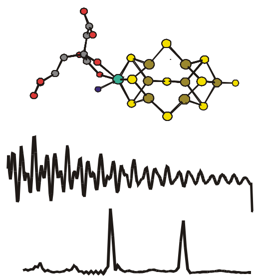
Basic Theory and Applications of EPR

Spying on unpaired electrons – What information can we get?



이홍인

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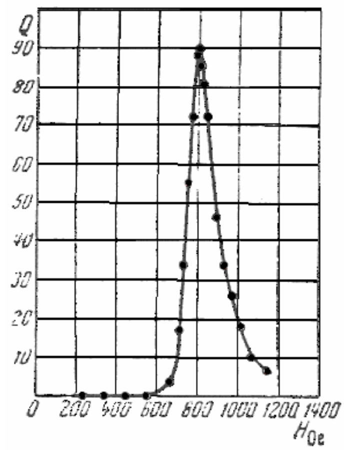
"But don't you see what this implies? It means that there is a fourth degree of freedom for the electron. It means that <u>the electron has spin, that it rotates</u>."

- George Uhlenbeck to Samuel Goudsmit in 1925 on hearing of the Pauli principle -



Zavoisky가 1944년에 얻은 최초의 EPR 스펙트럼
(시료: $CuCl_2.2H_2O$, 자장의 세기: 47.6G, 전자기파의 주파수: 133MHz)

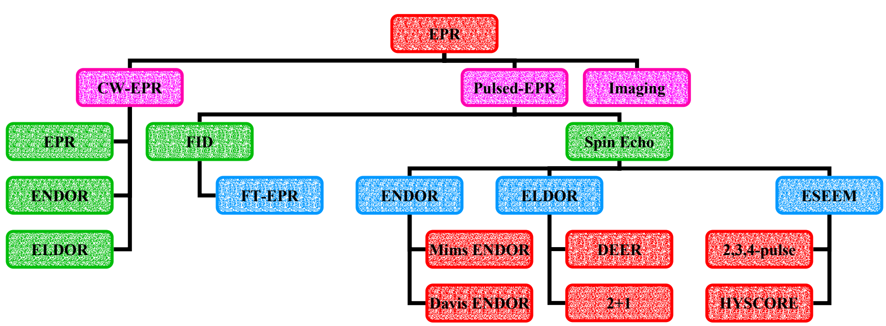
*"**There are spins everywhere**" is now a well known quote amongst EMR spectroscopists. It is born out by the huge list of topics at the right hand side. In some of these the use of EMR techniques is obviously minimal, history for example, in others such as biochemistry EMR's influence has been seminal. In topics such as imaging EMR is advancing at a rapid pace, particularly with recent advances in instrumentation and computing power. **For at least the next ten years we will see EMR following in the footsteps of NMR in instrumentation - moving to higher field/frequency machinery, and with a move from continuous wave (cw) to fourier transform (ft) measurements, possibly even eclipsing the former in time.** This will extend the list of topics even further. Another crumb from the physicist's plate will shortly be available - the use of force balence methods will enable the measurement of single spins on surfaces - the ultimate in detection sensitivity. There are also exciting arguments afoot among physicists concerning the very nature of the electron, (New Scientist, 14th October 2000, pp25), **Humphrey Maris of Brown University says he thinks he can cut an electron in two!** "*

- John Maher -

Applications

Anthropology, Archeology, Biochemistry, Biology, Chemical Reactions, Clusters, Colloids, Coal, Dating, Dosimetry, Electrochemistry, EPR Imaging, Excitons, Ferromagnets, Forensic Science, Gases, Gemmology, Geography, Geology, Glass, History, Inorganic Radicals, Materials Science, Medicine, Metal Atom Chemistry, Metalloproteins, Microscopy, Mineralogy, Organic Radicals, Organometallic Radicals, Paleontology, Photochemistry, Photosynthesis, Point Defects, Polymers, Preservation Science, Quantum Mechanics, Radiation Damage, Semiconductors, Spin Labels, Spin Traps, Transition Metals, Zoology

EPR Methodologies

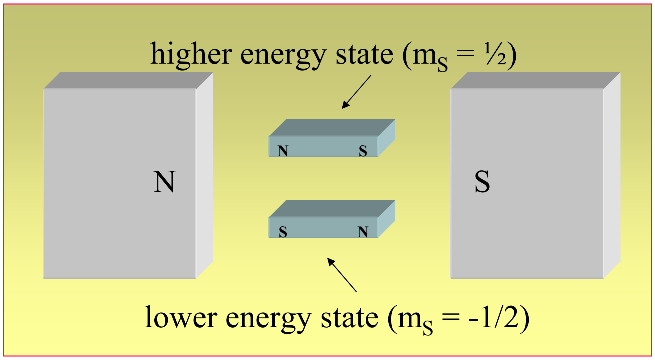


These are just scratches of modern EPR techniques.

What is EPR ?

Electron Pramagnetic Resonance (EPR)
Electron Spin Resonance (ESR)
Electron Magnetic Resonace (EMR)

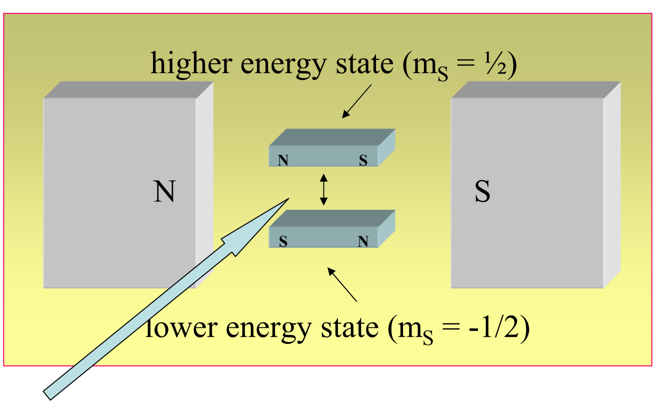
EPR ~ ESR ~ EMR



"Electron Zeeman Interaction"

What is EPR ?

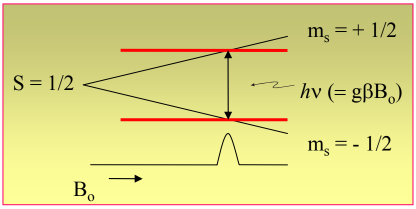
EPR is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field.



hν (microwave)

"Electron Zeeman Interaction"

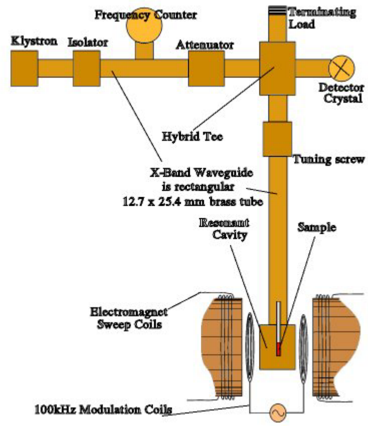
What is EPR ?



- ***h*** Planck's constant (6.626196 x 10^{-27} erg.sec)
- **ν** frequency (GHz or MHz)
- **g** g-factor (approximately 2.0)
- **β** Bohr magneton (9.2741 x 10^{-21} erg.Gauss^{-1})
- **B_o** magnetic field (Gauss or mT)

$$H = \beta \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{H}$$

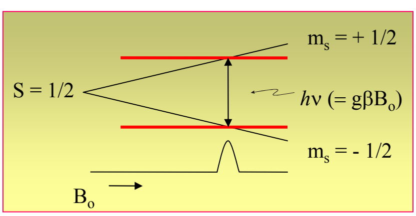
Selection Rule
$\Delta M_S = \pm 1$



Conventional CW EPR spectrometer Arrangement

"Electron Zeeman Interaction"

What is EPR ?



