Solid-state NMR of Paramagnetic Systems

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Outline

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

- 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

1.2 Overview & Recent Application

MAS SSNMR for Small Compounds

$^{13}$C for Cu(DL-Ala)$_2$

(a) 24 kHz

(a) 10 kHz

(c) 5 kHz

$^1$H for Mn(acac)$_3$

(d) 27.8 kHz

(e) 10 kHz

(f) 5 kHz

→ Sensitivity under VFMAS is comparable to that of diamagnetic SSNMR
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

SSNMR of Paramagnetic Metallo-proteins

Structural Information from Pseudo-contact Shifts

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

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

δPC = \frac{1}{12πε} (0.5 + 1.5 \cos 2θ) (χ_m - χ_0)

→ Distance Info (10-20 Å)

McDermott et al. JACS 127, 13816 (2005)

Bertini et al. JACS 129, 2219 (2007)
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

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?
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

"Rough" Definition of Paramagnetism for NMR Spectroscopists

Diamagnetic
- No unpaired electron spins in molecules
- No bulk spin magnetic moment without $B_0$

When $B_0$ is applied,
$$M = \chi H_0 = \chi B_0 / \mu_0$$
Magnetic susceptibility: $\chi$
- $\chi < 0$ & $\chi \sim 0$ (usually $\sim$ ppm)

Paramagnetic
- Unpaired electron spins orient randomly without $B_0$
- No bulk spin magnetic moment without $B_0$

$$M = \chi B_0 / \mu_0$$
- $\chi > 0$ & $\chi$ Small
- $\chi \sim C/T$
Atomic Magnetic Moment

- Atomic Magnetic Moment $\mu$
  
  There are two components in electronic magnetic moment in an atom or ion:
  - Spin component
    \[ \mu_s = \gamma_S h S = -g_S \mu_B S, \]  
    [2.1]
  - Orbital component
    \[ \mu_L = \gamma_L h L = -g_L \mu_B L , \]  
    [2.2]
  where $hS$ and $hL$ are spin and orbital angular momentum, respectively.
  
  The net atomic magnetic moment is
  \[ \mu = -g_S \mu_B S - g_L \mu_B L = -\mu_B (g_S S + g_L L), \]  
  [2.3]
  - $\mu_B$ denotes Bohr magneton ($e\hbar /2m$). $\mu_B$ is used as a “unit” to measure the electron magnetic moment.

- g-factor
  
  For electrons, g-factor $g$ is defined by
  \[ g\mu_B = -\gamma\hbar, \]  
  [2.4]
  where $g_S$ for a free electron spin is $g_e \sim 2.00$ ($\mu_S \sim -2\mu_B$).

Interactions of Electron Spins 1

- Electron Zeeman Interaction
  
  \[ H_{EZ} = - \mu \cdot B_0 = \mu_B (g_L L + g_e S) \cdot B_0. \]  
  [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 $g$ as
  \[ H_{EZ} = - \mu \cdot B_0 = \mu_B S \cdot g \cdot B_0, \]  
  [2.6]
  where $g$ is a 3-by-3 matrix that is defined by
  \[ \mu = S \cdot g \]  
  [2.7a]
  \[ (g)_{nm} = (S)_{n} (g_L L + g_e S) \_m/\{S(S+1)\} \]  
  [2.7b]  
  \[ g_{zz} = \frac{g_{xx} + g_{yy}}{2} \]

  Eq. [2.6] does not include $L$ apparently.
  
  The g-tensor is represented as an ellipsoid, as CSA tensor. When $g_L = 0$, $g = g_e$ (isotropic!).
**Interactions of Electron Spins 2**

**Fermi Contact Coupling**

The Hamiltonian for Fermi contact coupling with nuclear spin $I$ is given by

$$H_{\text{CON}} = A S \cdot I,$$

[2.8a]

with

$$A = 2 \mu_0 \gamma_L \hbar g_e \mu_B \rho,$$

[2.9]

$$\rho = |\phi_\beta|^2 - |\phi_\alpha|^2,$$

[2.10]

where $\phi_k$ 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 $\phi_k$ for the system to have non-zero $A$?

