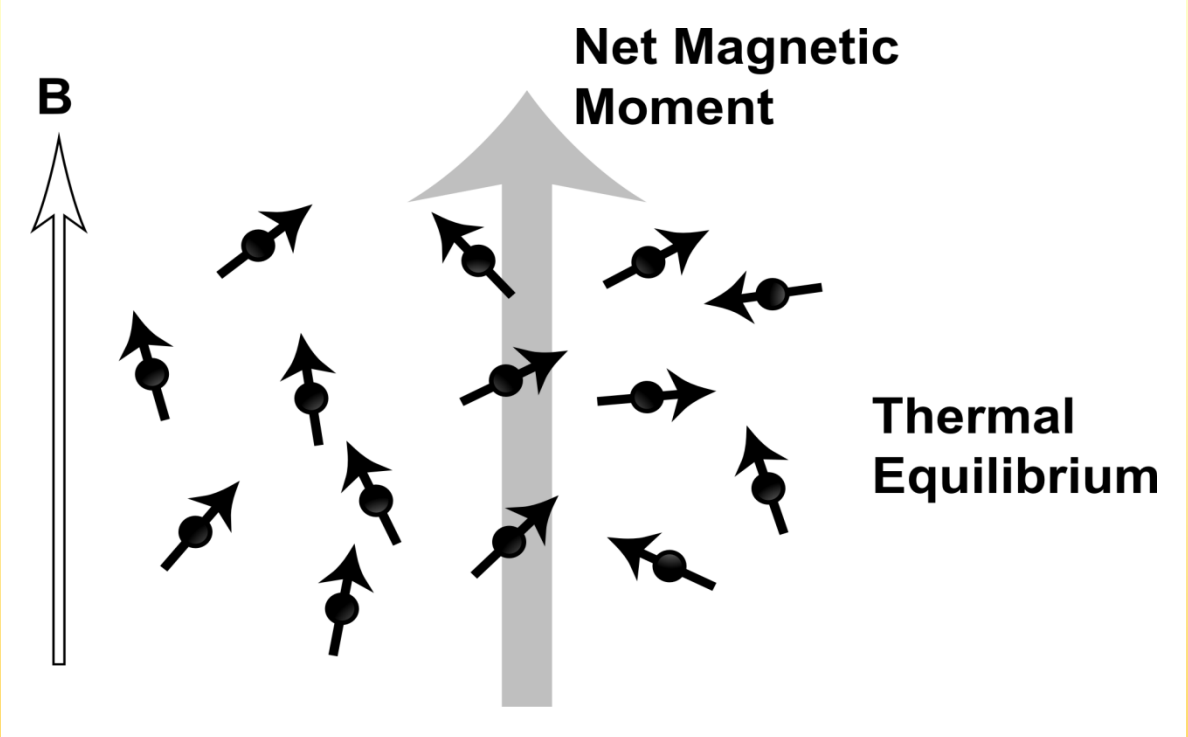


Basic aspects of NMR-spectroscopy



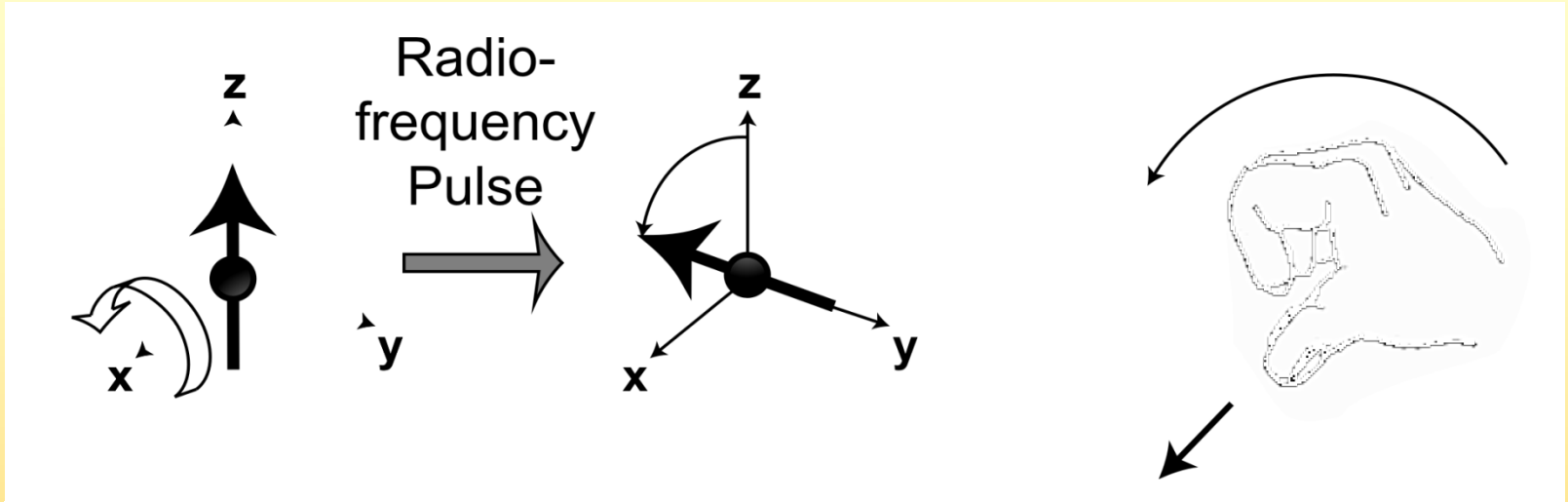
Without an external magnetic field all orientations are equal and the spins are randomly oriented

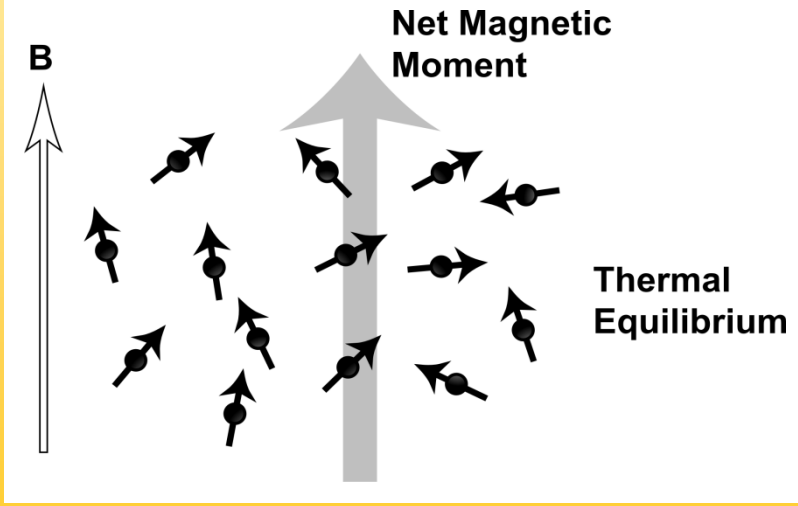
Basic aspects of NMR-spectroscopy



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



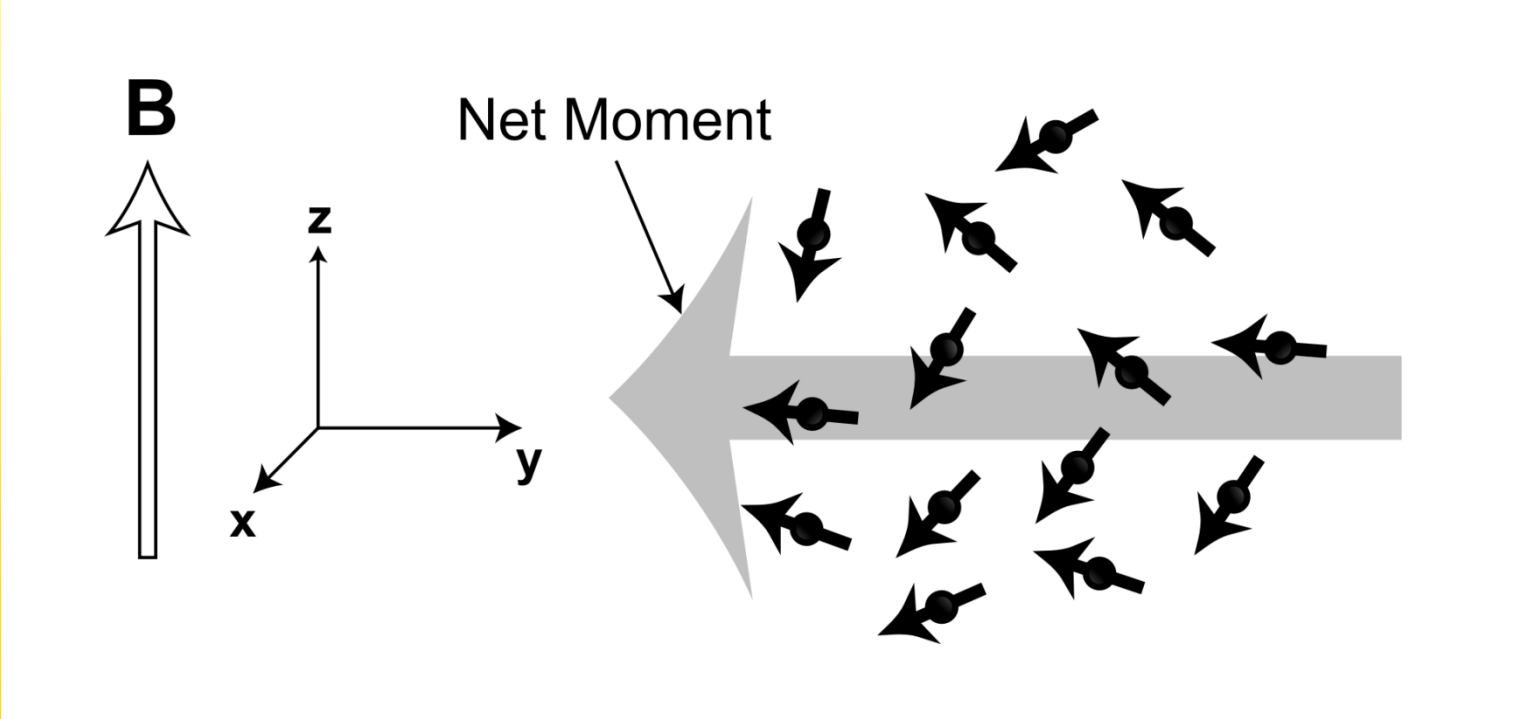


To do the experiment a radio frequency (RF) pulse turns every spin.

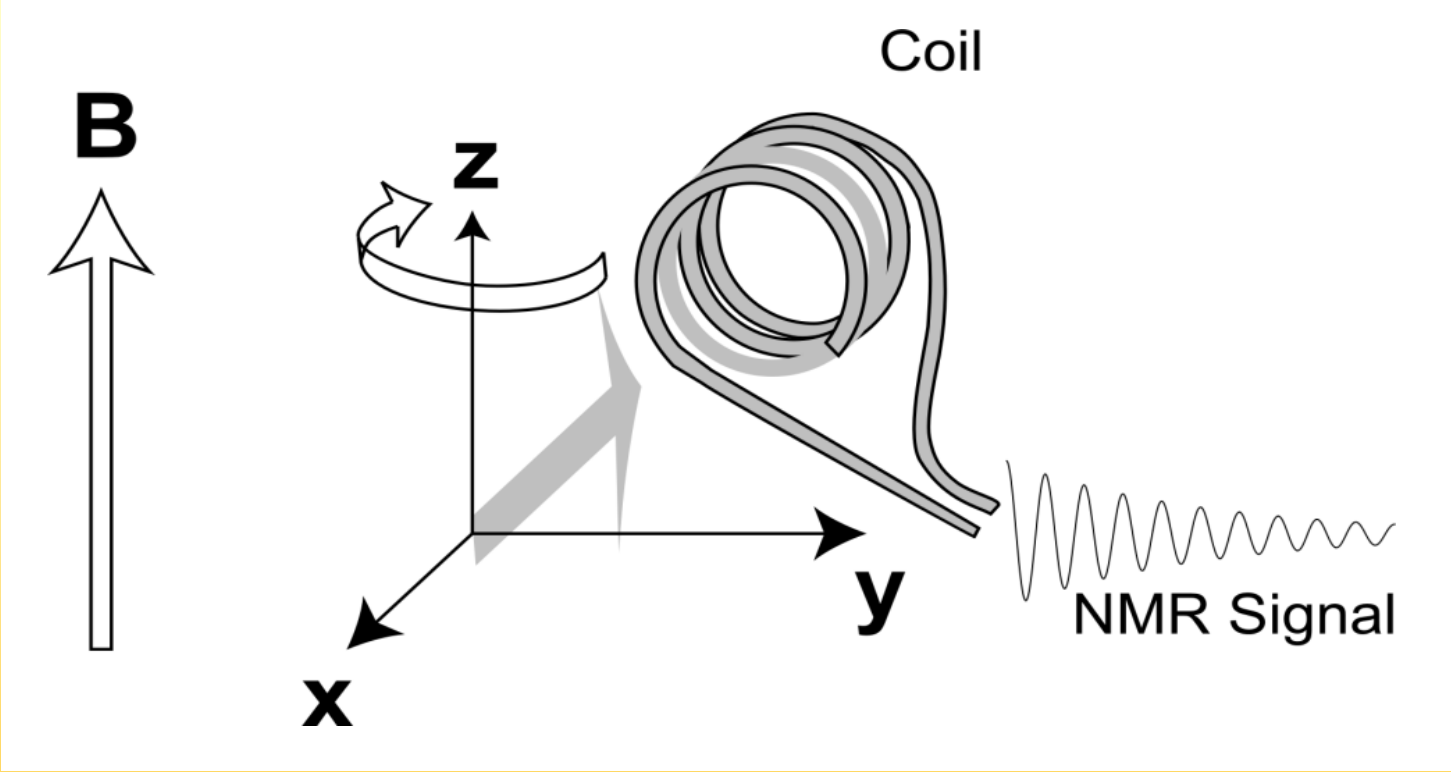
Note the rotation around the axis of the RF pulse field

Basic aspects of NMR-spectroscopy

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

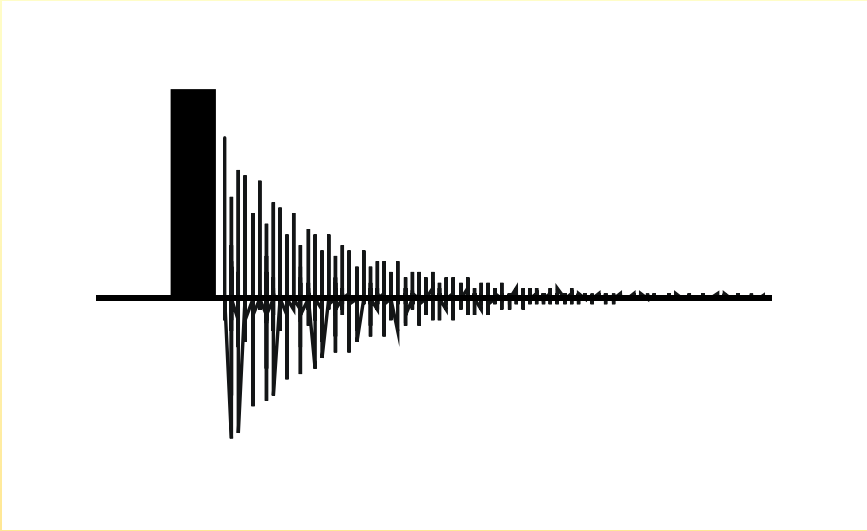


Multidimensional NMR-spectroscopy

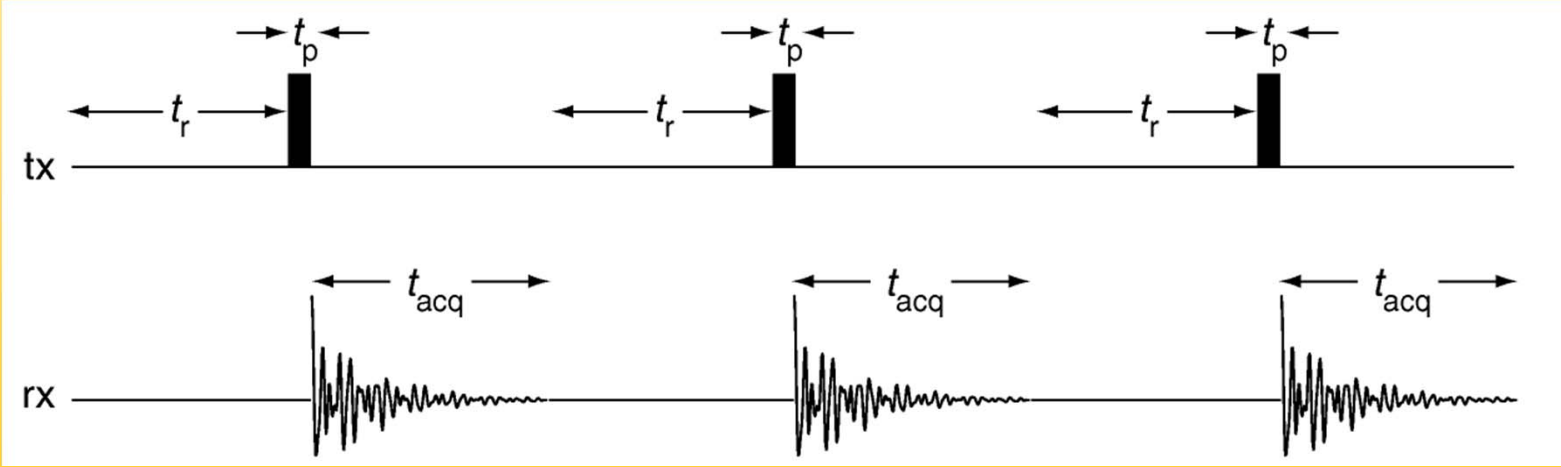


The precession that is still going on induces a current in the detection coil, the resulting signal is recorded

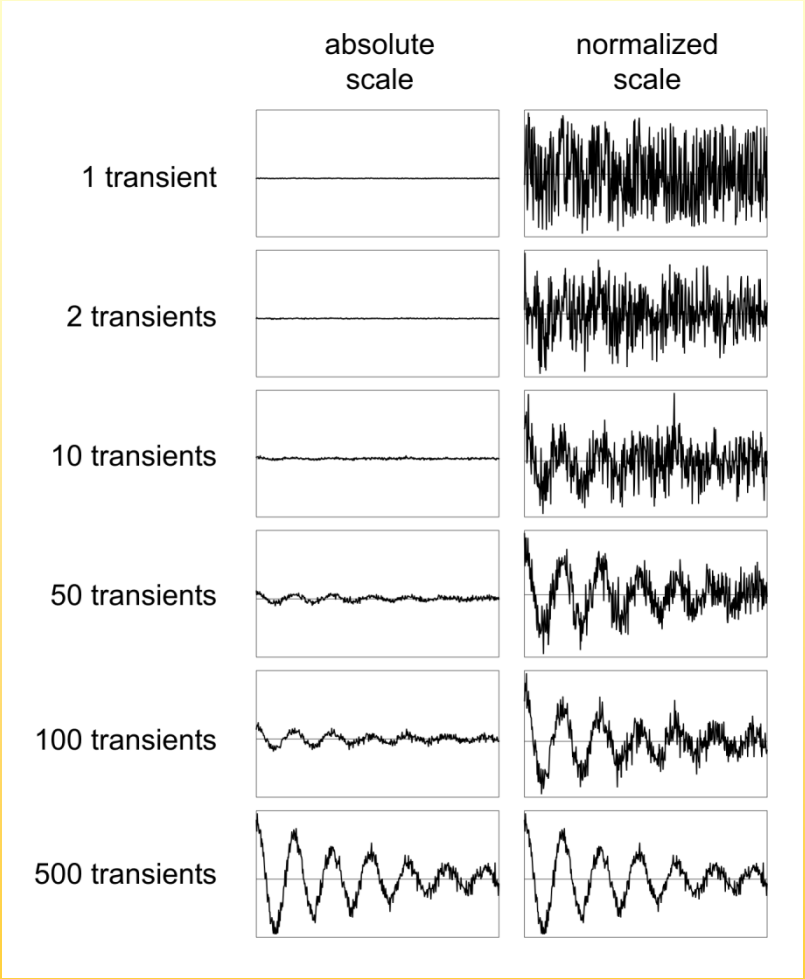
Basic aspects of NMR-spectroscopy



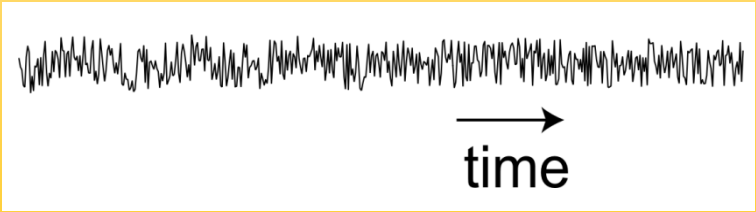
Thus the RF pulse starts the measurement which is then repeated…



NMR-parameter

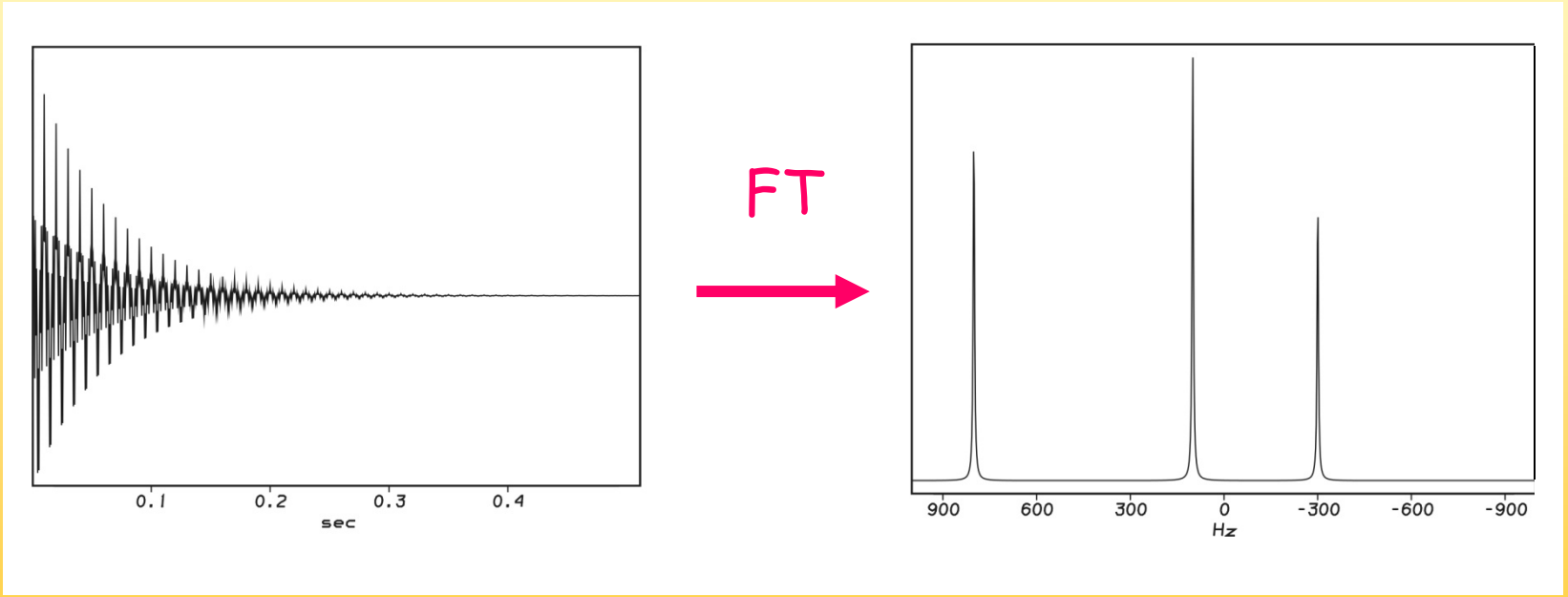


.... to get better signal-to-noise.