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Bruker EMX EPR spectrometer

"Electron Zeeman Interaction"

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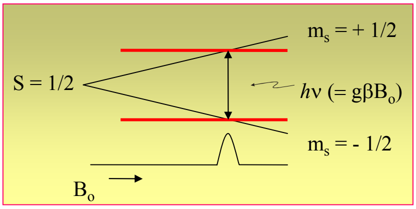

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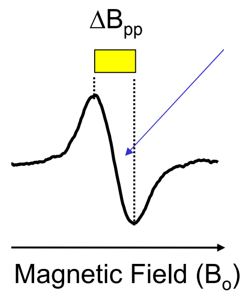


$H = \beta \mathbf{S} \cdot \mathbf{g} \cdot \mathbf{H}$

$\mathbf{g} = h\nu/\beta B_o$

Selection Rule
$\Delta M_S = \pm 1$

"Electron Zeeman Interaction"

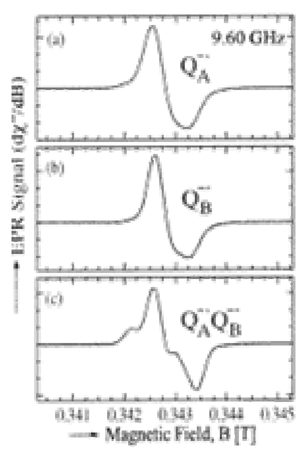
What is g ?

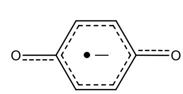
It is an inherent property of a system containing an unpaired spin.

Similar to the ***chemical shift*** observed in an NMR spectrum.

The g value for a single unpaired electron ***(free electron)*** has been calculated and experimentally determined. It is **2.0023192778 ± 0.0000000062 (=** g_e**)**. ***The g value for an S = 1/2 system is usually near g_e, but it is not exactly at g_e. Why not?***

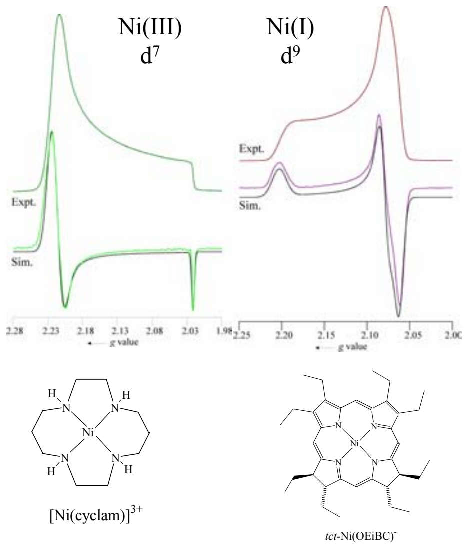
This is due to ***spin orbit*** coupling which determines both the value of ***g and its anisotropy*** (how far the 3 g values are from g_{av}. ***The g value can often be calculated and the value is characteristic for a particular spin system.***

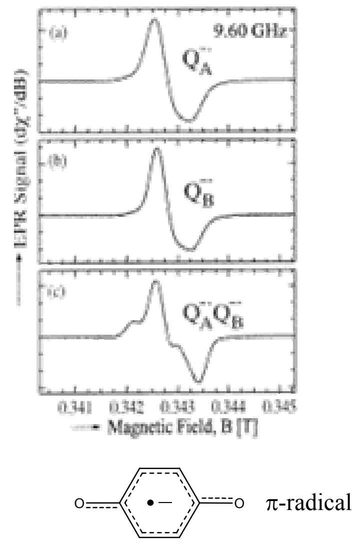




π-radical

What is g ?





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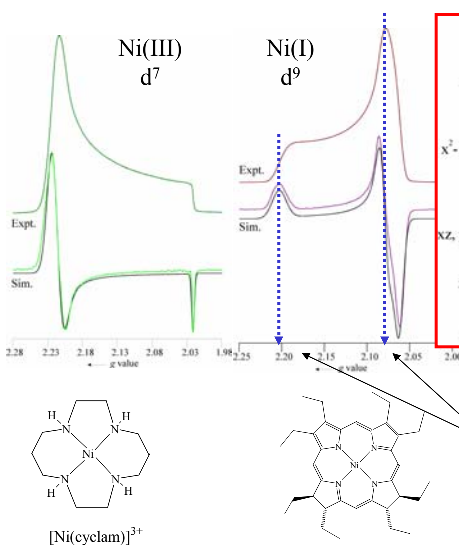
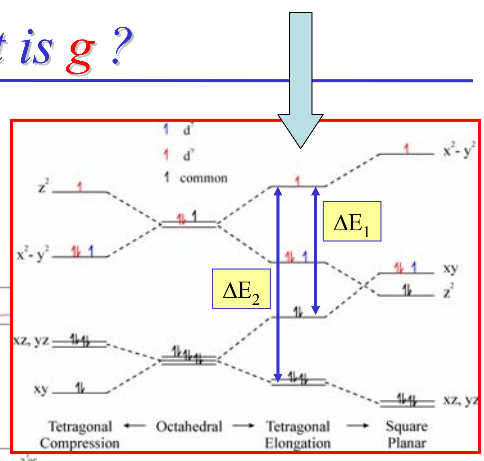




MO Scheme for Low-Spin $d^{7,9}$ Complexes

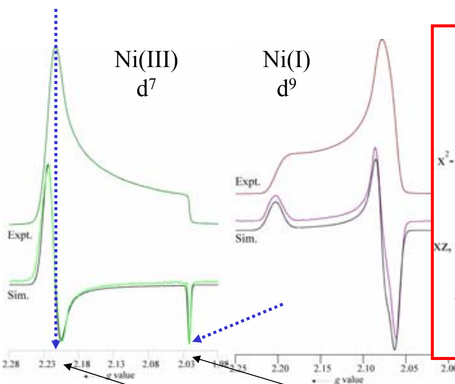
for d^9,

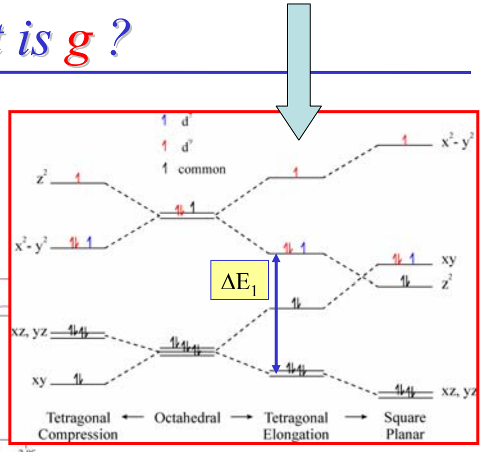
$g_z = g_e [1 + 8 \lambda / \Delta E_1] = g_\parallel$

$g_{x,y} = g_e [1 + 2\lambda / \Delta E_2] = g_\perp$

λ: spin-orbit coupling constant

What is g ?





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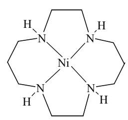
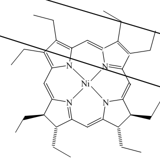


