

# NOESY Nuclear Overhauser Effect Spectroscopy

Dr. Antoni Jurkiewicz Department of Chemistry University of Chicago

The Nuclear Overhauser Effect (NOE) arises throughout RF saturation of one spin, the effect causes the perturbation via dipolar interactions with further nucleus spins [1, 2, 3]. This enhances the intensity of other spins. The effect is called the steady-state nuclear Overhauser effect. Since dipolar coupling interacts throughout space, it becomes a very useful tool to study the conformation of molecules. The energy diagram of two-spin system is shown in the following figure:

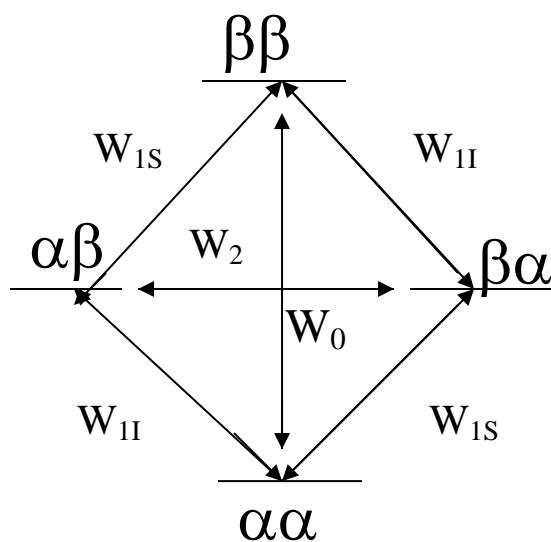


Figure 1. The energy and transitions in two-spin system.

$W_{1I}$  and  $W_{1S}$  are single quantum transition probability rates for saturated and observed spins, respectively.  $W_2$  and  $W_0$  are probability rates for double and zero quantum transitions. The  $W_{1I}$  and  $W_{1S}$  rates are responsible for the spin-lattice relaxation process. After saturation of the I-spin with RF, a population difference will be created across the energy levels that is different from that in the unperturbed state. This creates the Nuclear Overhauser Effect at unsaturated spin S. The magnitude of NOE can be calculated according to the following formula:

$$\epsilon_{NOE} = 1 + \frac{\gamma_I}{\gamma_S} \frac{(W_2 - W_0)}{W_0 + 2W_{1S} + W_2} \quad (1)$$

$\gamma_I$  and  $\gamma_S$  are gyromagnetic ratios for both spins. Both ratios are the same in case both spins are protons. One can notice that the magnitude of NOE depends strongly on  $W_2$  and  $W_0$  transitions. To induce the spin transition, a molecule should tumble or rotate with proper frequency similar to the difference between energy levels. The dynamics of such movements are expressed by the correlation time  $\tau_c$ . The correlation time might be viewed as the time that a molecule spends in the same place before it jumps to another position. This means that rapidly moving molecules have a short correlation time and slowly rotating ones have a long correlation time.

A very rough estimation of correlation time can be made according to following formula:

$$\tau_c \approx M_r \times 10^{-12} s \quad (2)$$

In our laboratory, users most often study small molecules with molecular weights below 1000. In such cases, often  $\omega\tau_c \ll 1$  ( $\omega$  is the Larmor frequency) and the condition is referred to as the “extreme narrowing limit.” The dipolar broadening effects are averaged to zero. The transition probability rates are:

$$W_{IS} \propto \gamma_I^2 \gamma_S^2 \frac{3\tau_c}{r^6} \quad (3.a)$$

$$W_2 \propto \gamma_I^2 \gamma_S^2 \frac{12\tau_c}{r^6} \quad (3.b)$$

$$W_0 \propto \gamma_I^2 \gamma_S^2 \frac{2\tau_c}{r^6} \quad (3.c)$$

Upon examination of formulas 1, 3.a, 3.b and 3.c one can notice that fast motion will induce  $W_2$  probabilities and slow motion will be preferred for  $W_0$ . Therefore, the magnitude and sign of NOE depends strongly on molecular dynamics. For slow motion, NOE might disappear or become negative. In consequence, experiments carried out at low temperatures might show a negative NOE if the molecule is small. Another important feature can be inferred from formula (1), being that the  $W_{IS}$  rate should be small in order to keep NOE large. This indicates that a longer spin-lattice relaxation time ( $T_1$ ) is more favorable to obtain a larger NOE. In order to lengthen  $T_1$ , the sample often needs to have paramagnetic oxygen removed by degassing.

