Spin Echo Dephasing

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General Comments Concerning Relaxation

Every person who has put a sample into an EPR spectrometer has performed a relaxation time experiment.
You assume that the sample comes to equilibrium by the time that you start collecting data.
A wise spectroscopist checks that the microwave power is selected to be in the linear response regime.

Any process that takes a spin from one resonant field to another may contribute to a measured relaxation curve.
A true T_1 requires coupling of electron spins to lattice thermal energies.

What can you learn by measuring spin echo dephasing in solids?

- Feasibility of doing pulse experiments that depend upon echo detection
- Local spin concentration
- Librational motion
- Proton spin concentration
- Methyl group types and concentrations
- Dynamic processes that average inequivalent nuclei
- Enhancement of echo dephasing by neighboring fast relaxing spin

A Spins and B Spins

A spins   B spins

Excited spins

time
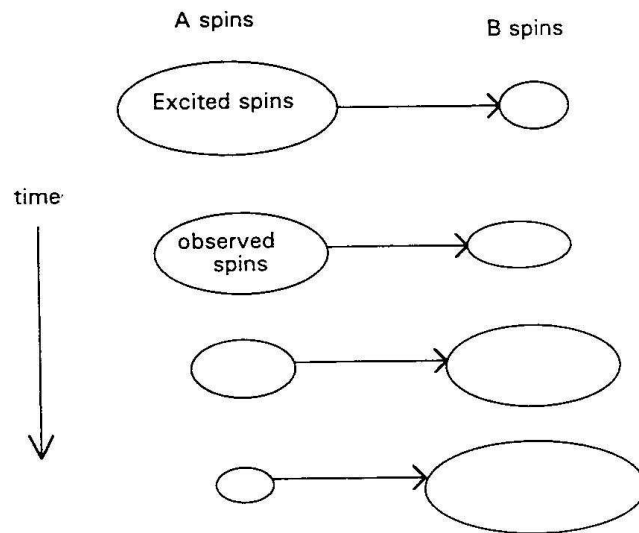
observed spins

The spins that are excited in an experiment are called the A spins. All other spins are designated as B spins. As a function of time A spins become B spins due to spectral diffusion, which decreases the number of A spins that can be observed.

Factors to consider in designing an experiment to measure dephasing

Want low resonator Q to minimize dead time

$$Q = 2\pi\nu\tau$$

where ν is microwave frequency

τ is the cavity ring-down time

To decrease excitation of echo modulation use longer pulses such as 40 and 80 ns

To decrease the effects of instantaneous diffusion use turning angles less than 90°

Measure temperature dependence to characterize dynamic processes.

Analysis of Spin Echo Decays

1. Fit to a stretched exponential

$$E(2\tau) = \exp\left[-\left(\frac{2\tau}{T_m}\right)^x\right]$$ Eq. (1)

τ is the time between pulses

T_m is the dephasing time constant

x   is a phenomenological parameter that depends
     on dephasing mechanism
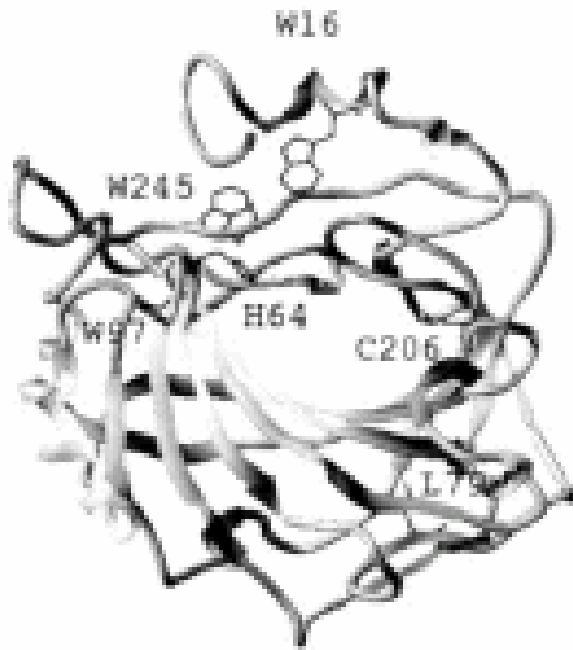   Values of x are between 0.5 and about 2.5.

For nuclear spin diffusion, $x > 2$
For a process that averages inequivalent environments, x varies from ~2
to 0.5 to 1 as the rate of the process increases.
For instantaneous diffusion $x = 1$
If dephasing is T_1 driven, $x = 1$

# Locations of Spin Labels on Human Carbonic Anhydrase II

The single native cysteine in HCAII was mutated to serine. Cysteines at the sites selected for spin labeling were introduced by site-directed mutagenesis.

M. Huber, M. Lindgren, P. Hammarstrom, L.-G. Martensson, U. Carlsson, G. R. Eaton, and S. S. Eaton, *Biophys. Chem.* **94**, 245 (2001).
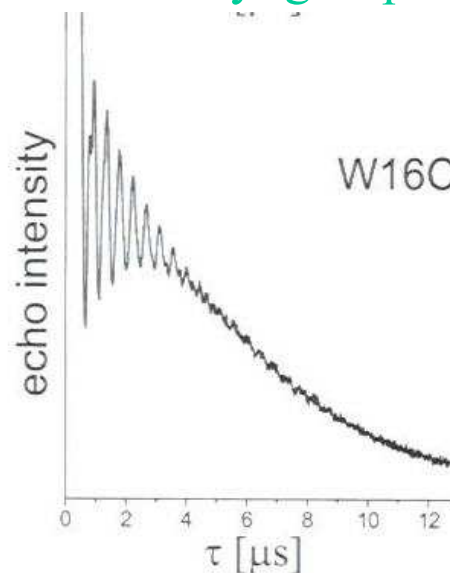
Comparison of Ways to Account for Modulation

