

# A BioNMR spektroszkópia alapjai

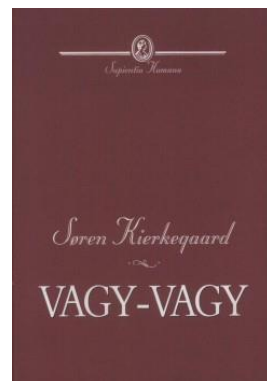
Az **NMR**-spektroszkópia szükséges feltétele

a **nullától különböző** magspin ( **$I \neq 0$** )

1)  $I=0$ , ha **mind** a protonok **mind** a neutronok száma **páros**: ( $^{12}\text{C}$ ,  $^{16}\text{O}$ )

2)  $I=1/2$ , ha tömegszáma páratlan ( $^1\text{H}$ ,  $^3\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{57}\text{Fe}$ ,  $^{113}\text{Cd}$ ) **vagy** a protonok, **vagy** a neutronok száma **páratlan**.

3)  $I=k$  ( $k=1,2,..$ ) **mind** a protonok **mind** a neutronok száma **páratlan** ( $^2\text{H}$ ,  $^{14}\text{N}$ )

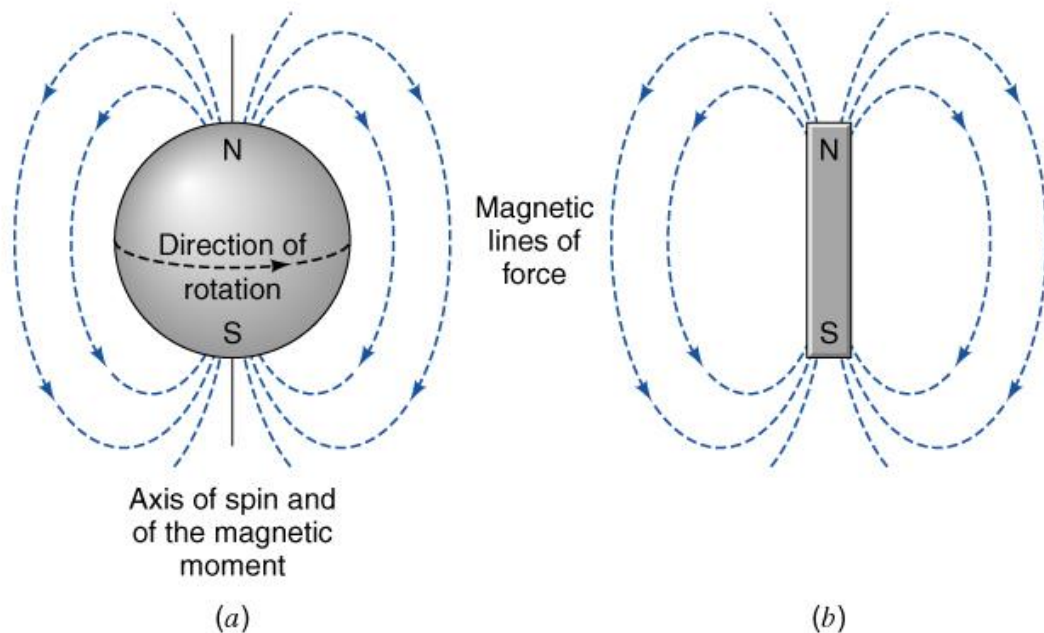


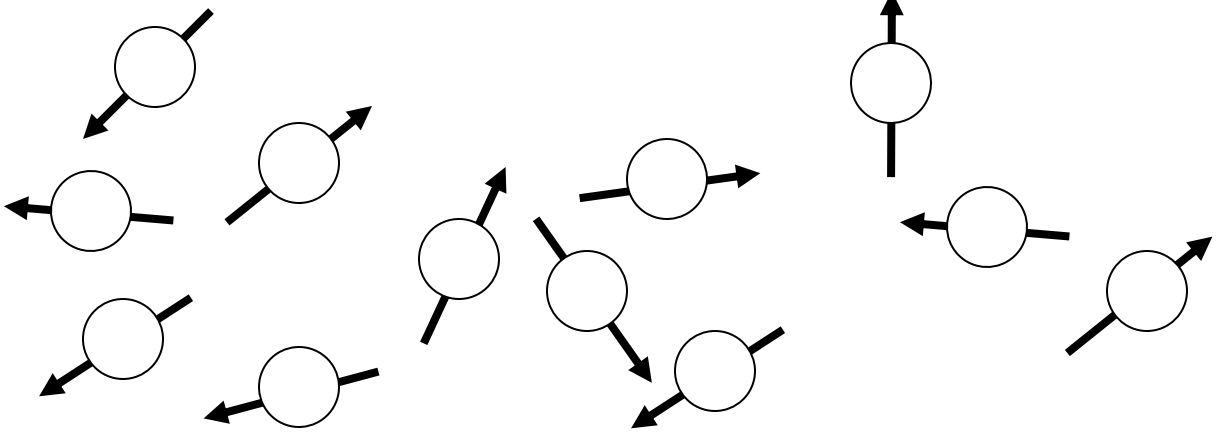
*klasszikus modell:*

atommag – egy **töltéssel** rendelkező részecske,  
– mely továbbá egy adott tengely mentén **forog**.

Töltéssel rendelkező **mag + forgás** = **köráram** létesítése  $\Rightarrow$   
köráram  $\Rightarrow$  **mágneses momentum** ( $\underline{\mu}$ ).

**“Bio-NMR”**: “Feles” spin kvantumszámú ( $I=1/2$ ) **magok**, ahol a  $\underline{\mu}$  egy mágneses dipólmomentum vektorral (rúd-mágnessel) modellezhető. A mágneses dipólmomentum ( $\underline{\mu}$ ) arányos a szögimpulzus-momentum vektorral ( $\underline{I}$ ). Giromágneses állandó ( $\gamma$ ) egy, az atommagra jellemző mennyiség:  $\underline{\mu} = \gamma \underline{I}$ .





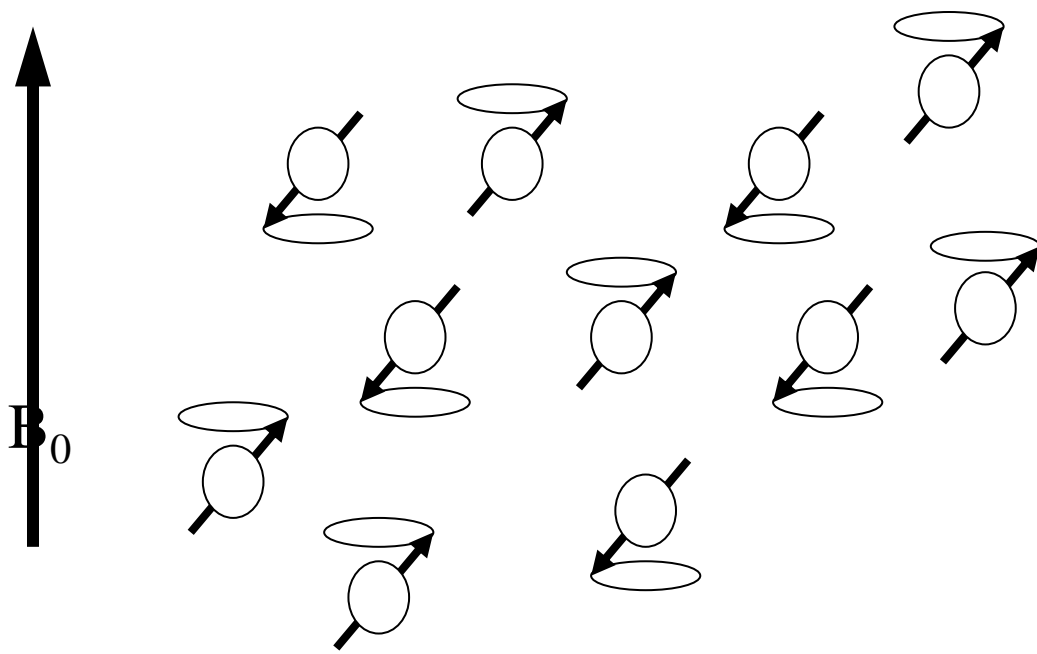
## Külső mágneses tér hiányában a magok spinjei rendezetlenül állnak

**kérdés:** Egy ilyen elemi dipólmomentum vektor ( $\underline{\mu}$ ) a külső tér-erőnek ( $\underline{B}_0$ ) megfelelően, azzal **párhuzamos** irányba rendeződik-e?

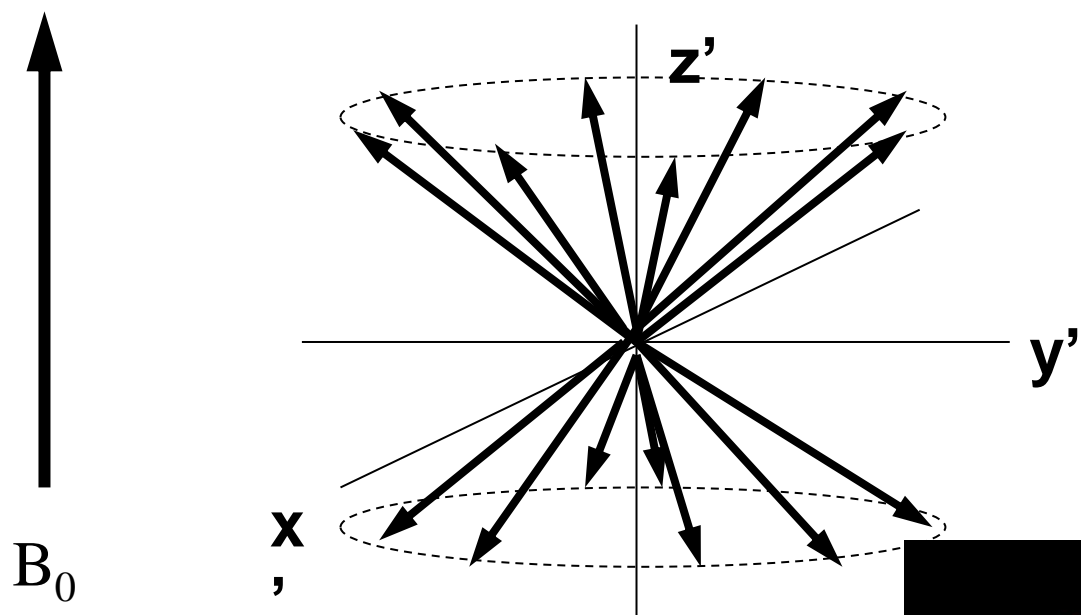
**válasz:** **Nem!**

**magyarázat:** Az említett atommagokhoz **mágneses** tulajdonsága mellett **forgó** mozgást is rendeltünk, ezért az elemi mágneses dipólmomentum vektorok ( $\underline{\mu}$ ) a külső sztatikus mágneses térre ( $\underline{B}_0$ ) merőleges sík szerinti forgó mozgást, úgynevezett **precessziót** fognak végezni.

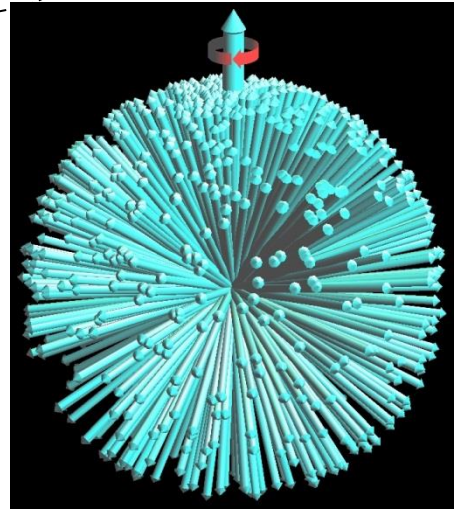




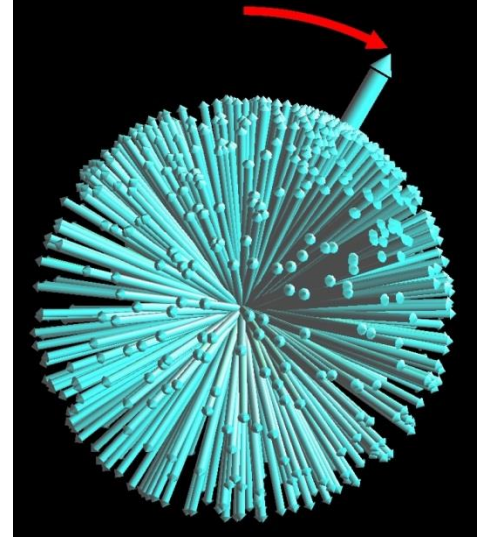
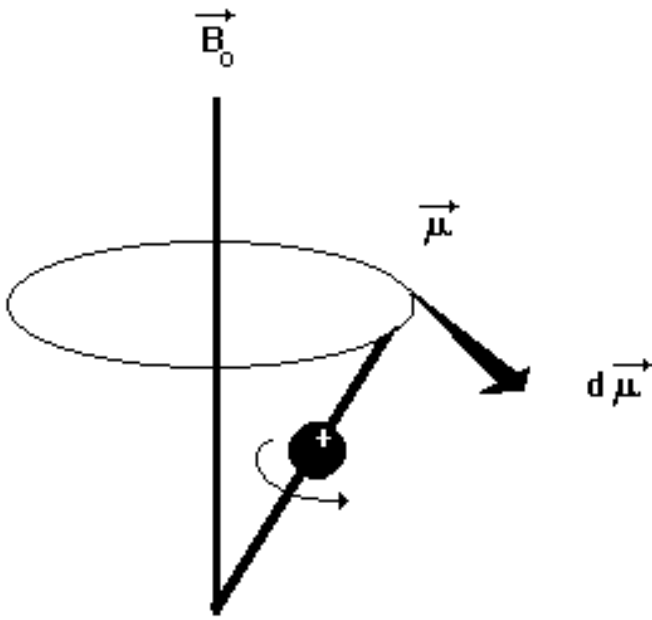
Külső mágneses tér hatására rendeződött és precesszáló magok



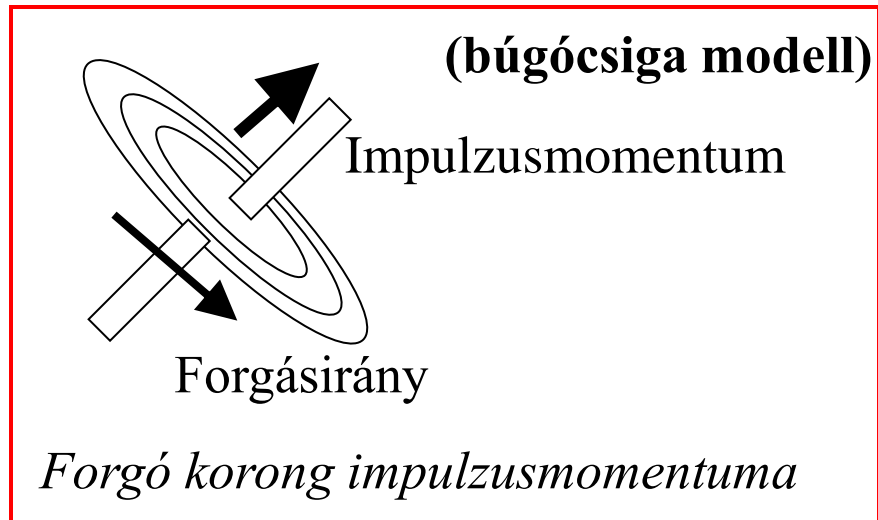
Külső mágneses tér hatására rendeződött és precesszáló spinek (közös origóból ábrázolva)



$B_0$  és  $B_1$  után a helyzet:



$$\frac{d\vec{\mu}}{dt} = -\gamma \vec{B} \wedge \vec{\mu}$$



$\omega_0$  szögsebesség nagysága arányos a külső statikus térerő nagyságával:  $\omega_0 = \gamma B_0$ .

Az  $\omega_0$  szögsebességgel arányos  $\nu_0$  (*Larmor*-frekvencia):  $\nu_0 = \omega_0/2\pi$ .

A mágneses dipólmomentum vektor ( $\mu$ ) **időbeli változását** a következő vektoriális szorzat írja le:

$$d\mu/dt = -\gamma \underline{B}_0 \otimes \underline{\mu}$$

A makroszkopikus-, globális- vagy mérhető-mágneszettség ( $\underline{M}$ ), a megfelelő elemi vagy mikroszkopikus mennyiség additív összege:  $\underline{M} = \sum \underline{\mu}_i$

Ennek segítségével a *Larmor*-precesszió átírható “makroszkopikus” alakba:  $d \underline{M} / dt = -\gamma \underline{B}_0 \otimes \underline{M}$

A  $\underline{\mu}_i$  vektoroknak kizárólag a  $\underline{B}_0$  -al párhuzamos, szokásosan “z” irányúnak nevezett komponensei adódnak konstruktívan össze. A termikus egyensúly állapotában tehát, a

$$\underline{M}_z = \sum (\underline{\mu}_z)_i$$

**Ha** tehát  $I = 1/2$  (pl.  $^1\text{H}$ ,  $^{13}\text{C}$ , stb.) **akkor** a  $(2I+1)=2$ , azaz két állapot;

két kvantumállapotot  $|\alpha\rangle$  és  $|\beta\rangle$   
 két energia  $(E_\alpha$  és  $E_\beta)$   
 két betöltöttségek  $(N_\alpha$  és  $N_\beta)$

$$N_\beta / N_\alpha = \exp(-\Delta E / kT).$$

Az egy átmenet (*Zeeman*-átmenet)

energiakülönbsége ( $\Delta E$ ):  $\Delta E = h\gamma B_0 / 2\pi$

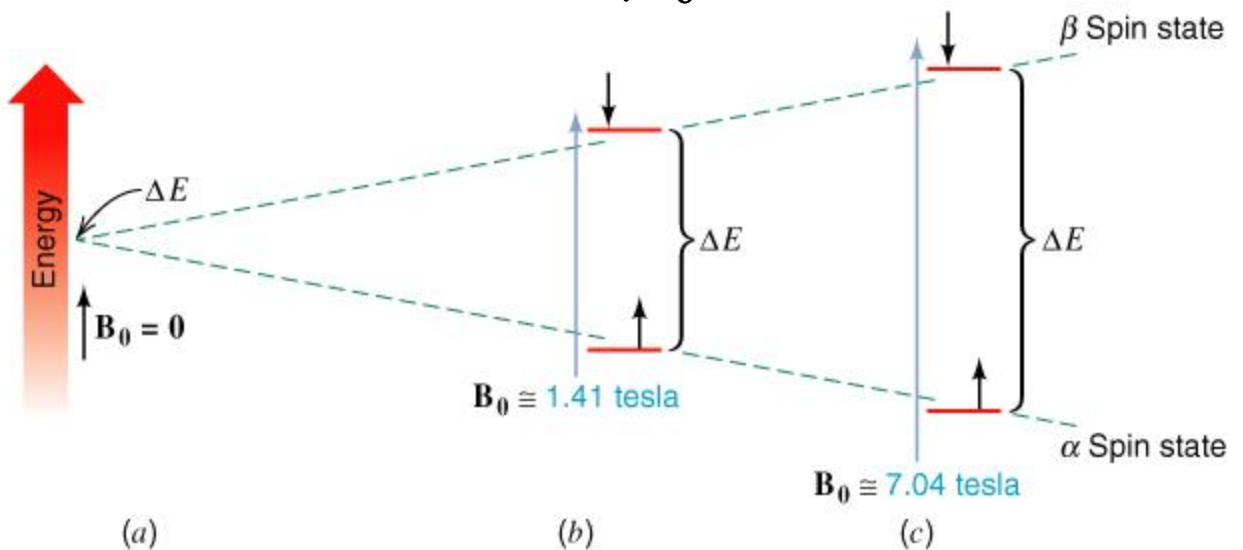
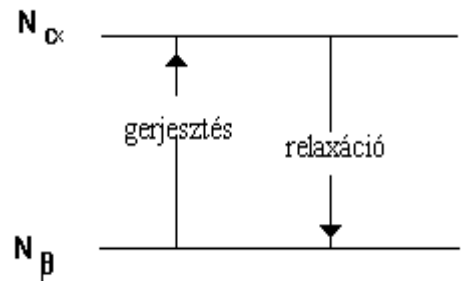


Figure 9.9 The energy difference between the two spin states of a proton depends on the strength of the applied external magnetic field,  $B_0$ . (a) If there is no applied field ( $B = 0$ ), there is no energy difference between the two states. (b) If  $B_0 \cong 1.41$  tesla, the energy difference corresponds to that of electromagnetic radiation of  $60 \times 10^6$  Hz (60 MHz). (c) In a magnetic field of approximately 7.04 tesla, the energy difference corresponds to electromagnetic radiation of  $300 \times 10^6$  Hz (300 MHz). Instruments are available that operate at these and even higher frequencies (as high as 800 MHz to 1 gigahertz).

# A BLOCH-egyenletek

**z-irányú** mágnesezettség időbeni alakulása:

$$dM_z/dt = -(M_z - M_0)/T_1$$

Az egyszerű differenciál-egyenletet megoldásaként a következő függvényt kapjuk:

$$M_z(t) = M_0(1 - \exp(-t/T_1))$$

**az x,y-síkban** zajló csillapított amplitúdójú precesszió alakulása:

$$dM_x/dt = (\omega_0 - \omega)M_y - M_x/T_2$$

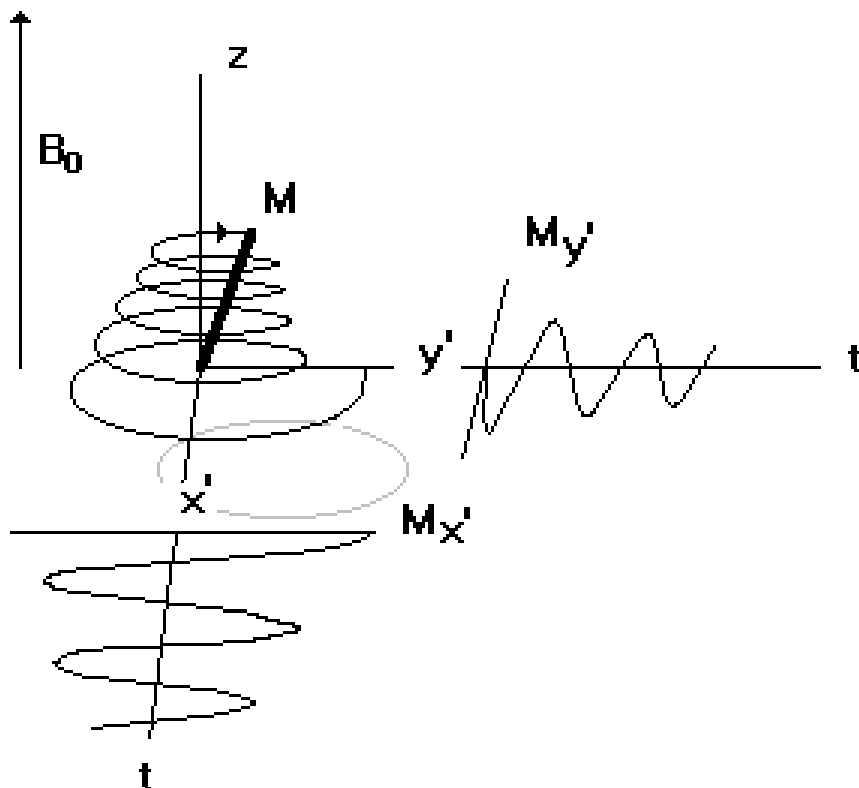
$$dM_y/dt = -(\omega_0 - \omega)M_x - M_y/T_2$$

Csatolt differenciál-egyenletrendszer megoldásaként a következőt kapjuk:

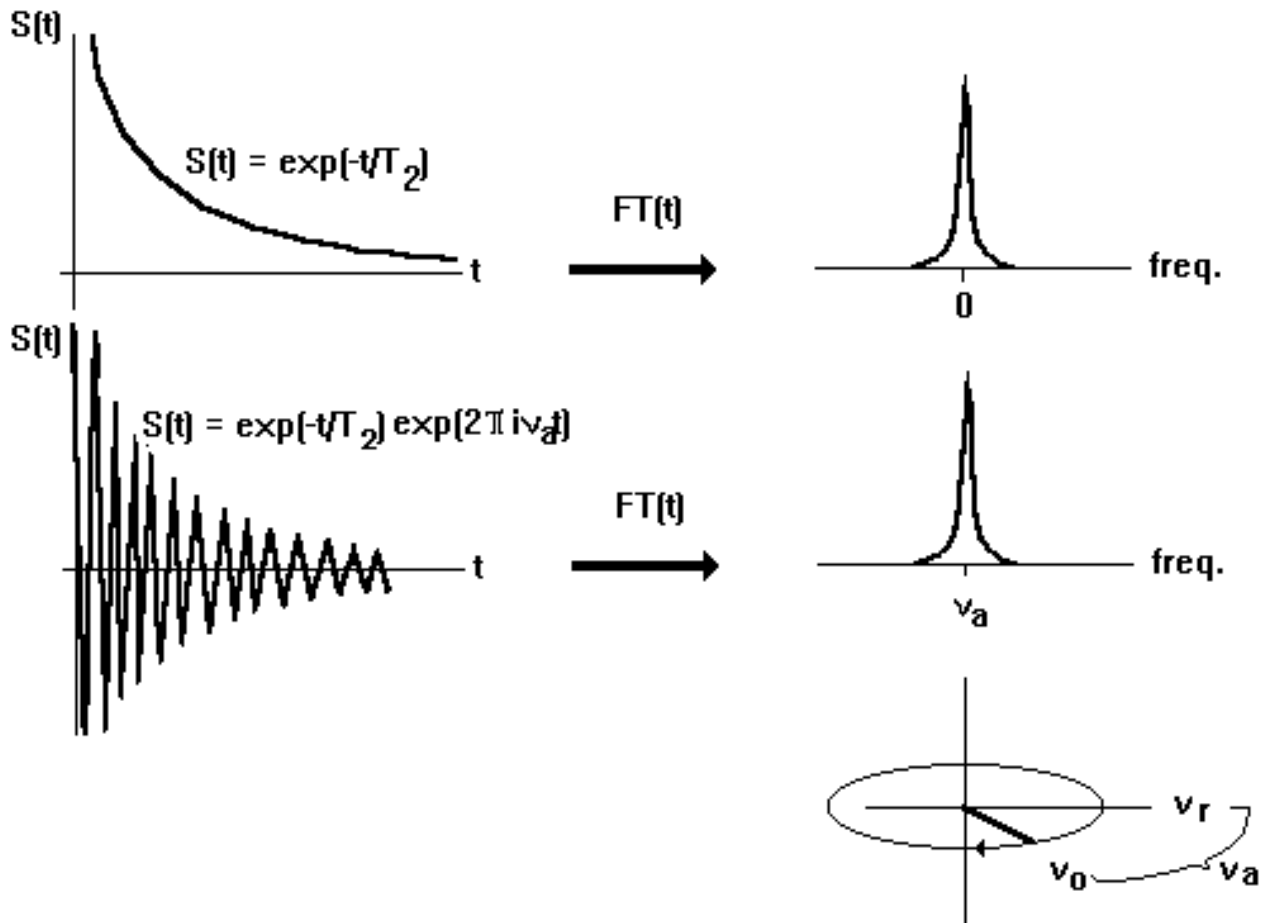
$$M_x(t) = M_0 \exp(-t/T_2) \sin(\omega_0 - \omega)$$

$$M_y(t) = M_0 \exp(-t/T_2) \cos(\omega_0 - \omega),$$

ahol  $(\omega_0 - \omega)$  a forgó referencia rendszerben a precesszió szögsebessége.



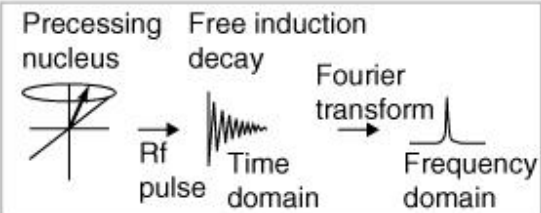
Ennek a függvény a *Fourier*-transzformáltja az NMR-spektrum.



