NMR course at the FMP:
NMR of organic compounds and small biomolecules
- III-

23.03.2009

Peter Schmieder
AG Solution NMR

The program

The product operator formalism

(the "PROF")

Basic principles

Building blocks

Two-dimensional NMR: The COSY

Peter Schmieder
AG Solution NMR

The
produkt operator formalism
(The "PROF")

Peter Schmieder
AG Solution NMR

The „PROF"

The „PROF" is a quantummechanical description of NMR-experiments based on the density matrix formalism

In the same way as quantum mechanics it can be introduced in an axiomatic way and does then consist of a set of rules

O.W. Sørensen et al.

Prog. NMR. Spectrosc. **16**, 163-192 (1983)

The „PROF"

When using those rules the mathemetics mainly consists of simple trigonometry and addition/subtraction. It is far more important to keep track of the calculations in order not make trivial mistakes.

trigonometric formula (1)

$$\cos^2\alpha + \sin^2\alpha = 1$$

$$\sin^2\alpha = 2 \sin\alpha \cos\alpha$$

$$\cos^2\alpha = \cos2\alpha - \sin2\alpha$$

$$\exp \pm i\alpha = \cos \alpha \pm i \sin \alpha$$

Peter Schmieder
AG Solution NMR

The „PROF"

$$\sin(\alpha+\beta) = \sin\alpha \cos\beta + \cos\alpha \sin\beta$$

$$\sin(\alpha-\beta) = \sin\alpha \cos\beta - \cos\alpha \sin\beta$$

$$\cos(\alpha+\beta) = \cos\alpha \cos\beta - \sin\alpha \sin\beta$$

$$\cos(\alpha-\beta) = \cos\alpha \cos\beta + \sin\alpha \sin\beta$$

trigonometric formula (2)

$$\sin\alpha \cos\beta = \tfrac{1}{2} [\sin(\alpha+\beta) + \sin(\alpha-\beta)]$$

$$\cos\alpha \sin\beta = \tfrac{1}{2} [\sin(\alpha+\beta) - \sin(\alpha-\beta)]$$

$$\cos\alpha \cos\beta = \tfrac{1}{2} [\cos(\alpha+\beta) + \cos(\alpha-\beta)]$$

$$\sin\alpha \sin\beta = \tfrac{1}{2} [\cos(\alpha-\beta) - \cos(\alpha+\beta)]$$

The „PROF"

In most calculations product operators are expressed in catesian coordinates

At the beginning of an experiment we have H_z

H_z

Type of nucleus (here hydrogen, in more general equations I or S)

direction (here cartesian coordinates)

Peter Schmieder
AG Solution NMR

The „PROF"

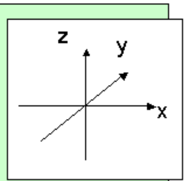
x,y-magnetization is then expressed as H_x or H_y or as C_x and C_y

During a calculation operators of chemical shift or coupling act on the operators the represent magnetization. According to certain rules other operators are thus created.

$$A \xrightarrow{\beta B} C \cos\beta + D \sin\beta$$

The „PROF“

$$I_z \xrightarrow{\beta I_x} I_z \cos\beta - I_y \sin\beta$$
$$I_z \xrightarrow{\beta I_y} I_z \cos\beta + I_x \sin\beta$$
$$I_x \xrightarrow{\beta I_x} I_x$$
$$I_x \xrightarrow{\beta I_y} I_x \cos\beta - I_z \sin\beta$$
$$I_y \xrightarrow{\beta I_x} I_y \cos\beta + I_z \sin\beta$$
$$I_y \xrightarrow{\beta I_y} I_y$$
$$I_z \xrightarrow{90° I_x} -I_y \qquad I_z \xrightarrow{90° I_y} I_x$$
$$I_x \xrightarrow{90° I_y} -I_z \qquad I_y \xrightarrow{90° I_x} I_z$$



$$I_x \xrightarrow{I_z \Omega\tau} I_x \cos\Omega\tau + I_y \sin\Omega\tau = I_x \cos 2\pi\delta\tau + I_y \sin 2\pi\delta\tau$$
$$I_y \xrightarrow{I_z \Omega\tau} I_y \cos\Omega\tau - I_x \sin\Omega\tau = I_x \cos 2\pi\delta\tau + I_y \sin 2\pi\delta\tau$$
$$I_z \xrightarrow{I_z \Omega\tau} I_z$$

$$I_{1x} \xrightarrow{I_{1z} I_{2z} \pi J_{12}\tau} I_{1x} \cos\pi J_{12}\tau + 2I_{1y} I_{2z} \sin \pi J_{12}\tau$$
$$I_{1y} \xrightarrow{I_{1z} I_{2z} \pi J_{12}\tau} I_{1y} \cos\pi J_{12}\tau - 2I_{1x} I_{2z} \sin\pi J_{12}\tau$$
$$2I_{1x} I_{2z} \xrightarrow{I_{1z} I_{2z} \pi J_{12}\tau} 2I_{1x} I_{2z} \cos\pi J_{12}\tau + I_{1y} \sin\pi J_{12}\tau$$
$$2I_{1y} I_{2z} \xrightarrow{I_{1z} I_{2z} \pi J_{12}\tau} 2I_{1y} I_{2z} \cos\pi J_{12}\tau - I_{1x} \sin\pi J_{12}\tau$$

$$\cos 2\alpha + \sin 2\alpha = 1$$
$$\sin 2\alpha = 2 \sin \alpha \cos \alpha$$
$$\cos 2\alpha = \cos 2\alpha - \sin 2\alpha$$
$$\exp \pm i\alpha = \cos \alpha \pm i \sin a$$

$$\sin(\alpha + \beta) = \sin \alpha \cos \beta + \cos \alpha \sin \beta$$
$$\sin(\alpha - \beta) = \sin \alpha \cos \beta - \cos \alpha \sin \beta$$
$$\cos(\alpha + \beta) = \cos \alpha \cos \beta - \sin \alpha \sin \beta$$
$$\cos(\alpha - \beta) = \cos \alpha \cos \beta + \sin \alpha \sin \beta$$

$$\sin \alpha \cos \beta = \tfrac{1}{2} [\sin(\alpha + \beta) + \sin(\alpha - \beta)]$$
$$\cos \alpha \sin \beta = \tfrac{1}{2} [\sin(\alpha + \beta) - \sin(\alpha - \beta)]$$
$$\cos \alpha \cos \beta = \tfrac{1}{2} [\cos(\alpha + \beta) + \cos(\alpha - \beta)]$$
$$\sin \alpha \sin \beta = \tfrac{1}{2} [\cos(\alpha - \beta) - \cos(\alpha + \beta)]$$

http://www.fmp-berlin.de/schmieder/teaching/vorlesung_nmr/pdf/rechenhilfen_produktoperatoren.pdf

Peter Schmieder
AG Solution NMR

The „PROF"

The name of the operators is derived from the type of magnetization they represent

H_z = longitudinal magnetization

H_x, H_y = in-phase magnetization

$H_{1x}H_{2z}$ = anti-phase magnetization

$H_{1x}H_{2y}$ = multiple quantum magnetization

} transverse magnetization

Beside those operators based on cartesian coordinates there are others which are not of interest for now

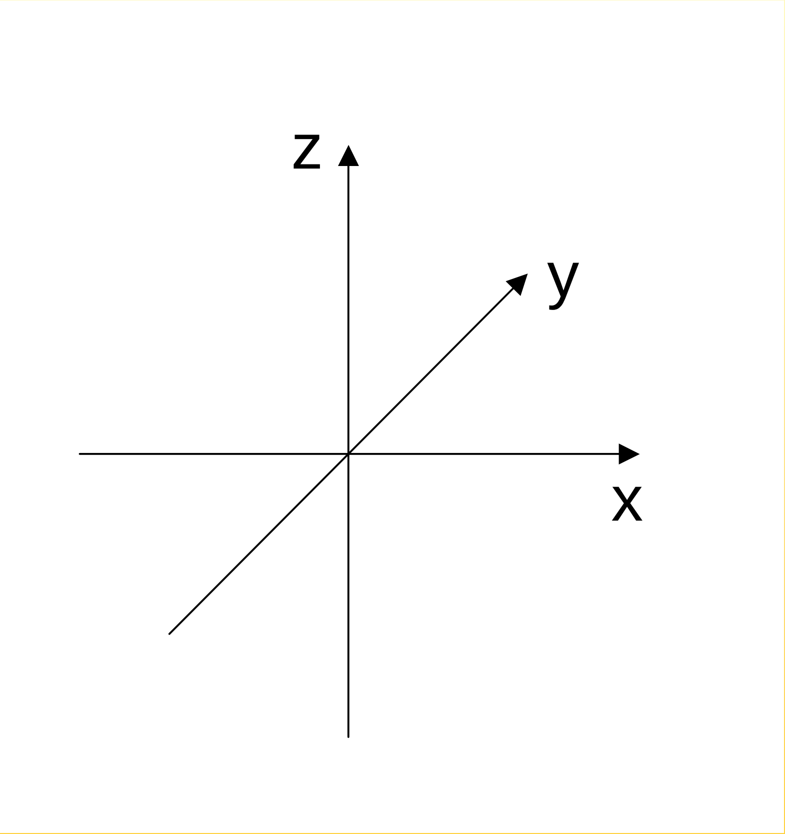
The „PROF“

Chemical shift and J-coupling (as long as it is weak coupling) can be calculated independently and in an arbitrary order.

That makes the calculation of „building blocks“ possible: the effect of these building blocks is then known and does not have to be re-calculated in more complex experiments

Peter Schmieder
AG Solution NMR

The „PROF"



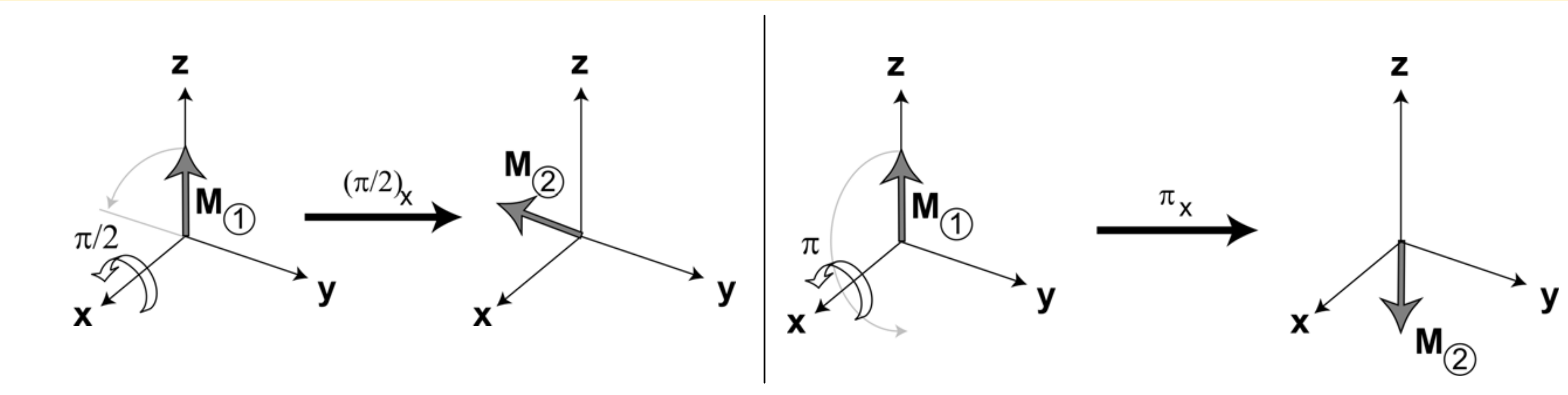
We use a „right-handed" coordinate system

The „PROF"

RF(radio-frequency)-pulses: the „x-Puls"

Remember:

Using the vector model 90° and 180° pulses hitting z-magnetization were described like this



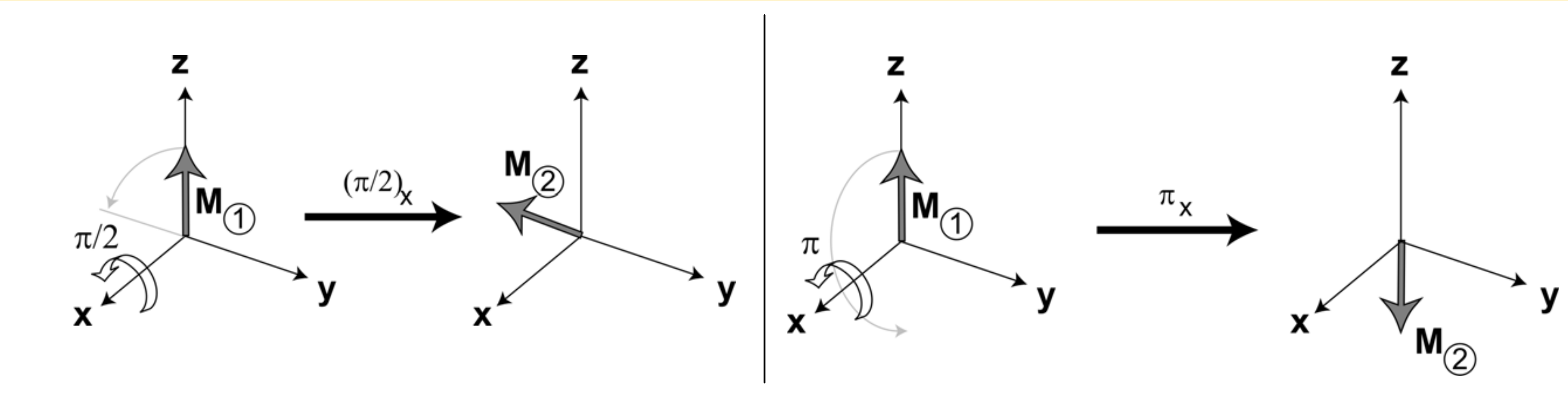
The „PROF"

The rule for the x-pulse looks like that

$$I_z \xrightarrow{\beta I_x} I_z \cos\beta - I_y \sin\beta$$

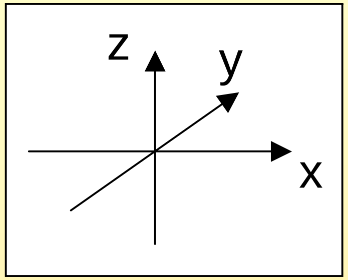
β = 90°: cos β = 0, sin β = 1, the result is $-I_y$

β = 180°: cos β = -1, sin β = 0, the result ist $-I_z$



Peter Schmieder
AG Solution NMR

The „PROF"



Rules for pulses from other directions are then easily derived

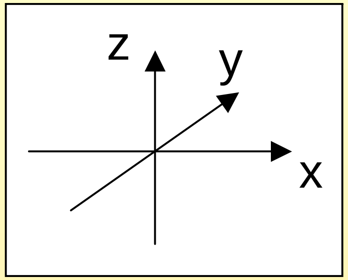
$$I_z \xrightarrow{\beta I_x} I_z \cos\beta - I_y \sin\beta$$

$$I_z \xrightarrow{\beta I_y} I_z \cos\beta + I_x \sin\beta$$

$$I_z \xrightarrow{\beta I_{-x}} I_z \cos\beta + I_y \sin\beta$$

$$I_z \xrightarrow{\beta I_{-y}} I_z \cos\beta - I_x \sin\beta$$

The „PROF"



Pulses do also act on transverse magnetization but not if both point in the same direction

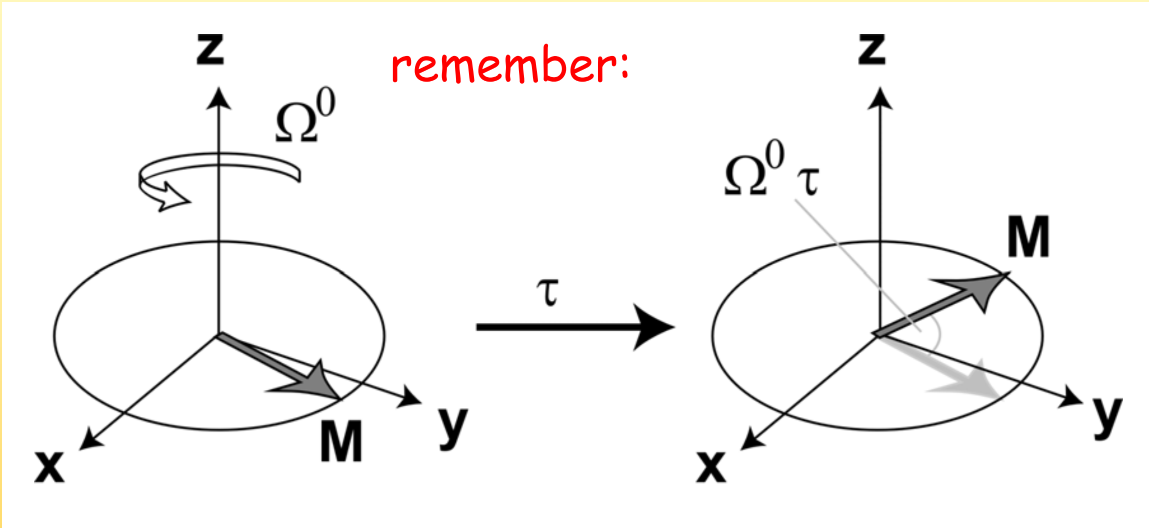
$$I_x \xrightarrow{\beta I_y} I_x \cos\beta - I_z \sin\beta$$

$$I_y \xrightarrow{\beta I_x} I_y \cos\beta + I_z \sin\beta$$

$$\left.\begin{array}{l} I_x \xrightarrow{\beta I_x} I_x \\ I_y \xrightarrow{\beta I_y} I_y \end{array}\right\}$$ no effect !!

