Agilent NMR System and BioPack

Automatic Spectral Compression for Faster 2-D and 3-D

Technical Overview

Introduction

Advantage statement

Automatic Spectral Compression (ASCOM) permits very rapid acquisition of 2-D and 3-D data without loss of information using standard data processing. Improvements of a factor of 2–5 are feasible for 2-D, and 4–25 for 3-D data sets. This capability is standard in BioPack for experiments run on an Agilent NMR system.

NMR and X-ray crystallography are the two methods currently used for determining the three-dimensional structure of biomolecules. NMR suffers from inherently low sensitivity compared to other analytical techniques, but the information it gives is unique and often only attainable using NMR techniques. To make the work of an NMR spectroscopist even harder, site-specific information on large molecules is very dense, only available by spreading the information over several dimensions in order to reduce spectral overlap. This necessitates much longer acquisition times as data sets are repeatedly acquired while one or more delays are incremented.

The topic of Fast Methods is getting a lot of attention in the NMR community. Most of the effort has been directed to cases where sensitivity is adequate, but the need for extra dimensions requires very long acquisition times (days). Different approaches, such as Projection NMR, Longitudinal Magnetization Optimization and Non-Linear Sampling have been shown to significantly reduce experiment time. Several of these require special processing which is unfamiliar to most users.

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Experimental

An old technique for reducing experiment time in a multi-dimensional NMR experiment is to set the indirect dimension spectral window smaller than that which is necessary to prevent folding. In cases where only a few peaks are well separated, this can easily cut the experiment time in half while retaining the same resolution. For example, in a ^{13}C HSQC, the spectral width in F1 could be halved, allowing the aromatic carbons to fold into the aliphatic region. Because the proton shifts are well separated, there is no overlap.

It is more challenging to permit folding when the peaks are not well separated, as might be the case in a ^{15}N-HSQC. So common practice has been to not fold or to only fold certain types of ^{15}Ns that are well separated. However, for many small to medium sized proteins, there is good potential for exploiting aggressive folding, under the restriction that all the peaks would still be resolved. However, this is an impossible task to do by eye.

This capability is now real and is incorporated into BioPack for use with an Agilent NMR System. Lescop, Schanda, Rasia, and Brutscher have developed software (ASCOM), which automates this difficult operation [1], and have contributed it to BioPack. It can operate with any pulse sequence and uses normal 2-D and 3-D FT processing software.

Results and Discussion

The ASCOM/BioPack program uses a peak pick list from a standard non-folded 2-D experiment to calculate the maximum amount of spectral folding possible without overlap. The ASCOM option is easily accessible from the BioPack menu (Figure 1).

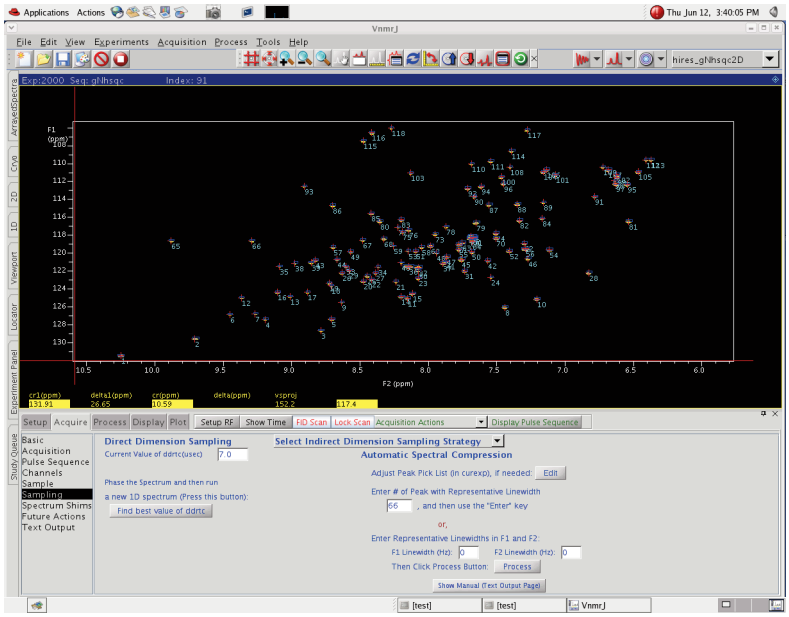

Figure 1. Sampling Page after ASCOM selection. The displayed spectrum is for an ^{15}N HSQC done with full 37 ppm F1 spectral window and 128 complex t1 increments. This experiment required 10 minutes of acquisition.

To begin, the normal 2-D spectrum is expanded such that the peaks displayed are the relevant peaks. Selecting the ASCOM option automatically performs a peak picking of the displayed 2-D spectrum. To ensure flexibility, the Edit button permits editing the peak list to remove any peaks not appropriate for analysis. The peak number for a peak having a representative linewidth can be entered, or alternatively, representative linewidths for peaks in F1 and F2. In either case, ASCOM calculates a new spectral width in F1 and a new number of t1 increments needed to give the same indirect dimension resolution as obtained with the displayed data, but with smaller spectral width in F1 and fewer t1 increments. In favorable cases, this can reduce the experiment time by a factor of five.