$[Ni(cyclam)]^{3+}$



tct-Ni(OEiBC)$^-$

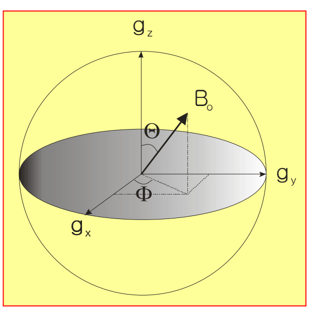
for d^7,

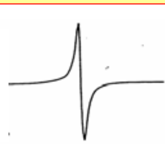
$g_z \sim g_e = g_{\parallel}$

$g_{x,y} = g_e\,[1 + 6\lambda / \Delta E_1] = g_{\perp}$

λ: spin-orbit coupling constant

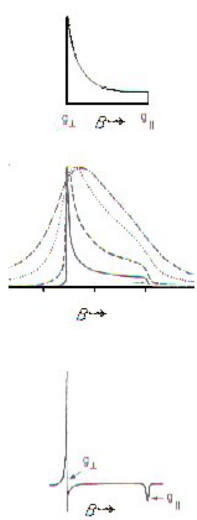
Powder Patterns of EPR Spectra



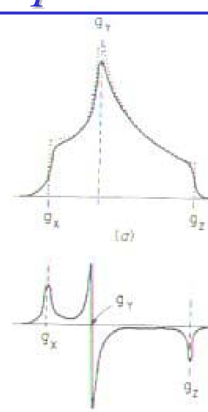


Isotropic ***g***: $g_x = g_y = g_z$

Powder*: randomly oriented samples such as frozen solutions, powders*



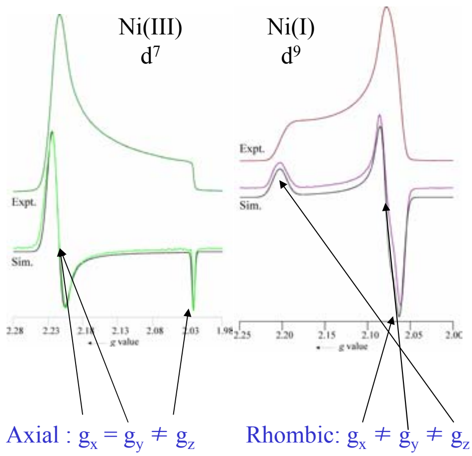
Axial ***g***: $g_x = g_y \neq g_z$

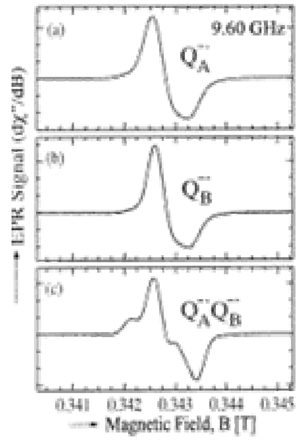


Rhombic ***g***: $g_x \neq g_y \neq g_z$

Even though we talk about ***g_x, g_y, and g_z****, the values should be more properly called* ***g_1, g_2, and g_3*** *unless we have evidence for the nature of the g tensor relative to the* ***molecular axes****.*

Powder Patterns of EPR Spectra



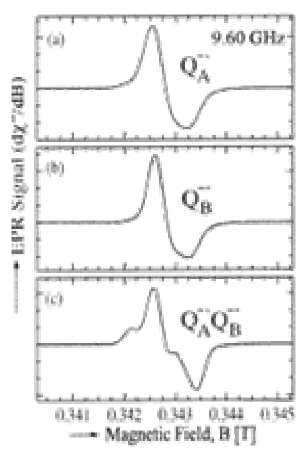


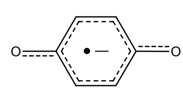
Near isotropic

Multifrequency EPR

Band	Frequency (GHz)	Resonance Field (G)
L	1.1	390
S	4.0	1,430
X	9.75	3,480
Q	34.0	12,100
W	94.0	33,500

Resonance field for g = 2





π-radical

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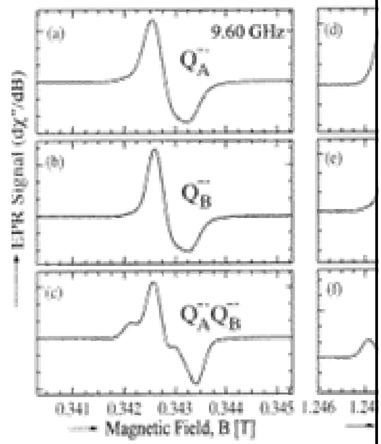

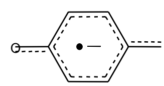

π-radical

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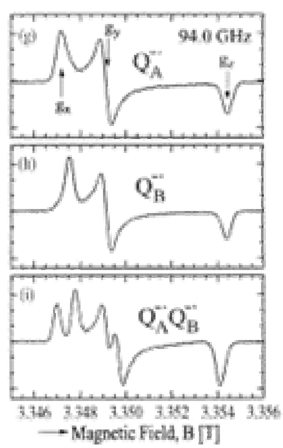
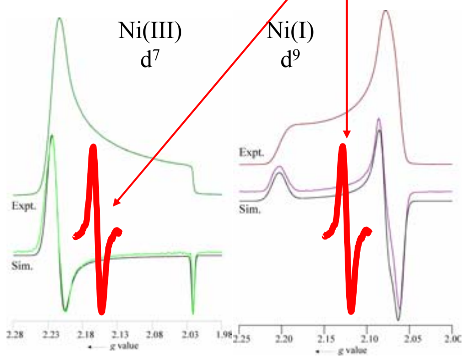
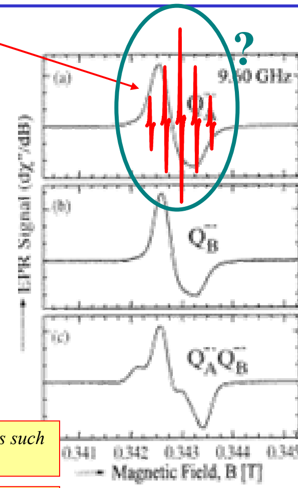
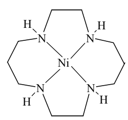


Bruker E680 W,X-band hybrid EPR spectrometer

Solution EPR





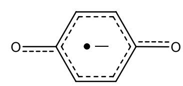


$[Ni(cyclam)]^{3+}$

***Powder**: randomly oriented samples such as frozen solutions, powders*

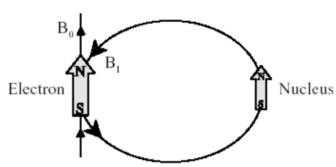
In solution**: when molecules are rapidly tumbling (within microwave time scale), **g-anisotropy is averaged out.

tct-Ni(OEiBC)⁻

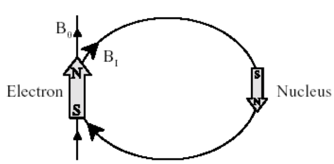


π-radical

Electron spin – Nuclear spin Interaction



$$B_{eff} = B_0 - B_{Ind}$$

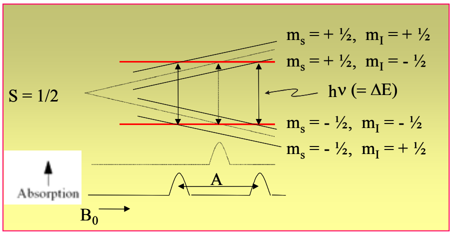


$$B_{eff} = B_0 + B_{Ind}$$

Isotope	Nuclear Spin (*I*)	% Abundance
^{1}H	1/2	99.9
^{2}H	1	0.02
^{12}C	0	98.9
^{13}C	1/2	1.1
^{14}N	1	99.6
^{15}N	1/2	0.37
^{16}O	0	99.8
^{17}O	5/2	0.037
^{32}S	0	95.0
^{33}S	3/2	0.76
^{51}V	7/2	99.8
^{55}Mn	5/2	100
^{56}Fe	0	91.7
^{57}Fe	1/2	2.19
^{59}Co	7/2	100
^{58}Ni & ^{60}Ni	0	68 & 26
^{61}Ni	3/2	1.19
^{63}Cu & ^{65}Cu	3/2	69 & 31
^{95}Mo & ^{97}Mo	5/2	16 & 9
^{183}W	1/2	14.4

"Hyperfine Interaction"

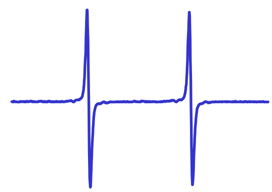
Electron spin – Nuclear spin Interaction



$H = \beta \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{H} + \mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}$

S=½;
I=½
Doublet

hfc (=A)



