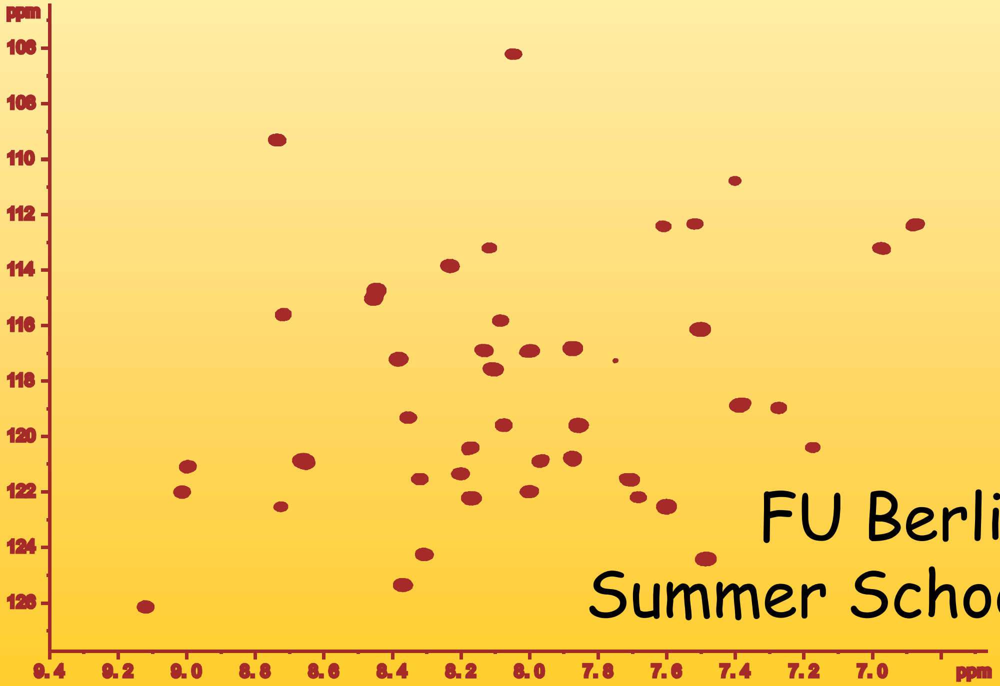
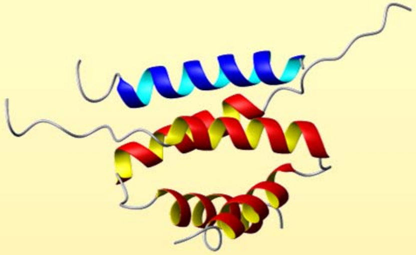
# How NMR works
# -
# an introduction



FU Berlin
Summer School 2014

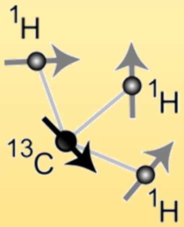
Monika Beerbaum
AG Solution NMR

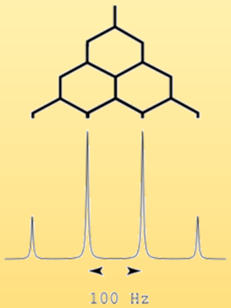
# the program



General aspects

Basic principles

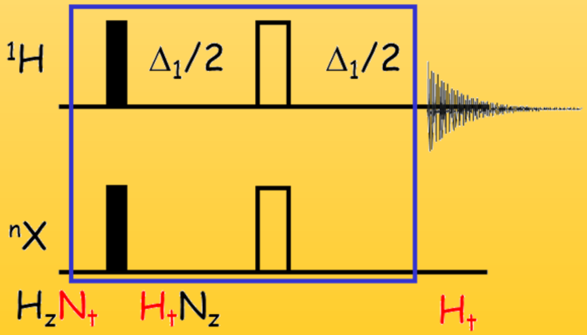


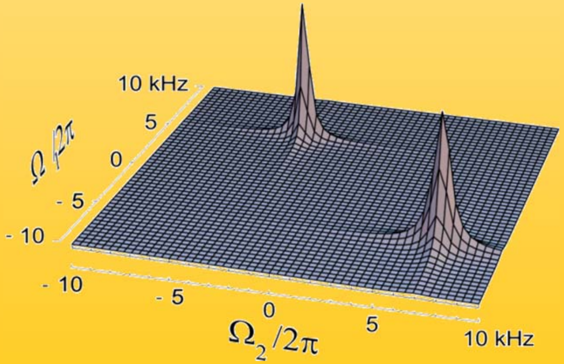


NMR parameters

Multidimensional NMR Spectroscopy

Understanding NMR experiments

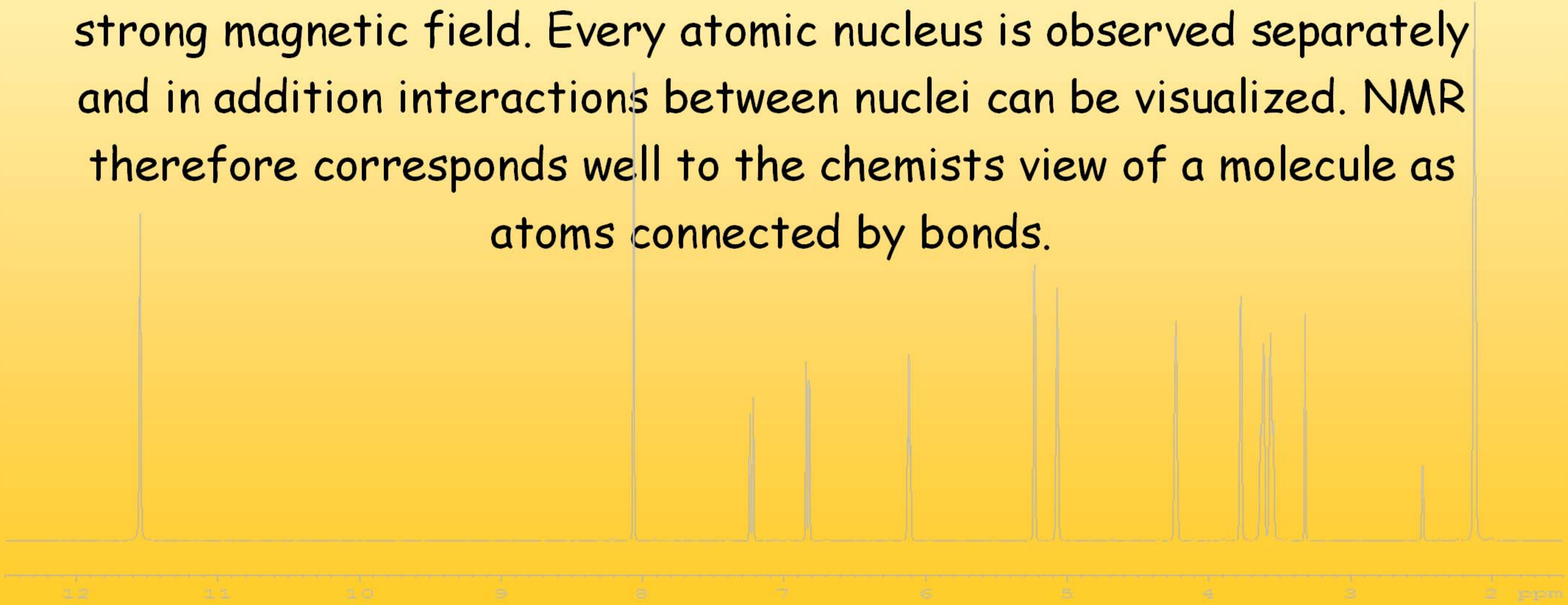




# General aspects of NMR-spectroscopy

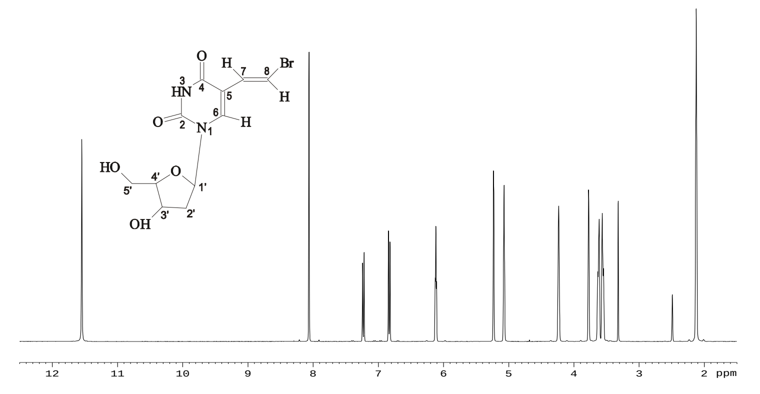
# Nuclear Magnetic Resonance

NMR-spectroscopy observes the resonance interaction of atomic nuclei with electromagnetic waves. The effect is only detectable in a strong magnetic field. Every atomic nucleus is observed separately and in addition interactions between nuclei can be visualized. NMR therefore corresponds well to the chemists view of a molecule as atoms connected by bonds.

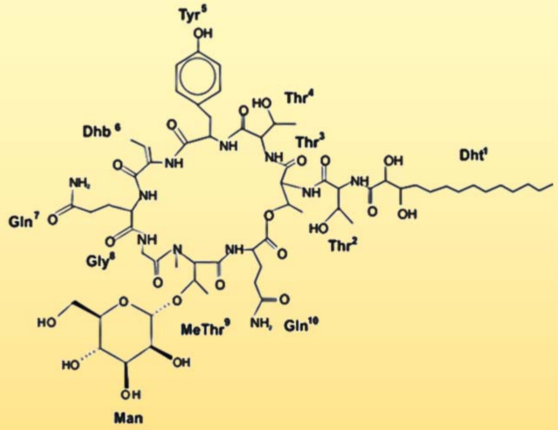


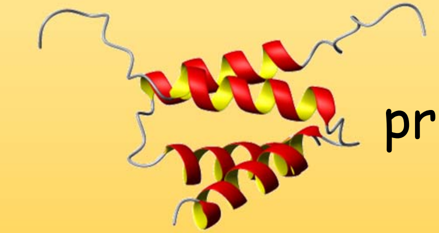
# general aspects: the idea

## Analytical method accompanying synthetic work

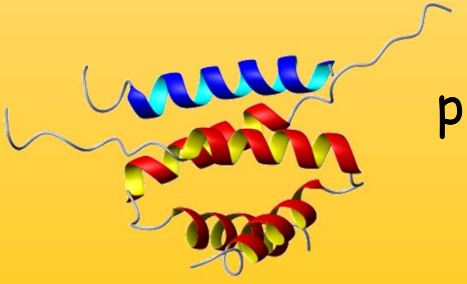


## Structure elucidation of natural compounds



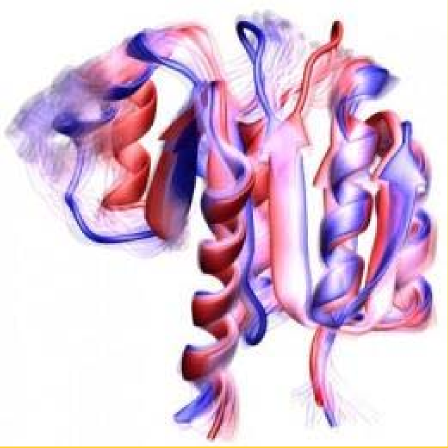


protein structure



protein ligand interaction

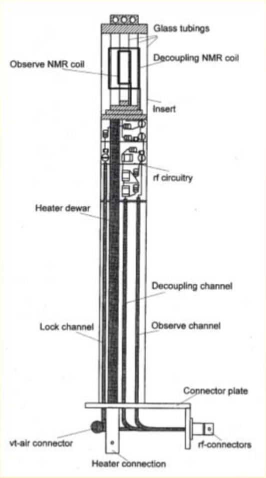
dynamics



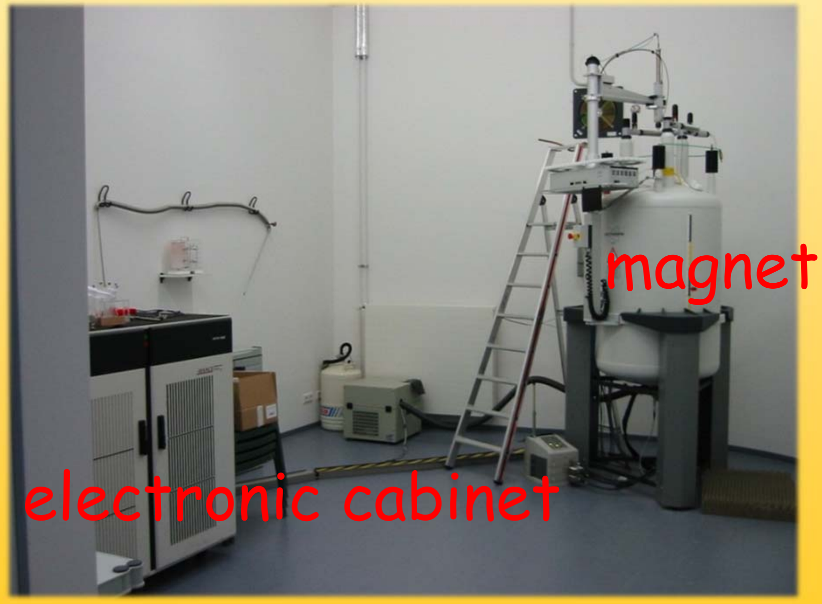
# general aspects: equipment

sample

probe head



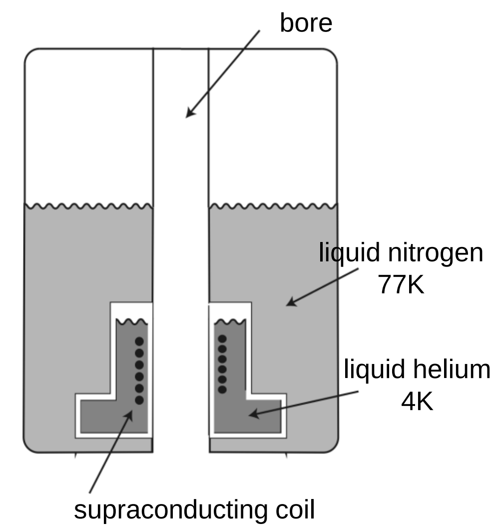
„Konsole“

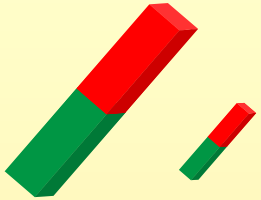


magnet

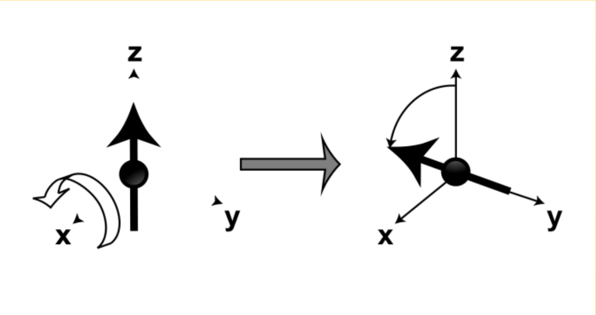
electronic cabinet

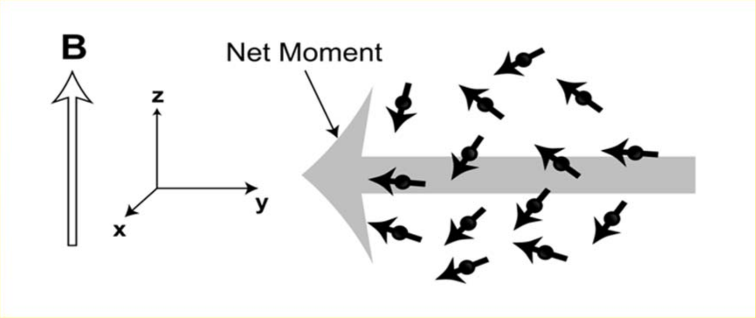
# general aspects: equipment



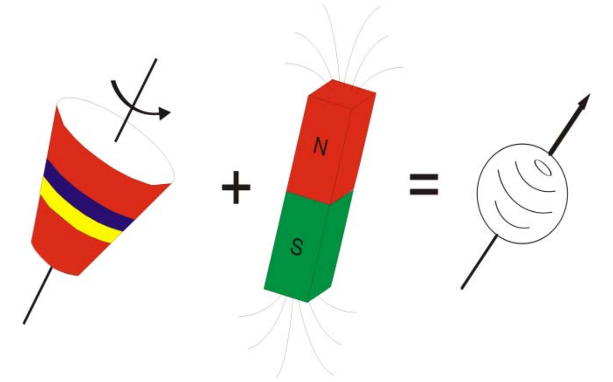


# Basic principles of NMR-spectroscopy

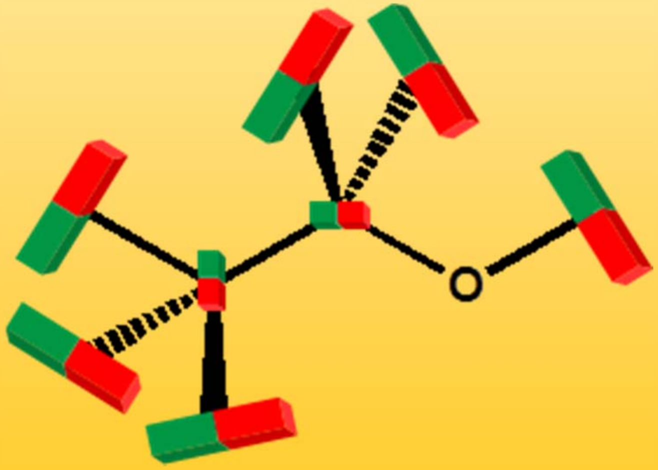




## basic principles: nuclear spins and magnetic moment



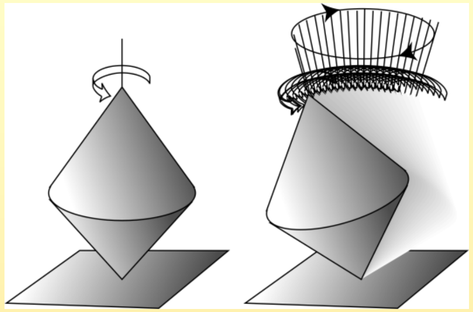
Ethanol $CH3-CH2-OH$



Atom nuclei have a quantum mechanical property that makes them behave like a combination of a spinner and a tiny bar magnet called spin.