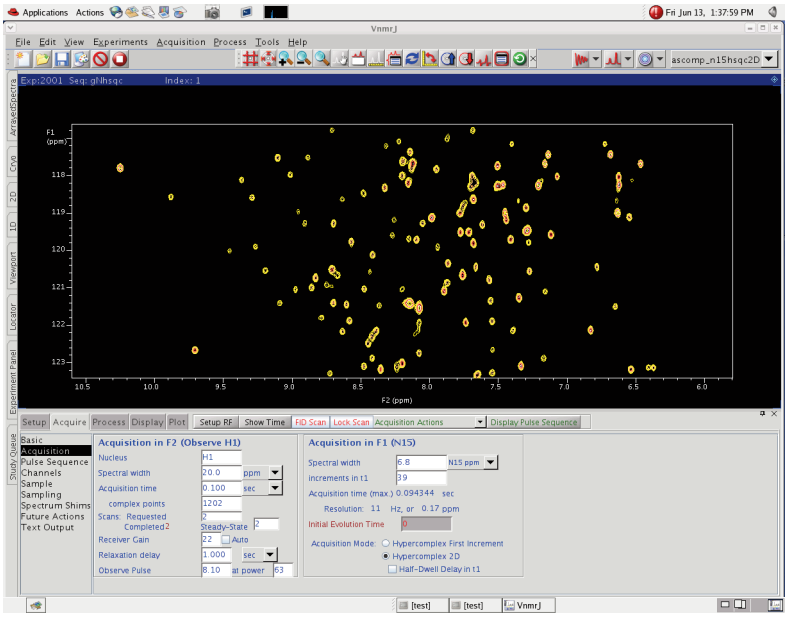

Figure 2. *^{15}N HSQC after ASCOM optimization. The displayed spectrum is for a ^{15}N HSQC done with a 6.8 ppm F1 spectral window and 39 complex t1 increments. This experiment required 3 minutes of acquisition.*

The reduced F1 window permits using a larger number of t1 increments, which results in a more adequately digitized indirect dimension. Linear prediction maybe used as well, to further enhance F1 resolution. Figure 3 shows a comparison of a slice in F1 for each of the data sets.

Separate 2-D experiments can be run for F1 F3 and F2 F3 2-D planes in a 3-D experiment to permit calculation of new spectral width values for each dimension. This can result in a reduction of time (for the same resolution) by a factor of 4–16. Linewidths can be much smaller, for example 15 Hz (@600 MHz) in both ^{1}H and ^{15}N dimensions.

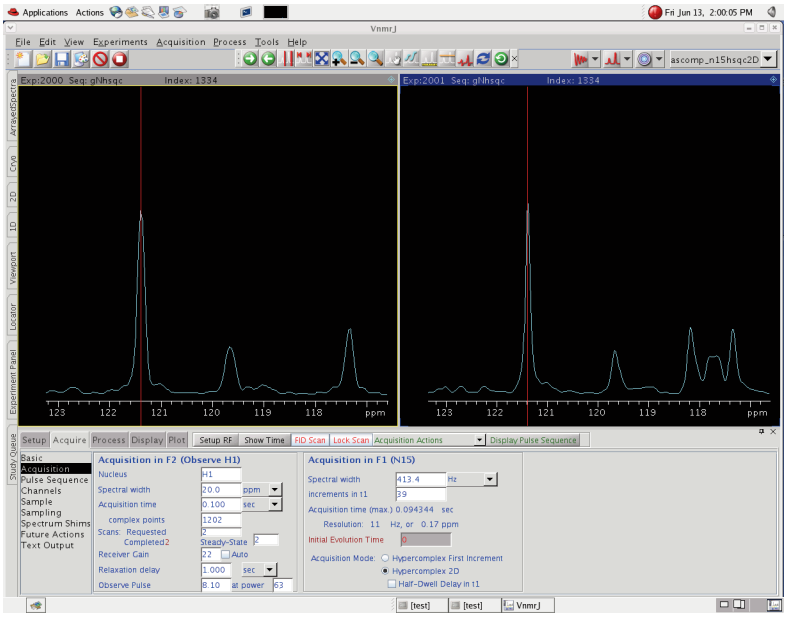

Figure 3. F1 slices for the same NH correlations for a full F1 (37 ppm) spectral width (left) and a reduced F1 (6.8 ppm) spectral width (right) from the two data sets. The linewidth of the largest peaks are 10.5 and 6.8 Hz, respectively. The additional lines in the spectrum at the right appear as a result of the intentional folding in F1.

Further speeding up of 3-D and 4-D experiments is possible by combining ASCOM optimization of indirect spectral widths with Fast experiments, such as SOFAST [2] or BEST [3], which feature relaxation delays that are in the 1 msec to 100 msec range. In this manner, it is possible to perform a high-resolution 4-D experiment in a few hours when sensitivity is not the limiting factor.

References

1. E. Lescop, P. Schanda, R. Rasia and B. Brutscher, J. Am. Chem. Soc., 129, 2756-2757 (2007).
2. P. Schanda and B. Brutscher, J. Am. Chem. Soc., 127, 8014-8015 (2005).
3. P. Schanda, H. Van Melckebeke and B. Brutscher, J. Am. Chem. Soc., 128, 9042-9043 (2006).

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