Protein dynamics from NMR Relaxation data

Clubb 3/11/15

Types of Motion Involved in Dynamics

NMR relaxation measurements provide information on dynamics at a wide range of time scales that is site specific:

Biomolecules are not static – it is often Structure AND Dynamics that determine Function:

- rotational diffusion ($\tau_2$)
- translational diffusion ($D$)
- internal dynamics of backbone and sidechains ($\tau_1$)
- degree of order for backbone and sidechains ($S^2$)
- conformational exchange ($R_{ex}$)
- interactions with other molecules ($k_{intra}$,$k_{inter}$)

Biomolecules are often not globular spheres:
- anisotropy ($D_{x}, D_{y}, D_{z}$)

All of these parameters are accessible through NMR measurements

Nitrogen-15 relaxation

$\mathbf{R}_1 = 1/T_1$ Longitudinal relaxation (decay back to z-axis)

$\mathbf{R}_2 = 1/T_2$ Spin-spin relaxation (dephasing in transverse plane)

$15\mathbf{N}\{\mathbf{H}\}$ heteronuclear NOEs
Heteronuclear steady state NOE (saturate amide proton and measure NOE effect on directly bonded nitrogen atom)
Measure $^{15}$N T1, $^{15}$N T2 and $^{1H-^{15}N}$ NOE

$^{15}$N T2 (R2)

$^{15}$N T1 (R1)

Steady state $^{1H-^{15}N}$ NOE

$\Delta = 17$ms

$\Delta = 86$ms

$\Delta = 138$ms

$\{H\} = (I - I_0)/I_0$

Where $I$ and $I_0$ are the intensity of the $^{15}$N resonance with and without saturation of the attached hydrogen atom.

$^{15}$N Relaxation $\rightarrow$ backbone motion

Record series of HSQC-type experiments in which peak intensity depends on rate of relaxation.

Fit relaxation data to motional model of protein or nucleic acid. (typically the “model free” model).

Extract rates of decay by fitting peak intensity as a function of delay time in each experiment.

Model Fitting

Dynamics

$10^{-12}$ to $10^{-3}$ sec

Model free analysis yields: $S^2$, $\tau_e$, $\tau_c$ and $R_{ex}$. 
Relaxation rates sample motions described by the spectral density function

The relaxation rate constants for dipolar, CSA and quadrupolar interactions are linear combinations of spectral density functions, J(ω). For example, one can derive the following equations for dipolar relaxation of a heteronucleus (i.e. 15N or 13C) by a proton:

\[
R_{1,N} = \frac{1}{T_{1,N}} = \frac{(d^2/4)[J(\omega_H-\omega_N) + 3J(\omega_N) + 6J(\omega_H+\omega_N)]}{\gamma_H/\gamma_N}
\]

\[
R_{2,N} = \frac{1}{T_{2,N}} = \frac{(d^2/8)[4J(0) + J(\omega_H-\omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H+\omega_N)]}{\gamma_H/\gamma_N}
\]

\[
\text{NOE}_{15N(1H)} = 1 + \frac{(d^2/4)(\gamma_H/\gamma_N)[6J(\omega_H+\omega_N) - J(\omega_H-\omega_N)] x T_{1,N}}{\gamma_H/\gamma_N}
\]

where \(d = (\gamma_H/\gamma_N)(h/8\pi)/r_{HN}^3\)

The J(ω) terms are “spectral density” terms that tell us what frequency of motions are going to contribute to relaxation. They have the form

\[
J(\omega) = \frac{\tau_c}{(1+\omega^2\tau_c^2)}
\]

and allow the motional characteristics of the system (the correlation time \(\tau_c\)) to be expressed in terms of the “power” available for relaxation at frequency \(\omega\):
Recall:

**Spectral Density Function** \( J(\omega) \): Power available from the lattice (molecular motions) to bring about relaxation via transition probabilities. It is a function of frequency \( \omega \). All measurable relaxation properties of a protein (T1, T2, NOE) can be expressed in terms of the spectral density function.

