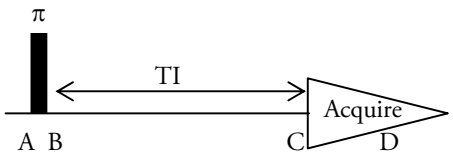
VI
RELAXATION

Lecture notes by Assaf Tal

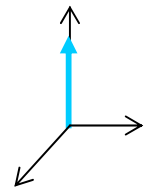
1. MEASURING T_1 AND T_2

1.1 T_1 - INVERSION RECOVERY

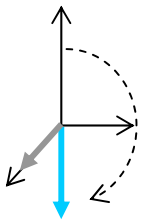
To measure T_1 of water, consider the following experiment:



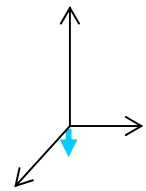
Let's go through what happens to the magnetization at each of the points outlined above.
A.) The magnetization is at thermal equilibrium,
B.) A hard π-pulse is used to flip the magnetization onto the –z axis.
C.) We wait a time TI. Longitudinal (T_1) relaxation kicks into effect.
D.) We excite the spin onto the xy-plane and measure. For the sake of simplicity, we can take the magnitude of the initial signal.



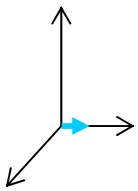
(A.) Thermal eq.



(B.) After π-pulse



(C.) After time TI



(D.) Precession (measure)

The amount of decay depends on the time TI we'd wait. We can solve the Bloch equations:

$$\begin{cases} \dfrac{dM_z}{dt} = -\dfrac{M_z - M_0}{T_1} \\ \text{initial condition: } M_z(t=0) = -M_0 \end{cases}$$

To solve, substitute:

$$Y = M_z - M_0$$
$$\frac{dY}{dt} = \frac{dM_z}{dt}$$
$$Y(0) = M_z(0) - M_0 = -2M_0$$

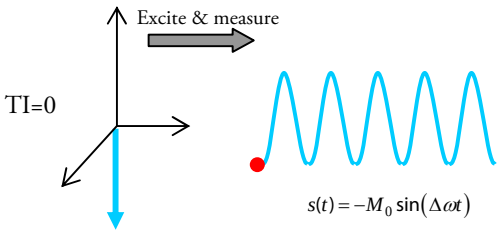
so:

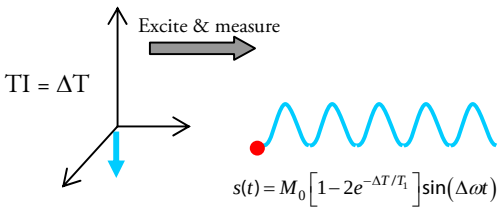
$$\frac{dY}{dt} = -\frac{Y}{T_1} \quad \Rightarrow \quad Y(t) = -2M_0 e^{-t/T_1}$$

Substituting back Y in terms of M_z, we recover the solution:

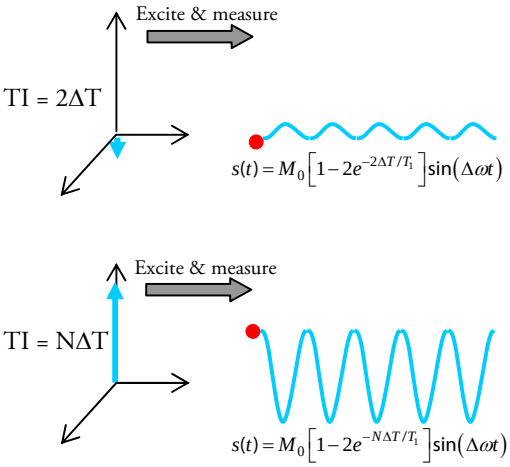
$$M_z(t) = M_0\left[1 - 2e^{-t/T_1}\right]$$

This will determine the amplitude of the signal after waiting a time TI. We can imagine a set of experiments done with different TIs. In each experiment, the maximal value of the signal is taken:

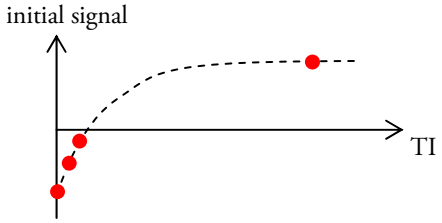




g



Next, you can imagine taking the initial amplitude of each decay and graphing it. You will then be able to directly observe the decay of M_z and deduce T_1:



By fitting this decay curve to

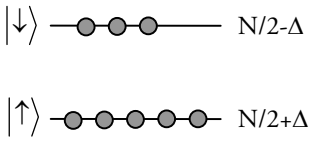
$$M_z(t) = M_0\left[1-2e^{-t/T_1}\right]$$

you can find T_1. This is called an **inversion recovery** (IR) experiment.

If our sample has multiple chemical shifts, a Fourier transform will yield a set of peaks, each recovering with its own unique T1 rate constant.

1.2 An Energy Level Look At T_1 Relaxation

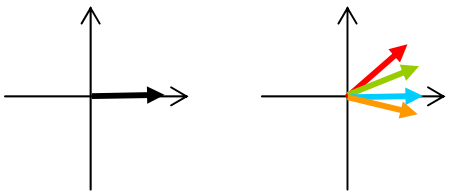
The Bloch sphere picture can be eschewed in favor of a more energy-level-diagram look at relaxation. The spin-1/2 system we'll be looking at has two possible states, "up" and "down", reflecting its alignment or anti-alignment with respect to the main B_0 field. Each level has a different energy which leads to a different Boltzmann distribution of spins. For example, if we had N spins, the "up" state would have slightly more than the "down" state:



These diagrams represent the populations, or diagonal terms of the density matrix which we've seen. Upon any disturbance of the system out of equilibrium – say, by excitation – the system will re-align itself within a time ~ T_1.

1.3 T_2 – Spin Echo Experiment

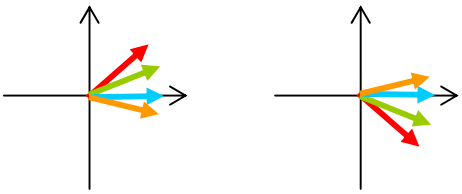
Imagine having a sample with spins having different offsets due to a combination of chemical shifts and inhomogeneity of B_0. Once you excite the spins from thermal equilibrium, they begin precessing at different rates, and eventually "spread out" in the xy-plane, due to **both B_0 inhomogeneity and a spread in chemical shifts**. This means that, if you were to acquire their signal, it would slowly die out because the spins would end up pointing in all sorts of directions and add up destructively (remember, the signal is a vector sum of the spins in the xy-plane):



Following excitation, all spins point in the same direction

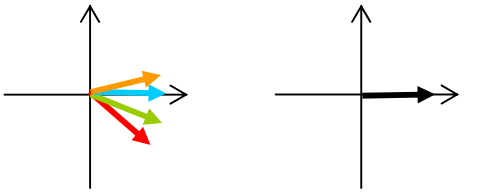
However, since each spin precesses at a different rate (= different colors), they end up dephasing

What would happen if we were to apply a 180 pulse along, say, the x-axis, after a time T? The pulse would invert our spins:



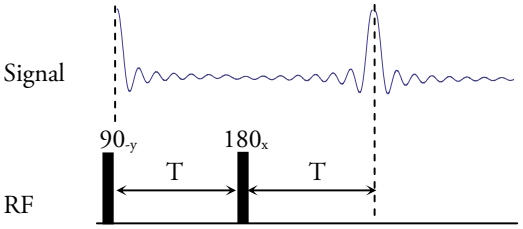
Before 180_x pulse After 180_x pulse

However, note the interesting part: if we were to wait an additional time T, the spins would end up re-aligning along the x-axis:



After 180_x pulse After additional time T

The reason for this can be understood by thinking of a particular spin: suppose a particular spin acquired some phase ϕ just prior to the 180 pulse. After the pulse, its phase would be $-\phi$. After a time T its phase would increase by ϕ once again, so its phase at the end would be $(-\phi)+\phi = 0$, i.e., it's back at the x-axis. If we'd continue acquiring throughout this experiment, we'd end up seeing the signal revive back again. This is called a **spin echo**. In terms of pulse sequences:



Now, the above drawing is a bit of a lie: in reality, the echo would be somewhat smaller than the original signal intensity. To see why, we need to divide the decay mechanisms into two:

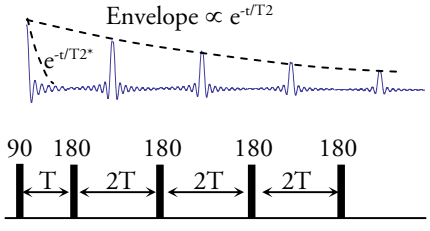
1. Decay due to microscopic T_2 effects, which cannot be reversed with a spin echo.
2. Decay due to a spatial spread of precession frequencies in the sample, as described above. This might come about because, for example, your main field is not perfectly homogeneous, $B_0 = B_0(\mathbf{r})$, leading to a precession frequency $\omega(\mathbf{r}) = \gamma B_0(\mathbf{r})$ (per chemical shift). This is sometimes called **inhomogeneous broadening**.

Each of these processes is characterized by its own decay constant. The microscopic decay is described by T_2 which we've already met. Inhomogeneous broadening leads to exponential-like decay in many cases and is denoted by T_2'. The combined rate, denoted $1/T_2$*, is under most circumstances given by the sum of rates:

$$\overbrace{\frac{1}{T_2^*}}^{\text{overall decay rate}} = \overbrace{\frac{1}{T_2}}^{\text{microscopic}} + \overbrace{\frac{1}{T_2'}}^{\text{inhomogeneous broadening}}$$

Only the inhomogeneous broadening is refocused by the 180° pulse. The microscopic fluctuations are unaffected, meaning T_2' decay will be refocused but T_2 will not, leading to:

What would happen if we were to give successive 180_x pulses, spaced 2T apart? One might initially think this pattern would repeat itself indefinitely, since the spins would dephase, get flipped (by the 180), rephase, dephase again, get flipped (by the 180), rephase, dephase, ... ad infinitum; in effect, there is relaxation that needs to be taken into account. But what relaxation? Because the 180 pulse refocuses spins with different precession frequencies, there are no B_0-inhomogeneity effects in the overall decay. Only the "true microscopic decay", T_2, plays a role here:



The decay after the excitation is determined by T_2^* (by both microscopic field fluctuations and field non-homogeneities), but the overall decay of the echoes is determined by T_2 alone. This furnishes us with a method of measuring the "true" T_2 microscopic decay of a sample.

1.4 Homonuclear Spin Echoes and J-Coupling

It is very important to realize that J-coupling evolution continues to evolve during a train of π pulses given on a homonuclear system, and is **not refocused** by them. This makes quantifying the T_2 decay of J-coupled species tricky. We will not spend any time on this topic, but you should keep in mind it's a non-trivial topic.

2. A Microscopic Look at T_1 and T_2

2.1 Relaxation is Caused By Fluctuating Fields

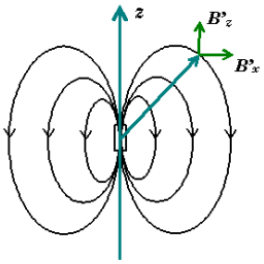
What causes relaxation? We have three mechanisms to account for:

$$\frac{dM_x}{dt} = \gamma M_y B_z - \gamma M_z B_y - \frac{M_x}{T_2}$$
$$\frac{dM_y}{dt} = \gamma M_z B_x - \gamma M_x B_z - \frac{M_y}{T_2}$$
$$\frac{dM_z}{dt} = \gamma M_x B_y - \gamma M_y B_x - \frac{M_z - M_0}{T_1}$$

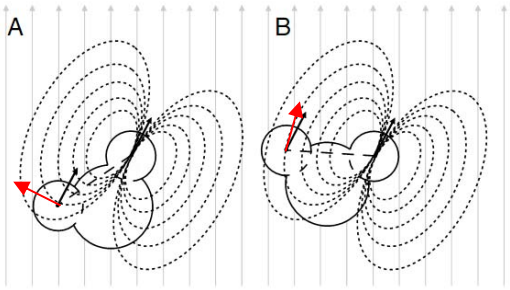
1. Red: why do M_x, M_y decay?
2. Green: why does M_z changes with a different time constant, T_1?
3. Blue: why does buildup occur?

