



# Electron Spin Relaxation Rates and the Use of Relaxation Enhancement to Measure Interspin Distances

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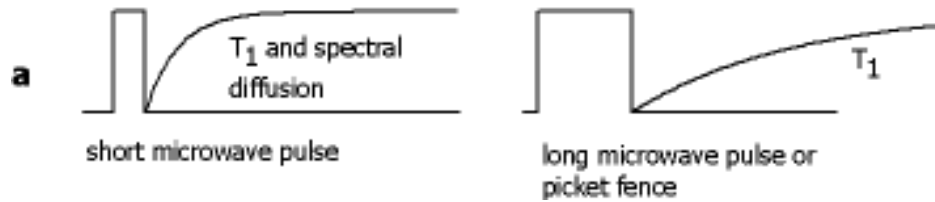


# Spin Lattice Relaxation

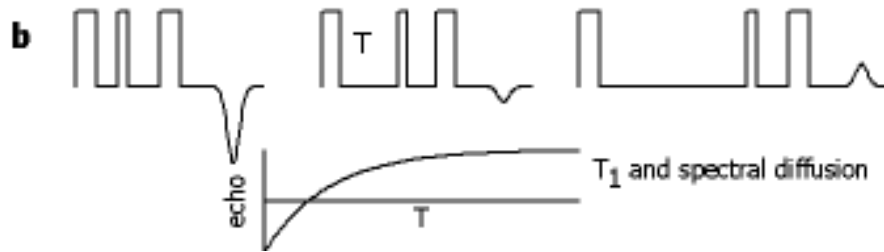
- Nitroxyls in glasses
- Other radicals in glasses
- Nitroxyls – faster tumbling
- Trityls at 20 °C
- Metal ions
  - Cu(II)
  - Vanadyl
  - Fe(III) heme
  - [4Fe-4S]<sup>+</sup>
- Distance measurements

## Methods to Measure Electron Spin Relaxation Times

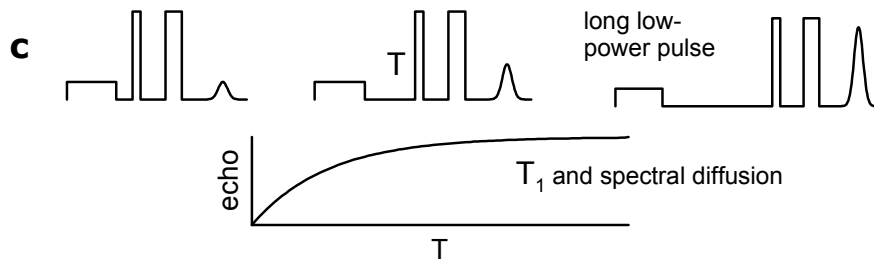
### Saturation Recovery



### Inversion Recovery



### Echo-Detected Saturation Recovery

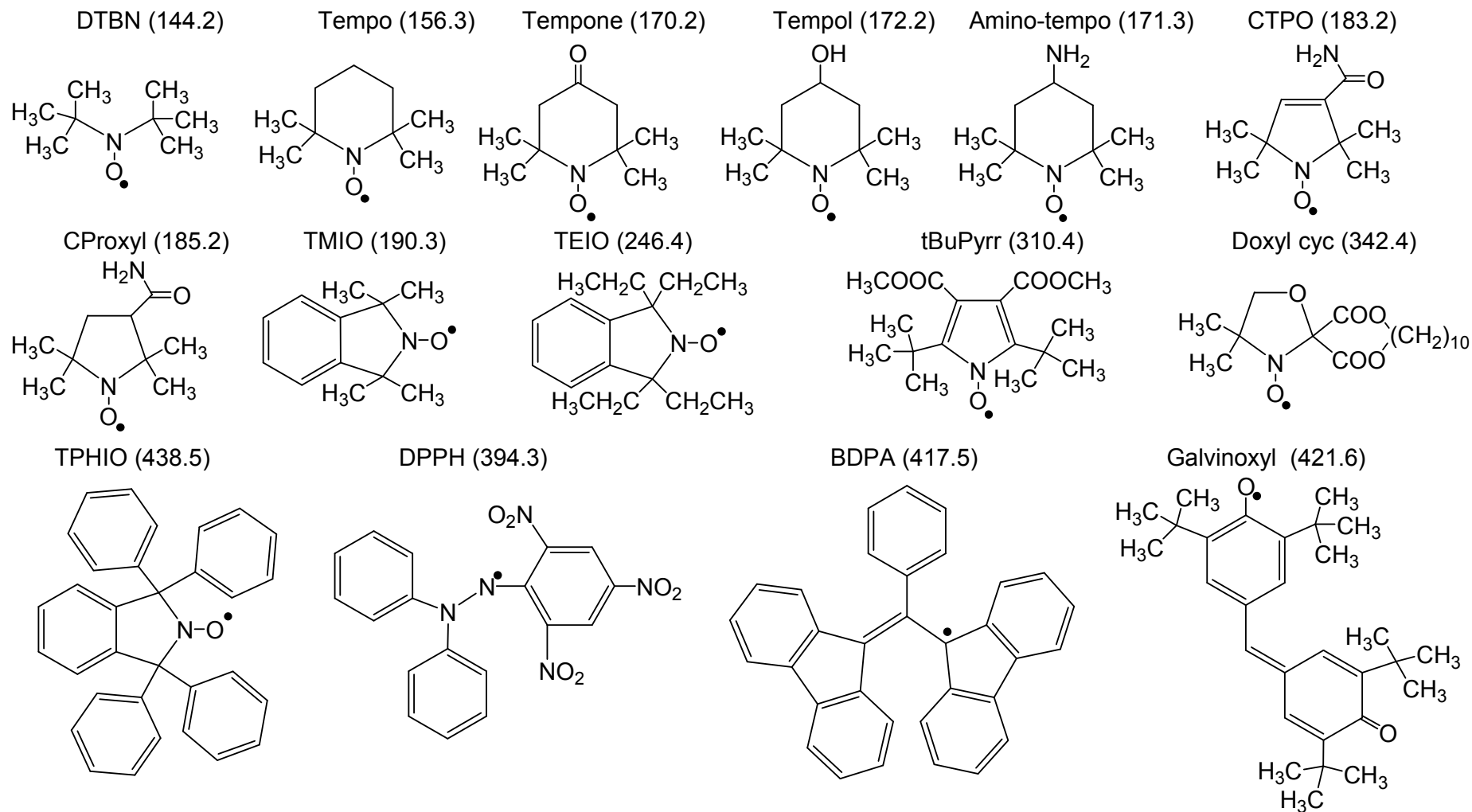


Any process that takes spins off resonance can contribute to a recovery curve.

The key to measuring  $T_{1e}$  is to separate its contribution from those of spectral diffusion processes.

Most EPR spectra are so broad that only a small fraction of the spins are saturated or inverted in an experiment.

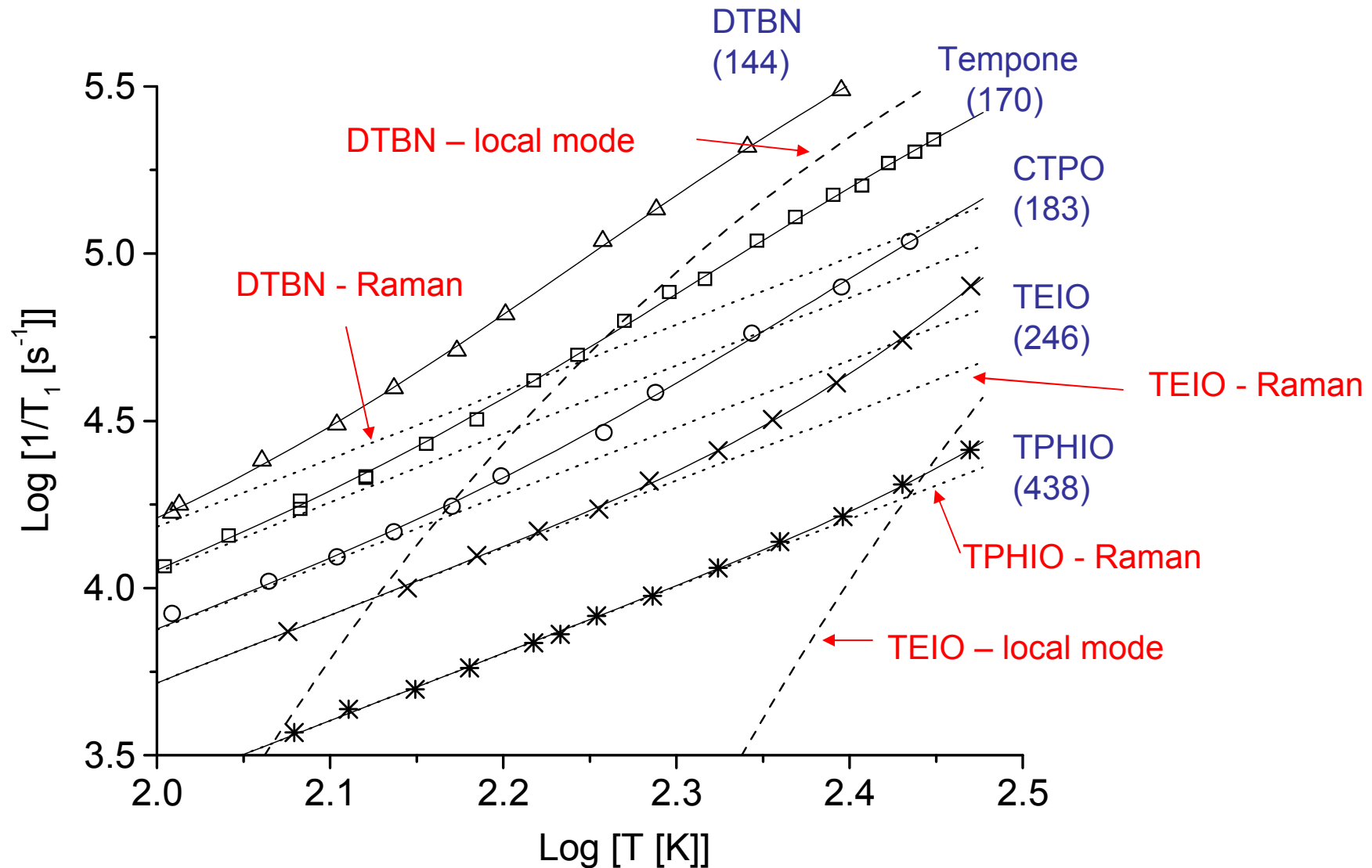
# Radicals Studied in Glassy Solvents



TMIO, TEIO, TPHIO prepared by Steven Bottle and students,  
Queensland University of Technology, Australia

