

VIŠEDIMENZIJSKA NMR SPEKTROSKOPIJA



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ISPIT:

- 2 parcijalna ispita, seminar, usmeni
- pristupanje parcijalnim ispitima je obvezno
- studenti koji postignu ukupno >60% na svakom od dva parcijalna ispita oslobođeni su pismenog dijela ispita
- studenti koji postignu ukupno 90-100% oslobođeni su ispita ako su održali i seminarsko izlaganje (ocjena **izvrstan, 5**)

ocjena:

- pismeni dio ispita (svi parcijalni ili redovni pismeni u ispitnom roku) – 50% ukupne ocjene
- usmeni dio ispita do 40% (izvodi se nakon održanog cijelog kolegija i obuhvaća kompletno gradivo)
- seminarsko izlaganje do 10 %

ukupna ocjena ispita nakon održanog kolegija:
kombinacija pismenog i usmenog dijela ispita te seminara

pismeni dio ispita	ocjena pismenog dijela ispita
50-60 %	dovoljan (2)
60-75 %	dobar (3)
75-90 %	vrlo dobar (4)
90-100 %	izvrstan (5)

usmeni dio: sukladno prosudbi nastavnika

CILJ KOLEGIJA:

- upoznavanje s osnovnim principima spektroskopije nuklearne magnetne rezonancije visoke rezolucije
- razumijevanje osnovnih parametara NMR
- pojašnjenje građe spektrometara NMR
- jednodimenzijske tehnike NMR
- korelacijska (dvo- i višedimenzijska) spektroskopija NMR
- osnovni principi spektroskopije NMR u čvrstom stanju
- interpretacija jedno- i dvo-dimenzijskih spektara
- značaj spektroskopije NMR u suvremenoj strukturnoj analizi
- upoznavanje sa spregnutom tehnikom LC-SPE-NMR

Nastavni sadržaj

- Spin jezgre i rezonancija
- Vektorski model
- Puls
- Pulsni slijed ili sekvenca
- Spinska jeka
- Koherencija
- Gradijenti magnetnoga polja

- Vremena opuštanja
- Kemijski pomaci i konstante sprega
- Spinski sustavi
- NMR spektrometri
- Korelacijska spektroskopija kroz kemijske veze
- Nuklearni Overhauser-ov efekt (NOE)
- Korelacijska spektroskopija kroz prostor

- Protokol za određivanje 2D strukture molekula
- Tehnike za određivanje konformacije i 3D strukture - konformacijska analiza (NMR + molekulsko modeliranje)
- Višedimenzijske tehnike NMR za određivanje strukture biomolekula (proteini)
- Interakcije molekula (ligand-receptor)
- NMR u čvrstom stanju - dipolne interakcije i anizotropija kemijskih pomaka
- Spregnute tehnike LC-NMR i LC-SPE-NMR

LITERATURA

Obvezna literatura:

1. T. D. W. Claridge, *High Resolution NMR Techniques in Organic Chemistry*, Elsevier, Amsterdam, 2009.
2. P. Novak i T. Jednačak, *Strukturna analiza spojeva spektroskopskim metodama*, TIVA Tiskara, Varaždin, 2013.
3. H. Friebolin, *Basic One- and Two-Dimensional NMR Spectroscopy*, VCH, Weinheim, 2005.
4. J. Keeler, *Understanding NMR Spectroscopy*, Wiley, Chichester, 2011.

Dopunska literatura:

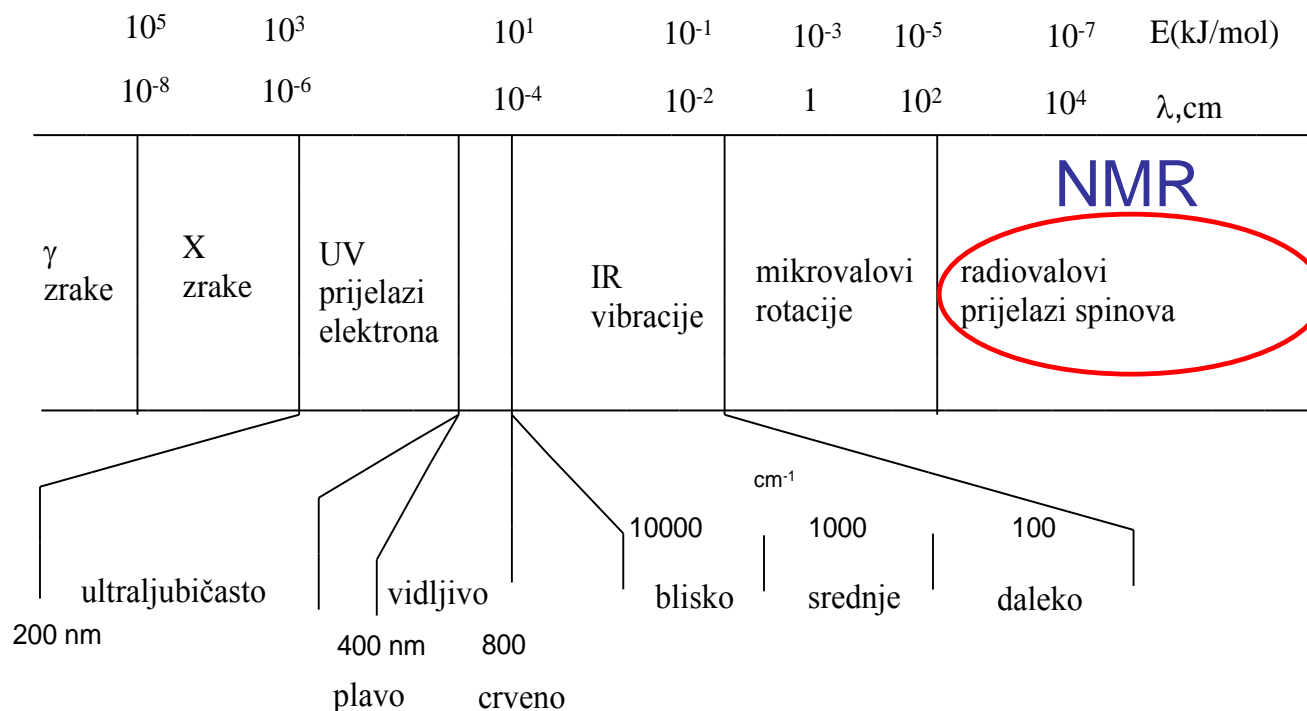
1. W.R. Croasmun, R.M.K. Carlson, *Two-dimensional NMR spectroscopy- Application for chemists and biochemists*, VCH, Cambridge, 1994.
2. M.J. Duer, *Introduction to Solid state NMR spectroscopy*, Wiley, 2007.
3. S. Berger i S. Braun, *200 and More NMR Experiments*, Wiley-VCH, Weinheim, 2004.
4. Jeremy N. S. Evans, *Biomolecular NMR Spectroscopy*, Oxford, 1995.
5. Znanstveni članci.

POVIJESNI RAZVOJ NMR-a

- 1924. W. Pauli - teorijski temelji NMR
- 1939. Rabi i sur. - dokaz o postojanju nuklearnog spina (molekularni snop LiCl)
- 1944. Nobelova nagrada iz fizike Rabiju
- 1946. Bloch (Stanford) i Purcell (Harvard)- prvi uspješni eksperimenti efekta NMR
- 1952. Nobelova nagrada iz fizike za NMR Blochu i Purcellu
- 1953. Prvi komercijalni NMR spektrometar (Varian 30 MHz)
- 1960. Spektrometri od 100 MHz
- 1970. Pulsni spektrometri s Fourierovom transformacijom
- 1980. Spektrometri od 400 MHz
- 1970.-1980. Dvodimenzijske metode i tehnike (2D NMR)

- 1970. Oslikavanje magnetnom rezonancijom (magnetic resonance imaging, MRI)
- 1991. Nobelova nagrada iz kemije za NMR, R. R. Ernst
- 1992. Magnet od 17.61 T (750) MHz
- 1995. Magnet od 21.14 T (900 MHz)
- 1999. Prvi komercijalni 900 MHz spektrometar
- 2002. Nobelova nagrada iz kemije za NMR, K. Wütrich
- 2003. Nobelova nagrada iz medicine za MRI, P. Lauterbur i P. Mansfield (fizičari)
- 2005. Magnet od 22.31 T (950 MHz)
- 2009. Magnet od 1000 MHz (1 GHz)
- 2015. Magnet od 1200 MHz (1.2 GHz), instalacija kreće ove godine

Spektar elektromagnetnoga zračenja



ICE NMR



Integrirani
NMR sustav-
INCA



BEST NMR



900 MHz



950 MHz



950 MHz



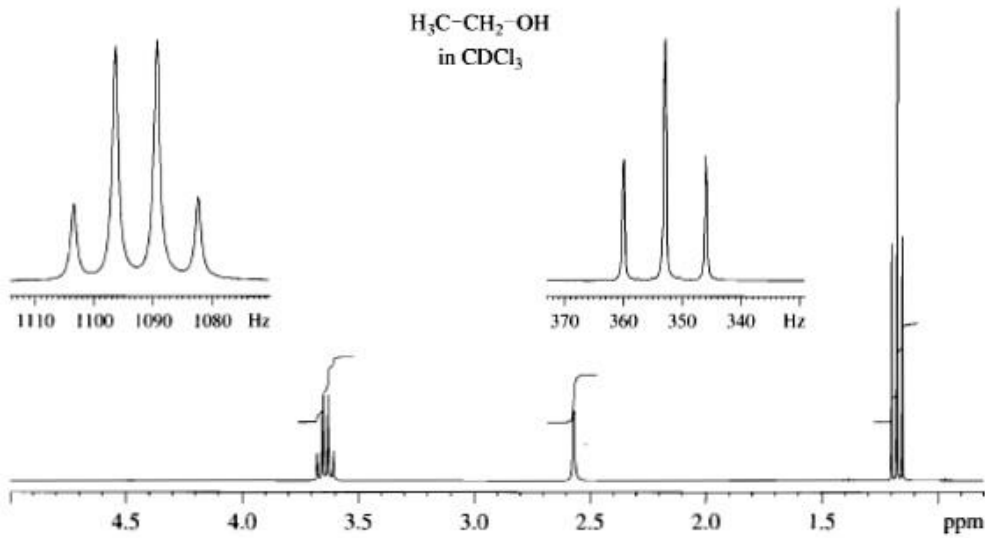




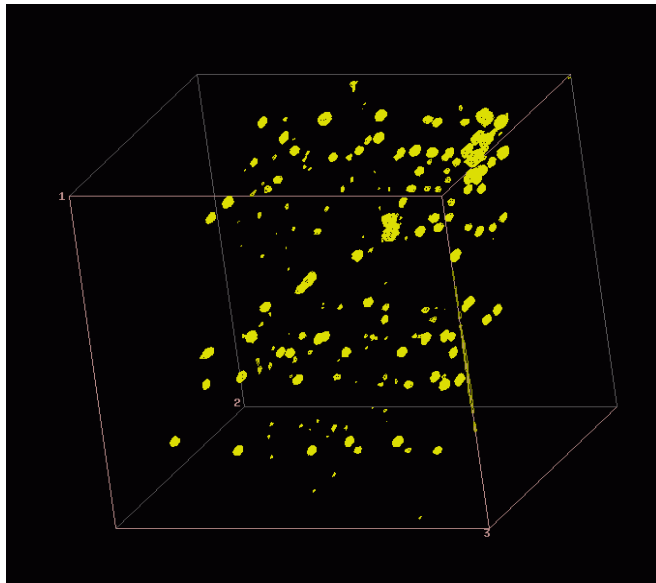
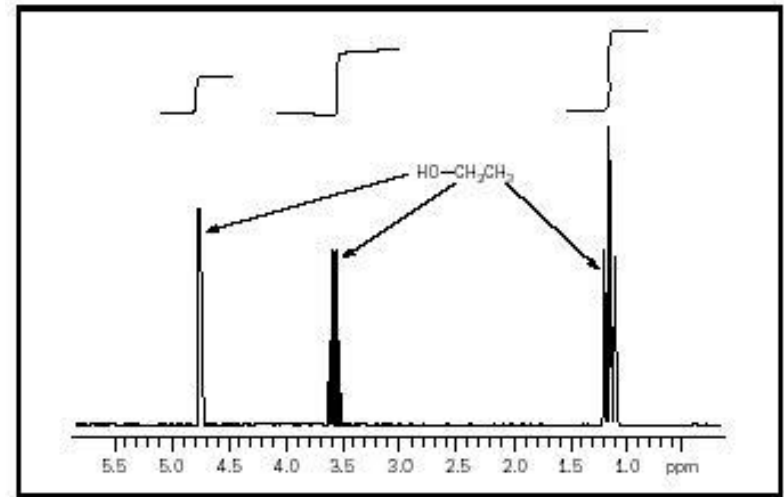
LC-SPE/ krio-NMR



