# Modern EPR Applications
# From Beer to DEER

Ralph T. Weber
Bruker BioSpin Corp. EPR Division

# What is EPR?

- EPR is Electron Paramagnetic Resonance.
- EPR is a form of magnetic resonance spectroscopy used to detect unpaired (or free) electrons.
- EPR is similar to NMR, but differs in that it measures unpaired electrons instead of nuclei.
- EPR is the only technique that unambiguously detects free radicals.
- EPR = ESR = EMR

# What is EPR?

- EPR samples have one absolute requirement…they must contain unpaired electrons.
- **Transition metal ions** - Fe, Cu, Mn, Co, Mo, Ni
- **Free Radicals** – Typically carbon, nitrogen or oxygen containing compounds.
- **Defect Centers** – Semiconductors, ionizing radiation, N centers in diamond.

# What is EPR?
# Who uses EPR?

- **Chemistry:**
  - Kinetics of radical reactions
  - Polymerization reactions
  - Spin trapping
  - Organo-metallic compounds
  - Catalysis
  - Petroleum research
  - Oxidations and reduction processes

# What is EPR?
# Who uses EPR?

- **Physics:**
  - Measurement of magnetic susceptibility
  - Transitions metal, lanthanide, and actinide ions
  - Conduction electrons in conductors and semiconductors
  - Defects in crystals (e.g. color centers in alkali-halides)
  - Optical detection of magnetic resonance, excited states
  - Crystal fields in single crystals
  - Recombination at low temperatures

# What is EPR?
# Who uses EPR?

- **Biology and Medicine:**
  - Spin label and spin probe techniques
  - Spin trapping
  - Free radicals in living tissues and fluids
  - Antioxidants, radical scavengers
  - Oximetry
  - Enzyme reactions
  - Photosynthesis
  - Structure of metalloprotein active sites
  - Photochemical generation of radicals
  - NO in biological systems

# What is EPR?
# Who uses EPR?

- **Industrial Research and QA/QC**:
  - Degradation of paints and polymers by light
  - Polymer properties and cross linking
  - Defects in diamond
  - Defects in optical fibers
  - Organic conductors
  - Influence of impurities/defects in semiconductors
  - Cigarette filter efficiency
  - Shelf life in fermented beverages
  - Behavior of free radicals in corrosion

# What is EPR?
# Who uses EPR?

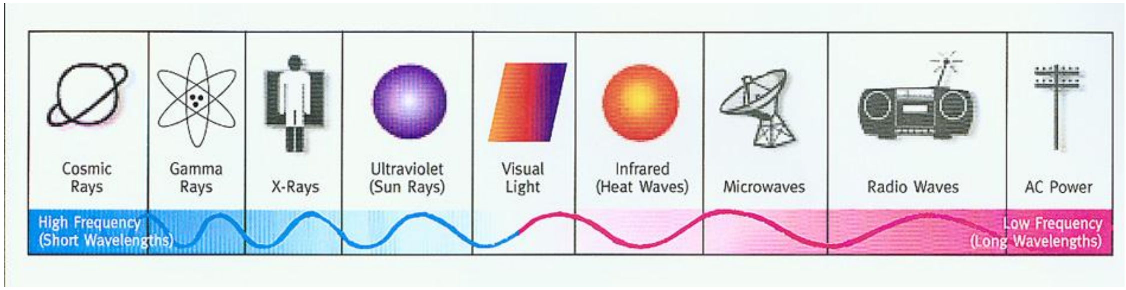
- **Ionizing radiation:**
  - Alanine radiation dosimetry
  - Detection of irradiated foods
  - Archaeological dating
  - Radiation effects and damage
  - Radiation effects on biological compounds

# What is EPR?

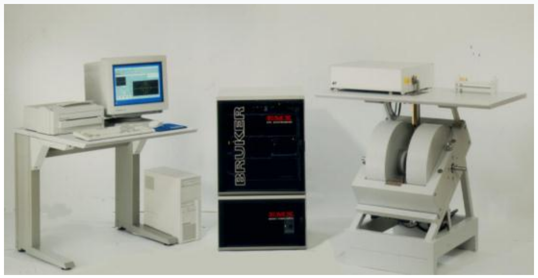
Some types of spectrometers:

- UV/Visible spectrophotometry
- * Infrared spectrometer
- * Nuclear Magnetic Resonance spectrometer
- * X-ray absorption spectrometer
- * Mass spectrometer
- * EPR spectrometer

* Manufactured by Bruker



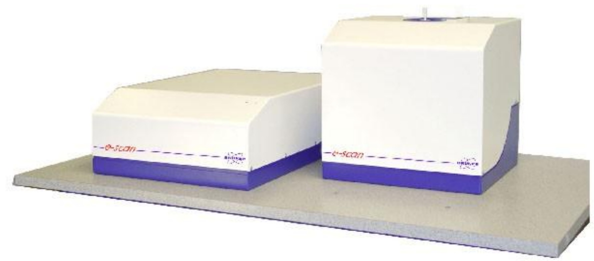
# What is EPR?
# What does an EPR spectrometer look like?



EMX

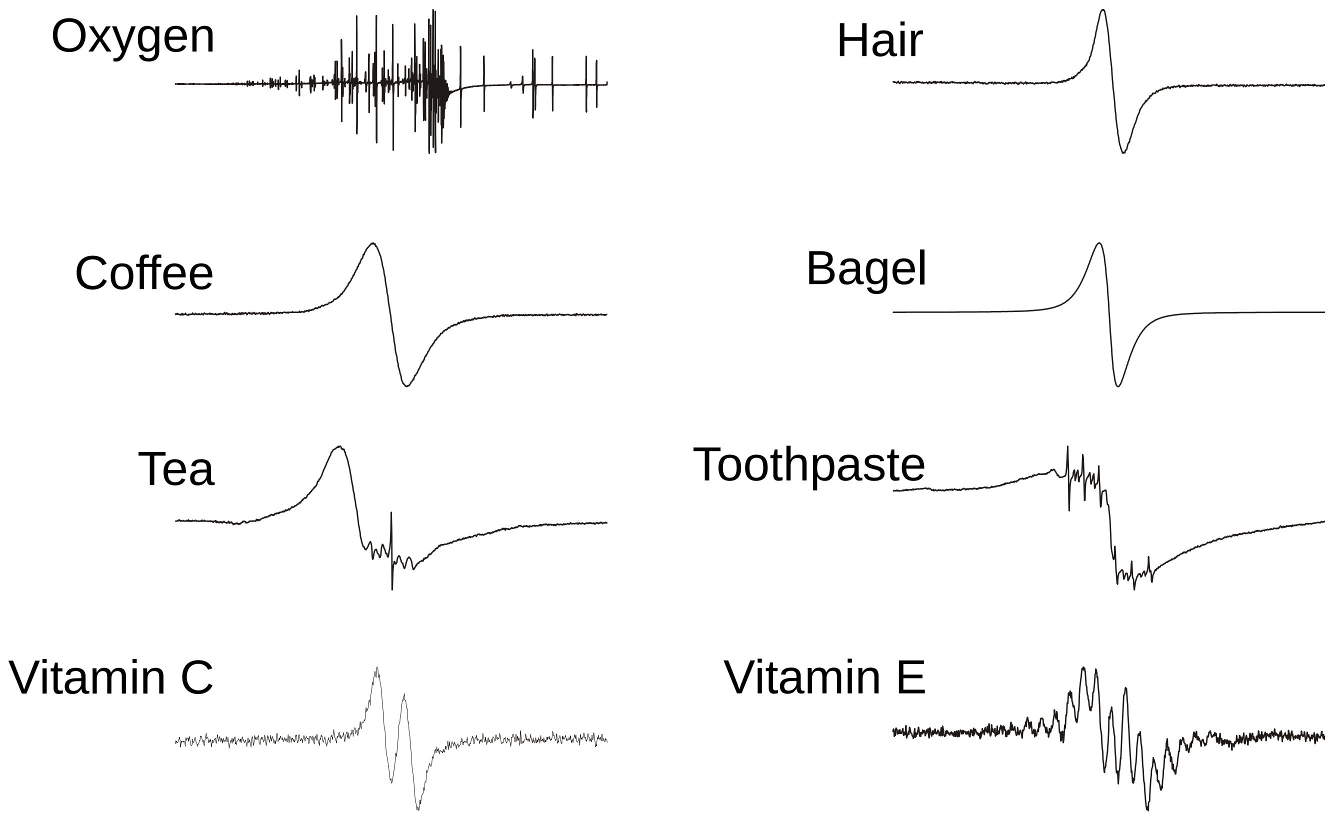
Elexsys E580


Elexsys E680



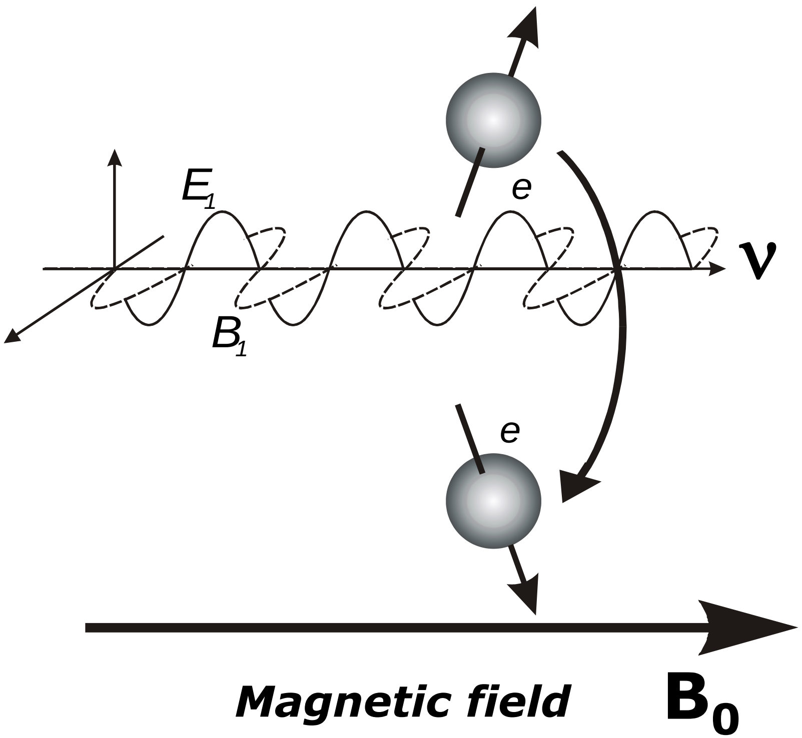
e-scan

# What is EPR?
# EPR Spectra of Commonly Found Substances



# What is EPR?

EPR is a magnetic resonance spectroscopy. Energy transitions are caused by the interaction of the unpaired electron with the magnetic component of microwave radiation (at a specific frequency) while an external magnetic field is applied to the sample.




$\Delta E = h\nu = g\,\mu_B\,B_0$

**h** = Planck's constant

**ν** = Microwave frequency

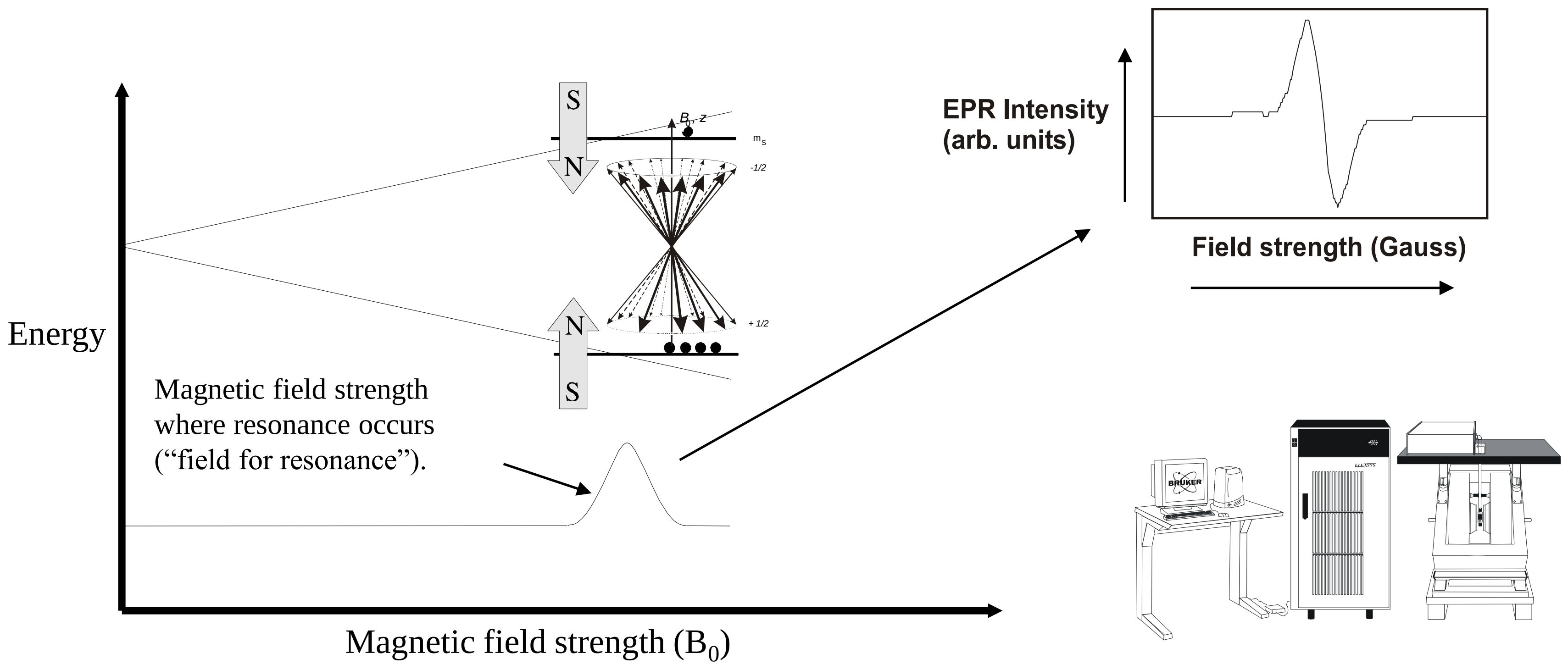
$\mu_B$ = Bohr magneton

$B_0$ = External magnetic field

**g** = g-factor of the sample

# What is EPR?

The EPR spectrometer provides a linear field sweep, while exposing the sample to a fixed frequency of microwave irradiation. The interaction of unpaired electrons with a magnetic field is known as the **Zeeman interaction**.



