Solid-State Nuclear Magnetic Resonance Spectroscopy for Structural Characterization of Proteins

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Introduction

Membrane proteins mediate a variety of important functions in biological systems, ranging from transmembrane transport to maintaining cellular shape, however many of these processes remain poorly understood. As of June 2020, approximately only 3.1% of all of Protein Data Bank protein structure entries corresponded to membrane proteins, despite making up approximately a third of the proteins known in living cells. There are a variety of techniques used to study the three-dimensional structure of membrane proteins, however these membrane proteins must be in the context of their native environment, with lipids, in order to fold to their biologically relevant conformers. Lipid mimetics, a method in which the native membrane in which these proteins reside are mimicked, commonly used in X-Ray crystallography and other structural methods due to the simplicity and uniformity it provides, may distort the structure of membrane proteins. Magic Angle Spinning Solid-State Nuclear Magnetic Resonance Spectroscopy (MAS ssNMR), another method for determining protein structure, can be performed on proteins expressed in their native cell environment and points toward MAS ssNMR as a more accurate technique to solve membrane protein structure. In addition, recent developments in MAS ssNMR indicate greater viability for use in solving the structure of membrane proteins.

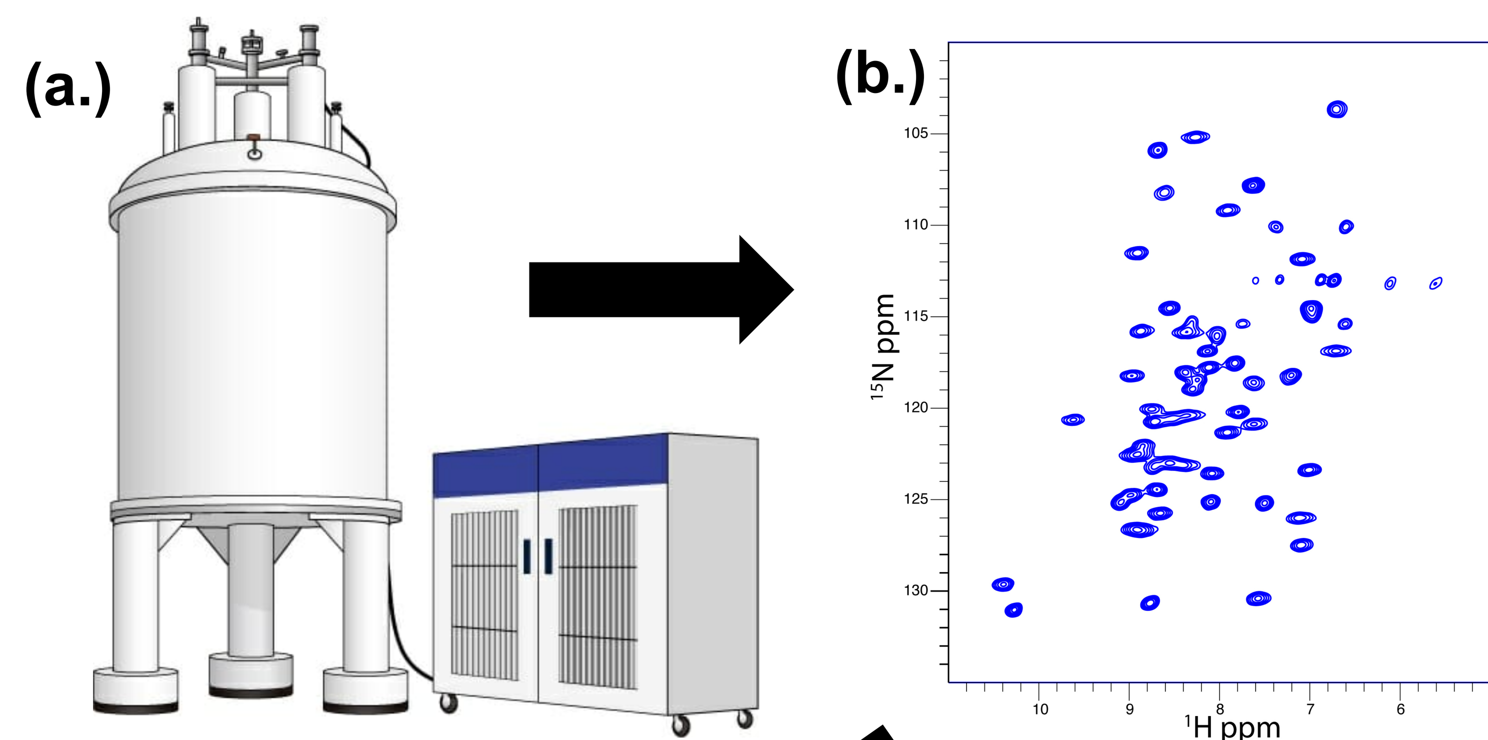

Figure 1. Representation of the general NMR workflow. **a.** NMR spectrometer and controller. **b.** NMR ^{1}H-^{15}N spectrum of a model protein. **c.** Three dimensional model of the protein from adapted from reference 1. Protein samples are placed in the spectrometer and a variety of spectra are collected. These spectra are analyzed and used to calculate the 3D structure of the protein.

Pulse Code

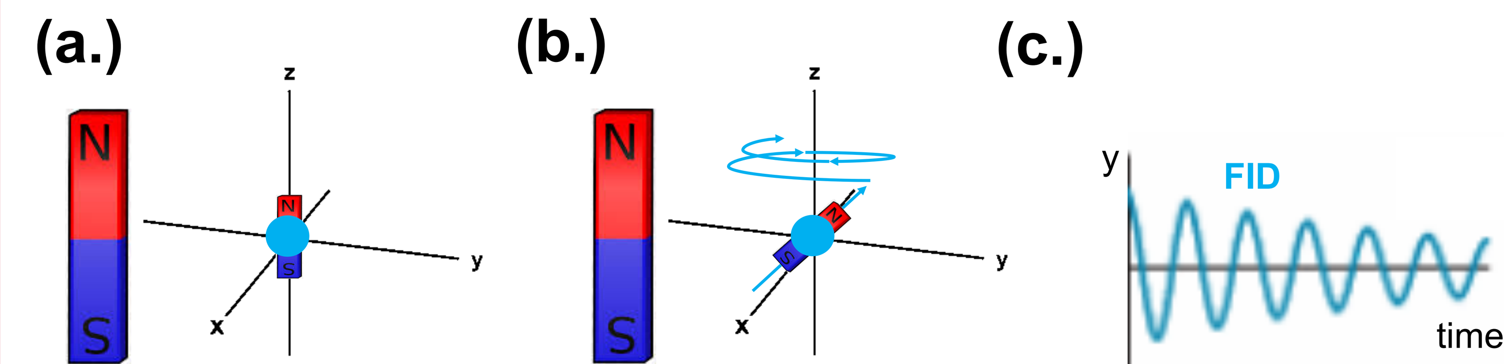

Figure 2. Cartoon representation of the acquisition of at atoms Free Induction Decay (FID) in NMR. Nuclei shown as a blue ball with magnetic poles. Large magnetic field represented as a large bar magnet. **a.** Nucleus magnetization at equilibrium. **b.** Nucleus magnetization processing back to equilibrium after being disturbed. **c.** FID spectra obtained from measuring nucleus magnetization along the y axis over time.

