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

- II -

16.03.2009

Peter Schmieder

AG Solution NMR

The program

CW vs. FT NMR

What is a pulse ?

Vectormodel

Water-flip-back

CW vs. FT

CW vs. FT

Two methods exist to record NMR spectra, which differ substantially in the way the spectrum is acquired:

CW-Technik

(continous wave)

FT-Technik

(fourier transform)

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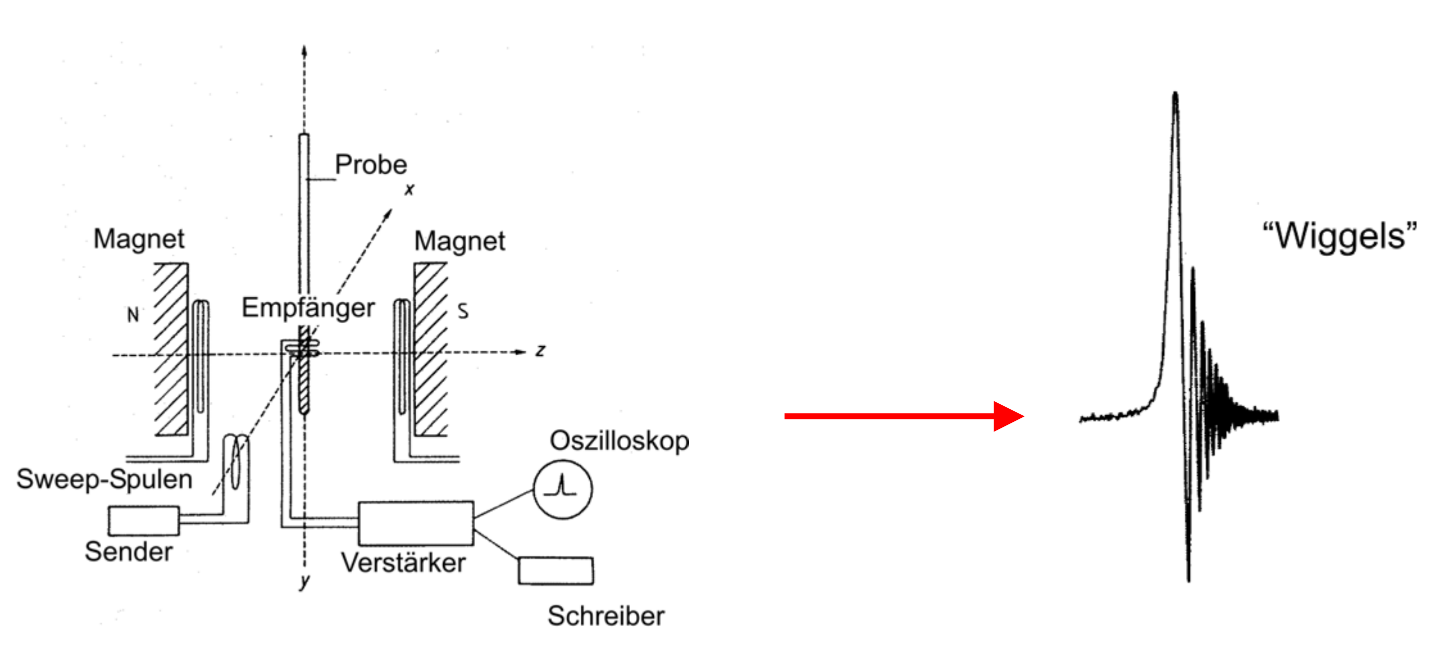
CW vs. FT

The CW-technique is the „typical“ methods, but it is hardly used these days because of the signal-to-noise problem in NMR



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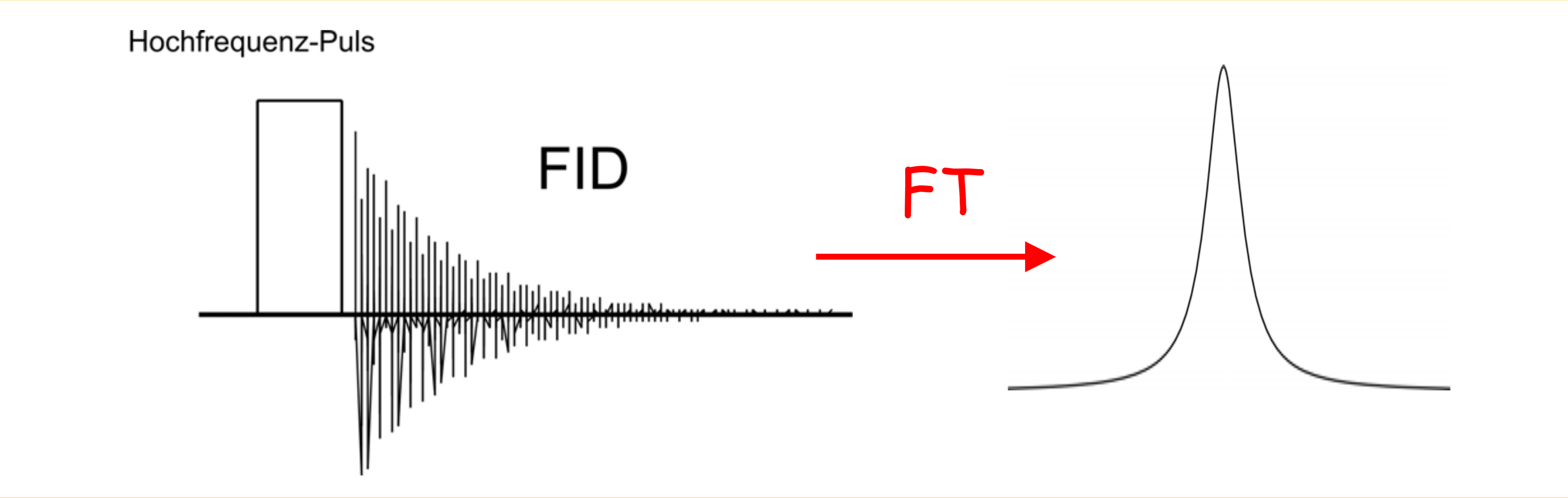
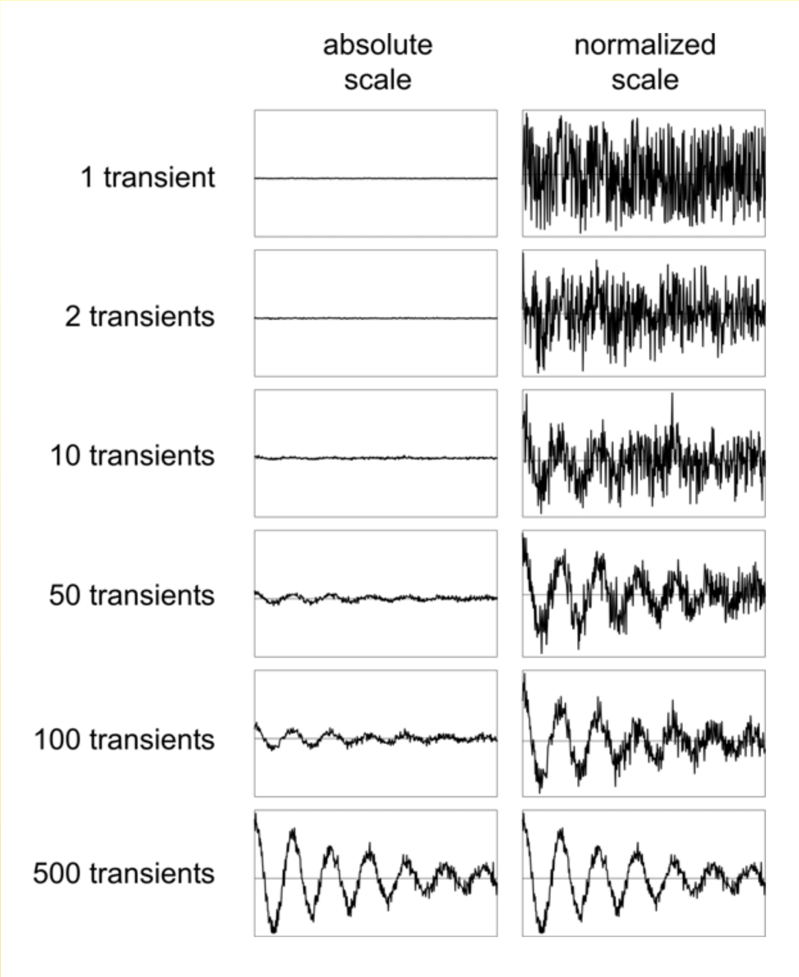
CW vs. FT

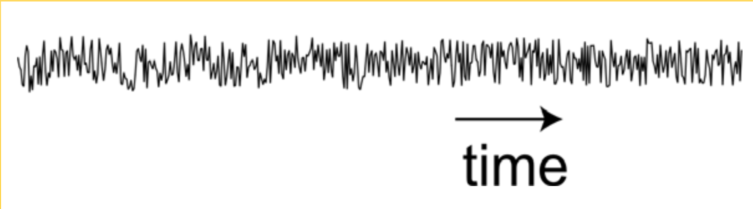
Modern NMR spectrometer all work with the FT-NMR-technique, which is also called „pulsed NMR" and which makes it easier to improve the signal-to-noise ratio...



CW vs. FT



.... by repetition and addition of the experiment



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What is a pulse

What is a pulse

With respect to the FT-technique a pulse is an important tool to manipulate spins.

But how can the relatively weak field of the pulse have such an effect when the much larger static field is always present.

This is a typical resonance phenomenon which can be explained with the concept of the „rotating coordinate system"

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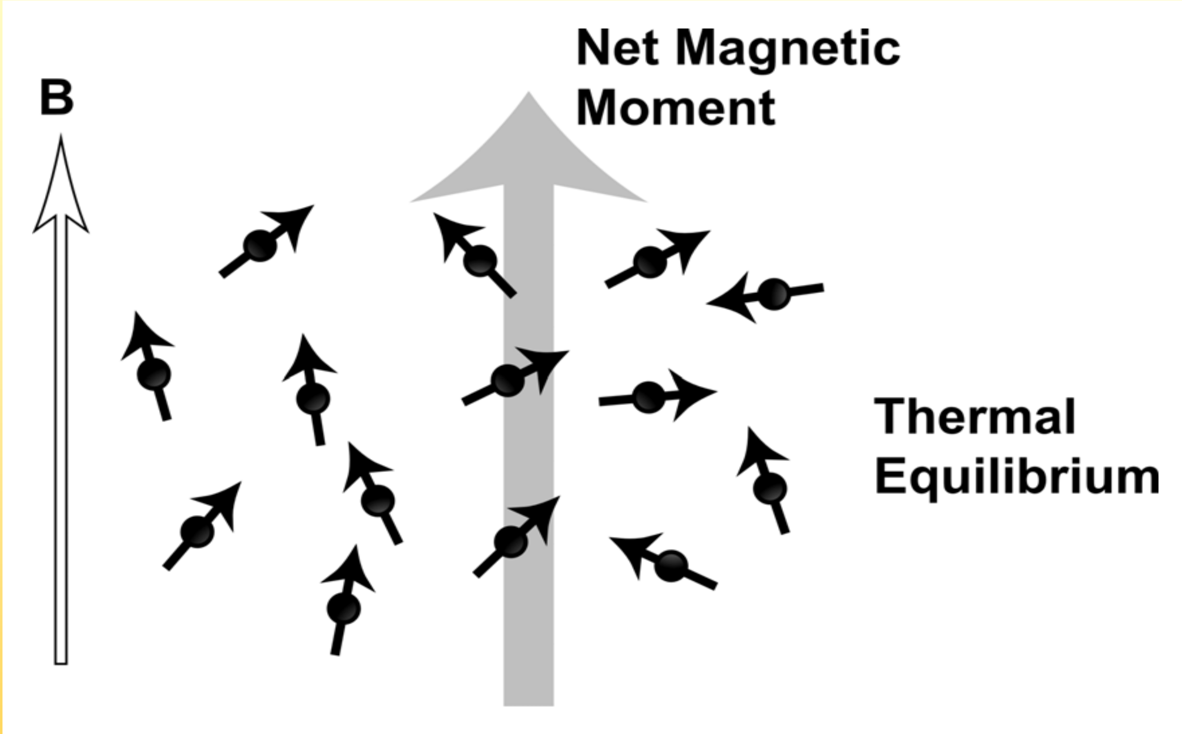
What is a pulse



Starting point is the z-magnetization that results from the Boltzmann distribution

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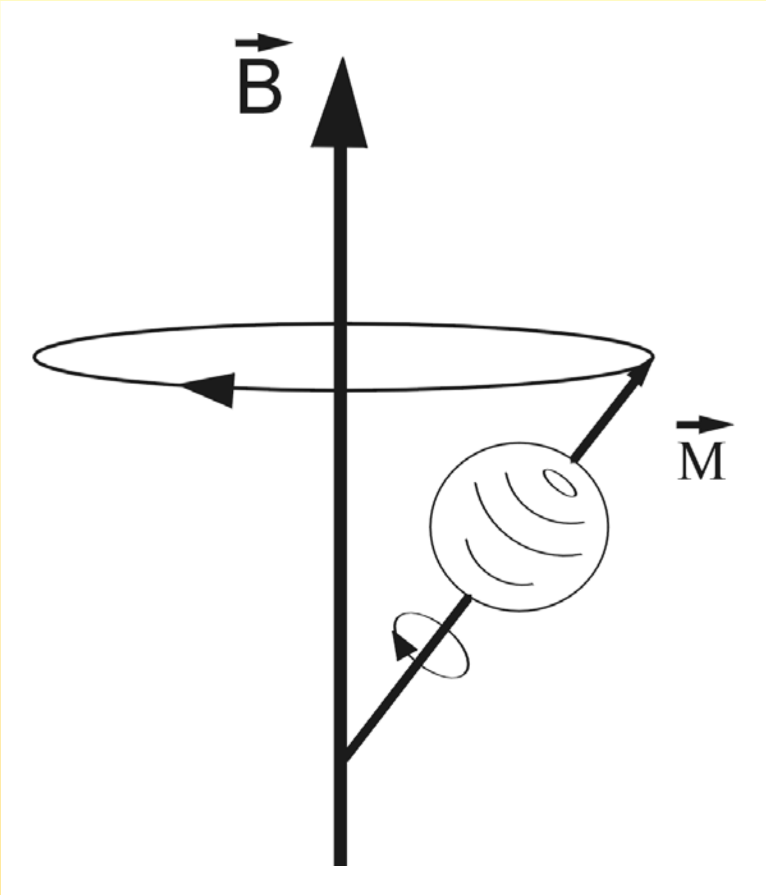
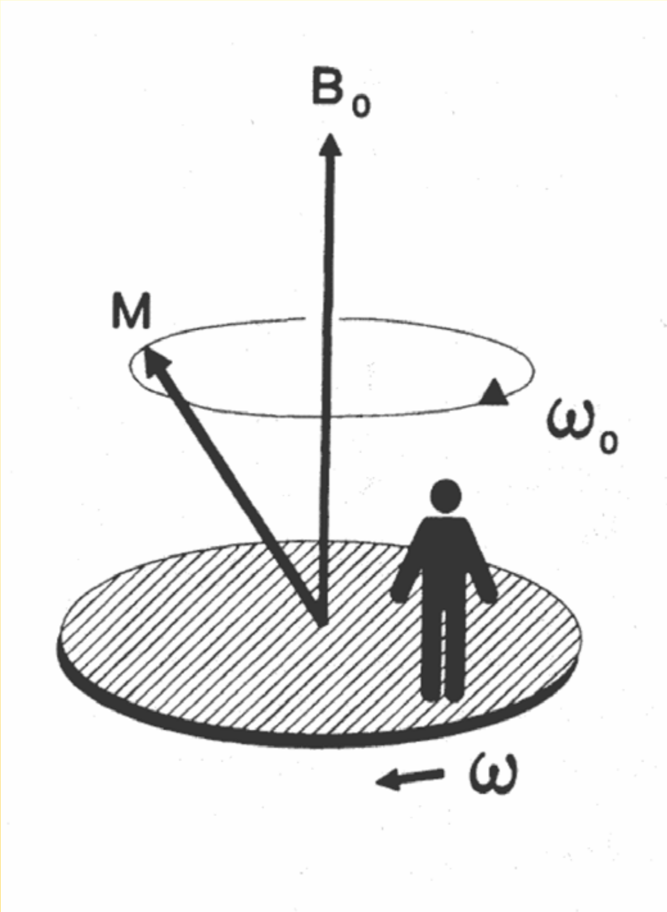
What is a pulse



The nuclear spin are rotating around the magnetic field provided by the magnet

What is a pulse



The concept of a rotating coordinate system is easy to grasp when we remind our self that we live on a rotating object our self. The observer rotates with a speed ω the spin with a speed ω_0

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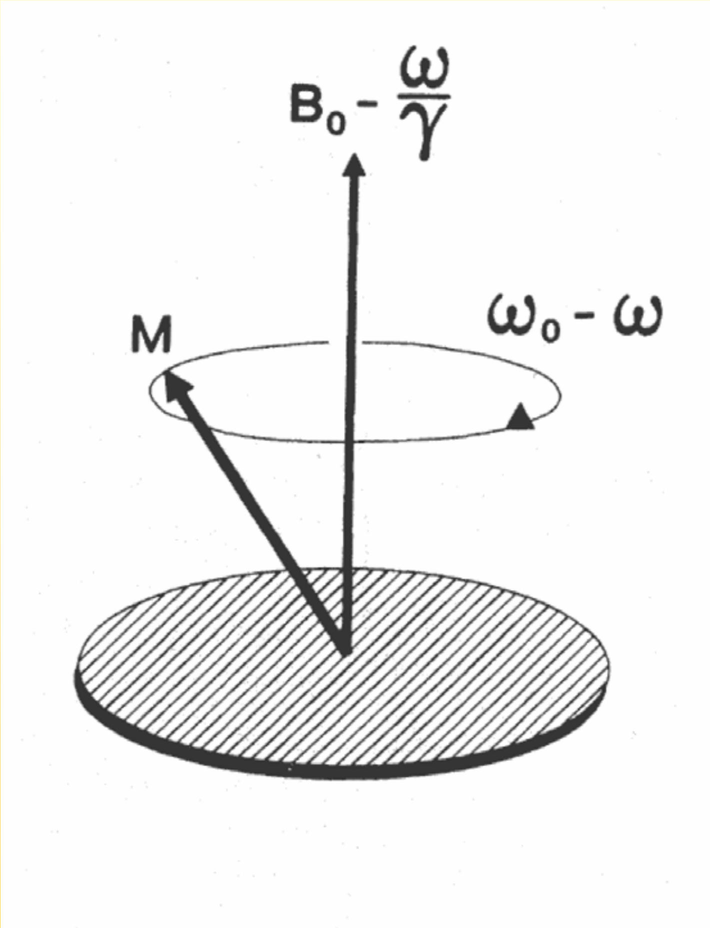
What is a pulse



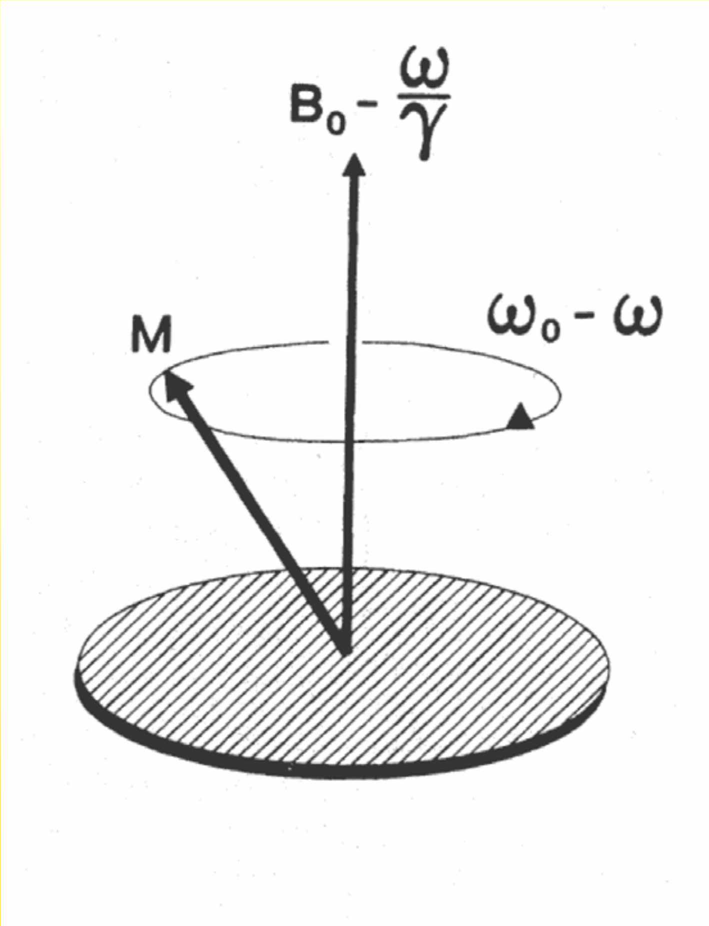
The movement of the spins (the rotation) is caused by the magnetic field

$$\omega_0 = 2\pi\, \nu_0 = \gamma\, B_0$$

Is the movement slower the observer has to conclude that the magnetic field is weaker

$$\omega_0 - \omega = \Omega = \gamma\, (B_0 - \omega/\gamma)$$

What is a pulse



Within this concept also negative frequencies are possible, i.e. rotations in the other direction

No movement means no magnetic field !!

