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Communication

Fast multi-dimensional NMR by minimal sampling

Ēriks Kupče, Ray Freeman *

Varian, Ltd., 6 Mead Road, Yarnton, Oxford, UK
Jesus College, Cambridge University, Cambridge, UK

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Abstract

A new scheme is proposed for very fast acquisition of three-dimensional NMR spectra based on minimal sampling, instead of the customary step-wise exploration of all of evolution space. The method relies on prior experiments to determine accurate values for the evolving frequencies and intensities from the two-dimensional 'first planes' recorded by setting $t_1 = 0$ or $t_2 = 0$. With this prior knowledge, the entire three-dimensional spectrum can be reconstructed by an additional measurement of the response at a single location (t_1^*, t_2^*) where t_1^* and t_2^* are fixed values of the evolution times. A key feature is the ability to resolve problems of overlap in the acquisition dimension. Applied to a small protein, agitoxin, the three-dimensional HNCO spectrum is obtained 35 times faster than systematic Cartesian sampling of the evolution domain. The extension to multi-dimensional spectroscopy is outlined.

Keywords: Agitoxin; Fast multi-dimensional NMR; HNCO; Minimal sampling; Resolving degeneracies

1. Introduction

One of the key turning points in the development of NMR was Jeener's introduction of two-dimensional spectroscopy [1], later extended to a vast armoury of different applications by Ernst [2]. This soon became routine in most fields of NMR. The standard procedure is to explore a sufficiently large number of evolution increments to satisfy the Nyquist sampling condition and the resolution requirements. The only drawback is that *multi*-dimensional spectra can be very time-consuming, because each and every evolution dimension needs to be mapped out with many digitization steps. The two-dimensional version has two key features. Spin coherences that evolve during a variable interval t_1 are *correlated* with responses detected during the ensuing acquisition interval t_2. Secondly, although the receiver is switched off during evolution, the frequencies of the evolving coherences can be determined by systematically incrementing t_1. Now, if we consider *correlation* and *indirect detection* separately, it becomes clear that the all-important correlation information could be obtained much more rapidly. At the interface between evolution and acquisition, it is the final phase of the evolving coherence that establishes the correlation; its frequency need not be measured in this manner because it can be determined from prior measurements. Consequently it is permissible to violate the Nyquist sampling condition in the evolution domain; in the limit only a single value of t_1 should suffice to determine all the correlations, thereby saving a considerable amount of spectrometer time.

We have exploited this idea to acquire and process three-dimensional NMR data much faster than the traditional methodology. Accurate information about the evolving frequencies is obtained from the two-dimensional 'first planes' of the three-dimensional spectrum, recorded by setting first $t_1 = 0$ and then $t_2 = 0$. In the absence of overlap in the acquisition dimension F_3, this is sufficient to reconstruct the full three-dimensional spectrum. When there *is* such overlap, the ambiguities can be resolved by recording the signal at a single location (t_1^*, t_2^*) where t_1^* and t_2^* are fixed values of the evolution times. By avoiding the customary systematic exploration of all of evolution

* Corresponding author. Address: Jesus College, Cambridge University, Cambridge, CB5 8BL, UK. Fax: +44 1223 336362.
E-mail address: rf110@hermes.cam.ac.uk (R. Freeman).

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space on a Cartesian grid, this procedure offers significant improvements in speed compared with existing sparse sampling methods [3–10], radial sampling [11–15] or projection–reconstruction NMR [16–25]. Note that all fast multi-dimensional sampling methods presuppose adequate intrinsic sensitivity; where this is not the case, full systematic sampling of the evolution domain is necessary. We treat the 'sampling-limited case' rather than the 'sensitivity-limited situation'.

The two-dimensional 'first planes' (F_1F_3 and F_2F_3) are used as a starting point; they are recorded by setting $t_2 = 0$ or $t_1 = 0$ in the three-dimensional pulse sequence. It is now common practice to record these planes during the preliminary setting-up procedure before embarking on the actual three-dimensional experiment, and they usually enjoy good sensitivity. This information is essential for two reasons—it provides accurate values for the frequencies used to reconstruct the final three-dimensional spectrum $S(F_1, F_2, F_3)$, and it serves to resolve any ambiguity in cross-peak assignment caused by degenerate chemical shifts in the F_3 dimension. Exact frequency degeneracies in the evolution dimensions (F_1 or F_2) are not resolved, nor would they be in the conventional three-dimensional methodology.

