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A novel optimization method is described for the acquisition of direct one-bond heteronuclear correlations. The RDSQC (Randomly optimized Direct correlation Single Quantum Coherence) experiment utilizes an optimization based on the randomly ordered sampling of a range of couplings. The random order of the $1/(2*(^1J_{CHmin}))$ delays removes the signal dependency on a single type of apodization, thus eliminating a significant portion of the F_1 artifacts induced in the accordion-optimized ADSQC experiment. Compared to the statically optimized GHSQC, the randomly optimized data maintains the desired signal intensity in most cases, with a small loss for the weakly coupled proton-carbon pairs and significant gains for the more strongly coupled pairs. Compared to the accordion-optimized ADSQC data, the randomly optimized data afforded similar signal-to-noise without the F_1 modulated artifacts simplifying spectral interpretation.

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Introduction.

Accordion-optimization [1] has commanded considerable recent interest, most of which has been applied to heteronuclear correlations for long-range data [2-8]. There has, additionally, been some attention given to direct correlation data[8,9]. Opposed to static optimization, [10] the direct and long-range applications of accordion-optimization benefit from sampling all of the desired couplings in a single experiment. Unfortunately, the utilization of the accordion-optimized experiments generates artifacts [11] that arise as a consequence of the variable delay. These responses can obscure the data so severely so as to hinder interpretation. It is then desirable to remove the artifacts so that the data become cleaner and more interpretable.

Bodenhausen and Ernst first noted these artifact responses in their original paper on accordion-optimization (almost twenty years ago) [1]. They observed that the diagonal peaks in their accordion experiment afforded the expected lineshape, however, the cross peaks produced shallow depressions in the baseline which were actually broad negative signals. The interferograms of the diagonal peaks decayed to zero while those of the cross peaks exhibited an echo-like shape.

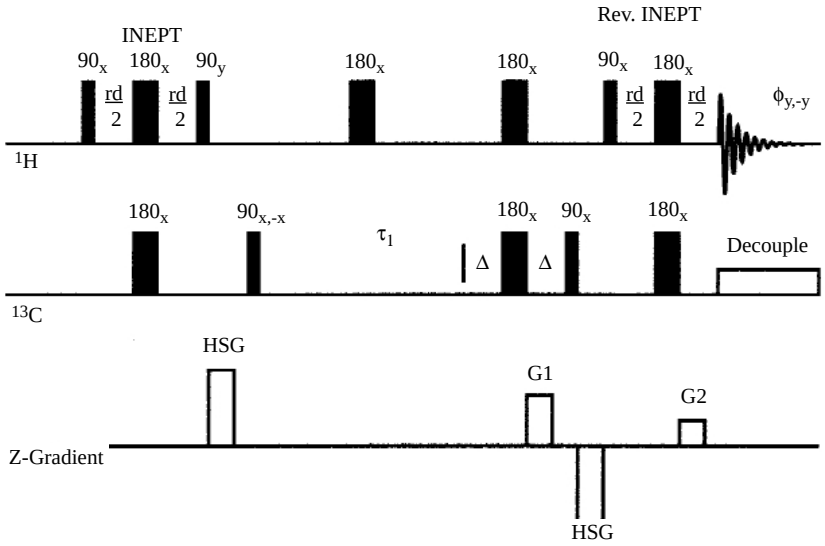


Figure 1. Pulse sequence for the randomly optimized RDSQC. A variation of the accordion-optimized ADSQC [9], this sequence also samples all couplings in a user-defined range. But unlike the ADSQC, the RDSQC samples these optimizations in a random order. Delays labeled rd are the randomly optimized delays, and were taken from a range of J_{1min} (inverse of twice the smallest $^1J_{CH}$ coupling) to J_{1max} (inverse of twice the largest $^1J_{CH}$ coupling). The random sequence was compiled in C with the command 'strand.' The delay Δ was set to an average of the $^1J_{CH}$ couplings and was used for the multiplicity-editing step. The gradient times G1 and G2 were set to a 4:1 ratio and the power levels were set to 10 and 5 G cm^{-1}, respectively. The phases were as shown in the figure.