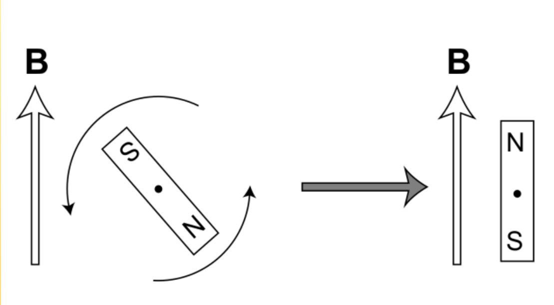
Different atom types have magnetic properties of different strength.

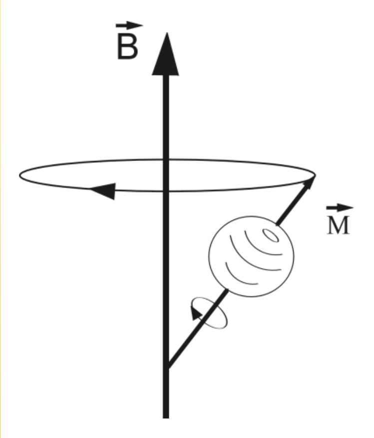
# basic principles: nuclear spins and magnetic moment



A spinner has an angular momentum whose axis is stable in three-dimensional space.

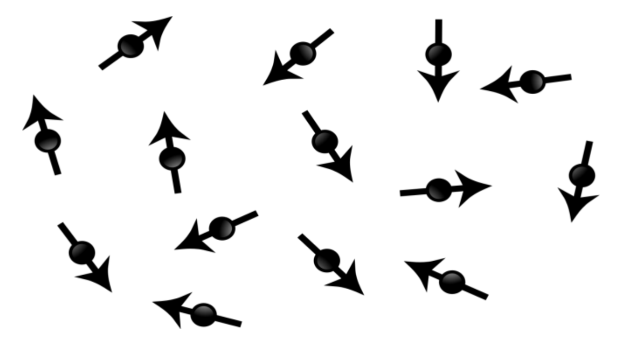
An alignment of the little bar magnet with an external magnetic field…

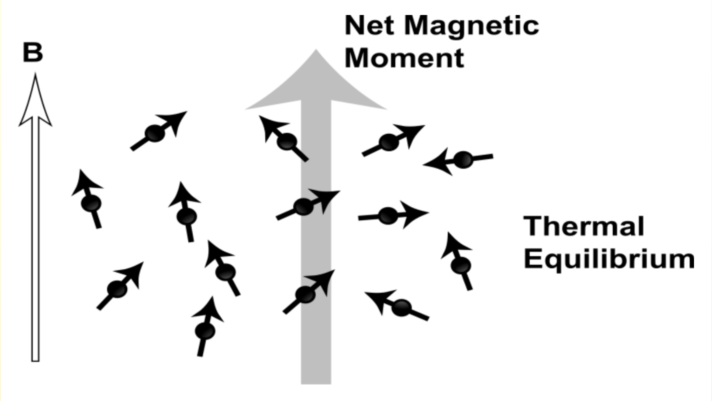




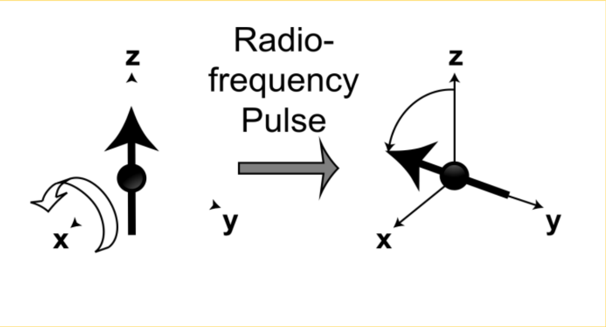
… is prevented by the properties of a spinner, a precession begins.

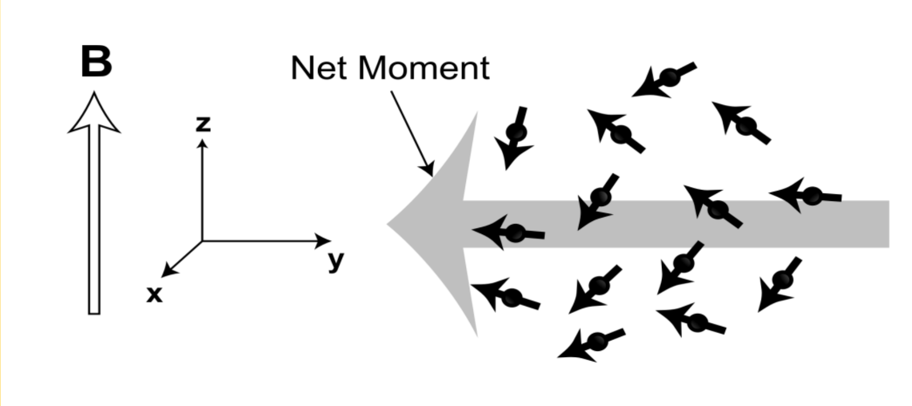
# basic principles: nuclear spins and magnetic moment





Without an external magnetic field the orientations of the spins are uniformly distributed in all directions. In a magnetic field they orientate preferably along the magnetic field.

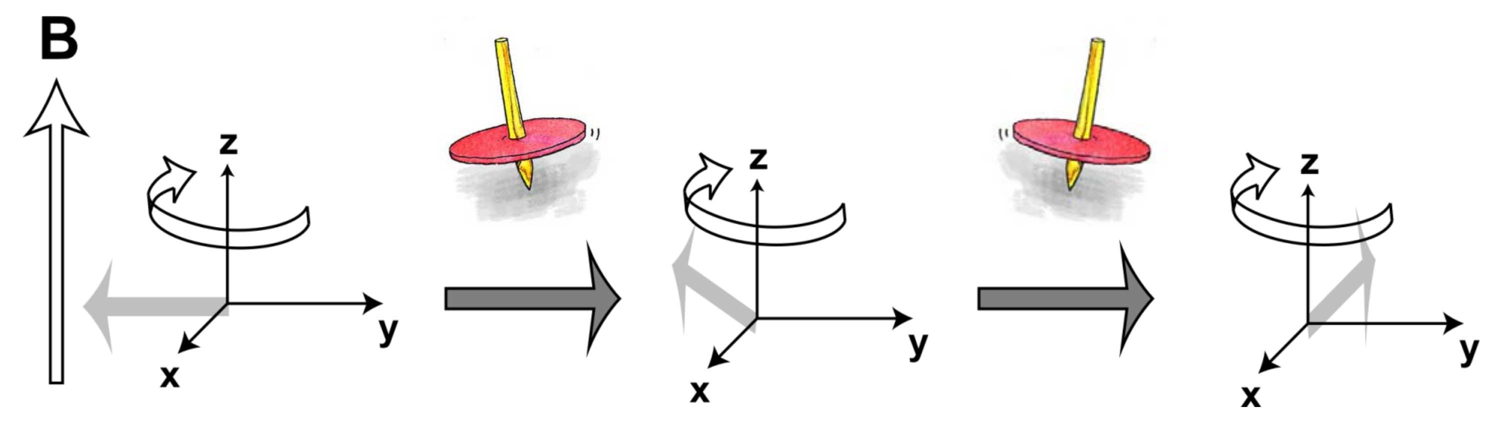




A “90deg pulse” (energy input) rotates every single spin and therefore the overall magnetization by 90deg.

# basic principles: nuclear spins and magnetic moment

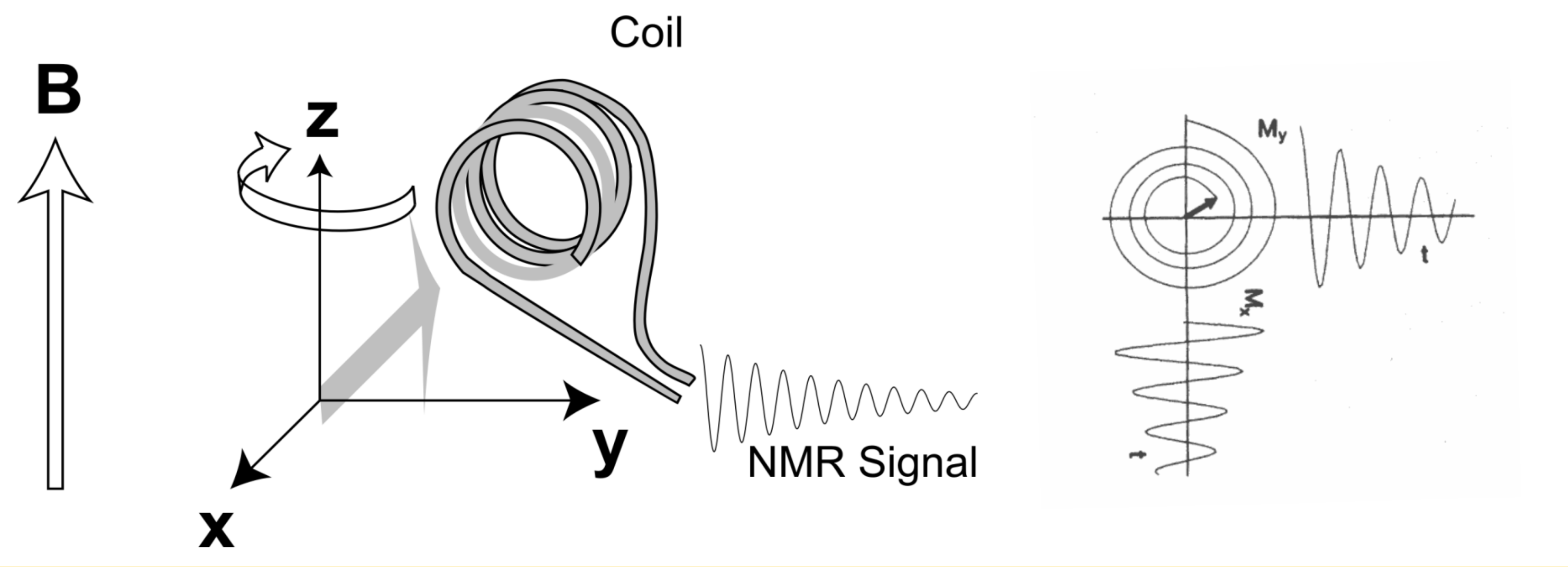
After the pulse the huge magnet tries to force the spins back to z. But as they behave like a spinner, they don´t move directly to z but start to process in the x,y-plane.



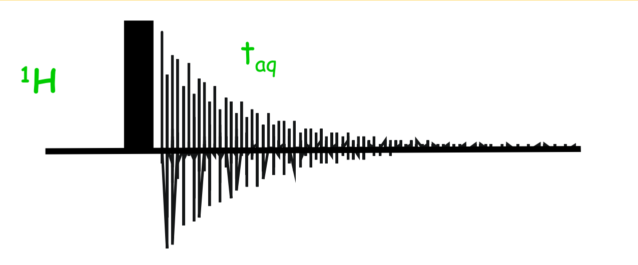
The rotation frequency depends mainly on the atom type and the field strength of the magnet.

$$\omega = \gamma B$$

# basic principles: nuclear spins and magnetic moment

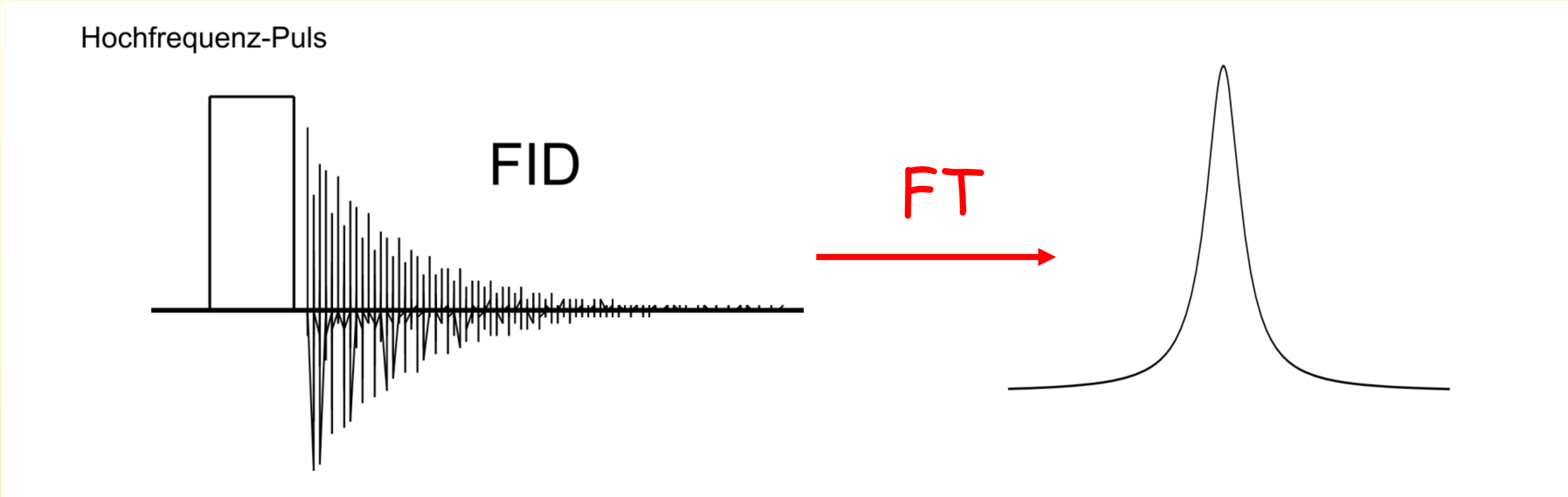


The rotating net magnetic moment induces a current in the detector coil outside the sample.



## basic principles: nuclear spins and magnetic moment

The signal called the FID is an overlap of the damped cosine waves of all different spins in the molecule.

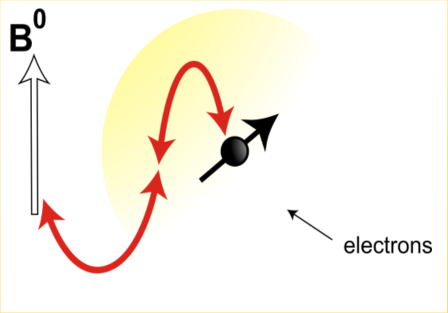


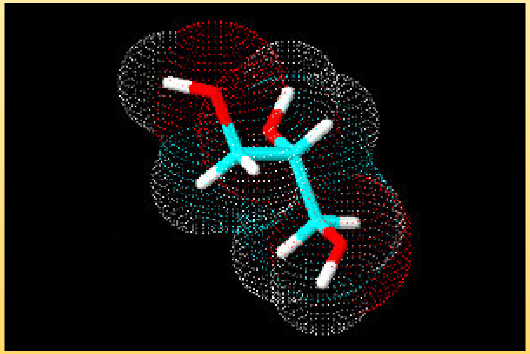
The Fourier transformation identifies all frequencies that are present in the FID.

