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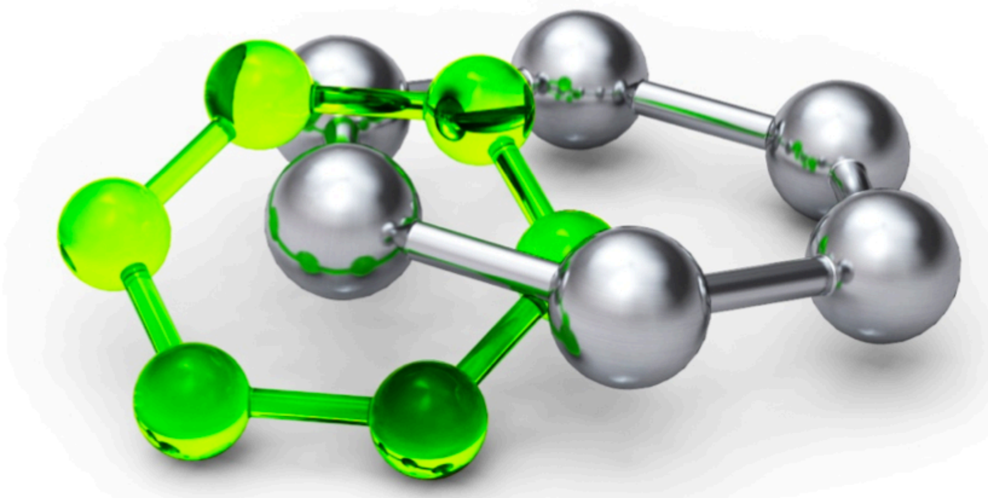

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2007–13
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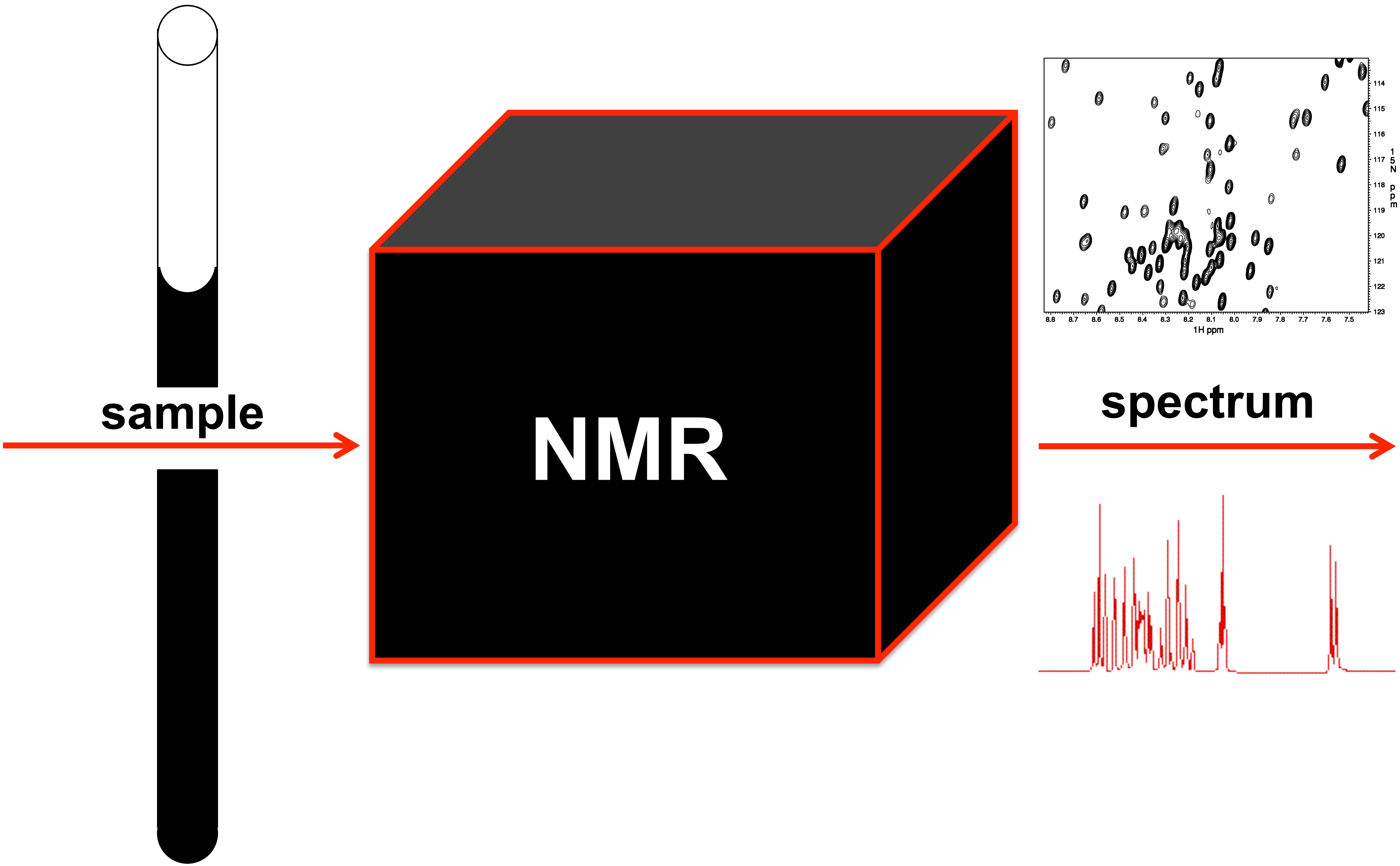
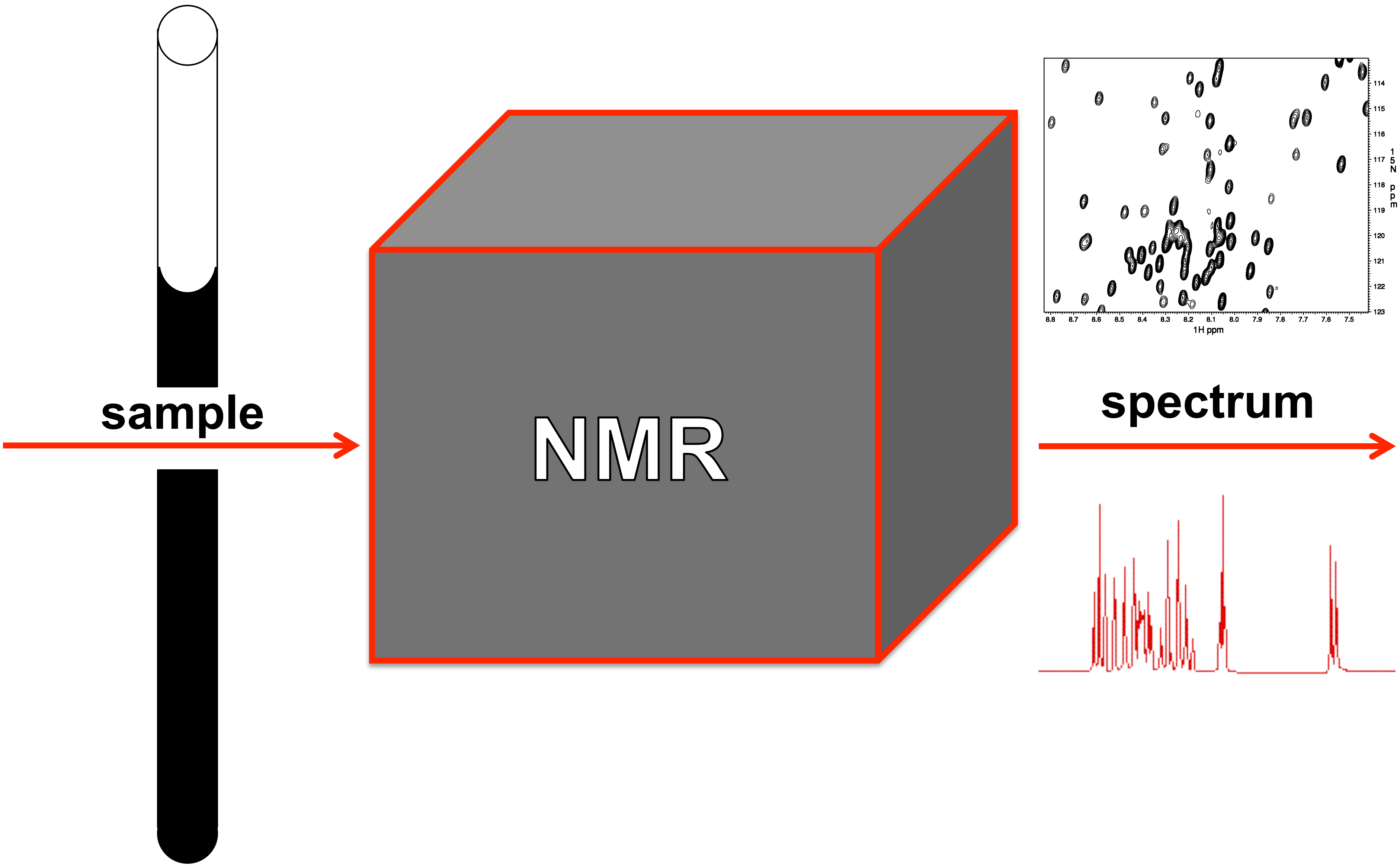
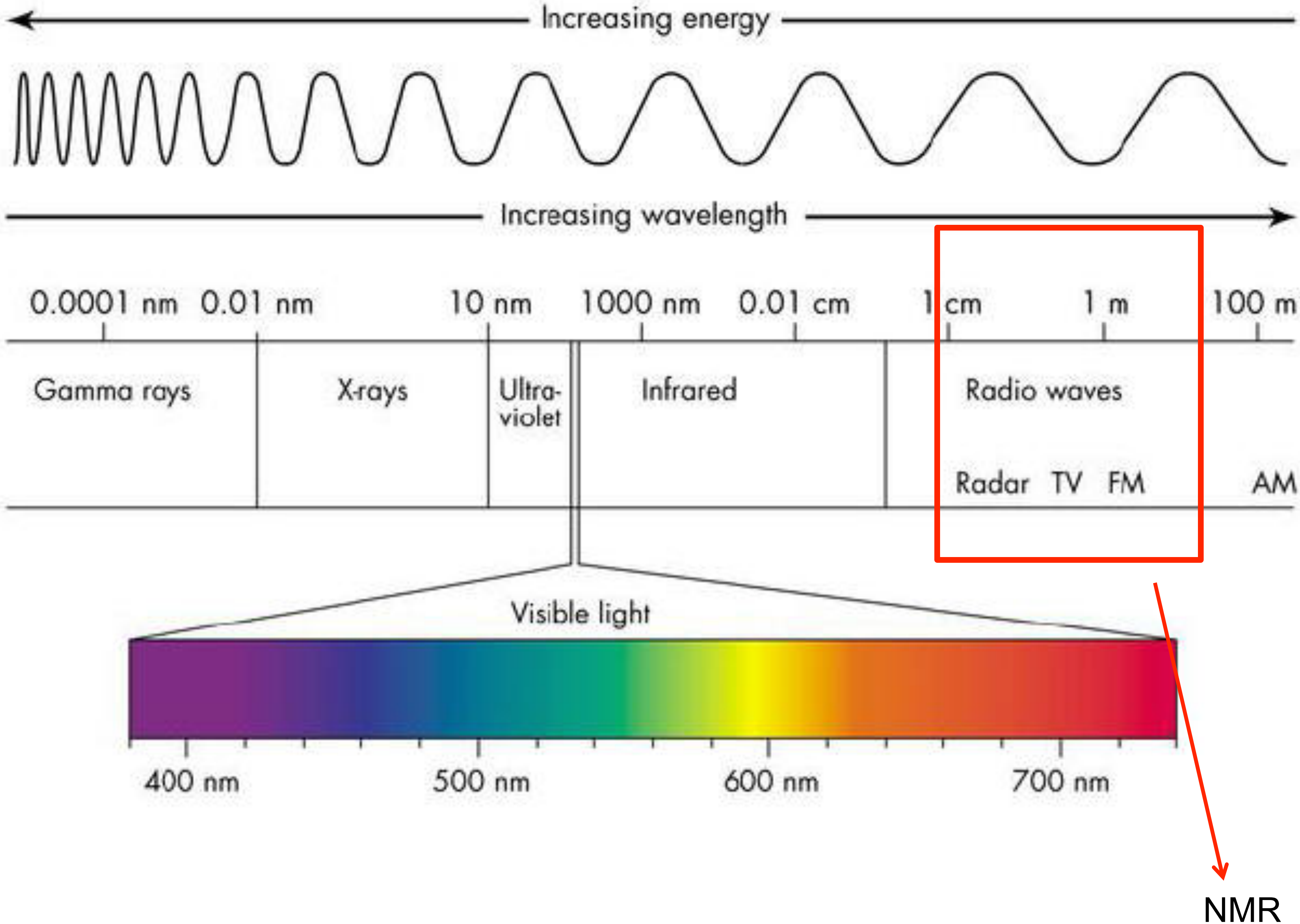
Obsah semináře

1) Úvod
2) Hardware – magnet, konsole (spektrometr)
3) Měření - Spiny
4) Zpracování spekter
5) NMR biomolekul
6) Interakce měřené pomocí NMR
7) Komplementární techniky
8) Závěr


sample
NMR
spectrum
1H ppm
15N ppm
8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5
114 115 116 117 118 119 120 121 122 123



sample
NMR
spectrum
1H ppm
15N ppm


Increasing energy
Increasing wavelength
0.0001 nm
0.01 nm
10 nm
1000 nm
0.01 cm
1 cm
1 m
100 m
Gamma rays
X-rays
Ultra-
violet
Infrared
Radio waves
Radar
TV
FM
AM
Visible light
400 nm
500 nm
600 nm
700 nm
NMR

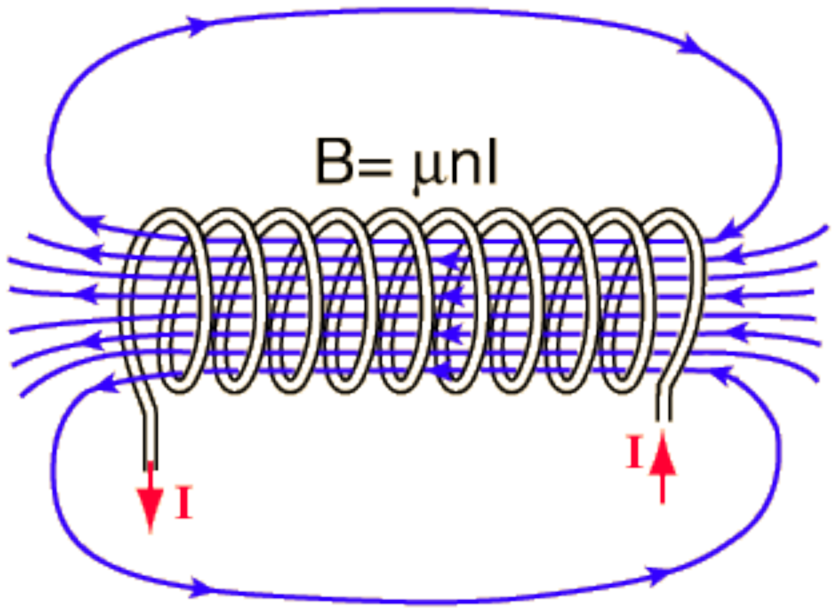
NMR hardware

1) Magnet
2) Spektrometr
3) Ovládací zařízení

NMR spektrometr

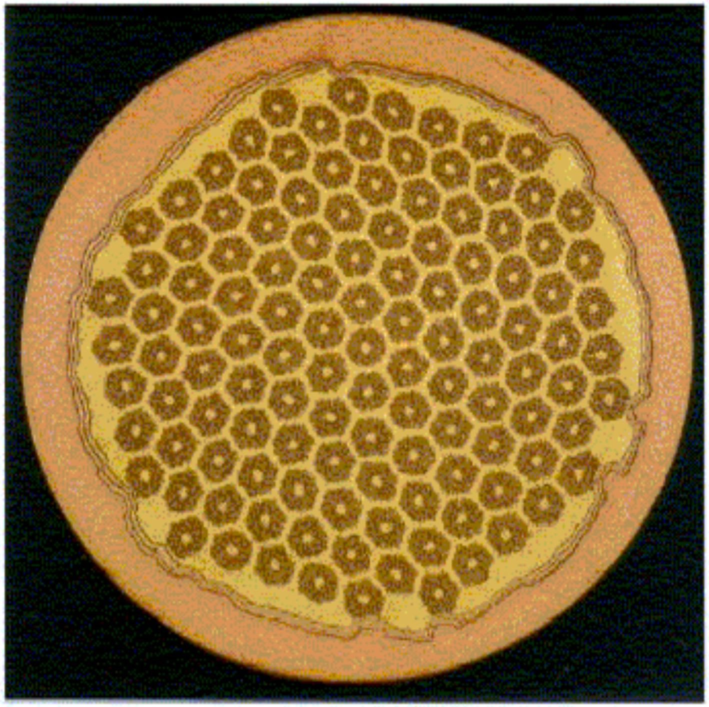
Magnetické pole země

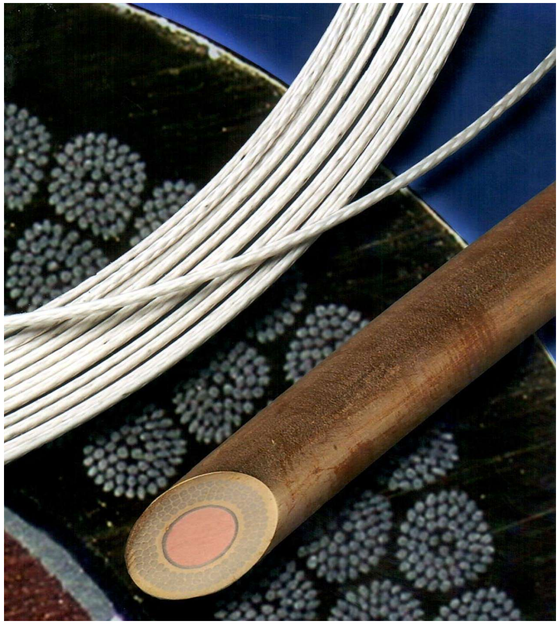
~ 50μT

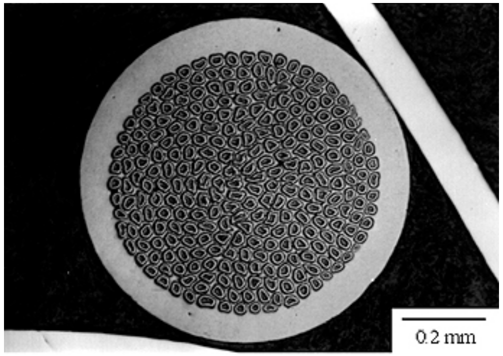


The magnetic field is concentrated into a nearly uniform field in the center of a long solenoid. The field outside is weak and divergent.

