

4th NMR Meets Biology Meeting

Introduction to NMR

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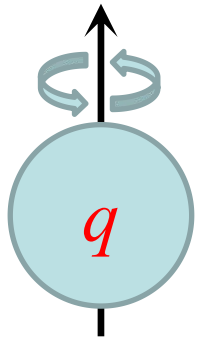
Khajuraho, India, 16-21 December 2018

Outline

- Angular moment (spin) and magnetic moment;
- Magnetic resonance phenomenon;
- Bloch equations;
- NMR pulses;
- FID, Fourier transform, 1D-NMR, 2D-NMR;
- T_1 and T_2 relaxation.

Angular momentum and magnetic moment

- In NMR we deal with spin magnetism. What is ‘**spin**’?
- Charged nucleus (or electron) is spinning:
there is angular momentum (spin) and magnetic moment
attention: this is a simple view, which is not (entirely) correct



The electric current for a charged particle moving around

$$I = \frac{qv}{2\pi r}$$

The magnetic moment is

$$\vec{\mu} = \frac{IS}{c} \vec{n} = \frac{qvr}{2c} \vec{n} = \frac{q}{2mc} \vec{J}$$

So, μ is proportional to *a.m.*; when *a.m.* is measured in \hbar units $\vec{\mu} = \frac{q\hbar}{2mc} \vec{S}$

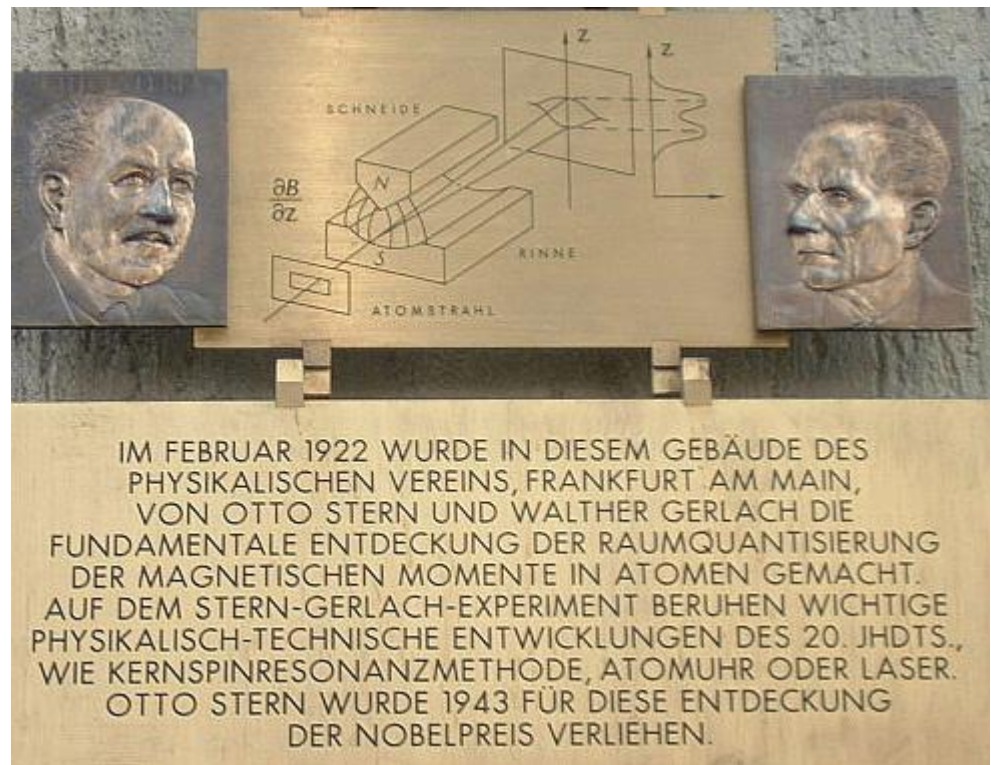
Quantum mechanics: this is not entirely correct (we are wrong by the **g-factor**)!

$$\vec{\mu}_e = g_e \mu_B \vec{S}, \quad \mu_B = -\frac{e\hbar}{2m_e c}, \quad g_e = 2 \left(1 + \frac{\alpha}{2\pi} + \dots \right) \approx 2.0023 \quad (\text{QED result})$$

$$\vec{\mu}_N = g_N \mu_N \vec{I}, \quad \mu_N = \frac{e\hbar}{2Mc}, \quad g_N \neq 1 \quad (g_p \approx 5.58, g_n = -\frac{2}{3} g_p \approx 3.83, \text{QCD result}) \quad 3$$

Angular momentum and magnetic moment

- Furthermore, QM tells us that S is **quantized**: angular moment measured in \hbar units cannot be an arbitrary number
- **Stern-Gerlach experiment**:
 - ✓ The beam of particles is deflected by inhomogeneous field
 - ✓ Reason: intrinsic magnetic moment (spin) of particles
 - ✓ In contrast to the classical expectation the distribution of the μ -vector is not continuous!
 - ✓ Spin is quantized



Angular momentum and magnetic moment

- Furthermore, QM tells us that S is quantized: angular momentum measured in \hbar units cannot be an arbitrary number
- S is integer (0, 1, 2, ...) or half-integer (1/2, 3/2, 5/2, ...) and $|S| = \{S(S+1)\}^{1/2}$
- Projection of S onto any axis in space varies in steps of 1 from $-S$ to S
Spin-1/2 particle (^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P ...): possible projections are $\pm 1/2$
Spin-1 particle: possible projections are $-1, 0, 1$
- When discussing spin-1/2 particles we can use a simplification (and forget about QM): spin magnetization is a classical **vector in the 3D-space**, which is changing by moving in external fields and due to spin relaxation
- As we can see from the density matrix description, this is correct (but only for two-level systems!): **fictitious spin** description

Magnetic resonance: classical viewpoint

- Motion of a classical magnetic moment in a constant magnetic field B_0

- Magnetic moment is $\vec{\mu}_N = g_N \mu_N \vec{J} = \gamma_N \vec{J}$
 γ_N is the **gyromagnetic ratio** (or magnetogyric ratio)

- We obtain a **torque**: $\frac{d\vec{J}}{dt} = -[\vec{\mu} \times \vec{B}_0] = -\gamma_N [\vec{J} \times \vec{B}_0]$

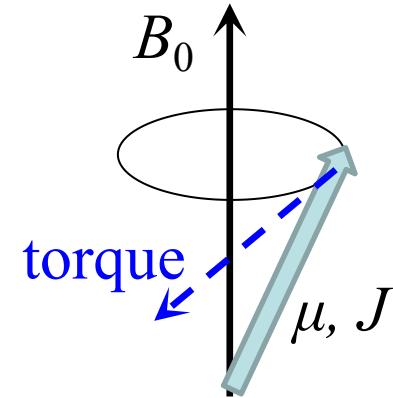
$$\frac{dJ_x}{dt} = -\gamma_N B_0 J_y, \quad \frac{dJ_y}{dt} = \gamma_N B_0 J_x, \quad J_z = \text{const}$$

- These equations tell us that: $J_x \propto \cos(\omega_0 t)$, $J_y \propto \sin(\omega_0 t)$, $J_z = \text{const}$
 $|J| = \text{const}$; the J -vector is rotating about B_0 at a frequency $\omega_0 = |\gamma_N| B_0$

This is **precession** of magnetic moment

The direction is given by the γ_N sign

- What happens to the magnetic moment if we apply an oscillating field?
- Typically this field is much weaker than B_0 : it does nothing except for some special cases

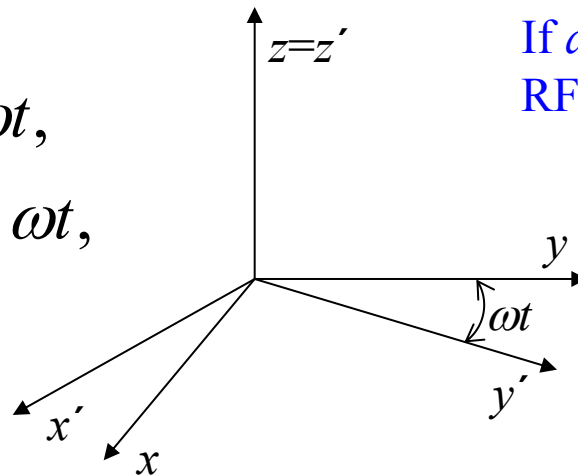


Magnetic resonance: classical viewpoint

- Let us apply an oscillating field perpendicular to B_0 : $B_{\perp} = 2B_1 \cos(\omega t)$
- To account for the effect of this field we can go

to the rotating frame:

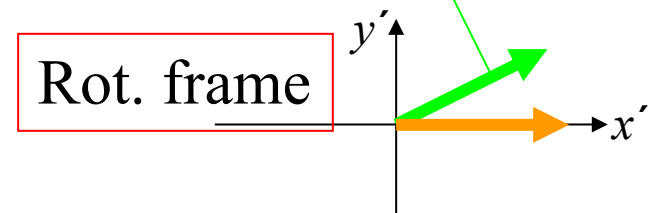
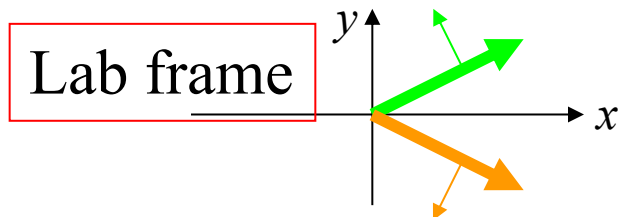
$$\begin{cases} x = x' \cos \omega t + y' \sin \omega t, \\ y = -x' \sin \omega t + y' \cos \omega t, \\ z = z' \end{cases}$$



If \vec{a} is a constant vector in the RF, in the LF we obtain

$$\frac{d\vec{a}}{dt} = [\vec{\omega} \times \vec{a}]$$

- Linear polarized field = 2 circularly polarized fields: two vectors rotating with the same speed in opposite directions



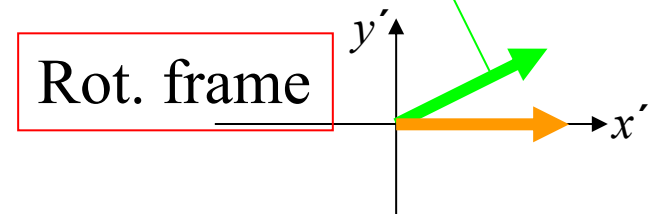
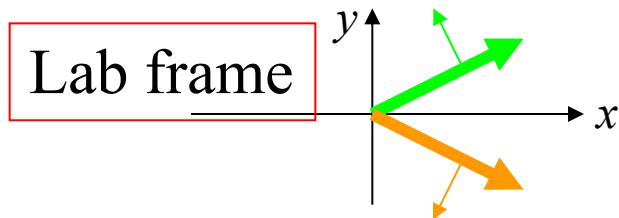
- At high ω the vector, which rotates with double frequency can be neglected. This is (usually) Ok for the description of resonance

Magnetic resonance: classical viewpoint

- What happens to the transverse field: $2B_1 \cos \omega t = 2B_1 (\cos \omega t, 0, 0)$
- Linear polarized field = 2 circularly polarized fields: two vectors rotating with the same speed in opposite directions

$$B_1 (\cos \omega t, \sin \omega t, 0) + B_1 (\cos(-\omega t), \sin(-\omega t), 0)$$

- Graphical representation in both frames:



- At high ω the vector, which rotates with double frequency can be neglected. The other vector does not move. This is (usually) Ok for the description of resonance

Magnetic resonance: classical viewpoint

- Equation of motion in the rotating frame

$$\vec{J} = J'_x \vec{i} + J'_y \vec{j} + J'_z \vec{k}$$

Here the components of J change and the basis vectors $\{i, j, k\}$ also change with time

- By taking derivative we obtain:

$$\begin{aligned} \frac{d\vec{J}}{dt} &= \frac{dJ'_x}{dt} \vec{i} + \frac{dJ'_y}{dt} \vec{j} + \frac{dJ'_z}{dt} \vec{k} + \\ &+ J'_x \frac{d\vec{i}}{dt} + J'_y \frac{d\vec{j}}{dt} + J'_z \frac{d\vec{k}}{dt} = \frac{\delta\vec{J}}{\delta t} + [\vec{\omega} \times \vec{J}] \end{aligned}$$

Here $\delta/\delta t$ is the time derivative in the rotating frame

- Equation of motion in the rotating frame

$$\frac{\delta\vec{J}}{\delta t} = \frac{d\vec{J}}{dt} - [\vec{\omega} \times \vec{J}] = -\gamma_N [\vec{J} \times \vec{B}] + [\vec{J} \times \vec{\omega}] = -\gamma_N [\vec{J} \times \vec{B}_{eff}]$$

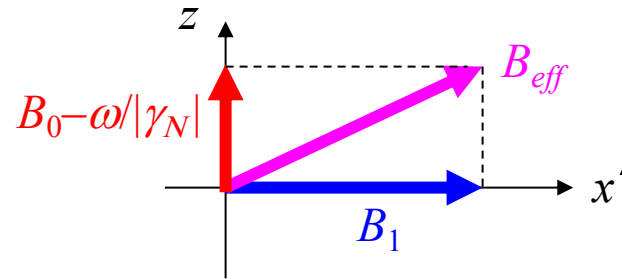
- Still the same equation but the new field equals to $\vec{B}_{eff} = \vec{B}_0 + \vec{\omega} / \gamma_N + \vec{B}_1$
- Superposition of the new B_0 and B_1 -fields gives a constant effective field B_{eff}

Magnetic resonance: classical viewpoint

- The **effective field** is the following vector $\vec{B}_{eff} = (B_1, 0, (\omega - \omega_0) / \gamma_N)$

- Precession **frequency** is

$$\Omega = \gamma_N B_{eff} = \sqrt{(\omega - \omega_0)^2 + \omega_1^2}$$



- If $\omega = \omega_0$ the precession axis is the x -axis \Rightarrow **variation of J_z reaches its maximum.** This variation rapidly decays with ω going to zero at $|\omega - \omega_0| \gg \omega_1$
- Resonance condition:** $\omega =$ (free precession frequency). **Weak B_1 is important!**
- Frequency range** (given by γ_N): radio-frequency, i.e., 300 MHz for ^1H @ 7 Tesla
- Resonance width** is given by condition $|\omega - \omega_0| \approx \omega_1$. If $\omega_1 \gg |\omega - \omega_0|$ the effective field is nearly parallel to the x -axis – **we are at resonance!**
- At resonance the precession frequency is ω_1 . If we switch on the resonant oscillating field for time period of τ_p the **flip angle** of magnetization is

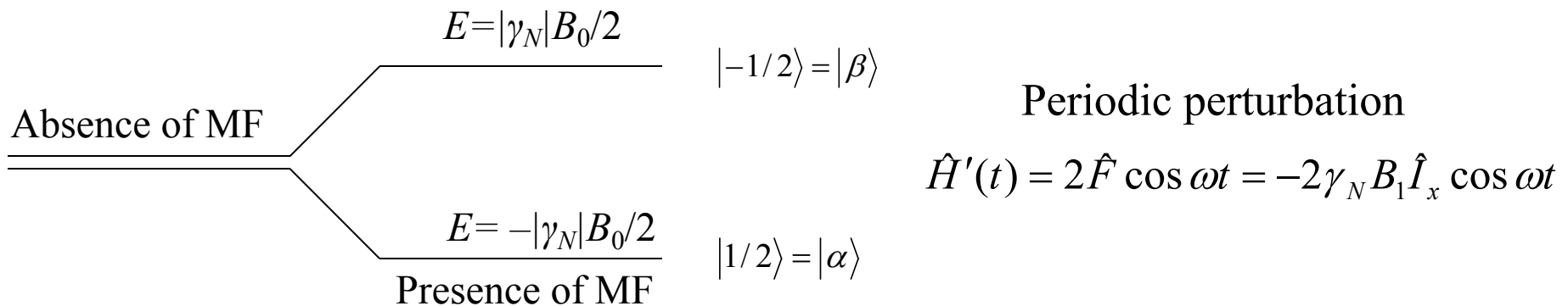
$$\Theta = \omega_1 \tau_p = \gamma_N B_1 \tau_p$$

Magnetic resonance: simplified quantum viewpoint

- The energy of interaction of the spin with an external field is

$$\hat{H}_N = -(\vec{\mu} \cdot \vec{B}_0) = -\hbar\gamma_N(\vec{B}_0 \cdot \hat{I})$$

- If B_0 is parallel to the Z-axis $E = -\hbar\gamma_N B_0 I_z$
- The degeneracy of the spin levels is lifted



- Fermi' golden rule tells us that there is a resonance when $\omega = \omega_0$

$$P_{\alpha\beta} = \frac{2\pi}{\hbar} |\langle \alpha | \hat{F} | \beta \rangle|^2 \delta(E_\alpha - E_\beta - \hbar\omega) \propto B_1^2 \delta(\hbar\omega_0 - \hbar\omega)$$

Macroscopic spin magnetization

- Up to now we discussed a single spin $\frac{1}{2}$, which is never the case in NMR
- At thermal equilibrium we have almost the same amount of spins pointing up and down: the energy gap between the spin levels is much smaller than kT

