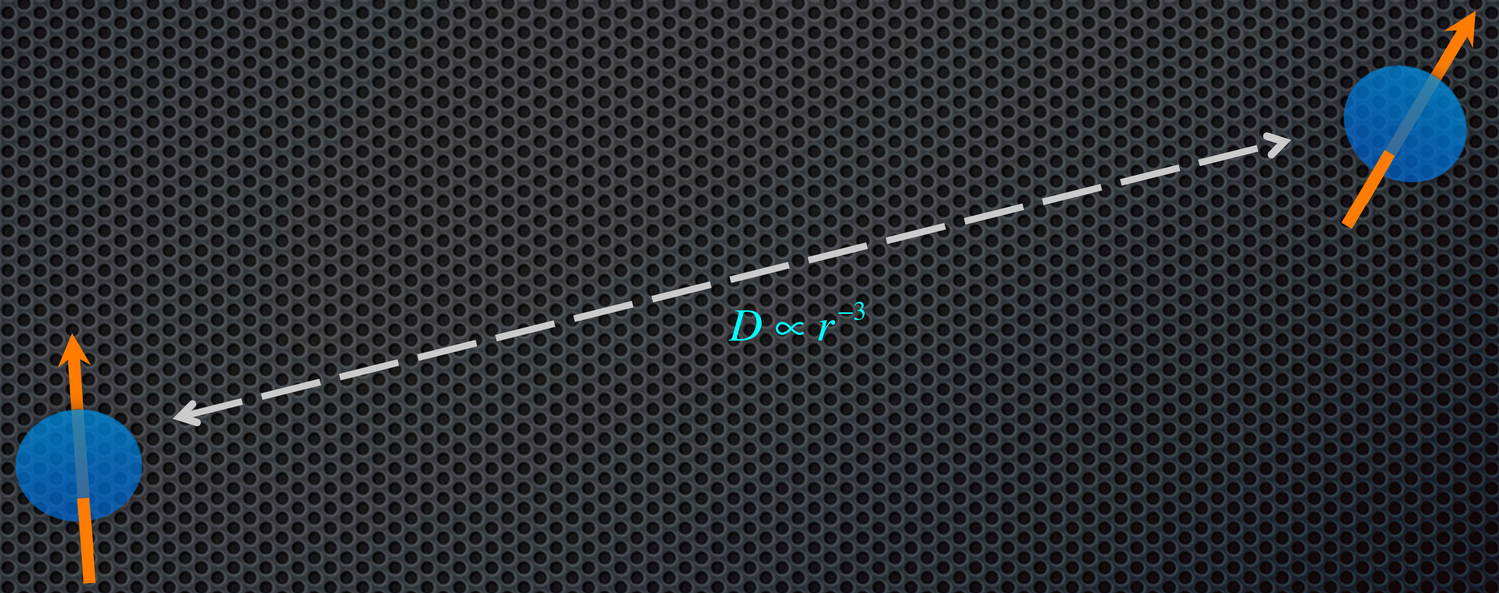
Electron Spin Resonance
in Biophysical Chemistry



Basic Principles

- Spin angular momentum vector $\hat{S}\hbar$, $\hat{I}\hbar$, $\hbar = \frac{h}{2\pi}$

  (Intrinsic Spin ; Purely QM !!)

- Magnetic Moment

$$\vec{\mu} = -\gamma \hbar \hat{S} = -g \beta \hat{S}$$

- γ: Gyromagnetic ratio
- β: Bohr magneton $= \frac{e\hbar}{2m}$ (β_e, β_N, β_B)
  - charge: e
  - mass: proton or ele.
- g: g factor (Dimensionless, intrinsic)

phys. meaning ↓

$$\rightarrow |\gamma| = \frac{g\beta}{\hbar} = \frac{ge}{2m} = \frac{\text{mag. moment } \mu}{\text{Int. Spin ang. momentum. } \hbar\hat{S}}$$

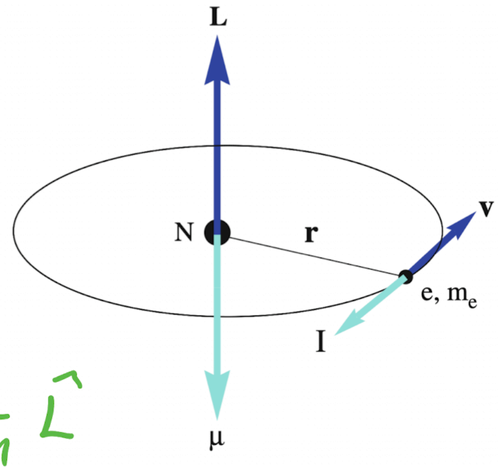
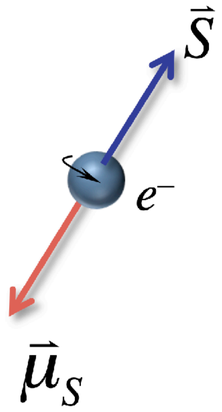


Fig. 1.1 Classical model illustrating relationship between angular momentum $L = m_e \cdot v \cdot r$ of electron, e, moving around a nucleus N and magnetic moment μ.

$$\mu = I \cdot A = \cdots = -\frac{e}{2m}\hat{L}$$



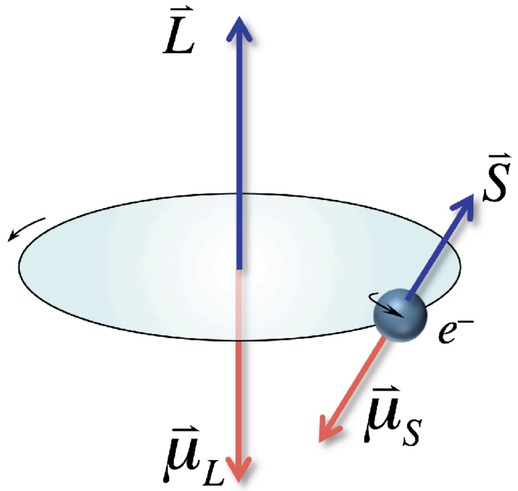
$$\mu_S = g_e \beta_e \sqrt{s(s+1)}$$

β_e = Bohr magneton
g_e = free electron g-value
s = spin angular momentum quantum number

The magnetic moment of a bound electron is determined by its total angular momentum ***J***

$$\mu = g\beta_e \sqrt{J(J+1)}$$

In atoms and molecules the electrons have both orbital and spin angular momentum. Each of these generates a magnetic dipole moment.



$$\mu_L = \beta_e \sqrt{l(l+1)}$$

$$\mu_S = g_e \beta_e \sqrt{s(s+1)}$$

The g-value depends on the spin-orbit coupling:

Examples.

Cu(II) in $Cu(acac)_2$	g=2.13
Ti(III) ions in solid TiO_2	g=1.96

TABLE 28.1 Parameters for Spin-Active Nuclei

Nucleus	Isotopic Abundance (%)	Spin	Nuclear g Factor g_N	Magnetogyric Ratio $\gamma/10^7$ (rad $T^{-1} s^{-1}$)
^1H	99.985	1/2	5.5854	26.75
^{13}C	1.108	1/2	1.4042	6.73
^{31}P	100	1/2	2.2610	10.84
^2H	0.015	1	0.8574	4.11
^{14}N	99.63	1	0.4036	1.93

$$\frac{m_p}{m_e} = \frac{1.67 \times 10^{-24}\ g}{9.1 \times 10^{-28}\ g} \sim 1835$$

$g_e = 2.0023$, $g_N = 5.5854$

$\beta_e = 9.274 \times 10^{-24}$ J/T

$\beta_N = 5.0504 \times 10^{-27}$ J/T

$\therefore \frac{\mu_e}{\mu_N} \sim 658 \Rightarrow \mu_e \cong 658\,\mu_N$

↪ better sensitivity per spin.

