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

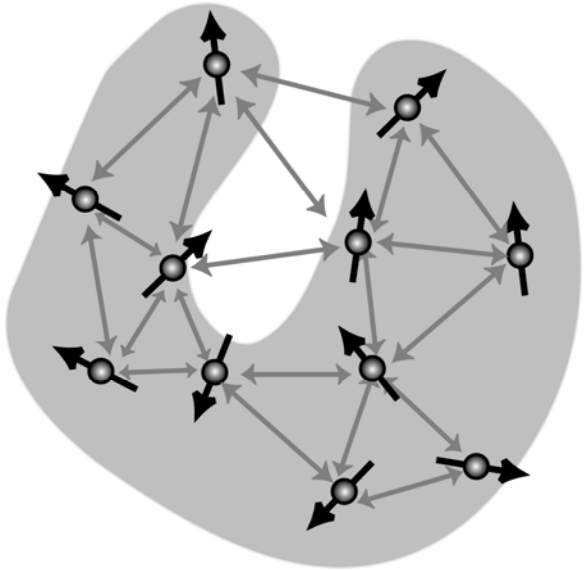
Christopher Jaroniec

Department of Chemistry

The Ohio State University

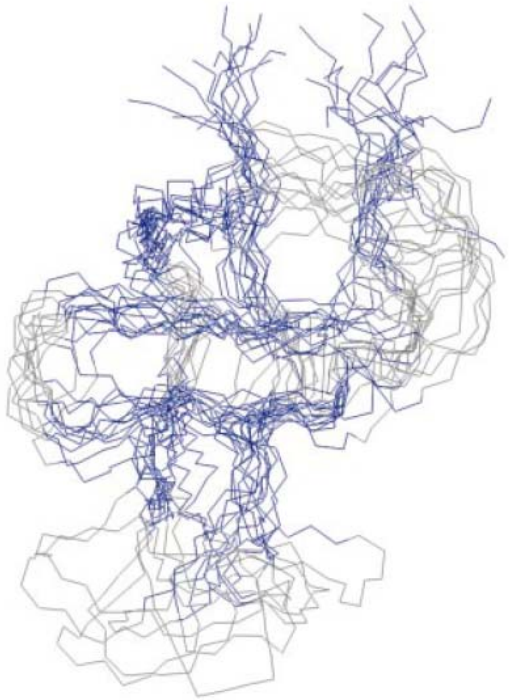
Dipolar Couplings and Molecular Structure

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



M.H. Levitt, "Spin Dynamics"

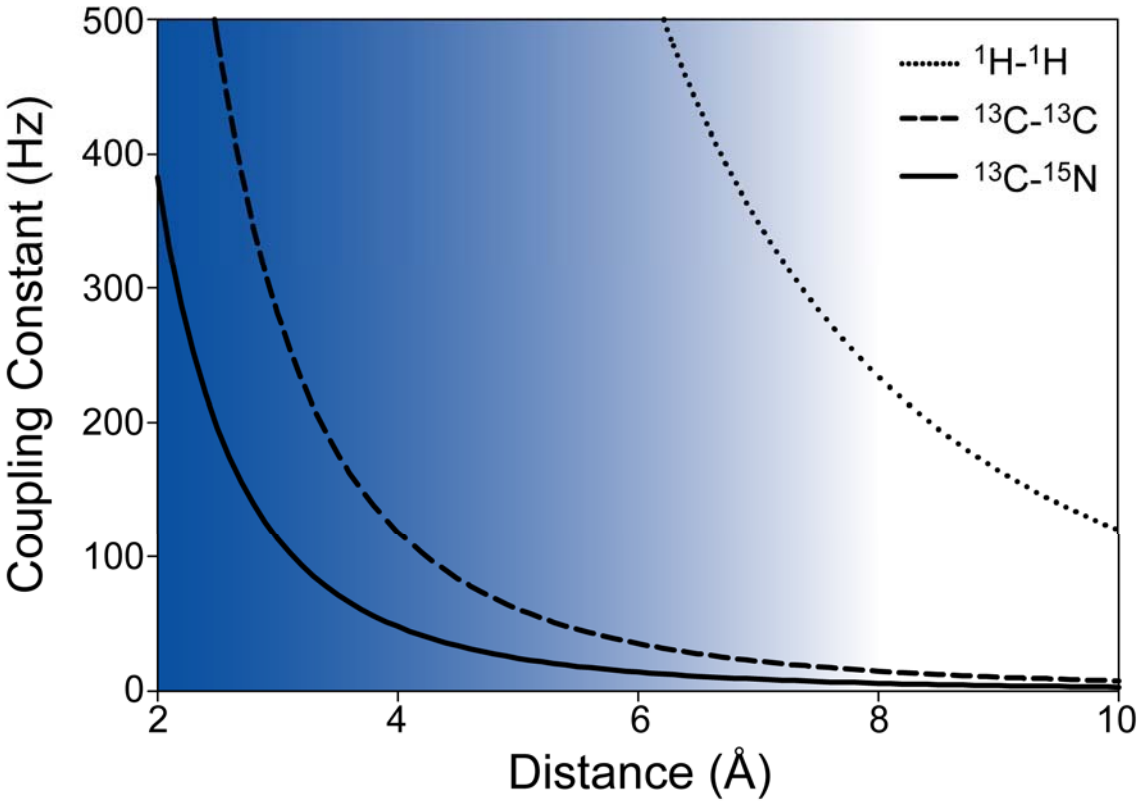
α-spectrin SH3 domain
(~300 ^{13}C-^{13}C restraints)

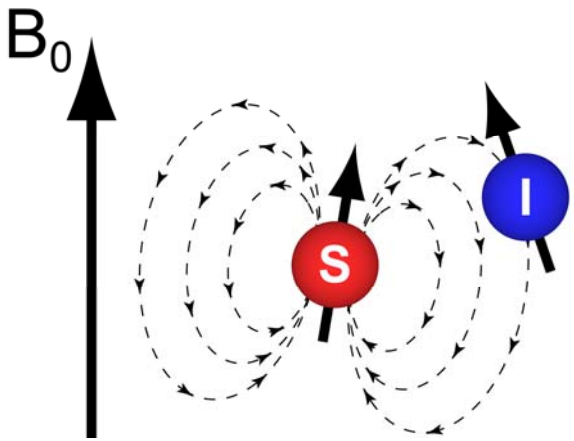


Castellani et al., Nature 420 (2002) 98

- **Dipolar coupling measurements are key for structural studies**
- **"Standard" methodology in solution NMR (e.g., NOESY); analogous methods emerging for MAS solid-state NMR**

Long-Range Restraints

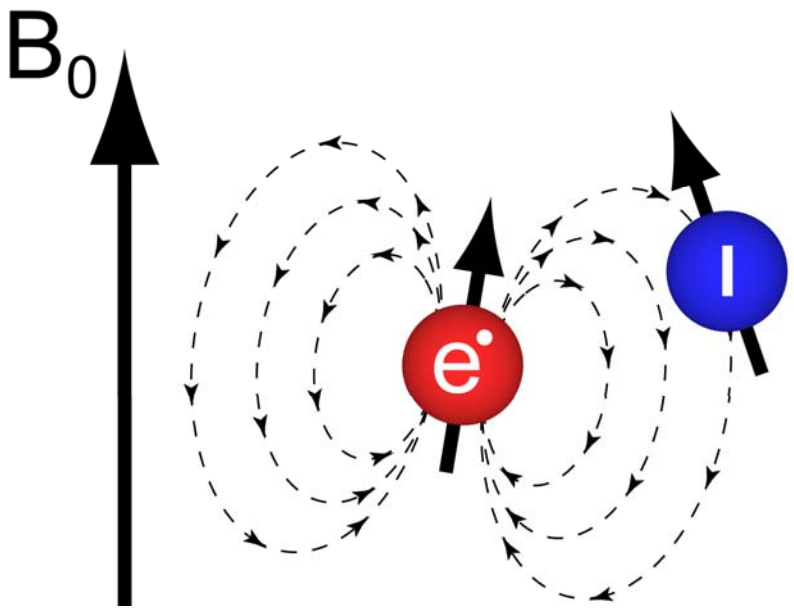




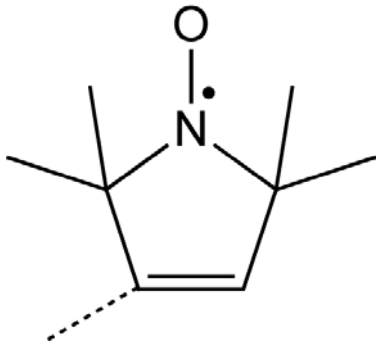
$$D_{IS} \propto \gamma_I \gamma_S / r_{IS}^3$$

- **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



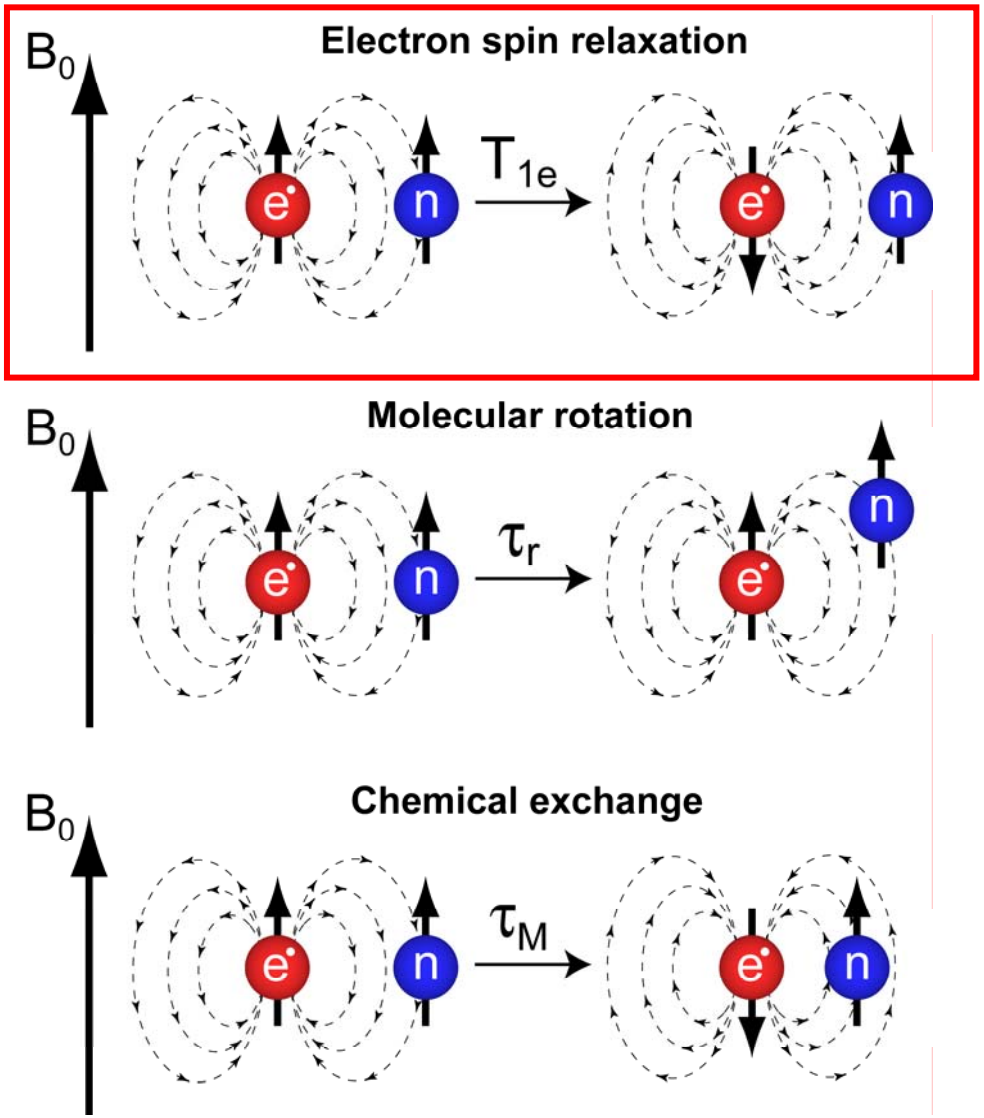
Nitroxide spin label



$$\left|\gamma_e / \gamma_H\right| \approx 660$$

- **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)

