NMR relaxation detects pervasive motions in proteins on many timescales

Dynamics research in the Zuiderweg Lab
1993-2010
Common NMR relaxation

Hinge motion  Allosteric motion
Residue motion  Domain motion  Protein Folding

NH  $T_1, T_2$

Residual Dipolar Coupling

$T_2, T_2$ CPMG  NH exchange
\[ E_{DD} = \mu_a \mu_b/r^3 \times [3\cos^2\theta - 1] \]

\[ \text{Relax}_{\text{Eff}} \sim \langle E_{DD}^2 \rangle \sim K/r^6 F(t_c) \]
\[ R \propto K \frac{1}{r_{ab}^6} F\left(\tau_{MOL},\omega_{NMR}\right) \]

\[ R \propto K \frac{1}{r_{ab}^6} \left[ \frac{S^2 \tau_C}{1 + \omega^2 \tau_C^2} + \frac{(1 - S^2) \tau_L}{1 + \omega^2 \tau_L^2} \right] \]
15N-1H vector (amide) Order parameters of Lysozyme
NMR Dynamics and Entropy

Model motion as a probability distribution $P(\theta)$

and compute averaged spherical harmonics

$$\langle P_2(\cos \theta_{0-\infty}) \rangle = \frac{1}{4\pi} \int_0^{2\pi} P_2(\cos \theta) P(\theta) \sin \theta \, d\theta$$

Straightforward substitution yields order parameter

$$S_{NMR}^2 \propto \left\langle \frac{3}{2} \cos^2 \theta_{0-\infty} - \frac{1}{2} \right\rangle^2 = \langle P_2 \cos \theta_{0-\infty} \rangle^2$$

and relaxation rate

$$R \propto K \frac{1}{r_{ab}^6} \left[ \frac{S^2 \tau_C^2}{1 + \omega^2 \tau_C^2} + \frac{(1 - S^2) \tau_L^2}{1 + \omega^2 \tau_L^2} \right]$$

Compare with experiment and adapt $P(\theta)$

Take best $P(\theta)$ and compute entropy from basic statistical mechanics

$$S_{CONFIG} = \int_0^{2\pi} P(\theta) \ln(P(\theta)) \sin \theta \, d\theta$$
Is the backbone really the best indicator of motion
Structure of Calmodulin with and without peptide

Smooth muscle myosin light chain kinase peptide
Dynamics of Calmodulin with and without peptide

Differences in order parameter (bound-free)

The NH may not see motions the C-C can

Differences in order parameter (bound-minus-free)

Dynamics of Calmodulin with and without peptide

Wang, T., King Frederick, K., Igumenova, T.I., Wand, A.J. and Zuiderweg, E.R.P.
MD: peptide-plane Dynamics is dominated by “crank-shaft” motions around the CA-CA direction. This would imply that CO-CA order parameter should be larger than N-H order parameters

Y. Pang, EZ, Biochemistry, 41, 2655 (2002)
However, we generally find the average CO-Ca order parameter to be smaller than the NH order parameter.

Data points are experimental.
Blue line is a 1.6 ns MD simulation using the CHARMM-param22 forcefield, binase in water.

Pang, A., Buck, M., and Zuiderweg, E.R.P. Backbone Dynamics of the Ribonuclease Binase Active Site Area using Multinuclear (^{15}N and ^{13}C) NMR Relaxation and Computational Molecular Dynamics, Biochemistry, 41, 2655-2666 (2002)
and the COCA order parameter decreases faster than the NH order parameter upon increase in temperature

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ratio $S^2_{NH}(303) / S^2_{NH}(278)$</th>
<th>Ratio $S^2_{COCA}(303) / S^2_{COCA}(278)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binase</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>0.97</td>
<td>0.87</td>
</tr>
<tr>
<td>Cyt-b5</td>
<td>0.99</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Thus, there must be significant motion affecting the CO-CA order more than the NH order parameter – in a rigid peptide plane, only a motion around the NH vector would do this.
However, such a correlated motion is unlikely. We currently favor the idea that there is sufficient flexibility in the peptide plane that re-orientational motions affecting the Ca atom would not necessarily affect the N-H bond vector.

There is QM and IR evidence that such may indeed be the case (Mannfors, B. E.; Mirkin, N. G.; Palmo¨, K.; Krimm, S. J. Phys. Chem. 2003, A107, 1825-1832.)

Better QM-based forcefields for MD simulations are needed to settle this issue.
In subsequent research, we have taken a more practical approach: how to get proper order parameters for the motions that affect the CO?

