

Supplementary Material: A Comparison of NCO and NCA Transfer Methods for Biological Solid-State NMR Spectroscopy

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1. Anisotropy and Asymmetry

Simulations demonstrating that all three transfer methods (adiabatic DCP, ^{OC}DCP, and EXPORT) behave reasonably with respect to anisotropy and asymmetry are shown in Figure 1. For reasonable tensor values, the transfer efficiency rarely varies by more than 10%.

2. Two-dimensional NCA and NCO spectra

Two-dimensional NCA and NCO spectra acquired using the [U-¹³C, U-¹⁵N] SH3 sample using adiabatic DCP and ^{OC}DCP are shown in Figure 2. This figure illustrates that the spectra acquired using the two different transfer methods are substantially the same, as would be expected based on the similar transfer efficiencies measured from 1D spectra. The spectra were acquired using a relatively old sample, so the peaks are not as well-resolved as previously published SH3 spectra.

3. Scripts

Included with this supplementary material are a series of files for generating and testing ^{OC}DCP pulses using SIMPSON. Each script file is executed using the command:

```
simpson script.in pulse_length
```

where `script.in` is the name of the input script and `pulse_length` is the desired length of the ^{OC}DCP pulse. Inside each script file are several parameters that should be set before execution; the most important of

these are the magic-angle-spinning frequency, the magnetic field (specified as the ¹H frequency), and the number of pulses to be generated at each stage. The default values for other variables should be adequate for most situations. As later scripts use the output of previous scripts, all scripts should be executed from within the same directory. In addition, the RF inhomogeneity file needs to be in the same directory.

Each script creates a subdirectory for its output. The individual shape files that are generated are labeled as `NCA/NCO-spinning frequency (Hz)-pulse length (in μ s)-nucleus-theoretical transfer efficiency.dat`. The theoretical transfer efficiency is used to distinguish different pulses. Although it is not guaranteed to be unique, it is precise enough (10 to 12 digits) that it functions reasonably well as a serial number.

3.1. General Scripts

`gauss8p` : RF inhomogeneity file. Needed for later stages of optimization and testing.

`shape2bruker.all` : TCL script to convert SIMPSON shape files (with amplitudes in Hz) into Bruker format (with amplitudes in percent of some maximum value). Executed as `"tclsh shape2bruker.all pulse_length ?<maxrf>?"` where `maxrf` is an optional parameter that can be used to ensure that all pulses are scaled to the same RF amplitude value. This script generates a new directory (either `Bruker-NCA-pulse_length` and/or `Bruker-NCO-pulse_length`) containing the output files.

3.2. NCA ^{OC}DCP Design

`NCA-OC1.in` : Initial script for generating NCA ^{OC}DCP pulses. A narrow band optimization for

