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Improved residual water suppression: WET180

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Abstract

Water suppression in biological NMR is frequently made inefficient by the presence of faraway water that is located near the edges of the RF coil and experiences significantly reduced RF field. WET180 (WET with 180° pulse-toggling) is proposed to cancel the faraway water contribution to the residual solvent signal. The pulse sequence incorporates a modification of the last WET selective pulse to accommodate insertion of a toggled 180° inversion pulse so that the original WET selective pulse angles are effectively preserved. Compared with existing WET methods, WET180 has the advantages of easy implementation, improved residual water suppression, clean spectral phase properties, and good signal intensity retention. WET180 is expected to be most useful in observing resonances close to water in samples containing biological molecules. In addition, the principle of WET180 can be applied in multidimensional experiments to improve residual water suppression and reduce artifacts around water.

Keywords

Biological NMR; Metabolomics; Residual water; Water suppression; WET180

Introduction

Water suppression is of keen interest in biological NMR. While a plethora of methods have been developed to suppress bulk water, faraway water (typically located in regions near the edges of the RF coil) frequently makes significant contributions to the residual water signals. Faraway water is difficult to suppress due to its reduced RF field and high probability of frequency offset that results from imperfect shimming of regions away from the RF coil center (Neuhaus et al. 1996; Mo and Raftery 2008). Residual water signals may not only limit receiver gain, but also obscure interesting nearby resonances. Thus faraway water suppression is especially important in the analysis of biofluids in metabolomics, when multiple components may appear near to the residual solvent signals and high-throughput NMR is essential. To reduce the residual water signals in the NMR spectra, low-pass filters

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(Marion et al. 1989) or other post-acquisition methods such as covariance NMR (Chen et al. 2007 and references therein) have been developed. Though they can be fast and efficient, the best suppression results should come from the data that contain minimal residual water signals.

Several pre-saturation based methods, most notably 1D NOESY, FLIPSY, PURGE and Pre-SAT180 have been shown to suppress residual water signal rather well (Neuhaus et al. 1996; Simpson et al. 2005; Mo and Raftery 2008). However, due to the saturation transfer effect, those methods may not be suitable for the detection of macromolecules, small molecules that bind to some macro-molecules in the solution, or molecules that experience fast proton exchange with water. Largely free of saturation transfer, WATERGATE (Piotto et al. 1992; Sklenar et al. 1993) type water suppression methods including excitation sculpting (Hwang and Shaka 1995), MEGA (Mescher et al. 1996) and SOGGY (Nguyen et al. 2007) are very efficient in eliminating residual water signals by selecting magnetizations that follow certain coherence pathways. Nevertheless, water selective pulses in those sequences are all under the influence of homonuclear coupling, and observed solute signal intensity might be further modulated.

WET type water suppression methods are preferred for observing small molecules that may bind with large molecules, as they do not suffer serious saturation transfer loss or homonuclear coupling during the water suppression period. Although the original WET sequence was developed to be B_1 insensitive (Ogg et al. 1994; referred as WET90 hereafter), it is frequently insufficient to suppress water from regions experiencing more than a 10% B_1 field attenuation, while in reality faraway region water can potentially experience up to a 10 fold attenuation in RF power compared to the bulk region (Szántay 1998).

Variants of WET have been proposed, most notably with modification of either the selective pulse train itself (Wu and Otting 2005) or the observe pulse. The latter is represented by a spatially selective composite pulse (Smallcombe et al. 1995; referred as WET-composite) or phase cycling of a 270° pulse for every three 90° observation pulses (Zhang et al. 2000; referred as WET270). While these approaches have shown improved residual water suppression, both WET-composite and WET270 lead to some signal losses due to the observe pulse's RF field inhomogeneity, and may require significant spectral phase corrections (Zhang et al. 2000; Mo and Raftery 2008).

