Pulse EPR Spectroscopy: ENDOR, ESEEM, DEER

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Stefan Stoll
University of Washington, Seattle
stst@uw.edu

Some References:
Books

Reviews
<table>
<thead>
<tr>
<th>Technique</th>
<th>Energy (eV)</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mössbauer</td>
<td>14400</td>
<td>$(^{57}\text{Fe})$</td>
</tr>
<tr>
<td>XAS/XES</td>
<td>7000</td>
<td>(Fe K-edge)</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>2</td>
<td>(600 nm)</td>
</tr>
<tr>
<td>IR/Raman</td>
<td>0.01</td>
<td>(800 cm$^{-1}$)</td>
</tr>
<tr>
<td>EPR</td>
<td>0.000004</td>
<td>(10 GHz = 40 μeV)</td>
</tr>
<tr>
<td>ENDOR etc.</td>
<td>0.000000004</td>
<td>(1 MHz = 4 neV)</td>
</tr>
</tbody>
</table>
Coupled spins

Crystallography view:
Structural cartoon

Magnetic resonance view:
System of coupled spins

1 unpaired electron spin on Fe$^{3+}$ ($S = 1/2$)
all magnetic nuclei ($^1H$, $^2H$, $^{14}N$, $^{15}N$, $^{13}C$, ...)
nonmagnetic nuclei invisible ($^{12}C$, $^{16}O$, $^{32}S$)
Information from cw EPR and pulse EPR

Pulse EPR: Set of high-resolution EPR techniques to determine local structure around a spin center (metal ion, metal cluster, or radical)

**CW EPR: low resolution**
- metal center, (ligands)
- strongly coupled magnetic nuclei

**Pulse EPR: high resolution**
- weakly coupled magnetic nuclei ($^1$H, $^{14}$N, $^{13}$C, etc)
- ligands in first and second ligand sphere
- hydrogen bonds
- distances to other spin centers

magnetic nuclei within a few Å electron spins with a few nm
Hidden details in solid-state CW EPR spectra

Origins of static line broadenings
1. anisotropies of $g$ tensor, $A$ tensor, $D$ tensor
2. site-to-site structural heterogeneity resulting in $g$, $A$, $D$ heterogeneity
3. unresolved splittings
   - hyperfine coupling to magnetic nuclei
   - coupling to other electron spins

Hidden structure

pulse EPR
1. Basics

CW vs. pulse EPR
Sample and spectrometer
Resonators and bandwidths
Pulses, excitation width
Orientation selection
FIDs and Echo
Deadtime, Relaxation

2. Interactions

Nuclear Zeeman interaction
Hyperfine interaction
Coupling regimes
Nuclear spectra
Quadrupole interaction

3. Experiments

Field sweeps
ENDOR
ESEEM
HYSCORE
DEER
Comparison CW and pulse EPR

**CW (continuous-wave) EPR**
- continuous excitation
- low microwave power (μW-mW)
- absorption spectroscopy
- measures steady-state response during excitation
- low resolution

**Pulse EPR**
- pulse excitation
- very high microwave power (W-kW)
- emission spectroscopy
- measures transient response after excitation
- high resolution

**EPR frequencies and fields**

- low-field EPR
- standard EPR
- high-field EPR

**EPR unit conversions**
- Energy units: 30 GHz = 1.00 cm\(^{-1}\) = 0.124 meV = 1.20 J/mol
- Field to frequency: 1 mT = 28 MHz @ \(g = 2\)
- Field units: 1 mT (millitesla) = 1 G (gauss)
How to make samples for EPR

Sample quantity and positioning
- know O.D. and I.D. of EPR sample tube
- fill no more than fits in the resonator

Things to watch out for:
(1) **Unwanted dioxygen**
- oxygen-sensitive samples
- dissolved dioxygen enhances relaxation
- important for liquid samples
- remove by freeze/pump/thaw, or Ar purging

(2) **Other paramagnetic centers**
- avoid paramagnetic impurities
- run controls on buffers and reagents
- use quartz (“fused silica”) tubes

(3) **Aggregation**
- due to slow freezing, solvent crystallization
- enhances relaxation, shortens $T_m$, $T_1$
- add glassing agent (glycerol, sucrose), freeze fast

(4) **Dielectric constant**
- high $\varepsilon_r$ solvents kill mw fields in resonator
- sensitivity loss
- worst: liquid water ($\varepsilon_r = 80$ at 20°C)
- frozen water: ($\varepsilon_r = 3.15$ at 0°C)

Sample concentration
magnetically dilute
- cw EPR: < 1 mM
- pulse EPR: ESEEM/ENDOR: max 5 mM
- DEER: less than 200 μM

Too concentrated?
- broadened spectra
- enhanced relaxation

Too dilute?
- Not enough signal.

