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Fast reconstruction of four-dimensional NMR spectra from plane projections

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Abstract

Four-dimensional NMR spectroscopy can be speeded up by a large factor by a projection-reconstruction technique related to that used in X-ray tomography. The limited amount of information recorded in a few suitably-tilted projection planes suffices to recreate the entire four-dimensional spectrum. The method is demonstrated by reference to the 500 MHz spectrum of 1 millimolar aqueous solution of ubiquitin by dispersing the responses of the three-dimensional HNCO spectrum into the C_α dimension of a four-dimensional HNCOCA spectrum obtained by projection-reconstruction.

Three-dimensional spectra of proteins are sometimes presented as two stereoscopic views – projections taken at slightly different angles of incidence. This suggests that a limited number of plane projections can define the entire spectrum, even when the latter is quite complex. Recently this concept has been exploited (Kupče and Freeman, 2003a, b; Kupče et al., 2003) to speed up the acquisition of three-dimensional NMR spectra by reconstructing them from a small number of projections. The procedure is analogous to that used in X-ray tomography (Hounsfield, 1973) where the physiological object to be recreated is continuous, in contrast to the discrete nature of an NMR spectrum. The method of projection relies on a well-established Fourier transform theorem (Bracewell, 1956; Nagayama et al., 1978) that relates a section through the origin of a time-domain signal $S(t_1, t_2)$ at an arbitrary angle α, to the projection of the corresponding frequency-domain spectrum $S(F_1, F_2)$ onto an axis through the origin inclined at the same angle α. An extension of this principle to a three-dimensional spectrum $S(F_1, F_2, F_3)$ indicates that when t_1 and t_2 are incremented simultaneously Fourier transformation generates a projection onto a tilted F_1F_2 plane (Kupče and Freeman, 2003a).

Projection-reconstruction ('PR-NMR') can also be applied to four-dimensional spectroscopy, a technique which often proves to be tediously slow by the traditional methodology. The process can be visualized by focusing attention on the three-dimensional data array $S(F_1, F_2, F_3)$ representing the evolution dimensions of a four-dimensional spectrum $S(F_1, F_2, F_3, F_4)$, leaving the direct-detection dimension (F_4) to the imagination (Figure 1). There are three distinct categories of projection to be considered. Those in the first category (the 'orthogonal' projections) are obtained by Fourier transformation of signals acquired by varying only one of the evolution-time parameters, keeping the other two fixed at zero. For example if t_1 is varied while $t_2 = t_3 = 0$, the resulting projection is onto a plane defined by the F_1 and F_4 axes. The two remaining orthogonal projections are on the F_2F_4 and F_3F_4 planes.

The second category involves simultaneous variation of two evolution times while the third is held at zero. For example, if t_1 and t_2 are varied in concert while $t_3 = 0$, quadrature detection in t_1 and t_2 followed by hypercomplex Fourier transformation of the appropriate combinations of signals generates two tilted projection planes. They are defined by F_4

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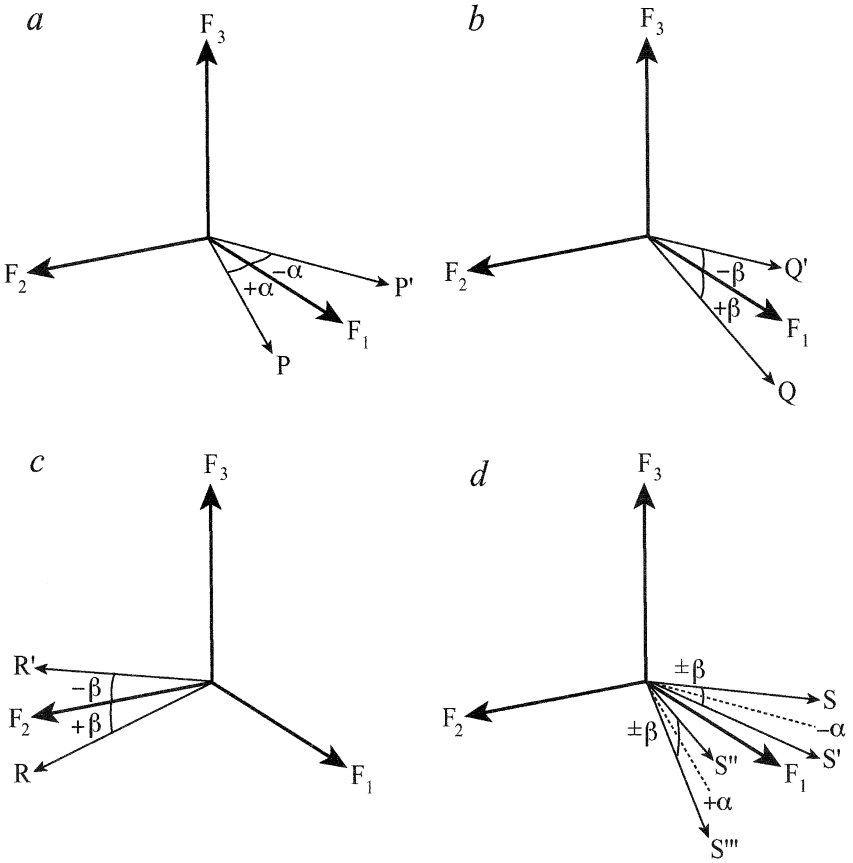