Magnet
- supravodivé solenoidy na bázi Nb a Sn ponořené do heliové a dusíkové lázně
- He-lázeň **~4 K** dále snížena J-T pumpou na **~2.1 K**
- v současnosti až 22 Tesla

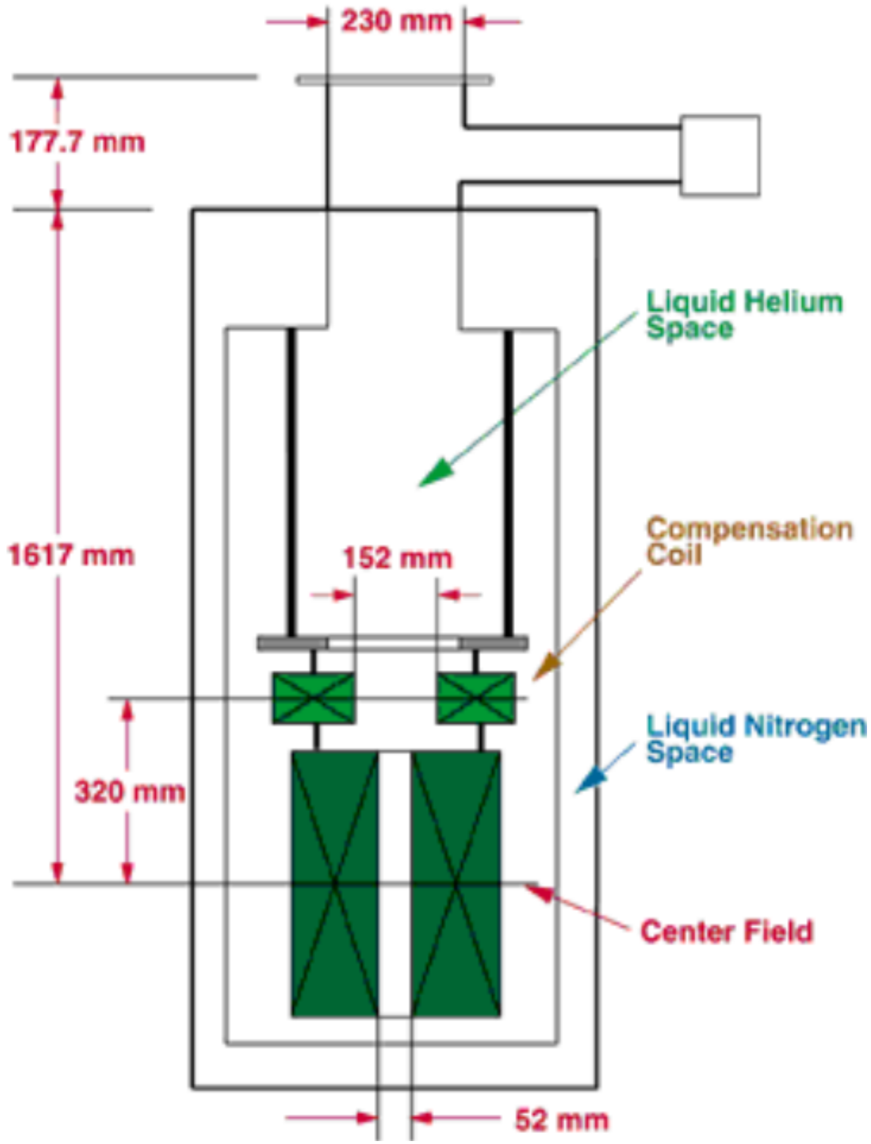


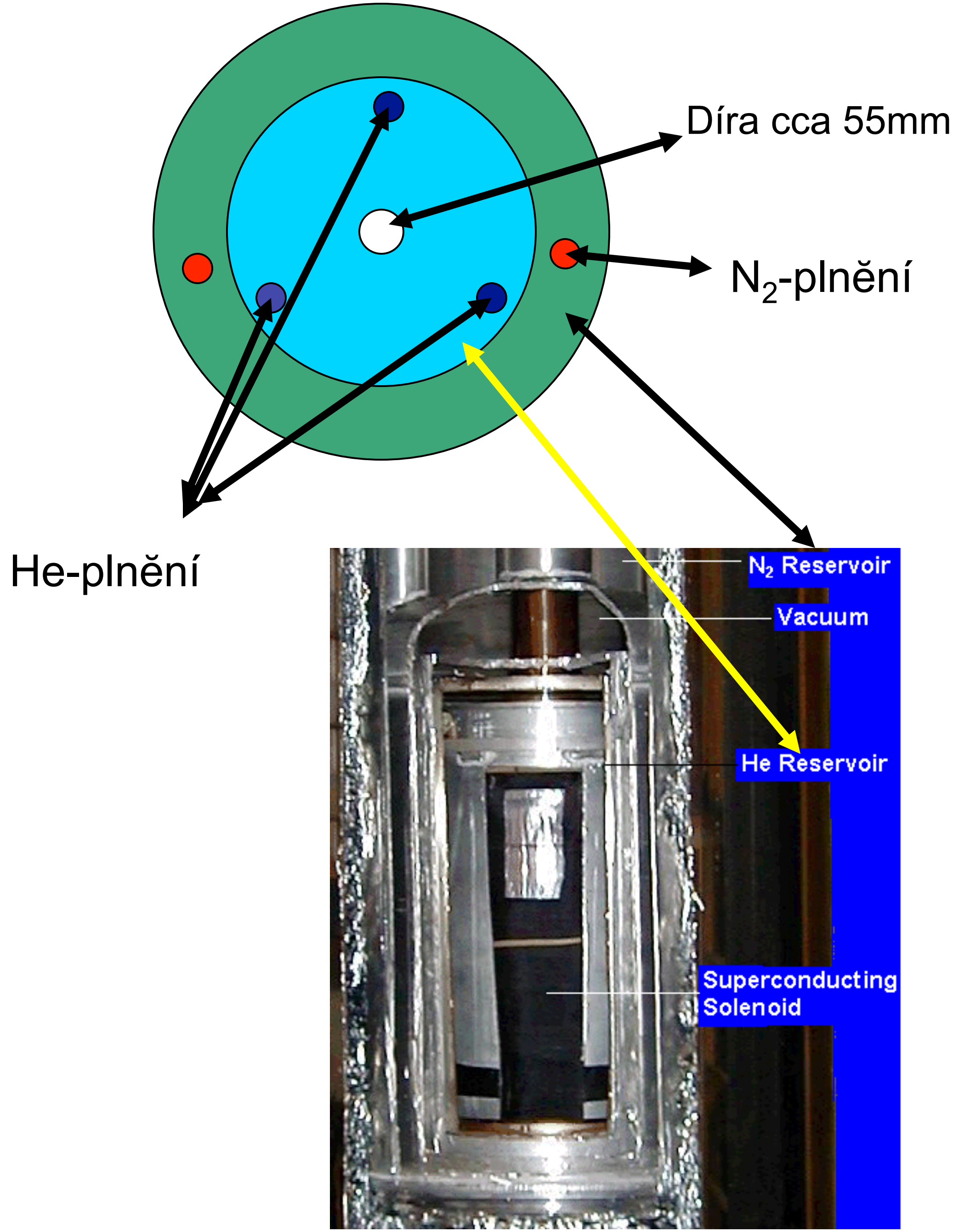




$(Nb, Ta)_3Sn$ supravodič o šířce 0.81 mm s 271 vlákny vnořenými do OFHC měděné matrice

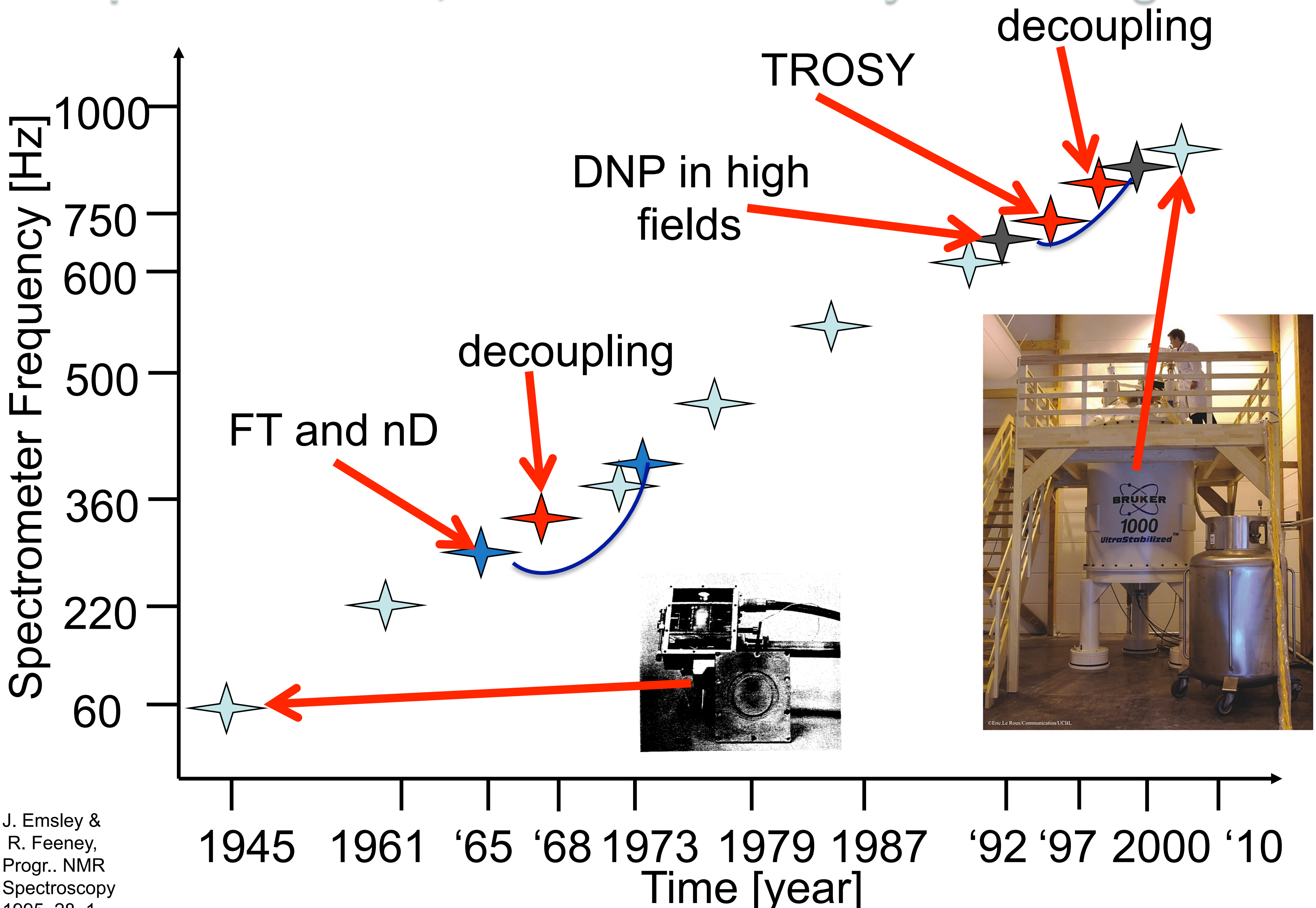
20T Superconducting Magnet





Díra cca 55mm
N_2-plnění
He-plnění
N_2 Reservoir
Vacuum
He Reservoir
Superconducting Solenoid

Zlepšení citlivosti, rozlišení a intenzity NMR magnetů



J. Emsley & R. Feeney, Progr.. NMR Spectroscopy 1995, 28, 1

Quench

an **abnormal** termination of magnet operation

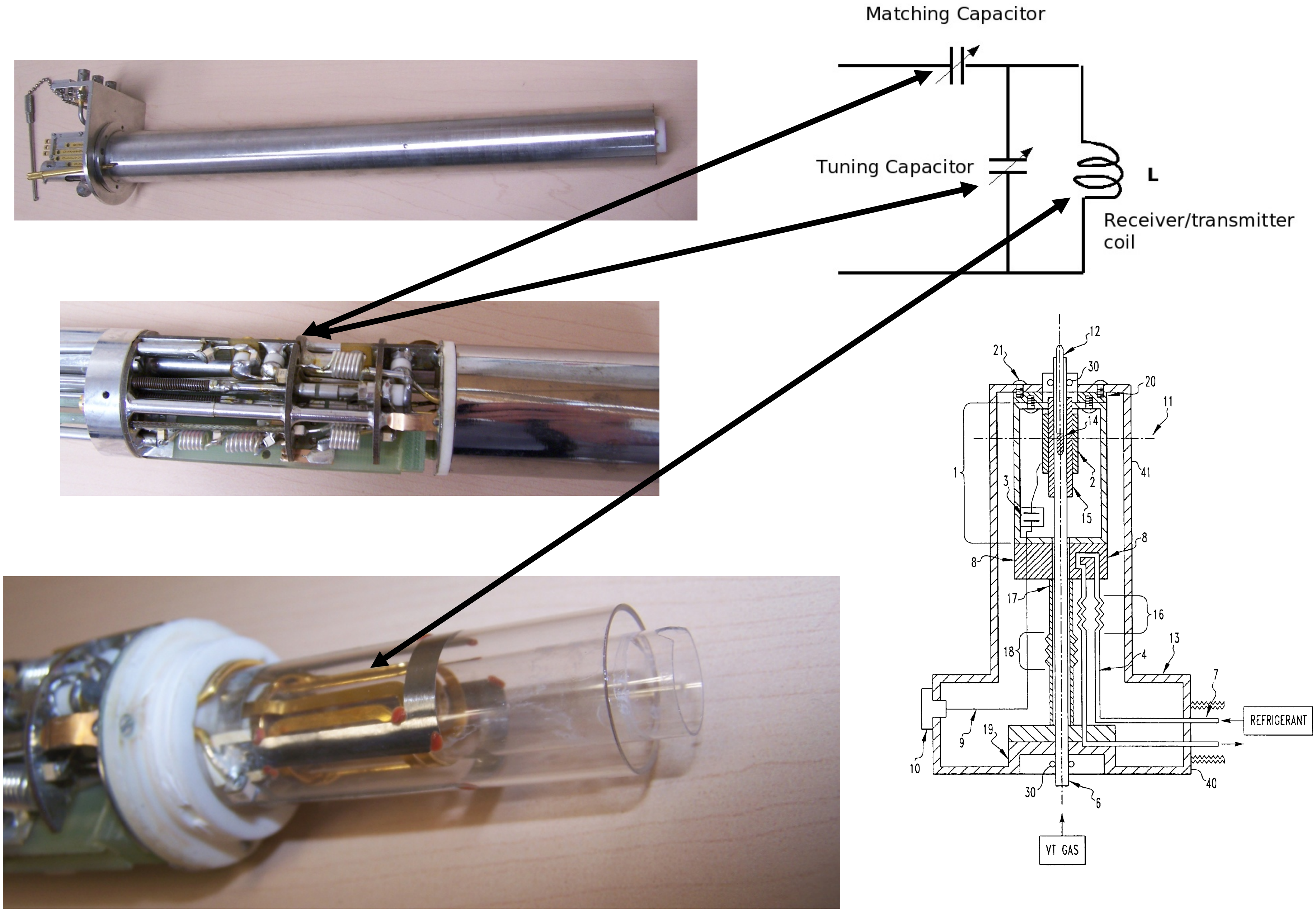
Occurs when part of the superconducting coil enters the normal (resistive) state.