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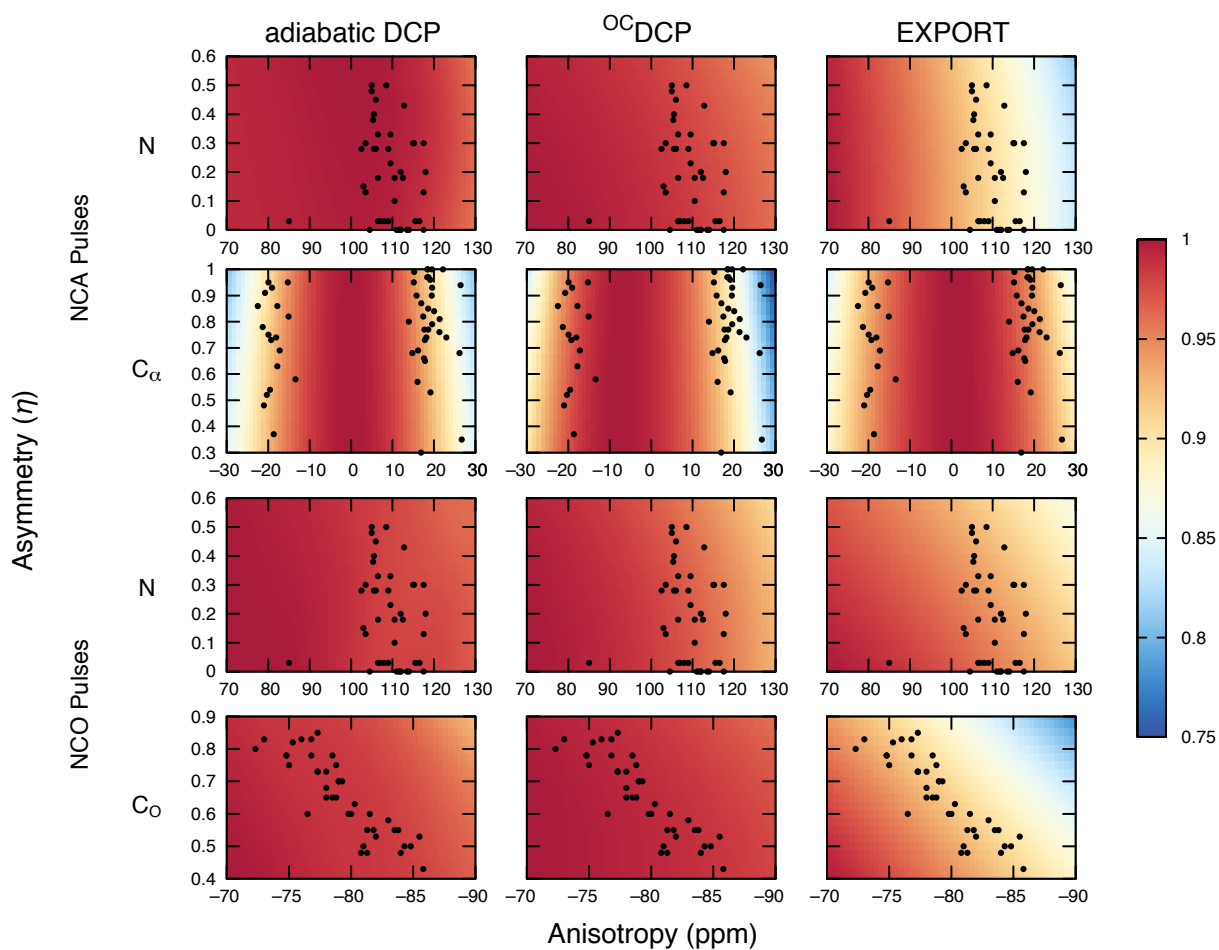


Figure 1: Simulated NCA and NCO normalized transfer efficiencies for adiabatic DCP, ^{OC}DCP , and EXPORT as a function of chemical shift anisotropy and asymmetry (η) at 700 MHz for 1H and with $\nu_r=14$ kHz. The simulations use a ^{13}C - ^{15}N two-spin system and a 8% gaussian RF inhomogeneity profile. The transfer efficiencies are normalized such that, for each graph, the maximum transfer efficiency is set to 1.0. The points on the graphs are representative anisotropy and asymmetry values as measured for the 56 residue $\beta 1$ immunoglobulin binding domain of protein G (GB1)^[1,2].

