Lecture 3. Relaxation Phenomena.

In this lecture, we continue with our tour of an NMR experiment. In the first lecture, we discussed the physical basis for the experiment. In the second lecture, we discussed the origins for the differences in Larmor frequency (or resonance frequency) between the various nuclei. In this lecture, we discuss relaxation, usually a favorite subject of graduate students and faculty, alike. Of course, this is not the relaxation one does at the beach, but is the process whereby the spins that were excited by an rf pulse return to their equilibrium population distributions. Understanding relaxation has many implications in modern NMR, not the least of which is how we do our experiments. For example, knowledge / measurement of relaxation phenomena is essential to determining molecular structure through the nuclear Overhauser enhancement or more recently, through the measurement of relaxation enhancement due to paramagnetic nuclei or electrons. Relaxation measurements form the basis for investigating the internal motions of proteins and nucleic acids, with dynamics on different timescales being manifested in different relaxation rates and processes. Finally, relaxation effects are exploited in all TROSY-based NMR experiments. TROSY was introduced into high resolution NMR spectroscopy as a method for line-narrowing that allows us to investigate high molecular weight biomolecules. In this lecture, we’ll discuss the physical basis for relaxation using (mostly) non-quantum mechanical arguments. At the end of this lecture, we will also discuss the effects of chemical exchange on the NMR spectrum.

3.1. Relaxation Theory

Phenomenological Relaxation.

As we saw before, relaxation can be introduced phenomenologically through the Bloch Equations. These equations propose two relaxation processes for decay of the NMR signal: transverse relaxation, occurring with a time constant of $T_2$ (and rate, $R_2$), and longitudinal relaxation, occurring with a time constant of $T_1$ (or rate, $R_1$). Transverse relaxation results in a loss of transverse magnetization without a return of the spin systems towards its equilibrium populations. Longitudinal relaxation is the process that returns the spin system to an equilibrium state with populations defined by the Boltzman equation.

Pictorially, $T_1$ and $T_2$ relaxation can be represented as follows. First, for $T_1$, consider the following thought experiment. We said that upon placing a sample in an external magnetic field, nuclear (and electron) spin angular momenta are quantized, or, alternatively, that the classical bar magnets used to describe a nuclear spin are aligned either parallel or anti-parallel to $B_0$. It is worth asking how long it takes for this process of spin alignment to reach equilibrium. To answer this question, we use the Bloch Equations (1.14- 1.16). In the absence of a pulse, the rate of change of $M_z$ is given by

$$\frac{dM_z}{dt} = \frac{-(M_z - M_0)}{T_1}.$$  \[3.1\]

Solving this differential equation by integration gives the $M_z$ value at any time, $t$, \[3.1\]
This is shown graphically in Figure 3.1. From these equations it is clear that $T_1$ is the time required to (re)establish $(e - 1)/e$ of the total magnetization along the $z$ axis. A similar picture is obtained for transverse magnetization, but $T_2$ has nothing to do with re-establishing the Boltzmann distribution. Instead, $T_2$, describes the relaxation of transverse magnetization. Immediately after excitation, the spins have a well-defined phase-relationship that is termed coherence. Coherence decays over time as the spins lose their phase relationship due to random fluctuations in the local magnetic environment. As the spins lose their phase coherence, the $x$- and $y$-components of the transverse magnetization become equilibrated, and any signal is lost. This process of phase coherence followed by decay of coherence is familiar to all of us. For example, all of your parents know where they were when Kennedy was shot; my generation remembers where they were when John Lennon was shot; each of us remember where we were when we learned about the 9/11 attacks. These sudden traumatic events in our culture are like the pulse applied to the spin system. As with the spins, there is a period following these events where all people share the same experience - we are, in effective, phase coherent. As time goes on, however, people randomly begin returning to the demands of their own lives and this shared experience is lost. In this (weak) analogy, the traumatic event is the pulse; immediately following the pulse, all spins have a defined relationship (called a phase). This phase relationship decays in time with a constant called $T_2$. Since $T_2$ represents a random process, it too is represented by an equation similar to Eqn 3.2, and $T_2$ is the time required for $(e - 1)/e$ of the transverse magnetization to decay.

In both longitudinal and transverse relaxation, random fluctuations in the local magnetic field result in random spin flips (i.e., relaxation). Longitudinal relaxation results in a flow of energy out of the spin system (as the energy that generated the excited spin system is dissipated into the lattice) and the Boltzmann distribution is re-established. On the other hand, transverse relaxation destroys the phase coherence but without a change in the overall energy of the system, i.e., without changing the net population levels or without re-establishing the Boltzmann distribution). If you are thinking that transverse relaxation sounds like a primarily entropic process, you would essentially be correct.
3.2. Experimental Determination of $T_1$ and $T_2$.

There are several methods to measure the $T_1$ and $T_2$ values of a spin system. The most common are the inversion-recovery and Carr-Purcell-Meiboom-Gill (CPMG) methods, respectively. We can get a good understanding how these experiments work using vectors and these are presented below.

**Inversion-Recovery for measuring $T_1$.** The inversion-recovery method was developed by Tom Mareci in the early ’70’s (then working with Ray Freeman, now at U. of Florida and the National High Magnetic Field Lab). The idea is the following; if we invert the magnetization by applying a 180° pulse to the spin system, then the magnetization will recover back to thermal equilibrium to re-establish the Boltzman distribution. $T_1$ represents the build-up of $M_z$ magnetization following the inversion. After a variable-length delay, $\tau$, the “size” of the $M_z$ magnetization is read by a 90° pulse. The pulse sequence and a vectorial interpretation of its affect on a spin system are shown in Figure 3.2. The magnetization at three different $\tau$-values is shown. For short values of $\tau$, $\tau << T_1$, the 180°-90° pulse sequence is essentially equivalent to a net 270° rotation about the x-axis and a very intense, inverted signal is detected (approaches $-M_0$). For intermediate $\tau$ values (B), $\tau \sim T_1$, the inverted signal has begun to relax, resulting in a smaller amount of magnetization along the negative z-axis and a less intense signal is detected. Finally, for longer values of $\tau$ (C), $\tau >> T_1$, the spin system has had time to relax and re-establish the Boltzman population distribution. Under these conditions, the spin system responds to the final 90° pulse as if it never “saw” the 180° pulse and the signal intensity approaches $M_0$.