# Nitroxyl Relaxation Rates in Sucrose Octaacetate Glass

Molecular weight in parentheses



In this "high-temperature limit" Raman process is proportional to T<sup>2</sup>

# Volume Scaling

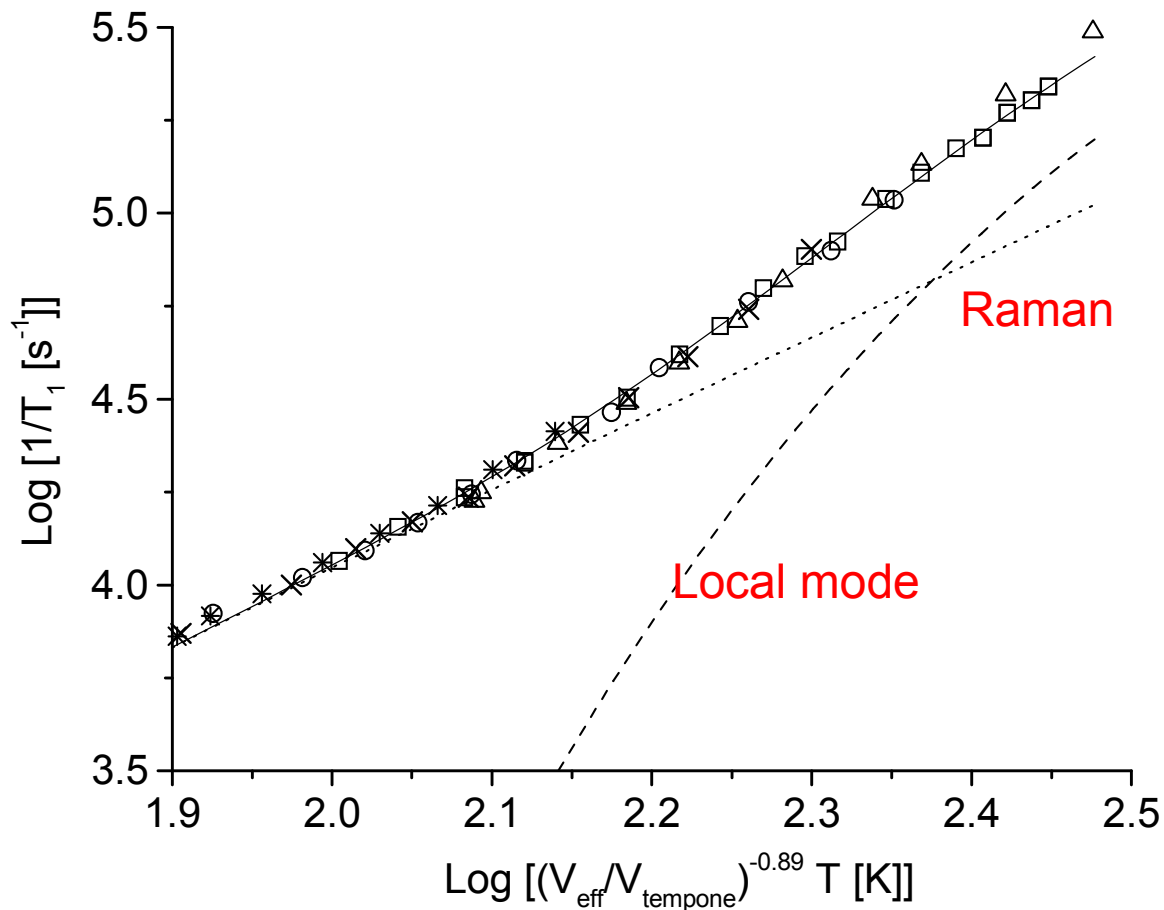
- $(V_{\text{eff}}/V_{\text{tempone}})^{\gamma} = \tau/\tau_{\text{tempone}}$   
 $\tau$  = tumbling correlation time  
 $\gamma = 4.7$  in decalin, 6.0 in mineral oil
- $(V_{\text{eff}}/V_{\text{tempone}})^{\gamma} = a_{\text{libration\_tempone}}/a_{\text{libration}}$   
 $a_{\text{libration}}$  = coefficient of libration process that averages components of the nitrogen hyperfine interaction  
 $\gamma = 3.5$  in sucrose octaacetate
- $(V_{\text{eff}}/V_{\text{tempone}})^{\gamma} = (C_{\text{Ram\_tempone}}/C_{\text{Ram}})^{-0.5}$   
 $1/T_1^{\text{Raman}} = C_{\text{Ram}} T^2$   
 $\gamma = 0.89$  in sucrose octaacetate or sorbitol

## $V_{\text{eff}}$ Derived from Tumbling, Libration, and the Raman Coefficient for Nitroxyls that Tumble Isotropically

Nitroxyl (mw/mw_tempone)	tumbling, decalin	tumbling, mineral oil	libration <sup>a</sup> , sucrose octaacetate	Raman
DTBN (0.85)	0.83	0.87	0.86	0.87
Tempo (0.92)	0.93	0.96	0.97	1.07
CTPO (1.08)			1.24	1.24
TMIO (1.12)	1.32	1.39	1.3	1.3
TEIO (1.45)	1.53	1.48	1.56	1.52
TPHIO (2.57)	2.27	2.17	1.56	1.52

<sup>a</sup> Calculated from the temperature dependence of nitrogen  $A_{zz}$

# Relaxation Rates in Sucrose Octaacetate Scaled by $V_{\text{eff}}$



$V_{\text{eff}}$  determined from tumbling correlation times and libration.

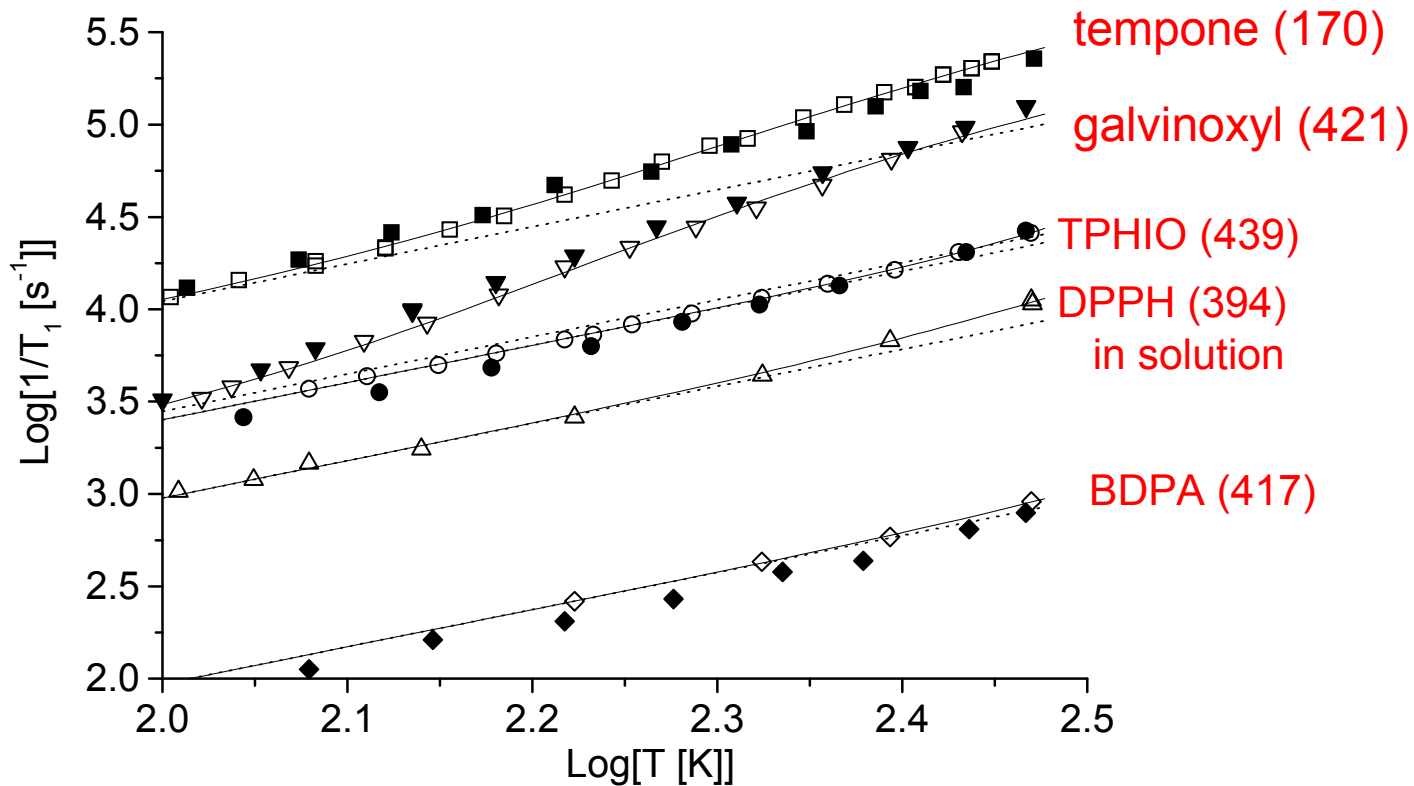
Plot includes points for DTBN, tempone, CTPO, TEIO, and TPHIO

Analogous plot is obtained in H-bonding sorbitol, but all rates are slower.

