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Effects of radiation damping for biomolecular NMR experiments in solution: a hemisphere concept for water suppression

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Abstract

Abundant solvent nuclear spins, such as water protons in aqueous solution, cause radiation damping in NMR experiments. It is important to know how the effect of radiation damping appears in high-resolution protein NMR because macromolecular studies always require very high magnetic field strengths with a highly sensitive NMR probe that can easily cause radiation damping. Here, we show the behavior of water magnetization after a pulsed-field gradient (PFG) using nutation experiments at 900 MHz with a cryogenic probe: when water magnetization is located in the upper hemisphere (having +Z component, parallel to the external magnetic field), dephasing of the magnetization by a PFG effectively suppresses residual water magnetization in the transverse plane. In contrast, when magnetization is located in the lower hemisphere (having -Z component), the small residual transverse component remaining after a PFG is still sufficient to induce radiation damping. Based on this observation, we designed ^1H - ^{15}N HSQC experiments in which water magnetization is maintained in the upper hemisphere, but not necessarily along Z, and compared them with the conventional experiments, in which water magnetization is inverted during the t_1 period. The result demonstrates moderate gain of signal-to-noise ratio, 0–28%. Designing the experiments such that water magnetization is maintained in the upper hemisphere allows shorter pulses to be used compared to the complete water flip-back and, thereby, is useful as a building block of protein NMR pulse programs in solution.

Keywords

NMR; radiation damping; relaxation; water; gradient

I. Introduction

In protein NMR in aqueous solution, pulse schemes to suppress the intensity of solvent water signals, without saturating water, have been developed to gain the relative intensity of protein signals (1–6). Sensitivity enhancement schemes to select only coherences of interest have frequently been inserted in such pulse schemes (7–9). In combination with these techniques, a set of pulsed-field gradients (PFGs), with positive and negative signs, often is applied during a t_1 period to dephase the water signal and to suppress generation of water transverse magnetization by radiation damping (7–12). These ideas are based on (I) aligning water magnetization to be parallel to the static magnetic field strength (+Z), or (II) temporally dephasing water when the water magnetization is located in the transverse (XY)

plane. These handlings of water magnetizations and selection of desired coherences are particularly important to suppress artifact peaks and in very fast multi-dimensional NMR experiments (5, 13–15).

Despite these efforts, residual water signals often remain during signal acquisition in practical applications. Reasons for this incomplete suppression of water may be imperfect radio-frequency pulses and inhomogeneity of the magnetic field. Difficulty with the suppression of a water signal becomes more evident with high-field magnets, in which radiation damping is severe. Even residual, but sufficiently *abundant*, water protons induce a voltage in the coil and cause an electromagnetic field that nutates the magnetization back toward the +Z axis (16–23). Instrumental techniques to reduce apparent radiation damping and the NMR processing schemes to read out the effect have been developed (24–29). However, it is known that even a pure -Z magnetization can cause radiation damping by leakage of applied radio-frequency field or thermal noise fluctuation of the coil (18, 30–32). Distant dipolar field is known to slow down complete dephasing of transverse magnetization when only Z-gradient is applied (21, 33). In biomolecular NMR experiments that require a high-magnetic field strength and a highly-sensitive cryogenic probe, characterization of the radiation damping and understanding of the behavior of water magnetization are important for better design of experiments (34–38).

In this manuscript, we demonstrate the radiation damping effect created by the small residual transverse component remaining after a pulse-field gradient at high magnetic field strength, in a way that readers can visually understand. Knowing that a low-pass filter is often used to remove the solvent water signal for individual free-induction decay (FID) in protein NMR data processing (39), the magnitude of the water signal is our most immediate concern, more so than the phase of water magnetization in the transverse plane. Our results show that when water magnetization is located in the upper hemisphere (having +Z component, parallel to the external magnetic field), dephasing of the transverse component of the water magnetization by a PFG effectively decreases the water signal intensity. This is because radiation damping works to decrease the transverse magnetization when the magnetization is located in the upper hemisphere. In contrast, when the water magnetization is located in the lower hemisphere (having -Z component, antiparallel to the external magnetic field), a small transverse component of the water magnetization induces radiation damping to magnify the transverse component. This result was confirmed by testing pulse sequences to allow water magnetization to always be above the XY plane (the upper hemisphere), but not necessarily along the Z-axis.