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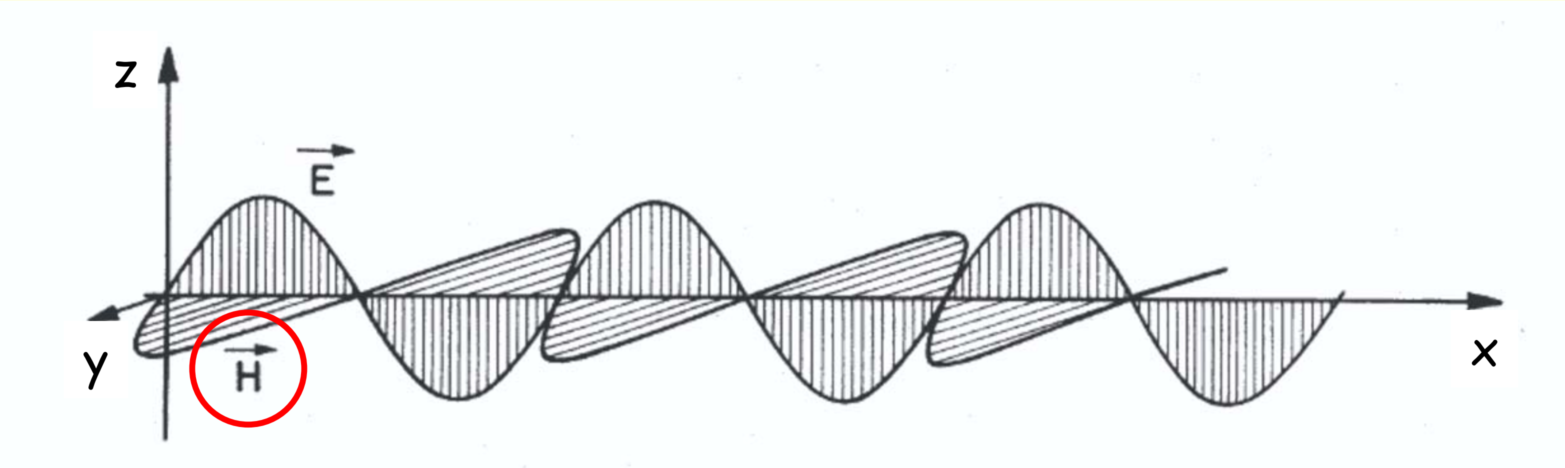
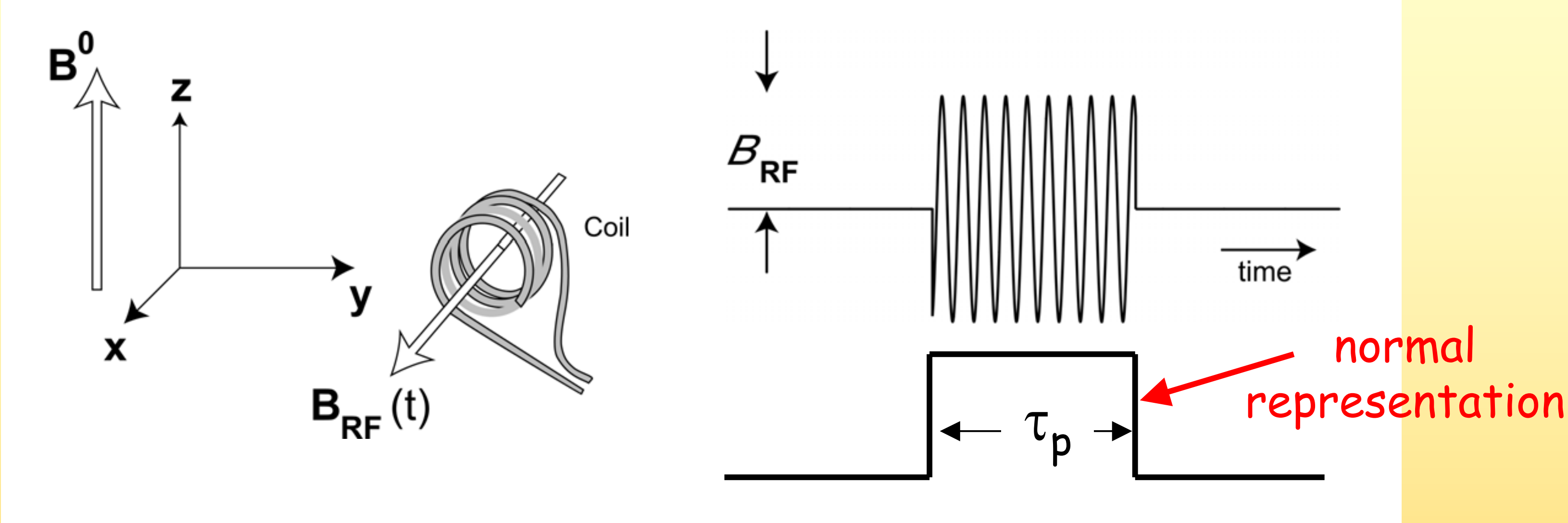
What is a pulse



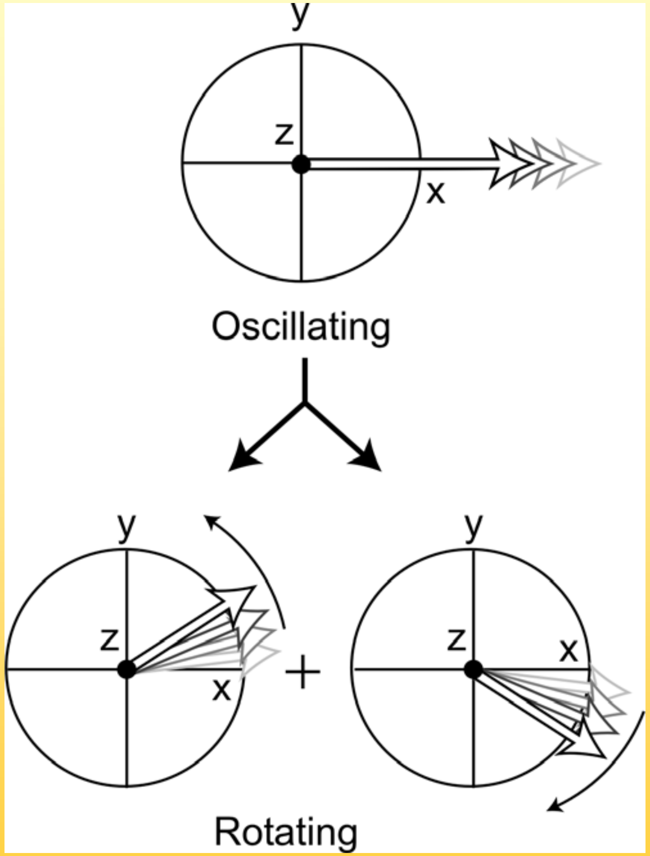
The pulse itself is the irradiation of radio waves. They are linearly polarized and have an electric and a magnetic component, only the latter is of interest here.

What is a pulse



The irradiation uses the coil, radio waves are sent to the sample in form of a „pulse", i.e. a short RF-burst. The field is in the x,y-plane

What is a pulse



A linearly polarized oscillation can be split into two components rotating in opposite directions. One component is quite fast in the rotation coordinate system and will be ignored.....

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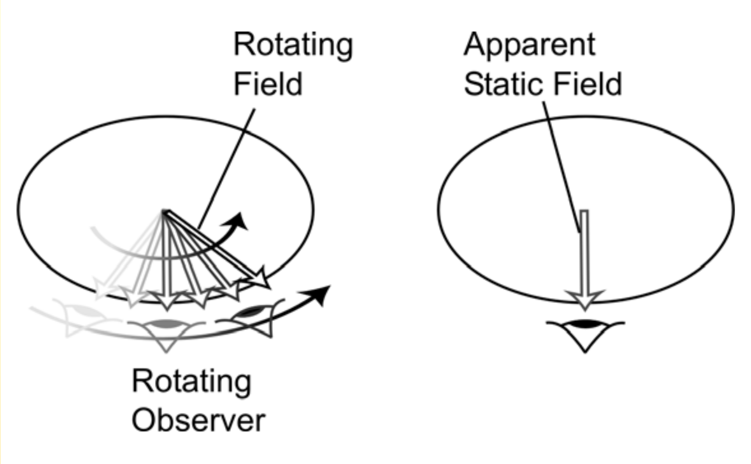
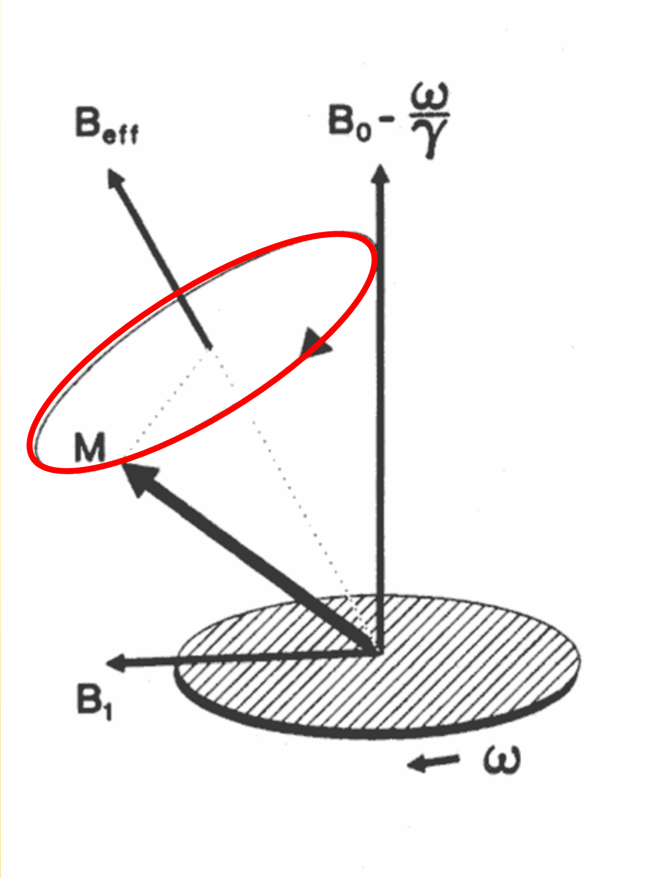
What is a pulse



.... while the other is almost static in the rotating coordinate system, it is „on resonance“ or at least close, it is called the B_1-field

What is a pulse



In the rotating coordinate system we then have a weakened static B_0 field and a relatively strong B_1 field which ends up being of similar strength close to resonance. The movement of the magnetization is now determined by the new „effective field", B_{eff}

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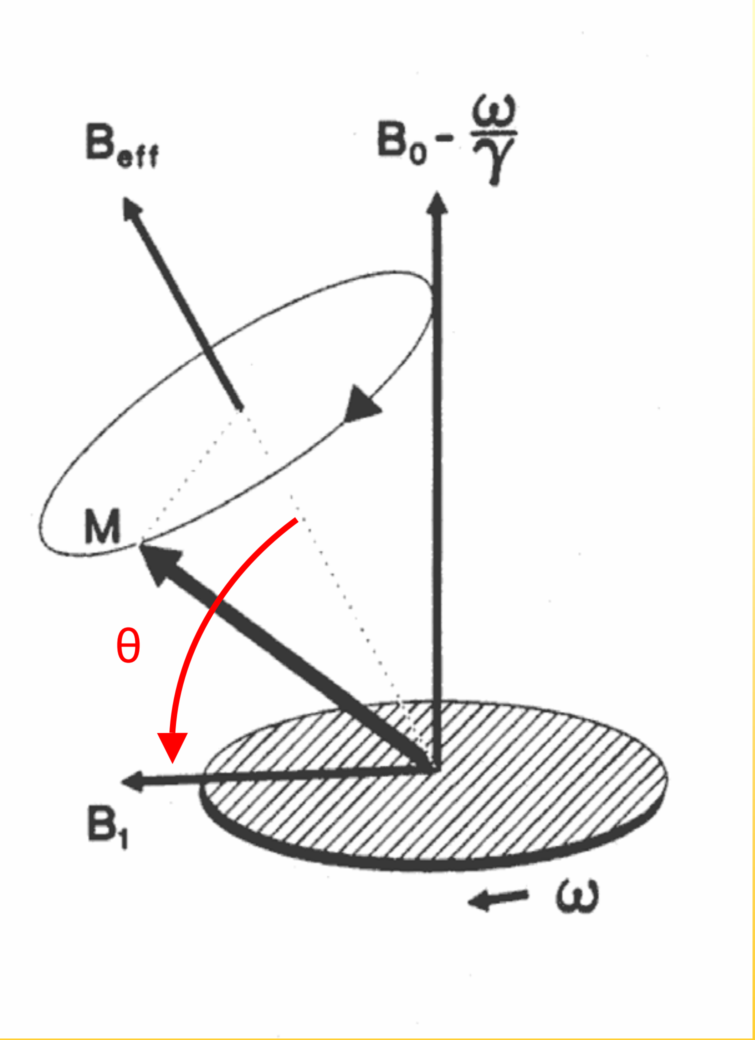
What is a pulse



Strength and tilt angle of the effective field B_{eff} are easily calculated

$$B_{eff} = \sqrt{(B_1)^2 + (B_0 - \omega/\gamma)^2}$$

$$\gamma B_{eff} = \sqrt{(\gamma B_1)^2 + \Omega^2}$$

$$\tan\theta = \frac{(B_0 - \omega/\gamma)}{B_1} = \frac{\Omega}{\gamma B_1}$$

$$B_{eff} \geq B_1$$

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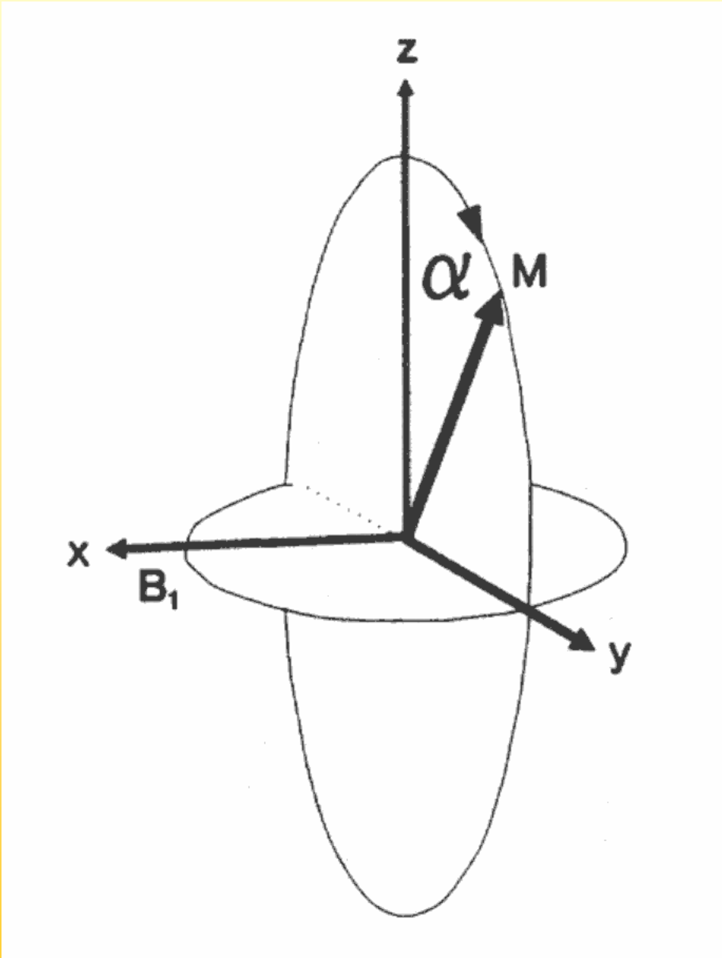
What is a pulse



If the frequency of the pulse matches that of the spin („on resonance") the main field vanished and the precession is only around the B_1-field.
The angle α is determined by the length and the strength of the pulse: A typicla value for a 90° Puls is 10 μsec

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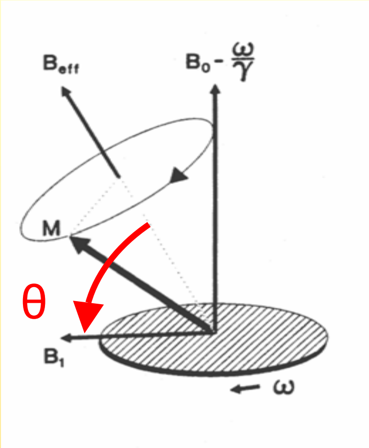
What is a pulse

frequency 600 MHz

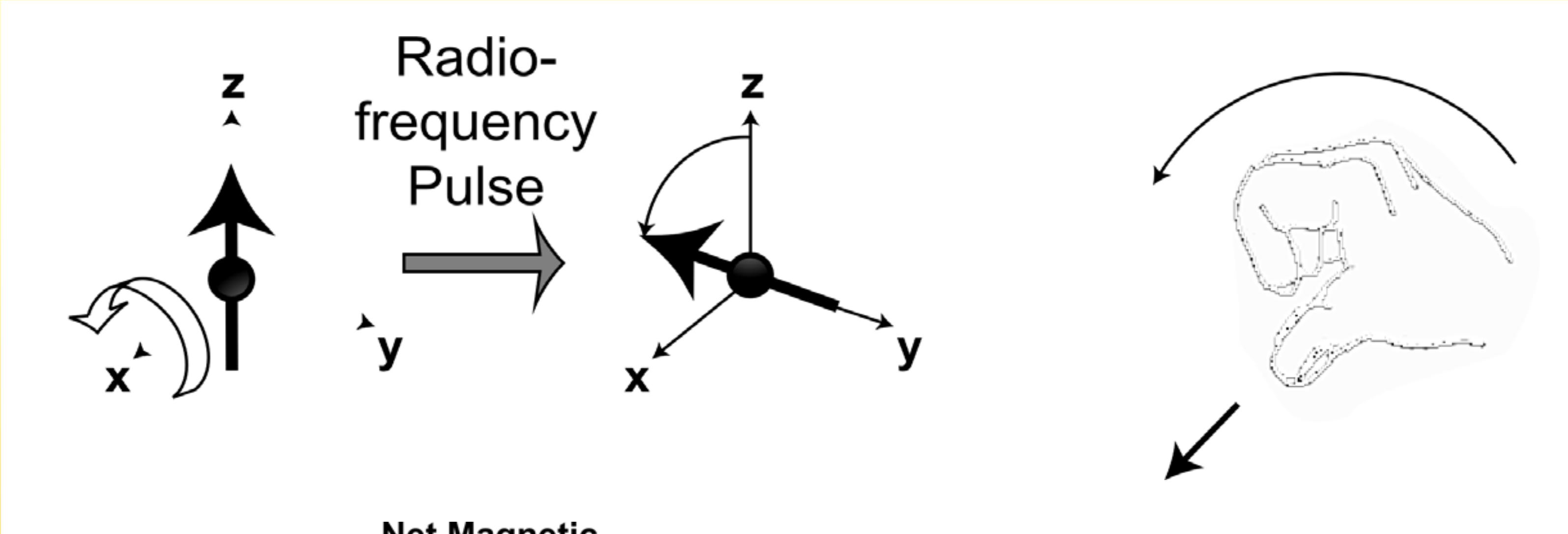
90° Puls = 10 μsec

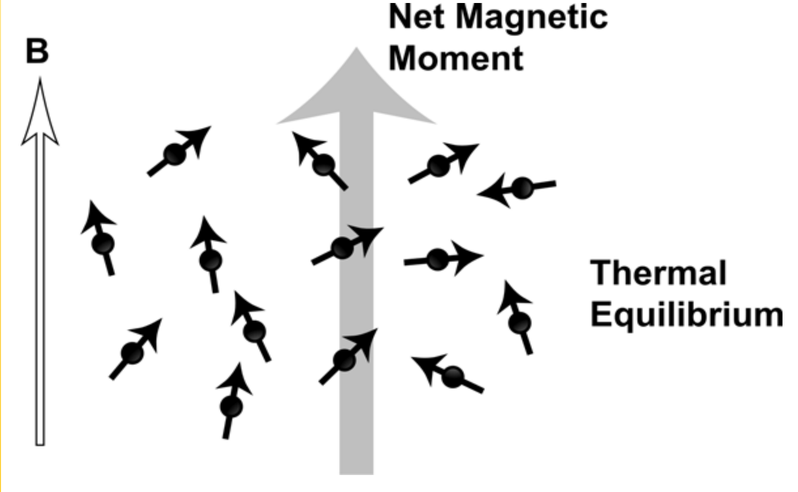
γB_1 = 25 kHz



3000 Hz 10 ppm	0 Hz 5 ppm	-3000 Hz 0 ppm
	on-resonance	
Ω = 3000	Ω = 0	Ω = -3000
tan θ = 0.12	tan θ = 0	tan θ = -0.12
θ = 6.8°	θ = 0	θ = -6.8°

What is a pulse





During a 90° pulse each spin is turned by 90°

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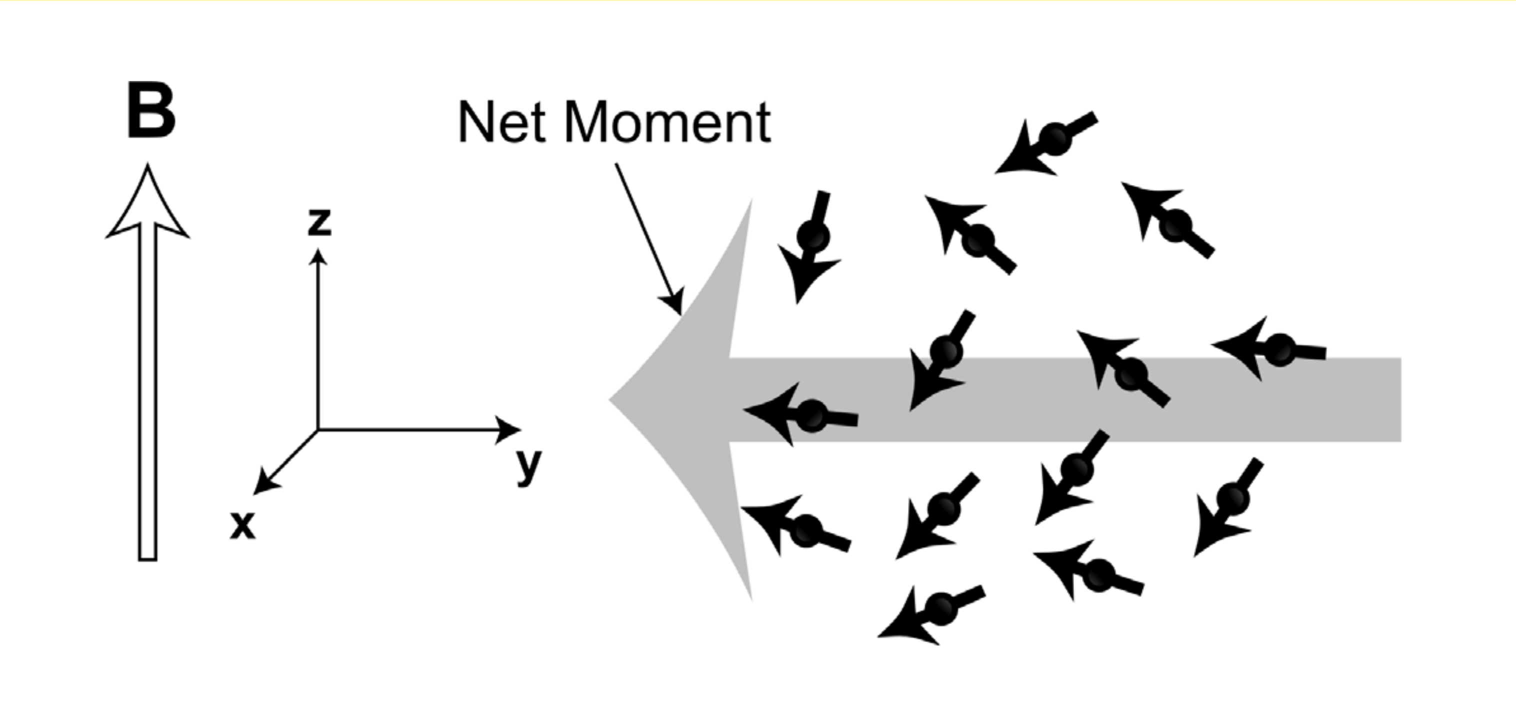
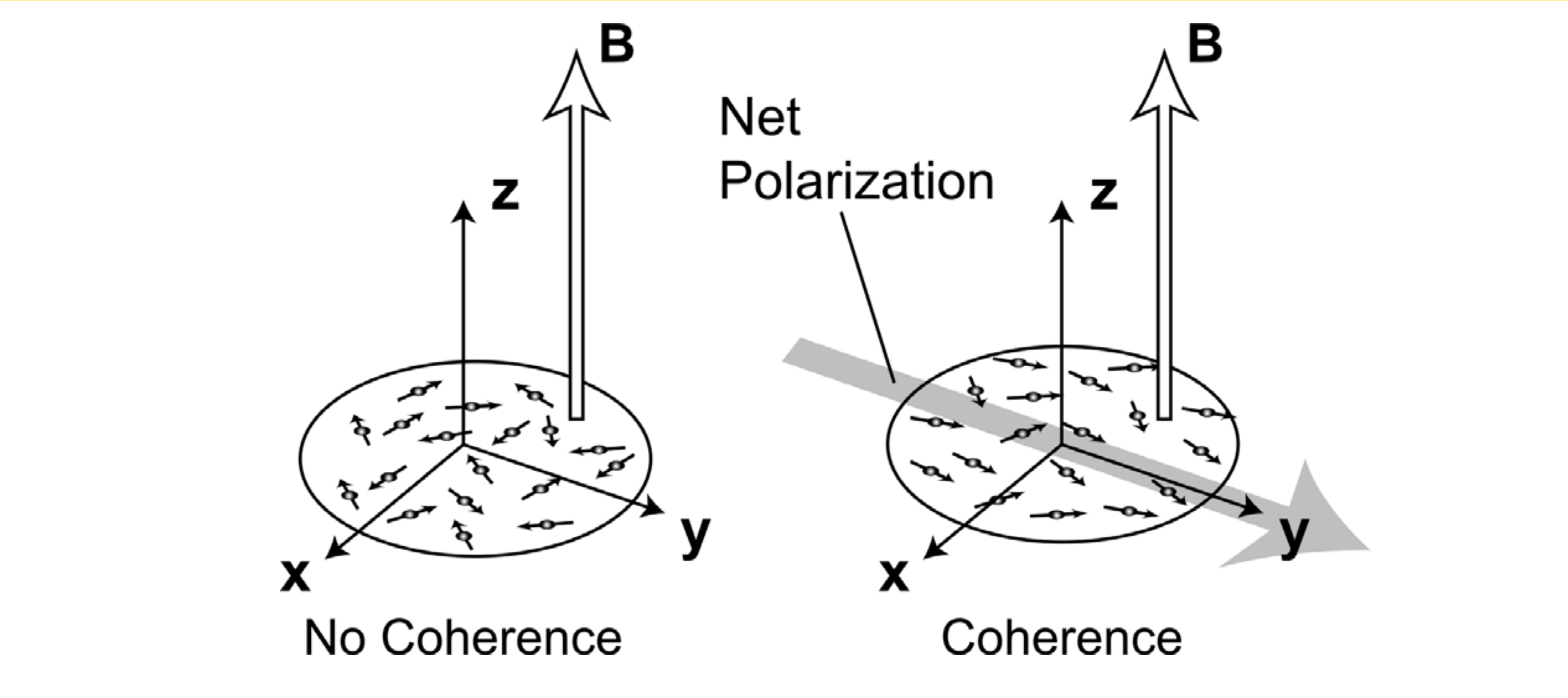
What is a pulse

The resulting magnetic moment is positioned in the x,y-plane, no z-magnetization is present



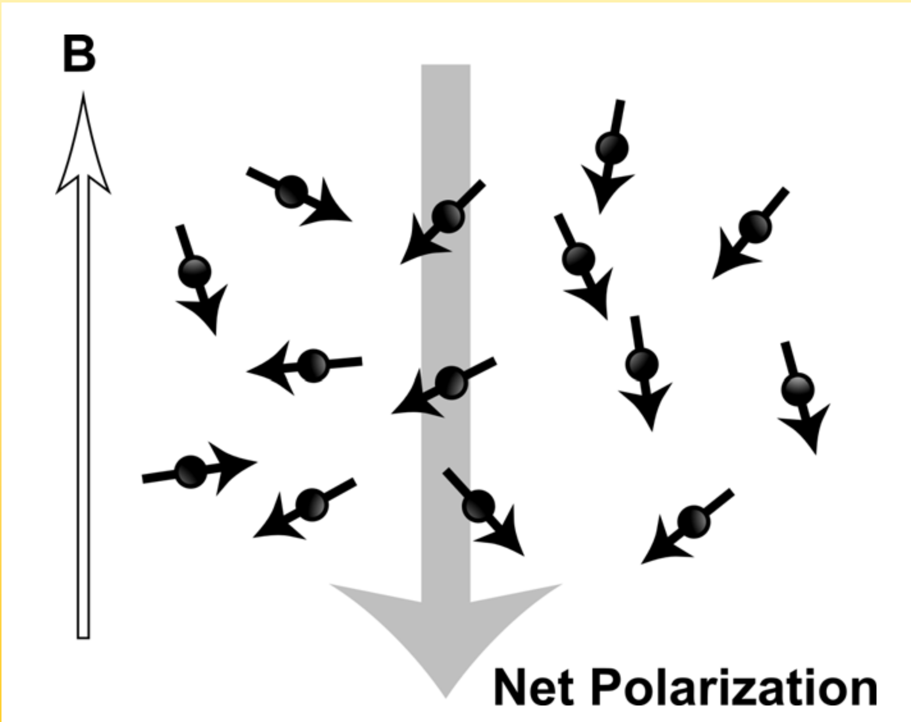
What is a pulse

But: This is not the same as a simple saturation that leads to equal occupation of both energy levels, here we have a coherent movement of the spins.



