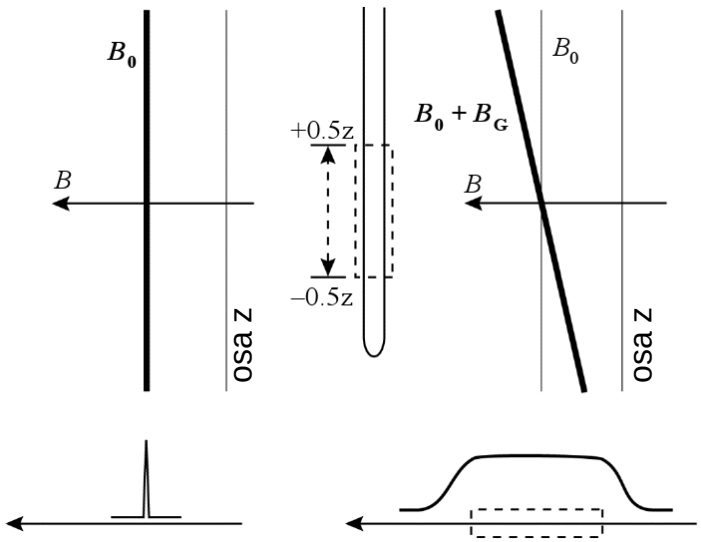
Gradient magnetického pole



Jiné B_0 - jiná Larmorova frekvence

Kódování pozice – frekvence

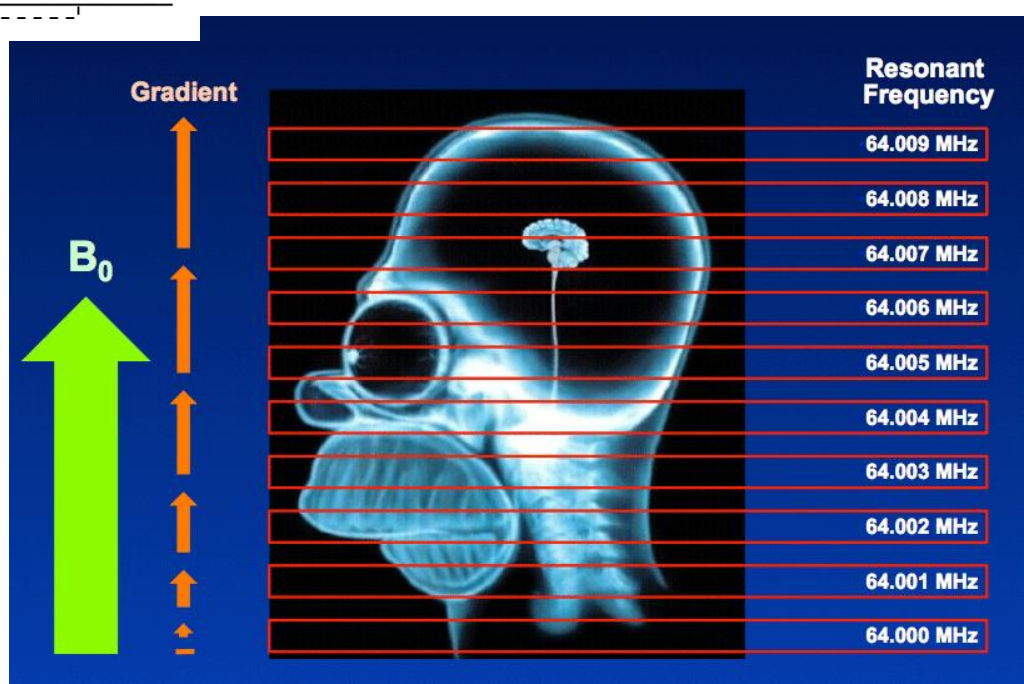
Intenzita signálu na dané frekvenci odpovídá počtu jader na dané pozici

ZOBRAZOVÁNÍ

Pomocí dalších cívek zajistíme, aby se magnetické pole B_0 měnilo lineárně v závislosti na poloze

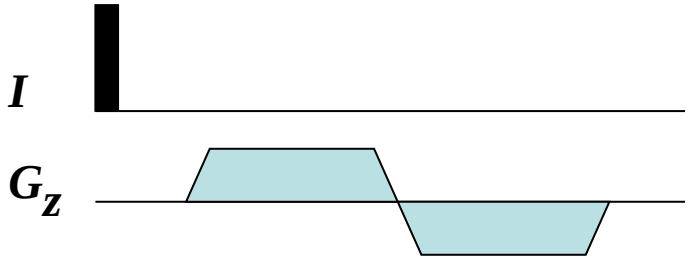
Gradient lze zapínat a vypínat

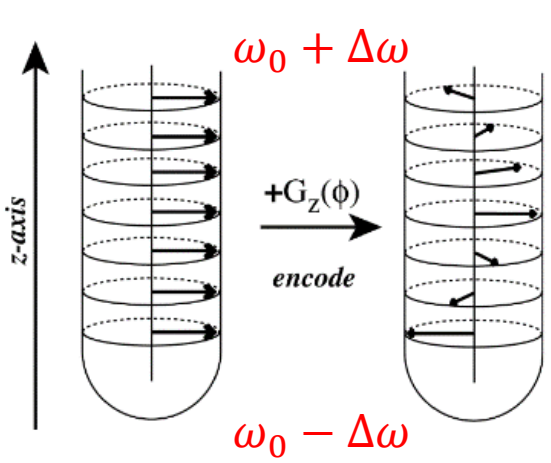
Gradientní pulsy



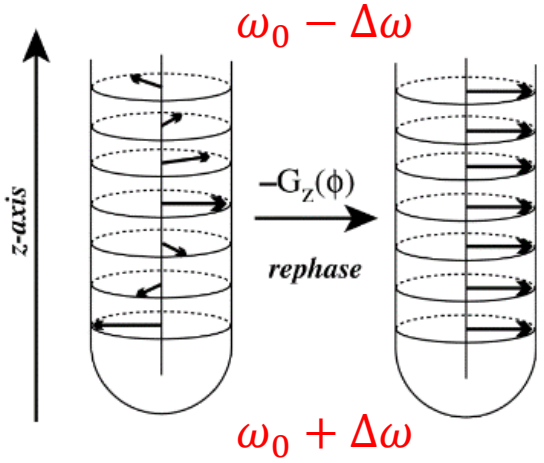
Využití gradientů v NMR

Gradientní echo





Změna polarity gradientu



dephasing

NMR signál (koherence) efektivně potlačen

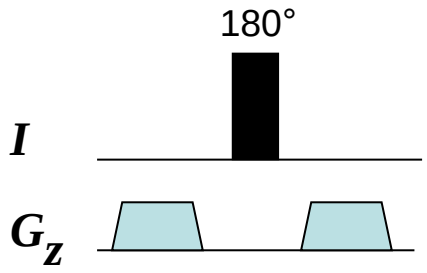
re-phasing

NMR signál (koherence) opět viditelný

ECHO

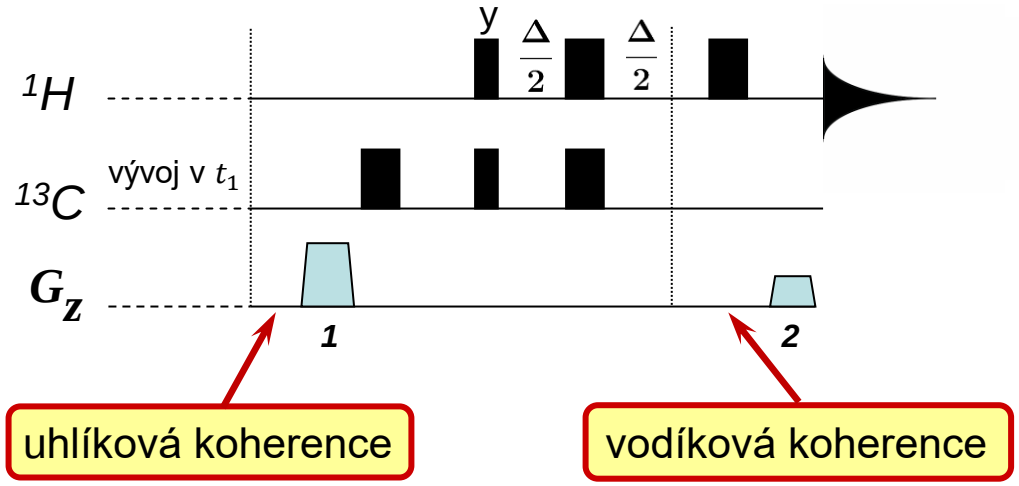
Využití gradientů v NMR

Potlačení nedokonalostí pulsu



- Změnu polarity zajišťuje 180° puls
- Při nedokonalém pulsu nedojde ke kompletní inverzi
- Gradienty zajistí, že se refokusuje jen invertovaná složka, ostatní se nesfázují a jsou tak potlačeny

Kontrola přenosu koherencí v HSQC experimentu



$\Omega_C + \gamma_C \Delta B(1)$

$\Omega_H + \gamma_H \Delta B(2)$

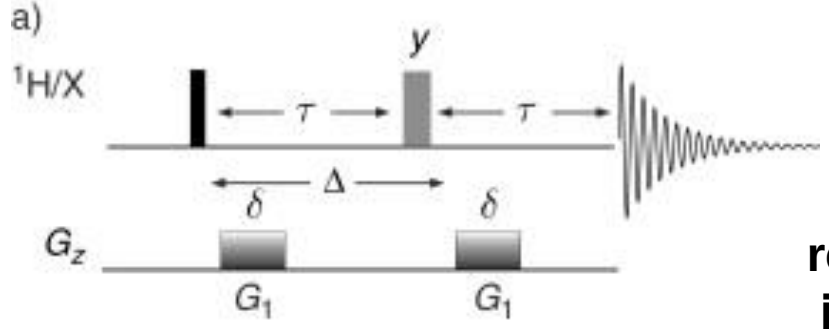
Pro účinný přenos musí platit

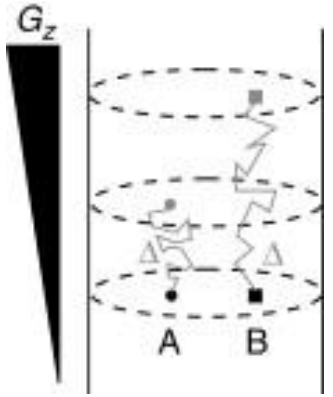
$$\frac{G_{z1}\tau_1}{G_{z2}\tau_2} = \frac{\gamma_H}{\gamma_C}$$

Vše ostatní je potlačeno

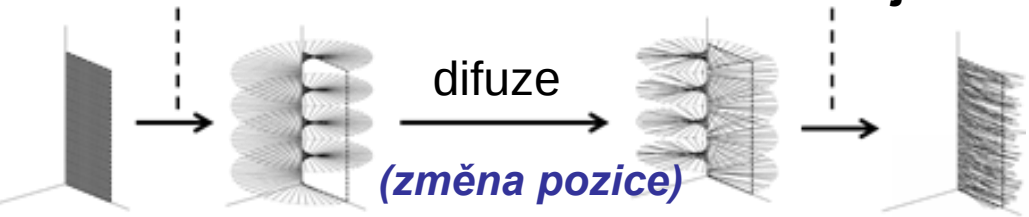
De-/re-phasing ovlivněn gyromagnetickým poměrem

Měření translační difuze

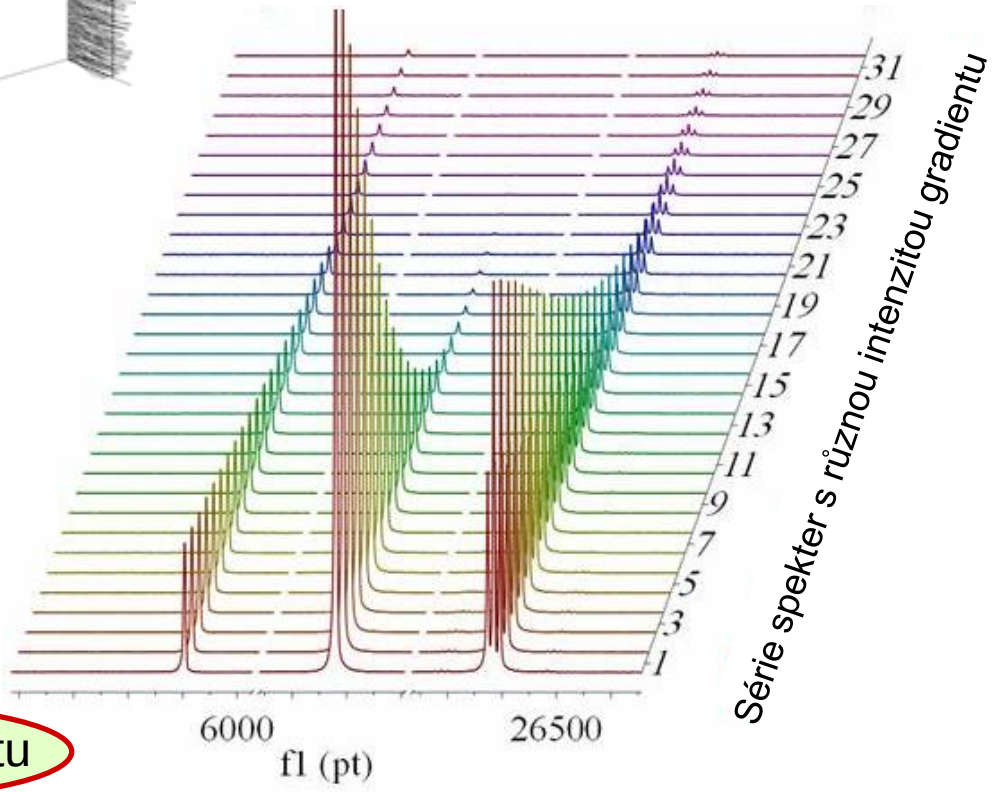




refokusováno jen částečně



bez difuze plně refokusováno



Stejskal-Tannor

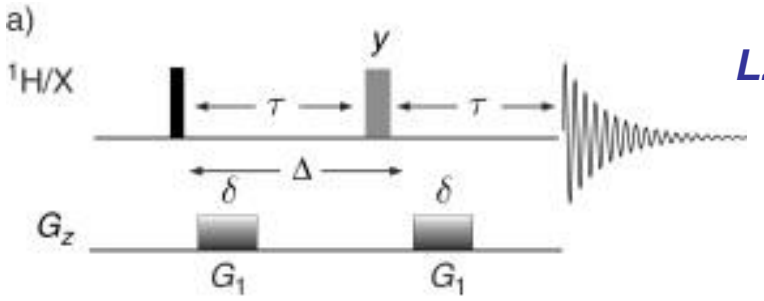
$$I(g) = I_0 \exp\left\{-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right\}$$