NMR visoke rezolucije

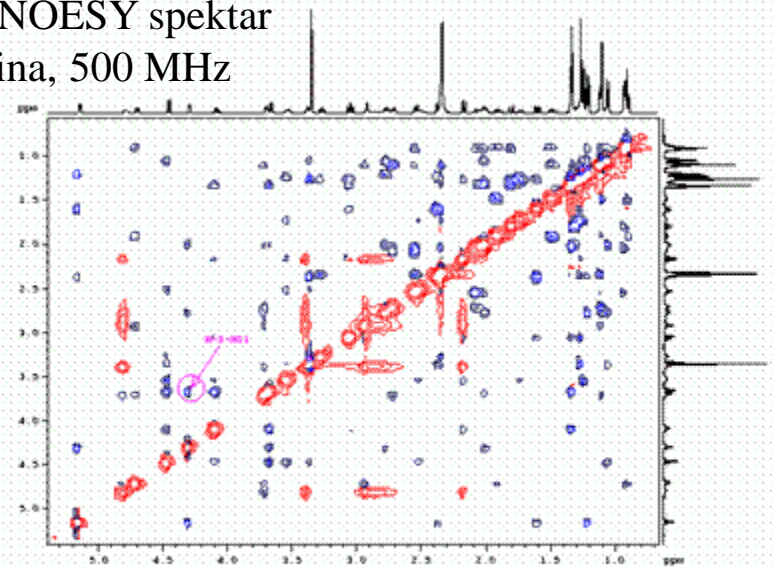


NMR spektar iste molekule, 300 MHz

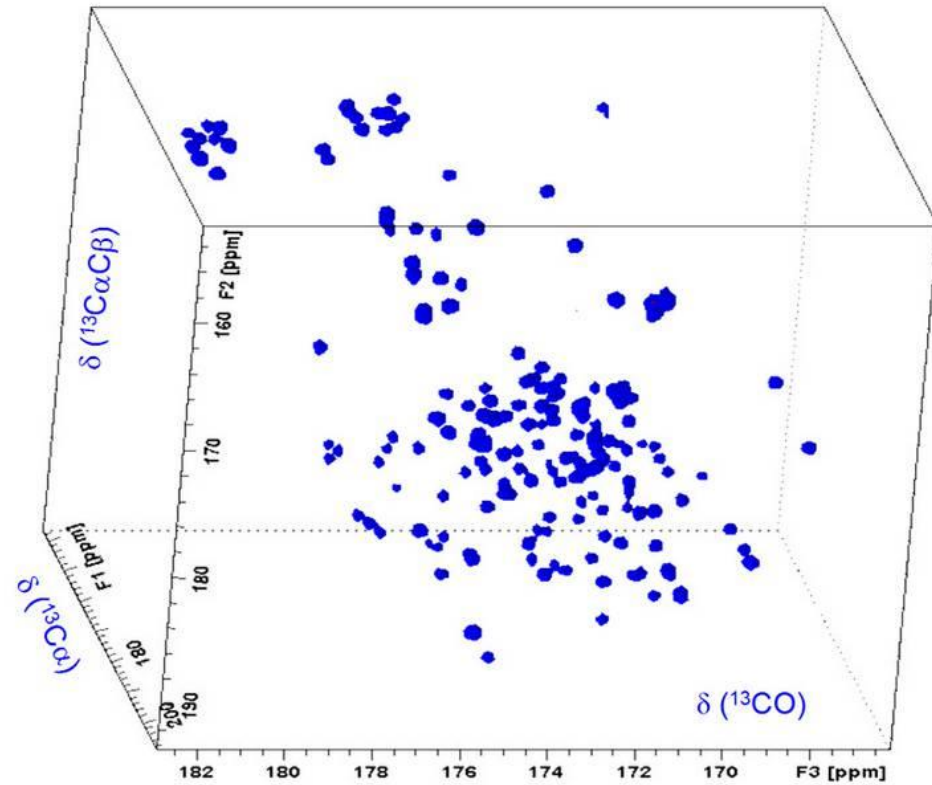
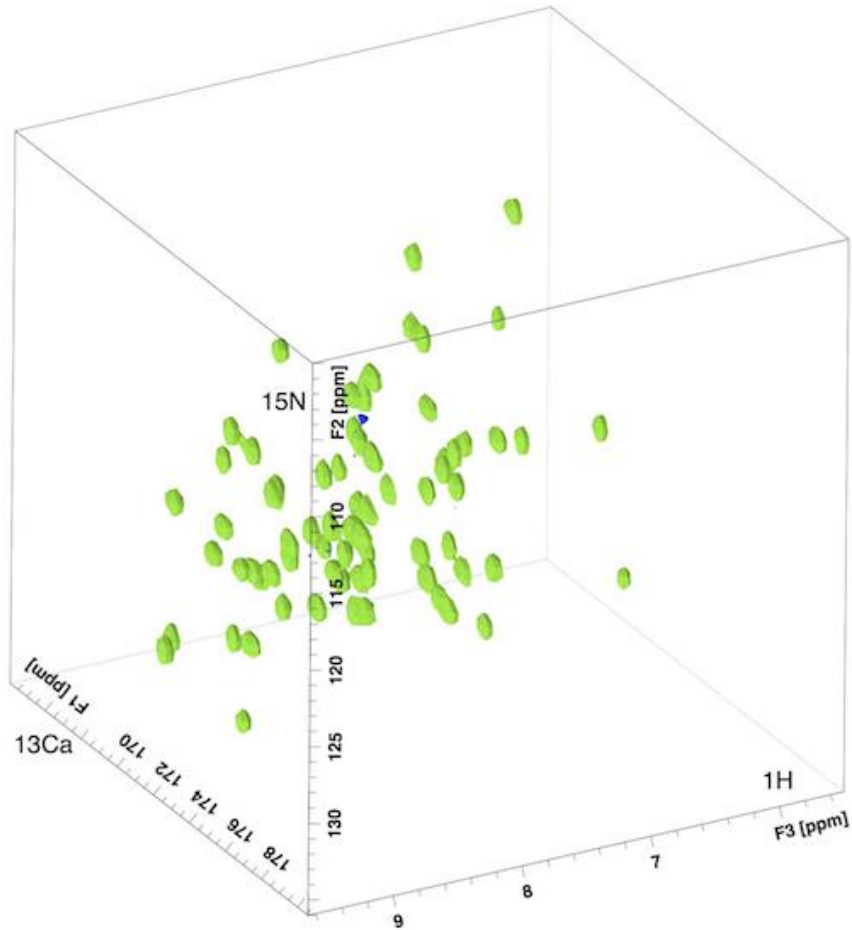


3D HMQC-TOCSY
NMR spektar
azitromicina
500 MHz

2D TPPI-NOESY spektar
azitromicina, 500 MHz



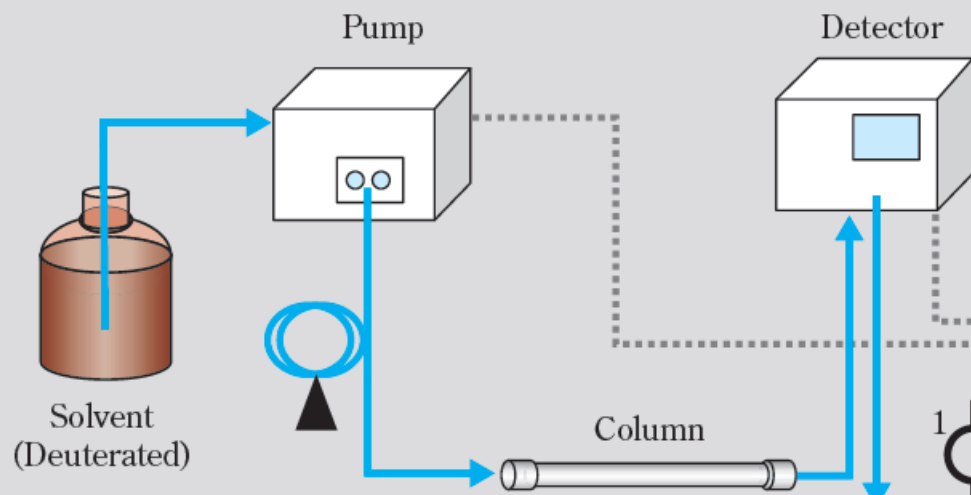
3D NMR spektri proteina



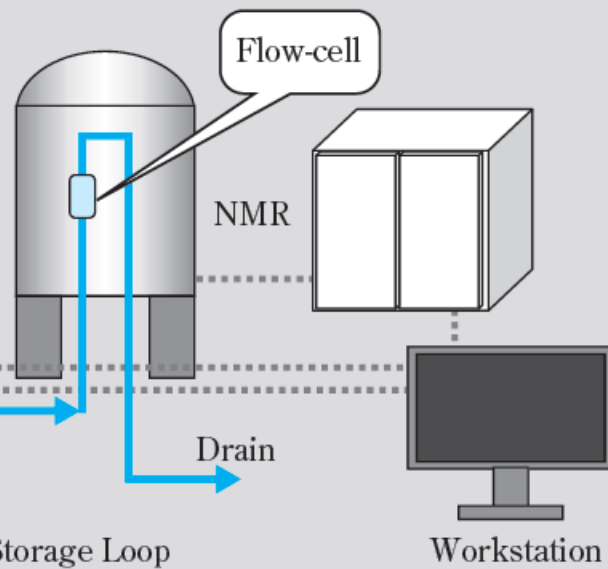
Sprega tekućinske kromatografije i NMR-a

