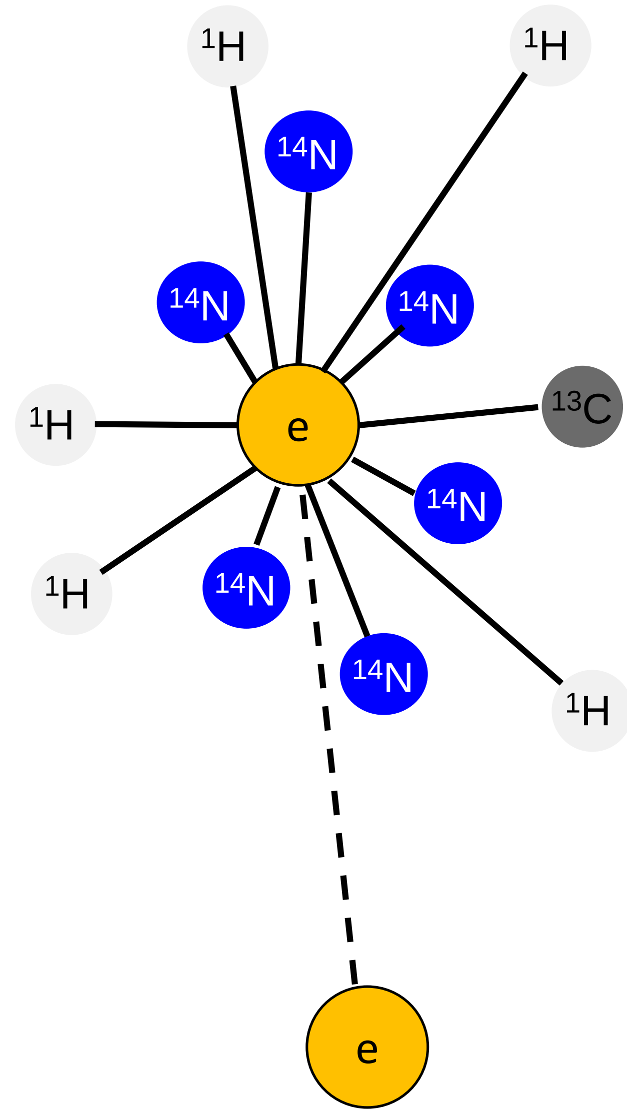
Pulse EPR Spectroscopy

Stefan Stoll
University of Washington, Seattle

4^{th} Penn State Bioinorganic Workshop
June 5, 2016



Goal

1. Learn what you can learn from pulse EPR

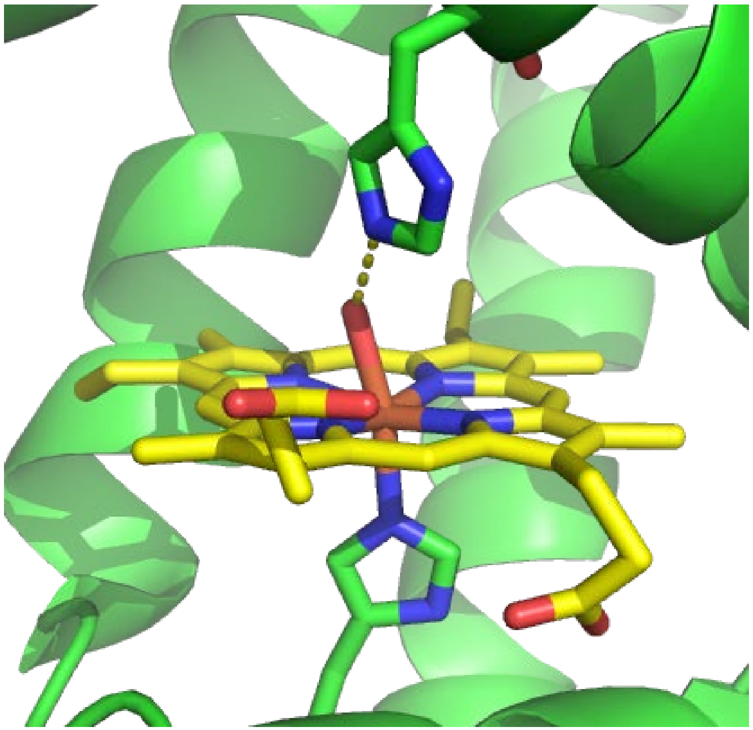
Contents

1. Pulse EPR basics
2. Magnetic nuclei and their interactions
3. Examples from the literature

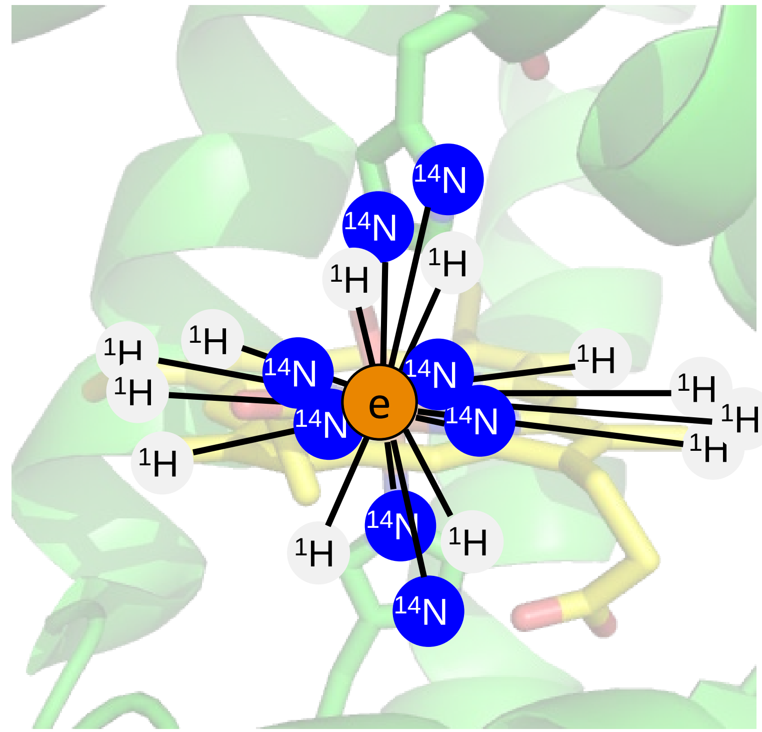
1. Pulse EPR basics

EPR sees spins and their coupling

Crystallography view:
structural cartoon



Magnetic resonance view:
system of coupled spins

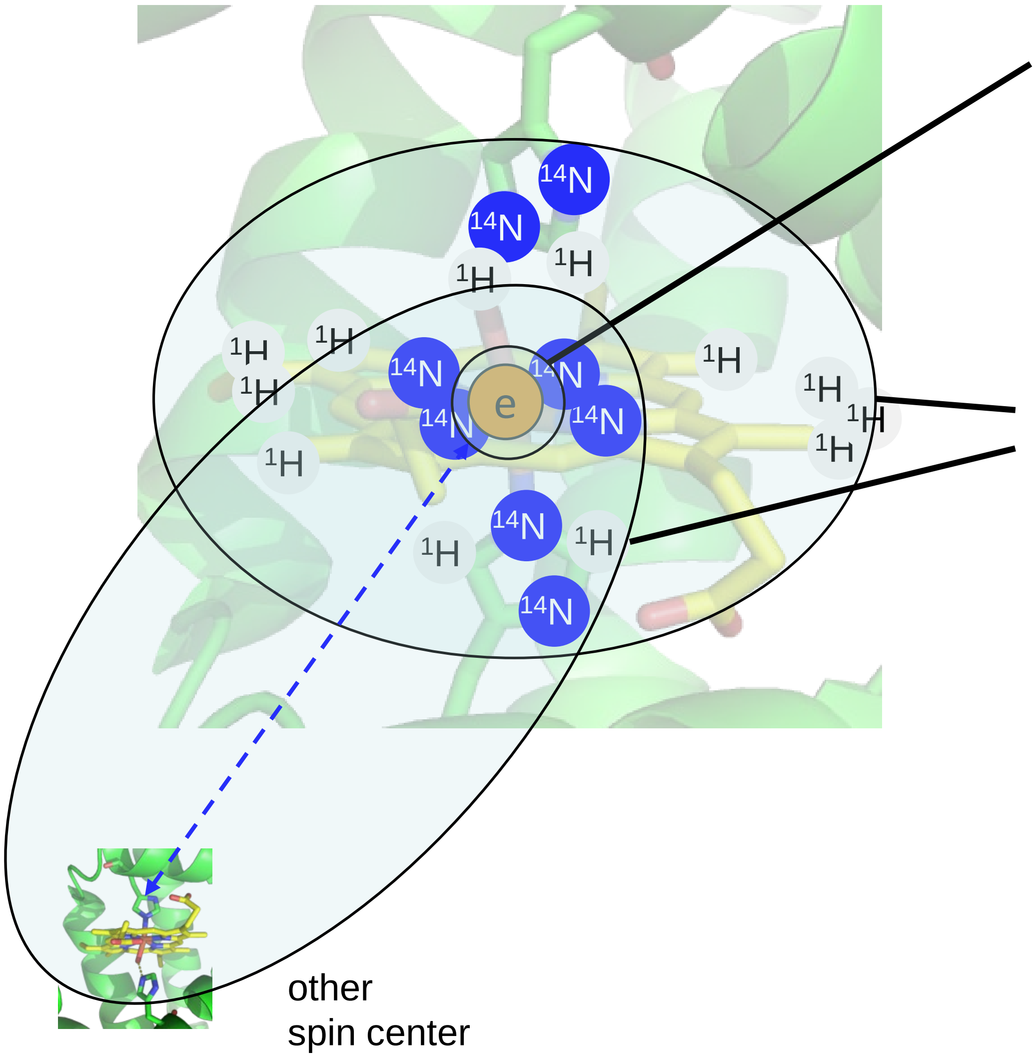


hydroxy-metmyoglobin (MbOH)
Fe(III) d^5 low-spin (S = 1/2)

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)



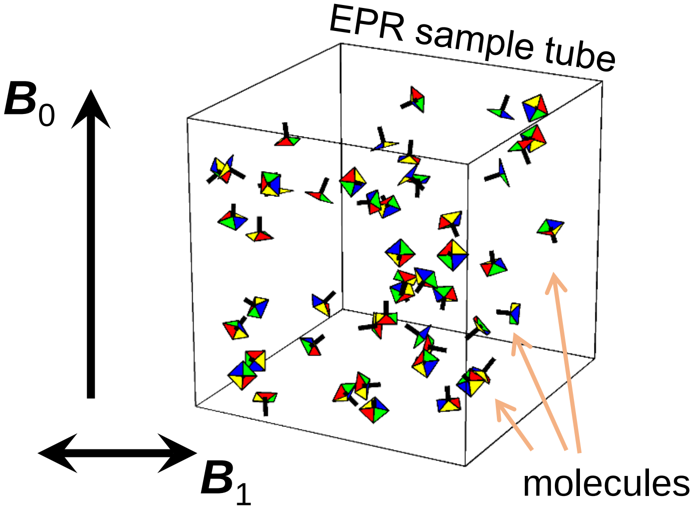
CW EPR: low resolution
- metal center, (ligands)
- strongly coupled magnetic nuclei

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

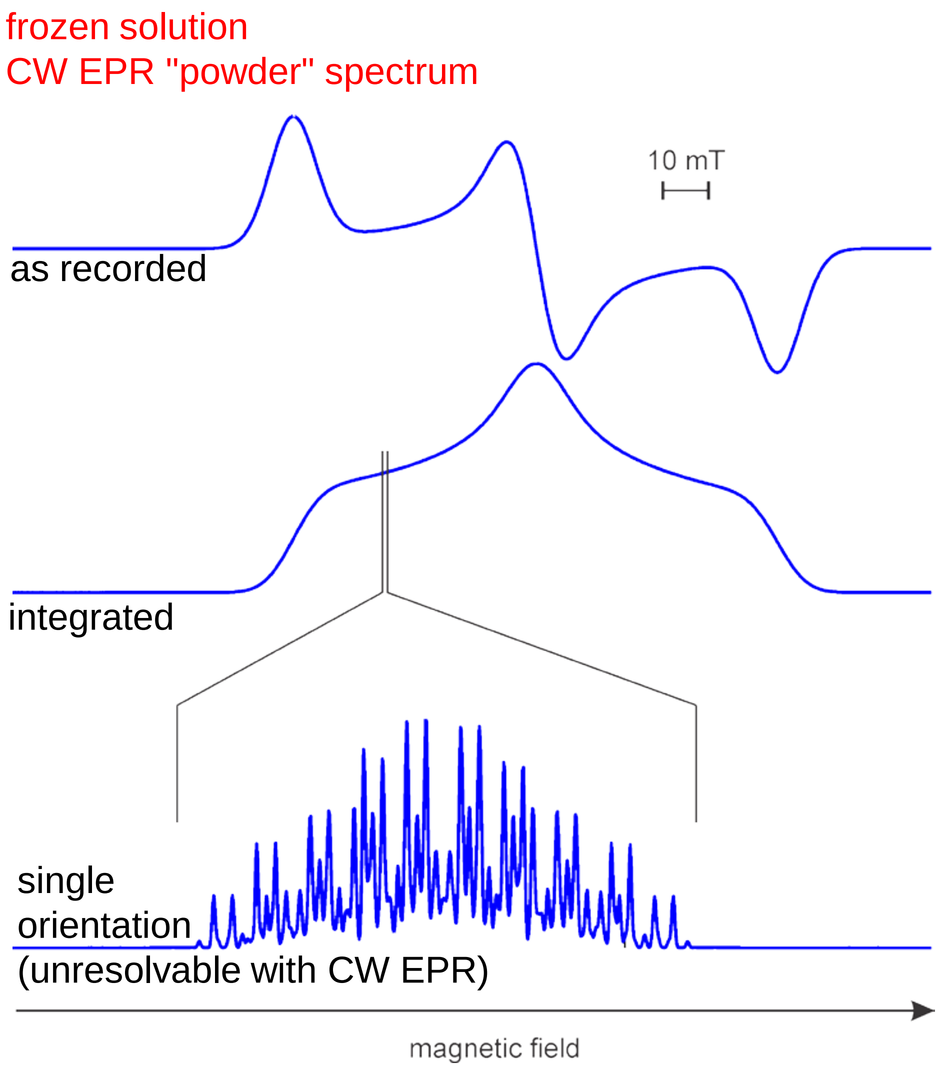
Pulse EPR is a solid-state spectroscopy

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.