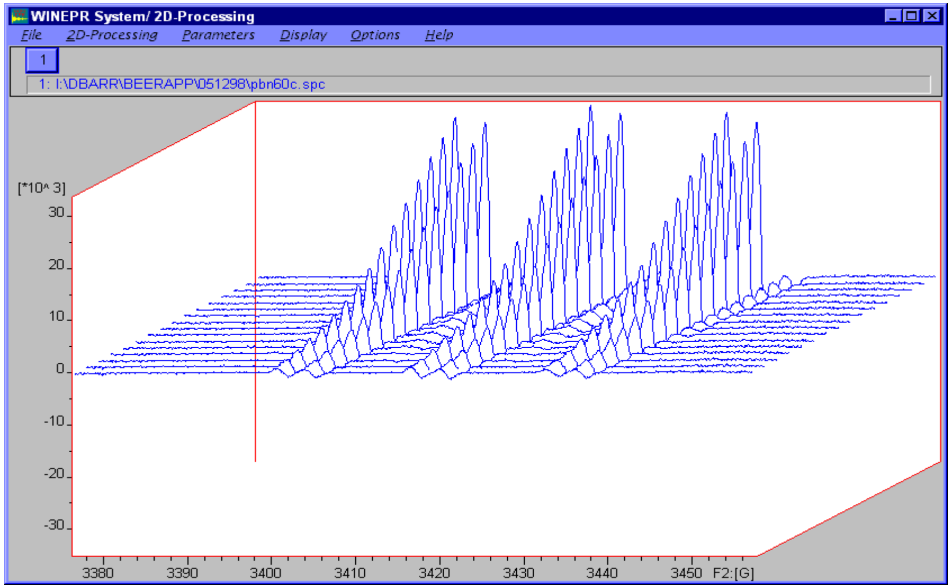
# What information does an EPR spectrum provide?

- Direct evidence for presence of “free” or unpaired electrons in a sample (*signal intensity*).
- Can indicate the type of sample (for example identifies a transition metal) (*g-factor*).
- Reveals the molecular structure and the environment near the electron (*hyperfine interactions*).
- Molecular motion in a sample with unpaired electrons (*line shape and/or line width*).

# Signal Intensity

# Signal Intensity

- If the sample's line width and the line shape remain constant the peak-to-trough intensity can be used as a measure of concentration.



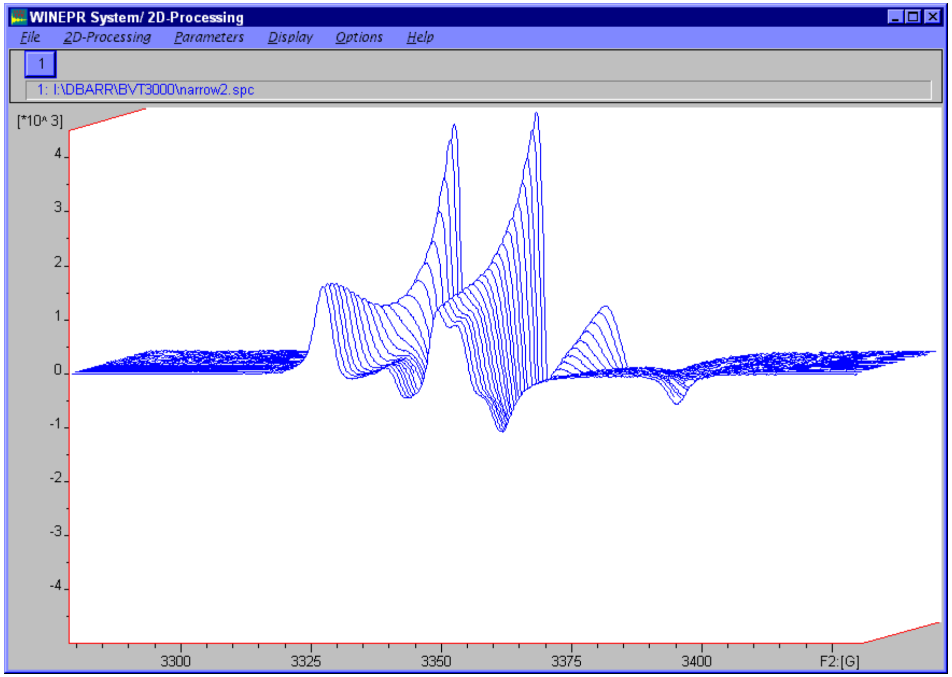
# Signal Intensity

- The integrated signal intensity is the absolute measure of concentration in the EPR sample.

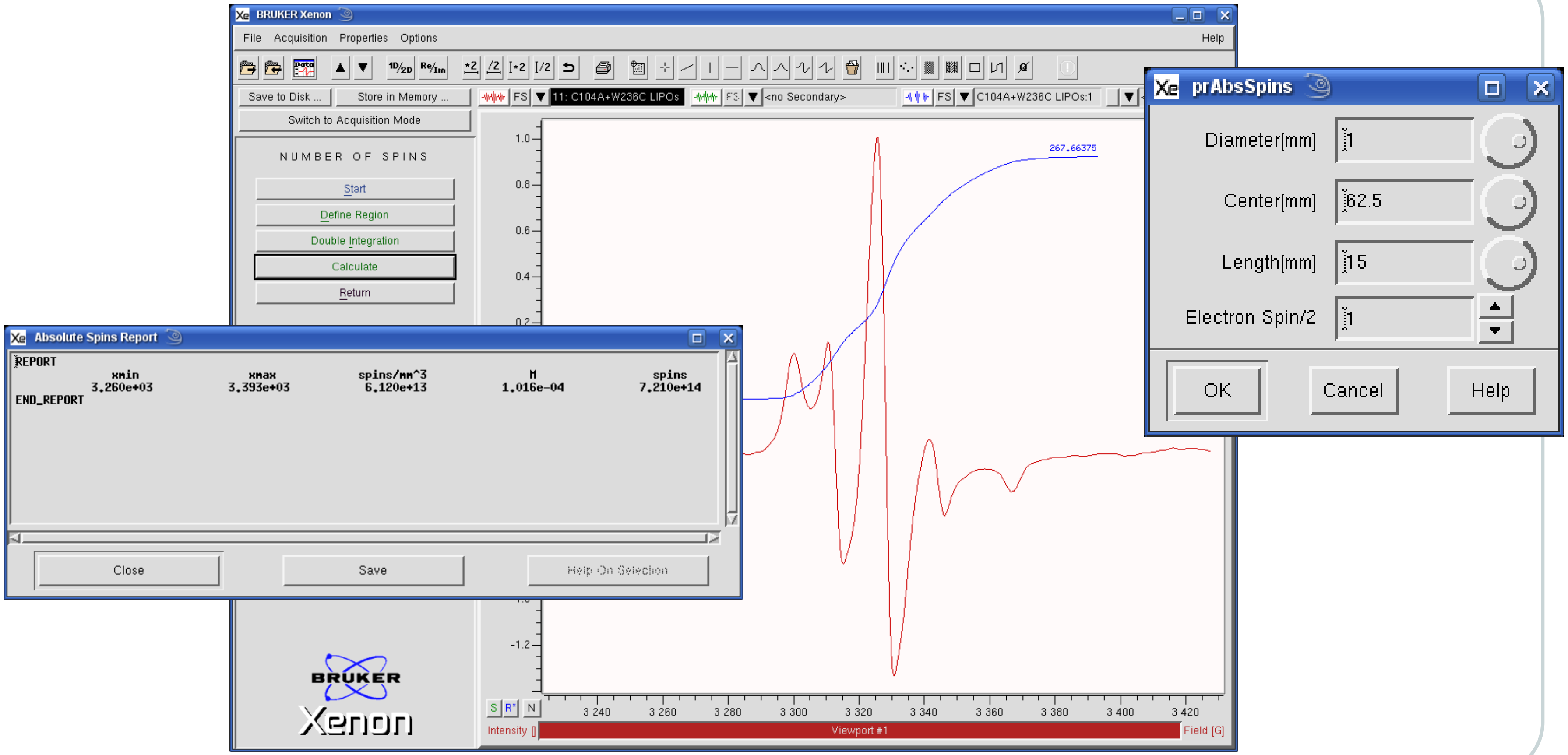
**Even though the line widths and peak heights of these samples are different the integrated intensity is the same.**

# Signal Intensity

- If the line width or line shape changes, the integrated intensity must be used to determine concentration.



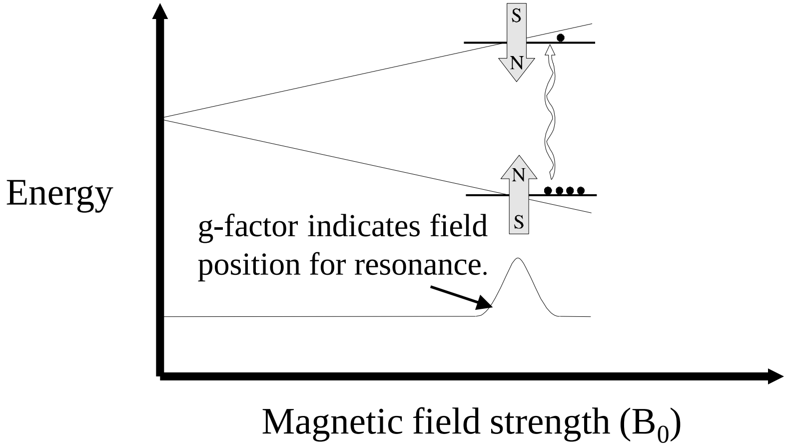
# Signal Intensity



# The g-factor

- The g-factor of an EPR sample determines the position in the magnetic field (at a given microwave frequency) where an EPR transition will occur.

$$g = \frac{h\nu}{\mu_B B_0}$$




h = Planck's constant

$\nu$ = Frequency

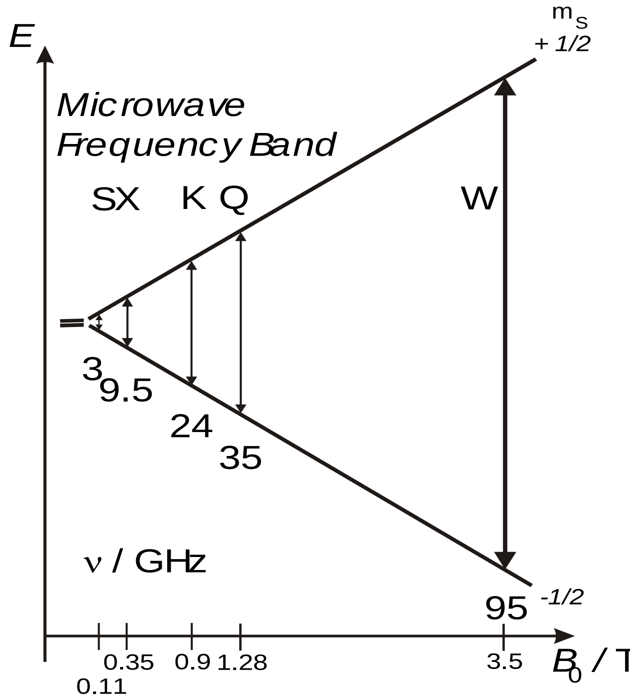
$\mu_B$ = Bohr magneton (mag. moment of electron)

$B_0$ = External magnetic field

g = g-factor of the sample

# The g-factor

- The g-factor is a constant but the field for resonance changes with microwave frequency.

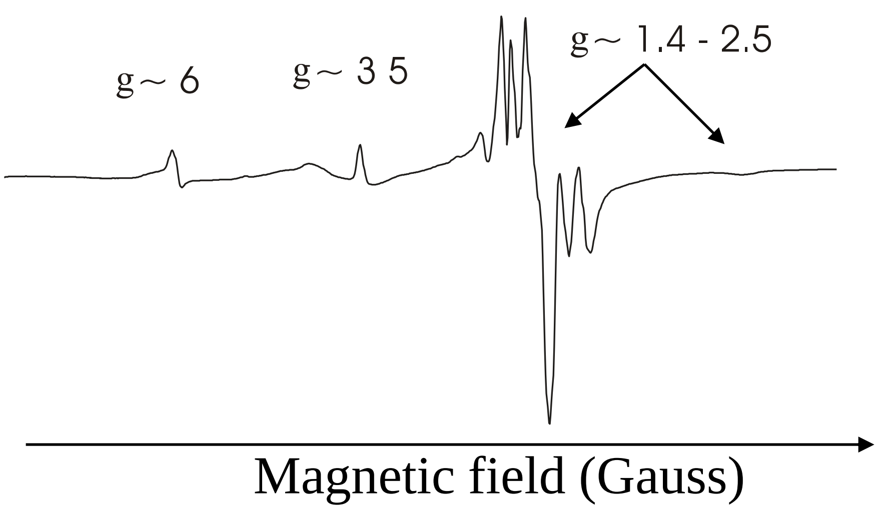



| Microwave Band | Microwave Frequency (GHz) | $B_0$ (for g=2) Gauss |
|---|---|---|
| L | 1.1 | 392 |
| S | 3.0 | 1070 |
| X | 9.5 | 3389 |
| K | 24.0 | 8560 |
| Q | 35.0 | 12485 |
| W | 94.0 | 33600 |

# The g-factor

- The **g-**factor helps us characterize the type of EPR sample we are measuring. For example, it can identify a specific metal ion, its oxidation state, spin state and coordination environment.

Cytochrome oxidase is a metalloprotein with more than one metal center. The g-values are used to identify and characterize the different centers.



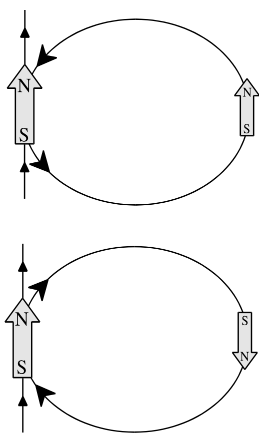
# Is it EPR or ESR?

(Electron Spin Resonance) or (Electron Paramagnetic Resonance)

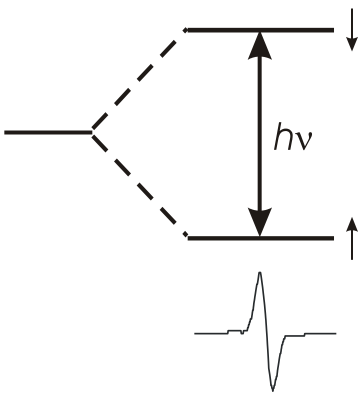
- The total magnetic moment of an electron ( *J* ) is defined by quantum numbers ( *L* ) and ( *S* ):
- *L* is the angular orbital momentum.
- *S* is the spin of the electron itself.
- Most organic radicals have their orbital angular momentum quenched. This is why many chemists call it ESR.
- Most metal complexes have substantial contributions from the orbital angular momentum to the paramagnetism. This is why many people studying metal ions call it EPR.

