

Nukleární magnetická rezonance

Sumární spin subatomárních částic (p,n,e)

0, 1/2, 1, 3/2, 2...

Celkový spin $I \Rightarrow 2I + 1$ orientací

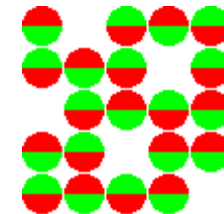
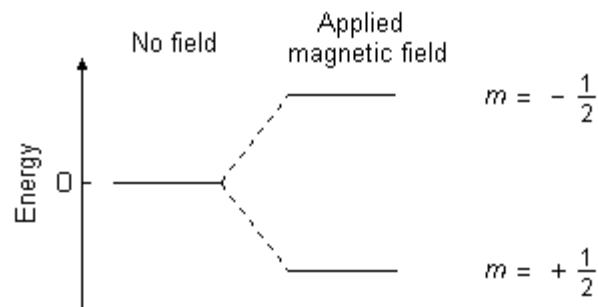
(dojde k rozštěpení v magnetickém poli)

Počet jader v jednotlivých orientacích

$$N_1/N_2 = \exp(-\Delta E/kT)$$

$$U_{\text{NMR}} = 0,99998$$

Energy levels for a nucleus with spin quantum number 1/2

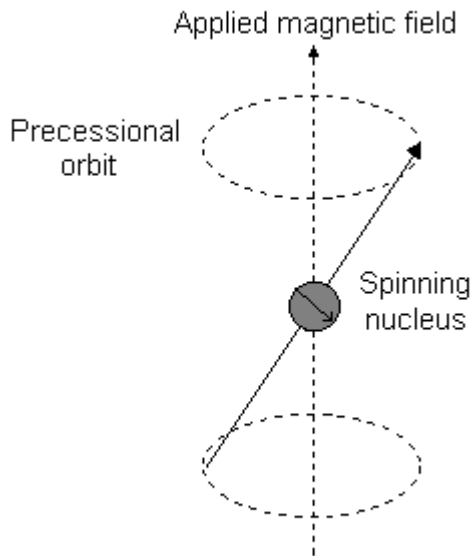


Nukleární magnetická rezonance

Magnetický moment rotujícího jádra μ

$$\mu = \gamma \hbar / 2\pi$$

γ gyromagnetický poměr

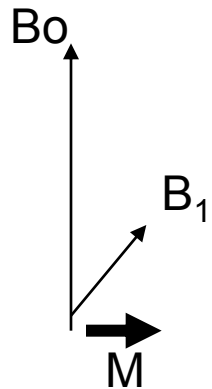
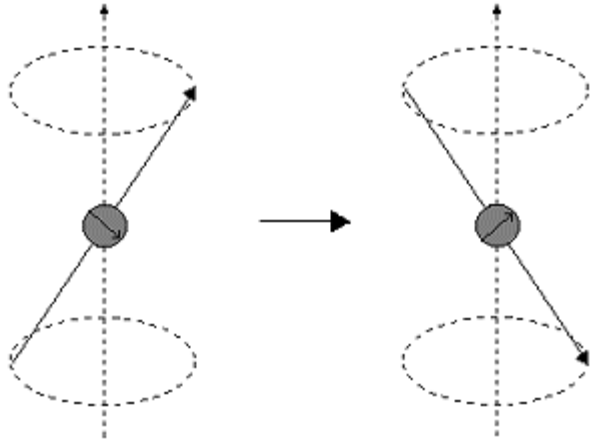


Umístění rotujícího magnetu v elektrickém poli

Frekvence rotace: $\nu_0 = \gamma B_0$

B_0 – indukce mg. pole

Nukleární magnetická rezonance



Rozdíl mezi dvěma energetickými hladinami

$$\Delta E = \gamma h B_0 = h \nu_0$$

pokud působíme elm. polem o frekvenci $\nu_1 = \nu_0$

Dojde k rezonanci a přechodu na vyšší Hladinu

$$\nu_0 = \gamma B_0$$

Nukleární magnetická rezonance (B = 2,3 T)

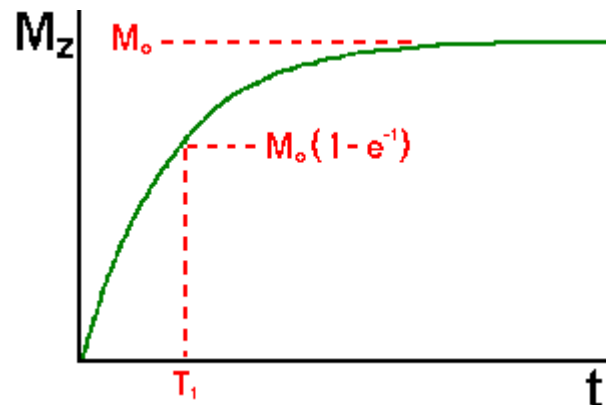
Jádro	I	ν_0 (MHz)	citlivost	% zastoupení
^1H	$\frac{1}{2}$	100	1	99,9
^{13}C	$\frac{1}{2}$	25	0,016	1
^{15}N	1	10	0,001	0,36
^{19}F	$\frac{1}{2}$	94	0,8	100
^{31}P	$\frac{1}{2}$	40	0,067	100
^{17}O	$\frac{5}{2}$	13	0,003	0,04

Nukleární magnetická rezonance

Spin-mřížková relaxace

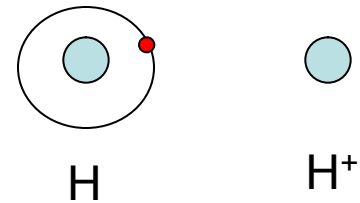
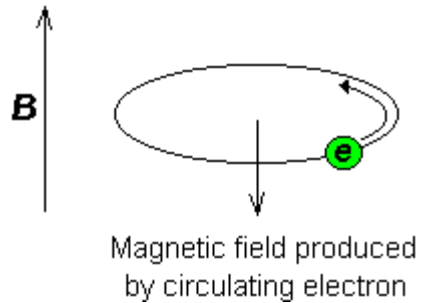
Jádro v excitovaném stavu se zbavuje energie relaxací

Relaxační čas (střední doba života jader v excitovaném stavu) – $T = 10^{-1} - 10^2 \text{s}$



Nukleární magnetická rezonance

Stínění jádra – chemické posuny



$$B = B_0 - \sigma B_0$$

$$\tau = 10 - \sigma$$

τ chemický posun (ppm)

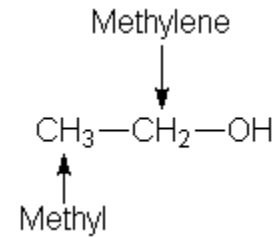
Nukleární magnetická rezonance

Chemické posuny

CH ₃	1
CH ₂	1,5
R ₂ NH	0,5-2
RNH ₂	1,5
ArCH ₃	2,5
-C≡CH	3
Ar-NH ₂	3,5 – 4,5
X-CH ₃	2,5 – 3,5
R-O-CH ₃	4
=C=CH ₂	5
ArH	7-8
RCOH	10
RCOOH	10-12

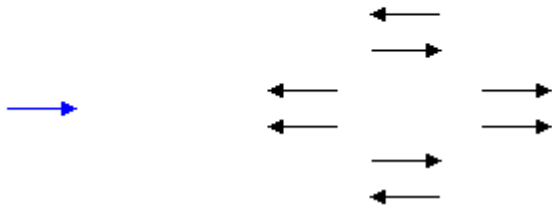
Nukleární magnetická rezonance

Multiplicita signálu – spin-spinová interakce



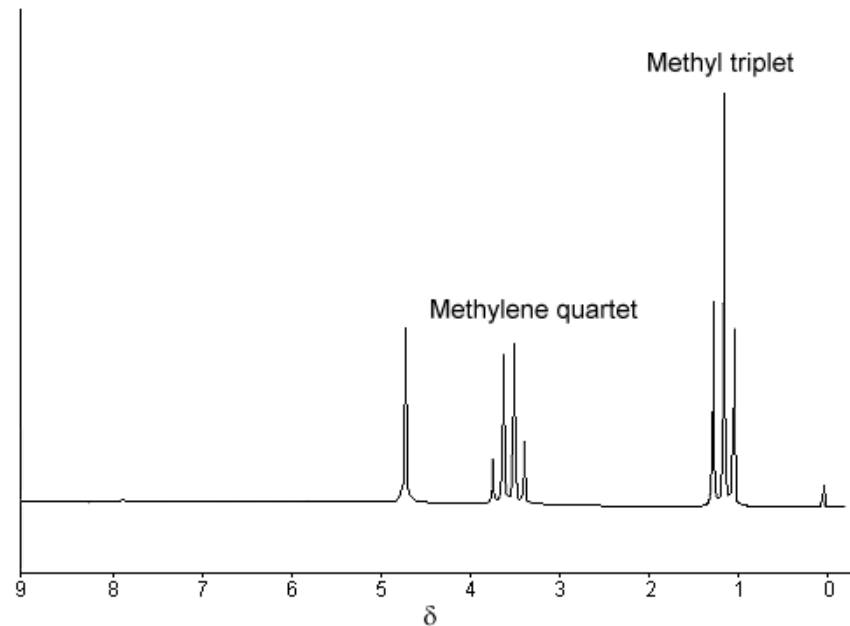
Applied field

Spin orientations of methylene protons



Multiplicita = $n+1$

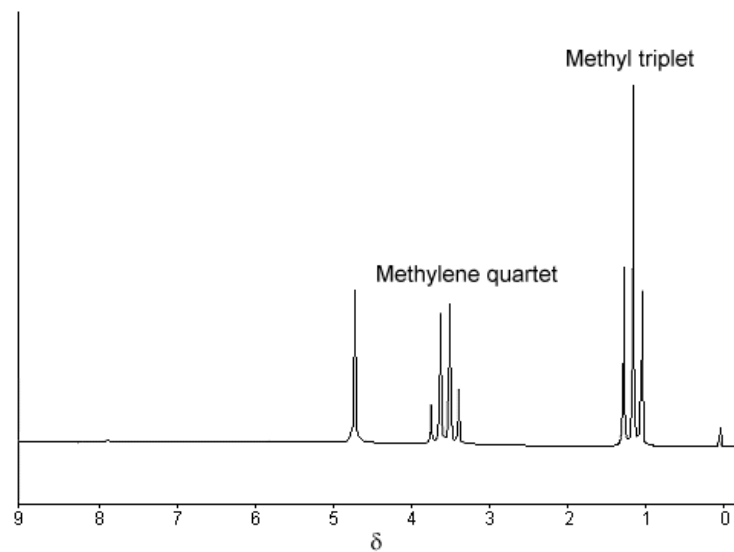
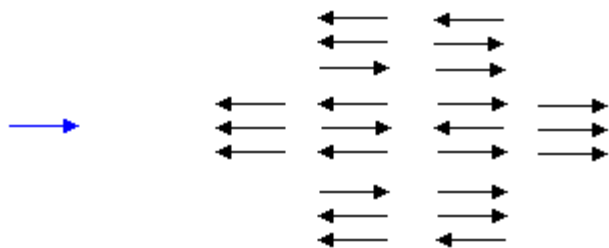
$(x + 1)^n$