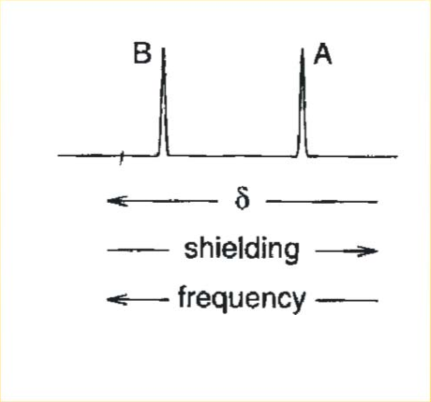
# Parameters in NMR-spectroscopy

# parameters: chemical shift

The chemical enviroment especially the electron cloud around every atom has an influence on the exact local magnetic field every atom feels. The more electrons the weaker the field.





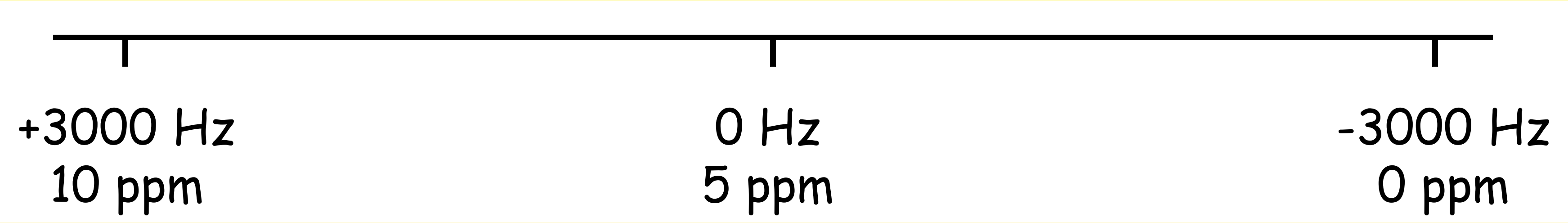


The resulting differences are orders of magnitude smaller than the overall rotation frequency.

# parameters: chemical shift

$^{1}H$, 600 MHz, 14.7 Tesla

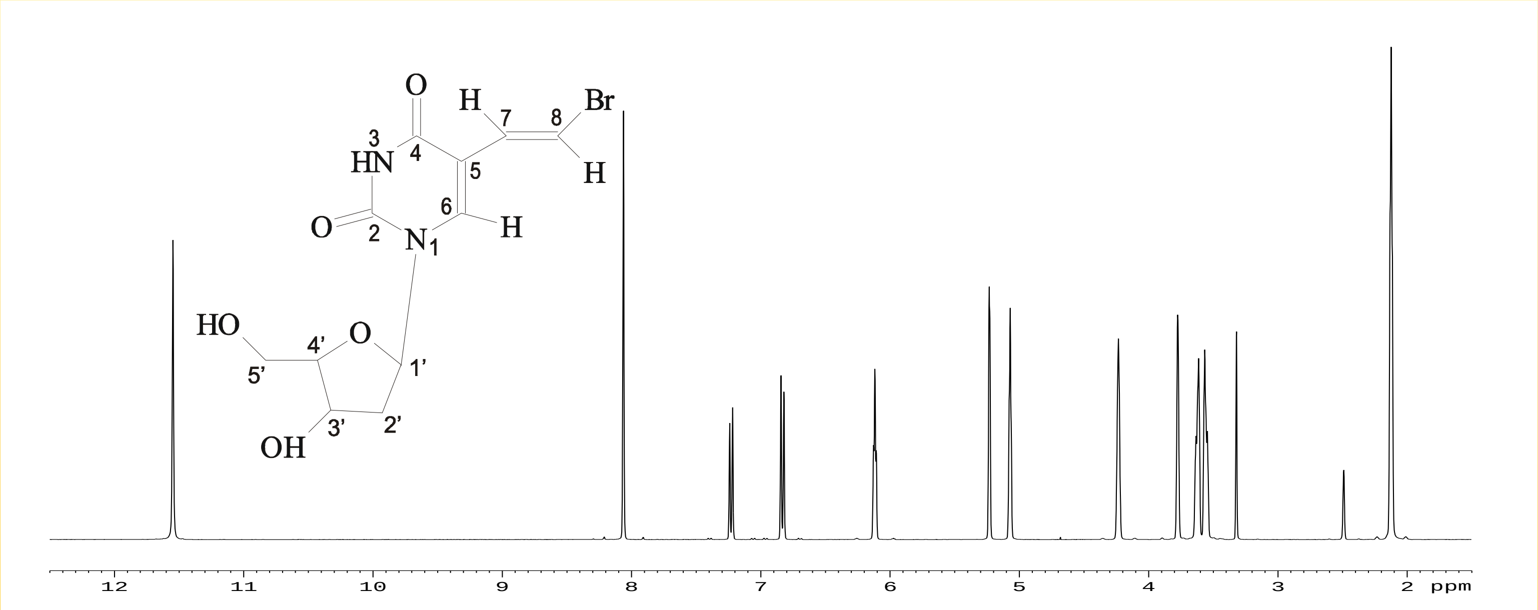
typical range for $^{1}H$



+3000 Hz
10 ppm

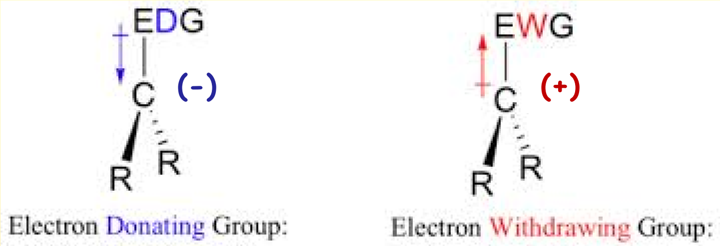
0 Hz
5 ppm

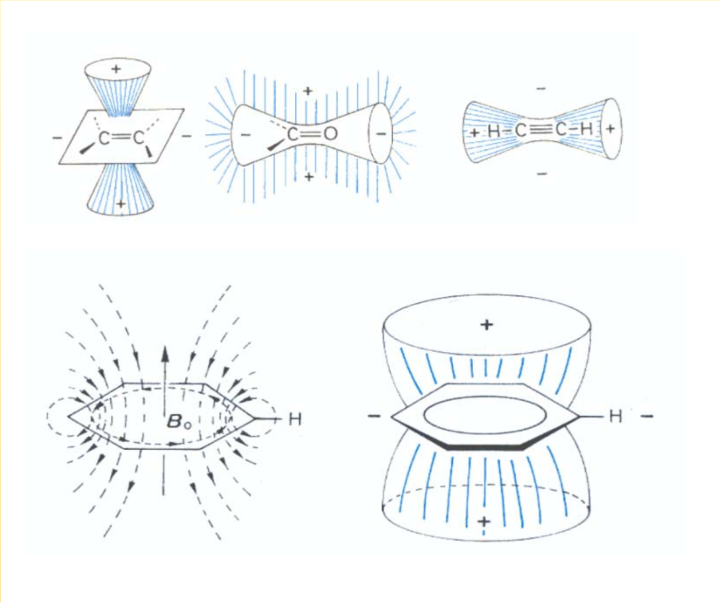
-3000 Hz
0 ppm



Each atom in the molecule gives rise to a resonance line.

# parameters: chemical shift

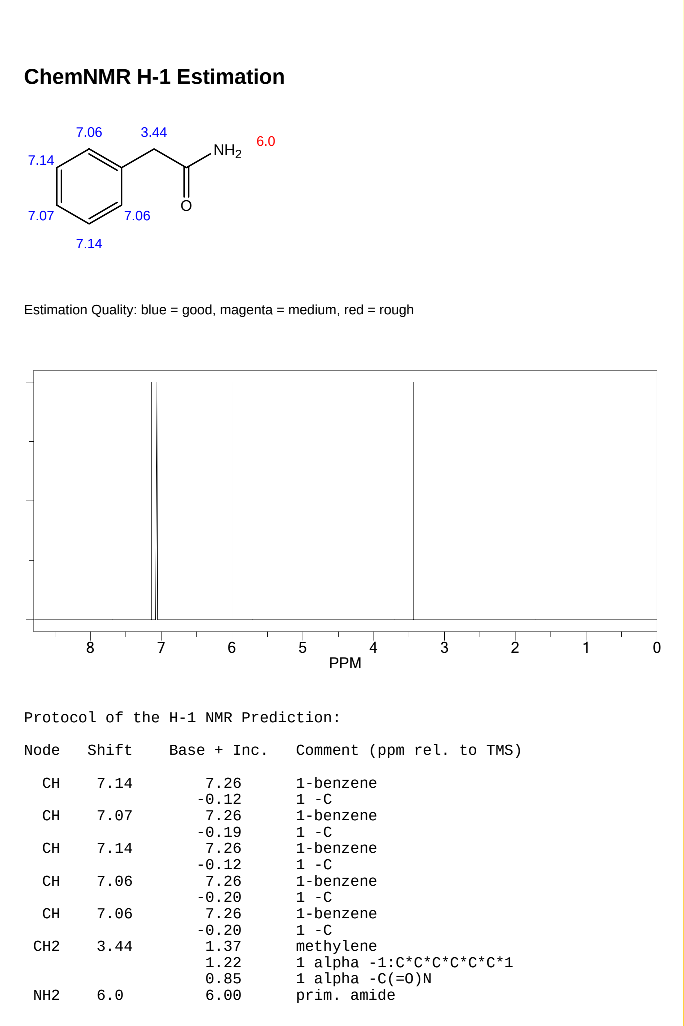




Besides the neighbourhood especially of electron withdrawing groups

anisotropy effects have influence on the chemical shift.

# parameters: chemical shift



**ChemNMR H-1 Estimation**

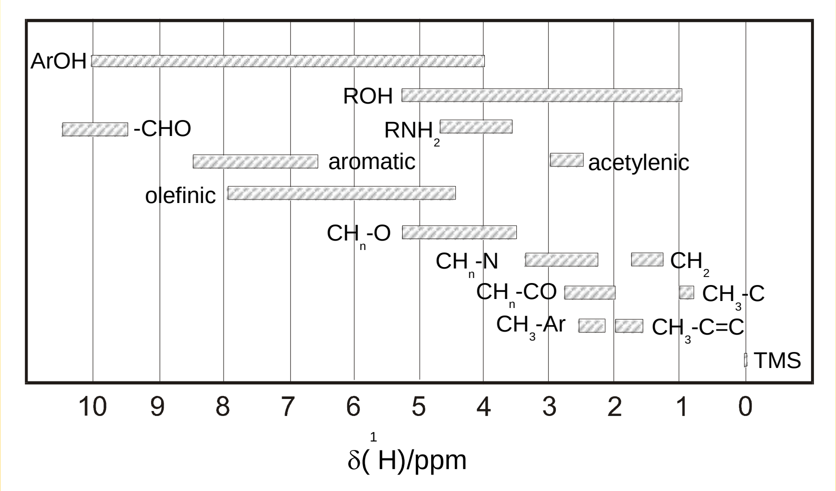
Estimation Quality: blue = good, magenta = medium, red = rough

```
Protocol of the H-1 NMR Prediction:

Node   Shift    Base + Inc.   Comment (ppm rel. to TMS)

  CH    7.14        7.26      1-benzene
                   -0.12      1 -C
  CH    7.07        7.26      1-benzene
                   -0.19      1 -C
  CH    7.14        7.26      1-benzene
                   -0.12      1 -C
  CH    7.06        7.26      1-benzene
                   -0.20      1 -C
  CH    7.06        7.26      1-benzene
                   -0.20      1 -C
 CH2    3.44        1.37      methylene
                    1.22      1 alpha -1:C*C*C*C*C*C*1
                    0.85      1 alpha -C(=O)N
 NH2    6.0         6.00      prim. amide
```

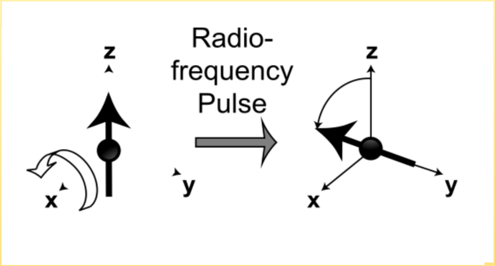
The chemical shift depends on the chemical environment.

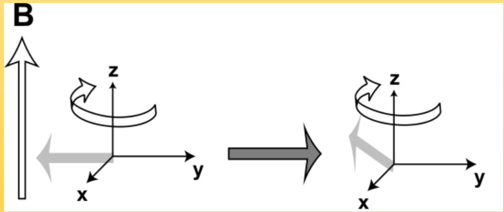


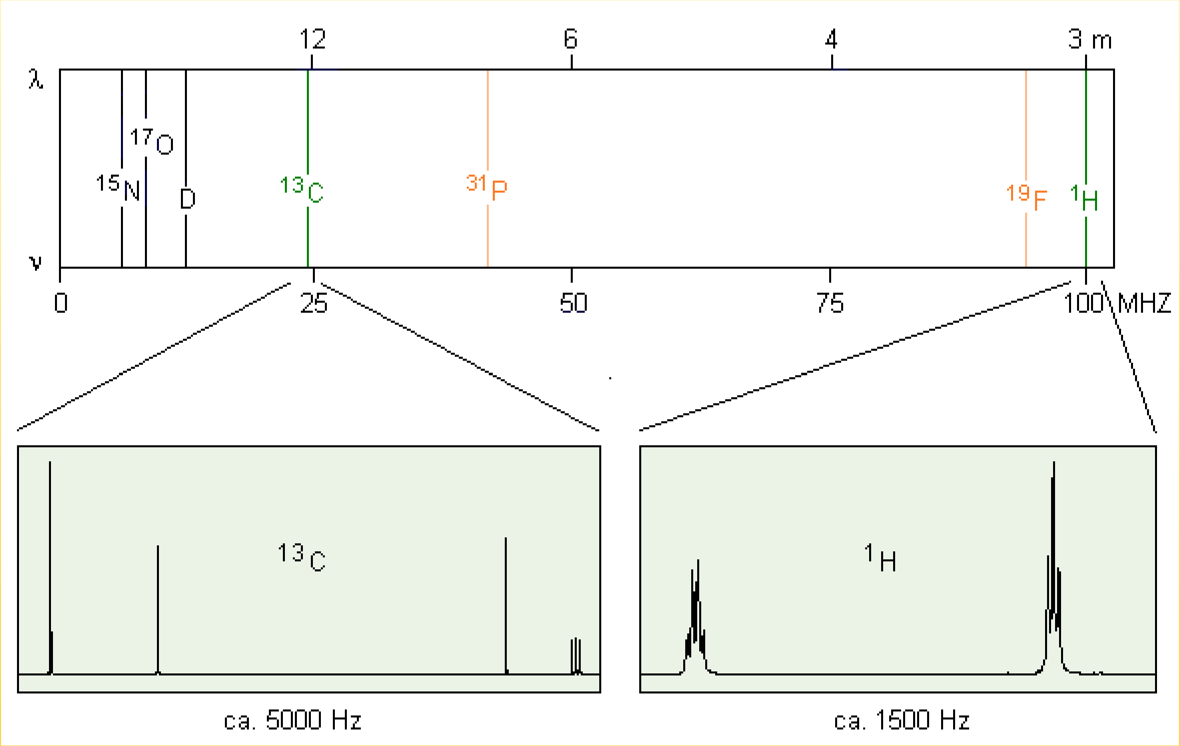
Programs like ChemDraw can predict spectra.

# parameters: chemical shift

The differences of the rotation speeds of different types of nuclei is very large compared to the differences between spins of the same type of nuclei.

