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 = 293K) is given by

$$\rho_{eq} = \frac{\exp(-H/kT) \text{trace} (\exp(-H/kT))}{2} = \frac{1}{2} (1 - \frac{H}{kT}) = \frac{1}{2} (1 - (-\gamma \hbar I_z B)/kT) = \frac{1}{2} (1 + 10^{-4} I_z)$$

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

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

$$\frac{1}{2} (1 + 10^{-4} I_z) \overset{180^\circ \text{I}_x}{\longrightarrow} \frac{1}{2} (1 - 10^{-4} I_z)$$

Relaxation is the phenomenon that the spins have the tendency to come back to their thermal equilibrium distribution $\rho_{eq}$. 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. 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 MHz * 10 ms = 6 000 000 times. This is the reason why despite of the small magnitude of the equilibrium polarization (10⁻⁴), NMR can still be observed with reasonable sensitivity.
The approach of thermal equilibrium is mainly induced by the following internal 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 ($\text{Energies/h} < 10^4$-$10^5$ Hz).
3. **electric quadrupolar interactions** of the nucleus with the non-constant 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 $\sim 200$ kHz for $^2$H and $\sim 3$ MHz for $^{14}$N).

### 1.2 The dipolar interaction

The dipolar interaction is the most important contribution to relaxation for biological macromolecules. A magnetic nucleus with gyromagnetic ratio $\gamma$ and spin $I$ is surrounded by a magnetic dipolar field $B_d$, which is given by the following equation:

$$B_d = -\frac{\gamma h \mu_0}{4 \pi r^3} \left( 1 - 3 \frac{\mathbf{r} \cdot (\mathbf{I} \cdot \mathbf{r})}{r^5} \right)$$

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

![Dipolar Field](image)

The dipolar energy $H_d$ of a second nucleus with gyromagnetic ratio $\gamma_2$ and spin $I_2$ in the field of the first nucleus with gyromagnetic ratio $\gamma_1$ and spin $I_1$ is then given as

$$H_d = -\mathbf{I}_2 \cdot B_d = \left( \gamma_2 \gamma_1 \right) \frac{h \mu_0}{4 \pi r^3} \left( I_1 - 3 \frac{\mathbf{r} \cdot (\mathbf{I}_1 \cdot \mathbf{r})}{r^5} \right) = \frac{\gamma_1 \gamma_2 h^2 \mu_0}{4 \pi r^3} \left( \mathbf{I}_1 \cdot \mathbf{I}_2 - 3 \frac{(\mathbf{I}_1 \cdot \mathbf{r})(\mathbf{I}_2 \cdot \mathbf{r})}{r^2} \right)$$

We notice that depending on the position of the second nucleus (distance vector $\mathbf{r}$), 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 $\mathbf{r}$ changes, whereas the length of $\mathbf{r}$ 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 T₂ measured by the Carr-Purcell-Meiboom-Gill experiment

A classical experiment to visualize T₂ is the spin-echo experiment with the Carr-Purcell-Meiboom-Gill (CPMG) sequence:

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

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° pulse is applied after time \( \tau \) (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 \( \tau \) (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 T₂ can be found from the envelope of the echoes:
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

\[ \Delta \nu = \Delta \omega / 2\pi = 1/(\pi T_2) \]
An example for this increase can be seen for the three molecules magainin (3 kDa), BPTI (7 kDa), and staphylococcal nuclease (18 kDa):

Fig.: one-dimensional proton spectra of magainin, BPTI, and staphylococcal 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 ~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 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.