LC-NMR

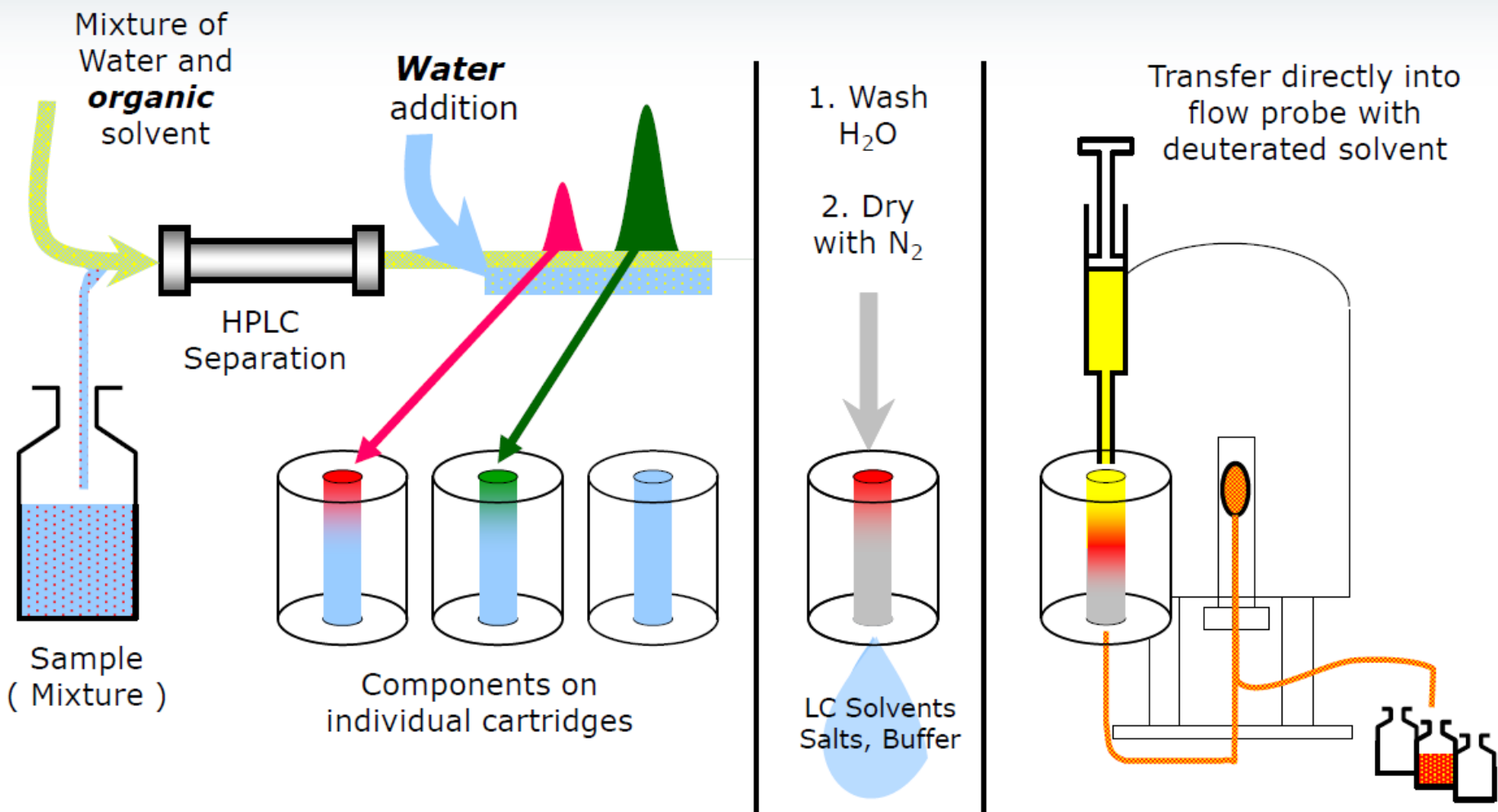
HPLC



NMR



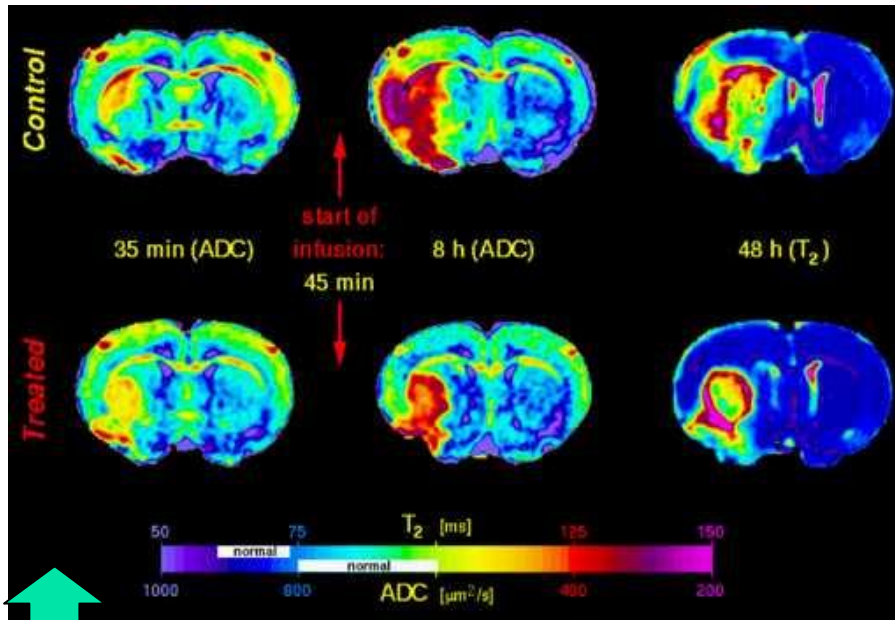
Ekstrakcija na čvrstoj fazi



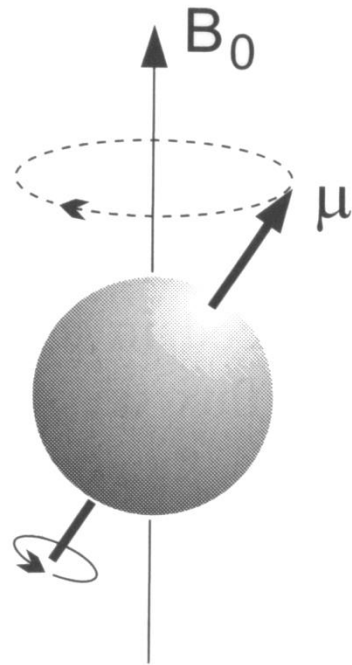
LC-SPE-NMR-MS sustav



MRI/MRS sustav



Spin jezgre i rezonancija

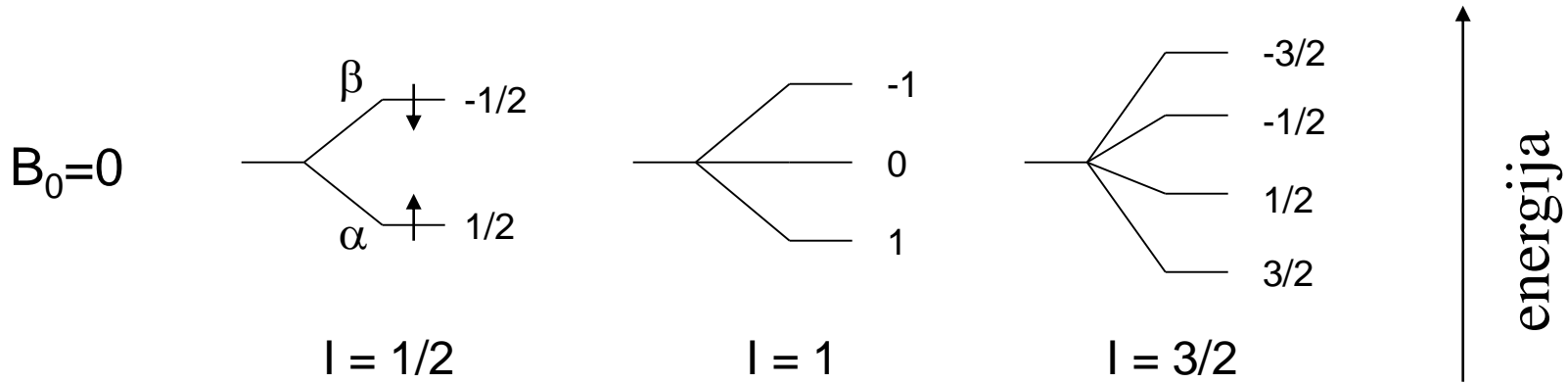


$$\mu = \gamma P$$

$$P = I \hbar / 2\pi$$

$$I = (0, 1/2, 1, 3/2, \dots)$$

Jezgra s kvantnim brojem I može imati $2I_n + 1$ orijentaciju

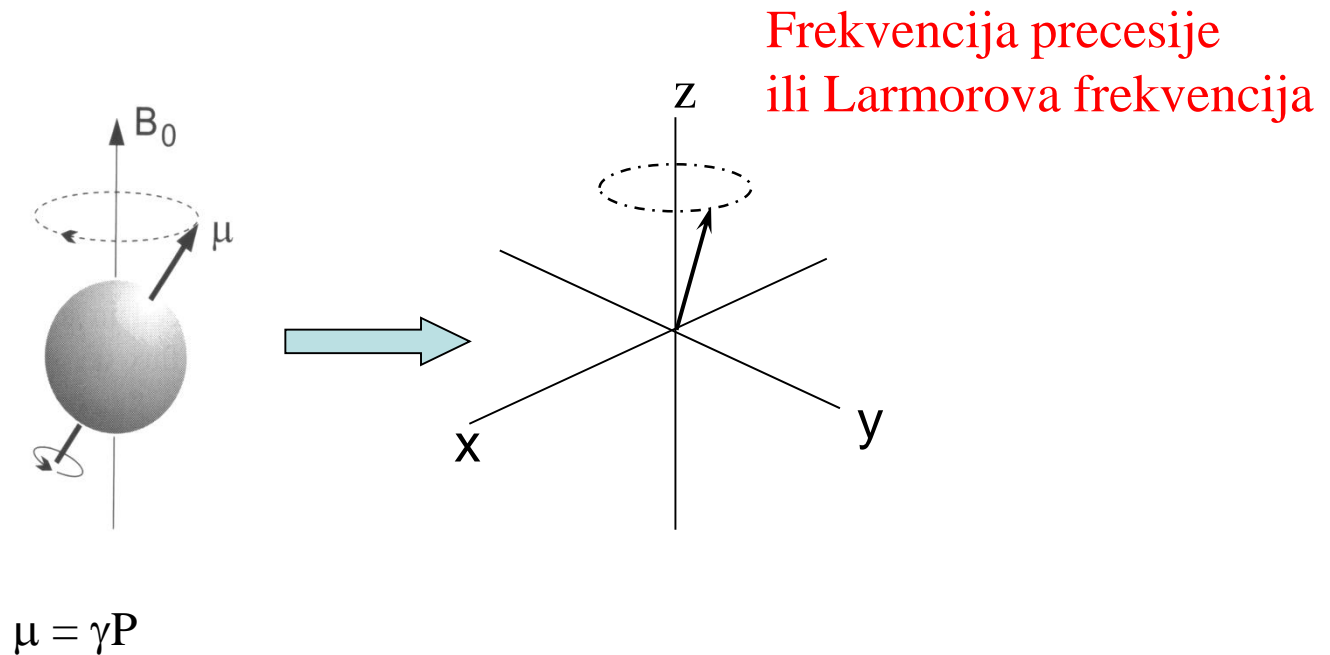


Svojstva nekih jezgri sa spinom $\frac{1}{2}$

izotop	Prorodna zastupljenost (%)	NMR frekvencija (MHz) $B_0=11.7$ T	Relativna osjetljivost
^1H	99.98	400.0	1.0
^3H	0	426.7	1.2 ^a
^{13}C	1.11	100.6	1.76×10^{-4}
^{15}N	0.37	40.5	3.85×10^{-6}
^{19}F	100	376.3	0.83
^{29}Si	4.7	79.5	3.69×10^{-4}
^{31}P	100	161.9	6.63×10^{-2}

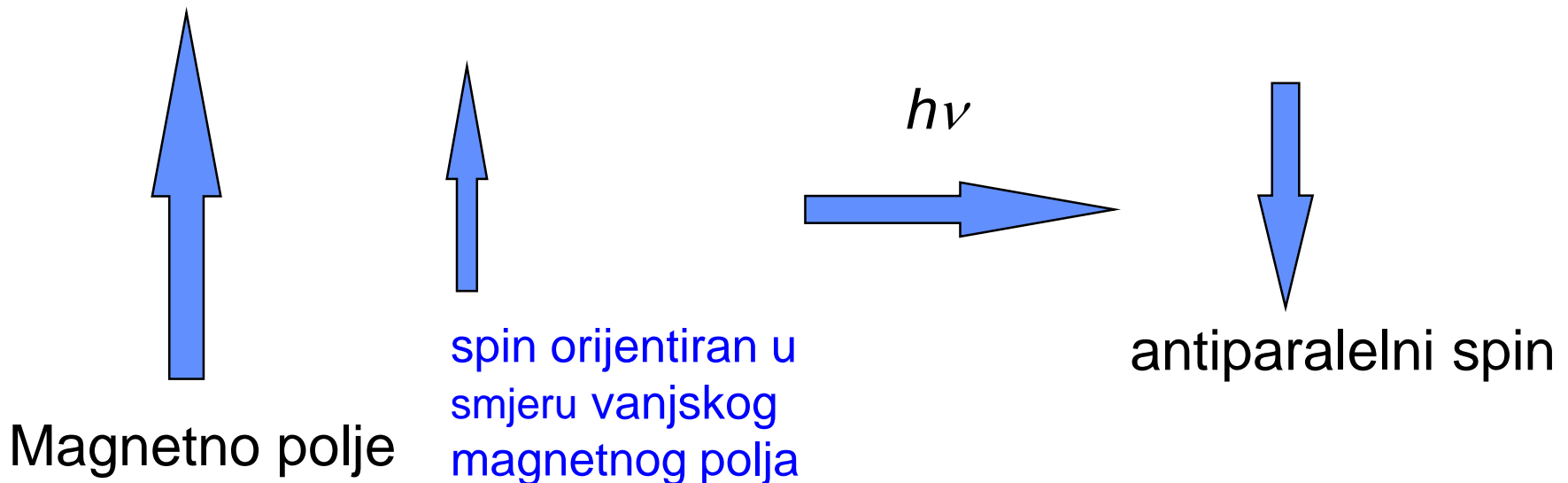
^aako ima 100% ^3H

Dogovorom je prihvaćeno da je smjer primjenjenog magnetnog polja smjer osi z u Cartesijevom koordinatnom sustavu

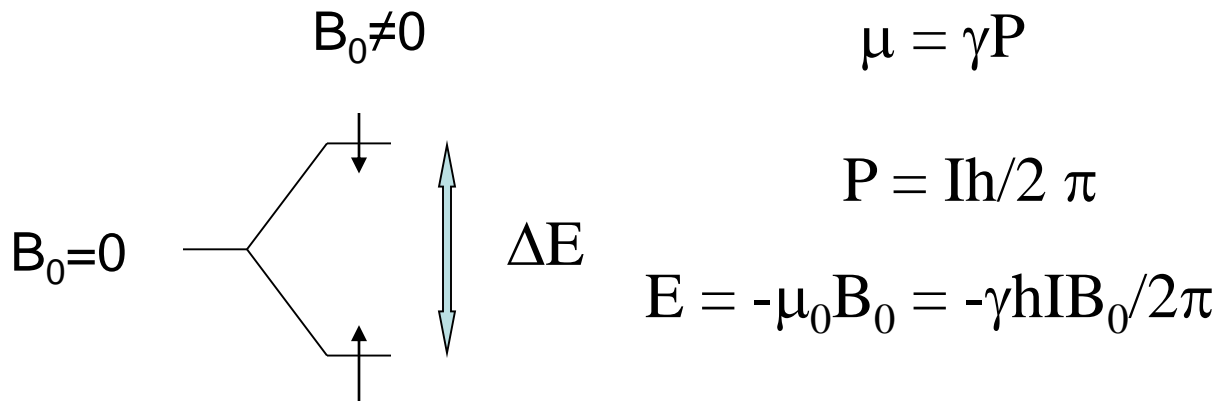


Utjecaj magnetnog polja

- Smjesti li se jezgra u vanjsko magnetsko polje B_0 , ona će se na smjer magnetnog polja usmjeriti paralelno ili antiparalelno.
- Kada energija zračenja koja djeluje na jezgru postane jednaka razlici energije između dva stanja spina, postiže se uvjet nazvan rezonancija.
- Energija potrebna za preskok spina karakteristična je za vrstu atomske jezgre.