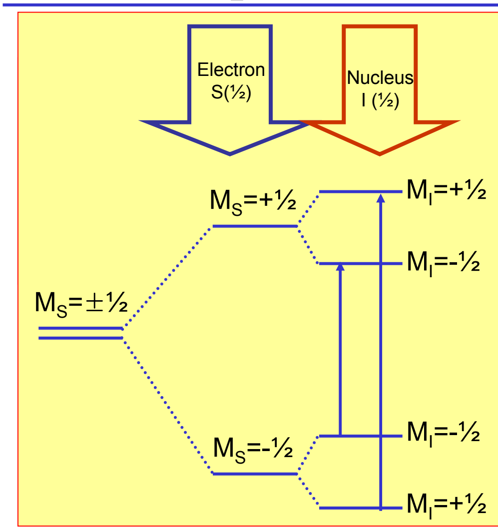
Magnetic Field ⟶

hfc: hyperfine coupling constant

Selection Rule
$\Delta M_S = \pm 1;\ \Delta M_I = 0$

"Hyperfine Interaction"

Electron spin – Nuclear spin Interaction



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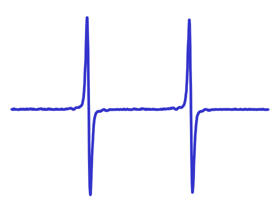

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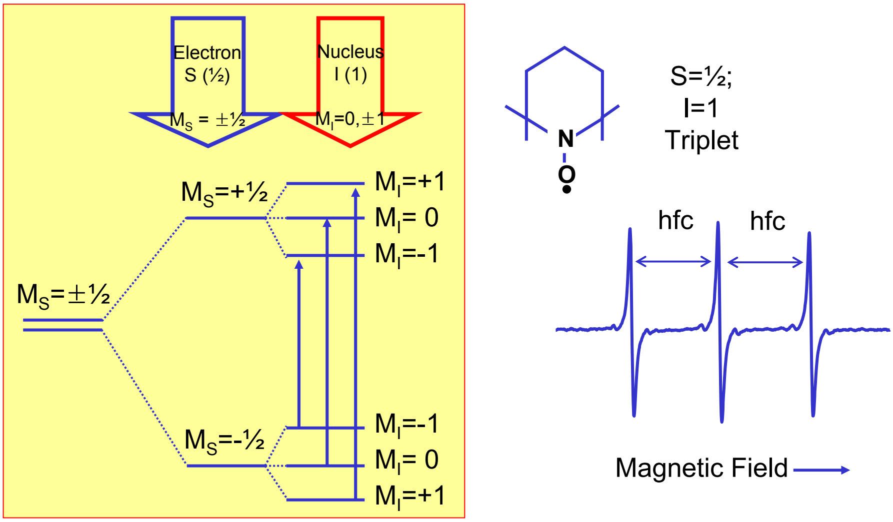

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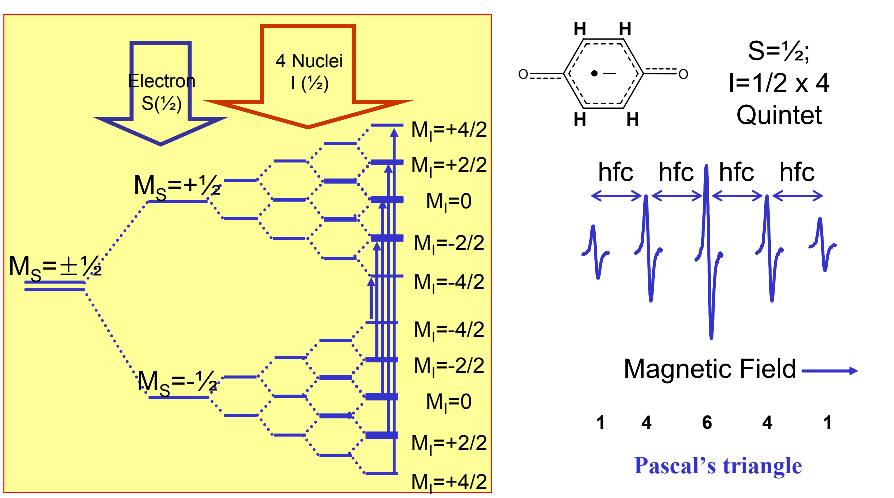
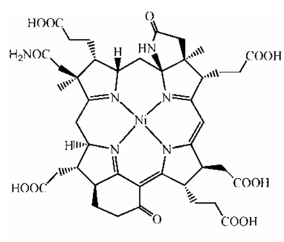


So far, we have considered the cases of hyperfine interactions in solutions or in the samples with very narrow g-anisotropy. How about powder samples?

Electron spin – Nuclear spin Interaction

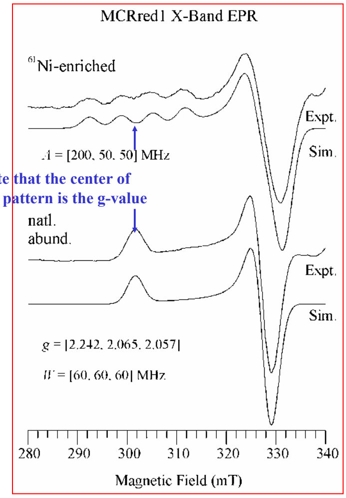
For ^{61}Ni, I = 3/2, so you expect (and see) 4 lines.

But the hyperfine splitting is unresolved in the $g_{\perp}$ direction.



F_{430}

Ni(I)
d^9



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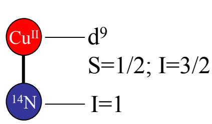
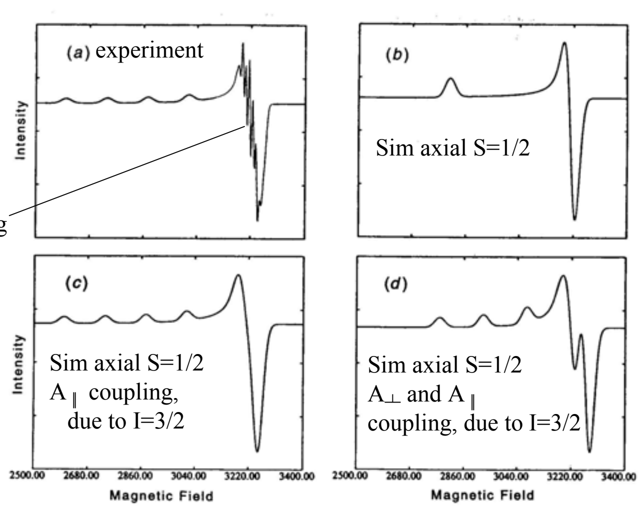
Electron spin – Nuclear spin Interaction

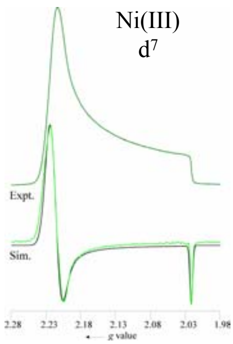
Superhyperfine Splitting

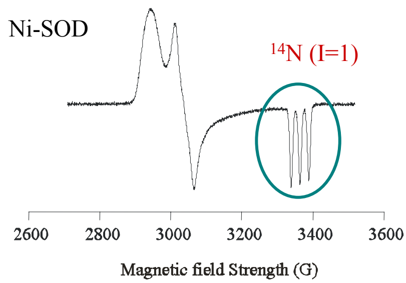


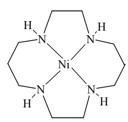


"Hyperfine Interaction"

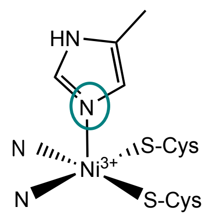
Electron spin – Nuclear spin Interaction







$[Ni(cyclam)]^{3+}$



Electron spin – Electron spin Interaction

When there is ***more than one unpaired electron (S>1/2)****, the interaction between the spins must be considered. This term can be very large. The Hamiltonian for a system with a spin > 1/2 is:* ***$H = D [S_z^2 - 1/3\ S(S+1)\ + E/D\ (S_x^2 - S_y^2)] + g_o \beta S\ H$***