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

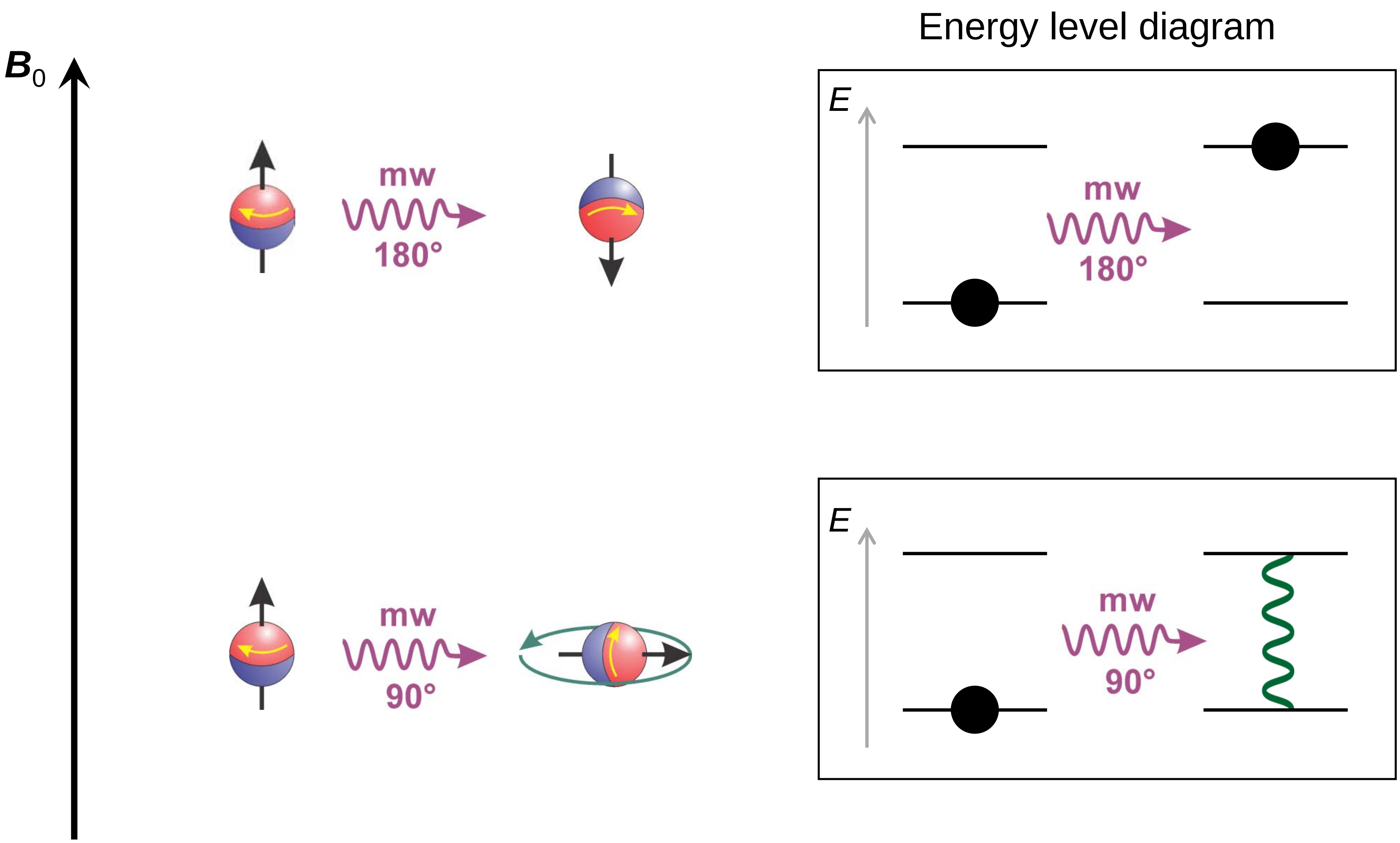
pulse EPR

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

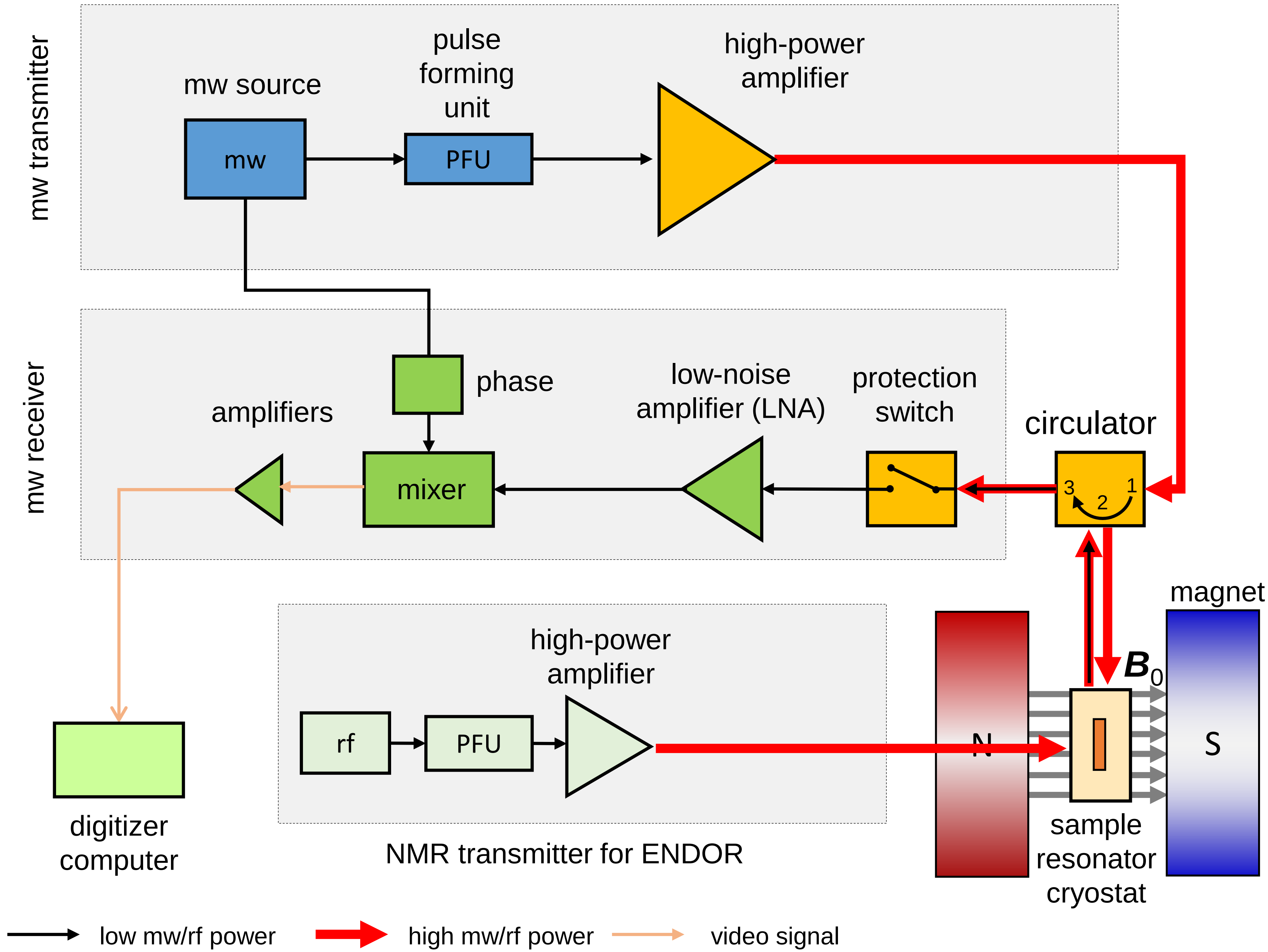
Pulse EPR
- pulse excitation
- very high microwave power (W-kW)
- emission spectroscopy
- measures transient response after excitation
- high resolution

Pulse EPR uses pulses to rotate electrons

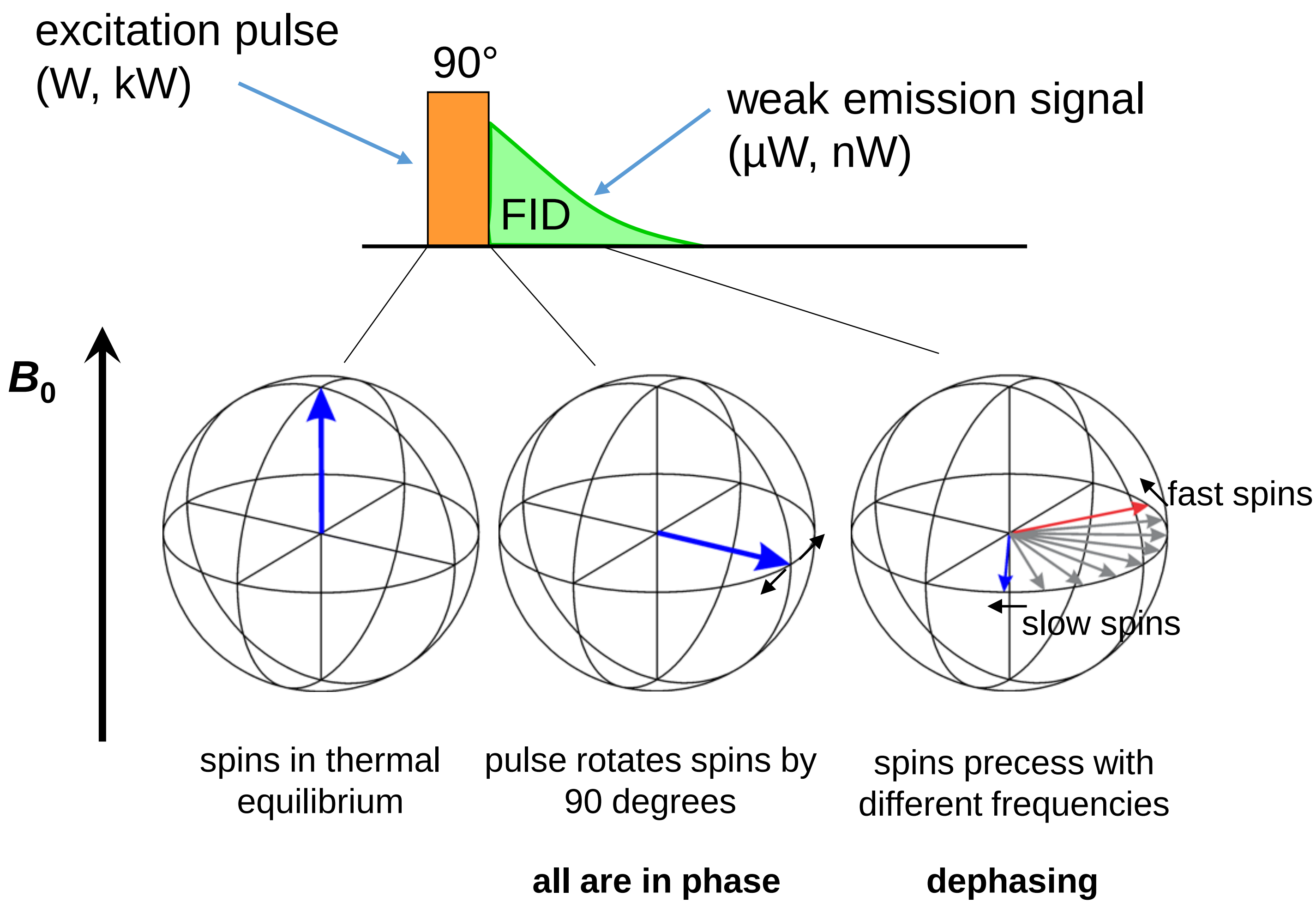


Spin-up and spin-down: stationary states
Spin-"sideways": non-stationary state (spins precess)

