Solid-State NMR Structural Studies of Proteins Using Paramagnetic Probes

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Protein Structure by MAS Solid-State NMR

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

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

M.H. Levitt, “Spin Dynamics”

Oschkinat et al., *Nature* 2002

- Conventional methods rely on measurements of \(^{13}\)C-\(^{13}\)C, \(^{13}\)C-\(^{15}\)N
  and \(^1\)H-\(^1\)H dipolar couplings via 2D or 3D correlation spectra
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Protein Structure by MAS Solid-State NMR

HET-s Prion Fibrils
Meier et al.
*Science 2008*

Anabaena Sensory Rhodopsin
Ladizhansky et al.
*Nature Methods 2013*

Type-III Secretion System Needle
Lange et al.
*Nature 2012*

• High-resolution structural and dynamic analysis possible for proteins up to ~300 aa – insights into function and mechanism
Long-Range Restraints Are Critical

- **Unambiguous** $^{13}\text{C}-^{13}\text{C}/^{13}\text{C}-^{15}\text{N}$ restraints $>5$-6 Å are often limited:
  
  small coupling magnitudes, low S/N, multispin effects

- Can be extended to $\sim8$-10 Å using $^{1}\text{H}-^{1}\text{H}$ couplings with fast MAS:
  Reif, Zilm, Rienstra, Meier, Pintacuda, and others
Solid-State NMR of Proteins Modified with Paramagnetic Tags

- Intentionally introduce paramagnetic centers at specific sites as long-range structural probes due to large $e^*-n$ couplings

\[ \left| \frac{\gamma_e}{\gamma_H} \right| \approx 660 \]
Paramagnetic Effects in MAS Solid-State NMR

- **Contact shift**: e\(^{-}\)-density at nucleus, negligible for e\(^{-}\)-n distances > \(~5\ \AA\)
- **Pseudocontact Shift (PCS)**: centers with large electron g-anisotropy (Co\(^{2+}\), lanthanides)
- **Paramagnetic Relaxation Enhancement (PRE)**: centers with small g-anisotropy (NO, Cu\(^{2+}\))
Nuclear Paramagnetic Relaxation

\[ \tau_c^{-1} = T_{1e}^{-1} + \tau_r^{-1} + \tau_M^{-1} \approx T_{1e}^{-1} \]

- Fluctuation of direction/intensity of dipolar field generated by electron spin at nucleus leads to enhanced nuclear relaxation
Nuclear Paramagnetic Relaxation

\[ \Gamma_1 \approx \frac{2C}{r_{en}^6} \left( \frac{3T_{1e}}{1 + \omega_n^2 T_{1e}^2} + \frac{7T_{1e}}{1 + \omega_e^2 T_{1e}^2} \right) \]

\[ C = \frac{1}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \gamma_n^2 g_e^2 \beta_e^2 S(S + 1) \]

\[ \Gamma_2 \approx \Gamma_1 \rho \approx \frac{C}{r_{en}^6} \left( 4T_{1e} + \frac{3T_{1e}}{1 + \omega_n^2 T_{1e}^2} + \frac{13T_{1e}}{1 + \omega_e^2 T_{1e}^2} \right) \]
Nuclear Paramagnetic Relaxation

- PRE effects can be large for nuclei ~20 Å from paramagnetic center
- Effects can be modulated by using different paramagnetic centers
- Transverse PRE directly proportional to $T_{1e}$ (i.e., slowest relaxing centers cause largest PREs)
- Longitudinal PRE largest when $1/T_{1e}$ ~ nuclear Larmor frequency (in angular units)
Typical $T_{1e}$ values @ RT are in the range $10^{-13}$ to $10^{-7}$ s

$T_{1e}$ values approximately the same for proteins in solution and hydrated proteins in solid phase @ RT

Spin Labeling of Proteins


- R1/R1’ side-chains placed at solvent-exposed aa K28 or T53
- Protein fold not affected
- “Diluted” in microcrystals with unlabeled/diamagnetic protein
Paramagnetic Protein Samples for SSNMR

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

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

3:1

Microdialysis (MPD:isopropanol)

Protein microcrystals

Pauli et al., *JMR* (2000)
McDermott et al., *JBNMR* (2000)
Franks et al., *JACS* (2005)
SSNMR of Spin Labeled GB1-T53C Mutant

Diamagnetic

$^{15}\text{N}$ (ppm)

$^{13}\text{C}$ (ppm)

SSNMR of Spin Labeled GB1-T53C Mutant

Diamagnetic

Spin-Labeled

SSNMR of Spin Labeled GB1-T53C Mutant

Diamagnetic

Spin-Labeled

SSNMR of Spin Labeled GB1-T53C Mutant

- Signals from nuclei within ~10-12 Å of spin label are suppressed by large transverse PRE effects (mainly during initial $^1$H-$^{15}$N CP)

Qualitative Long-Range Distance Restraints

Solution vs. Solid-State PRE

- Similar overall $^1$H$^N$ PRE profiles during CP/INEPT (main effect)
- PRE more pronounced in the solid state (for GB1 $\tau_{c,\text{solid}} \gg \tau_{c,\text{solution}}$)
Initial SSNMR Studies of $^{13}C,^{15}N$-Metalloproteins

