

Teoría y aplicaciones de la RMN de compuestos paramagnéticos

(preferiblemente proteínas ☺)

Alejandro J. Vila



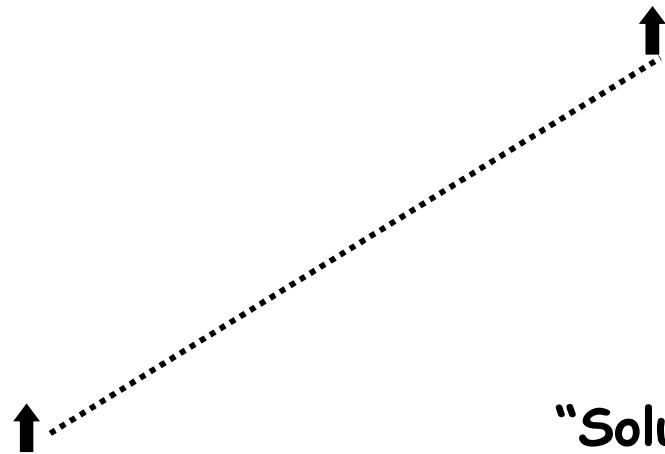
RMN de compuestos paramagneticos

- Teoria
- Tips prácticos
- Una aplicación

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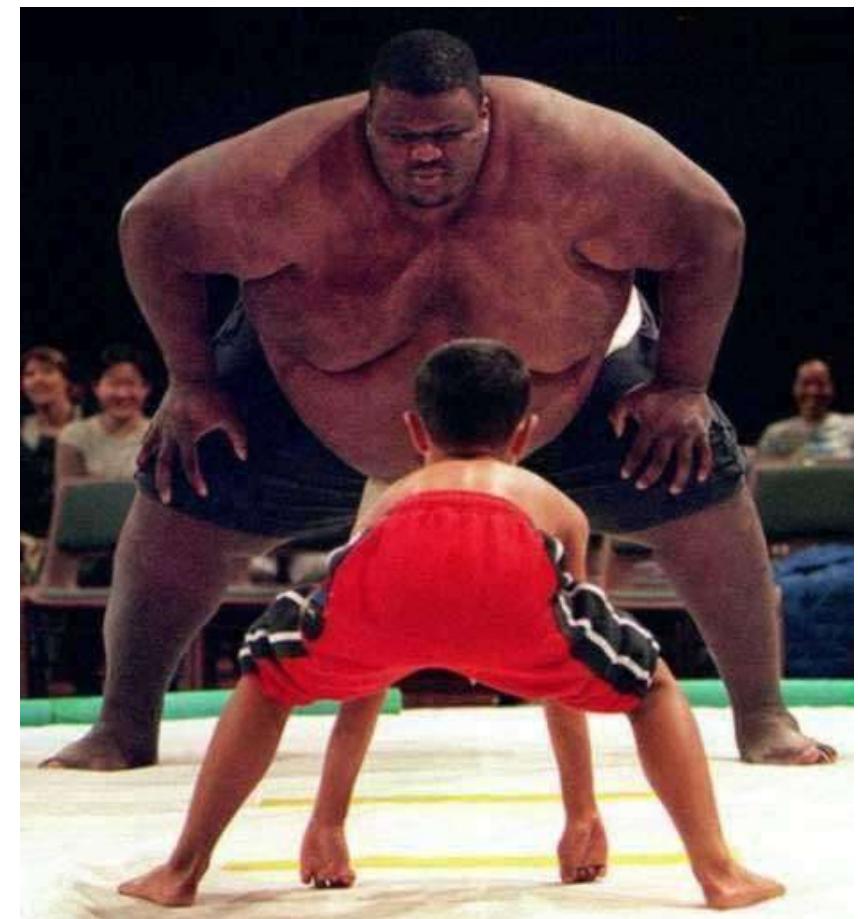
NMR of Paramagnetic Systems: The electron-nucleus interaction



Bertini, Luchinat, Parigi
“Solution NMR of Paramagnetic Molecules”,
2001, Elsevier

NMR of Paramagnetic Systems: The electron-nucleus interaction

$$\mu_{e^-} = 658 \mu_{1H}$$



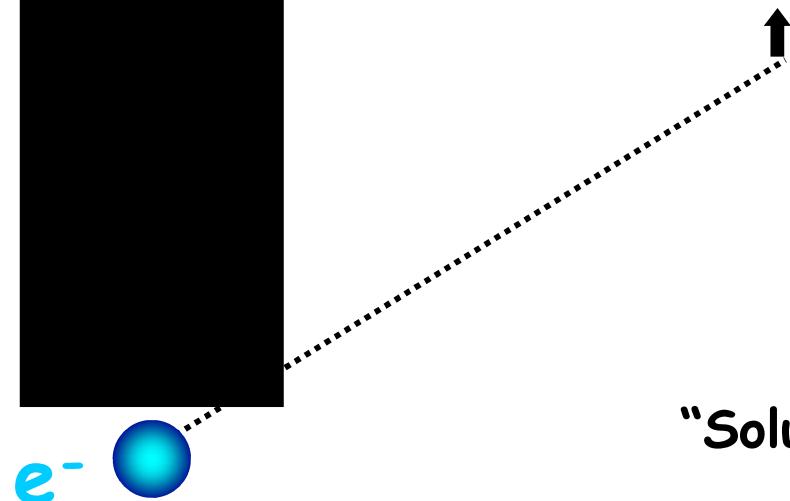
e^-

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Unpaired electrons affect:

- Chemical shifts
- Relaxation rates

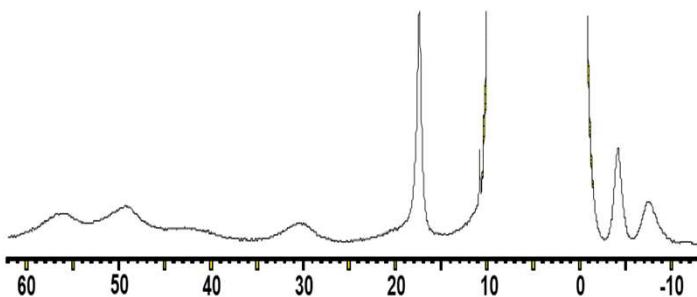
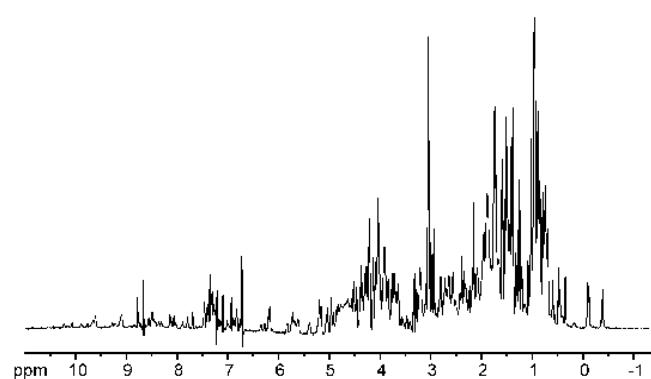
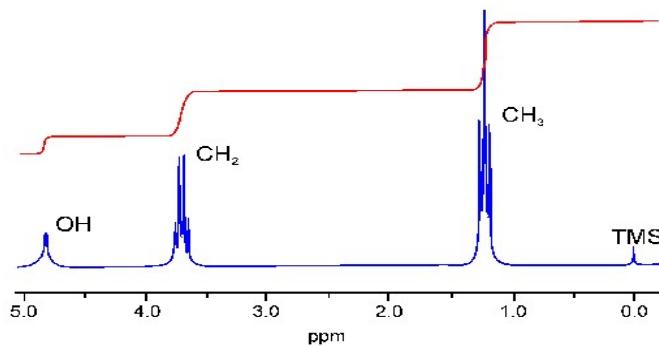


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Los electrones desapareados complican los espectros de RMN

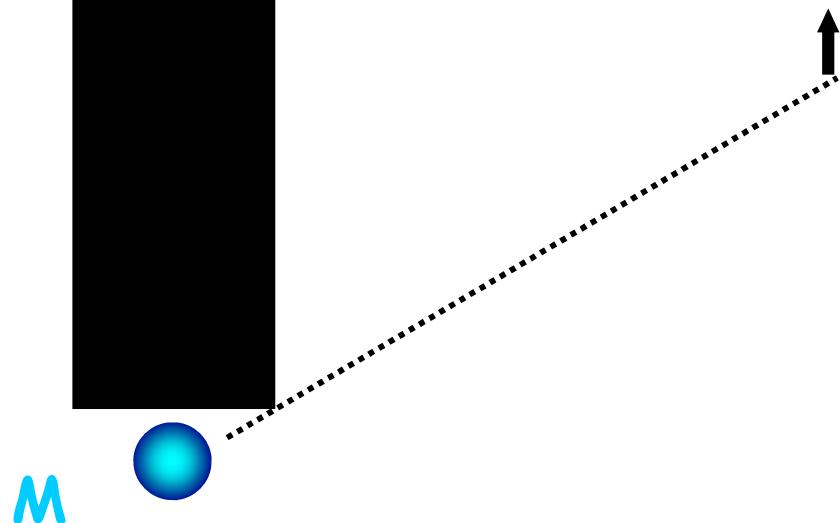
- ✓ Perturban el rango de desplazamientos químicos
- ✓ Incrementan la velocidad de relajación nuclear (\Rightarrow señales mas anchas)
- ✓ Disminuyen la intensidad del efecto NOE (si es que se ven!!)

Los electrones despareados complican los espectros de RMN



NMR of Paramagnetic Systems: The electron-nucleus interaction

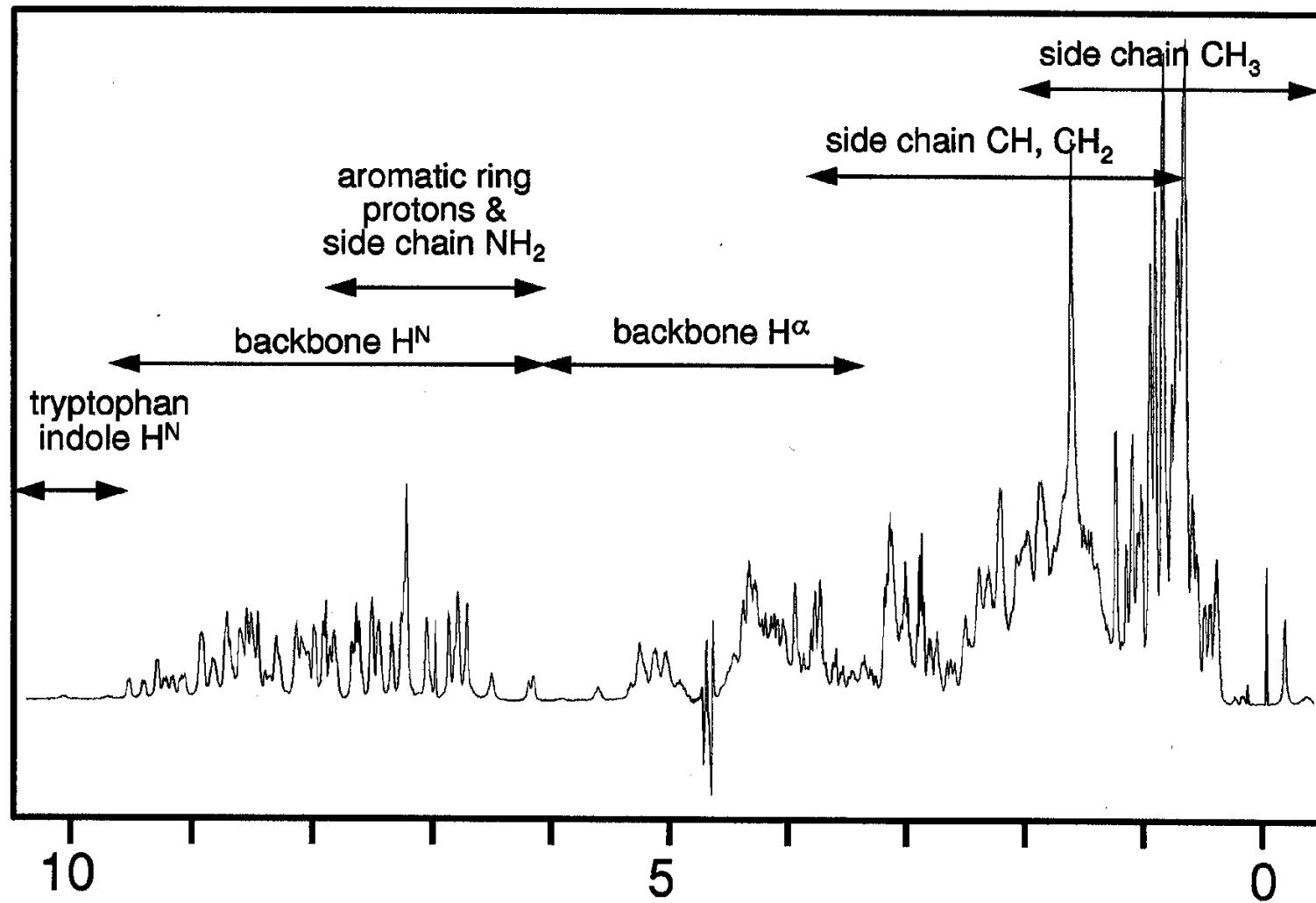
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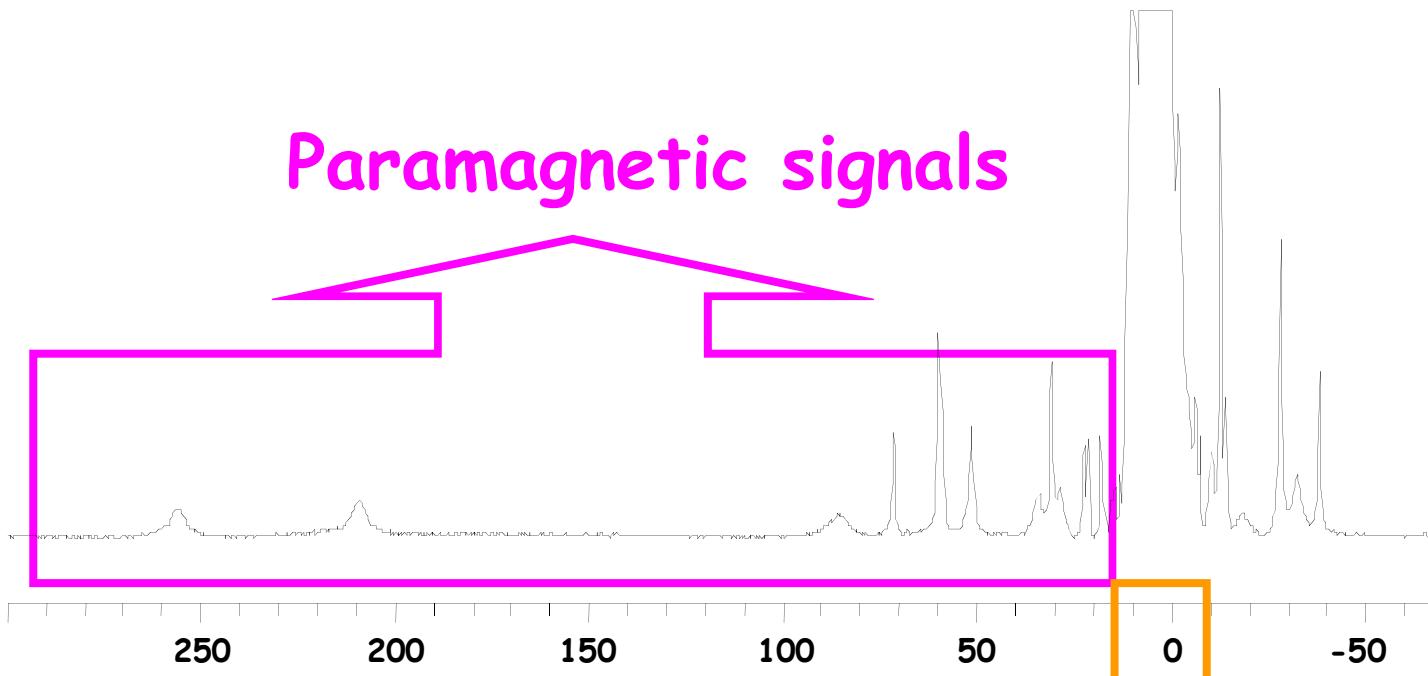
Unpaired electrons affect:

- **Chemical shifts**
- **Relaxation rates**

^1H NMR Spectrum of a Small Protein

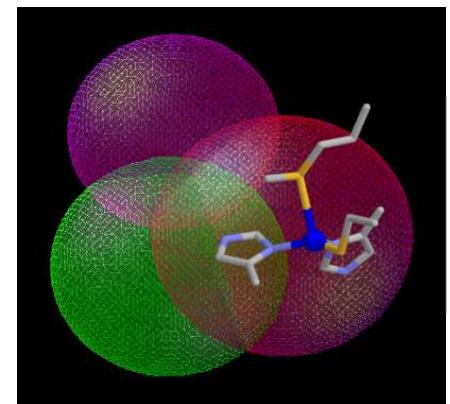
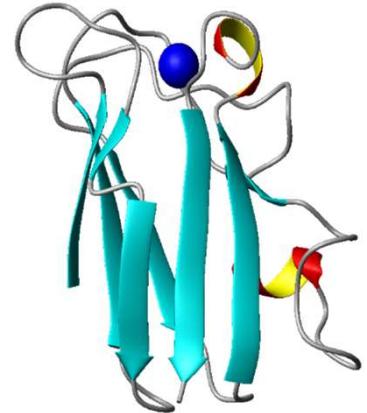


