Relaxation in NMR
Source of relaxation in NMR is fluctuation of nuclear interaction with respect to the external magnetic field.

Dipolar interaction:

\[ \frac{1}{N} \frac{1}{B_0} \]

CSA interaction:

\[ \frac{1}{11} \frac{2}{22} \frac{3}{33} B_0 \]

Other interactions: Quadrupolar, Paramagnetic, etc.

Relaxation in NMR
Where does the fluctuation come from?
• Random events:
  - Rotational diffusion
  - Translational diffusion
  - Vibrational/Librational motions
  - Conformational sampling/variability
• Non-random events:
  - Magic angle spinning
  - \( B_0 \) quenching

Correlation function
The behavior of the random fluctuation can be described by a correlation function.

What is a correlation function:
1. Correlation function of 2 variables is the expected value of their product. This could be evaluated as a function of space or time.
2. It is a measure of how quickly the two variables change as a function of time or space.

Correlation function
How does it relate to NMR relaxation?
Remember the master equation:
\[ \frac{d\rho(t)}{dt} = -i[H(t),\rho(t)] + \int [H(t'),\rho(t);H(t')\rho(t')] dt' \]

Taking the ensemble average, first term vanishes if \( H(t) \) is random:
\[ \frac{d\rho(t)}{dt} = \int [H(t'),\rho(t);H(t')\rho(t')] dt' \]

Expand \( H(t) \) in spin and time dependent operators:
[\text{Please provide the expanded equation here.]}

\[ \rho(t) = \sum_i \Psi_i(t) \sum_j \Psi_j(0) \sum_k \Psi_k(0) \sum_l \Psi_l(t) \]
Correlation function

How does it relate to NMR relaxation?

Substituting back:

\[
\tilde{\alpha}(t) = \int \tilde{\alpha}(t') \tilde{\alpha}(t'') \tilde{\alpha}(t'') dt'
\]

It describes the random function of time that contributes to changes in the spin density.

Correlation function

How quickly the NH dipole changes as a function of time with respect to the external magnetic field.

\[
C(t) \sim \langle \tilde{\alpha}_x(0) \tilde{\alpha}_x(t) \rangle
\]

LF=Laboratory frame, B_0 is static

In liquid it reduces to:

\[
C(t) = \frac{1}{2} \langle \tilde{\alpha}_x(0) \tilde{\alpha}_x(t) \rangle
\]

Relaxation in NMR

In NMR observed quantities: T_1, T_2, and NOE are determined by the Fourier transform of an appropriate time correlation function (the spectral density) evaluated at certain frequencies, thus certain magnetic fields.

\[
C(t)
\]

Relaxation in NMR

Lipari-Szabo Model

Free Approach

General approach
Lipari-Szabo Correlation function

Conditions:
• Overall and internal motions are independent
• \( C(t) = C_0(t) C_I(t) \)
• \( C_I(t) = \mathbf{P}_2(\mu(0) \cdot \mu(t)) \) in the molecular frame.
• \( C_I(0) = \mathbf{P}_2(\mu(0) \cdot \mu(0)) = 1 \)
• Area under the approximated correlation function is the same as the real one:
  \[
  \int_0^\infty (C_I(t) - S^2) dt = \int_0^\infty C_I(t) dt - S^2 \int_0^\infty dt
  \]

Isotropic Diffusion

Lipari-Szabo Correlation functions

\( C_I(t) = \mathbf{P}_2(\mu(0) \cdot \mu(t)) = S^2 + (1-S^2) e^{-t/\tau} \)

Isotropic Diffusion

\( C(t) = \exp(-6D_m t) = \exp(-t/\tau_c) \)

\( C(t) = C_0(t) C_I(t) = [S^2 + (1-S^2) \exp(-t/\tau_c)] \exp(-t/\tau_c) \)

Anisotropic Diffusion (Axial Symmetry)

\( C(t) = (1/5) \sum_{\mathbf{a}} \exp(-[6D_a + m^2(D_a - D)] |\mathbf{d}_a|^2) \exp(-t/\tau_c) \)

Non-isotropic Diffusion
Correlation functions

**Anisotropic Diffusion (Fully asymmetric)**

\[ C(t) = \frac{1}{5} \sum_{i=1}^{4} A_i \exp(-t/D_i) \]

\[ A_1 = \frac{d}{2} \]
\[ A_2 = \frac{0}{2} \]
\[ A_3 = \frac{d}{2} \]
\[ A_4 = \frac{d}{2} \]

\[ D_i = \frac{1}{3} (D_x + D_y + D_z) \]

\[ L^2 = \frac{1}{3} (D_x D_y + D_y D_z + D_z D_x) \]

\[ t_i = (t/\sqrt{D_i^2 - L^2}) \]

\[ t_i = (t/\sqrt{D_i^2 - L^2}) \]

\[ \text{Correlation function to relaxation rates} \]

\[ R_0 = \frac{\rho}{C(t)} \exp(-\rho) \]

\[ \text{Isotropic Diffusion} \]

\[ C(t) = \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ \alpha = \frac{\rho}{2} \]
\[ \beta = \frac{\rho}{2} \]

\[ \text{Isotropic Diffusion (Axial Symmetry)} \]

\[ C(t) = \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ \alpha = \frac{\rho}{2} \]
\[ \beta = \frac{\rho}{2} \]

