Fast Methods in NMR Using BioPack

Projection reconstruction 3-D and 4-D experiments

Application Note

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Introduction

One of the fastest evolving areas of NMR is the speeding up of data collection in multi-dimensional NMR. This step is the most time-consuming within the process of using NMR to obtain protein structure, for example. These experiments are often run for many days, consuming spectrometer time and limiting the possible throughput.

One new method generating considerable excitement is Projection Reconstruction (PR), the reduction of 3-D and 4-D experiments to sets of much faster 2-D experiments without loss of spectroscopic information. This is done by linking the two or three indirect dimension evolution times so that they evolve together, rather than one at a time. In addition to the orthogonal experiments where only one evolution time is incremented, anywhere from one to several dozen 2-D experiments are run with the linked evolution times. These 2-D experiments produce data equivalent to a projection of a higher-dimensional data set, or equivalently, a view of the higher-dimensional data from different angles. With enough angles, the information in the higher-dimensional data matrix can be unambiguously characterized.

Data from these experiments can be analyzed to produce a peak list with chemical shifts, or the 3-D or 4-D spectra themselves can be re-constructed. The primary purpose of many of these experiments is chemical shift assignment, so this objective can be reached in a much shorter period of time. This is a very important feature for NMR in the age of rapid protein production using the structural genomics programs active around the world.

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Instrumentation

These experiments typically involve indirect detection and the use of 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 in a VNMRS NMR Spectrometer. They are normally run at high field, 600–900 MHz for maximum sensitivity.

3-D Projection Reconstruction Experiments

A number of BioPack Triple-Resonance 3-D pulse sequences have been coded to permit linked acquisition of indirect dimensions to permit rapid protein 3-D experiments. These utilize an angle parameter. If the value of the angle is zero or 90 degrees, a normal F1F3 or F2F3 2-D experiment will be performed.

If the angle is between 0 and 90 degrees, the t1 and t2 evolution times are co-incremented at rates determined by the angle using a mathematical equation within the pulse program. Under favorable circumstances, it is possible that the chemical shifts characterized by F1, F2 and F3 for each amino acid residue may be determined from as few as one projection (or tilted) 2-D plane along with the orthogonal F1F3 and F2F3 2-D planes. More typically it may require several tilted planes.

Method

BioPack has an interface to manage the optimal collection of PR experiments. This page will be visible for any sequence using the angle variable (Figure 1).

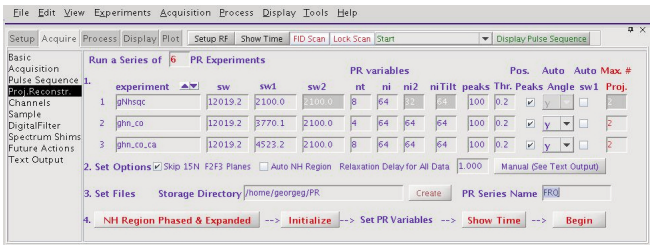

Figure 1. Projection reconstruction acquisition control panel.

Since the time of an individual 3-D experiment using one pulse sequence can be drastically reduced, it is possible and desirable to run several experiments in the same available period. Therefore, in a short time multiple chemical shift assignment triple-resonance experiments may be run in the PR mode.

Results and Discussion

The purpose of the panel is to specify the total number of pulse sequences to be used, the names of these sequences and the values controlling the 2-D acquisitions.

The user is free to modify the parameter values as desired. The number of transients is set relative to the intrinsic sensitivity of the sequence specified. A larger number of evolution time increments than normal may be used for better resolution since these are 2-D acquisitions for the orthogonal planes and each orthogonal plane is only acquired once, as opposed to the possibly several tilted planes.

Any of the experiments may be run just by setting the angle value. However, the best projection angles needed are not known in advance. Multiple 2-D experiments using different values could be set up and run manually, but this may result in more time used than optimal or necessary. One of the powerful features of BioPack's implementation of PR is automatic angle prediction [1]. The data are analyzed after the last orthogonal plane acquisition and after each tilted plane acquisition to obtain the most informative value of the projection angle for the next tilted plane acquisition, up to the maximum specified value. Often, fewer planes than the maximum value will suffice so that the experiment may be stopped and the next one in line begun. This produces the highest throughput possible.

The **Manual (See Text Output)** button produces a set of instructions for setting up a PR queue (Figure 2).

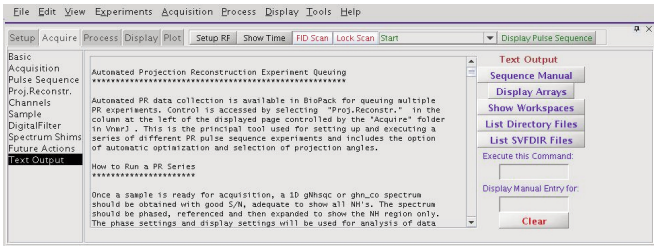

Figure 2. Projection reconstruction manual display.