Following the same reasoning as in Pre-SAT180 (Mo and Raftery 2008), one might expect that a 180° inversion pulse can be readily applied to the original WET sequence (referred as WET + 180°) so that residual water can be effectively canceled. However, such a simplistic method offers minimal benefit experimentally. Instead, an improved 1D sequence is achieved by altering the last pulse of the WET sequence to accommodate the introduction of the additional 180° inversion pulse (Fig. 1). We call this new sequence WET180, for its simplicity and the fact that a 180° inversion pulse is applied to cancel the residual water signal. We demonstrate that WET180 is a better sequence for suppressing the faraway water signal than the existing WET-composite or WET270 sequences.

Material and methods

After automatic gradient shimming, 1D NMR data for WET90, WET270, WET-composite, WET + 180 and WET180 (Fig. 1) were acquired using a standard 2 mM sucrose sample (with 0.5 mM DSS in 10% D₂O solution at room temperature), on a Bruker Avance DRX500 equipped with an inverse single-axis gradient probe. The acquisition time was 2.98 s (sweep width 11 ppm) with 2 s inter-scan delay. The proton hard 90° pulse width was 10.4 μs (24 kHz) and the WET selective pulse B₁ field strength was chosen to be 50 Hz at peak power unless otherwise stated. The 180° inversion pulse in WET180 was either a hard square pulse or an adiabatic inversion pulse. In the latter case, the pulse was 500 μs, shaped as a 20% smoothed CHIRP using a sweep width of 60 kHz at a peak power of 8.1 kHz.

All data were acquired with 16 scans following 8 dummy scans. For WET270, 16 FIDs using 90° and 270° observe pulses were acquired, processed separately, and scaled before summation (Mo and Raftery 2008). WET270 would also require different selective RF pulse strengths if individual best water suppression were to be desired for the 90° and 270° observe pulses.

All FIDs were zero-filled to 16 k complex points, exponentially line-broadened by 0.3 Hz, Fourier transformed and phased. First order baseline corrections were applied when needed. All WET sequence simulations were conducted using NMR-SIM (Bruker Bio-spin, Billerica, MA).

Results

Figure 2 summarizes a comparison of different 1D water suppression results using several WET-based methods, including WET with a 90° observe pulse (referred as WET90 hereafter), WET270 (Zhang et al. 2000), WET + 180, WET180 (Fig. 1) and WET with composite spatially selective observe pulses (referred as WET-composite). WET180 appears to give the best overall residual water suppression results evaluated by the following criteria: residual water size and line-width, the degree of peak distortion for proximate peaks of interest, overall spectral baseline and phase properties, peak intensity, and the ease of sequence set up and optimization of parameters.

In Fig. 2, both WET180 and WET-composite can reduce the faraway water signal with similar efficiency as they give several times smaller residual water signals and more than 50% reduction in residual water line-width compared to WET90. While overall baselines for all spectra seem to be reasonably flat, WET180 and WET-composite are especially better than others for suppressing the long tail (at the downfield side) of the water resonance.

The improvement of WET + 180 and WET270 over WET90 is smaller in reducing residual water size and line-width. In addition, WET + 180 appears to create some slight peak distortion on the residual water signal (negative intensity at the upfield side), and thus may severely obscure small peaks that happen to be in that region.

WET180, just like WET90, has good spectral phasing properties in that it has a small and predictable first order phase correction. On the other hand, WET-composite and WET270

results are less desirable. WET-composite typically requires a large first-order phase correction (30° in the current example) during spectral processing, and this phase correction is not linear across the spectrum, which may cause line-shape distortions for sharp peaks (Zhang et al. 2000). Moreover, the large first-order phase correction in WET-composite would need a large spectral baseline correction, which would be more problematic for multidimensional data. As for WET270, it would require separate data acquisition and processing for the scans involving 90° and 270° pulse excitations (Mo and Raftery 2008).

In setting up sequences involving WET type water suppression, the WET selective pulse RF field is typically calculated first and then fine-tuned experimentally so that the water signal is smallest. For WET270, the RF field has to be increased (up to 1 dB) and additionally tuned to obtain satisfactory water suppression for the scan involving the 270° pulse excitation (vide infra). In contrast, the acquisition and processing parameters for WET180 are the same as in WET90. In other words, the WET180 selective pulses can be optimized in the same way as WET90, thus no additional fine-tuning is needed.