The „PROF"

chemical shift:



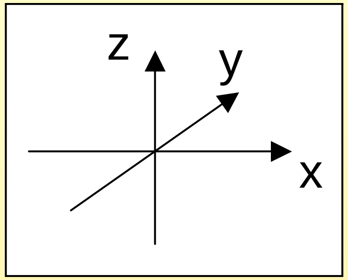
chemical shift Ω^0 acts for a time τ

$$I_x \xrightarrow{I_z\Omega^0\tau} I_x \cos \Omega^0\tau + I_y \sin \Omega^0\tau = I_x \cos 2\pi\delta\tau + I_y \sin 2\pi\delta\tau$$

angular frequency Ω^0 „normal" frequency δ (in hertz)

Peter Schmieder
AG Solution NMR

The „PROF"



Chemical shift acts only on transverse magnetization, I_z remains untouched,

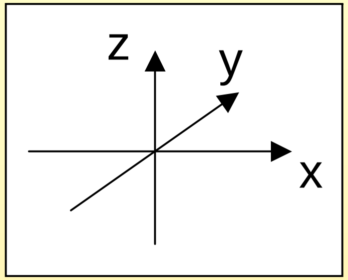
That is why it is sometimes called „z-pulses"

$$I_x \xrightarrow{I_z \Omega \tau} I_x \cos\Omega\tau + I_y \sin\Omega\tau$$

$$I_y \xrightarrow{I_z \Omega \tau} I_y \cos\Omega\tau - I_x \sin\Omega\tau$$

$$I_z \xrightarrow{I_z \Omega \tau} I_z$$

The „PROF"



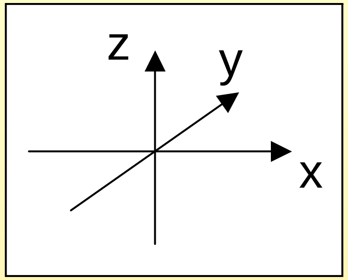
Scalar coupling leads to operators in which several operators are multiplied with each other: product operators

$$I_{1x} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} I_{1x}\cos\pi J_{12}\tau + 2I_{1y}I_{2z}\sin\pi J_{12}\tau$$

in-phase magnetization:
This part stays untouched

anti-phase magnetization:
coupling causes an modulation of transverse magnetization due to the coupling partner

The „PROF"



Scalar coupling acts on various kinds of transverse magnetization....

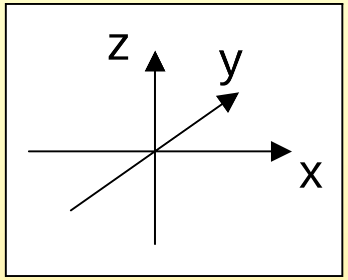
$$I_{1x} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} I_{1x}\cos\pi J_{12}\tau + 2I_{1y}I_{2z}\sin\pi J_{12}\tau$$

$$I_{1y} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} I_{1y}\cos\pi J_{12}\tau - 2I_{1x}I_{2z}\sin\pi J_{12}\tau$$

$$2I_{1x}I_{2z} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} 2I_{1x}I_{2z}\cos\pi J_{12}\tau + I_{1y}\sin\pi J_{12}\tau$$

$$2I_{1y}I_{2z} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} 2I_{1y}I_{2z}\cos\pi J_{12}\tau - I_{1x}\sin\pi J_{12}\tau$$

The „PROF"



... but not on longitudinal magnetization or on multiple quantum magnetization

$$I_{1z} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} I_{1z}$$

$$2I_{1x}I_{2y} \xrightarrow{I_{1z}I_{2z}\pi J_{12}\tau} 2I_{1x}I_{2y}$$

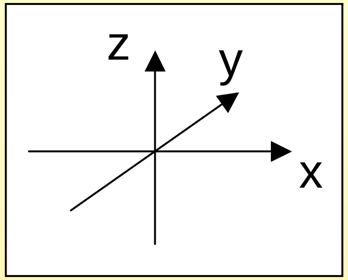
$$2I_{1x}I_{2y} \xrightarrow{I_{1z}I_{3z}\pi J_{13}\tau}$$

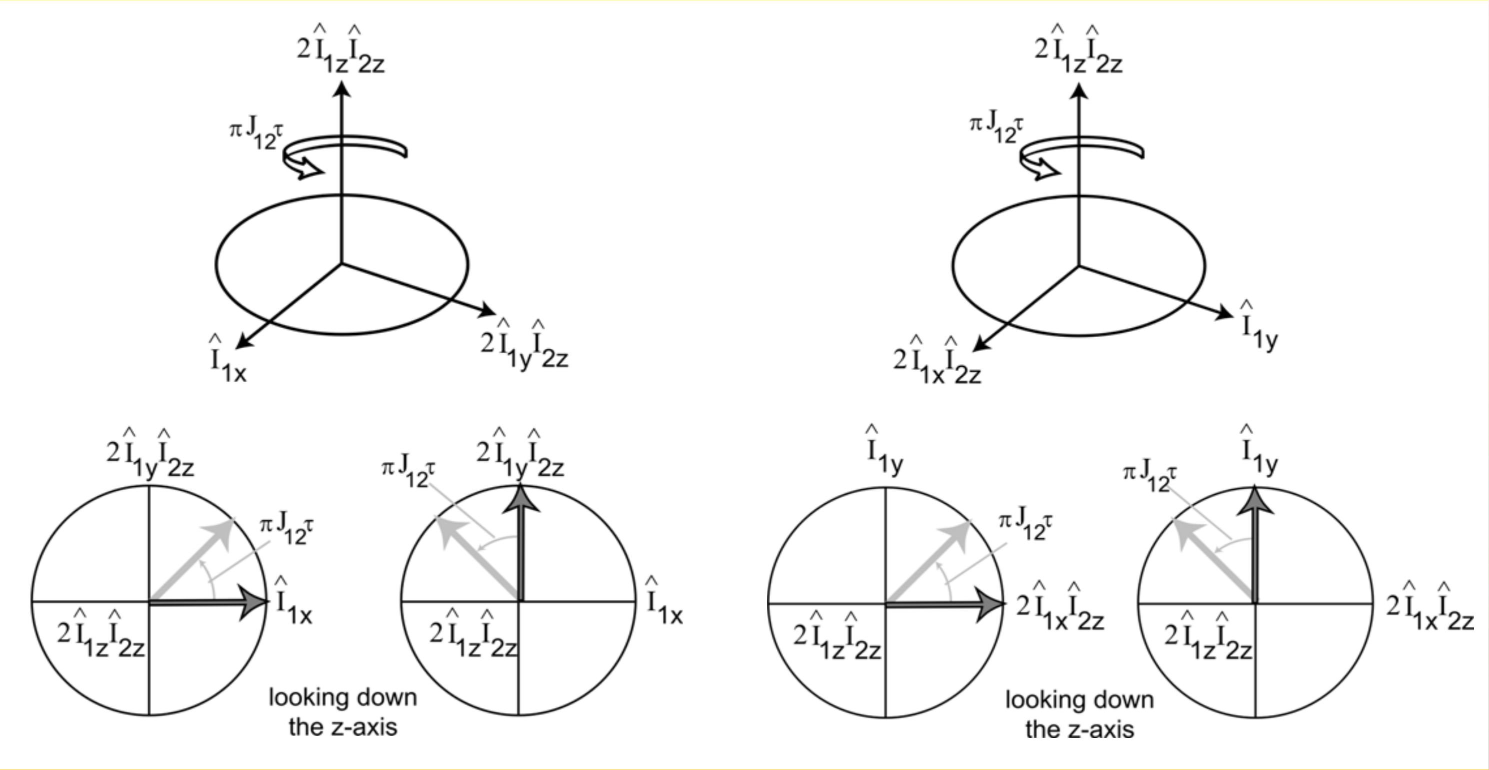
$$2I_{1x}I_{2y}\cos\pi J_{12}\tau + 2I_{1y}I_{2y}I_{3z}\sin\pi J_{12}\tau$$

Peter Schmieder
AG Solution NMR

The „PROF"

The evolution due to scalar coupling can be visiualized with cartesian coordinates

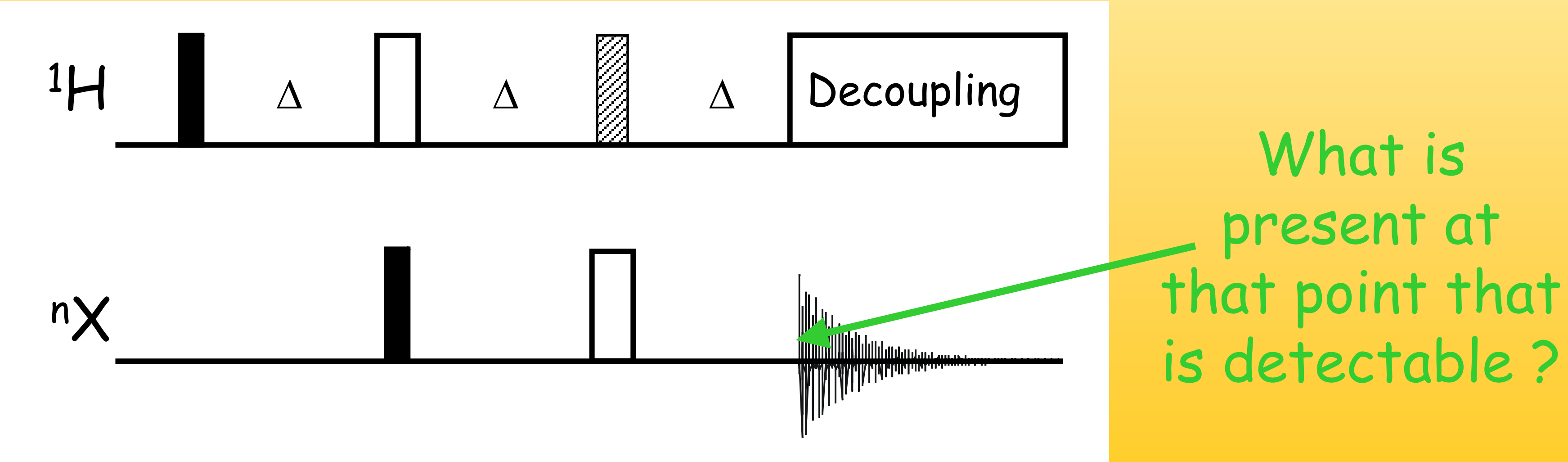




Peter Schmieder
AG Solution NMR

The „PROF“

Aim of the calculation is to derive what happens during a pulse sequence. Then we must ask what types of product operators lead to detectable signals during the acquisition



Peter Schmieder
AG Solution NMR

The „PROF“

Not detectable are longitudinal magentization (I_{1z}) or multiple quantum magnetization (z.B. $I_{1x}I_{2y}$) since the usual selection rules are still valid

Only transverse in-phase (I_{1x}) magnetization is detectable, anti-phase magnetization (e.g. $I_{1x}I_{2z}$) can evolve into something detectable during the acquisition time

The „PROF"

Let us now calculate what results from „in-phase" magnetization during the acquistion time under the influence of chemical shift and scalar coupling ?

$$I_{1x} \xrightarrow{I_z \Omega_1 t_{aq}} I_{1x} \cos\Omega_1 t_{aq} + I_{1y} \sin \Omega_1 t_{aq}$$

$$\xrightarrow{I_{1z} I_{2z} \pi J_{12} t_{aq}} I_{1x} \cos\Omega_1 t_{aq} \cos\pi J_{12} t_{aq} + \cancel{2 I_{1y} I_{2z}} \cos\Omega_1 t_{aq} \sin\pi J_{12} t_{aq}$$

$$+ I_{1y} \sin\Omega_1 t_{aq} \cos\pi J_{12} t_{aq} - \cancel{2 I_{1x} I_{2z}} \sin\Omega_1 t_{aq} \sin\pi J_{12} t_{aq}$$

$$= I_{1x} \cos\Omega_1 t_{aq} \cos\pi J_{12} t_{aq} + I_{1y} \sin\Omega_1 t_{aq} \cos\pi J_{12} t_{aq}$$

Peter Schmieder
AG Solution NMR

The „PROF“

We use $\Omega_1 = 2\pi\delta_1$ and we get (using our trigonometiric formulas)

$$I_{1x}\ \tfrac{1}{2}[\cos 2\pi(\delta_1 + J_{12}/2)t_{aq} + \cos 2\pi(\delta_1 - J_{12}/2)t_{aq}]$$

$$+\ I_{1y}\ \tfrac{1}{2}[\sin 2\pi(\delta_1 + J_{12}/2)t_{aq} + \sin 2\pi(\delta_1 - J_{12}/2)t_{aq}]$$ Imaginärteil !

