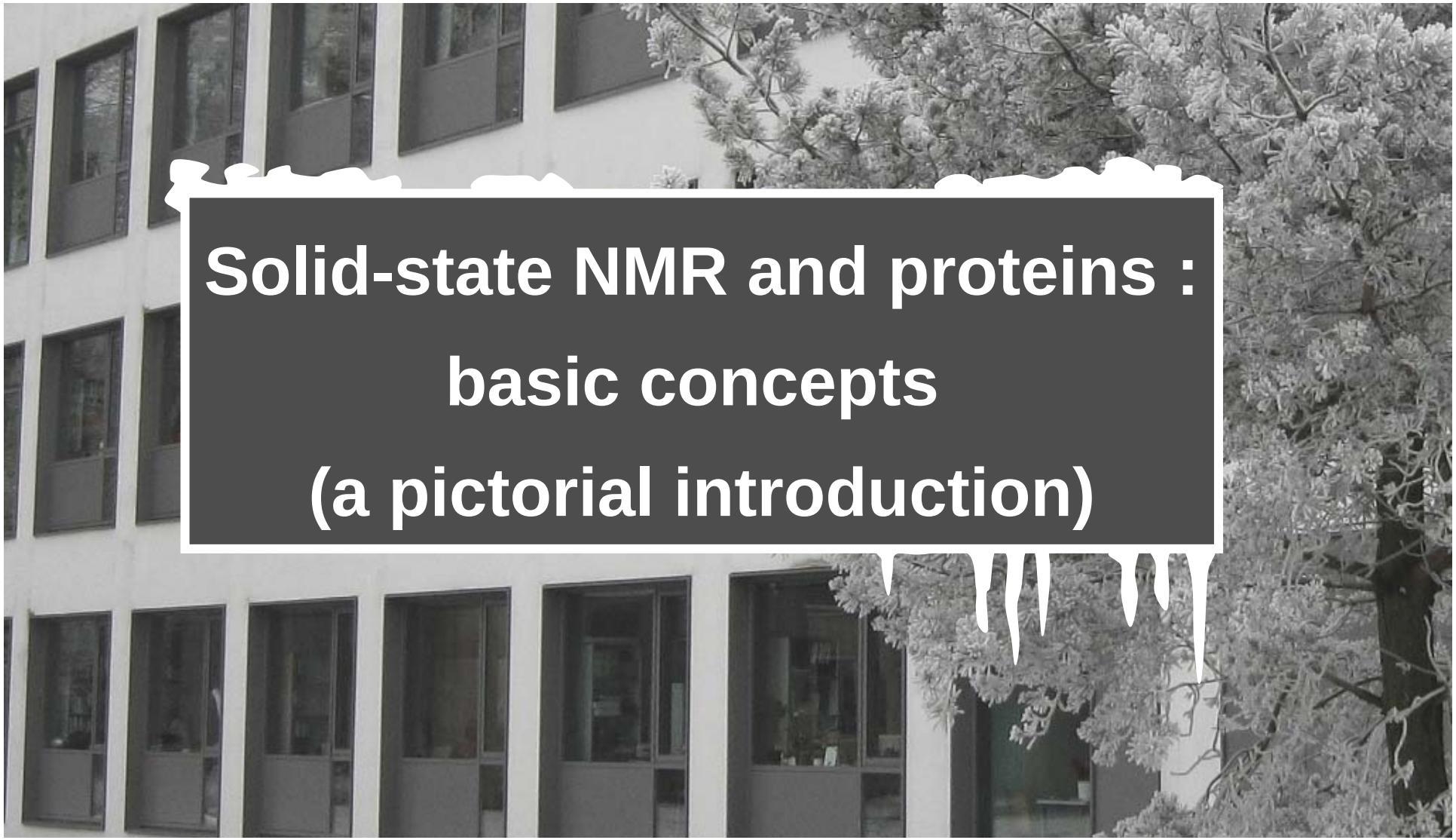


Solid-state NMR and proteins : basic concepts (a pictorial introduction)

Barth van Rossum, 16.02.2009

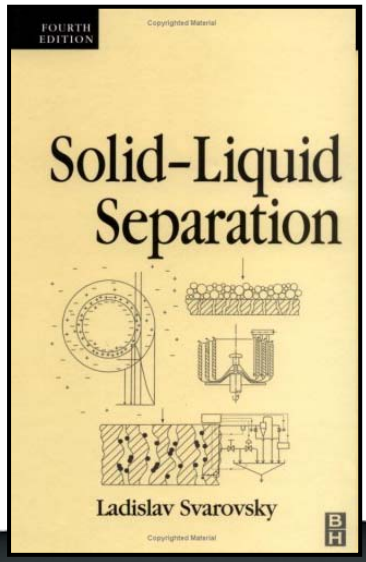
Solid-state and solution NMR spectroscopy have many things in common

Several concepts have been/will be discussed in the other lectures
(spin precession, RF pulses, 2D experiments, chemical shift assignment, etc….)

focus will be on:

→ differences between the two techniques

→ features unique for solid-state NMR spectroscopy



Overview

- chemical shift anisotropy

- magic-angle spinning (MAS)

- the CP-MAS experiment, decoupling

- cross polarization (CP) - part I

- assignment strategies: specific CP

- the dipolar coupling

- recoupling

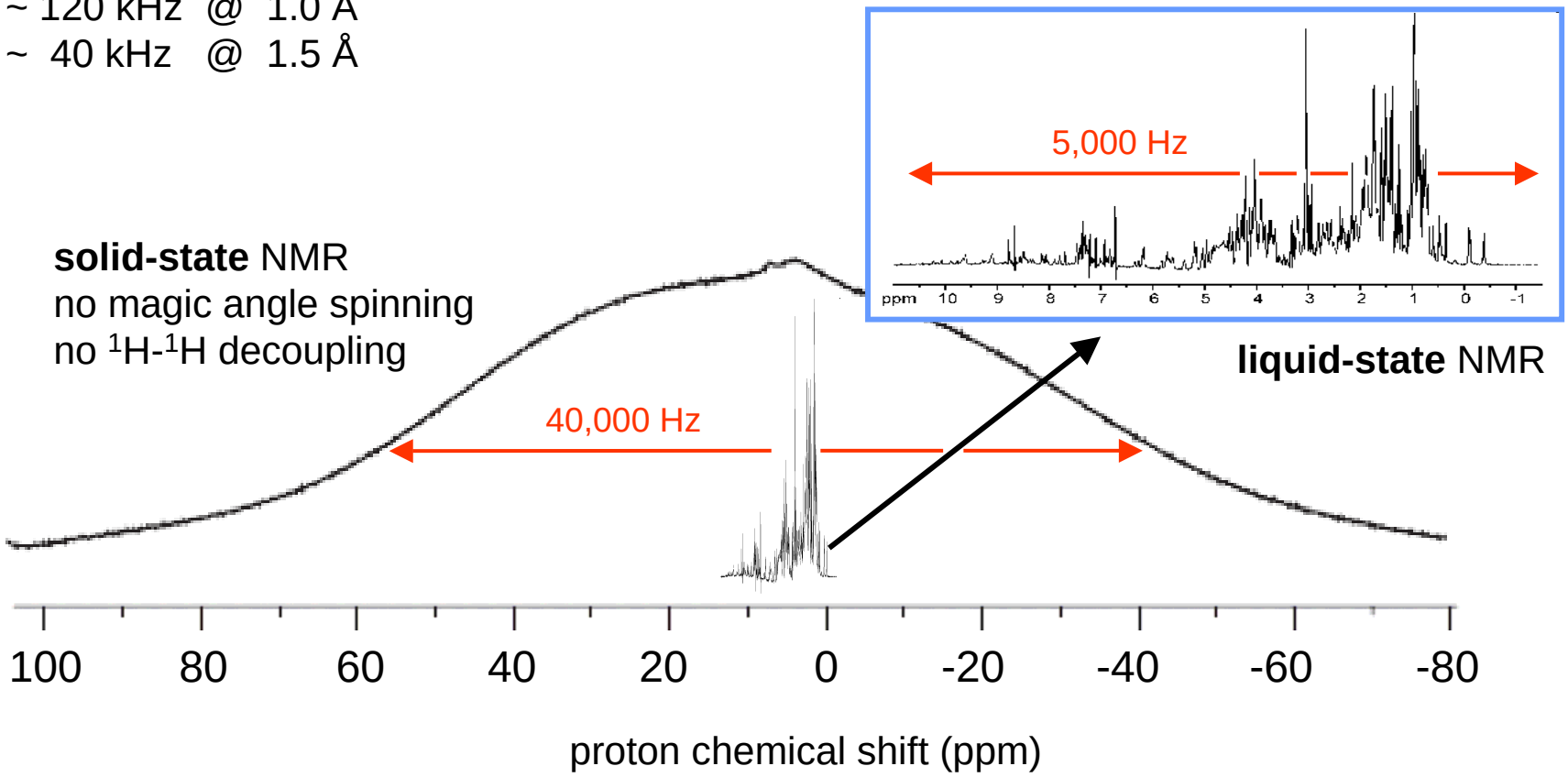
solids-state NMR vs. liquid-state NMR

solids	**liquids**
dipolar coupling 10 - 100 **kHz**	scalar coupling 10 – 100 **Hz**
anisotropic interactions	(mostly) isotropic interactions
^{13}C detection (^{1}H detection when deuterated)	^{1}H detection
sensitivity low	sensitivity high
requires magic-angle spinning	natural tumbling of molecules

solids-state NMR vs. liquid-state NMR

dipolar coupling between protons:

~ 120 kHz @ 1.0 Å
~ 40 kHz @ 1.5 Å

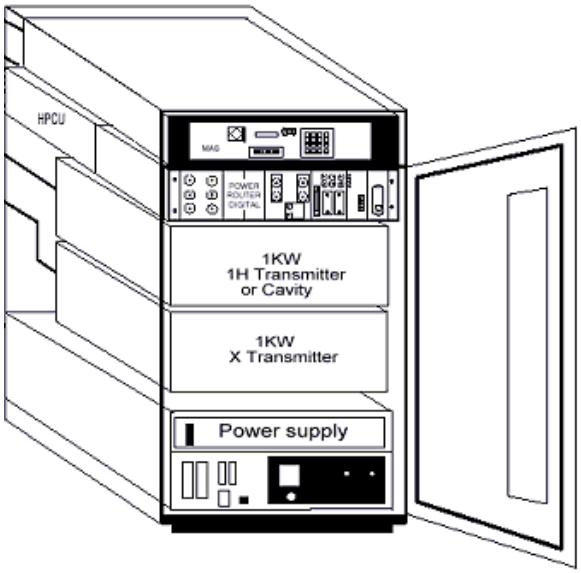


solids-state NMR vs. liquid-state NMR

Solid-state NMR uses high-power RF pulses (1000 W) to manipulate the spins

720,844 mm^3
(1 mW / mm^3)

67 mm^3
(15 W / mm^3)



15 mm^3
(70 W / mm^3)

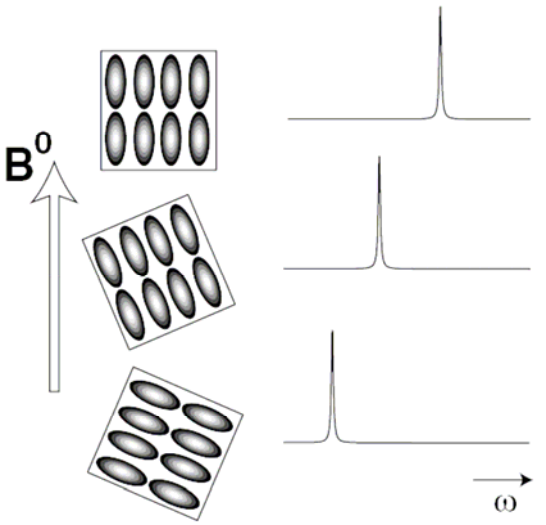
Solid-state NMR is brute force…

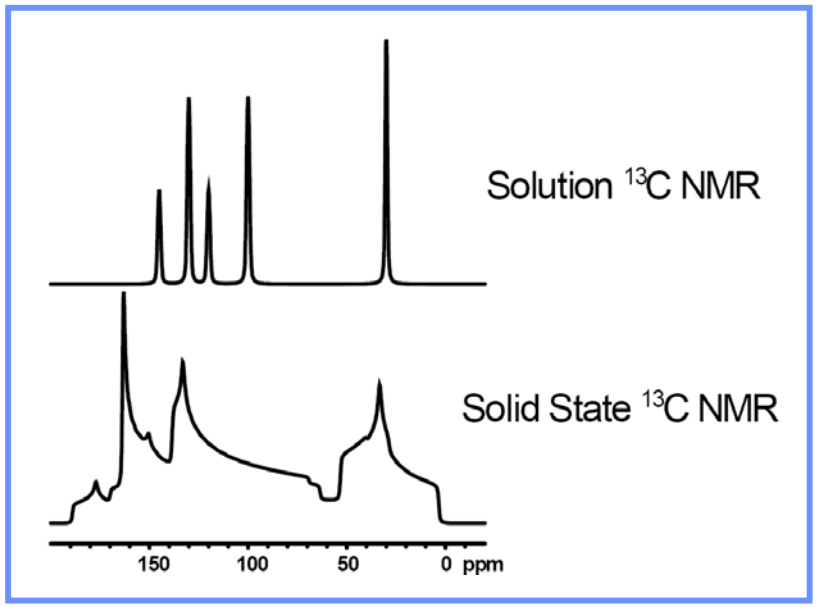
Anisotropic interactions

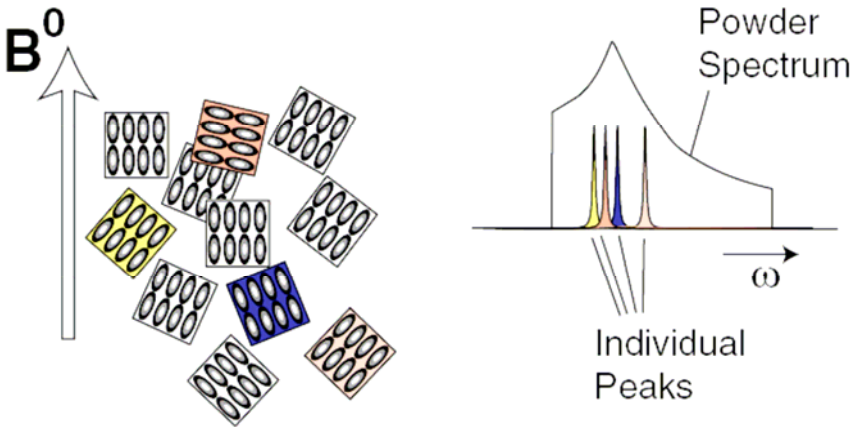
solids: interactions depend on orientation of molecule
these interactions are called anisotropic

→ limit resolution in NMR spectra of biological macromolecules

liquids: rapid random tumbling averages
anisotropic chemical shifts and couplings