The WET-composite sequence suppresses faraway water by applying spatially selective pulses (Bax 1985). Invariably, faraway solute signals are also suppressed, which leads to the reduction of interesting signal intensity. In the present experiments, this effect can be estimated using the signal intensity of DSS, which is also used for chemical shift referencing. In Fig. 2, WET-composite gives about 92% of WET90 intensity for DSS. Similarly, the use of WET180 with a square inversion pulse results in 94% signal intensity of WET90. The loss of 6% signal in WET180 is expected, due to spin-lattice relaxation, off-resonance effects and RF inhomogeneity of the 180° pulse. However, the loss can be reduced by using an adiabatic pulse inversion pulse that is less sensitive to RF inhomogeneity or off-resonance effect. The use of bipolar gradients (Wu et al. 1995) can reduce the required delay between the last WET pulse and detection during which spin-lattice relaxation takes place, thus spin-lattice relaxation can be minimized too.

The impact on peak intensity near the water signal by WET selective pulses was investigated by simulations. Since all sequences used the same selective RF powers for water suppression, the expected influence of WET selective pulses on peaks close to water would be very similar, if those peaks do not overlap with the tails of the residual water. With a selective RF field of 50 Hz (peak power), interesting peaks 50 Hz or more away from water resonance should be detected (Fig. 3). For the sucrose sample used in the current study, the residual water signal width at the level corresponding to 10% of the DSS maximum intensity is found to be about 30 Hz from the water center frequency for WET180 and WET-composite. For both sequences, the limiting factor in detecting an interesting peak near the water signal is the selective RF pulse strength (50 Hz in this case), rather than any residual water interference. On the other hand, the larger residual water line-width of 110 Hz for WET90 would severely hinder effective analysis for peaks near water.

Discussion

Intuitively, one would expect a simple addition of a 180° pulse to the WET sequence with concomitant phase cycling (WET + 180) would efficiently suppress the residual water

signal. However, it turned out not to be the case experimentally: first, with an extra 180° pulse, the required water selective pulses would have to be fine-tuned to slightly higher power (about 1 dB or so) if the smallest residual water signal for that particular scan is desired; second, the resulting spectrum does not appear to suppress residual water significantly better than the existing WET270 or WET composite pulse sequence.

Simulations shown in Fig. 3b demonstrate the less efficient water suppression obtained using WET + 180, which suffers from less tolerance to RF inhomogeneity. As the water signal itself is concerned, Fig. 3b also shows that water suppression is better if all the WET pulse angles increase by 10% (short dashed lines), which corresponds to an RF strength increase of 0.8 dB (assuming the pulse lengths are fixed). This explains our observation that the selective RF power had to be increased upon the addition of the 180° pulse for the WET + 180 sequence. The results are not surprising, since the extra 180° pulse, in combination with the last wet selective pulse (161°) would essentially make either a 341° or 19° pulse (assuming both pulses are aligned or perpendicular to each other), which is not the optimized 161° pulse angle in the original WET sequence.

Additionally, RF inhomogeneity may reduce the apparent overall selectivity of WET + 180 pulses. In the simulations shown in Fig. 3b, intensities of the peaks about 50–60 Hz away from the water resonance may become severely attenuated, because those signals from regions of slightly lower or higher RF field (Fig. 3b long and short dashed lines) will partially cancel those from the normal RF field (solid line). As a comparison, WET180 (Fig. 3c) behaves in the same way as WET90 (Fig. 3a): signals from slightly higher and lower RF field regions would sum constructively if the offset is more than 50 Hz.

