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Conventional and Exponential Sampling for 2D NMR Experiments with Application to a 2D NMR Spectrum of a Protein

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In two-dimensional NMR experiments on biological macromolecules, one is frequently compelled to acquire fewer points in t_1 than ideally required. This truncation of the signal in t_1 optimizes sensitivity at the expense of resolution (*1*) and may lead to a great loss of information through merging of cross peaks, cancellation between antiphase peaks, and "truncation artifacts." We (*2*) and others (*3, 4*) have shown how the maximum entropy method (MEM), when used to process conventionally acquired 2D NMR data, avoids many of the artifacts and alleviates the loss of resolution. Recently, however, we have attempted to optimize resolution and sensitivity in 2D NMR experiments by considering alternative methods of data acquisition. We have proposed (*5, 6*) a novel form of selective data sampling which we call exponential sampling. In this method many t_1 points would be acquired near the beginning of the experiment where the signal-to-noise ratio is high but some points, acquired with an exponentially decreasing frequency, would be acquired later in the experiment to aid the determination of high-resolution information. Such sampling would be used in conjunction with a data-processing method such as MEM; the conventional Fourier transform of data sampled in this way is unsatisfactory as it contains artifacts due to the many unmeasured points.

In this paper we extend our previous work where we demonstrated the use of exponential sampling in conjunction with MEM (*5, 6*) and show that exponential sampling should allow us to increase the available resolution in 2D NMR experiments. Further, as a practical demonstration we show that it can produce a large improvement in resolution in a 2D NMR spectrum of a protein. As in previous work (*5, 6*) the data were acquired conventionally and the exponential sampling was generated subsequently.

In deciding how to carry out a 2D NMR experiment the choice before one is often as follows: one may acquire (1) N t_1 points with $2m$ scans per point or, for example, (2) $2N$ points with m scans per point. Where signal-to-noise is poor one is normally forced to adopt course (1), that is, to truncate the data in order to optimize sensitivity. Once the possibility is available of (3) exponentially sampling, for example, N out of $2N$ points with $2m$ scans per point, the choice is wider. We have already shown, using one-dimensional models (*5, 6*), that exponential sampling of N points out of $2N$ is successful even when the signal-to-noise is very poor. Here (*7*) we show that course (3) is the best option compared to options (1) and (2). When acquiring a 2D NMR

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spectrum, accumulating $2m$ versus m scans per t_1 point results in a $\sqrt{2}$ improvement in signal-to-noise. We have simulated this effect by taking a simulated noise-free data set and adding two different amounts of Gaussian noise, one $\sqrt{2}$ less than the other. The data were simulated using the SIMPLTN program (*8*) and noise was added so that the standard deviation was either 71 or 50% of the first point of the FID. Figures 1 and 2 show the results obtained using both conventional (FT) or MEM processing. Figure 1a shows the FT of all 1024 points of the FID (acquisition time $\sim 5 \times T_2$) to which only 1% noise has been added. Figure 1b shows the FT of the first 128 points of the FID after adding 50% noise. No more resolution can be obtained from the data