![Image of two interacting spins](image)

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

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 $\omega$ which is given by $\gamma B_o$. 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 $\tau_c$, 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 $\tau_c$, the magnetic moment would have rotated by an angle

$$\phi = \omega \tau_c = \gamma B_o \tau_c$$

Because of the presence of the random dipolar field this angle $\phi$ is modified by an addition $\Delta \phi$ which is given by
\[
\Delta \phi = \pm \gamma B_d \tau_c = \pm \omega_d \tau_c
\]
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 \( t \):

\[
t = n \tau_c
\]
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.: Reif pg.5](image)

**Fig. 1.1.1** The drunkard’s random walk in one dimension.

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

\[
P_n(m) = \frac{n!}{\left(\frac{n+m}{2}\right)! \left(\frac{n-m}{2}\right)!} \left(\frac{1}{2}\right)^n
\]

This is the probability of finding the man at position \( m \times L \) 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 (\( x=0 \)).
Fig.: 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 $\gamma B_d \tau_c$ 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 $\delta$ of this random distribution of phases is given by

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

Now, when $\delta$ reaches the value $2\pi$ 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 $\delta$ has reached the value $2\pi$.

$$2\pi = \omega_d \left( \frac{T_2}{\tau_c} \right)^{\frac{1}{2}} \Rightarrow \frac{1}{T_2} = \left( \frac{\omega_d}{2\pi} \right)^2 \tau_c = \left( \frac{H_d}{\hbar} \right)^2 \tau_c$$

Thereby one can see that $T_2$ is inversely proportional to $\tau_c$ and it is directly proportional to the square of the (dipolar) interaction energy $H_d$. This is a very general principle, 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 as the molecules rotate. The characteristic time for this molecular Brownian rotation is called the molecular tumbling time or rotational correlation time $\tau_c$. For a spherical molecule of radius $a$ rotating in a liquid of viscosity $\eta$, $\tau_c$ is given by

$$\tau_c = \frac{4\pi a^3 \eta}{3kT} = \frac{V\eta}{kT}$$

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 T2 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:

$$\gamma_1 B_d = \omega_d = \frac{\gamma_1 \gamma_2 \hbar}{4\pi r^3}$$

$$\gamma_{15N} = -2.71 \cdot 10^7 \text{ (Ts)}^{-1}; \gamma_{1H} = 2.68 \cdot 10^8 \text{ (Ts)}^{-1}$$

$$\mu_o = 4\pi 10^{-7} \text{ VsA}^{-1}\text{m}^{-1}; T = \text{Vsm}^{-2}$$

$$\hbar = 1.054 \cdot 10^{-34} \text{ Js}$$

$$r = 1.01 \cdot 10^{-10} \text{ m}; \tau_c = 8 \cdot 10^{-9} \text{ s}$$

$$\omega_d / 2\pi = -1.18 \cdot 10^4 \text{ Hz}$$

$$\frac{1}{T_2} = \left(\frac{\omega_d}{2\pi}\right)^2 \tau_c = \frac{1}{(2\pi)^2} \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_1^2 \gamma_2^2 \hbar^2}{r^6} \tau_c$$

$$\frac{1}{T_2} = 1.1 \text{ Hz}$$

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 T2 corresponds to the time when the width of the phase distribution has reached a full circle overestimates T2. An exact calculation for two (dipolarly) interacting spins I and S at a distance r shows that

$$\frac{1}{T_{2I}} = \frac{1}{20} \left(\frac{\mu_0}{4\pi}\right)^2 \gamma_1^2 \gamma_2^2 \hbar^2 \left[ 4J(0,\tau_c) + J(\omega_I - \omega_S, \tau_c) + 3J(\omega_I, \tau_c) + 6J(\omega_S, \tau_c) + 6J(\omega_I + \omega_S, \tau_c) \right]$$

and by symmetry:
The function \( J(\omega, \tau_c) \) is called spectral density. It gives the probability of finding the frequency (energy) \( \omega \) in the heat bath that is provided by the thermal motion of the molecule. These motions are characterised by certain correlation times. For spherical molecules, \( J(\omega, \tau_c) \) has the following form:

\[
J(\omega, \tau_c) = \frac{\tau_c}{1 + \omega^2 \tau_c^2}
\]

where \( \tau_c \) is the rotational correlation time of the molecule.