But:  $T_1$  for spin labels on proteins is similar to free label:  $V_{\text{eff}}$  only includes atoms with significant spin density.



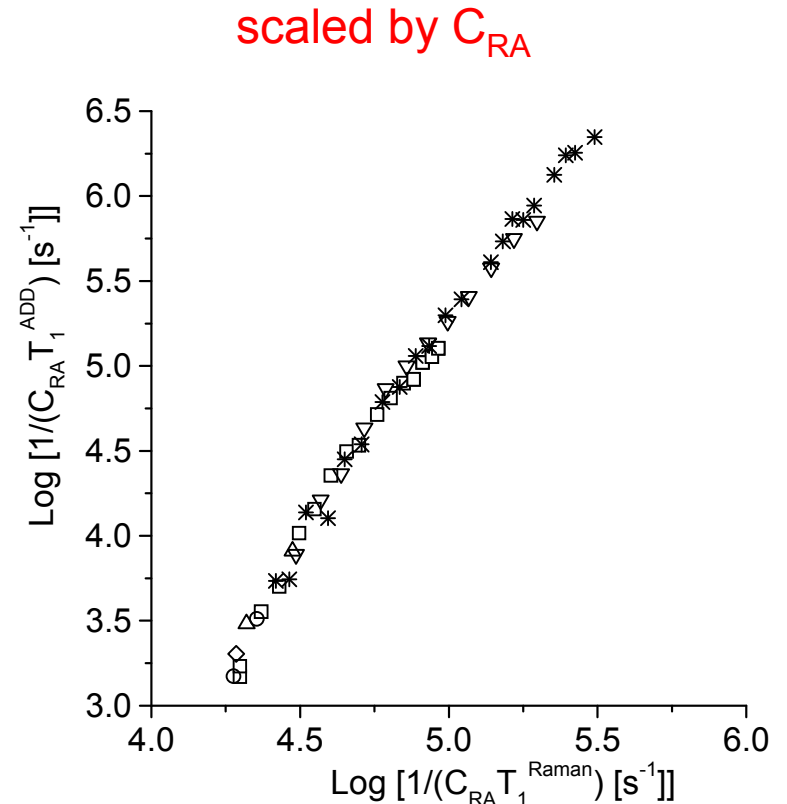
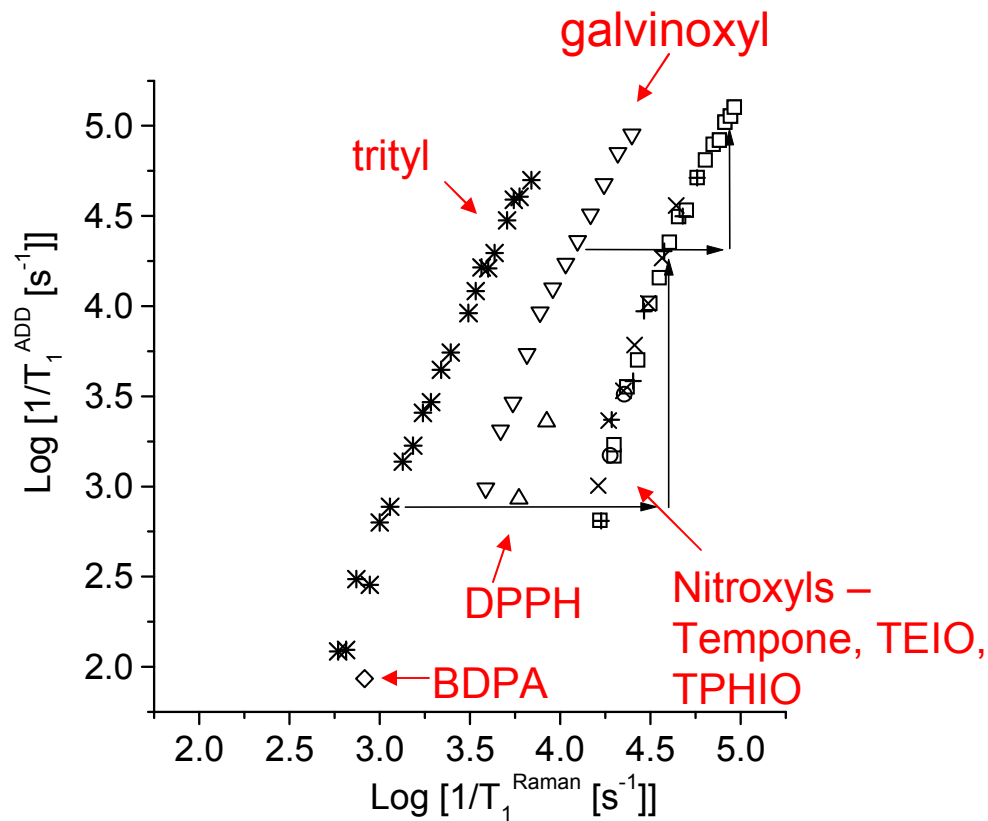
# Radicals in sucrose octaacetate



X-band, open symbols  
Q-band, solid symbols  
Dashed line, Raman process

Tempone and galvinoxyl:  
Raman process plus a large  
contribution from an additional  
process.

# Raman and local mode processes in glasses



$$\frac{1}{T_1^{\text{ADD}}} = \frac{1}{T_1} - \frac{1}{T_1^{\text{Raman}}}$$

Multiply both  $1/T_1^{\text{Raman}}$  and  $1/T_1^{\text{ADD}}$  by  $C_{\text{RA}}$

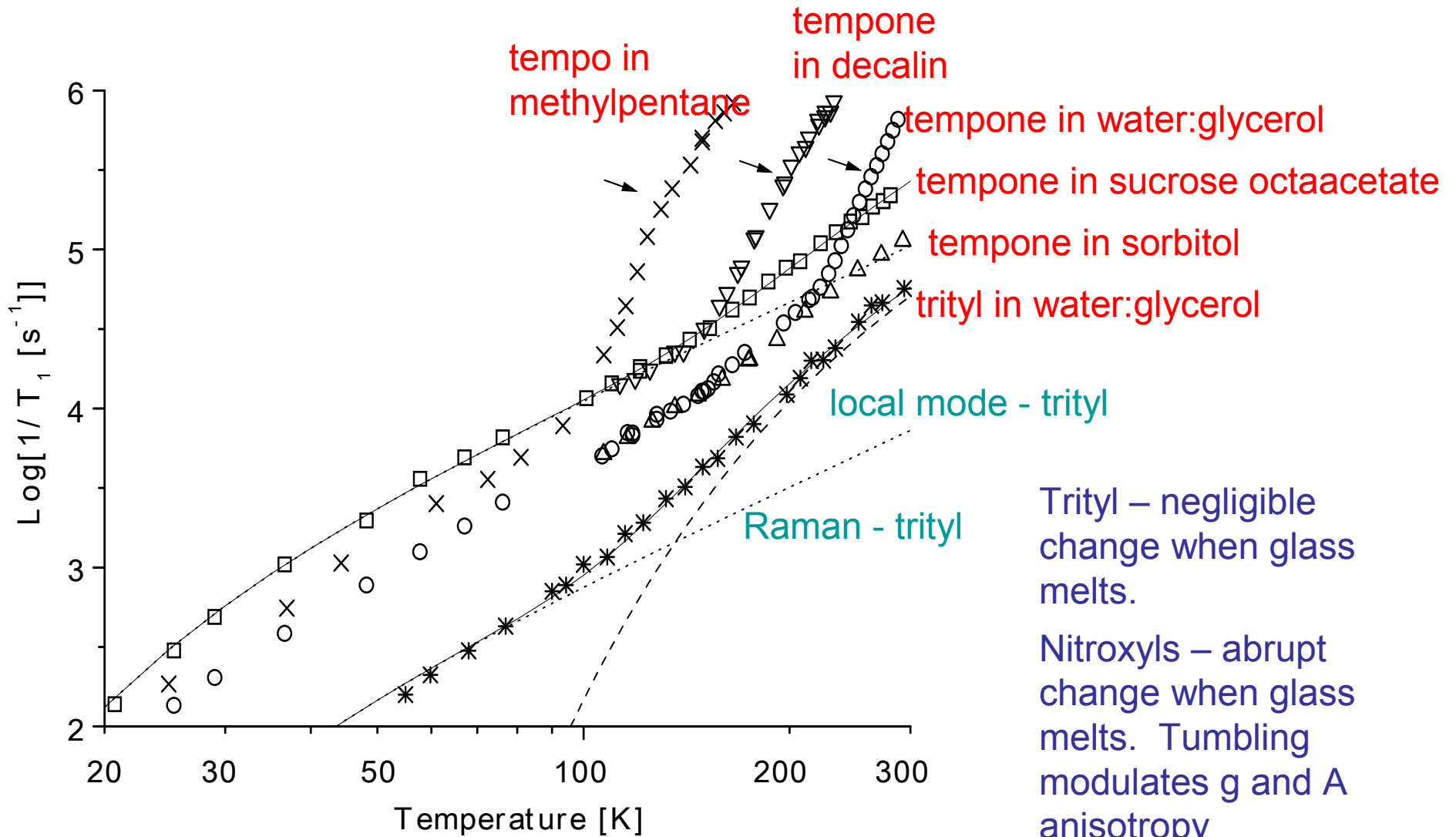
**Conclusion: contributions from Raman process and local mode are correlated.**

