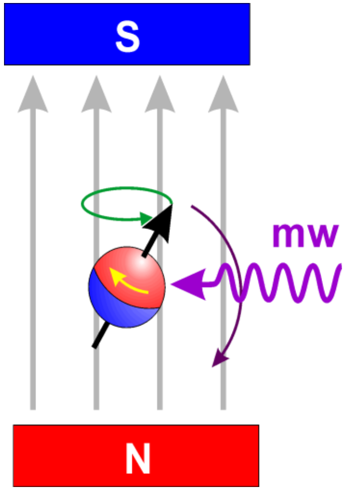
Pulse EPR Spectroscopy: ENDOR, ESEEM, DEER



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Some References:

Books

A. Schweiger, G. Jeschke, *Principles of Pulse Electron Paramagnetic,Resonance,* Oxford, 2001
M. H. Levitt, *Spin Dynamics - Basics of Nuclear Magnetic Resonance,* Wiley, 2008

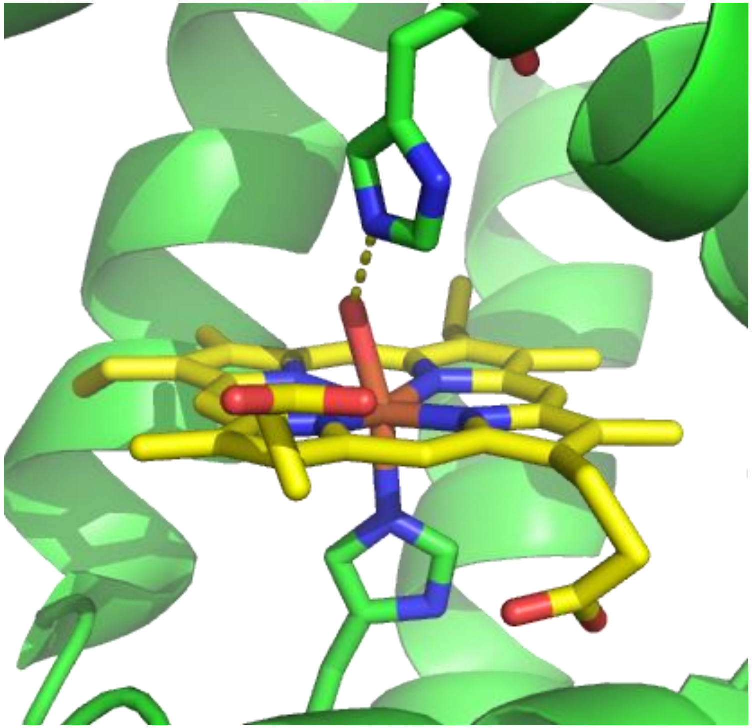
Reviews

W. B. Mims, *Electron Spin Echoes,* in: S. Geschwind (ed.), *Electron Paramagnetic Resonance,* Plenum, 1972, ch.4, 263-351
S. A. Dikanov, Yu. D. Tsvetkov, *Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopy,* CRC Press, 1992
Y. Deligiannakis, M. Louloudi, N. Hajiliadis, *ESEEM spectroscopy as a tool to investigate the coordination environment of metal centers,* Coord. Chem. Rev. 204, 1-112 (2000)

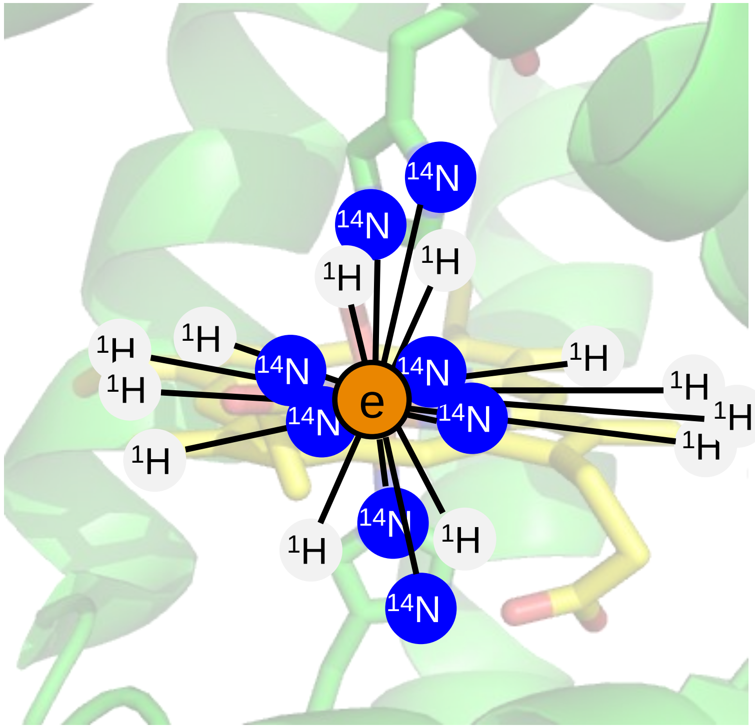
Mössbauer	14400 eV	(^{57}Fe)
XAS/XES	7000 eV	(Fe K-edge)
UV/Vis	2 eV	(600 nm)
IR/Raman	0.01 eV	(800 cm^{-1})
EPR	0.00004 eV	(10 GHz = 40 μeV)
ENDOR etc.	0.000000004 eV	(1 MHz = 4 neV)

Coupled spins

Crystallography view:
Structural cartoon



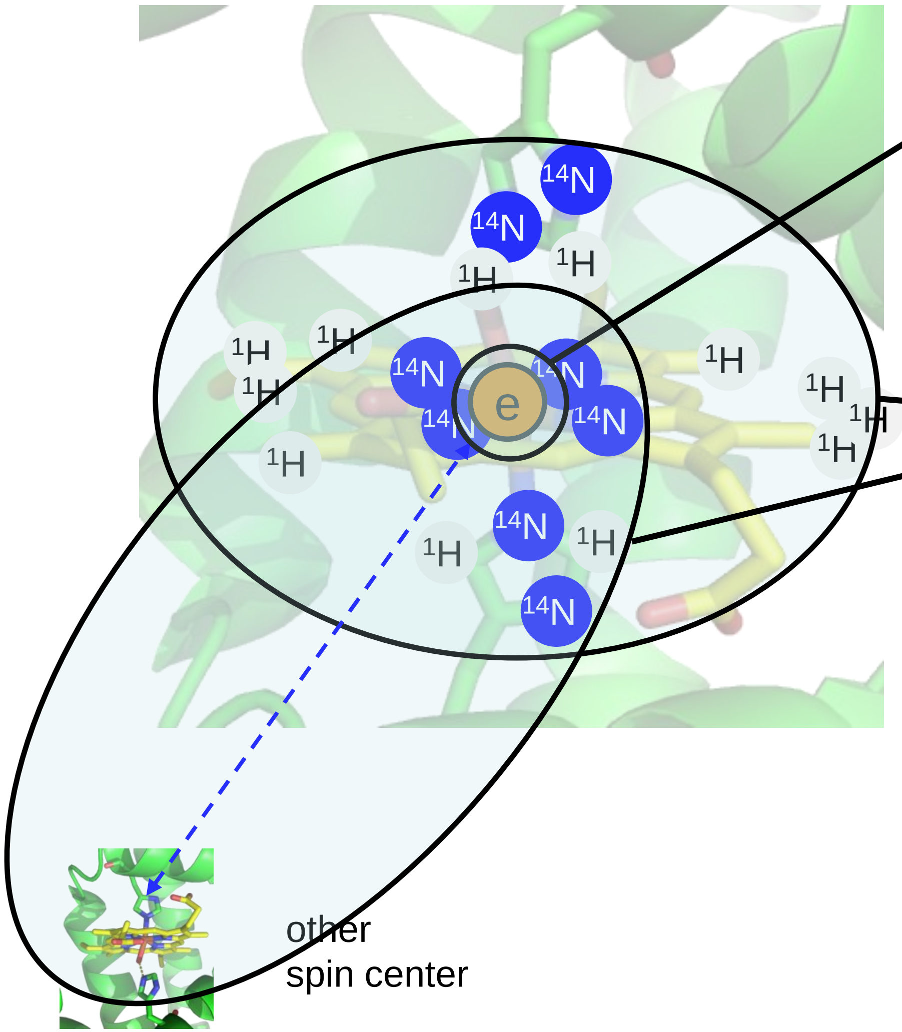
Magnetic resonance view:
System of coupled spins



1 unpaired electron spin on Fe^{3+} ($S = 1/2$)
all magnetic nuclei (^{1}H, ^{2}H, ^{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

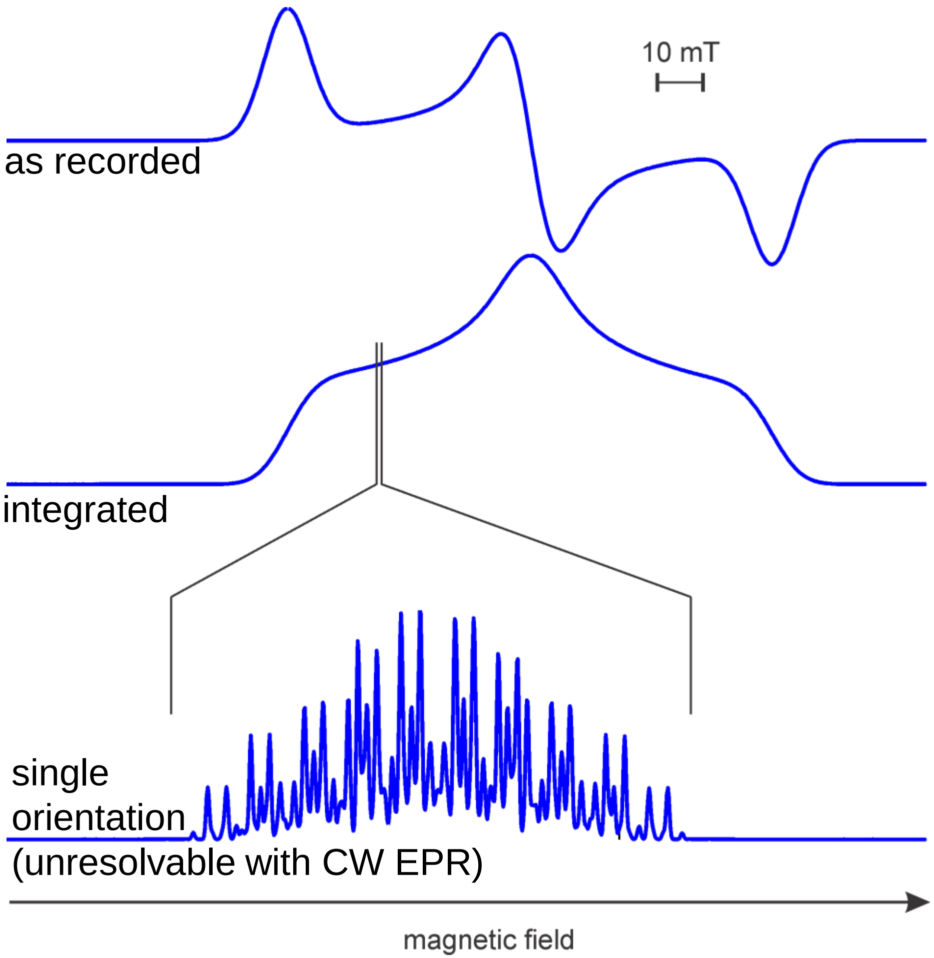
Pulse EPR: high resolution

- 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

frozen solution
CW EPR "powder" spectrum



Origins of static line broadenings

1. <u>anisotropies</u> of *g* tensor, *A* tensor, *D* tensor
2. site-to-site structural <u>heterogeneity</u> resulting in *g*, *A*, *D* heterogeneity
3. unresolved <u>splittings</u>
   - hyperfine coupling to magnetic nuclei
   - coupling to other electron spins

↓

Hidden structure

↓

pulse EPR

1. Basics

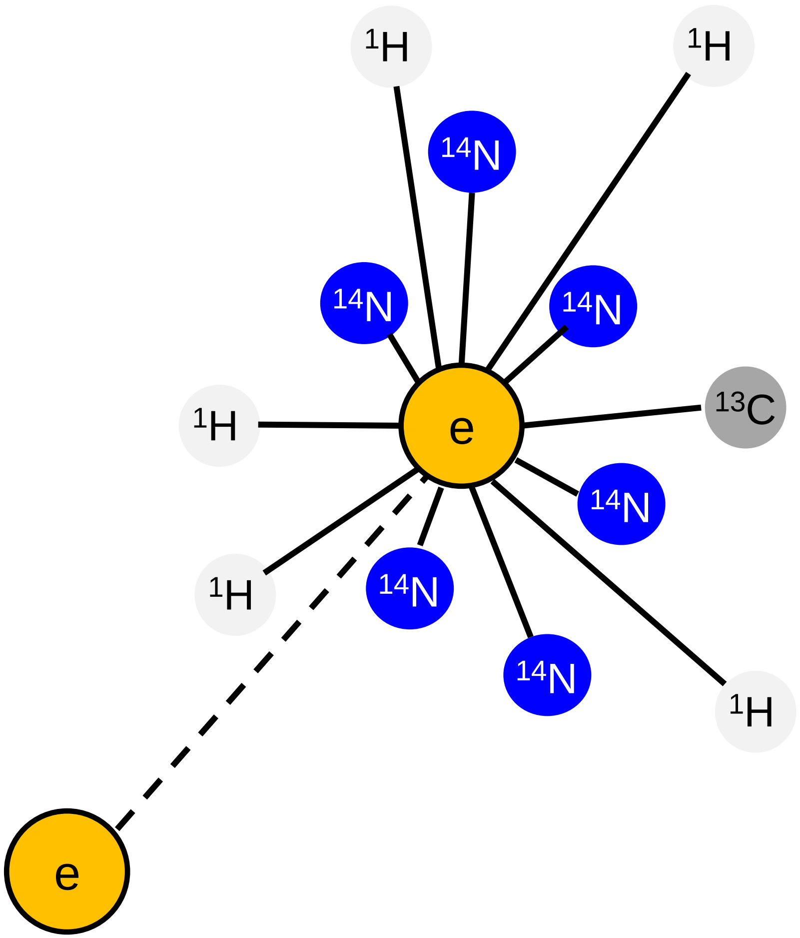
CW vs. pulse EPR
Sample and spectrometer
Resonators and bandwidths
Pulses, excitation width
Orientation selection
FIDs and Echo
Deadtime, Relaxation

2. Interactions

Nuclear Zeeman interaction
Hyperfine interaction
Coupling regimes
Nuclear spectra
Quadrupole interaction

3. Experiments

Field sweeps
ENDOR
ESEEM
HYSCORE
DEER

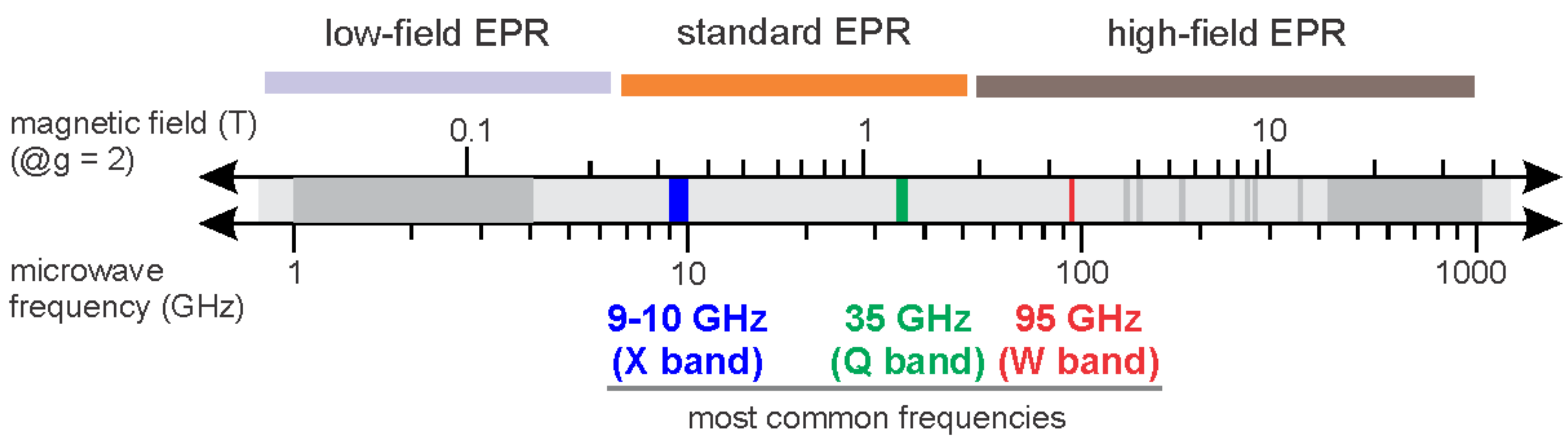


Comparison CW and pulse EPR

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



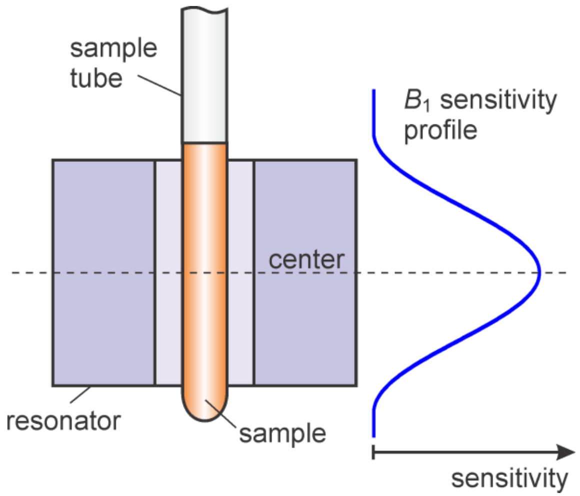
EPR unit conversions

- 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



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.

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

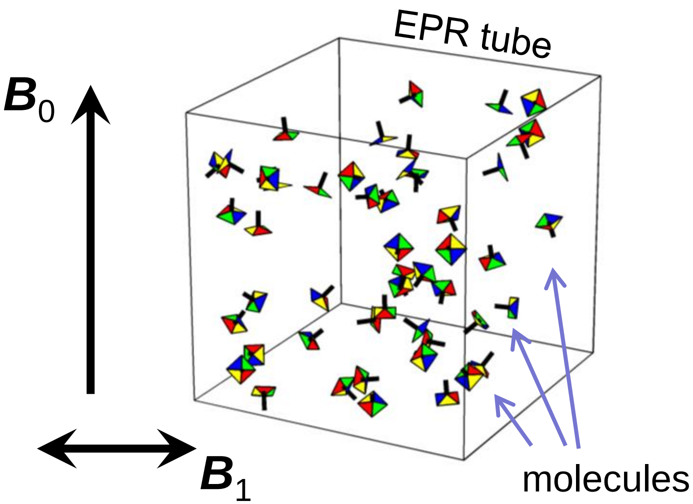
(3) Aggregation
- due to slow freezing, solvent crystallization
- enhances relaxation, shortens T_m, T_1
- add glassing agent (glycerol, sucrose), freeze fast

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

EPR measurement temperatures (approx)

organic radicals	30-200 K
mononuclear metal centers	5-40 K
oligonuclear metal clusters	2-10 K

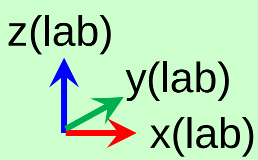
Frozen solutions; lab and molecular frame



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

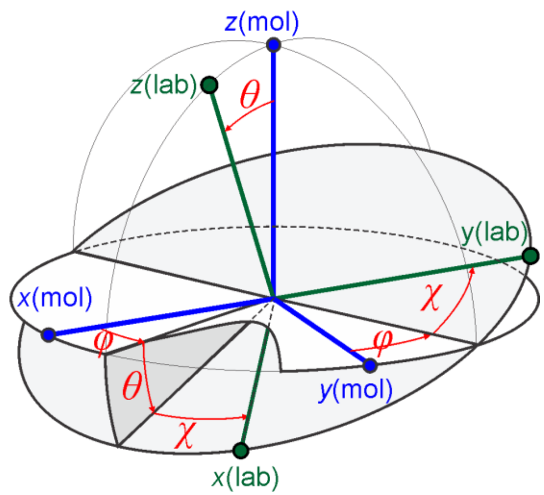
Frozen solution =
random uniform distribution of static orientations of the molecules, like a dilute powder.