Although this minimal sampling method is quite general, for clarity it is convenient to fix attention on the well-known HNCO pulse sequence, where the successive evolution of the (isotopically enriched) ^{15}N and ^{13}C spins is monitored by direct observation of the NH protons. For well-separated frequencies in the F_3 dimension, the required correlations are immediately obtained from the two-dimensional 'first planes' with no ambiguity, since each proton site is correlated with an ^{15}N site and a ^{13}C site. We concentrate here on the situation where two NH responses overlap; the case of multiple overlap is a straightforward extension of this treatment. Let the ^{15}N spins evolve for a fixed interval t_1^* and the ^{13}C spins for a fixed interval t_2^*. The existence of four *evolving* frequencies ν_{N1}, ν_{N2}, ν_{C1} and ν_{C2} associated with a single degenerate proton site suggests four possible cross-peaks, although clearly only two of these are genuine and two must be false. If the two overlapping NH peaks have significantly different intrinsic intensities, for example if one is strong and the other weak, assignment in the three-dimensional reconstruction is straightforward, because strong correlates with strong, and weak with weak.

In the more general case, where there are N overlapping proton peaks with comparable intensities, the assignment problem is more challenging. Four scans are performed, with the phases of the two radiofrequency coherence transfer pulses set (0°, 0°), (90°, 0°), (0°, 90°) and (90°, 90°). After Fourier transformation with respect to the acquisition time t_3, there are four proton intensity terms recorded with the four combinations of radiofrequency phases:

$$A_{0,0} = \sum_{i=1}^{N} P_i \cos \omega_{Ci} t_1^* \cos \omega_{Ni} t_2^* \tag{1}$$

$$A_{0,90} = \sum_{i=1}^{N} P_i \cos \omega_{Ci} t_1^* \sin \omega_{Ni} t_2^* \tag{2}$$

$$A_{90,0} = \sum_{i=1}^{N} P_i \sin \omega_{Ci} t_1^* \cos \omega_{Ni} t_2^* \tag{3}$$

$$A_{90,90} = \sum_{i=1}^{N} P_i \sin \omega_{Ci} t_1^* \sin \omega_{Ni} t_2^* \tag{4}$$

It is convenient to write (Eqs. (1)–(4)) as the linear combinations:

$$R(+) = A_{0,0} - A_{90,90} \tag{5}$$

$$I(+) = A_{90,0} + A_{0,90} \tag{6}$$

$$R(-) = A_{0,0} + A_{90,90} \tag{7}$$

$$I(-) = A_{90,0} - A_{0,90} \tag{8}$$

The key point is that these *experimental* quantities can also be *calculated* on the basis of the prior intensity and frequency information from the two-dimensional 'first planes'. (Note that at this stage in the processing, most of these ^{15}N and ^{13}C frequencies can be disregarded, having been previously assigned on the basis of non-overlapping NH signals, or because of intensity variations.) We consider the case $N = 2$. Two different 'candidate' sets, each consisting of four proton intensities, are calculated by substituting the known frequencies ν_{N1}, ν_{N2}, ν_{C1} and ν_{C2}, and the corresponding intensities into Eqs. (1)–(8). Although the two alternative sets could have been distinguished from a single measurement, we have used the hypercomplex data set to follow the variations. A typical example of twofold overlap is taken from the HNCO spectrum of agitoxin, described below. It has two possible cross-peak patterns (Fig. 1a). Table 1 sets out the intensity comparisons, and it is clear that set (1) gives a far better fit to the measured values than set (2), thus resolving the ambiguity. Since the assignment stage only has to distinguish between a small number of possibilities, the data processing is fast (a few seconds). Noise on the signal recorded at the location (t_1^*, t_2^*) does not affect the overall sensitivity; it only becomes a problem if it compromises the discrimination process.

Note that this protocol differs from the scheme suggested earlier for two-dimensional spectroscopy [26] where the overlap problem was left open. Had there been three overlapping NH signals, there would have been six possible 'candidates' as shown schematically in Fig. 1b. For the case of fourfold overlap there would be 24 possibilities, as indicated in Fig. 1c. The general case where N proton signals all fall at the same frequency, there are $N!$ different patterns.