- $\hat{S}_z |\alpha\rangle = +\frac{1}{2} |\alpha\rangle$

  $\hat{S}_z |\beta\rangle = -\frac{1}{2} |\beta\rangle$

- m_s : Electron spin Q.N.

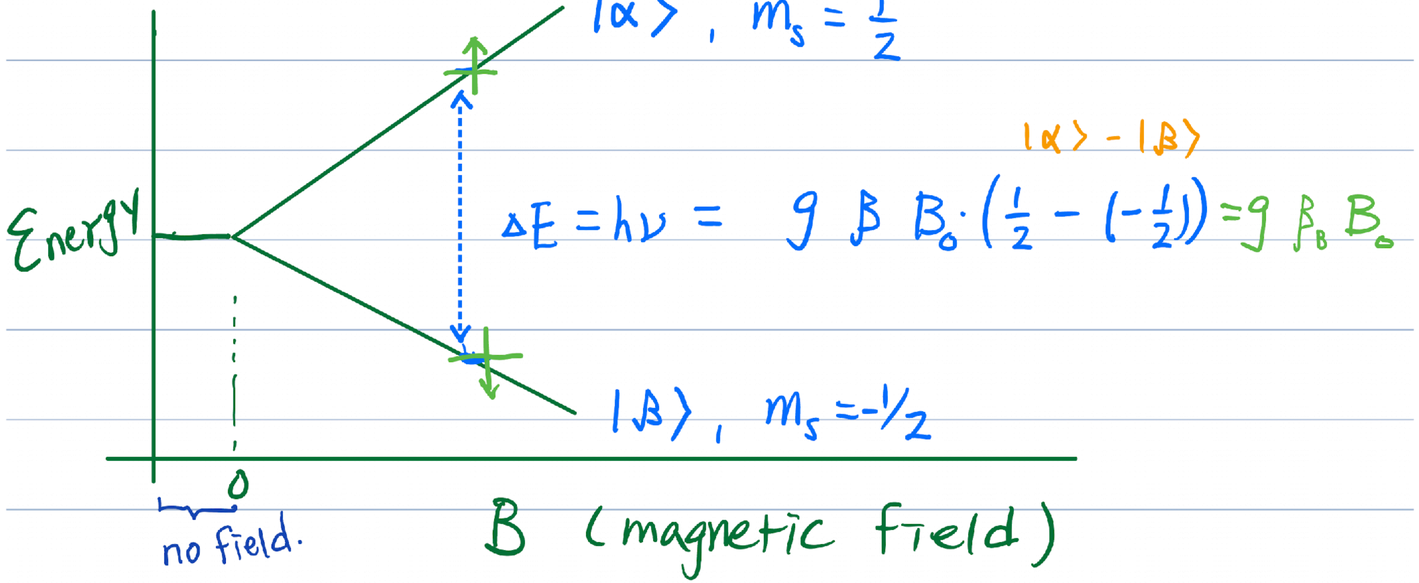
  e.g. $S = \frac{1}{2} \Rightarrow m_s = \pm 1/2$

- WHEN WE APPLY A STEADY MAGNETIC FIELD B ON A SPIN, THE INTERACTION BETWEEN B AND μ CAN BE EXPRESSED IN TERMS OF HAMILTONIAN:

$$\hat{\mathcal{H}} = -\vec{\mu} \cdot \vec{B}_0$$

→ If we only consider B (or H) in z direction,

$$\langle \hat{\mathcal{H}} \rangle = E = \gamma \hbar B_0 m_s = g \beta B_0 m_s$$



(Zeeman effect)

→ In order to induce transition between $|\alpha\rangle$ and $|\beta\rangle$, we apply an oscillating electromagnetic field to provide energy of frequency ν to satisfy the resonance condition,

→ In terms of angular frequency ω [rs^{-1}]

$\because \frac{\omega}{2\pi} = \nu \quad \Rightarrow \quad \omega = \gamma B_0 \propto B_0$

↑

Larmor Frequency.

[s^{-1}]. e.g. $\frac{1}{\mu s} \rightarrow MHz$.

Note:

$$2\pi\nu = \frac{g\beta}{\hbar} B_0$$

$$h\nu = g\beta B_0$$

- To induce the transition: tune ω as B_0 Fixed. or vice versa.

→ modern NMR: radio freq. pulse techniques.

e.g. $^1H \Rightarrow 500\ MHz \rightarrow 12\ Tesla.$

e.g. $\frac{B_0(NMR)}{B_0(EPR)} \sim \frac{\omega_N}{\gamma_N} \cdot \frac{\gamma_e}{\omega_e} = 658 \cdot \frac{0.5\,GHz}{9\,GHz} \sim 37 \quad \therefore B_0(EPR) \sim \frac{12}{37} = 0.33$ Tesla

e.g. Why not using NMR source (e.g. 500 MHz) to pump e^- spin ?

ESR and NMR are very different methods!

	electron	***proton***	***ratio***
Rest mass	m_e =9.1094×10^{-28} g	m_p =1.6726×10^{-24} g	5.446×10^{-4}
Charge	e=-4.80286×10^{-10}ESU	e=4.80286×10^{-10}ESU	-1
Spin angular momentum	$h/4\pi$	$h/4\pi$	1
Magnetic dipole moment	$\mu_S=g_e\,\beta_e\boldsymbol{S}$ g_e= 2.002322 $\beta_e=eh/4\pi m_e c$ = 9.274×10^{-24} J/T	$\mu_N=g_N\,\beta_N\boldsymbol{S}$ g_N= 5.5856 $\beta_N=eh/4\pi m_N c$ = 5.0504×10^{-27} J/T	658

$\Rightarrow \mu_e \cong 658\,\mu_N$

↪ better sensitivity per spin.

- ***Frequency:*** Factor 1000 larger in EPR ! *(GHz instead of MHz)*
- ***Relaxation Times:*** Factor 1000 000 smaller in EPR ! *(ns instead of ms)* = much higher technical requirements, but unique sensitivity to molecular motion
- ***Sensitivity :*** Factor 1 000 000 better than in NMR !! *(1nM instead of 1mM)* *An ideal case, though*

1000 (freq)x 658 ~ 10^6

The Basic ESR Experiment (conventional ESR)

- ESR is done from 1 to 300+GHz, up to 2000+ GHz
  - Machines are classified according to their **source frequency** :
    L (1.5), **S** (3.0), **C** (6.0), X (9), **Ku** (17), **K** (23), **Q** (36), **V** (50), **W** (95), **D**(140), **G**(180)
- Use **microwave transmission lines**
- Solid state [**Gunn diode** or DRO] or tube [**klystron**] sources
- **Temperatures** from 4K (heme and non-heme iron) to 310K+ (in vivo/vitro)
- **Sensitivity** : **Increases as (frequency)^2**, but limited by sample size, field homogeneity and component construction problems.
- Practically (at X-band): detect 10^{10} spins, a detectable concentration of ~10^{-9}M. (under the condition of 100 μL)