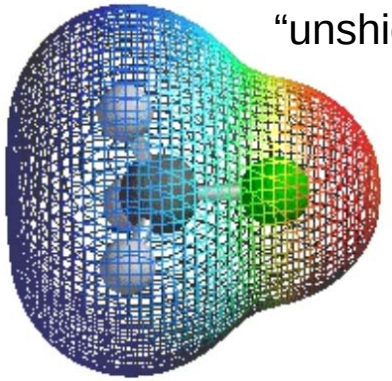


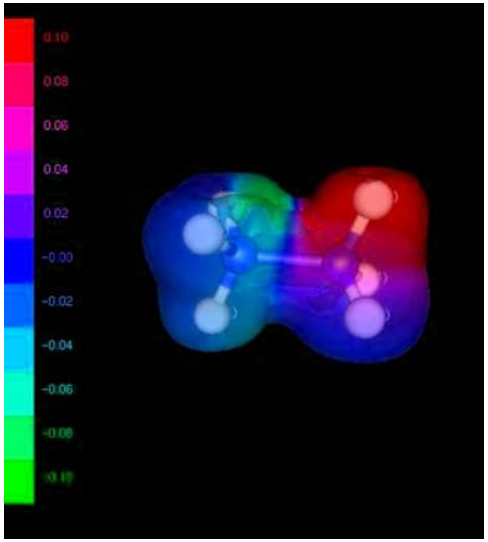
Chemical shift anisotropy (CSA)

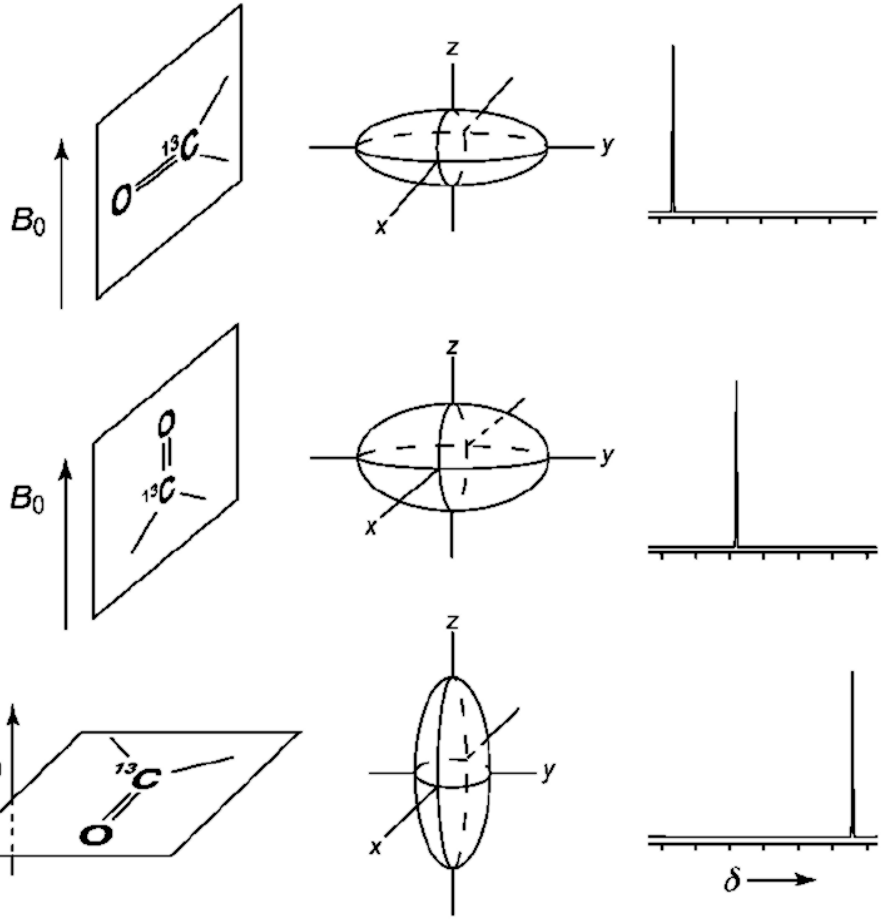
Electrons shield the nuclear spins from the external magnetic field

“unshielded”

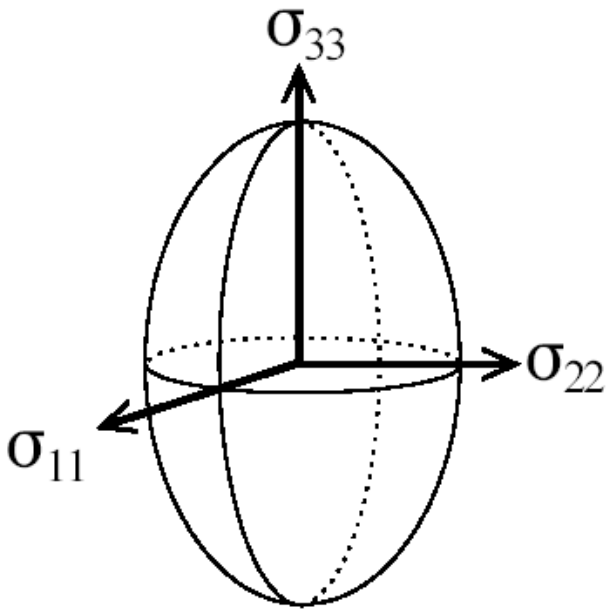
“shielded”







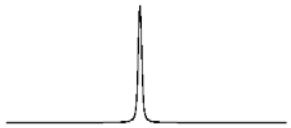
Chemical shift anisotropy (CSA)



$\sigma_{11} \leq \sigma_{22} \leq \sigma_{33}$

spherical symmetry

$\sigma_{11} = \sigma_{22} = \sigma_{33}$



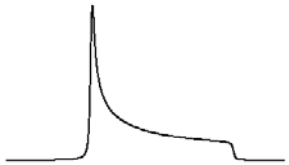
non-axial symmetry

$\sigma_{11} \neq \sigma_{22} \neq \sigma_{33}$

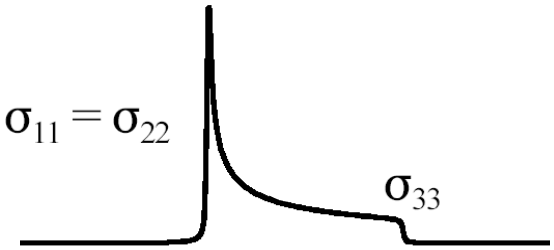
axial symmetry

$\sigma_{11} = \sigma_{22}$ (or)

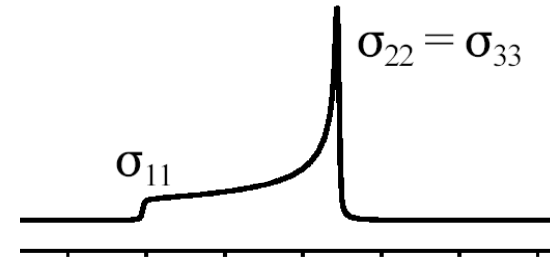
$\sigma_{22} = \sigma_{33}$



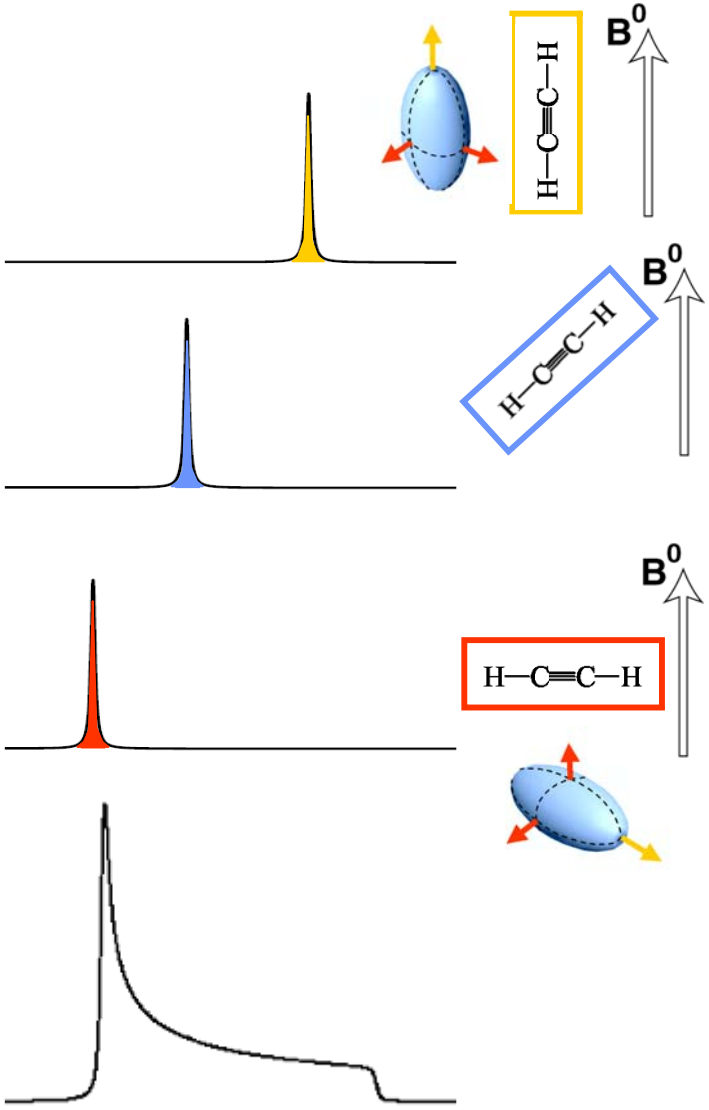
$\sigma_{11} = \sigma_{22}$
$\sigma_{11}, \sigma_{22} < \sigma_{33}$

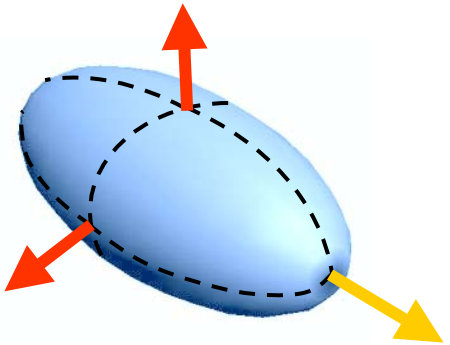


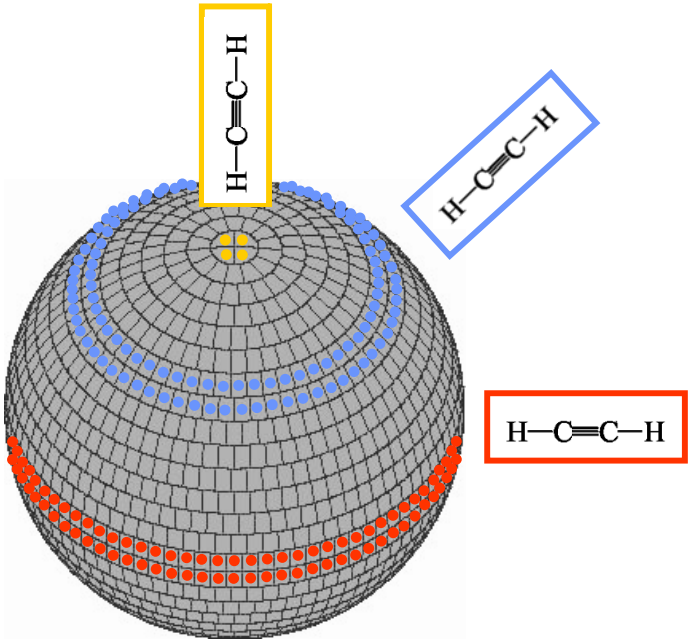
$\sigma_{22} = \sigma_{33}$
$\sigma_{22}, \sigma_{33} > \sigma_{11}$



Chemical shift anisotropy (CSA)

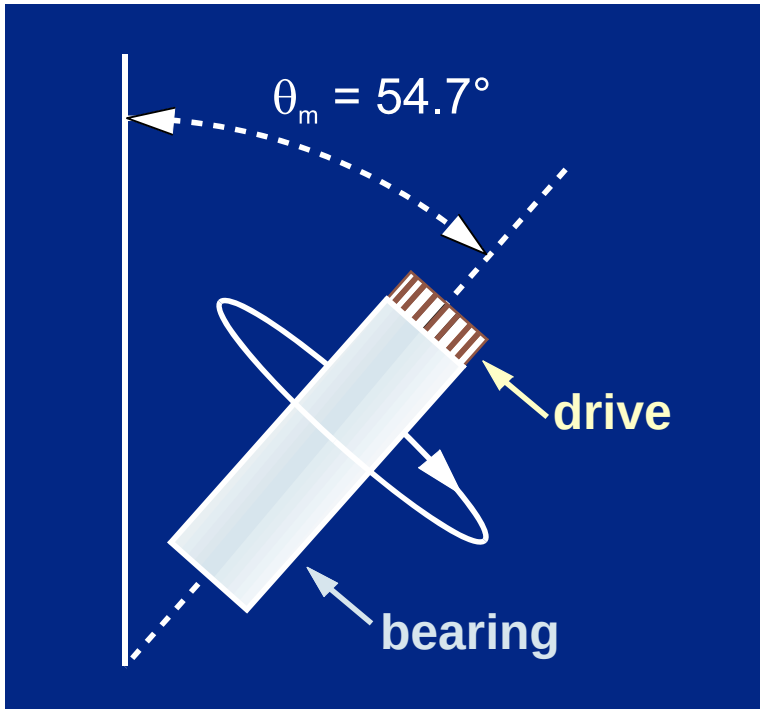


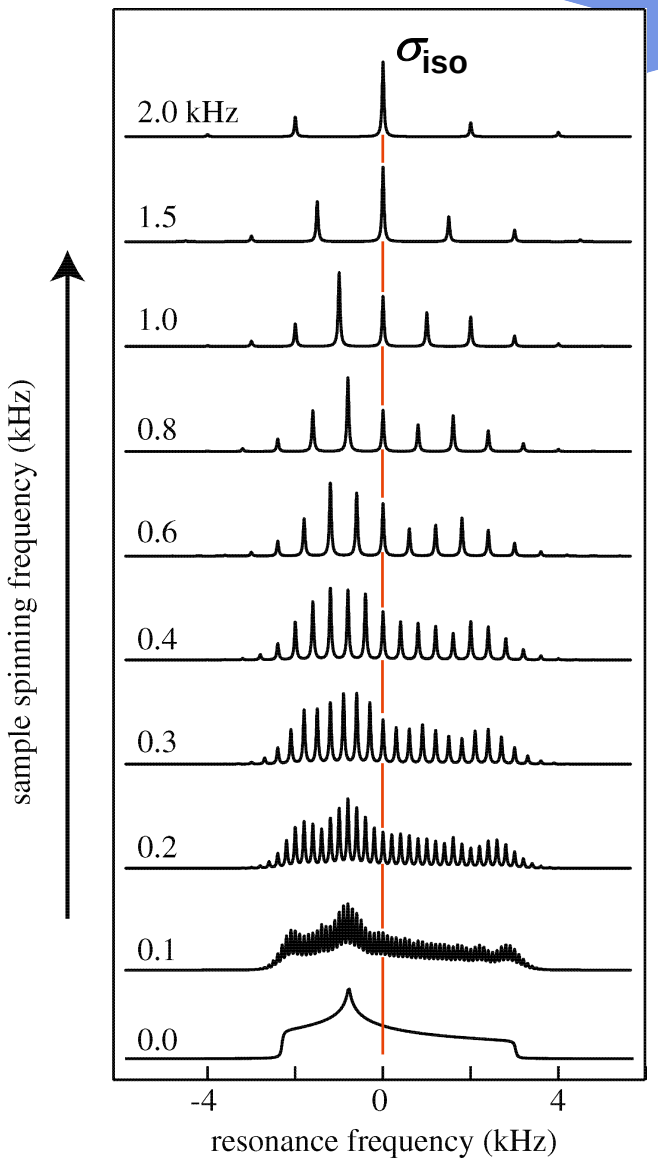




Magic-Angle Spinning (MAS)

