NMR Spectroscopy: Principles and Applications

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Relaxation

Lecture 9

Relaxation

Relaxation is the process by which the bulk magnetization reaches its equilibrium Value.

Features of Relaxation in NMR

Relaxation in NMR is slow – millisecond to several seconds and even minutes. This means we can manipulate transverse magnetization by multiple pulse-delay sequences. But wait long time between signal averaging scans.

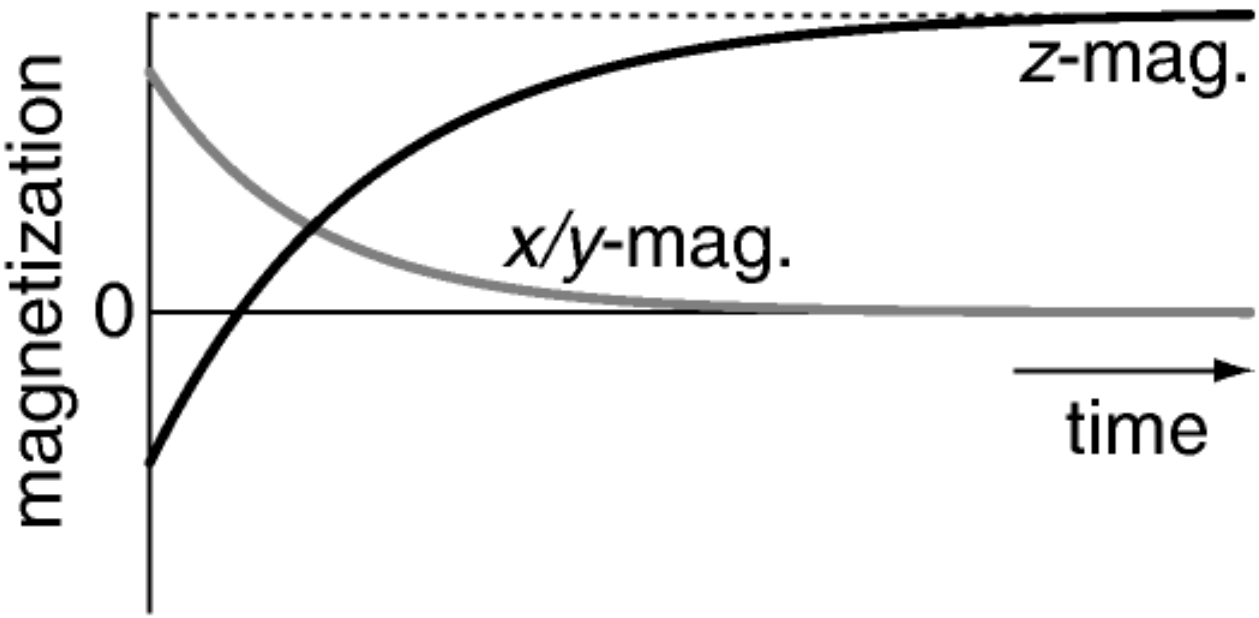
Long relaxation time means narrow spectral lines yielding high resolution spectrum.

NMR relaxation is sensitive to slow molecular motions and act as a probe for accessing such information.

Relaxation in the form of Nuclear Overhauser Effect (NOE) gives structural information of the molecule.

What is Relaxation?

Relaxation is the process by which the bulk magnetization reaches its equilibrium value. That is the z – magnetization grows back along the applied field axis (z-axis). Any transverse magnetization and other coherences decay over time.



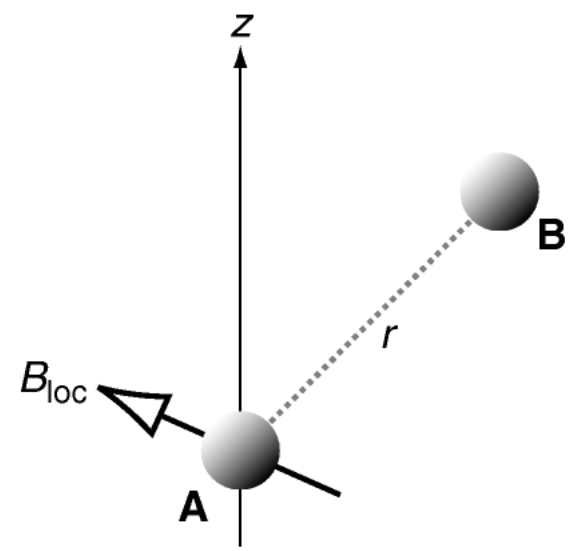
What is Relaxation?

In order to flip magnetization back along z-axis there should be frequency components in the vicinity of Larmor frequency present due to the relaxation process. The same process should also render the coherences to decay over time by introducing random phase disorder in them.

There are various naturally occurring interactions or mechanisms that lead to the generation of fluctuating local magnetic fields that cause relaxation.

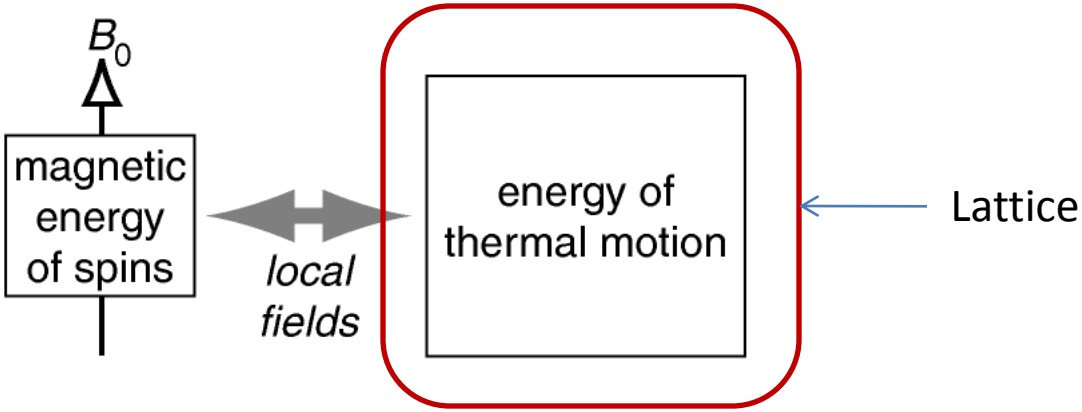
What is Relaxation?

Nuclear spins are tiny magnets and they interact with each other by producing magnetic fields at the site of the other spins. These magnetic fields change in magnitude and orientation (with respect to the applied field direction) due to thermal motions such as molecular rotations (gasses, liquids), collisions (gasses, liquids), and local centers of fluctuations and rotations (solids).



Spin-Lattice Relaxation

Thus the nuclear Zeeman interaction is coupled to the thermal energy of the whole system via motions and termed as lattice. Thus the Spin-Lattice interaction leads to the relaxation.



The lattice has many degrees of freedom with very large heat capacity compared to the spin energy, that energy flows from spins to lattice by bringing the spin energy in thermal equilibrium with the lattice. This asymmetry in the flow of energy (i.e spin system to lattice) bring about the generation of z- magnetization and no coherences at equilibrium.

Correlations of Random Motion

The nature of the time dependence of the local fields $B_{loc}(t)$ imposed by the random thermal motions is represented by a function called ***correlation function*** *characterized by a parameter called* ***correlation time****.*

Let us say in a collection of spins, the spin i experiences a local field $B_{loc,i}(t)$ and at a later time $t+\tau$, $B_{loc}(t+\tau)$, the ***correlation function G(t, τ)*** *is defined as the average over the collection of the product $B_{loc}(t)$ and $B_{loc}(t+\tau)$.*

$$G(t,\tau) = \frac{1}{N}\sum_{i=1}^{N} B_{loc,i}(t) B_{loc,i}(t+\tau)$$

$$= \overline{B_{loc}(t) B_{loc}(t+\tau)}$$

Correlations of Random Motion

We make a further assertion that the ***correlation function*** *does not depend on the absolute time but only the duration* τ *that has elapsed. The correlation function is then,*

$$G(\tau) = \frac{1}{N}\sum_{i=1}^{N} B_{loc,i}(0)B_{loc,i}(\tau)$$
$$= \overline{B_{loc}(0)B_{loc}(\tau)}$$

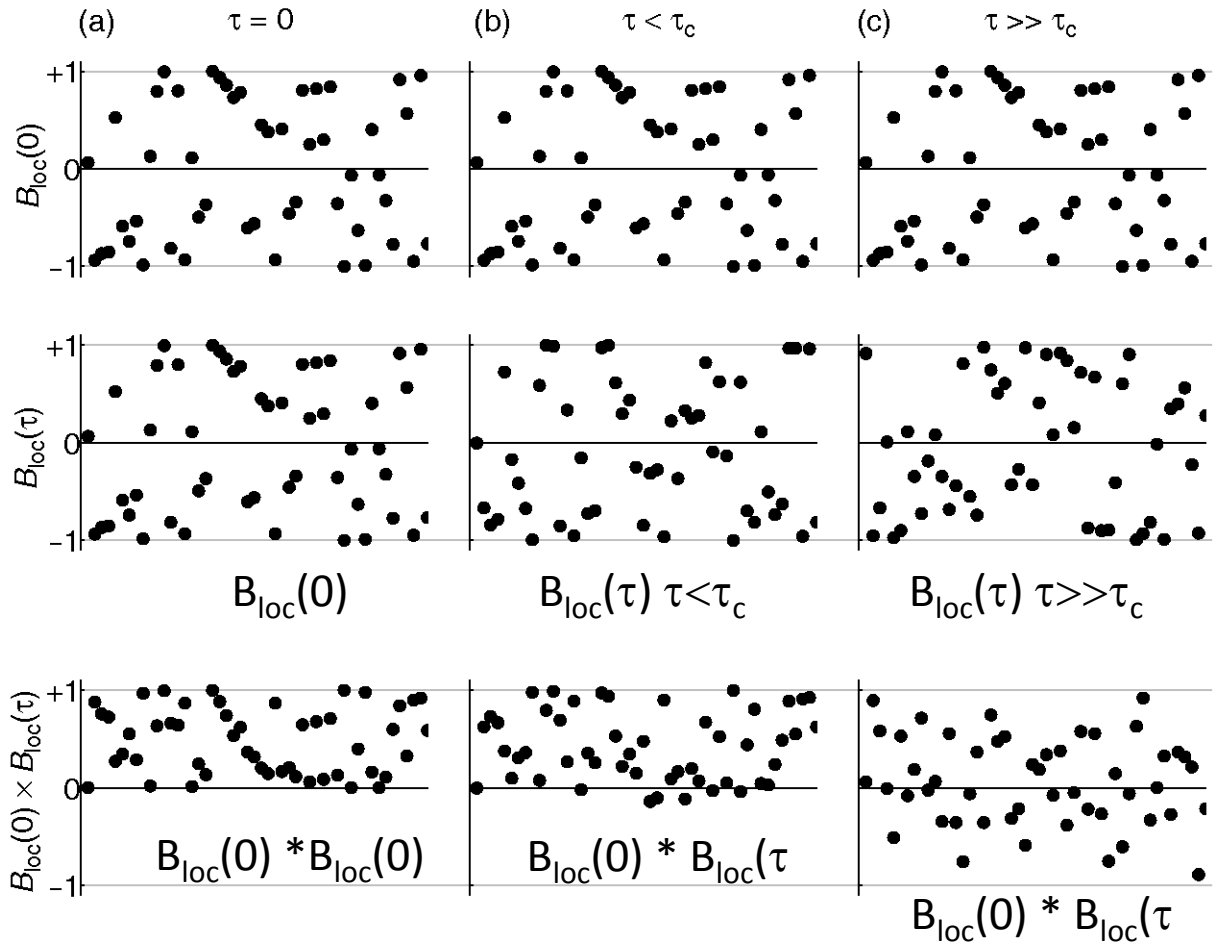
The function G(τ*) smoothly decays with a time constant called the* ***correlation time (*τ_c*)****. The correlation time is also defined as the average time taken for the molecule to rotate 1 radian away from the starting position.*

Visualizing Correlation Function

Let us follow, say 50 spins, by monitoring B_{loc} (set to vary between +1 to -1) at their sites for different values of τ.