Therefore, the inversion recovery sequence consists of a series of 1D experiments performed with increasing $\tau$ delays. Typically, about 7-10 delay values ranging from a few tenths of a second to several seconds (depending on the molecule being studied) are used and the data are

![Figure 3.2.](image-url)
analyzed according to the equation

\[ \ln \left( \frac{I(t) - I_0}{-2I_0} \right) = \frac{-t}{T_1} \]  

where \( I \) is the resonance intensity at time \( t \), and \( I_0 \) is the intensity following complete relaxation (the factor 2 in the denominator comes from boundary conditions placed on the integration of Eqn 3.1). The experimental data are fitted using linear regression; the slope is \(-1/T_1\) or \(-R_1\) (\( T_1 \) is time constant characterizing relaxation process; \( R_1 \) is the rate for that process). However, it is becoming increasingly common to fit the experimental data directly to Equation 3.3 using non-linear least squares regression.

**Measuring T\(_2\) using the CPMG Method.** \( T_2 \) measurements are made using a pulse sequence known as the Carr-Purcell-Meiboom-Gill (thankfully shortened to the CPMG sequence). In its simplest form, the CPMG is based upon a single spin echo: 90-\( \tau \)-180-\( \tau \)-acquire. At first glance, this sequence is similar to the inversion recovery sequence. However, its action on the bulk magnetization is easily distinguished using vectors (Figure 3.3). As shown in the Figure 3.3.

![Figure 3.3](image)

Figure, the effect of a 90-\( \tau \)-180-\( \tau \) sequence is to align the vectors along the -y axis. This realignment is known as a "spin echo" (for somewhat obvious reasons); and the amount of magnetization that is refocused is dependent on \( T_2 \). At short times, nearly all of the transverse magnetization is recovered (and measured) after the echo because there has been little relaxation; as time increases less magnetization is recovered in the echo (one can increase the \( \tau \) value, or, better yet, one can modify the sequence so that there are a number, \( n \), of \( \tau \)-180-\( \tau \) repeats in the sequence so that the sequence now is : 90-\( \tau \)-180-\( \{\tau \)-180-\( \tau \}\)_n). The rate of this decay is detected and plotted for analysis using either linear or non-linear forms.

### 3.3. A More Physical Description of Relaxation. The Solomon Equations.

The Bloch Equations are very useful for providing a semi-qualitative description of relaxation effects on the bulk magnetization (or individual spins). The problem with this phenomenological approach, however, is that it is not obvious that relaxation of the entire spin system should follow a mono-exponential decay, and it provides no insights into mechanisms of relaxation that would allow us to predict when it is important to take relaxation into account. In other words, it is too qualitative.

Now we will take a more physical / mechanistic look at relaxation. First, we will ask about the nature of relaxation from an excited state, i.e., is relaxation a spontaneous process for
nuclear spins? The rate of spontaneous relaxation goes as \((1/\omega)^3\). Since NMR transitions have \(\omega = 500,000,000\) (say), this corresponds to a rate of \(~10^{-27}\) spontaneous relaxation events per second, or to an excited state lifetime of \(~10^{26}\) sec. While this might not seem bad, recall that the life of the universe is about \(~10^{17}\) sec (compare to \(1.6 \times 10^8\) seconds for an average graduate career, and \(~10^5\) sec for this class), and you see that spontaneous relaxation of spin systems essentially doesn’t occur.

Therefore, we need to talk about stimulated relaxation of an excited spin system. Basically, as for any transition, including excitation, effecting a transition in stimulated relaxation requires coupling of the excited and ground states by a frequency in the surroundings (provided either by light or by motions of surrounding atoms / molecules) that matches the energy difference between the two states. Mathematically, the probability that this transition will occur is represented by a transition probability, \(W_{\beta\alpha}\), given by equation 3.3.

\[
W_{\beta\alpha} = J(\omega)\langle \alpha|\hat{\mathcal{H}}|\beta\rangle^2.
\]

In this equation, we are interested in the probability of starting in state \(\beta\) and switching to state \(\alpha\) by stimulated relaxation. \(\hat{\mathcal{H}}\) is a Hamiltonian operator that represents a physical mechanism capable of generating frequencies of the proper values, \(J(\omega)\) is the spectral density function, and the expression \(\langle \alpha|\hat{\mathcal{H}}|\beta\rangle\) is known as an overlap integral. The likelihood of stimulated relaxation is proportional to the size of the overlap integral, which is related to the amount of "overlap", or degree of similarity, between two states. When the two states have a definite (i.e., non-zero) overlap integral, this means that there is some region in physical space where the wave functions that describe the two states are similar; if the two states are identical then their overlap integral approaches unity. The transition probability is related to the square of this integral because transitions from \(\beta \rightarrow \alpha\) are as likely to occur as \(\alpha \rightarrow \beta\). The transition probability also depends on the spectral density function, which represents the “oscillator strength”, or number of oscillators at frequency \(\omega\) (if you’re not familiar with the concept of oscillator strength, read Turro’s “Modern Molecular Photochemistry”, or see below). Another way to think about \(J(\omega)\) is that it is conceptually identical to the power of light used in an absorption - the transition probability for an absorption increases as light power (or photon density) increases; similarly, the probability of a stimulated emission (or relaxation) increases as the spectral density at a particular frequency increases. Finally, for those with a spectroscopy background, this equation is Fermi’s Golden Rule as applied to NMR.

Spectral Density Functions. The spectral density plays a critical role in relaxation. There are several ways to introduce the spectral density. I’ll start with more qualitative and move towards more rigorous definitions.

Spectral Density as Oscillator Strength. One of the easiest ways to think about the spectral density is that it represents the number of oscillators giving rise to a particular frequency in the fluctuating magnetic field. In this way, the spectral density is the amount of power available from the surroundings to induce relaxation in your spin system.

The spectral density function has the general form shown in Equation 3.5, and the general
shape shown in Figure 3.4. Eqn 3.5 has the form of an absorptive Lorentzian. The important concept in this picture of the spectral density is the intensity of the spectral density function (or curve) at a given frequency. For instance, for the curve where $\omega \tau_c \sim 1$, there will be more efficient relaxation for spins when fluctuating fields with frequency log $\omega = 4$ are present than for the curve $\omega \tau_c << 1$. One way to interpret this is that spin systems with $\omega \tau_c \sim 1$ are tumbling with a certain rate (resulting in a particular value of the rotational correlation time), while spins with $\omega \tau_c << 1$ are tumbling much more rapidly.

$$J(\omega) \approx \frac{\tau_c}{1 + \omega^2 \tau_c^2} \quad [3.5]$$

On the other hand, if there are spectral densities for frequencies at log $\omega = 10$, they will be able to relax the spins with $\omega \tau_c << 1$, but won’t relax spins with $\omega \tau_c \sim 1$.