# Relaxation in Glasses

radical	$g_{\text{iso}}^{\text{a}}$	$\frac{(g - g_e)^2}{(g_{\text{tempone}} - g_e)^2}$	$C_{\text{RA}}$
tempone	2.0060	1.0	1.0
TPHIO	2.0059	1.1 to 0.85	1.0
galvinoxyl	2.0044	0.37 to 0.27	0.13
DPPH	2.0036	0.15 to 0.10	0.25
trityl	2.0026	0.011 to 0.0023	0.022
BDPA	2.0026	0.011 to 0.0023	0.040

<sup>a</sup>Range calculated for uncertainty in g values of  $\pm 0.0001$

# Impact of Tumbling on Relaxation at X-band



# Spectral Density Functions for Nitroxyl Tumbling

Bloembergen-Pound-Purcell

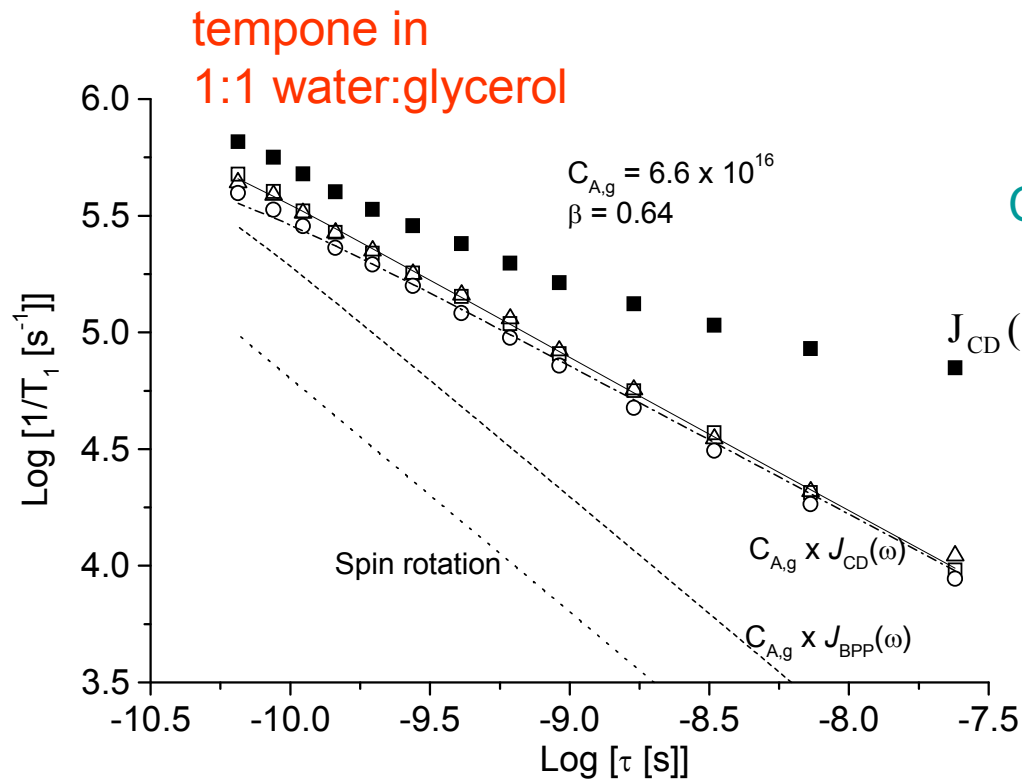
$$J_{\text{BBP}}(\omega) = \frac{\tau_R}{1 + (\omega\tau_R)^2}$$

Cole-Davidson distribution function

$$J_{\text{CD}}(\omega) = \frac{1}{\omega} \text{Re}\left[\frac{1}{(1 + i\omega\tau)^\beta}\right] = \frac{1}{\omega} \frac{\sin(\beta \cdot \arctan(\omega\tau))}{(1 + (\omega\tau)^2)^{\beta/2}}$$

$J_{\text{CD}}$  reduces to  $J_{\text{BBP}}$  when  $\beta = 1$ .

A smaller value of  $\beta$  indicates a broader distribution



Estimate the tumbling-dependent contribution to relaxation.

$1/T_1$  in water:glycerol -  $1/T_1$  in glycerol for

(□) tempone, (△) tempone- $d_{16}$ , (○)  $^{15}\text{N}$ -tempone.

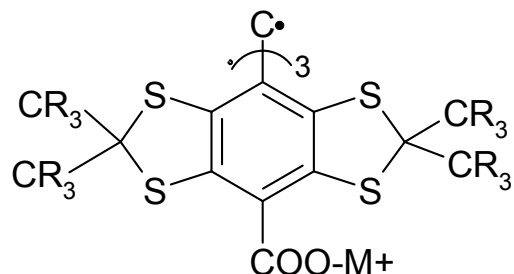
## Conclusions concerning relaxation for nitroxyl radicals

- For  $\tau > 2$  ns, local mode and Raman process dominate the relaxation. Rates are similar at X- and Q-band.
- For  $0.2 < \tau < 2$  ns, modulation of nitrogen hyperfine anisotropy and g anisotropy dominates the relaxation at X-band and S-band.
- Modulation of nitrogen hyperfine anisotropy has a greater effect at S-band than at X-band because the tumbling rates are closer to the S-band frequency.

# Factors that impact electron spin lattice relaxation for radicals

- Molecular flexibility and tumbling
- Rigidity of glassy solvent
- Deviation of g values from 2.003 due to spin-orbit coupling
- Dynamic processes, such as methyl rotation, occurring at a rate comparable to the EPR frequency
- **Molecular size**

## Relaxation Mechanisms for Triarylmethyl (Trityl) Radicals in Solution at 20° C.



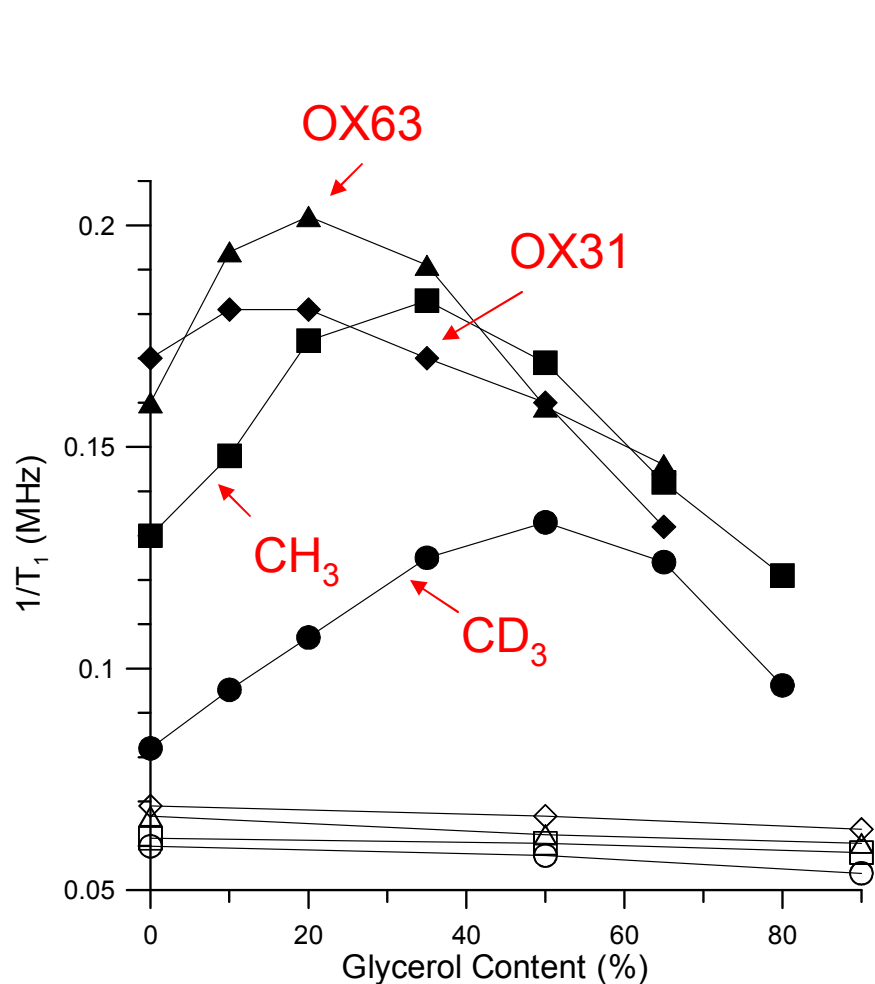
trityl-CH <sub>3</sub>	R = H
trityl-CD <sub>3</sub>	R = D
OX63	R = CH <sub>2</sub> CH <sub>2</sub> OH
OX31	R = CH <sub>2</sub> OCH <sub>2</sub> CH <sub>2</sub> OH

For these radicals g-values are close to 2, and the hyperfine coupling is weak. As a result, spin rotation and modulation of g and/or A anisotropy are ineffective relaxation mechanisms.

At frequencies above about 1 GHz spin lattice relaxation is dominated by a local vibrational mode. R group has little impact.

