8.2 The Nuclear Overhauser Effect

An important consequence of **DD relaxation** is the Nuclear Overhauser Effect, which can be used to determine intra- (and even inter-) molecular distances. The NOE effect is the change in population of one proton (or other nucleus) when another magnetic nucleus close in space is saturated by decoupling or by a selective 180 degree pulse. To understand this effect, we have to first consider the consequences of applying a second radio-frequency during an NMR experiment (decoupling).

**Double Resonance Experiments**

There are several types of NMR experiments that depend on the introduction of a second irradiation frequency \(B_1\), i.e. irradiation of a nucleus other than the one being observed.

There are two direct consequences of irradiating an NMR signal using the decoupler: decoupling and saturation:

1. **Decoupling.** Irradiation of a signal at the resonance frequency interferes with any coupling of the nucleus to others in the molecule. The effects of decoupling are almost instantaneous - once the decoupler is turned on coupling disappears on the order of fractions of a millisecond (assuming the decoupler power is high enough), when the decoupler is turned off, the coupling reappears on a similar time scale.

   - If the \(B_1\) frequency is on resonance and the power is high enough, then coupling can be completely suppressed. At weaker powers complicated effects arise. The most common experiment of this type is homonuclear decoupling in proton NMR spectra (HOMODEC), which is a simple and effective technique for establishing coupling relationships among protons. The experiment provides similar information to the 2D COSY experiment, but is less time consuming when only a few protons need to be assigned. For complex molecules homonuclear decoupling can become ineffective due to signal overlap, and 2D H-H correlation experiments such as COSY must be used. Similarly, heteronuclear decoupling provides information about the correlation between signals of different nuclei (e.g. proton and carbon signals), much in the way that CH-COSY experiments do.

   - If the \(B_1\) frequency is not exactly on resonance, then reduced couplings are observed. This is used in the **off-resonance decoupling (SFORD) experiment** (see Section 6), in which we irradiate somewhere upfield or downfield of the proton signals, and observe the \(^{13}\text{C}\) NMR spectrum, which shows much reduced \(J_{\text{CH}}\) values.

   - In **spin tickling** experiments one of the lines in a coupled multiplet is irradiated with very weak power. Lines in multiplets of other nuclei coupling to the irradiated one show additional splitting of individual lines in the multiplet which can be used to determine the relative signs of coupling constants.

   - **Broad band proton decoupling** is routinely used when observing heteronuclei to simplify spectra by removing the effects of proton coupling. Broad-band decoupling is almost always used when observing \(^{13}\text{C}\), but can also be helpful for observing \(^{19}\text{F}\), \(^{31}\text{P}\) and other nuclei.

2. **Saturation.** When a proton is irradiated transitions between \(\alpha\) and \(\beta\) states are induced, and the populations of the two states will tend to be equalized. The rate at which this occurs is a function of the strength of the decoupling field, but will in general be faster than \(T_1\) relaxation. If the field is powerful enough (i.e., if the induced transitions greatly exceed the rate of normal \(T_1\) relaxation), the populations of the \(\alpha\) and \(\beta\) states will become identical and the signal will disappear (become *saturated*). If the decoupler is turned off, normal signal intensity will return as a function of \(T_1\). The coupling will return to normal on a much shorter time scale.
The Nuclear Overhauser Effect

The alteration of normal spin population of a nucleus X by irradiation will cause the populations (and hence signal intensities) of other (non-irradiated) nuclei (A) to change provided that X is causing T₁ relaxation of A by the dipole-dipole mechanism. This is known as the Nuclear Overhauser Effect (NOE).

**Distinction between Decoupling and the NOE experiment.** In a decoupling experiment (HOMODEC) the B₁ irradiation must be on during acquisition of the FID (but not necessarily otherwise), and in an NOE experiment the decoupler is on during a delay period, but may be turned off during the acquisition of the FID.

**Origin of the NOE Effect.** When a proton is close in space to another proton (or any other nuclei with spin > 0), their magnetic dipoles interact (Dipole-Dipole interaction, DD). This interaction is distinct from J coupling, which is not a through space effect, but is mediated by polarization of bonding electrons in the molecule. The effects of DD interactions on the appearance of NMR spectra is completely averaged by the normal tumbling of molecules in solution if the medium is isotropic and viscosity is low enough to allow sufficiently fast molecular motion (short enough correlation time, τ_c). The DD interactions between protons do, however, dominate the ¹H T₁ relaxation processes in most molecules that contain more than one proton.