Pulse EPR spectrometer



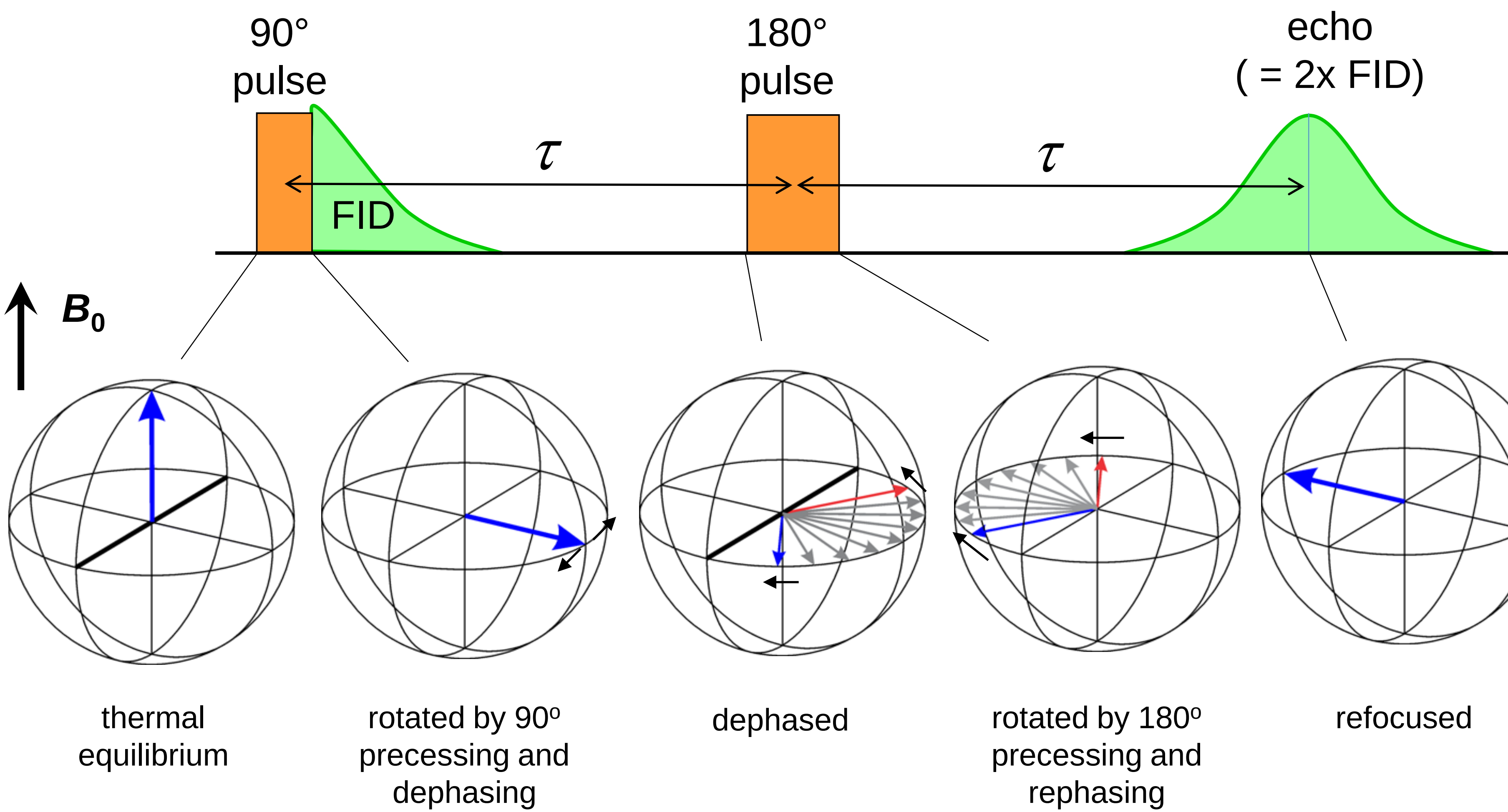
Free Induction Decay (FID)



signal intensity is proportional to vector sum of spins
in plane perpendicular to magnetic field

Electron Spin Echo (ESE)

two-pulse echo = primary echo = Hahn echo



2. Magnetic nuclei and their interactions

Magnetic nuclei and their interactions

Three effects that determine the energy of magnetic nuclei

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

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

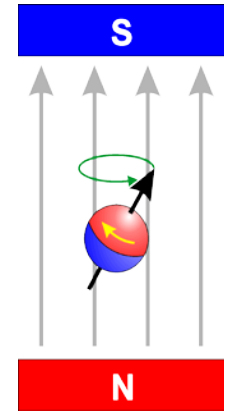
Contributions:
1. through-bond (isotropic; "Fermi contact")
2. through-space (anisotropic; dipolar)
(3. spin-orbit contribution)

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

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

1

Nuclear Zeeman Interaction



Nuclear precession/Larmor/Zeeman frequency:

$$\nu_\mathrm{I} = -(\mu_\mathrm{N}/h)\cdot g_\mathrm{n} B_0$$

nuclear Bohr magneton $5.0508\cdot 10^{-27}$ J/T

nuclear g factor

magnetic field

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

Example: ^{1}H with $B_0 = 0.350\ \mathrm{T}$ → $\nu_I = 14.9\ \mathrm{MHz}$ (electron: 9.8 GHz)

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

×6.5

opposite sign

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

Hyperfine coupling: 1. Fermi contact interaction

Electron-nucleus magnetic interaction due to small, but finite, probability of finding an unpaired electron *at position of nucleus* (s orbitals only!)

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

scales with g_{n}

spin density at position of nucleus

conventional units: MHz

Simple 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

Example: $A_{\mathrm{iso}}(^{1}\mathrm{H})$ = 20 MHz $\rightarrow$ 20/1420 = 1.4%

alternative: compare to quantum-chemical estimates

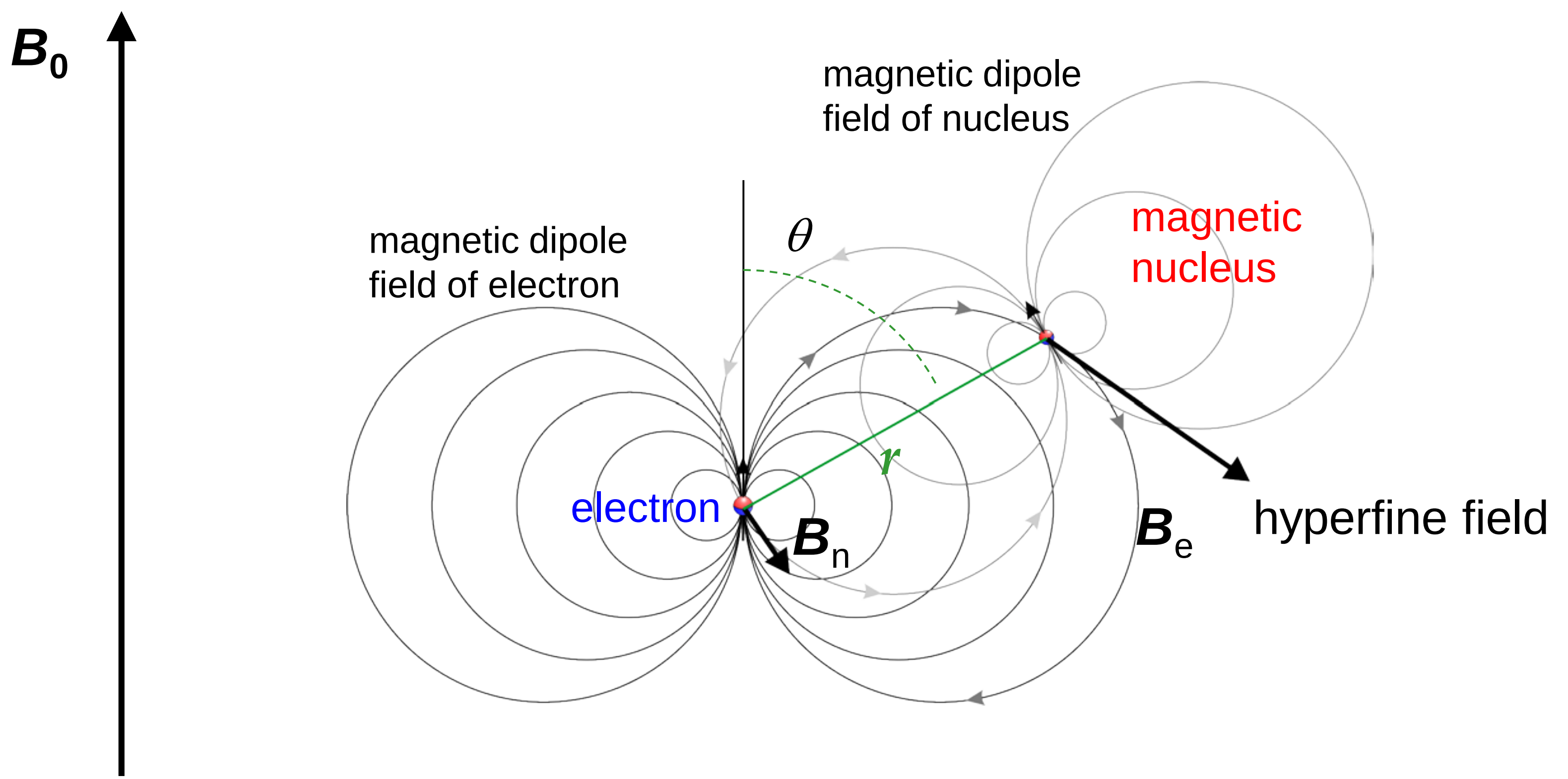
Reasons for non-zero A_{iso}

(1) ground-state open s shell
(2) valence-core spin polarization (e.g. 3d$\rightarrow$2s, 2p $\rightarrow$1s)

What you can learn from A_{iso}

(1) spin delocalization
(2) proximity of nucleus to metal ion

Hyperfine coupling: 2. Through-space dipolar coupling



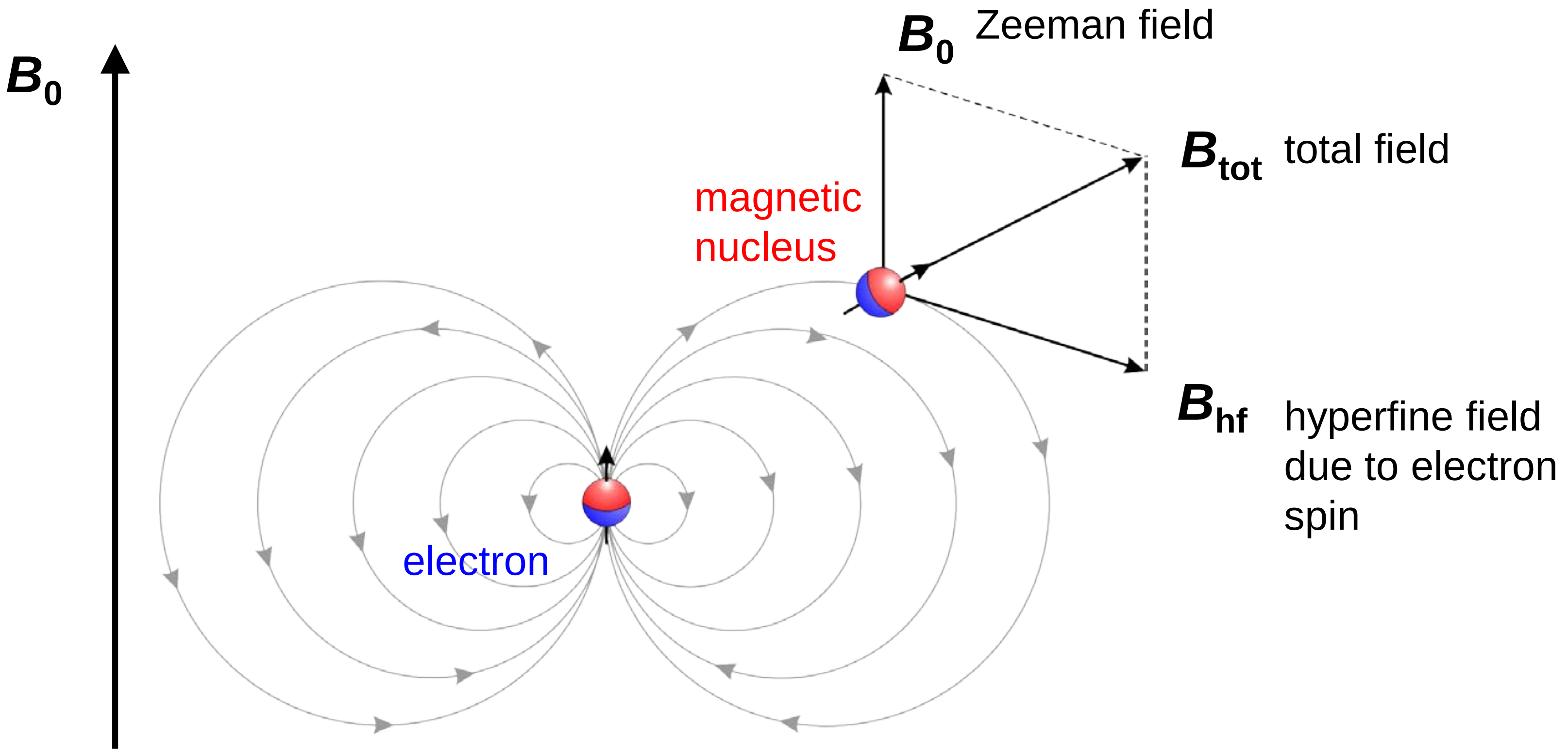
Dipolar coupling strength depends on

(1) $1/r^3$ (drops by 8 if r is doubled)

(2) θ (2x larger for $\theta = 0$ than for $\theta = \pi/2$)