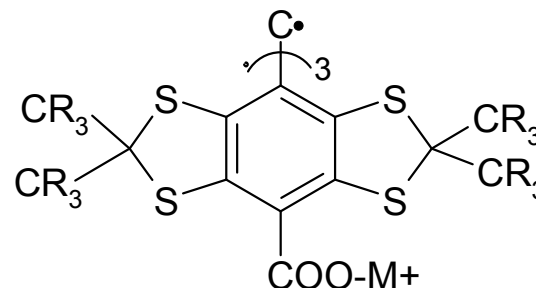


# Trityl at 250 MHz and 9 GHz, 20 °C



250 MHz, solid symbols  
X-band, open symbols

L. Yong et al., *J. Magn. Reson.* **152**, 156 (2001).



CH <sub>3</sub>	R = H
CD <sub>3</sub>	R = D
OX63	R = CH <sub>2</sub> CH <sub>2</sub> OH
OX31	R = CH <sub>2</sub> OCH <sub>2</sub> CH <sub>2</sub> OH

At 250 MHz the tumbling correlation time is  $\sim 1/\omega$ .

Modulation of electron-proton dipolar interaction dominates  $T_1$ .

For deuterated radicals, solvent protons dominate.

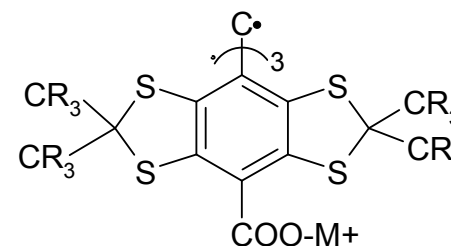
For natural isotope abundance radicals, both intramolecular and intermolecular electron-proton interactions contribute to relaxation.

# Trityls: impact of deuteration

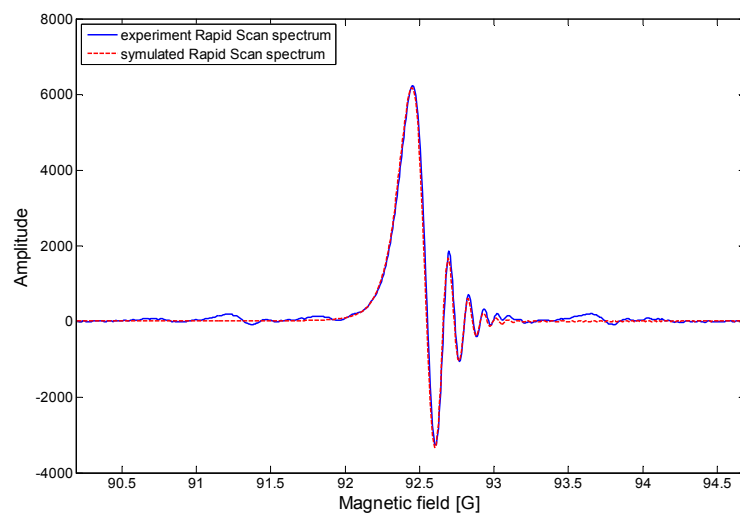
## Relaxation times in $\mu\text{sec}$ at 250 MHz

22 °C, 0.2 mM, pH ~ 8

	$T_1$ $\text{H}_2\text{O}$	$T_1$ $\text{D}_2\text{O}$	$T_2$ $\text{H}_2\text{O}$	$T_2$ $\text{D}_2\text{O}$
OX63	6.2	6.4	5.3	5.6
OX63-d <sub>24</sub>	7.4	8.7	6.9	7.6

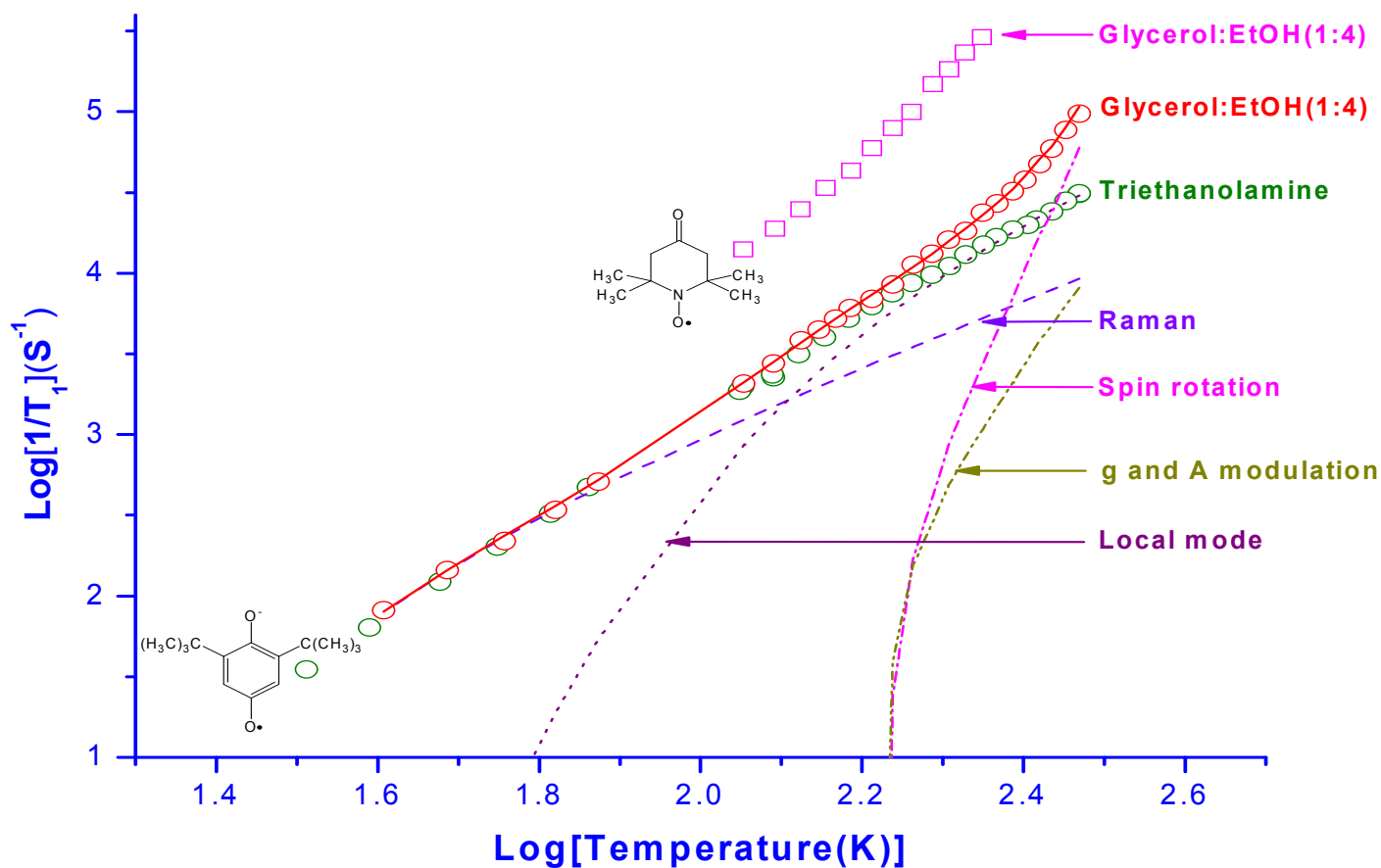


OX63                    R = CH<sub>2</sub>CH<sub>2</sub>OH  
 OX63-d<sub>24</sub>            R = CD<sub>2</sub>CD<sub>2</sub>OH



Rapid scan signal, 0.2 mM

# Semiquinone Radicals

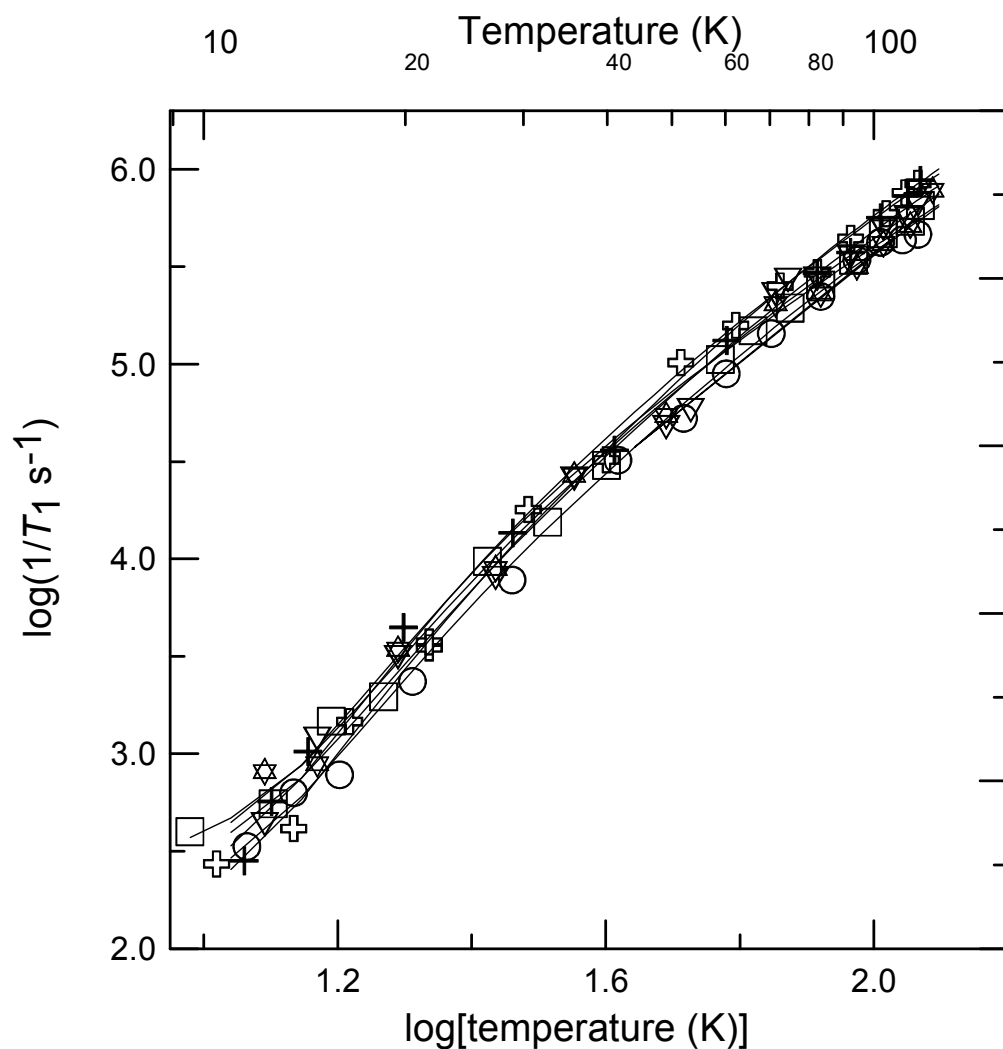


Relaxation rates are slower than for nitroxyls of similar size.  
 Modulation of hyperfine anisotropy is smaller contribution.