Band name	Typical frequency ν in GHz	Wavelength λ in mm	Energy $h\nu$ in reciprocal cm	Resonance field B at g = 2 in tesla
L-band	1	300	0.033	0.036
S-band	3	100	0.10	0.11
C-band	6	50	0.20	0.21
X-band	10	30	0.33	0.36
P-band	15	20	0.50	0.54
K-band	24	12.5	0.80	0.86
Q-band	35	8.6	1.2	1.25
U-band	50	6.0	1.7	1.78
V-band	65	4.6	2.2	2.32
E-band	75	4.0	2.5	2.68
W-band	90	3.3	3.0	3.22
F-band	110	2.7	3.7	3.93
D-band	130	2.3	4.3	4.64
G-band	180	1.67	6.0	6.43
J-band	270	1.11	9.0	9.64
No name	600	0.50	20	21.4
No name	1000	0.30	33	35.7

Sensitivity

$$\text{Net Absorption} \propto N_- - N_+$$

The ratio of populations at equilibrium is given by the Boltzmann distribution

$$\frac{N_+}{N_-} = e^{-\Delta E/k_B T} = e^{-g\mu_B B/k_B T}$$

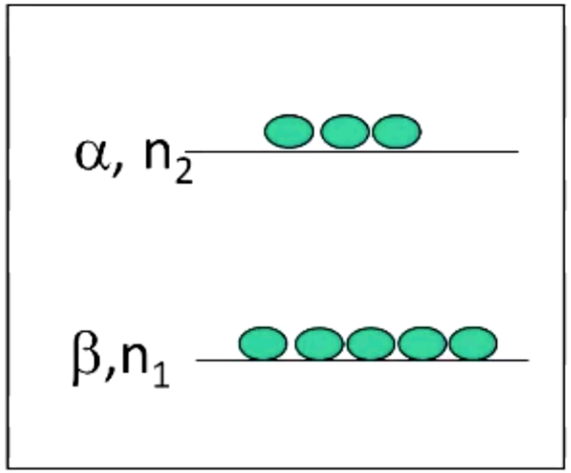
$$\frac{N_+}{N_-} \approx 1 - \frac{g\mu_B B}{k_B T} \longrightarrow N_- - N_+ = N_-\left[1 - \left(1 - \frac{g\mu_B B}{k_B T}\right)\right] = \frac{N g\,\mu_B B}{2\,k_B T}$$

- Net absorption increases with decreasing T, and with increasing magnetic field strength. (ΔN determines the bet absorption.)
- Higher frequency uses smaller waveguides, thus smaller sample volume, canceling the advantages (possibly).
- X-band can detect 10^{12} spins (10^{-12} moles) at room T. (on the order of 0.1 G wide)

The fractional excess population is:

$$\frac{n_2}{n_1} = e^{-\Delta E / kT}, \quad \Delta E = g\beta B_0$$

$$\frac{\Delta n}{n} = \frac{n_1 - n_2}{n_1 + n_2} = \frac{1 - e^{-\Delta E / kT}}{1 + e^{-\Delta E / kT}}$$



$$M_0 \propto n_1 - n_2$$

B_0 (T)

	Δn/n	300 K	77 K	1.5 K
e	9.5 GHz	0.0008	0.002	0.1508
~0.3 ^{1}H	14.5 MHz	0.0012x10^{-3}	0.00395x10^{-3}	0.2320 x10^{-3}
~3	95 GHz	0.0076	0.0253	0.9087
	145 MHz	0.0116x10^{-3}	0.0387x10^{-3}	2.3196x10^{-3}
~9.6	263 GHz	0.0210	0.0700	0.9996
	400 MHz	0.0320x10^{-3}	0.1066 x10^{-3}	6.3989x10^{-3}

• In an Equilibrium Spin System $N = N_\alpha + N_\beta$

→ Boltzmann Law:

$$\frac{N_\beta}{N_\alpha} = \exp(-\Delta E / kT)$$

for $\Delta E = h\nu = g \beta B_0$

$$\Rightarrow \frac{N_\beta}{N_\alpha} \approx 1 - \frac{\Delta E}{kT}$$

$$\therefore N_\alpha - N_\beta = N_\alpha \left[1 - \left(1 - \frac{\Delta E}{kT}\right)\right] = \frac{N_\alpha \Delta E}{kT}$$

$$\approx \frac{N}{2} \frac{\Delta E}{kT} \propto \frac{h\nu_0}{T} \text{ or } \frac{\beta B_0}{T}$$

① $T \downarrow$ or $\nu_0 \uparrow$ or $B_0 \uparrow \Rightarrow \Delta N \uparrow$

② $\nu_e \sim 658\, \nu_{1H}$ (at the same B_0)

a. Calculate the two possible energies of the ^1H nuclear spin in a uniform magnetic field of 5.50 T.

b. Calculate the energy ΔE absorbed in making a transition from the α to the β state. If a transition is made between these levels by the absorption of electromagnetic radiation, what region of the spectrum is used?

c. Calculate the relative populations of these two states in equilibrium at 300. K.

Solution

a. The two energies are given by

$$\begin{aligned} E &= \pm\frac{1}{2} g_N \beta_N B_0 \\ &= \pm\frac{1}{2} \times 5.5854 \times 5.051 \times 10^{-27}\ \text{J/T} \times 5.50\ \text{T} \\ &= \pm 7.76 \times 10^{-26}\ \text{J} \end{aligned}$$

b. The energy difference is given by

$$\begin{aligned} \Delta E &= 2(7.76 \times 10^{-26}\ \text{J}) \\ &= 1.55 \times 10^{-25}\ \text{J} \end{aligned}$$

$\sim 10^{-3}\ \text{cm}^{-1}$

$$\nu = \frac{\Delta E}{h} = \frac{1.55 \times 10^{-25}}{6.626 \times 10^{-34}} = 2.34 \times 10^{8}\ \text{s}^{-1}$$

This is in the range of frequencies called radio frequencies.

c. The relative populations of the two states are given by

$$\frac{n_\beta}{n_\alpha} = \exp\left(-\frac{E_\beta - E_\alpha}{k_B T}\right) = \exp\left(\frac{-2 \times 7.76 \times 10^{-26}\ \text{J}}{1.381 \times 10^{-23}\ \text{J K}^{-1} \times 300.\ \text{K}}\right) = 0.999963$$

$$\frac{n_\alpha - n_\beta}{\frac{1}{2}(n_\beta + n_\alpha)} \approx \frac{(1 - 0.999963)\, n_\alpha}{n_\alpha} = 3.7 \times 10^{-5}$$

or $\frac{n_\alpha - n_\beta}{n_\alpha + n_\beta} \approx \frac{n_\alpha (1 - 0.999963)}{2 n_\alpha} = 1.85 \times 10^{-5}$

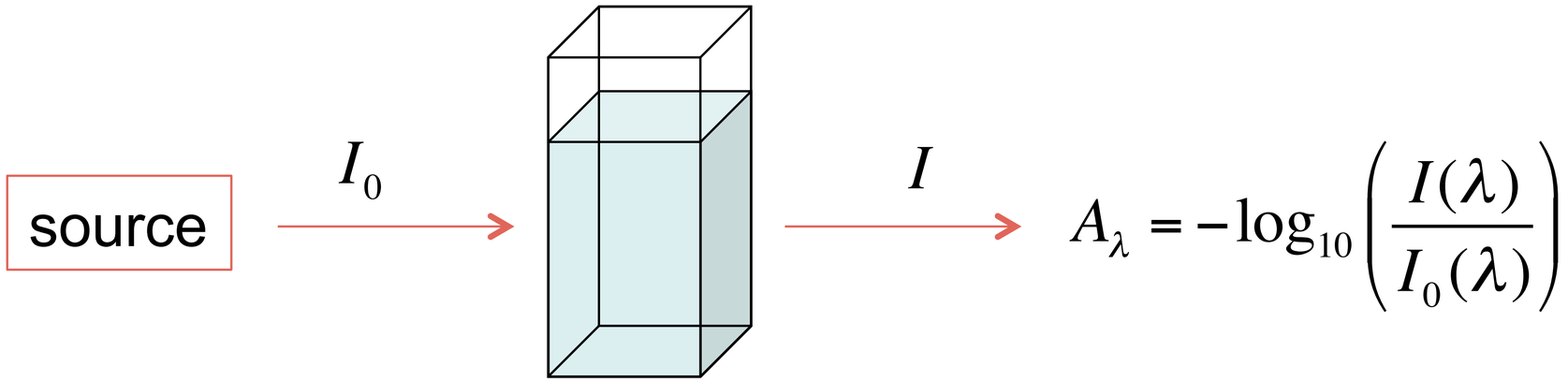
From this result, we see that the populations of the two states are the same to within a few parts per million. Note that observing the appropriate rules for significant figures, we would obtain a ratio of 1.00.