# Hyperfine Interactions

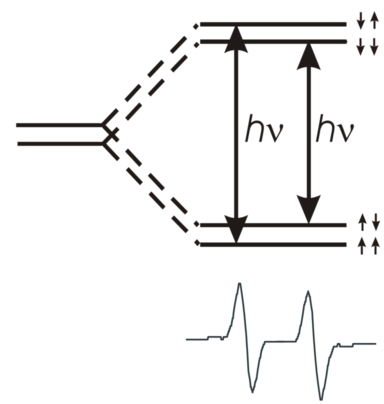
- Nuclei with spin influence the local magnetic field that the unpaired electron experiences.
- The magnetic moment of these nuclei will either add or subtract to the field of the laboratory magnet. This splits the absorption line into two lines centered about the g-factor.




Coupling can add or subtract to the field




No hyperfine

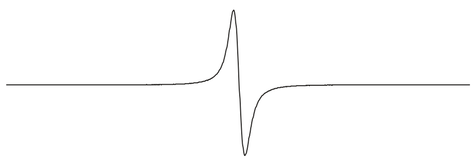



Hyperfine from spin=1/2 nucleus

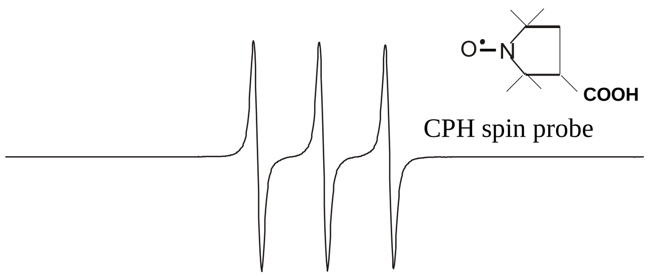
# Hyperfine Interactions

- Hyperfine interactions reveal neighboring nuclei.

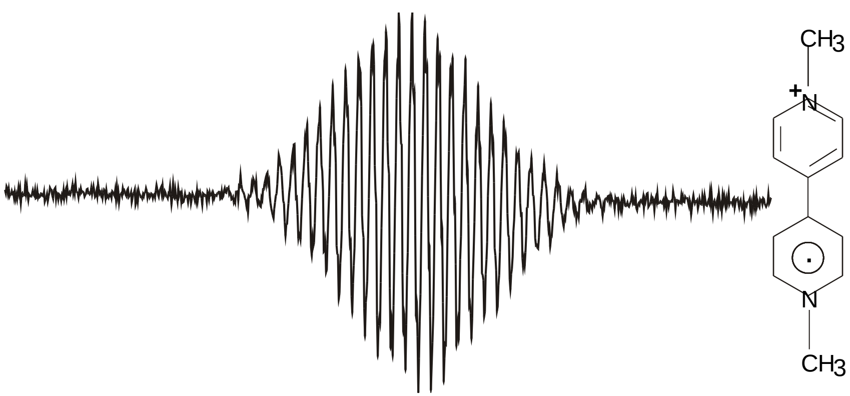
No hyperfine interaction

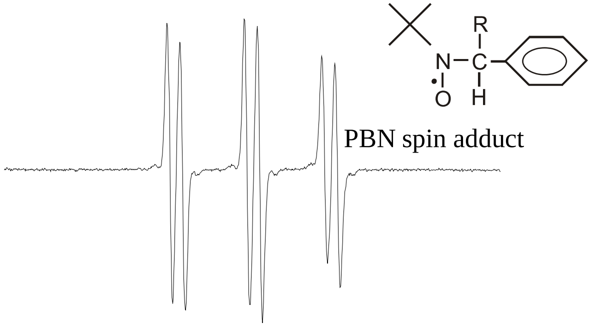


Simple hyperfine interaction




Multiple hyperfine interactions

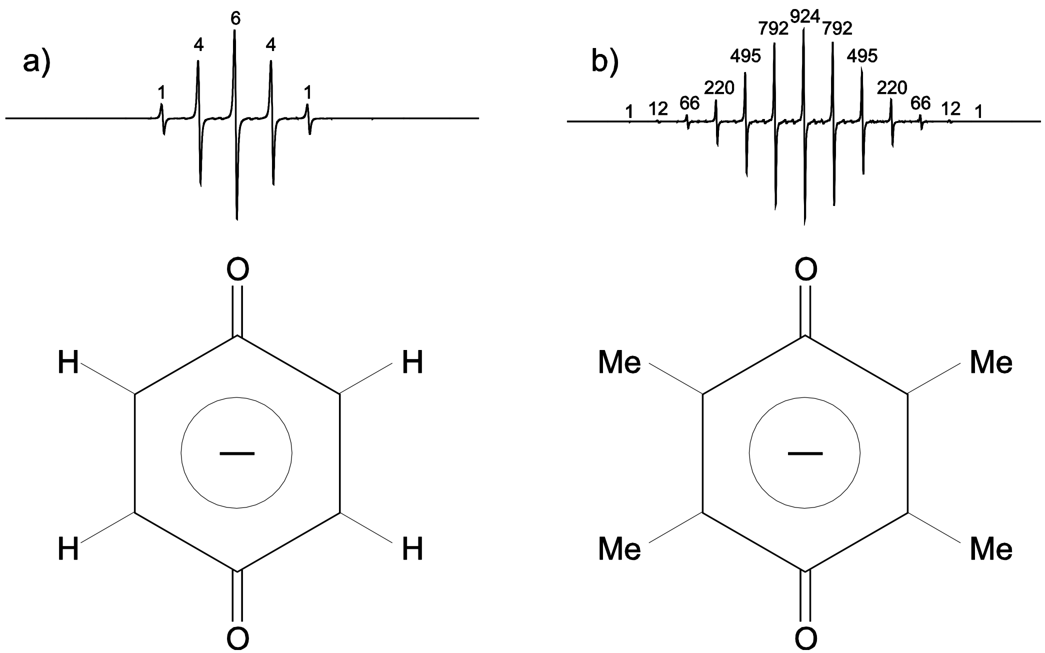


# Hyperfine Interactions

- The intensity pattern indicates the number of equivalent nuclei.

Binomial distribution for H hyperfine (Pascal's Triangle)




| No. of nuclei $n$ | No. of lines $2I+1$ | Binomial intensity ratios |
|---|---|---|
| 0 | 1 | 1 |
| 1 | 2 | 1 1 |
| 2 | 3 | 1 2 1 |
| 3 | 4 | 1 3 3 1 |
| 4 | 5 | 1 4 6 4 1 |
| 5 | 6 | 1 5 10 10 5 1 |
| 6 | 7 | 1 6 15 20 15 6 1 |

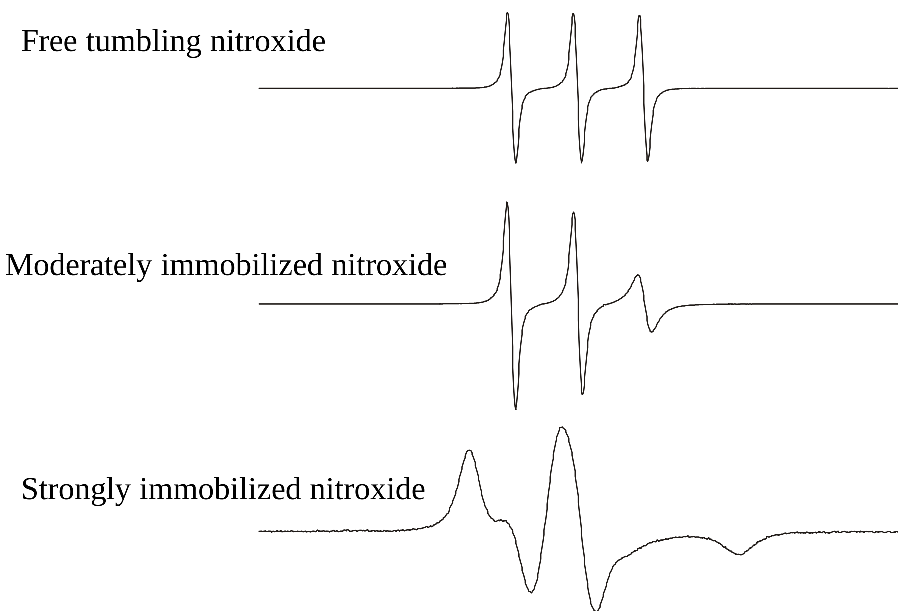
# Lineshapes

- Rapidly relaxing electrons cause broader lines, slower relaxation times result in sharper lines.
- Weak hyperfine interactions from more distant nuclei cause line broadening
- Anisotropic interactions cause line broadening.
- High concentrations of unpaired electrons  cause broadening via increased “spin-spin” relaxation.

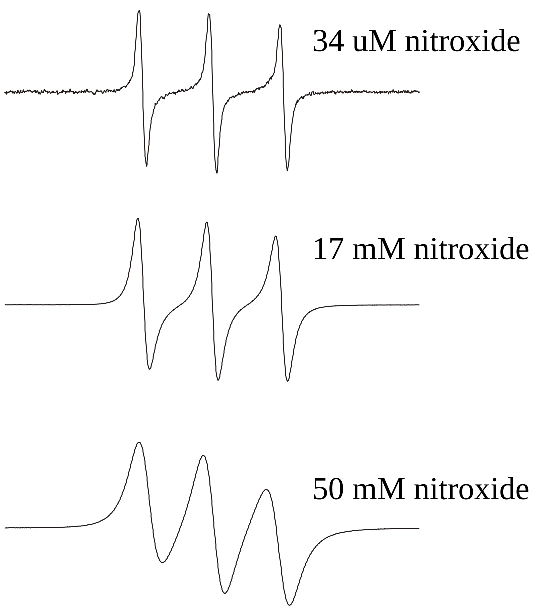
# Lineshapes

- Line shapes help evaluate molecular dynamics.

## Effects from molecular motion




## Effects from spin-spin relaxation



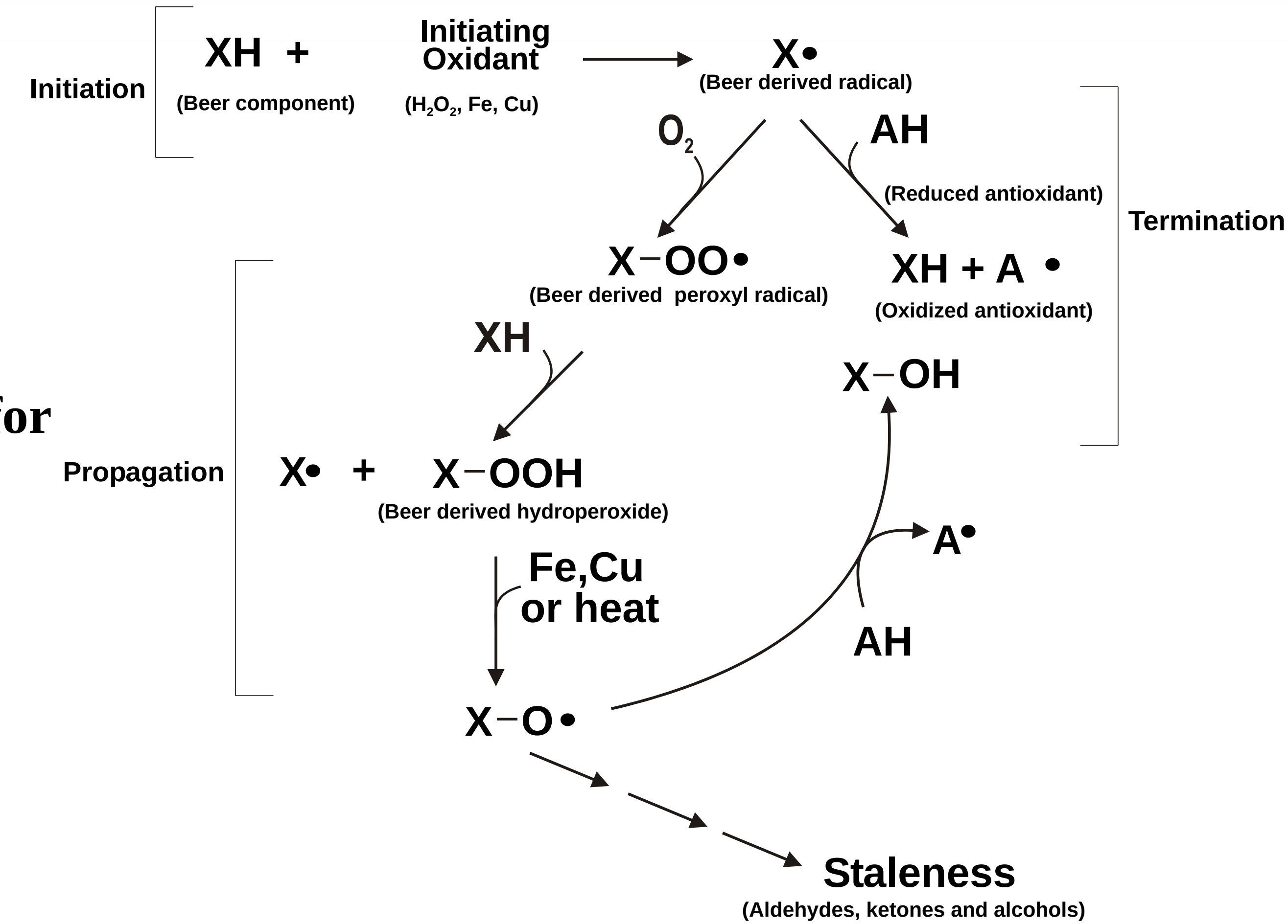
# CW Applications
# Beer

- Long distribution channels make fresh beer a challenge.
- Excess O2 and improper storage/processing promote free radical oxidation.
- Beer has antioxidants that protect it from premature staling
- When antioxidant levels get low, oxidation of beer components increases and stale flavors result.
- Breweries need to optimize antioxidants in their final product.