difůzní koeficient

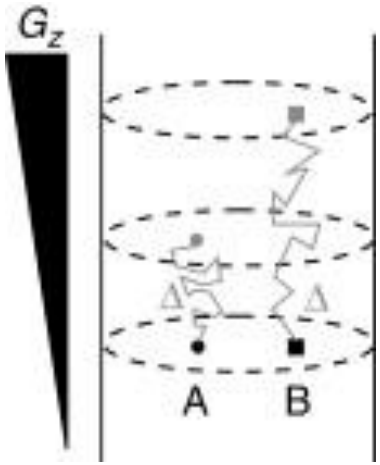
Intenzita gradientu

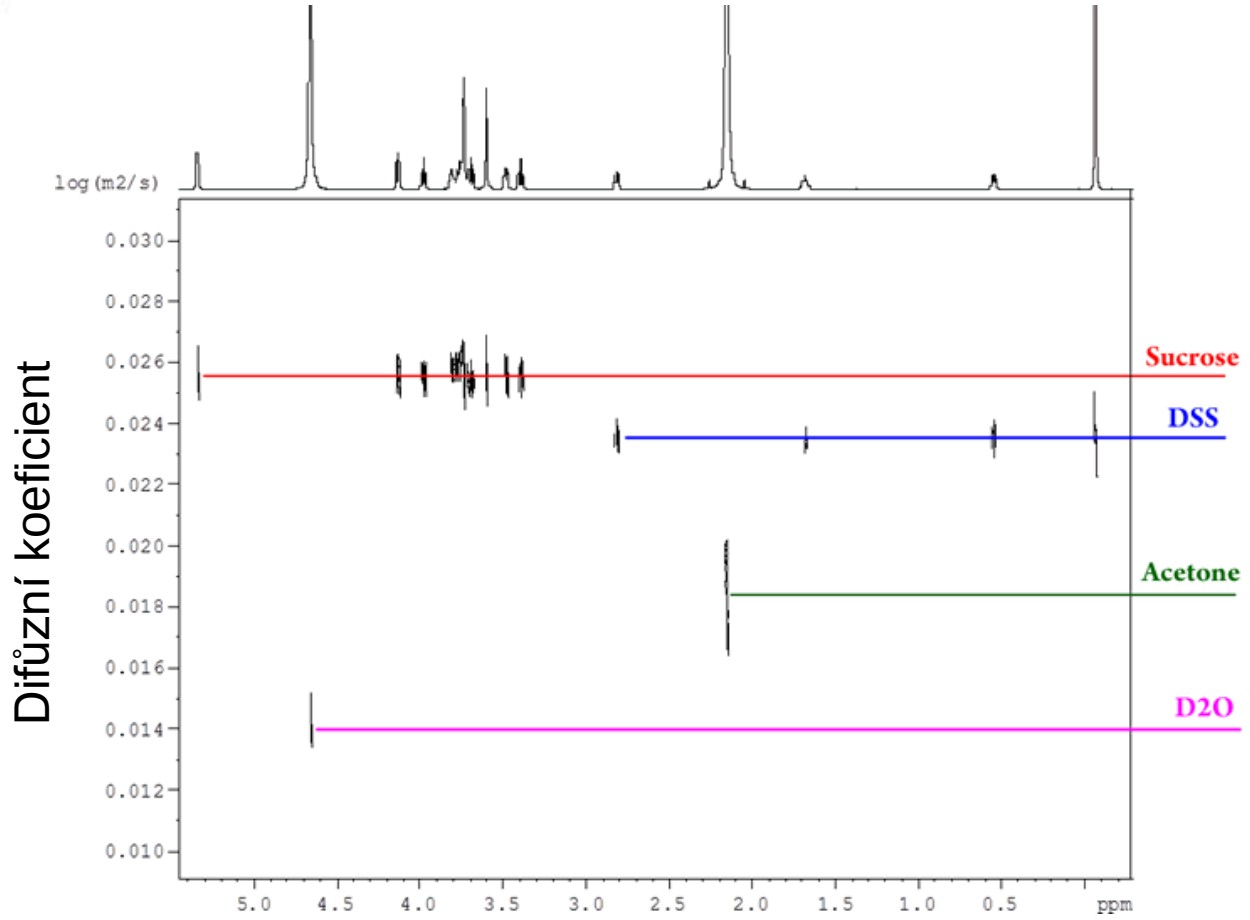
Translační difuze - DOSY

Diffusion Ordered SpectroscopY



Lze rozlišit jednotlivé složky směsi dle velikosti molekul



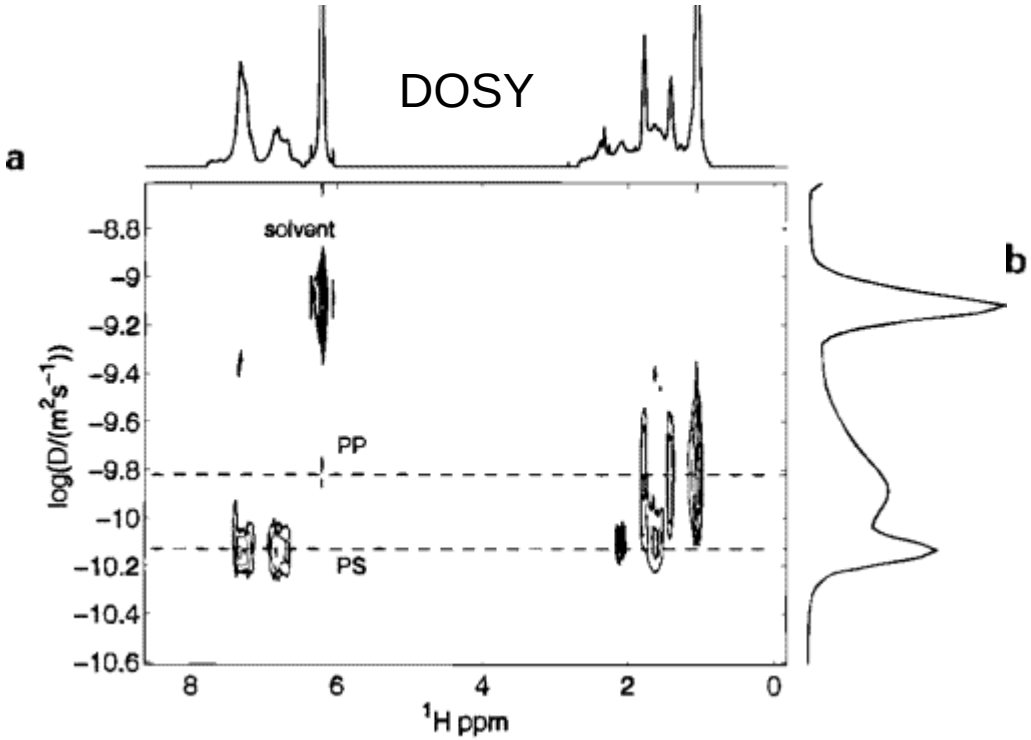


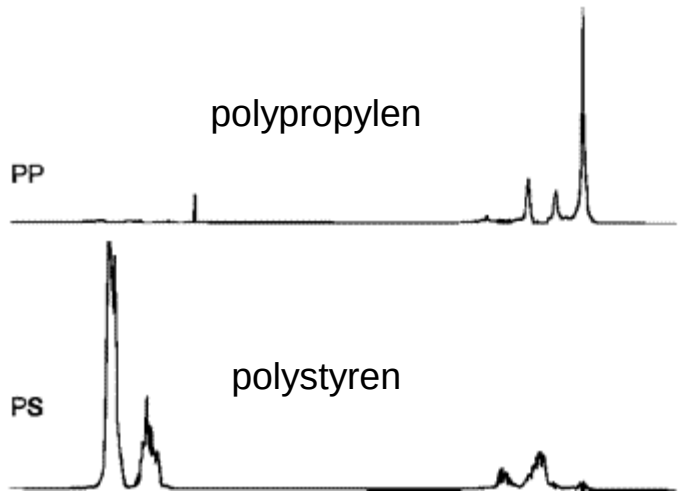
Translační difuze - informace

$$D = \frac{k_B T}{f} \qquad f = 6\pi\eta r_S \qquad V = \frac{4\pi}{3} r^3 \qquad D \propto \frac{1}{\sqrt[3]{M}}$$

viskozita hydrodynamický poloměr, objem molekulová hmotnost

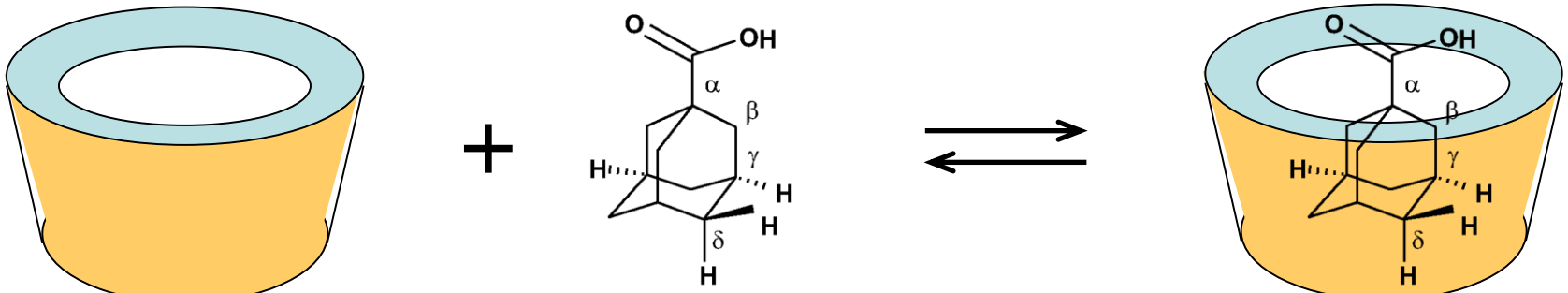
Komplexy, agregace, tvorba micell, solvatace, polydispersita polymerů

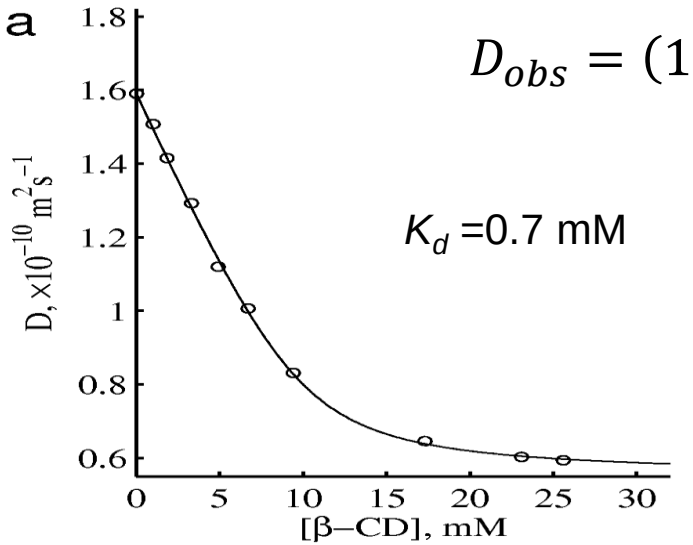




Translační difuze a výměna

Tvorba komplexu





$$D_{obs} = (1-p)\ D_{Free} + p\ D_{Bound}$$

K_d =0.7 mM

titrace

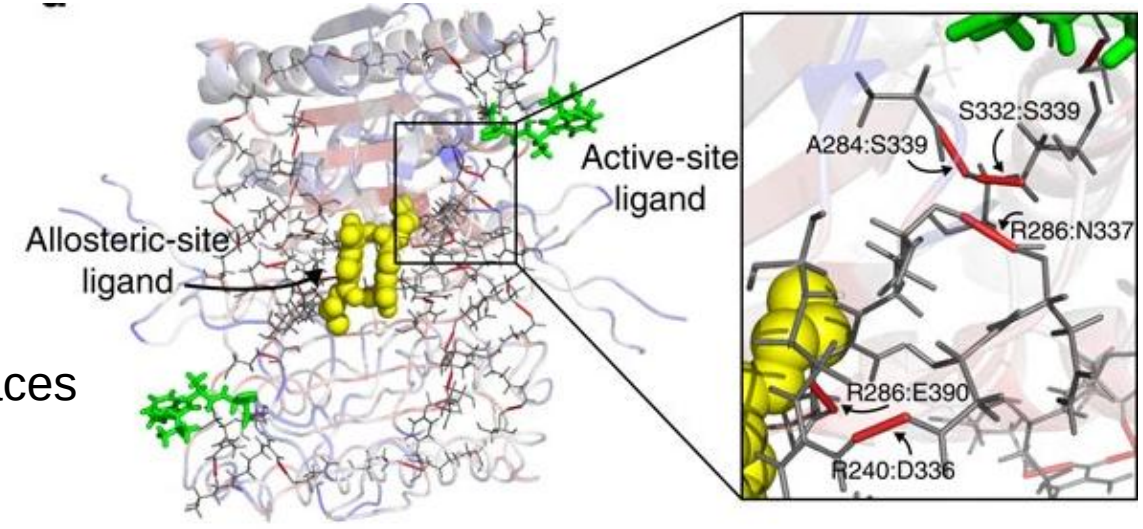
NMR of biomolecules

- highly selective response, can resolve individual atoms
- broad range of physicochemical characteristics
- molecules in solution – closer to physiological state
- insoluble structures, amyloids, membrane proteins – solid-state NMR

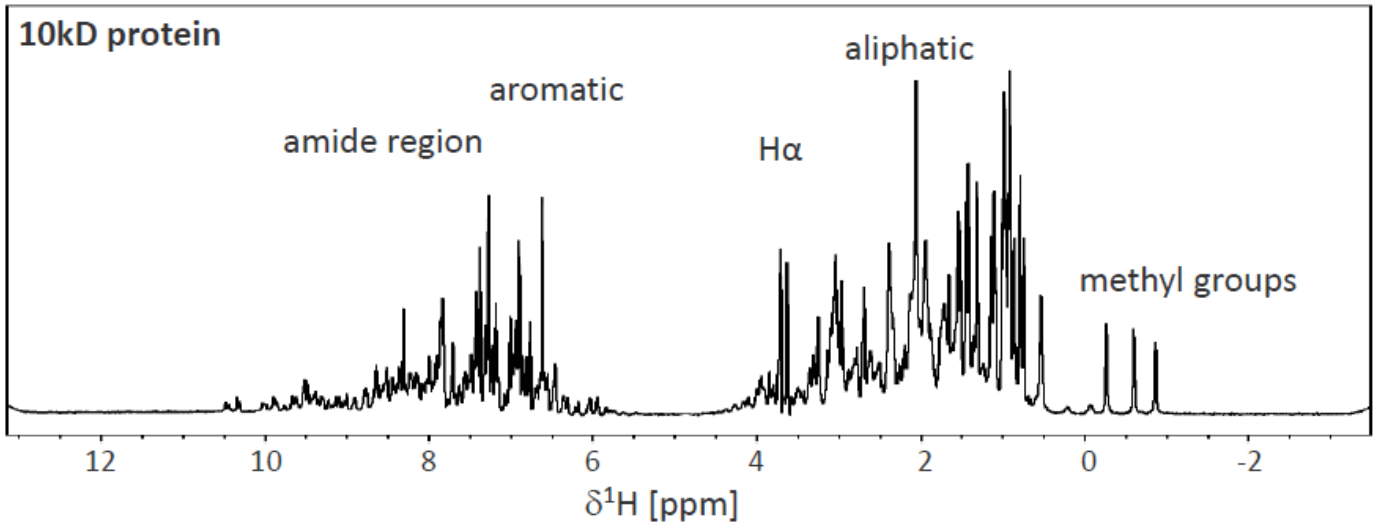
peptides and proteins ***nucleic acids*** ***oligosaccharides***

Available information

- 3D structure
- dynamics
- substrate identification
- complexation
- interaction surfaces / interfaces
- chemistry of active site
- protein folding process
- ...



Protein NMR



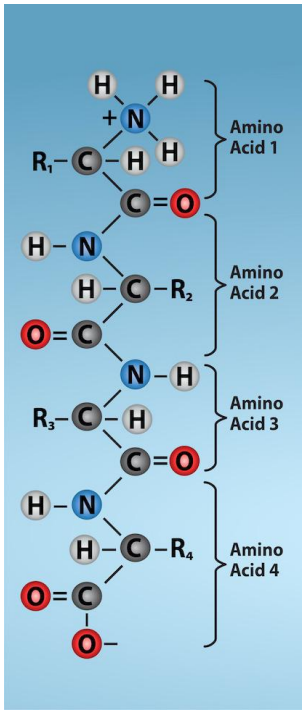
atoms in proteins	stable Isotope composition (natural abundance)
Hydrogen (H)	**^{1}H (99.9885%)** ^{2}H (0.0115%)
Carbon (C)	^{12}C (98.93%) **^{13}C (1.07%)**
Nitrogen (N)	^{14}N (99.632%) **^{15}N (0.368%)**

Spectral crowding resolved in multidimensional spectra

^{13}C/^{15}N (^{13}C/^{15}N/^{2}H) labelled samples

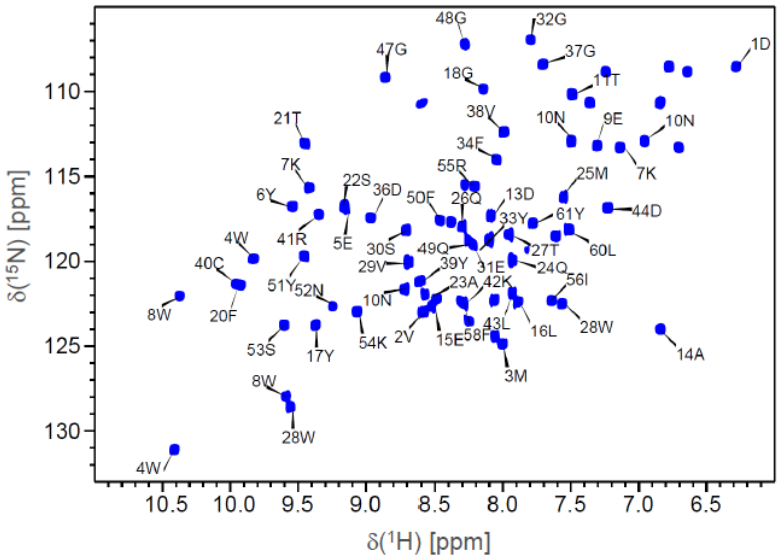
Protein expression in bacteria cells

- minimal media ($^{15}NH_4Cl$, $^{15}NH_4SO_4$ – solely nitrogen source, ^{13}C-glukose, ^{13}C-glycerol – solely carbon source)

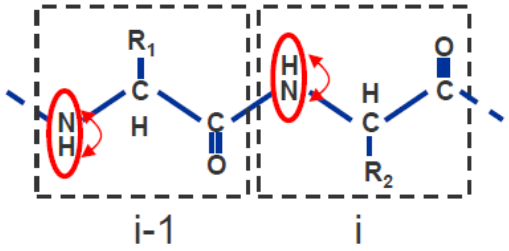


Multidimensional approaches

^{1}H-^{15}N 2D HSQC spectrum = protein fingerprint

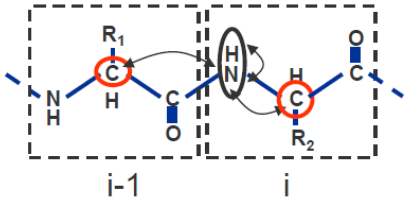


Each amino acid represented with one peak



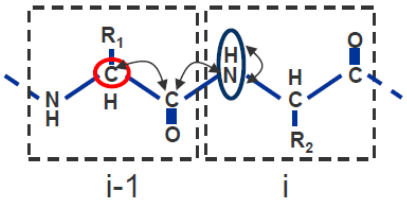
HNCA

signal for Cα of NH(i) and NH(i-1)