**EPR measurement temperatures** (approx)
- organic radicals 30-200 K
- mononuclear metal centers 5-40 K
- oligonuclear metal clusters 2-10 K
Frozen solutions; lab and molecular frame

Most common form of bioinorganic EPR samples: frozen aqueous solutions of proteins.

Frozen solution = random uniform distribution of static orientations of the molecules, like a dilute powder.

Lab frame
- fixed in laboratory
- $z(\text{lab})$ along static field $B_0$
- $x(\text{lab})$ along oscillating microwave field $B_1$

Relative orientation

Euler angles $\varphi, \theta, \chi$

Molecular frames
- fixed in molecules
- most commonly molecular symmetry frame or g tensor frame

$B_0$

$B_1$

$z(\text{lab})$
$y(\text{lab})$
$x(\text{lab})$

$z(\text{mol})$
$y(\text{mol})$
$x(\text{mol})$

$\varphi$
$\theta$
$\chi$
Pulse EPR spectrometer

- cw sources
- pulse formers
- high-power amplifier
- high-power attenuator
- circulator
- protection switch
- phase
- low-noise amplifier (LNA)
- digitizer
- computer
- amplifiers
- mixer
- mw transmitter
- mw receiver
- mw2
- mw1
- amplifier
- ENDOR transmitter
- high-power amplifier
- rf1
- PFU
- attenuation
- digitizer computer
- high mw power
- low mw power
- video signal
- magnet
- sample resonator cryostat
- B_0
Pulse EPR spectrometer
Resonators and bandwidth

Why to use a resonator?
+ concentrates microwave magnetic field ($B_1$) on sample; higher signal intensity
+ separates microwave electric field from sample; lower sample heating
- downside: works only for a very narrow range of frequencies

Types of resonators
1. dielectric (ring, split-ring)
2. cavity (rectangular, cylindrical)
3. loop-gap resonators

Resonator Q factor and bandwidth

$$Q = \frac{\nu_0}{\Delta \nu}$$

- resonator frequency
- bandwidth (undercoupled)

$Q$ factor; range: 100 - 10000

$\nu_0$ = 9.70 GHz
$Q_u$ = 1000

$cw$ EPR: high sensitivity
→ high $Q$, critically coupled

$pulse$ EPR: large bandwidth
→ high $Q$ + overcoupled, or low $Q$ + critically coupled
Microwave irradiation reorients spins

**Resonance condition:**

\[ h\nu_{\text{mw}} = g\mu_B B \]

- **Equilibrium:** Spin is aligned with the magnetic field.
- **Non-equilibrium:** Spin is not aligned with the magnetic field due to microwave irradiation.

**Rotating frame:**
- Follow precession (I = running along with the merry-go-round)
- Nutation

**Planck constant:** 6.626·10^{-34} J s

**Bohr magneton:** 9.274·10^{-24} J/T

**G factor**

\[ \frac{\nu_{\text{mw}}}{\text{GHz}} = \frac{B}{\text{mT}} \]

- 71.447732 GHz = \frac{B}{\text{mT}}
- 1 mT = 10 G
- 1 G = 2.8 MHz @ \( g = 2 \)
Pulses and excitation bandwidth

Rectangular pulse

- Excitation bandwidth = approx. distance between zeroes: $2/t_p$
  - Example: 10 ns pulse → 200 MHz

Microwave pulses (for electron spins)
- Frequency: 9-10 GHz, 34-36 GHz, 95 GHz
- Short: 5-20 ns
- Medium: 20 ns-200 ns
- Long: 200 ns-several μs

RF pulses (for nuclear spins)
- Frequency: 1-200 MHz
- Short: 10 μs
- Long: 100 μs
Spin gymnastics and Energy level diagrams

Classical description: Bloch equations (limited to a single spin)
Quantum description: Liouville-von Neumann equation (general)
Spectral width and pulse excitation bandwidth

- Only a small fraction of spins in the sample are excited.
- They have resonance frequencies close to the mw frequency.
- They have specific orientations → orientation selection.