Example: deuterium modulation in echo decays for spin-labeled carbonic anhydrase in deuterated solvent

Buried spin label, near many methyl groups.
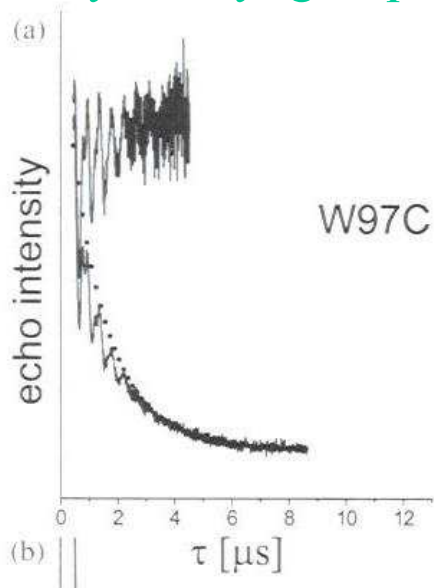
Surface spin label, near few methyl groups.

Decay obtained by fitting peaks was subtracted from data.

Spin-labeled HCA II in Deuterated Solvent

Method 1: Fit eq. (1) to maxima in echo decay. This gives shorter T_m and smaller x.

Method 2: Divide experimental data by simplified modulation function and fit eq. (1) to the resulting decay. A more accurate modulation function should give the most accurate results.

Method 3: Fit eq. (1) to experimental data at long τ values

	Method 1		Method 2		Method 3	
	T_M [μs]	x	T_M [μs]	x	T_M [μs]	x
L79C	1.4	1.0				
W97C	2.4	0.9	2.7	0.9		
C206	4.2	1.0	6.0	1.0	7.4	1.2
F176C	4.3	0.9	5.8	0.9	8.8	1.5
W245C	6.0	1.0	9.0	1.6	9.6	1.8
W16C	9.1	0.9	12.6	1.3	15.4	1.6

M. Huber, M. Lindgren, P. Hammarstrom, L.-G. Martensson, U. Carlsson, G. R. Eaton, and S. S. Eaton, *Biophys. Chem.* **94**, 245 (2001).
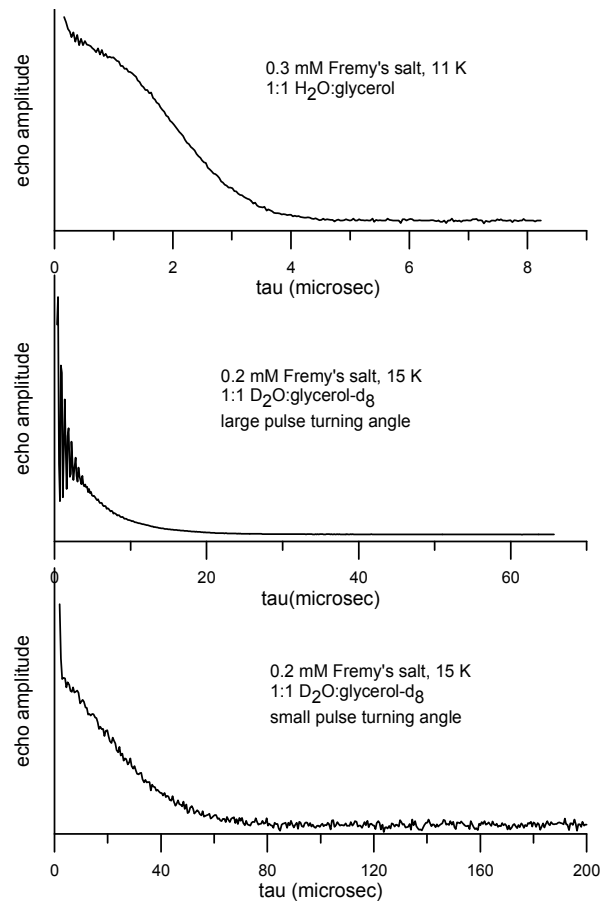
Analysis of Spin Echo Decays

2. Fit to a model appropriate for a particular dephasing mechanism

   a. Protons or other nuclear spins that contribute to nuclear spin diffusion
   b. Dynamic averaging of inequivalent nuclei
   c. Dipolar interaction with rapidly relaxing metal

Instantaneous Diffusion

•A spin contributes to two-pulse echo formation if the precession frequency for the spin remains constant during the time 2τ.

•If an event changes the precession frequency of a spin during the interval 2τ, then the second pulse does not exactly reverse the precession that occurred during the first time interval τ and the spin does not contribute to echo formation.

•If the second pulse flips both the spin that we are observing **and** a neighboring spin that is dipolar coupled to the observed spin, then the pulse changes the field at the observed (A) spin and hence the precession frequency of the A spin and causes it to not contribute to the echo. This effect is called instantaneous diffusion.

•Instantaneous diffusion increases with spin concentration and with the pulse turning angle and for the same spin concentration is more important for narrower spectra.

K. M. Salikhov and Yu. D. Tsvetkov in *Time Domain Electron Spin Resonance,* L. Kevan and R. N. Swartz, eds., Wiley, N.Y., 1979, ch. 6.

