J|A|C|S COMMUNICATIONS

Published on Web 06/24/2009

Accelerated Multidimensional NMR Data Acquisition by Varying the Pulse Sequence Repetition Time

Slobodan Macura*

Department of Biochemistry and Molecular Biology, College of Medicine, Mayo Clinic, Rochester, Minnesota 55905

Received February 12, 2009; E-mail: macura@mayo.edu

It has been recognized that the versatility of multidimensional (mD) Fourier transform (FT) NMR experiments[1] is enhanced when the data accumulation time is reduced. To date, such reduction has been achieved by numerous techniques, from evolution period shortening[2−4] and/or evolution parallelization[5,6] to the complete elimination of repetition time (T_R).[7]

To reduce the duration of mD experiments, I propose variation of the pulse sequence repetition time (VRT) in concert with sampling of evolution domain(s). In this approach, an experiment starts with the usual repetition time ($T_{R0} \approx 1 - 5T_1$) and ends with $T_R \ll T_1$, resulting in a considerable reduction of the length of data acquisition. If $T_{R0} \geq 5T_1$, equilibrium peak volumes are also preserved.

The total repetition time is the sum of the individual periods T_{Ri}, i.e., $N_S\sum_{i=1}^{N_1}T_{Ri}$, where N_S is the number of scans and N_1 the number of increments. With constant T_R, the total repetition time is $N_1N_ST_R$. In the VRT experiment, T_R becomes a function of evolution time t_1, i.e., $T_{Ri} = f(t_{1i})$. If $f(t_{1i})$ is chosen such that its time average $\bar{f}(t_1)$ is smaller than T_{R0}, the duration of the experiment is reduced by $T_{R0}/\bar{f}(t_1)$. Other delays unaffected by VRT, denoted by $\sum$d, render the total effect somewhat smaller, with a time reduction of $(T_{R0} + \sum d)/[\bar{f}(t_1) + \sum d]$.

The magnitude of the steady-state longitudinal magnetization depends on the repetition time T_R as $1 - \exp(-T_R/T_1)$. In the evolution domain, the magnetization decays exponentially as $\exp(-t_1/T_2)$ with relaxation time T_2. If T_R is modulated by an arbitrary function $f(t_1)$ in parallel with the evolution time, the normalized magnetization $M_r = M/M_0$ is given by

$$M_r(t_1) = \{1 - \exp[-f(t_1)/T_1]\}\exp(-t_1/T_2) \qquad (1)$$

The VRT term multiplies the t_1 free induction decay (FID) in analogy to the filtering function in data processing. Thus, eq 1 can be viewed as filtering along the evolution domain during data acquisition. In addition to the time savings, which depends on the time average $\bar{f}(t_1)$, one can select the VRT function to enhance resolution or achieve any other effect related to the line shape in the evolution domain. In 3D or higher-dimension experiments, line widths in the evolution domain are most often determined by the domain length rather than the natural line width, i.e., $t_1^{max}/T_2 \ll 1$. In this case, the line broadening caused by VRT is minimal (or negligible) compared with the broadening by subsequent filtering in the course of processing. In addition, the repetition time is often shorter than the relaxation time ($T_R < T_1$). When this is true, the T_2 term can be omitted, so to a first approximation, eq 1 can be simplified to

$$M_r(t_1) \approx 1 - \exp[-f(t_1)/T_1] \approx f(t_1)/T_1 \qquad (2)$$

Equation 2 shows that (with rapid pulsing) the magnetization amplitude during the evolution time follows the actual VRT function. This simplifies the selection of $f(t_1)$ to functions that would be used anyway to apodize the t_1 FID in the standard experiment. When $f(t_1)/T_1 > 1$, the full exponential form must be used in eq 2; eventually, with the VRT function decay, the linear approximation becomes valid again. Thus, when $f(t_1)/T_1 \geq 5$, the initial magnetization is constant [i.e., equal to the equilibrium magnetization ($M_r = 1$)], so it enters the linear approximation regime when $f(t_1) \ll T_1$.