What is a pulse

If we irradiate longer we go beyond the 90° angle and reach 180° at some point



The vector model

The vector model

The vector model is a semi-classical description of NMR experiments and works well with isolated spins, i.e. spins without scalar coupling.

The mathematical formalism attached to it are the Bloch equations

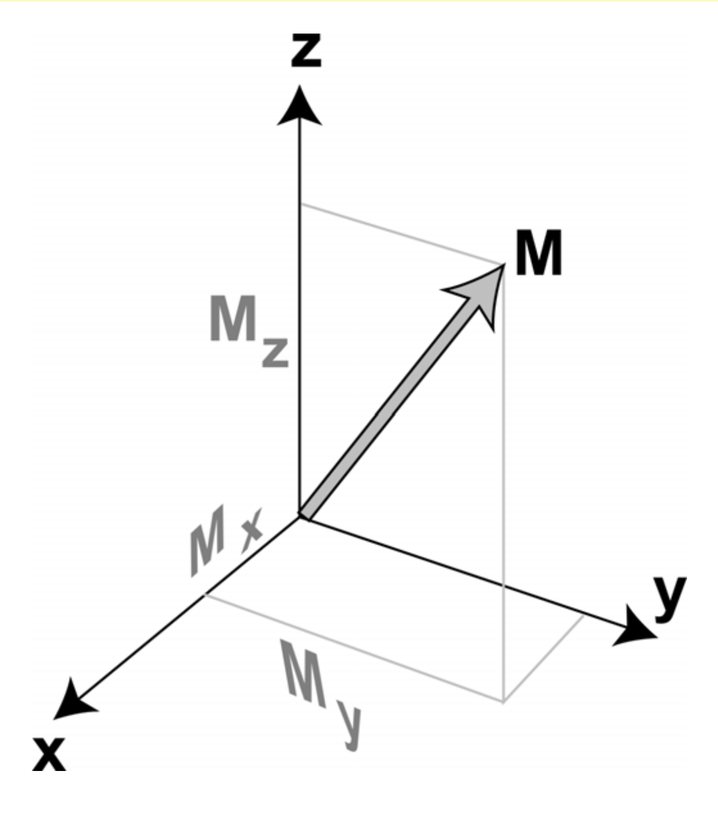
F. Bloch *Phys. Rev.* **70**, 460 – 474 (1946)

The vector model

In case of scalar coupling it is only usefull within certain limits. Since many multidimensional experiments are based on the transfer of magnetization via scalar coupling it is suitable there.

It is, however, well suited for the description of simple 1D pulse sequences and relaxation phenomena.

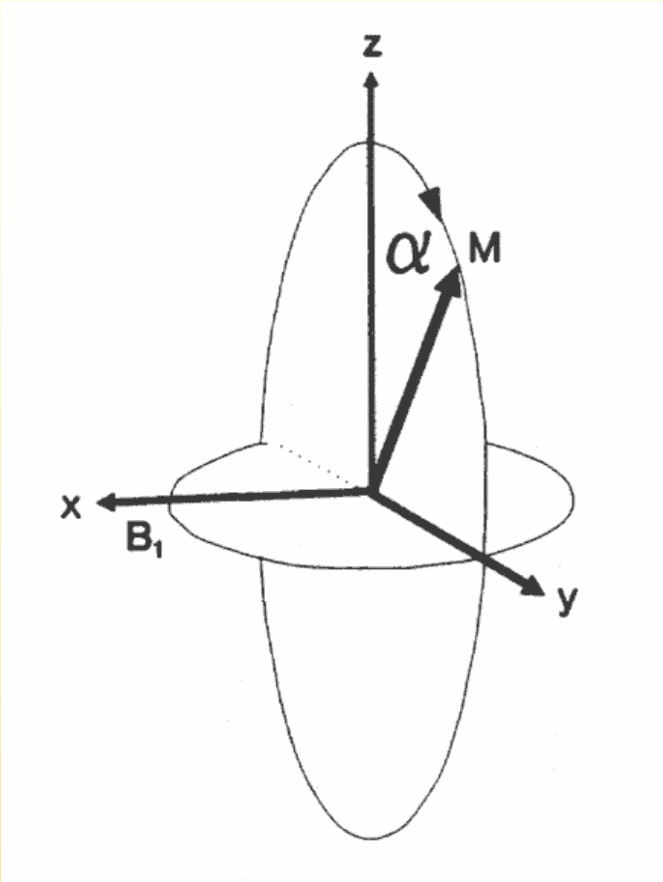
The vector model



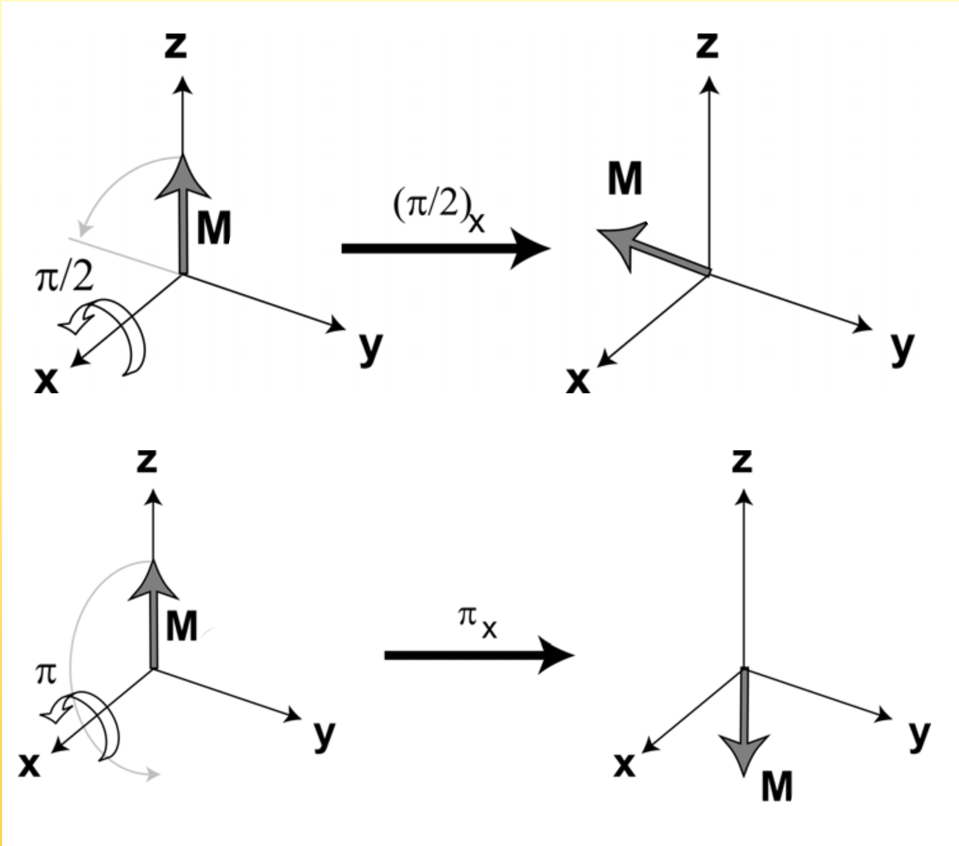
Magnetization is considered to be a vector that can be manipulated following the rules of vector manipulation using magnetic fields

The vector model

The first kind of magnetic field are pulses



The vector model

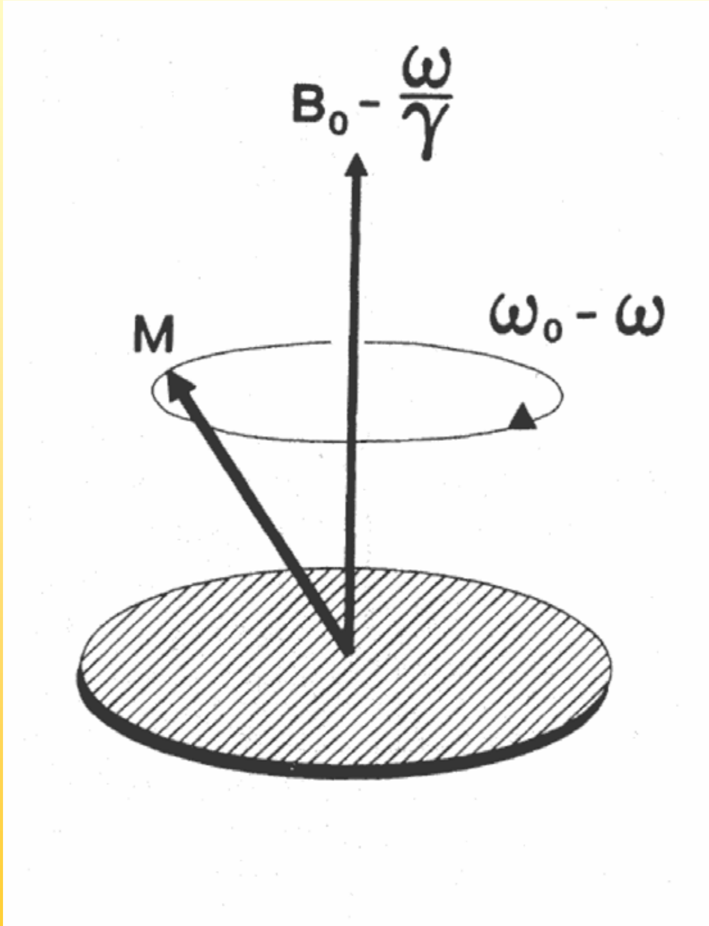


90° pulse from x: the vector turns around the x-axis to (-y)

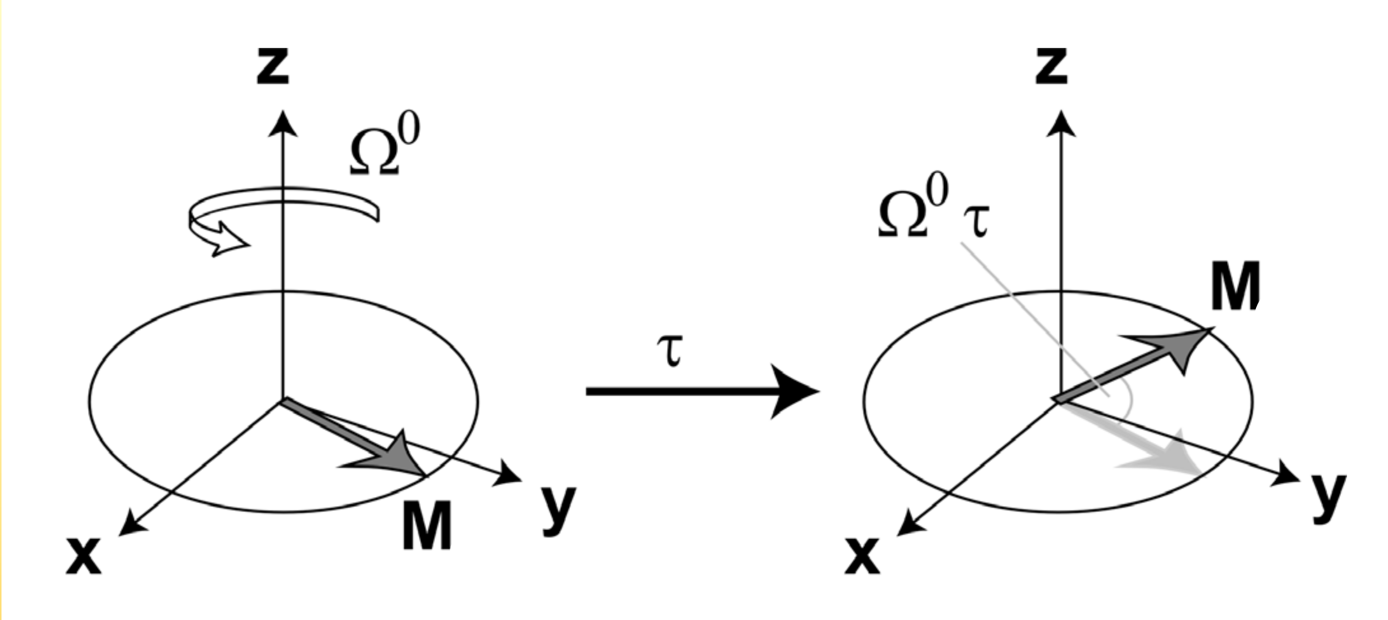
180° pulse from x: the vector turns around the x-axis to (-z)

The vector model

The second kind of magnetic field is the main or B_0-field, that causes chemical shift and scalar coupling



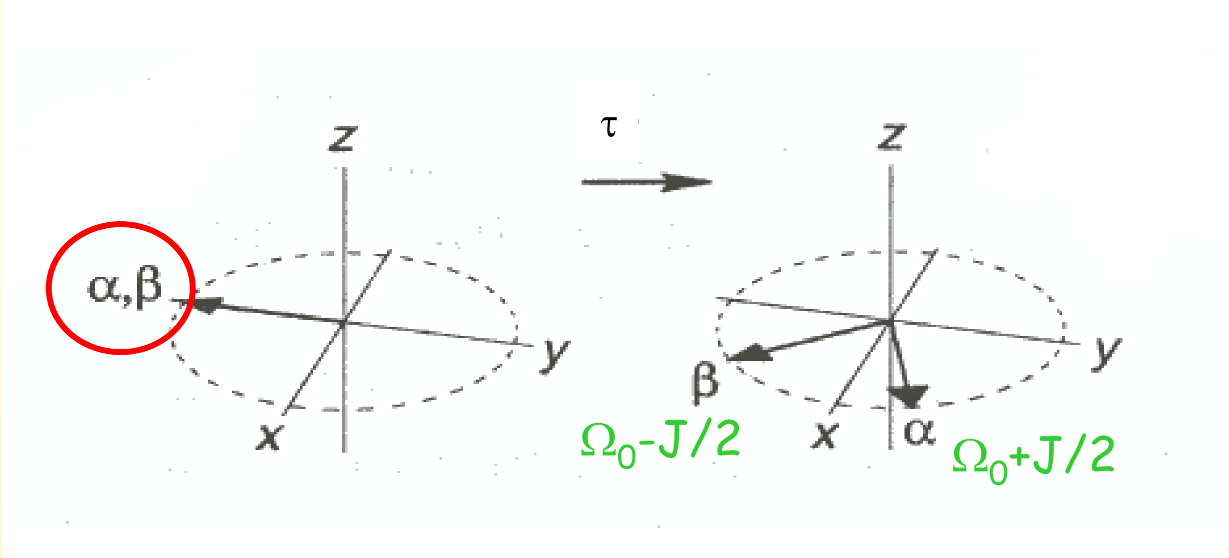
The vector model



Chemical shift only acts on x,y-magnetization and causes a rotation around the z-axis with a frequency Ω_0 (i.e. the chemical shift)

The vector model

α,β characterises the spin state of the „other" spin



Scalar coupling acts on x,y-magnetization as well and causes a rotation around the z-axis. The vector is split in two, one is moving J/2 faster, the other slower than Ω_0

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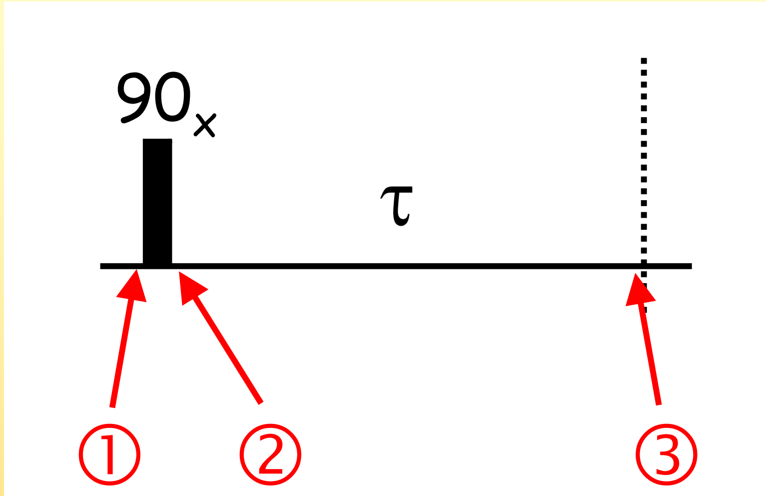
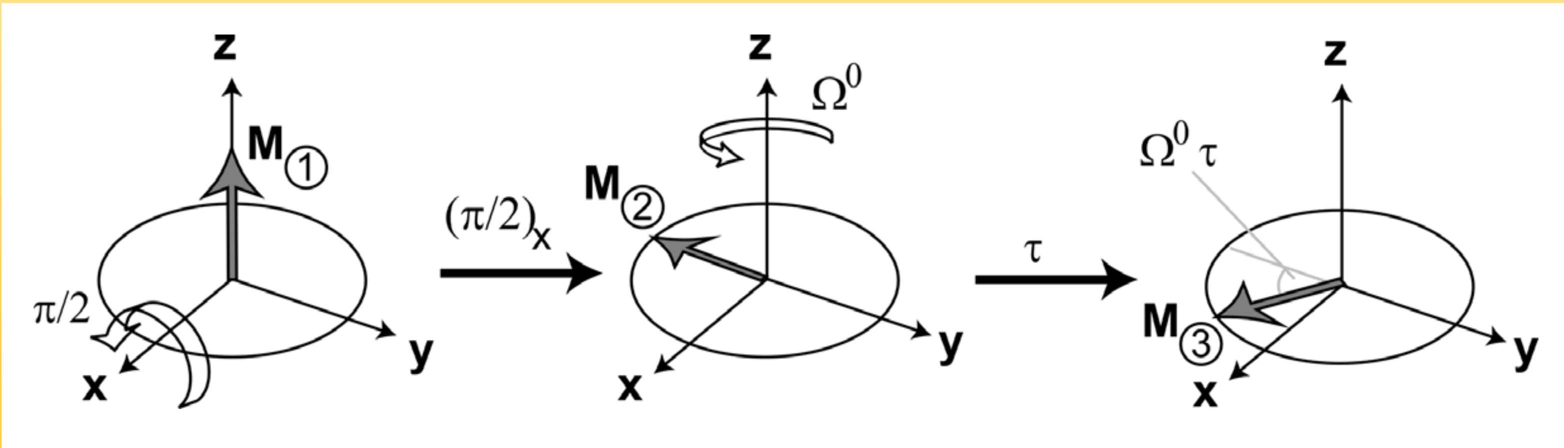
The vector model

With these tools we can now start to analyze simple NMR sequences which are often building blocks of more complicated experiments





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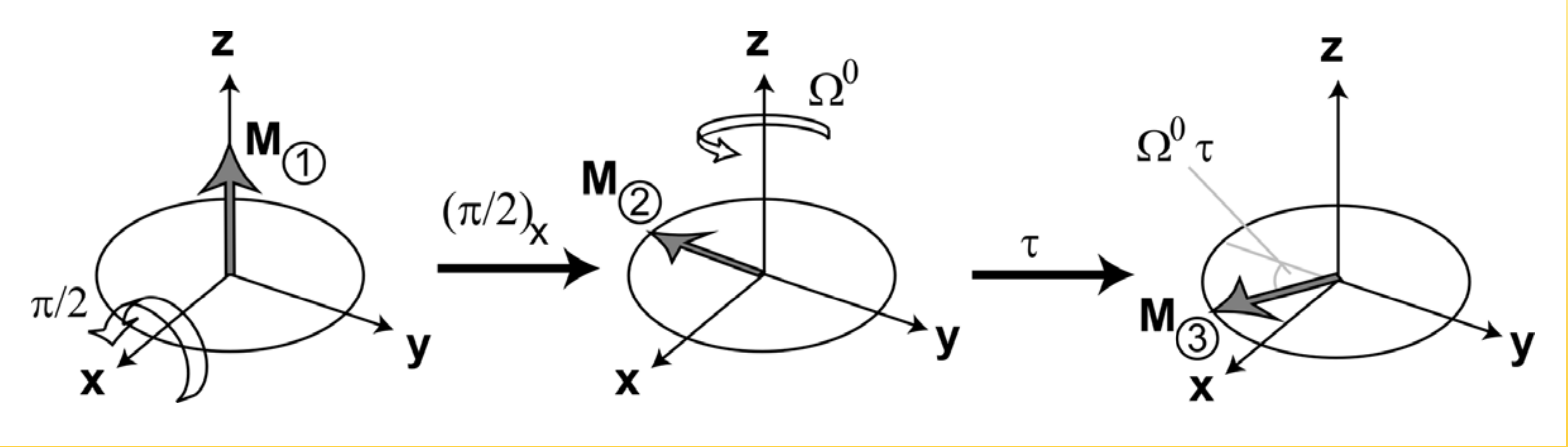
The vector model

Spins with different chemical shifts reach different positions during the delay τ. The will thus have different phases and no phase correction will be possible.



