Fast Methods in NMR Using BioPack

Ultra-fast multi-dimensional NMR

Application Note

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Introduction

The advent of cryogenically cooled probe technology and the use of high-field magnets have resulted in significantly higher NMR sensitivity. This has made the prospect of rapid throughput NMR real for many applications. For example, it is quite common for millimolar and sub-millimolar labeled protein samples to require only a few scans per increment in a 2-D or 3-D indirect detection experiment to achieve desired sensitivity. The total length of the experiment is then governed by the necessary phase cycling (minimized or eliminated by the use of gradients and non-quadrature detection), the spectral resolution desired in the indirect dimensions and the recycle delay needed to permit recovery of magnetization. For example, a 2-D HSQC (Heteronuclear Single Quantum Coherence) for ^{15}N in a 0.5 mM protein sample would need about 2–3 minutes for a 1 ppm F1 resolution at 2 scans per increment. This time, while fast, is too slow for studies of fast proton exchange or other kinetic processes.

The Brutscher [1,2] group in Grenoble has focused on development of ultra-fast methods of indirect detection where the recycle delay can be as short as a few milliseconds. The key to the NH-detected experiments is band-selection of the NH region using a shaped excitation. All pulses on protons are designed to only affect the NH protons and not affect the state of magnetization of aliphatic or water protons. This 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 1 msec (or essentially zero, since the acquisition time serves as the relaxation delay). The maximum acquisition time in any indirect dimension can be set for the desired resolution. This provides high quality data with good resolution. The dramatic reduction of the relaxation delay from ~1 sec to ~1 msec can makes an HSQC experiment drop from 2-3 minutes to only a few seconds.

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The main attraction of these experiments is for the study of fast processes such as exchange and dynamics. Of course, they are also of great use in the case of short sample lifetime.

Instrumentation

All of these experiments involve indirect detection and use, at a minimum, a ^{1}H-{^{15}N}-pfg probe and two channels of an Agilent NMR System. Typically, they use a ^{1}H-{^{13}C,^{15}N}-pfg probe, either room temperature, or for higher sensitivity, a cold probe and three to four RF channels. They are normally run at high field, 600–900 MHz for maximum sensitivity.

While the experiment works well, the natural question that comes to mind is what is the impact of this rapid pulsing and high decoupling duty cycle on a probe, and in particular, a cold probe where the coil must be maintained at a low temperature? Sample heating is not normally an issue in a Cold probe and is not an issue here either. However, the effect on the coil temperature must be carefully considered. Agilent's Cold Probes provide active compensation for coil heating. As energy is deposited in a coil by decoupling or rapid pulsing, the cold helium gas is dynamically regulated to restore the coil temperature to the desired value. While not instant in effect, the coil temperature never rises significantly, maintaining the Q and sensitivity. Cold probe systems lacking this dynamic feedback will not be able to cope with the coil heating and the NMR results will suffer, even though the probe may not be damaged.

Results and Discussion

The impact of preservation of Z magnetization is demonstrated in Figure 1, which shows comparative data for a recycle time of 10 msec and total time of 9 seconds for a complete 2-D SOFAST-HMQC. The data from a normal HSQC done in the same time (Figure 1, right) has considerably lower sensitivity than the ^{15}N SOFAST data (Figure 1, left). Figure 2 shows a portion of the data in stacked plot form for a more quantitative view of the comparison.

Of course, having user-friendly tools to set up and run complex experiments is just as important as the results they provide. Agilent's BioPack provides a convenient and robust way to set up SOFAST experiments. Going further, BioPack also includes BEST (Band-Selective Short Transient) versions of HSQC, and triple-resonance experiments HNCO, HNCA, HNCACB, HN(CO)CA, HN(CA)CO, and HN(CO)CACB. Also included are intra-residue versions HNCA, HN(CA)CO and HNCACB.