Since the original B_1 - and T_1 -insensitive WET pulse angles have been optimized, we realize that it is preferable to maintain those effective angles for the scan with the extra 180° pulse. The most straightforward approach is to apply the last selective 19° pulse with opposite phase to the subsequent 180° pulse. A rather small cost is that a small angle soft pulse (for a given power level) tends to be less selective than a large angle one. While one of the goals of WET180 is to suppress residual water efficiently so that nearby peaks can be easily identified and characterized, it is possible to lower the RF power for the last wet selective 19° pulse while the total pulse length remains the same as or shorter than that of the original WET 161° pulse. Further simulations using lower RF power indicate a similar water suppression result can be obtained (results not shown).

It can also easily be seen in Fig. 3c that slightly longer WET pulses would give slightly better suppression for water within 50 Hz of the carrier frequency. Experimentally, the required power or pulse-width change is very small, such that fine-tuned WET pulse lengths or RF powers can be applied directly to the scan that has the additional 180° pulse in WET180.

Similar to Pre-SAT180, bulk water is suppressed by the main sequence (WET), while the residual water signal contributed by faraway regions is further reduced by toggling of the 180° inversion pulse. Though the final spectrum after phase-cycling may contain very small

signals, the receiver gain is still dependent on the dynamic range of any individual scan. Thus a reasonable good shim and fine-tuned selective pulses are still necessary.

It is also possible to replace the high power 180° pulse with an adiabatic one. The advantages are that the adiabatic pulse would be insensitive to calibration, and that signal loss due to RF inhomogeneity and off-resonance effects would be minimized. However, the advantages of using an adiabatic pulse might be less apparent in WET180 than in Pre-SAT. First, successful use of WET will always require a good calibration and fine-tuning afterward for best water suppression. Moreover, WET180 is more likely to be used in the presence of large molecules, which invariably would have much faster relaxation rates than small molecules. Longer adiabatic pulses (500 μ s or longer) may introduce relaxation loss, and thus negatively impact sensitivity.

As such, the biggest drawback of WET180 is probably the longitudinal relaxation after the introduction of the 180° pulse. In a typical WET setting, there is an empirical delay of up to 10 ms, between the last WET pulse and read pulse. This delay has minimal impact if interesting magnetization is kept along +z axis for normal WET. However it may cause noticeable signal loss for the scan incorporating the 180° pulse, if the spin-lattice relaxation rates for the interesting peaks are very fast. Thus it might be necessary to reduce the delay, which consists of a gradient (duration about 2 ms), and subsequent gradient recovery time. While modern probes require just tens of microseconds for gradient recovery, spectral quality can be frequently improved with bipolar gradients, which efficiently overcome eddy currents and reduce gradient recovery time (Wu et al. 1995).

The spirit of WET180 modification needs to be heeded in multiple pulse sequences which may have built-in phase cycling to suppress faraway water (Zhang et al. 2000). Better water suppression can be achieved if we bear in mind the fact that different scans in those sequences may create a different initial water magnetization before or after the WET sequence. In practice, it would be beneficial to change either the first or the last WET selective pulse to accommodate the fate of water. For example, Fig. 4a and b show two 1D NOESY sequences with WET for water suppression during the mixing time, with pulse toggling for the selective 180° and the first WET pulses. For different scans, the first two hard 90° pulses can be in-phase or out-of-phase, creating an equivalent 180° or 0° pulse on water. In the latter case, the original WET pulse angles can be used ($\theta = 81.4^\circ$). In the scan with the effective 180° pulse by the first two hard 90° pulses, the water's fate can be reverted to the same state as in previous case by either adjusting a different θ pulse angle for the first WET selective pulse (Fig. 4a) or toggling on the selective 180° pulse (Fig. 4b). In Fig. 4a, the calculated θ pulse angle is 98.6° . However, it can be manually adjusted for best overall water suppression in practice and frequently it is found to be a smaller angle, presumably due to relaxation and radiation damping. Figure 4c middle trace shows a reduction of about 40% for the residual water signal with $\theta = 65^\circ$ for a lysozyme sample.

Alternatively, a selective 180° pulse can be toggled on before the first two hard pulses if they are in phase (resulting a 180° pulse for water) while the WET angles remain the same (Fig. 4b). Dramatic residual water reduction (more than 90% judged by signal height) is shown in the upper spectrum of Fig. 4c.

