

Notes on relaxation and dynamics

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1. Relaxation and dynamics

1.1 General Remark

Application of RF-frequency pulses changes the density matrix and moves the spins away from their thermal equilibrium distribution. E.g. for protons in a 14T magnetic field the density matrix at thermal equilibrium ($T = 293\text{K}$) is given by

$$\rho_{\text{eq}} = \frac{\exp(-\mathbf{H}/kT)}{\text{trace}(\exp(-\mathbf{H}/kT))} \approx \frac{1}{2}(\mathbf{1} - \mathbf{H}/kT) = \frac{1}{2}(\mathbf{1} - (-\gamma\hbar\mathbf{I}_z B)/kT) \approx \frac{1}{2}(\mathbf{1} + 10^{-4}\mathbf{I}_z) \quad (1.1)$$

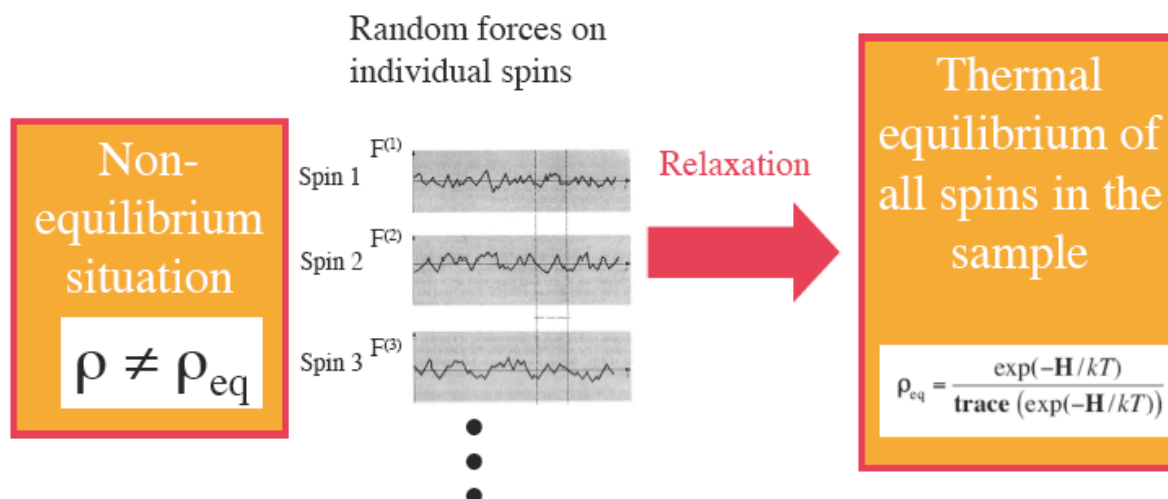
where $\mathbf{H} = -\gamma\hbar\mathbf{I}_z B$, $\exp(-\mathbf{H}/kT) \approx \mathbf{1} - \mathbf{H}/kT$ (since $\|\mathbf{H}\| \ll kT$), $\mathbf{1}$ is the unity matrix, $\text{trace } \mathbf{1} = 2$, $\text{trace } \mathbf{H} = 0$.

A 180° pulse around the x-axis (NOE-experiment) transfers this density matrix to

$$\frac{1}{2}(\mathbf{1} + 10^{-4}\mathbf{I}_z) \xrightarrow{180^\circ\mathbf{I}_x} \frac{1}{2}(\mathbf{1} - 10^{-4}\mathbf{I}_z) \quad (1.2)$$

Relaxation is the phenomenon that the spins have the tendency to come back to their thermal equilibrium distribution ρ_{eq} . The time constants for the return to thermal equilibrium are T_1 for the longitudinal magnetization (z-magnetization) and T_2 for the transverse magnetization (x,y-magnetization). For somewhat historical reasons T_1 is also called spin-lattice and T_2 is called spin-spin relaxation time.

For the relaxation phenomenon to happen, internal forces must act on the individual spins. In solution, the main source for these forces are random fields caused by the random (Brownian) motion of the molecule. Individual spins experience different random field fluctuations. The average of these fluctuations leads to the return to thermal equilibrium of the entire macroscopic ensemble of all spins.



The macroscopic relaxation rates can be calculated from a statistical description of the individual microscopic fluctuations. Thus the macroscopic relaxation rates contain information on the average dynamical behavior of individual spins and their surroundings

The approach of thermal equilibrium is mainly induced by the following internal forces (interactions):

1. **dipolar interactions** between the magnetic moments of the spins (typical energies in biomacromolecules $< 10^4$ - 10^5 Hz * h)
2. the **anisotropy of the chemical shift (CSA)**. Different orientations of the molecule in the solution lead to different shieldings, i.e. different Larmor frequencies. This results in internal forces on the spins (Energies/h $< 10^4$ - 10^5 Hz).
3. **electric quadrupolar interactions** of the nucleus with the inhomogeneous electric field produced by the electrons. This interaction is very large, but it is only observed for spins with $I > 1/2$. (quadrupolar energy/h ~ 200 kHz for ^2H and ~ 3 MHz for ^{14}N).

Remark 1: In NMR, the forces resulting from these random fields are rather weak compared to the force that the external magnetic field exerts on the spins. These weak forces lead to rather long relaxation times, e.g. for biological macromolecules in solution T_2 values are in the range of tens of milliseconds and T_1 values are on the order of seconds. Therefore we can observe the oscillations of the magnetic moments on the order of $600 \text{ MHz} * 10 \text{ ms} = 6\,000\,000$ times. This is the reason why despite of the small magnitude of the equilibrium polarisation (10^{-4}), NMR can still be observed with reasonable sensitivity.

Remark 2: The correlation between macroscopic relaxation phenomena and microscopic fluctuations is described in a very general way by the **fluctuation-dissipation theorem**: the macroscopic relaxation rates are proportional to (the Fourier transform of) the autocorrelation function of the fluctuating forces (see e.g. F. Reif). This is for example the case for the viscosity η of a medium (proportional to the autocorrelation of Brownian force fluctuations on individual particles) or the electrical resistance R (proportional to the autocorrelation of thermal voltage fluctuations in a conductor). The NMR theory of relaxation (Redfield theory) is the equivalent of the fluctuation-dissipation theorem applied to an ensemble of spins described by quantum statistics: the NMR relaxation rates are proportional to the autocorrelation function of the fluctuating microscopic fields. We shall see this in detail below.

1.2 The dipolar interaction

The dipolar interaction is the most important contribution to relaxation for biological macromolecules. A magnetic nucleus with gyromagnetic ratio γ and spin $\underline{I}$ is surrounded by a magnetic dipolar field $\underline{B}_d$ which is given by the following equation:

$$\underline{B}_d = -\frac{\gamma\hbar\mu_0}{4\pi r^3} \left\{ \underline{I} - 3 \frac{\underline{r}(\underline{I} \cdot \underline{r})}{r^2} \right\} \quad (1.3)$$

where $\underline{r}$ is the distance vector to the nucleus.

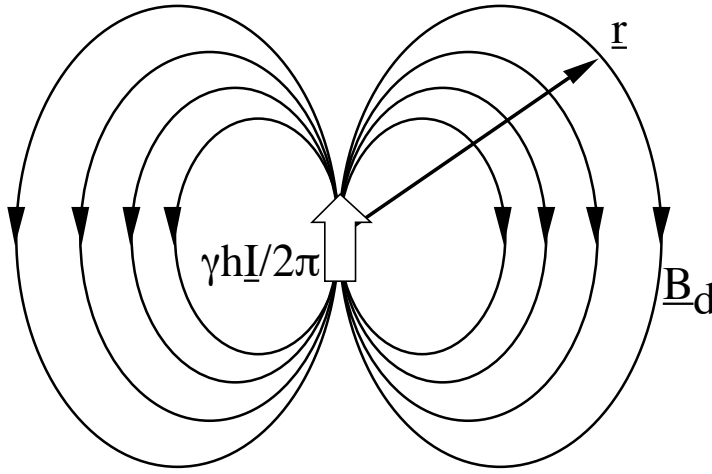


Fig.: dipolar field around nucleus with magnetic moment $\underline{\mu} = \gamma\hbar\underline{I}$

The dipolar energy H_d of a second nucleus with gyromagnetic ratio γ_2 and spin $\underline{I}_2$ in the field of the first nucleus with gyromagnetic ratio γ_1 and spin $\underline{I}_1$ is then given as

$$H_d = -\underline{\mu}_2 \cdot \underline{B}_{d1} = (\gamma_2\hbar\underline{I}_2) \cdot \frac{\gamma_1\hbar\mu_0}{4\pi r^3} \left\{ \underline{I}_1 - 3 \frac{\underline{r}(\underline{I}_1 \cdot \underline{r})}{r^2} \right\} = \frac{\gamma_1\gamma_2\hbar^2\mu_0}{4\pi r^3} \left\{ \underline{I}_1 \cdot \underline{I}_2 - 3 \frac{(\underline{I}_1 \cdot \underline{r})(\underline{I}_2 \cdot \underline{r})}{r^2} \right\} \quad (1.4)$$

We notice that depending on the position of the second nucleus (distance vector $\underline{r}$), the dipolar field and therefore the dipolar energy can have different values. If the two nuclei are part of the same molecule, then during the thermal movement of the molecule (Brownian rotation) the direction of $\underline{r}$ changes, whereas the length of $\underline{r}$ stays usually more or less constant. If the molecule has isotropic orientation in solution then the average of the dipolar interaction energy over all angles vanishes.

The Brownian rotation, however, leads to fluctuations in the dipolar field and dipolar interaction energy. These fluctuating fields can induce rotations of the spins. As the fluctuations are driven by the Brownian motion, this provides a mechanism to exchange energy between the spins and also between the spins and the mechanical motion of the molecule.

1.3 T_2 measured by the Carr-Purcell-Meiboom-Gill experiment

A classical experiment to visualize T_2 is the spin-echo experiment with the Carr-Purcell-Meiboom-Gill (CPMG) sequence:

$$90^\circ_x - [\tau - 180^\circ_y - \tau - \text{echo}]_n$$

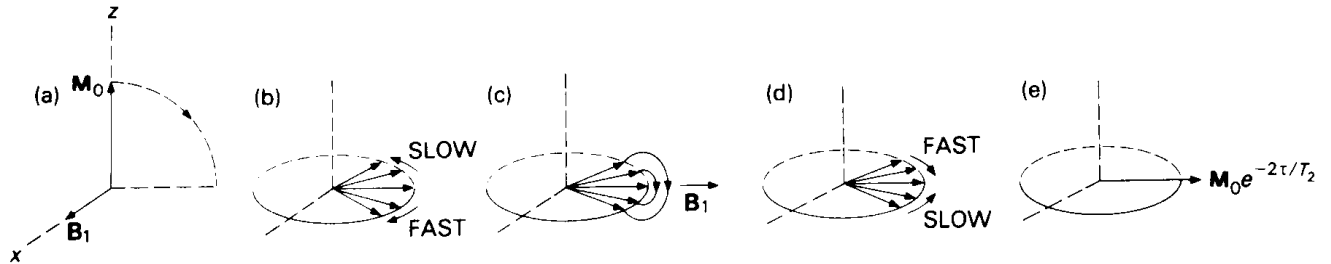


Fig. 3-14 The spin-echo effect
(a) a 90°_x pulse puts M_0 into the y' direction, (b) the spin isochromats^[11] fan out, (c) a 180°_y pulse interchanges slow and fast spins at time τ , (d) refocusing occurs, (e) the echo at time 2τ .

Fig.: Harris p 83.

An initial 90° pulse turns the equilibrium magnetization into the xy -plane. The magnetization vectors from different nuclei then begin to fan out (b) and the signal decays. A 180°_y pulse is applied after time τ (c) which has the effect of rotating all the magnetization vectors about the y -axis, or in other words reflecting them in the zy -plane, they continue to move in the same direction (d), and after a further time τ (e) they are again in phase in the y -direction, and the signal in the receiver coil is maximal. The process of refocusing by the 180° pulse can be repeated many times. The amplitudes of successive echoes decay exponentially and the true value of T_2 can be found from the envelope of the echoes:

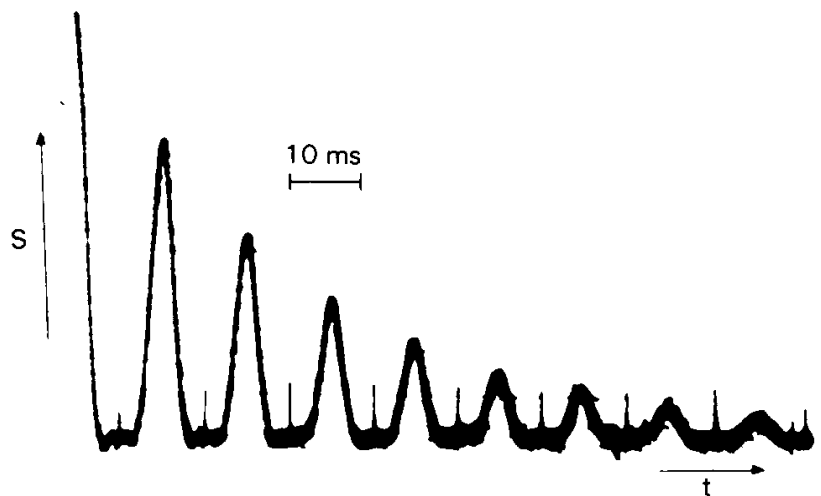


Fig. 3-15 The results of a CPMG experiment (see Eqn 3-33). Responses of the electronics to the 180° pulses are just visible between the echoes.