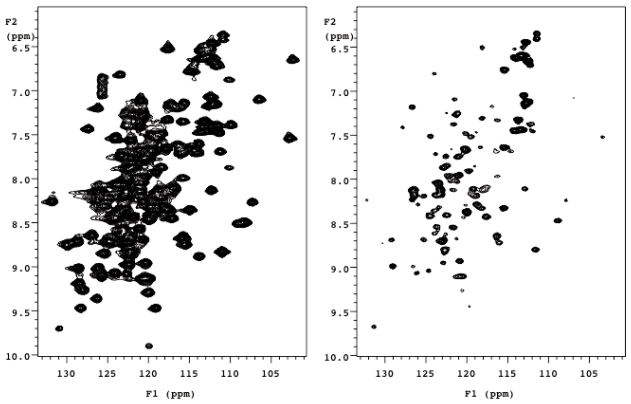

Figure 1. ^{15}N SOFAST-HMQC (left); Normal HSQC (right). Total acquisition time for each is 9 sec.

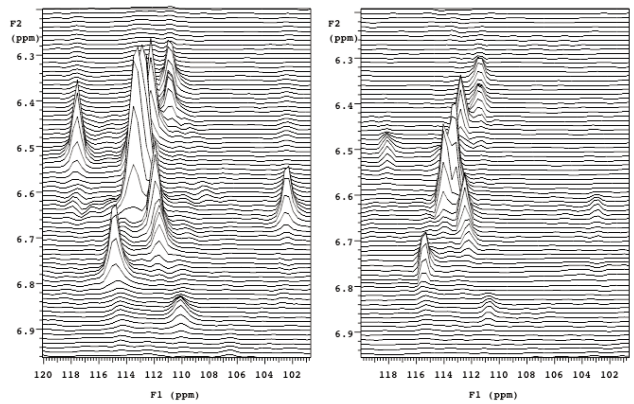

Figure 2. ^{15}N SOFAST-HMQC (left); Normal HSQC (right). Total acquisition time for each is 9 sec.

These experiments do very fast (~ a few minutes) 3-D experiments using the same principles as used for the SOFAST experiment in BioPack. The pulse sequences were designed to preserve non-NH magnetization along +Z by using region-selective pulses. All shapes are automatically created using Pbox internally in the sequences. These sequences are ideal for samples having limited lifetimes or in the study of kinetics.

Each of these experiments has the same or better performance in sensitivity as the standard BioPack experiments for normal (1 second) relaxation delays. Of course, they excel when used for obtaining 3-D data in a very short time. They are more sensitive by a factor of 2–4 for short relaxation delays (for example, 100 msec). The HN(CO)CA version gives about 50% better sensitivity even at 1 second relaxation delays because the Cbeta's are decoupled by a region-selective pulse during CA – > CO transfer.

Method

Fast and Ultra-Fast experiments are selected in BioPack using the drop-down Experiments menu in VnmrJ. Selecting a particular experiment runs a setup macro, which, in some cases, creates all the needed waveforms for pulsing and decoupling. In other cases, Pbox is used within the pulse sequence to create waveforms on-the-fly. Normal ^{15}N broadband decoupling during detection is typically used. 2-D sequences may be run as normal 2-D or, in the case of ^{15}N SOFAST-HMQC, both normal 2-D and IPAP 2-D, to permit for example, accurate measurement of residual dipolar couplings.

BioPack's VnmrJ Calibrations page (in the Setup folder) has been modified to include an Automatic BEST 2D button that will run a series of automatic 2-D experiments using triple-resonance assignment experiments with automatic processing and plotting (Figure 3). This takes about 12 minutes using the default 100 msec relaxation delay for ^{15}N HSQC, HNCO, HNCA, HN(CO)CA, HN(CA)CO, HNCACB and HN(CO)CACB (Figure 4).

These experiments could also be run as non-linearly sampled (NLS) versions by using the button on the Digital Filtering page of the Acquisition folder in VnmrJ for even faster experiments.

