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COMMUNICATIONS

Published on Web 01/08/2004

Generalized Reconstruction of *n*-D NMR Spectra from Multiple Projections: Application to the 5-D HACACONH Spectrum of Protein G B1 Domain

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Studies of biological macromolecules by NMR spectroscopy rely upon multidimensional, multinuclear experiments to separate the large number of resonances present and to provide correlations between these resonances to aid in their assignment. Traditionally, the number of different nuclei *n* that can be correlated in an experiment has been limited, however, by the need to sample all of the points in the *n*-D frequency space. This is achieved by stepping through the evolution dimensions systematically. Even with severely curtailed resolution, 3-D and 4-D experiments require days and weeks of instrument time, and higher-dimensional experiments have not been practical in reality. To overcome this problem, several groups have developed ideas for fast multidimensional NMR, the strengths and weaknesses of which have been summarized in recent reviews.[1,2]

Kupče and Freeman have recently proposed and demonstrated that one could reconstruct full 3-D[3,4] and 4-D spectra (personal communication) by collecting a small subset of 2-D spectra. For a 3-D reconstruction, two orthogonal projections with either t_1 or t_2 set to zero are used to create a "peak lattice." Degenerate peaks are then resolved by the addition of tilted projections, collected by incrementing t_1 and t_2 simultaneously at a fixed ratio. Here, we report the extension of this concept to spectra of arbitrary dimensionality, reconstructed from lower dimensional projections.

A point in an *n*-D orthogonal space is described by its coordinates (ω_0, ω_1...ω_{n-1}). The projection of such a point onto a tilted vector is equivalent to rotating the coordinate system and can be calculated by the simple relationship:

$$\omega_{\text{tilt}} = \sum_{i=1}^{n-1} \omega_i \cos \alpha_i, \text{ with } \sum_{i=1}^{n-1} \cos^2 \alpha_i = 1 \qquad (1)$$

where α_i is the angle between the tilt projection and the orthogonal axis of that dimension (Figure 1).

Analogous to the coordinate rotation, where the new ω_{tilt} represents a linear combination of different ratios of ω_1 to ω_{n-1}, the projection can be experimentally observed by evolving t_1 to t_{n-1} simultaneously. The maximum spectral width that the tilted vector needs to cover is:

$$sw_{\text{tilt}} = \sum_{i=1}^{n-1} sw_i \cos \alpha_i \qquad (2)$$

where sw_i is the spectral width of the indirect dimension *i* in the orthogonal space. The time increment needed for each evolution dimension to achieve this tilt is:

$$\Delta t_i = \cos \alpha_i / sw_{\text{tilt}} \qquad (3)$$

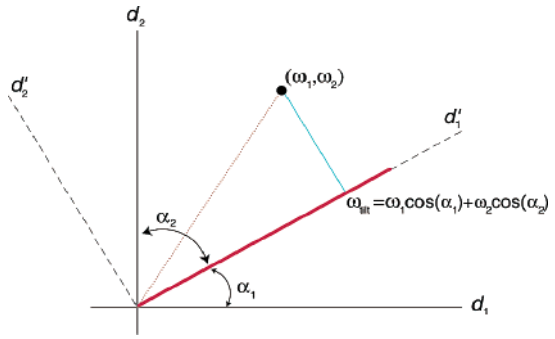

Figure 1. The projection of a point onto a vector is equivalent to rotating the coordinate system. For higher dimensional spectra and higher dimensional projections, the same concept can be used to determine projection points during reconstruction.

The directly observed dimension in any spectrum, represented here by the coordinate ω_0, is a special case, because it is used to separate signals and to reduce the complexity of the spectrum and cannot co-evolve with other dimensions. Thus, constructing an *n*-D spectrum from 2-D projections is equivalent to constructing (n − 1)-D spectra from 1-D vectors at different constant ω_0 values.