Anisotropic interactions can be suppressed using a technique called **magic-angle spinning**

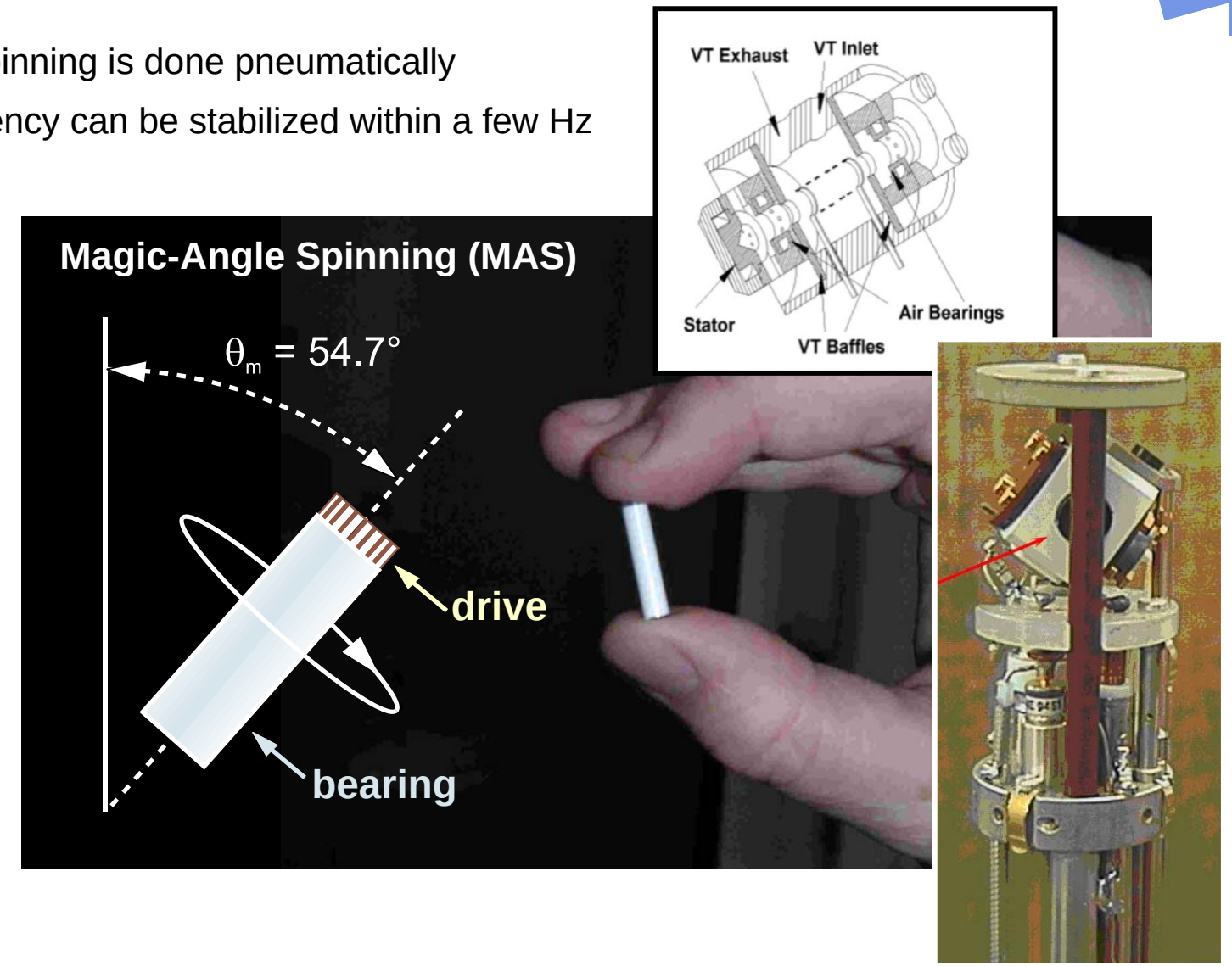




Magic-Angle Spinning (MAS)

magic-angle spinning is done pneumatically

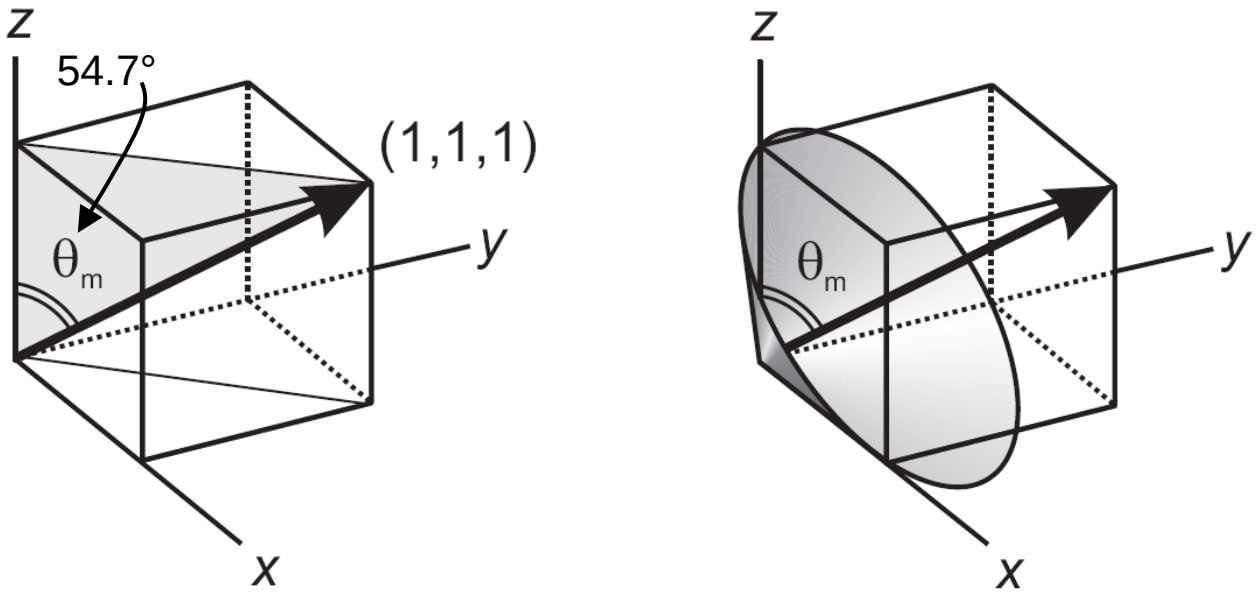
spinning frequency can be stabilized within a few Hz



Magic-Angle Spinning (MAS)

"magic angle" : the angle between the body diagonal of a cube and the *z*-axis

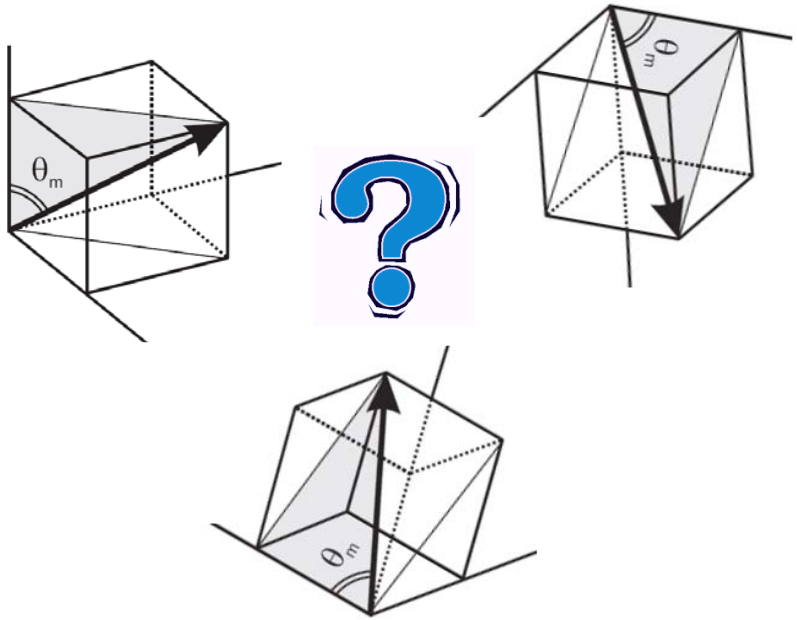
→ by rotation around this axis, a vector along *z* will cross the *x* and *y*-axes

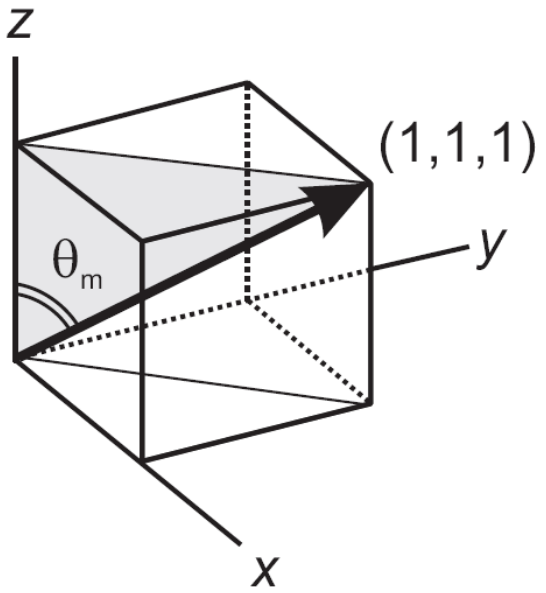


Magic-Angle Spinning (MAS)

note : for *any* rotation, one could construct a 1x1x1 cube around…

→ however, the *z*-axis is 'special' (B_0 direction!) and has to be one of the axes

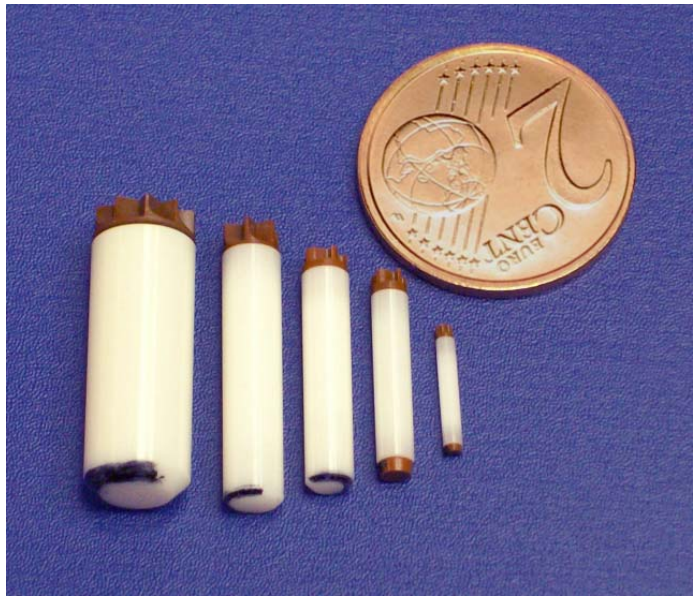




Magic-Angle Spinning (MAS)

Maximum spinning frequency depends on rotor diameter

some typical diameters:	4.0 mm	→	15 kHz	(1,400,000 x *g*)
	3.2 mm	→	25 kHz	(2,700,000 x *g*)
	2.5 mm	→	35 kHz	(3,500,000 x *g*)



(50,000 x *g*)…

Solid-state NMR is brute force…

Magic-Angle Spinning (MAS)

a 3.2 mm rotor spinning at 24 kHz…

… has a speed of 240 m/s when it would roll on the floor …

… and needs only 46 hours to roll around the earth…

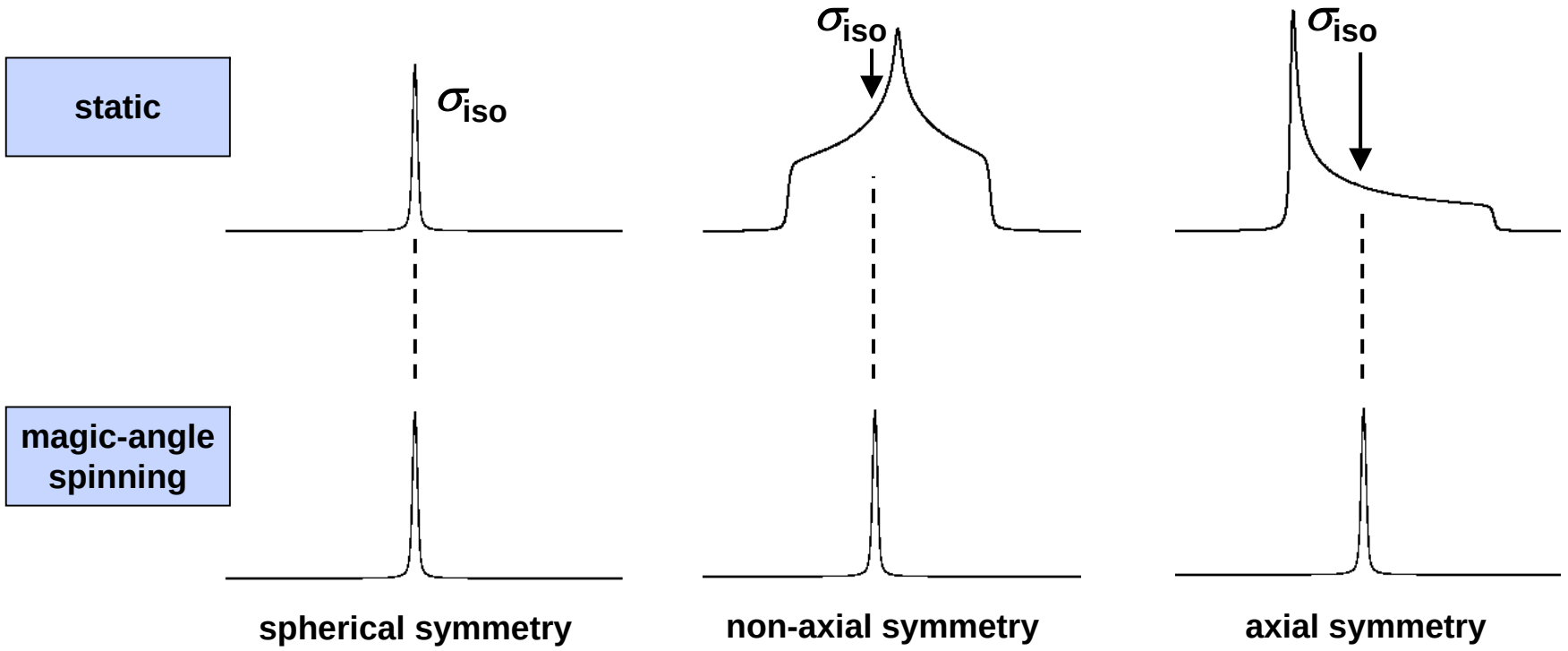
Chemical shift anisotropy (CSA)

$$\sigma_{iso} = (\sigma_{11} + \sigma_{22} + \sigma_{33}) / 3$$

σ_{iso} **= isotropic chemical shift**

- is what remains for very fast MAS
- is the shift as detected in liquid-state NMR