II. Concept

Although many papers described equations for radiation damping, here, a set of equations is described for the purpose to explain the data below. X, Y, and Z components of the magnetization are expressed as $M_X = M_0 \sin(\theta) \cos(\phi)$, $M_Y = M_0 \sin(\theta) \sin(\phi)$, and $M_Z = M_0 \cos(\theta)$. Here, polar angle, θ , is between 0 and 180°, and azimuthal angle, ϕ , is between 0 and 360° in spherical coordinates. In the presence of radiation damping, equations that contain the classic radiation damping rate, τ_R^{-1} , (but not the entire Bloch equation) are

described for the transverse and longitudinal magnetizations, assuming that the resonance frequency is located at the carrier (16, 18, 22),

$$dM_+(t)/dt = -M_+ \cos(\theta) / \tau_R \quad (1)$$

$$dM_z(t)/dt = M_0 \sin(\theta) (\sin(\theta) / \tau_R) \quad (2)$$

where τ_R is the radiation damping time constant. The equation is basically the same as those of Eq. (14) in Vlassenbroek et al (40), except for the phase term in Eq. (1), i.e., for the case of a tuned circuit with $t_0 = 0$, and with the carrier on resonance frequency.

Using this integration equation, $\int \frac{dx}{a^2 - x^2} = (1/a) \tanh^{-1}(x/a)$, one can derive

$$M_z(t) = M_0 \tanh \left(\frac{t}{\tau_R} + C \right) \quad (3)$$

For transverse magnetization, when the M_z that is given in Vlassenbroek et al (40) (Eq. (4)) is inserted to equation (1),

$$M_z(t) = M_0 \tanh \left(\frac{t - t_0}{\tau_R} - \log \left\{ \tan \left[\frac{\theta}{2} \right] \right\} \right) \quad (4)$$

The following form, similar to that of M_x in Vlassenbroek et al (40) but without the phase term is obtained.

$$-\log(M_x(t)) = \log \left(\cosh \left(\frac{t}{\tau_R} - C \right) \right) \quad (5)$$

$$M_x(t) = \text{sech} \left(\frac{t}{\tau_R} - C \right) \quad (5)'$$

The differential equations, Eqs. (1) and (2), give a simple picture of the time dependence of magnetization upon radiation damping. (I) $dM_z(t)/dt$ is always positive, indicating that radiation damping drives Z-magnetization towards equilibrium, i.e. minimizes the energy of the spin system, and indicating that the torque induced by the radiation damping rotates the inverted magnetization towards Z (Figure 1A) (20). (II) $dM_+(t)/dt$ changes the sign such that

the magnitude of the transverse magnetization decreases at $0 < \theta < 90^\circ$, but increases at $90^\circ < \theta < 180^\circ$ (Figure 1B). As a result, when $-Z$ magnetization at $\theta = 180^\circ$ recovers back to $+Z$ by radiation damping, the transverse magnetization increases the intensity until the $-Z$ magnetization hits $\theta = 90^\circ$ and decreases in intensity after that. Here, the effect of the phase term for $M_+(t)$ is ignored (commented later).

An important notion is that since the decay of the transverse magnetization is accelerated by the radiation damping in the range of $0 < \theta < 90^\circ$ (in the upper hemisphere), overall magnetization that is located at the upper hemisphere spontaneously decreases in the presence of radiation damping. To reduce the intensity of the transverse component of a water magnetization, the water magnetization needs to always be above the XY plane, but not necessarily exactly along the Z-axis.

