Ultra-fast NMR Spectroscopy in Cold Probes: A Powerful Combination To Study Dynamics in Bio-molecules

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Introduction

NMR spectroscopy is uniquely suited to the studying of dynamics of proteins. A significant challenge confronting NMR spectroscopists is that the 1-D NMR spectrum which can often be acquired in a few seconds cannot provide useful information at atomic resolution for large bio-molecules such as proteins. Researchers typically turn to 2-D NMR to achieve site-specific resolution but the experiments are usually too time consuming and are unable to perform at the fast time-scales required to reveal dynamics of interest within these molecules. The SOFAST NMR method[1] when combined with the performance of Varian cold probes has the potential to revolutionize the way NMR spectroscopists study fast protein dynamics.

Experimental

All experiments were conducted on a Varian 600MHz NMR Spectrometer using a 5mm Varian Triple Resonance Z-PFG cold probe.

Data shown in Figure 1 was acquired at the Institut de Biologie Structurale (ISB), Grenoble, France on a sample of ^{15}N-labeled Rb. capsulatus cytochrome C' (128 residues) in 90% H_2O / 10% D_2O.

Data shown in Figure 2 was acquired at the Central Drug Research Institute (CDRI), Lucknow, India on a sample of {^{15}N, ^{13}C}-labeled 1 mM ubiquitin in 90% H_2O / 10% D_2O

Discussion

A very fast 2-D NMR technique, the SOFAST-HMQC (band-Selective Optimized-Flip-Angle Short Transient Heteronuclear Multiple Quantum Coherence) was introduced in 2005[1]. The method is distinctive because it offers considerable advantage in speed and potentially increases the sensitivity per unit time of many conventional experiments. This allows the recording of 2-D NMR spectra of proteins in less than 10 sec, thereby lowering the barrier to real-time investigation of dynamic events in proteins.

The key to the experiment is the use of band-selection of the NH region through a shaped excitation that can give a variable flip angle from 0-140 degrees for the first pulse of the HMQC. This pulse preserves the +Z magnetization of the other protein protons, which serves as a reservoir of magnetization to rapidly relax the NH protons back to +Z during the acquisition time. This makes it possible to set the relaxation delay as short as 1msec (or zero, since the acquisition time effectively serves as the relaxation delay). The acquisition time in both dimensions can be set for the desired resolution in F1 and F2. This provides high quality data with excellent resolution as shown in Figure 1 (a).

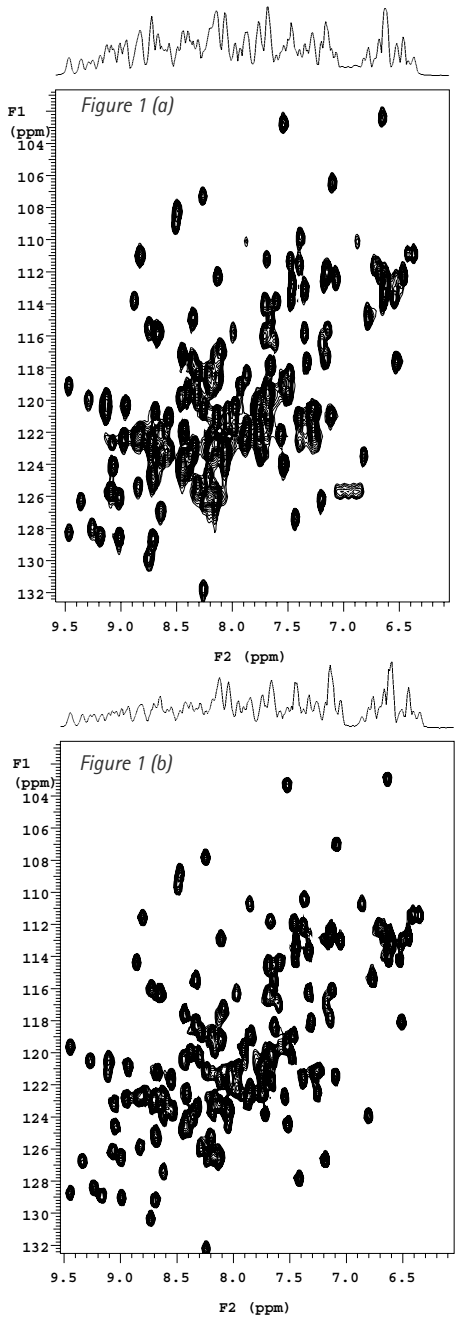

Figure 1
2-D ^{1}H-^{15}N correlation spectra of Rb. capsulatus cytochrome C' (128 residues) recorded on a Varian 600MHz NMR Spectrometer
a) SOFAST-HMQC with a recycle time of 1 msec and a total experimental time of 6 sec.
b) Standard HSQC using a recycle time of 1 sec and a total experimental time of 180 sec.
Data courtesy of Dr. Bernhard Brutscher, Institut de Biologie Structurale Jean-Pierre Ebel CNRS-CEA-UJF, Grenoble, France.