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

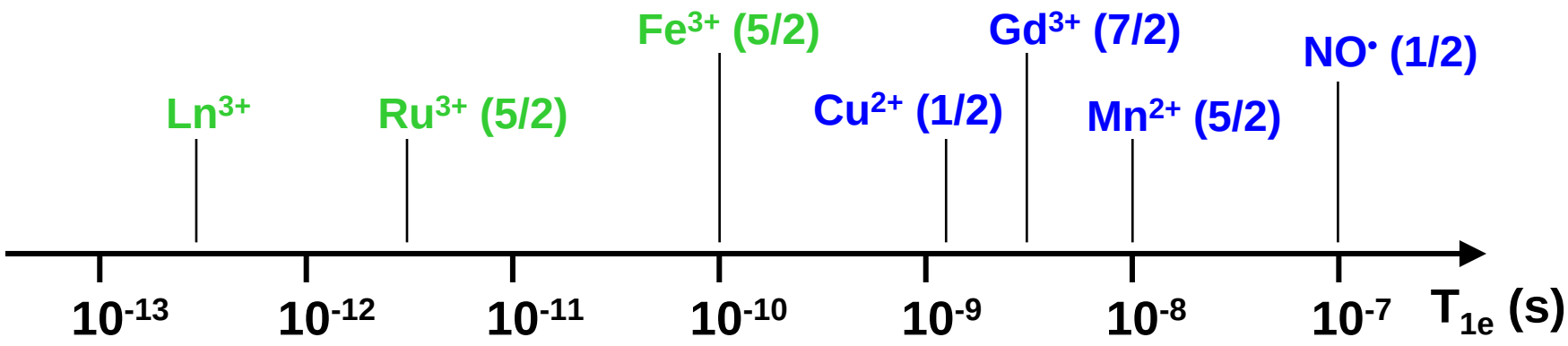
$$R_2, R_{1\rho} \approx \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left(4\tau_c+\frac{3\tau_c}{1+\omega_I^2\tau_c^2}+\frac{13\tau_c}{1+\omega_S^2\tau_c^2}\right)$$

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

- **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
Bertini & Luchinat, Coord. Chem. Rev. (1996)

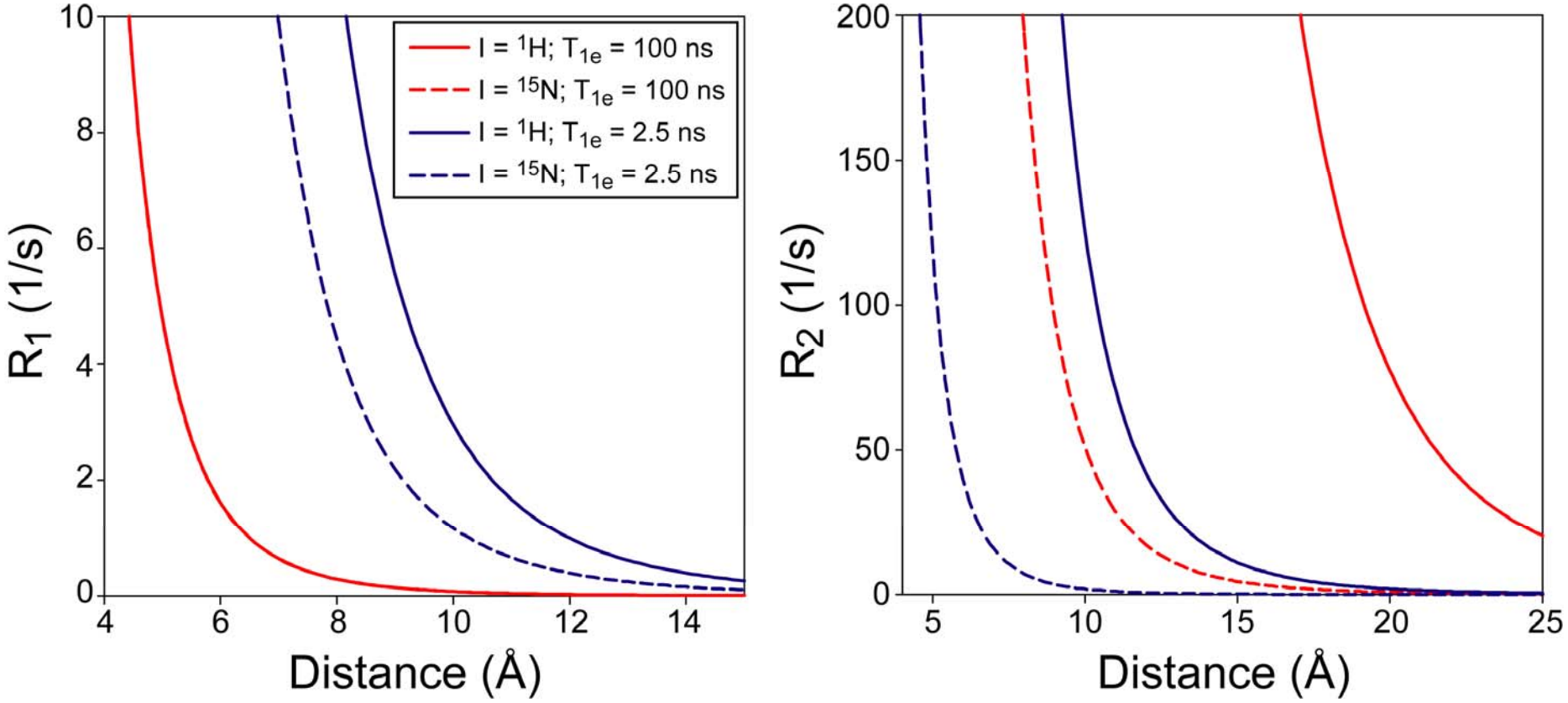
Electronic Relaxation Times



- 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

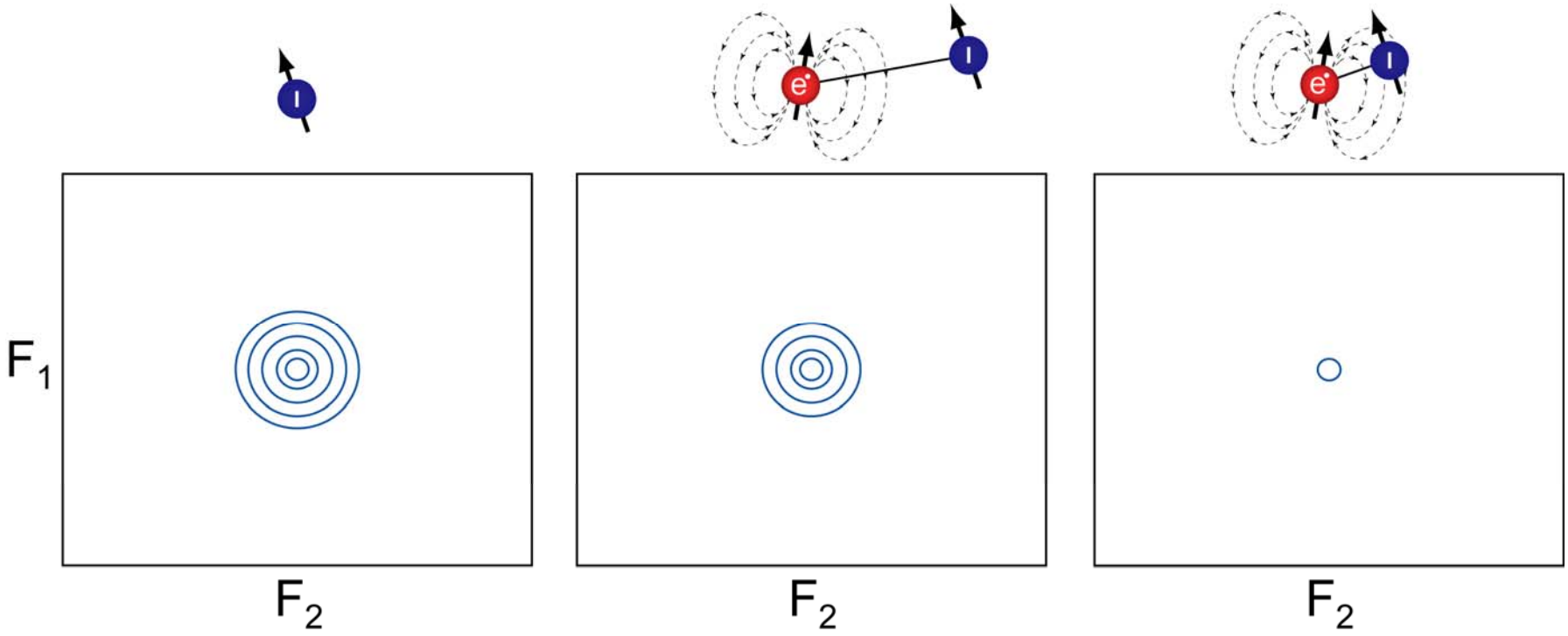
Bertini & Luchinat, Coord. Chem. Rev. (1996)
Eaton & Eaton, Biol. Magn. Res. (2000)

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



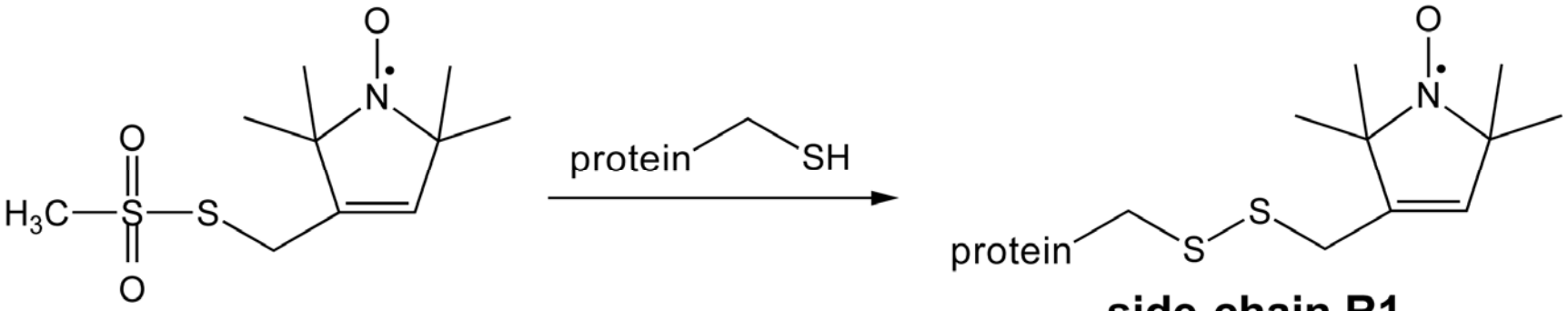
- **Longitudinal and transverse PRE varies strongly with T_{1e}: can be modulated by using different paramagnetic centers**
- **Significant PRE expected for distances of ~5-20 Å**

Simplest Version of Experiment



- **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

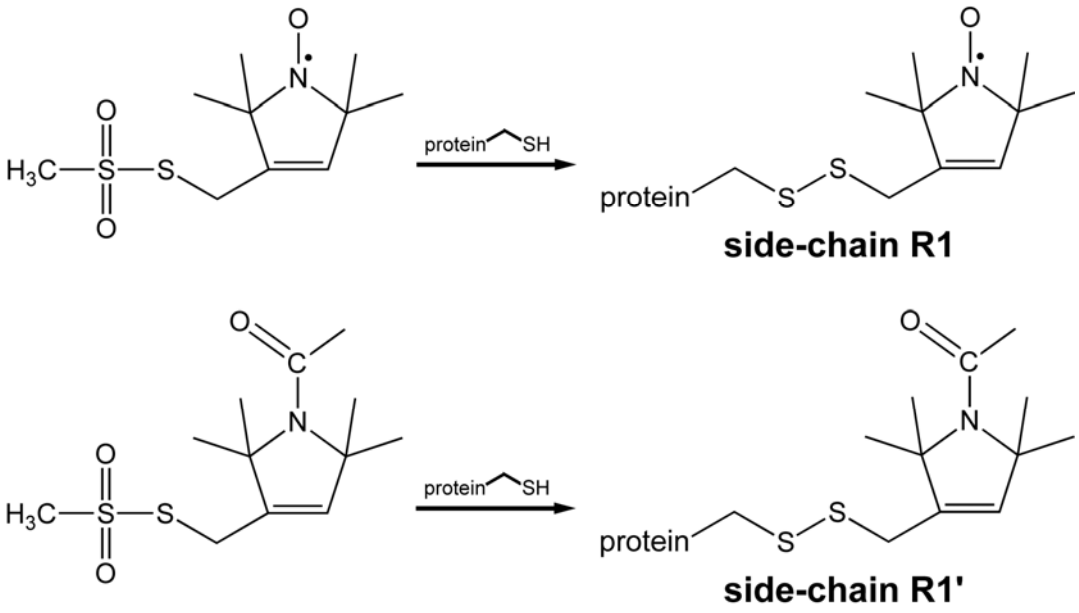


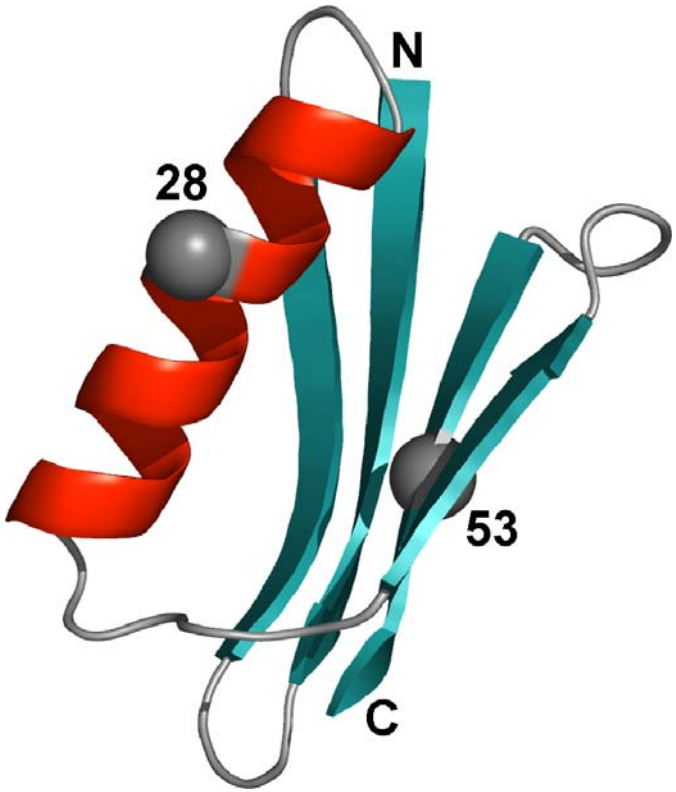
MTSL
Berliner et al. (1982)