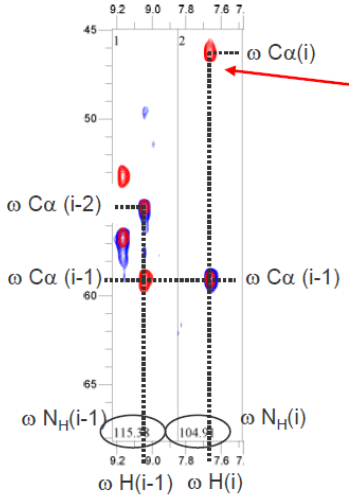


HN(CO)CA

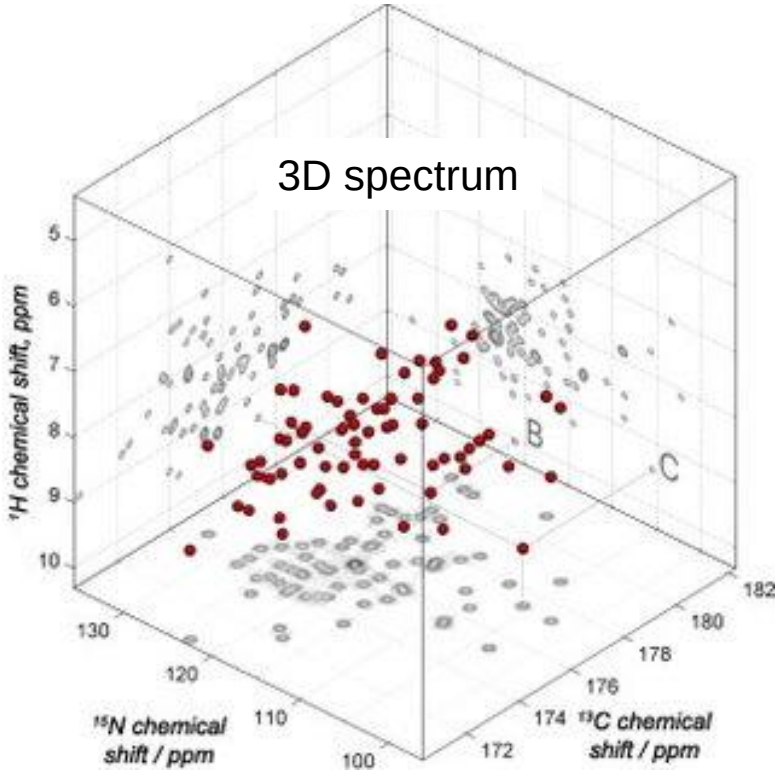
signal for Cα of NH(i-1)



HNCA / HN(CO)CA



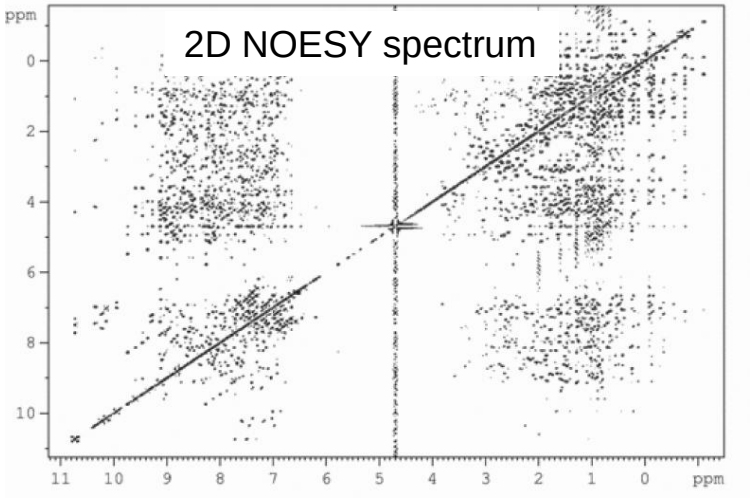
3D spectrum



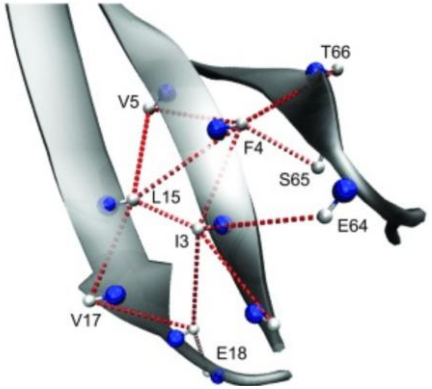
Structural information

Interpreting NOESY crosspeaks

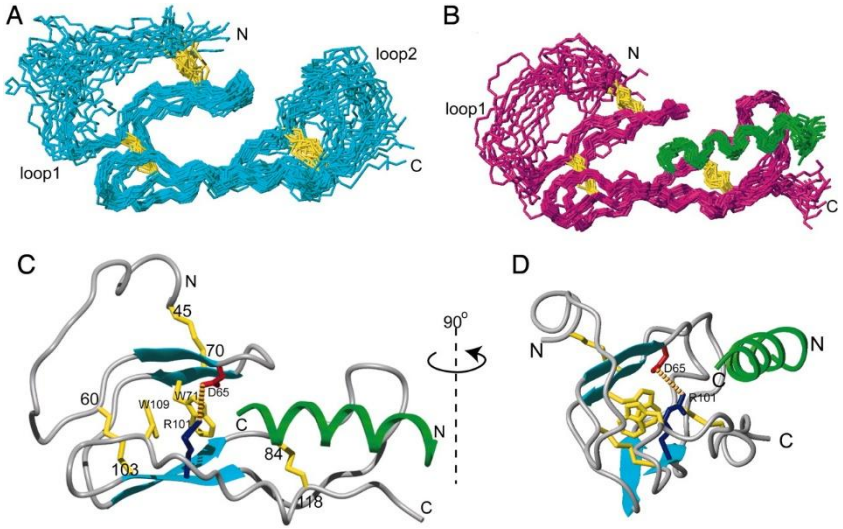
Crosspeak volume ⟷ distance



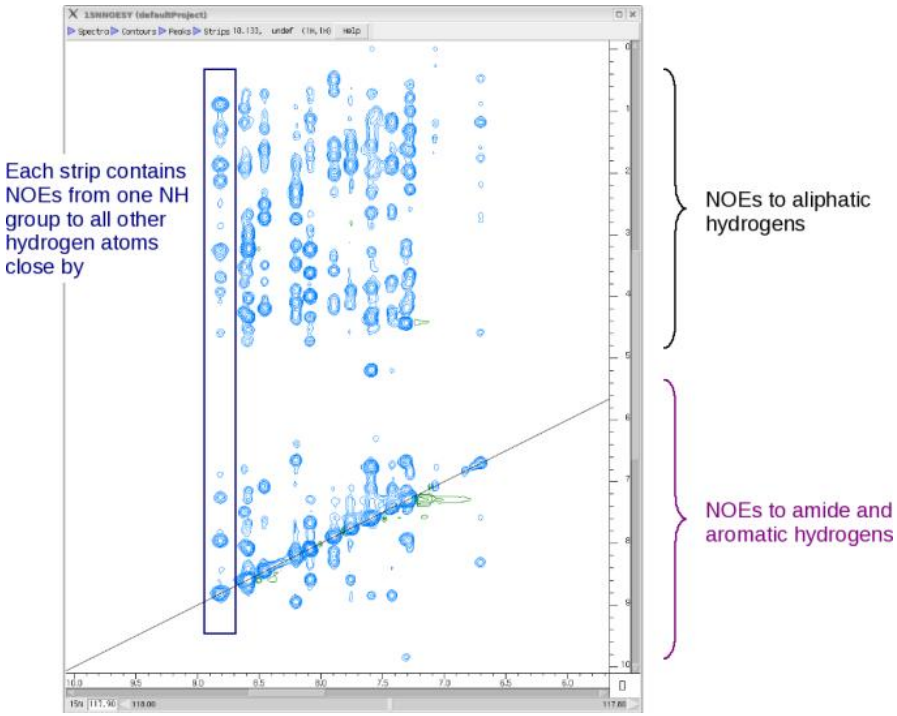
- *Thousands of restraints*
- *Minimization procedure*

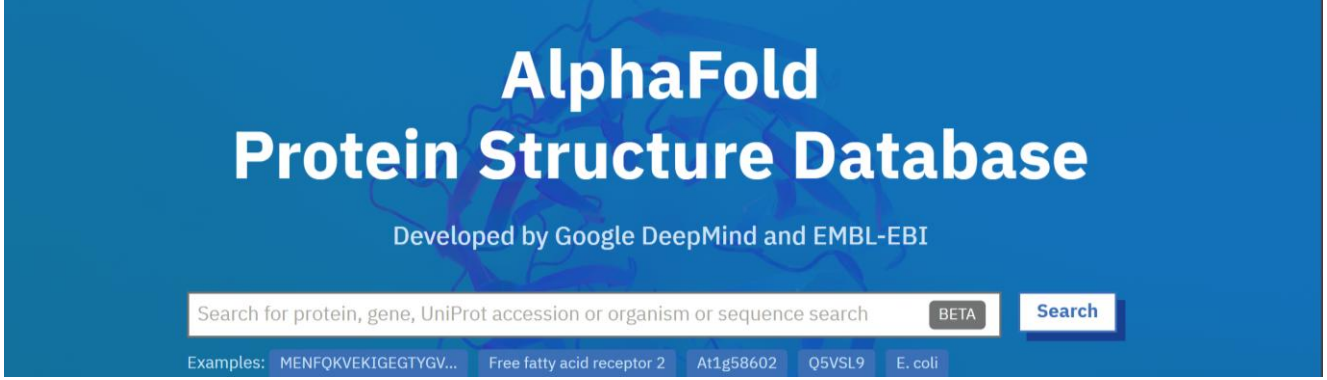


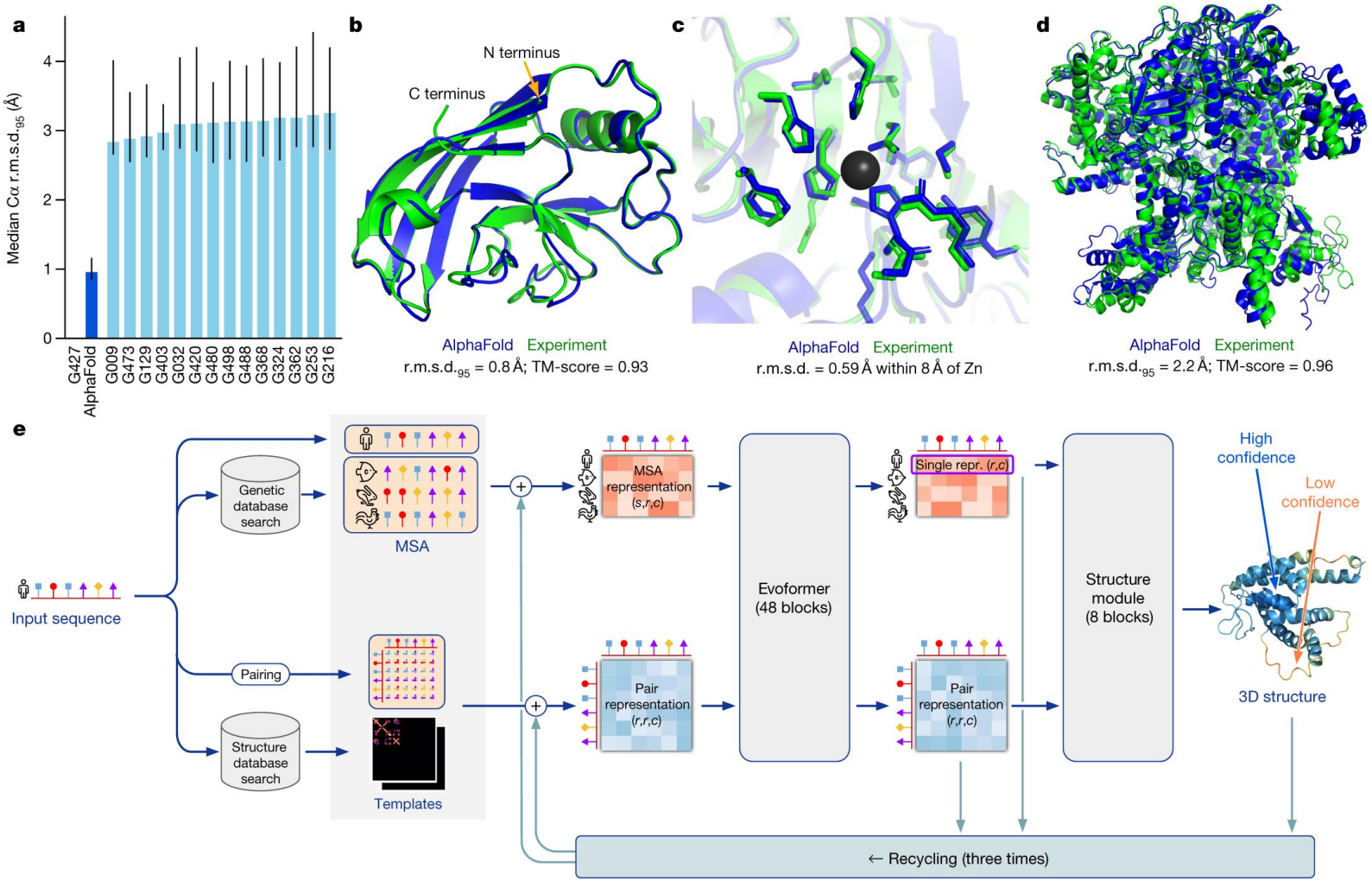
Ensamble of structures



3D NOESY-HSQC spectrum



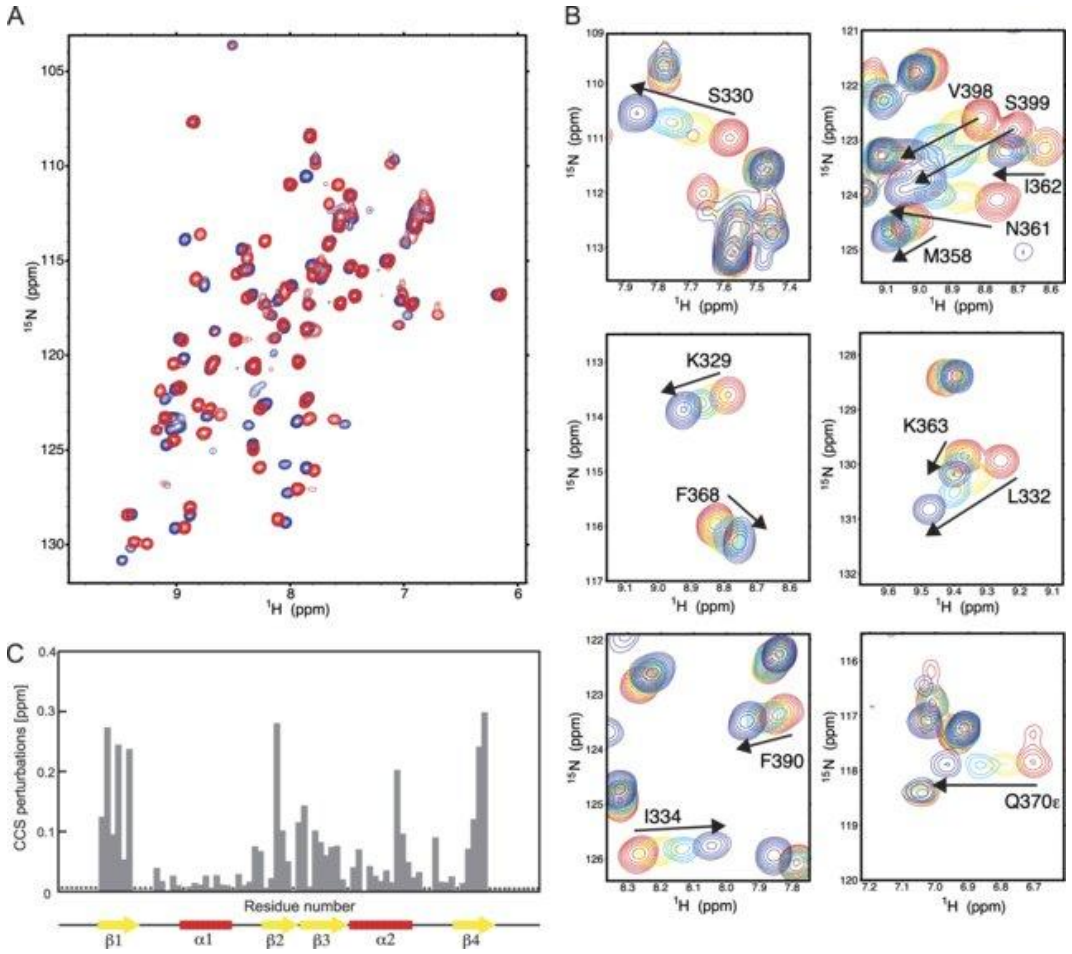

AlphaFold
Protein Structure Database
Developed by Google DeepMind and EMBL-EBI
Search for protein, gene, UniProt accession or organism or sequence search
BETA
Search
Examples: MENFQKVEKIGEGTYGV... Free fatty acid receptor 2 At1g58602 Q5VSL9 E. coli