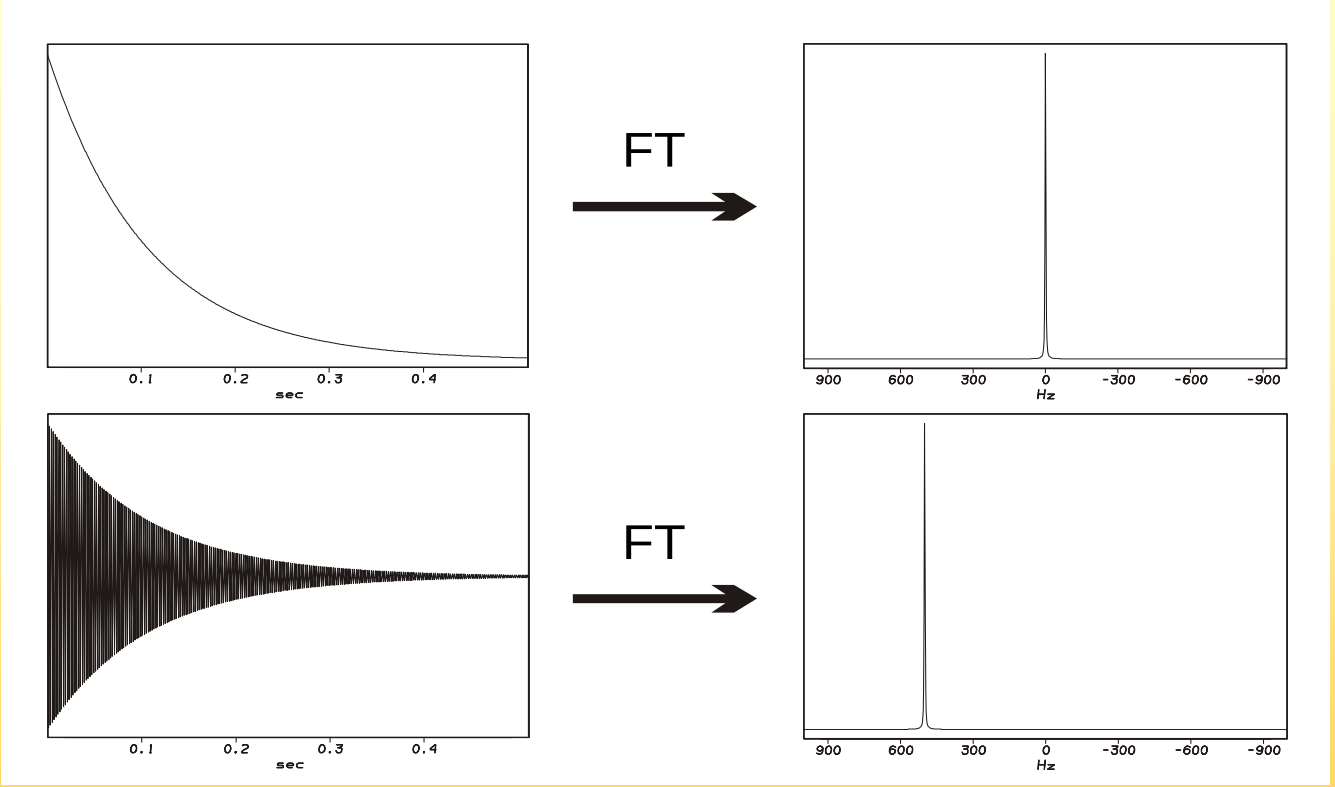


Basic aspects of NMR-spectroscopy

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

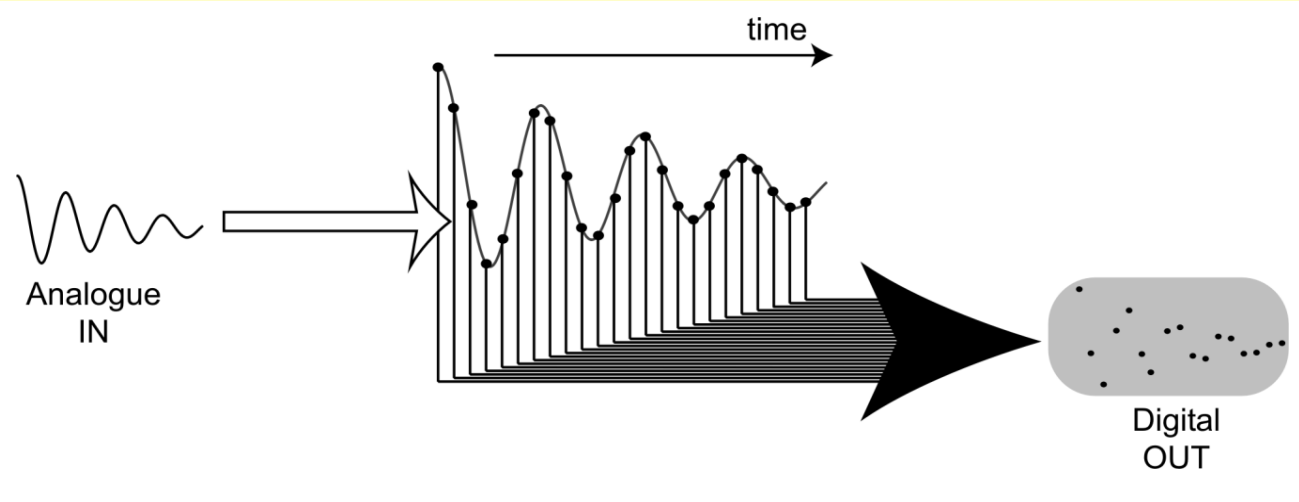


Basic aspects of NMR-spectroscopy

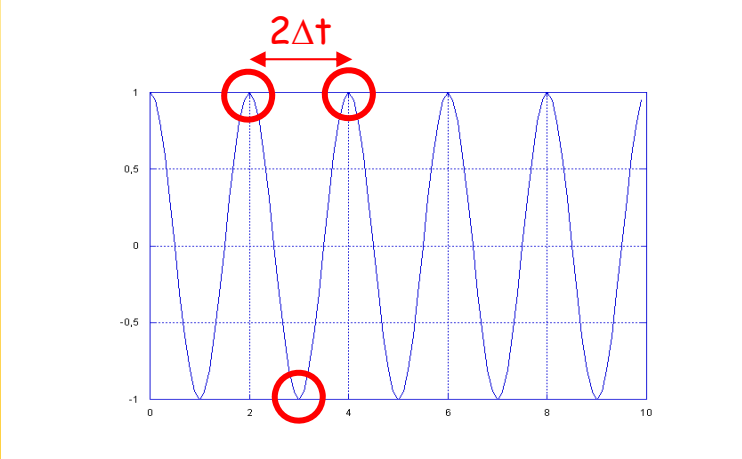


The decay determines the shape of the peak, the oscillation its position in the spectrum

Basic aspects of NMR-spectroscopy

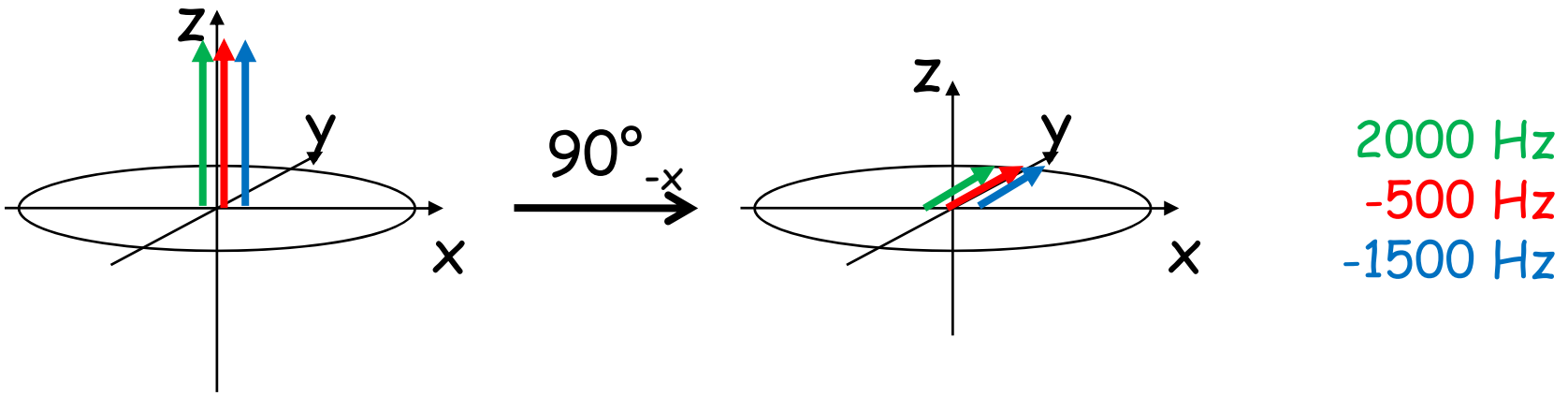


To perform the FT on a computer they need to be digitized which introduces some constraints on the experiments



Multidimensional NMR-spectroscopy

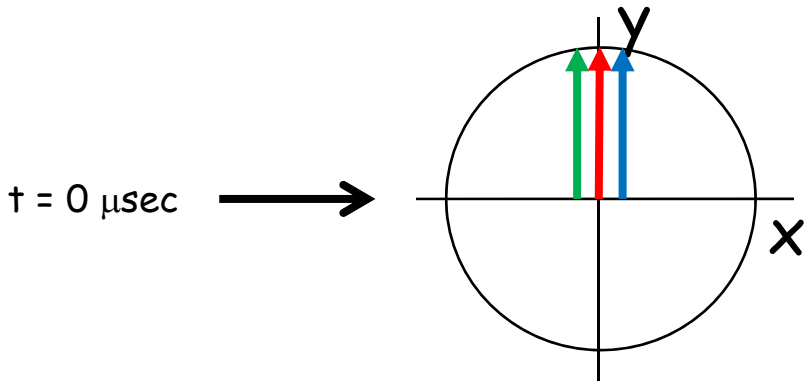
We take a closer look at that



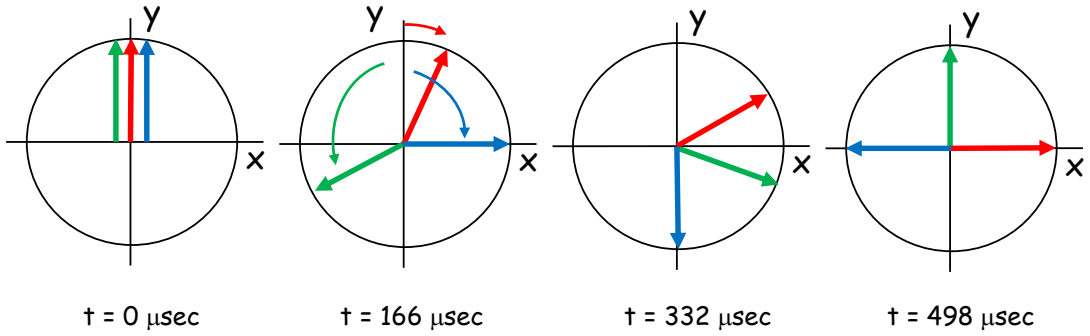
We record a spectrum using

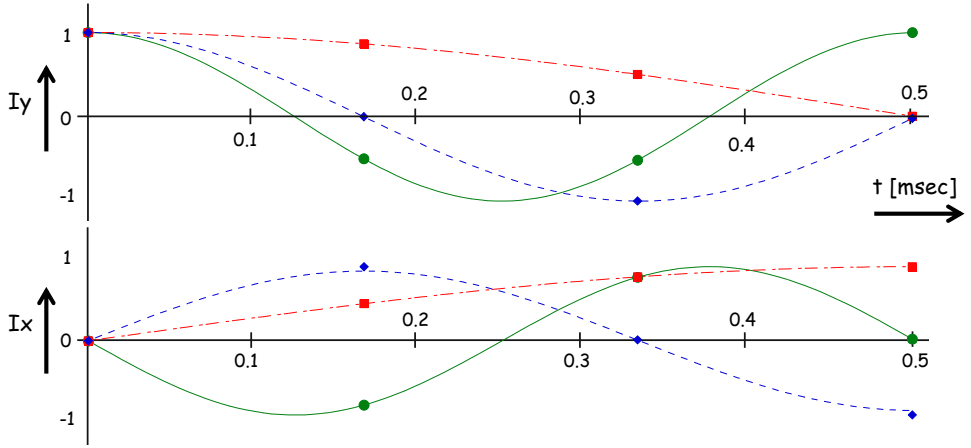
SW = 6000 Hz (+/- 3000),

which means a Δt = 166 µsec



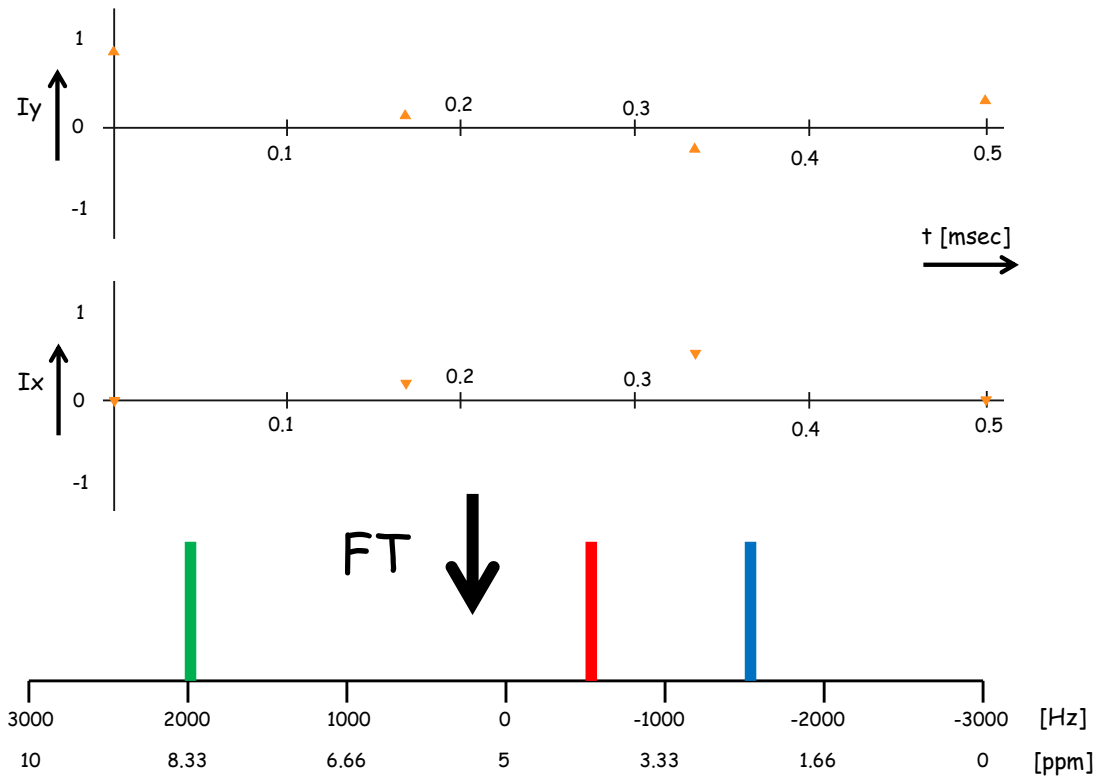
Multidimensional NMR-spectroscopy





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

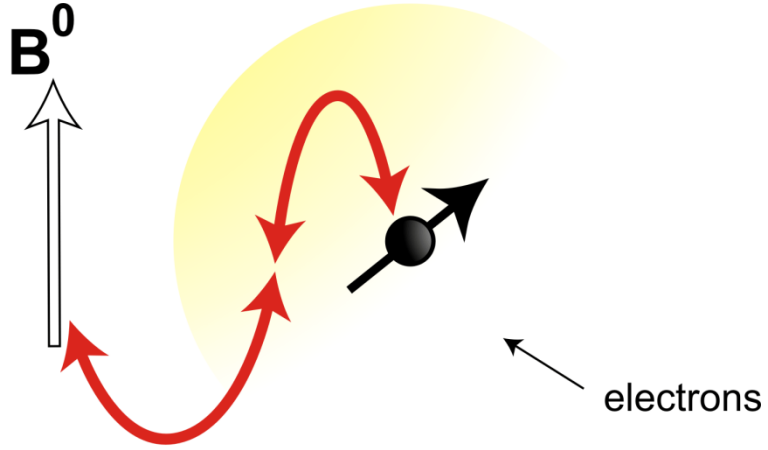
Kern	I	natürliche Häufigkeit	gyromagnetisches Verhältnis
^{1}H	1/2	99.98 %	26.75
^{12}C	0	98.89 %	0
^{13}C	1/2	1.11 %	6.73
^{14}N	1	99.63 %	1.93
^{15}N	1/2	0.37 %	-2.71
^{19}F	1/2	100 %	25.18
^{31}P	1/2	100 %	10.84
^{113}Cd	1/2	12.26 %	-5.96

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



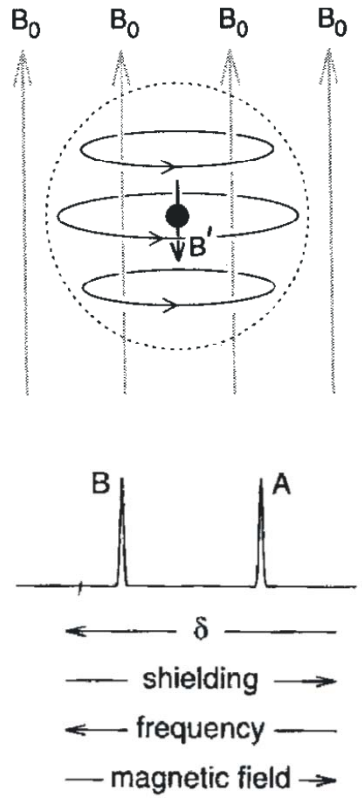
$$B_{eff} = (1 - \sigma)\, B_0$$

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

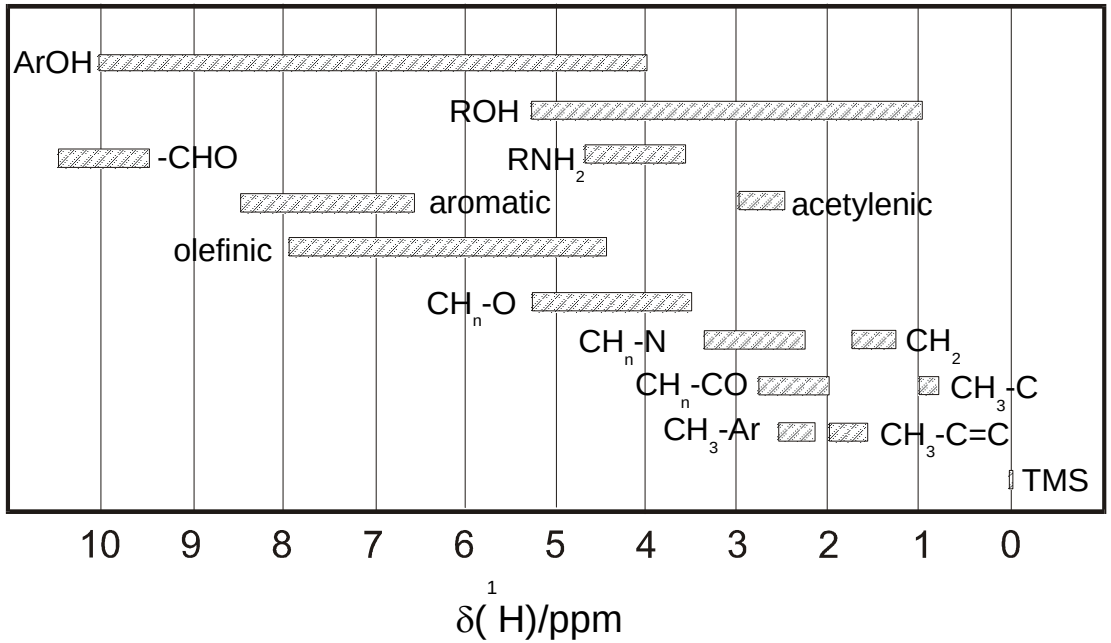
$$\delta = (\omega - \omega_{ref}) / \omega_0 \times 10^6$$

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

NMR-parameter

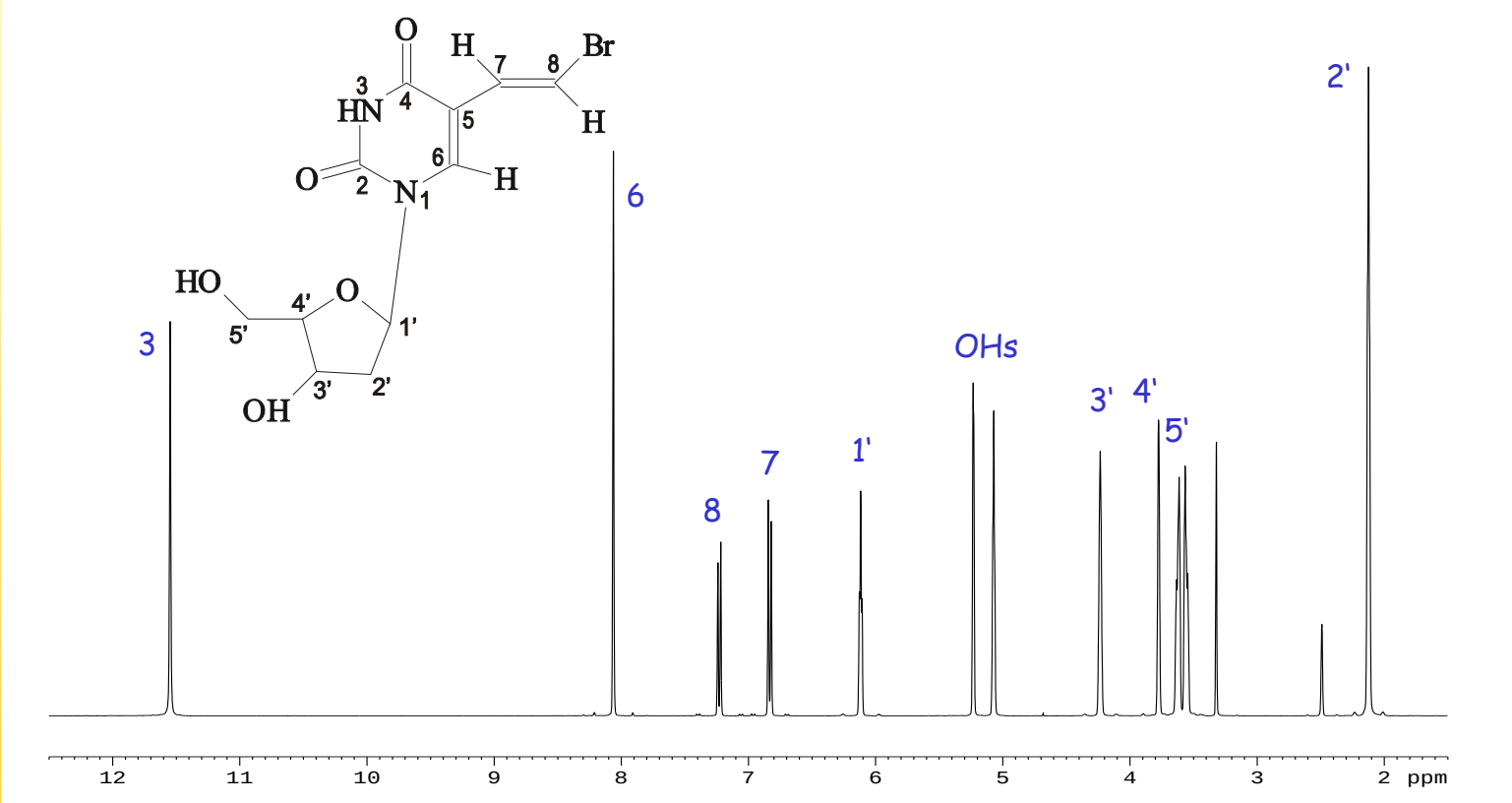


Chemical shift



NMR-parameter

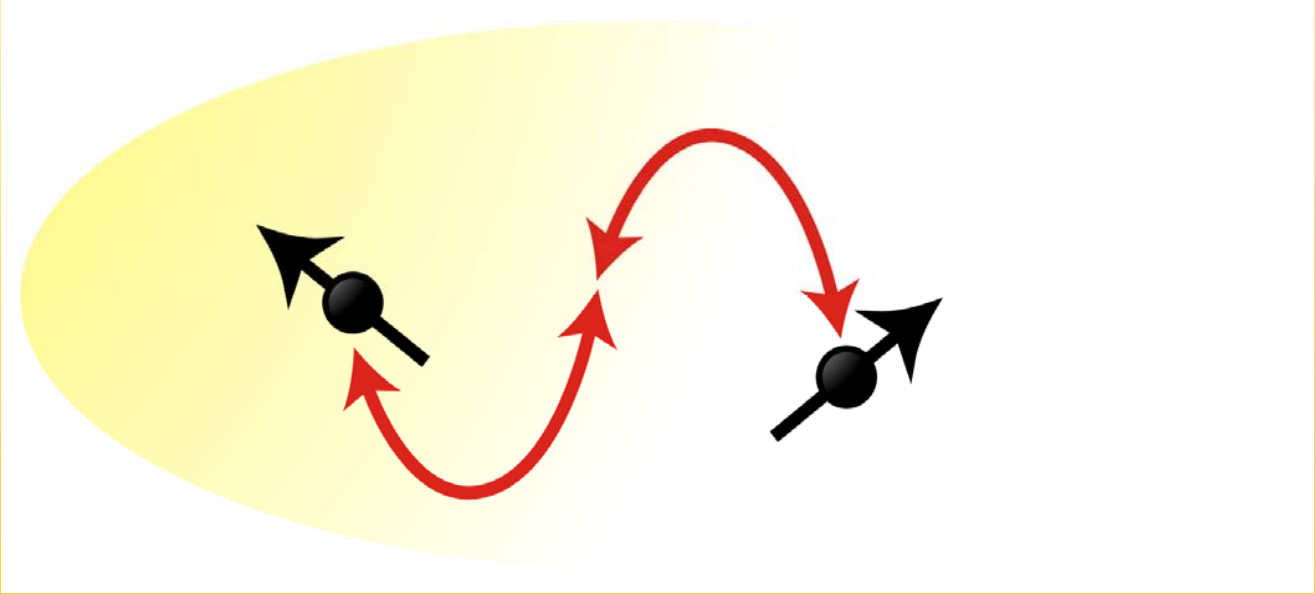
„Assignment" means to find the nucleus to each line in the spectrum