Conclusions

With the introduction of an inversion pulse and subsequent modification of the last selective WET pulse, WET180 should be the preferred 1D water suppression method in metabolomic NMR and ligand-protein studies when both macromolecules and small molecules are present and they may further interact or exchange with water.

WET180 has a clear advantage over existing WET-based methods in offering superior residual water suppression, better spectral phasing properties, and easy set-up and optimization. Because faraway water is efficiently cancelled in WET180, WET soft pulses can be made more selective with minimal perturbations to peaks in the regions close to water. Furthermore, the rationale of WET180 would be applicable to other multidimensional experiments that utilize WET sequence for the water suppression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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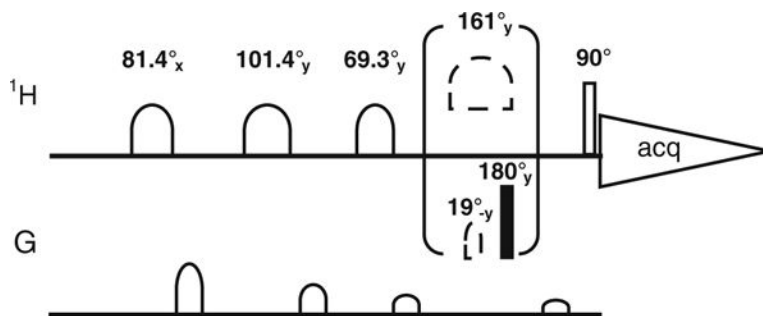


Fig. 1.

A schematic diagram for the T_1 - and B_1 -insensitive WET180 sequence. The first three WET selective pulses (represented by solid lines) have the original angles of 81.4°_x , 101.4°_y and 69.3°_y (with subscript x or y indicating relative pulse phases). The last WET selective pulse is either 161°_y or 19°_{-y} , with the latter followed by a high power square (or adiabatic) 180°_y pulse so that the net nutation angle for water remains 161° (dashed pulses in the bracket). If 19°_{-y} is replaced by 161°_y in the presence of the 180° pulse then the sequence is referred as WET + 180