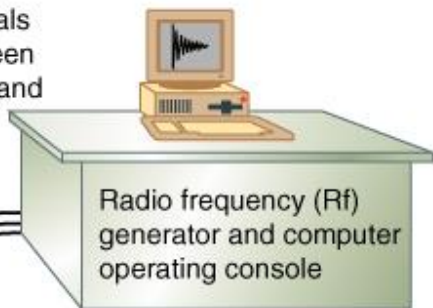
Superconducting magnet (cooled by liquid helium)



Sample tube spins within the probe coils in the hollow bore at the center of the magnet.



The radio frequency excitation pulse and resulting NMR signals are sent through cables between the probe coils in the magnet and the computer.



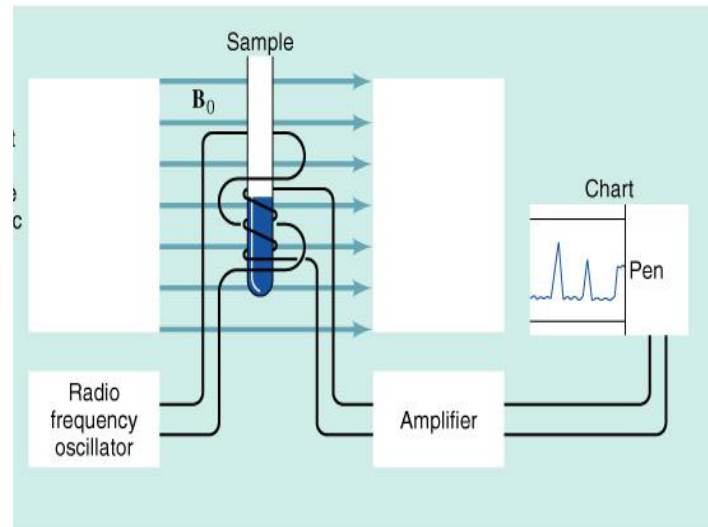
Radio frequency (Rf) generator and computer operating console

Fourier transformation of the signal from the time domain to the frequency domain occurs at the computer console.

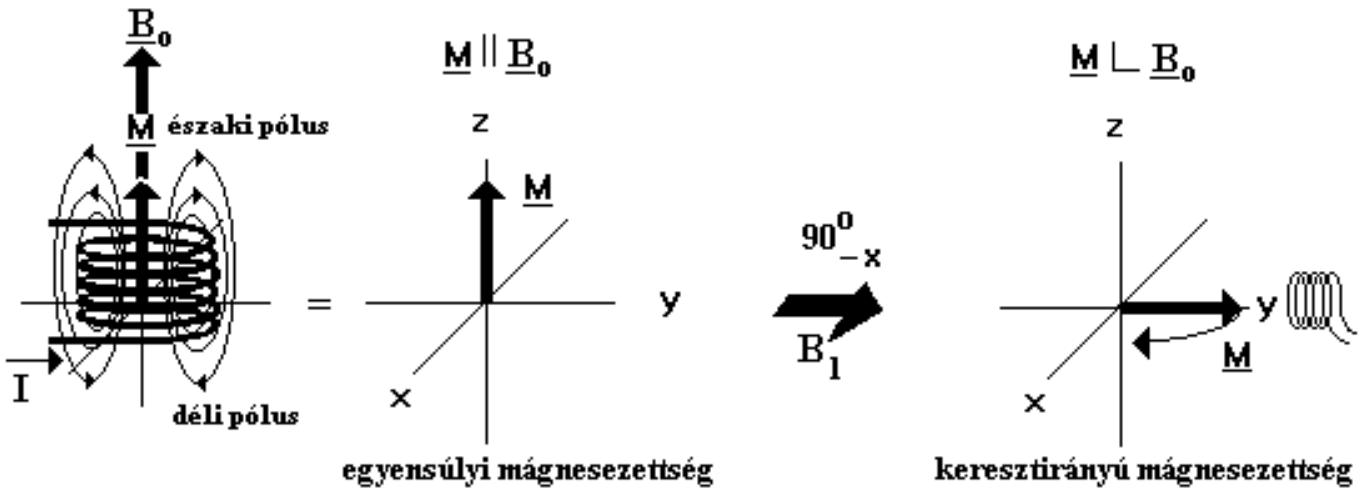




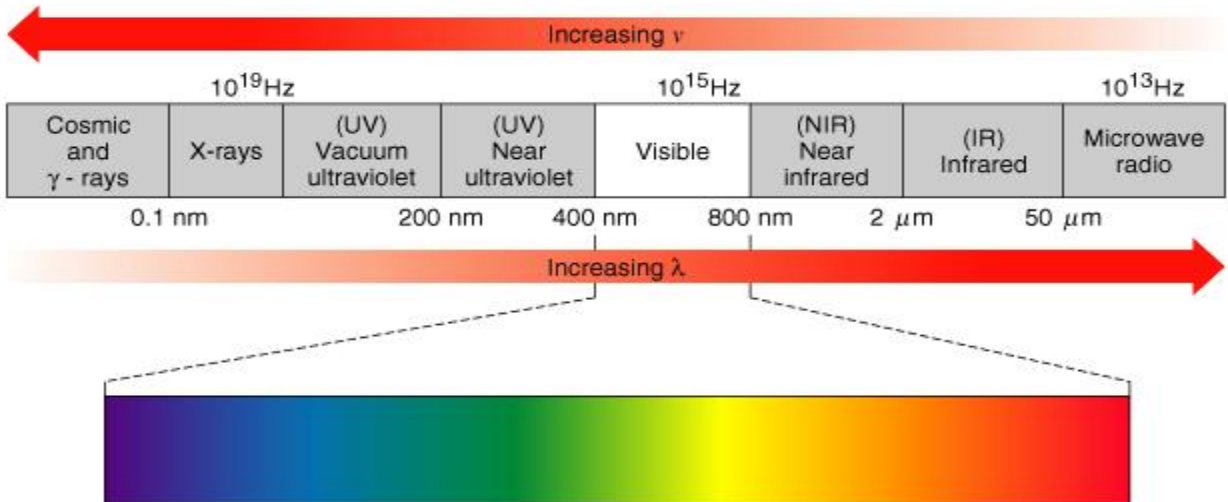
Courtesy Varian, Inc.

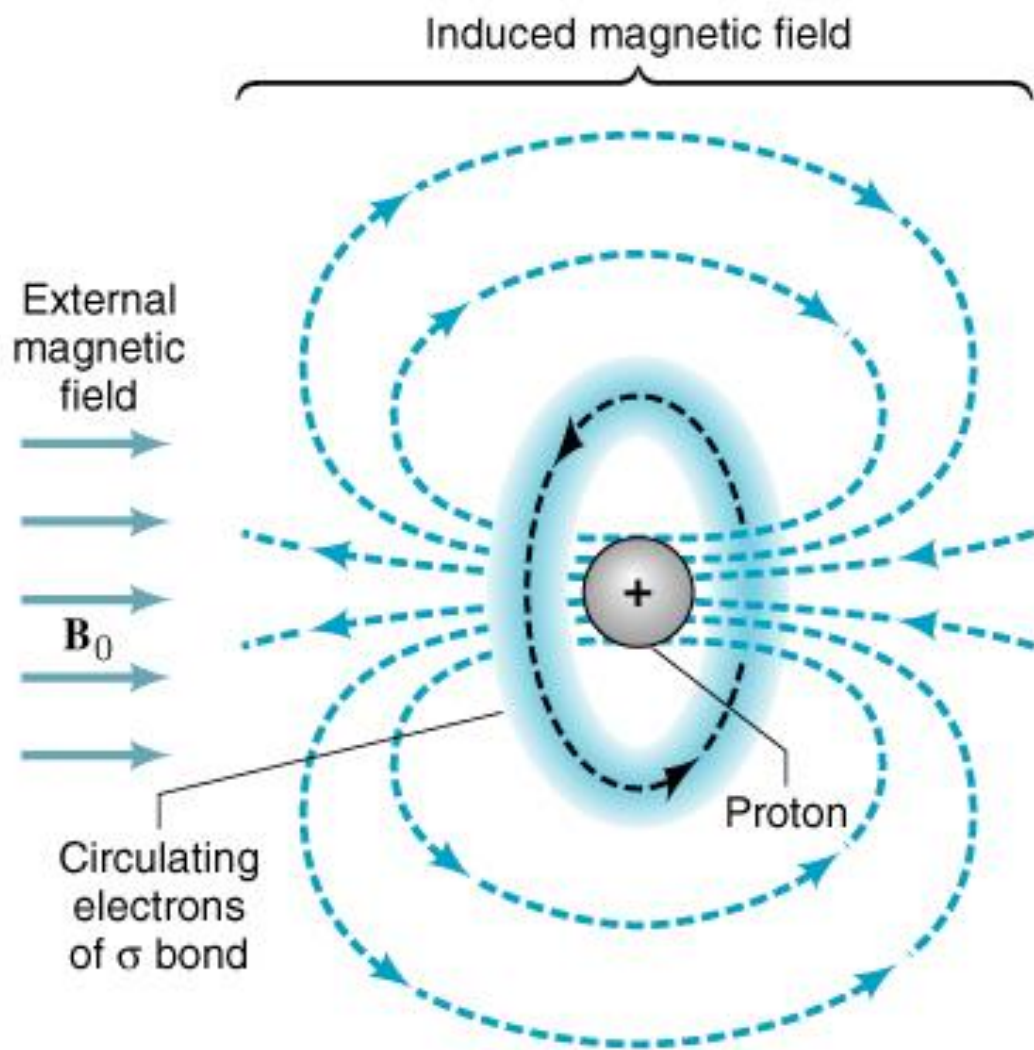


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Külső mágneses térben a makroszkopikus mágnesezettség gerjesztése annak precessziójához vezet, amely mérhető indukált feszültséget eredményez





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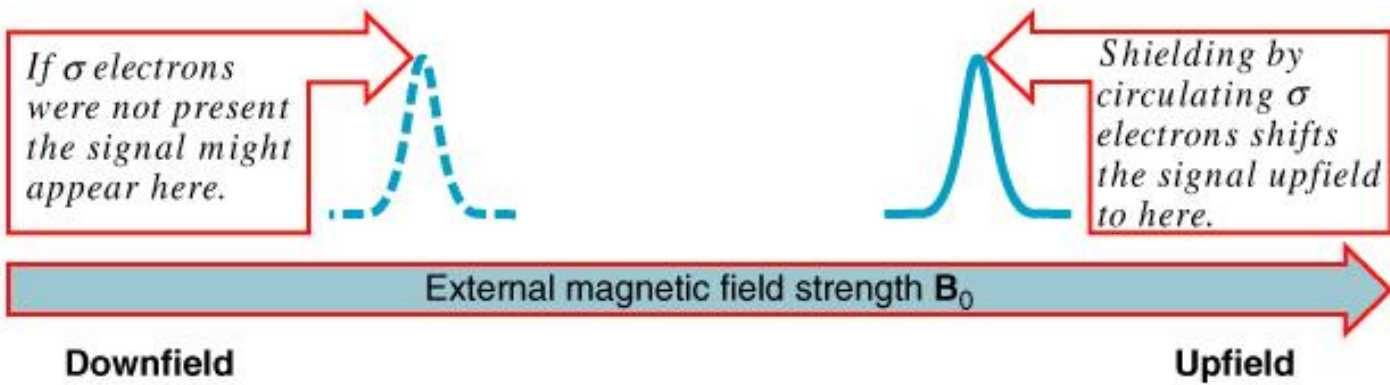
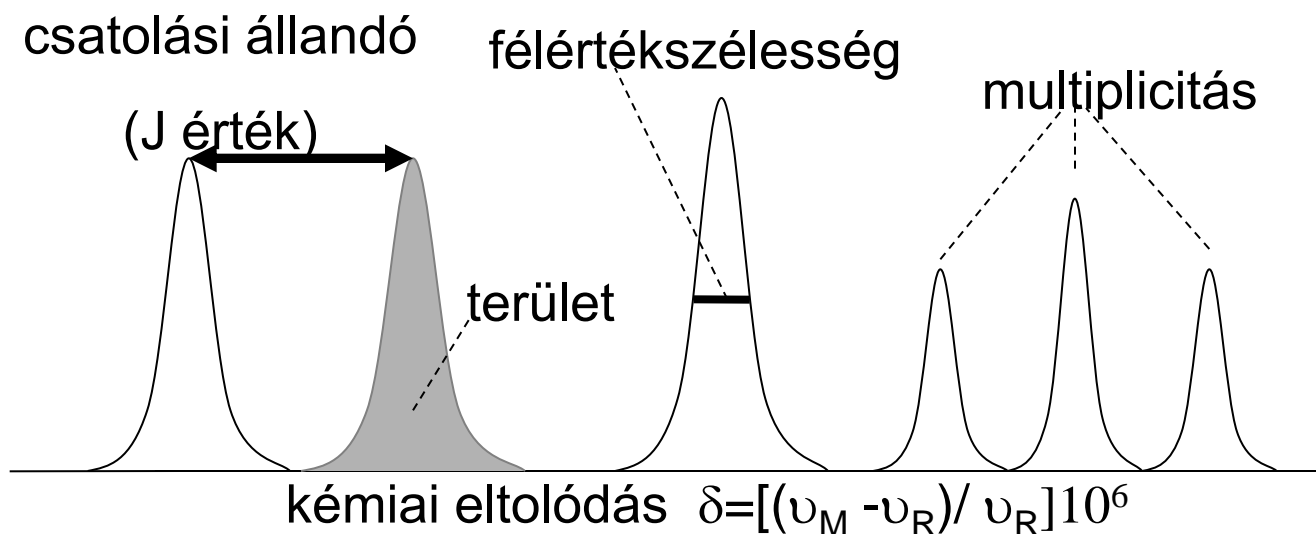


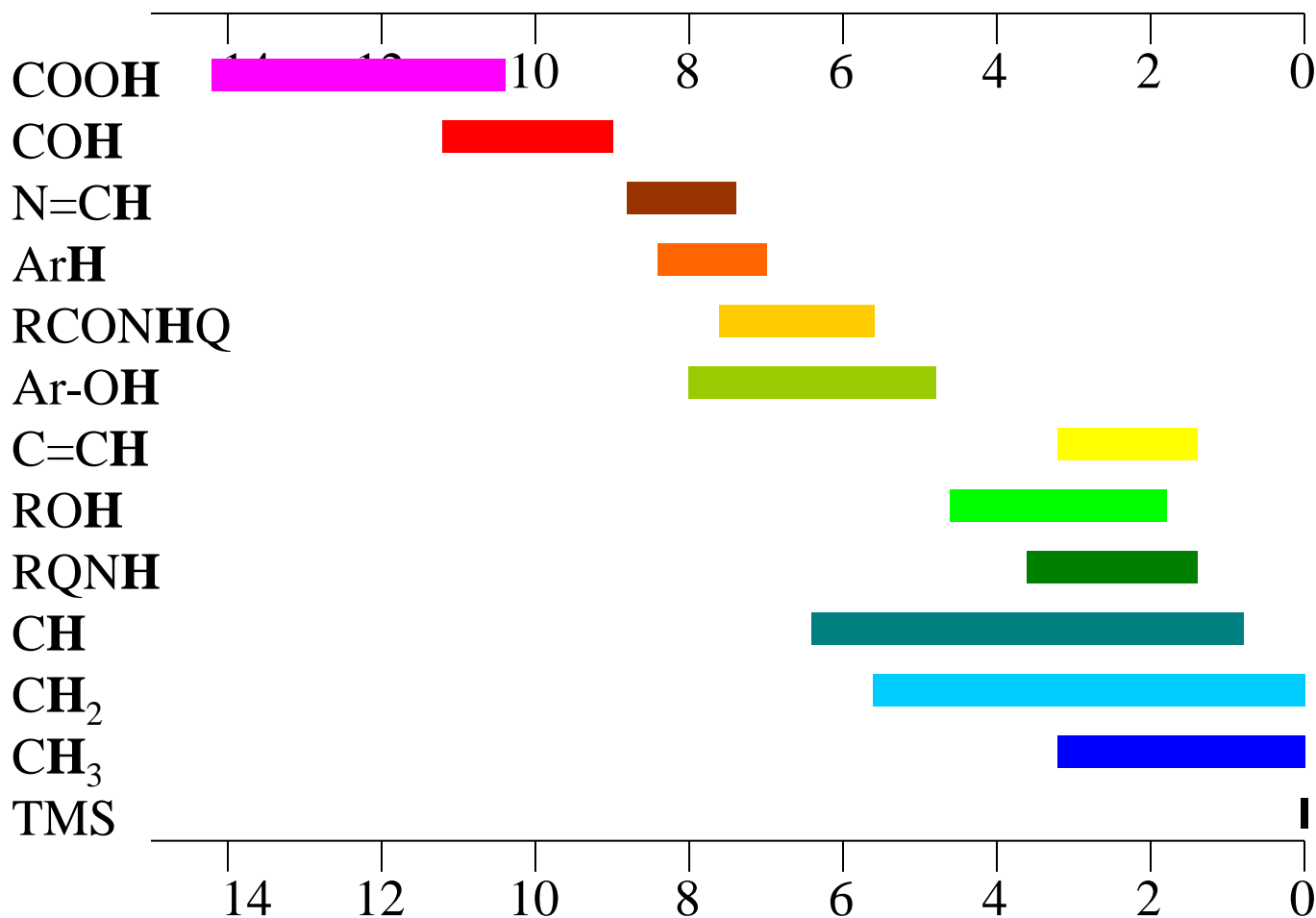
Figure 9.11 Shielding by  $\sigma$  electrons causes  $^1\text{H}$  NMR absorptions to be shifted to higher external magnetic field strengths.

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# A nagy felbontású NMR-spektrumok öt jellemző paramétere:



## Jellegzetes proton kémiai eltolódás értékek



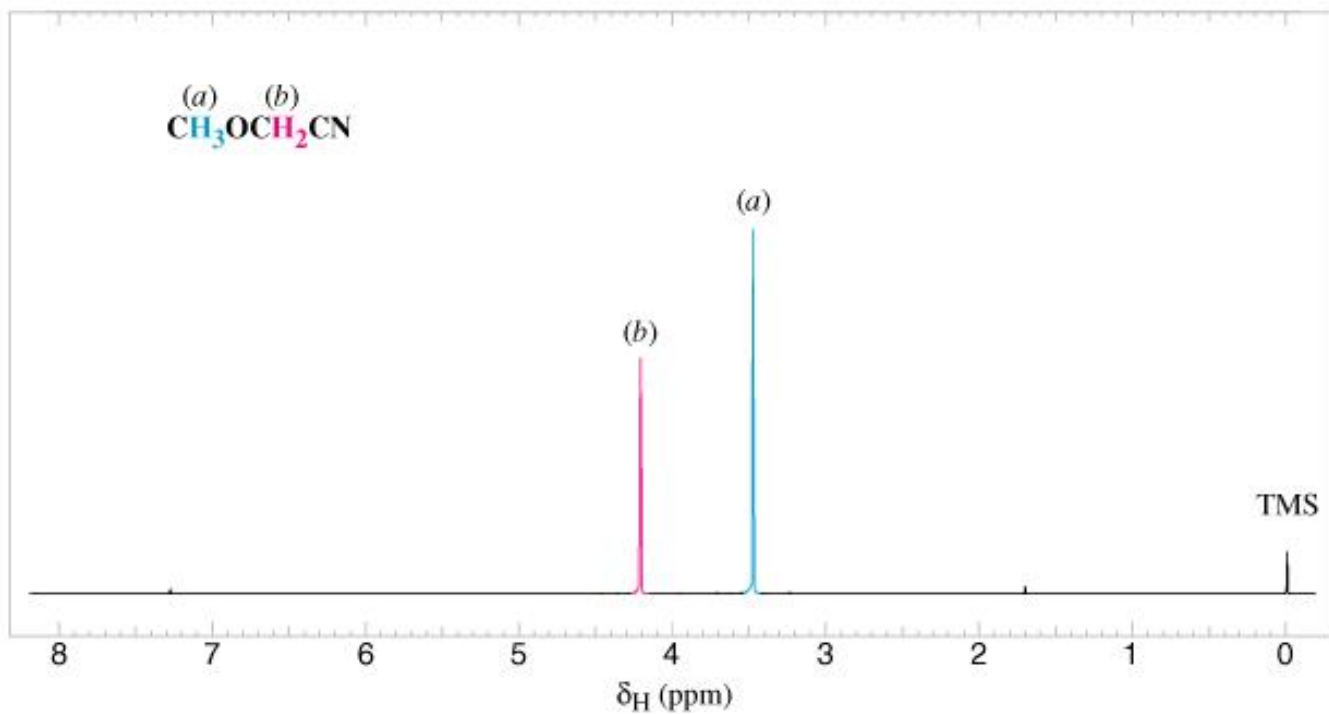
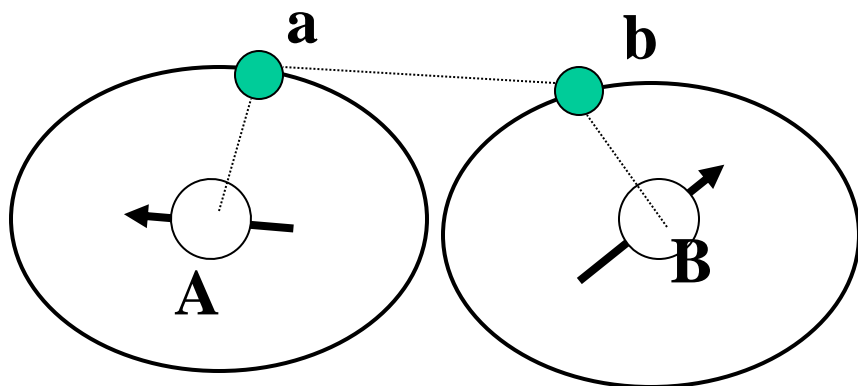


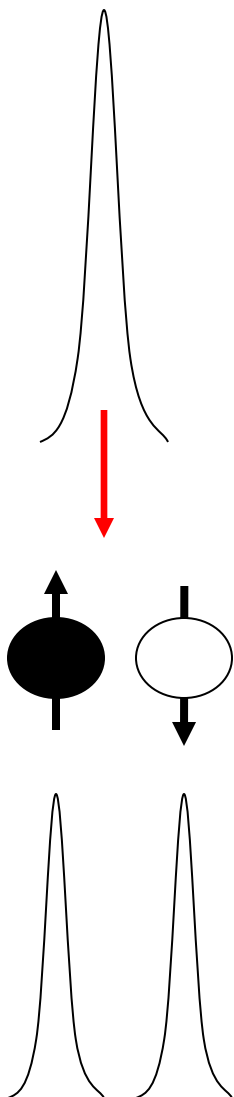
Figure 9.14 The 300 MHz  $^1\text{H}$  NMR spectrum of methoxyacetonitrile. The signal of the enantiotopic protons (b) is not split.

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# Spin-spin csatolás (a színkép finomszerkezete)

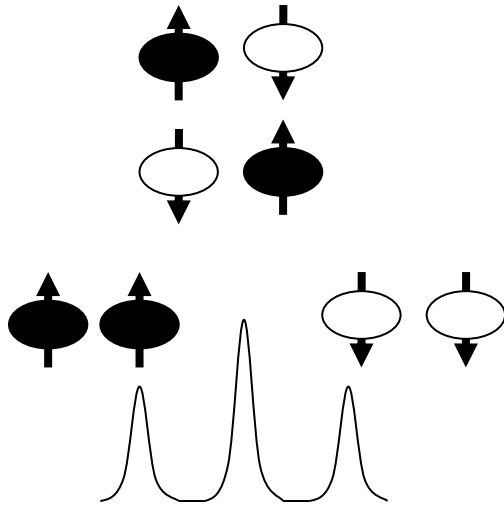


*A és B magok indirekt módon  
a és b elektronokon keresztül csatoltak.*

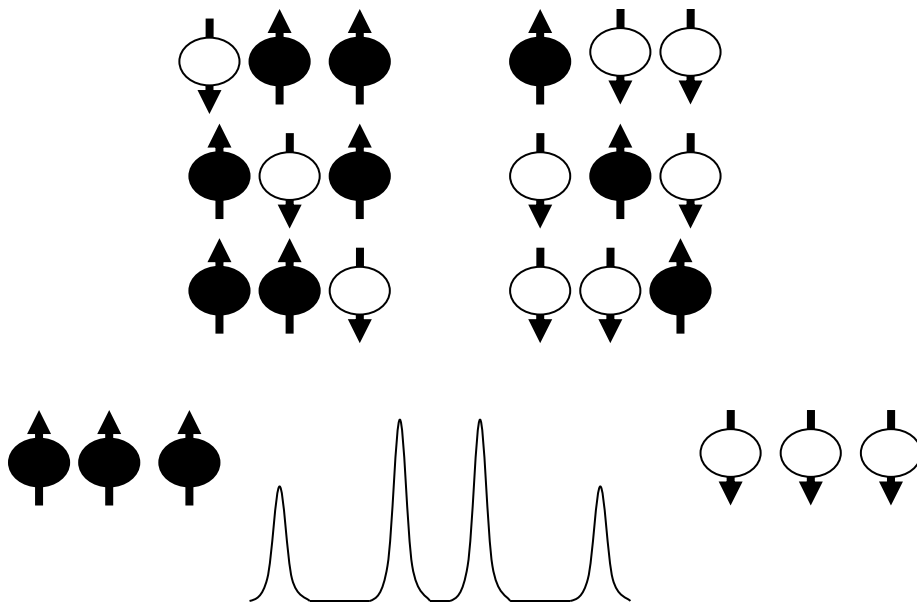


A jelenség a spin-spin felhasadás,  
a skaláris csatolás vagy  
a J-csatolás

**dublet mintázat**



**triplet mintázat**



**kvadruplet mintázat**

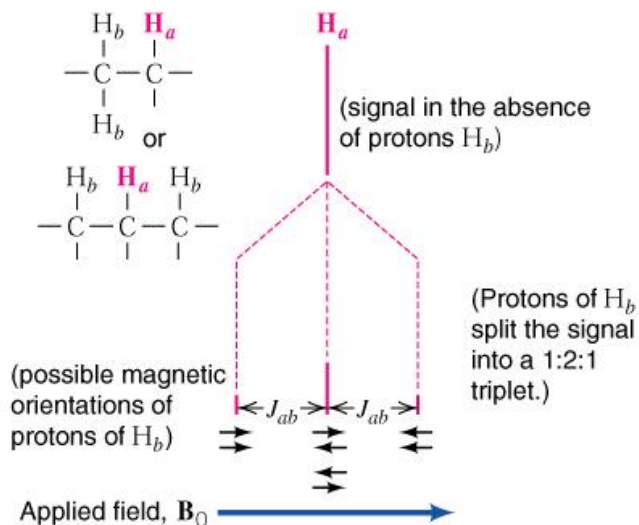
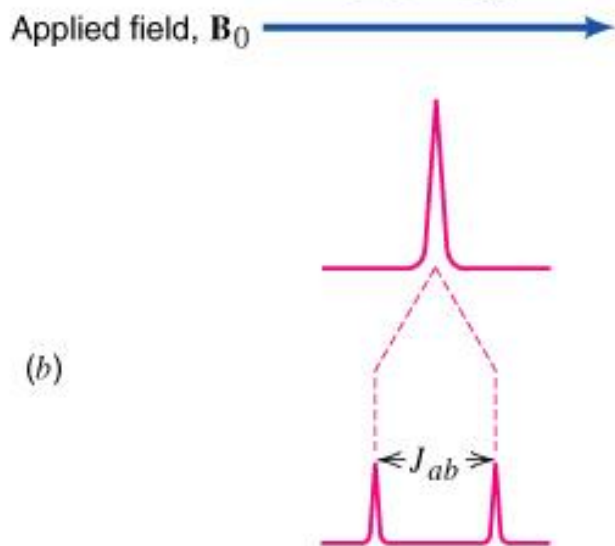
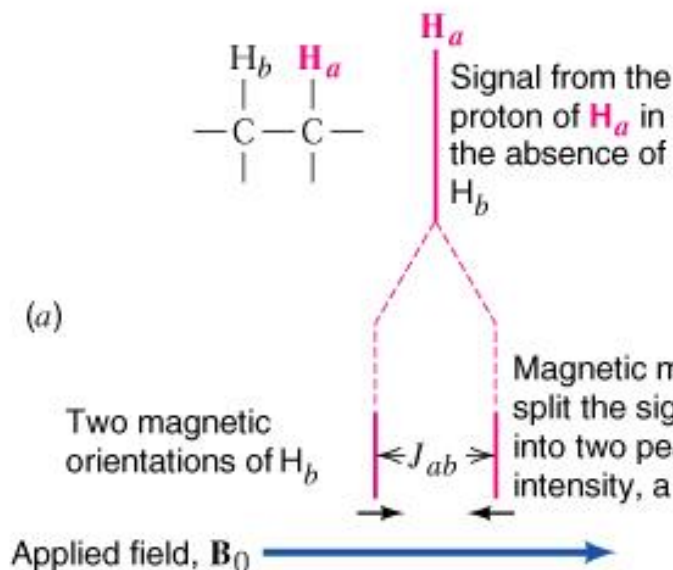


Figure 9.16 Two equivalent protons ( $H_b$ ) on an adjacent carbon atom split the signal from  $H_a$  into a 1:2:1 triplet.