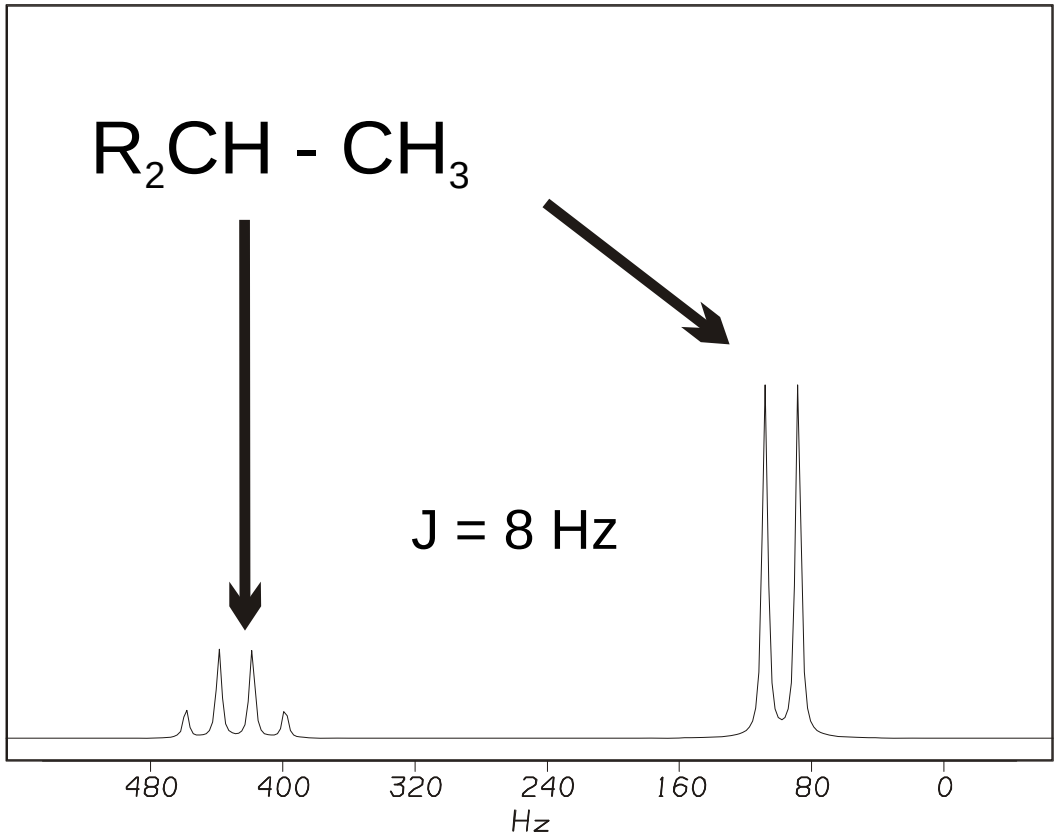
NMR-parameter

Scalar or J-coupling

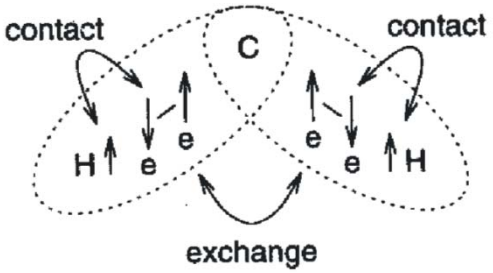
The electrons surrounding the nuclei do also establish an interaction between the nuclei



NMR-parameter

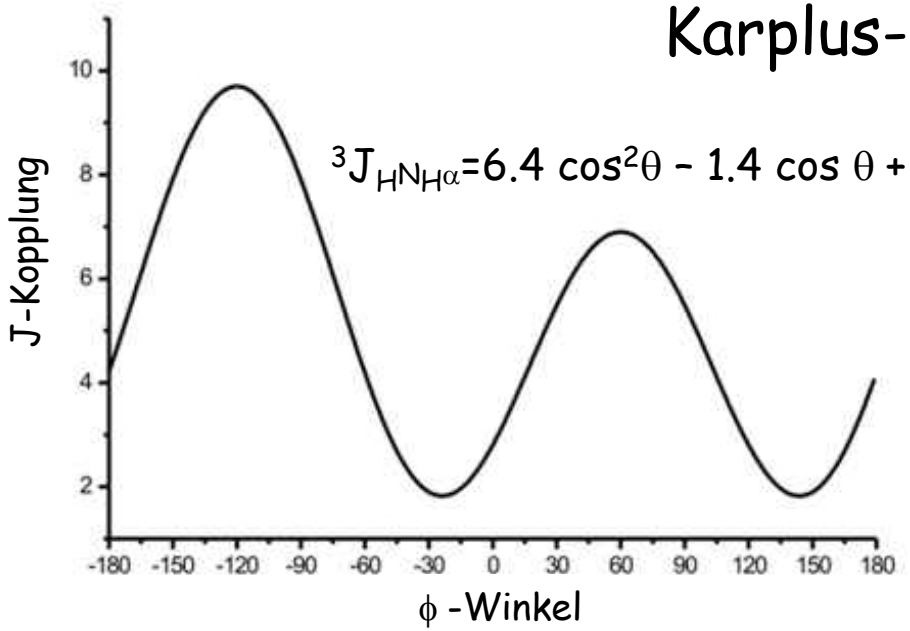


Scalar or J-coupling

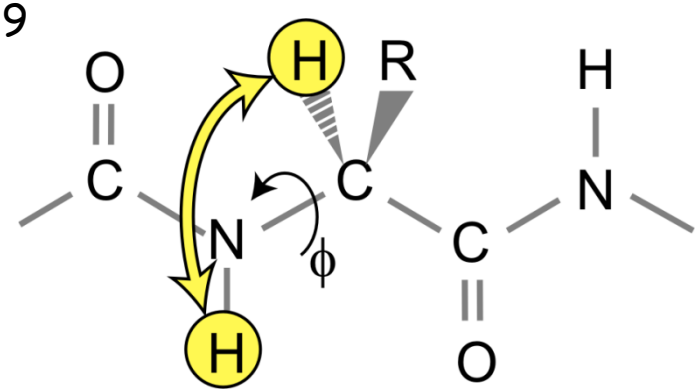


NMR-parameter

Karplus-equation



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

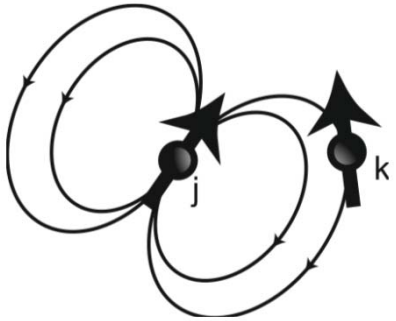


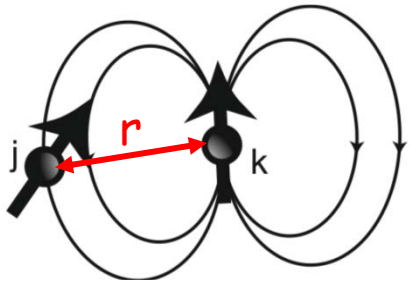
J-couplings yield structural information but are also important for the transfer of magnetization in multidimensional spectra

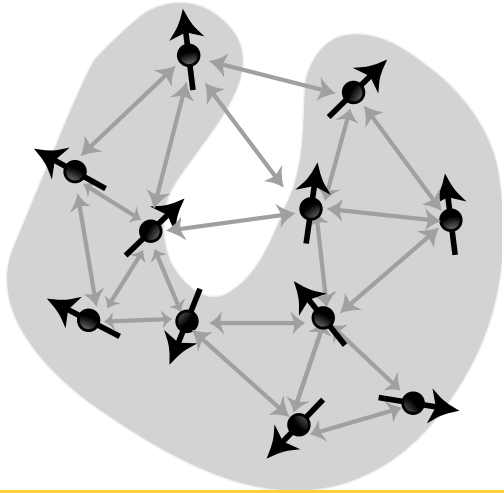
NMR-parameter

Dipol-Dipol Interaction

This mutual interaction is working through-space and results in an interaction network of spins. The size of the dipol-dipol coupling constant is much larger than that a scalar coupling and distant dependent



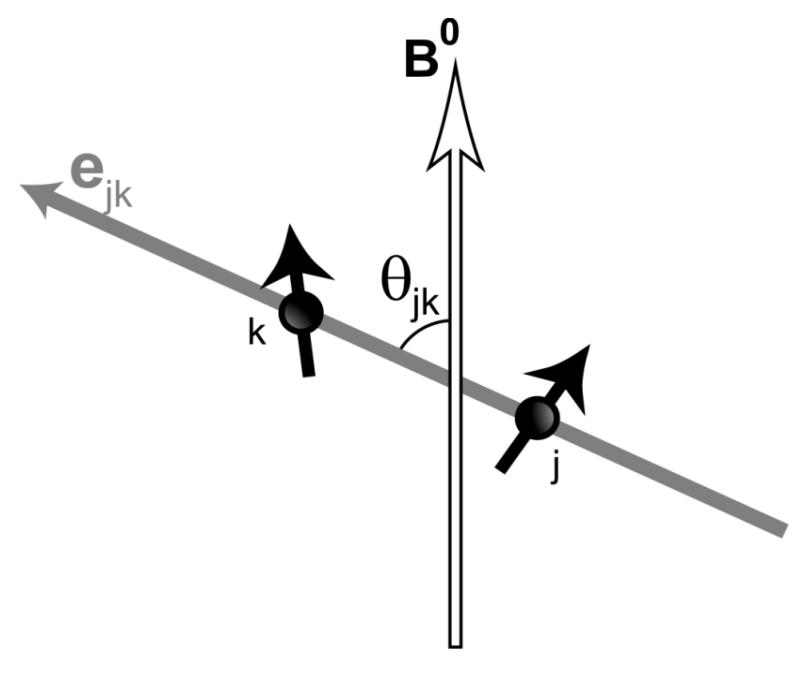




D_{HH} = -15000 Hz for r = 200 pm

NMR-parameter

While the dipol-dipol coupling constant is only dependent on the distance between the spins the size of the interaction does also depend on the orientation between the vector between the spins and the magnetic field.

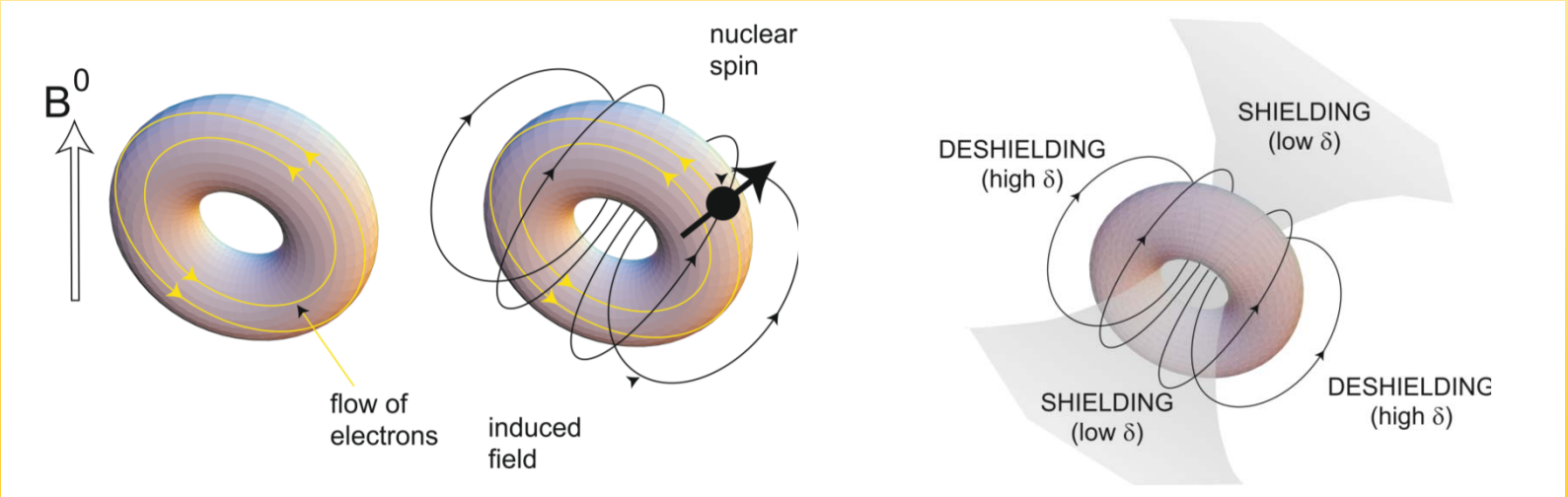


$$D \sim (3 \cos^2 \theta_{jk} - 1)$$

NMR-parameter

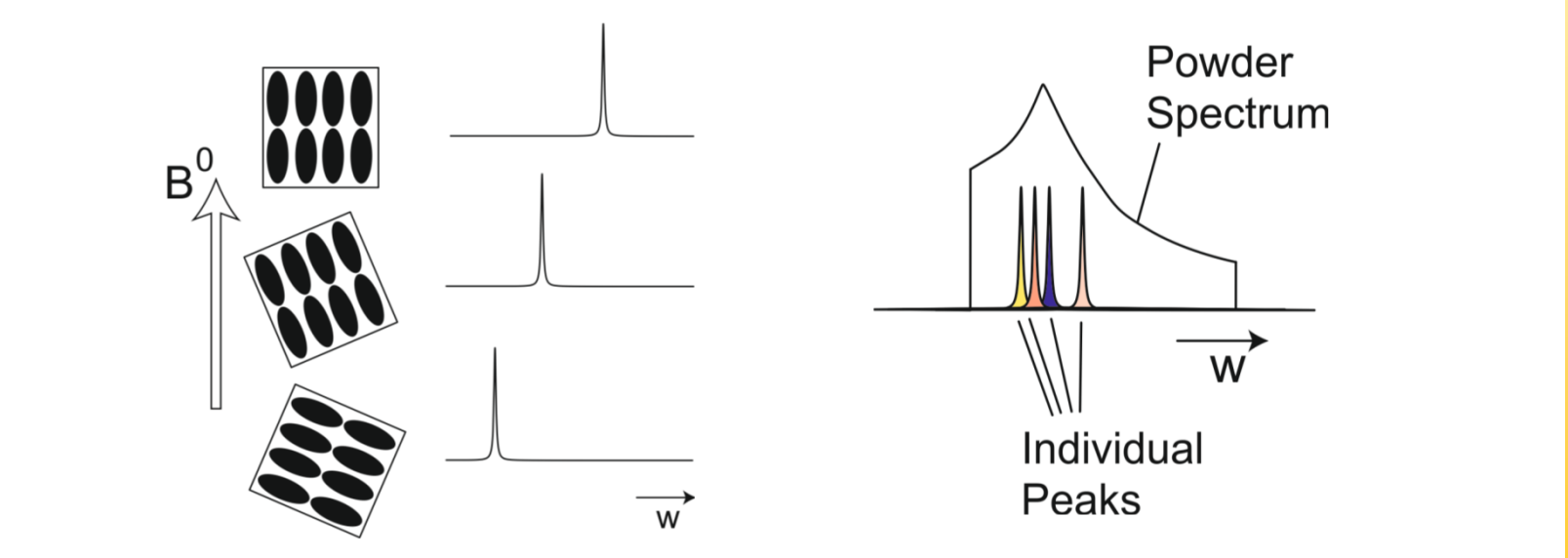
Chemical shift anisotropy (CSA)

When we first discussed chemical shift we assumed that the electrons would surround the nucleus spherically. This is usually not the case and the electrons create different additional field in each direction

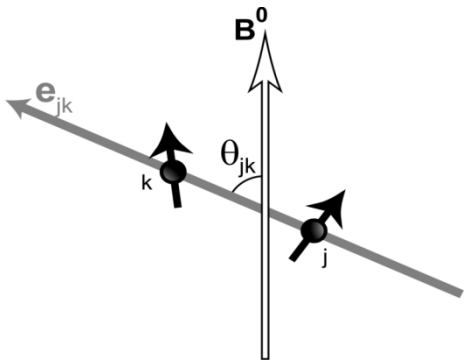


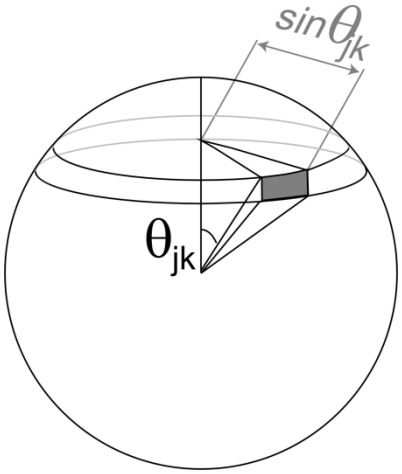
NMR-parameter

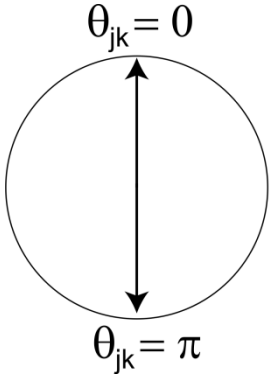
In a solid this results in a complicated pattern called a powder spectrum. In solution, the rapid reorientation of all molecules averages the effect of CSA and results in a single "isotropic" chemical shift

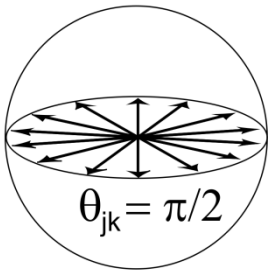


NMR-parameter









$D \sim (3 \cos^2 \theta_{jk} - 1)$

$$\int_0^{\pi} d\theta_{jk} \sin\theta_{jk} (3 \cos^2\theta_{jk} - 1) = 0$$

The same averaging takes places for the dipol-dipol-interaction. There is a distribution of orientations with many possibilities perpendicular to the field and only two with the field. Adding up all interactions leads to their cancelation.

NMR-parameter

Relaxation

Relaxation is the process during which the nuclei get rid of the energy transferred to the system by the RF pulse

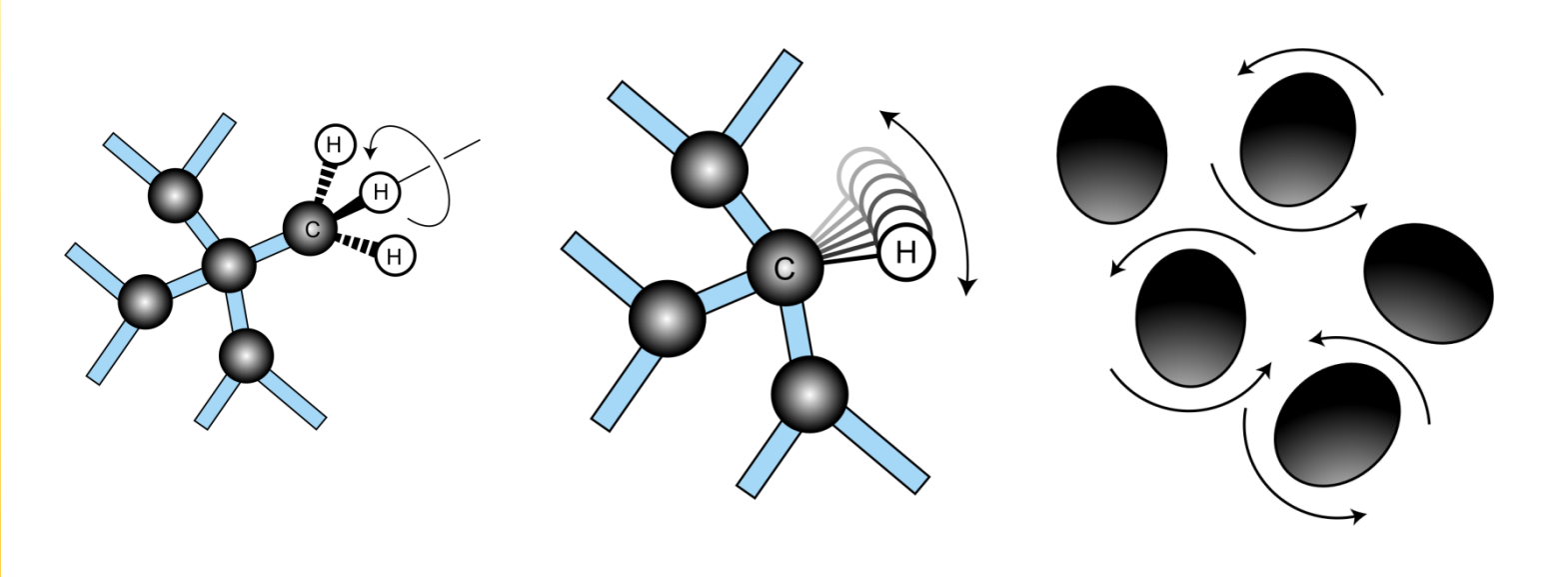
Contrary to other types of spectroscopy there are not many ways to create the necessary fluctuating magnetic field, except the movement of the molecule itself.

Thats why NMR-states are fairly long-lived!

If the dynamics of the molecule are the reason for relaxation, then we can learn something about the dynamics from analyzing relaxation

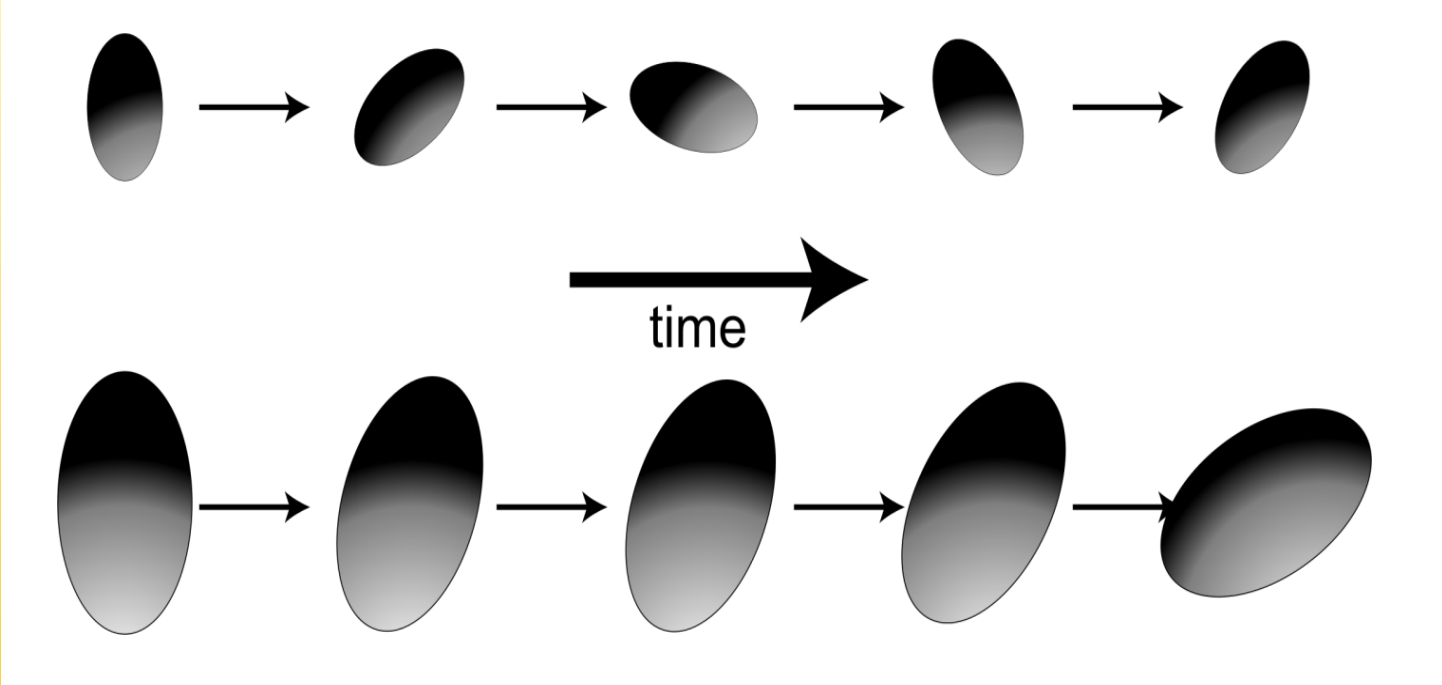
NMR-parameter

The movements can be within the molecule are of the molecule as a whole. They will be on different time scales in the range between picoseconds and milliseconds, sometimes even longer (seconds)



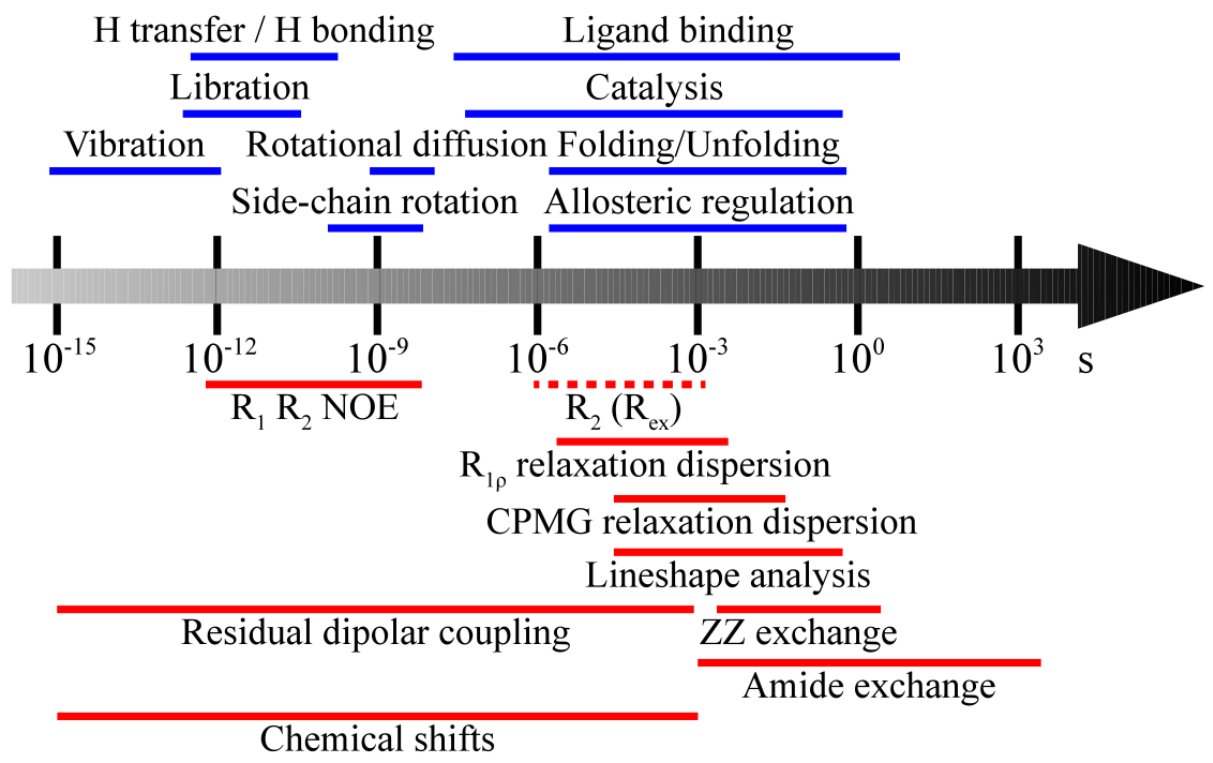
NMR-parameter

Larger Molecules move differently from smaller ones, they have other „correlation times" τ_c. That's why they will have different relaxation properties