Nukleární magnetická rezonance

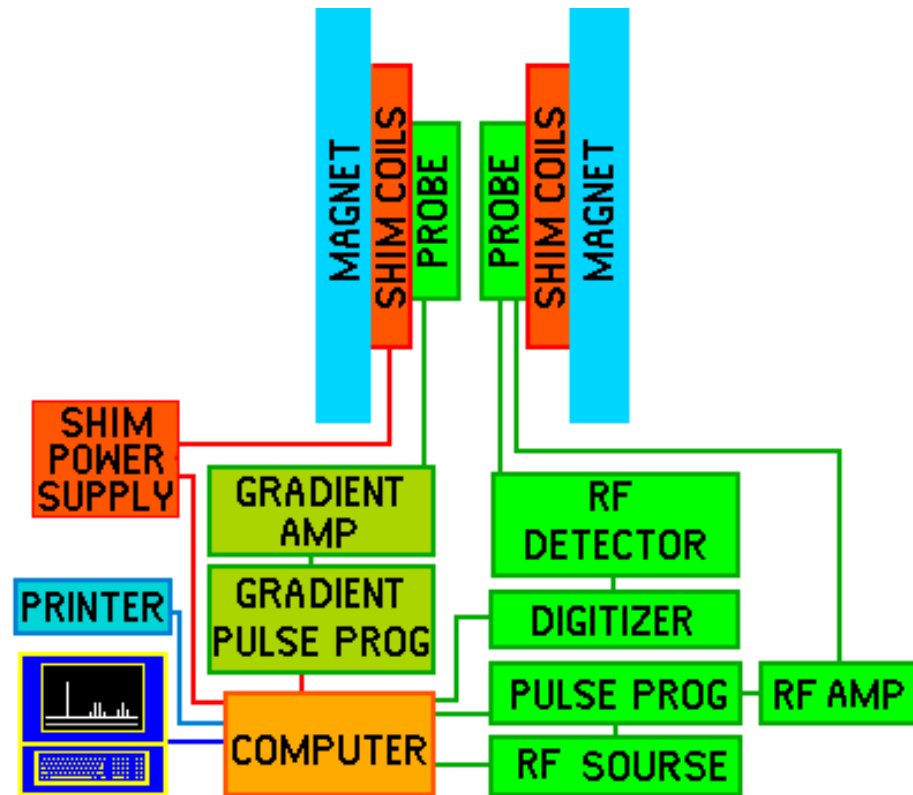
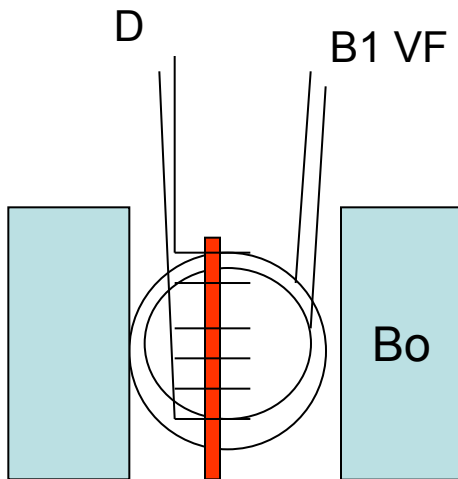
Applied field

Spin orientations of methyl protons

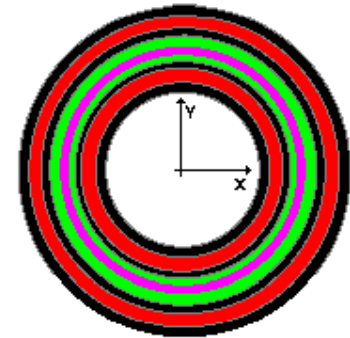
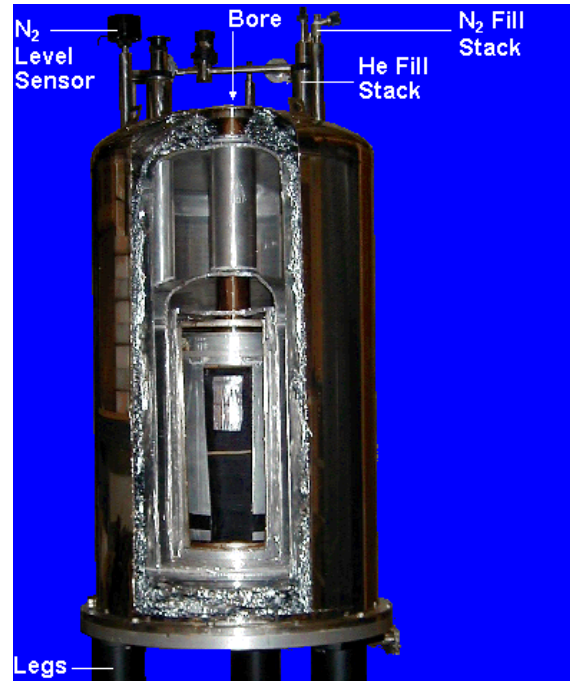


Nukleární magnetická rezonance

Přístrojové vybavení



Nukleární magnetická rezonance

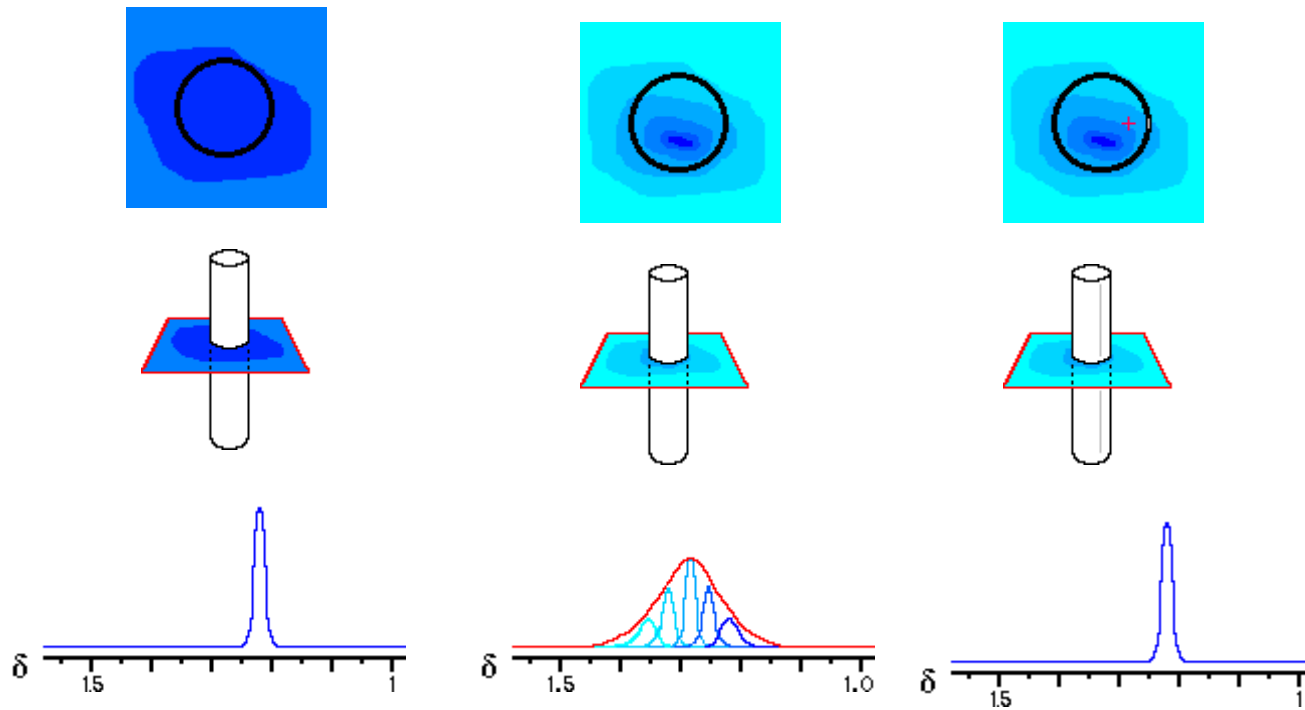


- Vacuum
- Liquid Helium
- Liquid Nitrogen
- Container & Support
- Superconducting Coil

