NMR of Paramagnetic Molecules

Kara L. Bren
University of Rochester

Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

Resources

- Bertini & Luchinat, “NMR of Paramagnetic Molecules in Biological Systems,” **1986**, Benjamin/Cummings: Menlo Park. ISBN: 0-8053-0780-X
- Bertini & Luchinat, “NMR of Paramagnetic Substances,” *Coord. Chem. Rev.* **1996**, *150*, 1-296.
- Banci, “Nuclear and Electron Relaxation. The Magnetic Nucleus-unpaired Electron Coupling in Solution,” **1991**, VCH: Weinheim. ISBN: 3-5272-8306-4
- Bren, “NMR Analysis of Spin States and Spin Densities,” in *Spin States in Biochemistry and Inorganic Chemistry: Influence on Structure and Reactivity*, (Eds: Swart & Costas), **2016**, Wiley: Chichester. DOI: 10.1002/9781118898277.ch16

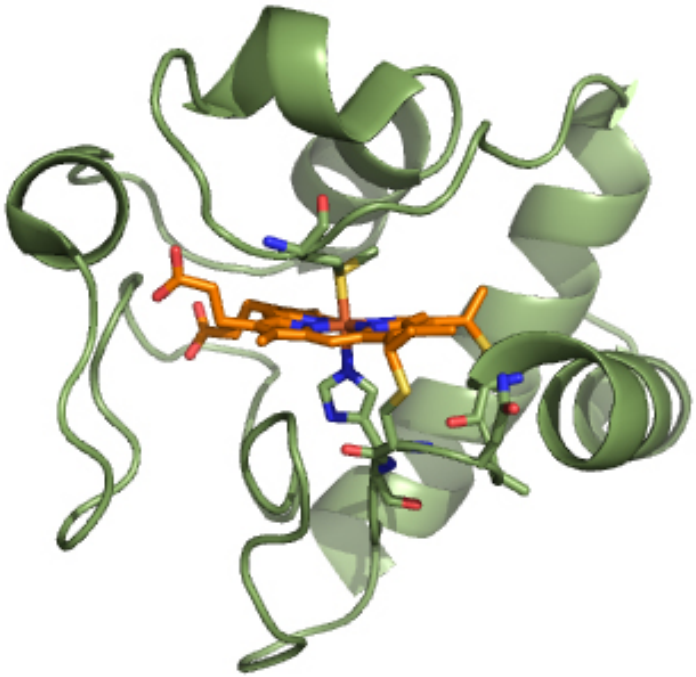
Outline

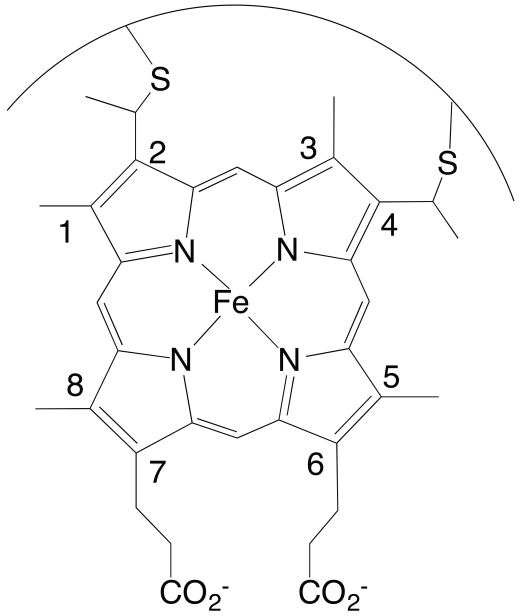
- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