This can **occur**

i) because the field inside the magnet is too large
ii) the rate of change of field is too large (causing eddy currents and resultant heating in the copper support matrix)
iii) or a combination of the two.
iv) a defect in the magnet can cause a quench.

MOVIE

NMR Sonda


Matching Capacitor
Tuning Capacitor
L
Receiver/transmitter coil
REFRIGERANT
VT GAS

Spektrometr

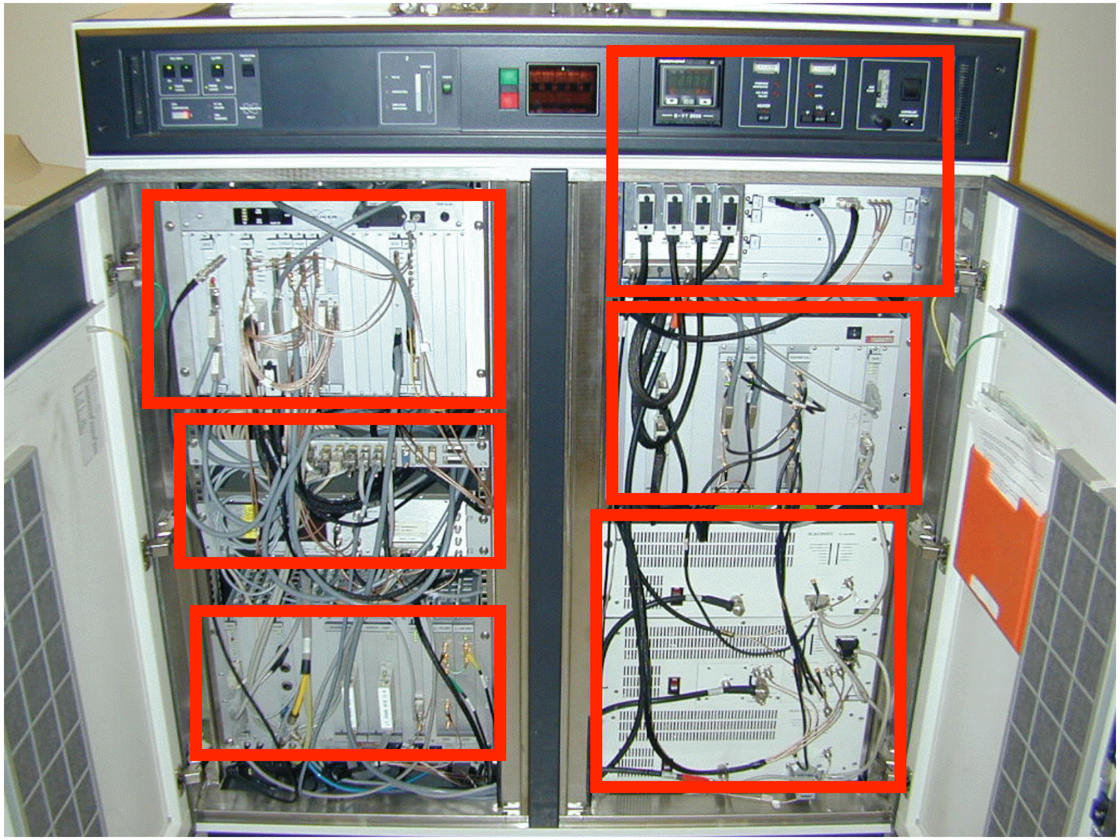
CBU

Control board unit

FGU

Frequency gen. u.

Shimms

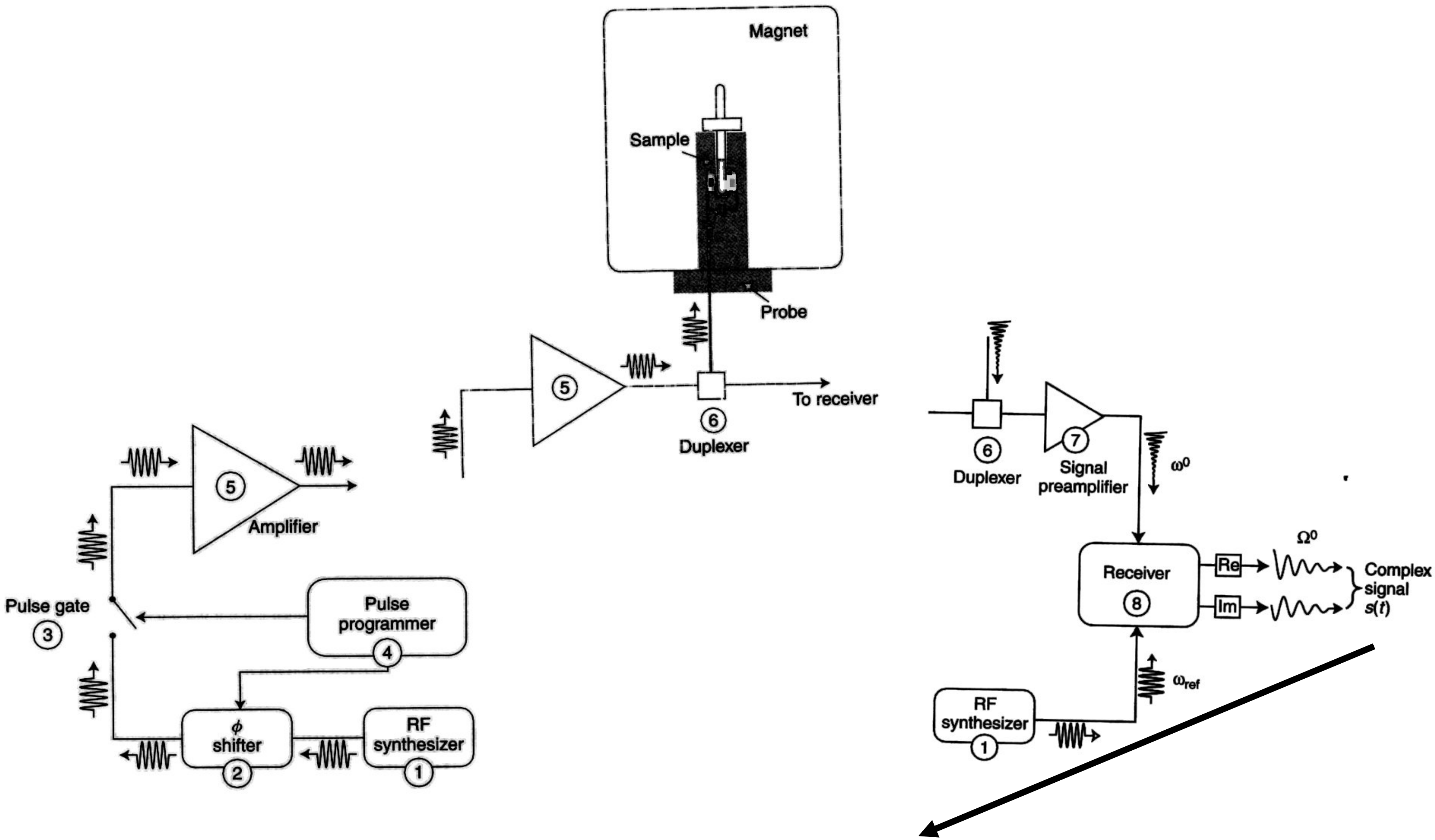


Temperature Unit

AcquisitionControler

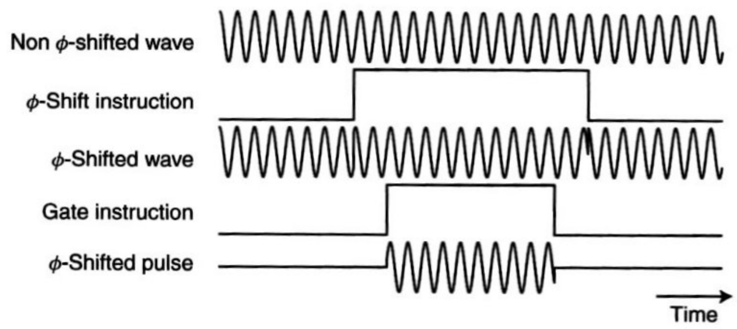
Transmitter

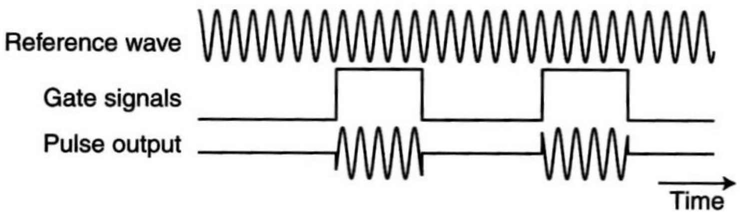
Spektrometr - přehled



Signál - $s_I(t)=\sum_l s_l(t)$

Pulz – jak je generován





R.f. phase	Jargon
$\phi = 0$	'x-pulse'
$\phi = \pi/2$	'y-pulse'
$\phi = \pi$	'$\bar{x}$-pulse' or '$-x$-pulse'
$\phi = 3\pi/2$	'$\bar{y}$-pulse' or '$-y$-pulse'

Pulzy:

a) tvrdé – 7-30 μs@-3~+3dB

b) selektivní – ms~s@>30db

c) adiabatické

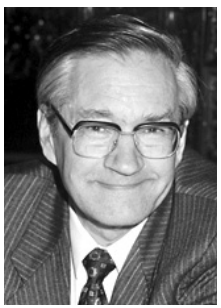
Trocha NMR-teorie

Isidor Isaac Rabi

Nobelova cena za *fyziku* v r. **1944**

“for his resonance method for recording the magnetic properties of atomic nuclei”


Bloch & Purcell *fyzika* **1952** “for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith”



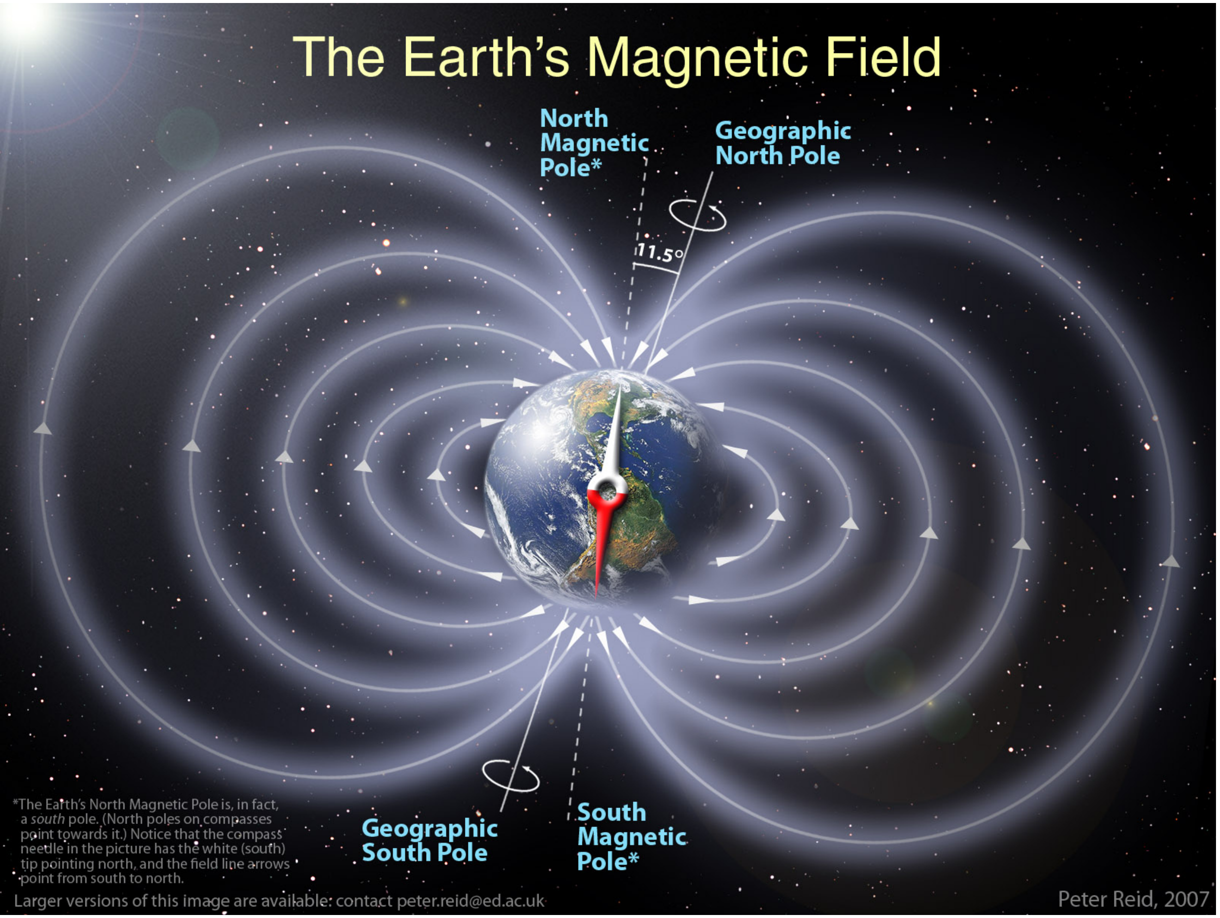
Ernst *chemie* **1991** ”for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy”

Wüthrich *chemie* **2002** “for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution”

Rezonanční podmínka $\omega_0 = -\gamma B_0$

Isotope	Ground state spin	Natural abundance	Magnetogyric ratio γ/rad s^{-1} T^{-1}	NMR frequency at 11.7433 T $(\omega^0/2\pi)$/MHz
^{1}H	1/2	~100	267.522×10^6	500.000
^{2}H	1	0.015%	$41\ 066 \times 10^6$	76.753
^{3}H	1/2	0	$285\ 349 \times 10^6$	533 320
^{10}B	3	19.9	$28\ 747 \times 10^6$	53 718
^{11}B	3/2	80.1%	85.847×10^6	−160 420
^{13}C	1/2	1.1%	67.283×10^6	125.725
^{14}N	1	99.6%	19.338×10^6	36 132
^{15}N	1/2	0.37%	$-27\ \ 26 \times 10^6$	50.684
^{17}O	5/2	0.04%	$-36.281\ \ 10^6$	+67 782
^{19}F	1/2	~100%	251.815×10^6	−470.470
^{23}Na	3/2	~100%	$70.808\ \ 10^6$	−132 259
^{27}Al	5/2	~100%	69.763×10^6	− 30 285
^{29}Si	1/2	4.7%	-53.190×10^6	99.336
^{31}P	1/2	~100%	108.394×10^6	−202.606
^{35}Cl	3/2	75.77%	10.610×10^6	−48 990
^{37}Cl	3/2	24.23%	8.832×10^6	−40.779
^{63}Cu	3/2	69.17%	71.118×10^6	132 577
^{65}Cu	3/2	30.83%	76.044×10^6	−142.018
^{107}Ag	1/2	51.84%	-10.889×10^6	+20.239
^{109}Ag	1/2	48.16%	12.518×10^6	+23.268
^{129}Xe	1/2	24.4%	74.521×10^6	+139.045
^{207}Pb	1/2	22.1%	55.805×10^6	−104 603
^{12}C	0	98.9%		
^{16}O	0	~100%		

Table of NMR-active nucleus propeties of arsenic			
	Isotope 1	**Isotope 2**	**Isotope 3**
Isotope	^{75}As		
Natural abundance /%	100		
Spin (I)	$^{3}/_{2}$		
Frequency relative to ^{1}H = 100 (MHz)	17.122710		
Receptivity, D^{P}, relative to ^{1}H = 1.00	0.0254		
Receptivity, D^{C}, relative to ^{13}C = 1.00	145		
Magnetogyric ratio, γ (10^{7} rad T^{-1} s^{-1})	4.596163		
Magnetic moment, μ (μ_N)	1.858354		
Nuclear quadrupole moment, Q/millibarn	314(6)		
Line width factor, 10^{56}l (m^{4})	0.13		