# CW Applications
# Beer

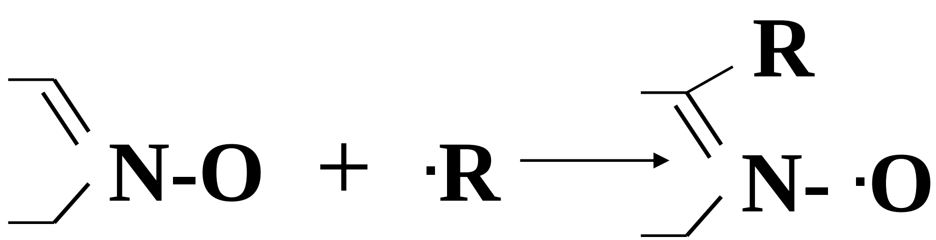
- As beer stales…EPR measures free radicals.

**Free radical mechanism for beer staling**



# CW Applications
# Beer

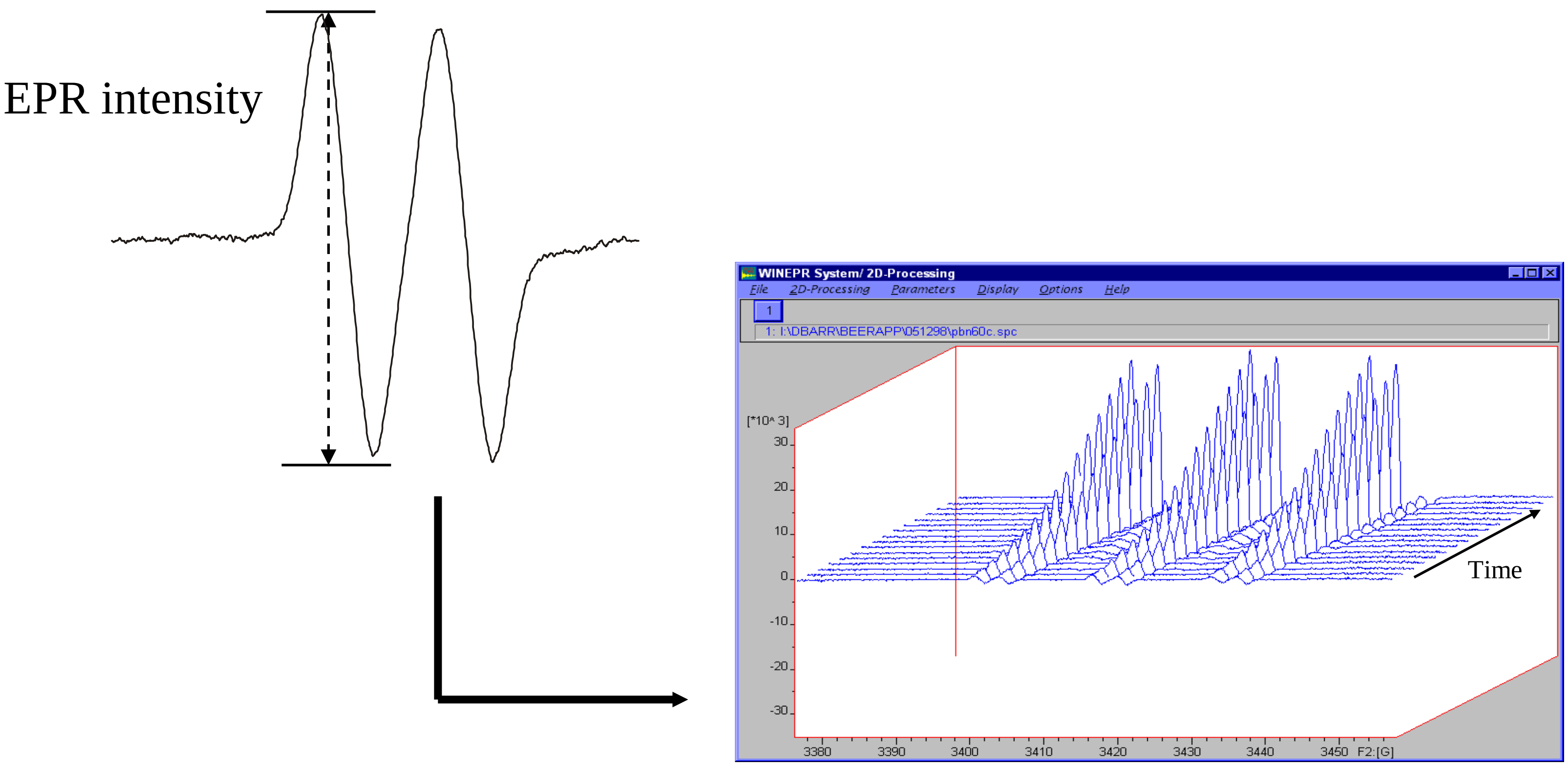
- Spin traps are nitrone or nitroso compounds used to "trap" short-lived free radicals that are otherwise not detectable.
- A covalent bond forms between the radical and the spin trap and a stable nitroxide radical is formed (often called a "spin adduct").
- The spin adduct is detected by EPR. Its spectrum provides a "signature" for the type of radical that was trapped.




(spin trap) (radical) spin adduct

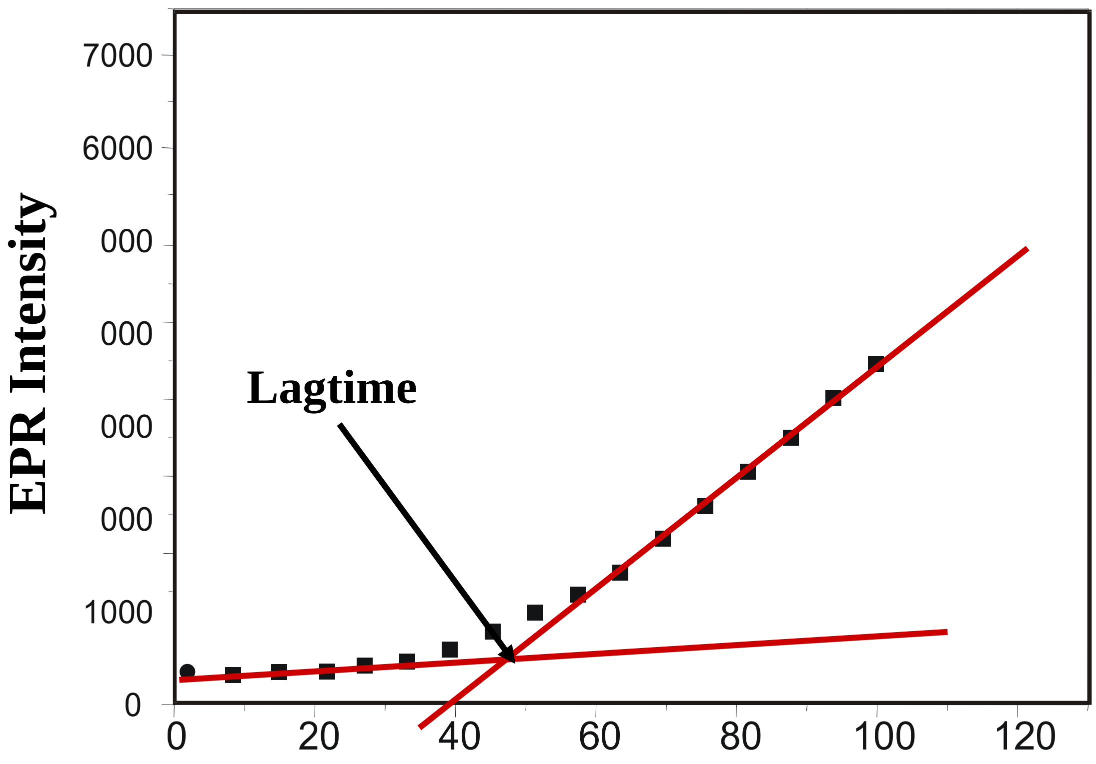
# CW Applications
# Beer

- Force age the beer at 60 C.



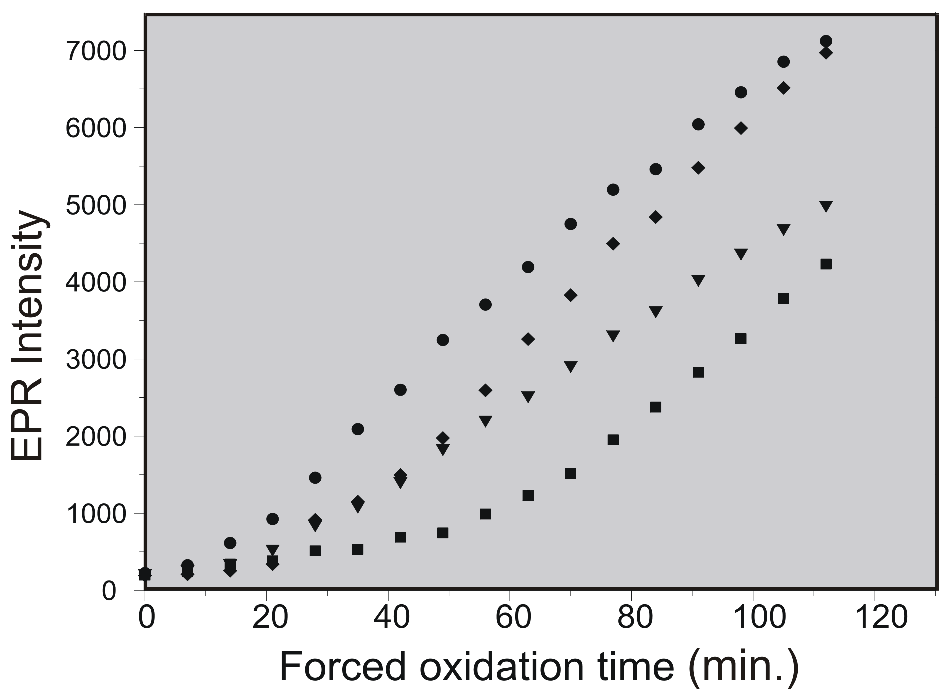
# CW Applications
# Beer

- Antioxidants in the beer cause a “lagtime”.



# CW Applications
# Beer

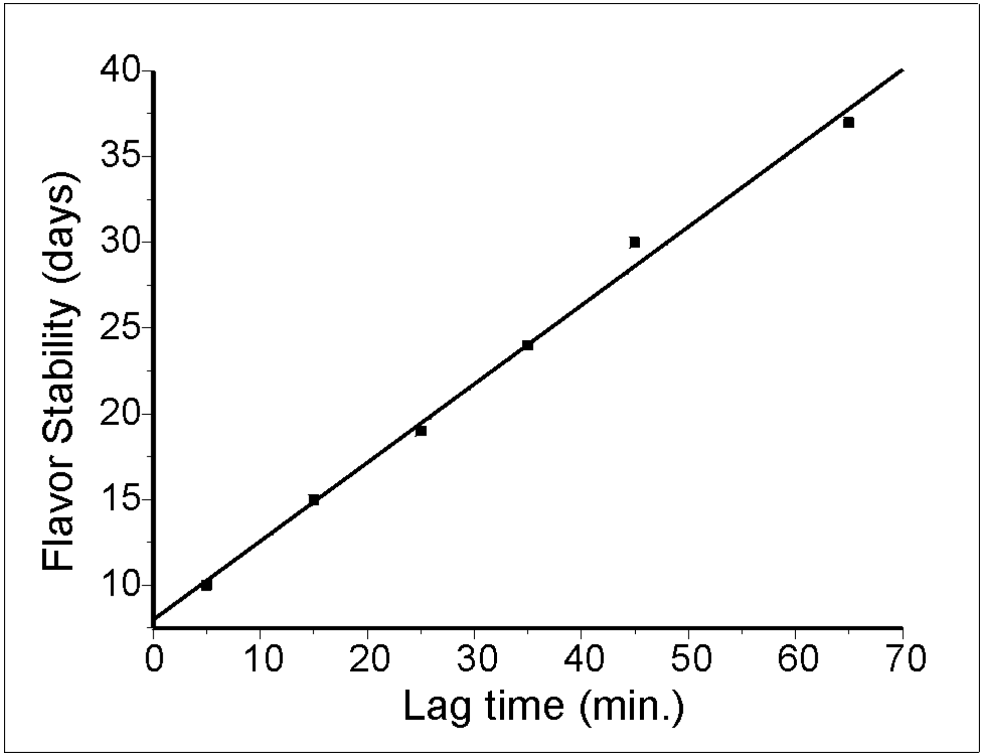
- Different beers have different lagtimes.




**Results from four popular American lagers**

# CW Applications
# Beer

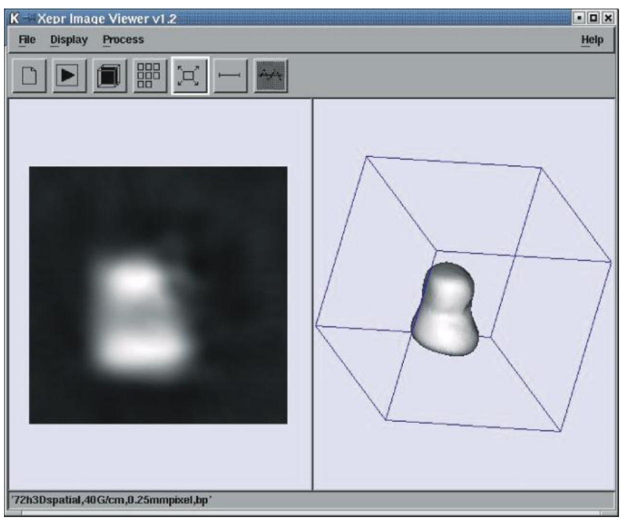
- Lagtimes correlate with flavor stability scores from sensory analysis.



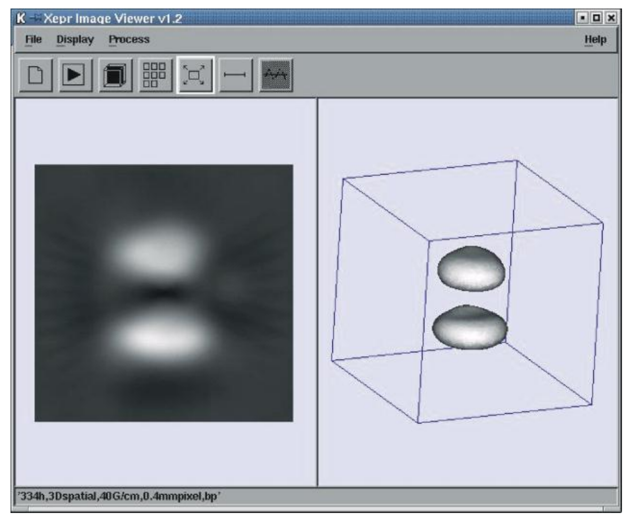
# CW Applications
# Imaging and Polymers

- EPR can be used to image radical distributions, just as NMR is used in MRI.

