Fast MAS Solid-State NMR of DC-SIGN Sediment

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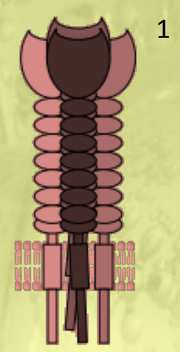
1. DC-SIGN

- Membrane protein found in dendritic cells (DCs).
- Forms tetramer with long repeating neck region and carbohydrate recognition domain (CRD).
- Binds to mannose rich pathogens like HIV to present to T-cells for destruction.
- However, HIV instead infects T-cell, and infection spreads to lymph nodes.
- Understanding of DC-SIGN could lead to prophylactic treatment of HIV.

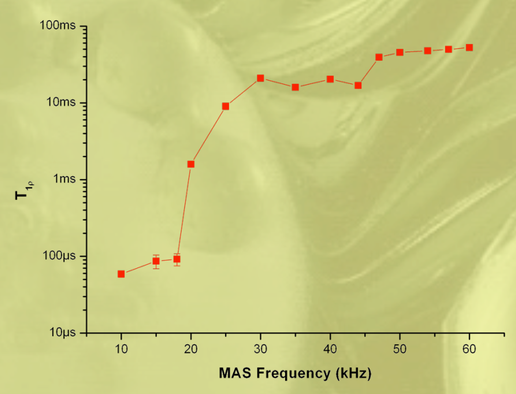


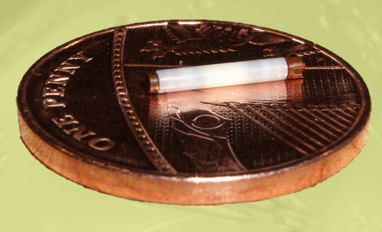
2. Solid-State NMR (SSNMR)

- Good for obtaining atomic level information on structure & dynamics of molecules.
- Detects resonant frequencies of different isotopes as their nuclear spins precess in a large magnetic field.
- Signal depends on chemical environment of individual isotopes, so can assign peaks by identifying effect of nearby atoms.
- No inherent size limitations due to molecular tumbling as in solution state NMR, so can look at large proteins.
- Usually use hydrated crystals, so small scale motions present, but no overall tumbling as in solution state.
- Requires homogenous sample, but membrane proteins notoriously hard to crystalise.
- Used 2 new innovations to look at DC-SIGN: Fast MAS and Ultracentrifugation.

3. Fast Magic Angle Spinning (MAS)

- Spinning the sample removes anisotropic dipolar coupling contributions, except those along the axis of rotation.
- By spinning at 54.7°, even these are removed due to $3\cos^2\theta - 1$ dependence of dipolar coupling, giving narrower lines.
- In the solid state, relaxation of transverse magnetisation in the rotating frame ($T_{1\rho}$) has incoherent contribution due to slow motions and coherent contribution due to static interactions.
- Fast MAS removes the coherent contribution (indicated by the plateau) enabling measurement of slow motions.

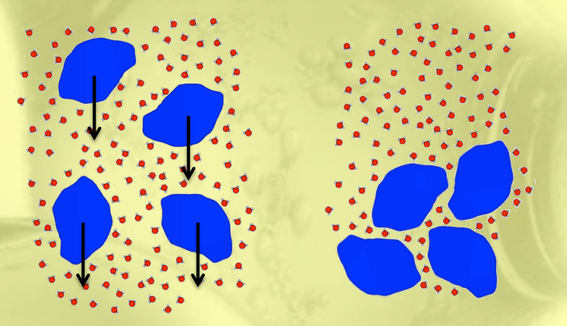




- Requires a small diameter rotor (here 1.3 mm) which holds up to 2 mg of hydrated sample.

