Fast Pure Shift NMR at Maximum Resolution

"Semi-real-time acquisition for fast pure shift NMR at maximum resolution". Peter Kiraly, Mathias Nilsson and Gareth A. Morris, J. Magn. Reson. **293**, 19-27 (2018). https://doi.org/10.1016/j.jmr.2018.05.012.

This manual describes the use of the band-selective semi-real-time pure shift NMR method, in which a passive spin network is inverted either by multi-frequency RSNOB pulses or by a WATERGATE-like pulse sandwich. The former is referred as srtACQn, and the latter as srtACQw. srtACQn requires prior knowledge of the passive spin frequencies, but delivers better resolution. Screen captures for the key steps are given after the list of instructions below. The package requires the WaveMaker software – available from the Bruker User Library, and part of the standard TopSpin 4.0.5 installation.

The pulse programs are provided with sensible default parameters, so the user can skip steps 4 to 8 of the protocol below in most applications. The details described in Fig.1 and in steps 4 to 8 are included to allow expert users more freedom. As an alternative to the protocol suggested below, one can follow the more typical procedure of reading in a parameter set for a semi-real-time experiment using `rpar`, and use `paracon` to adapt the parameter set to the local spectrometer configuration.

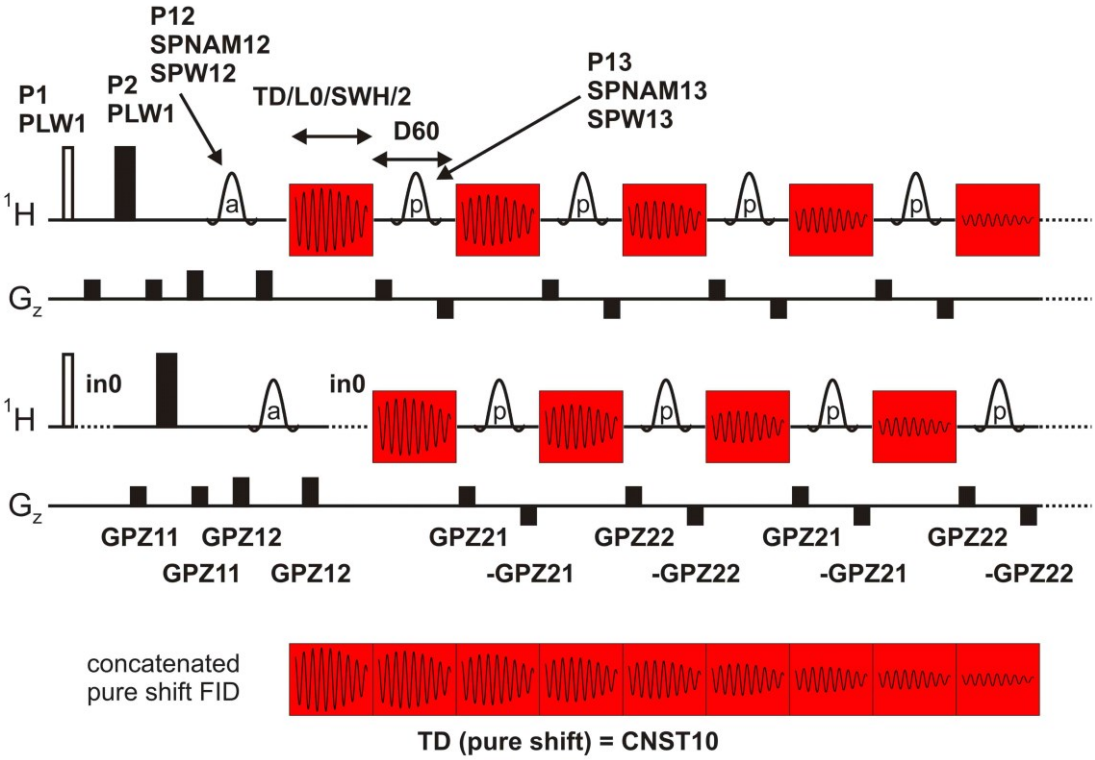

Figure 1 Pulse sequence diagram including TopSpin parameters. Shaped pulse symbols refer to selective 180° pulses applied to the observed, active (a) or to the decoupled, passive (p) spins. The passive spin inversion block is either a multi-frequency RSNOB pulse (srtACQn), or a soft 90° hard 180° soft 90° pulse sandwich (srtACQw). A pure shift "FID" is made by interleaving chunks of data acquired in two separate acquisitions, each of L0 chunks. The chunks to be interleaved are of duration 1/SWH (F1), where SWH (F1) is the spectral width in the indirect dimension of a 2D acquisition of just two increments (i.e. TD (F1) = 2). SWH (F1) should be large compared to the maximum value of J, and greater than the width of the widest multiplet to be decoupled. As with other pure shift methods that acquire data in chunks, SWH (F2) must be an integer multiple of SWH (F1). In each increment L0 chunks of data lasting slightly longer than 1/SWH (F1) are acquired, in order to allow some overlap between the data chunks in the two acquisitions. This is so that the first CNST11 and last CNST12 (both even numbers) data points of each chunk can be discarded in the data processing, to minimise problems caused by the digital signal processing. The final pure shift "FID" therefore contains CNST10 = 4 L0 SWH (F2)/ SWH (F1) data points; this will be the value of TD in the experiment where the FID is placed. Each data chunk that is acquired consists of CNST11 + CNST12 + 2 SWH (F2)/ SWH (F1) data points, so in each of the two increments acquired TD (F2) = L0 (CNST11 + CNST12 + 2 SWH (F2)/ SWH (F1)) and AQ (F2) = L0 [(CNST11 + CNST12)/(2 SWH (F2))+1/ SWH (F1)), where TD (F2) should be a power of 2 that is at least 256, and L0 should be an integer greater than 4. The delay D60 seconds is equal to 1/SWH (F1) – (CNST11+CNST12)/SWH (F2), and must be sufficient to accommodate the chosen passive spin inversion element (i.e. the value of TAUr calculated by the pulse program should be greater than zero).

All these parameters are set to sensible initial values by the relevant setup au_macro, but can be adjusted by the user bearing in mind the restrictions listed.