Remark: in the slow motion limit \( (\omega \tau_c \gg 1) \), the terms \( J(\omega, \tau_c) \) are very small compared to \( J(\omega \tau_c)=\tau_c \). In this case, \( 1/T_2 \) is always proportional to \( \tau_c \). In the slow motion limit, the crude estimate for \( 1/T_2 \) that we derived above is off from the exact value by a factor of \( 5/(2\pi)^2 \approx 0.13 \).

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 \( \omega_o \). 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 \( \omega_o \). 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 $\tau_c$.

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 $\tau_c$. For small molecules (short correlation time, $\omega \tau_c << 1$) 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, $\omega \tau_c >> 1$) the spectral density is very small at the position of $\omega$. 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-slective $T_1$) for two identical spins I (two protons) by dipolar coupling yields:

$$\frac{1}{T_1} = \frac{3}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_1^4 \hbar^2}{r^6} \left\{ J(\omega_1, \tau_c) + 4J(2\omega_1, \tau_c) \right\}$$

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° $I_x$-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° $I_x$ – (2) – $I$-$S$ spin-flip –(3):
Consider the table above and the distribution of states at equilibrium. We calculate for the ensemble average values of the observables (time point 1):

\[
\begin{align*}
\langle I_\alpha \rangle (1) & = \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I) \\
\langle I_\beta \rangle (1) & = \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\
\langle S_\alpha \rangle (1) & = \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\
\langle S_\beta \rangle (1) & = \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S)
\end{align*}
\]

where the \(\Delta P\)s are given from the Boltzmann distribution. We also notice that

\[
\frac{1}{2} (\langle I_\alpha \rangle - \langle I_\beta \rangle) = \langle I_z \rangle
\]

\[
\frac{1}{2} (\langle S_\alpha \rangle - \langle S_\beta \rangle) = \langle S_z \rangle
\]

and therefore

\[
\langle I_z \rangle (1) = \Delta P(I)
\]

\[
\langle S_z \rangle (1) = \Delta P(S)
\]

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

\[
\begin{align*}
\langle I_\alpha \rangle (2) & = \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\
\langle I_\beta \rangle (2) & = \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I) \\
\langle S_\alpha \rangle (2) & = \frac{1}{4} + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\
\langle S_\beta \rangle (2) & = \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S)
\end{align*}
\]

and therefore
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:

\[
\begin{align*}
\langle \langle I_z \rangle \rangle (3) &= 1 + \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(S) \\
\langle \langle I_z \rangle \rangle (3) &= 1 + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(S) \\
\langle \langle S_z \rangle \rangle (3) &= 1 - \frac{\Delta P(I)}{2} + \frac{\Delta P(S)}{2} + \frac{1}{4} - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} - \Delta P(I) \\
\langle \langle S_z \rangle \rangle (3) &= 1 - \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} + \frac{1}{4} + \frac{\Delta P(I)}{2} - \frac{\Delta P(S)}{2} = \frac{1}{2} + \Delta P(I)
\end{align*}
\]

and therefore

\[
\begin{align*}
\langle \langle I_z \rangle \rangle (3) &= \Delta P(S) = \langle \langle S_z \rangle \rangle (1) \\
\langle \langle S_z \rangle \rangle (3) &= -\Delta P(I) = -\langle \langle I_z \rangle \rangle (1)
\end{align*}
\]

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 $k_{\text{NOE}}$. $k_{\text{NOE}}$ is proportional to the square of the dipolar interaction times a function that depends on the rotational correlation time $\tau_c$ (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]:

$$
\frac{d}{dt} \begin{pmatrix} << I_z >> \\ << S_z >> \end{pmatrix} = \begin{pmatrix} -k_1(I) & -k_{\text{NOE}} \\ -k_{\text{NOE}} & -k_1(S) \end{pmatrix} \begin{pmatrix} << I_z >> - << I_z >>_{eq} \\ << S_z >> - << S_z >>_{eq} \end{pmatrix}
$$