2. Choice of evolution times

In setting up the minimal sampling experiment it is useful to have a guide to the best choice of the fixed evolution times, since these are free parameters. For the HNCO

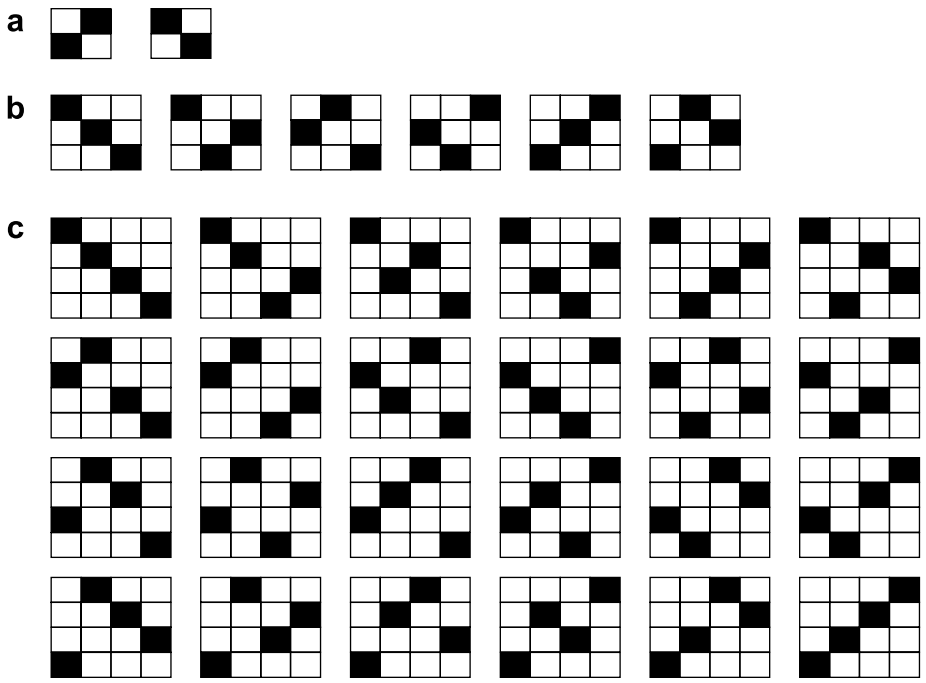

Fig. 1. Possible cross-peak patterns for (a) twofold, (b) threefold and (c) fourfold overlap of NH signals in an HNCO experiment. In each case the true pattern must be identified by matching the experimental NH intensities with those predicted from prior measurements of the ^{13}C and ^{15}N frequencies.

Table 1
Typical example of the matching of two sets of intensity calculations with the experimental measurements on the HNCO spectrum of agitoxin

Component	Measured	Calculated (1)	Calculated (2)
R^+	21.1	21.1	−27.9
I^+	0.0	−0.1	4.4
R^-	−22.2	−24.8	19.9
I^-	−17.8	−13.6	7.2

Clearly set (1) gives the better fit.

experiment outlined above, the expected NH intensities can be calculated for any combination of ^{13}C and ^{15}N reference frequencies. The danger arises if two such combinations predict similar NH intensities, that is to say, insufficiently distinct to make an unambiguous assignment. Since these predictions depend on the choice of t_1^* and t_2^*, we can calculate the evolution times most likely to give the best discrimination, and hence a definitive fit to the experimental data. The evolving frequencies ν_{N1}, ν_{N2}, ν_{C1} and ν_{C2} for the experimental case of agitoxin (described below) were used to obtain the graph shown in Fig. 2 for the ratio $t_2^*/t_1^* = 0.84$. This is a plot of the smallest of the maximum differences in peak intensities between the various combinations as a function of time measured along the tilted 40° axis. Peaks on this graph indicate the sampling times most likely to give a single unambiguous fit to the experimental NH intensities.

