Cross Polarization
Why don’t we normally detect protons in the solid-state

- Strong couplings between protons ($\delta_J > 20$kHz)
- Homogeneous interaction
  - Not readily averaged at moderate spinning speeds
- Methods for removing the couplings between protons during acquisition challenging
- Result
  - Typically we exploit low-$\gamma$ nuclei
Disadvantages of detecting low-$\gamma$ nuclei

- Natural abundance levels not always high
  - enrichment
- Low gyromagnetic ratio means the signal is attenuated
  - Solution transfer of polarization from protons to low $\gamma$-nuclei (INEPT?)
**1D $^1H/^{15}N$ INEPT NMR Spectrum**

$\tau/2$  

$H=2\pi J_{1z}l_{2z}+\Omega_1 l_{1z}+\Omega_2 l_{2z}$

$H_{CS}=\Omega_1 l_{1z}+\Omega_2 l_{2z}$

**QUESTION:**
What form does the $^{15}N$ signal take?
Why is INEPT not typically used in the solid-state?

- J-couplings exist in the solid-state why not use them
- Inhomogeneous broadening, short $T_2$ reduce sensitivity
- Could use re-focussed INEPT by $T_2$ problems still attenuate signal
Cross-polarisation

- In solid-state NMR we have other interactions we can exploit.
- Strong coupling between a bath of $^1H$ and low $\gamma$ nuclei.
Outline of what is happening

• Transfer of polarization from $^1$H to low-$\gamma$ nuclei
How does the transfer occur

Several models explain the behaviour:

1) Quantum mechanical
   - Coherent description of transfer of magnetization between two spins.

2) Thermodynamic
   - Coupling of a high temperature bath (proton, abundant magnetization) with a low temperature (low-γ nuclei) via the dipolar coupling and the equilibration of temperature.

3) Ingenious?
“Hahn’s Ingenious Concept¹” (1)

Normally two heteronuclear spins resonate at

\[ \omega_I = \gamma_I B_0 \quad \text{and} \quad \omega_S = \gamma_S B_0 \]

and pulses applied to I or S affect I or S.

If we apply resonant fields to I and S they precess with a frequency

\[ \Omega_I = \gamma_I B_{1I} \quad \text{and} \quad \Omega_S = \gamma_S B_{1S} \]

We can make the precession frequencies match by adjusting the frequency \( B_{1} \) of individual nuclei. When these conditions match we obtain the so called Hartmann-Hahn condition:

\[ \gamma_I B_{1I} = \gamma_S B_{1S} \]

¹) Principles of magnetic resonance, C.P. Slichter p277
“Hahn’s Ingenious Concept\(^1\)” (2)

Fulfilled Hartmann-Hahn condition

\[ \gamma_I B_{1I} = \gamma_S B_{1S} \]

I spin in close proximity to S spin so we have a strong heteronuclear dipolar coupling:

\[ H_{D,IS} = \frac{\mu_0}{4\pi} \frac{\gamma_I \gamma_S \hbar}{r_{12}^3} \left(1 - 3\cos^2 \theta\right) \left(2 \hat{i}_{1z} \hat{i}_{2z}\right) \]

Thus we can get resonant transfer of energy from the I to the S spin.

1) Principles of magnetic resonance, C.P. Slichter p277
Experimentally what is observed (1)

- The width of the matching condition is proportional to the strength of the dipolar coupling in both the static and MAS cross-polarisation experiment.
Experimentally what is observed (2)

- For a single coupling between I and S build-up is oscillatory
  \[ M_S = \frac{1}{2}(1 - \cos(\omega_{IS}t)) \]

- However, \( \omega_{IS} \) is orientation dependent and the efficiency is governed by the powder distribution. Result maximum efficiency is 72%.

- In reality many protons are coupled to a given low-\( \gamma \) nuclei and the build-up is not oscillatory and behaviour can be described as an exponential build-up.
Hartmann-Hahn Condition under MAS

• Under MAS the heteronuclear dipolar coupling is averaged, cross polarisation shouldn’t work!