Instantaneous Diffusion

0.3 mM Fremy's salt, 11 K
1:1 H_2O:glycerol

0.2 mM Fremy's salt, 15 K
1:1 D_2O:glycerol-d_8
large pulse turning angle

0.2 mM Fremy's salt, 15 K
1:1 D_2O:glycerol-d_8
small pulse turning angle

Protons in the solvent
dominate dephasing

When protons are replaced
by deuterons, instantaneous
diffusion makes a larger
contribution.

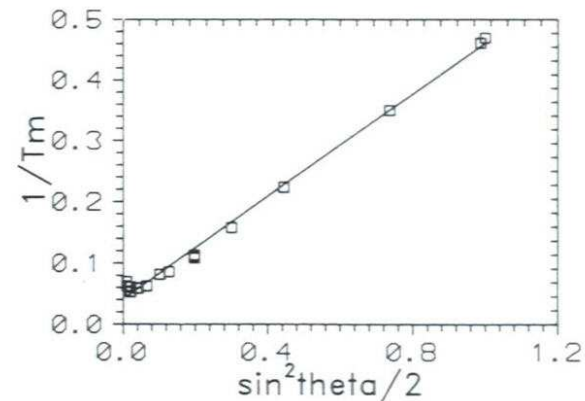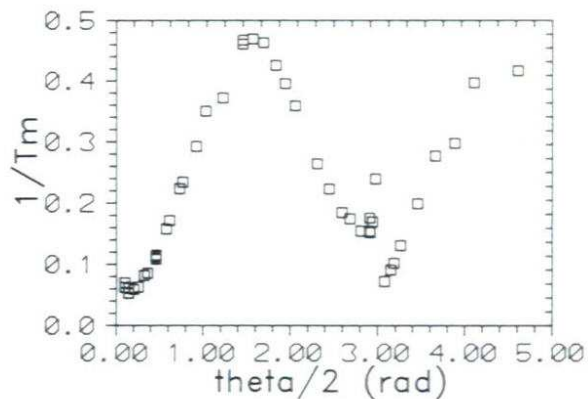Instantaneous diffusion is
decreased by making turning
angle smaller.

Note the difference in x-axis
scales for the 3 plots.

S. S. Eaton and G. R. Eaton, *Biol. Magn. Reson.* **19**, 29 (2000).

Instantaneous Diffusion

E' center in irradiated SiO_2 at room temperature

$$E(2\tau) = (\text{cons}\tan t)\exp(-2b\tau)$$

$$b = \frac{1}{T_m} + \gamma\left(\frac{4\pi^2 g\beta C}{9\sqrt{3}}\right)\sin^2\left(\frac{\theta}{2}\right)$$

τ is the time between pulses

$\theta/2$ is the turning angle for the first pulse

C is [spin] in spins/cm³

S. S. Eaton and G. R. Eaton, *J. Magn. Reson. A* **102**, 354-356 (1993).

Electron-electron Dipolar Interaction

In a sample of irradiated glassy SiO_2 the value of $1/T_m$ extrapolated to low turning angle was 25 μs.

Based on the slope of the plot, the spin concentration was ~6×10^{17} spins/cm^3.

Bloembergen et. al (1948) estimated that T_2 due to spin-spin dipolar interaction was given by

$$T_2 = \frac{hr^3}{2\pi\mu^2} \qquad T_2 = \frac{\left(6.626 \times 10^{-27}\, \text{erg sec}\right)\left(118 \times 10^{-8}\, \text{cm}\right)^3}{2(3.1416)\left(9.28 \times 10^{-21}\, \text{erg G}^{-1}\right)^2}$$

μ is the electron magnetic moment
$T_2 = 2 \times 10^{-5}$ sec ($= 20$ μs), which is in reasonable agreement with experiment.

N. Bloembergen, E. M. Purcell, R. V. Pound, *Phys. Rev.* **73**, 679 (1948).

Nuclear Spin Diffusion

Frequently, an unpaired electron is dipolar coupled to many surrounding nuclear spins.

If one of these nuclear spins flips, the dipolar coupling to the unpaired electron is changed, which changes the precession frequency for the unpaired electron.

Although the spin flip rate for an individual nuclear spin is relatively slow, the probability of some nuclear spin flipping is large, because of the large number of nuclear spins in typical organic materials.

The most common nuclear spin flip process is a flip-flop, $I_{1+}I_{2-}$.

The rate of this process increases proportional to the nuclear spin concentration.

Dipolar coupling is proportional to γ so proton spin diffusion is a more effective dephasing process than deuteron spin diffusion.

