Solid-state NMR of Paramagnetic Systems

Yoshitaka Ishii
Department of Chemistry,
University of Illinois at Chicago

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Outline

1. Background and Motivation
2. Basic Theory
3. Examples & Applications
4. Some Practical Aspects

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Sec. 1 Background & Motivation

1.1 Motivation of the Study?

1.2 Overview & Recent Applications

- Small Paramagnetic Systems
- Paramagnetic Proteins & Non-paramagnetic Proteins

1.3 The Problems in Paramagnetic Solid-state NMR

More than 1/3 of the Elements Show Paramagnetism in Periodic Table!

1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
		57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb		

Paramagnetic

Anti-Ferromagnetic

Diamagnetic

Ferromagnetic

Modified from http://www.aacg.bham.ac.uk/magnetic_materials/type.htm

Motivation

Many Potential Applications for Paramag Systems

- More than 1/3 of Elements in the Periodic Table Show Paramagnetism
- Nanoscience (Self-assembled structures)
- Molecular Electronics
- Drugs
- Metal-Protein Complex

Still Underdeveloped Spectroscopy

- Structural Information
- Assignment
- Sensitivity
- Resolution

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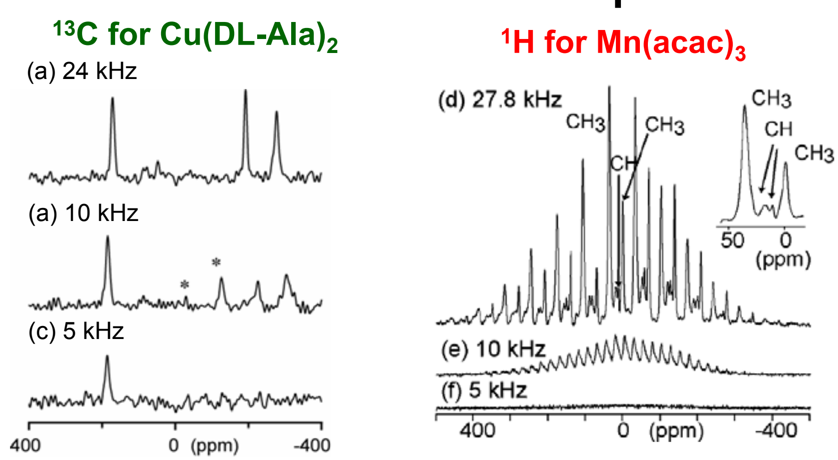
1.2 Overview & Recent Application

MAS SSNMR for Small Compounds



→ Sensitivity under VFMAS is comparable to that of diamagnetic SSNMR

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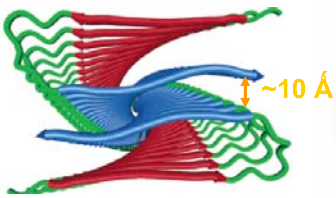
Ideal Structural Measurements for Biomolecules by Solid-state NMR?

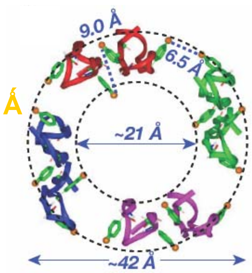
→ Paramagnetic Interactions?

- No extra cross peaks for resolution
- Detectable by shifts or relaxation
- Long-range distances (10 Å or longer)



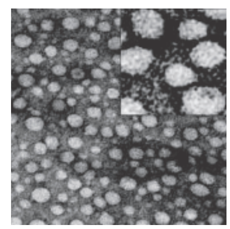
Amyloid Fibrils for Aβ(1-40)

Tycko et al. PNAS (2002) Biochemistry (2006)



PG-1 β-barrel in membrane

Hong et al. PNAS (2006)



Amyloid Intermediate & for Aβ(1-40)

Ishii et al. Nat Struct Biol (20

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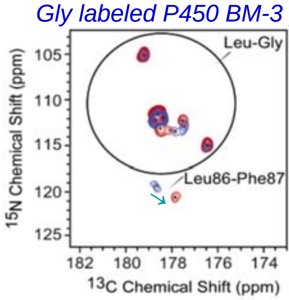
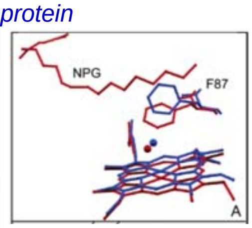
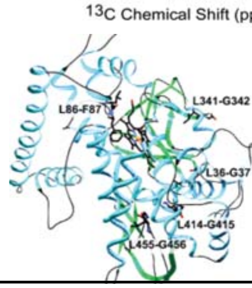
SSNMR of Paramagnetic Metallo-proteins

Structural Information from Pseudo-contact Shifts

Application to selectively ^{13}CO-Leu, ^{15}N-Phe, Gly labeled P450 BM-3 protein

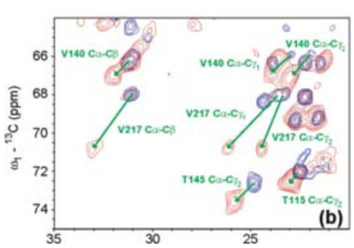






McDermott et al. JACS 127, 13816 (2005)

Application to uniformly ^{13}C-labeled Co(II)-MMP



Bertini et al . JACS 129, 2219 (2007)

$$\delta_{PC} = \frac{1}{12\pi r^3}(0.5 + 1.5\cos 2\theta)(\chi_{\parallel} - \chi_{\perp})$$

→ Distance Info (10-20 Å)

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Applications to Non-paramagnetic Proteins

Spin-labeled Protein

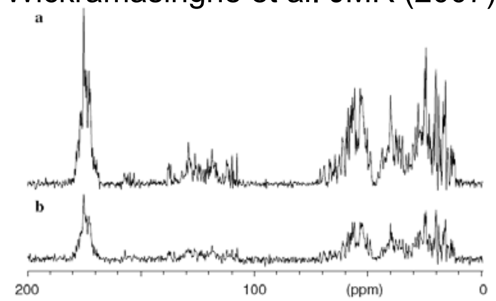
Jaroniec *et al*. JACS 129 **7502** (2007)

→

Long-range distance

Paramagnetic Doping for Protein microcrystals

Wickramasinghe et al. JMR (2007)



→ Sensitivity enhancement & Structural Info

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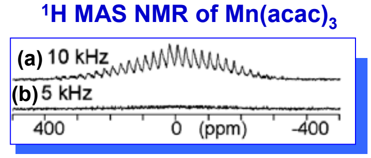
Challenges in Solid-state NMR for Paramagnetic Systems

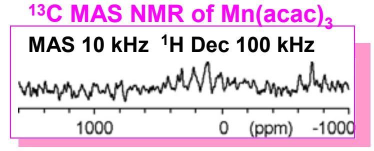
- Range of the Shifts Large
- Limited Resolution
- Assignment
- Requirement of Labeling
- Structural Information

→How can we solve these problems?