III. Effect of radiation damping on water magnetization after PFG

We qualitatively evaluated the effect of PFG on water magnetization that experiences radiation damping effect, using two simple NMR experiments: (A) apply a radio-frequency pulse at 180° flip angle and detect the FID signal immediately, and (B) apply the same radio-frequency pulse, followed by a PFG, and detect the FID (Figure 2). Data were recorded using a sample that contains 95% H_2O at a 900 MHz NMR instrument equipped with a cryogenic probe. In the ideal situation of Experiment (A), that is when the water proton magnetization is rotated exactly at 180° degree, there is no detectable water signal. However, practically, a small residual transverse component of the water magnetization exists after the inversion pulse, due to pulse imperfection or sample inhomogeneity. Such a small magnetization was detected as a FID signal (Figure 2A). Importantly, the FID did not necessarily exponentially decay to zero, but increased for a while due to a radiation damping field that was induced by the residual transverse magnetization (16, 18) (Figure 2A).

In Experiment (B) in which a PFG (10 G/cm, 2 ms) was applied prior to the signal detection, no significant signal was observed at an initial 5 ms of FID. This is conceivable because all or nearly all transverse magnetization is dephased by the PFG. However, in the middle of FID, the intensity of the water signal became large because radiation damping is triggered by partially *re-phased* water magnetization (Figure 2B). The phenomenon is typically explained by a leakage of applied radio-frequency field or thermal noise fluctuation of the coil, as proposed previously (18, 30–32), and the chaotic evolution of water magnetization has been described by the combination effect of PFG and distant dipolar field (21, 33). Here, we use a simple phrase, “partially *re-phased*”, without getting into the physical mechanism of the phenomenon, for the reason that there is a time lag in the generation of a small coherent transverse magnetization after PFG is applied. Overall, when the flip angle θ is $\sim 180^\circ$ at the start of the FID acquisition, *positive* $dM_+(t)/dt$ (Eq. (1)) increases M_+ until it passes the XY plane (i.e., $\theta = 90^\circ$). When θ is smaller than 90° , *negative* $dM_+(t)/dt$ (Eq. (1)) decreases M_+ (Figure 2B). The time delay that is required to generate a radiation damping effect on the water magnetization and the phase of the radiation damping in Experiment (B) depend on the strength and duration of the PFG and perhaps on the shimming of the probe (23, 37).

IV. Residual water intensity detected by ^1H nutation experiments

The above experiments characterized, qualitatively, the reappearance of water magnetization after PFG; however, the experiments using only one 180° pulse largely depend on the accuracy of the pulse width. Thus, sets of nutation experiments were next performed to systematically demonstrate the effect of radiation damping on water magnetization. In the nutation experiments, a series of FIDs were recorded after applying a nutation pulse, the duration of which is incremented like a t_1 evolution time. We performed three sets of experiments that detect FIDs: (A) without any pulse or PFG after the nutation, (B) with a PFG and a short pulse after the nutation, and (C) with a PFG after the nutation (Figure 3).

In Experiment (A), projection of the water magnetization in the transverse plane was detected as a FID immediately after the nutation pulse (Figure 3A). The nutation pulse was applied from Y so that the water magnetization on the carrier frequency was rotated on the Z-X plane. The receiver phase was set to X to show the X-magnetization in the real channel in Figure 3. As expected, the array of the FIDs in the real channel exhibited positive signals when the magnetization was rotated less than 180° , and negative signals when the nutation angle was in the range of 180° to 360° rotation (Figure 3A). FIDs were small in the imaginary channel because the water magnetization undergoes nutation on the Z-X plane. Shift of the phase of water magnetization occurred by radiation damping and became evident near every -180° nutation. Observed FIDs at the -180° nutation were basically similar to those in Figure 2A (data not shown).