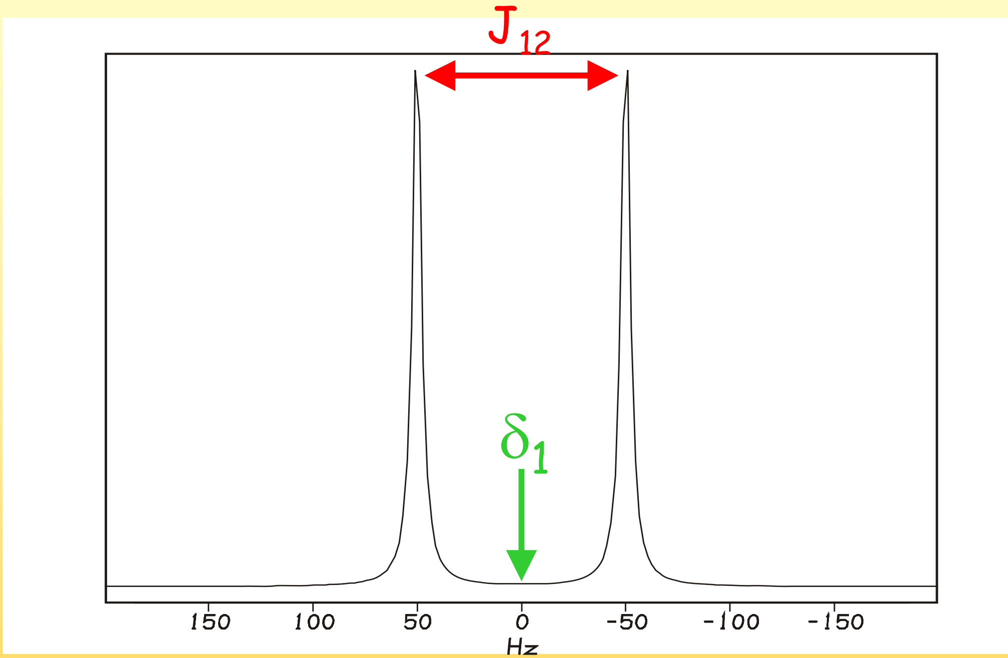
$$= I_1\ \tfrac{1}{2}[\exp i\,2\pi(\delta_1 + J_{12}/2)t_{aq} + \exp i\,2\pi(\delta_1 - J_{12}/2)t_{aq}]$$

„in-phase“ magnetization results in two lines with the same sign, seperated by J Hz and centered around δ_1 Hz

Peter Schmieder
AG Solution NMR

The „PROF"

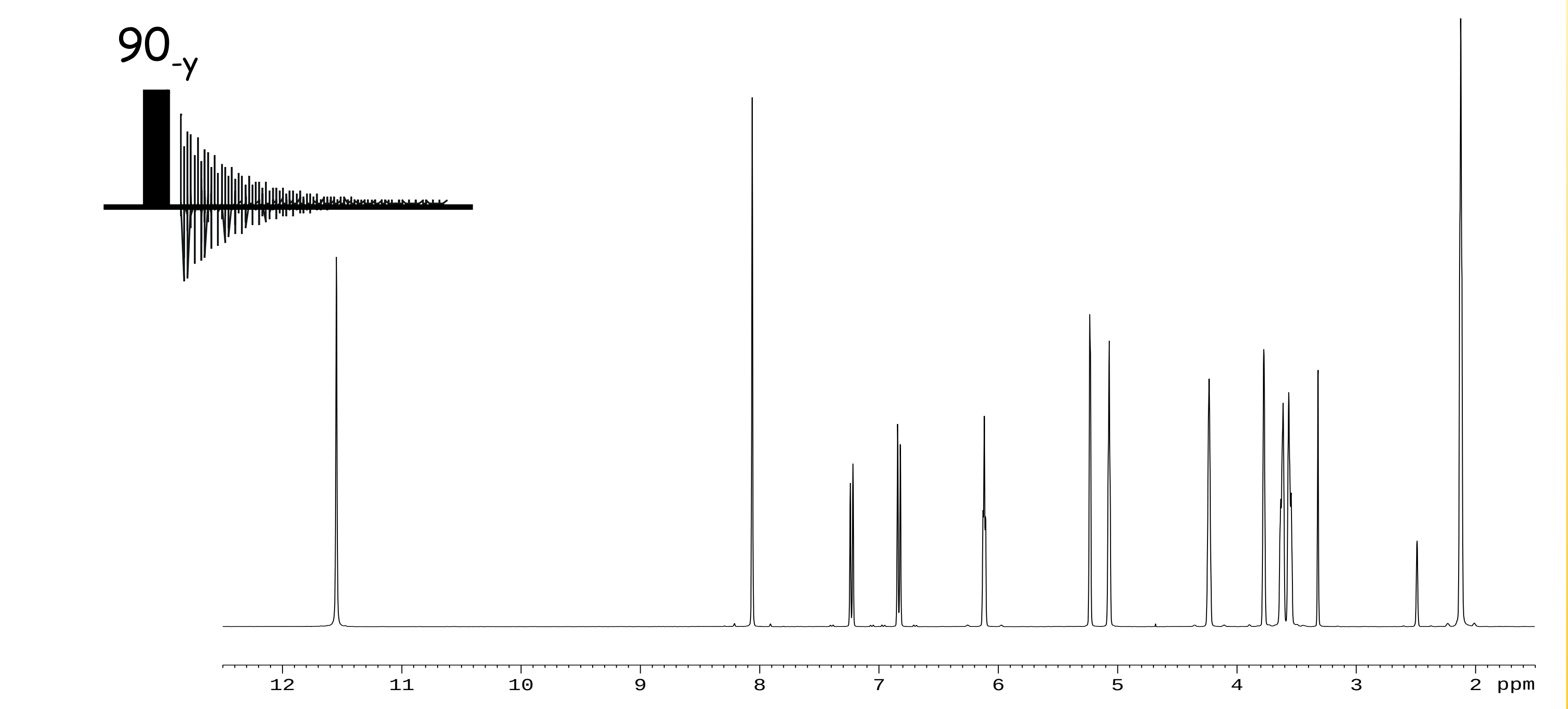
$I_{1x} \equiv$



Hence the name „in-phase" magnetization, since the result is an in-phase doublett

The „PROF"

This is what we get in a conventional 1D-NMR-experiment



The „PROF"

How about „anti-phase" magnetization ?

$$2I_{1x}I_{2z} \xrightarrow{I_z\Omega_1 t_{aq}} 2I_{1x}I_{2z}\cos\Omega_1 t_{aq} + 2I_{1y}I_{2z}\sin\Omega_1 t_{aq}$$

$$\xrightarrow{I_{1z}I_{2z}\pi J_{12}t_{aq}} 2I_{1x}I_{2z}\cos\Omega_1 t_{aq}\cos\pi J_{12}t_{aq} + I_{1y}\cos\Omega_1 t_{aq}\sin\pi J_{12}t_{aq}$$

$$+ 2I_{1y}I_{2z}\sin\Omega_1 t_{aq}\cos\pi J_{12}t_{aq} - I_{1x}\sin\Omega_1 t_{aq}\sin\pi J_{12}t_{aq}$$

$$= - I_{1x}\sin\Omega_1 t_{aq}\sin\pi J_{12}t_{aq} + I_{1y}\cos\Omega_1 t_{aq}\sin\pi J_{12}t_{aq}$$

The „PROF"

Again we use $\Omega_1 = 2\pi\delta_1$ and get

$$I_{1x}\ \tfrac{1}{2}[\cos 2\pi(\delta_1 + J_{12}/2)t_{aq} - \cos 2\pi\ (\delta_1 - J_{12}/2)t_{aq}]$$

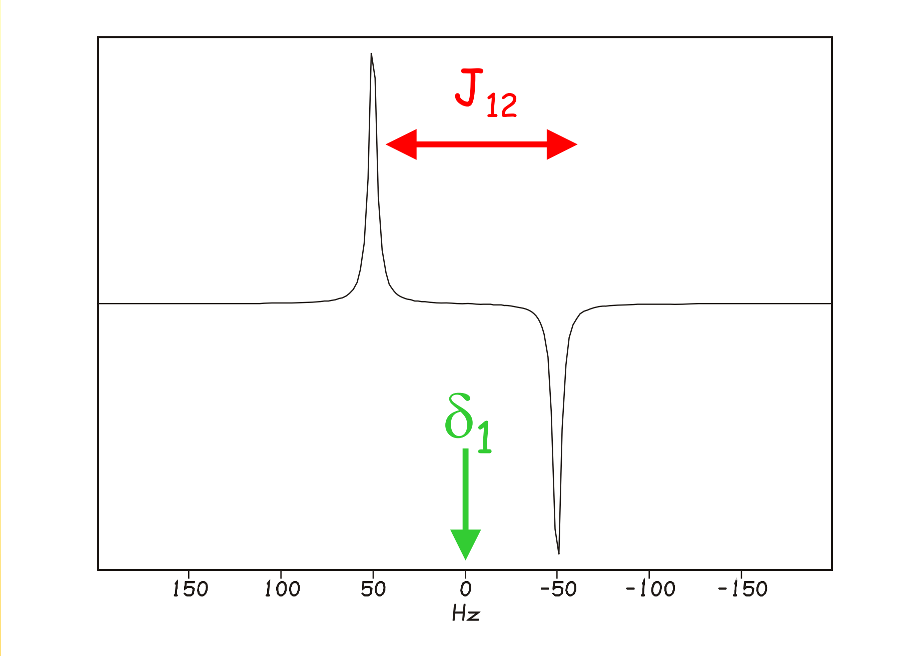
$$+\ I_{1y}\ \tfrac{1}{2}[\sin 2\pi(\delta_1 + J_{12}/2)t_{aq} - \sin 2\pi\ (\delta_1 - J_{12}/2)t_{aq}]$$ Imaginärteil !

$$= I_1\ \tfrac{1}{2}[\exp i\,2\pi(\delta_1 + J_{12}/2)t_{aq} - \exp 2i\,\pi(\delta_1 - J_{12}/2)t_{aq}]$$

„anti-phase" magnetization thus results in two lines with oposite sign, seperated by J Hz and centered around δ_1 Hz

The „PROF"

$$2I_{1x}I_{2z} \equiv$$



Hence the name „anti-phase" magnetization, since we obtain an anti-phase doublet

To create that type of magnetization we need a bit more then just one pulse !!

Peter Schmieder
AG Solution NMR

The „PROF“

a first summary

Produkt operators are used for a convinient calculation of complex pulse sequences

Usually the calculation is performed up to the end of the pulse sequence right before the acquistion.

The detectable operators that are present at that point give rise to well know signals during detection.

The calculation thus describes the result of the pulse sequence

Peter Schmieder
AG Solution NMR

The „PROF“

Since the calculation can get confusing quite quickly follow those rules

rule 1: calculate carfully, avoid sign or writing mistakes

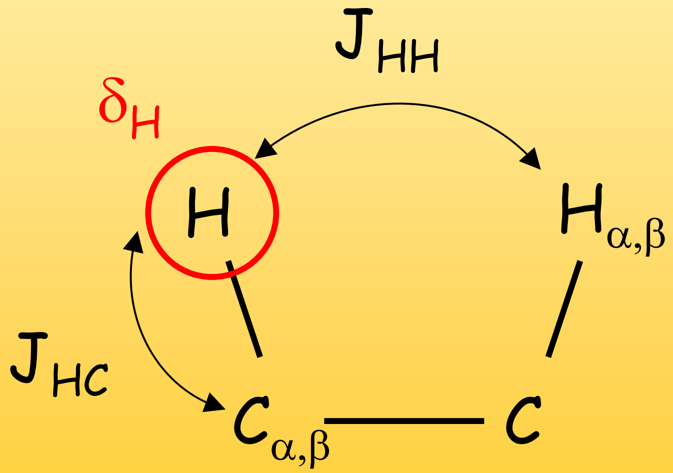
rule 2: always try to calculate seperable interactions seperately, i.e. chemical shift seperatly from scalar coupling

rule 3: do not re-caclulate “building blocks“

Building blocks

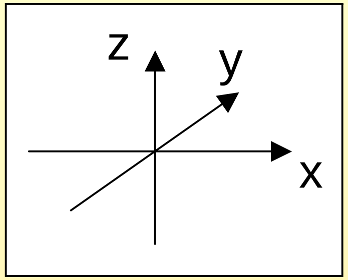
Building blocks

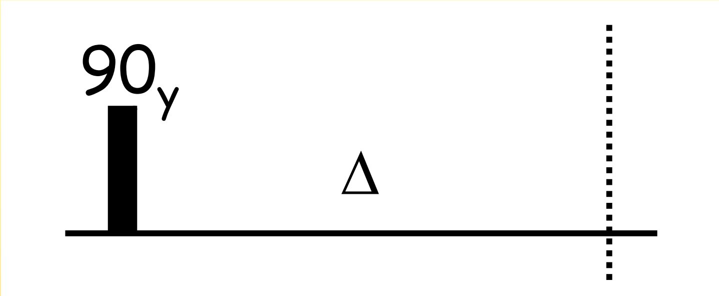
Now we know what kind of signals result from the detectable types of magnetization, now we can start to calculate "building blocks"



Peter Schmieder
AG Solution NMR

Building blocks





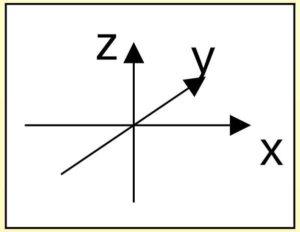
chemical shift: δ_H

$$H_x \xrightarrow{\delta_H \Delta} H_x \cos \Omega_H \Delta + H_y \sin \Omega_H \Delta$$

$$\xrightarrow{\delta_H t_{aq}} H_x \cos \Omega_H \Delta \cos \Omega_H t_{aq} + H_y \cos \Omega_H \Delta \sin \Omega_H t_{aq}$$

$$H_y \sin \Omega_H \Delta \cos \Omega_H t_{aq} - H_x \sin \Omega_H \Delta \sin \Omega_H t_{aq}$$

Building blocks



$$H_x \cos \Omega_H \Delta \cos \Omega_H t_{aq} + H_y \cos \Omega_H \Delta \sin \Omega_H t_{aq}$$

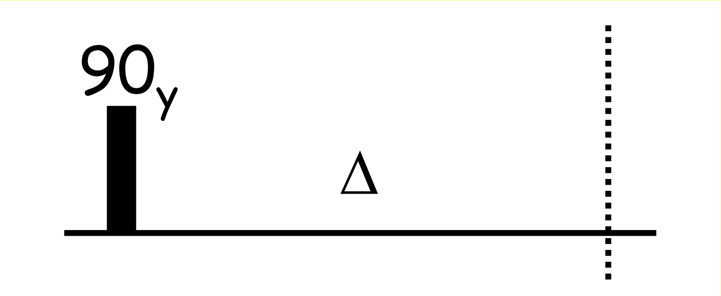
$$H_y \sin \Omega_H \Delta \cos \Omega_H t_{aq} - H_x \sin \Omega_H \Delta \sin \Omega_H t_{aq}$$

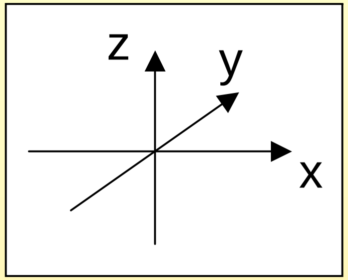
$$= H_x \cos \Omega_H(\Delta + t_{aq}) + H_y \sin \Omega_H(\Delta + t_{aq})$$

$$= H \exp i \; \Omega_H(\Delta + t_{aq}) = H \exp i(\Omega_H \Delta) \exp i(\Omega_H t_{aq})$$

Obviously all signals acquire a phase that is dependent on the chemical shift, no phase correction is thus possible in a simple 1D spectrum

Building blocks





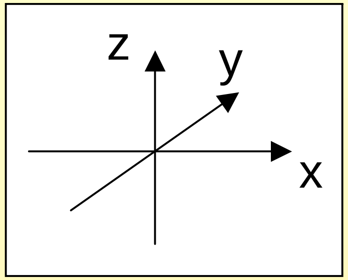
This is solved by keeping Δ short.

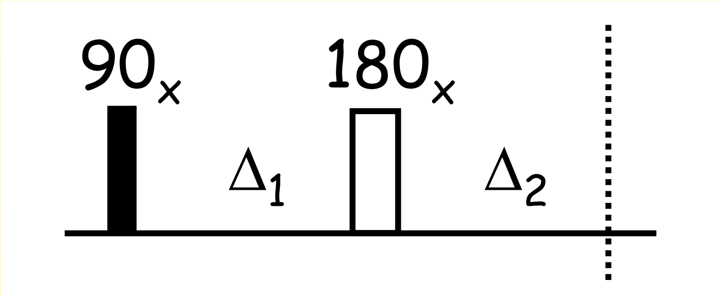
$\exp(\Omega_H\Delta) = 1 + \Omega_H\Delta$ for small values of $\Omega_H\Delta$

We see that in this case a linear phase correction (also called first order phase correction) is sufficient.