Lab frame
- fixed in laboratory
- z(lab) along static field $\boldsymbol{B}_0$
- x(lab) along oscillating microwave field $\boldsymbol{B}_1$

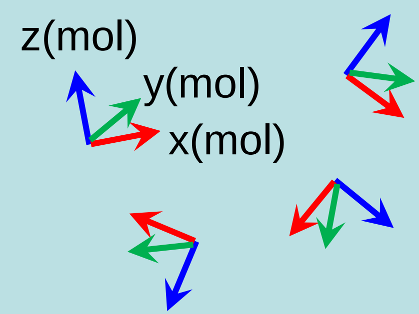


Relative orientation

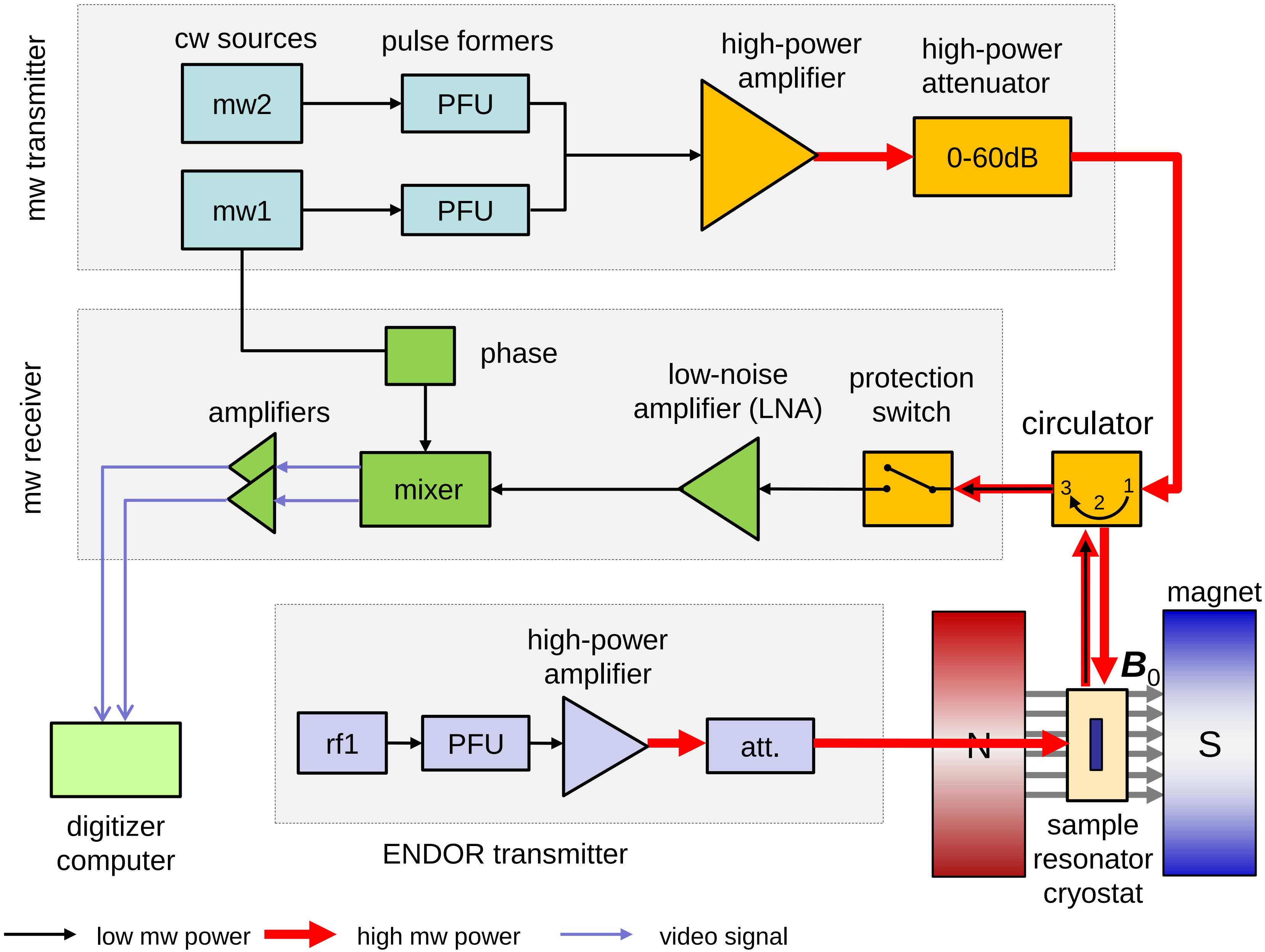
Euler angles φ, θ, χ



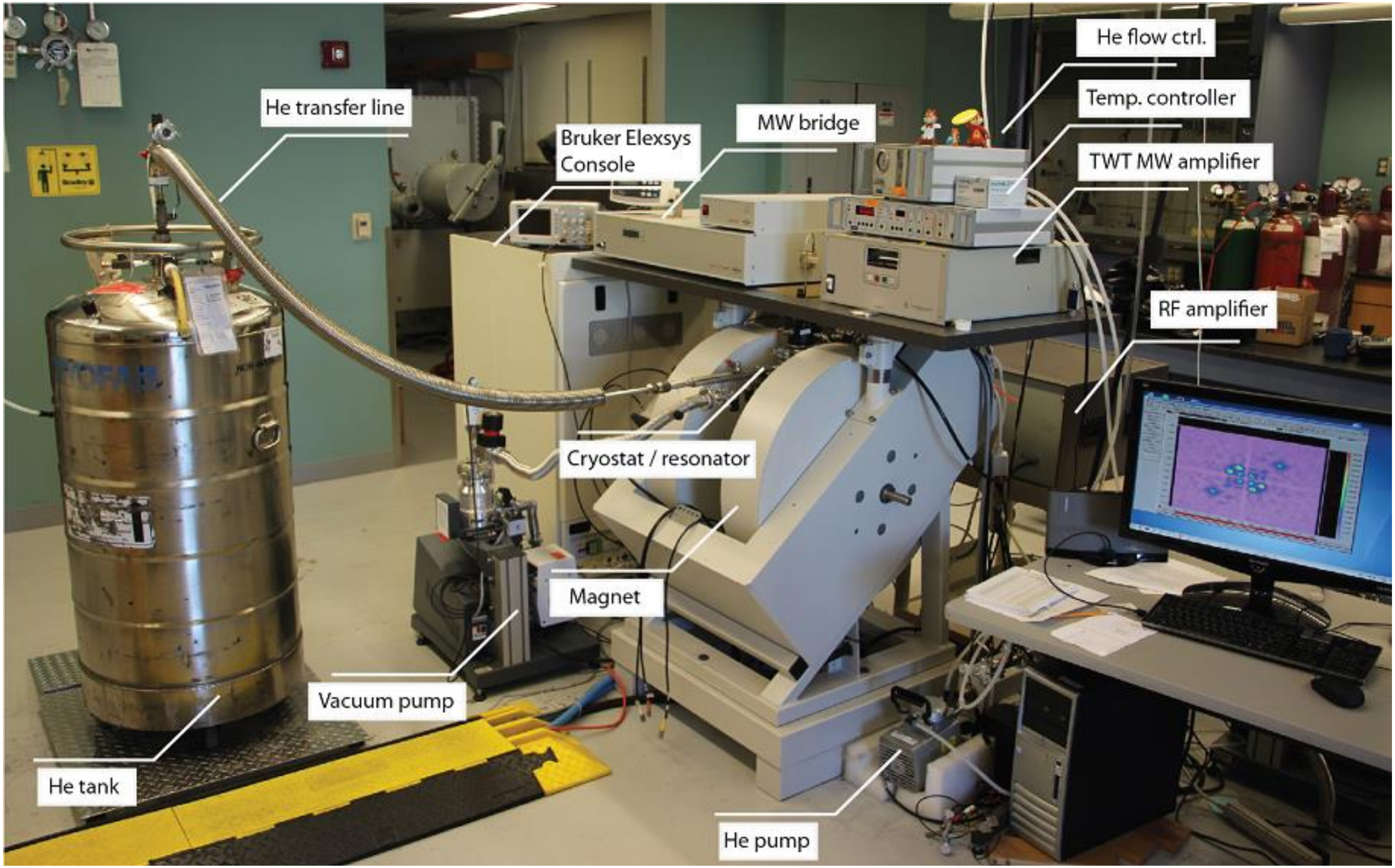
Molecular frames
- fixed in molecules
- most commonly molecular symmetry frame or g tensor frame



Pulse EPR spectrometer



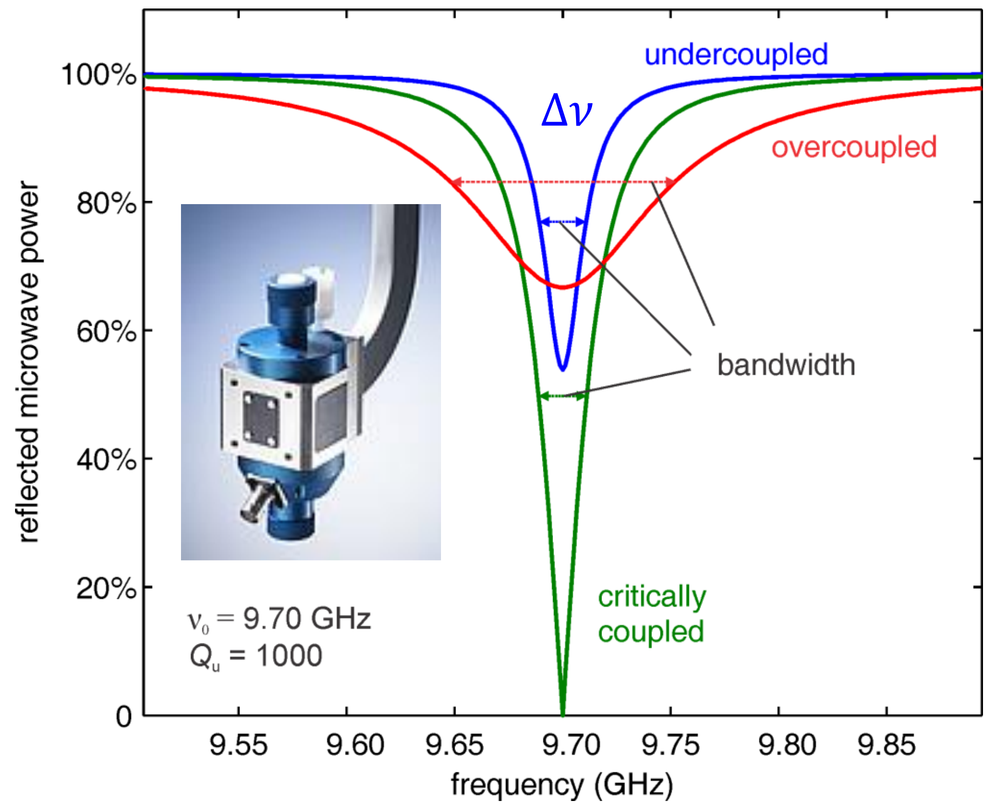
Pulse EPR spectrometer



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

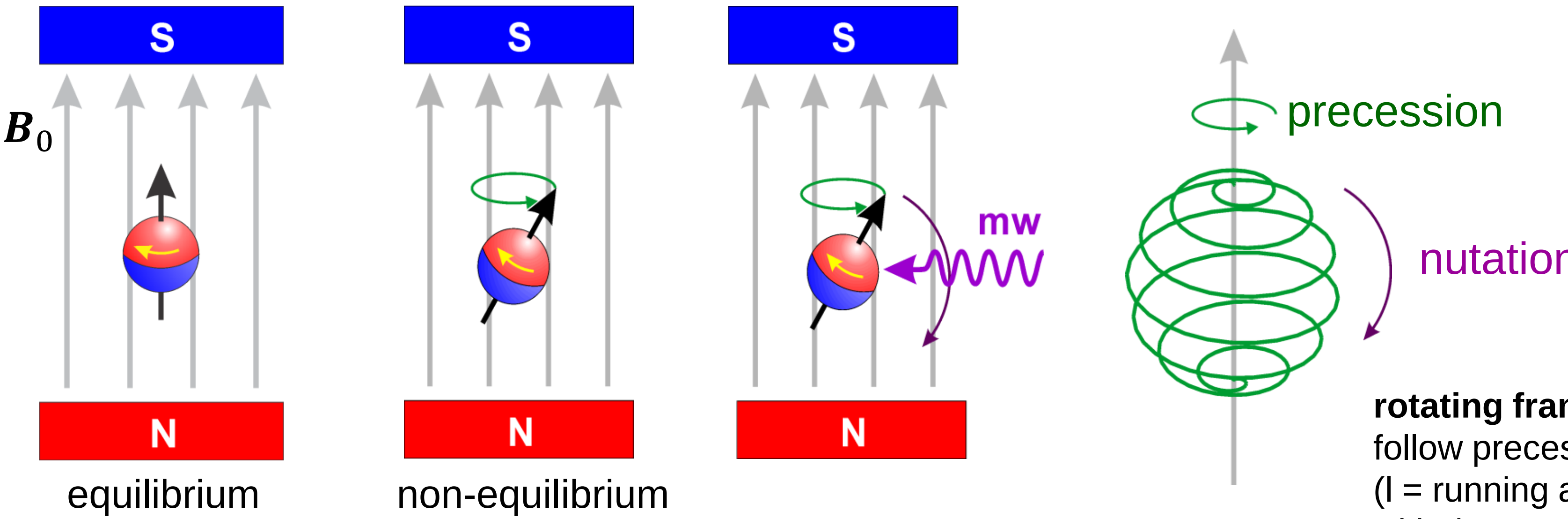
$$Q = \frac{\nu_0}{\Delta\nu}$$

- ν_0: resonator frequency
- $\Delta\nu$: bandwidth (undercoupled)
- Q: Q factor; range: 100 - 10000

cw EPR: <u>high sensitivity</u>
→ high Q, critically coupled

pulse EPR: <u>large bandwidth</u>
→ high Q + overcoupled, or low Q + critically coupled

Microwave irradiation reorients spins



rotating frame:
follow precession
(I = running along with the merry-go-round)

Resonance condition: mw frequency = precession frequency
(*Larmor* or *Zeeman* frequency)

$$h\nu_{\mathrm{mw}} = g\mu_{\mathrm{B}}B$$

Planck constant $6.626 \cdot 10^{-34}$ J s

mw frequency

g factor

Bohr magneton $9.274 \cdot 10^{-24}$ J/T

magnetic field

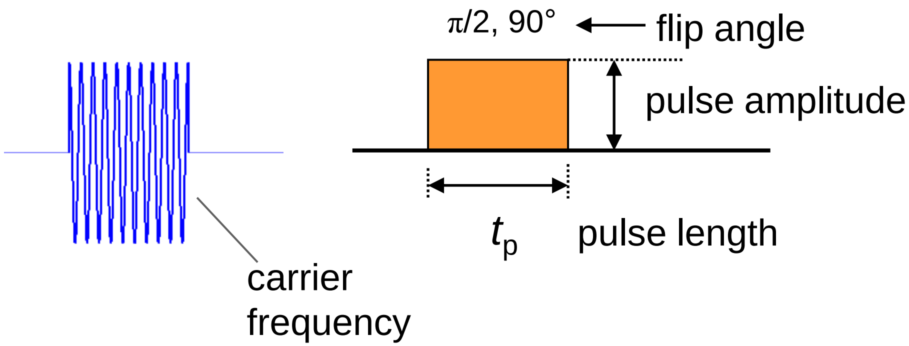
$$71.447732 \frac{\nu_{\mathrm{mw}}}{\mathrm{GHz}} = g \frac{B}{\mathrm{mT}}$$

1 mT = 10 G

1 G = 2.8 MHz @ g = 2

Pulses and excitation bandwidth

Rectangular pulse

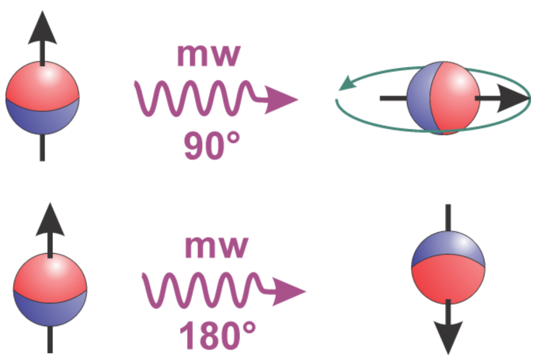


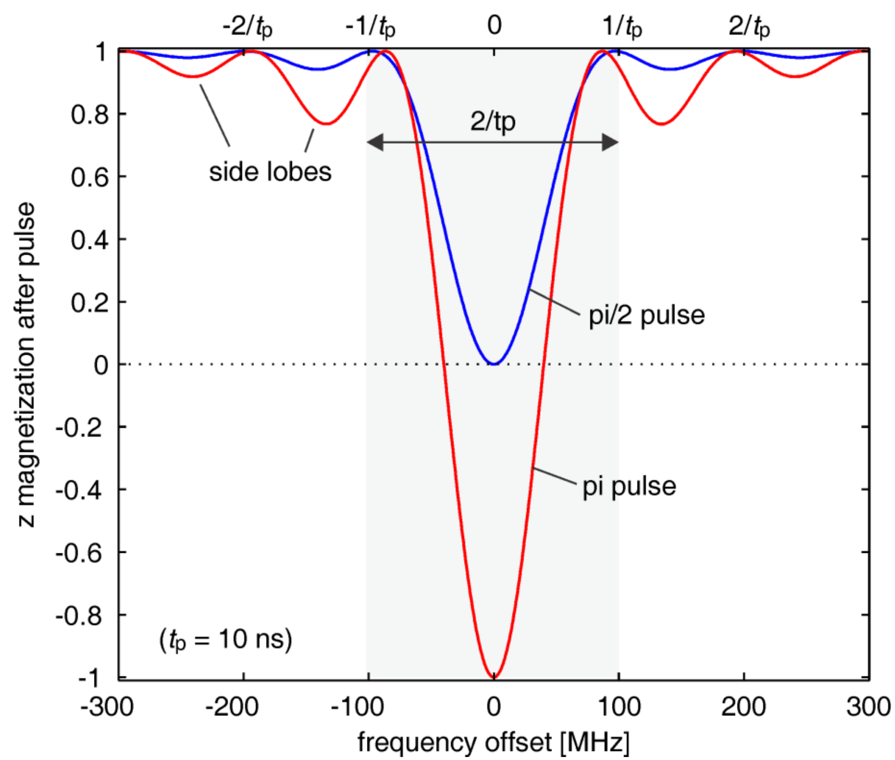
Microwave pulses (for electron spins)

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

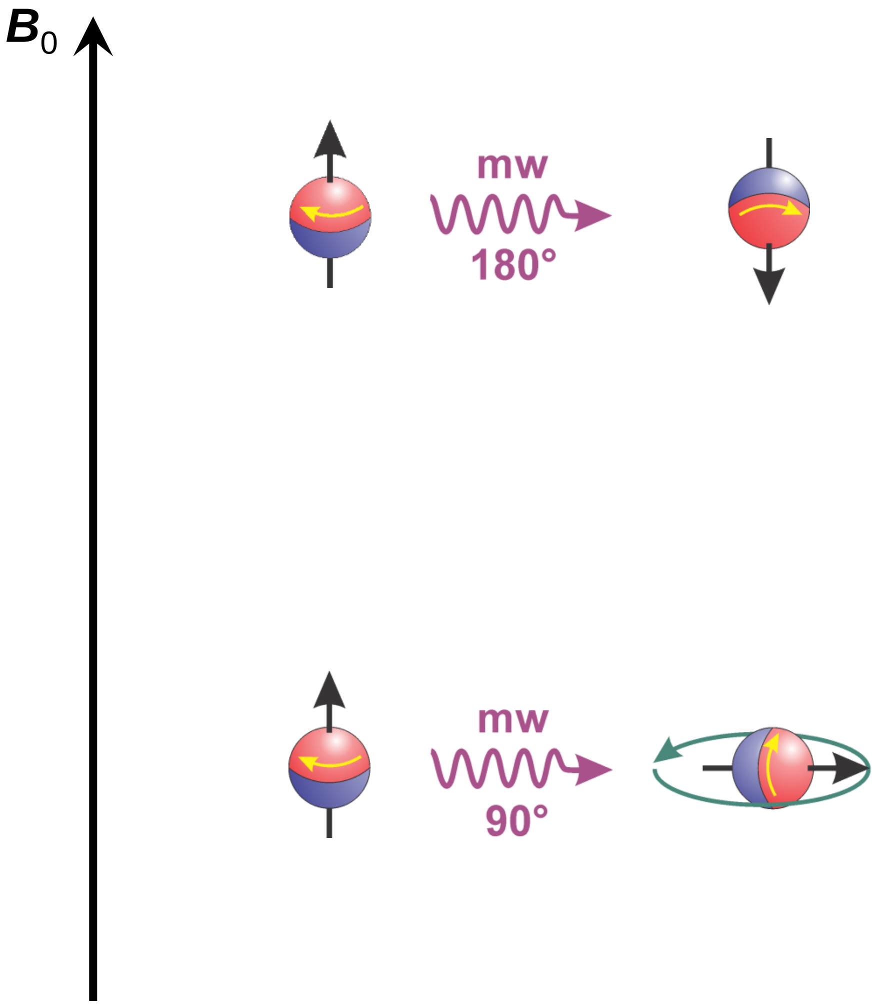




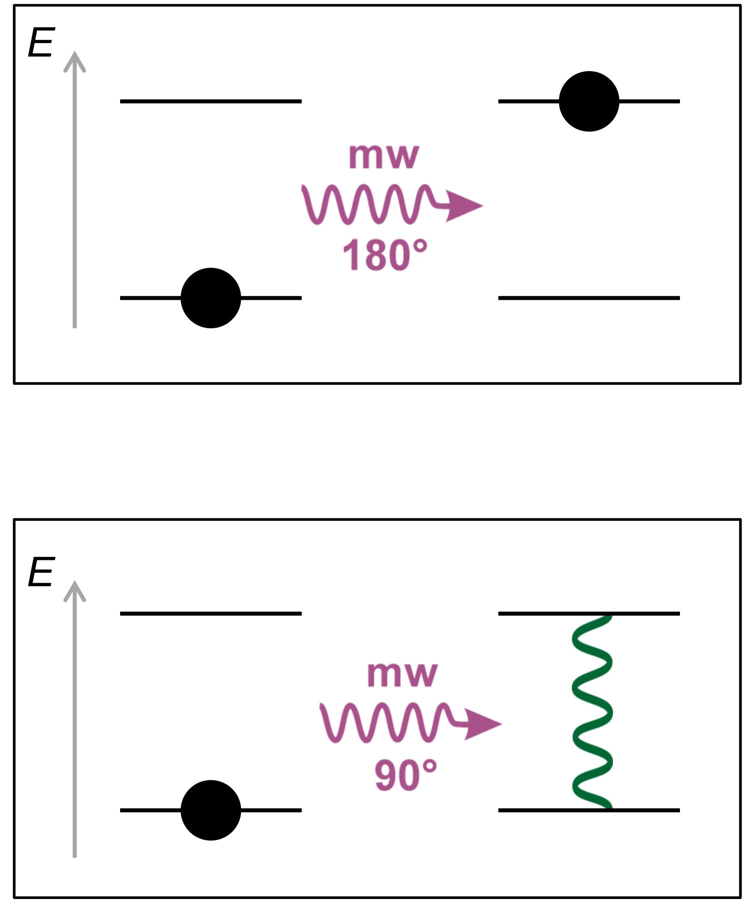
Pulse excitation bandwidth

- excitation bandwidth = approx. distance between zeroes: $2/t_p$ (for pi and pi/2 pulses)
- example: 10 ns pulse → 200 MHz