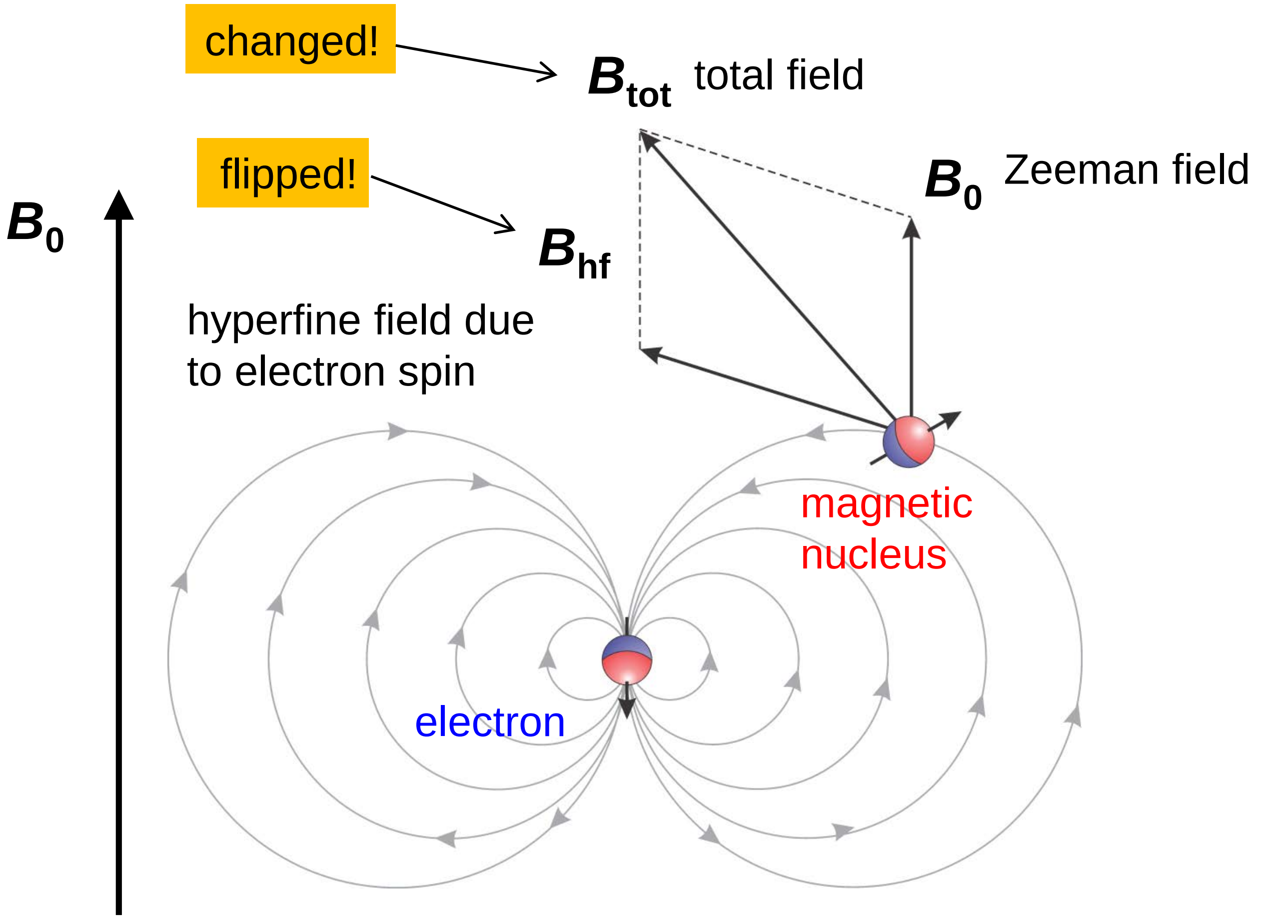
1+2

Combining Hyperfine and Zeeman: Local fields



at equilibrium, nuclear spin aligns along total field

Combining Hyperfine and Zeeman: Local fields



1+2

Nuclear frequencies and powder spectra

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

$$\nu_\mathrm{I} = -g_\mathrm{n}\mu_\mathrm{B}B_0/h$$

$$\nu(m_S) \approx |\nu_\mathrm{I} + m_S A|$$

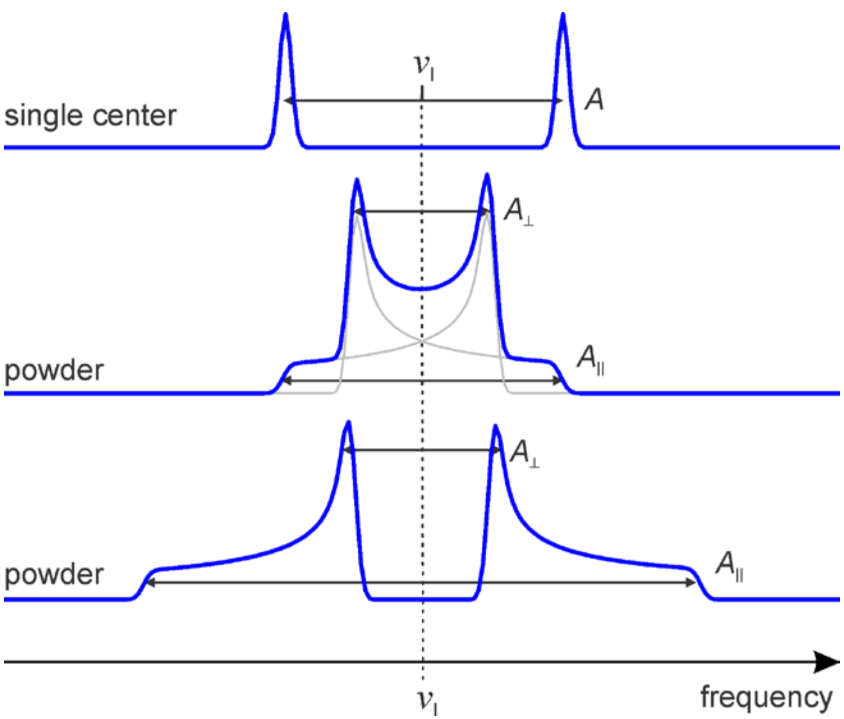
neglecting $m_S B$ term
(valid for weak and strong coupling only)

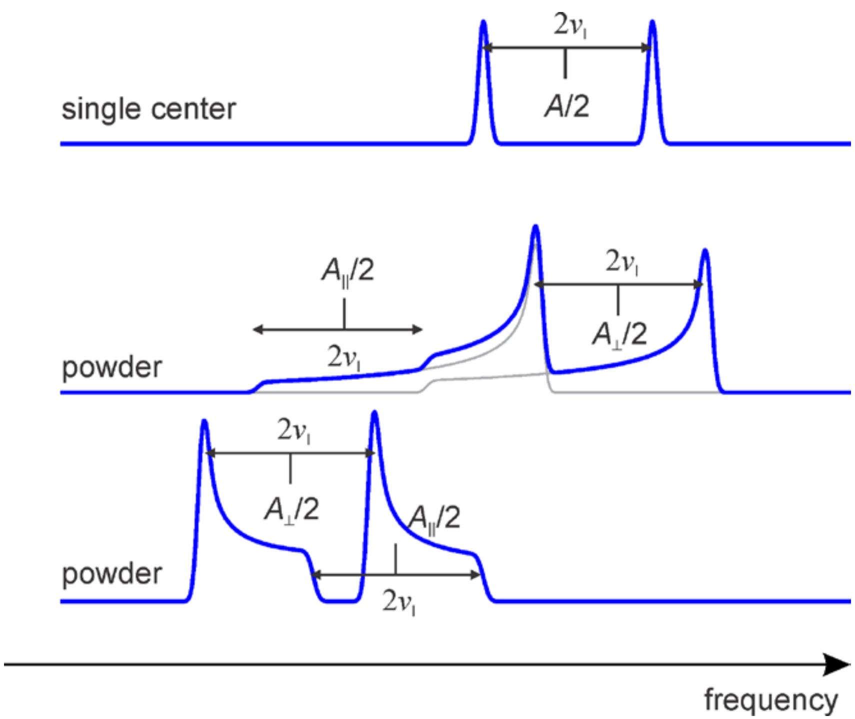
Weak coupling regime $|\nu_\mathrm{I}| \gg |m_S A|$

Strong coupling regime $|\nu_\mathrm{I}| \ll |m_S A|$

centered at ν_I, split by *A*

centered at *A*/2, split by $2\nu_\mathrm{I}$

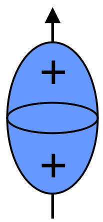




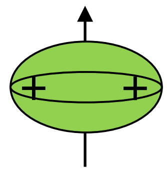
Nuclear Quadrupole Interaction

(1) Nuclei with spin>1/2 have electric quadrupole moment

- Nnonspherical spape, 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

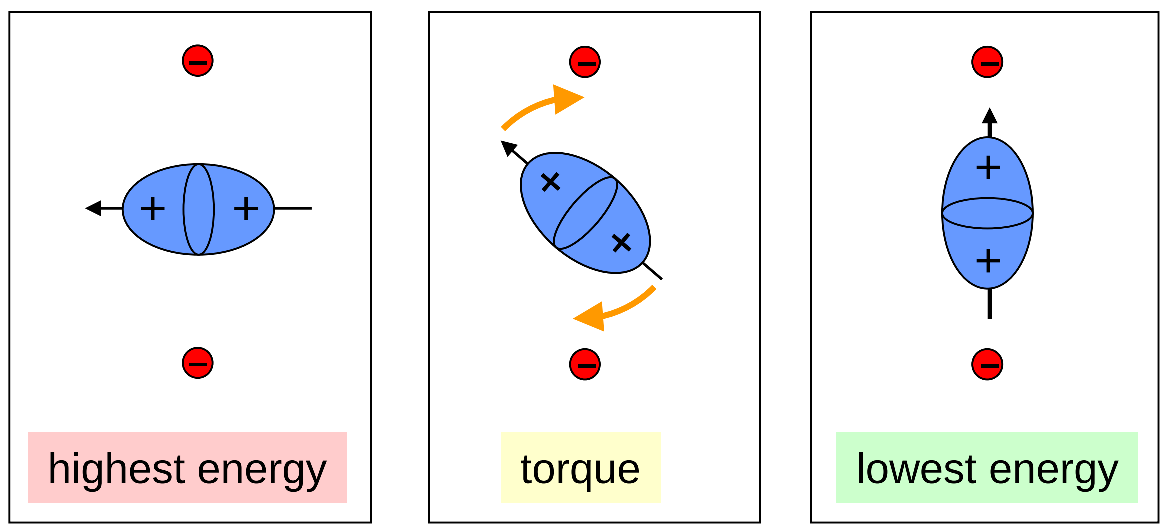
- Spin is tied to nuclear shape!

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

(3) Quadrupole nuclei have orientation-dependent energy

electric, not
magnetic interaction!

(4) This leads to additional splittings in spectra.



Types of pulse EPR experiments

Pulse EPR measures spin echo (or FID) amplitude as a function of…

Quantity	Experiment name	Result
Field	echo-detected field sweep FID-detected field sweep	EPR spectrum
Pulse delays	inversion recovery two-pulse ESEEM three-pulse ESEEM HYSCORE	relaxation times nuclear spectrum nuclear spectrum
RF frequency	Davies ENDOR Mims ENDOR	nuclear spectrum nuclear spectrum
MW frequency	ELDOR-detected NMR DEER	nuclear spectrum distance between spin centers

3. Examples

Stellacyanin: identification of Cu^{2+} imidazole ligand

^{14}N ESEEM

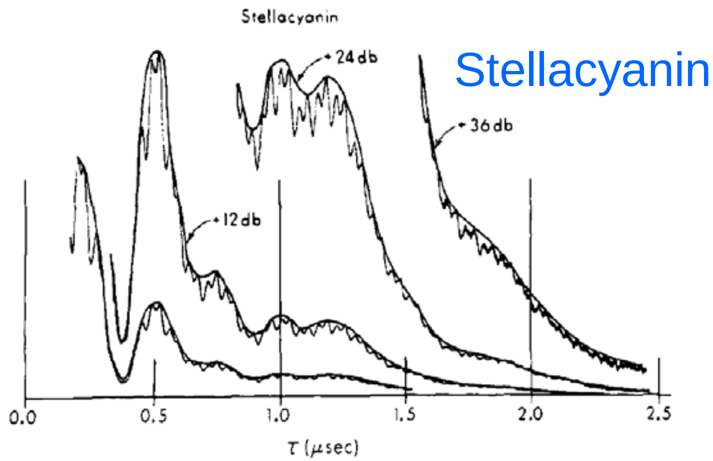


FIGURE 8: Envelope modulation function for stellacyanin. Conditions were similar to those in Figure 6. The low-frequency period is assigned to an imidazole ligand as in Figure 6.

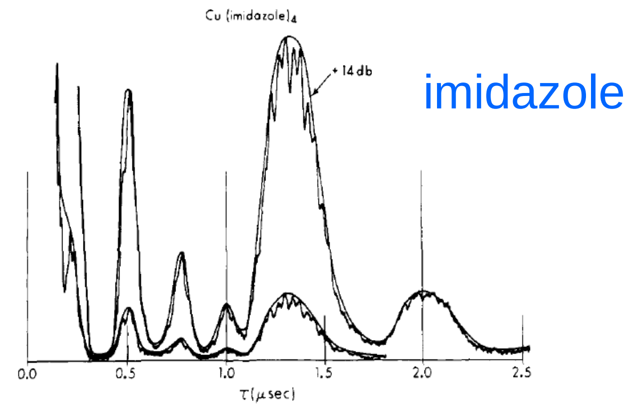


FIGURE 9: Envelope modulation function for the compound Cu(II)-(imid)$_4$. Conditions were similar to those in Figure 6. This curve is reproduced for comparison with the curve in Figure 10.