$E_\beta - E_\alpha \ll kT$, but $n_\alpha \approx n_\beta$

$\Rightarrow$ upward transition rate $\approx$ downward.

The EPR Experiment

In most spectroscopic experiments the absorbance is measured as a function of frequency.



In an EPR experiment the absorbance is very weak and this method is only feasible at very high magnetic fields.

Factors that lead to weak EPR signal intensity

The population difference between the spin states is small:

$$N_\alpha / N_\beta = \exp(-g_e \beta_e B_0 / kT)$$

$$\Delta N / N = 10^{-3} \text{ for } B_0 = 330 \text{ mT}$$

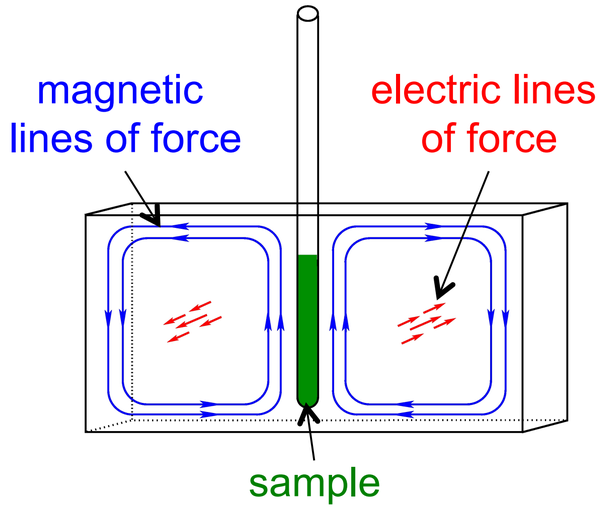
Spin relaxation:

- Fast relaxation causes line broadening
- If the relaxation is slow equalization of the populations can occur if the absorption rate is fast (power saturation)

The EPR Experiment

To overcome the problem of weak signals a resonator is used:

- The sample is placed in a resonant cavity such that it sits in the magnetic component of the resonant microwave field



$$B_1 \propto \sqrt{\frac{P_{\text{in}}\, Q}{\omega\, V_{\text{eff}}}}$$

Many other resonator designs are possible. Each has its advantages

- P_{in}: incident microwave power actually accepted by the resonator
- Q: higher Q → more energy builds up per cycle → larger B_1
- V_{eff}: "effective mode volume" at the sample location (smaller is better)
- ω: higher frequency tends to reduce B_1 for fixed P, Q, V (but other sensitivity factors increase with ω)

The EPR Experiment

The microwaves are usually brought to the resonator using a waveguide

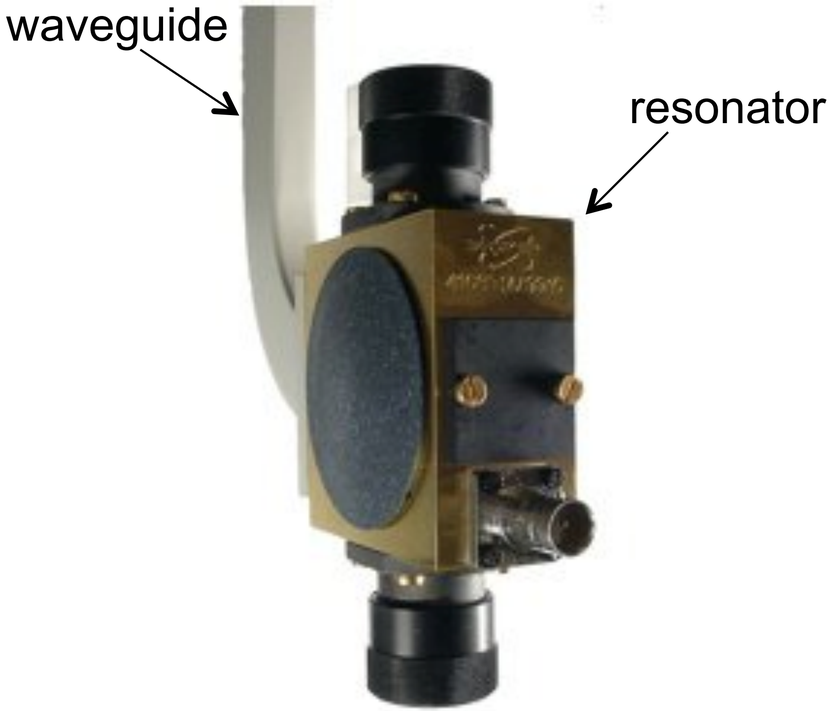
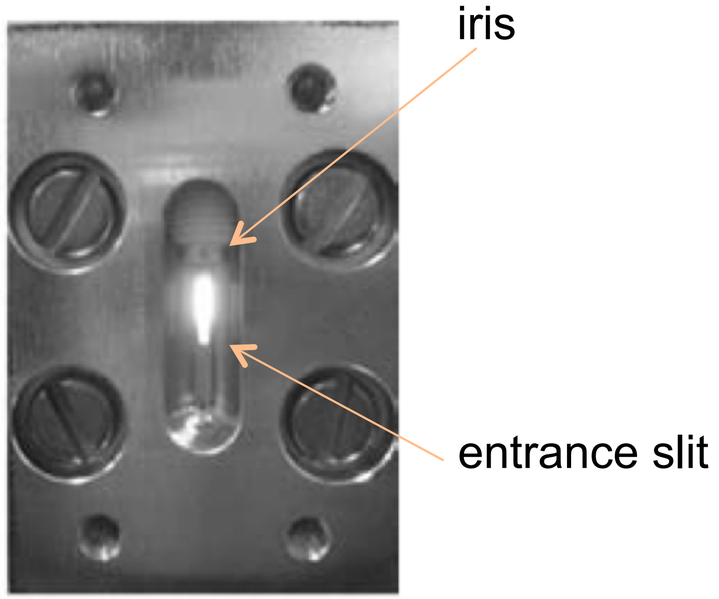


Image: Buker ER 4103TM cylindrical mode resonator
http://www.bruker.com/typo3temp/pics/e_75d2de1d39.jpg

An “iris” is placed at the entrance to the resonator to couple it.