Figure 1. The three evolution dimensions of a four-dimensional experiment; the direct detection axis F_4 is not shown. (a) If $t_3 = 0$, while t_1 and t_2 are incremented jointly with $\Delta t_2/\Delta t_1 = \tan\alpha$, then Fourier transformation generates tilted projections onto two planes defined by P or P' and the F_4 axis. (b) If $t_2 = 0$ while t_1 and t_3 are linked, with $\Delta t_3/\Delta t_1 = \tan\beta$, the two projection planes are defined by Q or Q' and the F_4 axis ($\alpha = 0°$). (c) If $t_1 = 0$ while t_2 and t_3 are linked, the two projection planes are defined by R or R' and the F_4 axis ($\alpha = 90°$). (d) If t_1, t_2 and t_3 are all linked together, there are four doubly-tilted projection planes defined by S, S', S'' or S''' and the F_4 axis. In the practical application described below, the F_1 axis corresponds to the C=O dimension, F_2 to the Cα dimension, and F_3 to the nitrogen-15 dimension.

and the vectors P or P' (Figure 1a) and they occur in symmetrically-related pairs tilted at $\pm\alpha$ where $\tan\alpha = \Delta t_2/\Delta t_1$ and Δt_2 and Δt_1 are the increments in the two linked dimensions. If t_1 and t_3 are linked while $t_2 = 0$, there are two projection planes inclined at $\pm\beta$ with $\alpha = 0°$, and defined by the vectors Q or Q' and the F_4 axis, with $\tan\beta = \Delta t_3/\Delta t_1$ (Figure 1b). If t_2 and t_3 are linked while $t_1 = 0$, there are two projection planes defined by F_4 and the vectors R or R', inclined at $\pm\beta$ with $\alpha = 90°$ (Figure 1c). This gives a total of six different projection planes in this category.

The third category are doubly-tilted projections. They correspond to simultaneous incrementation of t_1, t_2 and t_3 at rates determined by

$$t_1 = t\cos\alpha\cos\beta,$$
$$t_2 = t\sin\alpha\cos\beta,$$
$$t_3 = t\sin\beta.$$

Four complex planes are recorded with all eight permutations of 0° and 90° radiofrequency phase to give the usual quadrature detection in each evolution dimension, implemented according to the States-TPPI scheme (Cavanagh et al., 1996). Hypercomplex Fourier transformation of the appropriate combinations of signals generates a set of four doubly-tilted projection planes defined by the F_4 axis and the vectors S, S', S'' or S''', corresponding to tilt angles of $\pm\alpha$, $\pm\beta$. These provide four independent views of the four-dimensional spectrum (Figure 1d).

Certain 'reduced dimensionality' experiments also link evolution times together (Szyperski et al., 1993, 2002; Brutscher et al., 1995; Ding and Gronenborn,

2002; Kim and Szyperski, 2003; Kozminski and Zhukov, 2003) but in practice the evolution times are all incremented at the same rate. Successive evolution at chemical shifts Ω_A, Ω_B and Ω_C, with acquisition of the real and imaginary signal components in all three evolution dimensions generates a spectrum made up of components at the sum and difference frequencies ($\Omega_A \pm \Omega_B \pm \Omega_C$). These must be separated, for example by means of a G-matrix, peakpicking and a least-squares fitting procedure (Kim and Szyperski, 2003). By contrast, the PR-NMR method generates spectra in the familiar format; the only effect of intermodulation of chemical shifts is to create symmetrically related pairs of projections, defined by $\pm\alpha$ or $\pm\beta$.

PR-NMR introduces a new degree of freedom – the three evolution parameters are varied at different rates, so that arbitrary values of α and β can be selected (there is no particular merit in a choice of a 45° tilt angle). This permits the recording of projections tilted at any desired angle, and any residual ambiguities in the reconstruction process caused by accidental overlap can be resolved by taking a new set of projections at a different angle of incidence. Although this prolongs the data gathering, it is still far faster than the conventional mode, where all combinations of t_1, t_2 and t_3 must be explored systematically, with penalties in spectral resolution if the digitization is unduly restricted.