NMR-parameter

Depending on the time constant of the motion different relaxation mechanisms are of interest

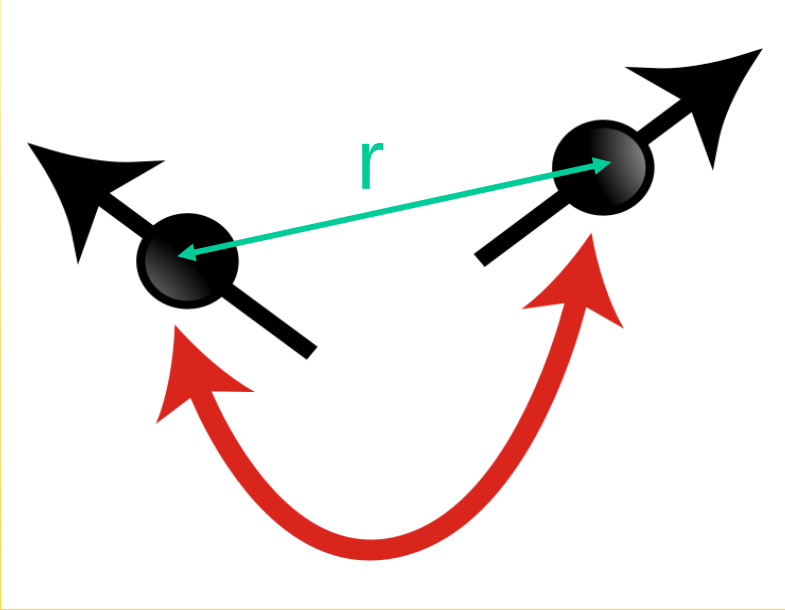


NMR-parameter

One particularly important relaxation phenomenon is the NOE-effect resulting from mutual relaxation of two spins. The importance of the effect results from the fact that it is distance dependent:

$$I(NOE) \sim 1/r^6$$

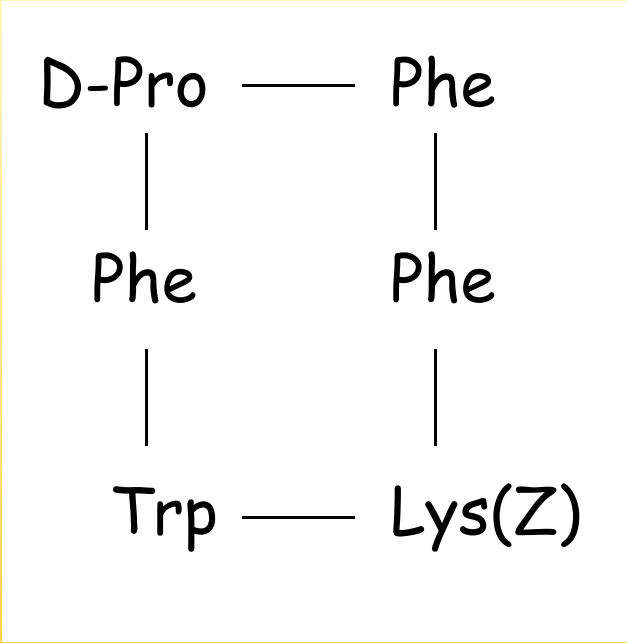
Because of the dependence on r^6 only short distances up to 500 pm can be determined

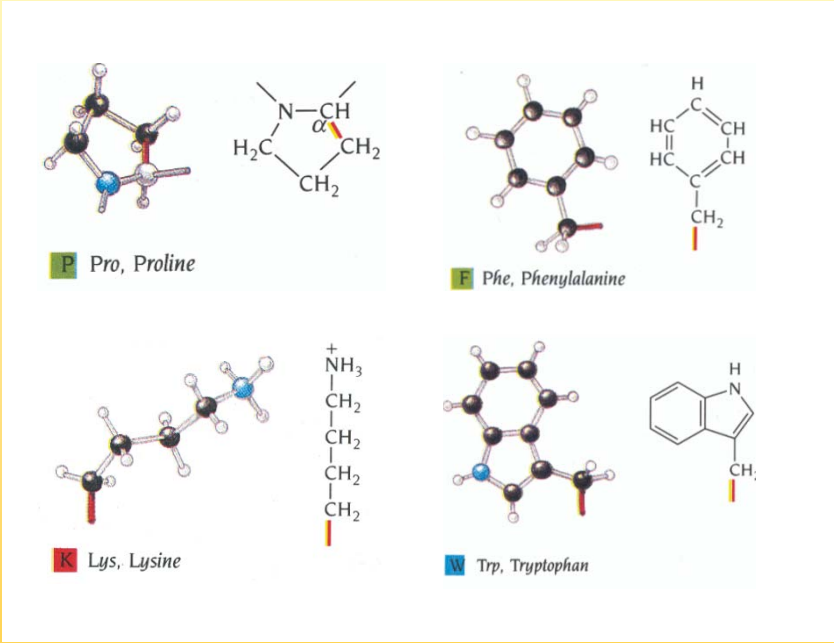


Multidimensional NMR-spectroscopy

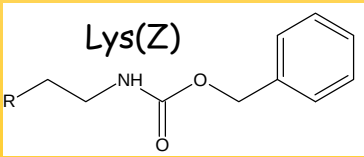
Multidimensional NMR-spectroscopy

Cyclic peptides are small peptides usuallywith a fixed conformation



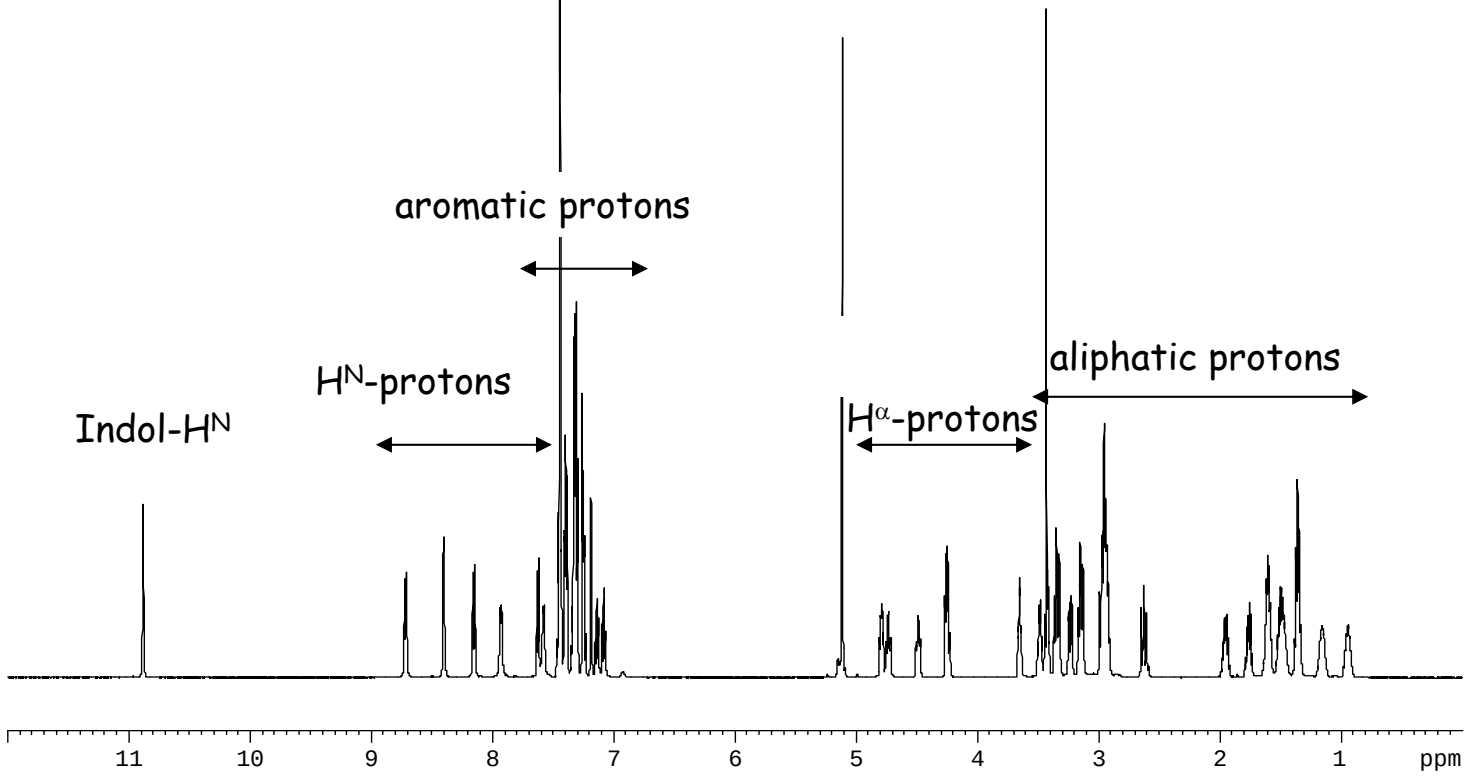


F3-008: *cyc*-(dP-F-F-K(Z)-W-F)

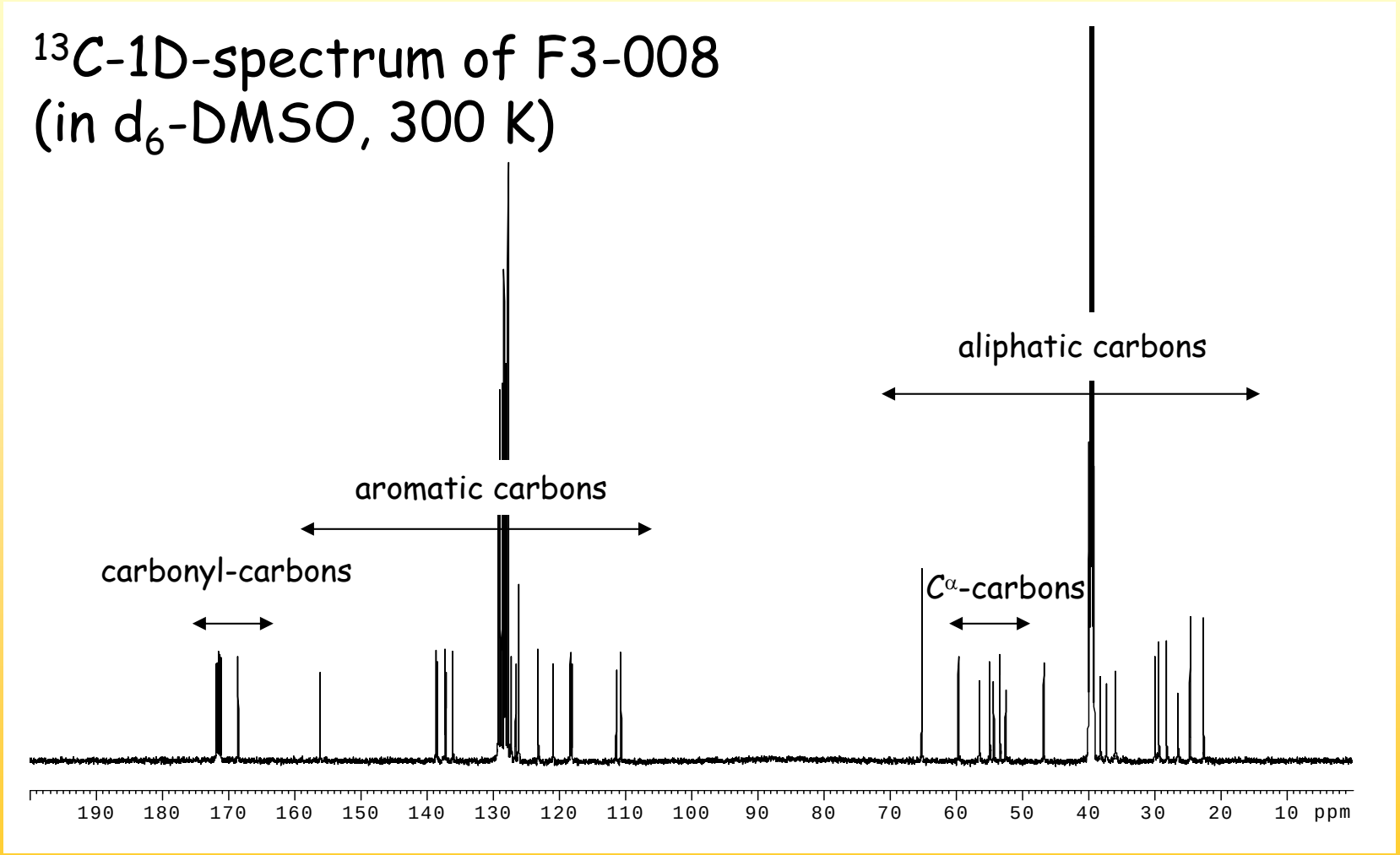


Multidimensional NMR-spectroscopy

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

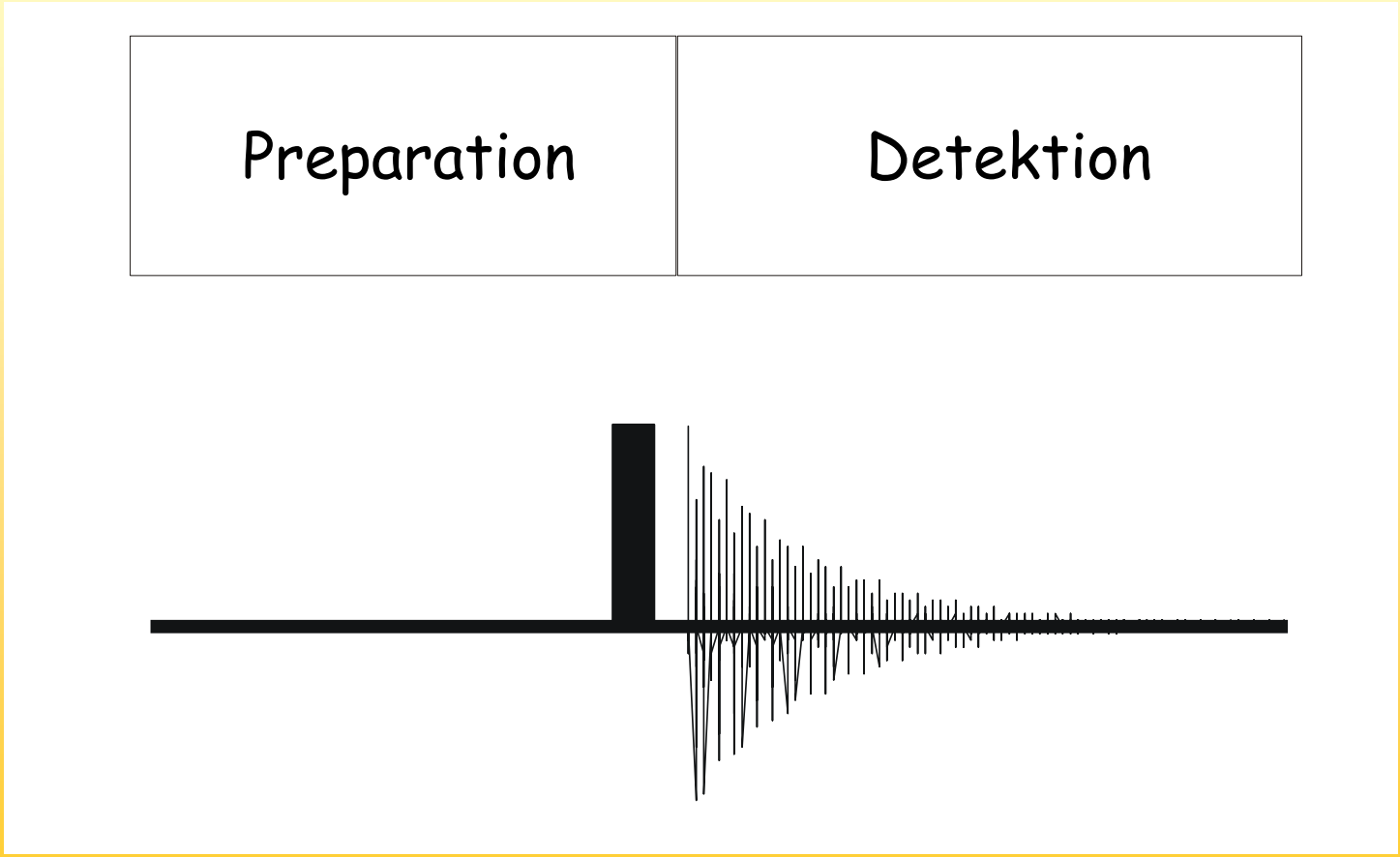


Multidimensional NMR-spectroscopy



Multidimensional NMR-spectroscopy

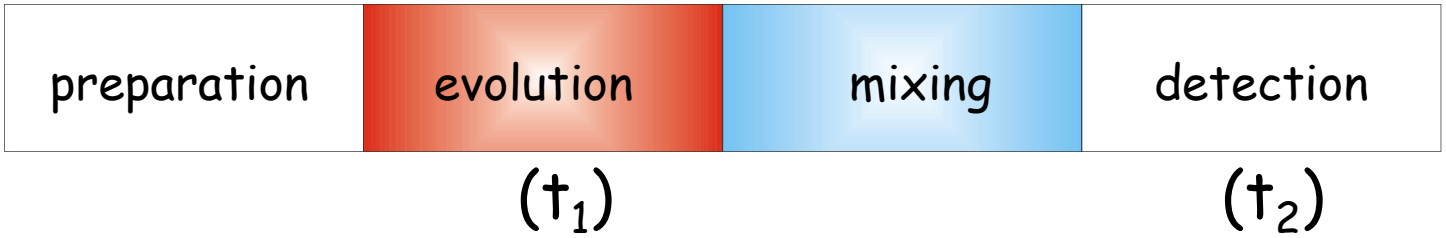
1D-NMR schematisch



Multidimensional NMR-spectroscopy

2D-NMR experiments contain two new elements:

evolution time and mixing time

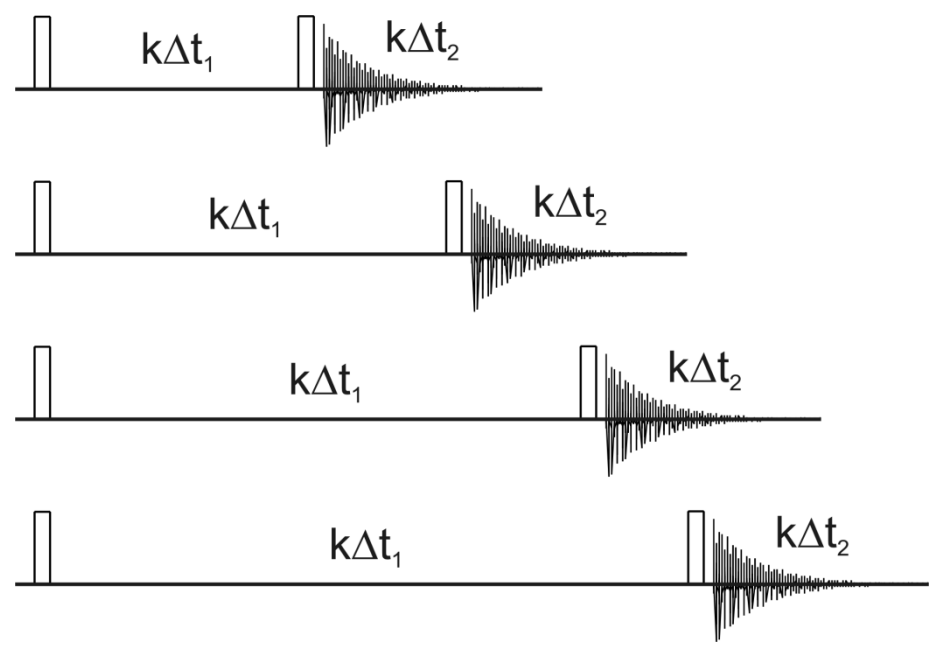


evolution time:
creation of a further frequency axis by indirect detection

mixing time:
transfer of magnetization via spin-spin interactions

Multidimensional NMR-spectroscopy

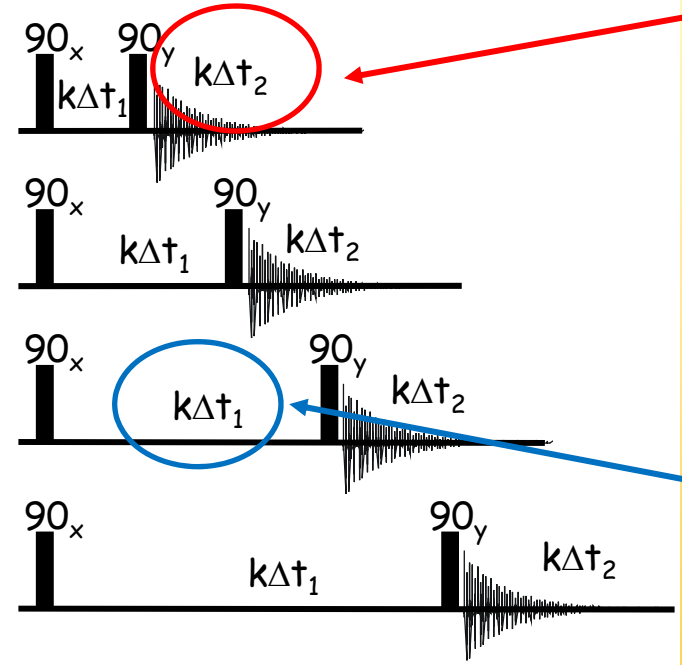
Evolution time



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

Multidimensional NMR-spectroscopy

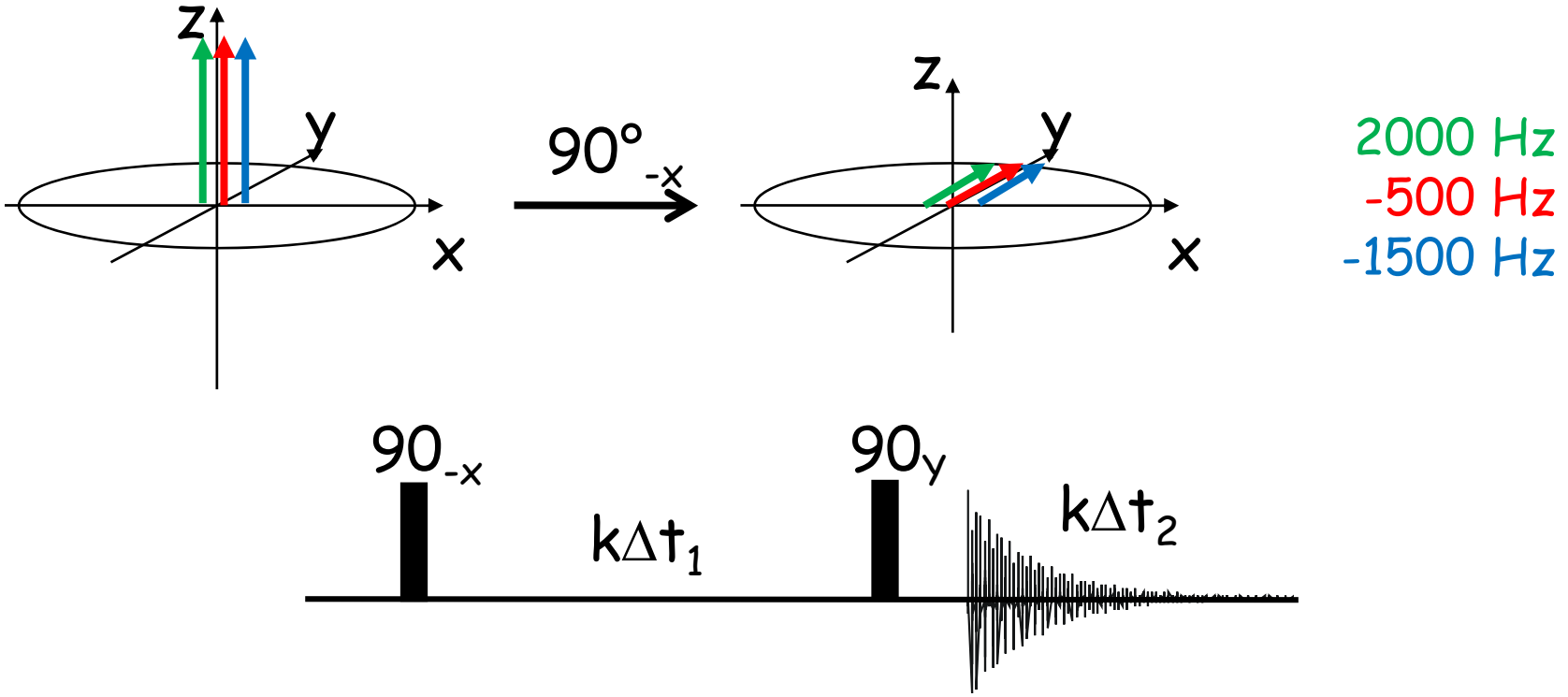
Evolution time



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

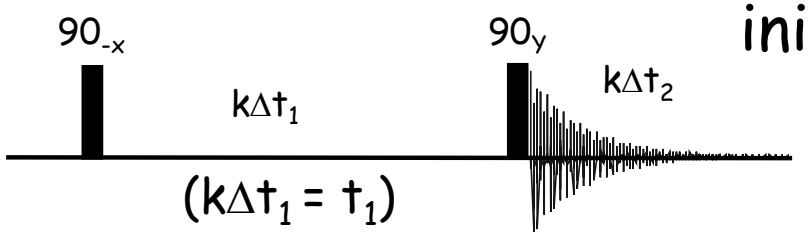
Multidimensional NMR-spectroscopy

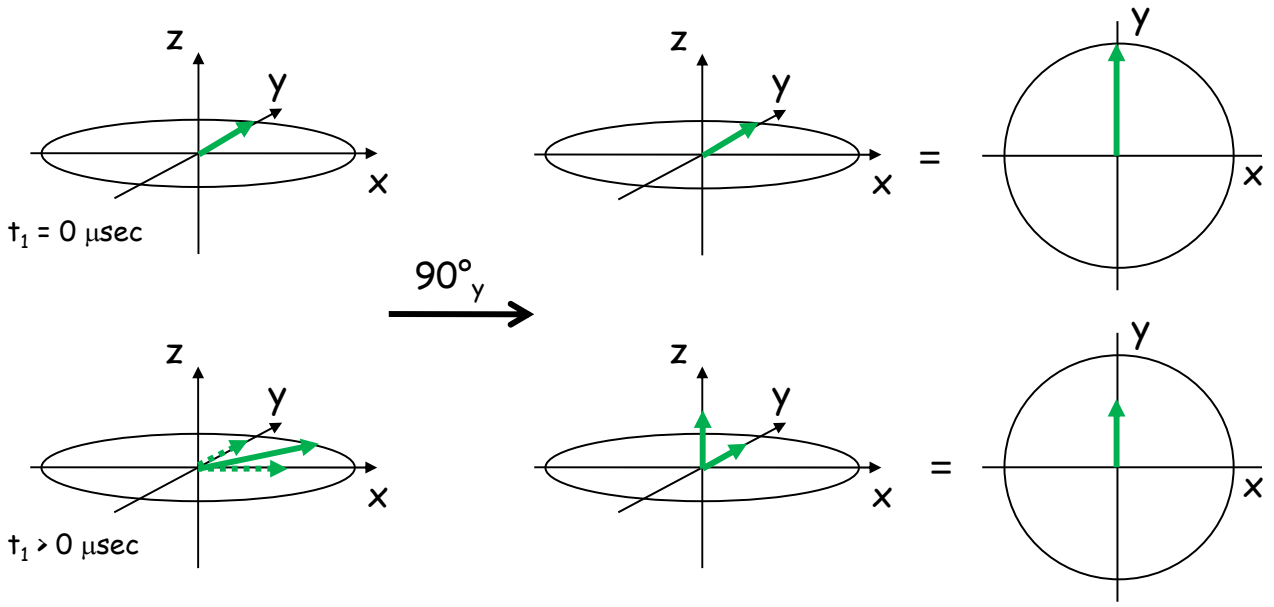
We take another closer look using the same three lines as in case of the 1D spectrum



