15N transverse relaxation experiments for proteins in solution at high-magnetic field strength

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\( ^{15}\text{N} \text{ R}_2 \text{ CPMG at high field} \)

\[
\begin{align*}
B_1 &> 5 \text{ kHz (~ 45 us)} \\
\tau_{\text{CP}} & = 0.5 \text{ ms} \\
\text{Total delay, ~ 50 - 100 ms}
\end{align*}
\]

(1) To reduce generation of antiphase component, \( \tau_{\text{CP}} \leq 0.5 \text{ ms} \)

\[
N_X \rightarrow N_X \cos \theta + N_Y H_Z \sin \theta
\]

(2) At high \( B_0 \), a larger \( \omega_{\text{rf}} = \gamma_N B_{\text{rf}} \) is needed to cover sufficient sw. However, application of high \( B_{\text{rf}} \) may cause probe heating or damage.

\[ \rightarrow \text{Since S/N is high at high magnetic field strength, total delay can be shorted than } T_2. \]
Guide line to choose a shortest total delay.

Assuming a simple two-point exponential fit, the fractional error is determined by S/N ($\Delta I_0/I_0$) and the relaxation delay, $T$. Thus, when the S/N is sufficient, you can reduce $T$.

(for example, when $T = 80$ ms is needed at 600 MHz, $T = 40$ ms may be sufficient at 900 MHz to get the similar protein $^{15}$N $R_2$ error, when S/N is more than 2 times better at 900)

$I(t) = I_0 \exp(-R_2 T)$

$R_2 = (\ln(I_0) - \ln(I(T)))/T$

$\Delta R_2 = \sqrt{ \left( \frac{\Delta I_0}{I_0} \right)^2 + \left( \frac{\Delta I(T)}{I(T)} \right)^2 } / T$

$\frac{\Delta R_2}{R_2} = \left( \frac{\Delta I_0}{I_0} \right) \sqrt{1 + \exp(2R_2 T)/(R_2 T)}$

$\frac{\Delta R_2}{R_2}$ : fractional error of $R_2$

Ishima et al. (2004) J. Biomol NMR
$^{15}$N $R_2$ CPMG dispersion at high field
- use the modified version by Hansen et al -

- 180° pulse
- $1/2 \nu_{\text{eff}}$
- $T_{\text{CP}}$ (constant time)


$B_1 > 5 \text{ kHz} \sim 45 \text{ us}$

$B_1 \leq 5 \text{ kHz}$ (two sets of data at different carrier)

$T_{\text{CP}} \sim 30-50 \text{ ms}$

$\nu_{\text{CP}} \sim 50-1000 \text{ Hz}$
CT- CPMG Relaxation dispersion experiment Analysis

Assuming a two-site exchange model

\[ p_a, p_b: \text{population } (p_a + p_b = 1) \]
\[ k_{ex} (=1/\tau_{ex}): \text{exchange rate} \]
\[ \delta\omega: \text{difference in chemical shifts} \]
\[ R_2^0: \text{intrinsic relaxation rate} \]

Quantitative analysis

Step 1: Fits for individual residues
Step 2: Group fits
  group of residues
  group of \( R_2 \) recorded by observing different nuclei

Qualitative analysis

\( R_2 \) RMSD or \( \Delta R_2 \) plot
CT- CPMG Relaxation dispersion experiment
Analysis - Step 1: Fits for individual residues

(1) Selection of residues for individual analysis
<Kovrigin et al, 2006; Jee et al, 2007>
Test whether the data fits best in which models, such as flat, fast exchange, and Carver-Richards equations.
(Careful that C-R equation does not reproduce very slow exchange)

<Ishima & Torchia 2005>
Select by $R_2$ RMSD by comparing with $R_2$ error
CT- CPMG Relaxation dispersion experiment

Analysis - Step 1: Fits for individual residues

Parameters, $p_a$, $k_{ex}$, and $\delta \omega$ are thought to be determined once the data sets from the two magnetic field strength are used for individual residue fit because they have different field dependence (see the graphs below). However, the parameters may not be still well optimized when both $R_2^0$s at two fields, i.e., $R_2^{0-1}$ and $R_2^{0-2}$, are variable parameters. This is because the two work for adjustable parameters.

$$p_a, k_{ex}, \delta \omega, R_2^{0-1}, R_2^{0-2}$$

**Fast exchange**

$$R_2 = R_2^0 + p_a p_b (\delta \omega)^2 k_{ex}/(k_{ex}^2 + (2\pi \nu_{CP})^2)$$

**Slow exchange**

$$R_2^a = R_2^{a0} + p_b k_{ex} - p_a k_{ex} \{\sin(\delta \omega/4\nu_{CP})/(\delta \omega/4\nu_{CP})\}$$
CT- CPMG Relaxation dispersion experiment
Analysis – Step 2: Group fits

(1) This step is done for a group of dispersion data sets,
   (a) recorded by observing different nuclei
   (b) recorded for a group of residues that are assumed to undergo correlated motions.

(2) Variable parameters in the group fits
   - uniform in a group: $p_a$ and $k_{ex}$
   - different in each residue: $\delta \omega$, $R_2^{0-1}$, $R_2^{0-2}$

Typical equations used for the fits
- Bloch-McConnell equation (McConnell, 1958, J Chem Phys)
CT- CPMG Relaxation dispersion experiment
Qualitative analysis

We may use R2 dispersion data qualitatively, for example, just to compare with the trends of the generalized order parameters. In this case, a plot of R2 RMSD or ΔR2 as residue number will be useful.

R2 RMSD is taken for each dispersion curve.
ΔR2 is the difference between R2\textsuperscript{eff, max} and R2\textsuperscript{eff, min}.
(Here, υ\textsubscript{eff} is the effective field strength (υ\textsubscript{eff} = 1/τ\textsubscript{CP})