^1H NMR Spectrum of a Paramagnetic Protein



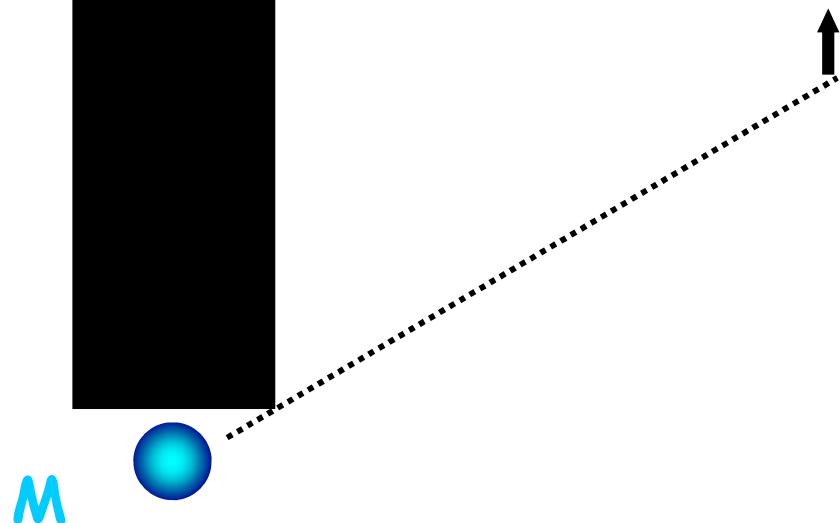
Selective probe in the metal site

Diamagnetic region



NMR of Paramagnetic Systems: The electron-nucleus interaction

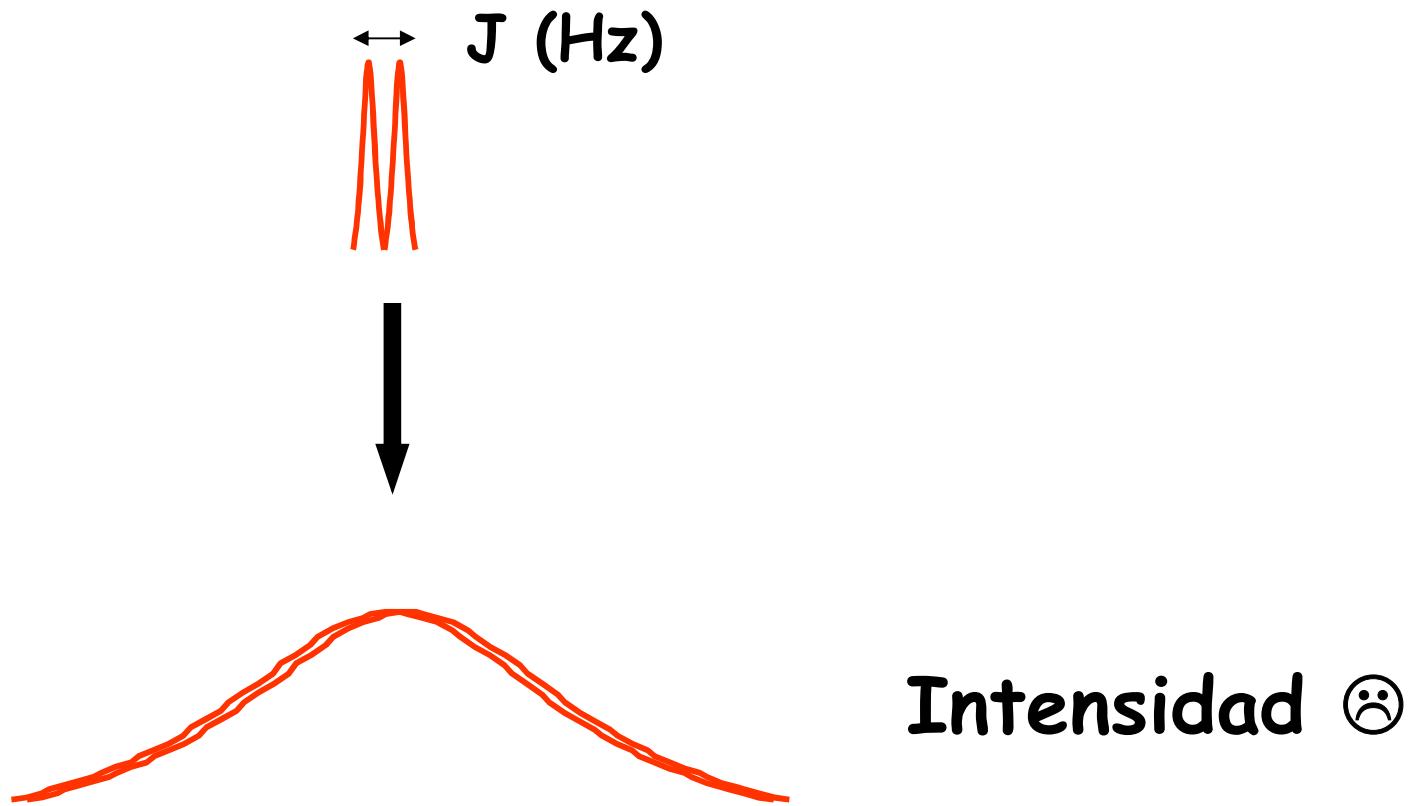
$$\mu_{e^-} = 658 \mu_{1H}$$



Unpaired electrons affect:

- Chemical shifts
- Relaxation rates ($R_2 > J!$)

Relaxation rates ($R_2 > J!$)



NMR of Paramagnetic Proteins

Electron-nucleus interaction

Fast relaxation rates

Broad lines

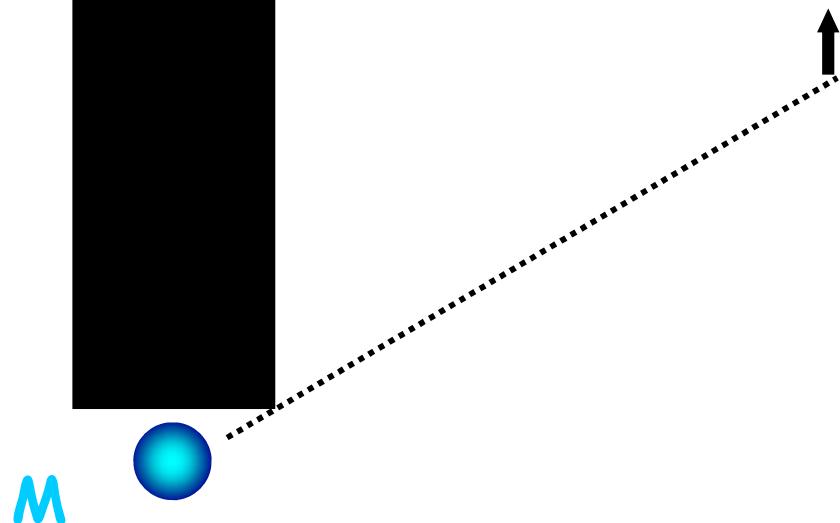
Low signal-to-noise

Large chemical shifts

Signal scrambling

NMR of Paramagnetic Systems: The electron-nucleus interaction

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Unpaired electrons affect:

- **Chemical shifts**
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NMR of Paramagnetic Systems: The electron-nucleus interaction

Unpaired electrons affect:

- Chemical shifts
- Relaxation rates

Mechanisms

$$a_c = \frac{\mu_0}{3S} \hbar \gamma_I g_e \mu_B \rho$$

Contact coupling

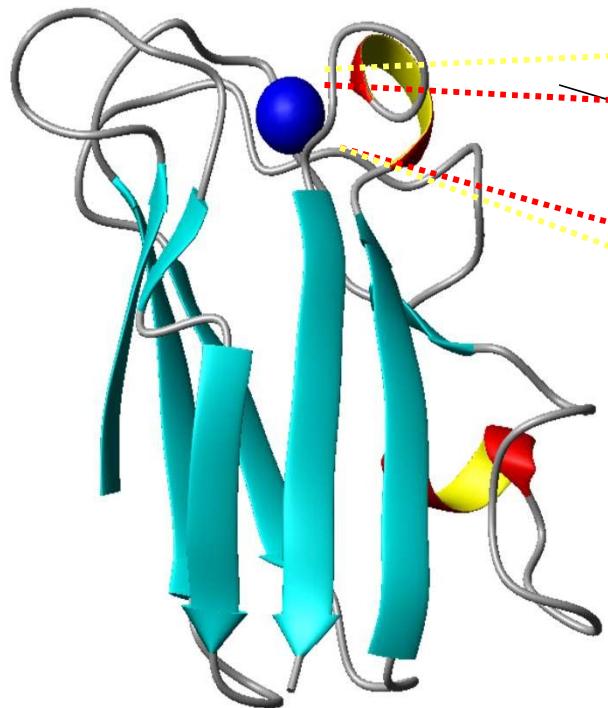
Electron spin density on the
nucleus

(\sim nuclear scalar coupling (J))

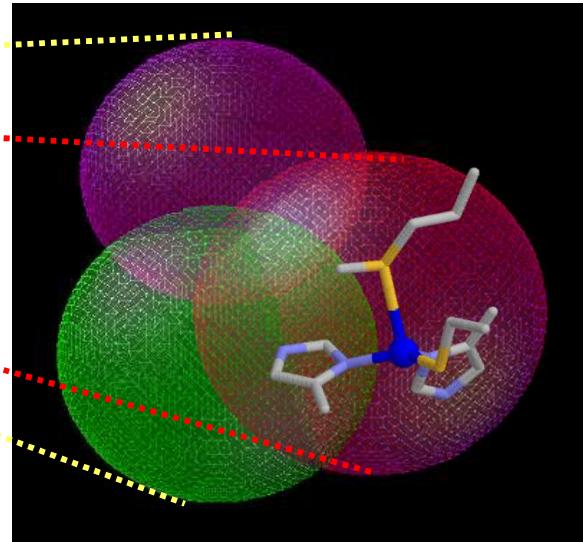
$$E = \frac{\mu_I \mu_S}{r_{IS}^3} (3 \cos^2 \theta - 1)$$

Dipolar coupling

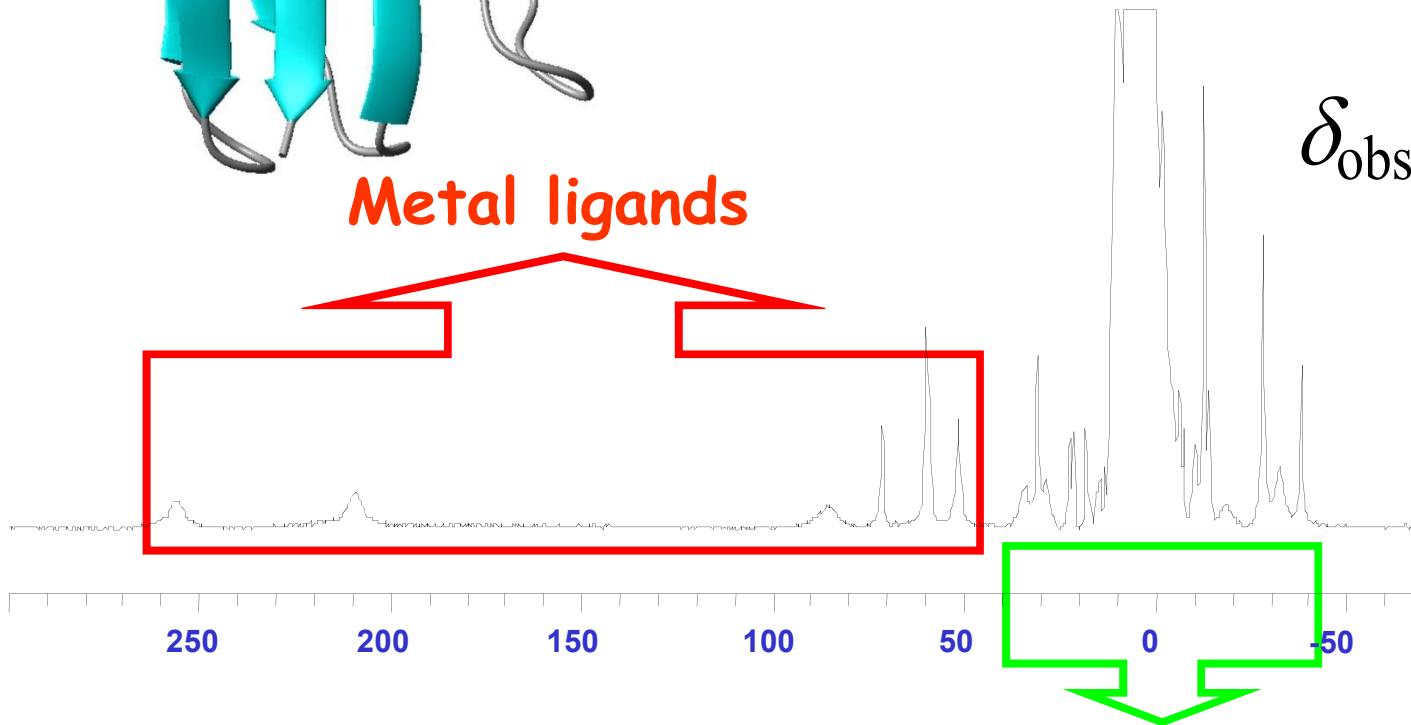
Electron - nucleus distance
(\sim nuclear dipolar coupling)



Metal ligands



$$\delta_{\text{obs}} = \delta_{\text{dia}} + \delta_{\text{con}} + \delta_{\text{pc}}$$



Nearby nuclei

The Contact shift

Through bonds

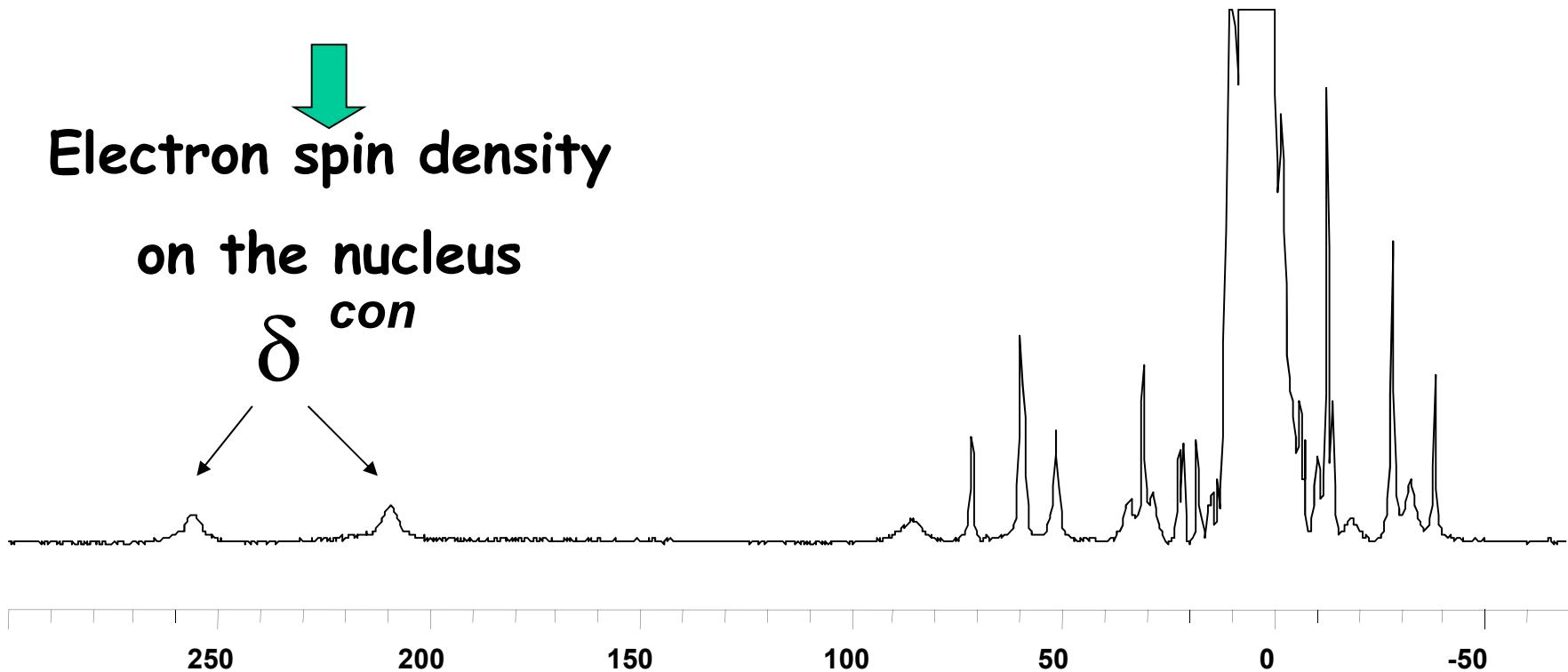
$$\delta^{con} = \frac{a_c}{\hbar\gamma_I B_0} \langle S_z \rangle \quad \rightarrow \quad a_c = \frac{\mu_0}{3S} \hbar\gamma_I g_e \mu_B \rho$$