Effects of Unpaired Electrons

Horse ferricytochrome *c*

S = 1/2

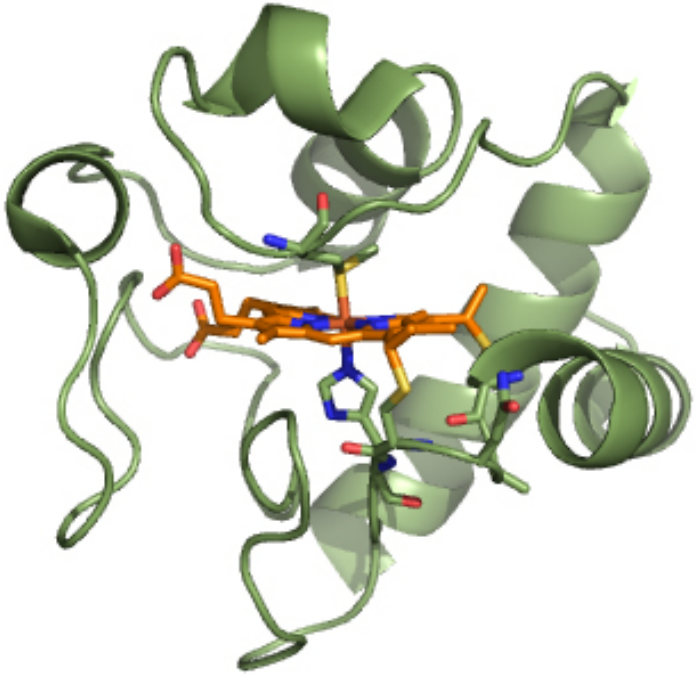


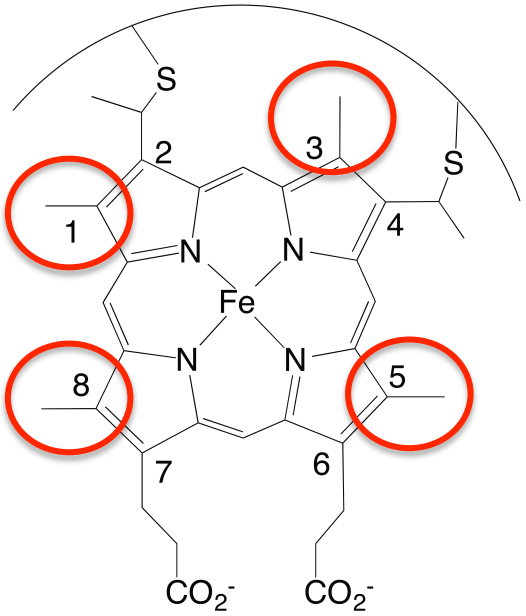


Effects of Unpaired Electrons

Horse ferricytochrome *c*

S = 1/2





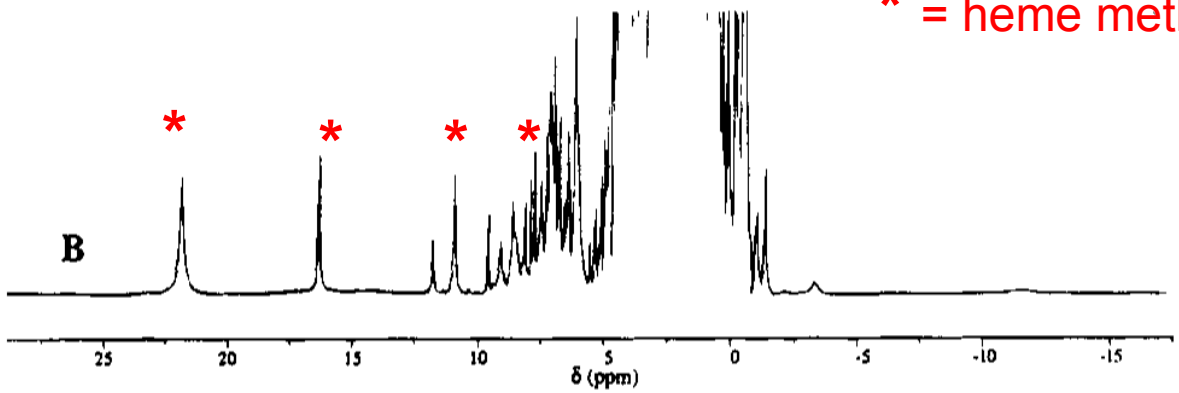
Heme methyls

Effects of Unpaired Electrons

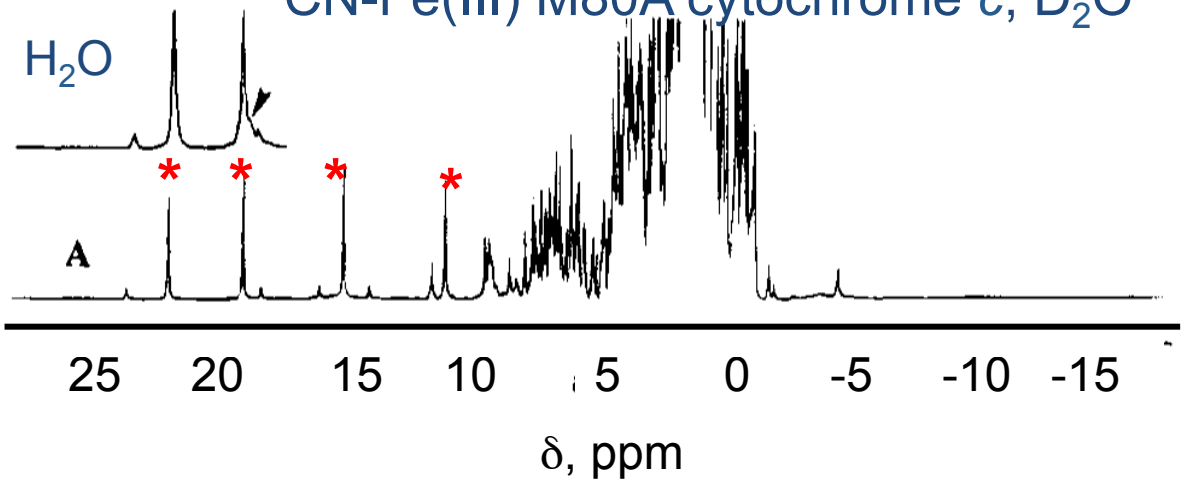
CN-Fe(III) cytochrome *c*, D_2O

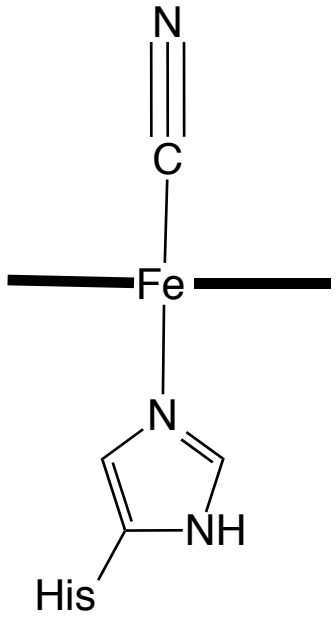
* = heme methyls

S = 1/2



CN-Fe(III) M80A cytochrome *c*, D_2O

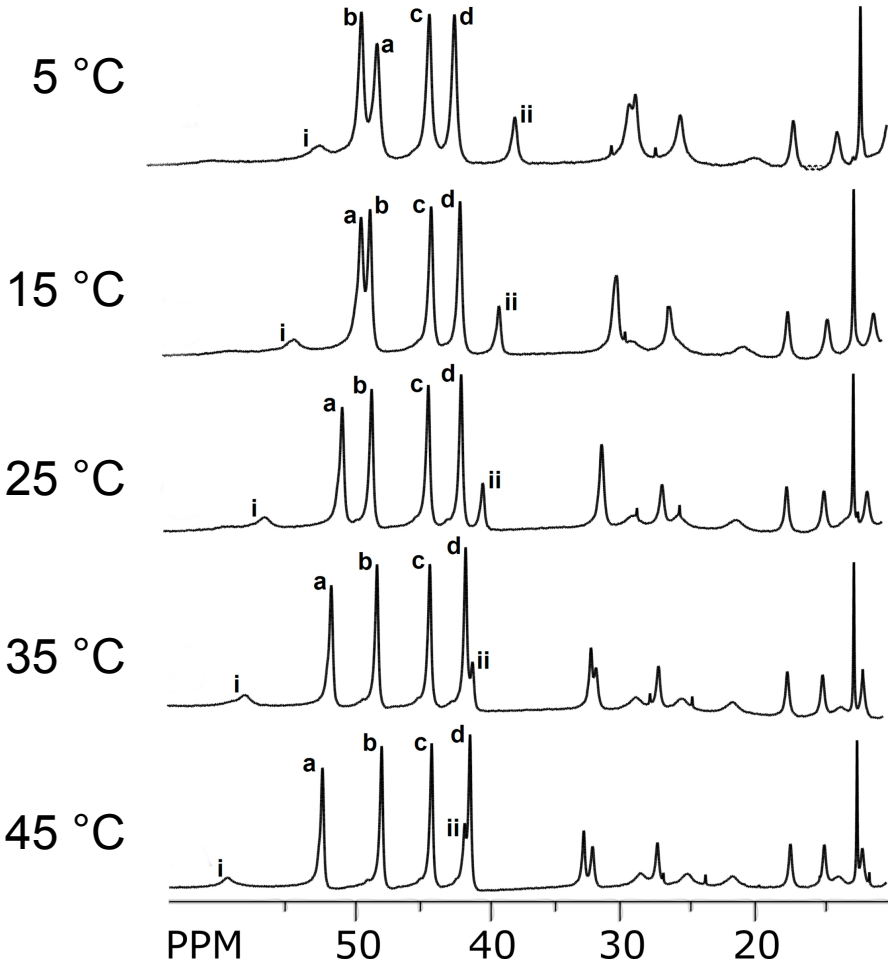




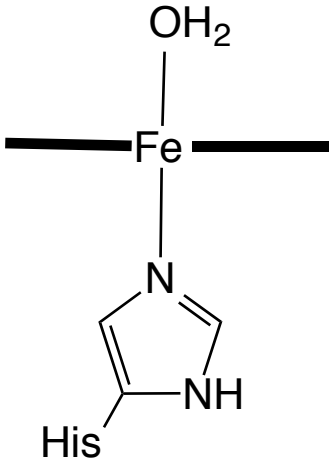
JACS **1995**, 8067

Effects of Unpaired Electrons

H. thermophilus Fe(III)M61A cyt *c*



S = 1/2, 5/2

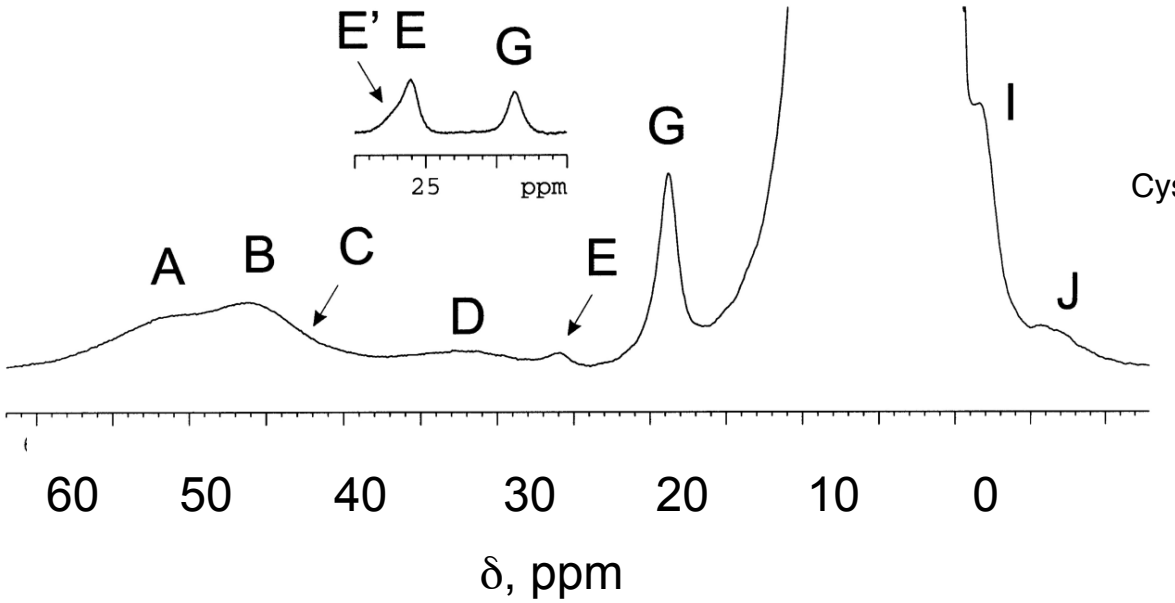


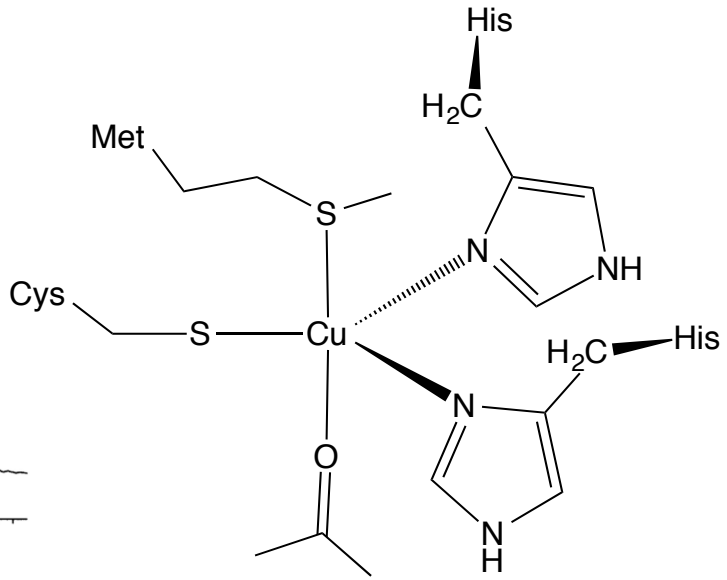
a,b,c,d = heme methyls

Effects of Unpaired Electrons

P. aeruginosa Cu(II) azurin

S = 1/2



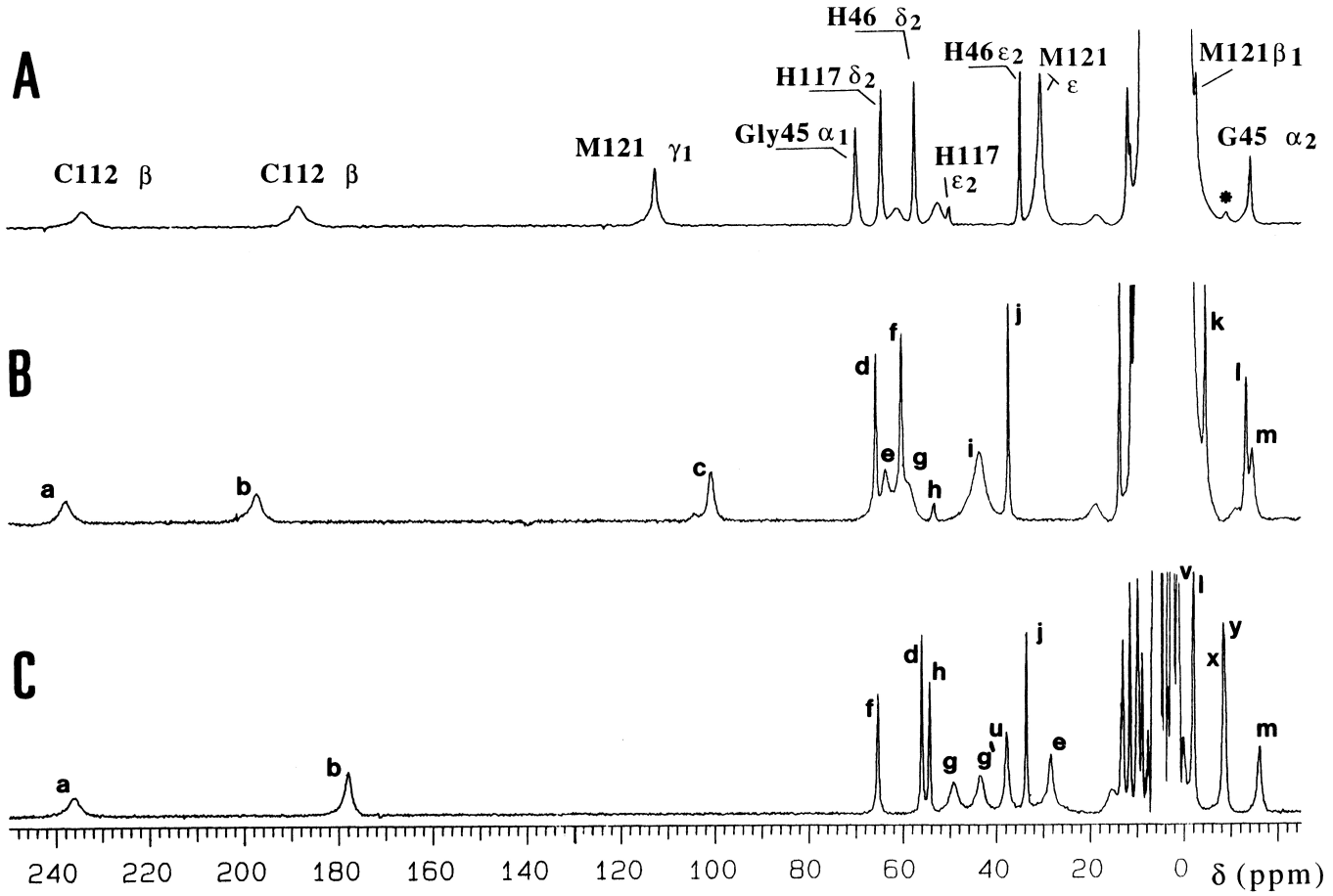


JACS **2000,** 3701

Effects of Unpaired Electrons

Ni(II) azurin

S = 1

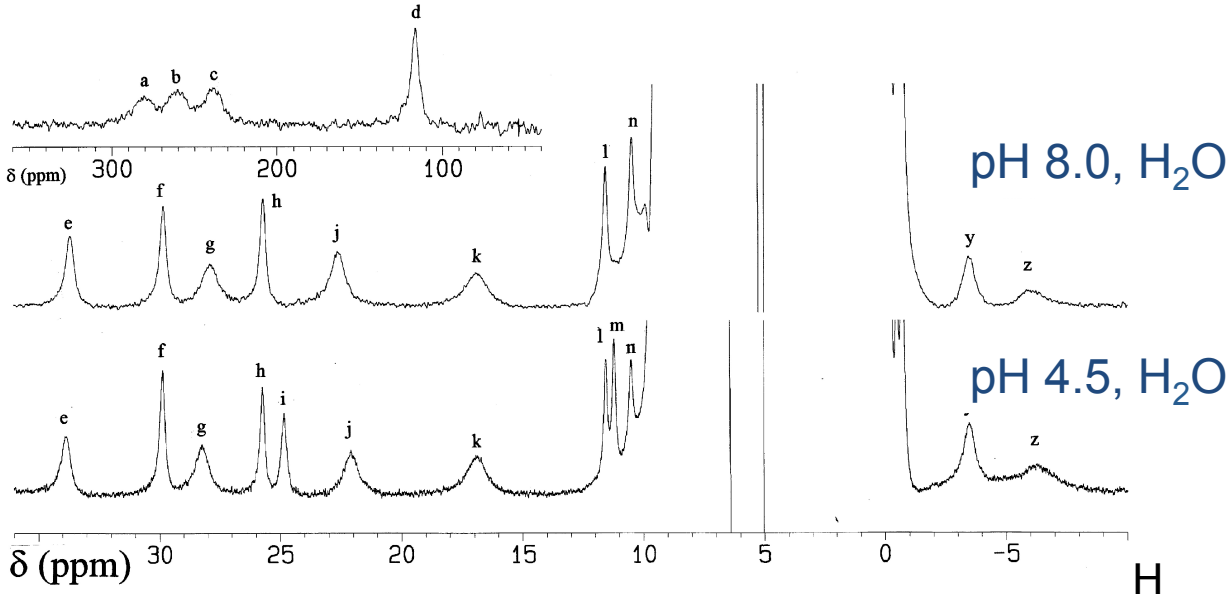


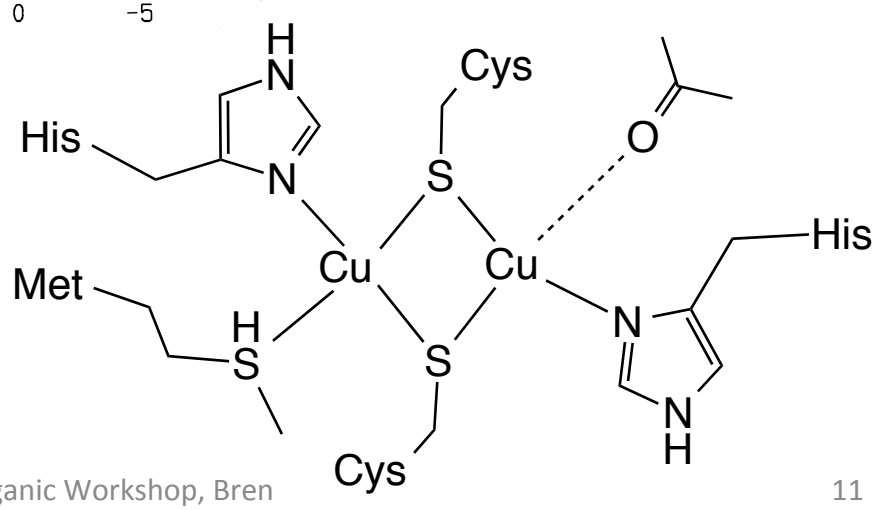
Biochemistry **1996**, 1810

Effects of Unpaired Electrons

T. thermophilus Cu_A domain

Cu(I)Cu(II) S = 1/2





JACS **1996,** 11658

Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

What Can We Learn?

- Metal oxidation state and spin state
- Electron spin relaxation time (estimate)
  - Presence of low-lying excited states
- Pattern and amount of electron spin delocalization onto ligands; hyperfine coupling constants
- Presence of hydrogen bonds
- Magnetic anisotropy, magnetic axes
- Structural refinement is possible
- (In addition, 3D structure, exchange phenomena, dynamics, etc.)

Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

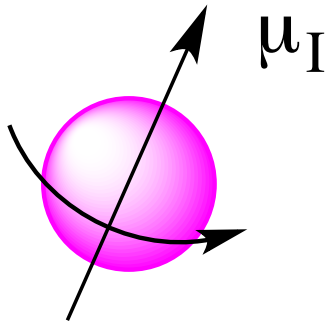
NMR Fundamentals

Some nuclei have spin angular momentum and an associated magnetic moment, μ_I.

$\mu_I = g_N (e/2m_p)\, I$

$g_N (^1H) = 5.5856947$

$|\mu_I| = (h/2\pi)\, I[I(I+1)]^{1/2}$



Need nucleus with non-zero spin $(I \neq 0)$

Examples: ^1H, ^{13}C, ^{15}N, ^{19}F, ^{31}P $(I = 1/2)$

^2H, ^{14}N, $(I = 1)$

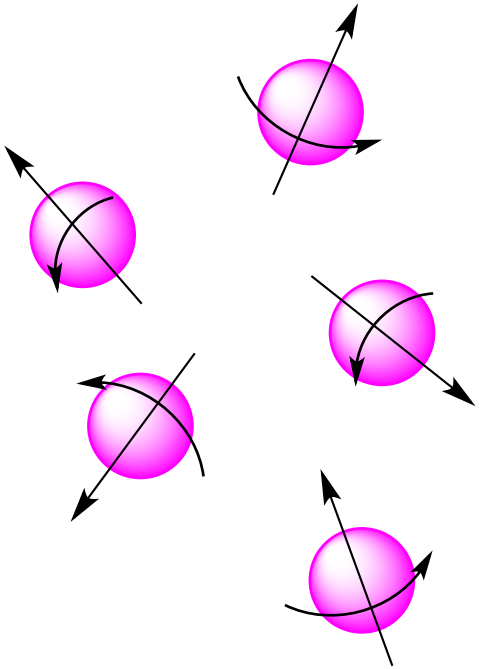
Herein we will base examples in nuclei with $I = 1/2$

NMR Fundamentals

A nucleus with spin I has states with associated M_I values where $M_I = -I, =I+1, \ldots I$

When $I = 1/2$, $M_I = \pm 1/2$

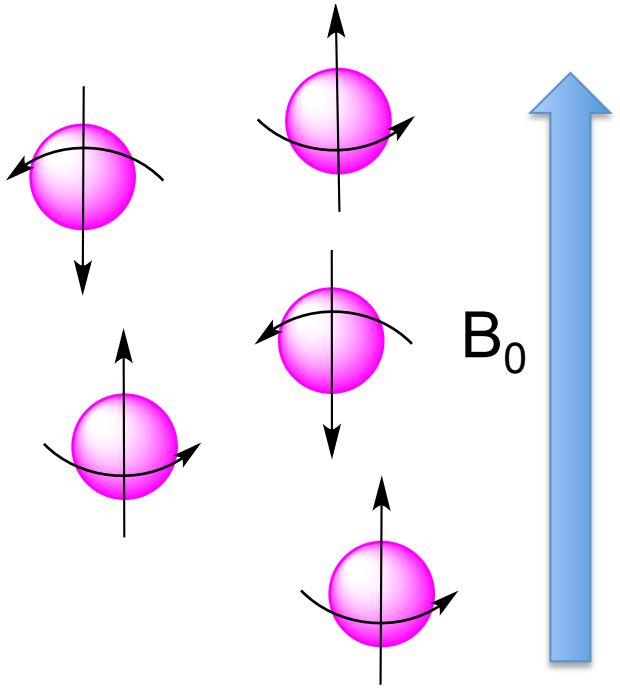
In the absence of a magnetic field, states with different M_I are degenerate and their magnetic moments μ orient randomly



NMR Fundamentals

Applying a magnetic field lifts this degeneracy. ^{1}H with $M_I = -1/2$ have a higher energy and ^{1}H with $M_I = +1/2$ have a lower energy.

The nuclear spins align with ($M_I = +1/2$) or opposed to ($M_I = -1/2$) the applied magnetic field:



The *z* component of the magnetic moment is shown and is $M_I h/2\pi$

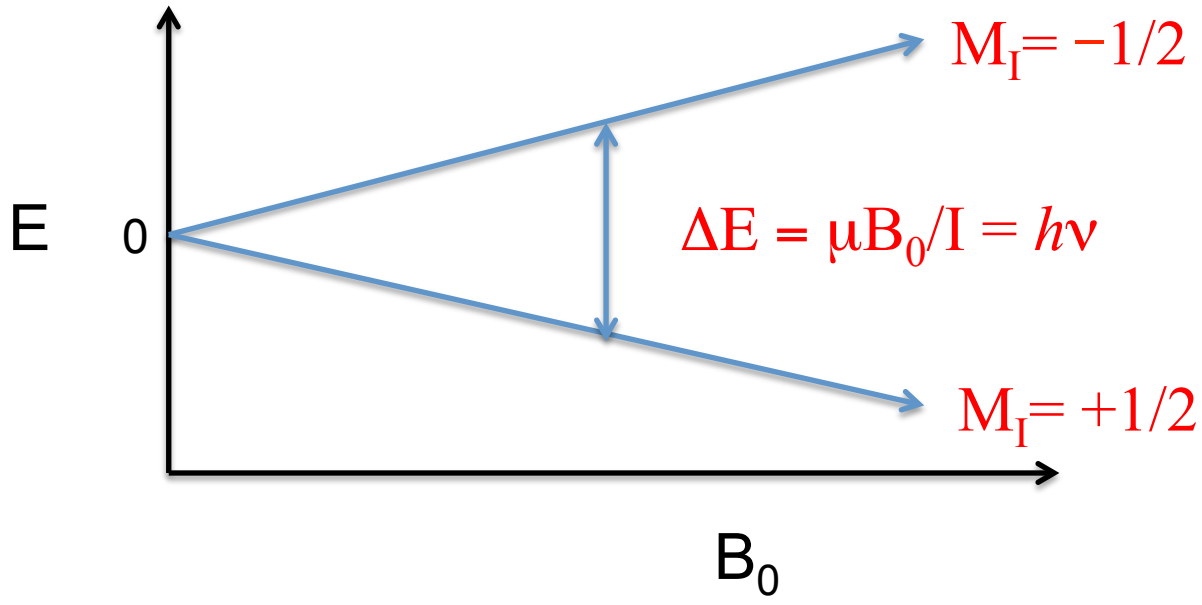
NMR Fundamentals

The energies of the nuclei in the magnetic field are:

$E = -M_I \mu B_0 / I$

Transitions may be induced between M_I states.