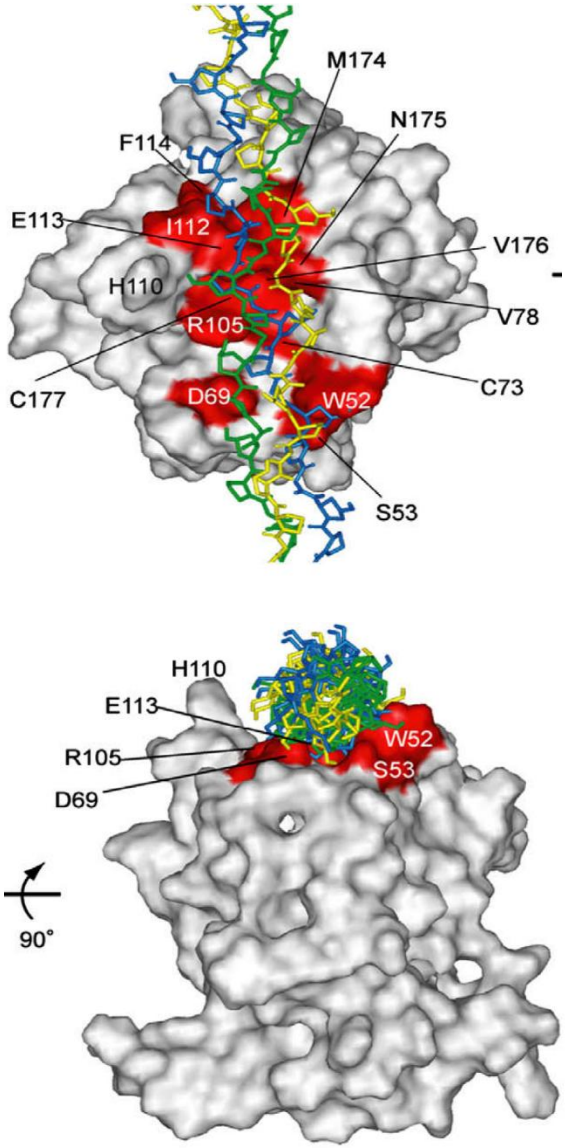

a
Median Cα r.m.s.d.95 (Å)
0
1
2
3
4
G427
AlphaFold
G009
G473
G129
G403
G032
G420
G480
G498
G488
G368
G324
G362
G253
G216
b
N terminus
C terminus
AlphaFold Experiment
r.m.s.d.95 = 0.8 Å; TM-score = 0.93
c
AlphaFold Experiment
r.m.s.d. = 0.59 Å within 8 Å of Zn
d
AlphaFold Experiment
r.m.s.d.95 = 2.2 Å; TM-score = 0.96
e
Input sequence
Genetic database search
MSA
Pairing
Structure database search
Templates
MSA representation (s,r,c)
Pair representation (r,r,c)
Evoformer (48 blocks)
Single repr. (r,c)
Pair representation (r,r,c)
Structure module (8 blocks)
High confidence
Low confidence
3D structure
← Recycling (three times)

Monitoring protein interactions

Chemical shift mapping during titration

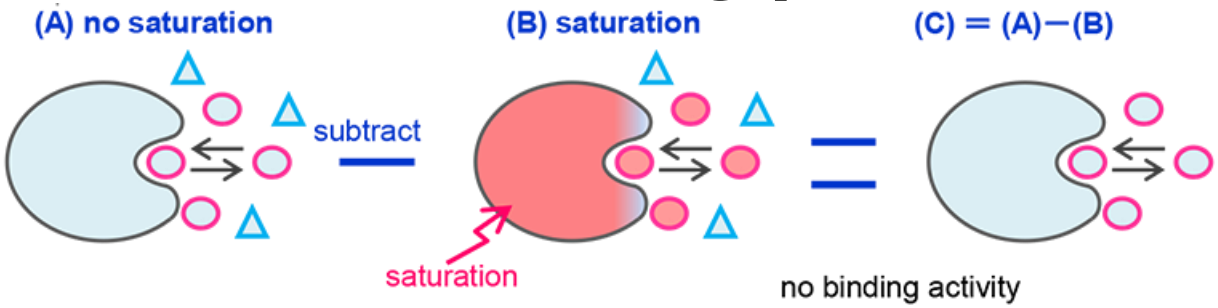
^{1}H-^{15}N HSQC





Fast Exchange between free and bound states averages chemical shifts

Monitoring protein interactions



STD: saturation transfer difference experiment for ligand screening

Drug discovery

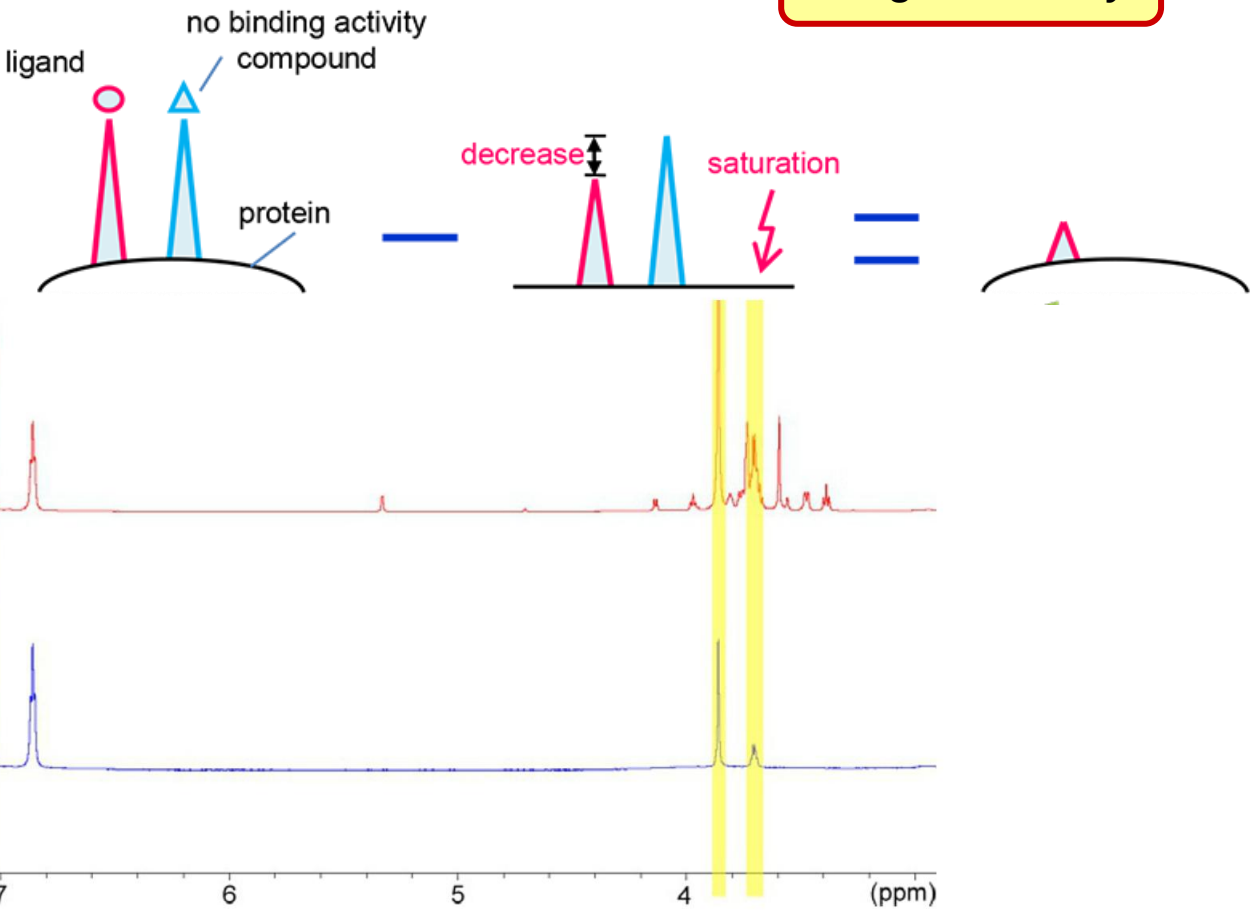


Fig. 7 (A) Reference spectrum of a sample containing 22 μ*M* HSA and a cocktail of (6 m*M* each): Naproxen, ANS, and sucrose. Peaks highlighted in *yellow* belong to Naproxen. Remaining peaks above 6 ppm belong to ANS, and remaining peaks below 6 ppm belong to sucrose. (B) STD NMR difference spectrum of the same sample, showing the presence of Naproxen and ANS signals. This shows that Naproxen and ANS are ligands for HSA, whereas sucrose is not.

Introduction to solid-state NMR

Samples

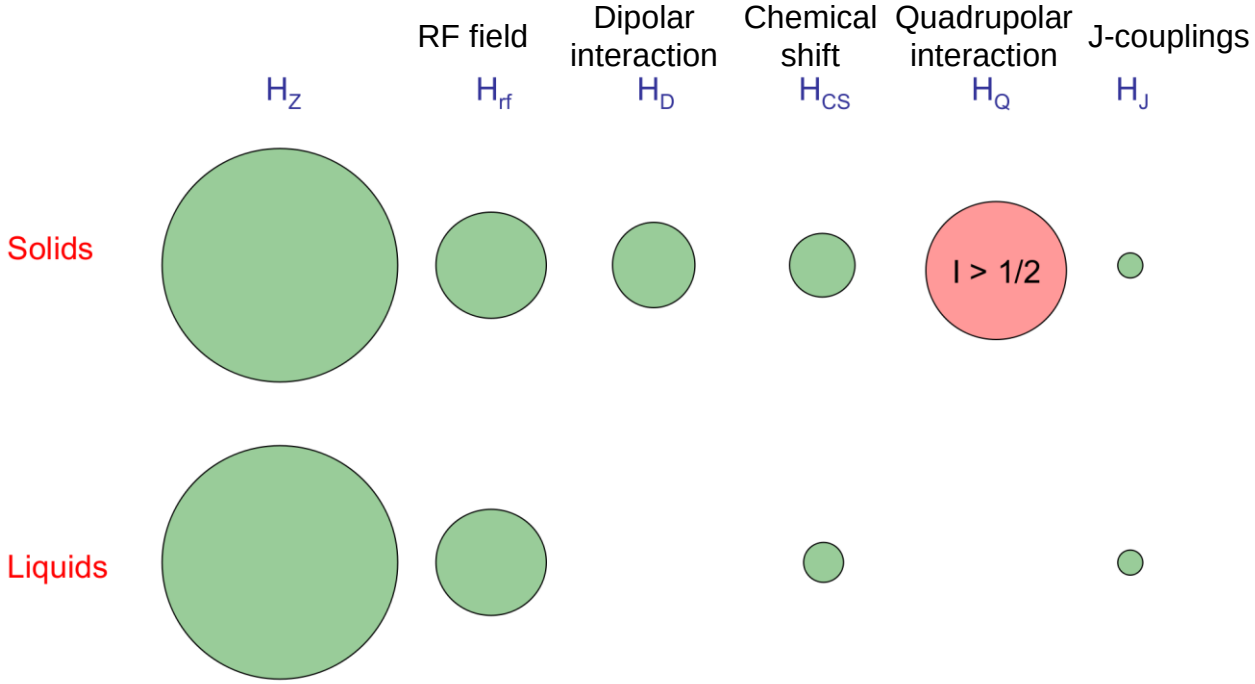
crystalline solids, polymers, amorphous materials, glasses, functional and porous materials (zeolites), insoluble proteins, large molecular assemblies,….

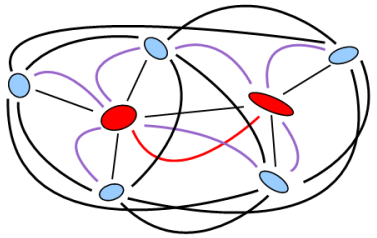
Methods

Magic angle spinning, cross-polarization, decoupling and recoupling methods, multiple-quantum correlations, wide-line excitations, magnetization transfers, multidimensional …

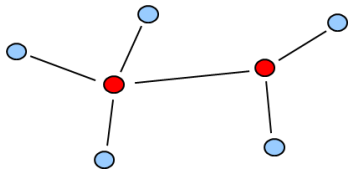
Information

Molecular conformation, non-covalent interactions, oxidation state or chemical reactivity, morphology of porous solids, properties of catalytic centers, dynamics in confinement, NMR crystalography, interpretation of electronic environment using DFT calculations, …



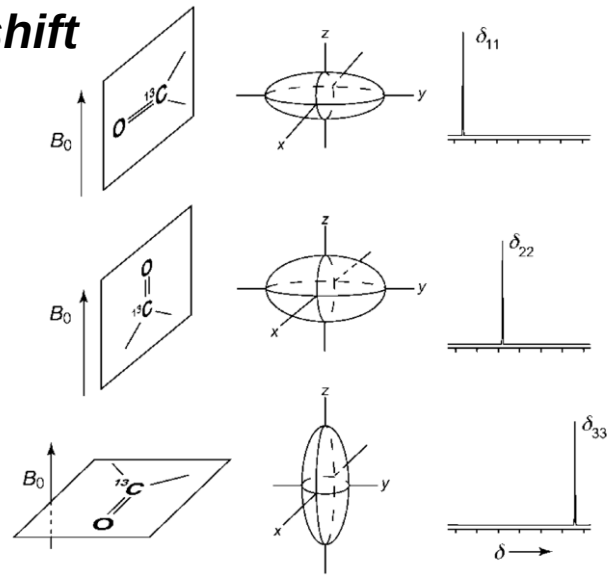


Dense network of anisotropic interactions

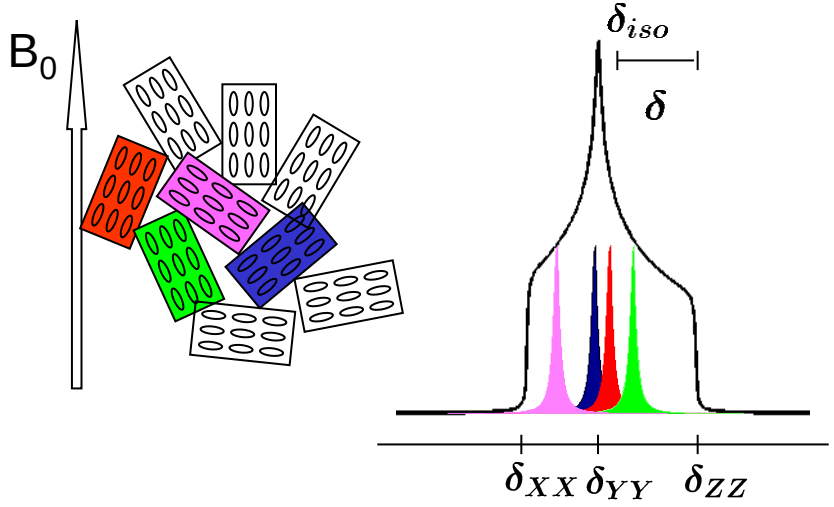


Anisotropy of interactions in solids

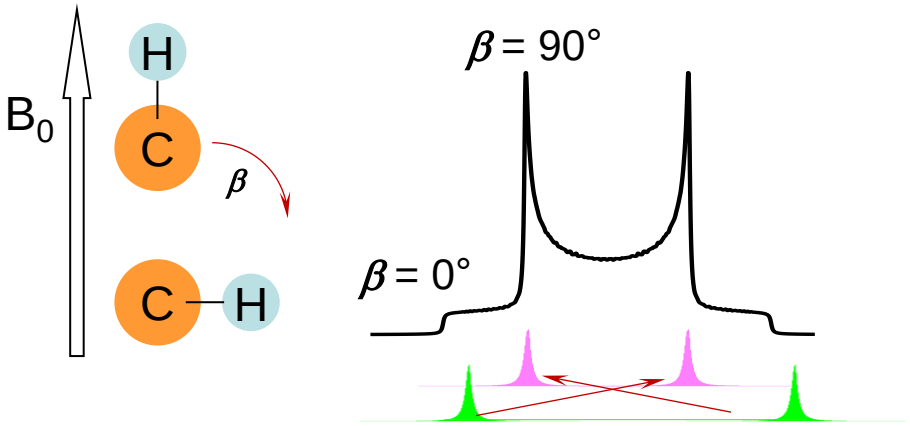
Chemical shift



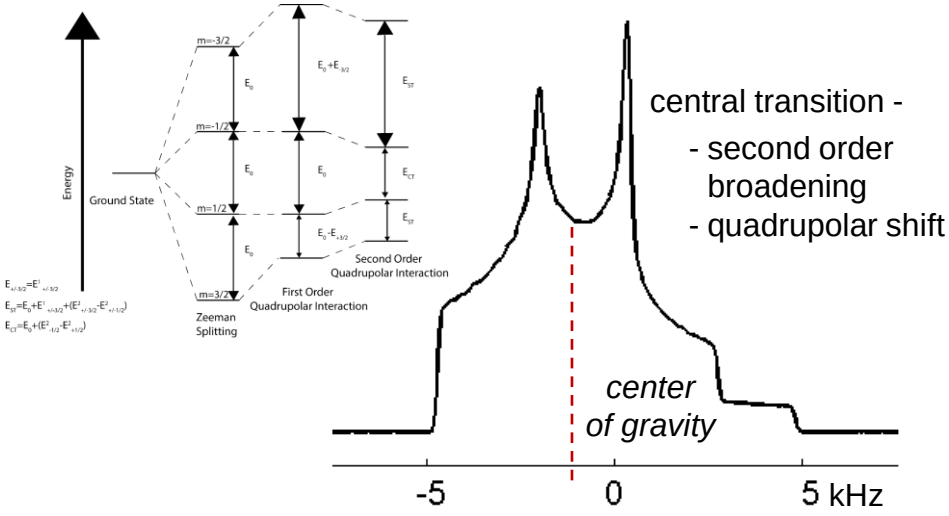
Powder sample



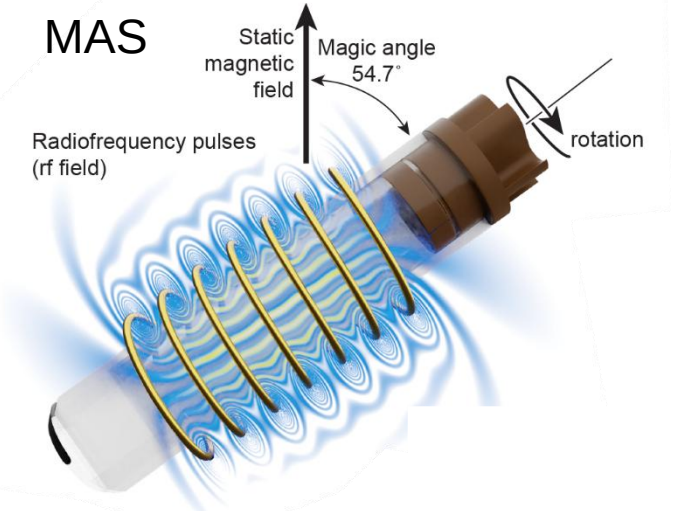
Dipolar interactions



Quadrupolar interactions



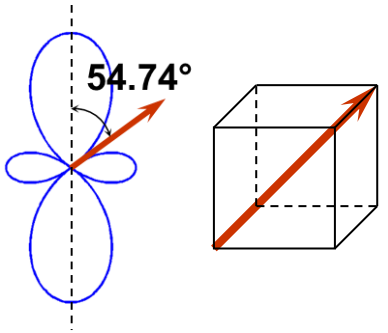
Recovering resolution – Magic Angle Spinning

