

# Nukleární magnetická rezonance

Sumární spin subatomárních částic (p,n,e)

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

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

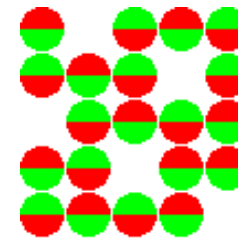
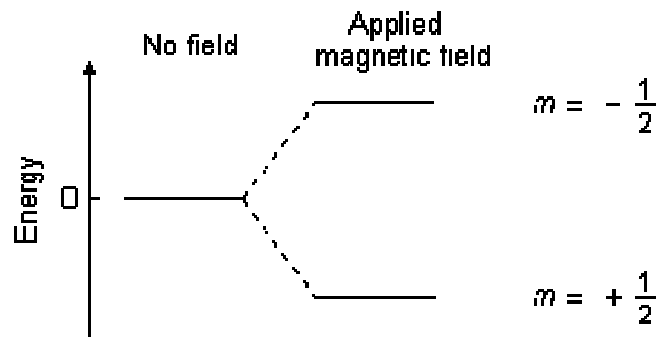
(dojde k rozštěpení v magnetickém poli)

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**

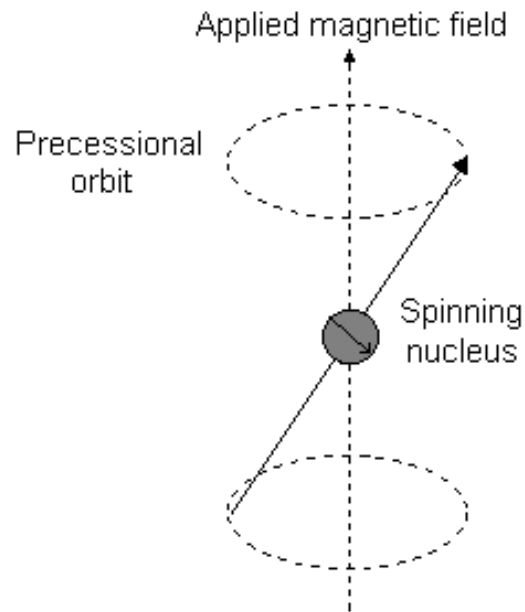


# Nukleární magnetická rezonance

Magnetický moment rotujícího jádra  $\mu$

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

$\gamma$  gyromagnetický poměr

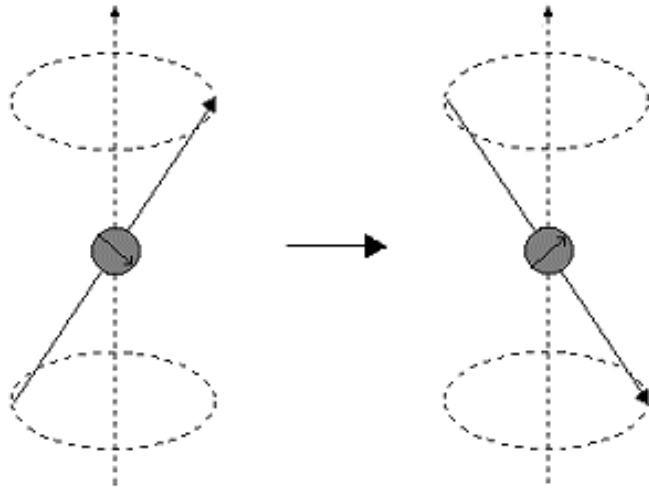


Umístění rotujícího magnetu v elektrickém poli

Frekvence rotace:  $\nu_0 = \gamma B_0$

$B_0$  – indukce mg. pole

# Nukleární magnetická rezonance



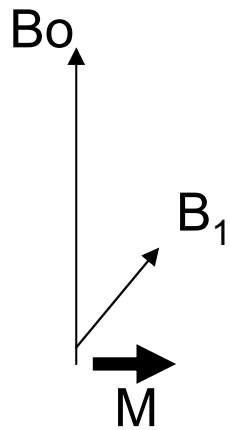
Rozdíl mezi dvěma energetickými hladinami

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

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)

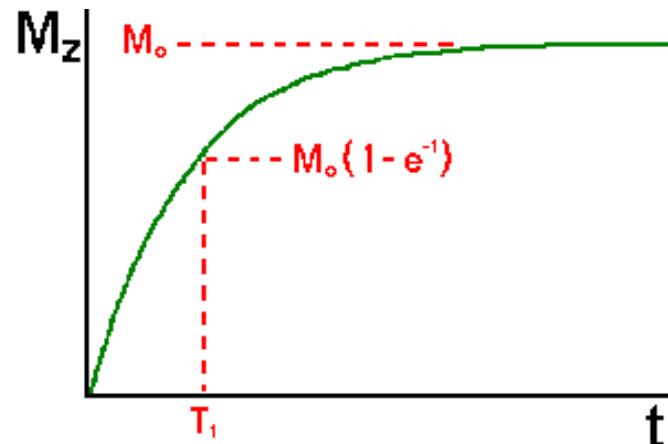
Jádro	I	$\nu_0$ (MHz)	citlivost	% zastoupení
$^1\text{H}$	$\frac{1}{2}$	100	1	99,9
$^{13}\text{C}$	$\frac{1}{2}$	25	0,016	1
$^{15}\text{N}$	1	10	0,001	0,36
$^{19}\text{F}$	$\frac{1}{2}$	94	0,8	100
$^{31}\text{P}$	$\frac{1}{2}$	40	0,067	100
$^{17}\text{O}$	$\frac{5}{2}$	13	0,003	0,04

# Nukleární magnetická rezonance

## Spin-mřížková relaxace

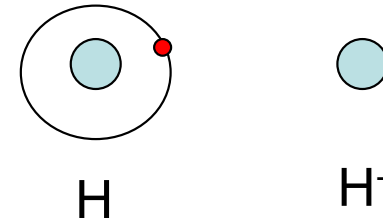
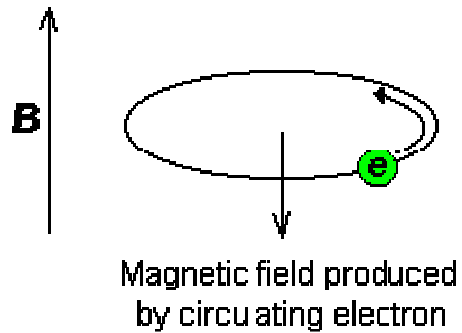
Jádro v excitovaném stavu se zbavuje energie relaxací

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



# Nukleární magnetická rezonance

Stínění jádra – chemické posuny



$$B = B_0 - \sigma B_0$$

$$\tau = 10 - \sigma$$

$\tau$  chemický posun (ppm)

# Nukleární magnetická rezonance

## Chemické posuny

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

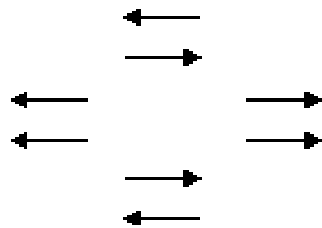
# Nukleární magnetická rezonance

Multiplicita signálu – spin-spinová interakce

Applied field

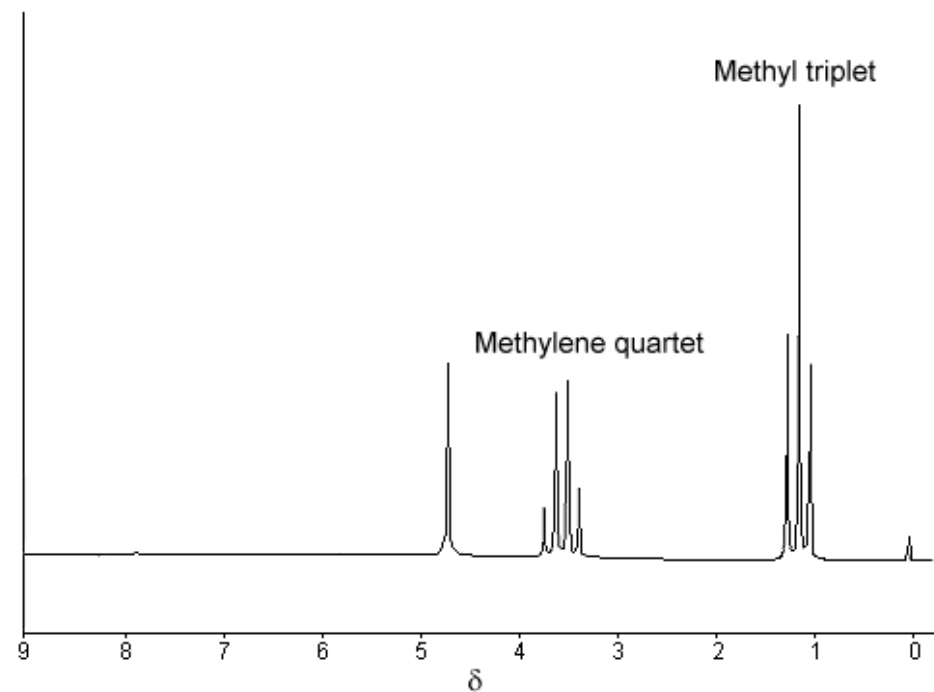
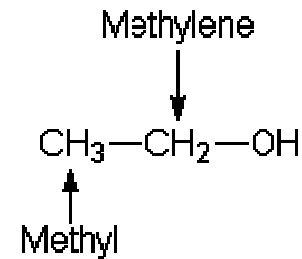


Spin orientations of methylene protons



Multiplicita =  $n+1$

$(x + 1)^n$



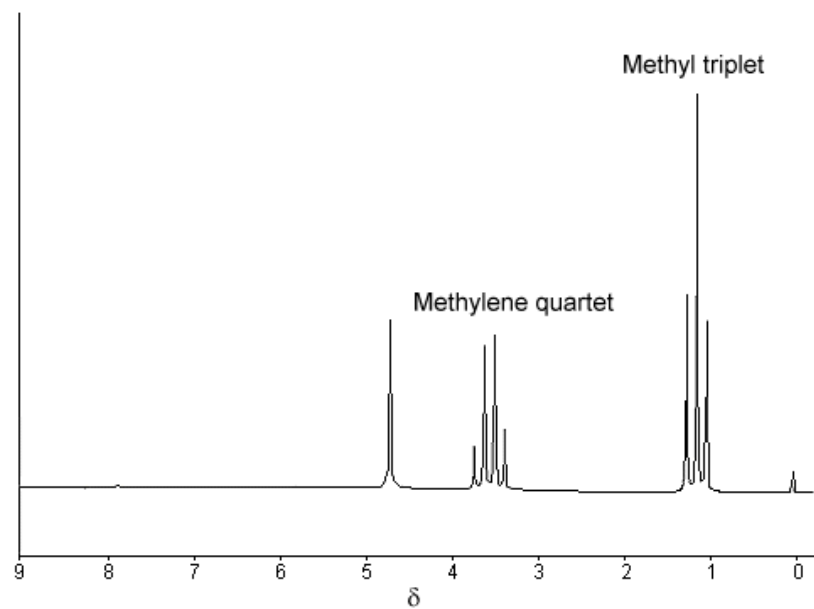
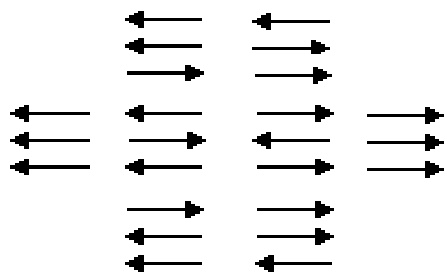