Fig.: Harris p. 83.

1.4 The linewidth increases with increasing molecular weight

A puzzle for many students of NMR is the fact that the line widths of the nuclear resonance lines are becoming larger when the molecular weight of the molecules in solution is increased. Remember that the linewidth is given by

$$\Delta\nu = \Delta\omega / 2\pi = 1/(\pi T_2) \quad (1.5)$$

An example for this increase can be seen for the three molecules magainin (3 kDa), BPTI (7 kDa), and staphyloccocal nuclease (18 kDa):

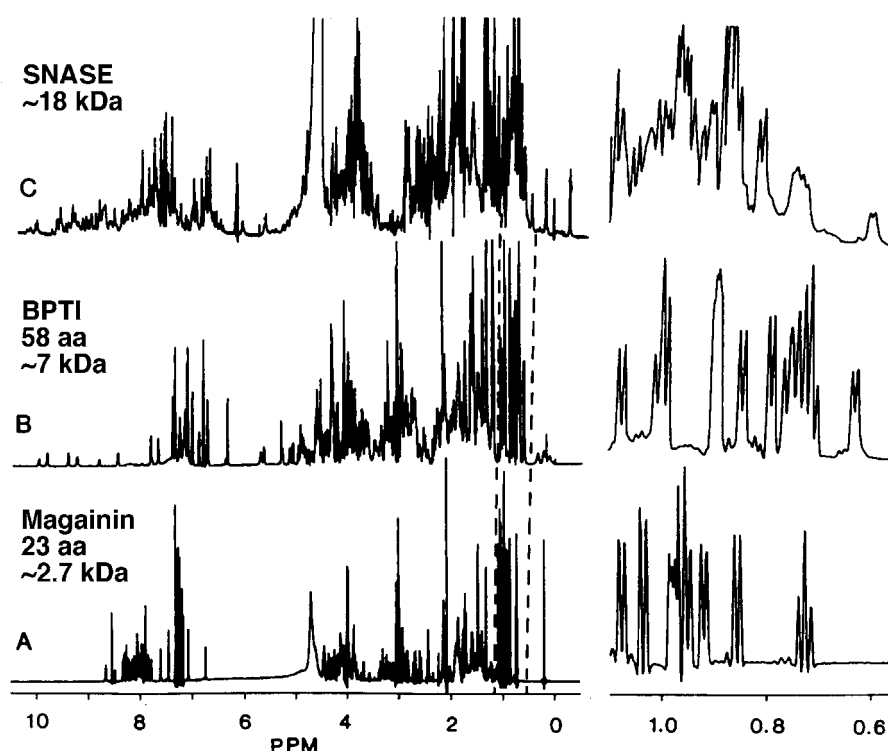


Fig.: one-dimensional proton spectra of magainin, BPTI, and staphyloccocal nuclease.

This increase in linewidth has very important consequences for high resolution NMR in solution:

1. It leads to unsolvable overlap problems
2. As the lines are becoming wider, their amplitudes decrease. Remember that the integral of the line stays constant. The smaller amplitude corresponds to a loss in the signal to noise ratio.
3. The speed of magnetization transfer in COSY experiments is given by the size of the scalar coupling constant J . The time required for this transfer is given by $\sim 1/J$. Only when the magnetization lives long enough, i.e. $T_2 > \sim 1/J$, the transfer can be carried out in an effective way. This means that for very large molecules, COSY experiments become completely inefficient.

1.5 A simple example of fluctuation-dissipation: why is T_2 for larger molecules inversely proportional to the molecular tumbling time?

A simple explanation for this behavior will be attempted in the following section. Consider a system of two spins A and B, which are close to each other in a molecule. The magnetic moment of spin B produces a dipolar magnetic field at the location of spin A. The Brownian motion of the molecule in solution has the effect that this dipolar magnetic field fluctuates. In principle there can be x,y, and z-components of the dipolar field at the location of A depending on the relative orientation of the two nuclei.

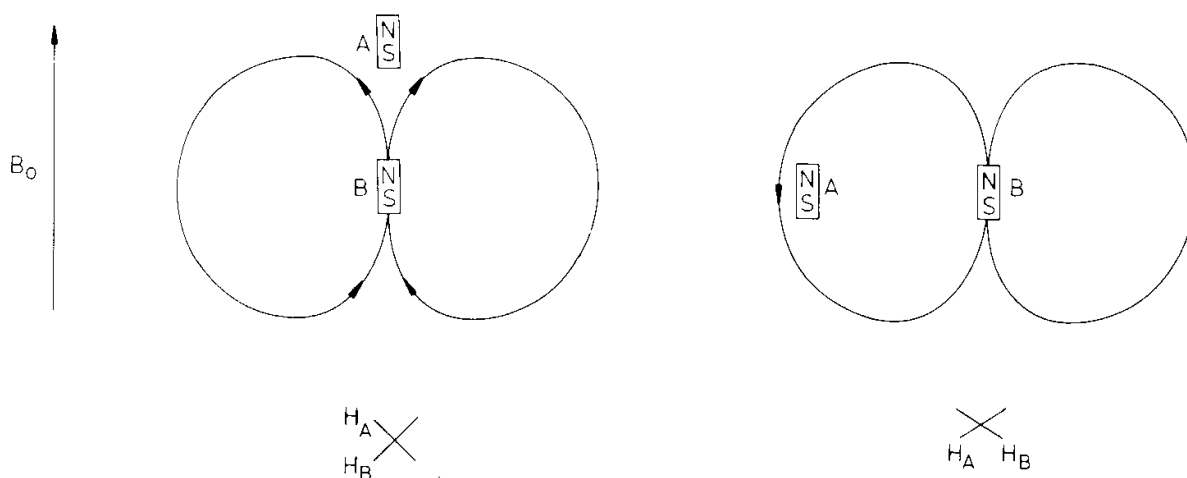


Figure 5.6 The interaction between two dipoles varies with their orientation.

Fig.: Derome p.102

If the dipolar field at the location of A is parallel to the external field, the magnetic moment of A will rotate a little faster, if the dipolar field is antiparallel, A will rotate a little slower than its normal Larmor frequency ω which is given by γB_0 . Now, let's make the simplification that the dipolar field has a constant size B_d and that the field can only have two orientations, i.e. parallel or antiparallel to z. At the same time we assume that the field stays constant in one direction for a characteristic time τ_c and that after this time it can either invert its direction or continue in the same orientation. This change is completely random and occurs with equal probability. Now let's also assume that the magnetic moment of A is in the xy-plane. In the absence of the dipolar field, after a time τ_c , the magnetic moment would have rotated by an angle

$$\phi = \omega \tau_c = \gamma B_0 \tau_c \quad (1.6)$$

Because of the presence of the random dipolar field this angle ϕ is modified by an addition $\Delta\phi$ which is given by

$$\Delta\phi = \pm \gamma B_d \tau_c = \pm \omega_d \tau_c \quad (1.7)$$

where the plus and minus sign depends on whether the dipolar field had been parallel or antiparallel to B_0 and where we have use the abbreviation $\omega_d = \gamma B_d$. Now, lets wait for a longer period t :

$$t = n \tau_c \quad (1.8)$$

In this case, the dipolar field had had n times the possibility to change its direction. The problem is identical to the random walk (or drunken man's walk) in one dimension. How far does the drunken man get if he only steps left and right by a length L of 1 m with equal probability and he repeats this process n times?

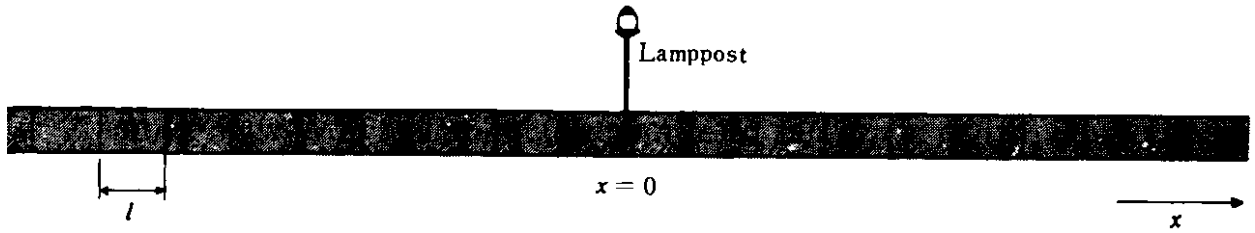


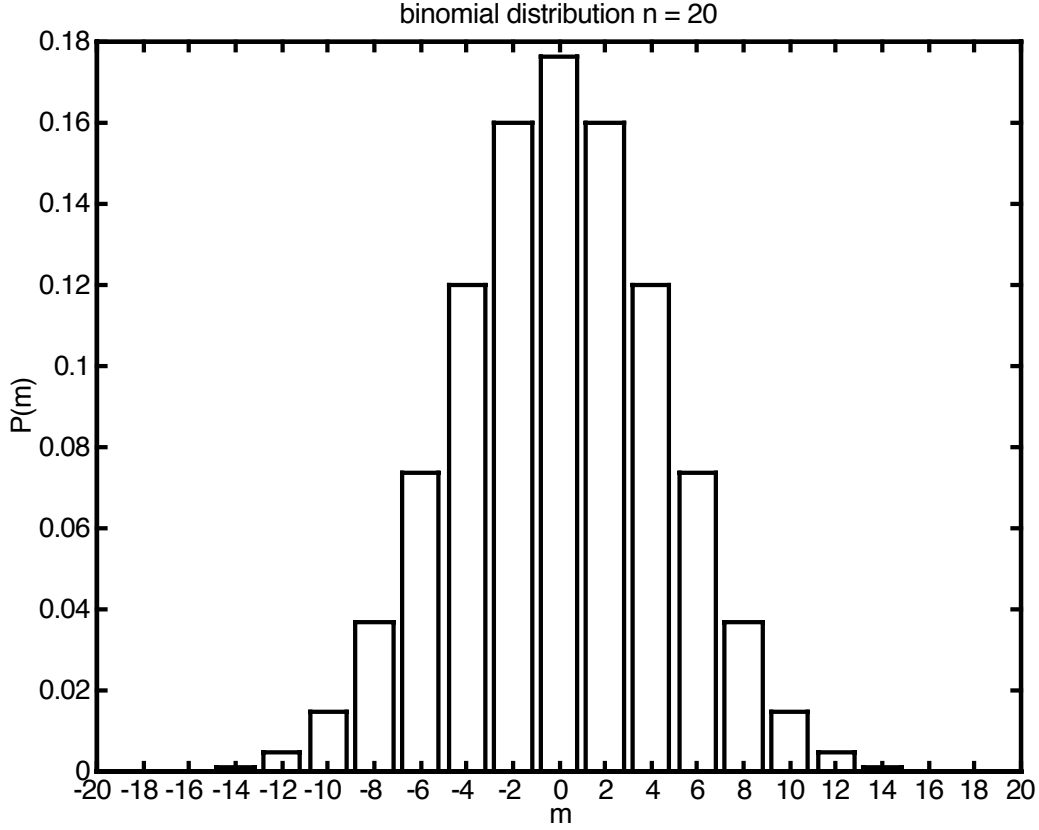
Fig. 1.1.1 The drunkard's random walk in one dimension.

Fig.: Reif pg.5

If this experiment is repeated many times (by many drunkards) the answer is the binomial probability distribution:

$$P_n(m) = \frac{n!}{\left(\frac{n+m}{2}\right)! \left(\frac{n-m}{2}\right)!} \left(\frac{1}{2}\right)^n \quad (1.9)$$

This is the probability of finding the man at position $m \cdot L$ after n steps with equal probability to left or right. The binomial distribution leads to the Gaussian distribution if the number of steps becomes very large. After their walk, some men will end up a little bit to the left, some men a little to the right, but the average position of all men after their walk is the spot where they started ($x=0$).



F

ig.: Binomial probability distribution for $n = 20$ steps with equal probability ($p = q = 0.5$) in left and right direction. The graph shows the probability $P_n(m)$ of a net displacement of m units to the right (adapted from Reif p. 11).

However, the width of the probability distribution or the root mean square displacement increases with the number of steps. For the binomial distribution the width is given by $n^{1/2}$. This means for 100 steps the mean square displacement is 10.

Now consider the situation for the dipolar coupling of the two spins A and B in a molecule in solution: of course we are not just dealing with a single molecule but with many molecules and we detect the nuclear moments of the spins A of all the molecules as a sum. After the time t , i.e. after doing n times the random addition of $\omega_d \tau_c$ to the phase angle of the single spins A, the phase angles of all the spins A of all the molecules will have a random distribution. The width δ of this random distribution of phases is given by

$$\delta = \omega_d \tau_c n^{1/2} = \omega_d \tau_c \left(\frac{t}{\tau_c} \right)^{1/2} = \omega_d (\tau_c t)^{1/2} \quad (1.10)$$

Now, when δ reaches the value 2π the spins A of all the molecules are more or less completely spread out in the xy plane. The result is that the sum of all the magnetic moments becomes 0 and the signal becomes zero.