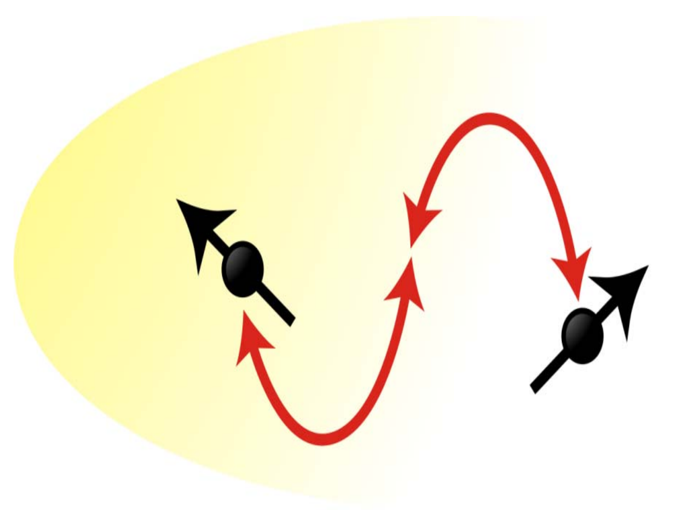






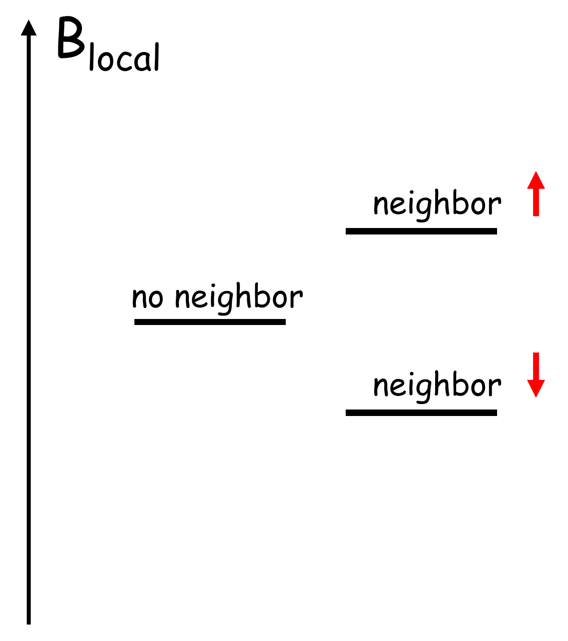
## parameters: scalar coupling

### scalar or J-coupling



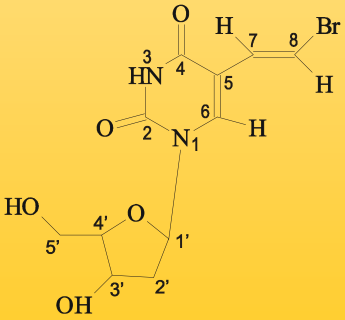
The electrons around the atom nuclei mediate an indirect spin-spin-interaction. Every little bar magnet feels it´s neighbor.

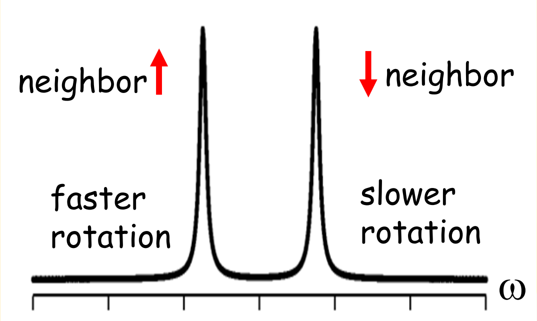
# parameters: scalar coupling



The local field for each nucleus depends on the orientation of its neighbor nucleus. A neighbor orientated with the magnetic field magnifies it, a neighbor orientated against the field reduces it.

$$\omega = \gamma B_{local}$$

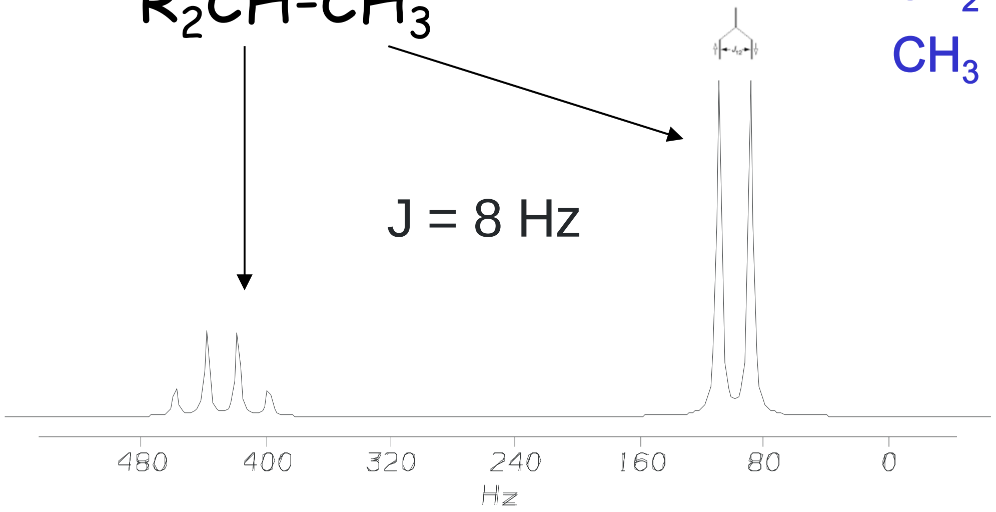




## parameters: scalar coupling

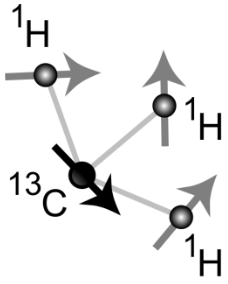
The splitting pattern of the signals give information about the multiplicity of neighbor groups. The integral is proportional to the number of protons that contribute to the signal.

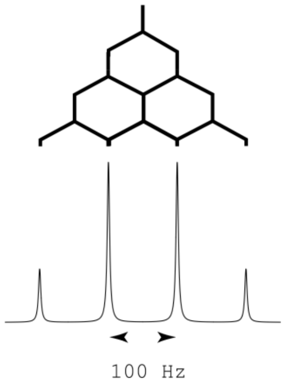
$R_2CH-CH_3$



**Signal splitting by neighbor groups:**

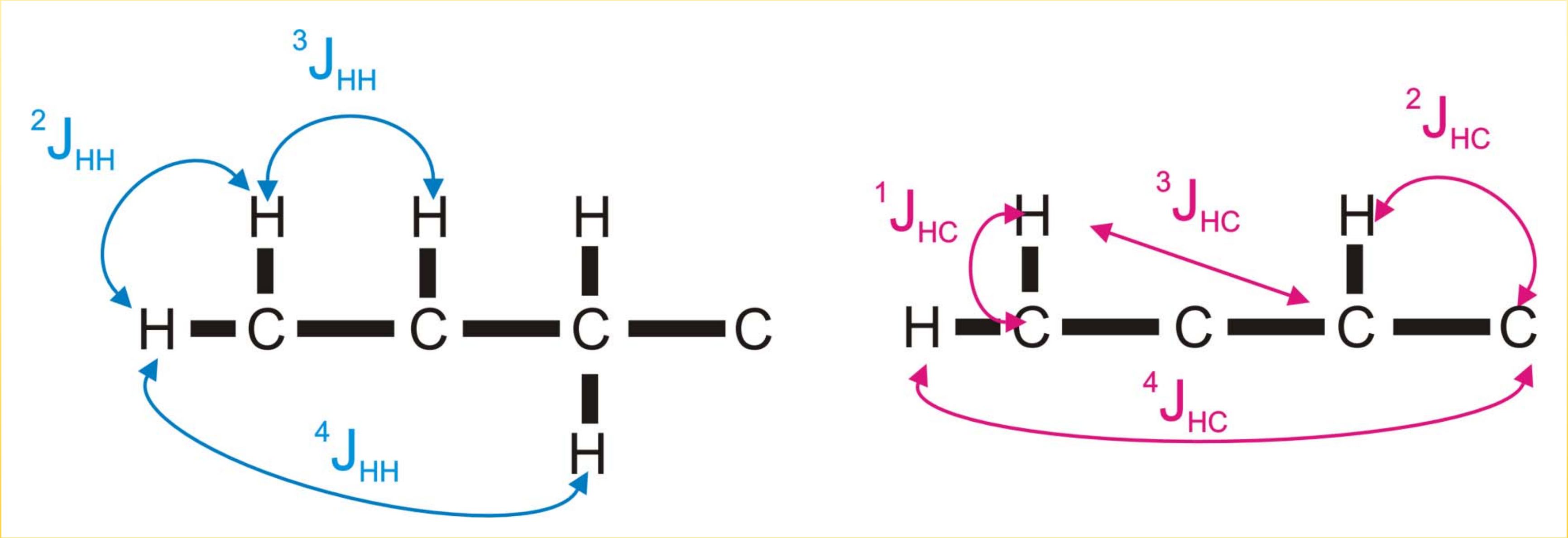
| | | |
|---|---|---|
| CH | doublet | 1:1 |
| $CH_2$ | triplett | 1:2:1 |
| $CH_3$ | quartett | 1:3:3:1 |





# parameters: scalar coupling

Coupling constants can either be homonuclear (between like nuclei) or heteronuclear (between different nuclei) and can either be direct (one-bond) or long-range (multiple bonds)



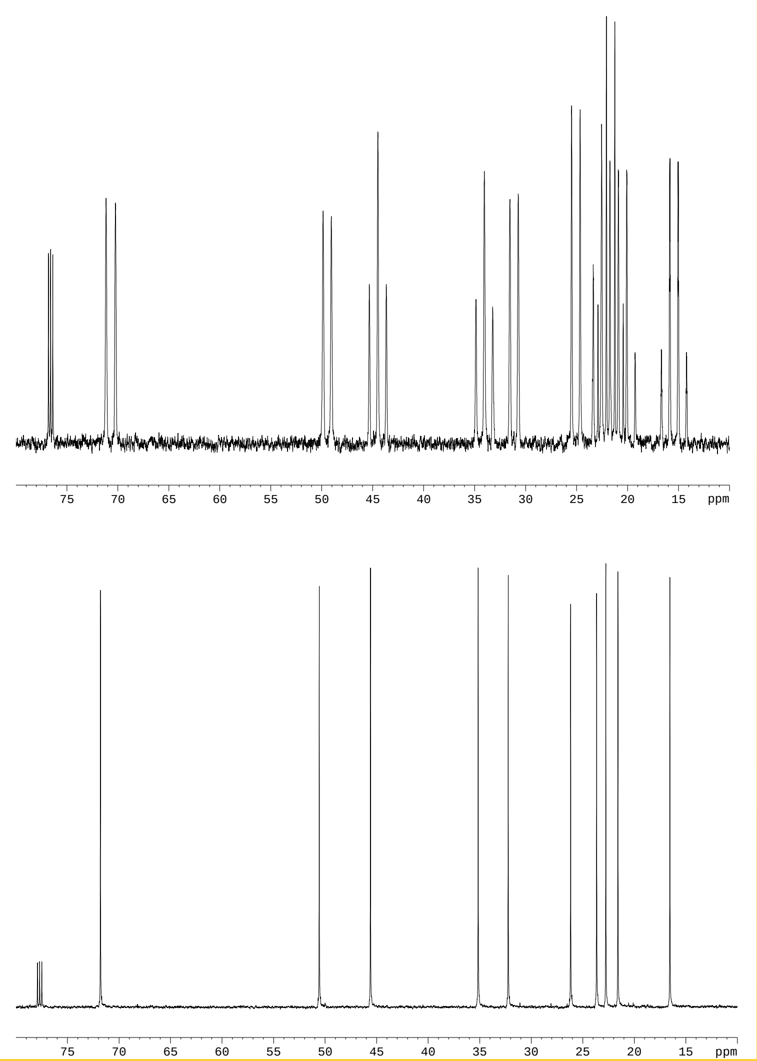
# parameters: scalar coupling

The magnitude of the coupling depends on the type of atoms involved, the distance between them and the orientation to each other.

Direct couplings are usually one order of magnitude larger than the so-called long-range couplings.

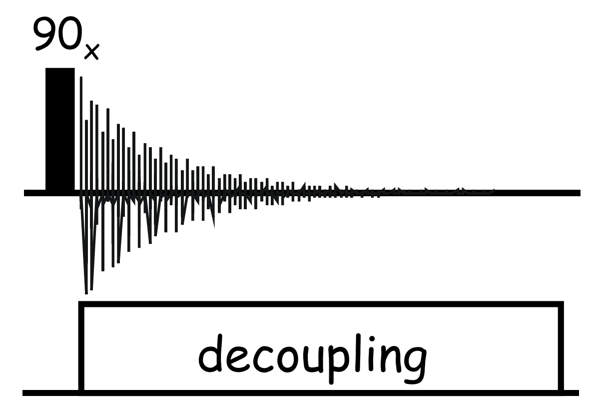
| $^1J_{HH}$ = 276 Hz | $^1J_{HC}$ = 125 .. 200 Hz | $^1J_{HN}$ = 60 .. 100 Hz |
|---|---|---|
| $^2J_{HH}$ = 0 .. 30 Hz | $^2J_{HC}$ = 0 .. 20 Hz | $^2J_{HN}$ = 0 .. 15 Hz |
| $^3J_{HH}$ = 0 .. 20 Hz | $^3J_{HC}$ = 0 .. 15 Hz | $^3J_{HN}$ = 0 .. 8 Hz |
| $^4J_{HH}$ = 0 .. 3 Hz | $^4J_{HC}$ = 0 .. 2 Hz | $^4J_{HN}$ = 0 .. 1 Hz |

# parameters: scalar coupling



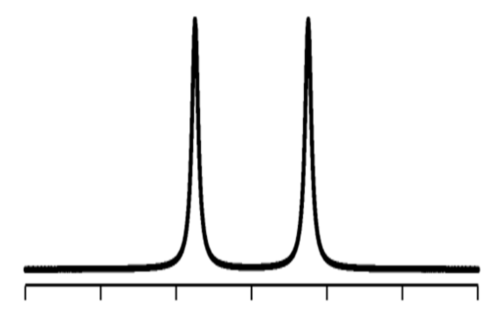
In case of different atom types it is possible to avoid the splitting by changing the direction of the neighbor bar magnet in a high frequency.

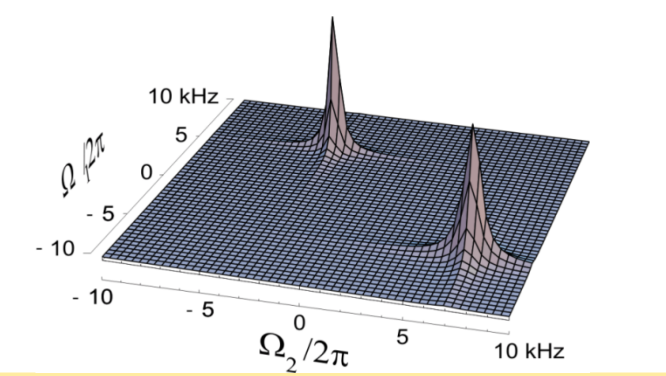
This technique is called decoupling.

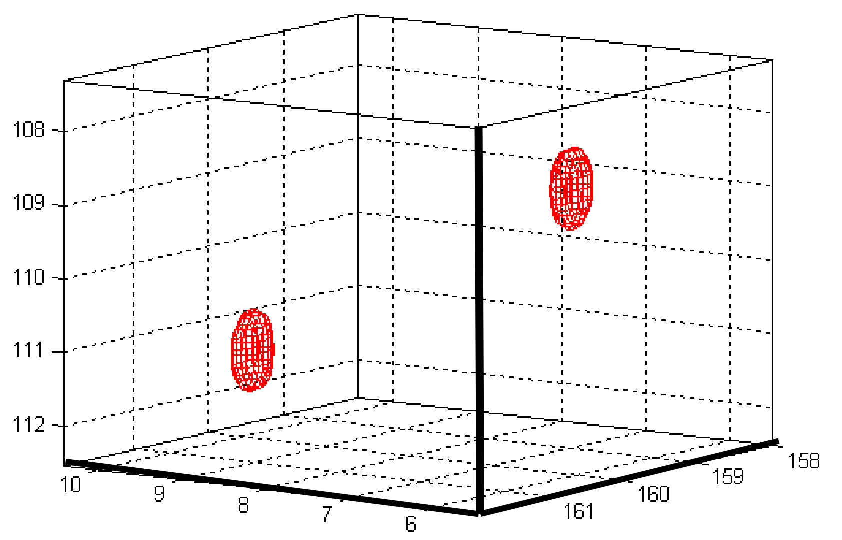


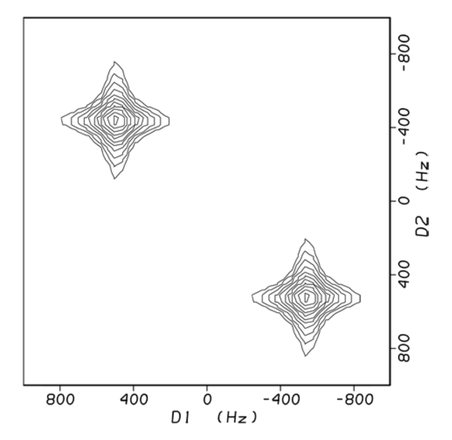
# Multidimensional NMR-spectroscopy

# multidimensional experiments: theory

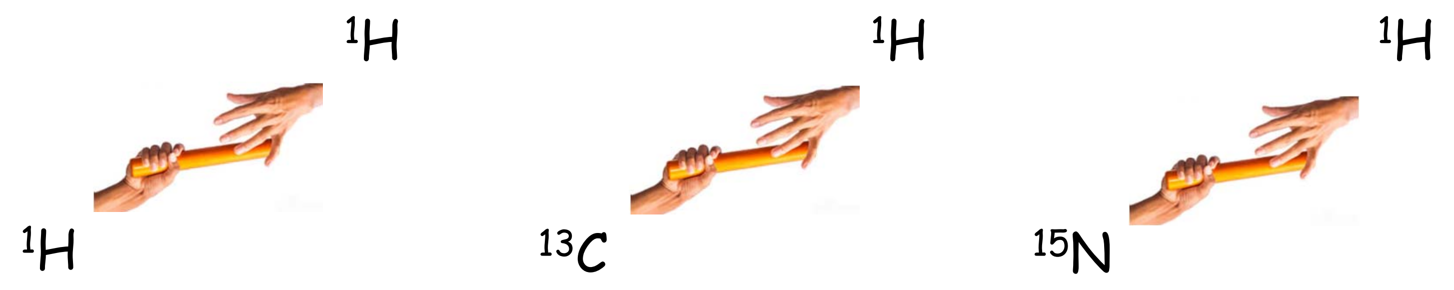








Information can be transferred from one spin to another by coupling like a baton.