Supravodivý magnet 7 T

Nukleární magnetická rezonance

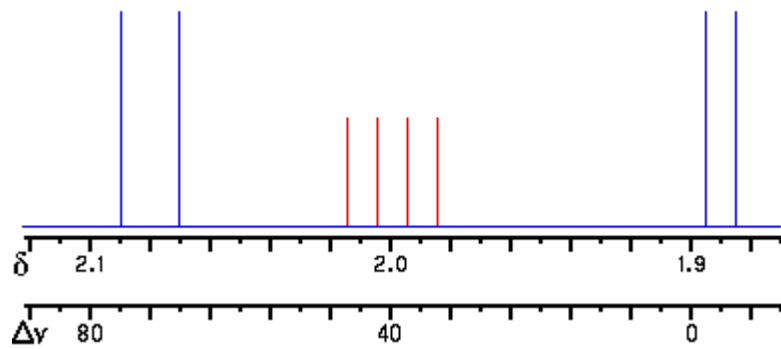
Vzorek – homogenizace pole



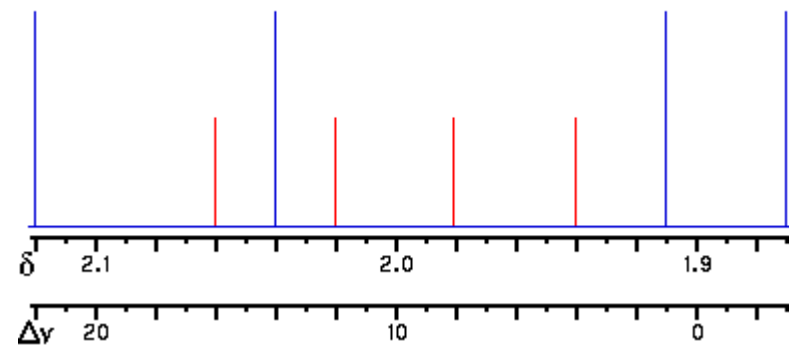
Nukleární magnetická rezonance

Volba přístroje

400 MHz



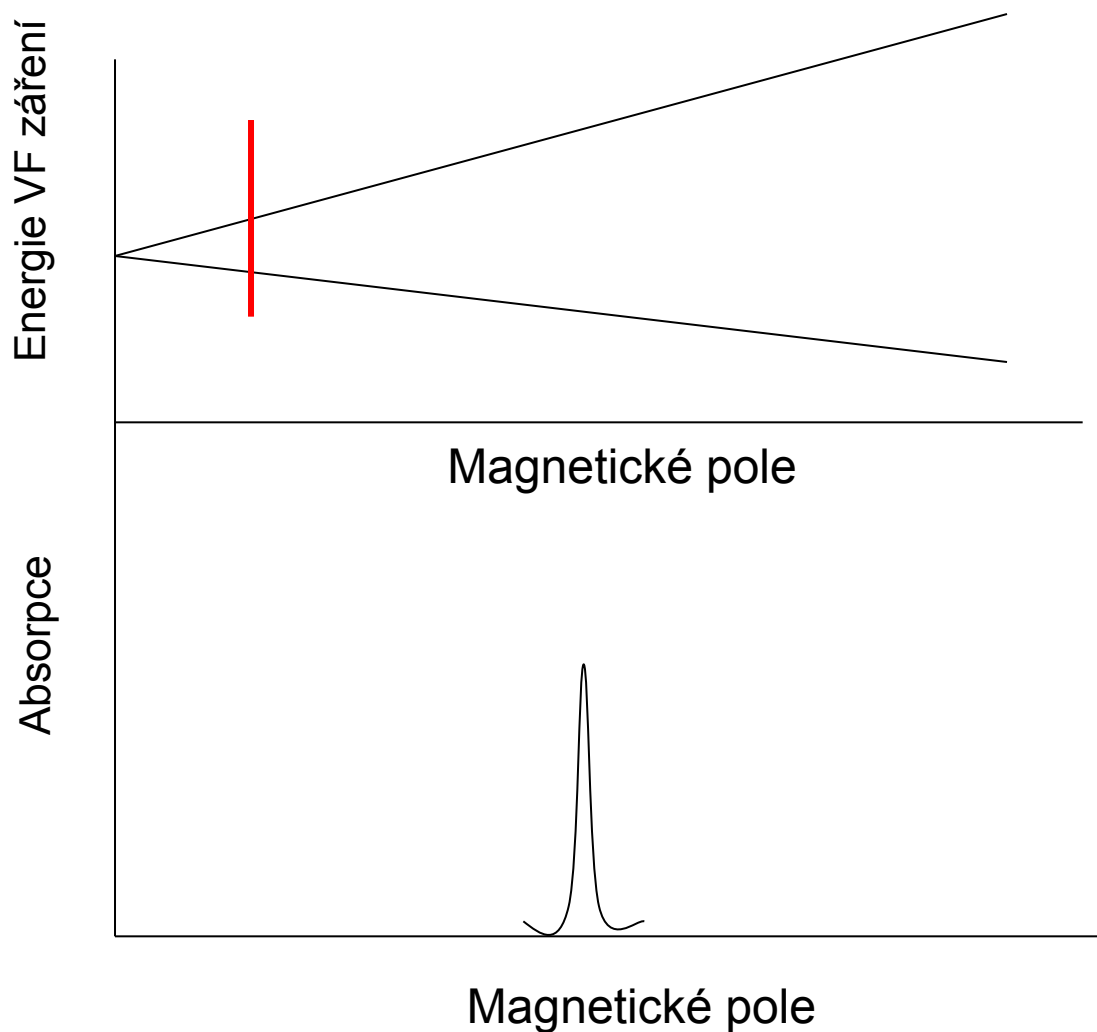
100 MHz



Atomy: 1,89 2,00 2,08

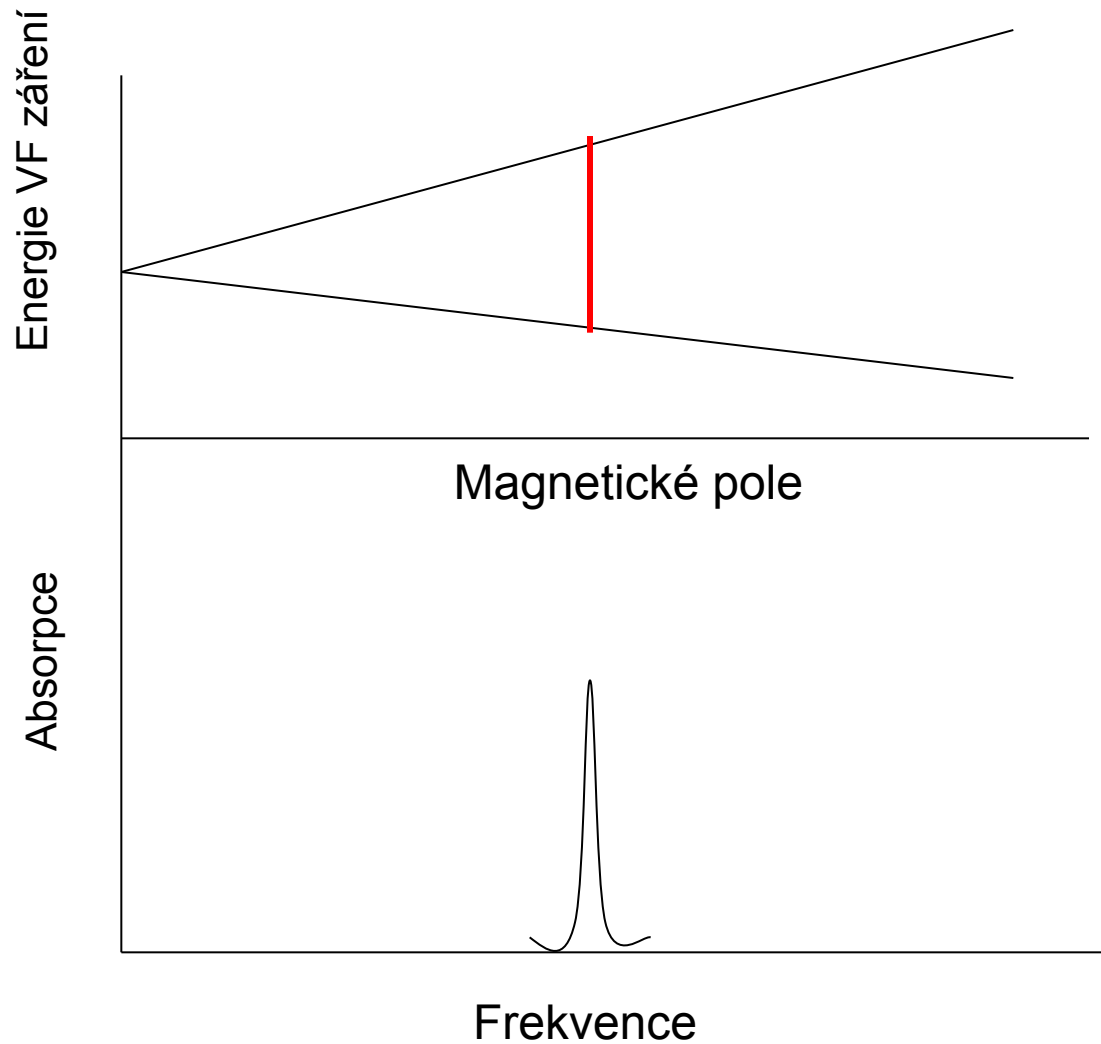
Nukleární magnetická rezonance

Metoda continuous wave (CW) – konstantní frekvence – mění se B_0



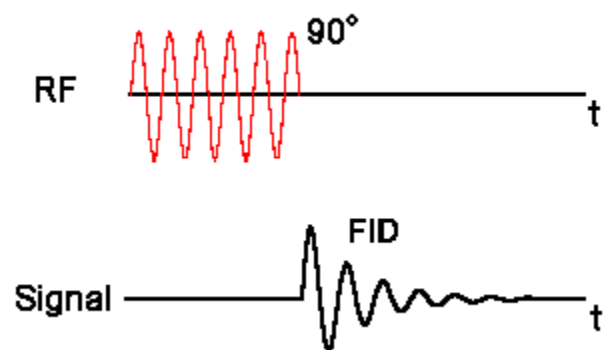
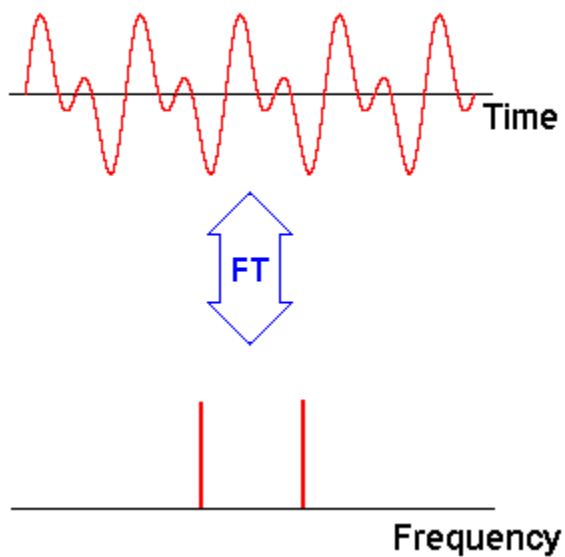
Nukleární magnetická rezonance

Metoda continuous wave (CW) – konstantní B_0 – mění se frekvence



Nukleární magnetická rezonance

Fourrierova transformace



Nukleární magnetická rezonance

Rozpouštědla – Deuterovaná

Acetone CD_3COCD_3

Chloroform CDCl_3

Methylnitrile CD_3CN

Water D_2O

Diethylether (DEE) $(\text{CD}_3\text{CD}_2)_2\text{O}$

Dimethyl Sulfoxide (DMSO) CD_3SOCD_3

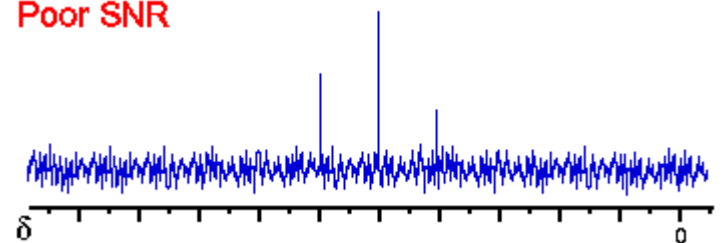
Ethanol $\text{CD}_3\text{CD}_2\text{OD}$

Atd.