Peter Schmieder
AG Solution NMR

Building blocks

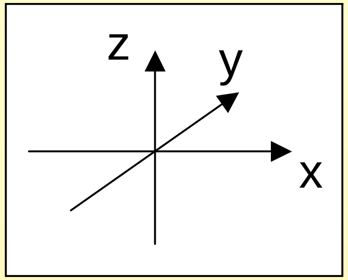


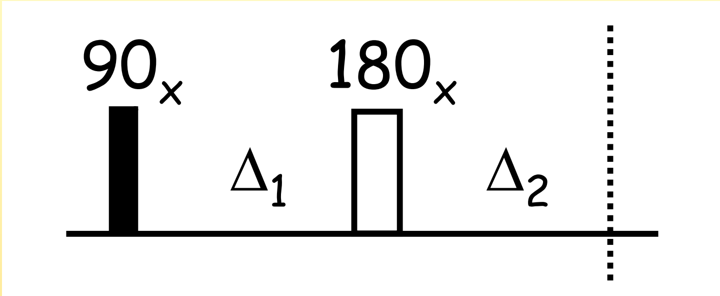


This sequence has already been inspected using the vector model, the result should be the same:

No chemical shift, homonuclear but no heteronuclear scalar coupling

Building blocks





First we do the calculation for chemical shift δ_H

$$H_z \xrightarrow{90^\circ H_x} -H_y \xrightarrow{\delta_H\Delta_1} -H_y \cos \delta_H\Delta_1 + H_x \sin \delta_H\Delta_1$$

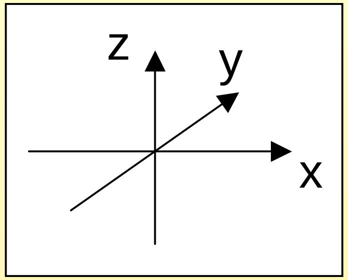
$$\xrightarrow{180^\circ H_x} H_y \cos \delta_H\Delta_1 + H_x \sin \delta_H\Delta_1$$

$$\xrightarrow{\delta_H\Delta_2} H_y \cos \delta_H\Delta_1 \cos \delta_H\Delta_2 - H_x \cos \delta_H\Delta_1 \sin \delta_H\Delta_2$$

$$H_x \sin \delta_H\Delta_1 \cos \delta_H\Delta_2 + H_y \sin \delta_H\Delta_1 \sin \delta_H\Delta_2$$

Peter Schmieder
AG Solution NMR

Building blocks



$H_y \cos \delta_H \Delta_1 \cos \delta_H \Delta_2 - H_x \cos \delta_H \Delta_1 \sin \delta_H \Delta_2$

$H_x \sin \delta_H \Delta_1 \cos \delta_H \Delta_2 + H_y \sin \delta_H \Delta_1 \sin \delta_H \Delta_2$

$= H_y \cos \delta_H (\Delta_1 - \Delta_2) + H_x \sin \delta_H (\Delta_1 - \Delta_2)$

if $\Delta_1 = \Delta_2 = \Delta$

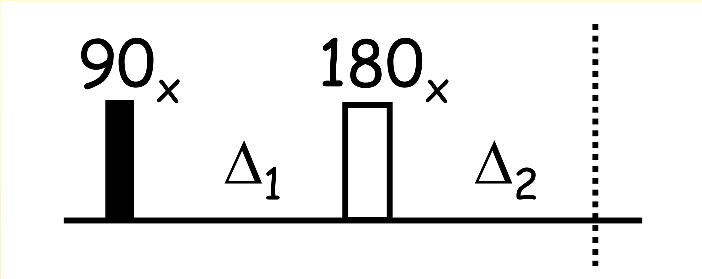
$= H_y$

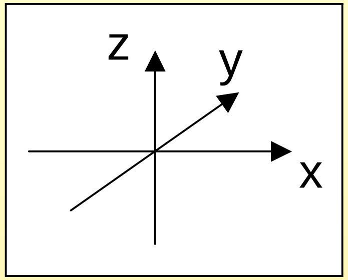
i.e. chemical shift has vanished in the end, it has been „refocussed“ !

(as we have seen in the vector model)

Peter Schmieder
AG Solution NMR

Building blocks





Now we calculate homonuclear coupling J_{HH}

$$H_{1z} \xrightarrow{90^\circ H_x} -H_{1y} \xrightarrow{\pi J_{HH}\Delta_1} -H_{1y} \cos \pi J_{HH}\Delta_1 + 2H_{1x} H_{2z} \sin \pi J_{HH}\Delta_1$$

$$\xrightarrow{180^\circ H_x} H_{1y} \cos \pi J_{HH}\Delta_1 - 2H_{1x} H_{2z} \sin \pi J_{HH}\Delta_1$$

$$\xrightarrow{\pi J_{HH}\Delta_2} H_{1y} \cos \pi J_{HH}\Delta_1 \cos \pi J_{HH}\Delta_2$$

$$- 2H_{1x} H_{2z} \cos \pi J_{HH}\Delta_1 \sin \pi J_{HH}\Delta_2$$

$$- 2H_{1x} H_{2z} \sin \pi J_{HH}\Delta_1 \cos \pi J_{HH}\Delta_2$$

$$- H_{1y} \sin \pi J_{HH}\Delta_1 \sin \pi J_{HH}\Delta_2$$

Peter Schmieder
AG Solution NMR

Building blocks

$$H_{1y} \cos \pi J_{HH}\Delta_1 \cos \pi J_{HH}\Delta_2 - 2H_{1x} H_{2z} \cos \pi J_{HH}\Delta_1 \sin \pi J_{HH}\Delta_2$$

$$-2H_{1x} H_{2z} \sin \pi J_{HH}\Delta_1 \cos \pi J_{HH}\Delta_2 - H_{1y} \sin \pi J_{HH}\Delta_1 \sin \pi J_{HH}\Delta_2$$

$$=$$

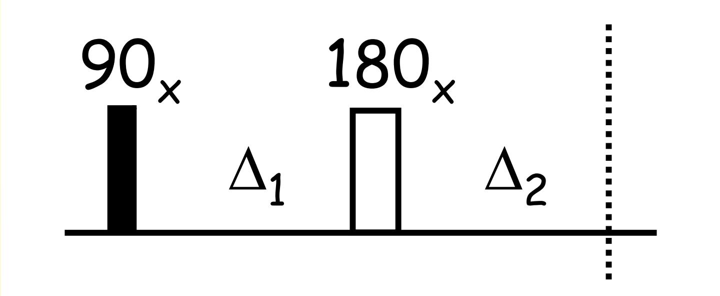
$$H_{1y} \cos \pi J_{HH} (\Delta_1 + \Delta_2) - 2H_{1x} H_{2z} \sin \pi J_{HH} (\Delta_1 + \Delta_2)$$

if $\Delta_1 = \Delta_2 = \Delta$

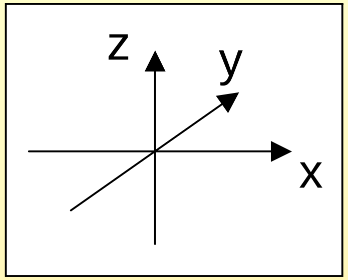
$$H_{1y} \cos \pi J_{HH}\, 2\Delta - 2H_{1x} H_{2z} \sin \pi J_{HH}\, 2\Delta$$

i.e. homonuclear coupling is still present at the end of the spin echo it has not been „refocussed"!

Building blocks



Finally heteronuclear coupling J_{HX}



$$H_z \xrightarrow{90^\circ H_x} -H_y \xrightarrow{\pi J_{HX}\Delta_1} -H_y \cos \pi J_{HX}\Delta_1 + 2H_x X_z \sin \pi J_{HX}\Delta_1$$

$$\xrightarrow{180^\circ H_x} H_y \cos \pi J_{HX}\Delta_1 + 2H_x X_z \sin \pi J_{HX}\Delta_1$$

$$\xrightarrow{\pi J_{HX}\Delta_2} H_y \cos \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2$$

$$- 2H_x X_z \cos \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$+ 2H_x H_z \sin \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2$$

$$+ H_y \sin \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

Building blocks

$$H_y \cos \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 - 2H_x X_z \cos \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$+ 2H_x H_z \sin \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 + H_y \sin \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$=$$

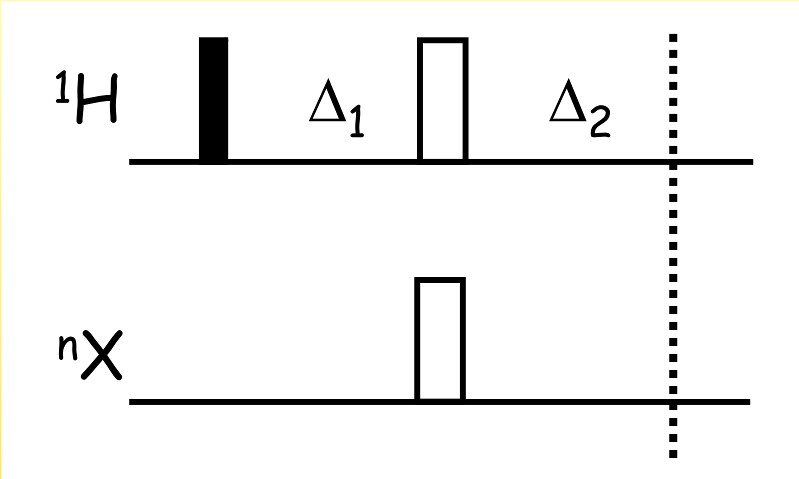
$$H_y \cos \pi J_{HX} (\Delta_1 - \Delta_2) - 2H_x X_z \sin \pi J_{HX} (\Delta_1 - \Delta_2)$$

if $\Delta_1 = \Delta_2 = \Delta$

$$H_y$$

i.e. heteronuclear coupling has vanished, it has been refocussed !

Building blocks



chemical shift: δ_H

scalar (J-) coupling: J_{HH}, J_{HX}

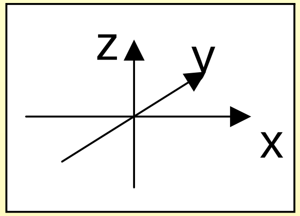
δ_H and J_{HH} are already known

δ_H refocussed if $\Delta_1 = \Delta_2$

J_{HH} not refocussed if $\Delta_1 = \Delta_2$

Peter Schmieder
AG Solution NMR

Building blocks



J_{HX} ?

$$H_z \xrightarrow{90^\circ H_x} -H_y \xrightarrow{\pi J_{HX}\Delta_1} -H_y \cos \pi J_{HX}\Delta_1 + 2H_x X_z \sin \pi J_{HX}\Delta_1$$

$$\xrightarrow[180^\circ X_x]{180^\circ H_x} H_y \cos \pi J_{HX}\Delta_1 - 2H_x X_z \sin \pi J_{HX}\Delta_1 \xrightarrow{\pi J_{HX}\Delta_2}$$

$$H_y \cos \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 - 2H_x X_z \cos \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$-2H_x H_z \sin \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 - H_y \sin \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$= H_y \cos \pi J_{HX}(\Delta_1 + \Delta_2) - 2H_x X_z \sin \pi J_{HX}(\Delta_1 + \Delta_2)$$

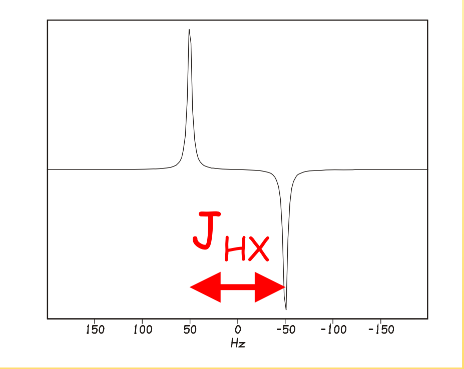
if $\Delta_1 = \Delta_2 = \Delta$: $H_{1y} \cos \pi J_{HX} 2\Delta - 2H_{1x} H_{2z} \sin \pi J_{HX} 2\Delta$

i.e. heteronuclear coupling is not refocussed any more !

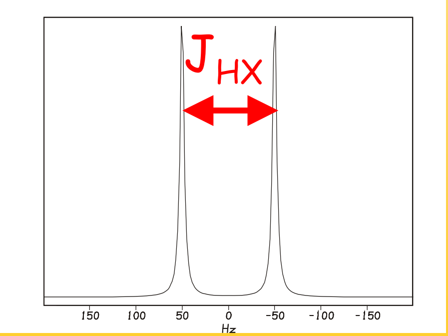
Building blocks

$$H_y \cos \pi J_{HX} 2\Delta - 2H_x X_z \sin \pi J_{HX} 2\Delta$$

If $\Delta = 1/4J_{HX}$ is choosen $2H_x X_z$ is obtained, i.e. an anti-phase signal, that is quite often utilized in complex pulse sequences



If $\Delta = 1/2J_{HX}$ is choosen H_{1y} is obtained, i.e. an in-phase signal



Building blocks

but…

$$H_y \cos \pi J_{HX} 2\Delta - 2H_x X_z \sin \pi J_{HX} 2\Delta$$

If Δ is set to a value short relative to 1/2J, then the coupling does hardly have an effect

$J = 5$ Hz, $2\Delta = 5$ msec << $1/2J = 100$ msec

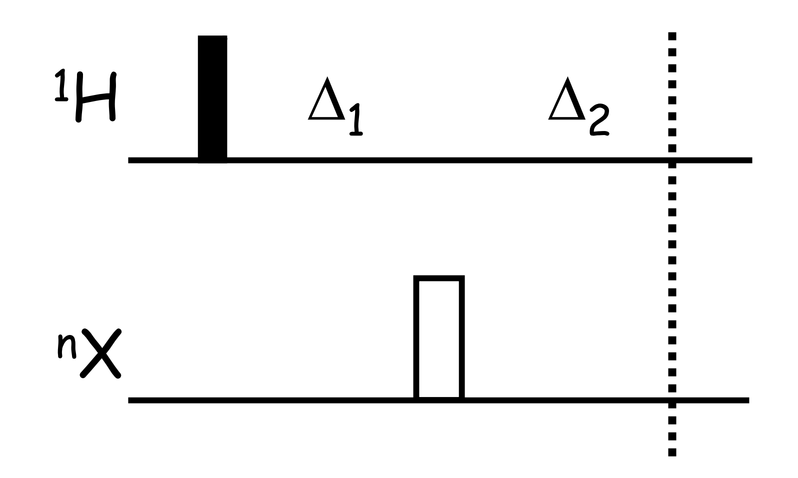
$\cos \pi J_{HX} 2\Delta = 0.99$

$\sin \pi J_{HX} 2\Delta = 0.08$

i.e. the coupling has not evolved noticably

Peter Schmieder
AG Solution NMR

Building blocks



chemical shift: δ_H

scalar (J-) coupling: J_{HH}, J_{HX}

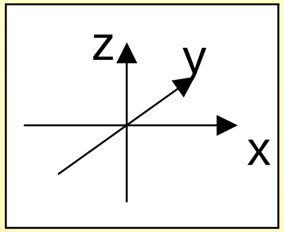
δ_H and J_{HH} are already known

δ_H not refocussed if $\Delta_1 = \Delta_2$

J_{HH} not refocussed if $\Delta_1 = \Delta_2$

Peter Schmieder
AG Solution NMR

Building blocks



J_{HX} ?

$$H_z \xrightarrow{90^\circ H_x} -H_y \xrightarrow{\pi J_{HX}\Delta_1} -H_y \cos \pi J_{HX}\Delta_1 + 2H_x X_z \sin \pi J_{HX}\Delta_1$$

$$\xrightarrow{180^\circ X_x} -H_y \cos \pi J_{HX}\Delta_1 - 2H_x X_z \sin \pi J_{HX}\Delta_1 \xrightarrow{\pi J_{HX}\Delta_2}$$

$$-H_y \cos \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 + 2H_x X_z \cos \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

$$-2H_x H_z \sin \pi J_{HX}\Delta_1 \cos \pi J_{HX}\Delta_2 - H_y \sin \pi J_{HX}\Delta_1 \sin \pi J_{HX}\Delta_2$$

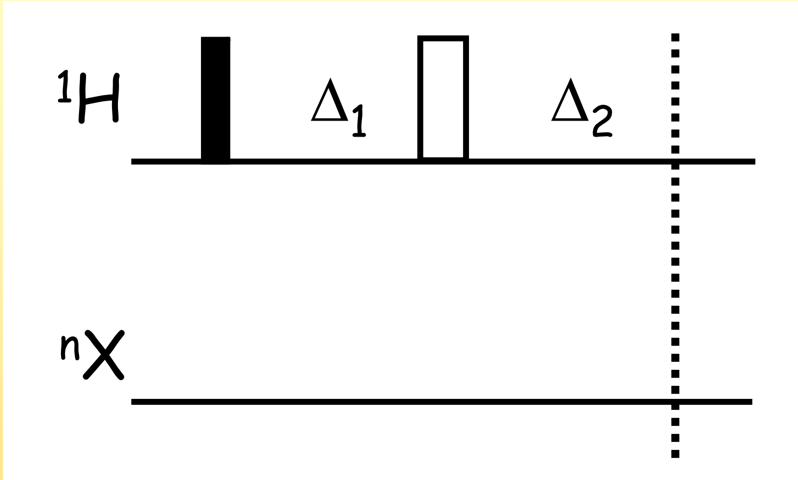
$$= -H_y \cos \pi J_{HX}(\Delta_1 - \Delta_2) - 2H_x X_z \sin \pi J_{HX}(\Delta_1 - \Delta_2)$$