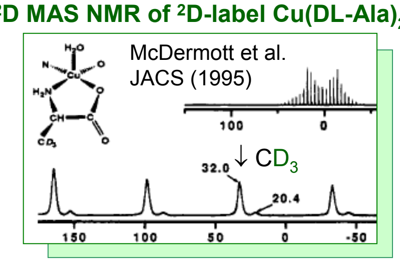
^{1}H MAS NMR of $Mn(acac)_3$



^{13}C MAS NMR of $Mn(acac)_3$



^{2}D MAS NMR of ^{2}D-label $Cu(DL\text{-}Ala)_2$



Sec. 2 Theory & Background

2.0 Definition of Paramagnetism

2.1 Thermal Averaging of Electron-nuclear Interactions

- Contact Coupling, e^{-}/n Dipolar Coupling, g-tensor
- Thermal Averaging of Electron Spin States
- Electron Spin Correlation Time
- Contact & Dipolar (Pseudo-contact) Chemical Shifts

2.2 Relaxation Properties

- Relaxation Mechanisms
- Electron Spin-correlation-time Dependence

2.3 Short Problem Solving Session

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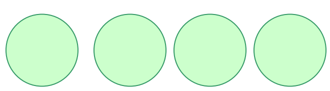
"Rough" Definition of Paramagnetism for NMR Spectroscopists

Diamagnetic



No unpaired electron spins in molecules

→ No bulk spin magnetic moment without B_0

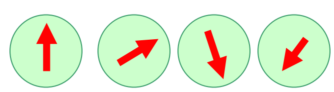
When $\boldsymbol{B}_0$ is applied,

$\boldsymbol{M} = \chi \boldsymbol{H}_0 = \chi \boldsymbol{B}_0/\mu_0$

Magnetic susceptibility: χ

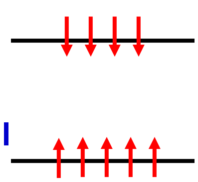
$\chi < 0$ & $\chi \sim 0$ (usually ~ ppm)

Paramagnetic



Unpaired electron spins orient randomly without B_0

→No bulk spin magnetic moment without B_0



$\boldsymbol{M} = \chi \boldsymbol{B}_0/\mu_0$

$\chi > 0$ & χ Small

$\chi \sim C/T$

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Atomic Magnetic Moment

- **Atomic Magnetic Moment** μ

There are two components in electronic magnetic moment in an atom or ion:

- ***Spin component***

$$\mu_s = \gamma_S \hbar \mathbf{S} = \boxed{-g_S \mu_B \mathbf{S},} \qquad [2.1]$$

- ***Orbital component***

$$\boldsymbol{\mu}_L = \gamma_L \hbar \mathbf{L} = \boxed{-g_L \mu_B \mathbf{L},} \qquad [2.2]$$

where $\hbar\mathbf{S}$ and $\hbar\mathbf{L}$ are spin and orbital angular momentum, respectively.
The net atomic magnetic moment is

$$\boldsymbol{\mu} = -g_S \mu_B \mathbf{S} - g_L \mu_B \mathbf{L} = -\mu_B(\boxed{g_S \mathbf{S}} + \boxed{g_L \mathbf{L}}), \qquad [2.3]$$

- μ_B denotes Bohr magneton ($e\hbar/2m$). μ_B is used as a "unit" to measure the electron magnetic moment.
- g-factor

For electrons, g-factor g is defined by

$$g\mu_B = -\gamma\hbar, \qquad [2.4]$$

where g_S for a free electron spin is $g_e \sim 2.00$ ($\boldsymbol{\mu}_S \sim -2\mu_B \mathbf{S}$).

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Interactions of Electron Spins 1

- **Electron Zeeman Interaction**

$$H_{EZ} = -\boldsymbol{\mu} \cdot \mathbf{B}_0 = \mu_B(g_L \mathbf{L} + g_e \mathbf{S}) \cdot \mathbf{B}_0. \qquad [2.5]$$

In general, handling the orbital contribution L is complicated. One simple way to include the orbital effect is to define the g tensor $\boldsymbol{g}$ as

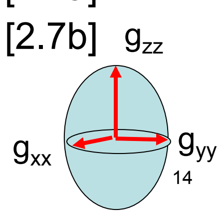
$$H_{EZ} = -\boldsymbol{\mu} \cdot \mathbf{B}_0 = \mu_B \mathbf{S} \cdot \boldsymbol{g} \cdot \mathbf{B}_0, \qquad [2.6]$$

where $\boldsymbol{g}$ is a 3-by-3 matrix that is defined by

$$\boldsymbol{\mu} = \mathbf{S} \cdot \boldsymbol{g} \qquad [2.7a]$$

$$(\boldsymbol{g})_{nm} = (\mathbf{S})_n (g_L \mathbf{L} + g_e \mathbf{S})_m / \{S(S+1)\} \qquad [2.7b]$$

Eq. [2.6] does not include $\boldsymbol{L}$ apparently.
The g-tensor is represented as an ellipsoid, as CSA tensor. When $g_L = 0$, $\boldsymbol{g} = g_e$ (isotropic!).



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Interactions of Electron Spins 2

Fermi Contact Coupling

The Hamiltonian for Fermi contact coupling with nuclear spin $\boldsymbol{I}$ is given by

$$\mathrm{H}_{\mathrm{CON}} = A\boldsymbol{S} \bullet \boldsymbol{I}, \qquad [2.8a]$$

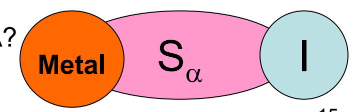
with

$$A = 2\mu_0 \gamma_L \hbar g_e \mu_B \boldsymbol{\rho}. \qquad [2.9]$$

$$\rho = |\phi_\beta|^2 - |\phi_\alpha|^2, \qquad [2.10]$$

where ϕ_k denotes the MO wave function (for the electron S) at Spin I when S takes the spin state k ($k = \alpha, \beta$).

Q. What kind of properties are needed for the MO ϕ_k for the system to have non-zero A?



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Interactions of Electron Spins 3

Pseudo Contact Coupling (Dipolar Coupling)

Like nuclear dipolar coupling, the Hamiltonian for electron-nuclear (e-n) dipolar coupling is given by

$$H_{\mathrm{PC}} = \boldsymbol{S} \bullet \boldsymbol{D} \bullet \boldsymbol{I}. \qquad [2.11]$$

If we can assume that the electron delocalizes at the atom or ion, in the high field approximation, eq. [2.11] yields

$$H_{\mathrm{PC}} = (d/r^3)(1-3\cos^2\theta) I_Z S_Z. \qquad [2.12]$$

What are r and θ?

When the g anisotropy is not negligible,

$$H_{\mathrm{PC}} = (\boldsymbol{\mu}/g_e) \bullet \boldsymbol{D} \bullet \boldsymbol{I}. \qquad [2.11b]$$

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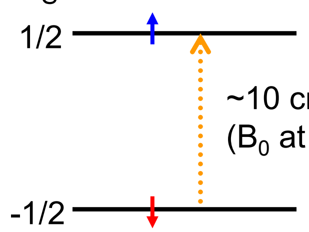
Thermal Averaging

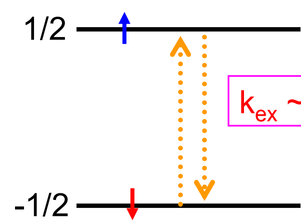
m_S Electron Zeeman (for S =1/2)



~10 cm^{-1} << 200 cm^{-1} = kT
(B_0 at 10 T) (T = 300 K)

& Electron spin relaxation is usually fast

→So $m_s = \pm$ ½ will be thermally mixed.