EPR absorption intensity

Frequency (GHz)

Spectral width: several GHz

- \( g = 1.83 \) pulse hits spins within 10-100 MHz of mw frequency
- \( g = 2.00 \) mw pulse at 9.5 GHz
- \( g = 2.55 \)
Orientation selection

Resonance frequency depends on orientation relative to field.
Different orientation → different resonance frequency
Free induction decay (FID)

- Spins in thermal equilibrium
- Pulse rotates spins by 90 degrees
- Spins precess with different frequencies

- All are in phase
- Dephasing

$B_0$

90° dead time
Dead time

**Dead time:**
- time after pulses during which power levels are too high to open the sensitive receiver
- due to 1) ringdown in cavity
  2) reflections in spectrometer
  3) recovery of receiver protection
- typical value: 100 ns at X-band
- shorter at higher frequencies
- affects all pulse EPR experiments

**Consequences**
- short values of $\tau$ cannot be accessed
- loss of broad lines
- phase distortions in spectrum
- spurious features in spectrum

1 kW = 1 mile
1 $\mu$W = 0.0016 mm
Two-pulse echo

also called primary echo or Hahn echo

90° pulse

180° pulse

two-pulse echo

(\(= 2x\) FID)

\(\tau\)

\(B_0\)

thermal equilibrium

rotated by 90° precessing and dephasing

dephased

rotated by 180° precessing and rephasing

refocused
Three-pulse echo

also called stimulated echo

- Thermal equilibrium
- Rotated by 90° precessing and dephasing
- Dephased
- "Stored" along -z, precessing
- Complex motion (approximate)
- Precessing and rephasing
- Refocused
Relaxation constants

$T_1$: longitudinal relaxation (spin-lattice relaxation)
$T_2$: transverse relaxation (spin-spin relaxation)
$T_m$: phase memory time (similar to $T_2$)

Spectral diffusion
- spin center randomly changes frequency during pulse sequence
- leads to dephasing and loss of signal
- contributes to $T_m$

cw EPR
- choose low mw power that avoids saturation
- choose scan rates, modulation amplitudes and frequencies that avoid passage effects

pulse EPR
- fast relaxation prevents long pulse experiment
- slow relaxation prevents fast repetition
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Magnetic nuclei and their interactions

Nuclear spin Hamiltonian (for one nuclear spin coupled to one electron spin):

\[ H_{\text{nuc}} = -g_n \mu_N B \cdot I + h S \cdot A \cdot I + h I \cdot P \cdot I \]

**Nuclear Zeeman interaction**
Magnetic interaction with external applied magnetic field (static or oscillating)

**Hyperfine interaction**
Magnetic interaction of nucleus with field due to electron spin

Two contributions:
1. through-bond (isotropic; "Fermi contact")
2. through-space (anisotropic; dipolar)

**Nuclear quadrupole interaction**
Electric interaction between nonspherical nucleus and inhomogeneous electric field

Only for nonspherical nuclei (spin > 1/2)!
Nuclear Zeeman Interaction

*magnetic* interaction of magnetic nucleus with external applied magnetic field (static, oscillating)

\[ \mathcal{H} = -B_0 \cdot \mu_{\text{nuc}} = -g_n \mu_N B_0 \cdot I \]

- magnetic field
- nuclear magnetic moment
- nuclear g factor

Nuclear precession frequency:

\[ \nu_I = -g_n \mu_N B_0 / \hbar \]

NMR: gyromagnetic ratio \( \gamma = g_n \mu_N / \hbar \)