- **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**

Kosen, Meth. Enzymol. (1989)
Hubbell & Altenbach, Curr. Op. Struct. Biol. (1994)

Spin Labeling of Protein GB1 (56 aa)

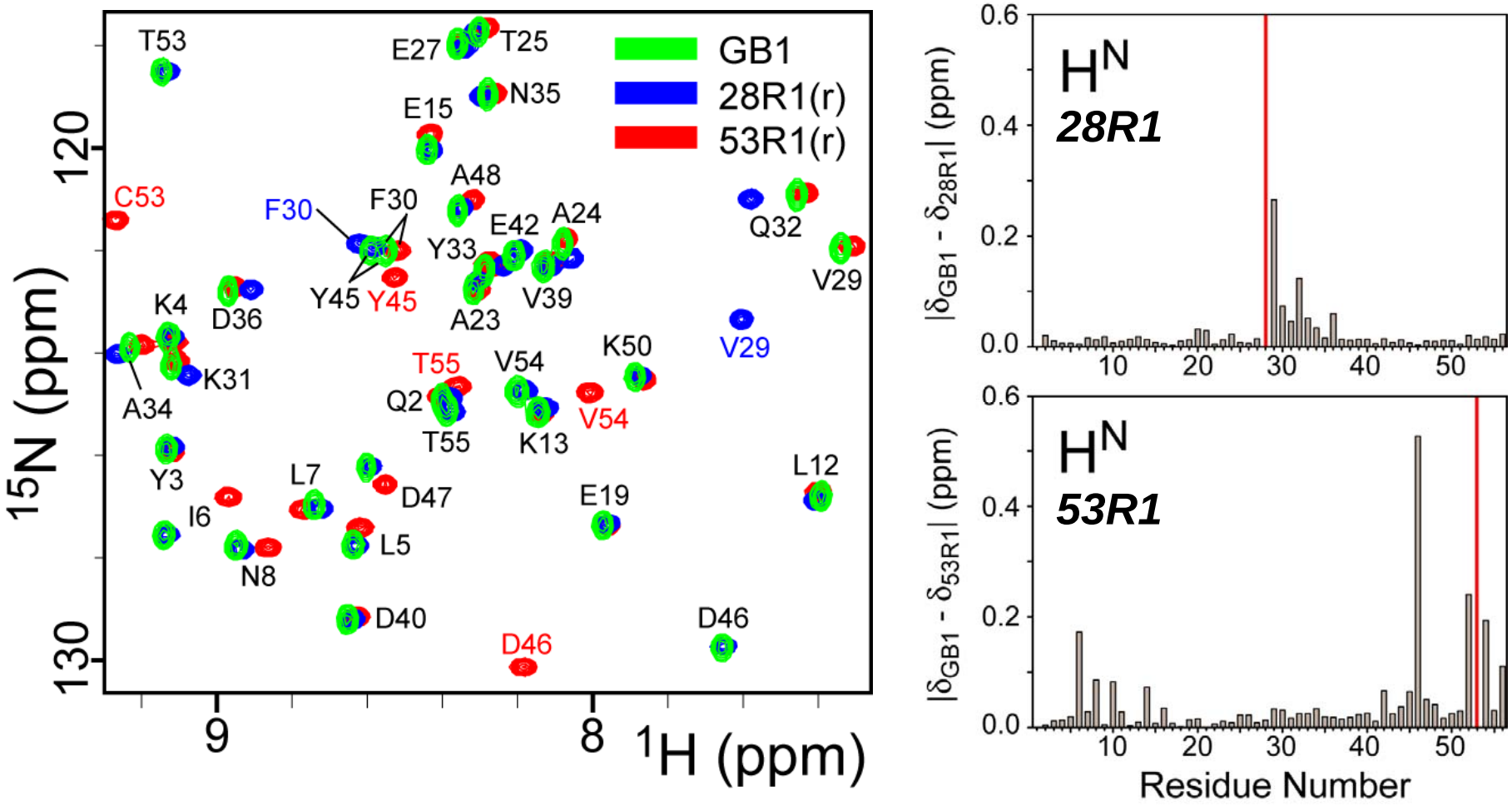




- **3D solution & X-ray structures known (Gronenborn et al., Science 1991)**
- **Excellent model system for SSNMR (Rienstra et al., JACS 2005)**
- **R1/R1' side-chain incorporated at solvent-exposed sites K28 & T53**

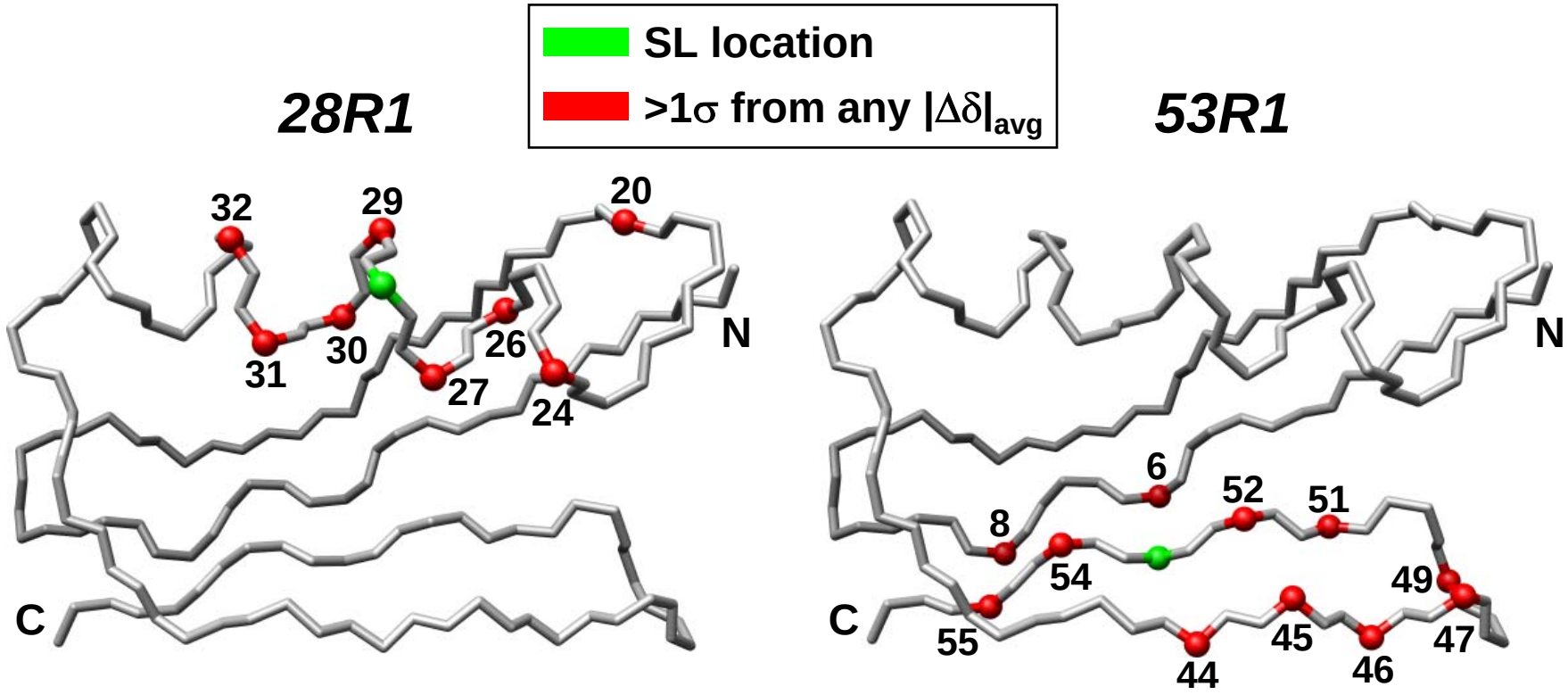
GB1 plasmid DNA:
A.M. Gronenborn (U. Pittsburgh)

No Major Effects on Protein Fold



- **Main CS differences ~±2 residues, and in spatial vicinity of R1-site**

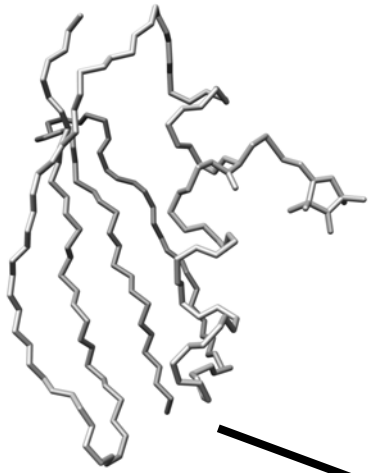
No Major Effects on Protein Fold



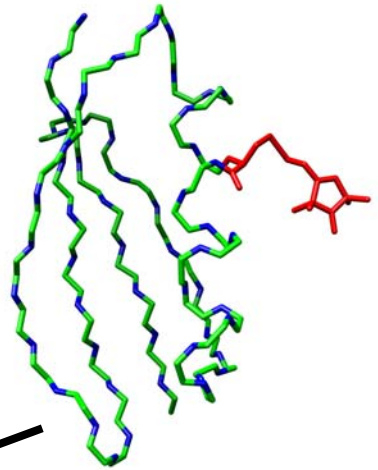
	$^{1}H^{N}$	^{15}N	$^{13}C\alpha$	$^{13}C\beta$	$^{13}C'$
$\lvert\Delta\delta_{28R1}\rvert_{avg}$ (ppm)	**0.02(4)**	**0.08(18)**	**0.03(4)**	**0.03(3)**	**0.02(4)**
$\lvert\Delta\delta_{53R1}\rvert_{avg}$ (ppm)	**0.05(8)**	**0.2(6)**	**0.06(7)**	**0.09(17)**	**0.10(21)**

SL Protein Samples for SSNMR

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



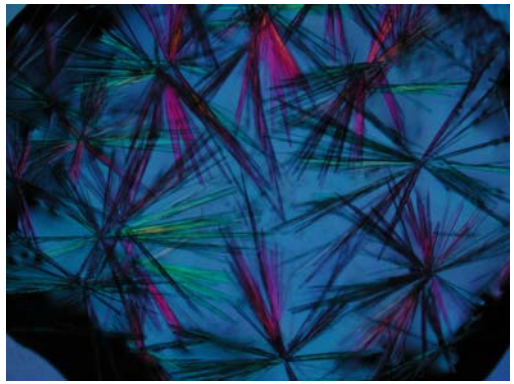
$^{13}C,^{15}N$ protein, R1



3:1

Microdialysis (MPD:isopropanol)

