A Brief Introduction to pulse EPR

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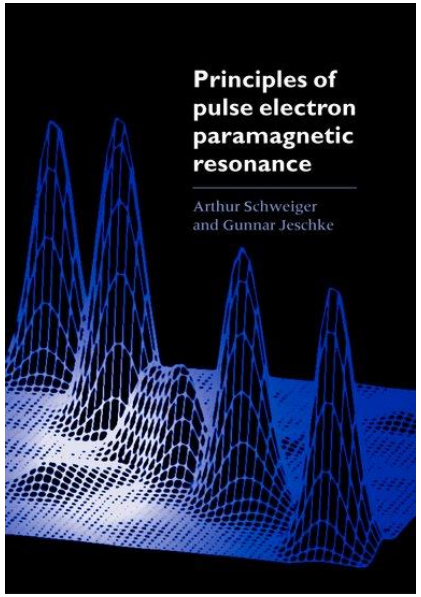
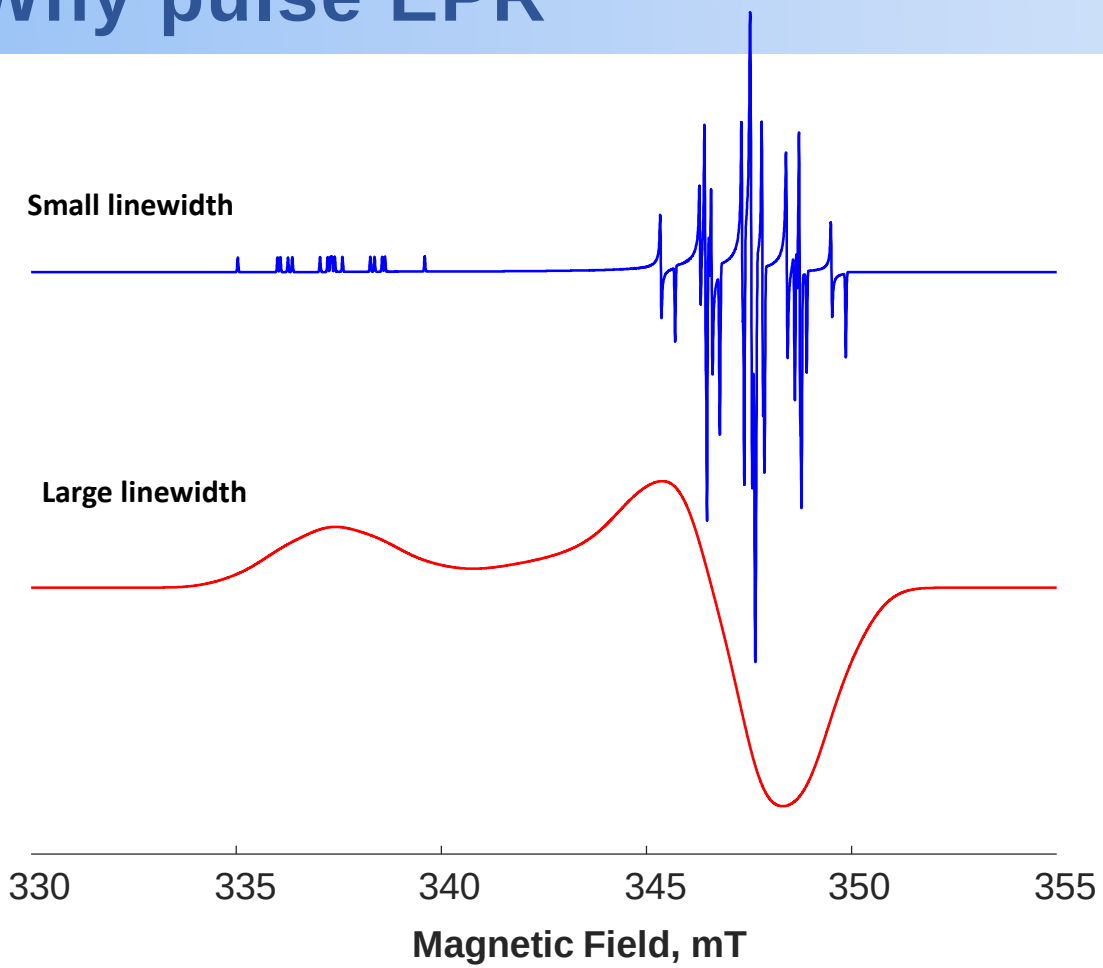


- Principles of pulse EPR
- Terminology
- Hyperfine interaction
- Examples

Why pulse EPR



All weak interactions are present in the CW EPR spectra, but they may be hidden within the linewidth.

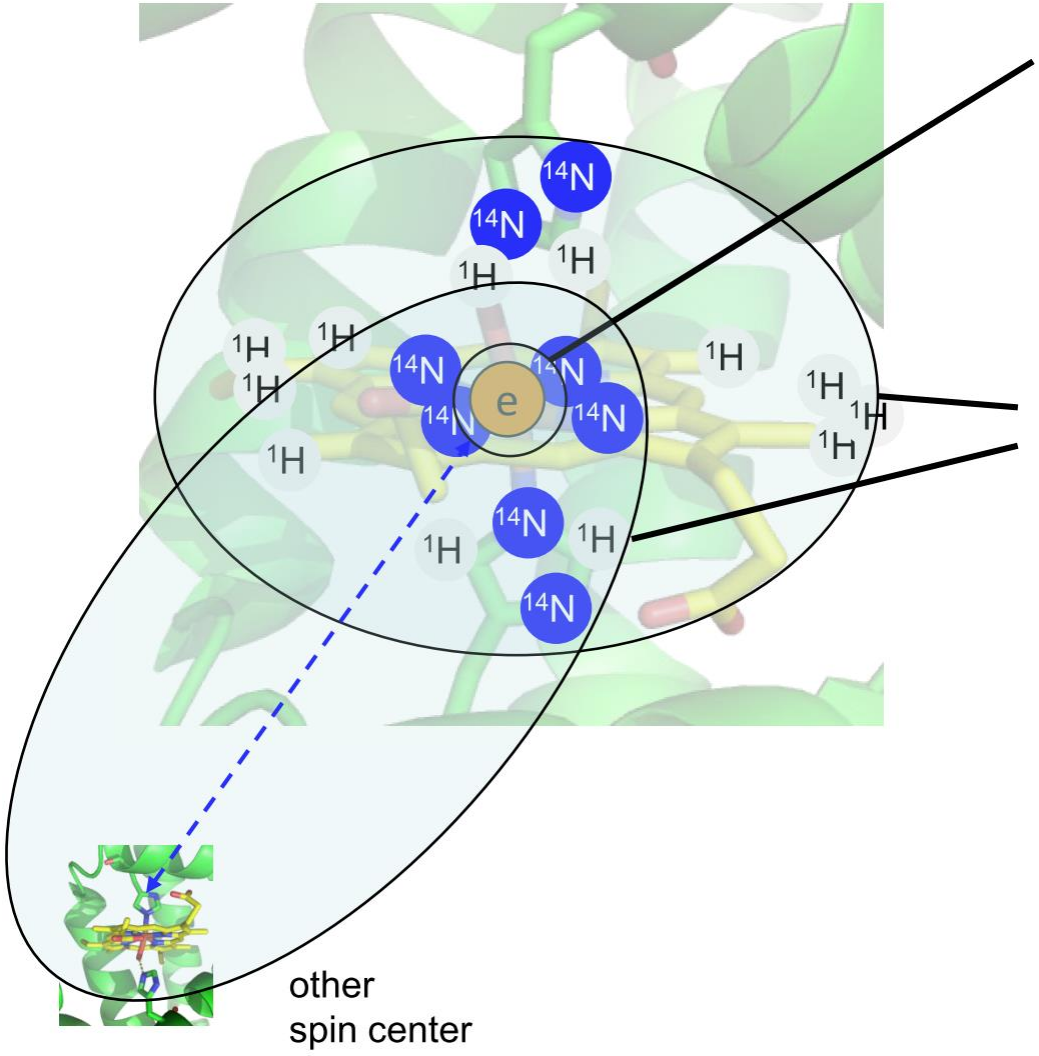
Pulse EPR methods allow to significantly improve spectroscopic resolution.

A hypothetical spectrum of an axial EPR spectrum containing 4x^{57}Fe (I=1/2)

Improved spectral resolution = Detect weaker magnetic interactions
= Obtain more precise information

Unpaired electron spin as a probe of the environment

Electron spin “senses” other nuclear and electron spins.



Close-range hyperfine interactions.
Can be studied by CW EPR.

Long-range hyperfine interactions (up to 5-6 Å)
Need pulse EPR.

Electron spin-spin interaction between spin centers (up to ~100 Å)
Shorter distances can be studied by CW EPR
Generally, pulse EPR required

CW EPR vs Pulse EPR: Key differences

Continues Wave EPR

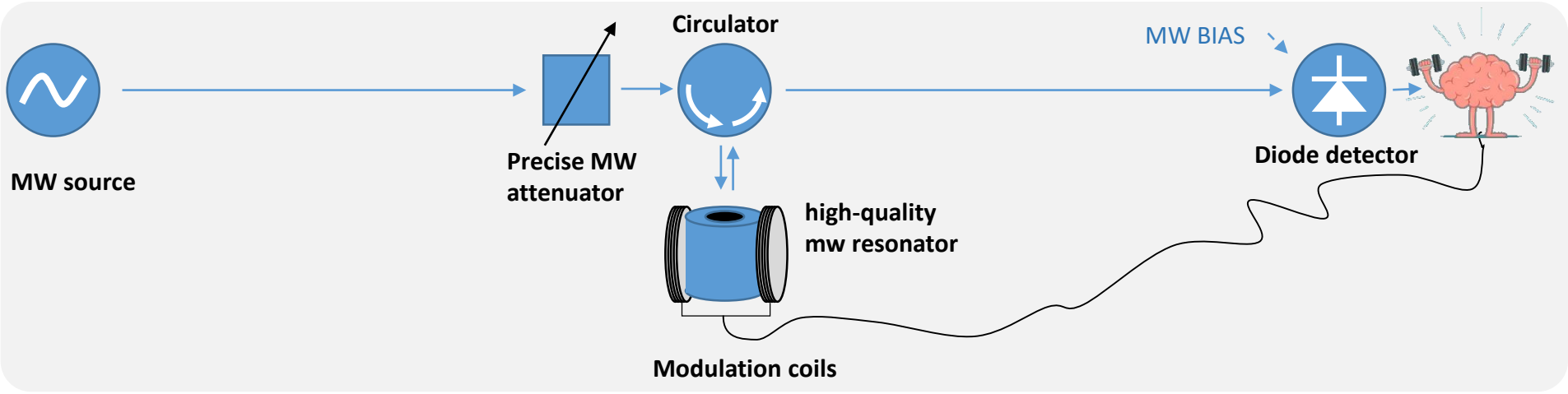
- Absorption spectroscopy
  Resonant absorption of microwaves
- Detection and excitation happen at the same time
- Direct output
  EPR spectrum
- Less susceptible to relaxation effects

Pulse EPR

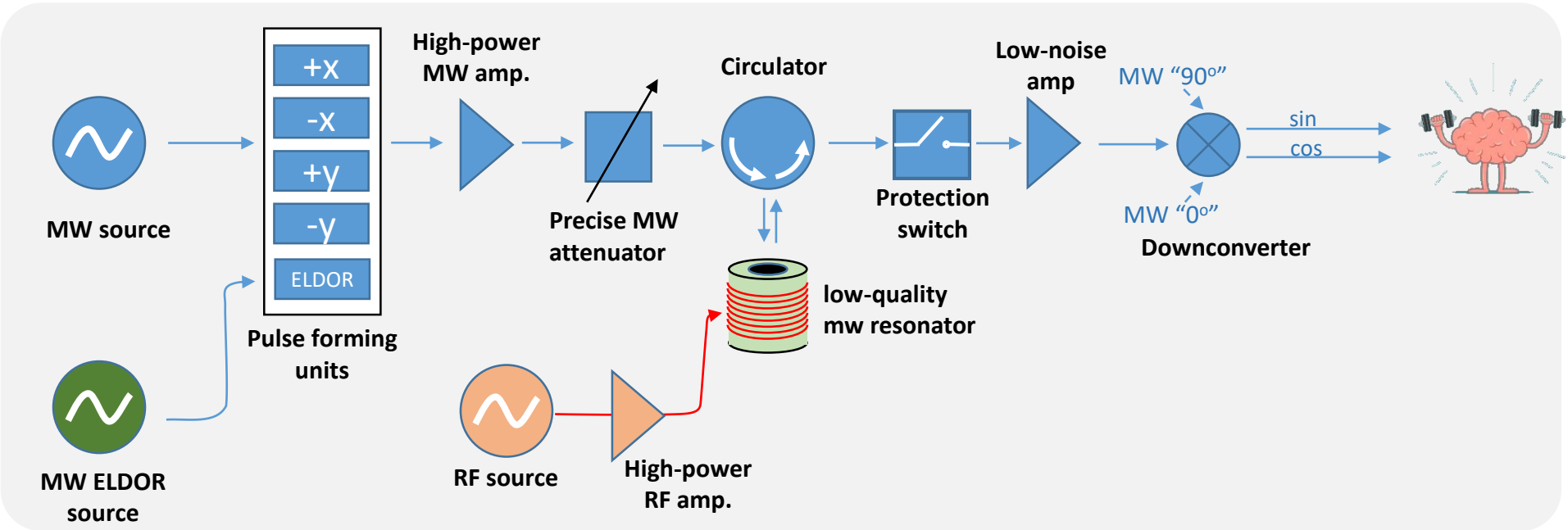
- Emission spectroscopy
  Sample transiently emits microwaves
- Detection and excitation are separate
- Complex outputs
  Most data require post processing
- Much more susceptible to relaxation effects
  You may not see a pulse EPR signals from a sample which has great CW EPR spectrum

Pulse EPR vs. CW EPR: Technique

CW EPR spectrometer ($):



Pulse EPR spectrometer ($$$$$):



Pulse EPR in a nutshell

We can turn a paramagnet into a **transient source** of MW irradiation by taking it out of its equilibrium state.

This transient signal produced by the paramagnet we detect.