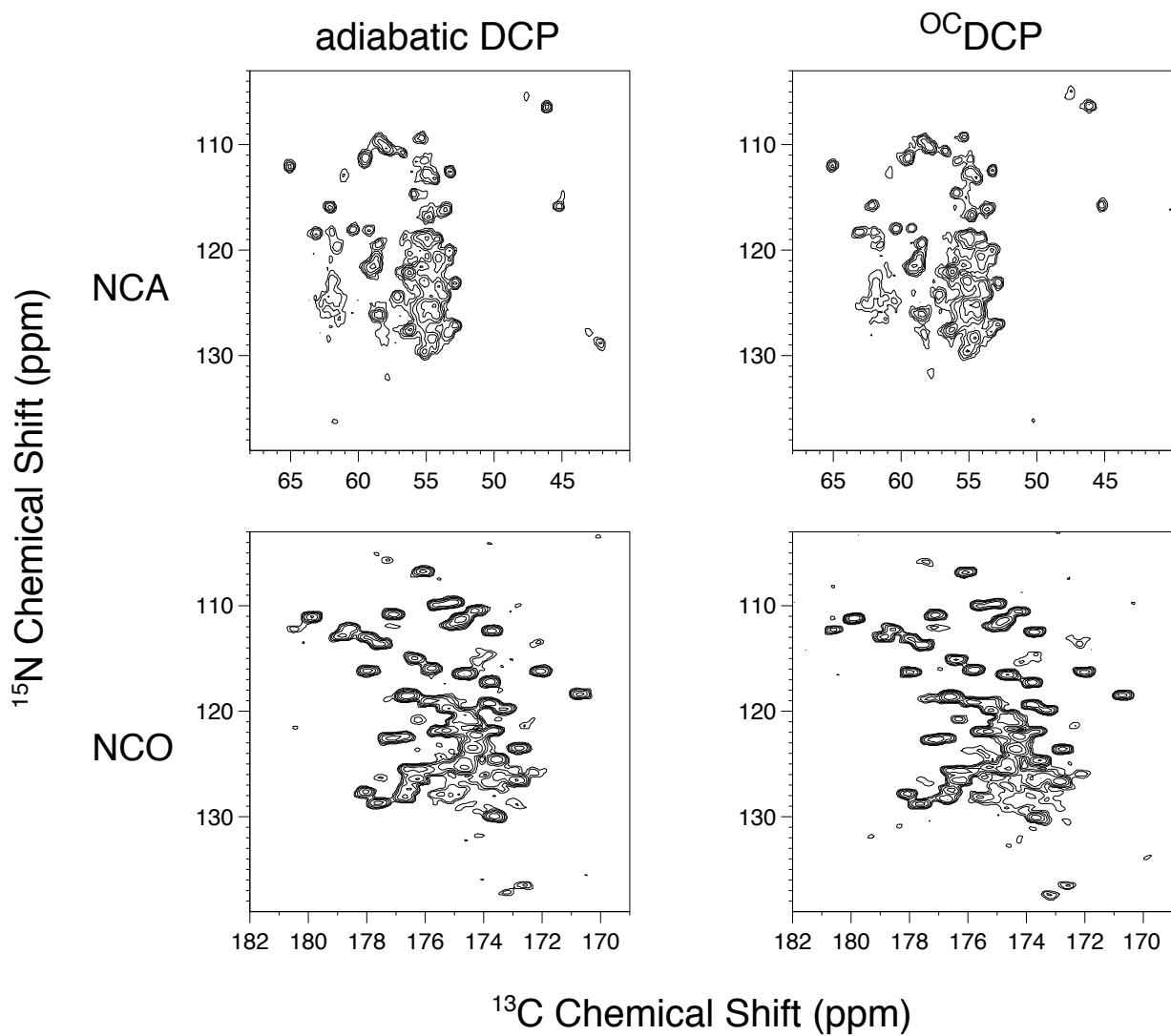


Figure 2: Two-dimensional NCA and NCO spectra of $[\text{U-}^{13}\text{C}, \text{U-}^{15}\text{N}]$ SH3 sample acquired using adiabatic DCP and ^{13}C DCP at 700 MHz for ^1H and with $\nu_r=14$ kHz. 192 increments were acquired in the indirect dimension with 16 scans per increment. Each experiment took approximately 2.5 hr to acquire.

^{15}N to ^{13}C transfer.

`NCA-OC2.in` : Uses output from previous script. Adds chemical shift offsets to ensure transfer over the desired chemical shift range.

`NCA-OC3.in` : Uses output from previous script. Adds an RF inhomogeneity profile.

`NCA-OC4.in` : Uses output from previous script. Adds loops over CSA values and asymmetry values.

