Magic-Angle Spinning (MAS)

magic-angle spinning is done pneumatically
spinning frequency can be stabilized within a few Hz
**Magic-Angle Spinning (MAS)**

Maximum spinning frequency depends on rotor diameter

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>Speed (kHz)</th>
<th>Force (x g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>15</td>
<td>1,400,000</td>
</tr>
<tr>
<td>3.2</td>
<td>25</td>
<td>2,700,000</td>
</tr>
<tr>
<td>2.5</td>
<td>35</td>
<td>3,500,000</td>
</tr>
</tbody>
</table>

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)

\[ \sigma_{\text{iso}} = \left( \sigma_{11} + \sigma_{22} + \sigma_{33} \right) / 3 \]

\( \sigma_{\text{iso}} \) = 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…)

À 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
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  \( \text{à} \)  1 ppm  =  700 Hz (\(^1\text{H}\))
\( \text{à} \)  1 ppm  =  175 Hz (\(^{13}\text{C}\))
\( \gamma_\text{H} : \gamma_\text{C} = 4 : 1 \)
\( \text{à} \)  12,000 Hz (MAS)  \( \sim \)  69 ppm (\(^{13}\text{C}\))
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)
à signals are coupled due to ‘rotational resonance effect’ (line splitting!)
à should generally be avoided

600 MHz à 1 ppm = 150 Hz (\(^{13}\)C)
18 kHz (MAS) ß à 120 ppm (\(^{13}\)C)

“Rotational resonance”
The CP-MAS experiment

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

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

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

$^1\text{H}$ 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
Chemical shift assignment: identification of side chains

assignment procedure is a two-step mechanism
1. identification of spin-systems (amino acids)
2. connecting spin-systems via backbone

\( ^{13}\text{C} \) chemical shifts are used to identify spin systems
(in liquids \(^1\text{H} \) shifts are used for identification)
Chemical shift assignment: identification of side chains

residues can be identified from characteristic $^{13}\text{C}^-^{13}\text{C}$ correlation patterns
Chemical shift assignment: sequential assignment

- specific or adiabatic CP
- typical building blocks for finding sequential connectivities

\[ \text{NCO transfer: inter-residual, connects sequential residues} \]
\[ \text{NCA transfer: intra-residual, transfer “within” residue} \]
Sequential assignment of L131-T132-I133 motive
Sequential assignment of L131-T132-I133 motive
Dipolar interaction

the dipolar coupling is the interaction between two magnetic moments

it depends on:
- the distance between the spins \( r \)
- the angle \( \theta \) between the magnetic field \( B \)
  and the vector connecting the spins

\[
D \propto \frac{1}{r^3} (3\cos^2 \theta - 1)
\]

\[
\left\{ \begin{array}{l}
\langle 3\cos^2 \theta - 1 \rangle = 0 \text{ in isotropic liquids} \\
\langle 3\cos^2 \theta - 1 \rangle \neq 0 \text{ in solids or oriented media}
\end{array} \right.
\]
Dipolar interaction

under magic angle spinning, the average angle
between the connection vector and B is the magic angle $\theta_m$

$$3\cos^2 \theta - 1 \neq 0$$

$$3\cos^2 \theta_m - 1 = 0$$
Dipolar interaction

The time average of every spin-pair has the connecting vector at the magic-angle time average is zero for $\theta = 54.7^\circ$

$$3\cos^2 \theta_m - 1 = 0$$

$\theta = 54.7^\circ$
Interaction under MAS

Similar considerations can be made for any interaction that contains the $3\cos^2 \theta - 1$ term, like the chemical shift interaction.

**Chemical shift interaction**

- Anisotropic part
- Isotropic part

$$3\cos^2 \theta - 1$$

**Dipolar interaction**

- Anisotropic part

$$3\cos^2 \theta - 1$$

Not orientation dependent
Dipolar interaction

Note: the time average is zero, **not** the dipolar coupling itself

the dipolar interaction is time dependent and oscillates around 0
Recoupling

The time-average of the dipolar coupling is zero but **not** the instantaneous coupling, it is oscillating around zero.
Recoupling

Recoupling techniques are useful for **short-range** transfer (e.g., for assignment of spin systems)

\[
D \propto \frac{1}{r^3} (3\cos^2 \theta - 1)
\]

however, distance information is more difficult to obtain if more than two spins interact in particular, for uniform isotope labelling, long range transfer is difficult due to **dipolar truncation** effects
Dipolar truncation

the phenomenon that weak couplings are ‘quenched’ by the strong couplings
à weak couplings contain the long-range distance information

\[ D \propto \frac{1}{r^3} (3\cos^2 \theta - 1) \]
**Dipolar truncation**

The phenomenon that weak couplings are ‘quenched’ by the strong couplings. Weak couplings contain the long-range distance information.

- Spin dilution

\[
D \propto \frac{1}{r^3} (3\cos^2 \theta - 1)
\]

Note: pair-wise labelling is maximal spin dilution
Dipolar truncation

the phenomenon that weak couplings are ‘quenched’ by the strong couplings
à weak couplings contain the long-range distance information

à spin dilution
à selective recoupling

\[ D \propto \frac{1}{r^3} (3\cos^2 \theta - 1) \]
Extensive $^{13}$C labelling to reduce dipolar truncation

amino-acid labelling bacteria grown on:
1,3-$^{13}$C glycerol (green)
or 2-$^{13}$C glycerol (red)

labelling mostly alternating
à strong couplings removed
à weak couplings not quenched

however, analytical treatment will still be extremely difficult
Extensive $^{13}$C labelling to reduce dipolar truncation

à less signals in the spectra
à partial suppression of $J$-couplings
à less assignment options