In Experiment (B), a PFG and a subsequent short radio-frequency pulse were applied after the nutation. In this way, we aimed to detect the Z-component of the water magnetization that remains after the nutation (Figure 3B). Similar to the case of Experiment (A), water magnetization on the carrier frequency was rotated on the Z-X plane by applying the nutation pulse from Y. Subsequent PFG was supposed to eliminate the transverse component of the water signal. Since a short pulse applied from $-Y$, the projection of $+Z$ magnetization was detected as a negative intensity at the start of the FID array. As expected, the intensity of the FIDs increased from $\theta = 0^\circ$ to 90° in the real channel, with the greatest negative intensity at time zero. Its imaginary component, which was orthogonal to the nutation plane, showed very small intensity when $\theta = 0^\circ$ to 90° (i.e., the magnetization before applying the PFG was in the upper hemisphere). Interestingly, at $90^\circ < \theta < 270^\circ$ (i.e., the magnetization before applying the PFG was in the lower hemisphere), significant signals were observed in both the imaginary channel and the real channel. Since PFG is supposed to eliminate the transverse component of the water signal, the PFG effect relates to the appearance of significant generation of water magnetization in the imaginary channel in the lower hemisphere.

In Experiment (C), only a PFG was applied after the nutation. In this case, no signals were expected to be detected if the gradient completely dephases the transverse component of the water magnetization and if radiation damping occurs. As expected, signal intensities were negligible when the nutated water magnetization was located in the upper hemisphere. In contrast, significant FID signals were observed when the nutated water magnetization was located in the lower hemisphere. This observation of significant FID signal in the lower

hemisphere in Experiment (C) is similar to that in Figure 2B. As discussed in the above section, we simply explain that this phenomenon is due to partial re-phasing of the water magnetization. Our major interest is the stark contrast in the observed FIDs when the nutated magnetization is in the upper hemisphere, compared to that in the lower hemisphere (Figure 3C). Such contrast occurs because a negative $dM_+(t)/dt$ decreases M_+ intensity at $0 < \theta < 90^\circ$, whereas a positive $dM_+(t)/dt$ increases M_+ at $90^\circ < \theta < 180^\circ$ (Eq. 1). Overall, these comparisons indicate how it is easy to reduce transverse magnetization of water when it is located in the upper hemisphere ($0 < \theta < 90^\circ$), but not in the lower hemisphere ($90^\circ < \theta < 180^\circ$).

V. Comparison of ^1H - ^{15}N HSQC experiments

The above results indicate that water magnetization should be maintained in the upper hemisphere, but not necessarily fully-along the +Z axis, for the purpose to decrease undesired behavior of water magnetization by radiation damping in conventional ^1H - ^{15}N HSQC experiments. In order to test this inference, we performed watergate ^1H - ^{15}N HSQC experiments with or without pulses that maintain water magnetization in the upper hemisphere (Figure 4). The watergate sequence used as a reference (Figure 4A) is not optimal for proteins that undergo amide-water proton exchange because the first water-selective pulse that is located soon after the first INEPT period is applied to flip “down” the water magnetization. The $-Z$ water magnetization is flipped up to $+Z$ by the 180° proton inversion pulse during the t_1 period. Thus, the water magnetization maintains the $+Z$ orientation at the starting of the acquisition period but the $-Z$ orientation during half of the t_1 period. To overcome this problem, selective pulses for water-signal excitation (Figure 4B) or a REBURP pulse for amide protons (Figure 4C) were inserted in every step of ^1H inversion to maintain water magnetization in the upper hemisphere. The REBURP pulse applied to invert amide signals, $700\ \mu\text{s}$ with a $4250\ \text{Hz}$ off-resonance, is not sufficiently long to invert only the amide proton but also perturbs water magnetization. However, the perturbation to the water magnetization is weak so that the water magnetization stays in the upper hemisphere and is not brought into the lower hemisphere.