- \( J(\omega) \) depends on how fast the macromolecule tumbles in solution. It therefore depends on the size and shape of the macromolecule, and the temperature and viscosity of the solution.

\[
J(\omega) = \frac{\tau_c}{1 + \omega^2 \tau_c^2}
\]

For spherically shaped macromolecule (isotropically tumbling)

\[
J(\omega) = \frac{\tau_c}{(1+\omega^2\tau_c^2)}
\]

Relaxation rates sample motions described by the spectral density function

\[
R_{1,N} = \frac{1}{T_{1,N}} = (d^2/4) \left[ J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) \right]
\]

On a 500MHz spectrometer:

- \( -450MHz \)
- \( -50MHz \)
- \( -550MHz \)

**Liparо-Szabo method to measure fast time scale from NMR data**

- Longitudinal (T1)
- Transverse (T2)
- 15N\{1H\}-NOEs

Fit relaxation data to obtain parameters that define the motion of the amide bond.

**Simplest case**

For an isotropic diffusion:

\[
J(\omega) = S^2 \tau_c (1 + \omega^2 \tau_c^2) / (1 + \omega^2 \tau_c^2)^2
\]

\[
1/\tau = 1/\tau_c + 1/\tau_e
\]

**\( S^2 \) and \( \tau_e \)**

- Order parameter \( S^2 \): Ranges from 0 to 1, with 0 meaning the N-H bond vector is completely flexible and 1 indicating that the bond vector is rigid.
- Time constant associated with fast motions \( \tau_e \): Normally in the picosecond range as proteins studied by NMR tumble much more slowly with \( \tau_c \) values in the nanosecond range.
• rotational diffusion ($\tau_c$)
• translational diffusion ($D$). In the above figure the protein is shown as an ellipsoid with distinct diffusion tensors that are parallel and perpendicular to the long axis of the protein.
Backbone Amide $^{15}$N NMR Relaxation

Backbone amide $^{15}$N spin relaxation is dominated by two interactions:

- $^{1}$H-$^{15}$N dipole-dipole (DD)
- $^{15}$N chemical shift anisotropy (CSA)

Additional contribution to transverse relaxation from conformational exchange

$$R_{1} = R_{1}^{DD} + R_{1}^{CSA}$$
$$R_{2} = R_{2}^{DD} + R_{2}^{CSA} + R_{ex}$$

The “additional” broadening seen in signals characterized by exchange is given by the $R_{ex}$ term.

Fitting of relaxation data

Slow motions ($\mu$s to ms)
- Conformational exchange ($R_{ex}$)
  reveals the presence of exchange process between different conformers that have distinct chemical environments. (chemical shifts).

This additional fitting parameter has units of sec$^{-1}$. It is the additional broadening of the $^{15}$N resonance caused by exchange.

Newer techniques are now available to measure $R_{ex}$ directly (relaxation compensated CPMG expts).
Simplest Case: Rex caused by two site exchange

\[
A \xrightarrow{k_{1}} B
\]

\[
R_{ex} = \frac{p_1 p_2 (\Delta \omega)^2}{k_{ex}} \left[ 1 - \frac{2}{k_{ex} \tau_{cp}} \tanh \left( \frac{k_{ex} \tau_{cp}}{2} \right) \right]
\]

- \(p_1\): population of A
- \(p_2\): population of B
- \(\Delta \omega\): frequency difference between A and B
- \(\tau_{cp}\): delay between pulses in cpmg expt.
- \(k_{ex}\): \(k_1/p_1 = k_{-1}/p_2\)

NtrC protein becomes active when it is phosphorylated. The two forms of the protein have different structures.
Question:

Does phosphorylation induce a structural change in NtrC?

or

Is there a pre-existing equilibrium between the different protein conformers that is shifted towards the phosphorylated form upon phosphorylation? (allosteric activation also called population model)

**Measured relaxation parameters**

The experiments were performed for NtrC in three different functional states: the inactive unphosphorylated form NtrC*, a partially active mutant form NtrC' [Asp^{86} \rightarrow Asn^{86}/Ala^{89} \rightarrow Thr^{89} (D86N/A89T)], and the fully active phosphorylated form P-NtrC' (J3).