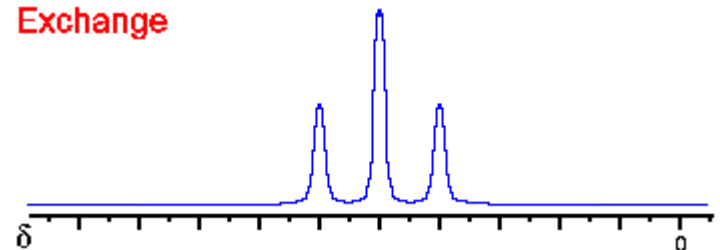
Signal-to noise ratio

Nízká a vysoká koncentrace

Poor SNR



Exchange



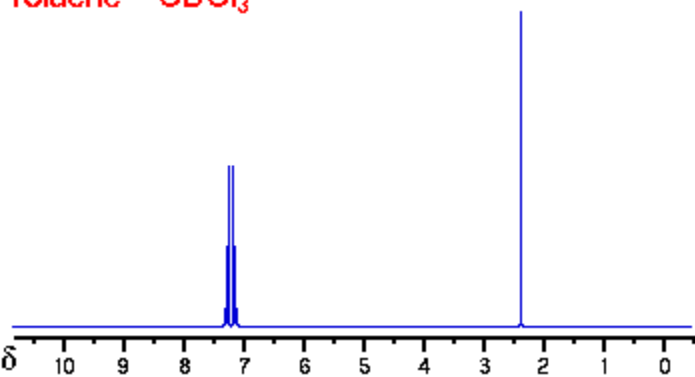
Nukleární magnetická rezonance

Chemické posuny

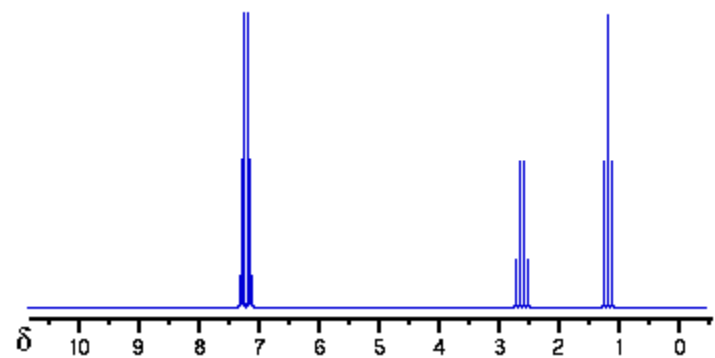
CH ₃	1
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R-O-CH ₃	4
=C=CH ₂	5
ArH	7-8
RCOH	10
RCOOH	10-12

Nukleární magnetická rezonance

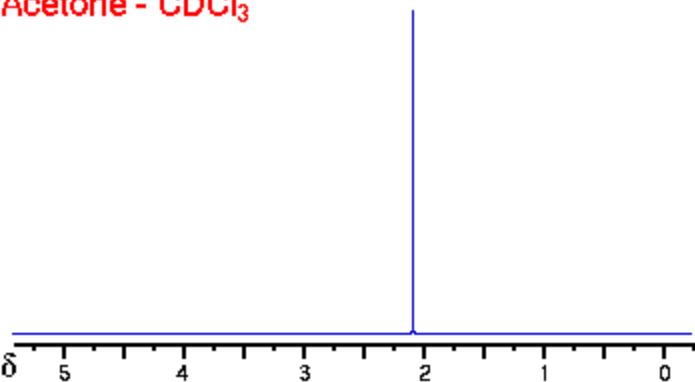
Toluene - CDCl_3



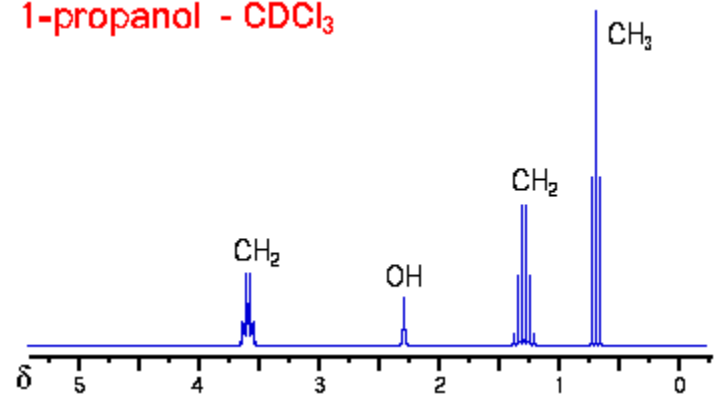
Ethyl benzene - CDCl_3



Acetone - CDCl_3

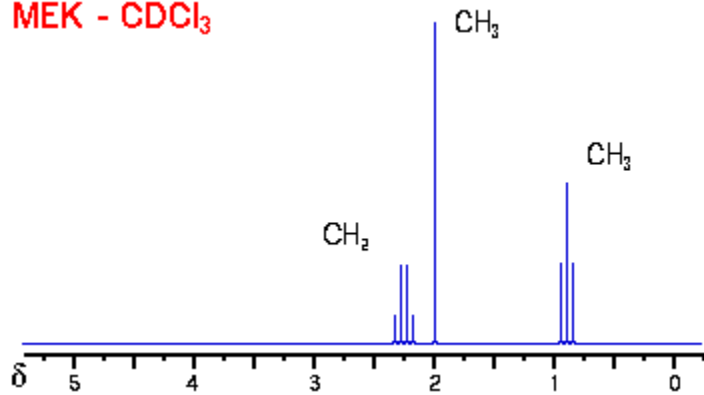


1-propanol - CDCl_3

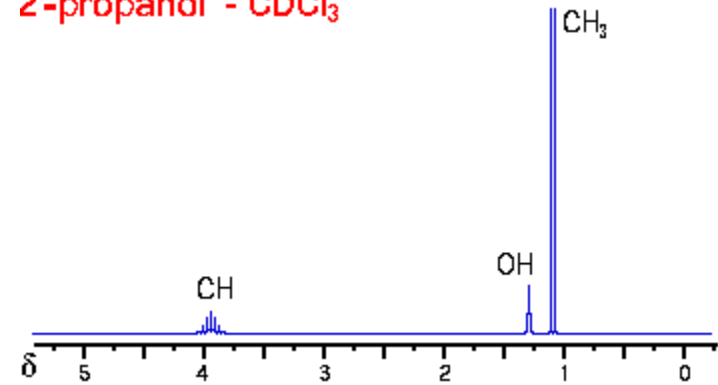


Nukleární magnetická rezonance

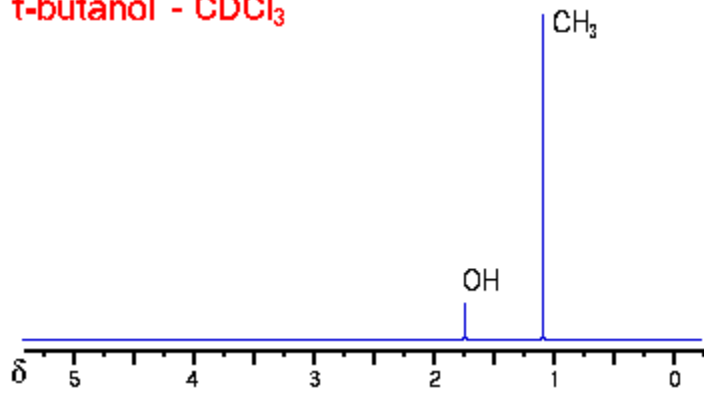
MEK - CDCl_3



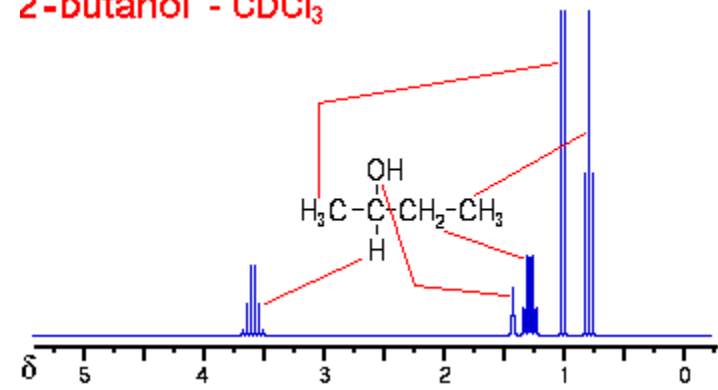
2-propanol - CDCl_3



t-butanol - CDCl_3

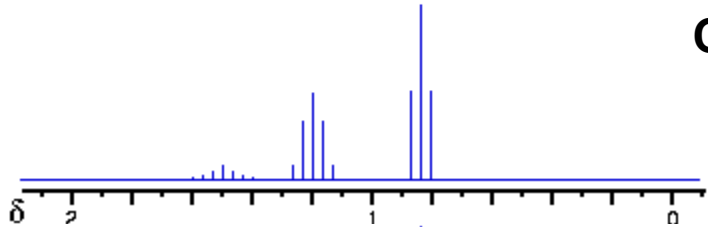
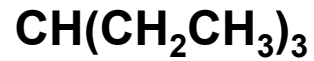