In column (a) τ=0, (b) $\tau<\tau_c$, and in (c) $\tau>>\tau_c$. The top row is B_{loc} at τ=0, the middle row is B_{loc} at τ, and the bottom row is product of the first and second. Note that:

$$\overline{B_{loc}(t)} = 0$$

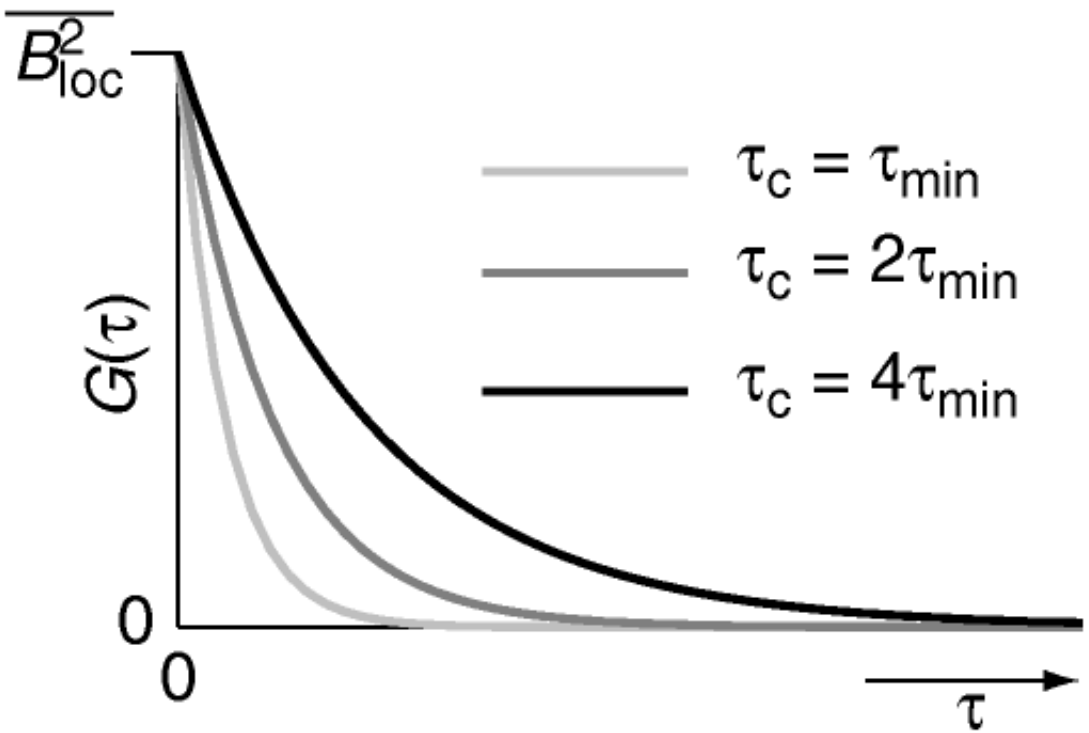


Visualizing Correlation Function

The product decays as the elapsed time increases. The function G(τ) thus looks as below for various τ_c.

G(τ) has maximum value at t=0.

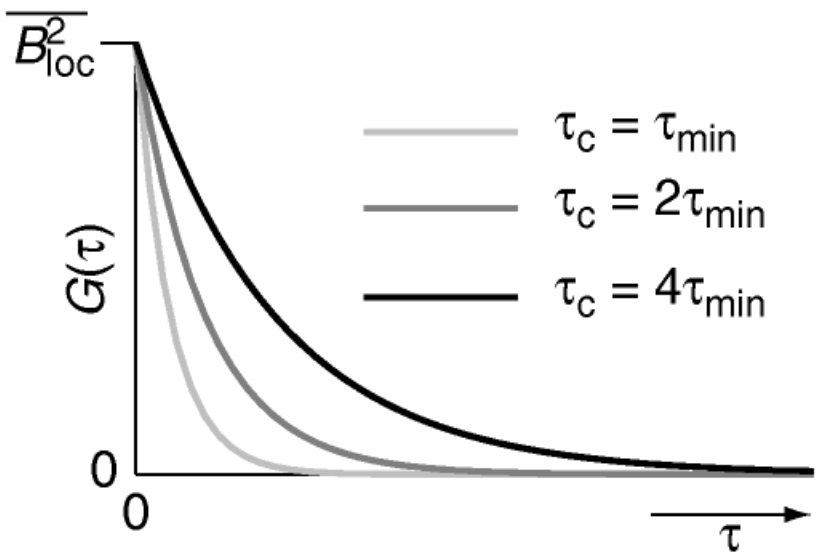
$$G(0) = \overline{B_{loc}(0)B_{loc}(0)}$$
$$= \overline{B_{loc}^2}$$
$$G(\tau) = \overline{B_{loc}^2}\exp(|\tau|/\tau_c)$$

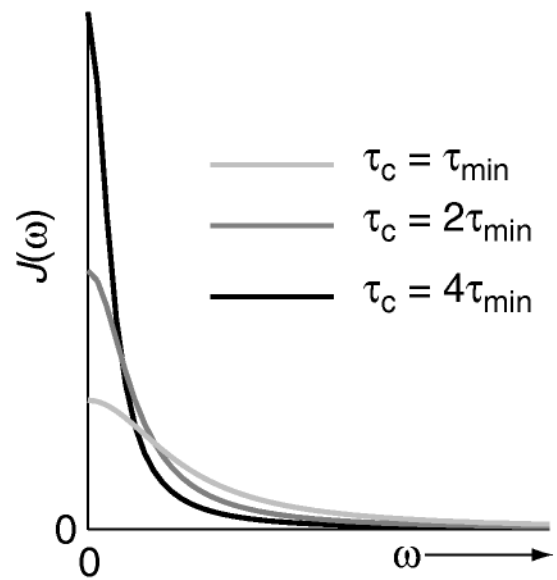


Spectral Density Function

The Fourier transform of the correlation function $G(\tau)$ is the spectral density function $J(\omega)$.

$$G(\tau) \xrightarrow{FT} J(\omega) \quad G(\tau) = \overline{B_{loc}^2} \exp(|\tau|/\tau_c) \quad J(\omega) = \overline{B_{loc}^2} \frac{2\tau_c}{1+\omega^2\tau_c^2}$$





The area under $J(\omega)$ is independent of τ_c.

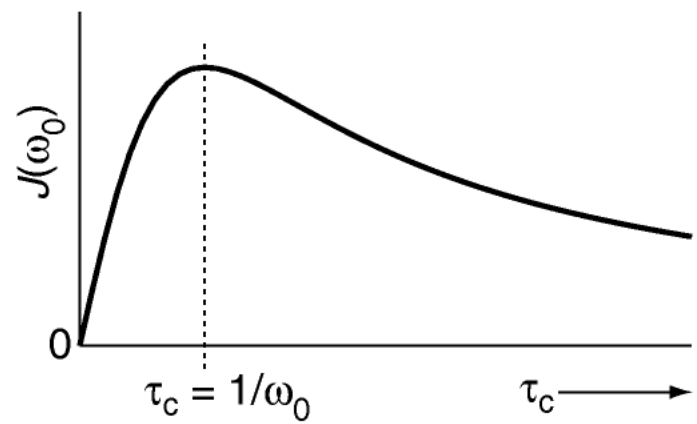
$$\int_0^\infty J(\omega)d\omega = \int_0^\infty \overline{B_{loc}^2} \frac{2\tau_c}{1+\omega^2\tau_c^2} d\omega = \pi \overline{B_{loc}^2}$$

Spectral Density at Larmor frequency

For the equilibrium z- magnetization to build up, there should be finite spectral density at the Larmor frequency, so that the spins can flip by utilizing the energy.

$$J(\omega_0) = \overline{B_{loc}^2}\, j(\omega_0)$$

$$j(\omega_0) = \frac{2\tau_c}{1+\omega_0^2\tau_c^2}$$



A plot of $J(\omega_0)$ vs τ_c shows a maximum when $\omega_0\ \tau_c \sim 1$. Thus, the longitudinal relaxation time of a given molecule depends on the spectrometer frequency.

Longitudinal Relaxation of Isolated Spin system

The z- magnetization of spin I=1/2 system is proportional to the population difference of the two states α and β.

$$M_z = \frac{1}{2}\gamma\hbar(n_\alpha - n_\beta)$$

At equilibrium the populations are given by the Boltzmann distribution.

$$n_\alpha^0 = \frac{1}{2}N\exp(-E_\alpha/k_BT) \quad n_\beta^0 = \frac{1}{2}N\exp(-E_\beta/k_BT)$$

$$n_\alpha^0 = \frac{1}{2}N(1-E_\alpha/k_BT) \quad n_\beta^0 = \frac{1}{2}N(1-E_\beta/k_BT) \because (E/k_BT) << 1$$

$$M_z^0 = \frac{\gamma^2\hbar^2 NB_0}{k_BT}$$

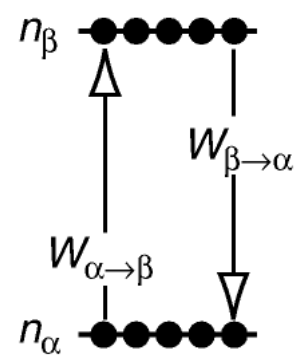
Longitudinal Relaxation of Isolated Spin system

Consider a two level system with a rate of transfer from $\alpha \leftarrow\rightarrow \beta$ is $W_{\alpha\beta}$. Then the rate of change of population of a level is given by the deviation from equilibrium of the populations of the levels and the transition rate.

$$\Delta n_\alpha = W_{\alpha\beta}(n_\beta - n_\beta^0) - W_{\alpha\beta}(n_\alpha - n_\alpha^0)$$

$$\Delta n_\beta = -W_{\alpha\beta}(n_\beta - n_\beta^0) + W_{\alpha\beta}(n_\alpha - n_\alpha^0)$$

$$\Delta M_z = -2W_{\alpha\beta}\left[(n_\alpha - n_\beta) - (n_\alpha^0 - n_\beta^0)\right] = -2W_{\alpha\beta}(M_z - M_z^0)$$