B_0

$\vec{M} = \sum_i \vec{\mu}_i = \chi \vec{B}_0$

$\chi = \frac{N \gamma_N^2 \hbar^2 I(I+1)}{3kT}$

Nuclear paramagnetism: the induced field is parallel to B_0

- We work with net magnetization of all spins; at equilibrium this is a vector parallel to B_0 (longitudinal magnetization)
- However, we do not measure the longitudinal component, but rotate M with RF pulses to obtain transverse magnetization and measure the signal from M_{\perp}

T₁ and T₂ relaxation

- Relaxation is a process, which brings a system to thermal equilibrium.
Physical reason: fluctuating interaction of spins with molecular surrounding
- For spins this means that $M_z = M_{||} = M_{eq}$ and $M_{\perp} = 0$

- There are two processes, which are responsible for relaxation

✓ Longitudinal, T₁, relaxation: M_{eq} is reached at $t \sim T_1$:

$$M_z = M_{eq} - (M_{eq} - M_0) \exp(-t / T_1)$$

✓ Transverse, T₂, relaxation: magnetization decays to zero at $t \sim T_2$:

$$M_{\perp} = M_0 \exp(-t / T_2)$$

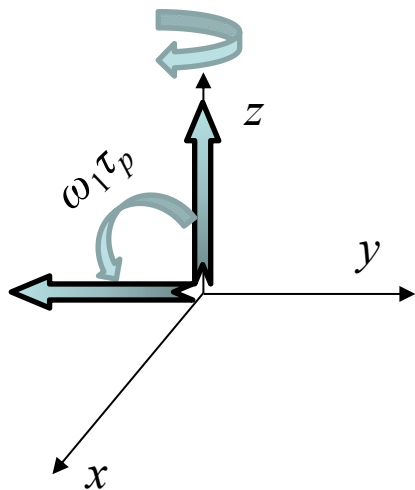
- Generally, $T_1 \neq T_2$.
- Taking all that into account we can write down equations describing precession+relaxation

Bloch equations and FID

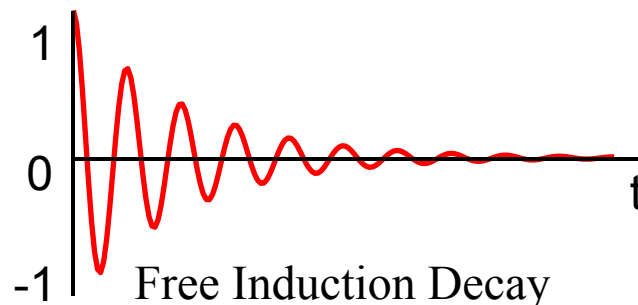
- We write down equations describing precession and add relaxation terms

$$\begin{cases} dM_x / dt = -(\omega_0 - \omega)M_y - M_x / T_2, \\ dM_y / dt = (\omega_0 - \omega)M_x - \omega_1 M_z - M_y / T_2, \\ dM_z / dt = \omega_1 M_y - (M_z - M_{eq}) / T_1 \end{cases}$$

- Now we can describe simplest NMR experiments. Example:
 M_z is flipped by 90 degrees by a resonant RF-pulse: $\varphi = \omega_1 \tau_p = \pi/2$
It starts rotating about the z -axis and decaying with T_2
We detect M_y (or M_x) and collect the Free Induction Decay (FID)



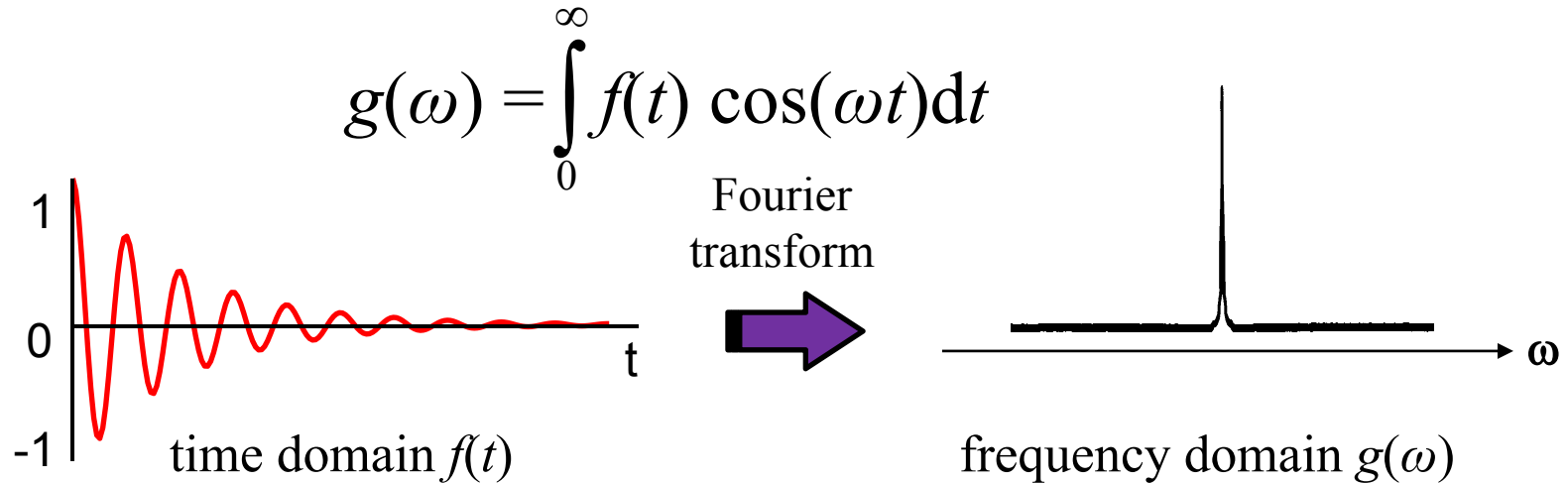
$$M_{\perp}(t) = M_0 \cos(\omega_0 t) \exp(-t / T_2)$$



How to obtain
the spectrum?

Fourier Transform NMR

- How do we obtain the spectrum – by performing the Fourier transform



- Some examples

$$\cos(\omega_0 t) \xrightarrow{\text{FT}} \delta(\omega - \omega_0) \quad \delta\text{-function, single freq.}$$

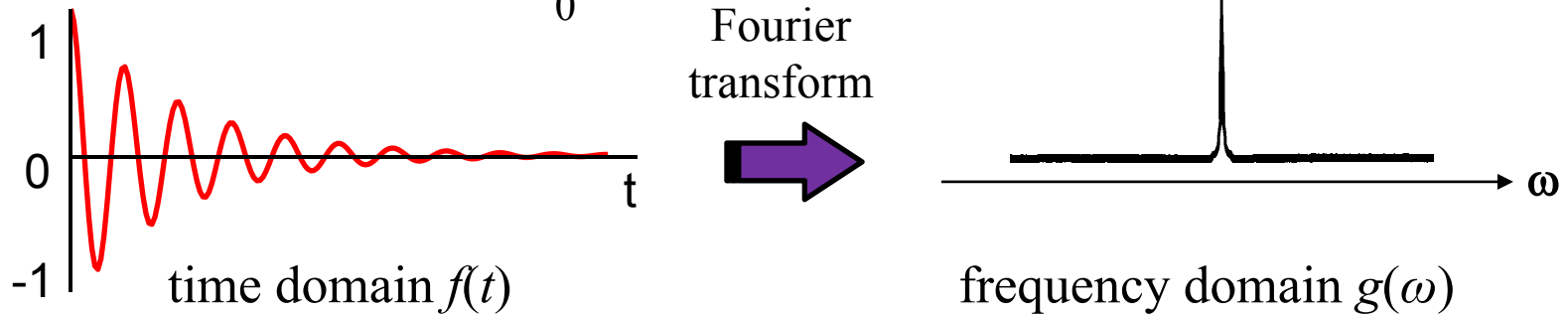
$$\exp(-t/T_2) \xrightarrow{\text{FT}} L(\omega) = \frac{T_2}{1 + \omega^2 T_2^2} \quad \text{Lorentzian at zero freq.}$$

$$M_0 \cos(\omega_0 t) \exp(-t/T_2) \xrightarrow{\text{FT}} L(\omega - \omega_0) \quad \text{Lorentzian at } \omega_0 \text{ freq.}$$