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Spin</th>
<th>%</th>
<th>( g_n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{63}\text{Cu})</td>
<td>3/2</td>
<td>69</td>
<td>+1.484</td>
</tr>
<tr>
<td>(^{65}\text{Cu})</td>
<td>3/2</td>
<td>31</td>
<td>+1.588</td>
</tr>
<tr>
<td>(^{53}\text{Cr})</td>
<td>3/2</td>
<td>9.5</td>
<td>-0.3147</td>
</tr>
<tr>
<td>(^{55}\text{Mn})</td>
<td>5/2</td>
<td>100</td>
<td>+1.3819</td>
</tr>
<tr>
<td>(^{57}\text{Fe})</td>
<td>1/2</td>
<td>2.1</td>
<td>+0.1806</td>
</tr>
<tr>
<td>(^{59}\text{Co})</td>
<td>7/2</td>
<td>100</td>
<td>+1.318</td>
</tr>
<tr>
<td>(^{61}\text{Ni})</td>
<td>3/2</td>
<td>1.1</td>
<td>-0.5000</td>
</tr>
</tbody>
</table>

no spin: \(^{56}\text{Fe}\), \(^{58}\text{Ni}\), \(^{60}\text{Ni}\), etc.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Spin</th>
<th>%</th>
<th>( g_n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{1}\text{H})</td>
<td>1/2</td>
<td>99.99</td>
<td>+5.58569</td>
</tr>
<tr>
<td>(^{2}\text{H})</td>
<td>1</td>
<td>0.01</td>
<td>+0.857438</td>
</tr>
<tr>
<td>(^{14}\text{N})</td>
<td>1</td>
<td>99.6</td>
<td>+0.403761</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>1/2</td>
<td>0.4</td>
<td>-0.566378</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>1/2</td>
<td>1.1</td>
<td>+1.40482</td>
</tr>
<tr>
<td>(^{17}\text{O})</td>
<td>5/2</td>
<td>0.04</td>
<td>-0.757516</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>1/2</td>
<td>100</td>
<td>+2.2632</td>
</tr>
</tbody>
</table>

no spin: \(^{12}\text{C}\), \(^{16}\text{O}\), \(^{32}\text{S}\), etc.
**Hyperfine coupling: 1. Fermi contact interaction**

\[ \mathcal{H} = h A_{\text{iso}} \mathbf{S} \cdot \mathbf{I} \]

**Origin:**
Small, but finite, probability of finding an electron at position of nucleus (s orbitals only!)

\[ A_{\text{iso}} = \frac{2 \mu_0 \mu_B \mu_N}{3} g_e g_n |\psi_0(r_n)|^2 \]

(SI units, \( A_{\text{iso}} \) in Hz)

\[ A_{\text{iso}} = \frac{1}{3} \frac{\mu_0 \mu_B \mu_N}{h} g_e g_n |\psi_0(r_n)|^2 \]

more general

(scales* with \( g_n \) spin density at position of nucleus)

Nucleus | Spin | \( A_{\text{iso}}(100\%) \)
---|---|---
\(^1\text{H}\) | 1/2 | 1420 MHz
\(^{14}\text{N}\) | 1 | 1811 MHz, 1538 MHz
\(^{15}\text{N}\) | 1/2 | -2540 MHz, -2158 MHz
\(^{13}\text{C}\) | 1/2 | 3777 MHz, 3109 MHz

alternative: compare to quantumchemical estimates

**Chemist's interpretation:**
spin population in atom-centered orbitals relative to 100% orbital occupancy via reference \( A_{\text{iso}} \)

**Reasons for non-zero \( A_{\text{iso}} \):**
1. ground-state open s shell
2. valence and core polarizations e.g. 3d\( \rightarrow \)2s, 3d\( \rightarrow \)1s
3. configurations with open s shell

Example:
\[ A_{\text{iso}}(^1\text{H}) = 20 \text{ MHz} \rightarrow 20/1420 = 1.4\% \]
Hyperfine coupling: 2. Through-space dipolar coupling

This assumes electron is localized.
In delocalized systems, integrate over electron spin density.
Combining Hyperfine and Zeeman: Local fields

$B_0$ Zeeman field

$B_{\text{tot}}$ total field

$B_{\text{hf}}$ hyperfine field due to electron spin

At equilibrium, nuclear spin aligns along total field.
Combining Hyperfine and Zeeman: Local fields

$B_0$ Zeeman field

$B_{\text{hf}}$ hyperfine field due to electron spin

$B_{\text{tot}}$ total field

Electron

Nucleus

changed!

flipped!
Hyperfine + Zeeman: Nuclear frequencies

External (Zeeman) magnetic field $B_0$ and total field acting on the nucleus $B_{\text{tot}}$.