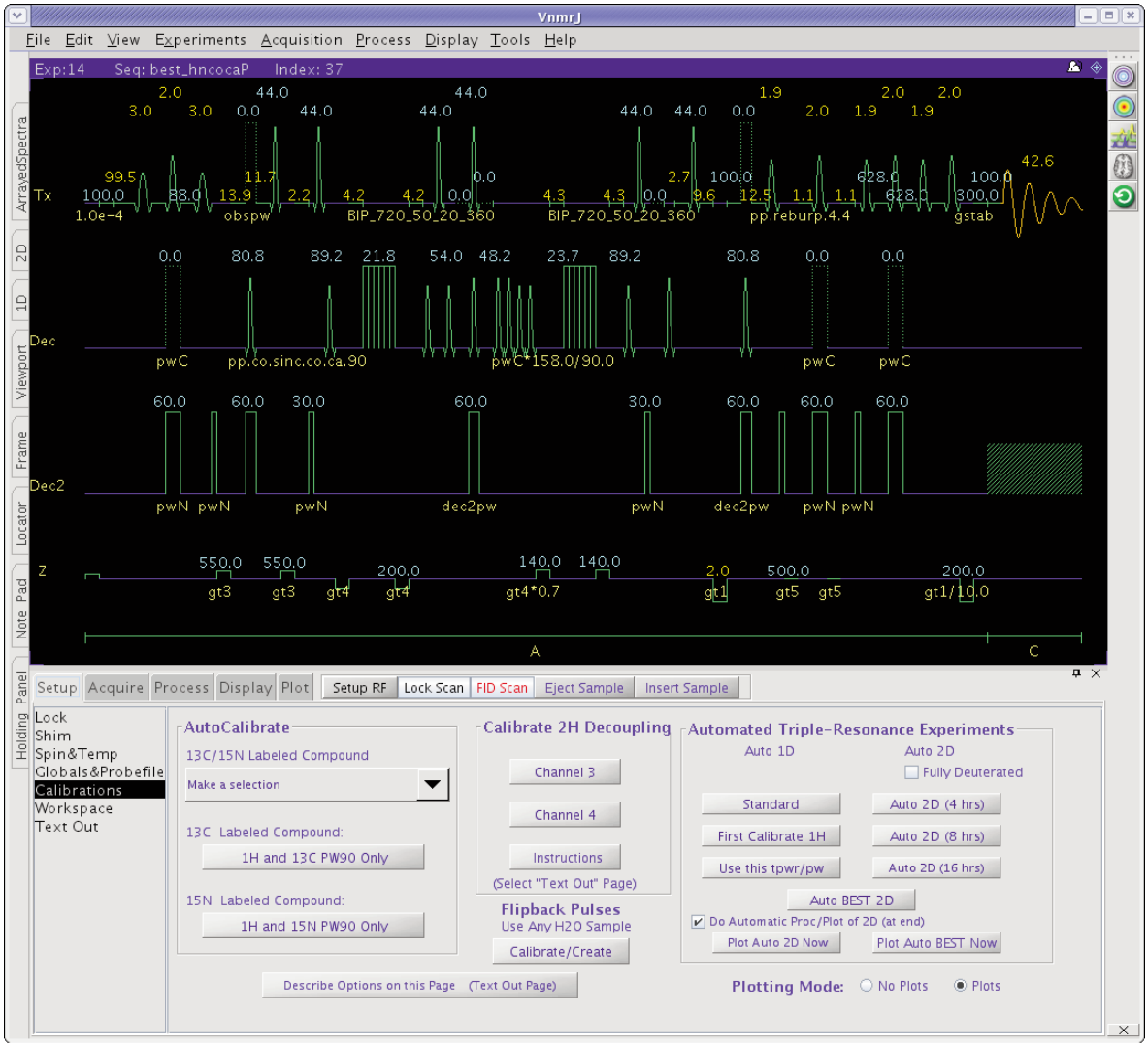

Figure 3. Calibrations page in BioPack. Note the Auto BEST 2D button, initiates automatic acquisition, processing, archival, and plotting of a series of BEST experiments. Note also, all ^{1}H pulses in this HN(CO)CA experiment are shaped for proper region selection to simultaneously permit NH protons to participate in magnetization transfer and keep aliphatic and water protons along +Z.