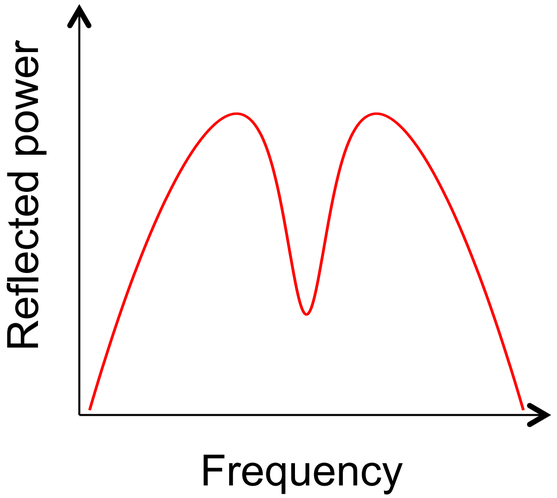


Hagen “Biomolecular EPR Spectroscopy” Fig. 2.6

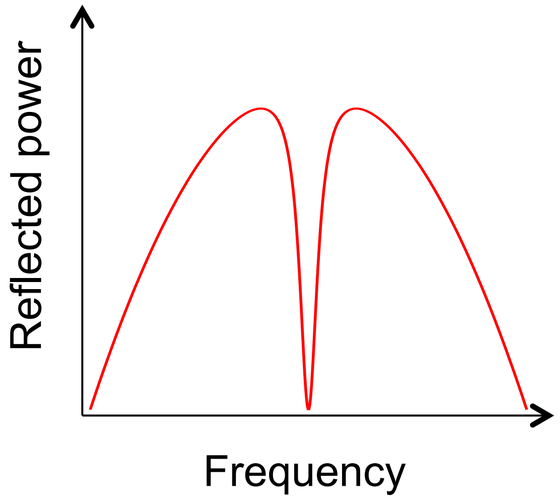
EPR Cavity Coupling

The source is critically coupled to the cavity so no power is reflected.

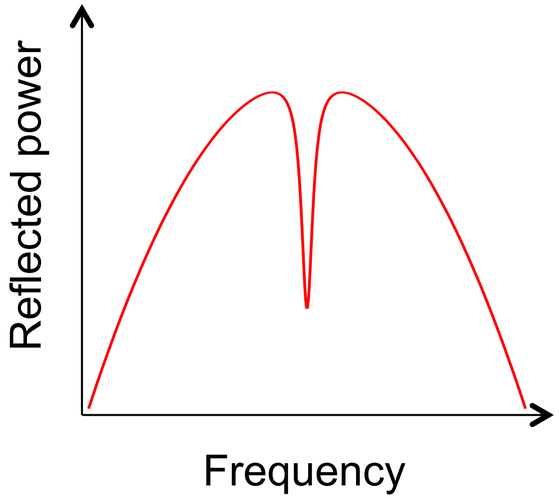
Over coupled



Critically coupled



Under coupled



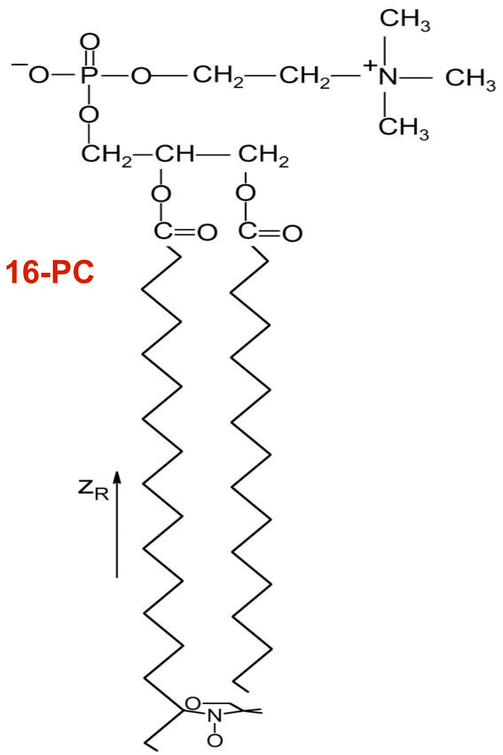
At critical coupling and exactly on resonance:

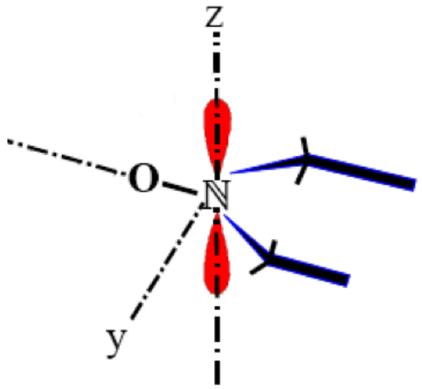
- The reflected power goes to (ideally) zero.
- That means the incident power is not reflected back toward the source.

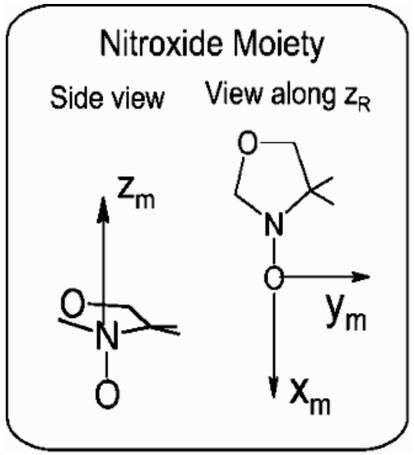
But that does not mean “all the microwave stays in the cavity forever.” It means:
The incident power is accepted by the resonator and then dissipated through losses.

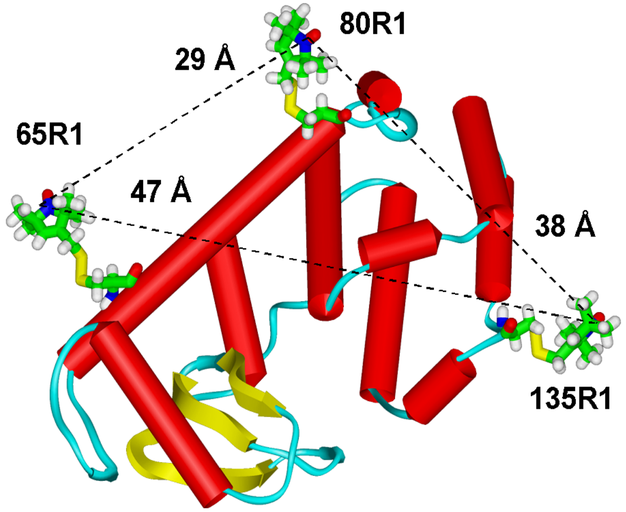
Spin label Electron Spin Resonance

Use spin label to probe the local environment in molecules









I=1/2 (for ^{15}N)
I=1 (for ^{14}N)

S=1/2

Structure and Stability of Nitroxides

Nitroxides are compounds containing the $>N^{\bullet}-O$ group which has an unpaired electron. The unpaired electron is located in a $2p_{\pi}$ (π^*) orbital of the nitrogen and the oxygen atom. Since there is also an N-O σ-bond and two electrons that fill a π-bonding orbital between these atoms, the effective N-O bond order is 1.5 (two center- three electron- bonding, 2c-3e). The structure of this group can be considered as a superposition of two mesomeric structures [34] (resonance)

Scheme 2.1 Mesomeric formula of the nitroxide group.