# Nukleární magnetická rezonance

Applied field

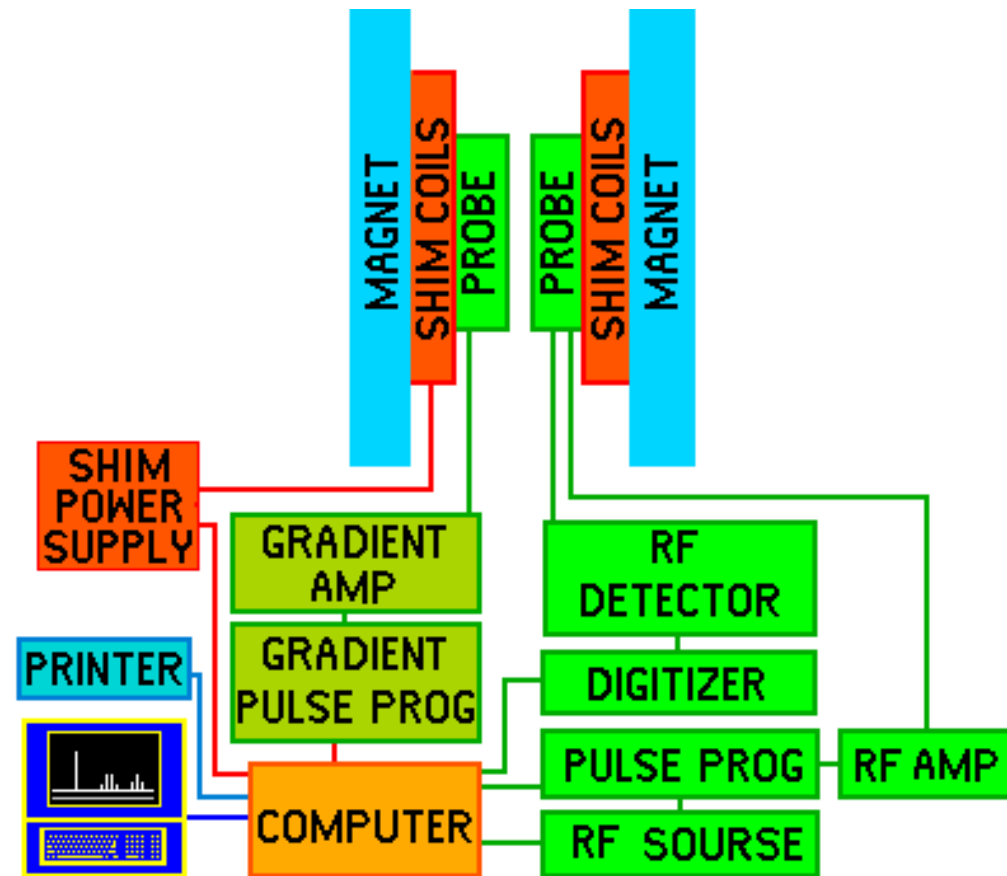
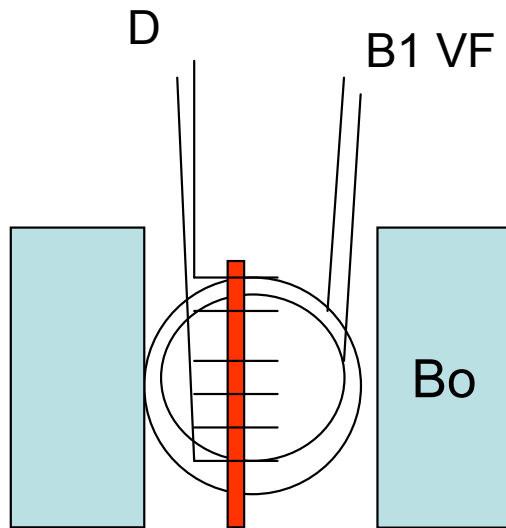


Spin orientations of methyl protons

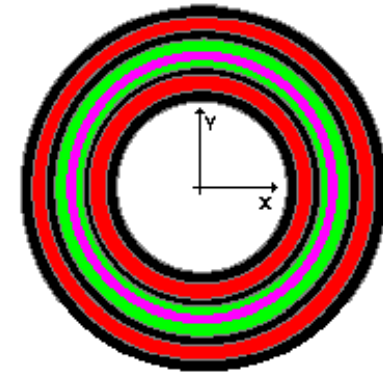
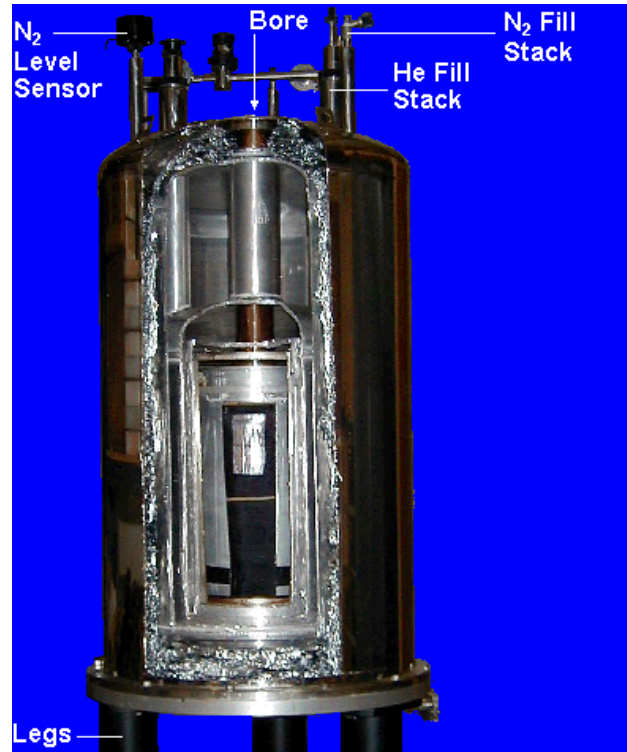


# Nukleární magnetická rezonance

Přístrojové vybavení



# Nukleární magnetická rezonance

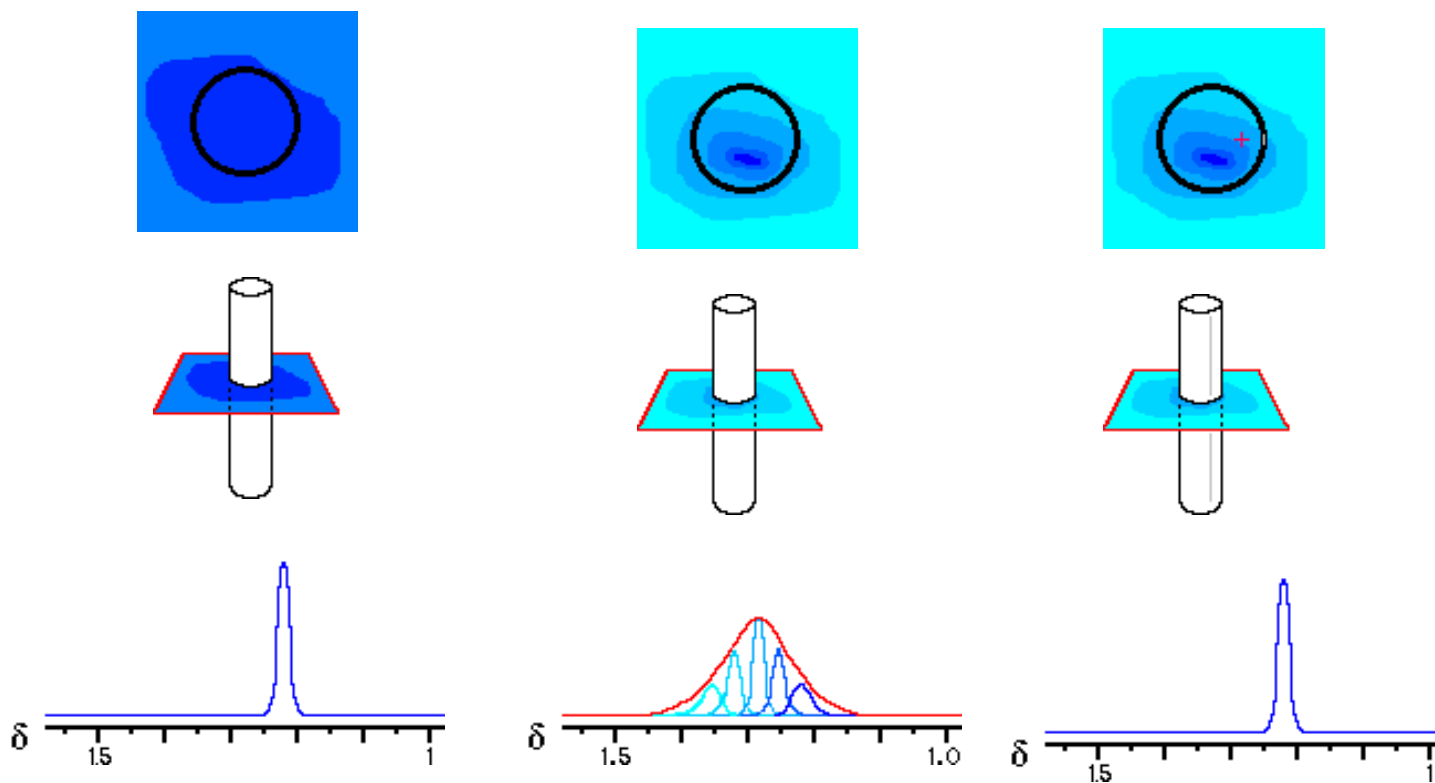


- Black** Vacuum
- Green** Liquid Helium
- Red** Liquid Nitrogen
- Grey** Container & Support
- Pink** Superconducting Coil