Spin gymnastics and Energy level diagrams

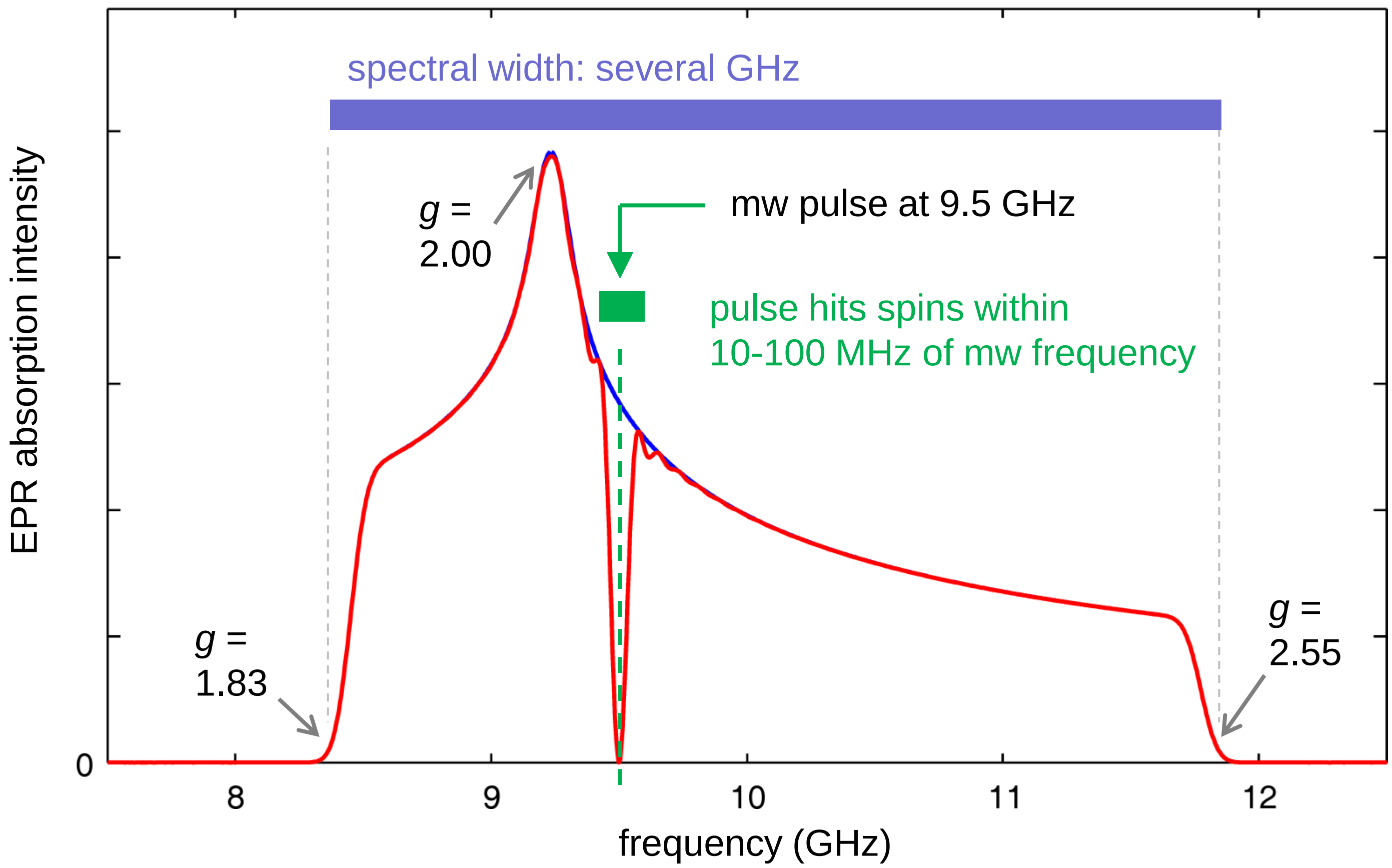


Energy level diagram



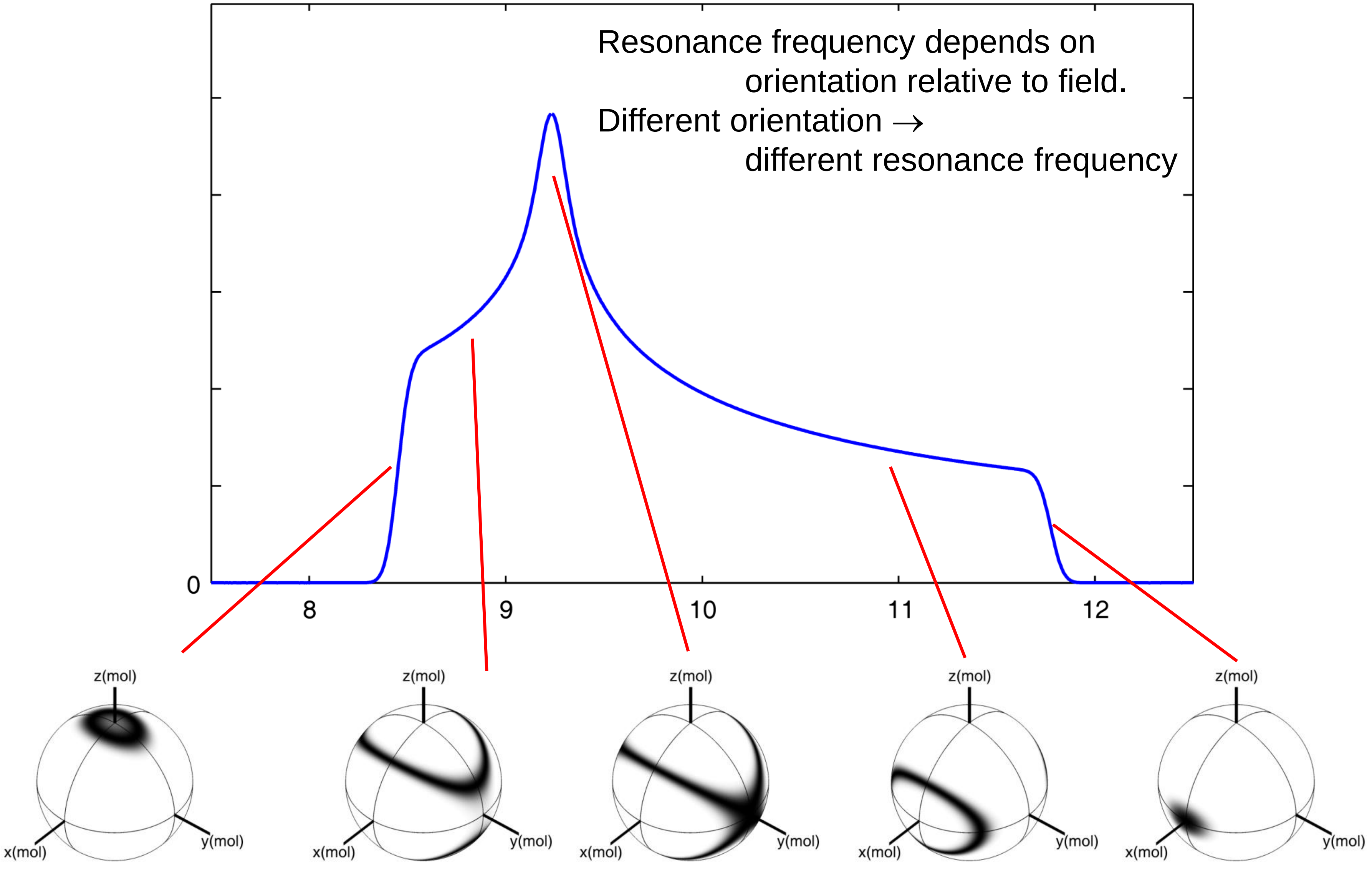
Classical description: Bloch equations (limited to a single spin)
Quantum description: Liouville-von Neumann equation (general)

Spectral width and pulse excitation bandwidth

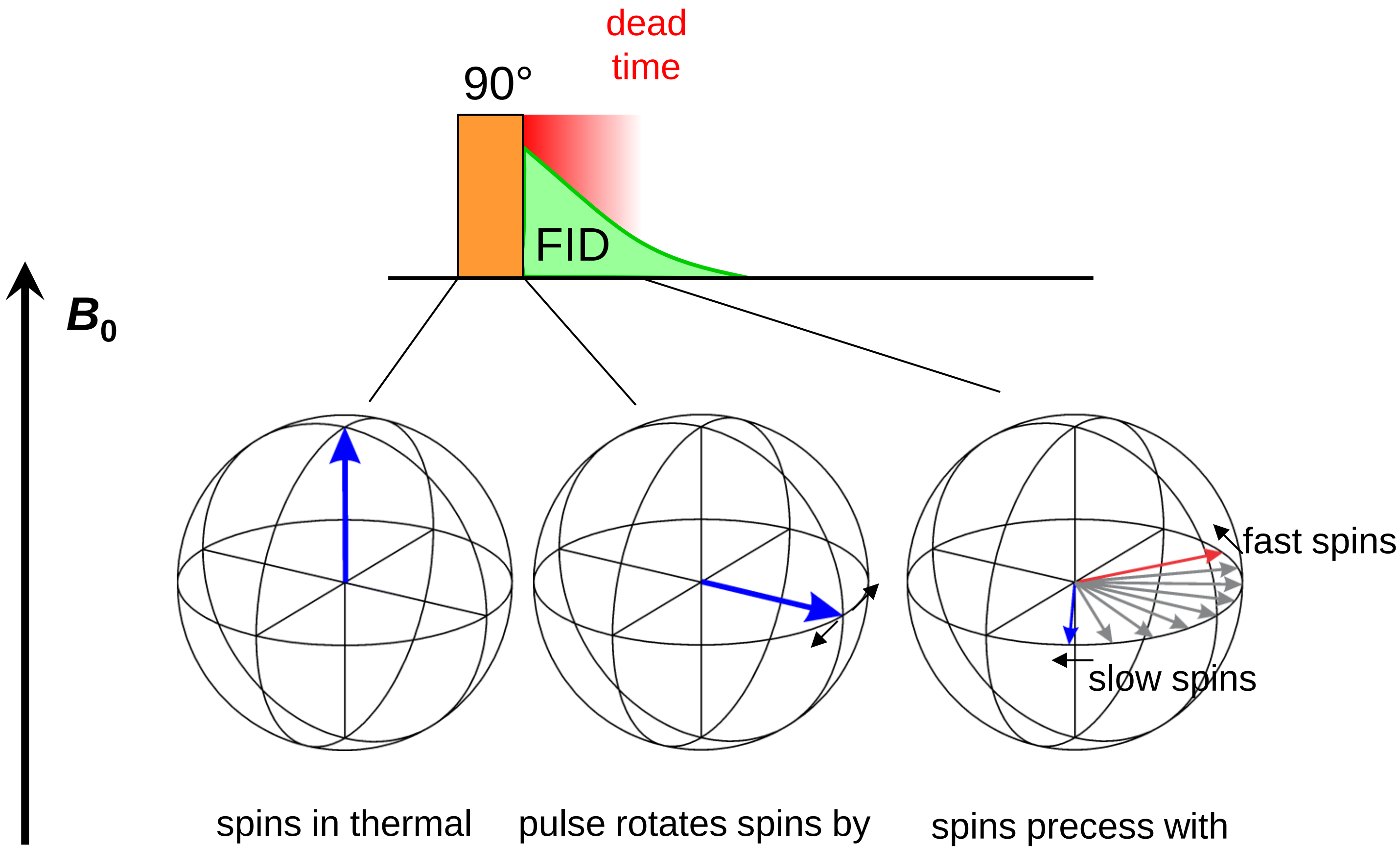


- Only a small fraction of spins in the sample are excited.
- They have resonance frequencies close to the mw frequency.
- They have specific orientations → orientation selection

Orientation selection



Free induction decay (FID)



spins in thermal equilibrium

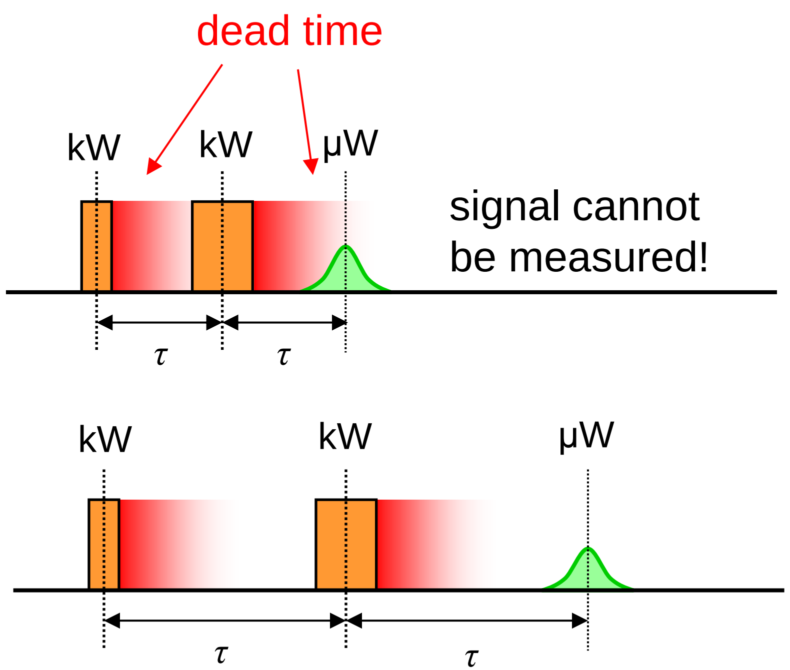
pulse rotates spins by 90 degrees

spins precess with different frequencies

all are in phase

dephasing

Dead time



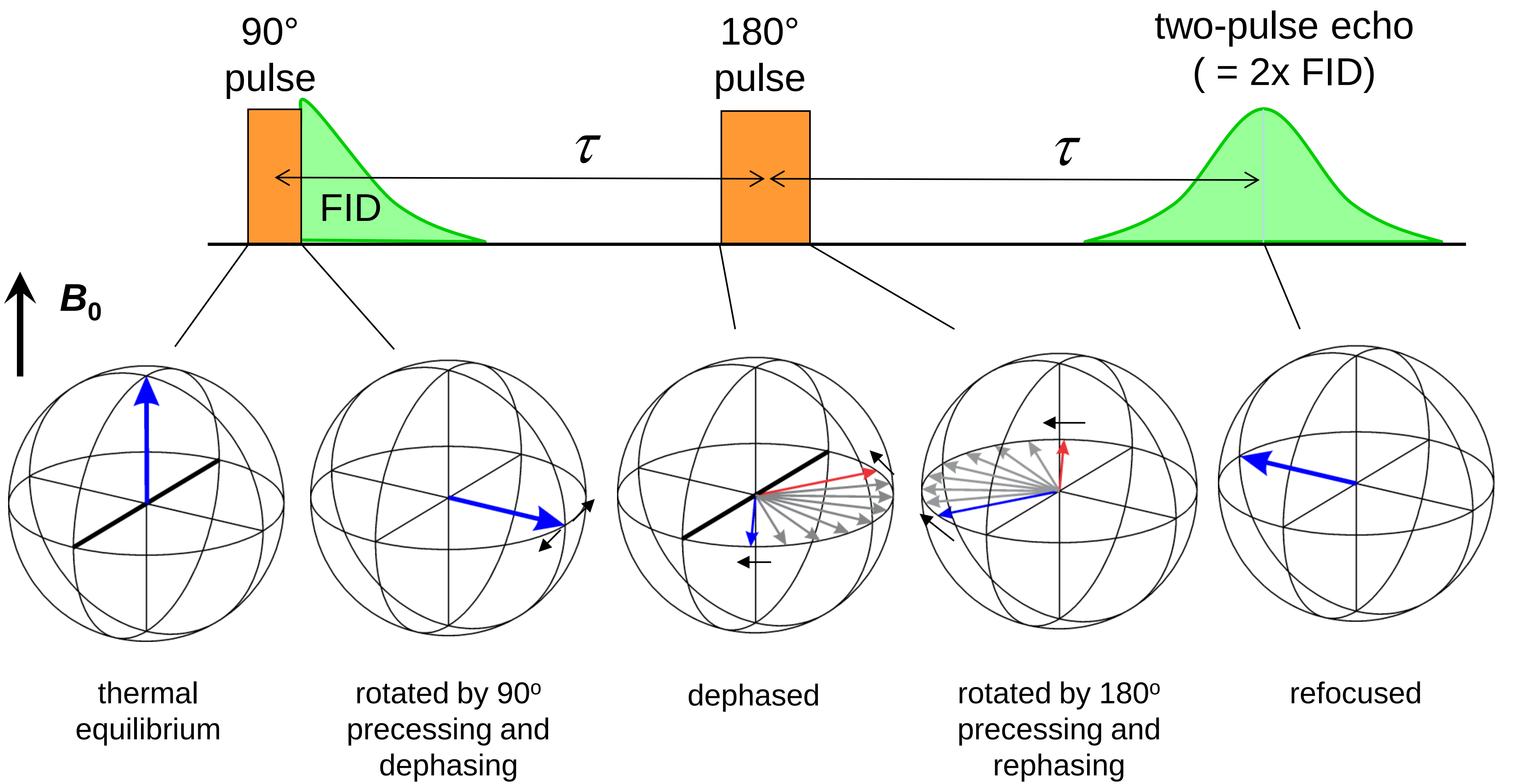
Dead time:
- time after pulses during which power levels are too high to open the sensitive receiver
- due to 1) ringdown in cavity
  2) reflections in spectrometer
  3) recovery of receiver protection
- typical value: 100 ns at X-band shorter at higher frequencies
- affects all pulse EPR experiments

Consequences
- short values of τ cannot be accessed
- loss of broad lines
- phase distortions in spectrum
- spurious features in spectrum

1 kW = 1 mile
1 μW = 0.0016 mm

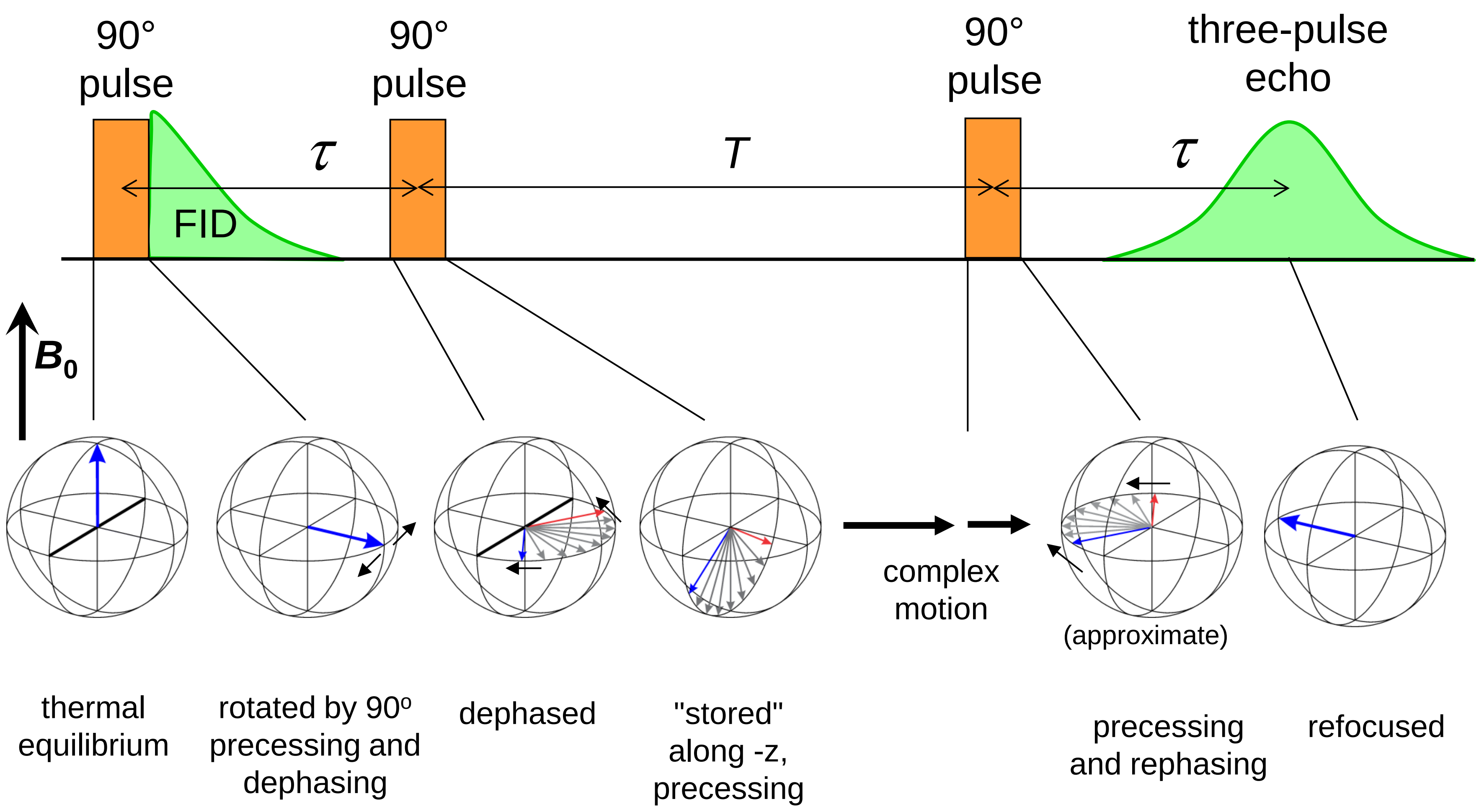
Two-pulse echo

also called primary echo or Hahn echo

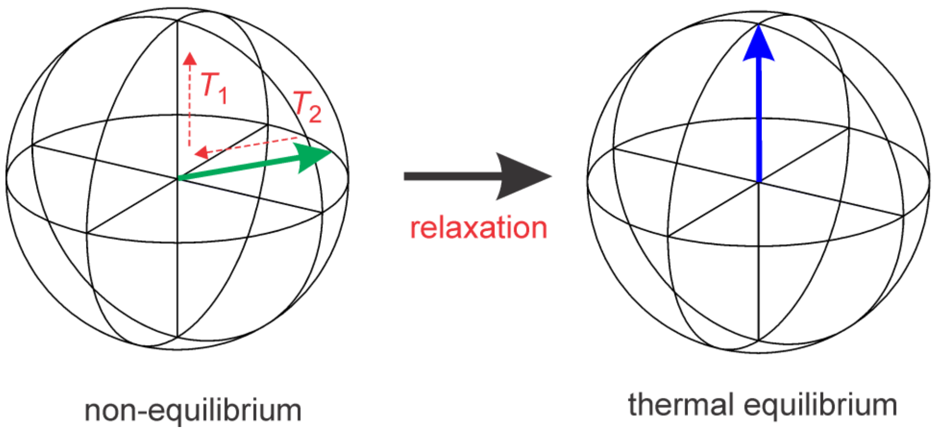


Three-pulse echo

also called stimulated echo



Relaxation



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)

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

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

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Deadtime, Relaxation

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Hyperfine interaction
Coupling regimes
Nuclear spectra
Quadrupole interaction

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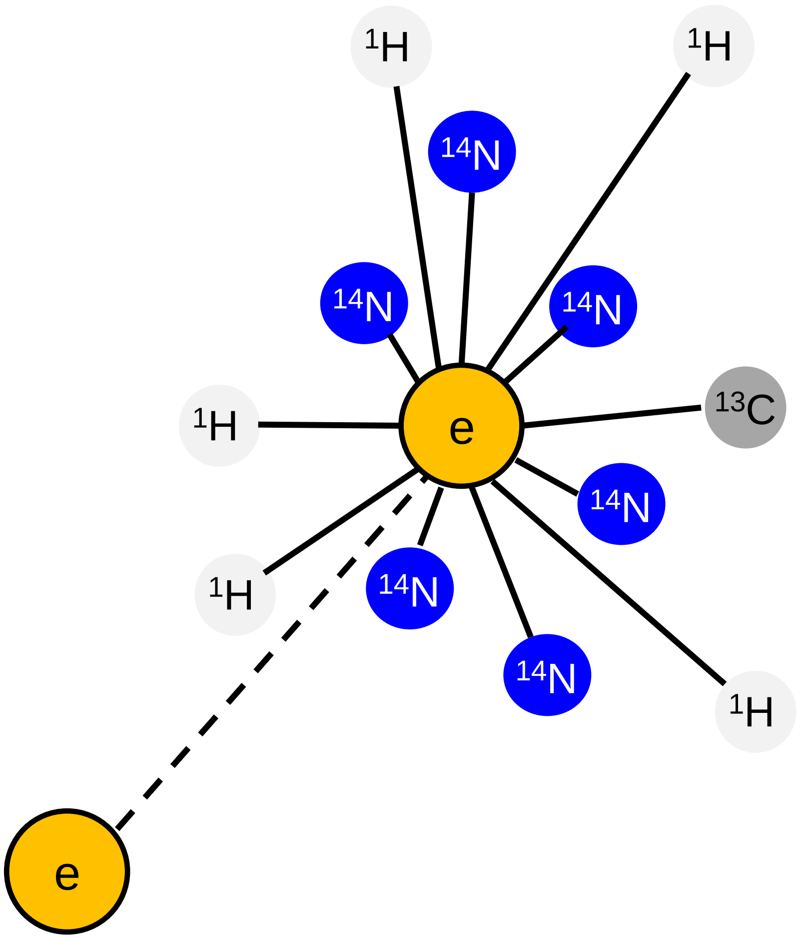


Magnetic nuclei and their interactions

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

$$\mathcal{H}_{\mathrm{nuc}} = -g_{\mathrm{n}}\mu_{\mathrm{N}}\boldsymbol{B}\cdot\boldsymbol{I} + h\,\boldsymbol{S}\cdot\boldsymbol{A}\cdot\boldsymbol{I} + h\,\boldsymbol{I}\cdot\boldsymbol{P}\cdot\boldsymbol{I}$$

B magnetic field
S electron spin
I nuclear spin

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

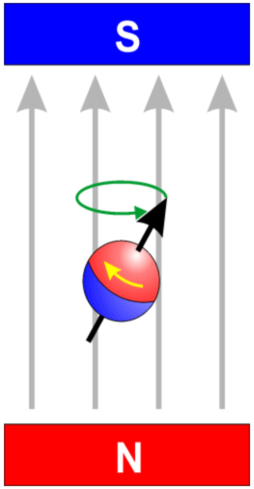
Only for nonspherical nuclei (spin > 1/2)!