The buildup part (#3) is difficult to explain and we'll have to leave it at that. The decay of **M**, with different transverse & longitudinal times, will be explained next.

The nuclear magnetic moments each create their own fields:

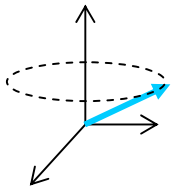


Now think about the following experiment: take two spins, fix one and move the other one about. Just by virtue of moving, the field "felt" by the spin changes:

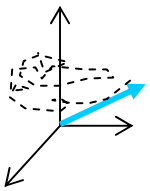


(Red: magnetic field)

Since all water molecules keep tumbling and moving around (a.k.a. brownian motion), each sees the main field + a fluctuation field created by the other spins. These fluctuating fields are what cause relaxation. You can think of the spin of a **single** molecule as "tumbling" on the surface a sphere:



Without fluctuating fields: precession



With fluctuating fields: precession + erratic "jumps" (not drawn to scale, etc.)

with the end result being the total magnetic moment decays back to equilibrium.

The fluctuating fields $\mathbf{B}_D$ felt by a spin can also be composed into transverse & longitudinal components:

$$\mathbf{B}_D(t) = \mathbf{B}_{D,\perp}(t) + \mathbf{B}_{D,\parallel}(t)$$

The longitudinal fluctuating field causes transverse relaxation and the transverse fluctuating field causes the longitudinal relaxation.

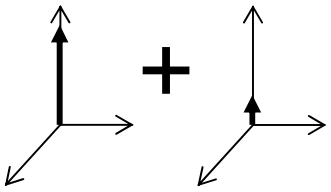
2.2 Transverse Relaxation (T_2)

Why does the transverse magnetization get "eaten up"? Let's work in the lab frame. Imagine first no fluctuating fields. A bunch of spins in an isochromat would all rotate with the same Larmor frequency, $\omega_0=\gamma B_0$.

Now imagine each spin feels a fluctuating field along the z-direction, so its precession frequency also becomes time dependent:

$$\omega(t)=\gamma(B_0+B_{D,\parallel}(t))$$

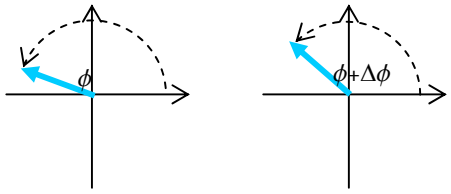
Total field =



Main B_0 field (large, ~ Tesla)

Smaller dipolar fluctuating longitudinal field (<<main)

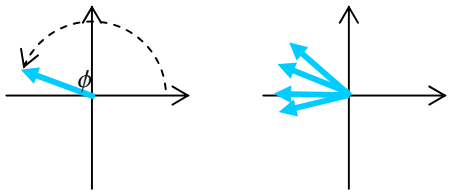
Imagine exciting a spin onto the xy plane. Without the fluctuating field, it would just execute precession and make a phase $\phi=\gamma B_0 t$ after precessing for a time t. With the fluctuating field along z the precessing frequency fluctuates as well, with the end result being a slightly different precessing frequency at the end, $\phi+\Delta\phi$, where $\Delta\phi$ depends on the exact nature of the fluctuations (imagine turning a wheel with a shaking hand):



No fluctuations ("Firm hand")

With fluctuations ("Shaky hand")
Note here $\Delta\phi<0$

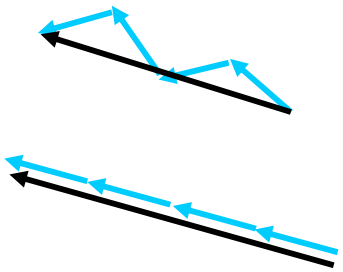
Now imagine a number of spins. In the absence of fluctuations they would all make the same angle. In the presence of fluctuations, they would fan out (remember, each spin feels a different fluctuation):



Many spins, no fluctuations. (microscopic view)

Many spins, fluctuations. (microscopic view)

This is what happens microscopically. Now, the **macroscopic** magnetization is the (vector) sum of the microscopic magnetization. What happens when you sum vectors that don't point in the same direction? They (partially) cancel out. Example:



Top: summing 4 vectors not pointing in the same direction. Bottom: all 4 vectors point in the same direction. In both cases, the "mini-vectors" (blue) all have the same size. You can now see why the magnetization in the plane decays:

The fluctuating z-field causes the spins to spread out (**dephase**), and hence add up destructively, leading to a decay of the macroscopic magnetization vector, **M**.