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

$$
k_1(I) = \frac{1}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \left\{ 3J(\omega_I, \tau_c) + J(\omega_I - \omega_S, \tau_c) + 6J(\omega_I + \omega_S, \tau_c) \right\}
$$

$$
k_1(S) = \frac{1}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \left\{ 3J(\omega_S, \tau_c) + J(\omega_S - \omega_I, \tau_c) + 6J(\omega_S + \omega_I, \tau_c) \right\}
$$

$$
k_{\text{NOE}} = \frac{1}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r^6} \left\{ 6J(\omega_I + \omega_S, \tau_c) - J(\omega_I - \omega_S, \tau_c) \right\}
$$

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 ...
inversion of spin I:
development from thermal equilibrium
\( t = 0 \)

some spin flips with next neighbors \( t = \Delta t \)

some spin flips with next neighbors \( t = 2\Delta t \)

some spin flips with next neighbors \( t = 3\Delta t \)

equal distribution at \( t = \infty \)

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 \( k_{\text{NOE}} \), the increase in z-magnetization on spin S due to NOE transfer from spin I can be approximated from the Solomon equation (\( dt \approx \Delta t = t_m \)): 
The amount of magnetization, which has started on $I_z$ and was transferred to $S_z$ during the mixing time, is given by:

$$\Delta \langle < S_z >> \rangle_{NOE} = -k_{NOE} \langle < I_z >>_{(t=0)} - < I_z >>_{eq} \rangle t_m$$

In the description of the NOESY experiment, the amount of $I_z$-magnetization at the beginning of the mixing period ($< I_z >>_{(t=0)}$) equals $-< I_z >>_{eq} \cos(\omega t)$. Therefore

$$\Delta \langle < S_z >> \rangle_{NOE} = k_{NOE} (\cos(\omega t_1) + 1) t_m < I_z >>_{eq}$$

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

$$\beta = -k_{NOE} t_m < I_z >>_{eq}$$

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 $\omega_I = \omega_S = \omega$. $k_{NOE}$ is then given as

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

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

$$\omega \tau_c = \sqrt{5/4} \approx 1.12$$

This means that for $B = 14$T, no NOE can be observed between protons if $\tau_c = 1.12 / (2\pi 600$ MHz) = 0.3 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 $\omega \tau_c >> 1$ (slow tumbling limit), the contribution of $J(2\omega, \tau_c)$ can be neglected and $k_{NOE}$ is simply given by

$$k_{NOE} = -\frac{1}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma^4 \hbar^2}{r^6} \tau_c$$
Fig.: NOE buildup rate $k_{\text{NOE}}$ for two protons at a distance of 2 Å for Larmor frequencies of 400, 600, 800 MHz.
From the homonuclear case, we have seen that $k_{\text{NOE}}$ 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. $^1\text{H}-^{15}\text{N}$ NOEs and to a lesser extent $^1\text{H}-^{13}\text{C}$ 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:

$$\text{NOE}([I]-S) = \frac{\langle S_z \rangle_{(\text{sat})}}{\langle S_z \rangle_{\text{eq}}}$$
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 $\langle \langle I_z \rangle \rangle = 0$:

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

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

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

And therefore:

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

$k_1$ 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 $\sim 0.5$ ns.
Fig: values of the steady state heteronuclear $^1$H-$^{15}$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 ($\sigma \parallel - \sigma \perp$) 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 ($^1$H) signal and observing changes in the $^{15}$N signal. The rate at which this occurs is the heteronuclear cross relaxation rate, $R_{HN}(H_z<->N_z)$; for long proton saturation, it reaches the steady state NOE($H_z<->N_z$) value. These are essentially the three experiments Nicholson et al 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:

$$R_N(N_z) = d^2\left[J(\omega_N - \omega_H) + 3J(\omega_N) + 6J(\omega_N + \omega_H)\right] + c^2J(\omega_N)$$

$$R_N(N_x) = 0.5d^2\left[4J(0) + J(\omega_N - \omega_H) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_N + \omega_H)\right] +$$

$$\frac{c^2}{6}\left[4J(0) + 3J(\omega_N)\right] + R_{ex}$$

$$NOE(H_z \leftrightarrow N_z) = 1 + \frac{\gamma_H d^2}{\gamma_N R_N(N_z)}\left[6J(\omega_N + \omega_H) - J(\omega_N - \omega_H)\right]$$

