



Reconstruction of the three-dimensional NMR spectrum of a protein from a set of plane projections

Ěriks Kupĉe^a & Ray Freeman^{b,*}

^aVarian, Inc., Eynsham, Oxford, U.K.; ^bJesus College, Cambridge, U.K.

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Abstract

Three-dimensional protein NMR spectra can be obtained significantly faster than by traditional methods by a projection-reconstruction procedure related to X-ray tomography. First, two orthogonal projections are acquired in quick two-dimensional experiments with the evolution parameters t_1 or t_2 set to zero. These projections define a three-dimensional lattice; all cross-peaks must lie on this lattice but not all lattice points are occupied. A third experiment with t_1 and t_2 incremented simultaneously and in a fixed ratio, generates a projection onto a tilted plane and thus establishes the positions of all the cross-peaks unambiguously. This projection-reconstruction technique has been tested on the 500 MHz three-dimensional HNCO spectrum of ubiquitin.

The recent flurry of publications on fast multidimensional NMR spectroscopy (Chen et al., 2000, 2003; Ding and Gronenborn, 2002; Szyperski et al., 2002; Kim and Szyperski, 2003; Frydman et al., 2002, 2003; Kupĉe and Freeman, 2003a–c; Kozminski and Zhukov, 2003) serves to highlight widespread concerns of NMR spectroscopists about the slow rate of data acquisition in the traditional Fourier transform mode (Jeener, 1971; Aue et al., 1976). The systematic step-by-step exploration of the evolution dimensions one by one is unnecessarily slow and can tie up an expensive spectrometer for days at a time, particularly for large molecules like proteins. The new fast techniques are based on the premise that the requisite information can be extracted from a much smaller number of measurements, at least for reasonably sparse spectra with a good signal-to-noise ratio.

The present communication suggests another approach to the speed problem. Consider, for simplicity, a three-dimensional spectrum. The NMR frequency space $S(F_1, F_2, F_3)$ can be regarded as a three-dimensional object, and can be reconstructed from a set of plane projections, just as a three-dimensional

anatomical image can be derived from a set of X-ray projections recorded at different angles of incidence (Hounsfield, 1973). Indeed the NMR ‘object’ presents a simpler challenge, being made up of discrete resonances reasonably well resolved from one another. Furthermore, the projections onto the F_2F_3 and F_1F_3 planes are readily obtained by setting $t_1 = 0$ or $t_2 = 0$ respectively. These are sometimes called the ‘first planes’ of a three-dimensional experiment and they have the advantage that the NMR signals are only moderately attenuated by spin-spin relaxation. These two orthogonal projections can be recorded very much faster than the full three-dimensional spectrum.

A projection is a two-dimensional map made up of pixels generated by a set of regularly spaced parallel rays. The intensity of a typical pixel is the total NMR absorption along the corresponding ray. This highlights a potential source of ambiguity for the reconstruction process. For relatively sparse spectra the projected intensity from a typical ray, combined with intensities measured in an orthogonal projection, may provide enough information to define the all the cross-peak intensities, but in the general case uncertainties can persist. Fortunately, just as in X-ray tomography, additional skew projections can be em-

*To whom correspondence should be addressed. E-mail: rf110@hermes.cam.ac.uk

ployed to help reconstruct the entire three-dimensional spectrum unambiguously.

There is a theorem (Bracewell, 1956; Nagayama et al., 1978) which states that the Fourier transform of a section through a two-dimensional time-domain signal passing through the origin and subtending an angle α with respect to the t_1 axis corresponds to a projection onto an axis through the origin of the frequency domain spectrum subtending the same angle α with respect to the F_1 axis. This theorem is readily generalized for three-dimensional data tables involving projection onto a tilted plane. If the evolution parameters t_1 and t_2 are incremented simultaneously with step sizes in the ratio $\Delta t_2/\Delta t_1 = \tan \alpha$, a hypercomplex transformation generates two new projections onto planes intersecting at the origin and tilted through angles $\pm\alpha$ with respect to the F_1F_3 plane. Although these new projections are also susceptible to ambiguities in the cross-peak intensities, they serve to lift the degeneracies that occurred when the 'first plane' projections alone were employed. In many cases just one tilt operation provides enough new intensity information to derive all the cross-peak intensities.

Simultaneous incrementation of two evolution parameters has already been used by several investigators (Brutscher et al., 1995; Ding and Gronenborn, 2002; Kim and Szyperski, 2003; Kozminski and Zhukov, 2003) but they employ quite different techniques for data processing. These earlier methods detect linear combinations of the chemical shifts that evolve in the two 'locked' dimensions, and these shifts must then be separated, for example by the G-matrix transformation (Kim and Szyperski, 2003). The projection-reconstruction processing scheme is simple and transparent, providing a direct comparison with the classic three-dimensional spectrum. It avoids some potential pitfalls of peak-picking routines, and needs no operator intervention. The sensitivity *per unit time* of the projection-reconstruction method is essentially the same as that of the conventional three-dimensional experiment. The resolution of the reconstructed three-dimensional spectrum is not degraded, being determined by the resolution in the plane projections, information that is well-digitized. In contrast, three-dimensional spectra recorded by the conventional technique often have poor resolution because sampling in the evolution dimensions is deliberately restricted in order to reduce the duration of the experiment.