Effective Lipari–Szabo order parameters and local correlation times for relaxation vectors of protein 13CO nuclei are extracted from a $^{13}$CO-$R_1$ experiment, a transverse $^{13}$CO CSA/$^{13}$CO-$^{13}$Ca CSA/dipolar cross correlation and a transverse $^{13}$CO CSA/$^{13}$CO–$^{15}$N CSA/dipolar cross correlation experiment. Given the global rotational correlation time from $^{15}$N relaxation experiments, the program COMFORD fits the $^{13}$CO data to an effective order parameter $S^2$ CO, an effective local correlation time and the orientation of the CSA tensor with respect to the molecular frame.

Wang, T., Weaver, D.S., Cai, S., Zuiderweg, E.R.P. 
We are trying to formulate a NMR relaxation paradigm that works for larger proteins.

The standard 15N-based paradigm does not work very well for proteins with $t_c > 15$ ns. The most problematic is the $1H \rightarrow 15N$ NOE experiment, which when properly performed, may take a month of instrument time. There also problems with the $R_2$ experiments – for the larger proteins one uses higher-field instruments which exacerbate $R_{ex}$ and the variations in $15N$ CSA relaxation.
A $^{15}$N paradigm suitable for larger proteins we try to develop uses $^{15}$N $R_1$

$R_2$ $^{15}$N-CSA/ $^{15}$N-$^1$H DD cross correlation

$R_1$ $^{15}$N-CSA/ $^{15}$N-$^1$H DD cross correlation

Model computations suggest that we can reliably fit for $\tau_c$, $S^2$, $\tau_{loc}$ and the $^{15}$N CSA out of of these upto $\tau_c$ 30 ns.

What is holding us back?

Lousy funding climate.
Experimental

$^{15}\text{N} R_1$: easy to run, easy on the probe, easy to interpret

$R_2$ $^{15}\text{N}$-CSA/ $^{15}\text{N}$-$^{1}\text{H}$ DD cross correlation:
easy to run, easy on the probe, easy to interpret

$R_1$ $^{15}\text{N}$-CSA/ $^{15}\text{N}$-$^{1}\text{H}$ DD cross correlation:
easy to run, easy on the probe, difficult to interpret
$R_1^{15}N$-CSA/ $^{15}N$-$^1H$ DD cross correlation:
Is difficult to interpret because 1H-1H NOE effects enter into the cross-relaxation matrix.

Earlier, we have tried to measure the NOEs and back-fit these in the relaxation matrix

More recently, we have been able to separate the effects using symmetric reconversion
We thus now have available the \( R_1 \) \(^{15}\text{N}\)-CSA/ \(^{15}\text{N}\)-\(^1\text{H}\) DD cross correlation rates, and are able to carry out the \(^{15}\text{N}\) relaxation on larger proteins.

What started as a nasty interference, the \(^1\text{H}\)-\(^1\text{H}\) relaxation rates extracted from the \( R_1 \) \(^{15}\text{N}\)-CSA/ \(^{15}\text{N}\)-\(^1\text{H}\) DD can be interpreted in terms of dynamics themselves:


The general applicability of this still needs to be further validated with MD runs.
Another approach to measure dynamics in proteins in a semi-quantitative way, is to just measure the intensity of cross peaks in HSQC, TROSY or HNCO.

Areas with low order parameters stand out with high peak intensities, areas with $R_{ex}$, stand out with low peak intensities.

This is almost exclusively due to variations in amide proton $R_2$ rates. Simulations show that dynamical effects on these rates have a much larger effect than variations in the local environment, provided one chooses an appropriate perdeuteration level.

An example is shown for the dynamical properties of a large protein (to be submitted)
Dynamical Properties of a 70 kDa protein

DnaK-Eco 1-605
NBD(ADP)-SBD(NRLLLTG)

Domain move with respect to each other

Local dynamics

HNCO Intensity / Flexibility

Residue

0 100 200 300 400 500 600 700

NBD Linker SBD LID

Disordered Tail

Local dynamics

Domains move with respect to each other
Common NMR relaxation

Hinge motion  Allosteric motion
Residue motion  Domain motion  Protein Folding

\[ \text{ps} \quad \text{ns} \quad \mu \text{s} \quad \text{ms} \quad \text{s} \]

NH \( T_1, T_2 \)

Residual Dipolar Coupling

\[ T_2, T_2 \text{CPMG}, \text{NH exchange} \]
Exchange Broadening and dynamics

\[ k_{\text{ex}} = 0 \]

\[ k_{\text{ex}} \ll |\omega_a - \omega_b| \]

\[ k_{\text{ex}} \sim |\omega_a - \omega_b| \]

\[ k_{\text{ex}} \gg |\omega_a - \omega_b| \]
How to detect milli/micro second dynamics