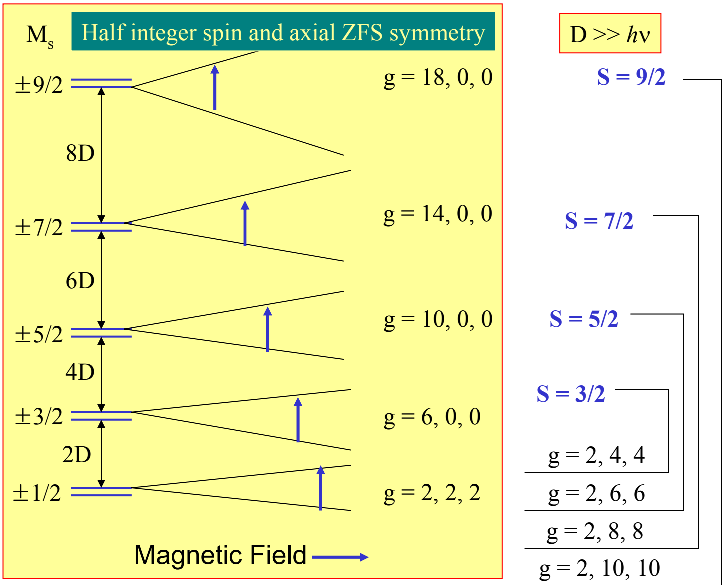
The new terms are D and E/D. D is called the ***zero-field splitting (ZFS) parameter****; E/D is the* ***rhombicity*** *(the ratio between D, the axial splitting parameter, and E, the rhombic splitting parameter, at zero field). The minimum value of E/D is 0 for an axial system. The maximum value is 1/2 for a rhombic system. The strength of the ZFS is determined by the nature of the ligands.*

So for a completely axial system (E/D = 0), $H = D [S_z^2 - 1/3\ S(S+1)] + g_o \beta S\ H$

Consider a case where S = 3/2, i.e., 4 unpaired electrons. These spins can interact to give a total spin moment, referred to as a system spin. There will be four sublevels for m_s, where S_z = -3/2, -1/2, 1/2, and 3/2.

*The energy for the + or -3/2 level will be: D[9/4-1/3(3/2*5/2)]= D[9/4-5/4]= D*
The energy for the + or - 1/2 level will be: -D.

Electron spin – Electron spin Interaction



The magnitude of the ZFS can be determined by EPR. The populations of each of the doublet has a ***Boltzmann distribution****. By lowering the temperature to the same energy range as the ZFS and by measuring the EPR amplitude of each doublet, a value of the ZFS can be obtained.*

Or we can measure ΔMs = ±1 transitions, such as +5/2 ↔ +3/2, at ***higher fields****.*

Interactions measured by EPR

$$H = \beta \mathbf{S \cdot g \cdot H} + \mathbf{S \cdot A \cdot I} + \mathbf{D[S_z^2 - 1/3\, S(S+1) + E/D(S_x^2 - S_y^2)]}$$

Electron Zeeman interaction (interaction of the spin with the applied field)
Spin orbit coupling

Hyperfine and superhyperfine interactions (electron spin-nuclear spin interaction)

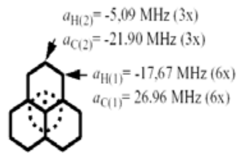
Spin-spin interaction

* Nuclear quadrupole interation can also be detacted.

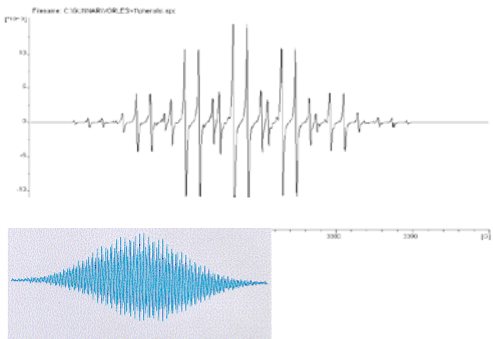
• High sensitivity (<1 μM to 0.1 mM)
• No background
• Definitive and Quantitative

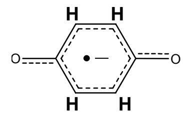
Electron spin – Nuclear spin Interaction

Phenalenyl radical

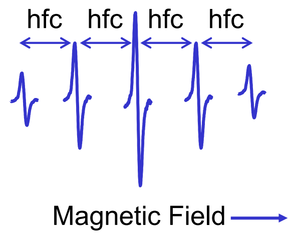


EPR spectrum:



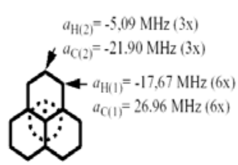


S=½;
I=1/2 x 4
Quintet

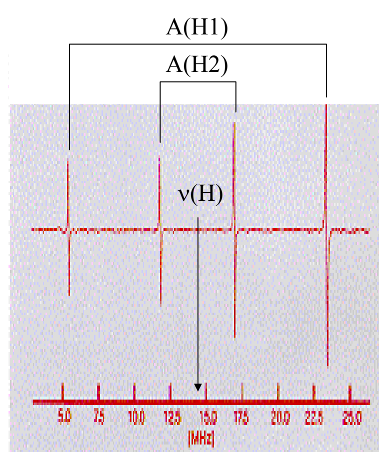


Electron Nuclear Double Resonance (ENDOR)

Phenalenyl radical



EPR spectrum:

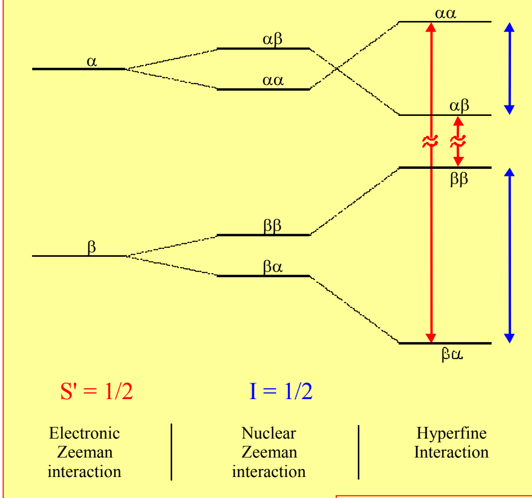


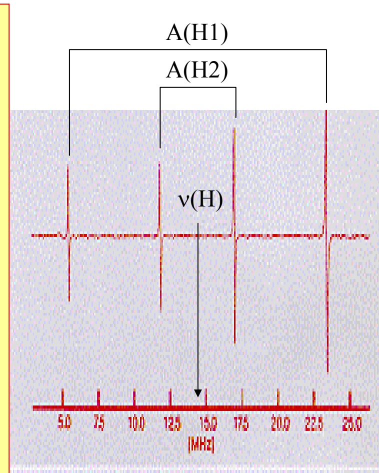
ENDOR Spectrum

$$H = \beta \mathbf{S} \cdot \mathbf{g} \cdot \mathbf{H} + g_n \beta_n \mathbf{I} \cdot \mathbf{H} + \mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I}$$

Electron Nuclear Double Resonance (ENDOR)

ENDOR = EPR-detected NMR



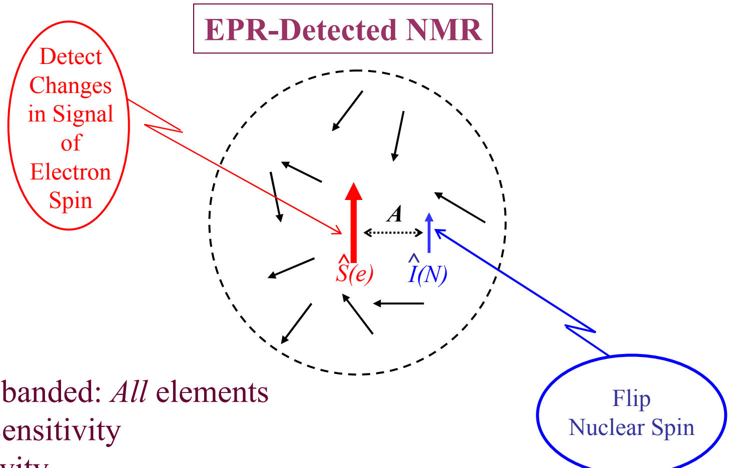


ENDOR Spectrum

$H = \beta\mathbf{S}\cdot\mathbf{g}\cdot\mathbf{H} + g_n\beta_n\mathbf{I}\cdot\mathbf{H} + \mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}$