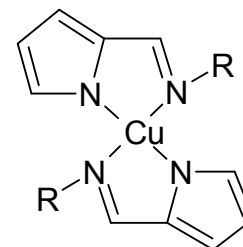
# Metal Ions

$\text{Cu}^{2+}$ ,  $\text{VO}^{2+}$ ,  $\text{Fe}^{3+}$  heme,  $[\text{4Fe-4S}]^+$

# CuL<sub>2</sub> Complexes in 2:1 toluene:CHCl<sub>3</sub>

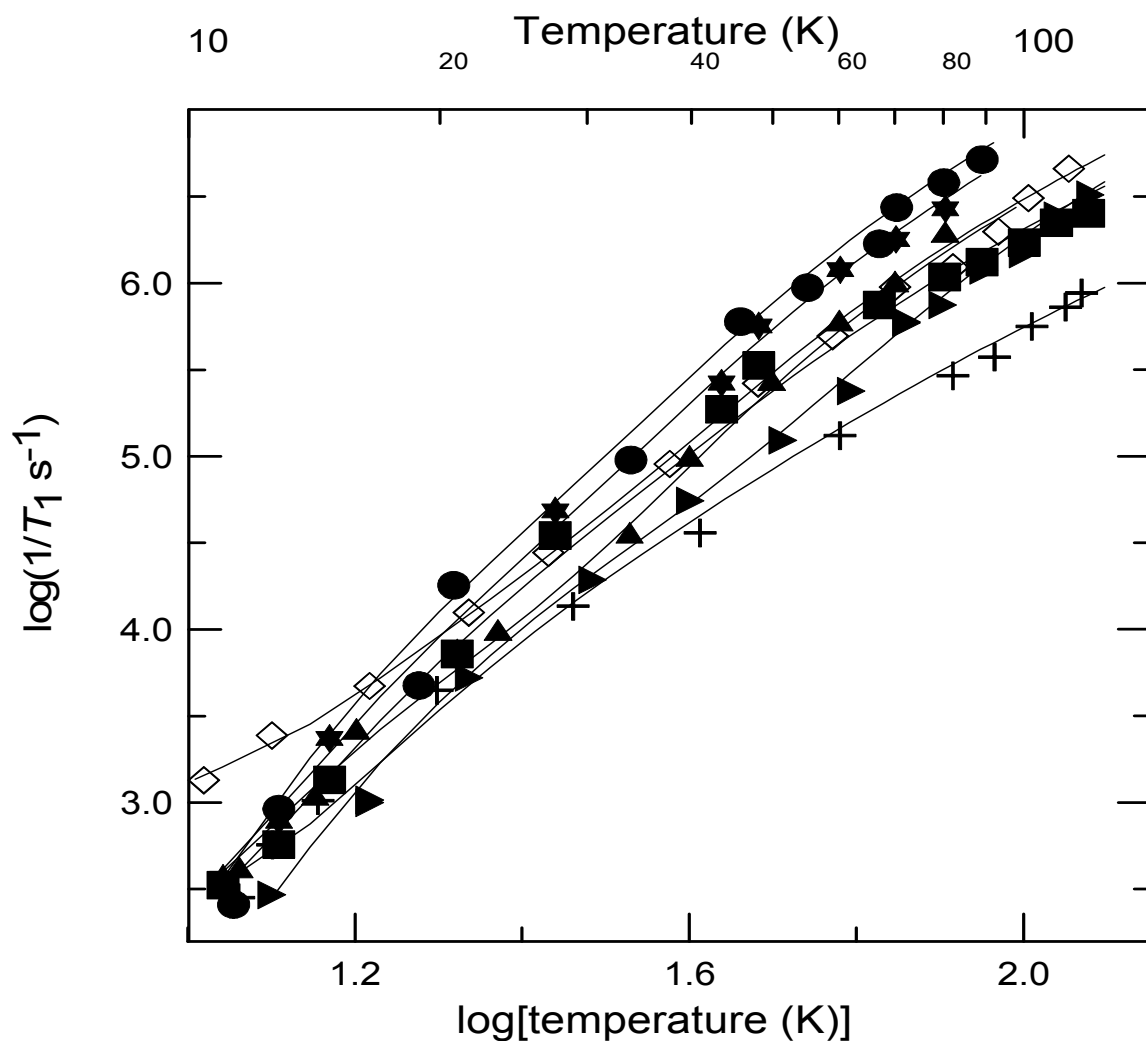


Fit line is sum of Raman and local mode.

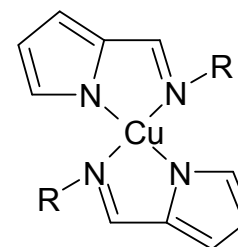


	R	Dihedral angle
+	H	0°
⊕	methyl	~25°
▽	n-butyl	27°
○	dibenzyl-methyl	33°
☆	benzyl	29°
□	2-adamantyl	36°

# Cu(II) Complexes

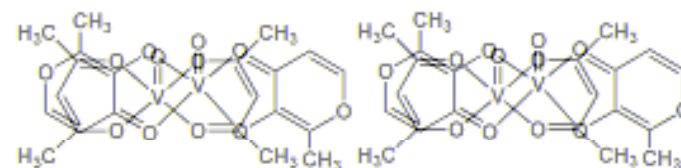
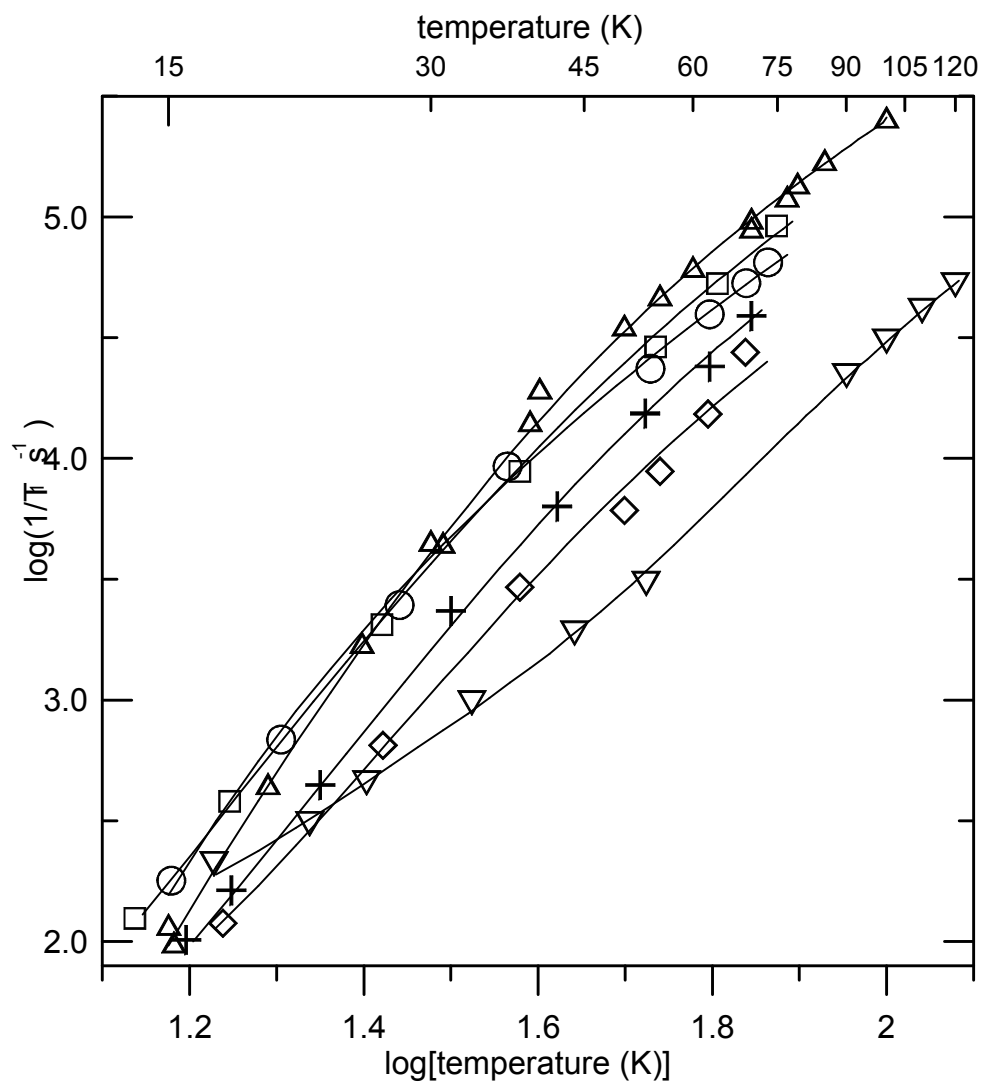


- $\text{Cu(aq)}^{2+}$
- ★  $\text{CuEDTA}$
- ▲  $\text{CuIm}_4$
- ◇  $\text{CuL}_2$ , R = t-butyl
- $\text{Cu(OH)}_4^{2-}$
- $\text{Cu(Melm)}_4$
- +  $\text{CuL}_2$  R = H



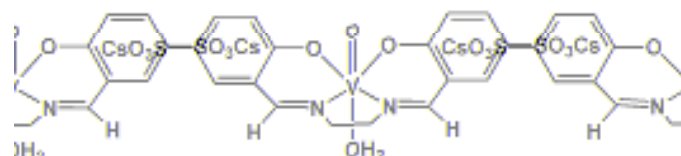
Solid lines are the sum of contributions from Raman and local mode processes.