How fast does **M** decay – what determines T_2? Quite simply: the rate of fluctuations. Fast fluctuations will result in lesser dephasing and hence slower decay.

An analogy might help see this: think of diffusion. Molecules randomly change their direction upon colliding with each other. It should be intuitively apparent that, the lower the concentration of your sample, the larger the diffusion. Here the story is the same: you can think of the spin as "diffusing" under the action of the fluctuating field – slower fluctuations mean "fewer collisions" and hence a "less dense" environment, leading to greater "diffusion" (dephasing, in our case).

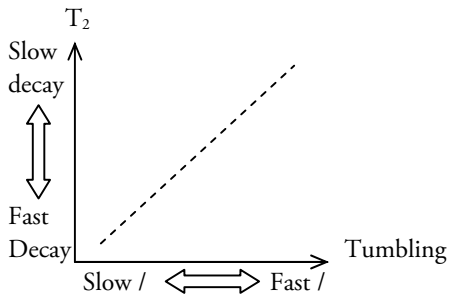
This directly relates to molecule sizes, because:

Large molecules
- → Tumble slowly
- → Slow fluctuations
- → Short T_2 (fast decay)

Small molecules
- → Tumble fast
- → Fast fluctuations
- → Long T_2 (slow decay)

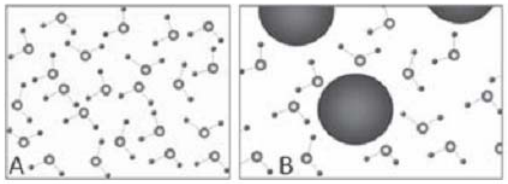
Hence, large molecules such as proteins have short T_2s, and as a result suffer from both broad linewidths (leading to a lack of spectral resolution) and smaller signal intensities (leading to lesser SNR). This is one of the reasons why the study of large proteins can be very challenging.

We can draw this graph:



Large mol. Small mol.

In tissue, water can be free (A) or in the vicinity of large macromolecules (B), which slow it down and lengthens its T_2:



Fast, long T_2 Slower, shorter T_2

In solids, where motion is greatly reduced, T_2 can be extremely short.

2.3 Longitudinal Relaxation (T_1)

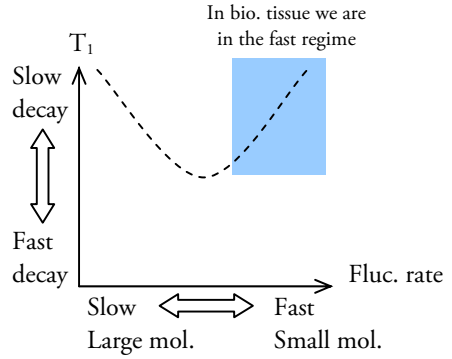
The x & y components of the fluctuating fields cause longitudinal relaxation. This can be easily understood if you can think of these fields as tiny "RF pulses" that tilt the magnetization.

Remember the idea: for an RF pulse to be successful, it needs to be **on resonance**. The rate of fluctuations determines whether the RF is on resonance or not: when the field fluctuates at the same frequency as the spin,

$$\omega_{fluctuations} = \omega_0 = \gamma B_0$$

the tiny "RF pulses" tilt the magnetization back to equilibrium much more efficiently, hence making T_1 shorter. Too fast or too slow – and you won't be on resonance anymore, diminishing the relaxation.

As before, we can draw:



In solids, for example, we saw T_2 is very short, but T_1 will be very long.

2.4 Application: Relaxation in Cancer

As an interesting application, let's apply our microscopic insight to understand why T_2 and T_1 values in tumors are larger than in regular tissue. Cancer is usually edematous: cells swell with water, making the macromolecule concentration lower, making the water molecules tumble faster, increasing T_2 and T_1.