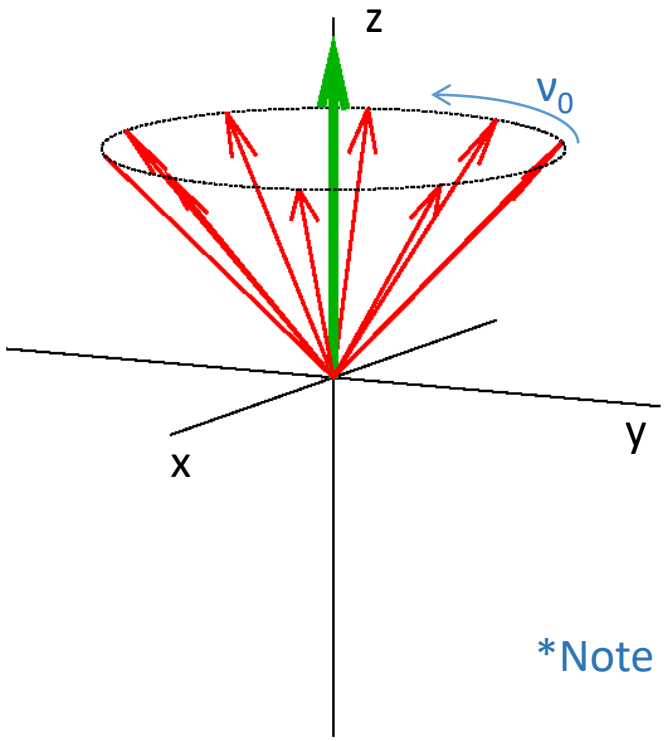
Time-evolution of an electron spin is affected by the magnetic environment (nuclear spins, other electron spins)

Observation of the outcome of the electron-spin evolution allows to assess information about spin-spin interactions

To get an idea about pulse EPR we need to think about what spins are doing during pulse EPR experiments

Spin acrobatics

A bunch of spins in an external magnetic field along “z” axis



Individual spins

Sum magnetization
(scale is not preserved)

sum of all the individual spins points along z direction

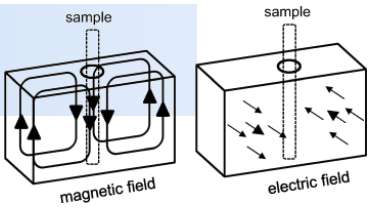
*Note that sum magnetization is stationary

Free electron in the absence of any other interactions.

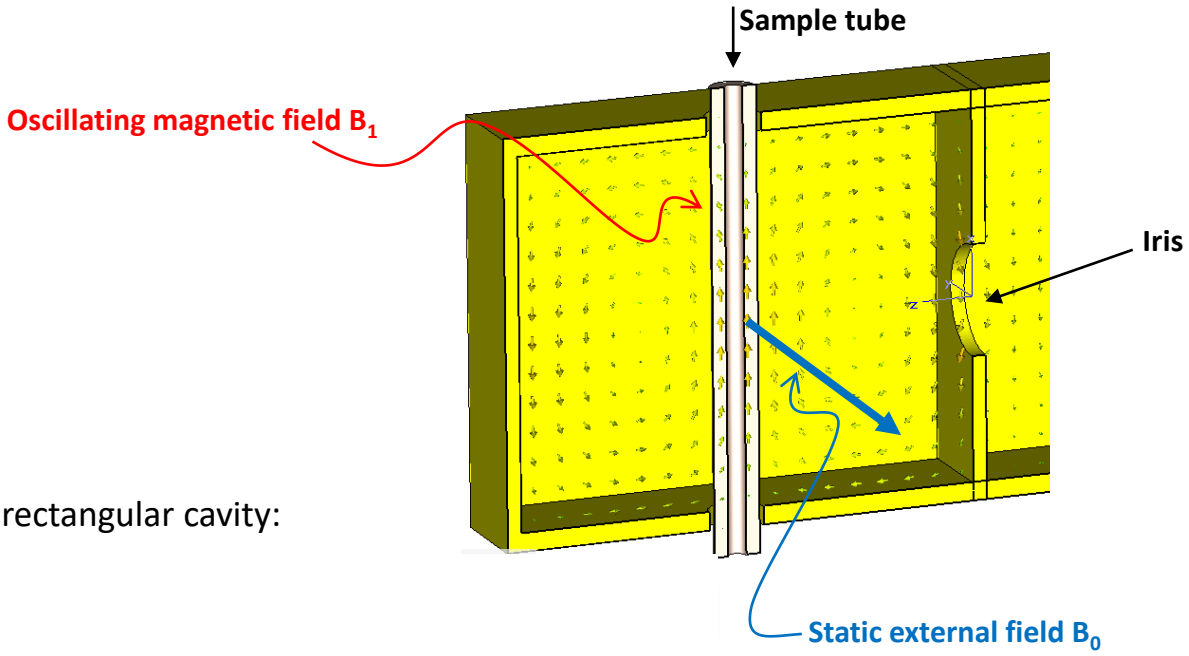
Precession frequency: $\nu_0 = g\mu_B B_0/h$

ν_0 – Larmor Frequency
g – g-value
μ_B – Bohr magneton
h – Planck Constant

Spin acrobatics



Microwave magnetic field inside the resonator (designated as B_1) is oscillating with ν_{mw}.



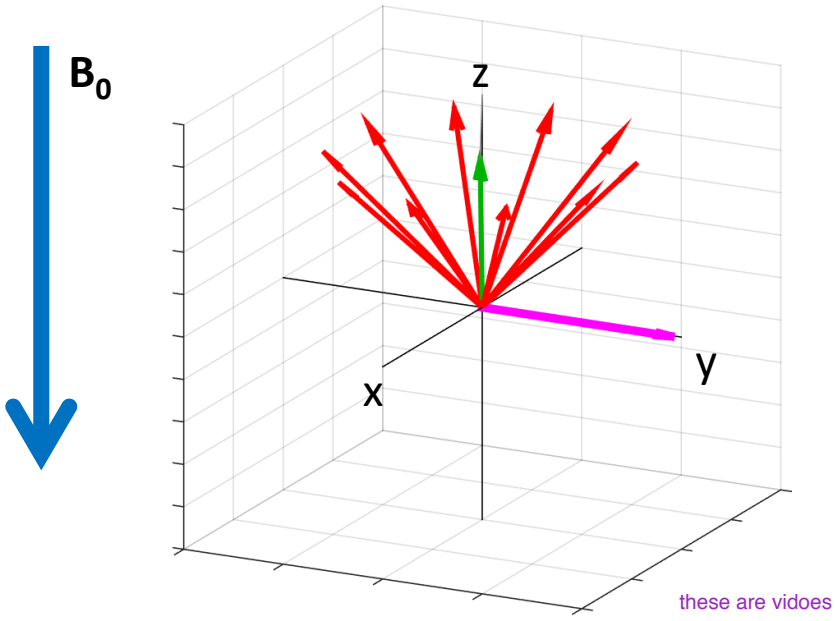
Simulation for a rectangular cavity:

Electron paramagnetic resonance event is when: $\nu_0 = \nu_{mw}$

Rotating Frame

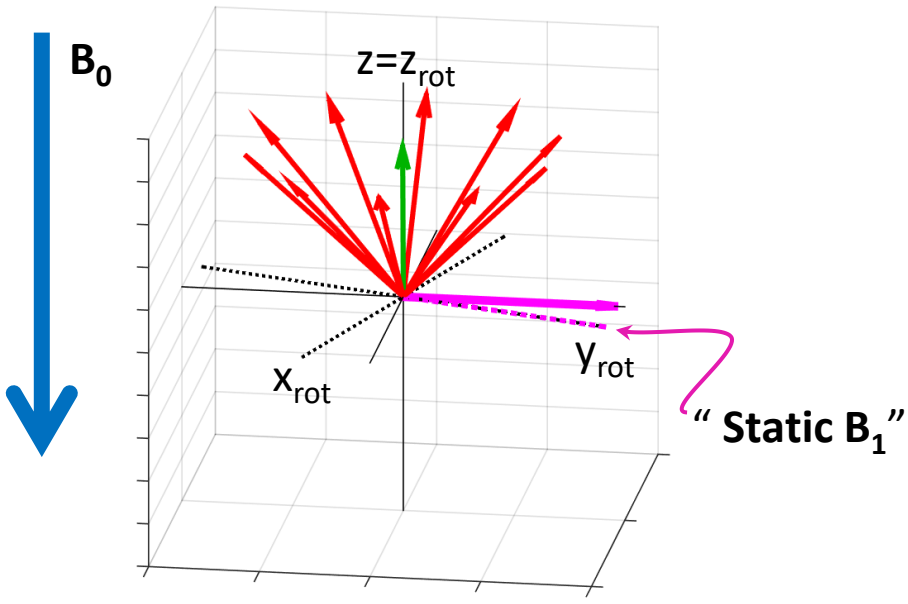
For in resonance condition, ($\nu_0 = \nu_{mw}$) we can simplify the picture by using a "rotating frame" formalism

Static frame



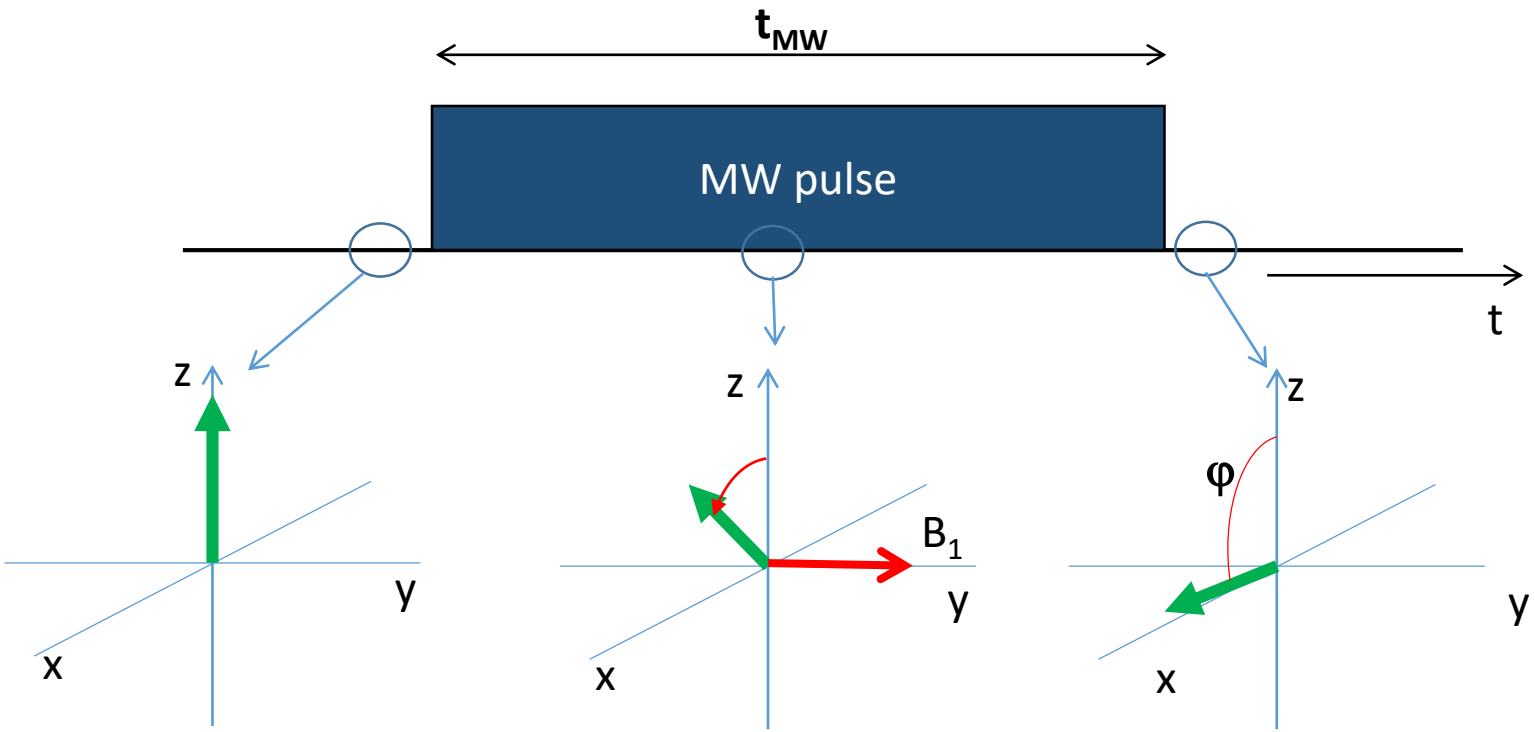
Rotating frame

omits processions around the z axis



For the rest of the class, we always work in rotating frame, so we reassign x_{rot}, y_{rot}, z_{rot} as x, y, z

MW Pulse



$$\varphi = \pi \nu_1 t_{MW}$$

$\nu_1 \sim \gamma B_1$

"π/2-pulse" : φ=90° x axis

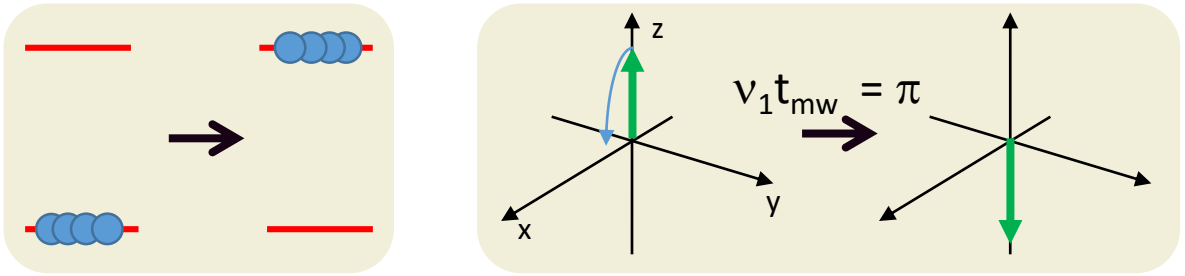
"π-pulse" : φ=180° -z direction

Higher microwave power = Higher B_1 = Faster precession

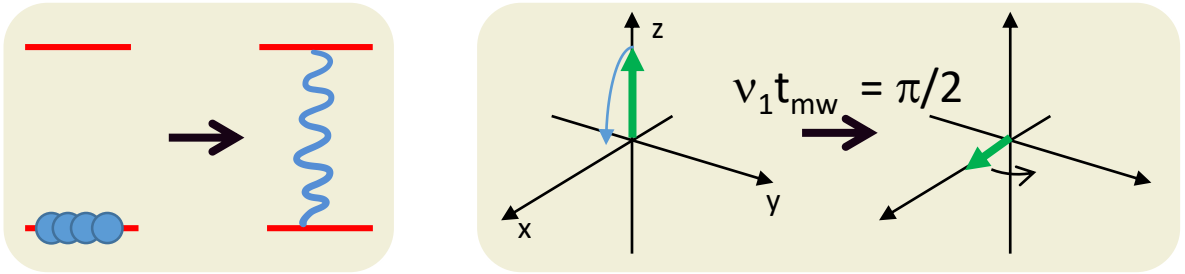
higher MW power = shorter π/2- and π- pulses