To understand the NOE effect, consider a pair of protons AX, close in space, but not J coupled to each other (J coupling is unrelated to the NOE effect, but complicates the discussion). Such a system has four energy states, corresponding to the αα, αβ, βα, and ββ spin states. The DD interaction of the protons will cause T₁ relaxation between the spin states with the transition probabilities ω₁ (for the single quantum relaxation αα/αβ, αα/βα, αβ/ββ and βα/ββ), ω₂ (for the double-quantum relaxation αα/ββ) and ω₀ (for the zero-quantum relaxation αβ/βα). In the graphic below there will be an excess population of Δ in the αα state, and a deficiency of -Δ in the ββ state.
8.2 The Nuclear Overhauser Effect

When the X-transition is irradiated, the populations of the αα and αβ states become equalized (saturated), as do the βα and ββ states. As relaxation occurs, the difference in these two populations depends crucially on which of the three relaxation processes dominates. If \( \omega_2 > \omega_1, \omega_0 \) then the βα/ββ population will tend to that of the ββ state, and the αβ/αα states will tend that of the αα state, hence there will be a larger population difference for the A transition (2Δ) than the equilibrium difference (Δ). Conversely, if \( \omega_0 \) dominates, then the βα/ββ will tend to the βα population, and the αβ/αα will tend to the αβ population, i.e. the population difference will tend to 0. It is important to recognize that the \( \omega_2 \) and \( \omega_0 \) processes only occur by mutual interaction the spins of A and X by the DD mechanism, not by other relaxation processes that involve other mechanism for producing fluctuating magnetic fields.

Shown below is an analysis of the population of a sample (population difference greatly exaggerated) if \( \omega_1 \) is the only relaxation pathway operative. First we irradiate the X transition, which will induce transitions of the X nucleus until the population is equalized. Then we turn off the decoupler and watch the sample.
The $\omega_2$ process simply reestablishes the normal population difference between the $\alpha$ and $\beta$ states for the $X$ nucleus. $A$ is not affected.

Now consider the situation when either the $\omega_0$ or the $\omega_2$ processes are the only ones operative. In the $\omega_0$ process, the dipolar interaction between $A$ and $X$ causes an $A$ nucleus to undergo an $\alpha \rightarrow \beta$ transition when the $X$ nucleus relaxes from $\beta \rightarrow \alpha$ ($\alpha_\beta \rightarrow \beta_\alpha$). The net result is that as $X$ returns to its normal population difference, it lowers the population difference for $A$. Thus, as the $X$ intensity decreases, the $A$ intensity decreases. If $X$ is irradiated continuously then the signal for $A$ will vanish (100% NOE). This is a negative NOE.
For the $\omega_2$ process, each time an $X$ nucleus relaxes from $\beta$ to $\alpha$ state, and $A$ nucleus also undergoes a $\beta$ to $\alpha$ transition ($\beta\beta \rightarrow \alpha\alpha$). This has the effect of increasing the population difference of $A$, i.e. an increase the area of $A$. This is a positive NOE. The phenomenon has sometimes been referred to as spin pumping - changing the population difference of $X$ pumps $A$ spins either from $\alpha$ to $\beta$ or $\beta$ to $\alpha$.

The reason we get NOE population changes is that the three dipolar relaxation pathways contribute to differing extents depending a number of factors. A key one is that the balance between $\omega_2$, $\omega_1$ and $\omega_0$ depends crucially on molecular motion ($\tau_c$). In mobile solvents molecular motion is much faster than the Larmor precession frequency ($\nu_0$). Under these conditions the double-quantum relaxation $\omega_2$ is more effective than $\omega_1$ or $\omega_0$, because there is a better match between $\tau_c$ and $2 \nu_0$ ($\omega_2$) than between $\tau_c$ and $\nu_0$ ($\omega_1$). If $\omega_2$ is the dominant relaxation process, then we get a positive NOE.
In real life, all three transition probabilities are finite. The equation governing the size of the NOE is shown below:

\[
\text{NOE} = 1 + \frac{\omega_2 - \omega_0}{2\omega_1 + \omega_2 + \omega_0} \cdot \frac{\gamma_X}{\gamma_A} \cdot X_{DD}
\]

Thus when \(\omega_2 > \omega_0\) the NOE will be positive, when \(\omega_0 > \omega_2\) the NOE will be negative, and if \(\omega_2 = \omega_0\) then there is no NOE. There will also be no NOE if the fraction of DD relaxation is small, The maximum NOE observable is reduced to the extent that \(T_1\) relaxation pathways other than DD between X and A are operative. This includes intermolecular DD processes (for example by solvent molecules or by dissolved dioxygen) and relaxation by the CSA mechanism (common for heavy nuclei) or QR mechanism (seen for quadrupolar nuclei).