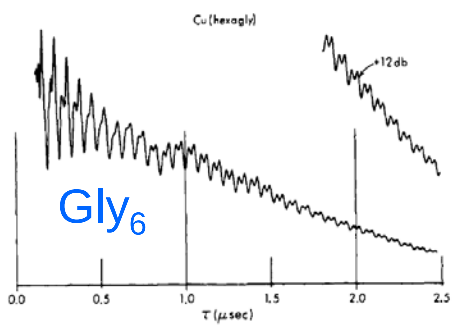


FIGURE 7: Envelope modulation function for Cu(II)-hexaglycine prepared at pH 6.0. Conditions were similar to those in Figure 4. The major features in the spectrum show the effect of proton coupling, similar to that seen for hydrated Cu(II) (Figure 4) or for Cu(II)-glycylglycine (Figure 5). The weak feature observed near 1 μs is ascribed to interaction with nitrogen atoms not directly ligated to copper.

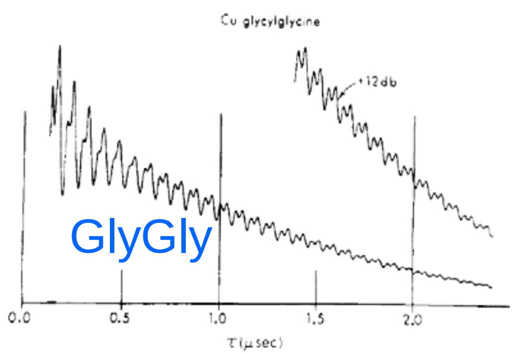


FIGURE 5: Envelope modulation function for Cu(II)-glycylglycine. Conditions were similar to those in Figure 4. The effect of proton coupling is almost the same as in Figure 4. Coupling between Cu(II) and the directly coordinating ^{14}N nucleus yields no visible effect in this experiment. This is because the term A *I·S* dominates other terms in that portion of the spin Hamiltonian that describes the interactions of the ^{14}N nucleus.

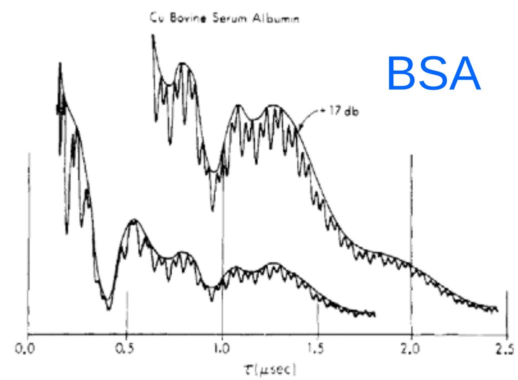
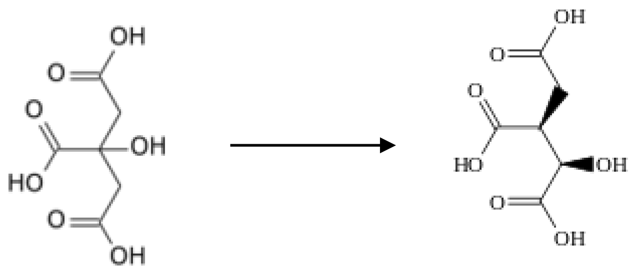


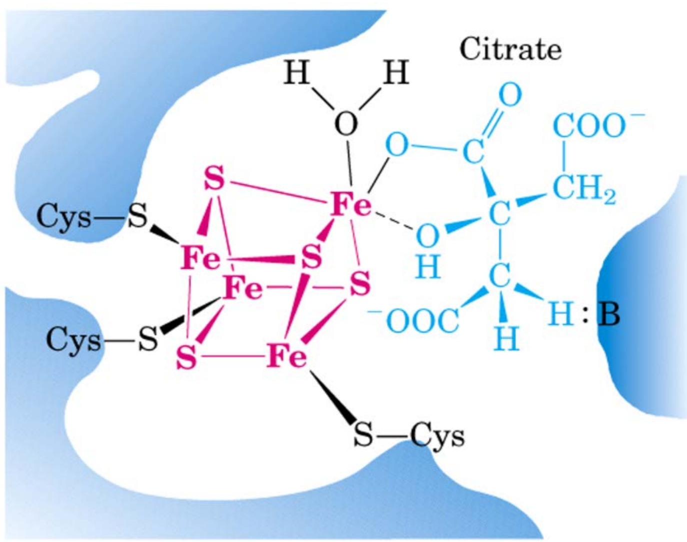
FIGURE 6: Envelope modulation function for the complex formed between Cu(II) and bovine serum albumin measured at the $g_\perp$ end of the EPR spectrum. Conditions were similar to those in Figure 4. The low-frequency period shown by the line joining the proton peaks is due to coupling with the ^{14}N nucleus belonging to the N-1 nitrogen in the imidazole ligand (^{14}N nuclei immediately adjacent to the Cu(II) ion do not contribute to the envelope in this type of experiment. (See Figure 5.))

Biochemistry **1976** 15 3863

Aconitase: substrate and water binding

^{17}O ENDOR





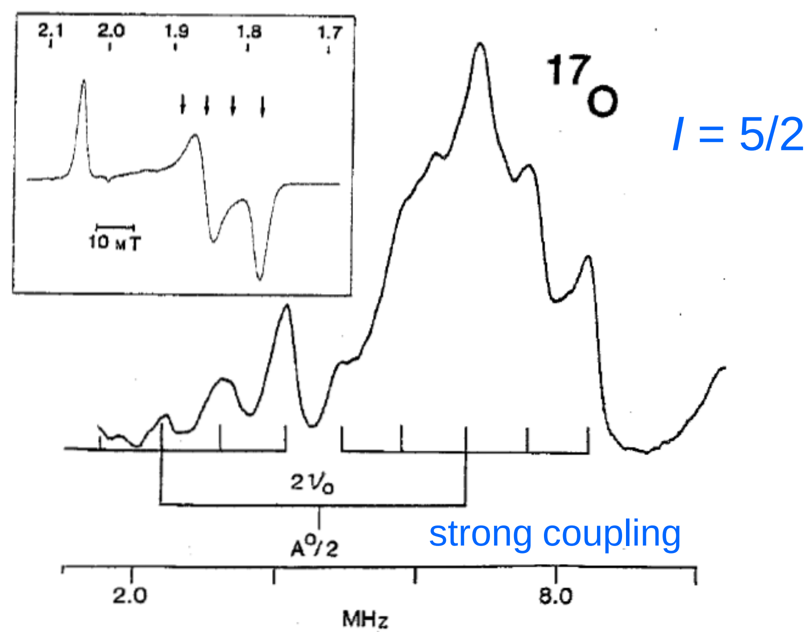
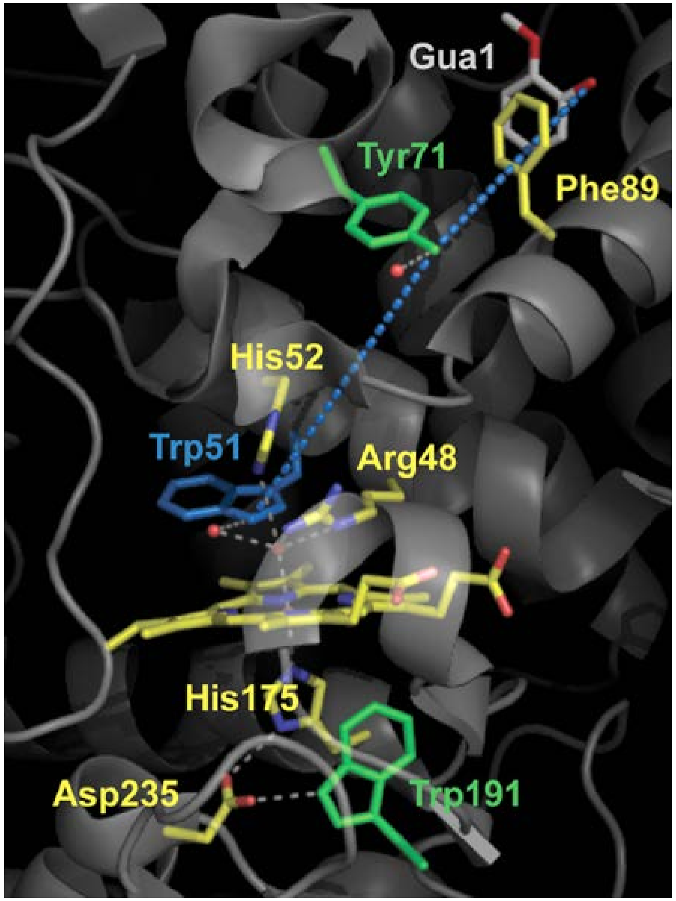


FIG. 1. *Inset*, EPR absorption derivative spectrum of $[4Fe\text{-}4S]^{1+}$ aconitase (0.16 mM) in the presence of citrate (0.1 mM) in 100 mM Hepes (pH 7.5) containing 13 mM potassium oxalate and 7 μM deazaflavin. Field positions at which ENDOR spectra were recorded are indicated, *g* values indicated at the top; microwave frequency, 9.2325 GHz. *Main figure*, ^{17}O ENDOR spectrum at $g = 1.88$ for $[4Fe\text{-}4S]^{1+}$ aconitase in the presence of citrate in $H_2{}^{17}O$ solution. A single-crystal ^{17}O ENDOR pattern (Equation 1) is indicated; the smaller splittings correspond to $3P°$. Experimental conditions: temperature, 2 K; magnetic field, 0.3650 tesla, modulation amplitude, ~0.3 millitesla; modulation frequency, 100 kHz; microwave frequency, 9.53 GHz; microwave power, 1 microwatt; time constant, 0.032 s; ENDOR scan rate, 3.0 MHz/s; 3432 scans. The background has been corrected by digital subtraction of a straight line.

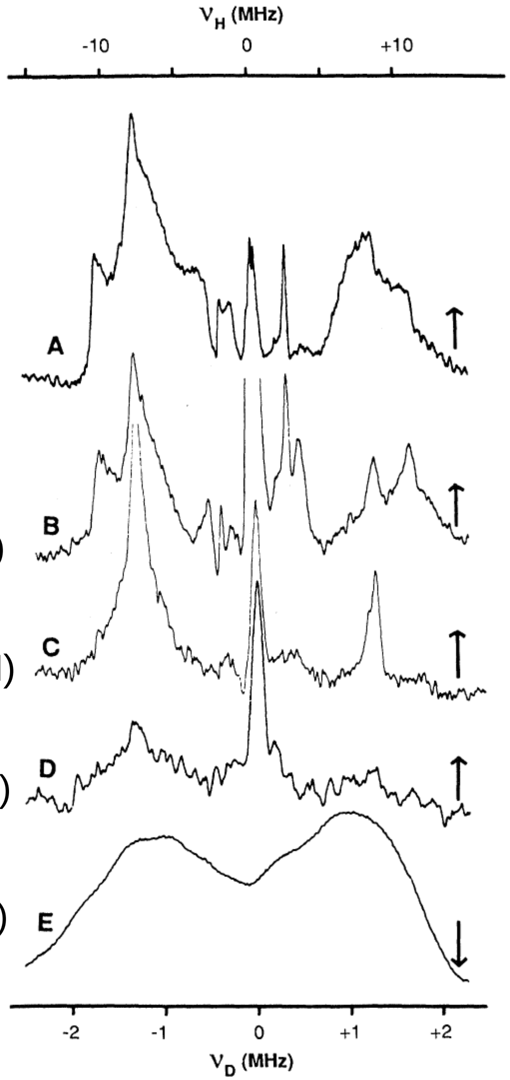
J.Biol.Chem. **1986** 261 4840

Cytochrome c peroxidase: tryptophan radical

H/D ENDOR



Compound ES



ES(H,H)

ES(W51F)

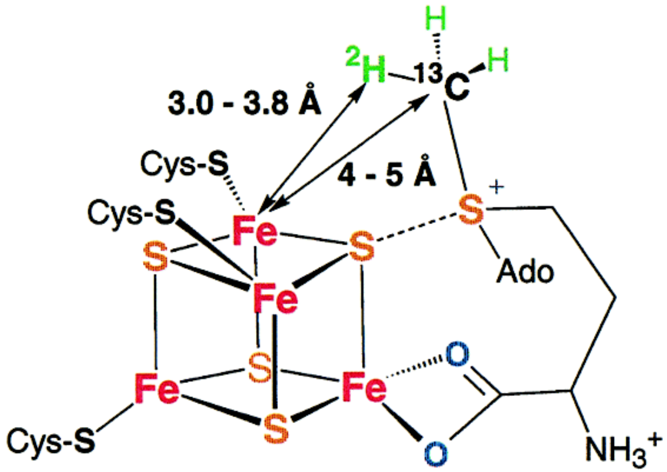
ES(d_8-Trp,H)

ES(d_8-Trp,D)

ES(d_8-Trp,D)