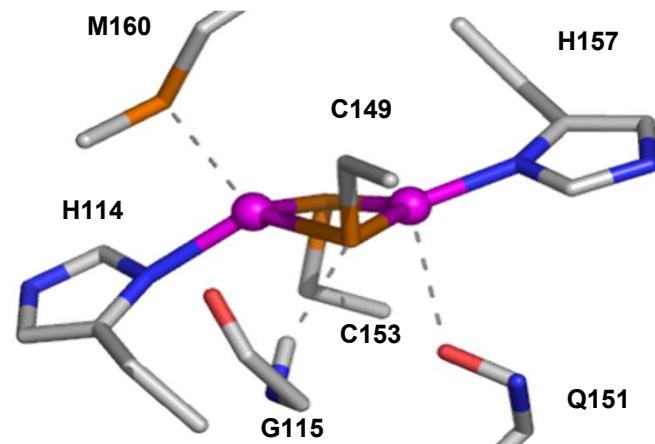
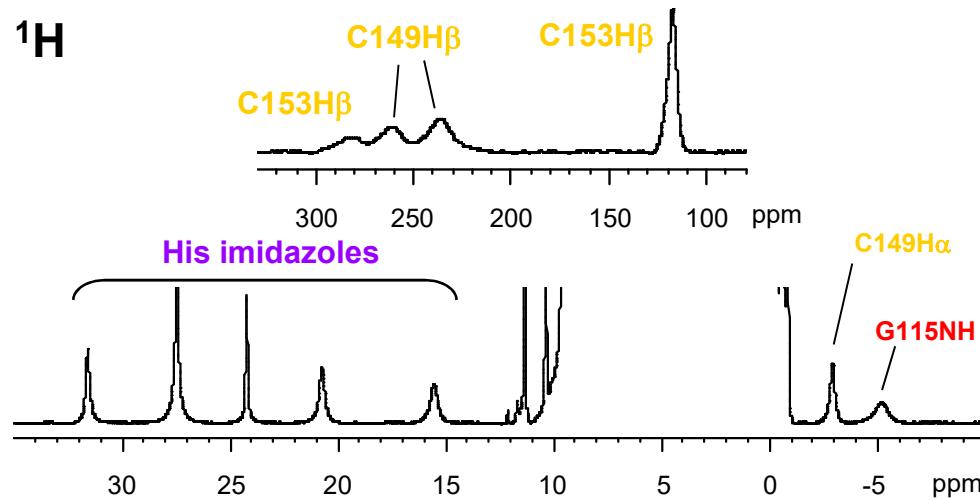
Electron spin density

on the nucleus

δ^{con}



^1H NMR - Cu_A

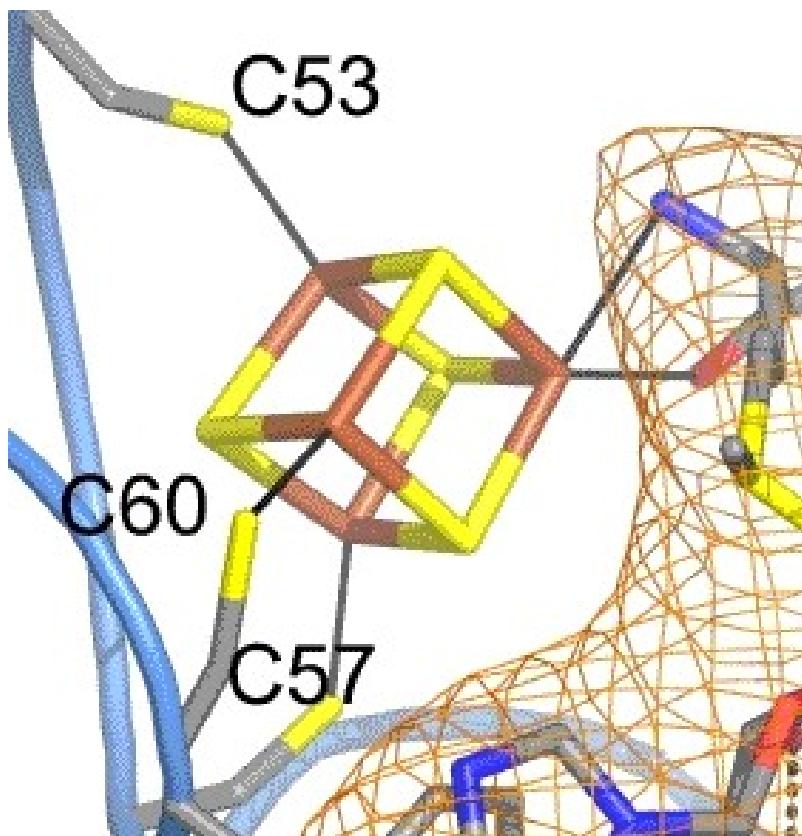


^1H Shifts in Cu_A are mostly of contact origin
Electron spin density

Cys @ CuA
His @ CuA

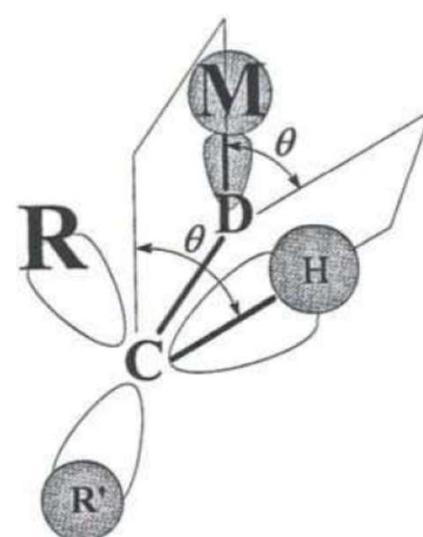
100 - 300 ppm
15 - 30 ppm

The Contact shift

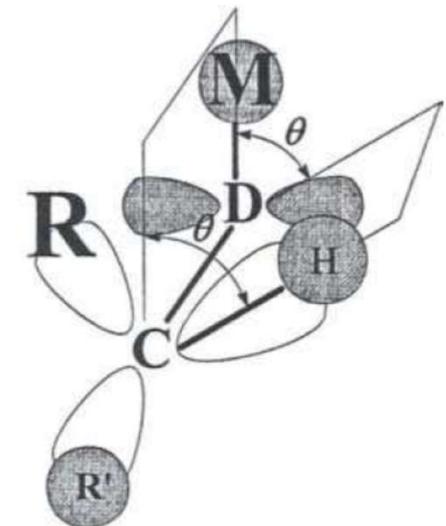


$$A/h = (\sin^2 \theta + a \cos \theta + b)$$

Karplus-like

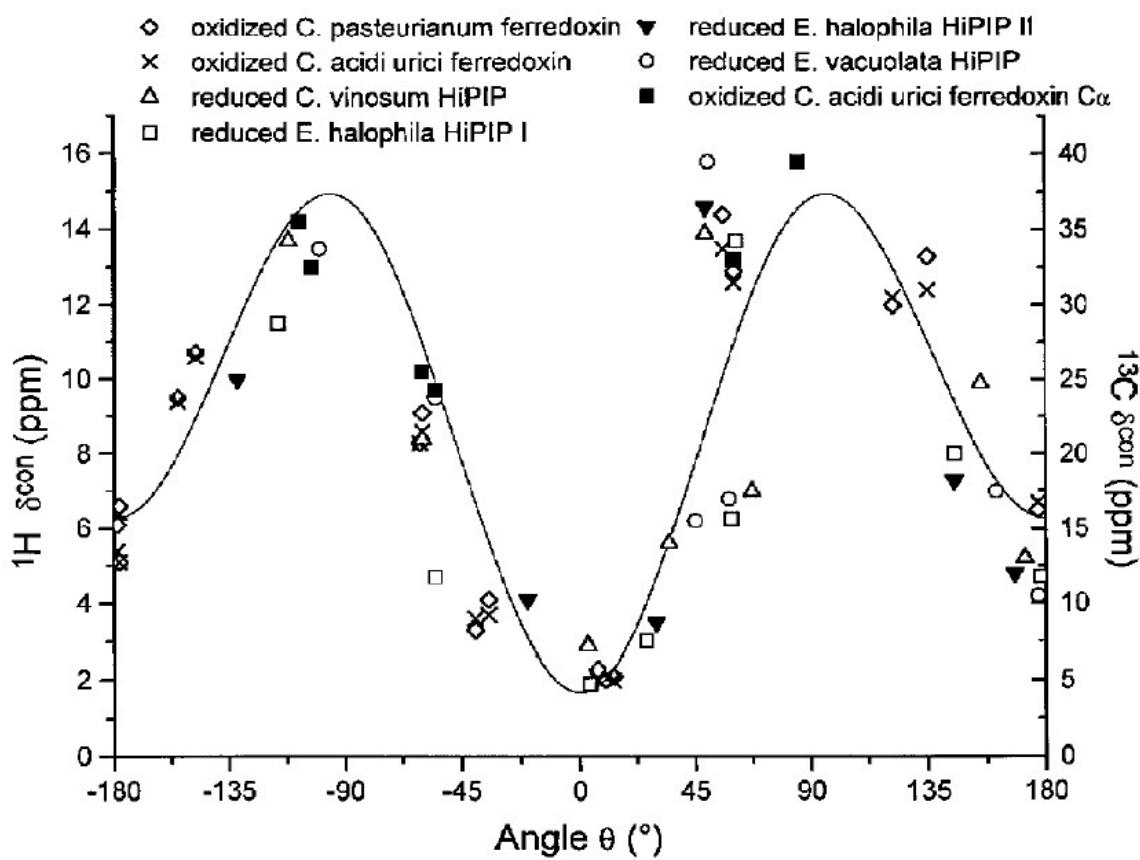


$$\delta \propto \cos^2 \theta$$

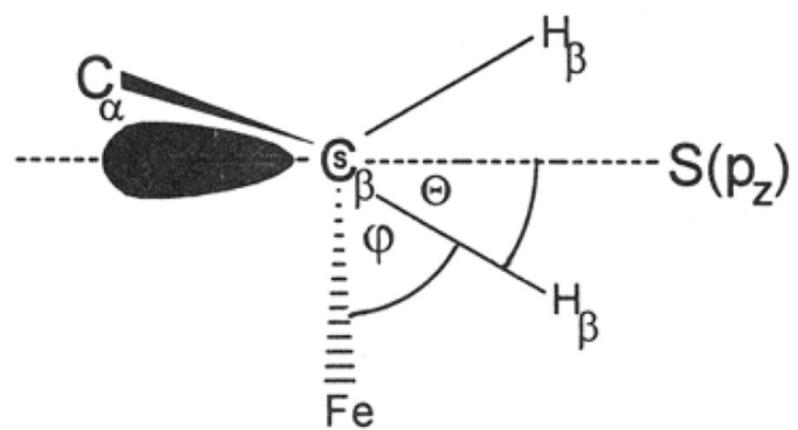


$$\delta \propto \sin^2 \theta$$

Dihedral angle dependence of hyperfine shifts of H β nuclei of Fe-bound Cys



$$A/h = (\sin^2 \theta + a \cos \theta + b)$$



$$a = 10.3$$

$$b = -2.2$$

$$c = 3.9$$

Bertini, Capozzi, Luchinat, Piccioli, Vila, JACS 1994

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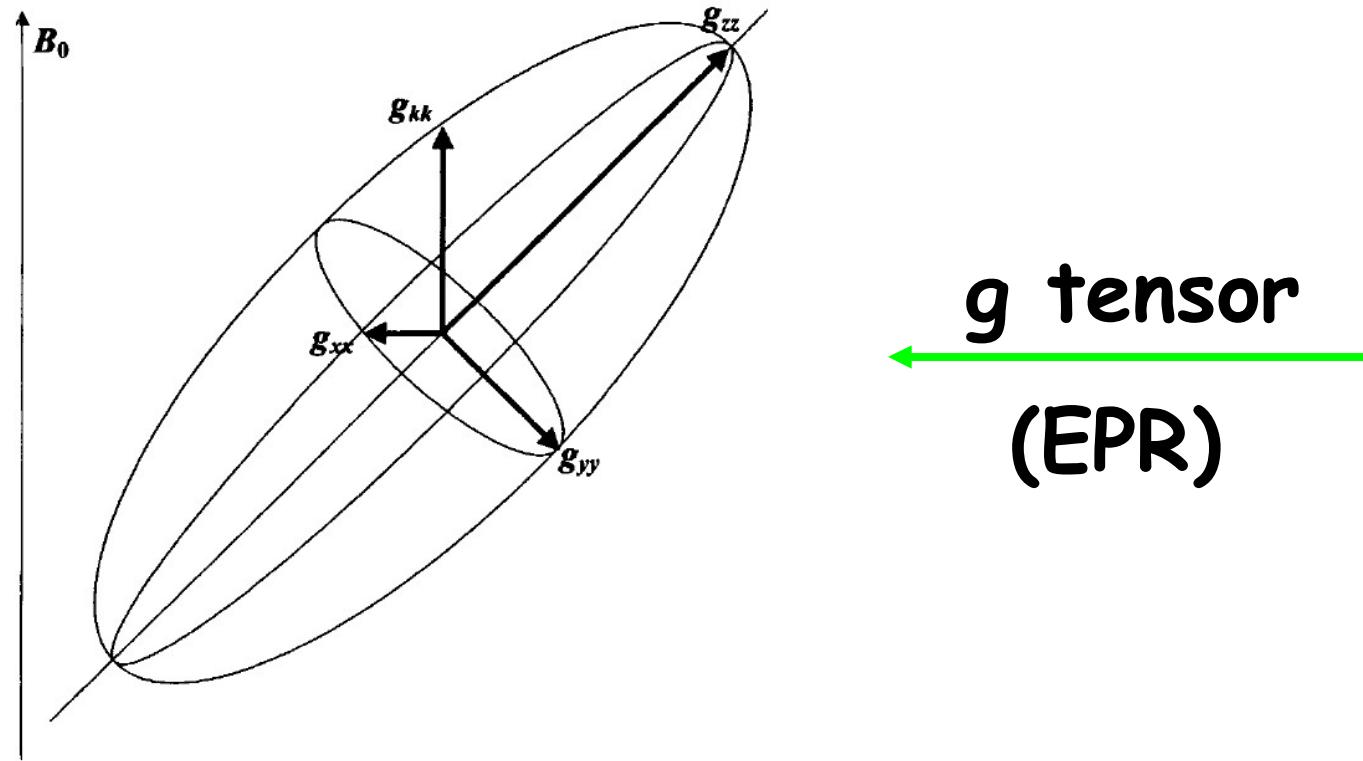
(\sim nuclear scalar coupling (J))

$$E = \frac{\mu_I \mu_S}{r_{IS}^3} (3 \cos^2 \theta - 1)$$

Dipolar coupling

Electron - nucleus distance
(\sim nuclear dipolar coupling)

The electron-nucleus interaction



The distribution of the electron density can be anisotropic (orientation-dependent)

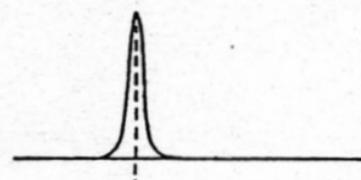
g Anisotropy on EPR spectra

(a) ISOTROPIC

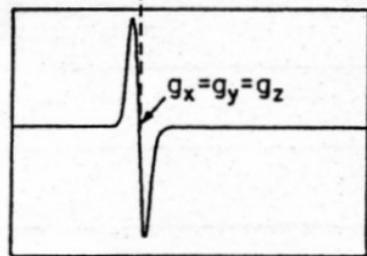
$$g_x = g_y = g_z$$



ABSORPTION



ABSORPTION
DERIVATIVE



MAGNETIC FIELD →

g Anisotropy on EPR spectra

(a) ISOTROPIC

$$g_x = g_y = g_z$$

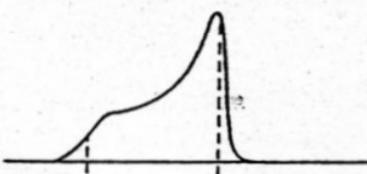
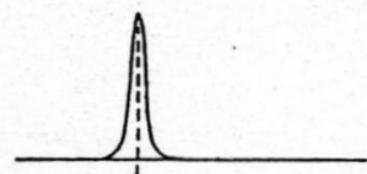


(b) AXIAL

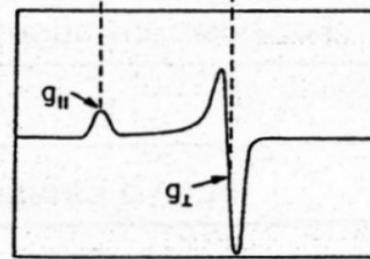
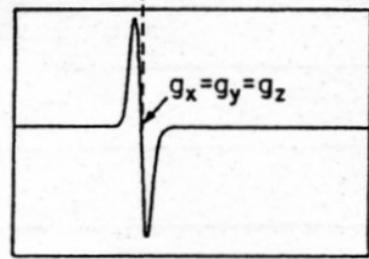
$$g_x = g_y < g_z$$



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MAGNETIC FIELD →

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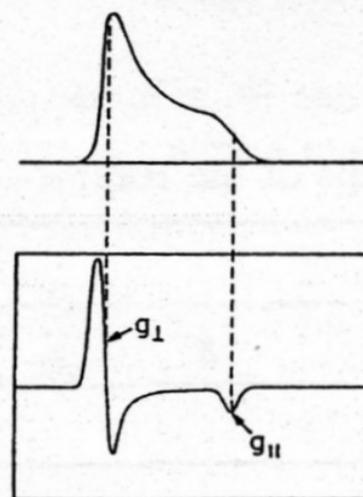
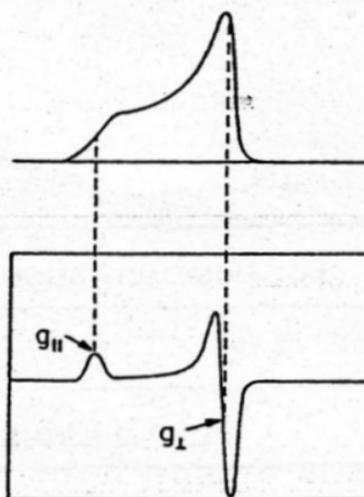
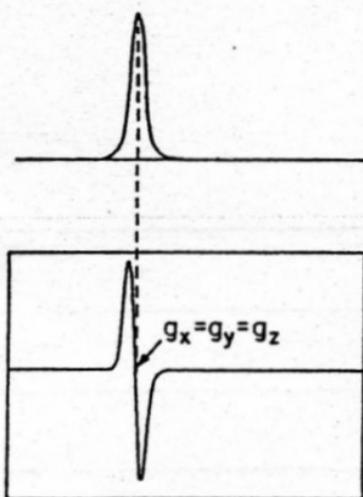


(c) AXIAL

$$g_x = g_y > g_z$$



ABSORPTION
DERIVATIVE



MAGNETIC FIELD \rightarrow

g Anisotropy on EPR spectra

(a) ISOTROPIC

$$g_x = g_y = g_z$$



(b) AXIAL

$$g_x = g_y < g_z$$



(c) AXIAL

$$g_x = g_y > g_z$$

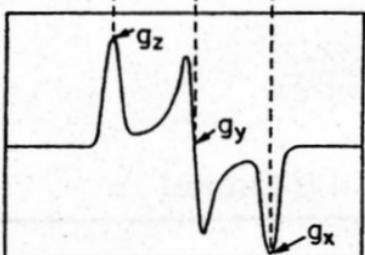
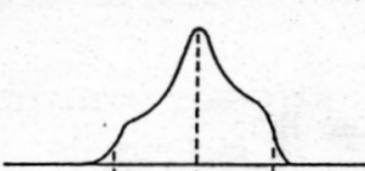
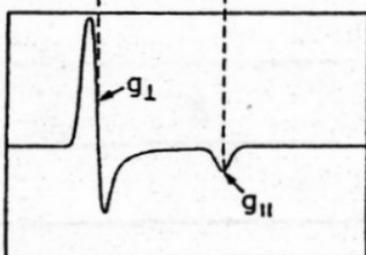
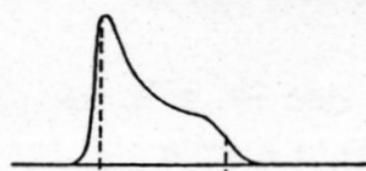
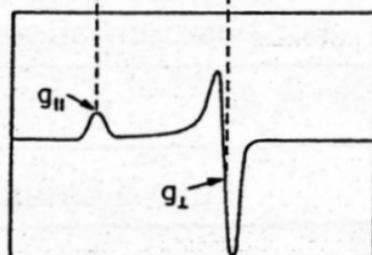
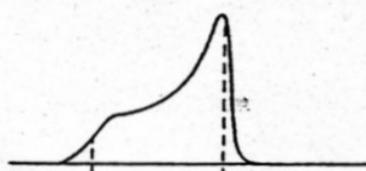
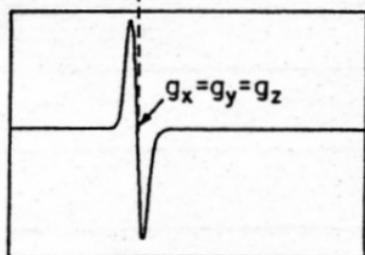
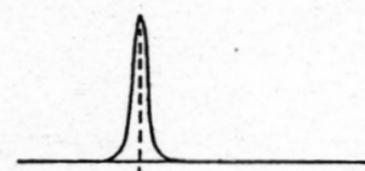