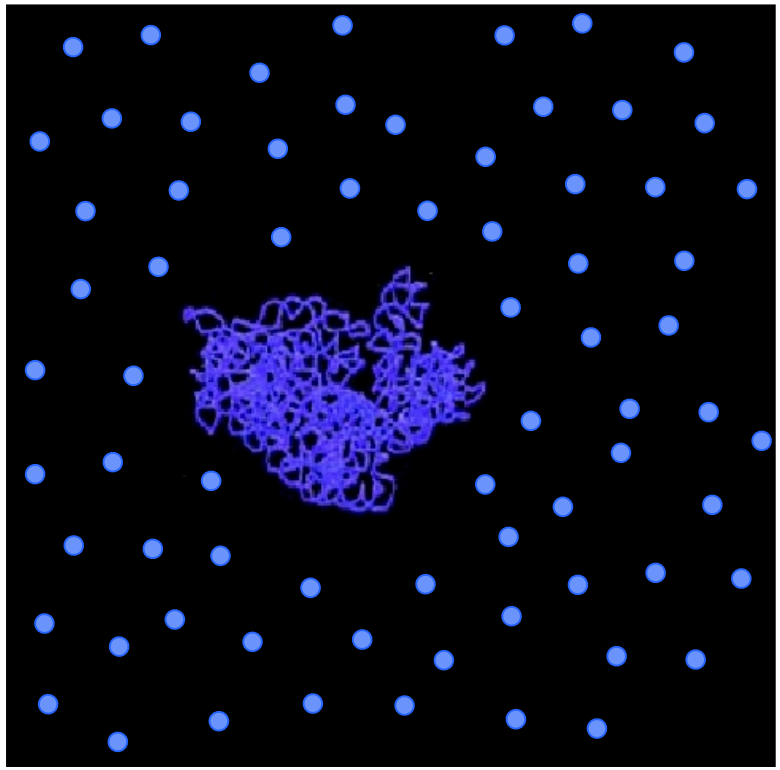
the isotropic shift does generally not coincide with maximum of the powder line shape

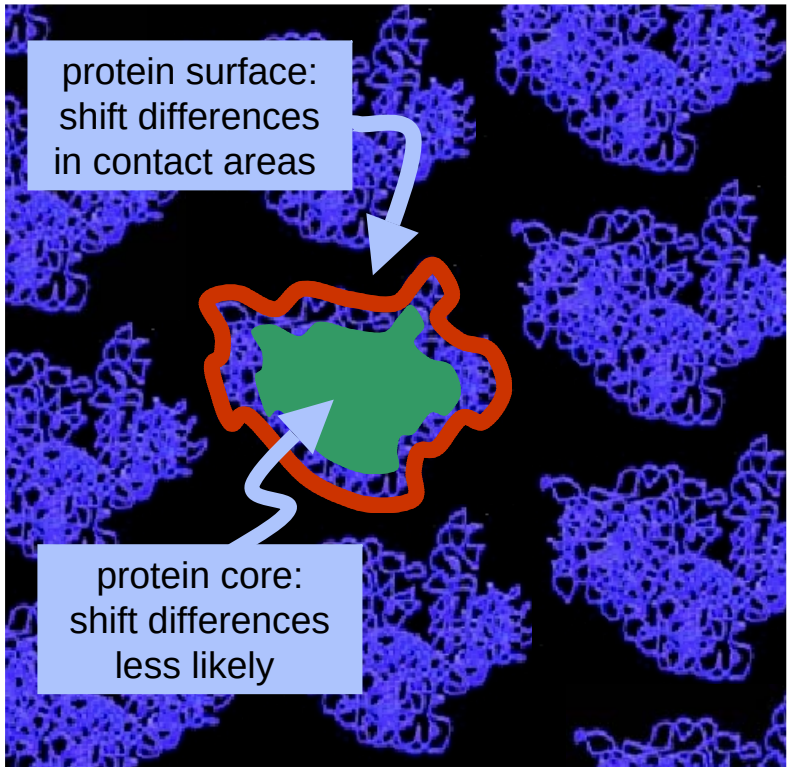


Isotropic chemical shift

chemical shifts of a protein determined by:

- folding of the protein
- interactions between proteins (solids!)
- 'environmental' factors (pH, temperature,solvent…)



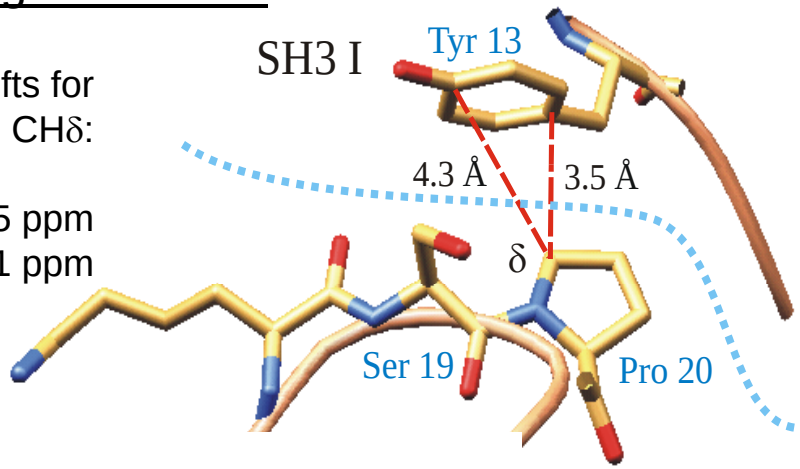


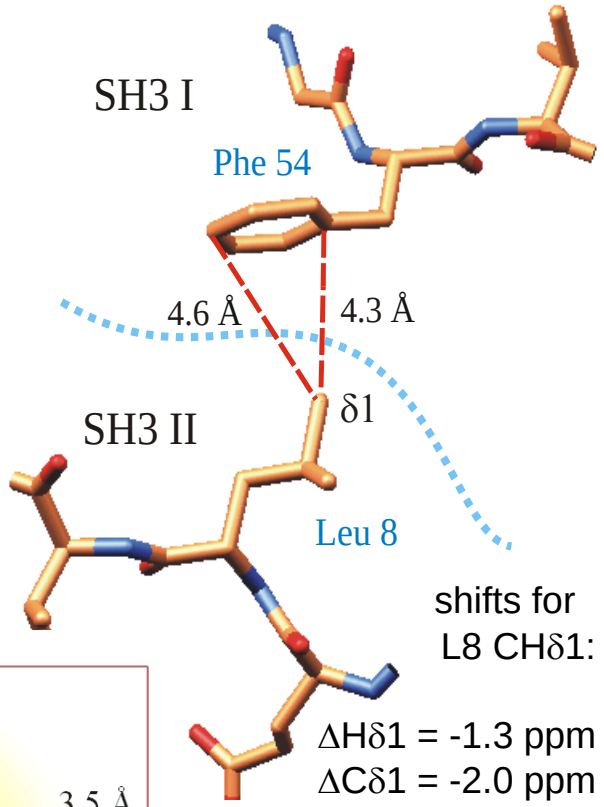
→ there is no such thing as *the* NMR chemical shift !!

‘Aggregation shifts’

shifts for
P20 CHδ:

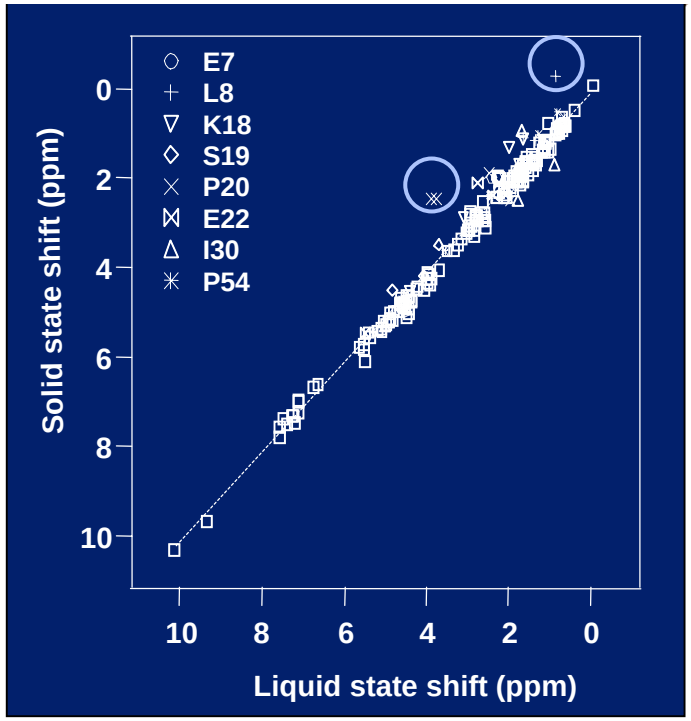
ΔHδ = -1.5 ppm
ΔCδ = -1.1 ppm



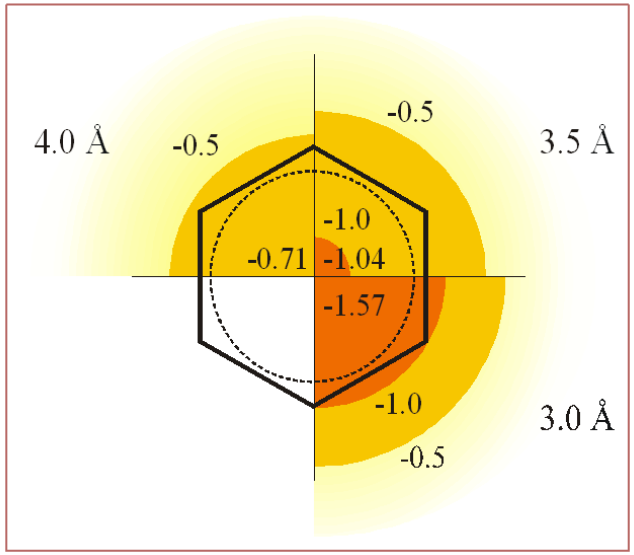


shifts for
L8 CHδ1:

ΔHδ1 = -1.3 ppm
ΔCδ1 = -2.0 ppm



SH3 II

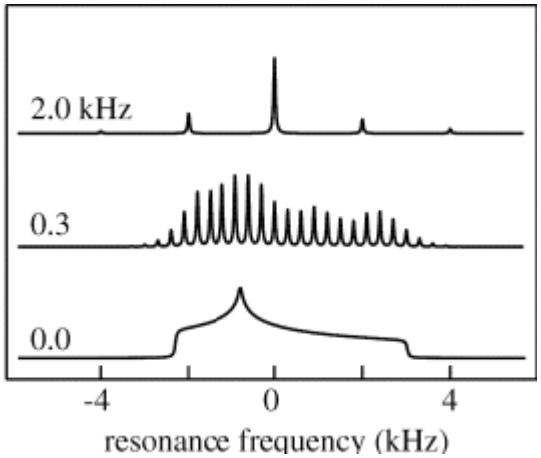


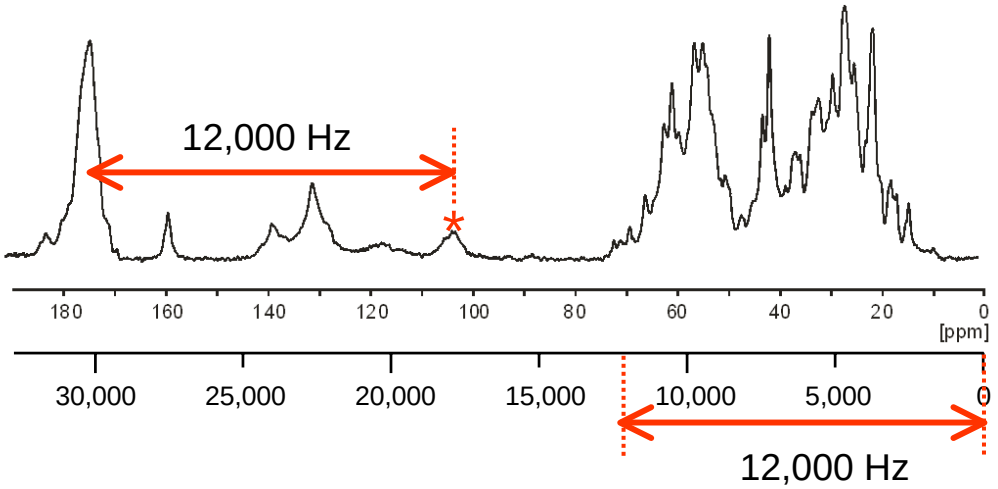
MAS: spinning side bands

What spinning frequency to use?

1. large enough to have most intensity in the centre band (isotropic shift)
2. remaining side bands should not interfere with other signals

example: spectrum recorded at 700 MHz, with 12 kHz MAS





700 MHz → 1 ppm = 700 Hz (^{1}H)
→ 1 ppm = 175 Hz (^{13}C)

$\gamma_H : \gamma_C = 4 : 1$

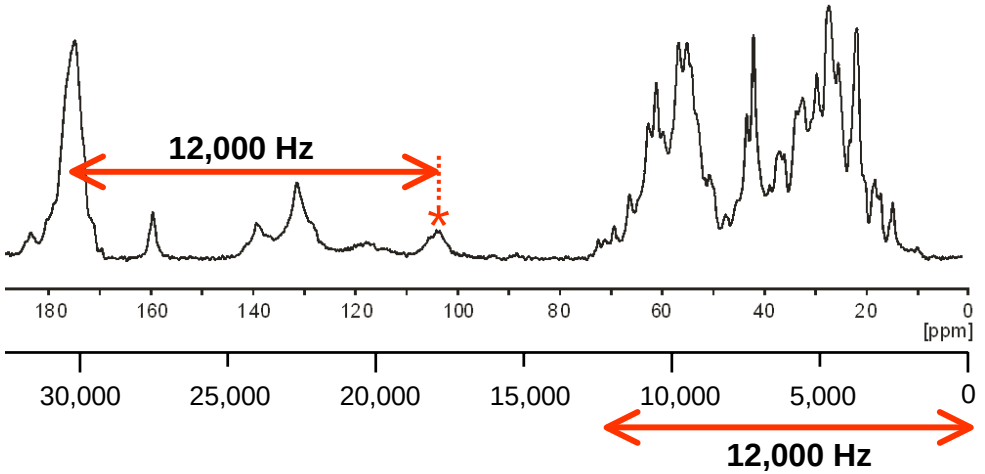
→ 12,000 Hz (MAS) ~ 69 ppm (13C)

MAS: spinning side bands

Spinning side bands are field dependent!