2-butanol - CDCl_3

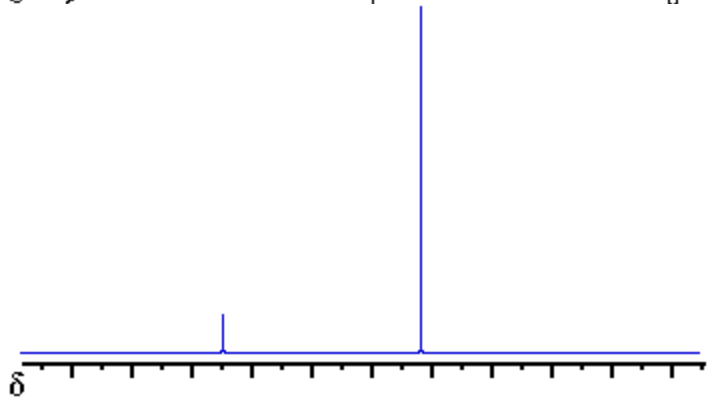


Nukleární magnetická rezonance

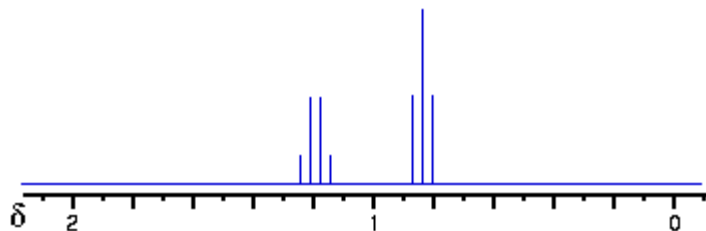
Spin-decoupling



Decoupling přes -CH₂-



Decoupling přes -CH--



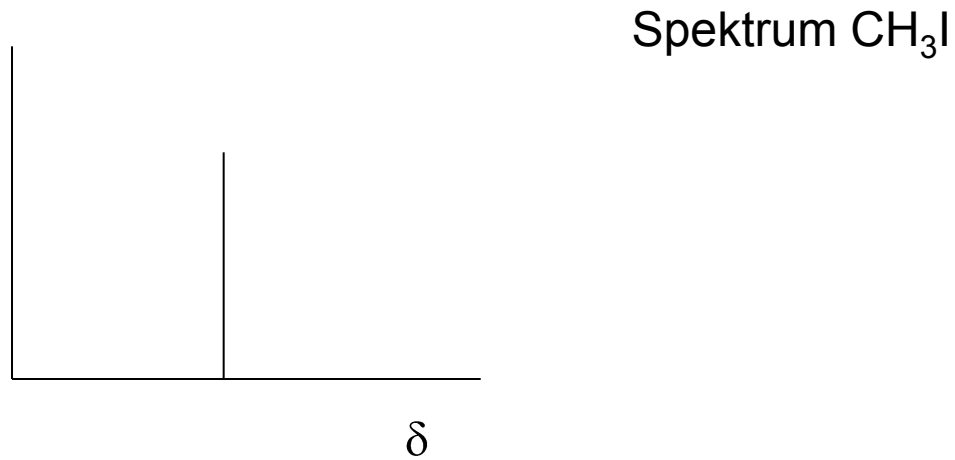
Nukleární magnetická rezonance- ^{13}C

Carbon-13 Chemical Shifts

Carbon-13* Environment	Chemical Shift Range (ppm)
CH_3CH_2	0-50
$-\text{C}\equiv\text{C}-$	60-100
$\equiv\text{C}-\text{X}$	70-170
C_6H_6	65
CHCl CHCl (cis)	71
Ar	120-150
CCl_4	97
COOR	170
COH	210
RCOR	220

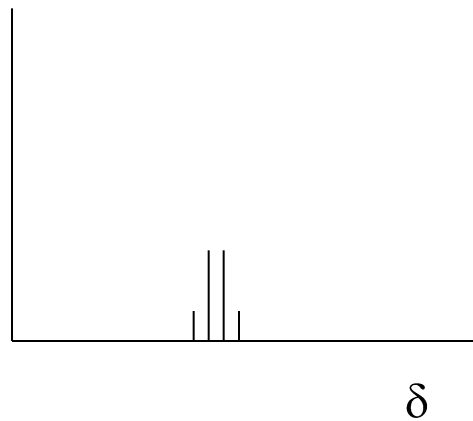
Nukleární magnetická rezonance- ^{13}C

Spin-spinová interakce mezi ^{13}C - ^1H



Nukleární magnetická rezonance- ^{13}C

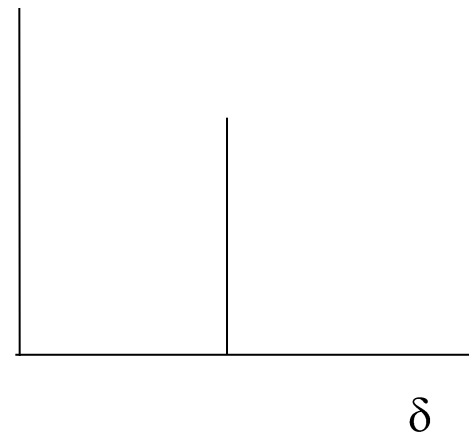
Spin-spinová interakce mezi ^{13}C - ^1H



Spektrum CH_3I

Interakce C – 3H

Decoupling přes H



Nukleární magnetická rezonance-¹⁵N



Nitrogen-14 Chemical Shifts _

Nitrogen-14* Environment	Chemical Shift Range (ppm)
NO ₂ Na	-355
NO ₃ ⁻ (aqueous)	-115
N ₂ (liquid)	-101
pyridine	-93
bare nucleus	0
CH ₃ CN	25
CH ₃ CONH ₂ (aqueous)	152
NH ₄ ⁺ (aqueous)	245
NH ₃ (liquid)	266

Nukleární magnetická rezonance- ^{31}P



Phosphorous-31 Chemical Shifts _

Phosphorous-31 Environment	Chemical Shift Range (ppm)
PBr_3	-228
$(\text{C}_2\text{H}_5\text{O})_3\text{P}$	-137
PF_3	-97
85% phosphoric acid	0
PCl_5	80
PH_3	238
P_4	450

Nukleární magnetická rezonance- ^{31}P

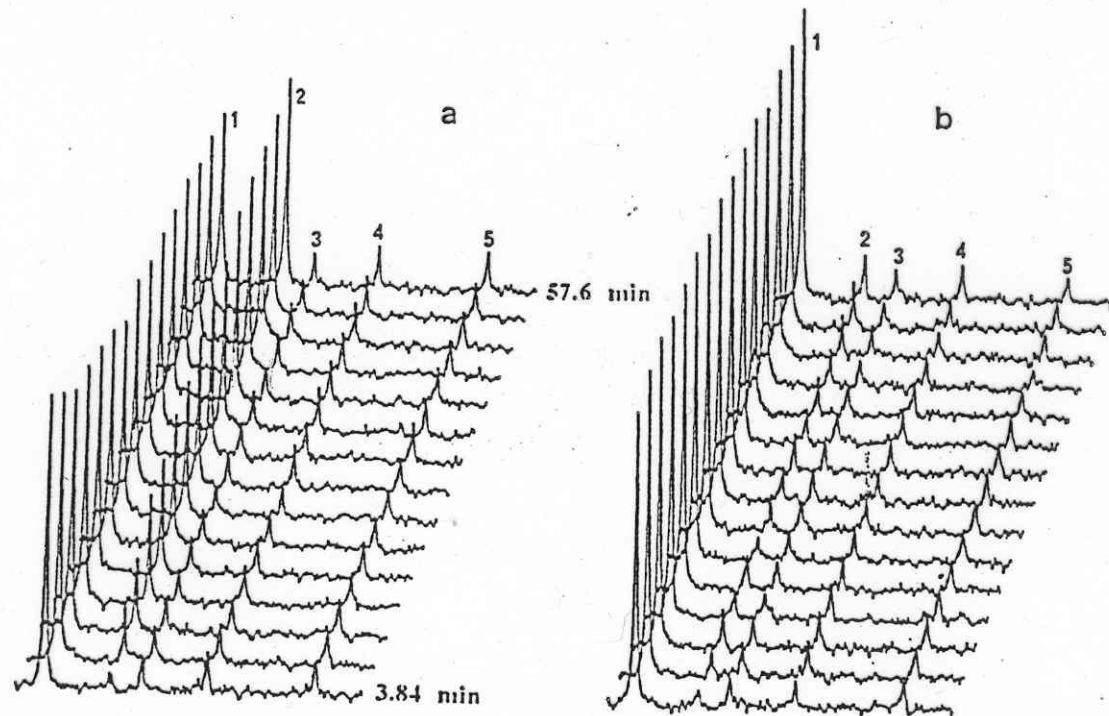


FIG. 1. Phosphocreatine synthesis by isolated rat skeletal muscle mitochondria followed by ^{31}P NMR spectroscopy: two series of typical spectra obtained during one hour with mitochondria (a) in the presence of 1 mM ATP or (b) in the presence of 1 mM ATP and 7 nmole/mg proteins of atractyloside. Peak assignments are (1) Pi, (2) PCr, (3), (4), and (5) γ -, α -, β - phosphorus of ATP respectively.

Nukleární magnetická rezonance- ^{31}P

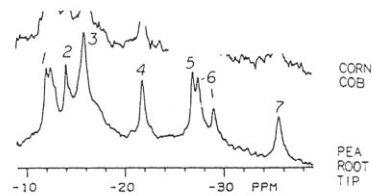


Fig. 12. ^{31}P -NMR spectra of living plant tissues, under oxygenated conditions. Peak assignments: 1, Glc-6-P; 2, cytoplasmic P; 3, vacuolar P; 4, γ -ATP; 5, α -ATP; 6, UDPG and nicotinamide adenine nucleotides; 7, β -ATP. [From Roberts (1984), with permission.]

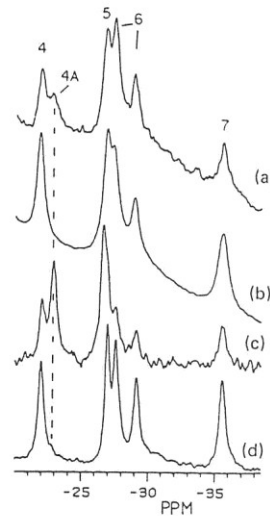


Fig. 19. ^{31}P -NMR partial spectra of the nucleotide region of (a) hypoxic maize root tips; (b) normoxic maize root tips; (c) extract of normoxic maize root tips, to which commercial ADP has been added; (d) extract of normoxic maize root tips, prior to addition of ADP. Peak assignments as in Fig. 12, except peak 4A, β -ADP (peak 5 will include a contribution from α -ADP, in addition to α -ATP). [From Roberts *et al.* (1985b), with permission.]