The rate of Change of M_z is written as

$$\frac{d}{dt}M_z(t) = -R_z\left[M_z(t) - M_z^0\right]$$

$$\frac{d}{dt}M_z(t) = -\frac{1}{T_1}\left[M_z(t) - M_z^0\right]$$

Longitudinal Relaxation of Isolated Spin system

The solution of the rate equation is straightforward.

$$\frac{d}{dt}M_z(t) = -R_z\left[M_z(t) - M_z^0\right]$$

$$\int \frac{1}{\left(M_z(t) - M_z^0\right)} dM_z(t) = \int -R_z dt$$

$$\ln\left(M_z(t) - M_z^0\right) = -R_z t + const.$$

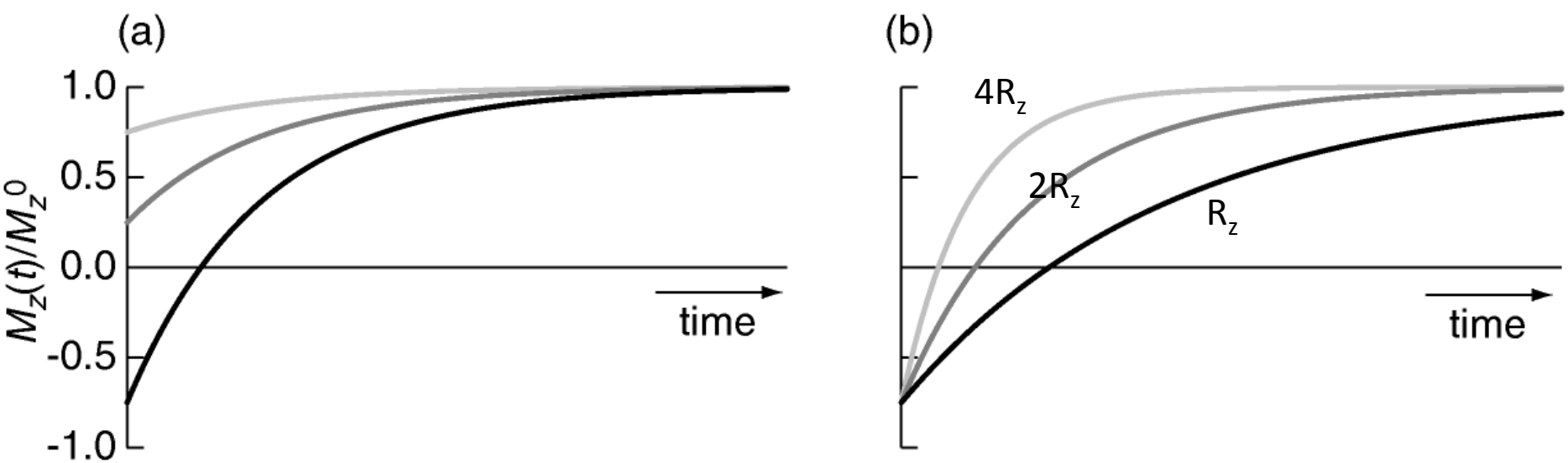
Setting t=0, we get the constant as ln[M_z(0)-M_z(t)]

$$\ln\left(\frac{M_z(t) - M_z^0}{M_z(0) - M_z^0}\right) = -R_z t \qquad \left(\frac{M_z(t) - M_z^0}{M_z(0) - M_z^0}\right) = \exp(-R_z t)$$

Longitudinal Relaxation of Isolated Spin system

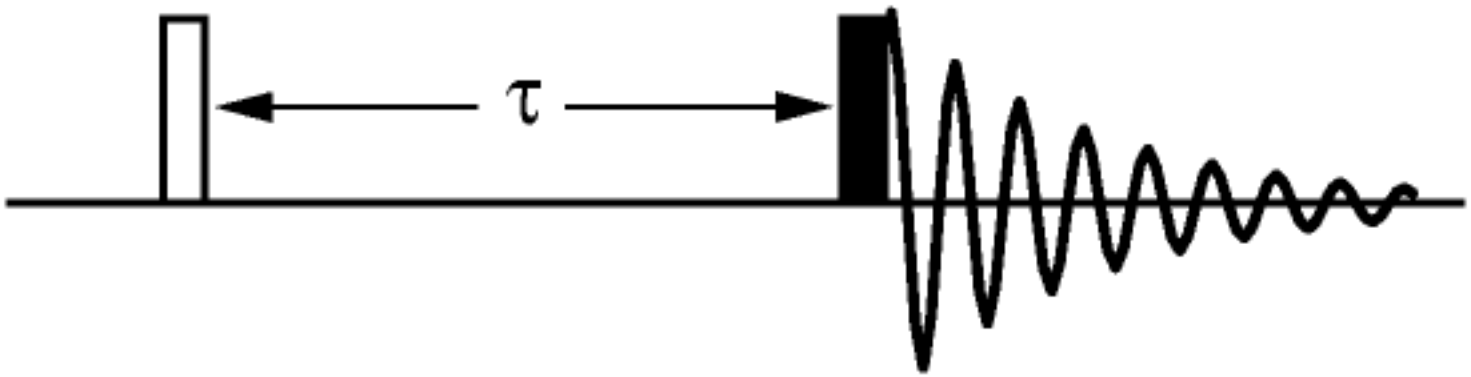
Plots show how the longitudinal magnetization vary with time (a) for a given rate constant and various starting point of $M_z(0)$ and in (b) the $M_z(0)$ was partial inversion and the curves are for various rate constants R_z.

$$\left(\frac{M_z(t)-M_z^0}{M_z(0)-M_z^0}\right)=\exp(-R_z t)$$

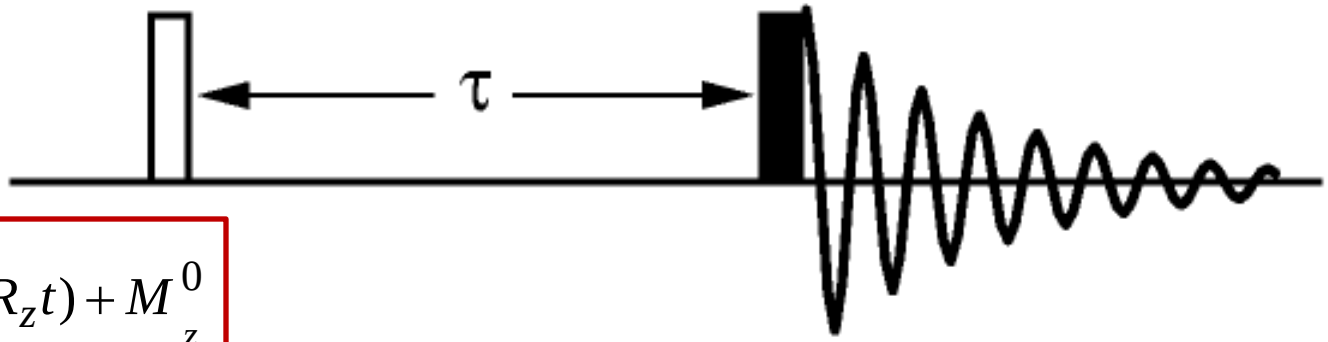


Inversion Recovery

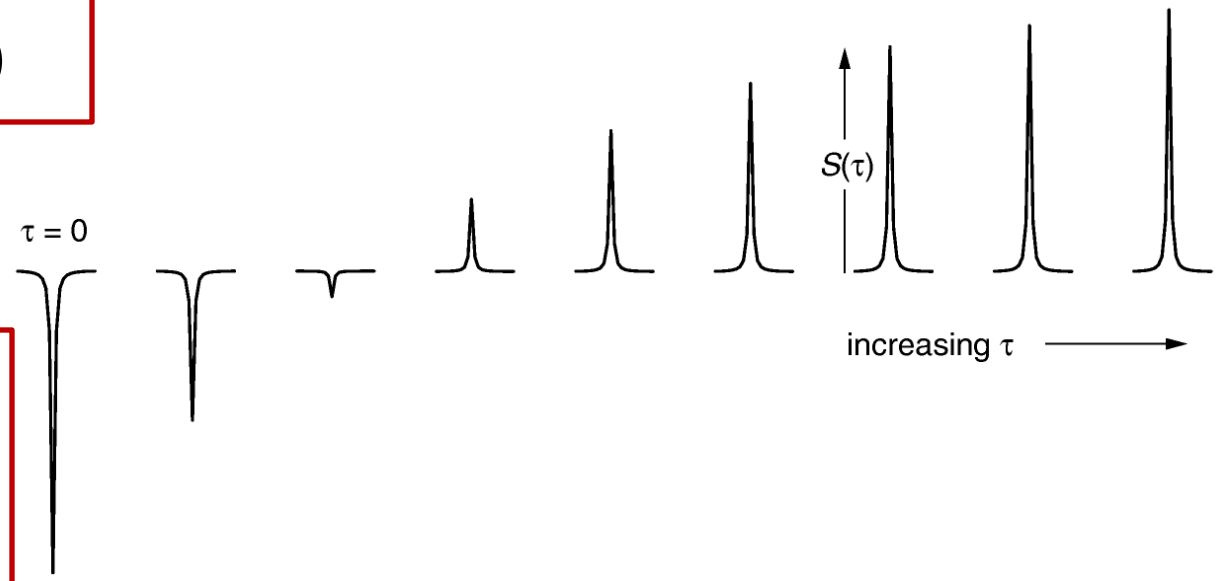
The rate constants R_z can be readily measured by inversion recovery experiment. Initially spins are inverted by a 180° pulse and allowed to recover for a time interval τ and a 90° pulse rotates the magnetization to the transverse plane for detection. By varying the inter pulse delay τ we trace the intensity profile of the recovery and the rate constant from the profile.