Cancer → Swelling
→ Lower macromol. concentration
→ Faster tumbling of water molecules
→ Larger T_1, T_2 (slower decay)

3. Cross Relaxation

3.1 Phenomenology

We have so far assumed a spin relaxes because of its "environment". In reality, this environment could very well be another nuclear spin in the same molecule. Two nuclear spins which are close enough can induce **cross relaxation**, in which the random tumbling of one creates fluctuating fields at the position of the other and vice-versa.

Let's look at longitudinal cross-relaxation. Assume two spins in the same molecule. In the absence of RF fields, assuming we are on-resonance, and neglecting cross-relaxation, each spin would relax with its own T_1 time constant:

$$\frac{dM_z^{(1)}}{dt} = -\frac{M_z^{(1)} - M_0^{(1)}}{T_1^{(1)}}$$
$$\frac{dM_z^{(2)}}{dt} = -\frac{M_z^{(2)} - M_0^{(2)}}{T_1^{(2)}}$$

This can be recast in matrix form:

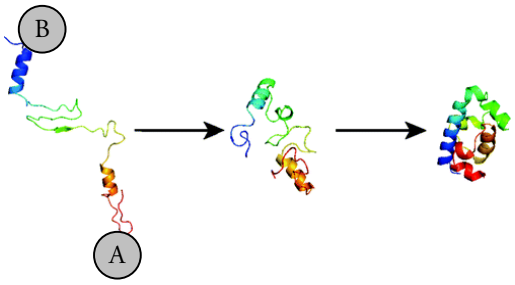
$$\frac{d}{dt}\begin{pmatrix} M_z^{(1)} \\ M_z^{(2)} \end{pmatrix} = -\begin{pmatrix} R_1^{(1)} & 0 \\ 0 & R_1^{(2)} \end{pmatrix}\begin{pmatrix} M_z^{(1)} - M_0^{(1)} \\ M_z^{(2)} - M_0^{(2)} \end{pmatrix},$$

where $R_1=1/T_1$. The presence of cross-relaxation implies the off-diagonal terms in the matrix are non-zero:

$$\frac{d}{dt}\begin{pmatrix} M_z^{(1)} \\ M_z^{(2)} \end{pmatrix} = -\begin{pmatrix} R_1^{(1)} & \sigma_1^{(21)} \\ \sigma_1^{(12)} & R_1^{(2)} \end{pmatrix}\begin{pmatrix} M_z^{(1)} - M_0^{(1)} \\ M_z^{(2)} - M_0^{(2)} \end{pmatrix}.$$

Just like with many other things in nature, cross relaxation is reciprocal, meaning the rate at which spin (1) relaxes because of spin (2) is the same as the rate at which (2) relaxes because of (1). Hence $\sigma_1^{(12)} = \sigma_1^{(21)}$. Cross relaxation can also occur between transverse magnetization states.

Cross relaxation has two very important roles in NMR: first, it is distance dependent. This makes it a highly useful tool in investigating molecular structure, and it is for this reason Kurt Wutrich was awarded the 2002 Nobel prize in chemistry. When a particular protein folds, for example, its different parts come close together and we can observe cross relaxation between peaks; while when it is unfolded, the cross-relaxation effects disappear. For example, in the folding protein diagram below, points A and B are initially far apart and no cross-relaxation will occur. In the final folded state they draw close together and cross-relaxation can be observed:



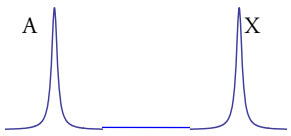
The proximity-dependent cross-relaxation effect is usually interpreted qualitatively and not quantitatively. It is observed when the distance is ~ 5 angstroms or less.

The second important use is for enhancing the polarization of a system. Unlike INEPT, this enhancement is not coherent and relies on modifying the thermal equilibrium state of the spins. We study this effect next.