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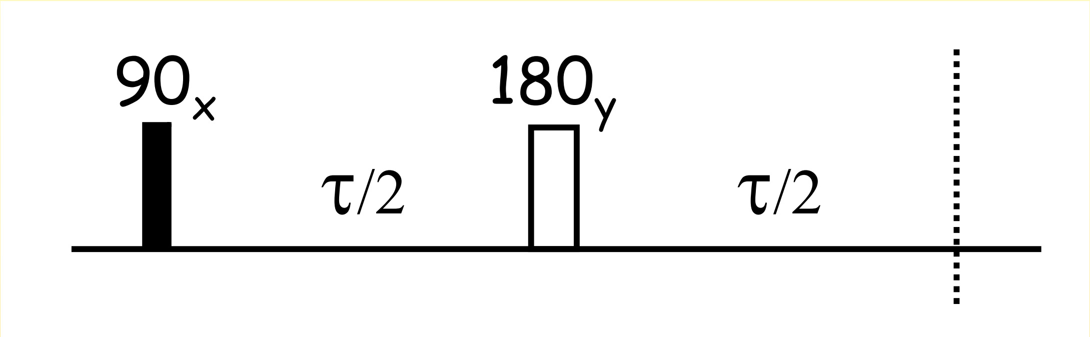
The vector model



A simple sequence to prevent the evolution of chemical shift is the „spin echo“ that is also an important building block in many advanced experiments

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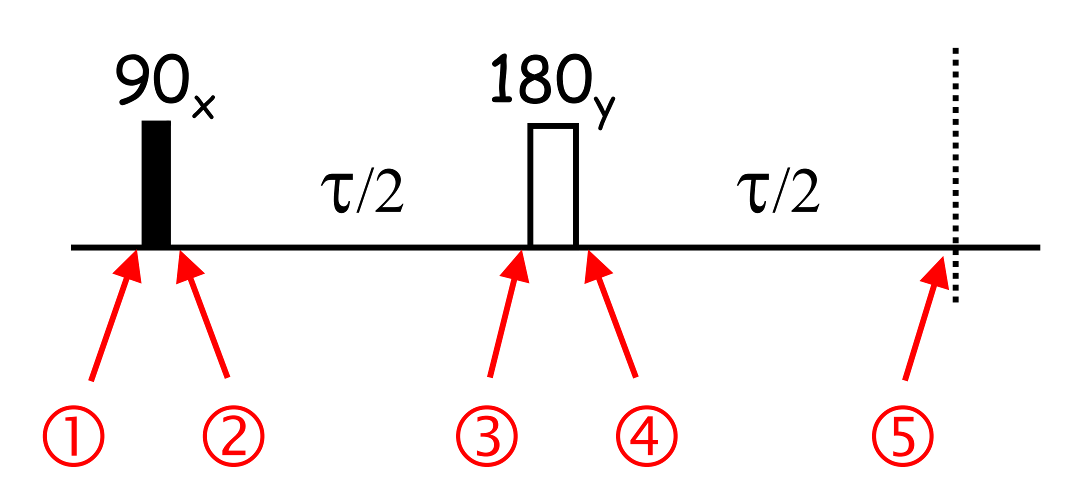
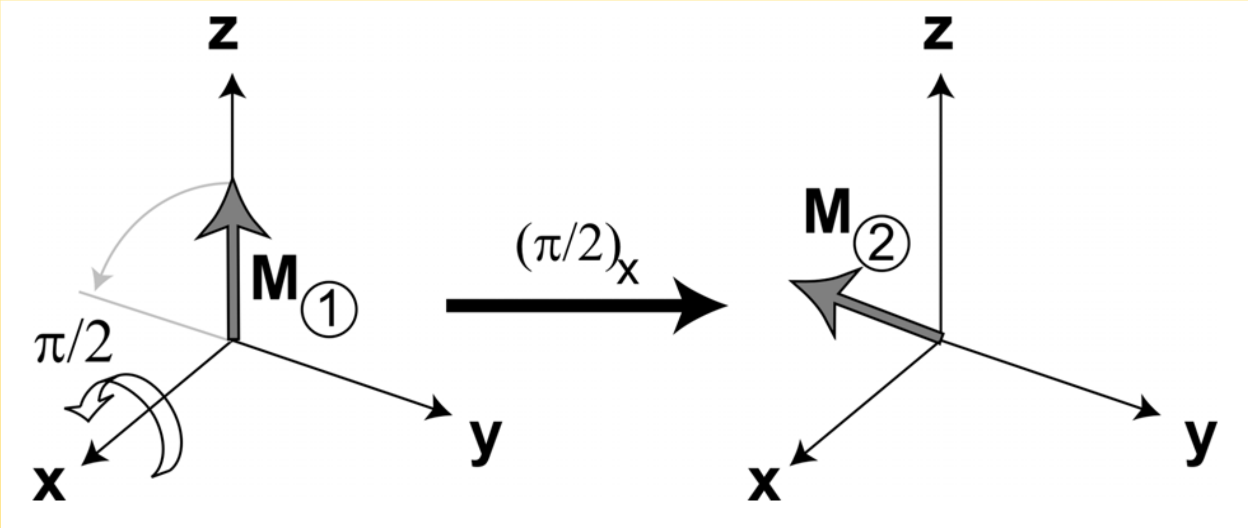
The vector model





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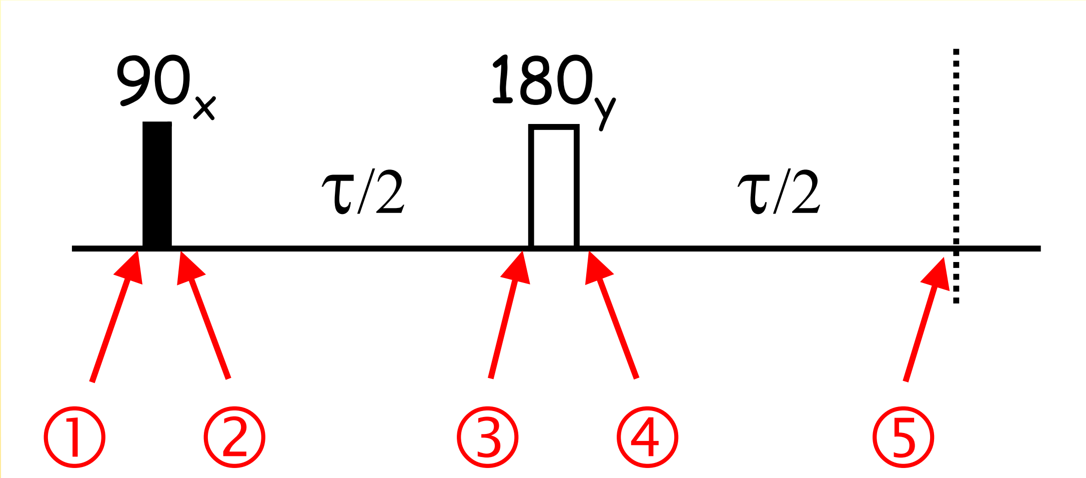
The vector model



We will first only consider chemical shift and will treat scalar coupling seperately

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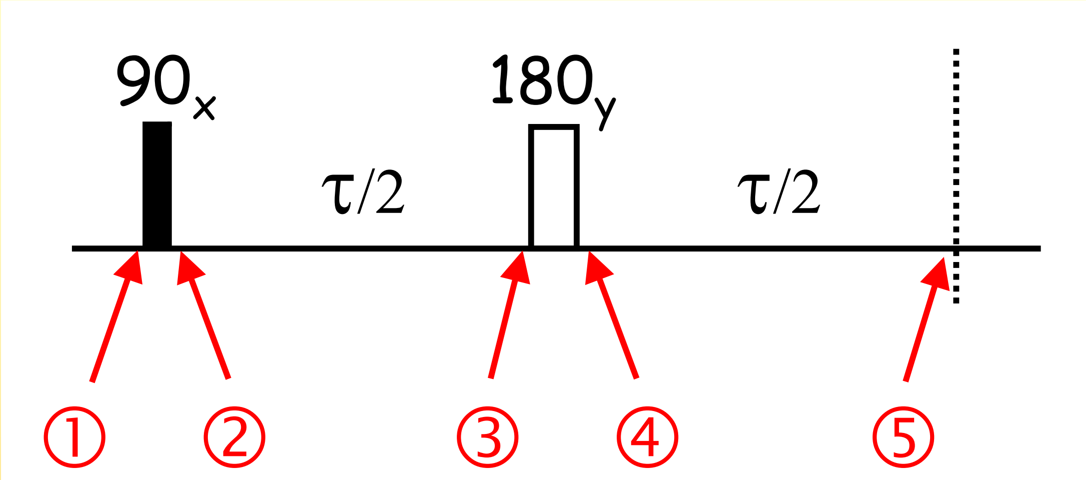
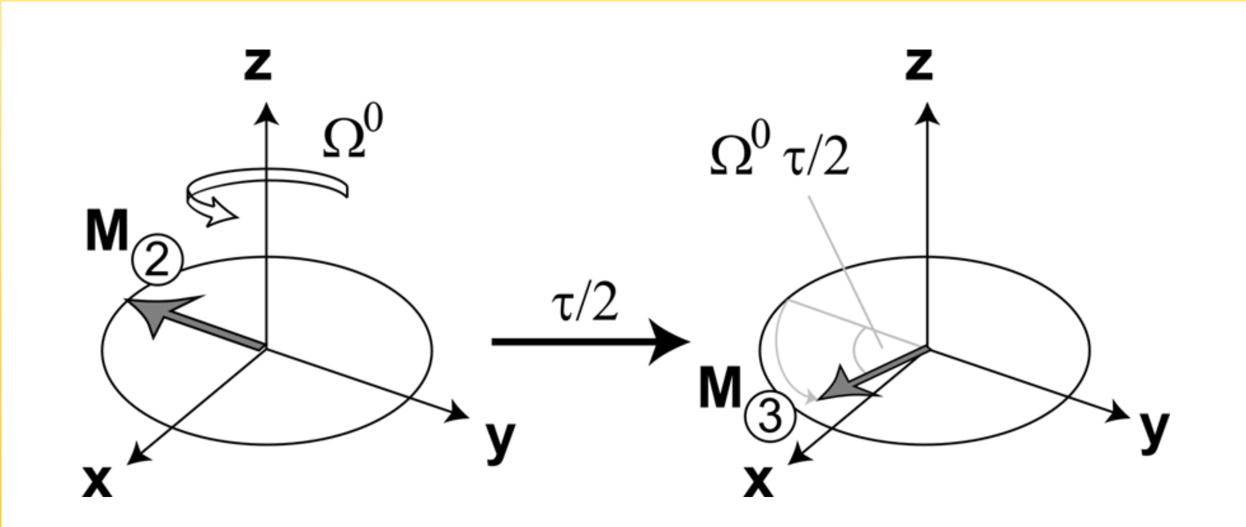
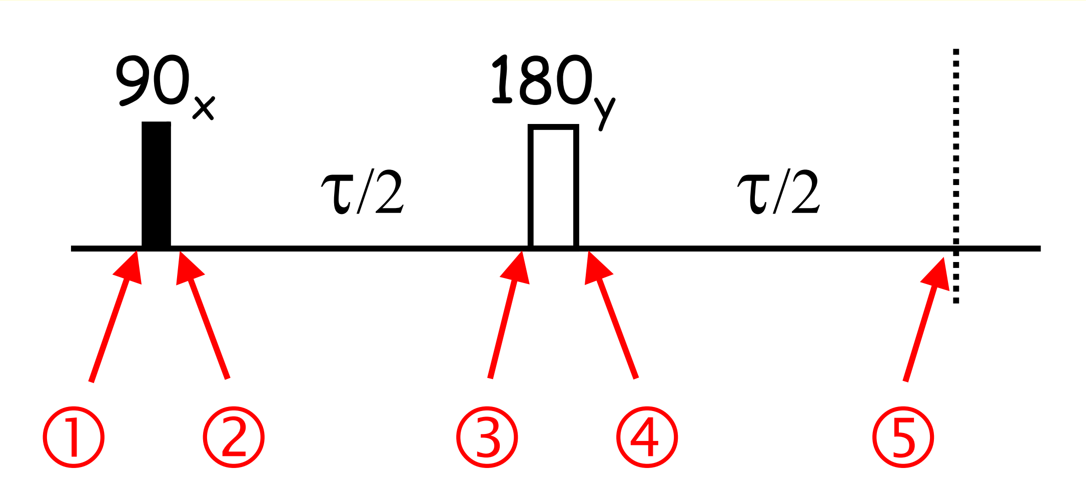
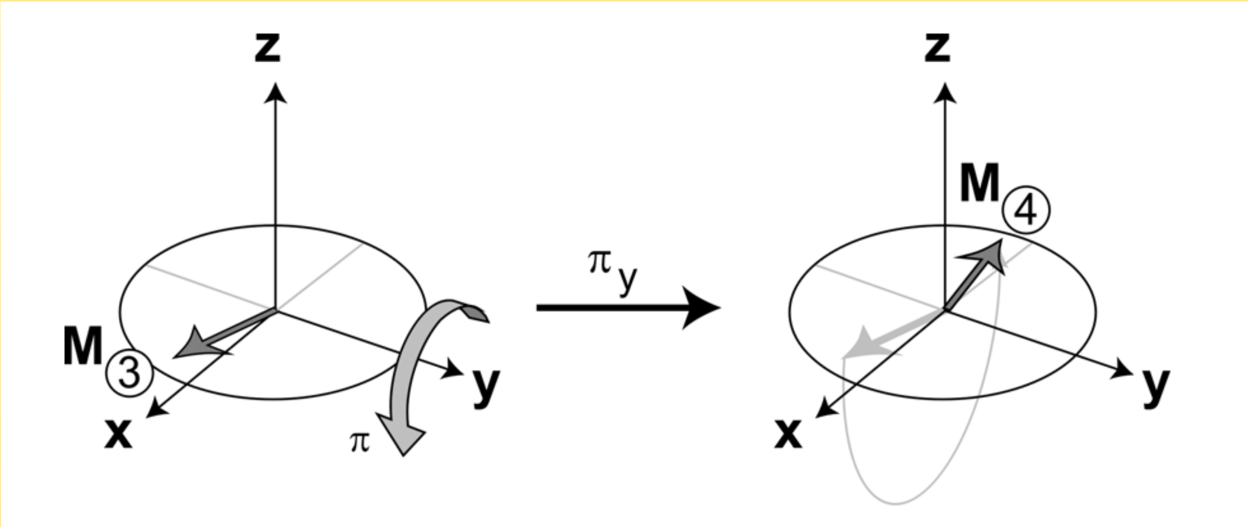
The vector model





The vector model





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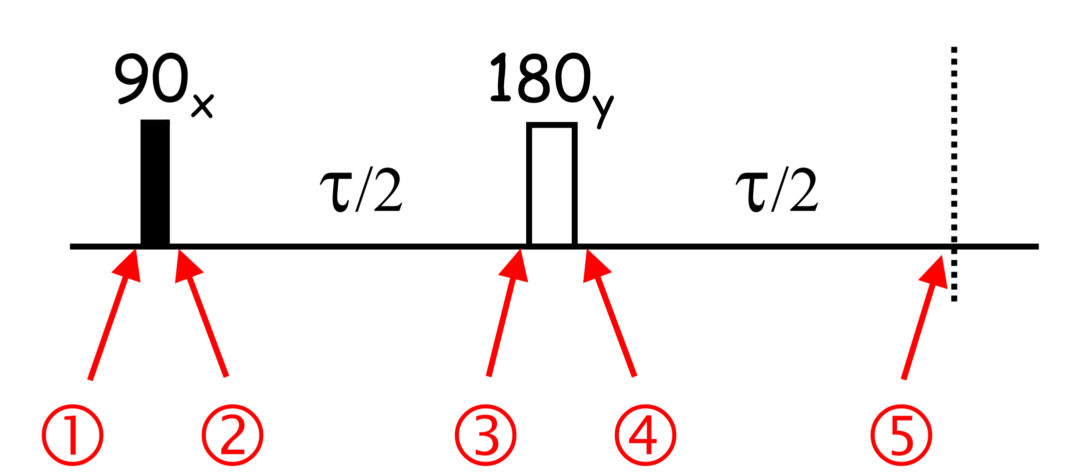
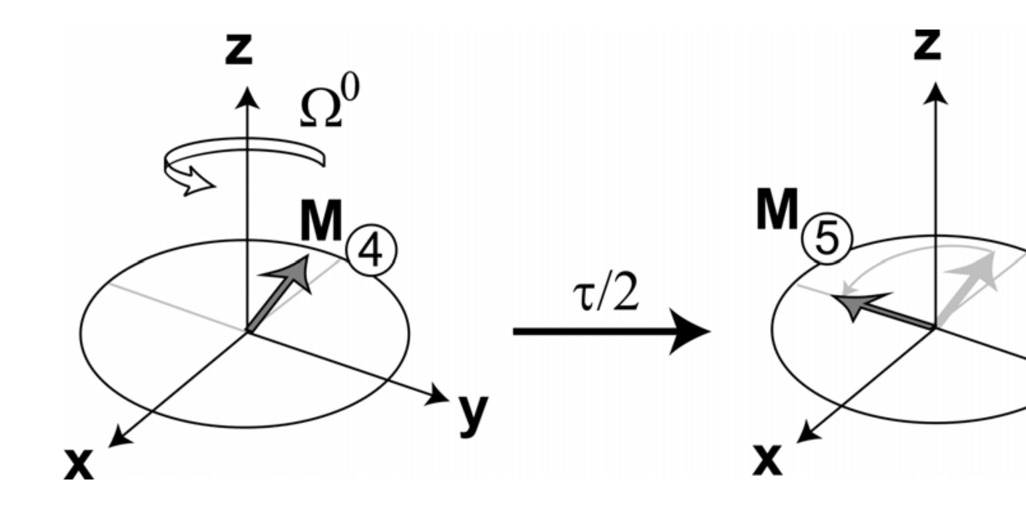
The vector model





The effect of chemcal shift has vanished !!

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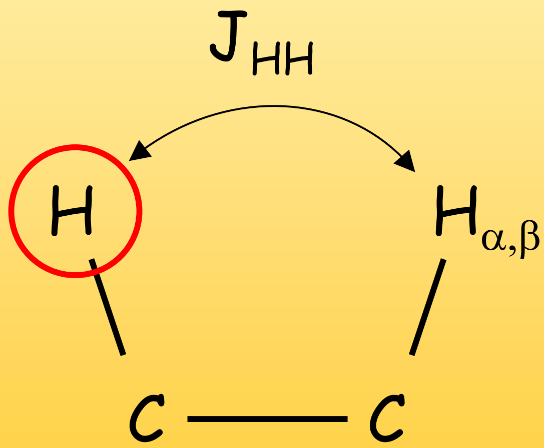
The vector model

Now we consider homonuclear scalar coupling. A nucleus „sees" the spin state of the other (α oder β) which results in two vectors of slightly different speed. The other spin experiences the 180° pulse as well



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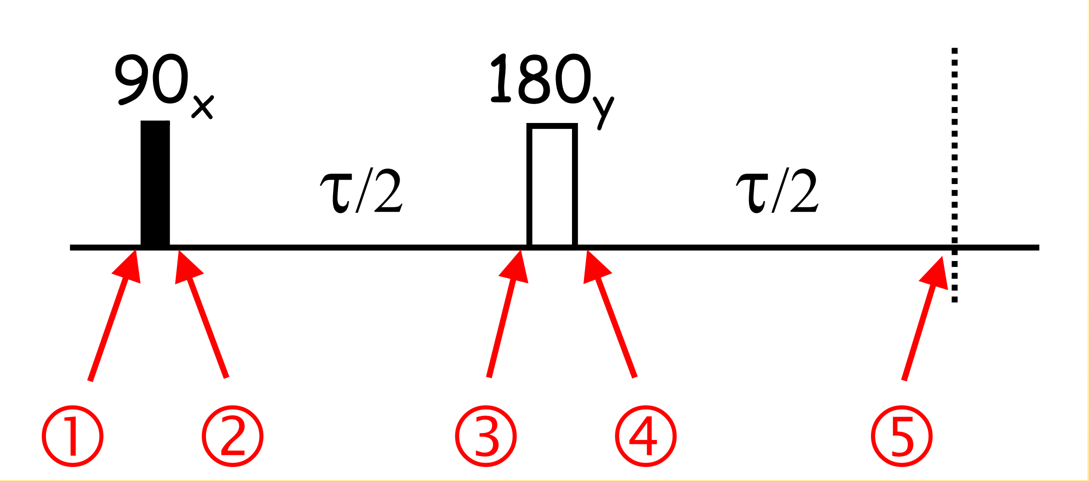
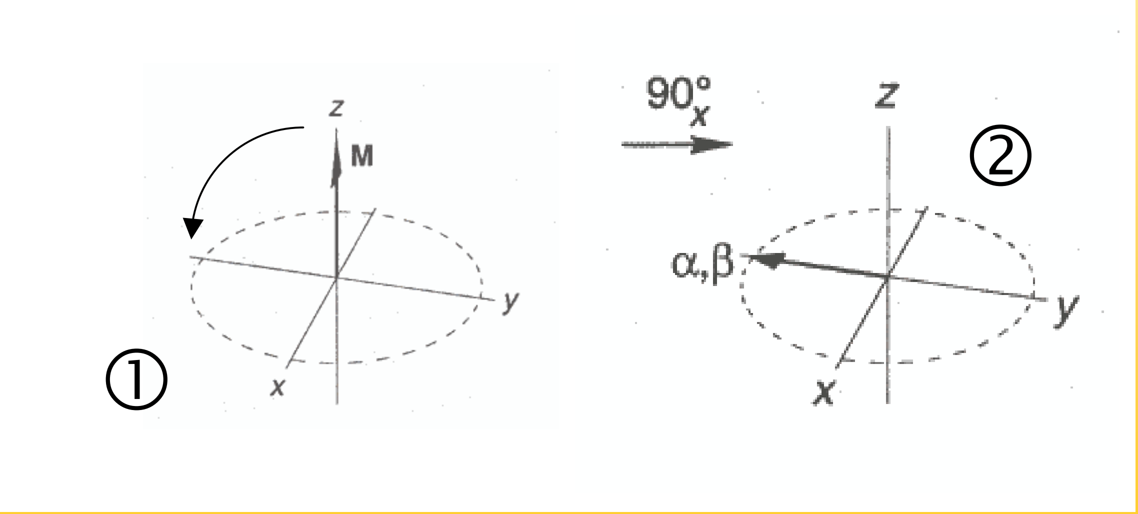
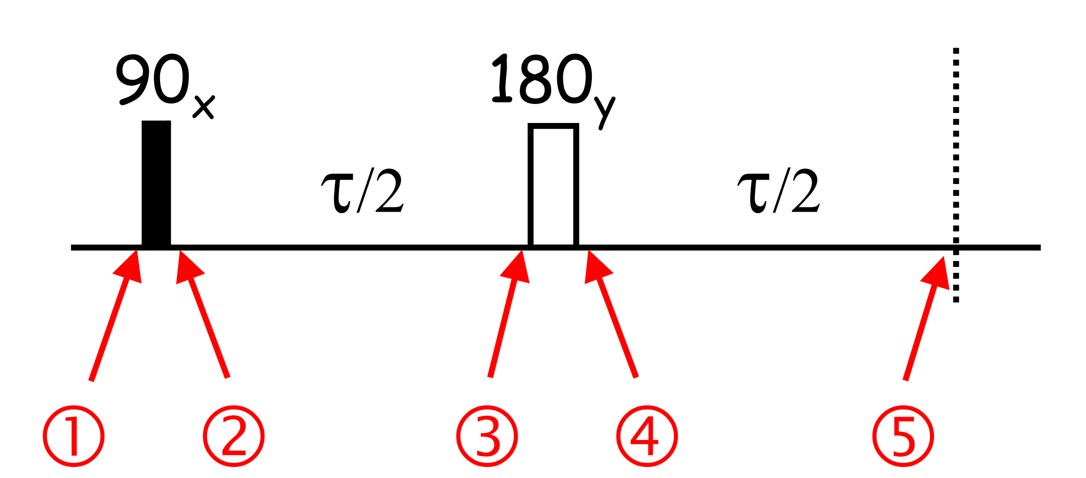
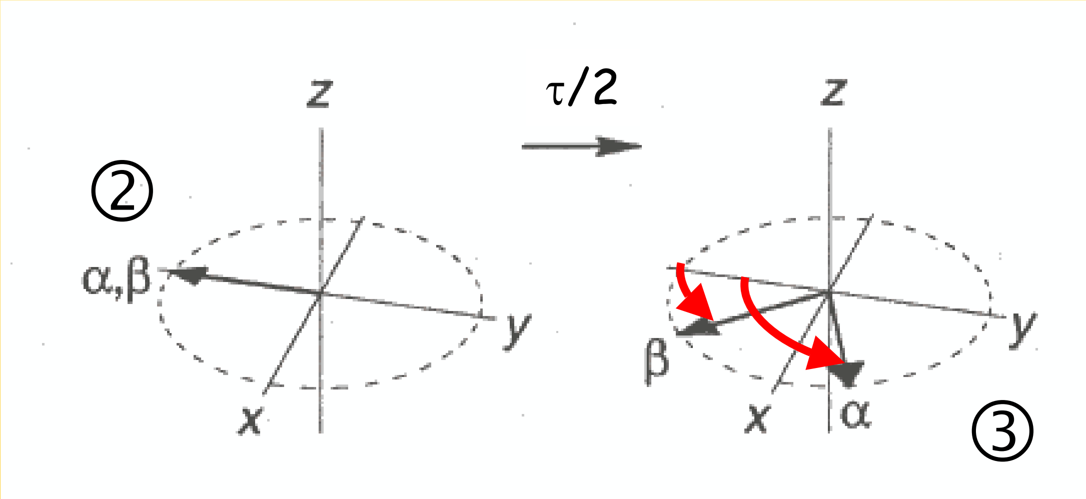
The vector model