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. $\omega_N$ and $\omega_H$ are the resonance frequencies of $^{15}$N and $^1$H. In a typical spectrometer, these are 50 and 500 MHz, respectively. The equations indicate that $^{15}$N 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 $R_{tx}$. Typically, motions on the order of ms to $\mu$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:

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

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 $\tau_m$ which is the same for all residues, a correlation time for internal motions, $\tau_e$ 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, $R_N(Nz)$, the transverse relaxation rate, $R_N(Nx)$, and the heteronuclear NOE($Hc<->Hz$), the three parameters, $\tau_m$, $\tau_e$, 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:

![Figure 1](image-url)

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

![Graph of $S^2$ and $R_{ex}$ vs residue number](image)

**Fig. 2**: Generalized order parameters ($S^2$) and $R_{ex}$, plotted as a function of residue number for the two complexes (1) DMP323 using three relaxation parameters, $T_1$, $T_2$ at 500 MHz and NOE at 600 MHz; (2) DMP323 using four relaxation parameters, $T_1$, $T_2$ at 500 MHz and NOE at 600 MHz; and (a) P9941 using three relaxation parameters, $T_1$, $T_2$ at 500 MHz and NOE at 600 MHz.

![Diagram of HIV-1 protease](image)

**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.
1.12 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), Abragam (1961), Redfield (1957).

1.12.1 Hamiltonian

\[ H = H_0 + H_1(t) \]

\( H_0 \): main Hamiltonian, discrete levels, time-independent
\( H_1 \): random, spin-lattice coupling

1.12.2 Liouville-von Neumann

\[ \frac{d\sigma}{dt} = -i[H, \sigma] \]

1.12.3 Transform operators to interaction picture

i.e. into the rotating frame

\[ \tilde{Q}(t) = \exp(iH_0t)Q \exp(-iH_0t) \]

\[ \frac{d\tilde{\sigma}}{dt} = -i[(\tilde{H} - H_0), \tilde{\sigma}] \]

\[ \Downarrow \]

\[ \frac{d\tilde{\sigma}}{dt} = -i[\tilde{H}_1(t), \tilde{\sigma}] \]

\( H_0 \) disappears!

1.12.4 Integrate once

\[ \tilde{\sigma}(t) = \tilde{\sigma}(0) - i \int_0^t [\tilde{H}_1(t'), \tilde{\sigma}(t')] dt' \]
put into original Liouville-von Neumann equation:

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

### 1.12.5 Take ensemble average (\(\overline{\cdot}\)) of all terms

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

(\(\tilde{H}_1(t)\) from random inner forces \(\Rightarrow \overline{H}_1(t) = 0\)). 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).

### 1.12.6 Introduce return to equilibrium ad hoc

\[\tilde{\sigma}(t) \rightarrow \tilde{\sigma}(t) - \tilde{\sigma}_{eq}\]

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.

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

### 1.12.7 Expand \(H_1\) in spin and time dependent part

\[H_1(t) = \sum_\alpha V_\alpha F_\alpha(t) = \sum_\alpha V_\alpha^* F_\alpha^*(t)\]

\(V_\alpha\) spin part

\(F_\alpha(t)\) random function of time
\[ \tilde{V}_\alpha(t) = \exp(iH_0 t)V_\alpha \exp(-iH_0 t) = \exp(i\omega_\alpha t)V_\alpha \]

such a decomposition is always possible =>

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

1.12.8 Use assumptions on random motions

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

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 \( |t-t'| \gg \tau_c \)

=> all \( \tilde{\sigma}(t) \) are nearly the same and they are nearly equal to \( \tilde{\sigma}(t) \)

=> can be omitted on \( \tilde{\sigma}(t) \)