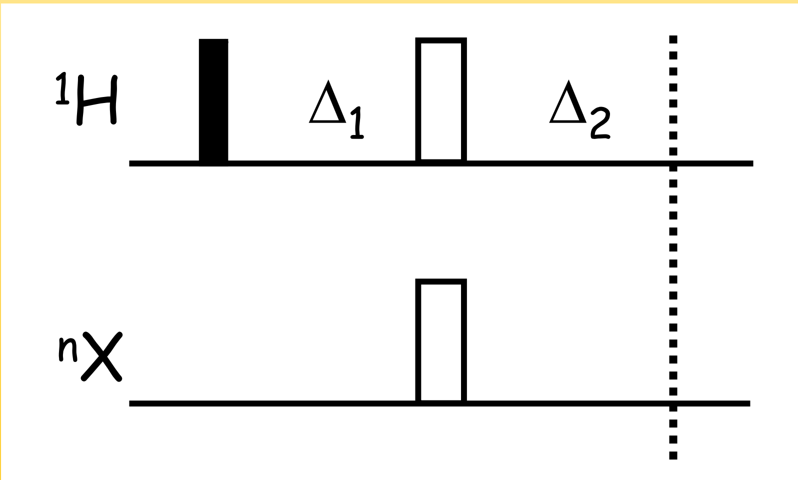
if $\Delta_1 = \Delta_2 = \Delta$: $-H_y$

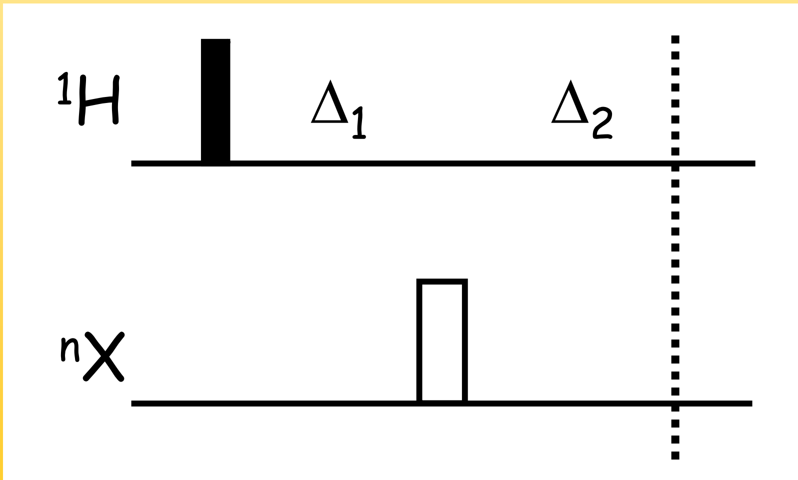
i.e. heteronucleare coupling is again refocussed !

Building blocks

Those „building blocks“ are now known and we do not need to recalculate them later on



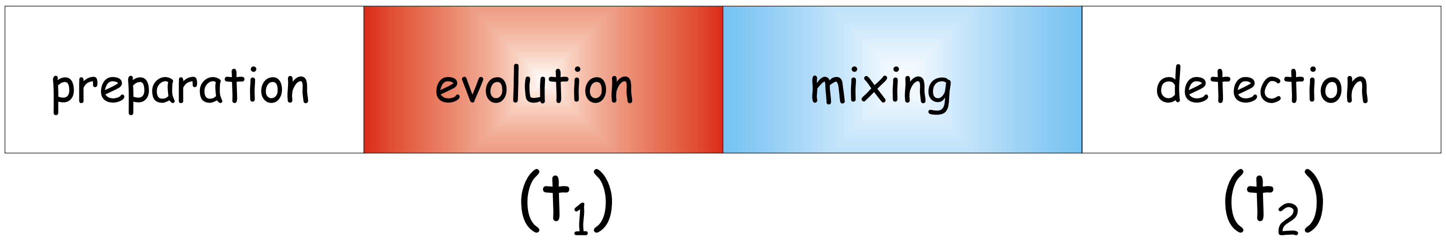




Two-dimensional NMR-spectroscopy: The COSY

2D NMR: COSY

2D-NMR sequences contain
two new elements:
evolution time and mixing time



evolution:
here a second time domain is created by indirect detection

mixing:
transfer of magentization from spin to spin via interactions between spins

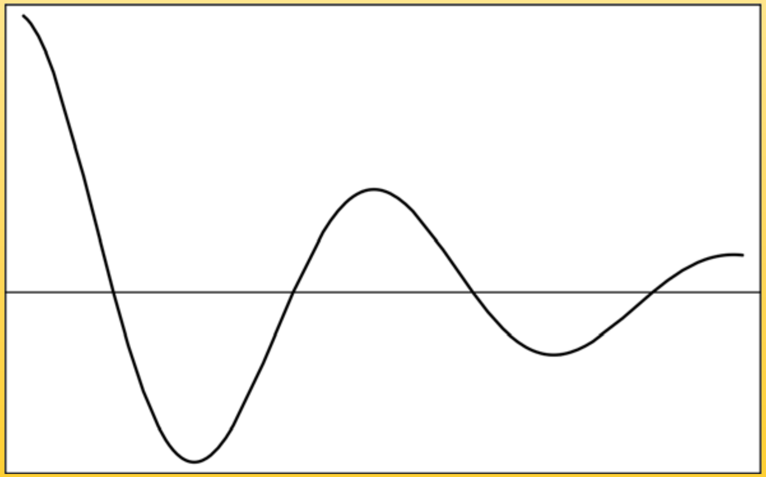
Peter Schmieder
AG Solution NMR

2D NMR: COSY

The NMR signale that is recorded during the acquisition is a damped cosine (we look only at the real part)

$$s(t)= \exp(-t/T_2)\exp(i\Omega_0 t)$$

$$s(t)= \exp((i\Omega_0 - 1/T_2)t)$$

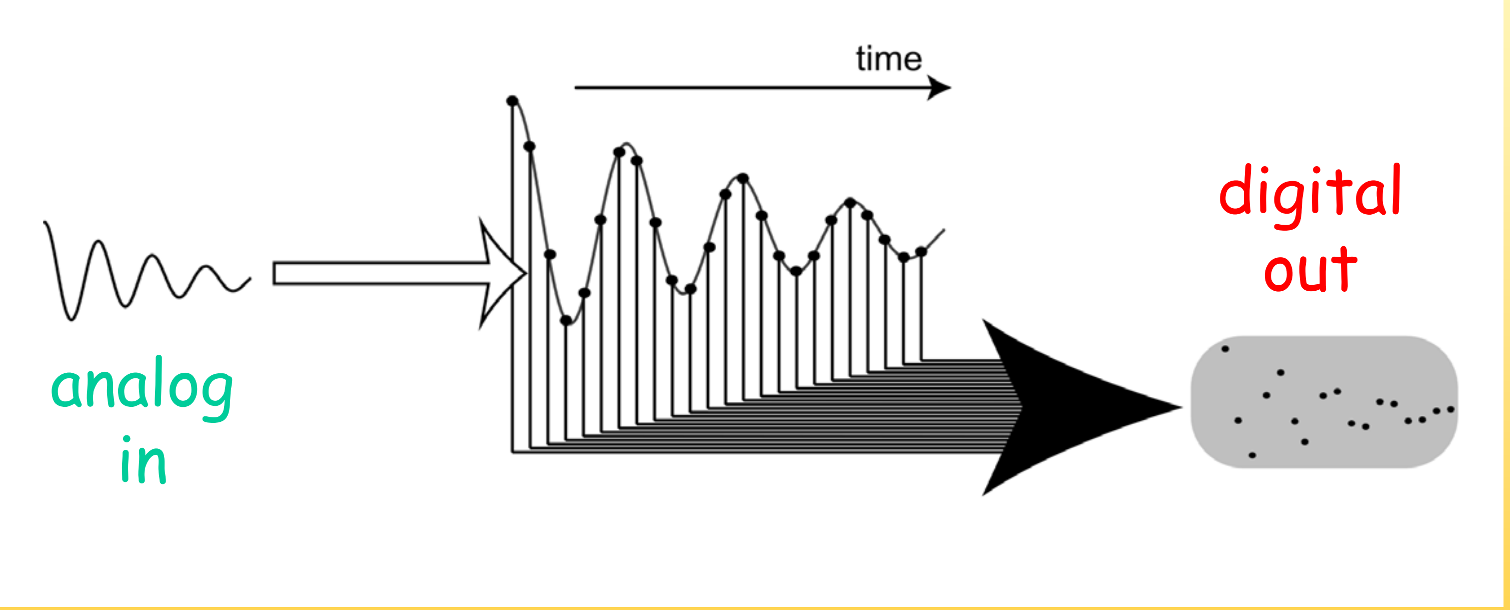


Peter Schmieder
AG Solution NMR

2D NMR: COSY

To analyze the signal we need to digitze it

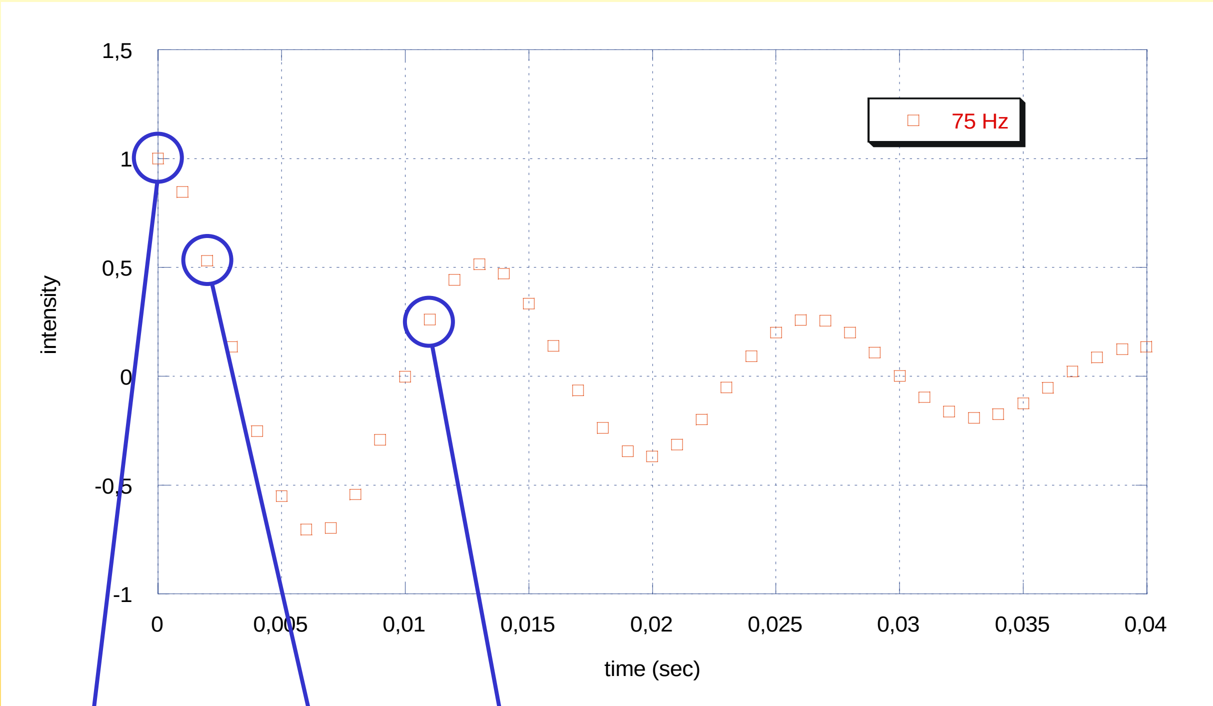
$s(t)= \exp((i\Omega_0 - 1/T_2)\,t)$



$s(k\Delta t)= \exp((i\Omega_0 - 1/T_2)\,k\Delta t)$

Peter Schmieder
AG Solution NMR

2D NMR: COSY



The FID is converted into a series of data points

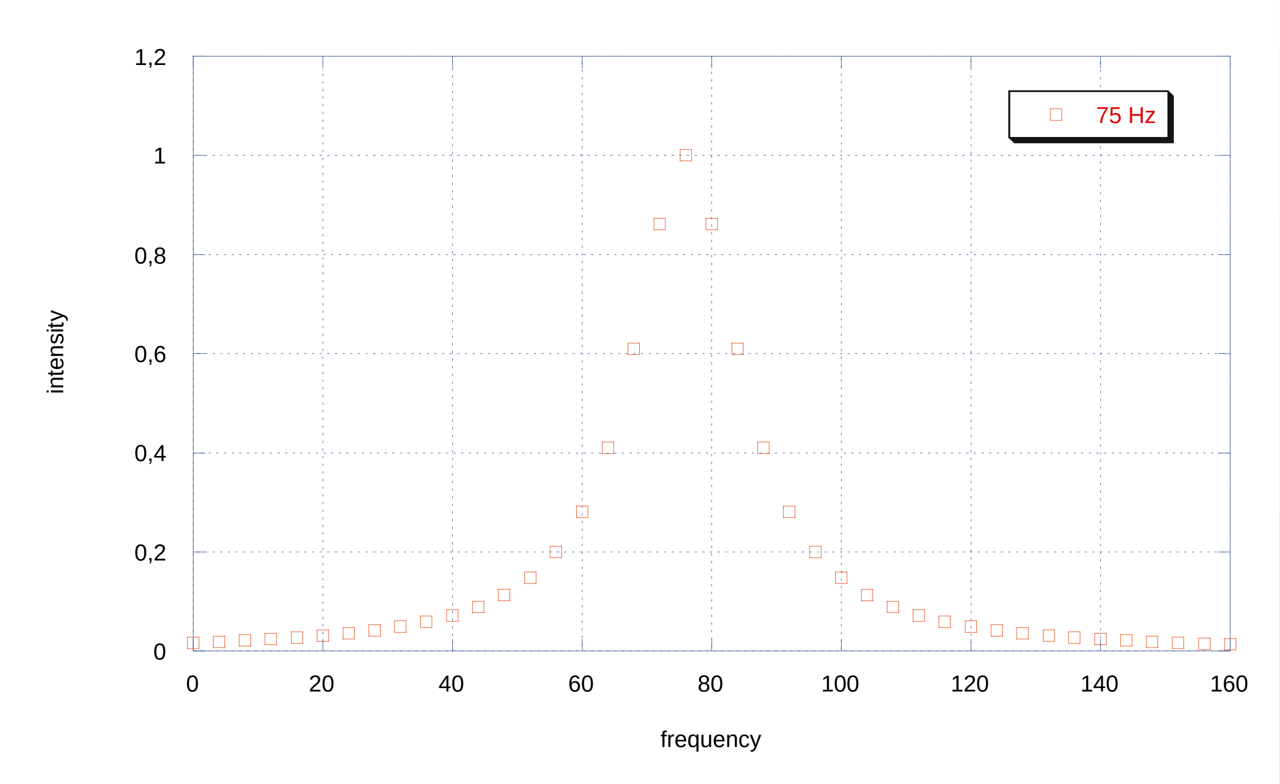
$$s(11\Delta t) = \exp((i\Omega_0 - 1/T_2)\, 11\Delta t)$$

$$s(2\Delta t) = \exp((i\Omega_0 - 1/T_2)\, 2\Delta t)$$

$$s(0\Delta t) = s(0) = \exp((i\Omega_0 - 1/T_2)\, 0) = 1$$

Peter Schmieder
AG Solution NMR

2D NMR: COSY

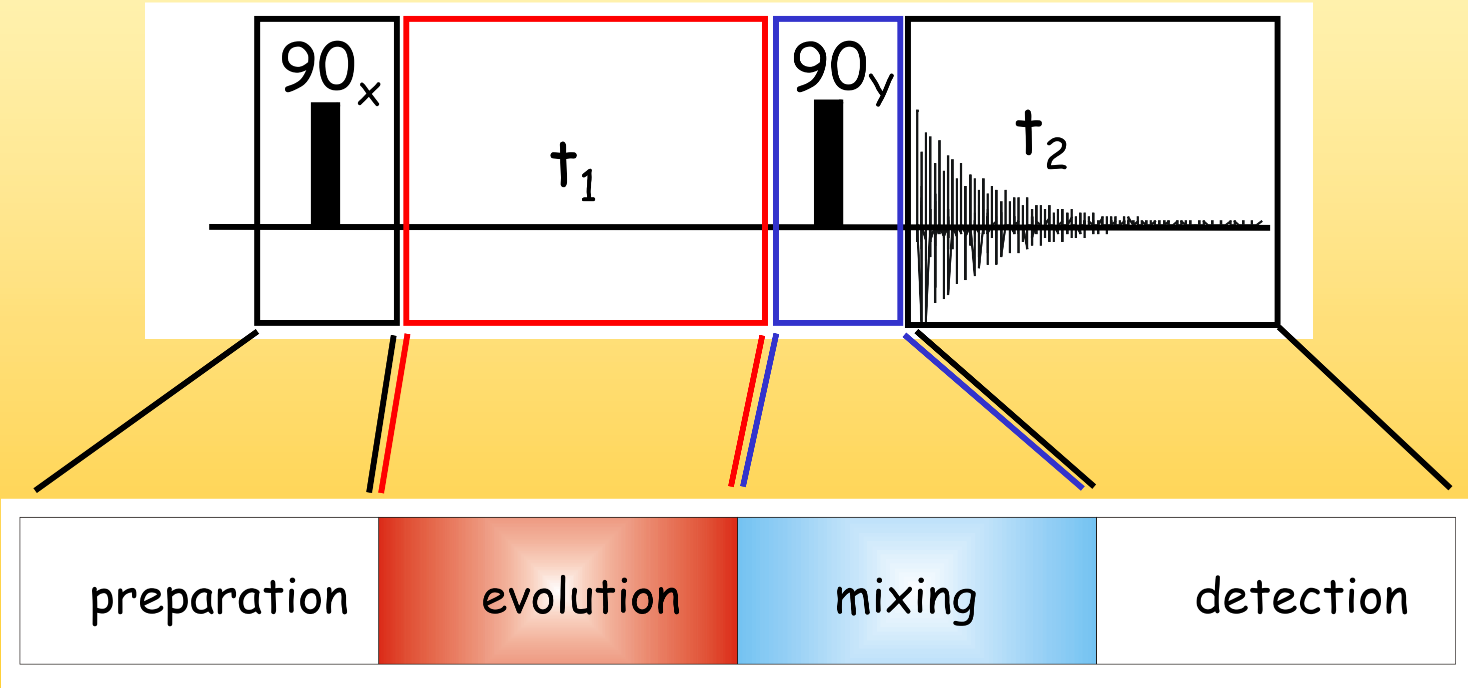


A digital Fourier transformation (DFT) converts that into another series of data points the spectrum