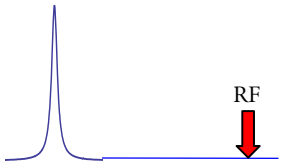
3.2 Nuclear Overhauser Effect (NOE)

The NOE effect was first suggested by Albert Overhauser in 1953, who suggested enhancing nuclear polarization by irradiating the electron spin transition in metals. His initial suggestion was met with great skepticism in the community, but was verified later that year by Carver and Slichter. Although initially suggested for electron-nuclear dipolar spin coupling, the NOE mechanism is equally valid for nuclear-nuclear dipolar spin coupling.

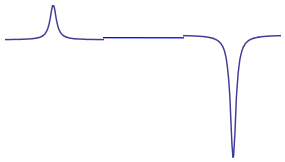
In the basic NOE experiment there are two transitions, A and X. When X is saturated by a continuous RF irradiation pulse, the magnitude of A changes. When it increases we say the NOE effect is positive; when it decreases, we say the NOE effect is negative. One often runs two experiments, one in which no irradiation is applied and one in which X is irradiated, and takes the difference spectrum:



Initially, both spins are not radiated prior to acquisition.

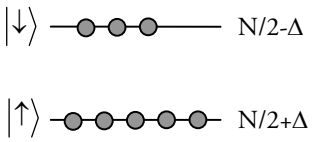


The X spin is irradiated, and the magnitude of A changes. Here it increases, meaning a positive NOE effect.

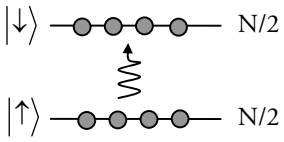


Taking the difference spectrum allows you to examine the NOE effect in greater detail.

The NOE is best thought of in terms of energy level diagrams. For a single spin-1/2, relaxation amount to transitions between the "up" and "down" states:



When we saturate a transition with an RF pulse we equilibrate the populations leading to no signal:



The NMR signal we observe (after excitation) will be proportional to the difference between the two levels. In the first case we'll observe the regular NMR line, and in the second case we won't observe a line at all. Of course, after we stop irradiating the populations of the second case will relax back to their Boltzmann distribution described by the first diagram and we'll see an NMR line again.

Two uncoupled spin-1/2s - say, a heteronuclear hydrogen pair - would be described by two such uncoupled diagrams. Here, N spins would be divided in proportion to their Boltzmann constants at the high temperature approximation, i.e. (neglecting normalization):

$$p_{\downarrow\downarrow} \propto 1-\frac{E_1}{kT}-\frac{E_2}{kT}$$
$$p_{\uparrow\downarrow} \propto 1+\frac{E_1}{kT}+\frac{E_2}{kT}$$
$$p_{\downarrow\uparrow} \propto 1-\frac{E_1}{kT}+\frac{E_2}{kT}$$
$$p_{\uparrow\uparrow} \propto 1+\frac{E_1}{kT}+\frac{E_2}{kT}$$

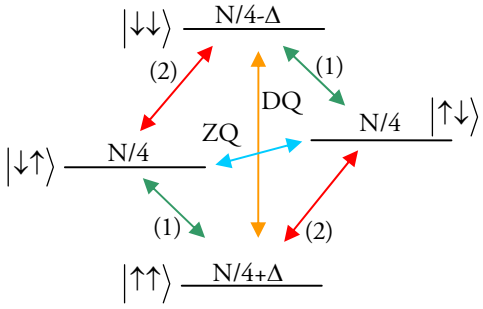
Now, since E_1 and E_2 only differ by the chemical shift (which, for homonuclear spins is extremely tiny), we can approximate $E_1 \approx E_2 \equiv E$, and obtain:

$$p_{\downarrow\downarrow} \approx 1 - \frac{2E}{kT}$$
$$p_{\uparrow\downarrow} \approx 1$$
$$p_{\downarrow\uparrow} \approx 1$$
$$p_{\uparrow\uparrow} \approx 1 + \frac{2E}{kT}$$

This means that, if we had N spins, they would have the following distributions:

$$N_{\downarrow\downarrow} \approx \frac{N}{4} - \Delta$$
$$N_{\uparrow\downarrow} \approx \frac{N}{4}$$
$$N_{\downarrow\uparrow} \approx \frac{N}{4}$$
$$N_{\uparrow\uparrow} \approx \frac{N}{4} + \Delta$$