Nukleární magnetická rezonance- ^{31}P

Měření intracelulárního pH
Pomocí posunu signálu
 ^{31}P

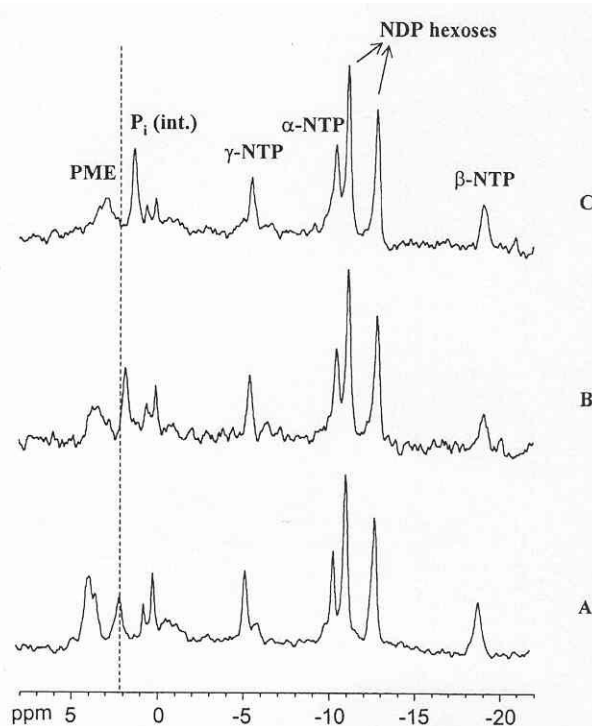


Fig. 8. In vivo ^{31}P NMR spectra of BHK21 cells (acquisition time of 3 min): (A) control conditions; (B) cells incubated with 60 mM lactate; (C) cells incubated with 10 mM ammonia. Assignments: PME, phosphomonoesters; P_i (int.), intracellular inorganic phosphate; NTP-nucleoside triphosphates, NDP-nucleoside diphosphates.

Nukleární magnetická rezonance- ^{31}P

Měření koncentrace nukleotidů
Pomocí ^{31}P

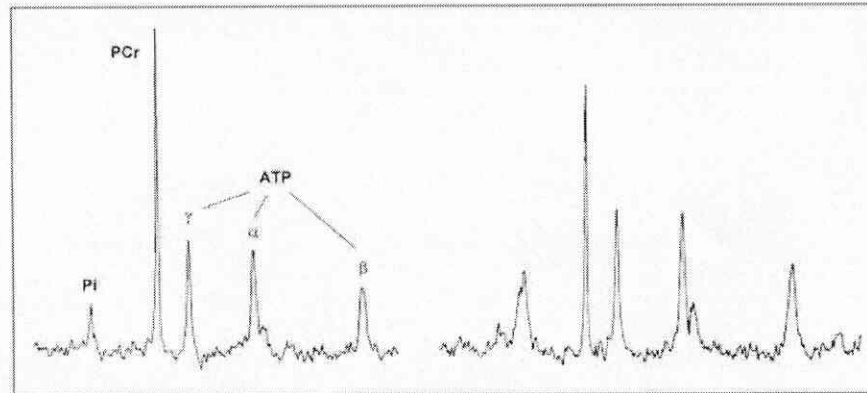


Figure 3 Typical ^{31}P NMR spectra from an isolated rat heart perfused with glucose only (right) or glucose and pyruvate (left). The major resonances, from left to right, report the relative heart contents of inorganic phosphate (Pi), PCr and the three phosphates of ATP (γ , α and β). Note the increase in PCr resonance area and fall in Pi resonance area when pyruvate is added to the glucose-containing buffer.

Nukleární magnetická rezonance- ^{31}P

Měření koncentrace
nukleotidů
Pomocí ^{31}P

IAA-iodacetát

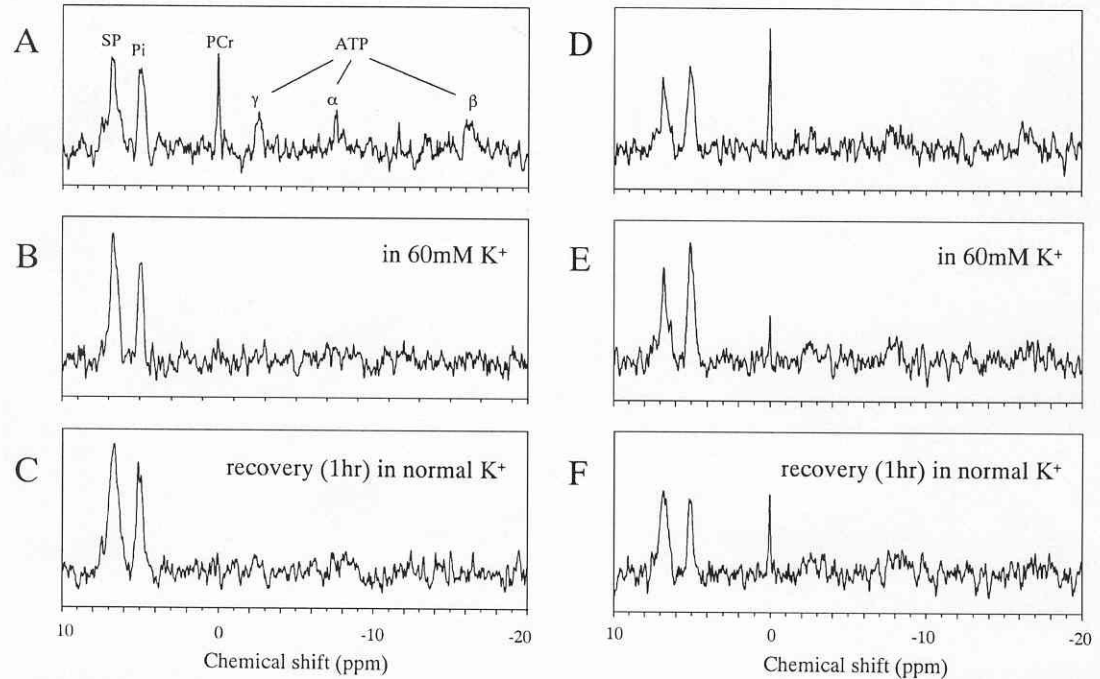


Fig. 1. ^{31}P NMR spectra of well-oxygenated rat brain slices pretreated with IAA and FC (IAA-pretreated neuron-rich slices) in ACSF containing lactate at 25 °C. A-C: ^{31}P NMR spectra from intact slices; A, under resting condition in normal K^+ ACSF; B, during high- K^+ stimulation for 8 min; C, after 60 min in normal K^+ ACSF. D-F: those from ischemic preconditioned slices, each corresponding to the conditions in A-C, respectively. Each spectrum represents an accumulation of 256 FIDs. Line broadening is 10 Hz. SP, sugar phosphates; Pi, inorganic phosphates; γ -, α -, and β -, 3 peaks of ATP.

Nukleární magnetická rezonance- ^{13}C

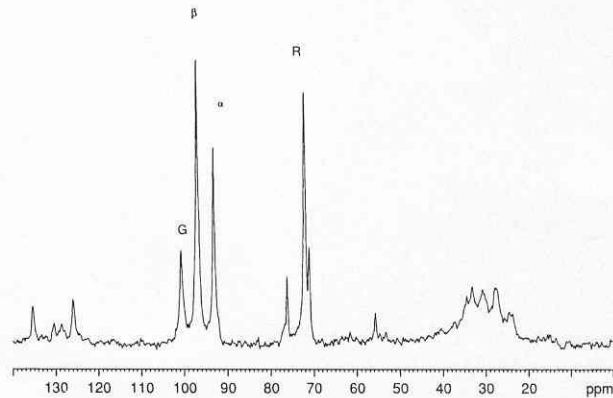


Fig. 7. Representative ^{13}C NMR spectrum acquired from a perfused rat heart under preischemic conditions after 30 min of labeled perfusion. The abbreviations denote $[1-^{13}\text{C}]$ glycogen (G), the α - and β -anomers of $[1-^{13}\text{C}]$ glucose (α and β), and $[2-^{13}\text{C}]$ ribose (R) used as standard.

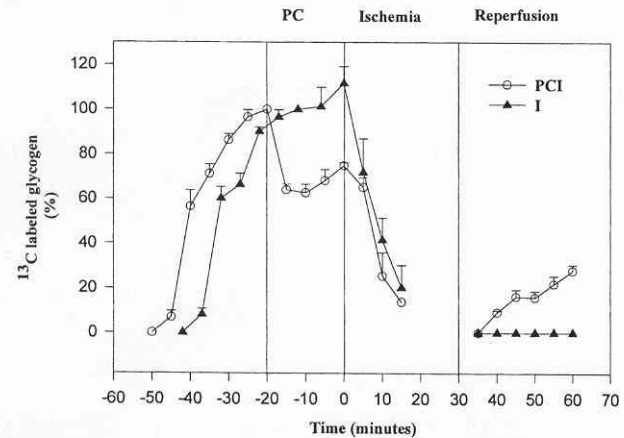


Fig. 8. ^{13}C -Labeling and mobilization of $[1-^{13}\text{C}]$ glycogen during protocol set I in preconditioned hearts (PCI, $n=6$, \circ) and ischemic controls (I, $n=6$, \blacktriangle). The labeled perfusion started at $t=-50$ min for the preconditioned hearts and $t=-42$ min for the controls. During the first 30 min of perfusion (stabilization), the behavior of the two groups was almost identical. $[1-^{13}\text{C}]$ -Glycogen was partially reduced by the first cycle of preconditioning ischemia and was not further reduced by subsequent cycles. In the PCI group, preischemic glycogen proved to be lower than that in Group I, with reduced $[1-^{13}\text{C}]$ glycogen mobilization during early ischemia ($t=0-15$ min). Only the PCI group resumed glycogen synthesis. Mean \pm S.E.M.

