Fast MAS Solid-State NMR of DC-SIGN Sediment

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- New Developments
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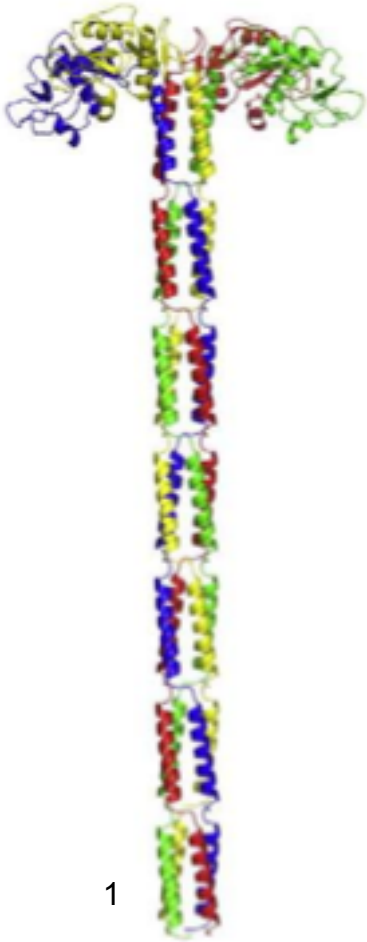
DC-SIGN

- Membrane protein found in dendritic cells and some types of macrophages.
- Receptor cells of this type traditionally bind to pathogens and present them to T-cells for destruction.
- DC-SIGN binds to HIV and presents that to T-cells, but instead of being digested the HIV infects the T-cell.
- Forms natural tetramer with long, repeating neck region and carbohydrate recognition domain (CRD).



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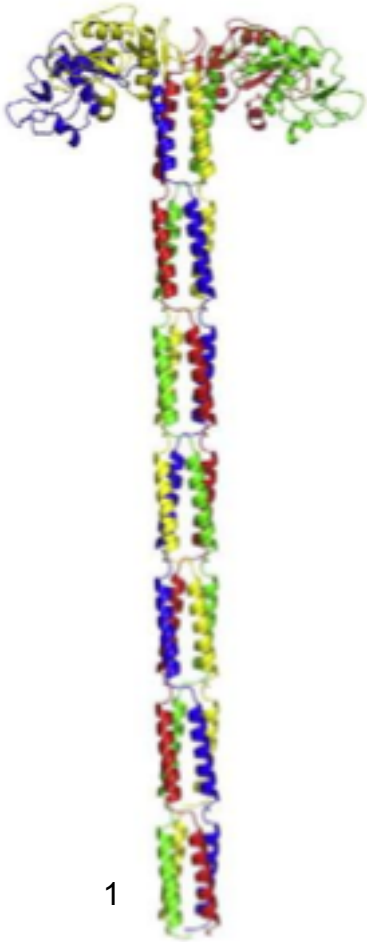
1. H. Feinberg *et al*., J. Mol. Biol., **394**, 613–620 (2009)

DC-SIGN

- Further understanding of these proteins could lead to prophylactic treatment for HIV.
- Want to try and get information on structure & dynamics at an atomic level.
- Solid-State NMR is appropriate method to try.



1

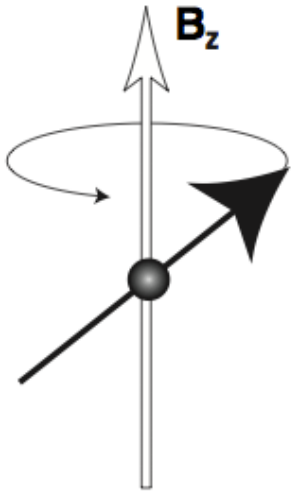
1. H. Feinberg *et al*., J. Mol. Biol., **394**, 613–620 (2009)

Introduction to Solid-State NMR

- Nuclear Magnetic Resonance (NMR) measures the Larmor frequency (ω_0) of the precession of the intrinsic nuclear spin of an atom around an external magnetic field (B_z).

$$\omega_0 = \gamma B_z$$

- Signal depends on gyromagnetic ratio of atom (γ), an inherent property of each isotope.