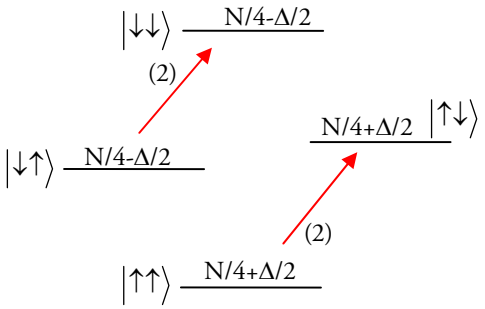
and the corresponding energy level diagram would be:



The red arrow indicates transitions of system (2), while the green arrow indicates transitions of system (1). These are called “single quantum transitions” since in each transition only one spin changes direction while the other remains unchanged. The spectrum, which will consist of two lines, represents the two possible single quantum transitions, (1) and (2).

The presence of cross-relaxation can be thought of as processes in which both spins change simultaneously. The blue line indicates a zero quantum transition, also called a "flip flop" transition, in which both spins change direction simultaneously. The total spin doesn't change, hence the name, "zero quantum transition." The orange line is a double quantum transition in which the spins flip from down/down to up/up and vice-versa. Once again, the name is self evident: the total angular momentum changes by two quanta.

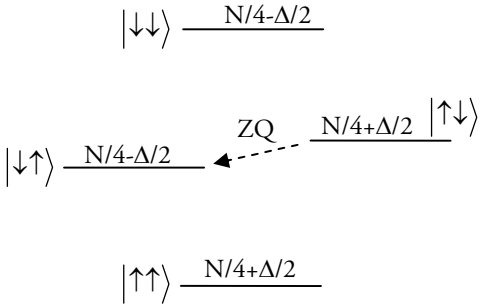
When we irradiate the transition corresponding to system (2), we equalize the corresponding populations and the corresponding line in the spectrum disappears:



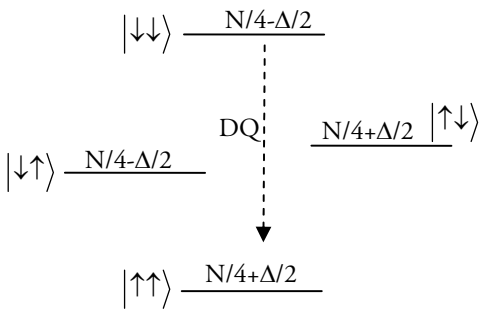
The population differences across (2) become 0, but the differences across (1) remain Δ. Note the total number of nuclei is conserved and equals N. However, as we try to take system (2) out of thermal equilibrium it tries to return to it, and it does so through all three possible mechanisms: the single quantum transition of system (2), the ZQ and the DQ transitions; the latter two also affect the population of system (1).

To be more precise, system (2) has **too many down** spins and **too few up** spins. To re-establish the Δ population difference between the $|\uparrow\downarrow\rangle$ and $|\uparrow\uparrow\rangle$ states, and the $|\downarrow\uparrow\rangle$ and $|\downarrow\downarrow\rangle$ states, the system can do one of three things:

- Relax via the single quantum transition (2).
- **Relax via the ZQ transition**. In this case, spins for system (2) would move from down to up, causing spins of system (1) to move from up to down, causing partial loss of signal for peak (1), leading to a **negative NOE**.



- **Relax via the DQ transition**. In this case, spins for system (2) would move from down to up, causing spins of system (1) to move from down to up with them. This would increase the size of peak (1), leading to a **positive NOE.**



Whether the NOE is positive or negative will thereform depend on the dominant relaxation mechanism. It turns out that in small, fast tumbling molecules, the DQ transition dominates and the NOE is positive. In large molecules, like proteins, the tumbling is slow and the ZQ transition dominates, and the NOE is negative.

How big is the NOE enhancement? This will depend at the nuclei observed and the molecular sizes. For example, for fast tumbling molecules, one can show that:

$$NOE = 1 + \frac{\gamma_1}{2\gamma_2}$$

depending on the nuclei investigated, so:

$$NOE_{H-H} \sim 1.5$$
$$NOE_{H-C} \sim 3$$
$$NOE_{H-15N} \sim -4$$

(the negative sign at the end is because the gyromagnetic ratio of 15N is negative)