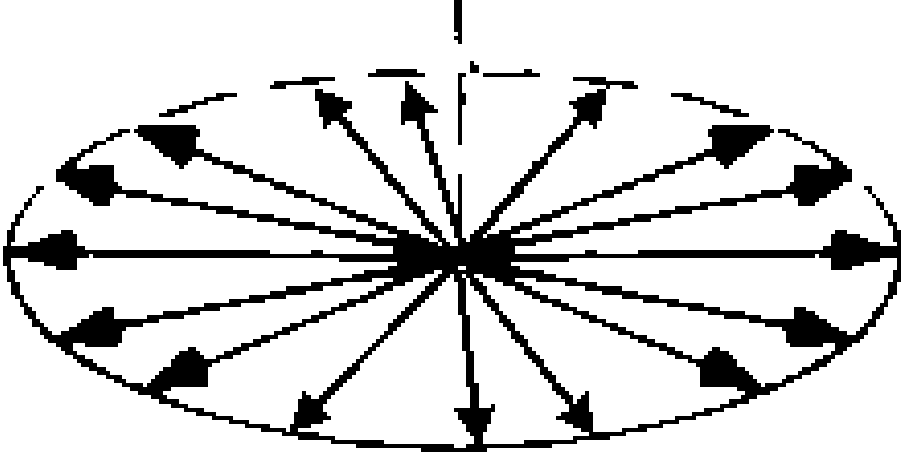


Fig.: the distribution of phase angles over the whole circle (pizza distribution) gives a net magnetic moment of zero for the whole ensemble of spins A.

To a first approximation, the relaxation time T_2 is the time when δ has reached the value 2π .

$$2\pi = \omega_d (\tau_c T_2)^{\frac{1}{2}} \Leftrightarrow \frac{1}{T_2} = \left(\frac{\omega_d}{2\pi} \right)^2 \tau_c = \left(\frac{H_d}{h} \right)^2 \tau_c \quad (1.11)$$

Thereby one can see that T_2 is inversely proportional to τ_c and it is directly proportional to the square of the (dipolar) interaction energy H_d . This is a very general principle resulting from the **fluctuation-dissipation** theorem, which would also hold if the system had been disturbed by a different interaction.

In reality, the dipolar field does not only jump between the $+z$ and $-z$ direction. The dipolar field moves continuously as the molecules rotate. The characteristic time for this molecular Brownian rotation is called the molecular tumbling time or rotational correlation time τ_c . For a spherical molecule of radius a rotating in a liquid of viscosity η , τ_c is given by

$$\tau_c = \frac{4\pi a^3 \eta}{3kT} = \frac{V\eta}{kT} \quad (1.12)$$

where V is the volume of the molecule. As the volume is proportional to the molecular weight, the rotational correlation time is proportional to the molecular weight. Therefore, T_2 is inversely and the line width is directly proportional to the molecular weight. As a rule of thumb, the rotational correlation time [given in ns] of a molecule in aqueous solution at room temperature is about half its molecular weight in kDa.

1.5.1 Example:

Estimate the dipolar T_2 for a proton which is 1.01 Å away from a ^{15}N nucleus with a rotational correlation time of 8 ns.

Answer: the dipolar frequency is derived from the dipolar coupling:

$$\begin{aligned}\gamma_1 B_{d2} &= \omega_d = H_d / \hbar = \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi r^3} \\ \gamma_{^{15}\text{N}} &= -2.71 \cdot 10^7 (\text{Ts})^{-1}; \gamma_{^1\text{H}} = 2.68 \cdot 10^8 (\text{Ts})^{-1} \\ \mu_0 &= 4\pi 10^{-7} \text{VsA}^{-1}\text{m}^{-1}; \text{T} = \text{Vs m}^{-2} \\ \hbar &= 1.054 \cdot 10^{-34} \text{Js} \\ r &= 1.01 \cdot 10^{-10} \text{m}; \tau_c = 8 \cdot 10^{-9} \text{s} \\ \omega_d / 2\pi &= -1.18 \cdot 10^4 \text{Hz} \\ \frac{1}{T_2} &= (\omega_d / 2\pi)^2 \tau_c = \frac{1}{(2\pi)^2} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \tau_c \\ \frac{1}{T_2} &= 1.1 \text{ Hz}\end{aligned}\tag{1.13}$$

The above simplifications have neglected a number of things:

1. the movement of the molecules is continuous and not a two-site random jump
2. there are also contributions of the dipolar field along the x- and y-axis which remove magnetization from the xy-plane
3. the assumption that T_2 corresponds to the time when the width of the phase distribution has reached a full circle overestimates T_2 .

An exact calculation for two (dipolarly) interacting spins I and S at a distance r shows that

$$\frac{1}{T_{2I}} = \frac{1}{20} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \left\{ 4J(0, \tau_c) + J(\omega_I - \omega_S, \tau_c) + \right. \\ \left. 3J(\omega_I, \tau_c) + 6J(\omega_S, \tau_c) + 6J(\omega_I + \omega_S, \tau_c) \right\}\tag{1.14}$$

and by symmetry:

$$\frac{1}{T_{2I}}(\omega_I, \omega_S) = \frac{1}{T_{2S}}(\omega_S, \omega_I)\tag{1.15}$$

The function $J(\omega, \tau_c)$ is called spectral density. It gives the probability of finding the frequency (energy) ω within the thermal motions of the molecule. For spherical molecules, $J(\omega, \tau_c)$ has the following form:

$$J(\omega, \tau_c) = \frac{\tau_c}{1 + \omega^2 \tau_c^2} \quad (1.16)$$

where τ_c is the rotational correlation time of the molecule.

Remark 1: in the slow motion limit ($\omega\tau_c \gg 1$), the terms $J(\omega, \tau_c)$ in Eq. 1.14 are very small compared to $J(0, \tau_c) = \tau_c$. Thus $1/T_2$ is simply proportional to τ_c . This holds also for other relaxation mechanism such as quadrupolar relaxation and CSA relaxation. Therefore T_2 s for different nuclei and from different relaxation mechanisms within a single molecule should be proportional to each other (provided there is no variation in local mobility and there is no additional line broadening from chemical exchange).

Remark 2: In the slow motion limit, the crude estimate for the heteronuclear $1/T_2$ that we derived above is off from the exact value by a factor of $5/(2\pi)^2 \approx 0.13$.

Remark 3: Redfield theory (see below, Eq. 2.16) shows that the spectral density is given by the Fourier transform of the autocorrelation function of the field fluctuations $F(t)$ resulting from the thermal motions:

$$J(\omega) = \int_0^\infty \overline{F(0)F^*(t')} \exp(i\omega t') dt' \quad (1.17)$$

where the overbar presents the ensemble average over all spins. Thus the relaxation rates, e.g. Eq. 1.14 are proportional to the square (autocorrelation) of the field fluctuations. This is (again) the central point of the fluctuation-dissipation theorem.

In an equilibrium situation, the autocorrelation of the field fluctuations $K(s) = \overline{F(t)F^*(t+s)}$ does not depend on the absolute value of the time t , but only on the difference s . $K(s)$ is a decaying function with a maximum at $s = 0$. In the case of rotational Brownian motion, the decay is monoexponential and the decay time is the rotational correlation time τ_c . The Fourier transform of the monoexponential decay gives Eq. 1.16.

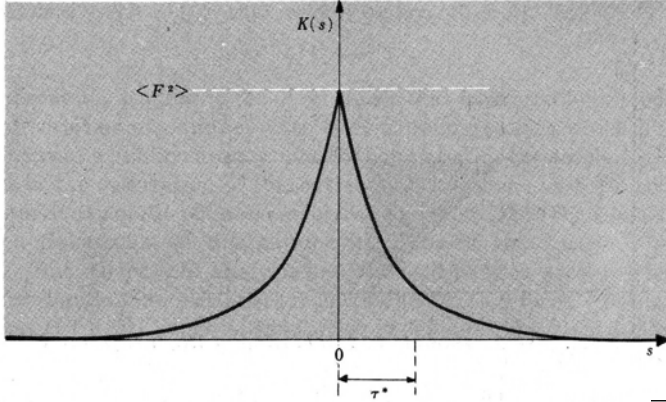


Fig.: Illustration of the correlation function $K(s) = \overline{F(t)F^*(t+s)}$ of a random function $F(t)$. (From Reif, pg. 571)

1.6 T_1 relaxation

The return to equilibrium along the z-axis of the spins involves transitions of the spins from the down to the up state and vice versa. Therefore in the random magnetic fields at the location of the nucleus I, there must be components that correspond to this transition, which happens at the frequency ω_0 . It can be shown that for dipolar relaxation the T_1 relaxation rate is proportional to the square of the dipolar field strength times the spectral density of the field fluctuations at frequency ω_0 . The spectral density $J(\omega, \tau_c) = \frac{\tau_c}{1 + \omega^2 \tau_c^2}$ has the following

appearance:

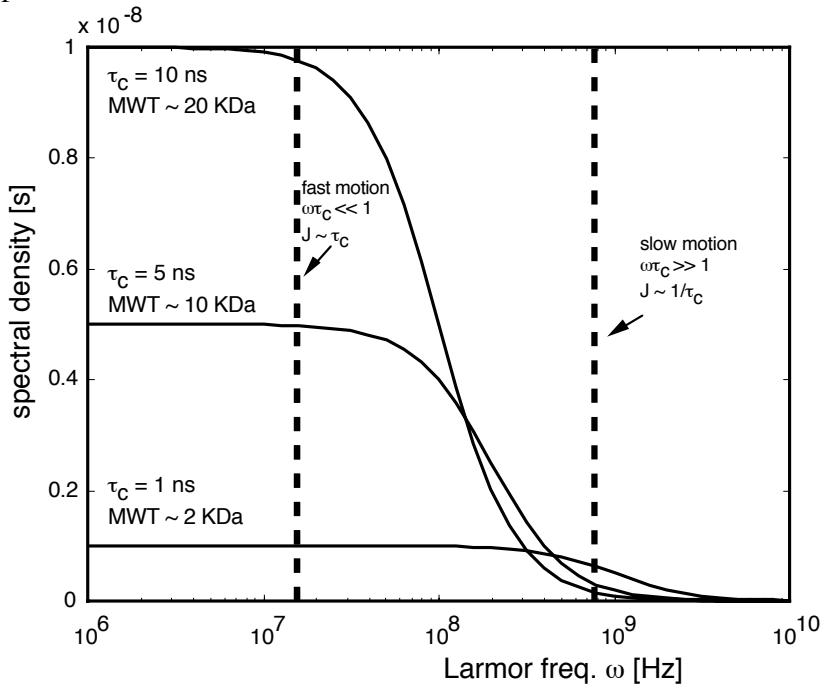


Fig.: the spectral density or the efficiency of relaxation for different Larmor frequencies and rotational correlation times τ_c .

What the graphic shows is that the spectral density is more or less constant from zero frequency up to a limiting frequency, which is given by $1/\tau_c$. For larger frequencies the spectral density goes to zero. The area under the spectral density function stays the same independent of τ_c . For small molecules (short correlation time, $\omega\tau_c \ll 1$) the function is spread out such that there is not much density at the position of the Larmor frequency. For large molecules (long correlation time, $\omega\tau_c \gg 1$) the spectral density is very small at the position of ω . Therefore both for small and large molecules T_1 relaxation is inefficient. Between the two limits, there is the most efficient T_1 relaxation, the “ T_1 minimum”. The exact calculation of T_1 (non-selective T_1) for two identical spins I (two protons) by dipolar coupling yields:

$$\frac{1}{T_1} = \frac{3}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^4 \hbar^2}{r^6} \{ J(\omega_I, \tau_c) + 4J(2\omega_I, \tau_c) \} \quad (1.18)$$

The behavior of the T_1 and T_2 as a function of the correlation time is shown in the following figure:

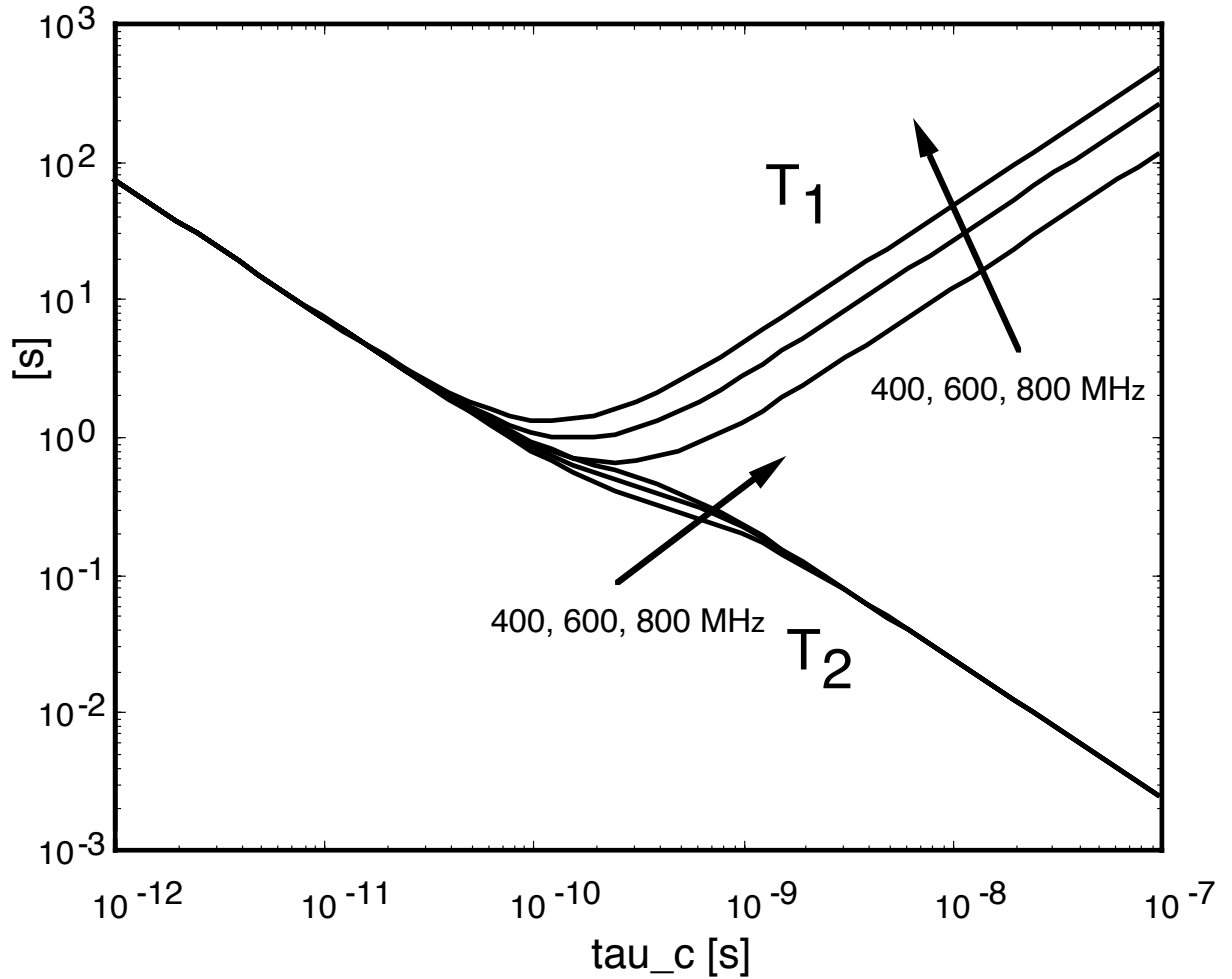


Fig.: T_1 and T_2 for two-spin system consisting of two protons with identical Larmor frequencies (400, 600 or 800 MHz) at a distance of 2 Å as a function of the correlation time.