The reconstruction method by Kupče and Freeman[3] can be generalized for *n*-D spaces and can be written for reconstruction from n − 1 orthogonal 2-D projections and *m*-tilted 2-D projections:

$$S_{n-\text{D}}(\omega_0,...\omega_{n-1}) = \min\begin{bmatrix} |S_1(\omega_0,\omega_1)|,...|S_{n-1}(\omega_0,\omega_{n-1})|, \\ |S_{\text{tilt},1}(\omega_0,\omega_{\text{tilt},1})|,...|S_{\text{tilt},m}(\omega_0,\omega_{\text{tilt},m})| \end{bmatrix} \qquad (4)$$

where $S_{\text{n-D}}$ represents the reconstructed spectrum and S_1...$S_{\text{tilt},m}$ represent the projections, and with ω_{tilt} for each tilted projection calculated by eq 1, using the set of angles that apply to that projection. The sensitivity of the reconstructed spectrum is limited by the projection with the lowest signal-to-noise ratio. It is important to note that this formula allows for the reconstruction of any arbitrary point in the *n*-D space directly, without reference to the rest of the space, which is useful for the analysis and visualization of a higher dimensional matrix. The same coordinate rotation concept can be applied for reconstruction from 3-D projections, projections of even higher dimensionality, or projections with different dimensionalities.

Simultaneously incrementing the evolution parameters has been employed by several groups to speed up multidimensional experiments.[1,5−8] The resulting intermodulation of chemical shifts can be disentangled, for example, by the G-matrix methodology to

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10.1021/ja039430q CCC: $27.50

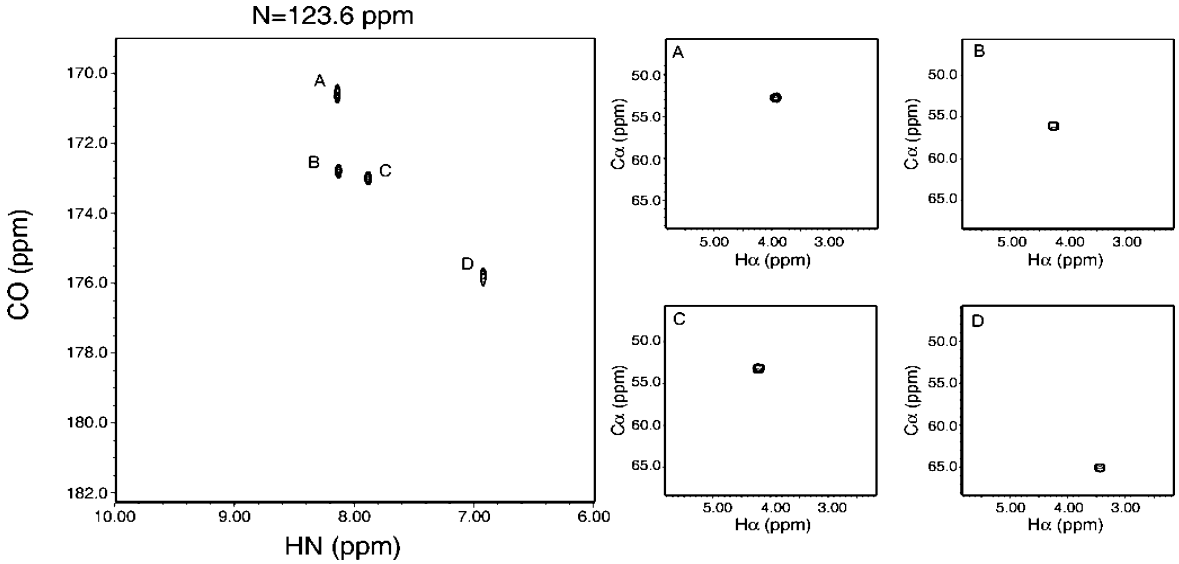

Figure 2. At left, a slice from the reconstructed 3-D HNCO spectrum. At right are the four $H^{\alpha}-C^{\alpha}$ planes from the reconstructed 5-D spectrum corresponding to the peaks in the HNCO slice, each showing a single peak. The resolution of the reconstructed 5-D spectrum was 512 × 64 × 64 × 64 × 64. All projections had 512 complex t_2 points for 4000 Hz spectral width. The spectral widths and numbers of complex points in the indirect dimensions were: for $HN-H^{\alpha}$, 2200 Hz, 20 points; for $HN-C^{\alpha}$, 3400 Hz, 16 points; for HN−C', 2000 Hz, 32 points; for HN−N, 2100 Hz, 32 points; for the diagonal, 4980 Hz, 17 points.