Rezonancija



h - Planckova konstanta
($6.62618 \times 10^{-34} \text{Js}$)

$$\Delta E = \gamma\hbar B_0/2\pi \quad \Delta E = h\nu$$

$$\nu = \gamma B_0/2\pi$$

Osnovni uvjet rezonancije

$$\nu_1 = \nu_0$$

Boltzmanova raspodijela

$$N_{\alpha} / N_{\beta} = e^{\Delta E/kT}$$

N- broj jezgri

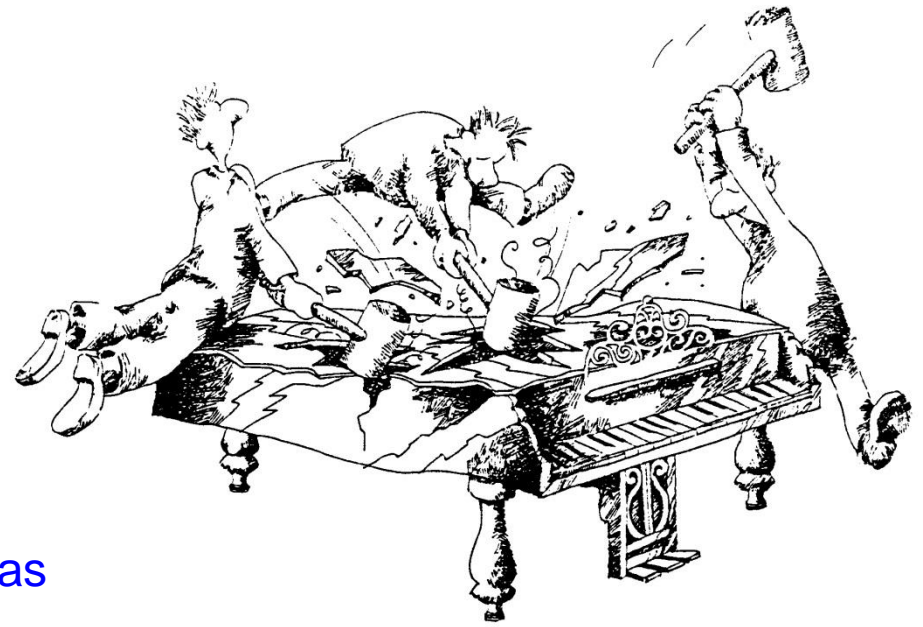
k- Boltzmanova konstanta ($1.38066 \times 10^{-23} \text{ JK}^{-1}$)

T- temperatura

Samo 1 jezgra na milijun u suvišku u osnovnom energijskom stanju !!



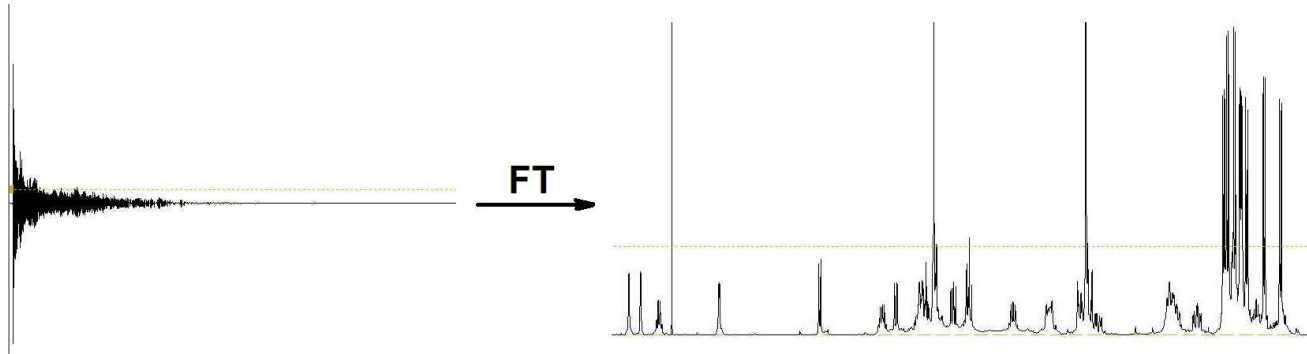
nekad



danas

vremenska domena

frekvencijska domena



$$f(\omega) = \int_{-\infty}^{+\infty} f(t) e^{i\omega t} dt$$

$$e^{i\omega t} = \cos \omega t + i \sin \omega t$$



realno

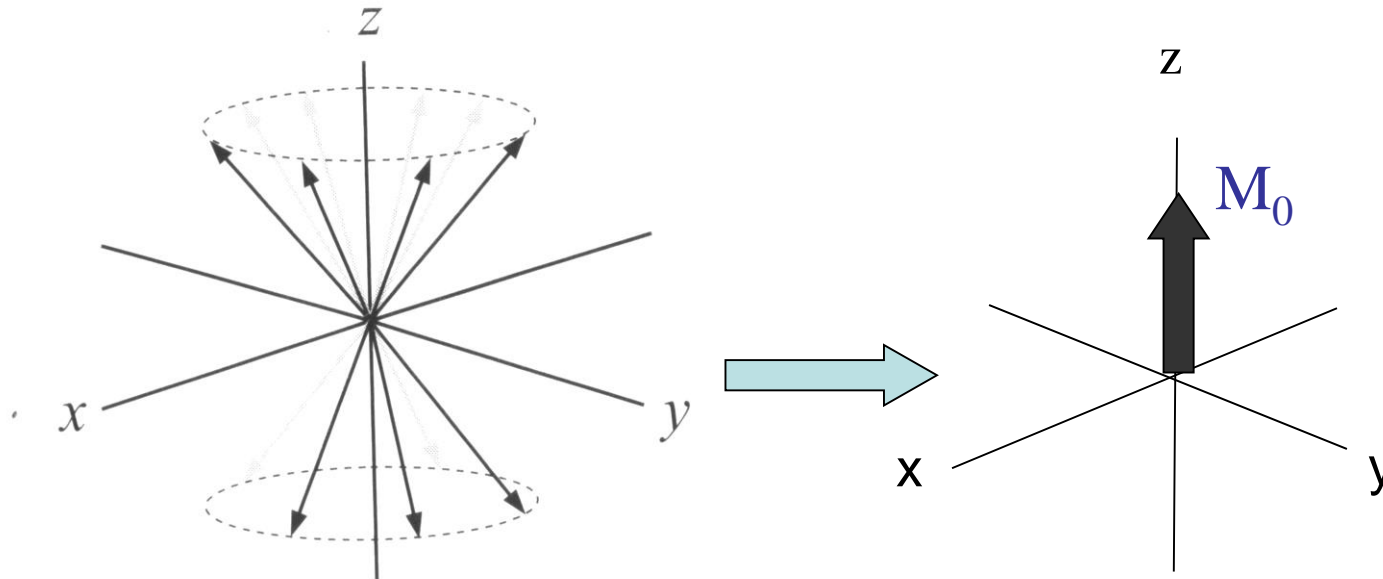


imaginarno

Apsorpcijski
sigal

Disperzijski
sigal

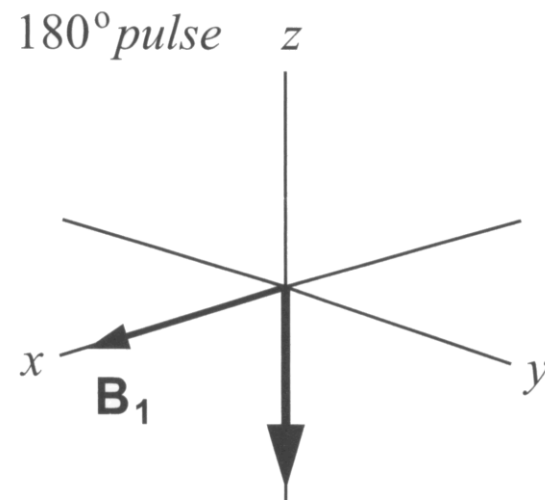
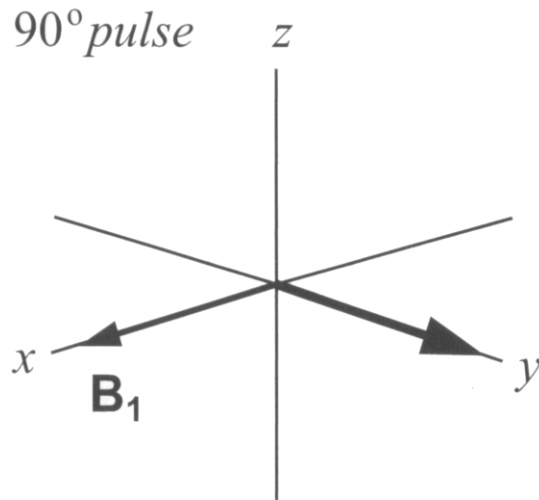
Vektorski model

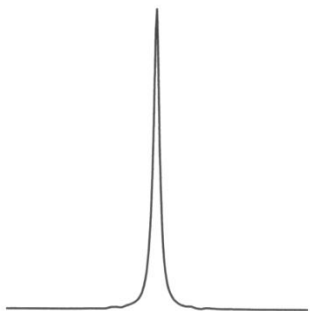
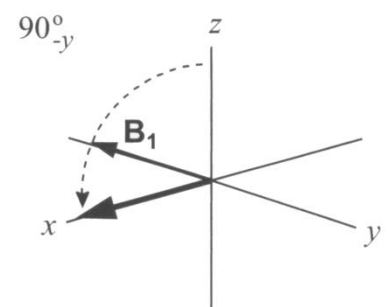
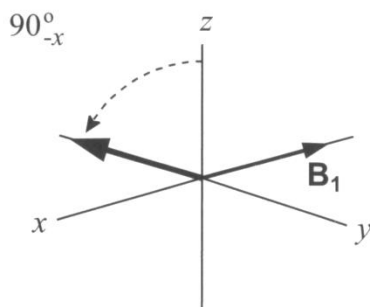
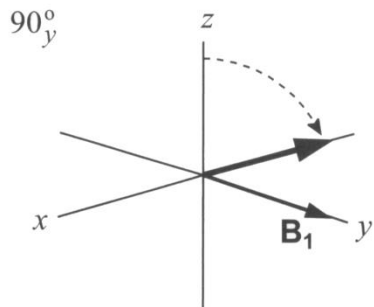
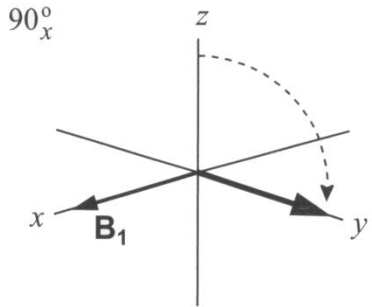


S obzirom da ima više istovrsnih spinova u stanju α vektor ukupne magnetizacije ima smjer $+z$

