MUSASHI: NMR Pulse Width Determination Method by Nonlinear Least Square Curve Fitting

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We have developed a new method named MUSASHI to determine the 90° ^{1}H pulse width in ^{1}H-NMR in an automated fashion using a nonlinear least square curve fitting algorithm. This method is very robust and provides an efficient mechanism for automatic pulse width calibration of spectrometers especially in the field of structural genomics by NMR.

Triple resonance NMR experiments are routinely used for solution structure analysis of proteins or in the field of structural genomics.[1] In the area of structural genomics, large numbers of ^{15}N, ^{13}C, and/or ^{2}H-labeled proteins are mass-produced. An extensive suite of double- and triple-resonance experiments is successively carried out for structure analysis. Inaccurate NMR pulse width settings drastically reduce the sensitivity of the resultant data. In comparison to a single pulse experiment, in multiple pulse experiments like triple resonance experiments, the accumulation of such inaccuracy throughout the pulse sequence result in substantial signal loss, even if the degree of the inaccuracy of each pulse width is small. Therefore, it is of utmost importance for optimum results to determine accurate 90° pulse widths and to use them in actual measurements. Traditional methods to obtain an accurate ^{1}H 90° pulse width rely on a series of nutation experiments that consists of a set of arrayed experiments with varying pulse widths. To reduce unfavorable effects caused by relaxation and radiation damping, the ^{1}H pulse width for the 360° flip-angle is usually searched and the quarter of the value for 360° is used for a 90° pulse width. Additionally, to obtain a more accurate value, it is required to perform an additional measurement around the 360° flip-angle with a smaller step. Needless to say, the signal completely disappears at the exact 360° pulse width as well as the signal intensity is diminished around the 360° pulse width. This implies that the spectrum with poor sensitivity is often used to seek the 360° pulse width. This may require longer experimental time to determine the pulse width, as the number of scans may have to be increased to acquire data with better SNR (signal-to-noise ratio) to overcome poor sensitivity. We developed an algorithm to determine the pulse width by means of a non-linear curve fitting method.[2,3] We named this MUSASHI (MUltiple Spectra Analyzing System with Hyper Intelligence). In this communication, we report this new program and demonstrate its application for ^{1}H pulse width determination.[4]

Prior to MUSASHI analysis, a nutation experiment is conducted. The Fourier transformed data in the direct observing axis is sent to the program. The parameters in the model function are optimized to minimize the difference between the model function and the acquired data. In such a model function, the signal intensities are a function of the independent parameters (duration of the pulse) as well as the value of the parameters to be determined (90° pulse width). In order to determine the 90° pulse width, we used a model formula being a multiple of a sinusoid and an exponential decay (Eq 1). Here, the signal intensity $f(t)$ is the function of time t and the other parameters of A, B, C, D, and ω:

$$f(t) = A\sin(\omega t + B)\exp(-t/C) + D \quad (1)$$

The exponential factor C in Eq 1 is the intensity loss due to $\boldsymbol{B}_1$ (external magnetic field) inhomogeneity. The curve of the integral of the acquired signals in the sampled region is fit using the model function (Eq 1) by nonlinear curve fitting.[5,6] The resulting values of A, B, C, D, and ω are obtained. Then, $2\pi/\omega$ is the 360° pulse width and $\pi/2\omega$ is the 90° pulse width.

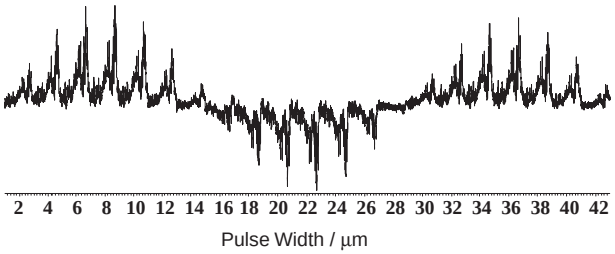

Figure 1. ^{1}H nutation data of chlorella ubiquitin. ^{1}H pulse width was varied from 2 to 42 μs in 2 μs step (totally 21 points).

With our new method,[7] ^{1}H pulse width of 7.10 μs for 90° was successfully obtained from the array data set[8] shown in Figure 1.

Two sets of arrayed experiment are performed in the conventional method, where the first data set is used to obtain a rough 360° pulse, followed by finer array to obtain an exact 360° pulse width. The conventional procedure requires three steps of decision making by a human or a computer; 1) to find the rough critical point, 2) to determine the range to search in the next experiments and 3) to find the point that will be the final results. In contrast, MUSASHI uses a table-list containing intensity modulation caused by varied pulse duration and the final result is provided by nonlinear curve fitting calculations. There is no room that a human or computer judges any critical value. This means anyone reaches the same results, so MUSASHI is very accurate in this point. In addition, MUSASHI is suitable for automation, as an accurate 90° pulse width is obtained from the only one arrayed data set. Therefore, automatic measurements no longer need the conditional branching to collect the second nutation data set.