The contributions of both structures to the ground state may be different, depending on the polarity of the medium and conjugation within the molecule. In apolar solvents both structures have the same weight while polar solvents will favor the charged mesomeric structure, leading to a higher charge density on the nitrogen (which can be observed as increased nitrogen hyperfine splitting in EPR spectra). The electronic structure is influenced in a similar way by π-complex formation with aromatic rings. Depending on the structure, the spin density on oxygen is ρ_o=0.58-0.72 and on nitrogen it is ρ_N=0.42-0.28 [35].The dipole moment of the N-O bond is 2.7 D and the distance is 1.26-1.29 A. The 2c-3e N-O bond has an energy of 419 kJ/mol, midway between the energy of an N-O single bond (230 kJ/mol) and N=O double bond (600 kJ/mol) [36]. As a result of the electron delocalization, nitroxides are relatively stable molecules. The energy gain from delocalization has been calculated as 126 kJ/mol [37].

$$BO_{\pi} = \frac{N_{\text{bonding}} - N_{\text{antibonding}}}{2}$$

In a nitroxide, the document's description corresponds to:

- $N_{\text{bonding}} = 2$ electrons in the π-bonding MO
- $N_{\text{antibonding}} = 1$ electron in the π* MO (this is the unpaired electron)

So:

$$BO_{\pi} = \frac{2-1}{2} = 0.5$$

Total bond order is then the sum of σ and π contributions:

$$BO_{\text{total}} = BO_{\sigma} + BO_{\pi} = 1 + 0.5 = 1.5$$

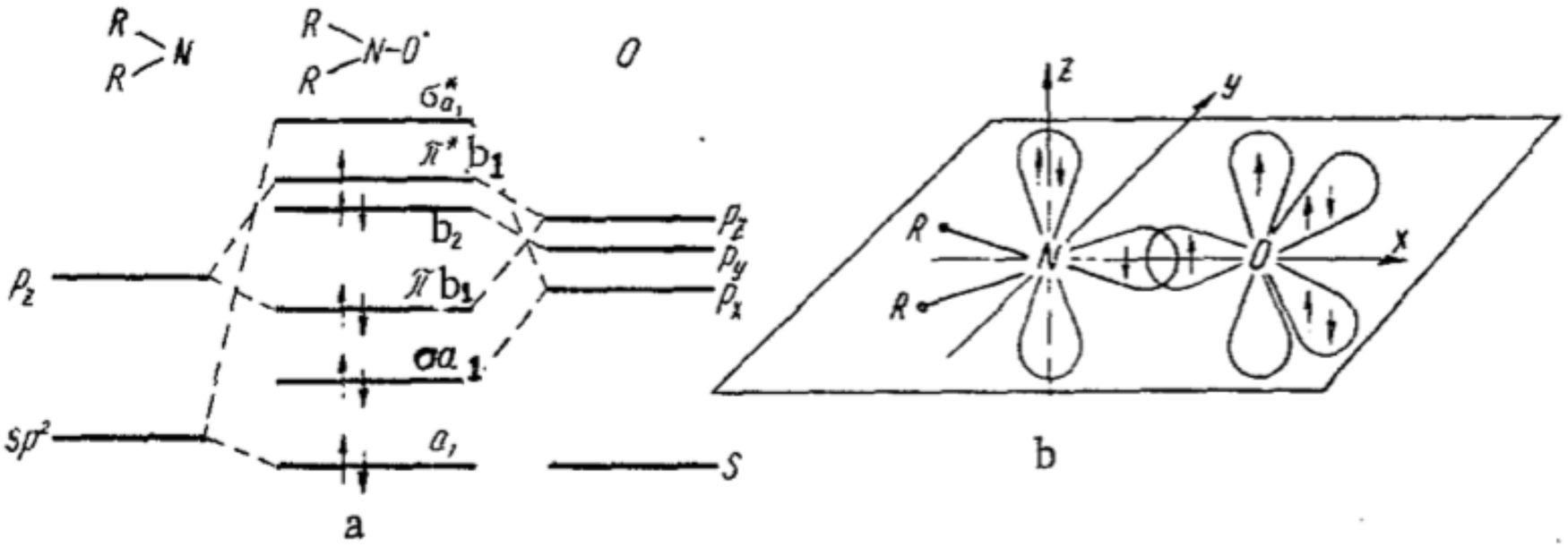
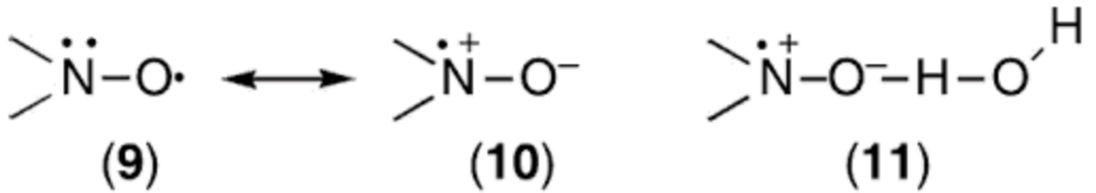


Fig. 2. Schematic representation of the energy levels (a) and electronic configuration (b) of the $\rangle$N—O· fragment of a free nitroxide radical [15].



(9) (10) (11)

derived nitroxides. The nitrogen hfs constants of nitroxides also exhibit a marked solvent dependence, because nitroxides exist as the result of the two mesomeric forms **9** and **10**; in apolar solvents, the two forms have the same weight and thus 50% of the spin density resides on the nitrogen atom and the remaining 50% on the oxygen. In contrast, polar solvents will favor form **10** with respect to **9**; as a result, an increase of the nitrogen splitting is observed, which is particularly evident for solvents where hydrogen bonding is possible (see structure **11**, Block 8.1).

The stability of nitroxides may be attributed to three factors: electron delocalization within the molecule, steric shielding of the paramagnetic center and stability towards disproportionation. If H–atoms in α-position to the NO moiety are available, disproportionation reactions will be favored, leading to diamagnetic substances. Thus, the majority of stable nitroxides are secondary amine N–oxides without α–hydrogen atoms. Bulky substituents on the α–carbon atoms do not only prevent disproportionation but also the tendency towards dimerization, particularly in the solid state. Thus steric hindrance is mainly responsible for kinetic stability. The more pronounced effect for stability is the electronic configuration of the N-O group. General structures for stable nitroxides are shown in Figure 2.9 with the simplest representative di-*tert*-butylnitroxide DTBN (a). The most common derivates stem from six-membered piperidine rings (b) and five-membered pyrrolidine (c), oxazolidine (d) pyrroline (e) rings. Since chemical structures of nitroxides are relatively complex, trivial names formed by abbreviations are usually employed.

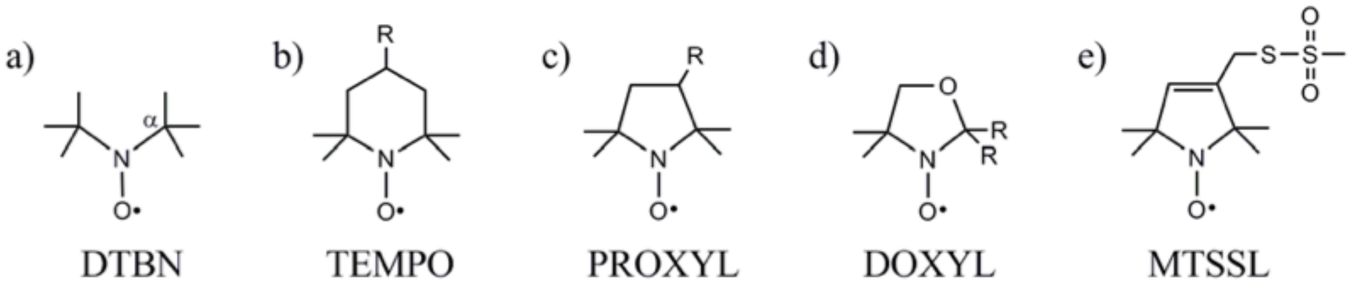


Figure 2.9 The main types of stable nitroxides contain no hydrogen atom at the α-carbon and are cyclic analogs of a) di-*tert*-butyl nitroxide, derivates of b) piperidine, c) pyrrolidine d) oxazolidine and e) pyrroline. Labeling reaction is possible with suitable functional group R. DTBN: di-*tert*-butyl nitroxide, TEMPO: 2,2,6,6-tetramethylpiperidine-*N*-oxyl, PROXYL: 2,2,4,4-tetramethylpyrrolidine-*N*-oxyl, DOXYL: 4,4-dimethyloxazolidine-*N*-oxyl, MTSSL: (*N*-oxyl-2,2,5,5 tetramethylpyrroline-3-methyl)methane thiolsulfonate.