Supravodivý magnet 7 T

# Nukleární magnetická rezonance

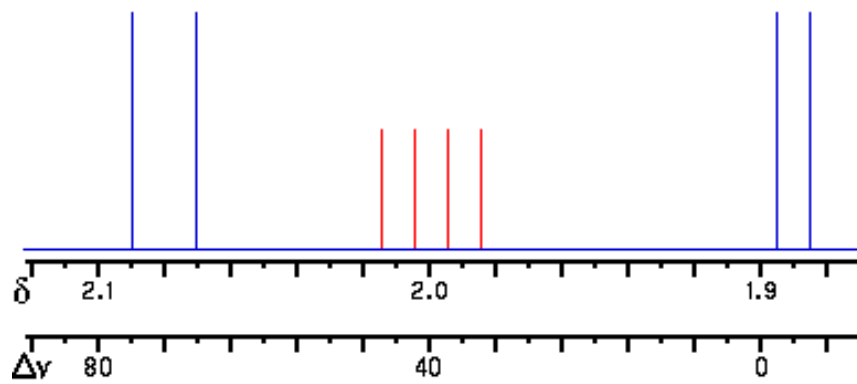
Vzorek – homogenizace pole



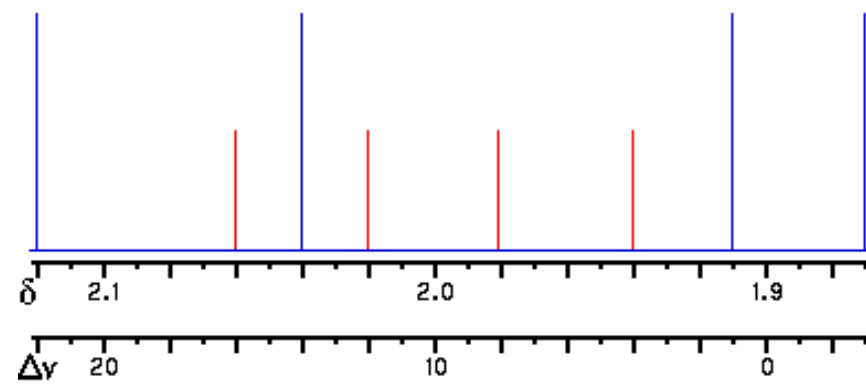
# Nukleární magnetická rezonance

Volba přístroje

400 MHz



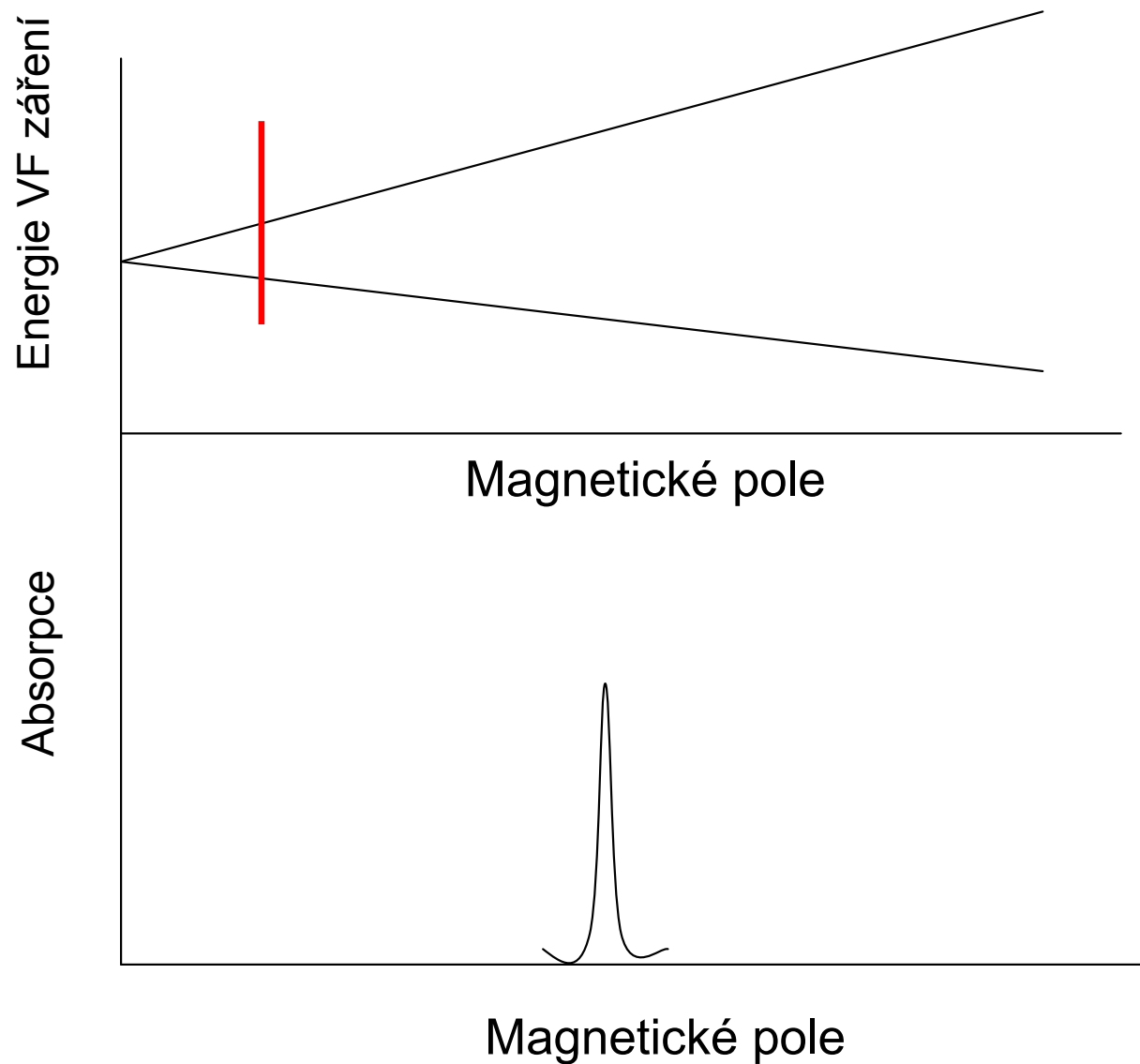
100 MHz



Atomy: 1,89 2,00 2,08

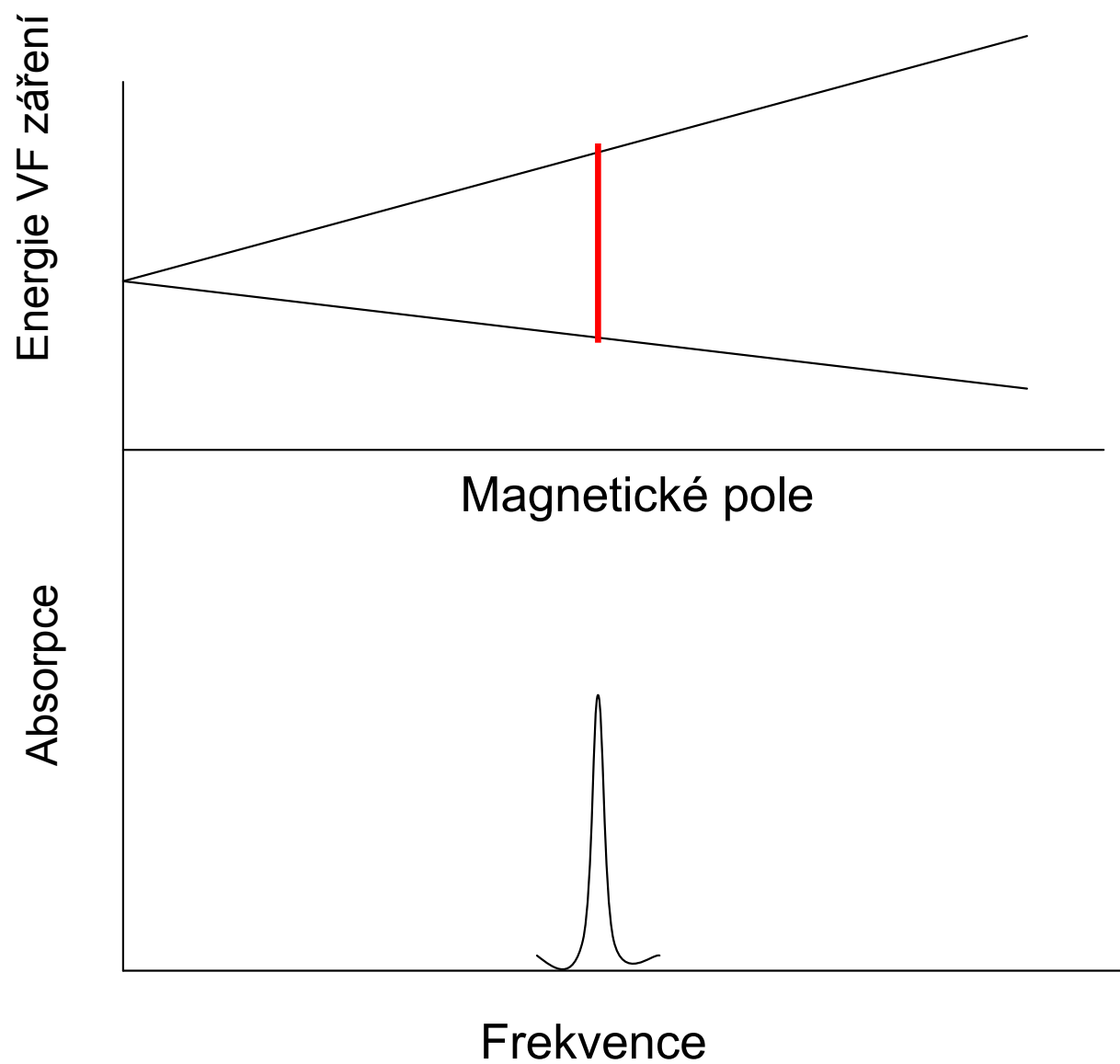
# Nukleární magnetická rezonance

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



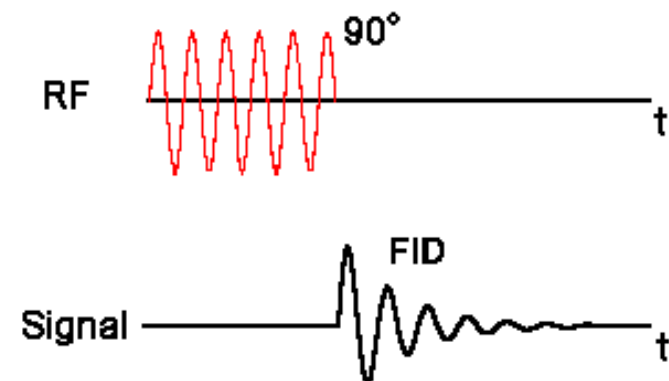
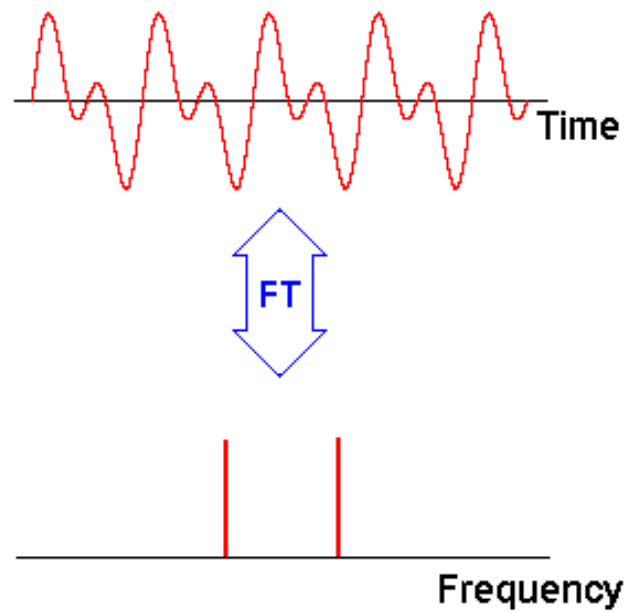
# Nukleární magnetická rezonance

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



# Nukleární magnetická rezonance

Fourrierova transformace





# Nukleární magnetická rezonance

Rozpouštědla – Deuterovaná

Acetone  $\text{CD}_3\text{COCD}_3$

Chloroform  $\text{CDCl}_3$

Methylnitrile  $\text{CD}_3\text{CN}$

Water  $\text{D}_2\text{O}$

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

Dimethyl Sulfoxide (DMSO)  $\text{CD}_3\text{SOCD}_3$

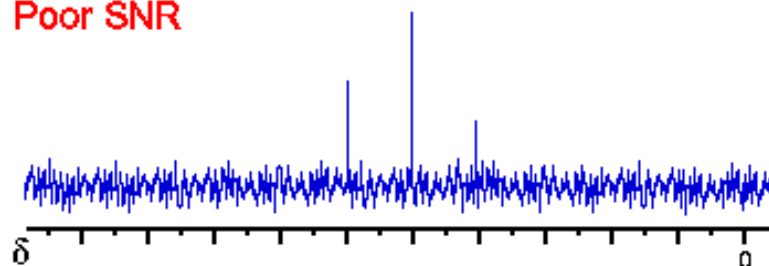
Ethanol  $\text{CD}_3\text{CD}_2\text{OD}$

Atd.

