Long Range Distance Restraints in Spin Labeled Proteins Probed by Solid-State NMR

Christopher Jaroniec

Department of Chemistry
The Ohio State University
Dipolar Couplings and Molecular Structure

\[ D_{IS} \propto \frac{\gamma_I \gamma_S}{r_{IS}^3} \]

\( \alpha \)-spectrin SH3 domain
(~300 \(^{13}\)C-\(^{13}\)C restraints)

M.H. Levitt, “Spin Dynamics”

- Dipolar coupling measurements are key for structural studies
- “Standard” methodology in solution NMR (e.g., NOESY); analogous methods emerging for MAS solid-state NMR
• Measurement of long-range (> ~5 Å) distances is critical (e.g., protein fold, intermolecular interactions, etc.)

• Complicated by small $D_{IS}$ and/or multi-spin effects
Studies of Paramagnetic Proteins

- Hyperfine coupling in general leads to contact & pseudocontact shifts, and enhanced nuclear spin relaxation (see Y. Ishii’s talk)

- Neglect contact & pc shifts for long-range measurements and paramagnetic species with small g-anisotropy

- Well-known effects: used in solution NMR of proteins since 1960’s
Nuclear Spin Relaxation Mechanisms

- Modulation of magnetic field at the nucleus leads to relaxation
Solomon Equations: Paramagnetic Relaxation Enhancement (PRE)

\[ R_1 \approx \frac{2}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma I^2 g_e^2 \mu_B^2 S(S+1)}{r^6} \left( \frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{7\tau_c}{1 + \omega_S^2 \tau_c^2} \right) \]

\[ R_2, R_{1p} \approx \frac{1}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma I^2 g_e^2 \mu_B^2 S(S+1)}{r^6} \left( 4\tau_c + \frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{13\tau_c}{1 + \omega_S^2 \tau_c^2} \right) \]

\[ \tau_c^{-1} = T_{1e}^{-1} + \tau_r^{-1} + \tau_M^{-1} \text{ (solution); } \tau_c^{-1} = T_{1e}^{-1} \text{ (solid); } |\omega_S| >> |\omega_I|; \ T_{1e} = T_{2e} \]

- \( R_1 \) and \( R_2 \) can be related to the electron-nucleus distance \( (r) \) if the electronic relaxation time constant \( (T_{1e}) \) is known

Solomon, Phys. Rev. 99 (1955) 559
Typical $T_{1e}$ values (solution/RT) are in the range $10^{-13}$ to $10^{-7}$ s (larger $T_{1e}$ = larger transverse PRE).

Exact $T_{1e}$'s under SSNMR conditions not available: one potential limitation to quantitative distance measurements.

Calculated SSNMR PRE (S=1/2, 500 MHz)

- Longitudinal and transverse PRE varies strongly with $T_{1e}$: can be modulated by using different paramagnetic centers
- Significant PRE expected for distances of ~5-20 Å
• Cross-peak intensity reduced by transverse PRE
• Distances between paramagnetic center and all nuclei can be monitored simultaneously via a simple 2D/3D correlation spectrum
Introduction of Nitroxide Spin Labels into Diamagnetic Proteins

- General method, works best for proteins with no native cysteines
- Cysteine introduced via site-directed mutagenesis, followed by reaction with thiol specific paramagnetic reagent (Hubbell, 1989)
- Used routinely for EPR studies; more recently in solution NMR

Spin Labeling of Protein GB1 (56 aa)

- 3D solution & X-ray structures known (Gronenborn et al., Science 1991)
- Excellent model system for SSNMR (Rienstra et al., JACS 2005)
- R1/R1’ side-chain incorporated at solvent-exposed sites K28 & T53

GB1 plasmid DNA:
A.M. Gronenborn (U. Pittsburgh)
No Major Effects on Protein Fold

• Main CS differences ~±2 residues, and in spatial vicinity of R1-site
No Major Effects on Protein Fold

| SL location | >1σ from any $|\Delta \delta|_{\text{avg}}$ |
|-------------|---------------------------------|
| $28R1$      |                                 |
| $53R1$      |                                 |

<table>
<thead>
<tr>
<th></th>
<th>$^1H^N$</th>
<th>$^{15}N$</th>
<th>$^{13}C\alpha$</th>
<th>$^{13}C\beta$</th>
<th>$^{13}C'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$</td>
<td>\Delta \delta_{28R1}</td>
<td>_{\text{avg}}$ (ppm)</td>
<td>0.02(4)</td>
<td>0.08(18)</td>
<td>0.03(4)</td>
</tr>
<tr>
<td>$</td>
<td>\Delta \delta_{53R1}</td>
<td>_{\text{avg}}$ (ppm)</td>
<td>0.05(8)</td>
<td>0.2(6)</td>
<td>0.06(7)</td>
</tr>
</tbody>
</table>
SL Protein Samples for SSNMR

$^{12}C,^{14}N$ protein, $R1'$

$^{13}C,^{15}N$ protein, $R1$

Microdialysis (MPD:isopropanol)

Protein Microcrystals (~2-4 mg)

Pauli et al., JMR (2000)
McDermott et al., JBNMR (2000)
Martin & Zilm, JMR (2003)
Franks et al., JACS (2005)
2D $^{15}$N-$^{13}$C$_\alpha$ Spectra of R1/R1’ Proteins