The ^1H - ^{15}N HSQC spectrum recorded using the hemisphere sequence with water-flip back pulses (Figure 4B) had 10% lower noise level in the two-dimensional spectrum and exhibited signal-to-noise (S/N) ratio increases ranging from 6–28 %, compared to that of the standard sequence (Figure 4A) (Table 1). Similar gain of S/N ratio was also obtained when a REBURP pulse was used in the hemisphere sequence (Figure 4C) (Table 1). All three sets of data were recorded using the same receiver gain (Table 1). In the standard sequence, the $-Z$ water magnetization that exists in the half of the t_1 period starts to generate the transverse component by the radiation damping, which brings a part of the bulk water signal to unexpected orientation. Thus, the standard sequence may suffer more amide-water exchange effect or contains more noise, originated from water protons, in the detected FID, compared to the hemisphere ones. The ^1H intensity of the first single FIDs of the two hemisphere experiments were approximately a half of the standard sequence (Table 1), demonstrating that the intensity of the remaining water signal in FID is smaller in the hemisphere sequences than the standard one.

^1H - ^{15}N HSQC spectra were also recorded using a coherence selection, echo and antiecho (EA), sequence (Figure 5). We compared the performance of (I) a standard EA sequence (Figure 5A) with (II) a standard EA sequence with a bipolar gradient during t_1 period (Figure 5B) and (III) with the hemisphere-type EA sequence (Figure 5C). In the latter, similar to the sequence of Figure 4C, a ^1H REBURP pulse was applied to invert amide protons and to maintain water magnetization in the upper hemisphere. In this comparison of the coherence selection versions of HSQCs, no significant gain of S/N ratio, but rather a 5% loss, was observed in the hemisphere experiment (Table 1). The lower S/N ratio is mainly from the signals that are located closer to the water chemical shift. Nevertheless, the magnitude of FIDs recorded at every 5 ms t_1 increment was smaller in the hemisphere version (Figure 6C) than others (Figures 6A and 6B), demonstrating reduction of residual water magnetization throughout the dataset acquisitions. The result is consistent with the time domain data of the HSQCs shown in Figure 7: signal intensity recorded in the hemisphere sequence (Figure 7C) at a relatively long t_1 evolution is smaller than others (Figures 7A and 7B). Overall, the hemisphere sequence reduced water magnetization better towards the end of (longer) t_1 evolution time.

VI. A hemisphere concept in ^1H - ^{15}N HSQC experiments

Water selective pulses and pulse field gradients are typically applied in biomolecular NMR experiments (1–11, 13). As described above, radiation damping has also been well investigated by many groups (16–21). In this manuscript, we illustrated the effects of severe radiation damping caused by residual water magnetization using simple nutation experiments. Our results indicate that maintaining water magnetization in the upper hemisphere (not necessarily aligned along +Z direction) is essential to suppress the severe effect of radiation damping when PFG is applied. A REBURP pulse to invert only amide protons but not water magnetization is typically long (> 2ms) and is difficult to incorporate into pulse sequences. In contrast, the REBURP pulse used in the hemisphere experiments is much *shorter* (0.7 ms) and *does* perturb water magnetization, having a trajectory such that water magnetization is located slightly above the XY plane at the end of the pulse. Such a short REBURP may be advantageous in future pulse scheme designs.

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Figure 1.

Typical trajectories of radiation damping for (A) Z-magnetization and (B) X-magnetization after applying 170°, 140°, 100°, 80°, 40°, and 10° radio-frequency pulses, assuming a magnetization that is located at the carrier frequency. The figure was generated using equation (14) in the reference of Vlassenbroeck et al (40) without the phase term. The trajectories for the magnetization started from $90^\circ < \theta < 180^\circ$ (in the lower hemisphere) are shown by black, and those started from $0^\circ < \theta < 90^\circ$ (in the upper hemisphere) are shown by red.

Figure 2.