Pintacuda, Giraud, Pierattelli, Bockmann, Bertini, Emsley, Angew. Chem. Int. Ed. 2007, 46, 1079

**Biomolecular Solid-State NMR**

Solid-State NMR Spectroscopy of a Paramagnetic Protein: Assignment and Study of Human Dimeric Oxidized Cu$^{II}$–Zn$^{II}$ Superoxide Dismutase (SOD)**

Guido Pintacuda, Nicolas Giraud, Roberta Pierattelli, Anja Böckmann, Ivano Bertini, and Lyndon Emsley*

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Paramagnetic Ions Provide Structural Restraints in Solid-State NMR of Proteins

Stéphane Balayssac,† Ivano Bertini,*†,‡ Moreno Lelli,† Claudio Luchinat,†,‡ and Massimiliano Maletta†,‡

Balayssac, Bertini, Lelli, Luchinat, Maletta, JACS 2007, 129, 2218
PRE Tuning by Other Paramagnetic Centers

Ermacora et al., *PNAS* (1992)

<table>
<thead>
<tr>
<th>Species</th>
<th>logK EDTA-M</th>
<th>S</th>
<th>$T_{1e}$ (ns)</th>
<th>$\Gamma_2^{NO}/\Gamma_2^{M}$</th>
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<tr>
<td>Zn$^{2+}$</td>
<td>16.68</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cu$^{2+}$</td>
<td>18.86</td>
<td>1/2</td>
<td>~2</td>
<td>~50</td>
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<td>Mn$^{2+}$</td>
<td>13.95</td>
<td>5/2</td>
<td>~10</td>
<td>~0.85</td>
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<tr>
<td>nitroxide</td>
<td>-</td>
<td>1/2</td>
<td>~100</td>
<td>1</td>
</tr>
</tbody>
</table>

- Tune longitudinal and transverse PREs by using paramagnetic centers with different electronic properties
Quantitative Restraints via EDTA-Cu$^{2+}$ Tags & R$_1$ PREs

Nadaud, Helmus, Kall & Jaroniec, J. Am. Chem. Soc. 2009, 131, 8108
Determination of Protein Fold

Ishita Sengupta
Philippe Nadaud
Rapid Acquisition of Relaxation Data

- GB1-28EDTA-Cu$^{2+}$
- 2D $^{15}$N-$^{13}$CO-S$^{3}$E @ 40 kHz MAS

• 1 mg (~150 nmol) $^{13}$C,$^{15}$N-GB1
• Experiment time: 7 min

- 40 kHz MAS, low-power RF & short recycle delays with Cu(II)-tags:
  full trajectories in ~12-48 hours vs. ~5-7 days with ~10 kHz MAS & 1 µmol protein

$^{15}$N Longitudinal PREs for GB1-EDTA-Cu$^{2+}$ Mutants

- >200 $^{15}$N PREs (4-5 per aa) for set of 6 Cu$^{2+}$/Zn$^{2+}$ GB1 mutants in ~2-3 weeks
Quantitative Long-Range Distance Restraints

\[ \Gamma_1^N = R_1^N (\text{Cu}^{2+}) - R_1^N (\text{Zn}^{2+}) \]

- Quantitative $^{15}$N-Cu$^{2+}$ distances in ~10-20 Å range accessible
Comparison of Experimental & Predicted PREs

- Backbone torsion angles fixed to GB1 values
- Conformation of EDTA-Cu\(^{2+}\) refined subject to PRE restraints
- Good agreement overall for PREs > \(~0.1\) s\(^{-1}\)
Effect of Intermolecular $^{15}$N-Cu$^{2+}$ Couplings on Longitudinal $^{15}$N PRE Measurements

- ~25%
- ~15%
- ~10%

• ~15-20% dilution of $^{13}$C,$^{15}$N-protein appears optimal

• Several elevated PREs observed even at ~10% dilution: $Cu^{2+}$ binding to endogenous surface Asp/Glu sites

Nadaud, Sengupta, Helmus & Jaroniec, *J. Biomol. NMR* 2011, 51, 293
Observation of Cu$^{2+}$ Sites by Solution NMR

- For super-stoichiometric [Cu$^{2+}$]/[protein] ratios the Cu$^{2+}$ ions bind to surface Asp and Glu side-chains

Nadaud, Sengupta, Helmus & Jaroniec, J. Biomol. NMR 2011, 51, 293
Refinement with X-ray Data and PREs

No PREs

• Torsions for helix & strands fixed to X-ray values, loops randomized

Collaboration with Charles Schwieeters
Refinement with X-ray Data and PREs

No PREs with ~230 PREs

- Torsions for helix & strands fixed to X-ray values, loops randomized

Collaboration with Charles Schwieters
Refinement with TALOS+ and PREs

- De novo calculation gives correct global fold with 1.8 Å bb RMSD vs. X-ray

$^1$H Detection: $^2$H, $^{13}$C, $^{15}$N-GB1 @ 60+ kHz MAS

0.35 mg (~50 nmol) DCN-GB1
800 MHz
~2 min

3D CONH: ~10 min

Relative Intensity (a.u.)