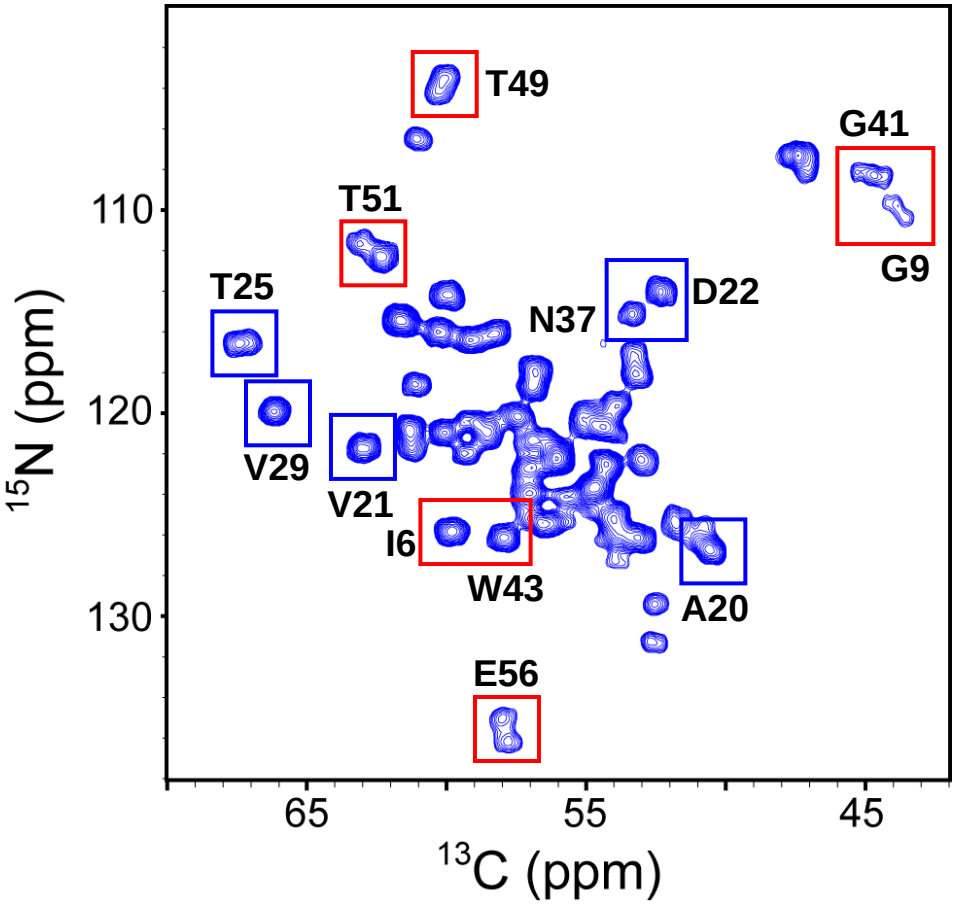
Protein Microcrystals (~2-4 mg)



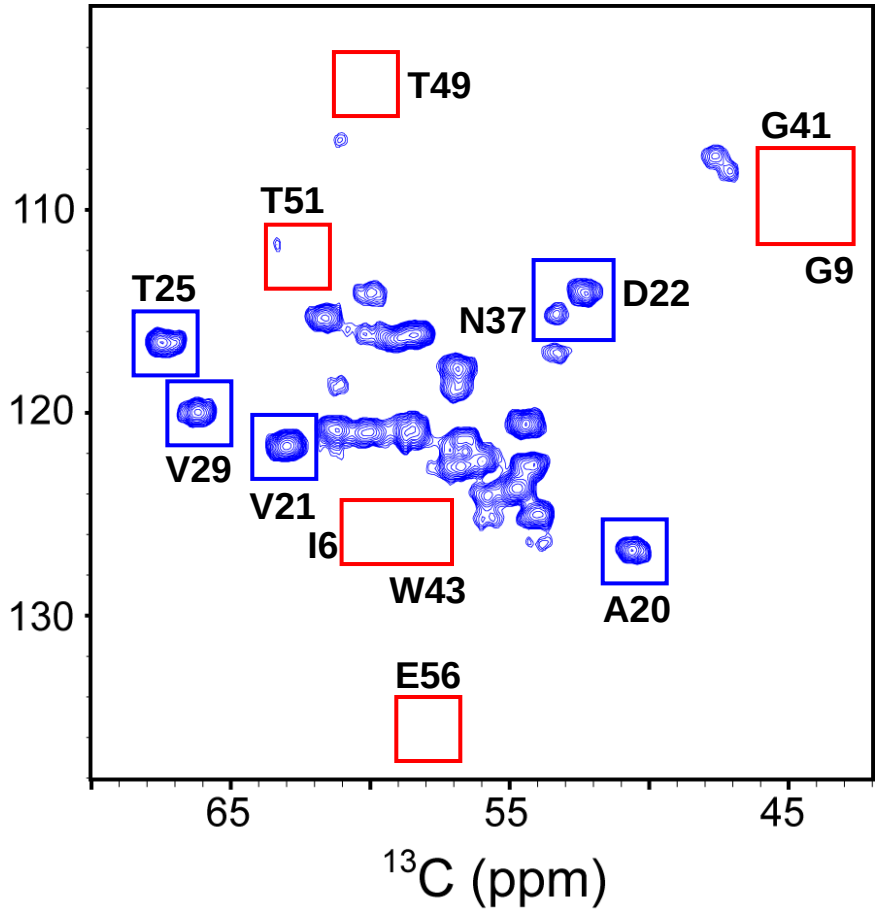
Pauli et al., JMR (2000)
McDermott et al., JBNMR (2000)
Martin & Zilm, JMR (2003)
Franks et al., JACS (2005)

2D ^{15}N-^{13}Cα Spectra of R1/R1' Proteins

Diamagnetic control (53R1')



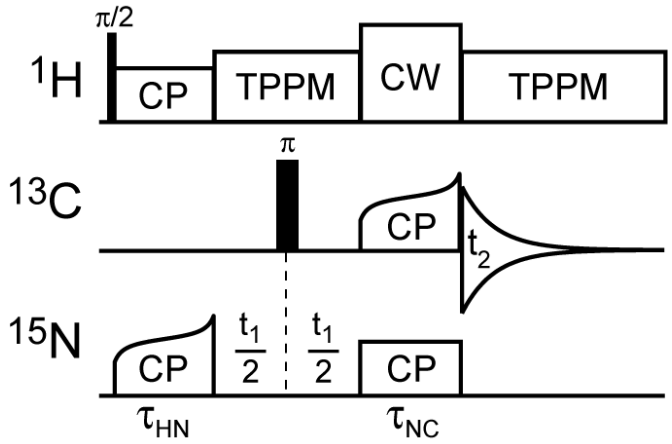
Spin-Labeled (53R1)



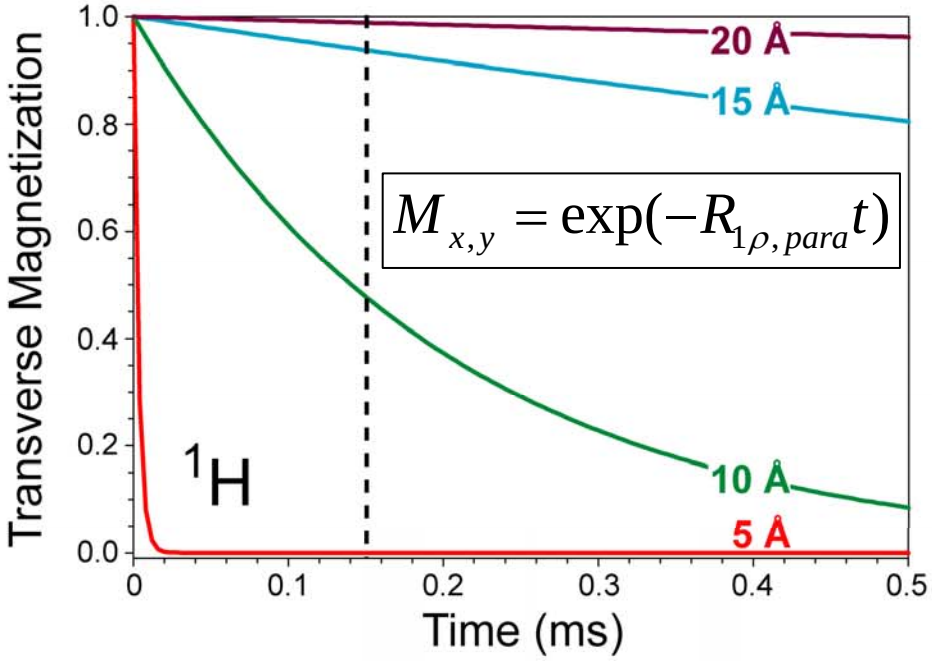
- **Significant variations in cross-peak intensity for R1-proteins**
- **No change in resonance frequencies; small changes in LW**

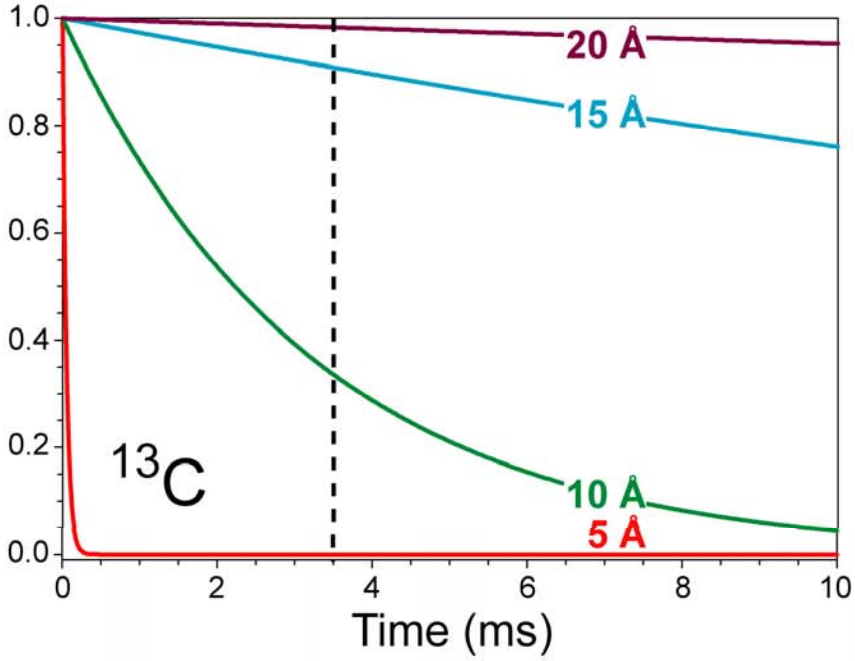
PRE Due to Spin Label (T_{1e} ~ 100 ns)

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

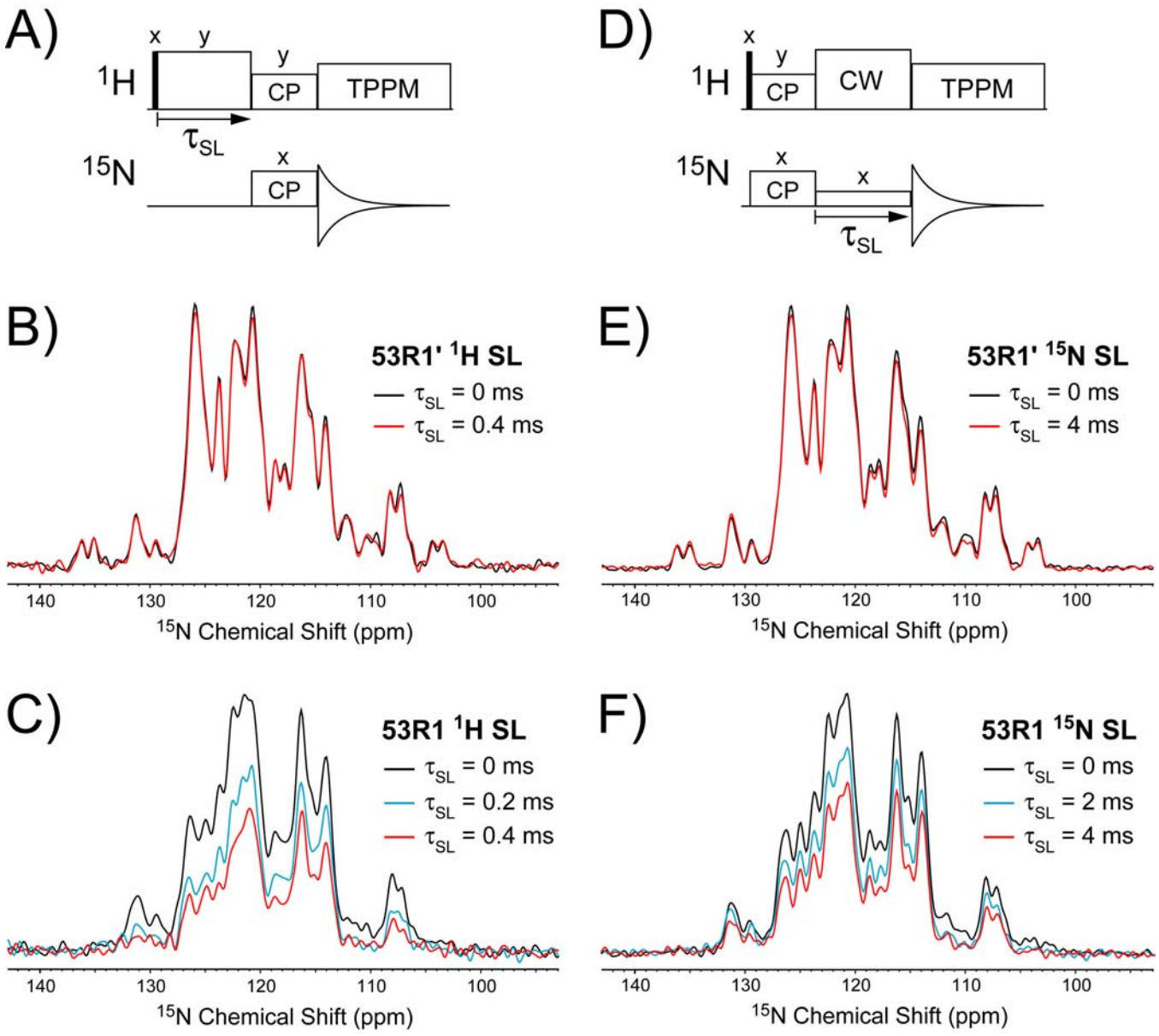


- **Cross-peaks from residues within ~10 Å of spin label are effectively suppressed during ^{1}H-^{15}N and ^{15}N-^{13}Cα CP transfers**