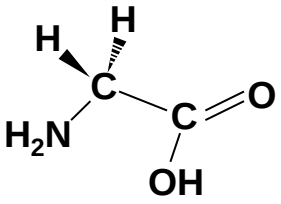


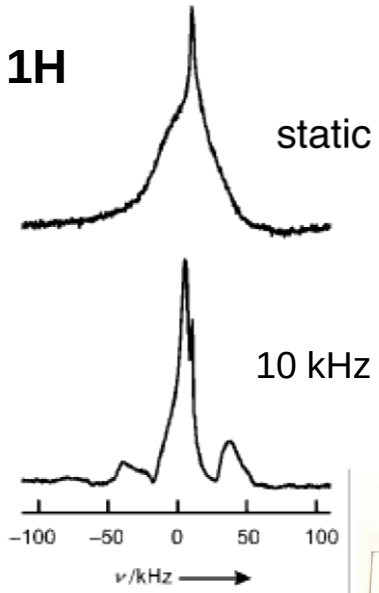
$$H_{int} = \text{spatial dependence} \cdot \text{spin operators}$$

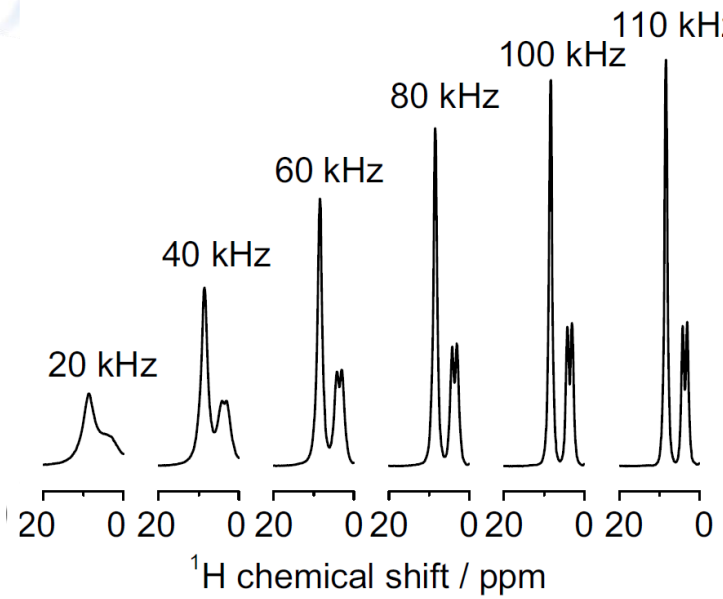
averaged to

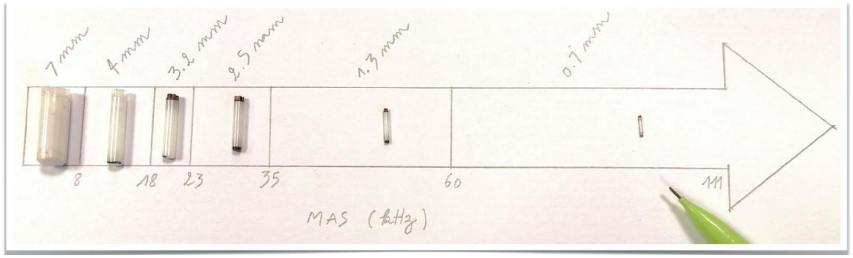
$$\frac{1}{2}\left(3\cos^2\beta_M - 1\right)$$

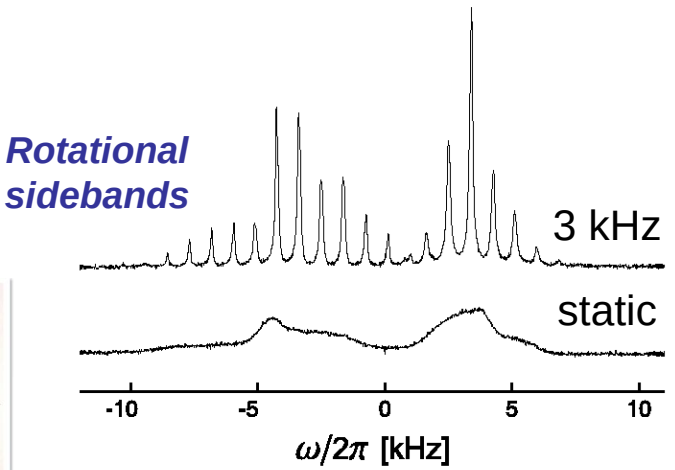




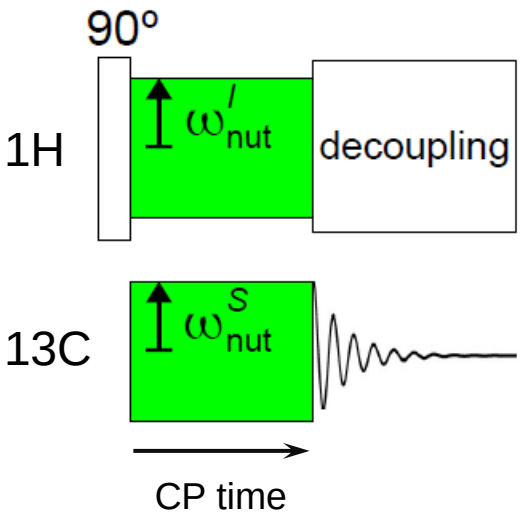




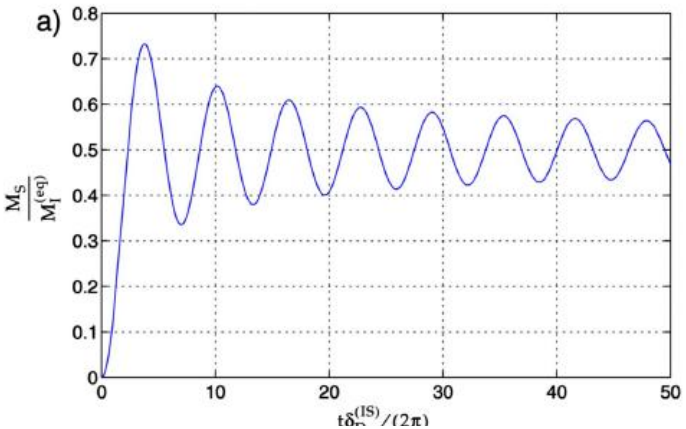




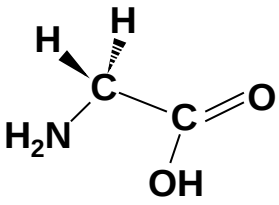
Enhancing signal – Cross-polarization

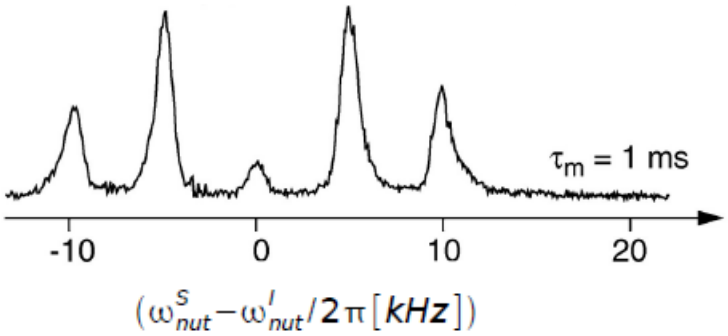


Polarization transfer from 1H to 13C mediated by direct dipole-dipole interaction



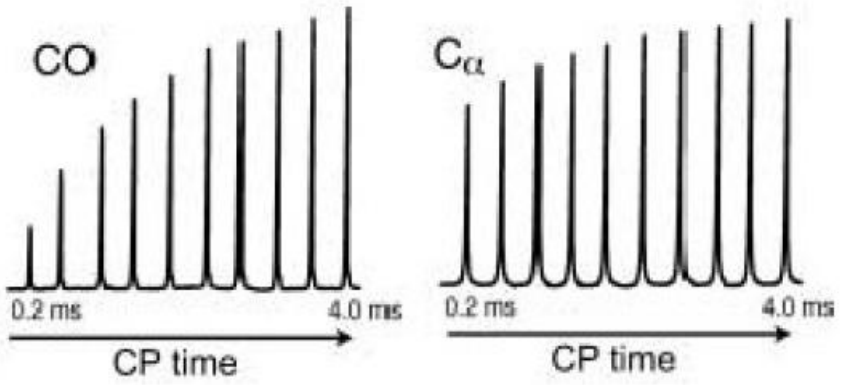
CP time





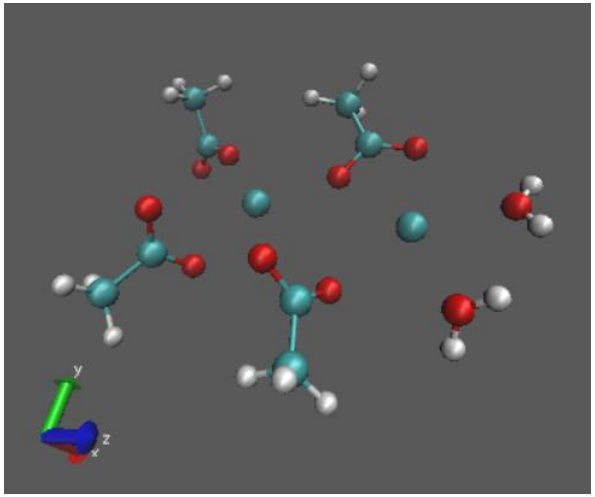
Hartmann-Hahn match

$$\omega_{nut}^{I} = \omega_{nut}^{S} \pm n\,\omega_r$$

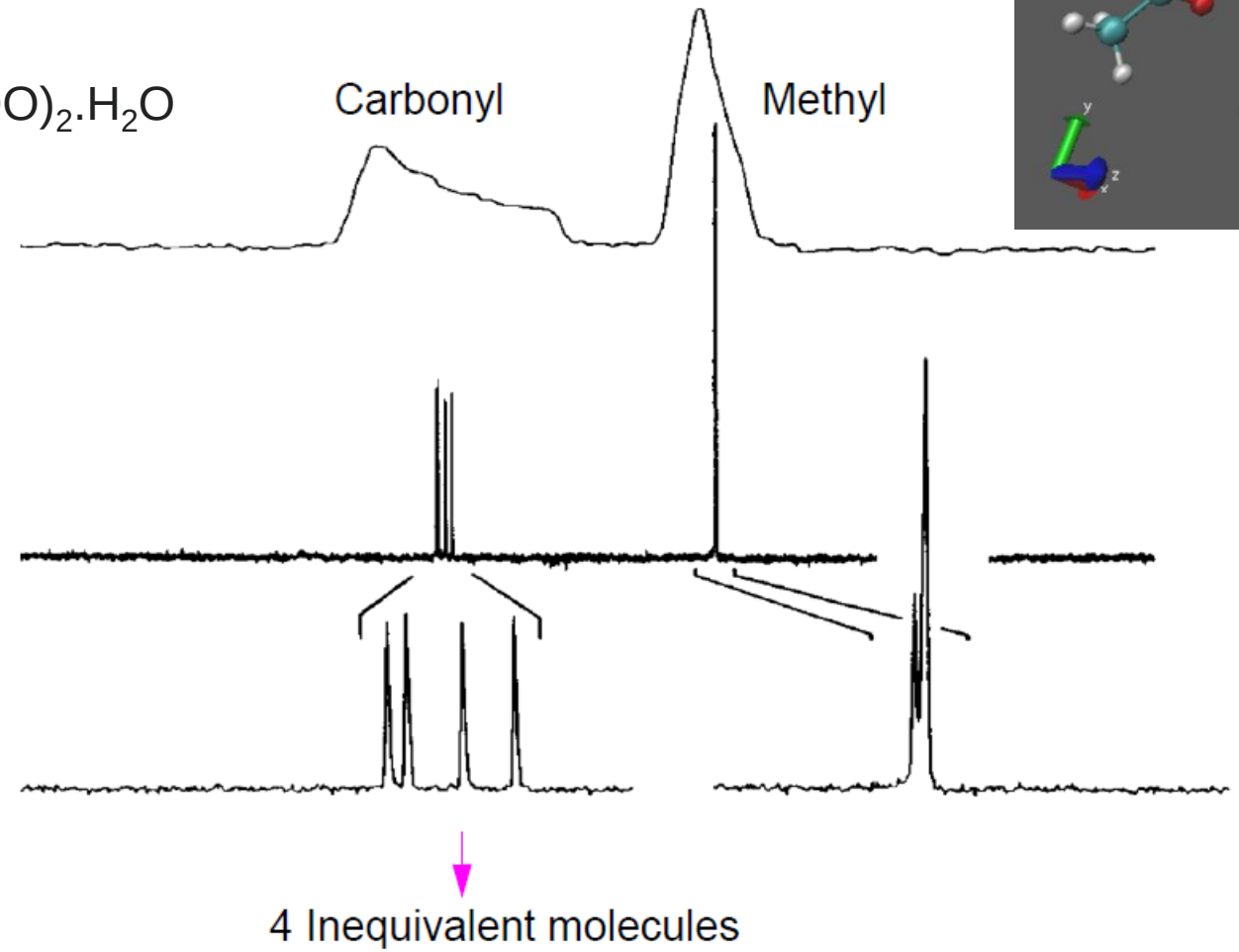


Buildup rate depends on dipolar coupling strength = distance

Non-equivalent positions in crystal



$(Ca(CH_3COO)_2.H_2O$

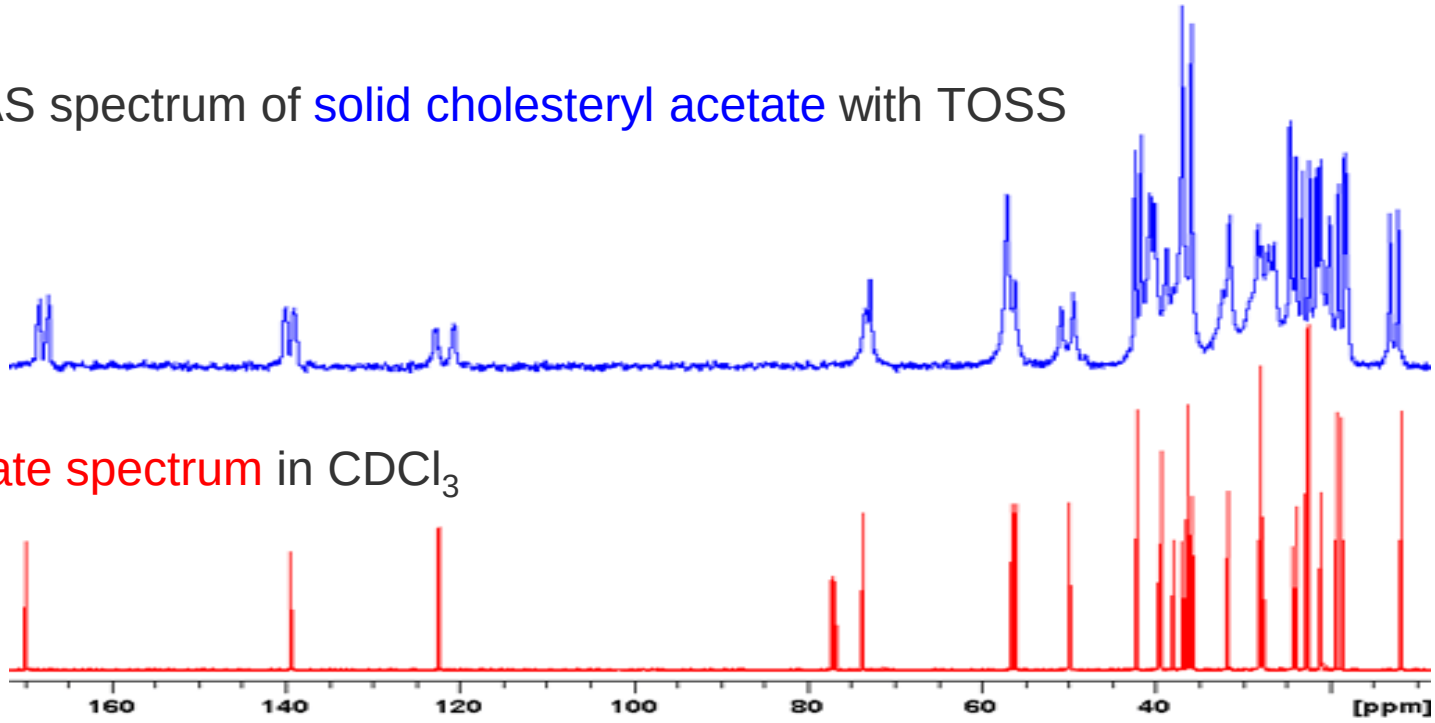


Přiřazení signálů a INADEQUATE

- přenesení ze spektra roztoku
- INADEQUATE
- další 2D SQ-DQ techniky (RFDR, C7, …)