For small molecules in low-viscosity solvents molecular motion is faster than \(\nu_0\) leading to \(\omega_2 > \omega_0\). A net positive NOE is expected. In fact, for such solutions the relationship \(\omega_2 : \omega_1 : \omega_0 = 12 : 3 : 2\). Under these conditions the maximum proton-proton NOE that can be seen is 50% \((\gamma_X = \gamma_A)\). What this means is that the sum of all of the NOE enhancements on a single proton cannot exceed 50%.
8.2 The Nuclear Overhauser Effect

One consequence of the fact that all NOEs on one proton cannot add up to more than 50% is that methyl groups as "receiver" will generally show rather small NOEs, because for any one proton in the CH$_3$ group, the main relaxation partners will be the other two protons within the methyl group. Remember that there is a $1/r^6$ distance dependence of DD relaxation. However methyl groups usually give well defined sharp peaks, typically in an uncrowded part of a spectrum, so even small NOE enhancements can be easily detected with modern pulse gradient NOE experiments.

The size of the NOE is also directly proportional to the ratio of the magnetogyric ratios of the the "sending" (X) and "receiving" (A) nuclei. Thus the smaller the $\gamma$ of a receiving nucleus, the larger will be the NOE produced by a irradiating the proton signals.

**Effect of Molecular Motion and Molecular Size on NOE.** For large molecules and/or high viscosity solvents (such as water or DMSO) the zero-quantum relaxation pathway is very efficient (molecular motion is slower than the Larmor precession frequency), and $\omega_0 > \omega_2$. Under these conditions negative NOEs approaching -100% can be observed. It is sometimes worthwhile to manipulate solvent viscosity and temperature to achieve negative NOE's, since these are inherently larger than the positive NOEs seen under conditions of fast molecular motion.
The Relay NOE Effect. Since the NOE is the consequence of population changes in nucleus X, and since the effect causes the populations of nearby nucleus A to change, it is clear that there can be secondary perturbations of populations (relay NOE effect, or spin diffusion) where A affects B, B affects C, and so on. In other words, when the population of a proton is changed by an NOE, this change can itself influence the populations of other protons near it. In the fast molecular motion regime, relay effects alternate in sign down a chain, when in the slow motion regime, direct and relay effects both have negative signs.

\[ \omega_2 \text{ dominates - positive NOE} \]
\[ \text{(small molecules, non-viscous media)} \]

\[ \text{irradiate X} \]

\[ X \quad A \quad B \quad C \]

\[ \text{positive NOE} \]
\[ X \downarrow, A \uparrow \]

\[ \text{negative NOE} \]
\[ A \uparrow, B \downarrow \]

\[ H_X \quad H_A \quad H_B \quad H_C \]

\[ H_B \text{ will show a small negative "relay" NOE once the population of } H_A \text{ has been perturbed by the direct NOE from } H_X. \]

\[ \omega_0 \text{ dominates - negative NOE} \]
\[ \text{(large molecules, viscous media)} \]

\[ \text{irradiate X} \]

\[ X \quad A \quad B \quad C \]

\[ X \downarrow, A \downarrow \]

\[ \text{negative NOE} \]
\[ A \downarrow, B \downarrow \]

\[ H_X \quad H_A \quad H_B \quad H_C \]

\[ H_B \text{ will show a negative "relay" NOE once the population of } H_A \text{ has been perturbed by the direct NOE, in spite of the fact that } H_B \text{ is too far away to be directly affected by } H_X. \] In favorable circumstances, this effect can propagate over several bonds.
To avoid false information from relay effects, irradiation times must be on the timescale of the $T_1$ of the A nucleus, or shorter, to minimize secondary population changes. False information is particularly easy to obtain when operating in the slow-motion regime, where both direct and relay NOEs have the same sign.