Component of hyperfine field perpendicular to external field (nonsecular)

$$B = (A_{||} - A_{\perp}) \sin \theta \cos \theta$$
$$= 3T_{\perp} \sin \theta \cos \theta$$

can be neglected at high field for small hfc

Component of hyperfine field parallel to external field (secular)

$$A = A_{||} \cos^2 \theta + A_{\perp} \sin^2 \theta$$
$$= A_{\text{iso}} + T_{\perp} (3 \cos^2 \theta - 1)$$

Nuclear frequencies:

$$\nu(m_s) = \sqrt{\nu_1 + m_s A)^2 + (m_s B)^2}$$

$$\nu_1 = -g_n \mu_N B_0 / h \quad m_s = \pm 1/2$$
Hyperfine vs. Zeeman: Three regimes

**Weak coupling**

$|B_0| \gg |B_{hf}|$

- Matching fields

**Intermediate coupling**

$|B_0| \approx |B_{hf}|$

- Angle between two total field vectors:
  \[
  \sin^2 \xi = \left( \frac{\nu_1 B}{\nu_\alpha \nu_\beta} \right)^2 = k
  \]

**Strong coupling**

$|B_0| \ll |B_{hf}|$

$k = \text{modulation depth parameter (important in ESEEM)}$
Nuclear frequencies and powder spectra

\[ \nu(m_S) = \sqrt{(\nu_1 + m_S A)^2 + (m_S B)^2} \]

\[ \nu(m_S) \approx |\nu_1 + m_S A| \]

\[ \nu_1 = -g_n \mu_B B_0 / h \]

neglecting \( m_S B \) term
(valid for weak and strong coupling only)

**Weak coupling regime** \( |\nu_1| \gg |m_S A| \)

centered at \( \nu_1 \), split by \( A \)

**Strong coupling regime** \( |\nu_1| \ll |m_S A| \)

centered at \( A/2 \), split by \( 2\nu_1 \)
Nuclear Quadrupole Interaction: Basics

(1) Some nuclei have electric quadrupole moment
- Nuclei with spin >1/2 are nonspherical, described by an electric quadrupole moment $Q$.

\[
\begin{array}{c}
Q > 0 \\
\text{prolate nucleus}
\end{array}
\quad
\begin{array}{c}
Q < 0 \\
\text{oblate nucleus}
\end{array}
\]

- Spin is tied to nuclear shape!

(2) Inhomogeneous electric fields in molecules: electric field gradient (EFG) at nuclei

(3) Quadrupole nuclei have orientation-dependent energy
- electric, not magnetic interaction!

\[
\begin{array}{c}
\text{Nucleus} \\
\text{Spin} \\
\text{Quadrupole moment (b)}
\end{array}
\]

\[
\begin{array}{ccc}
{^2}\text{H} & 1 & +0.00286 \\
{^{14}}\text{N} & 1 & +0.02044 \\
{^{33}}\text{S} & 3/2 & -0.0678 \\
{^{63}}\text{Cu} & 3/2 & -0.22 \\
{^{17}}\text{O} & 5/2 & -0.02558 \\
{^{55}}\text{Mn} & 5/2 & +0.33
\end{array}
\]

$1 \text{ b (barn)} = 100 \text{ fm}^2$
Nuclear Quadrupole Interaction: Mathematics