1.7 Spin-flips induced by Brownian motion, the nuclear Overhauser effect

Consider now a system of two spins I and S, which are in dipolar interaction. Spin I is moved away from thermal equilibrium by a $180^\circ I_x$ -pulse as described above. The dipolar field of the second nucleus S at the position of nucleus I fluctuates because of the Brownian motion of the molecule. These fluctuations generate transitions between the states of the IS spin system. The dominant transition is the so-called spin flip process where spin I changes from the up-state to the down-state and spin S from the down-state to the up-state and vice versa ($\downarrow\uparrow \leftrightarrow \uparrow\downarrow$). We look at the spin system for the following sequence of events: (1) – $180^\circ I_x$ – (2) – I-S spin-flip – (3):

	IS	IS	IS	IS
spin states at equilibrium (1)	$\uparrow\uparrow$	$\uparrow\downarrow$	$\downarrow\uparrow$	$\downarrow\downarrow$
probability	$\frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2}$	$\frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2}$	$\frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2}$	$\frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2}$
spin states after $180^\circ I_x$ pulse (2)	$\downarrow\uparrow$	$\downarrow\downarrow$	$\uparrow\uparrow$	$\uparrow\downarrow$
spin states after $\downarrow\uparrow \leftrightarrow \uparrow\downarrow$ spin flip (3)	$\uparrow\downarrow$	$\downarrow\downarrow$	$\uparrow\uparrow$	$\downarrow\uparrow$

Consider the table above and the distribution of states at equilibrium. We calculate for the ensemble average values of the observables (time point 1):

$$\begin{aligned}
 \langle\langle I_\alpha \rangle\rangle (1) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I) \\
 \langle\langle I_\beta \rangle\rangle (1) &= \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\
 \langle\langle S_\alpha \rangle\rangle (1) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\
 \langle\langle S_\beta \rangle\rangle (1) &= \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S)
 \end{aligned} \tag{1.19}$$

where the ΔP s are given from the Boltzmann distribution. We also notice that

$$\begin{aligned}
 \frac{1}{2}(\langle\langle I_\alpha \rangle\rangle - \langle\langle I_\beta \rangle\rangle) &= \langle\langle I_z \rangle\rangle \\
 \frac{1}{2}(\langle\langle S_\alpha \rangle\rangle - \langle\langle S_\beta \rangle\rangle) &= \langle\langle S_z \rangle\rangle
 \end{aligned} \tag{1.20}$$

and therefore

$$\begin{aligned} \langle\langle I_z \rangle\rangle (1) &= \Delta P(I) \\ \langle\langle S_z \rangle\rangle (1) &= \Delta P(S) \end{aligned} \quad (1.21)$$

After the $180^\circ I_x$ -pulse (time point 2), the following ensemble averages are calculated:

$$\begin{aligned} \langle\langle I_\alpha \rangle\rangle (2) &= \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\ \langle\langle I_\beta \rangle\rangle (2) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I) \\ \langle\langle S_\alpha \rangle\rangle (2) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\ \langle\langle S_\beta \rangle\rangle (2) &= \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S) \end{aligned} \quad (1.22)$$

and therefore

$$\begin{aligned} \langle\langle I_z \rangle\rangle (2) &= -\Delta P(I) \\ \langle\langle S_z \rangle\rangle (2) &= \Delta P(S) \end{aligned} \quad (1.23)$$

If the $\downarrow\uparrow \leftrightarrow \uparrow\downarrow$ spin flips between point (2) and (3) happened with a 100% probability the distribution of states would be the following:

$$\begin{aligned} \langle\langle I_\alpha \rangle\rangle (3) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\ \langle\langle I_\beta \rangle\rangle (3) &= \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S) \\ \langle\langle S_\alpha \rangle\rangle (3) &= \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\ \langle\langle S_\beta \rangle\rangle (3) &= \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I) \end{aligned} \quad (1.24)$$

and therefore

$$\begin{aligned} \langle\langle I_z \rangle\rangle (3) &= \Delta P(S) = \langle\langle S_z \rangle\rangle (1) \\ \langle\langle S_z \rangle\rangle (3) &= -\Delta P(I) = -\langle\langle I_z \rangle\rangle (1) \end{aligned} \quad (1.25)$$

Clearly the spin flips exchange the z-magnetizations between the two spins (trivial). If the probabilities $\Delta P(I)$ and $\Delta P(S)$ were equal, then the net effect from time point (1) to (3) would be that spin I is back to equilibrium whereas spin S has its equilibrium distribution inverted. The effect that z-magnetization can be exchanged between spins on the way to thermal equilibrium by means of the dipolar interaction is called the **nuclear Overhauser effect**.

1.8 The Solomon equation

The probability for the spin-flips is not 100%, but is given as a rate constant (per unit time). This constant is the NOE build-up rate k_{NOE} . k_{NOE} is proportional to the square of the dipolar interaction times a function that depends on the rotational correlation time τ_c (spectral density). The total return to equilibrium for the z-magnetizations of a two-spin system is described by the Solomon equation [Solomon, I. (1955). Phys. Rev. **99**, 559]:

$$\frac{d}{dt} \begin{pmatrix} \langle\langle I_z \rangle\rangle \\ \langle\langle S_z \rangle\rangle \end{pmatrix} = \begin{pmatrix} -k_1(I) & -k_{\text{NOE}} \\ -k_{\text{NOE}} & -k_1(S) \end{pmatrix} \begin{pmatrix} \langle\langle I_z \rangle\rangle - \langle\langle I_z \rangle\rangle_{\text{eq}} \\ \langle\langle S_z \rangle\rangle - \langle\langle S_z \rangle\rangle_{\text{eq}} \end{pmatrix} \quad (1.26)$$

In the case of an isolated, heteronuclear two-spin system, the rate constants $k_1(I)$, $k_1(S)$, k_{NOE} are given by

$$\begin{aligned} k_1(I) &= \frac{1}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \{ 3J(\omega_I, \tau_c) + J(\omega_I - \omega_S, \tau_c) + 6J(\omega_I + \omega_S, \tau_c) \} \\ k_1(S) &= \frac{1}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \{ 3J(\omega_S, \tau_c) + J(\omega_S - \omega_I, \tau_c) + 6J(\omega_S + \omega_I, \tau_c) \} \\ k_{\text{NOE}} &= \frac{1}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \{ 6J(\omega_I + \omega_S, \tau_c) - J(\omega_I - \omega_S, \tau_c) \} \end{aligned} \quad (1.27)$$

Remark: the approach to thermal equilibrium described by the Solomon equation, i.e by the NOE, i.e. by the spin-flips resembles very much the propagation of a heat wave. At time zero spin I is heated, then the heat dissipates to the next neighbor to the next neighbor to the next neighbor ...

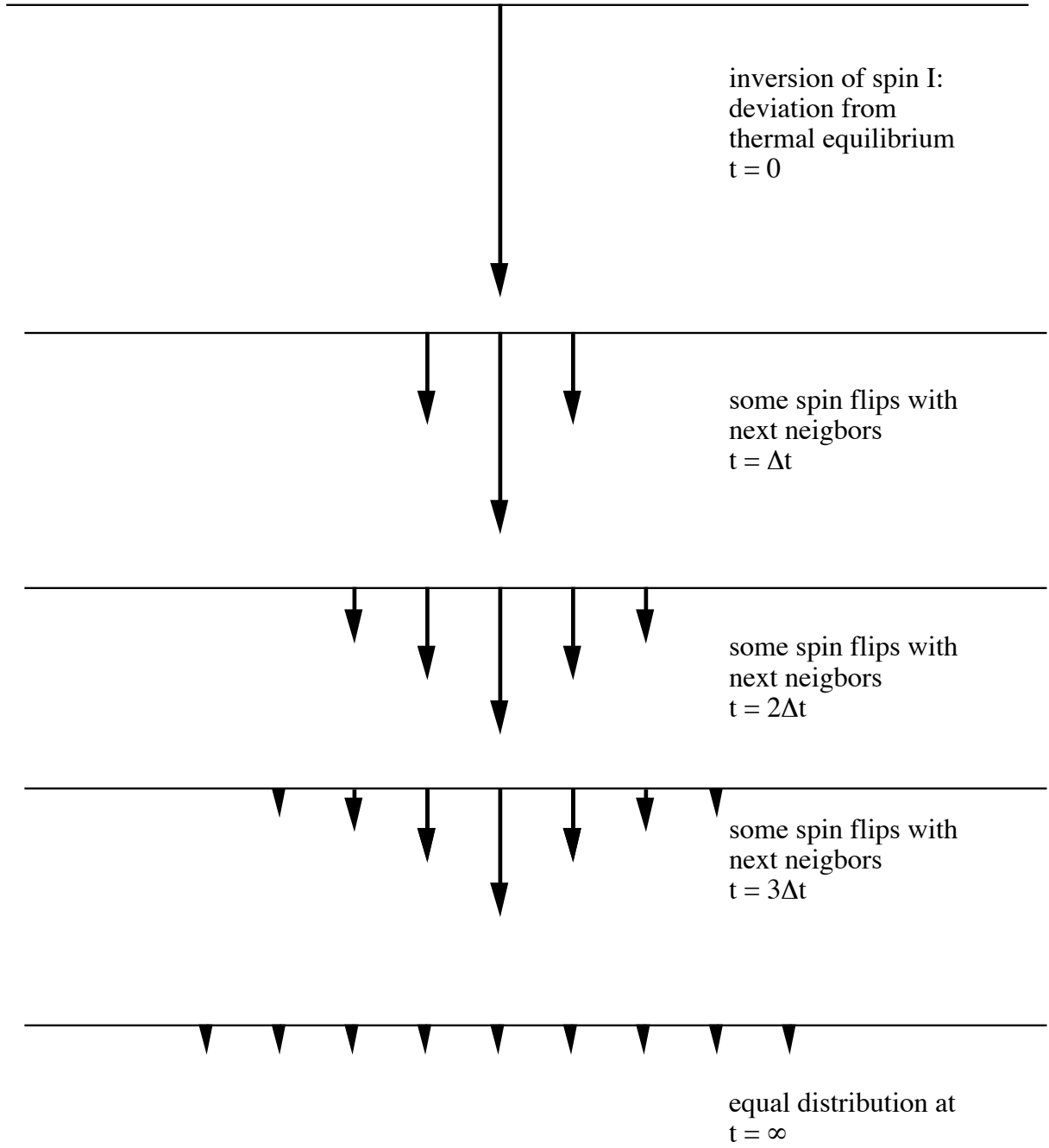


Fig.: approach to thermal equilibrium by NOESY spin-flips

1.8.1 Initial rate approximation

For NOE mixing times t_m , which are short compared to the NOE buildup rate k_{NOE} , the increase in z-magnetization on spin S due to NOE transfer from spin I can be approximated from the Solomon equation ($dt \approx \Delta t = t_m$):

$$\Delta \begin{pmatrix} \langle\langle I_z \rangle\rangle \\ \langle\langle S_z \rangle\rangle \end{pmatrix} \approx \begin{pmatrix} -k_1(I) & -k_{\text{NOE}} \\ -k_{\text{NOE}} & -k_1(S) \end{pmatrix} \begin{pmatrix} \langle\langle I_z \rangle\rangle - \langle\langle I_z \rangle\rangle_{\text{eq}} \\ \langle\langle S_z \rangle\rangle - \langle\langle S_z \rangle\rangle_{\text{eq}} \end{pmatrix} \Delta t \quad (1.28)$$

The amount of magnetization, which has started on I_z and was transferred to S_z during the mixing time, is given by:

$$\Delta \langle\langle S_z \rangle\rangle_{\text{NOE}} \approx -k_{\text{NOE}} \left(\langle\langle I_z \rangle\rangle_{(t=0)} - \langle\langle I_z \rangle\rangle_{\text{eq}} \right) t_m \quad (1.29)$$

In the NOESY experiment, the amount of I_z -magnetization at the beginning of the mixing period ($\langle\langle I_z \rangle\rangle_{(t=0)}$) equals $-\langle\langle I_z \rangle\rangle_{\text{eq}} \cos(\omega_I t)$. Therefore

$$\Delta \langle\langle S_z \rangle\rangle_{\text{NOE}} = k_{\text{NOE}} (\cos(\omega_I t_1) + 1) t_m \langle\langle I_z \rangle\rangle_{\text{eq}} \quad (1.30)$$

This means that the NOESY (I->S) cross-peak intensity (value β in the original equation of the NOESY experiment) is given by

$$\beta \approx -k_{\text{NOE}} t_m \langle\langle I_z \rangle\rangle_{\text{eq}} \quad (1.31)$$

Therefore the NOESY crosspeak intensity is proportional to the NOESY build-up rate k_{NOE} in the initial rate approximation. From the Solomon equation we realize that k_{NOE} is proportional to $\gamma_I^2 \gamma_S^2 / r^6$. It follows that the NOE is strongest for short distances and between protons (because protons have the largest gyromagnetic ratios).