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

1.12.9 Use one more assumptions on random motions

\[ F_\alpha(t)F^*_\beta(t') = G_{\alpha\beta}(|t-t'|) \]

\[ \frac{d\tilde{\sigma}(t)}{dt} = -\sum_{\alpha,\beta} \left[ V_\alpha, \left[ V^*_\beta, \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] \int_0^t \exp(i\omega_\alpha t - i\omega_\beta t') G_{\alpha\beta}(t-t') dt' \]

\[ \int_0^t \ldots dt' = \exp(i(\omega_\alpha - \omega_\beta) t) \int_0^t \exp(i\omega_\beta (t-t')) G_{\alpha\beta}(t-t') dt' \]

The integral can be extended to infinity since \( G_{\alpha\beta}(t) \to 0 \), for \( t \gg \tau_c \)
\[ \int_0^t \exp(i \omega_\beta (t - t')) G_{\alpha \beta}(t - t') dt' = \int_0^t \exp(i \omega_\beta \tau) G_{\alpha \beta}(\tau) d\tau \approx \int_0^\infty \exp(i \omega_\beta \tau) G_{\alpha \beta}(\tau) d\tau = J_{\alpha \beta}(\omega_\beta) \]

where the definition
\[ J_{\alpha \beta}(\omega) \equiv \int_0^\infty G_{\alpha \beta}(\tau) \exp(i \omega \tau) d\tau \]

has been used.

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

This is the principal result of Redfield's theory.

1.12.10 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 \( \hat{R} \) adopts the Redfield kite structure.

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

1. Relaxation is the process of return to thermal equilibrium
2. This return is mediated by magnetic field fluctuations, e.g. the dipolar fields. The fluctuations are the result of thermal motions of the molecules.
3. The relaxation effects depend on the correlation time $\tau_c$ of the fluctuating fields and are proportional to the square of the fluctuating field strength. For spherical motion, $\tau_c$ can be identified with the rotational correlation time of the molecules.
4. The transverse relaxation time $T_2$ relaxation is inversely proportional to the correlation time $\tau_c$. This means that the linewidth increases with increasing molecular weight. The longitudinal relaxation time $T_1$ increases for short and very long correlation times and goes through a minimum at $1/\omega$.
5. For biological spin 1/2 systems, the largest contribution to relaxation almost always comes from the internuclear dipolar fields.
6. The nuclear Overhauser effect is a dipolar relaxation effect. As all dipolar relaxation effects it is proportional to $1/r^6$ and is detectable between protons for distances < 0.5 nm. It also depends on $\tau_c$.
7. The correlation time dependence of $T_1$, $T_2$, and NOE can be used to study molecular motions on the picosecond to nanosecond time scale.
2. Exercises

1. The equilibrium distribution for spin I is given by

\[ \rho_{eq} = \frac{1}{2}(1 - \frac{H}{kT}) = \frac{1}{2}(1 - (\frac{\hbar I_z B}{kT}) = \frac{1}{2}(1 + \frac{10^{-4} I_z}{kT}) \]

Verify that tr \( \rho_{eq} = 1 \).

2. Approximately at which molecular weight in aqueous solution no NOE crosspeaks are observable? Hint: use the rule of thumb about rotational correlation times.

3. Calculate the magnetization pathway for the NOESY experiment for the transfer from I to S with the following “phase settings” of the pulses:

\( 90^\circ_x - \tau_1 - 90^\circ_x - t_m - 90^\circ_x - \text{Acquisition}(t_2) \)

4. The “imaginary part” (sine-modulated signal) of the \( \tau_1 \)-dimension in a two-dimensional experiment is acquired by recording a second FID with the phase of the first \( 90^\circ \)-pulse changed from \( x \) to \( y \). Calculate the magnetization pathway for the NOESY in this case:

\( 90^\circ_y - \tau_1 - 90^\circ_x - t_m - 90^\circ_x - \text{Acquisition}(t_2) \)
3. References

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.

\[
\Delta_{\text{opt}} = \frac{1}{2\pi J} \arctan \pi JT_2; \quad JT_2 \ll 1 \Rightarrow \text{no efficient transfer}
\]