## Polymer Degradation: X-Band Imaging



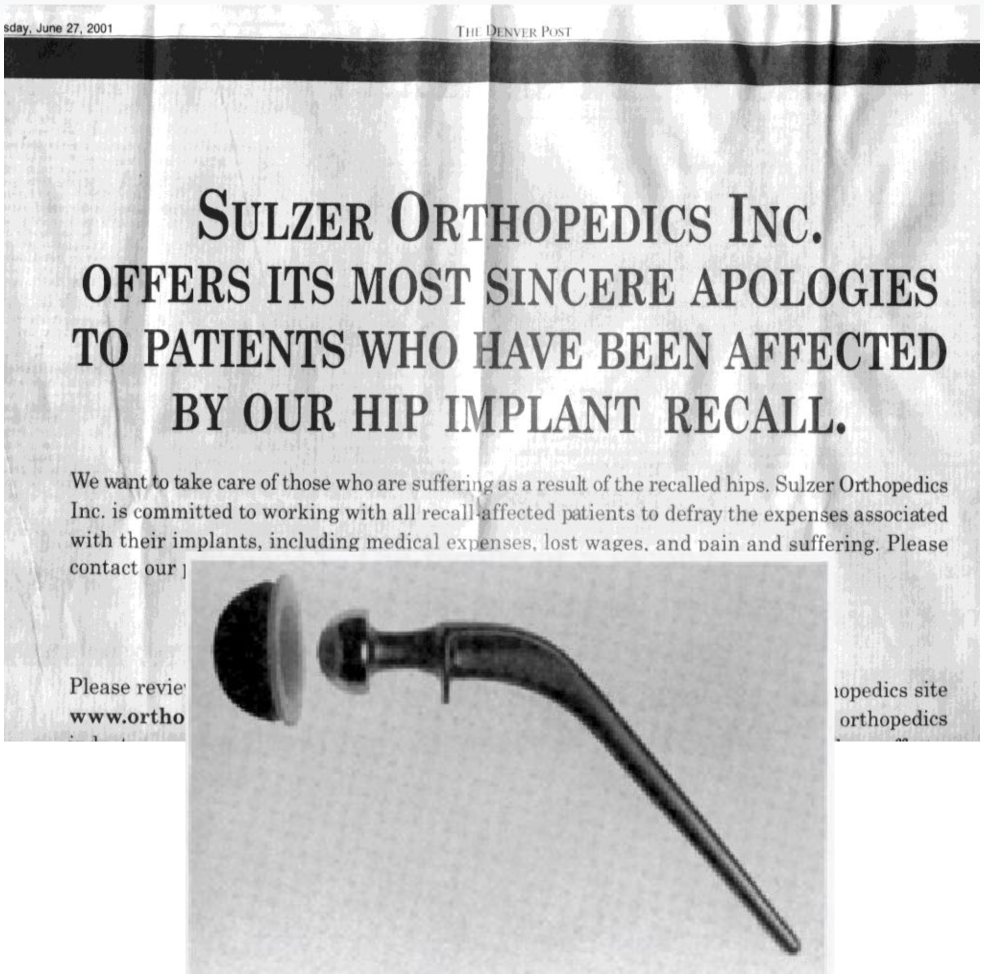

*3 days*




*14 days*

*In collaboration with S. Schlick*

# CW Applications
# Imaging and Polymers


sday, June 27, 2001 THE DENVER POST

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Please revie ... iopedics site
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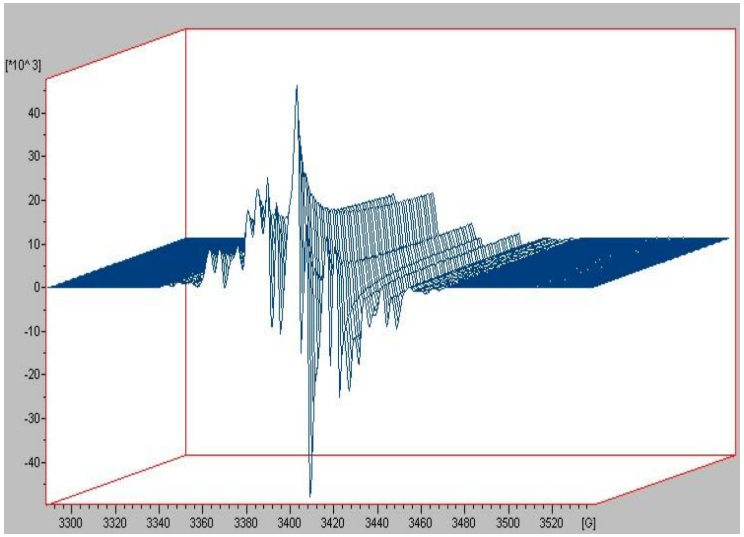
- Multi billion $ industry
- High growth
- Highly competitive
- Patients are outliving implants
- Oxidation is a problem.

# CW Applications
# Imaging and Polymers

- Cross-linking (via radical-radical recombination) improves wear properties.
- Incomplete recombination make the polymer susceptible to oxidative aging.
- Post irradiation heat treatment anneals radicals in the bulk material.
- EPR provides a rapid, direct method for evaluating the annealing process.

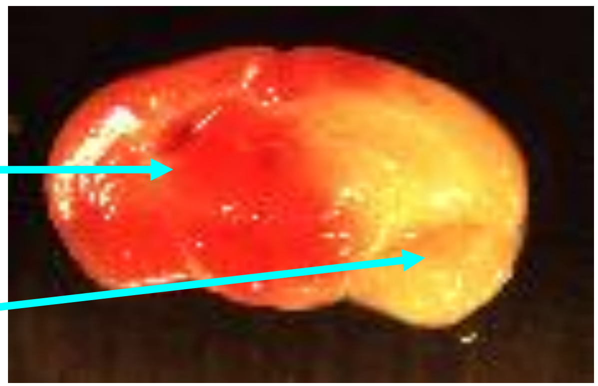



Heat treatment of irradiated UHMWPE

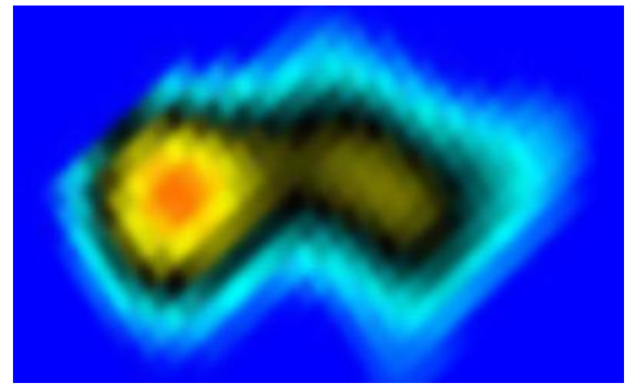
# CW Applications
# Imaging and in vivo Oximetry

Viable tissue

Dead tissue



Histological Image



*In vivo* EPR Image

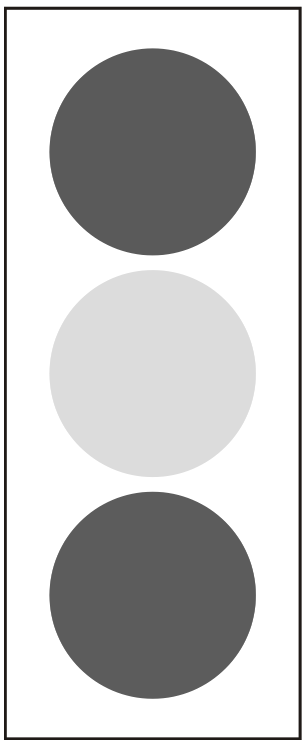
*Data and Images courtesy of J. Liu, S. Liu, and G. Timmins*

# CW Applications
# Multi-frequency EPR

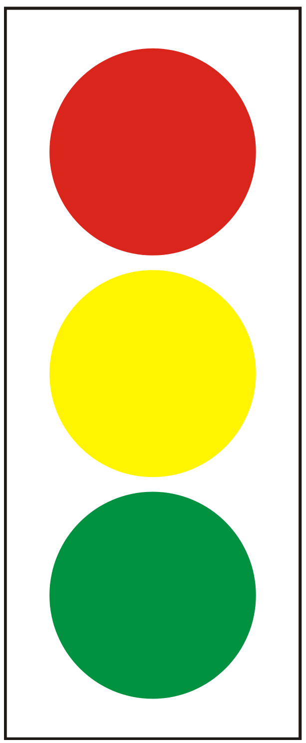
- X-band works very well.
  - It is sensitive.
  - Samples are a convenient size.
  - Much spectrometer operation can be automated.
- Multi-frequency EPR gives a more complete picture.
  - Appearance of EPR spectra depends strongly on the interplay of magnetic field dependent and independent interactions.
  - Multi-frequency EPR resolves the two contributions for an unambiguous interpretation.

# CW Applications
# Multi-frequency EPR

*X-band view of a traffic light*



*Multi-frequency view of a traffic light*

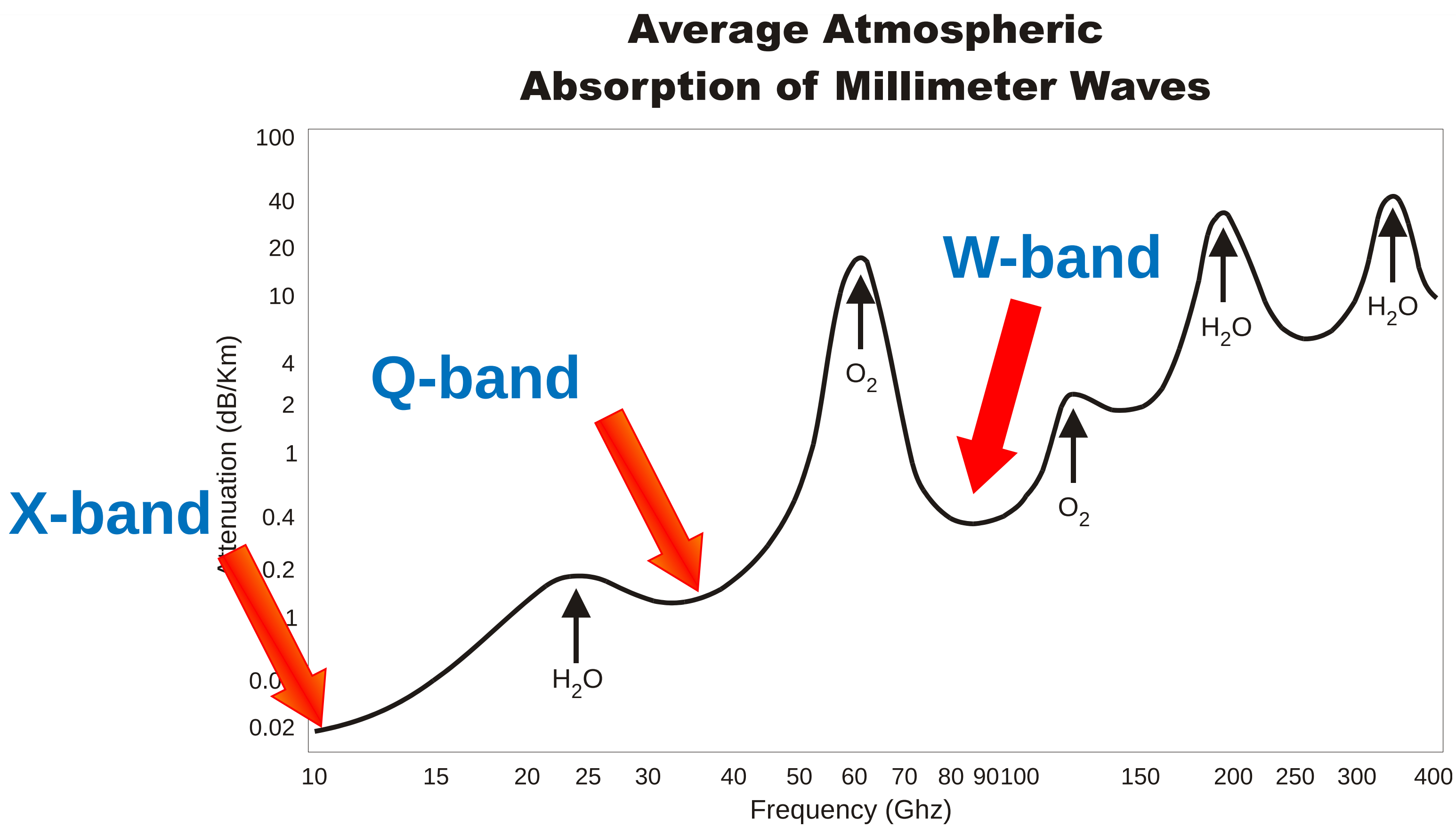


# CW Applications
# Multi-frequency EPR

| Microwave Frequency Band | Microwave Frequencies (GHz) | Common Microwave Frequencies for EPR (GHz) |
|---|---|---|
| L | 1-2 | ~1 |
| S | 2-4 | ~4 |
| C | 4-8 | |
| X | 8.2-12.4 | 9.2-9.9 |
| $K_u$ | 12.4-18 | |
| K | 18-26.5 | ~24 |
| Q | 26.4-40 | ~34 |
| V | 40-75 | |
| W | 75-110 | ~94 |

# CW Applications
# Multi-frequency EPR

- Common EPR frequencies are no accident.