(d) RHOMBIC

$$g_x \neq g_y \neq g_z$$

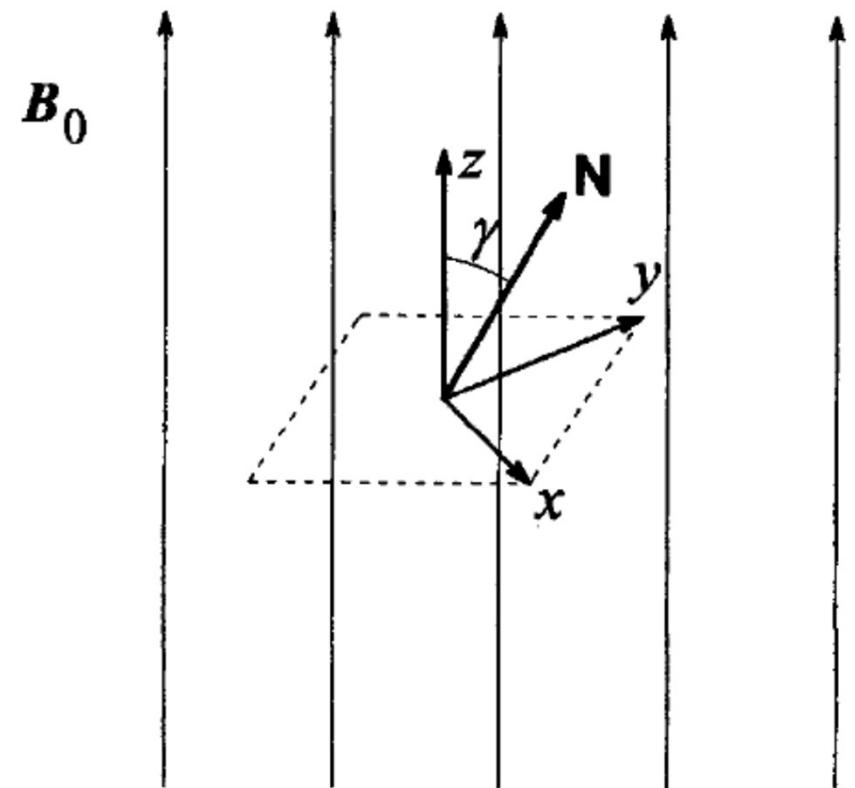


ABSORPTION
DERIVATIVE



MAGNETIC FIELD →

The Pseudocontact shift

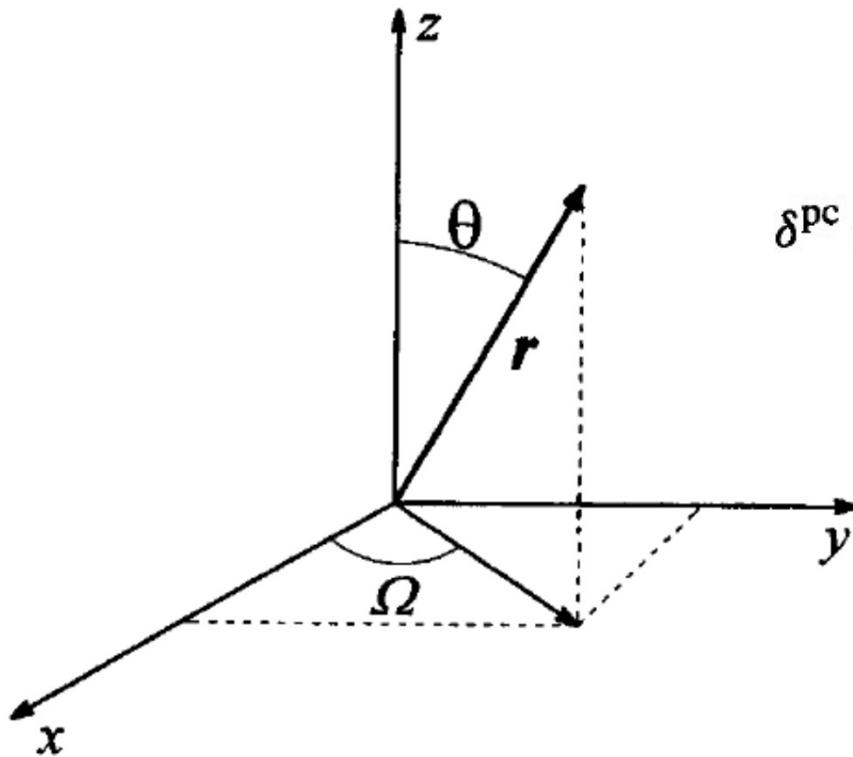


$$E^{\text{dip}} = -\frac{\mu_0}{4\pi} \hbar \gamma_I I_z(\mu) \frac{1}{r^3} (3 \cos^2 \gamma - 1).$$

Dipolar interaction between the nuclear and the electron magnetic moments

Orientation of the Metal-Nucleus vector with respect to B_0

The Pseudocontact shift



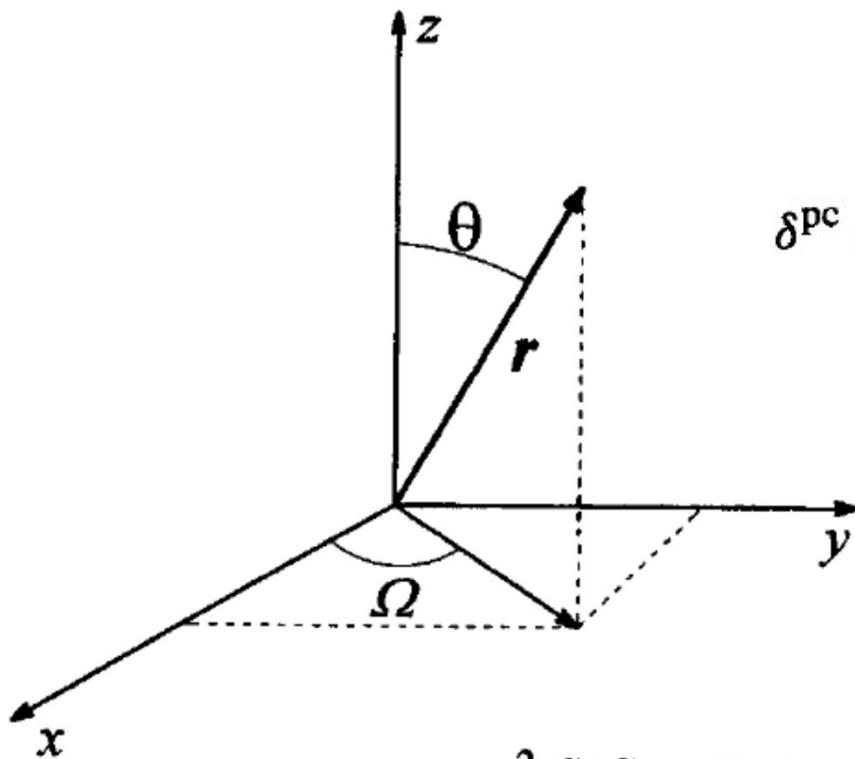
$$\delta^{\text{pc}} = \frac{\mu_0 \mu_B^2 S(S+1)}{4\pi 9kT} (g_{\parallel}^2 - g_{\perp}^2) \frac{1}{r^3} (3 \cos^2 \theta - 1)$$

Axial Symmetry

δ^{pc} does not depend on any nucleus parameter

$\delta^{\text{pc}} = 0$ for isotropic systems

The Pseudocontact shift



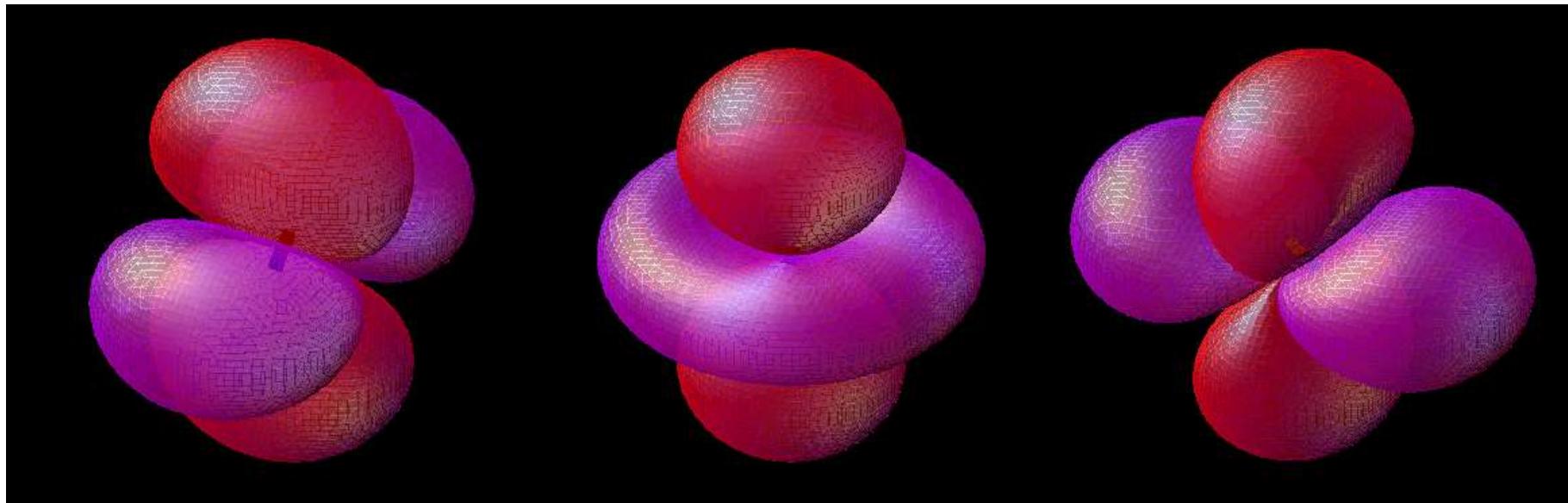
$$\delta^{\text{pc}} = \frac{\mu_0 \mu_B^2 S(S+1)}{4\pi} \frac{1}{9kT} (g_{\parallel}^2 - g_{\perp}^2) \frac{1}{r^3} (3 \cos^2 \theta - 1)$$

Axial Symmetry

$$\begin{aligned} \delta^{\text{pc}} = \frac{\mu_0 \mu_B^2 S(S+1)}{4\pi} \frac{1}{18kT} \frac{1}{r^3} & \left\{ [2g_{zz}^2 - (g_{xx}^2 + g_{yy}^2)] (3 \cos^2 \theta - 1) \right. \\ & \left. + 3(g_{xx}^2 - g_{yy}^2) \sin^2 \theta \cos 2\Omega \right\} \end{aligned}$$

Rhombic Symmetry

Pseudocontact shift constraints



Rhombic along x

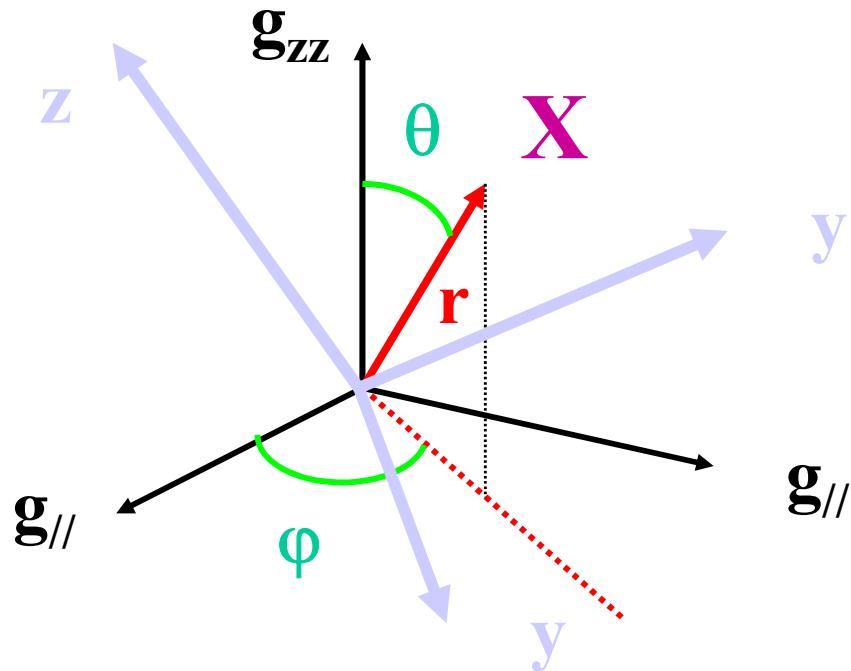
Axial

Rhombic along y

Isoshift surfaces

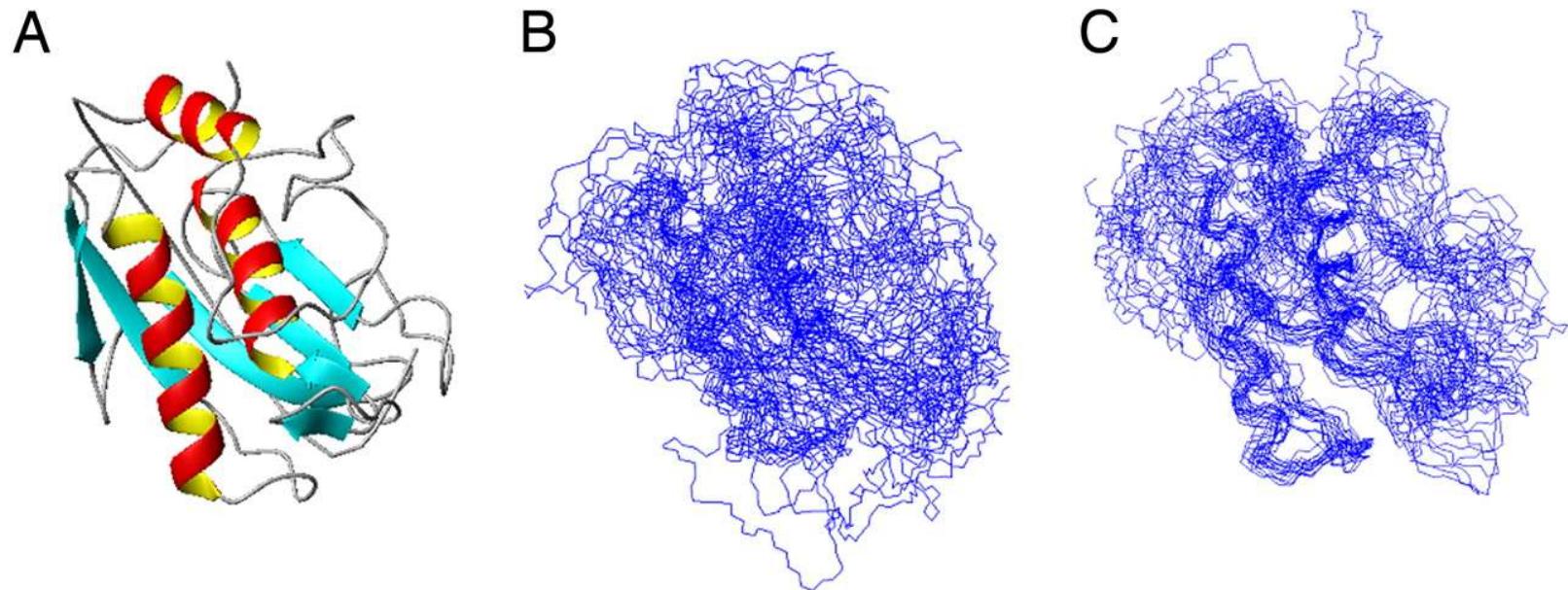
The pseudocontact shift provides structural parameters

$$\delta^{pc} = \frac{\mu_o}{4\pi} \frac{\mu_B^2 S(S+1)}{9kTr^3} \left[(3\cos^2 \theta - 1) (g_{//}^2 - g_{\perp}^2) \right]$$



The pseudocontact shift provides structural parameters

Families of 15 structures obtained without paramagnetic restraints (B) and with paramagnetic restraints (C); the reference X-ray structure (PDB ID code 1RMZ) is shown in A. The overall backbone RMSD (secondary structural elements plus loops) is 5....