Experiment setup:

1. Chose a region of the ^{1}H NMR spectrum to be detected (active region). There should not be any protons coupled to each other within this region. Set O1P to be the centre of the active region, and note the width of the desired active region in Hz. Make allowance for a transition region between the active and passive regions.
2. Acquire a conventional proton spectrum to be used for setup [ns=1 and ds=0 is sufficient].
3. With this proton spectrum as the active dataset, run the appropriate setup au-macro, `kp_set_srtACQn_r1` or `kp_set_srtACQw_r1`, from the command line.
4. If srtACQn is selected (to get the maximum resolution) then make a list of the chemical shifts of the passive spins. A selective 1D COSY experiment can be used, e.g. by using the standard TopSpin pulse program selcogp. The setup au-macro will prompt for user input. The user can call the au-macro `kp_setPL_r1` directly if the frequency list needs to be redefined.

   The bandwidth of each region containing passive spins is defined by CNST51. Sufficient gap should be left between the chosen frequency regions to avoid interference between them.

   If srtACQw is selected, then the user just needs to select the duration of the rectangular soft 90° pulse, which excites the active spins only. CNST51 is set to the inverse of the soft 90° pulse duration, which is a measure of its useful bandwidth.
5. Define the bandwidth of the active spin region, CNST50 (see documentation of WaveMaker for the definition of bandwidth of an RSNOB pulse).
6. Use the command `wvm -q` to create the desired pulse waveforms and update the relevant parameters in the dataset. The command `wvm -b` is then advisable, to check the inversion profile of the multi-frequency pulse waveform generated. Note that the TopSpin Bloch simulator is very useful, but does not always read the waveform parameters correctly. Set the “first offset” (e.g. -5000) and “end of calculated offset range” (e.g. +5000) to generate a display including all the selected frequency regions. The power and duration of the shape pulse (SP0 and P0) may also need to be set manually. Set the number of offsets calculated to be at least 500.

   There should be a zero excitation region in the middle of the excitation profile which

is as wide as the active spin region required. Each inversion region will have a mirror image on the opposite side of the centre of the spectrum, for Bloch-Siegert shift compensation; if there is unwanted overlap, adjust the region definitions.

7. Define the time interval between data acquisition blocks by setting D60 in seconds. This must be sufficient accommodate the passive spin inversion element, the gradient pulses (P17, D17) around it, and the CNST11+CNST12 data points that will be discarded during processing (see figure caption above). The user is warned if the gap is too small.
8. The parameters selected by the setup au-macro should work for most cases, but the user can customise the relevant parameters to get the best results for a given sample, as explained at Figure 7 below.
9. Run the experiment.
10. After the experiment has been completed, process the raw data using the au-macro `kp_srtACQproc_r1` (this is the same for both srtACQn and srtACQw experiments). The pure shift FID will be generated in the current experiment number plus 1000.
11. Process the 1D pure shift FID as normal; forward linear prediction can be useful to improve the digital resolution.