# CW Applications
# Multi-frequency EPR

- $\beta_e$ = Bohr magneton
- $S$ and $I$ = electronic and nuclear spin operators
- g = electronic g matrix
- $B_0$ = the externally applied magnetic field
- D = the ZFS (Zero Field Splitting) tensor
- A = the nuclear hyperfine matrix

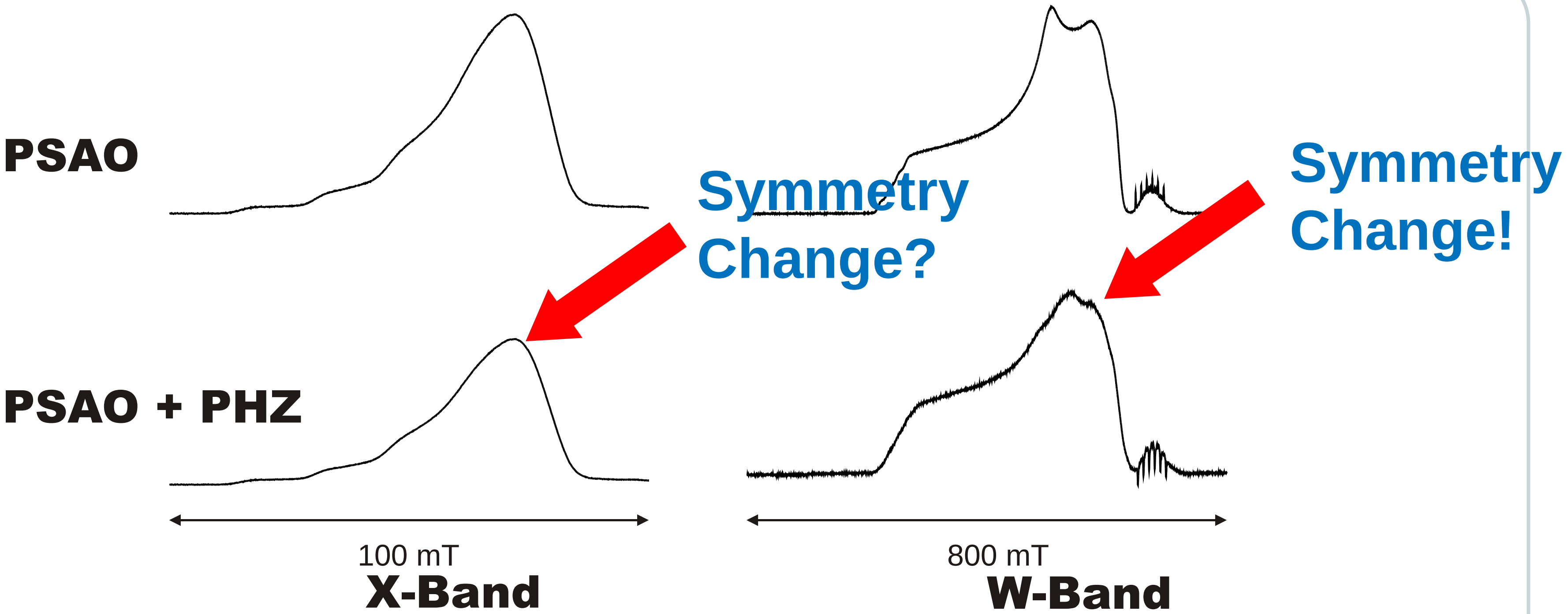
$$H = \underbrace{\beta_e S \cdot g \cdot B_0}_{\text{Magnetic Field Dependent}} + \underbrace{S \cdot D \cdot S + S \cdot A \cdot I}_{\text{Magnetic Field Independent}}$$

# CW Applications
# Multi-frequency EPR

- g-values
  - Identification of a paramagnetic species
  - Electronic state
  - Symmetry of site
- ZFS
  - Spin state
  - Valence state
  - Symmetry of site
- Hyperfine Couplings
  - Identity of nuclei
  - Number of nuclei
  - Distance of nuclei

# CW Applications
# Multi-frequency EPR

- Pea seedling amine oxidase and the inhibitor phenylhydrazine.

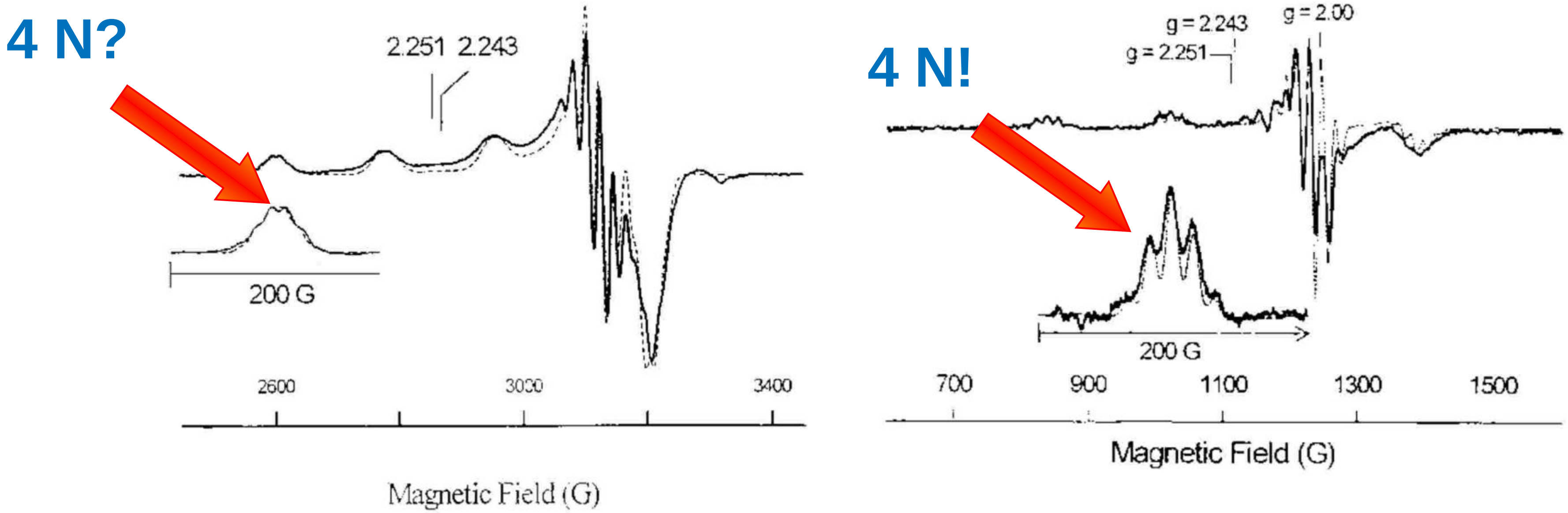



W-band shows symmetry change of the $Cu^{+2}$ site upon binding the inhibitor

Sample courtesy of Prof. J. L. McCracken

# CW Applications
# Multi-frequency EPR

- Type 2 $Cu^{+2}$ in pMMO (particulate Methane MonoOxygenase) from Methylomicrobium album BG8 grown with $^{15}N$ and $^{63}Cu^{+2}$.

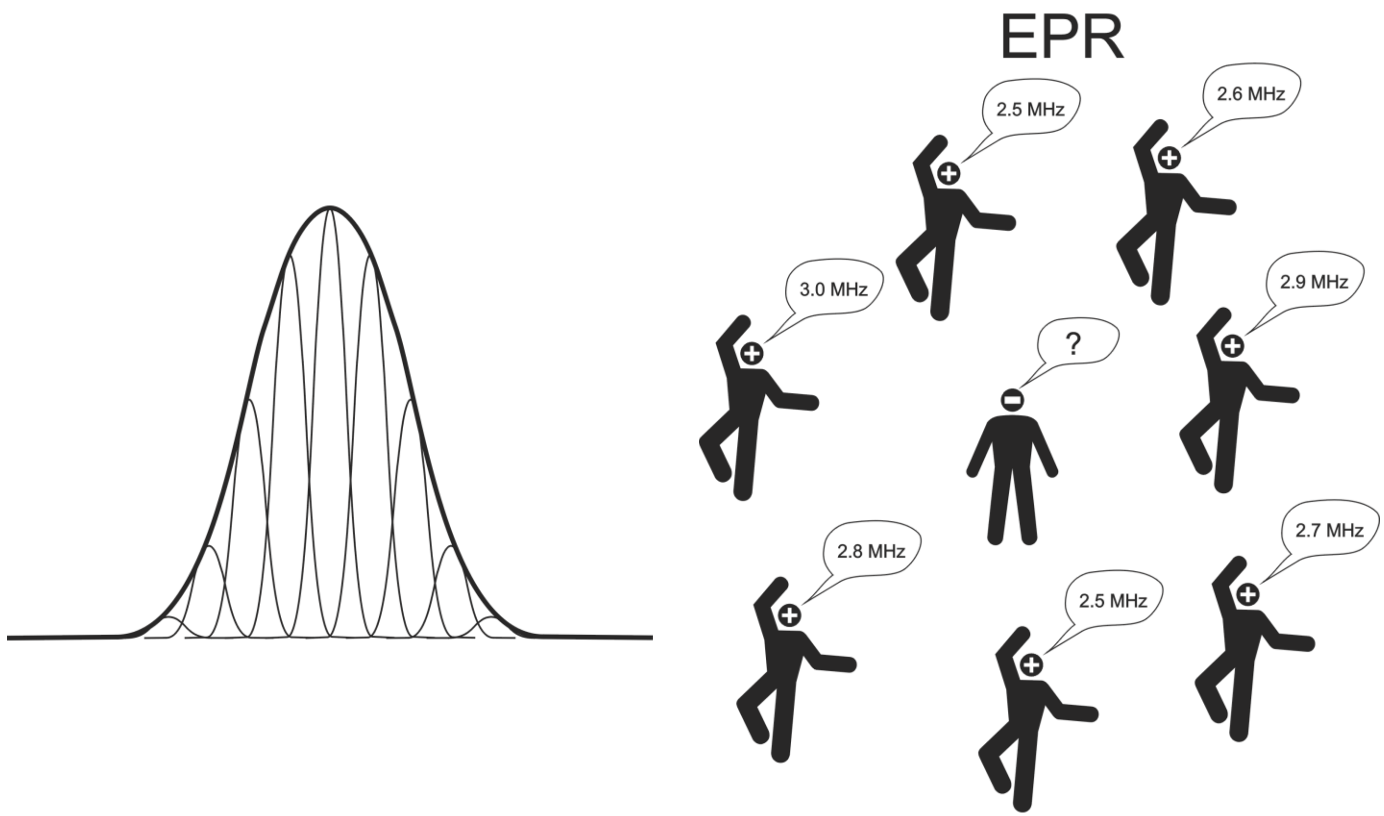



S-band spectrum shows the number of nitrogen ligands

Spectra courtesy of Prof. W.E. Antholine

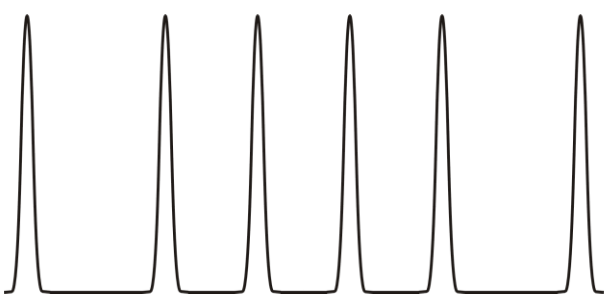
# Pulse Applications
# ESEEM and ENDOR

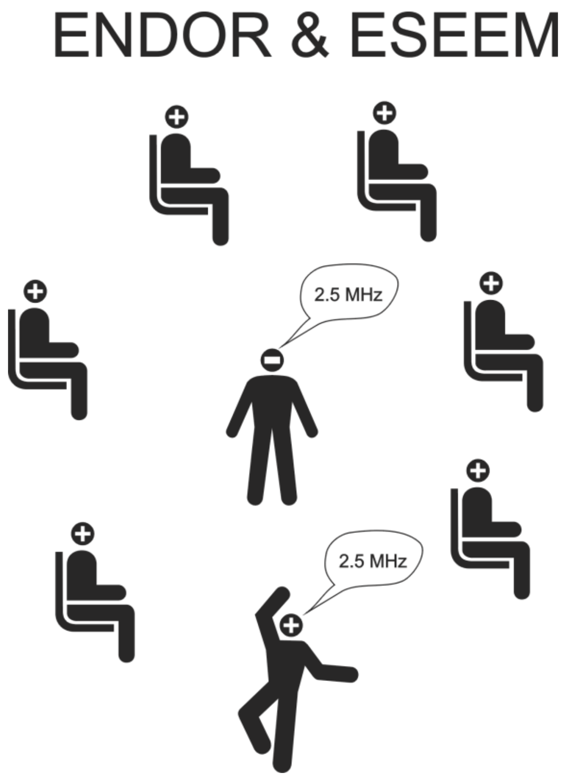
- Often details in an EPR spectrum can be obscured.



# Pulse Applications
# ESEEM and ENDOR

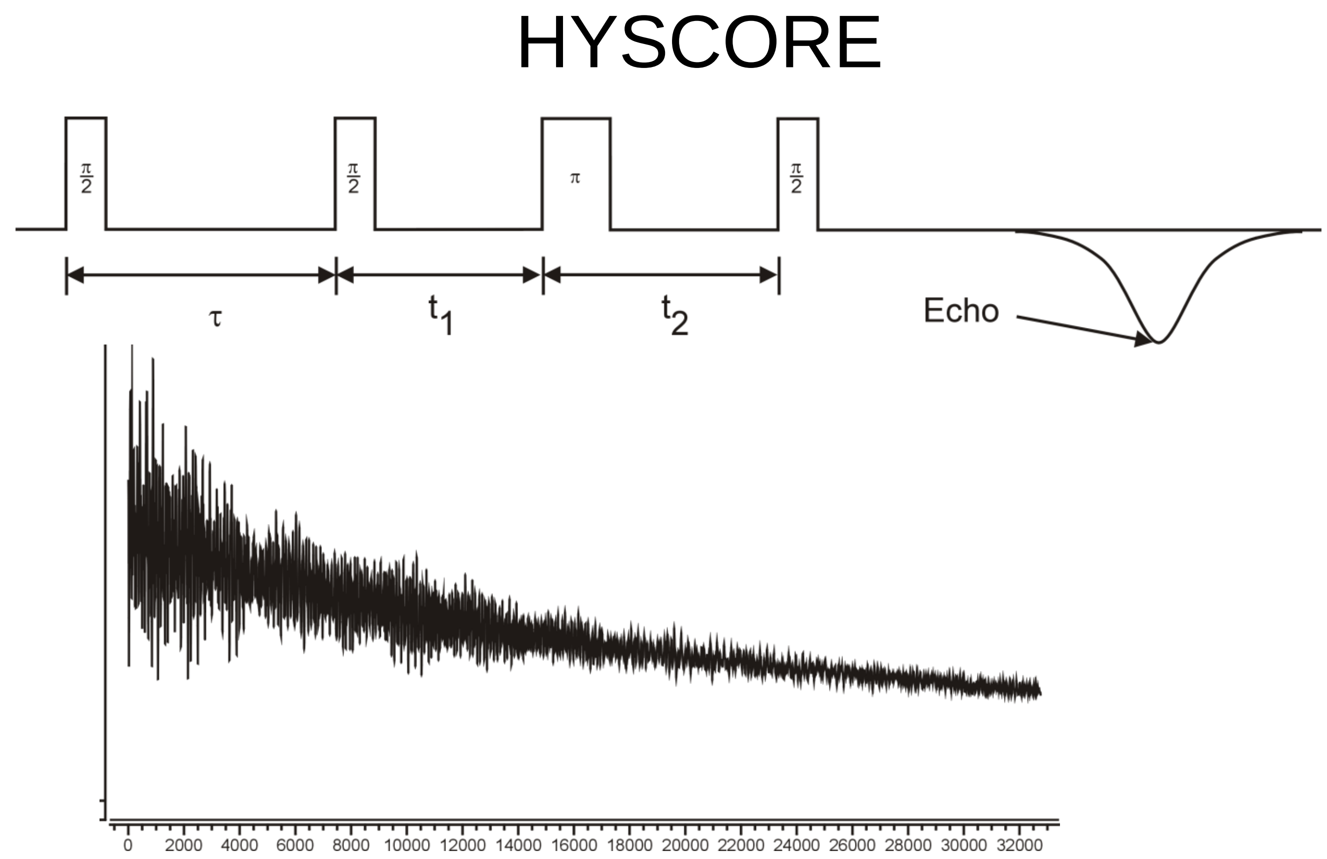
- ENDOR (Electron Nuclear Double Resonance and ESEEM (Electron Spin Echo Envelope Modulation) can restore the resolution.