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Figure 9.15 Signal splitting arising from spin-spin coupling with one nonequivalent proton of a neighboring hydrogen atom. A theoretical analysis is shown in (a) and the actual appearance of the spectrum in (b). The distance between the centers of the peaks of the doublet is called the coupling constant,  $J_{ab}$ . The term  $J_{ab}$  is expressed in hertz. The magnitudes of coupling constants are *not* dependent on the magnitude of the applied field and their values (in Hz) are the same, regardless of the operating frequency of the spectrometer.

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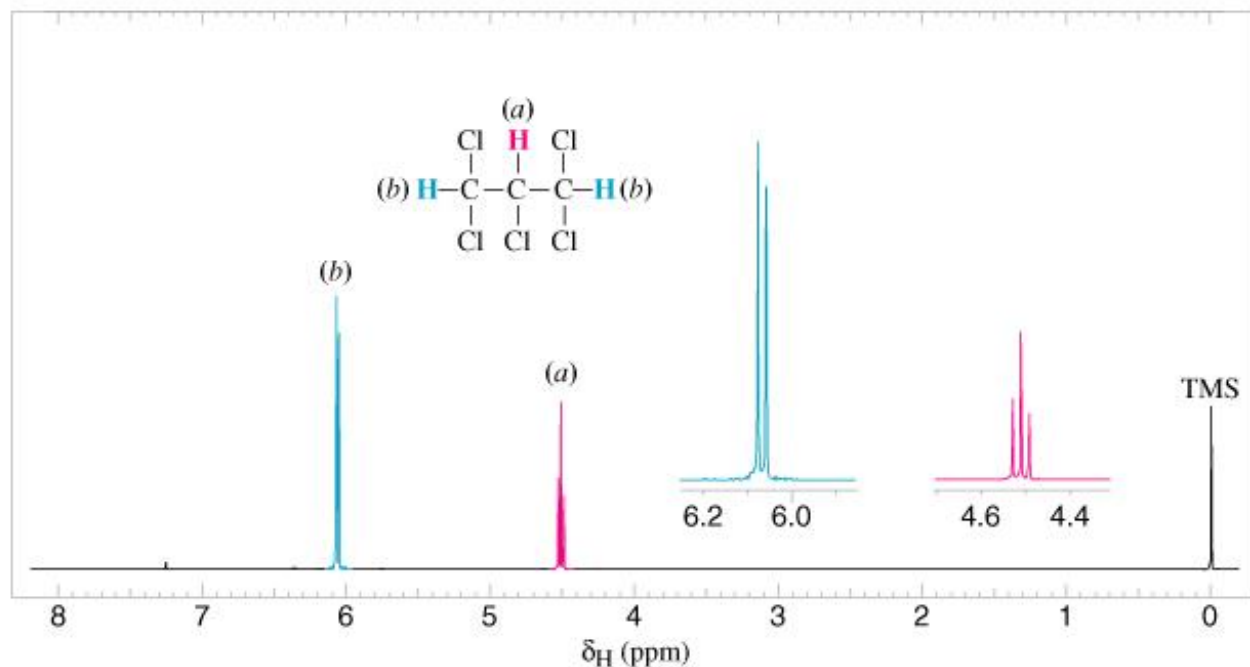
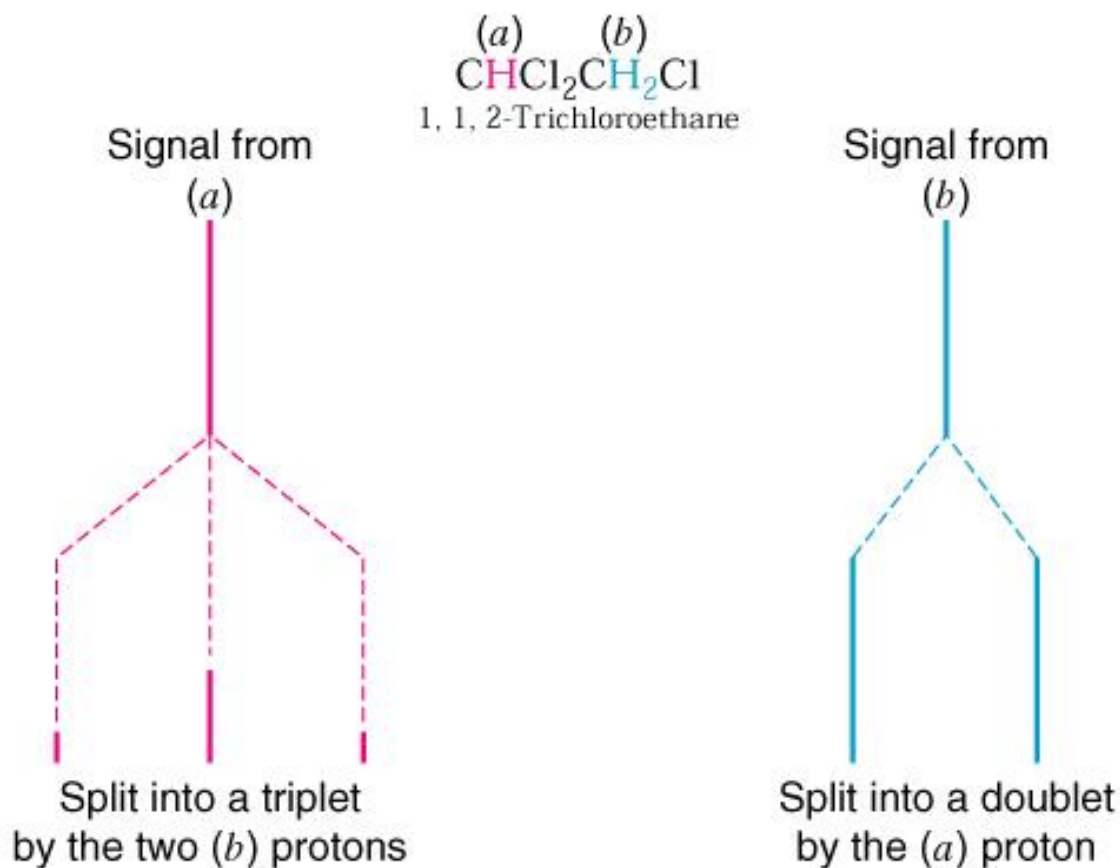


Figure 9.17 The 300 MHz  $^1\text{H}$  NMR spectrum of 1,1,2,3,3-pentachloropropane. Expansions of the signals are shown in the offset plots.

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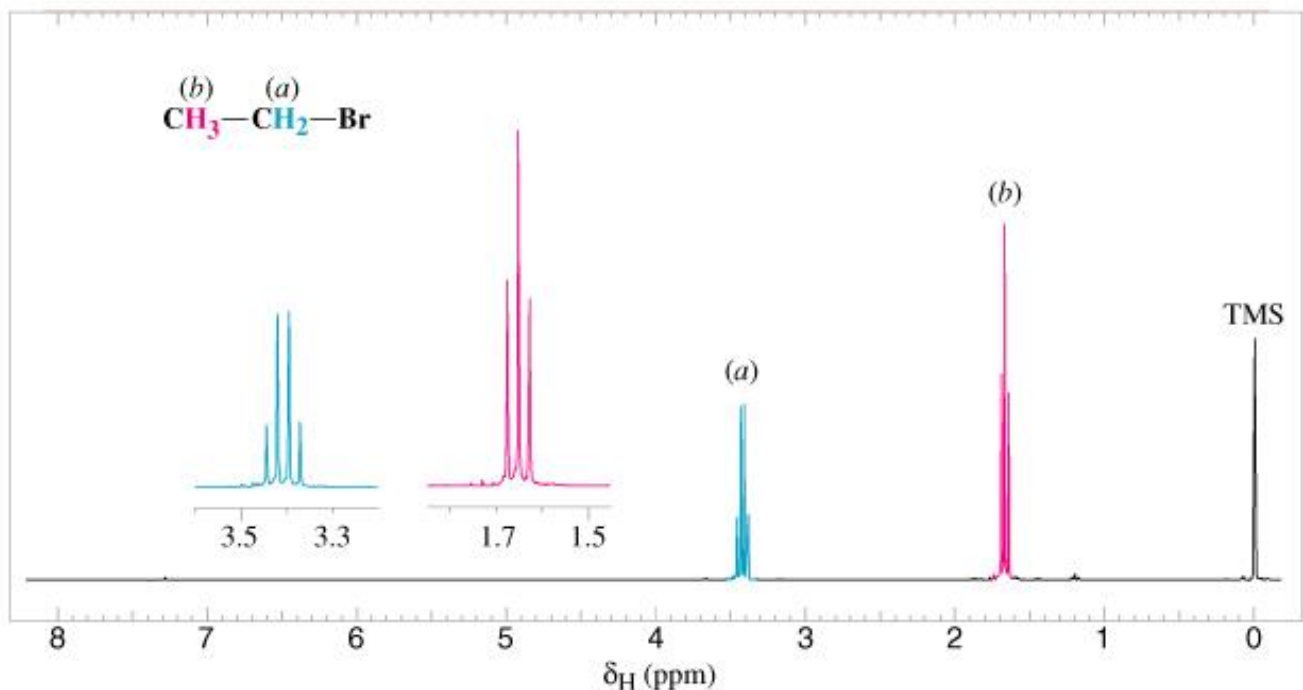


Figure 9.19 The 300 MHz  $^1\text{H}$  NMR spectrum of ethyl bromide. Expansions of the signals are shown in the offset plots

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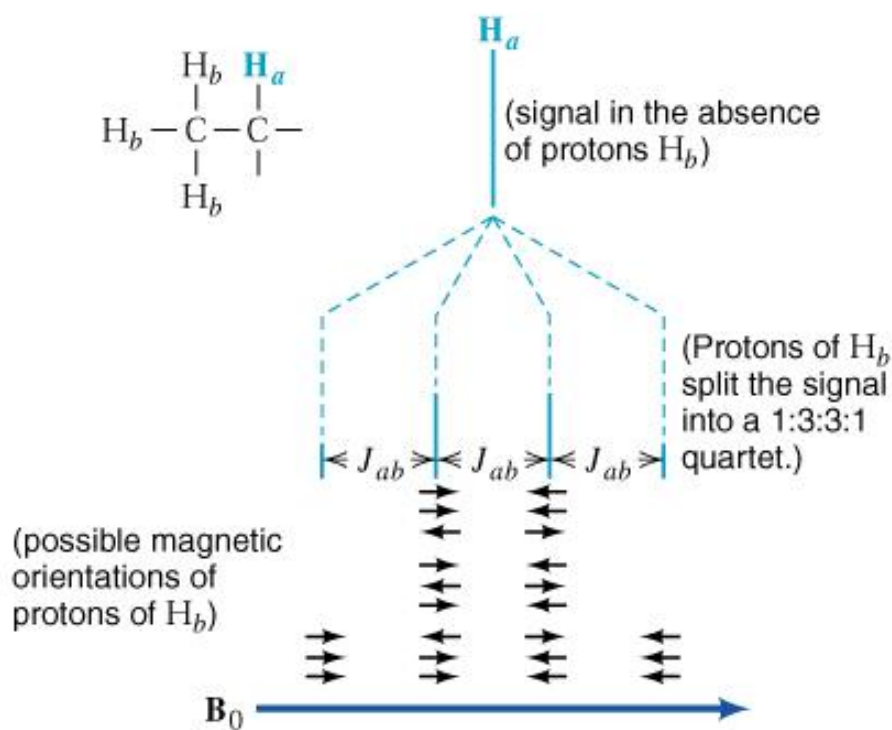


Figure 9.18 Three equivalent protons ( $\text{H}_b$ ) on an adjacent carbon split the signal from  $\text{H}_a$  into a 1:3:3:1 quartet.

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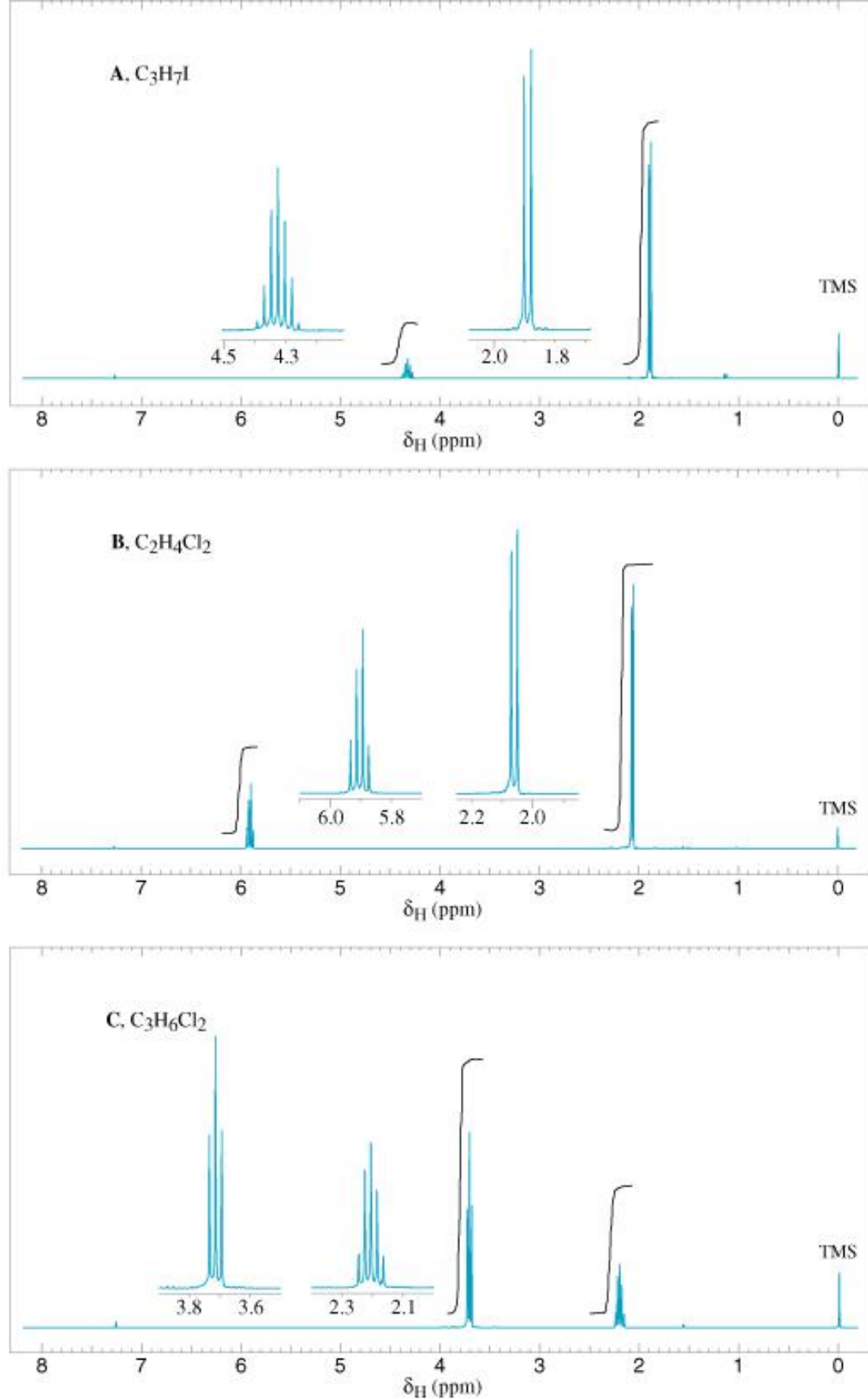
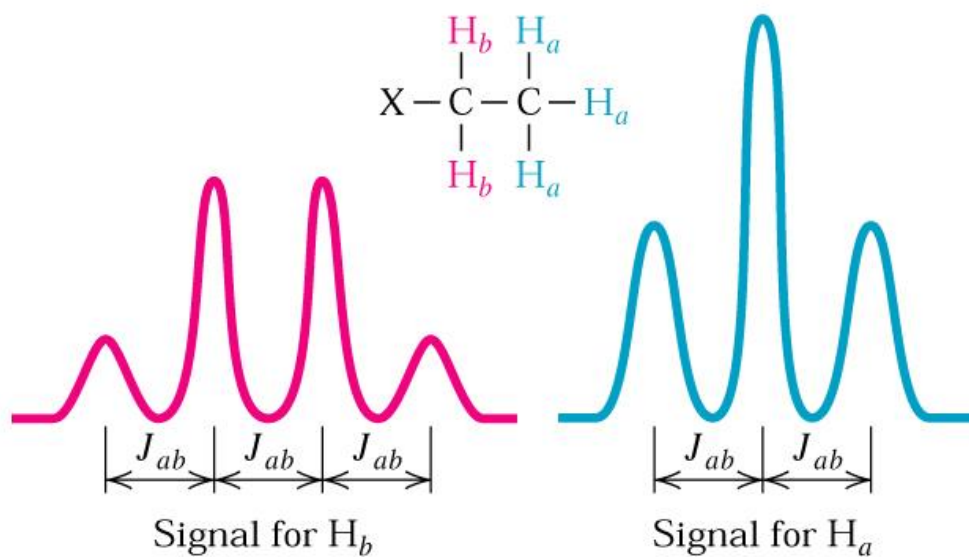


Figure 9.20 The 300 MHz <sup>1</sup>H NMR spectra for Problem 9.8. Expansions are shown in the offset plots.



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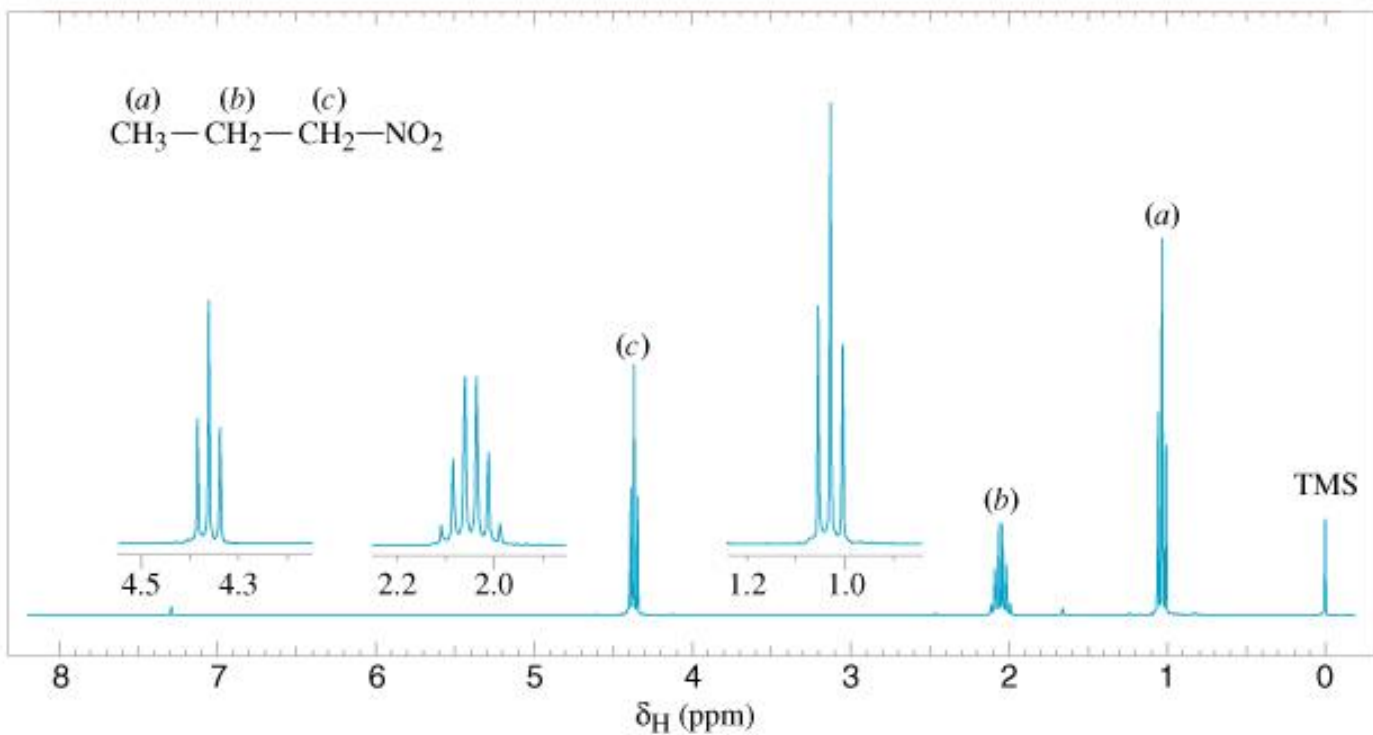


Figure 9.24 The 300 MHz  $^1\text{H}$  NMR spectrum of 1-nitropropane. Expansions of the signals are shown in the offset plots.

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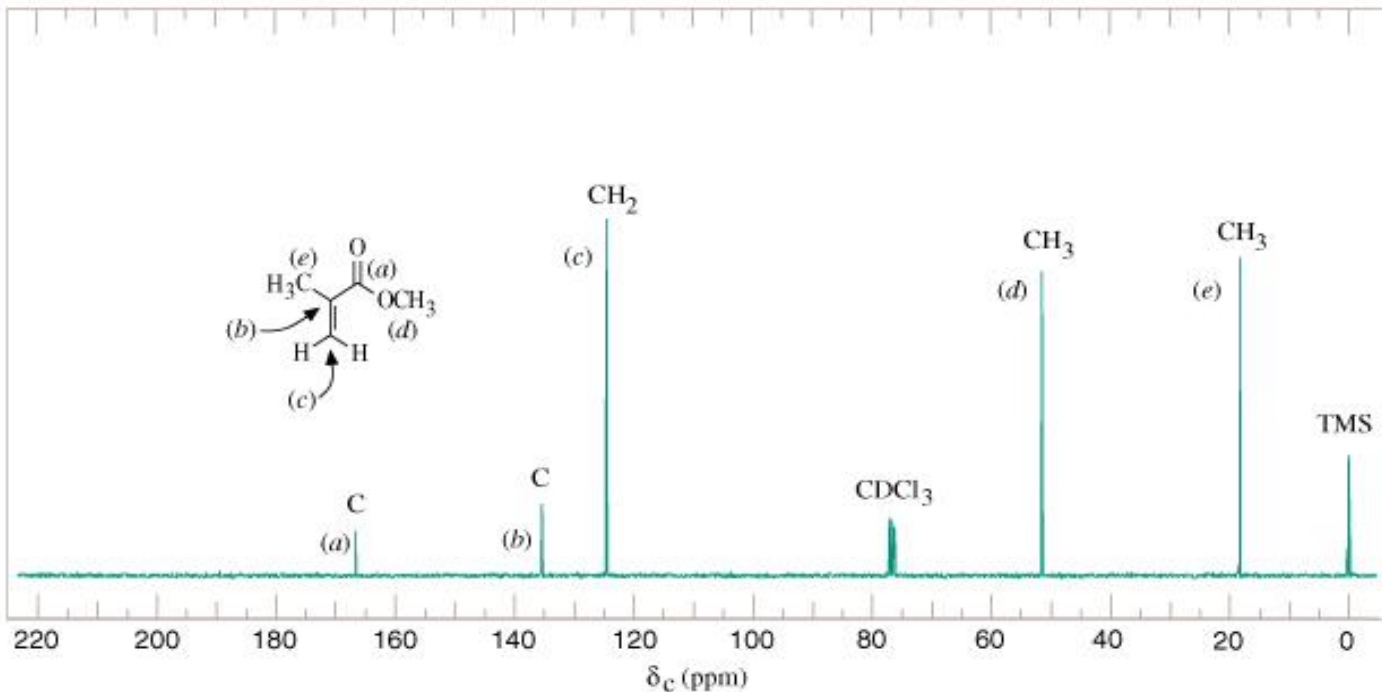
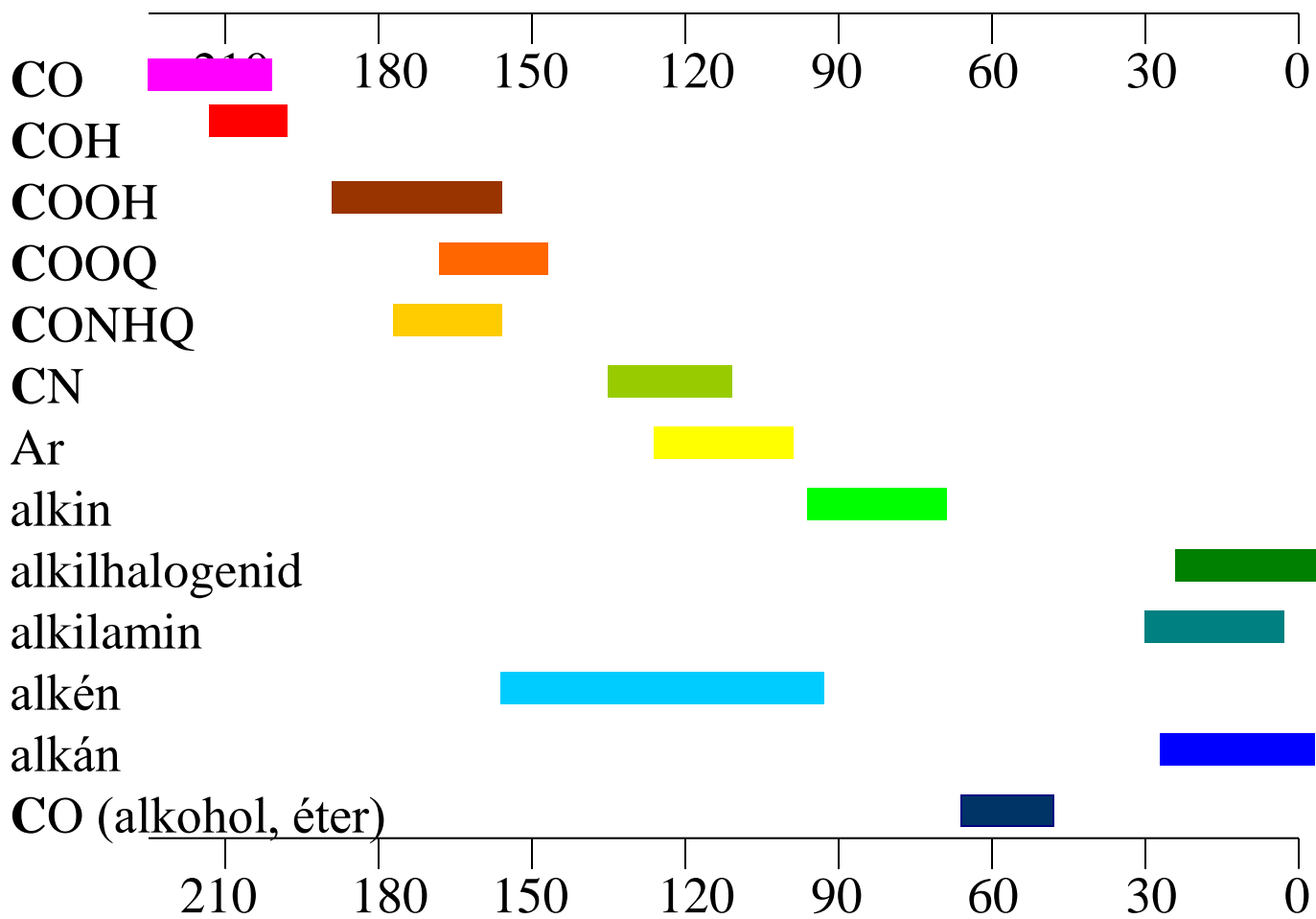


Figure 9.27 The broadband proton-decoupled <sup>13</sup>C NMR spectrum of methyl methacrylate. Information from the DEPT <sup>13</sup>C NMR spectra is given above the peaks.