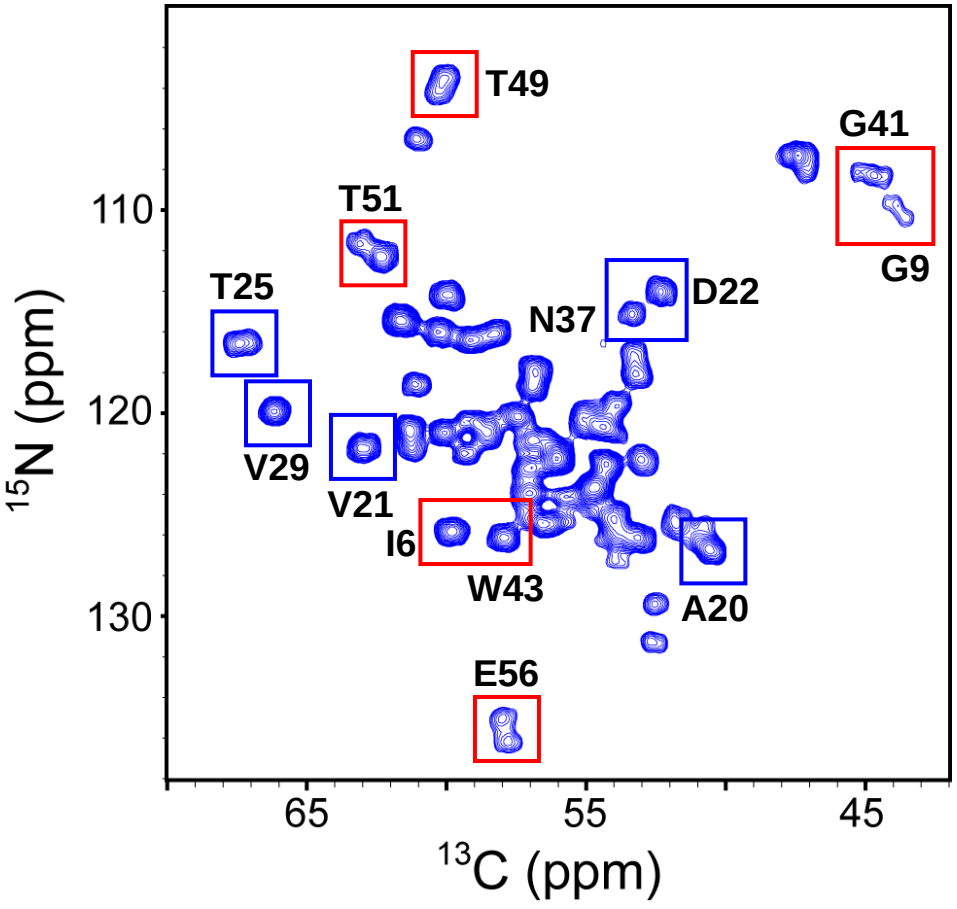


$^{1}H/^{15}N$ $T_{1\rho}$ Measurements

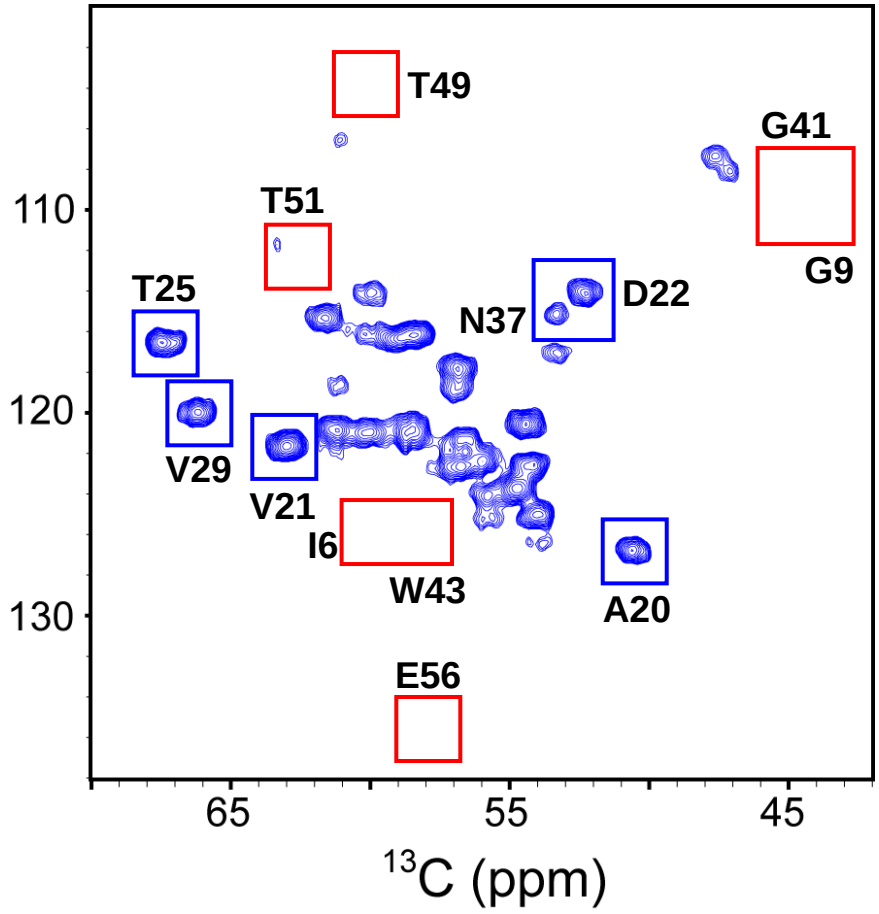


2D ^{15}N-^{13}Cα Spectra of R1/R1' Proteins

Diamagnetic control (53R1')

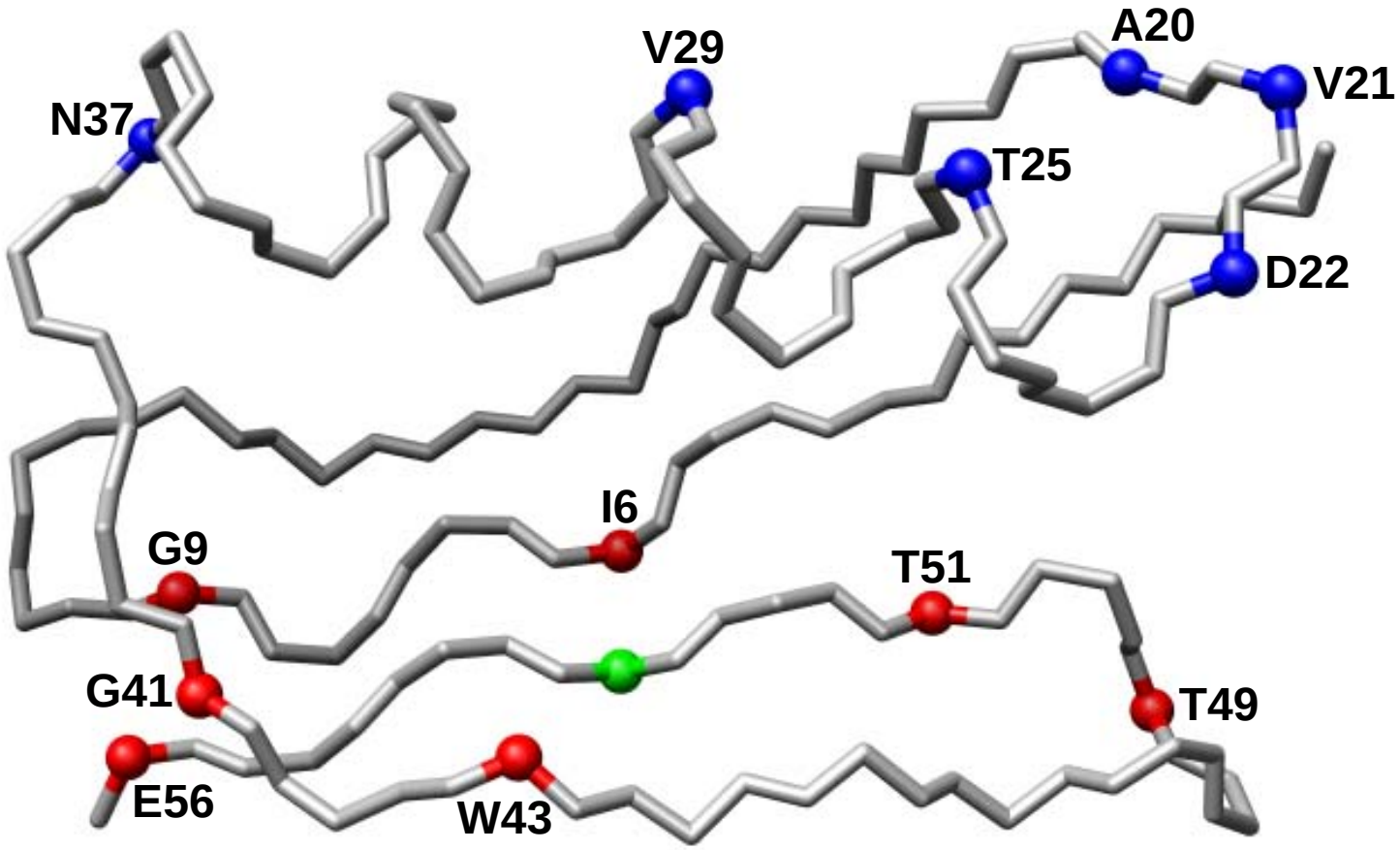


Spin-Labeled (53R1)



- **Significant variations in cross-peak intensity for R1-proteins**
- **No change in resonance frequencies; small changes in LW**