$k_{ex} \sim 1/\tau_C$

The next question:

- What is the thermal effect on contact coupling for NMR?
- How quickly can the averaging happen?

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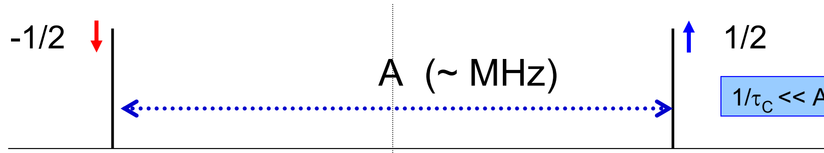
Thermal Averaging of Contact Coupling

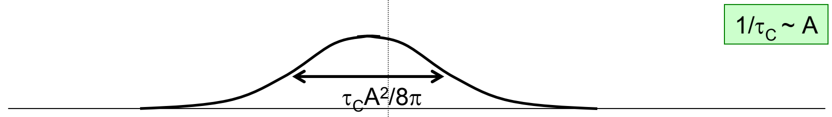
(Case 1) NMR spectrum of *I* <u>with slow</u> thermal averaging



$1/\tau_C$ << A

(Case 2) NMR spectrum of *I* <u>with moderate</u> thermal averaging

$1/\tau_C$ ~ A



(Case 3) NMR spectrum of *I* <u>with fast</u> thermal averaging

Q. Where do you expect the lines?

$1/\tau_C$ >> A

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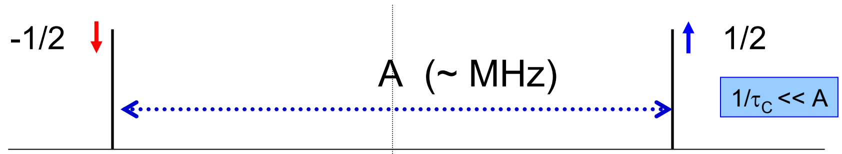
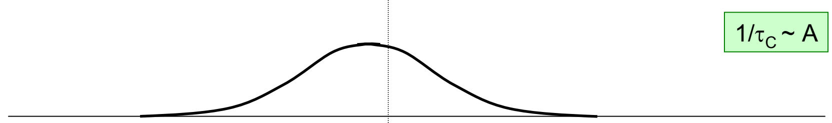
Thermal Averaging of Contact Coupling

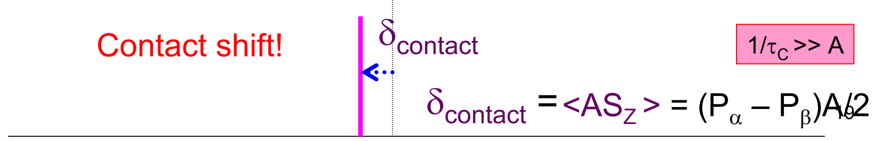
(Case 1) NMR spectrum of *I* with slow thermal averaging



(Case 2) NMR spectrum of *I* with moderate thermal averaging



(Case 3) NMR spectrum of *I* with fast thermal averaging



Calculation of Thermally Averaged Contact *Shift & Dipolar Shift*

	General	High field	Isotropic *g*
•	$H_{CON} = A\langle \boldsymbol{S} \rangle \cdot \boldsymbol{I}$ ~	$A\langle S_z \rangle I_z$	~ $A\langle S_z \rangle I_z$ [2.13]
•	$H_{PC} = \{\langle \mu \rangle / g_e\} \cdot \boldsymbol{D} \cdot \boldsymbol{I}$	~ $\{(\langle \mu \rangle / g_e) \cdot \boldsymbol{D}\}_z I_z$	~ $D\langle S_z \rangle I_z$ [2.14]

→ Let's obtain <μ> & <**S**> first.

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Magnetic Moment under Thermal Averaging

Case 1: For the isotropic g-tensor

$<\boldsymbol{S}> = Tr\{\boldsymbol{S}\exp(-H_{EZ}/kT)\}/Tr\{\exp(-H_{EZ}/kT)\}$ exp(-A)~ 1-A

$$\sim Tr\{\boldsymbol{S}(1-\underbrace{\frac{\mu_B g_e S_Z B_0}{kT}}_{H/kT})\}/Tr\{1-\frac{\mu_B g_e S_Z B_0}{kT}\}$$

$$=\sum_{\xi} <\xi|(\sum_{jkl}\boldsymbol{e}_j S_j)(S_Z g_e B_0)|\xi> \mu_B/\{(kT)Tr(1)\}$$

where $\boldsymbol{e}_j$ is an unit vector along the axis j (j =x, y, z) and |ξ> denotes a basis ket.

$$<\boldsymbol{S}> = \sum_{\xi}\sum_{jkl}\boldsymbol{e}_j g_e B_0 <\xi|S_j S_Z|\xi> \mu_B/\{(kT)Tr(1)\}$$

$$=\sum_{jkl} \boldsymbol{e}_Z g_e B_0\{S(S+1)\}\mu_B/(3kT)$$

$$= g_e\boldsymbol{B}_0\{S(S+1)\}\mu_B/(3kT) \quad [2.15]$$

In the high field approximation for the parameterized g tensor, <μ> is given by

$$<\mu> = \mu_B g_e <\boldsymbol{S}>$$

C: Curie factor

$$= g_e^{\,2}\boldsymbol{B}_0\{S(S+1)\}\mu_B^{\,2}/3kT = g_e^{\,2}C\boldsymbol{B}_0/T \quad [2.16]$$

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Magnetic Moment under Averaging 2

Case 2: A more general case

$<\boldsymbol{S}> = Tr\{\boldsymbol{S}\exp(-H/kT)\}/Tr\{\exp(-H/kT)\}$ exp(-A)~ 1-A

$$\sim Tr\{\boldsymbol{S}(1-\underbrace{\frac{\mu_B \boldsymbol{S}\cdot\boldsymbol{g}\cdot\boldsymbol{B}_0}{kT}}_{=H/kT})\}/Tr\{1-\frac{\mu_B \boldsymbol{S}\cdot\boldsymbol{g}\cdot\boldsymbol{B}_0}{kT}\}$$

$$=\sum_{\xi} <\xi|(\sum_{jkl}\boldsymbol{e}_j S_j)(S_k g_{kl} B_{0l})|\xi> \mu_B/(kT)Tr\{1\}$$

where $\boldsymbol{e}_j$ is an unit vector along the axis j (j =x, y, z) and |ξ> denotes a basis ket.