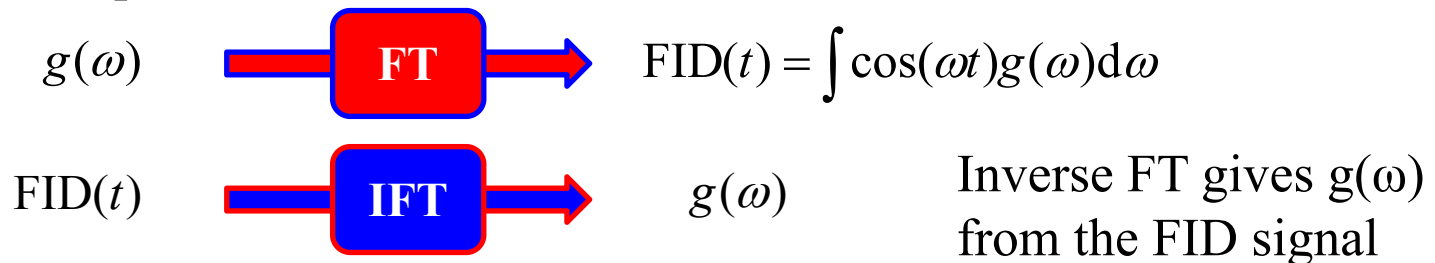
Fourier Transform NMR

- How do we obtain the spectrum – by performing the Fourier transform

$$g(\omega) = \int_0^{\infty} f(t) \cos(\omega t) dt$$



- Some examples



Widths of $g(\omega)$ and $FID(t)$ are inter-related: $\delta t \sim 1/\delta \omega$

- FT can be performed in a fast and efficient way (FFT algorithm)

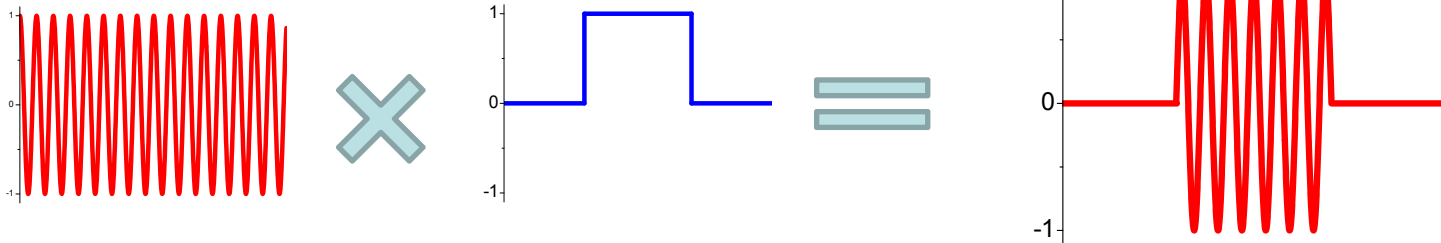
Fourier Transform NMR

- A few words about pulses
- RF-synthesizer produces a signal oscillating at the spectrometer reference frequency ω_{ref} :

$$S(t) \propto \cos(\omega_{ref}t + \psi(t))$$

The phase is time-dependent and can be precisely controlled


- Gating: the signal passes through the transmitter only for certain periods of time => we obtain an **RF-pulse**



- Parameters of the pulse:
 - ✓ frequency ω_{ref}
 - ✓ strength B_1 and duration τ_p provide the **flip angle** $\varphi = |\gamma_N|B_1\tau_p = \omega_1\tau_p$
 - ✓ **phase**: $\psi=0$ (x -pulse), $\psi=\pi/2$ (y -pulse), $\psi=\pi$ ($-x$ -pulse), $\psi=3\pi/2$ ($-y$ -pulse)

Fourier Transform NMR

- A few words about FID detection
- We measure the signal comparing it to the reference frequency ω_{ref} (recording oscillations at ~ 100 MHz frequency would be a disaster)

$$M(t) \propto \cos(\omega_0 t) e^{-t/T_2} \quad \omega_0 \Rightarrow \Omega_0 = \omega_0 - \omega_{ref} \quad M(t) \propto \cos(\Omega_0 t) e^{-t/T_2}$$


- Problem: no sensitivity to the sign of Ω_0 (ω_{ref} greater or smaller than ω_0)
- The solution is quadrature detection: the receiver provides a phase-shifted signal to obtain the information about the sign

$$S_A(t) = C \cdot \cos(\Omega_0 t) e^{-t/T_2}, \quad S_B(t) = C \cdot \sin(\Omega_0 t) e^{-t/T_2}$$

- We introduce ‘complex magnetization’, which contains full information

$$S(t) = S_A(t) + i \cdot S_B(t) = C \cdot \exp(i\Omega_0 t - t/T_2)$$

- Fourier transformation yields $S(\Omega) = C \int_0^{\infty} \exp(i\Omega_0 t - t/T_2) e^{-i\Omega t} dt$

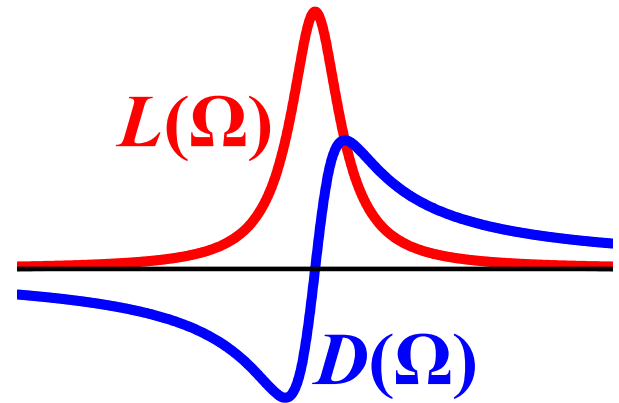
- Yet phasing is a problem

Fourier Transform NMR

- Fourier transformation yields $S(\Omega) = C \int_0^{\infty} \exp(i\Omega_0 t - t/T_2) e^{-i\Omega t} dt$
- Real and imaginary part of the signal (when C is real)

$$\text{Re}\{S(\Omega)\} = L(\Omega - \Omega_0) = \frac{T_2}{1 + (\Omega - \Omega_0)^2 T_2^2}$$

$$\text{Im}\{S(\Omega)\} = -D(\Omega - \Omega_0) = -\frac{(\Omega - \Omega_0) T_2^2}{1 + (\Omega - \Omega_0)^2 T_2^2}$$



- Generally, $C = |C| e^{i\psi}$ is a complex number because of a phase shift of the pulser and receiver

$$\text{Re}\{S(\Omega)\} \propto L(\Omega - \Omega_0) \cos \psi - D(\Omega - \Omega_0) \sin \psi$$

By varying the phase we can obtain the Lorentzian or purely dispersive line

The phase can be set and then kept the same

Back to relaxation

Before talking about 2D-NMR let us briefly discuss T_1 and T_2

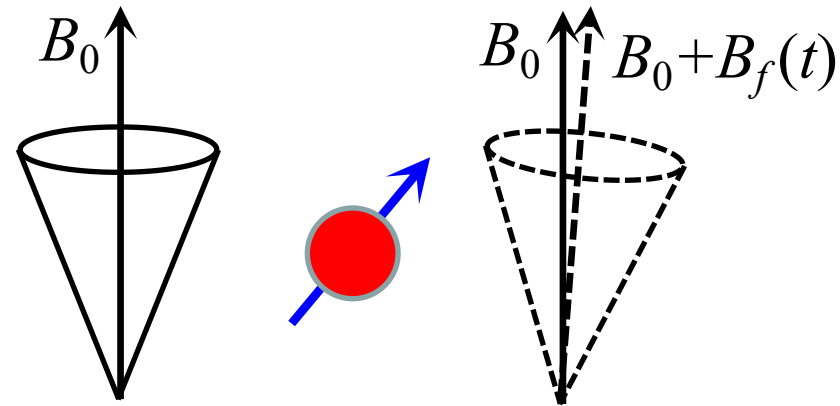
- ✓ Origin of T_1 and T_2
- ✓ Relaxation and molecular motion
- ✓ Measurement of the relaxation times

T_1 -relaxation

- T_1 -relaxation: precession in the B_0 field and a small fluctuating field $B_f(t)$

- The precession cone is moving

- Eventually, the spin can even flip



- Spin flips up-to-down and down-to-up have slightly different probability (Boltzmann law!): M_z goes to $M_{eq} \neq 0$