**NOE Basics**

The careful NOE study of $E$- and $Z$-crotonaldehyde (Rowan, McCammon, Sykes, *J. Am. Chem. Soc.*, 1974, 96, 4773) illustrates some important considerations in interpreting NOE data. The size of the NOE enhancement for a particular pair of proton is principally a function of two variables - the primary one is the distance between the "sending" and "receiving" protons (there is an $1/r^6$ dependence on distance). A secondary one requires consideration of what other protons are contributing to the DD relaxation of the "receiving" proton. If there are other protons that are closer than the "sending" one, then the "receiving" proton will of necessity show a smaller NOE enhancement. Thus when a Me group is the receiver, the NOE enhancement is always small (<5%) even when the "sending" proton is very close. This is because each methyl proton has two much closer DD coupled partners which provide the principal relaxation pathway, resulting in only a small contribution from more distant protons outside the methyl group.
The NOE data for crotonaldehyde allow the conclusion that the compound is mostly present in the s-trans conformation, since the s-cis would show rather different NOE enhancements.

**NOE Experiments**

The earliest NOE experimental method involved the straightforward process of decoupling one proton for a few seconds and then measuring a spectrum (Anet, F. A. L.; Bourn, A. J. R. *J. Am. Chem. Soc.* 1965, 87, 5250). Careful peak integrations were then used to determine which protons showed enhanced integrations, and thus were close in space to the decoupled one. Because of the inherently low accuracy of integrations, only large NOE effects could be reliably detected in this way. Thus methyl groups were almost always used as the "sender" rather than "receiver" because a large fraction of the relaxation of a methyl proton occurs from DD interactions with the other two protons of the methyl group, and only to a small extent by external protons.

Some typical structural problems addressed in this way are shown below. The most common application has been the determination of stereochemical and conformational relationships in relatively rigid molecules, since in conformationally mobile molecules NOE effects tend to be much smaller, and often were not reliably detectable by these direct methods.
With the development of stable spectrometers capable of precise difference spectroscopy an improved method for the measurement of NOE effects with higher accuracy became available (DNOE). Here a control spectrum, with the decoupler set to some innocuous frequency, is subtracted from the spectrum with irradiation of a specific multiplet. The resulting difference spectrum gives a large negative peak for the irradiated multiplet (it is saturated) and positive peaks for any proton whose area has been enhanced by the NOE interaction (occasionally these spectra are plotted with inverse intensities). Unaffected peaks are absent, or show a small sinusoidal oscillation due to small chemical shift mismatches caused by the decoupling process, which integrate to 0. NOE effects of less than 1% can be detected in this way.

A typical simple DNOE experiment on 7-methoxychromone is shown below. Spectrum A is a normal $^1$H NMR spectrum (200 MHz, CDCl$_3$ solvent). Spectrum B was obtained by preirradiating signal c with the decoupler before taking the spectrum. The decoupler was off during the acquisition. The middle spectrum is the difference between the two (B minus A) (MRC 1985, 23, 90). Assign all of the signals in the spectrum.
Difference NOE Experiment on 7-Methoxychromone

A

Normal

B - A

30%

2.3%

Irradiate H°

https://www.chem.wisc.edu/areas/reich/nmr/08-tech-02-noe.htm
The current methodology for obtaining NOE spectra involves a pulse gradient method in which the enhanced signals are directly detected, without the artifacts introduced by subtraction, leading to very high quality NOE spectra. A steroid example below from the original paper (J. Am. Chem. Soc. 1994, 116, 6037).
Measurement of H-H distances. The size of an NOE enhancement is strongly related to the distance between the two protons, but it is also a function of other relaxation processes operating on the "receiving" proton. Distances between protons are more directly related to the rate of buildup of the NOE enhancement. A series of experiments are carried out with increasing mixing times, and the increase in NOE enhancement is followed. The closest protons will show the most rapid build-up rates of the NOE. This sort of experiment, usually performed using the 2D NOESY technique, can map H-H distances in complicated molecules ranging from large natural products, to polypeptides, small pieces of DNA and even small proteins.