Inversion Recovery



$$M_z(\tau) = -2M_z^0 \exp(-R_z t) + M_z^0$$
$$= M_z^0\left(1 - 2\exp(-R_z t)\right)$$

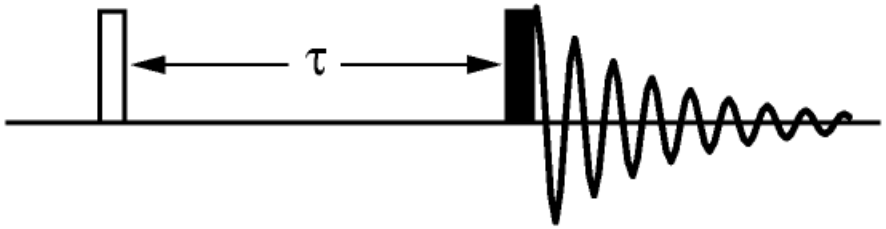


$$S(\tau) = -S^0\left(1 - 2\exp(-R_z \tau)\right)$$
$$\frac{S(\tau) + S(0)}{2S(0)} = \exp(-R_z \tau)$$
$$\ln\left(\frac{S(\tau) + S(0)}{2S(0)}\right) = -R_z \tau$$

A plot of LHS vs τ is a straight line with a slope R_z

Inversion Recovery

The rate constants R_z can be quickly estimated if we focus on getting a null signal by adjusting the t delay in the inversion recovery sequence.



$$S(\tau_{null}) = 0 = -S^0\left(1 - 2\exp(-R_z\tau_{null})\right)$$

$$\exp(-R_z\tau_{null}) = \frac{1}{2}$$

$$-R_z\tau_{null} = \ln\left(\frac{1}{2}\right)$$

$$R_z = \ln 2/\tau_{null}$$

$$\frac{1}{T_1} = R_z = \ln 2/\tau_{null}$$

$$T_1 = \tau_{null}/\ln 2$$

$$T_1 \cong 1.443 \times \tau_{null}$$

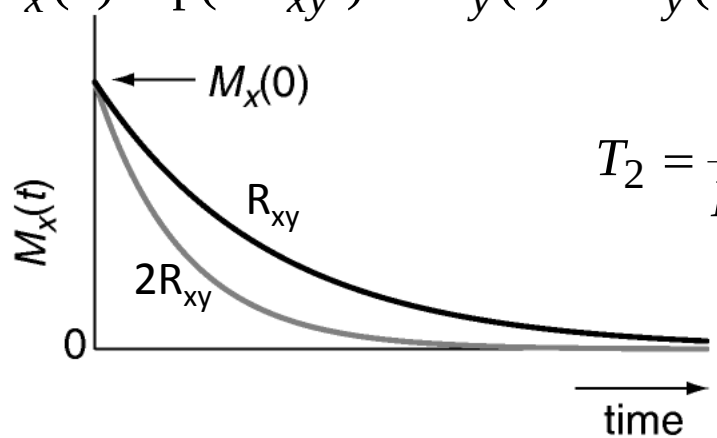
Transverse Relaxation

The magnetization in the transverse plane (more generally the coherences) decay to zero over time. The decay obeys a rate equation given as:

$$\frac{dM_x(t)}{dt} = -R_{xy}M_x(t) \quad \frac{dM_y(t)}{dt} = -R_{xy}M_y(t)$$

The solution of which is straightforward:

$$M_x(t) = M_x(0)\exp(-R_{xy}t) \quad M_y(t) = M_y(0)\exp(-R_{xy}t)$$



$$T_2 = \frac{1}{R_{xy}}$$

Transverse Relaxation

The decay of transverse magnetization or the coherences is an effect of the same random motion that caused a fluctuating local field B_{loc}. For the relaxation of the longitudinal magnetization the components in the transverse plane of this B_{loc} was important. We will see that the importance of the z-component on the relaxation of longitudinal magnetization when we consider more than a single spin system.

For the transverse magnetization and coherences, both the z-component and the transverse components of B_{loc} are required.

$$\overline{B_{loc,x}^2} = \overline{B_{loc,y}^2} = \overline{B_{loc,z}^2} = \overline{B_{loc}^2}$$

Transverse Relaxation

The transverse components of B_{loc} move the transverse magnetization towards the z- direction (like in T_1 process). In order for this process to be effective the fluctuations of B_{loc} should have spectral densities at the Larmor frequency. This is called ***non-secular*** *part of the relaxation. Here, the spin energy is transferred to the lattice.*

The z-component of B_{loc} acts like an offset term and de-phase the transverse magnetization/coherences. For this de-phasing to be random, it is not necessary for the fluctuations to be fast even very slow variations is sufficient. This is called the ***secular*** *part of the relaxation. Here the spin energy is exchanged among the spins themselves.*

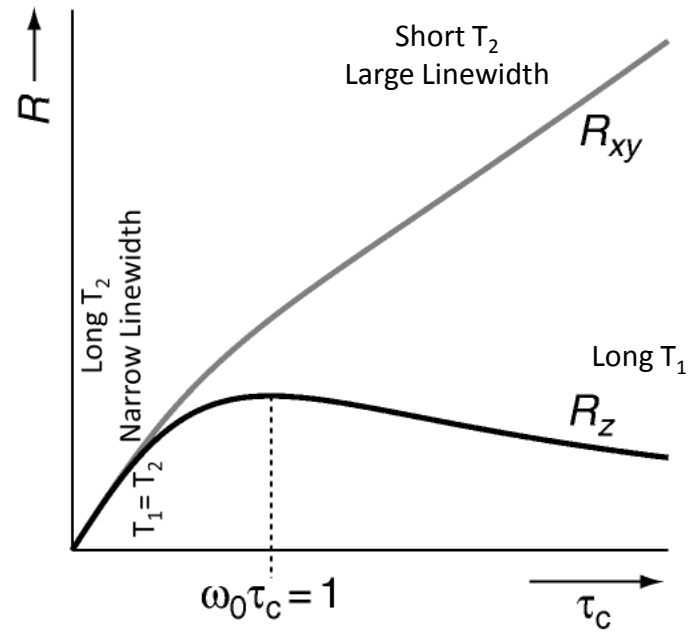
Thus both effect lead to decay of the transverse magnetization and any other coherences.

Transverse Relaxation

The relaxation rate constant can be shown to be:

$$R_{xy} = \underbrace{\frac{1}{2}\gamma^2 B_{loc}^2 \, j(0)}_{\text{Secular Part}} + \underbrace{\frac{1}{2}\gamma^2 B_{loc}^2 \, j(\omega_0)}_{\text{Non-Secular Part}}$$

$$R_{xy} = \underbrace{\frac{1}{2}\gamma^2 B_{loc}^2 \, j(0)}_{\text{Secular Part}} + \underbrace{\frac{1}{2} R_z}_{\text{Non-Secular Part}}$$



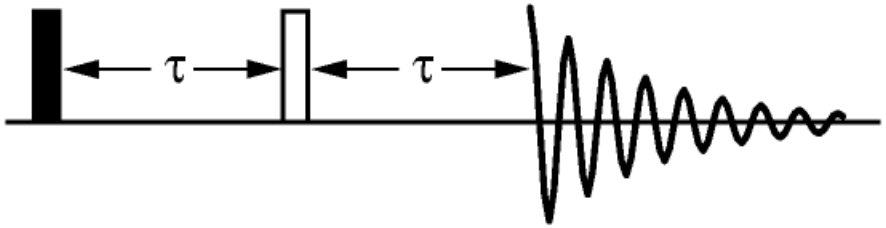
Plot of R_{xy} and R_z as a function of τ_c. For fast motion ($\omega_0\tau_c$<<1) both the rates are equal and in the slow motion limit ($\omega_0\tau_c$>>1) the R_z decreases whereas R_{xy} keeps increasing.

Transverse Relaxation

The linewidth in a 1D spectrum will yield the transverse relaxation rate constant. However, if the field is inhomogeneous, the linewidth would be broader than that caused by relaxation decay alone. However, the inhomogeneous broadening can be refocused by a spin-echo sequence and the T_2 time constant can be measured.

$$S(2\tau) = S(0)\exp(-R_{xy}2\tau)$$

$$\ln S(2\tau) = \ln S(0) - R_{xy}(2\tau)$$

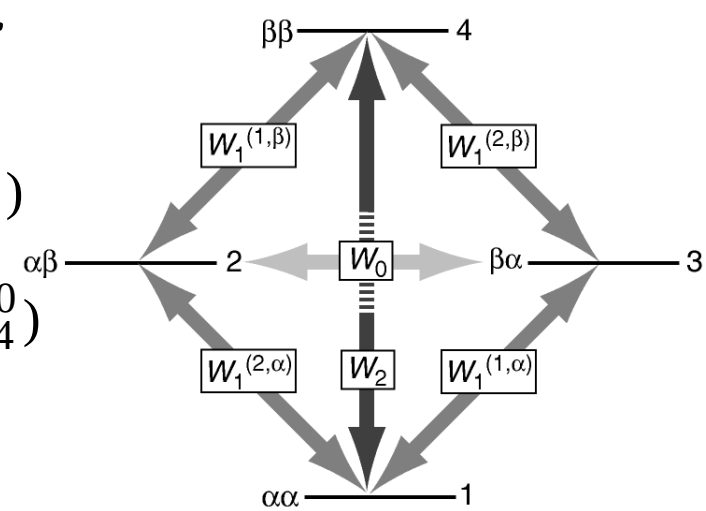


A plot of ***lnS(2τ)*** *with respect to* ***2τ*** *delay will be a straight line with a slope of* $-R_{xy}$.

Longitudinal Relaxation – Two Spins

So far we focused on isolated spins. Let us now consider a two-spin system. The possible transitions by relaxation is shown in the figure along with the transition probabilities that cause the populations in these levels to change. We can workout differential equations for the rate of change of populations.

$$\frac{dn_1}{dt} = -W_1^{(2,\alpha)}(n_1 - n_1^0) - W_1^{(1,\alpha)}(n_1 - n_1^0) - W_2(n_1 - n_1^0)$$
$$+ W_2^{(2,\alpha)}(n_2 - n_2^0) + W_1^{(1,\alpha)}(n_3 - n_3^0) + W_2(n_4 - n_4^0)$$



Longitudinal Relaxation – Two Spins

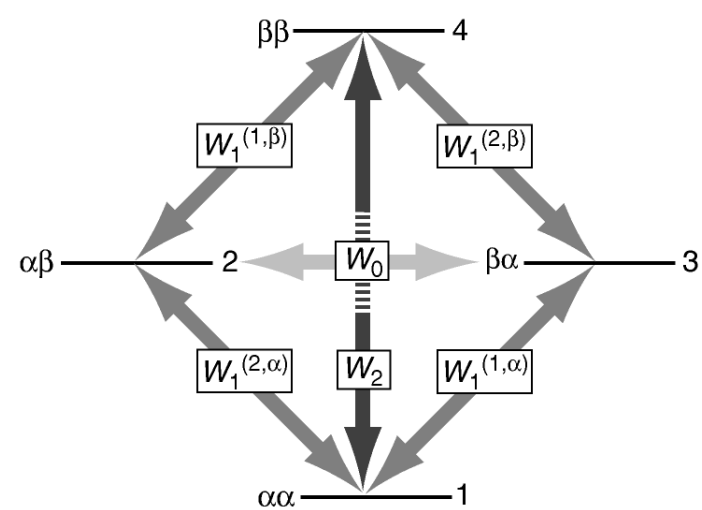
The expressions in terms of populations can be converted to z-magnetization:

$$I_{1z} = (n_1 - n_3) + (n_2 - n_4)$$
$$I_{2z} = (n_1 - n_2) + (n_3 - n_4)$$

Then the rate equation for magnetization is written after tedious calculations as:

$$\frac{dI_{1z}}{dt} = -R_z^{(1)}(I_{1z} - I_{1z}^0) - \sigma_{12}(I_{2z} - I_{2z}^0)$$
$$\frac{dI_{2z}}{dt} = -\sigma_{12}(I_{1z} - I_{1z}^0) - R_z^{(2)}(I_{2z} - I_{2z}^0)$$

$$R_z^{(1)} = W_1^{(1,\alpha)} + W_1^{(1,\beta)} + W_2 + W_0$$
$$R_z^{(2)} = W_1^{(2,\alpha)} + W_1^{(2,\beta)} + W_2 + W_0$$
$$\sigma_{12} = W_2 - W_0$$