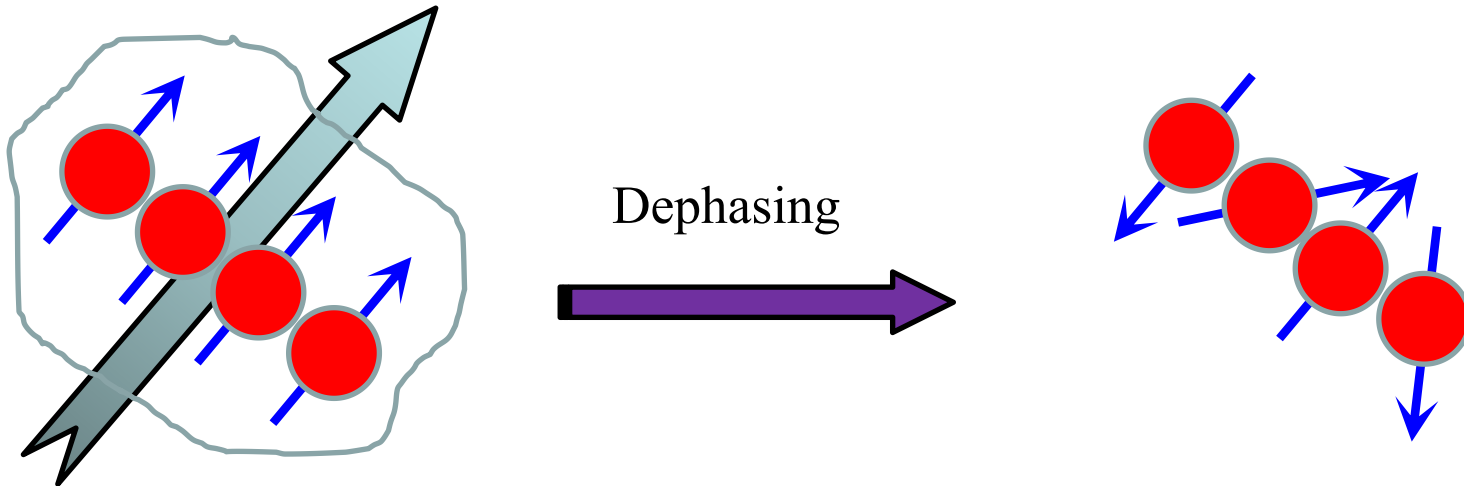
- General expression for the transition rate
$$W = \frac{1}{2T_1} = \frac{1}{\hbar^2} |V|^2 J(\omega)$$

$$J(\omega) = \frac{2\tau_c}{1 + \omega^2 \tau_c^2}$$

Noise spectral density at the transition frequency
 τ_c is the motional correlation time

T₂-relaxation

- T₂-relaxation: kicks from the environment disturb the precession
- Different spins precess differently and transverse net magnetization is gone



- Generally the T₂-rate

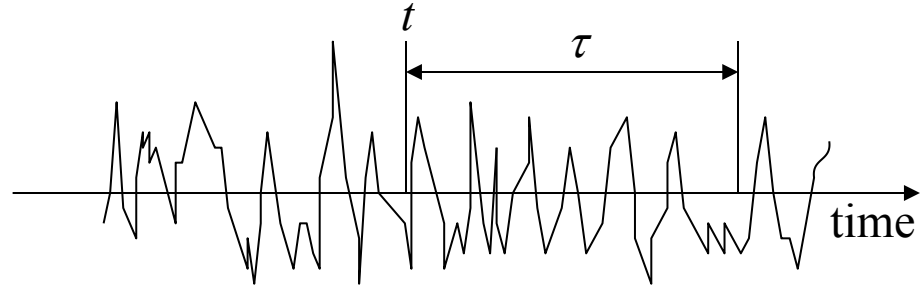
$$\frac{1}{T_2} = \frac{1}{T_2^a} + \frac{1}{T_2^{na}} = \frac{1}{T_2^a} + \frac{1}{2T_1}$$

Two contributions:
Adiabatic and non-adiabatic (T₁-related)

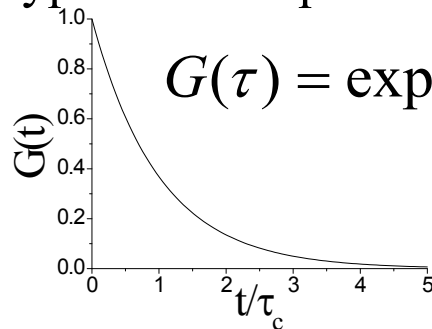
Noise spectral density

- Simple example: spins relaxed by fluctuating local fields, $\overline{B_f(t)}=0$
- However, the auto-correlation function is non-zero

$$\overline{f(t)f(t+\tau)} = G(\tau)$$



- Typical assumption

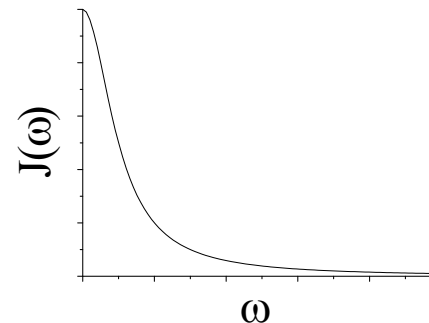


$$G(\tau) = \exp(-|\tau|/\tau_c)$$

- ✓ Exponential auto-correlation function
- ✓ τ_c comes into play

- Lorentzian-like noise spectral density

$$J(\omega) = 2 \int_0^{\infty} G(t) e^{-i\omega t} dt = \frac{2\tau_c}{1 + \omega^2 \tau_c^2}$$

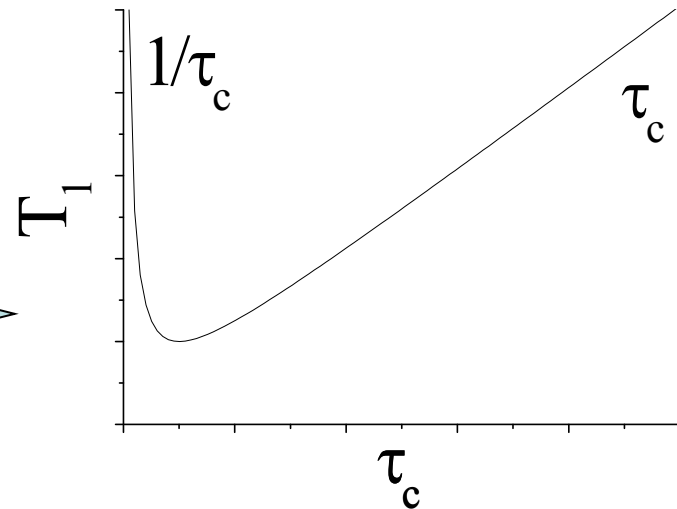


Expressions for T_1 and T_2

- Simple example: spins relaxed by fluctuating local fields, $\overline{B_f(t)}=0$
- The auto-correlation function is non-zero
- General expressions for T_1 and T_2

$$\frac{1}{T_1} = \gamma^2 (\overline{B_x^{*2}} + \overline{B_y^{*2}}) \frac{\tau_c}{1 + \omega^2 \tau_c^2},$$

$$T_1 \propto \frac{1 + \omega^2 \tau_c^2}{\tau_c}$$



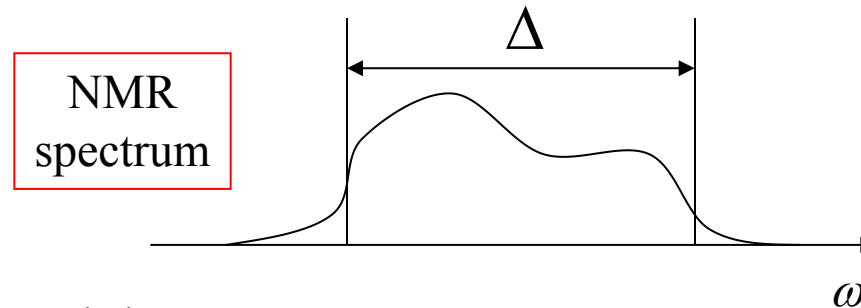
This dependence is explained by the $J(\omega)$ behavior

$$\frac{1}{T_2} = \frac{1}{T_2^a} + \frac{1}{T_2^{na}} = \frac{1}{T_2^a} + \frac{1}{2T_1} \quad \frac{1}{T_2^a} = \gamma^2 B_z^{*2} \tau_c$$



Inhomogeneous linewidth

- Problem: we need to discriminate two contributions to T_2
- Decay of the NMR signal **also** proceeds due to static **inhomogeneities** in the precession frequency ω_0 . This can be due to external field gradients and local static interactions.



- **Resulting rate** the signal decay

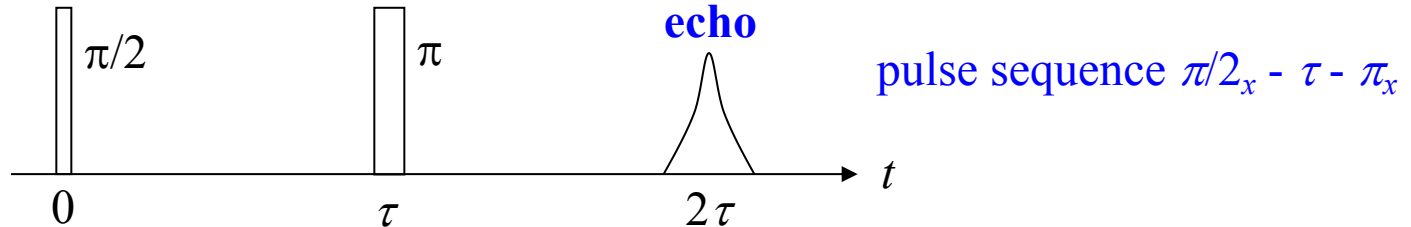
$$\frac{1}{T_2^*} = \frac{1}{T_2} + \Delta \approx \frac{1}{T_2^a} + \frac{1}{T_2^{na}} + \Delta$$