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## Jellegzetes <sup>13</sup>C kémiaieltolódás értékek



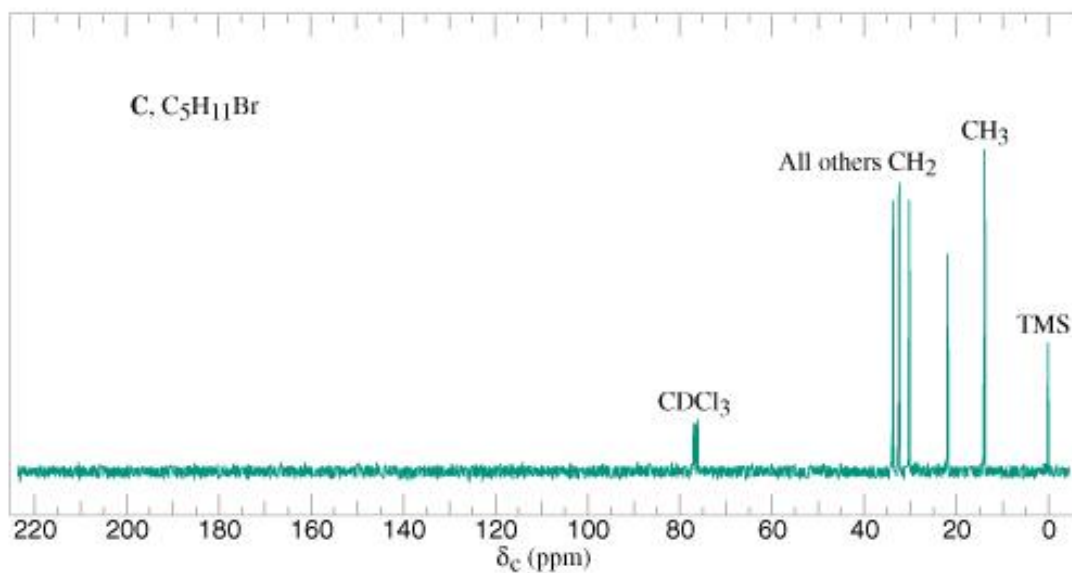
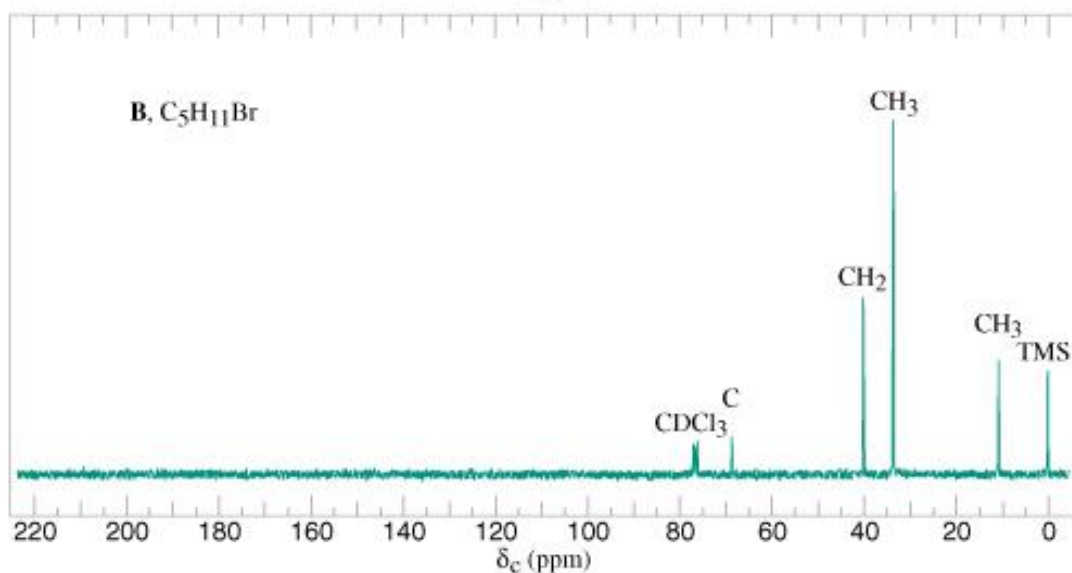
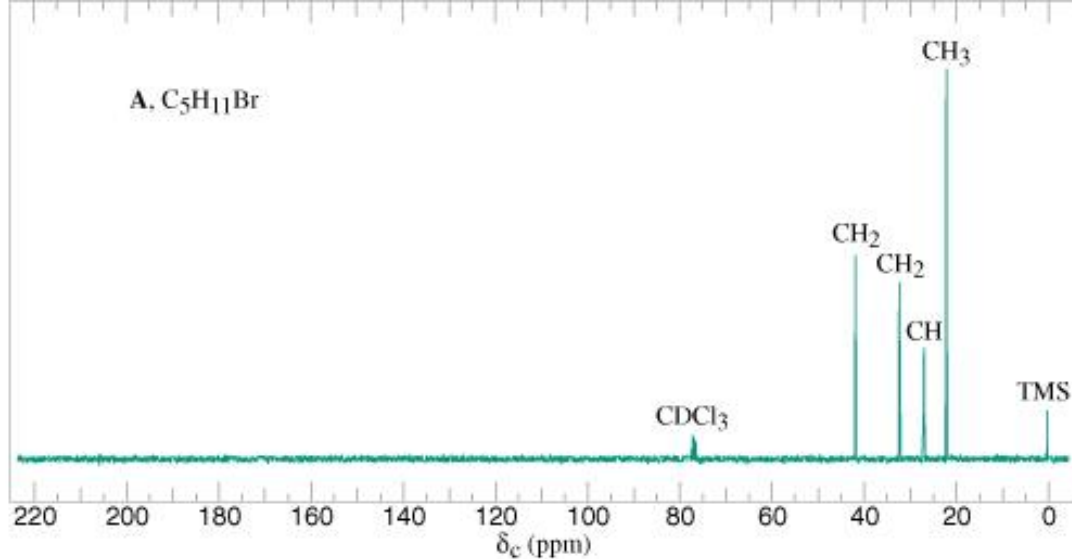
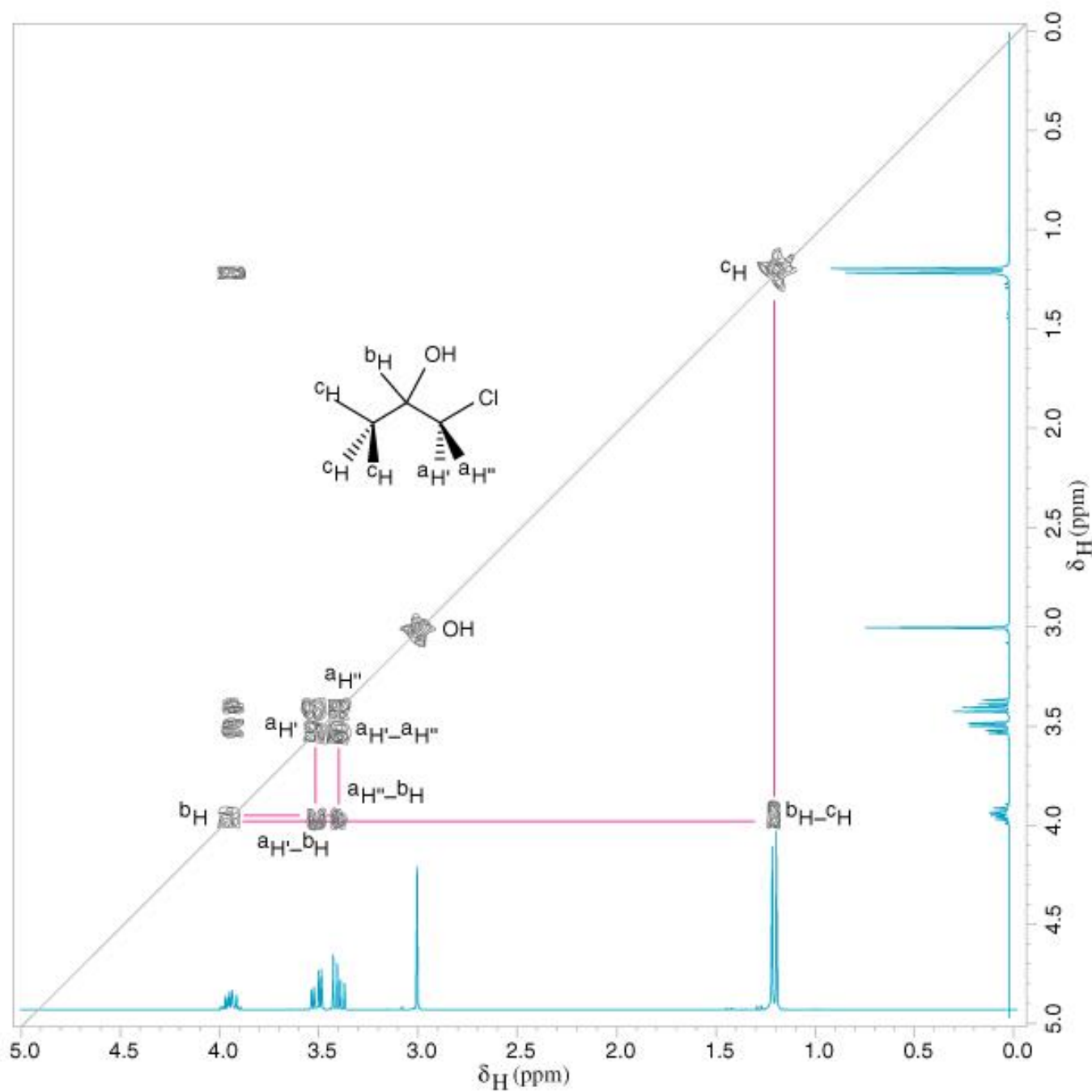
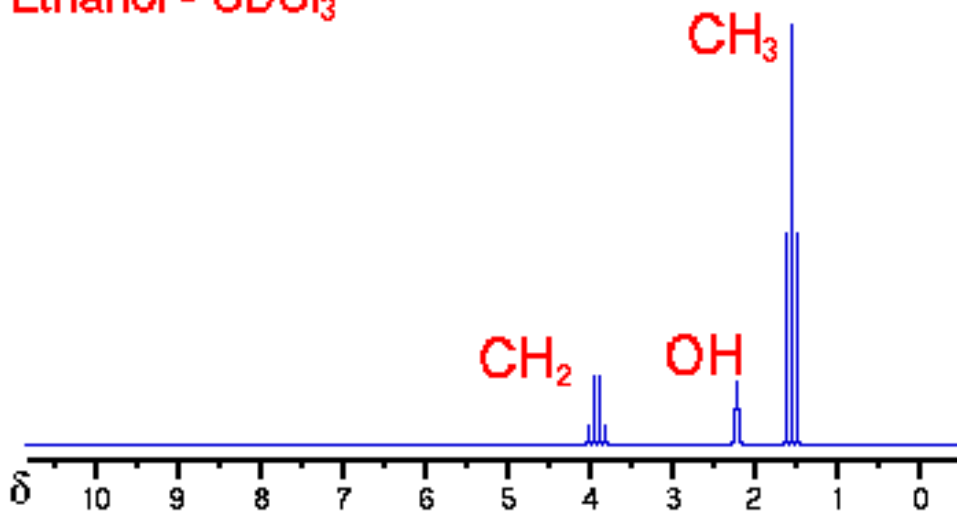


Figure 9.28 The broadband proton-decoupled  $^{13}\text{C}$  NMR spectra of compounds A, B, and C, Problem 9.11. Information from the DEPT  $^{13}\text{C}$  NMR spectra is given above the peaks.

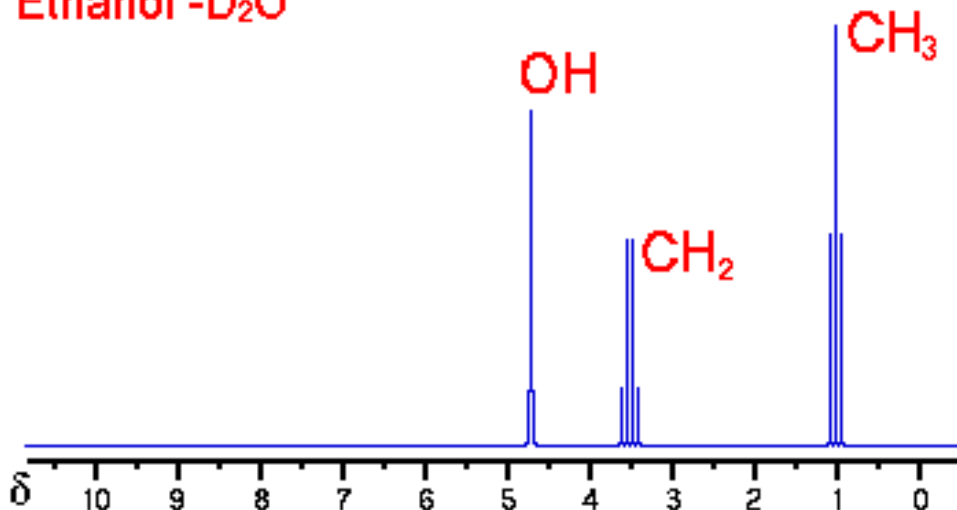


# Solvent dependence of chemical shifts

Ethanol -  $\text{CDCl}_3$

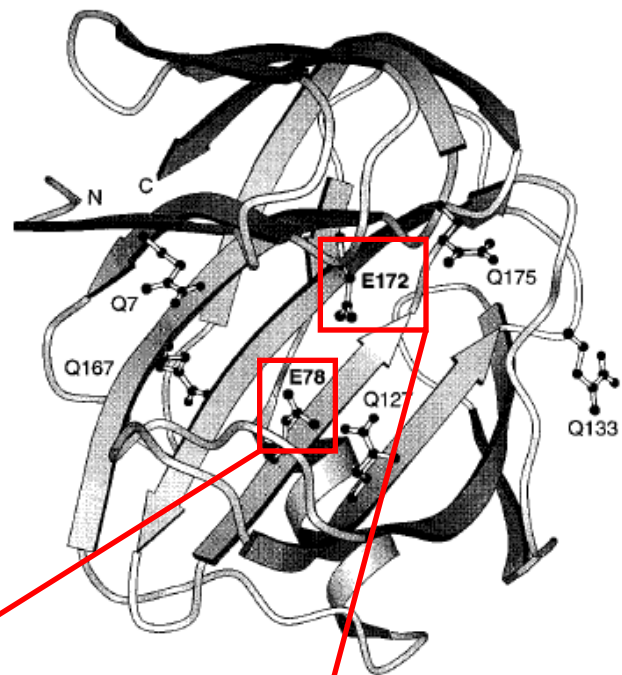


Ethanol -  $\text{D}_2\text{O}$



\*chemical shift changes with solvent used\*

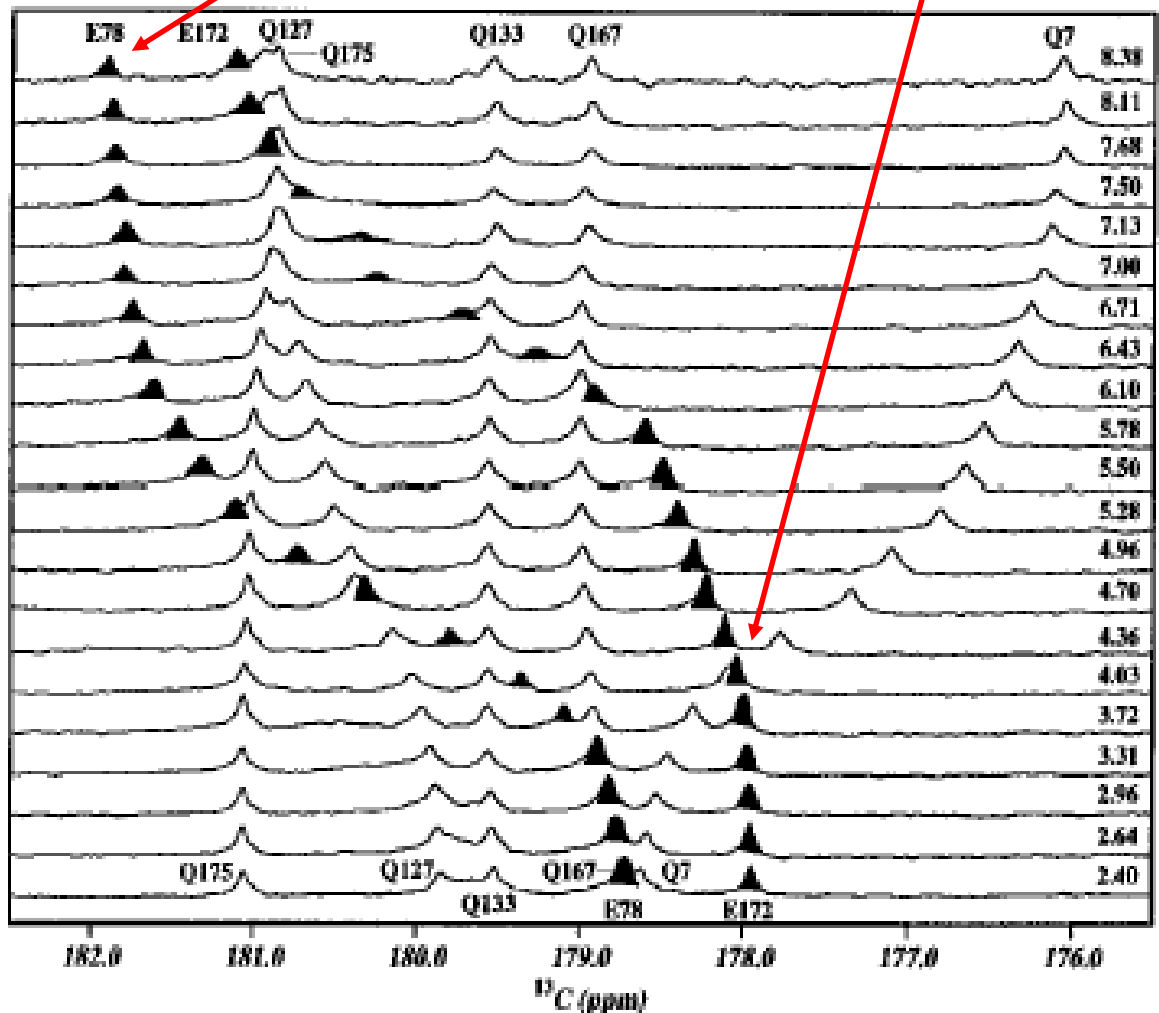
# pH dependence of chemical shifts



$^{13}\text{C}$ -NMR spectra of wild type BCX (xylanase from *Bacillus circulans*) recorded as function of pH at 25°C.

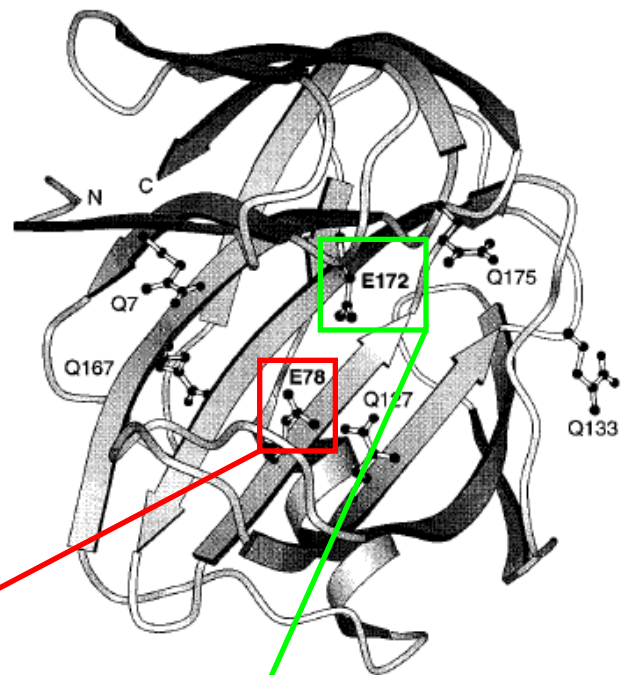
Peaks corresponding to E78 and E172 ; highlighted black to emphasize the titrat residues.

McIntosh, L.P. et al. *Biochemistry* 35 (1996) 9958.



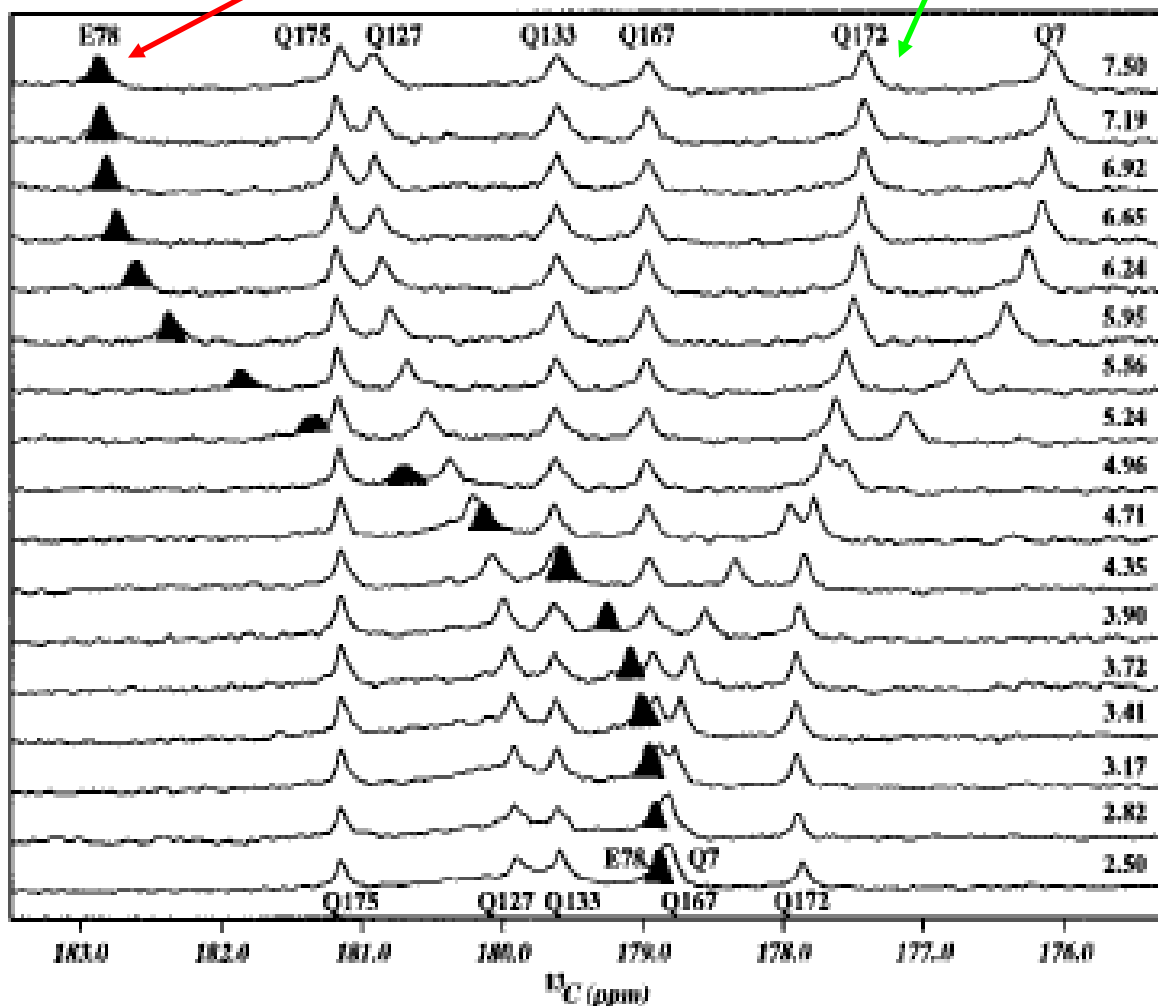


# pH dependence of chemical shifts

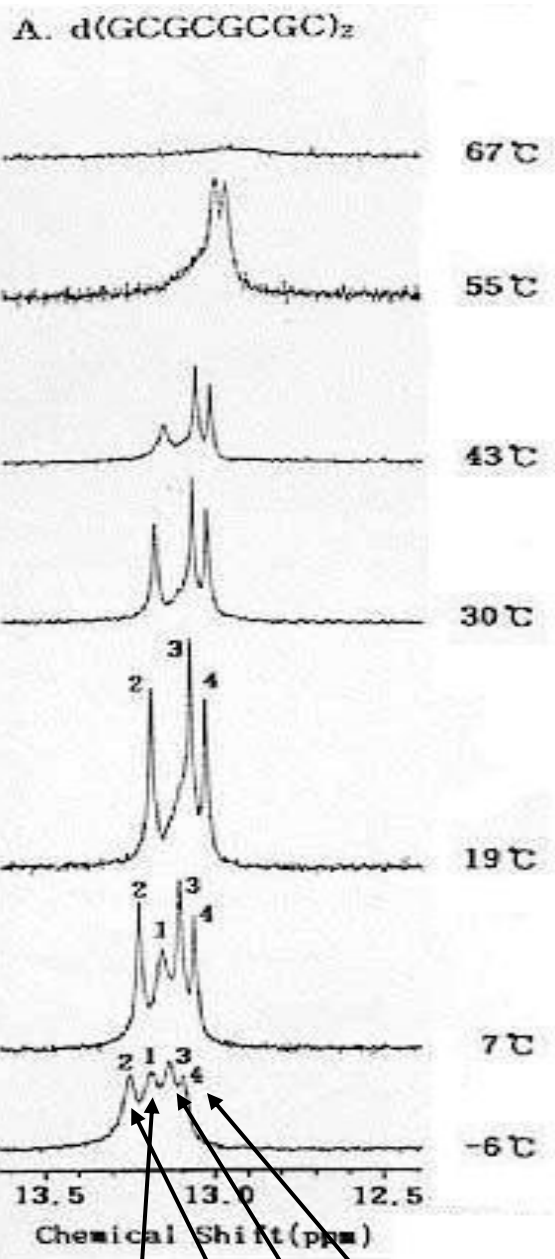


$^{13}\text{C}$ -NMR spectra of wild **E172Q**  
BCX recorded as function of pH at 25C  
The peak corresponding to E78 is  
highlighted in black.