Electron Nuclear Double Resonance (ENDOR)

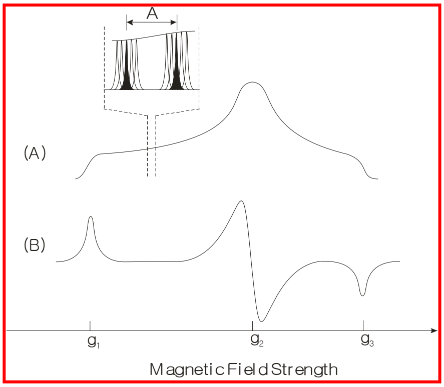
EPR-Detected NMR

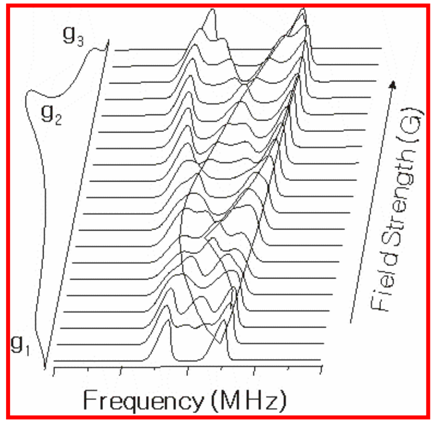


- Broad-banded: *All* elements
- High Sensitivity
- Selectivity

$H = \beta\mathbf{S}\cdot\mathbf{g}\cdot\mathbf{H} + g_n\beta_n\mathbf{I}\cdot\mathbf{H} + \mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}$

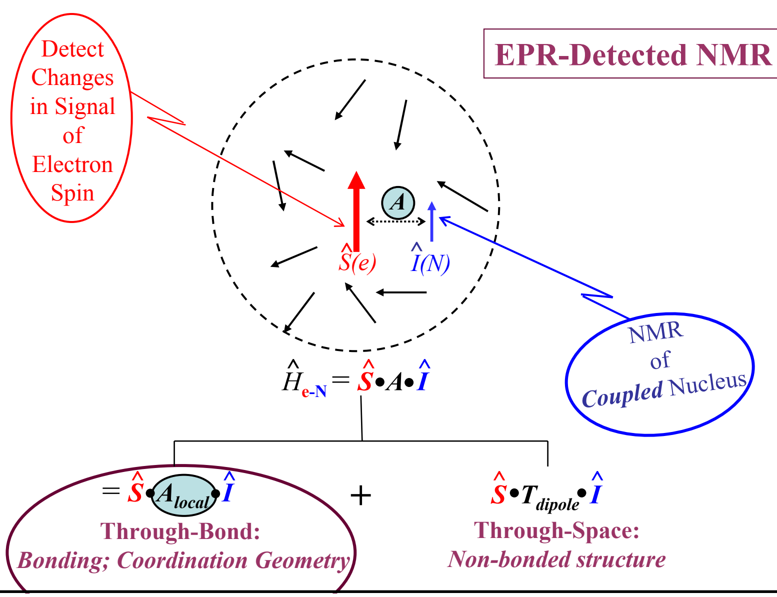
Electron Nuclear Double Resonance (ENDOR)





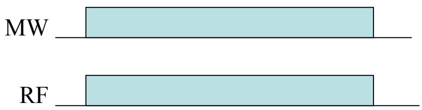
$H = \beta \mathbf{S \cdot g \cdot H} + g_n \beta_n \mathbf{I \cdot H} + \mathbf{S \cdot A \cdot I}$

Electron Nuclear Double Resonance (ENDOR)

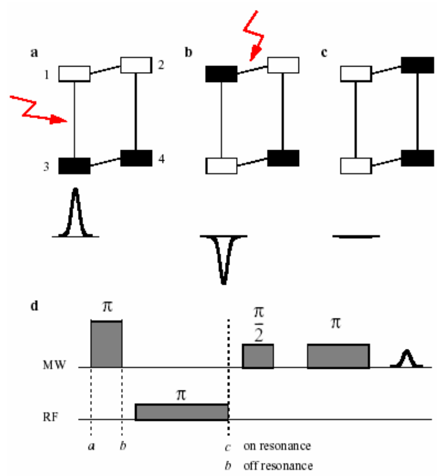


Electron Nuclear Double Resonance (ENDOR)

CW ENDOR

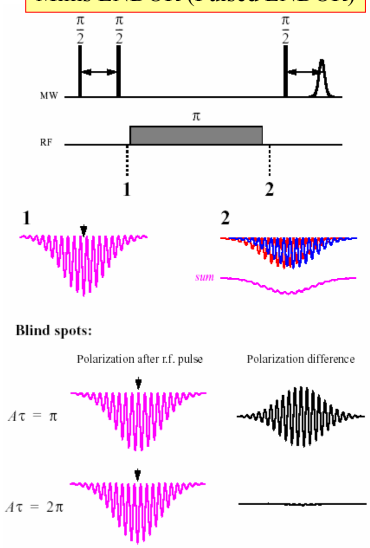


Davise ENDOR (Pulsed ENDOR)

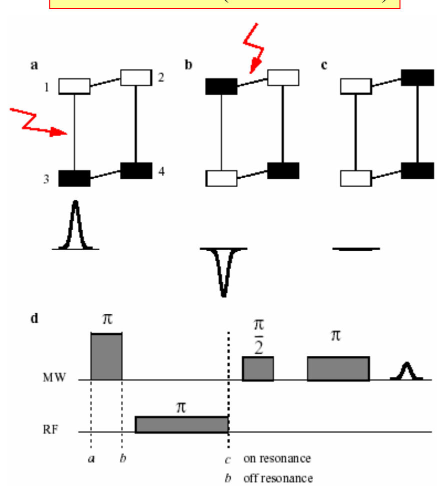


Electron Nuclear Double Resonance (ENDOR)

Mims ENDOR (Pulsed ENDOR)

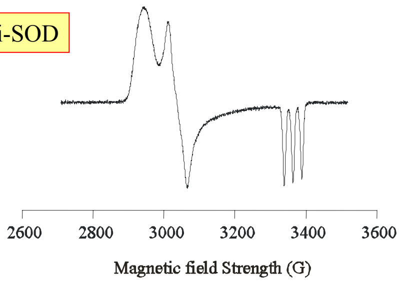


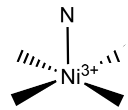
Davise ENDOR (Pulsed ENDOR)

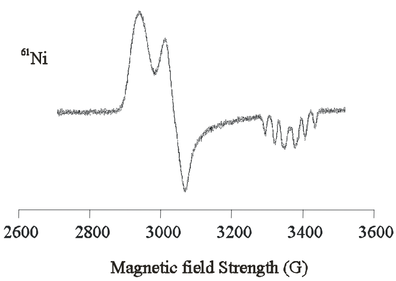


Applications - Metalloenzymes

Ni-SOD

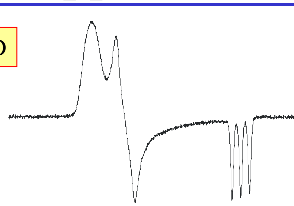


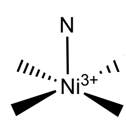




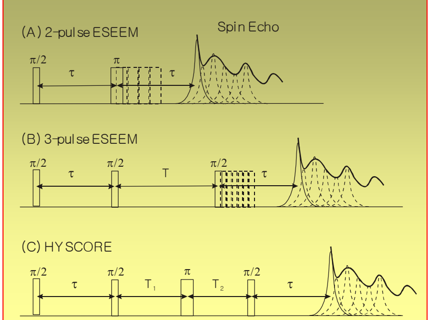
Applications - Metalloenzymes

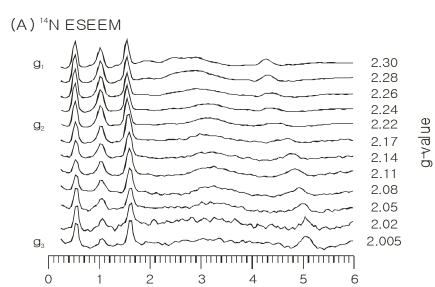
Ni-SOD





ESEEM:EPR-detected NMR w/o RF





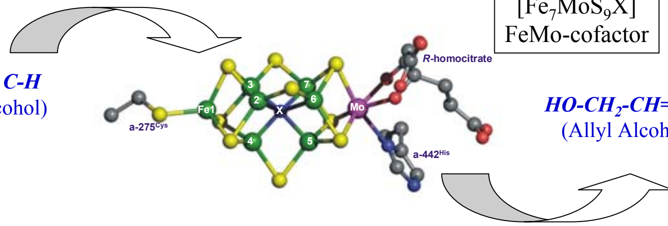
ESEEM (electron spin echo envelope modulation)