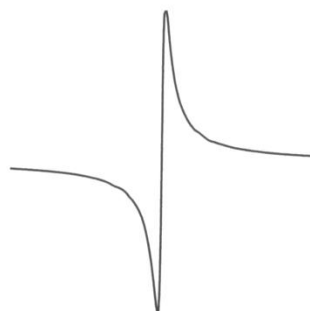
Radiofrekventni puls

Pulsni kut $\Phi = 360\gamma B_1 t_p / 2\pi$

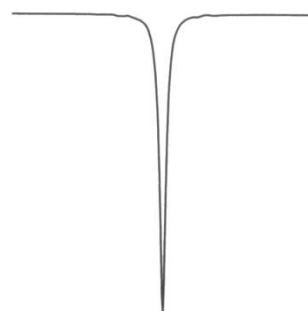




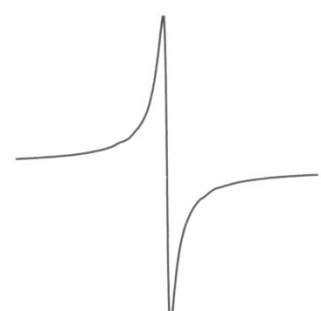
Pozitivna
apsorpcija



Pozitivna
disperzija

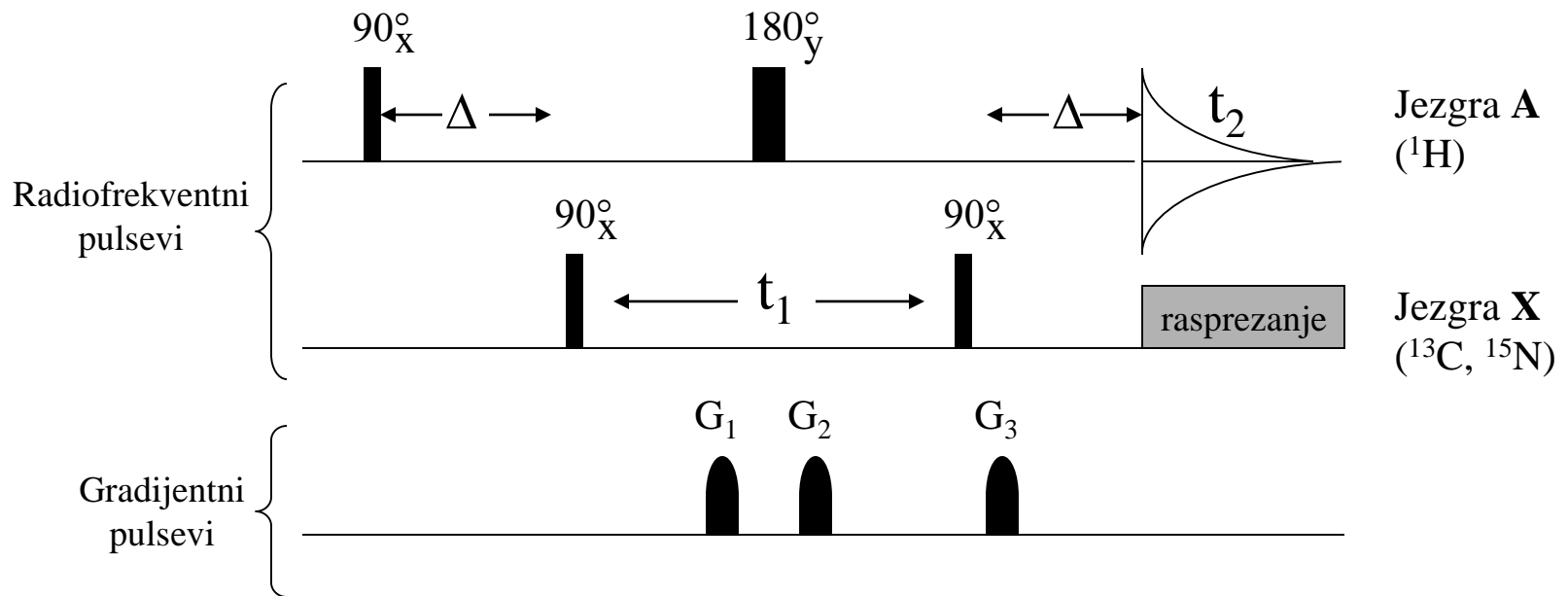


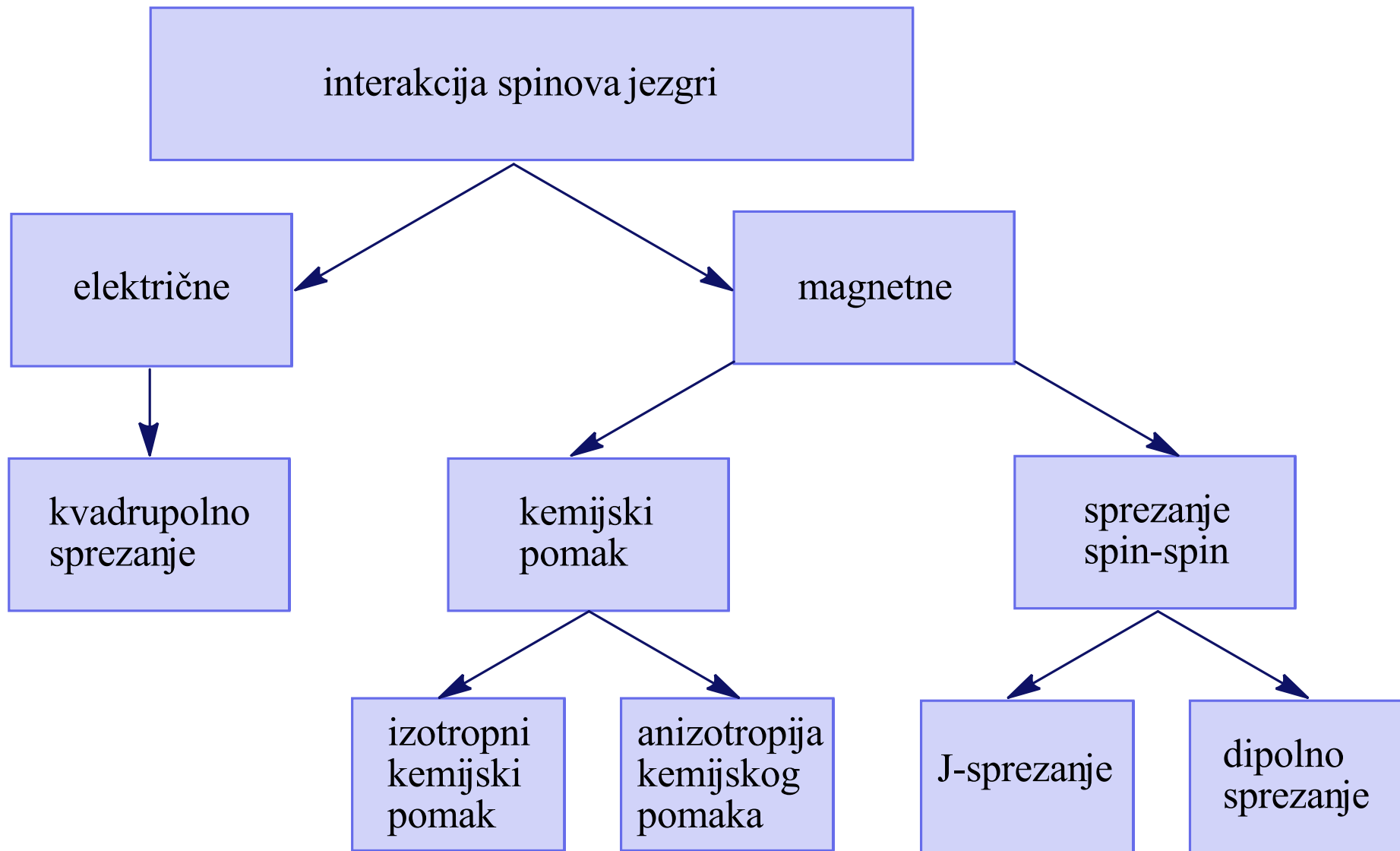
negativna
apsorpcija



Negativna
disperzija

Pulsni slijed

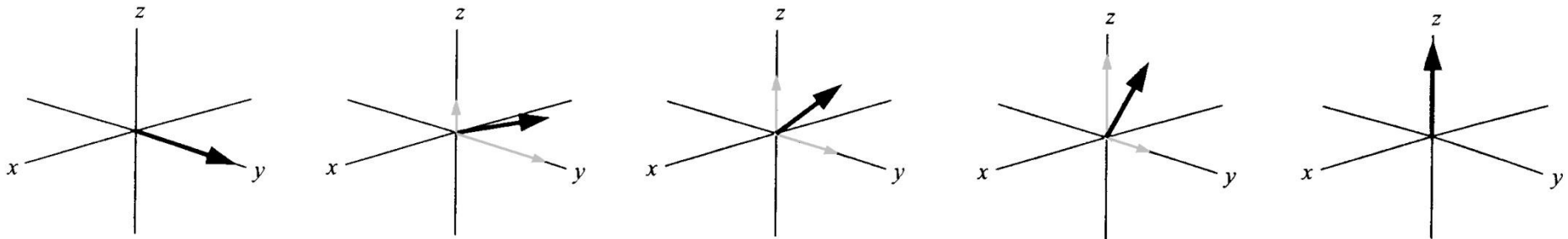




NMR parametri

a) Vremena opuštanja ili relaksacije

- vrijeme opuštanja spin-rešetka (longitudinalno), T_1
 - $dM_z/dt = (M_0 - M_z)/T_1$ Blochova jednažba
 - Intenzitet NMR signala

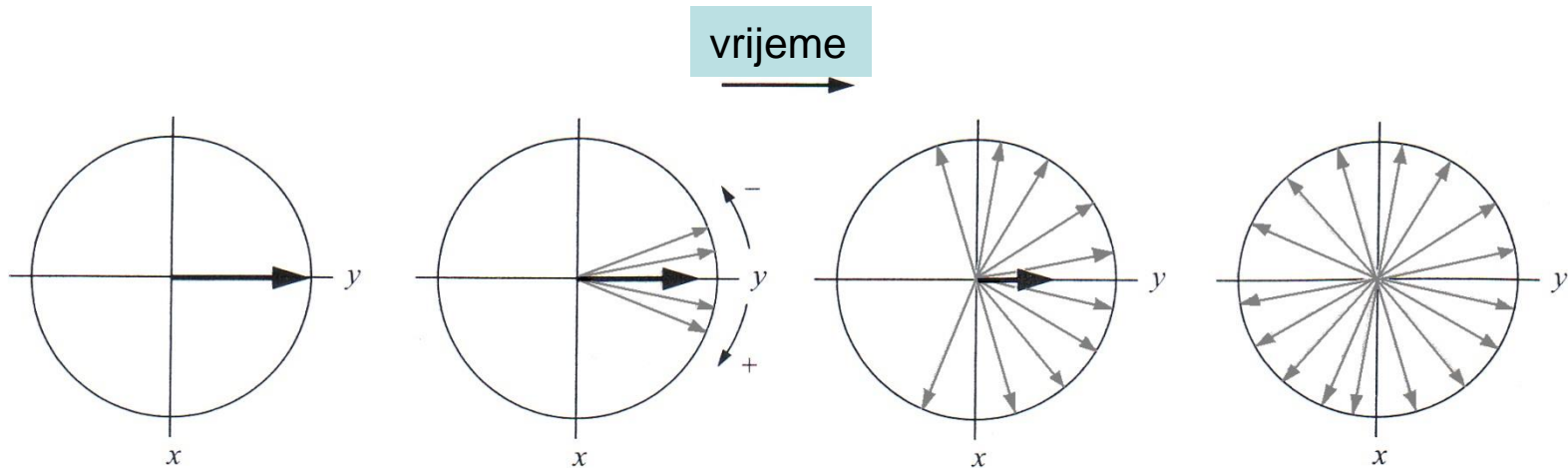


➤) vrijeme opuštanja spin-spin (transverzalno), T_2

➤ $dM_x/dt = -M_x/T_2$

➤ $dM_y/dt = -M_y/T_2$

➤ Širina NMR linija $\Delta\nu_{1/2} = 1/T_2$



Mehanizmi opuštanja spin-rešetka

$$\triangleright 1/T_1^{\text{uk}} = 1/T_1^{\text{DD}} + 1/T_1^{\text{CSA}} + 1/T_1^{\text{SR}} + 1/T_1^{\text{SC}}$$

DD- dipol-dipol (najučinkovitiji)