**Spectral Densities as a Correlation Function.** A more rigorous way to think about the spectral density function is that it represents the Fourier transform of the normalized autocorrelation function for the fluctuating local magnetic field.

In English, what this means is that we have to picture the local magnetic field as fluctuating about some average. Since the z-component is fixed, the fluctuating components are typically the orthogonal components, $B_x$, say. The long-term average of the x-component of the magnetic field is zero, $\langle B_x(t) \rangle = 0$, but the magnitude of this field is not, $\langle B_x^2(t) \rangle \neq 0$. The rate of these fluctuations is given by the autocorrelation function, $G(\tau)$,

$$G(\tau) = \langle B_x(t) \cdot B_x(t + \tau) \rangle \quad [3.6]$$

The autocorrelation function represents the average of the dot product of the fluctuating field at time $t$ with that at a later time, $t + \tau$. For short times, the field is likely to be similar at both times and the autocorrelation function will be non-zero (Figure 3.5). For longer values of $\tau$ the field may be fluctuating in the opposite direction at the two times, and the autocorrelation function will approach zero. From this, it follows that a rapidly fluctuating field will have a short autocorrelation time, whereas slowly fluctuating fields will have a longer autocorrelation time. Typically, the decay in the autocorrelation function is taken to be a simple exponential

$$G(\tau) = \langle B_x^2 \rangle \exp^{-|\tau|/\tau_c} \quad [3.7]$$
where $\tau_c$ is the correlation time.

The spectral density, $J(\omega)$, is the Fourier transform of the autocorrelation function,

$$J(\omega) = 2\int_0^\infty G(\tau) \exp\{i\omega\tau\}. \quad [3.8]$$

For isotropically tumbling molecules in solution the spectra density can be written as

$$J(\omega) = 2\langle B_x^2 \rangle \frac{\tau_c}{1 + \omega^2 \tau_c^2}. \quad [3.9]$$

Mostly, we work with the normalized spectral density, in which the first two terms of Eqn 3.9 are dropped, giving $J(\omega) = 2\langle B_x^2 \rangle J(\omega)$.

For molecules with short correlation times, i.e., tumbling rapidly, $J(\omega)$ is broad; molecules with a long correlation time, i.e., tumbling slowly, have narrow $J(\omega)$ (Figure 3.6).

**Solomon Equations.** Given this background, we can begin to introduce the Solomon Equations as a basis for understanding relaxation. The idea behind this model is that relaxation involves a change in the population of the the allowed energy levels. Therefore, the Solomon Equations are formulated as a kinetic expression. In other words, relaxation rates are formulated in terms of the transition probabilities and the populations of the various spin levels.

Figure 3.5. Random fluctuations in the magnetic field. (A). Local field fluctuations in $B_x$ sensed by two nuclei in different physical locations of the field. Random fluctuations in local field around a single point in space are compared for short time differences (B) and longer times (C). These figures are taken from Levitt's *Spin Dynamics* and obtained from his web site.

Figure 3.6. Comparing spectral densities for molecules with long and short rotational correlation times. Figures taken from Levitt's *Spin Dynamics* and obtained from this web site.
For a single, isolated spin, the equation for the rate change of the $|\alpha>$ and $|\beta>$ state populations is given as

$$\frac{dn_\alpha}{dt} = -W_1 n_\alpha + W_1 n_\beta \quad \text{and} \quad \frac{dn_\beta}{dt} = W_1 n_\alpha - W_1 n_\beta$$  \hspace{1cm} [3.10]

Note that the populations should really be represented as the difference from equilibrium, e.g., $(n_\alpha - n_\alpha^0)$ and $(n_\beta - n_\beta^0)$, but the additional terms complicate the equations without really adding substantially to the conceptual discussion. Since the z-component of the magnetization is proportional to the difference in the $|\alpha>$ and $|\beta>$ states, then it follows that

$$\frac{dM_z}{dt} = \frac{2\gamma h B_0}{k_B T} \left( \frac{dn_\alpha}{dt} - \frac{dn_\beta}{dt} \right) = -2W(M_z - 1).$$  \hspace{1cm} [3.11]

Eqn 3.11 can be integrated to give

$$M_z(\tau) = (M_z(0) - 1) \exp\{-2W\tau\},$$

which is the same as Eqn 3.2, if $T_1^{-1} = 2W$. This means that the spin-lattice relaxation rate is equal to twice the transition probability. More explicitly, we can write

$$T_1^{-1} = \gamma^2 \langle B^2 \rangle \frac{\tau_c}{1 + \omega^2 \tau_c^2}.$$  \hspace{1cm} [3.12]

**Solomon Equations for two spins.** For two non-scalar coupled spins, the solomon equations are a bit more complicated. Consider the two-spin system consisting of 4 energy levels (see Figure 3.7). For simplicity, we assume that the two spins are not scalar coupled. There are the single quantum transitions corresponding to the two different spins, I and S. The transition probabilities for these transitions are represented as $W_I$ and $W_S$, respectively. If we wanted to further distinguish between the two transitions of the I spin, for instance, we could write $W_I^1$ and $W_I^2$; at this point we will not distinguish these transitions but will do so in another discussion. In addition, there are two multiple quantum transitions that we haven’t yet considered: there is the $|\alpha\alpha> \leftrightarrow |\beta\beta>$ transition, which results from simultaneous flipping of both spins and is known as a double quantum transition with transition probability $W_2$. The second
pathway is this the zero-quantum (or flip-flop) transition $|\beta\alpha\rangle \leftrightarrow |\alpha\beta\rangle$ in which both spins flip simultaneously but in opposite directions ($W_0$). These are represented by the dashed arrows in Figure 3.7.