**Without CPMG**
- **Molecule N**: \( \omega = \omega_a \)
- **Molecule M**: \( \omega = \omega_b \)

**At t=0**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_b \)

**At t=\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_b \)

**At t=2\( \delta t \)**
- Molecule N: \( \omega = \omega_b \)
- Molecule M: \( \omega = \omega_a \)

**At t=3\( \delta t \)**
- Molecule N: \( \omega = \omega_b \)
- Molecule M: \( \omega = \omega_a \)

**At t=4\( \delta t \)**
- Molecule N: \( \omega = \omega_b \)
- Molecule M: \( \omega = \omega_a \)

**At t=5\( \delta t \)**
- Molecule N: \( \omega = \omega_b \)
- Molecule M: \( \omega = \omega_a \)

**At t=6\( \delta t \)**
- Molecule N: \( \omega = \omega_b \)
- Molecule M: \( \omega = \omega_a \)

**With CPMG**

**At t=0**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=2\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=3\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=4\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=5\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**At t=6\( \delta t \)**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)

**Apply \( \pi \) pulse**
- Molecule N: \( \omega = \omega_a \)
- Molecule M: \( \omega = \omega_a \)
Functional dynamics in the active site of the ribonuclease Binase

Wang, L., Pang, Y., Holder, T., Brender, J.R., Kurochkin, A, Zuiderweg, E.R.P.

Binase = Barnase

109 residues

Guanyl-specific ribonuclease

17 a.a. difference with barnase
Conformational exchange broadening in Binase
Binase MD averaged NMR structure

PDB Frame

Global Align Frame  Local Align Frames
RDC tensors deviate in all areas where we see exchange broadening, except for beta 3. This indicates that b3 is not moving.
Exchange broadening on the beta sheet is an induced effect.
Eliminating the induced broadening from the picture
Catalytic residues

Residues that interact with substrate

i.e. residues important to the protein function move

Where it matters it moves!
Maximum turnover rate for these enzymes is 1400 s$^{-1}$

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$k_{\text{cat}}$</th>
<th>$K_M$ ($\mu$M)</th>
<th>$k_{\text{cat}}/K_M$ ($\text{M}^{-1}\text{s}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binase</td>
<td>GpU</td>
<td>0.4</td>
<td>230</td>
</tr>
<tr>
<td>Barnase</td>
<td>GpU</td>
<td>4.3</td>
<td>150</td>
</tr>
<tr>
<td>Binase</td>
<td>Poly-I</td>
<td>141</td>
<td>80</td>
</tr>
<tr>
<td>Barnase</td>
<td>Poly-I</td>
<td>1413</td>
<td>130</td>
</tr>
</tbody>
</table>

from Schulga et al, Prot. Engin. 11, 775, 1998
NMR-detected dynamics is at roughly the same time scale

- Conformational exchange rates in Binase (s-1).
- Gln 28 $3.8 \times 10^3 \pm 600$
- Phe 55 $2.4 \times 10^3 \pm 120$
- Trp 70 $2.7 \times 10^3 \pm 200$
- Leu 97 $2.7 \times 10^3 \pm 700$
- Tyr 102 $4.7 \times 10^3 \pm 800$
- Ala 103 $6.1 \times 10^3 \pm 1200$
Functional Dynamics?

- Apparently, $k_{cat\ max}$ corresponds to the flap dynamics rate
- Apparently, $k_{cat\ max}$ reflects product release rate (exit rate)
GCTase, a negatively cooperative enzyme

Binding sites identical, Expect identical local binding free energies

First CTP
\[ \Delta G = -7.3 \text{ Kcal/M} \]

Second CTP
\[ \Delta G = -4.6 \text{ Kcal/M} \]

Thus:
2.7 Kcal/M of binding free energy lost on interface

$^{15}$N R$_2$ relaxation with and without exchange broadening suppression

$GCT$  $GCT(CTP)$  $GCT(CTP)_2$
Therefore, the allosteric free energy of negative cooperativity has an entropic component.
The allosteric free energy of positive cooperativity has an entropic component.

Students associated with dynamics research in the Zuiderweg lab:

Mark Fischer (1992-1998 Ph.D.)
Yuxi Pang (1997-2001 Ph.D.)
Daniel Weaver (2004-2009 Ph.D.)

Ananya Majumdar, Ph.D. (1997)
Tianzi Wang, Ph.D. (2001-2005)
Supported by

NSF MCB 9513355  2/1/96-1/31/99
Study of Isotropic and Anisotropic motions in proteins involving 13CO and 15ND NMR relaxation

NSF MCB 0135330  2/1/99-1/31/07
Motional modelling by NMR