Consider the three-dimensional HNCO experiment commonly employed for proteins uniformly enriched

in carbon-13 and nitrogen-15, in which proton polarization is transferred to N and then to CO before being returned to N and thence to H for detection. The F_1 axis shows the carbon-13 frequencies, the F_2 axis the nitrogen-15 frequencies and the F_3 axis the observed proton frequencies. This type of spectrum has the inherent simplicity that a given proton response is correlated with only one carbon and one nitrogen site. Of course proton responses often overlap in protein spectra. The information carried by the projections onto the F_1F_3 and F_2F_3 planes, obtained from the appropriate two-dimensional experiments, is examined one F_1F_2 plane at a time. A set of orthogonal traces drawn through the projection peaks along the F_1 and F_2 axes defines a two-dimensional lattice where cross-peaks could conceivably occur, but not all of these lattice points are occupied. Stated in another way, the intensities remain ambiguous, and some in fact are zero. These ambiguities can be resolved by measuring a suitably tilted projection.

There are several possible projection-reconstruction procedures; the simple scheme adopted here is best illustrated by a concrete example. The sample is a 1 millimolar aqueous solution of ubiquitin with 10% D_2O studied on a Varian Inova 500 spectrometer, using the popular HNCO pulse sequence (Kay et al., 1994). First, projections onto the F_1F_3 and F_2F_3 planes are measured and the data re-examined one F_1F_2 plane at a time (at a fixed proton frequency). A data array 'A' is generated by extending the projection peaks on the F_1 axis into parallel ridges running the entire length of the F_2 dimension (Figure 1). A second data array 'B' is created by drawing similar ridges in the F_1 dimension at every peak along the F_2 axis. At each point in the F_1F_2 plane the algorithm compares intensities in the 'A' and 'B' arrays and retains the lower value (McIntyre and Freeman, 1989; McIntyre et al., 1990). This leaves peaks only where the ridges intersect, representing all *conceivable* locations for the cross-peaks in this particular plane.

The contour map of Figure 2 shows the twelve responses from ubiquitin in the selected plane. The *actual* cross-peaks are identified by taking a tilted projection – in this case one inclined at 30° with respect to the F_1 axis. The projection peaks along this skew axis are used to create parallel ridges running across the F_1F_2 plane at 60° with respect to the F_1 axis. The true cross-peaks must all lie under these new ridges. When the lower-value algorithm is re-imposed on the twelve 'possible' cross-peaks, only four survive this operation (Figure 3). The process is then repeated for all F_1F_2

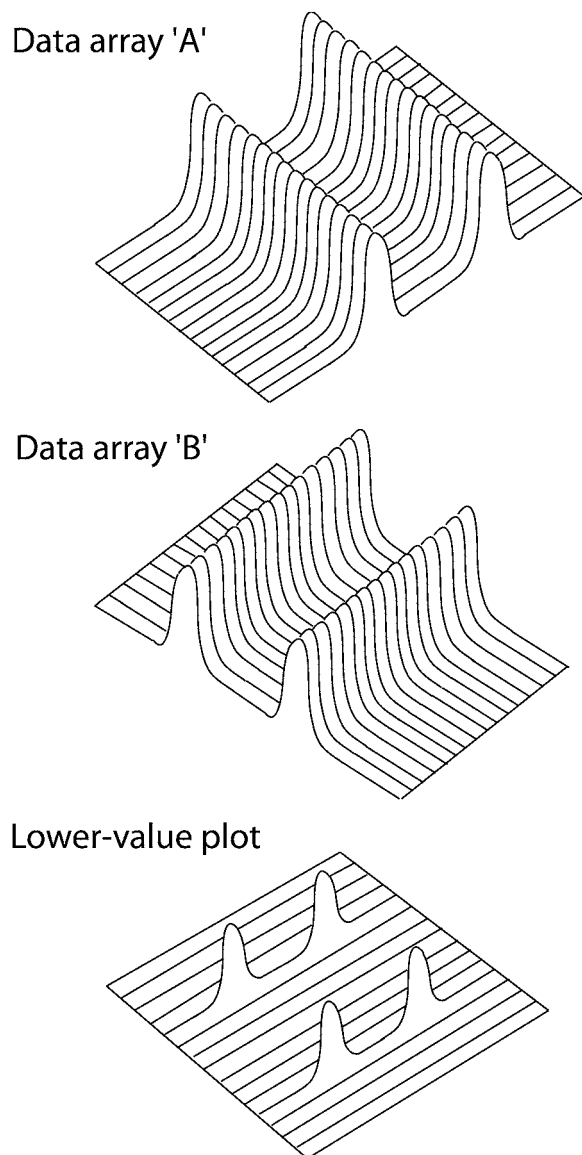


Figure 1. Schematic diagram showing how the experimental projections onto two orthogonal axes are extended into parallel ridges to form data arrays 'A' and 'B'. Intensities at corresponding locations in 'A' and 'B' are compared and the lower value retained. This leaves peaks at the intersections of the two sets of ridges.

