

## Module III, Lecture 04: Applications of Liquid State Relaxation Theory

This lecture gives an overview of the applications of Redfield theory in NMR and ESR spectroscopy. Two primary current applications are inter-atomic distance determination using the Overhauser effect and the determination of local structural order using the Lipari-Szabo restricted local motion model.

### Distance determination using nuclear Overhauser effect

The equation describing longitudinal relaxation and magnetization transport between two spins with a rotational diffusion modulated dipolar interaction was found in the previous lecture to be

$$\frac{d}{dt} \begin{bmatrix} \langle \hat{I}_Z \rangle - \langle \hat{I}_Z^{\text{eq}} \rangle \\ \langle \hat{S}_Z \rangle - \langle \hat{S}_Z^{\text{eq}} \rangle \end{bmatrix} = - \begin{bmatrix} \rho_{II} & \sigma_{IS} \\ \sigma_{IS} & \rho_{SS} \end{bmatrix} \begin{bmatrix} \langle \hat{I}_Z \rangle - \langle \hat{I}_Z^{\text{eq}} \rangle \\ \langle \hat{S}_Z \rangle - \langle \hat{S}_Z^{\text{eq}} \rangle \end{bmatrix} \quad (1)$$

$$\Downarrow$$

$$\begin{bmatrix} \langle \hat{I}_Z \rangle - \langle \hat{I}_Z^{\text{eq}} \rangle \\ \langle \hat{S}_Z \rangle - \langle \hat{S}_Z^{\text{eq}} \rangle \end{bmatrix} (t) = \exp \left( - \begin{bmatrix} \rho_{II} & \sigma_{IS} \\ \sigma_{IS} & \rho_{SS} \end{bmatrix} t \right) \begin{bmatrix} \langle \hat{I}_Z \rangle - \langle \hat{I}_Z^{\text{eq}} \rangle \\ \langle \hat{S}_Z \rangle - \langle \hat{S}_Z^{\text{eq}} \rangle \end{bmatrix} (0)$$

where the self-relaxation rates are:

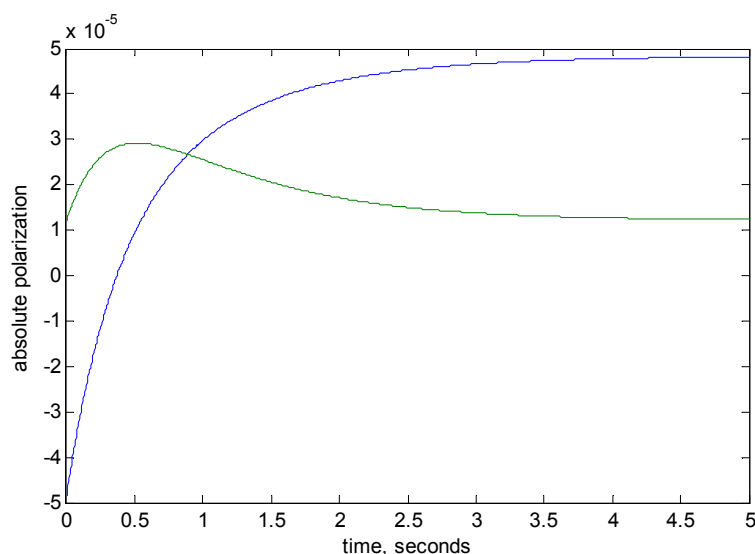
$$\rho_{II} = \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\tau_c}{r_{IS}^6} \left( \frac{3}{1 + \omega_I^2 \tau_c^2} + \frac{6}{1 + (\omega_I + \omega_S)^2 \tau_c^2} + \frac{1}{1 + (\omega_I - \omega_S)^2 \tau_c^2} \right) \quad (2)$$

$$\rho_{SS} = \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\tau_c}{r_{IS}^6} \left( \frac{3}{1 + \omega_S^2 \tau_c^2} + \frac{6}{1 + (\omega_I + \omega_S)^2 \tau_c^2} + \frac{1}{1 + (\omega_I - \omega_S)^2 \tau_c^2} \right)$$

and the cross-relaxation rate is:

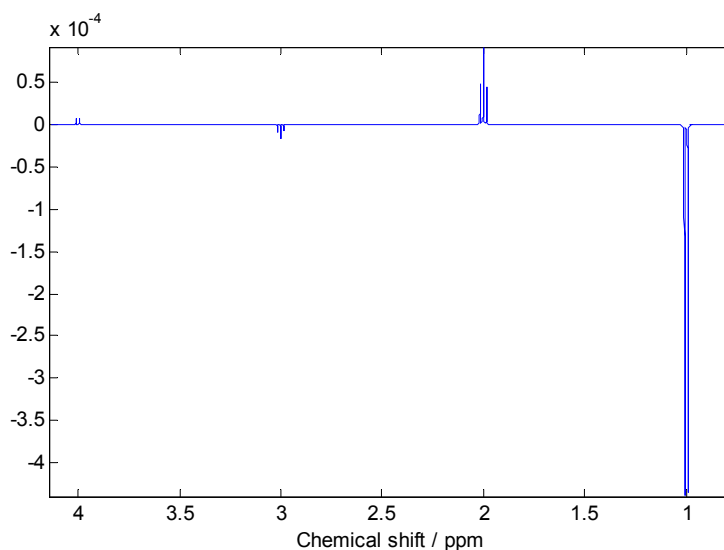
$$\sigma_{IS} = \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\tau_c}{r_{IS}^6} \left( \frac{6}{1 + (\omega_I + \omega_S)^2 \tau_c^2} - \frac{1}{1 + (\omega_I - \omega_S)^2 \tau_c^2} \right) \quad (3)$$