The selection rule is $\Delta M_I = \pm 1$



NMR Fundamentals

The energies of the nuclei in the magnetic field are:

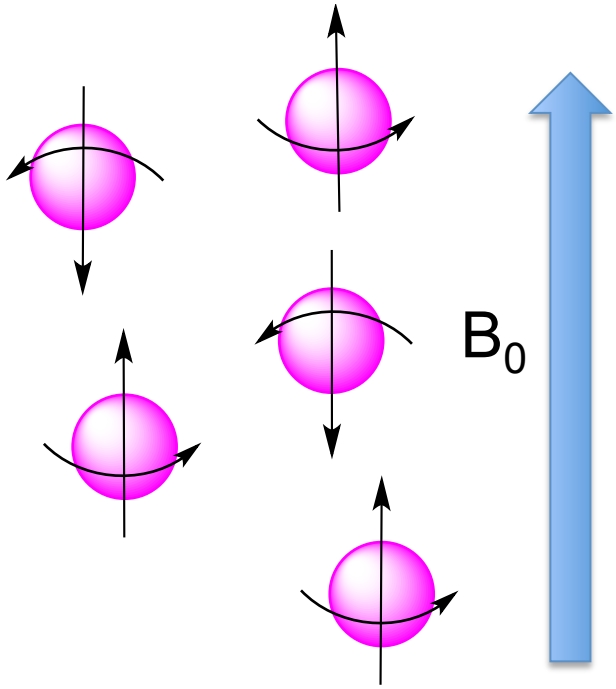
$E = -M_I \mu B_0 / I$

$\Delta E = \mu B_0 / I = h\nu$

Traditionally, the magnetogyric ratio γ (T^{-1} s^{-1}) is used in NMR:

$\gamma = \mu 2\pi / hI$, so

$$\Delta E = \gamma B_0 / 2\pi = h\nu$$

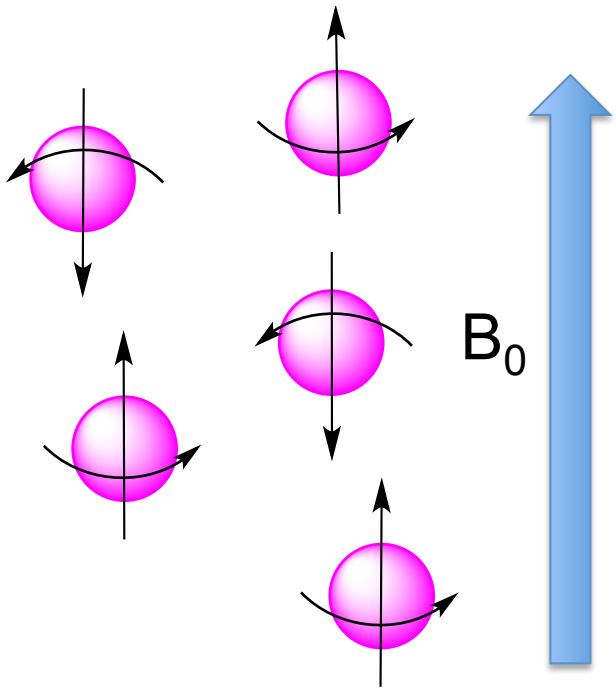


NMR Fundamentals

$$\Delta E = \gamma B_0/2\pi$$

A transition between M_I states corresponds to a "spin flip."

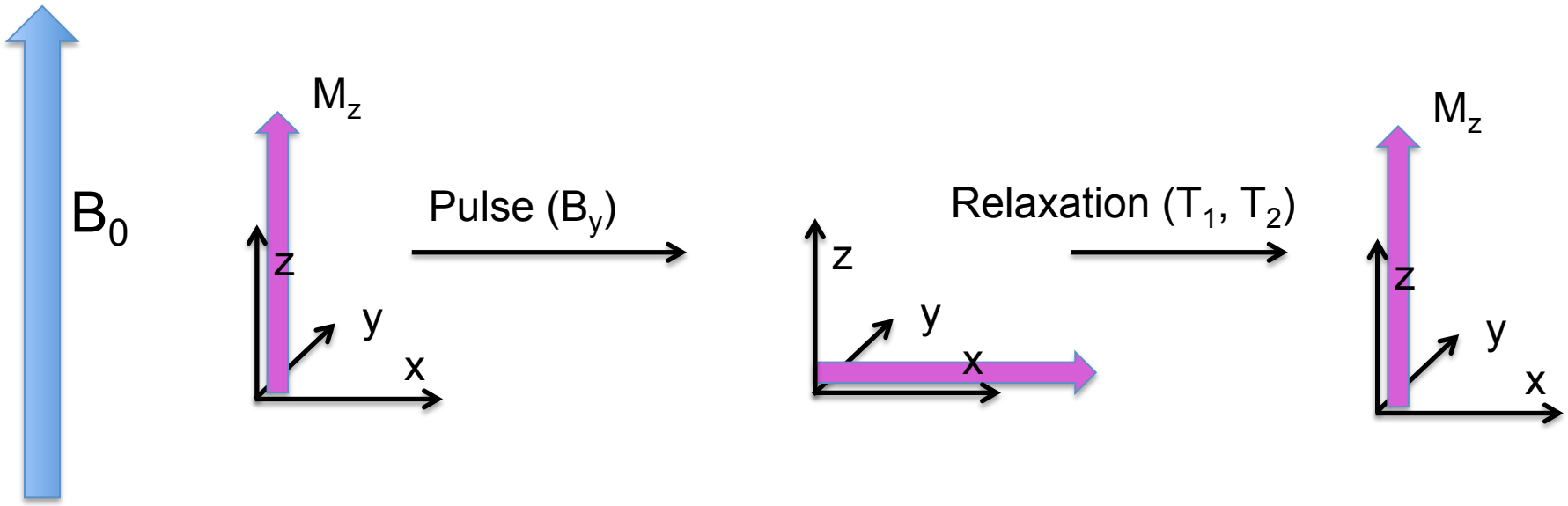
At equilibrium there is a small excess of spins aligned with the field:



$$\frac{N(-1/2)}{N(+1/2)} = \exp\left[-(E_{-1/2} - E_{+1/2})/k_B T\right]$$

NMR Fundamentals

We can consider a net magnetization vector for the sample. Exciting the sample decreases the different in up and down spins, tipping this vector, after which it returns to equilibrium:

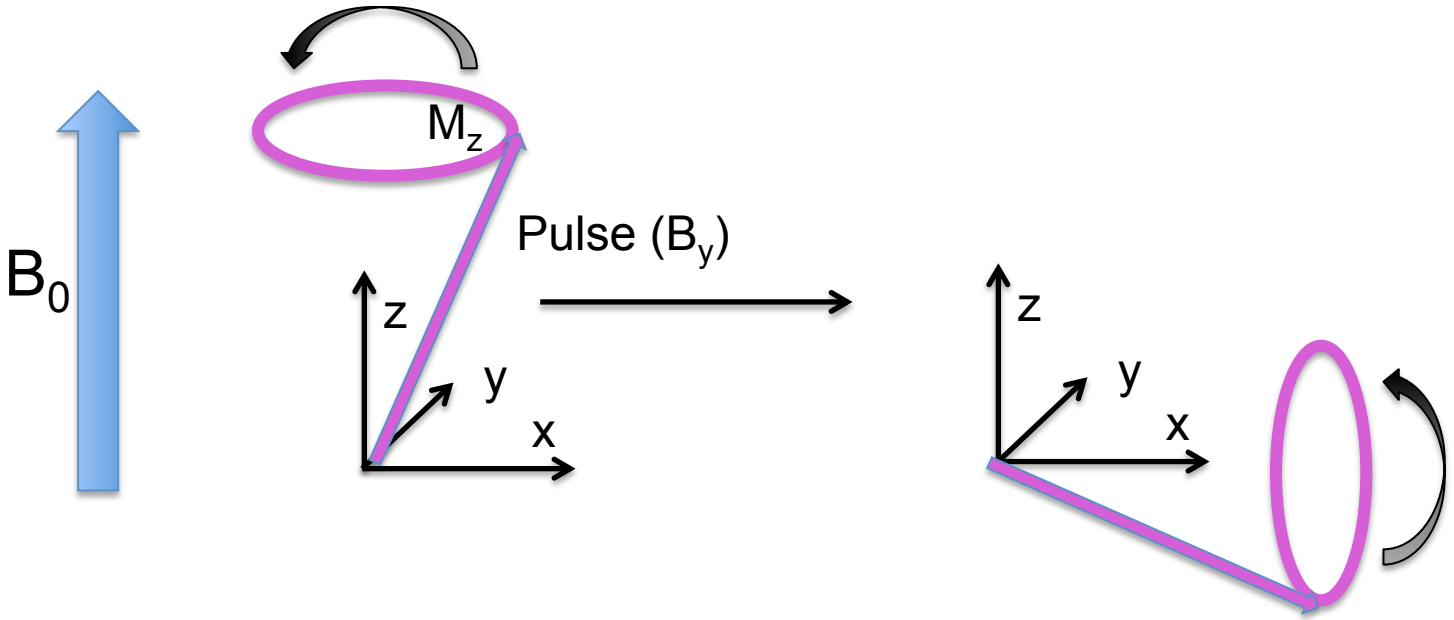


Pulse width is time of pulse; adjust time to change tip angle (i.e. 90˚ pulse)

NMR Fundamentals

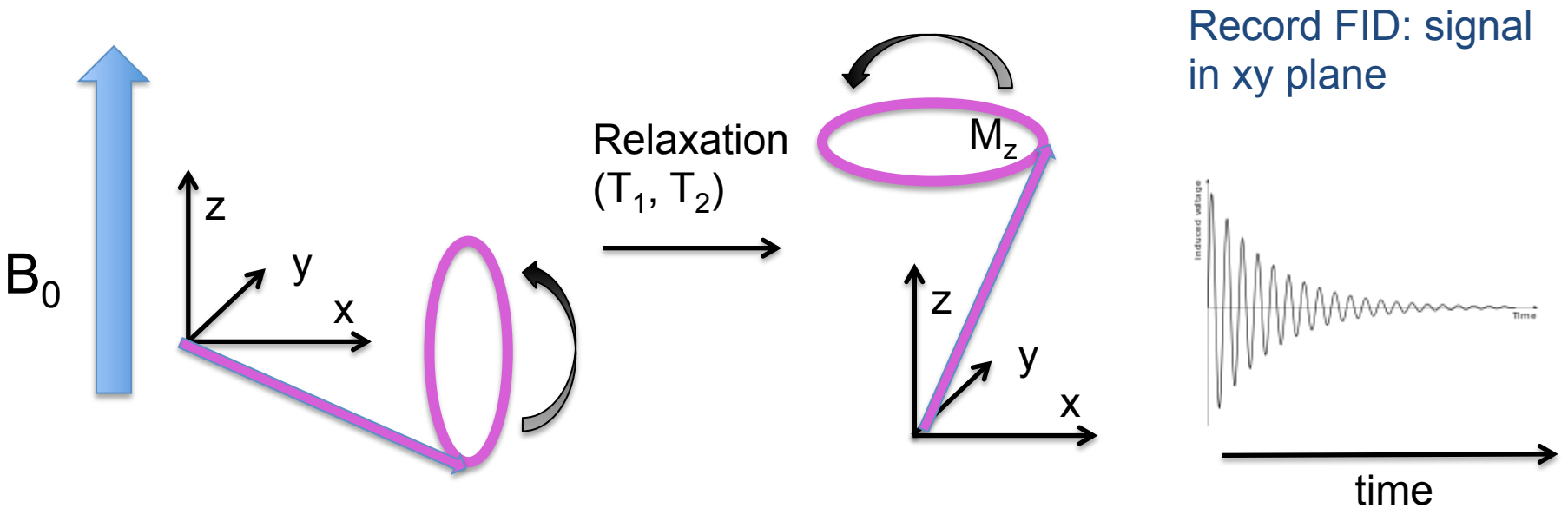
M_z is undergoing precession throughout this process – think of a cone rather than just the M_z vector tipping – precession frequency is the Larmor frequency:

$\omega_0 = \gamma B_0$ (rad) or $\nu_0 = \gamma B_0/2\pi$ (Hz) (Larmor equation)



NMR Fundamentals

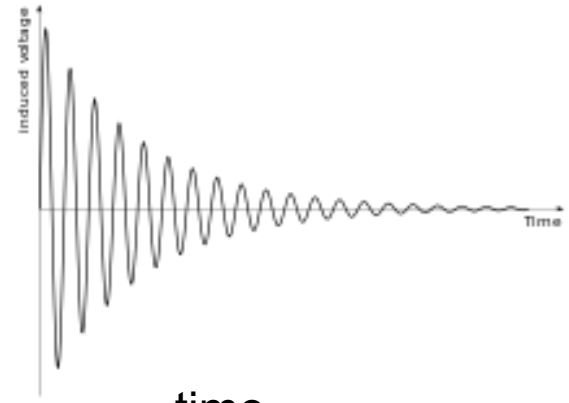
The observable signal is recorded in the *xy* plane during relaxation. T_1 = relaxation along *z* axis; T_2 = relaxation in *xy* plane. Precession frequency is observed.



NMR Fundamentals

The observable signal is recorded in the *xy* plane during relaxation. T_1 = relaxation along *z* axis; T_2 = relaxation in *xy* plane. Precession frequency is observed.

FID: time-domain signal in xy plane

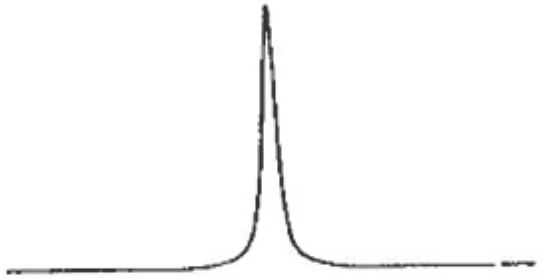


time

Fourier Transform

Line width

$\Delta\nu = 1/(\pi T_2)$



ν_0

frequency

NMR Fundamentals

The chemical shift results from small deviations from ν_0

Chemical shift:

$\delta\ (\text{ppm}) = 10^6\ [\nu(\text{obs}) - \nu(\text{ref})]/\nu(\text{ref})$

The chemical environment of nuclei (especially circulation of electrons) leads to deviations of $\nu(\text{obs})$ giving different chemical shifts.

Unpaired electrons can have very large effects on chemical shifts through different mechanisms.

Electrons vs. Nuclei

Quantity	Electron	^{1}H
Spin	$S = 1/2$	$I = 1/2$
Magnetic moment	μ_e	μ_I
Gyromagnetic ratio	$\gamma_e = \mu_B g_e/h$	$\gamma_I = \mu_I g_I/h$
Transition energy in field B_0	$h\nu = g_e \mu_B B_0$	$h\nu = h \gamma_I B_0/2\pi$
Transition between states	$M_S = \pm 1/2$	$M_I = \pm 1/2$

Electrons vs. Nuclei

Two major differences:

- Electrons have a larger magnetic moment
  - $|\mu_B| = 658\ |\mu_I(^1H)|$
  - Larger resonance frequency
  - More efficient relaxation
- Electrons are in orbitals
  - Delocalization
  - Spin-orbit coupling

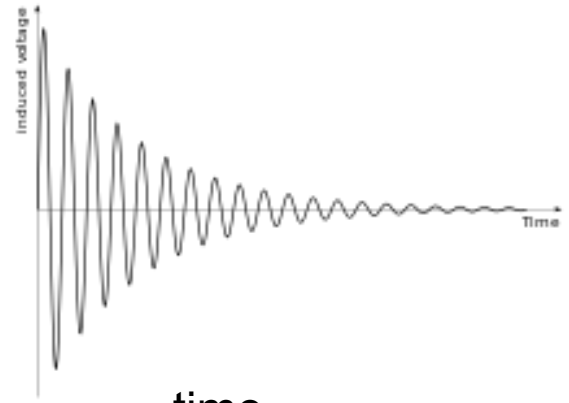
Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

Relaxation in NMR

The observable signal is recorded in the *xy* plane during relaxation. T_1 = relaxation along *z* axis; T_2 = relaxation in *xy* plane.

FID: time-domain signal in xy plane

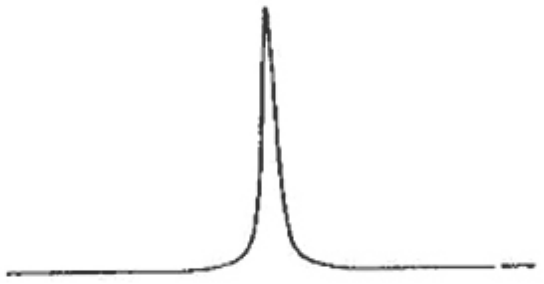


time

Fourier Transform

Line width

$\Delta\nu = 1/(\pi T_2)$



ν_0

frequency

Relaxation in NMR

The observable signal is recorded in the *xy* plane during relaxation. T_1 = relaxation along *z* axis; T_2 = relaxation in *xy* plane.