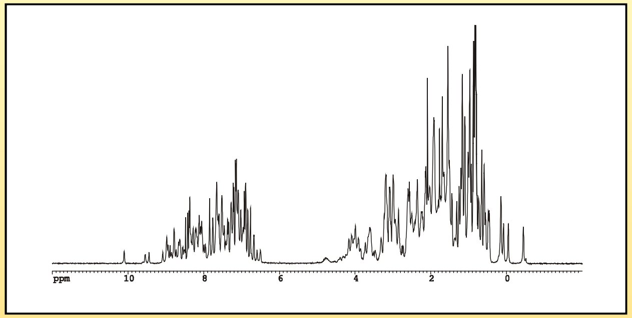


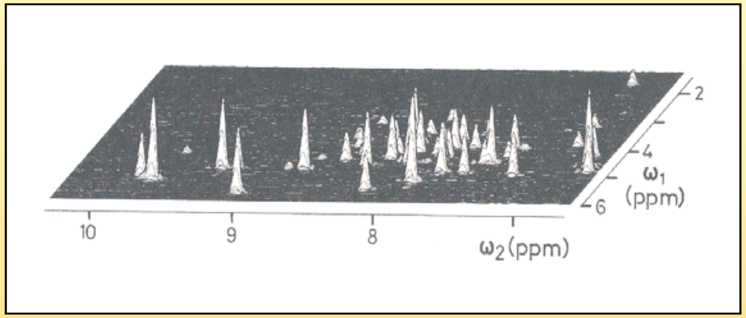
A 2D-spectrum is generated by storing the chemical shift of the first nucleus before the information transfer and detecting the chemical shift of the second nucleus after the transfer.

# multidimensional experiments: theory

1D-NMR:
2 Axes
intensity vs. frequency

2D-NMR:
3 Axes
intensity vs. freq. 1 vs. freq. 2





**better resolution:** signals are spread over a surface (2D) or over space (3D, 4D)

**more information:** Signals arise only due to interaction between nuclei. Appearance of a signal implies (according to the exp) chemical or spacial proximity of the nuclei.

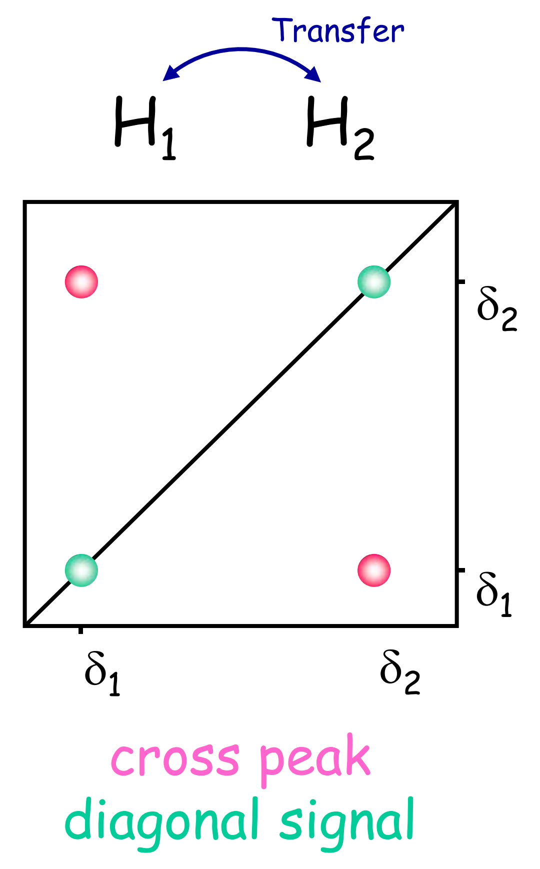
# homonuclear spectra

Transfer of magnetization takes place between like nuclei. Both axis exhibit the chemical shift of the same type of nucleus. If a transfer has taken place, the signal has different frequencies in the two dimensions:

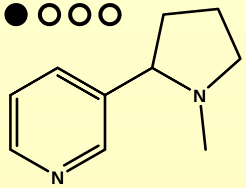
cross peak

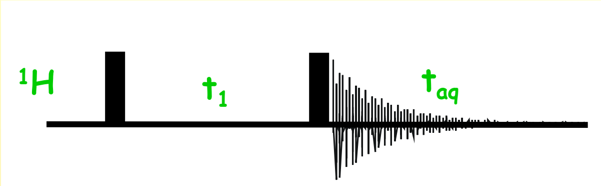
If no transfer has taken place, the shifts are the same in both dimensions:

diagonal signal

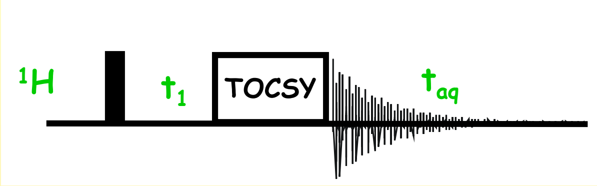


# multidimensional experiments: routine spectra

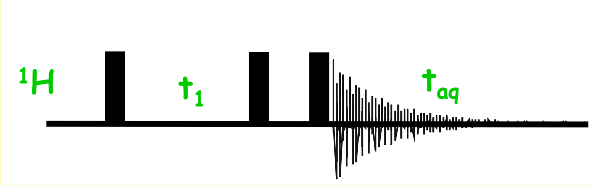




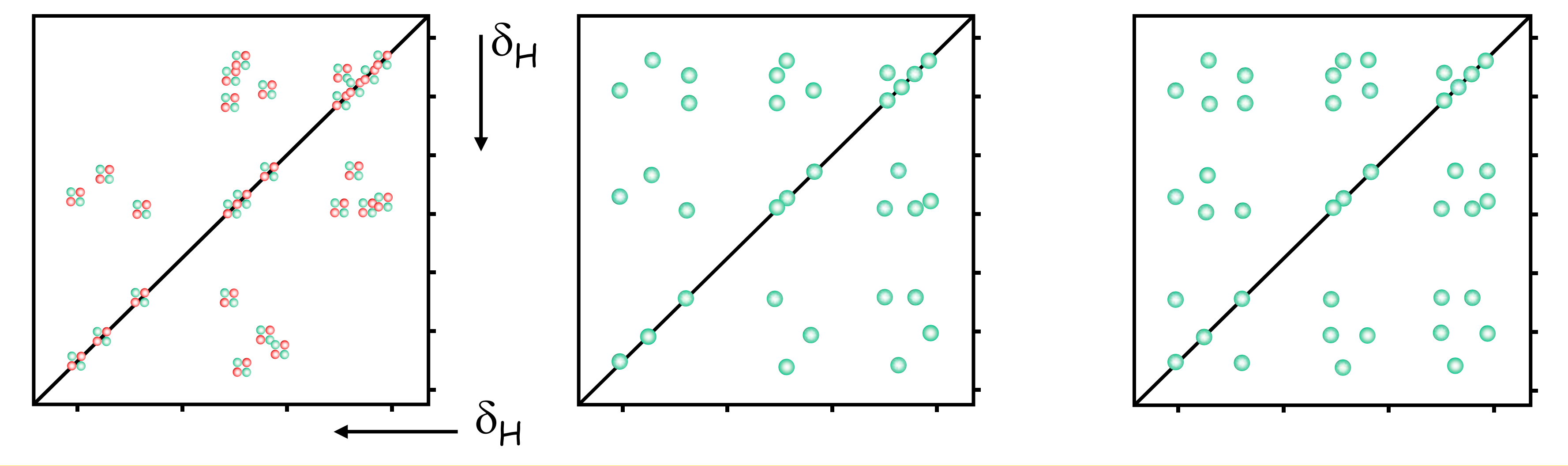
vicinal protons
(max. 3 bonds apart)



proton chains
(max. 3 bonds to the next proton)



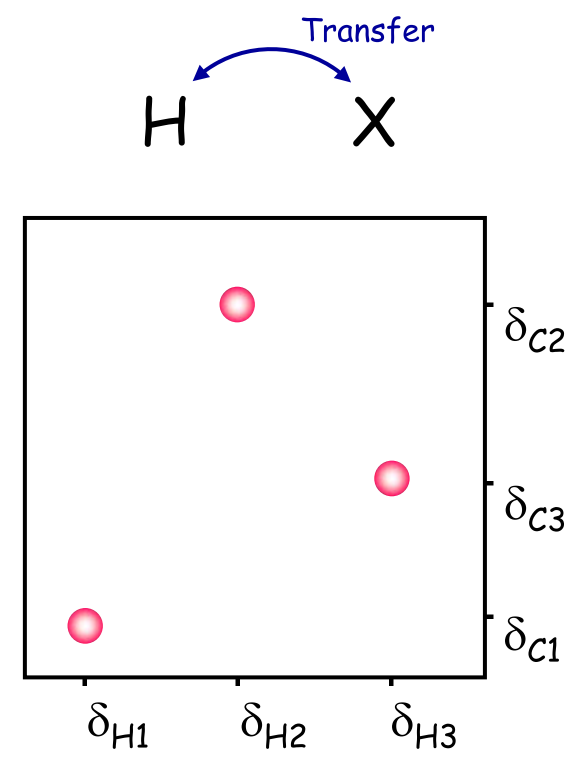
spacial nearby protons
(max. 5 Å)



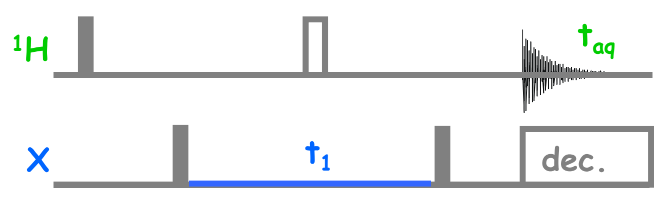
DQF-COSY TOCSY NOESY

# heteronuclear spectra

Transfer of magnetization takes place between nuclei of different types. The two axis show the chemical shift of the respective type of nucleus. If a transfer has taken place, a signal appears at the intersection of the two frequencies, without a transfer there is no signal.



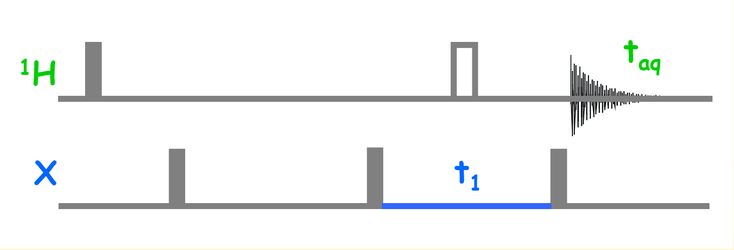
# multidimensional experiments: routine spectra



proton und heteronucleus

($^{13}$C or $^{15}$N)

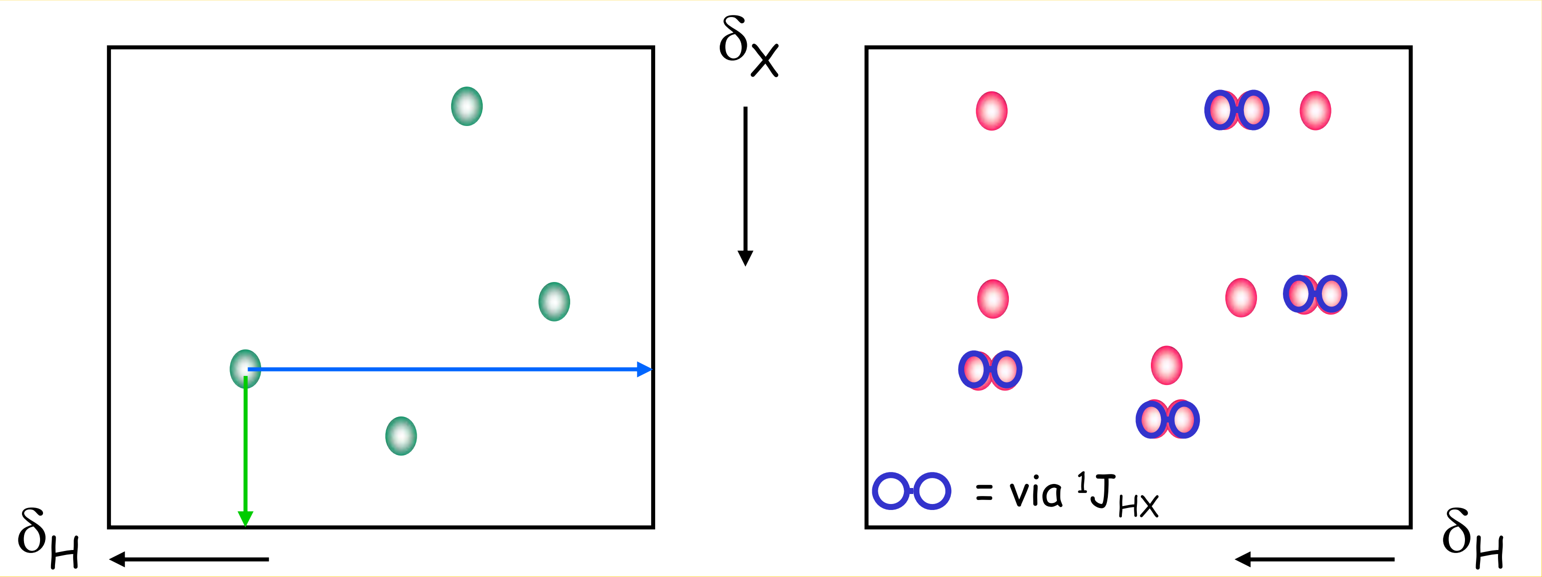
via one bond



protons und heteronucleus

($^{13}$C or $^{15}$N)