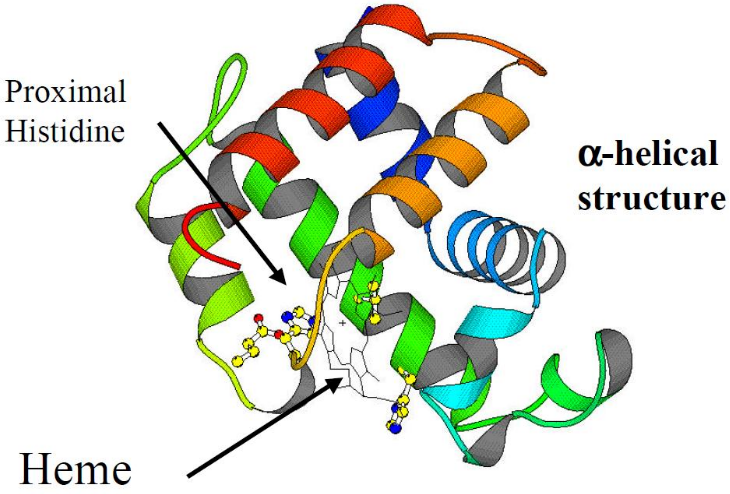
# Pulse Applications
# ESEEM and ENDOR

- The HYSCORE experiment.



# Pulse Applications
# ESEEM and ENDOR

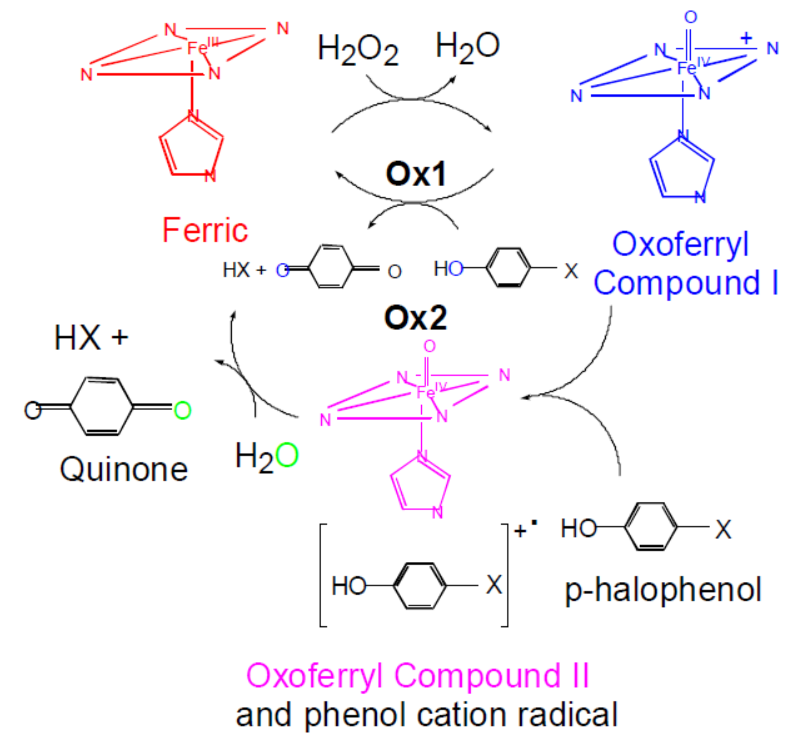
- DHP (Dehaloperoxidase) was first isolated from the marine worm *Amphitrite ornata*.
- DHP is a dimeric hemoglobin that also has significant peroxidase activity under physiological conditions.
- T.I Smirnova, R.T. Weber, M.F. Davis, and S. Franzen, JACS 130(7), 2128 (2008).




Courtesy of Prof. S. Franzen

# Pulse Applications
# ESEEM and ENDOR

- DHP dehalogenates halophenols.




Courtesy of Prof. S. Franzen

# Pulse Applications
# ESEEM and ENDOR

- Binding of the substrate TFP (2,4,6-trifluorophenol) cause a change in the EPR spectrum.
- Iron remains in the high spin state.
- What is happening at the iron site?

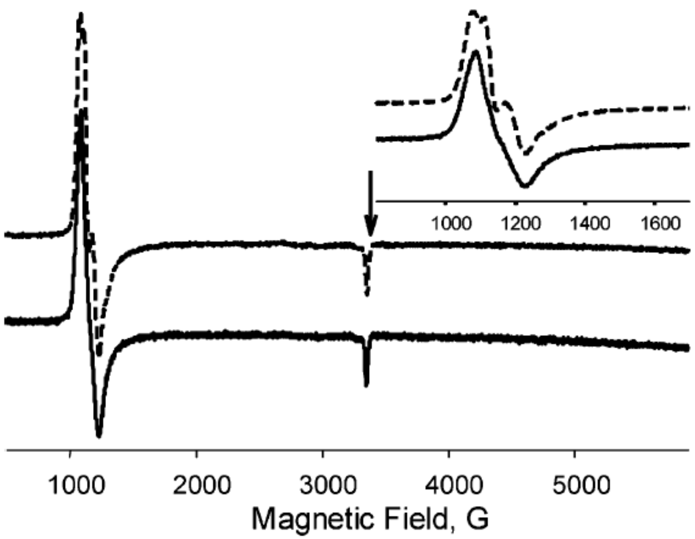



***Figure 1.*** CW X-band (9.5 GHz) EPR spectra of DHP in the absence (dotted line) and in the presence (solid line) of 10-fold excess of TFP at pH 6.0 and $T = 4$ K. Arrow indicates the field of HYSCORE experiment.

# Pulse Applications
# ESEEM and ENDOR

- Strongly coupled protons with A = 6.2 MHz.
- Weakly coupled protons with A ~ 2.5 MHz consistent with nonexchangeable heme and proximal histidine hydrogen atoms.
- Nitrogen resonances from the porphyrin ring and proximal histidine.
- Strongly coupled proton signals disappear upon addition of $D_2O$.
- Strongly coupled proton signals disappear upon binding of TFP.
- No $^{19}F$ resonances.

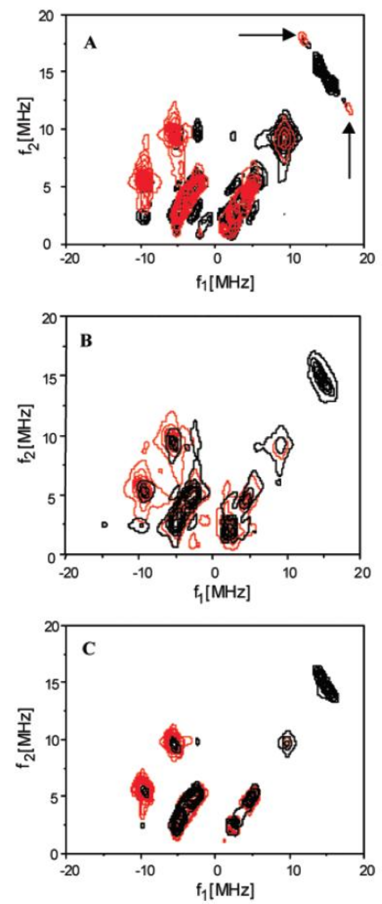



*Figure 2.* HYSCORE spectra of DHP in pH = 6.0 buffers prepared from $H_2O$ (A) and $D_2O$ (B) and DHP in $H_2O$ buffer with 10-fold excess of TFP (C). The spectra were recorded at magnetic field of 346 mT with $\tau$ =128 ns (red trace) and 100 ns (black trace) at $T$ = 4.5 K.

# Pulse Applications
# ESEEM and ENDOR

- Strongly coupled protons with A = 6.2 MHz are consistent with exchangeable heme-bound water.
- Binding of TFP results in a displacement of the heme-bound water.
- Transition from six- to five-coordinated iron.
- TFP does not bind directly to the heme iron.

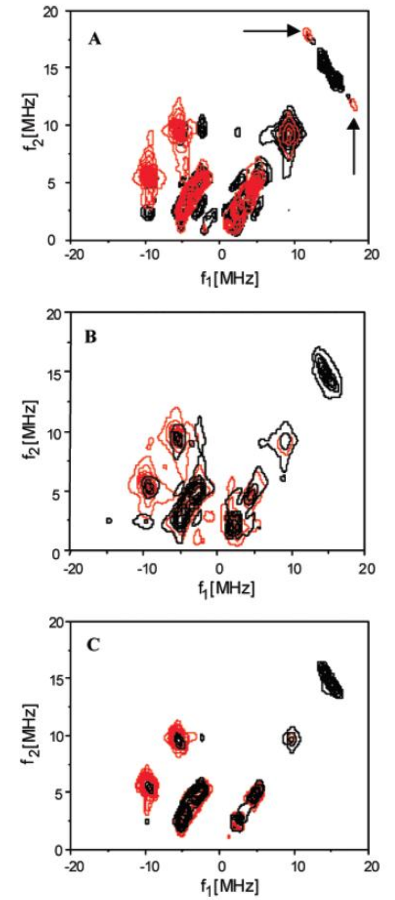
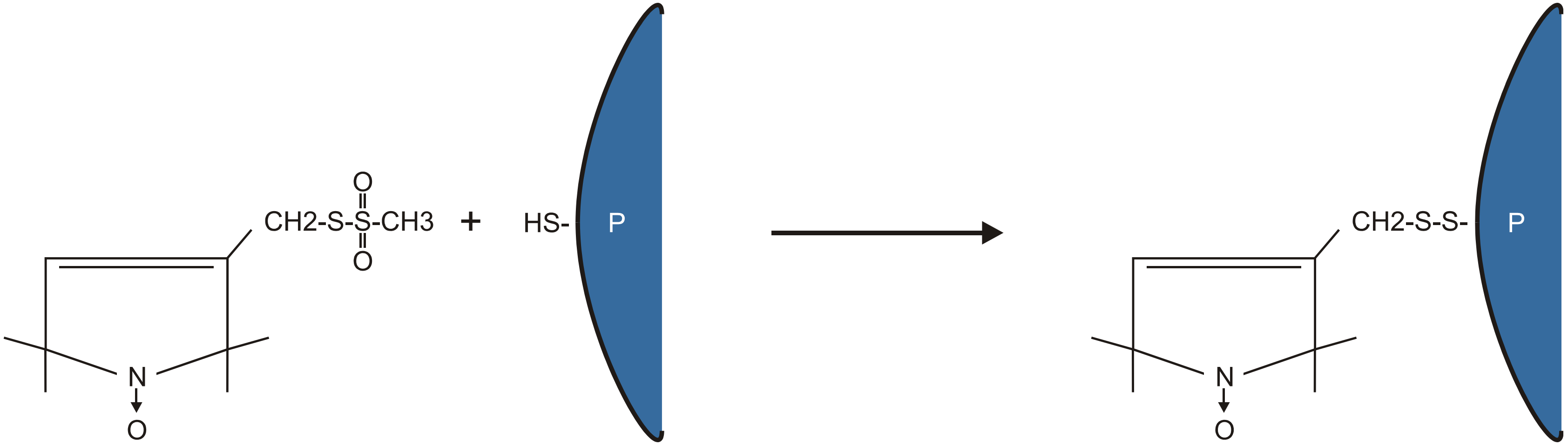



***Figure 2.*** HYSCORE spectra of DHP in pH = 6.0 buffers prepared from $H_2O$ (A) and $D_2O$ (B) and DHP in $H_2O$ buffer with 10-fold excess of TFP (C). The spectra were recorded at magnetic field of 346 mT with $\tau$ = 128 ns (red trace) and 100 ns (black trace) at $T$ = 4.5 K.