The following diagram shows the pulse sequence of the NOESY experiment.

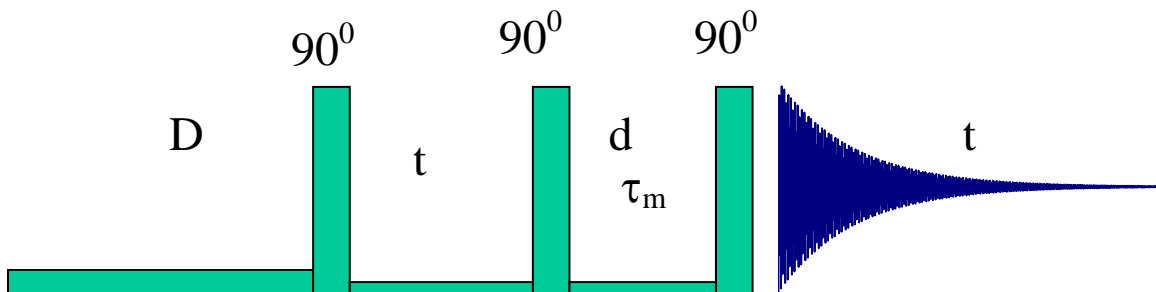


Figure 3. The NOESY pulse sequence

The first pulse equalizes the population differences across energy levels. Coherences evolve during the  $t_1$  period, encoding the phase in the second dimension. The second pulse transfers magnetization of all spins back to Z directions, enabling the mixing. During the mixing time  $\tau_m$ , NOE is created.  $\tau_m$  needs to be chosen carefully. Usually, it needs to be set close to the magnitude of the spin-lattice relaxation time. The last pulse converts the mixed magnetization to the xy plane and it is now ready for observation. The spectrum can be phased with both diagonal and cross peaks in order to be absorptive. In case of a long correlation time or slow motion, the cross peaks are positive and they can be in the same phase as diagonals, as shown in Figure 3.

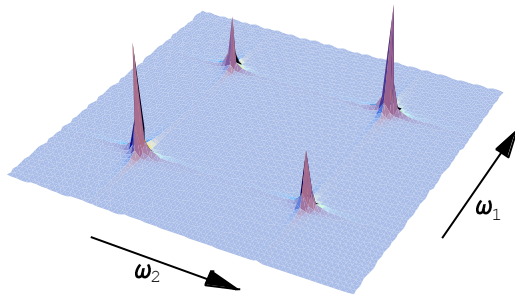


Figure 4. NOESY spectrum in case of a long correlation time, the cross peaks are positive.

When correlation time  $\tau_c$  is short or molecules experience fast motions, the cross peaks are negative and the following occurs (Figure 4):

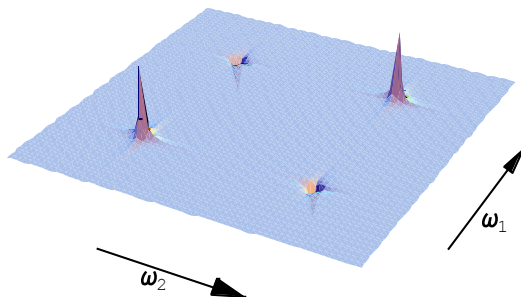


Figure 5. NOESY spectrum in case of a short correlation time, the cross peaks are negative.

COSY cross peaks might also appear in the spectrum, since they are dispersive it is rather easy to distinguish them.

Dr. Antoni Jurkiewicz Department of Chemistry University of Chicago

### [NOESY experiment setup.](#)

#### Further reading

1. Ernst R.R., Bodenhausen B., and Wokaun, A., "Principles of Nuclear Magnetic Resonances in One or Two Dimensions" Oxford University Press, 1992
2. Claridge, T.D., "High-Resolution NMR Techniques in Organic Chemistry"
3. Malcolm. H. Levitt, Spin Dynamics, John Wiley & Sons Ltd., 2001.