**Electric field gradient (EFG) at nucleus**

EFG is a 3x3 matrix $V$

Principal values $V_{xx}, V_{yy}, V_{zz}$

$|V_{zz}| \geq |V_{yy}| \geq |V_{xx}|$

$V_{xx} + V_{yy} + V_{zz} = 0$

Largest component $V_{zz} = eq$

Rhombicity

$$\eta = \frac{V_{xx} - V_{yy}}{V_{zz}}$$

$$0 \leq \eta \leq 1$$

sign of $q$ ambiguous for $\eta = 1$

**Spin Hamiltonian term**

Interaction of quadrupole moment with EFG

$$\mathcal{H} = \hbar \mathbf{I} \cdot \mathbf{P} \cdot \mathbf{I}$$

nuclear spin vector

quadrupole tensor

$$\mathbf{P} = \frac{e^2 Qq}{4I(2I-1)} \begin{pmatrix} -(1 - \eta) & 0 & 0 \\ 0 & -\eta(1 + \eta) & 0 \\ 0 & 0 & +2 \end{pmatrix}$$

Experimental parameters:

$$e^2 Qq/h$$ and $\eta$

quadrupole moment and EFG asymmetry

**Imidazole ligands:** EFG at $^{14}$N depends on electron populations of $2p_{x,y,z}$ orbitals

D$_2$O: $e^2 Qq/h = 0.213$ MHz, $\eta = 0.12$
Nuclear Quadrupole Interaction: $^{14}$N, $^2$H, $^{17}$O, $^{33}$S

$^2$H

$I = 1$ gives 2x3 lines

Length of H-bonds to semiquinones

Length of H-bonds to semiquinones

$K = a - \frac{b}{r_{O-D}^3}$

J. Biol. Chem. 2012 287 4662 link

$^{14}$N

EFG depends on electron populations $N_{x,y,z}$ of 2p$_{x,y,z}$ orbitals

very useful for imidazole ligands!

$^{33}$S

$I = 5/2$ gives 2x6 lines

mCoM reductase, $^{33}$S HYSCORE JACS 2005 127 17744 link

$^{17}$O

$I = 5/2$ gives 2x6 lines

Aconitase, $^{17}$O ENDOR J. Biol. Chem. 1986 261 4840 link
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EPR spectrum: Field sweep spectra

FID-detected field sweep

- works only if FID is longer than dead time
- use long microwave pulse

Echo-detected field sweep

Distortions due to tau-dependent nuclear modulation of echo amplitude
Relaxation measurements

$T_1$: Inversion recovery

measure echo intensity as a function of $t$

$V(t) = 1 - 2 \exp(-t/T_1)$

Other methods for $T_1$: saturation recovery, three-pulse echo decay

$T_2, \ T_m$: Two-pulse echo decay

measure echo intensity as a function of $\tau$

- approximately exponential decay
- phase memory, $T_m$, rather than $T_2$ is obtained
- best with small flip angles (avoids instantaneous diffusion)
Nuclear spectra: Mims ENDOR

**Mims ENDOR**: rf pulse frequency is varied

- rf pulse frequency is varied
  - mw: 90°, 180°
  - rf: echo

**Basics**
- use short hard mw pulses
- acquire echo intensity as function of rf pulse frequency

**Spectrum**
- echo intensity decreases whenever rf frequency is resonant with a nuclear transition
- upside-down representation

**Blind spots**
- intensity is modulated with τ-dependent sawtooth pattern, centered at Larmor frequency and with period 0.5/τ ("Mims holes")
- central hole at Larmor frequency!

works best for small hyperfine couplings less than about 1/τ (typically $^2\text{H}$, $^{13}\text{C}$)
Nuclear spectra: Davies ENDOR

Davies ENDOR: rf frequency is varied

Basics
- based on inversion recovery
- use medium/long mw pulses
- acquire echo intensity as function of rf pulse frequency

Spectrum
- fully inverted echo is baseline
- decrease in echo intensity when rf frequency is resonant with nuclear transition

Blindspots
- no $\tau$-dependent blindspots
- central hole at Larmor frequency
- width proportional to $1/t_p$
- suited for larger hf couplings
- for small couplings, use long pulses (narrower central hole)
ENDOR example: Weak coupling $^2$H, $^{13}$C

S-adenosyl-methionine (SAM) binding to [4Fe4S] cluster in pyruvate formate-lyase activating enzyme (PFL-AE)

Broderick & Hoffman
JACS 2002 124 3143 [link]
ENDOR example: Strong coupling $^{55}\text{Mn}$

$[\text{Mn}_4\text{Ca}]$ cluster in PSII

$S_2$: Mn(III,III,III,IV)

