Model-Free Approach to Internal Motions in Proteins

• Lipari & Szabo, JACS 104, 4546 (1982)
• Palmer AG, Kroenke CD, Loria JP, Meth. Enzymol. 339, 204-238 (2001)
Generalized Order Parameters and Internal Motion

Basis: fast internal motions scale interactions that are then modulated by molecular tumbling

Methyl rotation a model specific example:

\[ H_1(t) \propto (3\cos^2(\theta''(t) - 1) \]
\[ H_1(t) \propto (3\cos^2(\theta'(t) - 1)(3\cos^2(\theta - 1)/2) \]
\[ H_1(t) \propto (3\cos^2(\theta'(t) - 1)(-0.34) \]
\[ H_1(t) \propto (3\cos^2(\theta'(t) - 1) S \]

Order Parameter
• Scaling due to motions too fast to affect relaxation directly
• Efficiency of relaxation due to tumbling is reduced
• Scaling factor is an “order parameter” – 0 if isotropic, 1 if no internal motion

\[
J(\omega) = \frac{2}{5} \left( \frac{S^2 \tau_m}{1 + (\tau_m \omega)^2} + \frac{(1 - S^2)\tau}{1 + (\tau \omega)^2} \right)
\]

\[
\tau^{-1} = \tau_m^{-1} + \tau_i^{-1}, \text{ if } \tau_i \text{ is very short, it dominates } \tau
\]
• $S^2$ and $\tau_m$ are parameters often measured for proteins using $^{15}$N – $^1$H interactions where “r” is fixed at the bond length and $\gamma$s are known

• $^{15}$N $T_1$, $T_2$, and heteronuclear NOEs are usually measured.

• $\tau_m$ can be estimated from $T_1$, $T_2$ for a large molecule, $\omega$ $\tau_m$ is large here implying:

• $\frac{1}{T_2} \cong \frac{dd’}{4} \{2J(0)\}$, $\frac{1}{T_1} \cong \frac{dd’}{4} \{3J(\omega_N)\}$

• $J \cong S^2 \tau_m / (1 + (\tau_m \omega)^2)$, $(T_1 / T_2) \cong (\tau_m \omega_N)^2$

• Once $\tau_m$ is known, $S^2$ can be calculated from $T_1$, $T_2$ or NOE

• $S^2$ in a structured region is about 0.8, in loops less
Example from binding of phosphopeptides to SH2 domain *Biochemistry*, 33, 5987 (1994)

**Figure 9:** Schematic representations of the spatial distributions of residues requiring an $R_{ex}$ term or two-time-scale spectral density functions to fit the measured relaxation data. Residues which required an $R_{ex}$ term are highlighted in dark grey, and residues which required the two-time-scale spectral density function are shown in lighter grey. Residues which required neither of these spectral density functions or for which spectral density functions were not determined are white. The distribution for the uncomplexed form of PLC$\gamma$1C is shown in (a); the complexed form is shown in (b). The distributions are imposed on a preliminary structure of PLC$\gamma$1C in complex with pY1021 (Pascal et al., 1994). The phosphopeptide is not shown in the diagram. The figure was produced using SETOR (Evans, 1993).
Figure 3: Enhanced sensitivity pulse sequences employing pulsed field gradients for the measurement of (a) $^{15}\text{N} T_1$, (b) $^{14}\text{N} T_2$, and (c) $^{1}\text{H}-^{15}\text{N}$ NOE values. In all sequences, narrow and wide pulses indicate 90° and 180° pulses, respectively, darker gradient pulses indicate those used.
Figure 4: Region of the $^1$H-$^{15}$N shift correlation spectrum recorded with the pulse sequence used to determine $T_1$ as shown in Figure 3a and relaxation delay values ($\tau$) of (a) 5, (b) 246, and (c) 757 ms.