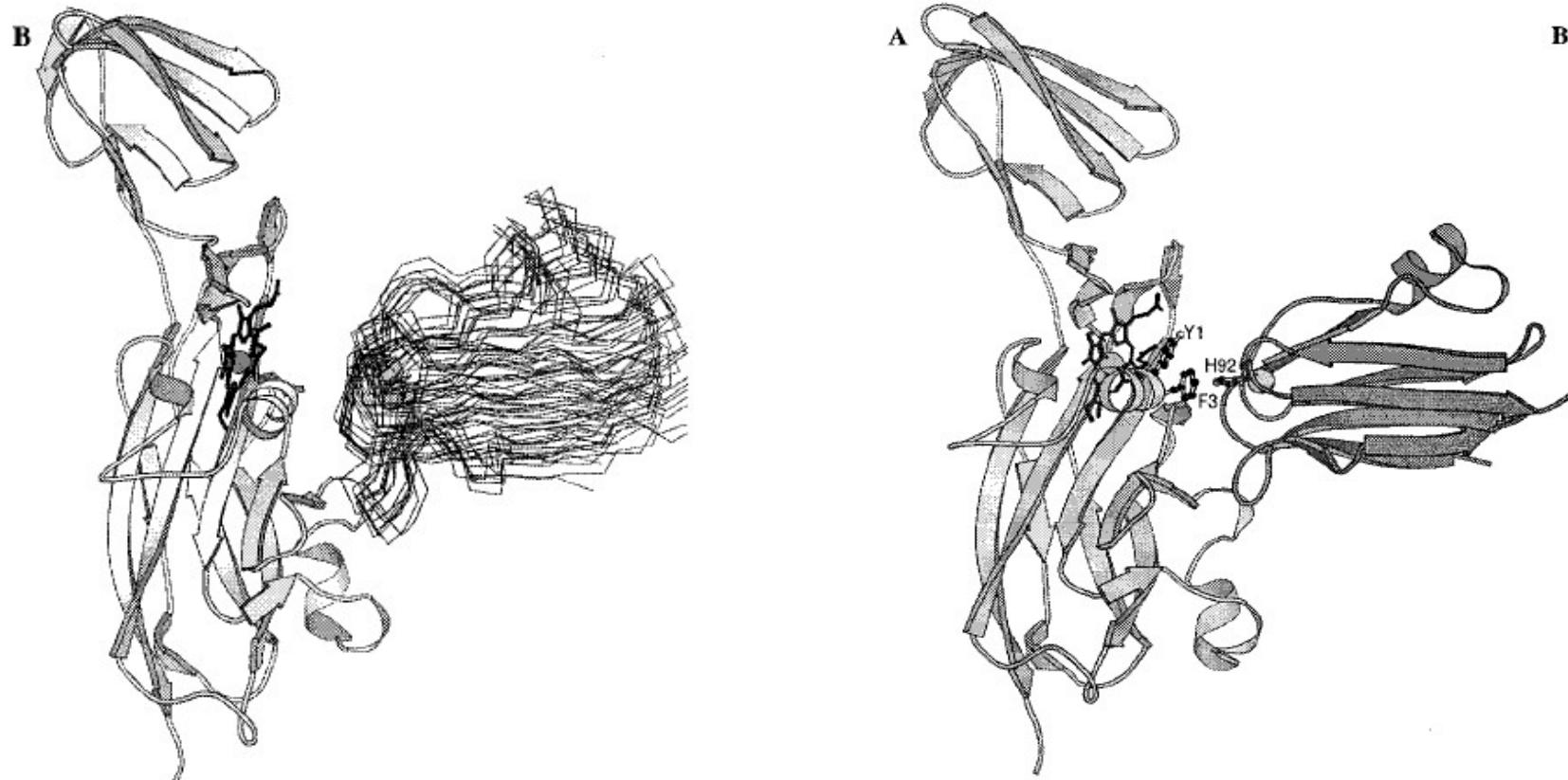


Stéphane Balayssac et al. PNAS 2008;105:45:17284-17289

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PNAS

Structural constraints based on pseudocontact shifts allow structure calculation

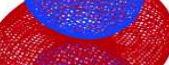
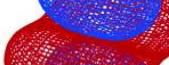
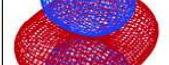
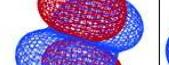


Plastocyanin-cytochrome f complex

Crowley et al (2001) J.Am.Chem.Soc. 123, 10444.

δ^{pc} allow identifying the binding interface and the relative orientation of the proteins

Lanthanide Probes in NMR provide distances

	f1 Ce	f2 Praseodymium	f3 Neodymium	f5 Samarium	f6 Europium	f7 Gadolinium	f8 Terbium	f9 Dysprosium	f10 Holmium	f11 Erbium	f12 Thulium	f13 Ytterbium
$J / 10^{-32} \text{ m}^3$	Ce 5/2 5.6	Pr 4 11.2	Nd 9/2 11.4	Sm 5/2 0.6	Eu 0 ~6	Gd 7/2 55.1	Tb 6 82.7	Dy 15/2 99.2	Ho 8 98.5	Er 15/2 80.3	Tm 6 50.0	Yb 7/2 18.0
PRE												
$\Delta\chi_{\text{ax}} / 10^{-32} \text{ m}^3$	2.1	3.4	1.7	0.2	-2.3	0	42.1	34.7	18.5	-11.6	-21.9	-8.3
$\Delta\chi_{\text{rh}} / 10^{-32} \text{ m}^3$	0.7	2.1	0.4	-0.1	-1.6	0	11.2	20.3	5.8	-8.6	-20.1	-5.8
PCS	 5 \AA	 5 \AA	 5 \AA	 5 \AA	 5 \AA		 10^{-7}	 10^{-7}	 10^{-13}	 10^{-13}	 10^{-13}	 10^{-13}
τ_e / s	10^{-13}				10^{-7}				10^{-13}			

Pintacuda, John; Su; Otting; Acc. Chem. Res. 2007, 40, 206-212

The hyperfine coupling is given by:

$$\delta^{exp} = \delta^{pdia} + \delta^{para}$$

$$\delta^{para} = \delta^{con} + \delta^{pc}$$

Through chemical bonds



Electron spin density
on the nucleus

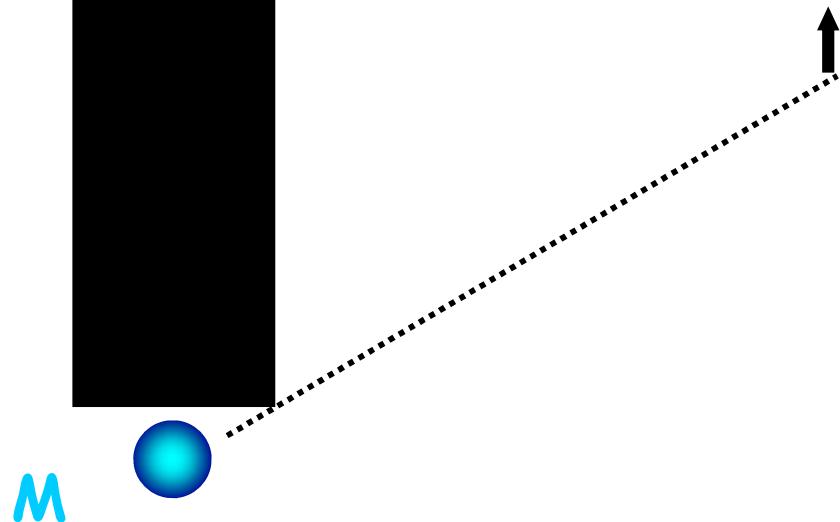
Dipolar interaction



Metal - nucleus distance
(anisotropy) r^{-3}

NMR of Paramagnetic Systems: The electron-nucleus interaction

$$\mu_{e^-} = 658 \mu_{1H}$$



Unpaired electrons affect:

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- Relaxation rates

Nuclear Relaxation in the presence of unpaired electrons

$$R_1 = 1 / T_1$$

$$R_1^{\text{exp}} = R_1^{\text{dia}} + R_1^{\text{para}}$$

$$R_2 = 1 / T_2$$

$$R_2^{\text{exp}} = R_2^{\text{dia}} + R_1^{\text{para}}$$

Contact contribution

Pseudocontact contribution

Curie contribution

Nuclear relaxation due to the electron-nucleus dipolar coupling

$$R_{1M} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6} \left[\frac{7\tau_s}{1+\omega_S^2 \tau_S^2} + \frac{3\tau_s}{1+\omega_I^2 \tau_S^2} \right]$$

$$R_{2M} = \frac{1}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6} \left[4\tau_s + \frac{13\tau_s}{1+\omega_S^2 \tau_S^2} + \frac{3\tau_s}{1+\omega_I^2 \tau_S^2} \right]$$

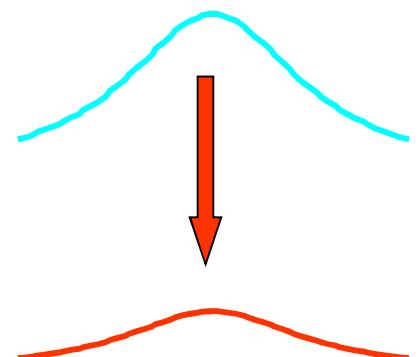
τ_s depends on the electronic structure of the metal ion

τ_s^{-1}

Electronic structure of the metal ion

Valence and spin state
Donor atoms (ligands)
Coordination geometry

Paramagnetic system	S	$\tau_s (s^{-1})$	small molecules ($\tau_r = 10^{-10} s^{-1}$)	large molecules ($\tau_r = 10^{-8} s^{-1}$)
Fe ³⁺ (H.S.)	5/2	10^{-9} - 10^{-11}	500-6000	500-3000
Fe ³⁺ (L.S.)	1/2	10^{-11} - 10^{-13}	2-50	1-60
Fe ²⁺ (H.S.)	2	10^{-12} - 10^{-13}	50-150	10-50
Co ²⁺ (H.S., 5-6 coord)	3/2	5×10^{-12} - 10^{-13}	20-200	10-2000
Co ²⁺ (H.S., 4 coord)	3/2	10^{-11}	200-300	200
Ni ²⁺ (5-6 coord)	1	10^{-12}	600-700	1000
Ni ²⁺ (4 coord)	1	10^{-10}	20-30	20
Cu ²⁺	1/2	10^{-9}	300-500	40-200
Ru ³⁺	1/2	10^{-11} - 10^{-12}	5-50	5-50
Re ³⁺	2	10^{-12} - 10^{-13}	50-150	10-50
Gd ³⁺	7/2	10^{-8} - 10^{-9}	5000-15000	800-5000
				100000-400000

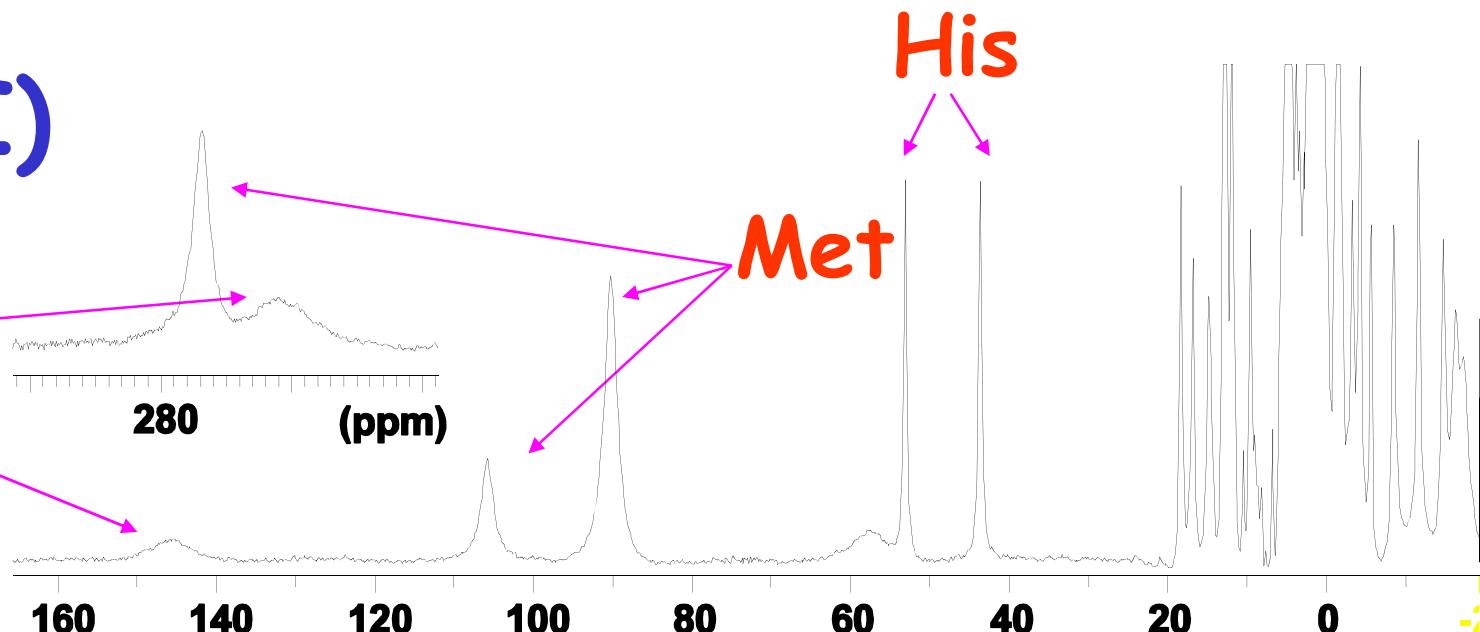
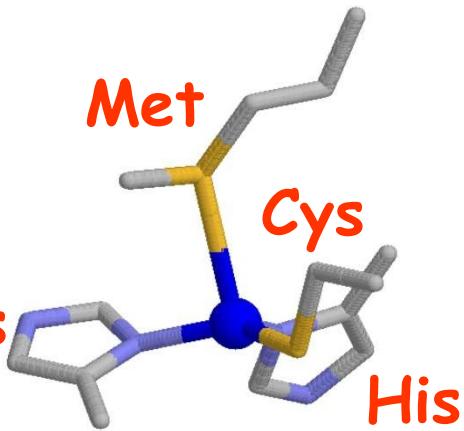


Co(II)

Cys

Met

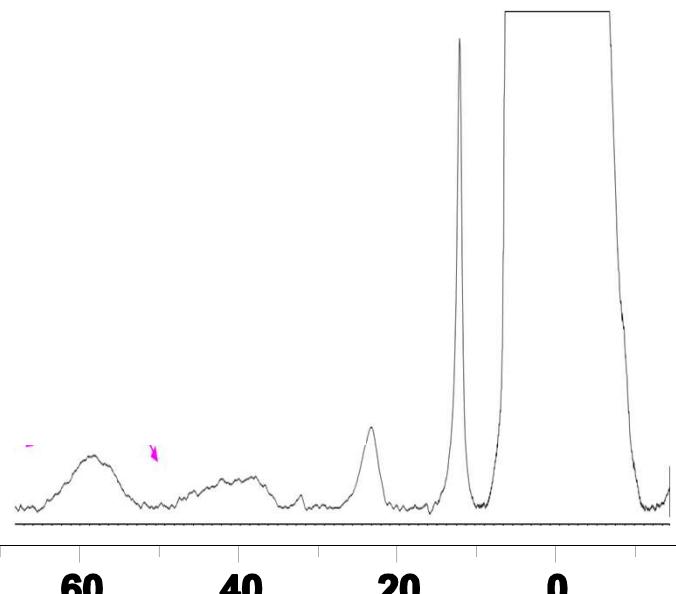
Cys



Cu(II)

Cys

Broadened beyond detection



NMR of Cu(II) proteins

Type 2 Cu(II)

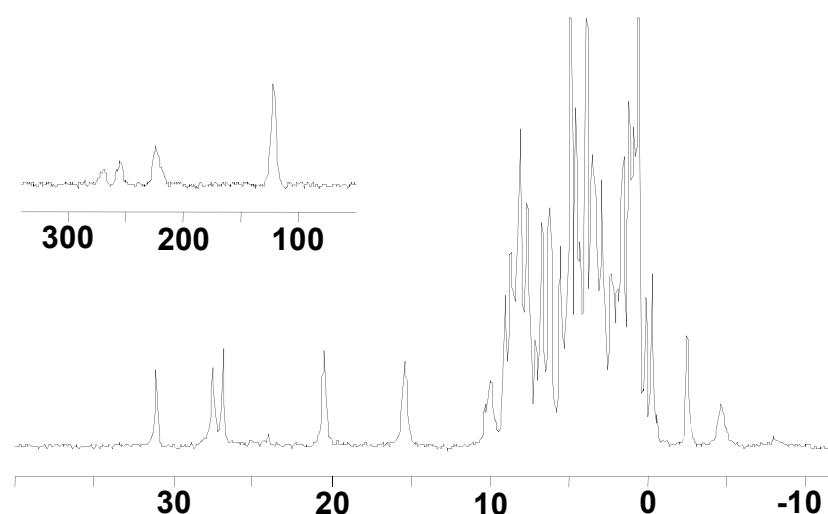
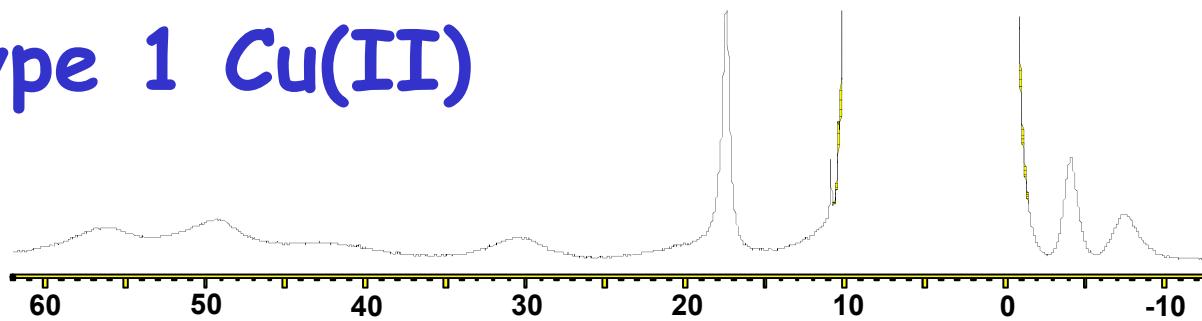
$$\tau_s 10^{-9} \text{ s}$$

Type 1 Cu(II)

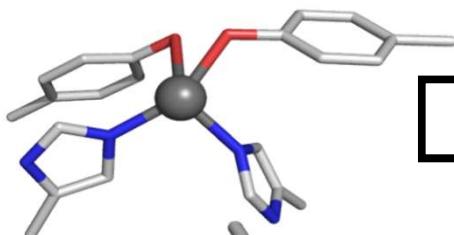
$$\tau_s 10^{-10} \text{ s}$$

Cu_A

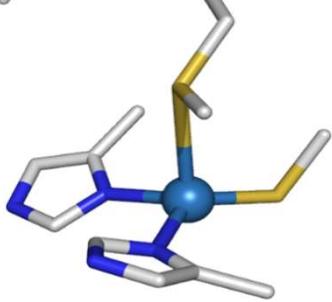
$$\tau_s 10^{-11} \text{ s}$$



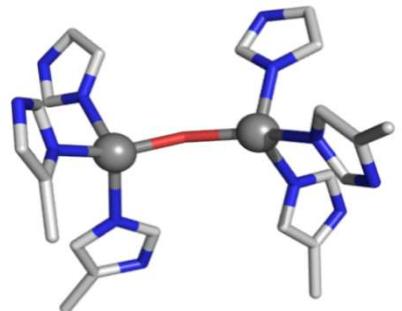
Electron Relaxation Times (τ_s) dominate Nuclear Relaxation



