PROTEIN DYNAMICS FROM NMR SPIN RELAXATION AT HIGH MAGNETIC FIELDS

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Introduction

NMR spin relaxation is a central tool in the investigation of protein dynamics spanning a wide range of timescales. Analysis of spin relaxation measurements at multiple magnetic field strengths is often necessary in order to distinguish between complex motional models and to access data quality. Also, quantitative measurements require both good spectral resolution and high sensitivity. Even so, only few high field (18.8 T or higher) measurements of protein spin relaxation have been reported to date. The aim of this work has been to practically approach the collection of quantitative ^{15}N T_1, T_2, and {^{1}H}-^{15}N NOE parameters of proteins in aqueous solution at high magnetic field. A series of pulse programs for these measurements, optimized for high field with a cryogenic probe, have been developed. Emphasis was placed on methods for minimizing ^{15}N off resonance effects, decoupling artifacts, and the influence of solvent on the accuracy of derived relaxation parameters. Special care was taken to prevent loss of data quality from both solvent saturation transfer and radiation damping, which can be especially prevalent with cryogenic probes.

Experimental

^{15}N spin relaxation data was collected for Human Ubiquitin at 298K on the 800 MHz Bruker Avance 2 spectrometer at NHMFL, equipped with a triple resonance cryogenic probe. The pulse sequences used were developed over the course of the project and are available to users of the 800 MHz spectrometer.

Results and Discussion

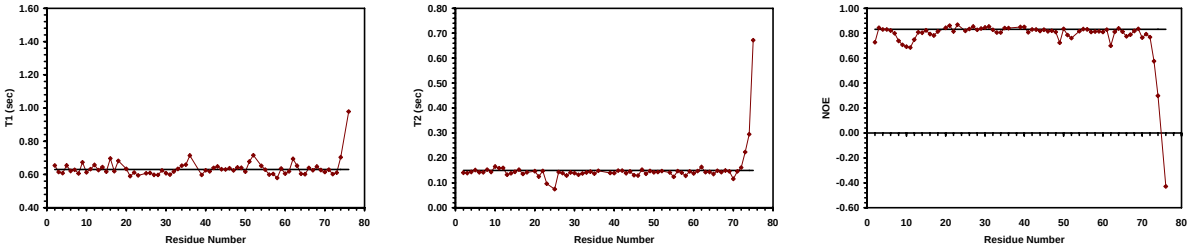

Figure 1: Ubiqitin ^{15}N T_1, ^{15}N T_2, and {^{1}H}-^{15}N NOE parameters collected at 800 MHz. Experimental data are shown as red diamonds and are compared to the theoretically predicted values (black bar) assuming an isotropically tumbling molecule with 4.2 ns rotational correlation time and internal motion in the extreme narrowing limit with a motional order parameter of $S^2 = 0.85$ at 298K.

NMR spin relaxation data acquired for Ubiquitin (Fig. 1) of static residues are in good agreement with theoretical predictions (black bars in Fig. 1), and with previously published values collected at 600 MHz. [1] Achieving high quality data required careful control of the water solvent magnetization in all experiments and interleaved data collection to minimize sample heating effects.

Conclusions

NMR relaxation methods have been developed and optimized, which will allow users to collect quantitatively accurate protein NMR spin relaxation data at high magnetic fields.

Acknowledgements

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References

[1] Lienin, S.F., *et al*., J. Am. Chem. Soc., **120**, 9870-9879 (1998).