We can write the kinetic equations describing the rate change of population for any of the levels as

$$\frac{dn_1}{dt} = -W_S n_1 - W_I n_1 - W_2 n_1 + W_S n_2 + W_I n_3 + W_S n_4$$

$$\frac{dn_2}{dt} = -W_S n_2 - W_I n_2 - W_0 n_2 + W_S n_1 + W_I n_4 + W_0 n_3$$

$$\frac{dn_3}{dt} = -W_I n_3 - W_S n_3 - W_0 n_3 + W_I n_1 + W_S n_4 + W_0 n_2$$

$$\frac{dn_4}{dt} = -W_S n_4 - W_I n_4 - W_2 n_4 + W_S n_3 + W_I n_2 + W_2 n_1.$$  \[3.13\]

Again, remember that each of these population should really be the difference between the population at a given time and that at thermal equilibrium. To complete the derivation of the Solomon equations, we need to introduce four “Zeeman” terms that represent specific magnetizations in terms of the populations.

The first of these Zeeman terms is the “identity” state, $E$, which is the sum of all populations. The second two terms, $I_z$ and $S_z$, represent the I spin and S spin magnetization as the sum of the population differences for the two I and S transitions. The final magnetization term, $2I_zS_z$ is a two-spin Zeeman term that is different in population differences for both the I and S spins. These four terms are written as

$$E = n_1 + n_2 + n_3 + n_4,$$

$$I_z = n_1 - n_3 + n_2 - n_4,$$

$$S_z = n_1 - n_2 + n_3 - n_4,$$

$$2I_zS_z = n_1 - n_3 - n_2 + n_4.$$  \[3.14\]

These Zeeman terms are part of a the set of product operators that are used to describe NMR; we will discuss them in more detail later. For now, we are interested in how these Zeeman terms evolve in time, meaning we will take their time derivatives as

$$\frac{dI_z}{dt} = -(W_I^\alpha + W_I^\beta + W_2 + W_0)I_z - (W_2 - W_0)S_z + (W_I^\alpha - W_I^\beta)2I_zS_z.$$  \[3.15\]

$$\frac{dS_z}{dt} = -(W_2 - W_0)I_z - (W_S^\alpha + W_S^\beta + W_2 + W_0)S_z - (W_S^\alpha - W_S^\beta)2I_zS_z.$$  \[3.16\]
\[
\frac{d}{dt} 2I_z S_z = -(W_I^\alpha - W_I^\beta)I_z - (W_S^\alpha - W_S^\beta)S_z - (W_I^\alpha + W_I^\beta + W_S^\alpha + W_S^\beta)2I_z S_z .
\] [3.17]

The total population does not vary with time, therefore \(\frac{dE}{dt} = 0\). Also, note that the exact equations for the evolution of these operators has been used, which takes into account the possibility that the two transitions of the I or S spin (e.g., I spin transition when S spin is in the \(|\alpha>\) versus \(|\beta>\) state) can have unique values (and when they don’t, then they simply add together).

In cases where this is not true, i.e., isotropically tumbling small molecules at low fields, then there is no contribution to the \(I_z\) or \(S_z\) states due to the two-spin order term. Finally, to incorporate the fact that we should use the difference with equilibrium populations, Eqn 3.14 should be written as

\[
\frac{dI_z}{dt} = -(W_I^\alpha + W_I^\beta + W_2 + W_0)(I_z - I_0) - (W_2 - W_0)(S_z - S_0) + (W_I^\alpha - W_I^\beta)2I_z S_z
\]

with similar expressions for the other two terms.

The Solomon equations say some important (and interesting!) things:

- The first term in Equation 3.15 \((2W_I + W_2 + W_0)\) describes the relaxation of the I spin through transitions involving the I spin. They are called self or auto-relaxation and generally given the symbol \(\rho_1\). Identical statements can be made regarding the second term of Eqn 3.16 for the S spin. We can write an expression for the single quantum transitions in terms of spectral densities as

\[
W_1 = \frac{3}{20} b^2 J(\omega_0)
\] [3.18]

where \(b = \frac{\mu_0 \gamma^2 \hbar}{4\pi \rho^3}\) and \(J(\omega_0)\) represents the spectral density at the Larmor frequency of the I or S spin and will be abbreviated as \(J(\omega)\). The odd 3/20 factor comes from the explicit derivation of this term which involves the spherical harmonics. The \(W_2\) and \(W_0\) terms can be written in terms of spectral densities as well:

\[
W_2 = \frac{3}{5} b^2 J(2\omega) \quad \text{and} \quad W_0 = \frac{1}{10} b^2 J(0).
\] [3.19] [3.20]

\(J(2\omega)\) and \(J(0)\) represent the spectral densities at twice the Larmor frequency and at zero frequency, respectively (see Figure 3.6). For molecules with short correlation times, i.e., small molecules, \(\omega^2 \tau_c^2 \rightarrow 0\) and the spectral densities essentially scale as the numerical coefficients, giving \(W_2 > W_1 > W_0\). On the other hand, for molecules with long rotational correlation times, i.e., large biomolecules or small molecules in viscous solutions, the order of the transition probabilities is reversed, \(W_0 > W_1 > W_2\).
and relaxation is dominated by the zero frequency spectral density, $J(0)$. Combining these expressions for the auto-relaxation gives

$$\rho_I = \frac{1}{10} b^2 \{ J(0) + 3J(\omega_1) + 6J(2\omega) \}. \quad [3.21]$$

- $\rho_I$ is dependent on the population difference of the I spin and the S spin population differences. In some cases, it may also depend on the two-spin term (for large molecules at high fields). Similar statements can be made for the S spin.
- The rate at which S spin magnetization is transferred to (or affects) the I spin is proportional to the $(W_2-W_0)$ term. This process is referred to as cross-relaxation, and is given the symbol $\sigma_{IS}$. The cross relaxation term can be written in terms of the spectral densities as

$$\sigma_{IS} = \frac{1}{10} b^2 \{ J(0) - 6J(2\omega) \}. \quad [3.22]$$

In the absence of $W_2$ (the $|\alpha\alpha\rangle \leftrightarrow |\beta\beta\rangle$ transitions) and $W_0$ (the $|\alpha\beta\rangle \leftrightarrow |\beta\alpha\rangle$ transitions), there is no cross-relaxation of the I and S spins.
- Relaxation involving the two-spin order term generates transfer of the $2I_zS_z$ magnetization into the I (or S) spin. This is non-zero only when the two I (or S) spin transitions experience different relaxation rates. This type of transfer is called cross correlation between different relaxation mechanisms (see below).
- The relaxation rates clearly depend on the rotational correlation time. Figure 3.9 shows plots for the auto- and cross-relaxation processes as a function of $\tau_C$. Since $R_1$ is the sum of these two processes (ignoring any cross-correlation terms), then it has the general shape shown in Figure 3.17.
- Longitudinal relaxation is proportional to the sum of the I and S spins, and occurs at rate $R_{\text{sum}} = \rho_I - \sigma_{IS}$, or

$$R_{\text{sum}} = \frac{3}{20} b^2 \{ J(\omega) + 4J(2\omega) \}. \quad [3.23]$$

The expression for $T_2$ is given by

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**Figure 3.8.** Plot of spectra density indicating the location of various spectra density terms. This figure is taken from Levitt’s *Spin Dynamics* book, and downloaded from his web site.
Clearly, relaxation of either the I or the S spin cannot be described purely as a single exponential, as done phenomenologically in the Bloch equations.