• As shown by Stejskal and Schaeffer transfer does occur as coupling is “not completely averaged but becomes time dependent”.

• Under these conditions the Hartmann-Hahn condition breaks down into:
  \[ \gamma_I B_{1I} = \gamma_S B_{1S} \pm n \]
  where \( n = \pm 1,2 \) (at longer mixing times \( n=0 \) is also visible)

Matching Condition for adamantane with a contact Time of 1 and 16ms respectively with 5 kHz MAS
Effects of dynamics on cross polarisation

Buildup of magnetization dependent on dynamics:

- $T_{HC} \rightarrow \mu s$ timescale
- $T_{1p} \rightarrow ms$ timescale

Profiles can be used to analyze dynamics and follow the following behavior

$$I_{ct} = I(0)e^{-ct/T_{1p}}\left[1 - e^{-ct/(1/T_{HC} - T_{1p})}\right]$$
Advantages of cross-polarisation

- The low-$\gamma$ polarization is enhanced by $\gamma_I/\gamma_S$
  - e.g. for $^{15}$N $\gamma_I/\gamma_S=10$, for $^{13}$C $\gamma_I/\gamma_S=4$

- As the polarization is derived from the protons, which typically relax faster than low-$\gamma$ nuclei the recycle delay of the experiment can typically be faster
Experimental difficulties applying CP

- In both static and MAS cross polarisation the width of the matching condition is proportional to the strength of the heteronuclear dipolar coupling.

- For weak couplings or mobile samples (where the dipolar coupling is averaged) width is small and experimentally it is difficult to match the Hartmann-Hahn condition.

- Small fluctuations in amplifier output can cause a mis-setting of the Hartmann-Hahn condition.

- Distribution of rf fields within the sample coil.
Improvements on the basic idea

- Ramped cross polarization

- Adiabatic cross polarization (100% transfer?)

- Multitude of others in literature.....
Application of CP

• Signal enhancement ✓
• Motional Filter
• Characterising Motions
  – $T_{HC}/T_{1p}$ ✓
  – $S_{HC}$
Application of CP

Excess ACh (Direct observation)

AChR - ACh

AChR + ACh

Difference

Direct

Cross Polarized

\( H_D \)

\( ^1H \)

\( ^{13}C \)

Isotropic

\( 0 \)

Restricted/Anisotropic

\( 20\,\text{kHz} \)
Coherent build-up of intensity enables the strength of the dipolar coupling between the 1H and 13C to be determined.

This can be used to determine an order parameter/measure of mobility for individual sites within the protein.

Lorieau and McDermott (2006) JACS 128:11505-12
Solid-state NMR spectra
Dipolar recoupling techniques and 2-dimensional NMR
Methods for transfer polarization and their application

Know how to manipulate anisotropic interactions to gain in resolution and sensitivity

These anisotropic interactions contain lots of structural/dynamic information

If we can selectively reintroduce these interactions we can obtain the information they encode

Need methods that selectively reintroduce these interactions when we perform MAS
Methods for transfer polarization and their application

Overview dipolar recoupling under magic angle spinning:

1) Heteronuclear dipolar couplings:
   a) Cross Polarization (moving from HX to XY)
   b) REDOR

2) Homonuclear dipolar recoupling methods
   a) selective (rotational resonance)
   b) broadband (proton driven spin diffusion/C7)
“Hahn’s Ingenious Concept\textsuperscript{1}” (1)

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\[ \frac{\omega_{II} - \omega_{I}}{\omega_{I}} \]

$^1H-^{13}C$

$^{15}N-^{13}C$
Weaker couplings:
Increased problems with relaxation
Applications assignment

NCA Experiment on microcrystalline ubiquitin.
Rotational Echo Double Resonance (REDOR)