- Significant variations in cross-peak intensity for R1-proteins
- No change in resonance frequencies; small changes in LW
PRE Due to Spin Label ($T_{1e} \sim 100$ ns)

Baldus et al., Mol. Phys. 95 (1998) 1197

- Cross-peaks from residues within $\sim 10$ Å of spin label are effectively suppressed during $^1H$-$^{15}N$ and $^{15}N$-$^{13}C_\alpha$ CP transfers

\[
M_{x,y} = \exp(-R_{1,\rho,para} t)
\]
$^{1}H/^{15}N$ T$_{1p}$ Measurements

**A)**

$^{1}H$

$^{15}N$

$\tau_{SL}$

**B)**

53R1 $^{1}H$ SL

- $\tau_{SL} = 0$ ms
- $\tau_{SL} = 0.4$ ms

**C)**

53R1 $^{1}H$ SL

- $\tau_{SL} = 0$ ms
- $\tau_{SL} = 0.2$ ms
- $\tau_{SL} = 0.4$ ms

**D)**

$^{1}H$

$^{15}N$

**E)**

53R1 $^{15}N$ SL

- $\tau_{SL} = 0$ ms
- $\tau_{SL} = 4$ ms

**F)**

53R1 $^{15}N$ SL

- $\tau_{SL} = 0$ ms
- $\tau_{SL} = 2$ ms
- $\tau_{SL} = 4$ ms
2D $^{15}$N-$^{13}$C$\alpha$ Spectra of R1/R1’ Proteins

- Significant variations in cross-peak intensity for R1-proteins
- No change in resonance frequencies; small changes in LW
Relation to GB1 Structure: 53R1

- Residues closest to R1 side-chain suppressed most effectively
Different set of lines suppressed in 28R1 relative to 53R1
Relation to GB1 Structure (28R1)

- Residues closest to R1 side-chain affected most significantly
Relation to GB1 Structure: Summary

Nadaud et al., JACS 129 (2007) 7502
Similar PRE profiles (\(^1\)H\(^N\) PRE during CP/INEPT is dominant)

PRE more pronounced in the solid-state (\(\tau_{c,\text{solid}} > \tau_{c,\text{solution}}\))
For R1 helix surface sites (e.g., 28R1):

\[
\begin{align*}
\chi_1 &= -60 \\
\chi_2 &= -60 \\
\chi_1 &= 180 \\
\chi_2 &= 60
\end{align*}
\]

\(\chi_5\) – little effect on distance
• Reasonable qualitative correlation between expected electron-nucleus distance and cross-peak intensity for $r_{en}$ up to $\sim 20 \text{ Å}$
Pulse Schemes for $T_1/T_{1ρ}$ Measurement

- Pseudo-3D: 2 chemical shift dimensions + relaxation (easily extended to pseudo-4D)
- Similar schemes for $^1H$ and $^{13}C$ relaxation measurements

Giraud et al., JACS 126 (2004) 11422
Preliminary Site-Resolved $^{15}$N $T_{1\rho}$ Measurements in 53R1
## Paramagnetic Metal Ions


![Diagram](image)

Ebright et al. (1992)

**EDTA-M binding:** Anderegg (1977), Powell (1979)

**T$_{1e}$ data:** Bertini & Luchinat, Coord. Chem. Rev. (1996)

<table>
<thead>
<tr>
<th>Ion</th>
<th>logK EDTA-M</th>
<th>S</th>
<th>T$_{1e}$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(II)</td>
<td>10.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>18.86</td>
<td>1/2</td>
<td>~1-5 x 10$^{-9}$</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>13.95</td>
<td>5/2</td>
<td>~10$^{-8}$</td>
</tr>
<tr>
<td>Gd(III)</td>
<td>17.30</td>
<td>7/2</td>
<td>~10$^{-8}$-10$^{-9}$</td>
</tr>
</tbody>
</table>
Preliminary Data: 53R1 vs. 53EDTA-Cu(II)

- Smaller $R_{2,para}$, larger $^{15}\text{N} \ R_{1,para}$ for Cu(II) as expected
- Similar $^{1}\text{H} \ R_{1,para}$ for R1 and Cu(II) - likely $^{1}\text{H}$ spin-diffusion
- Must be careful about metal ion exchange
Conclusions

• No fundamental limitations to MAS SSNMR studies of paramagnetic proteins

• $T_{1e}$ values in protein microcrystals appear to be similar to reported solution values

• Many potential applications:
  - Qualitative distance measurements up to ~20 Å in challenging biological systems
  - Spectral editing
  - Identification of ligand binding sites, …

• Tune magnitude of PRE by using different paramagnetic species

• Quantitative distance measurements?
Studies of Paramagnetic Solids

Small Molecules
(1980’s –)

• Bryant & co-workers, JACS (1983, 1986)
• Nayeem & Yesinowski, JCP (1988)
• Brough, Grey & Dobson, JACS (1993)
• McDermott & co-workers, JACS (1995)
• Heise et al., JACS (1999)
• Emsley & co-workers, JACS (2006)
• Polenova & co-workers, JPC (2006)

Proteins

• Bertini, Emsley & co-workers, Angew. Chem. (2007)
• Bertini & co-workers, JACS (2007)
• Ishii & co-workers, JMR (2007)
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