Introduction to Solid-State NMR

- Apply weak magnetic field (B_1) oscillating at resonance frequency ω_{rf} perpendicular to B_0.

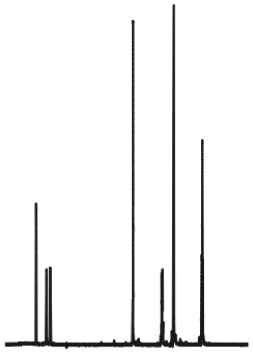
$$B_1 = |B_1| \cos(\omega_{rf} t + \varphi)$$

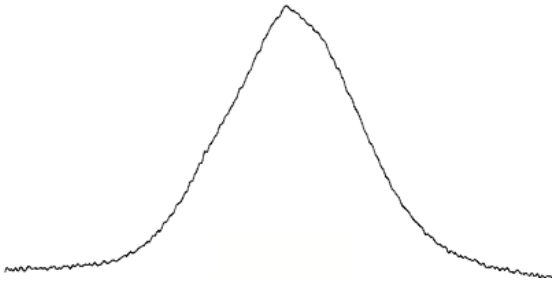
- Magnetisation of isotopes with $\omega_0 = \omega_{rf} \pm \omega_1$ (where $\omega_1 = \gamma B_1$) is rotated into xy plane.
- Free Induction Decay (FID) measured as magnetisation relaxes.
- Resonant frequency of atoms varies depending on chemical and electronic environment.

Introduction to Solid-State NMR

- Solid-State NMR (SS-NMR) requires a homogenous, solid sample.
- Advantages:
  - Not size limited like Solution-State NMR.
  - Not necessary to form high quality crystals.

  } Good for membrane proteins.
- Disadvantages:
  - Line broadening due static atoms.
    - Anisotropic interactions not averaged out.
    - Too many overlapping signals.

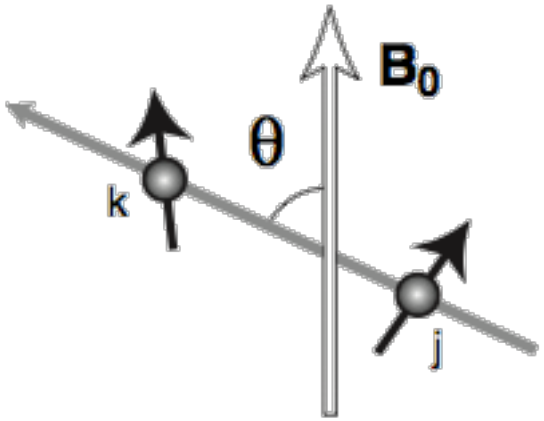




Introduction to Solid-State NMR

- Dipolar Coupling is major anisotropic interaction, depending on the angle between the line connecting two atoms and the direction of the magnetic field.

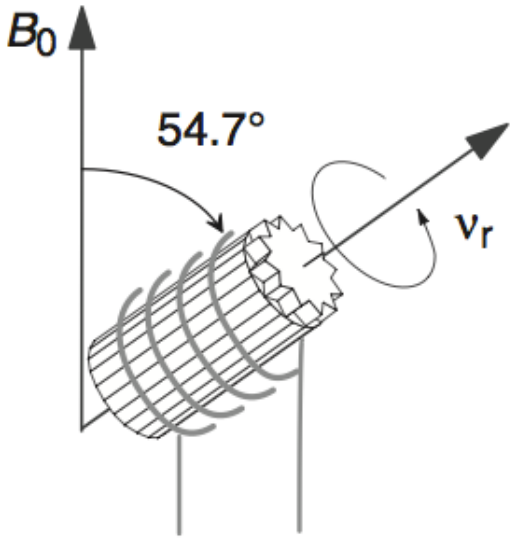
$$D = D_c \tfrac{1}{2} (3 \cos^2\theta - 1)$$