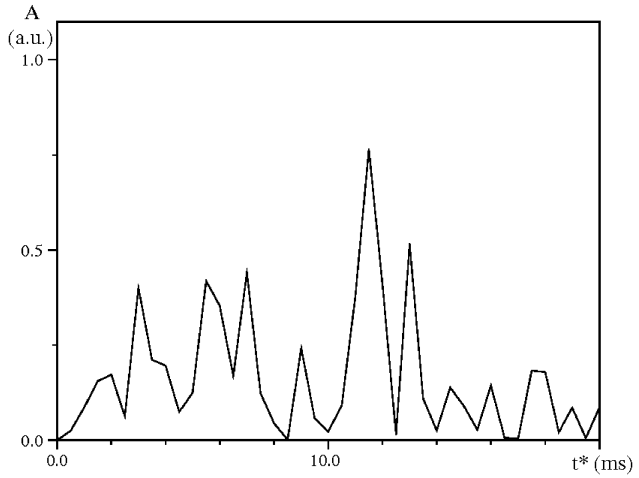

Fig. 2. Prediction of the most suitable sampling times for the HNCO experiment on agitoxin, calculated from the known ^{13}C and ^{15}N reference frequencies for the case $t_2^*/t_1^* = 0.84$. Maxima on this graph indicate the evolution times at which the various combinations give the most distinct results.

3. Experimental

The minimal sampling idea was tested on the HNCO spectrum of a 0.3 mM aqueous solution of a 4 kDa protein agitoxin, enriched in ^{15}N and ^{13}C, investigated on a 500 MHz spectrometer. In the HNCO spectrum there were six cases of twofold overlap of NH resonances, and two cases of threefold overlap. These ambiguities were resolved by matching calculated and experimental intensities. In general the resulting list of correlated ^{15}N, ^{13}C and ^{1}H frequencies may well be sufficient to solve the structural problem under investigation. In practice we reconstructed an *ersatz* three-dimensional HNCO spectrum based on the experimental frequencies and linewidths from the two-dimensional ^{15}N–^{1}H and ^{13}C–^{1}H planes. The projec-

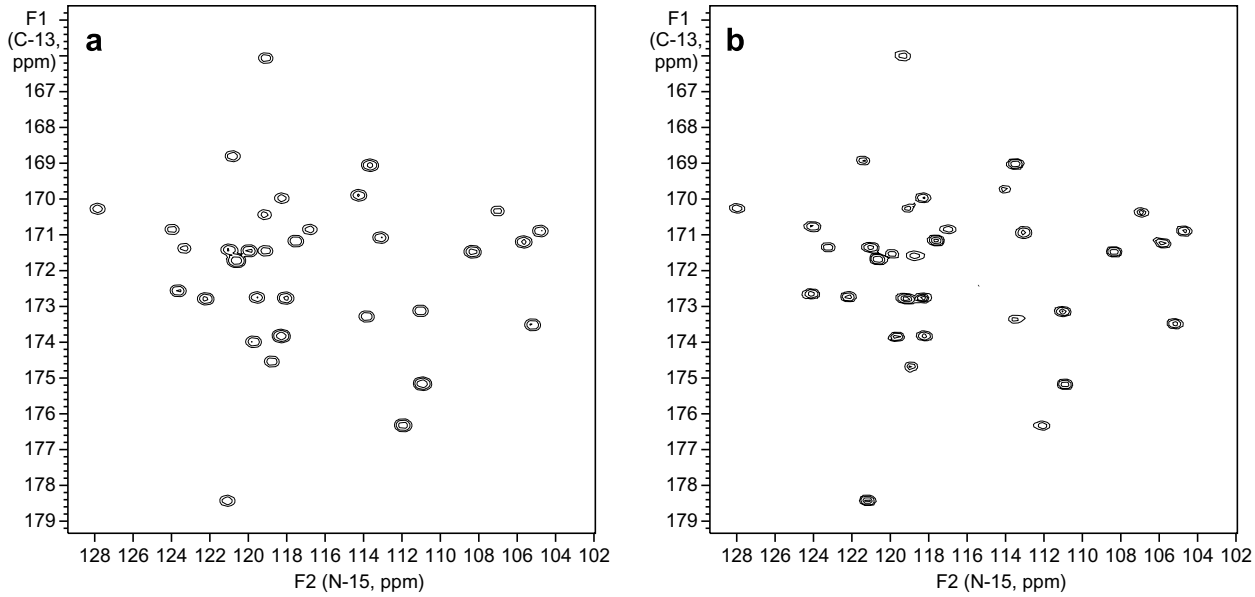

Fig. 3. The three-dimensional 500 MHz HNCO spectrum of 0.3 mM ($^{15}N/^{13}C$-enriched) agitoxin projected onto the ^{15}N–^{13}C plane (a) by the conventional method and (b) with minimal sampling. The conventional spectrum was run at a slightly different temperature, hence the slight differences in chemical shifts. The fixed evolution times were $t_1^* = 2.298$ ms and $t_2^* = 1.928$ ms. With four combinations of radiofrequency phase, four signal accumulation scans, and a relaxation delay of 1 s, the experimental duration was 18 s. The slow step is the acquisition of the two-dimensional 'first planes' which required an additional 18 min 40 s; no effort had been made to speed up the latter measurements, and there is scope for significant reduction in the overall duration.

