Communication

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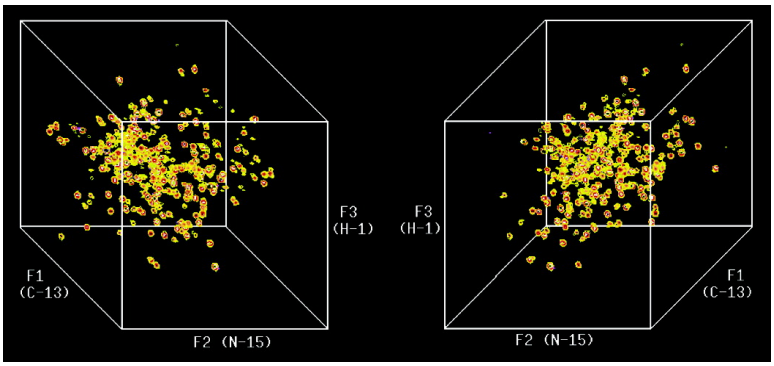

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Projection−Reconstruction of Three-Dimensional NMR Spectra

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We describe a technique for fast acquisition of three-dimensional NMR spectra by reconstruction from a set of plane projections, related to methods employed in X-ray tomography.[1] In principle, the internal structure of any three-dimensional object can be computed by measuring the X-ray absorption in several different directions. In an analogous manner, a three-dimensional NMR spectrum can be reconstructed from projections of the absorption intensity onto planes inclined at different angles. In contrast to the continuous nature of a physiological sample, NMR spectra are discrete and usually well-resolved, presenting a much more favorable case for reconstruction. Only a very small number of different projections suffice to recreate the entire three-dimensional spectrum, speeding up data acquisition by an order of magnitude.

The projection method relies on a Fourier transform theorem[2,3] which states that a section though the origin of a two-dimensional time-domain signal $S(t_1,t_2)$, subtending an angle α with respect to the t_1 axis, transforms as the projection onto a line through the origin of the two-dimensional frequency-domain signal $S(F_1,F_2)$, subtending the same angle α with respect to the F_1 axis. By extension, a three-dimensional NMR experiment where the evolution parameters t_1 and t_2 are incremented simultaneously and in the ratio $\Delta t_2/\Delta t_1 = \tan \alpha$, projects the three-dimensional NMR spectrum onto a plane tilted through an angle α with respect to the F_1F_3 plane. With the usual hypercomplex Fourier transformation, where real and imaginary signal components are acquired in both evolution dimensions[4] and suitably combined,[5] projections are obtained for both $+\alpha$ and $-\alpha$, providing two independent views of the three-dimensional array of NMR absorption peaks.[6] The special cases $t_1 = 0$ or $t_2 = 0$ (often used in setting up procedures) yield projections onto the orthogonal F_2F_3 and F_1F_3 planes, respectively. All such projections contribute to the task of recreating the full three-dimensional NMR spectrum. Several authors[7−10] have demonstrated that simultaneous incrementation of the evolution parameters can speed up multidimensional experiments, employing, for example, the G-matrix[7] to disentangle the resulting intermodulation of chemical shift frequencies. Projection−reconstruction gathers the raw data in an analogous manner but provides a clear pictorial representation of the spectra in the familiar format with no intermodulation effects. It emphasizes the importance of exploring differential rates of incrementation of t_1 and t_2, corresponding to different tilt angles for the projections. The processing is fast and straightforward, requiring no operator intervention other than the choice of tilt angle, which is not critical and could be automated if necessary.[6]

The reconstruction problem[11,12] reduces to finding a three-dimensional array of NMR peaks compatible with all the measured plane projections, just as two stereoscopic views of an object create a three-dimensional impression. An array $S(F_1,F_2,F_3)$ is built up one F_1F_2 plane at a time. The NMR frequencies observed in the projections recorded at $\alpha = 0°$ or 90° define an orthogonal lattice in this plane. All potential cross-peaks lie on this lattice, but not all such locations are occupied. The tilted projections impose further constraints that resolve these ambiguities.

A preliminary test[12] of the feasibility of the reconstruction method was carried out on the simple example of an HNCO spectrum,[13] where there are only a few NMR responses in any given F_1F_2 plane. Here, some more realistic applications are demonstrated: HNCA[14] and HN(CO)CA[15] spectra of 1 mM ubiquitin, uniformly labeled in carbon-13 and nitrogen-15, and the HNCO spectrum of an isotopically enriched 187-residue protein HasA.[16]

The 500-MHz HNCA spectrum of 1 mM ubiquitin was studied in aqueous solution (10% D_2O) on a Varian INOVA 500 spectrometer. Projections were acquired at $\alpha = 0°$ (carbon−proton plane) and 90° (the nitrogen−proton plane) and at tilt angles of ±30°. Figure 1 shows the carbon−nitrogen correlations in the form of strip plots. (Backbone assignments represent the key first step in protein structure determinations.[17]) Most protein studies involve several different radiofrequency pulse sequences. A certain economy of instrument time can be achieved by "borrowing" the nitrogen−proton projection ($\alpha = 90°$) from this HNCA measurement to use in a subsequent HN(CO)CA experiment on the same sample. Only two new projections are then needed, acquired at tilt angles $\alpha = \pm 60°$. The resulting spectra[6] are essentially indistinguishable from those recorded conventionally.