3.4. Relaxation Mechanisms

In the following section, I discuss the forms of the various Hamiltonians (discuss the various mechanisms, in English and not Math) involved in relaxation.

Dipole-Dipole Interactions. There is thermal fluctuation of all nuclei and electrons in every molecule. Associated with each NMR-active nucleus is a magnetic dipole, as we discussed in the first lecture. As this magnetic dipole fluctuates randomly from thermal motion, an oscillating magnetic field is generated. This field is capable of generating fluctuating magnetic fields ($B_s$) at the proper frequency to induce nearby nuclei to relax. Therefore, the dipole-dipole (or dipolar)

\[
R_2 = \frac{3}{20} b^2 \{ 3 J(0) + 5 J(\omega) + 2 J(2 \omega) \}. \tag{3.24}
\]

- Clearly, relaxation of either the I or the S spin cannot be described purely as a single exponential, as done phenomenologically in the Bloch equations.

Figure 3.5. The dipole-dipole interaction between two nuclei, I and S. In this figure, I and S are separated by a distance r, and the internuclear vector, $r_{IS}$, is oriented at an angle $\theta$ with respect to $B_0$. Random fluctuations in $r_{IS}$ generate a magnetic field, $B_s$, given as

\[
B_s \approx \frac{\gamma \hbar}{r_{IS}^3} (3 \cos^2 \Theta - 1)
\]

interaction occurs between two nuclei. A picture of this is shown in Figure 3.5, with the form of the Hamiltonian operator below. Since $B_s$ represents the field at spin I generated by spin S, the Hamiltonian for these two nuclei should be the product of the fields generated by I and S,
\[ \hat{H}_{DD} = C \left( \frac{\mu_0}{4\pi} \gamma_I^2 \gamma_S^2 \frac{\hbar^2 \tau_c}{r^6} \right) \]  \[3.25\]  

where \( C \) represents constants particular to homo- versus heteronuclear interactions (for instance, it is 6 for two protons), \( \gamma_I \) and \( \gamma_S \) are the gyromagnetic ratios of the two interacting nuclei, \( \mu_0/4\pi \) is the permittivity of free space (essentially how well charges interact in a vacuum), \( \hbar \) is Planck’s constant over \( 2\pi \), \( \tau_c \) is the molecular rotational correlation time, and \( r \) is the internuclear distance. 

The thing to remember about dipolar interactions is that they are extremely sensitive to distance, and to things with large \( \gamma \)s. Thus, we would predict that the primary relaxation pathway for \(^1\)Hs is dipole-dipole interactions with other protons. Similarly, a \(^{13}\)C nucleus is primarily relaxed by its attached \(^1\)Hs. Also, dipolar relaxation is mediated “through space”. In other words, the nuclei don’t need to be involved in a chemical-bond network for dipolar relaxation to be efficient.

Chemical Shift Anisotropy (CSA). In the last lecture, we presented the concept of anisotropy in chemical shielding. The magnetic field induced by this anisotropy will fluctuate with random motions within the molecule, and as the molecule tumbles. The Hamiltonian for this interaction is

\[ \hat{H}_{CSA} = \frac{2}{3} \gamma_A^2 B_0^2 (\Delta \sigma)^2 \tau_c \]  \[3.26\]  

From Equation 3.12, we see that the Chemical Shift Anisotropy (or CSA) contribution is strongly dependent upon the external field (to the square of the field!) and on the anisotropy in the shielding tensor \( \Delta \sigma = (\sigma_\parallel - \sigma_\perp) \). CSA is the primary relaxation mechanism for phosphates, and is becoming increasingly important in protein NMR as we move to higher fields. I’m not sure, but I would think that CSA is a significant contribution to relaxation in compounds with d orbitals.

From the Solomon equations we can write more explicit expressions for the relaxation time of a nitrogen or carbon nucleus using the transition probabilities or the spectral density functions. In solution, the dominant relaxation mechanisms are dipole-dipole and chemical shift anisotropy (see below). Therefore,

\[ R_1 = b^2 \left\{ 4J(\omega_H - \omega_X) + 3J(\omega_H + \omega_X) \right\} + c^2 J(\omega_X) \]  \[3.27\]  

\[ R_2 = \frac{b^2}{2} \left\{ 4J(0) + 4J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H) + 6J(\omega_H + \omega_X) \right\} + \frac{c^2}{6} \left\{ 4J(0) + 3J(\omega_X) \right\} + R_{ex} \]  \[3.28\]  

Note that \( c \) is a constant that scales the CSA contribution and is given by \( c = (\omega_H \Delta \sigma) / \sqrt{3} \); \( R_{ex} \) is a term that describes the effects of chemical exchange on the apparent transverse relaxation rate. It is included here for completeness, but can be neglected in many cases.

Scalar Interactions. Scalar coupling can cause relaxation in two ways. The first way arises
if $J_{IS}$ is time-dependent (called **Scalar Relaxation of the First Kind**). A second mechanism for scalar relaxation is when the spin state of nucleus S is fluctuating in time (i.e., due to its relaxation). This is called **Scalar Relaxation of the Second Kind**. The form for this Hamiltonian is

\[
\mathcal{H}_{SC} = \frac{8\pi J_{IS}^2 I_S (I_S + 1) \tau_{sc}}{3(1 + (\omega_I - \omega_S)^2 \tau_{sc}^2)}
\]  

[3.29]

where $J_{IS}$ is the scalar coupling constant, $(\omega_I - \omega_S)$ is the chemical shift difference between spins I and S, $I_S$ is the spin state of nucleus S, and $\tau_{sc}$ is the lifetime for exchange or for nucleus S for scalar relaxation of the first and second kinds, respectively.