Free-induction decays (FIDs) acquired after employing a ^1H 180° pulse at a 900 MHz NMR instrument with a cryogenic probe. A ^1H 180° pulse at 5.7 kHz $B_1/2\pi$ field strength was applied to a water proton signal at on-resonance frequency. FIDs were acquired (A) immediately after the 180° pulse, and (B) after the 180° pulse followed by a 10 G/cm smoothed-square (smsq)-shaped gradient for 2 ms. FIDs of both real and imaginary channels are shown in each figure. All experiments in this article were performed using 0.8 mM ^{15}N -labeled ubiquitin in 20 mM sodium formate at pH 4.5 with 5% D_2O at 16 $^\circ\text{C}$, in a 5 mm Shigemi tube with a sample height of 17 mm.

Figure 3.

Arrays of free-induction decays (FIDs) observed after ^1H nutation at 11.4 kHz $B_1/2\pi$ field strength with incremented time at 900 MHz. Water proton signal was located at on-resonance frequency. The radio-frequency pulse was incremented with a 4 μs step (which corresponds to approximately 16.3° angle rotation) until 512 μs with a pulse recycle delay of 5 s. The FIDs were acquired for up to 70 ms and were displayed as arrays without Fourier transformation. Thus, each FID looks like a spike in the array plots, when there is significant intensity. Note, even when generation of transverse magnetization is delayed in a FID, like in Figure 2B, such a delay is not visible in Figure 3. The experiments were performed in the following three ways: (A) FIDs were acquired immediately after the nutation, to observe the water signals in the X, Y projections; (B) FIDs were acquired after the nutation followed by a 700 μs smsq-shaped PFG with a subsequent 4 μs pulse, to observe the Z-component of the water signal; and (C) FIDs were acquired after the nutation followed by a 700 μs smsq-shaped PFG, to observe residual proton signals in the X, Y plane. Relative phases of the

nutations pulse and the receiver were Y and X, respectively, in experiment (A). The phase of the 4 μ s pulse after the PFG in experiment (B) was set to $-Y$. In each figure, FIDs of both real and imaginary channels are shown. Sine-modulated curves between (A) and (B), and also (B) and (C) indicate approximate positions of the water Z magnetization immediately after the nutations pulse. When the curve is positive (above the horizontal line), the water signal is located in the upper hemisphere at the end of the nutations.

Figure 4.

^1H - ^{15}N HSQC pulse schemes of (A) a standard water-gate sequence, (B) a hemisphere sequence with water-flip back pulses, and (C) a hemisphere sequence with a REBURP pulse. In (C), only the different pulse scheme (shown by square region in (B)) is described. Narrow (wide) black bars indicate rectangular ^1H and ^{15}N 90° (180°) pulses with 22.74 kHz and 5.56 kHz $B_1/2\pi$ field strengths, respectively. Gray sine-shapes on ^1H channel indicate 1.2 ms water-selective proton 90° pulses with a sine-shape. A shaped pulse on the ^1H channel in (C) indicates a 700 μs REBURP pulse applied at 4250 Hz off-resonance frequency. ^{15}N GARP decoupling was applied at 1.1 KHz $B_1/2\pi$ field strength during the acquisition. The phase cycling used is: $\Phi_1 = \{X, -X\}$, $\Phi_2 = \{X, X, -X, -X\}$, $\Phi_3 = \{4(X), 4(-X)\}$, and $\Phi_4 = \{-X, -X, X, X\}$, receiver = $\{2(X, -X), 2(-X, X)\}$. The delays used are $t_1 = 2.7$ ms, and $t_2 = 2.7$ ms. Gradient strengths are: $g_1 = 15$ G/cm (1.0 ms), $g_2 = 40$ G/cm (1.0 ms), and $g_3 = 25$ G/cm (1.0 ms). For quadrature detection of the indirect dimension, Φ_1 phase is incremented along with the receiver by 180° for each complex t_1 point. Note, a disadvantage of the experiment in Figure 4(A) is that water magnetization is located along $-Z$ during half of the t_1 period, so that transverse magnetization of the water magnetization is generated by radiation damping when the t_1 period becomes long. In Figure (B, C), the water magnetization always stays $Z = 0$.