A. Zecevic, G. R. Eaton, S. S. Eaton, and M. Lindgren, *Mol. Phys*. **95**, 1255 (1998).
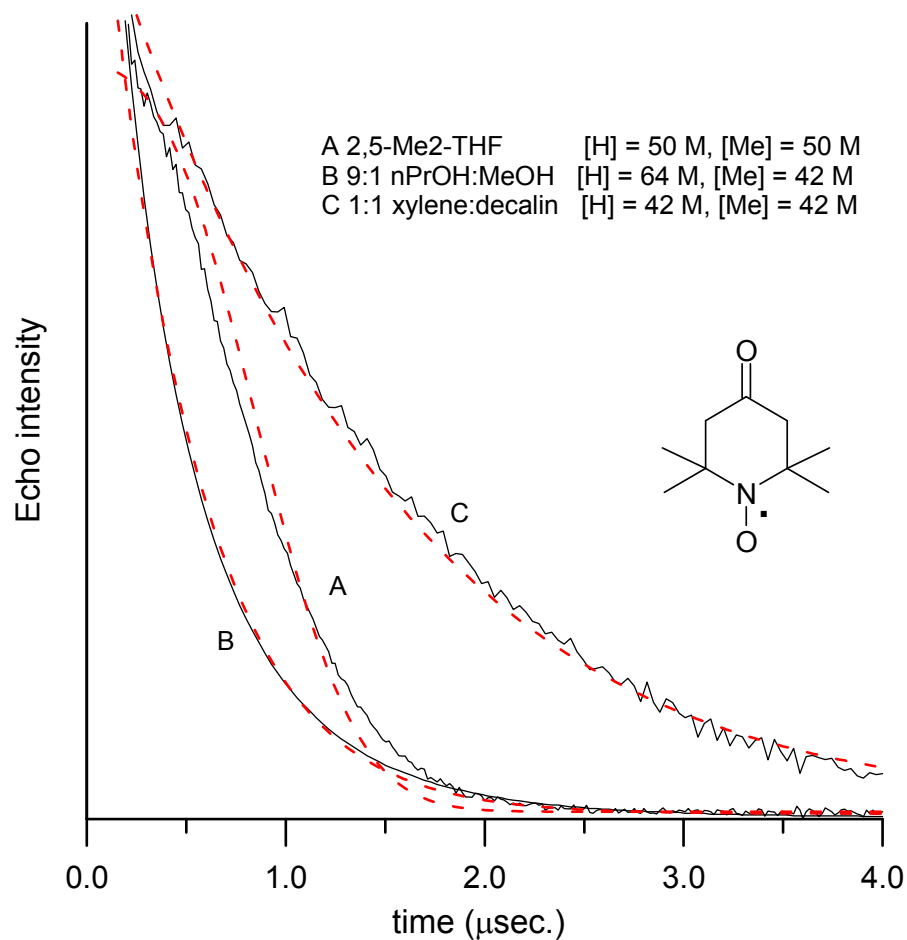
Nuclear Spin Diffusion
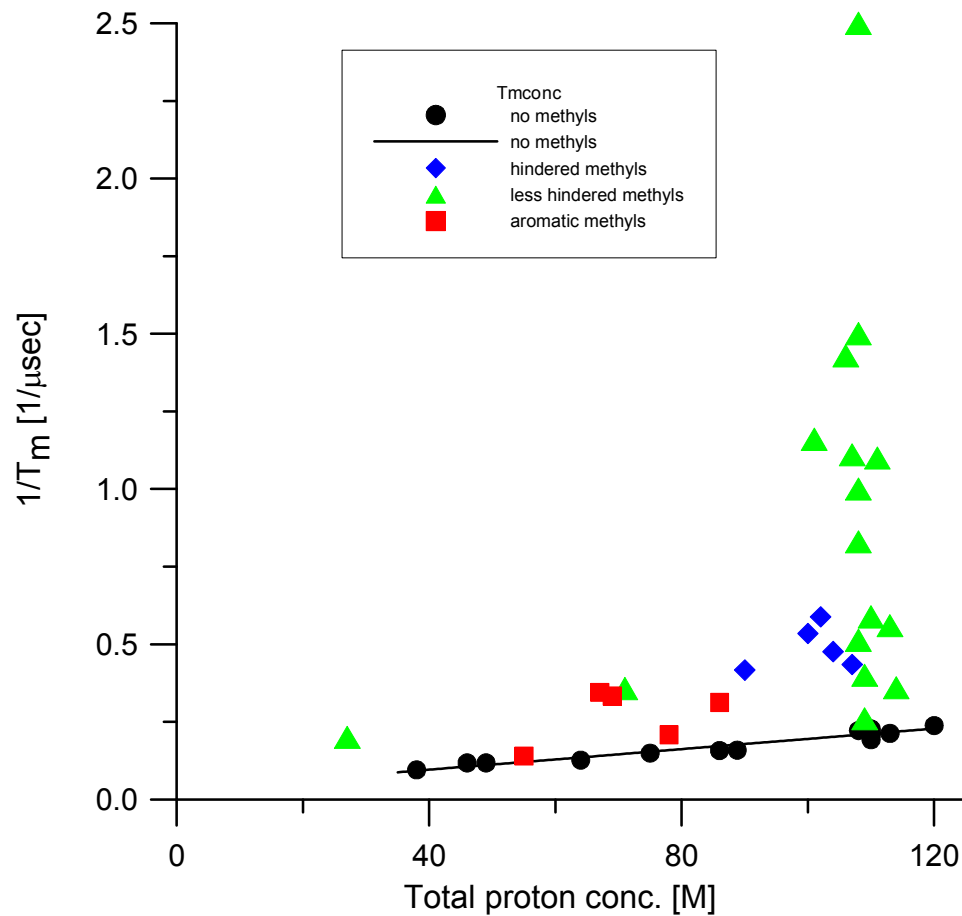
In the absence of methyl groups, $1/T_m$ increases monotonically with proton concentration.

The effects of methyl groups on dephasing depend upon the type of methyl group.

Effects of Methyl Groups

A 2,5-Me2-THF   [H] = 50 M, [Me] = 50 M
B 9:1 nPrOH:MeOH   [H] = 64 M, [Me] = 42 M
C 1:1 xylene:decalin   [H] = 42 M, [Me] = 42 M

Echo intensity

C

A

B

0.0   1.0   2.0   3.0   4.0

time (μsec.)

Echo decay curves are
substantially different
for tempone in these 3
solvents with approximately
the same concentration of
methyl protons.
Because the barrier to
rotation is lower, the
aromatic methyl groups
in xylene are less effective
in echo dephasing than the
methyls in n-propanol or
2,5-Me$_2$-THF.