1.9 Homonuclear NOE

For the homonuclear case (e.g. NOE between protons) we can set $\omega_I \approx \omega_S \approx \omega$. k_{NOE} is then given as

$$k_{\text{NOE}} = \frac{1}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma^4 \hbar^2}{r^6} \{ 6J(2\omega, \tau_c) - J(0, \tau_c) \} \quad (1.32)$$

For spherical Brownian motion, this function has a zero crossing at

$$\omega \tau_c = \sqrt{5/4} \approx 1.12 \quad (1.33)$$

This means that for $B = 14\text{T}$, no NOE can be observed between protons if $\tau_c \approx 1.12 / (2\pi 600\text{MHz}) = 0.3 \text{ ns}$. At correlation times larger than 0.3 ns, k_{NOE} becomes negative (but the cross peak in the NOESY spectrum will have the same sign as the diagonal peak). For $\omega \tau_c \gg 1$ (slow tumbling limit), the contribution of $J(2\omega, \tau_c)$ can be neglected and k_{NOE} is simply given by

$$k_{\text{NOE}} \approx -\frac{1}{10} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma^4 \hbar^2}{r^6} \tau_c \quad (1.34)$$

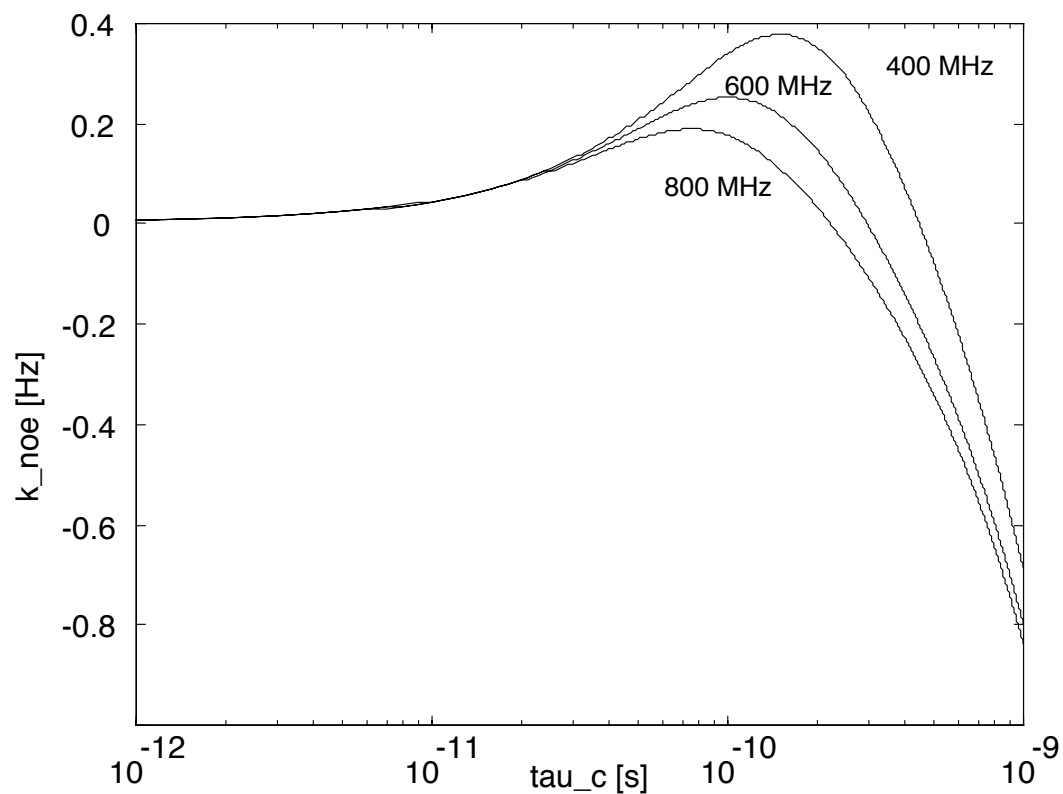


Fig.: NOE buildup rate k_{NOE} for two protons at a distance of $2 \text{ \AA}$ for Larmor frequencies of 400, 600, 800 MHz.

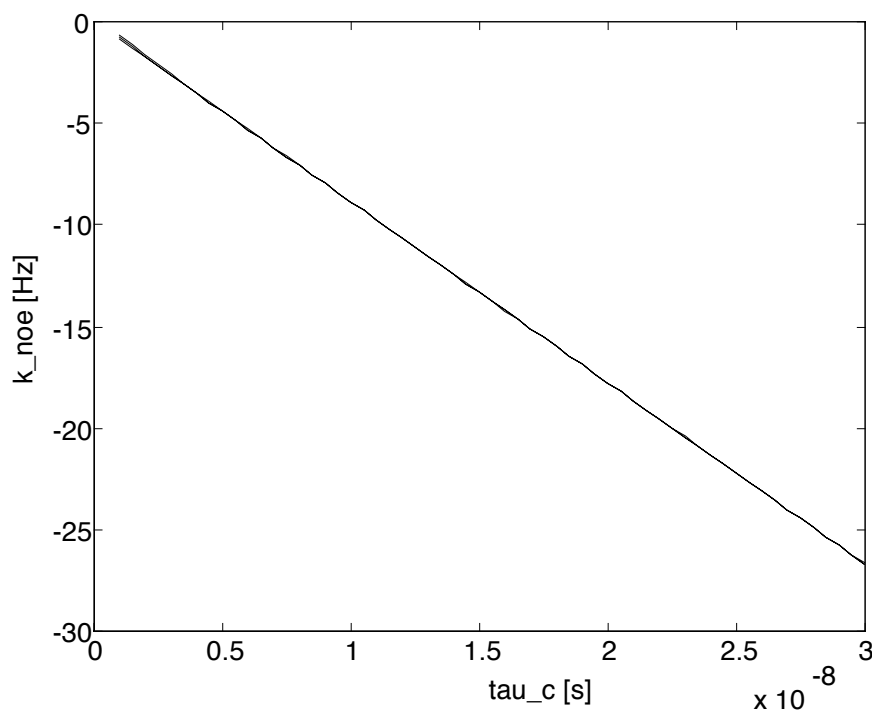


Fig.: NOE buildup rate k_{NOE} for two protons at a distance of $2 \text{ \AA}$ for Larmor frequencies of 400, 600, 800 MHz.

1.10 Heteronuclear NOE

From the homonuclear case, we have seen that k_{NOE} depends on the rotational correlation time of the internuclear vector of the two nuclei, which undergo dipolar relaxation. In principle, the rotational correlation time could be extracted from the intensities of the homonuclear proton-proton NOESY experiment. In practice, this is very difficult, because often the exact distances are not known and secondly many protons interact at equal strength since their distances are similar. The heteronuclear NOE between a proton and a directly bonded heteronucleus is much easier to interpret, because the one-bond distance is rather well determined and because this NOE is the dominant relaxation effect on the heteronucleus. $\{^1\text{H}\}$ - ^{15}N NOEs and to a lesser extent $\{^1\text{H}\}$ - ^{13}C NOEs are used to obtain information on the correlation times of the respective internuclear vectors in biological macromolecules. Usually the steady-state NOE effect is measured. The proton spin is often called I and the heteronuclear spin S. The steady state NOE enhancement compares the z-magnetization of the S-spin in thermal equilibrium to the z-magnetization of the S-spin at equilibrium when the I-spin is saturated:

$$\text{NOE}(\{I\}-S) = \frac{\langle\langle S_z \rangle\rangle_{(I\text{-sat})}}{\langle\langle S_z \rangle\rangle_{\text{eq}}} \quad (1.35)$$

Saturation of the I-spin means that its magnetization vanishes. This can be obtained by applying a large number of random RF-pulses on I. The z-magnetization of the S-spin for this case can be calculated as a steady state solution of the Solomon equation. For the steady state, the time-derivatives are simply set to 0. Because of the saturation of spin I, we use $\langle\langle I_z \rangle\rangle = 0$:

$$0 = \begin{pmatrix} -k_1(I) & -k_{\text{NOE}} \\ -k_{\text{NOE}} & -k_1(S) \end{pmatrix} \begin{pmatrix} -\langle\langle I_z \rangle\rangle_{\text{eq}} \\ \langle\langle S_z \rangle\rangle_{I\text{-sat}} - \langle\langle S_z \rangle\rangle_{\text{eq}} \end{pmatrix} \quad (1.36)$$

Solving for $\langle\langle S_z \rangle\rangle_{I\text{-sat}}$ yields:

$$0 = k_{\text{NOE}} \langle\langle I_z \rangle\rangle_{\text{eq}} - k_1(S) (\langle\langle S_z \rangle\rangle_{I\text{-sat}} - \langle\langle S_z \rangle\rangle_{\text{eq}})$$

$$\langle\langle S_z \rangle\rangle_{I\text{-sat}} = \frac{k_{\text{NOE}}}{k_1} \langle\langle I_z \rangle\rangle_{\text{eq}} + \langle\langle S_z \rangle\rangle_{\text{eq}} \quad (1.37)$$

And therefore:

$$\text{NOE}(\{I\}-S) = \frac{k_{\text{NOE}}}{k_1} \frac{\langle\langle I_z \rangle\rangle_{\text{eq}}}{\langle\langle S_z \rangle\rangle_{\text{eq}}} + 1 = \frac{k_{\text{NOE}}}{k_1} \frac{\gamma_I}{\gamma_S} + 1 \quad (1.38)$$

k_1 is the longitudinal relaxation rate ($1/T_1$). It can also contain contributions from other relaxation mechanisms besides the dipolar 1-bond relaxation. A significant contribution to k_1 for ^{15}N amide nuclei is the relaxation stemming from chemical shift anisotropy. The figure shows the behavior of the amide $\{^1\text{H}\}$ - ^{15}N -NOE as a function of the correlation time when this mechanism is taken into account. The effect clearly distinguishes between motions faster or slower than ~ 0.5 ns.

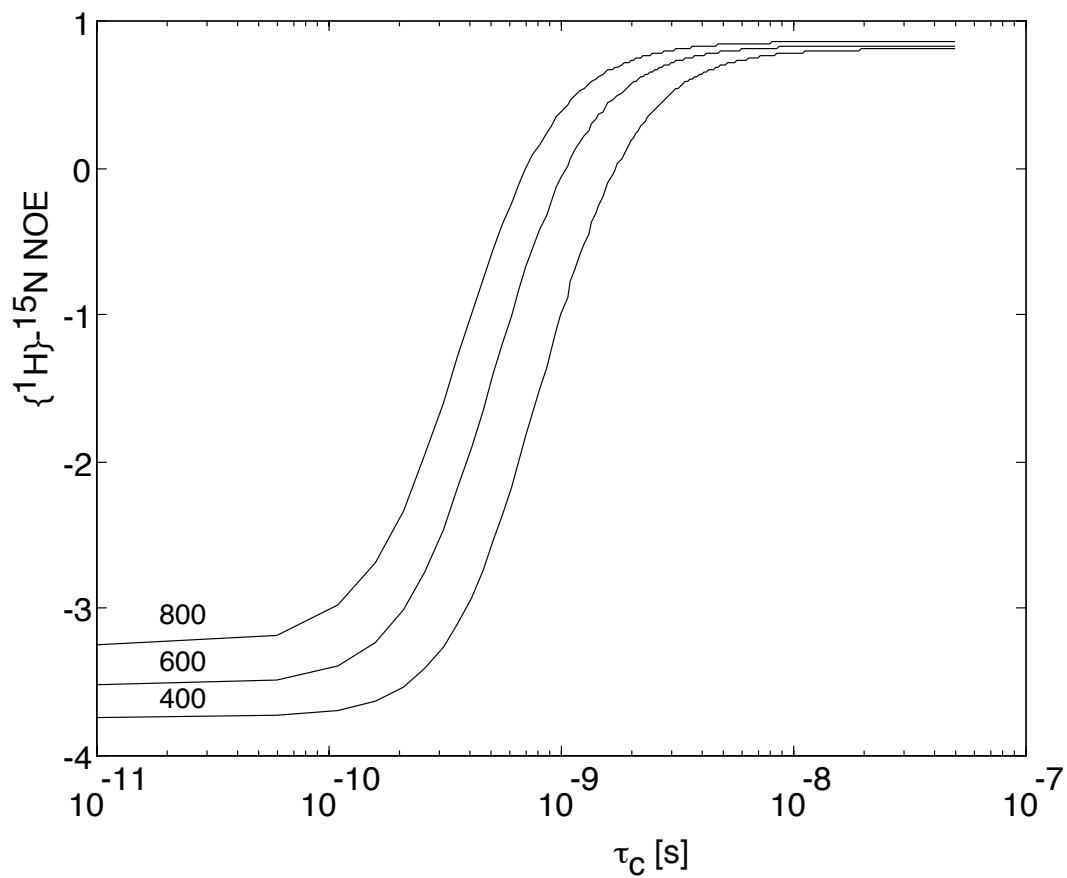


Fig: values of the steady state heteronuclear $\{^1\text{H}\}\text{-}^{15}\text{N}$ NOE as a function of correlation time for magnetic field strengths corresponding to 400, 600, and 800 MHz proton Larmor frequency. The effect of 160 ppm chemical shift anisotropy ($\sigma_{||} - \sigma_{\perp}$) on k_1 has been taken into account.