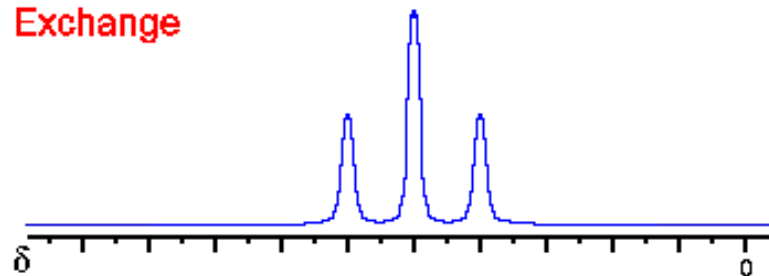
Signal-to noise ratio

Nízká a vysoká koncentrace

Poor SNR



Exchange



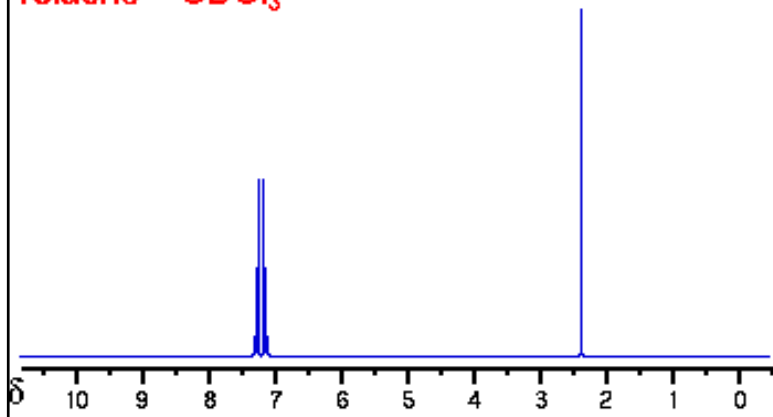
# Nukleární magnetická rezonance

## Chemické posuny

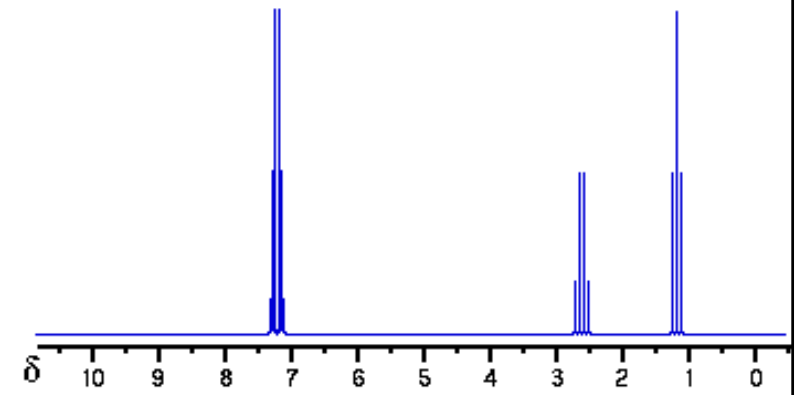
CH <sub>3</sub>	1
CH <sub>2</sub>	1,5
R <sub>2</sub> NH	0,5-2
RNH <sub>2</sub>	1,5
ArCH <sub>3</sub>	2,5
-C≡CH	3
Ar-NH <sub>2</sub>	3,5 – 4,5
X-CH <sub>3</sub>	2,5 – 3,5
R-O-CH <sub>3</sub>	4
=C=CH <sub>2</sub>	5
ArH	7-8
RCOH	10
RCOOH	10-12

# Nukleární magnetická rezonance

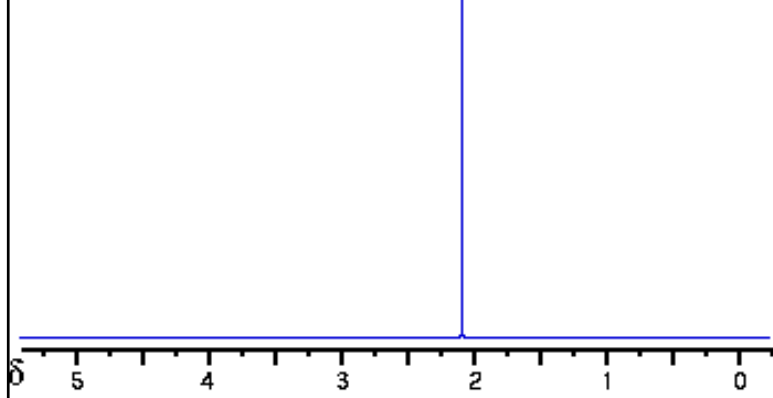
Toluene -  $\text{CDCl}_3$



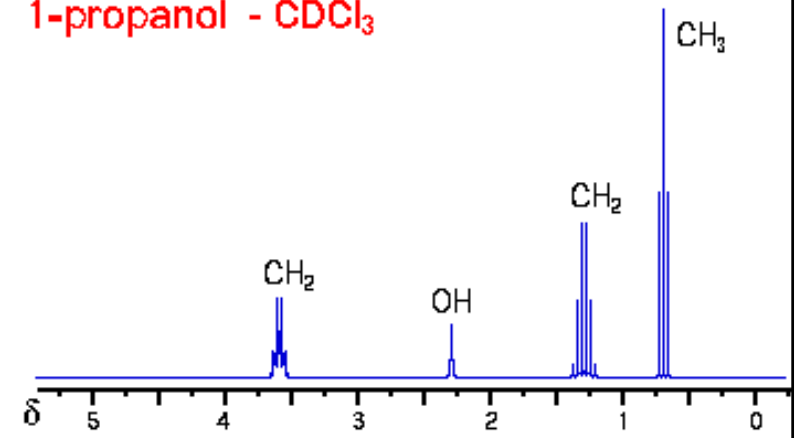
Ethyl benzene -  $\text{CDCl}_3$



Acetone -  $\text{CDCl}_3$

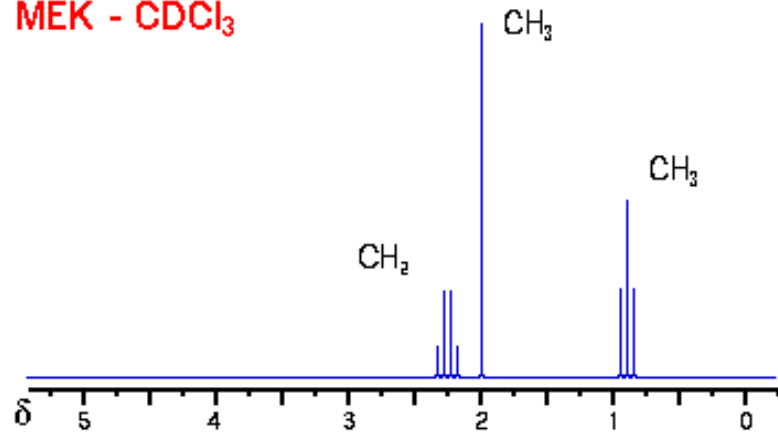


1-propanol -  $\text{CDCl}_3$

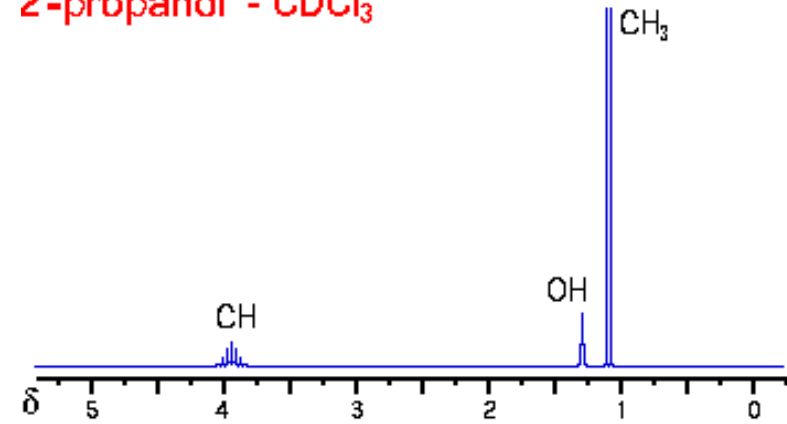


# Nukleární magnetická rezonance

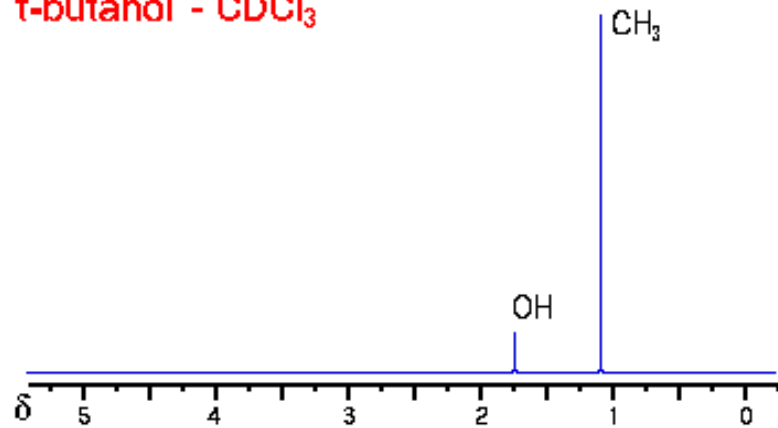
MEK -  $\text{CDCl}_3$



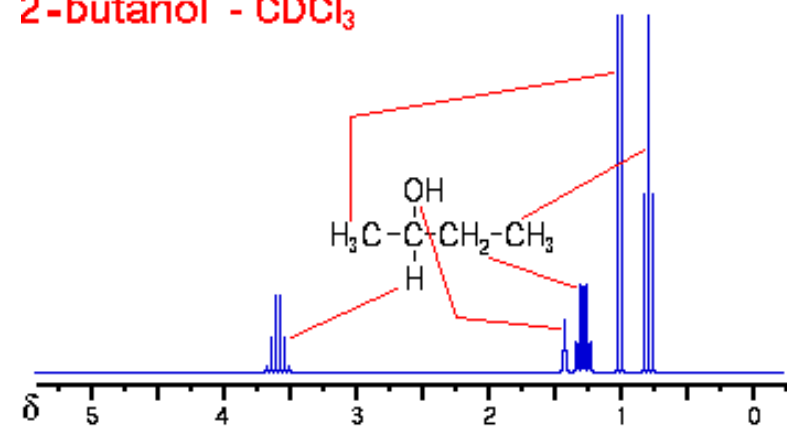
2-propanol -  $\text{CDCl}_3$



t-butanol -  $\text{CDCl}_3$

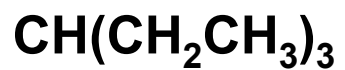
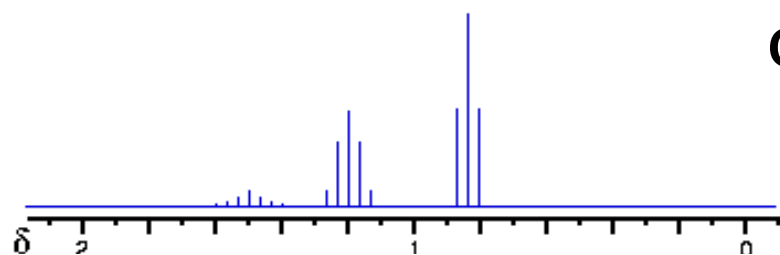