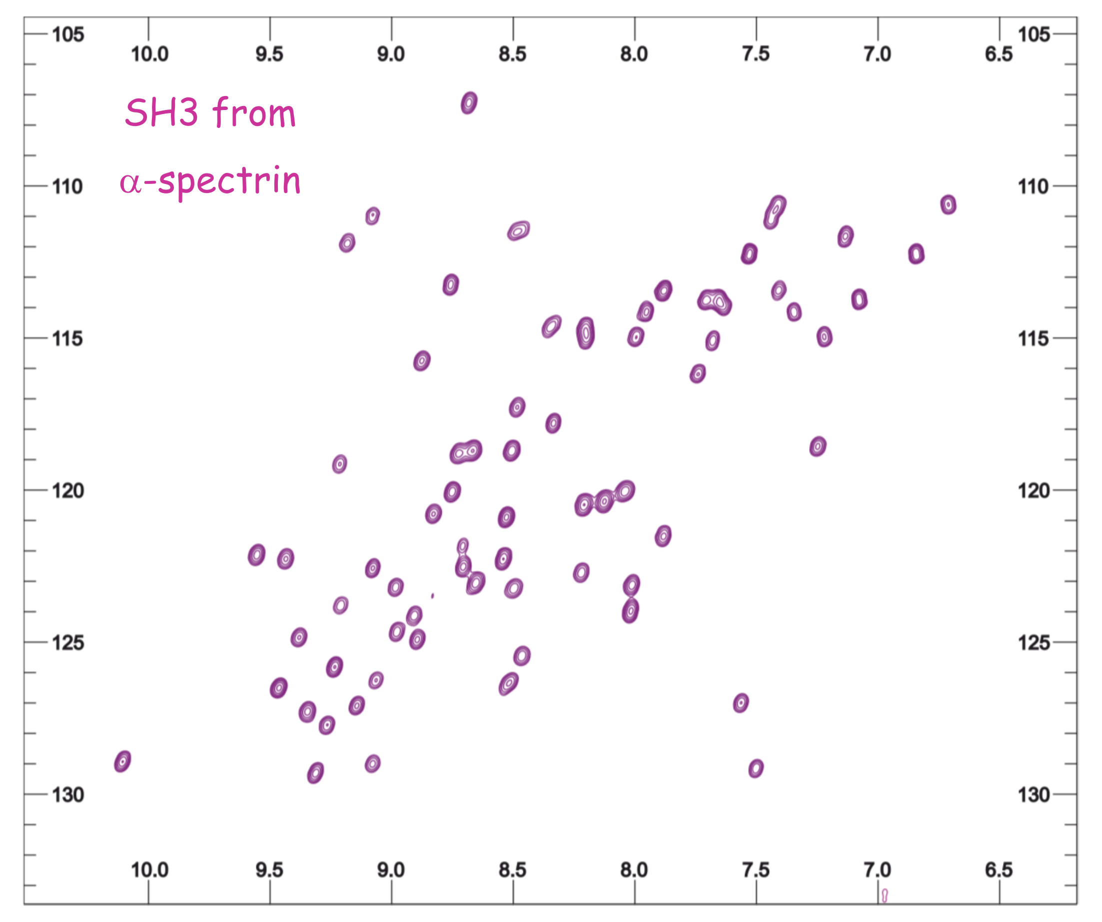
via two to four bonds



HMQC or HSQC

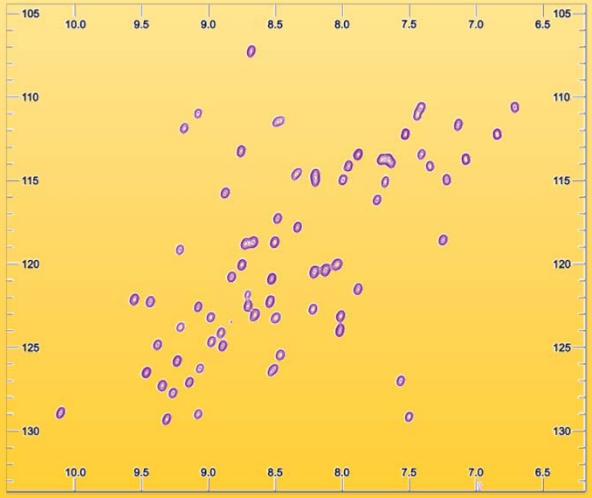
HMBC

# 15N-HSQC



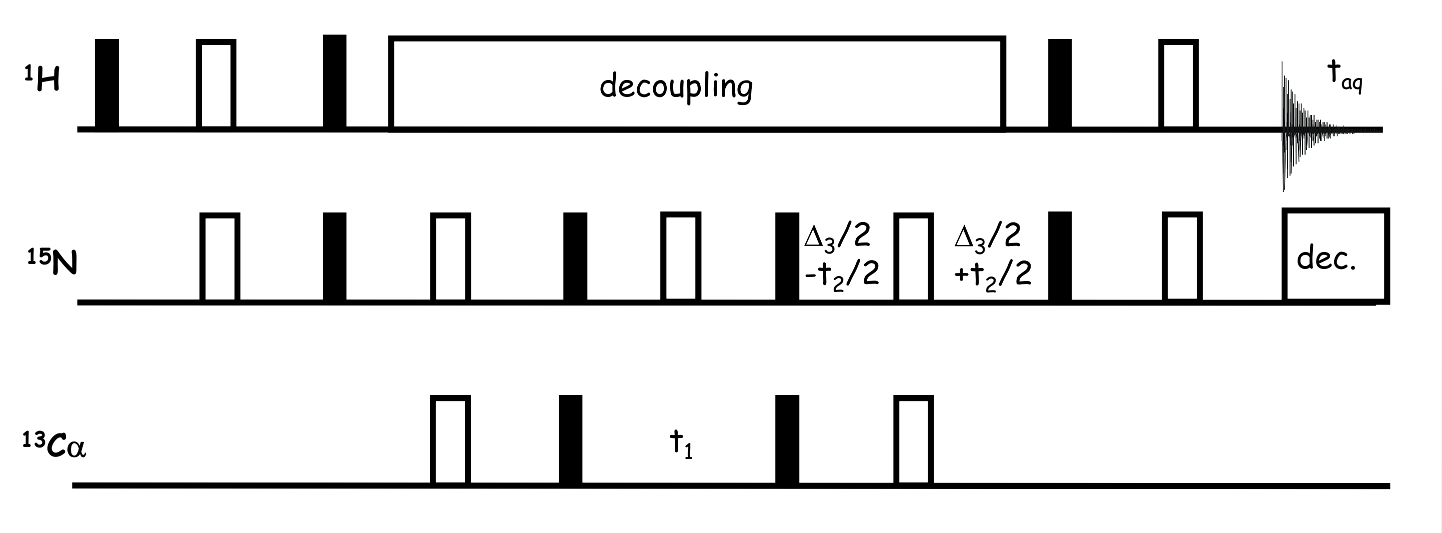
fingerprint spectrum of peptides and proteins

# Understanding NMR experiments: magnetization transfer and HSQC

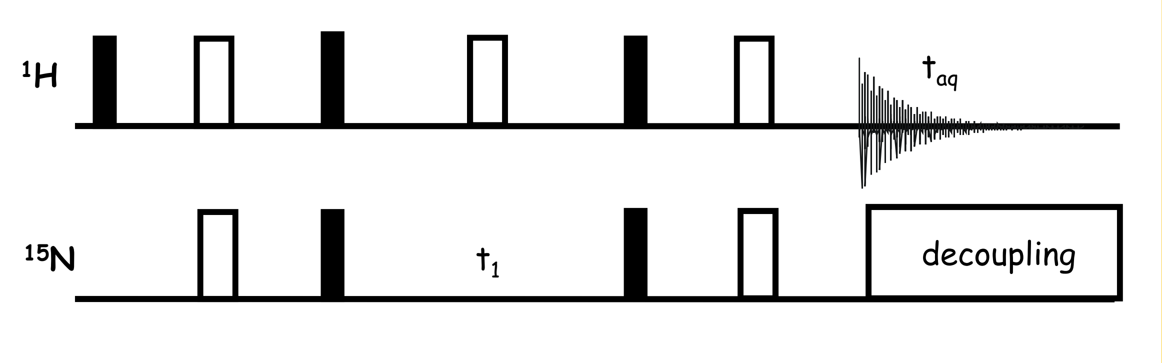


understanding NMR: magnetization transfer

## HNCA

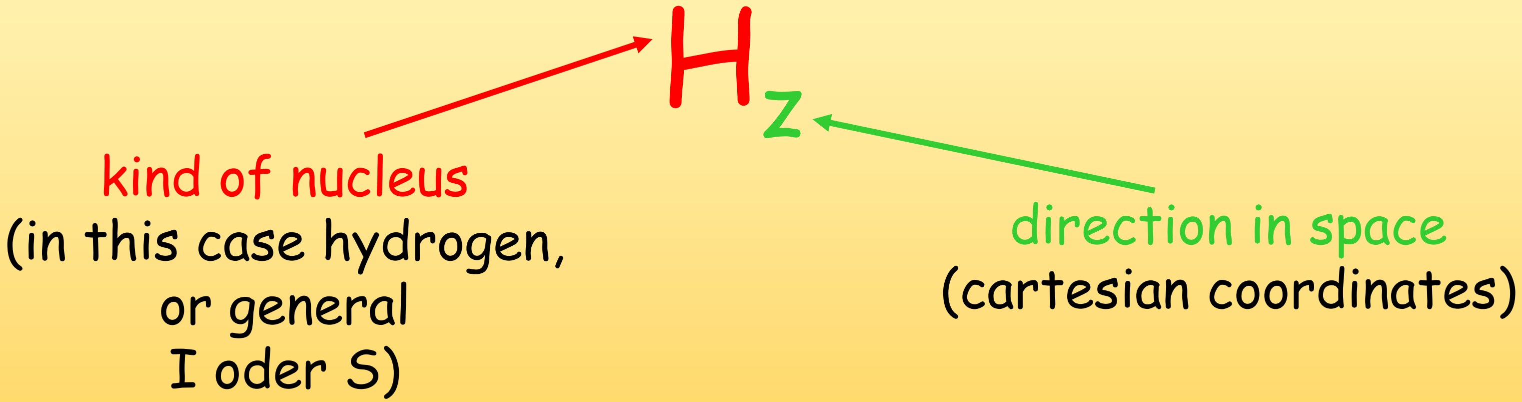


## HSQC



Spins and their magnetization can be expressed using cartesian coordinates.

Starting point of each experiment is $H_z$.

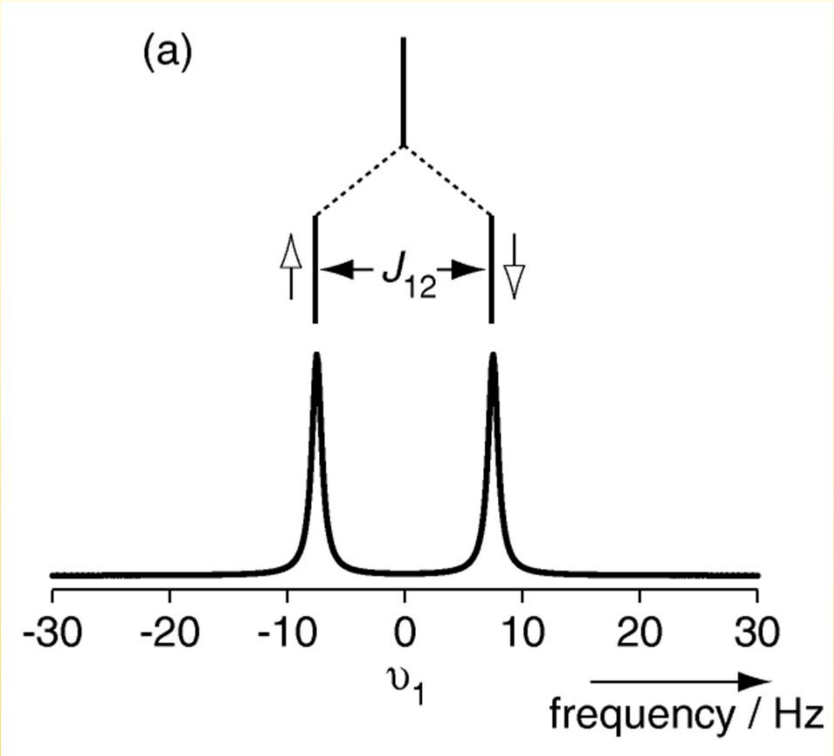


x,y-magnetization is described as $H_x$ or $H_y$,

or respectively as $C_x$ und $C_y$.

Or more generally t for transversal e.g. $H_t$, $N_t$, $I_t$.

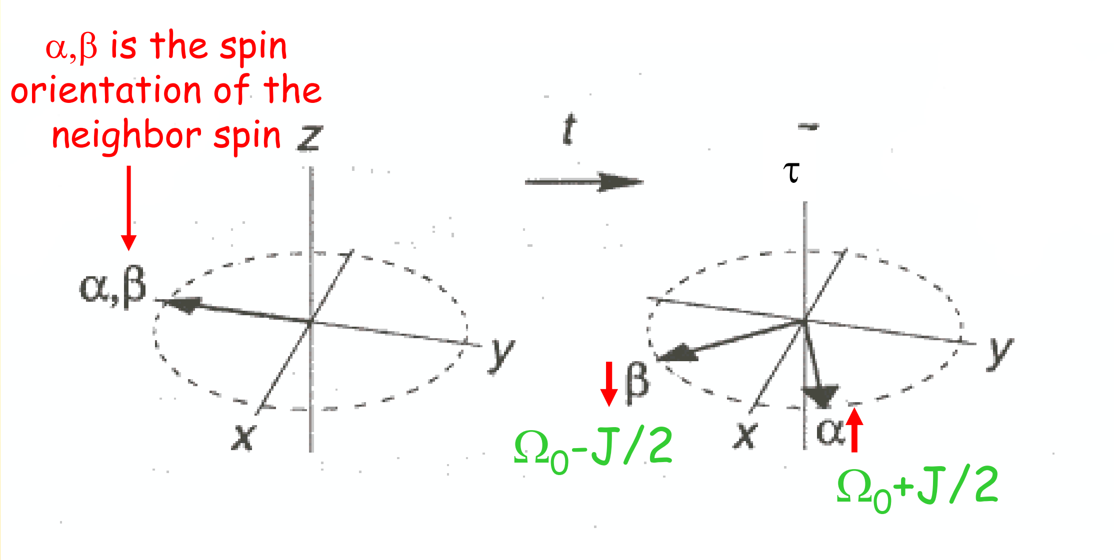
## understanding NMR: magnetization transfer



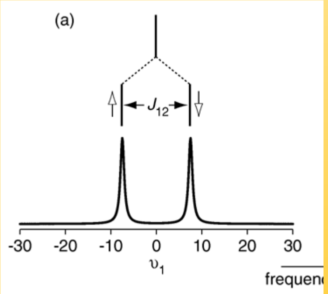
In 1D spectra coupling appears as splitting.
In multidimensional spectra one can use scalar coupling for information transfer, because it leads to types of magnetization where the spin states of different nuclei are "coupled to each other".

The splitting J is the difference of the rotation frequency in Hertz.

# understanding NMR: magnetization transfer



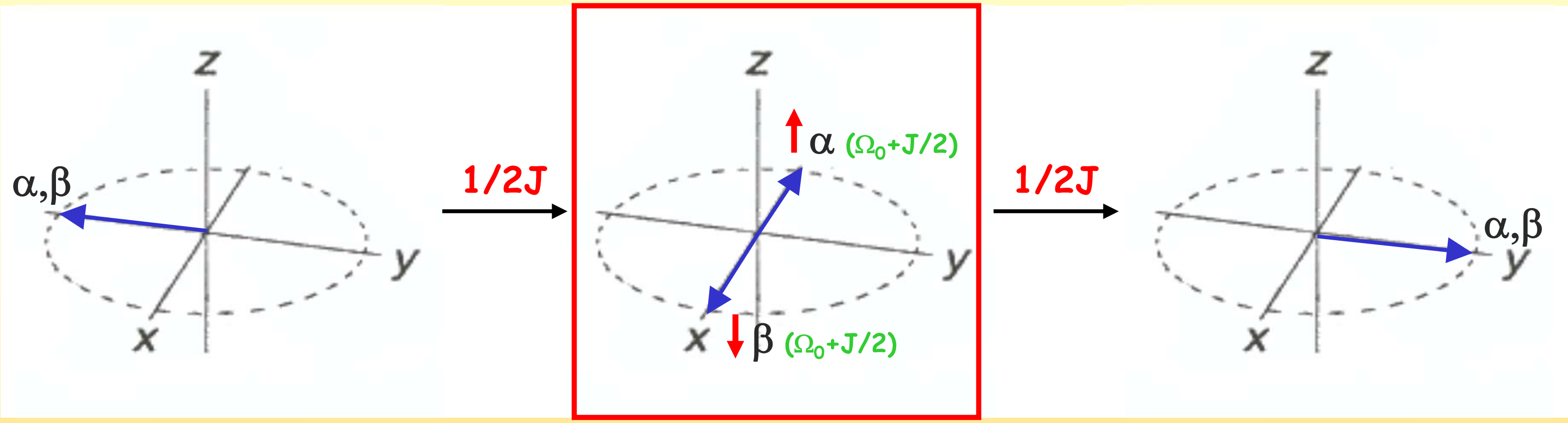
Scalar coupling acts on x,y-magnetization only and causes a rotation around the z axis.



The vectors rotate with $\Omega_0$ + J/2 and $\Omega_0$ - J/2.

As J is the difference of the rotation frequency in Hertz, 1/J is the time during which both components run out of phase by 360°.