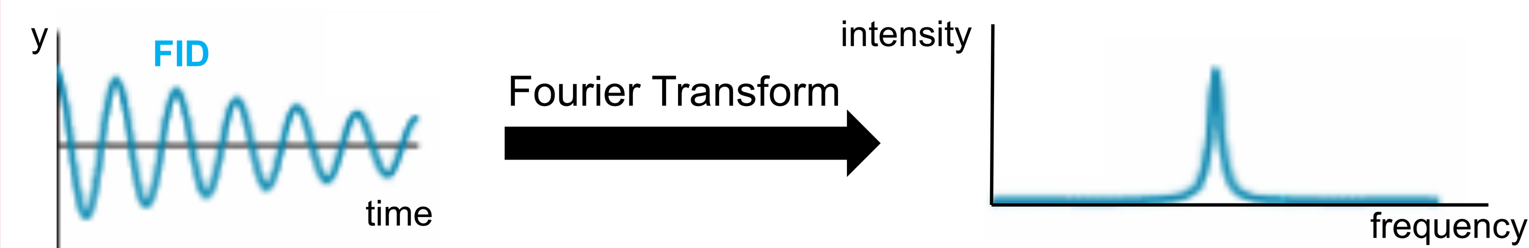

Figure 3. Demonstration of how a FID can be turned into more understandable data using a Fourier transform. Each peak corresponds to a single resonance.

Figure 4. Demonstration of how stacked 1D FIDs can create a 2D FID and be used to generate multidimensional spectra, modified from 2. Alternating conditions slightly between collection cycles allows for the collection of an indirect dimension. This can be used to show the resonance between atoms. **a.** Array of 1D FIDs lined up to make a 2D FID. **b.** Spectrum after mathematical transformation.

```
372  30m mc #0 to Start
373      F1PH(calph(p13, -90), caldel(d10, +in10) & caldel(d29, -in29))
374      F2PH(calph(ph5, +90), caldel(d0, +in0) & calclc(l10, +1) & caldel(d30, -in30))
```

Figure 5. Updated mc macro statement. This code controls the conditions used in the controller and thus the repeat acquisitions of 1D FID while changing the conditions slightly between runs to acquire a 2D or higher order FID.

References

[1.] Frericks Schmidt, H. L.; Sperling, L. J.; Gao, Y. G.; Wylie, B. J.; Boettcher, J. M.; Wilson, S. R.; Rienstra, C. M. Crystal Polymorphism of Protein GB1 Examined by Solid-State NMR Spectroscopy and X- Ray Diffraction. J. Phys. Chem. B 2007, 111 (51), 14362−14369.
[2.] Keeler, James. *Understanding NMR Spectroscopy*, John Wiley & Sons, Incorporated, 2010. ProQuest Ebook Central, https://ebookcentral.proquest.com/lib/umbc/detail.action?docID=819213.
[3.] Zhou DH, Nieuwkoop AJ, Berthold DA, et al. Solid-state NMR analysis of membrane proteins and protein aggregates by proton detected spectroscopy. *J Biomol NMR*. 2012;54(3):291-305.