$$<\boldsymbol{S}> = \sum_{\xi}\sum_{jkl}\boldsymbol{e}_j g_{kl} B_{0l} <\xi|S_j S_k|\xi> \mu_B/(kT)Tr\{1\}$$

$$=\sum_{jkl} \boldsymbol{e}_j g_{kl} B_{0l}\{\delta_{jk}S(S+1)/3\}\mu_B/(kT)$$

$$=\boldsymbol{g}\cdot\boldsymbol{B}_0\{S(S+1)/3\}\mu_B/(kT) \quad [2.17]$$

In the high field approximation for the parameterized g tensor, <μ> is given by

$$<\mu> = \mu_B \boldsymbol{g}\cdot<\boldsymbol{S}> = \boldsymbol{g}\cdot\boldsymbol{g}\cdot\boldsymbol{B}_0\{S(S+1)\}\mu_B^{\,2}/(3kT) \quad [2.18]$$

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Susceptibility Tensor

The susceptibility tensor χ is defined by

$$<\mu> = \chi \cdot \boldsymbol{B}_0/\mu_0. \quad [2.19]$$

From [2.18,19], we obtain

$$\chi = \{S(S+1)\mu_B{}^2/(3kT\mu_0)\}\boldsymbol{g}\cdot\boldsymbol{g} \quad [2.20]$$

Thus, the frame that diagonalizes g-tensor also diagonalizes χ. The principal values of the tensors χ and $\boldsymbol{g}$ are related as

$$\chi_{kk} = \{S(S+1)\mu_B{}^2/(3kT\mu_0)\}\ g_{kk}{}^2 \quad [2.21]$$
$$= (C/T\mu_0)g_{kk}{}^2,$$

where χ_{kk} and g_{kk} denote principal values for χ and $\boldsymbol{g}$.

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Calculation of Thermally Averaged Contact *Shift & Dipolar Shift*

Case 1: g-anitoropy neglected

- $\delta_{CON} = A<S_Z> \;= \boxed{A} g_e B_0\{S(S+1)\}\mu_B/(3kT)$ [2.22]

Isotropic shift

→ NOT Removable by MAS

- $\delta_{PC} = D(\theta)<S_Z> \;= \boxed{D(\theta,R)} g_e B_0\{S(S+1)\}\mu_B/(3kT)$

Anisotropic shift [2.23]

$D(\theta, R) = (1\text{-}3\cos^2\theta)/R^3$

→ Removable by MAS

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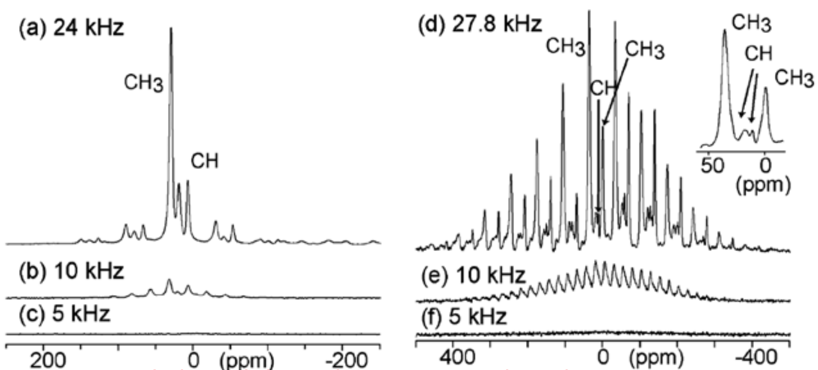
Anisotropic Shift for Paramagnetic Systems in ^{1}H VF-MAS NMR

Cu(II)(DL-Ala)$_2$ $S = 1/2$ **Mn(III)(acac)$_3$** $S = 2$



1/2(1+1/2) = 3/4 2(1+2) = 6

$\delta_{dipolar} \propto S(S+1)/R^3$ Q. What is R?

Thermally Averaged Hyperfine Shifts

Case 2: g-anitoropy NOT neglected

$$\delta_{CON} = A\langle S_z\rangle I_z$$

$$= \{A(\boldsymbol{g}\cdot\boldsymbol{B}_0)_z C/(\mu_B T)\} I_z$$

This is actually anisotropic ↑

$$= \frac{AB_0C}{\mu_B T}\{g_{xx}\sin^2\beta + g_{yy}\cos^2\beta\sin^2\alpha + g_{zz}\cos^2\beta\cos^2\alpha\}$$

[2.24]

$$\delta_{PC} = (\langle\mu\rangle/g_e\cdot\boldsymbol{D})_z I_z$$

$$= (C/g_e T)(\boldsymbol{B}_0\cdot\boldsymbol{g}\cdot\boldsymbol{g}\cdot\boldsymbol{D})_z I_z$$

(α,β,γ) denote Euler angles that define the g-tensor orientation with respect to the Lab frame

[2.25]

The tensor $(\boldsymbol{g}\cdot\boldsymbol{g}\cdot\boldsymbol{D})$ is NOT traceless ($g^2\boldsymbol{D}$ is traceless).

→ This term also includes both anisotropic and isotropic shifts

Bertini et al. "Solution NMR of Paramagnetic Molecules"

Yesnowski et al JCP 89, 4600 (1988)

Calculation of Thermally Averaged Hyperfine Shifts under MAS

Case 2: g-anitoropy NOT neglected

By averaging the diagonal elements of the tensors AC***g***/T and (C/g_eT)***g·g·D***, we obtain the isotropic shifts for contact and dipolar shifts as follows:

$$<\delta_{CON}> = \frac{AB_0C}{\mu_B T}\{g_{xx} + g_{yy} + g_{zz}\}/3 \quad [2.26]$$

$$<\delta_{PC}> = \frac{B_0Cd}{g_e \boxed{R^3 T}}\{(g_{zz}^2 - \frac{g_{xx}^2 + g_{yy}^2}{2})\frac{1-3\cos^2\eta}{3} + \left(\frac{g_{xx}^2 - g_{yy}^2}{6}\right)\sin^2\eta\cos 2\phi\} \quad [2.27]$$

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Distance Information from Isotropic PC Shifts

$$<\delta_{PC}> = \frac{B_0Cd}{g_e R^3 T}\{(g_{zz}^2 - \frac{g_{xx}^2 + g_{yy}^2}{2})\frac{1-3\cos^2\eta}{3} + \left(\frac{g_{xx}^2 - g_{yy}^2}{6}\right)\sin^2\eta\cos 2\phi\} \quad [2.27]$$

where η and ϕ are the polar and azimuthal angles of the dipolar vector with respect to the g-tensor frame (see Ref. below). The principal values g_{kk} can be obtained from EPR.

→ R, η, ϕ can be fitting parameters for structural studies!