`NCA-test.in` : Uses output from previous script. Used to test pulse performance.

3.3. NCO ^{OC}DCP Design

`NCO-OC1.in` : Initial script for generating NCO ^{OC}DCP pulses. A narrow band optimization for ^{15}N to ^{13}C transfer.

`NCO-OC2.in` : Uses output from previous script. Adds chemical shift offsets to ensure transfer over the desired chemical shift range.

`NCO-OC3.in` : Uses output from previous script. Adds an RF inhomogeneity profile.

`NCO-test.in` : Uses output from previous script. Used to simulate pulse performance.

4. Pulses

All pulses are designed to be applied with the same phase as a DCP pulse. For each pulse, there will be two shape files (one for the ^{15}N channel and one for the ^{13}C channel). The pulses are provided in separate directories for 600 and 700 MHz spectrometers.

4.1. Adiabatic DCP

The SIMPSON format shape files for the adiabatic DCP pulses are labeled as `AdiabaticDCP-spinning frequency (Hz)-N/CA/CO-ratio.dat`. The ratio indicates the ratio of the average RF amplitude to the spinning frequency (so, for $\nu_r=14$ kHz, a value of 3.5 corresponds to an average amplitude of 49 kHz). For Bruker format shapes (indicated by the `.bru` suffix) only a single file is provided for each spin (N, C_α , C_O). This is because in the Bruker format amplitudes are given as percentages and, consequently, it is not necessary to have separate files for shapes that only differ in amplitude.

The adiabatic shapes were generated using the Bruker ShapeTool. A rectangular shape was used for the N channel. For the C channel, the shape used was

“TangAmplitudeMod” with delta MAS set to 7000 Hz. For the C_α pulse, the dipolar coupling was set to 800 Hz, the RF field at match to 21000 Hz, and the Amplitude Scaling Factor to 50%. For the C_O pulse, the dipolar coupling was set to 1000 Hz, the RF field at match to 49000 Hz, and the Amplitude Scaling Factor to 85%.

4.2. ^{OC}DCP

The ^{OC}DCP shape files are labeled as `NCA/NCO-spinning frequency (Hz) - pulse length (in μs)-nucleus-theoretical transfer efficiency`.

The shape files are provided in SIMPSON format (indicated by the `.dat` suffix) and Bruker format (indicated by the `.bru` suffix).

The Bruker shapes have the same maximum RF amplitude (either 50 kHz or, for the “low-power” pulses, 25 kHz). To setup the pulses, first calibrate 50 kHz or 25 kHz RF fields for the ^{15}N and ^{13}C channels (i.e., 5 and 10 μs 90° pulses). Ideally, the ^{OC}DCP pulses should work with these power levels, but in practice it is highly recommended that you then further optimize the power levels of both channels for each pulse.

4.3. EXPORT

The EXPORT shape files are labeled as `EXPORT-spinning frequency (Hz) - pulse length (in μs)-nucleus-C`. The overall pulse length will be some multiple of 0.35 μs (as required on our spectrometers). The pulse length will be the length of two rotor periods (or as close as possible). The shape files are provided in SIMPSON format (indicated by the `.dat` suffix) and Bruker format (indicated by the `.bru` suffix).

References

- [1] B. J. Wylie, W. T. Franks, D. T. Graesser, C. M. Rienstra, Site-specific ^{13}C chemical shift anisotropy measurements in a uniformly ^{15}N , ^{13}C -labeled microcrystalline protein by 3D magic-angle spinning NMR spectroscopy, *J. Am. Chem. Soc.* 127 (2005) 11946–11947.
- [2] B. J. Wylie, L. J. Sperling, H. L. Frericks, G. J. Shah, W. T. Franks, C. M. Rienstra, Chemical-shift anisotropy measurements of amide and carbonyl resonances in a microcrystalline protein with slow magic-angle spinning NMR spectroscopy, *J. Am. Chem. Soc.* 129 (2007) 5318–5319. PMID: 17425317.