![Diagram showing metal, S, and I connected]

**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

$$H_{\text{PC}} = S \cdot D \cdot I.$$  

[2.11]

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

$$H_{\text{PC}} = (d/r^3)(1-3\cos^2 \theta) I_z S_z.$$  

[2.12]

What are $r$ and $\theta$?

When the g anisotropy is not negligible, $H_{\text{PC}} = (\mu g_e) \cdot D \cdot I.$  

[2.11b]
**Thermal Averaging**

$m_s$  Electron Zeeman  (for $S=1/2$)

$1/2$  $\uparrow$

$-1/2$  $\downarrow$

$\sim 10 \text{ cm}^{-1}$  $< 200 \text{ cm}^{-1} = kT$

($B_0$ at 10 T)  ($T = 300$ K)

$\&$  Electron spin relaxation is usually fast

→ So $m_s = \pm 1/2$ will be thermally mixed.

The next question:

♦ What is the thermal effect on contact coupling for NMR?

♦ How quickly can the averaging happen?

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

**Thermal Averaging of Contact Coupling**

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

$-1/2$  $\downarrow$

$1/2$  $\uparrow$

$A$  ($\sim$ MHz)

$1/\tau_C << A$

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

$1/\tau_C \sim A$

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

$1/\tau_C >> A$

Q. Where do you expect the lines?

$\tau_C A^2/8\pi$
Thermal Averaging of Contact Coupling

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

\[ 1/\tau_C << A \]

\[ 1/\tau_C \sim A \]

\[ 1/\tau_C >> A \]

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

\[ \delta_{\text{contact}} = \langle AS_Z \rangle = (P_\alpha - P_\beta)A/2 \]

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

Calculation of Thermally Averaged Contact Shift & Dipolar Shift

General | High field | Isotropic g
---|---|---
\[ H_{\text{CON}} = A \langle S \rangle \cdot I \sim A \langle S_z \rangle I_z \sim A \langle S_z \rangle I_z \] [2.13]

\[ H_{\text{PC}} = \langle \mu/g_e \rangle \cdot D \cdot I \sim \langle (\mu/g_e) \cdot D \rangle z \sim D \langle S_z \rangle I_z \] [2.14]

→ Let’s obtain \( <\mu> \) & \( <S> \) first.
Magnetic Moment under Thermal Averaging

Case 1: For the isotropic g-tensor

\[
\langle S \rangle = \text{Tr} \{ S \exp(-H_{\text{E2}} / kT) \} / \text{Tr} \{ \exp(-H_{\text{E2}} / kT) \}
\]

\[
\sim \text{Tr} \{ S (1 - \frac{\mu_B g_s S_z B_0}{kT}) \} / \text{Tr} \{ 1 - \frac{\mu_B g_s S_z B_0}{kT} \}
\]

\[
= \sum_j \langle \xi | (\sum_{jk} e_j S_j) (S_z g_s B_0) | \xi \rangle > \mu_B / ((kT)\text{Tr}(1))
\]

where \( e_j \) is an unit vector along the axis \( j \) (\( j = x, y, z \)) and \( |\xi\rangle \) denotes a basis ket.

\[
\langle S \rangle = \sum_j \sum_{jk} e_j g_s B_0 \langle \xi | S_j S_z | \xi \rangle > \mu_B / (3kT)
\]

\[
= g_s B_0 \{ S(S+1) \} \mu_B / (3kT)
\]

[2.15]

In the high field approximation for the parameterized g tensor, \( \langle \mu \rangle \) is given by

\[
\langle \mu \rangle = \mu_B g_s \langle S \rangle
\]

\[
= g_s B_0 \{ S(S+1) \} \mu_B^2 / 3kT = g_s^2 CB_0 / T
\]

[2.16]

Magnetic Moment under Averaging 2

Case 2: A more general case

\[
\langle S \rangle = \text{Tr} \{ S \exp(-H / kT) \} / \text{Tr} \{ \exp(-H / kT) \}
\]

\[
\sim \text{Tr} \{ S (1 - \frac{\mu_B S \cdot g \cdot B_0}{kT}) \} / \text{Tr} \{ 1 - \frac{\mu_B S \cdot g \cdot B_0}{kT} \}
\]

\[
= \sum_j \langle \xi | (\sum_{jk} e_j S_j) (S \cdot g \cdot B_0) | \xi \rangle > \mu_B / ((kT)\text{Tr}(1))
\]

where \( e_j \) is an unit vector along the axis \( j \) (\( j = x, y, z \)) and \( |\xi\rangle \) denotes a basis ket.