# understanding NMR: magnetization transfer

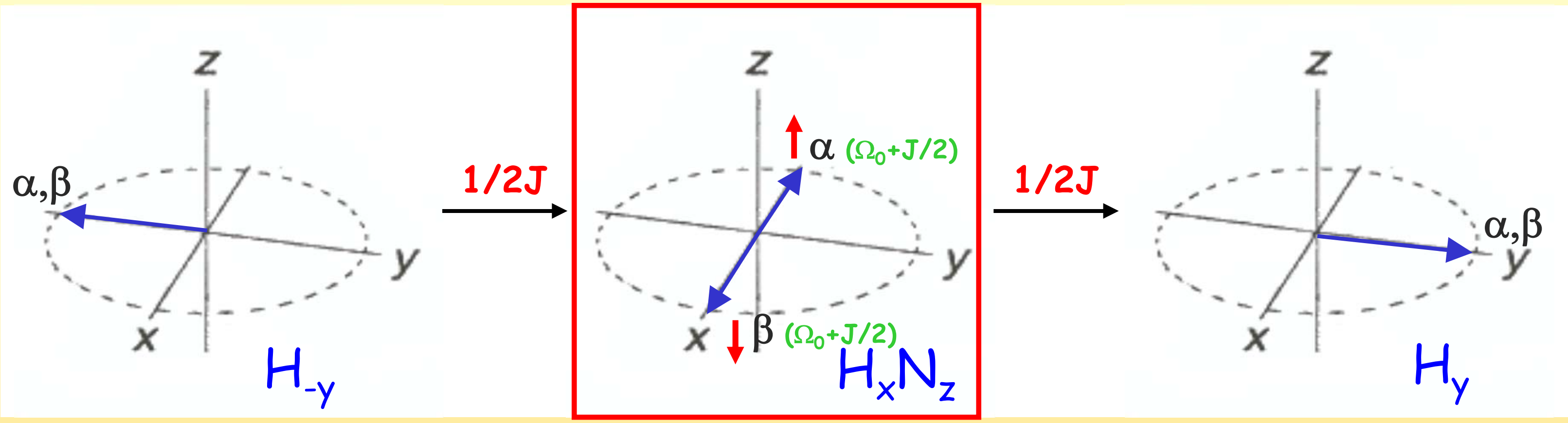


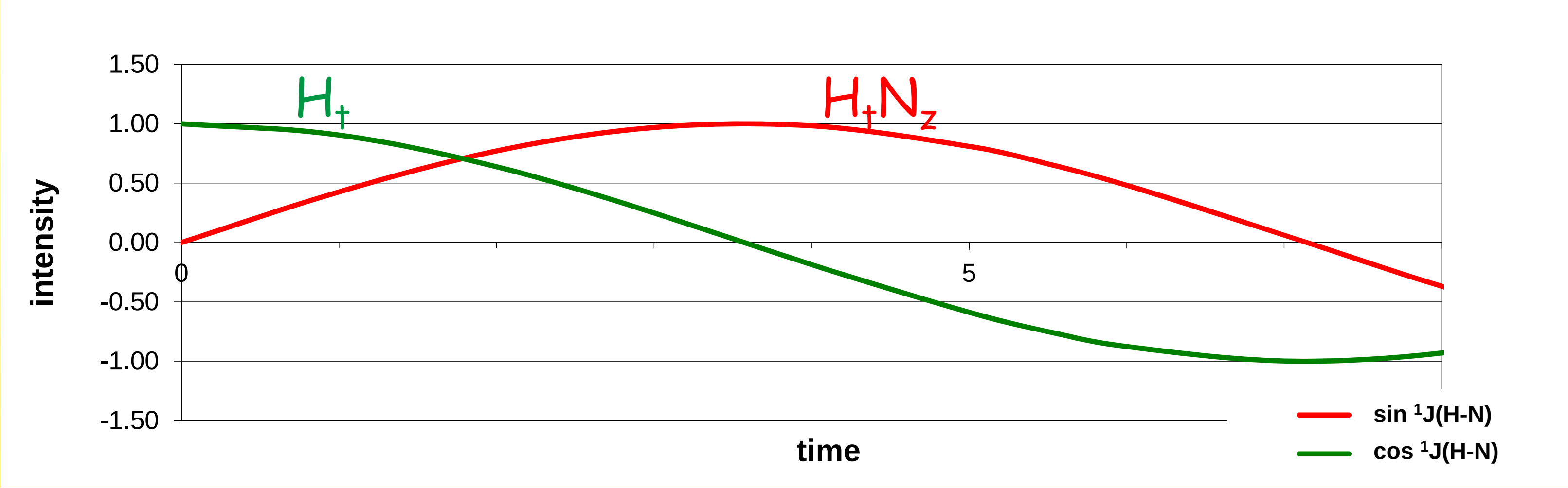
After a time period of 1/2J the spins are run out of phase by 180°.

At this point the effect of the coupling is maximal.

After a time of 1/J the faster spin has lapped the slower one exactly (360°), at this moment the effect of the coupling doesn´t exist.

# understanding NMR: magnetization transfer

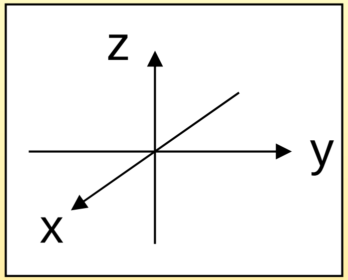




$$H_t\cos\pi Jt + H_tN_z\sin\pi Jt$$

# understanding NMR: magnetization transfer

Scalar coupling can only evolve between a nucleus in x,y-direction and another nucleus in z-direction.

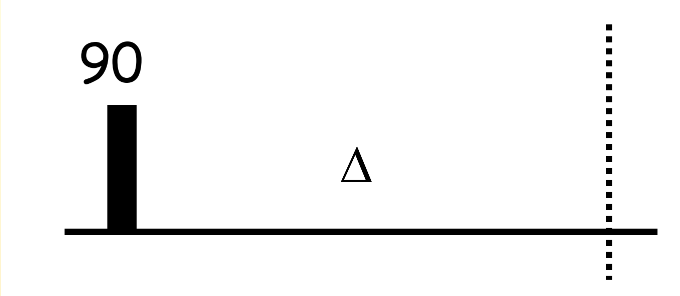


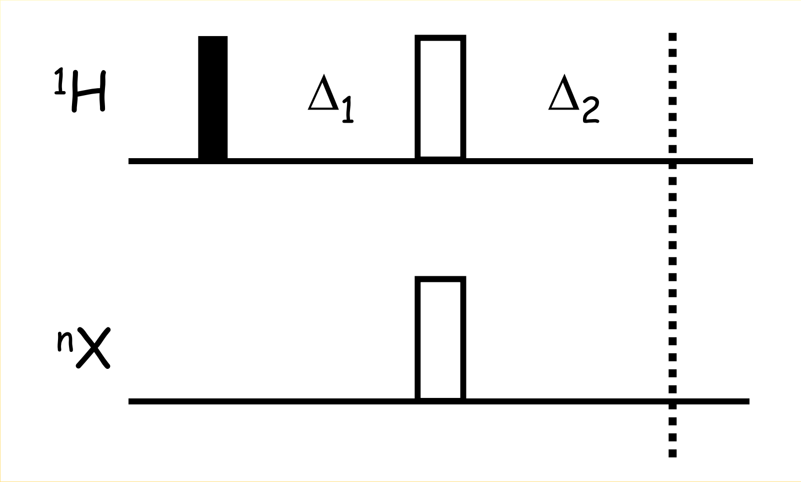
After 1/2J coupling time the completely coupled state $I_{1t}I_{2z}$ is reached.

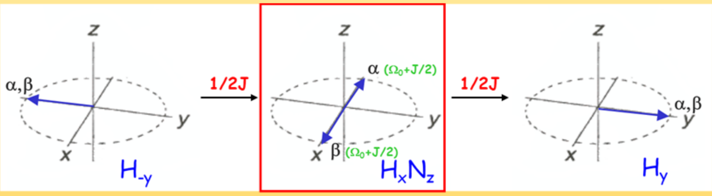
Without coupling or equally after 1/J pure (uncoupled) magnetization $I_{1t}$ is reached.

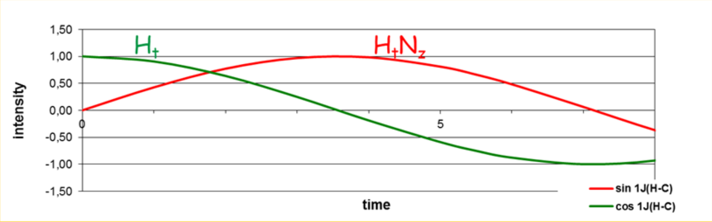
# understanding NMR: magnetization transfer

There are two possibilities to couple spins with each other to transfer magnetization (information):





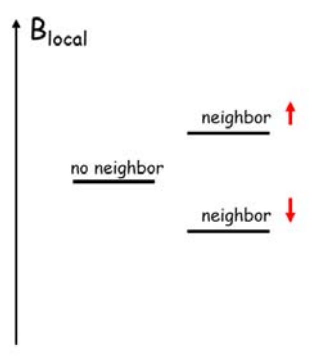




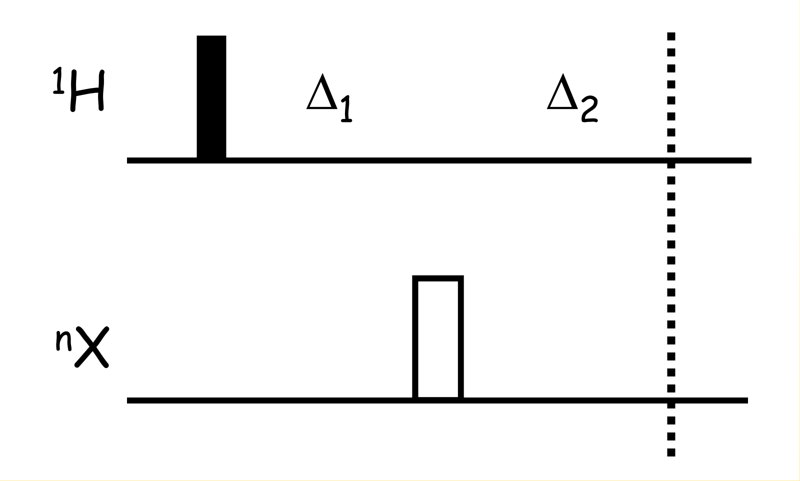
If we do this for 1/2J we yield the coupled state between H and X.

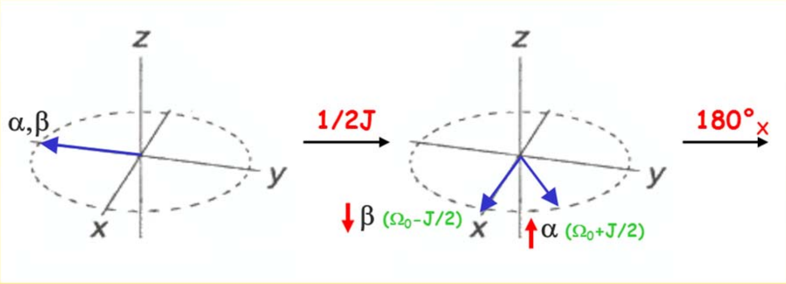
This way we can transfer magnetization, or information from H to X.

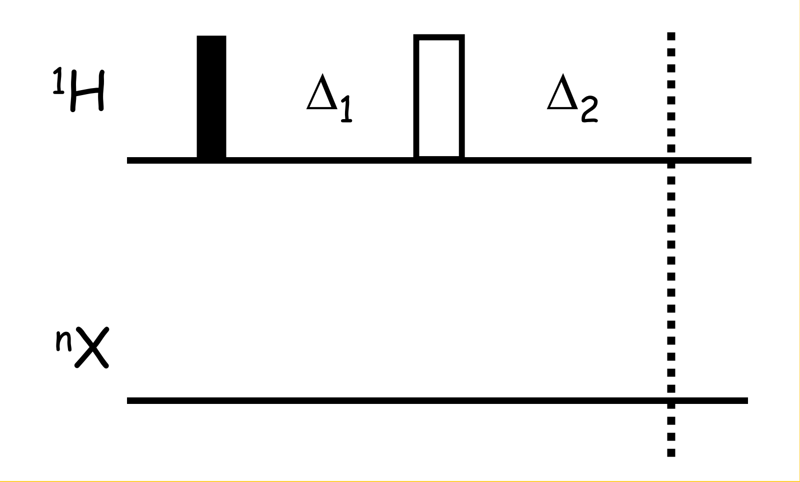
# understanding NMR: magnetization transfer

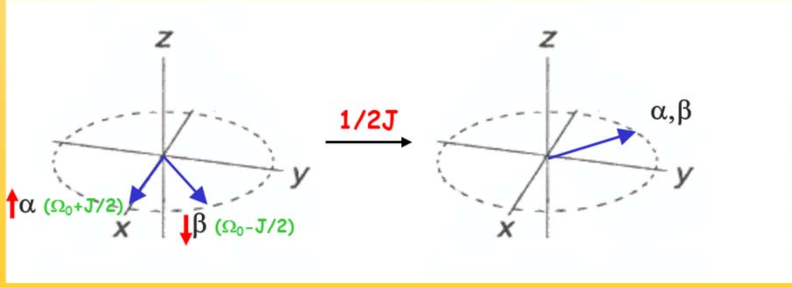


And two possiblilties to avoid coupling:



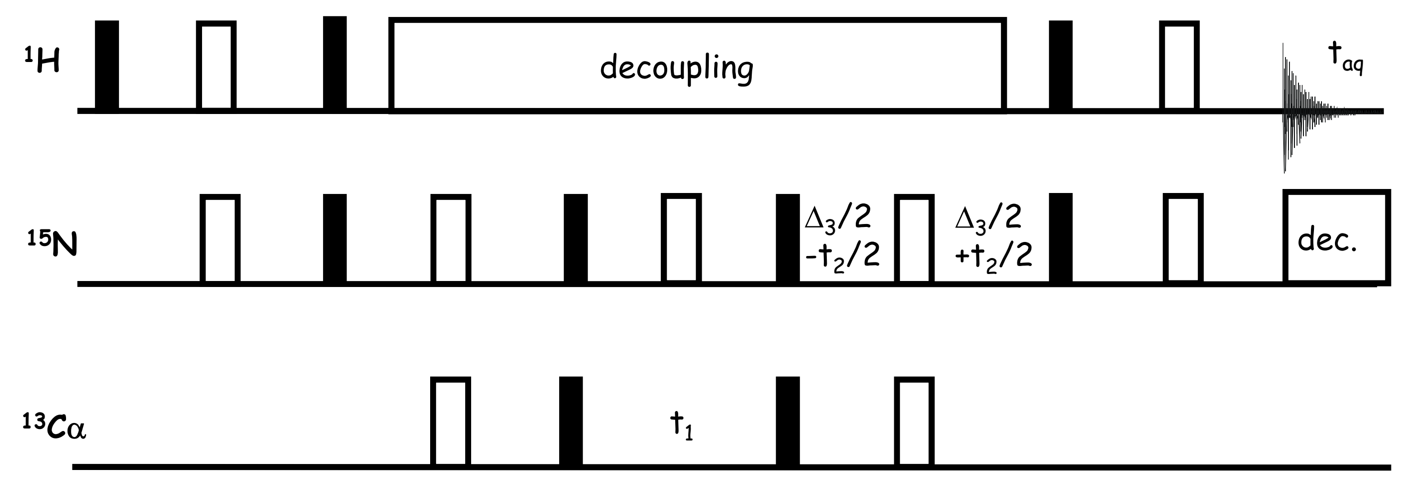




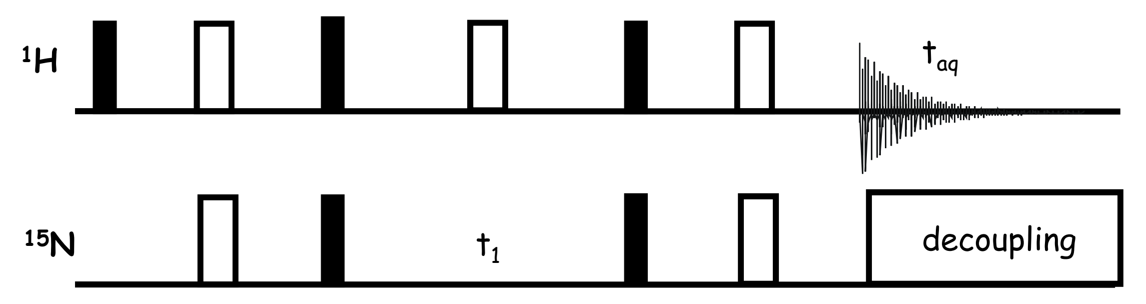


# understanding NMR: HSQC

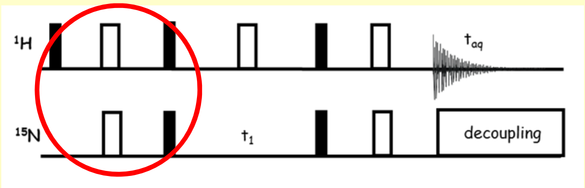
## HNCA

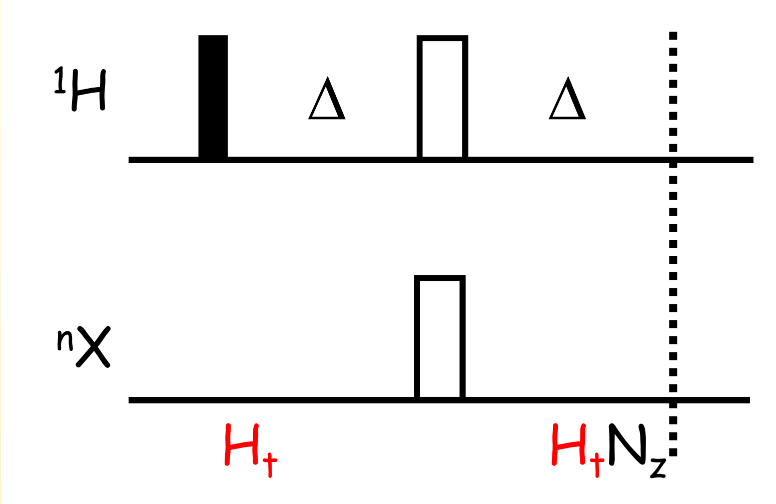


## HSQC



# understanding NMR: HSQC





1/2$J_{HX}$ evolution of scalar coupling

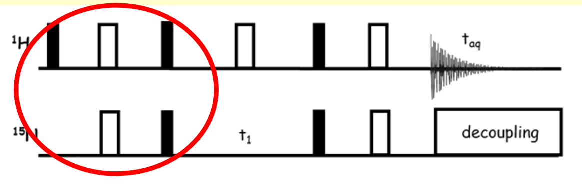
-> enables magnetization transfer

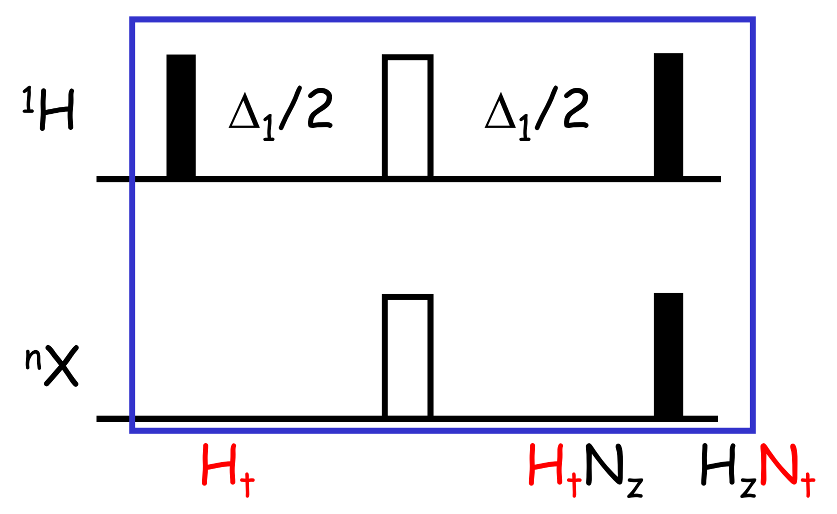
$1/(2\ ^1J_{HC}) = 3.6$msec ($J = 140$ Hz)

$1/(2\ ^1J_{HN}) = 5.5$msec ($J = 90$ Hz)

$\Delta_1$ is $1/(2\ ^1J_{HN})$ $H_tN_z$ is obtained. That means that the magnetization of each proton is bound to the magnetization of the hetero nucleus it is bound to.

# understanding NMR: HSQC



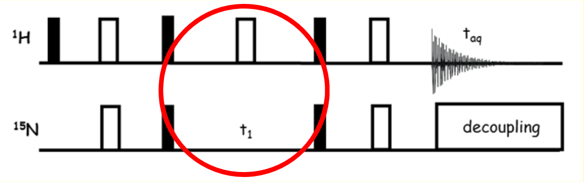


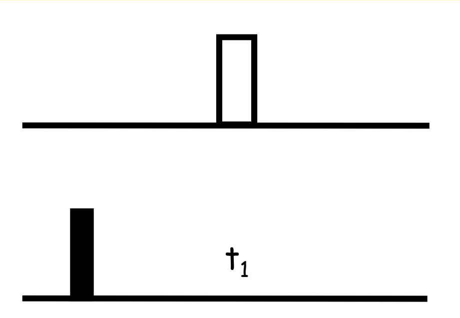
The two following 90°
pulses manage a
transfer from H to N (!!!)

$$H_x N_z \xrightarrow[90^\circ N_x]{90^\circ H_y} H_z N_y$$

This important building
block is called
INEPT-Sequence.

## understanding NMR: HSQC



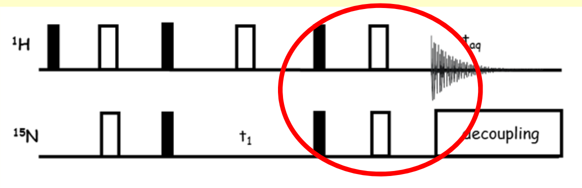


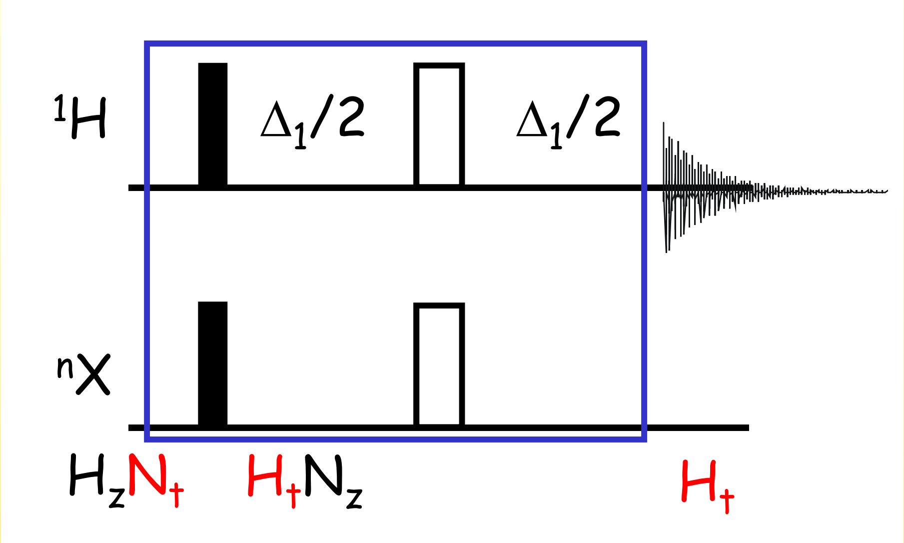
$H_z N_y$

During the evolution time $t_1$ the X nucleus rotates in the transversal plane
and its frequency is recorded.

The protons in z flip their orientation in the middle of the evolution time
-> no coupling effect

# understanding NMR: HSQC



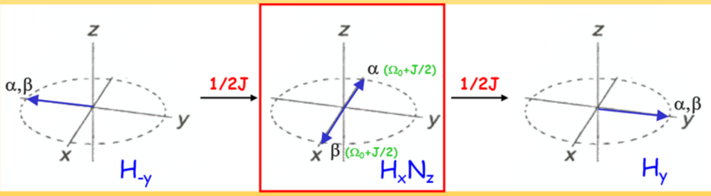


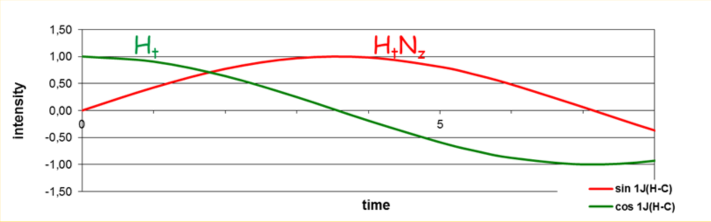
In the end the magnetization is the same as in a simple proton 1D but containing the information of the rotation frequency of nitrogen.

$$H_z N_y \xrightarrow[90^\circ\ N]{90^\circ\ H} H_x N_z$$

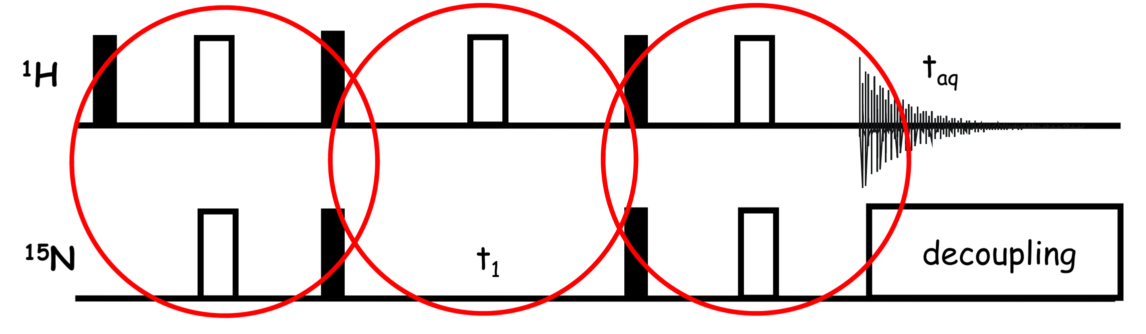
$$H_x N_z \xrightarrow[1/2J]{} H_y$$

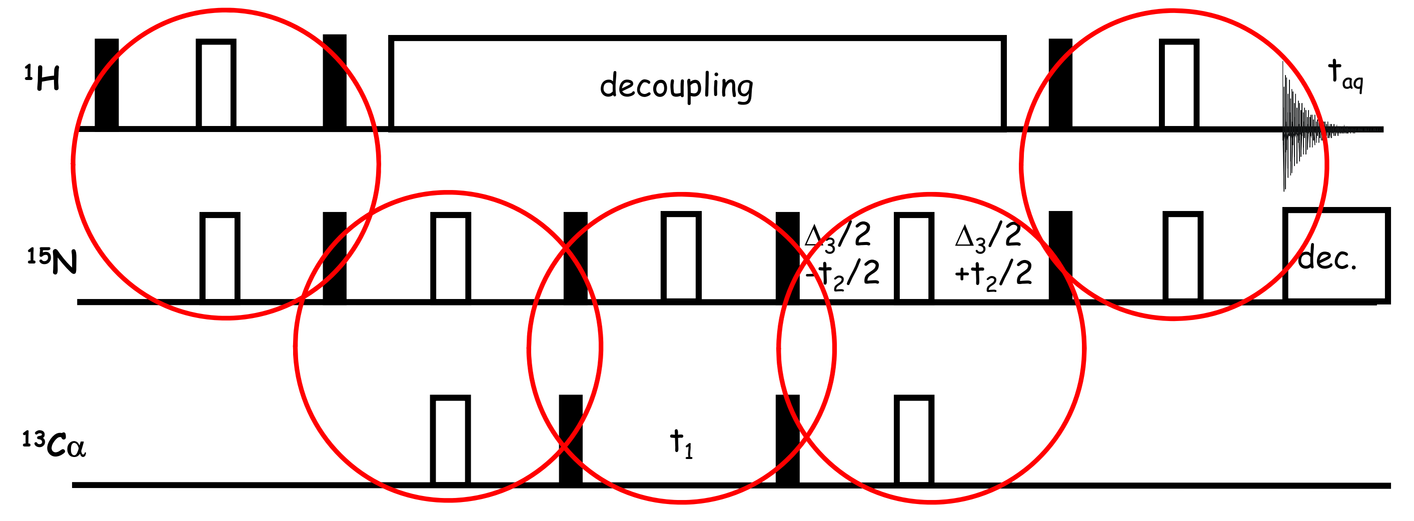
$$H_y \cos\Omega_N t_1$$



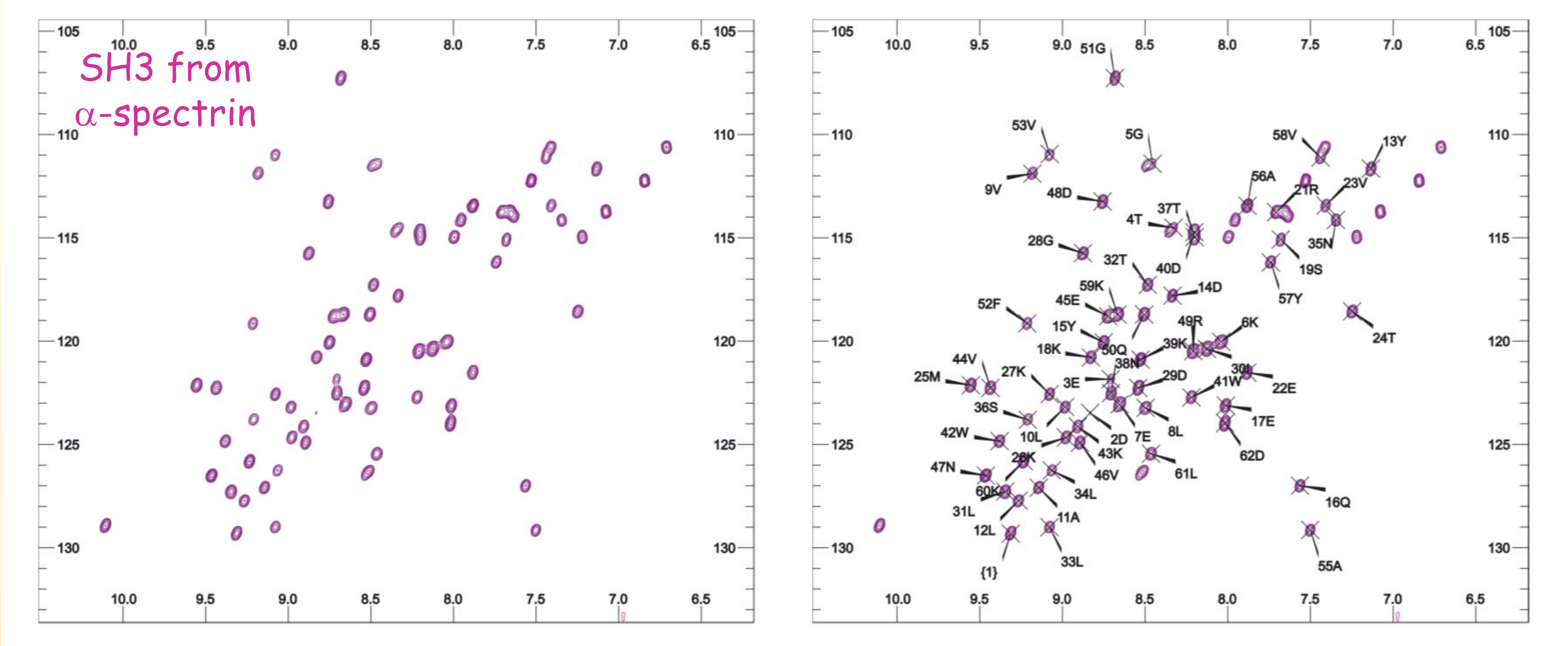


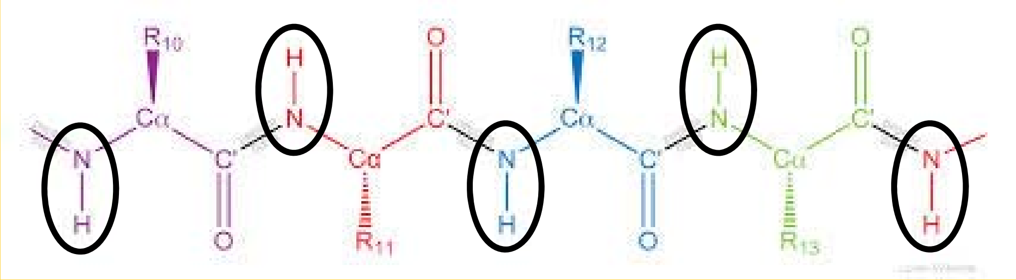
# understanding NMR: HSQC





# The result is the starting point for protein NMR.





# Thank You!