Longitudinal Relaxation – Two Spins

The rate equations imply that the relaxation of the z-magnetization of the two spins are coupled by the ***cross-relaxation*** *rate constant* σ_{12}*. This Cross-relaxation effect is know as* ***Nuclear Overhauser Effect (NOE)****.The term* R_z *is known as* ***auto-relaxtion*** *term.*

$$\frac{dI_{1z}}{dt} = -R_z^{(1)}(I_{1z} - I_{1z}^0) - \sigma_{12}(I_{2z} - I_{2z}^0)$$

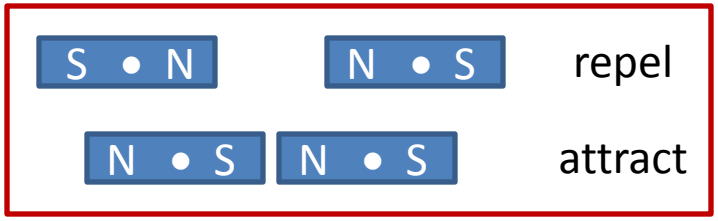
$$\frac{dI_{2z}}{dt} = -\sigma_{12}(I_{1z} - I_{1z}^0) - R_z^{(2)}(I_{2z} - I_{2z}^0)$$

$$\boxed{\begin{aligned} R_z^{(1)} &= W_1^{(1,\alpha)} + W_1^{(1,\beta)} + W_2 + W_0 \\ R_z^{(2)} &= W_1^{(2,\alpha)} + W_1^{(2,\beta)} + W_2 + W_0 \\ \sigma_{12} &= W_2 - W_0 \end{aligned}}$$

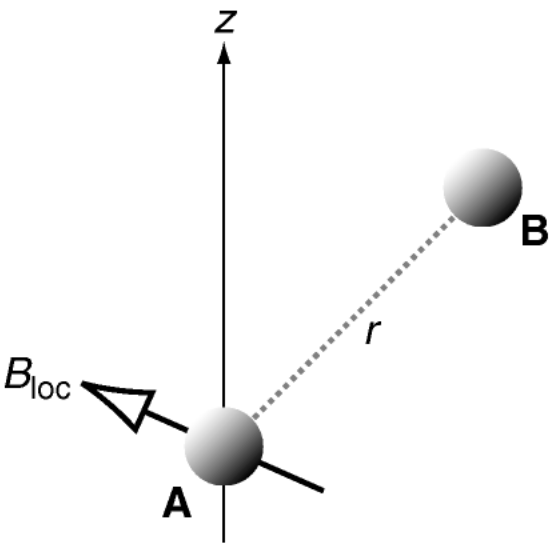
Dipole-Dipole Relaxation – Two Spins

The most ubiquitous relaxation mechanism in liquid state is the fluctuating local field induced by the ***dipole-dipole interaction*** *between spins.*

Each spin is a tiny magnet and is a dipole - two magnets brought together in one horizontal orientation repel and in the opposite horizontal orientation attract – indicating two distinct poles.



The interaction between two dipoles depend on the mutual distance between them and the orientation of the dipole-dipole vector with respect to the z-axis.



Dipole-Dipole Relaxation – Two Spins

With dipole-dipole interaction as the source of relaxation, all the transition probabilities are non-zero.

$$\frac{dI_{1z}}{dt} = -R_z^{(1)}(I_{1z} - I_{1z}^0) - \sigma_{12}(I_{2z} - I_{2z}^0)$$

$$\frac{dI_{2z}}{dt} = -\sigma_{12}(I_{1z} - I_{1z}^0) - R_z^{(2)}(I_{2z} - I_{2z}^0)$$

$$R_z^{(1)} = W_1^{(1,\alpha)} + W_1^{(1,\beta)} + W_2 + W_0$$

$$R_z^{(2)} = W_1^{(2,\alpha)} + W_1^{(2,\beta)} + W_2 + W_0$$

$$\sigma_{12} = W_2 - W_0$$

$$W_1^{(1)} = \frac{3}{40} b^2 j(\omega_{0,1}) \quad W_1^{(1)} = \frac{3}{40} b^2 j(\omega_{0,2})$$

$$W_2 = \frac{3}{10} b^2 j(\omega_{0,1} + \omega_{0,2}) \quad W_0 = \frac{1}{20} b^2 j(\omega_{0,1} - \omega_{0,2})$$

$$b = \frac{\mu_0 \gamma_1 \gamma_2 \hbar}{r^3} \qquad \mu_0 = 4\pi \times 10^{-7}\, Hm^{-1}$$

Permeability of vacuum

Dipole-Dipole Relaxation – Two Spins

We can now compute various relaxation rate constants:

$$\boxed{\begin{aligned} R_z^{(1)} &= W_1^{(1,\alpha)} + W_1^{(1,\beta)} + W_2 + W_0 \\ R_z^{(2)} &= W_1^{(2,\alpha)} + W_1^{(2,\beta)} + W_2 + W_0 \\ \sigma_{12} &= W_2 - W_0 \end{aligned}}$$

$$R_z^{(1)} = b^2\left[\frac{3}{20}\, j(\omega_{0,1}) + \frac{3}{10}\, j(\omega_{0,1}+\omega_{0,2}) + \frac{1}{20}\, j(\omega_{0,1}-\omega_{0,2})\right]$$

$$R_z^{(2)} = b^2\left[\frac{3}{20}\, j(\omega_{0,2}) + \frac{3}{10}\, j(\omega_{0,1}+\omega_{0,2}) + \frac{1}{20}\, j(\omega_{0,1}-\omega_{0,2})\right]$$

$$\sigma_{12} = b^2\left[\frac{3}{10}\, j(\omega_{0,1}+\omega_{0,2}) - \frac{1}{20}\, j(\omega_{0,1}-\omega_{0,2})\right]$$

Dipole-Dipole Relaxation – Two Spins

Let us focus on the cross-relaxation term:

$$\sigma_{12} = W_2 - W_0 \qquad \sigma_{12} = b^2\left[\frac{3}{10}j(\omega_{0,1}+\omega_{0,2}) - \frac{1}{20}j(\omega_{0,1}-\omega_{0,2})\right]$$

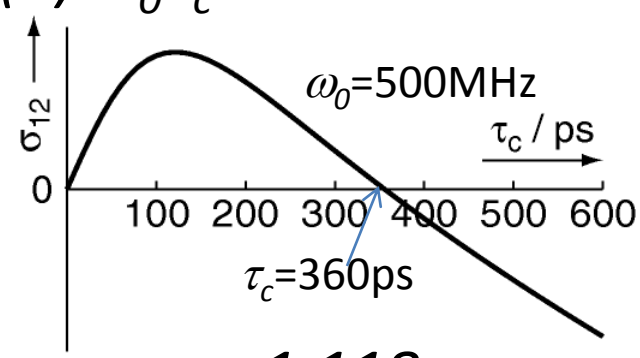
We can set $\omega_{0,1} = \omega_{0,2} = \omega_0$ *for homonuclear spins and then,*

$$\sigma_{12} = b^2\left[\frac{3}{10}j(2\omega_0) - \frac{1}{20}j(0)\right] \qquad j(\omega_0) = \frac{2\tau_c}{1+\omega_0^2\tau_c^2}$$

We can evaluate σ_{12} *for (a)* $\omega_0\tau_c \ll 1$ *and (b)* $\omega_0\tau_c \gg 1$

$$(a)\ \sigma_{12} = \frac{1}{2}b^2\tau_c \quad (b)\ \sigma_{12} = -\frac{1}{10}b^2\tau_c$$

$$(a)\ j(\omega_0) = 2\tau_c \quad (b)\ j(\omega_0 \neq 0) \approx 0 \quad j(0) = 2\tau_c$$

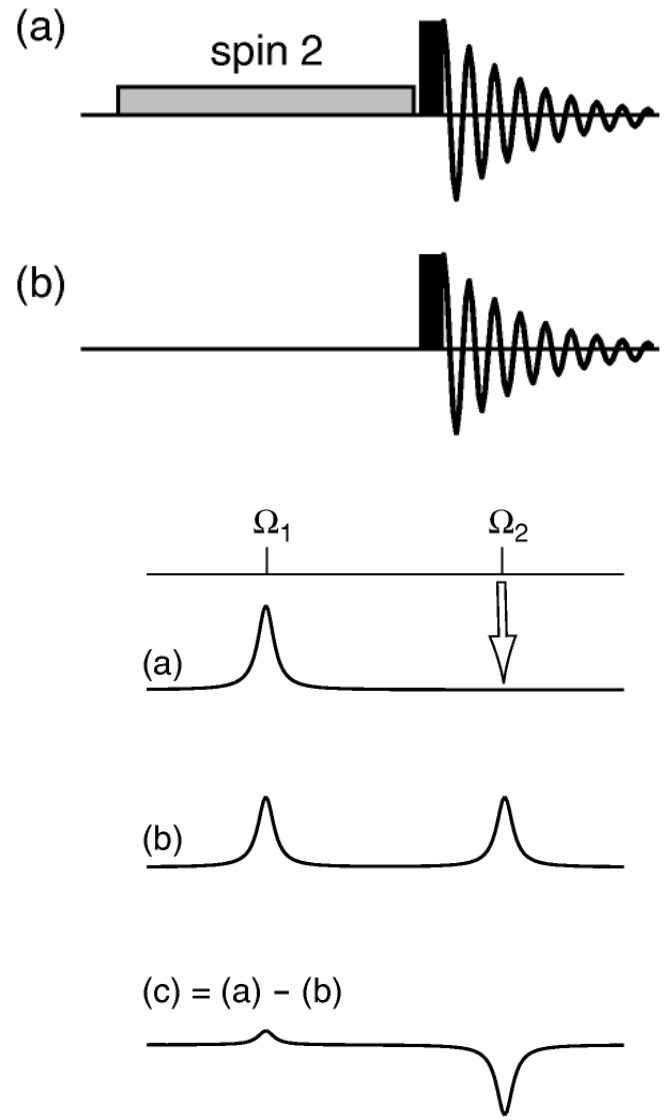


σ_{12} *is zero when* $W_2 = W_0$ *and happens when* $\omega_0\tau_c = 1.118$

NOE

Let us focus on an one-pulse experiment in which (a) prior to the pulse, one of the two spins is selectively irradiated continuously at its resonance so that the z-magnetization of this spin reaches the zero value – a situation termed ***saturation****. We then repeat the experiment (b) without the selective irradiation and subtract the results. This experiment is known as* ***steady-state NOE experiment****.*

If there is a cross relaxation between the spins then intensities of the un irradiated spin will be different and a NOE is observed. The NOE in spectrum (c) can be positive or negative depending on the sign of cross correlation rate (motional regime).