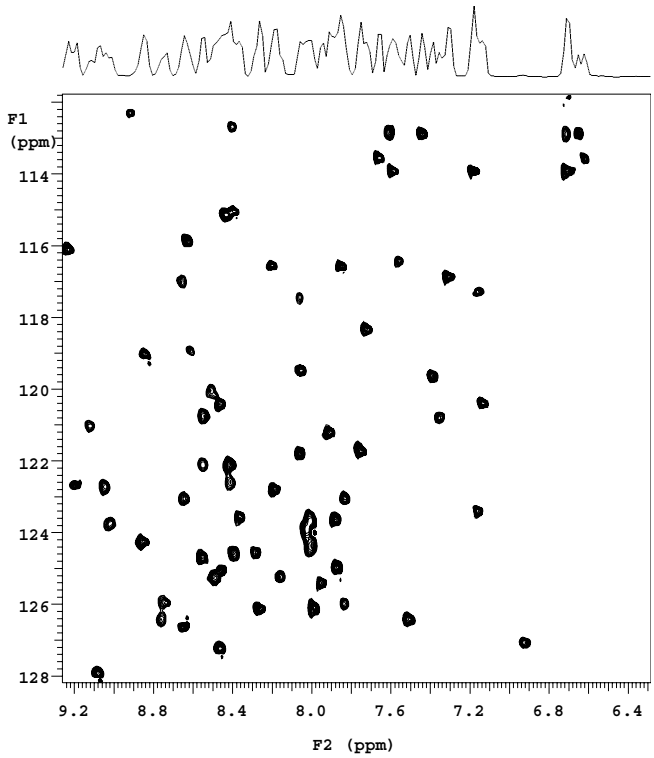

Figure 2 : SOFAST-HMQC collected on ^{15}N, ^{13}C labeled 1 mM ubiquitin (90% H_2O, 10% D_2O) using a 5mm Varian triple resonance Z-PFG cold probe. Experiment time was 8 sec. Data courtesy of Dr. Ashish Arora (Central Drug Research Institute, Lucknow, India).

Unfortunately most proteins that are involved in physiologically relevant biological processes are usually only available in extremely limited quantities. This problem is further exacerbated by the fact that protein samples enriched with at least one magnetically active isotope (^{15}N and/or ^{13}C) are required for the basic 2-D correlation experiments. Under these circumstances, researchers are increasingly resorting to the use of high sensitivity cold probe technology.

Combining cold probe technology with the ultra-fast NMR technique SOFAST can be a technological challenge. For instance, reducing the recycling delay in the SOFAST experiments means that the duty cycle of the NMR pulse sequence increases dramatically from the typical 8% to over 80% (RF pulses and ^{15}N decoupling). Imagine pushing this increased RF load into RF coil that needs to be maintained at cryogenic temperatures with a tight precision (higher then 0.1K stability) using a closed-cycle cryogenic refrigerator system. This technical challenge seemed significant enough for researchers at the Institute of Structural Biology (IBS) in Grenoble, France, to develop a modified version of the SOFAST experiment without ^{15}N decoupling with a reduced duty cycle just to ensure that the sequence could be run successfully on a cryogenic probe[2].

The benefits of being able to fully leverage cold probe technology for the ultra-fast SOFAST method are indeed significant, leading to a very careful evaluation of the technology by our Varian Applications and R&D teams. We have now demonstrated that it is possible to routinely run the SOFAST-HMQC experiments with ^{15}N decoupling without any special setup or hardware requirements on Varian cold probes. As an example, data recently collected on a newly installed 600 MHz Varian cold probe at the Molecular & Structural Biology Division, Central Drug Research Institute (CDRI) in Lucknow, India, is shown in Figure 2.

One can now envision maintaining the RF coil at 25K while continuously bombarding it with high-power RF pulses and decoupling, not merely for a few seconds, but hours. This advantage carries particular significance for laboratories that regularly perform an extended series of back-to-back 2-D experiments. Studies at the Varian NMR Applications Laboratory have shown that the duration of such power-intensive experiments can be extended from a few second 2-D experiment to series of 2-Ds taking several hours.

Conclusion:

The robust power handling capabilities of Varian cold probes coupled with the speed of the ultra-fast methods such as SOFAST-HMQC offers a powerful combination of technologies that promises to open new avenues for detailed exploration of protein dynamics through a significant increase in the throughput of such experiments. It also carries the potential to significantly increase the throughput of heteronuclear correlation experiments that may be conducted as part of ligand-receptor binding assays.

References:

1. Schanda, P., Brutscher, B. J. Am. Chem. Soc. 2005, 127, 8014-8015

2. Schanda, P., Kupcê, E., Brutscher, B. J. Biomol. NMR 2005, 33, 199–211