T_M of Spin Labeled HCA II at 40 K and Comparison with Information Concerning Probe Location

residue		L79C	W97C	C206	F176C	W245C	W16C
location		buried	buried	buried	at/near surface	surface/ intermed	surface
Probe mobility, CW, RT		rigid	rigid	rigid	mobile	rigid/ mobile	mobile
number of CH_3 in 5 to 10 Å shell		24	15	11	13	6	7
T_M [μs] in H_2O/glycerol		1.6	2.7	3.8	3.7	4.7	4.3

Spin Lattice Relaxation
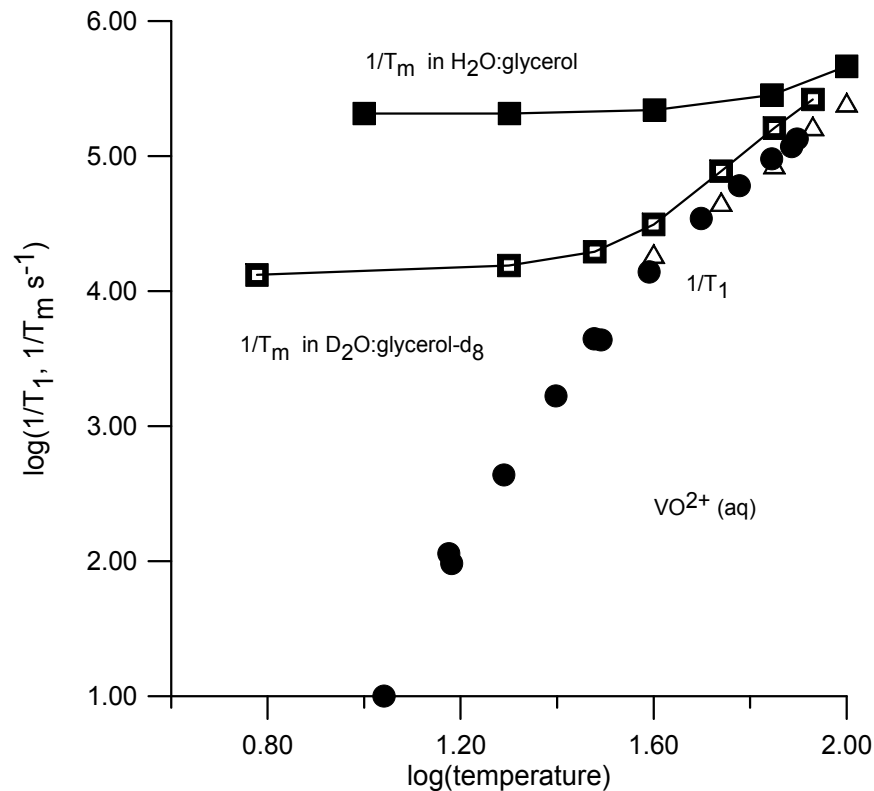
As temperature increases, $1/T_1$ increases and eventually becomes the dominant contribution to $1/T_{m.}$. The contribution from nuclear spin diffusion is smaller in deuterated solvent so $1/T_1$ dominates echo dephasing at lower temperature in deuterated solvent than in natural abundance solvent.
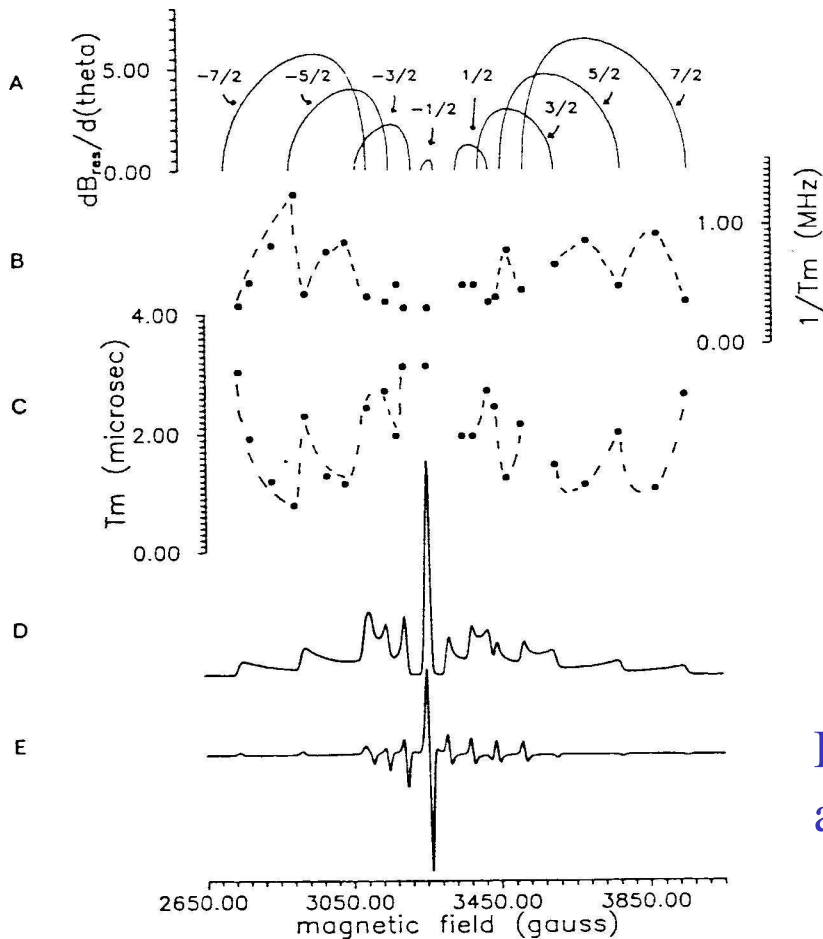
●   Saturation recovery

Δ   Inversion recovery

Vanadyl ion in 1:1 water:glycerol

G. R. Eaton and S. S. Eaton, *J. Magn. Reson.* **136**, 63 (1999).

Librations and Anisotropy in T_m

Vanadyl porphyrin in 9:1 toluene:THF

The change in resonance field per degree of rotational reorientation is largest at intermediate orientations of the molecule.