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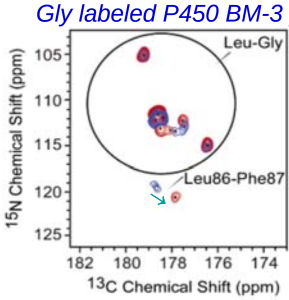
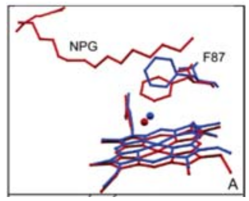
Yesnowski et al JCP 89, 4600 (1988)

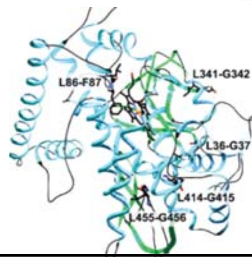
SSNMR of Paramagnetic Metallo-proteins

Structural Information from Pseudo-contact Shifts

Application to selectively ^{13}CO-Leu, ^{15}N-Phe, Gly labeled P450 BM-3 protein

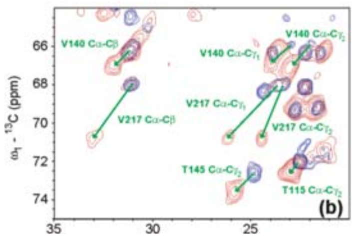






McDermott et al. JACS 127, 13816 (2005)

Application to uniformly ^{13}C-labeled Co(II)-MMP



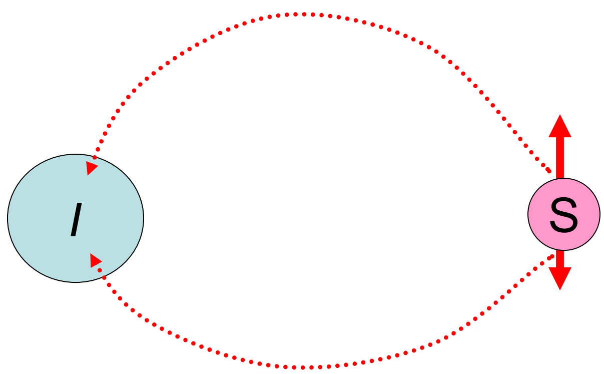
Bertini et al . JACS 129, 2219 (2007)

$$\delta_{PC} = \frac{1}{12\pi r^3}(0.5 + 1.5\cos 2\theta)(\chi_{\parallel} - \chi_{\perp})$$

30

Relaxation Properties

The main source of the paramagnetic relaxation in solids is thermally fluctuated fields due to hyperfine couplings.



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Correlation time

- We define the correlation time of the electron spin state τ_S as

  $C(t) = \langle S_z(t)S_z(0)\rangle = \langle S_z(0)^2\rangle \exp(-|t|/\tau_S)$ [2.28]

  τ_S is in the range of 10^{-13} to 10^{-8} s. This fluctuation can be introduced by electron spin relaxation, electron-electron spin couplings (dipolar & exchange couplings).

τ_S can be significantly different between samples in solids and solution (typically shorter in solids) because of intermolecular electron spin couplings.

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Paramagnetic Relaxation in Solution

- Two type of relaxation exists in solution:

Curie relaxation & Solomon relaxation (see the reference below for R_2).

$$R_1^{SL} = \frac{2S(S+1)}{15}\left(\frac{\mu_0 \hbar \gamma_I \gamma_S}{4\pi R^3}\right)^2 \left\{\frac{\tau_C}{1+(\omega_I-\omega_S)^2\tau_C^2} + \boxed{\frac{3\tau_C}{1+\omega_I^2\tau_C^2}} + \frac{6\tau_C}{1+(\omega_I+\omega_S)^2\tau_C^2}\right\} + \frac{S(S+1)}{3}\left(\frac{A}{h}\right)^2\left\{\frac{\tau_C}{1+\omega_S^2\tau_C^2}\right\}$$

Dominant term in solids when $\tau_C >> 1/\omega_S \sim 10^{-12}$

$$R_1^{Curie} = \frac{S^2(S+1)^2}{5}\frac{\gamma_S^2 B_0^2}{(3kT)^2}\left(\frac{\mu_0 \hbar \gamma_I \gamma_S}{4\pi R^3}\right)^2\left\{\frac{3\tau_r}{1+\omega_I^2\tau_r^2}\right\}$$

~ 0 in solids

where τ_r is the rotation correlation time of the molecule, $1/\tau_C = 1/\tau_S + 1/\tau_r$.

(t_r for protein ~ns)

In solids, $\tau_r \sim \infty$. → $R_1^{Curie} \sim 0$ & $\tau_C \sim \tau_S$.

Bertini "Solution NMR of paramagnetic Molecules"

Emsley JACS 129 14118 (2007)

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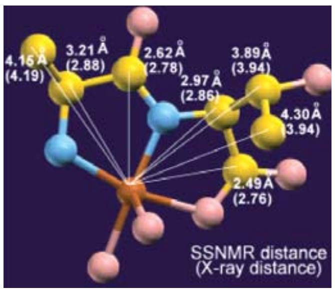
Structural Info from Paramagnetic R_1

- $R_1^{SL} \propto 1/R^6$ → Distance information

Cu(II)-^{13}C distance determination
using ^{13}C R_1 measurements
for unlabeled Cu(Ala-Thr)



→ Seven ^{13}C-Cu distances were determined without requirements of ^{13}C-labeled samples

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R_2 Paramagnetic Relaxation

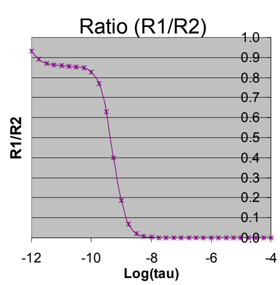
Solomon relaxation

$$R_2^{SL} = \frac{S(S+1)}{15}\left(\frac{\mu_0\hbar\gamma_I\gamma_S}{4\pi R^3}\right)^2\left\{\boxed{4\tau_c} + \frac{\tau_C}{1+(\omega_I-\omega_S)^2\tau_C^2} + \boxed{\frac{3\tau_C}{1+\omega_I^2\tau_C^2}} + \frac{6\tau_C}{1+\omega_S^2\tau_C^2} + \frac{6\tau_C}{1+(\omega_I+\omega_S)^2\tau_C^2}\right\}$$
$$+\frac{S(S+1)}{3}\left(\frac{A}{h}\right)^2\left\{\tau_C + \frac{\tau_C}{1+\omega_S^2\tau_C^2}\right\}$$

Dominant dipolar terms in solids
(First term dominant
when $\tau_C < 10^{-9}$.)

$R_2^{SL} \propto \gamma_I^2$

→ Even if ^{1}H Signals are very broad, ^{13}C, ^{15}N signals may be observable.



Sec. 3 Examples & Applications

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^{1}H & ^{13}C High Resolution Paramagnetic SSNMR

Problems **Paramagnetic Shifts Are Large (^{1}H & ^{13}C)**

- **Fundamental RF methods fail** **(^{1}H-^{1}H or ^{1}H decoupling, CP)**
  - ♦ ^{2}D labeling (Dobson et al. 1990; Oldfield et al.)
  - ♦ Resolution under MAS at ~10 kHz in a few cases (^{1}H Yesinowski et al. 1988; ^{13}C McDermott et al. 1995, ^{13}C Kohler et al. 2001)

  → *Labeling required & Limited sensitivity/resolution*
- **Numerous sidebands due to large anisotropic shifts**
- **Assignments are difficult**

  → *Selective ^{2}D or ^{13}C-labeling required*
- **Sophisticated experiments rarely attempted** (2D, Distance)
  - ♦ 2D ^{13}C/^{13}C correlation (Terao et al. 1999; Emsley et al. 2000)

  → *^{13}C labeled samples even for small molecules*

Low Resolution/Sensitivity, Assignment Difficult

→ *Only handful studies over 30 years* before 2000

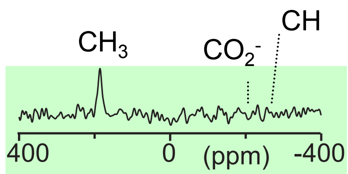
Very Fast Magic Angle (VFMAS) Changes the Situations !