1.11 Dynamical information from relaxation experiments

The relaxing way to study motion (adapted from G. Wagner, 1995, *nature structural biology*, 2, 255-257)

^{15}N NMR relaxation studies have emerged as a powerful approach for the determination of the motional properties of molecules in solution. In an ^{15}N relaxation experiment, one creates non-equilibrium spin order and records how this relaxes back to equilibrium. At equilibrium, the magnetization of the ^{15}N spins is aligned along the external field, and this alignment can be inverted by radio frequency pulses. The spins will relax back to equilibrium along the direction of the magnetic field with a longitudinal relaxation time, T_1 , or the longitudinal relaxation rate $R_N(N_z)$. The magnetization can also be oriented perpendicular to the external magnetic field. The relaxation time of this spin order back to equilibrium is called the transverse relaxation time, T_2 , and the rate can be denoted as $R_N(N_x)$. A third relaxation parameter is the so-called heteronuclear nuclear Overhauser effect (NOE). This is measured by saturating the proton (^1H) signal and observing changes in the ^{15}N signal. The rate at which this occurs is the heteronuclear cross relaxation rate, $R_{\text{HN}}(H_z \leftrightarrow N_z)$; for long proton saturation, it reaches the steady state NOE($H_z \leftrightarrow N_z$) value. These are essentially the three experiments Nicholson *et al*¹ perform to learn about mobility in HIV protease. Other spin orders could be created and the relaxation analyzed. The relaxation of the spin orders is due to rotational diffusive motions of the nitrogen atom and the orientation of its chemical bonds (N-H bond) relative to the external field. The molecular motions cause the ^{15}N nucleus to experience energy fluctuations inducing transitions between the Zeeman energy levels - and result in relaxation. Thus, the relaxation of spin orders is related to rotational motions of the amide group; this includes the overall, and internal motions of the HIV protease. The effect of the motions on relaxation rates is described with a spectral density function $J(\omega)$. This function describes the relative distribution of the frequencies of the rotational diffusive motions. For the three parameters measured by Nicholson and colleagues, these relations are:

$$\begin{aligned}
 R_N(N_z) &= d^2 [J(\omega_N - \omega_H) + 3J(\omega_N) + 6J(\omega_N + \omega_H)] + c^2 J(\omega_N) \\
 R_N(N_x) &= 0.5 d^2 [4J(0) + J(\omega_N - \omega_H) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_N + \omega_H)] + \\
 &\quad \frac{c^2}{6} [4J(0) + 3J(\omega_N)] + R_{\text{ex}} \\
 \text{NOE}(H_z \leftrightarrow N_z) &= 1 + \frac{\gamma_H d^2}{\gamma_N R_N(N_z)} [6J(\omega_N + \omega_H) - J(\omega_N - \omega_H)]
 \end{aligned} \tag{1.39}$$

In these relations, d and c represent the strengths of the dipolar interaction between the proton and nitrogen, and the chemical shift anisotropy; they also represent the main relaxation mechanisms for ^{15}N in proteins. ω_N and ω_H are the resonance frequencies of ^{15}N and ^1H . In a typical spectrometer, these are 50 and 500 MHz, respectively. The equations indicate that ^{15}N

¹ L. Nicholson, T. Yamazaki, D.A. Torchia, S. Grzesiek, A. Bax, S.J. Stahl, J.D. Kaufman, P.T. Wingfield, P.Y.S. Lam, P.K. Jadhav, C.N. Hodge, P.J. Domaille, and C.-H. Chang: Flexibility and function in HIV-1 protease. *Nature Structural Biology* 2, 274-279, 1995.

relaxation is primarily sensitive to very fast motions in the order of the frequencies of protons and nitrogens as well as the sum and difference thereof. However, the transverse relaxation rates can also be influenced by slow conformational exchange, represented by R_{ex} . Typically, motions on the order of ms to μ s would have an effect on the transverse relaxation rates. To measure dynamics from such relaxation experiments one has to perform further experiments, or alternatively one can make assumptions about an analytical form of the spectral density function that contains a sufficiently small number of parameters can be fitted to the experiments. The most commonly used analytical form for a spectral density function has been proposed by Lipari and Szabo which is a sum of two Lorentzian shapes:

$$J(\omega) = S^2 \frac{\tau_m}{1 + (\omega\tau_m)^2} + (1 - S^2) \frac{\tau}{1 + (\omega\tau)^2} \quad (1.40)$$

with $\tau^{-1} = \tau_m^{-1} + \tau_e^{-1}$

Accepting that this is a reasonable assumption, the relaxation rates depend on only one global and two local parameters, the overall correlation time τ_m which is the same for all residues, a correlation time for internal motions, τ_e and an order parameter S^2 which is a measure of the amplitude of the internal motions. With the measurement of three relaxation parameters, the longitudinal relaxation rate, $R_N(N_Z)$, the transverse relaxation rate, $R_N(N_X)$, and the heteronuclear NOE($H_Z \leftarrow N_Z$), the three parameters, τ_m , τ_e , and S^2 , can readily be fitted.

The following figure shows the measured T_1 , T_2 , and NOE values for the HIV-1 protease in complex with two different inhibitors:

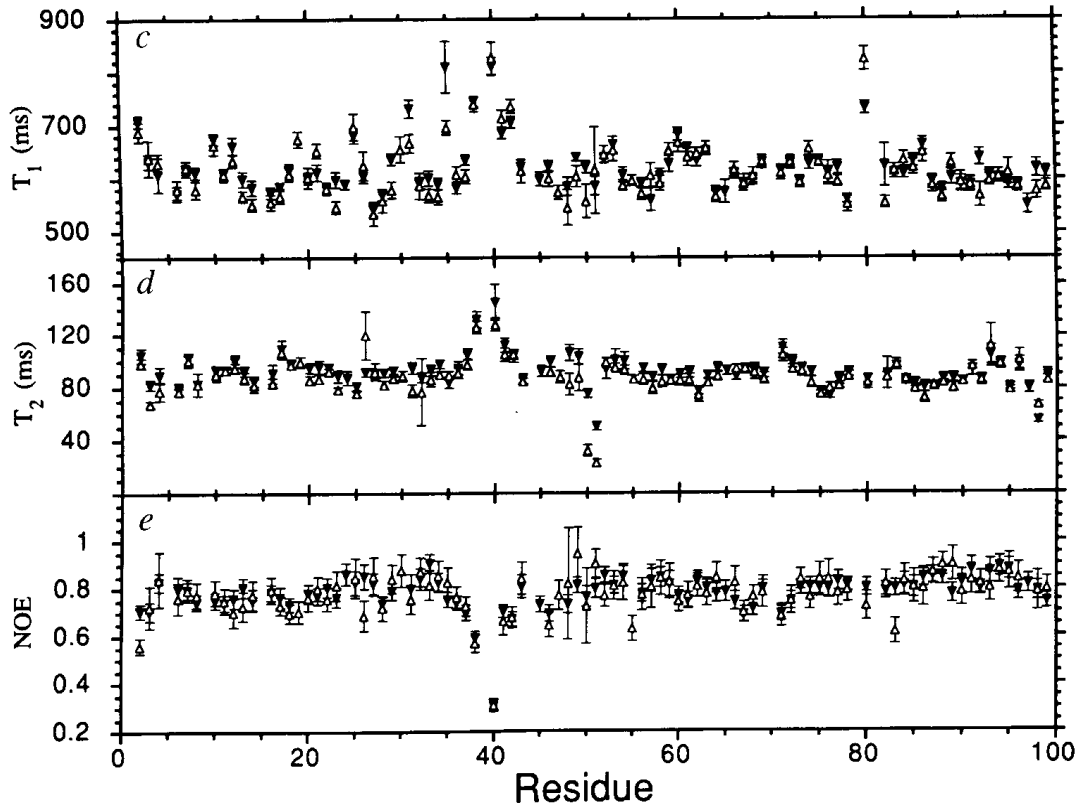


Fig. 1 Inhibitors *a*, DMP323 and *b*, P9941, their respective inhibition constants, K_i , and experimental relaxation parameters *c*, T_1 ; *d*, T_2 ; and *e*, NOE values of HIV-1 protease/inhibitor complexes, DMP323 ($\blacktriangledown$) and P9941 ($\triangle$).

A fit of the parameters S^2 and R_{ex} is shown in the next figure (Fig. 2). Clearly visible is the enhanced mobility around residues 38-42. These residues are at the hinges of the two enzyme flaps that have to open in order to allow the binding of an inhibitor or substrate to the protease active site.

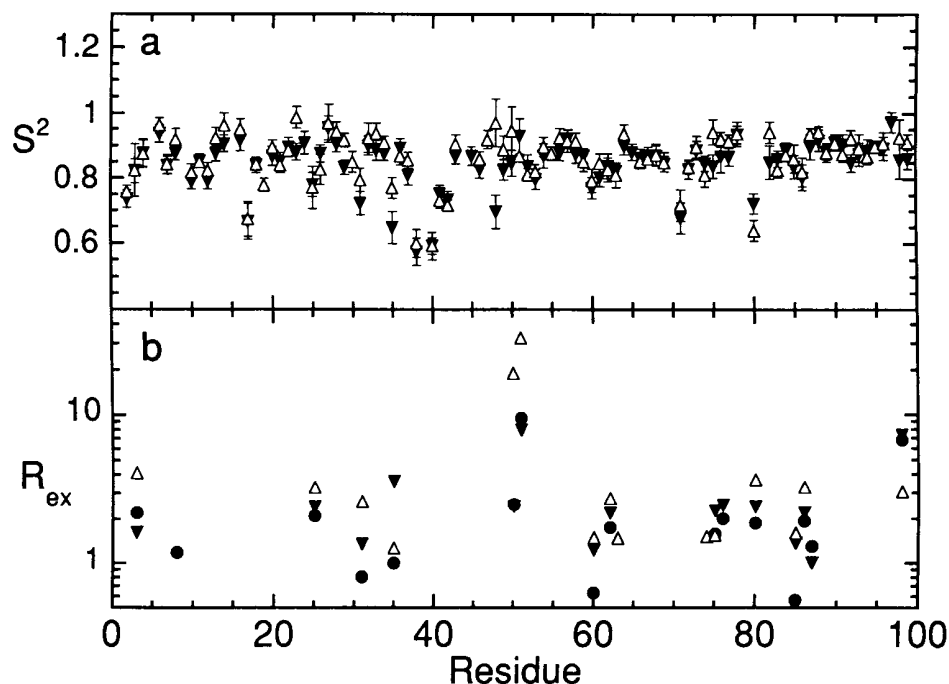


Fig. 2 a, Generalized order parameters (S^2) and b, the chemical exchange contribution to $1/T_2$ (R_{ex}), plotted as a function of residue number for the two complexes (▼) DMP323 using three relaxation parameters, T_1 , T_2 at 500 MHz and NOE at 600 MHz; (●) DMP323 using four relaxation parameters, T_1 , T_2 at 500 MHz and T_{ρ} , NOE at 600 MHz; and (Δ) P9941 using three relaxation parameters, T_1 , T_2 at 500 MHz and NOE at 600 MHz.

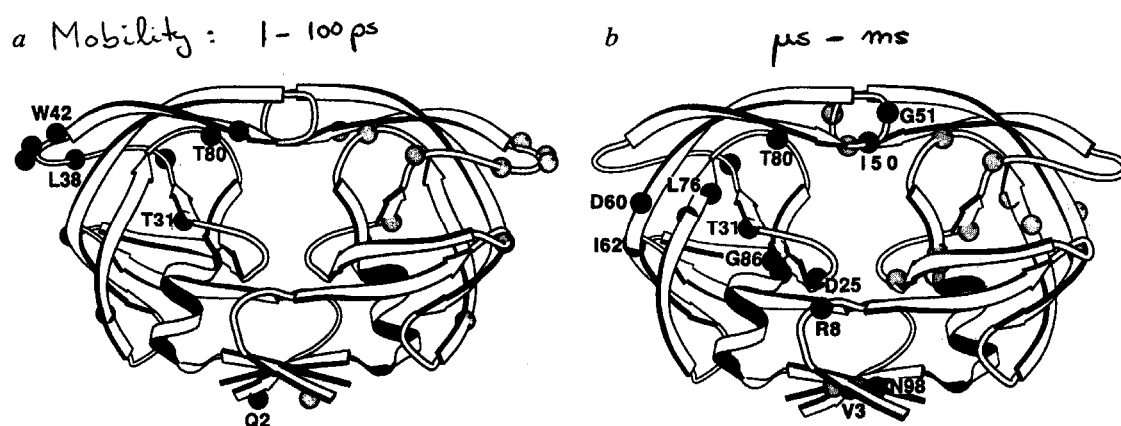


Fig.: The mobility of HIV-1 protease as determined by NMR relaxation experiments. The mobility of the two enzyme flaps makes it possible for the substrate to enter into the active site of the protease. All three figures from Nicholson et al. (1995) Flexibility and function in HIV-1 protease. Nature Structural Biology 2, 274-279.