Nuclear Zeeman Interaction

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

$$\mathcal{H} = -\boldsymbol{B}_0 \cdot \boldsymbol{\mu}_{\mathrm{nuc}} = -g_{\mathrm{n}}\mu_{\mathrm{N}}\boldsymbol{B}_0 \cdot \boldsymbol{I}$$

magnetic field — nuclear magnetic moment — nuclear g factor — nuclear Bohr magneton $5.0508 \cdot 10^{-27}$ J/T



Nuclear precession frequency: $\nu_{\mathrm{I}} = -g_{\mathrm{n}}\mu_{\mathrm{N}}B_0/h$

NMR: gyromagnetic ratio $\gamma = g_{\mathrm{n}}\mu_{\mathrm{N}}/\hbar$

Nucleus	*Spin*	*%*	g_n
^{63}Cu	3/2	69	+1.484
^{65}Cu	3/2	31	+1.588
^{53}Cr	3/2	9.5	- 0.3147
^{55}Mn	5/2	100	+1.3819
^{57}Fe	1/2	2.1	+0.1806
^{59}Co	7/2	100	+1.318
^{61}Ni	3/2	1.1	- 0.5000

no spin: ^{56}Fe, ^{58}Ni, ^{60}Ni, etc.

Nucleus	*Spin*	*%*	g_n
^{1}H	1/2	99.99	+5.58569
^{2}H	1	0.01	+0.857438
^{14}N	1	99.6	+0.403761
^{15}N	1/2	0.4	- 0.566378
^{13}C	1/2	1.1	+1.40482
^{17}O	5/2	0.04	- 0.757516
^{31}P	1/2	100	+2.2632

^{1}H / ^{2}H: ×6.5

^{14}N / ^{15}N: opposite sign

no spin: ^{12}C, ^{16}O, ^{32}S, etc.

Hyperfine coupling: 1. Fermi contact interaction

$$\mathcal{H} = h\, A_{\mathrm{iso}}\, \boldsymbol{S} \cdot \boldsymbol{I}$$

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

one (unpaired) electron

$$A_{\mathrm{iso}} = \frac{2}{3}\frac{\mu_0 \mu_{\mathrm{B}} \mu_{\mathrm{N}}}{h} g_{\mathrm{e}} g_{\mathrm{n}} |\Psi_0(\boldsymbol{r}_{\mathrm{n}})|^2$$

(SI units, A_{iso} in Hz)

scales* with g_{n}

spin density at position of nucleus

more general

$$A_{\mathrm{iso}} = \frac{1}{3}\frac{\mu_0 \mu_{\mathrm{B}} \mu_{\mathrm{N}}}{h} g_{\mathrm{e}} g_{\mathrm{n}} \sigma_{\alpha-\beta}(\boldsymbol{r}_{\mathrm{n}}) \langle S_z \rangle^{-1}$$

* possible isotope effect for ^{1}H/^{2}H

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

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

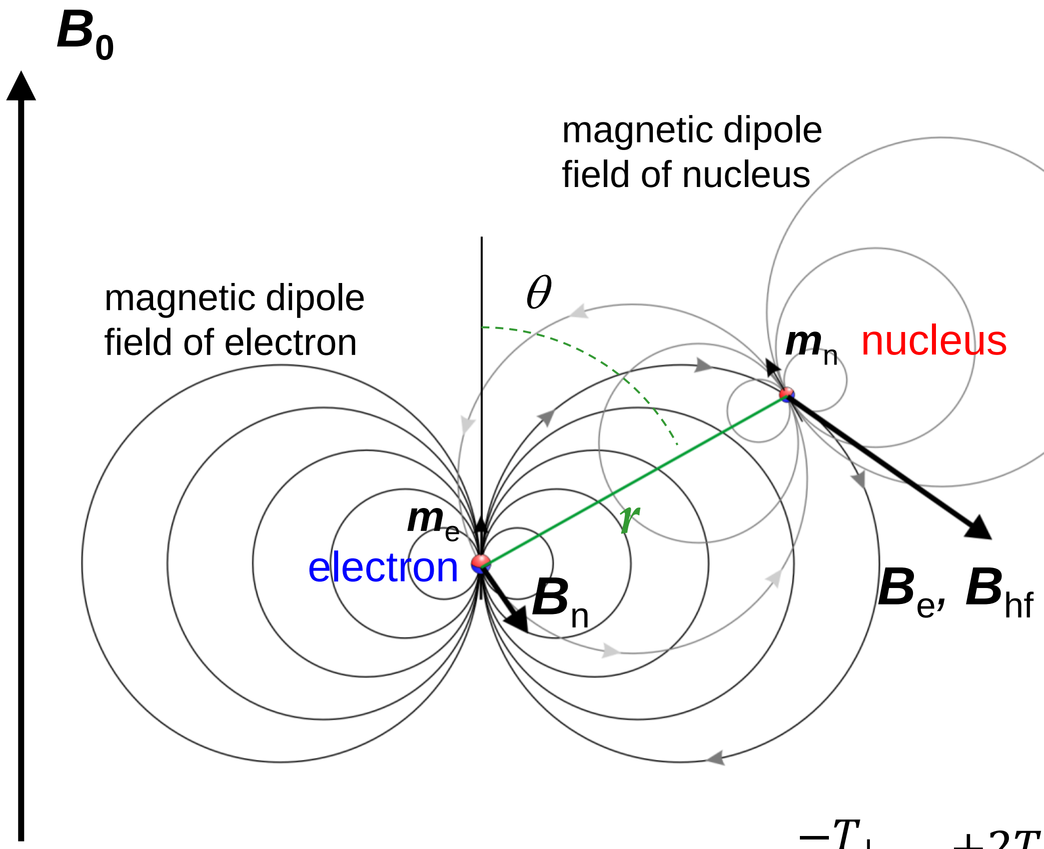
alternative: compare to quantumchemical estimates

Reasons for non-zero A_{iso}

(1) ground-state open s shell
(2) valence and core polarizations
e.g 3d→2s, 3d→1s
(3) configurations with open s shell

Example:
A_{iso}(^{1}H) = 20 MHz → 20/1420 = 1.4%

Hyperfine coupling: 2. Through-space dipolar coupling



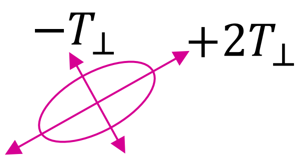
$$\mathcal{H} = h\,\boldsymbol{S}\cdot\boldsymbol{T}\cdot\boldsymbol{I}$$

electron spin (S) — nuclear spin (I)

$$\boldsymbol{T} = T_\perp \begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & +2 \end{pmatrix}$$

$$T_\perp = \frac{\mu_0}{4\pi h}\mu_B\mu_N \cdot \frac{g_e g_n}{r^3}$$

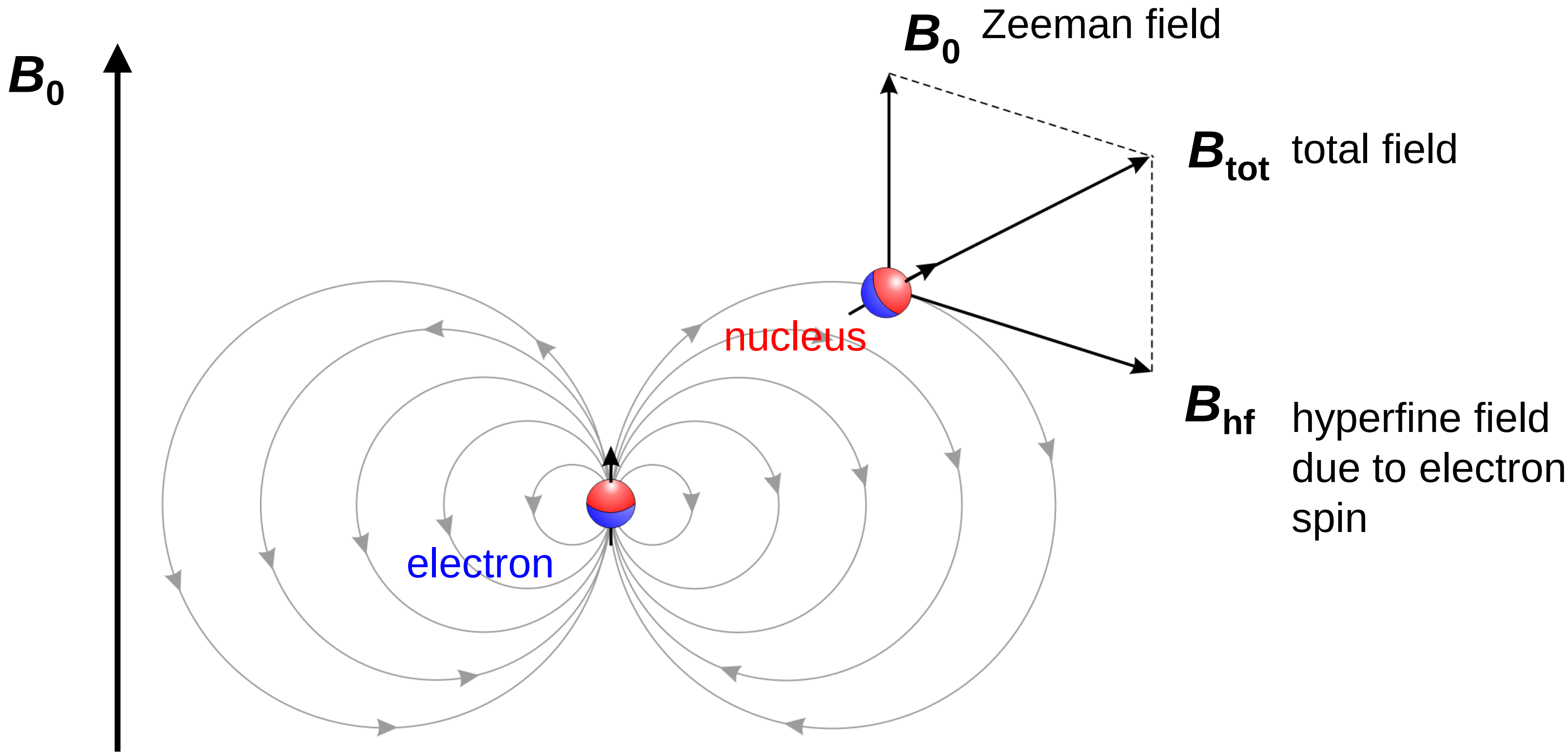
- orientation dependence
- distance dependence



T = dipolar hyperfine matrix
eigenvalues: principal values

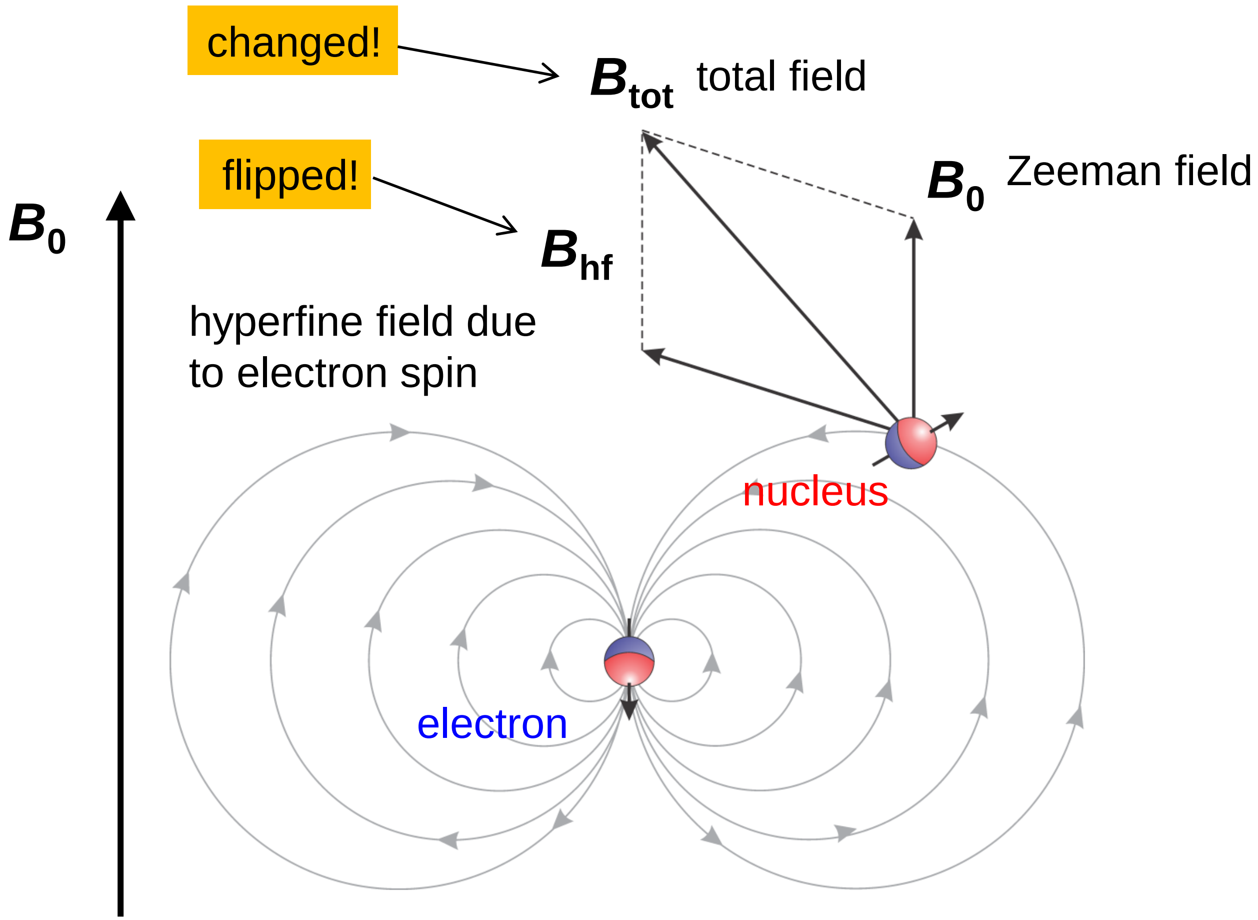
This assumes electron is localized.
In delocalized systems, integrate over electron spin density.

Combining Hyperfine and Zeeman: Local fields

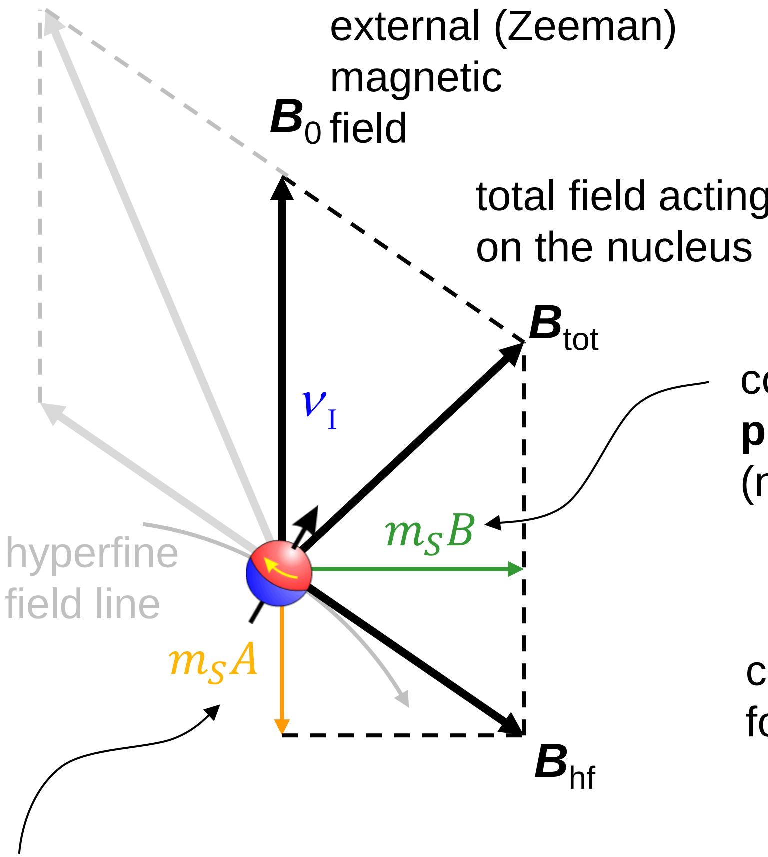


at equilibrium, nuclear spin aligns along total field

Combining Hyperfine and Zeeman: Local fields



Hyperfine + Zeeman: Nuclear frequencies



component of hyperfine field **perpendicular** to external field (nonsecular)

$$\begin{aligned} B &= \left(A_{||} - A_{\perp}\right) \sin\theta \cos\theta \\ &= 3T_{\perp} \sin\theta \cos\theta \end{aligned}$$

can be neglected at high field for small hfc

component of hyperfine field **parallel** to external field (secular)

$$\begin{aligned} A &= A_{||}\cos^2\theta + A_{\perp}\sin^2\theta \\ &= A_{\mathrm{iso}} + T_{\perp}(3\cos^2\theta - 1) \end{aligned}$$

Nuclear frequencies:

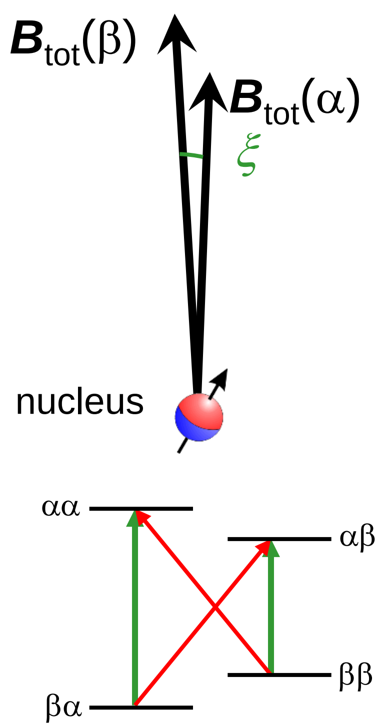
$$\nu(m_S) = \sqrt{(\nu_{\mathrm{I}} + m_S A)^2 + (m_S B)^2}$$

$$\nu_{\mathrm{I}} = -g_{\mathrm{n}}\mu_{\mathrm{N}}B_0/h \qquad m_S = \pm 1/2$$

Hyperfine vs. Zeeman: Three regimes

Weak coupling

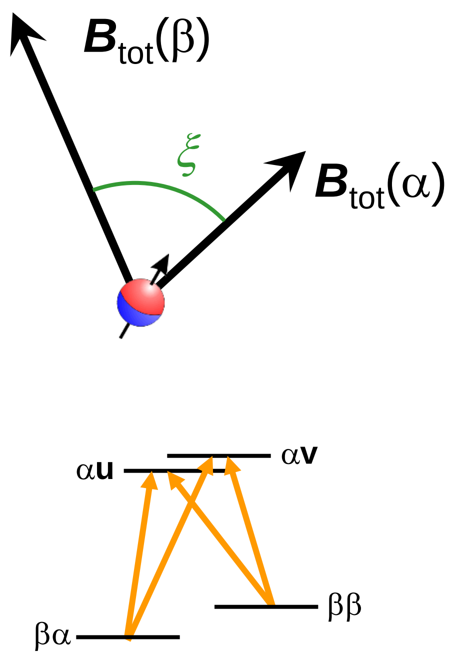
$|\boldsymbol{B}_0| \gg |\boldsymbol{B}_{\mathrm{hf}}|$



Intermediate coupling

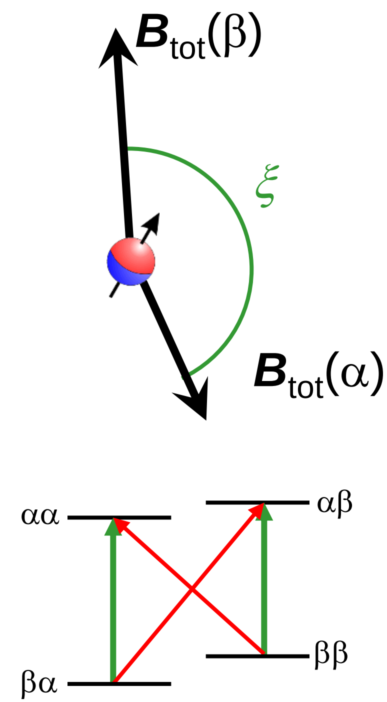
$|\boldsymbol{B}_0| \approx |\boldsymbol{B}_{\mathrm{hf}}|$

matching fields



Strong coupling

$|\boldsymbol{B}_0| \ll |\boldsymbol{B}_{\mathrm{hf}}|$



angle between two total field vectors:

$$\sin^2\xi = \left(\frac{\nu_{\mathrm{I}}B}{\nu_\alpha\nu_\beta}\right)^2 = k$$

k = modulation depth parameter (important in ESEEM)

Nuclear frequencies and powder spectra

$$\nu(m_S) = \sqrt{(\nu_I + m_S A)^2 + (m_S B)^2}$$

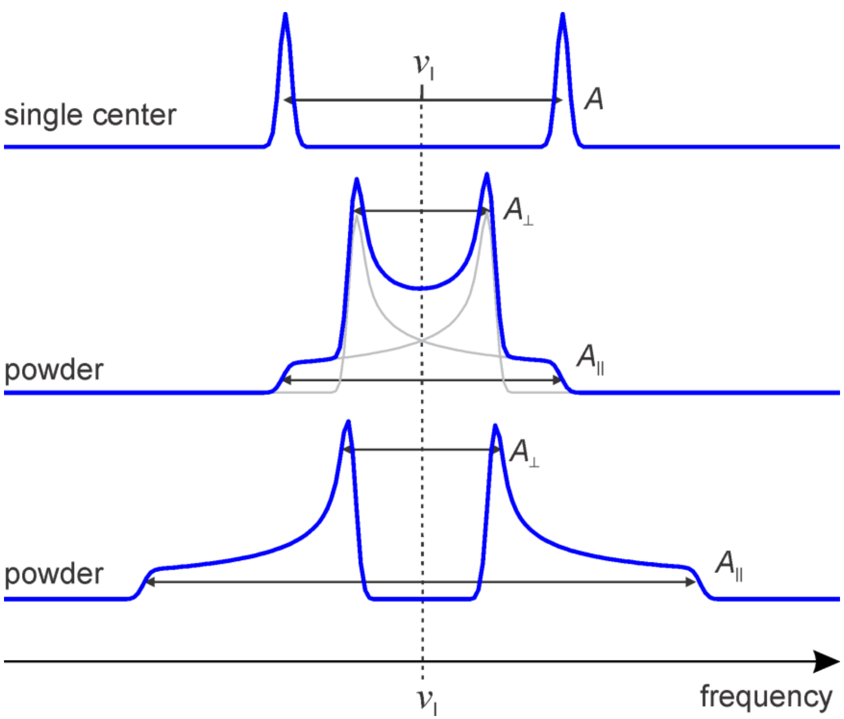
$$\nu(m_S) \approx |\nu_I + m_S A|$$

$\nu_I = -g_n \mu_B B_0 / h$

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

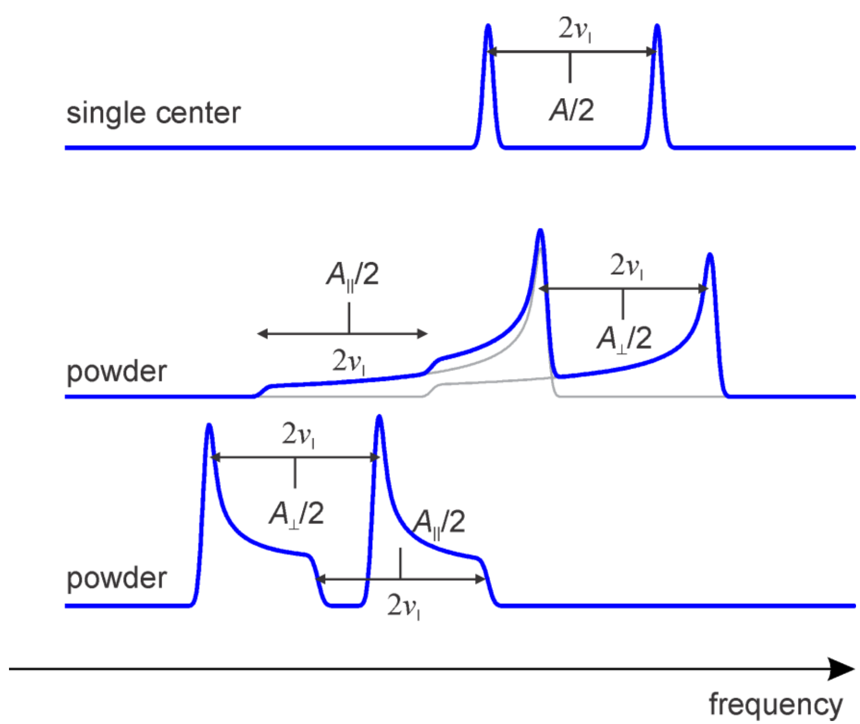
Weak coupling regime $|\nu_I| \gg |m_S A|$

centered at ν_I, split by *A*



Strong coupling regime $|\nu_I| \ll |m_S A|$

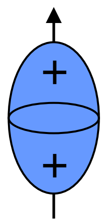
centered at *A*/2, split by $2\nu_I$



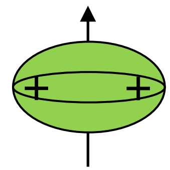
Nuclear Quadrupole Interaction: Basics

(1) Some nuclei have electric quadrupole moment

- Nuclei with spin >1/2 are nonspherical, described by an electric quadrupole moment Q.