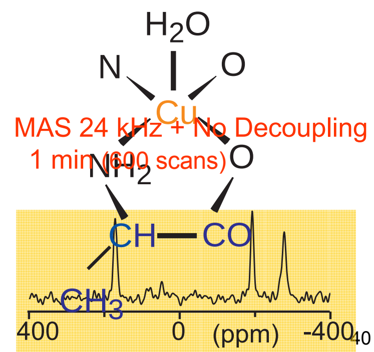
^{13}C High Resolution Paramagnetic SSNMR

Problems **Paramagnetic Shifts Are Large (^1H & ^{13}C)**

- **^1H (^1H-^1H) RF decoupling ineffective**
  → ***Decoupling by Very Fast MAS***
- **Numerous sidebands**
  → ***Removal by Very Fast MAS***
- **CP ineffective**

Cu(DL-Ala)$_2$ 1 min (600 scans)
^{13}C MAS at 5 kHz





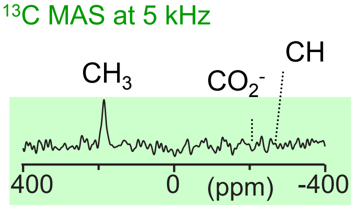
40

^{13}C High Resolution Paramagnetic SSNMR

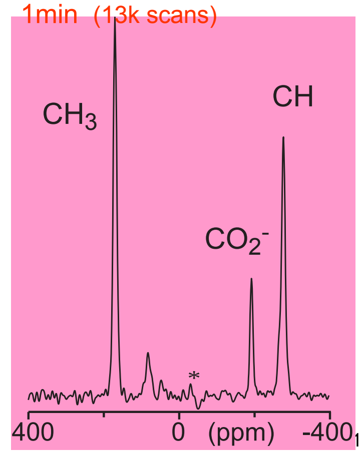
Problems **Paramagnetic Shifts Are Large (^1H & ^{13}C)**

- **^1H (^1H-^1H) RF decoupling ineffective**
  → ***Decoupling by Very Fast MAS***
- **Numerous sidebands**
  → ***Removal by Very Fast MAS***
- **CP ineffective**
  → ***Recoupling-based transfer using strong RF fields***

Cu(DL-Ala)$_2$ 1 min (600 scans)
^{13}C MAS at 5 kHz

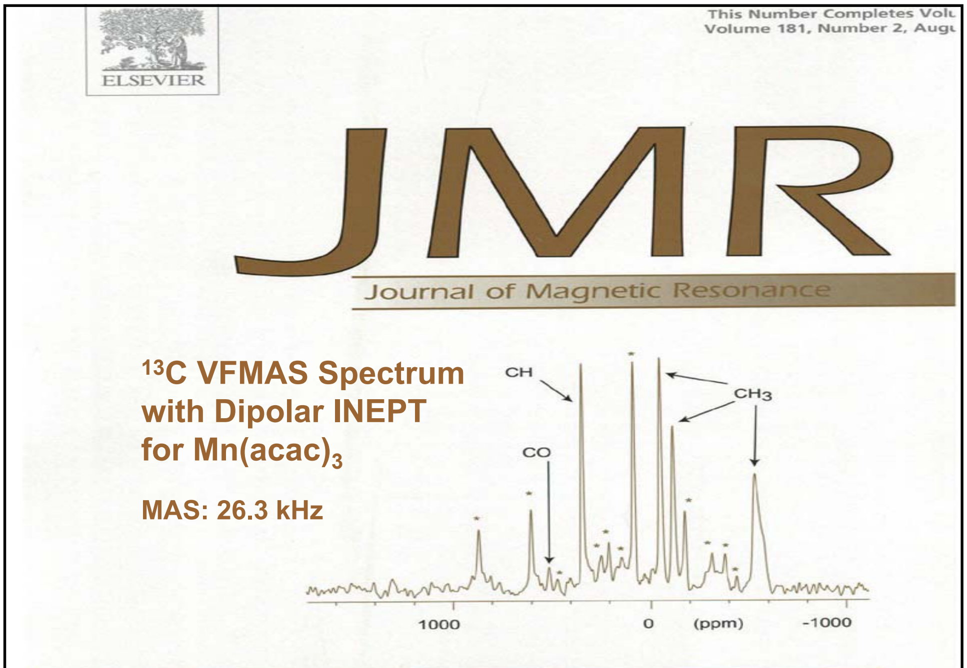


MAS 24 kHz + Dipolar INEPT
1min (13k scans)



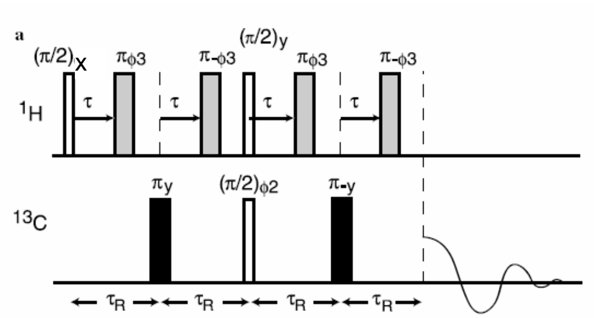
1

• Ishii et al. JACS **125**, 3438-3439 (2003)



Wickramasinghe & Ishii J. Magn. Reson. (2006)

Dipolar INEPT Pulse Sequence



Frydman et al JMR (2001)
Ishii et al. JMR (2006)

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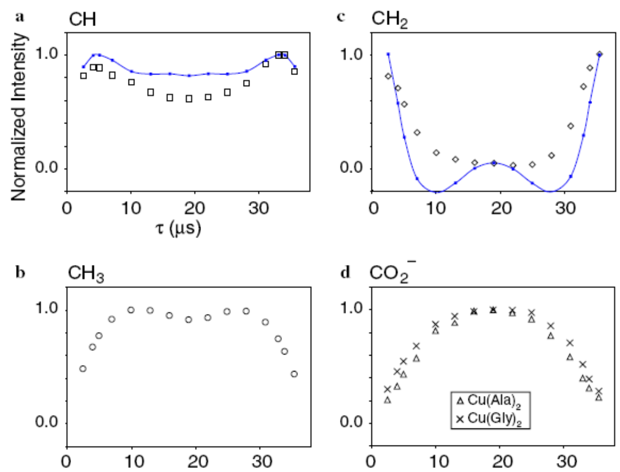
Assignment using Dipolar INEPT