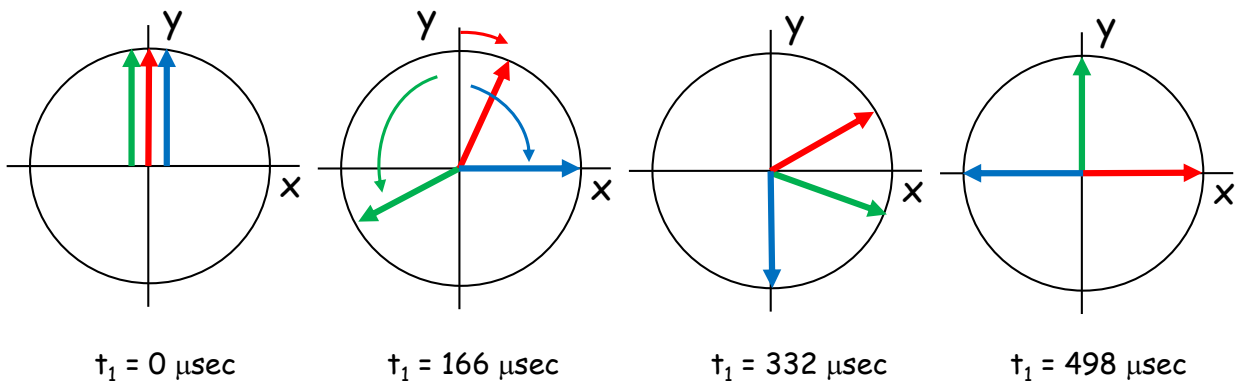
Multidimensional NMR-spectroscopy

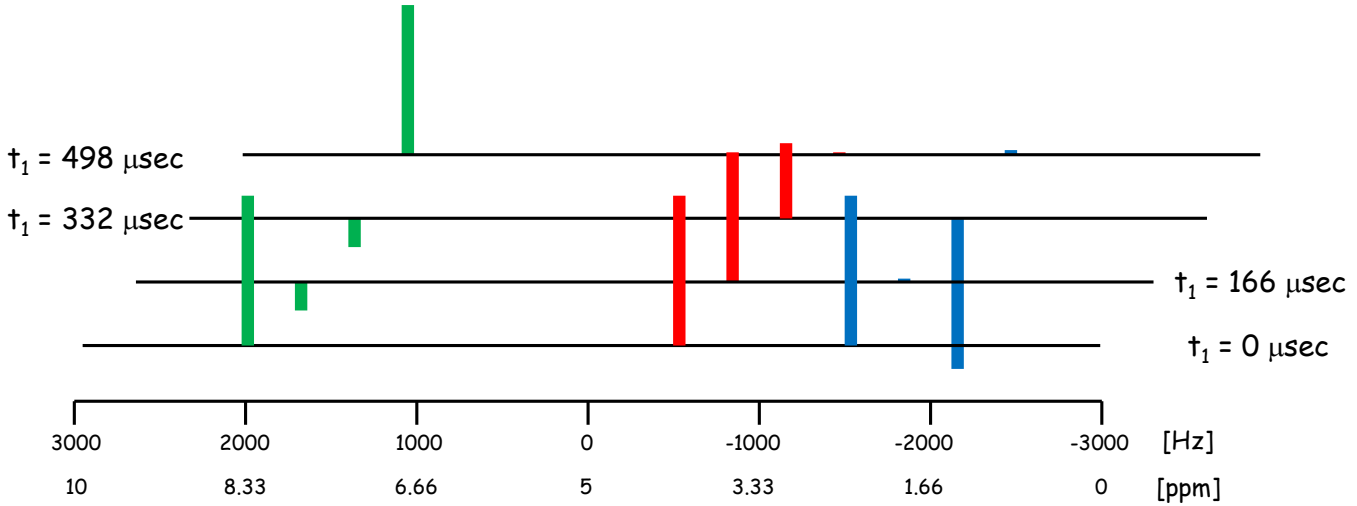
One can see that the initial intensity in t_2 depends on the frequency





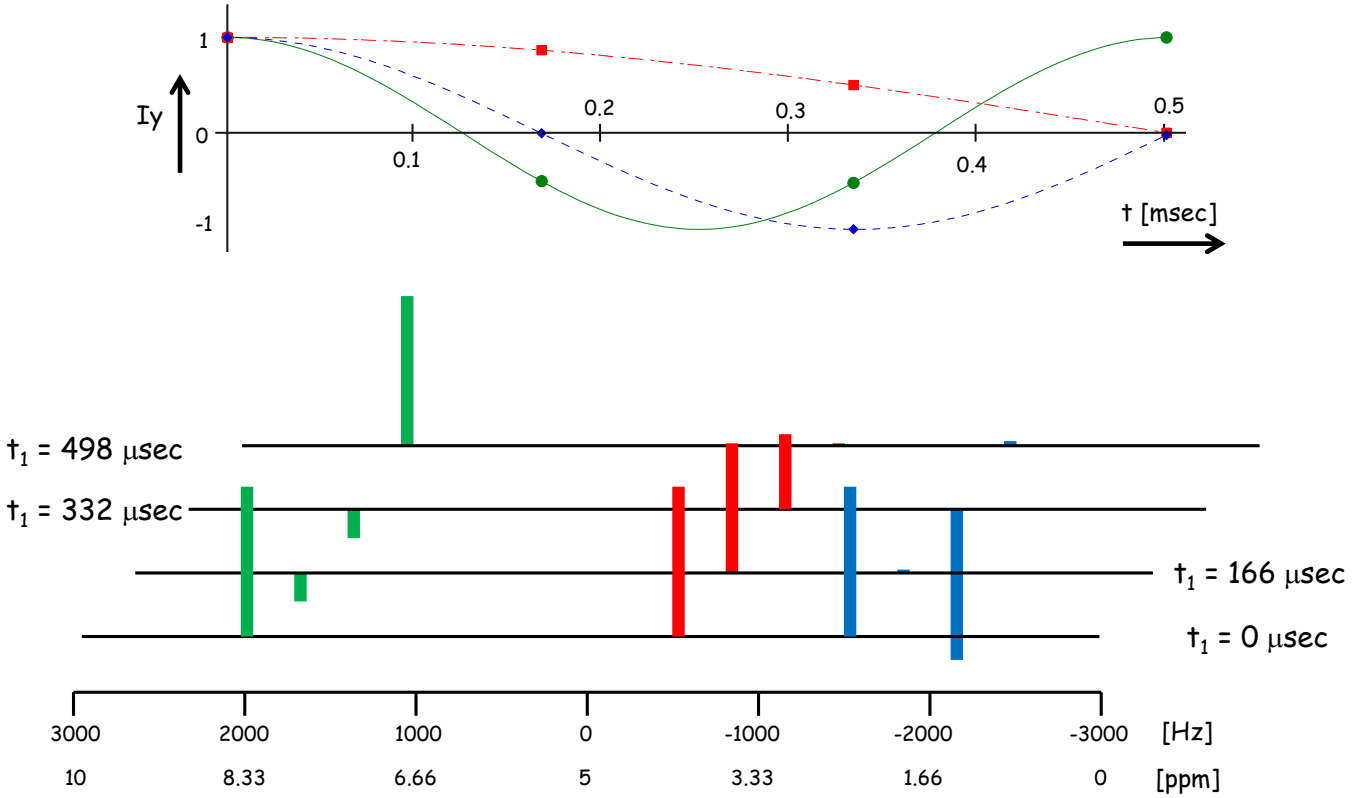
Multidimensional NMR-spectroscopy





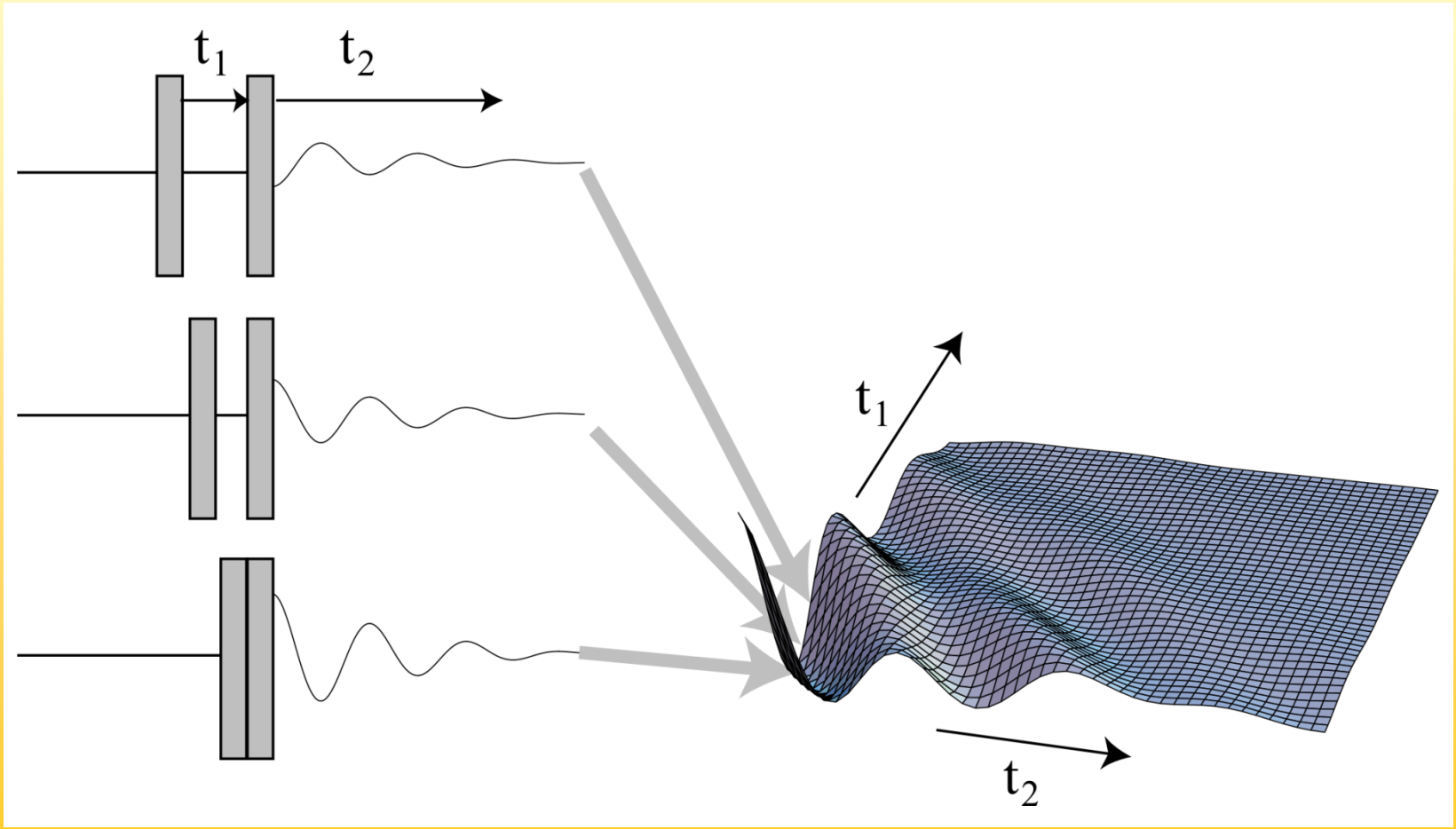
Multidimensional NMR-spectroscopy

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



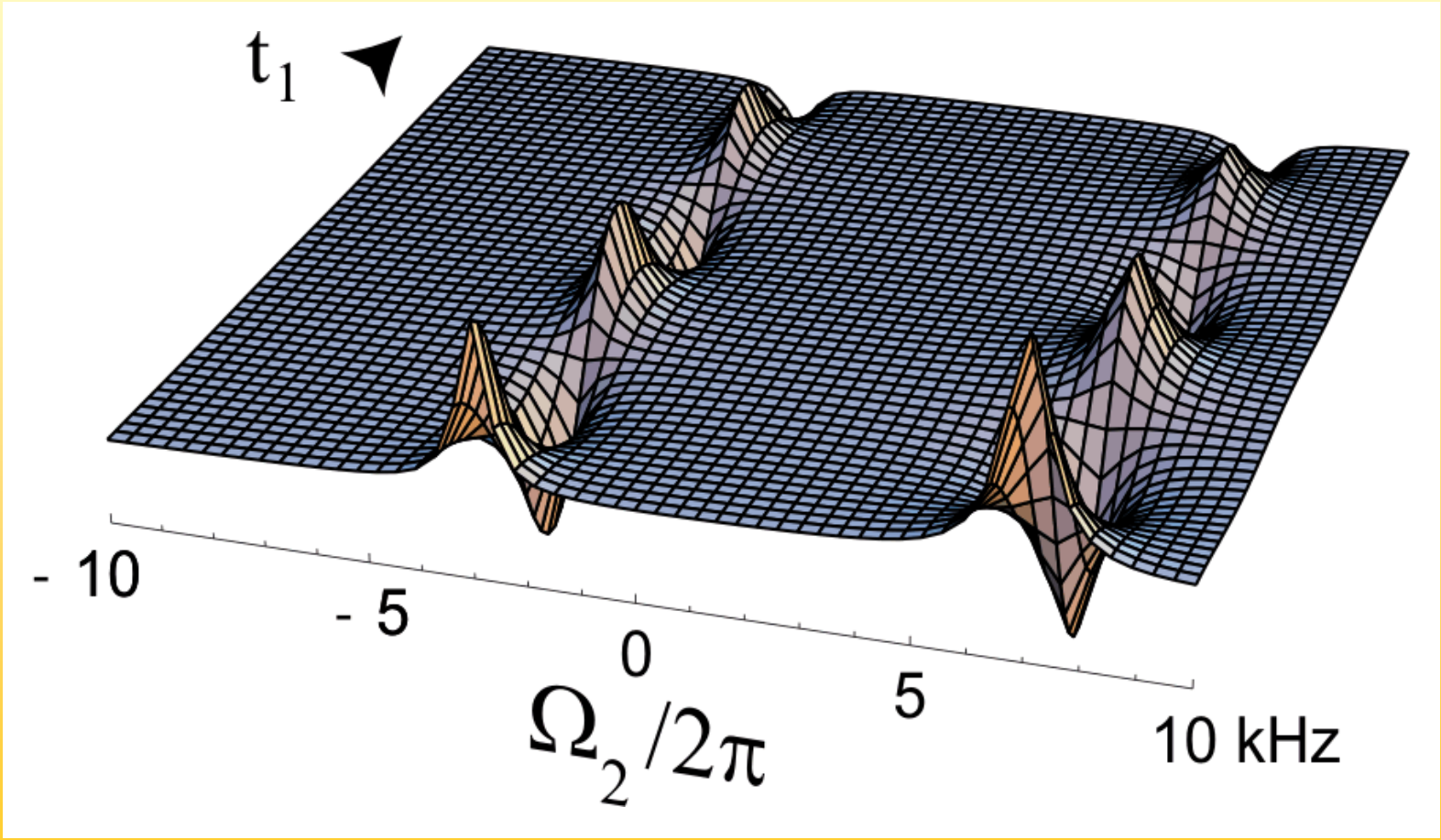
Multidimensional NMR-spectroscopy

We obtain a two-dimensional FID



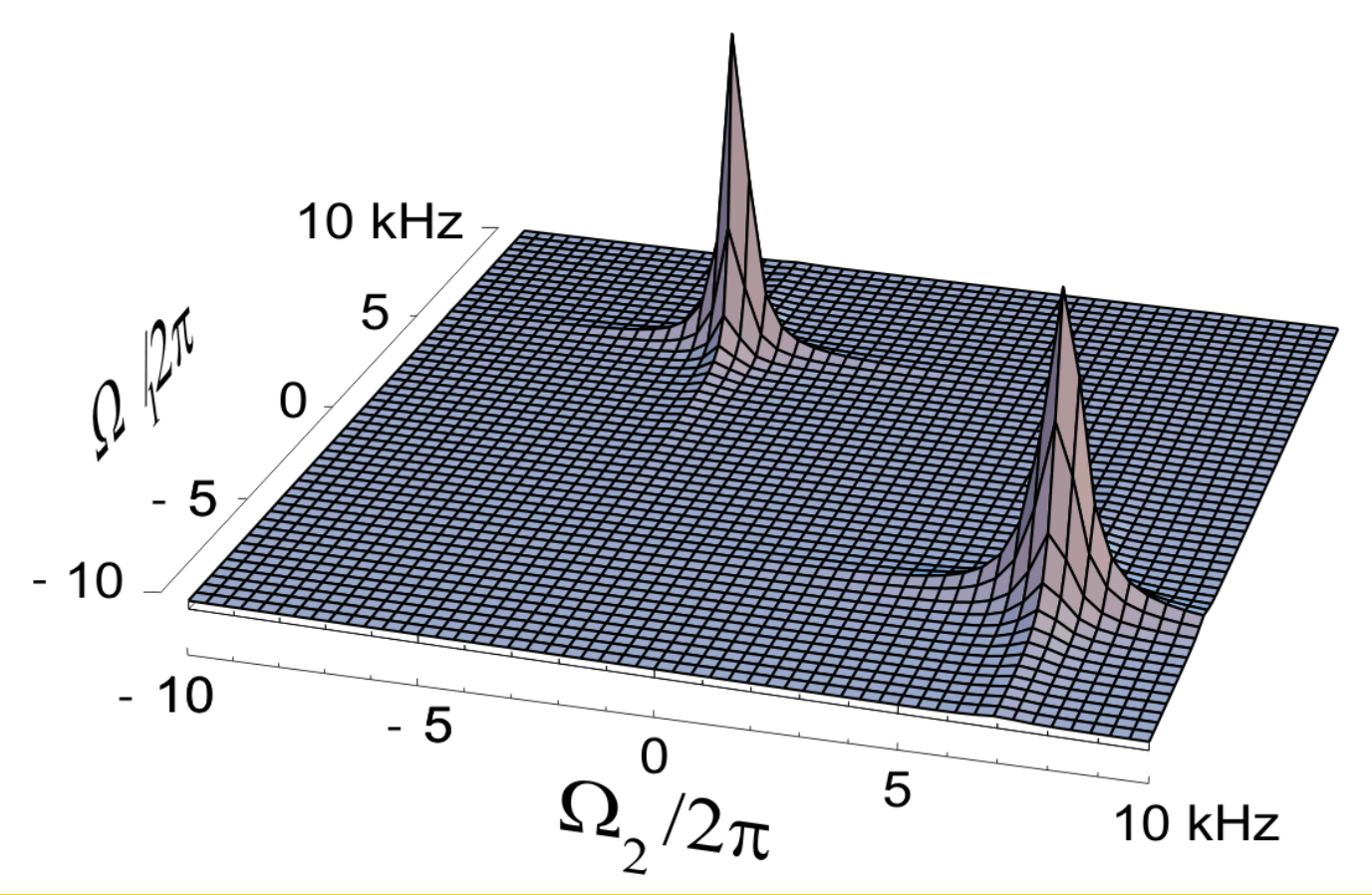
Multidimensional NMR-spectroscopy

a first FT results in an „interferogram"