\[
\langle S \rangle = \sum_j \sum_{jk} e_j g_s B_0 \langle \xi | S \cdot S_n S_z | \xi \rangle > \mu_B / (kT)\text{Tr}(1)
\]

\[
= g \cdot B_0 \{ S(S+1) / 3 \} \mu_B / (kT)
\]

[2.17]

In the high field approximation for the parameterized g tensor, \( \langle \mu \rangle \) is given by

\[
\langle \mu \rangle = \mu_B g_s \langle S \rangle
\]

\[
= g \cdot g \cdot B_0 \{ S(S+1) \} \mu_B^2 / (3kT)
\]

[2.18]
Susceptibility Tensor

The susceptibility tensor $\chi$ is defined by

$$<\mu> = \chi \frac{B_0}{\mu_0}.$$ \[2.19\]

From [2.18, 19], we obtain

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

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

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

= \frac{C}{T\mu_0} g_{kk}^2,$n

where $\chi_{kk}$ and $g_{kk}$ denote principal values for $\chi$ and $g$.

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Calculation of Thermally Averaged Contact Shift & Dipolar Shift

Case 1: g-anitoropy neglected

- $\delta_{\text{CON}} = A <S_Z>$
- $\delta_{\text{PC}} = D(\theta) <S_Z>$

\[2.22\]

Isotropic shift

$\rightarrow$ NOT Removable by MAS

\[2.23\]

Anisotropic shift

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

$\rightarrow$ Removable by MAS
Anisotropic Shift for Paramagnetic Systems in $^1$H VF-MAS NMR

Cu(II)(DL-Ala)$_2$

$S = 1/2$

Mn(III)(acac)$_3$

$S = 2$

$\delta_{\text{dipolar}} \propto S(S+1)/R^3$

Q. What is $R$?

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

$2(1+2) = 6$

Thermally Averaged Hyperfine Shifts

Case 2: g-anisotropy NOT neglected

$\delta_{\text{CON}} = A< S_z > I_z$

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

This is actually anisotropic

$= \frac{A B_0 C}{\mu_B T} \{ g_{xx} \sin^2 \beta + g_{yy} \cos^2 \beta \sin^2 \alpha + g_{zz} \cos^2 \beta \cos^2 \alpha \}$

[2.24]

$\delta_{\text{PC}} = (\langle \mu >/g_e \cdot \mathbf{D})_z I_z$

$= (C / g_e T)(B_0 \cdot \mathbf{g} \cdot \mathbf{g} \cdot \mathbf{D})_z I_z$

[2.25]

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

$\rightarrow$ 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-anisotropy NOT neglected

By averaging the diagonal elements of the tensors \( ACg/T \) and \((C/g_e T)g\cdot g\cdot D\), we obtain the isotropic shifts for contact and dipolar shifts as follows:

\[
<\delta_{\text{CON}}>=\frac{AB_0C}{\mu gT}\{g_{xx}+g_{yy}+g_{zz}\}/3 \quad [2.26]
\]

\[
<\delta_{\text{PC}}>=\frac{B_0Cd}{g_{gR}^2T}\left\{\left(\frac{g_{xx}^2+g_{yy}^2}{2}\right)-\frac{1}{3}\cos^2\eta \right\}
+\left(\frac{g_{xx}^2-g_{yy}^2}{6}\right)\sin^2\eta\cos2\phi \quad [2.27]
\]

Distance Information from Isotropic PC Shifts

\[
<\delta_{\text{PC}}>=\frac{B_0Cd}{g_{gR}T^3}\left\{\left(\frac{g_{xx}^2+g_{yy}^2}{2}\right)-\frac{1}{3}\cos^2\eta \right\}
+\left(\frac{g_{xx}^2-g_{yy}^2}{6}\right)\sin^2\eta\cos2\phi \quad [2.27]
\]

where \(\eta\) and \(\phi\) 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.

→ \(R, \eta, \phi\) can be fitting parameters for structural studies!