ESE-ENDOR Amplitude

(d) $S_2$-NH$_3$

ESE-ENDOR Amplitude

(c) $S_2$-untreated

JACS 2000 122 10926
Nuclear spectra: ESEEM

electron spin echo envelope modulation

Two-pulse ESEEM: $\tau$ is varied

- modulation of echo amplitude as a function of interpulse delay(s)
- modulation with nuclear resonance frequencies and their combinations
- modulation due to hyperfine coupling of electron spin with surrounding nuclei
- modulation depth depends on hyperfine coupling, quadrupole coupling, nuclear Zeeman

Three-pulse ESEEM: $T$ is varied

- modulation depth/amplitude

- modulation depth/amplitude

- modulation depth/amplitude
(1) Electron spin flip induces nuclear precession

- Electron spin flip inverts hyperfine field at nucleus.
- This changes the total local field and the quantization direction of the nucleus.
- The change is sudden on the timescale of the nucleus.
- The nucleus will precess around the new field direction.
ESEEM: Pictorial model

(2) Nuclear precession modulates electron precession

- precessing nucleus causes a time-dependent field along z felt by the electron
- electron precession frequency becomes time dependent

ESEEM: Data processing

1. **Raw data: ESEEM time trace**
   - Time scale: 0 to 6 μs
   - Frequency scale: 0 to 20 MHz

2. **Fit exponential decay**
   - Time scale: 0 to 6 μs

3. **Subtract exponential decay**
   - Time scale: 0 to 6 μs

4. **Fourier transform, magnitude**
   - Frequency scale: 0 to 20 MHz

5. **Zero filling**
   - Time scale: 0 to 10 μs

6. **Apply apodization window**
   - Time scale: 0 to 6 μs
Nuclear spectra: ENDOR vs. ESEEM

ENDOR

\[ \nu_\alpha \quad \nu_\beta \]

equal intensities

Two-pulse ESEEM

\[ \nu_\alpha - \nu_\beta \quad \nu_\alpha + \nu_\beta \]

\[ k/2 \quad k/2 \]

\[ \nu_\alpha \quad \nu_\beta \quad -k/4 \quad -k/4 \]

\[ T_m \text{ decay (fast)} \]

sum and difference frequencies

no blind spots

Three-pulse ESEEM

\[ \frac{k}{4} (1 - \cos \omega_\beta \tau) \]

\[ \frac{k}{4} (1 - \cos \omega_\alpha \tau) \]

\[ \nu_\alpha \quad \nu_\beta \]

\[ T_1 \text{ decay (slower)} \]

no sum and difference frequencies

blind spots

\( \tau \) adds to dead time
Nuclear spectra: ENDOR vs. ESEEM

ENDOR:
- maximum intensity at $\theta = 90^\circ$
- minimum intensity at $\theta = 0^\circ$

ESEEM:
- no intensity along principal axes
- maximum intensity off-axis

difficult to measure broad lines with ESEEM!
only central part visible!

Situations for best intensities
ESEEM enhanced by nuclear state mixing; most intense in matching regime, i.e. low nuclear frequencies

ENDOR enhanced by hyperfine enhancement, most intense for high nuclear frequencies
HYSCORE: A two-dimensional ESEEM experiment

HYSCORE: $t_1$ and $t_2$ is varied

HYSCORE = hyperfine sublevel correlation

$\pi$ pulse should be as short as possible

2D time domain (TD)

echo intensity as a function of $t_1$ and $t_2$

2D frequency domain (FD)

only 1st and 2nd quadrant are shown
HYSCORE: Data processing

Time-domain data

\( t_2 \) baseline correction  \( t_1 \) baseline correction

2D windowing

Spectrum

2D Fourier transform

2D zero filling

taper towards zero
HYSCORE: Spectra

Weak coupling

\[ \nu_{\alpha} \]
\[ \nu_{\beta} \]

Strong coupling

\[ \nu_{\alpha} \]
\[ \nu_{\beta} \]

First quadrant

\[ \nu_{\alpha} \]
\[ \nu_{\beta} \]

Second quadrant

\[ \nu_{\alpha} \]
\[ \nu_{\beta} \]