planes until the entire three-dimensional spectrum has been reconstructed.

The time saving is appreciable. The two orthogonal projections required 9 min 46 s, and 9 min 40 s of data accumulation, while the tilted projection took 9 min 45 s, a total duration of 29 min 11 s to reconstruct the entire three-dimensional spectrum. For comparison, the corresponding conventional three-dimensional spectrum of ubiquitin required 18 h

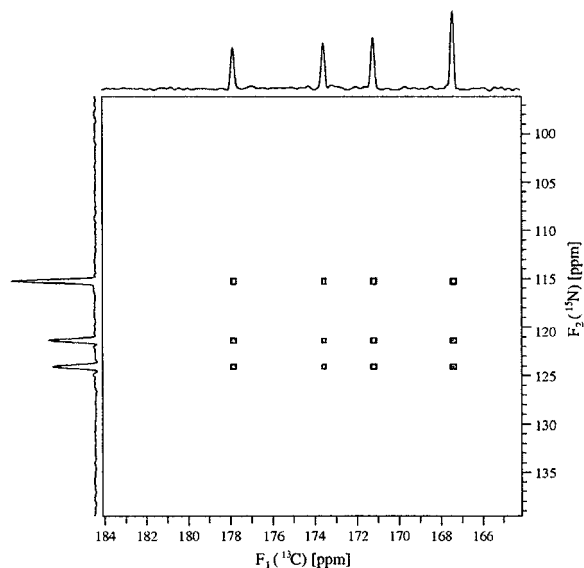


Figure 2. A selected F_1F_2 plane from the HNCOC spectrum of ubiquitin derived from the two orthogonal projections shown along the top and left margins. The intensity contours indicate twelve possible locations for C-N cross-peaks, but more information is required before the true cross-peaks can be identified. Overall experimental duration: 19 min 26 s.

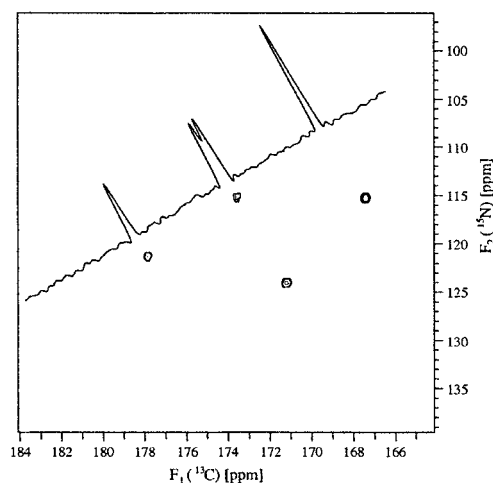


Figure 3. The same selected F_1F_2 plane of ubiquitin with the twelve 'potential' cross-peaks constrained by information from the 30° projection. Only four true C-N cross-peaks have survived this operation. The tilted projection required 9 min and 45 s of data accumulation.

and 54 min, almost 39 times longer. Figure 4 compares three typical F_1F_2 planes derived by projection-reconstruction with the corresponding planes from the full three-dimensional experiment. The projection-reconstruction spectra were twice as finely digitized as