**NOE in Carbon-13 NMR Spectroscopy**

$^{13}$C spectra are commonly measured with noise-modulated $^1$H decoupling. In most molecules the C-H carbons are relaxed almost entirely by the DD mechanism. Decoupling of the protons thus gives an NOE of the carbon signals. The carbons achieve a population difference like that of protons, so that much larger NOE's are observed, as high as 199% if the carbon is relaxed 100% by the DD mechanism.
The energy levels of four spin states for a $^{13}\text{C}$-$^1\text{H}$ pair is shown. Decoupling the protons equalizes the populations of the CβHβ and CβHa states, as well as the CaHa and CaHβ states. If $\omega_2$ dominates, then the population difference between the Ca and Cβ energy levels is determined by the energy difference between the CβHβ and CαHα states, which is four times as large as the energy difference between the CaHa and CβHa states, hence one expects a much large NOE enhancement than for the H-H situation.

**Coupled $^{13}\text{C}$ NMR Spectra with NOE.** The measurement of undecoupled $^{13}\text{C}$ NMR spectra is usually very time consuming since many of the carbon signals are split into complex multiplets, and there is no NOE enhancement of signal intensities. However, a nearly maximum NOE enhancement can be achieved by use of *gated decoupling*, in which the decoupler is kept on during a delay period when the NOE enhancement builds up, but turned off during acquisition of the FID, so that fully coupled spectra are obtained. This works because the decoupling effect turns on and off nearly instantaneously (microseconds), whereas the NOE enhancement builds up and decays on the time scale of $T_1$ (seconds).

**Integration of Carbon Spectra.** $^{13}\text{C}$ NMR spectra cannot usually be accurately integrated since there are several effects which change the areas of the peaks:

1. Spectra are often run under *saturation conditions* with insufficient delay time between pulses for full recovery of magnetization. Since $T_1$ of carbons vary between 0.1 to $>100$ sec, individual pulses have to be as
much as 500 seconds apart ($5T_1$) to permit complete relaxation of all carbons if accurate integrations are to be obtained.

2. The Nuclear Overhauser Effect increases the area of individual peaks depending on the extent to which DD relaxation versus other pathways relax a particular carbon.

Spectra with minimal NOE enhancement can be obtained by using the inverse gated decoupling technique, in which the decoupler is on only during the short acquisition time, but off otherwise, so that only a small NOE enhancement builds up.

An alternative technique for obtaining integrable spectra is to use the relaxation reagent Cr(acac)$_3$, which will shorten $T_1$ for all carbons by the action of unpaired electrons on the chromium. This will both reduce the saturation problems (by decreasing $T_1$) and reduce or eliminate the NOE enhancement (by reducing or eliminating proton-carbon DD relaxation). Unfortunately, it is not feasible to add Cr(acac)$_3$ to all samples.

Below a series of $^{13}$C NMR spectra which illustrate the problems in achieving accurate integrations of $^{13}$C NMR signals, whose area can be strongly affected both by saturation effects (for quaternary carbons with very long $T_1$ values), and by the NOE enhancement.
8.2 The Nuclear Overhauser Effect

A number of heteronuclei have negative gyromagnetic ratios. Such nuclei will have the sign of the NOE reversed, leading to reduction in intensity, nulled peaks, or negative signals if proton-X DD relaxation is present and proton decoupling is being used. Some common spin 1/2 nuclei with negative $\gamma$ are $^{15}$N, $^{29}$Si, and $^{119}$Sn. If spectra of these nuclei are taken with proton decoupling, then the NOE will reduce the intensity of the signals, or even make them negative. It usually advantageous to take such spectra with pulse techniques that involve polarization transfer from proton to the heteronucleus to minimize the negative NOE.

400 s pulse delay. The areas are much improved, but still far from ideal because of differential NOE enhancements. 16 transients, acquisition time 1 s, total time: 6416 sec. (From Abraham and Loftus)

400 sec pulse delay with gated decoupling. The areas are now nearly ideal. 16 transients, acquisition time 1 s, total time: 6416 sec. (From Abraham and Loftus)

With Cr(acac)$_3$ as relaxation agent. Greatly improved areas in a much shorter time. 1000 transients, acquisition time 1 s, no pulse delay, total time: 1000 sec (From Abraham and Loftus)

Heteronuclear NOE
For most quadrupolar nuclei (⁶Li is a rare exception) the principal relaxation pathway is quadrupolar relaxation, so that little or no NOE can be detected. Even many spin ½ nuclei with large chemical shift ranges (e.g., ⁷⁷Se, ¹⁹⁹Hg, ¹²⁵Te), show no NOE as a result of proton decoupling because the principal relaxation pathway is the **CSA mechanism** (Chemical Shift Anisotropy).