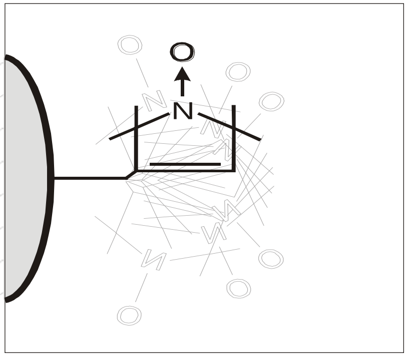
# CW & Pulse Applications
# Site Directed Spin Labeling



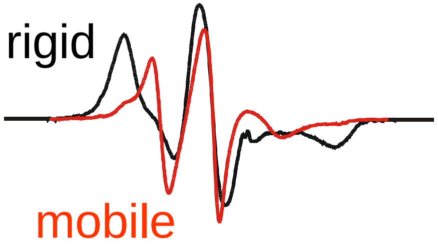

Courtesy of Prof. Eduardo Perozo

# CW & Pulse Applications
# Site Directed Spin Labeling

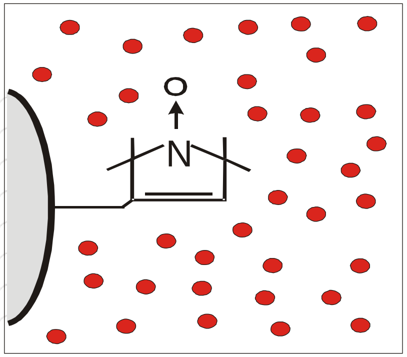
## Probe Mobility



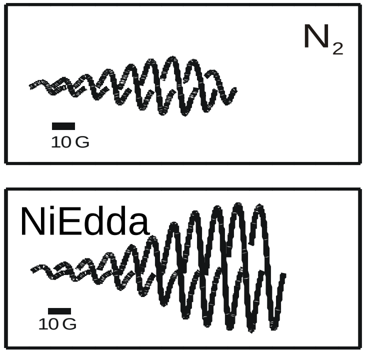

From line-shape analysis:




## Solvent Accessibility



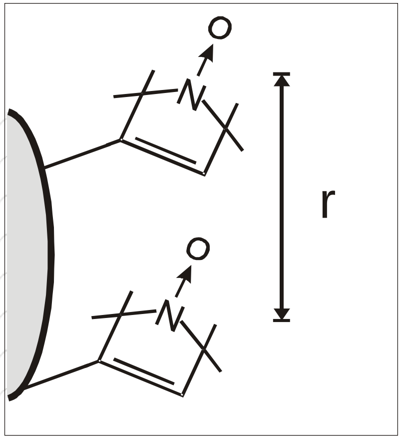

From power saturation:




Courtesy of Prof. Eduardo Perozo

# CW & Pulse Applications
# Site Directed Spin Labeling

## Works up to 1.5 nm

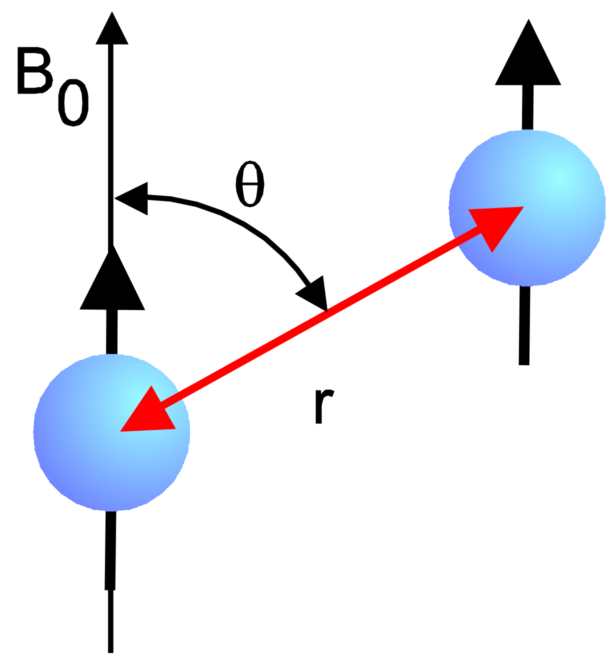



From dipolar broadening:


Courtesy of Prof. Eduardo Perozo

# CW & Pulse Applications
# Site Directed Spin Labeling

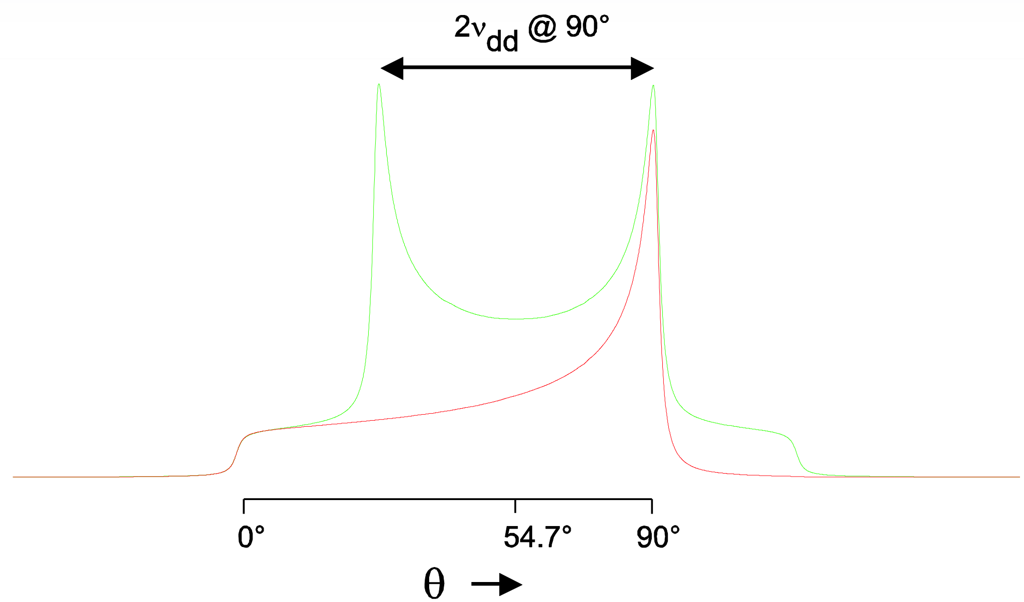
- Electron-electron dipolar interaction.




$$\nu_{dd}(r,\theta) = \frac{\mu_0 g_1 g_2 \mu_B}{2h} \cdot \frac{1}{r^3} \cdot (3\cos\theta^2 - |1)$$

# CW & Pulse Applications
# Site Directed Spin Labeling

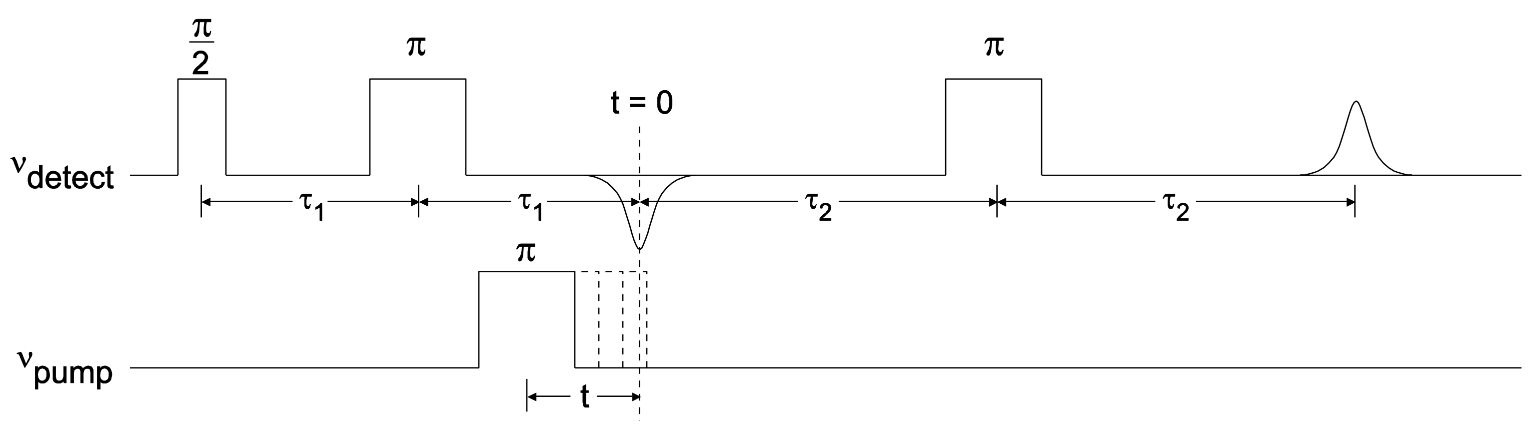
- Electron-electron dipolar interaction.
- Pake pattern.




$$r = \sqrt[3]{\frac{52.16\ \text{MHz}}{\nu_{dd}(90^\circ)}}\ \text{nm}$$

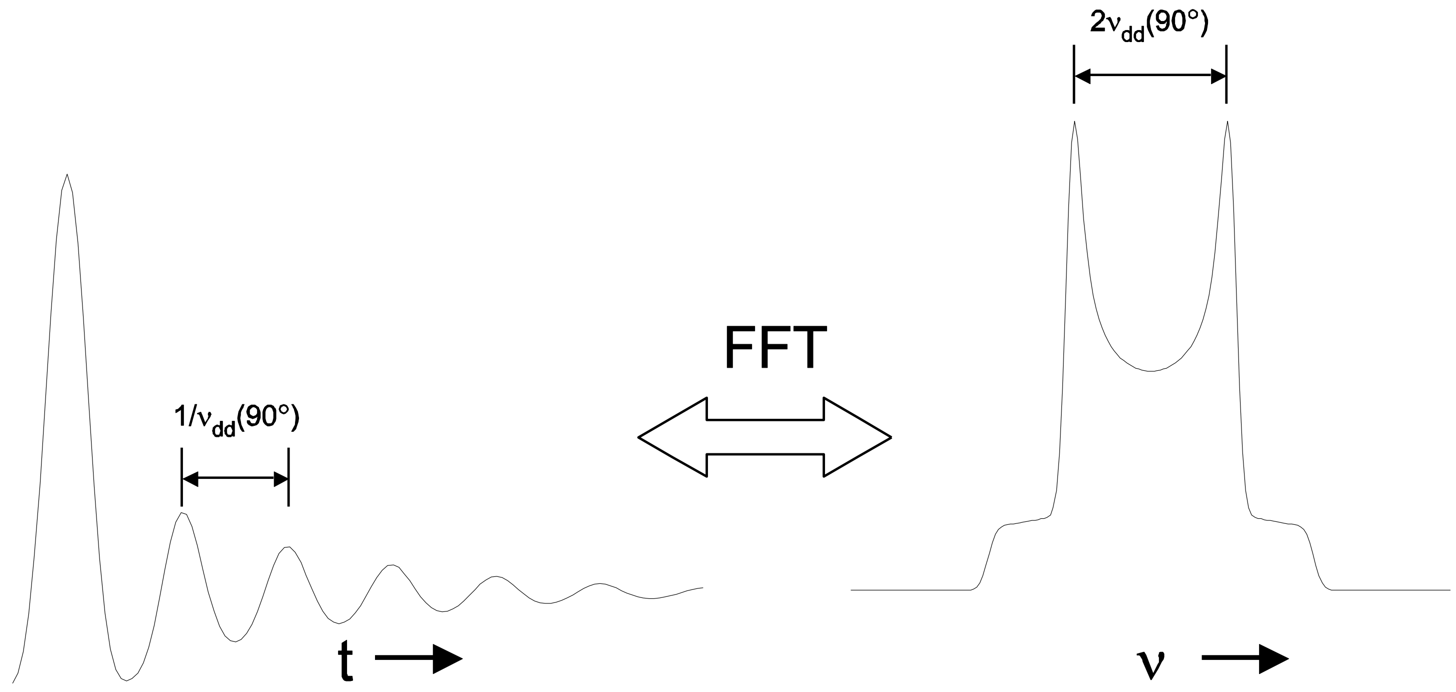
# CW & Pulse Applications
# Site Directed Spin Labeling

- DEER (Double Electron-Electron Resonance).
- Also called PELDOR (Pulse Electron Double Resonance).
- Works for distances from 2-8 nm.
- Pulse sequence.

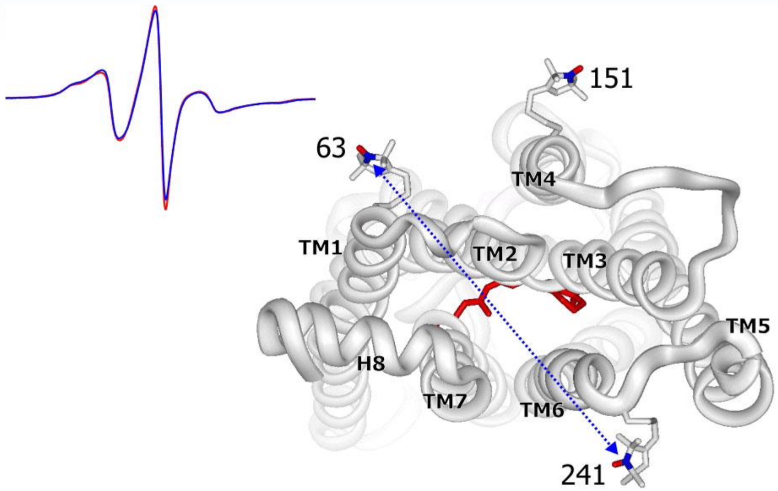


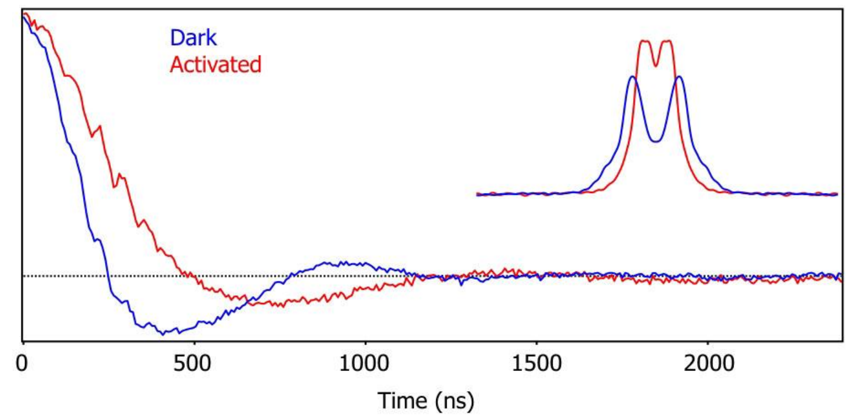
# CW & Pulse Applications
# Site Directed Spin Labeling

- Time and frequency domain behavior of DEER data.



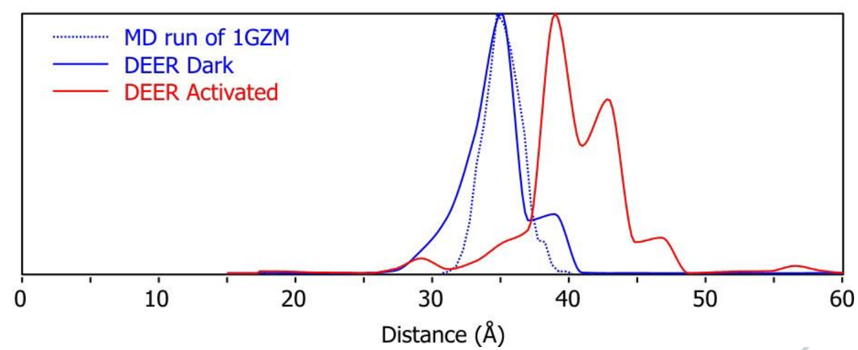
# CW & Pulse Applications
# Site Directed Spin Labeling




**Rhodopsin: activated - dark**

**$\Delta r = 6$ Å**




**Data courtesy Prof. Wayne Hubbell**

Innovation with Integrity