\[ \text{From correlation function to relaxation rates} \]

\[ R_0 = \frac{\rho}{C(t)} \exp(-\rho) \]

\[ \text{Isotropic Diffusion} \]

\[ R_0 = \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ \alpha = \frac{\rho}{2} \]
\[ \beta = \frac{\rho}{2} \]

\[ \text{Relaxation Rates} \]

\[ 1/T_1 = \frac{d}{2} \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ 1/T_2 = \frac{0.5}{2} \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ T_1 = \frac{1}{(1/\rho)^2} \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]

\[ T_2 = \frac{0.5}{(1/\rho)^2} \{ [S_f^2 + S_s^2/(1-S_s^2)] \exp(\alpha t) + (1-S_f^2) \exp(-\alpha t) \} \exp(-\beta t) \]
Protein Backbone Relaxation Analysis

Protocol

- Estimate the overall correlation time
  - Eliminate residues experiencing large amplitude fast internal motions (NOE < 0.6)
  - Eliminate residues undergoing conformational exchange (low T2 values)

- Choose the proper diffusion model
  - Isotropic (D_iso)
  - Axially symmetric (D_0, D_+, D_-)
  - Fully asymmetric (D_x, D_y, & D_z)

- For non-isotropic model diffusion tensor must be defined
- Protein structure
- Local order parameter and fast internal correlation times
- Residue specific fitting

Isotropic Diffusion

Non-isotropic Diffusion
Human GAIP

**Diffusion Tensor Fitting**

<table>
<thead>
<tr>
<th>R°</th>
<th>q°</th>
<th>q'°</th>
<th>tₑ (ns)</th>
<th>D₁/D₂</th>
<th>D₂/D₃</th>
<th>χ²</th>
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<tbody>
<tr>
<td>96.8</td>
<td>-31.0</td>
<td>11.80</td>
<td>1.69</td>
<td>2.07</td>
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<td></td>
</tr>
<tr>
<td>96.5</td>
<td>-31.6</td>
<td>11.75</td>
<td>1.70</td>
<td>2.05</td>
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<td></td>
</tr>
<tr>
<td>94.4</td>
<td>-37.3</td>
<td>11.72</td>
<td>1.47</td>
<td>4.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94.5</td>
<td>-37.3</td>
<td>11.70</td>
<td>1.48</td>
<td>4.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inertia ratio (I⁄Iₚ): 2.49

Estimated D₁/D₃: 1.89

**Human GAIP**

**Aggregation State**

\[
R_{obs} = x \cdot R(\tau_{mono}) + (1-x) \cdot R(\tau_{dimer})
\]

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>tₑ (ns)</th>
<th>% Dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>8.7</td>
<td>46</td>
</tr>
<tr>
<td>0.2</td>
<td>8.7</td>
<td>34</td>
</tr>
</tbody>
</table>
Things to consider in a single field experiments

- Sample aggregation
  - Measure $T_2$ at different sample concentrations
  - Substantially high average order parameters
  - Poor overall fit of the diffusion parameters

- Error estimates
  - Possible use of reproducibility
  - Goodness of the fit

- Sample stability
  - Interleave and scramble the data whenever possible
Things to consider in multiple fields experiments

- **Temperature effect**
  - Use a single TSP sample to calibrate the temperature of the probe under the exact experimental conditions
  - Interleave and scramble the data whenever possible
  - Heat compensation

- **Error estimates**
  - Consistent procedure throughout all the different field strengths
  - Possible use of reproducibility and signal to noise normalization
  - Goodness of the fit

- **Sample stability**
  - No accidental correlation in sample decay and field strengths

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**Human Ubiquitin**

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**Temperature dependent of Ubiquitin $^{13}$C relaxation**

15°C - 47°C

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**Typical decay curves for $^{13}$C relaxation data**
Typical decay curves for $^1^3$C relaxation data

1. Interaction tensors

- Cross-correlated relaxation: $C^i(CSA) \cdot C^j(DD)$
- Cross relaxation: $C^i \cdot C^j$
- Auto-correlated relaxation

\[ R = R^{CSA} + R^{DD} \]

Assumptions:
- Uniform CSA
- Isotropic overall diffusion
- One effective correlation time and order parameter

General NMR relaxation rate equations:

\[ R^{CSA} = \frac{1}{T_1^{CSA}} \left( \frac{1}{[CSA]} \right) \]
\[ R^{DD} = \frac{1}{T_2^{DD}} \left( \frac{1}{[DD]} \right) \]
Isolation of CSA contribution from field dependent $2R_2 - R_1$ values

\[ 2R_2 - R_1 = \frac{4}{3} g_0^2 \Delta \gamma B_0 + \sum \frac{4}{3} \sum \frac{4}{3} 2g_2 (b_0) = 2g_0^2 \delta (\alpha, \beta) \]

\[ \Delta = \delta \chi + \delta \mu \frac{3}{2} \delta \nu \]

\[ \delta \chi = \frac{3}{2} \delta \nu \frac{1}{3} \delta \mu \]

Typical $2R_2 - R_1$ values as a function of $B^2$

Comparison of effective order parameters
Protons within 3Å distance

Correlation times vs. temperatures from $^{15}$N and $^{13}$C data sets

Effective Model-Free order parameters

Changes in average order parameter as a function of temperature