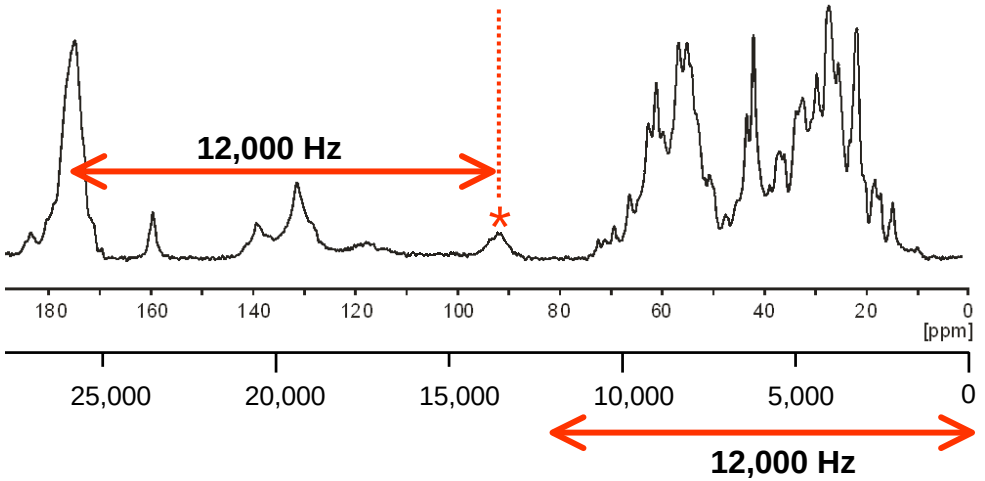
700 MHz → 1 ppm = 700 Hz (^{1}H)
→ 1 ppm = 175 Hz (^{13}C)

12,000 Hz (MAS) ←→ ~69 ppm (^{13}C)



600 MHz → 1 ppm = 600 Hz (^{1}H)
→ 1 ppm = 150 Hz (^{13}C)

12,000 Hz (MAS) ←→ 80 ppm (^{13}C)

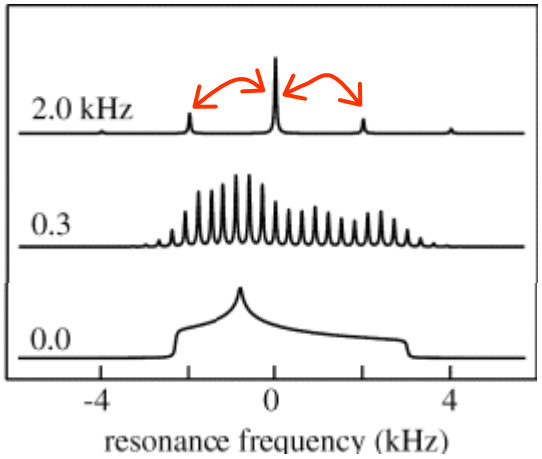


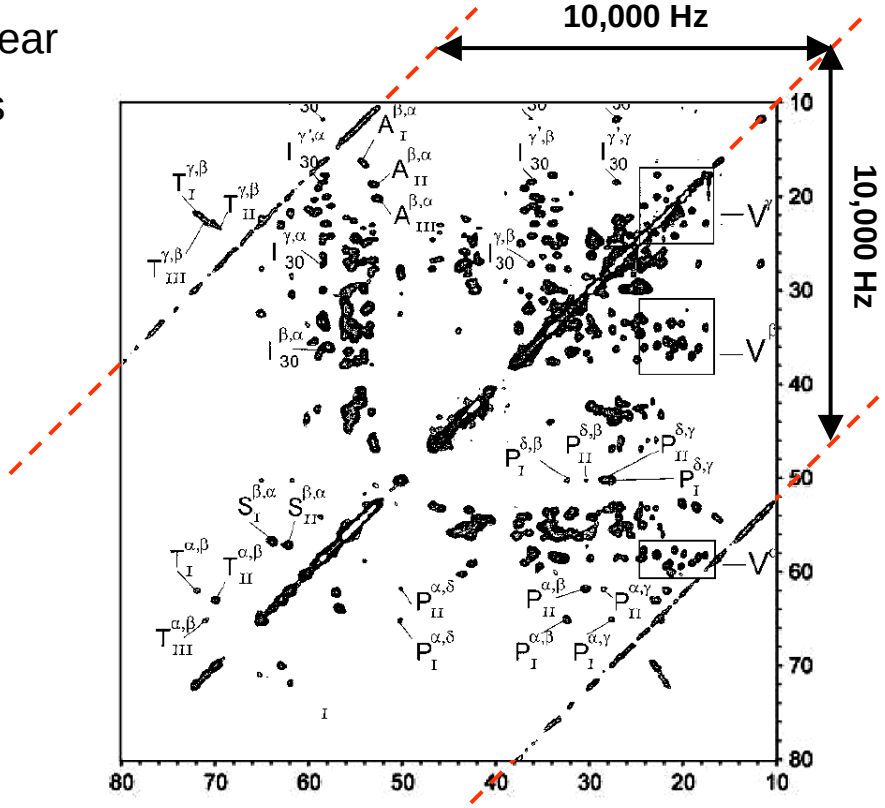
MAS: spinning side bands

Care should be taken when choosing the MAS frequency to avoid overlap and rotational resonance

In 2D spectra, side-bands appear as additional diagonal patterns

side bands diagonals in 2D spectra are not “artefacts”



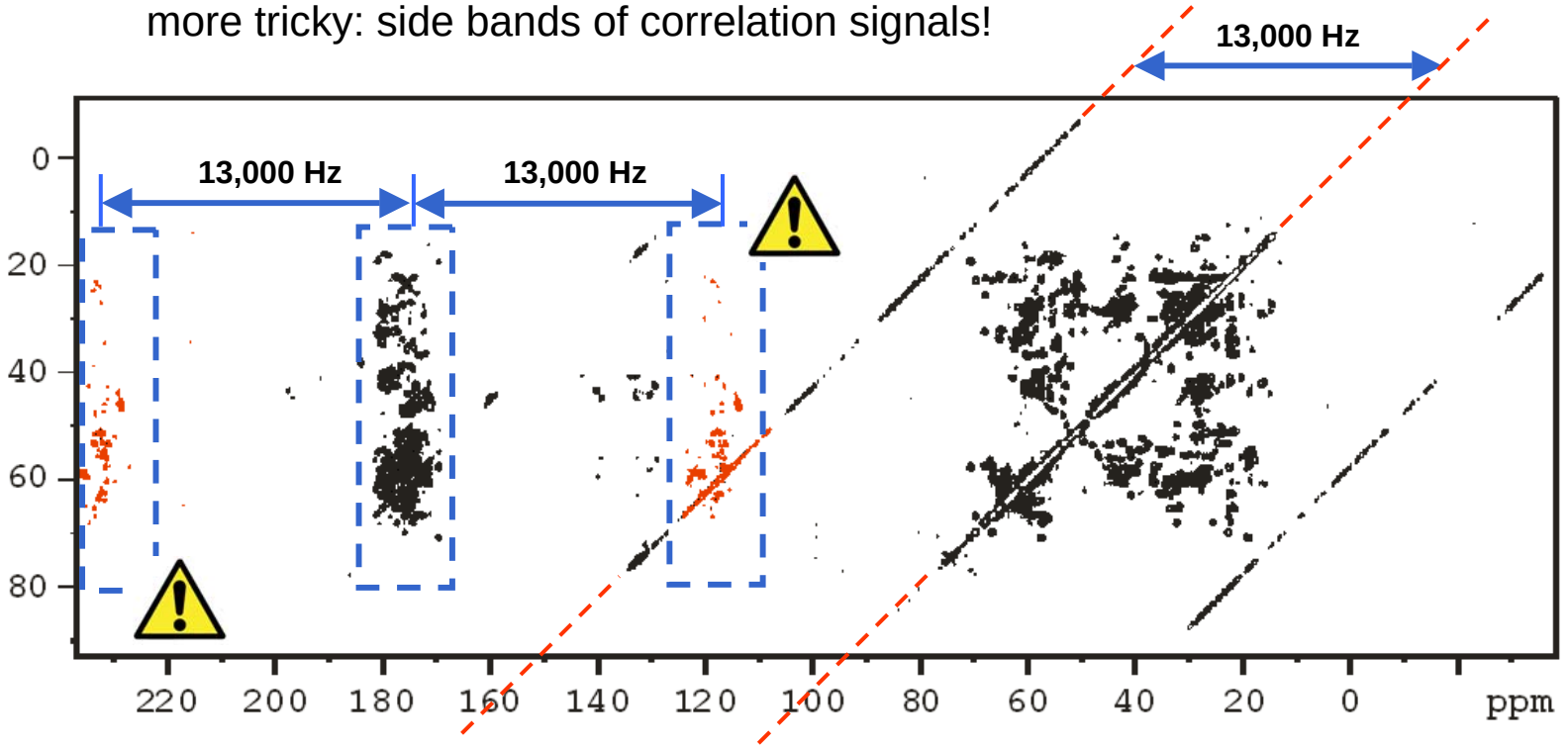


(2D spectrum recorded at 750 MHz, with 10 kHz MAS)

MAS: spinning side bands

Care should be taken when choosing the MAS frequency
to avoid overlap and rotational resonance

more tricky: side bands of correlation signals!



(2D spectrum recorded at 900 MHz, with 13 kHz MAS)

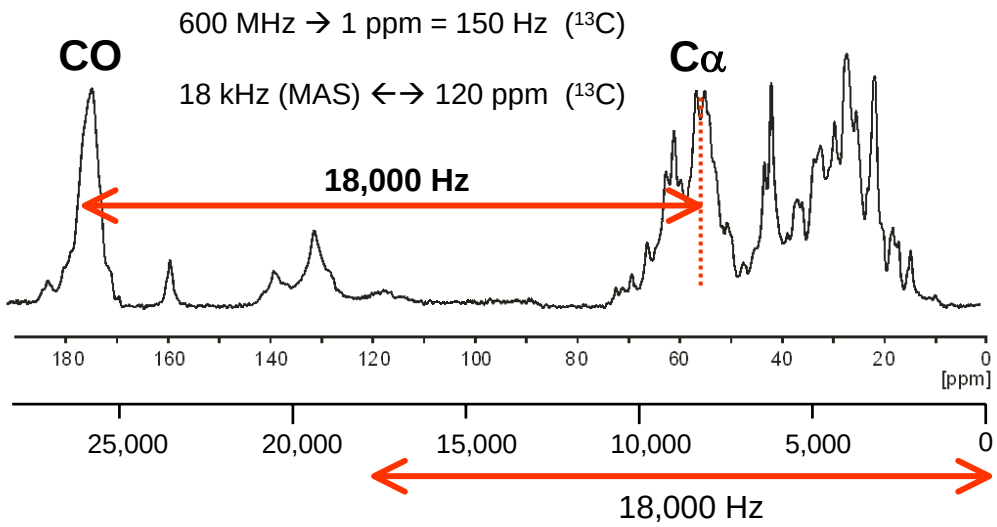
MAS: spinning side bands

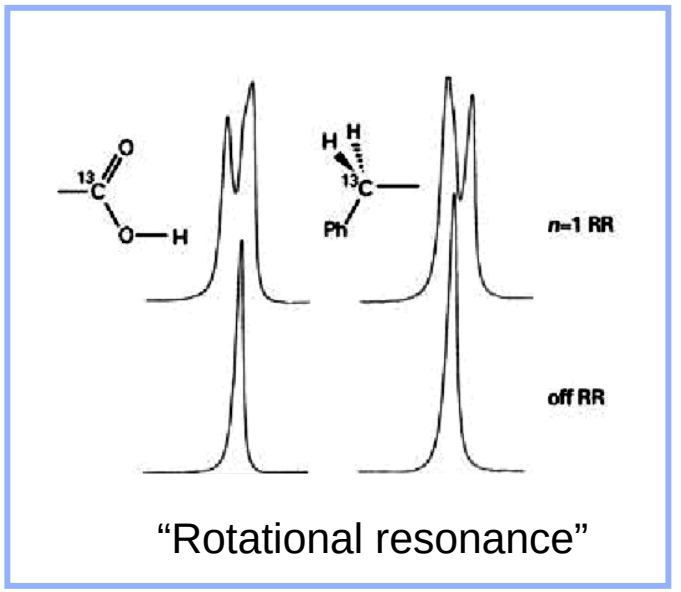
Care should be taken when choosing the MAS frequency
to avoid overlap and rotational resonance

MAS frequency matches the chemical shift difference of two signals (in Hz)

→ signals are coupled due to 'rotational resonance effect' (line splitting!)

→ should generally be avoided





"Rotational resonance"

Magic-Angle Spinning (MAS)

MAS mimics orientation averaging in liquids by imposing a collective reorientation of all molecules around a special axis

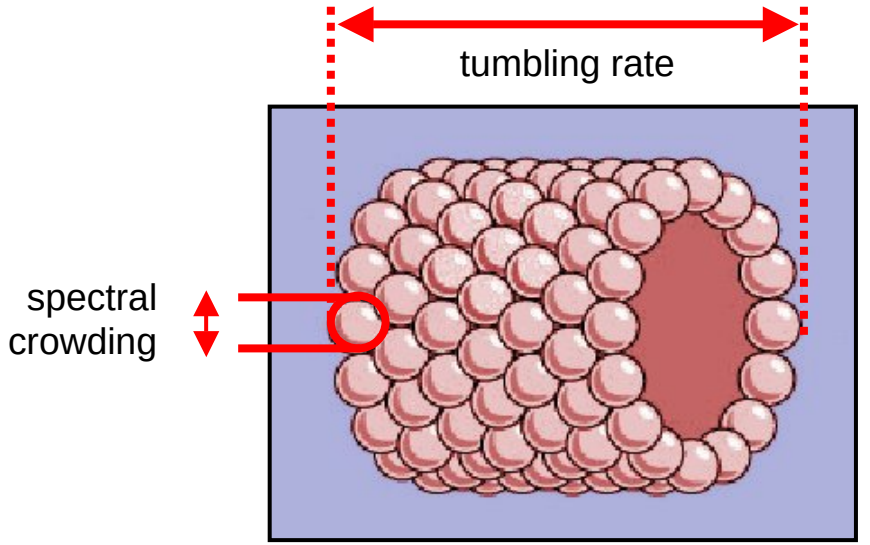
→ "tumbling rate" not dependent on size

what's more:

- without MAS, only single crystals give a high solid-state NMR resolution
- with MAS, you **do not need crystals**

However, it helps to have some sort of local order …

complex size versus protein size



solids *and* liquids:

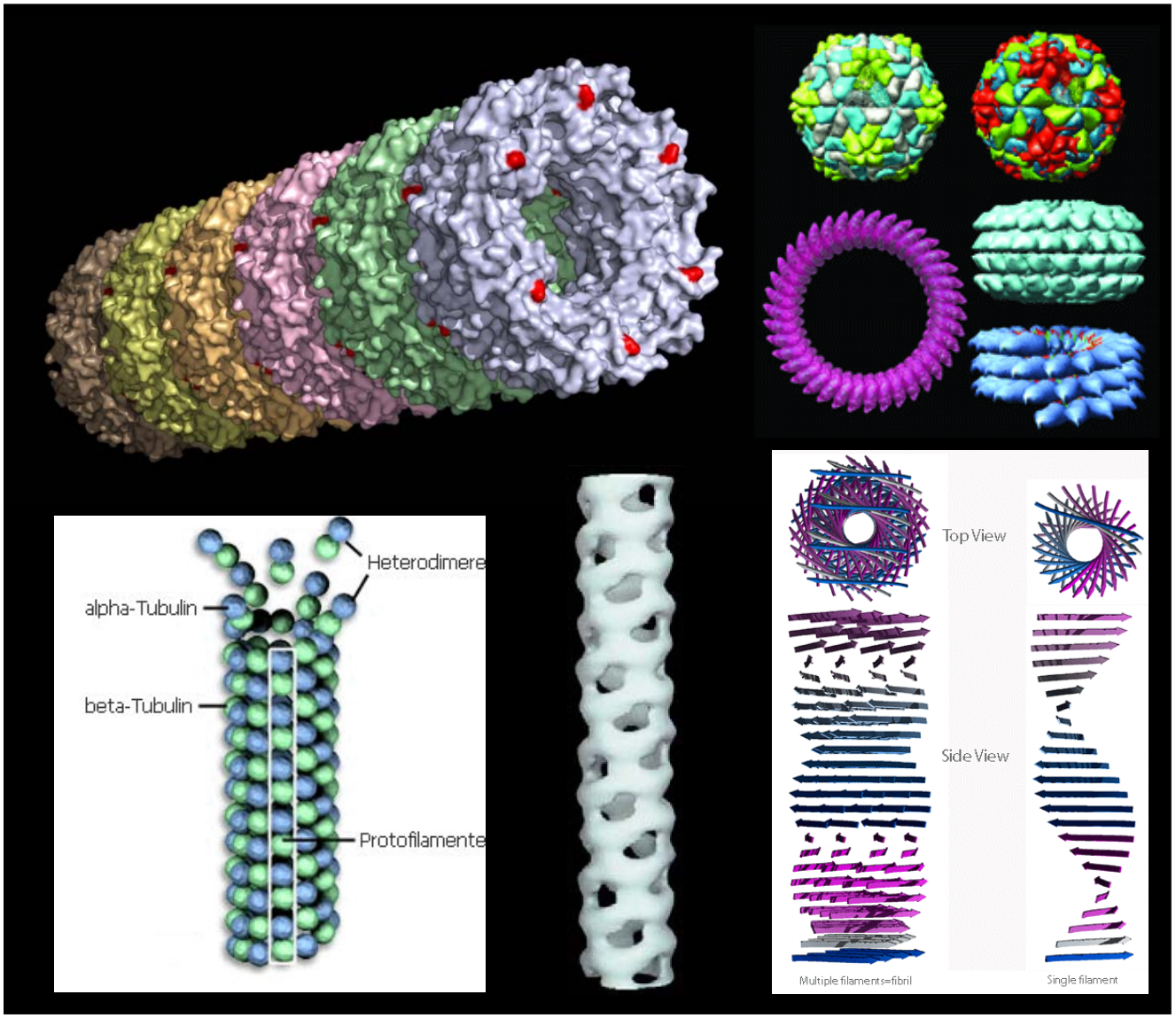
→ protein size determines spectral crowding

liquids:

→ complex size determines tumbling rate

solids:

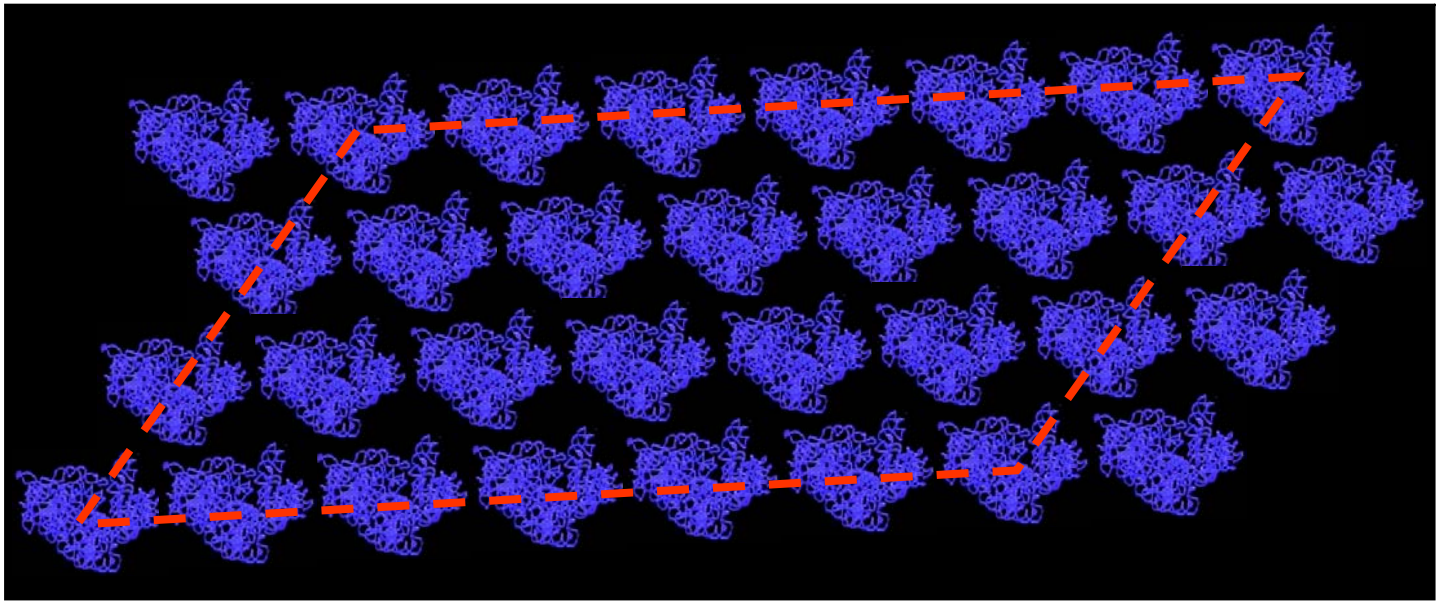
→ no upper limit for complex size

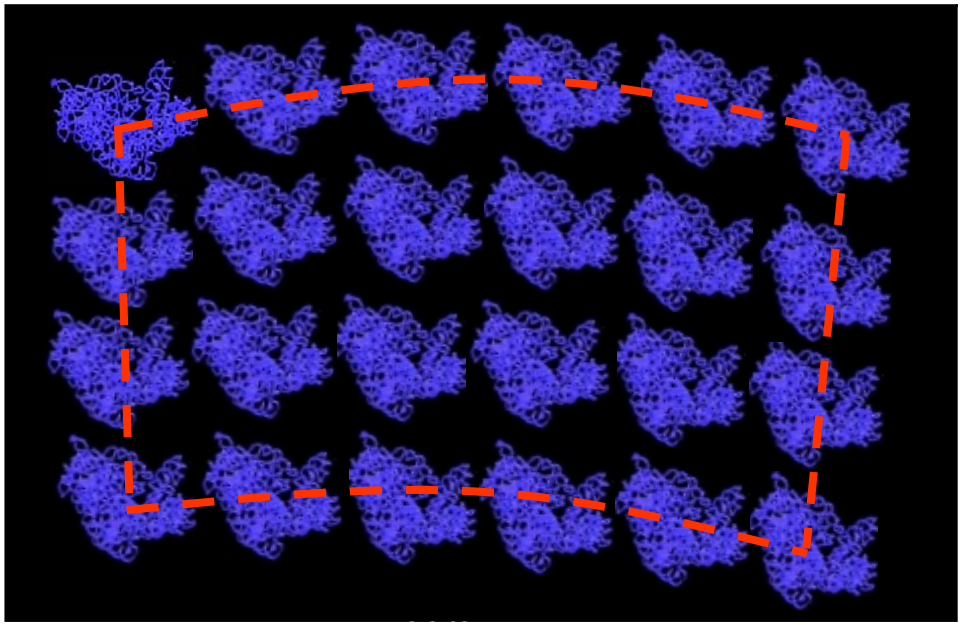


systems with high native symmetry not good enough for X-ray crystallography

- non-crystallographic symmetry
- rotation symmetry
- combination of rotation and translation: helical symmetry

X-ray crystallography requires unit-cell that repeats in all directions and is related by translation symmetry only



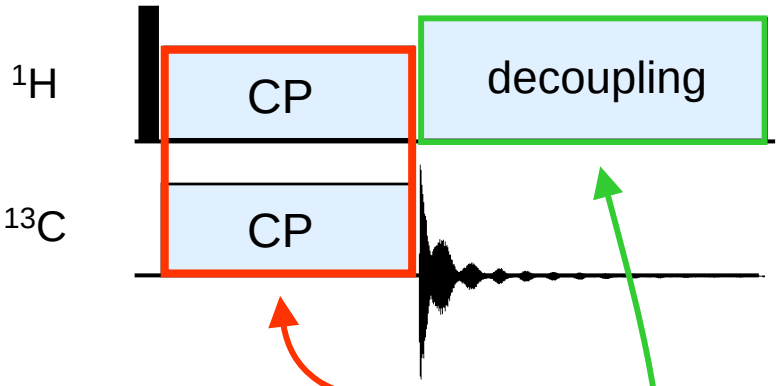


Ordered system provides better NMR resolution. But: no need for crystals in the ‘classical’ sense

X-ray:	long-range order required, single-crystals required
MAS NMR:	no long-range order required, - short-range order sufficient - non-crystallographic symmetries

The CP-MAS experiment

the standard MAS NMR experiment to detect ^{13}C

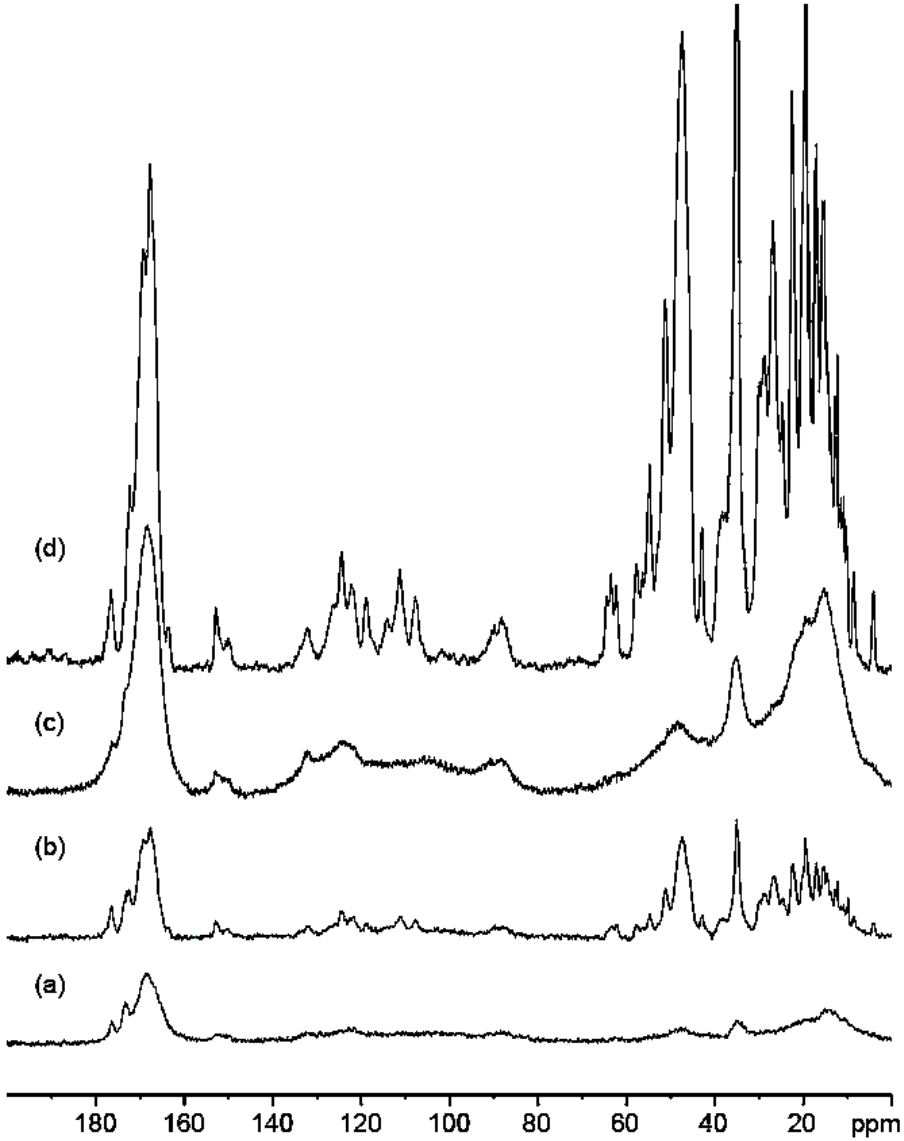


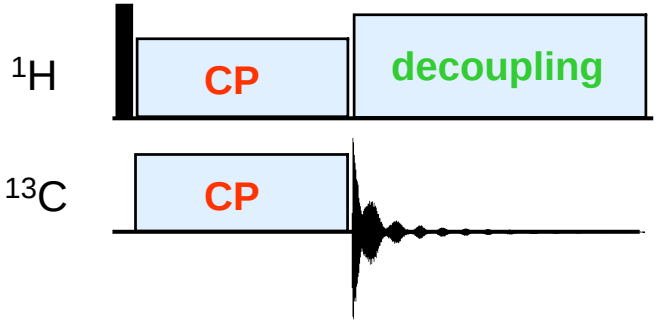
^{1}H used to enhance ^{13}C signal via cross-polarization (CP)

^{1}H ‘removed’ during data acquisition (decoupling)

^{1}H only used to get more signal

Effect of CP and decoupling on sensitivity





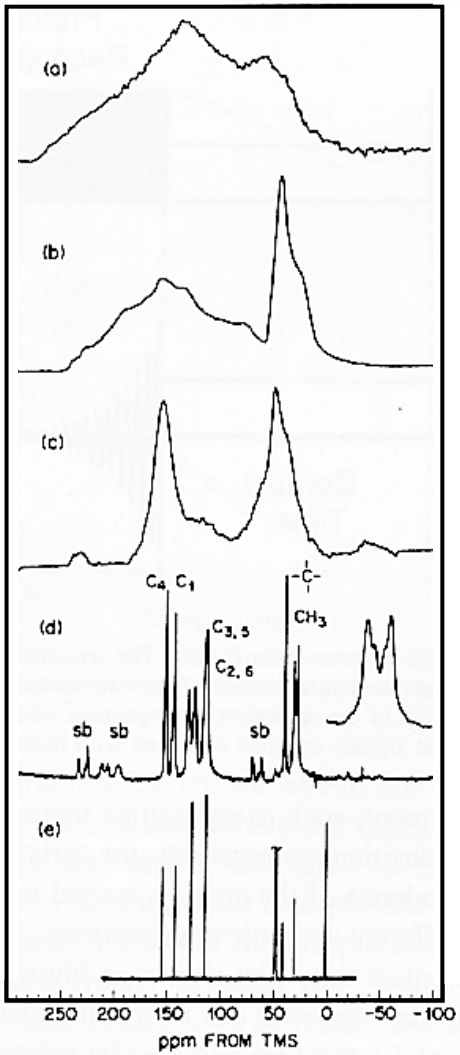
← **CP** + **decoupling**

← **CP** (no decoupling)