Assumption here: $T_1 = T_2$ (simplest case – fast motion)

Relaxation must be induced by exchange of energy at the resonance frequency ν

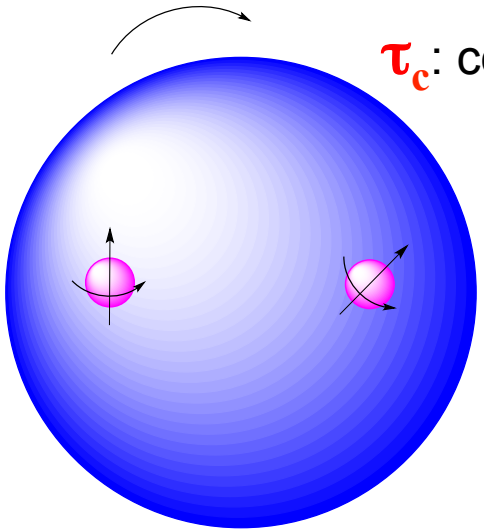
Energy provided by fluctuating magnetic field – here we'll consider unpaired electrons as a source

Relaxation Mechanisms

- Dipole-dipole
  - Most important
  - Through-space interaction between magnetic moments and fluctuating magnetic field
  - Modulated by molecular tumbling
- Quadrupolar ($I>1/2$), scalar
- Spin rotation
- Chemical shift anisotropy
- Others…

Dipole-dipole Relaxation

- The relaxation of one spin (dipole) by through-space interactions with other dipoles
- A fluctuating field is needed, and this is generated by molecular motion
- Relaxation rate depends on $\mu_1^2\mu_2^2$, r^{-6}, τ_c



τ_c: correlation time

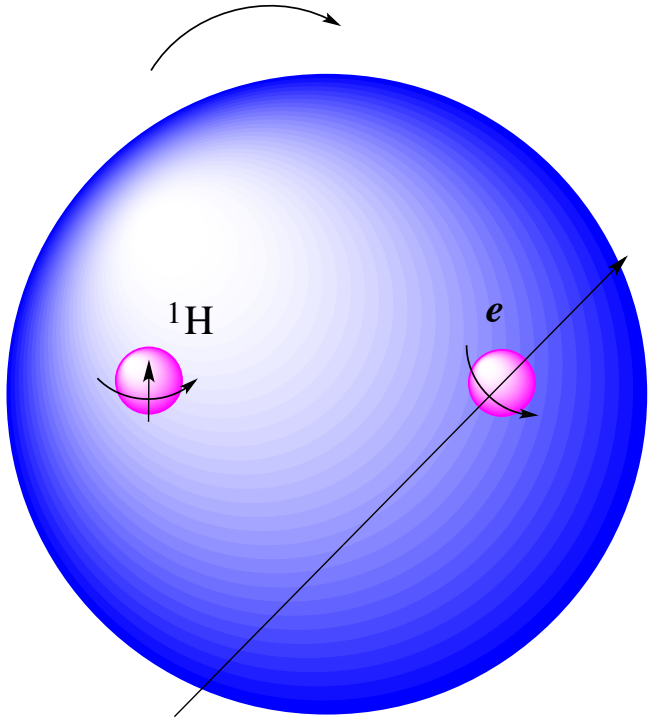
$$\tau_c = 4\pi a^3 \eta / 3kT$$

Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

Dipole-dipole Relaxation

- Depends on $\mu_1^2\mu_2^2$, r^{-6}, τ_c
- An unpaired electron's μ is 658x greater than μ for ^{1}H.
- Unpaired electron has a large impact on ^{1}H relaxation



For dipole-dipole nuclear relaxation by unpaired electron:

$$T_{1,2}^{-1} = R_{1,2} = \frac{4}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_c$$

Note dependence on S, γ_I, r^{-6}, τ_c

But there is more to τ_c …

Correlation Time

The correlation time τ_c actually contains three contributions:

1. molecular tumbling time (τ_r), 2. electron spin relaxation time (τ_s), 3. chemical exchange (τ_M).

The overall correlation τ_c time is:

$$\tau_c^{-1} = \tau_r^{-1} + \tau_s^{-1} + \tau_M^{-1}$$

Assuming no chemical exchange:

$$\tau_c^{-1} = \tau_r^{-1} + \tau_s^{-1}$$

In a diamagnetic system, no chemical exchange:

$$\tau_c^{-1} = \tau_r^{-1}$$

Correlation Time

In a paramagnetic molecule, especially if it is not too large (large means long τ_r), τ_s usually dominates τ_c

τ_r ranges from 10^{-9} s (small protein) to 10^{-7} s (large for NMR)

τ_s ranges from 10^{-13} s to 10^{-8} s; but values 10^{-13} to 10^{-10} most feasible for high-resolution NMR.

Thus $\tau_r^{-1} \ll \tau_s^{-1}$ and τ_s dominates τ_c *for metalloproteins*.

Example: Fe(III)cytochrome *c* (MW = 12 kDa):

$$\tau_c^{-1} = \tau_r^{-1} + \tau_s^{-1} = (10^{-9}\ \mathrm{s})^{-1} + (10^{-13}\ \mathrm{s})^{-1}$$

$$\tau_c = 10^{-13}\ \mathrm{s} = \tau_s$$

Correlation Time

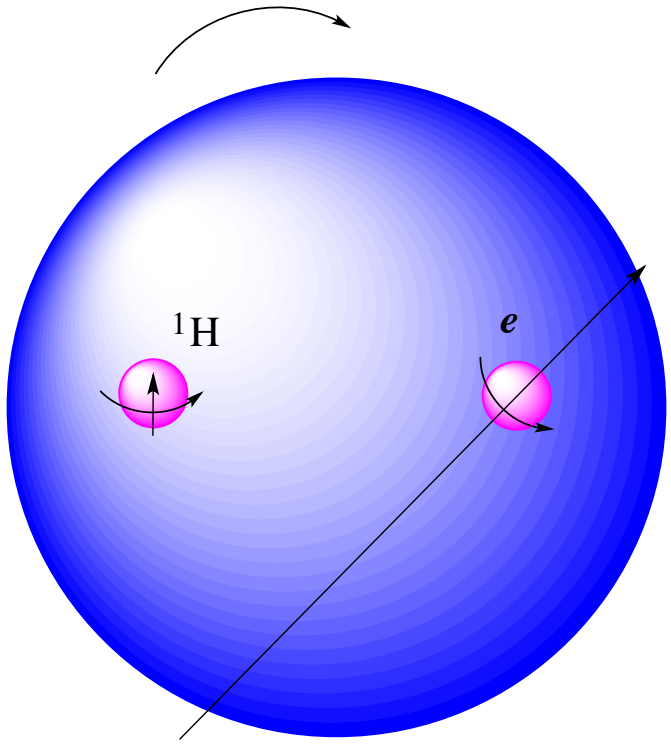
In a paramagnetic molecule, especially if it is not too large (large means long τ_r), τ_s usually dominates τ_c

Note for small molecules, especially with long τ_s, instead you may have $\tau_c = \tau_r$

Small Cu(II) complex, $\tau_s = (10^{-8}\ \mathrm{s})$, $\tau_r = (10^{-12}\ \mathrm{s})$

In this case, $\tau_s^{-1} < \tau_r^{-1}$ and $\tau_c = \tau_r$.

Dipole-dipole Relaxation – e^-/nucleus



$$R_{1,2} = \frac{4}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_c$$

$\tau_c = \tau_s$

For NMR of metalloproteins, variations in τ_s are the most important factor determining line widths. It also can be important for small molecules.

Long τ_s => large $R_{1,2}$ => small $T_{1,2}$ => large $\Delta\nu$ (broad line)

Electron Spin Relaxation Times τ_s

$$R_{1,2} = \frac{4}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_s$$

Metal	Ox state	S	C.N.	τ_s (s)	Linebroadening (Hz)*
Fe	+3	1/2	5,6	10^{-12} - 10^{-13}	0.5-20
Fe	+3	5/2	4,5,6	10^{-9} - 10^{-11}	200-12000
Fe	+2	2	4	10^{-11}	150
Fe	+2	2	5,6	10^{-12} - 10^{-13}	5-20
Cu	+2	1/2	any	(1-5) x 10^{-9}	1000-5000
Mn	+2	5/2	4,5,6	10^{-8}	100000
Mn	+3	2	4,5,6	10^{-10} - 10^{-11}	150-1500

Coord. Chem. Rev. **1996**, *150*, p. 84

*for ^{1}H 5 Å from metal

Paramagnetic Relaxation Enhancement

$$R_{1,2} = \frac{4}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_s$$

- Measure distances (r) and/or refine structures by measuring effects of a paramagnetic probe on line widths and relaxation times ($R_{1,2}$)
- Need to be in regime where dipolar relaxation dominates
- Other effects on relaxation:
  - Contact, applicable mostly for small molecules (rapid tumbling) with large τ_s (slow electron relaxation)
  - Curie, most relevant for very large molecules with high S
  - Effects of quadrupolar nuclei

Contact Relaxation

Contact:
(in absence of exchange phenomena)

$$R_{1,2} = 2/3\ S(S+1)\ (A/h)^2\ \tau_s$$

Dipolar:
(when $\tau_s^{-1} >> \tau_r^{-1}$)

$$R_{1,2} = 4/3 \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2\, g_e^2\, \mu_e^2\, S(S+1)}{r^6}\ \tau_s$$

- Depends on S, A^2, and τ_s
- Never depends on τ_r (why not)?
- May be in effect when hyperfine coupling is strong over a number of bonds (so not extremely small r)
- Will be in effect when τ_s/τ_r is relatively large
- May be in effect for very large A values.

What can I do with these broad peaks?

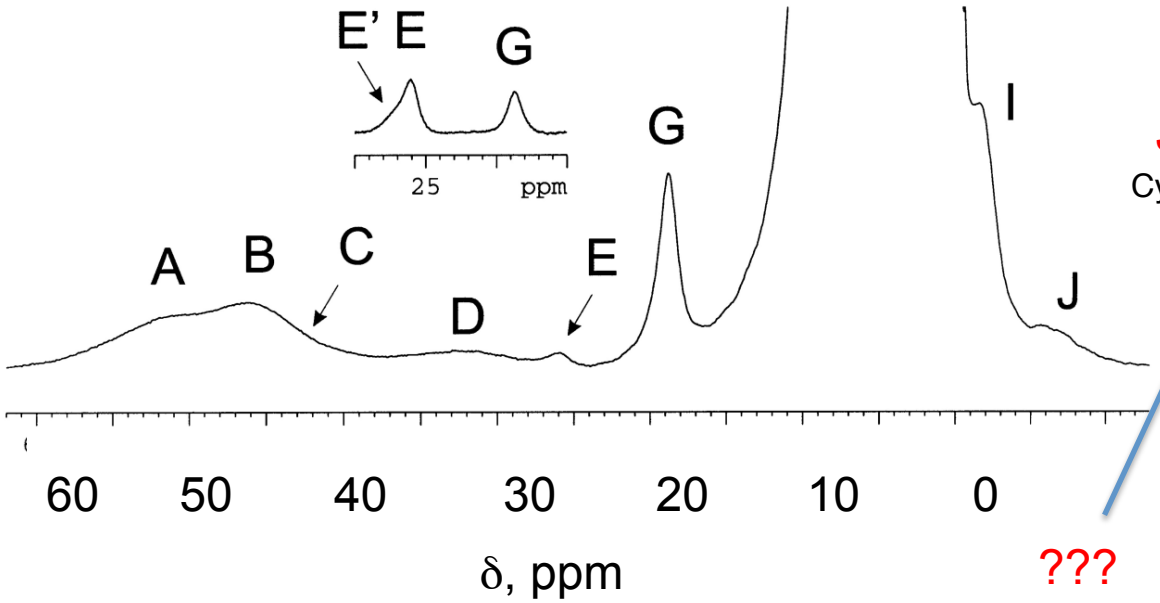
- Try some cool tricks, and learn what you can!
- Change metal or metal oxidation state (although you may want to study the native metal in a particular oxidation state, of course).
- Take advantage of the situation! Fast relaxation times means you can use a short acquisition time and recycle time, and get many scans in a short period of time while enhancing intensity of broad peaks
- Increase the temperature – faster tumbling (shorter τ_r) and faster electron relaxation (shorter τ_s) (and faster exchange if present) can help significantly.
- Detect nuclei other than ^{1}H – especially ^{13}C, ^{15}N, or ^{2}H. Relaxation enhancement and line widths depend on γ^2.

Finding Hidden Peaks

P. aeruginosa Cu(II) azurin

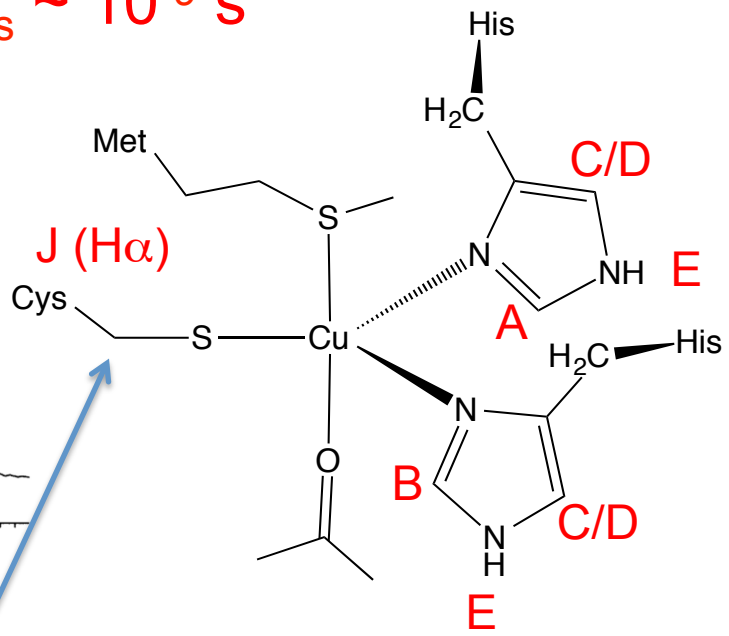
$S = 1/2$

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



J (Hα)

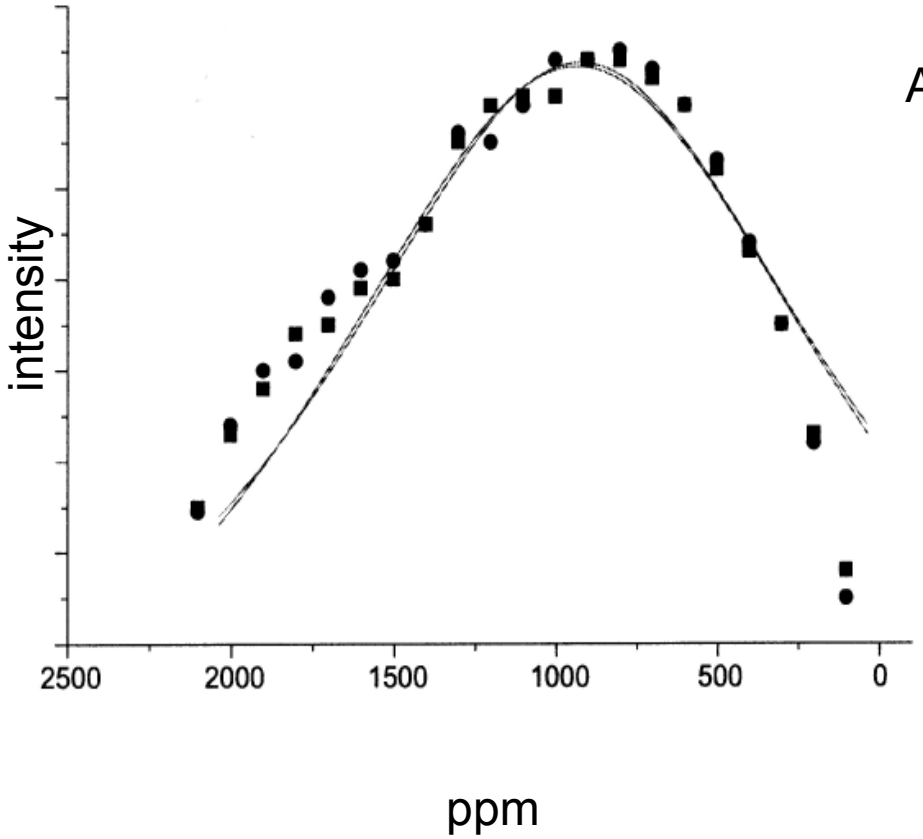
???



JACS **2000,** 3701

Finding Hidden Peaks

Saturation transfer experiment



Az Cu(I)* + Az Cu(II) ⇌

Az Cu(II)* + Az Cu(I)

Saturate Cys ^{1}H in diamagnetic Cu(I), observe change in intensity in spectrum of Cu(II)

JACS **2000,** 3701

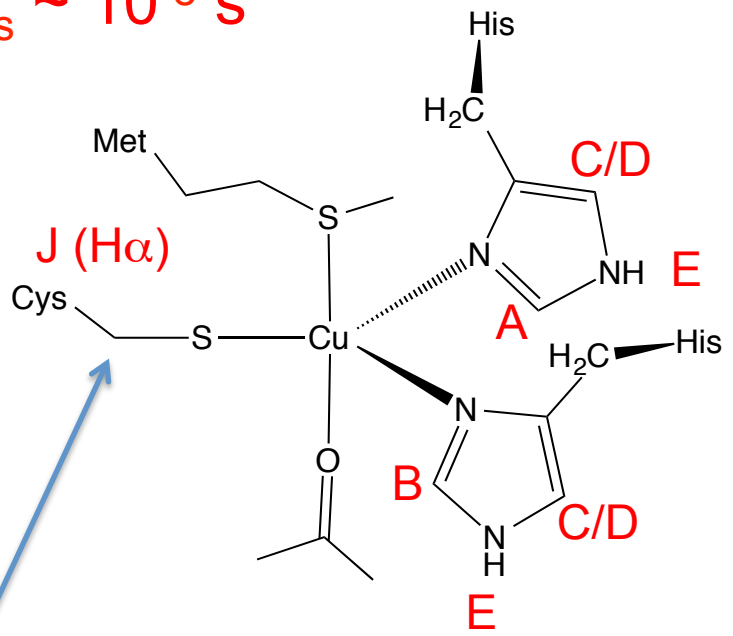
What can I do with these broad peaks?