As an example of scalar relaxation of the first kind, consider the case of an amide nitrogen. In this system, the $^{15}$N is scalar coupled to its attached $^1$H ($^1J_{NH} \sim 91$ Hz). However, the amide proton undergoes exchange with solvent and the scalar coupling constant “fluctuates” during this exchange process ($^1J_{NH} \rightarrow 0$ when the $^1$H is not bound to $^{15}$N).

**Unpaired Electrons (or Paramagnetic Sites).** This is really a case of dipole-dipole relaxation, but is often considered separately because there are not unpaired electrons in most biological and organic molecules, and because the $\gamma$ for an electron is so large. Therefore, we would expect this effect to be strongly distance dependent. Also, note that relaxation of one coupling partner by an unpaired electron can lead to scalar relaxation of the second kind. The effects of a paramagnetic center on relaxation of a given nucleus is given by the Solomon-Bloembergen equation (Eqn 3.29) in which $S$ is the nuclear angular momentum quantum number for the paramagnetic center, $g_e$ is the gyromagnetic ratio for the unpaired electron, $\mu_B$ is the Bohn magneton, and $\omega_e$ is the electron Larmor frequency.

\[
R_{1, e} = \frac{2}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \gamma_N g_e^2 \frac{\mu_B^2 S(S + 1)}{r^6} \left[ \frac{7\tau_c}{1 + (\omega_c t_c)^2} + \frac{3\tau_c}{1 + (\omega_N t_c)^2} \right]
\]  

[3.30]

**Cross Correlations.** We have discussed the major relaxation pathways for organic and biological molecules. The only one we haven’t really discussed is quadrupolar relaxation. In addition, we need to make the point that all of these relaxation mechanisms involve the generation of randomly fluctuating magnetic fields. Therefore, it isn’t too surprising that different relaxation mechanisms can interact (or cross-correlate) with each other. In other words, although we have isolated each of these contributions to the relaxation, in reality, there are often multiple relaxation pathways available to a single nucleus, and some of the various pathways may actually be correlated. As a rough analogy, consider the effect of two musicians playing the same note, but slightly out of tune. In addition to the distinct oscillation frequencies generated by their instruments, there is a third frequency that arises from the interactions of the two pressure waves. This loosely corresponds to how two relaxation mechanisms can interact to produce additional relaxation.

Specific examples of cross-correlated relaxation include the interaction between the dipole-dipole mediated relaxation of the two $^1$H in a $^{13}$C labeled molecule. This cross-correlation can be used to measure the angle between these two CH bond vectors in the sugars of RNA and in...
amino acids. Another manifestation of cross-correlated relaxation is seen in the interference between the dipole-dipole and chemical shift anisotropy relaxation mechanisms in an NH bond. This effect forms the basis for the TROSY effect, which is currently being used to increase the molecular weight limit of systems that can be studied by NMR. Because of its importance we will spend a few minutes discussing the origin of the line-narrowing effects.

Consider an isolated NH spin system. If we observe the $^{15}$N transitions without $^1$H decoupling we see a doublet with resonance frequencies $\omega_\alpha = \omega_N + \frac{1}{2} J_{NH}$ and $\omega_\beta = \omega_N - \frac{1}{2} J_{NH}$; the $\alpha$ and $\beta$ superscripts indicating that the I spin transition is coupled to the $|\alpha\rangle$ or $|\beta\rangle$ S-spin state, respectively. The Hamiltonian operators for the dipole-dipole and chemical shift anisotropy relaxation mechanisms, in a frame centered on the line connecting the I and S spin vectors, are

$$\hat{\mathcal{H}}_{DD} = \left(\frac{\gamma I S}{r^3}\right)\left[2I_z S_z - I_x S_x - I_y S_y\right]$$

and

$$\hat{\mathcal{H}}_{CSA} = \frac{1}{3} \gamma I \Delta \sigma [2B_z I_z - B_x I_x - B_y I_y]$$

where the spin operators I, S, and IS were introduced above (in the discussion of the Solomon Equations), B is the magnetic field, and $\Delta \sigma = \sigma_\parallel - \sigma_\perp$ is the shielding anisotropy. These two Hamiltonians contribute to longitudinal and transverse relaxation by contributing to the time-derivatives of $I_z$, $S_z$, and $2I_z S_z$ (see Goldman, Further Reading). For the TROSY effect, which stands for transverse-relaxation optimized spectroscopy, we are obviously more interested in the effects of the cross-correlated relaxation on $T_2$. The transverse relaxation operators, known as raising operators, are represented by $\hat{I}_+ = \hat{I}_x + i\hat{I}_y$. Again, recall that we are interested in the transverse relaxation of the two components of the I-spin doublet arising from scalar coupling to the S spin. The time-dependence of the transverse operators are given by

$$\frac{dI_+^\alpha}{dt} = -\frac{i}{2} I_+^\alpha - (\lambda + \eta) I_+^\alpha - \mu I_+^\beta, \text{ and}$$

$$\frac{dI_+^\beta}{dt} = -\frac{i}{2} I_+^\beta - (\lambda - \eta) I_+^\beta - \mu I_+^\alpha$$

where the $\lambda$, $\eta$, and $\mu$ terms are given by

$$\lambda = D \tau_c \left\{ 4(1 + \alpha^2) + \frac{3(1 + \alpha^2)}{1 + \omega_I^2 \tau_c^2} + \frac{1}{1 + (\omega_I - \omega_S)^2 \tau_c^2} + \frac{3}{1 + \omega_S^2 \tau_c^2} + \frac{6}{1 + (\omega_I + \omega_S)^2 \tau_c^2} \right\}$$

$$\eta = 2 \alpha D \tau_c \left\{ 4 + \frac{3}{1 + \omega_I^2 \tau_c^2} \right\}, \text{ and}$$

$$\mu = D \tau_c \left\{ 4(1 + \alpha^2) + \frac{3}{1 + \omega_I^2 \tau_c^2} \right\}.$$
and the D and $\alpha$ terms are given by

$$D = \frac{1}{20} \frac{\gamma_I \gamma_S^2 \hbar^2}{r^6} \quad \text{and} \quad \alpha = -\left(\frac{2}{3}\right) \frac{B \Delta \sigma r^3}{\gamma_S \hbar}. \quad [3.36]$$

The $m$ terms represent cross-relaxation of one component of the doublet by the other component, and, since $\mu \ll J$, this term generally does not contribute to relaxation in biomolecules. Similarly, the first term in Eqns 3.31 and 3.32 (which are proportional to $J$), do not contribute to relaxation. Thus, the transverse relaxation rates for the $I$ spin transitions are

$$R_2^\alpha = \lambda + \eta \quad \text{and} \quad R_2^\beta = \lambda - \eta. \quad [3.37]$$