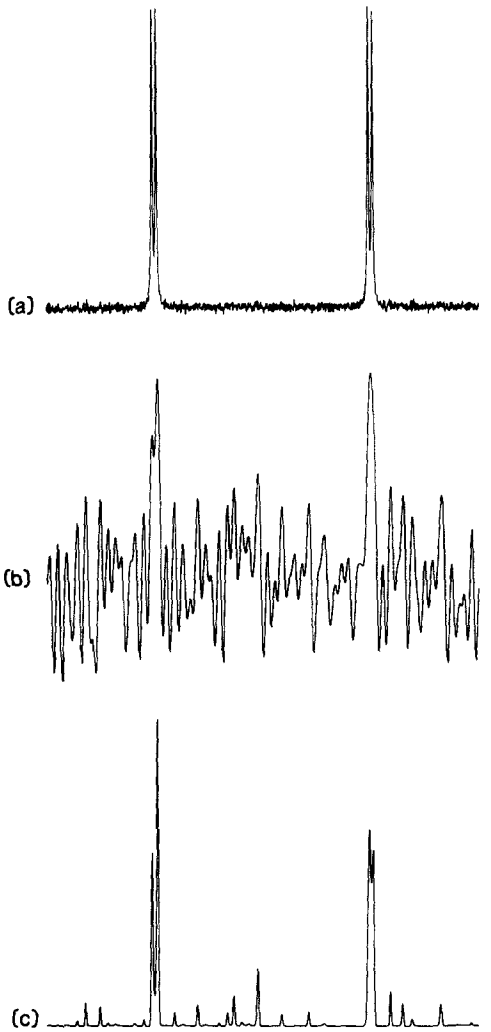

FIG. 1. Simulated spectra of an AX spin system produced by (a) a conventional Fourier transform (FT) of a 1024 point FID after adding noise having a standard deviation equal to 1% of the first point; (b) a conventional FT of the first 128 points of the FID after adding 50 times more noise; and (c) a MEM reconstruction using the same data as in (b).

even if resolution enhancement is used (not shown). This, therefore, is the conventional result when sensitivity has been optimized by truncating the data. Figure 1c shows the MEM result obtained from the same data; it offers some improvement in resolution but the intensities are distorted. Figure 2a shows the FT of 256 points after adding 71% noise; a matched filter has been used to optimize the sensitivity. This is the conventional result when an attempt has been made to achieve better resolution at the expense of a worse signal-to-noise ratio. The MEM result using the same data is shown in Fig. 2b; it is not a success. By contrast, Fig. 3 shows the MEM result obtained from an FID where 128 points have been sampled exponentially out of 256 after adding 50% noise. This is clearly the best result. It has much improved resolution, although the intensities are still distorted, and it represents option (3). We have used simulated data to study this question to avoid instrumental factors that might confuse the issue, such as t_1 noise (*9*). We next show that this option works in a real situation.

We have chosen to demonstrate the method with a NOESY spectrum of the small protein BPTI (basic pancreatic trypsin inhibitor) in H_2O. In previous work with 2D data sets we have used MEM data processing in both dimensions and this remains our intention for spectra of biological macromolecules in the future. At present, however, we are limited by the computing facilities available to us so we have carried out this work as follows. First, Fourier transformation was carried out in the t_2 dimension, thus there is no attempt to avoid t_2 ridges, baseline distortions, etc., using MEM.

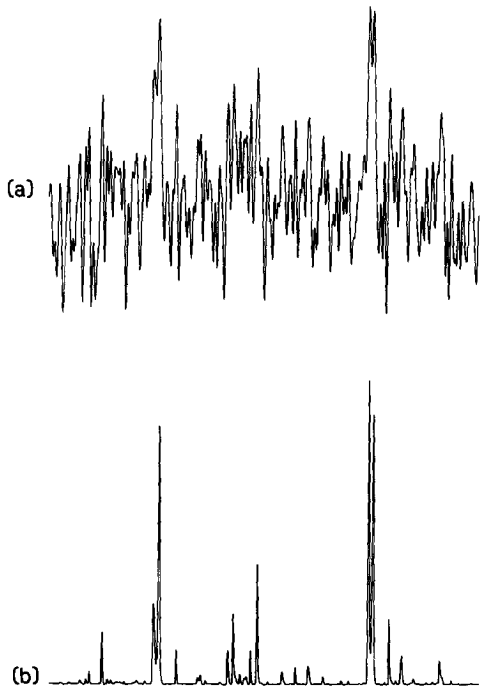

FIG. 2. Simulated spectra of an AX spin system produced by (a) a conventional FT of the first 256 points of the same FID as used in Fig. 1 but after adding noise having a standard deviation equal to 71% of the first point and using matched filtration; and (b) a MEM reconstruction using the same data.

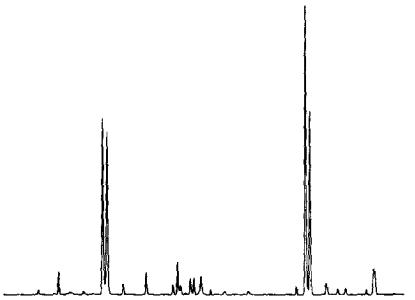

FIG. 3. A simulated spectrum of an AX spin system produced by a MEM reconstruction using 128 points exponentially sampled out of 256 points of the same FID as used in Figs. 1b and 1c. All spectra in Figs. 1–3 have the same digital resolution.