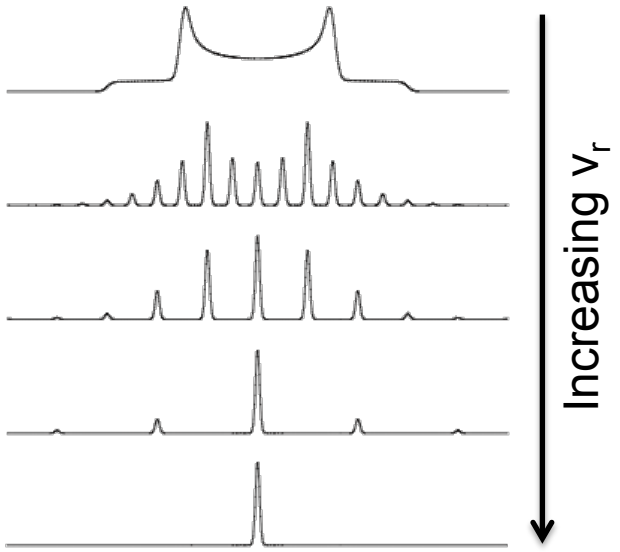


- Can average out the dipolar interactions by rotating the sample.
- But interactions aligned with the axis of rotation remain.

Introduction to Solid-State NMR

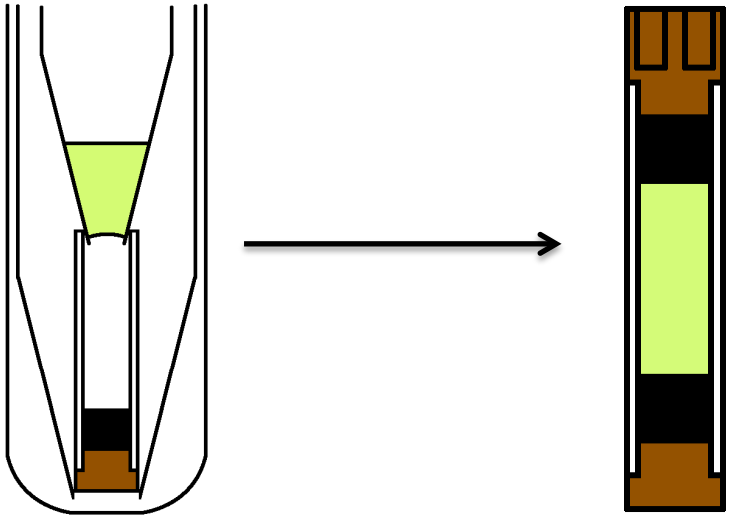
- Magic Angle Spinning removes the remaining contributions along the axis of rotation by rotating at the so-called 'Magic Angle' of 54.7°, as $3\cos^2(54.7) - 1 = 0$.
- Dipolar interactions are averaged out over a complete rotation, removing broadening effects.





Introduction to Solid-State NMR

- Sample held in rotor; narrow tube that spins inside the probe.
- Want homogenous sample close to natural environment, so normally hydrate sample by suspending in buffer.
- Can't just use lyophilised solid in NMR as each molecule will be locked in slightly different configuration.
- Rotor packed (filled) using centrifuge.



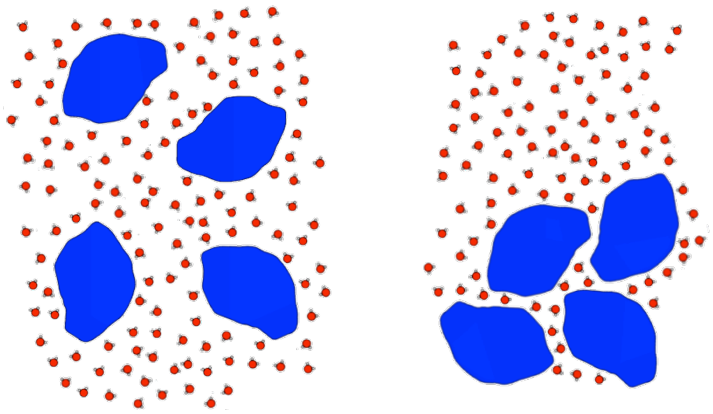
New Developments

- Sample Preparation by Ultra-Centrifugation
  - Feasibility Study with DC-SIGN
- Fast MAS
  - Development of method and application to DC-SIGN

- Will combine these two state of the art methods.