Terminology

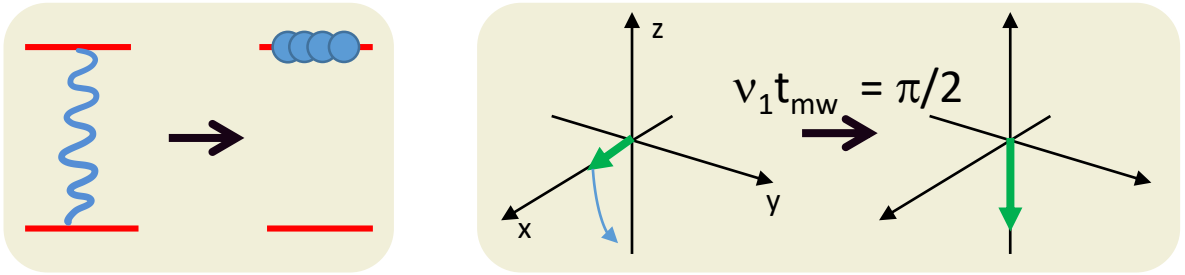
polarization transfer a.k.a. π-pulse



create **coherence** state a.k.a. $\pi/2$-pulse



coherence to **polarization** transfer



Text

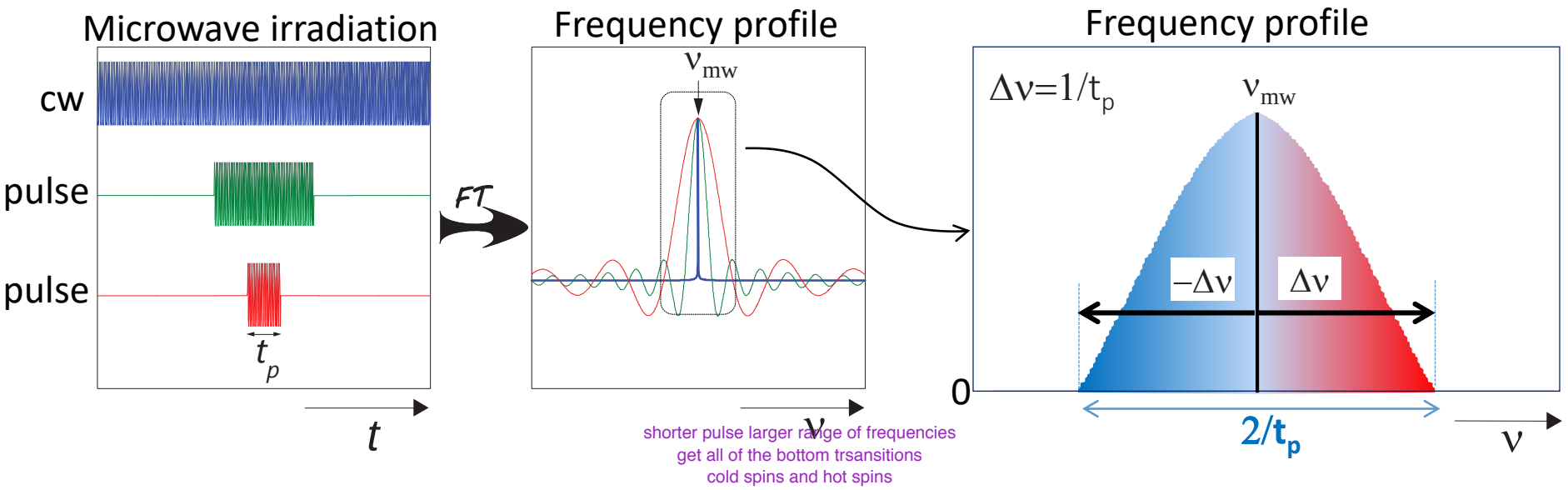
Polarized state is when all spins are in the same state

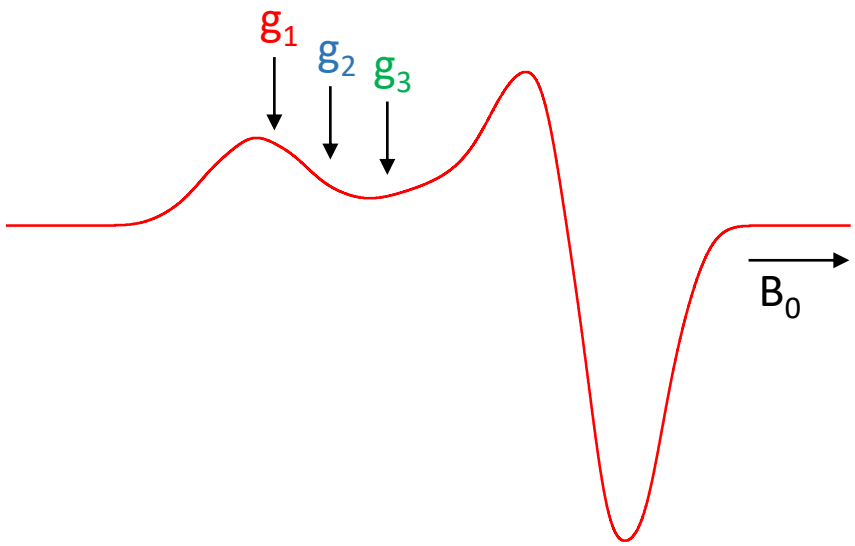
Coherence is a state in which spins oscillate between two states. Sum magnetization *precess* in xy-plane

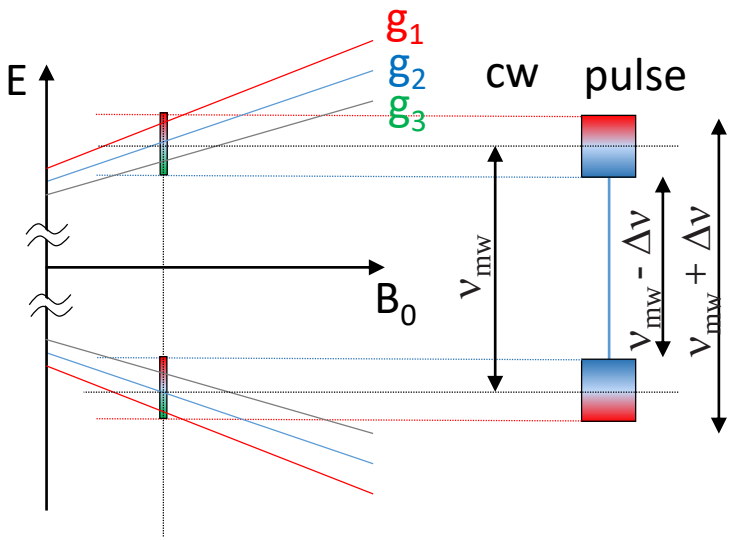
Transition moment is a probability of changing the state under irradiation

Detection of EPR signal means observation of sum magnetization on **x** and **y** axis, representing "*real*"/"in phase" and "*imaginary*"/"out of phase" channels respectively.

Microwave Pulse Bandwidth

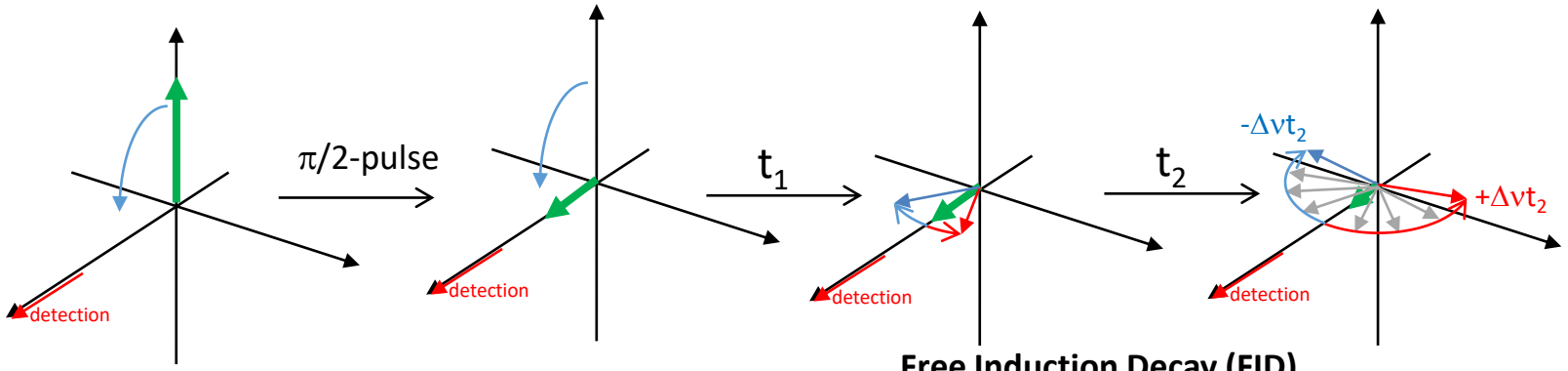


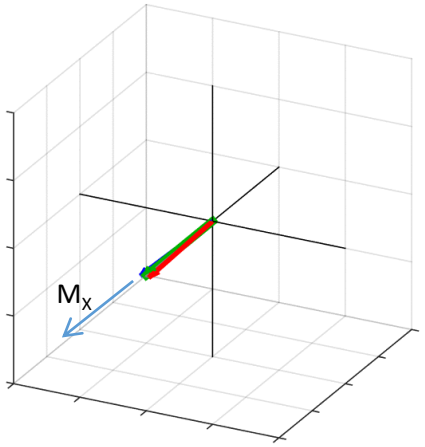




Free induction decay

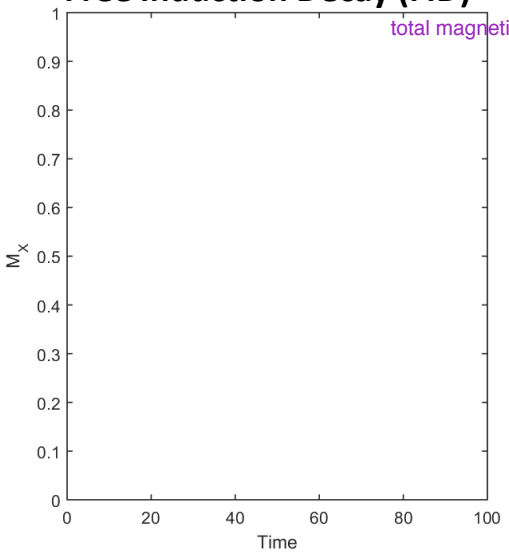
So, MW pulse moves an assemble of spins from equilibrium





Free Induction Decay (FID)

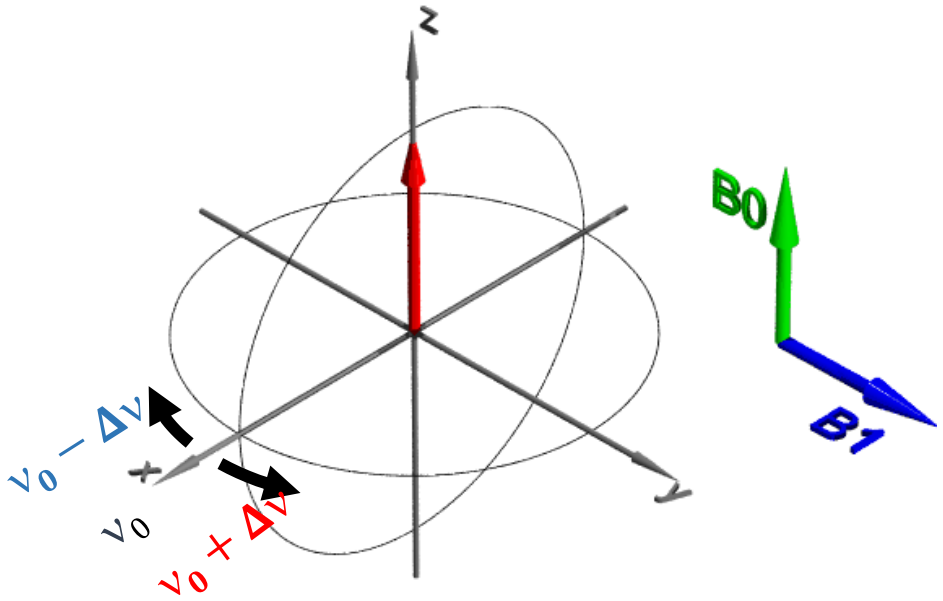
total magnetization decays as time goes on



As we are still tuned to only **one frequency** – Larmor Frequency, spins with different frequencies will appear precessing