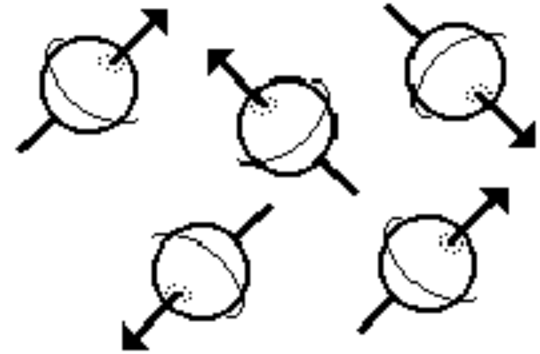


Figure 1
Randomly oriented nuclear magnetic moments

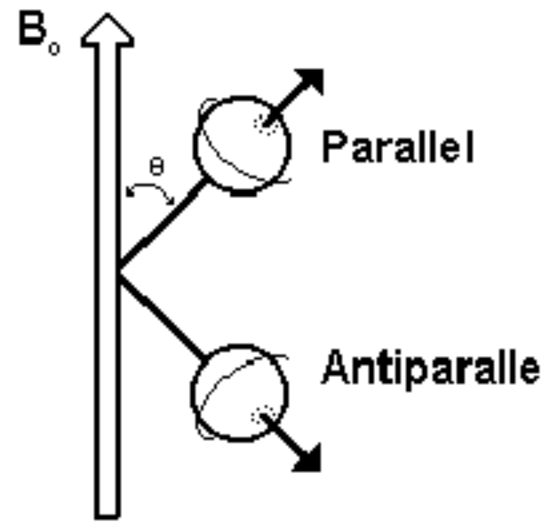


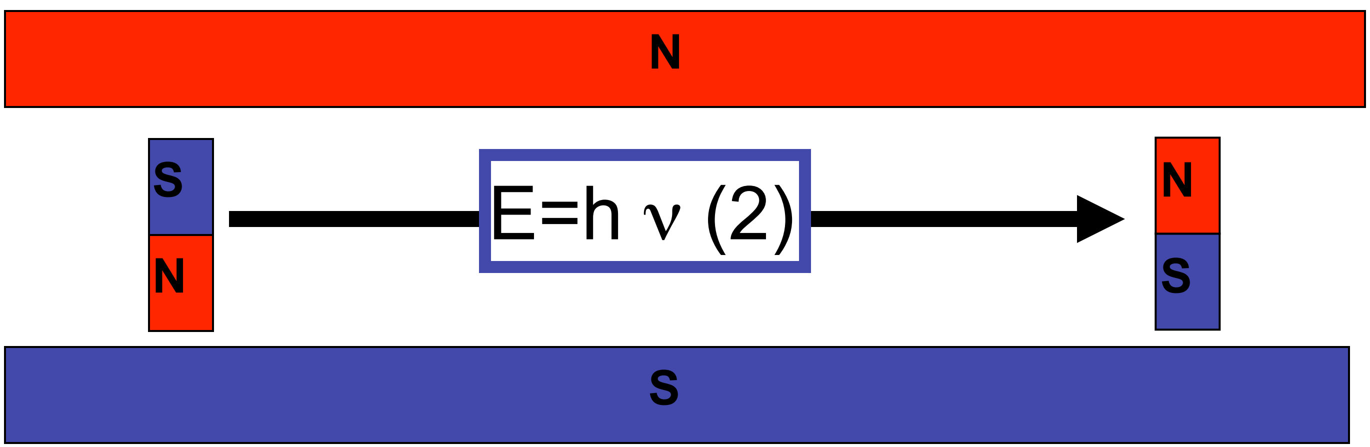
Figure 2
Nuclear magnetic moments in the presence of an external field

NMR - Refresh

1) nuclear spin ≠ 0 (^{1}H, ^{13}C, ^{15}N, ^{31}P)
   - number of neutrons **and** the number of protons **both even** ⇒ **NO** nuclear **spin**
   - number of neutrons **plus** the number of protons **odd** ⇒ **half-integer spin** (i.e. ½, 3/2, 5/2)
   - number of neutrons **and** the number of protons **both odd** ⇒ **integer spin** (i.e. 1, 2, 3)

2) $\nu = \gamma * B$ **(1)** - when placed in a magnetic field of strength **B**, a nuclei with a net spin can absorb a photon, of frequency ν. The frequency ν depends on the gyromagnetic ratio, γ of the nuclei

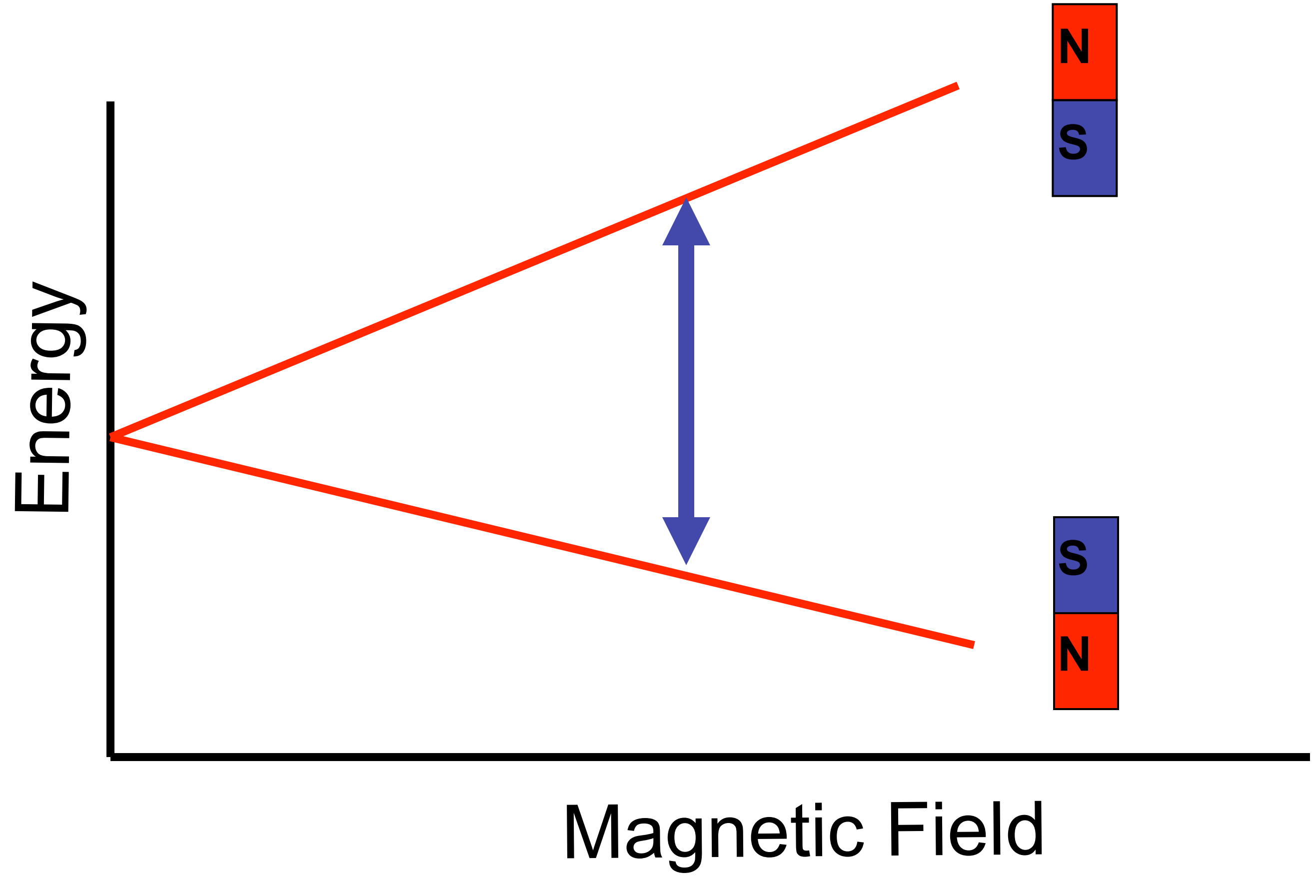
3) from quantum mechanics we know that nucleus with spin ***I*** can have ***2I +1*** orientations ⇒ **nuclei with a spin ½** can have **two orientations in** an external **magnetic field**– **low / high energy**

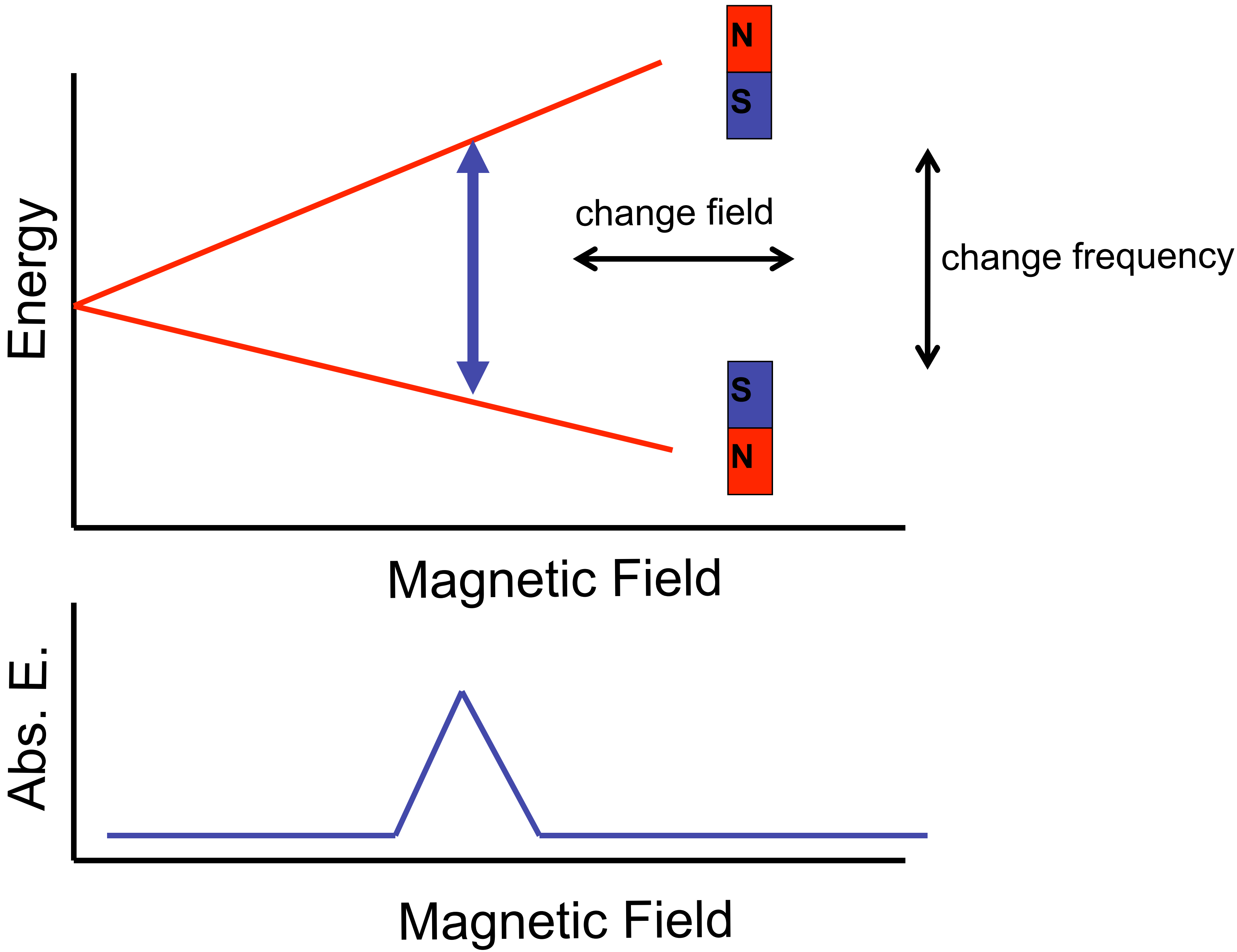


Nuclear Magnetic Resonance

Refresh

From (1) and (2): $E = h \gamma B$




N
S
change field
change frequency
Energy
S
N
Magnetic Field
Abs. E.
Magnetic Field

CW vs. Fourier transform NMR

Problem of NMR

the magnitude of the energy changes in NMR spectroscopy small ⇒ **sensitivity is a major limitation**

Solution I.

increase sensitivity by recording many spectra, and then add them together; because **noise is random**, it adds as the square root of the number of spectra recorded.

For example, if **100** spectra of a compound were recorded and summed, then the ***noise would increase*** by a factor of **10**,

but the ***signal would increase*** in magnitude by a factor of **100**

⇒ large increase in sensitivity.

However, if this is done using a **CW-NMR**, the time needed to collect the spectra is very large (one scan takes **2** - **8** minutes).

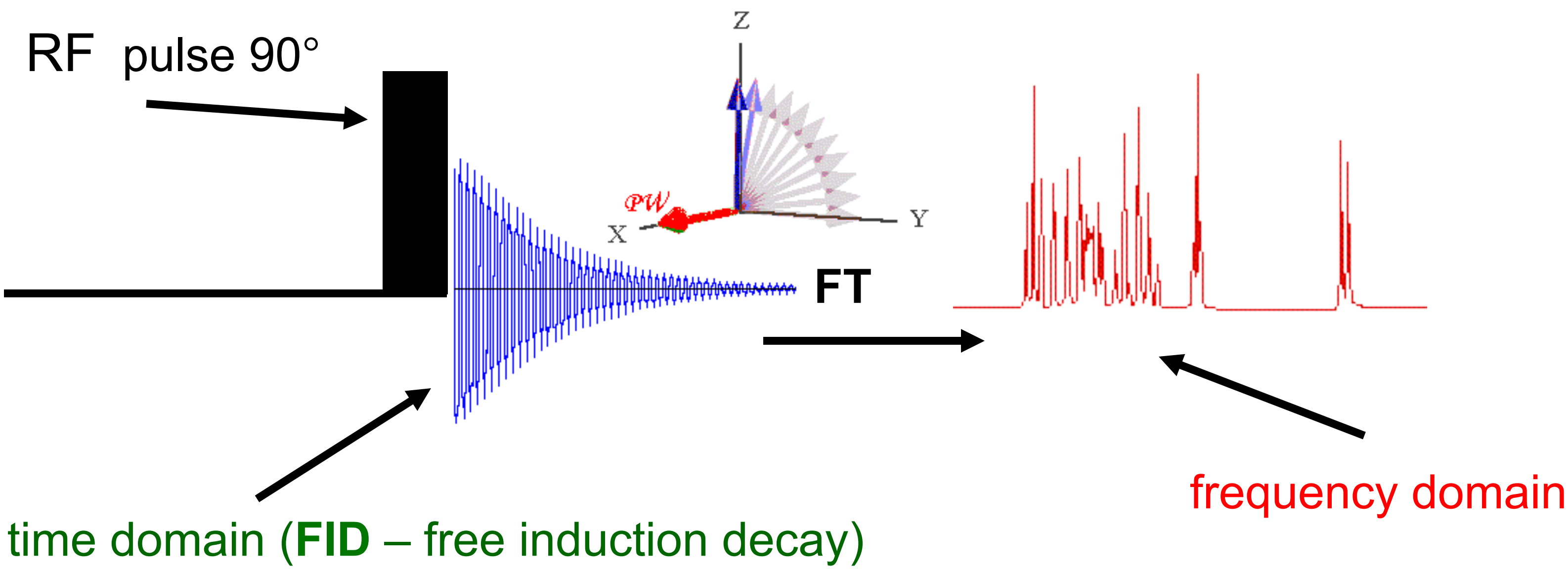
CW vs. Fourier transform NMR

Solution II.

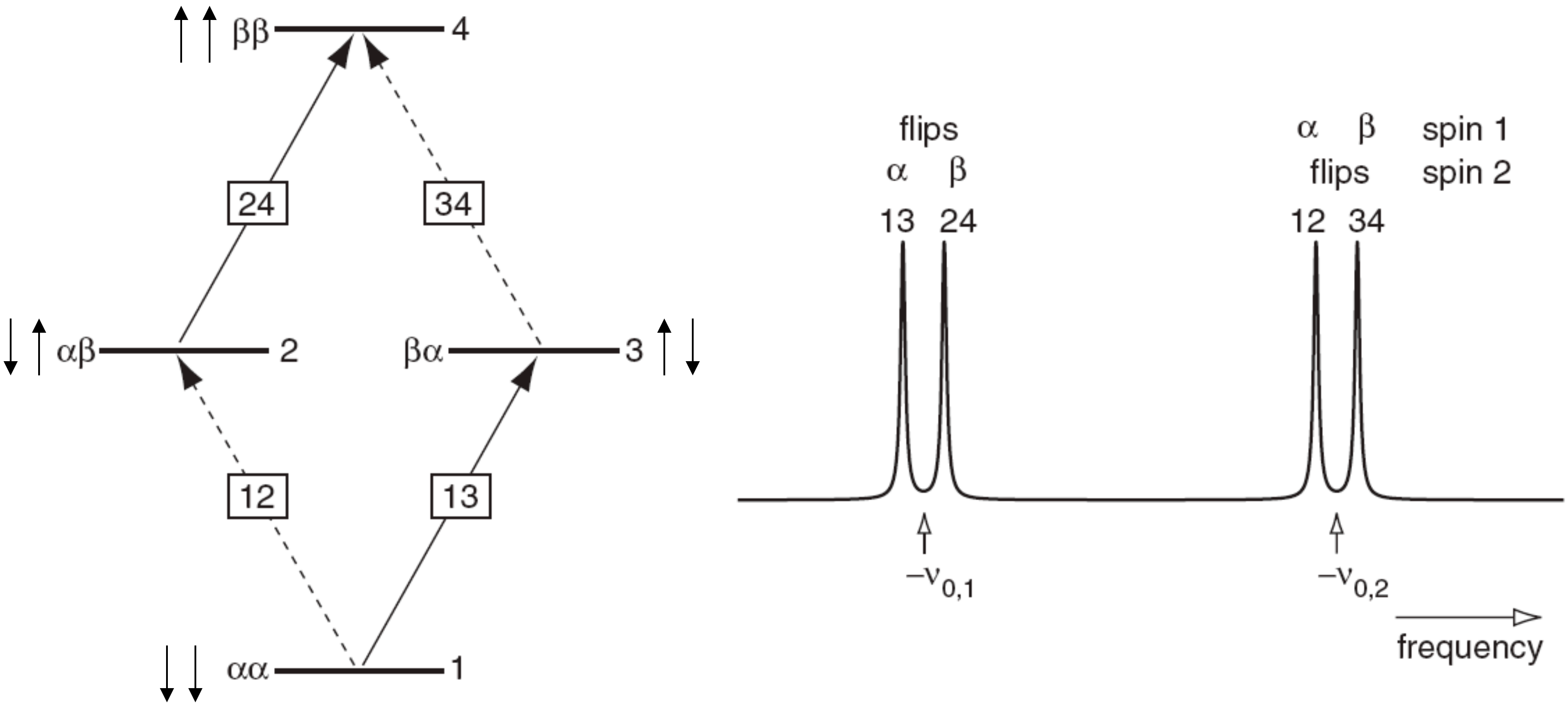
FT-NMR ⇒ ***all frequencies*** in a spectrum are ***irradiated simultaneously*** with a radio frequency pulse.