2-butanol -  $\text{CDCl}_3$

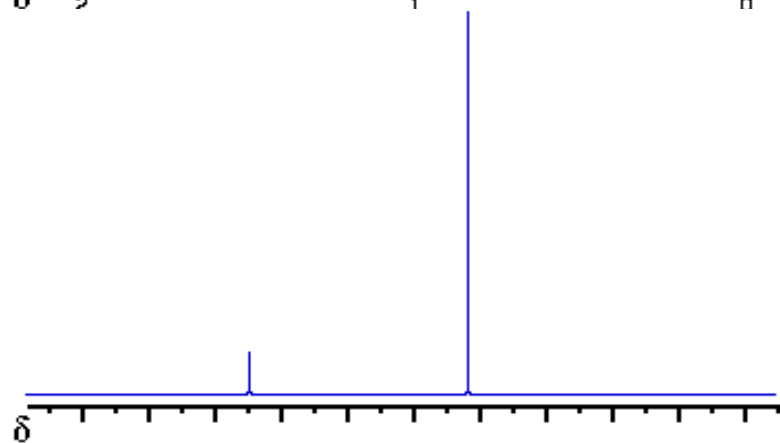


# Nukleární magnetická rezonance

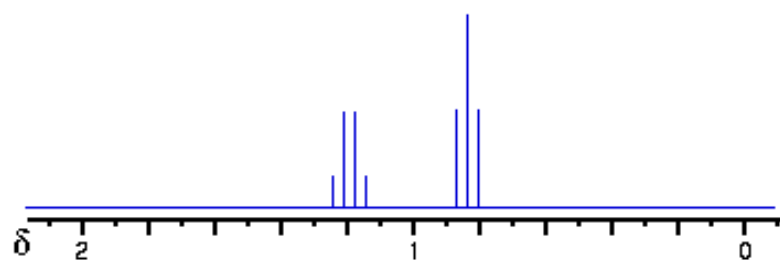
Spin-decoupling



Decoupling přes  $-\text{CH}_2-$



Decoupling přes  $-\text{CH}-$



# Nukleární magnetická rezonance- $^{13}\text{C}$

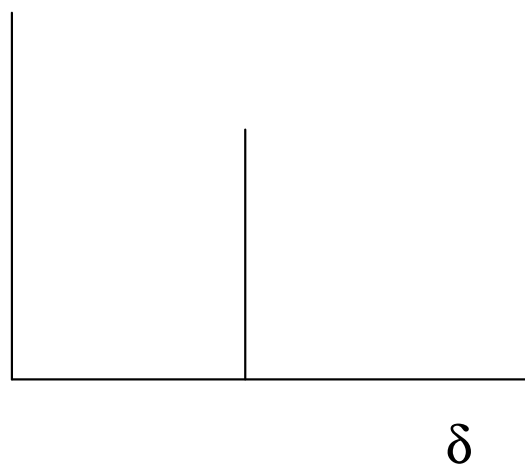
## Carbon-13 Chemical Shifts

Carbon-13* Environment	Chemical Shift Range (ppm)
$\text{CH}_3\text{CH}_2$	0-50
$-\text{C}\equiv\text{C}-$	60-100
$\equiv\text{C}-\text{X}$	70-170
$\text{C}_6\text{H}_6$	65
$\text{CHCl}$ $\text{CHCl}$ (cis)	71
$\text{Ar}$	120-150
$\text{CCl}_4$	97
$\text{COOR}$	170
$\text{COH}$	210
$\text{RCOR}$	220

# Nukleární magnetická rezonance- $^{13}\text{C}$

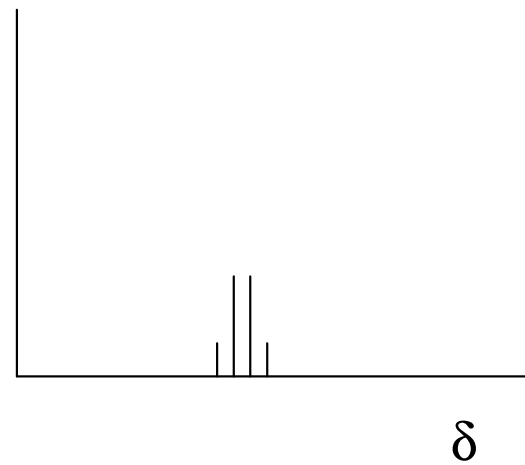
Spin-spinová interakce mezi  $^{13}\text{C}$ - $^1\text{H}$

Spektrum  $\text{CH}_3\text{I}$



# Nukleární magnetická rezonance- $^{13}\text{C}$

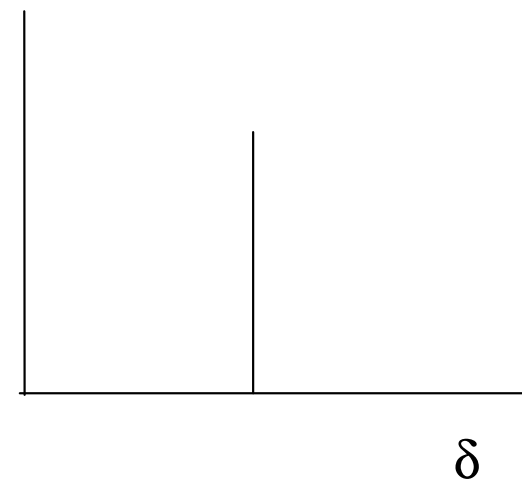
Spin-spinová interakce mezi  $^{13}\text{C}$ - $^1\text{H}$



Spektrum  $\text{CH}_3\text{I}$

Interakce C – 3H

Decoupling přes H





# Nukleární magnetická rezonance-<sup>15</sup>N



## Nitrogen-14 Chemical Shifts \_

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

# Nukleární magnetická rezonance- $^{31}\text{P}$



## Phosphorous-31 Chemical Shifts \_

<b>Phosphorous-31 Environment</b>	<b>Chemical Shift Range (ppm)</b>
$\text{PBr}_3$	-228
$(\text{C}_2\text{H}_5\text{O})_3\text{P}$	-137
$\text{PF}_3$	-97
85% phosphoric acid	0
$\text{PCl}_5$	80
$\text{PH}_3$	238
$\text{P}_4$	450

# Nukleární magnetická rezonance- $^{31}\text{P}$

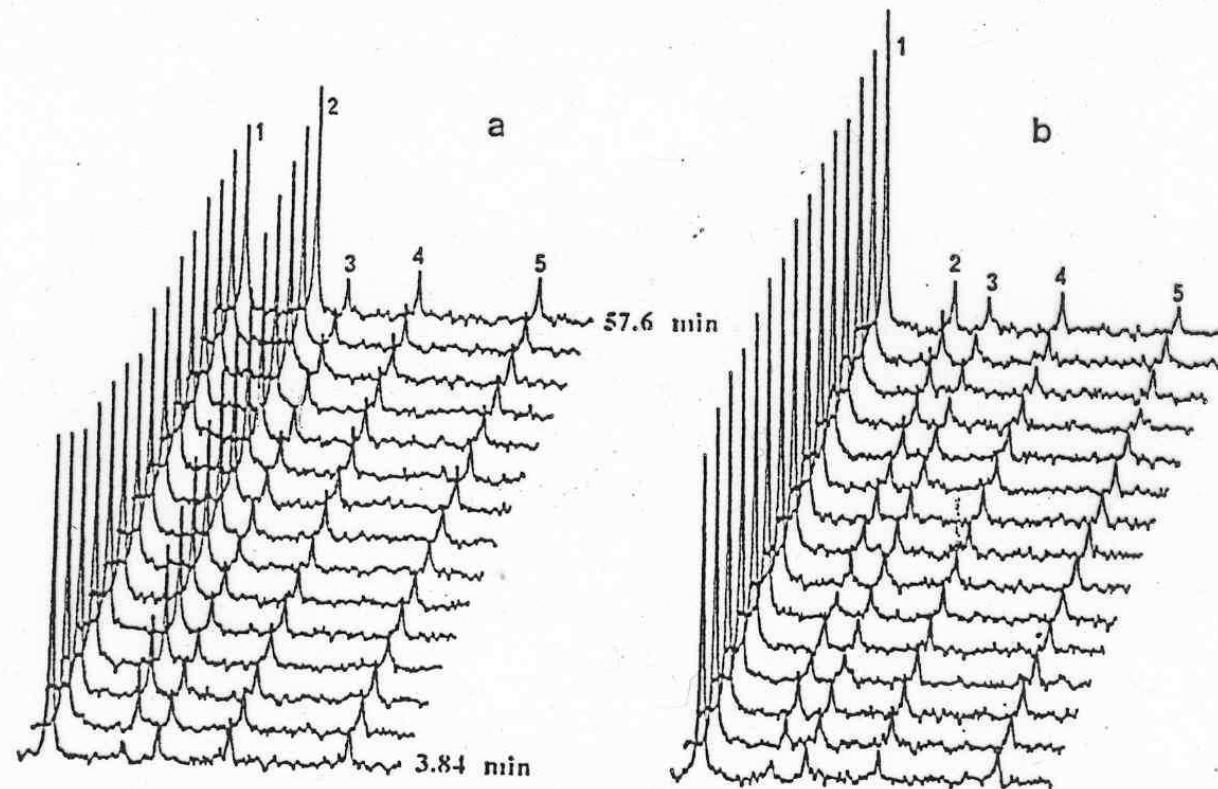


FIG. 1. Phosphocreatine synthesis by isolated rat skeletal muscle mitochondria followed by  $^{31}\text{P}$  NMR spectroscopy: two series of typical spectra obtained during one hour with mitochondria (a) in the presence of 1 mM ATP or (b) in the presence of 1 mM ATP and 7 nmole/mg proteins of atractyloside.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

# Nukleární magnetická rezonance- $^{31}\text{P}$

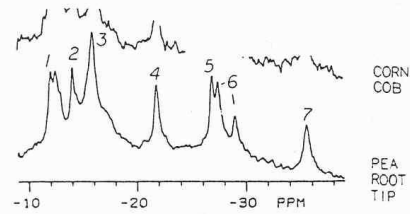


Fig. 12.  $^{31}\text{P}$ -NMR spectra of living plant tissues, under oxygenated conditions. Peak assignments: 1, Glc-6-P; 2, cytoplasmic  $\text{P}_i$ ; 3, vacuolar  $\text{P}_i$ ; 4,  $\gamma\text{ATP}$ ; 5,  $\alpha\text{ATP}$ ; 6, UDPG and nicotinamide adenine nucleotides; 7,  $\beta\text{ATP}$ .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