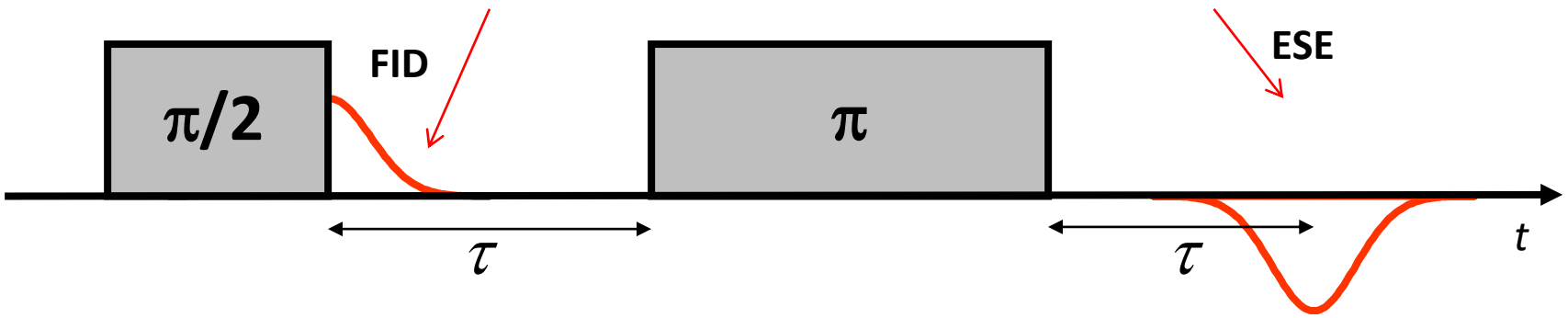
Hahn Echo experiment

FID – *free induction decay*
ESE – *electron spin echo*



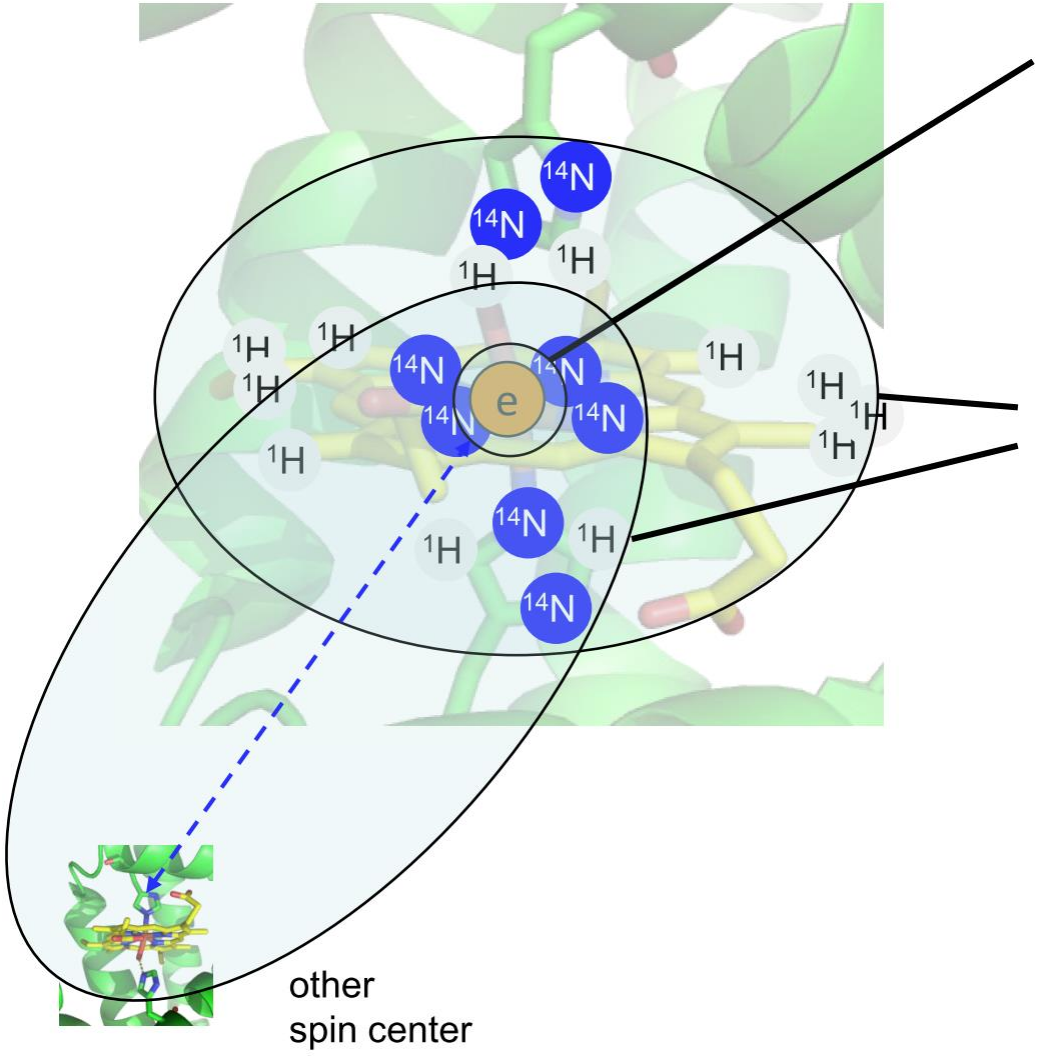
cold spins and hot spins combine again in the -x direction and that is the ESE

Sample irradiates MW with an intensity ~|M_x|



Unpaired electron spin as a probe of the environment

Electron spin “senses” other nuclear and electron spins.



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ESEEM / ENDOR = paramagnetic NMR

Key components of the spin-Hamiltonian that directly contribute to ESEEM/ENDOR:

Nuclear Zeeman interaction: $H_N = g_n\beta_n B_0 I$

$\nu_n = g_n\beta_n B_0$

Nuclear Quadrupole interaction (for I>1/2): $H_Q = IPI$

parametrization: $K = P_z/2$

$\eta = P_y - P_x/P_z$

$$P = \begin{pmatrix} P_x & & \\ & P_y & \\ & & P_z \end{pmatrix} \qquad P_x + P_y + P_z = 0$$

this is what we look at most of the time

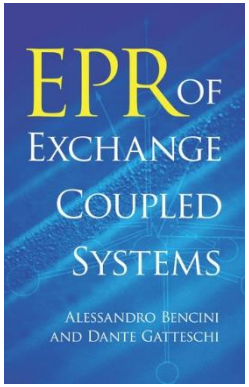
Apparent* Hyperfine Interaction: $H_{HF} = SAI$

parametrization: $A_{iso} = (A_x + A_y + A_z)/3$

$A_{anis} = [A_x, A_y, A_z] - a_{iso}$

Text

$$A = \begin{pmatrix} A_x & & \\ & A_y & \\ & & A_z \end{pmatrix}$$



* In the case of a complex multi-spin systes, apparent hyperfine is not directly related to the spin density. Look up "spin-projection coefficients" to learn more.

Z	A	E	Spin I	Nat. Abund. [%] (Half-life)	calc. X-Band ENDOR Freq. [MHz at 0.350 T] (free nucleus)	$g = \mu / (I\ \mu_N)$
0	1	n	1/2		10.2076432	–3.8260854
1	1 2 3	H H H	1/2 1 1/2	99.9885 0.0115 (12.32 y)	14.9021186 2.28756617 15.8951945	5.58569468 0.857438228 5.95792488
6	13	C	1/2	1.07	3.747940	1.404824
7	14 15	N N	1 1/2	99.636 0.364	1.077197 1.511043	0.40376100 –0.56637768
8	17	O	5/2	0.038	2.02098	–0.757516
9	19	F	1/2	100.0	14.01648	5.253736
25	53 55	Mn Mn	7/2 5/2	(3.74 E6 y) 100.0	3.8296 3.701688	1.4354 1.38748716
26	57	Fe	1/2	2.119	0.483548	0.18124600
27	59 60	Co Co	7/2 5	100.0 (1925 d)	3.527 2.027	1.322 0.7598
28	61	Ni	3/2	1.1399	1.33399	–0.50001
29	63 65	Cu Cu	3/2 3/2	69.15 30.85	3.961568 4.2359	1.484897 1.5877

Bruker Almanac

Isotropic Hyperfine interaction

Fermi contact term:

$$A_{iso} = \frac{\mu_0}{3h} \langle S_z \rangle^{-1} g \mu_B g_n \mu_n \sigma_{\alpha-\beta}$$

Text

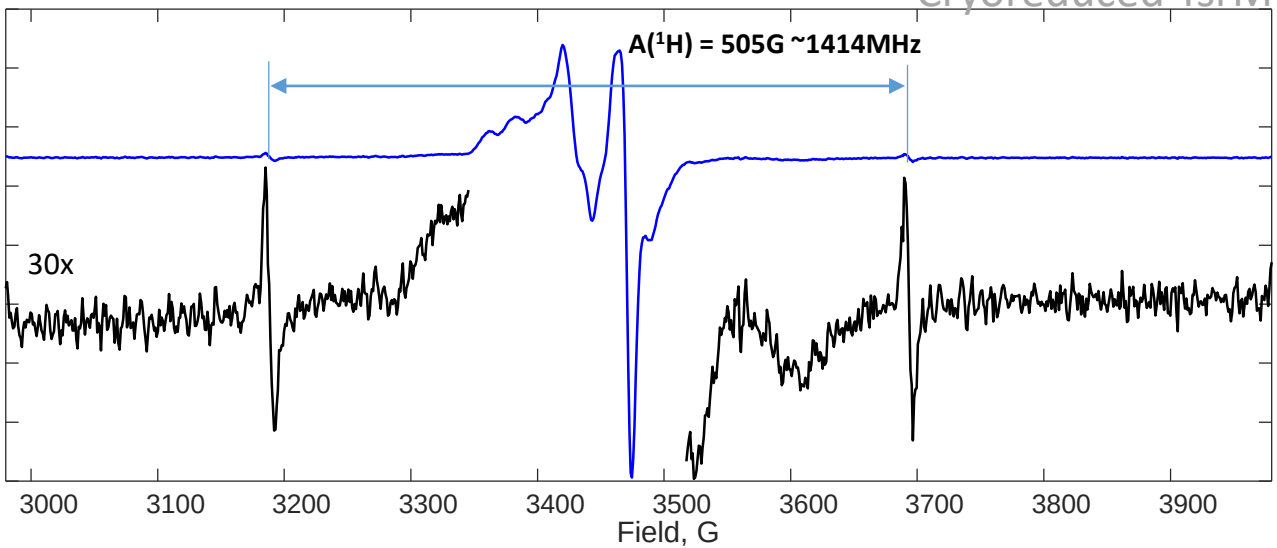
scales with g_n

spin density at the nucleus

100% spin density centered at the nucleus:

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

Cryoreduced TsrM



Anisotropic Hyperfine coupling

Through space dipolar coupling constant:
(approximate description)

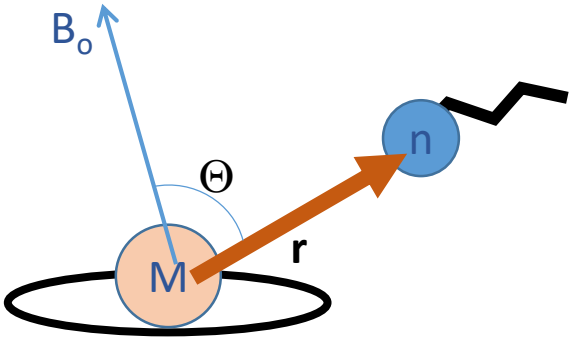
$$a = \frac{\mu_0}{4\pi} g\mu_B g_n \mu_n \frac{1}{r^3} [3\cos^2(\theta) - 1]$$

scales with g_n

scales with $1/r^3$

Coupling constant **a** depends on the angle between $\vec{\mathbf{B}}_0$ and $\vec{\mathbf{r}}$ (Θ)

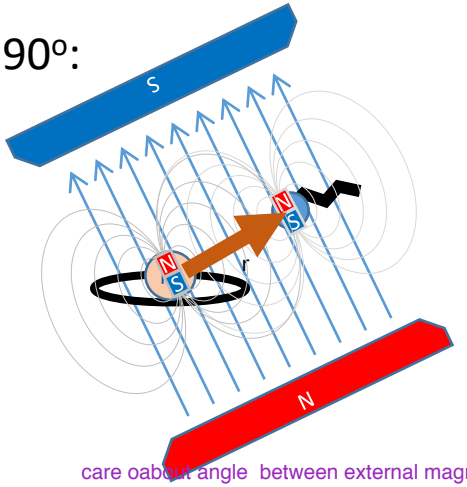
appoximated as points in space



Commonly used parameter: $T = \frac{\mu_0}{4\pi} \frac{g\mu_B g_n \mu_n}{r^3}$

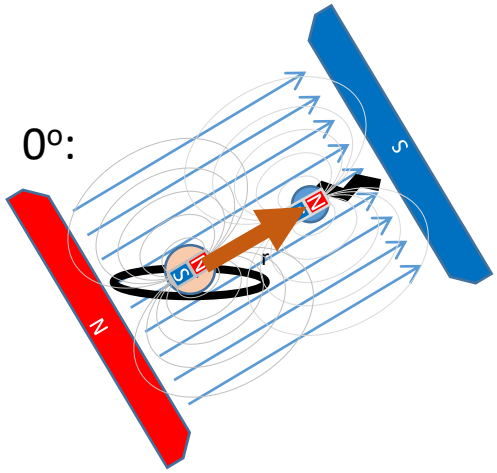
Anisotropic Hyperfine coupling

90°:

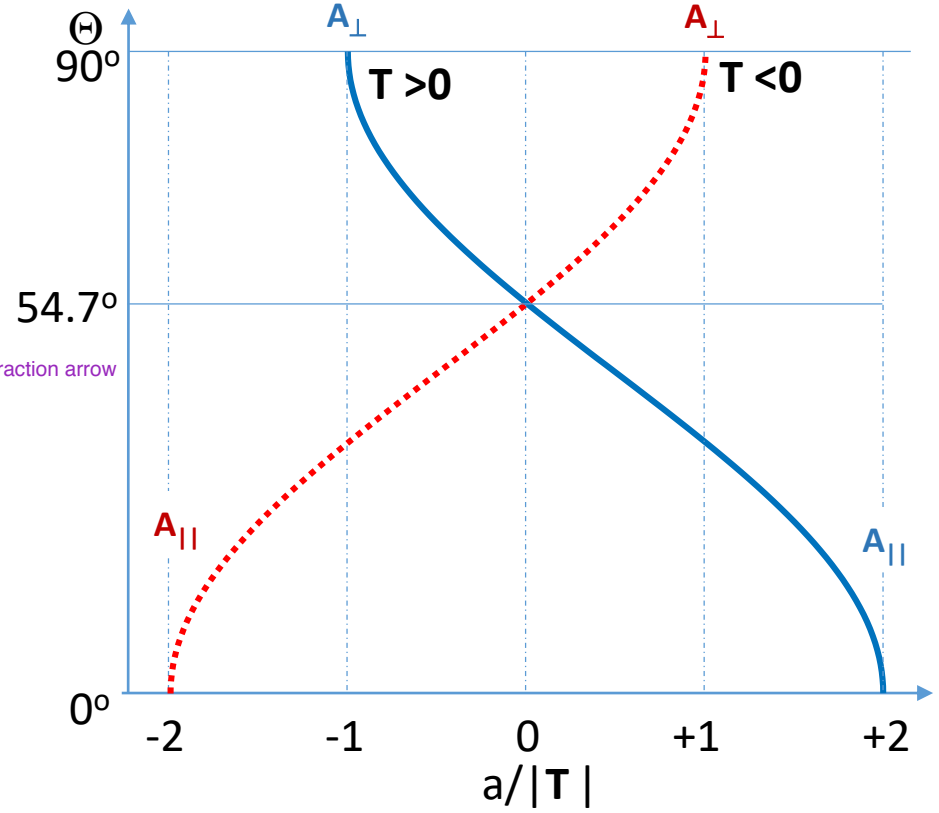


care oabout angle between external magnetic field and the interaction arrow

0°:



$a = T\,(3\cos^2(\Theta)-1)$



Pure dipole-dipole interaction gives pure axial HF coupling constant.