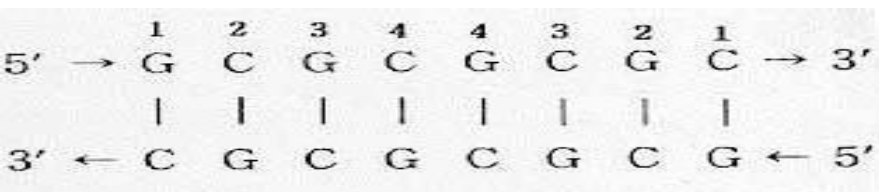
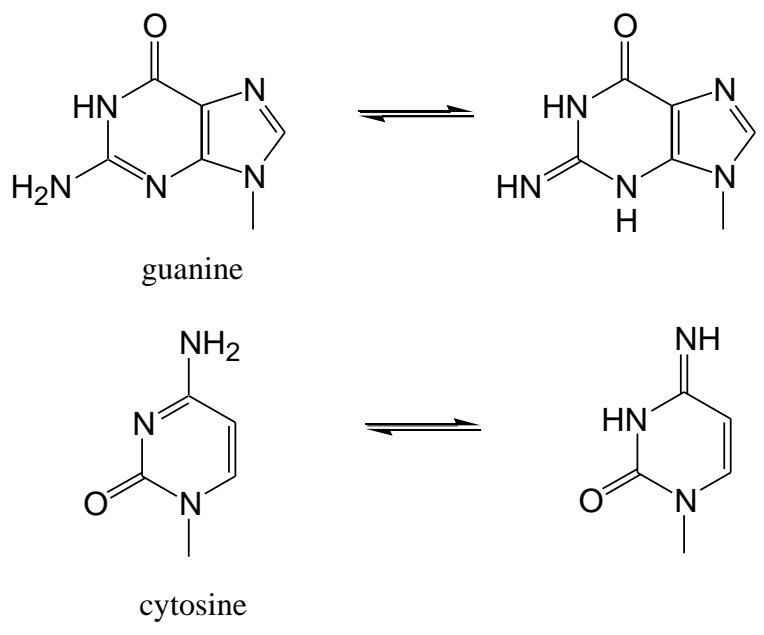
McIntosh, L.P. et al. *Biochemistry* 35 (1996) 9958.



# Temperature dependence of chemical shifts



double stranded  $\longleftrightarrow$  single stranded  
 (imino protons exchange with water protons)

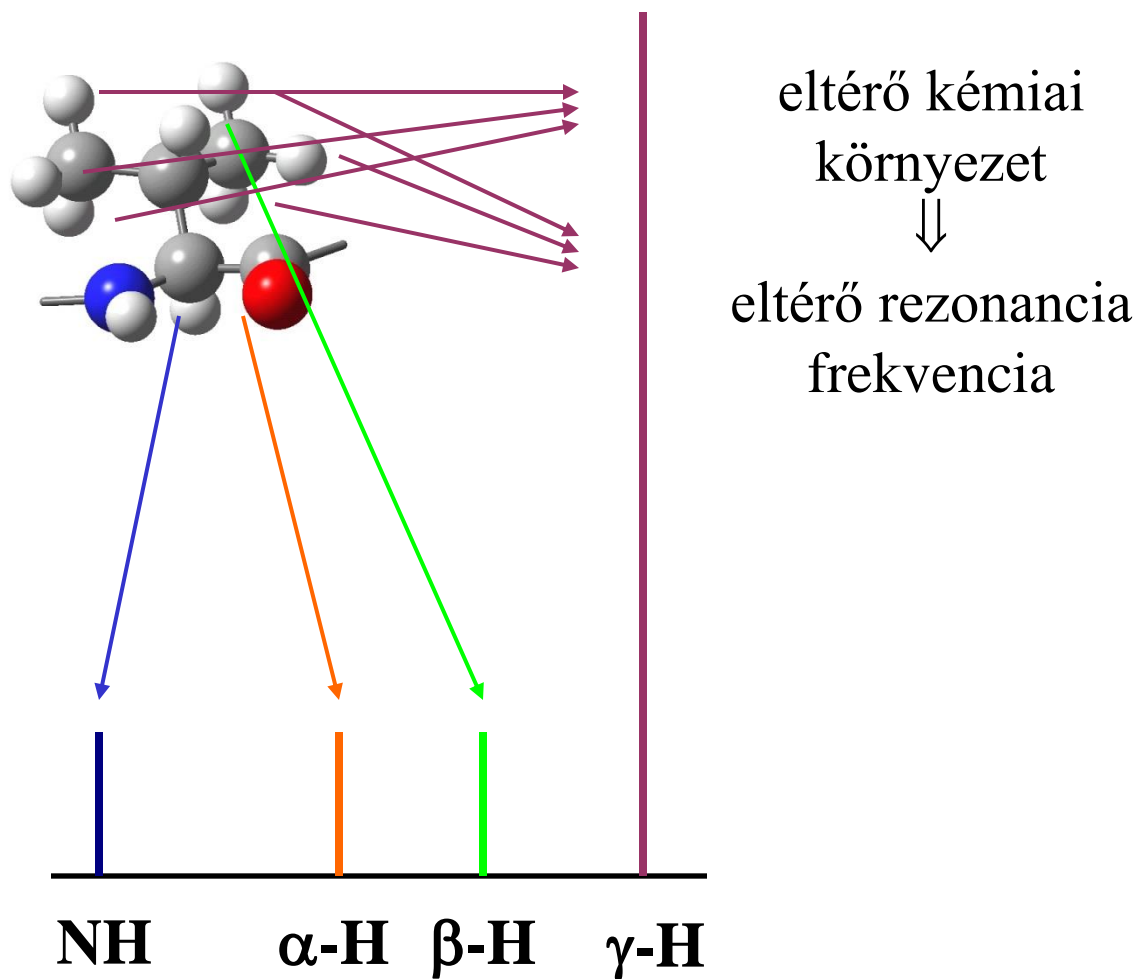


imino resonance signals of d(GCGCGCGC)<sub>2</sub> in water

# Peptid és fehérje

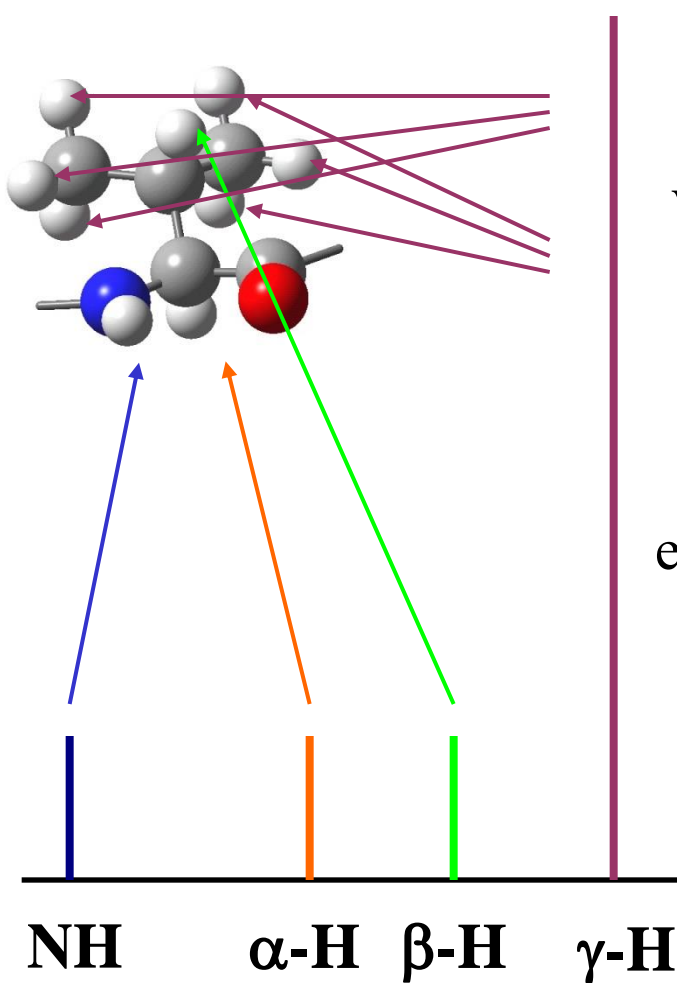
## NMR-spektroszkópia

*alapjelenség*



# Peptid és fehérje NMR-spektroszkópia

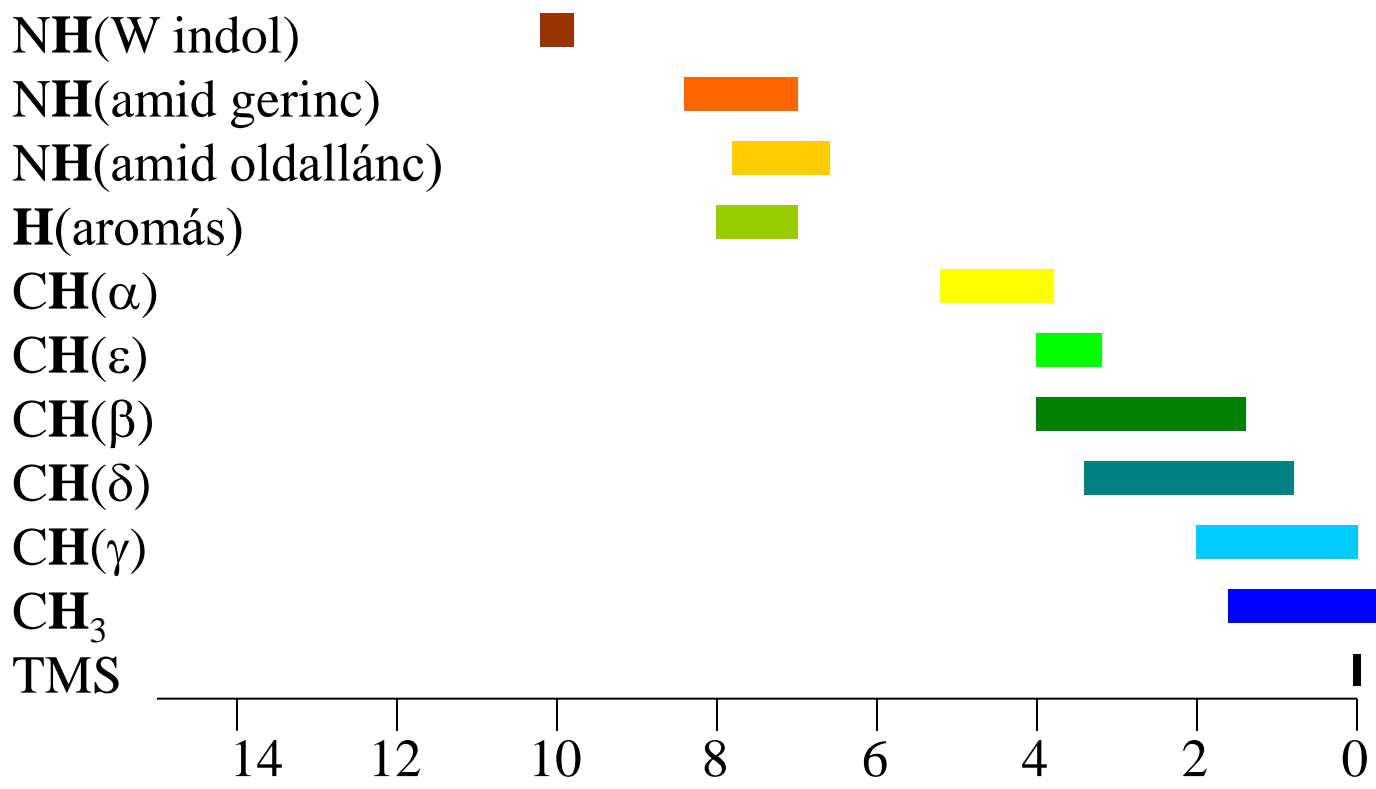
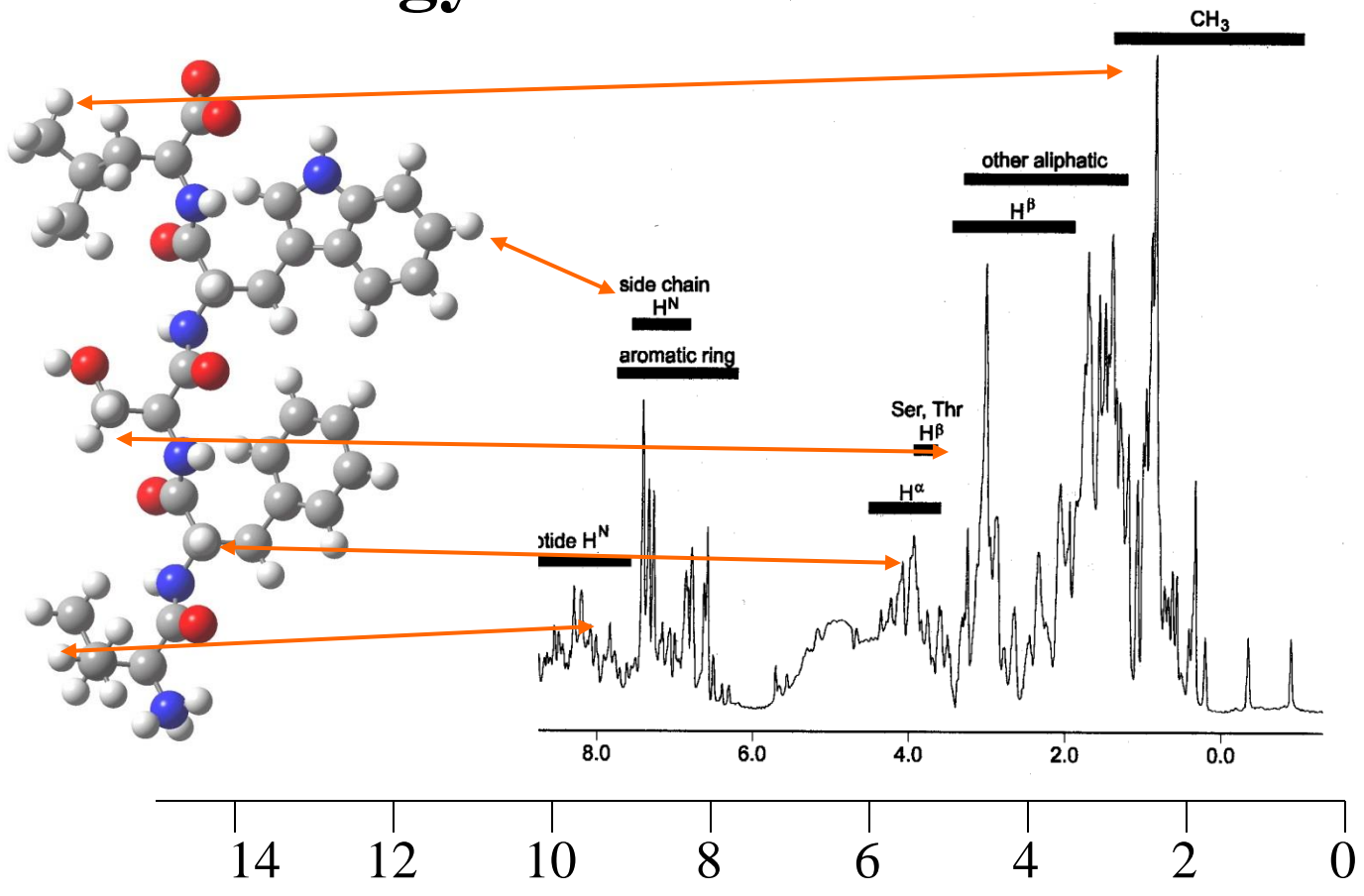
## *1. jelhozzárendelés*



a jelhozzárendelés  
vagy spektrum asszignáció  
ez előbbi megfigyelésen  
alapszik

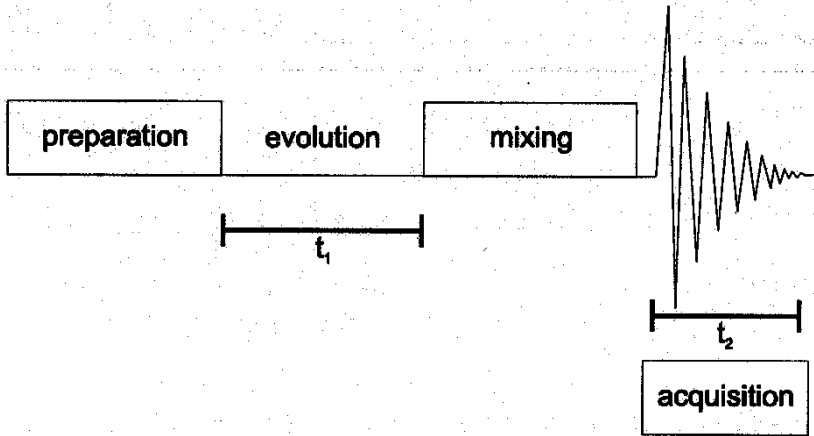
eltérő rezonancia frekvencia  
↓  
eltérő kémiai környezet

# Homonukleáris NMR-spektroszkópia egy dimenzióban

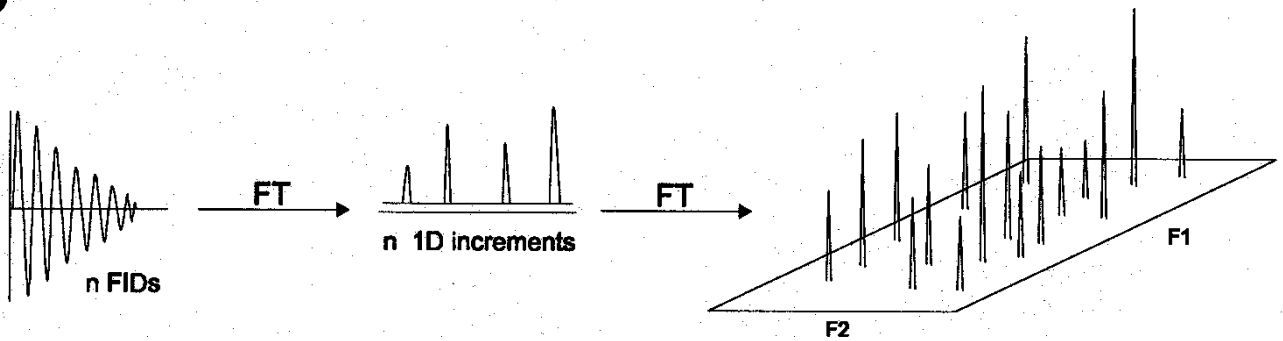


# Homo- vagy Heteronukleáris NMR-spektroszkópia két dimenzióban

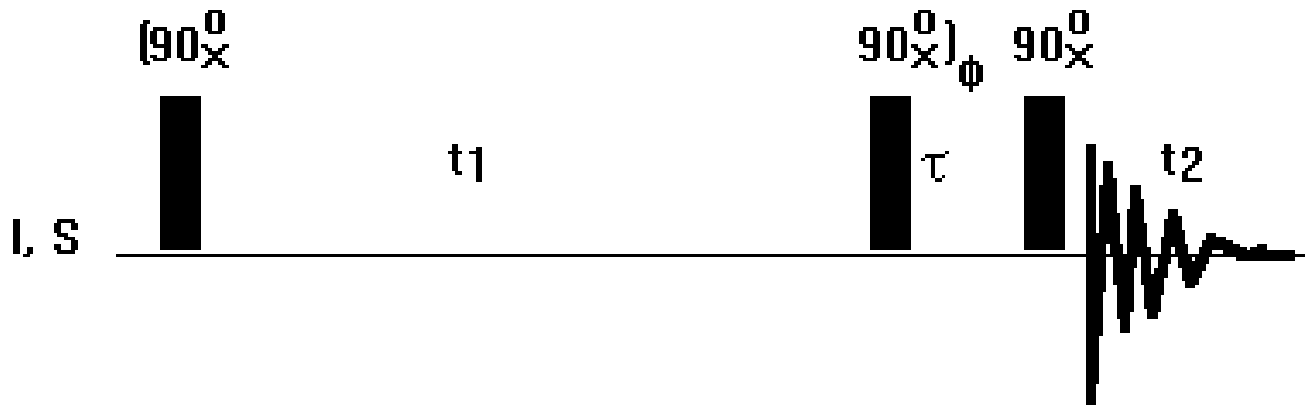
**A**



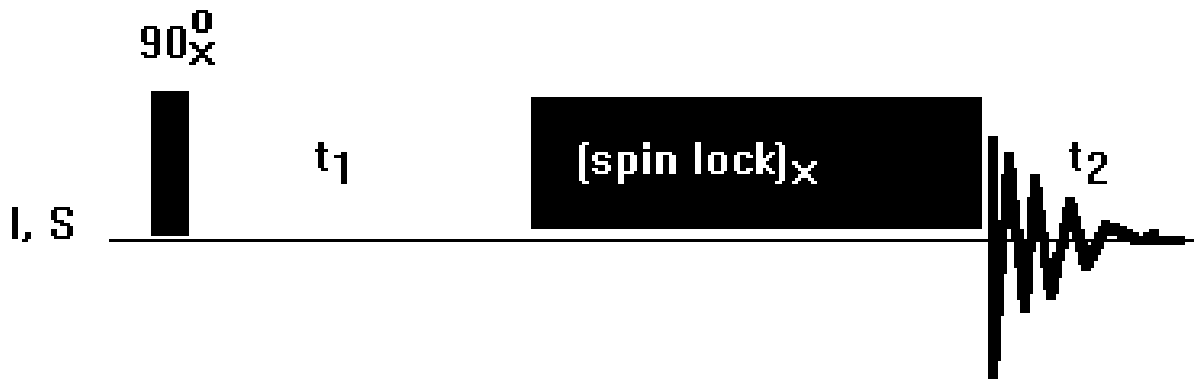
**B**



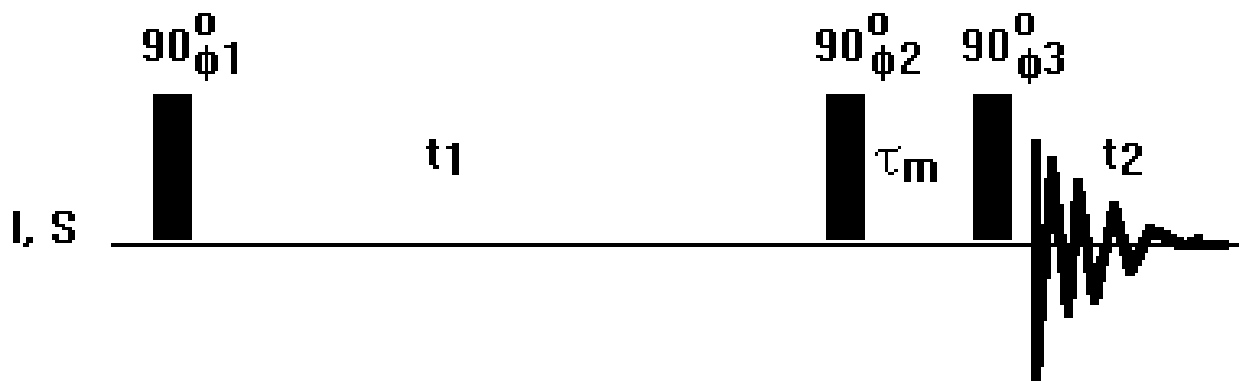
# DQF-COSY = Double-Quantum Filtered-CORrelated SpectroscopY



# TOCSY = Total-Correlated SpectroscopY



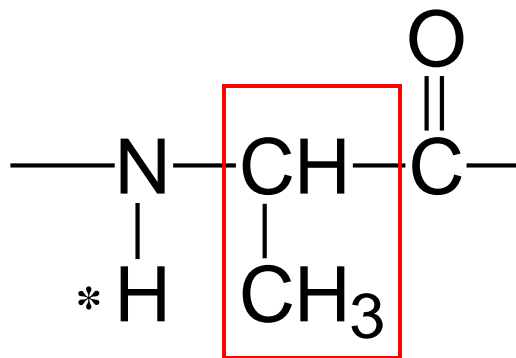
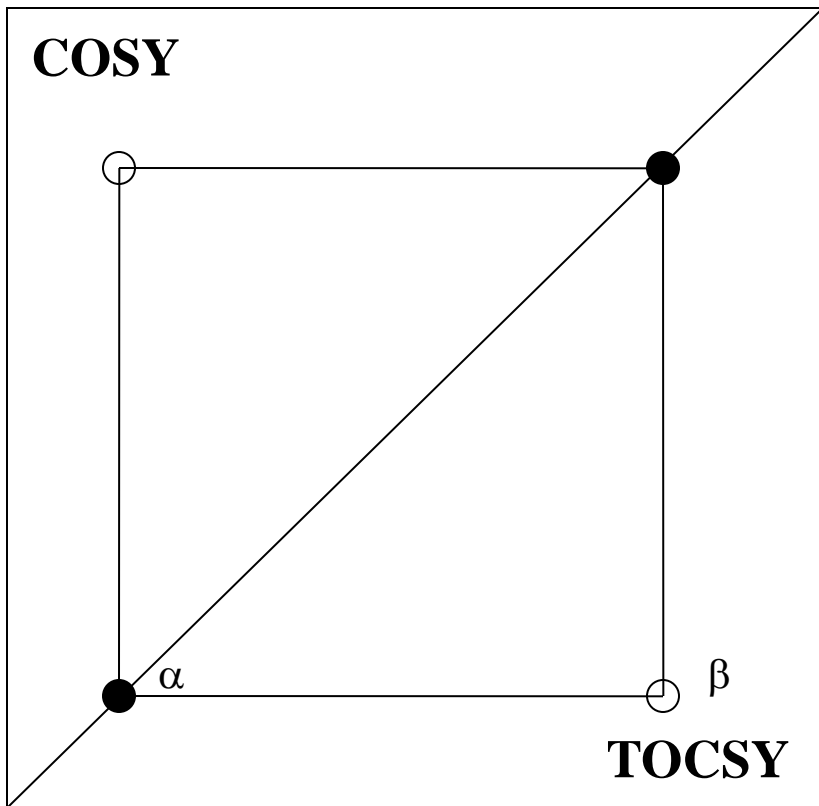
# NOESY = Nuclear Overhauser Effect SpectroscopY



**Ala**

$A_3X$

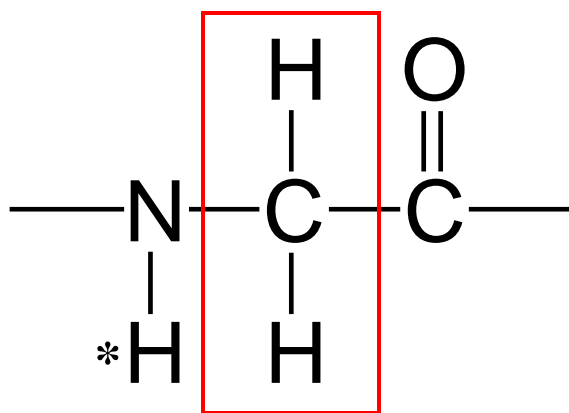
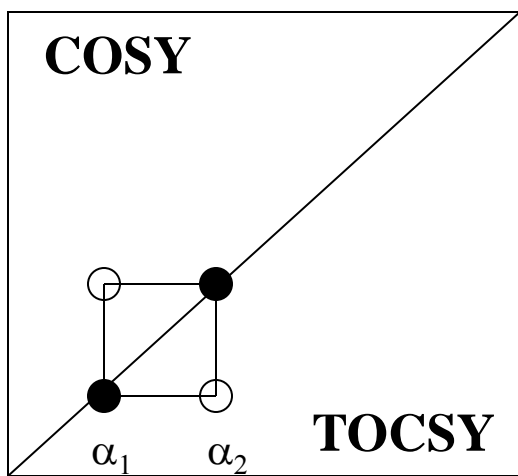
**COSY**