Steady-State NOE (Homo-Nuclear)

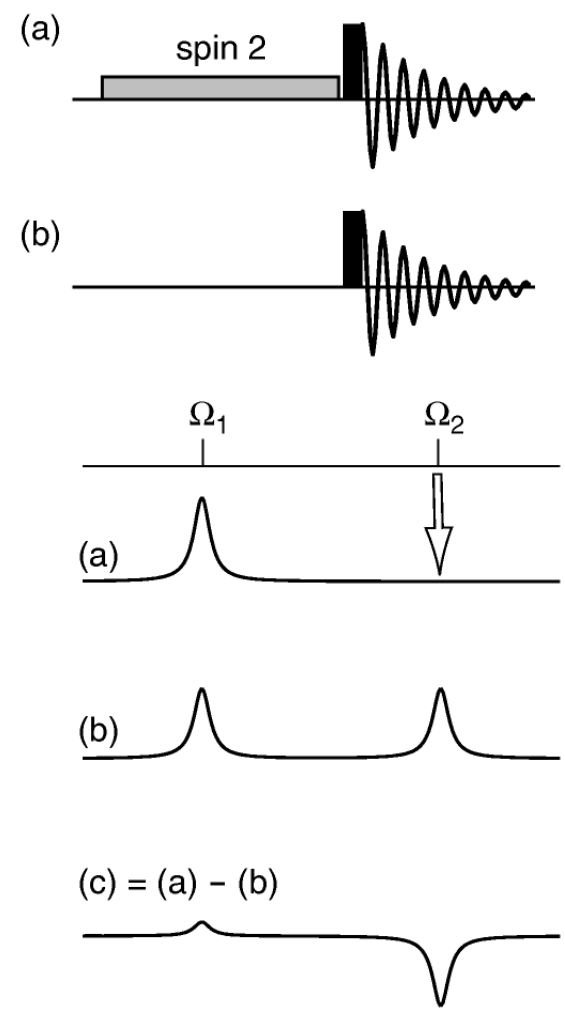
*In the **steady-state NOE experiment**, the spins reach a steady state of z-magnetization,($I_{1z,ss}$, $I_{2z,ss}$=0)before the pulse. At steady state,*

$$\frac{dI_{1z}}{dt} = -R_z^{(1)}(I_{1z} - I_{1z}^0) - \sigma_{12}(I_{2z} - I_{2z}^0) = 0$$

$$R_z^{(1)}(I_{1z,ss} - I_{1z}^0) = \sigma_{12} I_{2z}^0$$

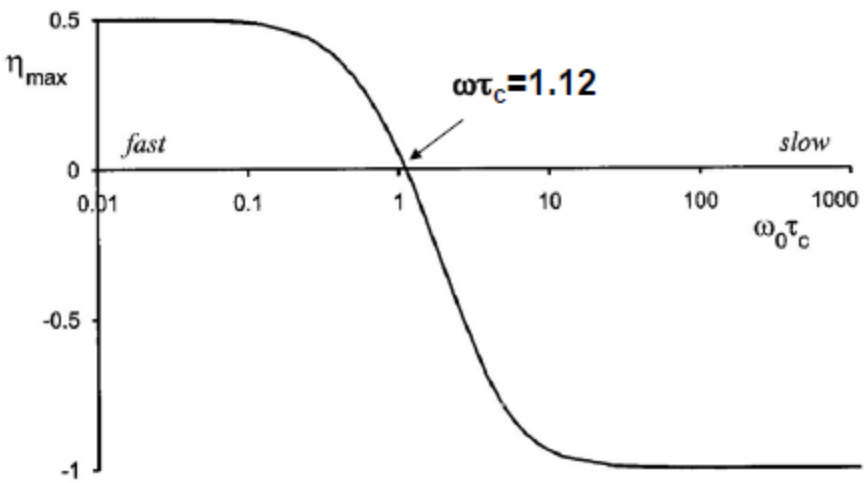
$$\boxed{I_{1z,ss}} = \frac{\sigma_{12}}{R_z^{(1)}} I_{2z}^0 + I_{1z}^0 = \boxed{I_{1z}^0\left(1 + \frac{\sigma_{12}}{R_z^{(1)}}\right) \text{ with } I_{1z}^0 = I_{2z}^0}$$

$$\text{NOE enhancement } \boxed{\eta_{ss} = \frac{I_{1z}^0\left(1 + \frac{\sigma_{12}}{R_z^{(1)}}\right) - I_{1z}^0}{I_{1z}^0} = \frac{\sigma_{12}}{R_z^{(1)}}}$$

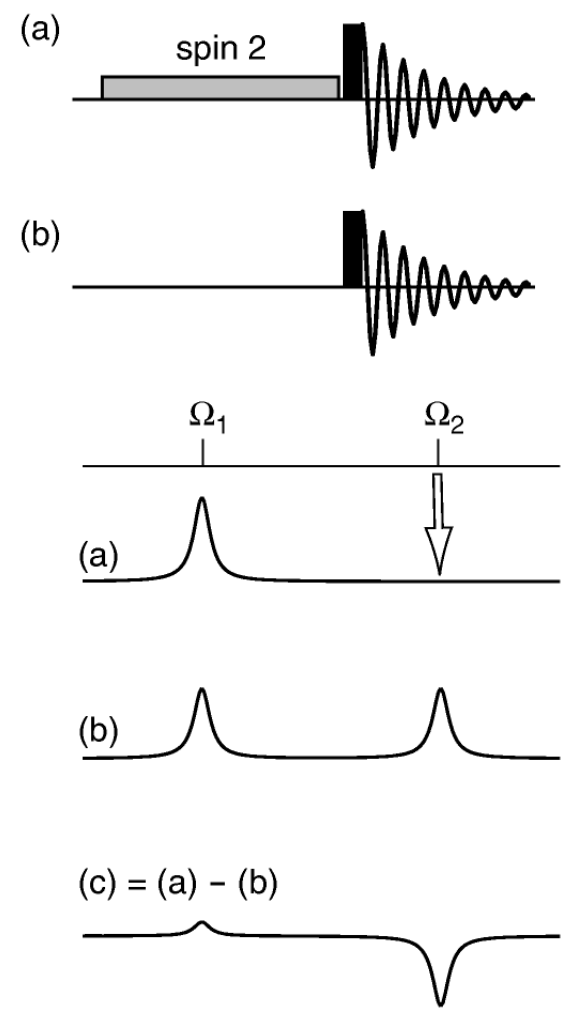


Steady-State NOE (Homo-Nuclear)

*In the **steady-state NOE experiment**, a plot of the NOE enhancement vs $\omega_0\tau_c$ is illustrative.*



$$\eta_{ss} = \frac{\sigma_{12}}{R_z^{(1)}}$$



Steady-State NOE (Hetero-Nuclear)

*In the **steady-state NOE experiment**, if the spins are of different nuclei (I and S) and say the I spins are irradiated, the steady state of z-magnetizations are $I_{z,ss}$=0, and $S_{z,ss}$. At steady state,*

$$\frac{dS_z}{dt} = -R_z^{(S)}(S_z - S_z^0) - \sigma_{IS}(I_z - I_z^0) = 0$$

$$R_z^{(1)}(S_{z,ss} - S_z^0) = \sigma_{IS} I_z^0$$

$$S_{z,ss} = \frac{\sigma_{IS}}{R_z^{(S)}} I_z^0 + S_z^0 = \boxed{S_z^0\left(1 + \frac{\gamma_I}{\gamma_S}\frac{\sigma_{IS}}{R_z^{(S)}}\right) \text{ with } \frac{S_z^0}{I_z^0} = \frac{\gamma_S}{\gamma_I}}$$

$$\text{NOE enhancement } \boxed{\eta_{ss} = \frac{S_z^0\left(1 + \frac{\gamma_I}{\gamma_S}\frac{\sigma_{IS}}{R_z^{(S)}}\right) - S_z^0}{S_z^0} = \frac{\gamma_I}{\gamma_S}\frac{\sigma_{IS}}{R_z^{(S)}}}$$

Transient NOE (Homo-Nuclear)

One could also monitor the relaxation evolution if one or both spins are inverted and allowed to come back to equilibrium. Such an experiment is called Transient NOE experiment. Assuming homonuclear case and setting $R_z^{(1)}=R_z^{(2)}$ *and* $I_{1z0}=I_{2z0}$*, we can write the rate equations as,*

$$\frac{dI_{1z}(t)}{dt} = -R_z(I_{1z}(t) - I_z^0) - \sigma_{12}(I_{2z}(t) - I_z^0)$$

$$\frac{dI_{2z}(t)}{dt} = -\sigma_{12}(I_{1z}(t) - I_z^0) - R_z(I_{2z}(t) - I_z^0)$$

$$\chi_1(t) = I_{1z}(t) - I_z^0 \quad \chi_2(t) = I_{2z}(t) - I_z^0 \text{ and using } \frac{dI_z^0}{dt} = 0$$

$$\frac{d\chi_1(t)}{dt} = -R_z\chi_1(t) - \sigma_{12}\chi_2(t)$$

$$\frac{d\chi_2(t)}{dt} = -\sigma_{12}\chi_1(t) - R_z\chi_2(t)$$

Transient NOE (Homo-Nuclear)

The solutions can be easily obtained from the sum and difference equations,

$$\frac{d(\chi_1+\chi_2)}{dt} = -(R_z+\sigma_{12})(\chi_1+\chi_2),\ (\chi_1+\chi_2) = (\chi_1(0)+\chi_2(0))e^{-(R_z+\sigma_{12})t}$$

$$\frac{d(\chi_1-\chi_2)}{dt} = -(R_z-\sigma_{12})(\chi_1-\chi_2),\ (\chi_1-\chi_2) = (\chi_1(0)-\chi_2(0))e^{-(R_z-\sigma_{12})t}$$

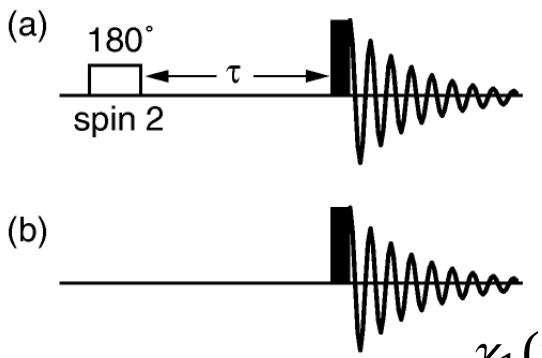
$$\chi_1(t) = \chi_1(0)\left(e^{-(R_z+\sigma_{12})t} + e^{-(R_z-\sigma_{12})t}\right) + \chi_2(0)\left(e^{-(R_z+\sigma_{12})t} - e^{-(R_z-\sigma_{12})t}\right)$$

$$\chi_2(t) = \chi_1(0)\left(e^{-(R_z+\sigma_{12})t} - e^{-(R_z-\sigma_{12})t}\right) + \chi_2(0)\left(e^{-(R_z+\sigma_{12})t} + e^{-(R_z-\sigma_{12})t}\right)$$

The deviations $\chi(t)$ from equilibrium value evolves in a multi-exponential way.