Sample Preparation by Ultra Centrifugation

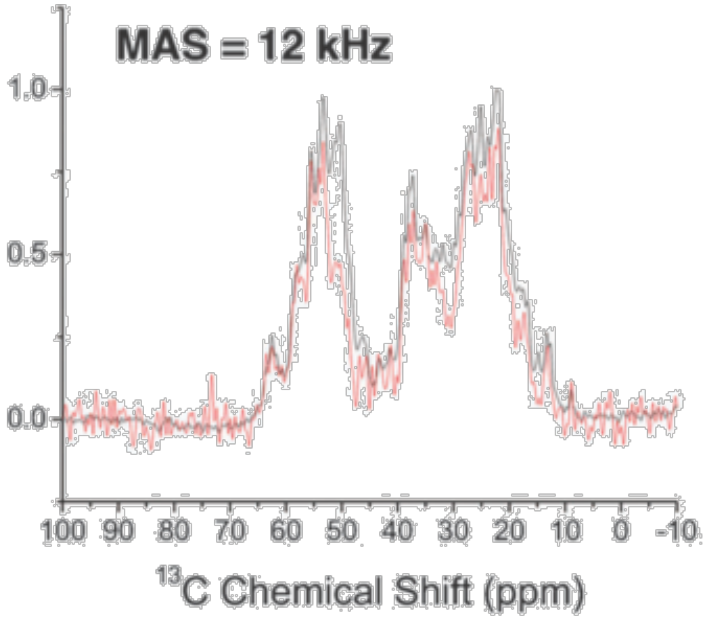
- Recently been proposed[2] that instead of directly using solid, can use very high concentration solution in an ultra centrifuge to form sediment directly into rotor.
- Works for large molecules (>10 kDa), as these are driven outwards into hydrated sediment.
- Most proteins investigated so far have been approximately spherical.



2. I. Bertini *et al.,* Proc. Natl. Acad. Sci. U. S. A., **108**, 10396-10399 (2011)

Sample Preparation by Ultra Centrifugation

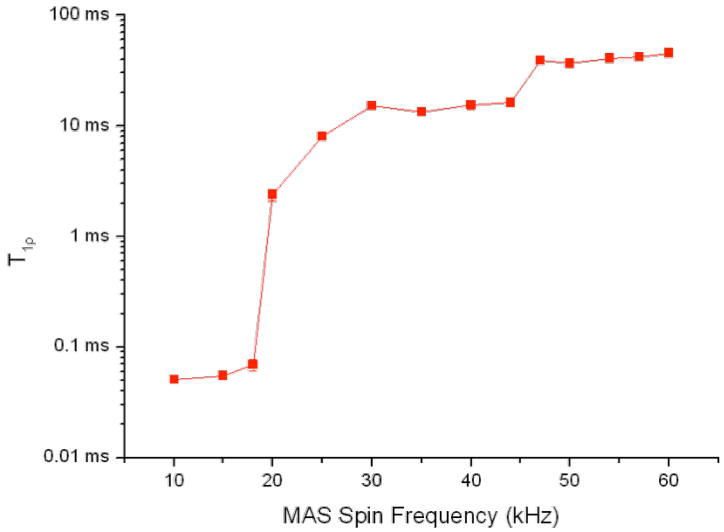
- Been shown that spectra from UC (red line) can be as good as those from crystalline solids (black line), but without having to crystallise solids[3].



3. I. Bertini *et al.,* Phys. Chem. Chem. Phys., **14**, 439-447 (2012)

Fast MAS

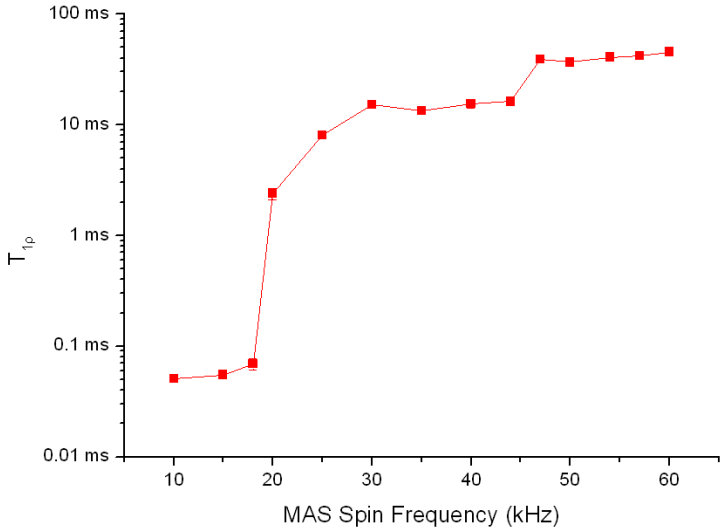
- It has recently been shown[4] that performing MAS at high spinning frequencies further removes incoherent contributions to transverse magnetisation relaxation time in bulk amides.
- Have verified this for bulk carbonyl signals in protein GB1.