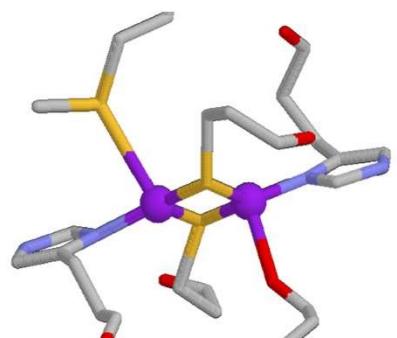
T2: $\tau_s \sim 10^{-9}$ s



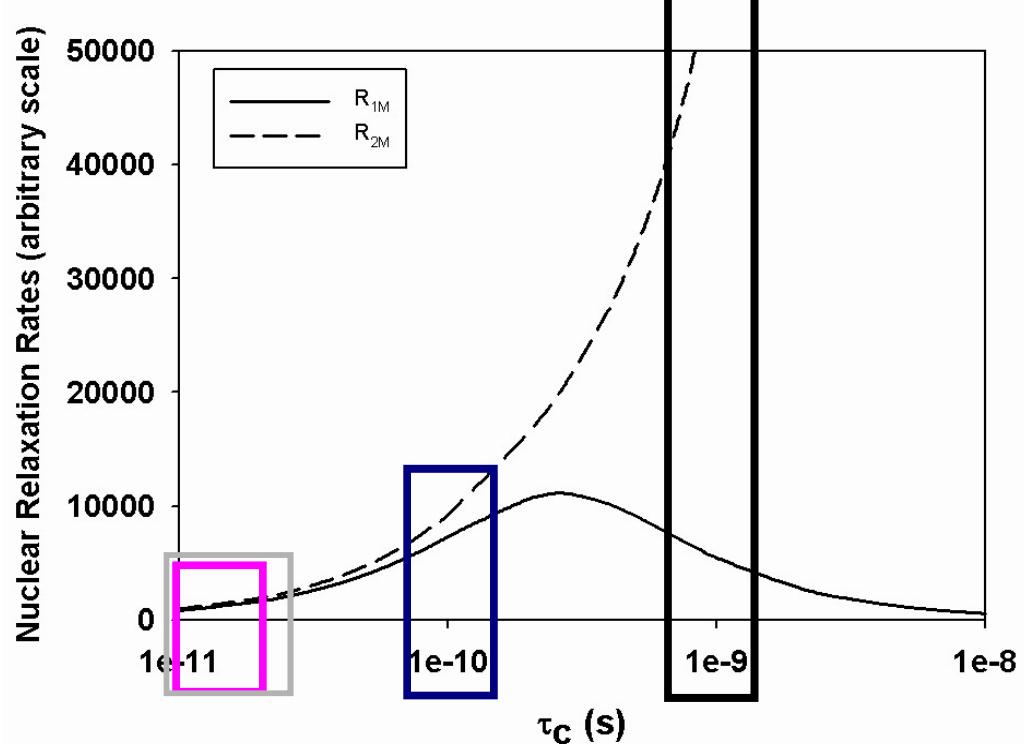
T1: $\tau_s \sim 10^{-10}$ s



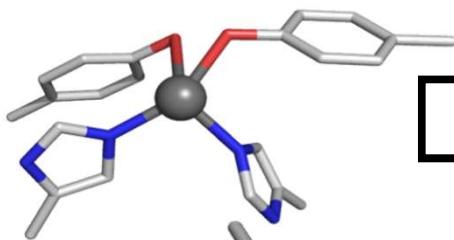
T3: $\tau_s \sim 10^{-11}$ s



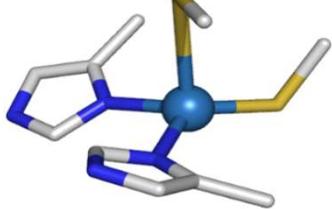
CuA: $\tau_s \sim 10^{-11}$ s



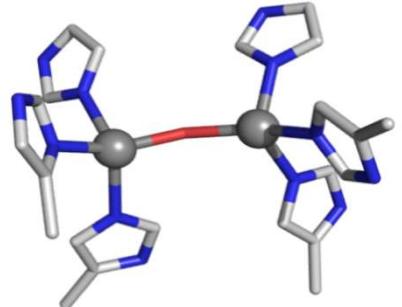
Electron Relaxation Times (τ_s) dominate Nuclear Relaxation



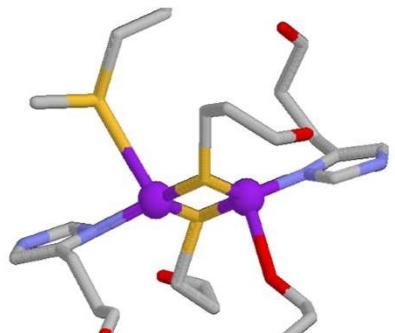
T2: $\tau_s \sim 10^{-9}$ s



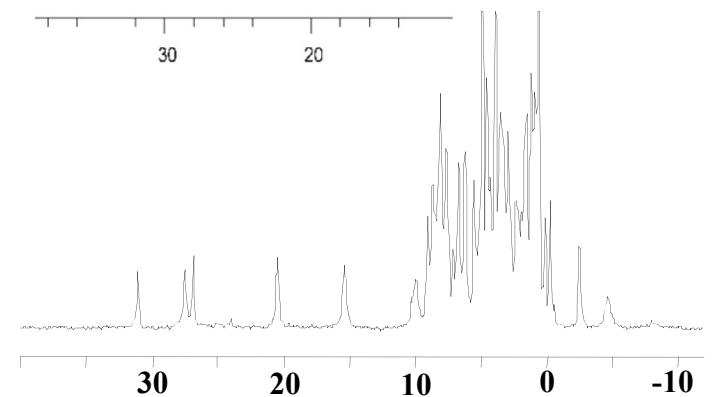
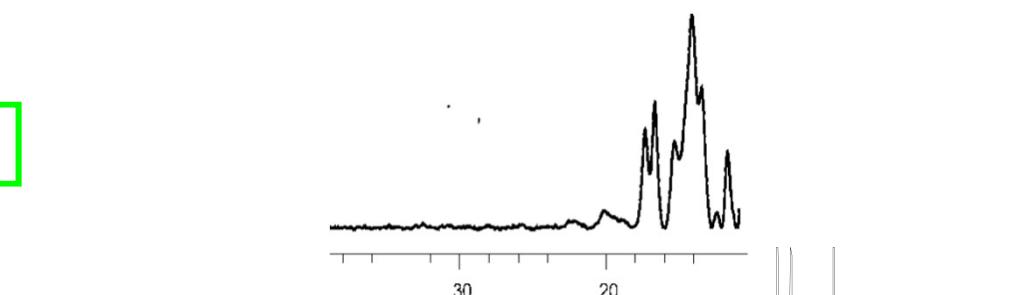
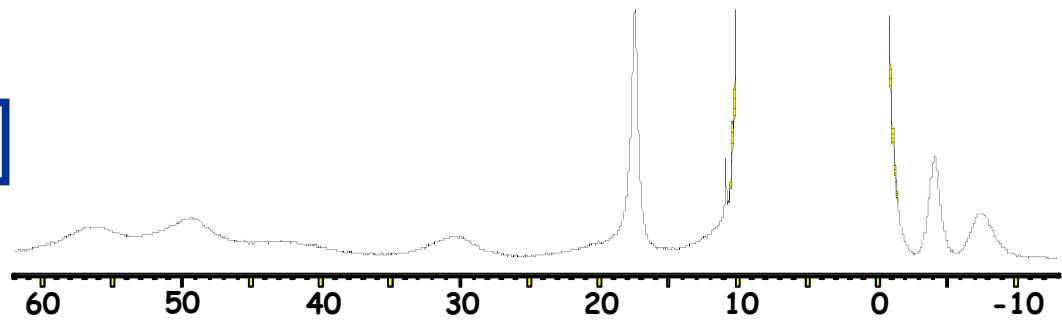
T1: $\tau_s \sim 10^{-10}$ s



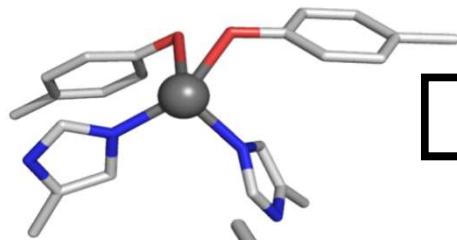
T3: $\tau_s \sim 10^{-11}$ s



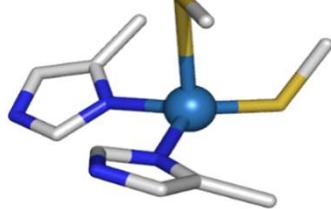
CuA: $\tau_s \sim 10^{-11}$ s



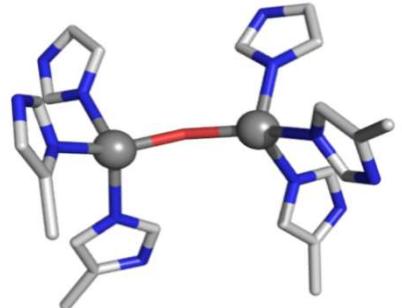
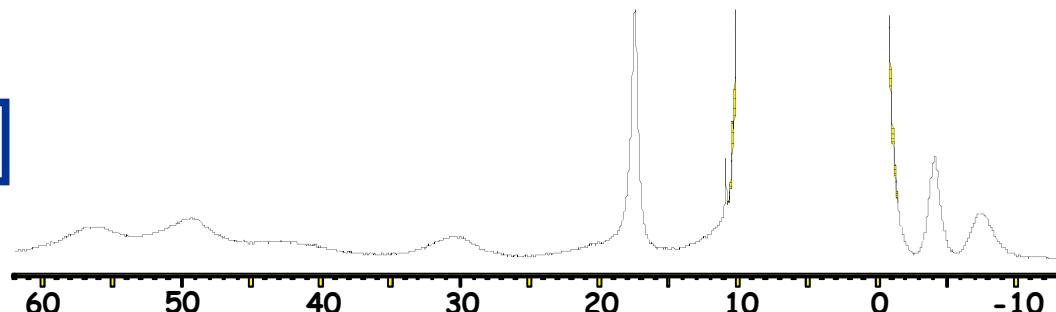
Electron Relaxation Times (τ_s) dominate Nuclear Relaxation



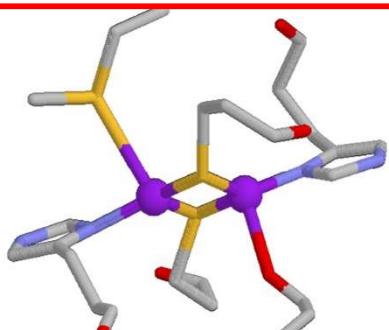
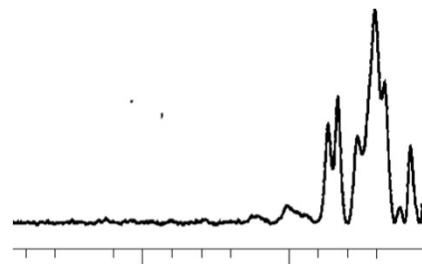
T2: $\tau_s \sim 10^{-9}$ s



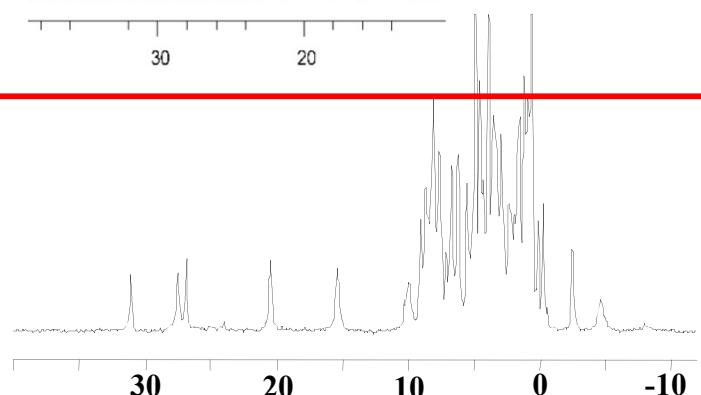
T1: $\tau_s \sim 10^{-10}$ s



T3: $\tau_s \sim 10^{-11}$ s



CuA: $\tau_s \sim 10^{-11}$ s



NMR of Paramagnetic Proteins

Electron-nucleus interaction

Fast relaxation rates

Broad lines

Fast pulsing

Low signal-to-noise

Selectivity

Large chemical shifts

Signal scrambling

Higher resolution

Electron spin density

RMN de compuestos paramagneticos

- Teoria
- Tips prácticos
- Una aplicación

Practical hints

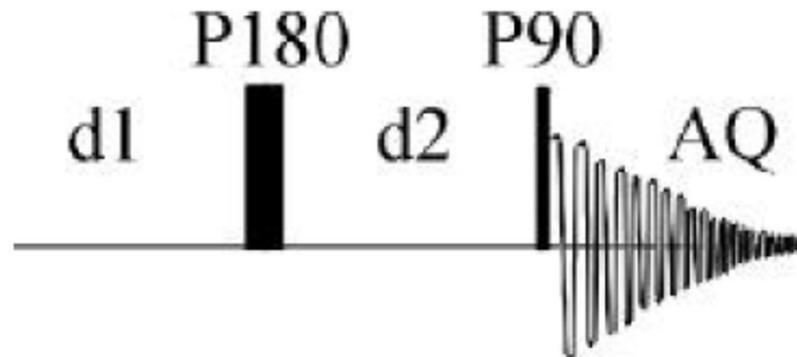
1. Record 1D ^1H , ^{13}C and ^{15}N spectra under different conditions
(Selective residue labeling)
2. Study the different contributions to shifts and relaxation
3. Record tailored homonuclear and heteronuclear experiments to assign resonances

Practical problems with paramagnetic samples

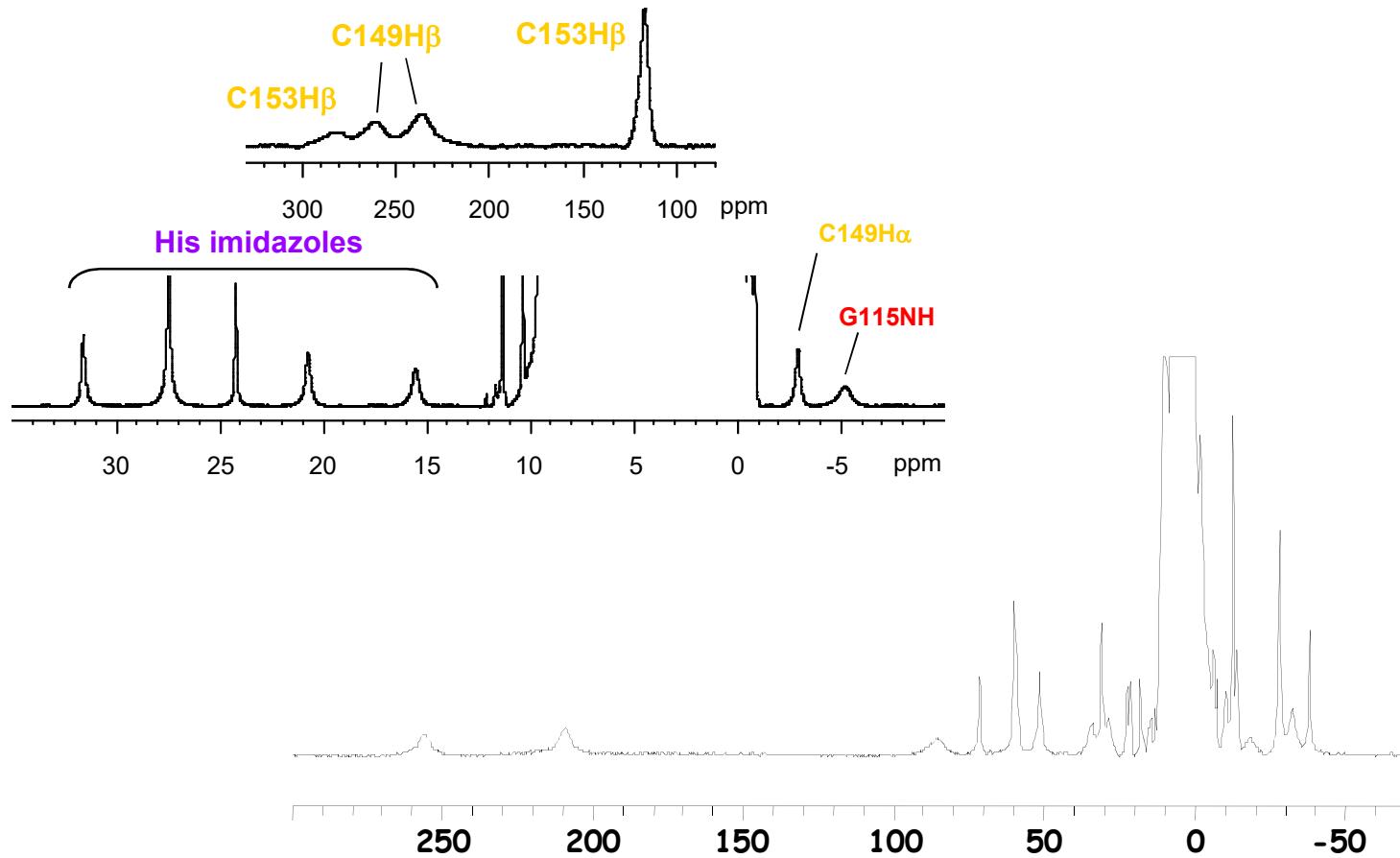
1. The excitation of a large range of chemical shifts
2. J-couplings cannot be measured
3. Relaxation can be faster than magnetization transfer

Practical hints for recording NMR spectra on paramagnetic samples

Super WEFT (Water Eliminated Fourier Transform)