Why does this have a 120,000 Hz line width?

1. It is very close to Cu(II)
2. But…line width can't be explained by dipolar relaxation
3. Contribution from contact relaxation because of large *A*
4. Observation consistent with efficient spin delocalization onto Cys residue

$S = 1/2$

$\tau_s \sim 10^{-8}$ s



800, 850 ppm
120,000 Hz(!)

JACS **2000,** 3701

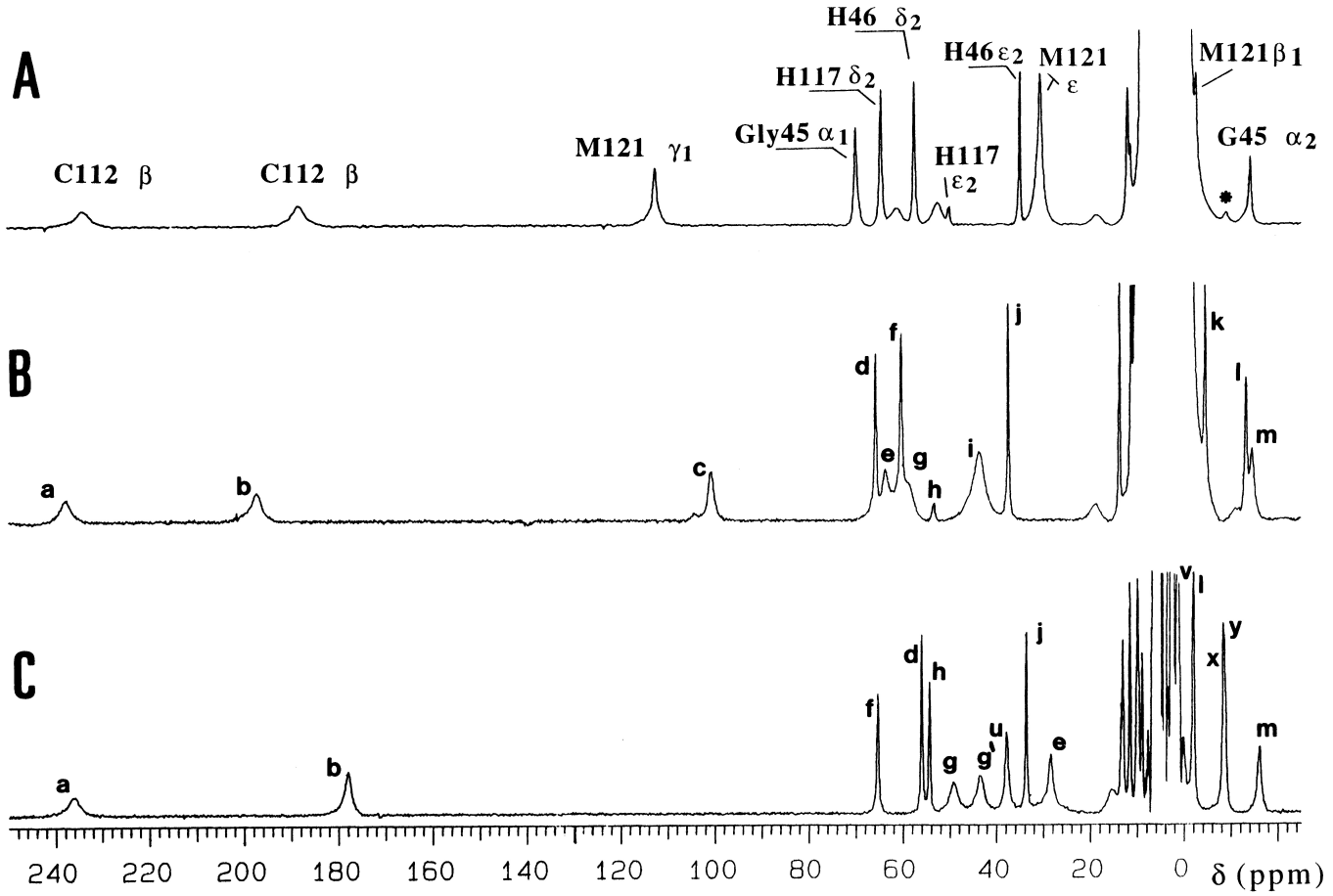
What can I do with these broad peaks?

- Try some cool tricks, and learn what you can!
- Change metal or metal oxidation state (although you may want to study the native metal in a particular oxidation state, of course).
- Take advantage of the situation! Fast relaxation times means you can use a short acquisition time and recycle time, and get many scans in a short period of time while enhancing intensity of broad peaks
- Increase the temperature – faster tumbling (shorter τ_r) and faster electron relaxation (shorter τ_s) (and faster exchange if present) can help significantly.
- Detect nuclei other than ^{1}H – especially ^{13}C, ^{15}N, or ^{2}H. Relaxation enhancement and line widths depend on γ^2.

Metal Substitution

Ni(II) azurin

$S = 1, \tau_s \sim 10^{-12}$ s

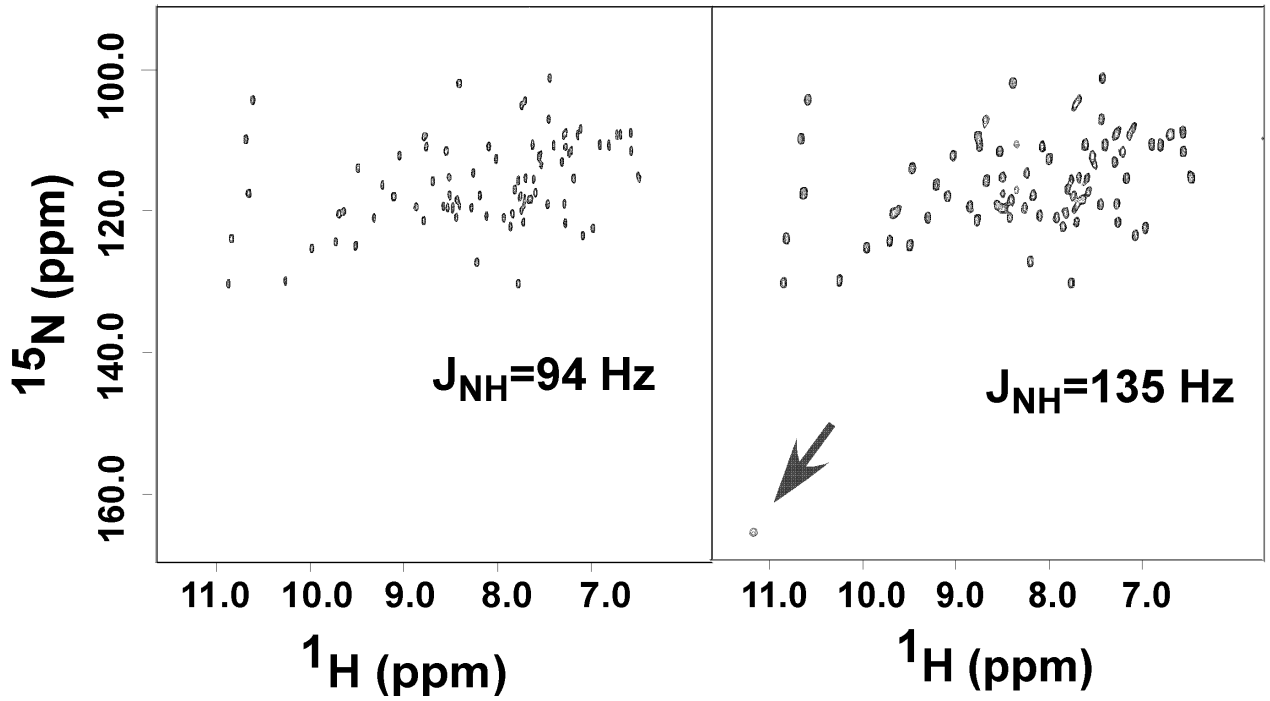


Biochemistry **1996**, 1810

What can I do with these broad peaks?

- Try some cool tricks, and learn what you can!
- Change metal or metal oxidation state (although you may want to study the native metal in a particular oxidation state, of course).
- Take advantage of the situation! Fast relaxation times means you can use a short acquisition time and recycle time, and get many scans in a short period of time while enhancing intensity of broad peaks
- Increase the temperature – faster tumbling (shorter τ_r) and faster electron relaxation (shorter τ_s) (and faster exchange if present) can help significantly.
- Detect nuclei other than ^{1}H – especially ^{13}C, ^{15}N, or ^{2}H. Relaxation enhancement and line widths depend on γ^2.

Finding Hidden Peaks



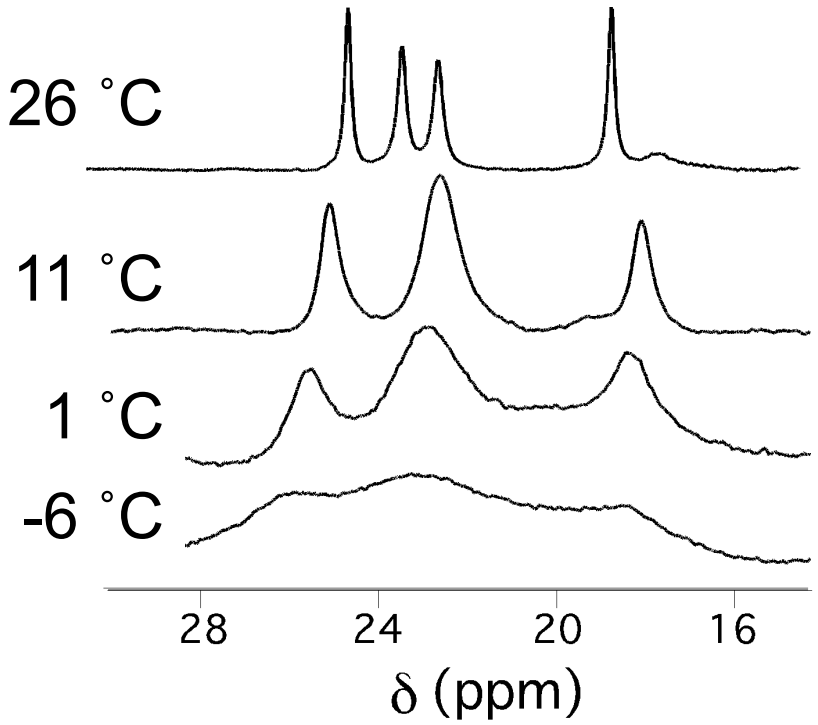
^{1}H-^{15}N HSQC spectra of 1 mM *Hydrogenobacter thermophilus* [*U*-^{15}N]-ferricytochrome c_{552} showing the effect of a decreased INEPT delay on intensity of the peak correlating the heme axial His δHN nuclei.

From *Encyclopedia of Inorganic Chemistry* DOI: 10.1002/0470862106.ia319

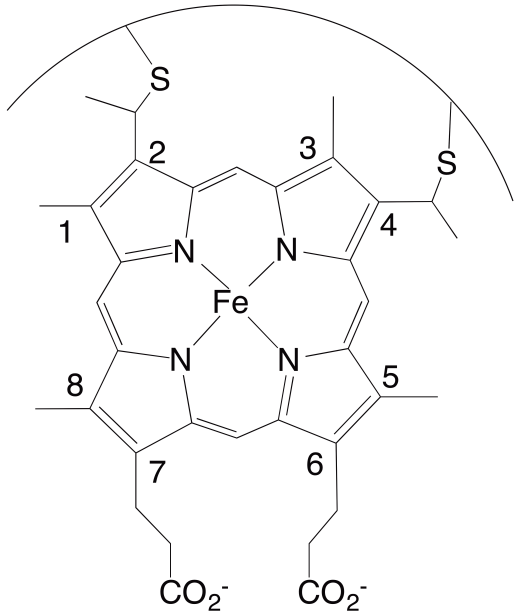
What can I do with these broad peaks?

- Try some cool tricks, and learn what you can!
- Change metal or metal oxidation state (although you may want to study the native metal in a particular oxidation state, of course).
- Take advantage of the situation! Fast relaxation times means you can use a short acquisition time and recycle time, and get many scans in a short period of time while enhancing intensity of broad peaks
- Increase the temperature – faster tumbling (shorter τ_r) and faster electron relaxation (shorter τ_s) (and faster exchange if present) can help significantly.
- Detect nuclei other than ^{1}H – especially ^{13}C, ^{15}N, or ^{2}H. Relaxation enhancement and line widths depend on γ^2.

Effect of Temperature



^{1}H resonances of heme methyl groups of *Hydrogenobacter thermophilus* ferricytochrome c_{552}

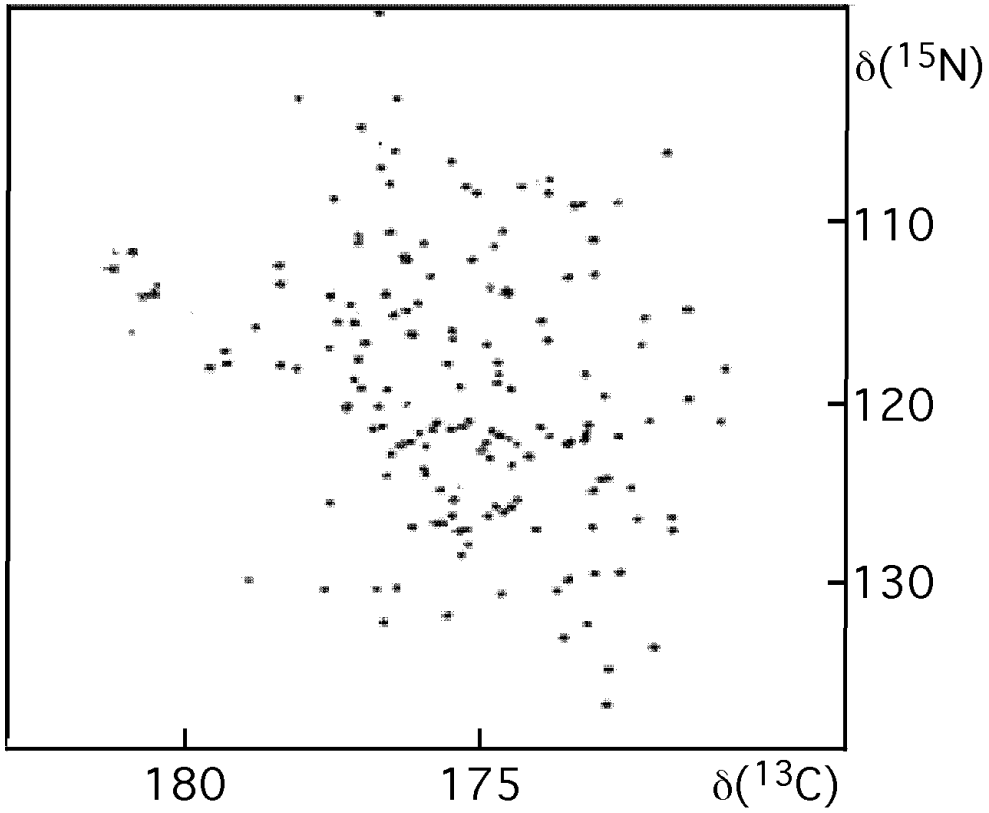


From *Encyclopedia of Inorganic Chemistry* DOI: 10.1002/0470862106.ia319

What can I do with these broad peaks?

- Try some cool tricks, and learn what you can!
- Change metal or metal oxidation state (although you may want to study the native metal in a particular oxidation state, of course).
- Take advantage of the situation! Fast relaxation times means you can use a short acquisition time and recycle time, and get many scans in a short period of time while enhancing intensity of broad peaks
- Increase the temperature – faster tumbling (shorter τ_r) and faster electron relaxation (shorter τ_s) (and faster exchange if present) can help significantly.
- Detect nuclei other than ^{1}H – especially ^{13}C, ^{15}N, or ^{2}H. Relaxation enhancement and line widths depend on γ^2.