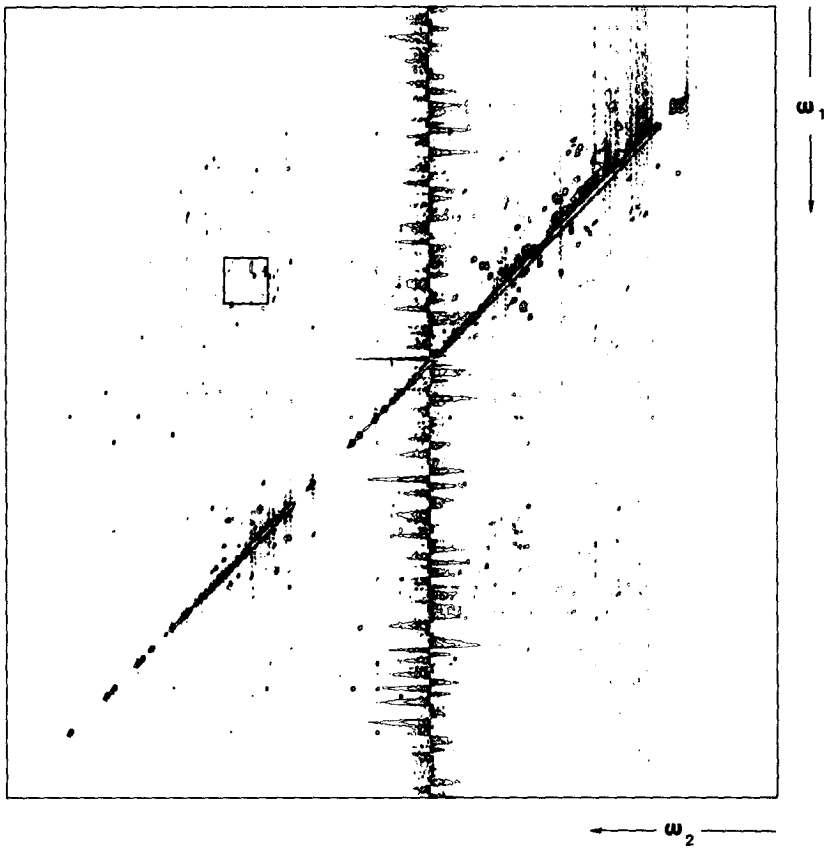

FIG. 4. A NOESY spectrum acquired at 400 MHz on a Bruker AM400 instrument of the protein basic pancreatic trypsin inhibitor (BPTI) in H_2O:D_2O, 9:1, v/v. The H_2O signal was suppressed by irradiation during the relaxation delay. There are 2048 points in ω_2 and 1024 points (zero-filled from 512 points) in ω_1. The square shows the region which is expanded in Fig. 5.

Second, after transposition of the matrix, a series of columns was chosen for MEM data processing. The standard deviation of the noise used in the MEM reconstructions (*10, 11*) was found by recording a t_1 point with no pulses and no water irradiation to give a file of noise. The standard deviation of this noise was multiplied by $\sqrt{n/2}$, where n is the size of the first Fourier transform in t_2. This value was then used when processing the columns.

We illustrate the results using a small section of the spectrum (Fig. 4) and have not assigned individual NOE cross peaks. In this work we are not attempting to resolve information within cross peaks in ω_1, only to resolve and locate cross peaks correctly.

Figure 5a shows the result obtained after a conventional FT of the first 128 points in t_1, zero-filled to 1024 points, which represents the case where the data have been truncated in t_1. Multiplication by a sinebell, shifted by 45°, was used in t_1 and no filter function was used in t_2. Figure 5b shows the MEM result using the same 128