Reason: $\delta\omega \cdot \delta t \sim 2\pi$

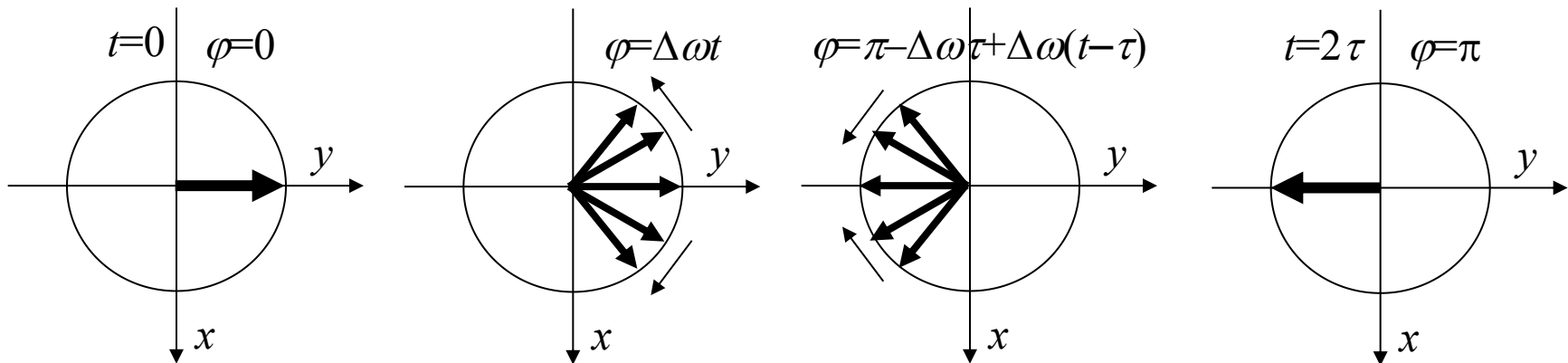
- The **first two contributions** are the same for all the molecules and thus define the **homogeneous linewidth**.
- The **last contribution** defines the **inhomogeneous linewidth**.
- In **solids** usually $T_2^* \ll T_2$

T₂-measurement: spin echo

- Large **inhomogeneous linewidth** means very fast dephasing of the spin
- However, dephased magnetization can be **focused back** by pulses



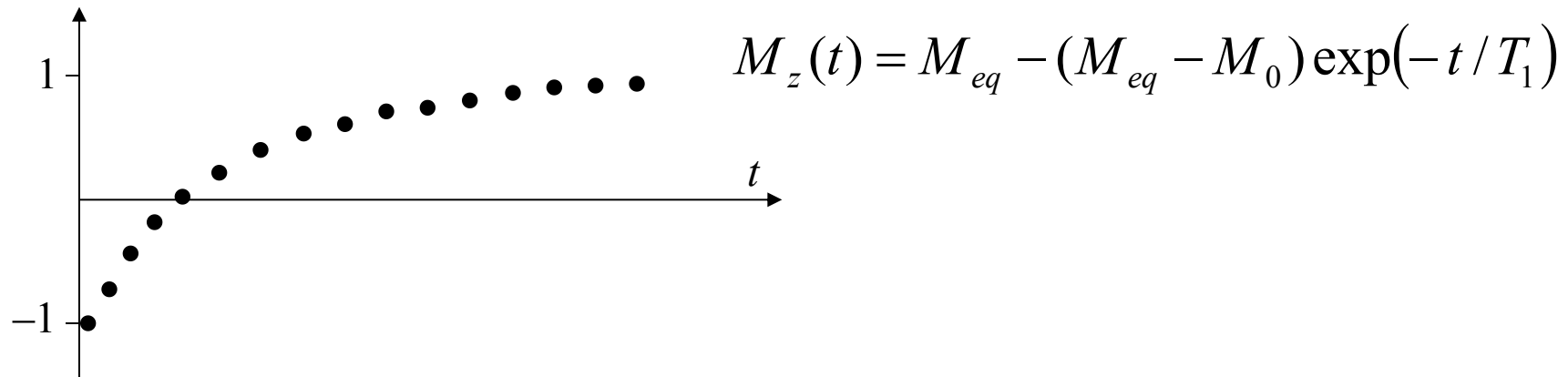
- Explanation: let us divide system into **isochromates** having the same frequency ω_0 . Their offsets are $\Delta\omega = \omega_0 - \omega$. At certain time they all have different phases



- But at $t=2\tau$ all have the same phase: there is an ‘**echo**’!
- The spin echo signal decays with T₂

T_1 -measurement: inversion-recovery

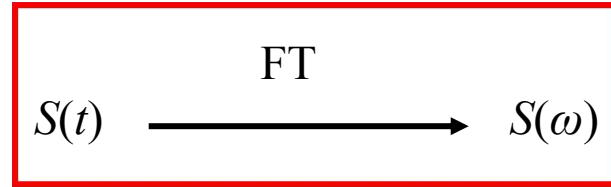
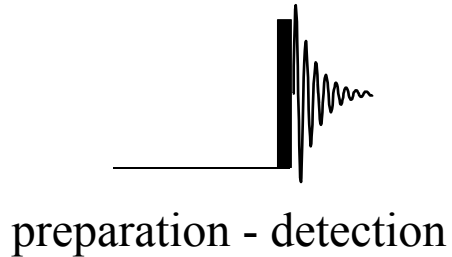
- Determination of T_1 is often quite important as well
- Standard method is **inversion-recovery**
- First we turn the spin(s) by pulse (usually $\pi/2$ or π) and then look how system **goes back to equilibrium** (recovers z-magnetization). If the pulse is a π -pulse magnetization will be **inverted** (maximal variation of m-n) and then **recovered**
- Equation for M_z is as follows:



- The kinetic trace (t -dependence) gives **T_1 -time**
- To detect magnetization at time t one more $\pi/2$ -pulse is applied, the sequence is then $\pi_x - t$ (variable) - $\pi_x/2$ - measurement
- Spin echo can be used for detection as well, the pulse sequence is then $\pi_x - t$ (variable) - $\pi_x/2$ - τ - π_x - τ - measurement
- The sequence should be repeated with different delays t

1D-NMR

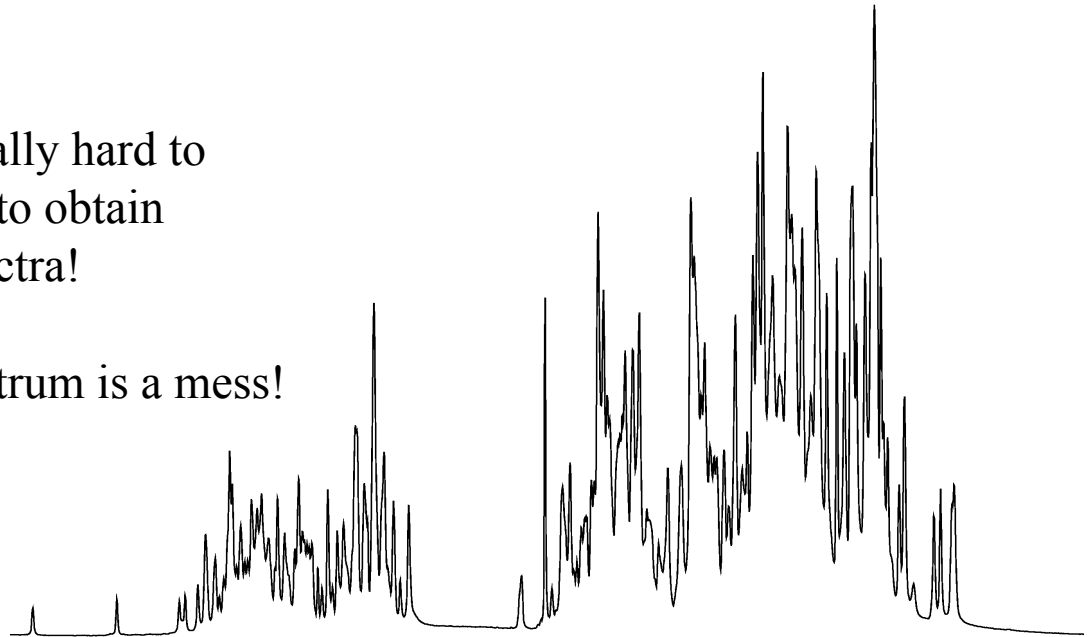
- 1D-NMR experiment (simplest case)



- Why is it not enough?

For large proteins it is really hard to assign NMR signals and to obtain information from the spectra!