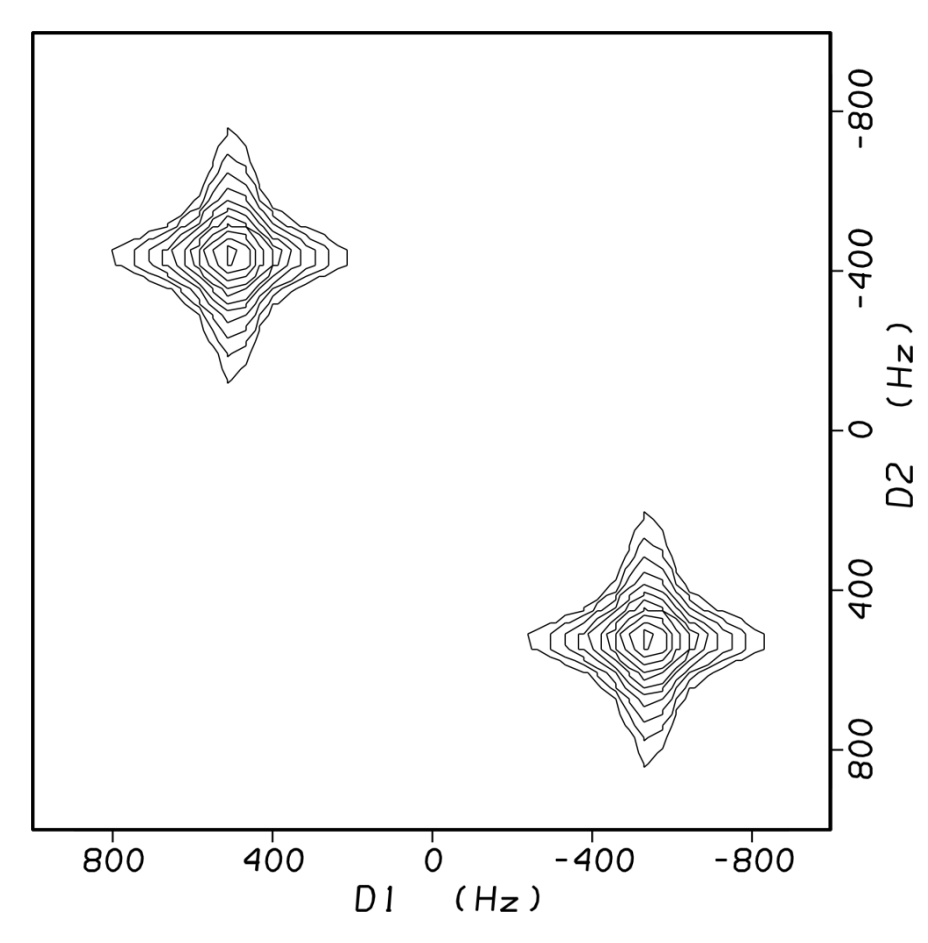


Multidimensional NMR-spectroscopy

a second FT yields the two-dimensional spectrum

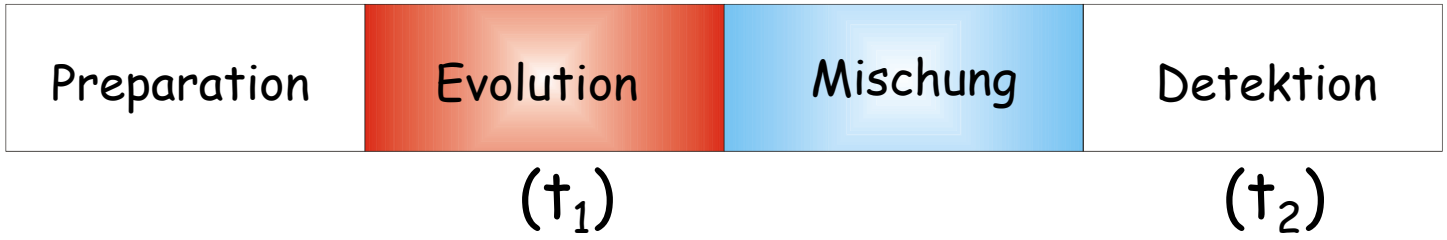


Multidimensional NMR-spectroscopy



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

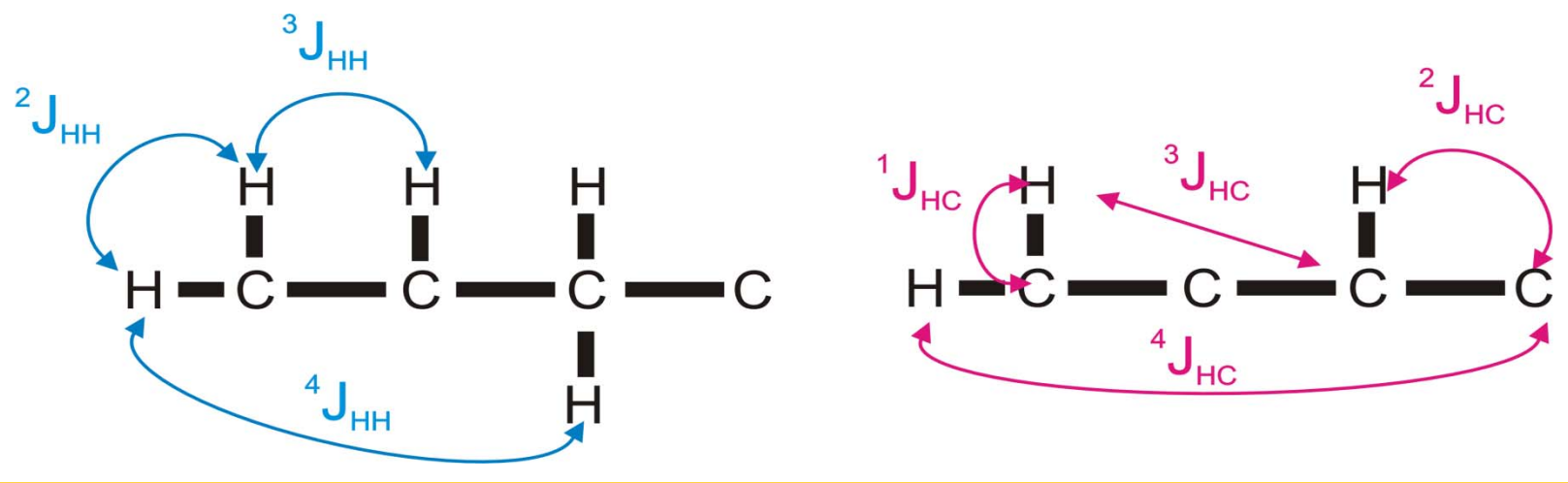
Multidimensional NMR-spectroscopy



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

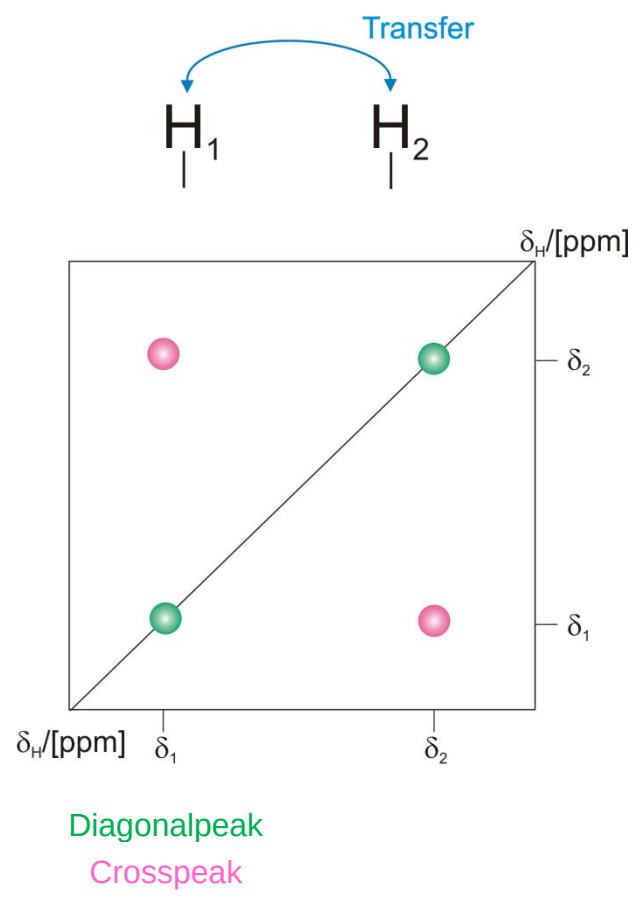
homonuclear spectra

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:

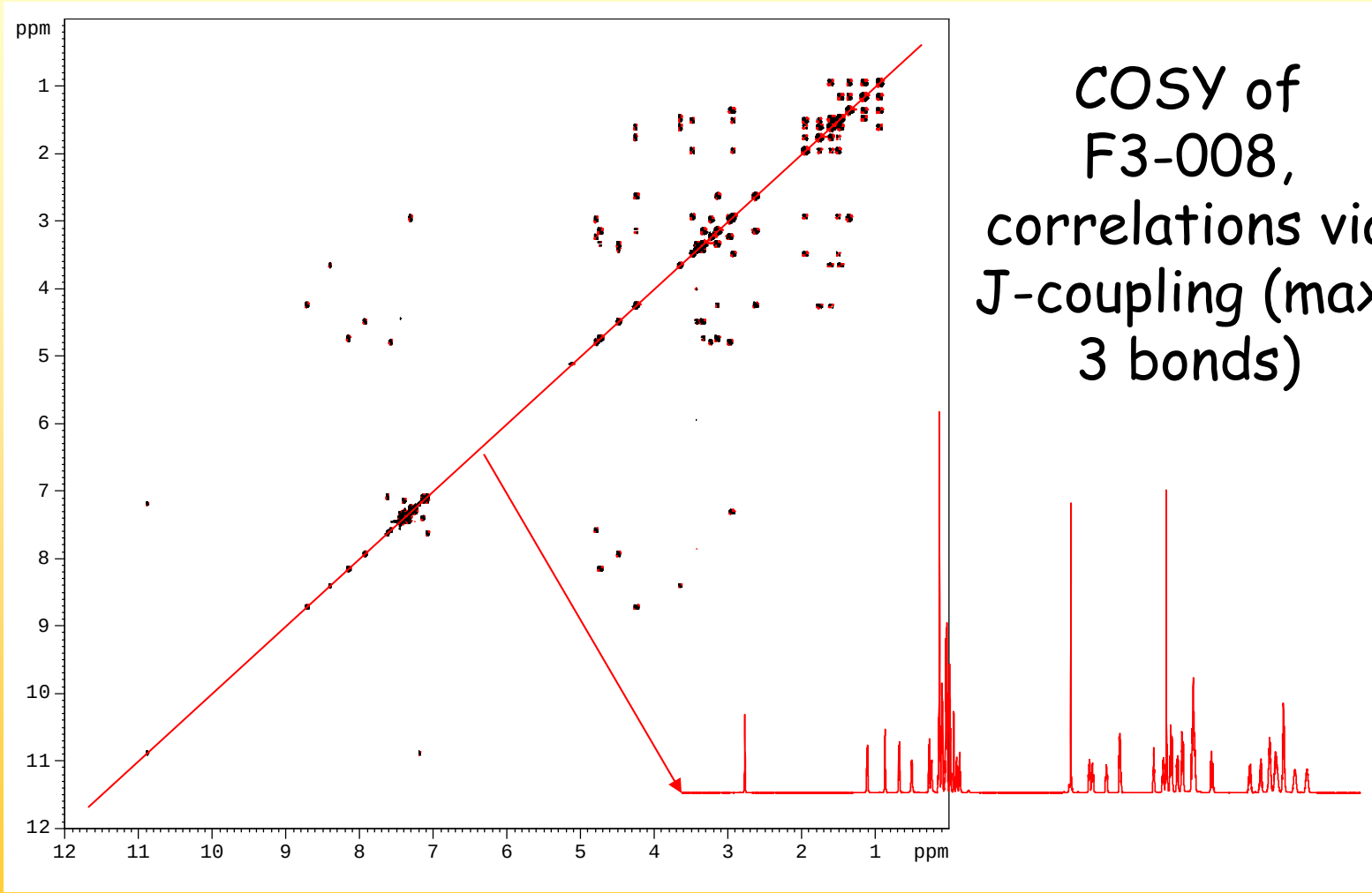
Crosspeak

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

Diagonalpeak



Multidimensional NMR-spectroscopy

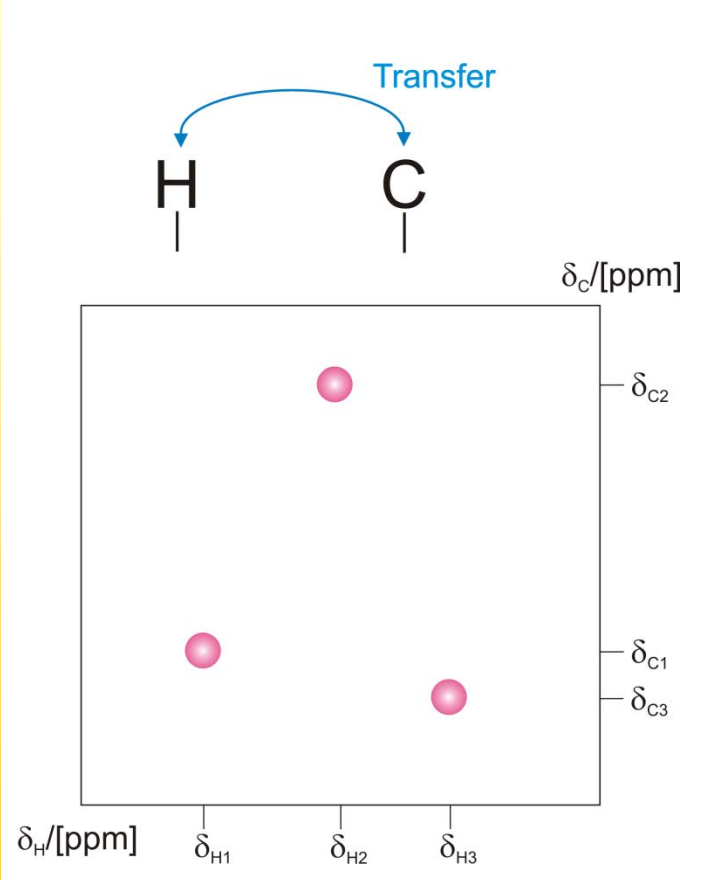


COSY of F3-008, correlations via J-coupling (max. 3 bonds)

Multidimensional NMR-spectroscopy

heteronuclear spectra

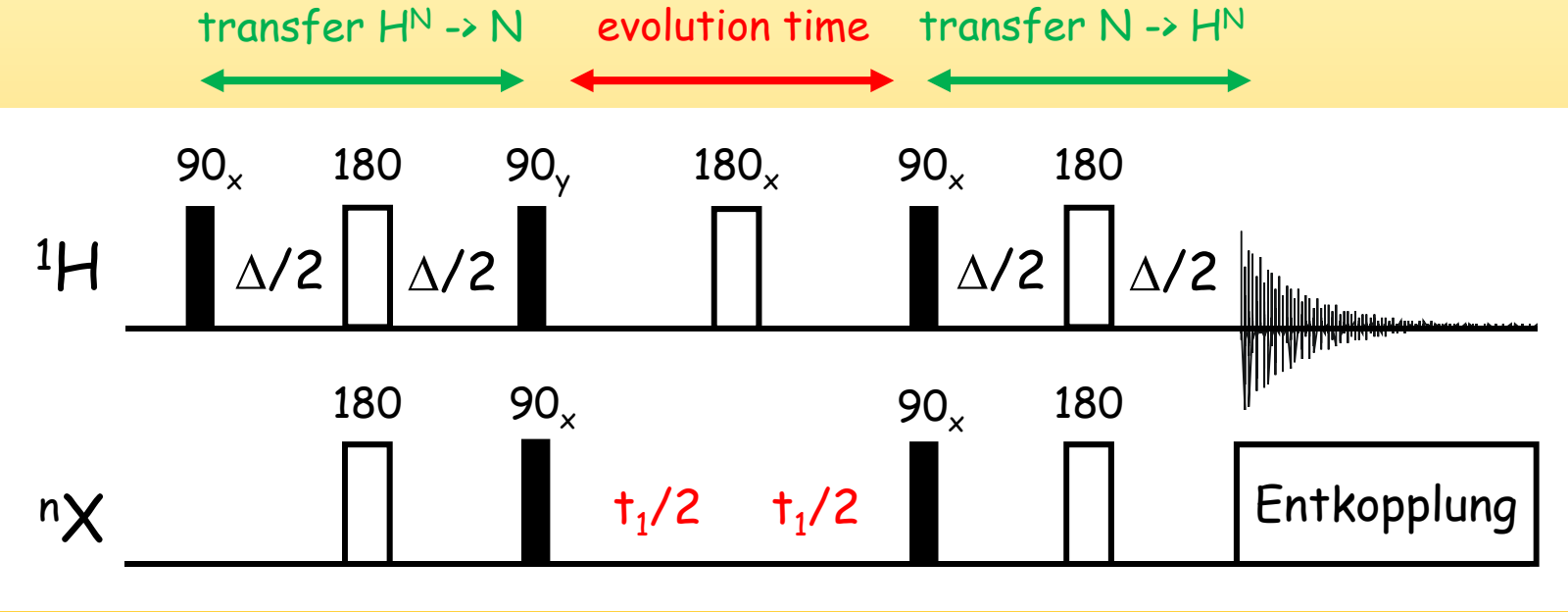
Here the transfer takes place between different types of nuclei und thus both axes exhibit different chemical shifts. If there is no transfer then there will be no peak, but if there is, the peak appears at the intersection of the chemical shifts of the two nuclei involved.



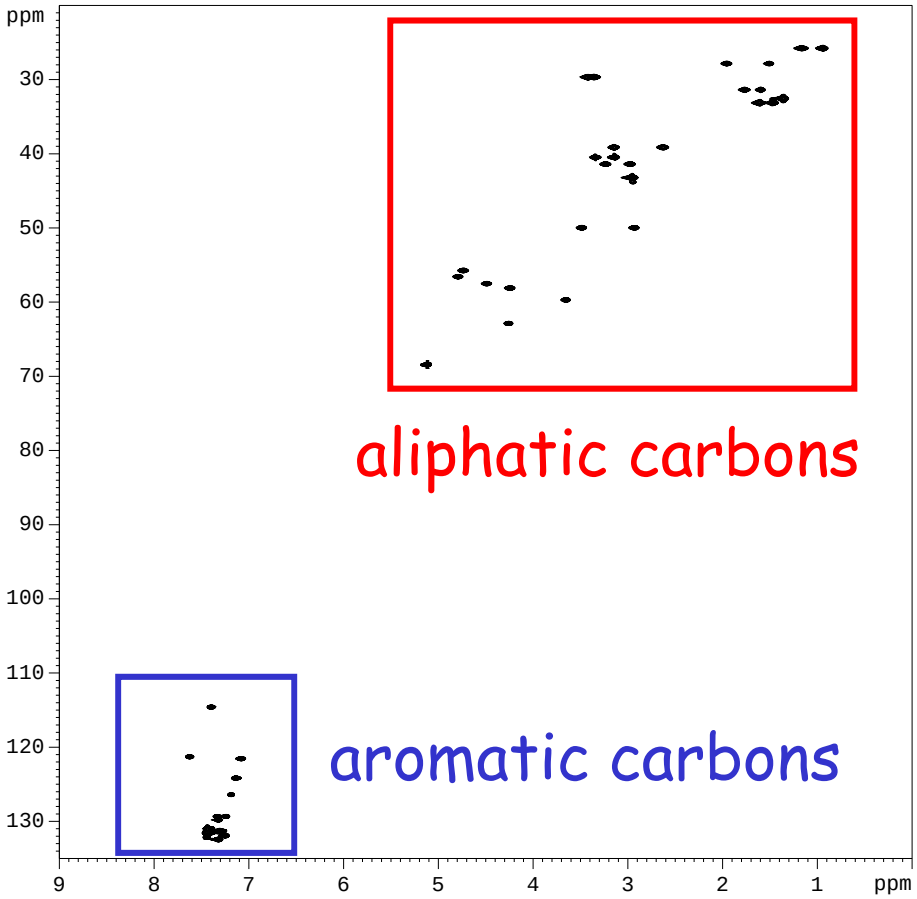
Multidimensional NMR-spectroscopy

Pulse sequence of the HSQC

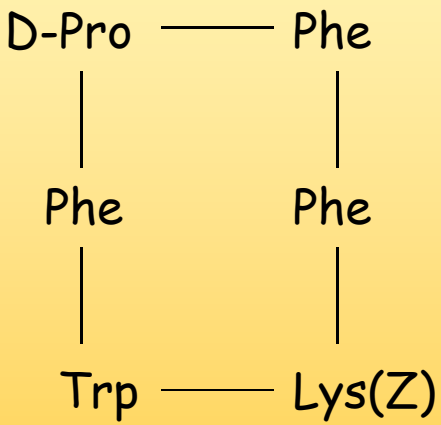
To create more complex spectra the number of pulses in the experiment increases, there order and timing matters but can be controlled very precisely by the hardware.



Multidimensional NMR-spectroscopy



^{13}C-HSQC of F3-008

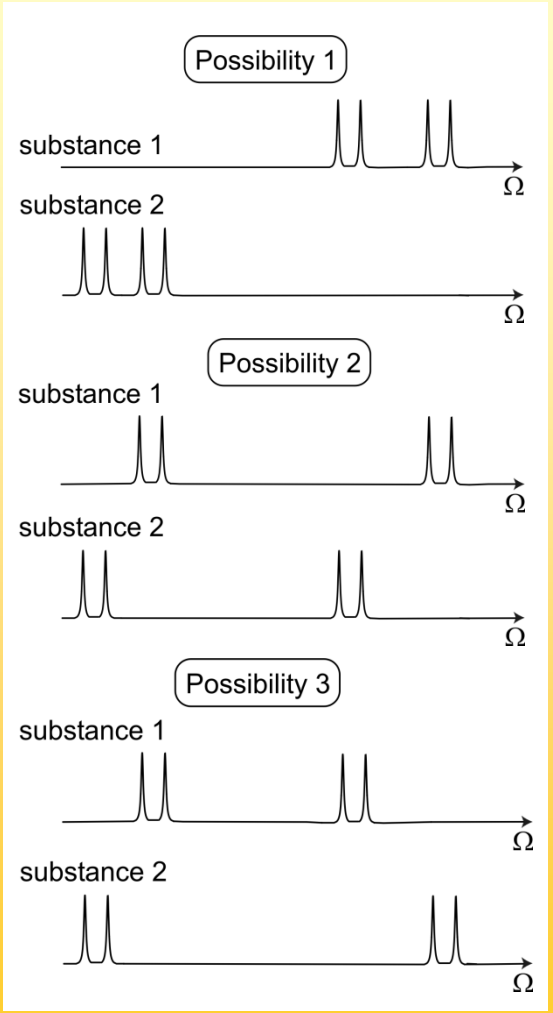


Multidimensional NMR-spectroscopy

A simple example:

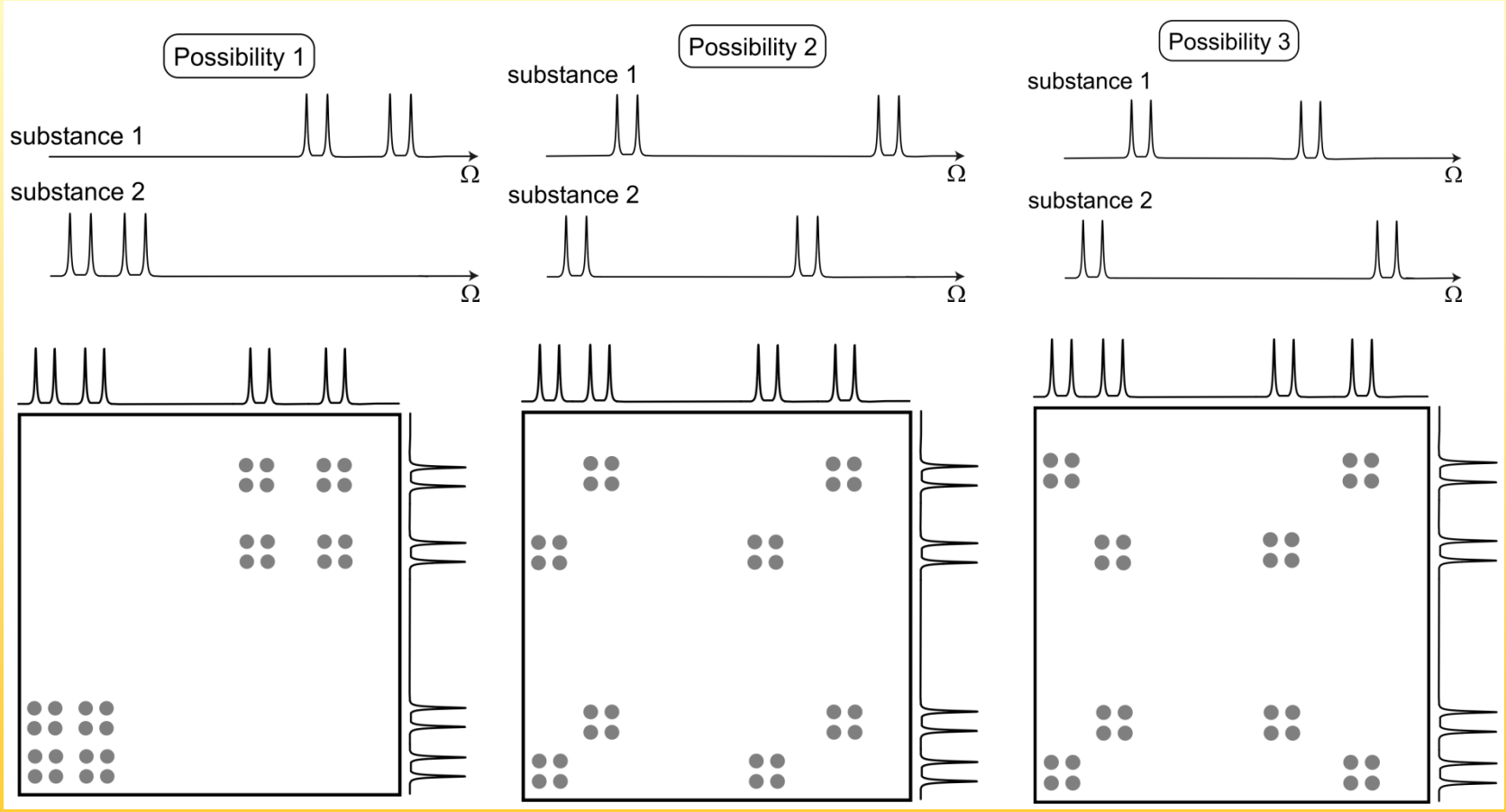
?

An assignment using 1D is not possible...



Multidimensional NMR-spectroscopy

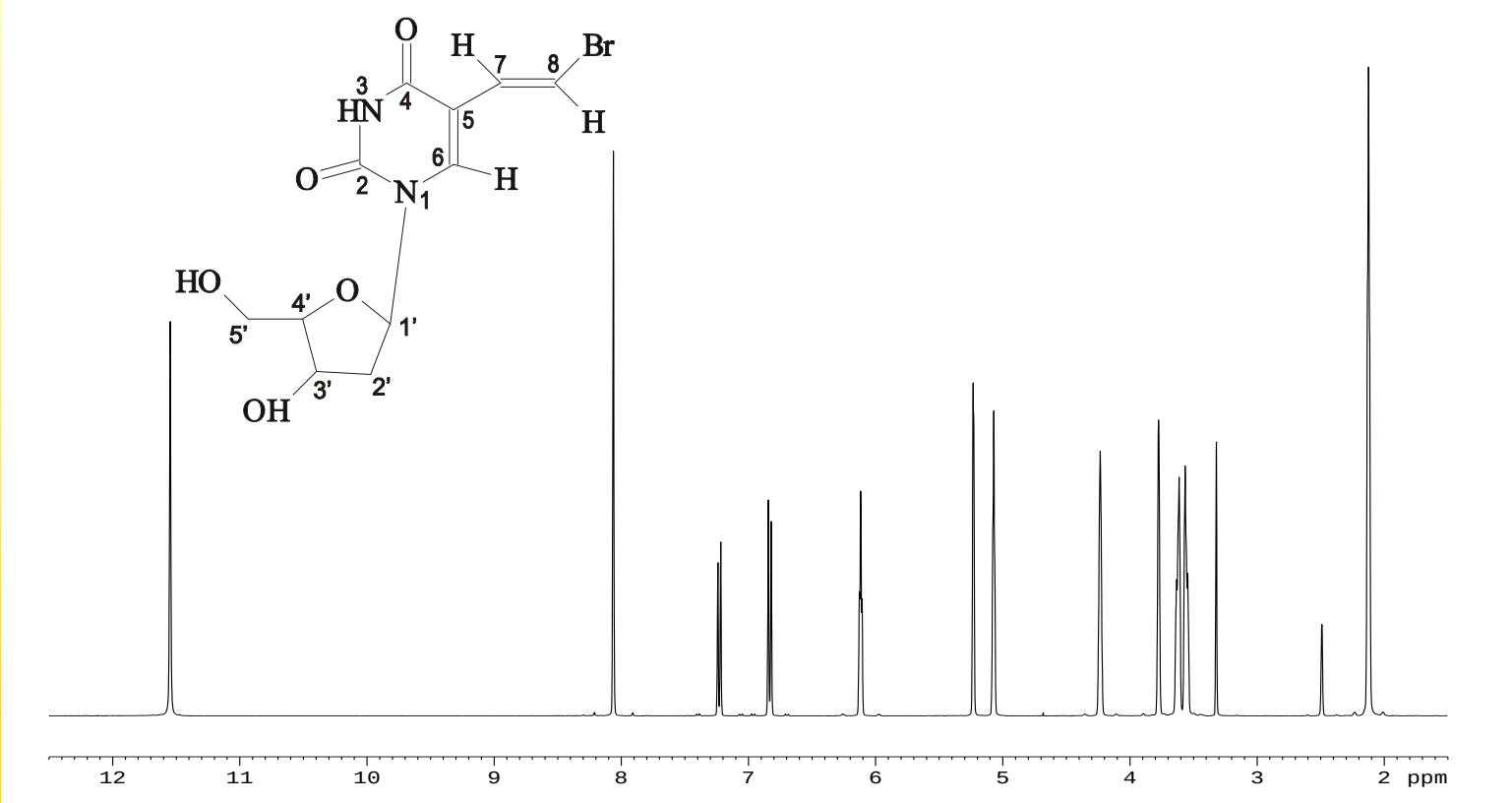
....but easy in 2D.