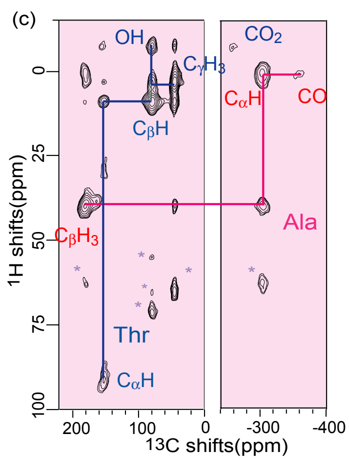
Effective transfer-time (τ) dependence of signal intensities



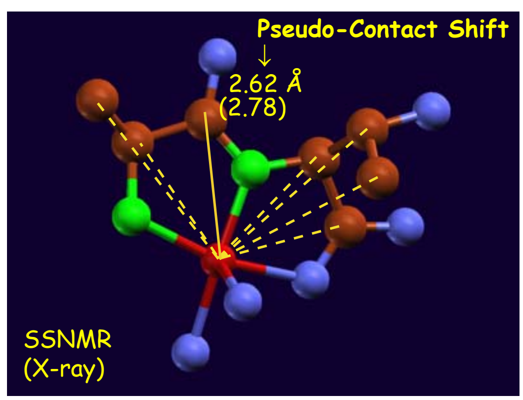
44

Distance Measurements for Cu(Ala-Thr)

With CP (ct = 0.5 ms)



Exp Time ~ 30 hours



Agree Well !

[Ishii et al. JACS 2003, JMR 2006
Wickramasinghe et al JPC B 2007]

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Structural Information ?

♦ Pseudo-Contact (Anisotropic) Shift

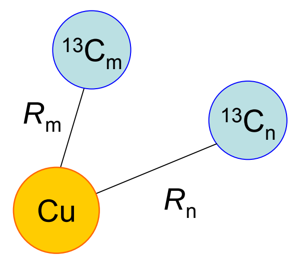
$$\Delta \equiv |\sigma_{11} - \sigma_{iso}| = cS(S+1)/R^3$$

♦ Paramagnetic Relaxation Time: T_1

$$1/T_1 = k\, S(S+1)\tau_S / \{(1+\omega_I^2 \tau_S^2) R^6\}$$

R: Metal-^{13}C Distance
S: Electron Spin Number
τ_S: Electron Spin Correlation Time
ω_I: ^{13}C NMR Frequency
c, k: Known Constant

→ $\{(T_1^m)/(T_1^n)\}^{1/6} = (R_m/R_n)$



No Labeling Necessary !

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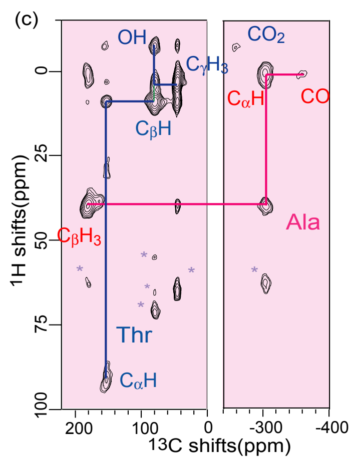
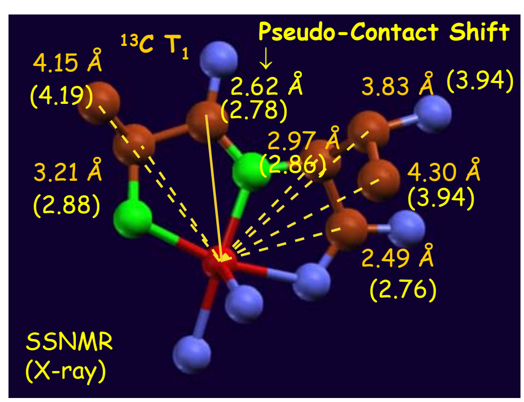
Distance Measurements for Cu(Ala-Thr)

With CP (ct = 0.5 ms)



Exp Time ~ 30 hours



All Agree Well !

[Wickramasinghe et al JPC B 2007]

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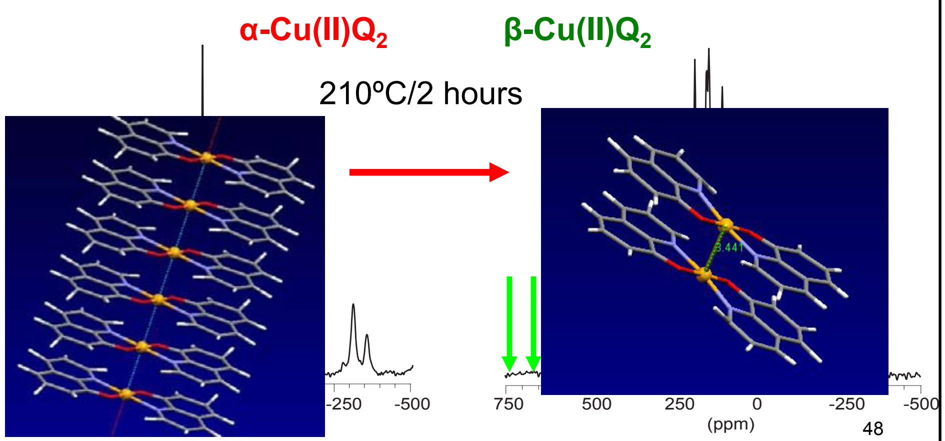
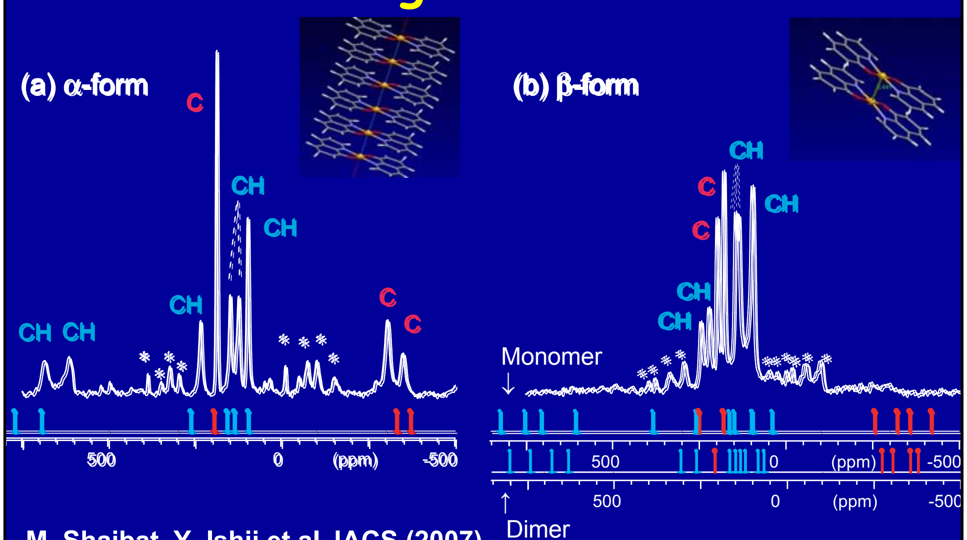
^{13}C VFMAS Characterization of Solid-State Reaction for $Cu(II)(8\text{-quinolinol})_2$

Anti-cancer drugs for leukemia



What is the origin of the difference?