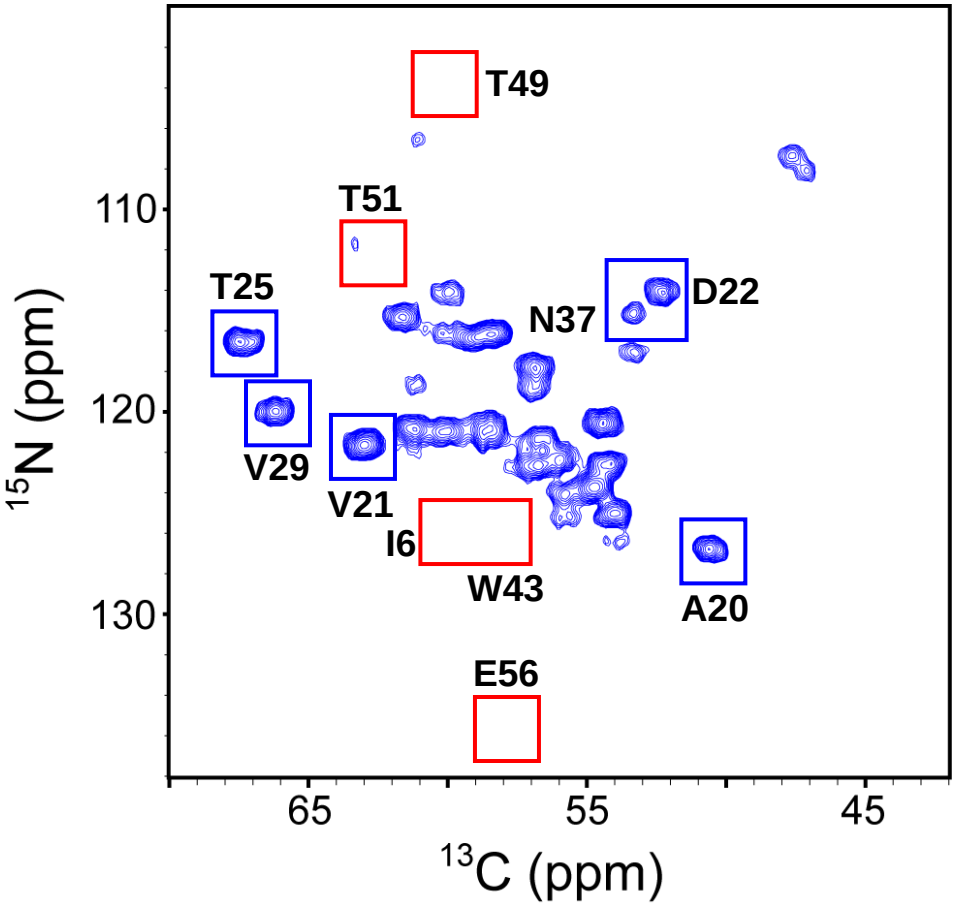
Relation to GB1 Structure: 53R1



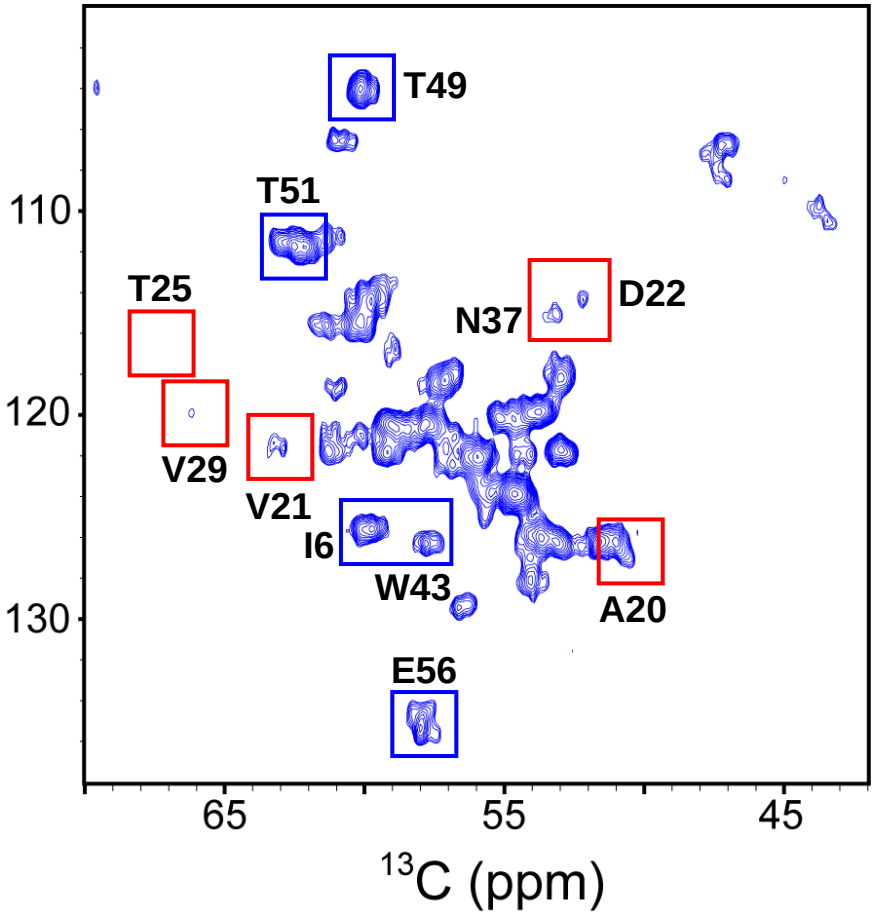
- **Residues closest to R1 side-chain suppressed most effectively**

2D ^{15}N-^{13}Cα Spectra of 53R1/28R1

Spin-Labeled (53R1)

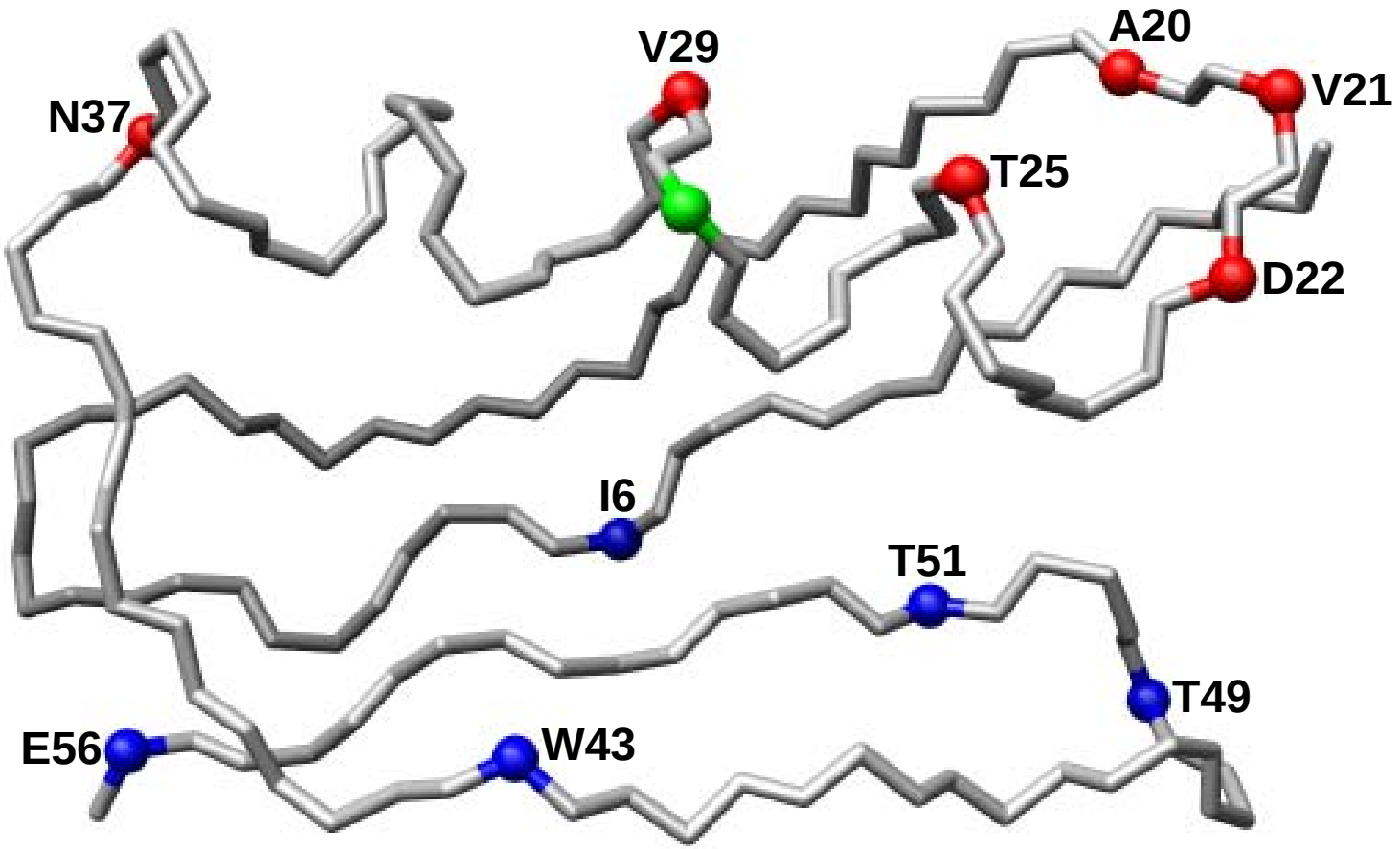


Spin-Labeled (28R1)



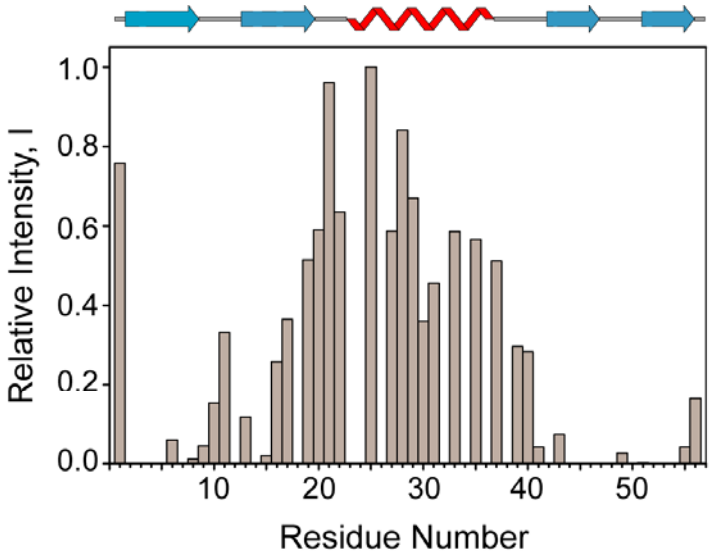
- **Different set of lines suppressed in 28R1 relative to 53R1**

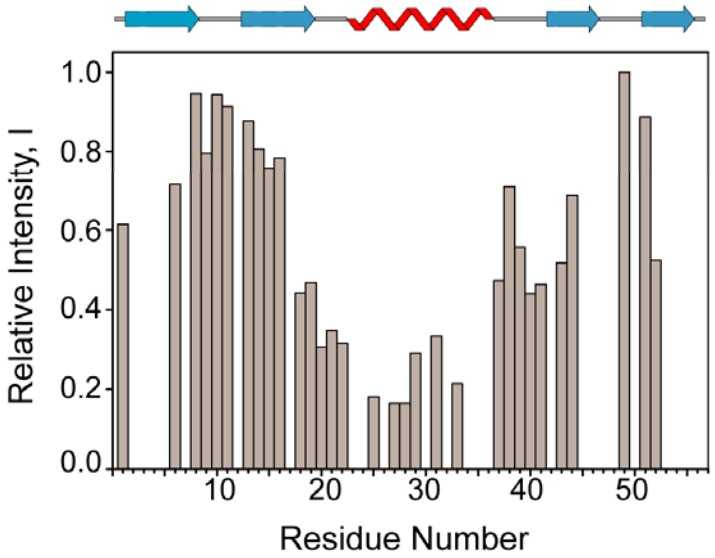
Relation to GB1 Structure (28R1)

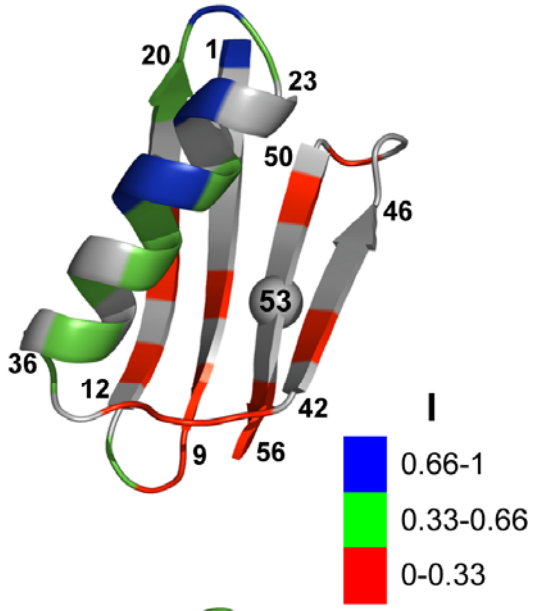


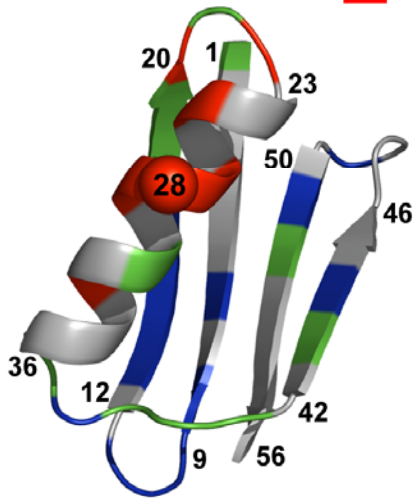
- **Residues closest to R1 side-chain affected most significantly**