^{13}C CP-MAS spectrum of solid cholesteryl acetate with TOSS

solution state spectrum in $CDCl_3$

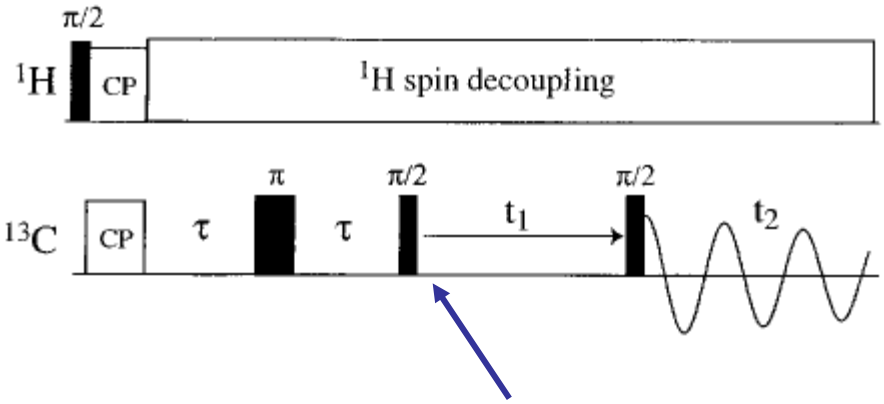


http://chem.ch.huji.ac.il/nmr/techniques/solid/solid.html

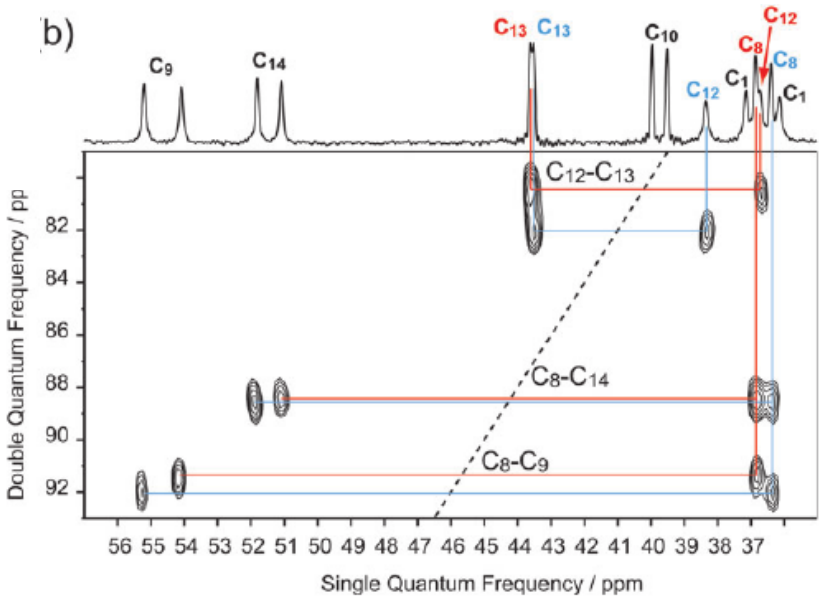
Přiřazení signálů a INADEQUATE

- přenesení ze spektra roztoku
- INADEQUATE
- další 2D SQ-DQ techniky (RFDR, C7, …)

Incredible Natural Abundance DoublE QUAntum Transfer Experiment

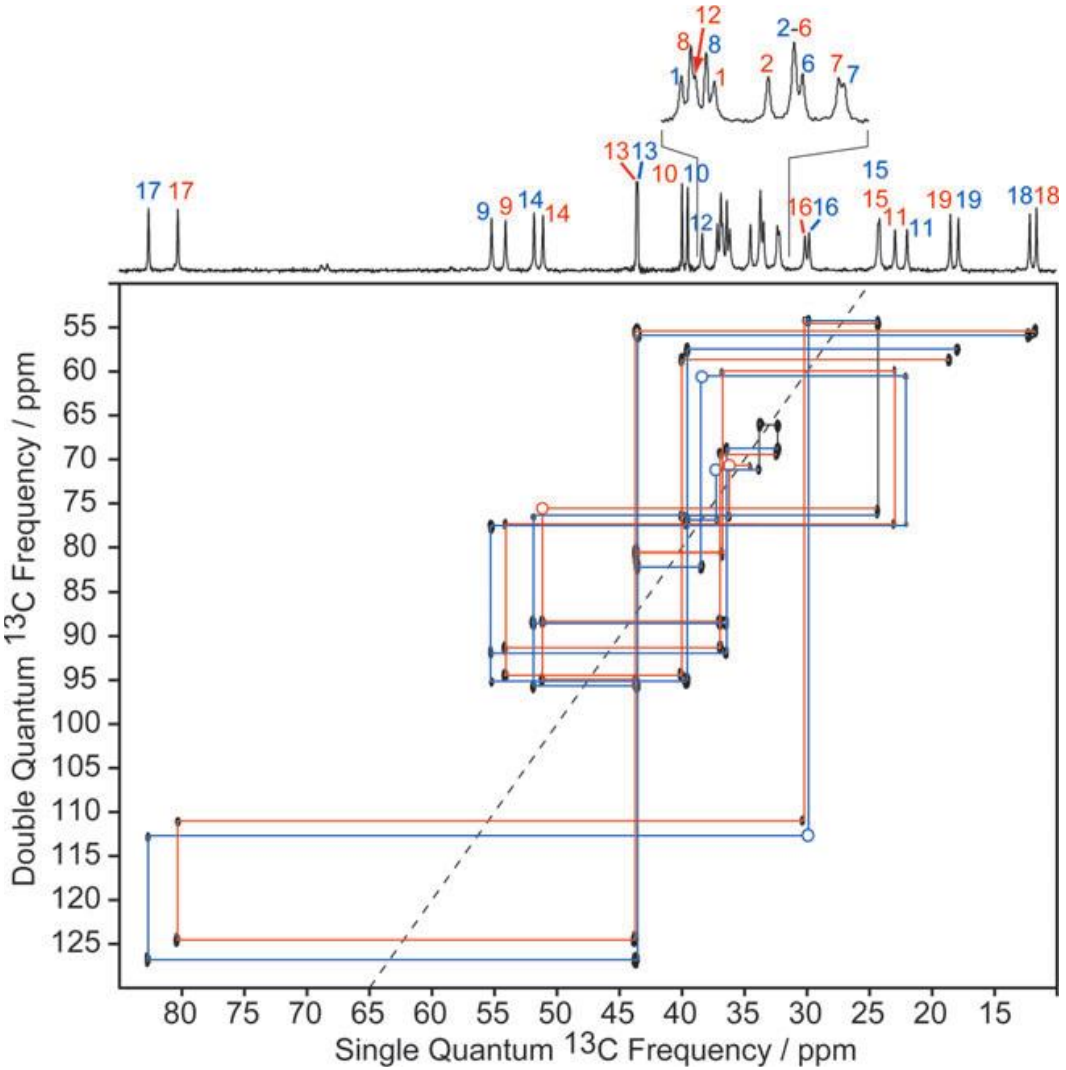


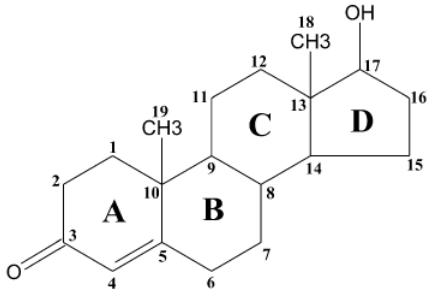
dvoukvantová koherence



vzniká mezi sousedními uhlíky s J vazbou nebo DD interakcí

Polymorfismus krystalů





(a) α-testosteron

červená

V

(b) β-testosteron

modrá

NMR krystalografie

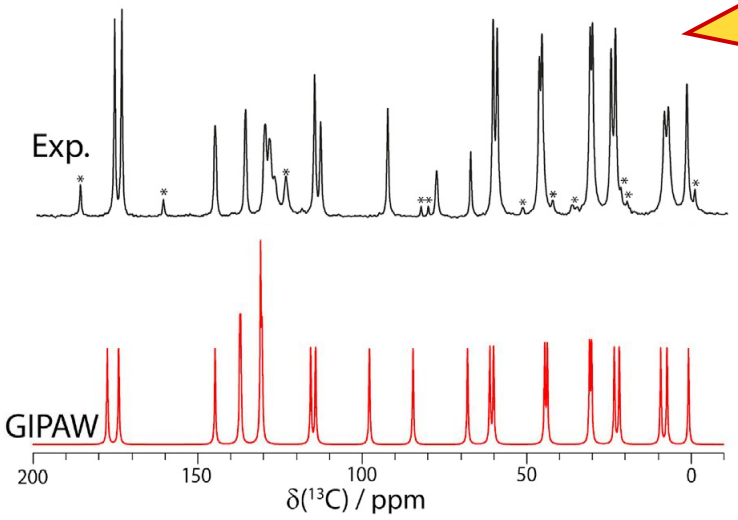
NMR data

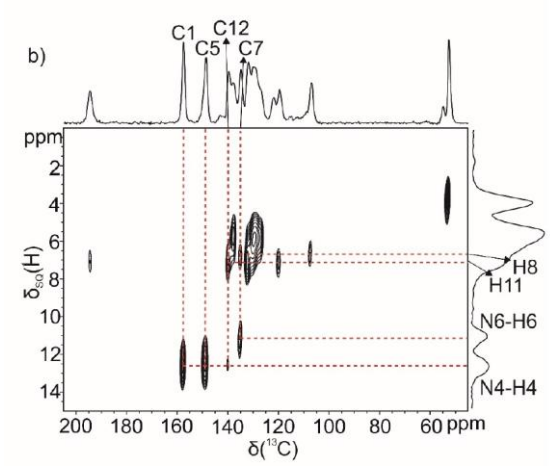
Generátor krystalových struktur

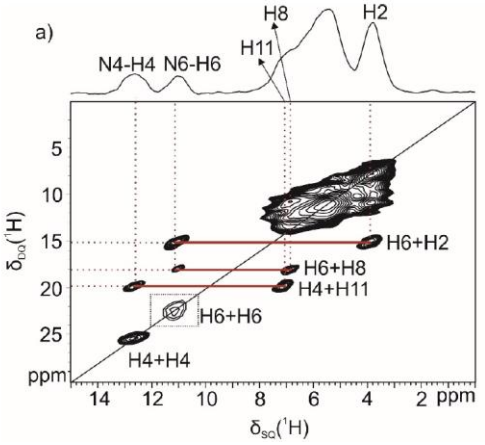
DFT GIPAW výpočet chemických posunů

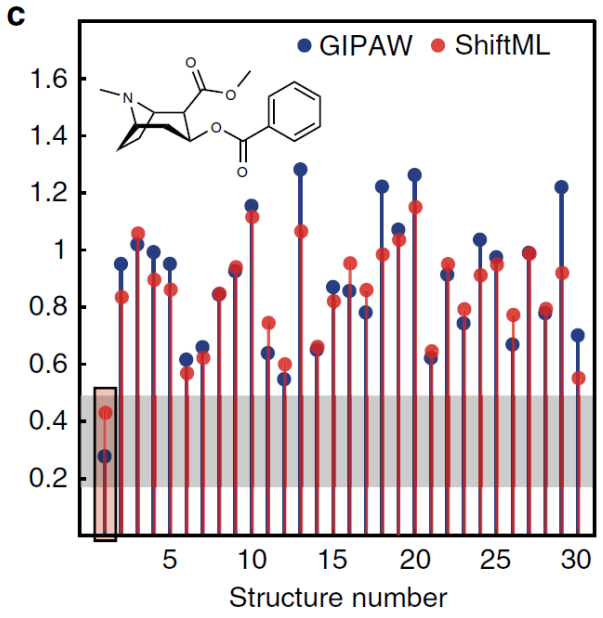
Porovnání s experimentem

$$RMSE_{\mathrm{NMR}} = \sqrt{\frac{1}{N}\sum_{i}^{N}\left(\delta_{calc,i} - \delta_{\mathrm{exp,i}}\right)^2}$$





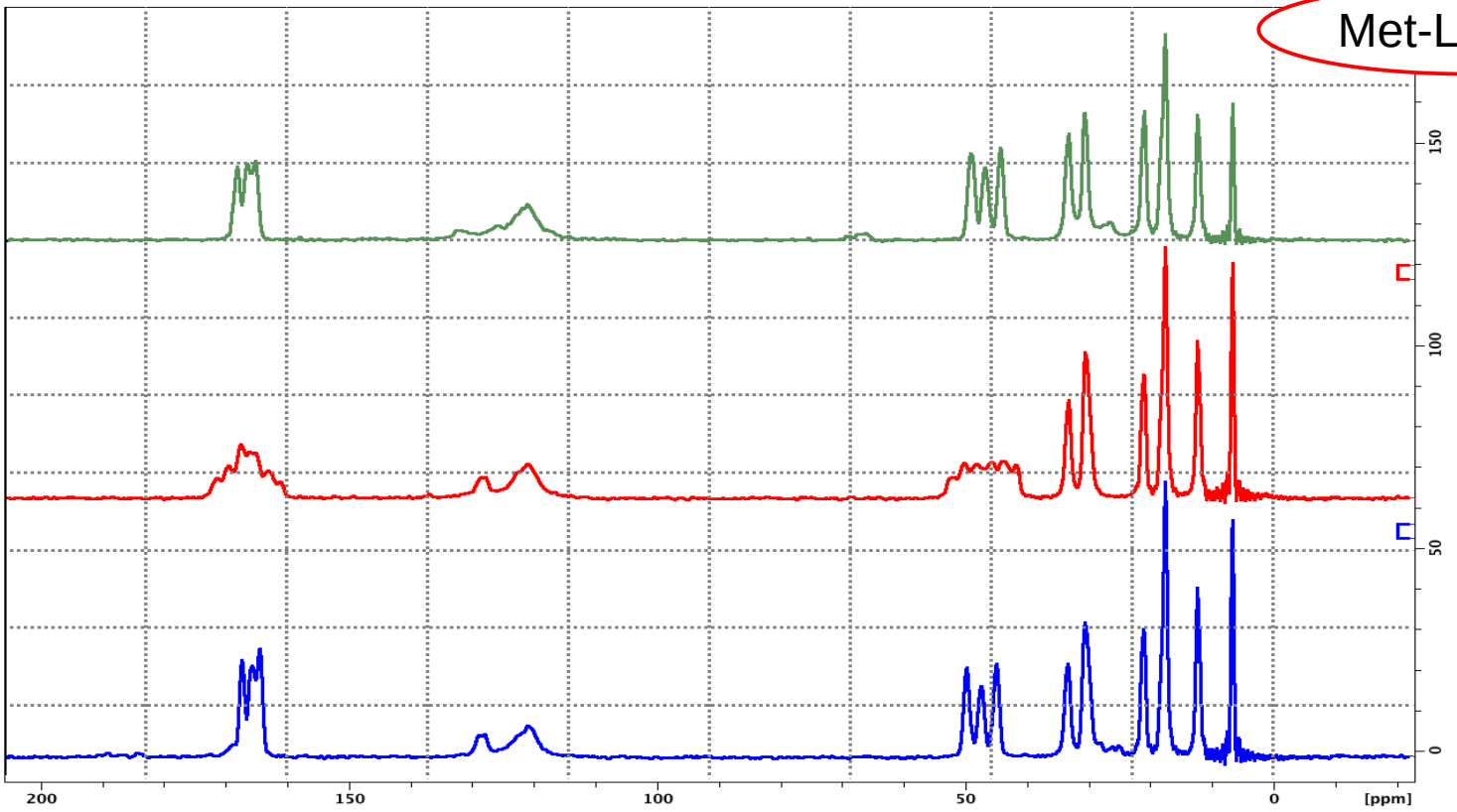




Homonukleární dipolární recoupling

Rotational Resonance $\Delta\omega_{iso} = n\,\omega_R$

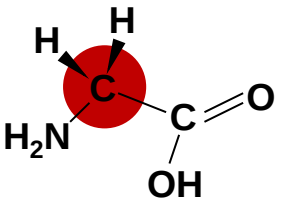
spinová rotace ***prostorová rotace***
chem. posun ***MAS***