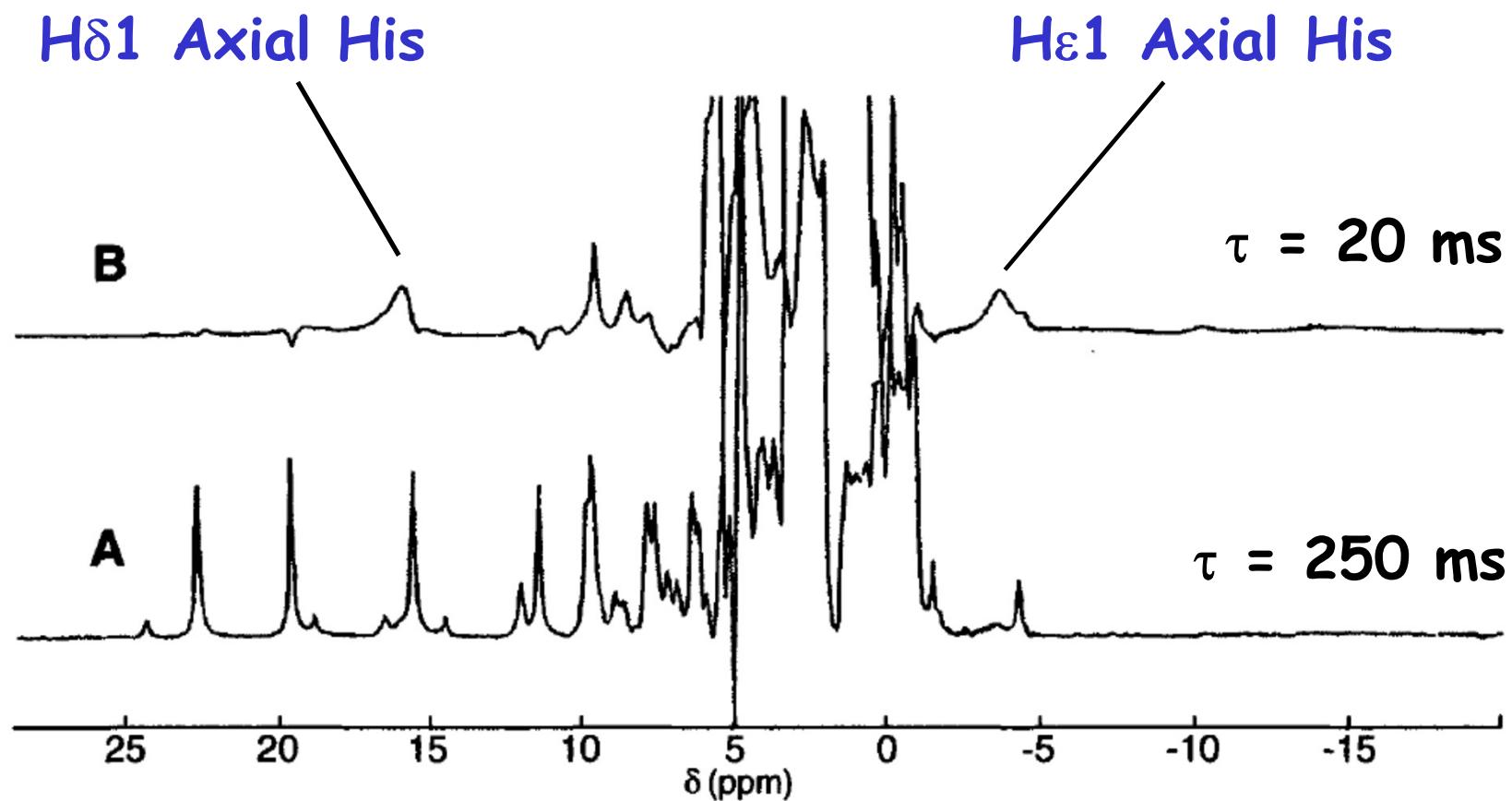


1H NMR spectra



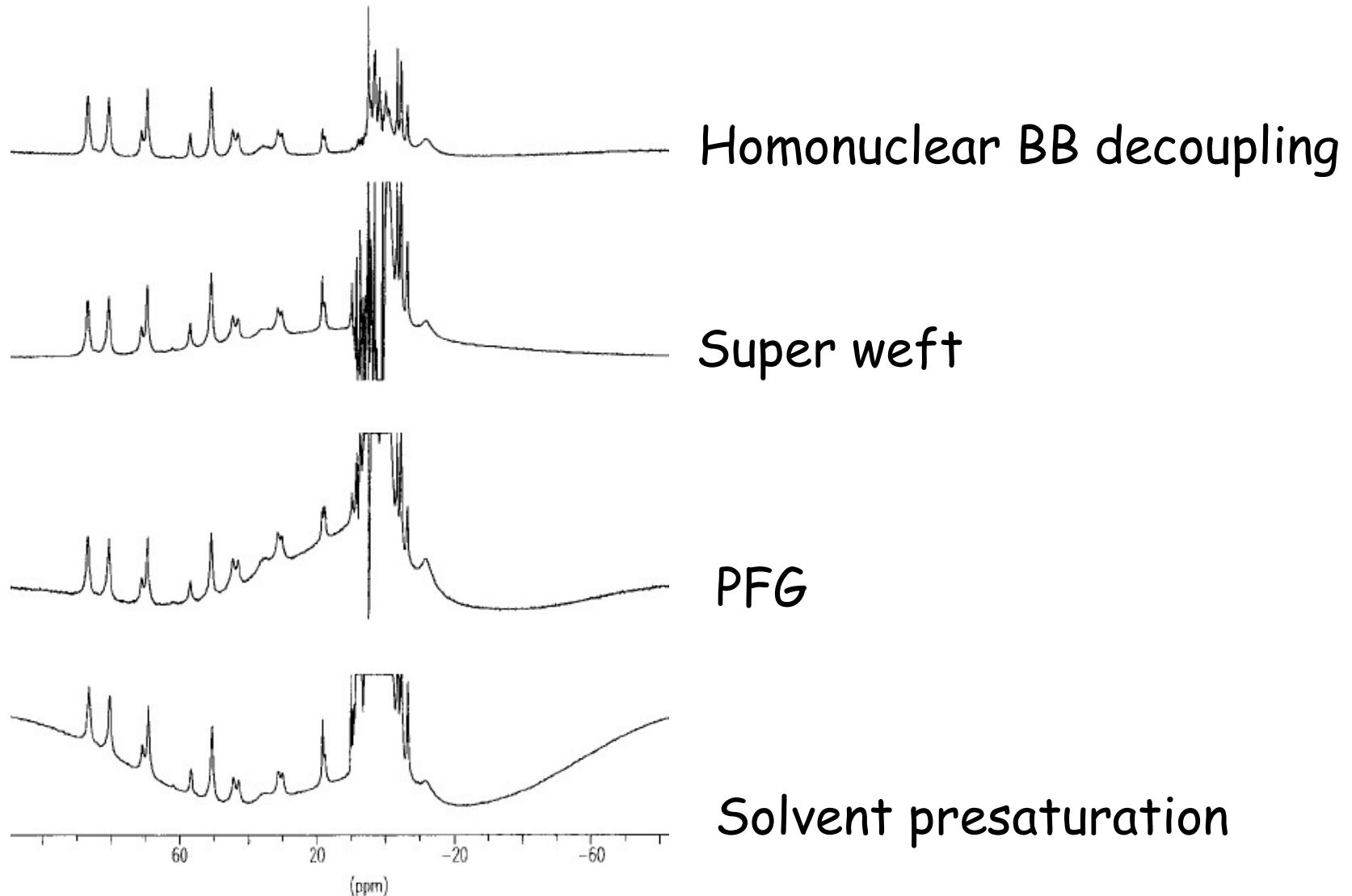
Explore different spectral widths and recycle rates

Met80Ala cytochrome c - Cyanide adduct



Bren et al. (1995) JACS, 117, 8067

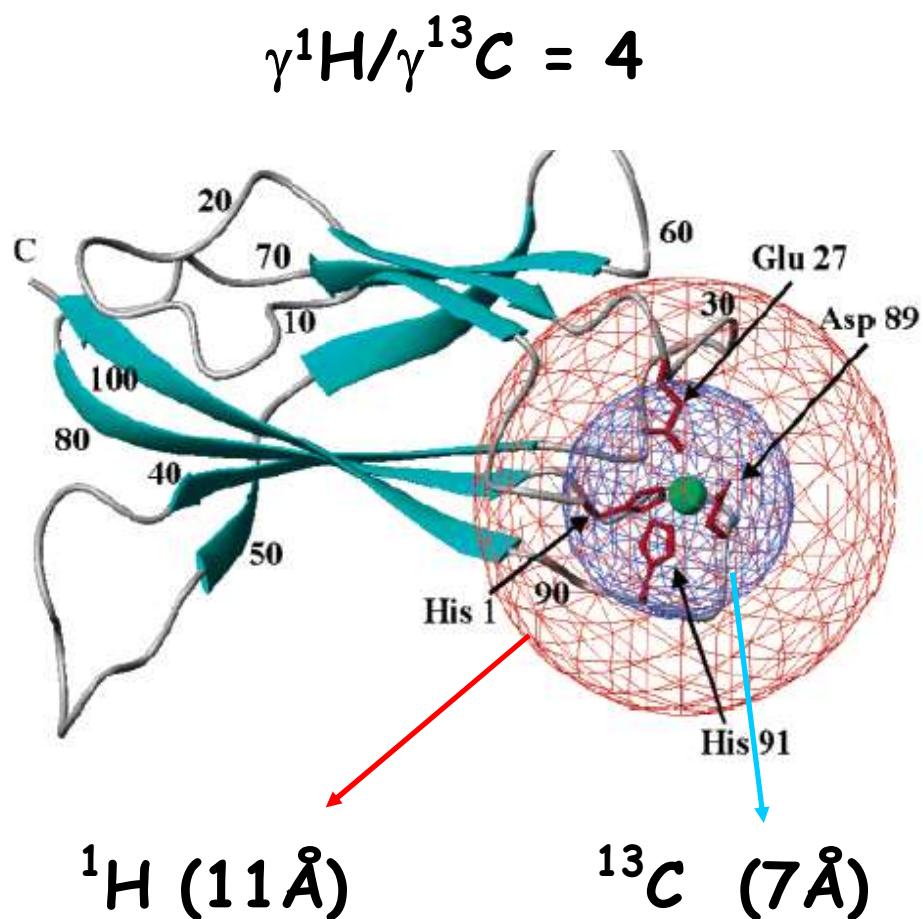
Solvent suppression



Bondon & Mouro, JMR 134, 154-157 (1998)

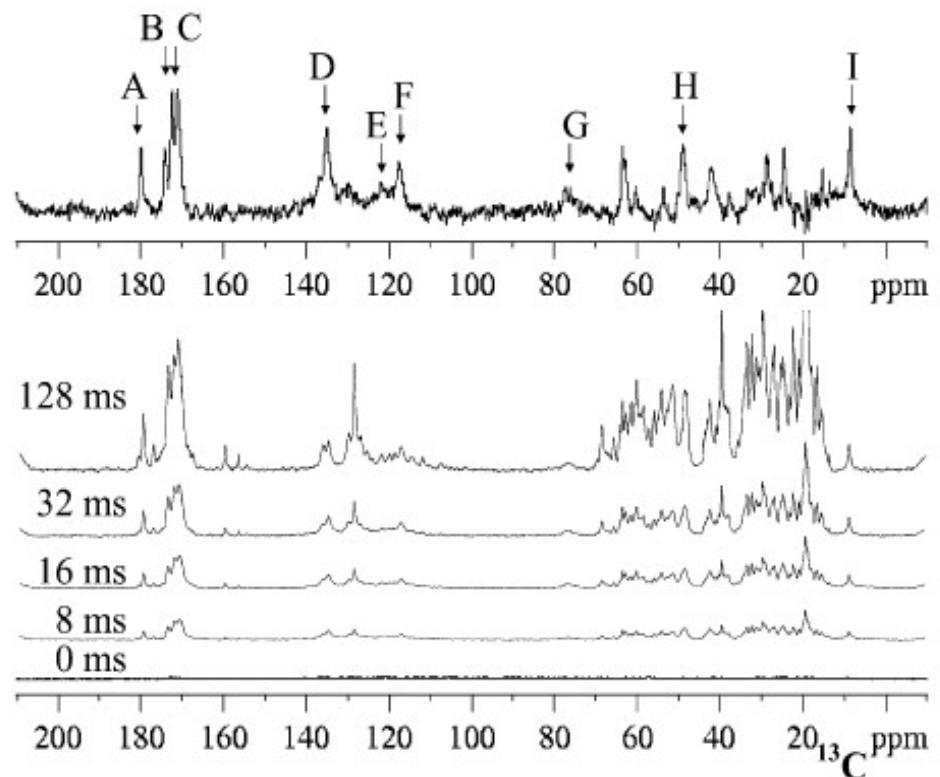
^{13}C Direct Detection

A Strategy for the NMR Characterization of Type II Copper(II)
Proteins: the Case of the Copper Trafficking Protein CopC
from *Pseudomonas Syringae*



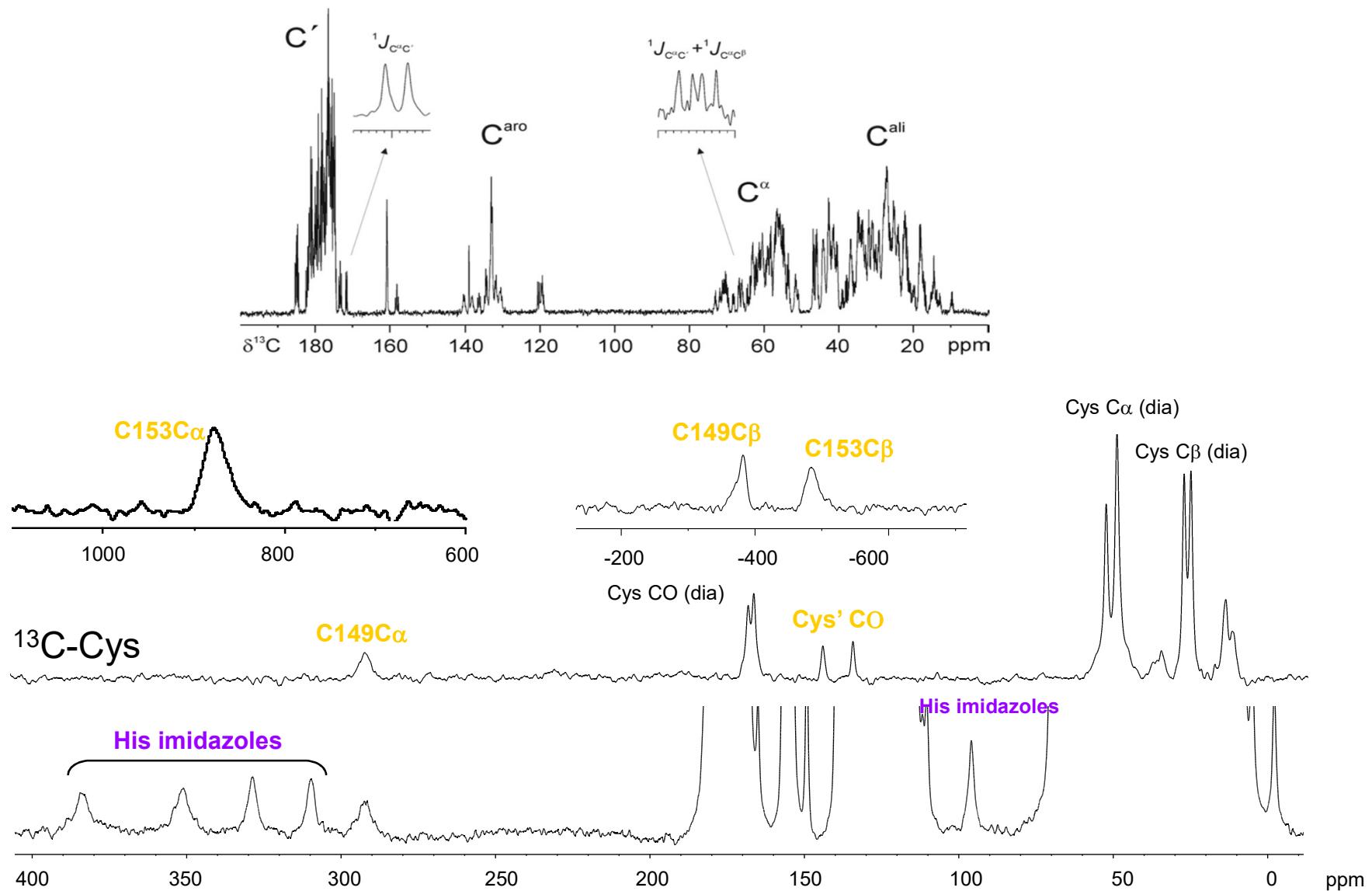
$$\text{Cu(II)}, \tau_s = 3 \times 10^{-9}\text{s}$$

Saturation Recovery ^{13}C Spectra



Arnesano *et al* (2003) *J.Am.Chem.Soc.* 125, 7201.