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 $^1\text{H}-^{13}\text{C}$ and ~ 93 Hz for $^1\text{H}-^{15}\text{N}$. 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)

\[ J \frac{T_2}{T_2} << 0.5 \Rightarrow \text{no efficient transfer} \]

\[ ^1J_{\text{NC}} \alpha \cdot T_2(C_\alpha) = 0.15 \text{ !! for } 30 \text{ kDa} \]

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 $I$:

\[
[I_x, I_y] = I_x \cdot I_y - I_y \cdot I_x = iI_z
\]

[and cyclic permutations thereof (x->y->z->x)]

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

\[
I_x = \frac{1}{2} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}, I_y = \frac{1}{2} \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix}, I_z = \frac{1}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}
\]

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

\[
I_x \cdot I_x = I_x^2 = I_y^2 = I_z^2 = \frac{1}{4}
\]

\[
I_x \cdot I_y = -I_y \cdot I_x = \frac{1}{2} I_z
\]

[and cyclic permutations thereof (x->y->z->x)]

4.4 Irreducible representation of the dipolar interaction

The dipolar Hamiltonian (in angular momentum units)

\[
H_d = \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi r^3} \left( I_1 \cdot I_2 - 3(I_1 \cdot \vec{r})(I_2 \cdot \vec{r}) / r^2 \right)
\]

can be written as

\[
H_d = \chi_d \sqrt{\frac{4}{5}} \sum_{m=-2,2} \sum_i V_{m,i}^* = \chi_d \sum_{m=-2,2} D_{0m}^2 (\varphi, \theta, \psi) \sum_i V_{m,i}^*
\]

with \( \chi_d = \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4\pi^3} \)

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

The spin part of the Hamiltonian and its characteristic frequencies are given by
V_{0,1} = -2 J_z S_z; \omega_{0,1} = 0
V_{0,2} = \frac{1}{2} J_z S_z; \omega_{0,2} = \omega_i - \omega_s
V_{0,3} = \frac{1}{2} J_z S_z; \omega_{0,3} = \omega_s - \omega_i
V_{1,1} = \frac{\sqrt{6}}{2} J_z S_z; \omega_{1,1} = \omega_i
V_{1,2} = \frac{\sqrt{6}}{2} J_z S_z; \omega_{1,2} = \omega_s
V_{2,1} = -\frac{\sqrt{6}}{2} J_z S_z; \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}

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 ($\omega \tau_c >> 1$), 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 $V_{0,1} = -2 J_z S_z$ will give rise to a zero frequency spectral density. Therefore, the summation in the Redfield sum over the indices $\alpha$ and $\beta$ reduces to the case where $\alpha = \beta = (0,1)$

$$\frac{d}{dt} \tilde{\sigma}(t) = -\sum_{\alpha,\beta} \left[ V_{\alpha} \left[ V_{\beta}^* \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] \right] J_{\alpha\beta}(\omega \beta) =$$

$$= \left[ V_{(0,0)} \left[ V_{(0,1)}^* \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] J_{(0,1)(0,0)}(\omega_{(0,1)}) \right] =$$

$$= \left[ -2 J_z S_z; \tilde{\sigma}(t) - \tilde{\sigma}_{eq} \right] J_{(0,1)(0,0)}(0)

Note that the indices $\alpha$ and $\beta$ 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 $\alpha, \beta$ corresponds to a distinct frequency $\omega_{m,i}$ and vice versa. Therefore we can just make the identification $\alpha = (m, i_\alpha)$ and $\beta = (m, i_\beta)$.

We need to find an expression for $J_{(0,1)(0,0)}(0)$. 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 $Y_{2m}(\theta, \phi)$. The random functions $F_{\alpha}(t)$ are therefore given by

$$F_{\alpha}(t) = \chi_d \sqrt{\frac{4\pi}{5}} Y_{2m_{\alpha}}(\theta(t), \phi(t))$$
For spherical diffusion, the correlation function \( F_\alpha(t)F_\beta^*(t') = G_{\alpha\beta}(|t-t'|) \) is given by

\[
F_\alpha(t)F_\beta^*(t') = \frac{\chi_d^2}{5} Y_{2m_\mu}(\theta(t), \phi(t)) Y_{2m_\mu}^*(\theta(t'), \phi(t')) = \frac{\chi_d^2}{5} \delta_{m_\mu m_\mu} \exp(-|t-t'|/\tau_c)
\]