In words, the transverse relaxation rate for the $I$ spin coupled to the $|\alpha\rangle$ state of the $S$ spin is governed by the sum of the DD ($\lambda$) and CSA ($\eta$) terms whereas the $I$ spin coupled to the $S$ spin $|\beta\rangle$ state is governed by the difference (this is because the formal treatment includes the cross correlations via perturbation theory; the additional energy / interaction, etc., generally manifests itself as a sum and difference to the effect that is being perturbed). Therefore, $R_2^\alpha > R_2^\beta$ and the two lines have different linewidths (Figure 3.10). This effect is proportional to the magnetic field; as the field increases, the size of the CSA term increases leading to additional broadening of the $\omega_I^\alpha$ transition and narrowing of the $\omega_I^\beta$ transition. For carbons in aromatic rings, this interference effect is maximal at around $\sim 185$ MHz (carbon Larmor frequency, or 17.6T). For aliphatic carbons, which have substantially smaller CSA, the field would be significantly higher). For amide nitrogens the field optimum appears to be around 1.1 GHz, or $\sim 25$ T, but no one has been able to confirm this experimentally yet since the highest fields are only around 900 MHz at this time.

Although not explicitly stated in the discussion above, the derivation of the interference effects assumes that the principal axis of the shielding tensor ($\sigma_{||}$) is parallel to the line connecting the two spins involved in the dipolar relaxation. This effect can be generalized to include any angle by multiplying the transverse relaxation rate by $(3\cos^2\theta - 1)/2$; when the shielding tensor and the dipolar vector are colinear, as above, then $\theta = 0$ and the angular term is unity and the

**Figure 3.10.** $^{15}$N 1D NMR spectrum showing the two lines of the doublet arising from scalar coupling to its attached proton. For the $|\alpha\rangle$ transition, the DD and CSA terms constructively interfere and increase the transverse relaxation rate, leading to the broadened transition. For the $|\beta\rangle$ state, the two mechanisms destructively interfere and the resonance is narrowed. This figure is taken from Levitt's *Spin Dynamics* book, and downloaded from his web site.
interference effect is maximal; for other angles, the angular term reduces the extent of interference. This is the case for CH groups in sugars and other aliphatics, for instance.

The interference effects are evident in 2D experiments. Figure 3.11 shows $^1$H-$^{15}$N correlations from a 2D experiment is shown in the top panel. This experiment is performed with spin decoupling in both the $^1$H and $^{15}$N dimension, and this has the effect of averaging the cross correlation effects in the single line. In the middle panel (b), the $^1$H-$^{15}$N correlation peak is collected without spin decoupling, resulting in 4 resonances with different linewidths; the resonance in the upper left has constructive interference in both $^1$H and $^{15}$N dimensions and is quite broad. The resonances in the upper right and lower left have constructive interference in one dimension and destructive in the other, while the resonance in the lower right has destructive interference in both dimensions, resulting in substantial line narrowing. This line narrowing effect is exploited in the TROSY experiment, where a specific sequence of pulses with desired amplitudes and phases selects just the $\omega^\beta_I$ transition while filtering out the $\omega^\alpha_I$ transition. We will discuss this experiment further in a future lecture.

Chemical Exchange.

As a spectroscopic technique, NMR is fundamentally time-dependent and is therefore sensitive to molecular motions. We have been discussing molecular motions on the nano- and picosecond timeframe, as they are directly related to relaxation of the nuclear spins. Most of the time these motions are of questionable importance for functional studies in biomolecules. On the other hand, molecular motions on the microsecond - millisecond ($\mu$s/ms) timescale also impact on the NMR experiment in many ways, primarily in terms of the observed resonance frequency and linewidth.

In this section, some of the fundamental concepts of exchange effects are introduced, particularly as they build on our previous discussions of resonance frequency and relaxation. It should be pointed out that studies of molecular conformational changes have been performed in many different fields and typically are used to investigate the kinetics and energetics of intramolecular reorganization. In addition, these studies form the foundation for one of the more interesting areas in modern biophysics: functional dynamics.
3.5. Chemical exchange effects on resonance frequency.

Consider the following simple case of a single spin-1/2 nucleus that is found in a molecule exhibiting two different conformations, a and b, during the time-course of an NMR experiment. As a consequence of this exchange, the nucleus finds itself in two chemically (and magnetically) different environments, giving rise to chemical shifts $\delta_a$ and $\delta_b$ in conformations a and b, respectively. We can assume that the interconversion rates are given as shown in Figure 3.12. Exchange like this can be observed in organic molecules due to chair-boat interconversions in cyclohexanes or “envelope” movements in cyclopentanes, in inorganic complexes fluctuating between different geometries or ligands, and in biological molecules. In the latter case, conformational exchange is often seen in ring-flips in phenylalanines and tyrosines or as conformation changes associated with binding or releasing a ligand. There can also be more subtle cases of chemical exchange arising from chemical reactions (H-D exchange), or in the formation of weak van der Waals complexes.

We can start with two limiting cases. First, we assume the exchange is very slow, such that there are effectively two non-interconverting populations. Under these conditions, we expect resonance frequencies for two separate molecules (some of the resonances may overlap if the magnetic environment is fairly similar in the two molecules). The intensity of the resonances from state A or B will depend on the populations of the two states, $p_A$ and $p_B$, respectively, and the relaxation behavior of the resonances from each molecule or state are as described above and indicated by $R_{1,A}$ and $R_{2,A}$ with similar notation for the B state. Without proof, the conditions for this situation to hold true are $k_{ex}^{-1} \ll \Delta \delta$ where $k_{ex} = k_1 + k_{-1}$ and $\Delta \delta = \delta_A - \delta_B$. When these conditions are valid we say that we are in slow exchange on the NMR time scale.

For the second limiting case, we assume that the exchange is very rapid such that $k_{ex}^{-1} \gg \Delta \delta$ (of course we are really only interested in the magnitude of the difference although sometimes we might be interested to know the absolute sign of the difference when the population of one state is very low). Here, we are in fast exchange on the NMR time scale. Essentially NMR observables are represented by the population-weighted average. For example, the resonance frequency is given by

$$\delta_{obs} = \frac{\omega_A p_A + \omega_B p_B}{p_A + p_B}$$  \[3.38\]

where $\omega_A(B)$ and $p_A(B)$ represent the resonance frequency and population in state A or B, respectively.