$$Hamiltonian = g\beta HS_z - g_N\beta_N HI_z + a \cdot I \cdot S$$

Electron Zeeman | Nuclear Zeeman | Hyperfine

$$\Delta E = h\nu = g\beta H \qquad \text{(for e-, 1/2-(-1/2)=1)}$$

$$\frac{\Delta E_e}{\Delta E_n} = \frac{g_e\beta_e H}{g_n\beta_n H} \sim 658$$ (more sensitive (per spin) than NMR)

$$\Delta E = h\nu = g\beta H^* \qquad (m_I=0)$$

$$= g\beta H\left(\frac{1}{2} - \left(-\frac{1}{2}\right)\right) + \left(\frac{1}{2}a - \left(-\frac{1}{2}a\right)\right) \qquad (m_I=1)$$

$$= g\beta H + a$$

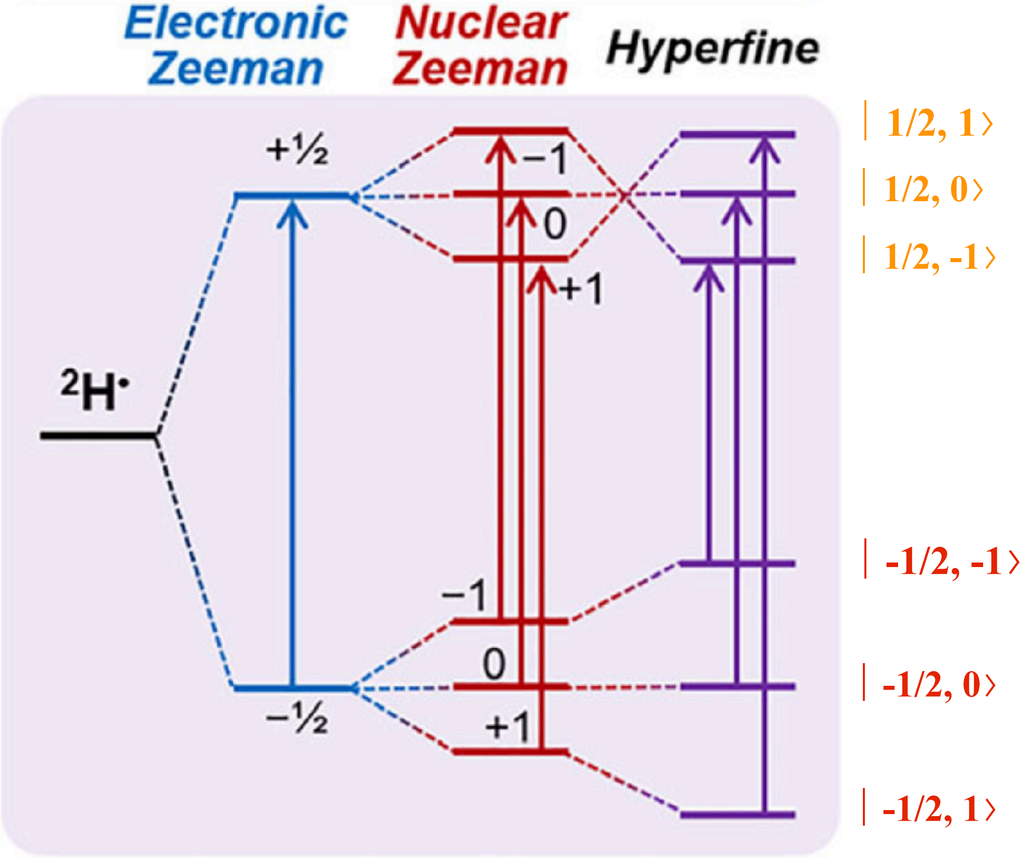
$$H = H^* - \frac{a}{g\beta} \quad (m_I=1) \qquad \left(for \ \ |\alpha_e, m_I = 1\rangle \Leftrightarrow |\beta_e, m_I = 1\rangle\right)$$

$$H = H^* + \frac{a}{g\beta} \quad (m_I=-1) \qquad \left(for \ \ |\alpha_e, m_I = -1\rangle \Leftrightarrow |\beta_e, m_I = -1\rangle\right)$$

Overall:

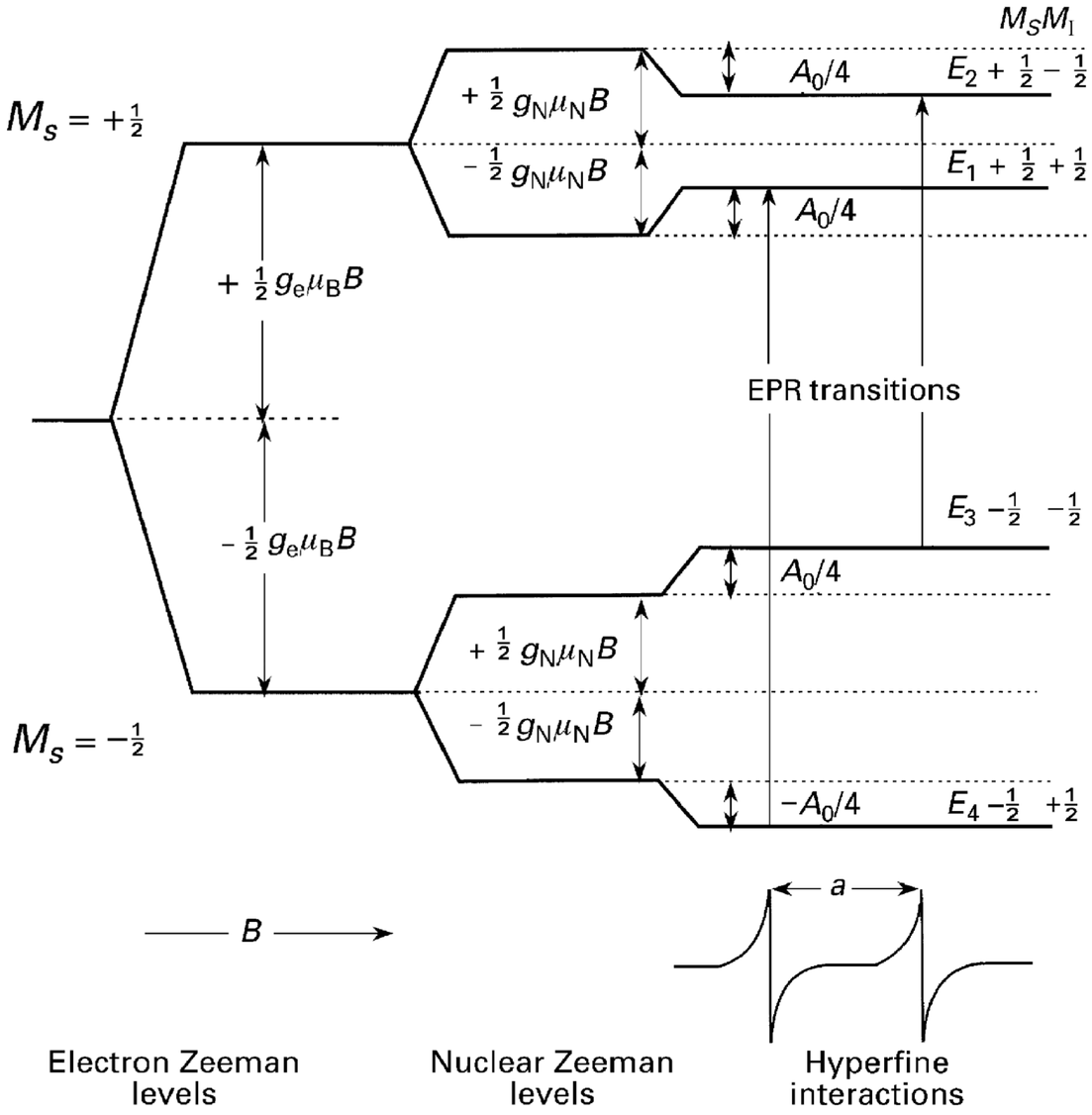
$$H = H^* - \frac{a}{g\beta} m_I$$

in terms of angular freq, ω:
ω=γH for γ=2πgβ/h (gyromagnetic ratio)



$$H = H^* - m_I A \qquad \text{for} \qquad A = \frac{a}{g\beta}$$

[Gauss]



$$E_1 = \tfrac{1}{2} g_e \mu_B B - \tfrac{1}{2} g_N \mu_B B + \tfrac{1}{4} h A_0 \qquad M_S = +\tfrac{1}{2},\ M_I = +\tfrac{1}{2}$$

$$E_2 = \tfrac{1}{2} g_e \mu_B B + \tfrac{1}{2} g_N \mu_N B - \tfrac{1}{4} h A_0 \qquad +\tfrac{1}{2}\ -\tfrac{1}{2}$$

$$E_3 = -\tfrac{1}{2} g_e \mu_B B + \tfrac{1}{2} g_N \mu_N B + \tfrac{1}{4} h A_0 \qquad -\tfrac{1}{2}\ -\tfrac{1}{2}$$

$$E_4 = -\tfrac{1}{2} g_e \mu_B B - \tfrac{1}{2} g_N \mu_N B - \tfrac{1}{4} h A_0 \qquad -\tfrac{1}{2}\ +\tfrac{1}{2}$$

$$\Delta M_I = 0 \quad \text{and} \quad \Delta M_S = \pm 1$$

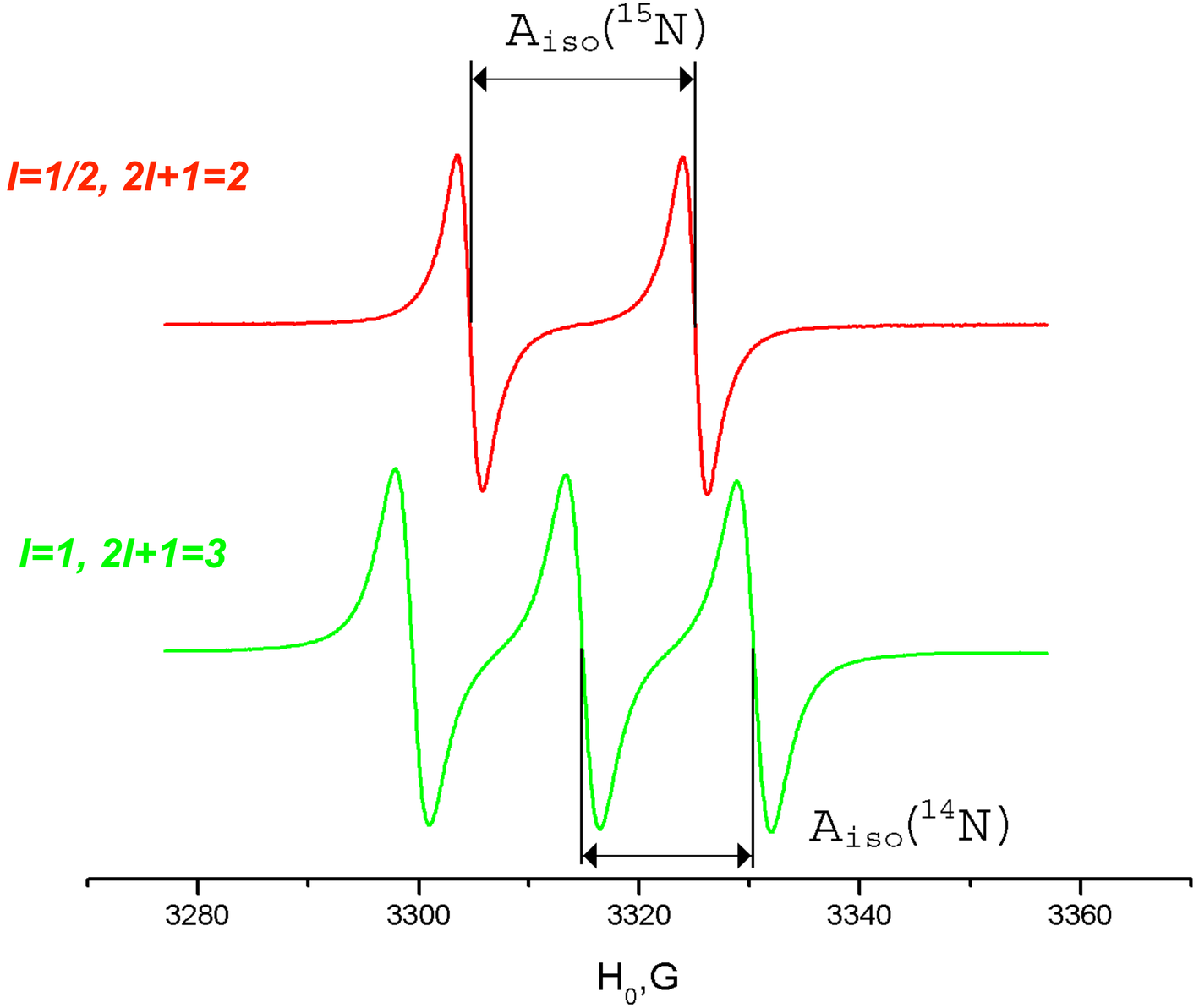
Thus two resonance transitions can occur at

$$\Delta E_A = E_1 - E_4 = g_e \mu_B B + \tfrac{1}{2} h A_0$$

$$\Delta E_B = E_2 - E_3 = g_e \mu_B B - \tfrac{1}{2} h A_0$$

$$B_1 = \frac{h\nu}{g_e \mu_B} - \frac{h A_0}{2 g_e \mu_B} = \frac{h\nu}{g_e \mu_B} - \frac{a}{2}$$

$$B_2 = \frac{h\nu}{g_e \mu_B} + \frac{h A_0}{2 g_e \mu_B} = \frac{h\nu}{g_e \mu_B} + \frac{a}{2}$$


$A_{iso}(^{15}N)$
I=1/2, 2I+1=2
I=1, 2I+1=3
$A_{iso}(^{14}N)$
3280
3300
3320
3340
3360
H_0,G