Assuming a room temperature  $^1\text{H}$ - $^{13}\text{C}$  spin system in a 600 MHz magnet with inter-nuclear distance of 1.03 Angstrom and a rotational correlation time of 100 ps with proton magnetization inverted at time zero, we get the following curves for the time dependence of the longitudinal magnetization:



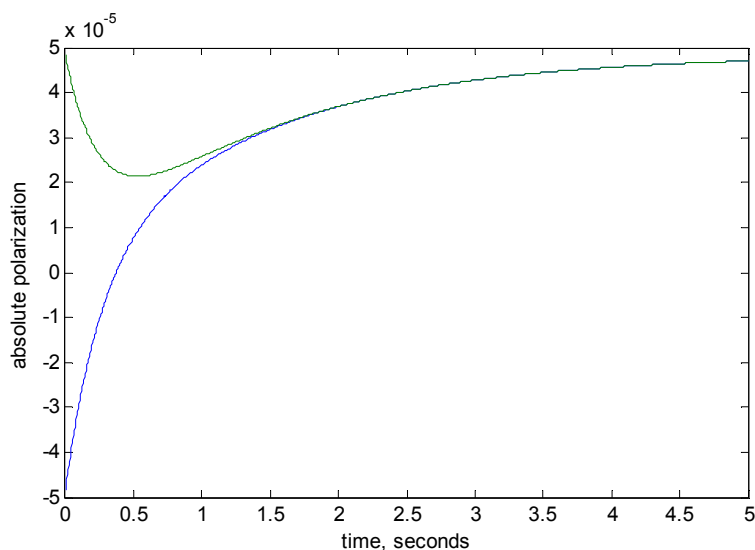
Several things in this figure are worth noting. Firstly, the room temperature equilibrium polarization of  $^{13}\text{C}$  is approximately a factor of four smaller than that of protons, a consequence of the smaller magnetogyric ratio. Secondly, an enhancement of  $^{13}\text{C}$  polarization, by nearly a factor of three may be obtained via the Overhauser effect if the nearby proton is inverted. Thirdly, the three relaxation parameters  $\rho_{II}$ ,  $\rho_{SS}$  and  $\sigma_{IS}$  may be extracted by fitting. Inter-atomic distance and rotational correlation time may then be determined by solving Equations (2) and (3).

A manual NOE measurement experiment involves inverting the source spin, waiting for a variable “mixing” time and running a pulse-acquire experiment. Thermal equilibrium NMR spectrum is typically subtracted from the data to bring it into the form stipulated by Equation (1). A typical picture (a chain of four protons with 2 Angstrom spacing at 600 MHz and a correlation time of 200 ps) is given below



The peaks are integrated, the integrals plotted against time and fitted to Equation (1), in most cases directly with respect to the distance and the correlation time.

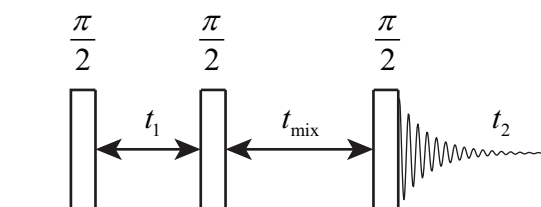
As expected from the appearance of Equation (3), homonuclear Overhauser effect becomes negative for long correlation times. Longitudinal magnetization dynamics in a room-temperature  $^1\text{H}$ - $^1\text{H}$  spin system in a 600 MHz magnet with the a distance of 2.00 Angstrom and a correlation time of 1 ns is shown below



In multi-nuclear systems the two-spin approximation described above is often invalid and the distances resulting from such fitting runs can only be viewed as approximate.