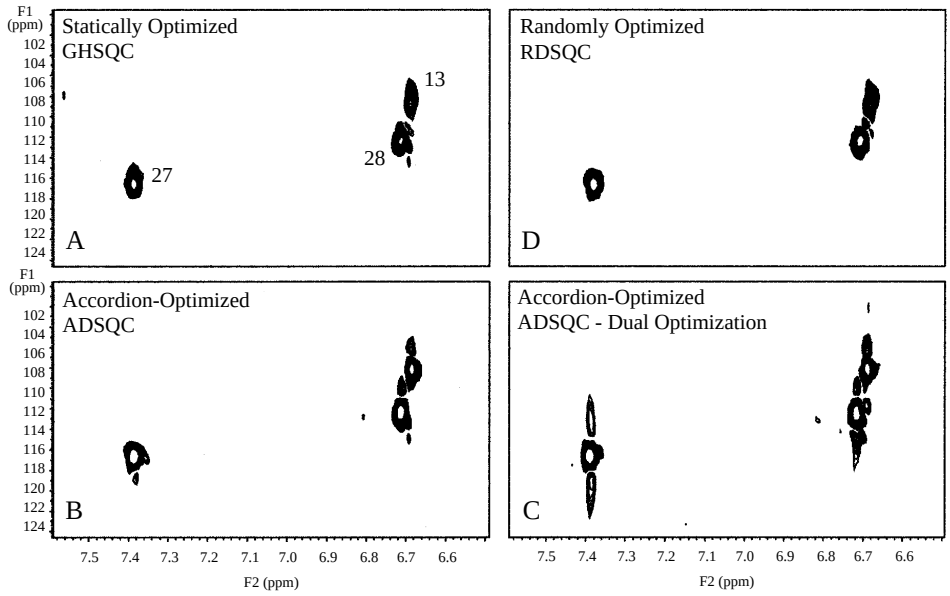


Figure 2. Contour plots of the ceftiofur (**1**) 13-, 27- and 28-position responses for the different optimization techniques of direct heteronulear correlation data. All data were acquired, processed, and plotted with identical parameters when possible. [A]: The statically optimized GHSQC was acquired with a $^1J_{CH}$ delay of 140 Hz. [B]: The accordion-optimized ADSQC was accordion optimized for only the reverse INEPT transfer, and sampled a range from 120 to 220 Hz. The heteronuclear coupling spread into the F_1 domain is created by the accordion-optimization. The triplet's negative wings are produced when a gaussian apodization is used on an echo-type interferogram in t_1. [C]: The dual ADSQC was accordion-optimized for both the INEPT and the reverse INEPT transfers, similar to the accordion-HMQC [8]. This experiment produces significantly more of the F_1 artifacts. [D]: The echo-type interferograms in t_1 can be removed when the accordion steps are sampled randomly. The r andomly optimized RDSQC samples all of the same optimizations in the ADSQC, but instead places them in a random order. A common gaussian function can be then applied proior to t_1 transformations.

A recent report by Zangger and Armitage described in detail the accordion-optimized artifacts in their accordion-HMQC experiment [8]. The modulated responses are actually the heteronuclear couplings spread into a triplet in the inverse-detected F_1 domain. The report further extracted the coupling magnitudes *via* a complex computation. This usage of the heteronuclear coupling information justified their presence as useful data. Unfortunately, a "triplet" created by the hetero-nuclear coupling will reduce the intensity of the main correlation to form the "wings" or outside responses of the triplet. Additionally, a triplet response in the F_1 domain can hinder interpretation in crowded regions or lead to signal cancellation when a negative wing and positive direct overlap.

Accordion-optimized direct correlation experiments such as the ADSQC [9] or accordion-HMQC [8] contain these artifact responses at appreciable intensity. The accordion-HMQC is optimized with two accordion delays affording strong direct correlation responses as well as the heteronuclear triplet spread into the F_1 domain. This was useful for the extraction of the coupling magnitudes. The ADSQC experiment, however, is accordion-optimized in the reverse-INEPT step only. The initial INEPT transfer is optimized statically so as to minimize artifact responses. Unfortunately, the direct responses still exhibit the unwanted artifact response spread into F_1, albeit at lesser intensities as compared to the accordion-HMQC. It would be desirable to obtain direct correlation data that is free of these responses to simplify interpretation. The randomly optimized RDSQC was thus designed to eliminate the artifacts present in the accordion-optimized direct correlation data.

Results.