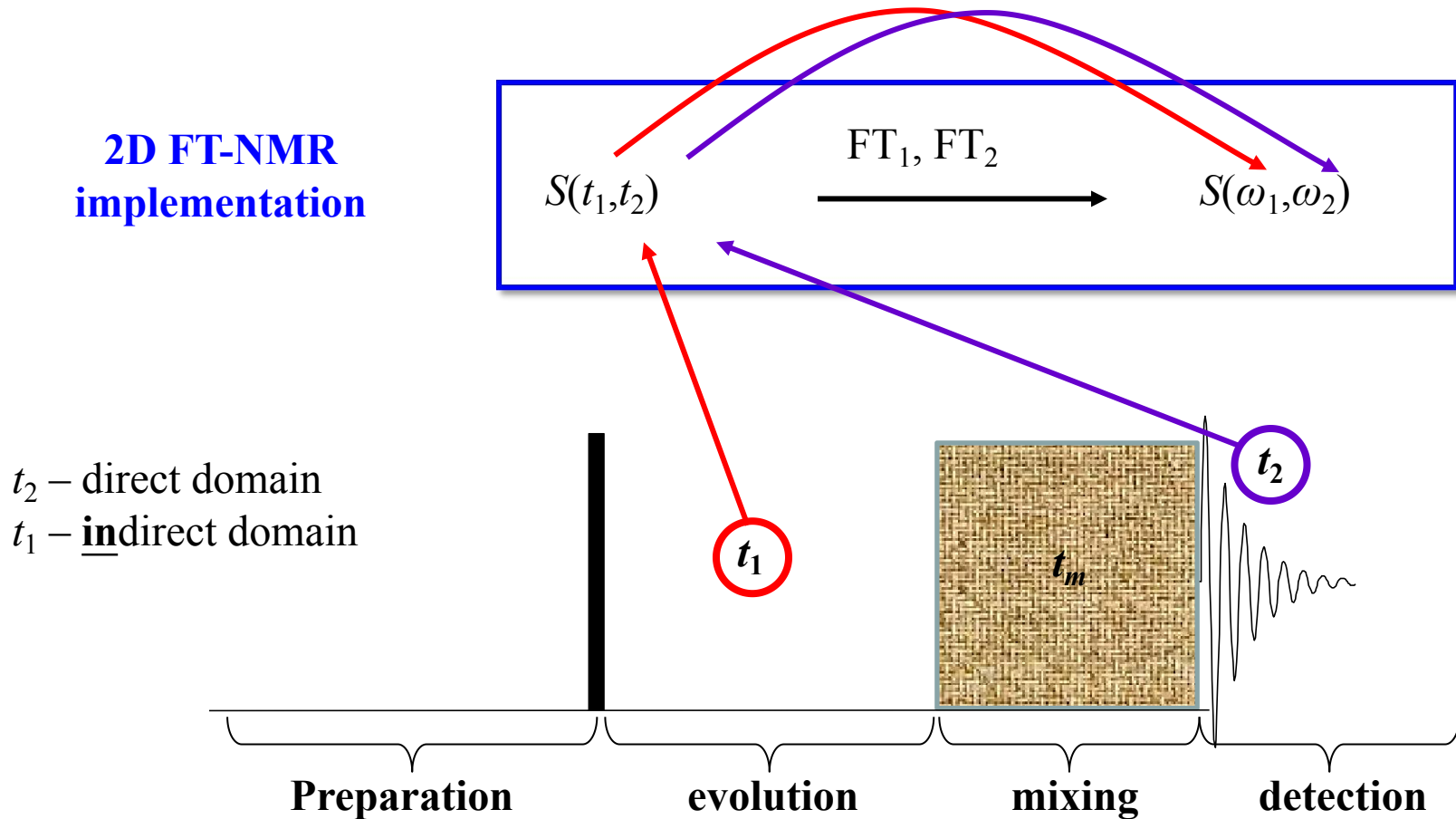
Too many peaks \Rightarrow Spectrum is a mess!



2D-NMR

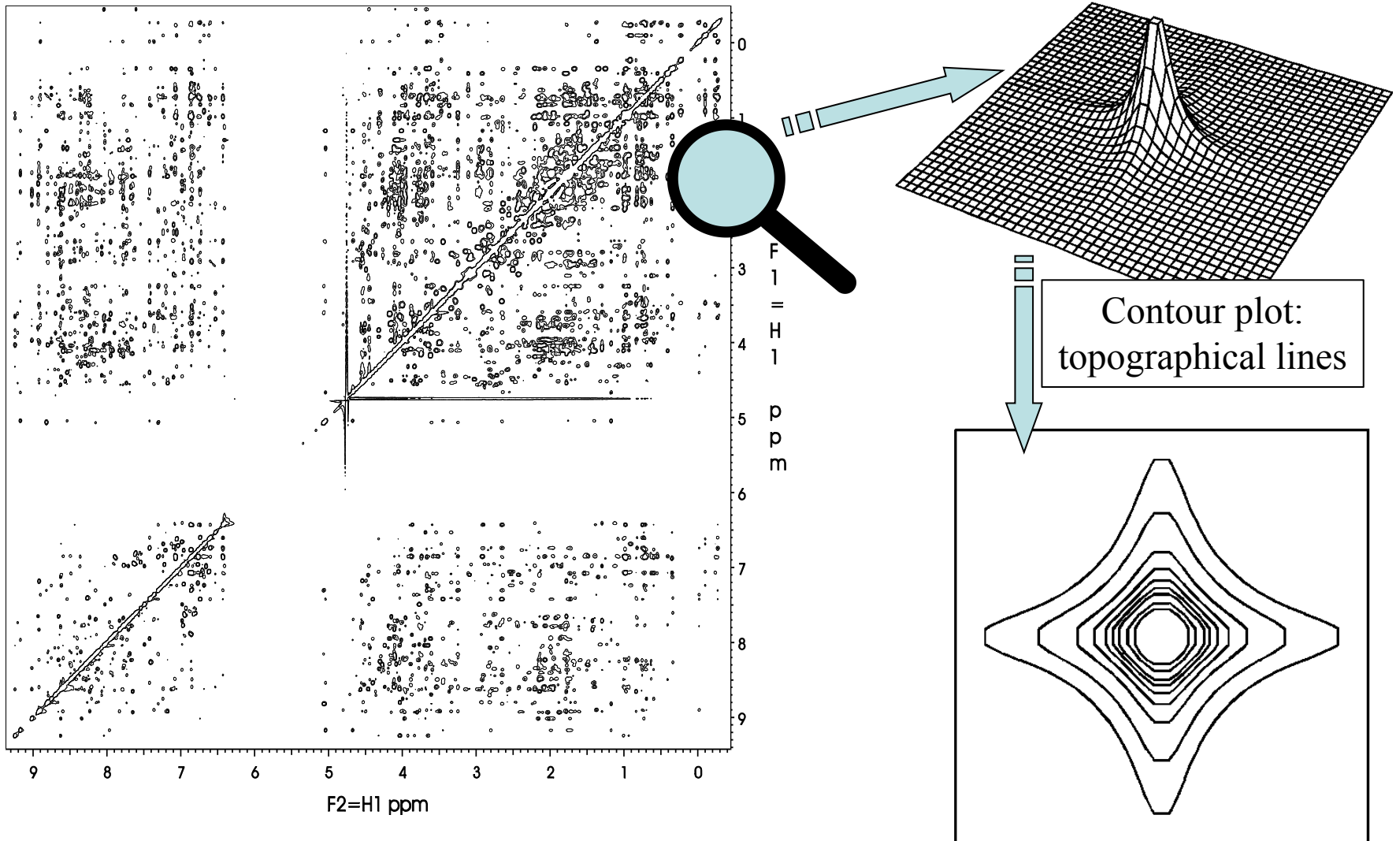
- Idea: adding a second dimension for improved resolution

2D FT-NMR implementation



It is up to you how to design preparation and mixing: decide, what you want to know about your molecules!