$1/T_m$ is largest at intermediate orientations and smallest along the principal axes.

Field-swept echo detected spectrum at 50 K

First-derivative CW spectrum at 20 K

J.-L. Du, K. M. More, S. S. Eaton, G. R. Eaton, Isr. J. Chem. 32, 351 (1992).

Dephasing due to averaging of equivalent couplings

When rate of averaging is slow relative to inequivalence
     CW spectrum shows inequivalent resonances
     T_m is long
When rate of averaging is comparable to inequivalence
     CW spectrum shows one broad signal
     T_m is short
When rate of averaging is fast relative to inequivalence
     CW spectrum shows averaged resonance
 T_m is long

Methyl Group Rotation – Nitroxyls

Two-pulse spin echo decays in 1:1 water:glycerol.
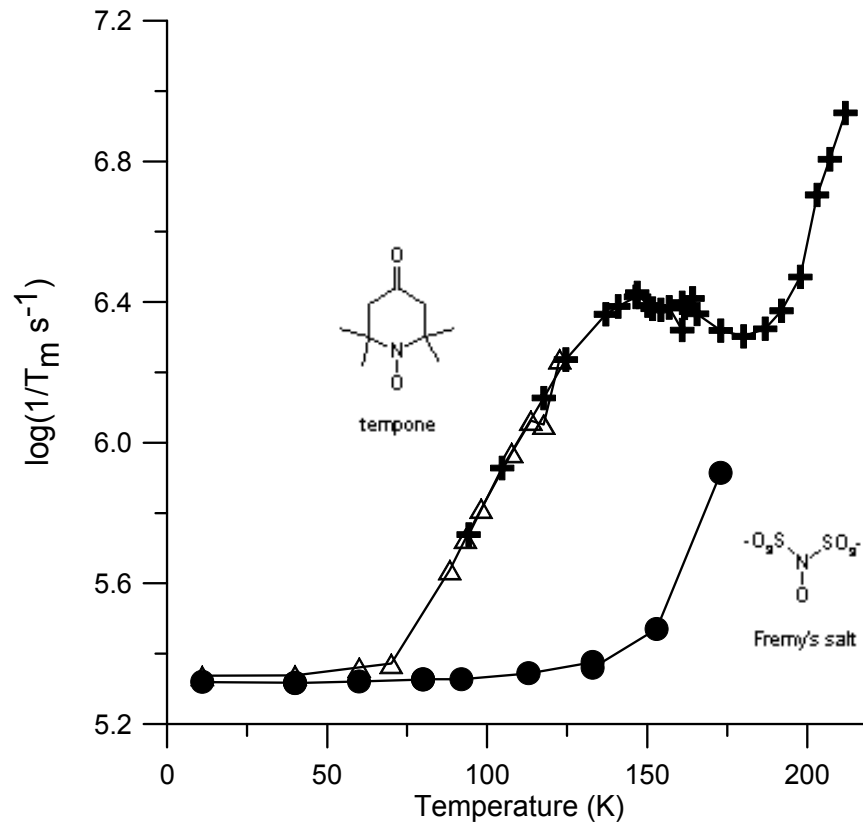
$1/T_m$ for Fremy's salt is approximately independent of temperature below about 120 K.
The temperature dependence of $1/T_m$ for tempone is due to rotation of the ring methyl groups at rates comparable to the difference between the hyperfine splittings that are averaged by the rotation. The hyperfine coupling to the methyl protons is too small to resolve in CW lineshapes. The activation energy is 2.0 kcal/mole.