Once a sample is ready for acquisition, a 1-D ghn_co spectrum is obtained with good S/N, adequate to show all NHs.

The number of PR experiments desired is entered in the top entry box and then their names in the experiment column, scrolling if more than 3 sequences are to be used.

The **Begin** button is selected to start the experiments. While the experiments are running, histograms are displayed showing the optimum angle used for the next experiment for the best chance of resolving overlapped peaks. The analysis files have the full chemical shift assignments.

4-D Projection Reconstruction Experiments

A set of 18 BioPack Triple-Resonance pulse sequences has been submitted by Ron Venters, Brian Coggins, and Pei Zhou of Duke University to permit linked acquisition of indirect dimensions allowing for rapid acquisition of 4-D PR experiments [2]. These have angle parameters similar to the angle for 3-D experiments. Since there is one extra dimension there are two angles for converting a 4-D sequence into a 2-D version. Additionally, there are several 4-D → 3-D sequences, in which case only one angle is needed. If the value of either of these angles is zero or 90 degrees, an orthogonal 2-D projection will be collected. If the projection angles are between 0 and 90 degrees, the t1, t2 and t3 evolution times are co-incremented. Because of the higher dimensionality, the number of tilted planes required to remove all ambiguity is typically larger than in the 3-D versions.

Method

These experiments have parameter sets in which the best angle combinations have already been determined. The user just selects the number of tilted planes and this sets up the list of permissible angles. BioPack has a dedicated interface to manage the optimal collection of these 4-D PR experiments.

The Acquire/Acquisition page is optimized for these experiments (Figure 3). The box at the left shows the detection dimension (usually NH protons). The PR Acquisition boxes show the active number of increments and spectral windows for the 2-D experiment.

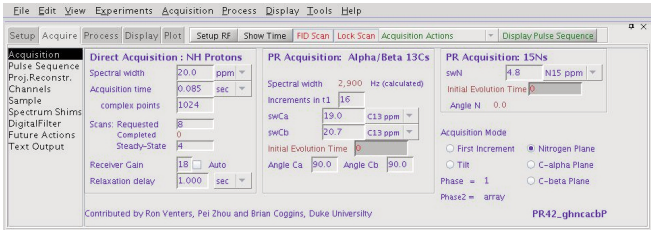

Figure 3. Acquisition panel for the PR42_ghncacbP experiment.

All of the angles and the spectral width values are set by operator choice in the Acquisition Mode section at the lower right. The sequences are named PR42... or PR43..., indicating 4-D → 2-D or 4-D → 3-D PR experiments, respectively.

Management of acquisition of multiple 2-D experiments with different tilt angles, suitable for spectral reconstruction, is done using the Proj.Reconstr. page in the Acquire folder in the VnmrJ interface (Figure 4).

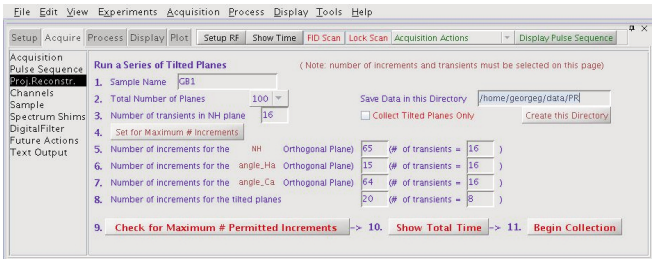

Figure 4. Projection reconstruction acquisition control panel for PR42_ghacanhP.

The first experiment's parameters will be set when the PR queue begins. While 3-D PR queuing is set up for multiple pulse sequences, this queuing is for different angle combinations for the same pulse sequence. The user just uses a menu to select a total number of tilted planes.

The **Begin** button is used to start acquisition.

While the above 3-D automatic process may have an unknown number of tilted planes acquired, the 4-D acquisition is defined prior to starting, so the **Show Total Time** button gives an accurate total time.

Projection Reconstruction data processing is done with the Duke program PRCALC [3].

Conclusion

Projection Reconstruction experiments offer a new way of speeding up the data collection process in multi-dimensional NMR. The versatility of the VnmrJ pulse programming language reduces the task of running these experiments to just a choice of angle(s). The intuitive and tailored interface permits fast and robust setup of the experiment queue and makes it easy for users unfamiliar with this area to get started.

References

1. Software by Eriks Kupce, Agilent Technologies, Inc.
2. Ronald A. Venters, Brian E. Coggins, Doug Kojetin, John Cavanagh, and Pei Zhou. *JACS* 127(24), 8785-8795 (2005).
3. B. Coggins, and P. Zhou, *J.Biomolecular NMR*, 34, 179–195 (2005).

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