The vector model





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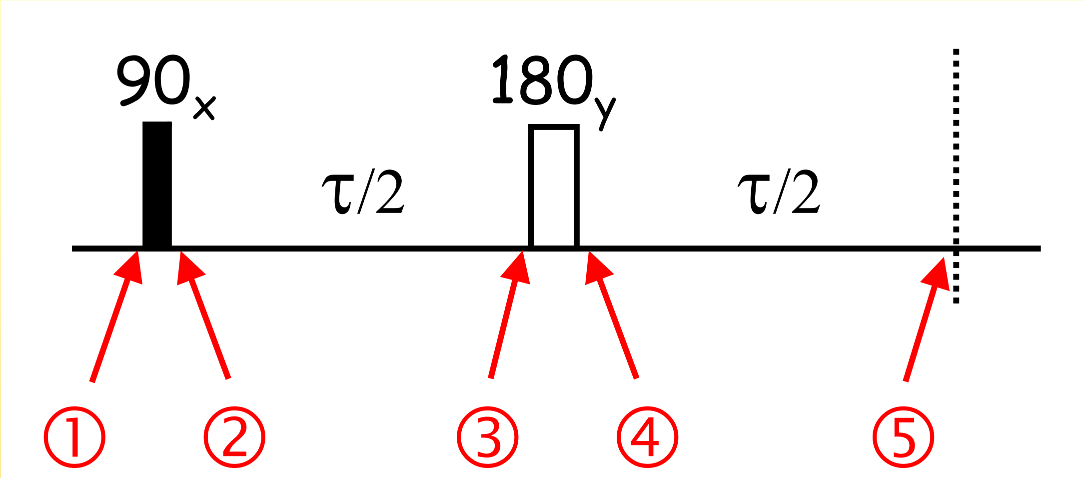
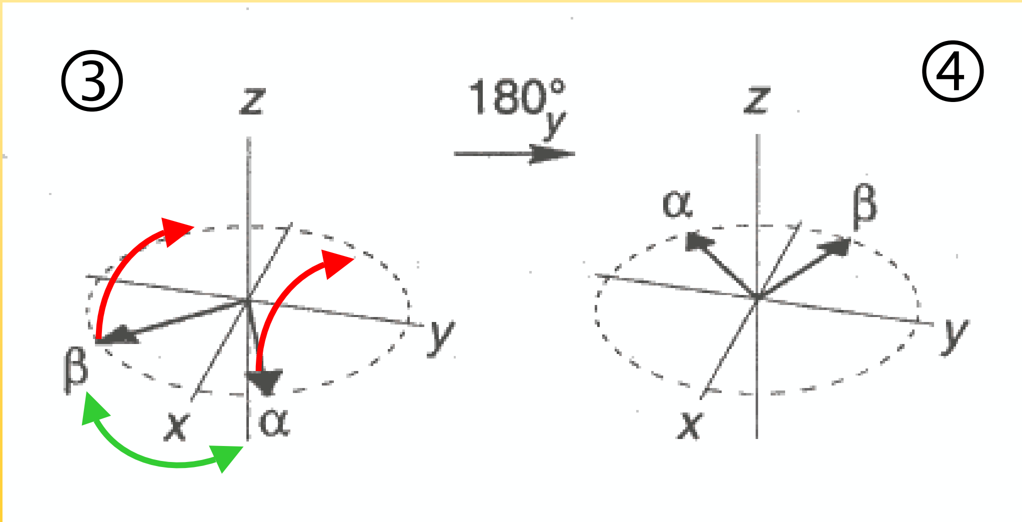
The vector model





α,β are exchanged

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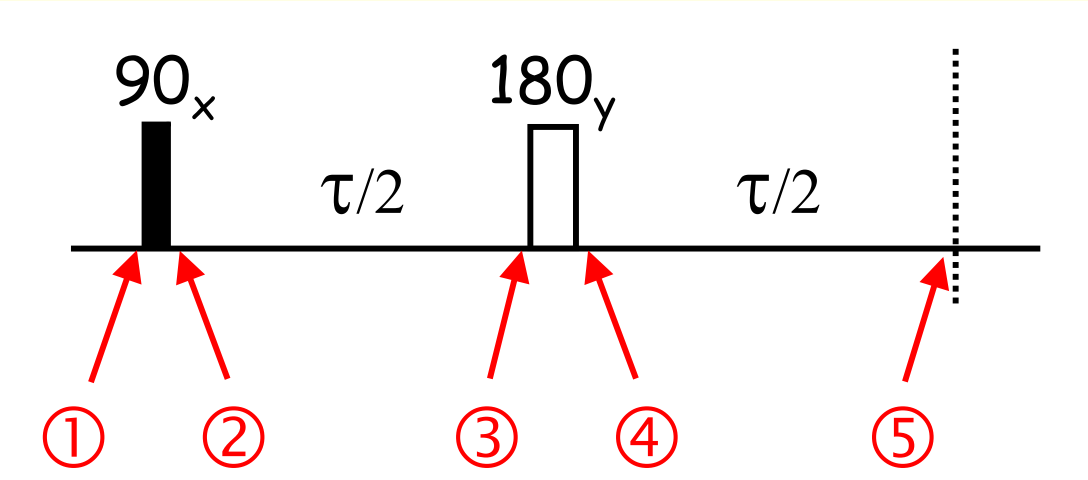
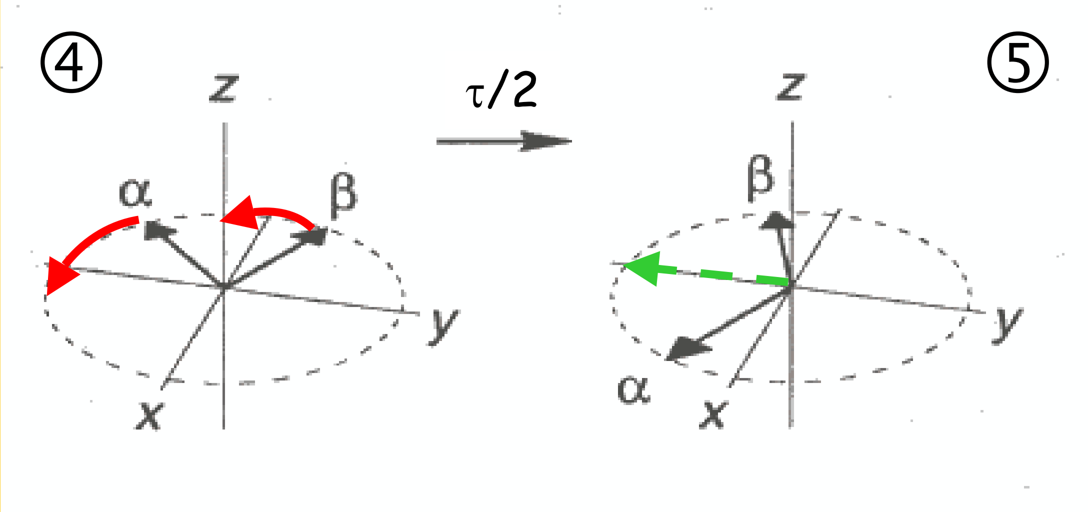
The vector model





While the effect of chemical shift is still refocussed the coupling is not

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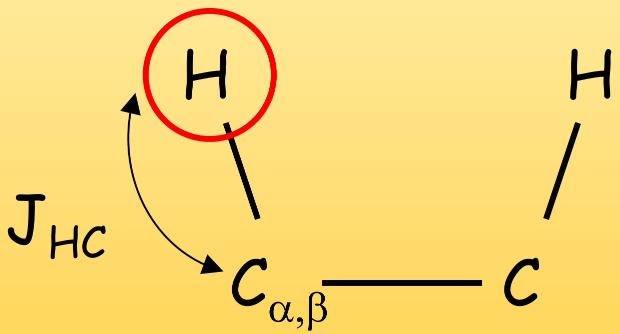
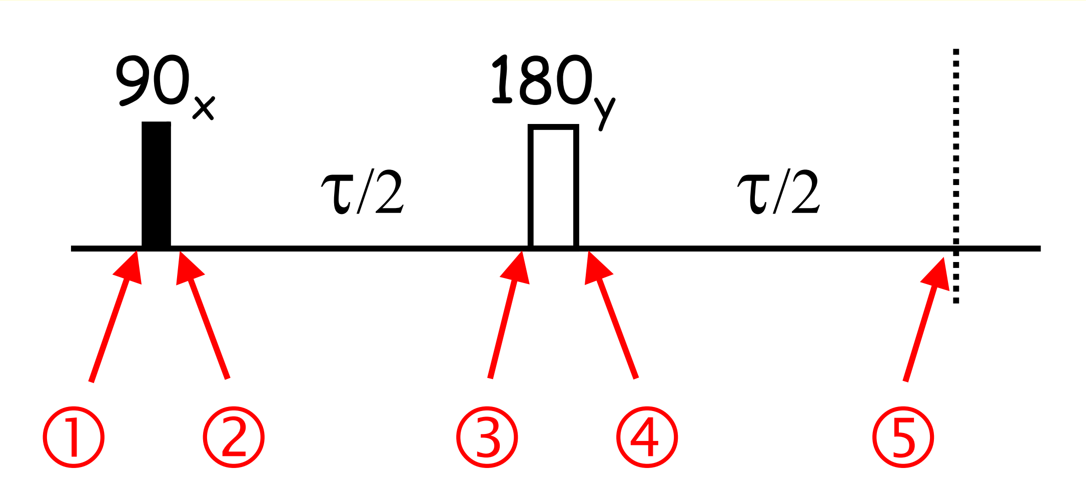
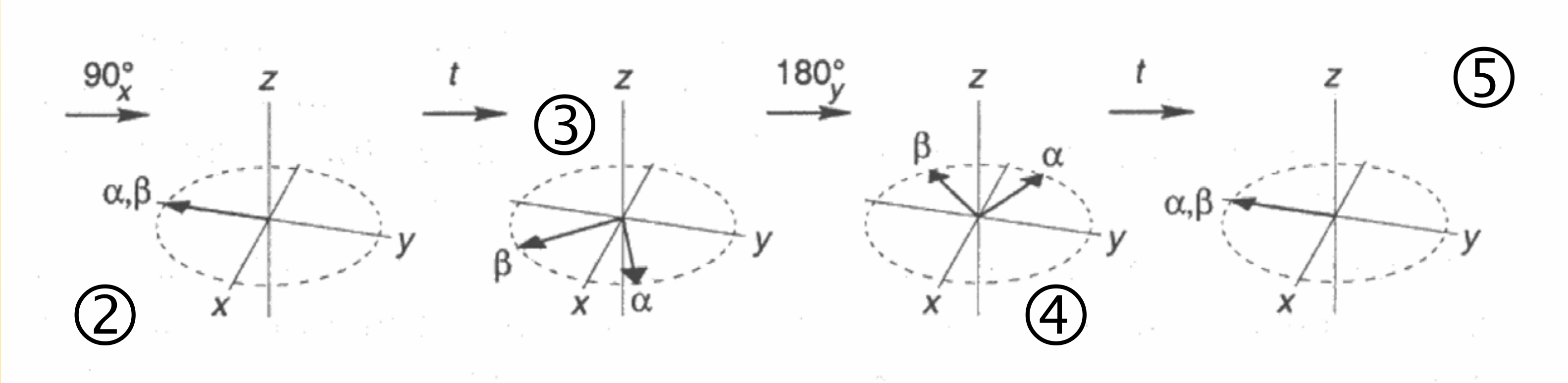
The vector model

In the case of heteronuclear scalar coupling the "other", the coupled spin is not hit by the 180° pulse



The vector model





Now both the effect of chemical shift AND scalar coupling are refocussed !!

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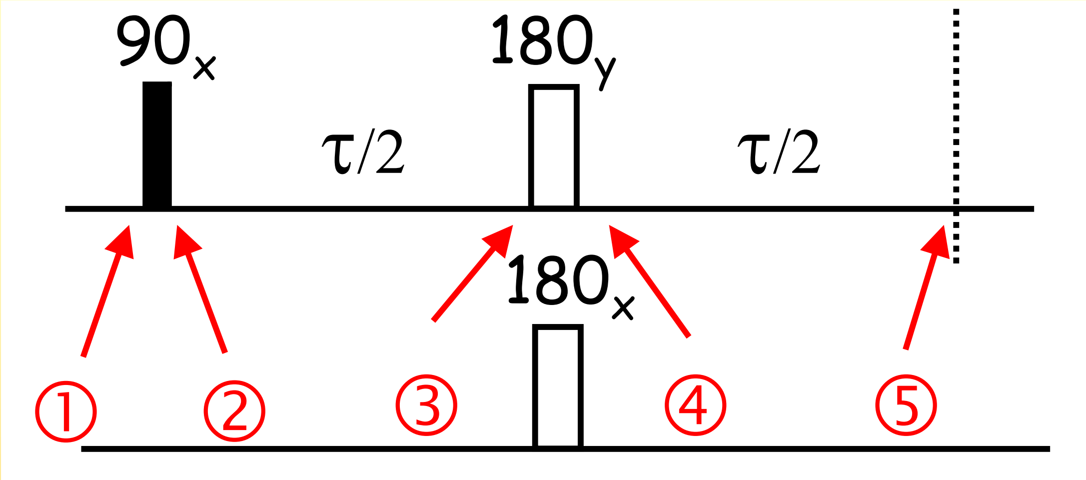
The vector model



To affect the coupled spin we have to add a pulse. Now the the coupled spin is again hit by the pulse, the situation is comparable to the homonuclear case

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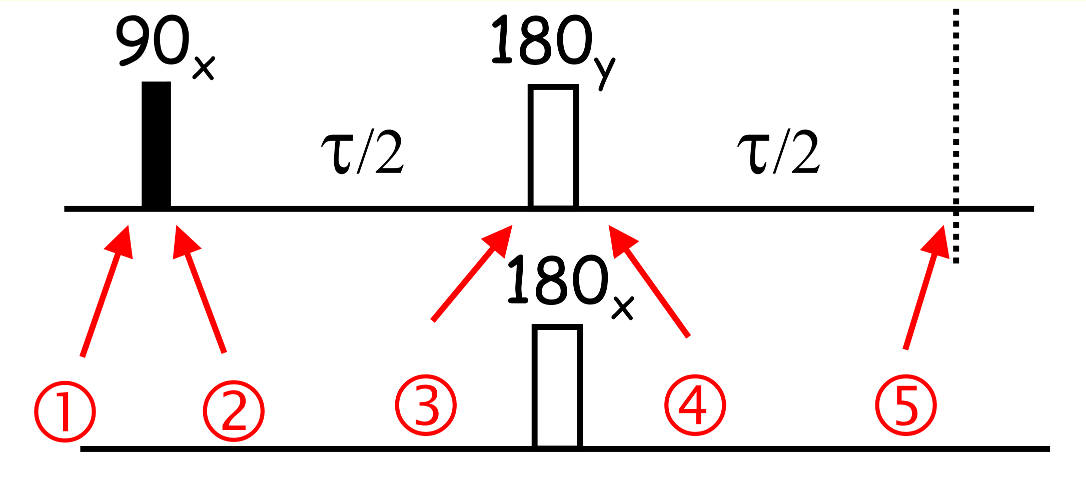
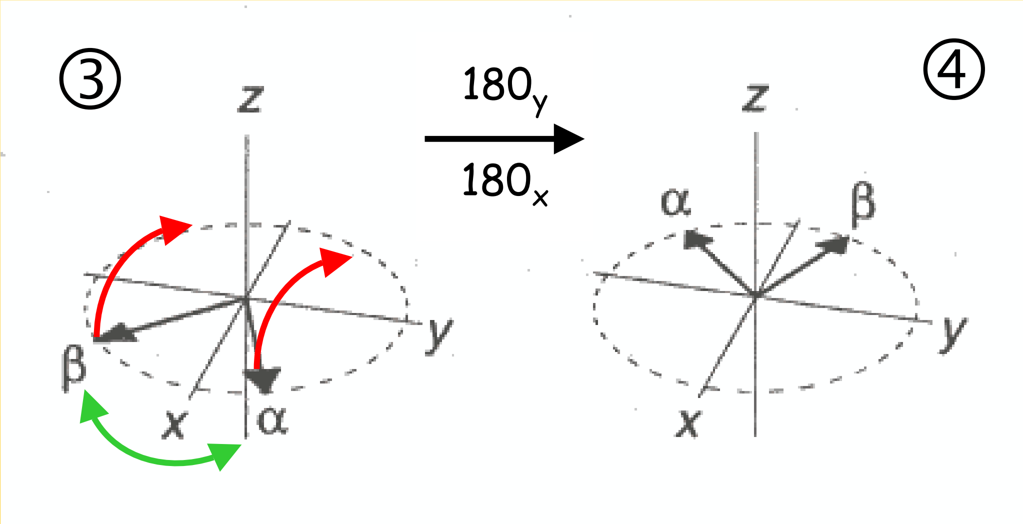
The vector model





α,β are exchanged

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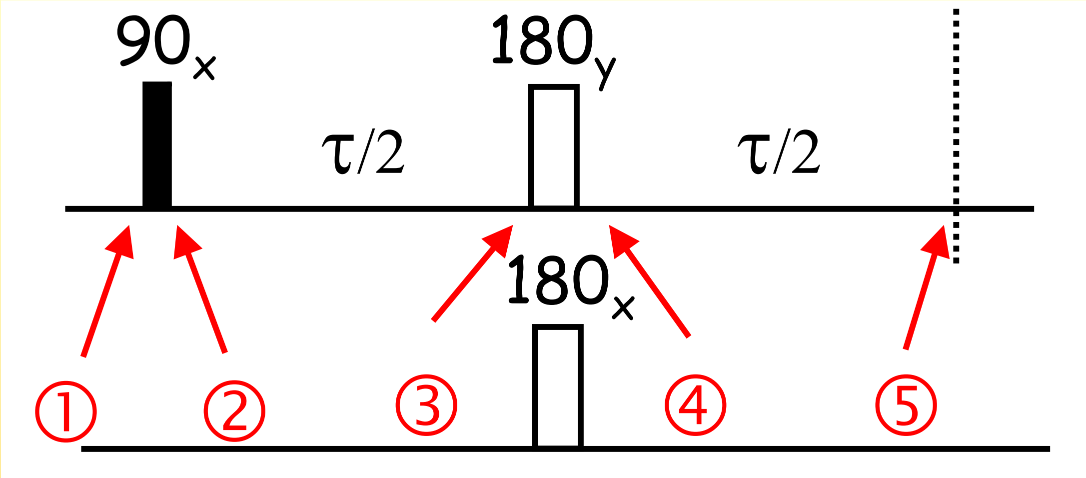
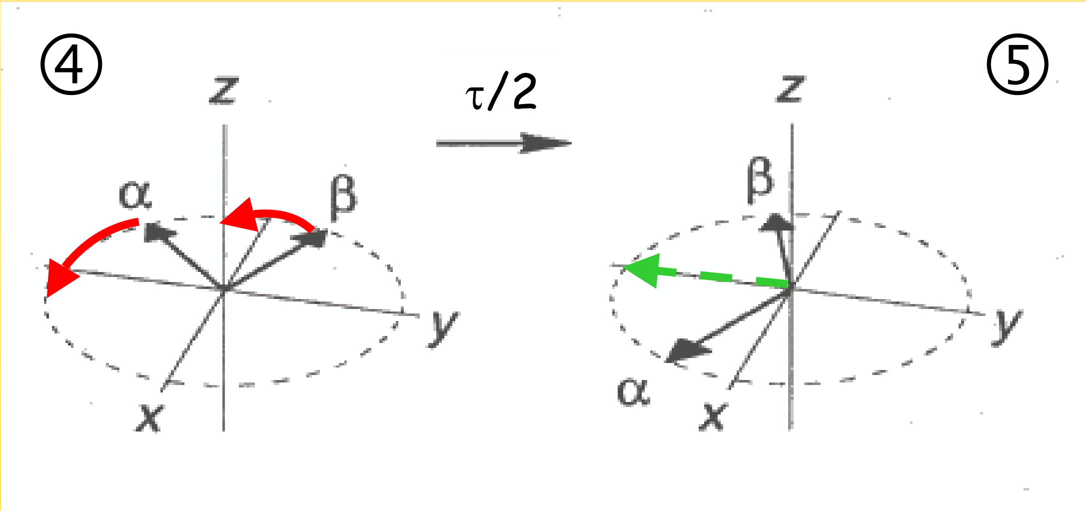
The vector model

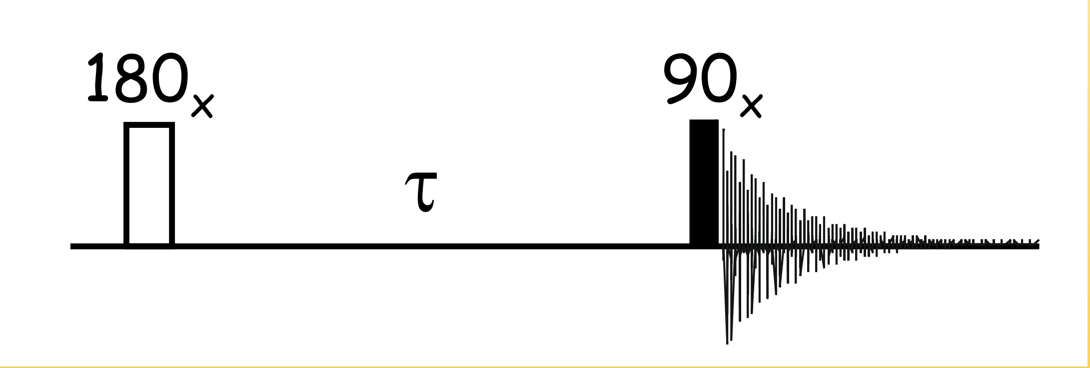




Again, the effect of chemical shift is refocussed but the coupling is not !