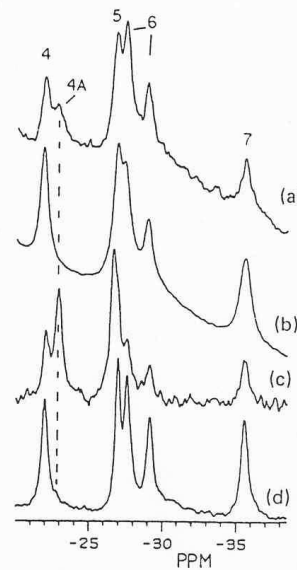


Fig. 19.  $^{31}\text{P}$ -NMR partial spectra of the nucleotide region of (a) hypoxic maize root tips; (b) normoxic maize root tips; (c) extract of normoxic maize root tips, to which commercial ADP has been added; (d) extract of normoxic maize root tips, prior to addition of ADP. Peak assignments as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

# Nukleární magnetická rezonance- $^{31}\text{P}$

Měření intracelulárního pH  
Pomocí posunu signálu  
 $^{31}\text{P}$

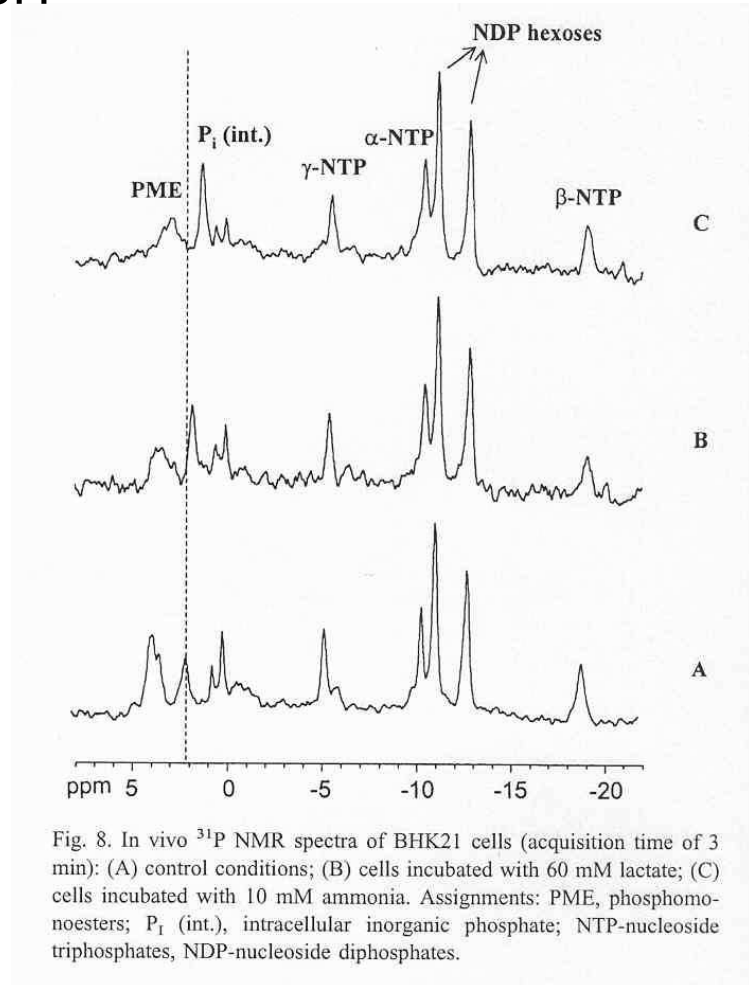


Fig. 8. In vivo  $^{31}\text{P}$  NMR spectra of BHK21 cells (acquisition time of 3 min): (A) control conditions; (B) cells incubated with 60 mM lactate; (C) cells incubated with 10 mM ammonia. Assignments: PME, phosphomonoesters;  $\text{P}_i$  (int.), intracellular inorganic phosphate; NTP-nucleoside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

# Nukleární magnetická rezonance- $^{31}\text{P}$

Měření koncentrace nukleotidů  
Pomocí  $^{31}\text{P}$

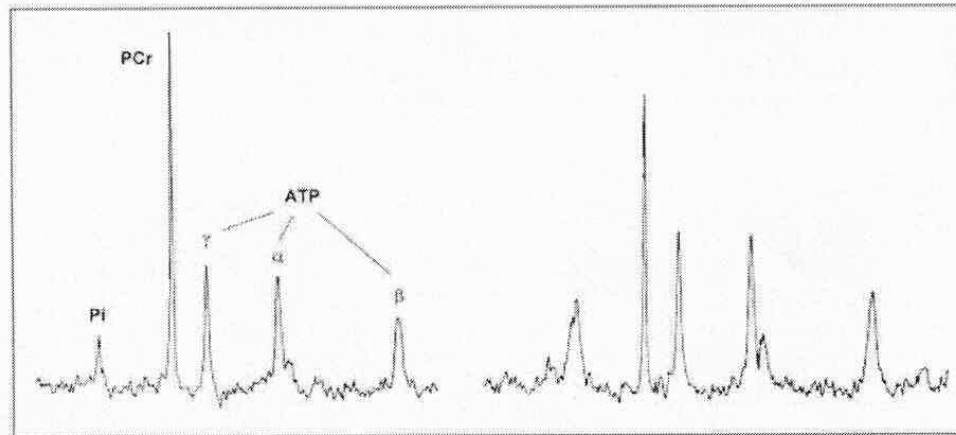


Figure 3 Typical  $^{31}\text{P}$  NMR spectra from an isolated rat heart perfused with glucose only (right) or glucose and pyruvate (left). The major resonances, from left to right, report the relative heart contents of inorganic phosphate (Pi), PCr and the three phosphates of ATP ( $\gamma$ ,  $\alpha$  and  $\beta$ ). 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- $^{31}\text{P}$

Měření koncentrace  
nukleotidů  
Pomocí  $^{31}\text{P}$

IAA-iodacetát

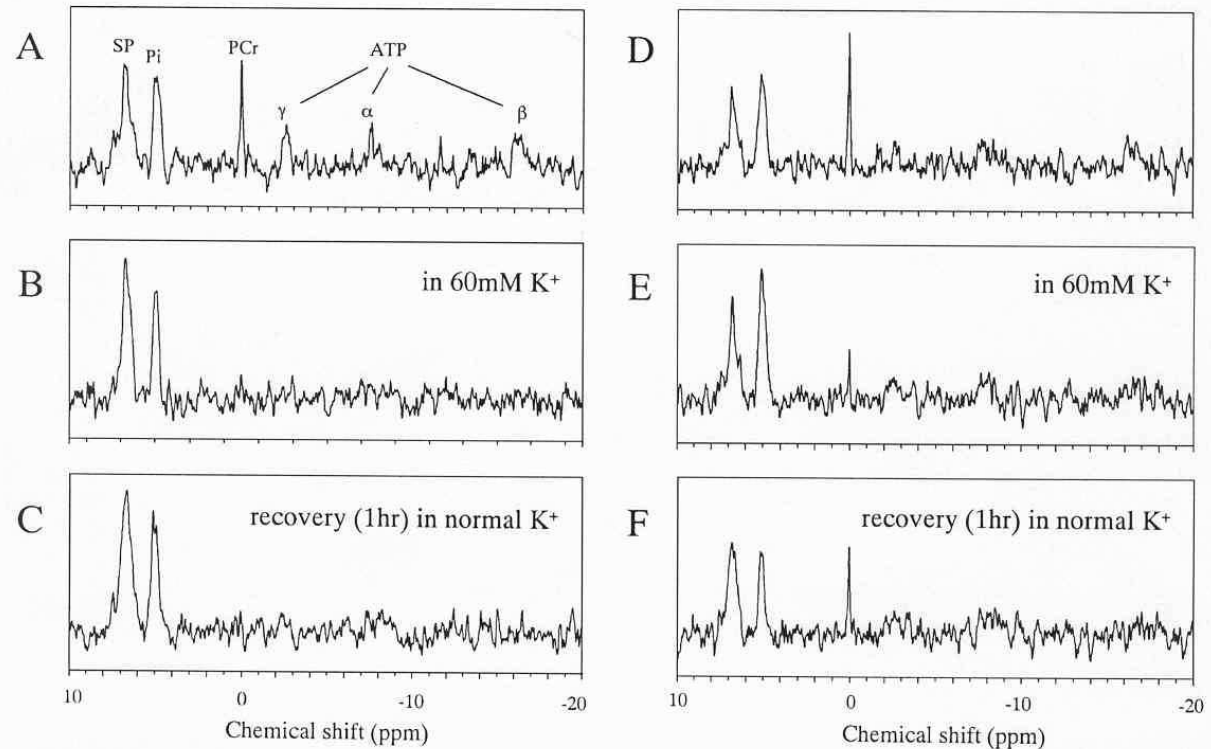


Fig. 1.  $^{31}\text{P}$  NMR spectra of well-oxygenated rat brain slices pretreated with IAA and FC (IAA-pretreated neuron-rich slices) in ACSF containing lactate at 25 °C. A-C:  $^{31}\text{P}$  NMR spectra from intact slices; A, under resting condition in normal  $\text{K}^+$  ACSF; B, during high- $\text{K}^+$  stimulation for 8 min; C, after 60 min in normal  $\text{K}^+$  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;  $\text{P}_i$ , inorganic phosphates;  $\gamma$ -,  $\alpha$ -, and  $\beta$ -, 3 peaks of ATP.

# Nukleární magnetická rezonance- $^{13}\text{C}$

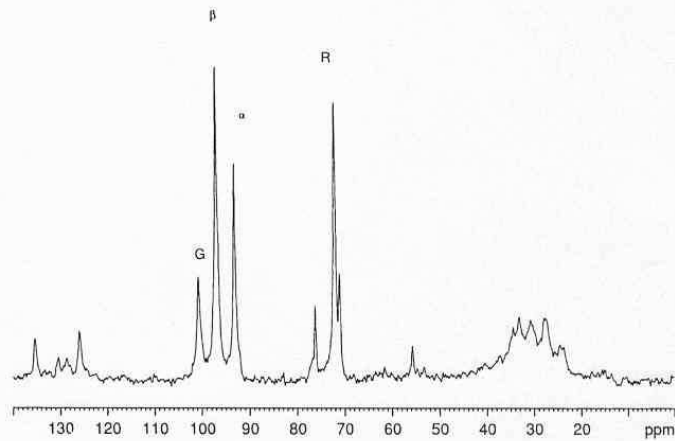


Fig. 7. Representative  $^{13}\text{C}$  NMR spectrum acquired from a perfused rat heart under preischemic conditions after 30 min of labeled perfusion. The abbreviations denote  $[1-^{13}\text{C}]$ glycogen (G), the  $\alpha$ - and  $\beta$ -anomers of  $[1-^{13}\text{C}]$ glucose ( $\alpha$  and  $\beta$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

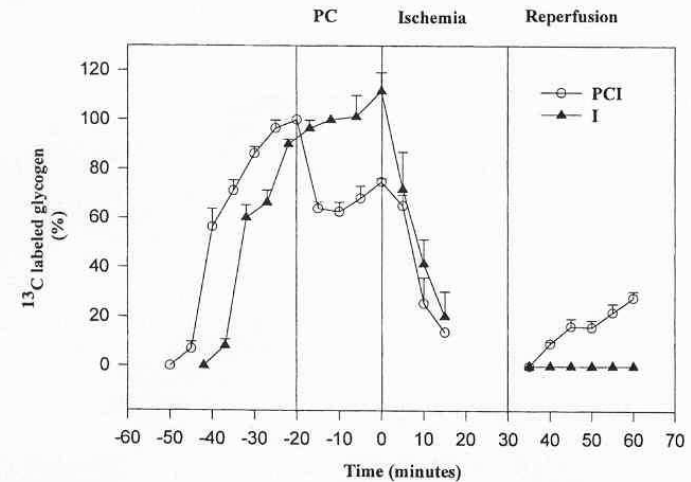


Fig. 8.  $^{13}\text{C}$ -Labeling and mobilization of  $[1-^{13}\text{C}]$ glycogen during protocol set I in preconditioned hearts (PCI,  $n=6$ ,  $\circ$ ) and ischemic controls (I,  $n=6$ ,  $\blacktriangle$ ). The labeled perfusion started at  $t=-50$  min for the preconditioned hearts and  $t=-42$  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- $^{15}\text{N}$