Following the pulse, the nuclei return to thermal equilibrium.
A ***time domain*** emission signal is recorded by the instrument as the nuclei relax.

A ***frequency domain*** spectrum is ***obtained by Fourier transformation***.



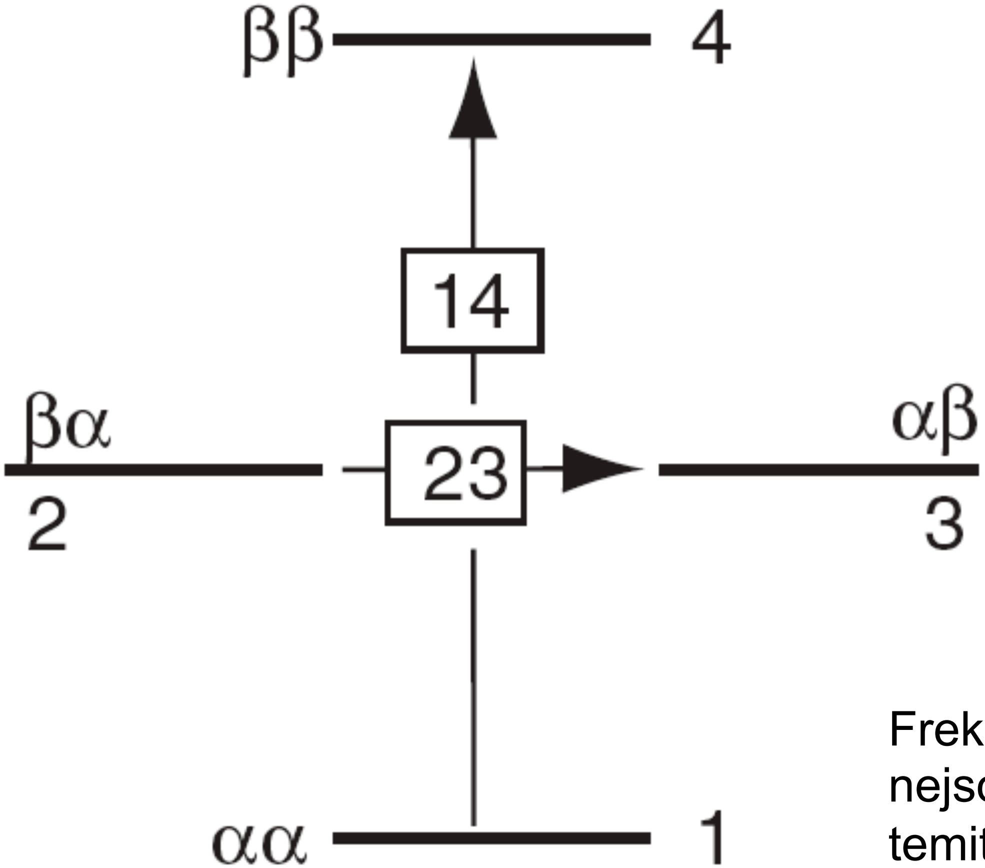
Jednokvantové přechody



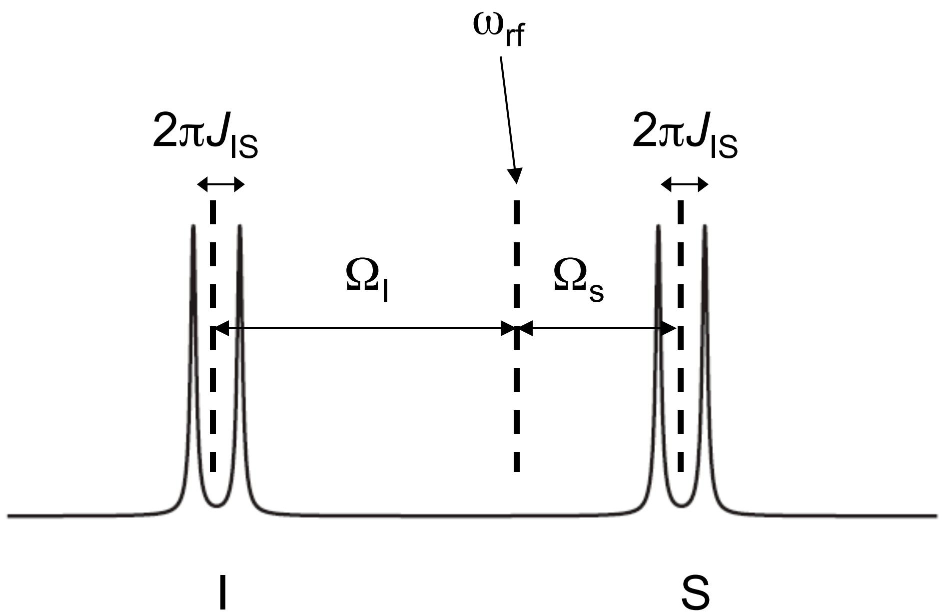
1) Larmorova frekvence spinu 2 > než spinu 1

2) Kapling J_{12} je pozitivní

Dvoukvantový a nulkvantový přechod



Frekvence těchto přechodů nejsou ovlivněny kaplingem mezi temito dvěma spiny



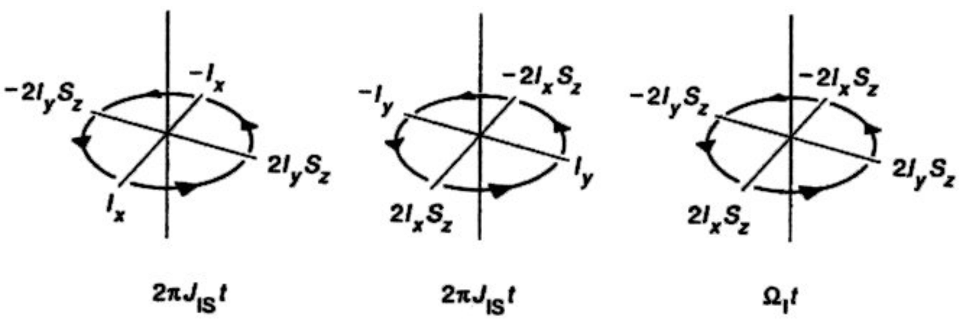
NMR spektrum jader I a S se spinem ½. Ω_s a Ω_I jsou rezonanční frekvence (rad/s), ω_{rf} je budící frekvence a J_{IS} je spin-spinová kaplingová konstanta.

Transformace a (vybraná) pravidla produktových operátorů pro spinový systém **IS** vlivem skalárního kaplingu J_{IS}, resonanční frekvence Ω_I a v časové periode t.

$$I_x \xrightarrow{\pi J_{IS} t} I_x \cos \pi J_{IS} t + 2 I_y S_z \sin \pi J_{IS} t$$

$$I_y \xrightarrow{\pi J_{IS} t} I_y \cos \pi J_{IS} t - 2 I_x S_z \sin \pi J_{IS} t$$

$$I_z \xrightarrow{\pi J_{IS} t} I_z$$



$$2I_x S_z \xrightarrow{\pi J_{IS} t} 2I_x S_z \cos \pi J_{IS} t + I_y \sin \pi J_{IS} t$$

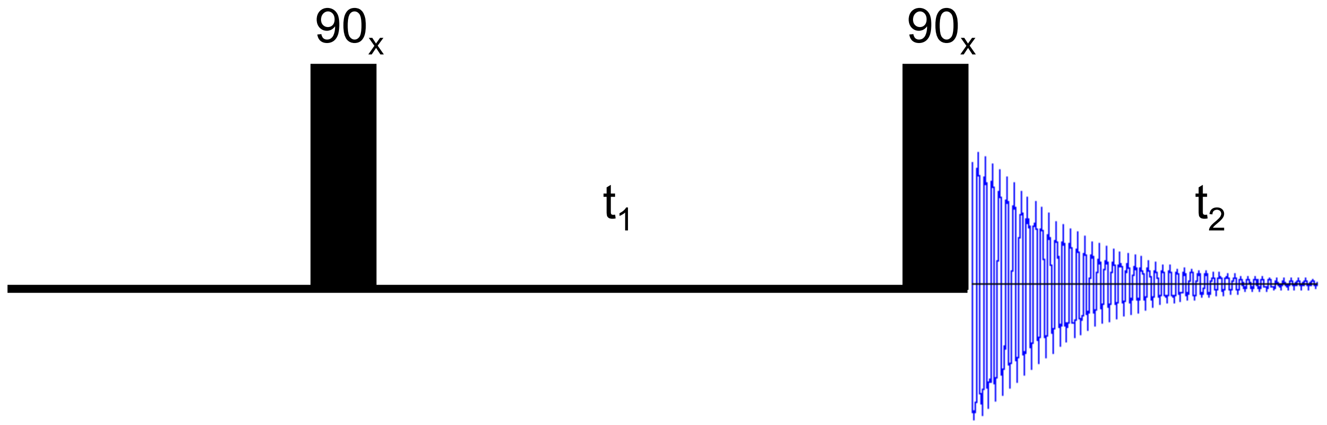
$$2I_y S_z \xrightarrow{\pi J_{IS} t} 2I_y S_z \cos \pi J_{IS} t - I_x \sin \pi J_{IS} t$$

$$2I_z S_z \xrightarrow{\pi J_{IS} t} 2I_z S_z,$$

COSY – základní 2D experiment

Obecně všechny 2D experimenty:

Příprava→Evoluce→Mixing→Detekce



$$I_z \xrightarrow{(90^\circ_x)_{IS}} \xrightarrow{\Omega_I t_1} \xrightarrow{\pi J_{IS} t_1}$$

$$-I_y \cos\Omega_I t_1 \cos\pi J_{IS} t_1 + 2I_x S_z \cos\Omega_I t_1 \sin\pi J_{IS} t_1$$

$$+I_x \sin\Omega_I t_1 \cos\pi J_{IS} t_1 + 2I_y S_z \sin\Omega_I t_1 \sin\pi J_{IS} t_1$$

$$\xrightarrow{(90^\circ_x)_{IS}}$$

$$-I_z \cos\Omega_I t_1 \cos\pi J_{IS} t_1 - 2I_x S_y \cos\Omega_I t_1 \sin\pi J_{IS} t_1$$

$$+I_x \sin\Omega_I t_1 \cos\pi J_{IS} t_1 - 2I_z S_y \sin\Omega_I t_1 \sin\pi J_{IS} t_1$$

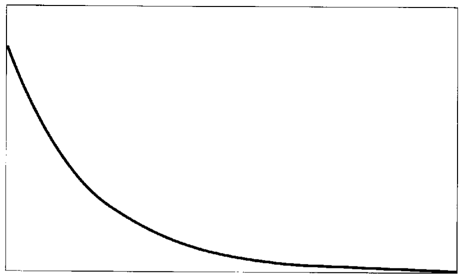
$$I_x \sin\Omega_I t_1 \cos\pi J_{IS} t_1 - 2I_z S_y \sin\Omega_I t_1 \sin\pi J_{IS} t_1$$

$$= I_x \frac{1}{2}[\sin(\Omega_I + \pi J_{IS})t_1 + \sin(\Omega_I - \pi J_{IS})t_1]$$

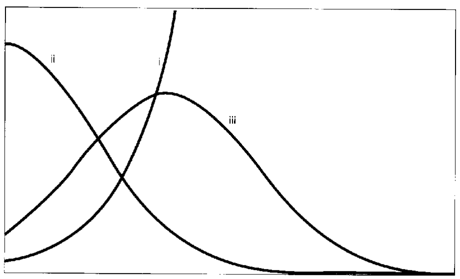
$$-2I_z S_y \frac{1}{2}[\cos(\Omega_I + \pi J_{IS})t_1 - \cos(\Omega_I + \pi J_{IS})t_1].$$

Zpracování NMR-signálu

Zpracování NMR dat



(a)



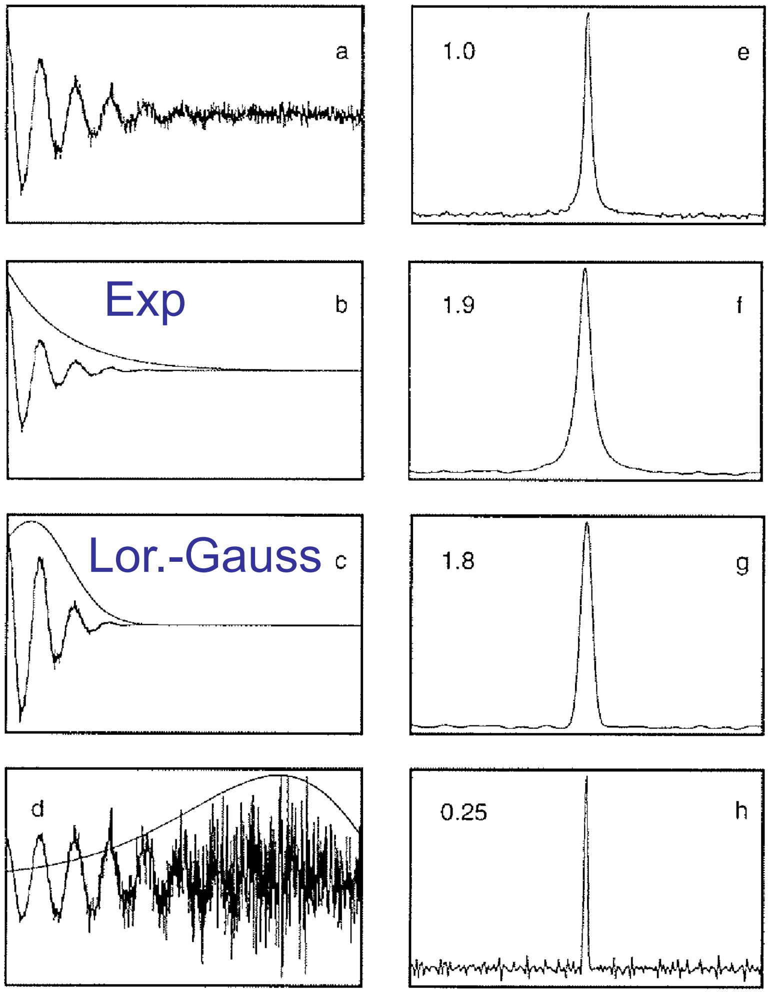
(b)

Okénkové funkce:

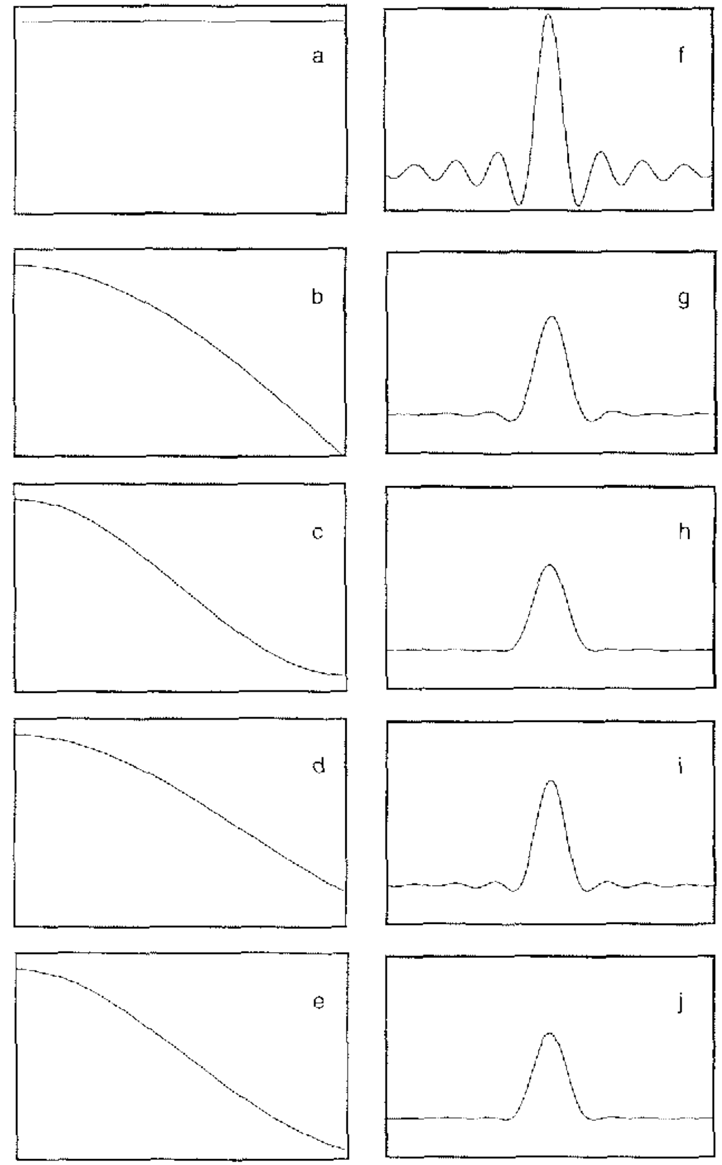
1) zvýšení S/N poměru

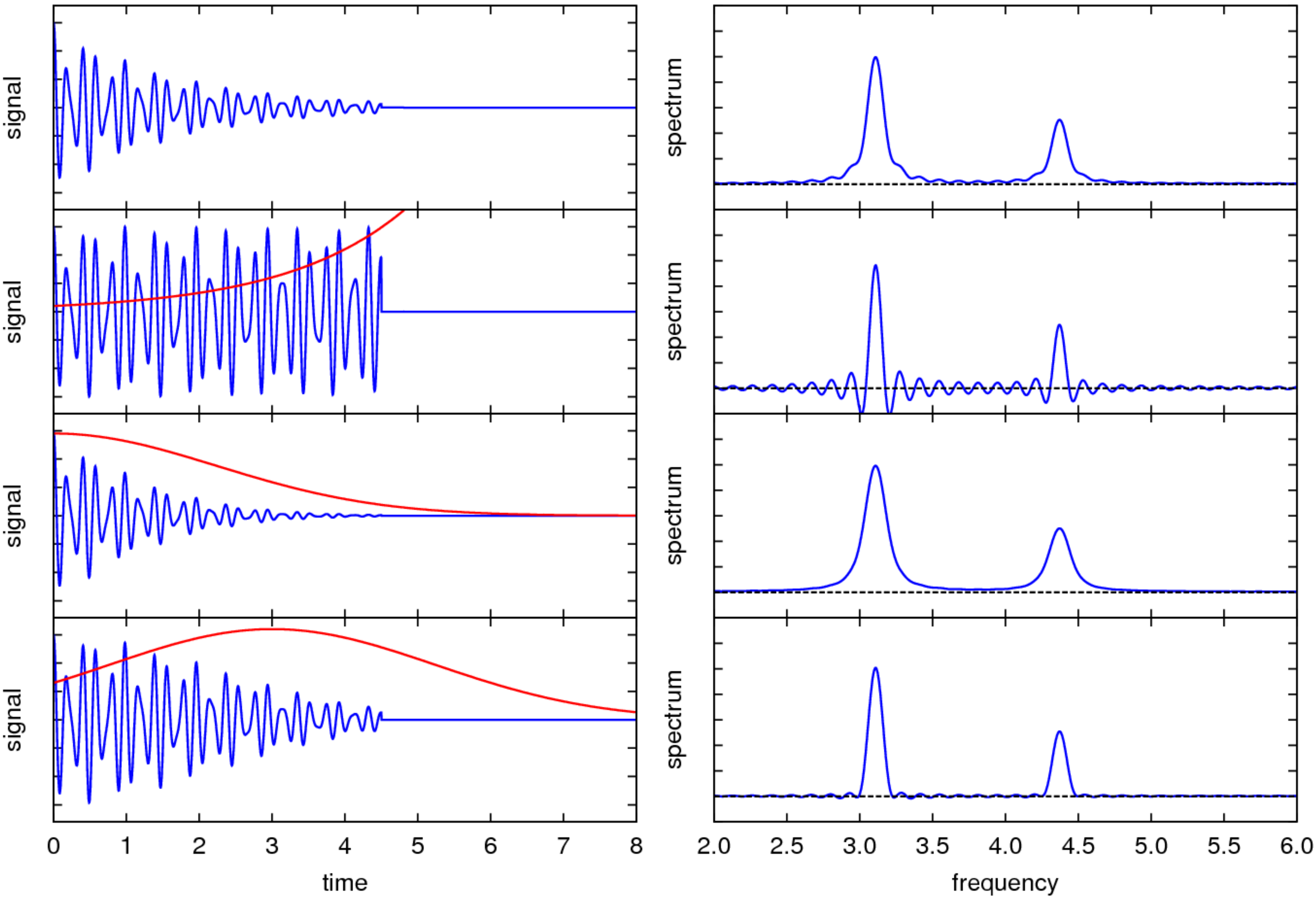
2) zvýšení rozlišení

Zpracování NMR dat – okénkové funkce - apodizace



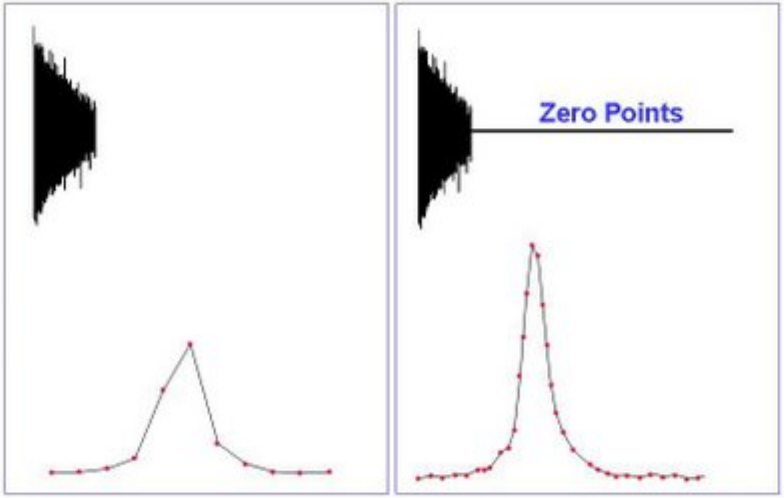
Kaiserova o. f.ce



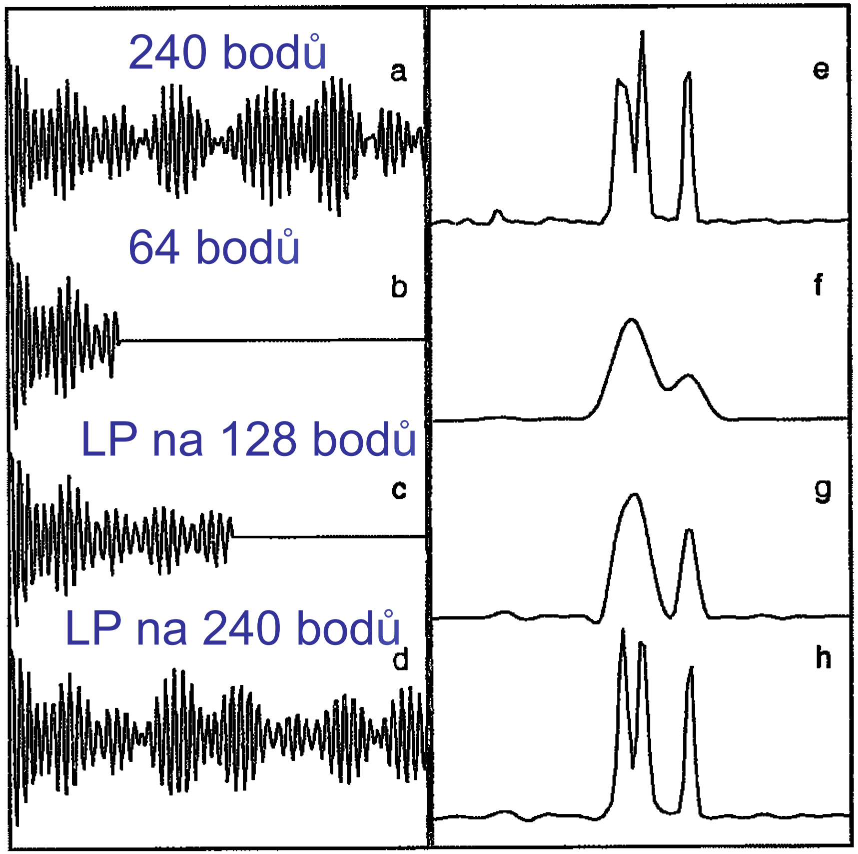

signal
signal
signal
signal
0
1
2
3
4
5
6
7
8
time
spectrum
spectrum
spectrum
spectrum
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
frequency

Zpracování NMR dat – Zero Filling, Lineární predikce

Zero filling



Lineární predikce



Zpracování NMR dat – shrnutí

I) Potlačení solventu

II) Okénková f.ce

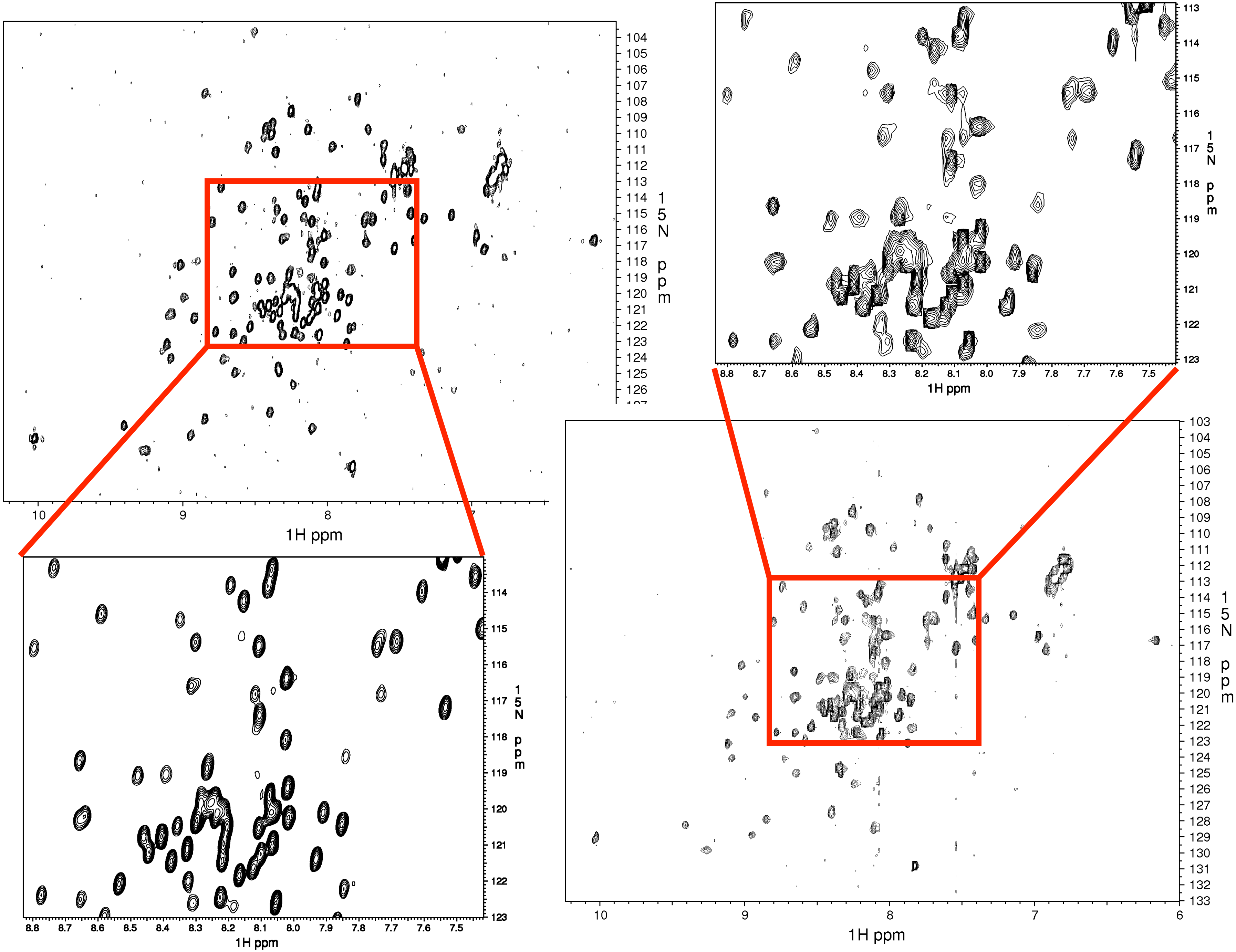
III) Zero-filling

IV) FT

V) Transpozice

```
|nmrPipe -fn POLY -time \
|nmrPipe -fn SP -off 0.33 -end 0.98 -pow 2 -c 1.0   \
|nmrPipe -fn ZF -size 2048 \
|nmrPipe -fn FT -auto \
|nmrPipe -fn PS -p0 -76.0 -p1 0.0 -di \
|nmrPipe -fn EXT -x1 11.0ppm -xn  6.0ppm -sw \
|nmrPipe -fn POLY -ord 3 -auto \
|nmrPipe -fn TP \
```

F2


1H ppm
15N ppm
1H ppm
15N ppm
1H ppm
15N ppm
1H ppm
15N ppm

NMR coby nástroj pro studium struktury a dynamiky biomol

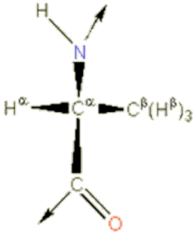
Composition of the Earth's Crust, Seawater, and the Human Body*

Earth's Crust		Seawater		Human Body†	
Element	%	Compound	m*M*	Element	%
O	47	Cl^-	548	H	63
Si	28	Na^+	470	O	25.5
Al	7.9	Mg^{2+}	54	C	9.5
Fe	4.5	SO_4^{2-}	28	N	1.4
Ca	3.5	Ca^{2+}	10	Ca	0.31
Na	2.5	K^+	10	P	0.22
K	2.5	HCO_3^-	2.3	Cl	0.08
Mg	2.2	NO_3^-	0.01	K	0.06
Ti	0.46	HPO_4^{2-}	<0.001	S	0.05
H	0.22			Na	0.03
C	0.19			Mg	0.01

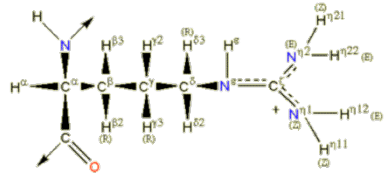
*Figures for the earth's crust and the human body are presented as percentages of the total number of atoms; seawater data are millimoles per liter. Figures for the earth's crust do *not* include water, whereas figures for the human body do.

†Trace elements found in the human body serving essential biological functions include Mn, Fe, Co, Cu, Zn, Mo, I, Ni, and Se.