Applications - Metalloenzymes

Nitrogenase

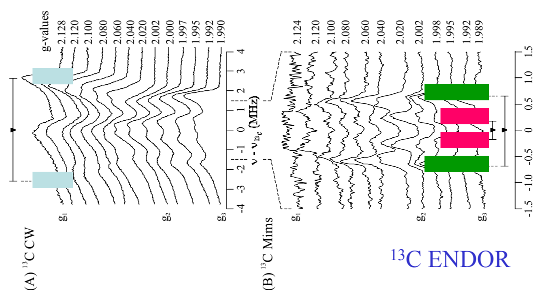
HO-CH$_2$-C≡C-H
(Propargyl Alcohol)

[Fe_7MoS_9X]
FeMo-cofactor



HO-CH$_2$-CH=CH$_2$
(Allyl Alcohol)

2e$^-$ + 2H$^+$



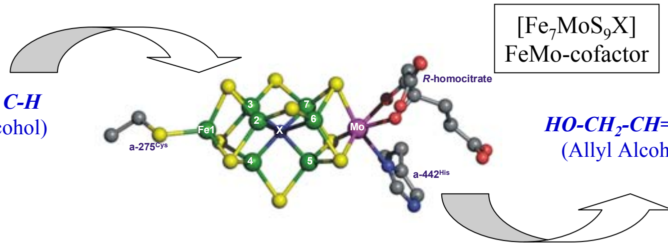
^{13}C ENDOR

Applications - Metalloenzymes

Nitrogenase

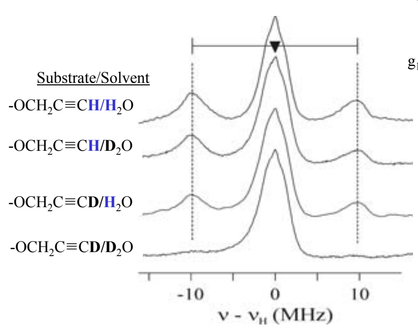
HO-CH$_2$-C≡C-H
(Propargyl Alcohol)

[Fe_7MoS_9X]
FeMo-cofactor

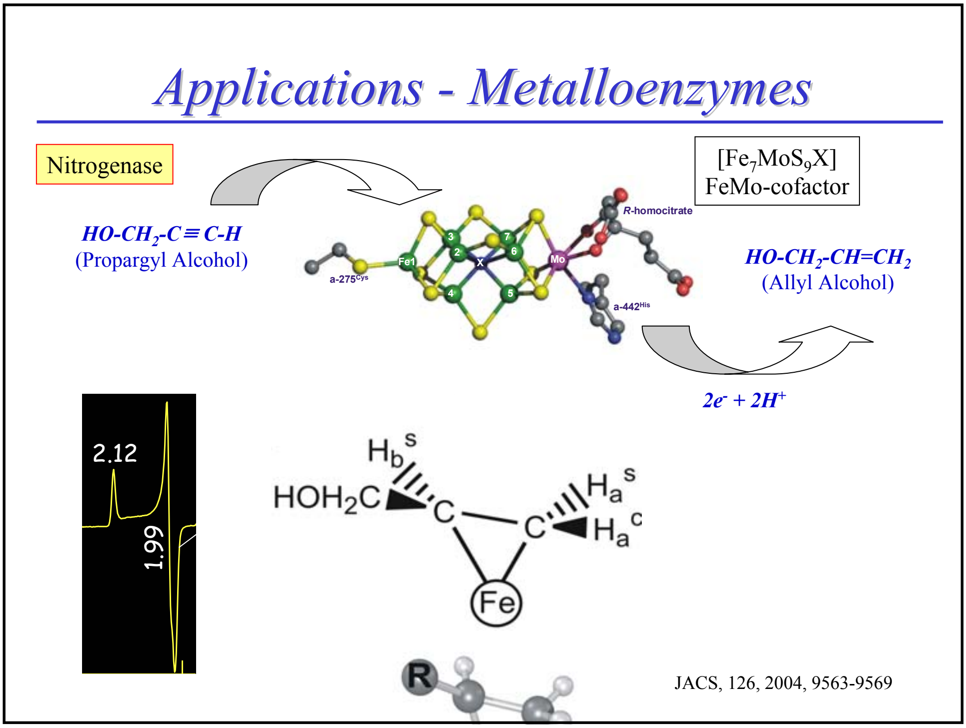


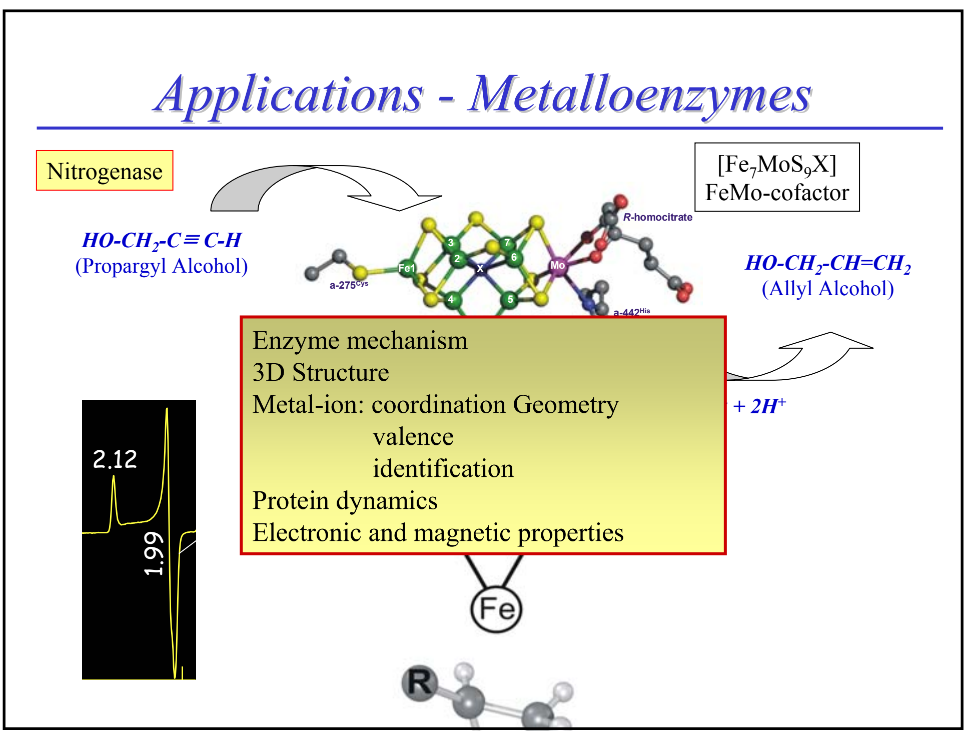
HO-CH$_2$-CH=CH$_2$
(Allyl Alcohol)

2e$^-$ + 2H$^+$



^{1}H ENDOR


Applications - Metalloenzymes
Nitrogenase
$[Fe_7MoS_9X]$
FeMo-cofactor
$HO-CH_2-C\equiv C-H$
(Propargyl Alcohol)
R-homocitrate
Fe1
X
Mo
a-275^{Cys}
a-442^{His}
$HO-CH_2-CH=CH_2$
(Allyl Alcohol)
$2e^- + 2H^+$
2.12
1.99
HOH_2C
H_b^s
H_a^s
H_a^c
C
C
Fe
R
JACS, 126, 2004, 9563-9569