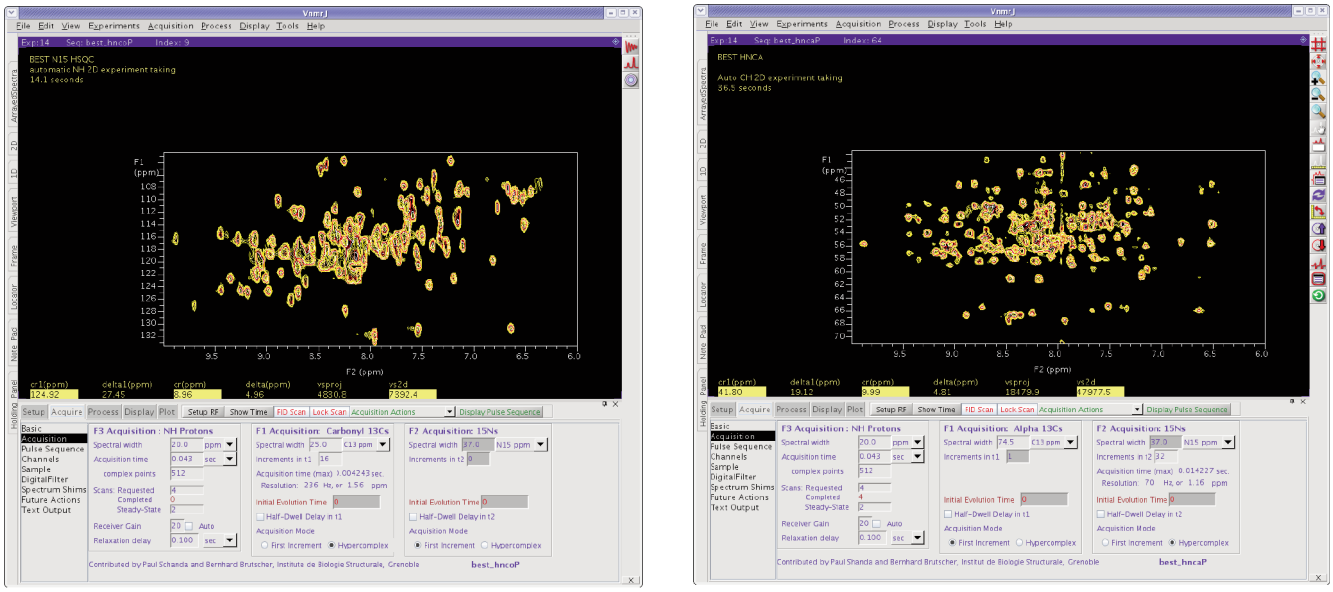

Figure 4. Displays produced during automatic BEST 2D experiment for HNCO and HNCA. The protein used was ~1 mM NuiA in H_2O run in a Salt-Tolerant ^{13}C-enhanced HCN Cold probe on a 600 MHz Agilent NMR System. Note the HNCO and the HNCA were completed in 18 seconds and 36 seconds, respectively.

Conclusion

This application note demonstrates the power of Ultra-Fast Multi-Dimensional NMR. While these experiments are cutting-edge and complex, Agilent's BioPack makes their set up and running very easy.

References

1. P. Schanda and B. Brutscher, SOFAST, *J.Am. Chem.* So. 2005, 127, 8014.
2. E. Lescop; P. Schanda; B. Brutscher, B. BEST,. *J.Magn.Reson.* 2007, 187, 163-169.

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