Supplementary Text for
A Suite of Solid-State NMR Experiments to Utilize Orphaned Magnetization for Assignment of Proteins Using Parallel High and Low Gamma Detection

A. Gallo[a, †], W. T. Franks[a, b, †], J. R. Lewandowski[a, *]

[a]Department of Chemistry, University of Warwick, Gibbet Hill Road, CV4 7AL, Coventry, UK

[b]Department of Physics, University of Warwick, Gibbet Hill Road, CV4 7AL, Coventry, UK

[†]Authors have contributed equally

*Corresponding author J.R.Lewandowski@warwick.ac.uk

Nomenclature

All the pulse sequences described in the main text can be downloaded from http://wrap.warwick.ac.uk/116953 or the authors' website. The pulse sequences are named by their intended polarization pathway, where upper-case letters indicate that there is chemical shift evolution for that portion of the pathway, and lower-case letters indicate that there is not. Square brackets indicate simultaneous (or sequential) acquisitions. CA, CB, CO, and Cali refer to the α-carbon, β-carbon, carbonyl, and all aliphatic carbons, respectively. The number of upper-case nuclei indicates the number of dimensions along a particular pathway, where the square brackets indicate a split in polarization pathway. Within the brackets, the order of the listing indicates which direct acquisition occurs first. So, [C,NH] indicates that the ^{13}C is detected first, as in Figure 1a, where [NH,C] indicates that the ^{1}H is detected first, as in Figure 1b. The type of ^{13}C–^{13}C mixing is indicated after the polarization pathway, examples in this work are RFDR, DREAM, and scalar coupling schemes (COSY and TOCSY). All heteronuclear polarization transfers were accomplished via CP in this work. The prefix "war" (for University of Warwick) is used to differentiate these experiments from other similarly

named pulse sequences in our library. The Bruker pulse-program names (pulse durations (p2), power levels (pl2), delays, etc.) are consistent by type with other pulse programs in our library. For example, p10, plw10, plw20, and spnam0 will always refer to an $^{1}H–^{13}C$ CP (as opposed to an $^{1}H–^{15}N$ or $^{15}N–^{13}C$ CP) period for these pulse sequences. Finally, the "MULREC" suffix is added to differentiate these experiments from similar experiments that do not use a second receiver.

For example, the pulse program "war.hCANH" is expanded into "war.hCA[C,NH]_RFDR_AFTER" indicating that this a 3D that encodes the $^{13}C^{\alpha}$, ^{15}N, and $^{1}H^{N}$ chemical shifts AND also a 2D $C^{\alpha}–CX$ experiment. The initial ^{1}H excitation is not allowed to evolve. If the experiment were "war.hCA[NH,C]_RFDR_MULREC", then the ^{13}C mixing and the ^{13}C acquisition would have been saved until the hCANH 3D had been finished.

The transition from the single receiver experiment into the dual receiver experiment is straightforward. One takes the (optimized) 3D experiment, and changes the pulse sequence name into the [3,2]D version of the experiment. In the acquisition parameters (accessed with the command 'eda') there is an "M" button. This button will create the parent and child(ren) datasets. The variable array "mulexpno" is populated by this button, and the child datasets are created. For the parent experiment, mulexpno[0] is -1, and mulexpno[1(,2,3…n)] is the "EXPNO" for the child experiment(s). The parent dataset is the only place that the numbers matter, but for good lab practice and bookkeeping we set the child experiment's mulexpno[0] to the parent experiment. For the parent 3D experiment, the sweep width for the indirect ^{13}C dimension may need to be wider depending on the 2D experiment being acquired, but all other acquisition parameters are the same. In the child experiment, the routing (command "edasp") is changed so that the ^{13}C channel is the acquisition (f1) channel, and the second receiver routing is removed. The direct dimension acquisition is set for a $^{13}C–^{13}C$ 2D (*i.e.* 400 ppm, ~30 ms acquisition). The acquisition dimensions for the child are still set so that it appears to be a

3D. The experiment will not run properly when the child is defined as a 2D. All indirect dimensions are set to be ^{13}C with the SW of the carbon dimension in the parent dataset. The F2 (middle) acquisition should be set to 1 point, and F1 (right-most) is set to the same number of points as the ^{13}C dimension of the parent. These parameters are to avoid confusion during processing, and to avoid the need to redefine the acquisition parameters after acquisition. For processing, the 3D processing is standard, but the 2D dataset needs to be changed into a 2D processing buffer, which can be done with a standard 2D processing command. Despite the recommendations above, the F2 dimension may not be interpreted by Topspin properly, so the command "s td" is used to correct the acquisition parameters to match the suggestions above (changing the middle dimension to be 1 may be needed).

Before acquiring a dataset, we found it necessary to run the command "ii restart" in the parent experiment. This ensured that the child acquisition parameters were communicated back to the parent dataset. Without this step it sometimes the time allocation became garbled between the two experiments, and then decoupling during the child experiment acquisition became very long. Running "ii restart" before acquisition resolved the timing problems.