Nukleární magnetická rezonance- ^{15}N

Asimilace amoniaku sledovaná
Pomocí ^{15}N

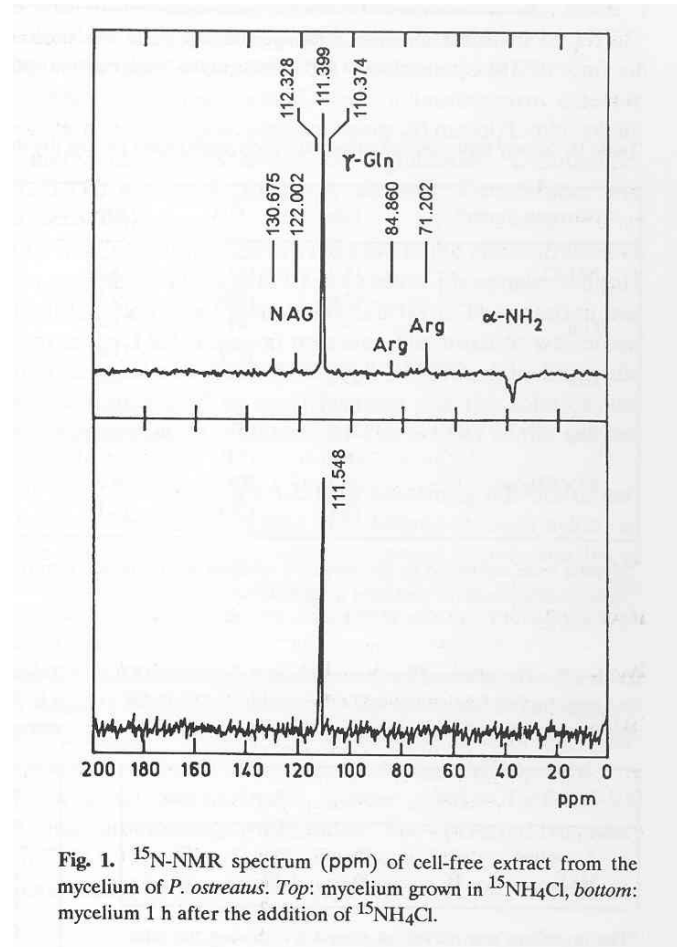
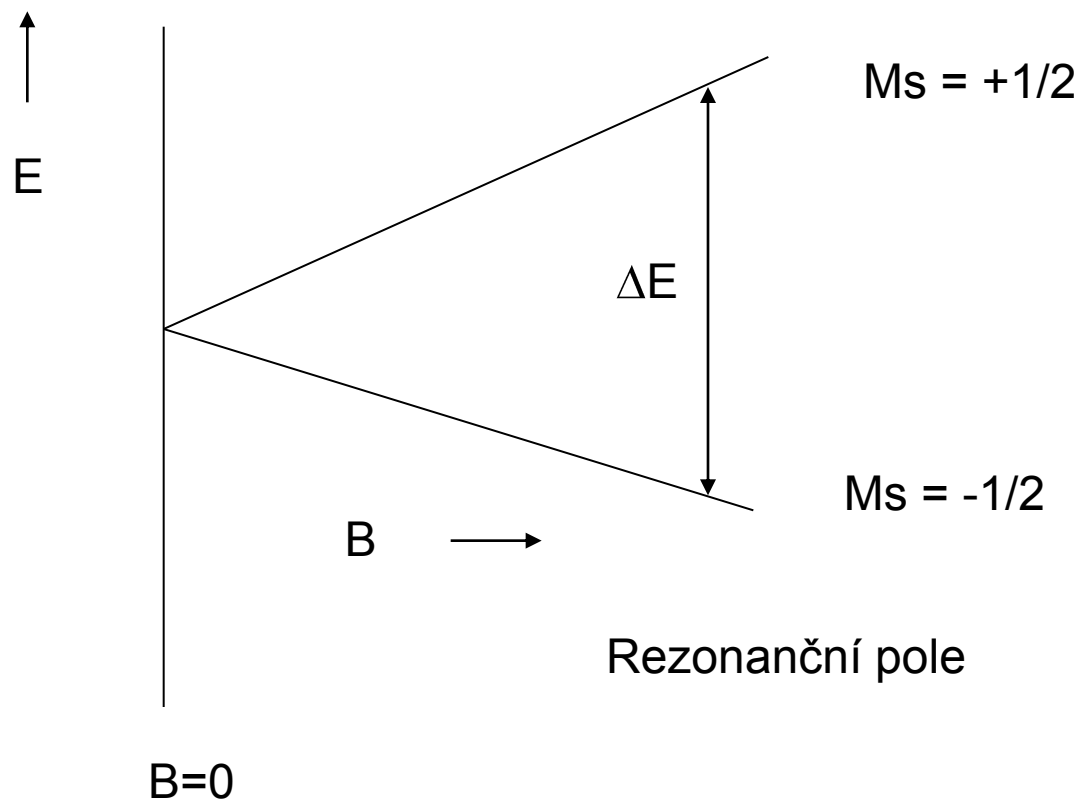


Fig. 1. ^{15}N -NMR spectrum (ppm) of cell-free extract from the mycelium of *P. ostreatus*. Top: mycelium grown in $^{15}\text{NH}_4\text{Cl}$, bottom: mycelium 1 h after the addition of $^{15}\text{NH}_4\text{Cl}$.

EPR – elektronová spinová rezonance

Nepárový elektron



EPR – elektronová spinová rezonance

$$\Delta E = h\nu = g\mu B_0$$

ΔE rozdíl energií

h is Planck constant

ν is frekvence mikrovln

g faktor spin-orbitální interakce

B magnetické pole

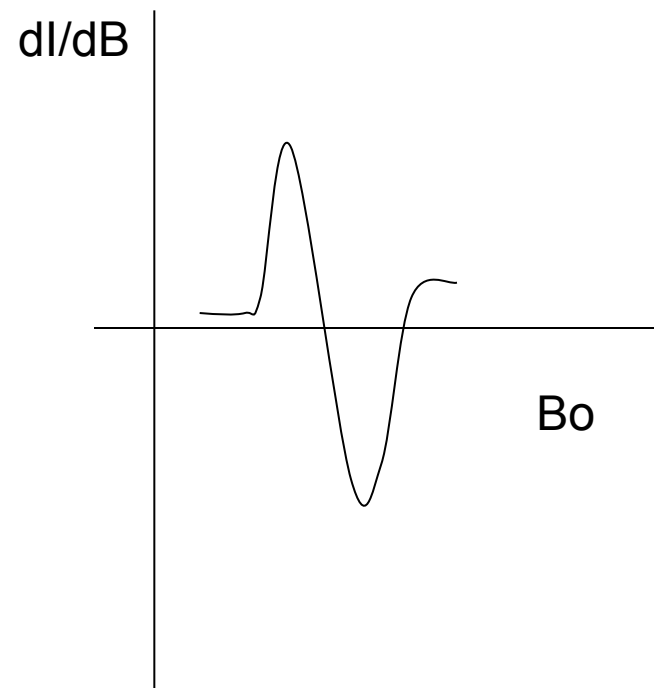
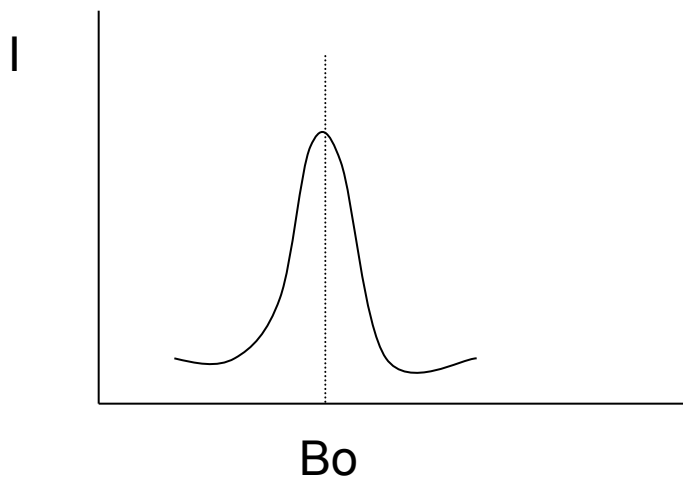
Počet jader v jednotlivých orientacích

$$N_1/N_2 = \exp(-\Delta E/kT)$$

$$EPR = 0,989$$

$$B_0 = 0,1 - 0,5 \text{ T}; \quad \nu = 10 - 100 \text{ GHz}$$

EPR – elektronová spinová rezonance



EPR – elektronová spinová rezonance

Interakce s protony

1 proton $I = \frac{1}{2}$ multiplicita = $n + 1$, intenzita píků $(x + 1)^n$

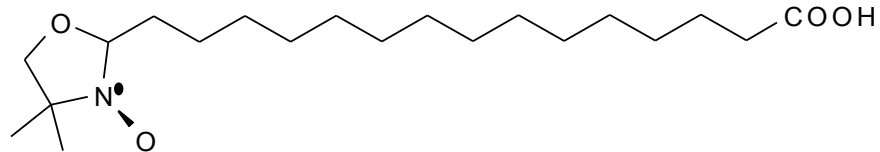
1:2:1

1:3:3:1

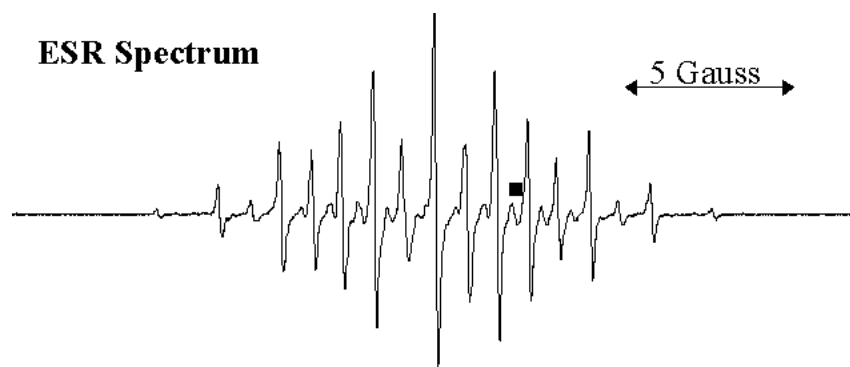
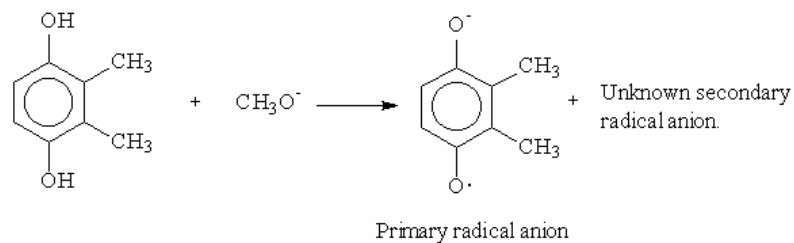
EPR – elektronová spinová rezonance

