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

<table>
<thead>
<tr>
<th></th>
<th><strong>solids</strong></th>
<th><strong>liquids</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>dipolar coupling</td>
<td>10 - 100 kHz</td>
<td>scalar coupling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 – 100 Hz</td>
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<tr>
<td>anisotropic interactions</td>
<td></td>
<td>(mostly) isotropic interactions</td>
</tr>
<tr>
<td>13C detection</td>
<td>(1H detection when deuterated)</td>
<td>1H detection</td>
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<tr>
<td>sensitivity low</td>
<td></td>
<td>sensitivity high</td>
</tr>
<tr>
<td>requires magic-angle spinning</td>
<td></td>
<td>natural tumbling of molecules</td>
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</table>
solids-state NMR vs. liquid-state NMR

dipolar coupling between protons:

~ 120 kHz @ 1.0 Å
~ 40 kHz @ 1.5 Å

solid-state NMR
no magic angle spinning
no $^1$H-$^1$H decoupling

liquid-state NMR

proton chemical shift (ppm)
solids-state NMR vs. liquid-state NMR

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

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
Chemical shift anisotropy (CSA)

spherical symmetry
\[ \sigma_{11} = \sigma_{22} = \sigma_{33} \]

non-axial symmetry
\[ \sigma_{11} \neq \sigma_{22} \neq \sigma_{33} \]

axial symmetry
\[ \sigma_{11} = \sigma_{22} \quad \text{(or)} \]
\[ \sigma_{22} = \sigma_{33} \]

\[ \sigma_{11} \leq \sigma_{22} \leq \sigma_{33} \]
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

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

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

- \( \sigma_{\text{iso}} = \text{isotropic chemical shift} \)
- 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:

\[ \Delta H \delta = -1.5 \text{ ppm} \]
\[ \Delta C \delta = -1.1 \text{ ppm} \]

shifts for L8 CHδ1:

\[ \Delta H \delta 1 = -1.3 \text{ ppm} \]
\[ \Delta C \delta 1 = -2.0 \text{ ppm} \]
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 $\rightarrow$ 1 ppm = 700 Hz ($^1$H)
$\rightarrow$ 1 ppm = 175 Hz ($^{13}$C)
$\gamma_H : \gamma_C = 4 : 1$

$\rightarrow$ 12,000 Hz (MAS) $\sim$ 69 ppm ($^{13}$C)
**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”.
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

600 MHz → 1 ppm = 150 Hz (\(^{13}\)C)
18 kHz (MAS) ←→ 120 ppm (\(^{13}\)C)
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 …

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 $^{13}\text{C}$

$^1\text{H}$ 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

- (a) (no CP, no decoupling)
- (b) (no CP) decoupling
- (c) CP (no decoupling)
- (d) CP + 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 $^1\text{H}-^1\text{H}$ 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 (\(^1\text{H}\) and \(^{13}\text{C}\)) are ‘spin-locked’

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

\[
\begin{align*}
(\omega_0 / 2\pi) &\sim 500 \text{ MHz} \\
\omega_0 &= \gamma_H B_0 \\
(\omega_1 / 2\pi) &\sim 50 \text{ kHz} \\
\omega_1 &= \gamma_H B_1
\end{align*}
\]
Cross polarization (CP)

![Diagram of CP process]

Since $B_0$, $\gamma_H$ and $\gamma_C$ are all fixed, the Zeeman splitting is different for $^1H$ and $^{13}C$...

...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”

\[
\frac{N_2}{N_1} = \exp \left( \frac{-(E_2 - E_1)}{kT} \right)
\]

low T \rightarrow large population differences \rightarrow high polarization

high T \rightarrow small population differences \rightarrow low polarization
“Boltzmann’s ingenious concept”

- Equal temperature
  - Large splitting
  - Large population differences
  - High polarization
  - Small splitting
  - Small population differences
  - Low polarization
Cross polarization (CP)

in equilibrium:
levels separated by $\omega_0$
$\rightarrow$ occupation of levels according to real Boltzmann distribution

during spin lock:
levels separated by $\omega_1$
with $\omega_1 \ll \omega_0$
$\rightarrow$ levels are ‘compressed’
$\rightarrow$ 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 $^1\text{H}$ and $^{13}\text{C}$ (Hartmann-Hahn matching), polarization can be exchanged with conservation of energy

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

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

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

coupling to lattice reduces spin temperature → $^{13}\text{C}$ 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