Relation to GB1 Structure: Summary



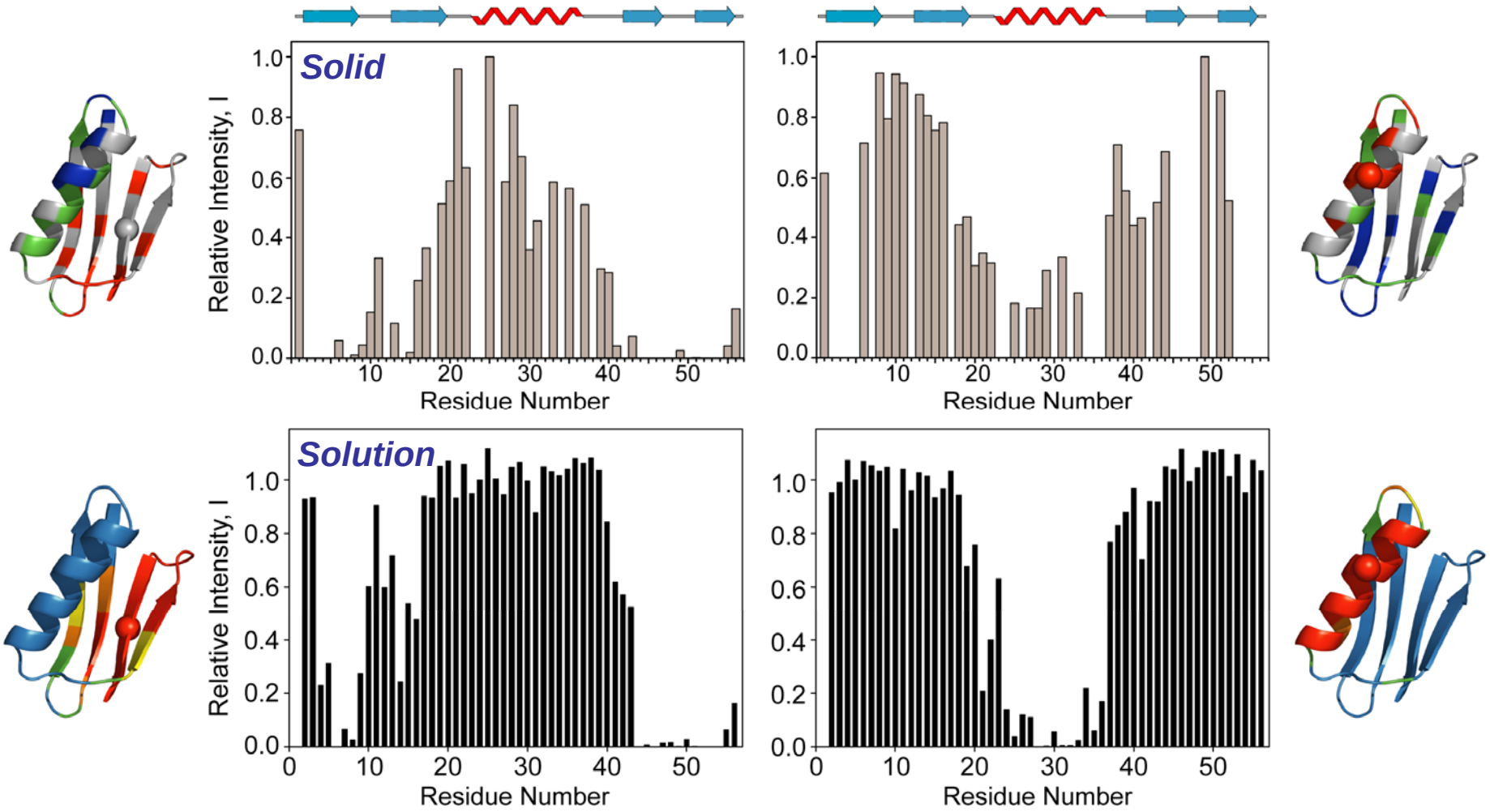






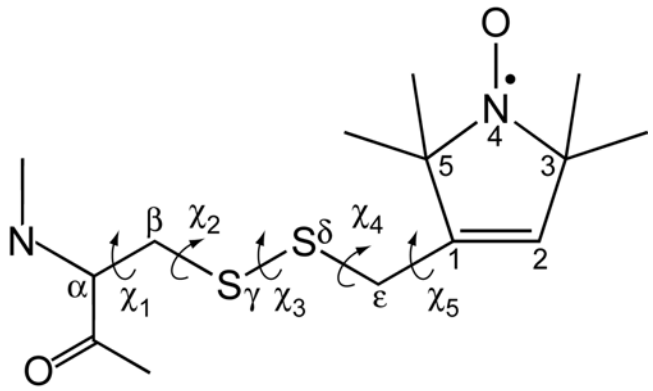
Nadaud et al., JACS 129 (2007) 7502

Solution vs. Solid-State PRE



- **Similar PRE profiles ($^1H^N$ PRE during CP/INEPT is dominant)**
- **PRE more pronounced in the solid-state ($\tau_{c,solid} > \tau_{c,solution}$)**

Estimated SL-Nucleus Distances

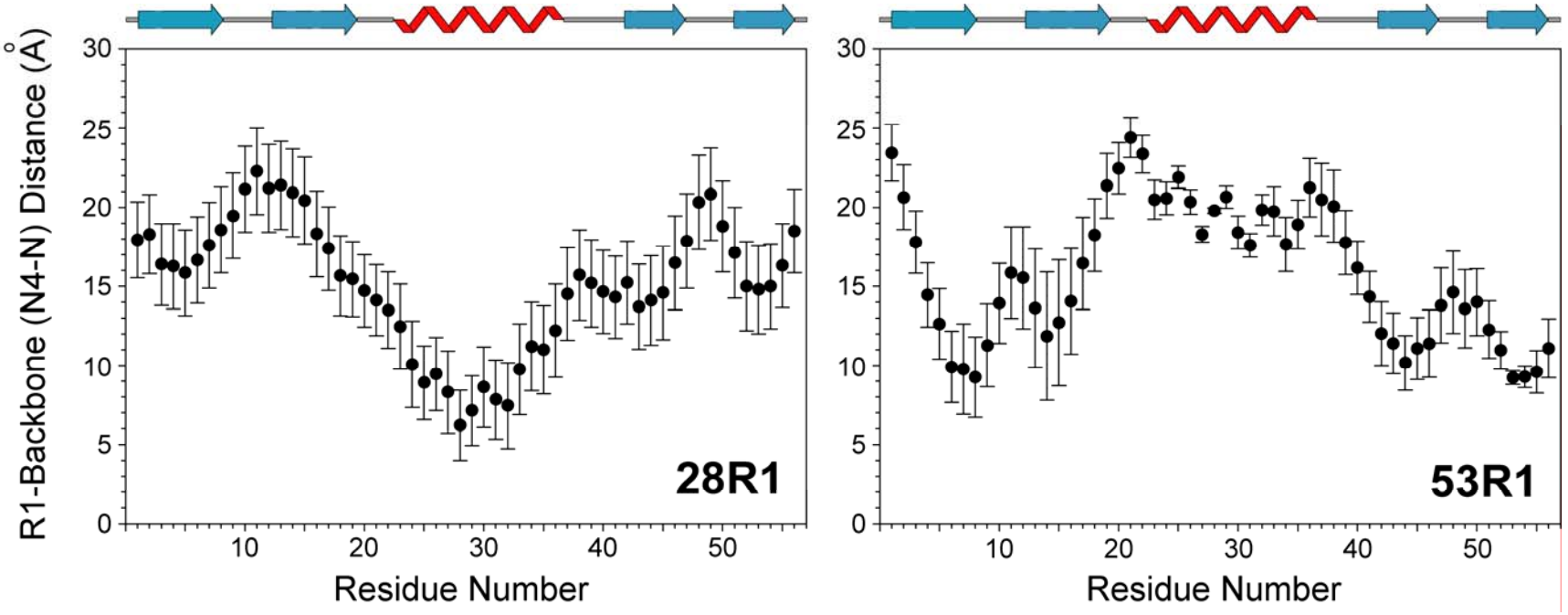


Langen et al., Biochemistry 39 (2000) 8396

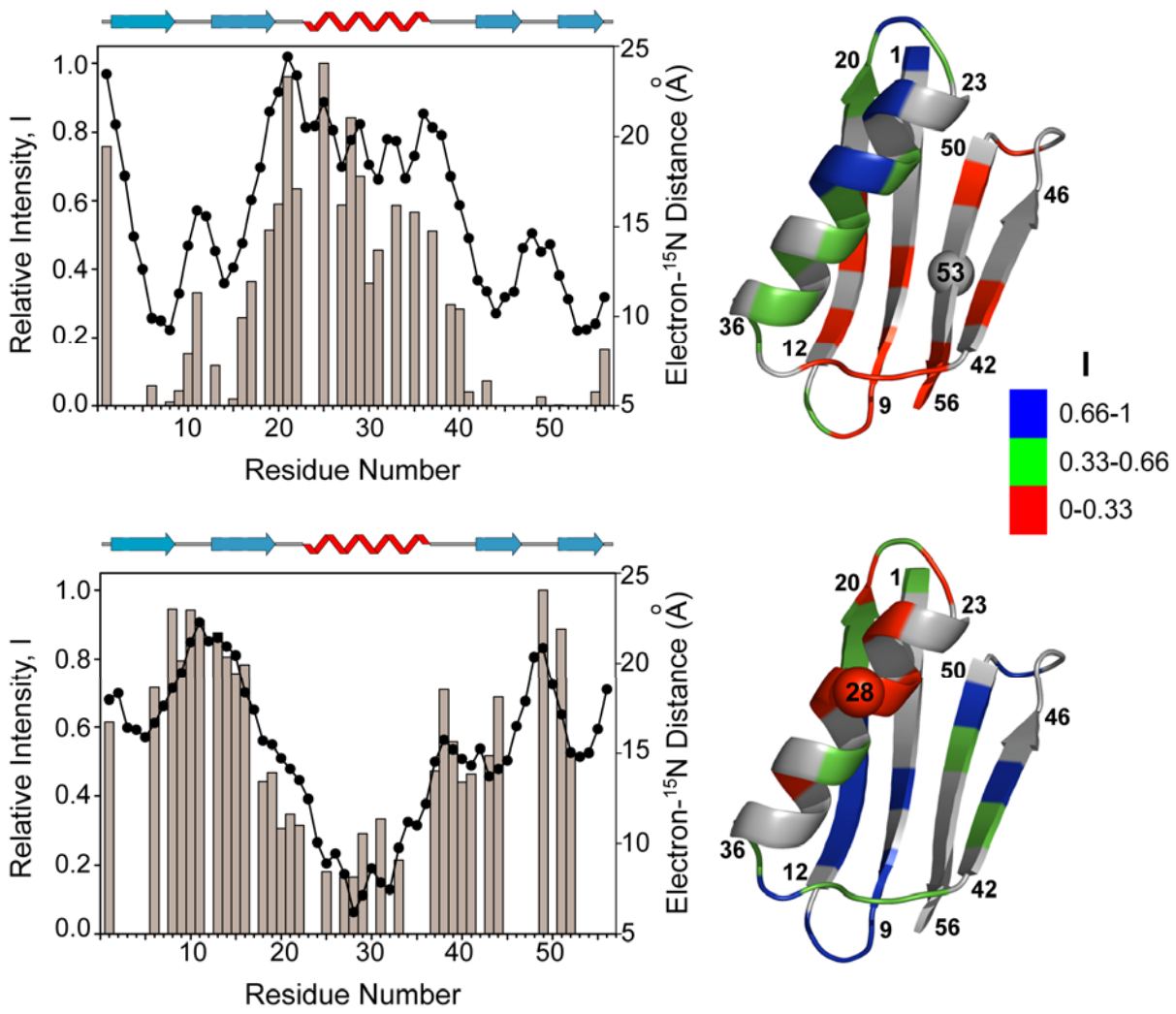
For R1 helix surface sites (e.g., 28R1):

$$\begin{cases} \chi 1 = -60 \\ \chi 2 = -60 \end{cases} \text{ or } \begin{cases} \chi 1 = 180 \\ \chi 2 = 60 \end{cases}$$