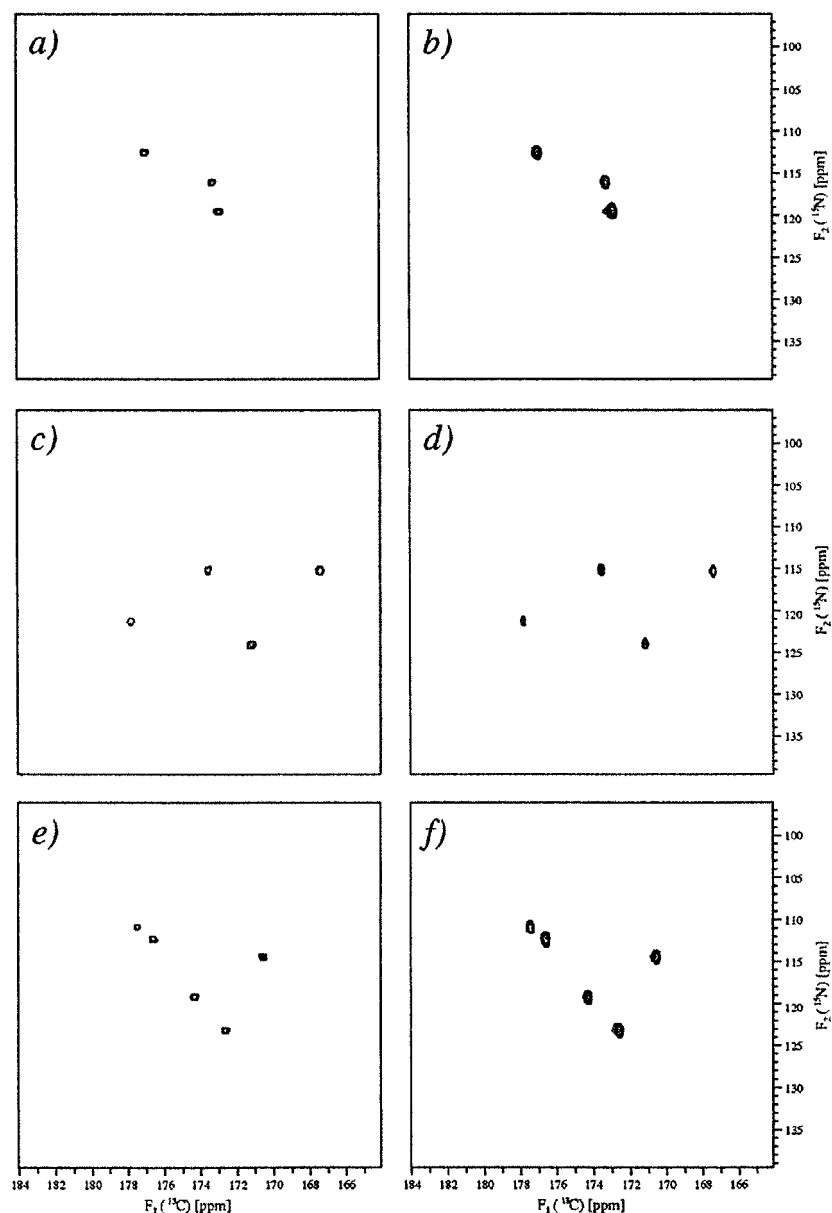


Figure 4. Selected F_1F_2 planes from the ubiquitin spectrum, comparing projection-reconstruction (left) with the conventional method (right). The planes representing C-N correlations were extracted at proton frequencies 7.28 ppm for (a) and (b); 8.31 ppm for (c) and (d); 8.77 ppm for (e) and (f). The full conventional spectrum required 18 h 54 min of data accumulation.

the conventional spectra; otherwise the two versions are indistinguishable.

The data acquisition process is essentially equivalent to three separate two-dimensional measurements, and can be completed much faster than the full three-dimensional experiment where the two evolution dimensions must be explored independently, one step at a time. If higher resolution is desired, the signals in

the t_1 and t_2 dimensions can be followed for longer periods without involving an unrealistically long experiment. This could revive interest in F_1F_2 sections, which, in HNC0 spectra, show the carbon-nitrogen correlations. This display mode is little studied by the conventional methodology because of poor resolution.

For more complex spectra a different processing scheme may be required. As before, each F_1F_2 plane is

processed separately. Suppose there are M projection peaks on the F_1 axis and N projection peaks on the F_2 axis; in general, M and N can be quite large numbers. As described above, a two-dimensional lattice is constructed to define potential cross-peak co-ordinates. The intensity of each projection peak defines the sum of the intensities along the corresponding trace, so there are $M + N$ simultaneous equations governing the intensities of MN (potential) cross-peaks, a notoriously underdetermined situation when M and N are large.

The additional information is extracted from a third projection onto a tilted plane. In principle, a program could be written to calculate undesirable choices of tilt angle – those that would catch many of the lines in *enfilade* and thus provide fewer new simultaneous equations governing the intensities. The worst conceivable situation occurs when the intersections in the F_1F_2 plane form a *regular* lattice, which in reality is quite unlikely. It may well turn out that only a single tilted projection is required in many cases, but further skew projections can be obtained if necessary. In this manner a self-consistent picture of the entire three-dimensional spectrum can be constructed.

This communication is intended to demonstrate the principle of the projection-reconstruction method by reference to a simple case, but there is every reason to believe that the technique can be applied quite generally. The spectra were deliberately run at a relatively low field (protons at 500 MHz); better results would be anticipated in a high-field spectrometer. Although the three-dimensional case is easier to visualize, four-dimensional spectra could also be treated by projection-reconstruction, with similar savings in instrument time. All multidimensional spectra

can in principle be reduced to sets of plane sections; indeed the usual practical method for displaying the results of high-dimensional experiments is to plot selected planes.

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