produce a set of "base spectra".[8] These base spectra, in fact, represent tilted projections at fixed angles. Taking the GFT 5-D HACACONH experiment, for example, base spectra 1−8 are projections at angles (60°/120°, 60°/120°, 60°/120°, 60°) relative to the orthogonal C^{α}, H^{α}, C′, and N axes, respectively. Depending upon spectral complexity, however, it may be possible to reconstruct a spectrum with less than the $2^k - 1$ planes needed to determine chemical shifts as described in the GFT methodology.[8]

Although chemical shift information can be extracted from projected spectra through a curve-fitting procedure,[8] reconstructing the *n*-D matrix offers several advantages. First, it offers a "conventional" way of data presentation and examination. With a handful of tilted projections, peak shape can also be approximated.[3] Furthermore, real peak intensities, which are important in several types of experiments such as relaxation studies, could be extracted from the projections through a more elaborate reconstruction procedure, which we are evaluating.[9]

To illustrate these concepts, we have reconstructed the 5-D HACACONH spectrum of the 56-residue protein G B1 domain from four orthogonal 2-D planes ($H^{\alpha}-HN$, $C^{\alpha}-HN$, C′−HN, N−HN) collected in four separate experiments and eight additional tilted planes (corresponding to base spectra 1−8 in the GFT method) collected in a single experiment by co-evolution of H^{α}, C^{α}, C′, and N, using a 2 mM $^{15}N/^{13}C$ double labeled sample. Details of the pulse sequence and experimental setup will be described elsewhere. A C program, PR-CALC, was written to reconstruct spectra of arbitrary dimensionality from projections of arbitrary dimensionality and orientation. Linear interpolation was used to allow application of eq 4 to discrete spectra. Since it is rather cumbersome to reconstruct and visualize the complete 5-D matrix, we have devised the following strategy to analyze the data from these experiments. First, a 3-D HNCO spectrum was produced by co-evolving C' and N to give ±45° projections, followed by reconstruction with the orthogonal C'−HN, N−HN projections. The HNCO spectrum contained the expected 51 peaks corresponding to residues not preceded by glycine, with no degeneracy. 2-D $H^{\alpha}-C^{\alpha}$ planes from the 5-D matrix corresponding to these peak positions at fixed HN, N and C′ chemical shifts were then reconstructed to yield the positions in the C^{α} and H^{α} dimensions (Figure 2). All but one plane contained single peaks located according to these chemical shifts, with the one plane containing an artifact in addition to the real peak. The degeneracy in that slice was removed by the addition of an extra tilted projection crossing the N and C^{α} dimensions at a 30° angle with respect to C^{α}. The four orthogonal and eight tilted planes were collected in 87 min; the additional ±45° tilted projection for the independent HNCO reconstruction required 12 min.

For proteins much larger than protein G B1 domain, more tilted projections could be used to resolve peak degeneracies. Indeed, with a few more tilted planes, 3-D HNCA and HN(CO)CA spectra have been reconstructed for a 20 kDa protein.[4] Sensitivity limitations will likely be improved by the development of new technologies, such as cryogenic probes. These results suggest that projection−reconstruction has exceptional potential. The procedure is simple and transparent, functions using conventional equipment, allows for reconstruction at high resolution, and is exceedingly fast.

Acknowledgment. We thank Prof. Ray Freeman for encouragement and helpful discussions and for providing a preprint of a paper in press, and we thank Prof. Lewis Kay for proofreading this manuscript. This work was supported by NIH Grant AI055588-01 to P.Z. B.E.C. is the recipient of an NSF Graduate Research Fellowship.

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JA039430Q