Peter Schmieder
AG Solution NMR

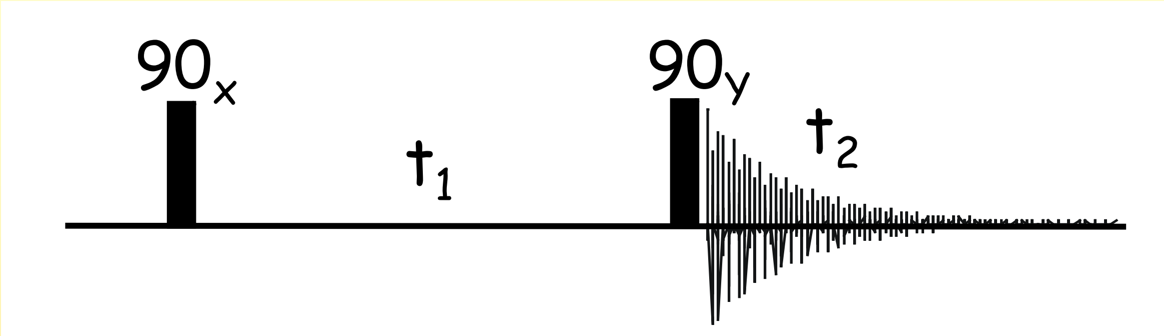
2D NMR: COSY

The simplest two-dimensional spectrum is the COSY



Peter Schmieder
AG Solution NMR

2D NMR: COSY



We consider two protons with $\Omega_{H1} = 2\pi\delta_{H1}$ and $\Omega_{H1} = 2\pi\delta_{H2}$

$$H_{1z} \xrightarrow{90^\circ H_x} -H_{1y} \xrightarrow{2\pi\delta_H t_1} -H_{1y}\cos 2\pi\delta_{H1}t_1 + H_{1x}\sin 2\pi\delta_{H1}t_1$$

not detectable

$$\xrightarrow{90^\circ H_y} -H_{1y}\cos 2\pi\delta_{H1}t_1 - H_{1z}\sin 2\pi\delta_{H1}t_1$$

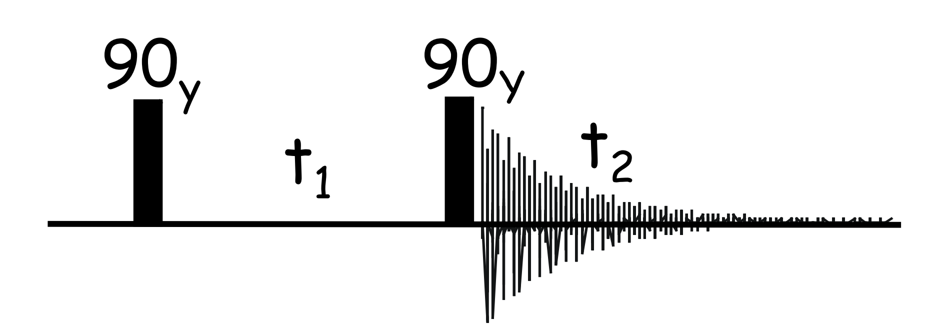
$$\xrightarrow{2\pi\delta_H t_2}$$

$$-H_{1y}\cos 2\pi\delta_{H1}t_1 \cos 2\pi\delta_{H1}t_2 + H_{1x}\cos 2\pi\delta_{H1}t_1 \sin 2\pi\delta_{H1}t_2$$

$$-H_1 \cos 2\pi\delta_{H1}t_1 \exp 2\pi\delta_{H1}t_2$$ Quadraturdetektion

Peter Schmieder
AG Solution NMR

2D NMR: COSY



To achieve quadrature detection in the indirect dimension we do a second experiment

$$H_{1z} \xrightarrow{90^\circ H_y} H_{1x} \xrightarrow{2\pi\delta_H t_1} H_{1x} \cos 2\pi\delta_{H1}t_1 + H_{1y} \sin 2\pi\delta_{H1}t_1$$

$$\xrightarrow{90^\circ H_y} -H_{1z} \cos 2\pi\delta_{H1}t_1 + H_{1y} \sin 2\pi\delta_{H1}t_1$$

not detectable

$$\xrightarrow{2\pi\delta_H t_2}$$

$$H_{1y} \sin 2\pi\delta_{H1}t_1 \cos 2\pi\delta_{H1}t_2 + H_{1x} \sin 2\pi\delta_{H1}t_1 \sin 2\pi\delta_{H1}t_2$$

$$H_1 \sin 2\pi\delta_{H1}t_1 \exp 2\pi\delta_{H1}t_2$$

2D NMR: COSY

Taken together we have a „hypercomplex" signal

$H_1 \cos 2\pi\delta_{H1}t_1 \exp 2\pi\delta_{H1}t_2$ und $H_1 \sin 2\pi\delta_{H1}t_1 \exp 2\pi\delta_{H1}t_2$

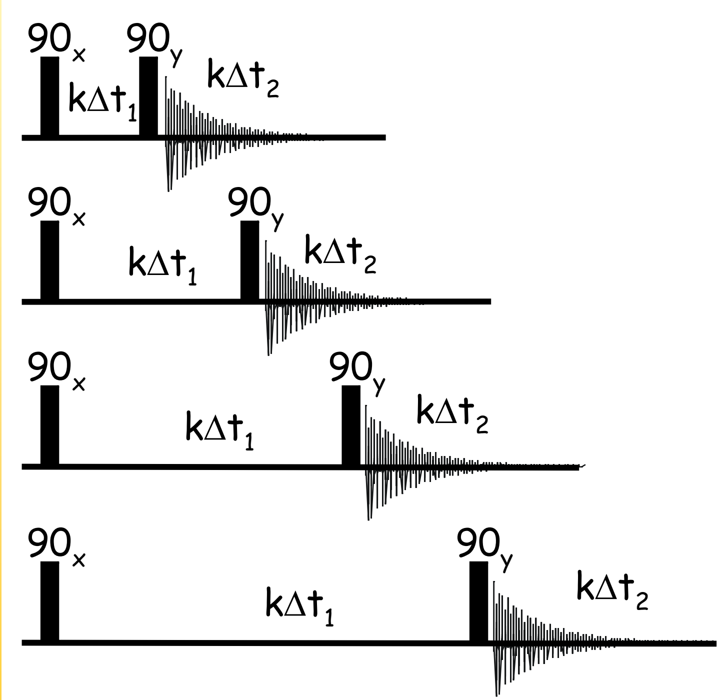
$= H_1 \exp 2\pi\delta_{H1}t_1 \exp 2\pi\delta_{H1}t_2$

$= H_1 \exp 2\pi\delta_{H1}(k\ \Delta t_1)$

$\times \exp 2\pi\delta_{H1}(k\ \Delta t_2)$

This is done by the incrementation of Δt_1

This is done by the ADC

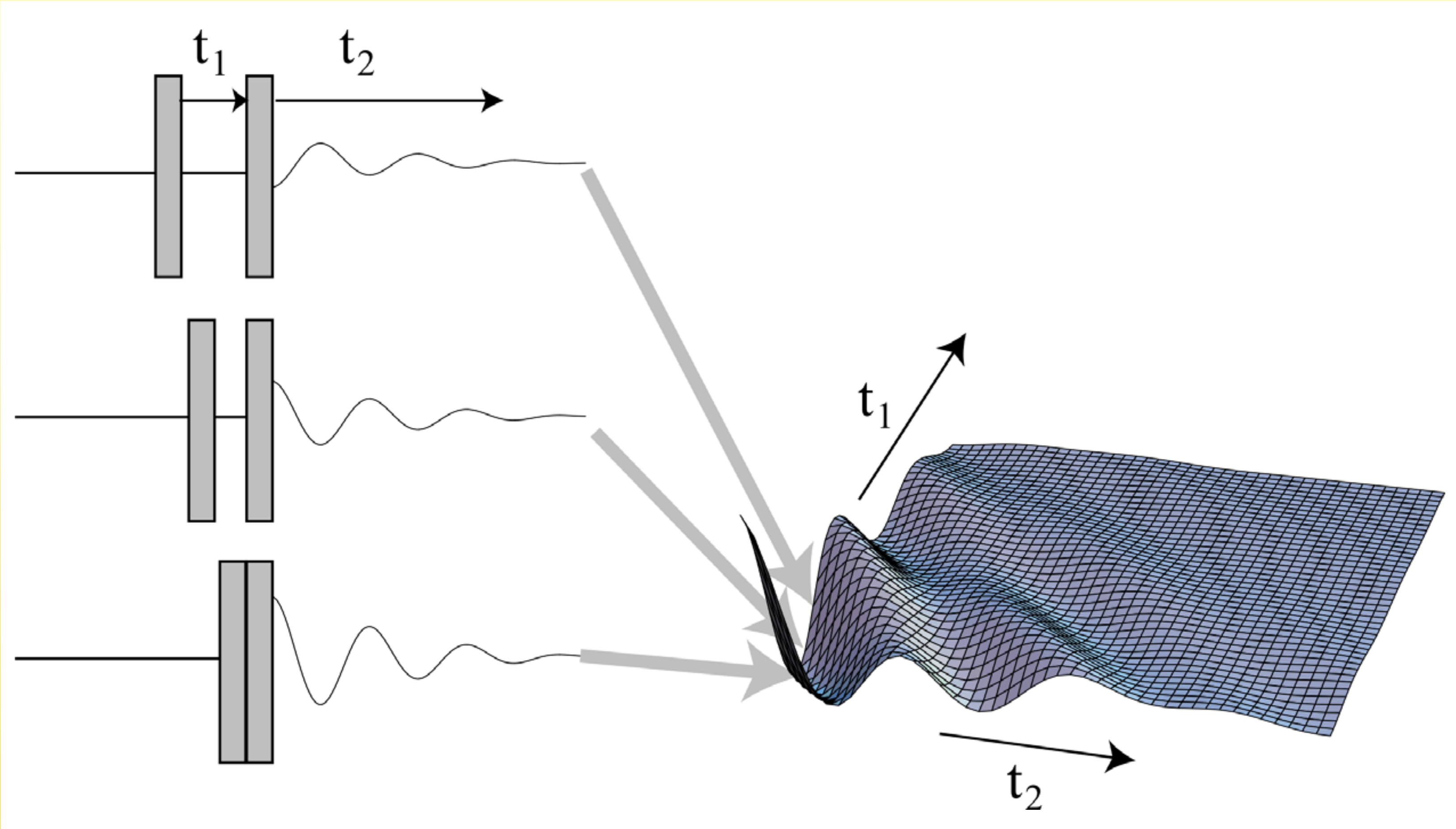


We obtain a two-dimensional area of data points .....

Peter Schmieder
AG Solution NMR

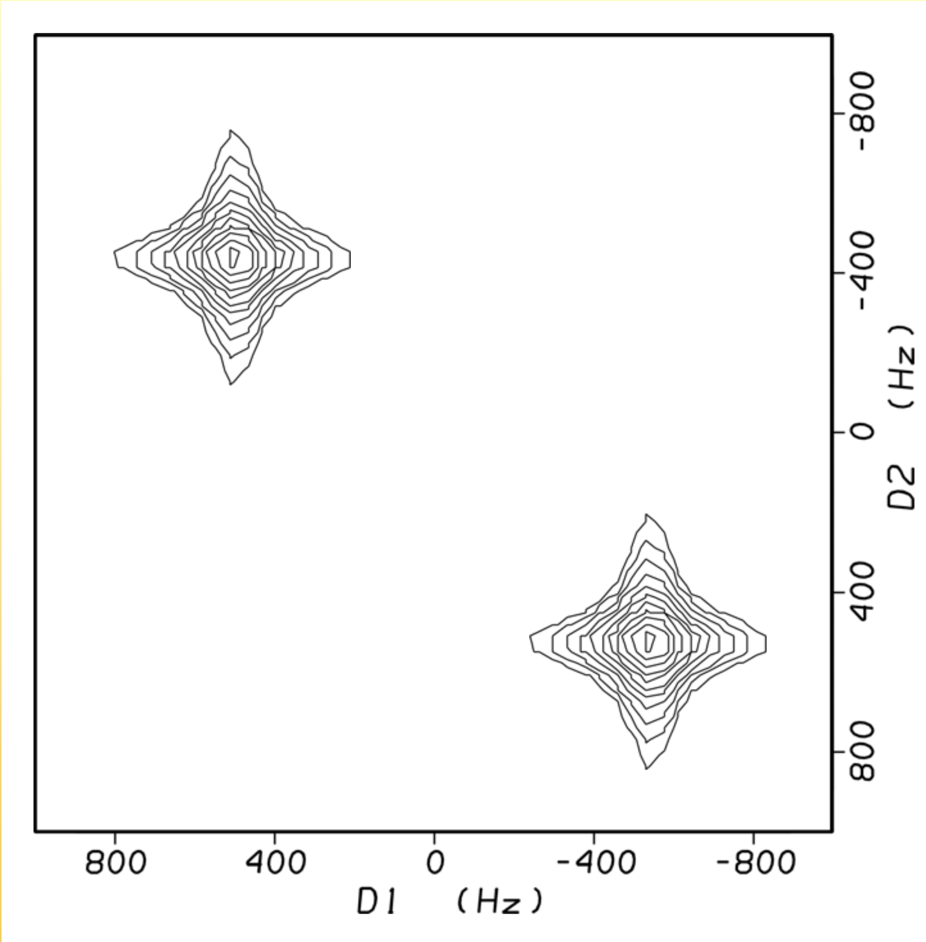
2D NMR: COSY

..... a two-dimensional FID



Peter Schmieder
AG Solution NMR

2D NMR: COSY



After two FTs we get our well known 2D spectrum

2D NMR: COSY

But up to know we have the same information on both axes

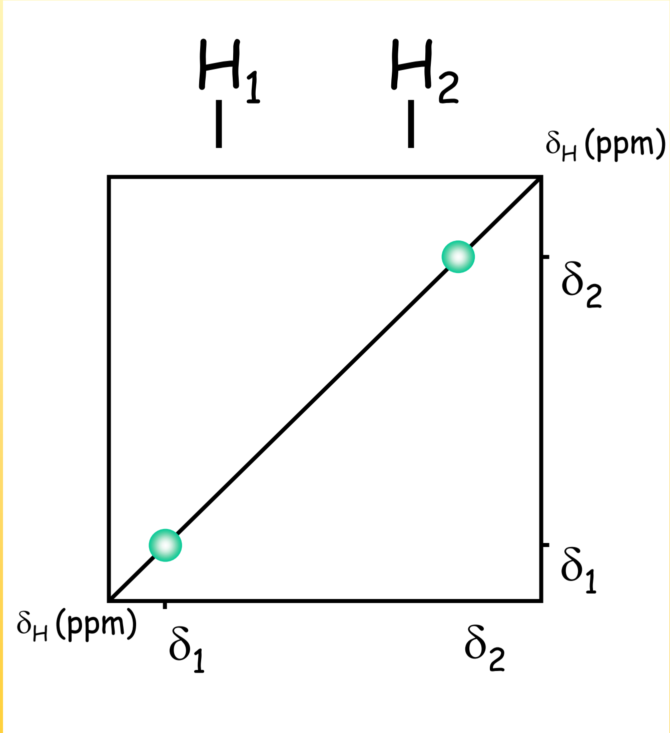
$H_1 \exp 2\pi\delta_{H1} t_1 \exp 2\pi\delta_{H1} t_2 =$