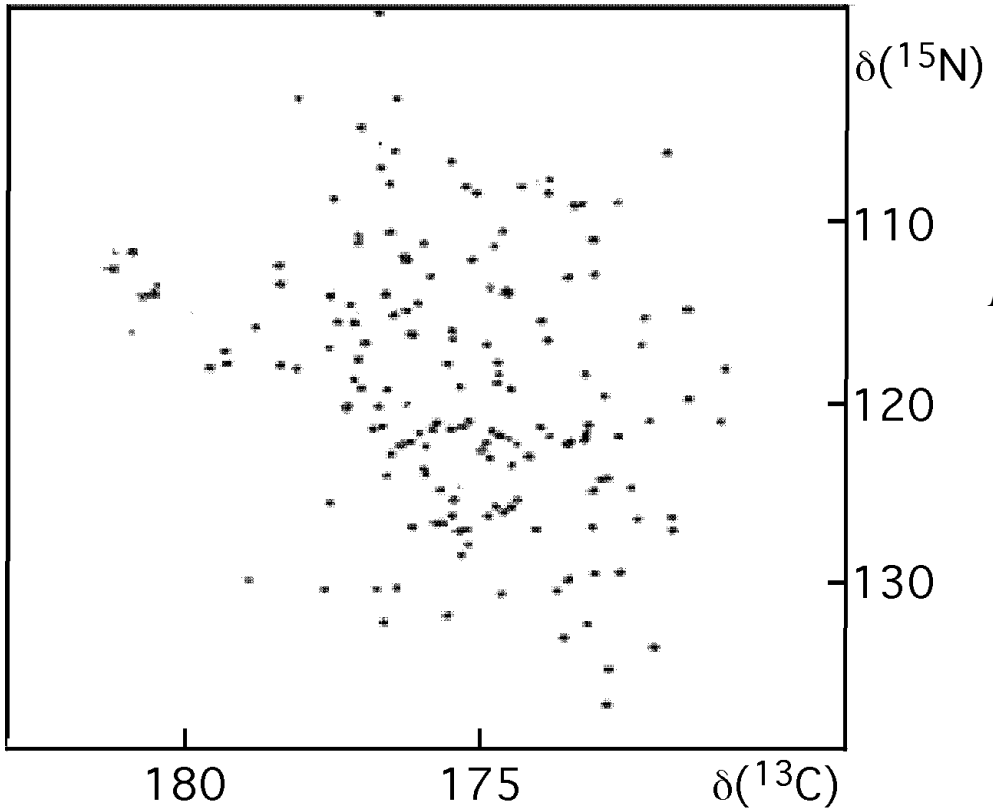
Detection of ^{13}C



2-D in-phase-anti-phase spectrum correlating backbone ^{13}C and ^{15}N nuclei, with detection of ^{13}C (CON-IPAP experiment). Data were acquired on a 1.5 mM sample of ^{13}C,^{15}N labeled reduced monomeric superoxide dismutase (*see* ID779-) with a 14.1 T Bruker Avance spectrometer equipped with a cryogenically cooled probehead optimized for ^{13}C detection at 298 K

Prog. Nucl. Magn. Reson. Spectrosc. **2006**, *48*, 25.

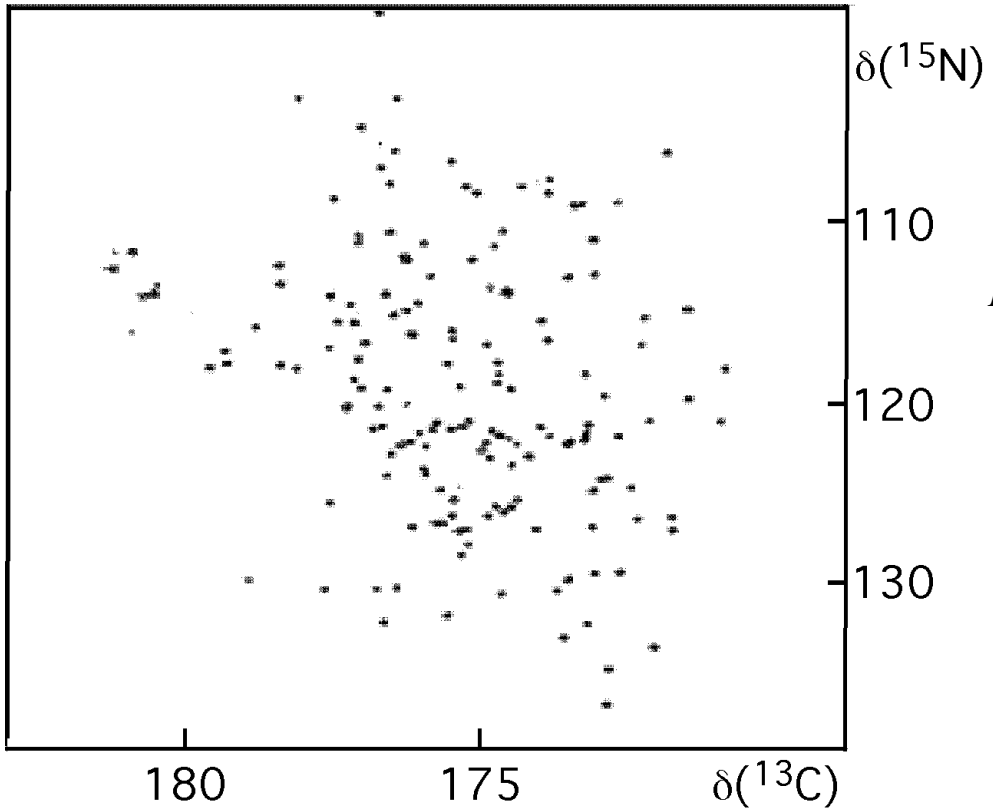
Detection of ^{13}C



$$R_{1,2} = 4/3 \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_s$$

Prog. Nucl. Magn. Reson. Spectrosc. **2006**, *48*, 25.

Detection of ^{13}C



$$R_{1,2} = 4/3 \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_e^2 S(S+1)}{r^6} \tau_s$$

Prog. Nucl. Magn. Reson. Spectrosc. **2006**, *48*, 25.

Relaxation Summary

- Properties of metal site have a profound effect on relaxation and thus line widths, with τ_s being most important
- A number of systems show minimal line broadening – especially LS Fe(III), 4-coordinate HS Ni(II), 5- and 6-coordinate HS Co(II), 5- and 6-coordinate HS Fe(II). Also – most Ln(III), (not Gd(III)), Ru(III), and many bi- and multinuclear sites
- Line broadening is diminished for low γ nuclei (by γ^2 factor)
- Relaxation enhancement provides information on molecular and electronic structure
- We have ways to deal with it!

Outline

- Resources
- Examples of effects on spectra
- What we can learn (why bother?)
- NMR fundamentals (review)
- Relaxation mechanisms in NMR
- Effects of unpaired electrons on relaxation
- Effects of unpaired electrons on chemical shifts

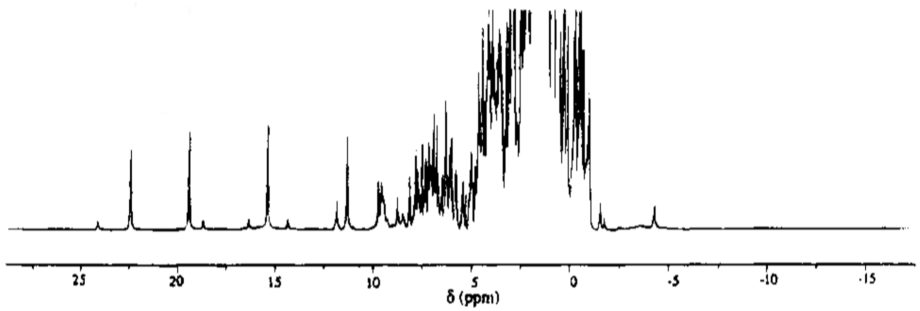
Paramagnetic Chemical Shifts

- Values are not necessarily related to amount of line broadening
- Can be higher or lower than diamagnetic (positive or negative; to high frequency or low frequency)
- Are caused by two primary mechanisms: contact (through-bond) and dipolar (through-space)
- Unlike with relaxation, both contact and dipolar often play a role
- Contain information on electronic structure, molecular structure, spin delocalization, magnetic anisotropy

Paramagnetic Chemical Shifts

$\delta_{obs} = \delta_{para} + \delta_{dia}$

$\delta_{para} = \delta_{con} + \delta_{pc}$



δ_{dia} is shift in isostructural diamagnetic molecule

δ_{con} is contact shift – through-bond electron-nucleus interaction

δ_{pc} is pseudocontact (also called dipolar) shift – through-space electron-nucleus interaction

Contact Shift

$$\delta_{con} = [(2\pi A/h)\, g_e\, \beta_e\, S\,(S+1)]/[3\, k_B T\, \gamma_I]$$

Note dependence on:

- Hyperfine coupling constant (A)
- Spin ($S\,(S+1)$)
- Temperature ($1/T$)
- Value γ_I in denominator cancels with part of A

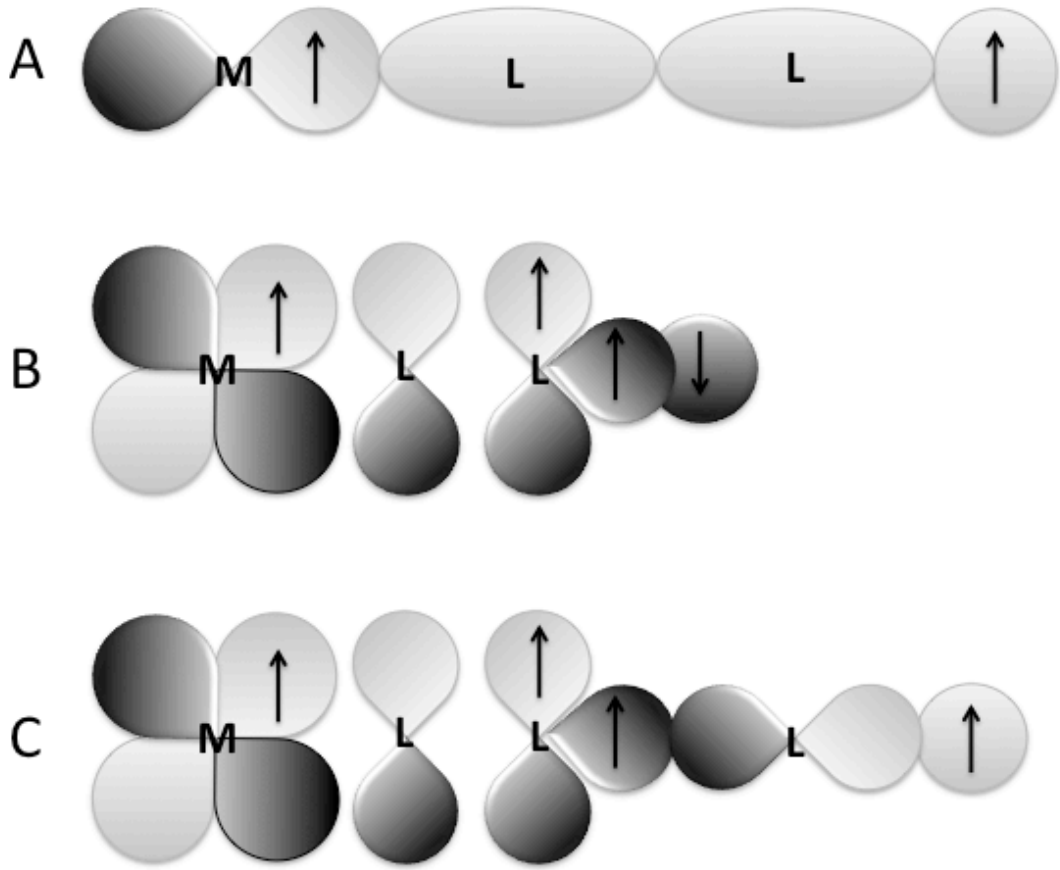
Contact Shift

$$\delta_{con} = [(2\pi A/h)\, g_e\, \beta_e\, S\,(S+1)]/[3\, k_B T\, \gamma_I]$$

- Reflects electron spin density at the nucleus
- Sign (+/-) reflects positive or negative spin density
- Often dominant for nuclei 1-3 bonds from metal
- Can be significant over more bonds in π system
- Independent of nucleus
- Temperature dependence ($1/T$) helps identify these peaks, but deviations from ideal are common.
- Can be used to estimate A

Paramagnetic Chemical Shifts

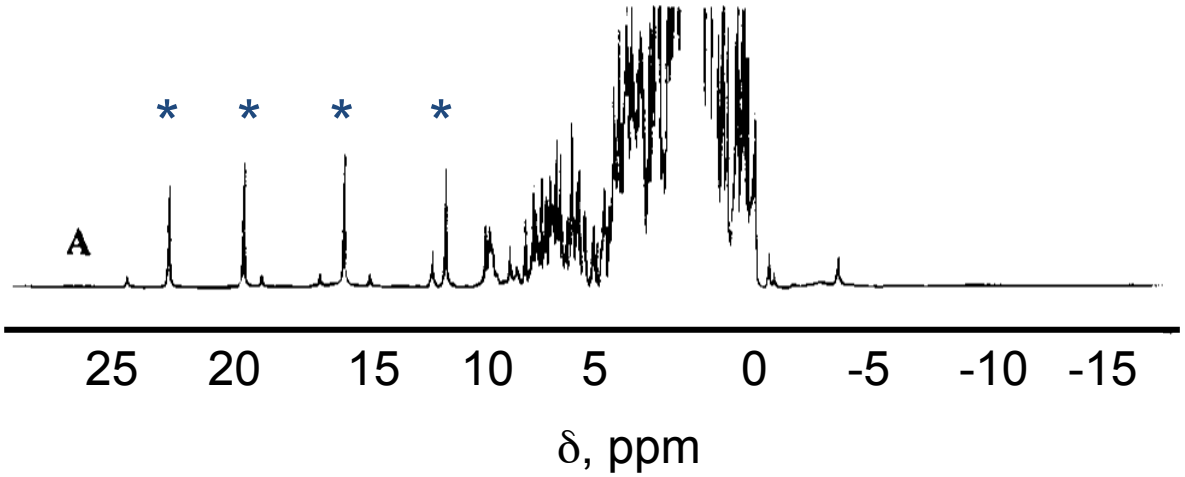
Sign of δ_{con} can change depending on delocalization mechanism

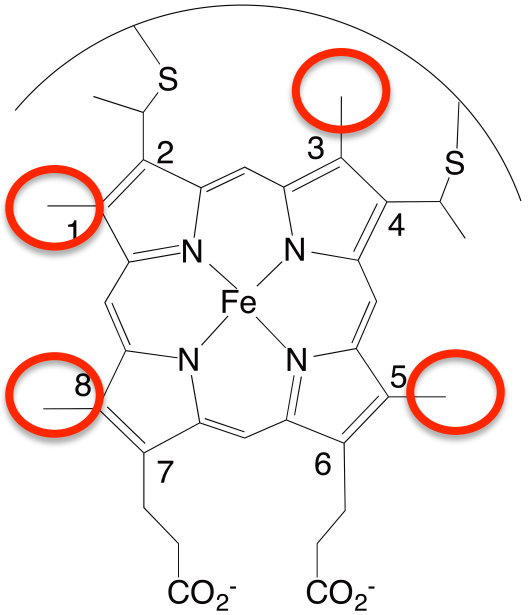


From *Encyclopedia of Inorganic Chemistry* DOI: 10.1002/0470862106.ia319

Contact Shift Examples

CN-Fe(III) M80A cytochrome *c*, D_2O



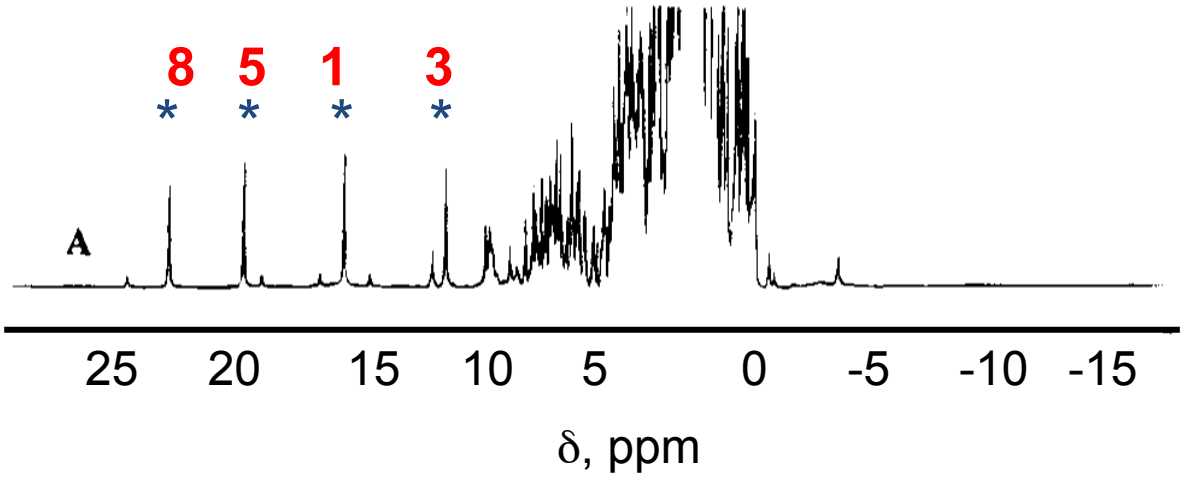


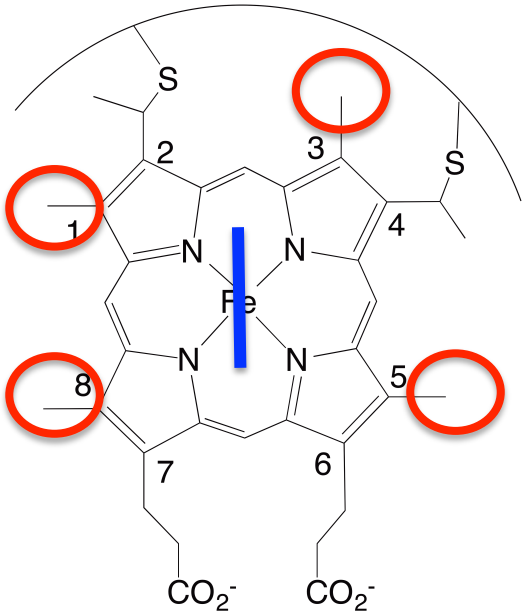
Heme methyl protons have large, positive spin density through 1) delocalization through π system, 2) polarization through two nuclei (C, H) outside of π system