K. Nakagawa, M. B. Candelaria, W. W. C. Chik, S. S. Eaton, and G. R. Eaton *J. Magn. Reson.* **98**, 81 (1992).

Methyl Group Rotation – Cu^{2+} Complexes
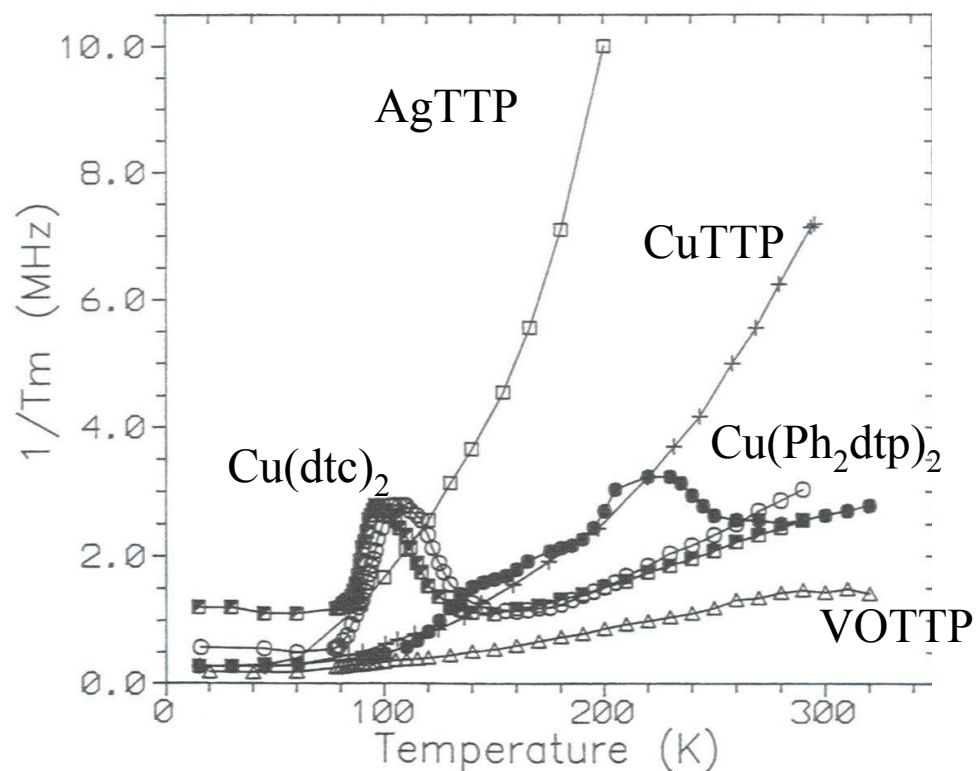
The effects of methyl rotation at a rate comparable to differences between the hyperfine interactions can also seen in Cu^{2+} complexes such as bis(diethyldithiocarbamato)-copper, $Cu(dtc)_2$.

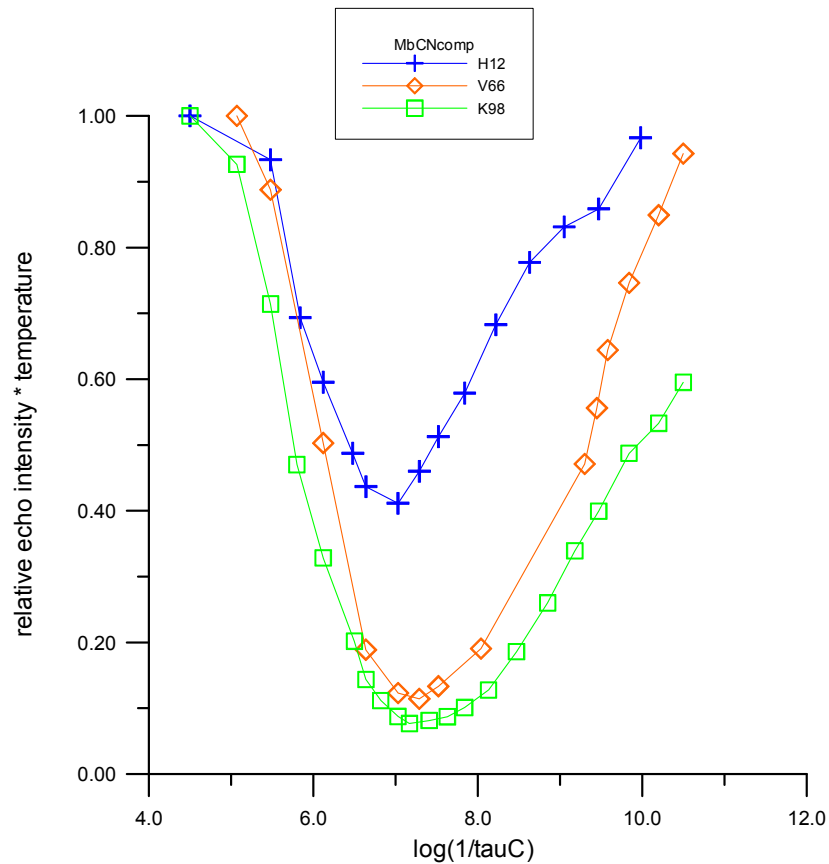J.-L. Du, G. R. Eaton, and S. S. Eaton, *Appl. Magn. Reson*. **6**, 373 (1994).