$Q > 0$
prolate nucleus
egg shaped



$Q < 0$
oblate nucleus
burger shaped

Nucleus	*Spin*	*Quadrupole moment (b)*
^{2}H	1	+0.00286
^{14}N	1	+0.02044
^{33}S	3/2	- 0.0678
^{63}Cu	3/2	- 0.22
^{17}O	5/2	- 0.02558
^{55}Mn	5/2	+0.33

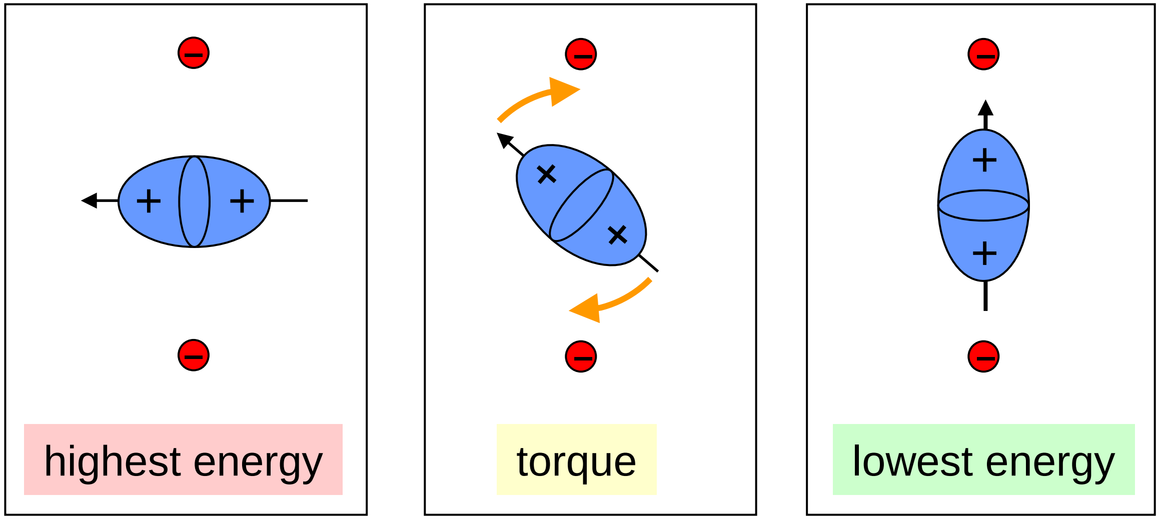
1 b (barn) = 100 fm^2

- 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!



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 $\eta = 1$

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

Spin Hamiltonian term

Interaction of quadrupole moment with EFG

$$\mathcal{H} = h\,\boldsymbol{I} \cdot \boldsymbol{P} \cdot \boldsymbol{I}$$

nuclear spin vector ($\boldsymbol{I}$)

quadrupole tensor ($\boldsymbol{P}$)

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

Experimental parameters:

e^2Qq/h and η

quadrupole moment (Q)

EFG strength (q)

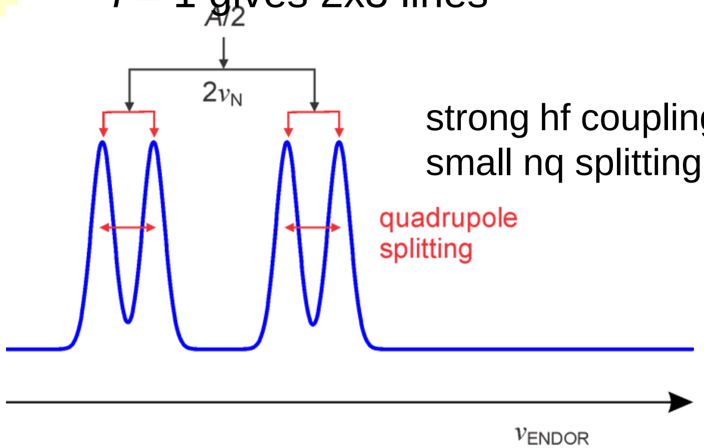
EFG asymmetry (η)

D_2O: e^2Qq/h = 0.213 MHz, η = 0.12

Nuclear Quadrupole Interaction: ^{14}N, ^{2}H, ^{17}O, ^{33}S

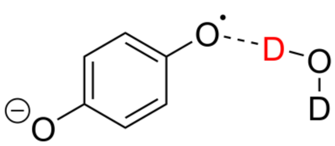
^{2}H

I = 1 gives 2x3 lines



strong hf coupling
small nq splitting

Length of H-bonds to semiquinones



$$K = a - \frac{b}{r^{3}_{\mathrm{O-D}}}$$

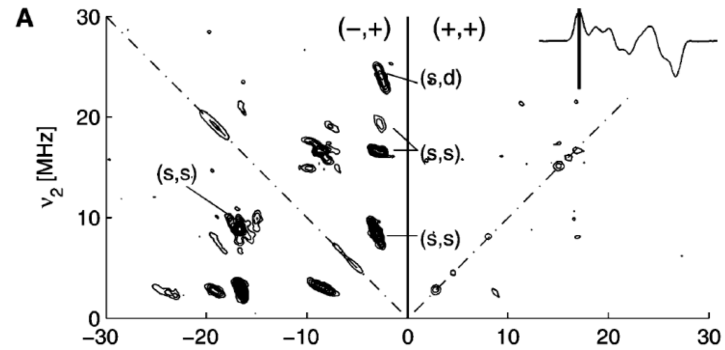
J. Biol. Chem. **2012** 287 4662 link

^{14}N

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

very useful for imidazole ligands!

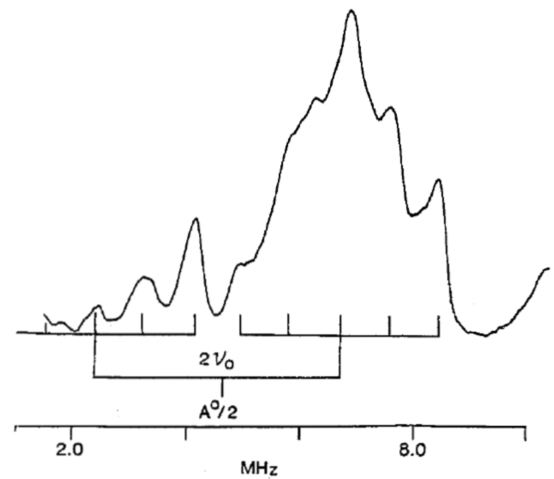
^{33}S



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

1. Basics

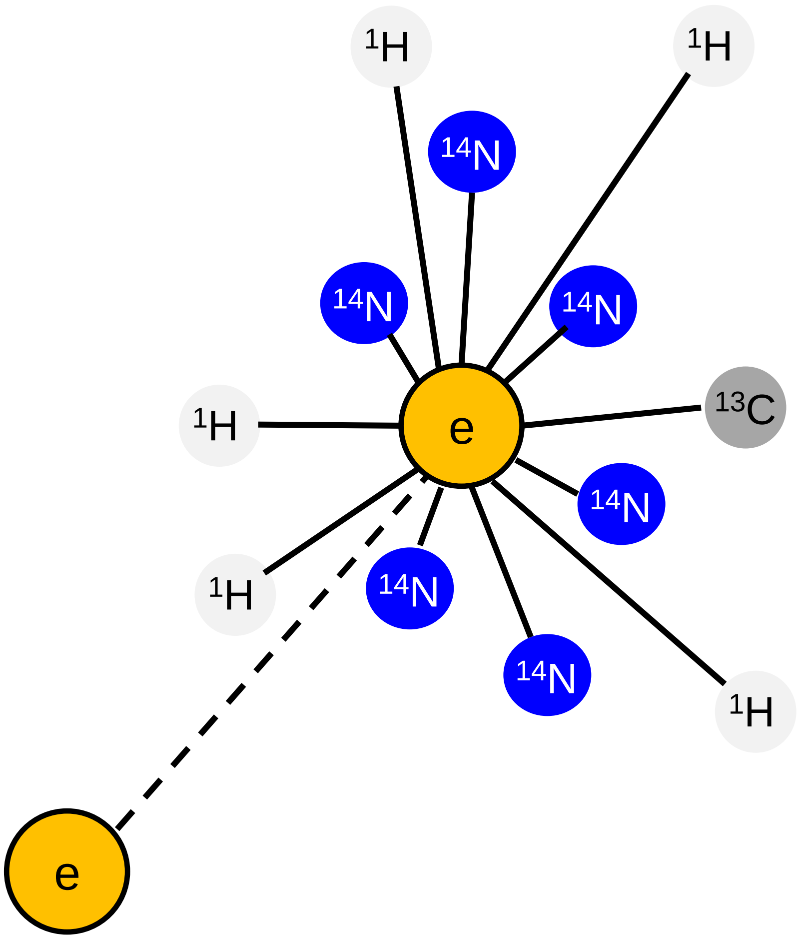
CW vs. pulse EPR
Sample and spectrometer
Resonators and bandwidths
Pulses, excitation width
Orientation selection
FIDs and Echo
Deadtime, Relaxation

2. Interactions

Nuclear Zeeman interaction
Hyperfine interaction
Coupling regimes
Nuclear spectra
Quadrupole interaction

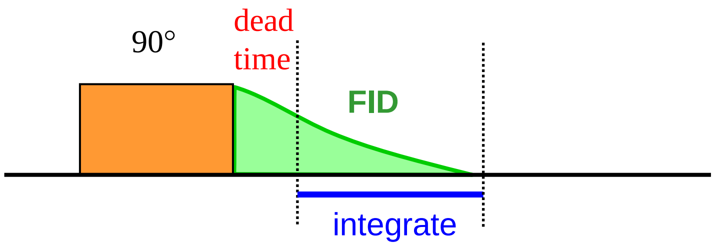
3. Experiments

Field sweeps
ENDOR
ESEEM
HYSCORE
DEER



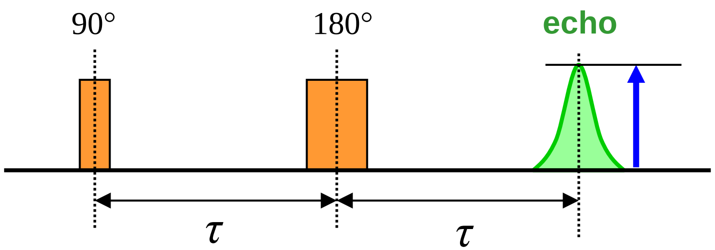
EPR spectrum: Field sweep spectra

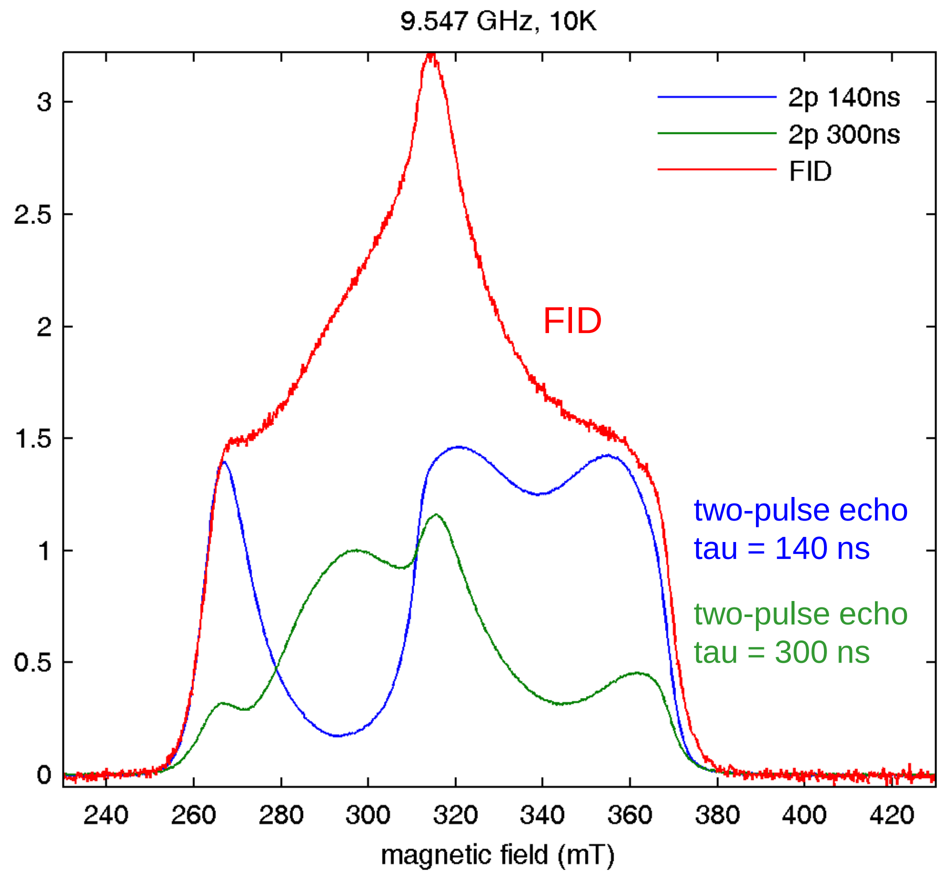
FID-detected field sweep



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

Echo-detected field sweep





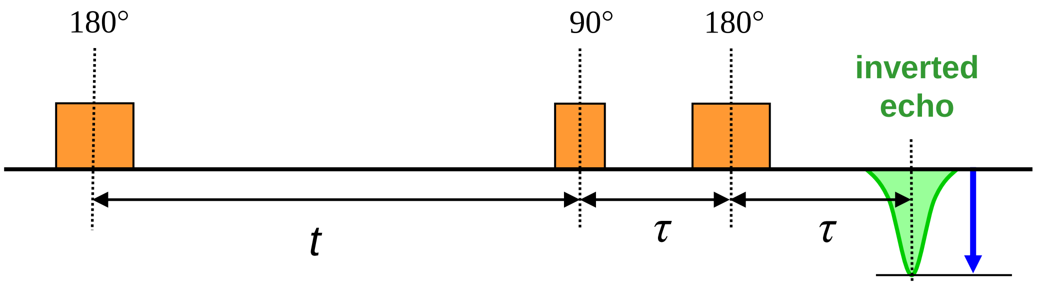
Alexey Silakov

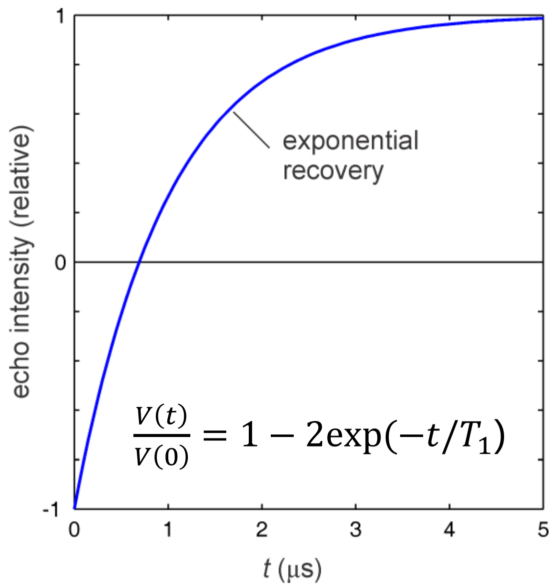
Distortions due to tau-dependent nuclear modulation of echo amplitude

Relaxation measurements

T_1: Inversion recovery

measure echo intensity as a function of t

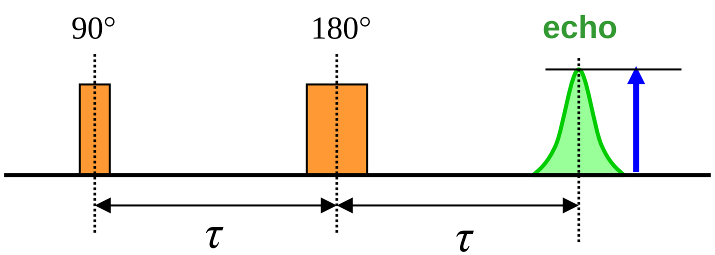


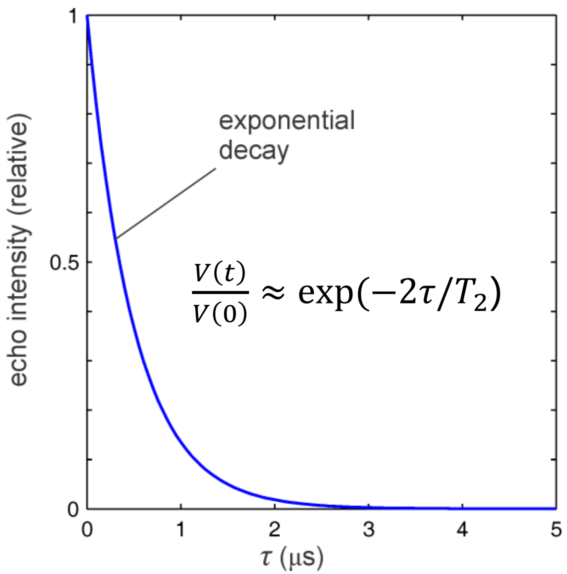


Other methods for T_1: saturation recovery, three-pulse echo decay

T_2, T_m: Two-pulse echo decay

measure echo intensity as a function of τ

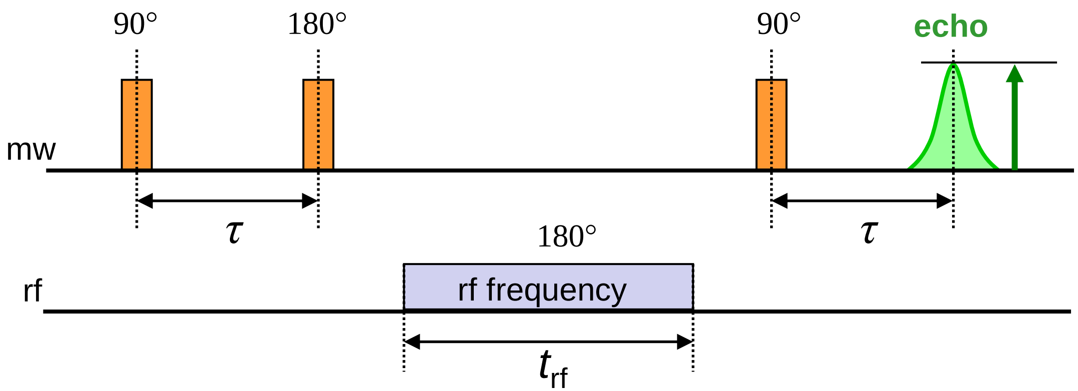


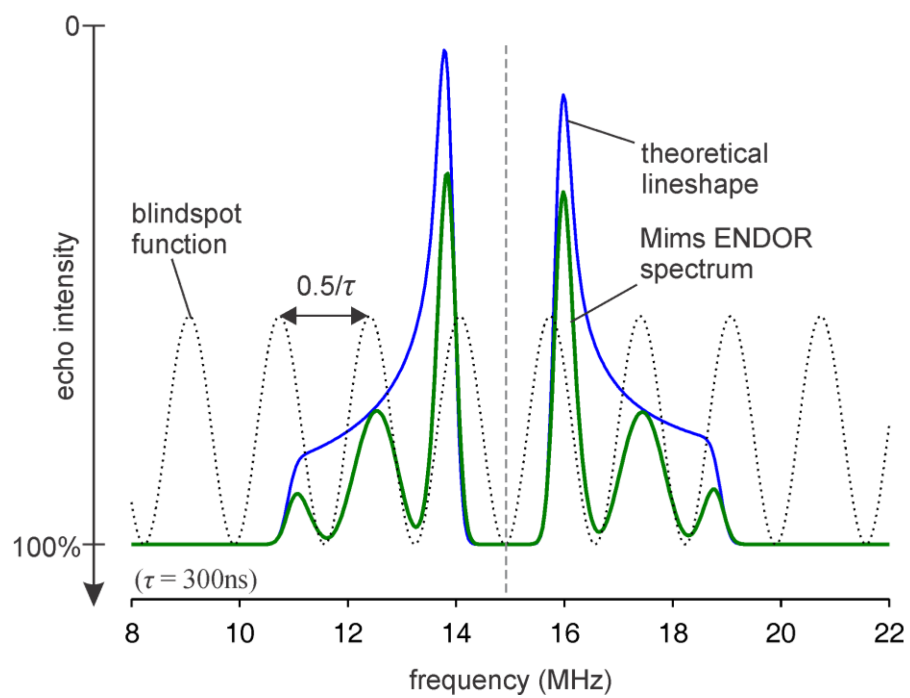


- approximately exponential decay
- phase memory, T_m, rather than T_2 is obtained
- best with small flip angles (avoids instantaneous diffusion)