JACS **1995**, 8067

Contact Shift Examples

CN-Fe(III) M80A cytochrome *c*, D_2O





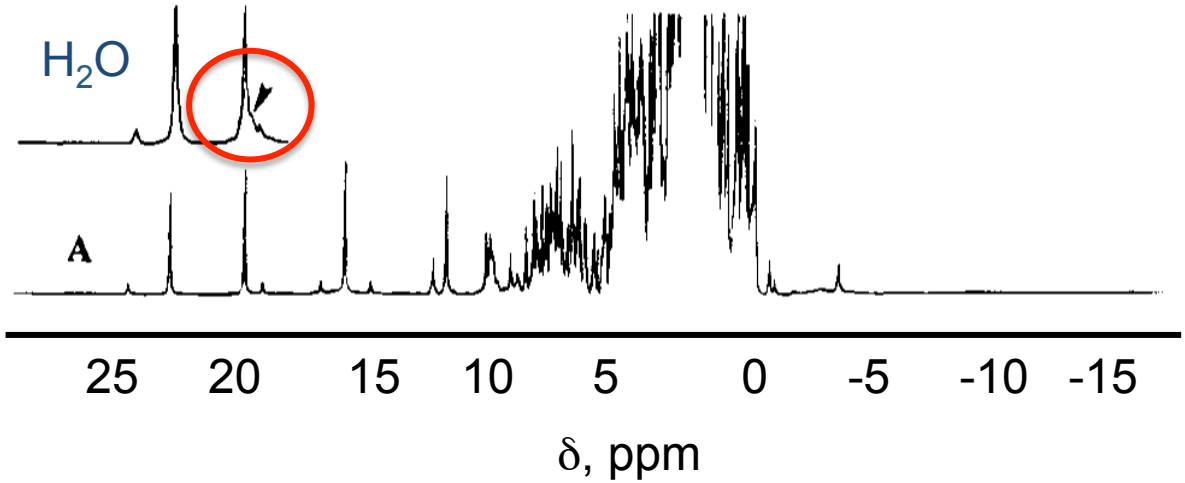
Heme methyl proton shift pattern reflects axial His orientation

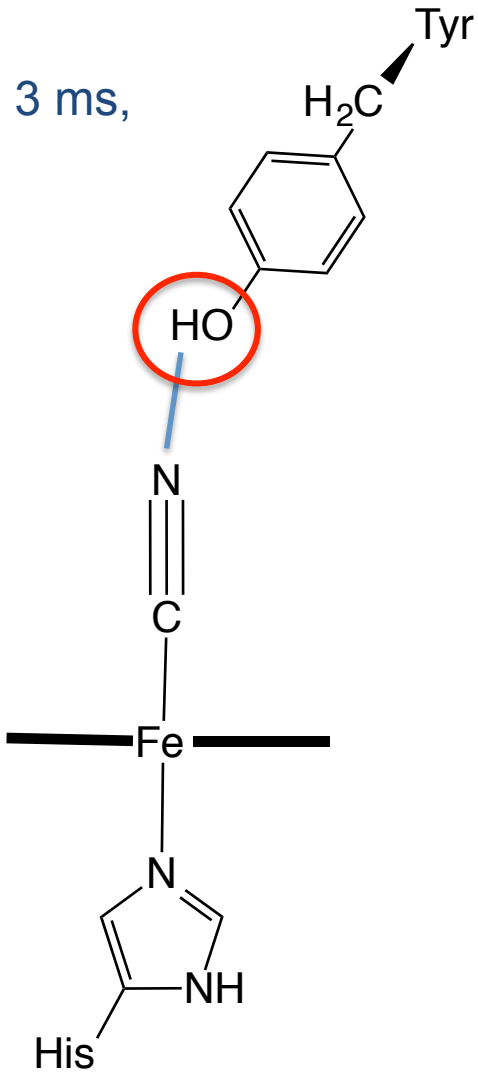
JACS **1995**, 8067

Contact Shift Examples

(Also T_1 = 9 ± 3 ms,
So r ~ 4 Å)

CN-Fe(III) M80A cytochrome *c*, D_2O





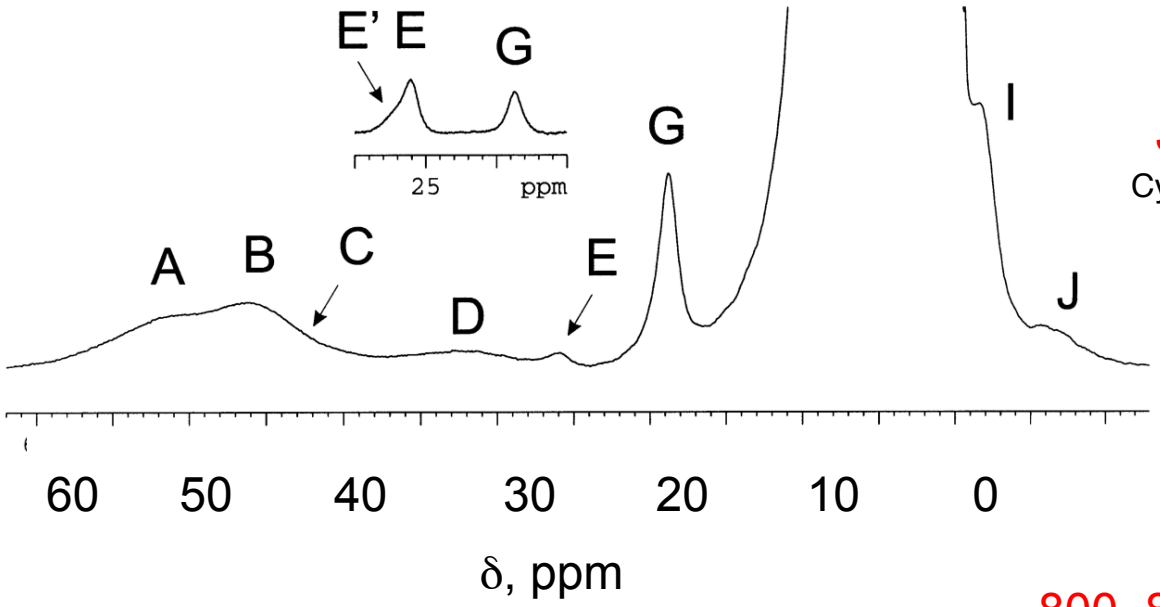
Tyr67 OH δ_{para} has a ~5 ppm contribution from δ_{con}, supporting H-bonding

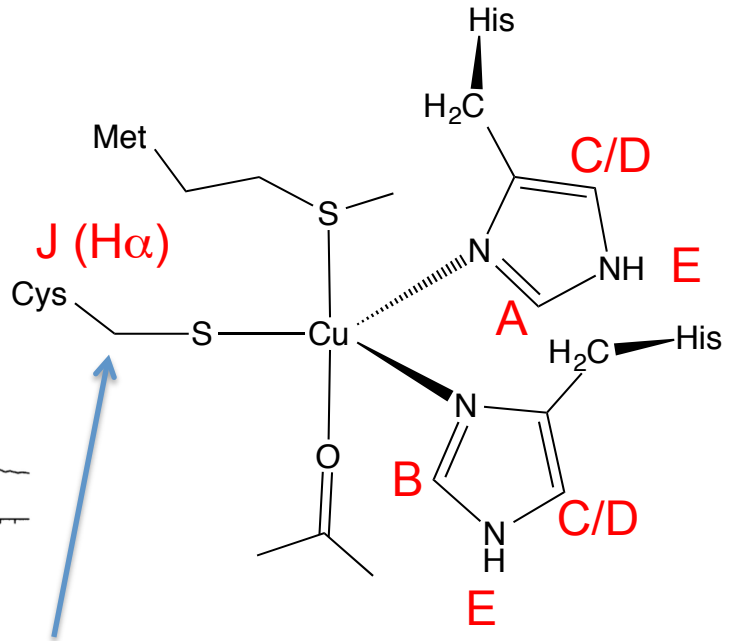
JACS **1995**, 8067

Contact Shift Examples

P. aeruginosa Cu(II) azurin

S = 1/2



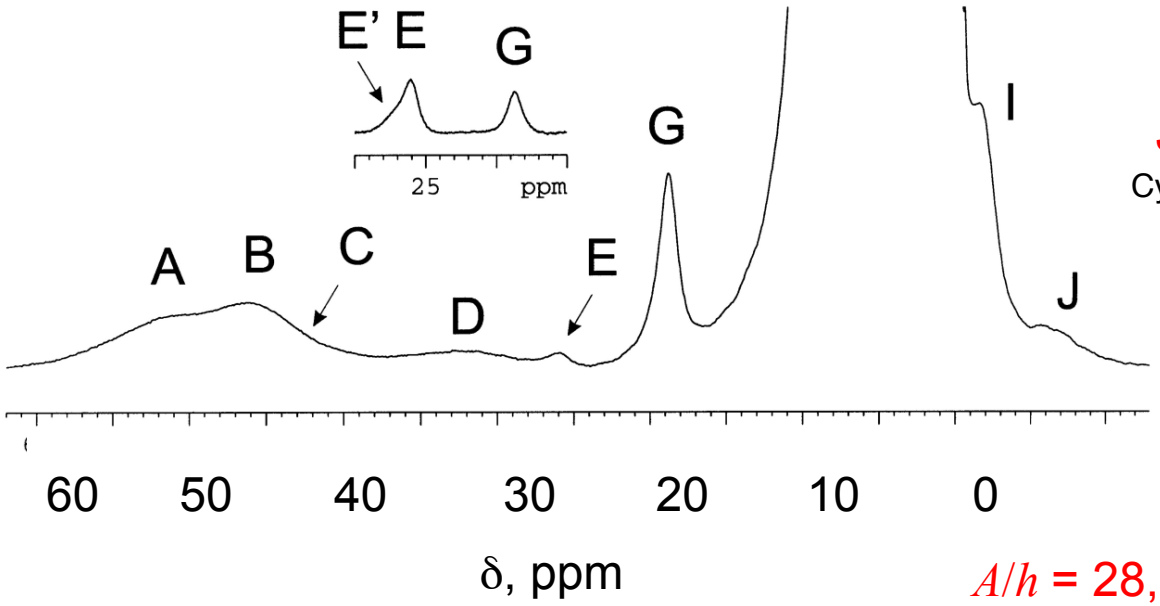


800, 850 ppm

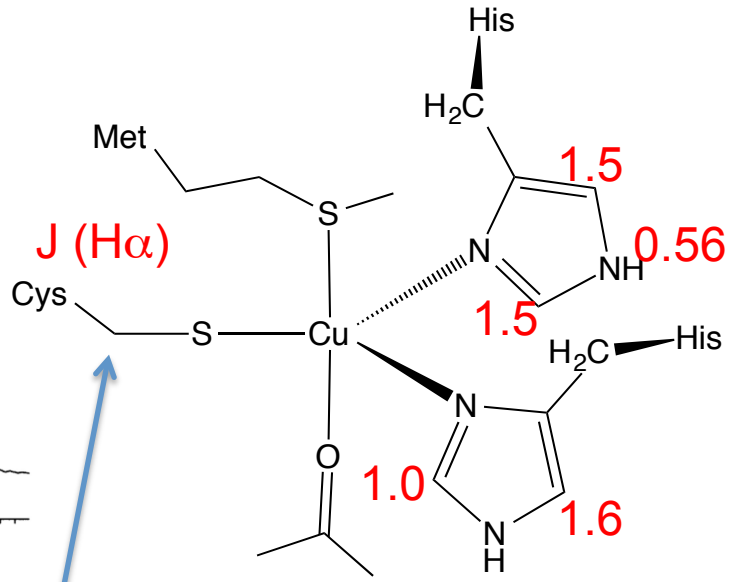
JACS **2000,** 3701

Contact Shift Examples

P. aeruginosa Cu(II) azurin



A/h values



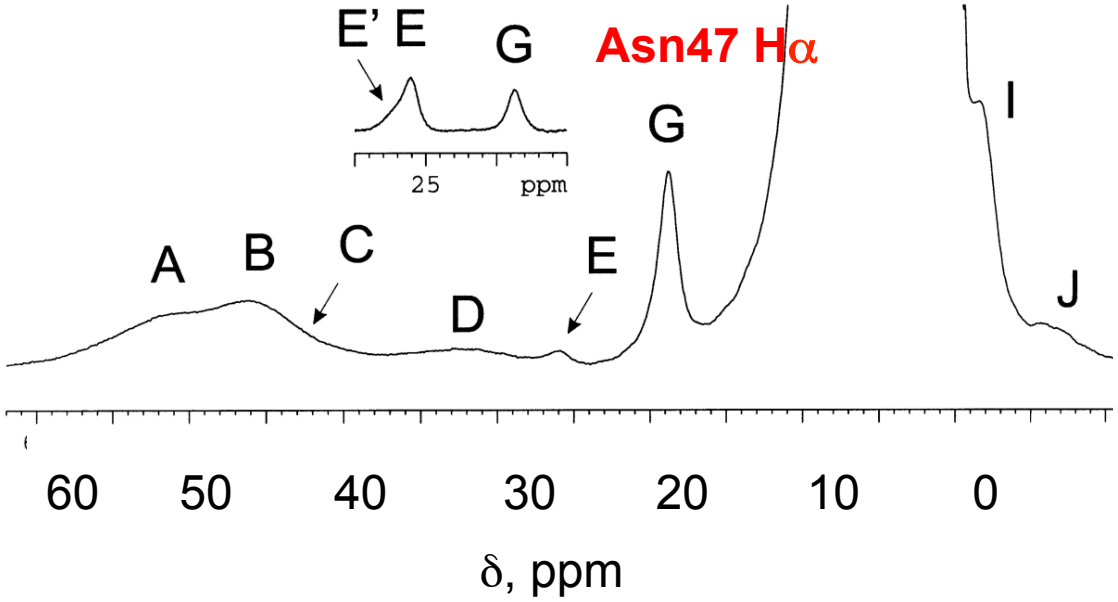
A/h = 28, 27 MHz
2% unpaired spin density