^{1}H Detected Experiments

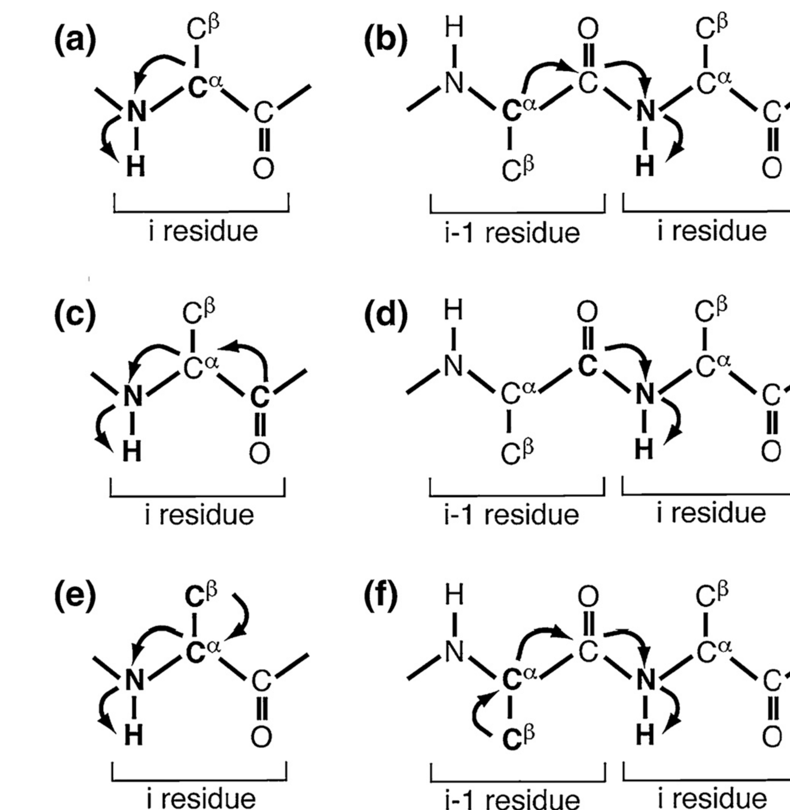

Figure 6. Transfer pathways of spin polarizations for 6 proton detected triple resonance experiments for protein backbone assignments from 3. Primary ^{1}H-^{13}C cross polarization step is omitted. Bold font show nuclei whose chemical shift was allowed to evolve. The resonances from this series of experiments has been assigned on GB1.
a. C_αNH **b.** $C_\alpha(C_o)$NH
c. $C_o(C_\alpha)$NH **d.** C_oNH
e. $C_\beta C_\alpha$NH. **f.** $C_\beta C_\alpha(C_o)$NH

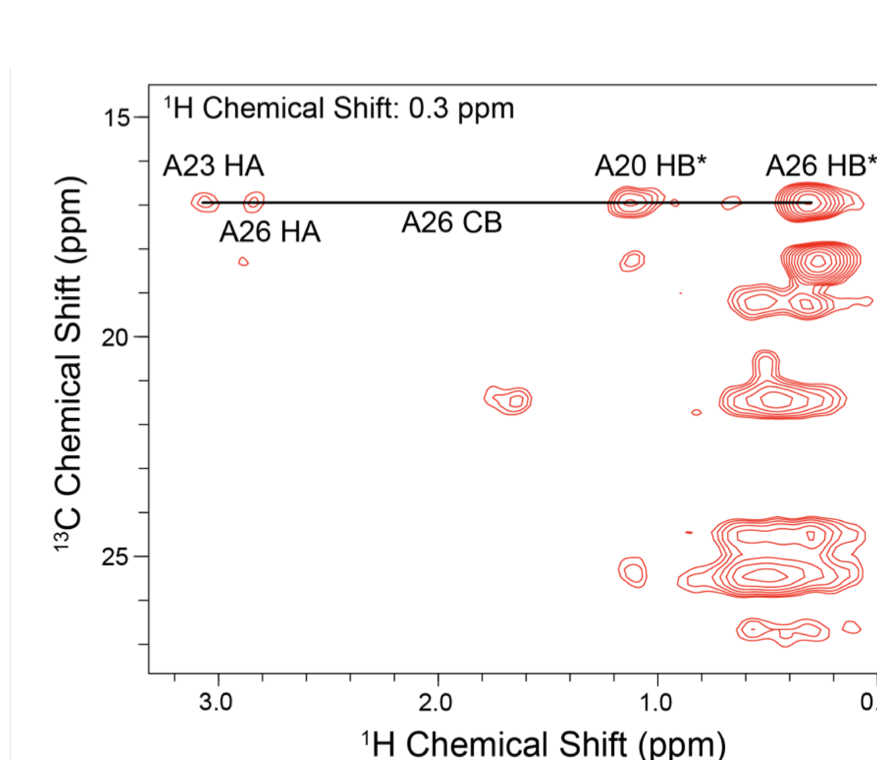

Figure 7. An HC plane of a ^{13}C-^{1}H-^{1}H spectrum of the GB1 model protein. Through space correlations with A26 C_β. This along with other proton detected experiments are now being tested at very fast magic angle spinning speeds (105kHz) to see what what resonances can be observed with them. This, along with other experiments could prove useful in identifying both intra- and inter-residue resonances.

Summary

- NMR Pulse code was updated. This makes the code easier to read for future students and keeps the code from becoming outdated.
- The backbone resonances of the GB1 protein were assigned using proton detected NMR experiments without deuteration.

Future Directions

- Continue studying proton detected experiments on the GB1 model protein to determine what long range resonances can be observed.
- Use these new proton detected experiments to solve a *de novo* structure of a protein.

Acknowledgements

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