Science **1989** 245 738

PFL-AE: coordination of S-adenosyl-methionine

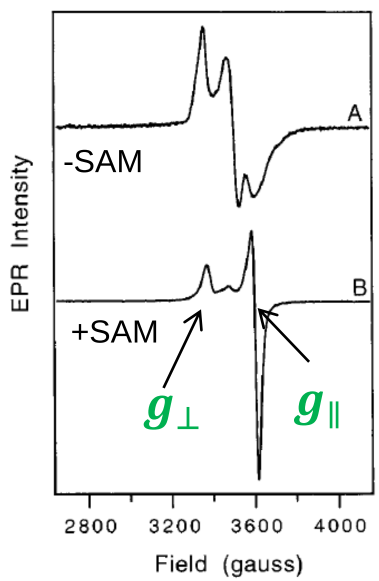


^{2}H ENDOR

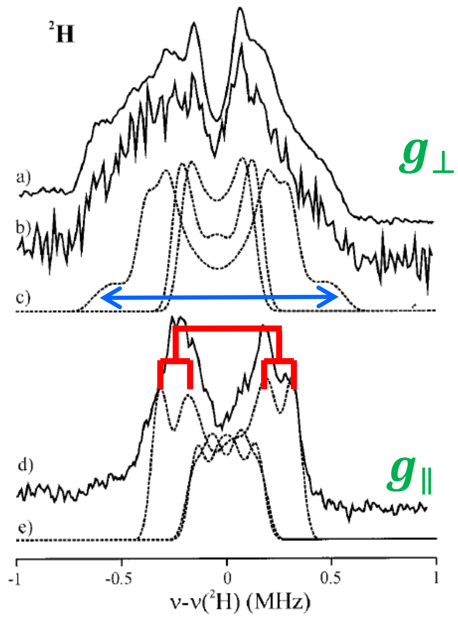
^{13}C ENDOR

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

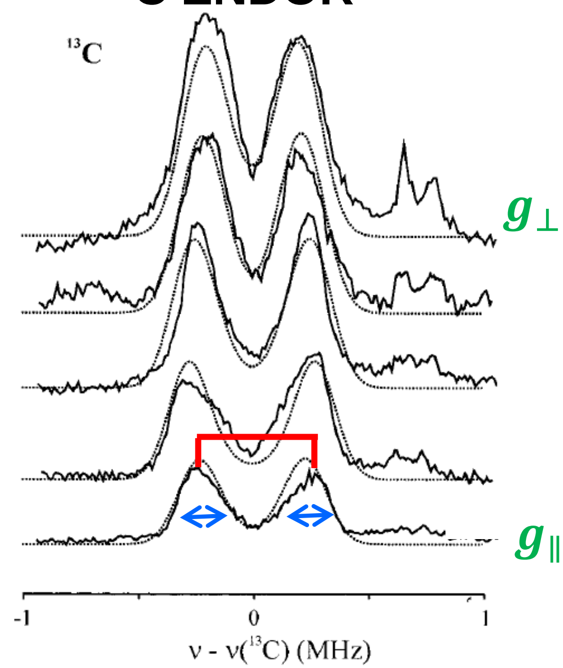
CW EPR



^{2}H ENDOR



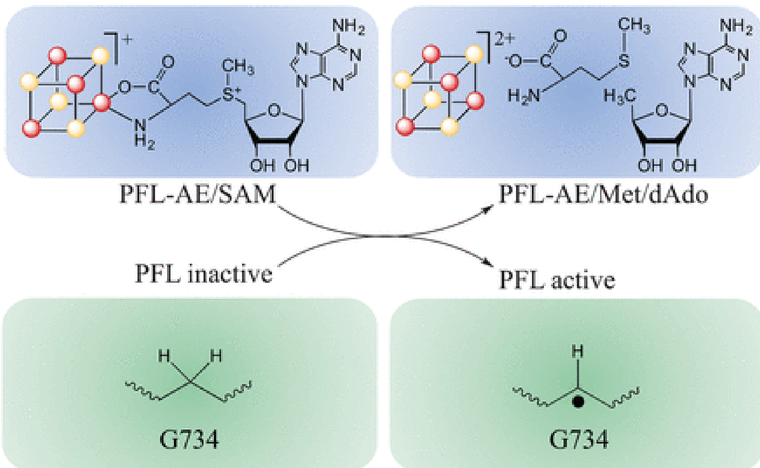
^{13}C ENDOR

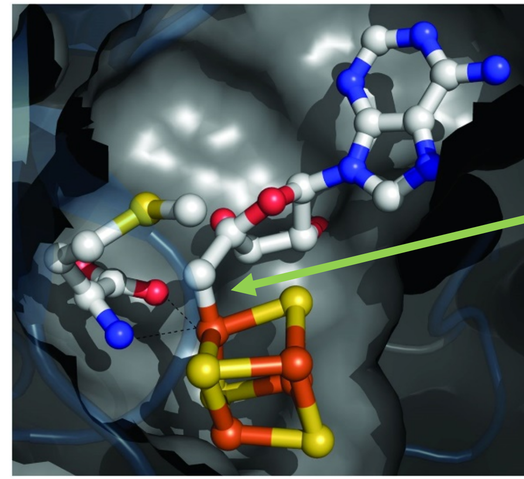


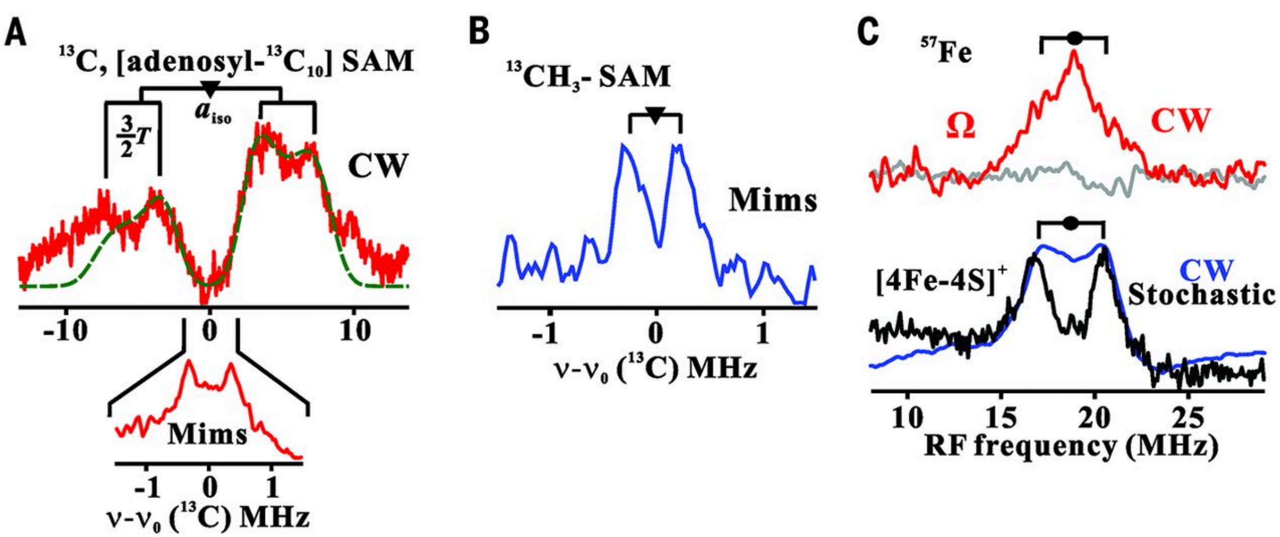
JACS **2002** 124 3143

Pyruvate formate-lyase activating enzyme: Fe-C bond

^{13}C ENDOR



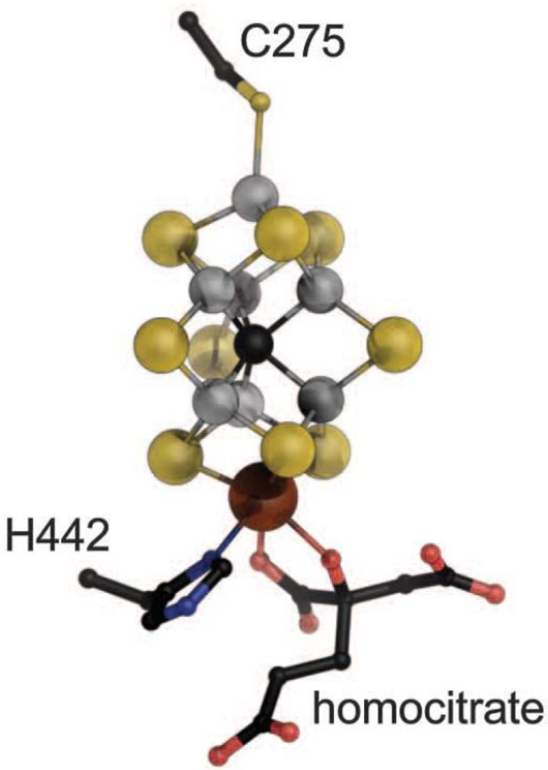




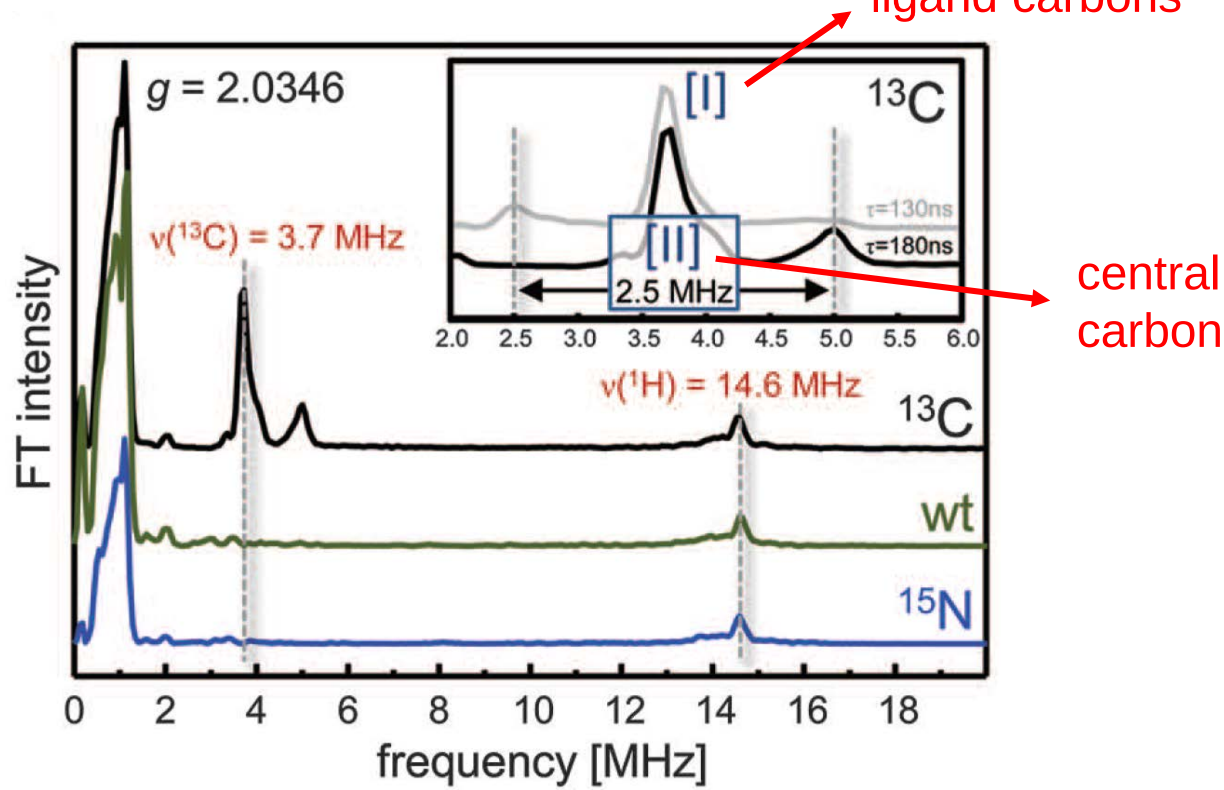
Science **2016** 352 822

Nitrogenase FeMo cofactor: interstitial carbon

^{13}C, ^{15}N ESEEM



X-band 3-pulse ESEEM

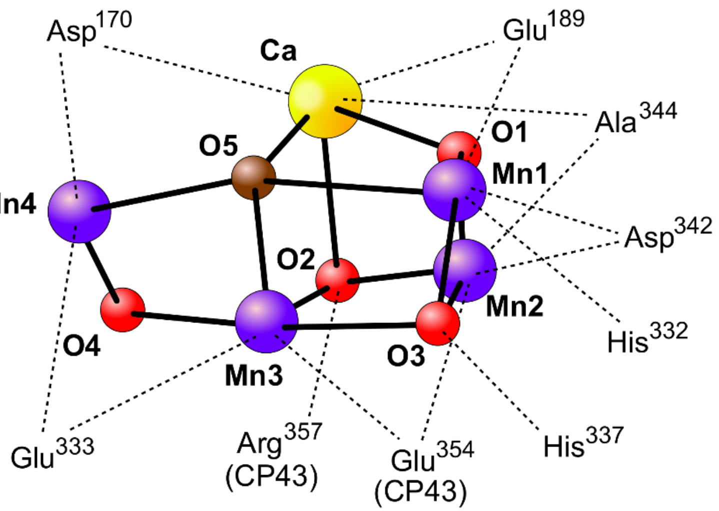


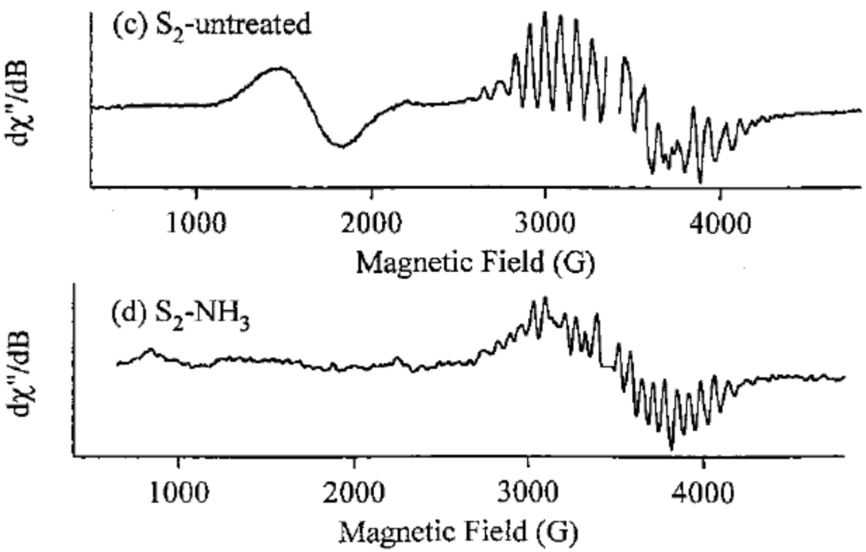