4. J. Lewandowski *et al.*, J. Am. Chem. Soc., **133**, 16762-16765 (2011)

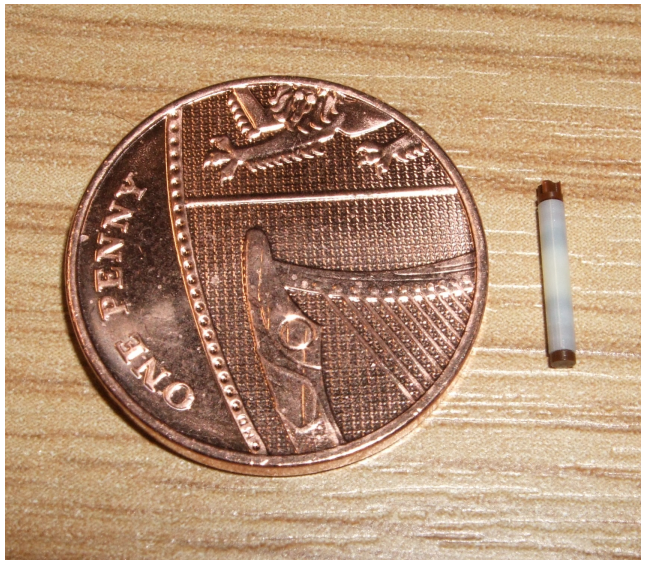
Fast MAS

- The resulting signal is primarily due to incoherent contributions due to dynamics and motion of the protein.
- Further averaging achieved by applying spin-lock fields.
- Allows better measurement of fully protonated protein (no deuteration).



Fast MAS

- In order to spin at high frequencies, need a very small sample rotor.
- Used 1.3 mm rotor, capable of spinning up to 67 kHz.
- Can hold 2-3 mg sample.