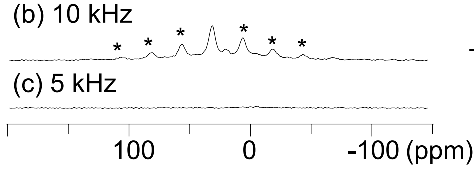
M. Shaibat, Y. Ishii et al JACS (2007)

Collaboration with Dr. A. de Dios & L Casabianca at Georgetown

^1H MAS spectra of $Cu(DL\text{-}Ala)_2$

Sample: 13 mg (~50 μmol),
Exp time: 20 ms (4 scans)

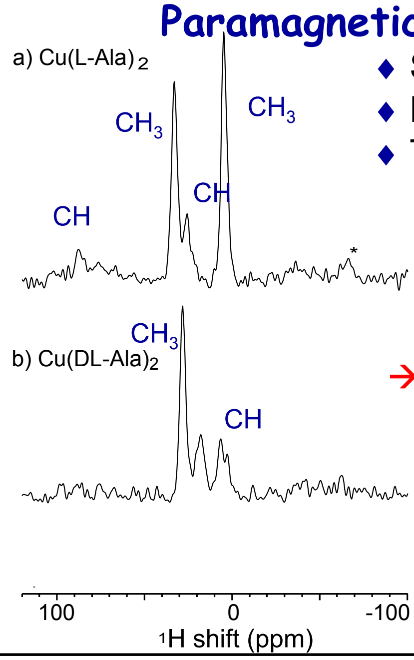
- S/N x14, Compared with 10 kHz
- S/N 800 for CH_3



→ Numerous Sidebands !

Ishii *et al*. JACS 2005

High-Sensitivity ^1H SSNMR for Paramagnetic Systems ?



- Sample: ~ 5 μg (20 nmol)
- Difference Spectra
- Total Exp Time 4 mins

- Sensitivity Compared with Diamagnetic Systems
  → x10-12

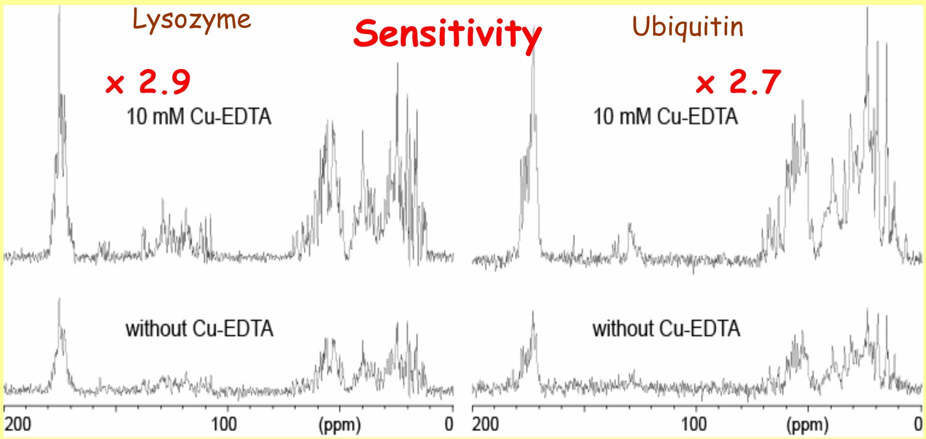
→ Recycle Delays Only 3 ms

Sensitivity $\propto (T_2/T_1)^{1/2}$

→ Solid-State NMR Analysis Possible in a Nano-Mol Scale

[Ishii *et al.* JACS 2005]

^{13}C SSNMR CPMAS Spectra of Protein Microcrystals at 40 kHz MAS



2 hour exp

4 hour exp

Wickramasinghe et al. JMR (2007)

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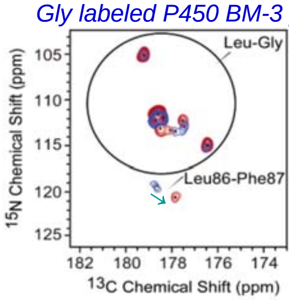
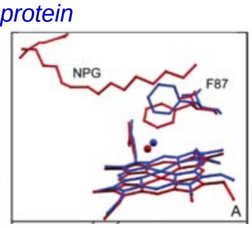
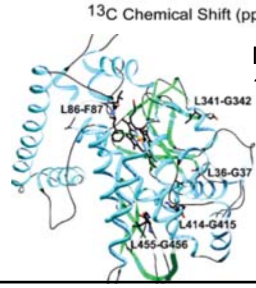
SSNMR of Paramagnetic Metallo-proteins

Structural Information from Pseudo-contact Shifts

Application to selectively ^{13}CO-Leu, ^{15}N-Phe, Gly labeled P450 BM-3 protein

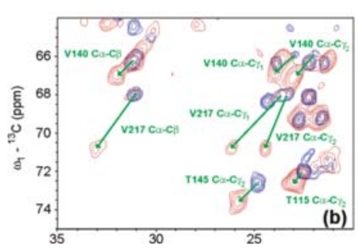






McDermott et al. JACS 127, 13816 (2005)

Application to uniformly ^{13}C-labeled Co(II)-MMP



Bertini et al . JACS 129, 2219 (2007)

$$\delta_{PC} = \frac{1}{12\pi r^3}(0.5 + 1.5\cos 2\theta)(\chi_{\parallel} - \chi_{\perp})$$

53

4. Experimental Aspects

- Temperature Dependence of Shifts
  → Line broadening due to temperature distribution

- Choose optimum spinning ($\Delta T \propto 1/T$)
- Enough VT Air & Optimize Line Shape for Standards ($Cu(DL\text{-}Ala)_2$ & Lead Nitrate)

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Practical Protocols to Examine Paramagnetic Systems

(1) ^{1}H VFMAS
→ Check Line shape & ^{1}H T_1

(2) ^{13}C Dipolar INEPT (with two τ values)
→ Line shape & ^{13}C Assignments

(3) ^{13}C 1 pulse & Inversion recovery
→ ^{13}C T_1 (Distances)

(4) 2D $^{13}C/^{1}H$ correlation → ^{1}H Assignments

55

Selected References

Paramagnetic Solution NMR

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Paramagnetic SSNMR

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- K. Liu, D. Ryan, K. Nakanishi, and A. McDermott, J. Am. Chem. Soc., 1995. **117**(26): p. 6897-6906.
- N.P. Wickramasinghe, M. Shaibat, L.B. Casabianca, A.C. de Dios, J.S. Harwood, and Y. Ishii, J. Chem. Phys., In press.

Relaxation

- A. Abragam, *Principles of nuclear magnetism*. International series of monographs. 1961, New York: Oxford University Press.
- Emsley et al. J. Am. Chem. Soc. 2007. **129** 14118-14119.

Magnetism

- C. Kittel "Introduction to Solid-state Physics"
- C.P. Slichter, "*Principles of Magnetic Resonance"*

Conclusion

- Paramagnetic interactions are potentially useful for obtaining structural information for biomolecules!
- Long-range distance constraint can be obtained
- Structural analysis is possible for
  - *Small unlabeled paramagnetic compounds*
  - *Labeled paramagnetic proteins.*