Science **2011** 334 940

Photosystem II: Structural model of OEC

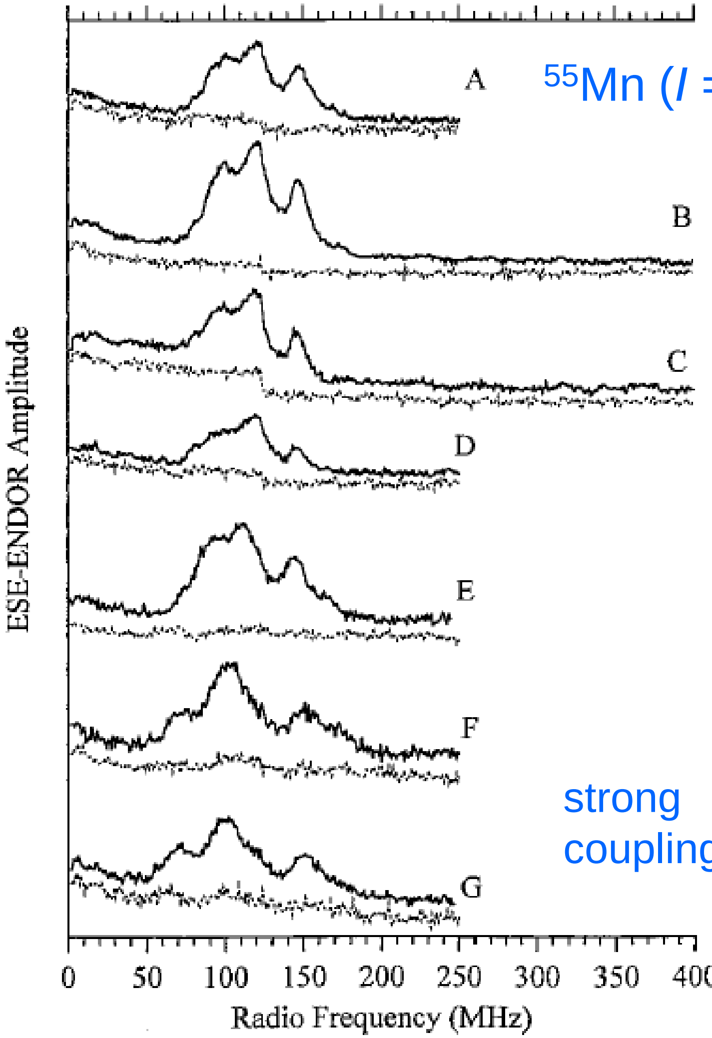
^{55}Mn ENDOR

[Mn_4Ca] cluster in PSII





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



^{55}Mn (I = 5/2)

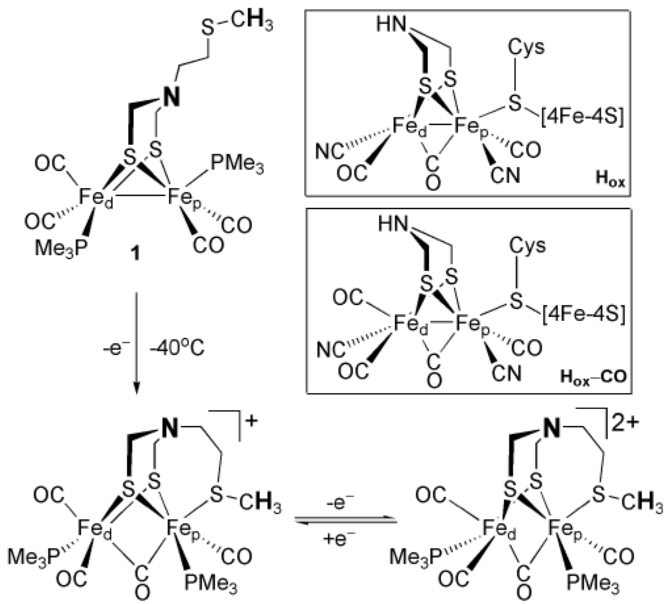
strong coupling

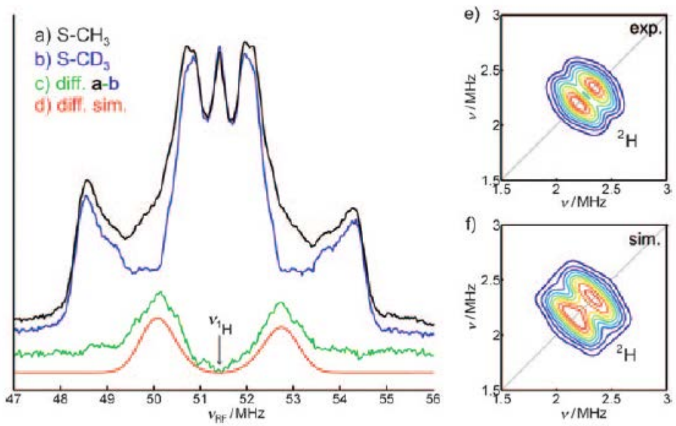
JACS **2000** 122 10926

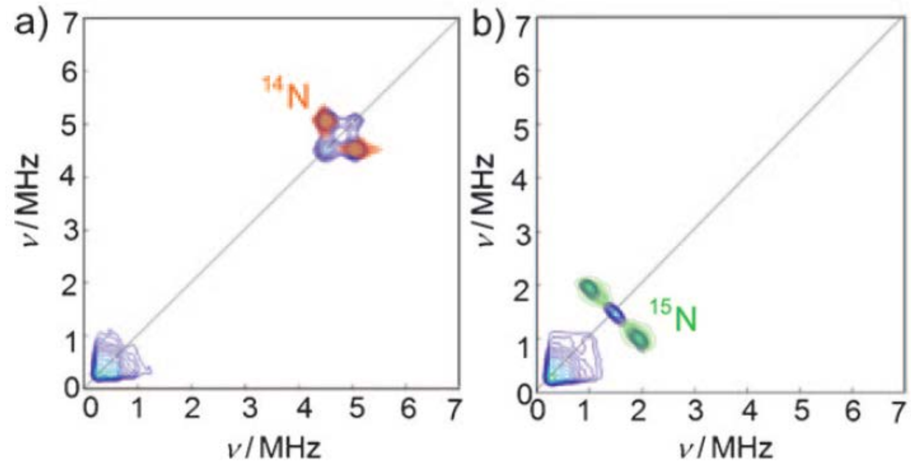
[FeFe] hydrogenase: azadithiolate

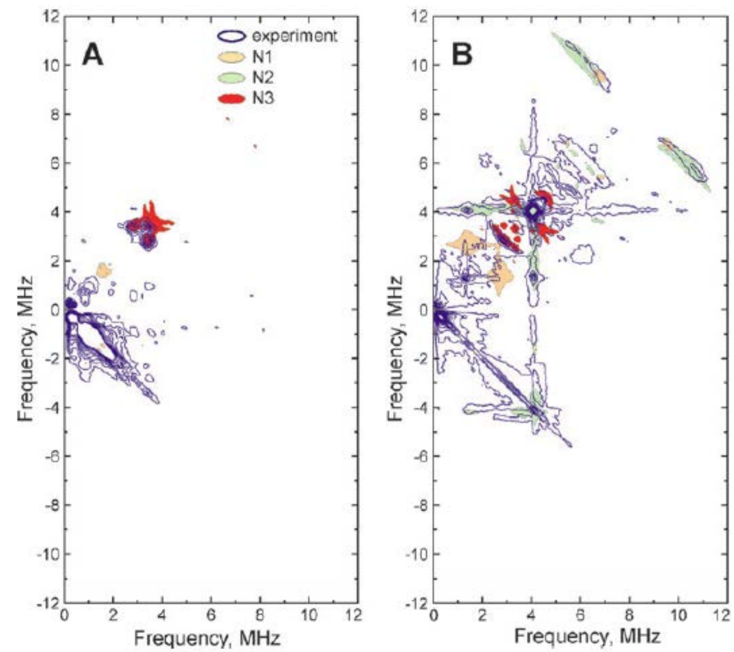
[FeFe] hydrogenase + model

$^{14}N/^{15}N$ HYSCORE









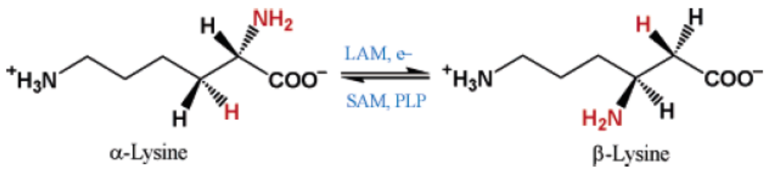
Angew. Chem. **2011** 50 1

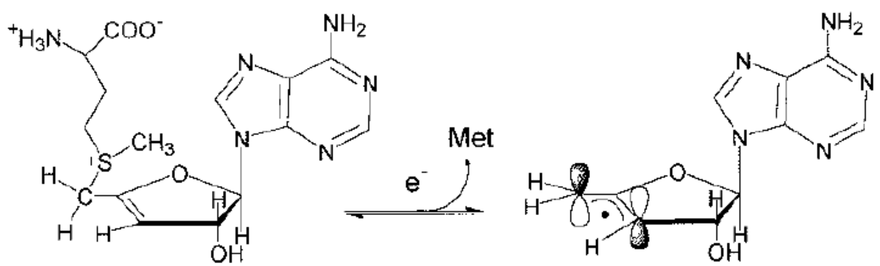
PCCP **2009** 11 6592

LAM: trapping of radical intermediate

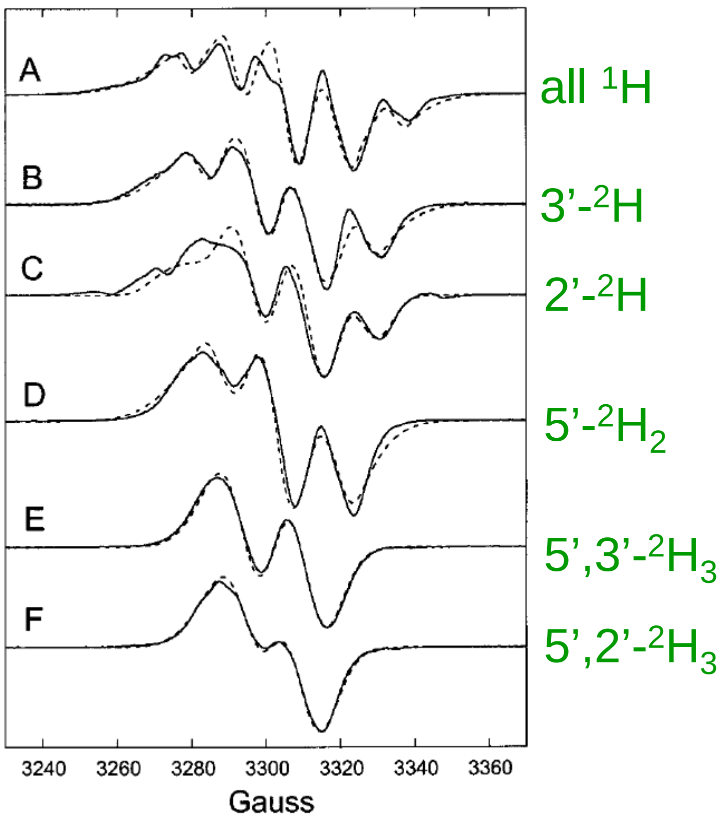
CW EPR resolved
No need for pulse EPR

LAM = Lysine 2,3-aminodismutase





anhydroadenosyl
radical (anSAM)