Various experiments exist for the observation of direct heteronuclear correlations. Both the GHSQC and GHMQC are statically optimized experiments. The accordion-optimized ADSQC contains a variable delay in the reverse INEPT step and is statically optimized in the INEPT step. The accordion-HMQC and the dual-ADSQC are both accordion-optimized experiments with the variable delay in both transfer steps. These latter two experiments afford excellent signal intensity yet suffer in F_1 modulated artifacts. Last, the RDSQC employs random optimization in both transfer steps.

The pulse sequence for the Randomly optimized Direct correlation Single Quantum Coherence (RDSQC) experiment is shown in Figure 1. The delays labeled rd are randomly optimized based on a range of one-bond couplings. They are set to cover a user-defined range such as 120 – 220 Hz (for systems containing both small and large heteronuclear coupling constants). The delays labeled Δ are set to an average of the $^1J_{CH}$ couplings, such as 150 or 160 Hz (for the same systems as described above). This element is used for multiplicity editing and need not be set with

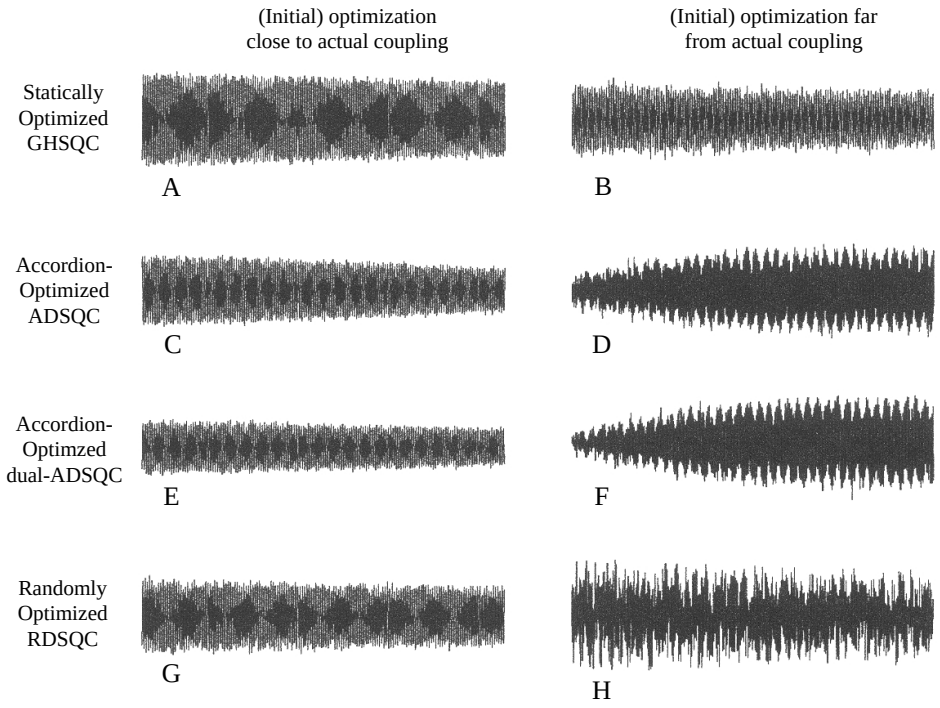


Figure 3. Display of the t_1 interferograms acquired from the different optimization techniques of direct heteronuclear correlation data. [A and B]: Statically optimized GHSQC. [C and D]: Accordion-optimized ADSQC. [E and F]: Dual-ADSQC. This experiment was accordion-optimized in both the INEPT and reverse INEPT delays, similar to the accordion-HMQC [8]. [G and H]: Randomly optimized RDSQC. The left column exhibits the fid response from a methyl group (H-20/C-20) whose $^1J_{CH}$ coupling is close to the actual/initial optimization. The right column contains the fids from a response (H-29/C-29) whose $^1J_{CH}$ coupling is far from the actual/initial optimization. The GHSQC and RDSQC data exhibited similar shaped fids for both responses. The ADSQC and dual-ADSQC fids, however, differ tremendously due to the decrementation of the variable delay away from [C/E] and toward [F/H] the actual coupling. The latter forms an echo-type interferogram, which, upon gaussian apodization, will afford small, negative bands in the F_1 frequency domain. The RDSQC interferograms form a more acceptable and consistent shape, and hence, afford no such artifacts.