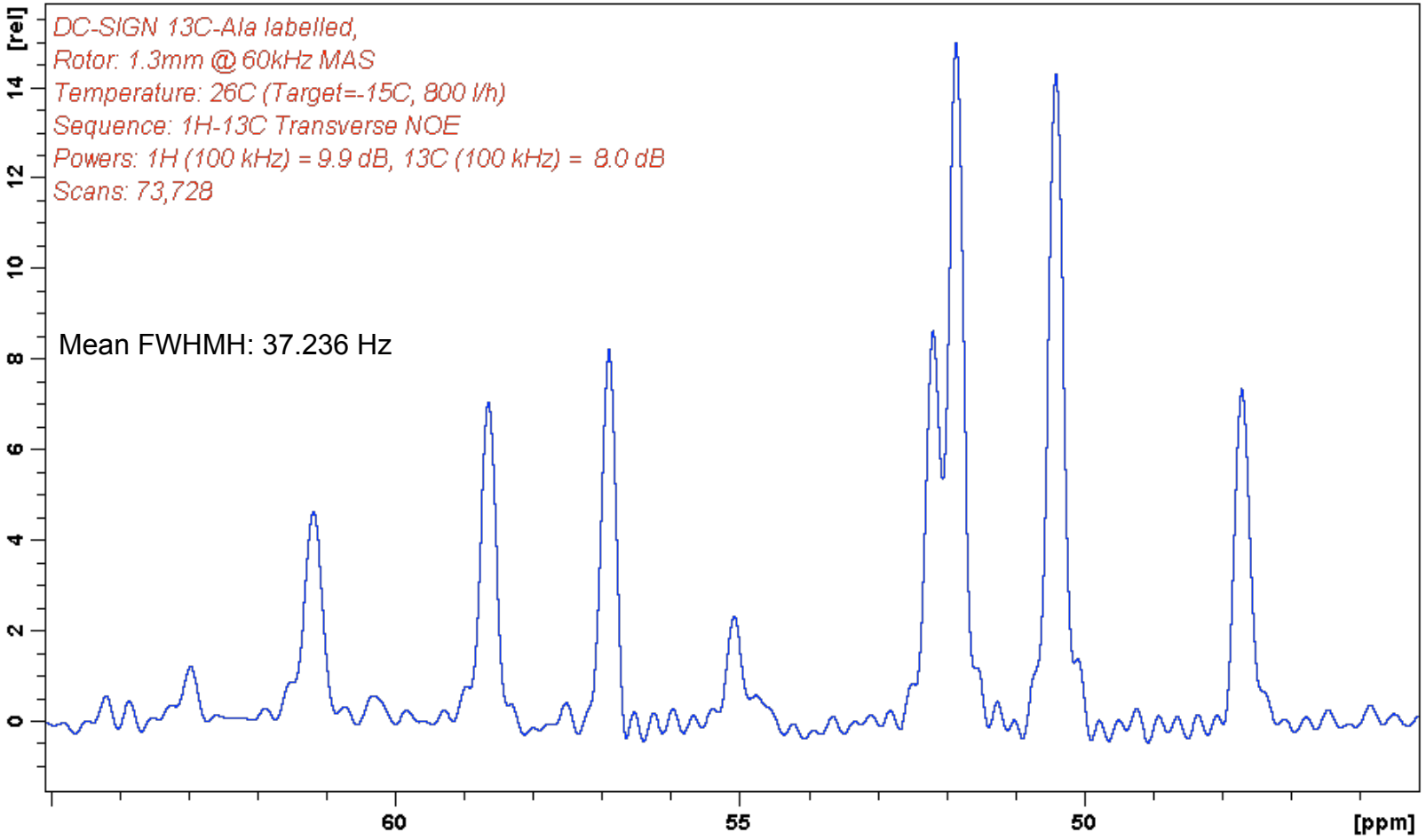
Fast MAS

- Used 600 MHz magnet (14.1 Tesla).
- Also needed cooling unit due to frictional heating.