Transient NOE (Homo-Nuclear)

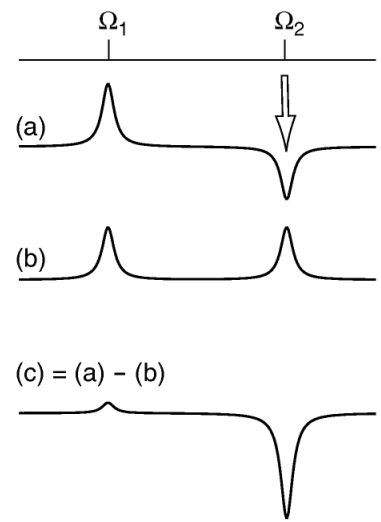
Let us say that we (a) selectively invert spin 2 alone and wait for a time t before applying the 90° pulse, and in (b) skip the inversion pulse and subtract the signals.



$$\chi_1(0) = 0$$

$$\chi_2(0) = -I_{2z} - I^0_{2z} = -2I^0_{2z}$$

$$\chi_2(0) = -2I^0_z$$



$$\chi_1(t) = -2I^0_z\left(e^{-(R_z+\sigma_{12})t} - e^{-(R_z-\sigma_{12})t}\right)$$

$$\chi_2(t) = -2I^0_z\left(e^{-(R_z+\sigma_{12})t} + e^{-(R_z-\sigma_{12})t}\right)$$

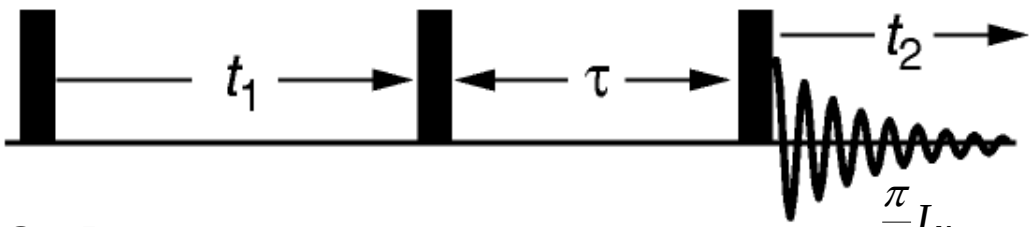
$$\text{NOE } \eta_I = \frac{I_{1z}(t) - I^0_z}{-2I^0_z} = \frac{\chi_1(t)}{-2I^0_z} = \left(e^{-(R_z+\sigma_{12})t} - e^{-(R_z-\sigma_{12})t}\right)$$

$$I_{2z}(t) = I^0_z\left[1 - 2\left(e^{-(R_z+\sigma_{12})t} + e^{-(R_z-\sigma_{12})t}\right)\right]$$

The return to equilibrium of the inverted magnetization of spin 2 cannot be characterized by a single rate constant T_1.

Two-Dimensional NOE Experiment NOESY

We can get the NOE signal between all the spins in a molecule by performing a 2D Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiment. We retain only the z-component after the second pulse. Let us only focus on offset evolution.



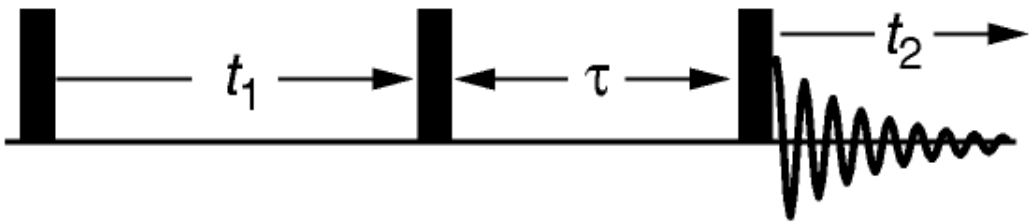
$$I_{1z} \xrightarrow{\frac{\pi}{2}I_x} -I_{1y} \xrightarrow{\Omega_1 t_1 I_{1z}} -I_{1y}\cos(\Omega_1 t_1) + I_{1x}\sin(\Omega_1 t_1) \xrightarrow{\frac{\pi}{2}I_x} -I_{1z}\cos(\Omega_1 t_1)\ldots$$

$$I_{2z} \xrightarrow{\frac{\pi}{2}I_x} -I_{2y} \xrightarrow{\Omega_2 t_1 I_{1z}} -I_{2y}\cos(\Omega_2 t_1) + I_{2x}\sin(\Omega_2 t_1) \xrightarrow{\frac{\pi}{2}I_x} -I_{2z}\cos(\Omega_2 t_1)\ldots$$

The z- magnetization after the second pulse relaxes back and cross-relaxation converts part I_{1z} to I_{2z} and vice versa The third 90° pulse rotates the z magnetization to detectable signals.

Two-Dimensional NOE Experiment NOESY

Since during t_1 evolution, the spins are frequency labeled, when the cross relaxation inter-convert magnetizations between the spins that appear as a cross-peak.



$$\chi_1(0) = -\cos(\Omega_1 t_1) I_{1z} - I_{1z}^0$$

$$\chi_2(0) = -\cos(\Omega_2 t_1) I_{2z} - I_{2z}^0$$

Let's focus on the part that has cosine frequency label

$$I_{1z}(\tau) = -\cos(\Omega_1 t_1) I_{1z}\left(e^{-(R_z+\sigma_{12})\tau} + e^{-(R_z-\sigma_{12})\tau}\right)$$ Diagonal Peak

$$-\cos(\Omega_2 t_1) I_{2z}\left(e^{-(R_z+\sigma_{12})\tau} - e^{-(R_z-\sigma_{12})\tau}\right)$$ Cross Peak

$$I_{2z}(\tau) = -\cos(\Omega_1 t_1) I_{1z}\left(e^{-(R_z+\sigma_{12})\tau} - e^{-(R_z-\sigma_{12})\tau}\right)$$ Cross Peak

$$-\cos(\Omega_2 t_1) I_{2z}\left(e^{-(R_z+\sigma_{12})\tau} + e^{-(R_z-\sigma_{12})\tau}\right)$$ Diagonal Peak

Two-Dimensional NOE Experiment NOESY

Schematic 2D NOESY spectrum is shown. The axial peaks arise from the terms that don't have cosine modulation with respect to t_1.

$$I_{1z}(\tau) = -\cos(\Omega_1 t_1) I_{1z}\left(e^{-(R_z+\sigma_{12})\tau} + e^{-(R_z-\sigma_{12})\tau}\right) \quad \text{Diagonal Peak}$$

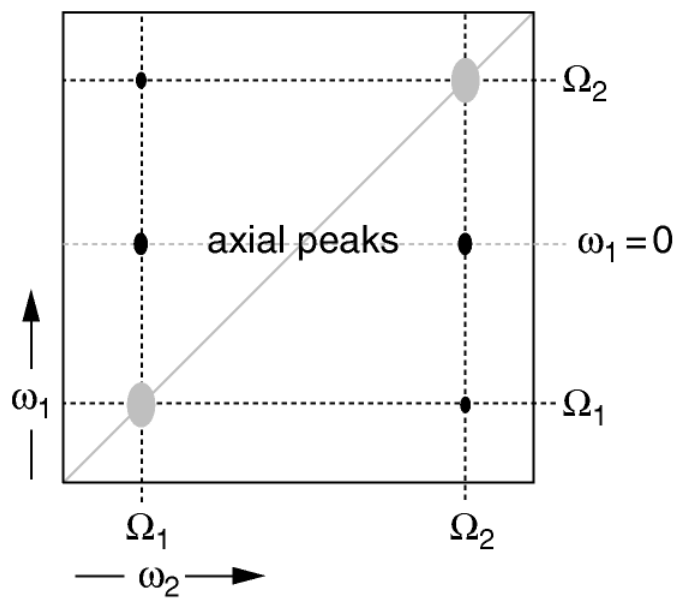
$$-\cos(\Omega_2 t_1) I_{2z}\left(e^{-(R_z+\sigma_{12})\tau} - e^{-(R_z-\sigma_{12})\tau}\right) \quad \text{Cross Peak}$$

$$I_{2z}(\tau) = -\cos(\Omega_1 t_1) I_{1z}\left(e^{-(R_z+\sigma_{12})\tau} - e^{-(R_z-\sigma_{12})\tau}\right) \quad \text{Cross Peak}$$

$$-\cos(\Omega_2 t_1) I_{2z}\left(e^{-(R_z+\sigma_{12})\tau} + e^{-(R_z-\sigma_{12})\tau}\right) \quad \text{Diagonal Peak}$$

$$\chi_1(0) = -\cos(\Omega_1 t_1) I_{1z} - I_{1z}^0$$

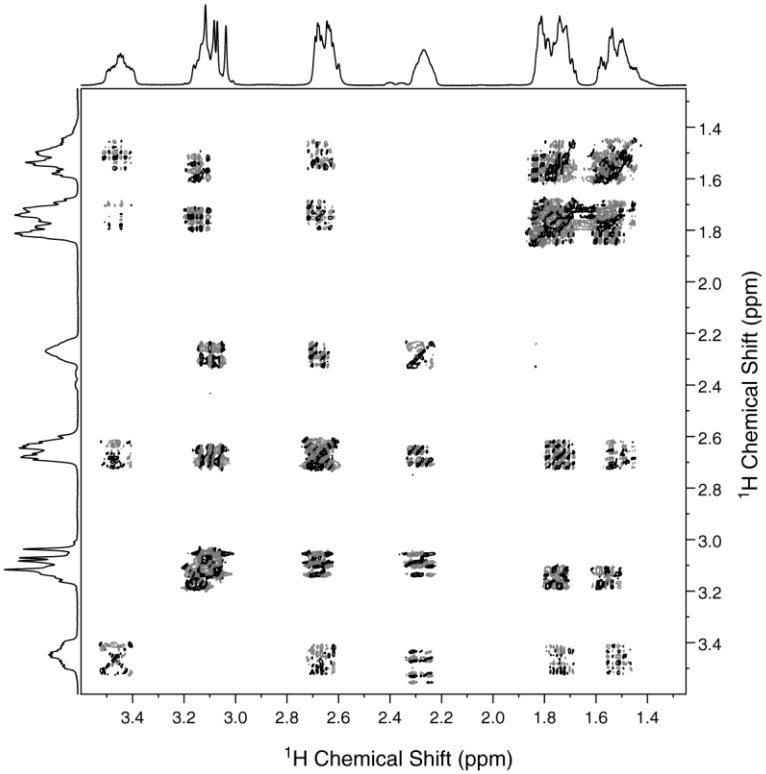
$$\chi_2(0) = -\cos(\Omega_2 t_1) I_{2z} - I_{2z}^0$$