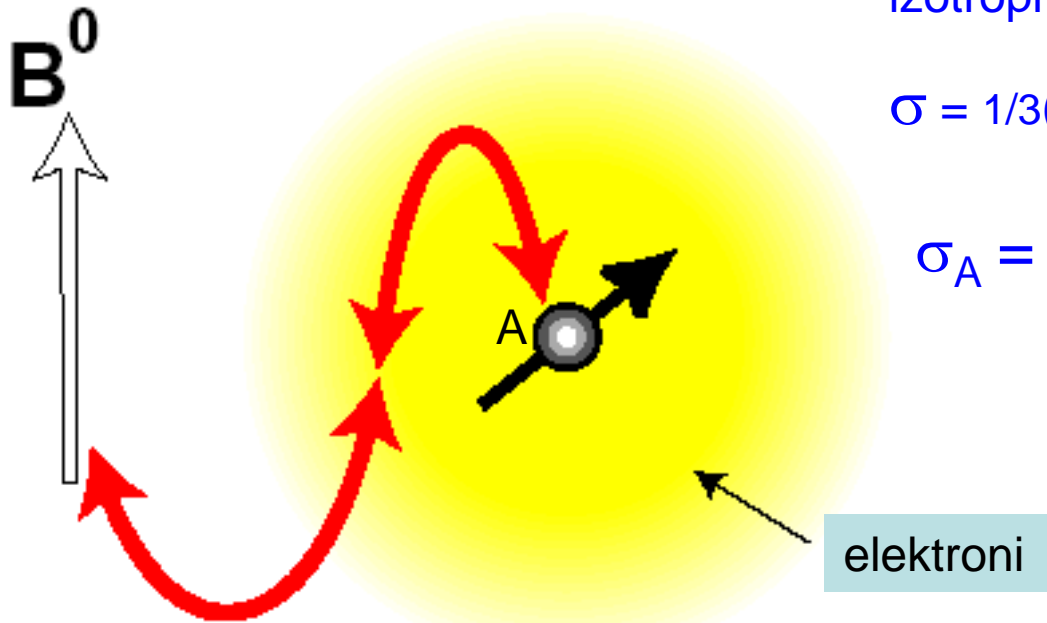
CSA- anizotropija kemijskog pomaka

SR- spinska rotacija (rotori npr. CH₃)

SC- skalarna sprega (bliska Larmorova frekvencija ¹³C-
³⁷Br)

b) Kemijski pomak, nuklearno zasjenjenje

Levitt



Konstanta zasjenjenja za izotropni medij

$$\sigma = 1/3(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$$

$$\sigma_A = \sigma^{\text{dia}} + \sigma^{\text{para}} + \sum_{A \neq X} \sigma_X$$

Elektroni koji okružuju jezgru A stvaraju magnetno polje (zasjenjenje) koje utječe na ukupno magnetno polje koje osjećaju jezgre

- $B_{\text{ef}} = B_0 - B_{\text{ind}}$
- $B_{\text{ind}} = \sigma_{\text{ef}} B_0$
- $B_{\text{ef}} = B_0 - \sigma_{\text{ef}} B_0 = (1 - \sigma_{\text{ef}}) B_0$

$$\nu = \gamma B_0 / 2\pi$$

Uvjet rezonancije

$$\nu = \gamma (1 - \sigma_{\text{ef}}) B_0 / 2\pi$$

Referentna δ Skala

$$\begin{aligned} \delta_{\text{uzorak}} &= \nu_{\text{uzorak}} - \nu_{\text{referentno}} / \nu_{\text{referentno}} \\ &= \Delta\nu \text{ (Hz)} / \nu_{\text{referentno}} \text{ (MHz)} \end{aligned}$$

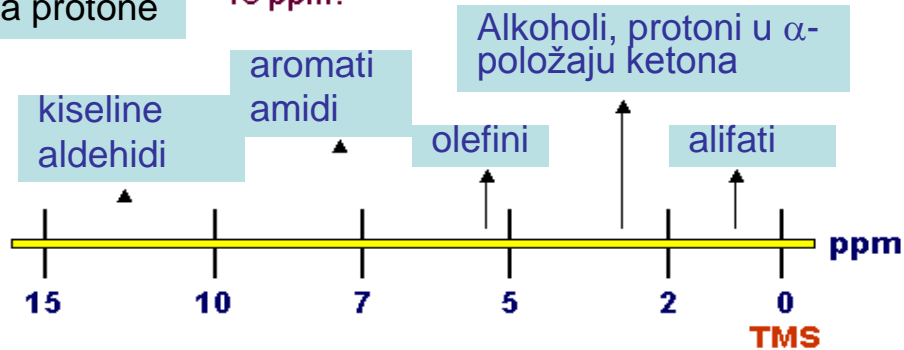
Skala u ppm!

$$\delta(\text{ppm}) = 10^6 \times (\nu - \nu_{\text{ref}} / \nu_{\text{osc}})$$

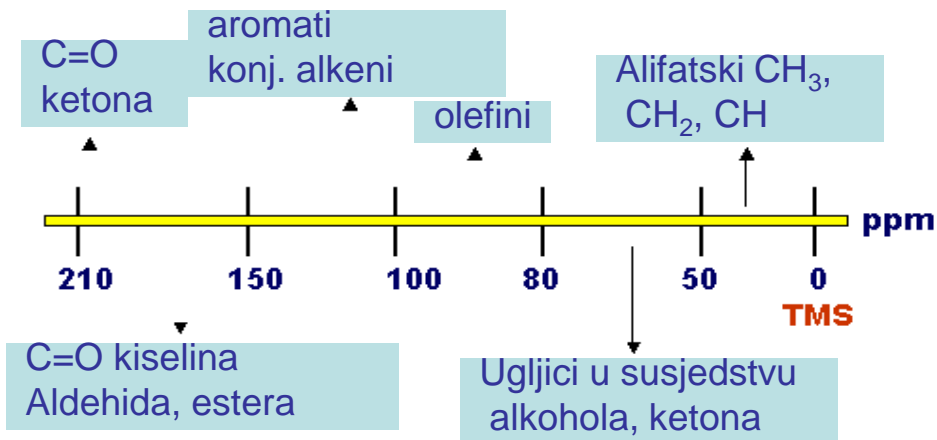
- Izračunajte kemijski pomak za protone u CHBr_3 , CH_2Br_2 i CH_3Br ako su položaji signala u ^1H NMR spektru snimanom na 90 MHz instrumentu ($B_0 = 2.11\text{T}$) redom 614 Hz, 441 Hz i 237 Hz
 - 6,82 ppm
 - 4,90 ppm
 - 2.63 ppm
- Pri kojoj frekvenciji bi rezonirali ti protoni na instrumentu koji radi na $B_0 = 7.05\text{ T}$?
 - 2046 Hz
 - 1470 Hz
 - 789 Hz

Skala za različite jezgre

Za protone ~ 15 ppm:

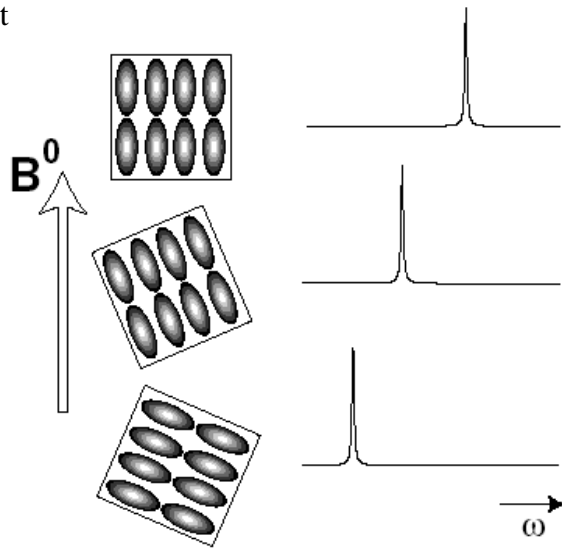


Za ugljike ~ 220 ppm:



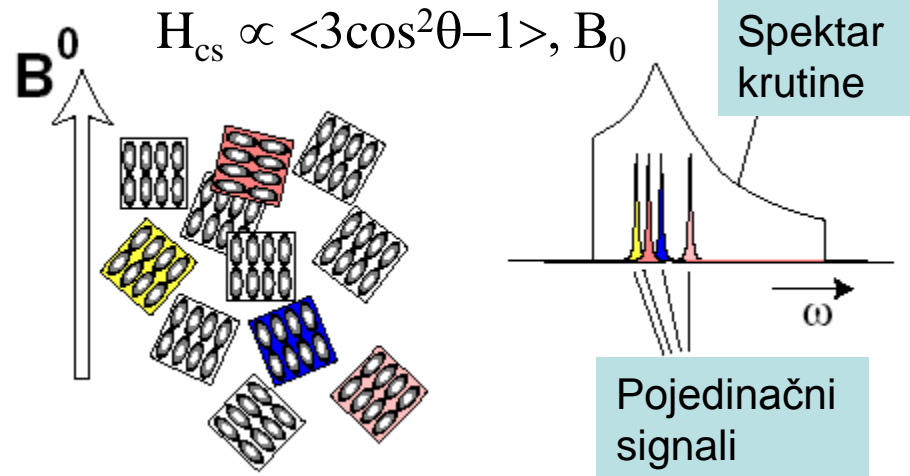
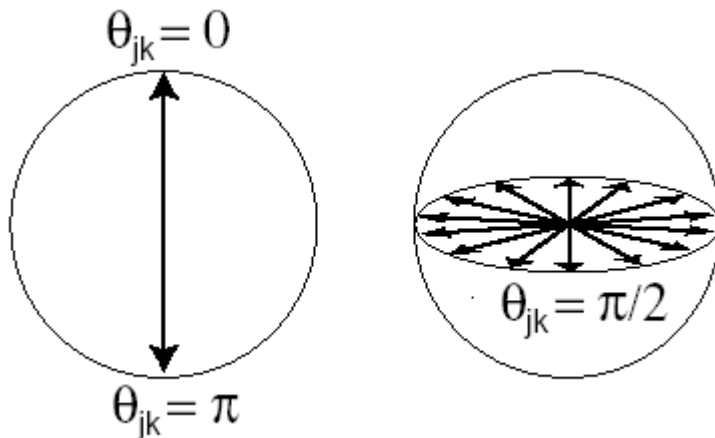
Anizotropija kemijskog pomaka (CSA)

Levitt

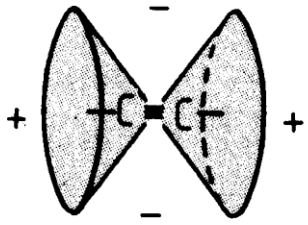


U NMR-u tekućina gibanje (tumbanje) molekula uzrok je simetričnosti vremenski uprosječenog elektronskog zasjenjenja. U NMR-u čvrstog stanja, molekule se ne gibaju izotropno pa kemijski pomak ne ovisi samo o kemijskom identitetu atoma nego i o prostornom odnosu između molekule i vanjskog magnetnog polja.

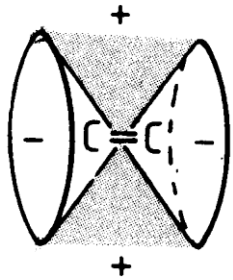
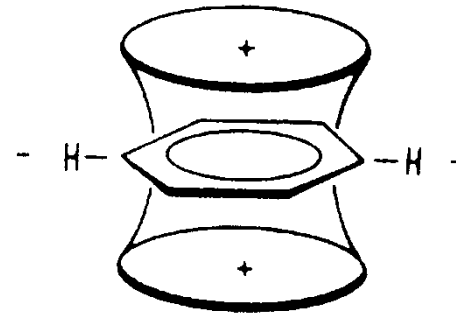
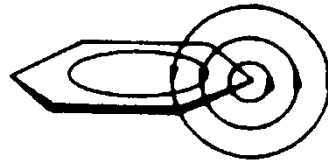
Vjerojatnost orijentacije