Table 1

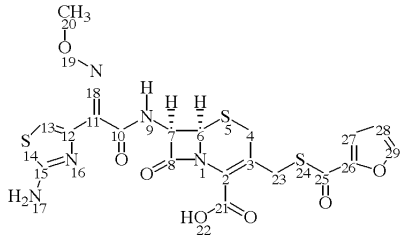
Signal-to-noise data calculated from the traces of the 2D contour plots of the different optimization techniques for direct heteronuclear correlation data. The responses with the strongest intensities have been bolded. Experiments were acquired, processed and plotted with identical parameters. An initial statically optimized direct correlation data (GHSQC) set would normally be acquired with an optimization of 140 Hz. The accordion-optimized ADSQC afforded greater intensity for the more strongly coupled proton-carbon pairs with a minor loss in the aliphatic region. Still better, the dual-ADSQC, with both INEPT and reverse-INEPT transfers optimized with accordion delays, afforded some of the strongest intensities. Unfortunately, this data set exhibited exceptionally strong F_1 modulated responses. The randomly optimized RDSQC, however, afforded good signal-to-noise while exhibiting none of the F_1 modulated artifacts that plague the accordion-optimized data.

Position	Coupling $^1J_{CH}$ (Hz)	GHSQC 140 Hz	ADSQC 120-220 Hz	Dual-ADSQC 120-220 Hz	RDSQC 120-220 Hz
4	130.5	**67.8**	50.7	45.1	49.8
23	144.2	**67.2**	65.4	59.5	61.3
20 O-Me	142.6	502.1	421.3	**512.5**	484.7
6	179.6	74.9	92.4	**117.9**	97.8
7	155.8	76.7	81.6	**86.6**	78.7
13	191.5	43.5	**102.9**	101.4	82.0
28	181.1	78.2	**137.4**	99.8	104.6
27	180.1	85.3	122.7	**167.6**	134.3
29	208.3	--	50.2	**56.7**	48.6

the randomized delay. Setting these delays in a random manner creates significant artifacts through multiplicity editing errors. Optimizing Δ for a static value works well. Alternatively, the 180° ^{1}H pulse and both Δ delays can be removed to acquire the data without multiplicity editing.

Inherently, the RDSQC experiment will simultaneously record better signal intensity for some responses and worse signal intensity for others when compared to a statically optimized experiment such as the GHSQC or GHMQC. This is due to the fact that a range of couplings is being sampled. If a GHSQC experiment is optimized for a value of *X*, and a proton-carbon pair is coupled by the same value, the data will afford a response with strong intensity. However, if a second proton-carbon pair is coupled by a magnitude far different from *X*, a weak or even no response may be observed. The statically optimized data will inherently afford different response intensities for different couplings based on the optimization employed. In contrast, the RDSQC samples all of the desired optimizations in a single experiment affording an average intensity for all responses, which may be better or worse than the static variant for a given response.

The data acquired with the statically optimized GHSQC, the accordion-optimized ADSQC, the dual accordion-optimized ADSQC and the randomly optimized RDSQC produce very different intensities for the responses in ceftiofur, **1**. Table 1 lists the signal-to-noise taken from the F_1 traces for the three



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experiments. The strongest responses for the different experiments have been bolded. The GHSQC experiment afforded considerable signal-to-noise for the smaller $^1J_{CH}$ coupled responses such as the 4 and 23 position methylenes and also the 20-methyl, and a lesser intensity for the more strongly coupled responses, specifically the 29-position furanyl response. This illustrates the direct relationship of the static optimization of 140 Hz for spin systems containing both large and small heteronuclear coupling constants. The correlations for the 13-, 27- and 28-position responses are shown in Figure 2A. The clean correlations are consistent with static optimization.

As compared to the static GHSQC data, the accordion-optimized ADSQC data afforded significant improvement for the larger $^1J_{CH}$ coupled responses (13, 27, 28 and 29). There was also the expected (but still acceptable) decline in intensity for the aliphatic responses. Since the GHSQC was optimized for only a single coupling close to these aliphatic responses, they exhibited strong correlations. The ADSQC, however, sampled an entire range of couplings and averaged the intensities so that all direct correlations were observed with more uniform intensities. The 13-, 27- and 28-position contours for the ADSQC data are shown in Figure 3B. The accordion delays create the negative artifacts modulated in the F_1 domain.