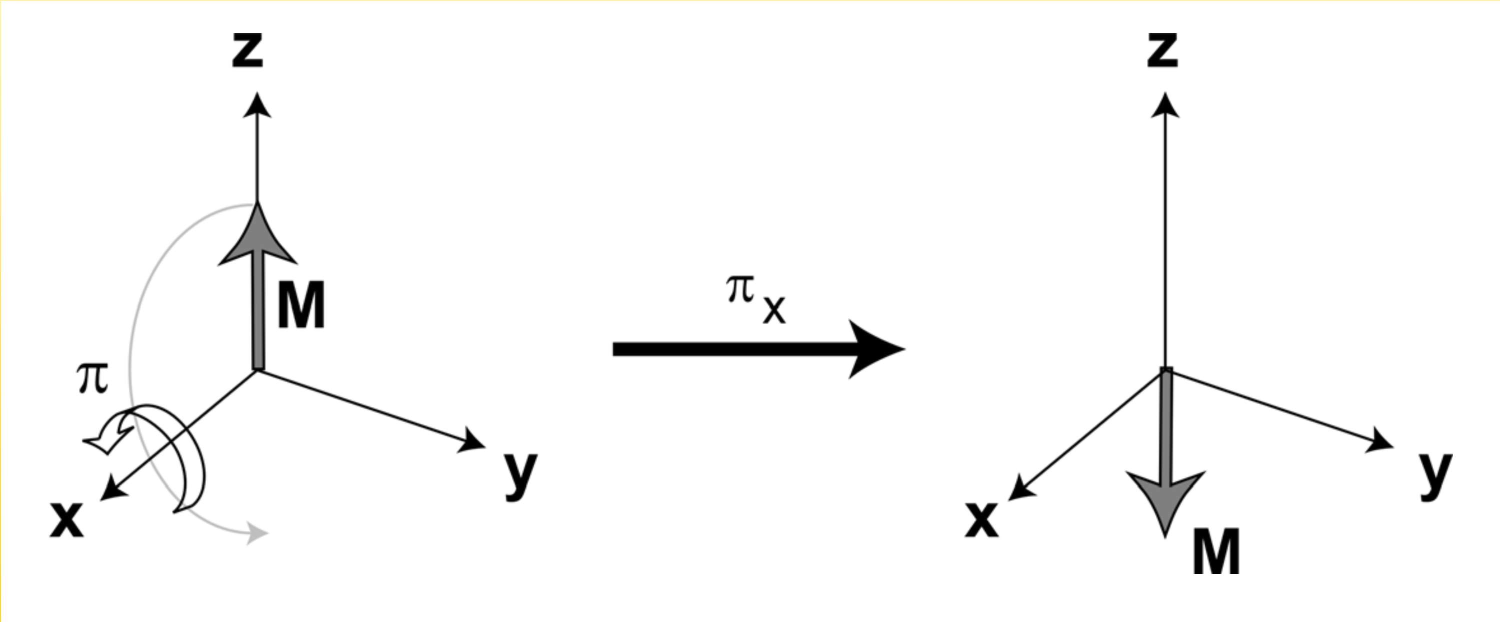
The vector model

Using the vector model experiments for the determination of relaxation times can be explained. The inversion recovery experiment is used to determine longitudinal T_1-relaxation times



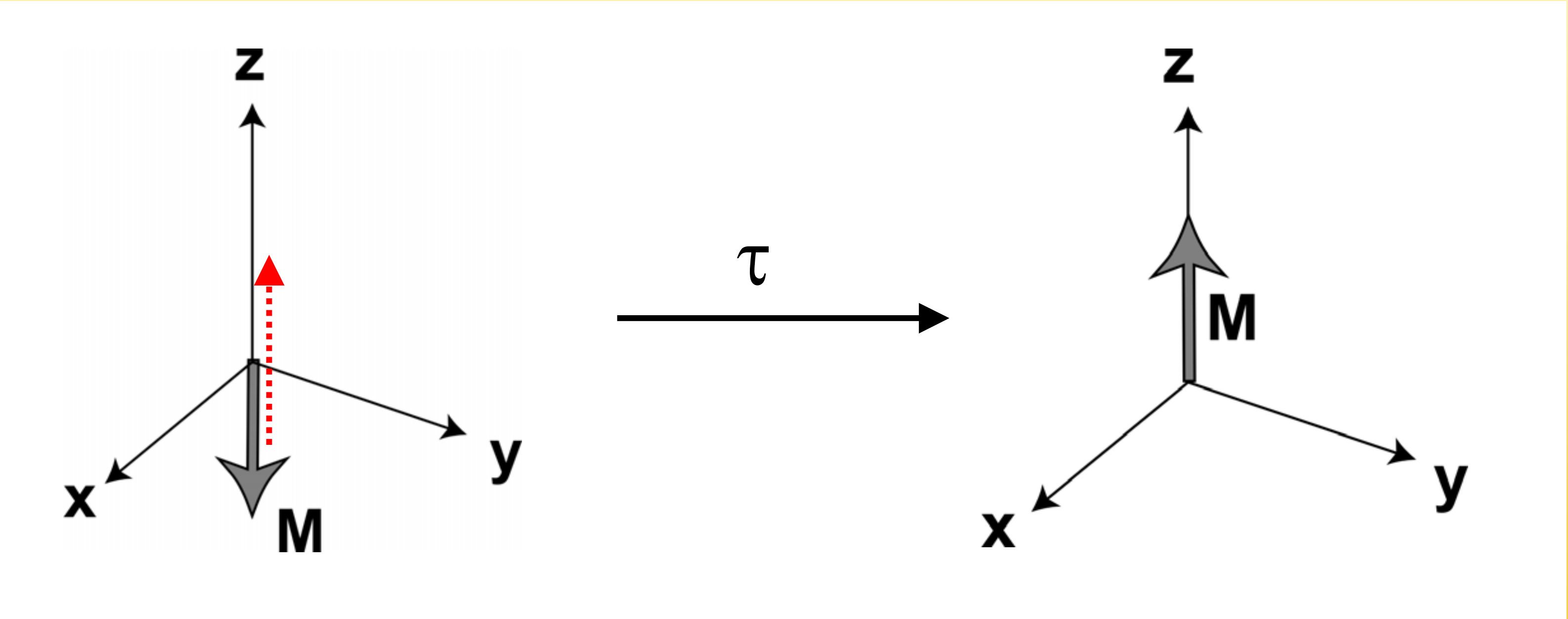
The vector model

The effect of the 180° pulse is known, it rotates the magnetization to the (-z)-axis.



The vector model

Then we wait for a certain amount of time (τ) while the magnetization returns to the (+z) direction



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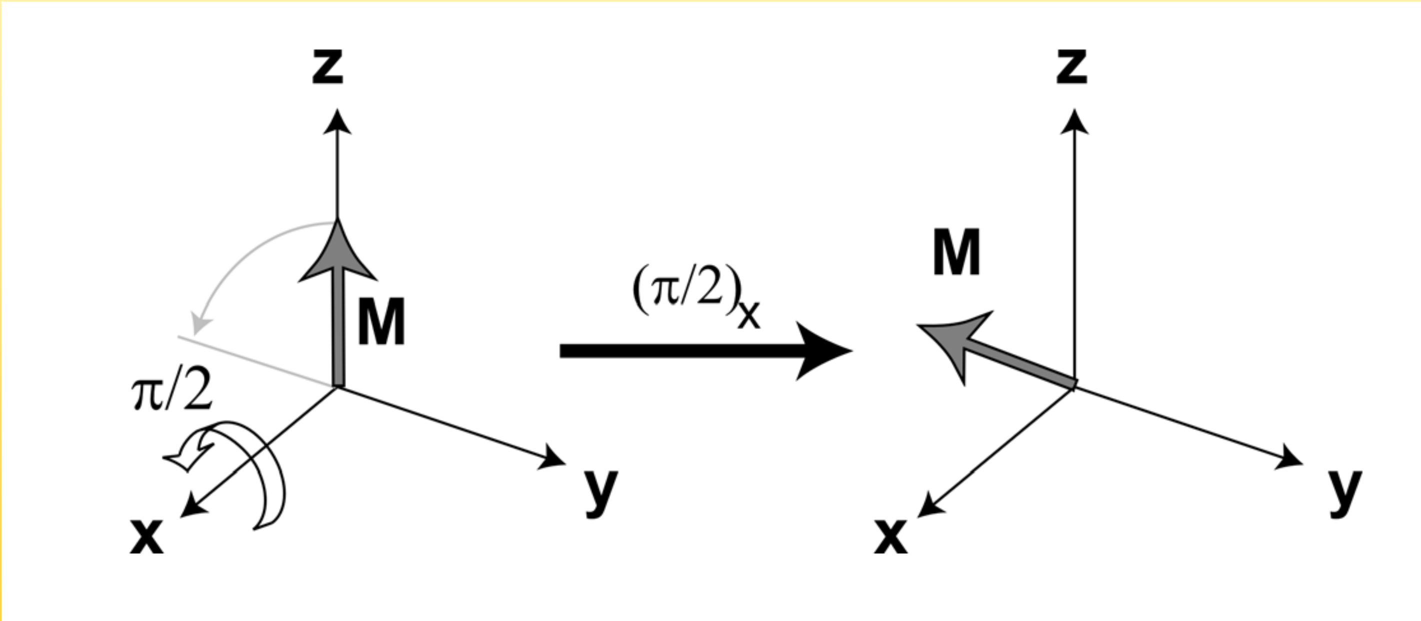
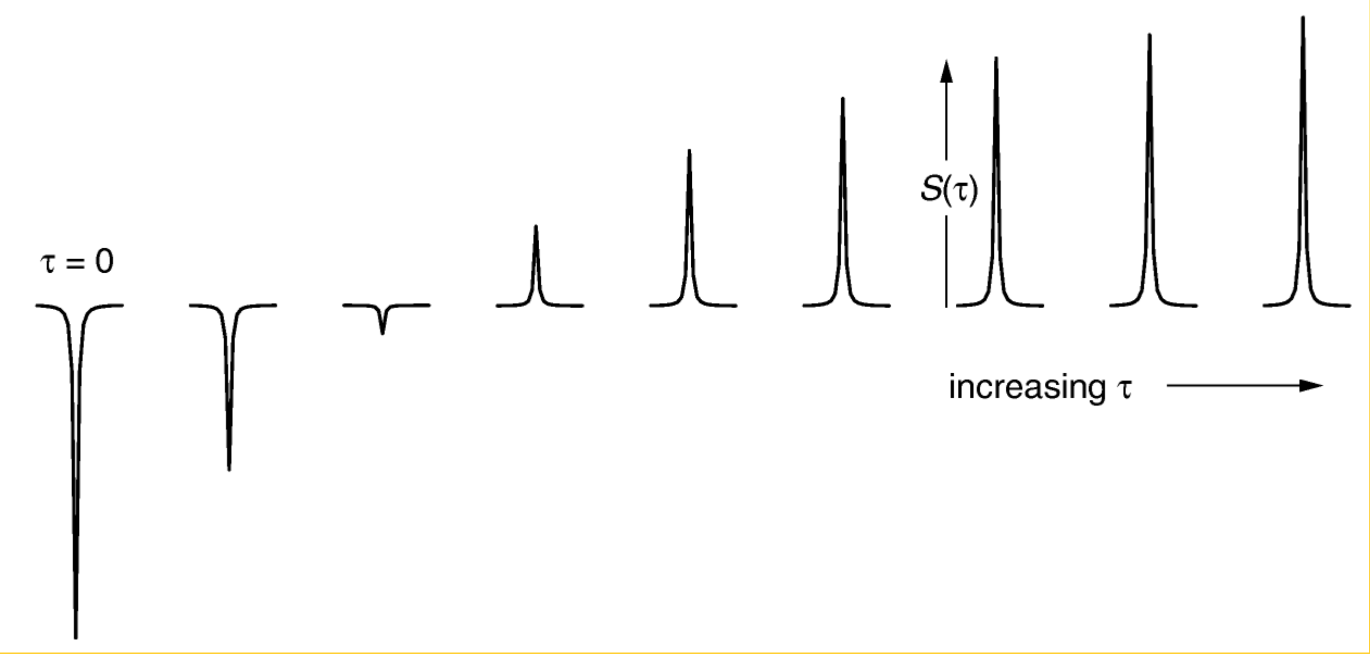
The vector model

What magentization is present after τ is tested using a 90° pulse that converts the z-magnetization present into detectable magnetization



The vector model

A series of experiments with different values for τ is recorded and the relaxation time can be deterimened by fitting to a theoretical curve.



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Water flip back pulses

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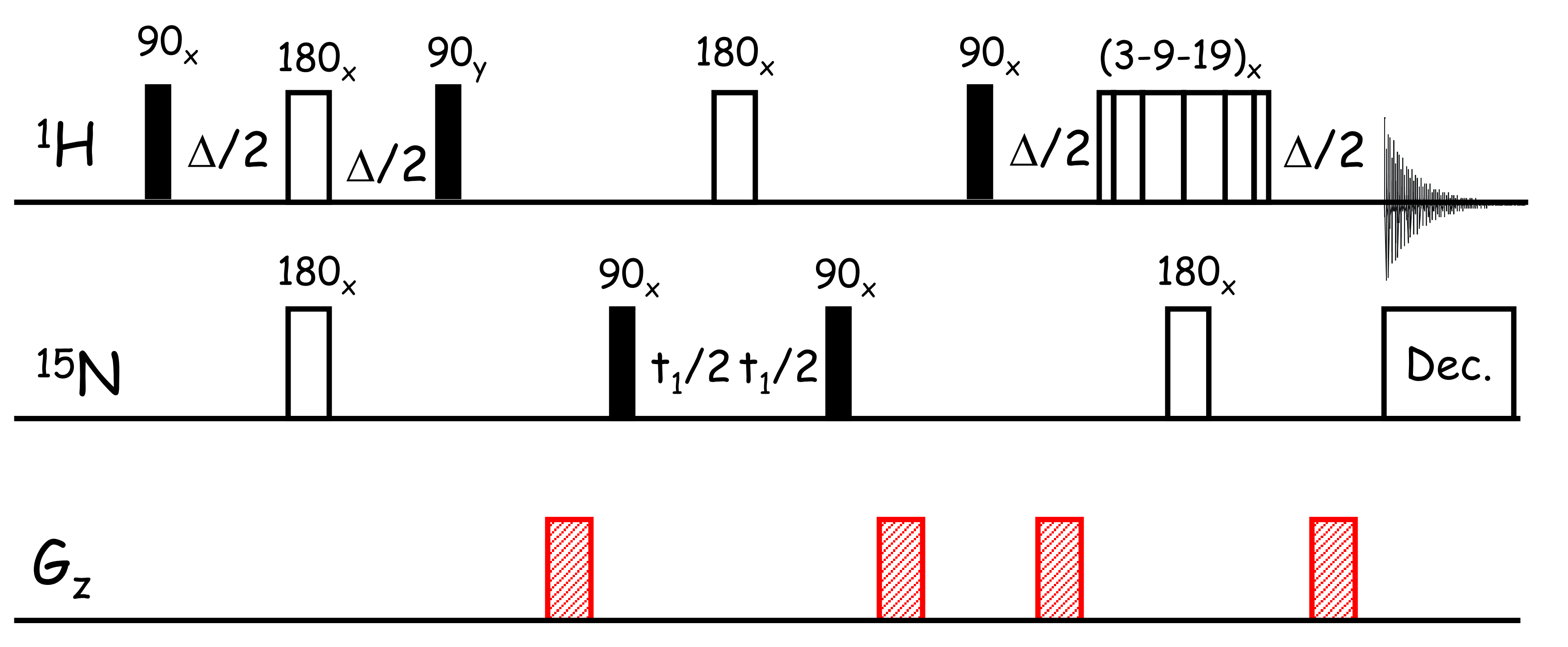
Water flip back pulses

One of the most important experiments at least for proteins is the ^{15}N-HSQC that correlates amide protons with the directly attached nitrogen



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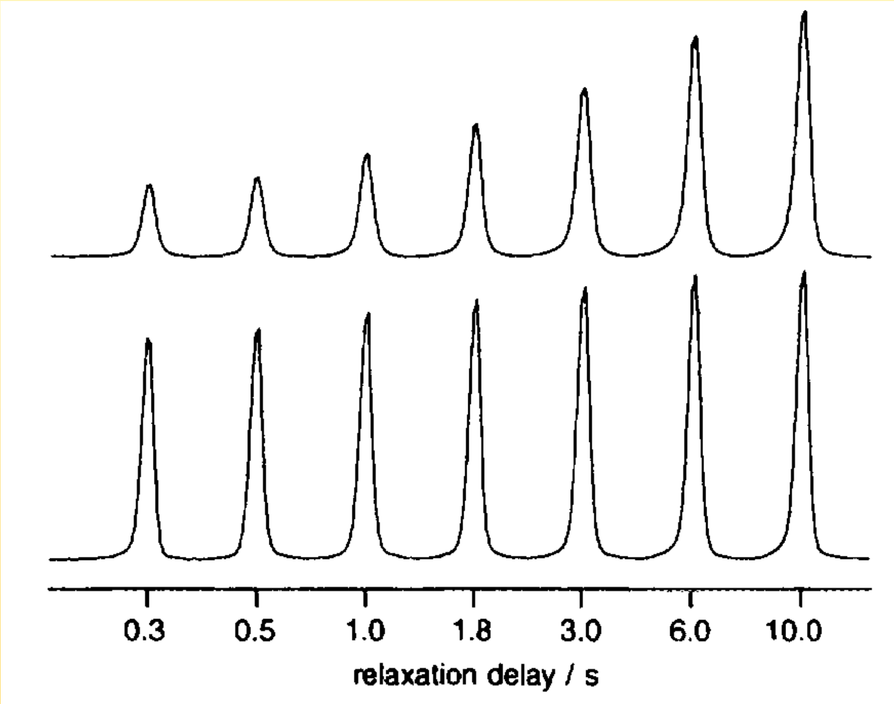
Water flip back pulses

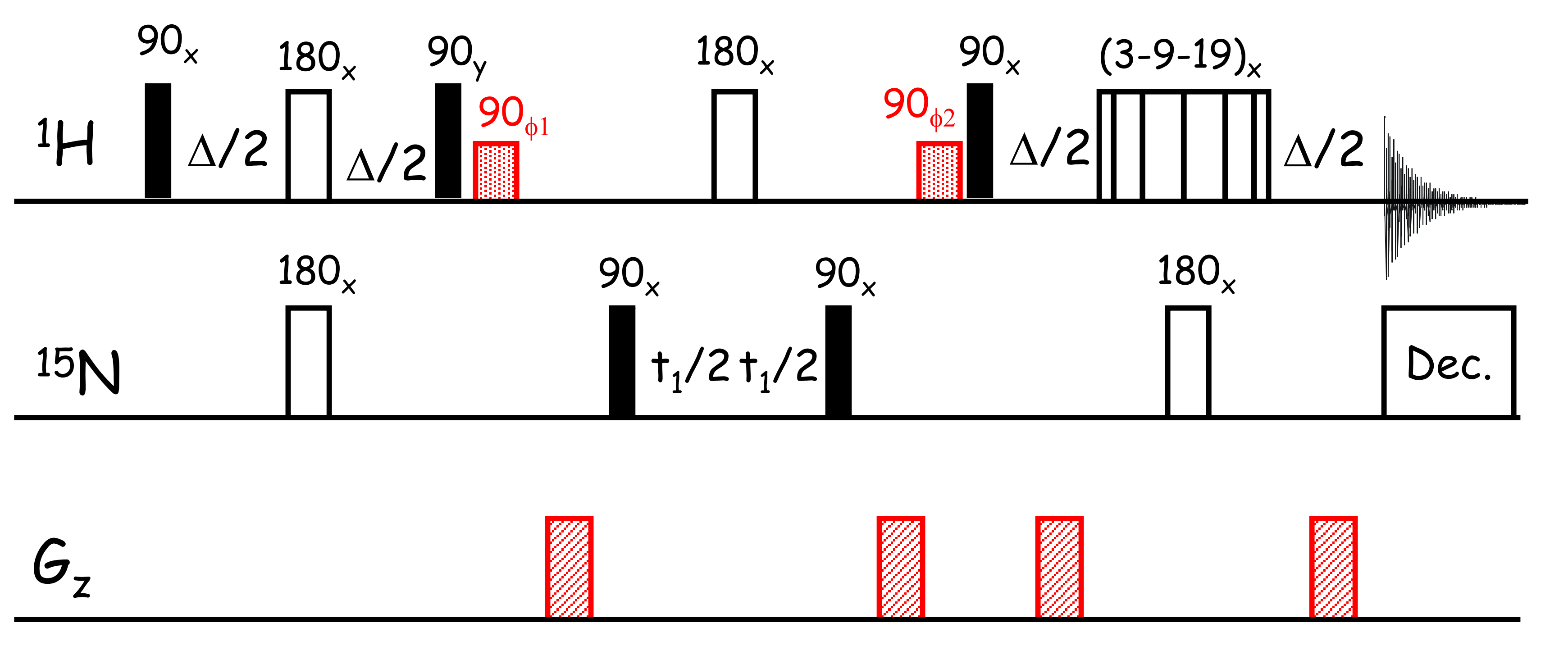
It was shown that the behaviour of the water, even though it is theoretically suppressed by the pulse sequence, has an influence on the signal intensity.