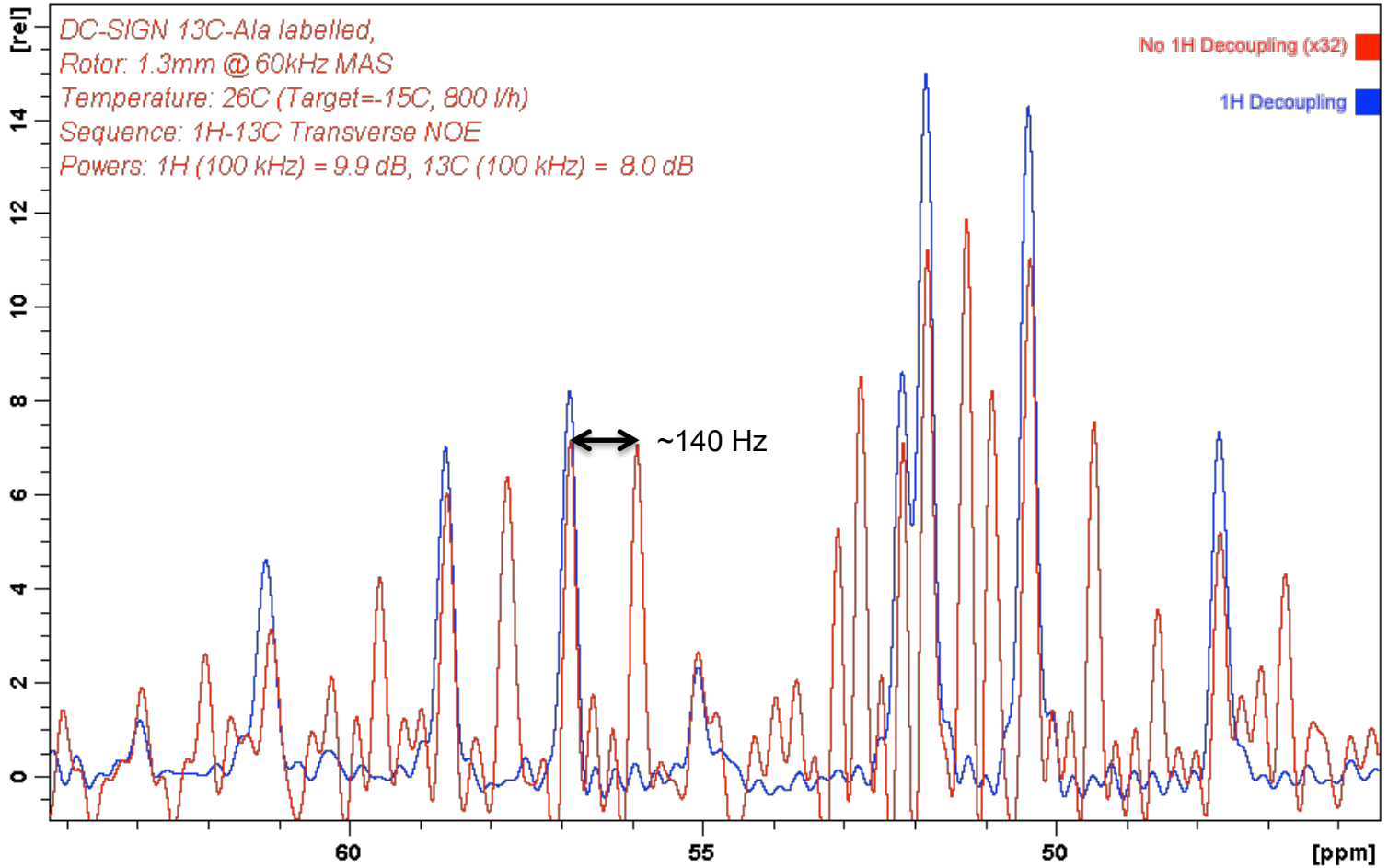
Results

- Looked at 156 kDa segment (4 x residues 62-404) using ^{13}C labelled Alanine (26 residues per monomer segment).
- Sample fully protonated.

