Long-range structure restraints – residual dipolar couplings

Isotropic molecular tumbling averages the dipolar fields from nuclear spins to zero. (As we have seen earlier, macromolecules have short $T_2$ relaxation times in part because this averaging takes longer.)

For two spins rigidly held in place (as in a solid), the dipolar field of one spin will change the Larmor frequency of the other. In analogy to scalar couplings, the spins will show doublets due to dipolar couplings. In contrast to scalar couplings that are mediated through chemical bonds, dipolar couplings are through-space interactions. (They also present the mechanism behind NOEs.)

Placing molecules in an anisotropic environment with residual motions (e.g. a liquid crystal) leads to incomplete averaging of the dipolar couplings and, hence, to residual dipolar couplings (RDCs).

Here is a robust way of creating an anisotropic environment in an NMR tube:

Styrene is polymerised with divinylbenzene for branching and dibenzoylperoxide as the radical initiator. A cylindrical polystyrene stick is introduced into the NMR tube together with CDCl₃. The polymer starts to swell and stretches owing to the constraints of the glass wall of the tube.

From left to right: unswollen polymer stick in a standard 5-mm NMR tube, polymer stick directly after polymerization, free polymer stick completely swollen, polymer stick swollen in the NMR tube.

After a few days, the gel is fully equilibrated. The actual sample molecule can be added to the polymer together with the solvent or after the polymer is already swollen. Diffusion at room temperature overnight is enough to measure RDCs.
RDCs are measured as the difference of one-bond couplings measured in the isotropic and anisotropic phases:

\[ \Delta\text{RDC} = |\mathcal{J}_{\text{HC}}| + |\mathcal{D}_{\text{HC}}| \]

The peaks are split by $^{1}J_{\text{HC}} + ^{1}D_{\text{HC}}$. Since RDCs can be positive or negative, the splitting can be increased or decreased. RDCs do not lead to additional splittings of cross-peaks that are already split by $J$-couplings (why not?).

RDCs depend on the angle between the axes of the probability tensor and the vector connecting the interacting spins (I and S in the picture below).

The probability tensor is an ellipsoid spanned by three orthogonal principal axes which indicate the probability of alignment with the $B_0$ axis. The probability tensor is determined by the shape of the molecule (due to steric interactions with the alignment medium) and by the charge distribution of the molecule (if the alignment medium carries charges).
The RDC can be written as

\[ D = - (3/16\pi^3)\gamma_I\gamma_S\mu_0 h/R^3(P_x r_x^2 + P_y r_y^2 + P_z r_z^2) \]

where \(\gamma_I\) and \(\gamma_S\) are the gyromagnetic ratios of spins I and S, \(\mu_0\) and \(h\) are constants (the permeability of vacuum and Planck’s constant), \(R\) is the internuclear distance and \(r_x, r_y\) and \(r_z\) are the unit vectors describing the orientation of the I-S vector in the principal axis frame of the probability tensor (Concepts Magn. Res. 21A, 10 (2004)).

Most frequently, a less intuitive description is used that involves the so-called alignment tensor. The alignment tensor presents the deviation from a spherical tensor, i.e. the three principal components \(A_x, A_y\) and \(A_z\) of the alignment tensor are simply given by

\[ A_x = P_x - 1/3, \quad A_y = P_y - 1/3, \quad A_z = P_z - 1/3. \]

RDCs can be measured for many spin pairs, relating each internuclear vector to the common coordinate system defined by the alignment tensor. In this way it is possible to determine the relative orientation of different parts of a molecule that are located far from one another.

Example:

Cyclosporin A is a cyclic polypeptide that is soluble in CDCl\(_3\) but barely in water. It is a drug that prevents the rejection of foreign organ implants by the immune system. The crystal structure is somewhat different the NMR structure determined by NOEs. RDCs measured in a stretched gel in CDCl\(_3\) agree only poorly with the crystal structure (B in the figure below). The agreement is better with the NMR structure (C in the figure below). Refinement of the NMR structure against the measured RDCs yields good agreement between the experimental RDCs and RDCs back-calculated from the final structure (A in figure). The refined structure (in D) is a bit different from the structure in C, but still in reasonable agreement with the NOEs.