J. Stonehouse et al. *JMR A* **107**, 178-184 (1994)

Water flip back pulses

Therefore, additional pulses were implemented to control the water: water-flip-back-pulses to keep the water in the (+z)- or (−z)-direction all the time



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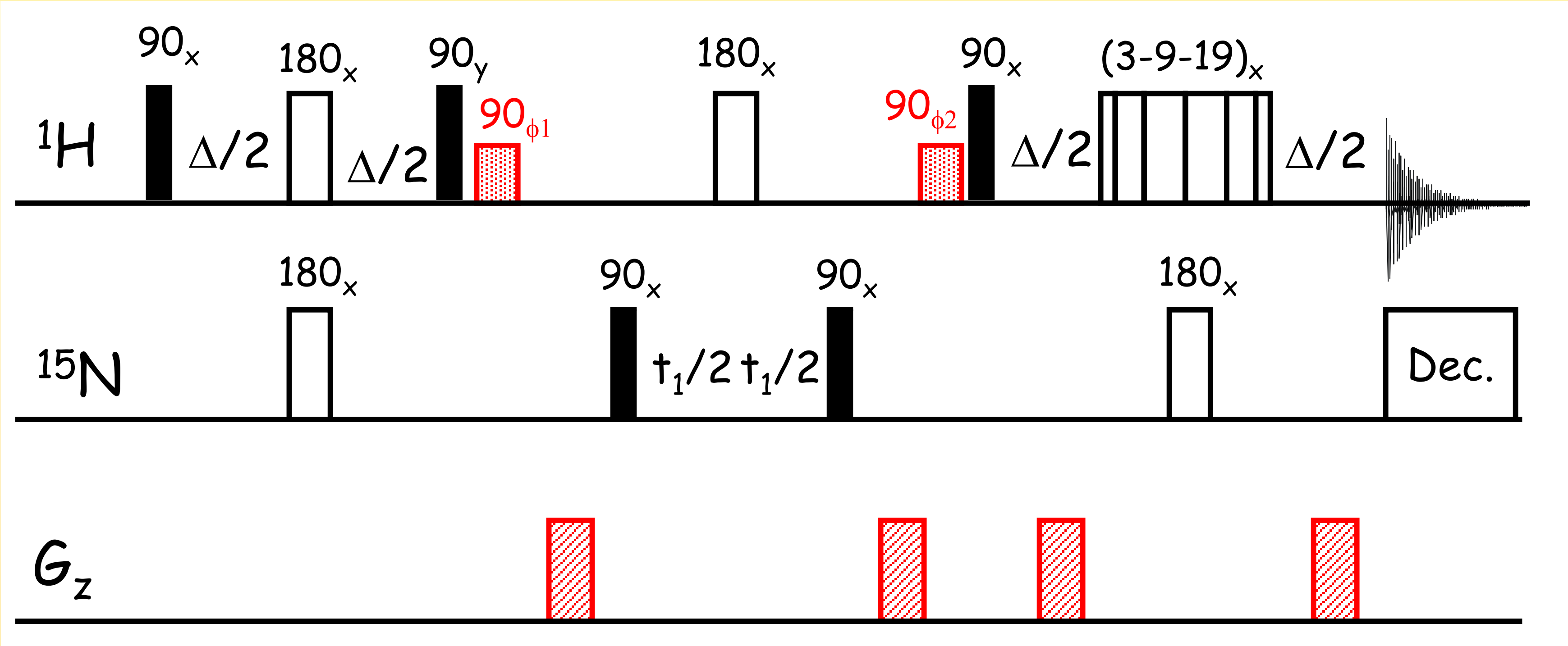
Water flip back pulses

Using the vector model we can now follow the movement of the water and find out what we have to choose for $\phi 1$ and $\phi 2$ to achieve our goal



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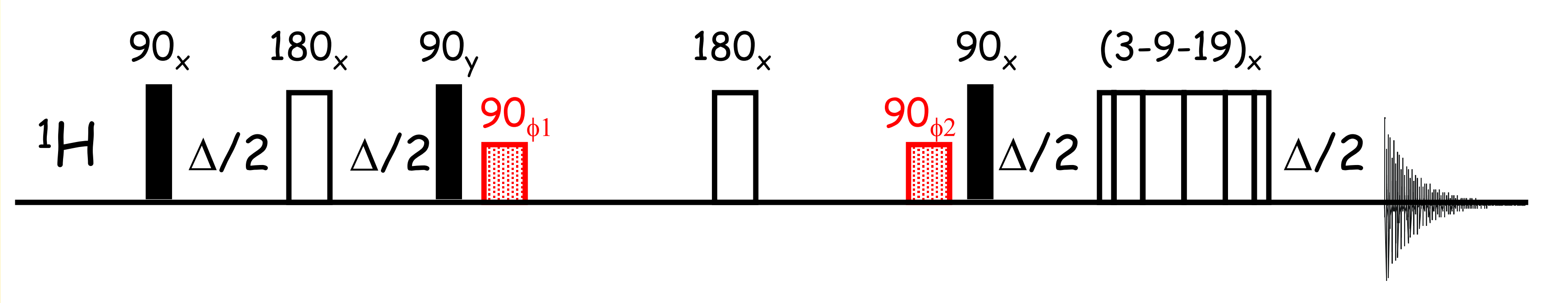
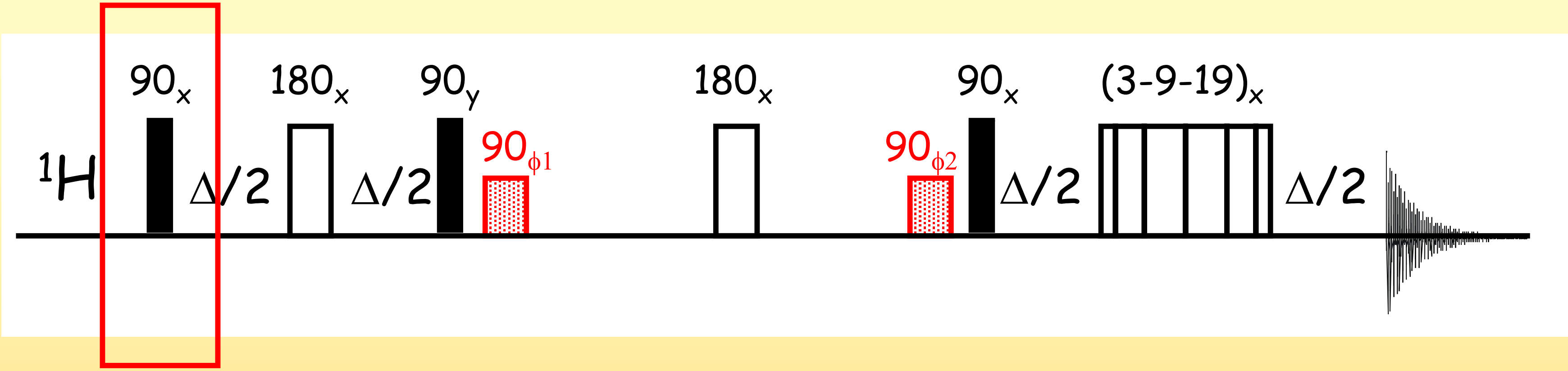
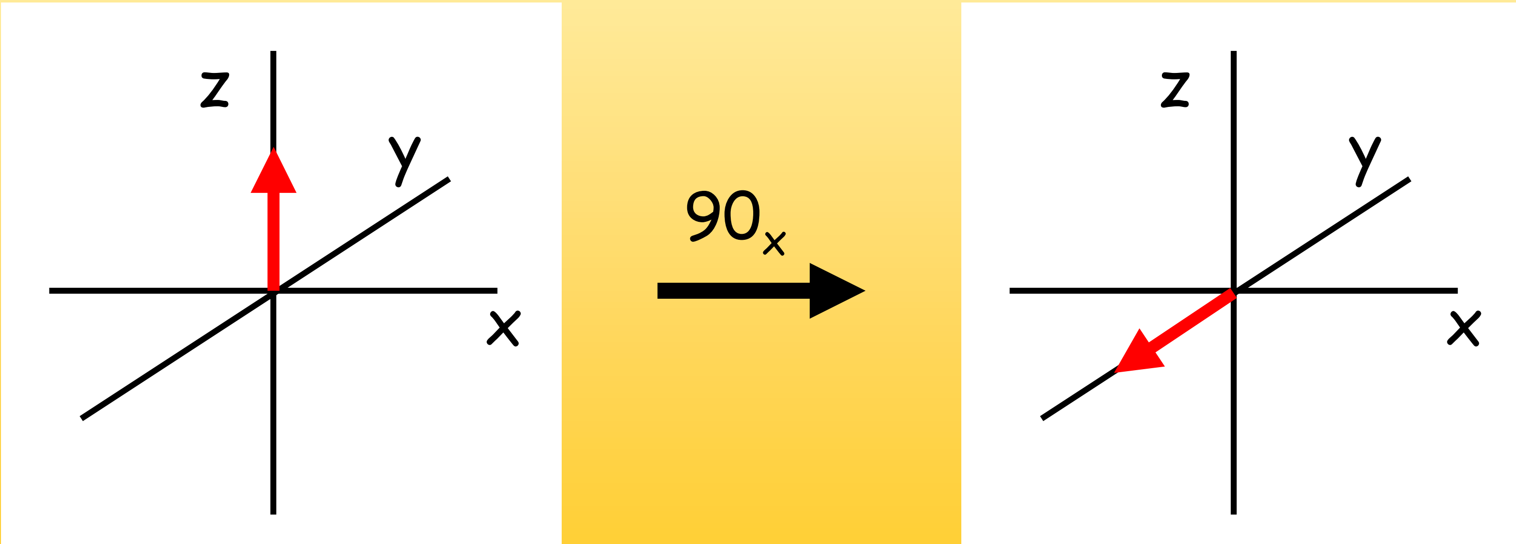
Water flip back pulses



When considering the water resonance we need only look at the proton channel. And since we are sitting on resonance, we do not have to take chemical shift into account but can concentrate on the pulses.

Water flip back pulses





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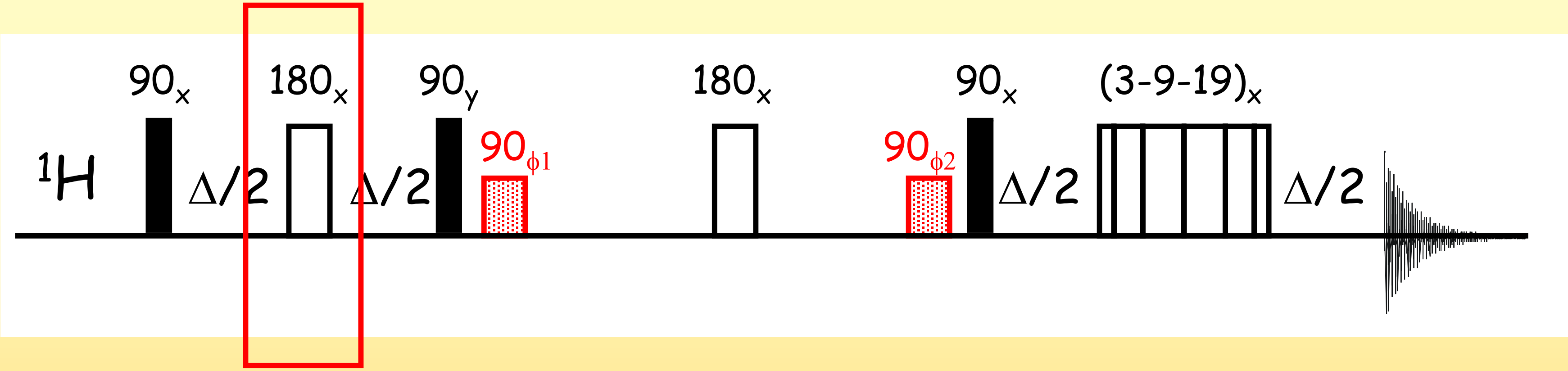
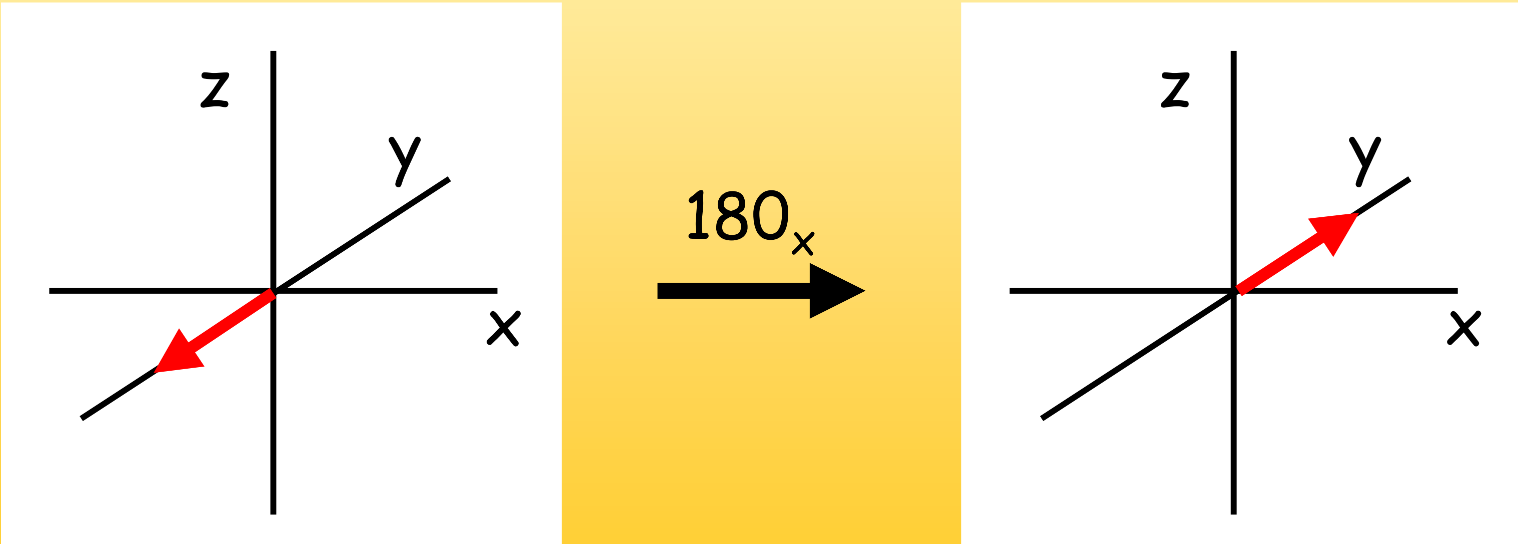
Water flip back pulses





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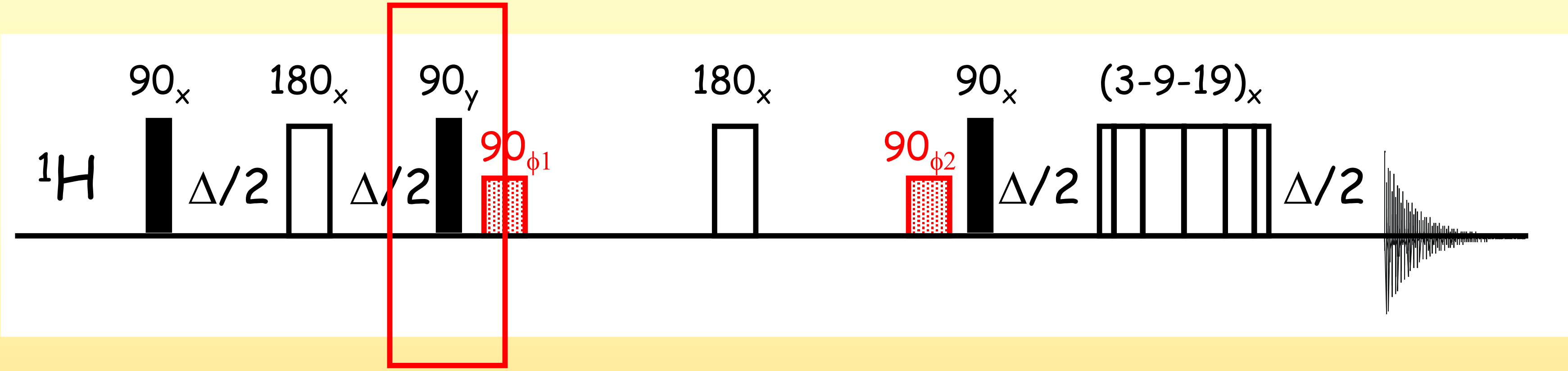
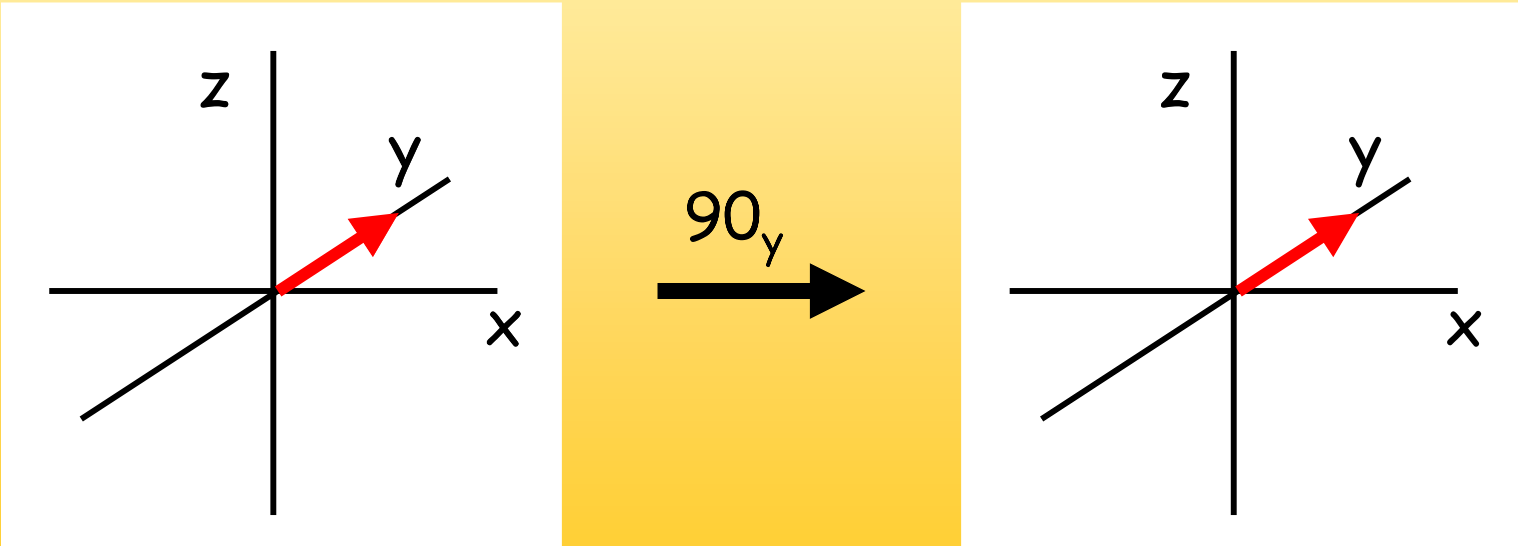
Water flip back pulses





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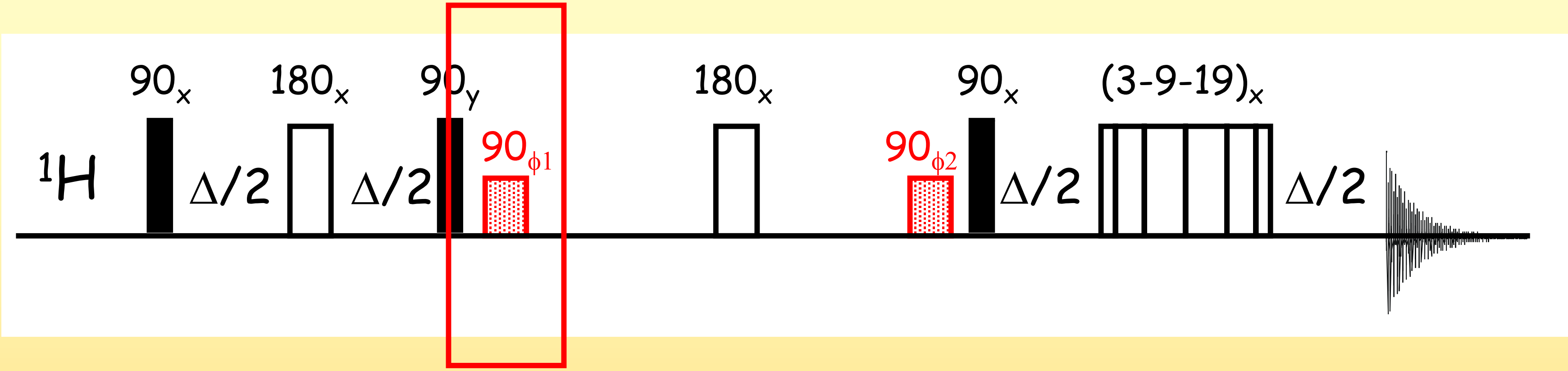
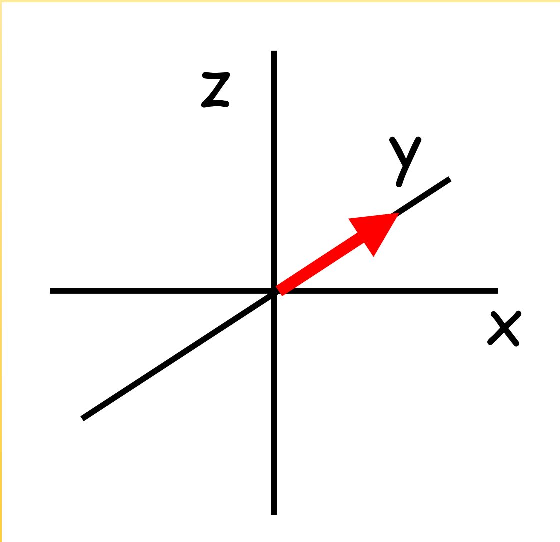
Water flip back pulses





If we now want to get the water back to the z-axis before the gradient after the $90_{\phi1}$ pulse we see:

$\phi1 = x$

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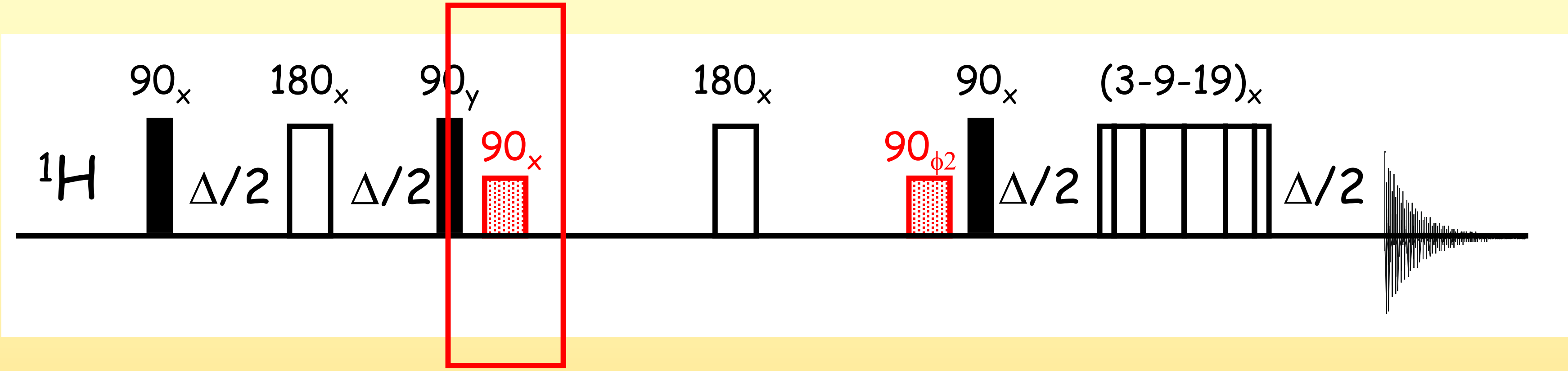
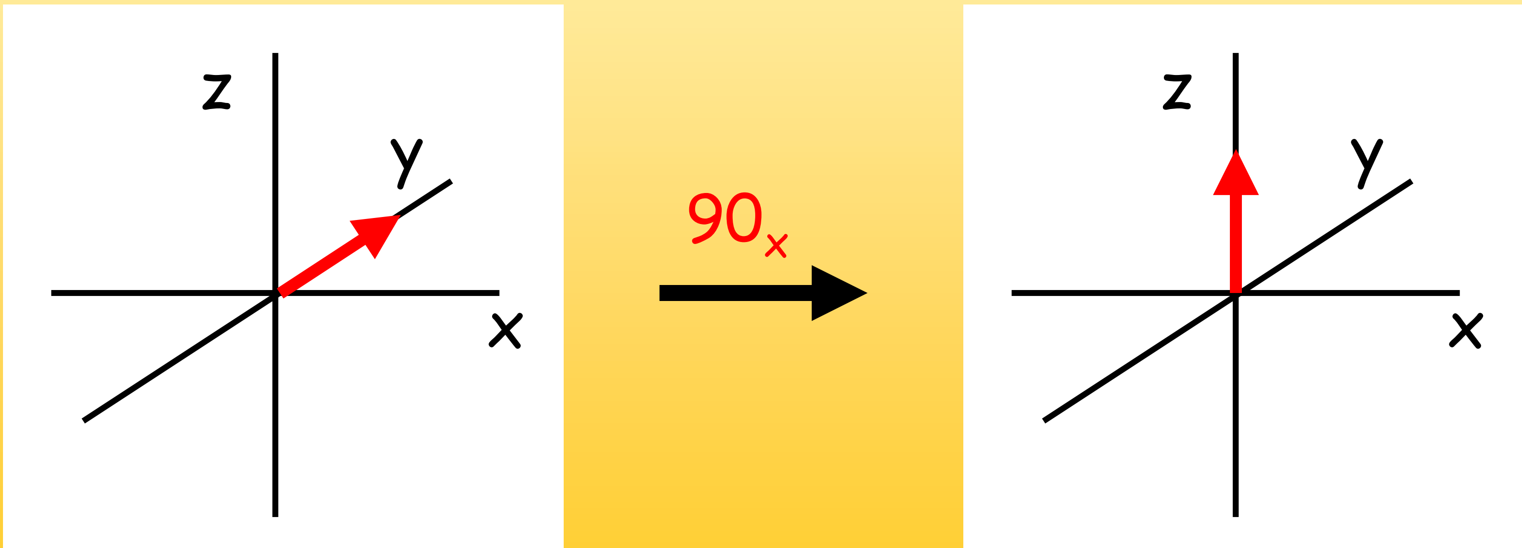
Water flip back pulses