# V(IV) Complexes



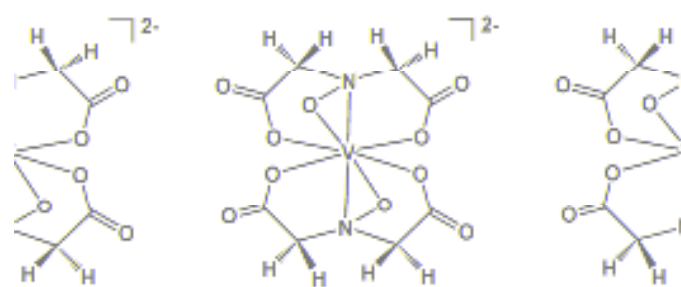
1 2

1 2



3

3

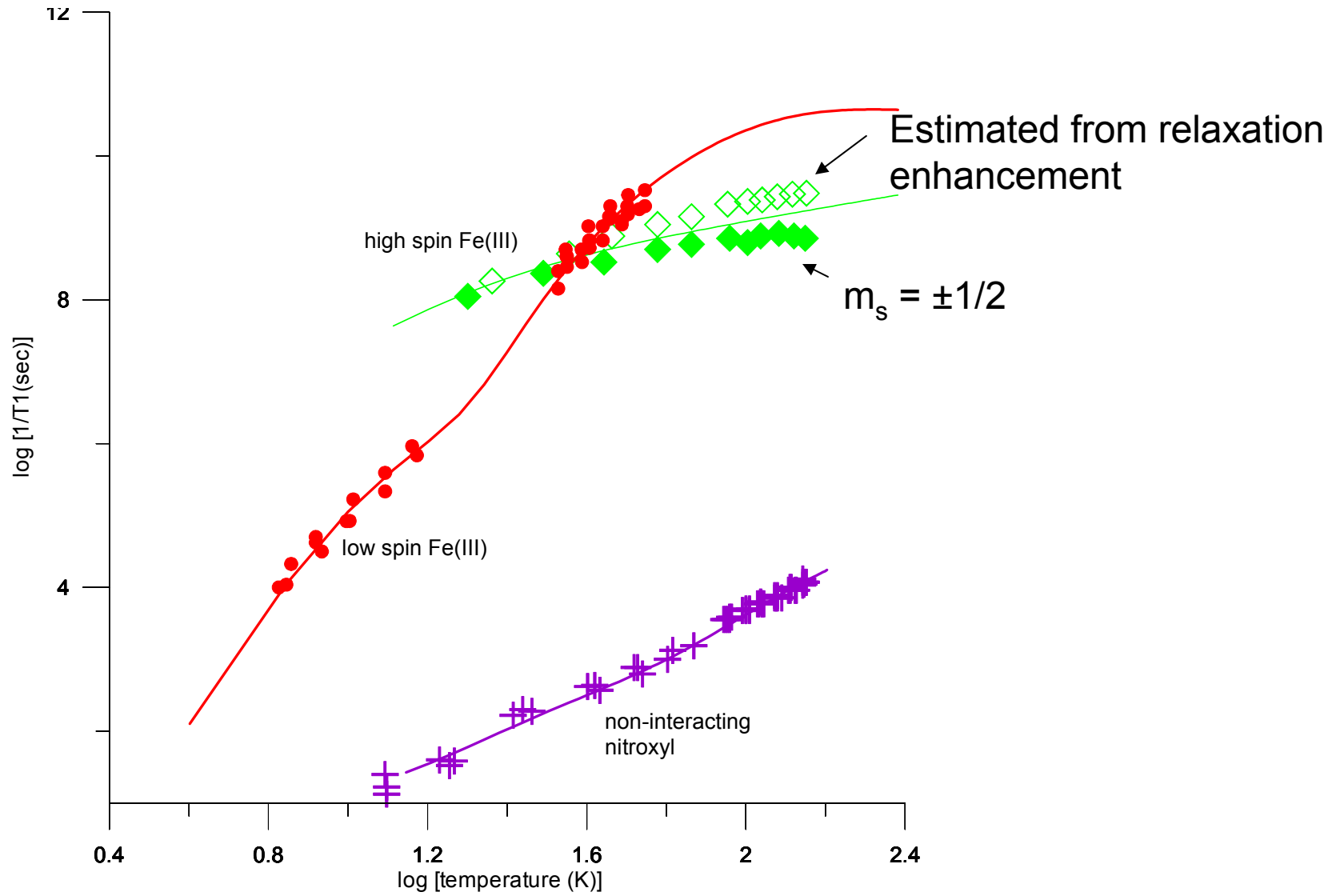


4

4

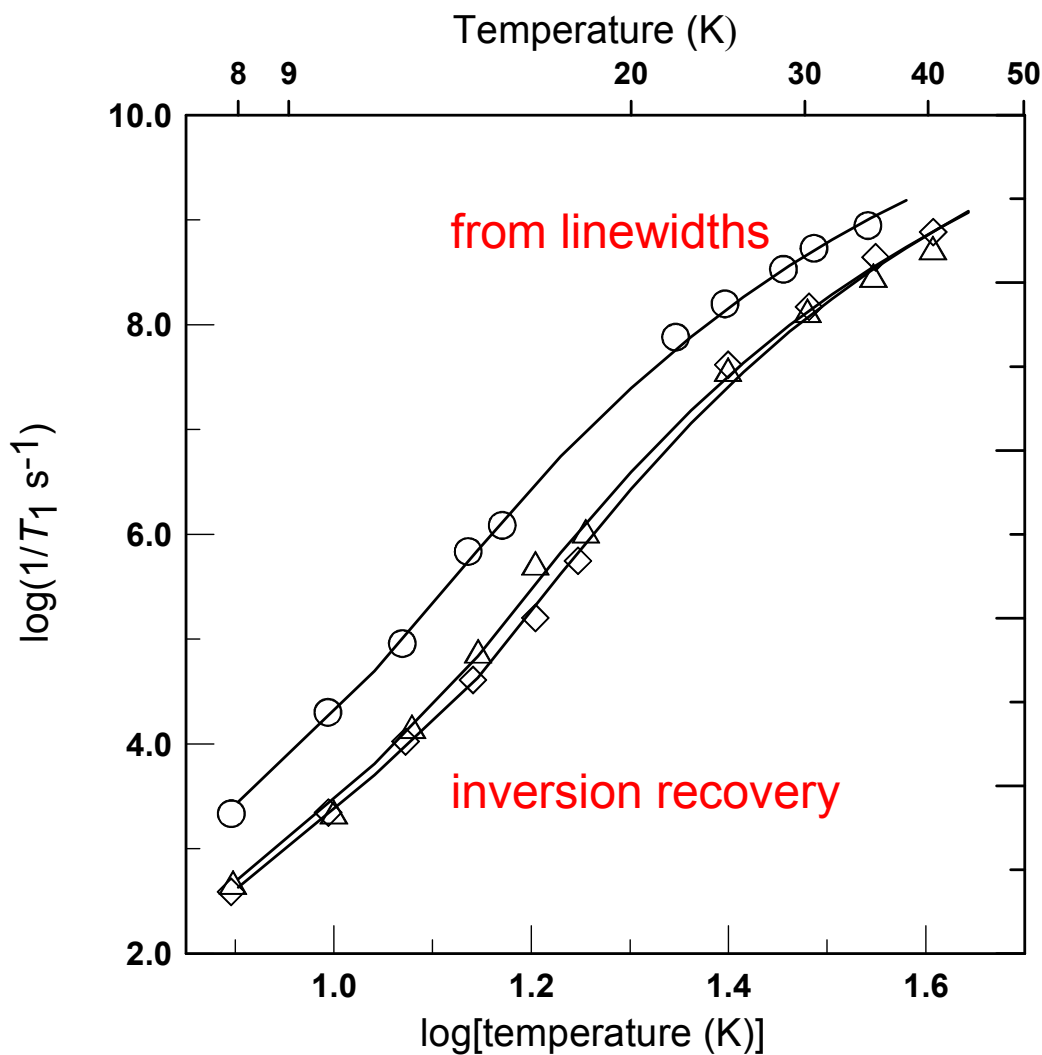
- △ aquo VO<sup>2+</sup>
- 1
- 2
- + 4
- ◇ 3
- ▽ vanadylporphyrin