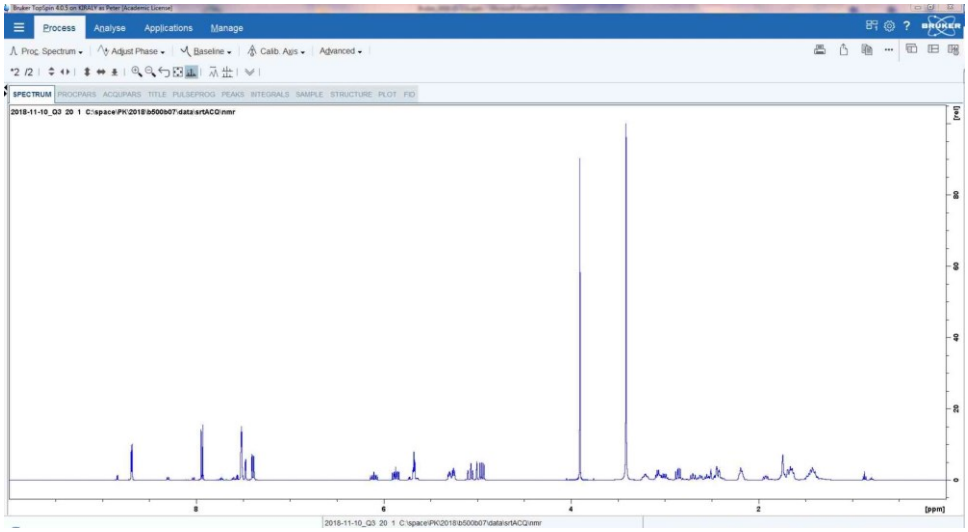

Figure 2 Conventional ^{1}H NMR spectrum, where O1P has been set to the middle of the desired active spin region of the pure shift experiment.

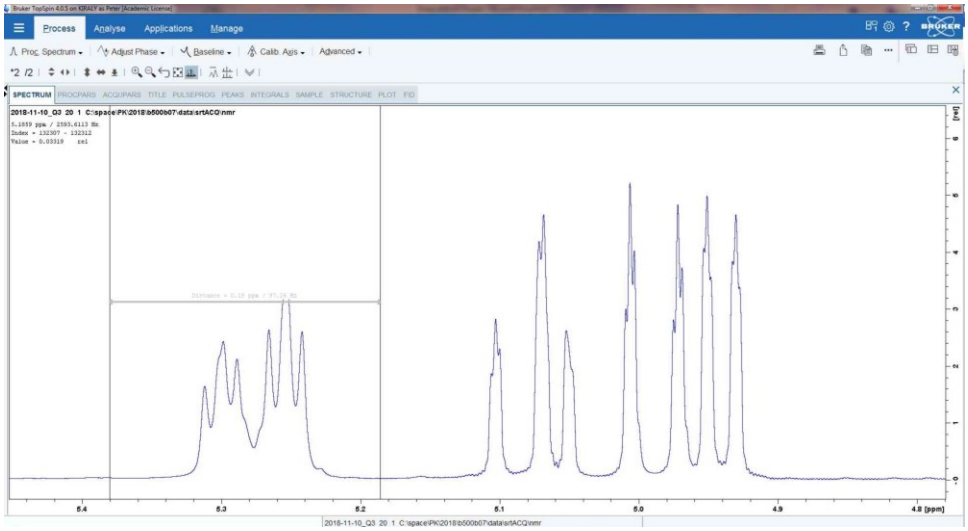

Figure 3 Zoom showing the overlapping multiplets in the active spin region. For this example a 50-100 Hz RSNOB pulse is suitable for selecting the region between the cursors.