$H_1 \exp 2\pi\delta_{H1} (k\ \Delta t_1)$

$\exp 2\pi\delta_{H1} (k\ \Delta t_2)$

(the same for H_2)

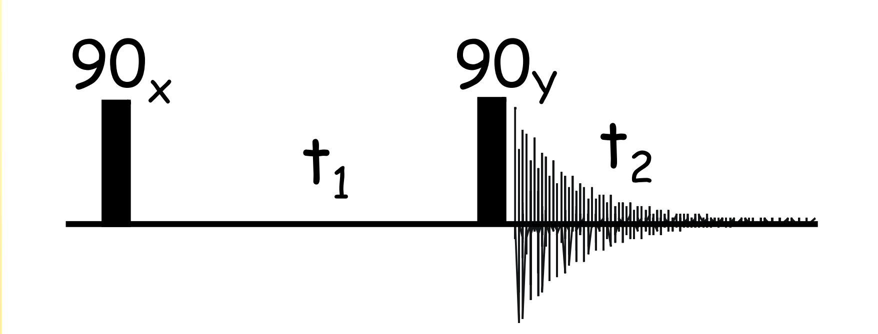
We have created a second dimension by incrementing during the evolution time but our spectrum has only a diagonal



Peter Schmieder
AG Solution NMR

2D NMR: COSY

Now we remember that mixing is achieved via coupling and we calculate for J_{HH} as well



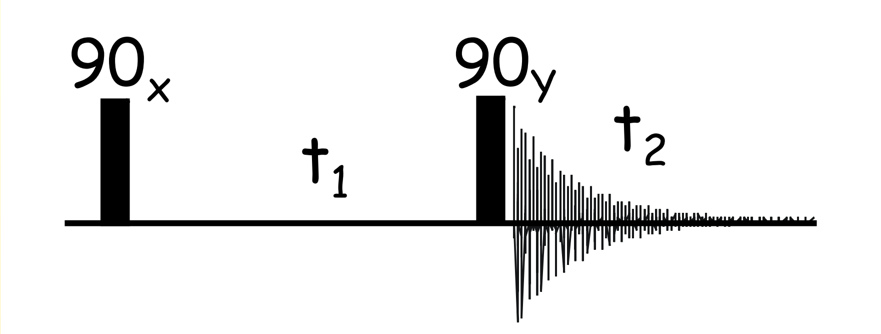
$$H_{1z} \xrightarrow{90^\circ H_x} -H_{1y} \xrightarrow{2\pi\delta_H t_1} -H_{1y}\cos 2\pi\delta_{H1}t_1 + H_{1x}\sin 2\pi\delta_{H1}t_1$$

Watch this operator !!

$$\xrightarrow{\pi J_{HH} t_1}$$

$$-H_{1y}\cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 + 2H_{1x}H_{2z}\cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$$
$$+ H_{1x}\sin 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 + 2H_{1y}H_{2z}\sin 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$$

2D NMR: COSY



Then the second 90° pulse

Here is where the transfer takes place !!

$\xrightarrow{90^\circ H_y}$

$-H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 - 2H_{1z}H_{2x} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$

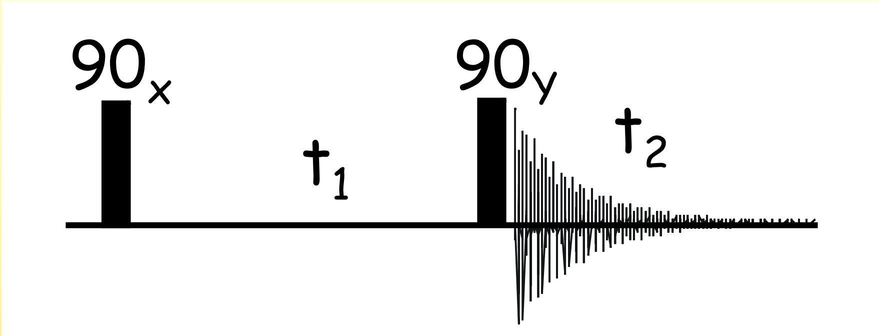
$-H_{1z} \sin 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 + 2H_{1y}H_{2x} \sin 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$

not detectable

two types of dectable magnetization remain

$-H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 - 2H_{1z}H_{2x} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$

2D NMR: COSY



Then we start the acqusition

$$- H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 - 2H_{1z} H_{2x} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1$$

$$\xrightarrow{\delta_H t_2} - H_{1y} \cos 2\pi \delta_{H1}t_1 \cos \pi J_{HH}t_1 \cos 2\pi \delta_{H1}t_2$$

$$+ H_{1x} \cos 2\pi \delta_{H1}t_1 \cos \pi J_{HH}t_1 \sin 2\pi \delta_{H1}t_2$$

$$- 2H_{1z} H_{2x} \cos 2\pi \delta_{H1}t_1 \sin \pi J_{HH}t_1 \cos 2\pi \delta_{H2}t_2$$

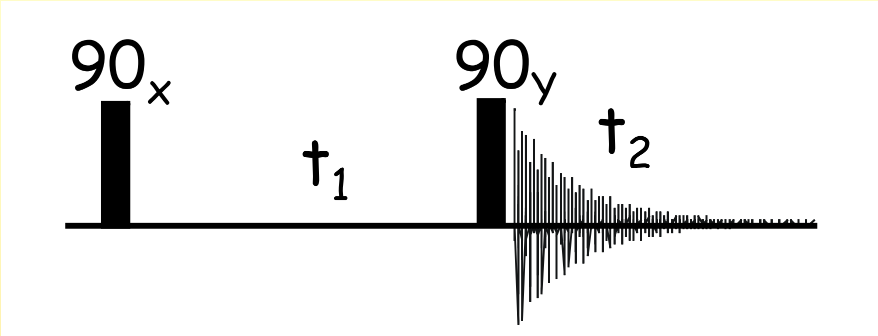
$$- 2H_{1z} H_{2y} \cos 2\pi \delta_{H1}t_1 \sin \pi J_{HH}t_1 \sin 2\pi \delta_{H2}t_2$$

H1 !

H2 !

Peter Schmieder
AG Solution NMR

2D NMR: COSY



we focus on detectable magnetization

$\xrightarrow{\pi J_{HH} t_2}$

$$- H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 \cos 2\pi\delta_{H1}t_2 \cos \pi J_{HH}t_2$$

$$+ H_{1x} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 \sin 2\pi\delta_{H1}t_2 \cos \pi J_{HH}t_2$$

Im !

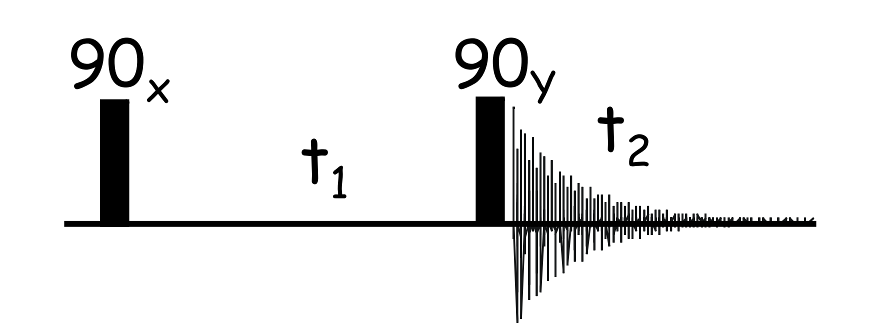
$$- H_{2y} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1 \cos 2\pi\delta_{H2}t_2 \sin \pi J_{HH}t_2$$

$$+ H_{2x} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1 \sin 2\pi\delta_{H2}t_2 \sin \pi J_{HH}t_2$$

Im !

The caclulation for the second FID (1. pulse 90°$_y$) is omitted !

2D NMR: COSY



As a result of our calculation we get: (only the real part is given)

$$- H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 \cos 2\pi\delta_{H1}t_2 \cos \pi J_{HH}t_2$$

$$- H_{2y} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1 \cos 2\pi\delta_{H2}t_2 \sin \pi J_{HH}t_2$$

In t_1 immer die Verschiebung von H1

In t_2 die Verschiebung von H1 oder H2

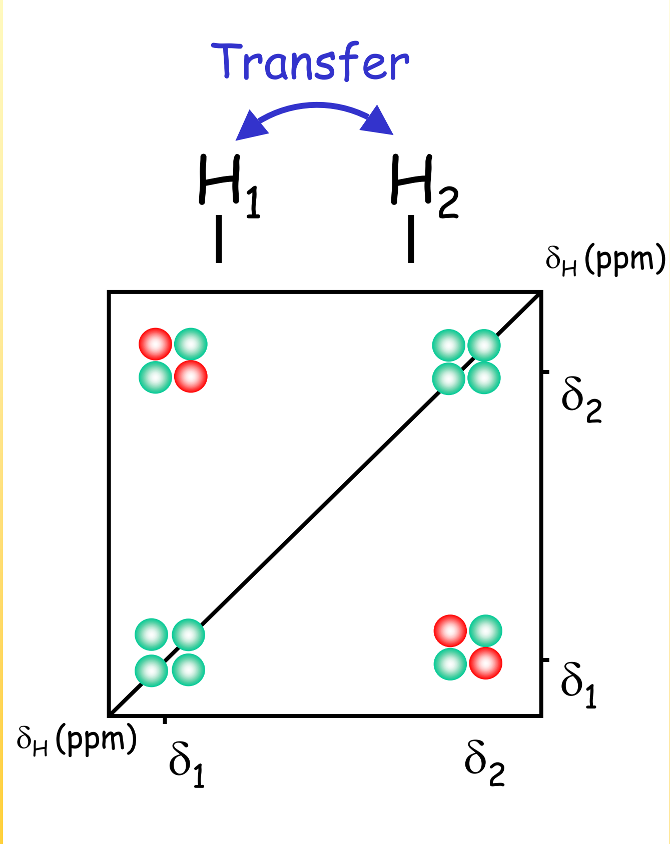
Peter Schmieder
AG Solution NMR

2D NMR: COSY

Now something new has shown up:

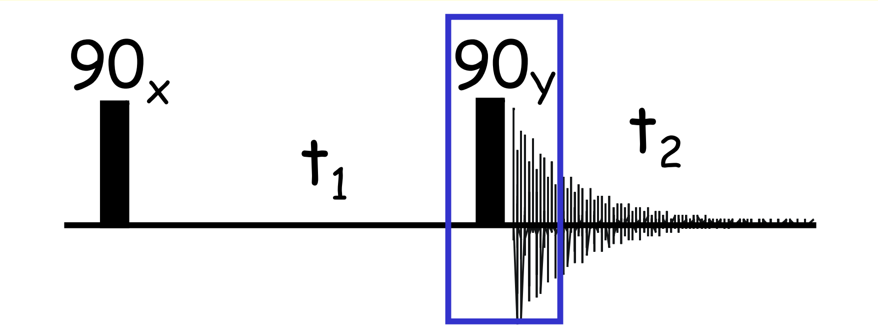
There are signals that have been labeled with different chemical shifts in both dimensions: the crosspeaks

A transfer of magnetization has taken place



Peter Schmieder
AG Solution NMR

2D NMR: COSY



COrrelation SpectroscopY =

COSY

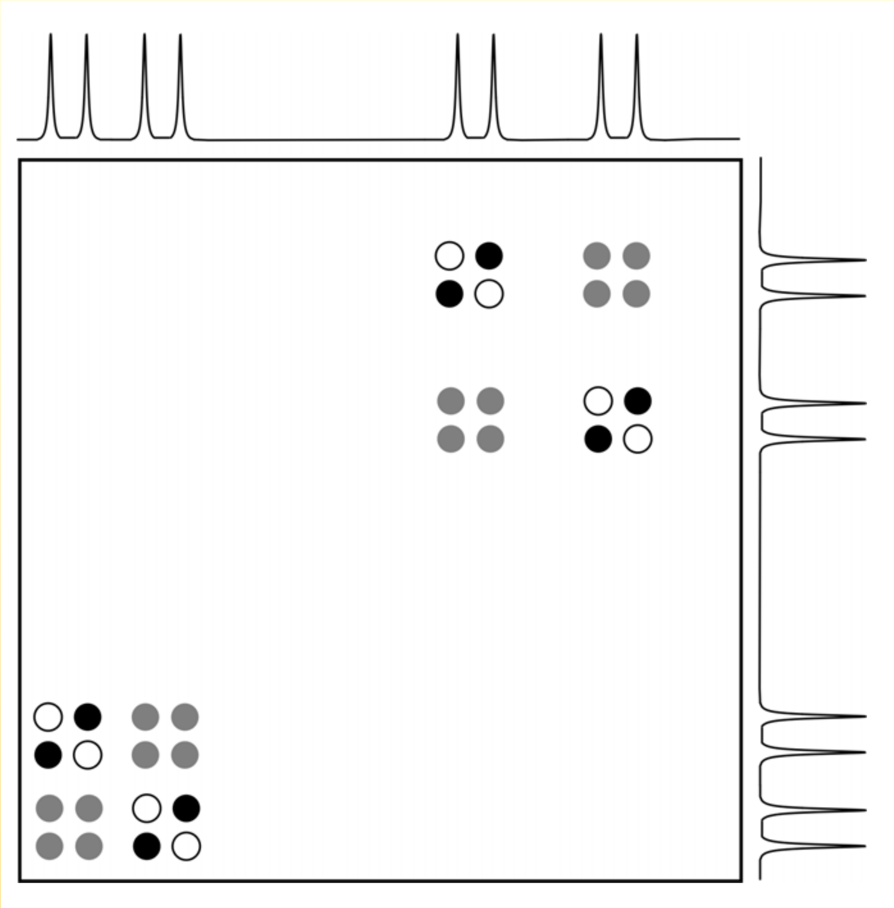
The „mixing time" is a simple 90° pulse in this case.

It causes a transfer of magnetization.

Cross peaks in a 2D spectrum thus indicate the presence of a scalar coupling between the two nuclei whose frequencies intersect at the position of the cross peak.