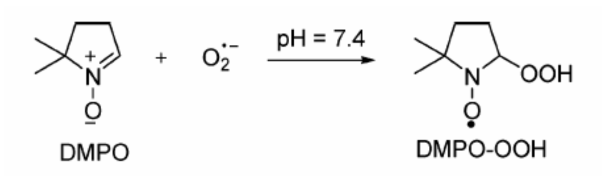

Applications - Metalloenzymes
Nitrogenase
$[Fe_7MoS_9X]$
FeMo-cofactor
$HO-CH_2-C\equiv C-H$
(Propargyl Alcohol)
R-homocitrate
Fe1
X
Mo
a-275^{Cys}
a-442^{His}
$HO-CH_2-CH=CH_2$
(Allyl Alcohol)
Enzyme mechanism
3D Structure
Metal-ion: coordination Geometry
valence
identification
Protein dynamics
Electronic and magnetic properties
$+ 2H^+$
2.12
1.99
Fe
R

Applications – Trapping Free Radicals

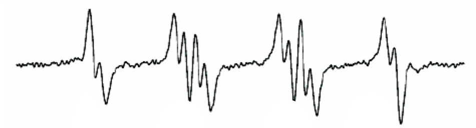
Spin-trapping

Species: superoxide, hydroxyl, alkyl, NO

Spin-trapping agentss: DMPO, PBN, DEPMPO, Fe-DTCs



Oxidative stress
Cell signaling
Photoreaction
:
:

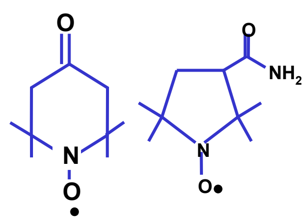


A_N = 1.42 mT, A_H^{β}, = 1.134 mT, and A_H^{γ} = 0.125 mT.

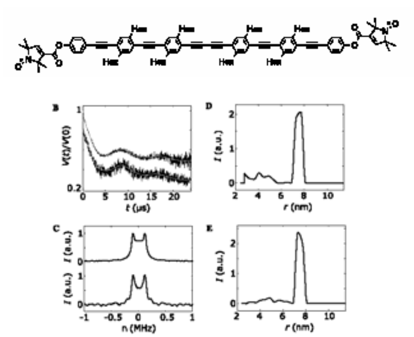
Applications – Distance measurement

Spin-labelling

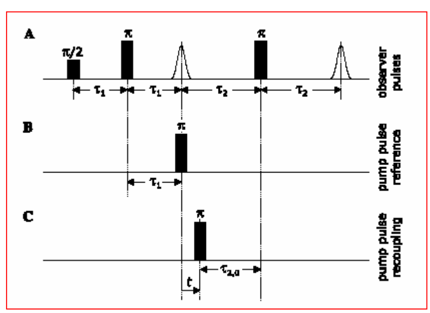
Species: TEMPO….



Membrane protein
::



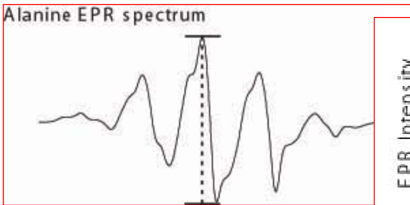
JMR, 169, 2004, 1-12

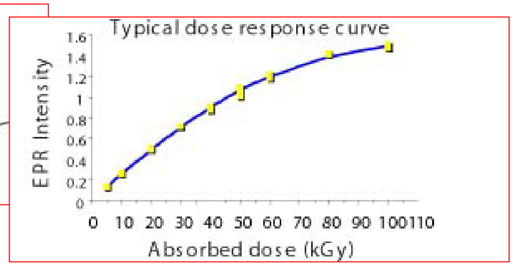


DEER (Double electron electron resonance)

Applications – Radiation Dosimetry

Alanine dosimetry

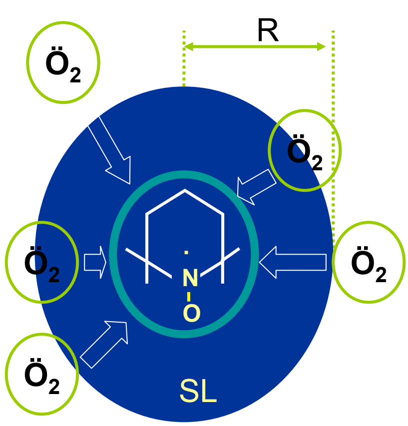




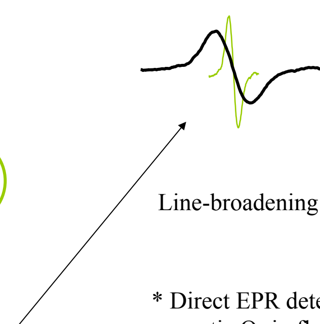
Alanine forms a very stable free radical when subjected to ionizing radiation, such as gamma-ray, e-beam, and X-ray.

Applications – EPR Imaging

Oximetry



Bimolecular collision between SL and oxygen leads to Heisenberg spin exchange



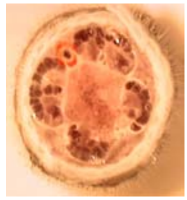
Line-broadening

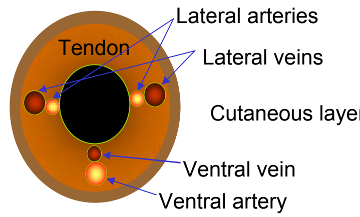
* Direct EPR detection of paramagnetic O_2 in fluid is not possible.

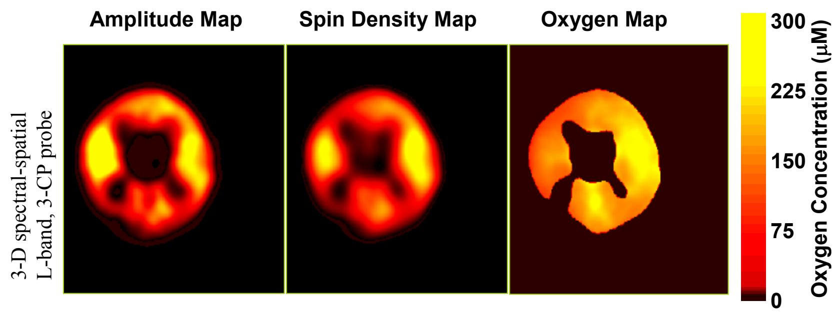
Applications – EPR Imaging

Oximetry

Mapping of
arterio-venous
oxygenation
in a rat tail, *in vivo*







Sendhil Velan, S., Spencer, R.G.S., Zweier, J. L. & Kuppusamy P. Magn. Reson. Med. 43, 804-809 (2000)

Applications

There are spins everywhere.

- 재료 연구 EPR
  - C_{60}
  - 자성체
  - 레이저 재료
  - 반도체 특성
  - 유기 전도체
  - 고분자 특성
  - 광섬유 특성
  - 고온 초전도체
- 물리 연구 EPR
  - 결정장
  - 광학 특성
  - 자화도 측정
  - 결정의 결점
  - 분자의 여기 상태
  - 전•반도체의 전도 전자
  - 전이 금속, 란탄족, 악틴족 이온
- 방사선 연구 EPR
  - 식품의 방사선 효과
  - 고고학
  - 생물체의 방사선 영향
- 생물, 의학 연구 EPR
  - 광합성
  - 조영제
  - 발암 기작
  - 노화 현상
  - 생체내 NO
  - 항산화 작용
  - 생분자의 운동
  - 효소 반응 및 구조
- 화학 연구 EPR
  - 촉매
  - 금속착물
  - 유기금속
  - 스핀 트랩
  - 고분자 반응
  - 산화-환원 반응
  - 라디칼 반응의 속도론

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