Využití v biochemii

- Studium radikálů (peroxydy)
- Studium konformace – spinové značky
- studium hemu

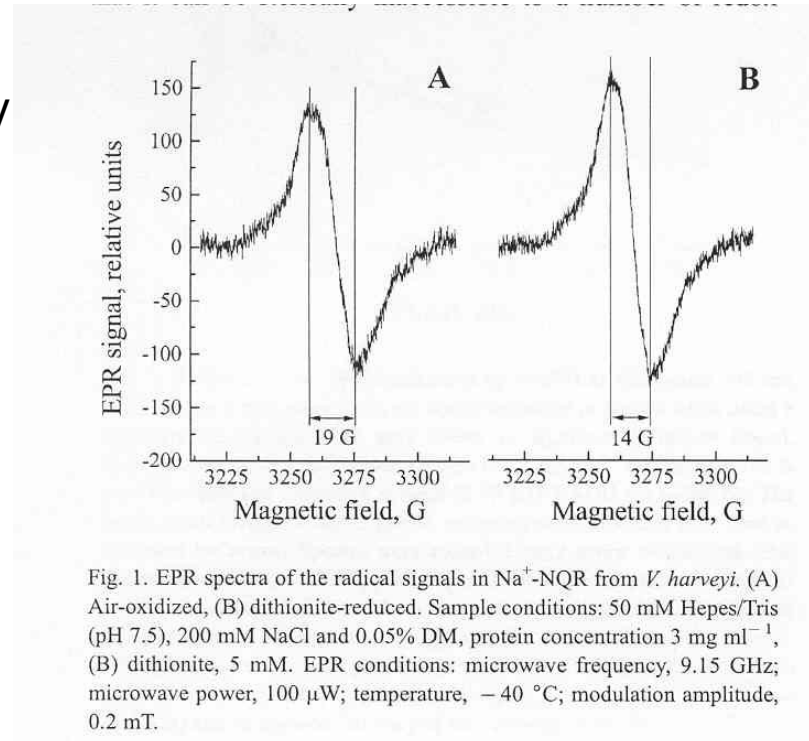


EPR – elektronová spinová rezonance



EPR – elektronová spinová rezonance

EPR spektrum NADH-UQ reduktasy
signál UQ
Signál 2Fe2S potlačen,
Signál FMN radikálu interferuje



EPR – elektronová spinová rezonance

EPR spektrum ferredoxinu
z mořské řasy
(3Fe-4S) $1+ / 0$
Typický signál

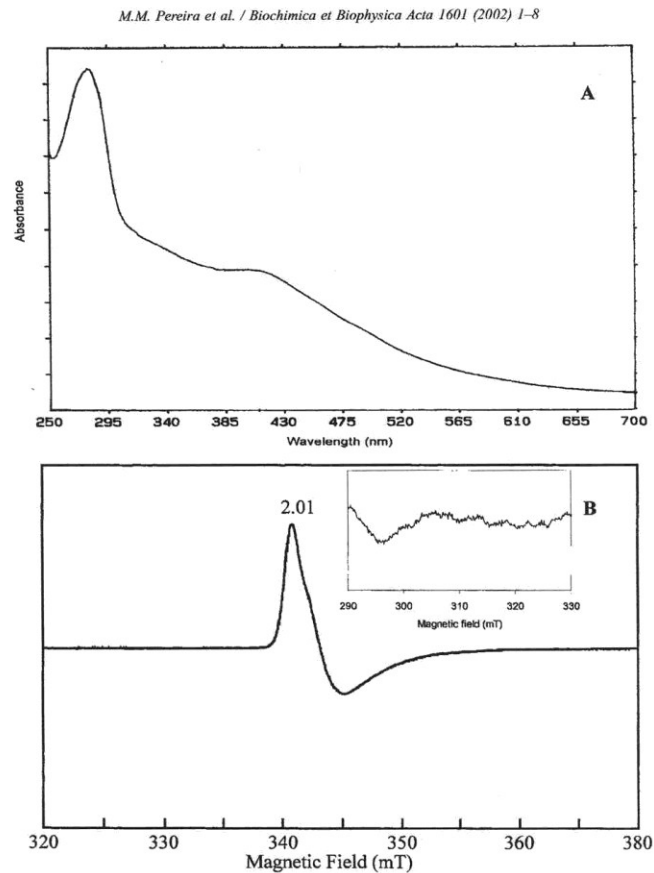


Fig. 2. (A) UV-visible spectrum of the oxidised ferredoxin from *R. marinus*. (B) EPR spectra of the ferredoxin from *R. marinus* in oxidised form and perpendicular mode (main spectrum) and reduced form and parallel mode (insertion). EPR conditions—temperature: 10 K; microwave frequency: 9.64 GHz (perpendicular mode) and 9.43 GHz (parallel mode); microwave power: 2.4 mW; modulation amplitude: 0.9 mT. Protein concentration 50 μ M (A) and 100 μ M (B).

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EPR spektrum cyt bc1 – mutant
M183K, M183H

Fe³⁺ hem (3,78 = cyt bL 3,44, cyt
bH,
2,94 bis-His cyt b nebo c1)

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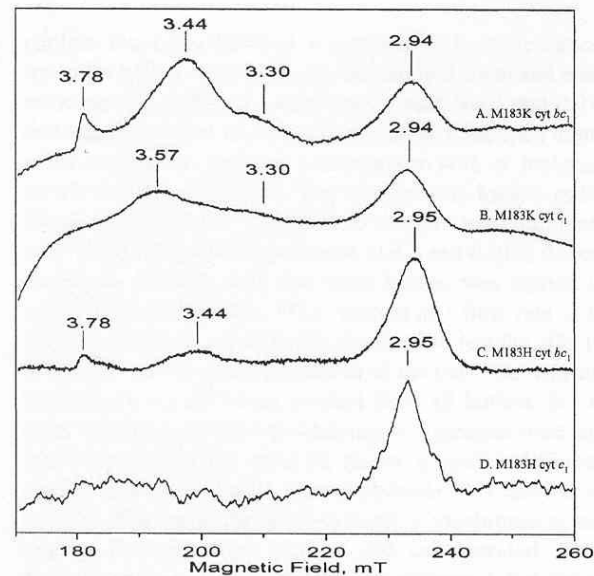


Fig. 2. X-band EPR spectra of *Rb. capsulatus* cytochromes. (A) Cytochrome *bc*₁ complex containing M183K cytochrome *c*₁ (39 μ M). (B) Purified M183K cytochrome *c*₁ (148 μ M). (C) Cytochrome *bc*₁ complex containing M183H cytochrome *c*₁ (29 μ M). (D) Purified M183H cytochrome *c*₁ (24 μ M). The buffer conditions are: 100 mM Tris–DCl (pD = 8.0), 10 mM NaCl, 0.1% dodecyl maltoside and 55% *d*₃-glycerol for (A) and (C); 25 mM Tris–DCl (pD 8.0), 10 mM NaCl, 0.25% cholate and 55% *d*₂-ethylene glycol for (B); 25 mM Tris–DCl (pD 8.0), 10 mM NaCl, 1% cholate and 55% *d*₃-glycerol for (D). All spectra were recorded at 10 K and instrument settings were: number of scans, 5; microwave power, 10 mW; modulation amplitude, 1.0 mT; microwave frequency, 9.59 GHz.

EPR – elektronová spinová rezonance

EPR spektrum oxidace radikálů
LDL vyvolaných H₂O₂

Spektrum radikálu tokoferolu (A)

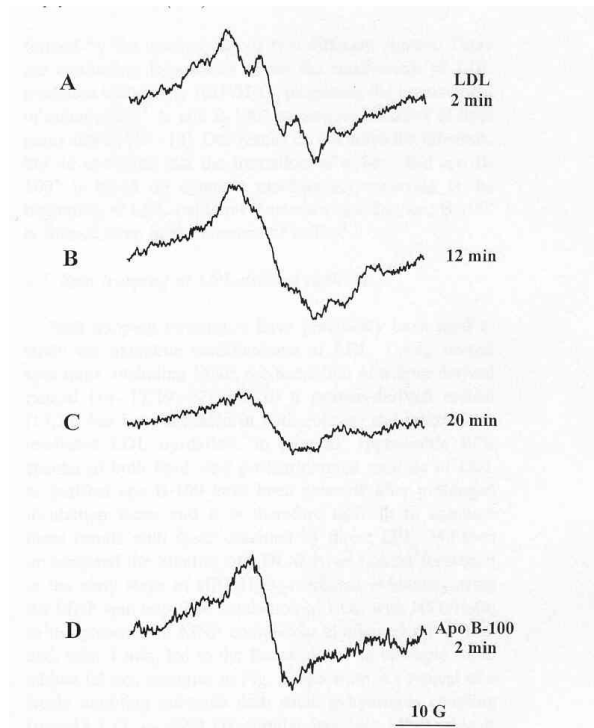


Fig. 1. EPR spectra at 37 °C of LDL and purified apo B-100 treated with HRP/H₂O₂. (A) Spectrum of LDL (1.5 mg/ml) recorded after 2 min of incubation with HRP (10 μM) and H₂O₂ (2.5 mM) in phosphate-buffered saline, DTPA (0.1 mM), pH 7.4; (B) sample (A) after 12 min of incubation, and (C) sample (A) after 20 min of incubation. (D) Spectrum of apo B-100 (4 mg/ml) recorded after 2 min of incubation with HRP (10 μM) and H₂O₂ (2.5 mM), in 10 mM Tris-HCl and 10 mM sodium deoxycholate, DTPA (0.1 mM), pH 9.0. Spectrometer conditions were as follows: modulation amplitude, 5 G; time constant, 82 ms; sweep time, 21 s; number of scans, 20.