The colour indicates the agreement (blue) or disagreement (red) with the experimental RDCs (measured in Hz).
Additional notes on relaxation

Different mechanisms can lead to nuclear relaxation. All of them depend on the rotational correlation time \( \tau_c \).
The mechanism discussed earlier that depends on the anisotropic electronic environment of the nuclear spin is called chemical shift anisotropy (CSA).

A second, and often more important, mechanism is due to dipole-dipole (DD) relaxation. DD relaxation is observed if a nuclear spin is near another spin (nuclear or electron spin) that changes its position relative to the observe-spin as the molecule tumbles in solution. DD interactions decay with \( 1/r^6 \) (like NOEs), where \( r \) is the internuclear distance. For example, the effective magnetic field of a \(^{15}\text{N}\)-spin is strongly affected by the dipolar field of a directly bonded \(^1\text{H}\).

DD interactions also depend on \( \gamma_1^2 \gamma_5^2 \), where \( \gamma_1 \) and \( \gamma_5 \) are the gyromagnetic ratios of the spins. Therefore, protons are particularly effective sources of DD relaxation. Paramagnetic impurities (oxygen, metal ions) enhance relaxation even more.

The \( T_1 \) and \( T_2 \) relaxation times can be used to derive \( \tau_c \). \( \tau_c \) is directly proportional to the molecular weight of the molecule, i.e. can be used to distinguish monomers from dimers or to assess aggregation in general.

\(^{15}\text{N}\)-relaxation measurements are very popular with proteins:
- there is one NH group per amino acid (namely the backbone amide),
- making proteins with \(^{15}\text{N}\) is inexpensive,
- \(^{15}\text{N}\)-HSQC-type experiments can be used to transfer magnetization from protons to nitrogens for enhanced sensitivity,
- scalar couplings between \(^{13}\text{N}\) are usually negligibly small so that \( T_2 \) relaxation can be measured in spin-echo experiments without having to worry about coupling evolution, and
- the relaxation is easy to understand because it is dominated by the dipolar field from the directly attached proton.
Two simple $^{15}$N-HSQC-type sequences to measure $T_1$ and $T_2$ of the $^{15}$N-spins of a protein:

$T_1^{(15N)}$

$^{1H}$

\[ 90^\circ \Delta 180^\circ \Delta 90^\circ \]

\[ 90^\circ \Delta \Delta \tau \] 

$\tau = $ relaxation delay.

$T_2^{(15N)}$

$^{1H}$

\[ 90^\circ \Delta 180^\circ \Delta 90^\circ \]

\[ 90^\circ \Delta \Delta \tau/2 \tau/2 \tau_1 \Delta \Delta \]

\[ \Delta = 1/(4^{1}J_{HN}) \]

$\Delta = 1/(4^{1}J_{HN})$, $\tau = $ relaxation delay.
Here is an experimental example:

(A) shows $R_1 = 1/T_1$ and (C) shows $R_2 = 1/T_2$ for a protein domain (residues 500 – 633) with mobile termini (as evidenced by the decreased $T_1$ times and increased $T_2$ times).

Exercises:

1) Why does a $^{15}$N spin in a NH group relax more quickly than a $^{15}$N-spin in a ND group? How much faster approximately would one expect the relaxation to be ($\gamma_H/\gamma_D \approx 6.5$)?

2) Protein expression in *E. coli* in D$_2$O leads to perdeuterated proteins. Amide protons can be reintroduced by dissolving the protein in water, whereas CD bonds are stable. Why are amide proton signals narrower in a perdeuterated protein?
This is used to measure $^{15}\text{N}$-HSQC spectra of proteins with high molecular weight.

3) Integrate the Watergate sequence into the $T_1^{(15\text{N})}$ pulse sequence without making the experiment any longer.

4) Phase cycle: What is the effect of changing the sign of the first $90^\circ(\text{H})$ pulse in the $T_1^{(15\text{N})}$ pulse sequence? What is the effect of changing the sign of the first $90^\circ(15\text{N})$ pulse? How can this be used to observe only magnetization that has been excited by the first $90^\circ(\text{H})$ pulse and been transferred to the $^{15}\text{N}$ spins?