To assess the feasibility of studying larger molecules by the new technique, experiments were performed on a 1.7 mM aqueous solution (10% D_2O) of HasA, where the three-dimensional HNCO spectrum is rich in absorption peaks. Since the number of required projections depends on the complexity of the spectra, it is advantageous to operate at high magnetic field. In this instance, spectra were recorded at 700 MHz with projections taken at $\alpha =$ 0°, ±30° ±60°, and 90°, requiring a total duration of 33 min 40 s. Figure 2 compares two typical F_1F_2 planes obtained by the conventional methodology (left) with the corresponding planes recreated from projections (right). The reconstruction technique generates identical patterns of carbon−nitrogen cross-peaks and also offers better resolution.[6]

The instrument time required for projection−reconstruction depends on the number of required projections N, a parameter determined by the complexity of the spectrum. For a typical case $N = 4$, with 32 evolution steps in t_1 and t_2 (incremented simultaneously), compared with a conventional three-dimensional experiment with 64 × 32 increments in t_1 and t_2 (incremented independently), the theoretical speed advantage would be a factor of 16, reducing a day-long measurement to about an hour. Although the sensitivity *per unit time* is the same as that of the conventional mode, it is reduced in proportion to the square root of the

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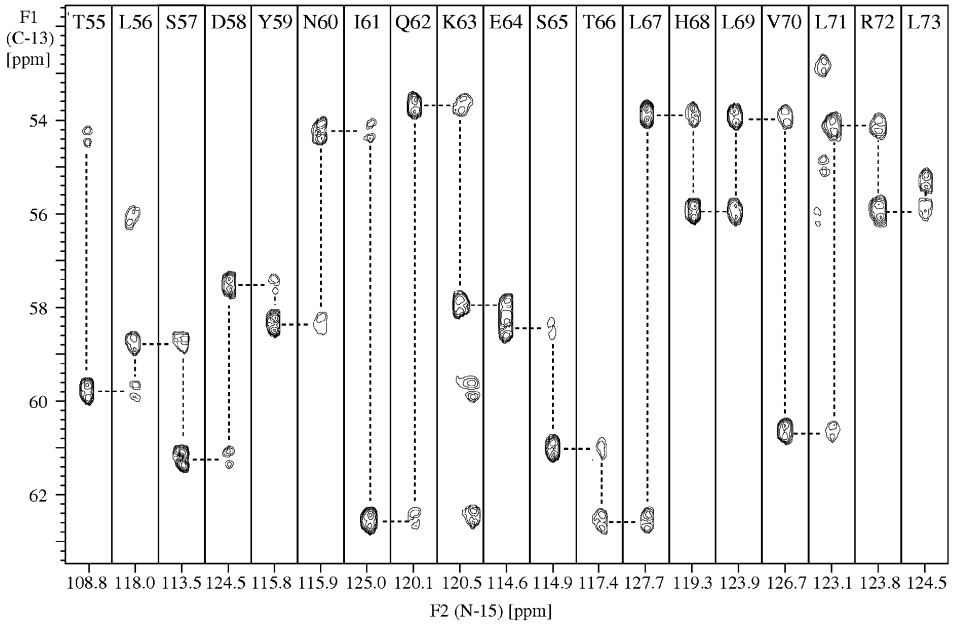

Figure 1. Strip plots of the 500-MHz HNCA spectrum of 1 mM aqueous solution (10% D_2O) of ubiquitin, uniformly labeled in carbon-13 and nitrogen-15. These spectra were reconstructed from plane projections tilted at $\alpha = \pm 30°$ with respect to the F_1F_3 (carbon−proton plane) and at $\alpha = 0°$ and 90°. Note the doublet character of the cross-peaks, attributed to carbon−carbon couplings; this reduces the sensitivity. Total experimental time, 1 h 30 min.

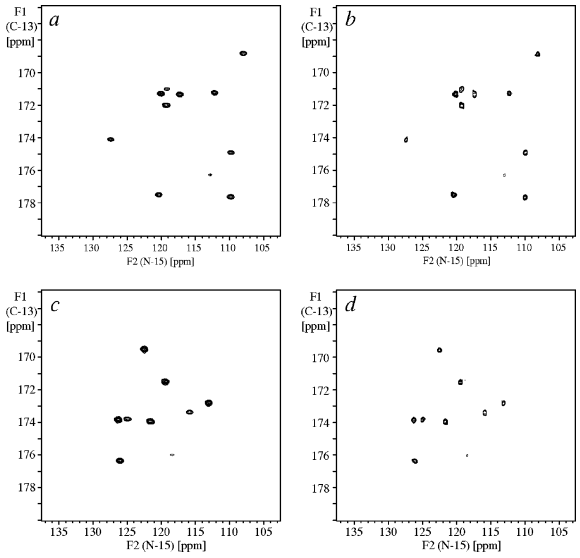

Figure 2. Comparison of conventional (left) and reconstructed spectra (right) from 700-MHz three-dimensional HNCO experiments on a 187-residue protein HasA. These two typical F_1F_2 planes show carbon−nitrogen correlations. Reconstruction involved data from six different projections, $\alpha = 0°$, $\pm 30°$, $\pm 60°$, and 90°, with respect to the carbon−proton F_1F_3 plane. Total experimental duration for the reconstructed spectra, 33 min 40 s (to be compared with 11 h for the conventional mode).

experimental duration. Consequently, projection−reconstruction can realize its full theoretical speed advantage only if the inherent sensitivity of the sample is already adequate.[6]

Four-dimensional spectra are also amenable to projection−reconstruction, using simultaneous incrementation of three evolution parameters, t_1, t_2, and t_3, at different rates. The speed advantage is predicted to be correspondingly higher than that in three-dimensional spectroscopy. The results can be presented as a set of two-dimensional contour plots or as a table of correlated frequency coordinates. Work along these lines is in progress.

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Supporting Information Available: Comparison of conventional and reconstructed HN(CO)CA spectra of ubiquitin, and practical considerations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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