![Diagram of induced fit and population model](image_url)
Differences in slow-time scale dynamics

The two conformers are in fast exchange

- Single set of resonances observed for apo and phosphorylated forms. Therefore the two forms must be in fast exchange with one another.

- Rex values are a measure of the broadening caused by the exchange process.

Structural differences

Apo-form vs. phosphorylated
Mutations that activate the protein shift the equilibrium towards the active conformation

Superimposed HSQC spectra

Unphosphorylated D86N mutant that shows intermediate activity

Apo-form

Phosphorylated form

Fig. 4. Relationship between chemical shift changes and activity of the following NtrC forms (36): V151 (gray), D54E (red), D66N (green), D66N/A80T (gold), D66N/A80T/V151 (blue), and P-NtrC (cyan) in respect to wild-type NtrC (black). (A) Signals for D86 in 1H-15N HSQC spectra of five NtrC variants are superimposed. Larger chemical shift changes (shift of the signal to the lower right corner) coincide with increased activity of the corresponding mutants (36). This collinear behavior is observed for a number of residues, as shown in B: D66 (36), D11 (36), D10 (+), M81 (36), and V91 (36). Chemical shifts are shown relative to P-NtrC and therefore reflect the relative population between inactive and active conformers for each mutant.
Measuring Slow Chemical Exchange Processes
(ligand binding, cis/trans proline isomerization)

Two-site exchange

\[
\begin{align*}
A \quad & \xrightleftharpoons[k_a]{k_b} \quad B \\
\text{IsdC:heme} \quad & \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} \quad \text{IsdC + heme}
\end{align*}
\]

\[k \ll \Delta \omega, \quad \tau \gg 1/\Delta \omega\]

Fig. 1.50 Typical spectra reflecting exchange at different rates relative to the chemical shifts. (Reprinted from Ref. 34 with permission.)
Heme binding kinetics from ZZ-exchange NMR

\[ \text{IsdC:heme} \xrightleftharpoons{k_{\text{off}}}^{k_{\text{on}}} \text{IsdC + heme} \]

(holo) (apo)

Table S1: Summary of the average \( k_{\text{on}}, k_{\text{off}}, \) and \( k_{d} \) values obtained using the H2O/C N-exchange experiment

<table>
<thead>
<tr>
<th>System</th>
<th>( k_{\text{on}} ) (s(^{-1}))</th>
<th>SD</th>
<th>( k_{\text{off}} ) (s(^{-1}))</th>
<th>SD</th>
<th>( k_{d} ) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>H76</td>
<td>5.52</td>
<td>0.50</td>
<td>5.67</td>
<td>0.42</td>
<td>1.93×10(^{5})</td>
</tr>
<tr>
<td>W77/1</td>
<td>3.42</td>
<td>0.21</td>
<td>4.88</td>
<td>0.33</td>
<td>1.66×10(^{5})</td>
</tr>
<tr>
<td>G80</td>
<td>7.84</td>
<td>0.61</td>
<td>7.01</td>
<td>0.56</td>
<td>2.38×10(^{5})</td>
</tr>
<tr>
<td>S107</td>
<td>5.90</td>
<td>0.42</td>
<td>2.63</td>
<td>0.18</td>
<td>8.94×10(^{6})</td>
</tr>
<tr>
<td>G115</td>
<td>6.31</td>
<td>0.44</td>
<td>4.67</td>
<td>0.35</td>
<td>1.59×10(^{5})</td>
</tr>
<tr>
<td>G127</td>
<td>8.30</td>
<td>0.76</td>
<td>5.82</td>
<td>0.53</td>
<td>1.98×10(^{6})</td>
</tr>
</tbody>
</table>

*Standard deviations were calculated from 150 repeats of a Monte-Carlo procedure.

* Calculated based on a \( K_{d} \) of 0.34 μM.

Robson SA, Peterson R, Bouchard I S, Villarreal VA, Clubb RT. J Am Chem Soc. 2010; 132:9522-