# Fe(III) heme





# [4Fe-4S]<sup>1+</sup> in Electron Transfer Flavoprotein Ubiquinone Oxidoreductase



- *Rhodobacter*
- ◇ porcine
- △ human

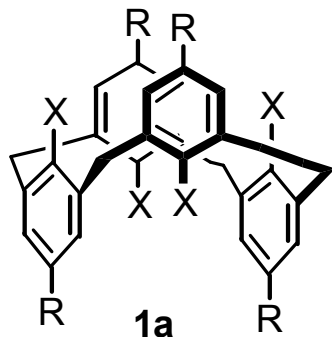
Orbach energies (K)

<i>Rhodobacter</i>	175
Human	210
Porcine	225

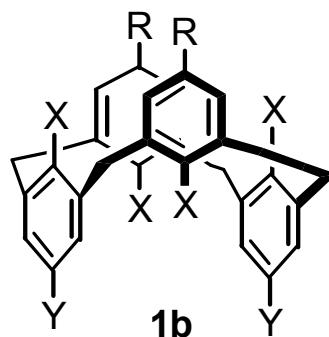
# Relaxation Enhancement and Interspin Distances

# Dinitroxyls and Tetranitroxyl

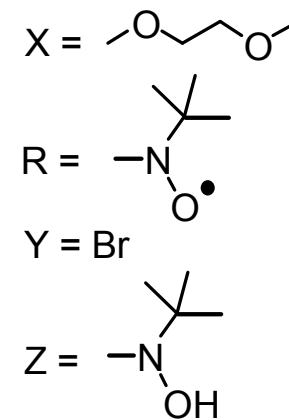
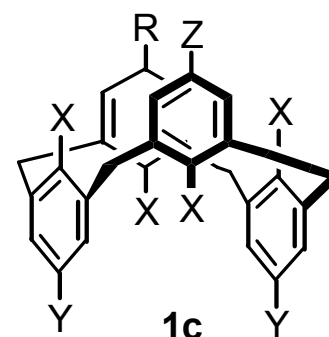
tetradiradical



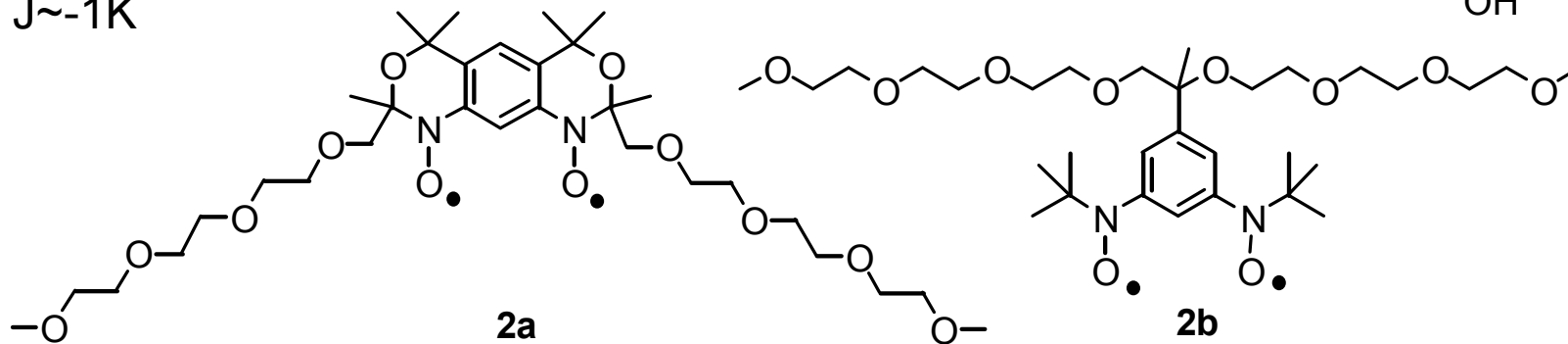
diradical



monoradical



$J \sim -1\text{K}$



$J \sim 300\text{K}$

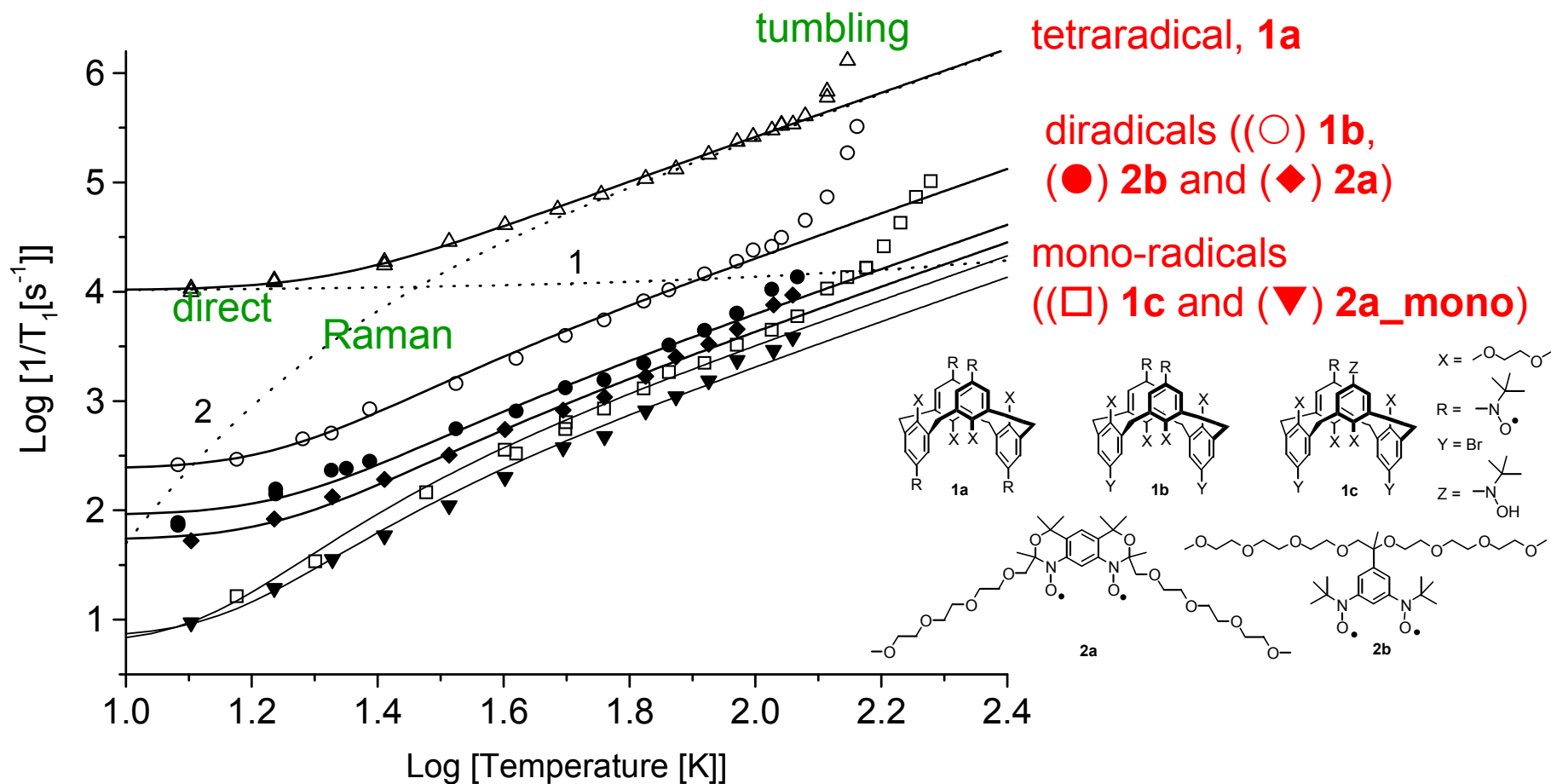
diradical

diradical

$r \sim 5 \text{ to } 7 \text{ \AA}$

Prepared by Andrzej Rajca and co-workers, University of Nebraska

# Mono-, di- and tetra-nitroxyls in 4:1 toluene:CHCl<sub>3</sub>



Direct process -  
increased local  
spin concentration

Raman -  
increased  
modulation of  
spin-spin  
interaction

Tumbling -  
increased modulation  
of spin-spin  
interaction

# Relaxation Enhancement due to Interaction with Rapidly Relaxation Paramagnetic Center

- Quantitative analysis of increase in  $1/T_1$  due to dipolar interaction with faster relaxing spin
  - Measure SR curve for slowly relaxing spin in the absence of interaction at a series of temperatures
  - Determine relaxation rates for rapidly relaxing spin as function of temperature
  - Analyze SR curve for slowly relaxing spin interacting with rapidly relaxing spin taking account of the anisotropy of the dipolar interaction.
- Examples
  - Spin label interacting with low-spin Fe(III) in myoglobin
  - Spin label interacting with high-spin Fe(III) in myoglobin
  - Flavosemiquinone interacting with  $[4\text{Fe-4S}]^+$  in electron transfer flavoprotein ubiquinone reductase

# What Else Can You Learn from Relaxation Times?

- The nature of the spin system - e.g., is there a low-lying excited state?
- Measure collisions between species – e.g., oximetry.
- Predict utility for MRI contrast reagents.
- Predict utility for EPR spin label and spin probe broadening reagents.
- Local concentration of spins - are spins uniformly distributed or aggregated?
- Motions of paramagnetic species.

## Further Information

*Biological Magnetic Resonance* , vol. 19, 2001

Distance Measurements by EPR

*Biological Magnetic Resonance*, vol. 23, 2004

Biomedical ESR - Part A: Free Radicals, Metals, Medicine, and Physiology

*Biological Magnetic Resonance*, vol. 24, 2004

Biomedical ESR - Part B: Methodology, Instrumentation and Dynamics