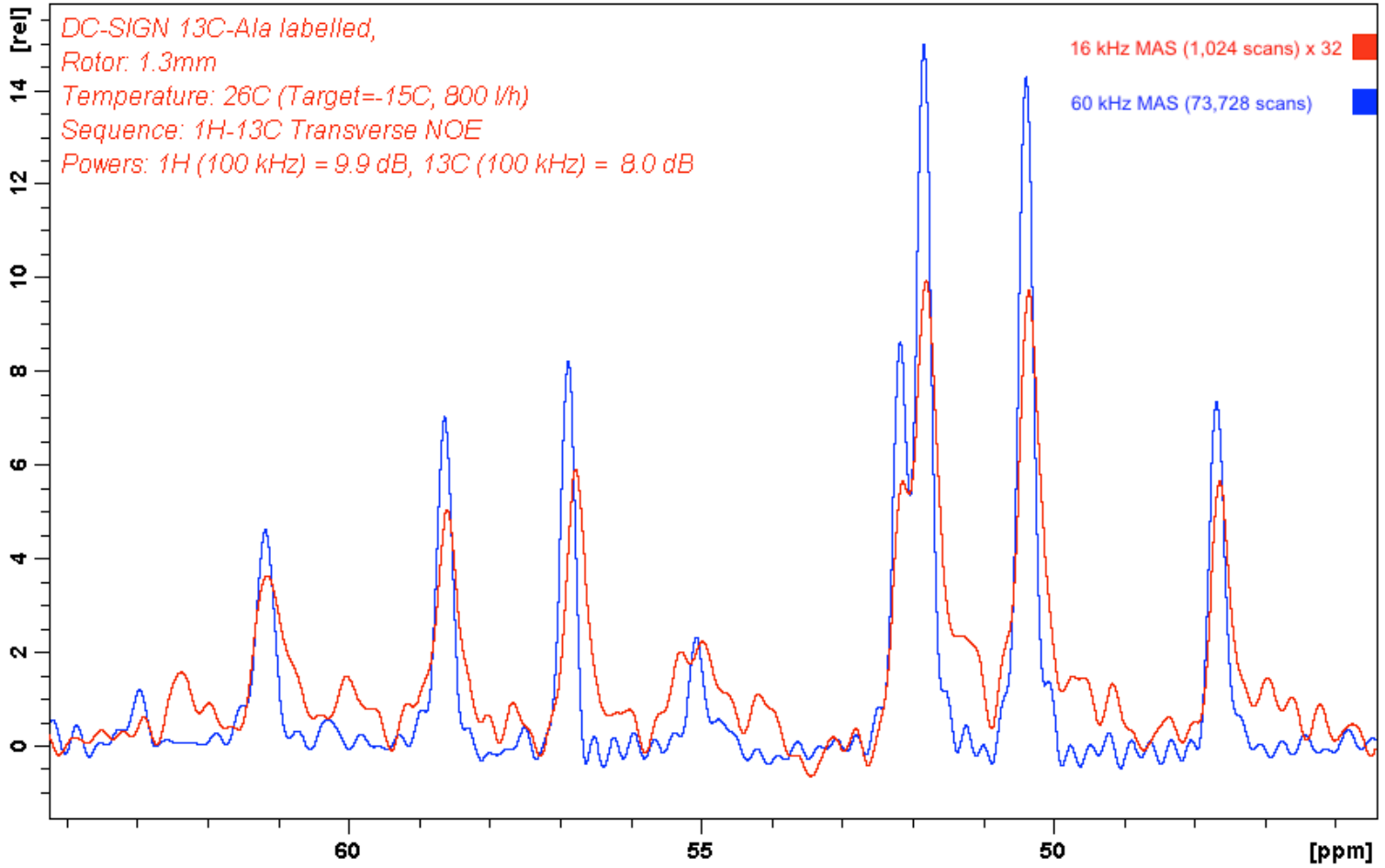
Results


DC-SIGN 13C-Ala labelled,
Rotor: 1.3mm @ 60kHz MAS
Temperature: 26C (Target=-15C, 800 l/h)
Sequence: 1H-13C Transverse NOE
Powers: 1H (100 kHz) = 9.9 dB, 13C (100 kHz) = 8.0 dB
Scans: 73,728
Mean FWHMH: 37.236 Hz
[rel]
0
2
4
6
8
10
12
14
60
55
50
[ppm]

Results


DC-SIGN 13C-Ala labelled,
Rotor: 1.3mm @ 60kHz MAS
Temperature: 26C (Target=-15C, 800 l/h)
Sequence: 1H-13C Transverse NOE
Powers: 1H (100 kHz) = 9.9 dB, 13C (100 kHz) = 8.0 dB
No 1H Decoupling (x32)
1H Decoupling
~140 Hz
[rel]
0
2
4
6
8
10
12
14
60
55
50
[ppm]

Results


DC-SIGN 13C-Ala labelled,
Rotor: 1.3mm
Temperature: 26C (Target=-15C, 800 l/h)
Sequence: 1H-13C Transverse NOE
Powers: 1H (100 kHz) = 9.9 dB, 13C (100 kHz) = 8.0 dB
16 kHz MAS (1,024 scans) x 32
60 kHz MAS (73,728 scans)
[rel]
0
2
4
6
8
10
12
14
60
55
50
[ppm]

Conclusions

- Ultra Centrifugation been shown to work for an elongated protein; DC-SIGN.
- Fast MAS reduces line width.
- Narrow line widths indicate highly ordered structure, but resolving of J-coupling when ^{1}H-^{13}C coupling included indicates high mobility.

Future Work

- Further data collection with longer relaxation time
  - Less truncation
  - Narrower lines
- Use INEPT pulse sequence
  - Look at mobility through J-coupling
- Obtain solution spectrum
  - Allow direct comparison between methods
- Assign residues
  - Possible conformational information

Acknowledgements

- Thanks to:
  - Józef Lewandowski
  - Dan Mitchell
  - Steven Brown
  - Jonathan Lamley

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  - MOAC
  - EPSRC