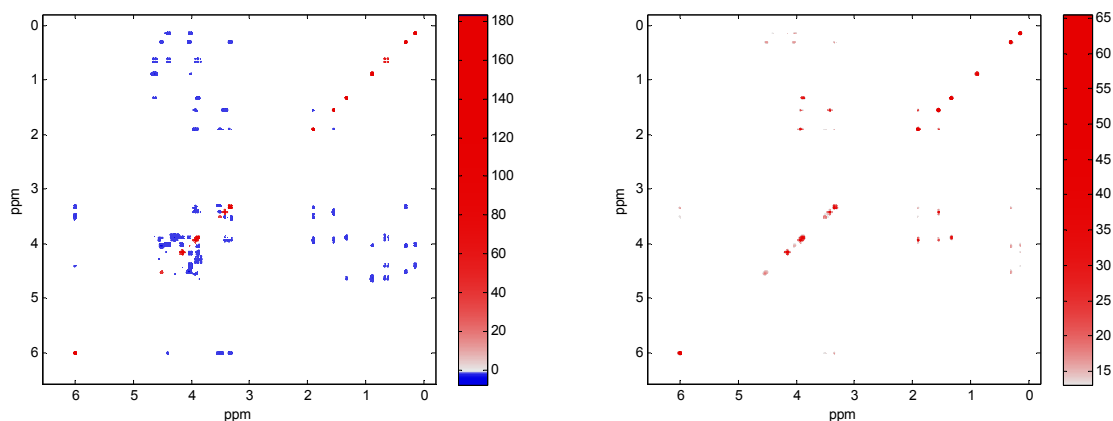
### NOESY spectroscopy

NOESY is a two-dimensional NMR experiment that is sensitive to the longitudinal magnetization transfer via the Overhauser effect. The block diagram of the experiment is



A fairly complicated phase cycle and gradient selection process is used to ensure that only the desired magnetization transfer paths are manifested in the recorded signal – see the literature and Spinach source code for further information on the implementation of NOESY sequences. Typical mixing times are between 50 and 500 ms, depending on the relaxation times present in the system.

NOESY spectra of sucrose in a 400 MHz magnet with mixing time of 500ms and correlation times of 200 ps (non-viscous solvent, left) and 1 ns (viscous solvent, right) are shown below. Note the sign and the amplitude of cross-peaks in both cases.



Because the spin systems are usually complex and many magnetization transfer paths are present, NOESY peaks are not quantitatively related to distances – in protein structure determination they are binned into “strong”, “medium” and “weak”. They are, however, a reliable indication of spatial proximity.

### Lipari-Szabo analysis of local motion

Both isotropic and anisotropic rotational diffusion are not good approximations in systems exhibiting local as well as global motion. A typical situation in a protein is global tumbling with a rotational correlation time of a few nanoseconds and more or less restricted local motion of each amino acid residue within the secondary and tertiary protein structure. Under the reasonable assumption that the two motions are uncorrelated with one another, the following expression emerges for the Fourier transform of the correlation function:

$$J(\omega) = \frac{S^2 \tau_C}{1 + \omega^2 \tau_C^2} + \frac{(1 - S^2) \tau}{1 + \omega^2 \tau^2}, \quad \tau = \left( \frac{1}{\tau_C} + \frac{1}{\tau_{\text{int}}} \right)^{-1} \quad (4)$$

where  $\tau_C$  is the correlation time of the global rotational diffusion,  $\tau_{\text{int}}$  is the correlation time of the local motion and  $S^2$  is called *order parameter*. It can be interpreted as a fraction of the full body angle that is spanned by the restriction cone of the internal motion. The spectral density in Equation (4) is used as a drop-in replacement for the isotropic rotational diffusion spectral density:

$$J(\omega) = \frac{\tau_C}{1 + \omega^2 \tau_C^2} \quad (5)$$

It is easy to see that Equation (4) reduces to Equation (5) in the limit of perfect internal order, *i.e.* when  $S^2 = 1$  and in the limit of completely unrestricted internal motion when  $S^2 = 0$ .

In practical measurements the order parameters are extracted from the simultaneously measured longitudinal relaxation rates, transverse relaxation rates and NOE transfer rates at several different magnetic fields. In terms of the corresponding spectral densities, and including CSA as well as DD relaxation mechanism, those rates are:

$$\begin{aligned} R_{1,I} &= \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{1}{r_{IS}^6} (3J(\omega_I) + 6J(\omega_I + \omega_S) + J(\omega_I - \omega_S)) + \frac{2\gamma_S^2 (\Delta\sigma_I)^2 B_0^2}{15} J(\omega_I) \\ R_{1,S} &= \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{1}{r_{IS}^6} (3J(\omega_S) + 6J(\omega_I + \omega_S) + J(\omega_I - \omega_S)) + \frac{2\gamma_S^2 (\Delta\sigma_I)^2 B_0^2}{15} J(\omega_S) \\ R_{2,I} &= \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{20} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{1}{r_{IS}^6} (4J(0) + 3J(\omega_I) + 6J(\omega_S) + 6J(\omega_I + \omega_S) + J(\omega_I - \omega_S)) + \frac{\gamma_S^2 (\Delta\sigma_I)^2 B_0^2}{45} (4J(0) + 3J(\omega_I)) \\ R_{2,S} &= \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{20} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{1}{r_{IS}^6} (4J(0) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S) + J(\omega_I - \omega_S)) + \frac{\gamma_S^2 (\Delta\sigma_S)^2 B_0^2}{45} (4J(0) + 3J(\omega_S)) \\ \sigma_{IS} &= \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{1}{r_{IS}^6} (6J(\omega_I + \omega_S) - J(\omega_I - \omega_S)) \end{aligned} \quad (6)$$

The fitting proceeds using non-linear least squares method; assuming that enough field points are available, correlation times and order parameters may be extracted for a variety of global and local rotational diffusion models.