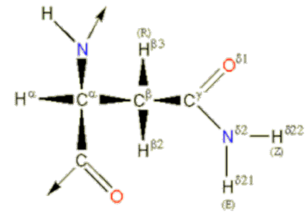
L-Alanine
(Ala)



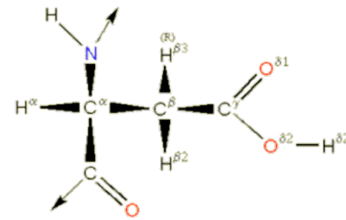
L-Arginine
(Arg)



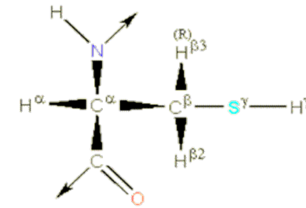
L-Aspargine
(Asn)



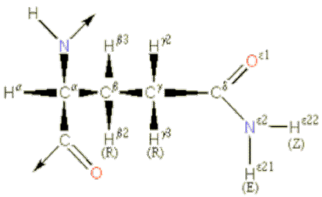
L-Aspartic Acid
(Asp)



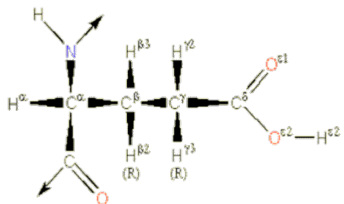
L-Cysteine
(Cys)



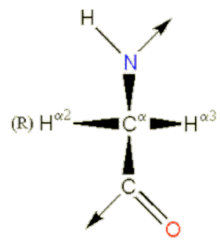
L-Glutamine
(Gln)



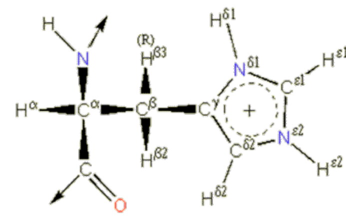
L-Glutamic Acid
(Glu)



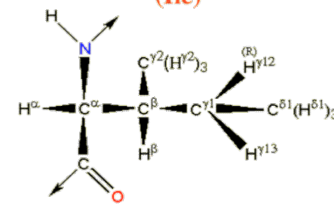
Glycine
(Gly)



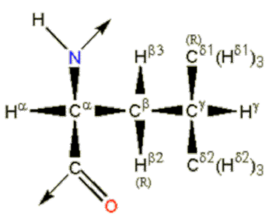
L-Histidine
(His)



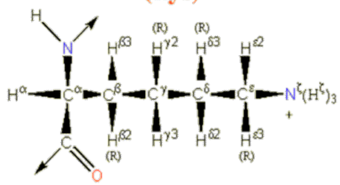
L-Isoleucine
(Ile)



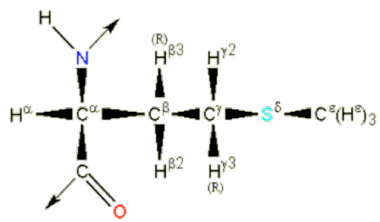
L-Leucine
(Leu)



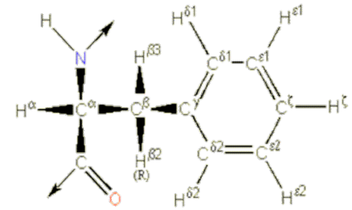
L-Lysine
(Lys)



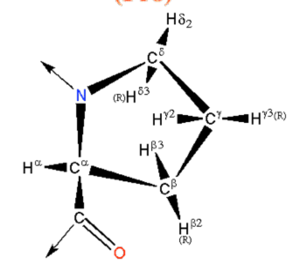
Methionine
(Met)



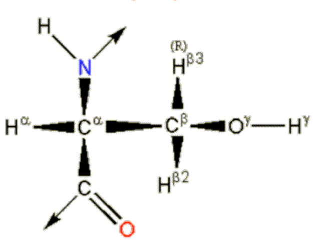
L-Phenylalanine
(Phe)



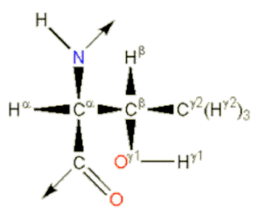
L-Proline
(Pro)



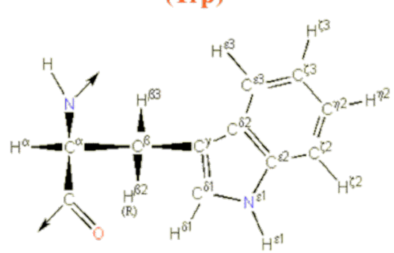
L-Serine
(Ser)



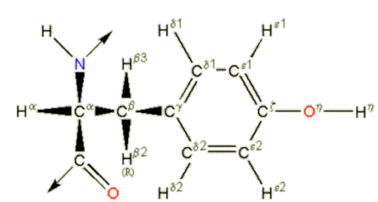
L-Threonine
(Thr)



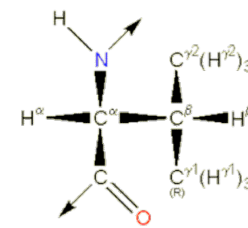
L-Tryptophan
(Trp)



L-Tyrosine
(Tyr)



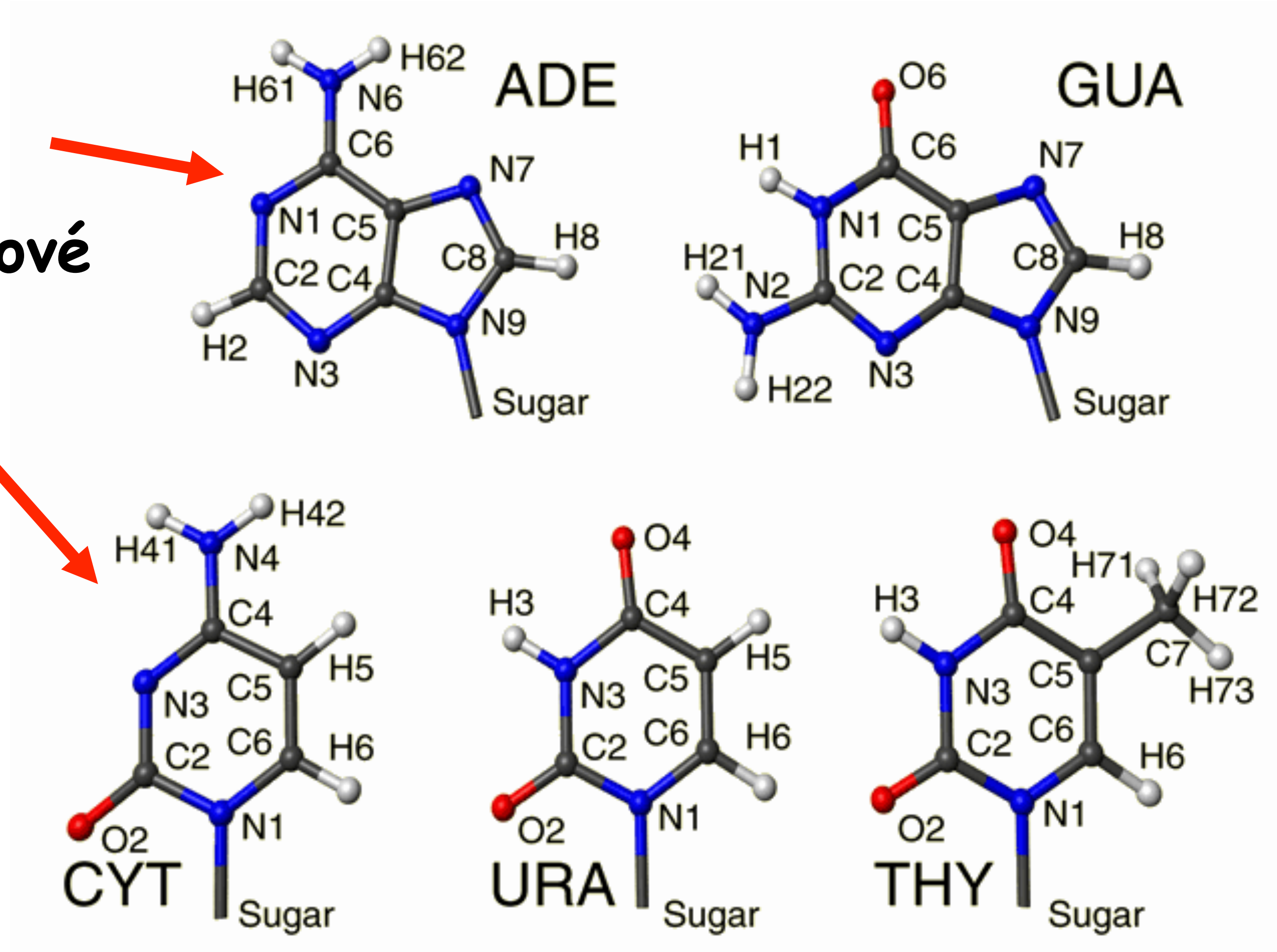
L-Valine
(Val)