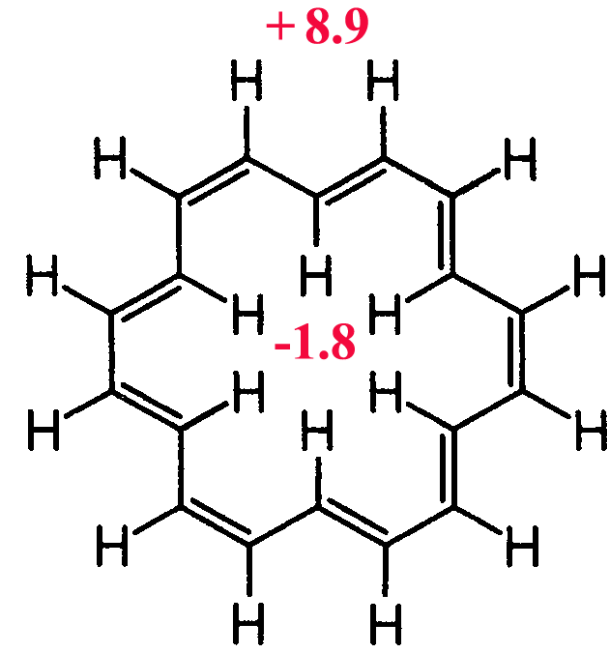
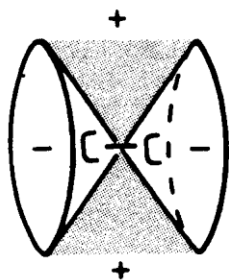
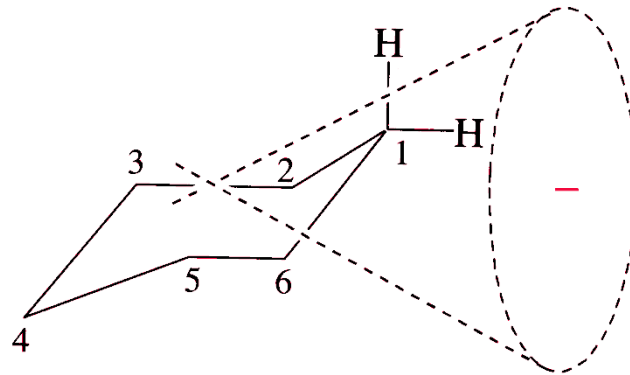
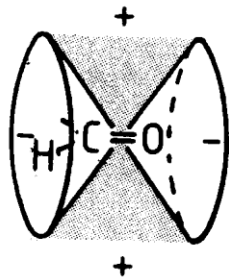
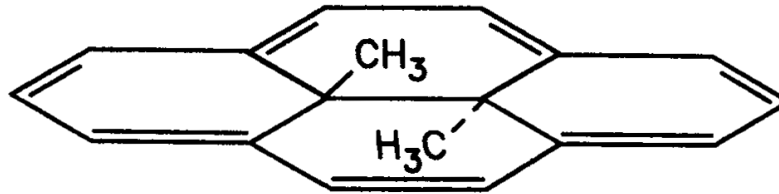
Anizotropija kemijskog pomaka i prstenaste struje



B_0 ↑

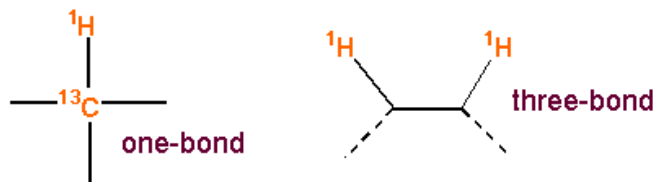


$$\delta(\text{CH}_3) = -4.25$$

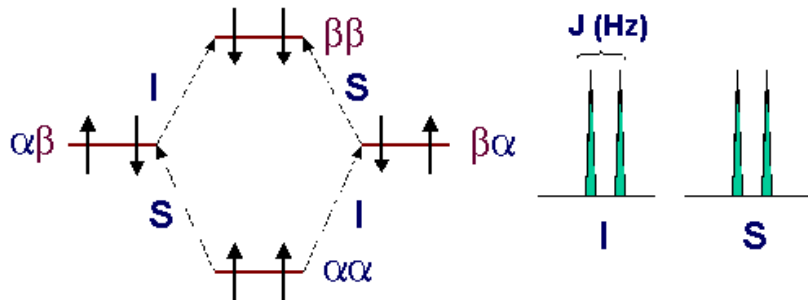


c) Konstanta sprege spin-spin

Spinsko stanje susjedne jezgre može utjecati na energetske razine promatrane jezgre. Za takve jezgre kažemo da su međusobno spregnute preko jedne ili više kemijskih veza



Energetski dijagram. Svaki spin sada ima dvije podrazine ovisno o stanju spina s kojim je u sprezi



Razlika između dvije linije dubleta se zove konstanta sprege J i ima jedinicu Hz

Način sprežavanja bitan je za identifikaciju spinskog sustava u molekuli i za određivanje njene strukture

Energetske razine

$\beta\beta$ —————

$\alpha\beta$ —————

$\beta\alpha$ —————

$\alpha\alpha$ —————

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

$\beta\beta$ —————

$\alpha\beta$ —————

$\beta\alpha$ —————

$\alpha\alpha$ —————

$^1\text{H}-^{13}\text{C}$

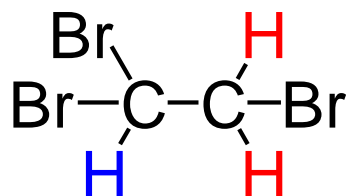
Konstanta sprege spin-spin, J(Hz)

$${}^nJ_{AX} = {}^nJ_{AX}^{OD} + {}^nJ_{AX}^{SD} + {}^nJ_{AX}^{FC}$$

OD – orbitalno-dipolarna interakcija orbitalnog momenta elektrona i spina jezgre

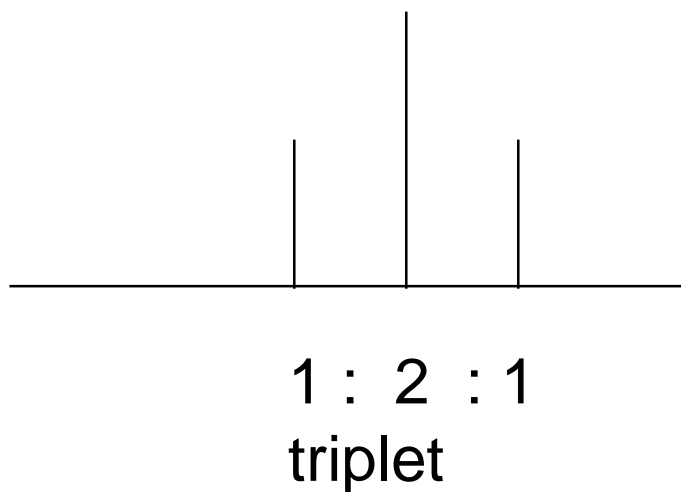
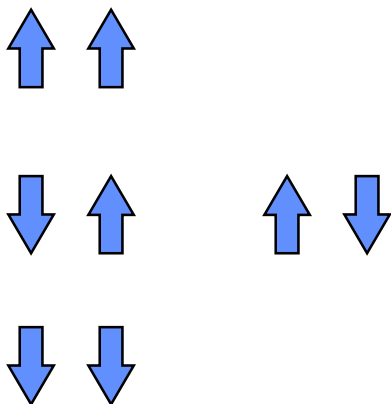
SD – spin-dipolarna interakcija spinova elektrona i jezgre

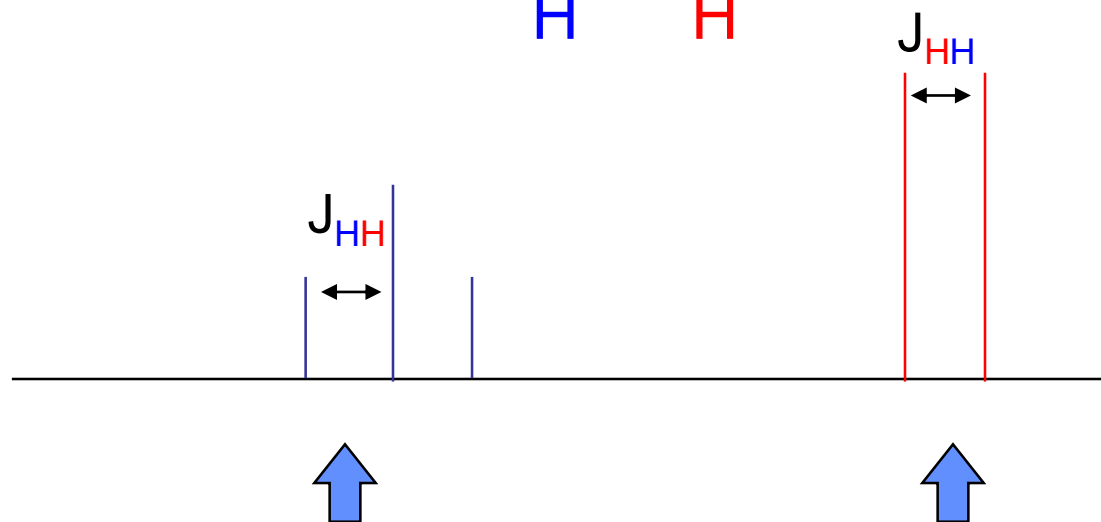
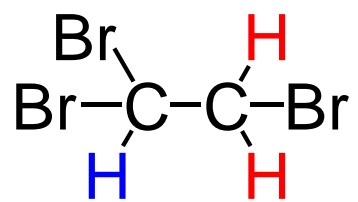
FC – Fermijev kontaktni član (interakcija magnetnih momenata jezgre i s elektrona)



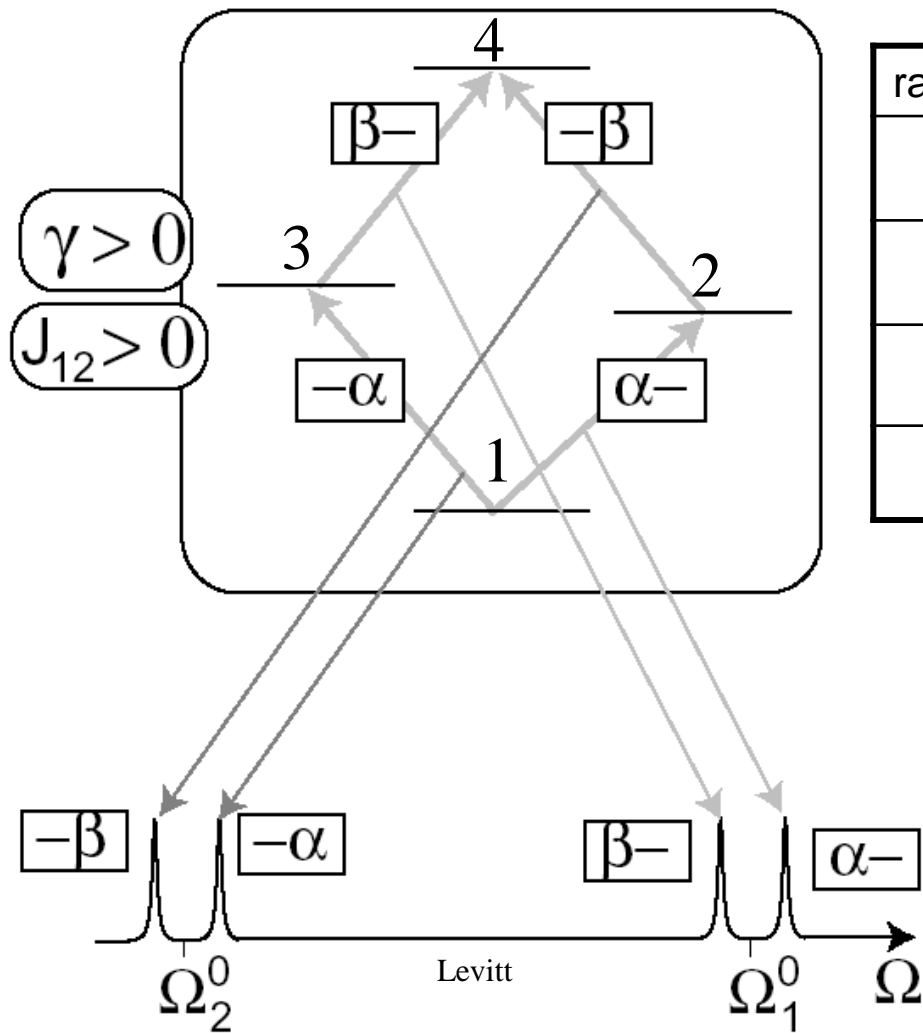
Promotrimo -CHBr₂ proton u molekuli.