Applications of NMR-spectroscopy

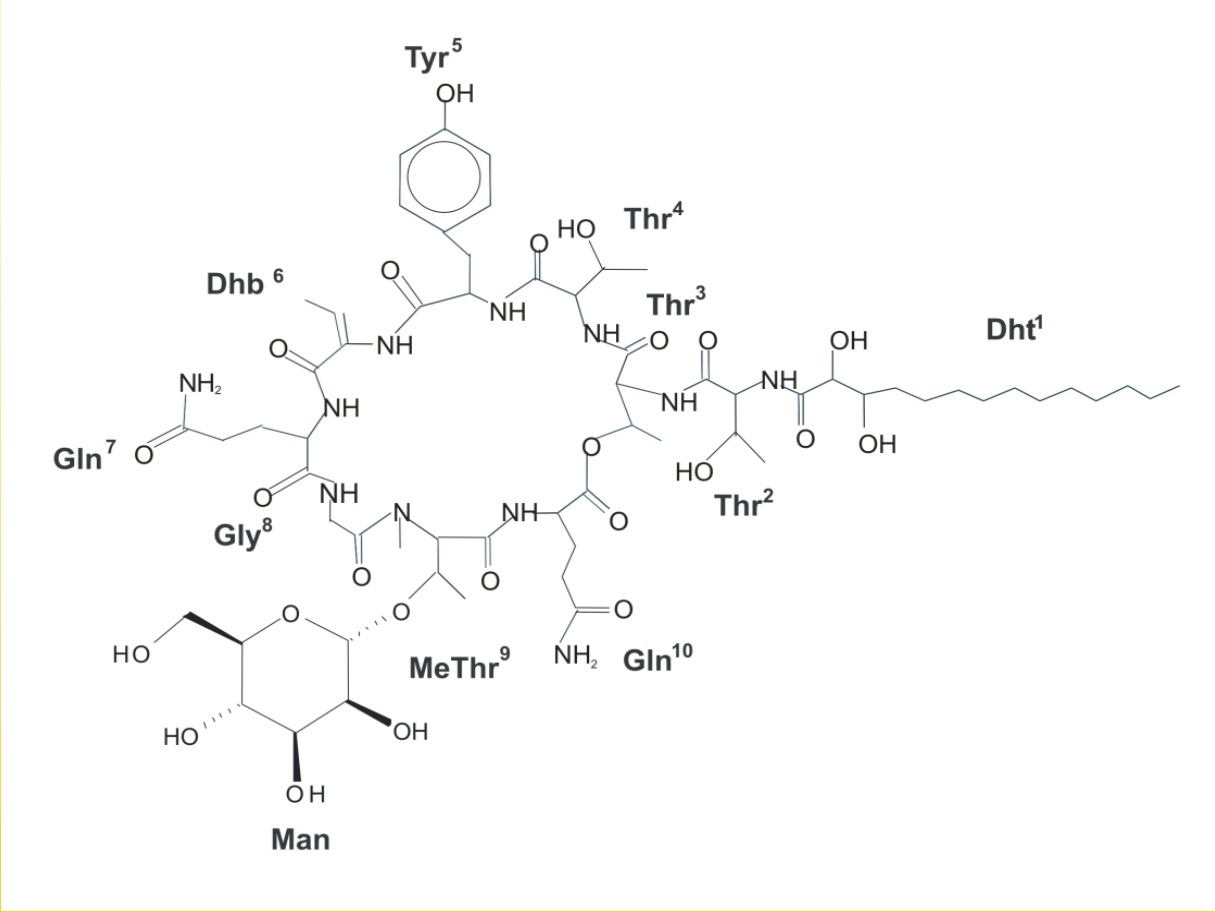
Applications of NMR-spectroscopy

NMR as an analytic method during synthetic work



Applications of NMR-spectroscopy

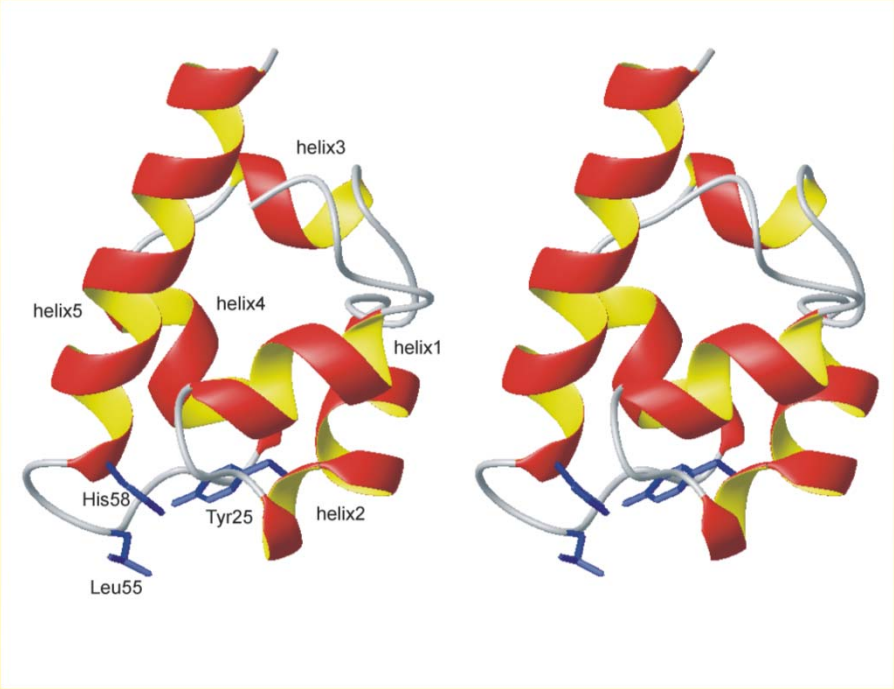
Determination of the constitution of natural products



Applications of NMR-spectroscopy

Determination of 3D structures of proteins

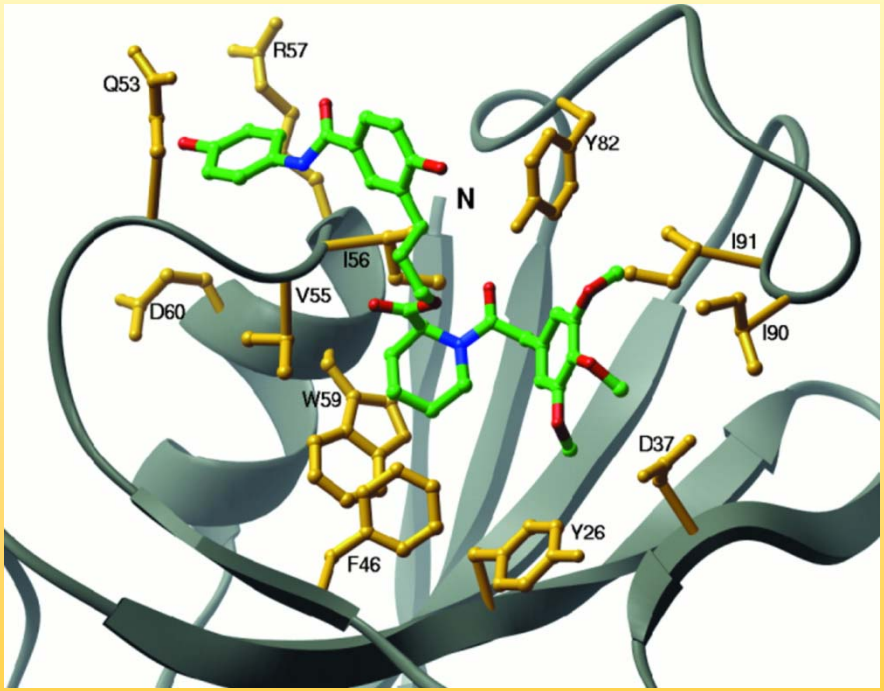
Using NMR 3D structures of proteins can be determined either in solution of in the solid state



Applications of NMR-spectroscopy

Detection of intermolecular interactions

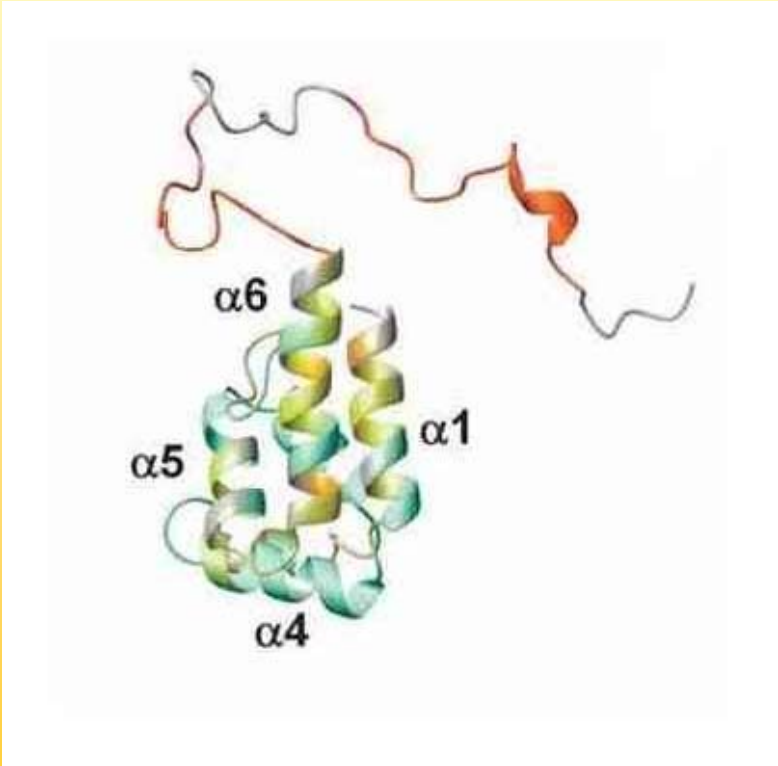
NMR can be used for the detection of protein-ligand interactions



Applications of NMR-spectroscopy

Investigation of dynamic phaenomena

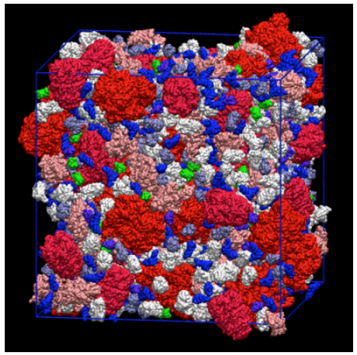
Using the NMR the mobility of proteins (and other molecules) can be investigated

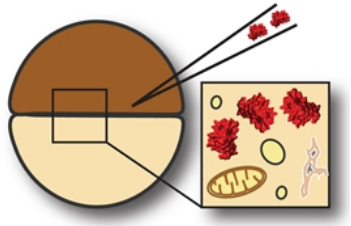


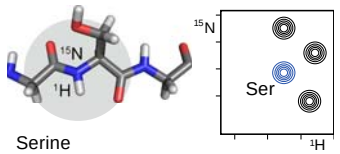
Applications of NMR-spectroscopy

Detection of processes in living cells

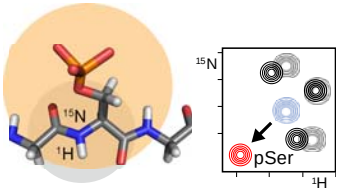
Using in-cell-NMR-spectroscopy changes and processes within a living cell can be visualized.







Serine



phospho-Serine

Detection of protein-ligand-interactions using NMR-spectroscopy

NMR and protein-ligand interactions

NMR-spectroscopy is well suited for the investigation protein-ligand-interactions. Since such an interaction changes the magnetic environment of the nuclei, it can be detected by a change in the chemical shifts (or other NMR parameters).

Of particular importance is that also relatively weak interactions that would not be observed in many biological assays can be detected. Often strong interactions are more difficult to observe.

The investigations can be used for a detailed study of one particular interaction or for the "screening" of ligand-libraries to find novel interaction partners.

NMR and protein-ligand interactions

Differentiation „strong" and „weak" binding:

Strong binding: protein and ligand form a unit

Weak binding: ligand is almost independent from the protein

Differentiation „small" und „large" molecules:

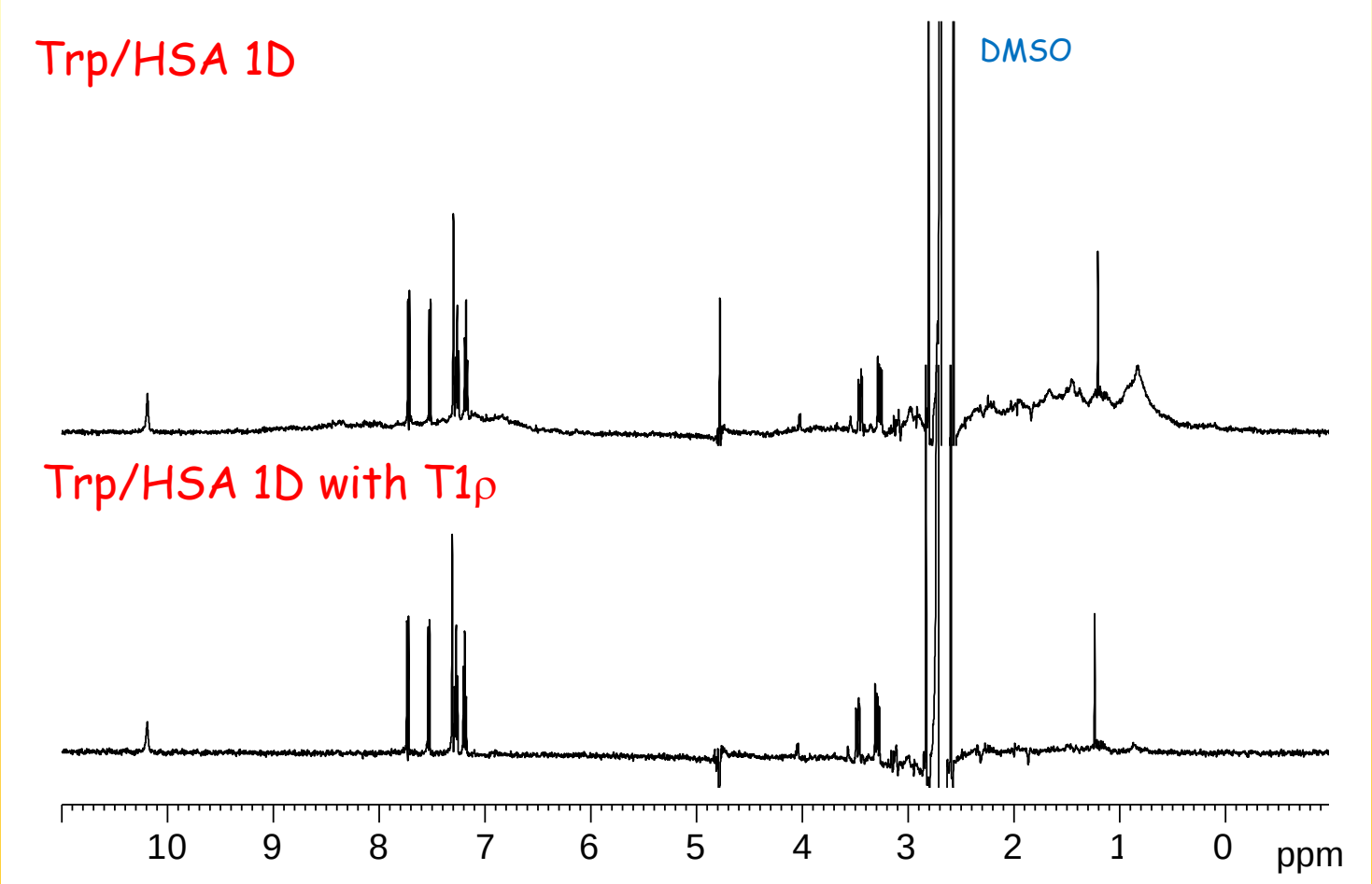
Translational diffusion

Rotational diffusion:

relaxation, NOE-effect, spin-diffusion

NMR and protein-ligand interactions

An example is the T1ρ-filter: L-Trp und Human Serum Albumin



NMR and protein-ligand interactions

In principle there are two ways to conduct the experiments: observation of properties of the ligand („ligand-observed") or observation of properties of the protein ("protein-observed")

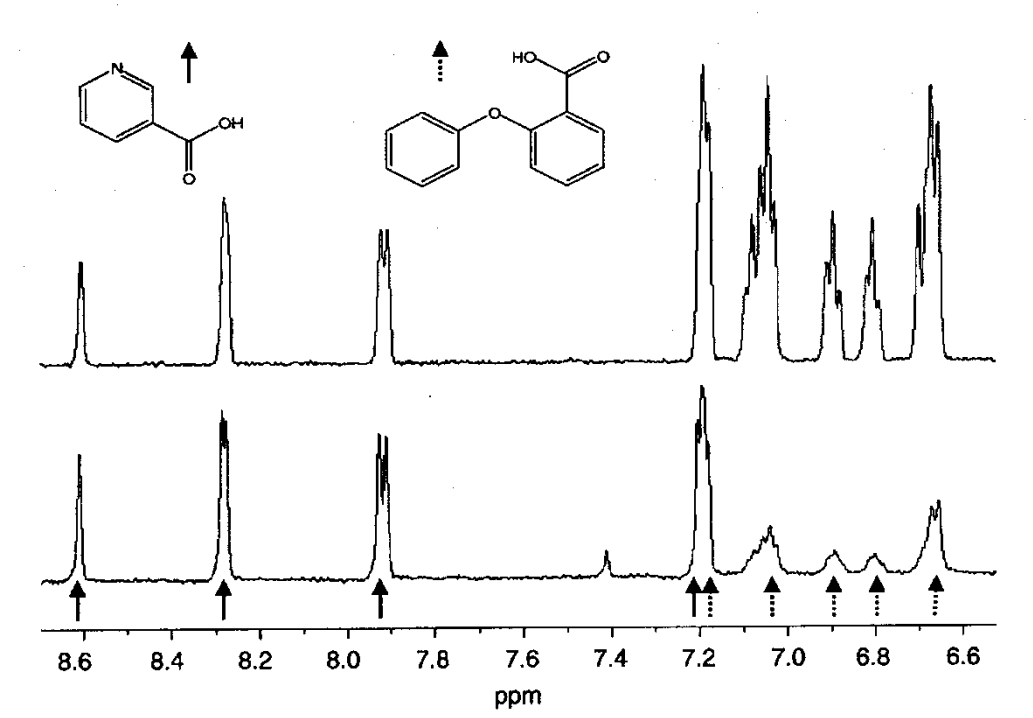
Ligand observation does not required labeling of the protein, only a small amount of protein and is suitable also for very large proteins but less informative regarding the interaction site.

Protein observation requires labeled protein and a resonance assignment which means an increased effort and a size limit on the protein, but information on the interaction site can be obtained.

Ligand-detected methods

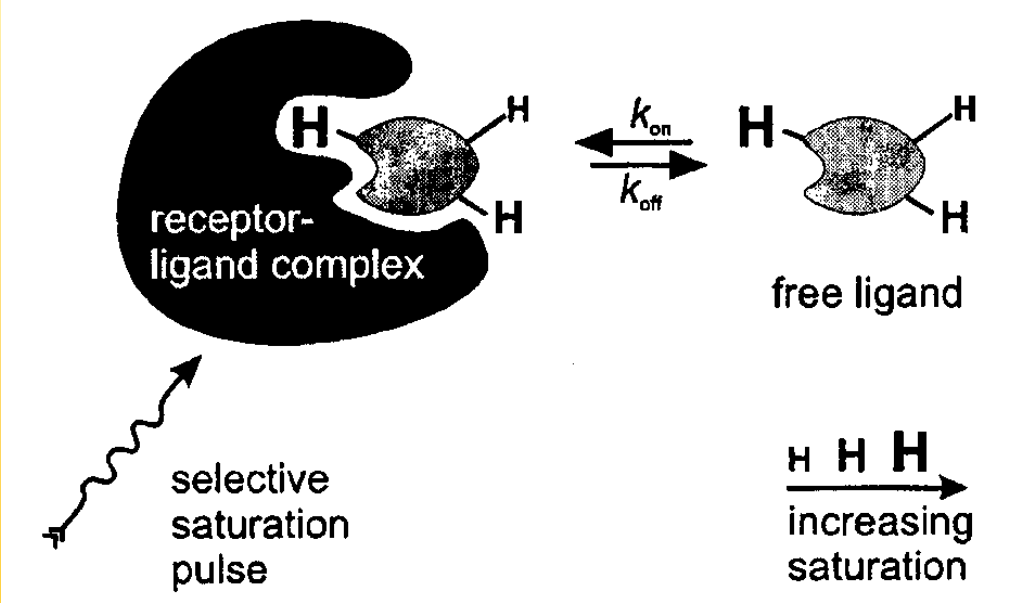
NMR and protein-ligand interactions

Ligand-detected methods are based on the fact that an interaction between ligand and protein transfers some of the proteins properties onto the ligand.

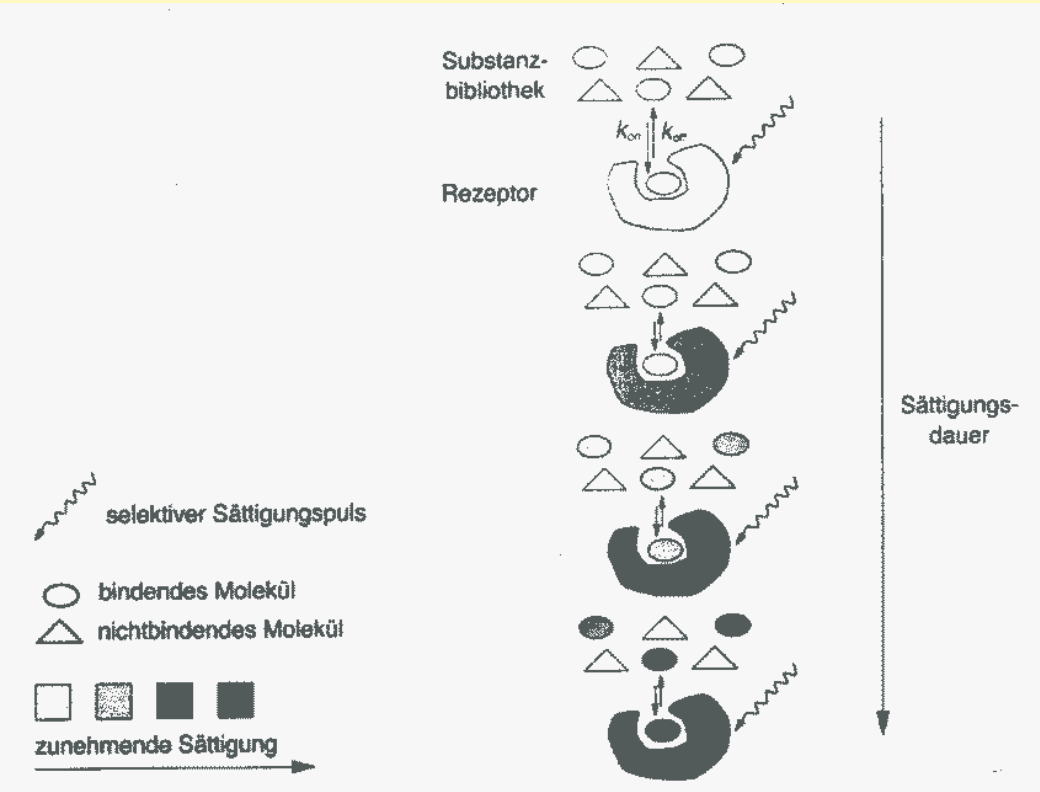


NMR and protein-ligand interactions

A very popular method are the STD-experiments (Saturation Transfer Difference), which are based on the difference between two 1D spectra, one recorded with protein irradiation, one without