---

**Figure 3.12.** Chemical exchange typically deals with a molecule that can exist in two different chemical, and therefore, magnetic states. This could be due to intramolecular torsion angle dynamics as in the chair-boat conformers, or it might be due to protonation-deprotonation reactions, etc.
The effect of chemical exchange on linewidth is to effectively increase the rate of transverse relaxation. This can be visualized as a more rapid decrease in the magnetization vector of spin a when it flips to spin b. Therefore, one conclusion to come out of the Bloch Equations is a modification of $T_2$ to include the exchange, as shown in Equation 4.16:

$$\frac{1}{T_2} = \frac{1}{T_2^0} + \frac{1}{\tau_a}$$

[3.39]

This equation says that the transverse relaxation rate is increased above the normal relaxation rate ($1/T_2^0$) by the exchange rate for $a \rightarrow b$ (which equals the inverse of the lifetime of state a ($1/\tau_a$)). Of course, a similar expression exists for the effect of chemical exchange on the b transition. Thus, as the lifetime of the a state decreases, we see an increased linewidth, similar to what would be observed for more rapid relaxation. Note that this is not a true relaxation process.

### 3.6. General expression for chemical exchange.

A general expression for the effect of exchange on two coupled resonances has been derived by McConnell and presented by Sandstrom. I recapitulate that presentation here. From the Bloch equations and a consideration of basic chemical kinetics, the spectrum is be given by

$$\nu = \frac{(-C_0)\left\{P\left[1 + \tau\left(\frac{p_b}{T_{2a}} + \frac{p_a}{T_{2b}}\right)\right] + QR\right\}}{P^2 + R^2}$$

[3.40]

where

$$P = \tau\left[\frac{1}{T_{2a}T_{2b}} - 4\pi^2 \Delta \nu^2 + \pi^2 \delta \nu^2\right] + \frac{p_a}{T_{2a}} + \frac{p_b}{T_{2b}},$$

$$Q = \tau[2\pi \Delta \nu - \pi \delta \nu (p_a - p_b)],$$

$$R = 2\pi \Delta \nu \left[1 + \tau\left(\frac{1}{T_{2a}} + \frac{1}{T_{2b}}\right)\right] + \pi \delta \nu \tau\left(\frac{1}{T_{2b}} - \frac{1}{T_{2a}}\right) + \pi \delta \nu (p_a - p_b),$$

and

$$\delta \nu = \nu_a - \nu_b, \ \Delta \nu = 0.5(\nu_a + \nu_b) - \nu, \ and \ \tau = \frac{p_a}{k_a} = \frac{p_b}{k_b}.$$  

These intimidating equations are used to model both frequency and linewidth changes as a function of the frequency difference between the two spins (see Figure 3.13), $\delta \nu$, the population of the two states, $p_a$ or $p_b$ (which are the fraction populations and are related as $p_a + p_b = 1$), the inherent linewidths of the two states (related to the transverse relaxation rates), and the lifetime of a given state, $\tau = p_a/k_a$, for instance. Recall, of course, that the observed exchange rate $k_{ex} = k_a + k_b$ and that these rates (and the populations) are temperature dependent.
Finally, the constant in front of Eqn 3.40 is used to calculate the absolute scale of the signal and is given as

\[ C_0 = \frac{i\gamma B_0 N\mu^2}{kT} \]

where \( N \) is the number density of the nuclei (number of nuclei per unit volume) and \( \mu \) is the observable component of the bulk magnetization.

Figure 3.13 shows simulations of chemical exchange on the linewidths and resonance frequency on a single spin undergoing two-site exchange, e.g., this is one resonance that experiences two different chemical and magnetic environments. There following comments are worth understanding with the help of these figures:

- under slow exchange conditions the system gives two resonances at \( \omega_a \) and \( \omega_b \), with intrinsic linewidths and transverse relaxation rates. In the example given here, the

**Figure 3.13.** Simulations of exchange based on Eqn 3.40. Top uses \( T_{2a} = T_{2b} = 0.5 \) s; \( \sigma_0 = 40 \) Hz, and \( p_a = 0.33 \) for a series of 10 \( k_{ex} \) values; the series on the bottom uses \( p_a = 0.5 \) otherwise identical parameters. The figures to the right are slices from the \( p_a = 0.33 \) simulation at the indicated \( k_{ex} \) values.
transverse relaxation rates are equal in the two sites. Under these slow exchange conditions, we see relatively sharp lines,

- As the exchange rate begins to increase, the immediate effect is to broaden the transitions for each state. The broadening decreases the overall resonance intensity because we are not destroying the total of the resonances in either state, simply causing them to jump more quickly. The effective linewidth for a given resonance is

\[
v_a = \frac{T_{2a}}{1 + T_{2a}} \left[ 1 + \frac{4\pi^2 T_{2a}^2 \delta \nu^2}{(1 + T_{2a} k_a)^2} \right].
\]

- As the exchange rate increases, resonances can broaden beyond the limits of detection (see the \( k_{\text{ex}} = 32 \) panel on the right side of Figure 3.13), causing them to “disappear”. Under these conditions, we are in the intermediate exchange regime, where \( k_{\text{ex}} \sim 2\pi/\delta \omega \).

- As the exchange rate increases further, we enter into the fast exchange regime and the two resonances begin to coalesce into a single resonance with at the population-weighted average position. This can be seen from Eqn 3.40 as all terms involving \( \tau \) (i.e., the lifetime of the A or B state) becomes negligible. The spectrum is given by

\[
v = \frac{p_a T_{2a} + p_b T_{2b}}{T_{2a} + T_{2b}} + 4\pi^2 (p_a v_a + p_b v_b - v)^2.
\]

- The averaging can easily be seen for \( p_a = p_b = 0.5 \) (series at the bottom of Figure 3.13). For these conditions the coalesced line is found at \( v = 0 \).

- Recall that a rate constant reflects the energy of activation relative to the ambient temperature. This implies that temperature provides an extremely interesting approach to altering the observed exchange rates, and this has been done numerous times. As you might expect, at lower temperatures, one typically experiences slow exchange conditions whereas high temperatures typically correspond to fast exchange.

- By measuring the rate of exchange as a function of temperature, one can arrive at activation parameters for the particular exchange reaction of interest.