Reconstruction of the target spectrum is achieved by finding the only data array $S(F_1, F_2, F_3, F_4)$ that is compatible with all the measured projections. There are several possible algorithms for this purpose, but a simple superposition routine (Kupče and Freeman, 2003a) combined with a lower-value test (McIntyre and Freeman, 1989; McIntyre et al., 1990) has been found to work well for spectra with an adequate signal-to-noise ratio. The results would normally be presented as selected two-dimensional sections through the four-dimensional spectrum. Once allowance has been made for the shorter measurement time, the sensitivity of the PR-NMR technique matches that of the conventional mode. The spectral resolution is not compromised; indeed it can be improved if necessary by sacrificing part of the speed advantage. Four-dimensional PR-NMR employs the familiar standard radiofrequency pulse sequences without modification, simply linking the evolution times together, two or three at a time.

NMR spectroscopists have recourse to a higher frequency dimension when a simpler version of the spectrum is not fully resolved. For example, it may well happen that there is overlap of CO resonances in a three-dimensional HNCO spectrum (Kay et al., 1994) that can be resolved by recording the corresponding four-dimensional HNCOCA spectrum (Yang and Kay, 1999) to take advantage of the dispersal by the Cα frequencies. We illustrate this principle by reference to the 500 MHz spectra of 1 millimolar aqueous solution (10% D_2O) of ubiquitin, uniformly labelled in carbon-13 and nitrogen-15. The three-dimensional HNCO spectrum was recorded by the conventional method. One of the F_1F_2 planes was selected, containing five responses, representing the carbon-nitrogen correlations at a proton frequency of 8.77 ppm. Although there is in fact no actual overlap here, spreading out these five signals into a fourth frequency dimension demonstrates the feasibility of resolving degenerate resonances when they do arise.

The four-dimensional HNCOCA experiment recorded the projections onto the three orthogonal 'first planes' F_1F_4, F_2F_4 and F_3F_4 by varying t_1 with $t_2 = t_3 = 0$, by varying t_2 with $t_1 = t_3 = 0$, and by varying t_3 with $t_1 = t_2 = 0$, respectively. For the next stage, given the choice between recording six tilted projections or four doubly-tilted projections, we selected the latter. The three evolution times were linked together as described above, and signals were acquired with all eight permutations of 0° and 90° radiofrequency phase. Each signal was sampled as 60 complex data points, extended by linear prediction to 128 points, and then zero-filled to 512 points. Hypercomplex Fourier transformation of the appropriate combinations of these eight responses gave four doubly-tilted projections at $\alpha \pm 30°$, $\beta \pm 60°$ (Figure 1d). Together with the first-plane projections these supplied enough information to reconstruct the entire four-dimensional HNCOCA spectrum. (In fact the doubly-tilted projections alone would have sufficed.)

This made it possible to extract five different F_1F_3 planes, all taken at the same proton frequency (8.77 ppm) but at different Cα frequencies, thus separating the five responses as illustrated in Figures 2b–f. The total experimental time was only 38 min, whereas a full four-dimensional HNCOCA spectrum recorded by the conventional method could take as long as seven days, depending on the choice of resolution conditions (Yang and Kay, 1999). This is a speed advantage of well over 200, considerably extending the range of applicability of four-dimensional NMR spectroscopy. Apart from the linking of the evolution times there was no modification of the actual pulse