$A_x=A_y=A_\perp$ $A_z=A_{||}$

“NMR” spectrum for S=1/2, I=1/2

Total hyperfine coupling (including isotropic part)

$$\boldsymbol{A = a_{iso} + T(3\cos^2\Theta - 1)}$$

“Anisotropy at Θ” $B = 3T sin\Theta cos\Theta$

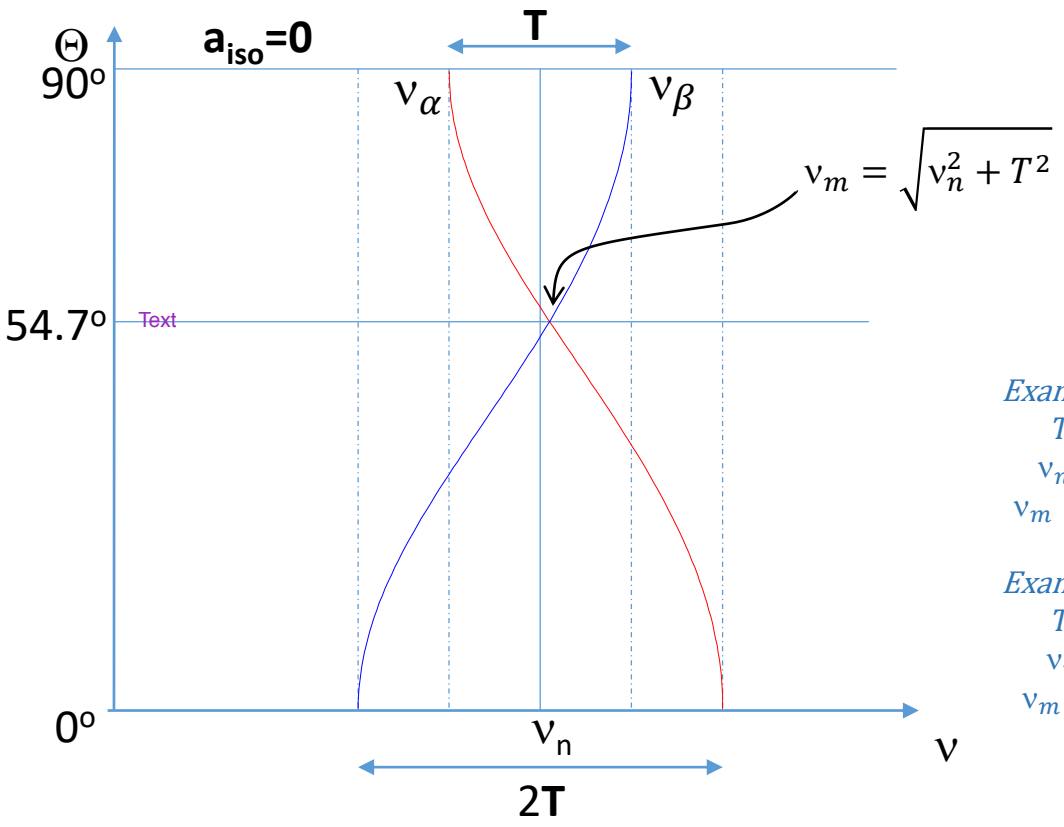
NMR frequencies

α : [M_S= + 1/2] β-> [M_S= + 1/2]

$$\nu_\alpha = \sqrt{\left(\nu_n + \frac{A}{2}\right)^2 + \frac{B^2}{2}}$$

$$\nu_\beta = \sqrt{\left(\nu_n - \frac{A}{2}\right)^2 + \frac{B^2}{2}}$$

Θ	B
0°	0
45°	1.5 T
54.7°	1.4 T
90°	0



Example 1:
$T = 1\ MHz$
$\nu_n = 10\ MHz$
$\nu_m = 10.05\ MHz$

Example 2:
$T = 1\ MHz$
$\nu_n = 3\ MHz$
$\nu_m = 3.16\ MHz$

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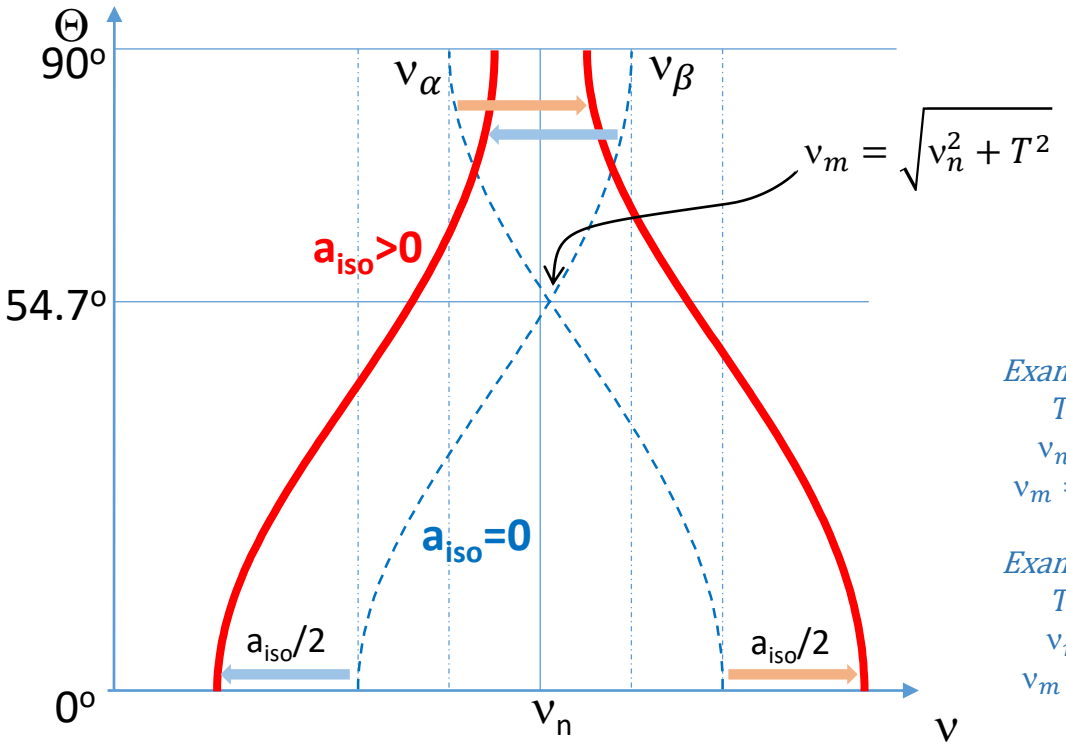

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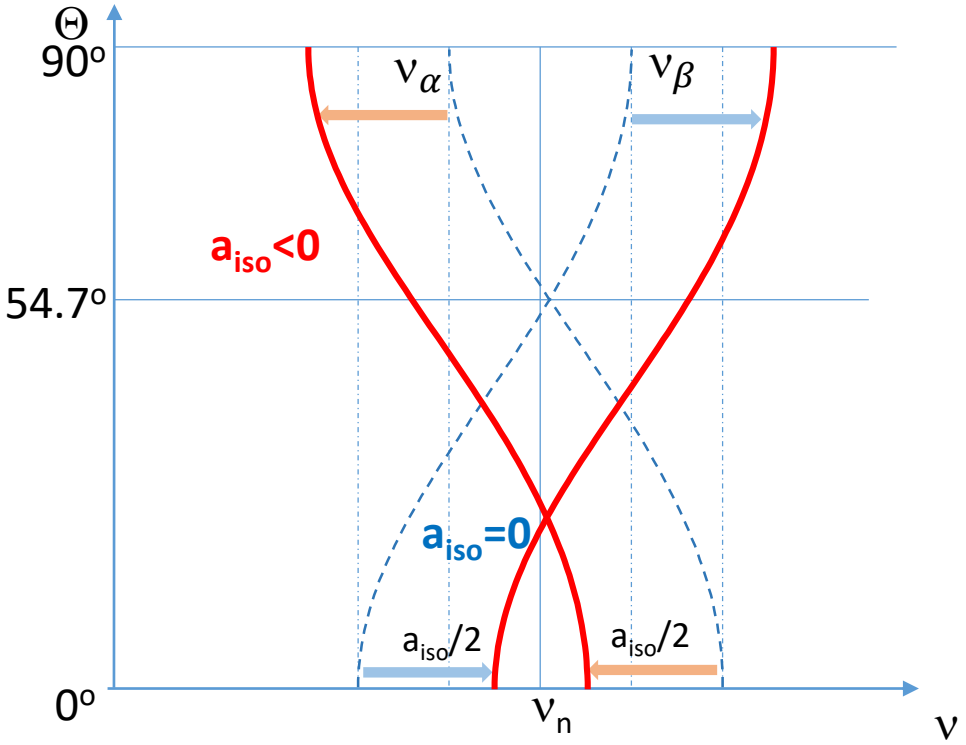
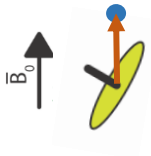


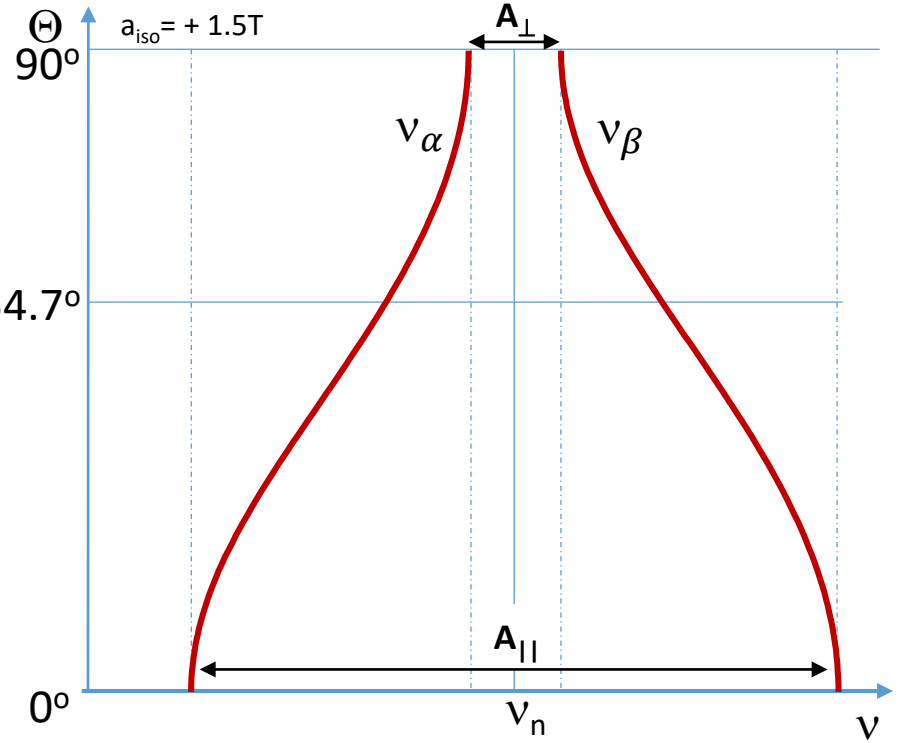
“NMR” spectrum for S=1/2, I=1/2

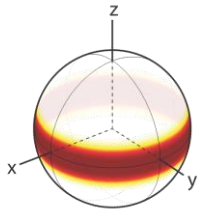
Single orientation:

almost none parallel but lots more normal to magentic field

thats how you have to avage if you do randm frozen sample (not crystal)

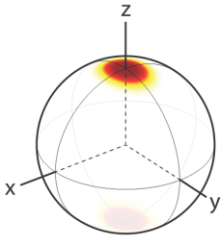






All orientations in xy plane will produce the same ν_α and ν_β

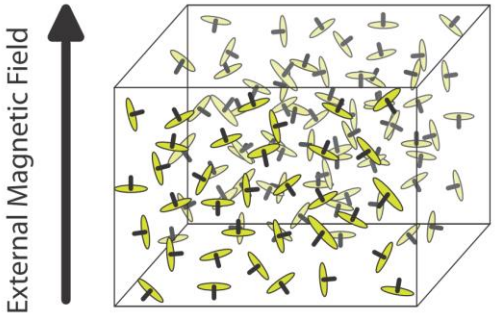
Maximum intensity

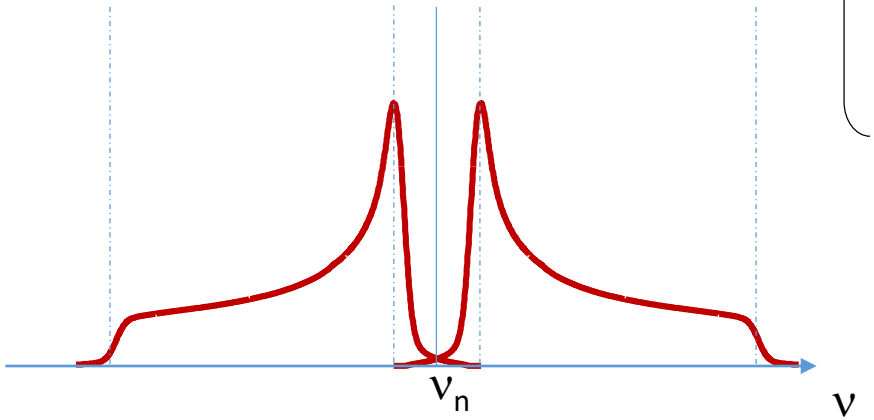


There is only one particular orientation of the molecule for these ν_α and ν_β

Very little intensity

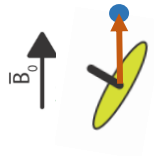
“Powder-averaging” all orientation:

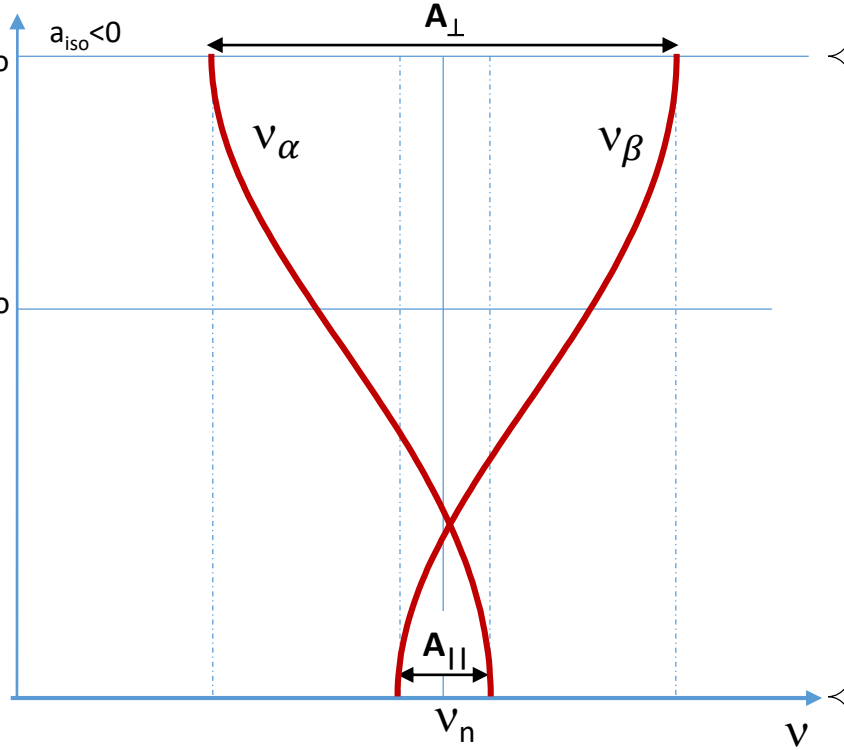


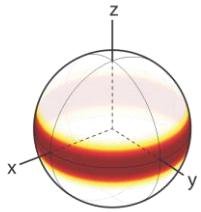


“NMR” spectrum for S=1/2, I=1/2

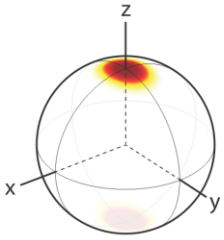
Single orientation:





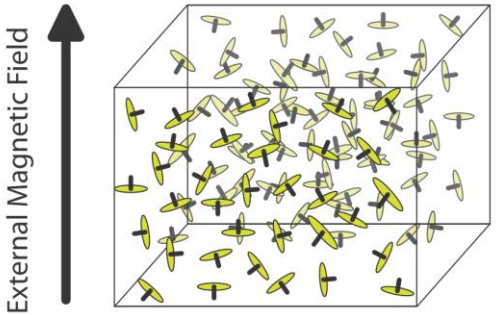


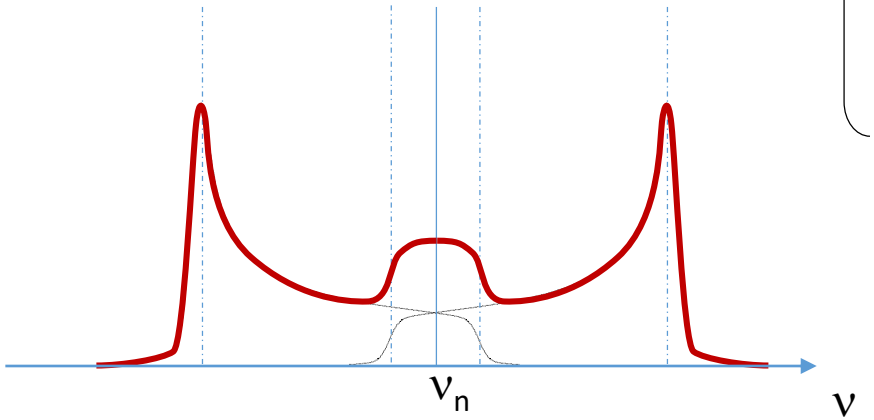
All orientations in xy plane will produce the same ν_α and ν_β
Maximum intensity



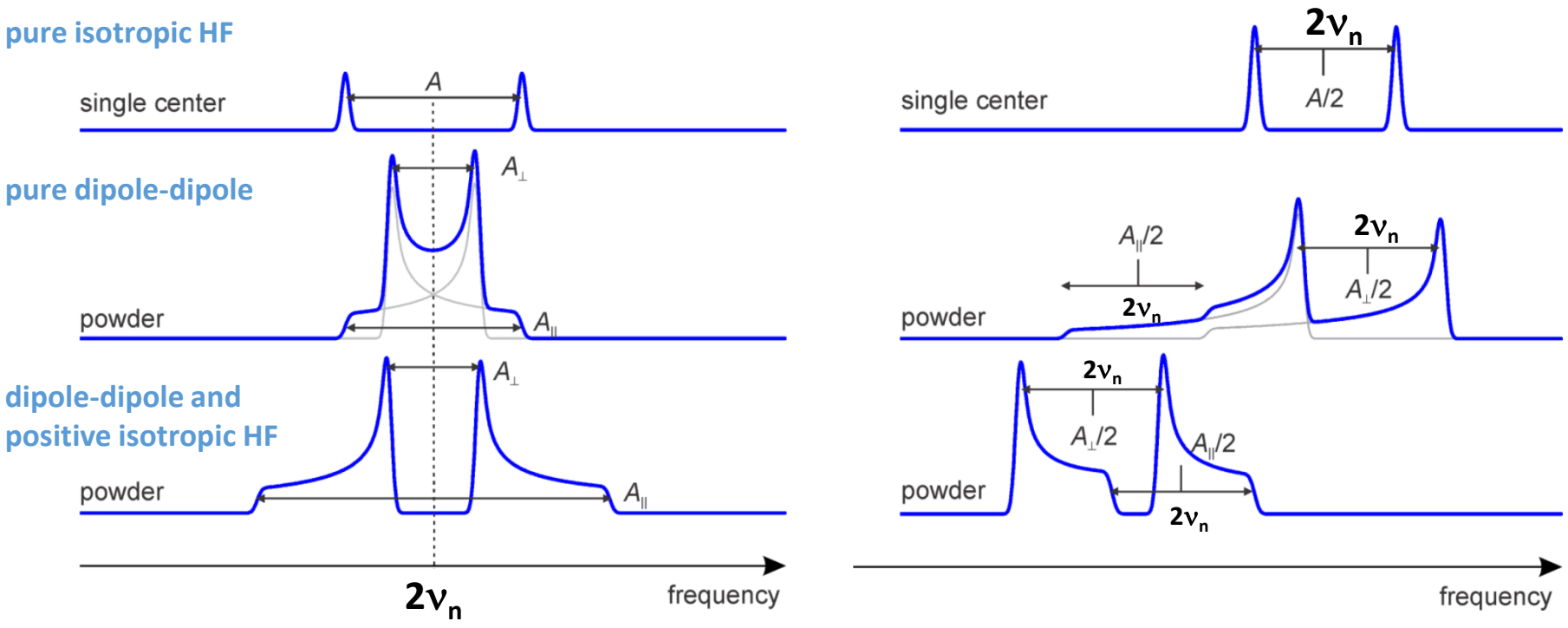
There is only one particular orientation of the molecule for these ν_α and ν_β
Very little intensity

“Powder-averaging” all orientation:



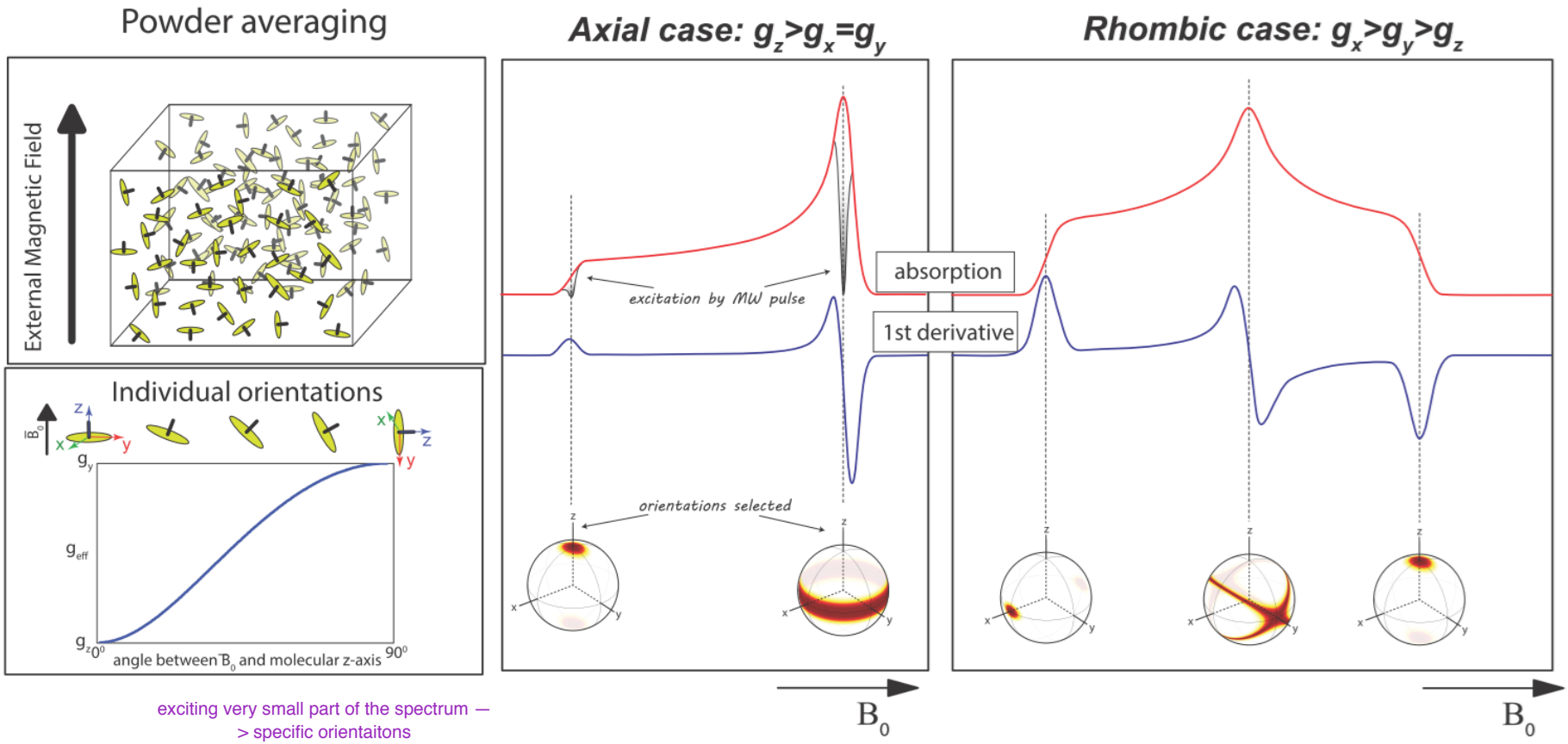


“NMR” spectrum for S=1/2, I=1/2



Orientation selectivity

Broad EPR signals: MW pulses excite a fraction of the EPR spectrum
: a fraction of molecules selected that have certain orientation

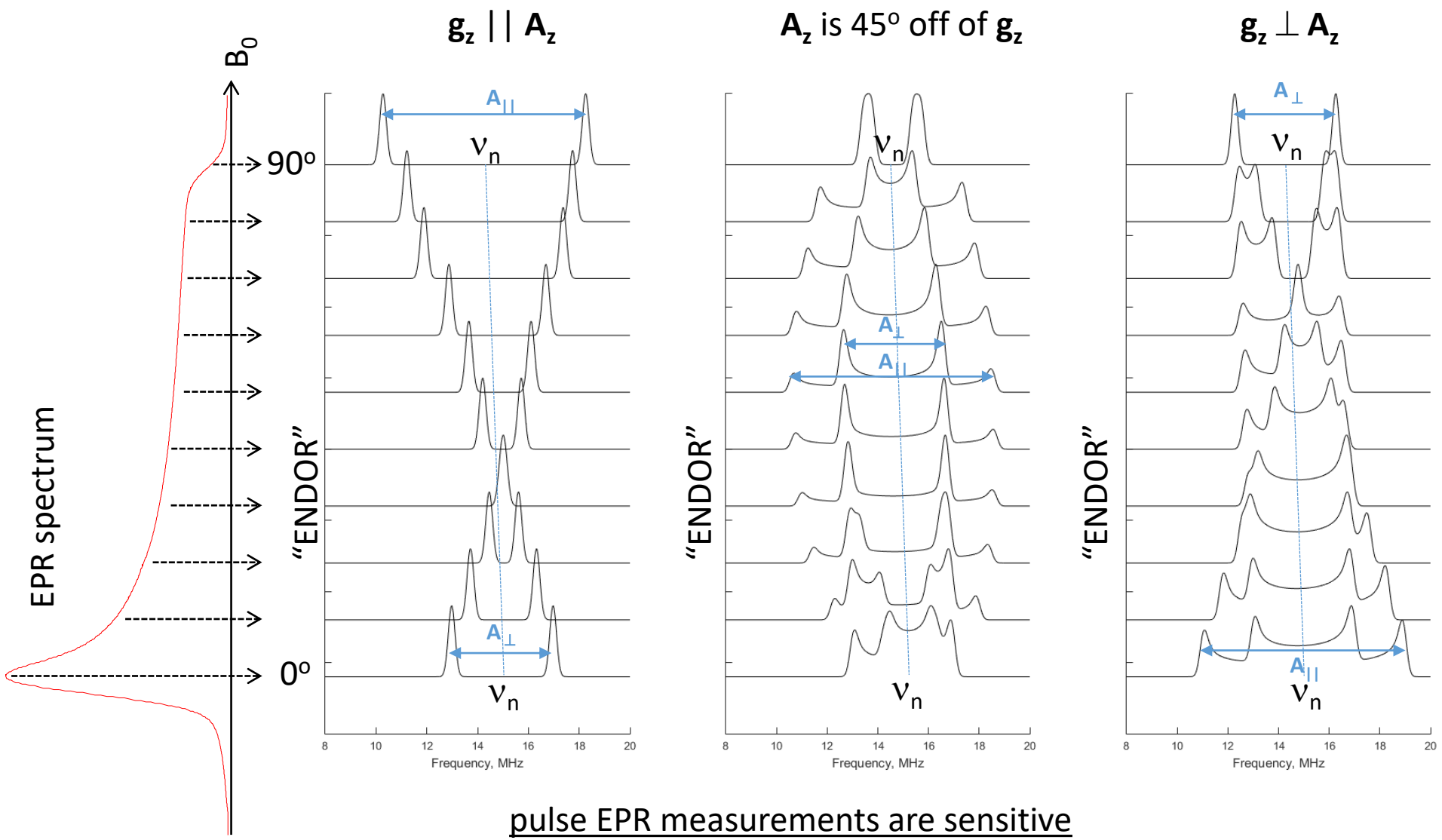


exciting very small part of the spectrum —
> specific orientaitons

For such cases it is possible to do “orientation-selective” pulse experiments.