A variation of the ADSQC experiment, the dual-ADSQC utilized accordion-optimization in both the INEPT and reverse INEPT elements. The dual optimization is similar to the accordion-HMQC. This experimental configuration produces, on average, the best signal-to-noise for the direct correlations, but also produces the most intense F_1 artifacts. Six of the strongest responses in **1** were observed in the dual-ADSQC data set as shown in Table 1. Correlations for this data are shown in Figure 2C. The extreme F_1 modulated artifacts can easily be observed in the overlap between the 13- and 28-position responses. Because of the magnitude of the modulation, the ADSQC sequence was originally published with only the reverse INEPT optimized for accordion decrementation. The dual-ADSQC data is only shown here for comparison purposes.

Alternately, the randomly optimized data afforded similar signal-to-noise to the ADSQC for all but one response and significantly better intensity for the more strongly coupled responses than the GHSQC. The intensities are still less than the dual-ADSQC, but the RDSQC data provided virtually no F_1 artifacts. The 13-, 27- and 28-position contours are shown in Figure 2D. This experiment also sampled the same $^1J_{CH}$ ranges as the ADSQC and dual-ADSQC. In short, the RDSQC experiment afforded a cleaner data set than either the ADSQC or the dual-ADSQC experiment thereby simplifying interpretation.

Discussion.

Accordion-optimized direct correlation data such as acquired with the ADSQC (Accordion-optimized Direct correlation Single Quantum Coherence) and accordion-HMQC experiments differ greatly from that of statically optimized data (GHSQC and GHMQC) in that the former produce intense F_1 modulated responses.

A second potential source for the F_1 modulated response could arise from incorrect apodization of the individual t_1 fids. Consider the interferograms in a normal, statically optimized experiment such as a GHSQC. Two very similar scenarios result which are dependent upon the actual $^1J_{CH}$ coupling of the proton-carbon pair and the optimization used to sample the data. The first is when the actual coupling constant and the experimental optimization are close. The resulting t_1 fid (Figure 3A) will start with strong intensity and lessen with minor decay as a function of relaxation. The second scenario arises when the coupling is far from the optimization, resulting with a somewhat weaker intensity to start but also with minor decay due to relaxation (Figure 3B). Both t_1 fids will exhibit a similar shape and can be weighted using a gaussian apodization. The 2D contour *via* a transformed fid of the second scenario is shown in Figure 2A.

The accordion-optimized data will also produce two scenarios. These, however, afford very different results. Unlike the above example, accordion-optimization samples a range of couplings. Here, the first scenario exists when the actual $^1J_{CH}$ coupling is

sampled at the beginning of the accordion range. The t_1 fid (Figure 3C) is now dependent upon both relaxation and also the different coupling being sampled at every increment. As the sampled coupling decrements away from the actual $^1J_{CH}$ coupling constant, the interferogram will decay more rapidly. This is similar to the type of response afforded from the statically optimized experiments but with more decay in intensity. The t_1 fid in Figure 3C is from an accordion-optimized ADSQC experiment where the actual coupling is near the beginning of the accordion range. The more rapid decay is due to the response dependency on the sampled optimization.

The second scenario exists when the actual coupling constant is far from the initial point in the accordion range. This type of interferogram will also exhibit typical relaxation decay; however, there will be little initial signal response as the optimizations in the initial increments will not sample the heteronuclear pair effectively. As the experiment decrements toward the actual coupling the signal response will build in intensity. This will form an echo-type t_1 fid and is shown in Figure 3D. When a gaussian weighting function is employed, this echo-type behavior will produce negative responses spread equidistant in the inverse domain, as shown in the contour plot in Figure 2B.

The dual-ADSQC experiment, similar to the accordion-HMQC experiment, produces the same two scenarios as the ADSQC. The major difference is that the second scenario echo-type fid is more exaggerated. This behavior is shown in Figure 3F. The beginning of the interferogram is smaller and also increases to a larger intensity.

The RDSQC experiment utilizes a randomization function to sample all of the same optimizations as accordion-optimized data, but instead of being decremented, the $^1J_{CH}$ coupling based delays are placed into a random order. Opposite from the accordion-optimized scenarios above, the randomly optimized data will afford only a single scenario. Regardless of the actual coupling of the proton-carbon pair, the random ordering will sample all proton-carbon pairs with similar shaped fids. Examples are shown in Figure 3G and H. Being a random optimization, Figure 3G was obviously not taken from a response whose coupling is close to the initial (or distant) optimization, but is instead taken from the same 20-methyl group used in Figures 3A, C and E. The response in Figure 3H is also from the same correlation as used in Figures 3B, D and F. It is clear that the fids decay in a manner more similar to the statically optimized GHSQC data than the accordion-optimized ADSQC data.