Nuclear spectra: Mims ENDOR

Mims ENDOR: rf pulse frequency is varied





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

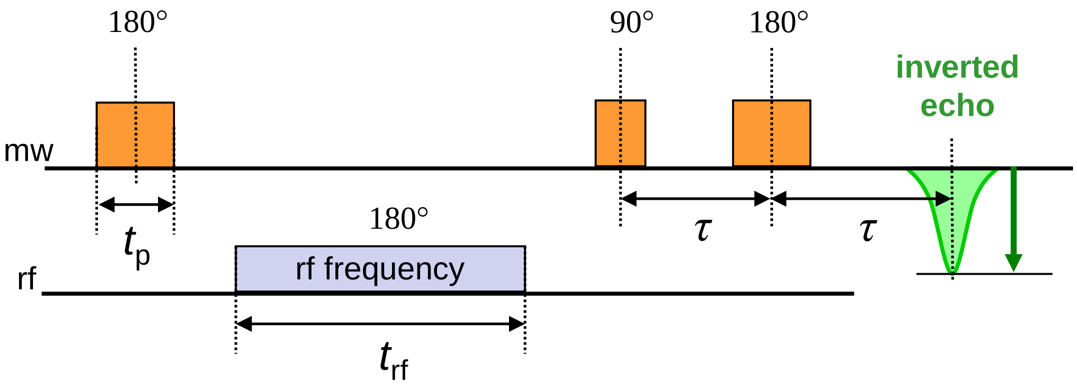
Blind spots

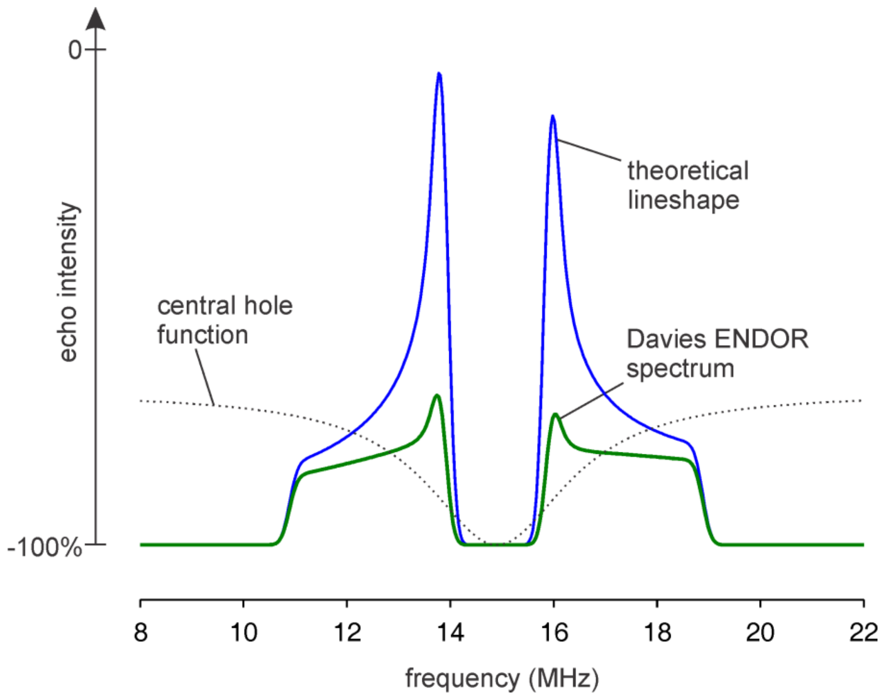
- intensity is modulated with τ-dependent sawtooth pattern, centered at Larmor frequency and with period $0.5/\tau$ ("Mims holes")
- central hole at Larmor frequency!

works best for small hyperfine couplings less than about $1/\tau$ (typically ^{2}H, ^{13}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

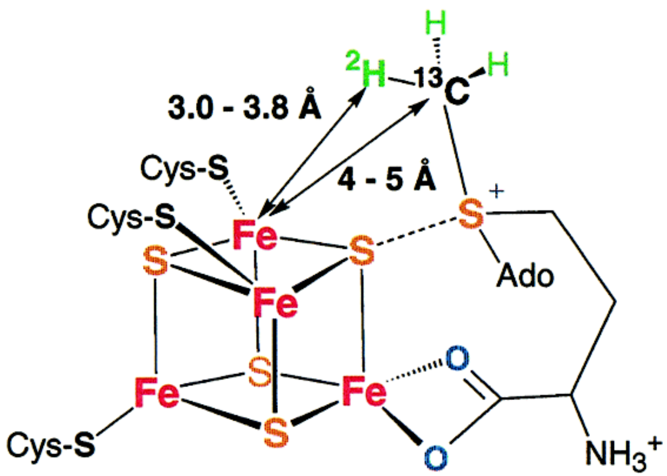
Spectrum

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

Blindspots

- no τ-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)

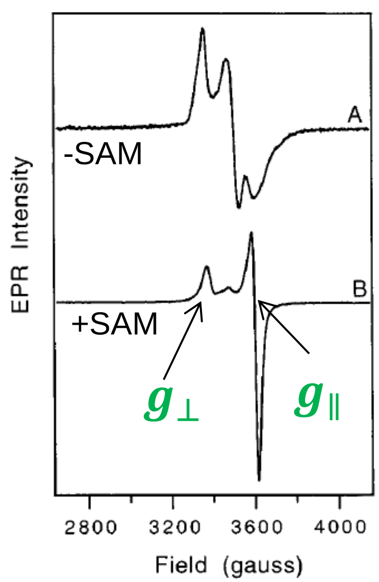
ENDOR example: Weak coupling ^2H, ^{13}C



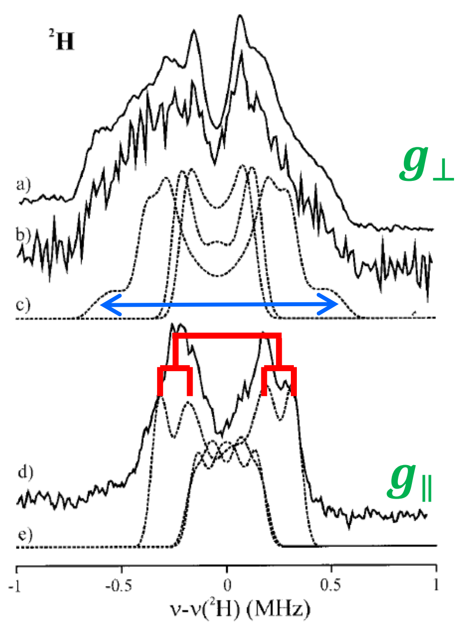
S-adenosyl-methionine (SAM)
binding to [4Fe4S] cluster in
pyruvate formate-lyase activating enzyme (PFL-AE)

Broderick & Hoffman
JACS **2002** 124 3143 link

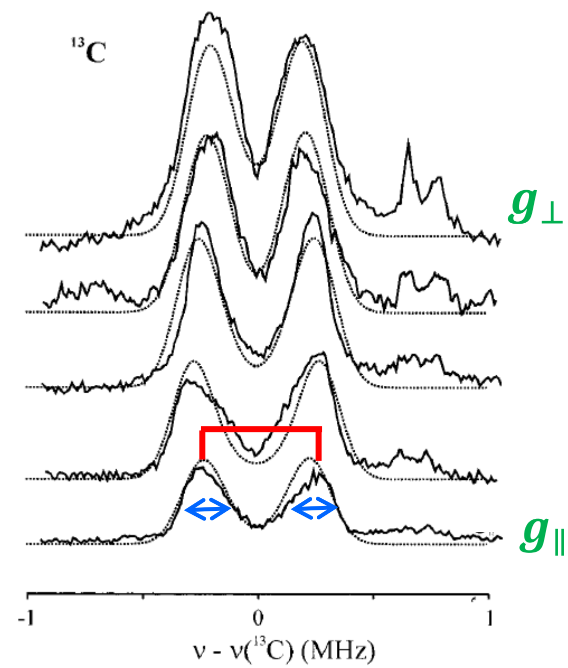
CW EPR



^2H ENDOR

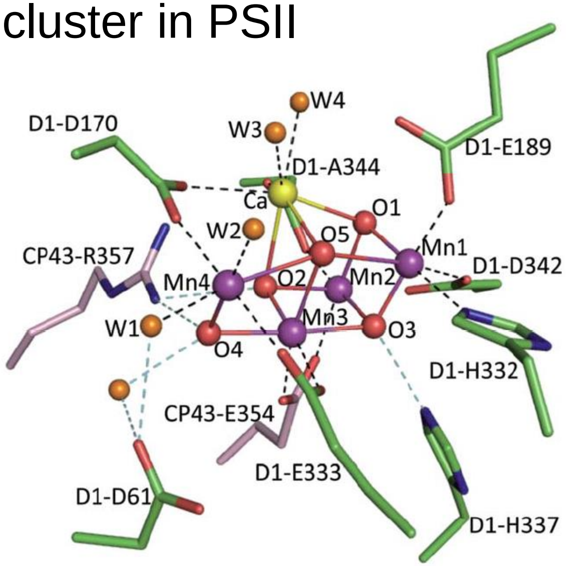


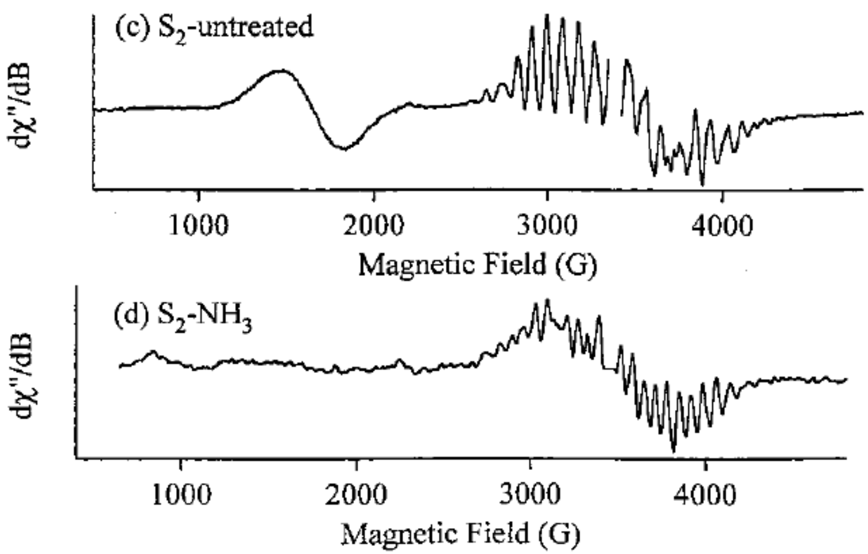
^{13}C ENDOR



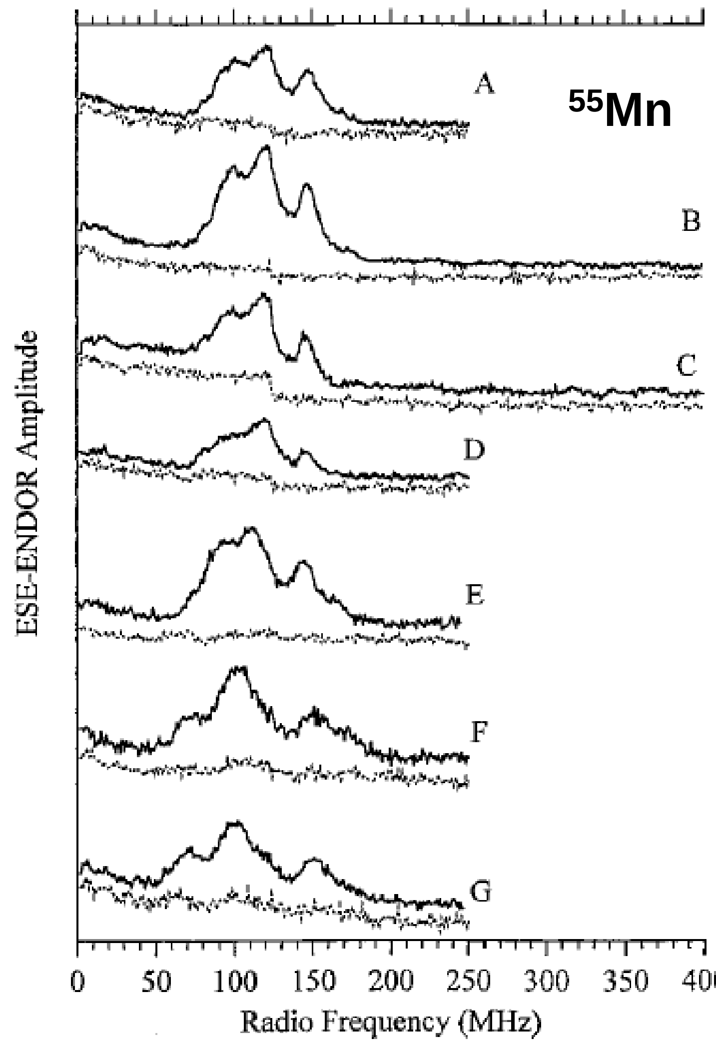
ENDOR example: Strong coupling ^{55}Mn

[Mn_4Ca] cluster in PSII





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

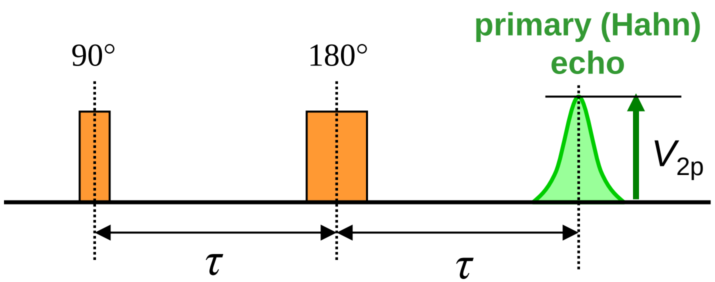


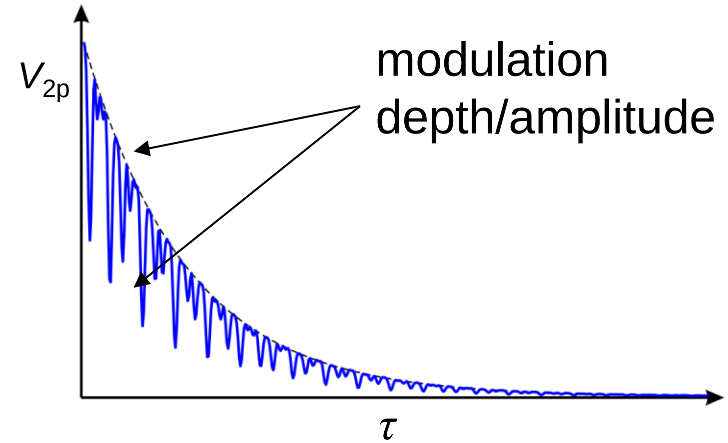
JACS **2000** 122 10926

Nuclear spectra: ESEEM

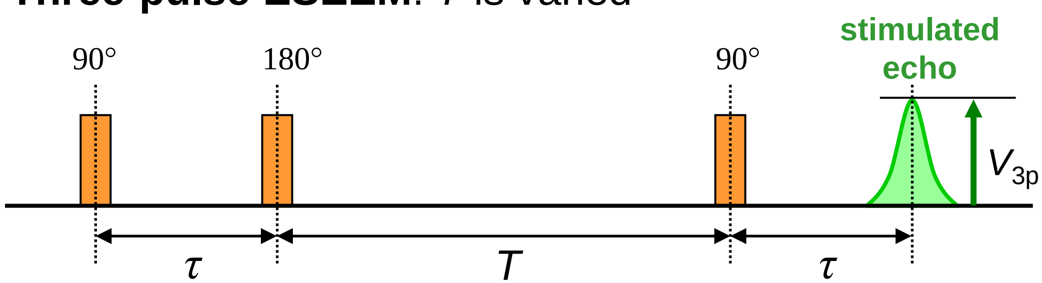
electron spin echo envelope modulation

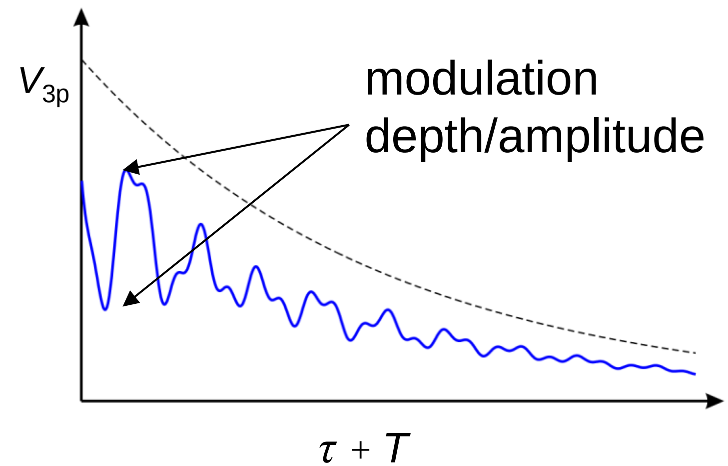
Two-pulse ESEEM: τ is varied





Three-pulse ESEEM: T is varied

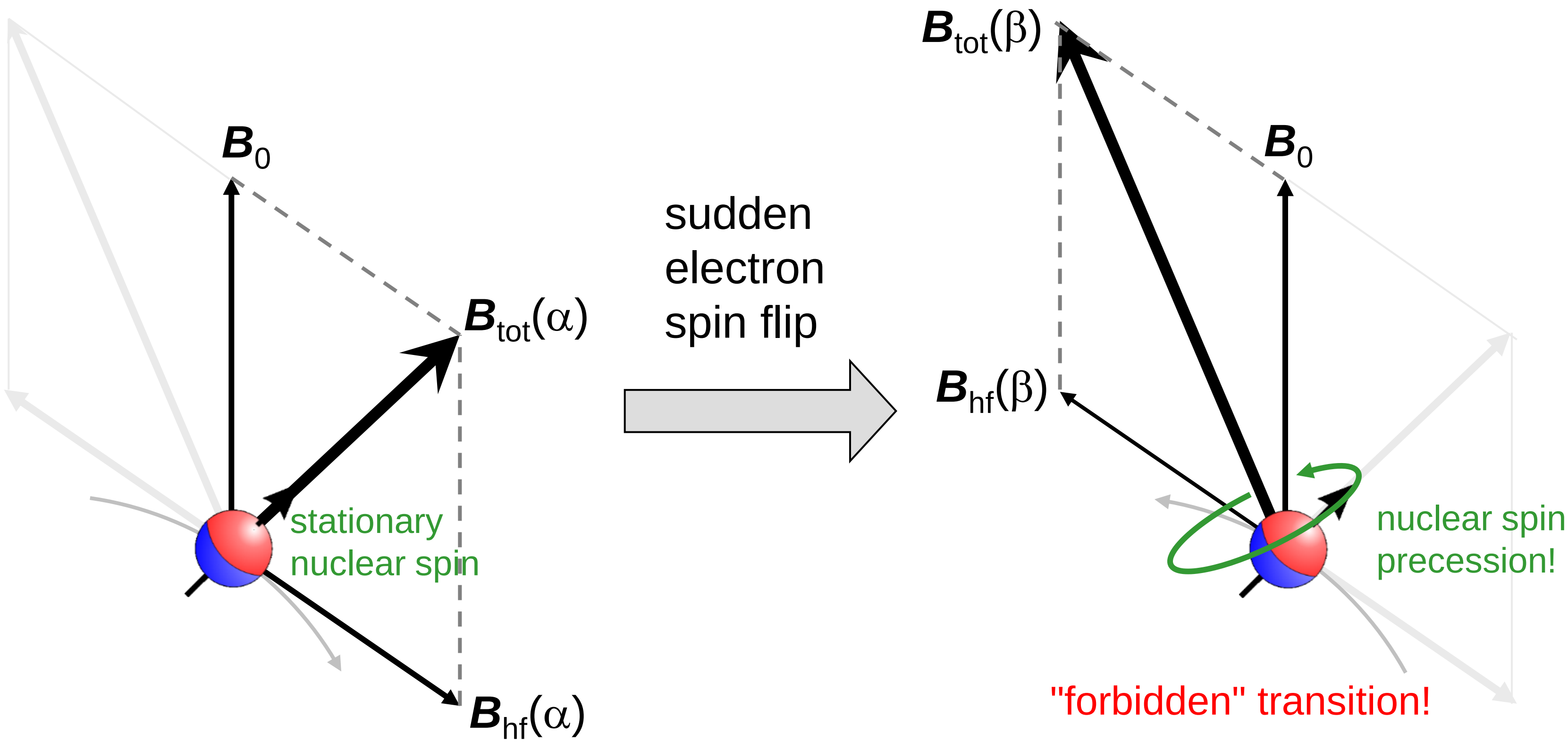




- modulation of echo amplitude as a function of interpulse delay(s)
- modulation with nuclear resonance frequencies and their combinations
- modulation due to hyperfine coupling of electron spin with surrounding nuclei
- modulation depth depends on hyperfine coupling, quadrupole coupling, nuclear Zeeman