2D-NMR spectrum of a protein

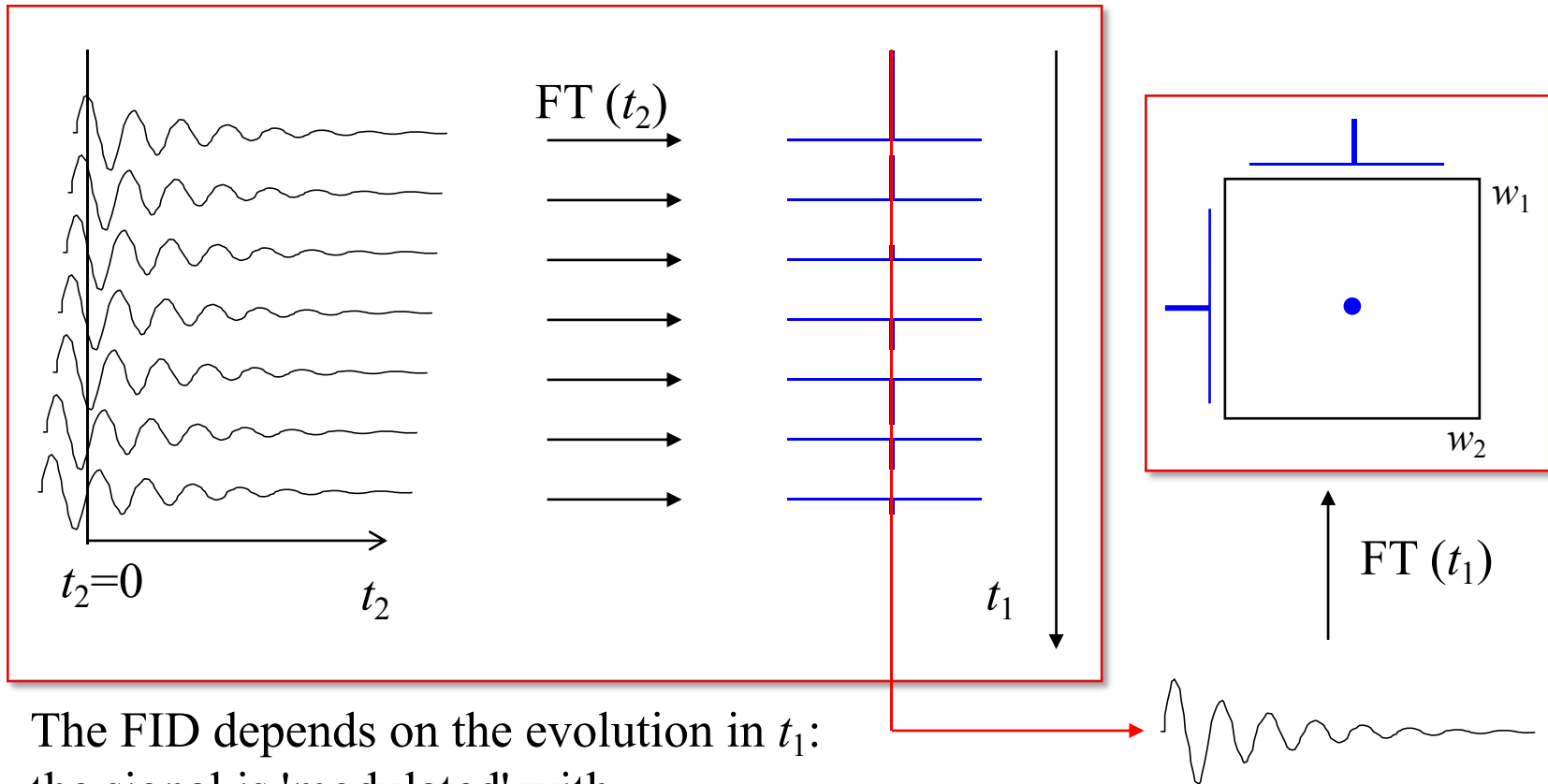


In 2D the peaks become resolved

Direct and indirect domain

Direct time domain (acquisition, t_2): FID detection

Indirect time domain (evolution time, t_1 in 2D): FIDs are collected for different t_1 times



The FID depends on the evolution in t_1 :
the signal is 'modulated' with ω_1

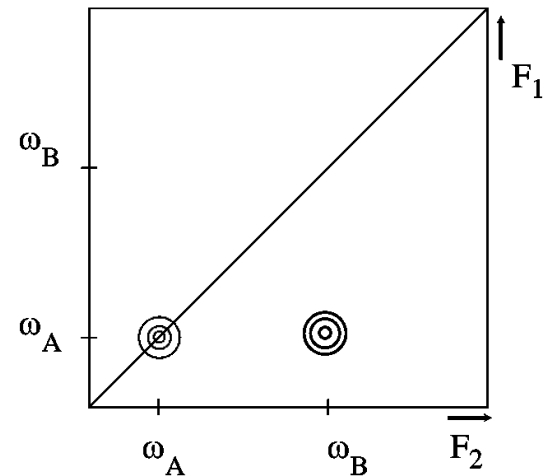
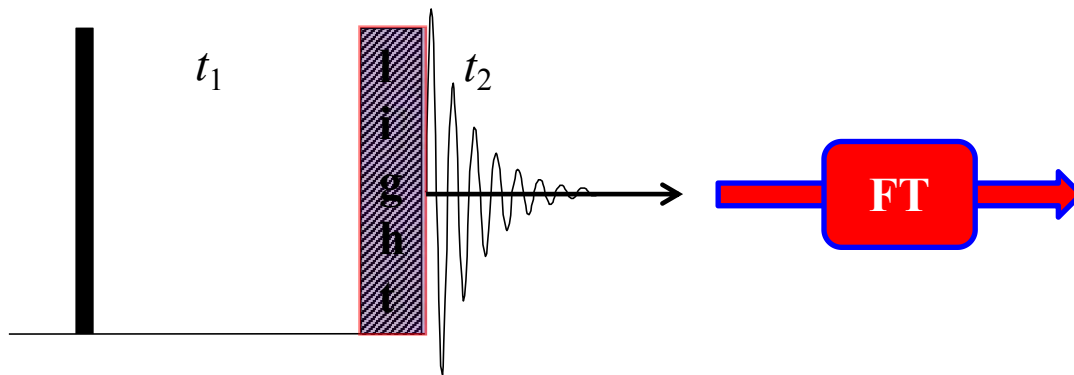
For simplicity here the frequency ω is the same in t_1 and in t_2

Cross-peaks and diagonal peaks

Simple example: **SCOTCH** experiment

Spin **CO**herence **T**ransfer in (photo) **CH**emical reactions

Reaction $A \xrightarrow{h\nu} B$ with a proton at ω_A in A which resonates at ω_B in B.



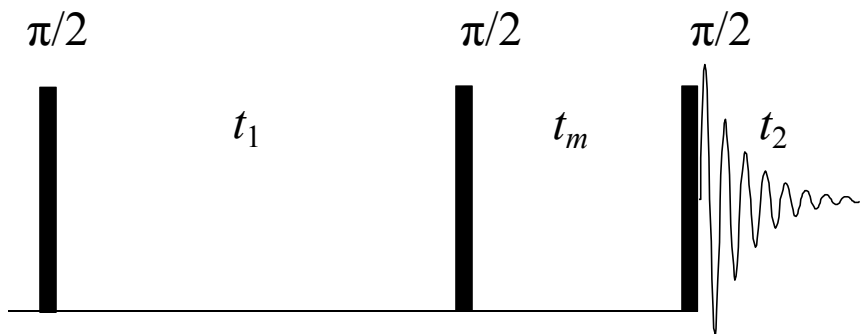
M magnetization evolves with the frequency ω_A in t_1 . After the light pulse, the frequency changes to ω_B .

FT provides a 2D spectrum with a peak at ω_A in F_1 and ω_B in F_2

The **cross-peak** comes from $A \rightarrow B$ and $\omega_A \neq \omega_B$

When conversion $A \rightarrow B$ is incomplete the **diagonal peak** stays

Example: NOESY experiment



Cross-peaks come from NOE during the mixing period

SY = **S**pectroscop**Y**

How does it work?

$$I_{1z} \xrightarrow{\pi/2_x} -I_{1y} \xrightarrow{t_1} -I_{1y} \xrightarrow{\pi/2_x \text{ NOE}} -I_{1z} \xrightarrow{\pi/2_x} I_{2y}$$

Transverse magnetization has gone from spin 1 to spin 2

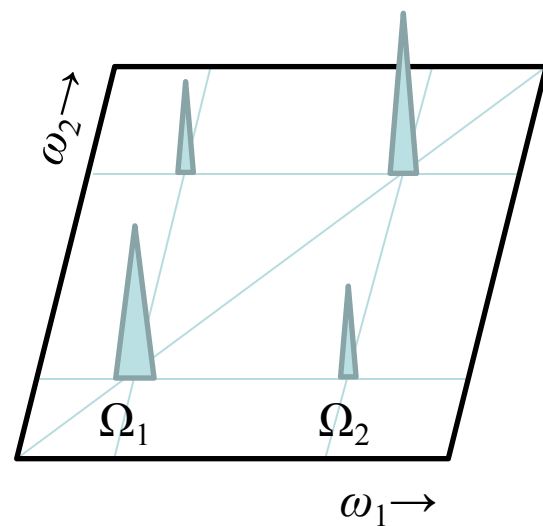
The efficiency of transfer is (simplified)

$$\sin(\omega_1 t_1) * \sin(\omega_2 t_2)$$

A cross-peak will appear in the **NOESY-spectrum**

The cross-peak gives information on NOE \Rightarrow distance between the spins

The same method can be used to study chemical exchange (EXSY)



Summary

- ✓ NMR is working with magnetic moments of nuclei
(originating from their spins)
- ✓ Simple theory (Bloch equations) allows one to understand
basic experiments
- ✓ 1D & 2D NMR concepts are introduced