Orientation selectivity

Orientation selective measurements (I=1/2, axial case, A_{iso}=0):



pulse EPR measurements are sensitive
to relative orientation between **g** and **A** tensors

Most common types of experiments

Three general classes of pulse EPR experiments:

- Electron spin echo envelop modulation (ESEEM) experiments
  - 2-pulse, 3-pulse, 4-pulse ESEEM
  - Hyperfine sublevel correlation (HYSCORE)

  ← Time-domain
  Takes advantage of hyperfine-mixed forbidden spin transitions

- Double resonance
  - Electron nuclear double resonance (ENDOR)
  - ELDOR-detected NMR

  ← Frequency-domain
  Perturbation of an NMR / EPR transition changes EPR signal

  - Electron-electron double resonance (ELDOR/DEER)

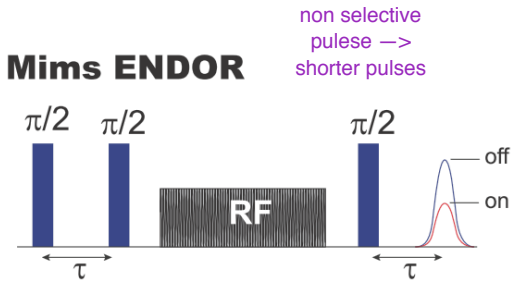
  ← Time-domain
  Perturbation of one spin center affects the other spin center

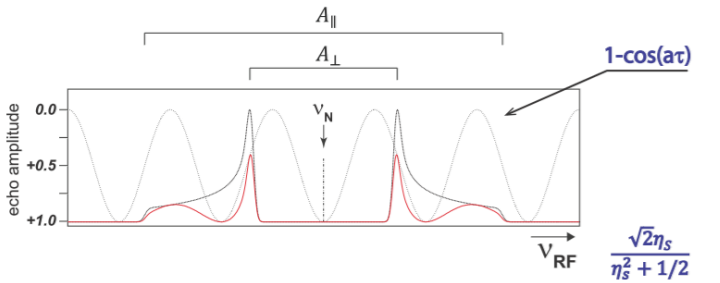
- Relaxation studies (broadly defined)

We are not going to go into details of each experimental setup.
Let's focus on what we get out of such experiments

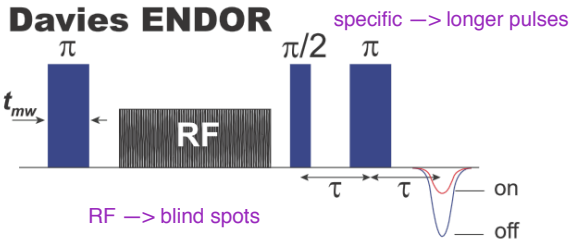
Most popular methods

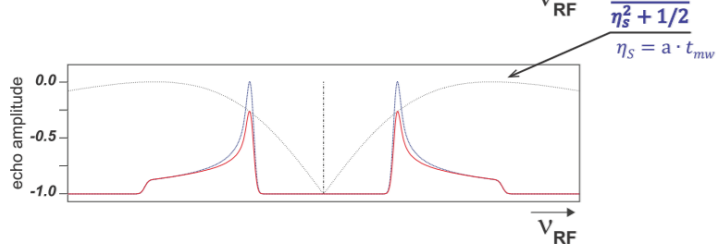
☺ Weaker, mostly isotropic HF couplings; Short pulses – good signal
☹ Periodic blind spots



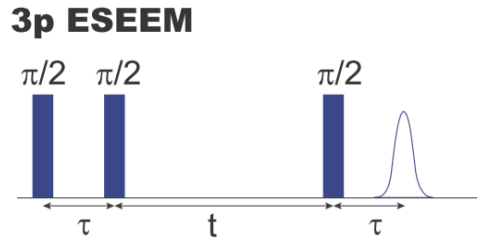


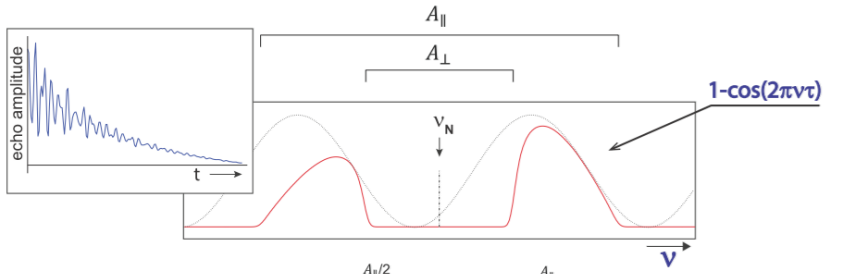
☺ Strong, mostly isotropic HF couplings
☹ Needs Selective pulses; Relaxation is an issue



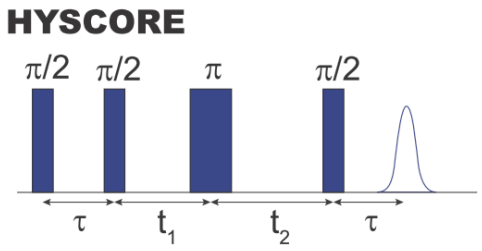


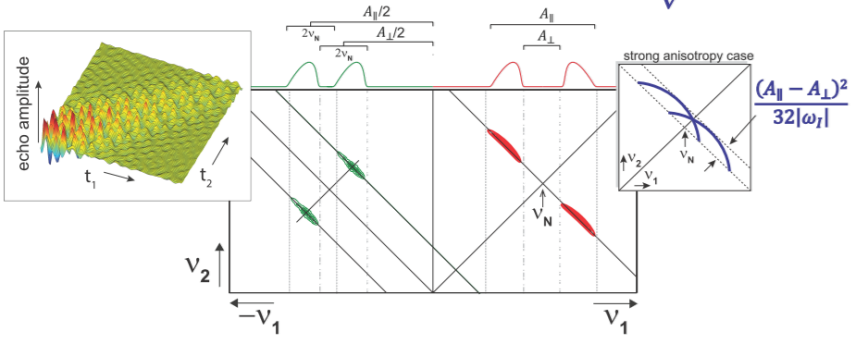
☺ Weaker anisotropic HF couplings
☹ Requires decent anisotropy





☺ Weaker anisotropic HF couplings; Superior resolution
☹ Requires decent anisotropy. Long accumulation times





Note, in all cases spectra are affected by "blindspots"

… a bit more about ESEEM

modulation depth (k) defines whether we will see an ESEEM signal or not.

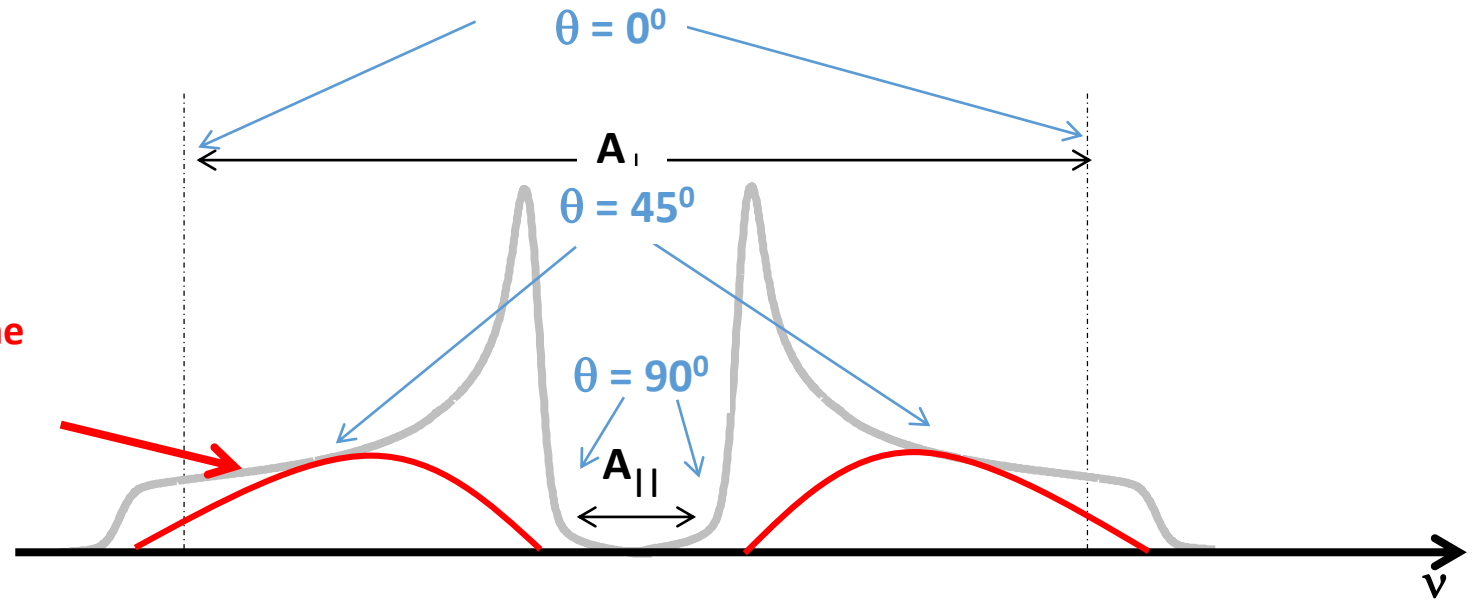
forbidden transitions

$$k = \left(\frac{B\nu_I}{2\pi\nu_\alpha\nu_\beta}\right)^2 \qquad \boldsymbol{B} = 3T\,sin\,\theta cos\theta$$

if $\theta = 0^0 \rightarrow$ B = 0, k=0
if $\theta = 90^0 \rightarrow$ B = 0, k=0
if $\theta = 45^0 \rightarrow$ B,k are maximum

If B = 0, no ESEEM signal!

Actual shape of the powder averaged ESEEM signal



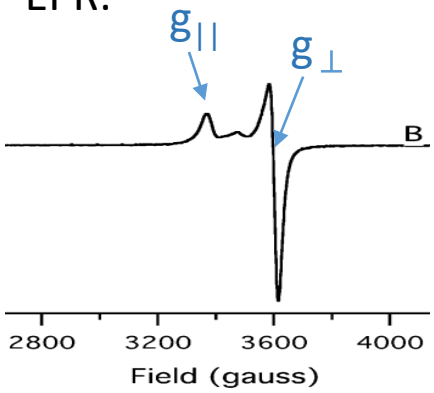
ENDOR Example

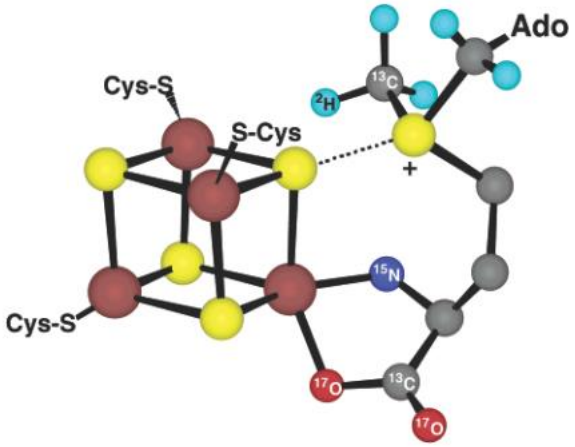
Inorg. Chem. 2005, 44, 727–741
Inorganic Chemistry
Article

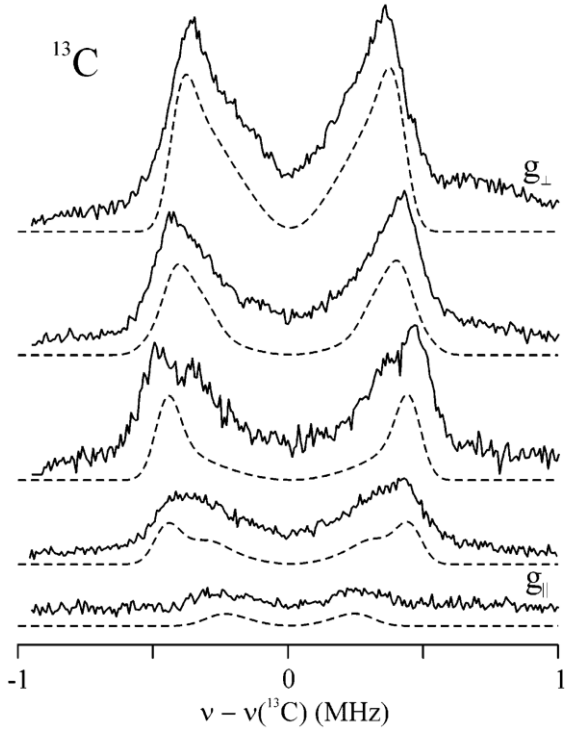
Spectroscopic Approaches to Elucidating Novel Iron–Sulfur Chemistry in the "Radical-SAM" Protein Superfamily

Charles J. Walsby,[†,‡] Danilo Ortillo,[§] Jian Yang,[§] Mbako R. Nnyepi,[§] William E. Broderick,[§] Brian M. Hoffman,[‡] and Joan B. Broderick*[,§]

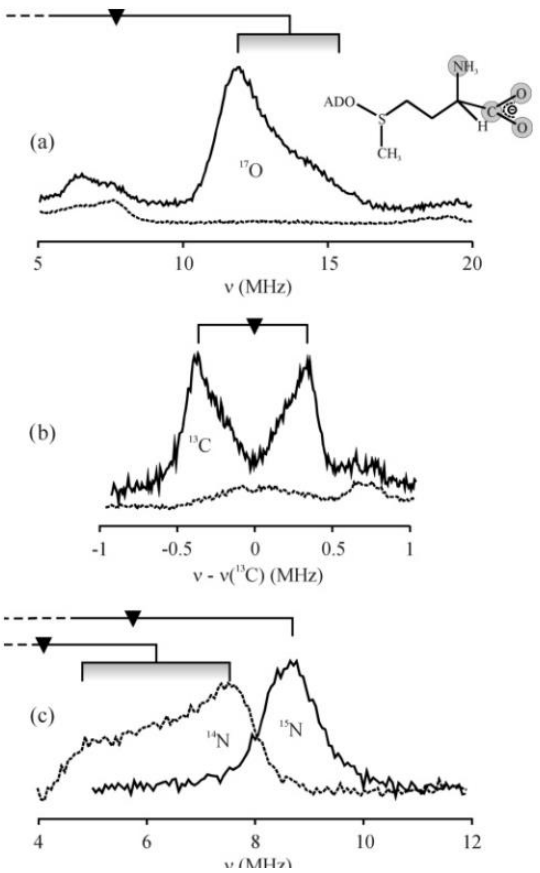
EPR:







$\mathbf{A}(^{13}C) = [-1.1, 0.95, 0.82]$ MHz and Euler angles, relative to the g-tensor frame, of $\theta = 30°$ and $\varphi = 0°$. The ^{13}C tensor can be decomposed into the sum of an isotropic component, $a_{iso}(^{13}C) = 0.22$ MHz, and two mutually perpendicular dipolar tensors, $\mathbf{T}(^{13}C) = [-2T, T, T] = [-1.28, 0.64, 0.64]$ MHz and $\mathbf{t}(^{13}C) = [t, -2t, t] = [-0.04, 0.09, -0.04]$ MHz. The first of these is assigned to the through-space dipolar interaction between the cluster spin and the ^{13}C nucleus. The latter is derived from a local interaction with electron spin density on the carbon atom itself with the ^{13}C nucleus.