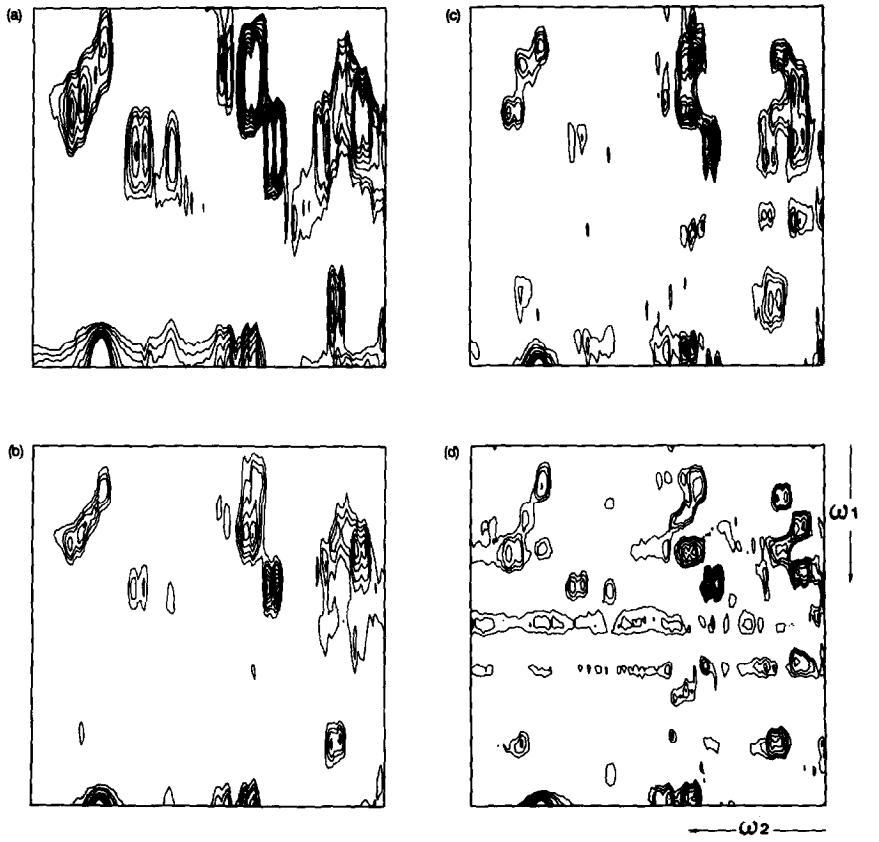

FIG. 5. A region of the spectrum of BPTI produced by (a) a conventional FT of the first 128 points in t_1 multiplied by a sinebell shifted by 45°, zero-filled to 1024 points; (b) a MEM reconstruction using the same 128 points; (c) a MEM reconstruction using 128 points exponentially sampled out of the first 256 points in t_1; and (d) a conventional FT of 512 points in t_1, using the same filter function as for (a). All spectra have the same digital resolution and are contoured at the same levels (0.1 to 1.3% of the maximum diagonal peak, not shown here).

points; it is a slight improvement. Figure 5c, however, shows the MEM result obtained by sampling 128 points exponentially from the first 256 points in t_1. It is dramatically better, as it is very similar to the result obtained by FT of 512 points in t_1, shown in Fig. 5d. The last spectrum was obtained using the same filter function as for Fig. 5a. All the spectra have the same digital resolution. It is noteworthy that a better result is not obtained if one samples 128 points exponentially out of 512 points (not shown), although the FT of 256 points (also not shown) shows slightly less resolution (broader lines) than does that of 512 points. This result is in accord with previous work (*6*). Thus the result in Fig. 5c is remarkably close to the assumed "right answer," that is, the conventional FT of 512 points, Fig. 5d. This represents, for the sample used, an effective recording time of only 4.5 h as opposed to 18 h. Alternatively, in a situation where much less sample was available (a 20 m*M* sample was used here), exponential sampling could render feasible the recording of useful spectra not otherwise obtainable.

In future work we plan to explore the use of exponential and other selective sampling schemes to reveal intra-cross-peak structure. Exponential sampling is suited to NOESY and other experiments where the useful signal (i.e., that giving rise to cross peaks) is at least predominantly cosine modulated in t_1. It should be useful in conjunction with homonuclear experiments obtained using isotropic mixing (TOCSY) (*12*) and more recent variants (HOHAHA) (*13*) on larger molecules, for which the in-phase nature of the cross peaks is an advantage, to obtain through bond-coupling information. In the case of sine-modulated signals (COSY and modifications thereof) alternative sampling schemes are required and are under investigation.

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