← (no CP) **decoupling**

← (no CP, no decoupling)

Effect of MAS and decoupling on ^{13}C resolution



← no MAS, no decoupling

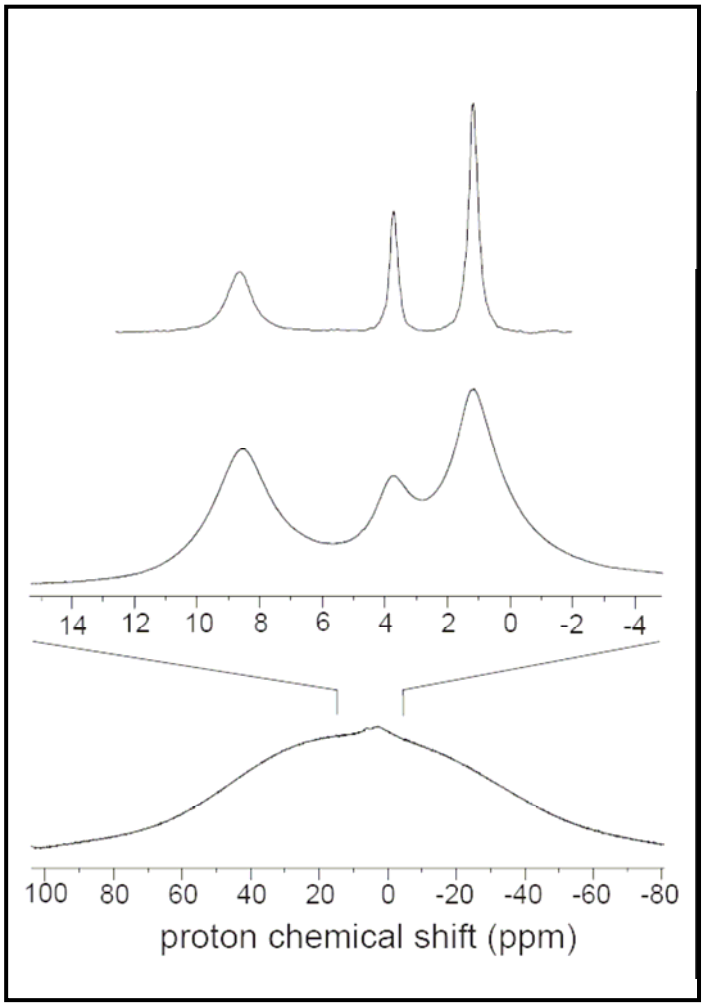
← no MAS, decoupling

← MAS, no decoupling

← MAS, decoupling

← solution NMR

Effect of MAS and decoupling on ^{1}H resolution



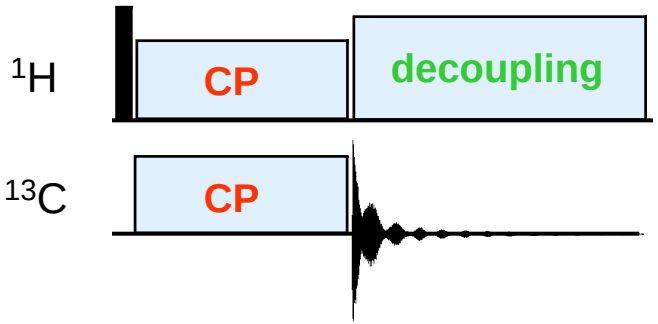
← MAS, ^{1}H-^{1}H decoupling

← MAS, no decoupling

← no MAS, no decoupling

Cross polarization (CP)

'classical' description of cross-polarization uses concept of **spin temperature**

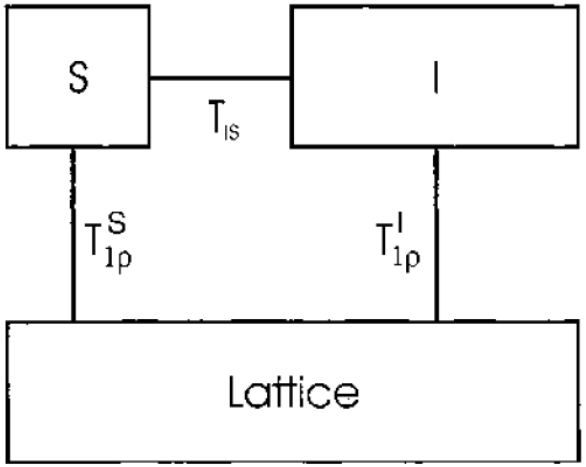


this approach is valid as long:

1. system contains a large number of spins
2. strong ^{1}H-^{1}H dipolar couplings are present

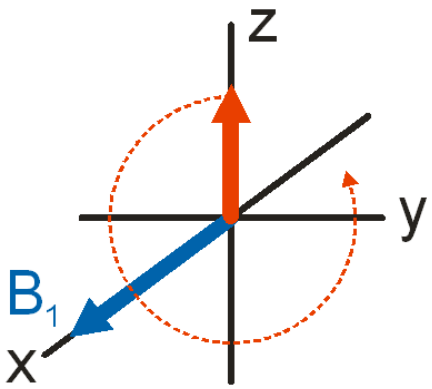
thermodynamic approach:

- polarization exchange between two reservoirs with different spin temperature coupled to a large reservoir ('lattice')
- relaxation to equilibrium

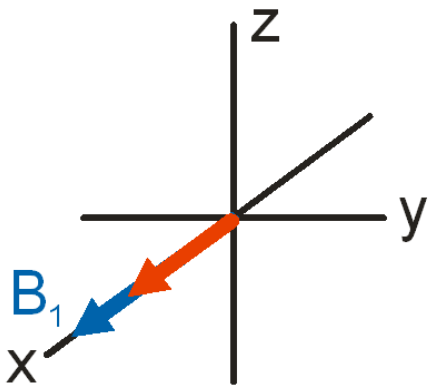


Cross polarization (CP)

during CP, both spin-types (^{1}H and ^{13}C) are '**spin-locked**'

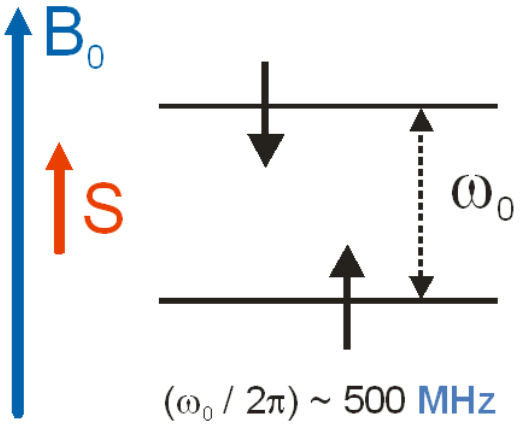


pulse :
spins rotate



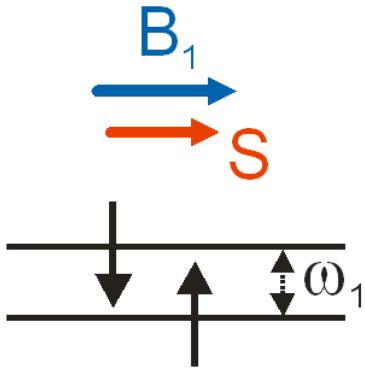
spin-lock pulse:
spins are trapped

like the Zeeman interaction, the spin-lock pulse gives rise to a **splitting** (spin up, spin down)



$(\omega_0 / 2\pi) \sim 500$ MHz

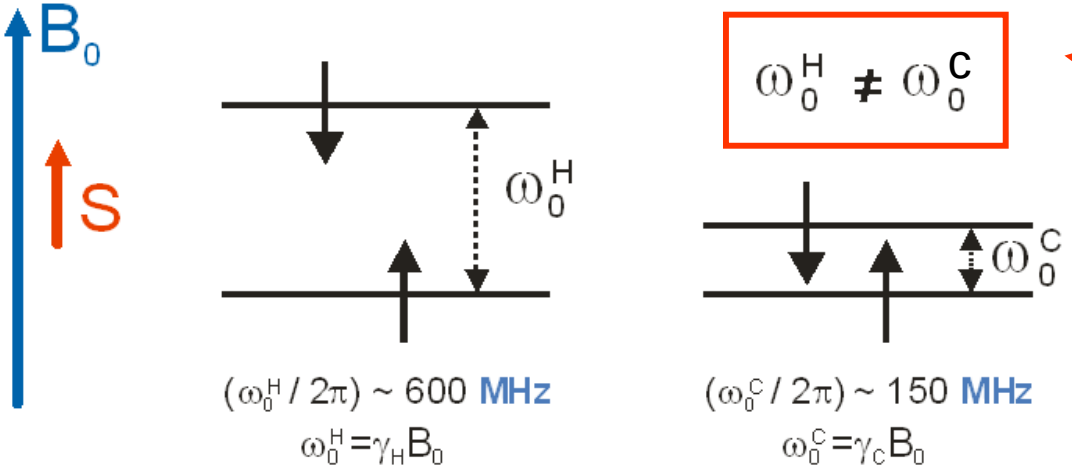
$\omega_0 = \gamma_H B_0$



$(\omega_1 / 2\pi) \sim 50$ kHz

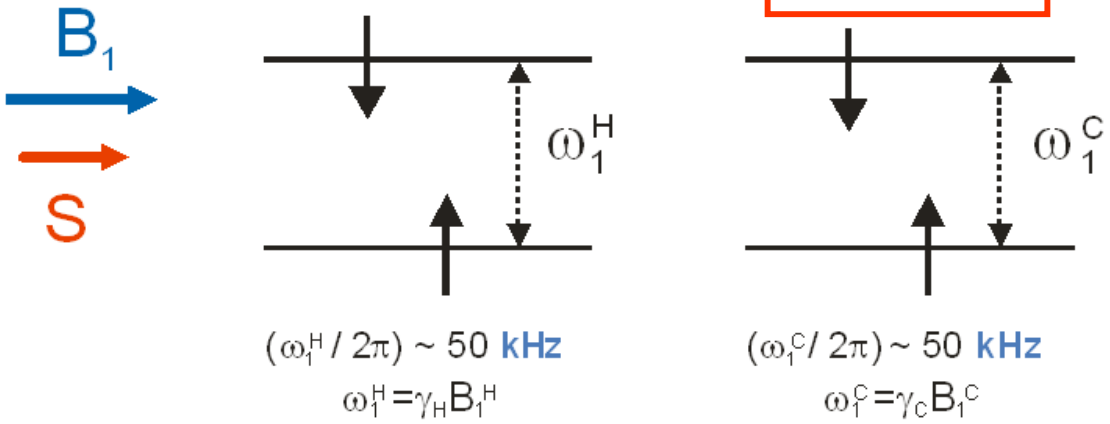
$\omega_1 = \gamma_H B_1$

Cross polarization (CP)



$\omega_0^H \neq \omega_0^C$

since B_0, γ_H and γ_C are all fixed, the Zeeman splitting is different for ^1H and ^{13}C …



$\omega_1^H = \omega_1^C$

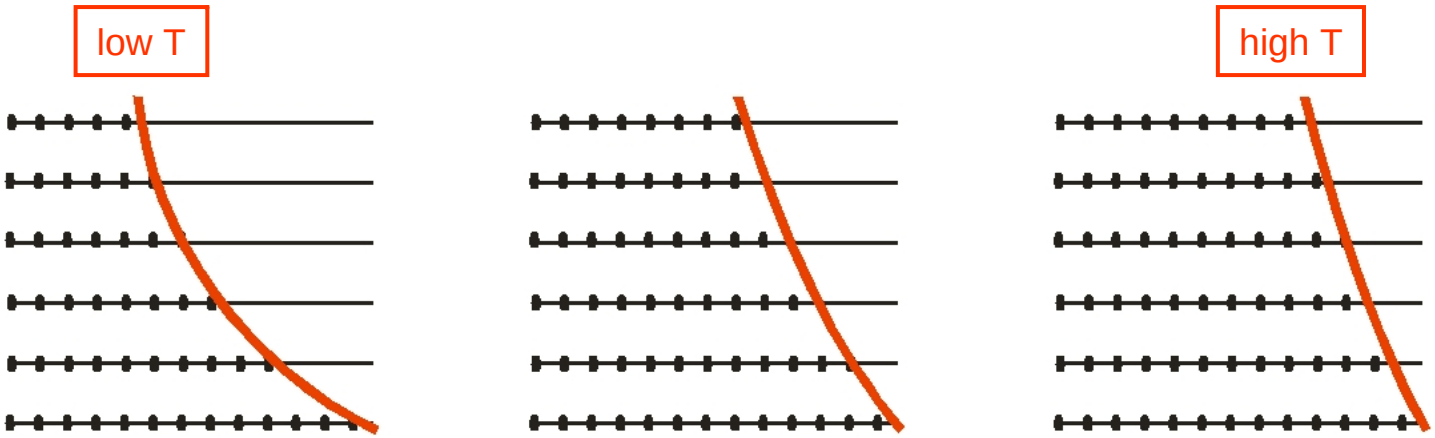
… however, the spin-lock fields B_1^H and B_1^C can be **chosen** so that the splitting for ^1H and ^{13}C becomes equal

Hartmann-Hahn Matching

“Boltzmann’s ingenious concept”

$$\frac{N_2}{N_1} = \exp\frac{-(E_2 - E_1)}{kT}$$

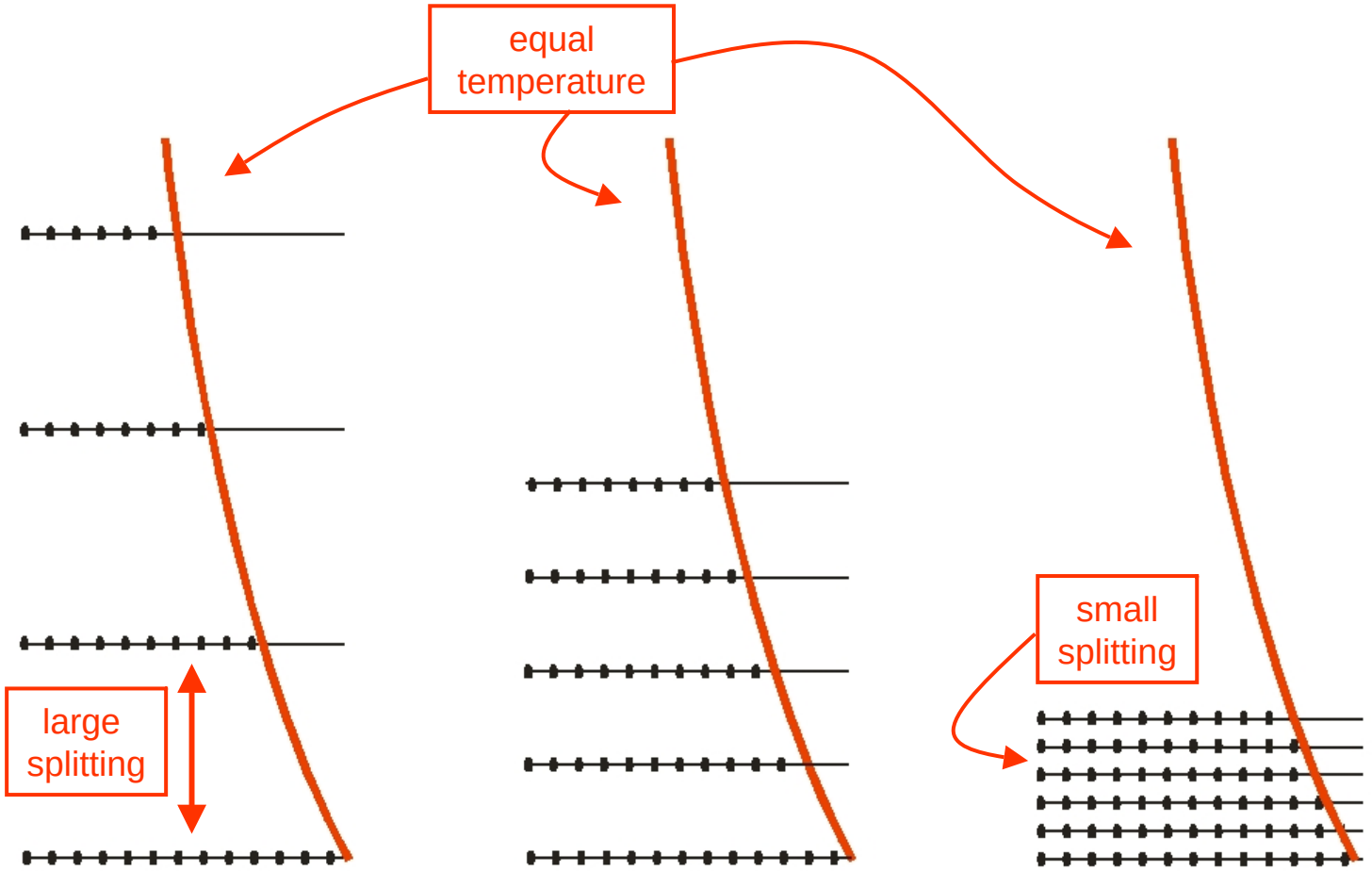
mhhh..