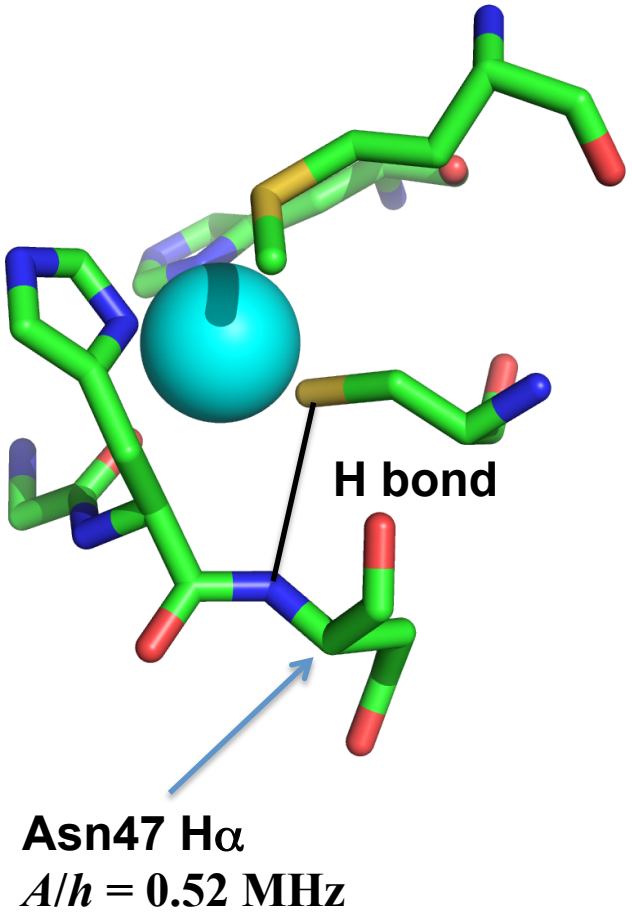
JACS **2000,** 3701

Contact Shift Examples

P. aeruginosa Cu(II) azurin

A/h values



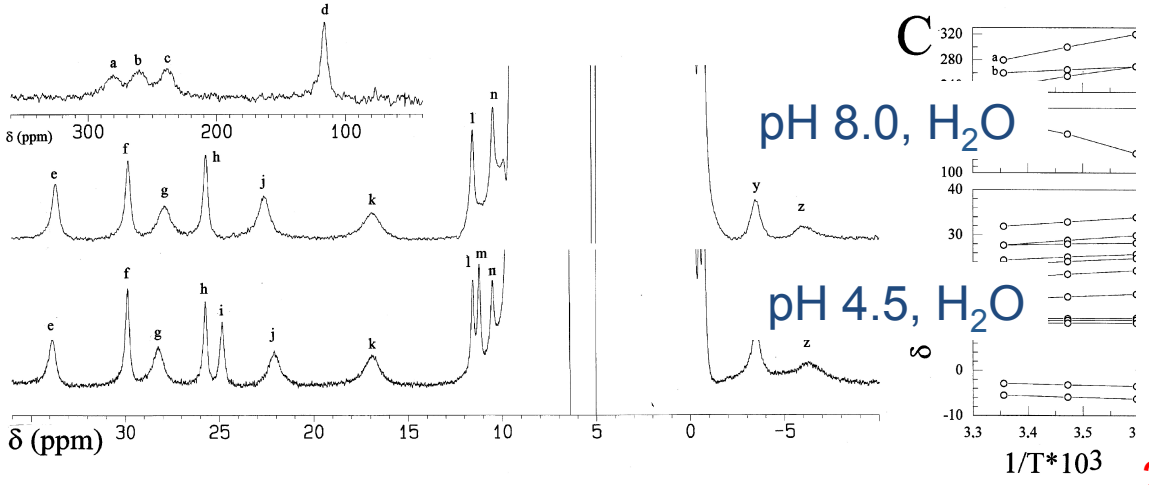


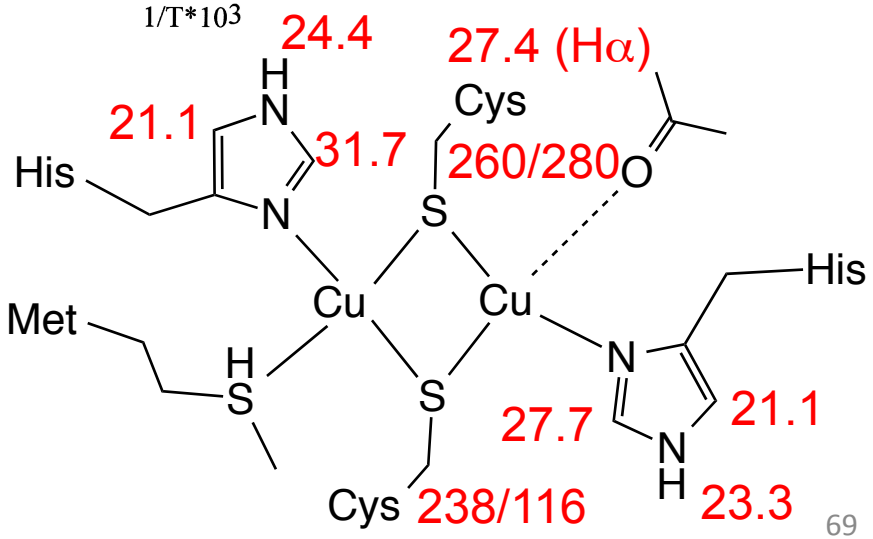
JACS **2000,** 3701

Contact Shift Examples

T. thermophilus Cu_A domain

Cu(I)Cu(II) S = 1/2





JACS **1996,** 11658

Paramagnetic Chemical Shifts

$\delta_{obs} = \delta_{para} + \delta_{dia}$

$\delta_{para} = \delta_{con} + \delta_{pc}$

δ_{dia} is shift in isostructural diamagnetic molecule

δ_{con} is contact shift – through-bond electron-nucleus interaction

δ_{pc} is pseudocontact (also called dipolar) shift – through-space electron-nucleus interaction

Pseudocontact Shift

$$\delta_{pc} = (12\pi r^3)^{-1}[\Delta\chi_{ax}(3\cos^2\theta - 1) + 3/2\ \Delta\chi_{rh}(\sin^2\theta\cos 2\Omega)]$$

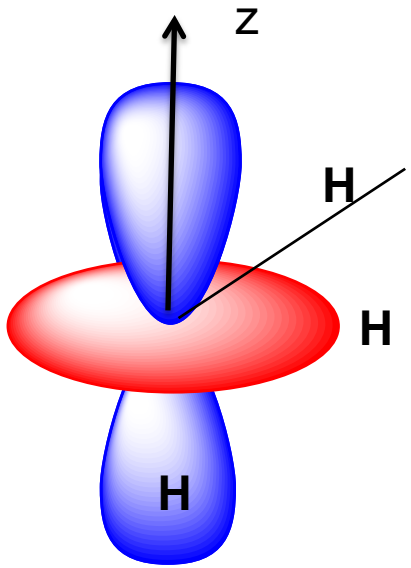
Note dependence on:

- Magnetic anisotropy ($\Delta\chi_{ax}$, $\Delta\chi_{rh}$)
- Position/structure (r, θ, Ω) in magnetic axis system

Pseudocontact Shift

$$\delta_{pc} = (12\pi r^3)^{-1}[\Delta\chi_{ax}(3\cos^2\theta - 1) + 3/2\ \Delta\chi_{rh}(\sin^2\theta\cos 2\Omega)]$$

Axial system ($\Delta\chi_{rh} = 0$); r = 5 Å, $\Delta\chi_{ax} = 3 \times 10^{-32}$ m^3



$\theta = 54.7^\circ$; 0 ppm

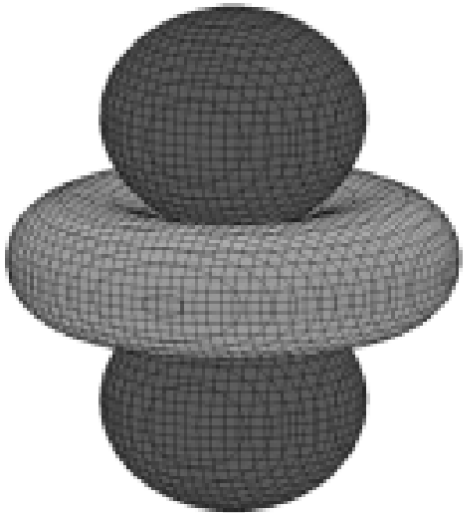
$\theta = 90^\circ$; −6.4 ppm

$\theta = 180^\circ$; +12.7ppm

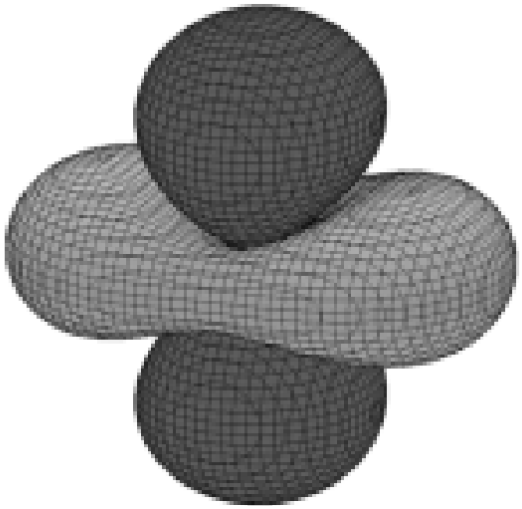
Note position as well as distance determines magnitude and sign of shift

Pseudocontact Shift

Isopseudocontact shift surfaces



$\Delta\chi_{rh} = 0$



$\Delta\chi_{rh} = 1/3\ \Delta\chi_{ax}$

Positive shifts are in dark gray, negative in light gray.

From *Encyclopedia of Inorganic Chemistry* DOI: 10.1002/0470862106.ia319

Pseudocontact Shift

Assuming $\Delta\chi_{rh} = 0$ and nucleus in axial position ($\Omega = 0^{\circ}$)

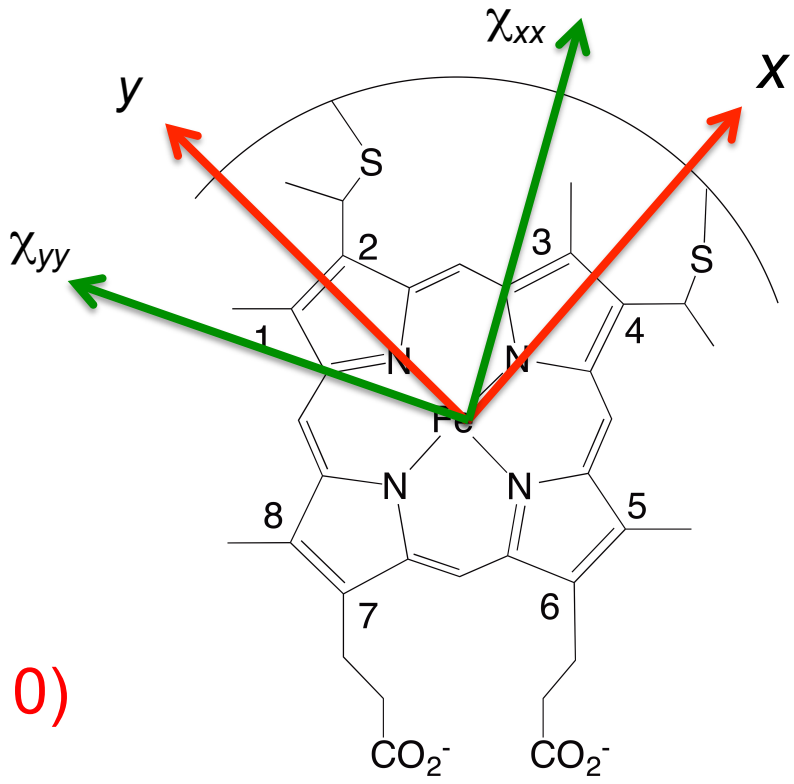
Metal ion	S or J	$\Delta\chi_{ax}$ (10^{-32} m^3)	δ_{pc} (ppm) r = 7 Å
Fe(II)	2	2.1	1.1
Fe(III) (LS)	1/2	2.4	1.3
Fe(III) (HS)	5/2	3.0	1.6
Co(II) (HS, 5-6 coord.)	3/2	7	3.7
Co(II) (HS, 4 coord.)	3/2	3	1.6
Cu(II)	1/2	0.6	0.3
Gd(III)	7/2	0.2	0.1
Ce(III)	5/2	2	1.0
Tb(III)	6	35	19

Coord. Chem. Rev. **1996**, *150*, 1

Magnetic Axes Determination Using δ_{pc}

$$\delta_{pc} = (12\pi r^3)^{-1}[\Delta\chi_{ax}(3\cos^2\theta - 1) + 3/2\ \Delta\chi_{rh}(\sin^2\theta\cos 2\Omega)]$$

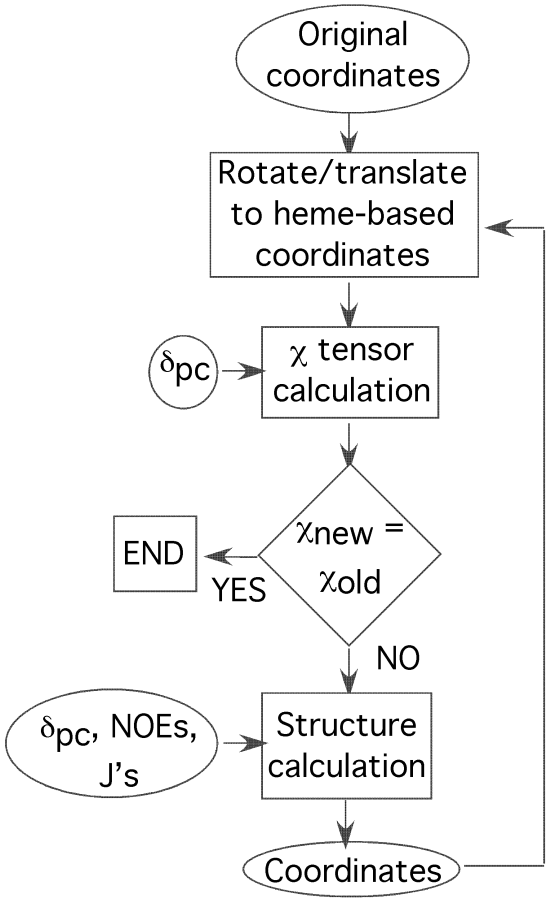
- Measure δ_{obs}
- Subtract diamagnetic component
- Fit resulting shifts to above equation to determine χ orientation and anisotropy

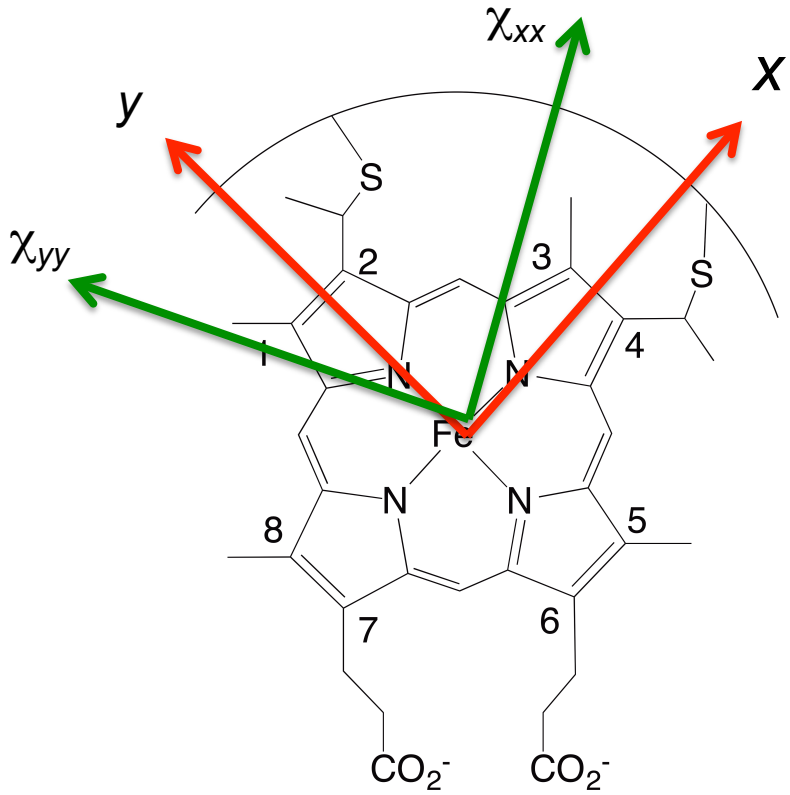


z axis out

$$\delta_{pc} = \delta_{obs} - \delta_{dia}\ (\text{when } \delta_{con} \sim 0)$$

Structure Refinement with δ_{pc}





z axis out

From *Encyclopedia of Inorganic Chemistry* DOI: 10.1002/0470862106.ia319

J. Biol. Inorg. Chem. **1996**, *1*, 117

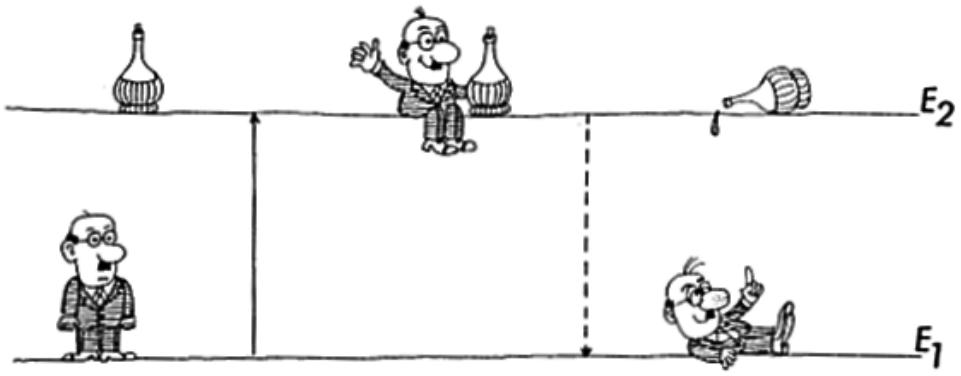
Take-home Messages

- Yes, you can take NMR spectra of paramagnetic molecules
- These spectra are rich in information content – line widths, relaxation times, and chemical shifts all reflect electronic and molecular structure
- The electronic relaxation time is key in determining line broadening extent
- Magnetic anisotropy determines magnitude of pseudocontact shifts

Acknowledgments

Bren Group

Rory Waterman
Brandy Russell
Linghao Zhong
Xin Wen
Lea Michel
Ravinder Kaur
Sarah Bowman
Mehmet Can
Ben Snyder
Jesse Kleingardner
Matthew Liptak
Rebecca Smith

Mentors

Harry Gray
Ivano Bertini
Lucia Banci
Paola Turano
Claudio Luchinat
Gerd La Mar