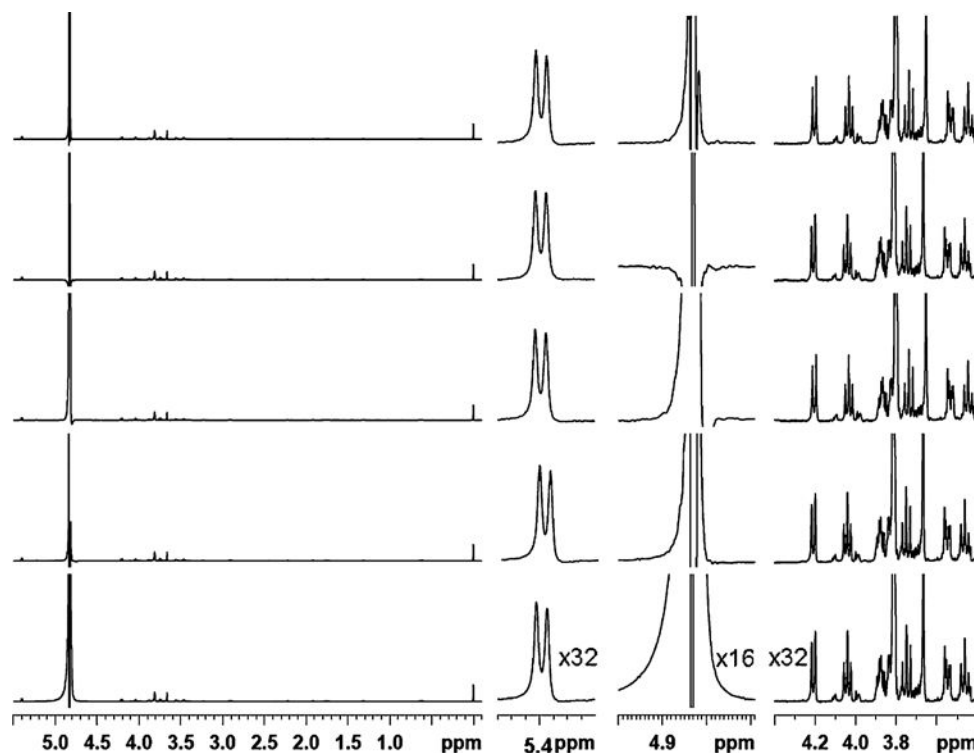


Fig. 2. Water suppression results for several variants of WET. From the top to the bottom are: WET-composite (with a spatially selective composite observe pulse ($90_x 90_y 90_{-x} 90_{-y}$)), WET180 (Fig. 1 sequence with a square 180° inversion pulse), WET + 180 (WET with the simple addition of a 180° pulse), WET270, WET90 (WET with a 90° observe pulse). Regions containing sucrose peaks and water are expanded on the right hand side. Minor distortions for the anomeric peak are caused by the eddy current from the gradient amplifier in this particular set-up. For the ease of operation and comparison, all WET selective pulses were one lobe SINC shaped and had the same RF peak power (50 Hz). For WET270, FIDs were acquired and processed separately for the scans with 90° and 270° observe pulses before summation as they required different first order phase corrections. All high power pulse RF fields were 24 kHz

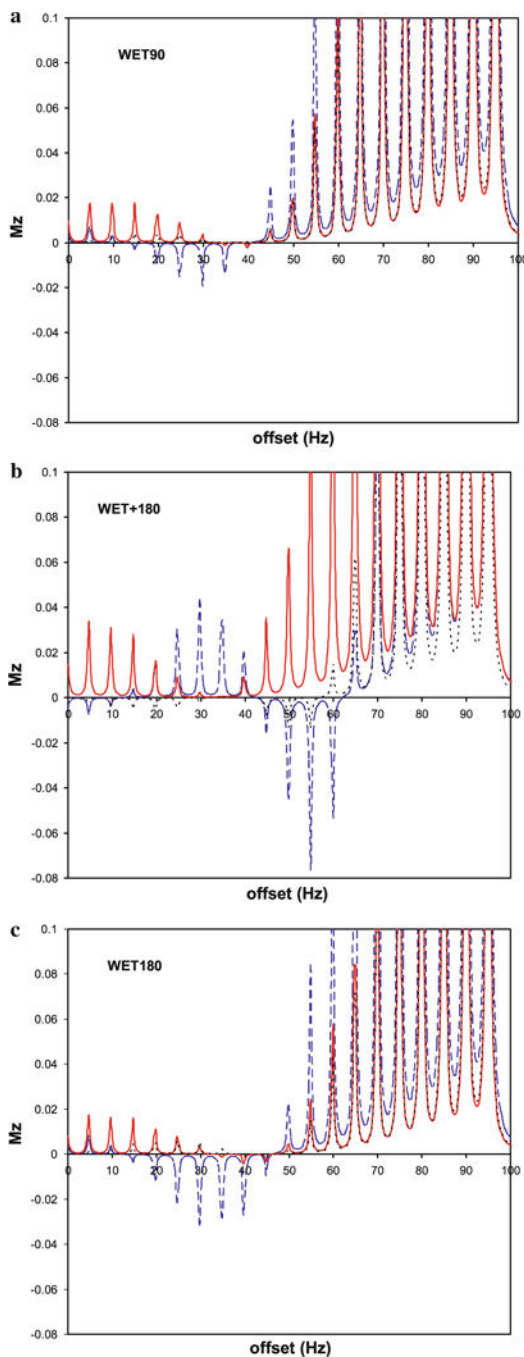


Fig. 3. Simulated z-magnetization (represented by peak height) as a function of offset and RF inhomogeneity, after WET selective pulses for water suppression and a hard 180° pulse if applicable. WET selective pulses were set to 90% (long dashed line), 100% (solid line) or 110% (short dashed line) of the intended angles to mimic the effect of RF inhomogeneity. (a) Original B_1 - and T_1 -insensitive WET: $81.4^\circ_x - 101.4^\circ_y - 69.3^\circ_y - 161^\circ_y$. (b) WET + 180 for the scan with the simple addition of a hard 180° pulse in a). (c) WET180 for the scan that has the last WET pulse modified to 19°_{-y} and immediately followed by a hard 180°_y pulse,