2. A quick derivation of the master equation

according to M. Goldman (1993) with special thanks to Bloembergen, Purcell, Pound (1948), Wangsness and Bloch (1953), Bloch (1956), Solomon (1955), Redfield (1957), Abragam (1961), Ernst, Bodenhausen, Wokaun (1987).

2.1 The total Hamiltonian

$$H = H_0 + H_1(t) \quad (2.1)$$

H_0 : main Hamiltonian, discrete levels, time-independent

H_1 : random, spin-lattice coupling

2.2 Liouville-von Neumann equation

$$\frac{d\sigma}{dt} = -i[H, \sigma] \quad (2.2)$$

2.3 Transform operators to interaction picture

i.e. into the rotating frame

$$\begin{aligned} \tilde{Q}(t) &= \exp(iH_0 t) Q \exp(-iH_0 t) \\ \frac{d\tilde{\sigma}}{dt} &= -i[(\tilde{H} - H_0), \tilde{\sigma}] \\ &\Downarrow \\ \frac{d\tilde{\sigma}}{dt} &= -i[\tilde{H}_1(t), \tilde{\sigma}] \end{aligned} \quad (2.3)$$

H_0 disappears!

2.4 Integrate once

$$\tilde{\sigma}(t) = \tilde{\sigma}(0) - i \int_0^t [\tilde{H}_1(t'), \tilde{\sigma}(t')] dt' \quad (2.4)$$

put into original Liouville-von Neumann equation:

$$\frac{d\tilde{\sigma}(t)}{dt} = -i [\tilde{H}_1(t), \tilde{\sigma}(0)] - \int_0^t [\tilde{H}_1(t), [\tilde{H}_1(t'), \tilde{\sigma}(t')]] dt' \quad (2.5)$$

2.5 Take ensemble average ($\overline{\quad}$) of all terms

$$\frac{d\overline{\tilde{\sigma}}(t)}{dt} = - \int_0^t \overline{[\tilde{H}_1(t), [\tilde{H}_1(t'), \tilde{\sigma}(t')]]} dt' \quad (2.6)$$

($\tilde{H}_1(t)$ from random inner forces $\Rightarrow \overline{\tilde{H}_1(t)} = 0$). This equation is exact. It shows nicely the second order nature of relaxation theory, i.e. time derivatives are proportional to the square of the disturbing Hamiltonian.

Remark: Taking the ensemble average “seems strange, since the very concept of density matrix is intimately linked to the ensemble average. That a further averaging is necessary stems from the fact that the operator $\tilde{H}_1(t)$ is a random function of time; in different parts of the system otherwise identical, simulated by different members of a Gibbs ensemble, it may have a different history, resulting in a different density matrix $\tilde{\sigma}$.” (M. Goldman, 1993).

2.6 Introduce return to equilibrium ad hoc

$$\tilde{\sigma}(t) \rightarrow \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \quad (2.7)$$

This forces $\tilde{\sigma}(t)$ to end up at $\tilde{\sigma}_{eq}$. This hat trick is necessary because the lattice is not treated quantum-mechanically.

$$\frac{d\overline{\tilde{\sigma}}(t)}{dt} = - \int_0^t \overline{[\tilde{H}_1(t), [\tilde{H}_1(t'), \tilde{\sigma}(t') - \tilde{\sigma}_{eq}]]} dt' \quad (2.8)$$

2.7 Expand H_1 in spin and time dependent part

$$H_1(t) = \sum_{\alpha} V_{\alpha} F_{\alpha}(t) = \sum_{\alpha} V_{\alpha}^+ F_{\alpha}^*(t) \quad (2.9)$$

V_{α} spin part

$F_{\alpha}(t)$ random function of time

$$\tilde{V}_{\alpha}(t) = \exp(iH_0 t) V_{\alpha} \exp(-iH_0 t) = \exp(i\omega_{\alpha} t) V_{\alpha} \quad (2.10)$$

such a decomposition is always possible $\Rightarrow$

$$\frac{d\tilde{\sigma}(t)}{dt} = - \sum_{\alpha, \beta} \int_0^t \overline{\left[\tilde{V}_{\alpha}(t), \left[\tilde{V}_{\beta}^+(t'), \tilde{\sigma}(t') - \tilde{\sigma}_{eq} \right] \right]} F_{\alpha}(t) F_{\beta}^*(t') dt' \quad (2.11)$$

2.8 Use assumptions on random motions

1. $F_{\alpha}(t) F_{\beta}^*(t') \rightarrow 0$, when $|t - t'| \gg \tau_c$
2. $\tilde{\sigma}(t')$ varies slowly on time scale of τ_c

This has the following consequences:

1. $\tilde{\sigma}(t')$ can be replaced by $\tilde{\sigma}(t)$ in the integral
 2. all members of the ensemble experience many random events, when $|t - t'| \gg \tau_c$
- $\Rightarrow$ all $\tilde{\sigma}(t)$ are nearly the same and they are nearly equal to $\overline{\tilde{\sigma}(t)}$
- $\Rightarrow$ — can be omitted on $\tilde{\sigma}(t)$
- $\Rightarrow$

$$\frac{d\tilde{\sigma}(t)}{dt} = - \sum_{\alpha, \beta} \int_0^t \left[\tilde{V}_{\alpha}(t), \left[\tilde{V}_{\beta}^+(t'), \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] \times \overline{F_{\alpha}(t) F_{\beta}^*(t')} dt' \quad (2.12)$$

2.9 Use one more assumptions on random motions

$$\overline{F_{\alpha}(t) F_{\beta}^*(t')} = G_{\alpha\beta}(|t - t'|) \quad (2.13)$$

$$\begin{aligned} \frac{d\tilde{\sigma}(t)}{dt} &= - \sum_{\alpha,\beta} \left[V_{\alpha}, \left[V_{\beta}^+, \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] \int_0^t \exp(i\omega_{\alpha}t - i\omega_{\beta}t') G_{\alpha\beta}(t-t') dt' \\ \int_0^t \dots dt' &= \exp(i(\omega_{\alpha} - \omega_{\beta})t) \int_0^t \exp(i\omega_{\beta}(t-t')) G_{\alpha\beta}(t-t') dt' \end{aligned} \quad (2.14)$$

The integral can be extended to infinity since $G_{\alpha\beta}(t) \rightarrow 0$, for $t \gg \tau_c$

$$\begin{aligned} \int_0^t \exp(i\omega_{\beta}(t-t')) G_{\alpha\beta}(t-t') dt' &= \int_0^t \exp(i\omega_{\beta}\tau) G_{\alpha\beta}(\tau) d\tau \approx \\ &\approx \int_0^{\infty} \exp(i\omega_{\beta}\tau) G_{\alpha\beta}(\tau) d\tau = J_{\alpha\beta}(\omega_{\beta}) \end{aligned} \quad (2.15)$$

where the definition

$$J_{\alpha\beta}(\omega) \equiv \int_0^{\infty} G_{\alpha\beta}(\tau) \exp(i\omega\tau) d\tau \quad (2.16)$$

has been used.

=>

$$\boxed{\frac{d\tilde{\sigma}(t)}{dt} = - \sum_{\alpha,\beta} \left[V_{\alpha}, \left[V_{\beta}^+, \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] \exp(i(\omega_{\alpha} - \omega_{\beta})t) J_{\alpha\beta}(\omega_{\beta})} \quad (2.17)$$

This is the principal result of Redfield's theory!

2.10 Secular approximation

The secular approximation retains only terms with $\omega_\alpha = \omega_\beta$ in the Redfield equation, since other terms are assumed to oscillate rapidly on the time scale of the changes of the density matrix. This approximation leads to a system of coupled linear differential equations, which is easily solved by standard methods of linear algebra. The relaxation supermatrix $\hat{\hat{R}}$ adopts the Redfield kite structure.

$$\frac{d\tilde{\sigma}(t)}{dt} \approx - \sum_{\substack{\alpha, \beta \\ \omega_\alpha = \omega_\beta}} \left[V_\alpha, \left[V_\beta^+, \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] J_{\alpha\beta}(\omega_\beta) \equiv -\hat{\hat{R}}(\tilde{\sigma}(t) - \tilde{\sigma}_{eq}) \quad (2.18)$$

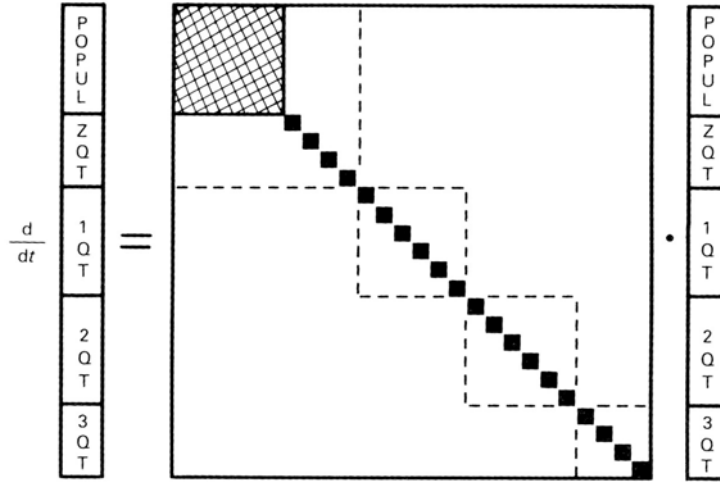


FIG. 2.3.1. Matrix representation of the relaxation superoperator. The Redfield matrix $\mathbf{R}$ has a block structure due to the neglect of non-secular contributions. The first block couples the populations and off-diagonal elements with $\Delta M = 0$ (ZQTs). Each of the following blocks couples one order ΔM of off-diagonal elements (single-quantum transitions (1QTs), double-quantum (2QTs) and higher-order transitions) among themselves. In the absence of degenerate transitions, there is no transverse cross-relaxation, and all off-diagonal elements relax independently. In this case, the Redfield matrix consists of one block coupling the populations, and a tail of diagonal elements (so-called 'Redfield kite').

From Ernst, Bodenhausen, Wokaun 1987, pg. 54

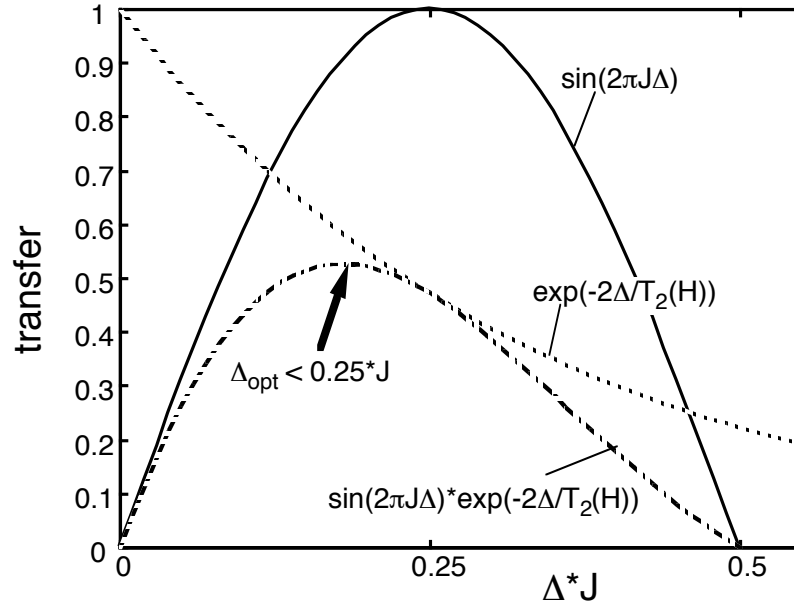
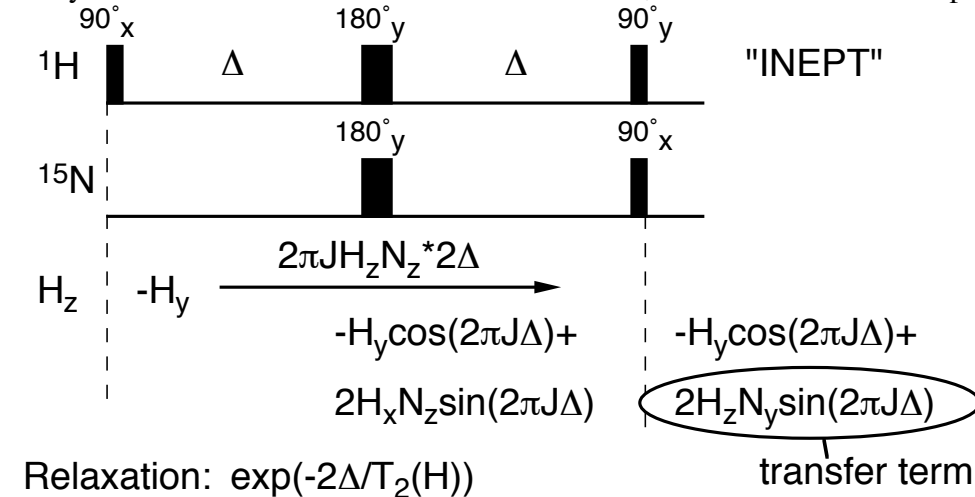
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4. Appendix

4.1 Efficiency of scalar magnetization transfer in the presence of relaxation

In the presence of relaxation the efficiency of the scalar magnetization transfer depends strongly on the ratio of the transfer speed J and the decay rate $1/T_2$. If the magnetization has decayed too fast at the end of the transfer interval, no efficient transfer is possible.