ESEEM: Pictorial model

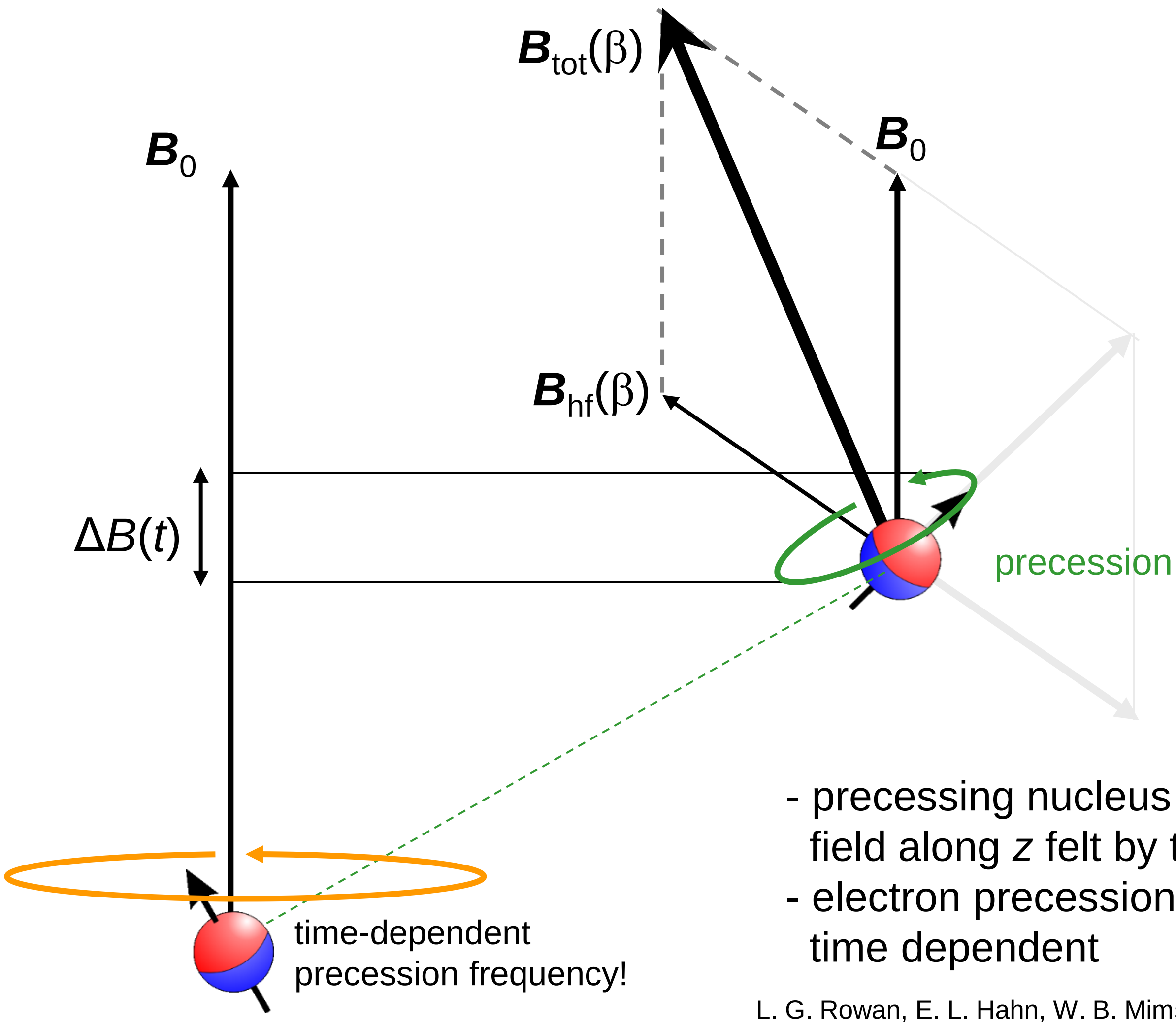
(1) Electron spin flip induces nuclear precession



- 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

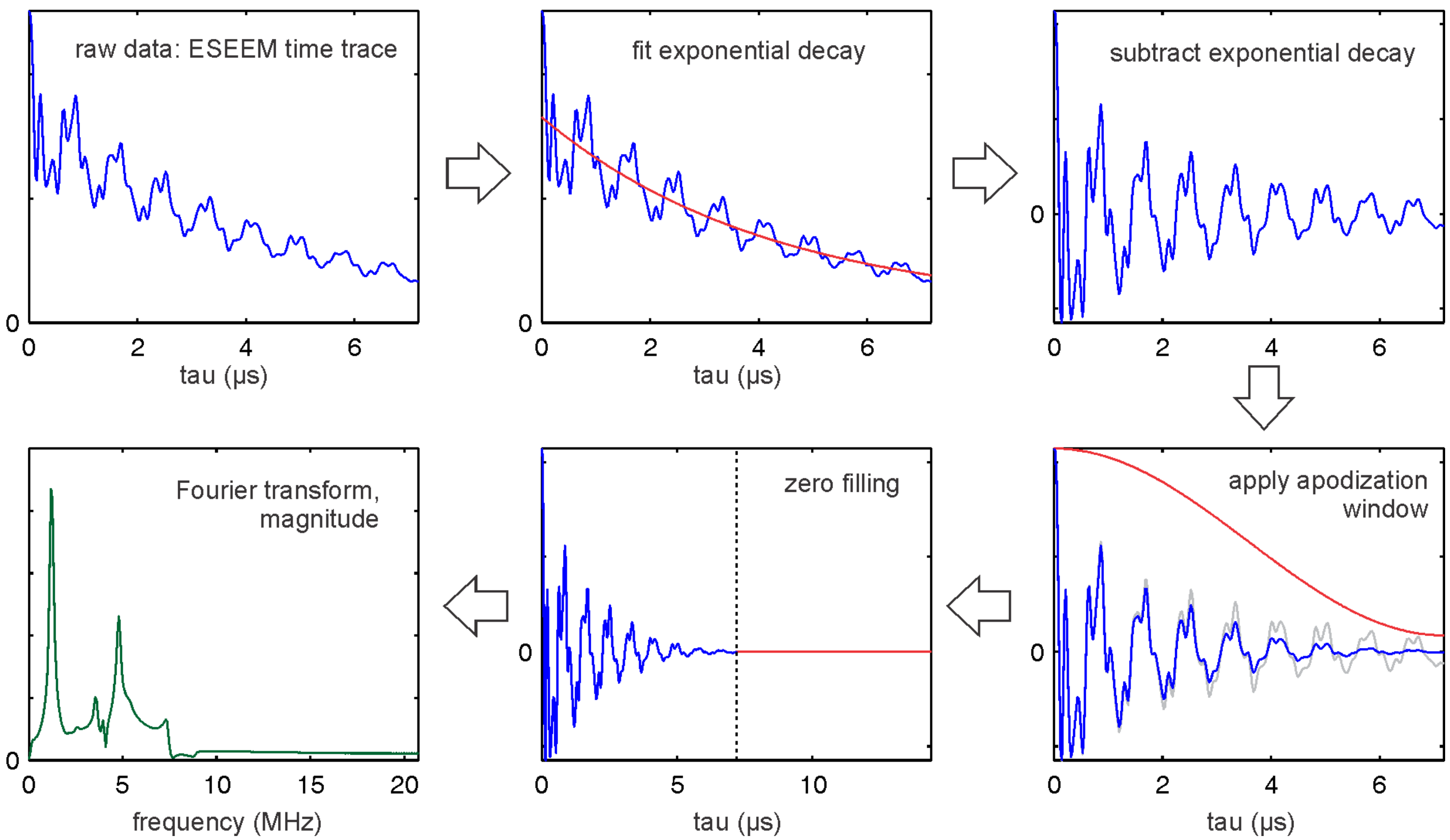
(2) Nuclear precession modulates electron precession



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

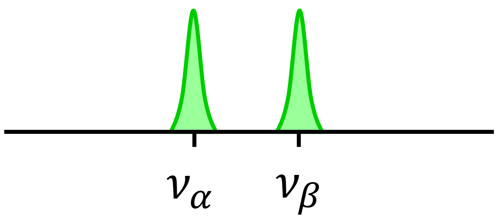
L. G. Rowan, E. L. Hahn, W. B. Mims, Phys. Rev. A 137, 61-71, 1965
D. Grischkowsky, S. R. Hartmann, Phys. Rev. B 2, 60-74,1970
S. A. Dikanov, Yu. D. Tsvetkov, ESEEM Spectroscopy, CRC Press, 1992

ESEEM: Data processing



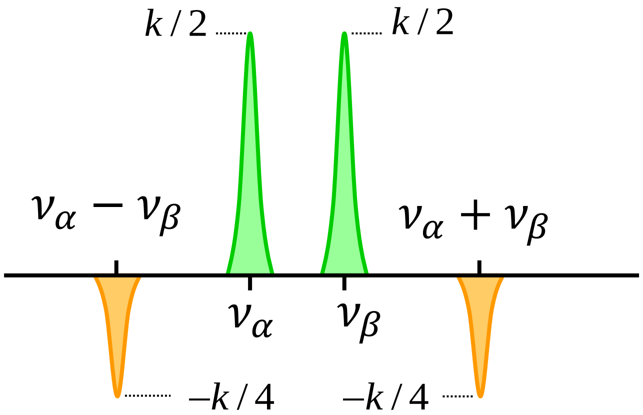
Nuclear spectra: ENDOR vs. ESEEM

ENDOR



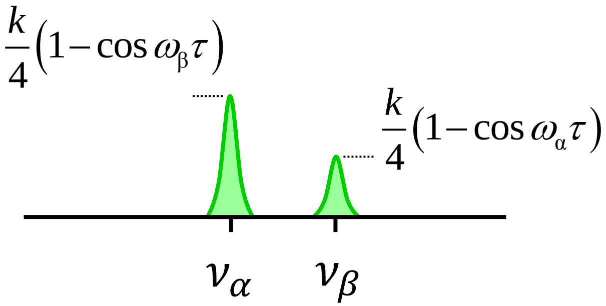
equal intensities

Two-pulse ESEEM



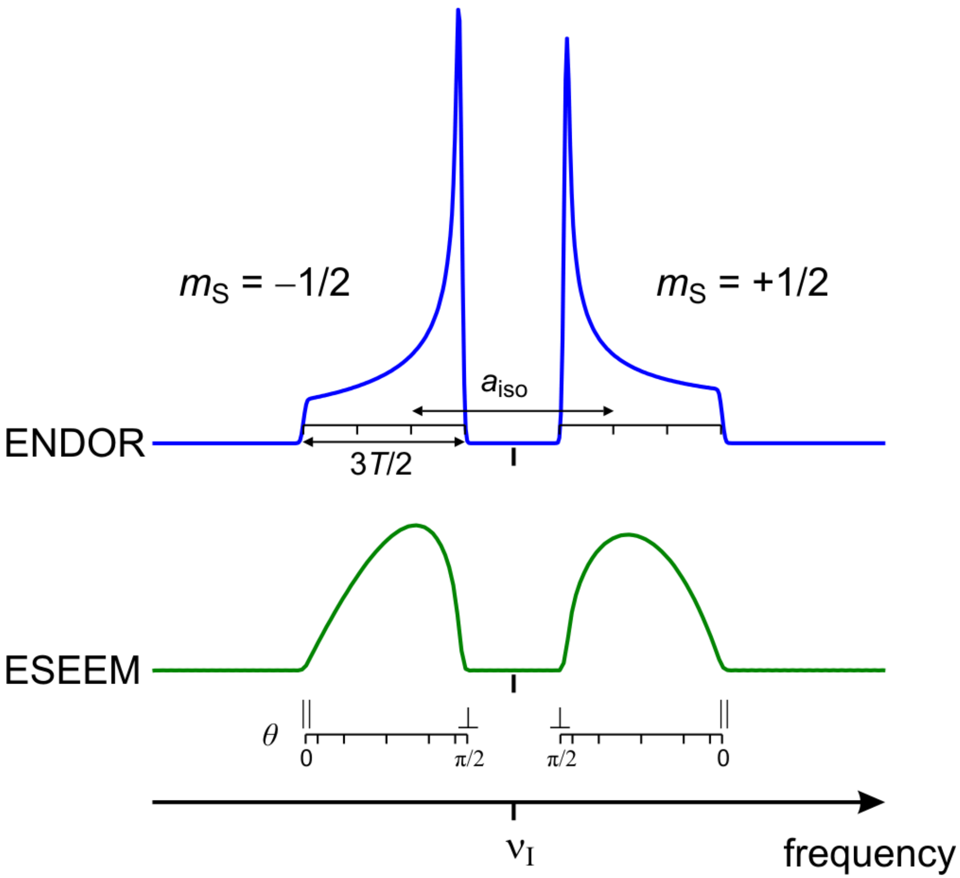
T_m decay (fast)
sum and difference frequencies
no blind spots

Three-pulse ESEEM



T_1 decay (slower)
no sum and difference frequencies
blind spots
τ adds to dead time

Nuclear spectra: ENDOR vs. ESEEM



ENDOR:

- maximum intensity at $\theta = 90°$
- mimimum intensity at $\theta = 0°$

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

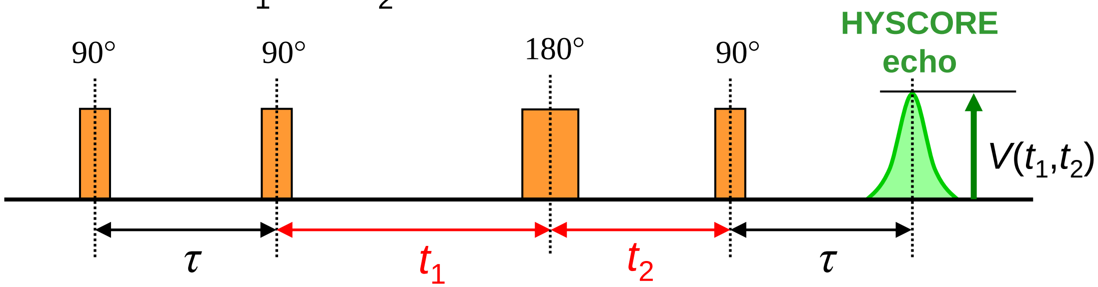
ESEEM enhanced by nuclear state mixing; most intense in matching regime, i.e. low nuclear frequencies

ENDOR enhanced by hyperfine enhancement, most intense for high nuclear frequencies

HYSCORE: A two-dimensional ESEEM experiment

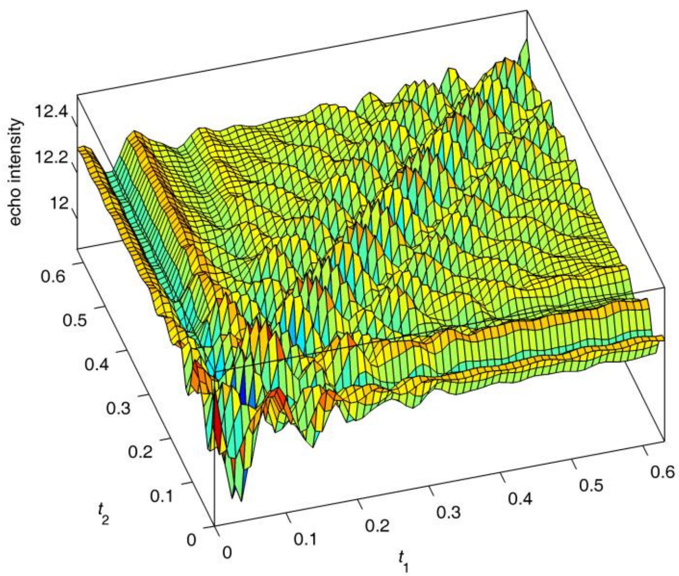
HYSCORE = hyperfine sublevel correlation

HYSCORE: t_1 and t_2 is varied



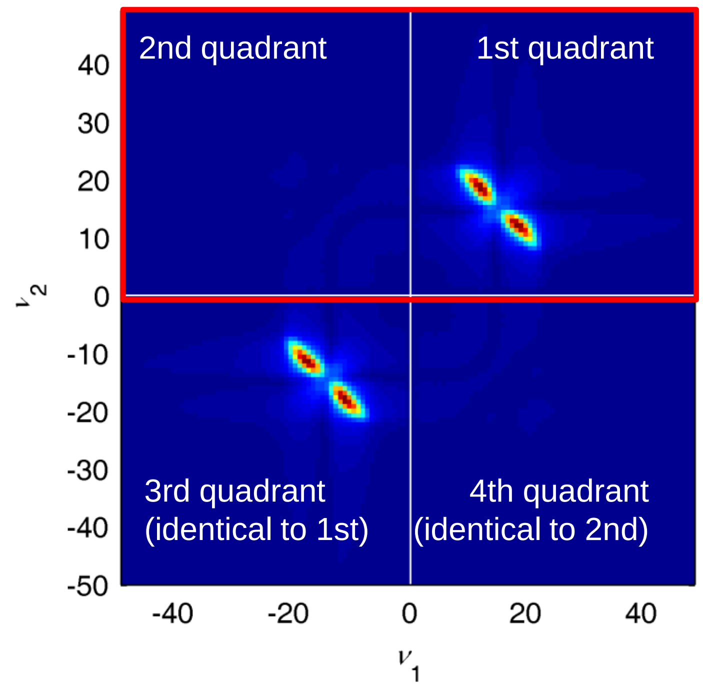
π pulse should be as short as possible

2D time domain (TD)



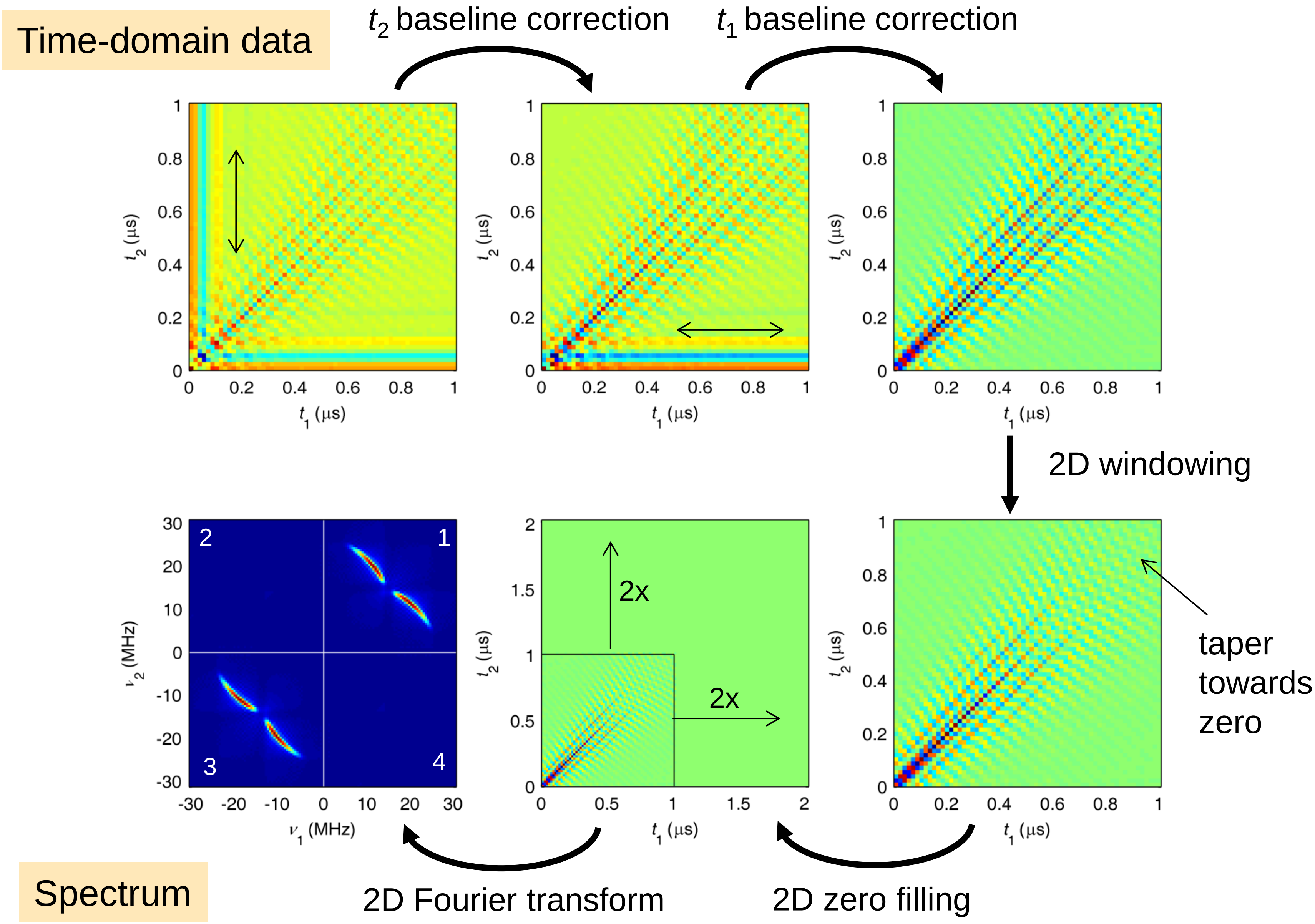
echo intensity as a function of t_1 and t_2

2D frequency domain (FD)



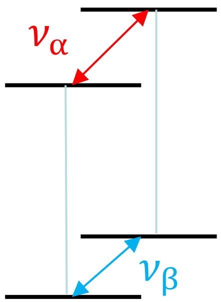
only 1st and 2nd quadrant are shown

HYSCORE: Data processing

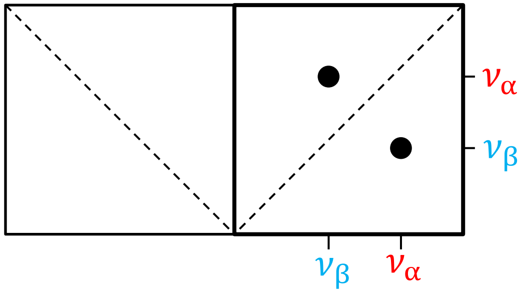


HYSCORE: Spectra

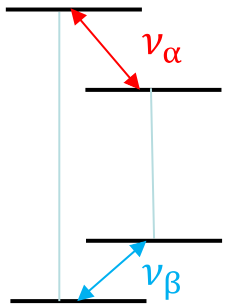
Weak coupling



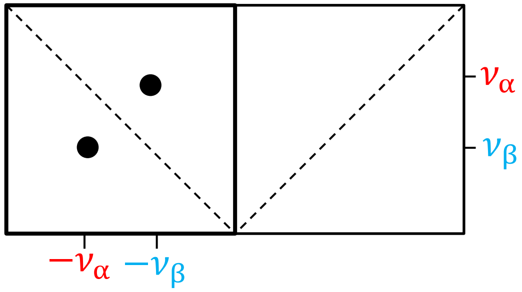
first quadrant



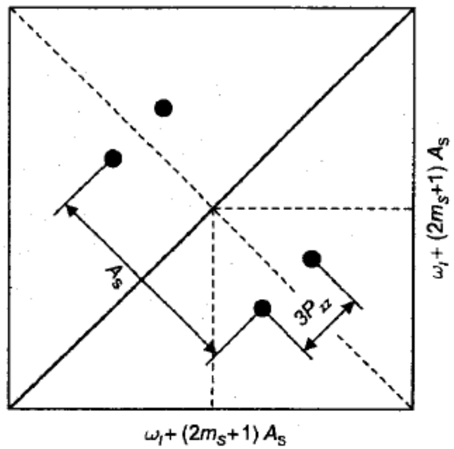
Strong coupling



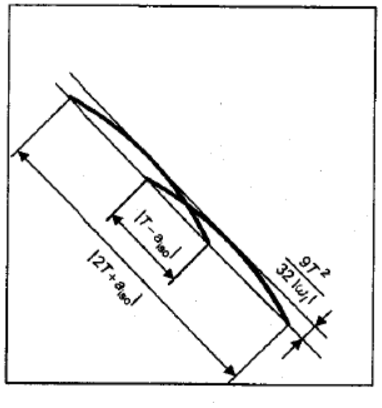
second quadrant



Quadrupole splittings



Powder spectra



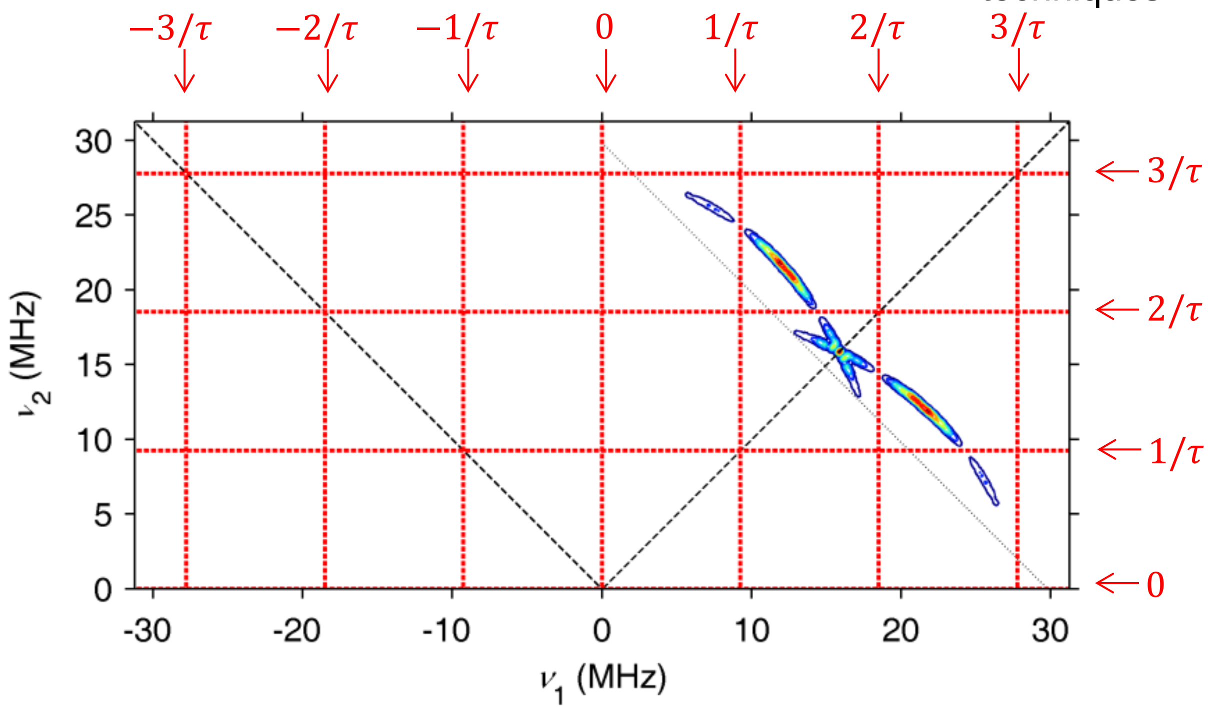
HYSCORE: Blind spots

Blind spots:
- τ-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

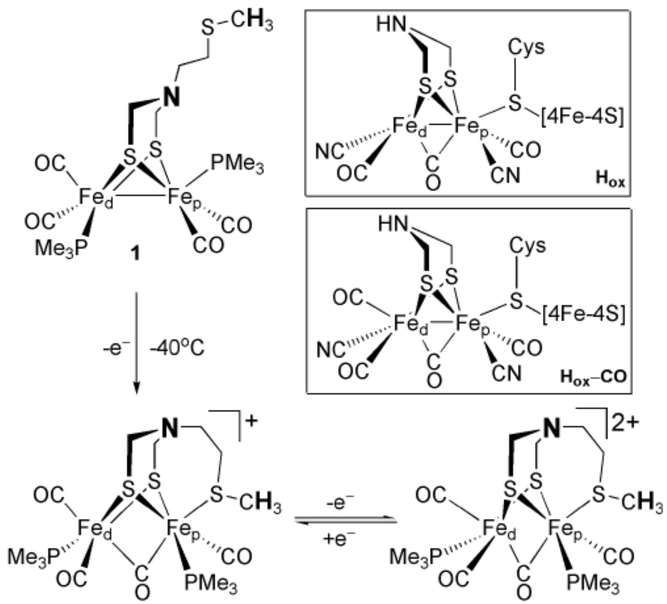
Consequences:
- peaks are missing
- peaks are distorted
- danger of wrong assignment