Figure 5: Examples of (a) $T_1$ and (b) $T_2$ decay curves for Ser 44 (+) and Leu 80 (X) in the uncomplexed (solid lines) and pY102L peptide-complexed (dashed lines) forms. The curves indicate best fits to single-exponential decays. Error bars, if shown, would be smaller than the size of the characters used to indicate the data points.
Changes in Order Parameters on Complexation

**Figure 7**: Plot of the order parameter $S^2$ (solid line) and $R_{ex}$ (solid diamonds) as a function of residue number for (a) the uncomplexed form of SH2 and (b) the complexed form of SH2.
Other Contributions to $T_2$ can Complicate Analysis

- Chemical exchange effects: $R_{ex}$
- Anisotropic tumbling of macromolecule
- $^{15}$N $T_1$, $T_2$, and heteronuclear NOEs over determine system when internal motions are fast and isotropic model applies - $T_1/T_2$ ratio for certain residues will be inconsistent
- $R_{ex}$ can be evaluated through $B_0^2$ dependence or relaxation compensated Carr-Purcell experiments: Loria JP, Rance M, Palmer AG, JACS 121, 2331-2332 (1999)

\[
\frac{1}{T_2} \cong \left(\frac{dd'}{4}\right)\{2J(0)\} + R_{ex} = \left(\frac{dd'}{4}\right)\{2J(0)\} + \Theta_{ex} B_0^2
\]
Cross Correlation Effects

• TROSY - Pervushin, Riek, Wider & Wuthrich, PNAS 94, 12366 (1997)
• TROSY na CRINEPT - Riek, Pervushin & Wuthrich TIBS, 25, 462 (2000)
• Cα-N torsion angles - Reif, Hennig & Griesinger Science, 276, 1230-1233 (1997)
TROSY
Transverse Relaxation Optimized Spectroscopy

K. Pervushin, R. Riek, G. Wider, K. Wuthrich
PNAS 94 12366 (1997)

Spin relaxation with interference between two contributions

\[ f(t)V = f_1(t)V_1 + f_2(t)V_2 \]

\[ \frac{1}{T_{1,2}} \propto |V_1|^2 J_{11}(\omega) + |V_2|^2 J_{22}(\omega) + |V_1 V_2| J_{12}(\omega) \]

\[ J_{ij}(\omega) = \int f_i(t + \tau) f_j(t) \exp(i\omega\tau) \]
$^{15}\text{N} \text{ CSA and } ^1\text{H} - ^{15}\text{N} \text{ Dipole Interactions Interfere}$

$^{15}\text{N} - \overset{\uparrow}{-1^\text{H}} - \overset{\uparrow}{^1\text{H}}$

$\alpha$ - fields interfere

$\beta$ - fields reinforce

$\nu - ^{15}\text{N}$

$\nu_0$ - 1 GHz

width
Deuteration and TROSY Greatly Improve Resolution

Differential Line
Broadening due to cross-correlation
Other Cross-Correlated Relaxation Phenomena
A general approach

\[ \frac{1}{T_{1,2}} \propto |V_1|^2 J_{11}(\omega) + |V_2|^2 J_{22}(\omega) + |V_1 V_2| J_{12}(\omega) \]

\[ J_{ij}(\omega) = \int f_i(t + \tau) f_j(t) \exp(i\omega\tau) \]

- If motions are uncorrelated, latter integral is zero
- Correlated example: 2 \( ^\alpha \) protons on a \(^{13}\text{C} \) methylene
The effects are geometry dependent

- Use in structure determination: Reif, Hennig, Griesinger, Science, 276, 1230-1233 (1997)

2Q coherence split by both $^1$Hs

$\beta\beta$, $\alpha\beta, \beta\alpha$, $\alpha\alpha$
Example: Acyl Chain Rotation in Lipid Bilayers

\[ H_{\text{eff}} = f(t)V \]
Figure 2. The 50.3-MHz $^{13}$C difference spectrum of sonicated DMPC vesicles containing unenriched and 90% enriched 2-$^{13}$C-MA; 7200 transients requiring 6 h were collected for each spectrum.
Figure 3. Data from coupled spin-relaxation experiments. Vertical scale is normalized so that the equilibrium $^{13}$C intensity is 1; (□), (○), and (□): $\nu_1, \nu_3,$ and $\nu_4$ following $^{13}$C inversion; (+), $\nu_4$ following $^1$H inversion. Solid lines are the calculated fit of the rotational isomerization plus torsion model described in the text.
Methyl-TROSY
another example of cross-correlation effects
V. Tugarinov, R. Sprangers and L.E. Kay

Double and zero quantum coherences between $^{13}\text{C}$ and $^{1}\text{H}$ evolve with the effects of coupling to the remaining 2 protons

Proton coupled ZQ (H-C) spectrum
Pulse sequence for HZQC

\[ \text{\( ^1\text{H} \)} \]

\[ \text{\( ^{13}\text{C} \)} \]

\[ \text{\( ^2\text{H} \)} \]

\[ \text{PFG} \]

WALTZ-16
Comparison of HMQC and HZQC Data