Asimilace amoniaku sledovaná  
Pomocí  $^{15}\text{N}$

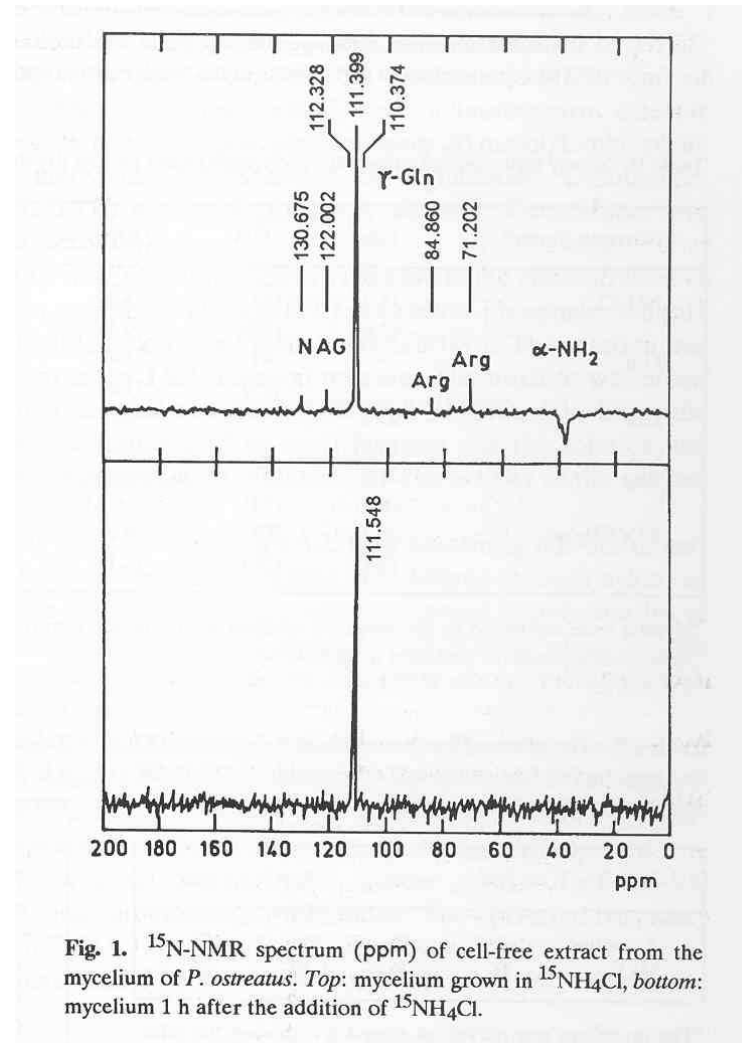
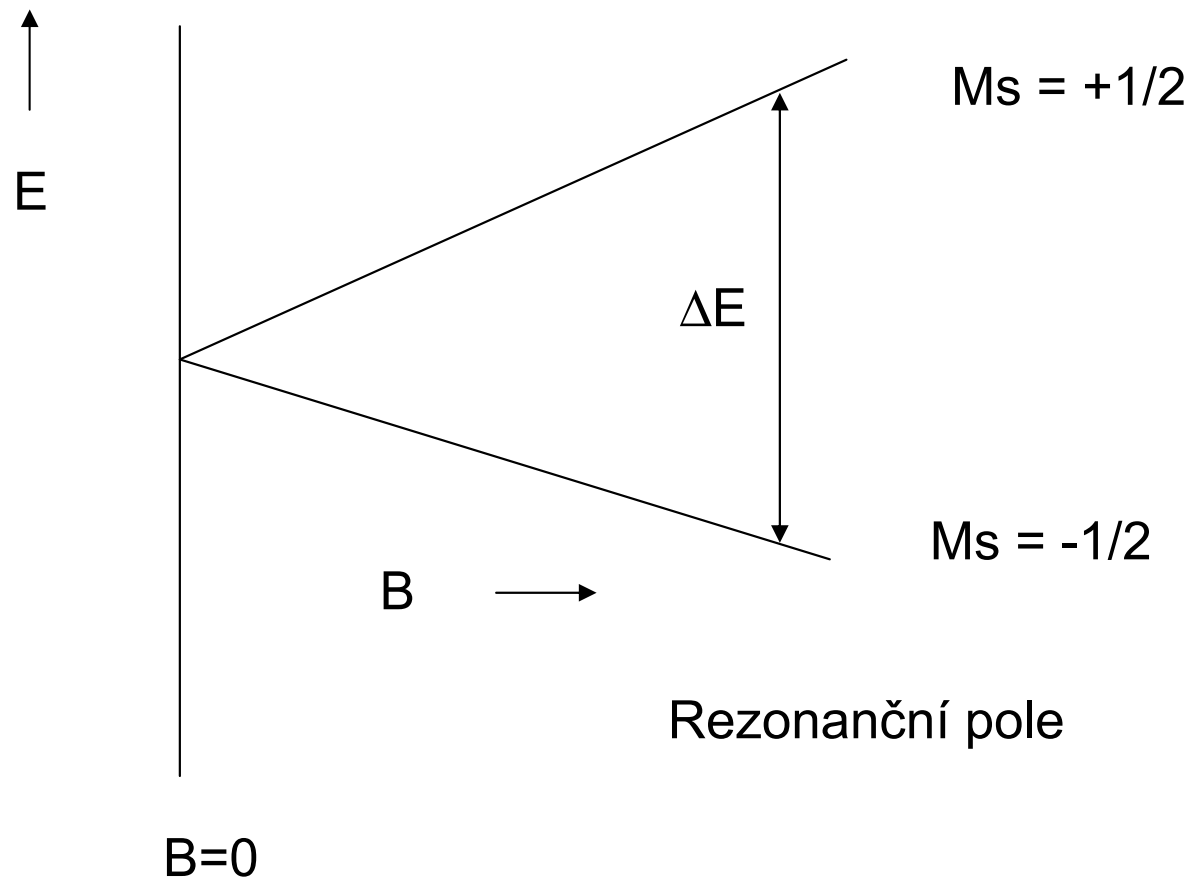


Fig. 1.  $^{15}\text{N}$ -NMR spectrum (ppm) of cell-free extract from the mycelium of *P. ostreatus*. Top: mycelium grown in  $^{15}\text{NH}_4\text{Cl}$ , bottom: mycelium 1 h after the addition of  $^{15}\text{NH}_4\text{Cl}$ .