I used a 10 mM sucrose solution in D_2O (prepared by two cycles of freeze-drying and dissolving in "100 percent" D_2O) to demonstrate the basic properties of the VRT method. At 600 MHz and 25 °C (using a Bruker Avance spectrometer equipped with a cryoprobe), the residual HOD protons were characterized by $T_1 = 20.4$ s and $T_2 = 0.50$ s; with repetition time of 12 s and an evolution time of 0.13 s, these relaxation times satisfy the conditions under which eq 2 was derived [$f^{max}(t_1)/T_1 \leq 0.6$, $t_1^{max}/T_2 \leq 0.26$]. I implemented the VRT method by substituting variable-delay lists for the fixed delays (T_R and t_m) in the pulse sequences. When both T_R and t_m were varied, I used the variable counter list as well. To eliminate interferences of residual transverse magnetization from previous scans, a small gradient pulse at the beginning of the relaxation delay (or the end of acquisition) was inserted.

Figure 1 shows the results of an experimental test of eq 2 in which traces of t_1 FIDs (solid lines) of the residual HOD signal are compared with the respective $f(t_1)$ values (dashed lines) obtained from a series of VRT−TOCSY experiments on the sucrose sample. As expected, the FIDs of the residual solvent signal followed the respective VRT functions rather closely.

To test the VRT method further, I implemented it in a NOESY−HSQC 3D experiment in which I modulated T_R and t_m. Again I used 10 mM sucrose, where the natural abundance makes the solution 0.1 mM in ^{13}C. In addition to the low ^{13}C concentration, the nuclear Overhause effects (NOEs) in sucrose are comparatively weak (and positive), rendering detection of cross-peaks somewhat challenging. Figure 2 shows the Glc-H_1 ^1H vector from a series of VRT−NOESY−HSQC 3D spectra recorded with different VRT functions. The VRT modulation was applied in both the t_1 and t_2 domains, implying that both T_R and t_m were continuously shortened by departure of either t_1 or t_2 from zero (t_3 is the detection time). Noticeable in Figure 2 is the line narrowing and improved signal-to-noise ratio (SNR) of the diagonal (Glc-H_1 → Glc-C_1) in the VRT experiments relative to an otherwise identical experiment recorded with constant T_R. The SNR for the cross-peak (Glc-$H_{2,4}$ → Glc-H_1 → Glc-C_1) remained almost constant, even though the experiments took half the time. Here the narrowing of the diagonal line was caused by shortening of the mixing time rather than by T_R modulation, in complete analogy to the ACCORDION experiment.[8] The cross-peak was affected only minimally because its buildup curve is rather flat around 1 s.

In theory, the VRT method accelerates data acquisition at the expense of SNR: a shorter T_R at the outskirts of the indirect domain weakens the observable signal with no effect on the noise. However,

 10.1021/ja9011063 CCC: $40.75

because of the finite length of delays unaffected by VRT (acquisition, mixing, dephasing/rephasing, etc.), the signal is appreciable even with $f(t_1) = 0$. This means that data apodization must be applied in any case, thus attenuating any unfavorable VRT effects.

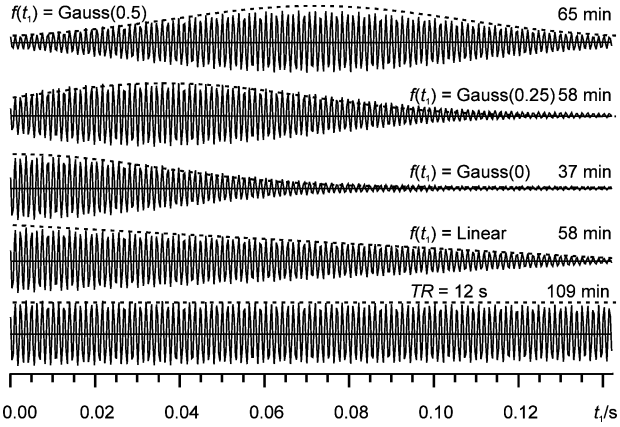

Figure 1. Free induction decay of the residual HOD signal (10 mM sucrose in D_2O) in a series of VRT−TOCSY experiments with the indicated VRT functions. Numbers of minutes indicate total durations of the experiments. The number in the Gaussian VRT function indicates the fractional position of the maximum within the time domain. In each case, $f^{max} = 12$ s.