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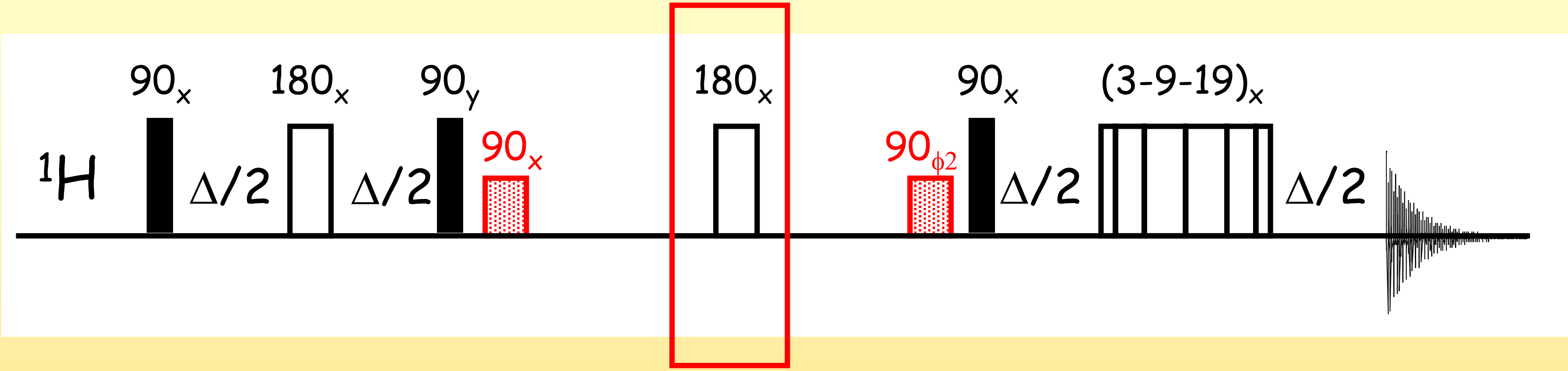
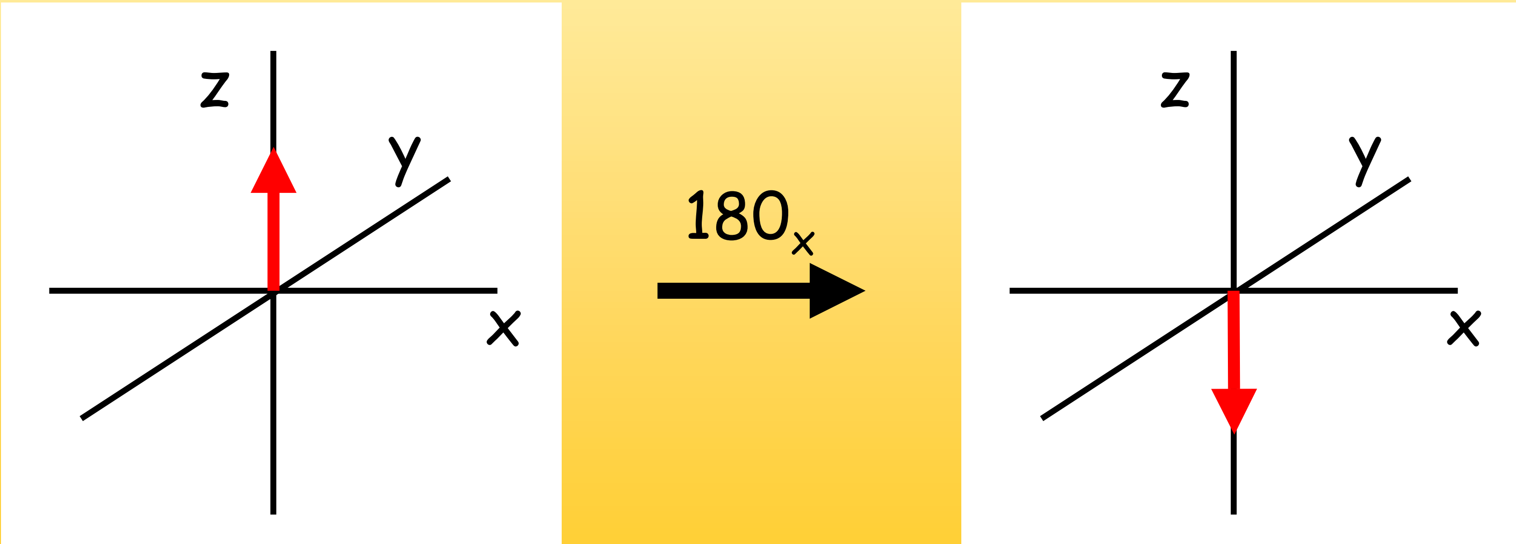
Water flip back pulses





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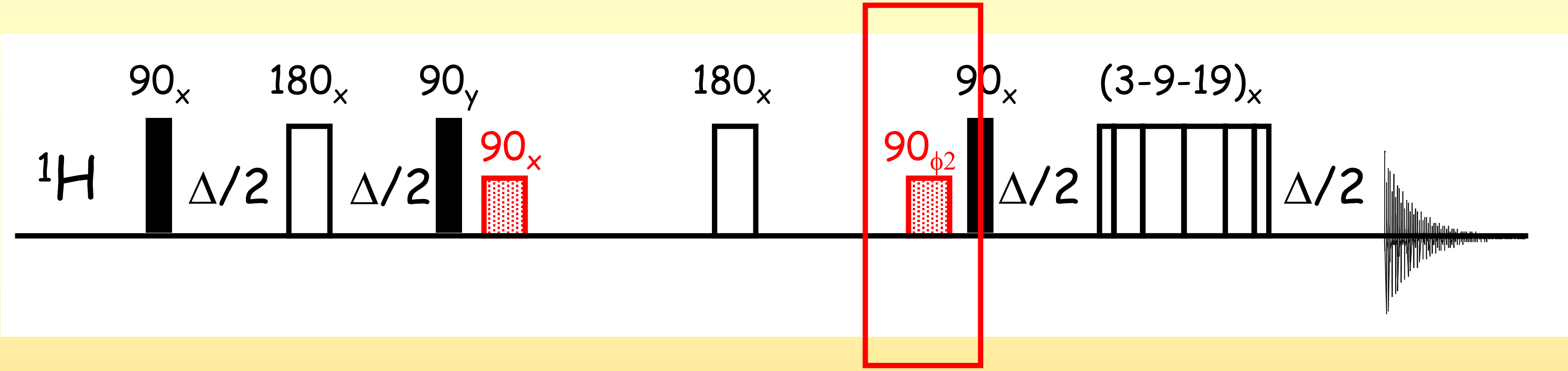
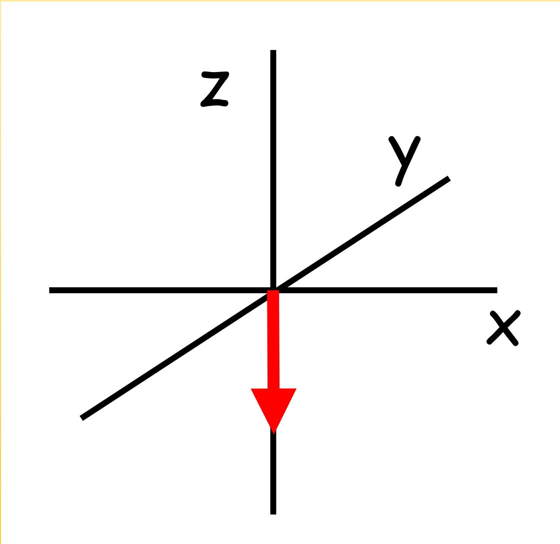
Water flip back pulses





The $(3\text{-}9\text{-}19)_x$ does nothing to the water, so the two following pulses have to turn the water to +z:

$\phi 2 = x$

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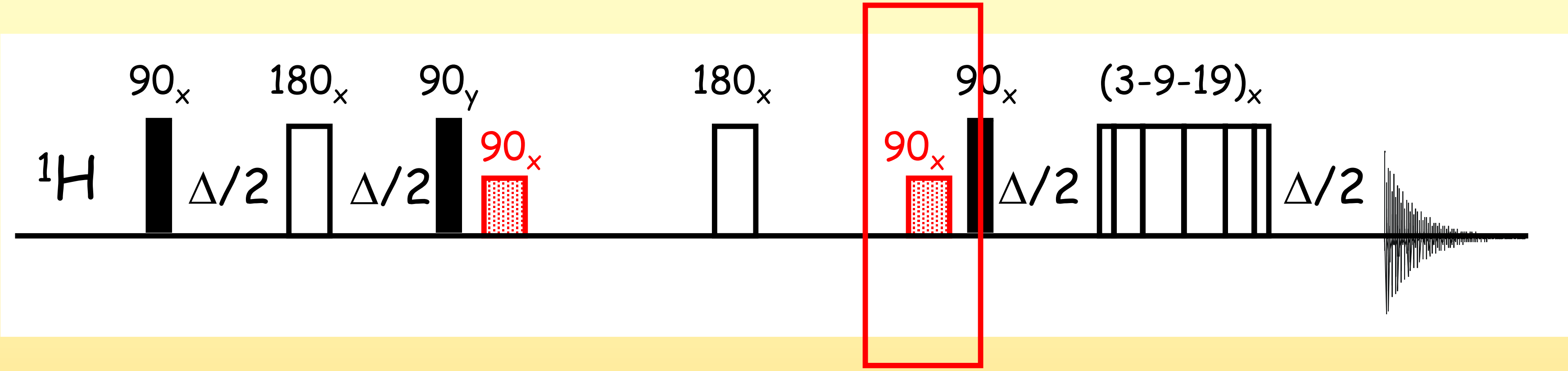
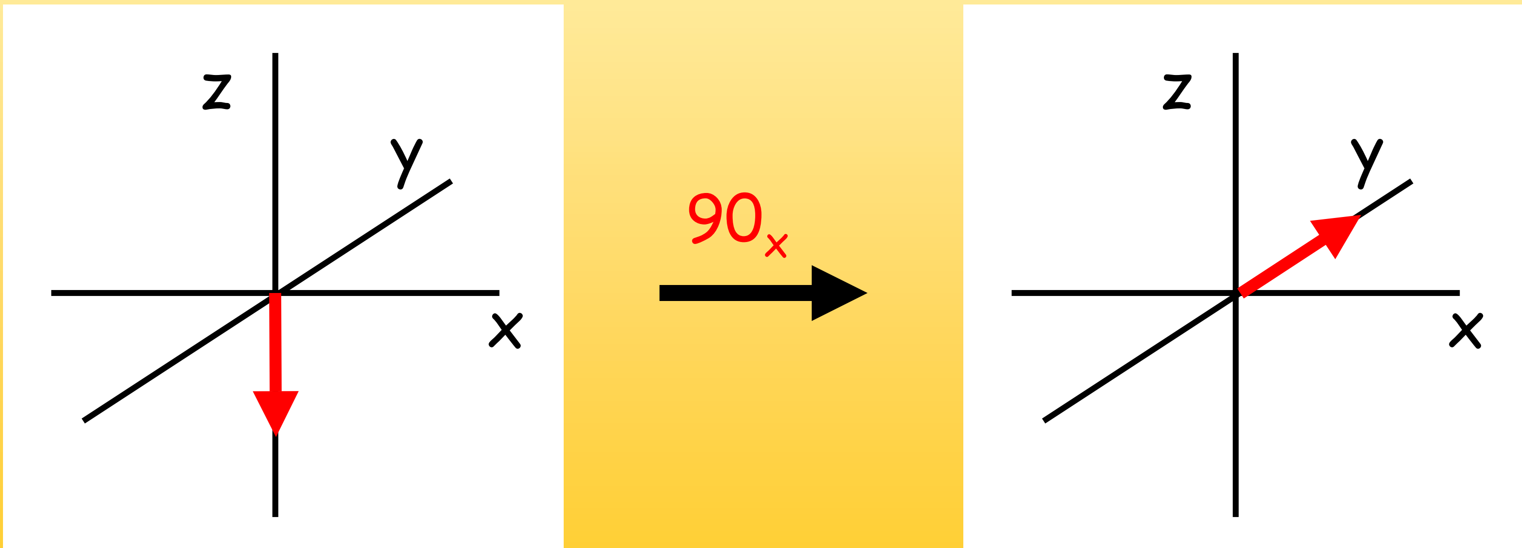
Water flip back pulses





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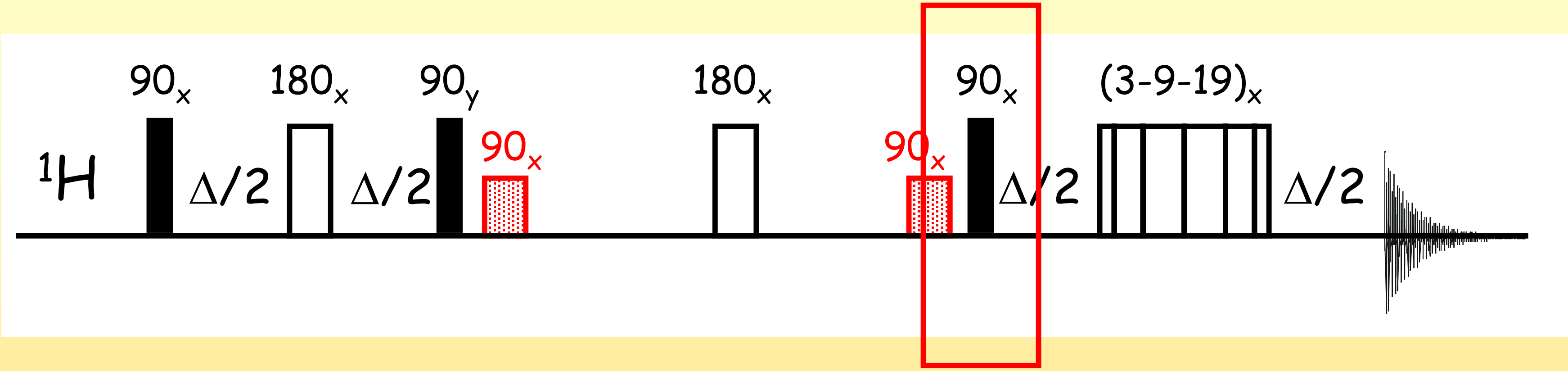
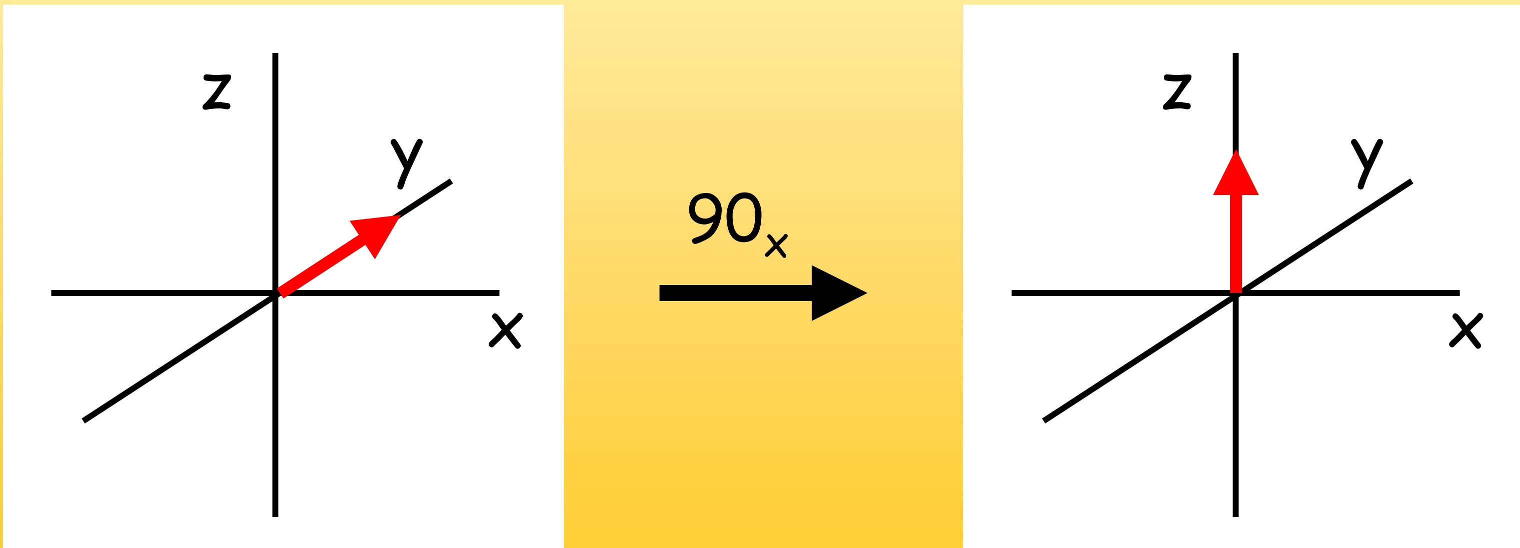
Water flip back pulses





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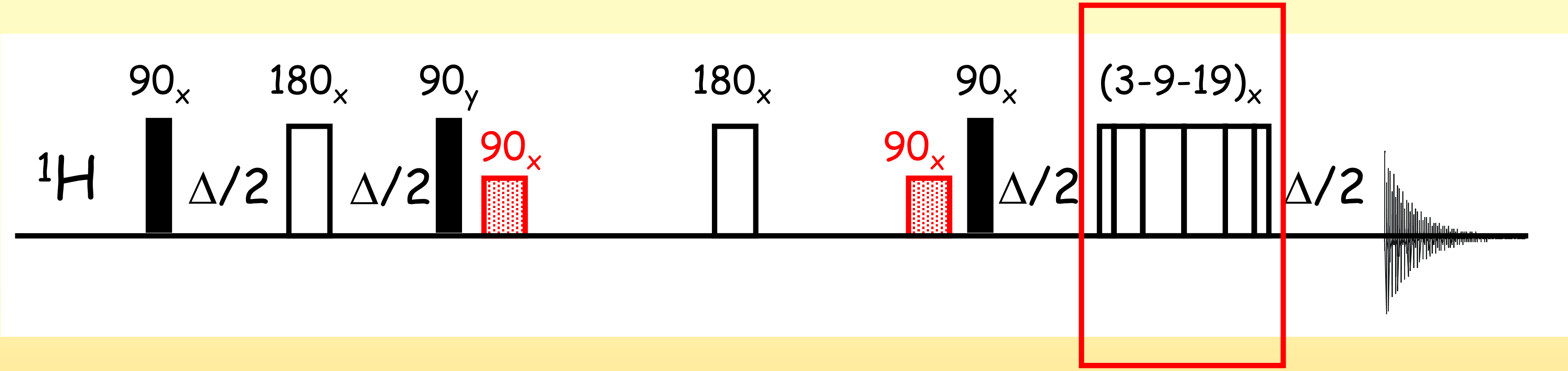

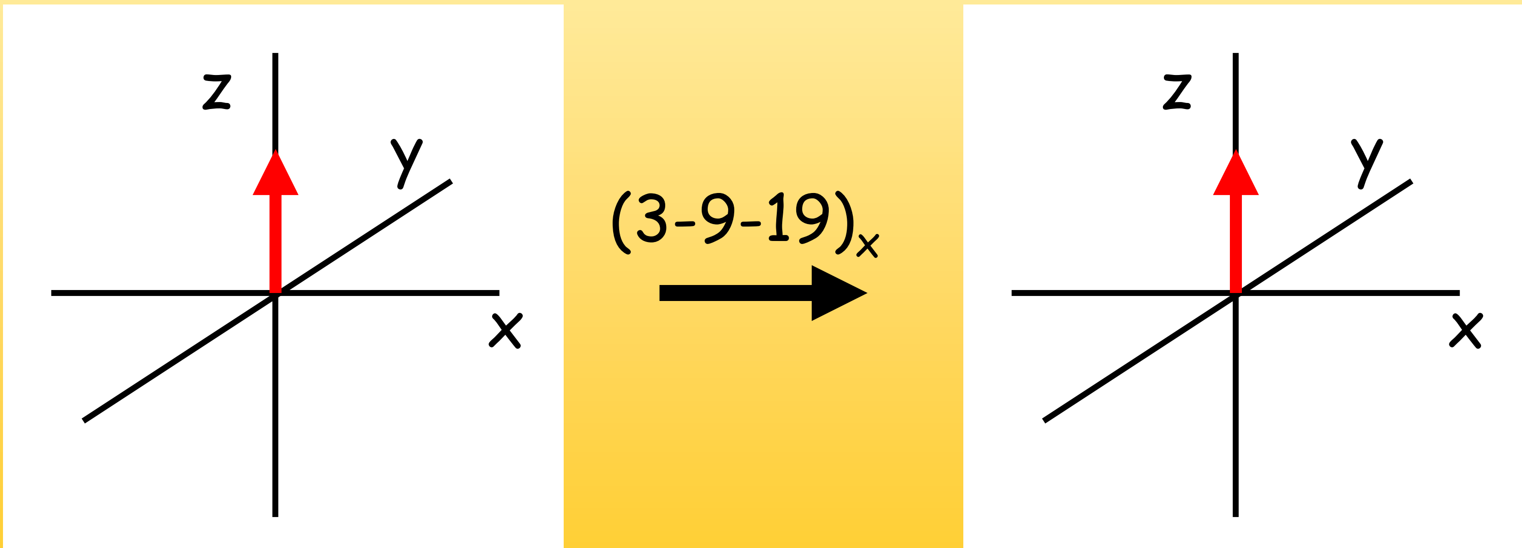

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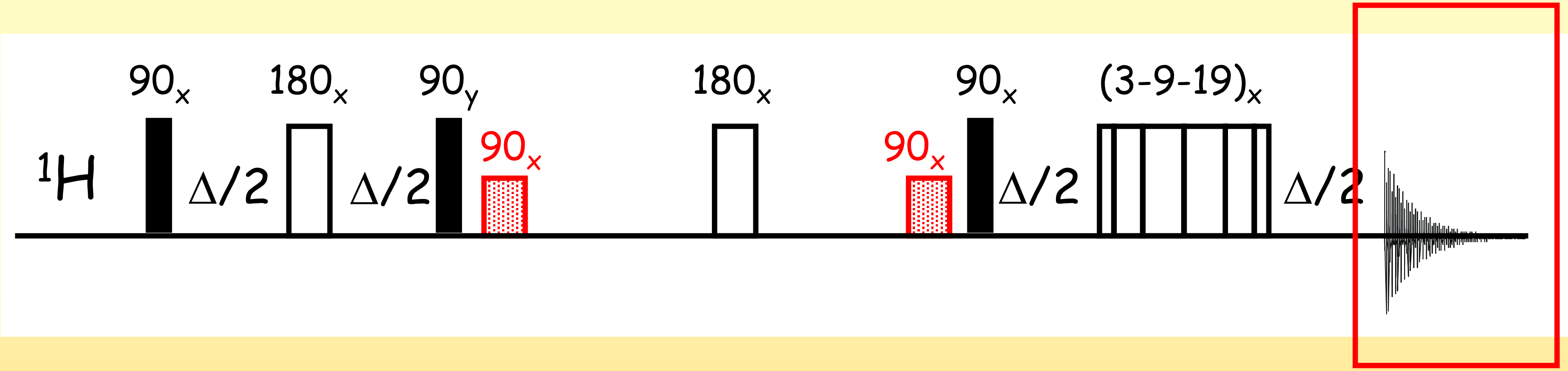

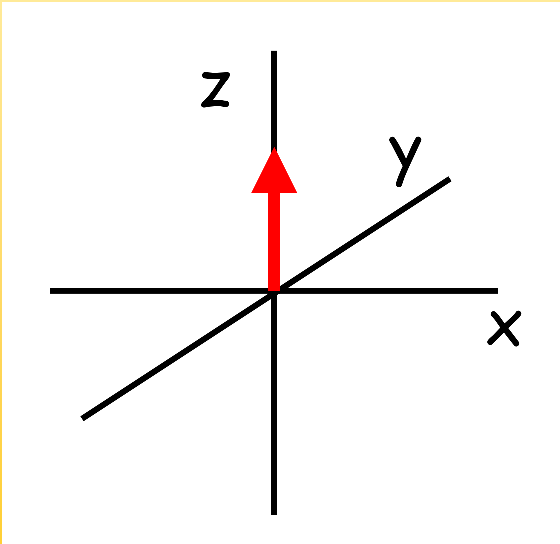

At the beginning of the aqcuisition we have achieved our goal, water points to the +z direction !

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That's it

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