MUSASHI takes an advantage of curve-fitting method. This fitting method results in the pulse width determination with very high resolution.[4] The pulse width can successfully be obtained as long as the data sampling fulfills the Nyquist's theory. Therefore,

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MUSASHI can provide the pulse width with the fewer total number of the data points, providing a mechanism to obtain accurate pulse widths in a rapid fashion compared to conventional methods. This provides a robust procedure in conjunction with automation to obtain complex multinuclear experiments[4] with highly accurate pulse widths.

Our method uses not only the data points around 360° pulse width but also the data points with other flip angle. This causes higher sensitivity than conventional methods. Additionally, MUSASHI has the option to use integral of the signals. Typical medium sized proteins (MW ≈ 10000) approximately have typically about one hundred amide protons and several hundreds aliphatic protons in the molecule. Using the integral option, MUSASHI provides a valuable mode to obtain the pulse width based on the region of interest, i.e., amide or aliphatic. Since the large number of protons are sampled, more accurate results are obtained compared to conventional techniques that rely on peak heights. In addition, the change of signal intensities through the total components of the arrayed experiment are considered, therefore not only the data with poor signal-to-noise ratio (SNR) around 360° pulse but those with high SNR are used to determine the width. This also contributes to determine the pulse width in a shorter time.

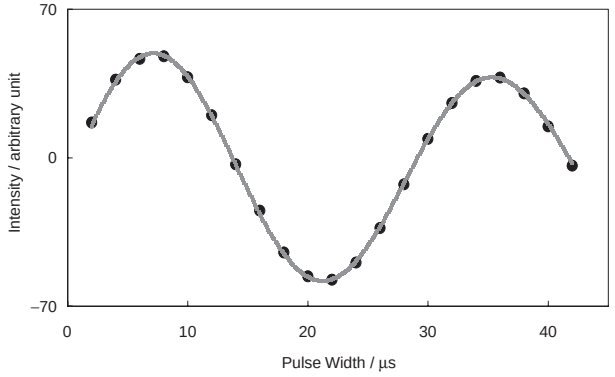

Figure 2. Calculated fit using MUSASHI (curve) and the intensity of the spectrum (closed circle) from the nutation experiment shown in Figure 1. The parameters determined by MUSASHI were $A = 59.96$ (arbitrary unit), $\omega = 0.2229\,\mathrm{rad/\mu s}$, $B = -0.05422$ rad, $C = 126.1\,\mu\mathrm{s}$, and $D = -7.384$ (arbitrary unit).

As we described above, we developed a new method MUSASHI to determine 90° pulse widths. MUSASHI drastically reduces the total time required to determine the pulse widths. To date, we have exercised this procedure on more than ten different protein samples, MUSASHI has proven to be very robust and reliable, as it provides quite reasonable pulse widths (data not shown) and they were used for the further double- and triple-resonance experiments. This method is helpful for protein NMR experiments requiring complicated experimental settings especially in structural genomics. MUSASHI opens the door for the full-automatic NMR spectrometer calibration with minimal human intervention for structural genomics.[9,10]

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References and Notes

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4 MUSASHI can be applied for the ^{13}C and ^{15}N pulse width determination for labeled proteins in solution. This will be described elsewhere (Asakura et al., submitted).
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6 Available from http://www.ici.ro/camo/unconstr/lbfgs.htm
7 MUSASHI was written in Percival macro language for Delta-NMR version 4.3 (JEOL Ltd., Akishima, Japan and JEOL USA Inc., Peabody, MA, USA). The original LBFGS nonlinear curve fitting routine[6] was written in FORTRAN and was ported to Percival. The calculations were done on MacOS 10.3.7 on a PowerBook G4 (Apple Computer Inc., Cupertino, CA, USA).
8 0.9 mM $^{13}C/^{15}N$ uniformly (≈98%) labeled chlorella ubiquitin solution was purchased (Chlorella Industry Co. Ltd., Tokyo, Japan). Data was obtained on a JEOL JNM-ECA600 spectrometer (JEOL LTD., Akishima, Japan) operating at 14.1 T. The temperature of the solution was set to 25 °C. A set of arrayed single pulse experiment varying pulse width from 2 to 42 μs in 2 μs step with solvent suppression was carried out.
9 Delta-NMR[7] for Red-hat Linux, Microsoft Windows and Apple MacOSX is available from http://www.jeol.com/. Delta-NMR supports not only JEOL's data format but also the other formats. This means that data acquired on systems other than JEOL's can be processed by Delta-NMR and MUSASHI. Although the current version of Delta-NMR (version 4.3) does not contain MUSASHI, we have a plan to port MUSASHI to Delta-NMR in the coming major revision of Delta-NMR. This new version of Delta-NMR also will be available from the same address shown above. For those that need help to install it, please have a contact with the author to whom corresponding should be addressed.
10 Because the critical part of MUSASHI was ported from an open-source[6,7] and we here describe the idea of 90° pulse determination, we believe that a professional programmer or a graduate student in physical or analytical chemistry having knowledge of computer programming can implement a program equivalent to MUSASHI on his or her own system (on his or her favorite language or operating system) in a relatively short time. We are pleased to provide further information for those who wish to build their own upon the request to the author to whom corresponding should be addressed.

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