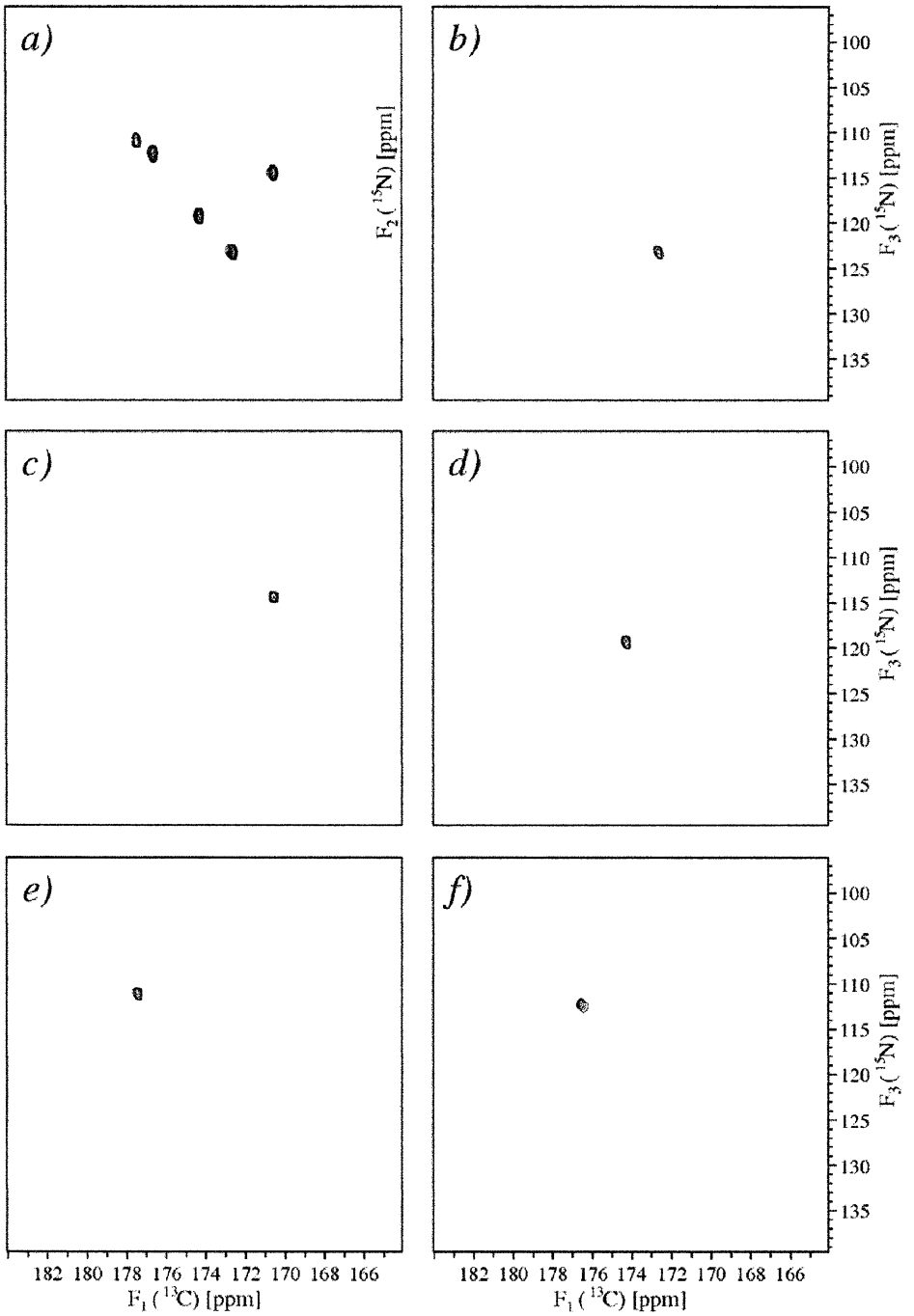

Figure 2. (a) The conventional three-dimensional HNCO spectrum of ubiquitin showing five carbon-nitrogen correlations in the F_1F_2 plane, taken at a proton frequency of 8.77 ppm. These resonances are then separated by recording different F_1F_3 planes from a four-dimensional HNCOCA experiment obtained by projection-reconstruction. The Cα frequencies are (b) 62.0 ppm, (c) 60.9 ppm, (d) 60.0 ppm, (e) 58.1 ppm, (f) 54.6 ppm. This demonstrates (in principle) how overlap in the three-dimensional spectrum could be resolved in the four-dimensional version.

sequence, so there is every reason to believe that PR-NMR is directly applicable to other four-dimensional experiments. The application to very crowded protein spectra has yet to be demonstrated experimentally, but given adequate sensitivity, there is no fundamental reason why this should not be feasible. After all, the anatomical samples examined by X-ray tomography are intrinsically 'crowded'; it is just a question of measuring enough projections.

The question of the minimum number of projections required to solve the general four-dimensional problem will be deferred to a later analysis because there are several possible solutions. To cite just two examples – three orthogonal planes F_1F_4, F_2F_4 and F_3F_4 define all the possible cross-peak positions, and one doubly-tilted plane $(F_1F_2F_3)F_4$ will resolve any ambiguities, provided there is no overlap in the doubly-tilted plane. Alternatively, information from two orthogonal planes can be combined with that from two doubly-tilted planes to define the full four-dimensional spectrum.

The same principles can be generalized to n dimensions, where $n > 4$. Take the case of five-dimensional NMR, which at the present time is seldom studied by the conventional method because of the obvious time constraints. With four linked evolution dimensions and the fifth dimension devoted to direct detection, three independent tilt angles (α, β and γ) must be defined. The simplest procedure is to increment all four evolution times t_1, t_2, t_3 and t_4 together, and this mode by itself provides sufficient information to solve the entire problem. With quadrature detection in all four evolution dimensions the fully-linked experiment requires the recording of eight complex planes. Hypercomplex Fourier transformation of the appropriate combinations of signals generates a set of eight triply-tilted projections at $\pm\alpha$, $\pm\beta$ and $\pm\gamma$. Despite this additional complexity, the speed advantage over conventional spectroscopy is calculated to be appreciably higher than that of the four-dimensional case demonstrated above. PR-NMR adds a useful new weapon to the growing armoury of fast multidimensional NMR methodology (Freeman and Kupče, 2003) opening the way for dynamic studies and spectroscopy in higher dimensions.

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