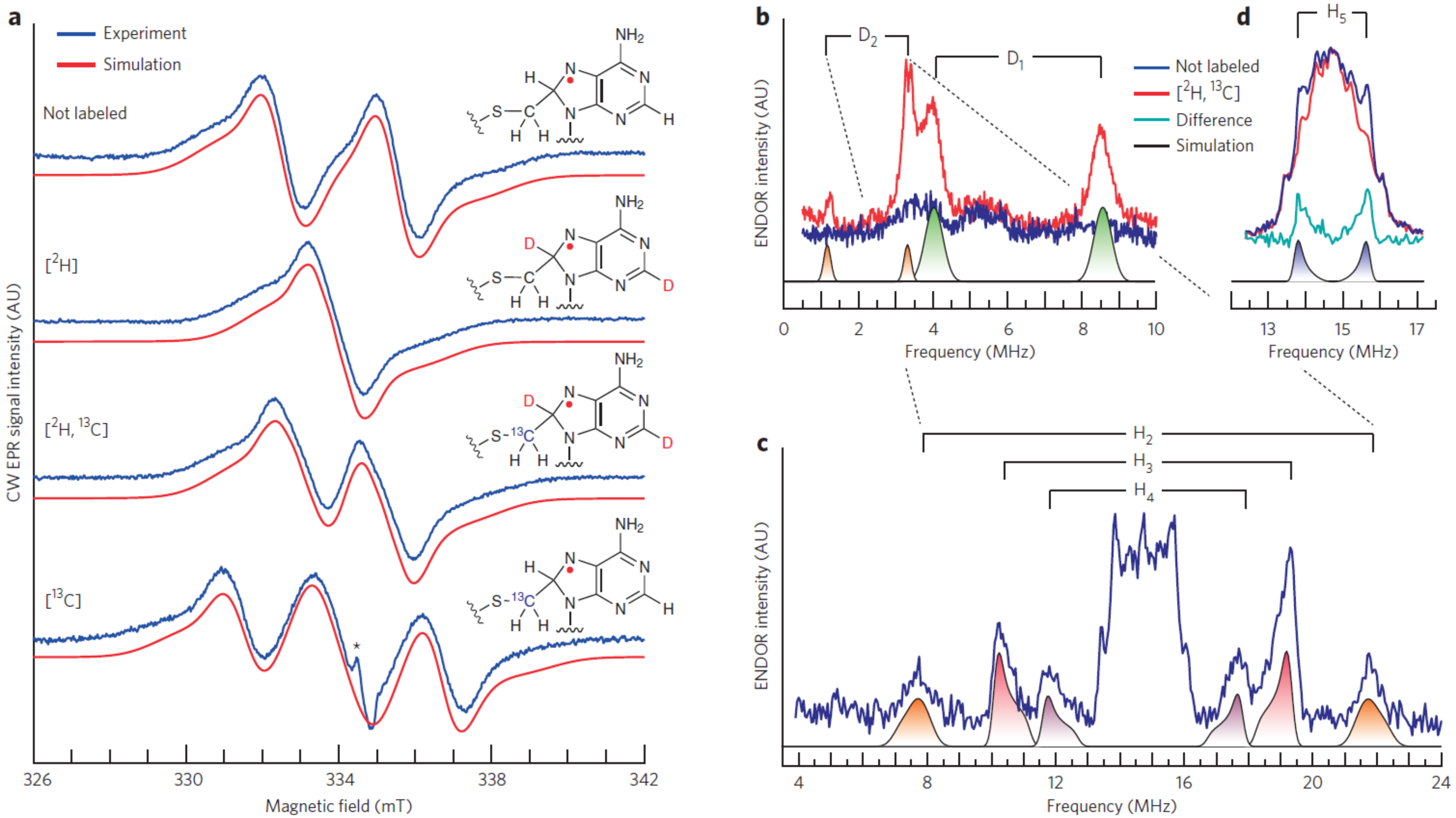


Biochem. **2001** 40 7773

Cfr: Radical substrate intermediate

Resolved CW EPR

Pulse EPR

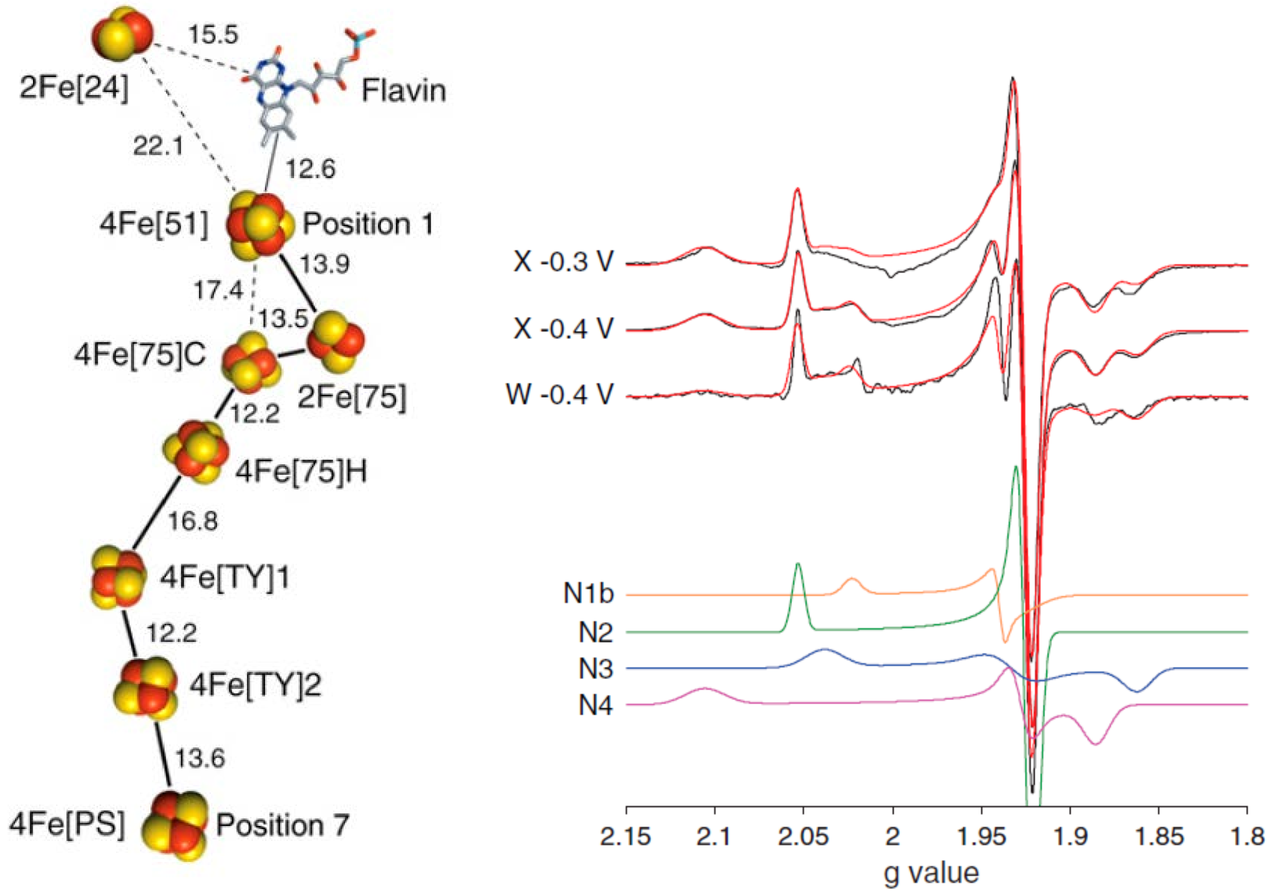


Nat. Chem. Bio. **2013**, 9, 422

Complex 1: Assignment of FeS clusters

FeS-FeS DEER

Assignment of FeS cluster EPR signals to sites in crystal structure

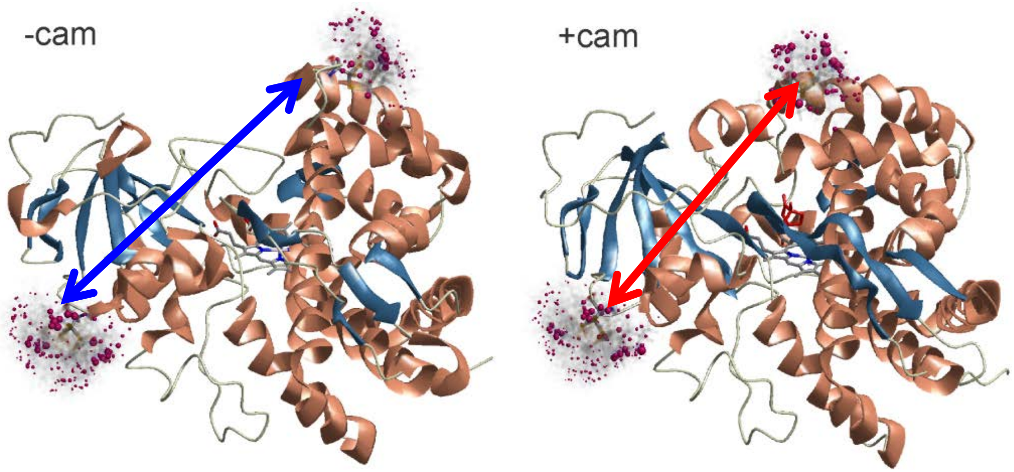


	Ohnishi (3, 4)	Hirst (5, 6)	
	A	B	C
2Fe[24]			
4Fe[51]	N3	N3	N3
2Fe[75]	N1b	N1b	N1b
4Fe[75]C	N4	(N5)	(N5)
4Fe[75]H	(N5)		
4Fe[TY]1		N4	
4Fe[TY]2			N4
4Fe[PS]	N2	N2	N2

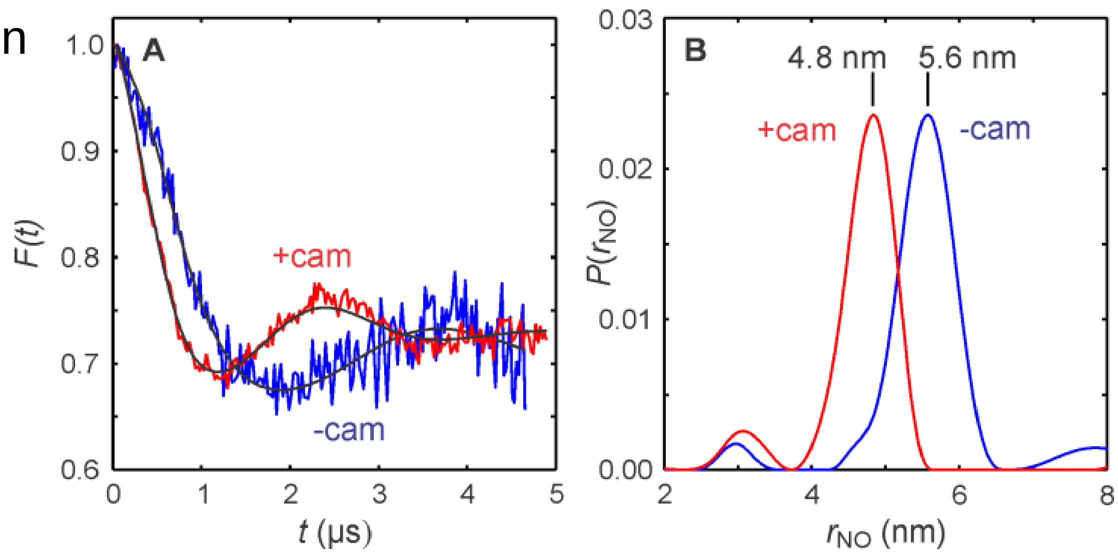
PNAS **2010** 107 1930
Annu.Rev.Biochem. **2013** 82 551

Cytochrome P450cam: conformational change

Nx-Nx DEER



time-domain signal

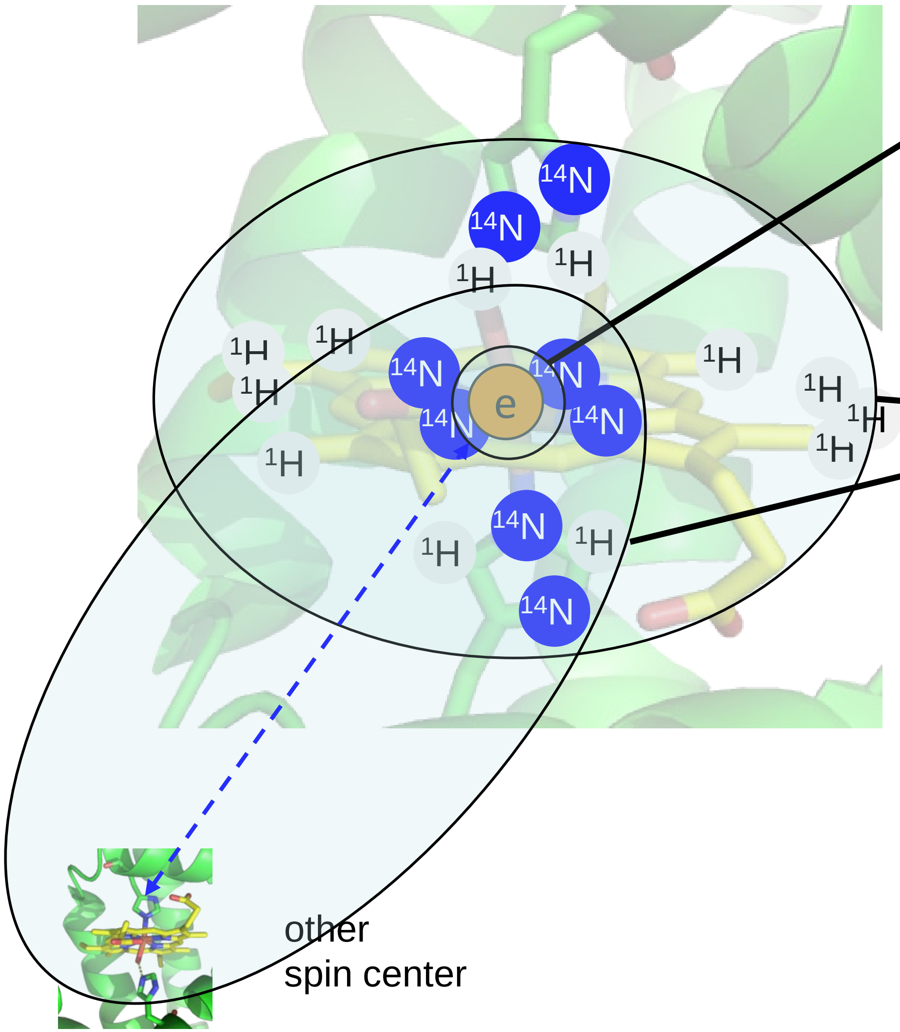


distribution distribution

PNAS **2012** 198 12888

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

What you can learn from EPR data

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