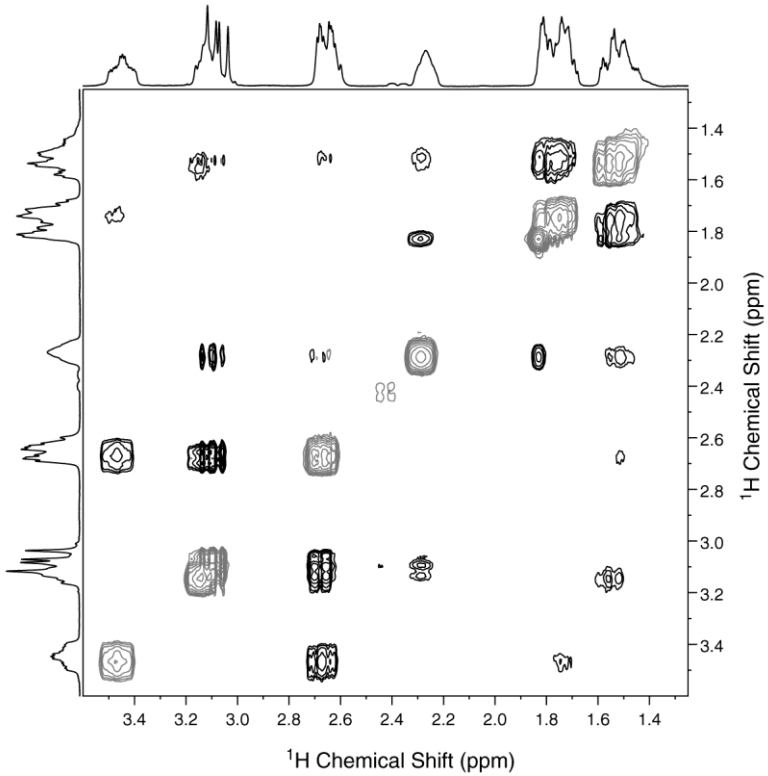
Two-Dimensional NOE Experiment NOESY

The NOESY cross-peaks indicate a non-vanishing cross-relaxation rate that depends on the distance between the spins ($\sigma \propto r^{-6}$). Weaker cross peak imply longer distance.

DQFC



NOESY



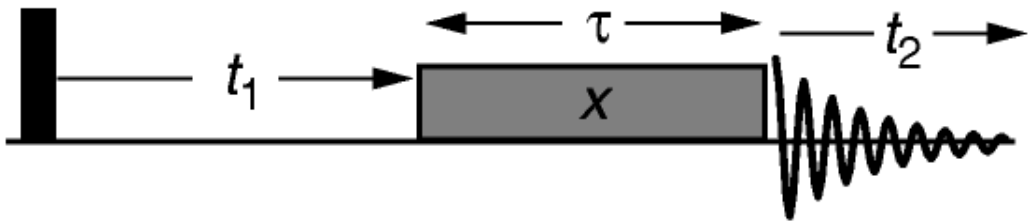
Two-Dimensional NOE Experiment ROESY

When the product $\omega_0 \tau_c$ ~ 1.18 the cross relaxation term vanishes and thus the NOESY experiment does not yield any cross peak in this motional regime. From Stokes formula, the correlation time of a molecule can be roughly estimated from its molecular weight A. molecular weight of 1000 corresponds to a correlation time of 0.5 ns with . Small molecules with molecular weights in the range of 700-900 Daltons fall in the zero – NOESY - cross-peak regime when probed using a 500 MHz spectrometer.

If the transverse magnetizations are spin-locked then they can also cross relax and this cross relaxation term will give rise to cross peak. Such an experiment is called ***Rotating frame Overhauser Spectroscopy*** *–* ***ROESY****. The ROESY cross relaxation term does not vanish for any correlation time.*

Two-Dimensional NOE Experiment ROESY

*The **Rotating frame Overhauser Spectroscopy** – **ROESY** is a simple sequence that uses spin-locking field during the mixing time.*



We can write the rate equations of the x- component of the magnetization during the spin-lock field as:

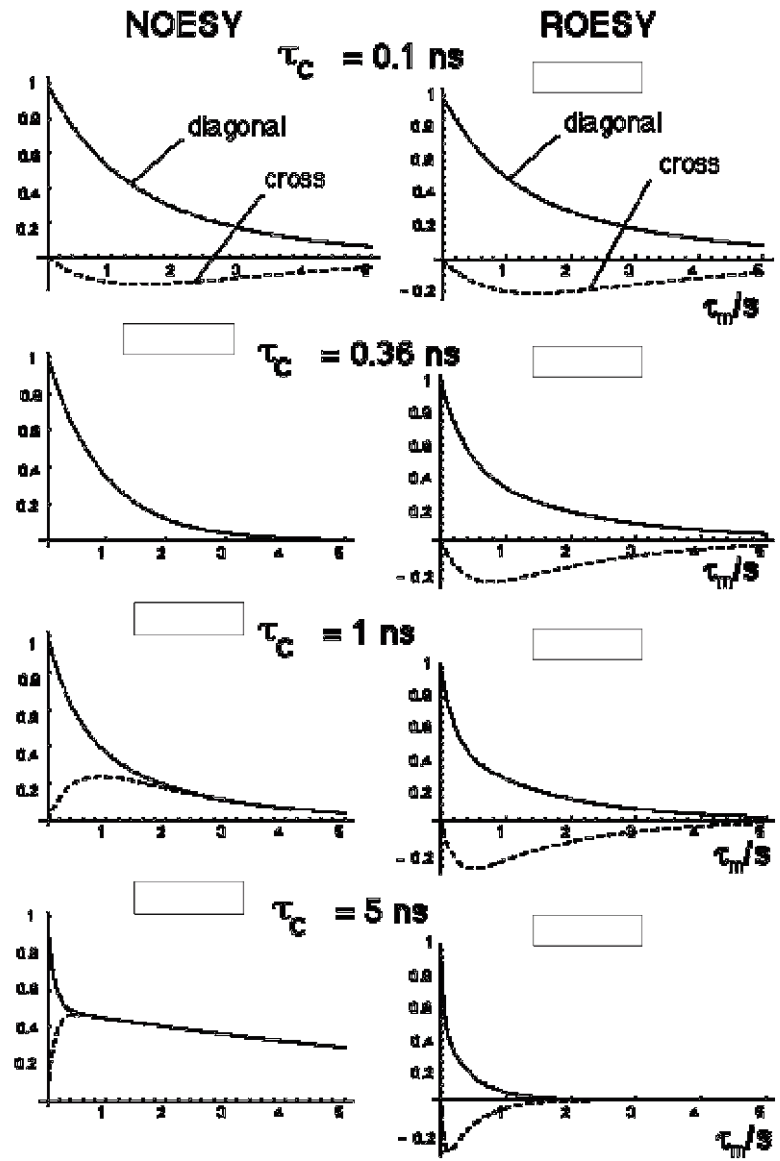
$$\frac{dI_{1x}(t)}{dt} = -R_{xy}I_{1x}(t) - \sigma_{xy}I_{2x}(t) \qquad \frac{dI_{2x}(t)}{dt} = -\sigma_{xy}I_{1x}(t) - R_{xy}I_{2x}(t)$$

$$\sigma_{xy} = b^2\left[\frac{1}{10}j(0) + \frac{3}{20}J(\omega_0)\right]$$

σ_{xy} is a sum of two positive terms and does not vanish for any correlation time.

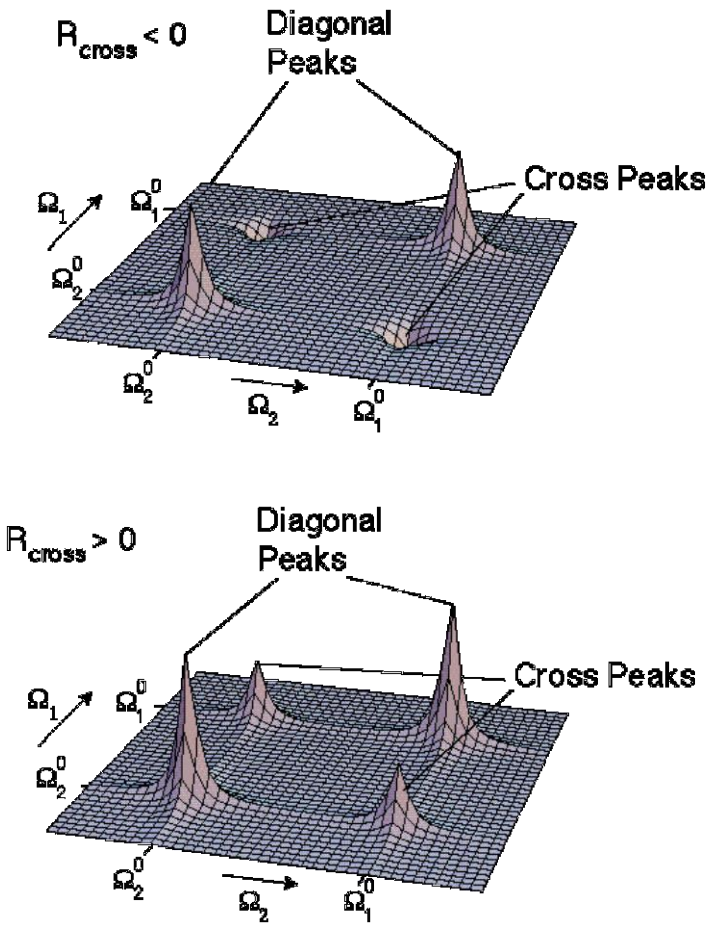
Two-Dimensional NOE Experiment NOESY vs ROESY

Comparison of diagonal- and cross-peak amplitudes in NOESY and ROESY as a function of the mixing time for a set of rotational correlation times. The distance between the two protons set to be 0.2nm and the field strength is 11.74T (500 MHz). For tc =0.36 ns the NOESY cross-peak is zero and above that correlation time the NOESY cross-peak amplitude is inverted and appear with the same sign as the diagonal.

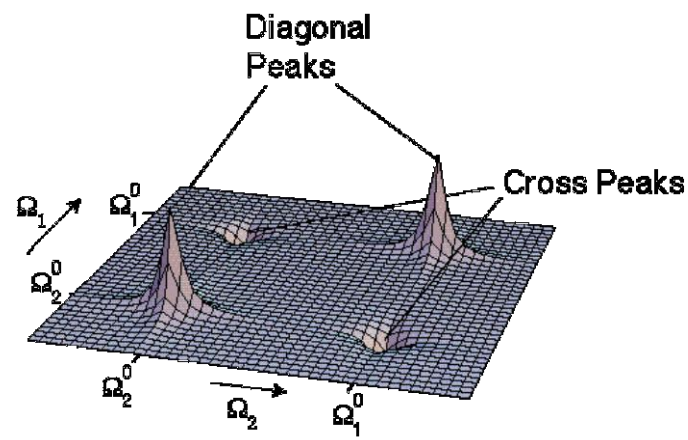


Two-Dimensional NOE Experiment
NOESY vs ROESY

NOESY



ROESY



Further Reading

We have only briefly touched the subject of relaxation in NMR. There are detailed chapters in the books we have recommended on this topic and several review articles. One can, perhaps, take a first dive in this branch by starting with the following article:

A Primer For Nuclear Magnetic Relaxation in Liquids

Concepts in Magnetic Resonance Part A ***17A****: 86-116, (2003).*