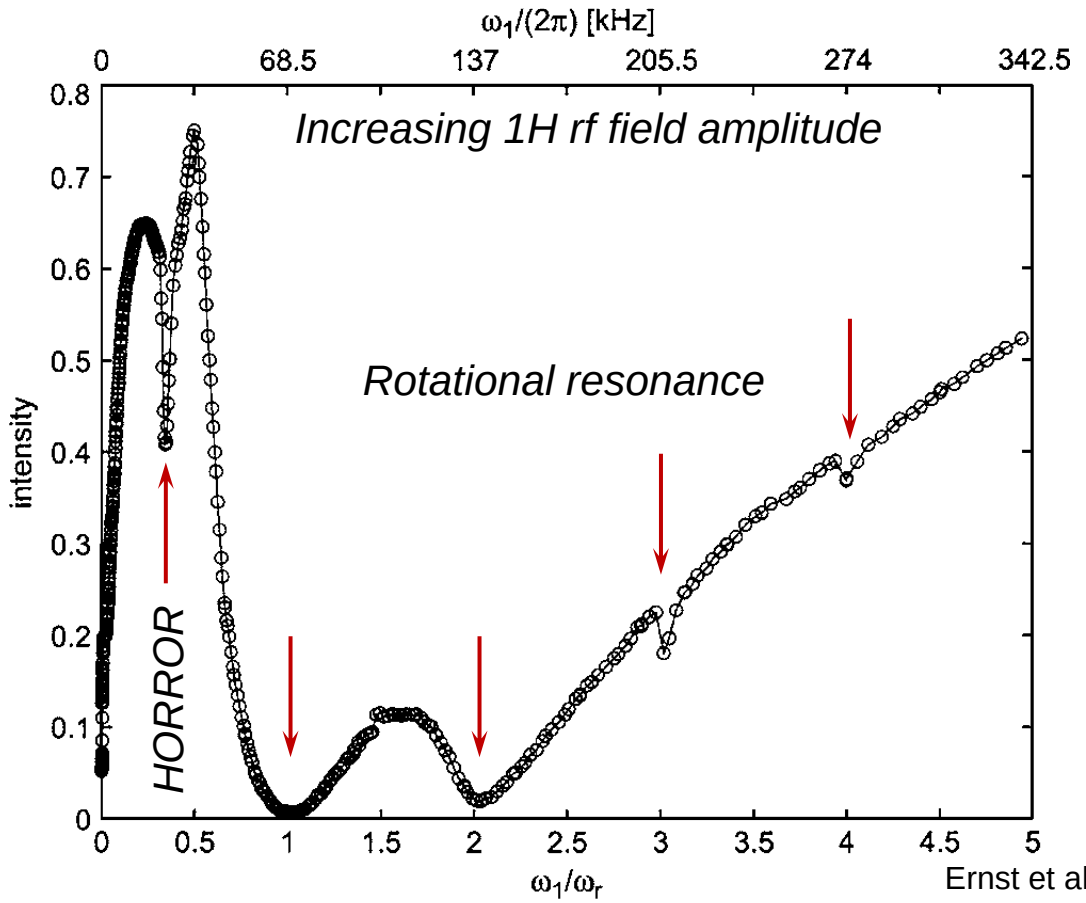


Heteronuclear decoupling at elevated MAS

1H decoupling and resonance conditions

MAS frequency of 68.4 kHz
1.3 mm rotor
600 MHz spectrometer





Ernst et al., JCP, 2005, 064102

Průměrný Hamiltonián a pulzní sekvence

Laboratorní systém

$e^{-iH_Z t}$

$$H^L = H_Z + H_{int} + H^L_{RF}(t)$$

frekvence

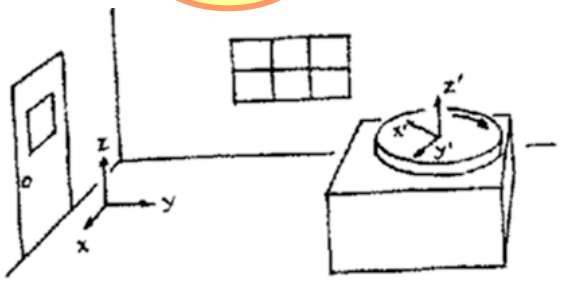
časově proměnná amplituda (pulsní sekvence)

Rotující systém

$e^{-iH_{RF} t}$

$$H^R = H_{int} + H_{RF}(t)$$

proměnná pouze amplituda



„Interakční“ systém $H^I(t)$ – *studium přímého vlivu H_{RF} na interní Hamiltonián*

průměrný Hamiltonián

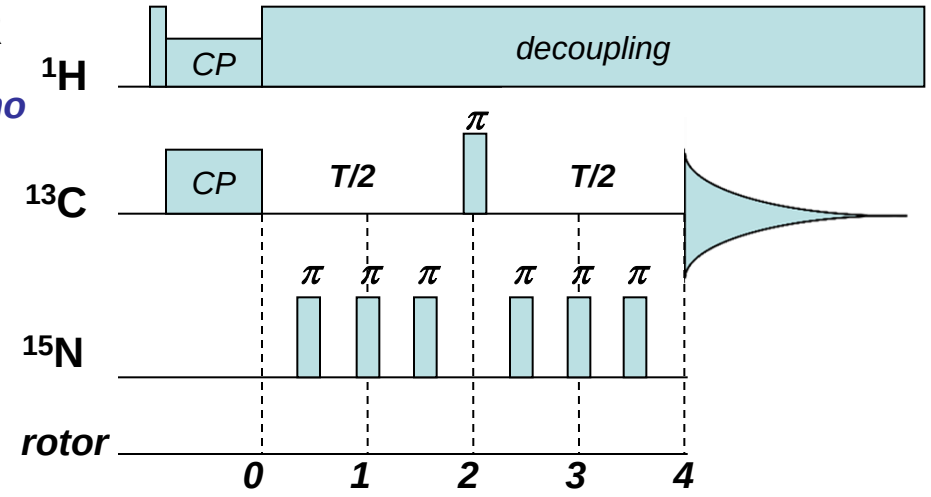
$$\overline{H} = \frac{1}{T}\int_0^T H^I(t)\,dt$$

pomocí vhodné pulzní sekvence lze vytvořit *Hamiltonián* požadovaného tvaru

Heteronuclear dipolar recoupling

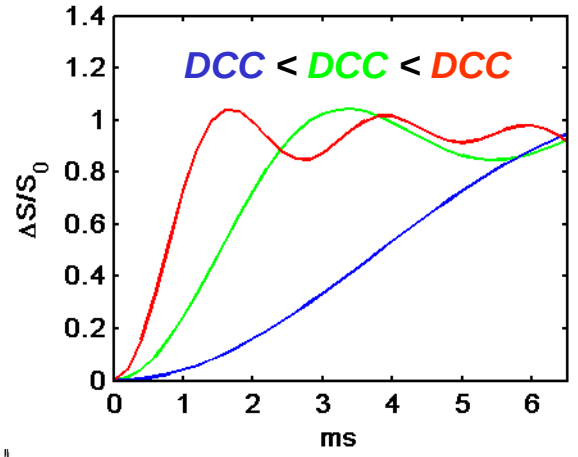
REDOR

Rotational Echo Double Resonance



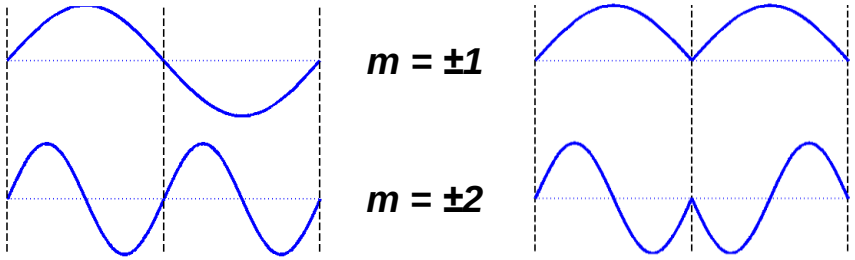
Purpose

- Measure internuclear distances
- Assess motional averaging



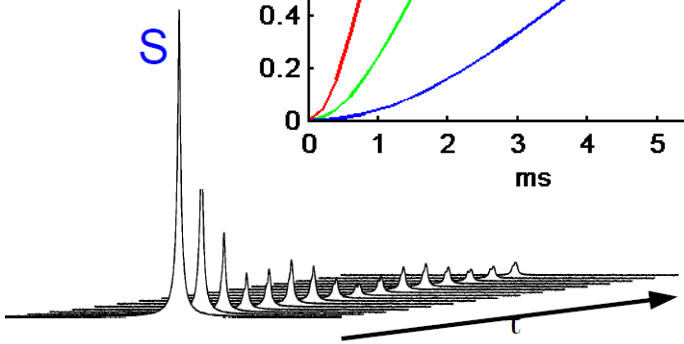
Principle

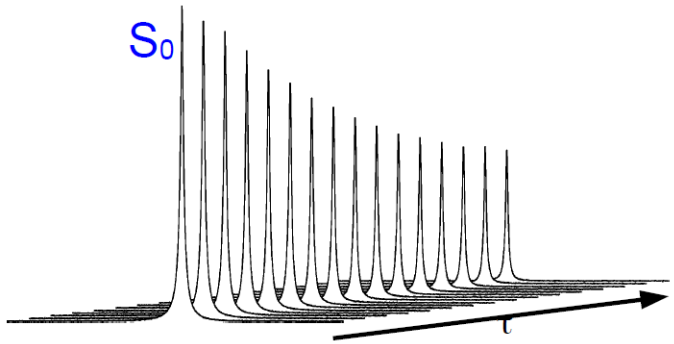
$$H^I_{DD}(t) = \sum_{m=-2}^{+2} \omega^m_{DD} \exp(im\omega_R t)\ I_z S_z$$