so that the effective pulse angle of the last WET pulse remains 161°_y for water. All WET selective pulse blocks were followed by a 2 ms gradient and 2 ms delay. An additional delay of 8 ms was applied before the final observe pulse. All WET selective pulses had RF peak strength of 50 Hz. The high power proton pulse RF was 25 kHz. For the ease of simulation, relaxation was ignored during all pulses or delays

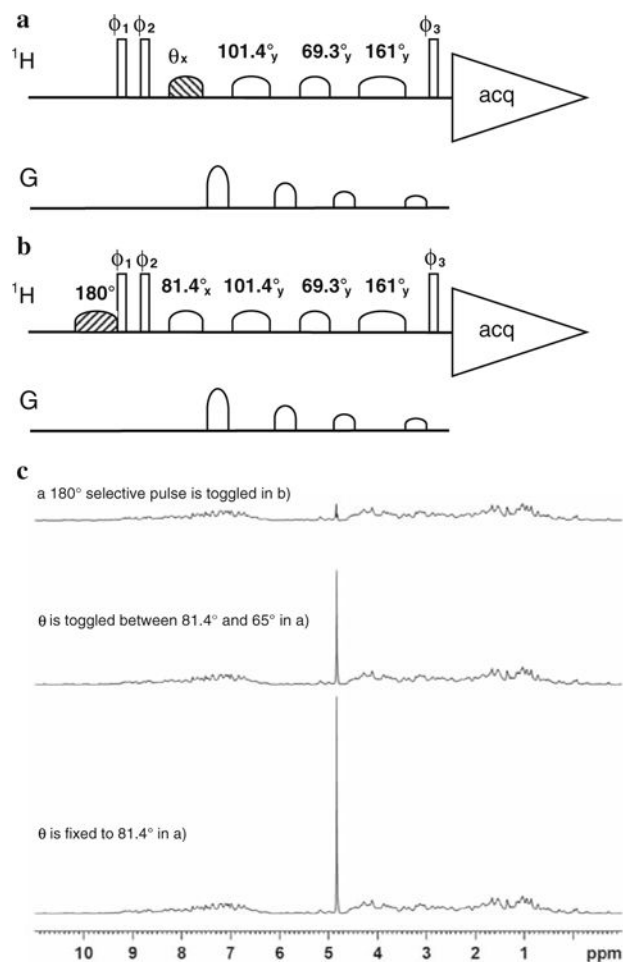


Fig. 4.

(a) A 1D NOESY sequence with WET water suppression during mixing time. θ is 81.4° for scans that have first two hard pulses with 180° or 90° phase shifts with respect to each other (resulting in a 0° or 90° pulse for water), or a variable angle smaller than 98.6° if the two pulses are in phase (resulting an equivalent 180° pulse for water). (b) Another alternative 1D NOESY sequence with constant WET selective pulse angles. However, a selective 180° is toggled on if the first two high power pulses are in phase. (c) Demonstration of improved residual water suppression by a 5 mM lysozyme in 10% D_2O at room temperature. Lower trace: 1D NOESY with normal WET angles (θ is fixed to 81.4°). Middle trace: residual water was readily reduced by more than 40% if θ was toggled between 81.4° and 65° , using the method described in a). Upper trace: residual water was reduced by more than 90% using the pulse sequence in b). Standard NOESY phase cycles were: $\phi_1 = x, -x$; $\phi_2 = 8(x), 8(-x)$; $\phi_3 = x, x, -x, -x, y, y, -y, -y$; receiver: $x, -x, -x, x, y, -y, -y, y, -x, x, -x, -y, y, y, -y$