On je pod utjecajem spinova susjednih protona. Postoje 4 moguće kombinacije spinova:





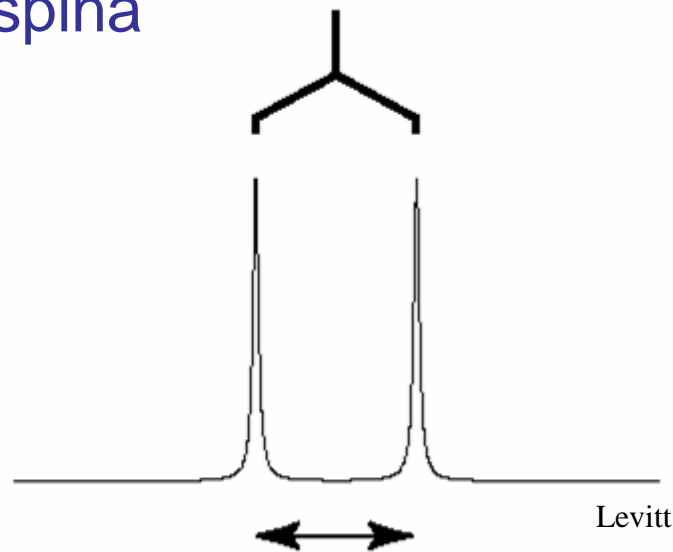
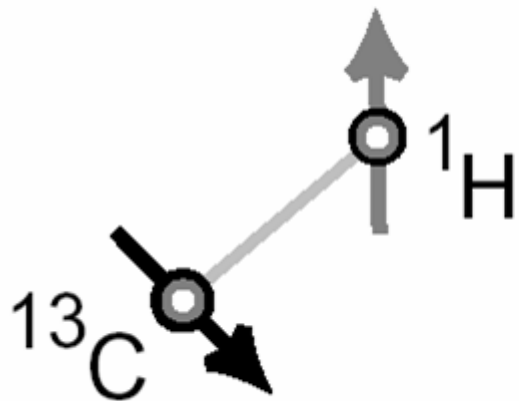
Homonuklearna sprega J : 2 spina



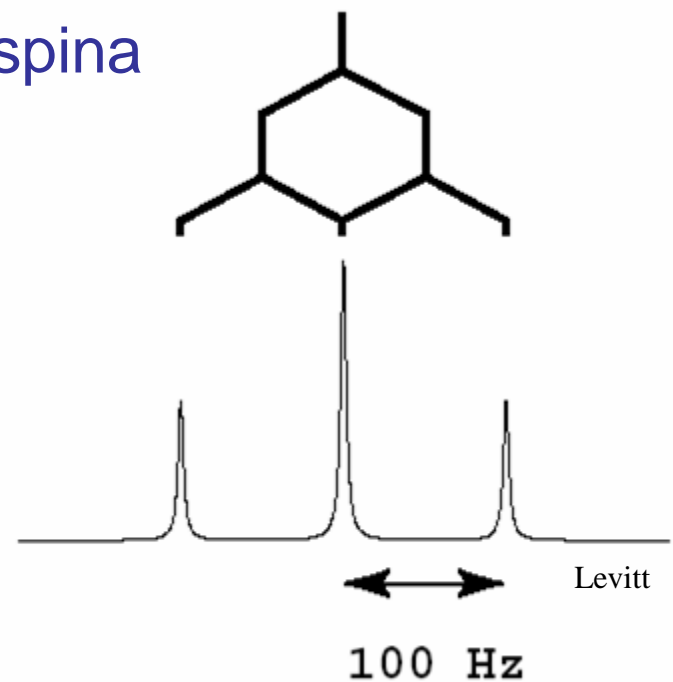
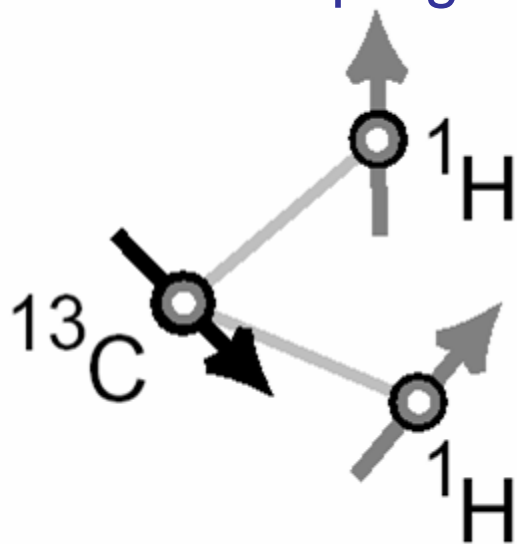
razina	Spinsko stanje	energija
1	$\alpha\alpha$	$-\frac{1}{2}v_0^1 - \frac{1}{2}v_0^2 + \frac{1}{4}J_{12}$
2	$\alpha\beta$	$+\frac{1}{2}v_0^1 - \frac{1}{2}v_0^2 - \frac{1}{4}J_{12}$
3	$\beta\alpha$	$-\frac{1}{2}v_0^1 + \frac{1}{2}v_0^2 - \frac{1}{4}J_{12}$
4	$\beta\beta$	$+\frac{1}{2}v_0^1 + \frac{1}{2}v_0^2 + \frac{1}{4}J_{12}$

prijelaz	energija
1→2	$v_0^1 - \frac{1}{2}J_{12}$
3→4	$v_0^1 + \frac{1}{2}J_{12}$
1→3	$v_0^2 - \frac{1}{2}J_{12}$
2→4	$v_0^2 + \frac{1}{2}J_{12}$

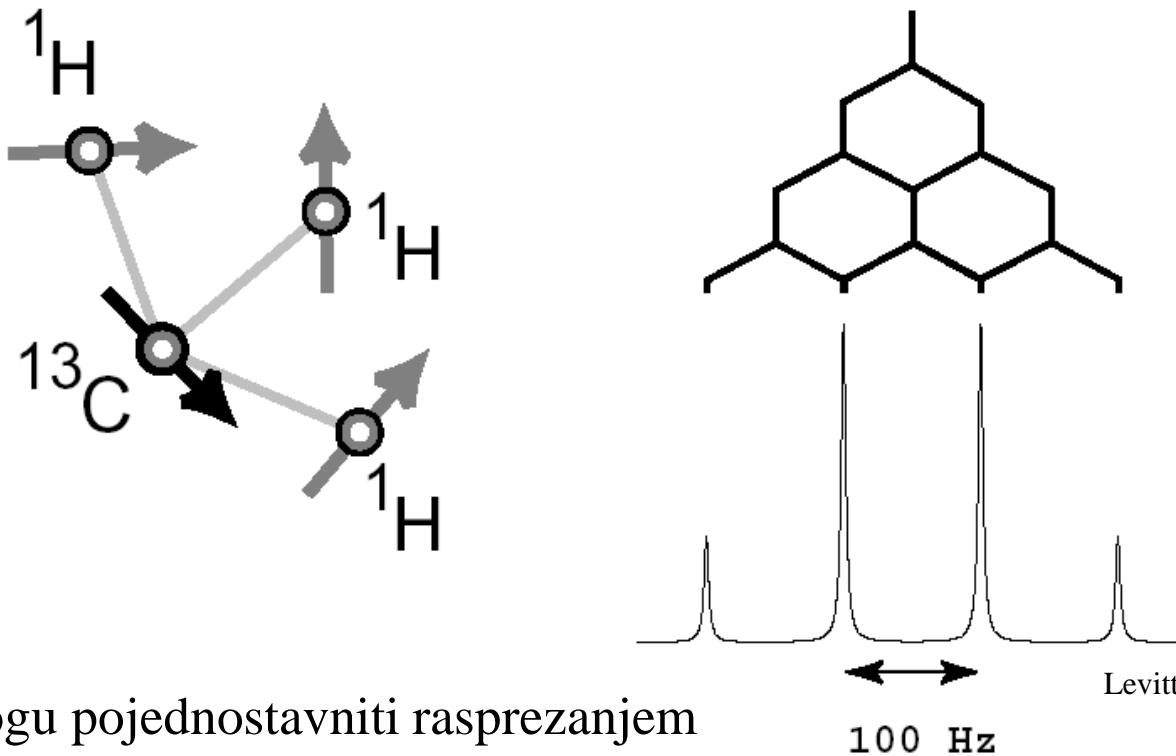
Heteronuklearna sprega J : 2 spina



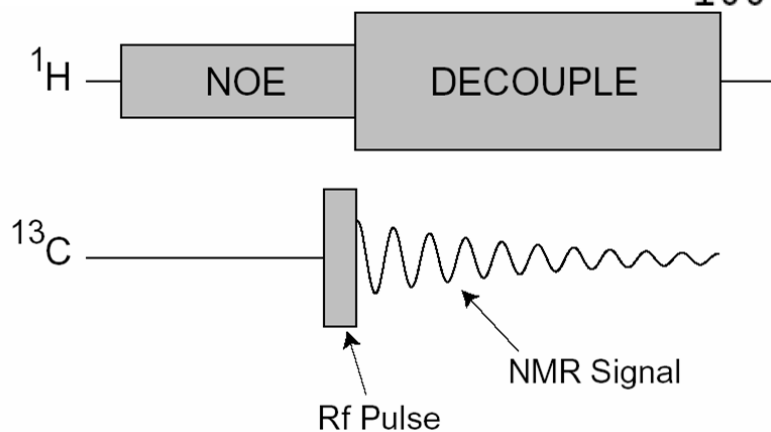
Heteronuklearna sprega J : 3 spina



Heteronuklearna sprega J : 4 spina



Spektri se mogu pojednostavniti raspredanjem



Pascalov trokut

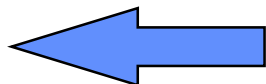
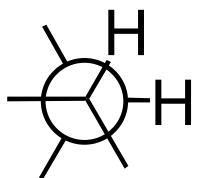
relativni odnosi intenziteta signala za $l=1/2$:

				1												
				1		1										
				1		2		1								
				1		3		3		1						
				1		4		6		4		1				
				1		5		10		10		5		1		
				1		6		15		20		15		6		1

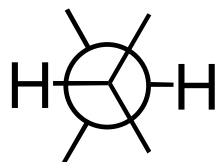
relativni odnosi intenziteta signala za $l=1$:

				1				
				1	1	1		
			1	2	3	2	1	
		1	3	6	7	6	3	1
1	4	10	16	19	16	10	4	1

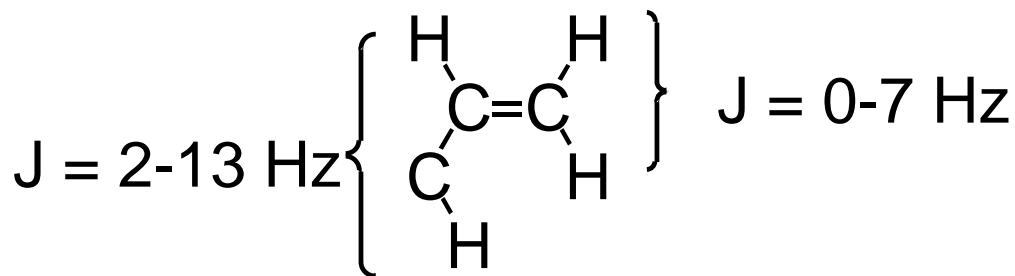
Konstante sprega spin-spin



$J = 2-6 \text{ Hz}$



$J = 5-14 \text{ Hz}$



cis - $J = 2-15 \text{ Hz}$

trans - $J = 10-21 \text{ Hz}$

Ovisnost sprege $^1J_{\text{CH}}$ i hibridizacije

$$^1J_{\text{CH}} \sim 5 (\%s)$$

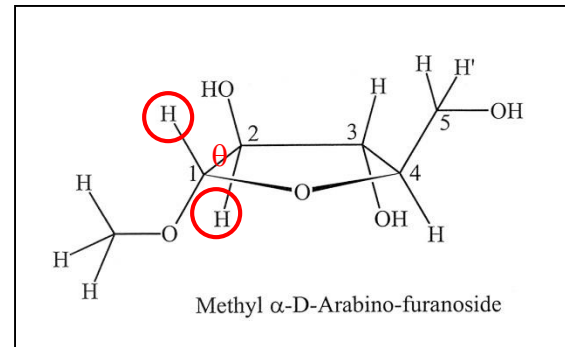
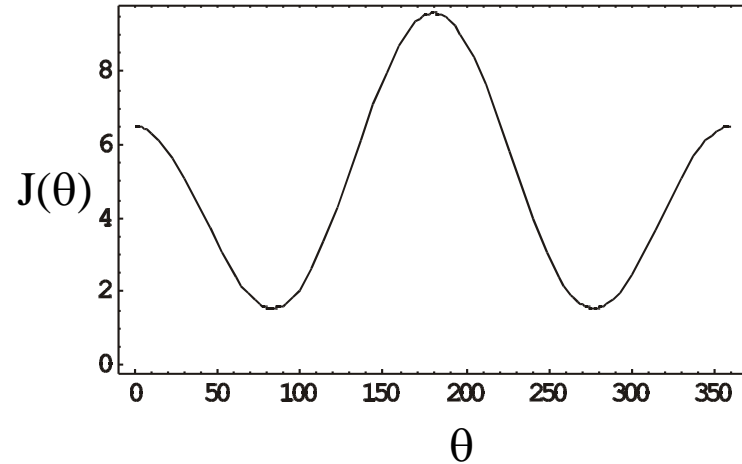
		%s	J/Hz	J/Hz _{exp}
etan	sp ³	25	125	124.9
eten	sp ²	33	165	156.4
etin	sp	50	250	249.0

Spin-spin sprege kroz tri veze

Martin Karplus je pokazao da vicinalna sprega između ^1H atoma ovisi o diedarskom kutu između njih. Ova relacija se može iskazati pomoću **Karplusove jednadžbe**:

$$J(\theta) = A \cos^2(\theta) + B \cos(\theta) + C$$

A , B , i C su empirijski određeni parametri.



Sprega J omogućuje procjenu molekulske konformacije!