Testing of a 900 MHz H/C/N NMR PISEMA Probe For Use With Membrane Proteins In Bicelles

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Introduction

Several leading groups have recently shown the value of triple-resonance $^{1}H/^{13}C/^{15}N$ capability to enable several novel and promising methods, including three dimensional correlations and ^{13}C decoupling. Measurements of the ^{13}C and/or ^{15}N chemical shift and $^{1}H–^{13}C/^{15}N$ heteronuclear dipolar coupling frequencies for nearly all side chain sites enabled the complete three-dimensional structures of proteins to be determined with triple-resonance approaches. Of particular interest are dipolar chemical shift correlations in anisotropic media. The latter can be utilized to determine structures of membrane proteins. One of the best ways to get the correlated dipolar and anisotropic chemical shift data is the PISEMA technique [*1*]. The technical challenges arise largely from the requirement of very high rf field strengths during the ^{1}H-^{15}N CP, as well as the need for very high proton decoupling with acceptably low sample heating. The results reported thus far in an 11.7 T magnet using a home-built probe capable of only 50 kHz field strengths at the three frequencies simultaneously (but without magnetic compensation and with high E fields) were nonetheless extremely promising [*1*]. The ^{13}C chemical shifts and the ^{1}H-^{13}C and ^{13}C-^{15}N dipolar coupling frequencies were measured, which provided orientation constraints complementary to those derived from the ^{15}N backbone sites. A number of techniques have been demonstrated to partially address the resolution limitations one expects from a dense network of homonuclear couplings in $^{13}C/^{13}C$ directly bonded carbons. Though not previously applied to the PISEMA and related experiments, gradients have proven essential in most other NMR techniques – for coherence selection, solvent suppression, and reduction in number of phase cycles in multi-dimensional spectra. This suggests gradients, if available, will also be useful in NMR methods for highly aligned membrane proteins.

There is strong interest in extending such capabilities to higher fields for greatly improved resolution and S/N. There is also strong interest in making such methods, including the use of gradients in solids NMR, available to the broader research community by development of suitable probes by an experienced probe vendor for use in the more widely available narrow-bore (NB) high-field magnets. The 900 MHz spectrometer housed within the FSU Magnet Lab represents an ideal system to test our prototype PISEMA probe.

Experimental

During our first visit to the facility the main objectives were to (a) push the probe to find any underlying weaknesses and correct them on site if need be, and (b) characterize the performance of the probe by examining individual nutation plots of each of the three channels and then perform ^{13}C and ^{15}N CP experiments on some simple compounds.

Results and Discussion

Substantial improvements were made in the coils and circuit designs during the Phase II SBIR development of a 4-mm PISEMA probe compatible with a 900 MHz narrow-bore magnet compared to lower frequency versions [*2*]. An inner bifilar magnetically compensated solenoid is used for the ^{13}C and ^{15}N channels with a novel doubly balanced circuit using capacitively shortened transmission-line tuning elements that permits operation at power levels and efficiency substantially higher than previously reported for high-field NB probes. Calculations from preliminary bench test data indicated π/2 pulse widths of 3.3 μs should be obtained at power levels of 241 W, 464 W, and 1570 W for ^{1}H, ^{13}C, and ^{15}N respectively. Other experimental data and calculations suggested the probe should be capable of handling considerably higher power levels. After the usual cleanup, we were able to obtain the desired plots. For example, we obtained 3.3, 3.3, and 4 μs for π/2 pulse widths at power levels of ~200 W, 700 W, 800 W for ^{1}H, ^{13}C, and ^{15}N respectively. The corresponding ^{13}C and ^{15}N CP experiments also worked well.

Conclusions

Overall, the probe performed as expected other than excessive ^{1}H backgrounds, which should now be remedied. We plan to test out the probe's gradient coil and run a simple PISEMA experiment during our next visit.

Acknowledgements

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References

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