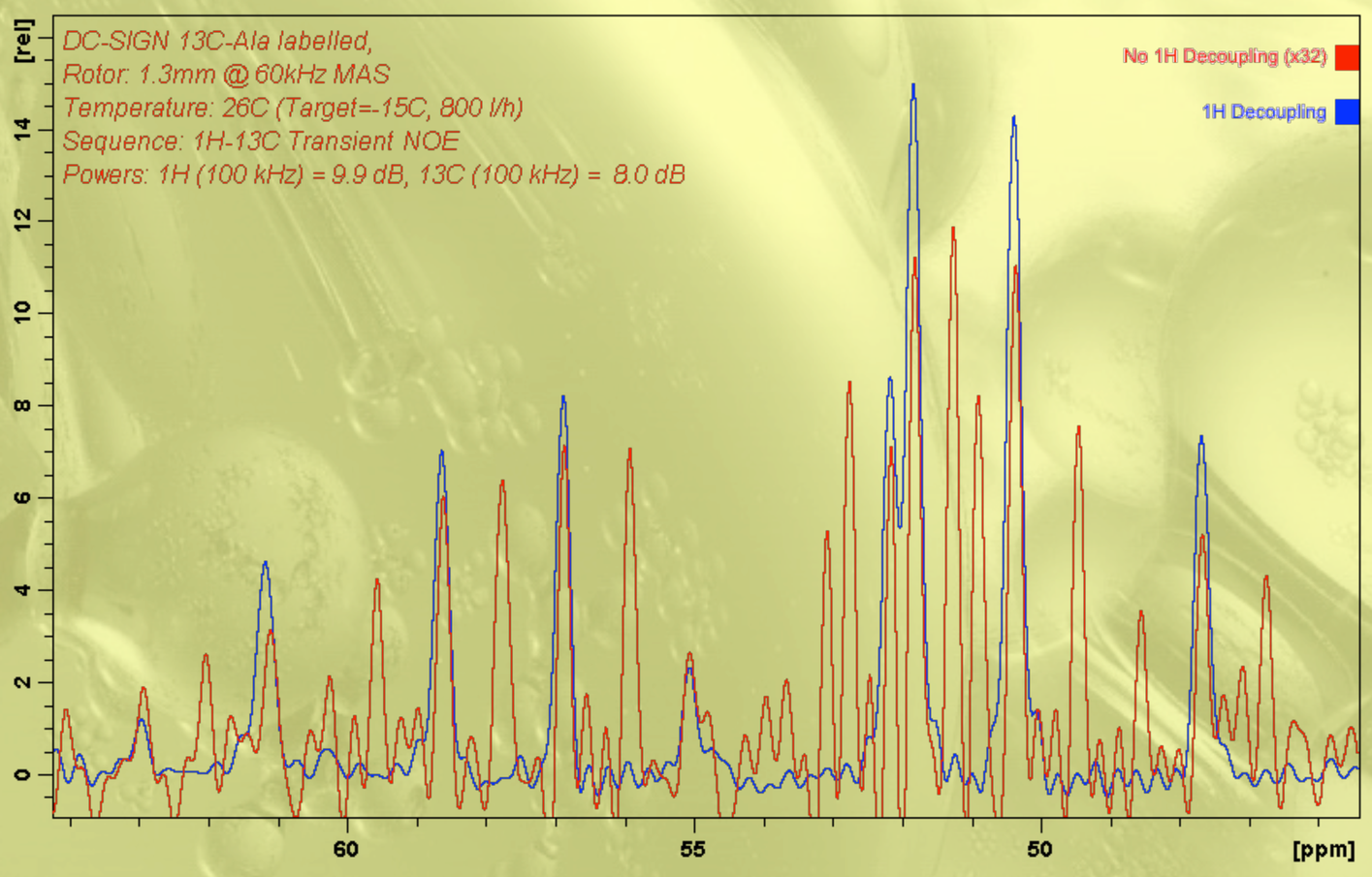
4. Ultracentrifugation (UC)

- Ultracentrifugation of highly concentrated solutions results in sediments recently shown to yield highly resolved SSNMR spectra comparable to crystalline samples[3].
- Provides way of obtaining homogenous solid sample of large proteins which are hard to crystallize.
- Previous work mainly done on rounded molecules; part of this work is a feasibility test for elongated molecules.



5. Results – Combining State of the Art Preparation Method and SSNMR Technique

- DC-SIGN segment used weighed 156,000 kDa (residues 62-404, corresponding to neck region and CRD) and was sparsely labeled with ^{13}C on the backbone of each Alanine residue (26 residues in total in 12 distinguishable chemical environments).
- Spectra show narrow line widths, averaging 37.2 Hz, which is comparable to spectra from crystalline samples, and indicate highly ordered structure and very homogenous sample.
- J-coupling (140 Hz H-Cα) is observed in ^{1}H-^{13}C coupled spectra, indicating high nanosecond mobility of molecule despite ordered structure.



1D ^{13}C spectrum with signal enhanced by heteronuclear Nuclear Overhauser Effect (NOE). Blue and red spectra were respectively obtained with and without the 1H heteronuclear decoupling.

Summary:

- A combination of sparse labeling, ultracentrifugation and fast MAS yields high quality spectra comparable to ones using crystalline samples.
- This has been shown for a large 156 kDa elongated protein, extending the state of the art and paving a way for further studies.

6. References

1. Pölmann *et al.*, Trends Immunol., **22**, 643-646 (2001)
2. J. Lewandowski *et al.*, J. Am. Chem. Soc., **133**, 16762-16765 (2011)
3. I. Bertini *et al.,* Proc. Natl. Acad. Sci. U. S. A., **108**, 10396-10399 (2011)

7. Acknowledgments

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- Background image © Chris Becker.