Dephasing of X through the recoupling of the XY heteronuclear dipolar coupling

\[ \frac{\pi}{2} \gamma \text{ Spin Lock} \]

\[ ^1\text{H} \]

\[ \pi \text{ Decouple} \]

\[ X \]

\[ \pi \ \pi \ \pi \ \pi \ \pi \ \pi \]

\[ Y \]

\[ \omega_r \]
Averaging of anisotropic interactions

Anisotropic interactions oscillate with a $\omega_r$ and $2\omega_r$ dependence under MAS e.g. CSA

$$\omega_{CSA}(\alpha, \beta, \gamma) = C_1 \cos(\omega t - \gamma) + S_1 \sin(\omega t - \gamma) + C_2 \cos(2\omega t - 2\gamma) + S_2 \sin(2\omega t - 2\gamma)$$

If we want to selectively reintroduce them under MAS we want to find a way of selectively disrupting this averaging.
Spin/Spatial Interactions

Anisotropic interactions are time dependent under MAS

Can interfere with MAS selectively disturbing averaging process

Example apply $\pi$ pulses during rotor cycle results in non-zero contribution from heteronuclear dipolar coupling

\[ \omega \sim 2D_{IS}(\sin(\omega_r) + \sin(2\omega_r)) \cdot I_k \]
Dephasing curves – distance measurements

Example dephasing data here.

1.54 Å

2.5 Å

4.0 Å
Distances in biomolecules

Murphy et al. 2001
Transferred echo double resonance (TEDOR)

INEPT in the solid-state?
Homonuclear recoupling methods

a. Dipolar assisted rotary recoupling (DARR)

b. Proton driven spin diffusion/Rotation resonance (selective)

c. Radio frequency driven recoupling

d. C7/POST-C7

e. HORROR/DREAM
Homonuclear recoupling under MAS

1. Rotation resonance (selective)
Rotational resonance – lineshape

[Diagram showing spectra and rotational resonance equations]
Rotational resonance

\[ \omega_r = \delta_2 - \delta_1 \]

Same chemical shift

Different chemical shift
Magnetization exchange experiments

\[ \omega_r = \delta_2 - \delta_1 \]

[Image of a molecule with carbon-13 labels and a spectrum.]

[Graphs showing polarization transfer over time for different transitions.]
Two active spins with chemical shift equal to MAS frequency, (n=1, rotation resonance condition)

Short distance, 1 bond coupling to passive spin

$\omega_r = \Delta \Omega_{12}$

Distance (Å)
Rotational resonance - applications

\[ \frac{\langle \Delta \Sigma \rangle}{\langle \Delta \Sigma \rangle_0} \]

\[ \frac{\langle \Delta \Sigma \rangle}{\langle \Delta \Sigma \rangle_0} \]

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Rotation Resonance - Summary

1. Advantages
   a. Recoupling selective
   b. When sensitivity/analysis allows geometries can be determined with high resolution

2. Disadvantages
   a. Only one spin pair recoupled at once
   b. Makes analysis of large labelled molecules difficult
   c. On resonance lines are broadened reducing resolution and sensitivity
Proton driven spin diffusion

- Interactions between $^1\text{H}$ and low $\gamma$ nuclei remain
- Transfer driven by:
  - heteronuclear coupling between $^1\text{H}$ and low $\gamma$ nuclei
  - $^1\text{H}-^1\text{H}$ homonuclear interactions not averaged by MAS
Spin diffusion

Assignment and structure determination of SH3
Problems with PDSD

- At high fields/MAS frequencies
  - Chemical increases meaning $^1\text{H}-^1\text{H}$ couplings are no longer homogeneous
  - MAS more effectively averages $^1\text{H}-^1\text{H}$ couplings
- Results in attenuation of PDSD
  - Requires longer mixing times
  - Reduced sensitivity due to $T_1$ relaxation
Dipolar assisted rotary resonance recoupling (DARR)

Takegoshi, 2001
Comparison of PDSD and DARR

DARR/PDSD mixing time (ms)

Intensity (a.u.)

RFDR mixing time (ms)

C=O

DARR 20 ms  RFDR 4 ms

ring  α  β

DARR 1s  RFDR 16 ms

$^{13}$C Chemical Shift (ppm)