no π-pulses

with π-pulses every halft of rotor period

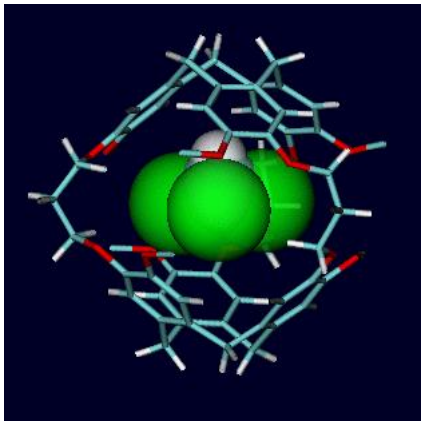




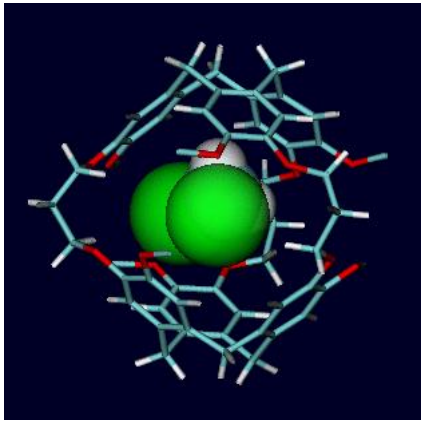
Dynamika supramolekulárních komplexů

Kryptofan-E

$CHCl_3$

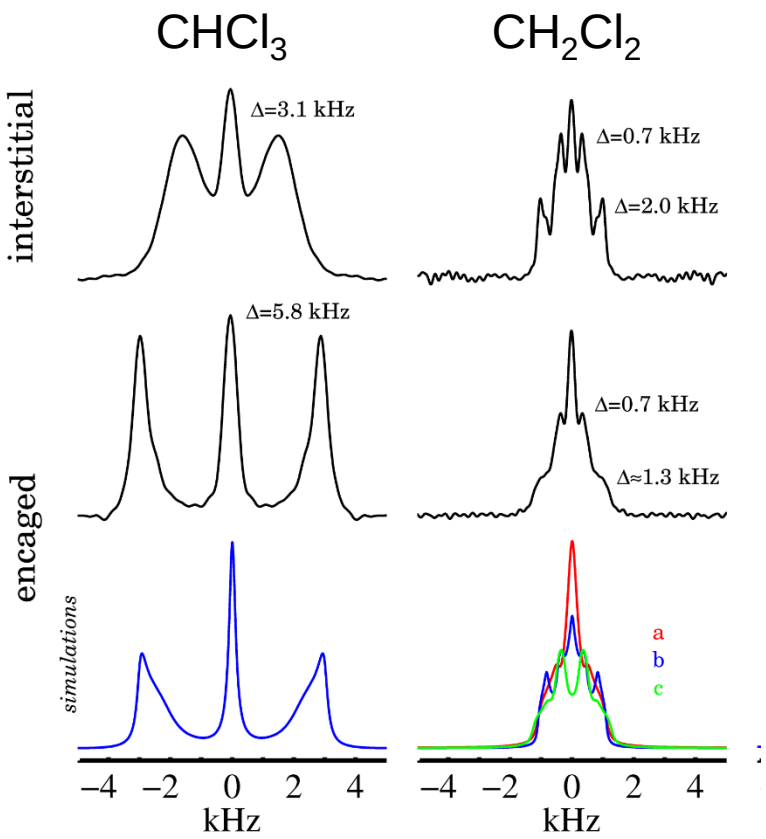


CH_2Cl_2



DD interakce vystředovaná pohybem

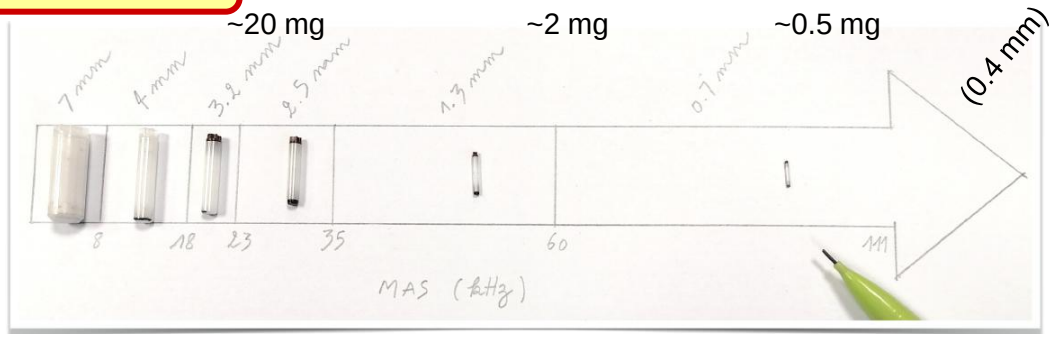
Statická hodnota 21 kHz

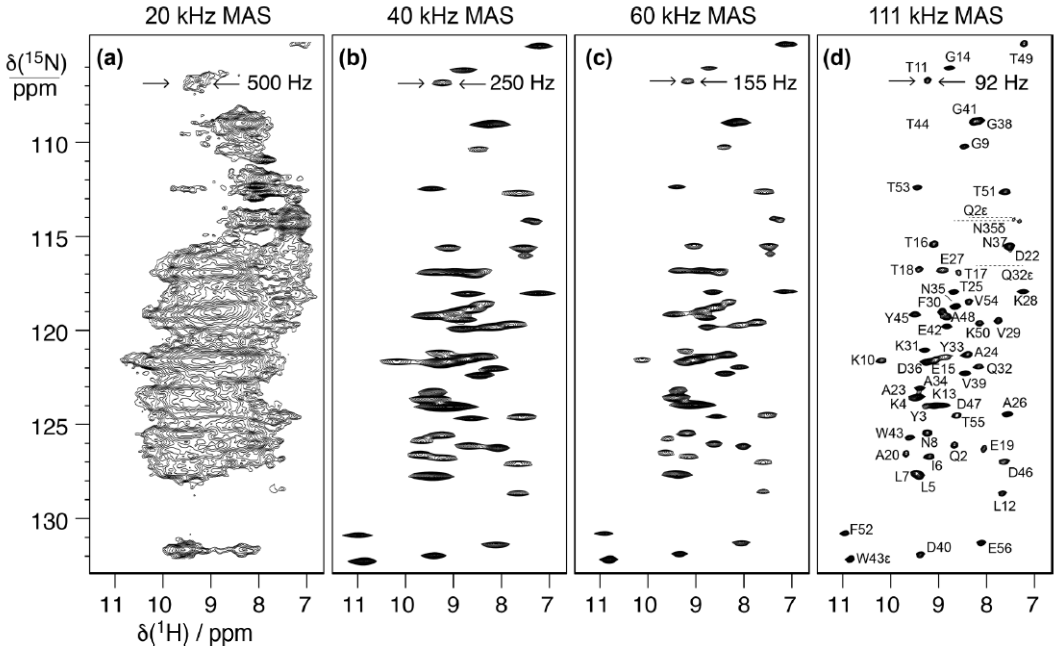


<DCC> = 19 kHz 3–5 kHz

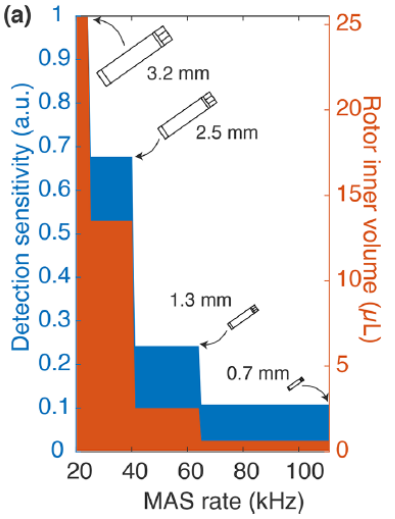
Biological solid-state NMR

Faster MAS

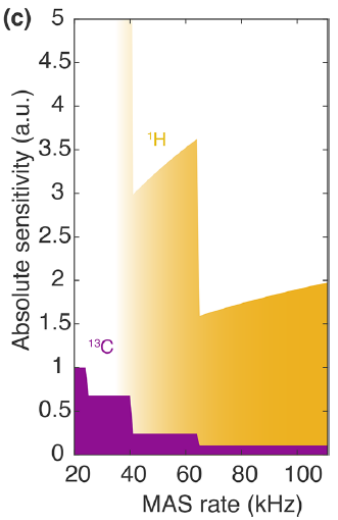




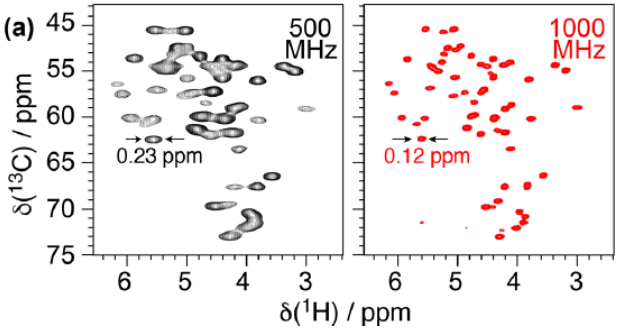
Advantage of smaller coils



Advantage of 1H detection



Higher B_0

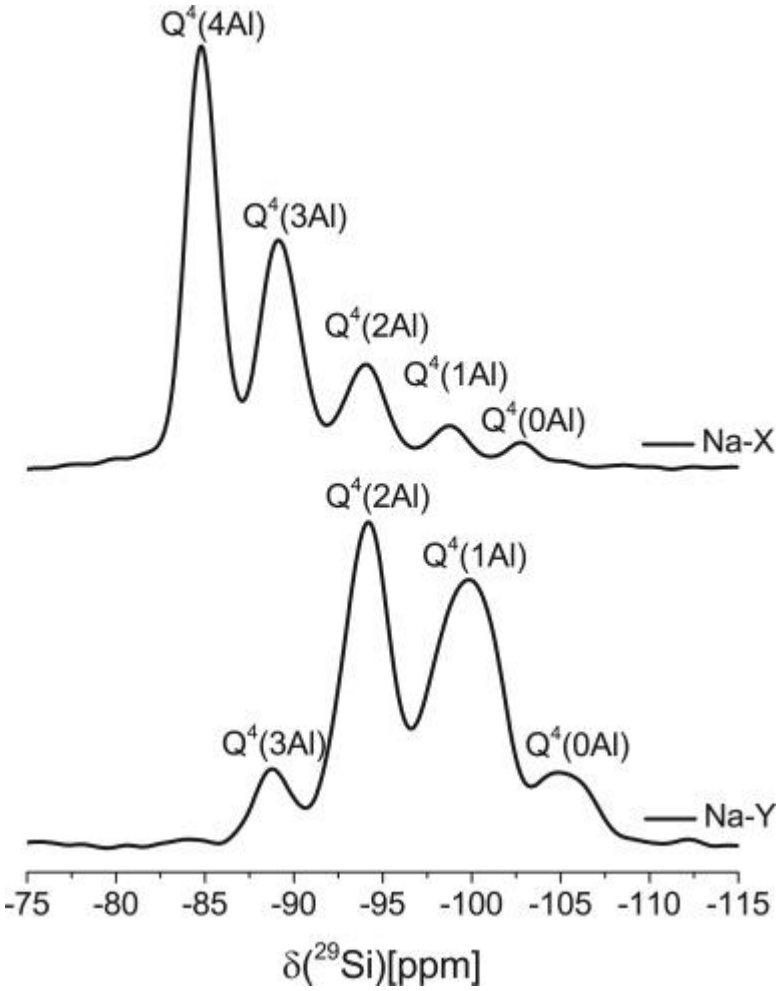


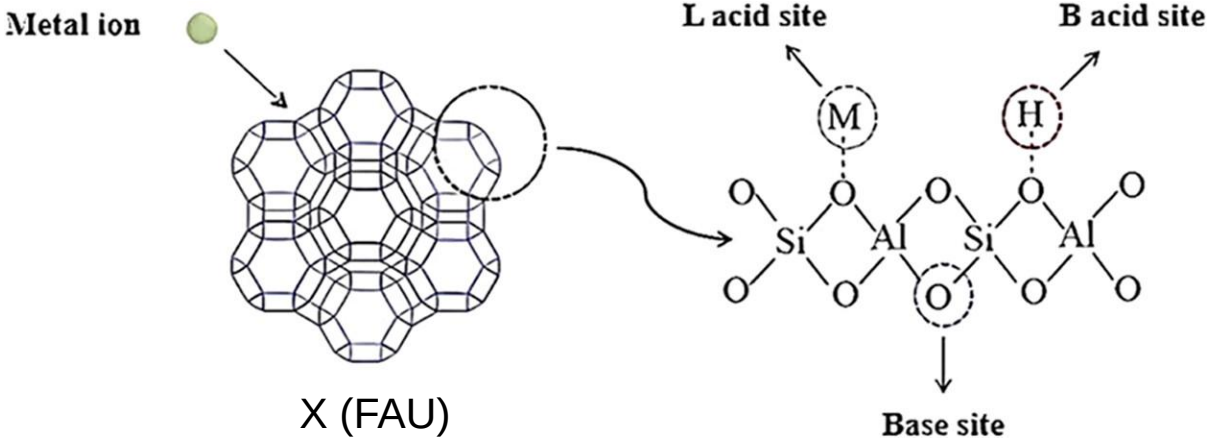
T. Le Marchand *et al.*, *Chem. Rev.* (**2022**), DOI: 10.1021/acs.chemrev.1c00918

NMR spectra of zeolites

^{29}Si MAS

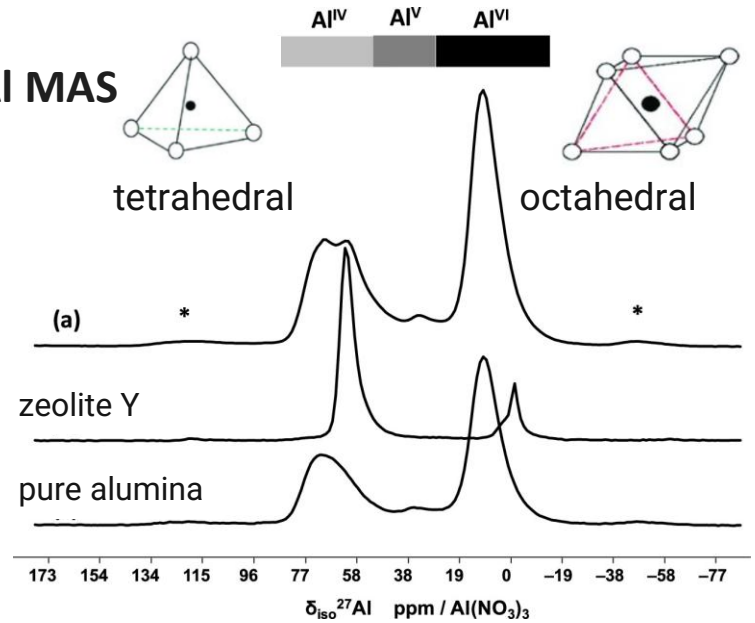
Na-X (top, Si/Al =1.3) and
Na-Y (bottom, Si/Al =2.7).





X (FAU)

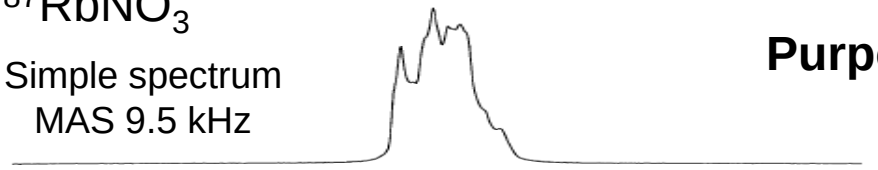
^{27}Al MAS



MQ MAS experiment

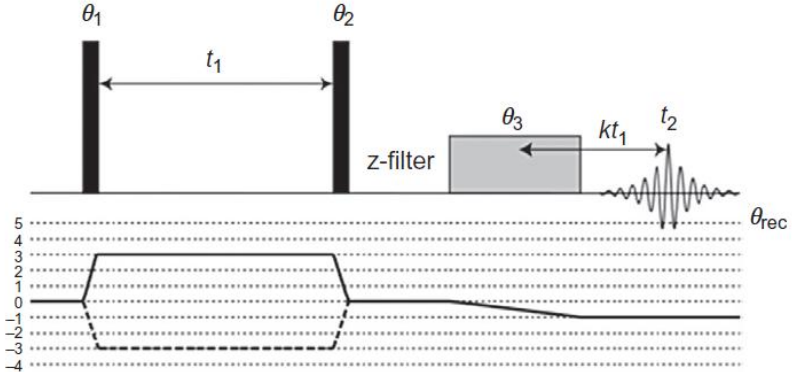
$^{87}RbNO_3$

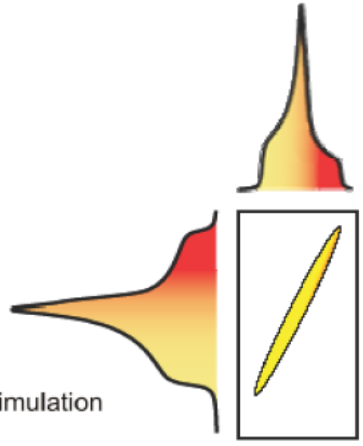
Simple spectrum
MAS 9.5 kHz



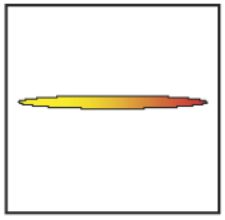
Purpose recover high resolution by removing effect of quadrupolar broadening

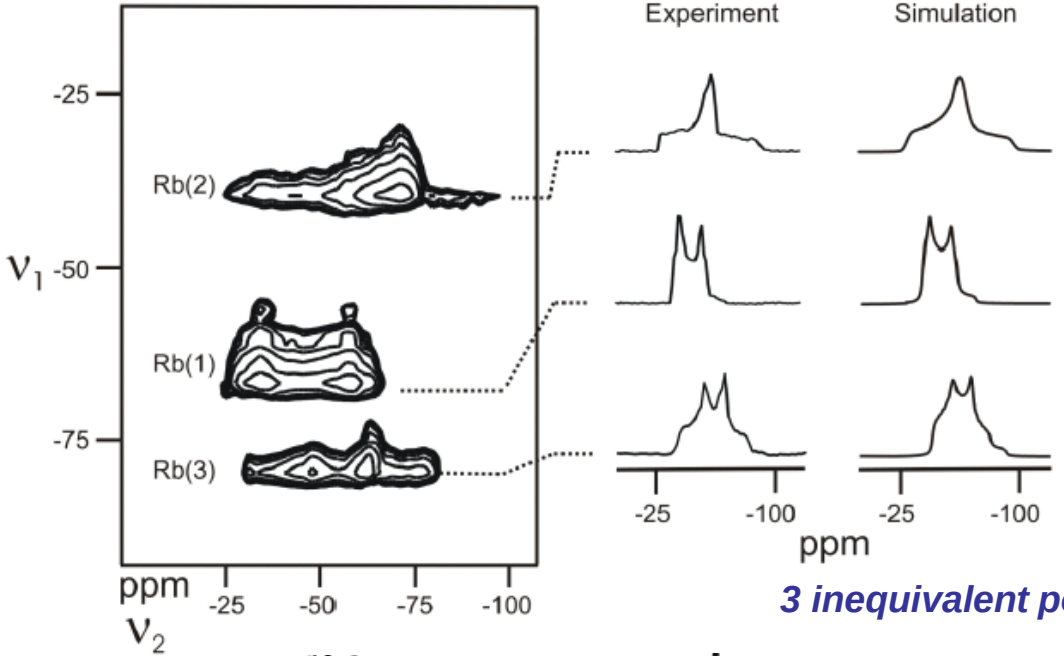
2D experiment that correlates MQ with SQ

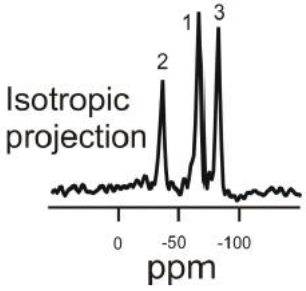


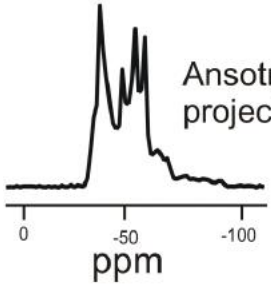


Shearing
Transformation



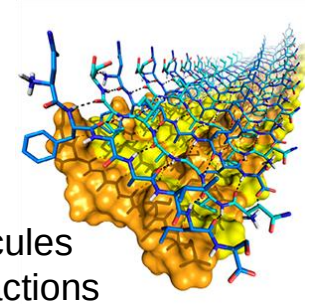






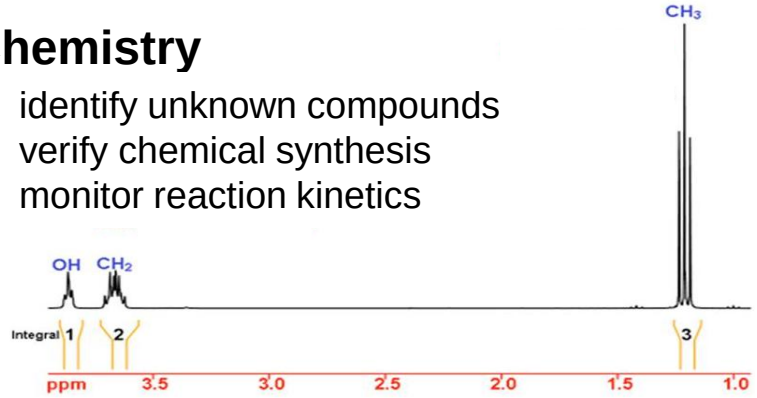
3 inequivalent positions of Rb atom

Nuclear Magnetic Resonance



Chemistry

- identify unknown compounds
- verify chemical synthesis
- monitor reaction kinetics

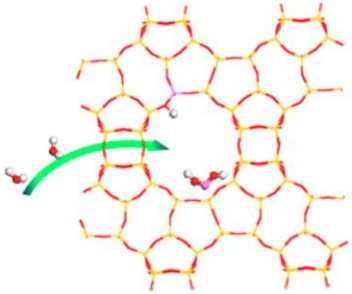


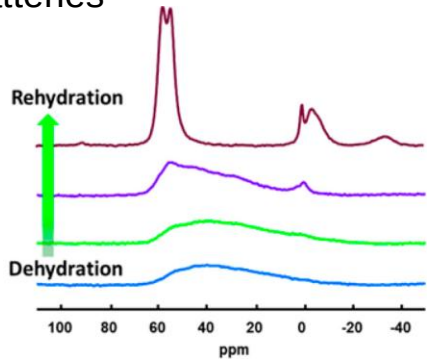
Structural biology

- determine 3D structures of biomolecules
- insights into their function and interactions
- drug design

Material science

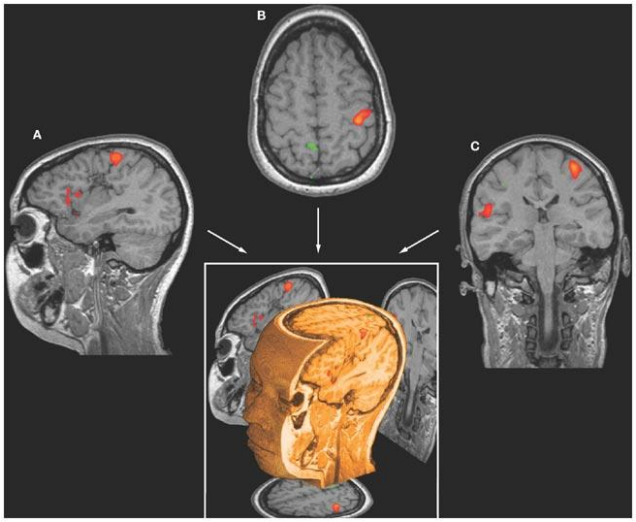
- polymers, nanomaterials, amorphous materials
- Characterisation of functional materials - catalysts
- energy storage materials - batteries





Medicine

- non-invasive diagnostic imaging
- brain disorders, tumours, and musculoskeletal injuries angiography, functional imaging of brain
- metabolomics (diagnostics and treatment monitoring)



Environmental science

- Pollutants (aerosols)
- soil composition (organic matter)

Pharmaceutic industry

- Drug formulations, quality control
- Polymorphs and bioavailability