Solid-State NMR Structural Studies of Proteins Using Paramagnetic Probes

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Protein Structure by MAS Solid-State NMR

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

α-spectrin SH3 domain
(~300 $^{13}$C-$^{13}$C restraints)

M.H. Levitt, “Spin Dynamics”


• Conventional approaches rely on measurements of through-space $^{13}$C-$^{13}$C & $^{13}$C-$^{15}$N dipolar couplings in 2D/3D correlation spectra
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• High-resolution structural and dynamic analysis is now possible for proteins up to ~300 aa and multi-MDa assemblies of smaller subunits
Long-Range Distances Are Most Critical

- One bottleneck is the collection of large numbers of unambiguous >~5-6 Å distance restraints: small couplings, multispin effects, low S/N
Solid-State NMR of Proteins Modified with Paramagnetic Tags

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

\[ \left| \frac{\gamma_e}{\gamma_H} \right| \approx 660 \]
Paramagnetic Effects in MAS SSNMR Spectra

- **Contact shifts**: $e^*$-density at nucleus, negligible for $e^*$-$n$ distances $> \sim 5$ Å
- **PCS**: centers with large electron g-anisotropy (e.g., Co$^{2+}$, lanthanides)
- **Relaxation**: centers with small g-anisotropy (e.g., nitroxides, Cu$^{2+}$, Mn$^{2+}$, Gd$^{3+}$)
Nuclear Paramagnetic Relaxation

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

Solomon, Phys. Rev. 99 (1955) 559

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

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

\[ \Gamma_2 \approx \Gamma_1 \rho \approx \frac{C}{r_{en}^6} \left( 4T_{1e} + \frac{3T_{1e}}{1 + \omega_n^2 T_{1e}^2} + \frac{13T_{1e}}{1 + \omega_e^2 T_{1e}^2} \right) \]
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 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
Concept for PRE Based Protein SSNMR

- Electron-nucleus distances monitored simultaneously via resonance intensities in simplest 2D or 3D SSNMR spectra

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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

GB1 (56 aa)
Paramagnetic Protein Samples for SSNMR

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

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

3:1

Microdialysis (MPD:isopropanol)

Protein microcrystals

Pauli et al., JMR (2000)
McDermott et al., JBNMR (2000)
Martin & Zilm, JMR (2003)
Franks et al., JACS (2005)
SSNMR of Spin Labeled GB1-T53C Mutant

Diamagnetic

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

Initial SSNMR Studies of $^{13}$C,$^{15}$N-Metalloproteins


DOI: 10.1002/anie.200603093

**Biomolecular Solid-State NMR**

**Solid-State NMR Spectroscopy of a Paramagnetic Protein: Assignment and Study of Human Dimeric Oxidized Cu$^{	ext{II}}$–Zn$^{	ext{II}}$ 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†,‡

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

Species logK EDTA-M S T₁e (ns) Γ₂NO/Γ₂M
--- -------- ------ -------- ---------------
Zn²⁺ 16.68  -      -          -
Cu²⁺ 18.86 1/2    ~2        ~50
Mn²⁺ 13.95 5/2    ~10       ~0.85
nitroxide -      1/2      ~100     1

• Tune longitudinal and transverse PREs by using paramagnetic centers with different electronic properties

Ermacora et al., *PNAS* (1992)
Quantitative Restraints via EDTA-Cu$^{2+}$ Tags & R$_1$ PREs

Nadaud et al. JACS 2009, 131, 8108; Nadaud et al. JACS 2010, 132, 9561
Determination of Protein Fold
15N Longitudinal PREs for GB1-EDTA-Cu2+ Mutants

• >200 15N PREs (4-5 per aa) for set of 6 Cu2+/Zn2+ 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 \(^{15}\text{N-}\text{Cu}^{2+}\) distances in ~10-20 Å range accessible
Comparison of Experimental & Predicted PREs: Limited Data Set from Initial Studies

- Backbone torsion angles fixed to GB1 values
- Conformation of EDTA-Cu$^{2+}$ refined subject to PRE restraints
- Good agreement for PRE values > ~0.1 s$^{-1}$

Effect of Intermolecular Electron-Nucleus Interactions on PRE Measurements

- ~15-20% dilution of $^{13}\text{C},^{15}\text{N}$-protein in natural abundance matrix appears to be optimal, though not critical; several elevated PREs observed at ~10% dilution (secondary Cu$^{2+}$ binding site)

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 appear to bind to surface Asp and Glu side-chains

Nadaud, Sengupta, Helmus & Jaroniec, J. Biomol. NMR 2011, 51, 293
Rapid Acquisition of SSNMR Protein Spectra

- Fast data acquisition facilitated by rapid MAS, low-power RF & Cu(II)-tags (same idea as PACC approach introduced before by Ishii and others)

Sample: 1 mg (~150 nmol)
Experiment time: 7 min!

GB1-28EDTA-Cu^{2+}

Rapid 3D SSNMR: Sequential Assignments

- Complete “backbone walk” from two 3D SSNMR spectra of ~3 h each
- Sensitivity can be further improved with deuterated proteins & $^1$H detection ...

Refinement with X-ray Data and PREs

No PREs

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

Collaboration with C. Schwieters (NIH)
Refinement with X-ray Data and PREs

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

No PREs

With ~230 PREs

Collaboration with C. Schwieters (NIH)
Refinement with TALOS+ and PREs

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

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

Sengupta et al. J. Biomol. NMR 2015, 61, 1
• Signals from nuclei within ~10 Å of Cu$^{2+}$ center strongly attenuated due to transverse PREs
PCS Measurements in Co$^{2+}$ Tagged Proteins

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

Rajith Arachchige
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

![Graph and molecular structure image]

Li, Pilla, Yang et al. *JACS* 2013, 135, 8294
4MMDPA: Su, Otting et al. *JACS* 2008, 130, 10486
Oligomeric State of Membrane-Bound 7-Helix Sensory Rhodopsin from PREs
SSNMR of Spin Labeled Human PrP23-144 Fibrils
Higher Order huPrP23-144 Fibril Architecture

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

Intermolecular PREs

\[ \sim 5 \text{ nm} \]
Solvent Interfaces via $^{15}$N PREs with Cu$^{2+}$-EDTA: Similar to earlier work by Ishii, Reif

- Control
- 200 mM Cu(II)-EDTA

- A118
- I138

$^{15}$N Longitudinal PRE (s$^{-1}$)

- < 0.1 s$^{-1}$ (protected)
- 0.1 - 0.25 s$^{-1}$
- > 0.25 s$^{-1}$ (exposed)
- n.d.

Darryl Aucoin
• 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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