Interaction with Low Spin Fe(III) - Calculated
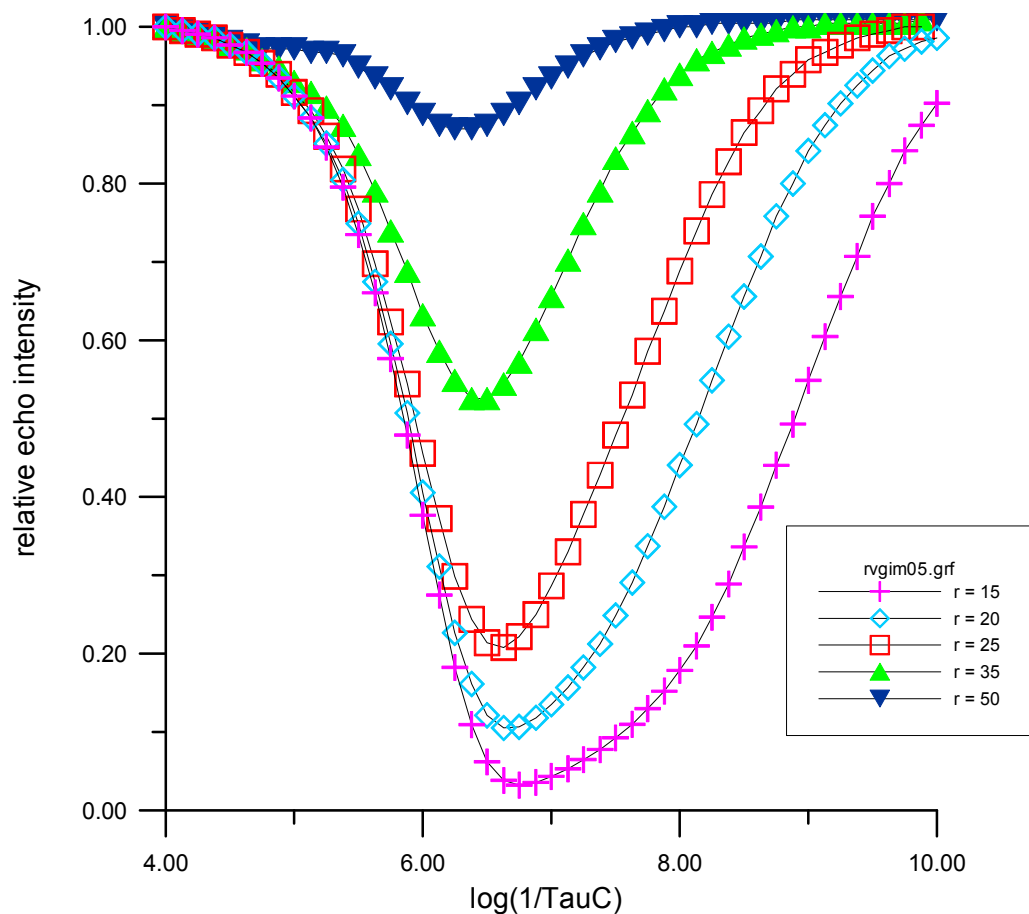
Relative echo intensity calculated for a nitroxyl spin label interacting with low-spin met myoglobin Fe(III)-imidazole at $\tau = 500$ ns for a range interspin distances.
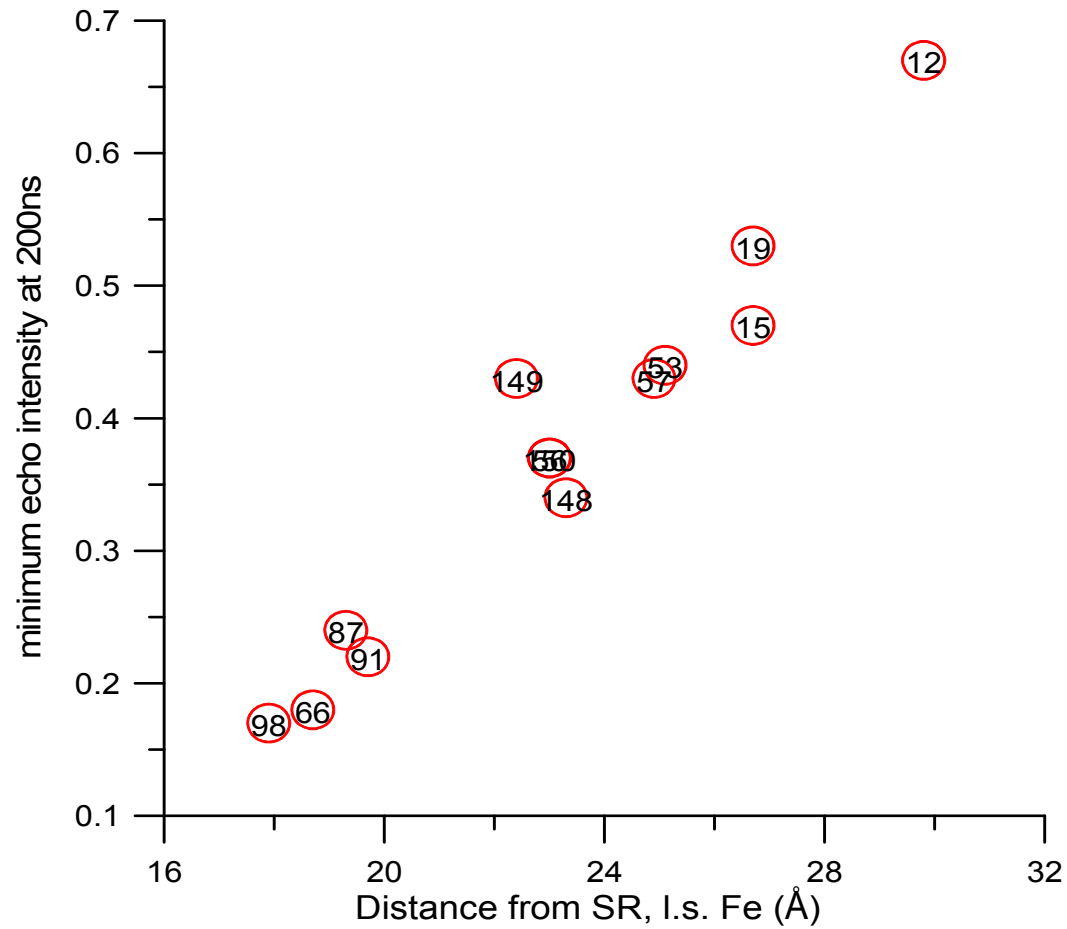
Interaction with Low Spin Fe(III) – Experimental

Relative intensity of two-pulse spin echoes for three spin-labeled variants of metmyoglobin.

Values of Fe(III) relaxation rates as a function of temperature were used to estimate the τ_C.

Correlation between minimum echo intensity and Fe-nitroxyl interspin distance

Summary of Dynamic Processes

The two-pulse spin echo decay time, T_m, provides information concerning a range of dynamic processes including:

Libration of molecules in glasses
Averaging of inequivalent nuclei
Averaging of dipolar interaction with metal ion

Acknowledgements