$\chi 5$ – little effect on distance

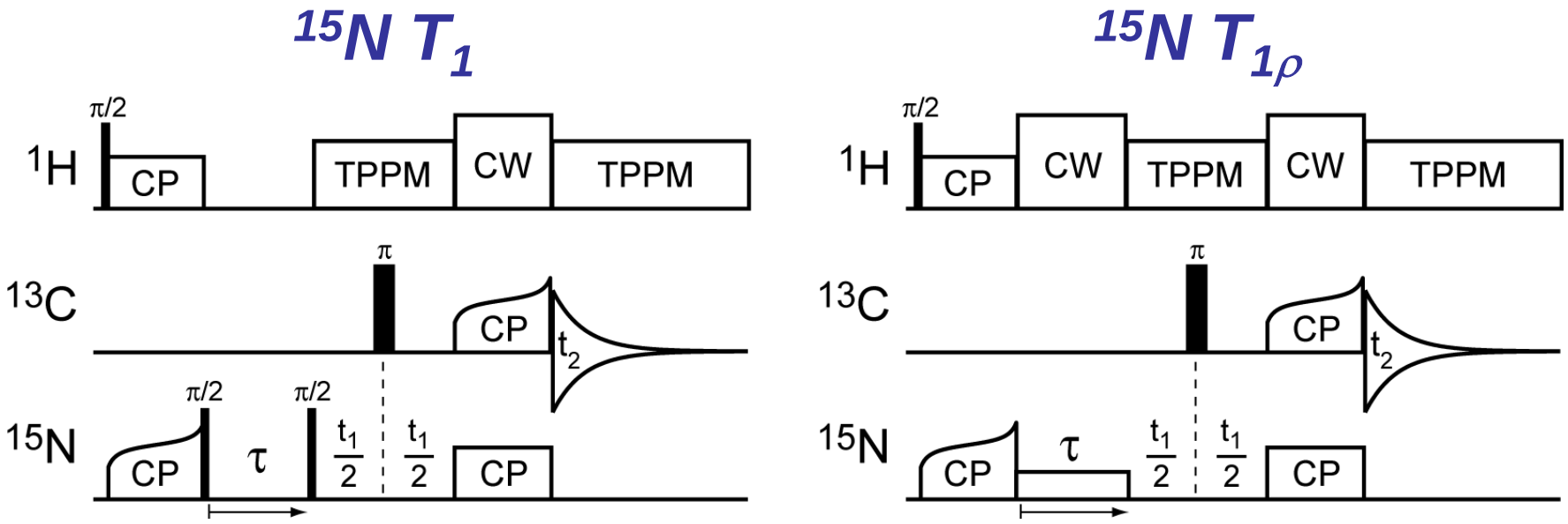


PRE vs. Estimated SL Distances



- **Reasonable qualitative correlation between expected electron-nucleus distance and cross-peak intensity for r_{en} up to ~20 Å**

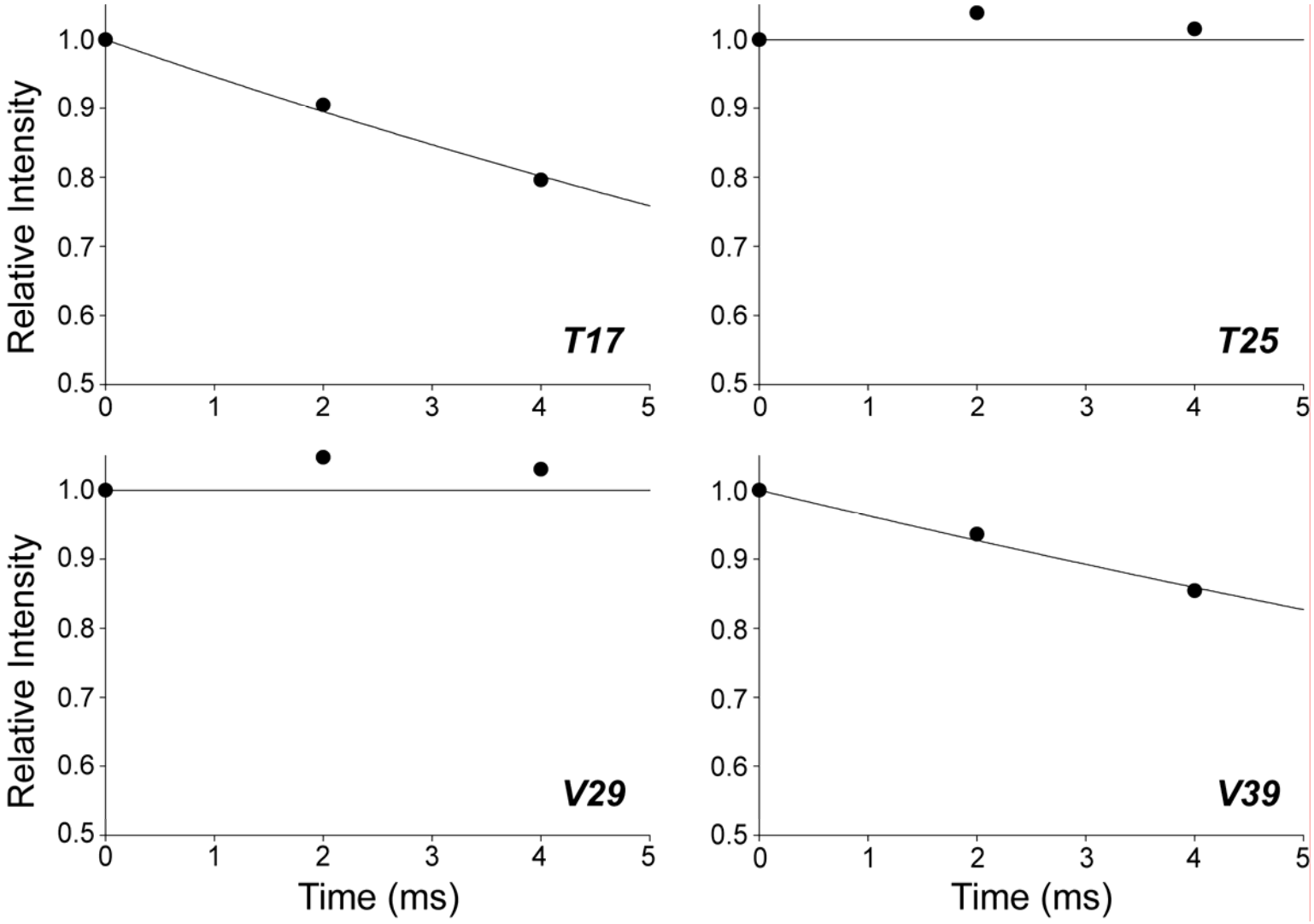
Pulse Schemes for $T_1/T_{1\rho}$ Measurement



Giraud et al., JACS 126 (2004) 11422

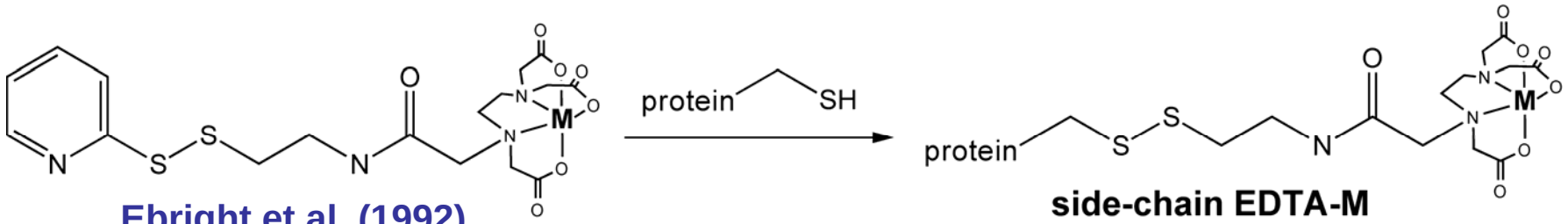
- **Pseudo-3D: 2 chemical shift dimensions + relaxation (easily extended to pseudo-4D)**
- **Similar schemes for ^{1}H and ^{13}C relaxation measurements**

Preliminary Site-Resolved ^{15}N $T_{1\rho}$ Measurements in 53R1



Paramagnetic Metal Ions

Griesinger & co-workers, Magn. Res. Chem. (2006)

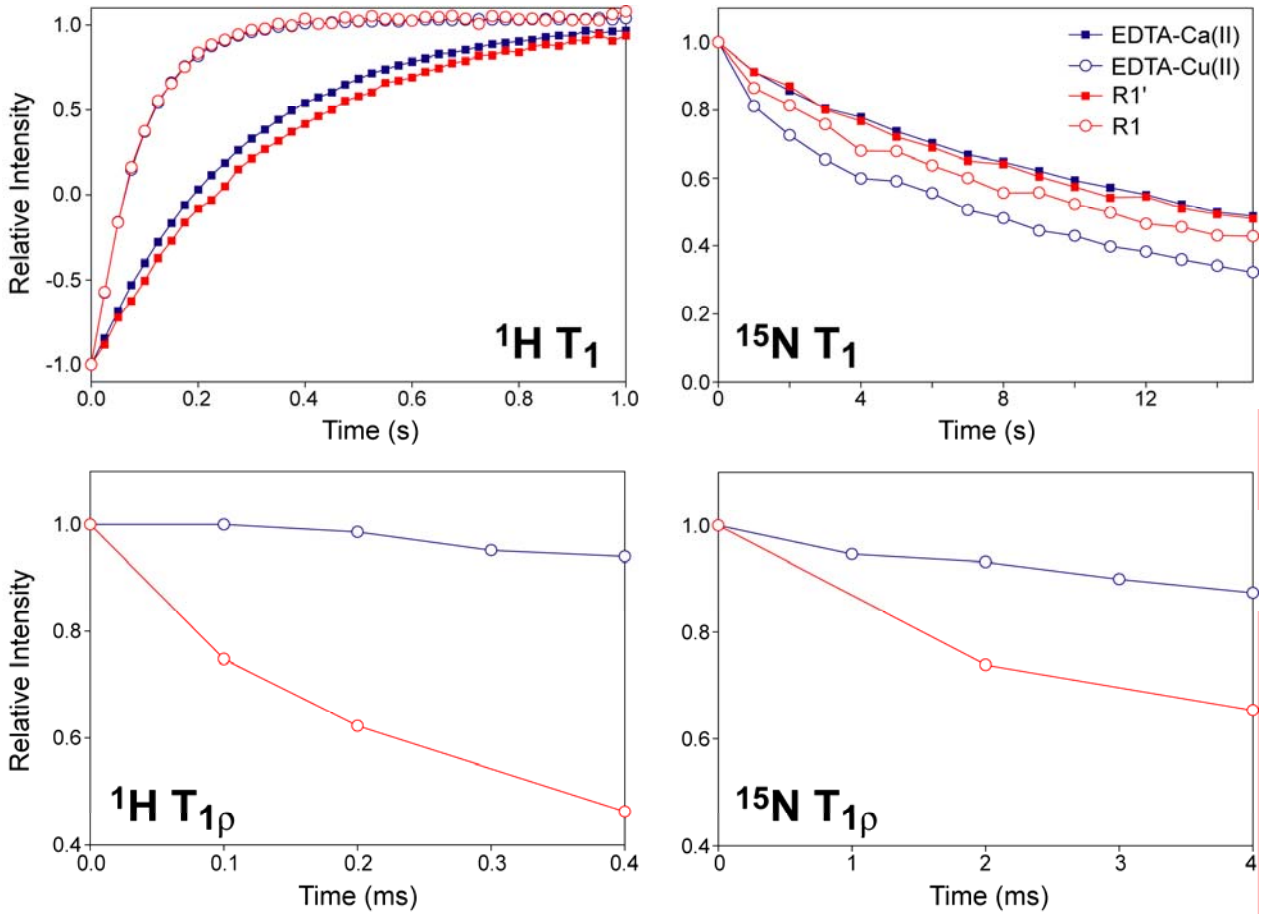


Ebright et al. (1992)

Ion	logK EDTA-M	S	T_{1e} (s)
Ca(II)	10.70	-	-
Cu(II)	18.86	1/2	~1-5 x 10^{-9}
Mn(II)	13.95	5/2	~10^{-8}
Gd(III)	17.30	7/2	~10^{-8}–10^{-9}

EDTA-M binding: Anderegg (1977), Powell (1979)
T_{1e} data: Bertini & Luchinat, Coord. Chem. Rev. (1996)

Preliminary Data: 53R1 vs. 53EDTA-Cu(II)



- Smaller $R_{2,para}$, larger ^{15}N $R_{1,para}$ for Cu(II) as expected
- Similar ^{1}H $R_{1,para}$ for R1 and Cu(II) - likely ^{1}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)
- Walter & Oldfield, Chem. Comm. (1987)
- Nayeem & Yesinowski, JCP (1988)
- Campbell & Haw, Inorg. Chem. (1988)
- Groombridge & Perkins, Chem. Comm. (1991)
- Brough, Grey & Dobson, JACS (1993)
- McDermott & co-workers, JACS (1995)
- Heise et al., JACS (1999)
- Ishii & co-workers, JACS (2003, 2005), JMR (2006)
- Emsley & co-workers, JACS (2006)
- Polenova & co-workers, JPC (2006)

Proteins

- McDermott & co-workers, JACS (1998, 2005), Biochem. (1999)
- Bertini, Emsley & co-workers, Angew. Chem. (2007)
- Bertini & co-workers, JACS (2007)
- Ishii & co-workers, JMR (2007)

Acknowledgments

- Philippe Nadaud
- Jonathan Helmus
- Nicole Höfer (visiting, U. Limerick)

- Angela Gronenborn (U. Pittsburgh)
- Ad Bax (NIH)
- Chad Rienstra (UIUC)

$$$

THE OHIO STATE UNIVERSITY

alzheimer's association™