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

\[
J_{\alpha\beta}(\omega) = \frac{\chi_d^2}{5} \int_0^\infty \exp(-\tau / \tau_c) \exp(i\omega \tau) d\tau = \frac{\chi_d^2}{5} \delta_{m_\mu m_\mu} \left[ \frac{\tau_c}{1 + \omega^2 \tau_c^2} + i \frac{\omega \tau_c}{1 + \omega^2 \tau_c^2} \right]
\]

And we realize that

\[
J_{(0,0),(0)}(0) = \frac{\chi_d^2}{5} \tau_c
\]

Therefore

\[
\frac{d\tilde{\sigma}(t)}{dt} = -4[I_zS_z, \tilde{\sigma}(t) - \tilde{\sigma}_{eq}] \frac{\chi_d^2}{5} \tau_c
\]

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

\[
\frac{d\tilde{\sigma}(t)}{dt} = -4[I_zS_z, \tilde{\sigma}(t)] \frac{\chi_d^2}{5} \tau_c
\]

Therefore, it is easy to see that a density matrix which proportional to \( I_x \) at time 0, will always be proportional to \( I_x \).

\[
\tilde{\sigma}(0) = a(0)I_x \Rightarrow \tilde{\sigma}(t) = a(t)I_x
\]

Putting this “Ansatz” into the Redfield equation yields

\[
I_x \frac{da(t)}{dt} = -4[I_zS_z, a(t)I_x] \frac{\chi_d^2}{5} \tau_c = -4I_zS_z a(t) \frac{\chi_d^2}{5} \tau_c = -I_z a(t) \frac{\chi_d^2}{5} \tau_c
\]
or

\[
\frac{da(t)}{dt} = -\frac{k}{5} \tau_c a(t) = -\frac{1}{T_2} a(t)
\]

\[\Rightarrow a(t) = a(0) \exp(-t/T_2)\]

\[
\frac{1}{T_2} = \frac{k}{5} \tau_c = \left( \frac{\gamma_1 \gamma_2 \hbar \mu_0}{4 \pi \nu} \right)^2 \frac{\tau_c}{5}
\]

This expression for $T_2$ corresponds to textbook expressions in the slow tumbling limit. The complete expression for spectral density terms at non-zero frequencies is derived in an analogous manner.
Can my protein be studied by 2D/3D/4D NMR?

No labels available:
1D in H₂O

$T_2 \text{H}^N$ from 1-1 echo


$\Phi = x, y, -x, -y$

$\text{Rec} = x, -x, x, -x$

$T = \frac{1}{4\delta}$

(no J_{HHN} dephasing)

1D-oneone experiment - NEF 308K

$T_2 = \frac{2(\Delta A - \Delta B)}{\ln(I_A/I_B)}$

$T_2 = 13 \text{ ms}$

$I_A/I_B = 0.65$

$\Delta A = 0.1 \text{ ms}$

$\Delta I = 2.9 \text{ ms}$

$T = 85 \mu\text{s} \ @ \ 600 \text{ MHz}$
Structure determination feasible

Without deuteration:

If $T_2 \geq 12$ ms, $\tau_c \leq 15$ ns, $MWT < 30$ kDa

With deuteration:

Secondary structures $\leq 110$ kDa
Tertiary structures $\leq 60$ kDa

\[
\tau_c[nS] = \frac{1}{5T_2[s]}
\]

$T_2 = 30$ ms $\Rightarrow \tau_c = 6.6$ ns

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$\tau_c[nS] \sim \frac{1}{2} MWT[kDa]$ @ 20°C

FIG. 1. NMR spectra of 3.5 mM samples at 20°C and 4.7 T in the absence and absence of 10 mM C13APR.

The position of the THF internuclear proton resonance of C13APR (H-3D) is marked.