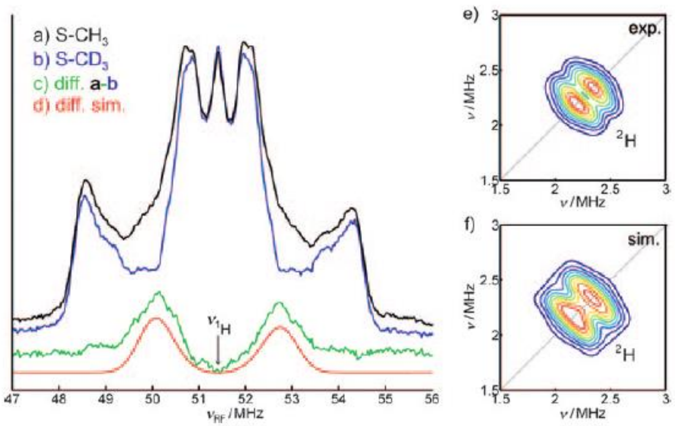
Remedies:
- acquire spectra with several different tau values
- use blind-spot free advanced techniques

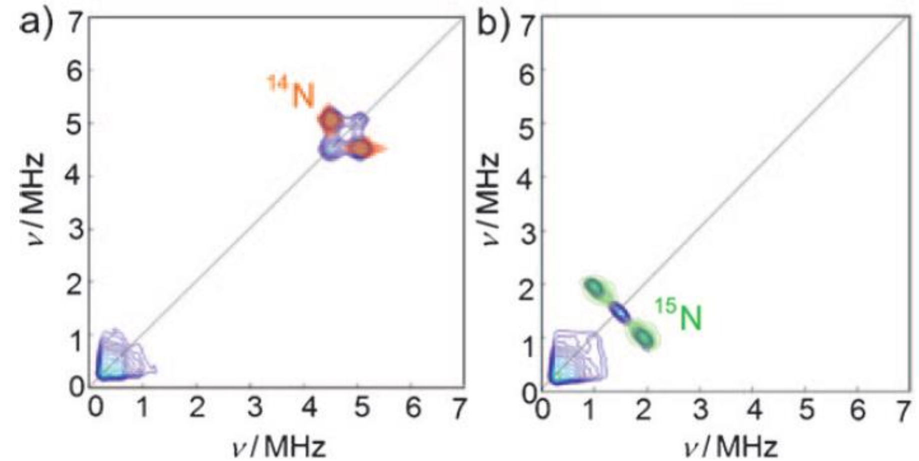


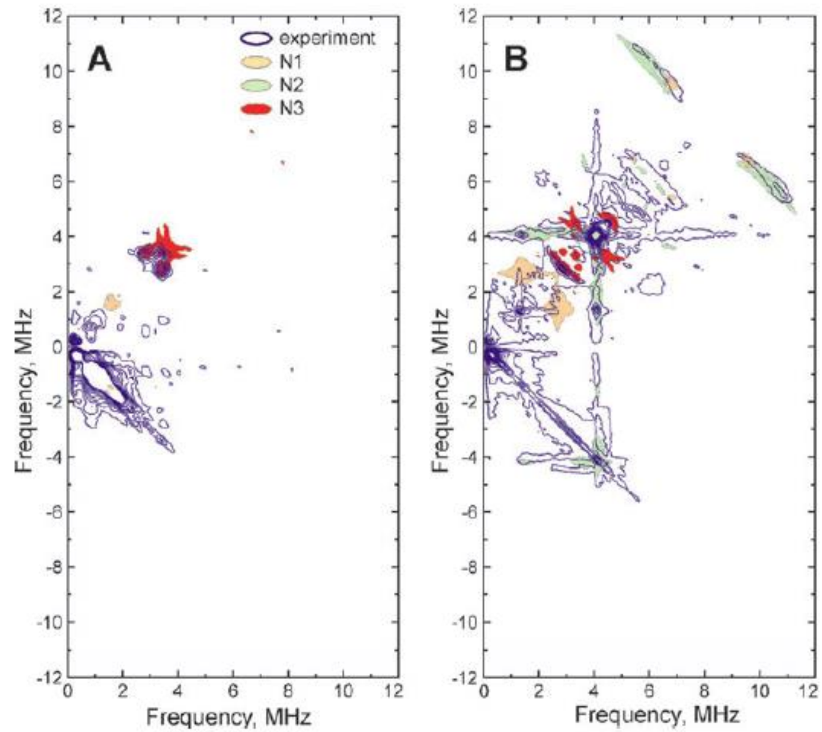
HYSCORE: Example

[FeFe] hydrogenase + model





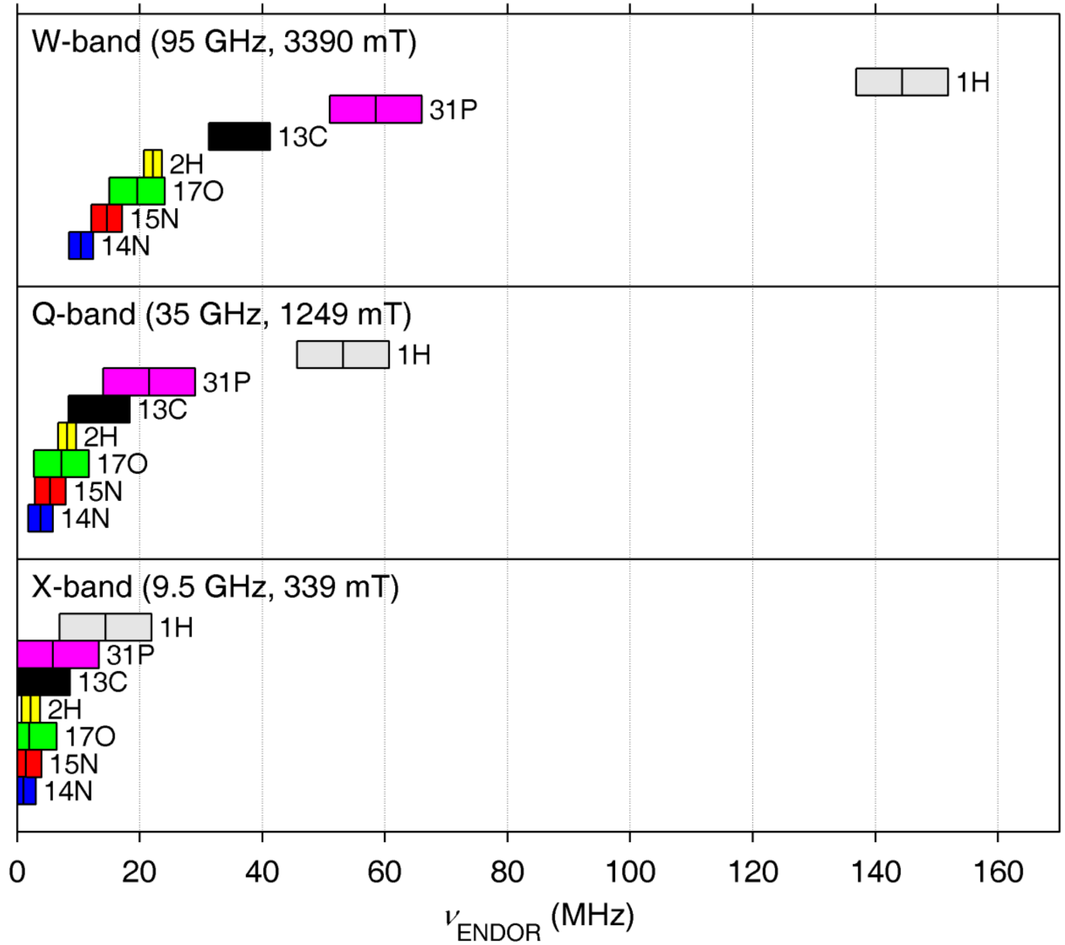




Angew. Chem. **2011** 50 1
PCCP **2009** 11 6592

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

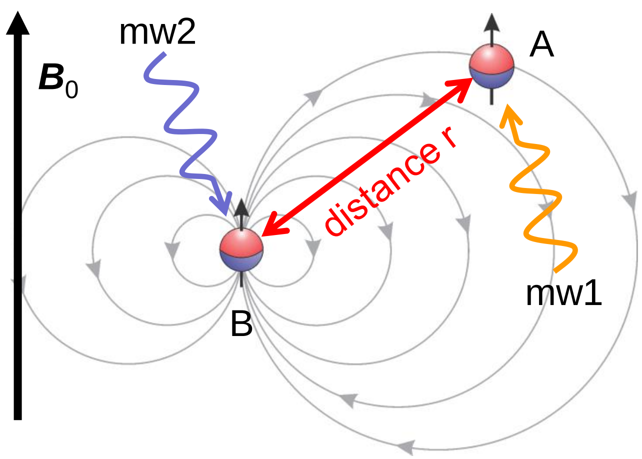
Disadvantages
- less signal for strongly anisotropic systems
- less available power
- longer pulses

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

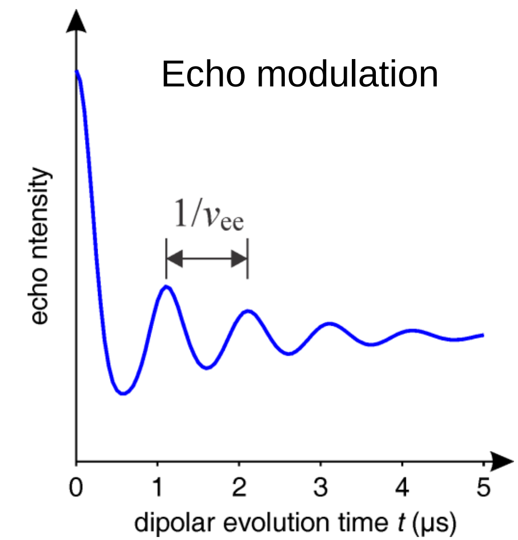
DEER: Distances between electron spins

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



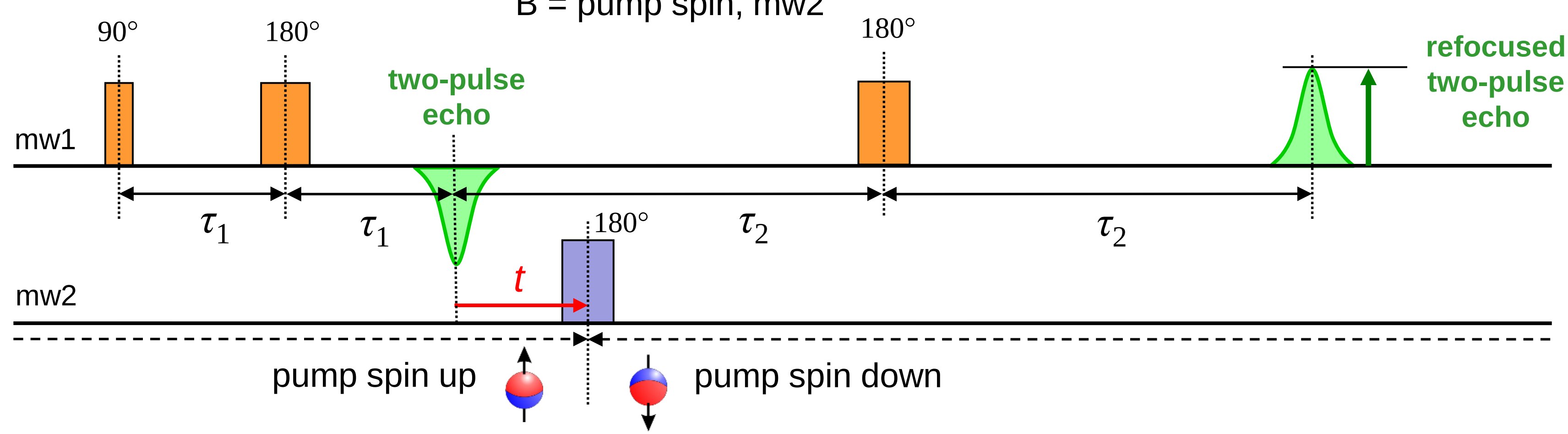
Dipolar coupling between two electron spins analogous to dipolar hyperfine coupling

$$\nu_{ee} = \frac{\mu_0 \mu_B^2}{4\pi h} g_A g_B \frac{1}{r^3}$$



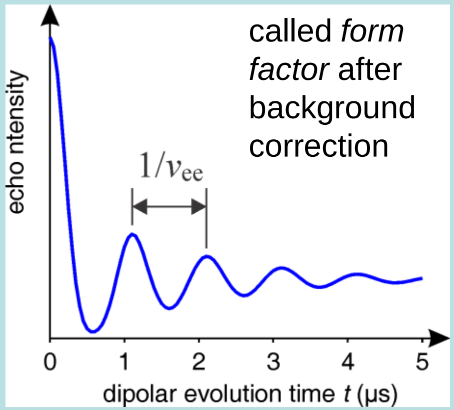
4-pulse DEER: t is varied

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



DEER: Data analysis

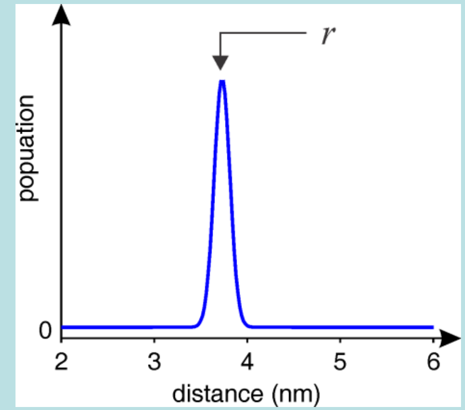
Echo modulation



Least-squares analysis

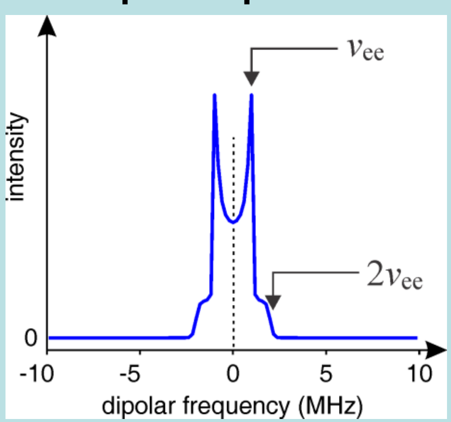
(Gaussian fit or Tikhonov regularization)

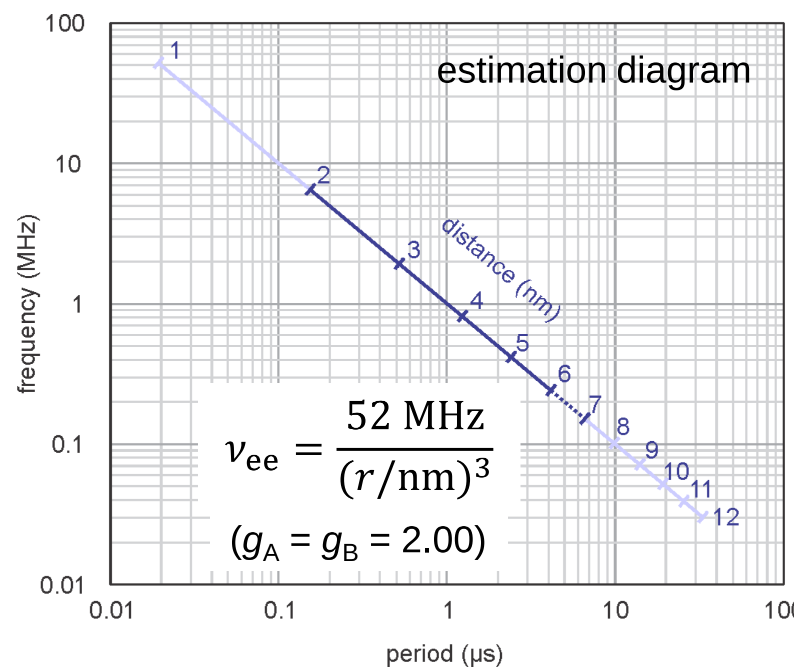
Distance distribution



Fourier transform

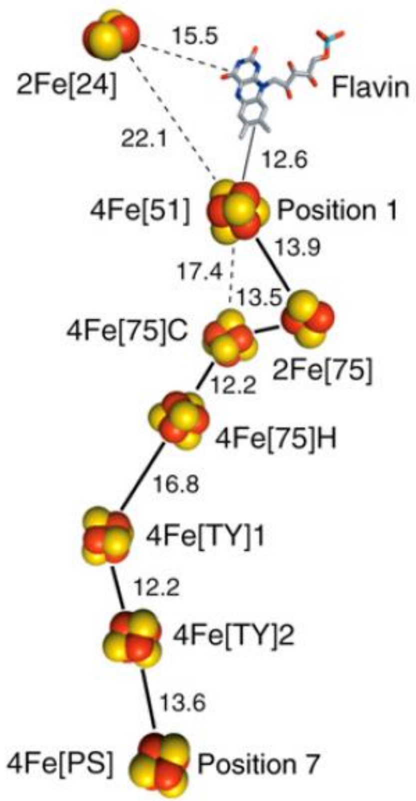
Dipolar spectrum





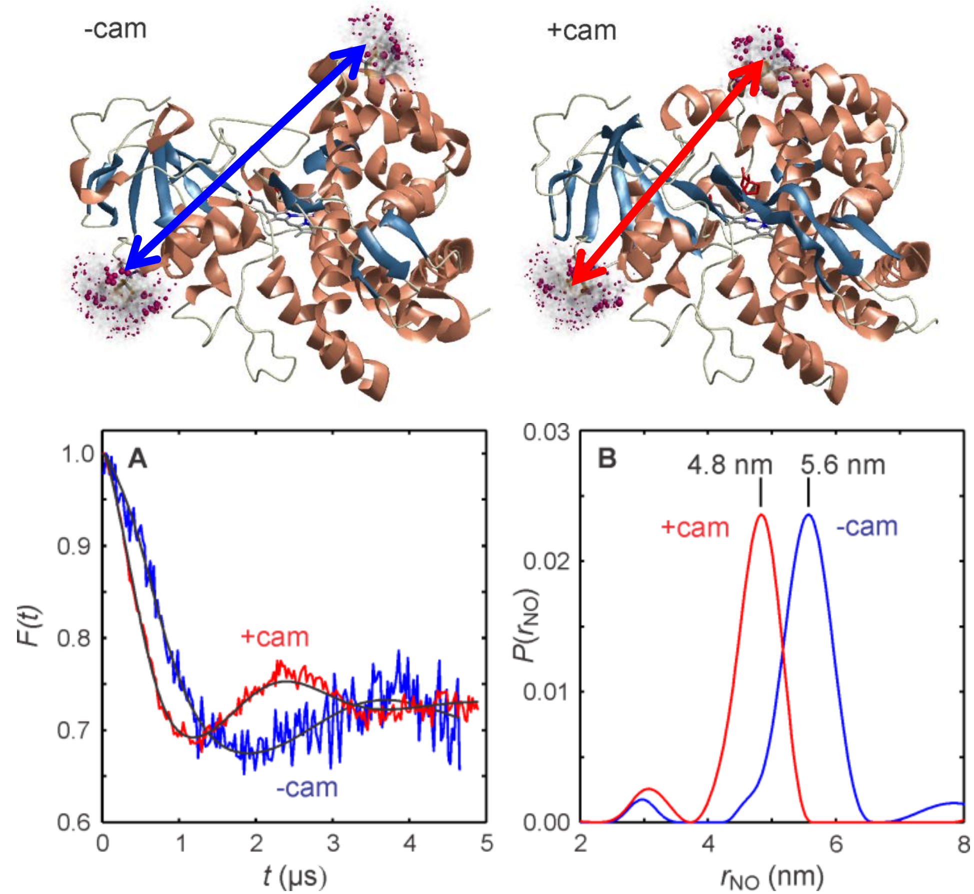
DEER: Examples

Arrangement of iron-sulfur clusters



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

Conformational change upon substrate binding



Cytochrome P450cam
Stoll et al; PNAS **2012** 109 12888 link

What you can learn from EPR data

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