A statically optimized GHSQC data set is normally weighted with a gaussian function in both dimensions prior to transformation. This method affords clean data with no artifactual responses. The interferograms derived with static optimization (Figures 3A and B) obviously can benefit from the gaussian apodization. Alternately, the interferograms arising *via* the accordion-optimization (Figures 3C to 3F) cannot benefit from any single apodization. The application of a gaussian function on an echo-type fid will produce the negative F_1 bands as shown in Figures 2B and C. As a solution, the randomly optimized data provides t_1 fids that are more similar to the statically optimized fids which can easily benefit from a gaussian weighting function while simultaneously producing no artifacts.

EXPERIMENTAL

The data acquired for Table 1 and Figures 2 and 3 utilized a sample of ceftiofur (**1**) for the testing and comparisons of the different optimization techniques. This compound is a semi-synthetic cephalosporin developed for the treatment of bovine respiratory disease, and is a useful example because of the aliphatic, aromatic, and furanyl moieties present. The $^1J_{CH}$ couplings range from 130.5 (position 4) to 208.3 Hz (position 29). The t_1 fids in Figures 3A, C, E and G were taken from the 20-methyl group, and the fids in Figures 3B, D, F and H were taken from the H-29/C-29 furanyl response. These responses were used for the fid display because of the actual $^1J_{CH}$ couplings of the proton-carbon pairs. The methyl group $^1J_{CH}$ was 142.6 Hz and the furanyl response exhibited a $^1J_{CH}$ coupling of 208.3 Hz which were close to the ends of the accordion range employed in the ADSQC and RDSQC experiments. The methyl group coupling was also the closest to the static optimization of 140 Hz that was used in the GHSQC experiment.

The sample of ceftiofur was prepared as a near-saturated solution of **1** dissolved in 150 µL dimethylsulphoxide-d_6 (Isotec, 99.996% D). All NMR experiments were acquired on a Varian INOVA 400 MHz NMR spectrometer, operating at a proton frequency of 399.80 MHz, and equipped with a Nalorac Z•Spec™ MIDG-400-3 gradient micro inverse-detection NMR probe. The 90° pulse lengths were as follows; 6.3 µs at 49 dB (63 dB max) for ^1H, and 11.9 µs at 59 dB (63 dB max) for ^{13}C. RDSQC experimental parameters are given in the caption of Figure 1. The data in Figures 2 and 3 were acquired with 2 transients per 1024 increments and apodized with a gaussian weighting function prior to transformation. Data were acquired with multiplicity editing. The RDSQC experiment functions well with linear prediction, though the ADSQC may in some instances function improperly with linear prediction due to the inconsistent shape of the t_1 fids.

The optimization values were calculated as follows. The step-size between points was determined by $[1/(2*((^1J_{CHmin}-{}^1J_{CHmax})/ni))]$, where $^1J_{CHmin}$ is the smallest coupling in the desired range, $^1J_{CHmax}$ is the largest coupling in the desired range, and ni is the number of experimental increments. This value is then successively subtracted from the starting point of $[1/(2*(^1J_{CHmin})]$. When taken in order, this series is the same as the decrementation of the variable delay in the ADSQC and dual-ADSQC experiments. For the RDSQC, however, the series was then randomized with the C programming command "srand." The srand random generator provides a reproducible (otherwise known as pseudo-random) set of variable numbers based on the array of values supplied to the function. Hence, any series of coupling optimizations could be randomized concentrating the experimental optimizations in the range of actual couplings so as to maximize signal intensity. The randomization function did not produce additional t_1 noise. The RDSQC data are also very reproducible.

REFERENCES AND NOTES

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[10] Static optimization refers to the experimental optimization for only a single coupling per experiment. Accordion-optimization is the integrated sampling of a range of couplings in a decremented/incremented manner. Random optimization is the random ordering of the accordion-optimized delays.

[11] Zangger and Armitage described the F_1 modulated signal spread into the inverse domain in detail. (See reference 8) This heteronuclear triplet response is not the desired information in the RDSQC experiment and has henceforth been labeled an "artifact."