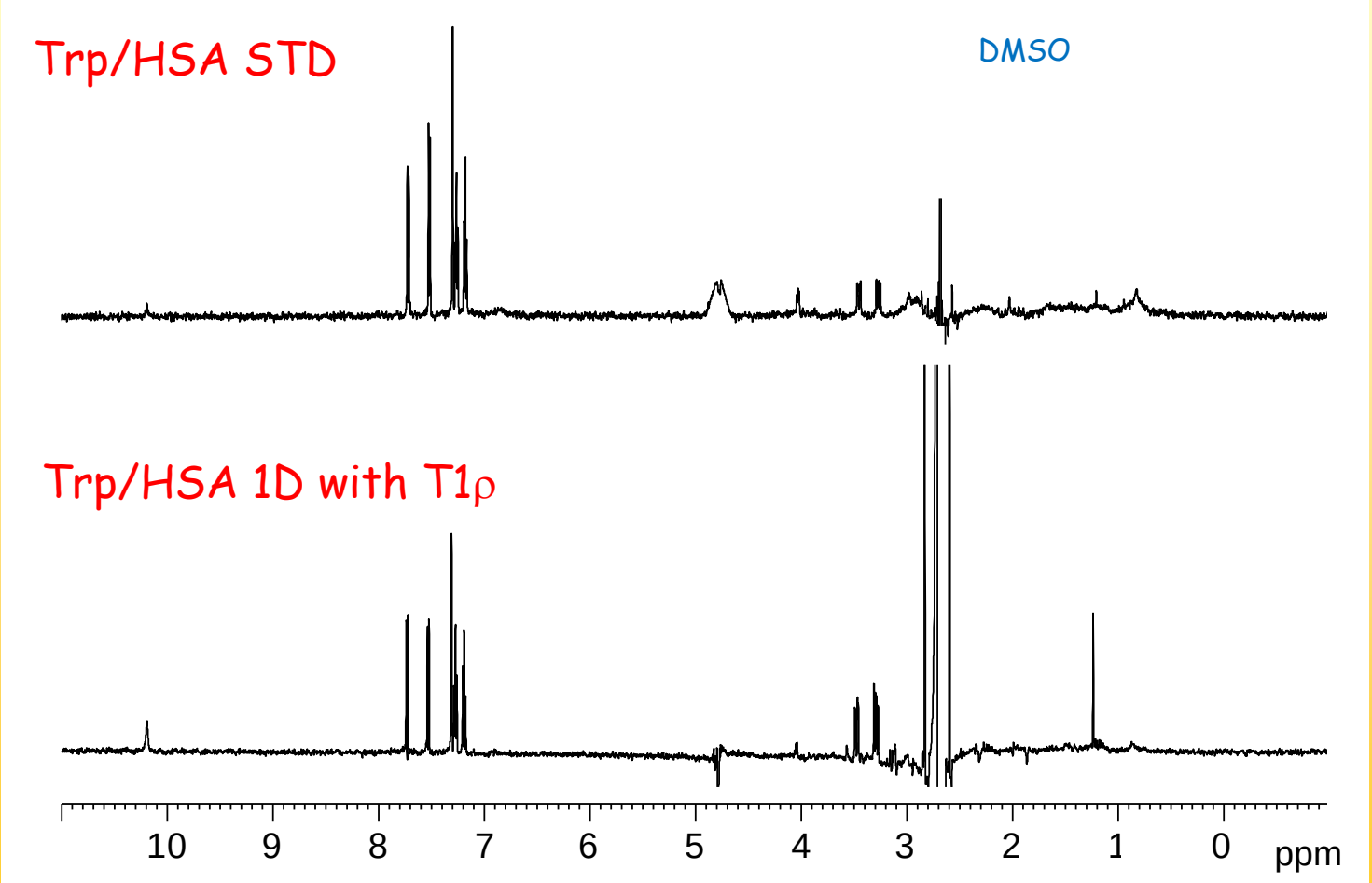
NMR and protein-ligand interactions



Spin-diffusion spreads the saturation quickly within the protein (the larger the protein is, the better) and also to bound ligands, even if bound only weakly. Unbound ligands are unaffected. The difference contains only binding ligands, the protein is suppressed with a $T_{1\rho}$ filter

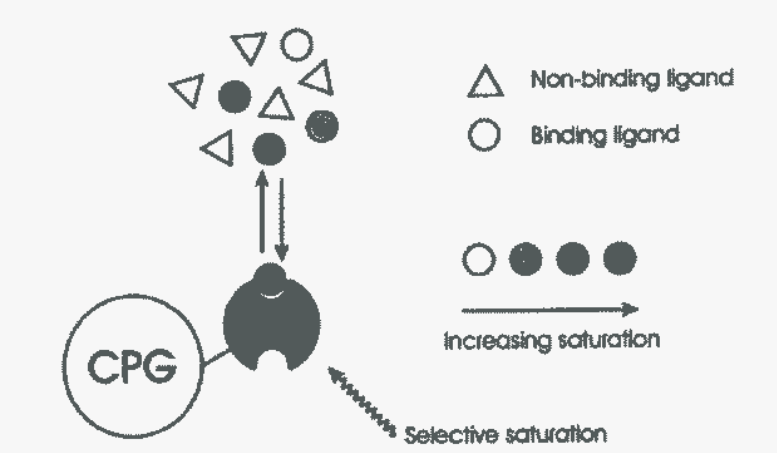
NMR and protein-ligand interactions

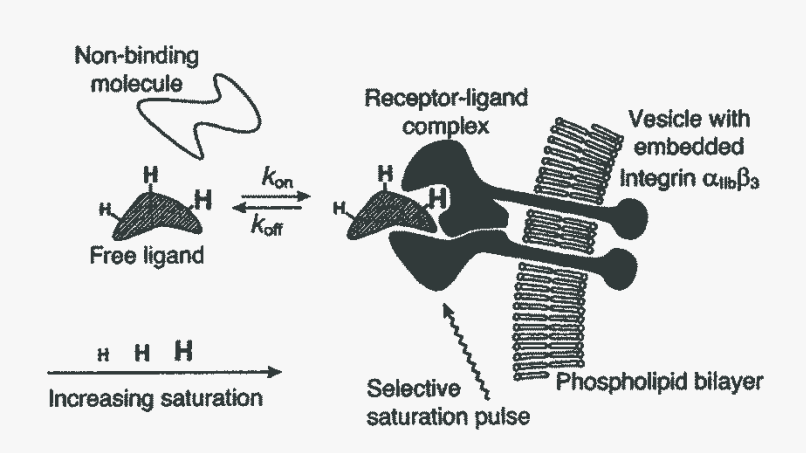
As an example: L-Trp binds to Human Serum Albumin



NMR and protein-ligand interactions

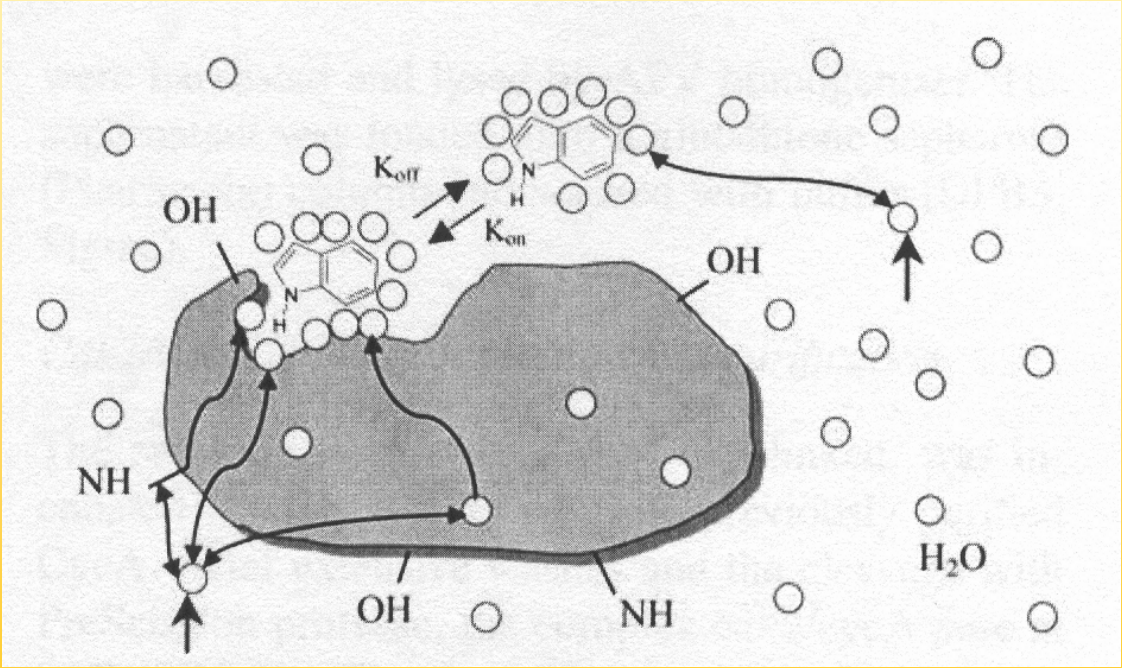
The method can also be used in solid-state NMR and using solubilized receptors.





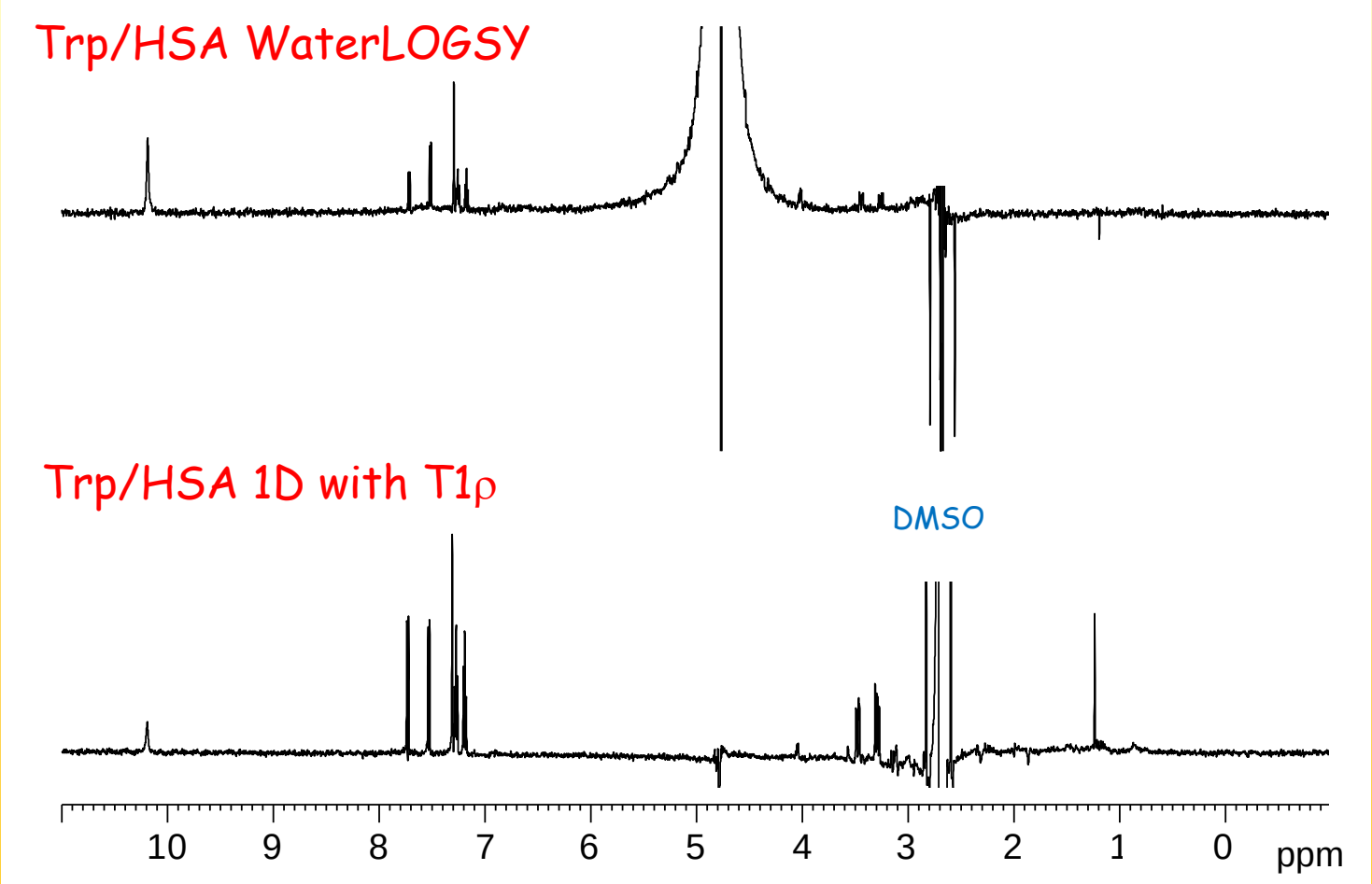
NMR and protein-ligand interactions

Another method to detect small ligands bound to proteins is the WaterLOGSY. Here differences in the hydration of bound and unbound ligands are used.



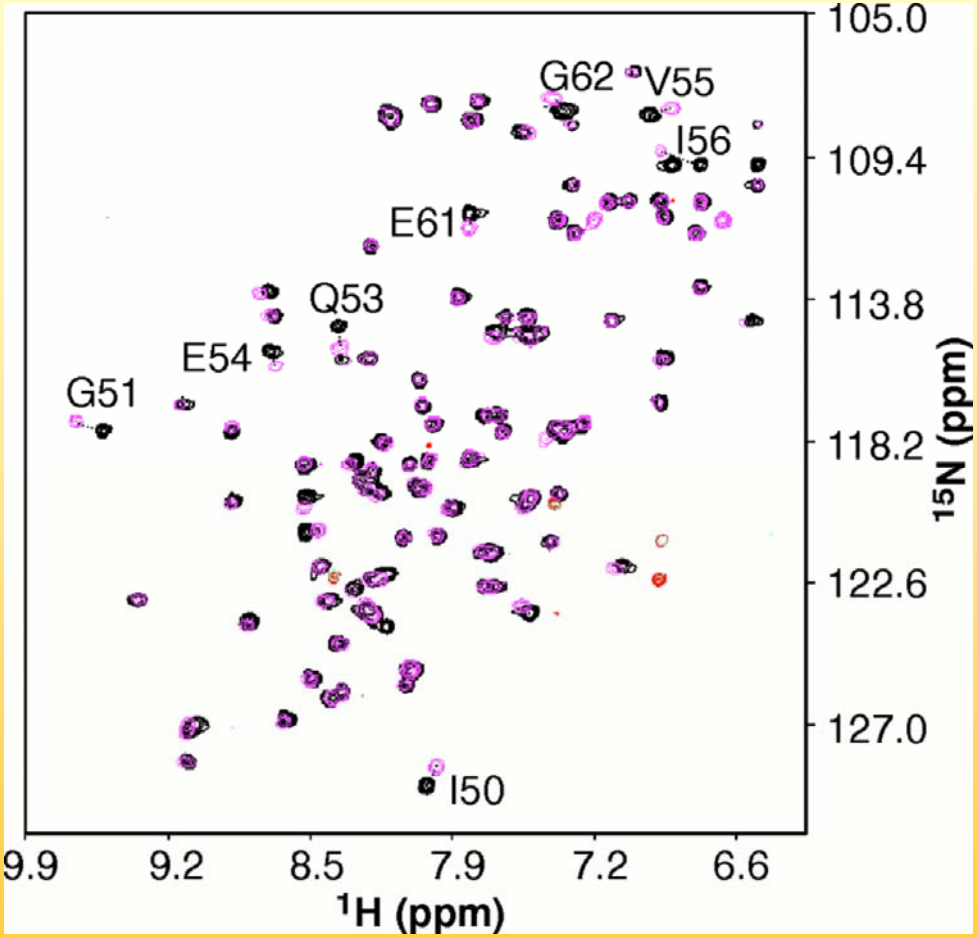
NMR and protein-ligand interactions

As an example: L-Trp binds to Human Serum Albumin



Protein-detected methods

NMR and protein-ligand interactions



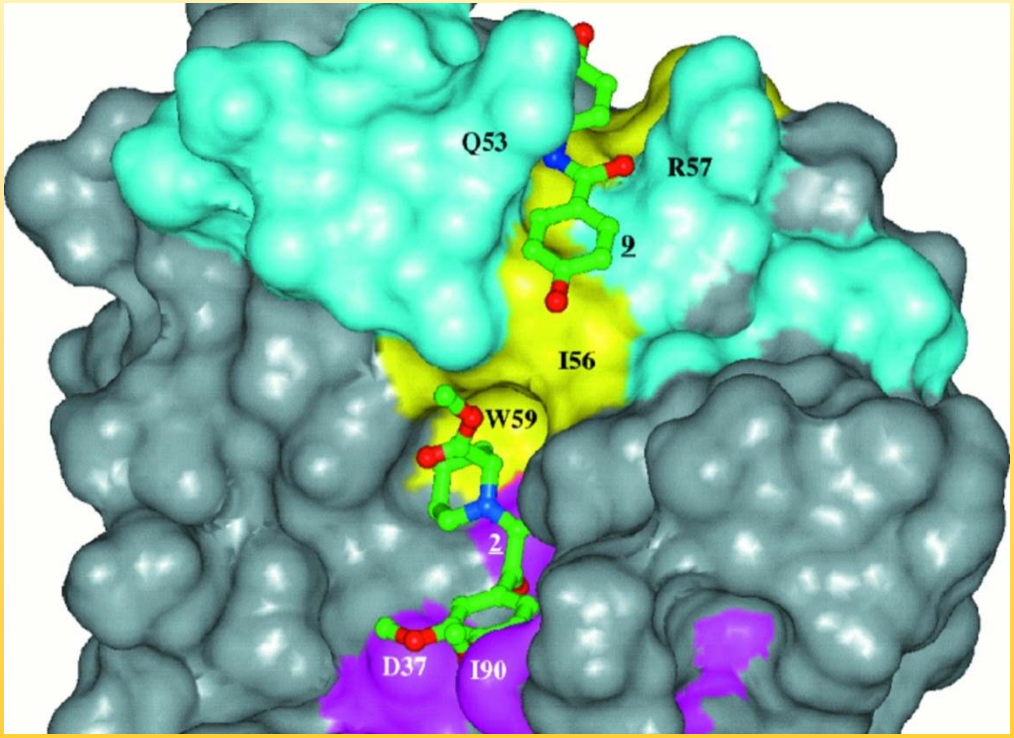
The most famous method is called

„SAR-by-NMR"

in which a $^{1}H,^{15}N$-HSQC is recorded with and without ligand(s) and interactions are detected by a change in the spectrum (shift of peaks, disappearance of peaks)

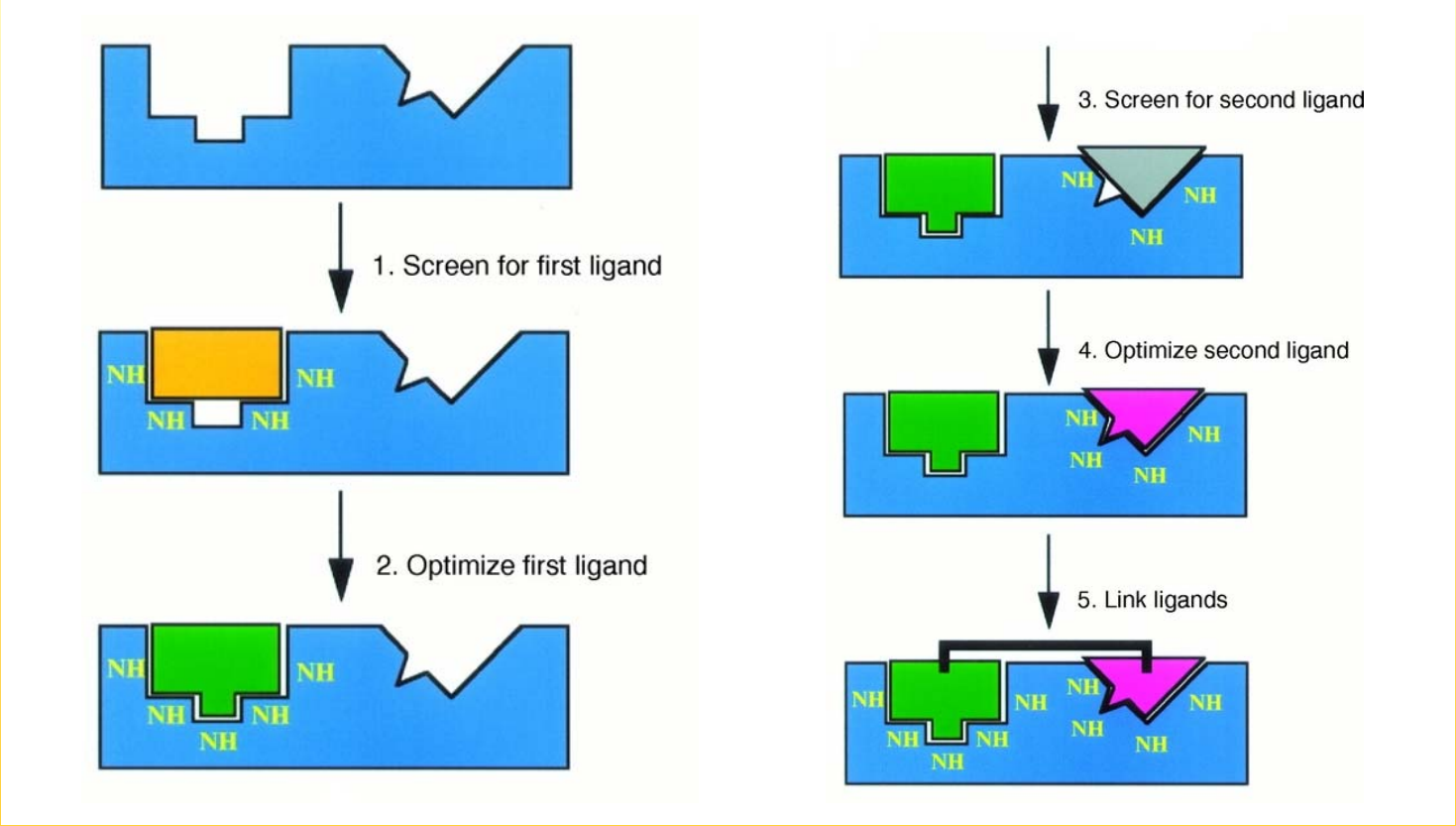
NMR and protein-ligand interactions

Here it is also possible to define the interaction site on the protein, a structure can be determined or a model can be created.

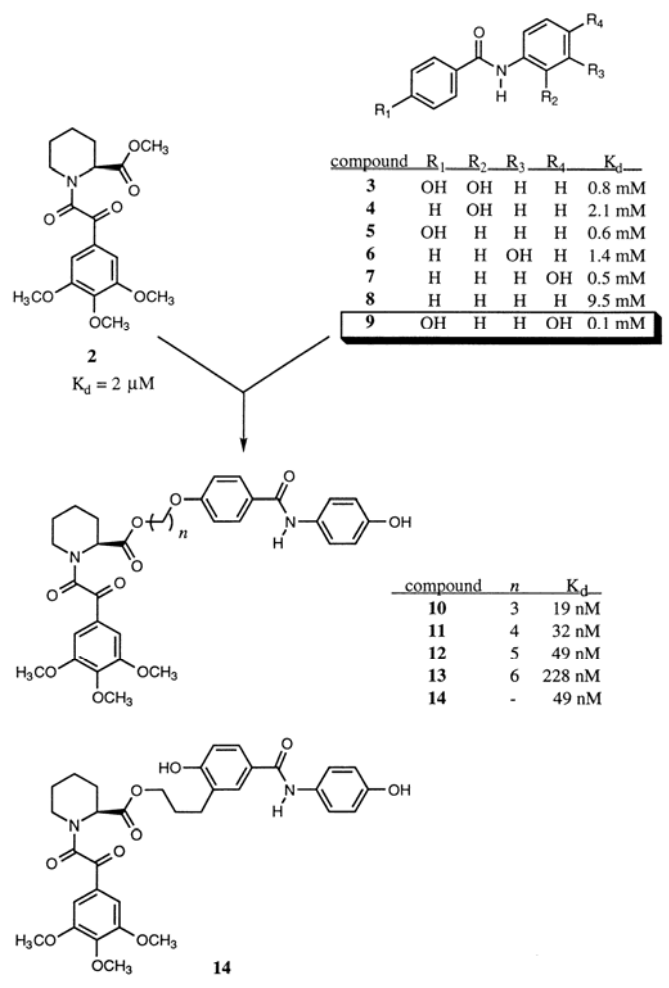


NMR and protein-ligand interactions

Several weakly binding ligands are identified that bind at adjacent sites and are subsequently combined to larger ligands



NMR and protein-ligand interactions



compound	R_1	R_2	R_3	R_4	K_d
3	OH	OH	H	H	0.8 mM
4	H	OH	H	H	2.1 mM
5	OH	H	H	H	0.6 mM
6	H	H	OH	H	1.4 mM
7	H	H	H	OH	0.5 mM
8	H	H	H	H	9.5 mM
9	OH	H	H	OH	0.1 mM

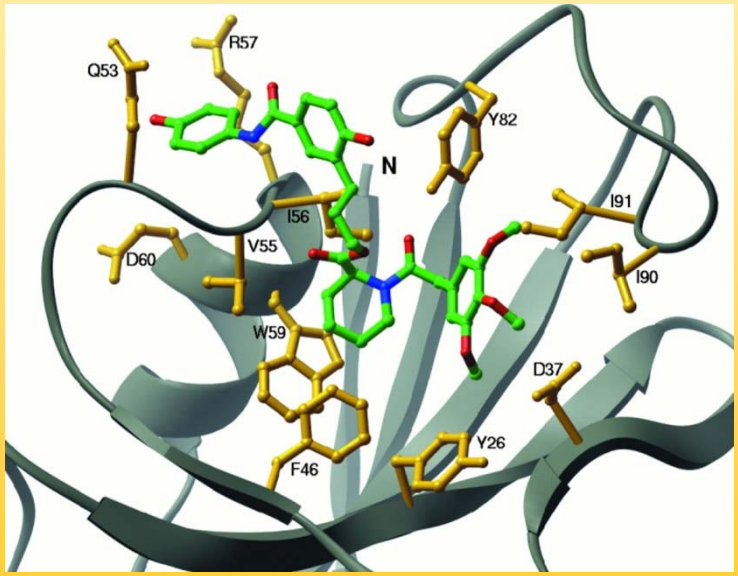
2

K_d = 2 µM

compound	n	K_d
10	3	19 nM
11	4	32 nM
12	5	49 nM
13	6	228 nM
14	-	49 nM

14

This will (hopefully) lead to tight binding ligands with a novel chemistry



That's it

http://schmieder.fmp-berlin.info/teaching/lehre_unis_berlin.htm