# EPR – elektronová spinová rezonance

Nepárový elektron



# EPR – elektronová spinová rezonance

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

$\Delta E$  rozdíl energií

$h$  is Planck constant

$\nu$  is frekvence mikrovln

$g$  faktor spin-orbitální interakce

$B$  magnetické pole

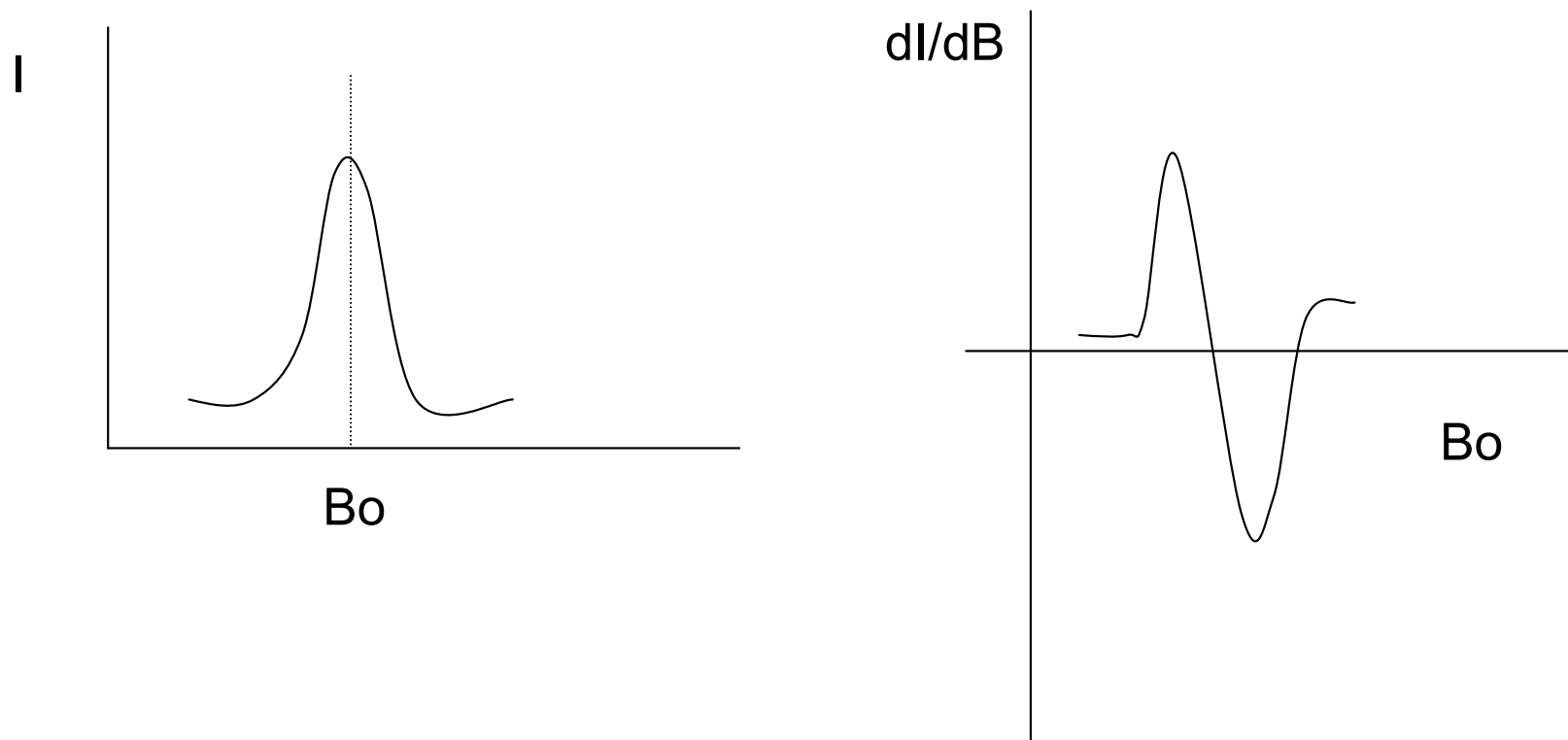
Počet jader v jednotlivých orientacích

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

$$g_{EPR} = 2,0036$$

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

# EPR – elektronová spinová rezonance



# EPR – elektronová spinová rezonance

Interakce s protony

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

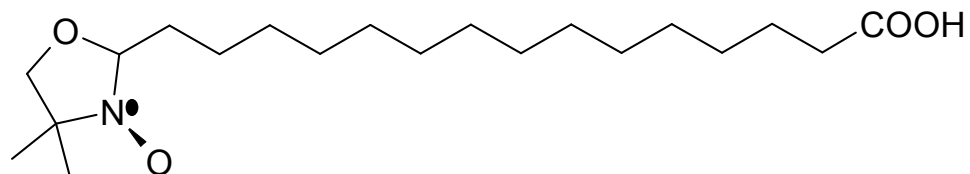
1:2:1

1:3:3:1

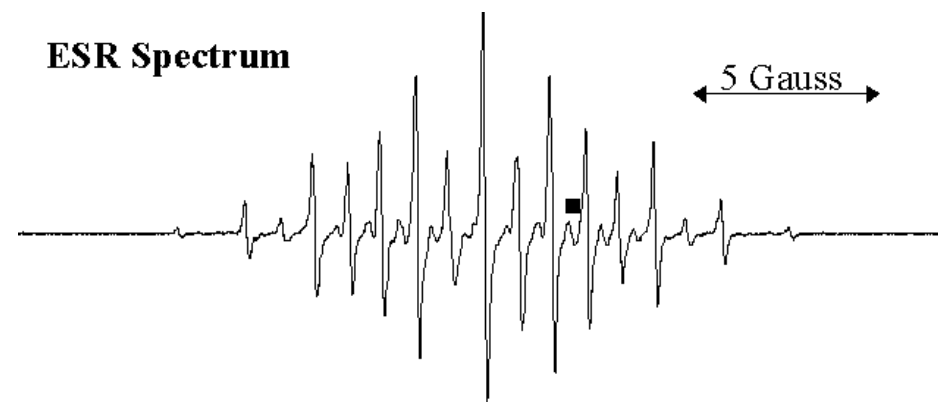
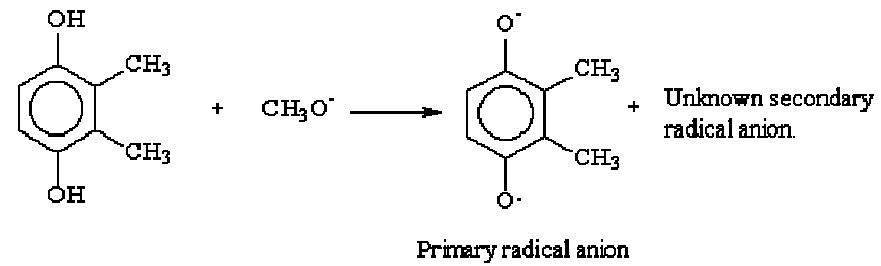
# EPR – elektronová spinová rezonance

Využití v biochemii

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

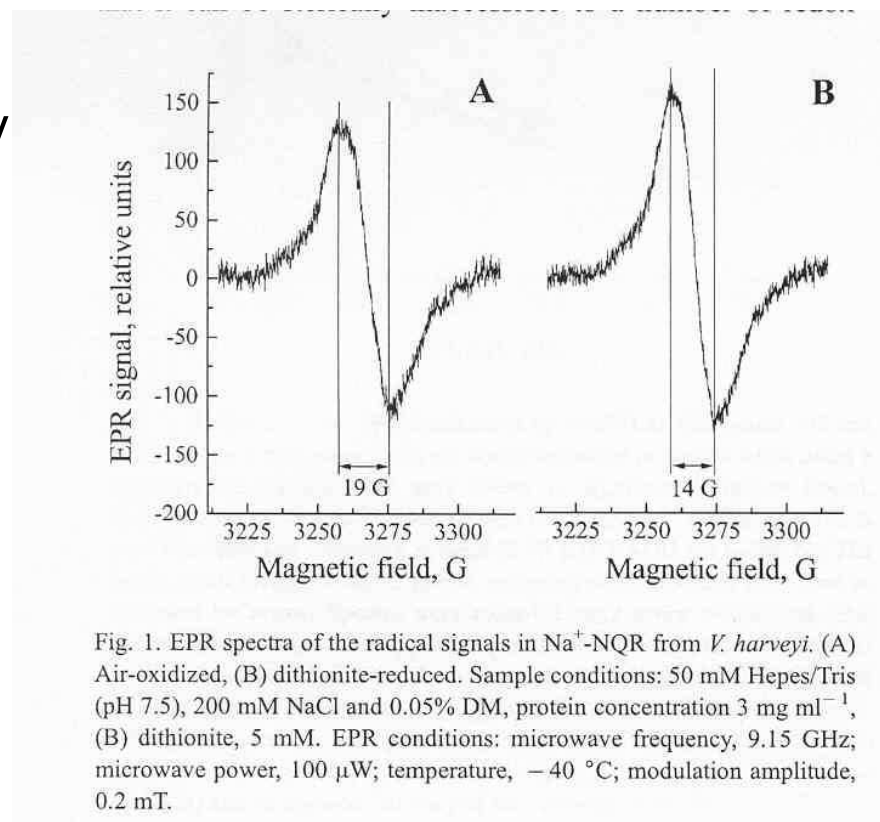


# EPR – elektronová spinová rezonance



# EPR – elektronová spinová rezonance

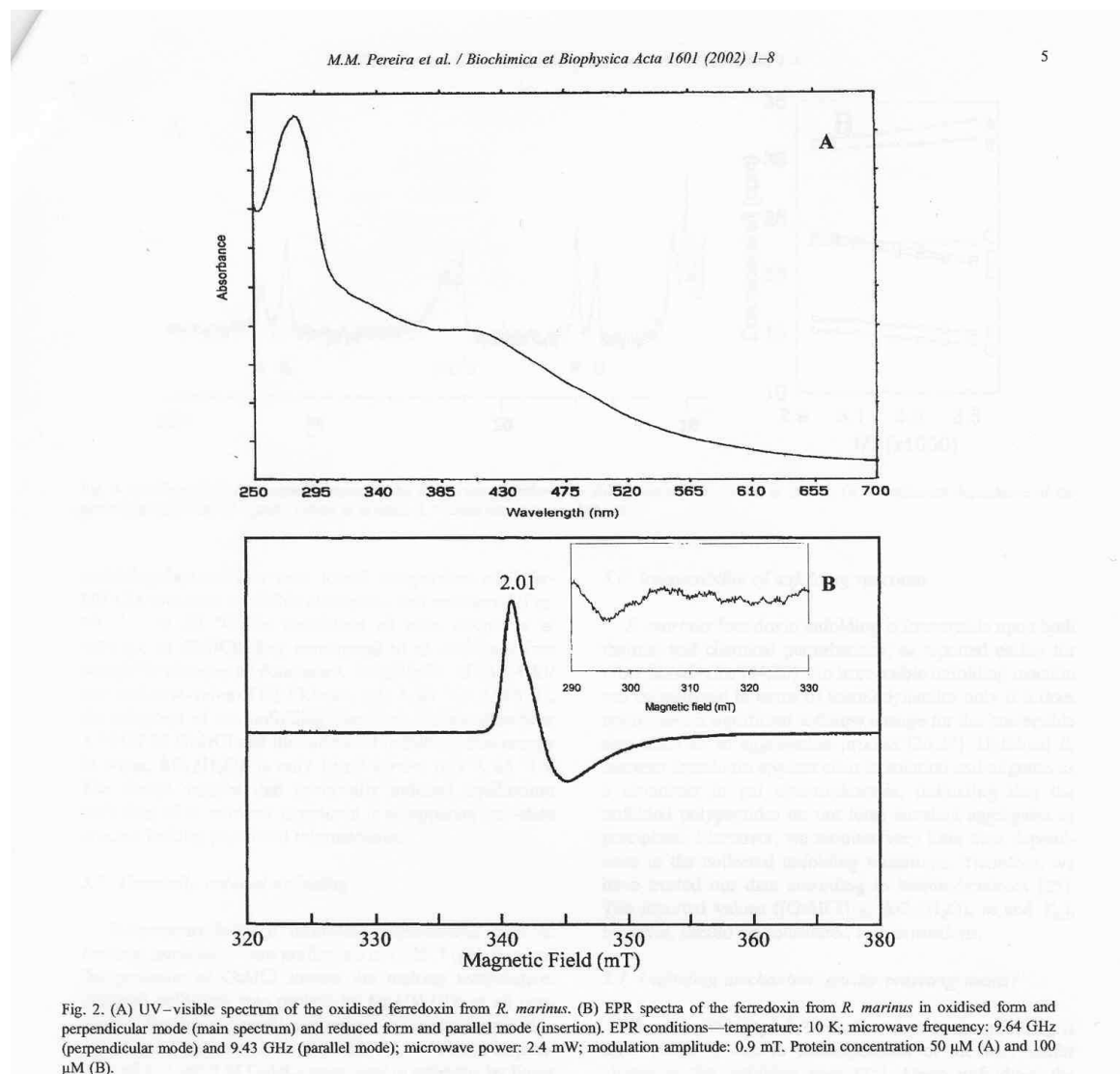
EPR spektrum NADH-UQ reduktasy  
signál UQ  
Signál 2Fe2S potlačen,  
Signál FMN radikálu interferuje





# EPR – elektronová spinová rezonance

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



# EPR – elektronová spinová rezonance

EPR spektrum cyt bc1 – mutant  
M183K, M183H

Fe<sup>3+</sup> hem (3,78 = cyt bL 3,44, cyt  
bH,  
2,94 bis-His cyt b nebo c1)

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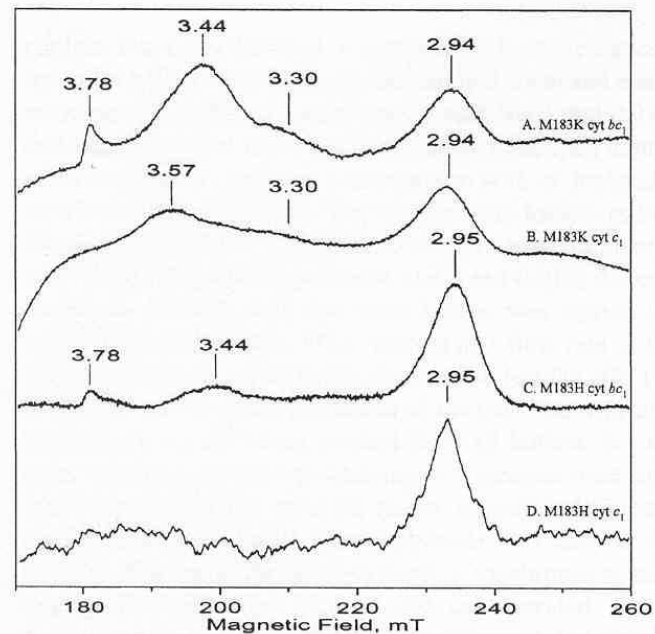


Fig. 2. X-band EPR spectra of *Rb. capsulatus* cytochromes. (A) Cytochrome  $bc_1$  complex containing M183K cytochrome  $c_1$  (39  $\mu\text{M}$ ). (B) Purified M183K cytochrome  $c_1$  (148  $\mu\text{M}$ ). (C) Cytochrome  $bc_1$  complex containing M183H cytochrome  $c_1$  (29  $\mu\text{M}$ ). (D) Purified M183H cytochrome  $c_1$  (24  $\mu\text{M}$ ). 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 H<sub>2</sub>O<sub>2</sub>

Spektrum radikálu tokoferolu (A)