**Gly**

$AX$

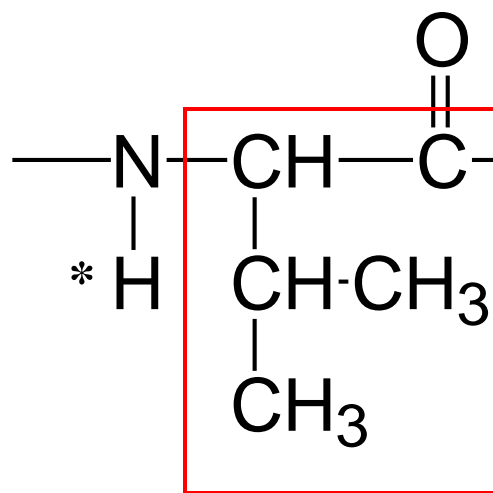
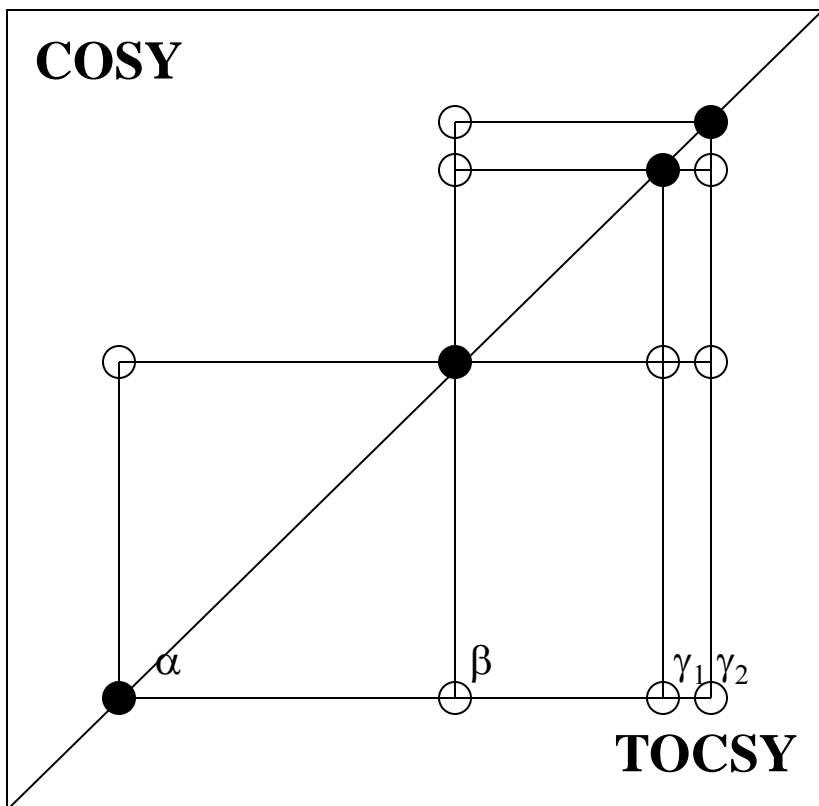
**COSY**





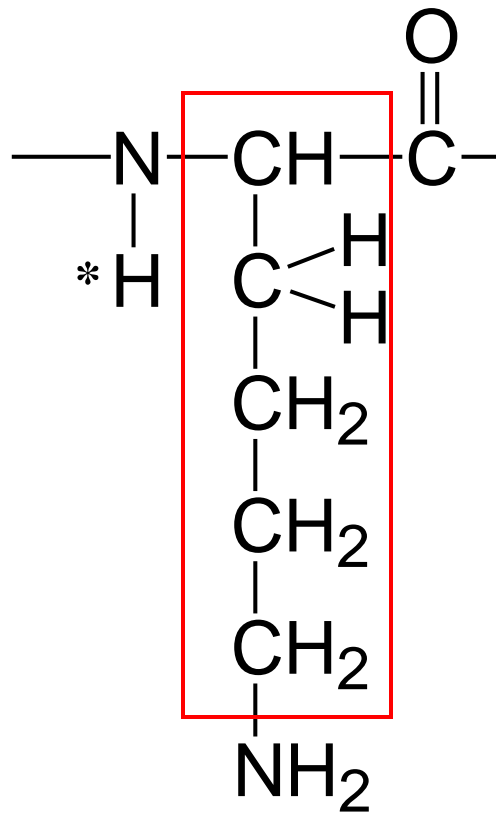
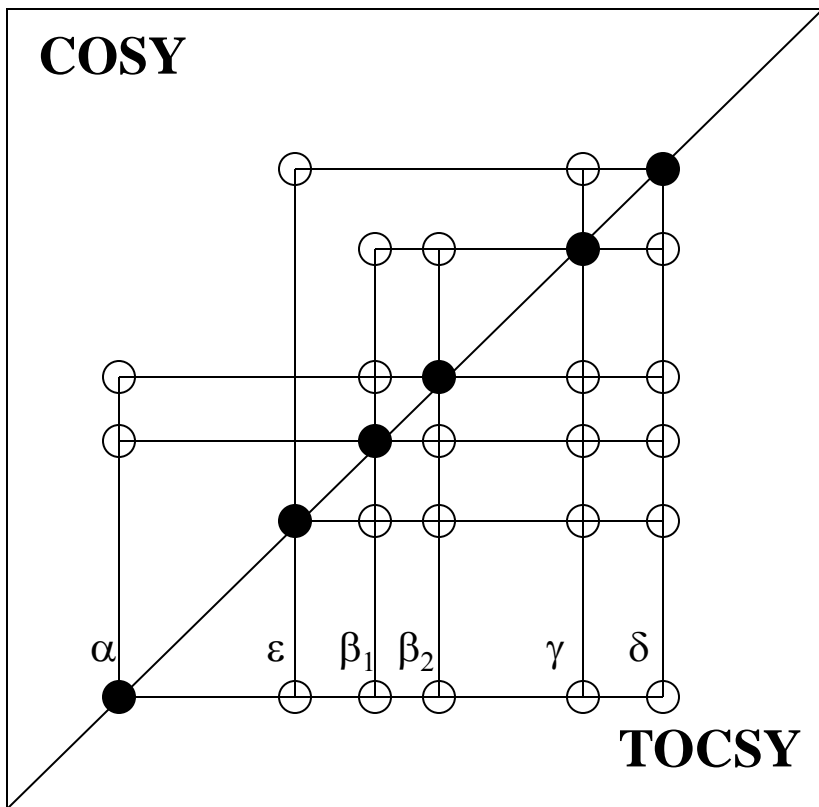
Val

$A_3B_3MX$



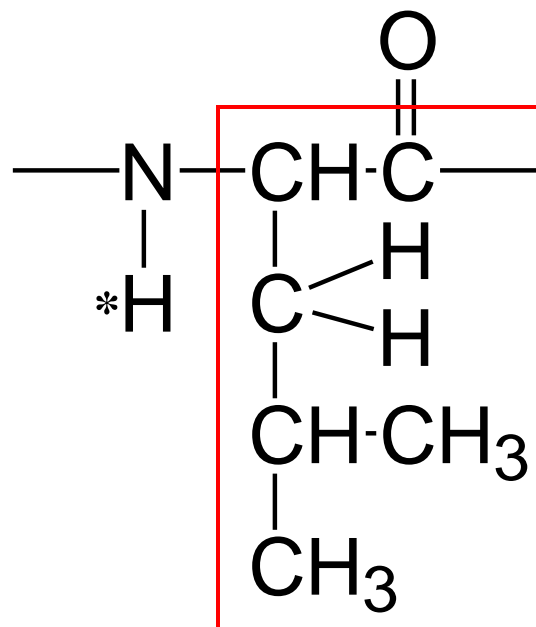
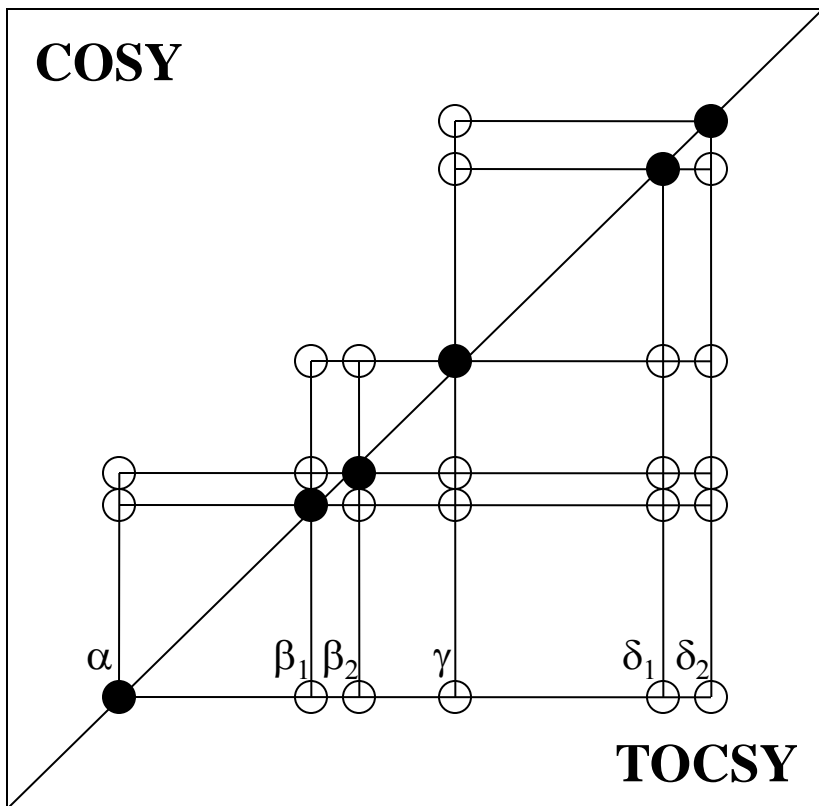
Lys

$A_2(F_2T_2)MPX$



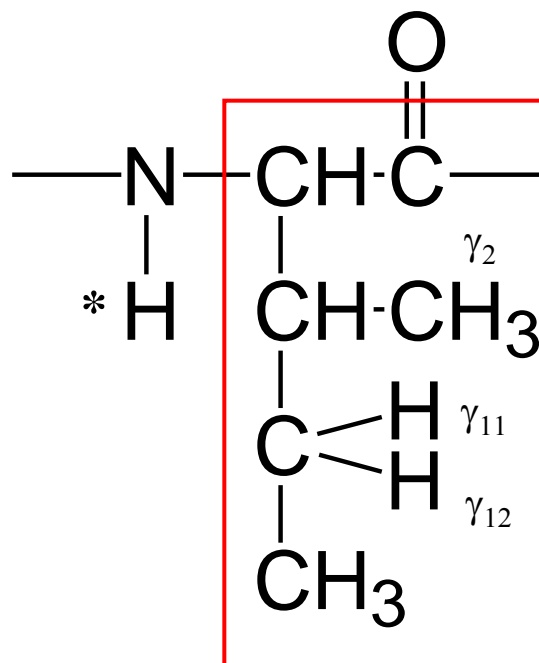
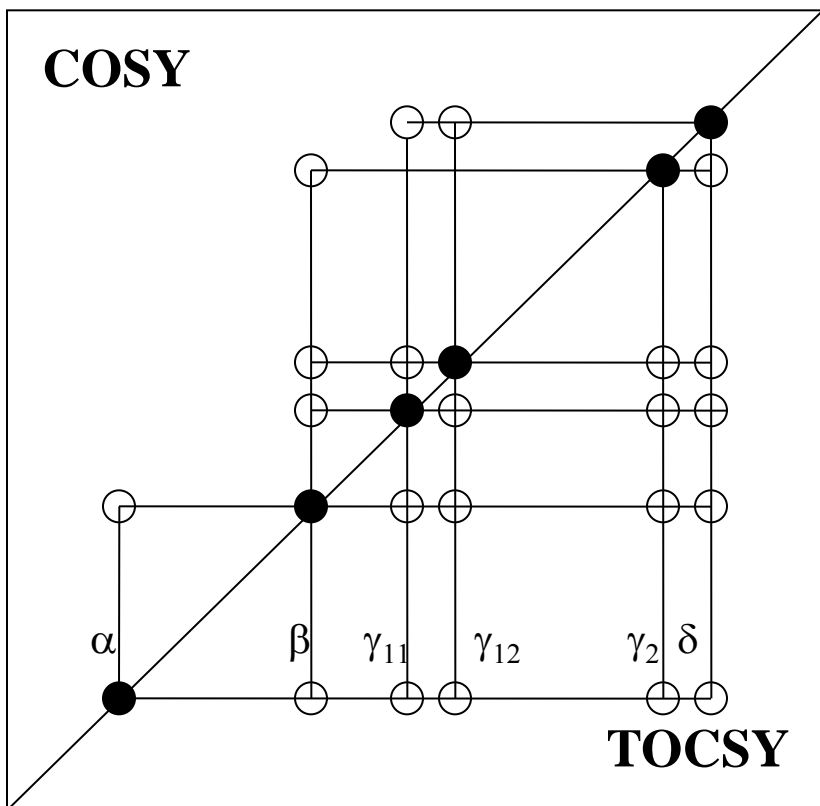
Leu

$A_3B_3MPTX$



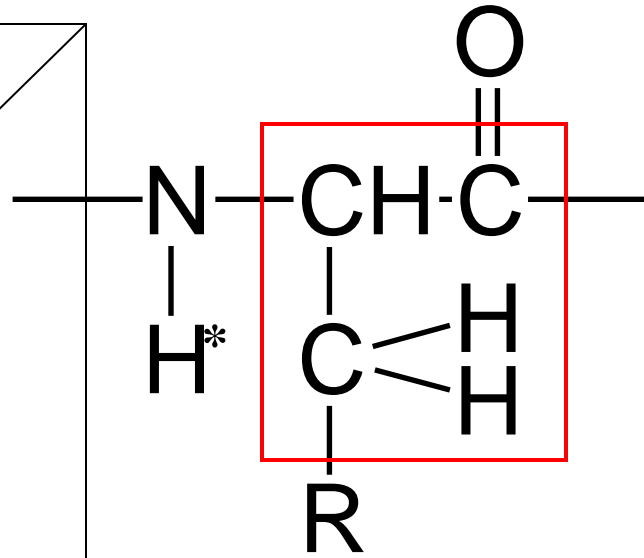
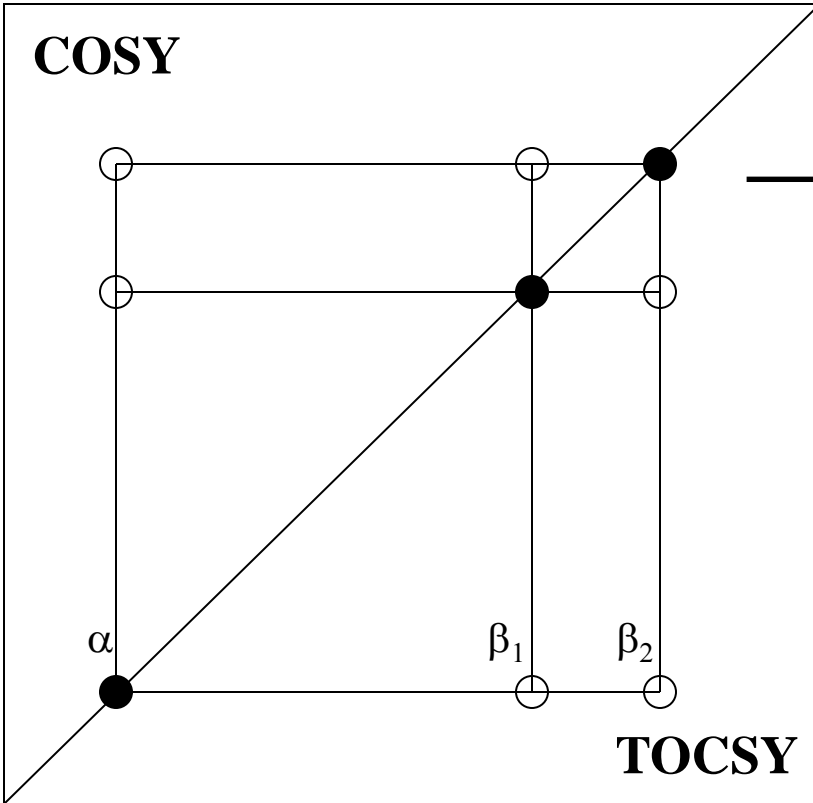
Ile

$A_3MPT(B_3)X$



**J típus**

**AMX**



R = OH

**Ser**

SH

**Cys**

COOH

**Asp**

CONH<sub>2</sub>

**Asn**

C<sub>6</sub>H<sub>5</sub>

**Phe**

C<sub>6</sub>H<sub>5</sub>OH

**Tyr**

C<sub>3</sub>H<sub>3</sub>N<sub>2</sub>

**His**

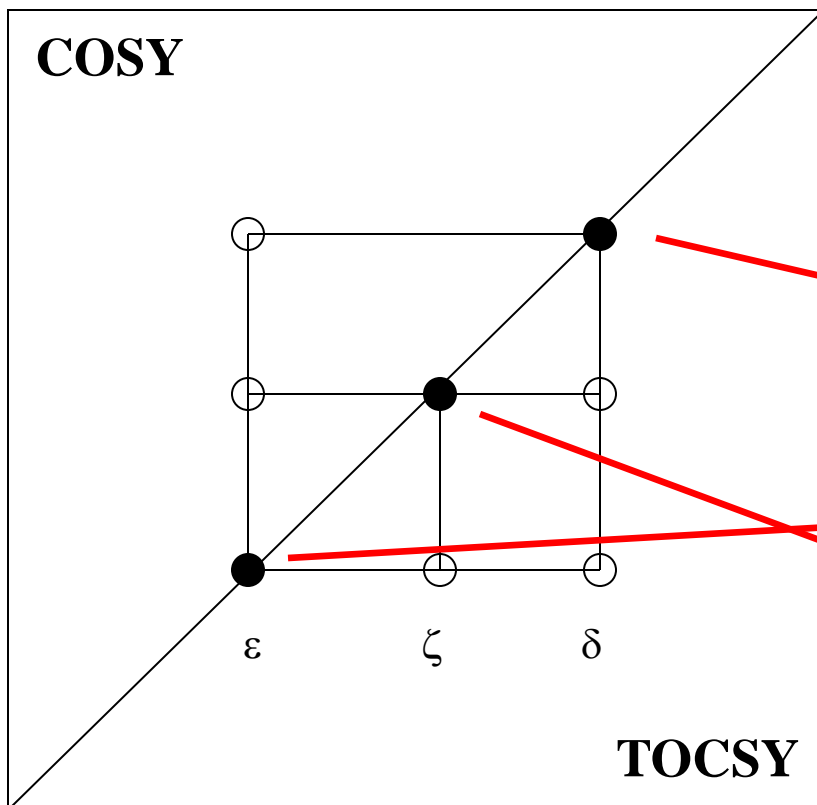
C<sub>8</sub>H<sub>6</sub>N<sub>1</sub>

**Trp**

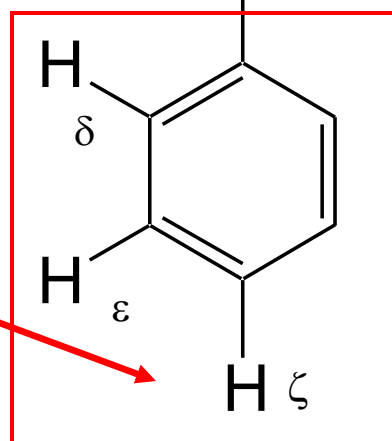
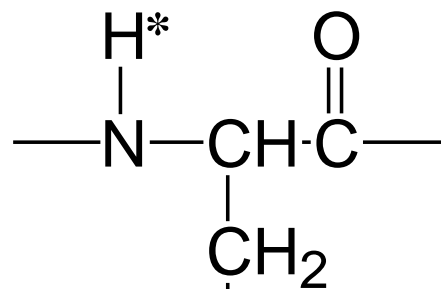
# Phe gyűrű

AA'XX'M

COSY



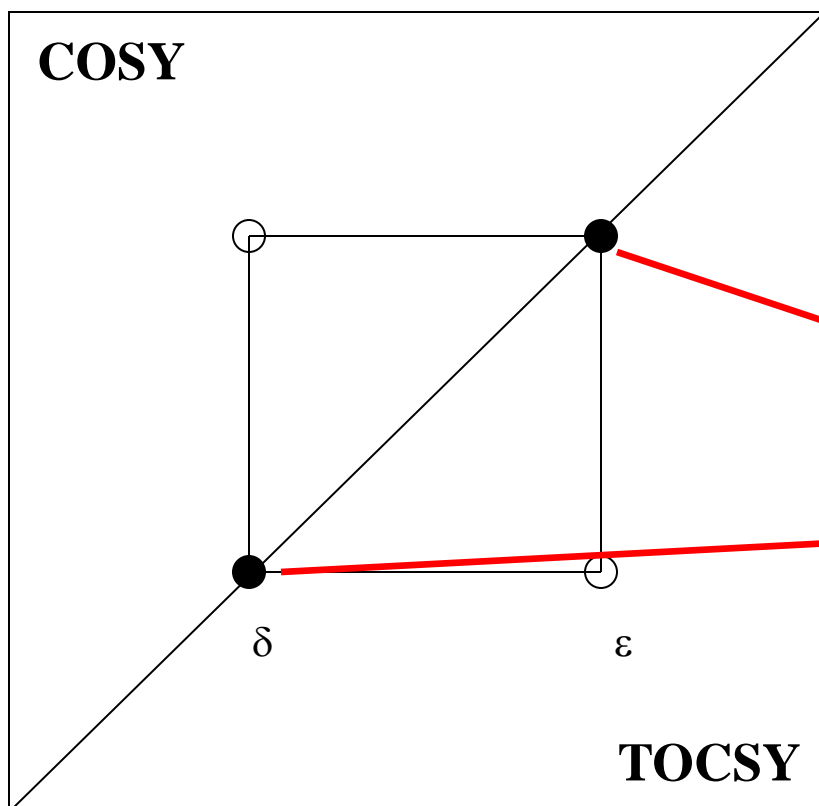
TOCSY



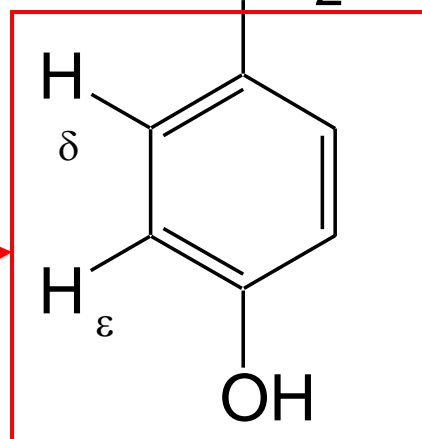
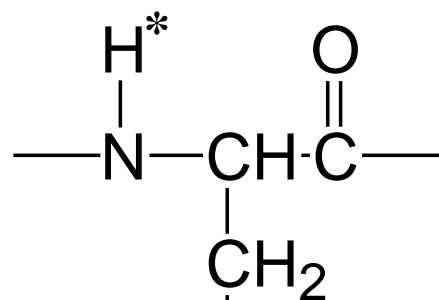
# Tyr gyűrű

AA'XX'

COSY

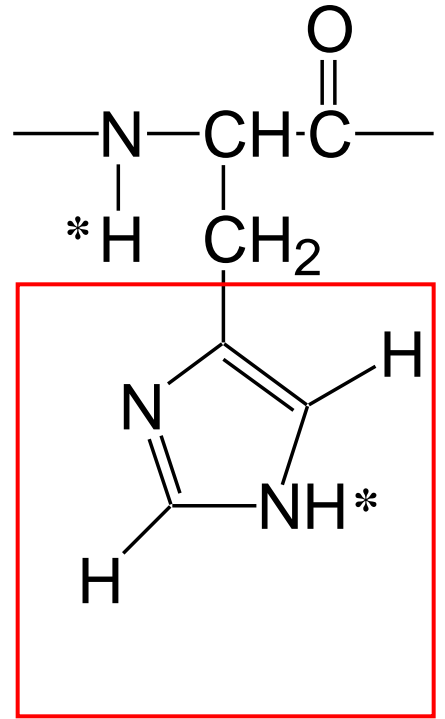
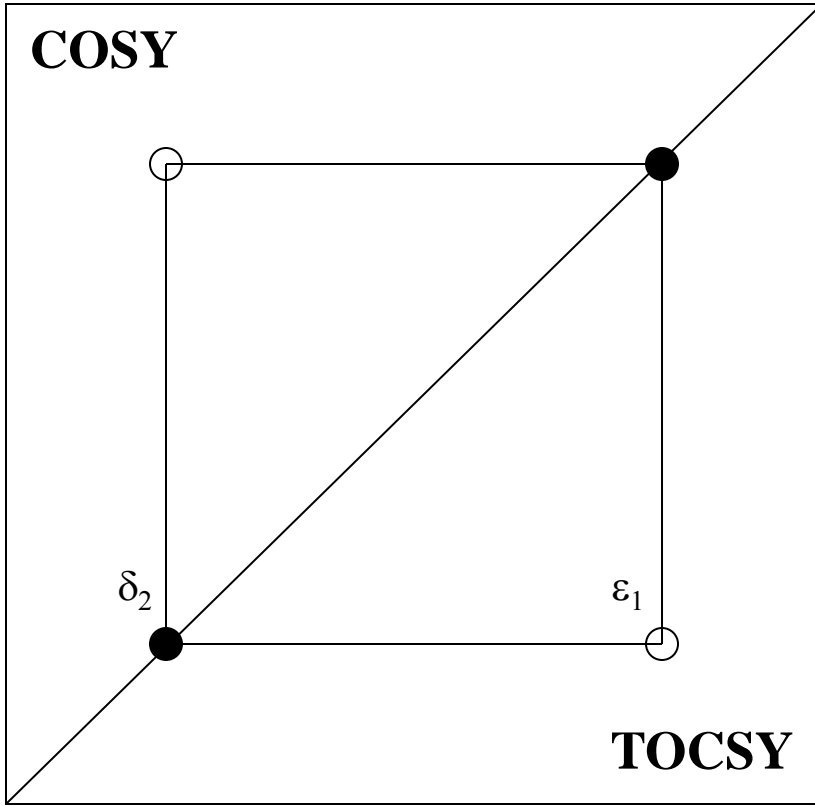


TOCSY



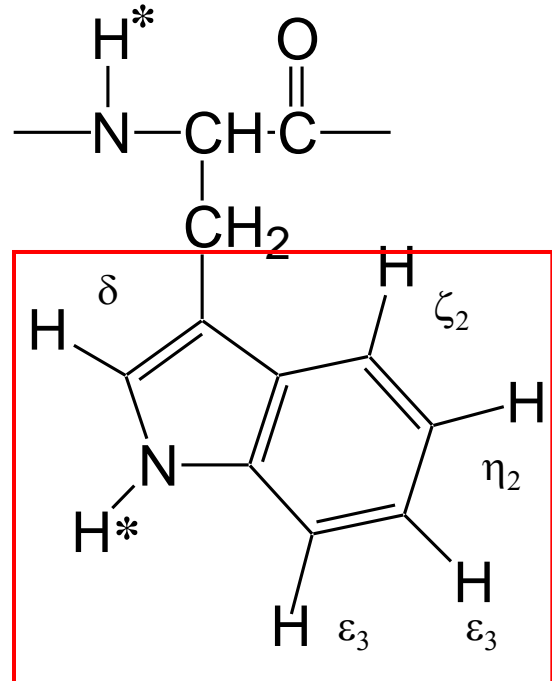
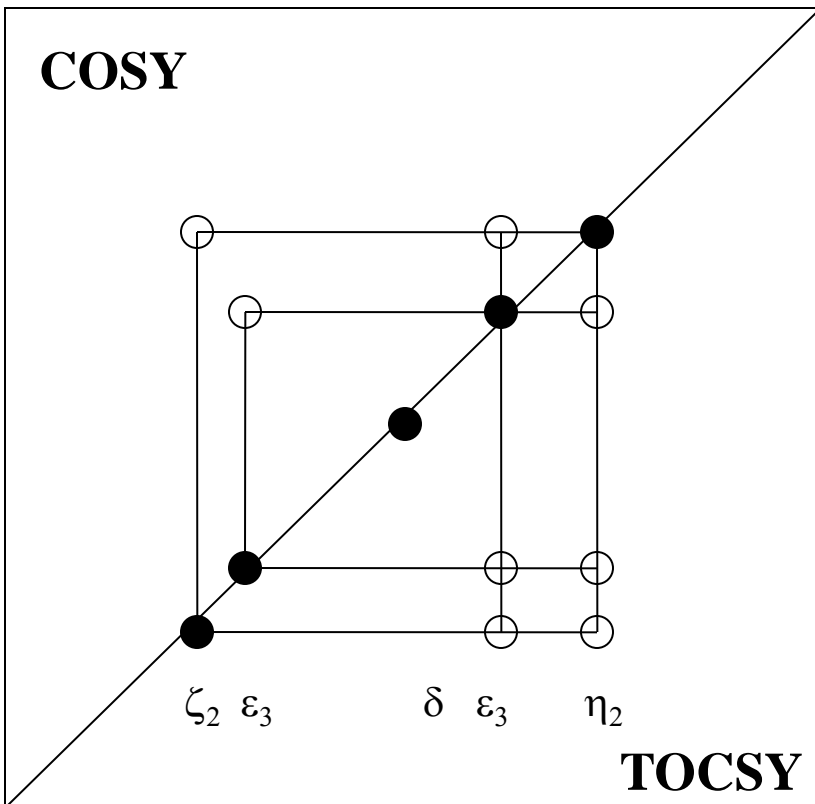
# His gyűrű