low T → large population differences → high polarization

high T → small population differences → low polarization

“Boltzmann’s ingenious concept”



large splitting → large population differences → high polarization
small splitting → small population differences → low polarization

Cross polarization (CP)

in equilibrium:

levels separated by ω_0

→ occupation of levels according to real Boltzmann distribution

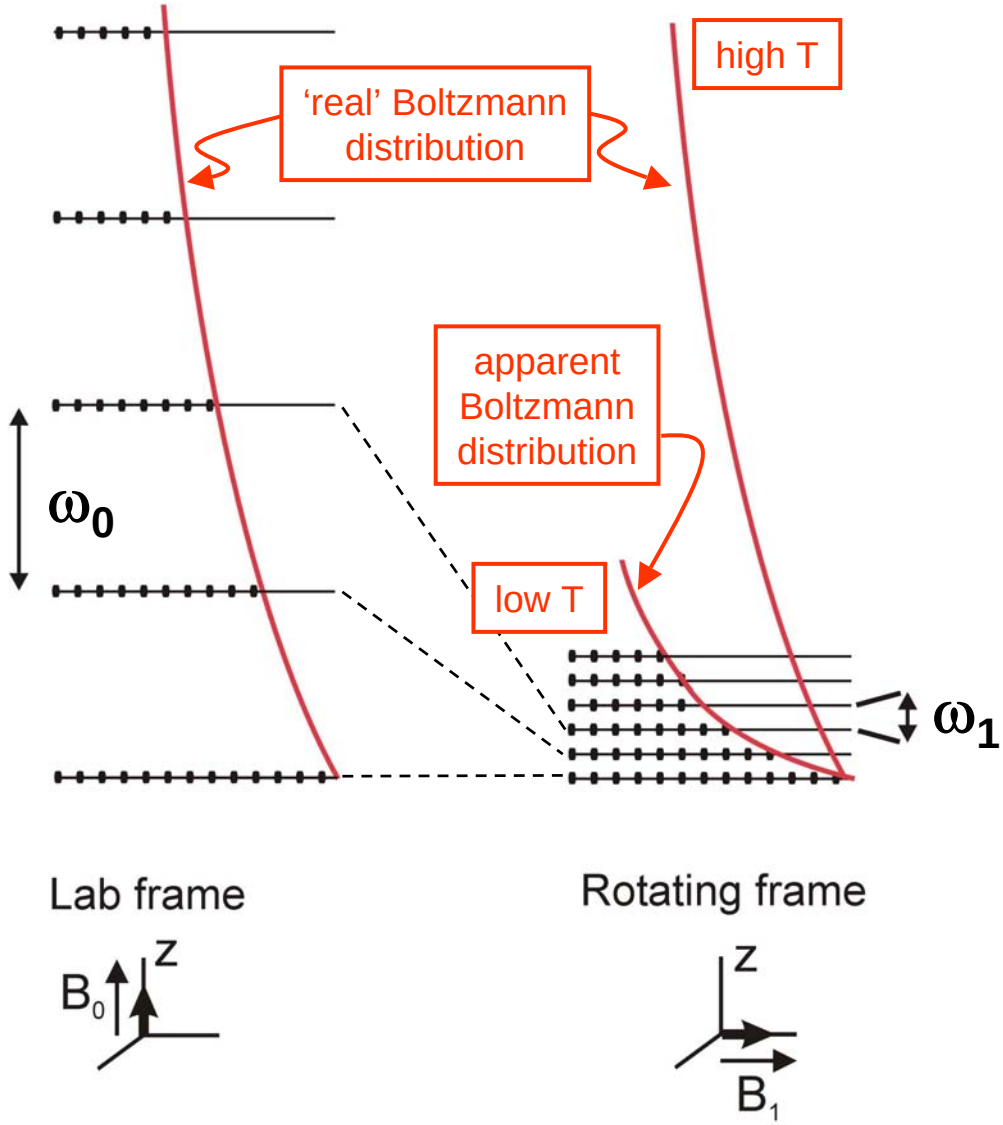
during spin lock:

levels separated by ω_1

with $\omega_1 << \omega_0$

→ levels are 'compressed'

→ occupation of levels looks like a Boltzmann distribution, but **one for much lower T**



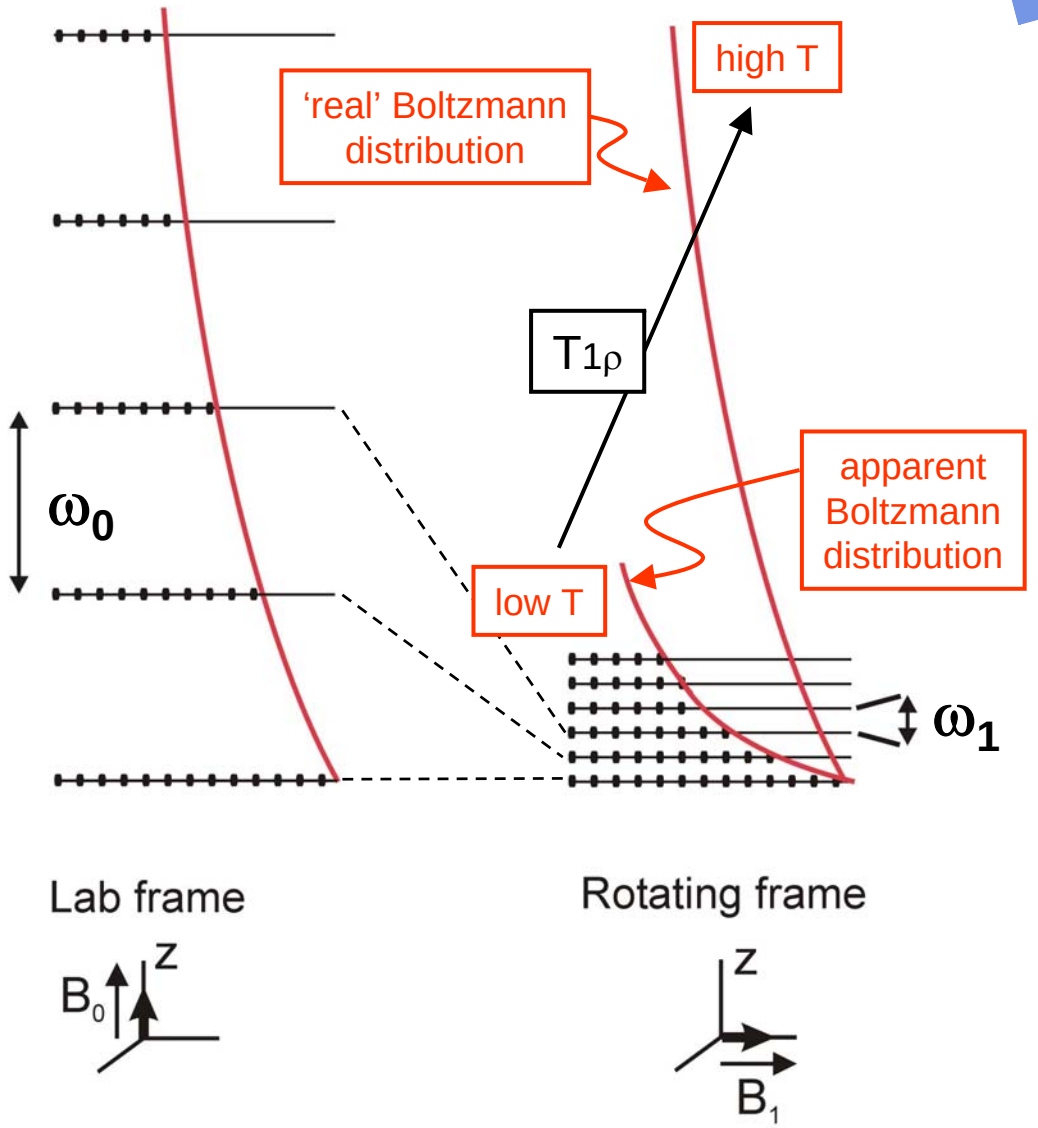
Cross polarization (CP)

spin locking ^{1}H

lowers the spin-temperature

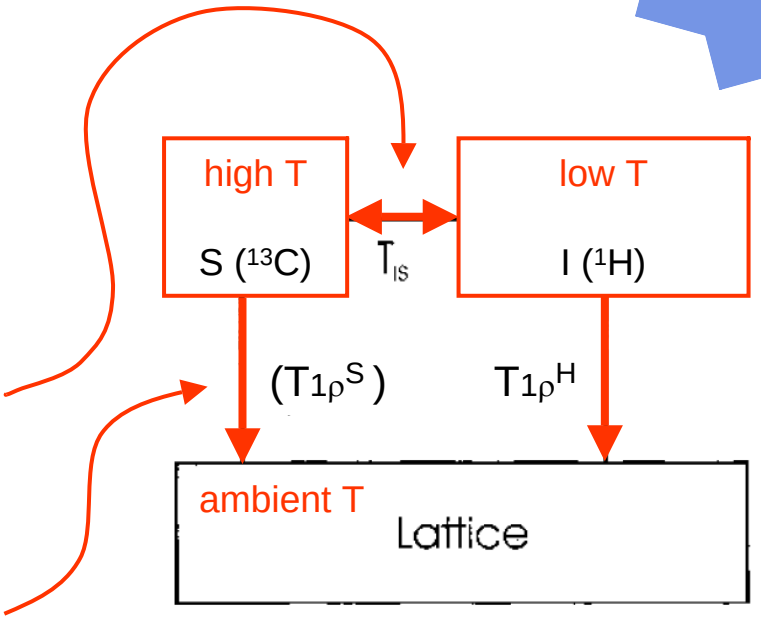
during spin lock:

relaxation back to the normal temperature with $T_{1\rho}$



Cross polarization (CP)

by matching energy splitting for ^{1}H and ^{13}C (Hartmann-Hahn matching), polarization can be exchanged with conservation of energy

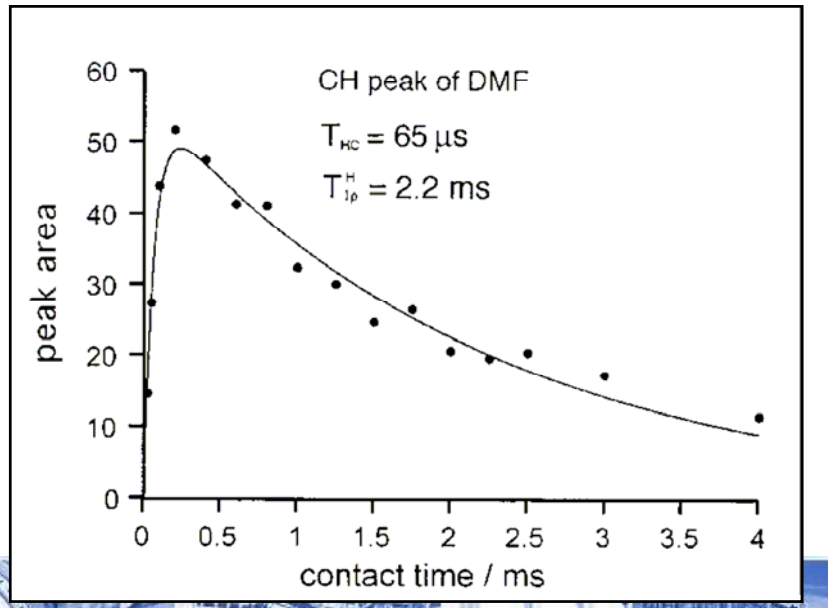


heteronuclear (^{1}H - ^{13}C) dipolar interaction couples ^{1}H and ^{13}C polarization reservoirs

homonuclear interaction (mostly ^{1}H- ^{1}H) provides coupling to lattice ($T1\rho$ relaxation)

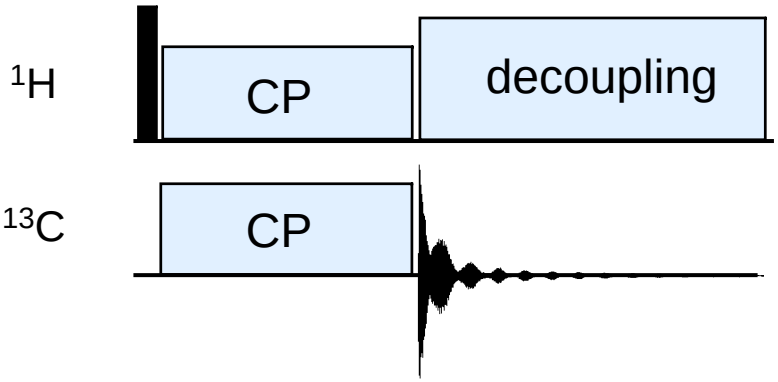
equilibration lowers ^{13}C spin temperature
→ ^{13}C polarization first increases (short CP)

coupling to lattice reduces spin temperature
→ ^{13}C polarization relaxes (long CP)



Cross polarization (CP)

'classical' description of cross-polarization
uses concept of spin temperature



this approach is valid as long:

1. system contains a large number of spins
2. strong ^{1}H-^{1}H dipolar couplings are present

however…

CP also works for an isolated ^{1}H-^{13}C pair

CP also works with suppression of ^{1}H-^{1}H coupling during spin lock