Yesnowski et al JCP 89, 4600 (1988)
SSNMR of Paramagnetic Metallo-proteins

*Structural Information from Pseudo-contact Shifts*

Application to selectively $^{13}\text{C}-\text{Leu}, ^{15}\text{N}-\text{Phe},$ Gly labeled P450 BM-3 protein

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

Relaxation Properties

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

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

- We define the correlation time of the electron spin state $\tau_S$ as
  
  \[ C(t) = \langle S_z(t)S_z(0) \rangle = \langle S_z(0)^2 \rangle \exp(-|t|/\tau_S) \]  
  [2.28]

$\tau_S$ 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).

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

Paramagnetic Relaxation in Solution

- Two type of relaxation exists in solution:
  
  Curie relaxation & Solomon relaxation (see the reference below for $R_2$).

\[
R_{1,\text{Curie}} = \frac{2S(S+1)}{15} \left( \frac{\mu_0 B_f \gamma_S}{4\pi R^3} \right)^2 \left( \frac{\tau_C}{1 + (\omega_1 - \omega_2)^2 \tau_C} \right) + \frac{6\tau_C}{1 + (\omega_1 + \omega_2)^2 \tau_C^2} \]

\[
R_{1,\text{Sol}} = \frac{S(S+1)}{3} \left( \frac{A}{R} \right)^2 \left( \frac{\tau_C}{1 + \omega_2^2 \tau_C^2} \right) + \frac{3\tau_C}{1 + (\omega_1 + \omega_2)^2 \tau_C^2} \]

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

In solids, $\tau_r \sim \infty \rightarrow R_{1,\text{Curie}} \sim 0$

& $\tau_C \sim \tau_S$.

Dominant term in solids when $\tau_C > 1/\omega_2 \sim 10^{-12}$

Bertini “Solution NMR of paramagnetic molecules”
Emsley JACS 129 14118 (2007)
**Structural Info from Paramagnetic $R_1$**

- $R_1^{SL} \propto \frac{1}{R^6} \rightarrow$ Distance information

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

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

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**$R_2$ Paramagnetic Relaxation**

*Solomon relaxation*

$$R_2^{SL} = \frac{S(S+1)}{15} \left( \frac{\mu_0 n_f \gamma I}{4aR^2} \right)^2 \left\{ \frac{4\tau_C}{1 + (\omega_1 - \omega_2)^2 \tau_C^2} + \frac{3\tau_C}{1 + \omega_5 \tau_C^2} \right\} + \frac{S(S+1)}{3} \left( \frac{A}{\hbar} \right)^2 \left\{ \frac{\tau_C}{1 + \omega_5 \tau_C^2} + \frac{2\tau_C}{1 + (\omega_1 + \omega_2)^2 \tau_C^2} \right\}$$

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

$R_2^{SL} \propto \gamma_1^2$

$\rightarrow$ Even if $^1$H Signals are very broad, $^{13}$C, $^{15}$N signals may be observable.
Sec. 3 Examples & Applications

3.1 Small Paramagnetic Systems
   3.1.1 Moderate MAS
   3.1.2 Very-Fast MAS
   3.1.3 Structural Information

3.2 Paramagnetic Proteins
   3.2.1 Structural Information

3.3 Non-Paramagnetic Proteins → Talk on Thursday
   3.3.1 Examples
   3.3.2 Structural Information
   3.3.3 Sensitivity Enhancement

1H & 13C High Resolution Paramagnetic SSNMR

Problems: Paramagnetic Shifts Are Large (1H & 13C)

- Fundamental RF methods fail (1H-1H or 1H decoupling, CP)
  - 2D labeling (Dobson et al. 1990; Oldfield et al.)
  - Resolution under MAS at ~10 kHz in a few cases (1H Yesinowski et al. 1988; 13C McDermott et al. 1995, 13C Kohler et al. 2001)
    → Labeling required & Limited sensitivity/resolution
- Numerous sidebands due to large anisotropic shifts
- Assignments are difficult
  → Selective 2D or 13C-labeling required
- Sophisticated experiments rarely attempted (2D, Distance)
  - 2D 13C/13C correlation (Terao et al. 1999; Emsley et al. 2000)
  - 13C labeled samples even for small molecules

Very Fast Magic Angle (VFMAS) Changes the Situations!
**13C High Resolution Paramagnetic SSNMR**