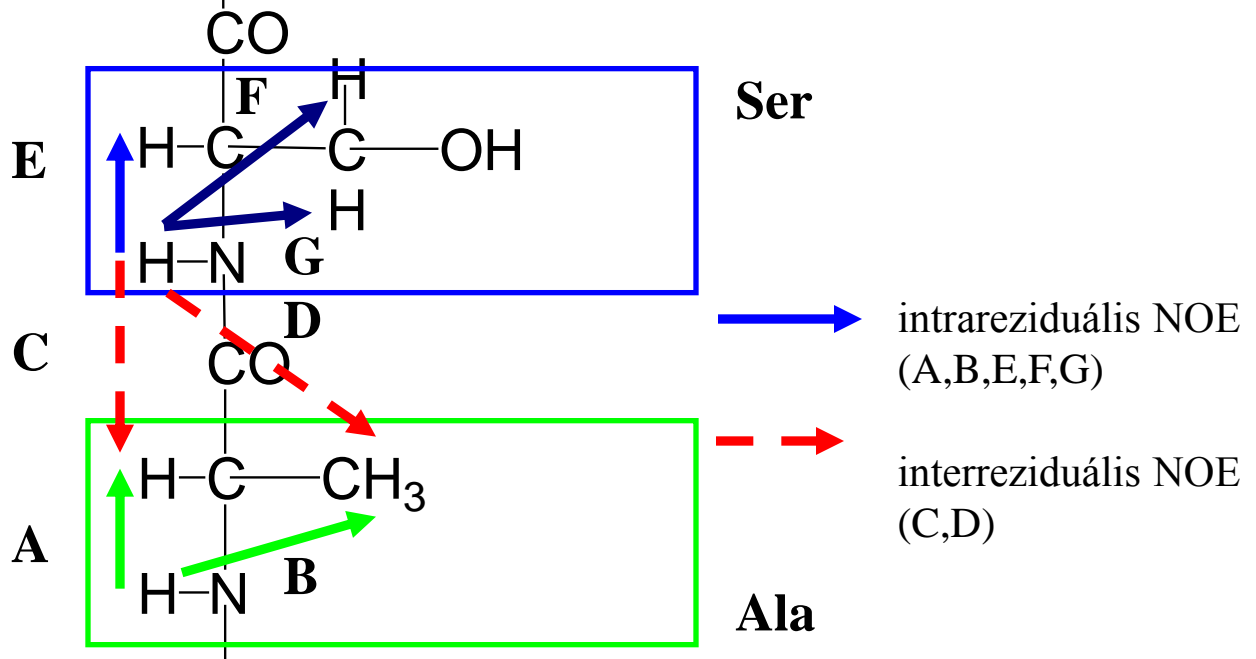
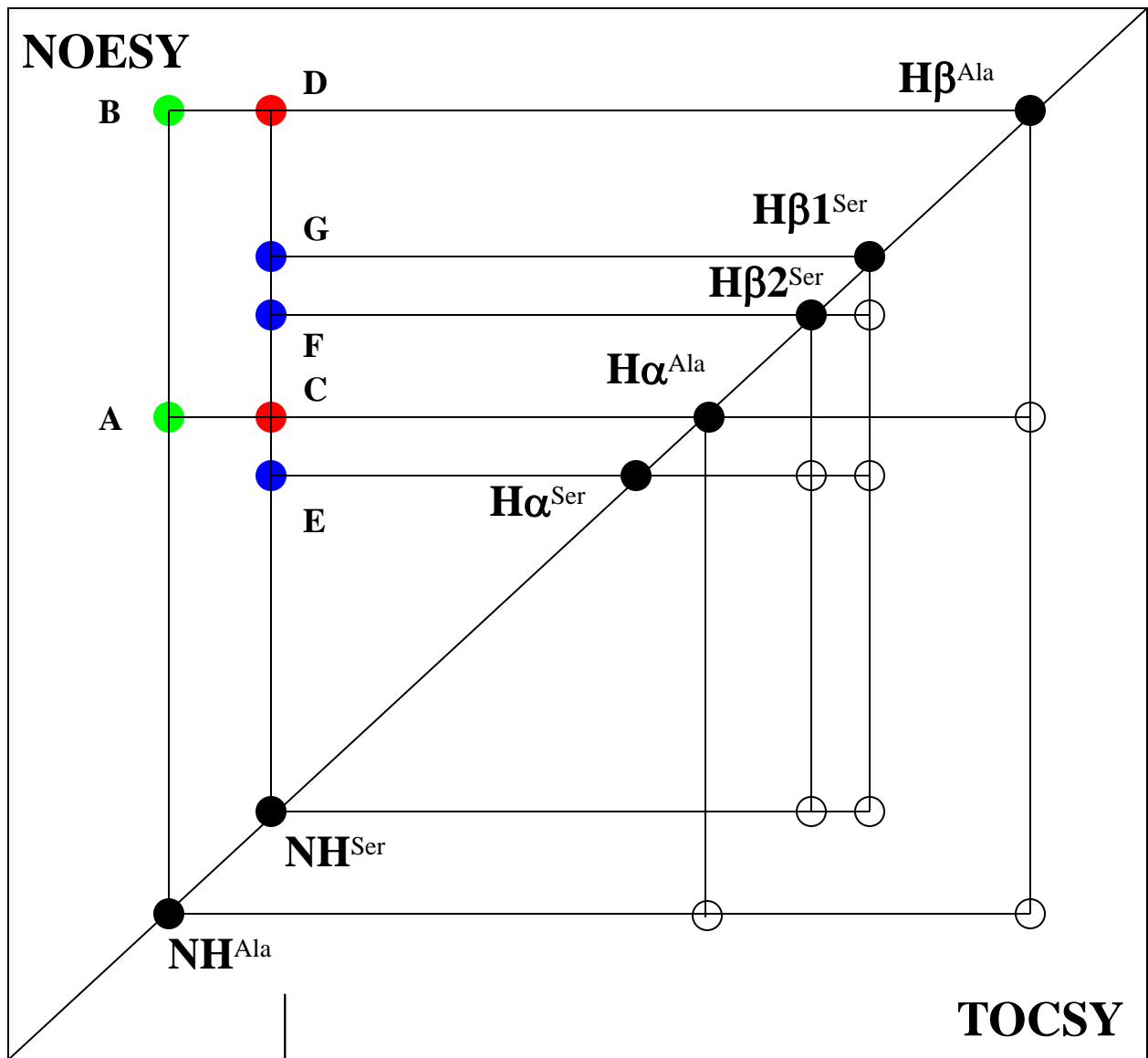
AX



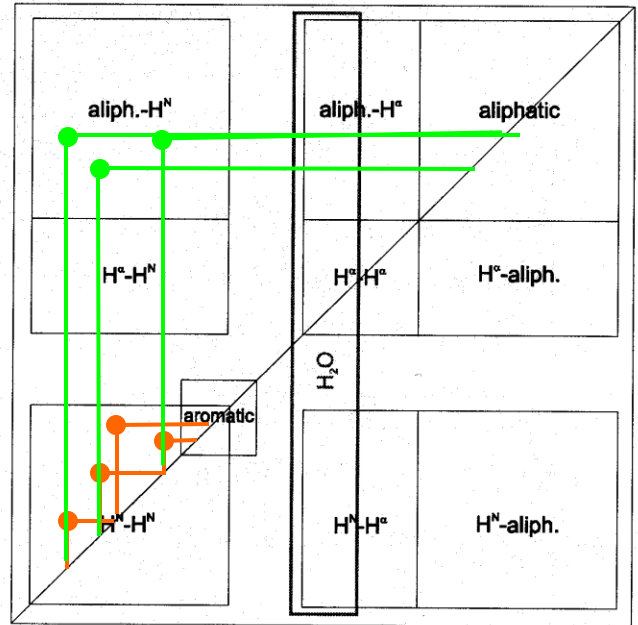
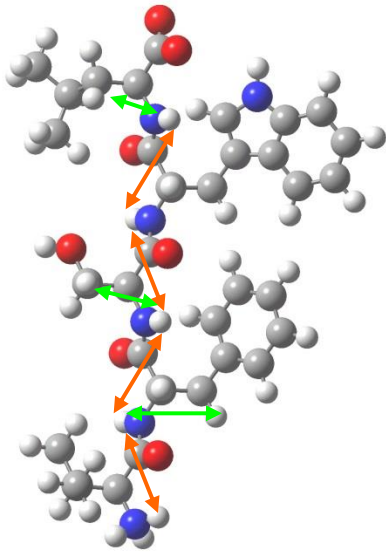
# Trp gyűrű

A(X)MP + A

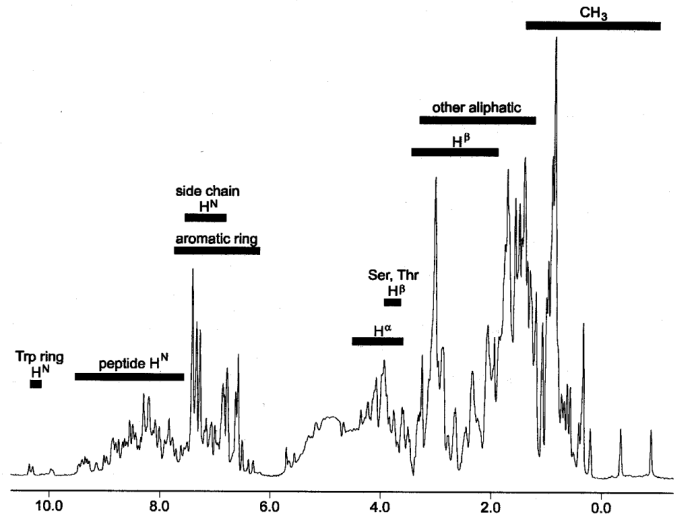




# Homonukleáris NMR-spektroszkópia



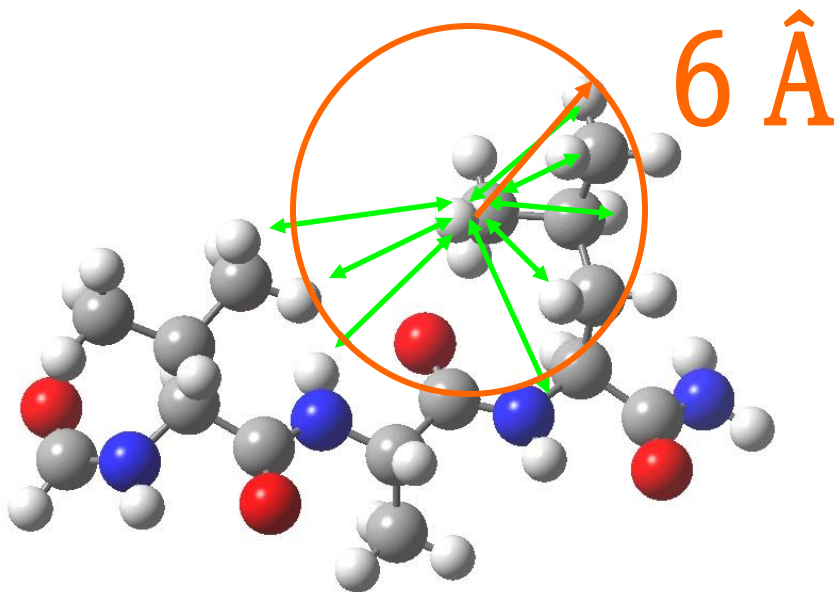
## Jelhozzárendelés



# Peptid és fehérje

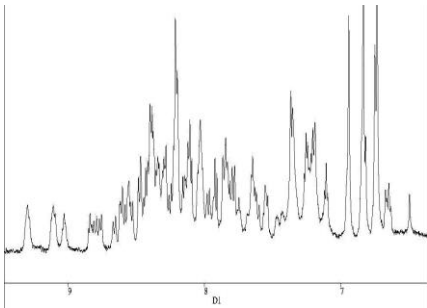
## NMR-spektroszkópia

### 2. szerkezetmeghatározás



Távolság jellegű  
adatok  
(NOE)  
⇓  
3D-szerkezet

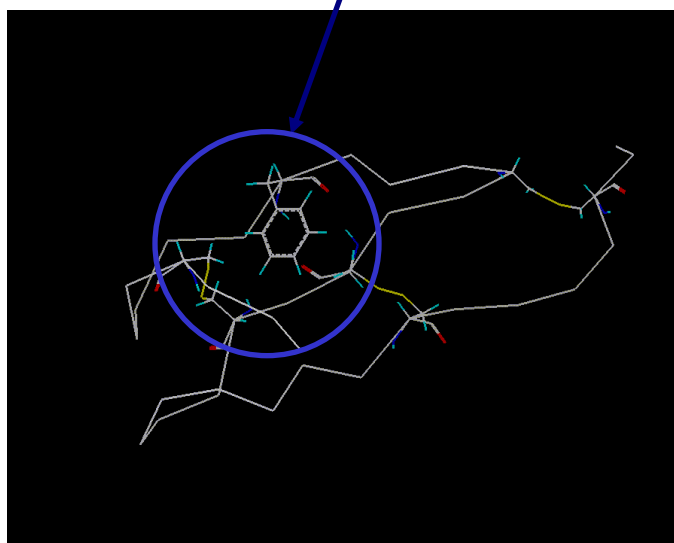
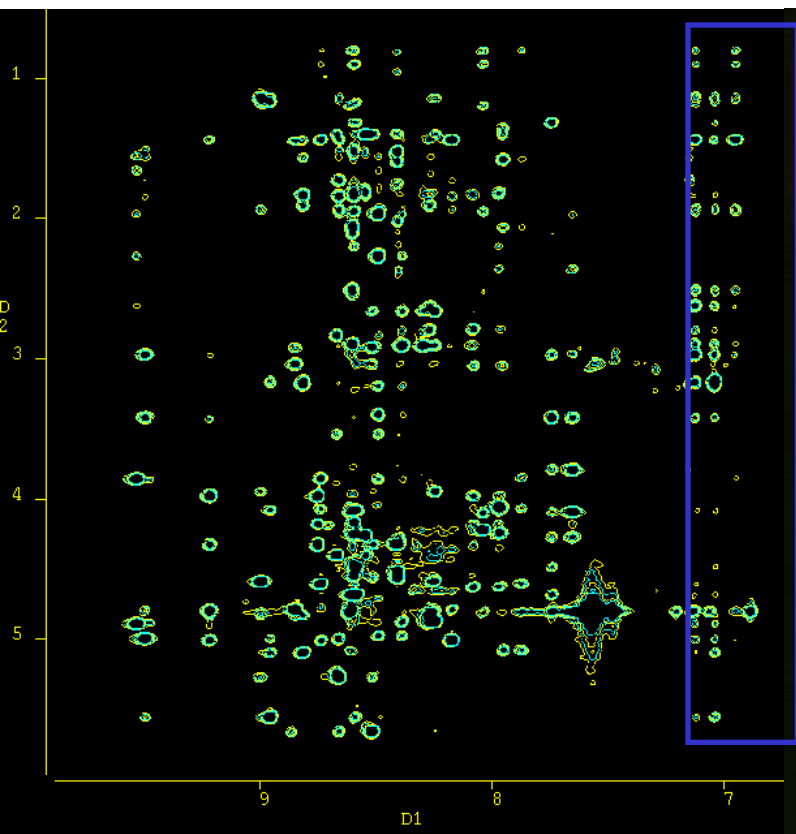




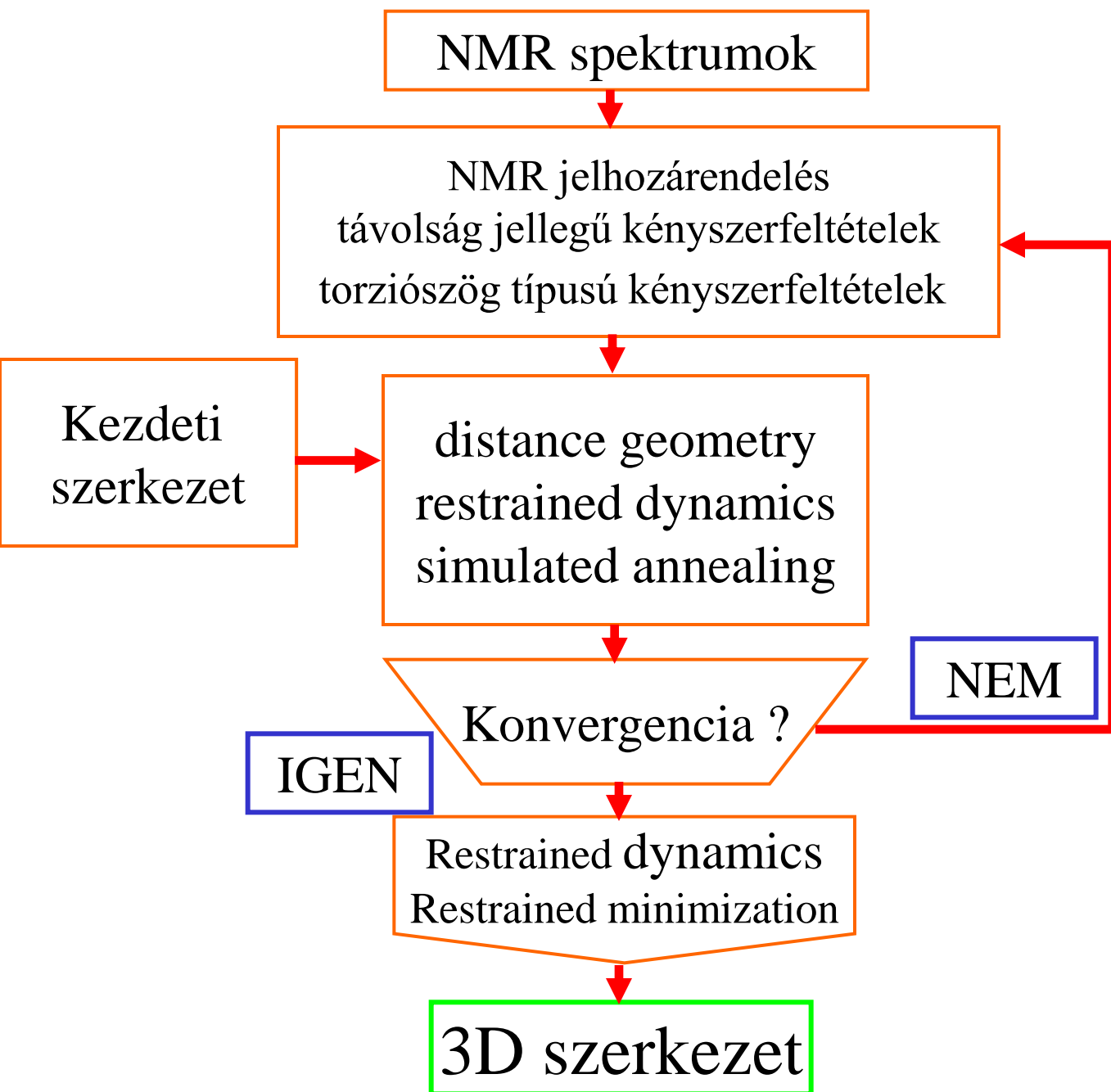
# $^1\text{H}$ - $^1\text{H}$ -NOESY



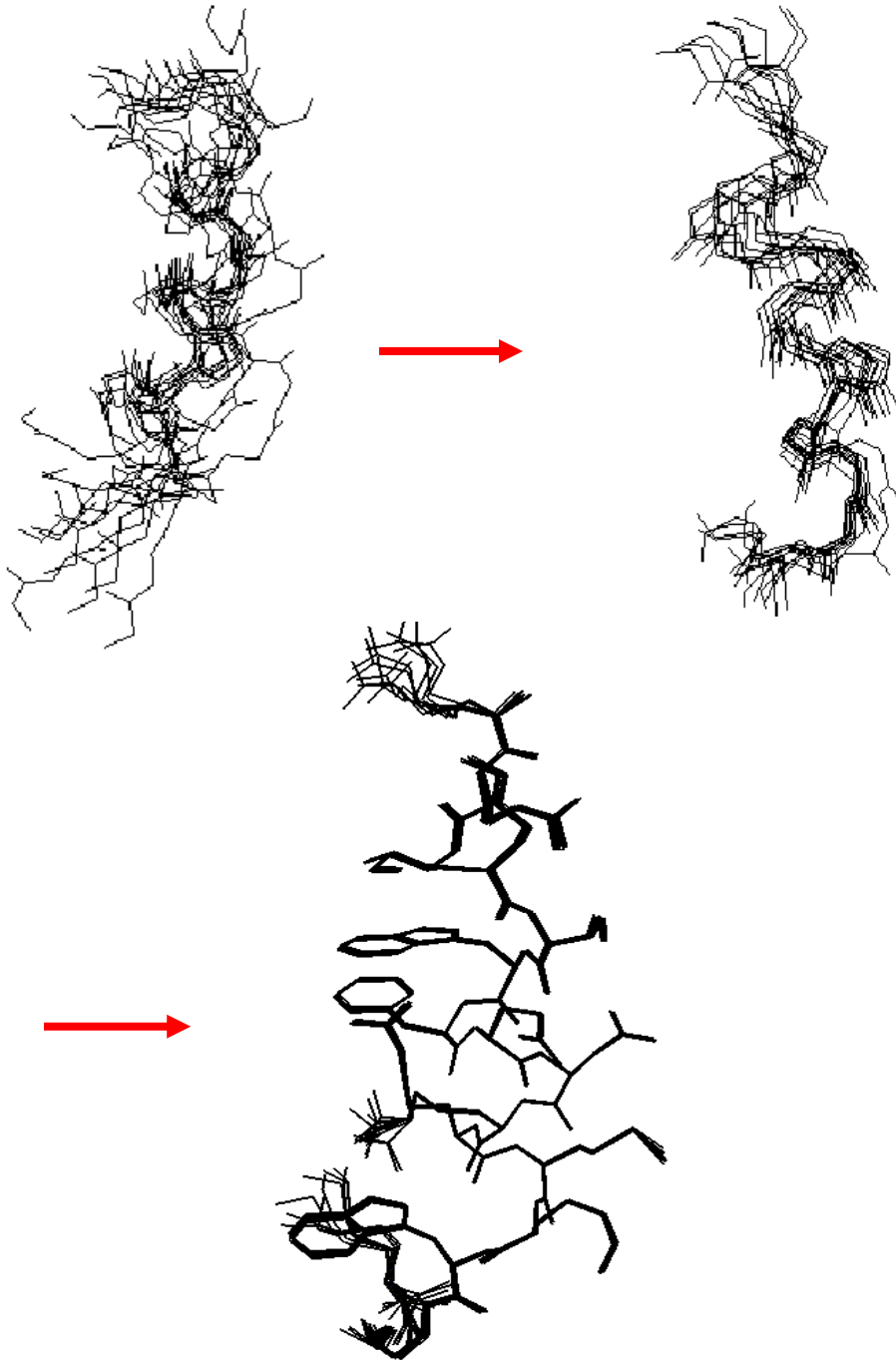
# Schistocerca gregaria kimotripszin inhibitor (SGCI) hidrofób magja



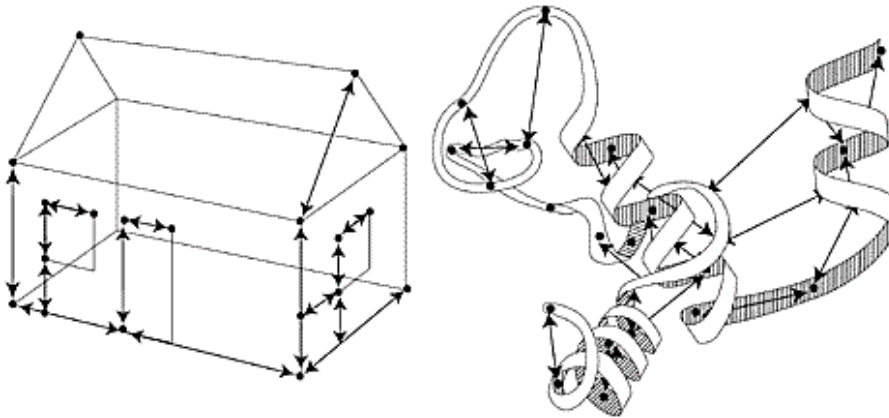
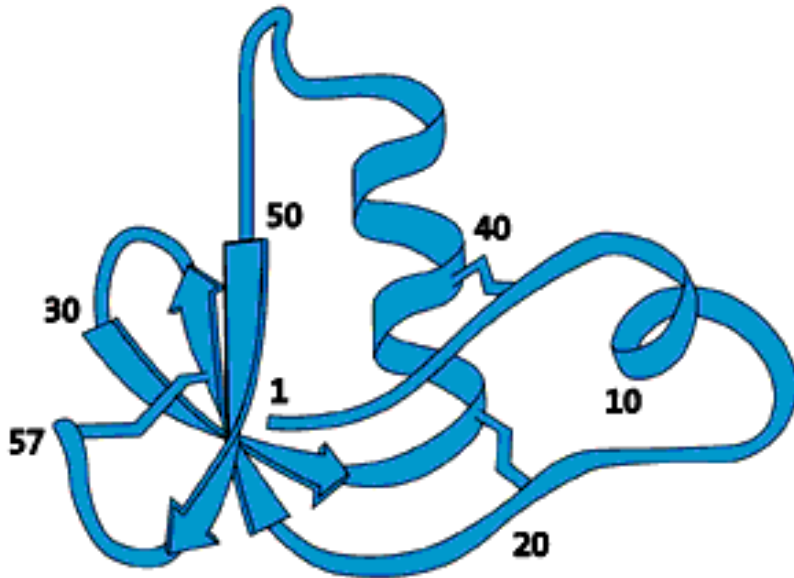
# Makromolekulák 3D szerkezetének meghatározása NMR adatok alapján