In the VRT experiment, the spin system never reaches the steady state because a distinct T_R characterizes each evolution increment. Thus, acquisition of more scans at any given increment would require cycling through T_R values rather than through the pulse phase and/or receiver phase. However, most often this is unnecessary because the T_R changes between successive evolution increments are rather small. For example, with linear increments in VRT and N_1, the T_R change $[f(0)/N_1]$ is usually less than the SNR in a given FID. Consequently, in most experiments, implementation of VRT requires only replacing the fixed T_R by a variable delay. If the acquisition domain is short, then the residual transverse magnetization should be removed by a small gradient after the acquisition. If a gradient is not available, then random modulation of the whole set of phases between successive scans (keeping the relative phases constant within the scan or as required by phase rotation) will send potential artifacts into the background noise. The only apparent limitation of the VRT method is that it cannot be implemented in experiments that require a long T_R (e.g., for preirradiation).

The VRT method works well for molecules of all sizes (small molecules to macromolecules), often cutting the total experiment time in half without any artifacts. In fact, in small- and medium-sized molecules where there is no spin diffusion, the VRT method can shorten the mD acquisition time without affecting the spectrum at all. In such cases, ^{13}C-bound protons can be characterized by shorter T_1's than ^{12}C-bound protons (used for T_R estimate), so the T_R's tend to be overestimated.

The VRT method can be applied in numerous forms. For example, by a suitable choice of the VRT function, one could either sculpt the evolution line shapes or use the function as a T_1 filter to selectively broaden the lines with long T_1's or transmit T_1 information into evolution line widths. VRT could be applied in concert with sampling of one or all of the evolution domains, with the mixing period, or with any of their combinations. The ability of the VRT method to preserve the equilibrium magnetization by

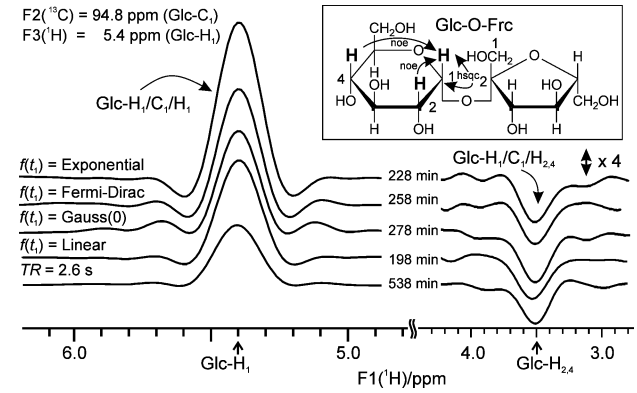

Figure 2. Proton vector from VRT−NOESY−HSQC 3D spectra of 10 mM sucrose in D_2O taken at the F2(^{13}C) frequency of Glc-C_1 and F3(^{1}H) of Glc-H_1. Because of the low digital resolution in the 3D spectra of Glc-$H_{2,3}$, the resonances are not resolved. Magnetization flows from $H_{2,3}$ to H_1 via an NOE (in sucrose, the NOE is positive, thus giving a negative cross-peak) and then, for frequency labeling, via single-quantum coherence between H_1 and C_1, as indicated by the arrows in the structure. The total experiment time is expressed in minutes. Experiments were performed with the VRT functions indicated at the left and used to vary both T_R and t_m simultaneously. In each case, $f^{max} = 2.6$ s.

starting the experiment with $f(0) \geq 5T_1$ is particularly useful in quantitative mD spectroscopy (e.g., EXSY, NOESY), as will be shown elsewhere.

Because of its close analogy to filtering, the VRT method should be used with caution, as with all filtering functions. In addition, an extremely fast repetition rate can introduce spectral distortions specific to the spectrometer (the VRT function should acknowledge a duty cycle limitation for a given system) and the sample (sample overheating due to duty-cycle shortening). However, the principal advantage of the VRT is in reduced data acquisition time in mD experiments with minimal alteration of existing pulse sequences.

It is noteworthy that VRT can be combined with other acceleration methods for even better results. For example, one could easily create the VRT−SOFAST hybrid, in which the excitation pulse width is modified in concert with the changing repetition time. This combination of methods will enable a smooth transition from the normal 2D regime (low speed, no distortion) through the VRT−SOFAST regime to the SOFAST regime.

Acknowledgment. I thank Dr. Stanimir Vuk-Pavlović for useful suggestions and critical reading of the manuscript.

Supporting Information Available: Acquisition and processing details for Figures 1 and 2, plots of the VRT functions used, and ^{1}H−^{15}N VRT−HMQC spectra of ubiquitin. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA9011063