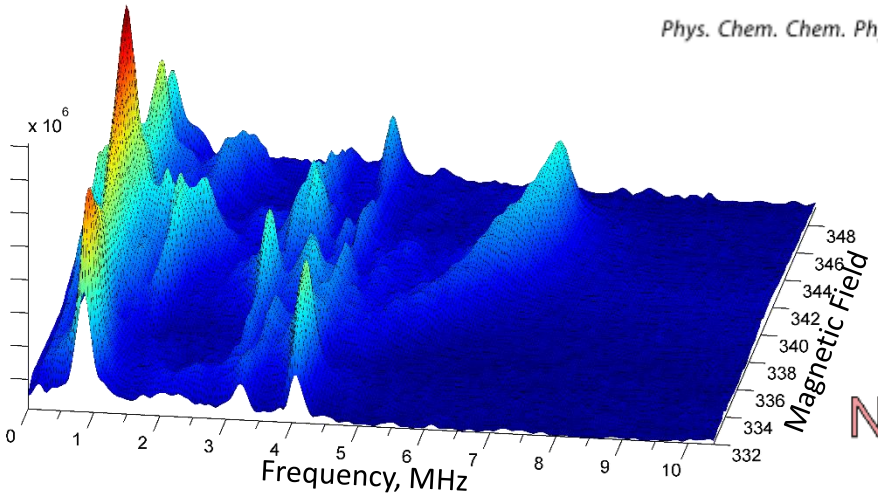
https://doi.org/10.1021/ic0484811

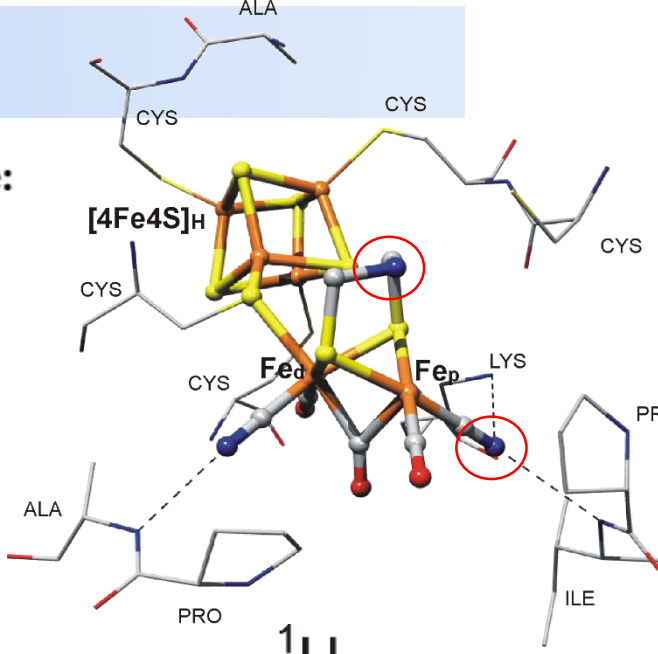
ESEEM Example

^{14}N HYSCORE investigation of the H-cluster of [FeFe] hydrogenase: evidence for a nitrogen in the dithiol bridge†

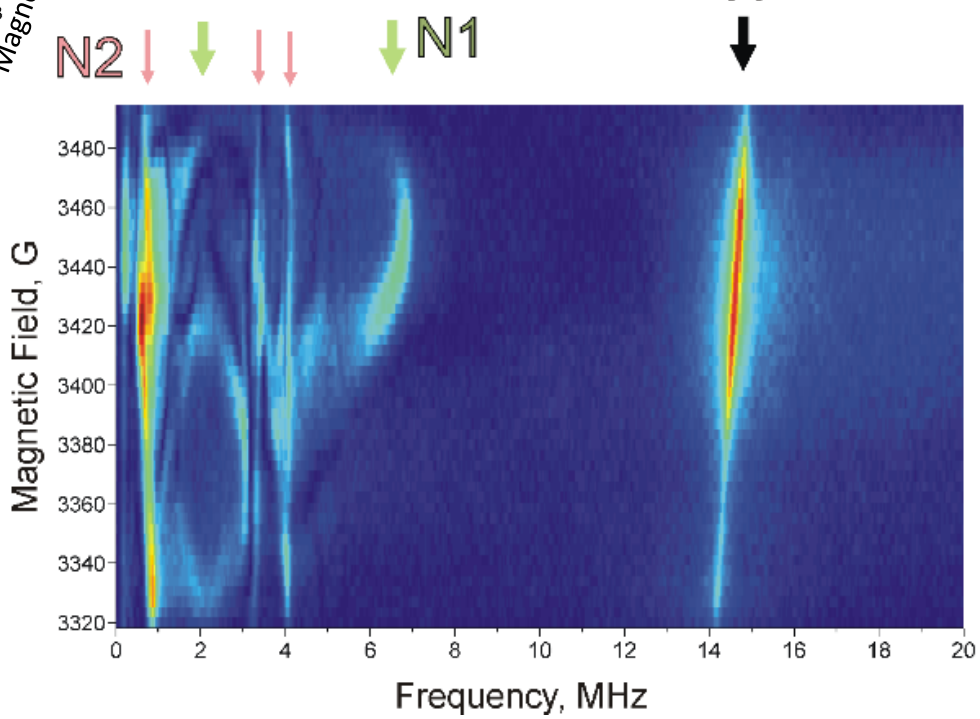
Alexey Silakov,* Brian Wenk, Eduard Reijerse and Wolfgang Lubitz*

Phys. Chem. Chem. Phys., 2009, 11, 6592–6599





Top view



	hyperfine interaction				quadrupole interaction	
	A_{xx}	A_{yy}	A_{zz}	$\lvert A_{iso}\rvert$	K	η
N2	1.5	3.8	-0.4	1.53	0.95	0.34
N1	1.0	1.9	1.4	1.43	1.23	0.13

https://doi.org/10.1039/B905841A

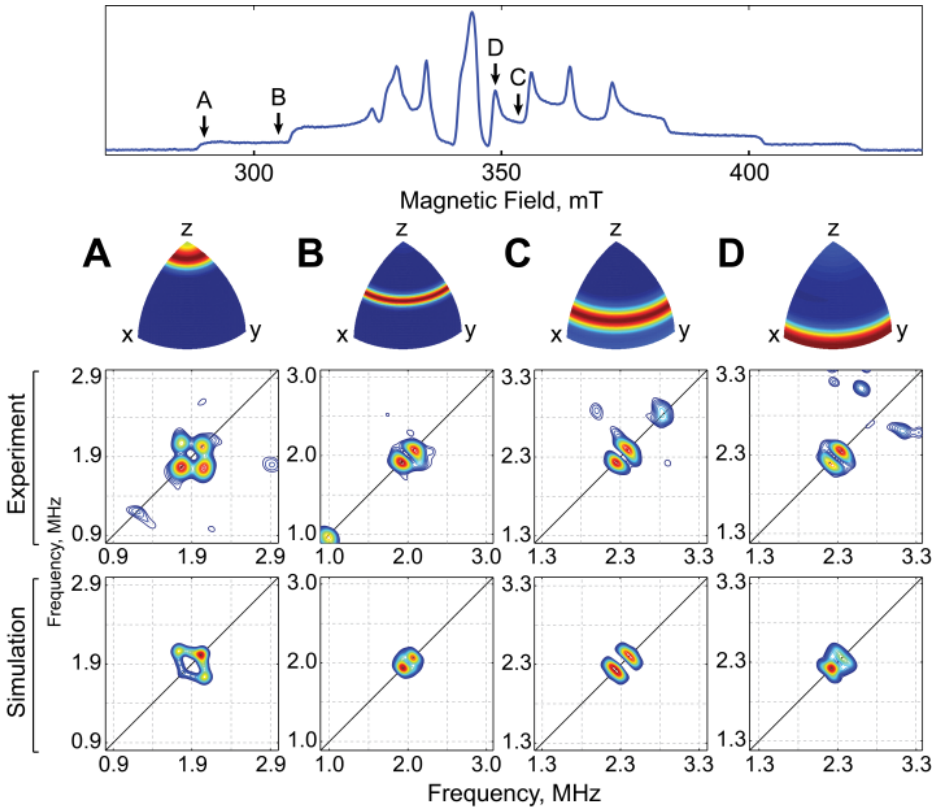
HYSCORE example

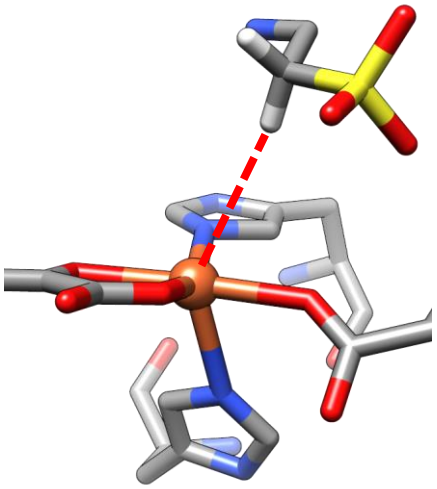
Vanadyl as a Stable Structural Mimic of Reactive Ferryl Intermediates in Mononuclear Nonheme-Iron Enzymes

Inorganic Chemistry

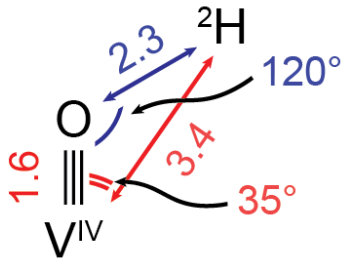
Cite This: *Inorg. Chem.* 2017, 56, 13382-13389

Ryan J. Martinie,[†] Christopher J. Pollock,[†] Megan L. Matthews,[§] J. Martin Bollinger, Jr.,[*,†,‡] Carsten Krebs,[*,†,‡] and Alexey Silakov[*,†]





TauD PDB:1OS7



TauD•(V^{IV}O)•d$_4$-taurine•succinate complex

^{2}H Hyperfine coupling

[0.26, 0.26, -0.68] ± 0.05 [0, 35, 15] ± 10°

https://doi.org/10.1021/acs.inorgchem.7b02113

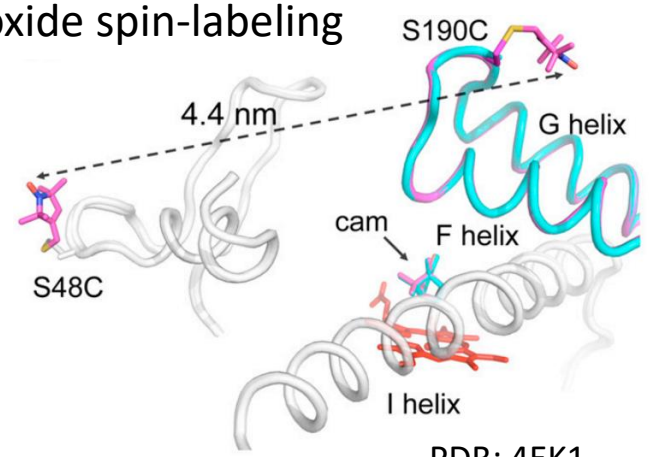
DEER example

Double electron–electron resonance shows cytochrome P450cam undergoes a conformational change in solution upon binding substrate

Stefan Stoll[a,1,2], Young-Tae Lee[a,1], Mo Zhang[a], Richard F. Wilson[b], R. David Britt[a,3], and David B. Goodin[a,3]

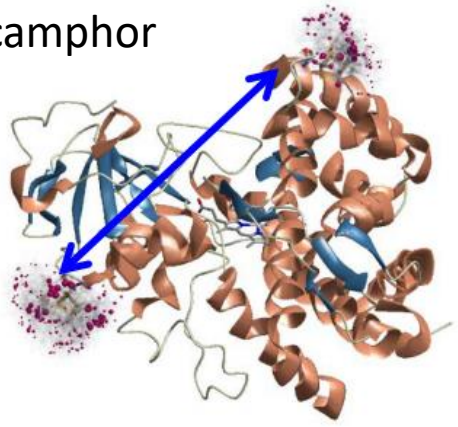
12888–12893 | PNAS | August 7, 2012 | vol. 109 | no. 32

Nitroxide spin-labeling

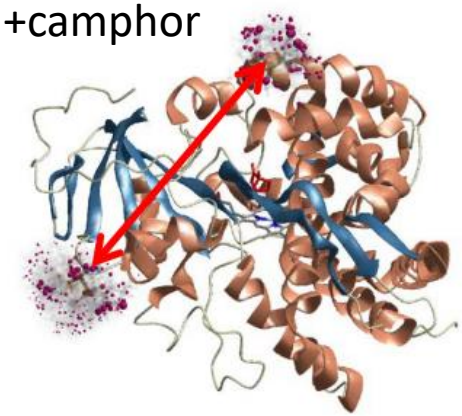


PDB: 4EK1

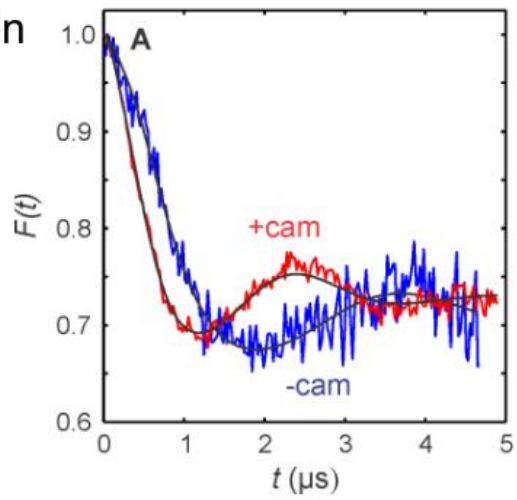
- camphor

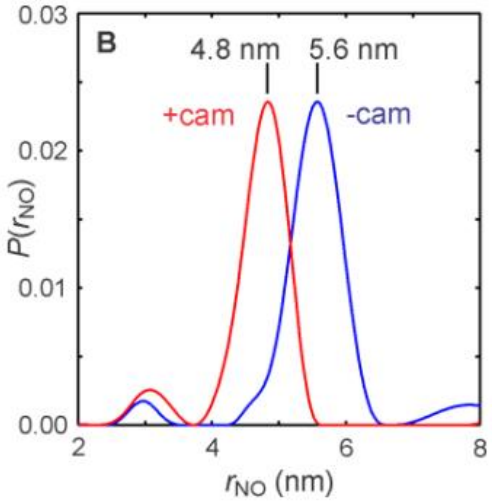


+camphor



time-domain signal





distance distribution

https://doi.org/10.1073/pnas.1207123109