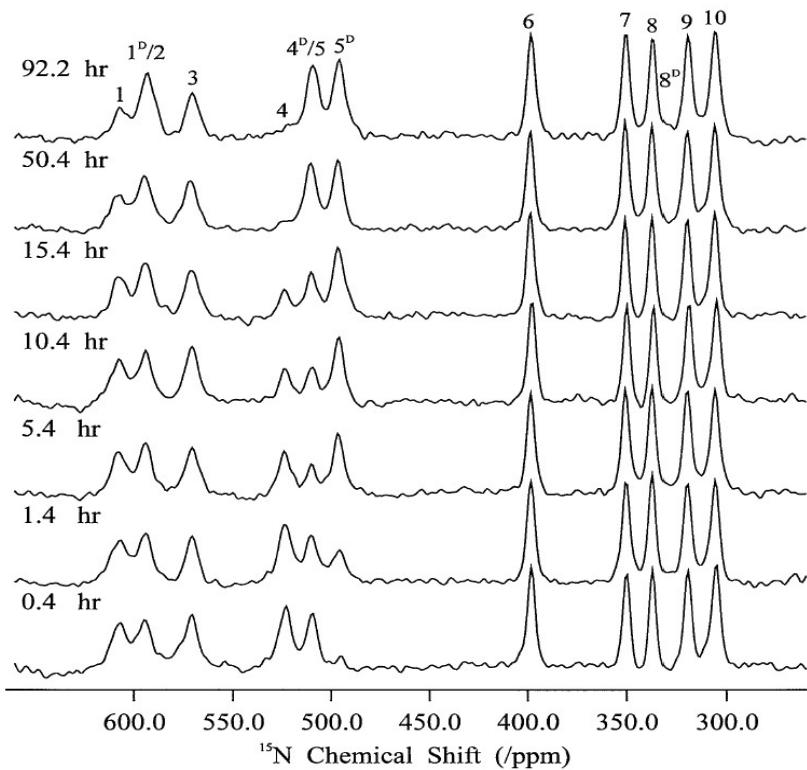
^{13}C Direct Detection



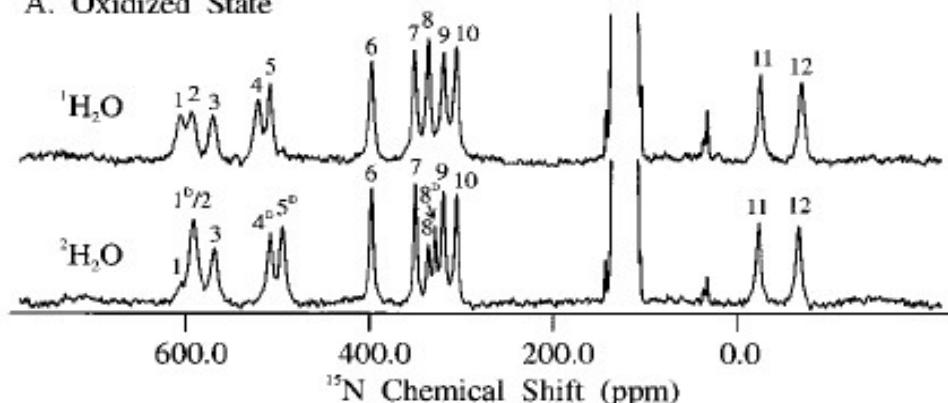
^{15}N Direct Detection

Amplification of One-Bond $^1\text{H}/^2\text{H}$ Isotope Effects on
 ^{15}N Chemical Shifts in *Clostridium pasteurianum*
Rubredoxin by Fermi-Contact Effects through
Hydrogen Bonds

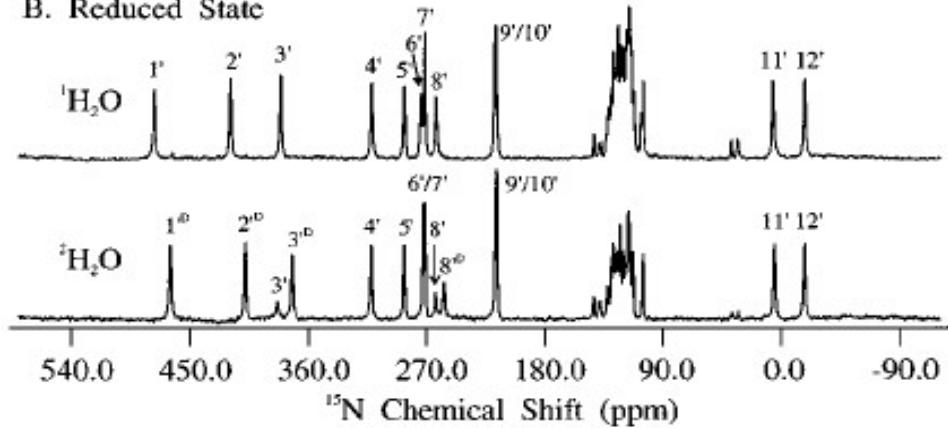
$$\gamma^1\text{H} / \gamma^{15}\text{N} = 10$$



A. Oxidized State



B. Reduced State

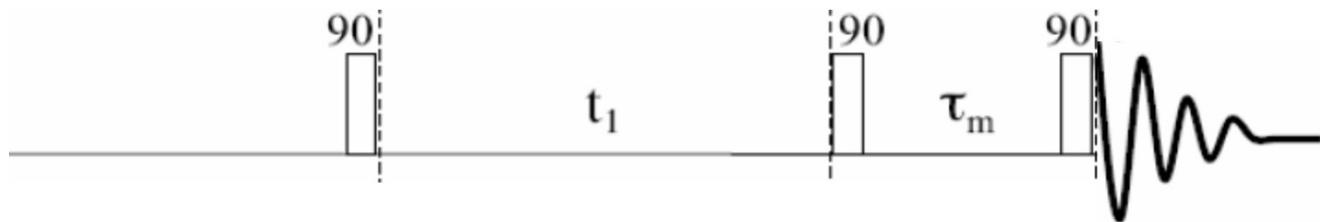


Xia et al (1998) J.Am.Chem.Soc. 120, 4893.

Hydrogen bonds

^1H signal assignments

NOESY



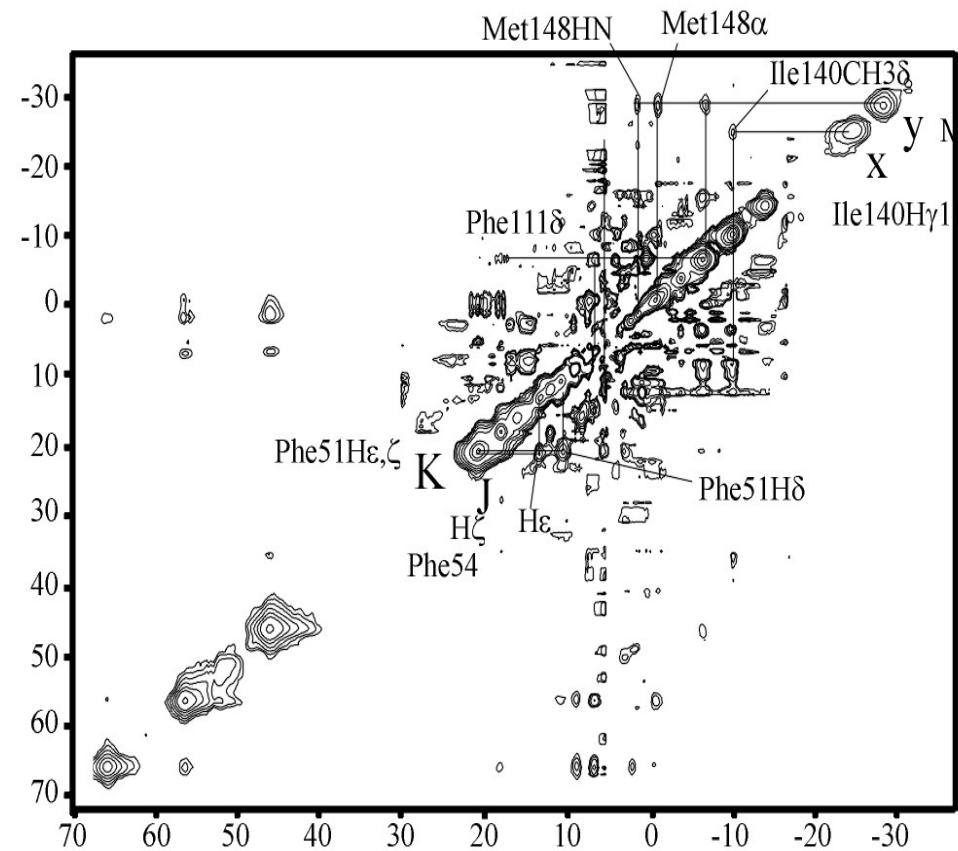
Longitudinal ^1H magnetization during mixing time
(optimized according to T1 values)

Transverse ^1H magnetization (T2) during:

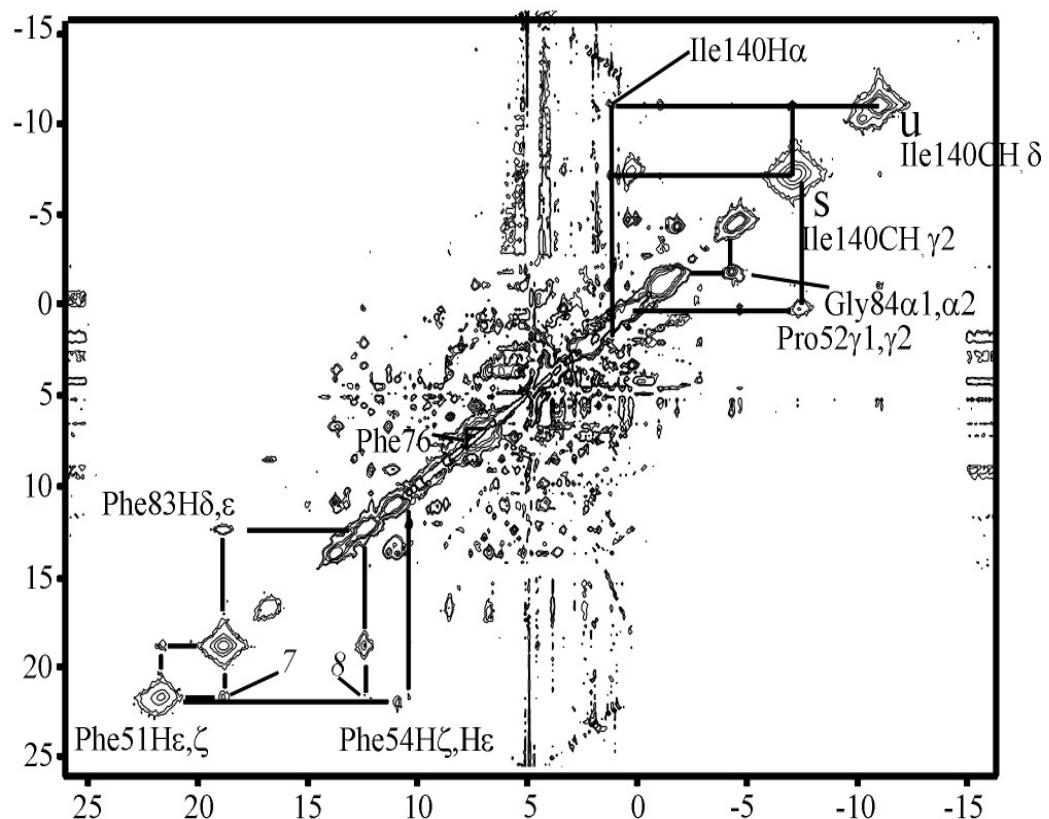
- Evolution time (t_1)
- Acquisition (5-10 ms)

Optimize Number of Scans (max.) and number of points in the indirect dimension

NOESY



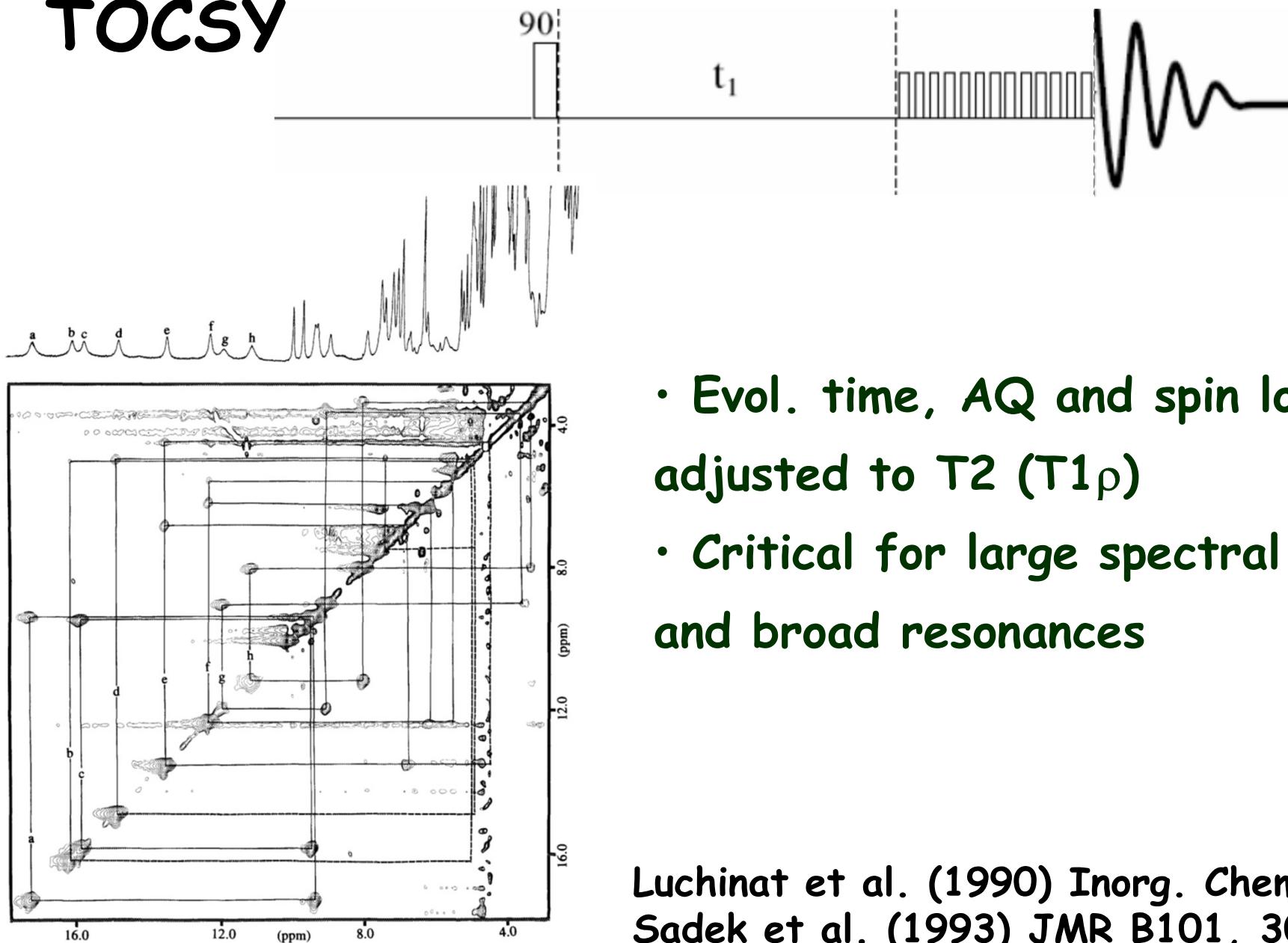
$\tau_M = 7 \text{ ms}$



$\tau_M = 15 \text{ ms}$

Donaire et al. (1998) Biochemistry

TOCSY

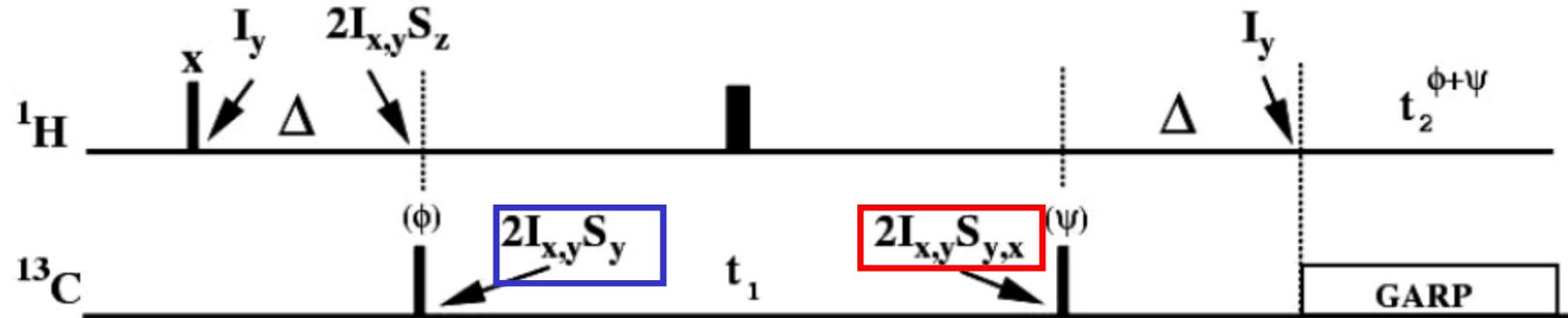


- Evol. time, AQ and spin lock time adjusted to T_2 ($T_{1\rho}$)
- Critical for large spectral widths and broad resonances

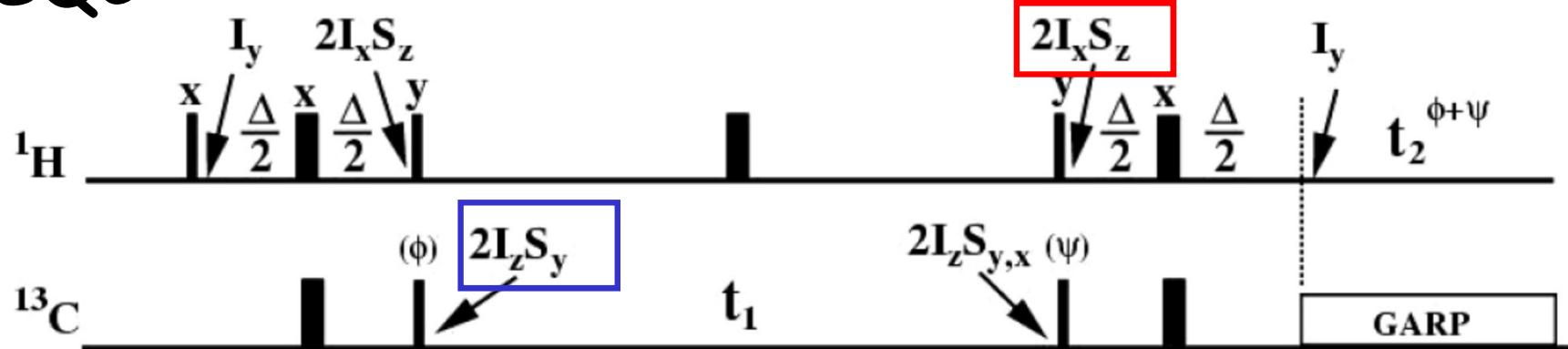
Luchinat et al. (1990) Inorg. Chem. 29, 4351
Sadek et al. (1993) JMR B101, 309

Inverse Detection Experiments

HMQC



HSQC



Adjust Δ 's to ^1H T2

General guidelines

- Select the simplest possible pulse sequence
- Shorten magnetization transfer delays according to relaxation rates
- Shorten or eliminate gradients
- Avoid anti-phase components when $\Delta\nu > J$
- Avoid unnecessary digital resolution
- Maximize the number of scans