**Problems.** Paramagnetic Shifts Are Large (1H & 13C)

- 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)

13C MAS at 5 kHz

MAS 24 kHz + No Decoupling
1 min (13k scans)

**• Ishii et al. JACS 125, 3438-3439 (2003)**
$^{13}$C VFMAS Spectrum with Dipolar INEPT for Mn(acac)$_3$

MAS: 26.3 kHz

Dipolar INEPT Pulse Sequence

Frydman et al JMR (2001)
Ishii et al. JMR (2006)
Assignment using Dipolar INEPT

Effective transfer-time ($\tau$) dependence of signal intensities

Distance Measurements for Cu(Ala-Thr)

With CP (ct = 0.5 ms)

Exp Time ~ 30 hours

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

♦ Pseudo-Contact (Anisotropic) Shift
\[ \Delta \equiv |\sigma_{11} - \sigma_{\text{iso}}| = cS(S+1)/R^3 \]
♦ Paramagnetic Relaxation Time: \( T_1 \)
\[ \frac{1}{T_1} = kS(S+1)\tau_S / \left( (1 + \omega_I^2\tau_S^2)R^6 \right) \]

\( R \): Metal-\(^{13}\)C Distance  
\( S \): Electron Spin Number  
\( \tau_S \): Electron Spin Correlation Time  
\( \omega_I \): \(^{13}\)C NMR Frequency  
\( c, k \): Known Constant

\[ \rightarrow \left( \frac{T_1^m}{T_1^n} \right)^{1/6} = \left( \frac{R_m}{R_n} \right) \]

No Labeling Necessary!

Distance Measurements for \( \text{Cu(Ala-Thr)} \)

With CP (ct = 0.5 ms)

\[ \begin{align*}
\text{Cu} & \quad \text{CO}_2 \\
\text{Ala} & \quad \text{Thr}
\end{align*} \]

Exp Time ~ 30 hours

[Wickramasinghe et al JPC B 2007]
**13C VFMAS Characterization of Solid-State Reaction for Cu(II)(8-quinolinol)₂**

Anti-cancer drugs for leukemia

α-Cu(II)Q₂  β-Cu(II)Q₂

210°C/2 hours

What is the origin of the difference?

(a) α-form  (b) β-form

M. Shaibat, Y. Ishii et al JACS (2007)
Collaboration with Dr. A. de Dios & L Casabianca at Georgetown
$^1$H MAS spectra of Cu(DL-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$_3$

(b) 10 kHz
(c) 5 kHz → Numerous Sidebands!

Ishii et al. JACS 2005

High-Sensitivity $^1$H 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]
**13C SSNMR CPMAS Spectra of Protein Microcrystals at 40 kHz MAS**

#### Sensitivity

**Lysozyme**

- **x 2.9**
- 10 mM Cu-EDTA
- Without Cu-EDTA

**Ubiquitin**

- **x 2.7**
- 10 mM Cu-EDTA
- Without Cu-EDTA

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*

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

McDermott et al. JACS 127, 13816 (2005)

Bertini et al. JACS 129, 2219 (2007)

\[
\delta_{PC} = \frac{1}{12\tau^2}(0.5 + 1.5 \cos 2\theta)(\chi_\parallel - \chi_\perp)
\]
4. Experimental Aspects

- Temperature Dependence of Shifts
  \[ \text{Line broadening due to temperature distribution} \]

- Choose optimum spinning \((\Delta T \propto 1/T)\)
- Enough VT Air & Optimize Line Shape for Standards (Cu(DL-Ala)₂ & Lead Nitrate)

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**Practical Protocols to Examine Paramagnetic Systems**

1. \(^1\)H VFMAS
   \[ \rightarrow \text{Check Line shape & } ^1H \ T_1 \]

2. \(^{13}\)C Dipolar INEPT (with two \(\tau\) values)
   \[ \rightarrow \text{Line shape & } ^{13}\text{C Assignments} \]

3. \(^{13}\)C 1 pulse & Inversion recovery
   \[ \rightarrow ^{13}\text{C } T_1 \text{ (Distances)} \]

4. 2D \(^{13}\)C/\(^1\)H correlation \(\rightarrow ^1\text{H Assignments} \)
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.