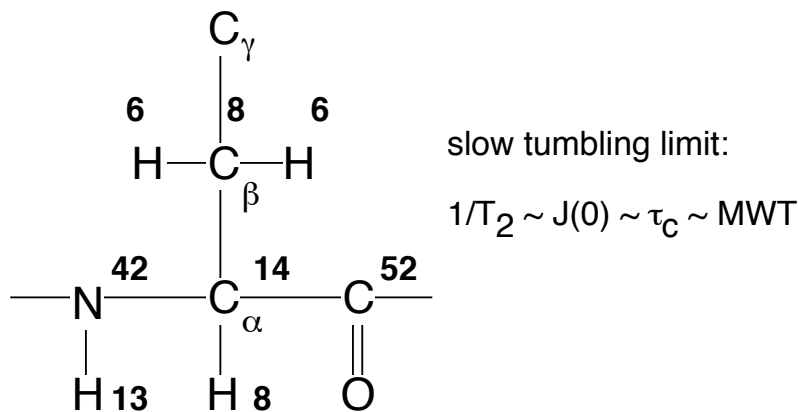


$$\Delta_{opt} = \frac{1}{2\pi J} \operatorname{atan} \pi J T_2; J T_2 \ll 1 \Rightarrow \text{no efficient transfer}$$

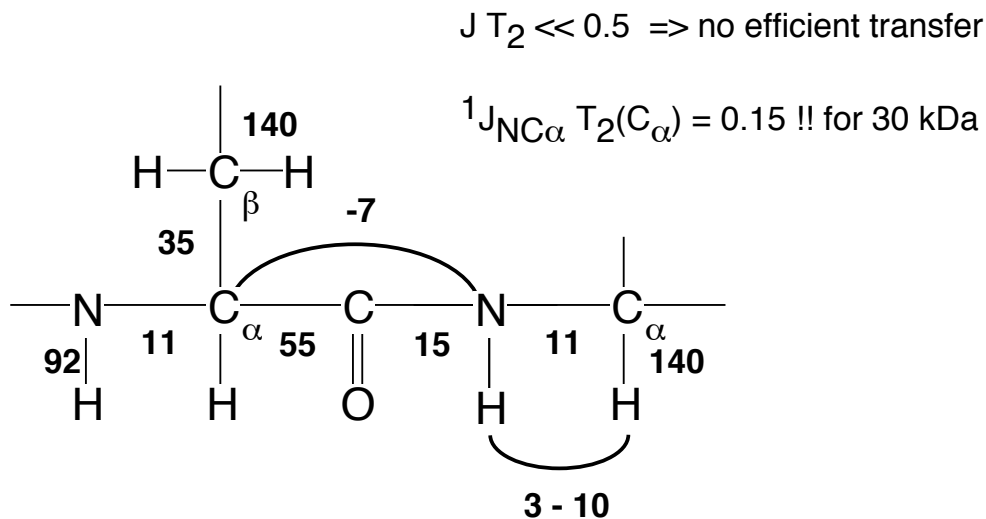
Fig.: efficiency of scalar magnetization transfer by INEPT

4.2 The size of the scalar couplings

The size of the coupling constants between protons that are connected via three bonds varies in the range from ~ 3 to 10 Hz. This means that it takes between 0.1 to 0.3 s to transfer magnetization completely via the proton-proton three-bond J-couplings. The size of the heteronuclear one-bond coupling constants is usually much larger, e.g. ~ 140 Hz for ^1H - ^{13}C and ~ 93 Hz for ^1H - ^{15}N . This means that the magnetization transfer takes ~ 7 ms for $^1\text{H} \rightarrow ^{13}\text{C}$ and ~ 11 ms for $^1\text{H} \rightarrow ^{15}\text{N}$.



Typical T_2 -values (ms) for a ($\tau_c \sim 15$ ns) protein
(MWT ~ 30 kDa)



J-values (Hz) of a protein

Fig.: T_2 -values for a 30 kDa protein and J-couplings of a protein.

4.3 Some product operator gymnastics

The following commutator rules hold for any spin I:

$$\begin{aligned} [I_x, I_y] &= I_x \cdot I_y - I_y \cdot I_x = iI_z \\ &\text{[and cyclic permutations thereof (x} \rightarrow \text{y} \rightarrow \text{z} \rightarrow \text{x)]} \end{aligned} \quad (4.3.1)$$

For a spin-1/2 particle the angular momentum operators can be represented by 2 x 2 matrices, which are called Pauli matrices:

$$I_x = \frac{1}{2} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}, I_y = \frac{1}{2} \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix}, I_z = \frac{1}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \quad (4.3.2)$$

In addition to the canonical commutator rules, the following relations hold for spin-1/2:

$$I_x \cdot I_x = I_x^2 = I_y^2 = I_z^2 = \frac{1}{4} \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \quad (4.3.3)$$

$$\begin{aligned} I_x \cdot I_y &= -I_y \cdot I_x = \frac{1}{2} iI_z \\ &\text{[and cyclic permutations thereof (x} \rightarrow \text{y} \rightarrow \text{z} \rightarrow \text{x)]} \end{aligned} \quad (4.3.4)$$

4.4 Irreducible representation of the dipolar interaction

The dipolar Hamiltonian (in angular momentum units)

$$H_d = \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi r^3} \left\{ \underline{I}_1 \cdot \underline{I}_2 - 3 \frac{(\underline{I}_1 \cdot \underline{r})(\underline{I}_2 \cdot \underline{r})}{r^2} \right\} \quad (4.4.1)$$

can be written as

$$\begin{aligned} H_d &= \chi_d \sqrt{\frac{4\pi}{5}} \sum_{m=-2,2} Y_{2m}(\vartheta, \varphi) \sum_i V_{m,i}^+ = \chi_d \sum_{m=-2,2} D_{0m}^2(\varphi, \vartheta, \psi) \sum_i V_{m,i}^+ \\ &\text{with } \chi_d = \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi r^3} \end{aligned} \quad (4.4.2)$$

and $Y_{2m}(\vartheta, \varphi)$ is the second order spherical harmonic with angular arguments that correspond to the polar angles of the internuclear distance vector $\underline{r}$, and $D_{0m}^2(\varphi, \vartheta, \psi)$ is a Wigner rotation matrix.

The spin part of the Hamiltonian and its characteristic frequencies are given by

$$\begin{aligned}
 V_{0,1} &= -2I_z S_z; \omega_{0,1} = 0 \\
 V_{0,2} &= \frac{1}{2} I_+ S_-; \omega_{0,2} = \omega_I - \omega_S \\
 V_{0,3} &= \frac{1}{2} I_- S_+; \omega_{0,3} = \omega_S - \omega_I \\
 V_{1,1} &= \frac{\sqrt{6}}{2} I_+ S_z; \omega_{1,1} = \omega_I \\
 V_{1,2} &= \frac{\sqrt{6}}{2} I_z S_+; \omega_{1,2} = \omega_S \\
 V_{2,1} &= -\frac{\sqrt{6}}{2} I_+ S_+; \omega_{2,1} = \omega_I + \omega_S \\
 \text{for } m \neq 0: V_{-m,i} &= (-1)^m V_{m,i}^*; \omega_{m,i} = -\omega_{-m,i}
 \end{aligned} \tag{4.4.3}$$

4.5 Example: heteronuclear T_2 from Redfield theory

We want to use the Redfield equation and calculate an expression for the T_2 for a heteronuclear two spin-system IS due to dipolar interaction under the assumption of spherical diffusion. We further assume the slow motion limit ($\omega\tau_c \gg 1$), such that all spectral densities besides $J(0)$ can be neglected.

An inspection of the operators for the dipolar interaction shows that only the term $V_{0,1} = -2 I_z S_z$ will give rise to a zero frequency spectral density. Therefore, the summation in the Redfield sum over the indices α and β reduces to the case where $\alpha = \beta = (0,1)$

$$\begin{aligned}
 \frac{d\tilde{\sigma}(t)}{dt} &\approx - \sum_{\substack{\alpha, \beta \\ \omega_\alpha = \omega_\beta = 0}} \left[V_\alpha [V_\beta^+, \tilde{\sigma}(t) - \tilde{\sigma}_{eq}] \right] J_{\alpha\beta}(\omega_\beta) = \\
 &= - \left[V_{(0,1)} [V_{(0,1)}^+, \tilde{\sigma}(t) - \tilde{\sigma}_{eq}] \right] J_{(0,1)(0,1)}(\omega_{(0,1)}) = \\
 &= - \left[-2I_z S_z, [-2I_z S_z, \tilde{\sigma}(t) - \tilde{\sigma}_{eq}] \right] J_{(0,1)(0,1)}(0)
 \end{aligned} \tag{4.5.1}$$

Note that the indices α and β are used to label different parts of the random Hamiltonian corresponding to distinct rotational frequencies in the rotating frame. If we look at the expansion of the dipolar Hamiltonian, we see that every pair of indices (m,i) corresponds to a distinct frequency $\omega_{m,i}$ and vice versa. Therefore we can just make the identification $\alpha = (m_\alpha, i_\alpha)$ and $\beta = (m_\beta, i_\beta)$.

We need to find an expression for $J_{(0,1)(0,1)}(0)$. From the expansion of the dipolar interaction, we see that the rotational diffusion of the molecule leads to a time-dependent variation of the spherical harmonical functions $Y_{2m}(\vartheta, \varphi)$. The random functions $F_\alpha(t)$ are therefore given by

$$F_\alpha(t) = \chi_d \sqrt{\frac{4\pi}{5}} Y_{2m_\alpha}(\vartheta(t), \varphi(t)) \tag{4.5.2}$$

For spherical diffusion, the correlation function $\overline{F_\alpha(t)F_\beta^*(t')} = G_{\alpha\beta}(|t - t'|)$ is given by

$$\begin{aligned}\overline{F_\alpha(t)F_\beta^*(t')} &= \chi_d^2 \frac{4\pi}{5} \overline{Y_{2m_\alpha}(\vartheta(t), \varphi(t)) Y_{2m_\beta}^*(\vartheta(t'), \varphi(t'))} = \\ &= \frac{\chi_d^2}{5} \delta_{m_\alpha m_\beta} \exp(-|t - t'|/\tau_c)\end{aligned}\quad (4.5.3)$$

Such that $J_{\alpha\beta}(\omega)$ is given by

$$\begin{aligned}J_{\alpha\beta}(\omega) &= \int_0^\infty \overline{F_\alpha(\tau)F_\beta^*(0)} \exp(i\omega\tau) d\tau = \frac{\chi_d^2}{5} \delta_{m_\alpha m_\beta} \int_0^\infty \exp(-\tau/\tau_c) \exp(i\omega\tau) d\tau \\ &= \frac{\chi_d^2}{5} \delta_{m_\alpha m_\beta} \frac{1}{1/\tau_c - i\omega} = \frac{\chi_d^2}{5} \delta_{m_\alpha m_\beta} \left[\frac{\tau_c}{1 + \omega^2 \tau_c^2} + i \frac{\omega \tau_c}{1 + \omega^2 \tau_c^2} \right]\end{aligned}\quad (4.5.4)$$

And we realize that

$$J_{(0,1)(0,1)}(0) = \frac{\chi_d^2}{5} \tau_c \quad (4.5.5)$$

Therefore

$$\frac{d\tilde{\sigma}(t)}{dt} \approx -4 \left[I_z S_z, \left[I_z S_z, \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] \frac{\chi_d^2}{5} \tau_c \quad (4.5.6)$$

The equilibrium magnetization $\tilde{\sigma}_{eq}$ contains only longitudinal or unity operators which commute with $I_z S_z$ and can be omitted in this equation.

$$\frac{d\tilde{\sigma}(t)}{dt} \approx -4 \left[I_z S_z, \left[I_z S_z, \tilde{\sigma}(t) \right] \right] \frac{\chi_d^2}{5} \tau_c \quad (4.5.7)$$

From the commutator algebra (Eqs. 4.3.1, 4.3.3), it is easy to see that a density matrix which proportional to I_x at time 0, will always be proportional to I_x (I and S are assumed to be spin 1/2).

$$\tilde{\sigma}(0) = a(0)I_x \Rightarrow \tilde{\sigma}(t) = a(t)I_x \quad (4.5.8)$$

Putting this “Ansatz” into Eq. 4.5.7 yields

$$\begin{aligned}I_x \frac{da(t)}{dt} &\approx -4 \left[I_z S_z, \left[I_z S_z, a(t)I_x \right] \right] \frac{\chi_d^2}{5} \tau_c = \\ &= -4 \left[I_z S_z, \left[I_z S_z, I_x \right] \right] a(t) \frac{\chi_d^2}{5} \tau_c = \\ &= -4 \left[I_z S_z, iI_y S_z \right] a(t) \frac{\chi_d^2}{5} \tau_c = -I_x a(t) \frac{\chi_d^2}{5} \tau_c\end{aligned}\quad (4.5.9)$$

or

$$\begin{aligned}\frac{da(t)}{dt} &\approx -\frac{\chi_d^2}{5} \tau_c a(t) = -\frac{1}{T_2} a(t) \\ \Rightarrow a(t) &\approx a(0) \exp(-t/T_2)\end{aligned}\tag{4.5.10}$$

$$\frac{1}{T_2} \approx \frac{\chi_d^2}{5} \tau_c = \left(\frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi r^3} \right)^2 \frac{\tau_c}{5}$$

This expression for T_2 corresponds to textbook expressions (Eq. 1.14) in the slow tumbling limit. The complete expression for spectral density terms at non-zero frequencies is derived in an analogous manner.