# Egy helikális fragmens szerkezetének finomítása

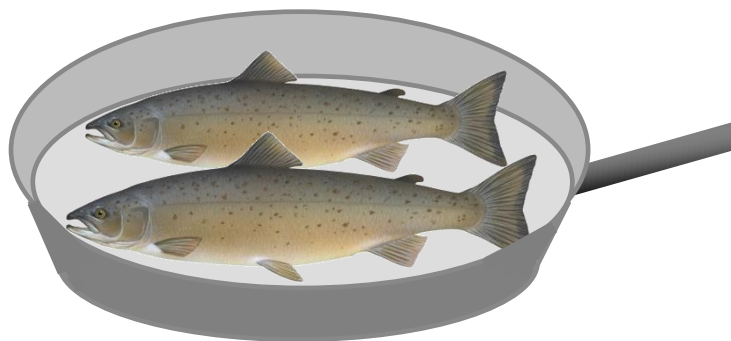


# Makromolekulák 3D szerkezetének Meghatározása NMR adatok alapján



# Miért NMR?

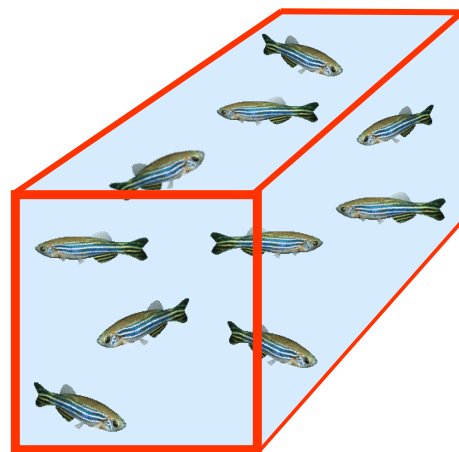
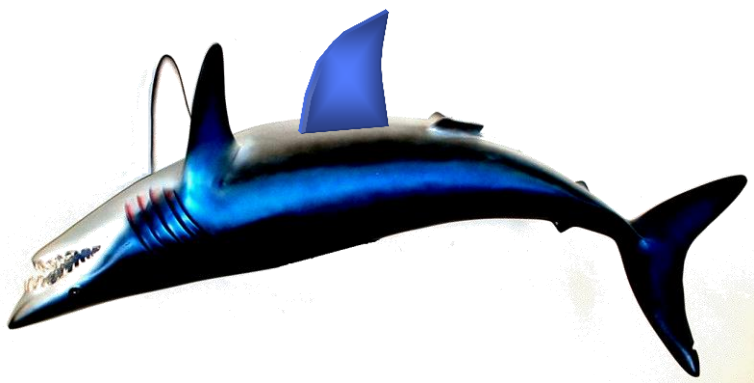
Powerful modern structural tools  
for looking at complexes



Electron microscopy ~190

Crystallography ~  
45,000 structures

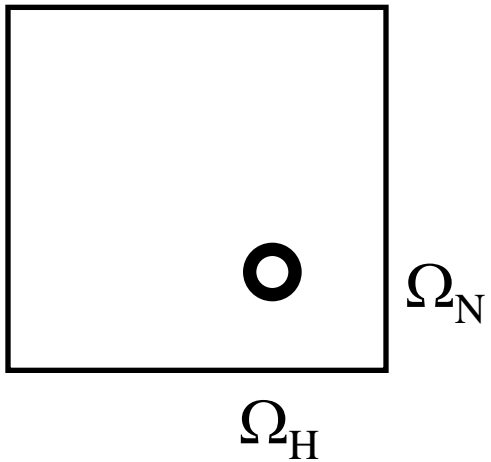
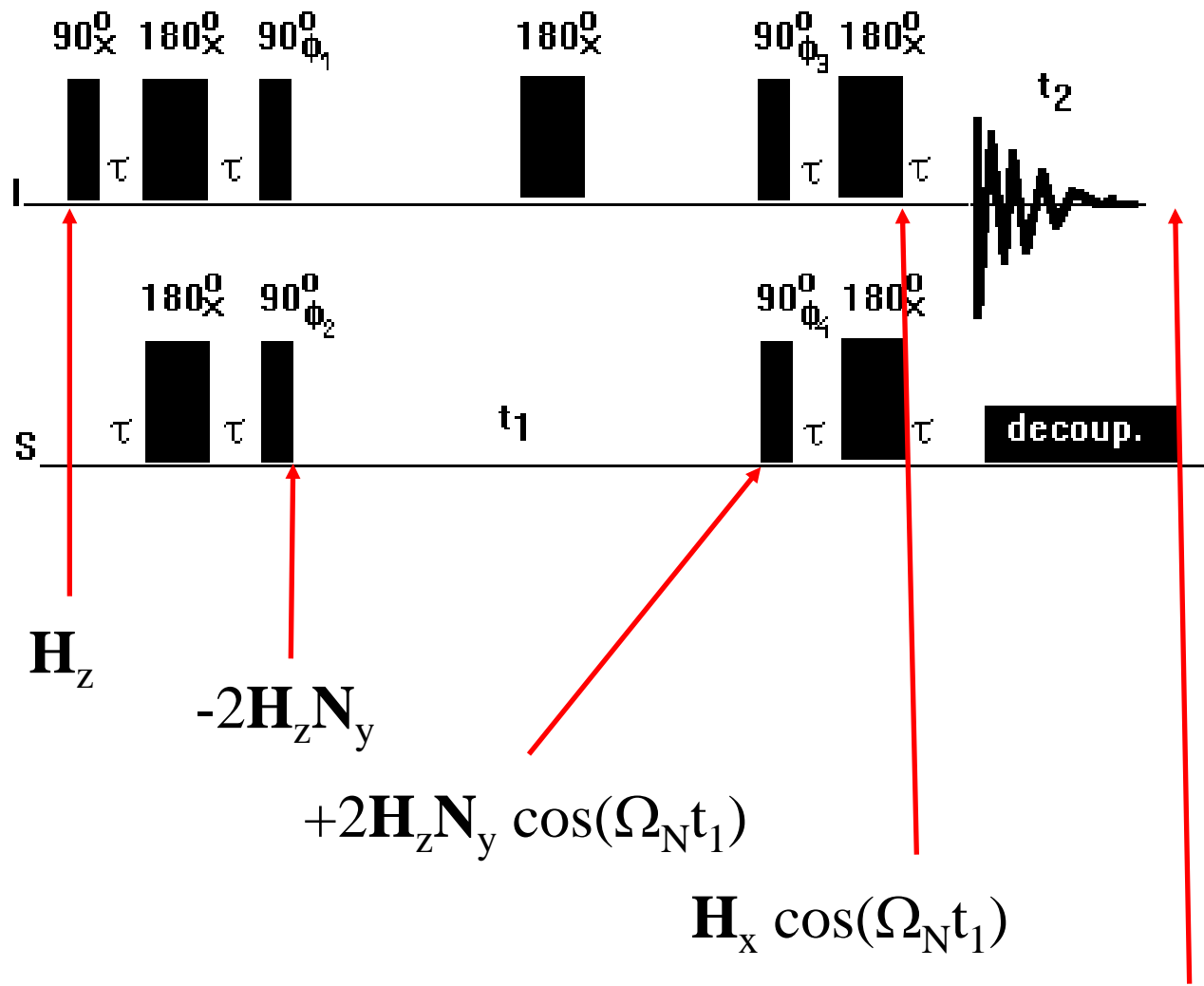
Modelling



Nuclear Magnetic  
Resonance ~7,500  
- can also give  $K_d$   
and  $k$

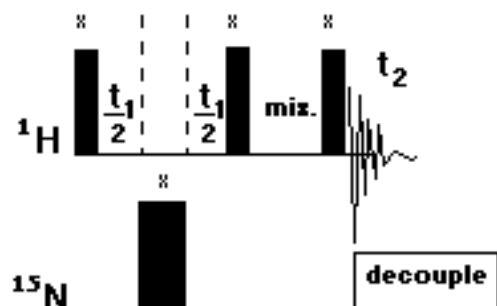
# Heteronukleáris egyszeres-kvantum koherencia spektrum

HSQC = Heteronuclear Single-Quantum Coherence

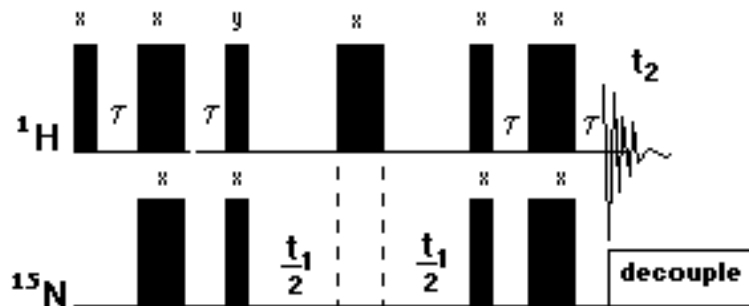


# 3D-NOESY-HSQC

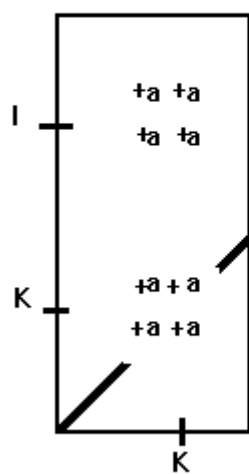
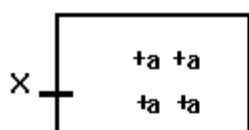
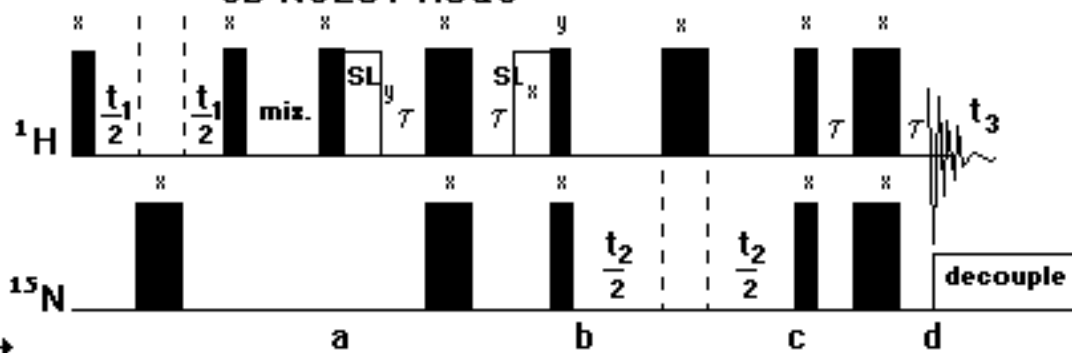
2D NOESY



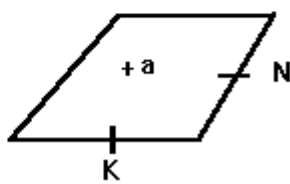
2D HSQC



3D NOESY-HSQC



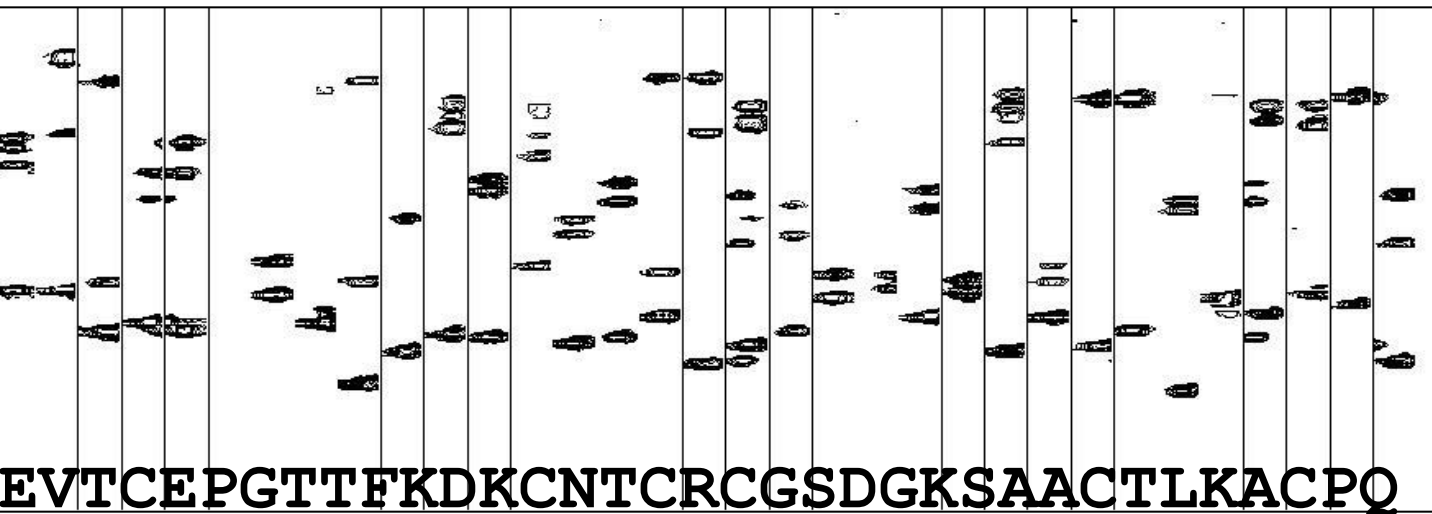
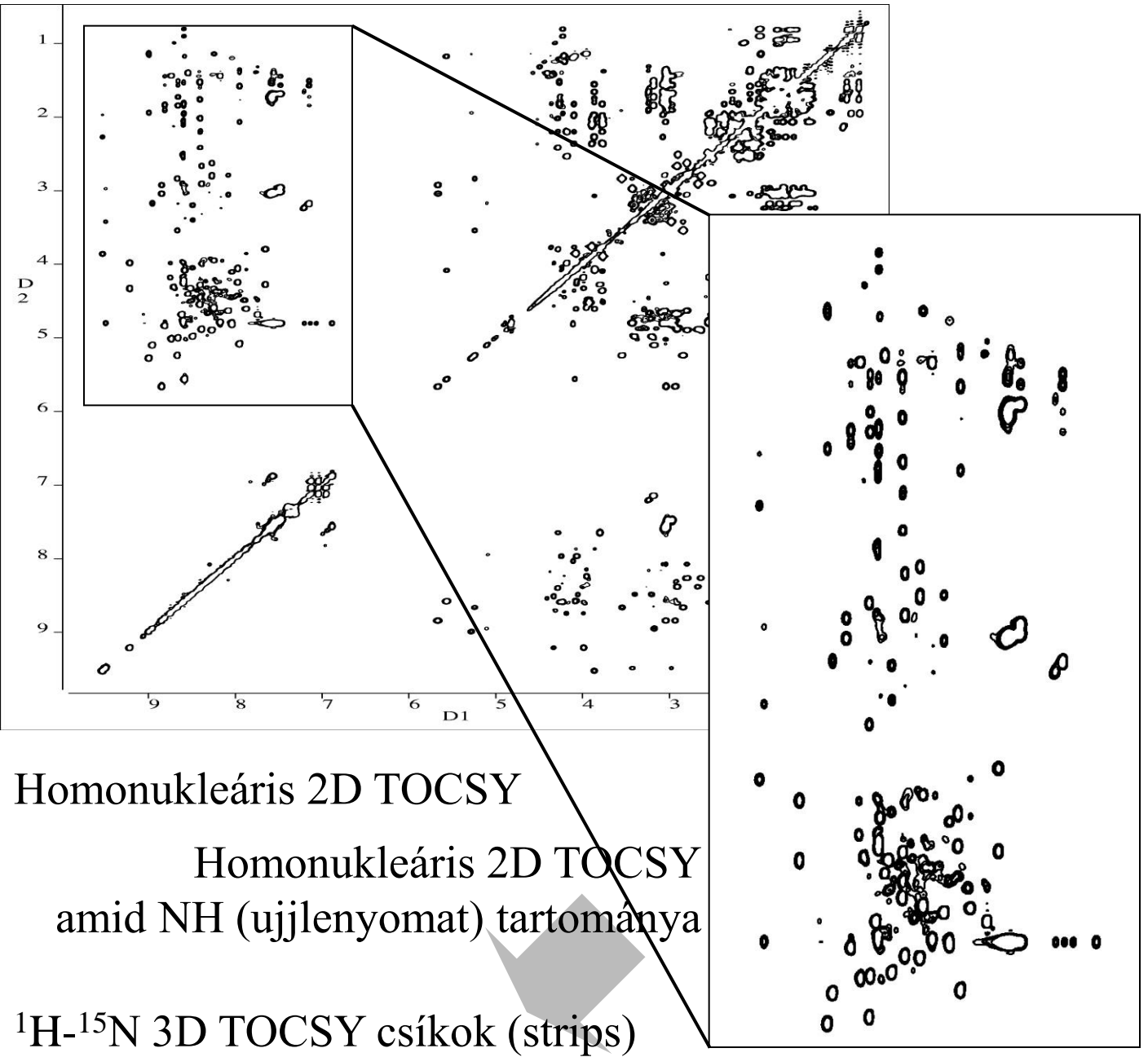
NOESY



HSQC



# 2D és 3D NMR-spektrumok összevetése



# A proteomika és genomika korát éljük

több mint 800 organizmus teljes genom ismert  
az emberi genom  $\approx 30\,000$  gén  $\Rightarrow$  fehérje

“a gyümölcsök nem maguktól érnek meg “  
új közelítésmód szükséges:  
szerkezeti genomika (**structural genomics**)

## Mi a szerkezeti genomikában az NMR szerepe:

14 000 fehérje szerkezet található a PDB, ebből  
17% NMR (2200) és 82% Röntgen

*(2001 január)*

### célkitűzés:

~~minden  
szerkezet~~

minden fehérjecsald egy-egy  
reprezentatív elemének  
meghatározása

# A program:

Nagy mennyiségű fehérje expresszálása

- Európa (*Heinemann Nat. Struct. Biol. 2000*)
- Japán (*Yokoyama et al. Prog. Biophys.Mol.Biol.2000*)
- USA (NIH) 7 kiemelt centrum  
(*Terwillinger Nat. Struct. Biol. 2000*)

Legfontosabb eszközök: - röntgenkristallográfia  
- NMR spektroszkópia



Az N

- k
- a
- a

sa

onosítása

n

- membran fehérje esetén  
Japan, RIKEN (39 spektrométer)  
pl. hélix köteg (helical bundle) fehérjék  
75 millió \$/év  
a teljes genom  
15 milliárd Ft/év  
akár 20-25 % ilyen fehérje

# Az NMR mint szerkezetmeghatározó eszköz:

- NOE alapú közelítés (mérethatár <50kDa)  
(nem deuterált de  
 $^{15}\text{N}$  és  $^{13}\text{C}$  jelölt minta <25kDa)

molekulatömeg



vonalszélesség



spektrális felbontás

## Kedvező tény:

fehérjék átlagos doménjének mérete:

$\approx 17\text{kDa}$  ( $\approx 150$  aminosav)

## Hatékonyság:

csúcs: egy 90 aminosav hosszú fehérje esetében  
plazmidtól a 3D szerkezetig mindössze 30 nap

*(Kozlov et al. J.Biomol.NMR 2000)*

stratégia: automatizáció

## Az NMR mint „rosta” (screening tool):

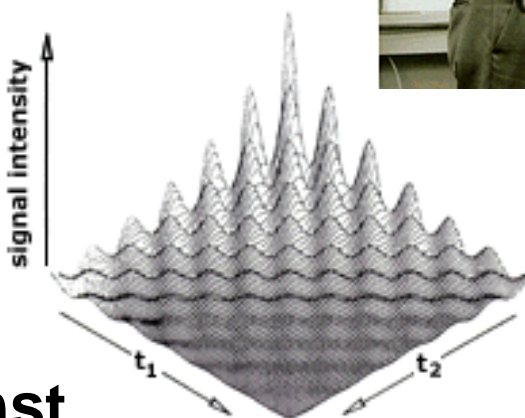
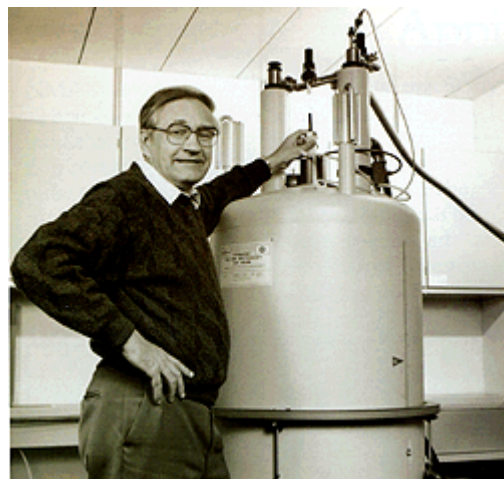
- az amidok NH frekvenciáin keresztül kiszűrjük a fehérje rendezetlen részeit

(CLEANEX *Hwang et al. J.Am.Soc.Chem. 1997, 119, 6203*)

- a kémiai eltolódásban rejlő információk kiaknázása ( $^1\text{H}$ - $^{15}\text{N}$  correlation (e.g. HSQC))

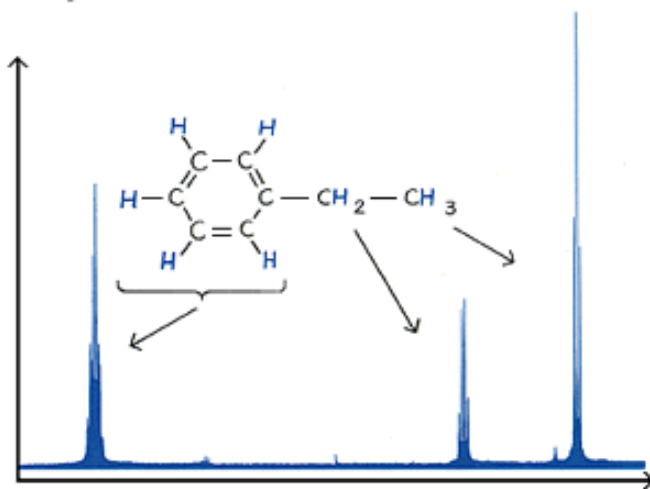


# The Nobel Prize in Chemistry 1991



Richard R. Ernst

"for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy"



*A proton NMR spectrum of a solution containing a simple organic compound, ethyl benzene. Each group of signals corresponds to protons in a different part of the molecule.*



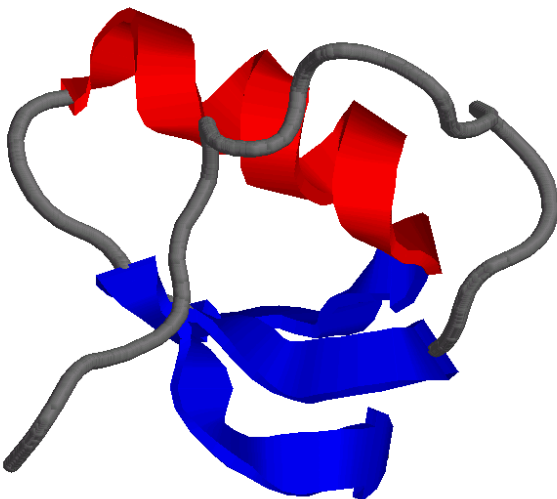
# The Nobel Prize in Chemistry 2002



**Kurt Wüthrich**



**"for the development  
of methods for  
identification and  
structure analyses of  
biological  
macromolecules"**



# Magnetic Resonance Imaging (MRI) a gyógyászatban

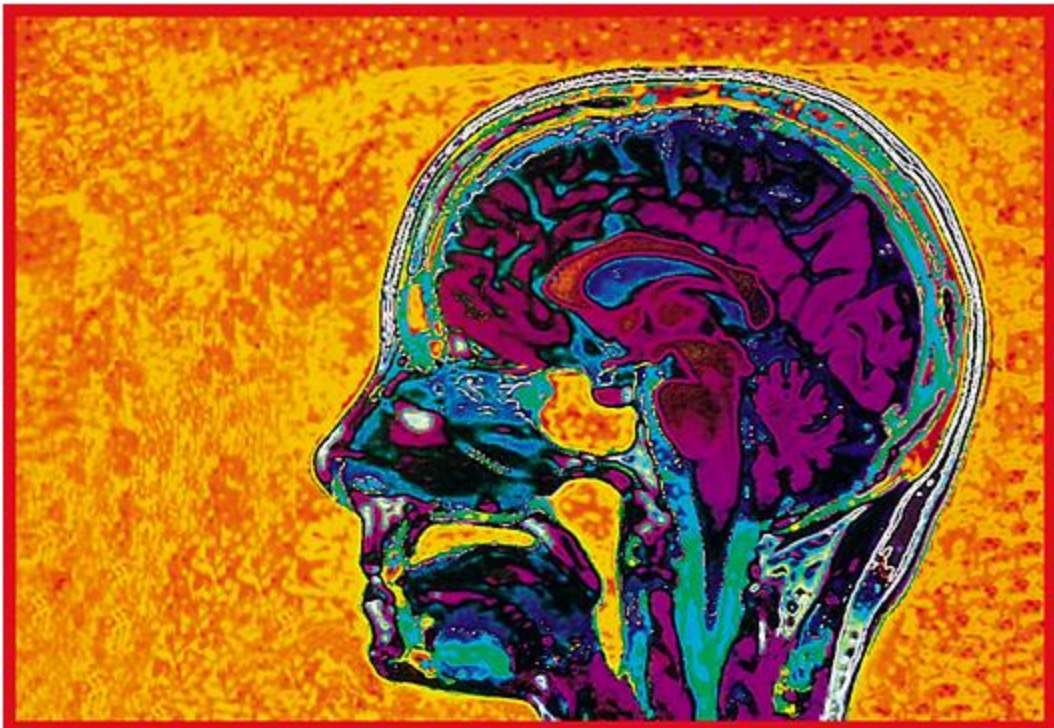
## **előnyök:**

- nem használ ionizáló sugárzást mint a röntgen
- nem kell festék vagy kontrasztanyagot bevinni
- „lágy-szövetek” kontrasztosabbak

**kivitelezés:** tipikus  $^1\text{H}$ -NMR kísérlet ahol a szöveteket felépítő sejtek protonjait figyeljük meg.

**képképzés függ:** az adott szövetben lévő protonok számától,  
az adott protonok relaxációs idejétől  
(T1 spin-mátrix relaxációs idő és  
T2 spin-spin relaxációs idő)

**felhasználási terület:** tumor sejtek, ödémák,  
koros elváltozások azonosítása



Harry Sieplinga/The Image Bank

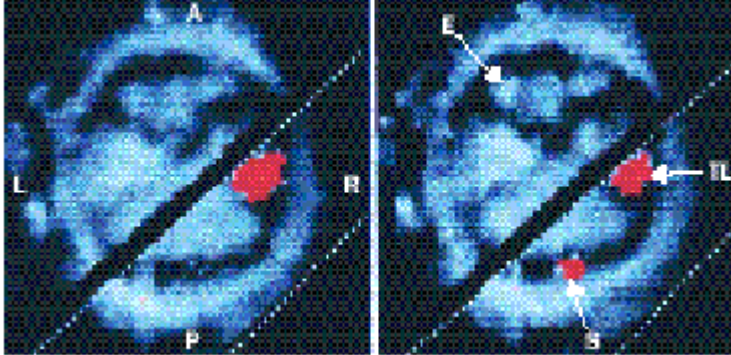
$^{31}\text{P}$ -NMR sejt-metabolizmusok követése





# The Nobel Prize in Physiology or Medicine 2003

**Paul C. Lauterbur**



The Lancet, 1999 354: 645-646



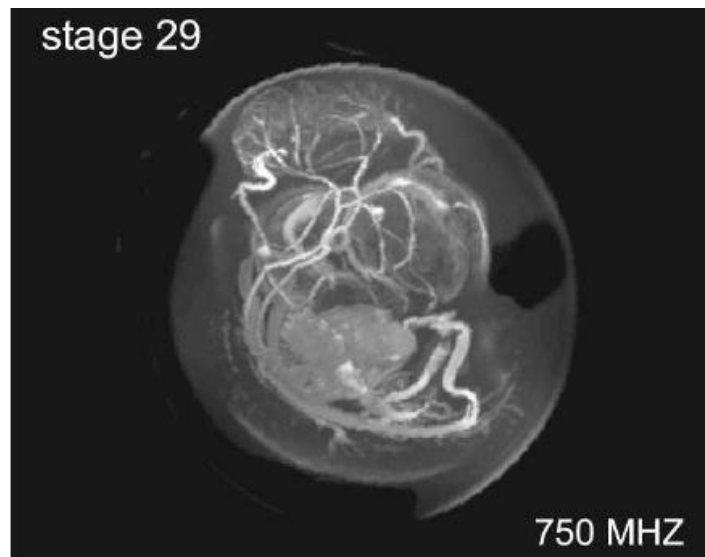
photo University of Illinois

**"for their discoveries concerning magnetic resonance imaging"**

**Sir Peter Mansfield**



photo PRB



RepProgPhys, 2002 65: 1489-1511