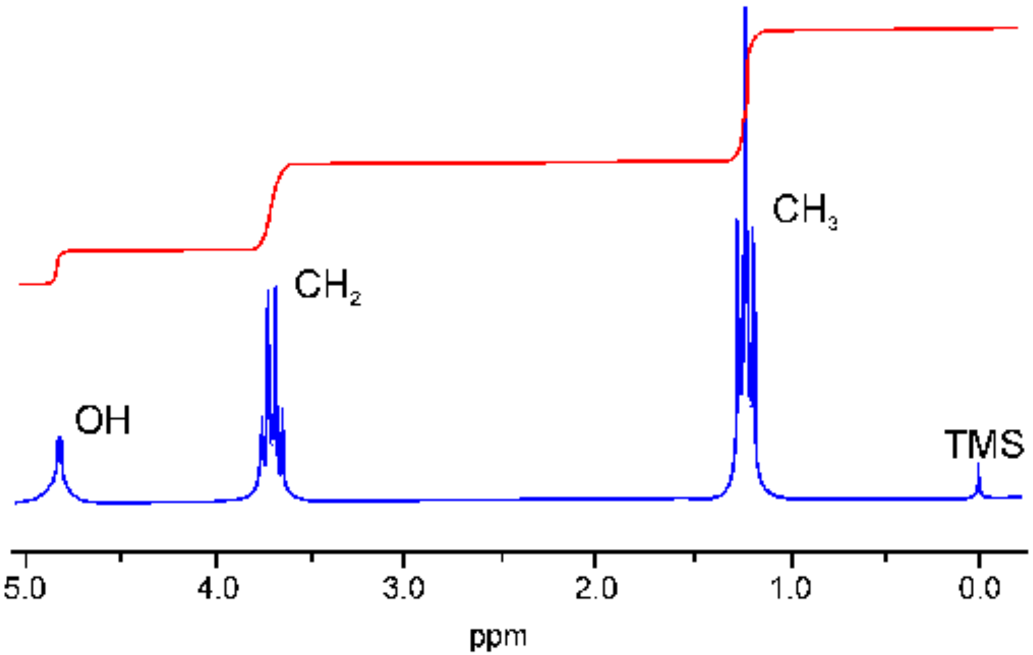
Báze nukleových kyselin

i) purinové

ii) pyrimidinové

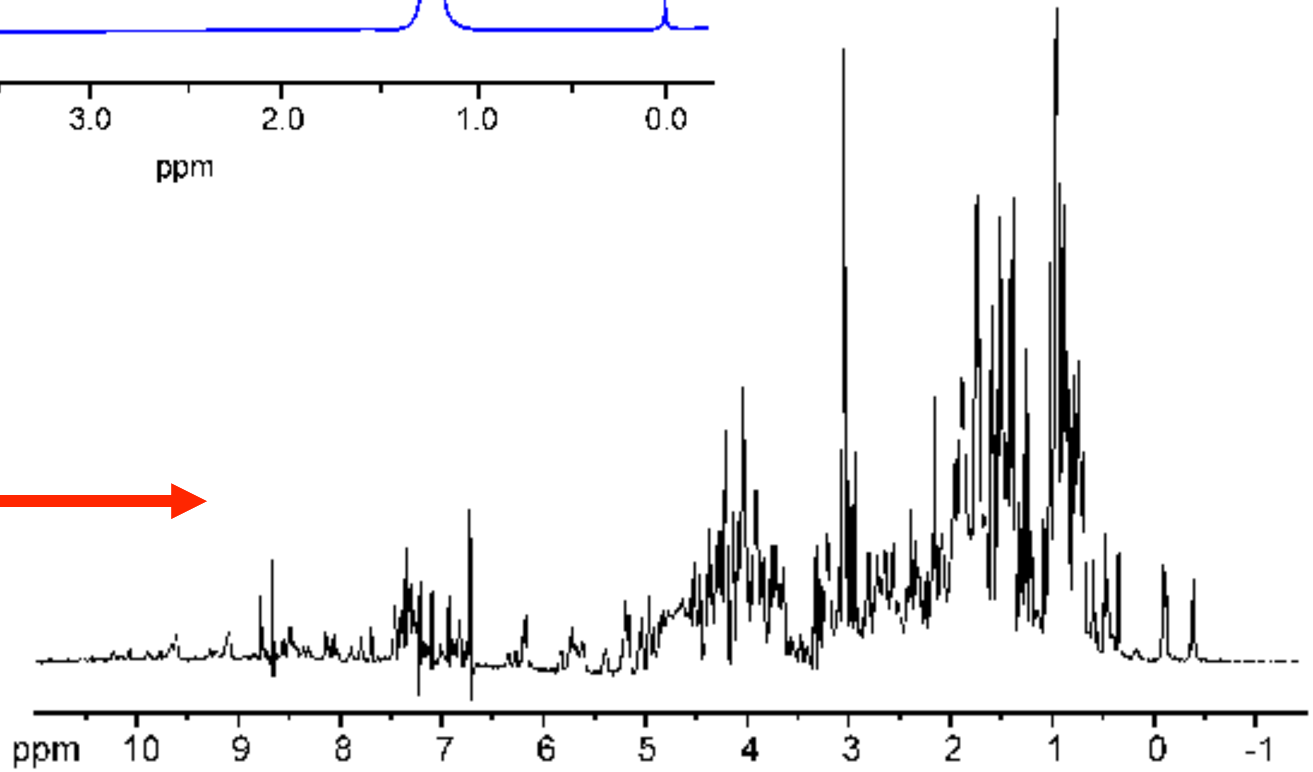


Každý vodík (proton) = 1 NMR signál

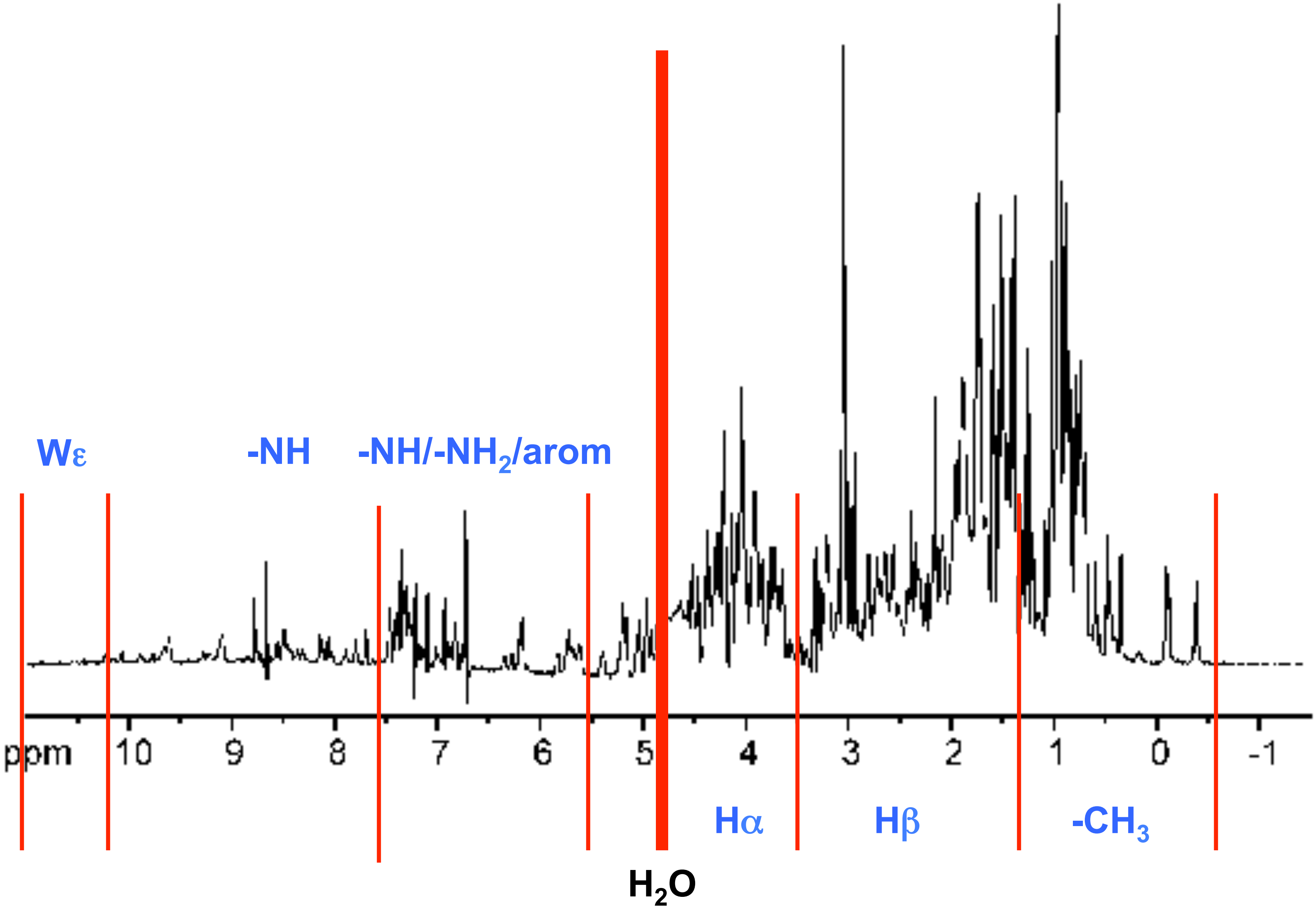


EtOH

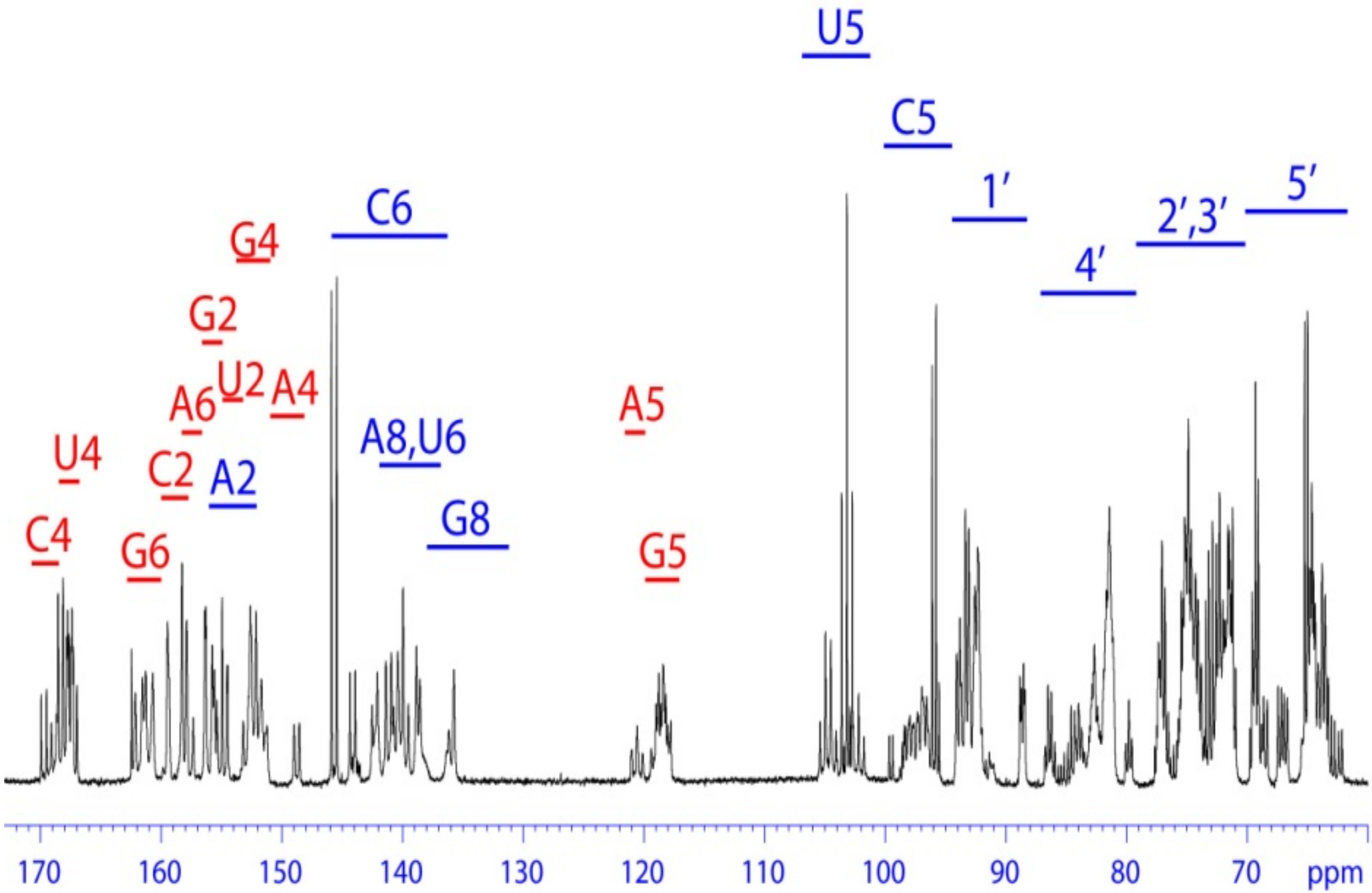
protein



Každý (nevyměnitelný) vodík (proton) = 1 NMR signál



Každý (nevyměnitelný) vodík (proton) = 1 NMR signál



NMR

Komplexní metoda:

0) Příprava vzorku

a) Měření

b) Zpracování

c) Interpretace - přiřazení rezonančních frekvencí

- změny chem. posunu vlivem interakce

- dynamika

- strukturní výpočty

d) Strukturu nevidíme (přímo) !ale počítáme!

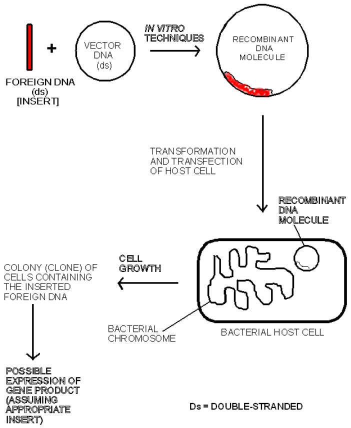
e) **X-Ray**, **NMR**, **cryoEM**

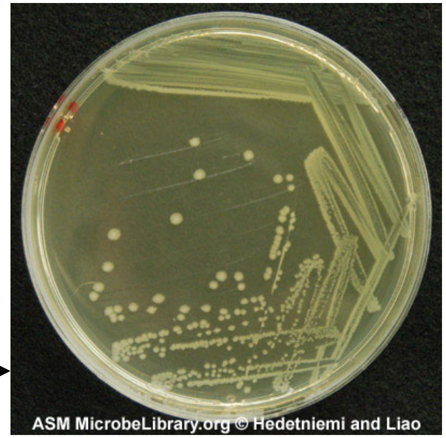
f) Moderní výzkum je komplexní => nutnost užití i jiných fyzikálně-chemických metod

Samples:

1) Small organic molecules	- synthesis	- costly
2) Peptides (10-40 aa)	- synthesis	- costly
3) Proteins (40-200 aa)	- $^{13}C/^{15}N$ enriched media	- costly
4) Large proteins > 200 aa	- $^{2}H/^{13}C/^{15}N$ enriched media	-pretty costly

If high concentrations (>10mM) can be used measurements in natural abundance can be performed and the sample goes from ***costly*** to ***affordable***

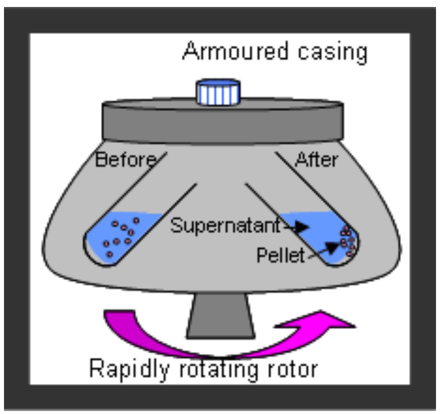




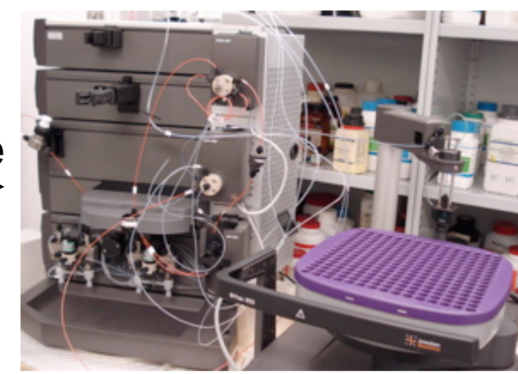
$^{15}NH_4Cl$, 37°C

^{13}C-glukosa

supernatant odstraníme odstředěním



ultra-centrifugace

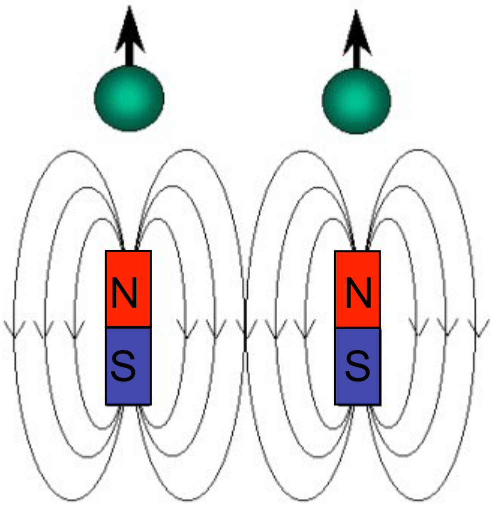


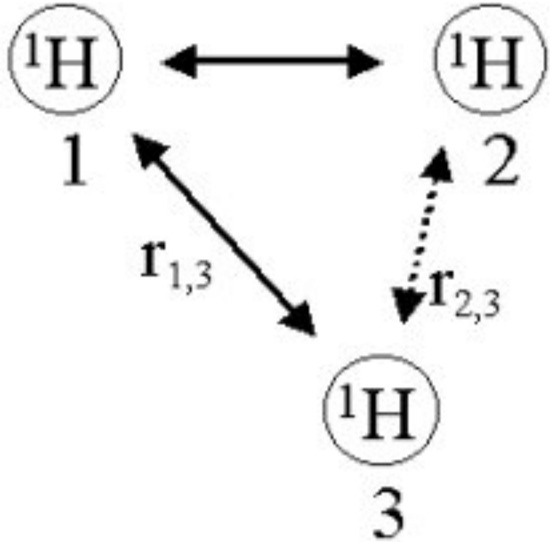
Nuclear Overhauser Effect (SpectroscopY) = NOE(SY)

i) caused by dipolar coupling between nuclei.
ii) the local field at one nucleus is affected by the presence of another nucleus.
iii) the result is a mutual modulation of resonance frequencies.
iv) the NOE operates through space.
v) the intensity of the interaction is a function of the distance between the nuclei according to the following equation: $I = A(1/r^6)$, I is the intensity, A is a scaling constant, and r is the distance between the nuclei

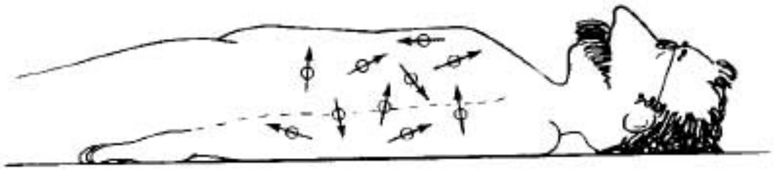
vi) the NOE provides a link between an experimentally measurable quantity, I, and internuclear distance

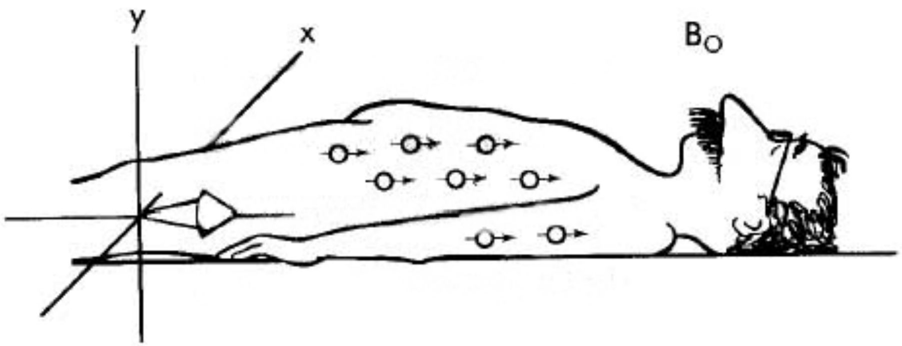
vii) NOE is only observed up to ~6Å

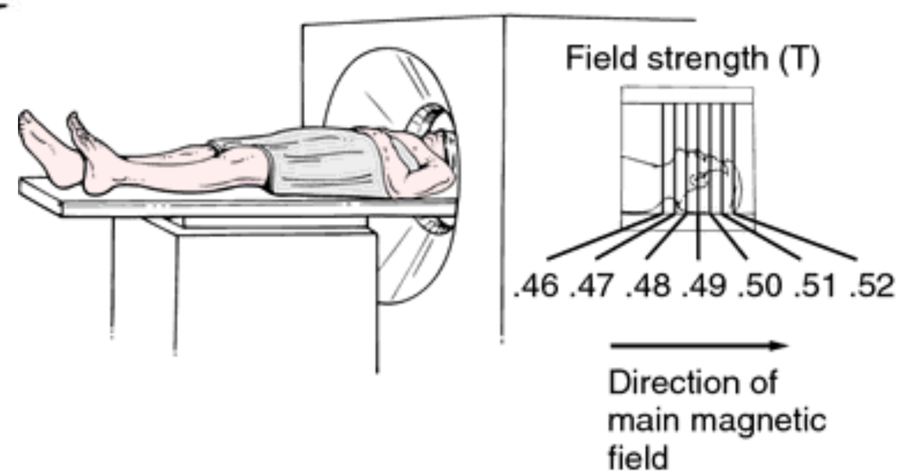


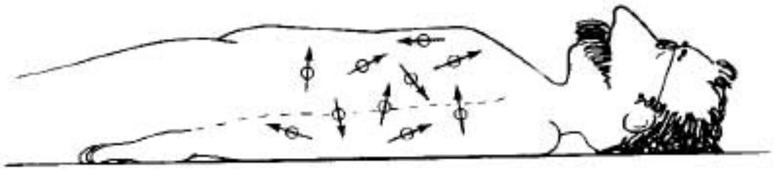


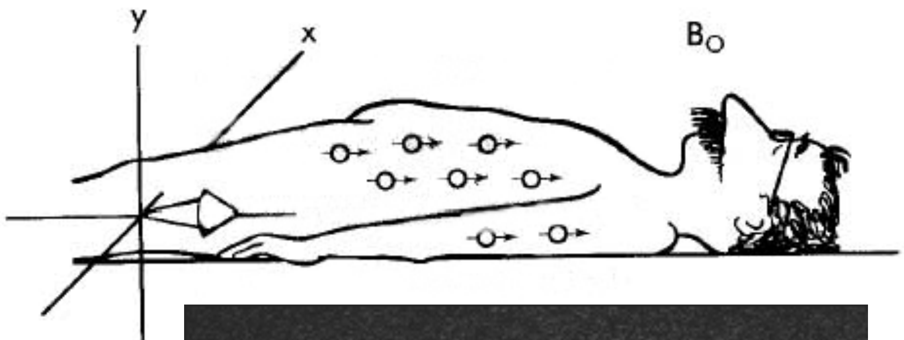
MRI je taky NMR

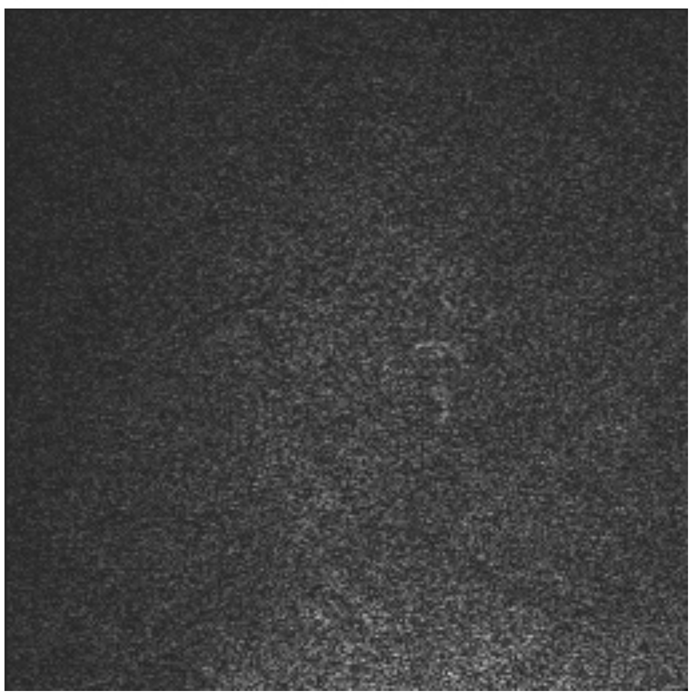


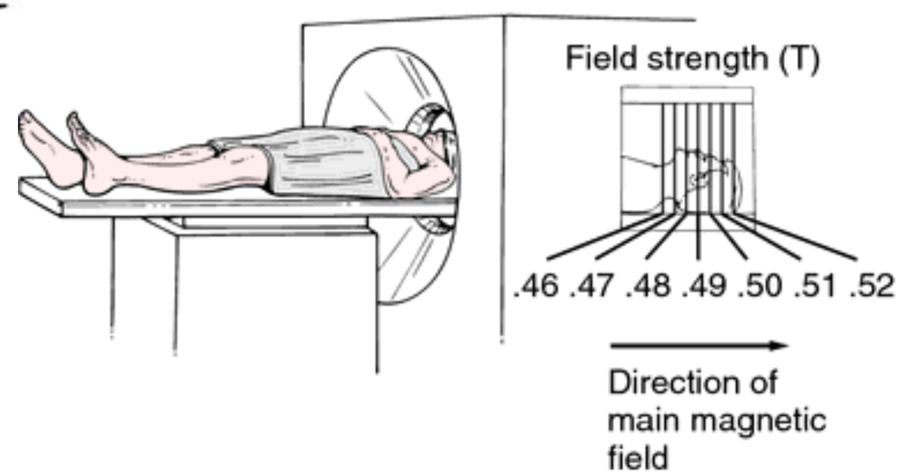

y
x
Bo


Field strength (T)
.46 .47 .48 .49 .50 .51 .52
Direction of
main magnetic
field




y
x
B_O




Field strength (T)
.46 .47 .48 .49 .50 .51 .52
Direction of main magnetic field