- Full relaxation trajectories:
  ~3 hours via 2D’s
  ~14 hours via 3D’s

Dwaipayan Mukhopadhyay

• Quantitative $^1$H-$^{2+}$ distances can be measured on protonated background

Direct Estimation of Electron $T_1$ From PRE Data

\[
\frac{\Gamma_2^H}{\Gamma_1^N} \approx \frac{\gamma_H^2}{2\gamma_N^2} \left( \frac{4T_1e + \frac{3T_1e}{1 + \omega_H^2 T_1e^2} + }{1 + \omega_H^2 T_1e^2} \right) \left( \frac{\frac{3T_1e}{1 + \omega_N^2 T_1e^2} + }{7T_1e} \right)
\]

Average for 14 residues with largest PREs ($\Gamma_1^N > 0.1 \text{ s}^{-1}$)

$T_{1e} \approx 2.5 \text{ ns}$
(range 1.8 – 3.1 ns)

- Good agreement with $T_{1e}$’s determined for several Cu$^{2+}$ metalloproteins in solution at ambient temp (Banci et al, Mag Res Rev 1986)

$T_{1e}: 1.8$-$5.7 \text{ ns (avg. 2.8 ns)}$

Oligomeric State of Membrane-Bound 7-Helix Sensory Rhodopsin from PREs

Wang, Munro, Kim, Jung, Brown & Ladizhansky, JACS 2012, 134, 16995
PrP23-144 Amyloid Strains and Species Barriers

Jones & Surewicz, Cell 2005, 121, 63

- Ordered ~30-residue C-terminal parallel-in-register amyloid β-core

Helmus et al. PNAS 2008, 105, 6284; JACS 2010, 132, 2393; JACS 2011, 133, 13934; Jones et al. JBC 2011, 286, 42777
$^{13}$C-$^{13}$C \& $^{13}$C-$^{15}$N Distances in huPrP23-144 Fibrils

- $\sim70$ $^{13}$C-$^{13}$C and $^{13}$C-$^{15}$N distances $>3$ Å
- Assignments and structural restraints facilitated by use of specifically methyl labeled samples ($A^\beta_\gamma^2L^\delta_2V^\gamma_2 \& M^\varepsilon_1^\delta_1$)

Theint Theint
SSNMR of Spin Labeled huPrP23-144 Fibrils

- Signals from nuclei within ~10-12 Å of spin label effectively attenuated
Structural Model of huPrP23-144 Amyloid Core

- 176 total restraints (71 $^{13}$C-$^{13}$C/$^{15}$N distances >3 Å; 59 PREs; 46 bb torsions)
Higher Order Fibril Architecture

\[ \eta = \frac{0.48 \cdot MPL}{MW} \approx 1.99 \]

Intermolecular PREs

\( ^{15}N \) Longitudinal PRE (s⁻¹)

Counts

Mass / Length (kDa/nm)

100 nm

\(~30 \text{ nm}\)

\(~6 \text{ nm}\)

Horizontal Distance (nm)
Model of huPrP23-144 Fibril
Solvent Interfaces via $^{15}$N PREs with Cu$^{2+}$-EDTA

Aucoin et al., in preparation
Compact High-Affinity Cu^{2+} Binding Tags

Synthesis based on: Lacerda et al., *Polyhedron* (2007)

Ishita Sengupta  Min Gao  Rajith Arachchige
PRE Measurements: 28TETAC-Cu$^{2+}$ GB1

- Signals from nuclei within ~10 Å of Cu$^{2+}$ center strongly attenuated due to transverse PREs

Sengupta et al. J. Biomol. NMR 2015, 61, 1
PCS Measurements in Co$^{2+}$ Tagged GB1

$$\delta_{PCS} = \frac{1}{12\pi r_{en}^3} \left[ \Delta \chi_{ax} \left( 3 \cos^2 \theta - 1 \right) + \frac{3}{2} \Delta \chi_{rh} \sin^2 \theta \cos 2\phi \right]$$

Similar work on lanthanide binding bidentate tags: Ubbink et al.
Structure determination of a Co^{2+} metalloprotein aided by PCS restraints

Bertini, Bhaumik, De Paepe, Griffin, Lelli, Lewandowski, Luchinat JACS 2010, 132, 1032
Structure determination with PCS restraints from 4MMDPA-Co^{2+} proteins and CS-Rosetta

Li, Pilla, Yang et al. JACS 2013, 135, 8294
4MMDPA: Su, Otting et al. JACS 2008, 130, 10486
Summary

- Paramagnetic tags can be used as unique structural probes in MAS solid-state NMR with many potential applications to biological solids:
  - Quantitative long-range distance measurements
  - Protein fold determination
  - Probing intermolecular contacts
  - Spectral editing & sensitivity enhancement
  - Identification of ligand binding sites
  - …
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