Peter Schmieder
AG Solution NMR

2D NMR: COSY



We will now take a look at the fine structure of the peaks

2D NMR: COSY

$$- H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 \cos 2\pi\delta_{H1}t_2 \cos \pi J_{HH}t_2$$

$$- H_{2y} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1 \cos 2\pi\delta_{H2}t_2 \sin \pi J_{HH}t_2$$

Let's use our trigonometric formulas

$$= -H_{1y} \tfrac{1}{2} [\cos 2\pi(\delta_{H1} + J_{HH}/2)t_1 + \cos 2\pi(\delta_{H1} - J_{HH}/2)t_1] \times$$

$$\tfrac{1}{2} [\cos 2\pi(\delta_{H1} + J_{HH}/2)t_2 + \cos 2\pi(\delta_{H1} - J_{HH}/2)t_2]$$

$$-H_{2y} \tfrac{1}{2} [\sin 2\pi(\delta_{H1} + J_{HH}/2)t_1 - \sin 2\pi(2\pi\delta_{H1} - J_{HH}/2)t_1] \times$$

$$\tfrac{1}{2} [\sin 2\pi(\delta_{H2} + J_{HH}/2)t_2 - \sin 2\pi(\delta_{H2} - J_{HH}/2)t_2]$$

Peter Schmieder
AG Solution NMR

2D NMR: COSY

$$= -H_{1y}\,\tfrac{1}{2}\,[\cos 2\pi(\delta_{H1} + J_{HH}/2)t_1 + \cos 2\pi(\delta_{H1} - J_{HH}/2)t_1] \times$$

$$\tfrac{1}{2}\,[\cos 2\pi(\delta_{H1} + J_{HH}/2)t_2 + \cos 2\pi(\delta_{H1} - J_{HH}/2)t_2]$$

$$-H_{2y}\,\tfrac{1}{2}\,[\sin 2\pi(\delta_{H1} + J_{HH}/2)t_1 - \sin 2\pi(2\pi\delta_{H1} - J_{HH}/2)t_1] \times$$

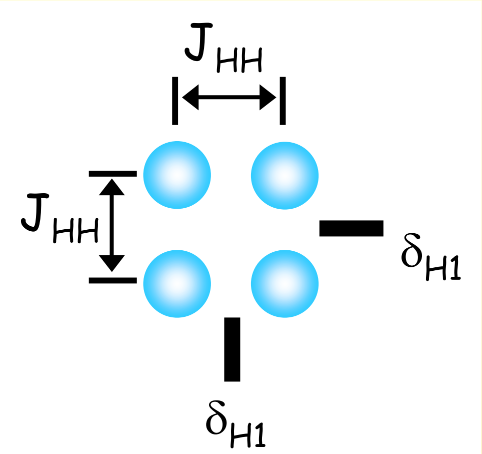
$$\tfrac{1}{2}\,[\sin 2\pi(\delta_{H2} + J_{HH}/2)t_2 - \sin 2\pi(\delta_{H2} - J_{HH}/2)t_2]$$

We get a total of 8 peaks, 4 with a cosine and with δ_{H1} in both dimensions, 4 with a sine and with different chemical shifts in both dimensions

Peter Schmieder
AG Solution NMR

2D NMR: COSY

The first four are all positive (in-phase), they have δ_{H1} in both dimensions (diagonal signal) and all are resulting from cosine functions



$$+ H_1 \cos 2\pi(\delta_{H1} + J_{HH}/2)t_1 \cos 2\pi(\delta_{H1} + J_{HH}/2)t_2$$

$$+ H_1 \cos 2\pi(\delta_{H1} + J_{HH}/2)t_1 \cos 2\pi(\delta_{H1} - J_{HH}/2)t_2$$

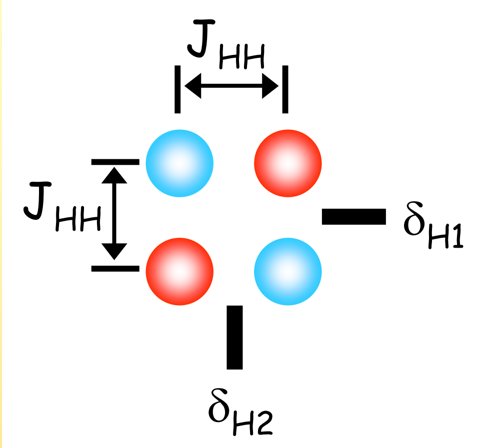
$$+ H_1 \cos 2\pi(\delta_{H1} - J_{HH}/2)t_1 \cos 2\pi(\delta_{H1} + J_{HH}/2)t_2$$

$$+ H_1 \cos 2\pi(\delta_{H1} - J_{HH}/2)t_1 \cos 2\pi(\delta_{H1} - J_{HH}/2)t_2$$

Peter Schmieder
AG Solution NMR

2D NMR: COSY

The second four have alternating signs (anti-phase), different chemical shifts (δ_{H1} and δ_{H2}) in the two dimensions (cross peaks) and all result from sine functions



$$+ H_2 \sin 2\pi(\delta_{H1} + J_{HH}/2)t_1 \sin 2\pi(\delta_{H2} + J_{HH}/2)t_2$$

$$- H_2 \sin 2\pi(\delta_{H1} + J_{HH}/2)t_1 \sin 2\pi(\delta_{H2} - J_{HH}/2)t_2$$

$$- H_2 \sin 2\pi(\delta_{H1} - J_{HH}/2)t_1 \sin 2\pi(\delta_{H2} + J_{HH}/2)t_2$$

$$+ H_2 \sin 2\pi(\delta_{H1} - J_{HH}/2)t_1 \sin 2\pi(\delta_{H2} - J_{HH}/2)t_2$$

Peter Schmieder
AG Solution NMR

2D NMR: COSY

We thus have a problem with phase

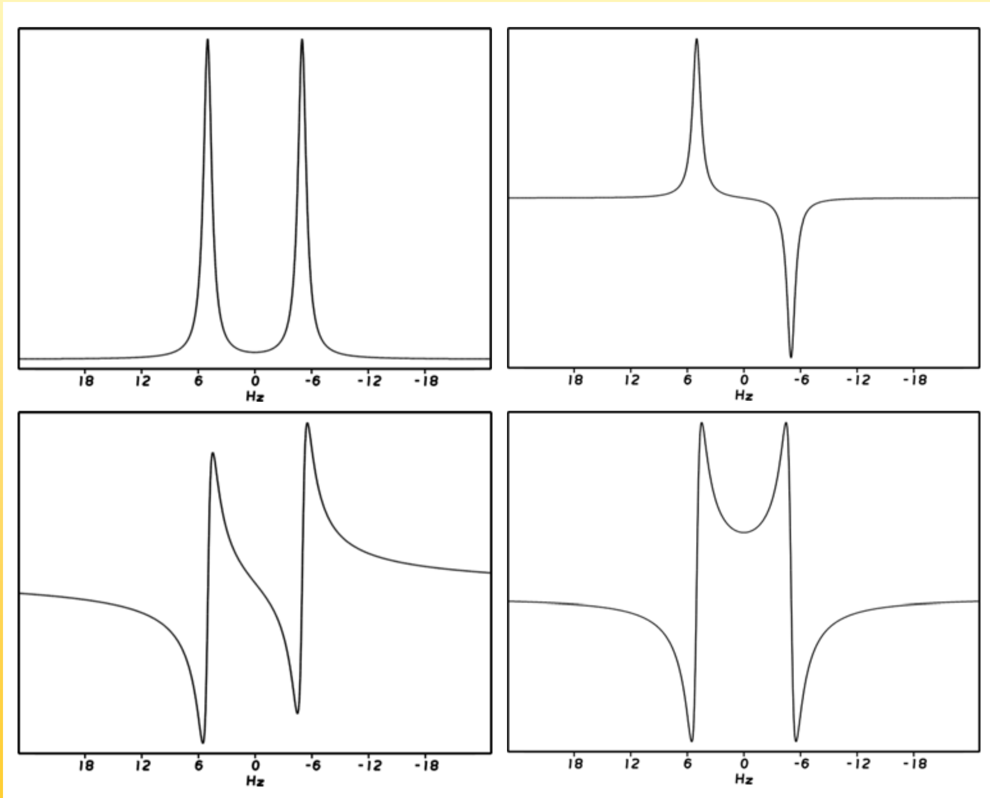
in-phase   anti-phase

absorbtiv

$\Delta\phi$ = 90°

cos vs. sin

dispersiv



2D NMR: COSY

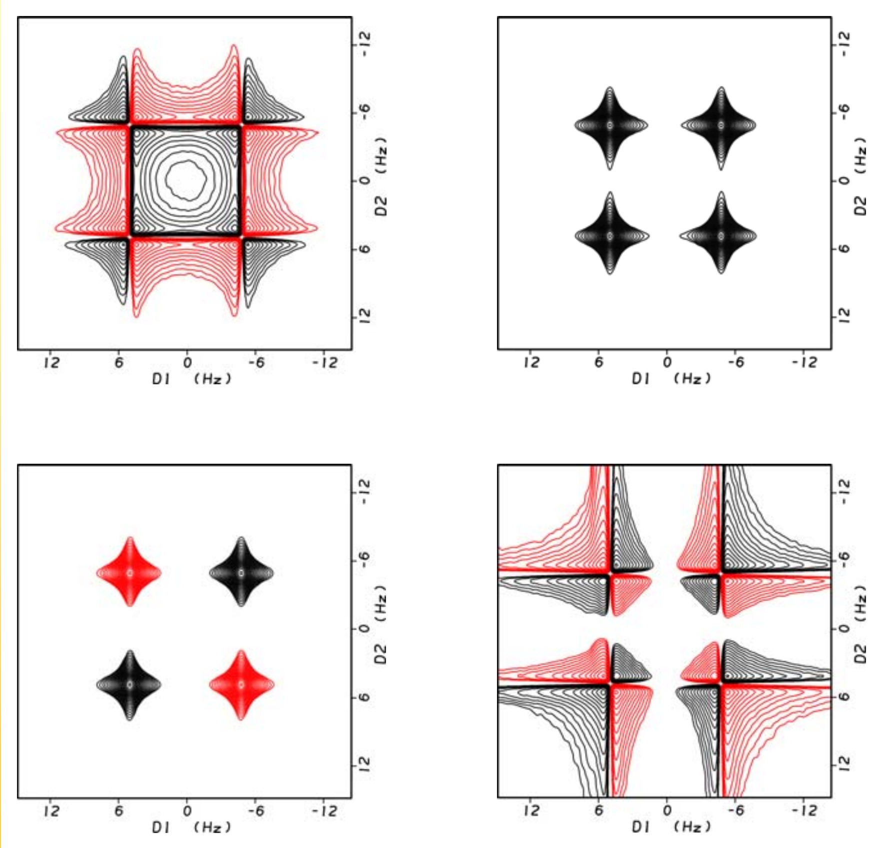
either

diagonal peak
absorptiv,
cross peak
dispersiv

or

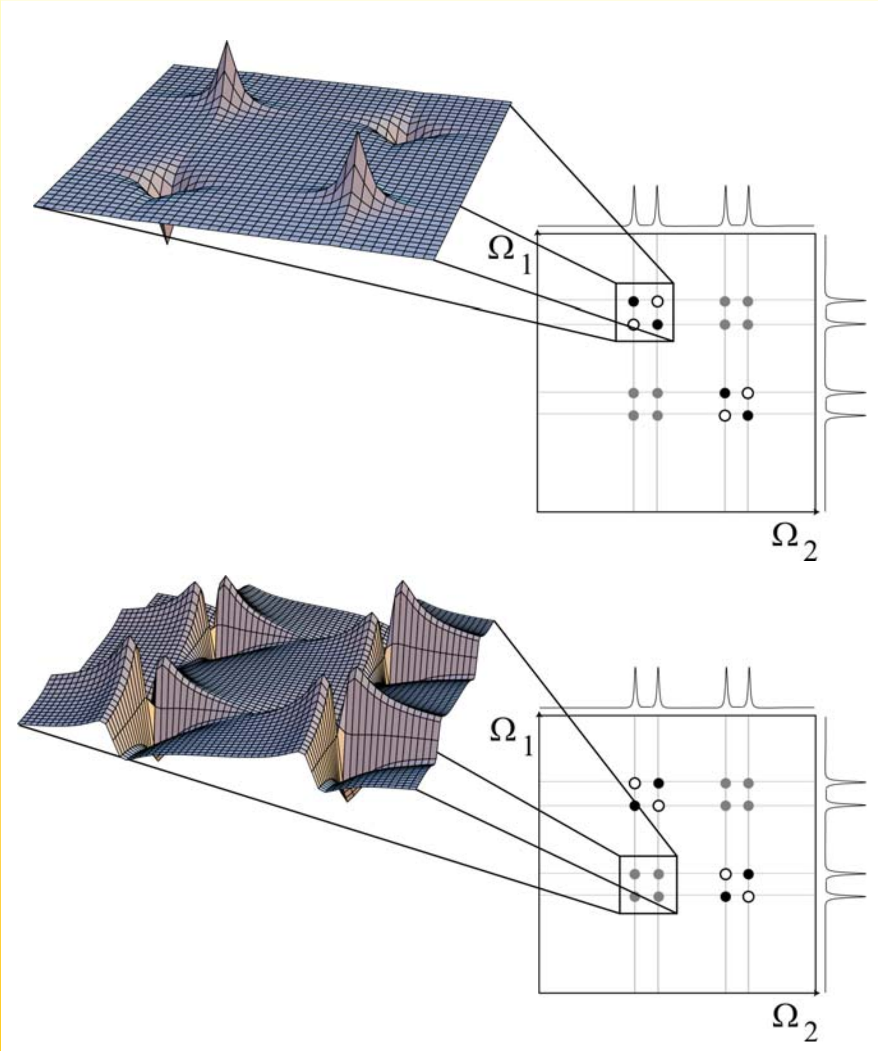
diagonal peak
dispersiv,
cross peak
absorptiv

cross peak   diagonal peak



Peter Schmieder
AG Solution NMR

2D NMR: COSY



That means:

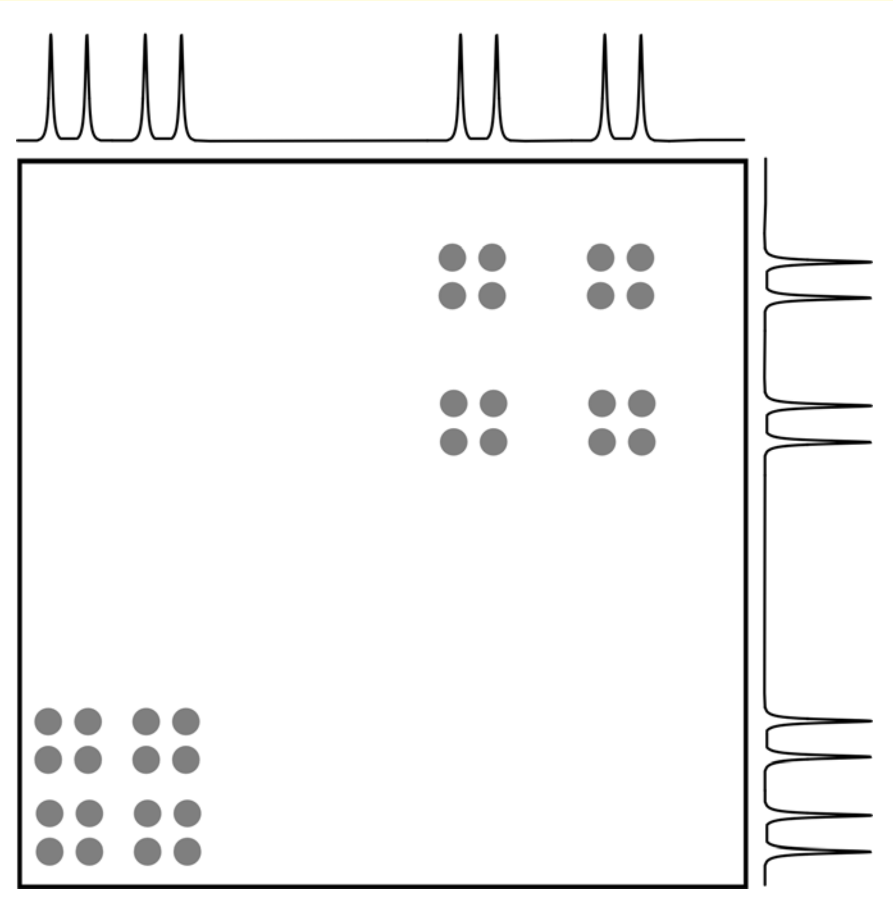
<u>either</u>

the diagonal is braod an the cross peaks can vanish because of overlap

<u>or</u>

the cross peaks are braod and the signals with different signs cancel

2D NMR: COSY



In the regular COSY the solution is a magnitude calculation

We have seen already that the DQF-COSY is another solution for that problem

Peter Schmieder
AG Solution NMR

That's it

www.fmp-berlin.de/schmieder/teaching/selenko_seminars.htm