Figure 5.

^1H - ^{15}N HSQC pulse schemes of standard echo-antiecho (EA) sequences (A) without and (B) with bipolar gradient during the acquisition, and (C) a hemisphere sequence. In (B) and (C), only different schemes (in a square region) are shown. The same notations used in Figure 2 are used to draw the pulse sequences. The phase cycling used is: $\phi_1 = \{X, -X\}$, $\phi_2 = \{X, X, -X, -X\}$, $\phi_3 = \{Y, Y, -Y, -Y\}$, and receiver = $\{X, -X, -X, X\}$. The delays used are $\tau_1 = 2.7$ ms, $\tau_2 = 1.7$ ms (delays for a REBURP pulse and gradient). Gradient strengths are: $g_1 = 25$ G/cm (1.0 ms), $g_2 = 40$ G/cm (1.0 ms), $g_3 = 2.5$ G/cm (1.0 ms), $g_4 = -1$ G/cm (1.0 ms), $g_5 = 16.2$ G/cm (0.5 ms). Gradients g_2 and g_5 are used for coherence selection. In (B), bipolar gradient during t_1 evolution was applied at 1G/cm strength. Quadrature detection in the indirect dimension is obtained using the sensitivity enhancement approach by recording two sets of spectra by alternating the sign of g_2 gradient and ϕ_3 phase at each t_1 increment. ϕ_1 phase and the receiver phase are incremented by 180° for each complex t_1 point.

Figure 6.

Arrays of FIDs of echo-antiecho ^1H - ^{15}N HSQC experiments of a ubiquitin sample. Signals were recorded using standard echo-antiecho sequences (A) without and (B) with bipolar gradient during the acquisition, and using (C) a hemisphere sequence. A total of 8 FIDs were recorded with t_1 increments of 5 ms up to 40 ms, for each echo and antiecho FID at 900 MHz. A single scan was acquired to observe FID signals.

Figure 7.

Time-domain points of echo-antiecho ^1H - ^{15}N HSQC experiments recorded using standard echo-antiecho sequences (A) without and (B) with bipolar gradient during the acquisition, and using (C) a hemisphere sequence. A total of 200 FIDs, for each echo and antiecho, were recorded with t_1 increments of $340\ \mu\text{s}$, from 0 to 68 ms, at 900 MHz. Observed intensities are clearly different among the three data sets at longer t_1 period (marked by circles). The HSQC experiments were acquired with spectral widths of 2941.2 and 14097.74 Hz for F_1 and F_2 dimensions, respectively. The resolutions of the acquired spectra were 9.38 and 13.76 Hz, respectively. Eight scans were accumulated to observe protein signals in two-dimensional spectra.

Table 1

Comparison of signal-to-noise (S/N) ratios of peaks recorded using different ^1H - ^{15}N HSQC sequences.

Pulse Sequences ¹⁾	Noise	S/N of peaks at different ¹ H chemical shift (ppm)				Ave S/N	First point Intensity (a.i.)	
		6.11	7.02	7.62	8.78	9.25		
Water-gate sequence								
Standard (Figure 4A)	53448	708	978	1265	782	842	915	8000
Hemisphere with water flip-back pulses (Figure 4B)	48611	811	1253	1468	1008	896	1087	4000
Hemisphere with a REBURP pulse (Figure 4C)	50794	783	1245	1667	1012	1000	1141	5000
Echo-AntiEcho sequence								
Standard (Figure 5A)	211874	1179	1793	2123	1227	1227	1509	7000
Standard with bipolar gradient (Figure 5B)	209989	1142	1809	2142	1238	1190	1504	6000
Hemisphere with a REBURP pulse (Figure 5C)	213561	936	1451	2153	1076	1123	1423	7000

¹⁾Different receiver gains are used between watergate sequence and echo-antiecho sequence. However, uniform receiver gain was used among each sequence.