tion of this spectrum onto the ^{15}N–^{13}C plane is shown in Fig. 3, where it is compared with the same spectrum obtained by the conventional method. The minimal sampling data were gathered at fixed evolution times $t_1^* = 2.298$ ms and $t_2^* = 1.928$ ms, requiring only an 18-s experiment. The measurement of the two-dimensional 'first planes' (^{15}N–^{1}H and ^{13}C–^{1}H), recorded by the conventional method, required 8 min 40 s, and 10 min, respectively, although there is considerable scope for shortening these experiments. At present they are the limiting factor; when these times are included, there is still a 35-fold speed advantage, as the corresponding conventional spectrum (with 32×64 evolution increments) required an 11-h experiment.

As with all new methodology, it remains to be seen just how far this technique can be extended to very crowded spectra, such as those of large proteins. In more complex spectra, repetition of the measurements at different settings of t_1^* or t_2^* may be necessary to obtain a more effective discrimination between the superposed evolution patterns of Eqs. (1)–(4). Note that there is considerable scope for repeating the measurement at several different settings of t_1^* or t_2^* without significantly affecting the overall speed advantage, since the slow stage is the acquisition of the two-dimensional 'first planes'.

4. Multi-dimensional spectra

Extension to higher dimensions should be straightforward, with correspondingly greater advantages in speed compared with the traditional methodology. The NHCOCA experiment would be an obvious candidate. For four-dimensional spectroscopy there would be three fixed evolution intervals t_1^*, t_2^* and t_3^*, and eight orthogonal intensities would be recorded, with the radiofrequency transfer pulse phase set to (0°, 0°, 0°); (0°, 0°, 90°); (0°, 90°, 0°); (0°, 90°, 90°); (90°, 0°, 0°); (90°, 0°, 90°); (90°, 90°, 0°) and (90°, 90°, 90°). These sets of intensities must be matched with calculated sets from pre-recorded orthogonal planes. Each cross-peak would have one of four possible patterns (Fig. 4). Extension to five-dimensional spectroscopy would accordingly involve measurement of 16 orthogonal components and 8 possible patterns of cross-peaks. Such patterns can easily be generated and explored numerically, making the method amenable to automation. This promises access

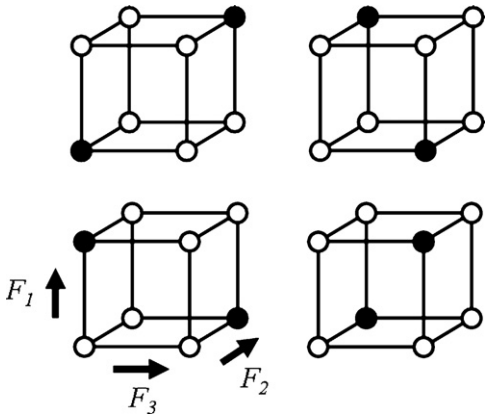

Fig. 4. Possible cross-peak patterns for four-dimensional NMR when there are two overlapping resonances of the observed spins. Assignment is based on matching the observed intensities with one of the four possible sets predicted from pre-recorded orthogonal planes.

to higher-dimensional spectra of biological molecules in experiments with acceptable durations.

5. Conclusions

Minimal sampling allows a three-dimensional spectrum ($F_1F_2F_3$) to be reconstructed from the orthogonal 'first planes' (F_2F_3) recorded with $t_1 = 0$, and (F_1F_3) recorded with $t_2 = 0$. One additional measurement at a location (t_1^*, t_2^*) where t_1^* and t_2^* are fixed values, serves only to discriminate any ambiguous assignment of cross-peaks, caused by degenerate chemical shifts in the acquisition dimension F_3. By drastically reducing the time required for data collection, this promises to provide the result much faster than conventional methodology or the existing sparse sampling schemes, provided that sensitivity is adequate. We call the method 3D-SPEED (single-point evaluation of the evolution domain). There is every reason to believe that higher-dimensional versions will offer even greater advantages in speed.

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