Quadrupole splittings

\[ \omega_{+}(2m_{s}+1)A_{s} \]

Powder spectra
HYSCORE: Blind spots

Blind spots:
- $\tau$-dependent intensity factor: $\sin(\pi \nu_1 \tau) \sin(\pi \nu_2 \tau)$
- intensity drops to zero at frequencies that are multiples of $1/\tau$
- both dimensions, all quadrants

Example:
$\tau = 120$ ns
$1/\tau = 8.33$ MHz

Consequences:
- peaks are missing
- peaks are distorted
- danger of wrong assignment

Remedies:
- acquire spectra with several different tau values
- use blind-spot free advanced techniques
HYSCORE: Example

[FeFe] hydrogenase + model

Angew. Chem. 2011 50 1
PCCP 2009 11 6592
ENDOR/ESEEM at higher fields and frequencies

ENDOR/ESEEM frequency ranges for common isotopes:

**Advantages**
- separation of isotopes
- weak coupling regime for large hyperfine couplings
- increased sensitivity for low-gamma nuclei
- larger spin polarization
- larger orientation selectivity

**Disadvantages**
- less signal for strongly anisotropic systems
- less available power
- longer pulses

**Pulse EPR power**
- X-band 9-10 GHz 1000 W
- Q-band 34-36 GHz 10 W
- W-band 95 GHz 0.4 W
- D-band 130 GHz 0.125 W
- G-band 263 GHz 0.020 W
DEER: Distances between electron spins

DEER = double electron-electron resonance
(also called PELDOR = pulse electron double resonance)

Dipolar coupling between two electron spins analogous to dipolar hyperfine coupling

\[ \nu_{ee} = \frac{\mu_0 \mu_B^2}{4\pi \hbar} g_A g_B \frac{1}{r^3} \]

4-pulse DEER: \( t \) is varied

A = probe spin, mw1
B = pump spin, mw2

Echo modulation

B = probe spin, mw1
A = pump spin, mw2

pump spin up
pump spin down
DEER: Data analysis

Echo modulation

called form factor after background correction

1/ν_{ee}

dipolar evolution time \( t (\mu s) \)

Dipolar spectrum

ν_{ee}

dipolar frequency (MHz)

Distance distribution

Least-squares analysis

(Gaussian fit or Tikhonov regularization)

Estimation diagram

\[ \nu_{ee} = \frac{52 \text{ MHz}}{(r/\text{nm})^3} \]

\( g_A = g_B = 2.00 \)
DEER: Examples

Arrangement of iron-sulfur clusters

Conformational change upon substrate binding

Complex 1 (NADH:quinone oxidoreductase)
Hirst et al; PNAS 2010 107 1930 [link]
Annu.Rev.Biochem. 2013 82 551 [link]

Cytochrome P450cam
Stoll et al; PNAS 2012 109 12888 [link]
### What you can learn from EPR data

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Structural information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPR spectrum (CW or pulse)</strong></td>
<td>type of spin center (metal, radical)</td>
</tr>
<tr>
<td>g tensor</td>
<td>spin quantum number</td>
</tr>
<tr>
<td>hyperfine</td>
<td>delocalization of spin onto ligands</td>
</tr>
<tr>
<td>zero-field splitting</td>
<td>coordination geometry</td>
</tr>
<tr>
<td>relaxation times</td>
<td>oxidation state, spin multiplicity</td>
</tr>
<tr>
<td><strong>Nuclear spectra (ESEEM/ENDOR)</strong></td>
<td></td>
</tr>
<tr>
<td>nuclear Zeeman frequency</td>
<td>type of ligand nuclei</td>
</tr>
<tr>
<td>isotropic hyperfine</td>
<td>ligand protonation states</td>
</tr>
<tr>
<td>anisotropic hyperfine</td>
<td>location of protons</td>
</tr>
<tr>
<td>nuclear quadrupole</td>
<td>oxidation state assignment in clusters</td>
</tr>
<tr>
<td><strong>Dipolar spectra (DEER)</strong></td>
<td>coordination mode of ligands</td>
</tr>
<tr>
<td>dipolar coupling</td>
<td>distance between spin centers</td>
</tr>
</tbody>
</table>