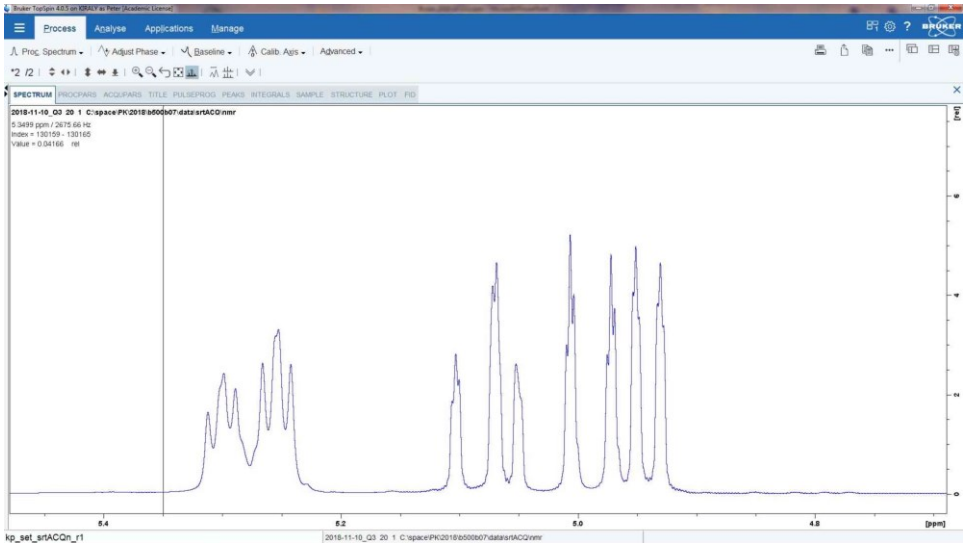

Figure 4 Call the experiment setup au-macro `kp_set_srtACQn_r1` to convert the parameter set to semi-real-time pure shift NMR, using multi-frequency RSNOB pulses for passive spin inversion. All parameters will be set to sensible initial values, but the chemical shifts of the passive spins need to be defined by answering the popup questions.

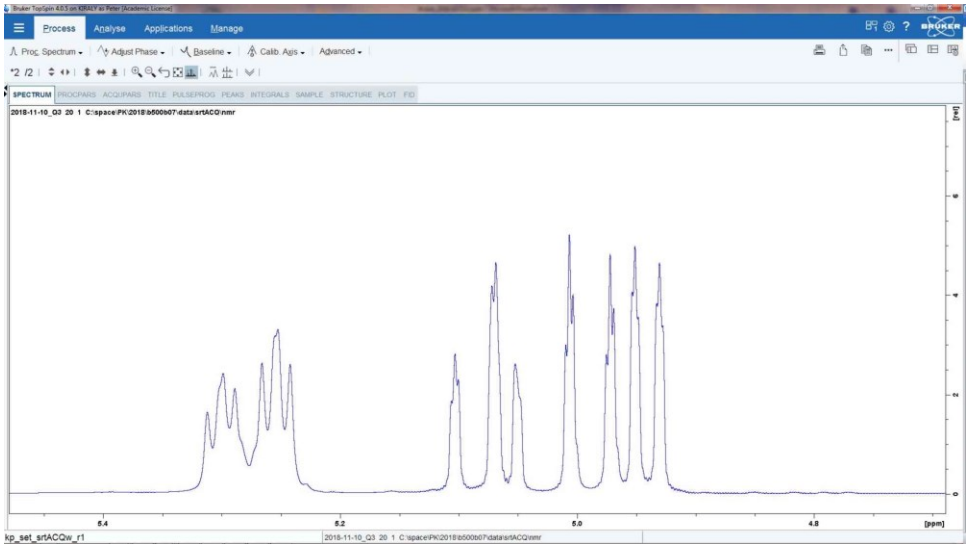

Figure 5 Call the experiment setup au-macro `kp_set_srtACQw_r1` to convert the parameter set to semi-real-time pure shift NMR, using WATERGATE-like passive spin inversion. All parameters will be set to sensible initial values.

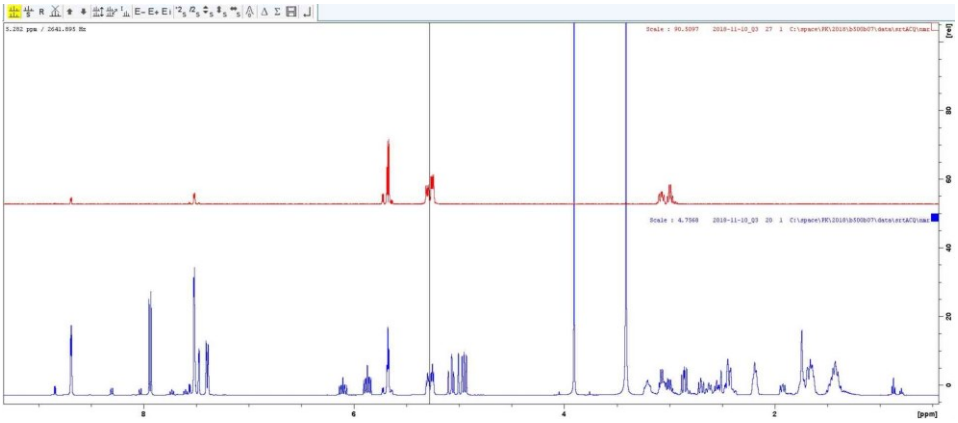

Figure 6 Example showing the results of a selective 1D COSY experiment (red) above the conventional proton spectrum (blue). The cursor is set to the selected region (active spins for the pure shift experiment), and all the other peaks are caused by coherence transfer via *J*-coupling to the spins of the selected region. The small intensity peaks in the aromatic region may correspond to protons with very small couplings, which are not resolved in the conventional proton spectrum. Such peaks may be ignored, because there is no benefit from decoupling unresolved couplings. In this example, two passive spin regions were accordingly identified, at 3.02 ppm and 5.68 ppm.

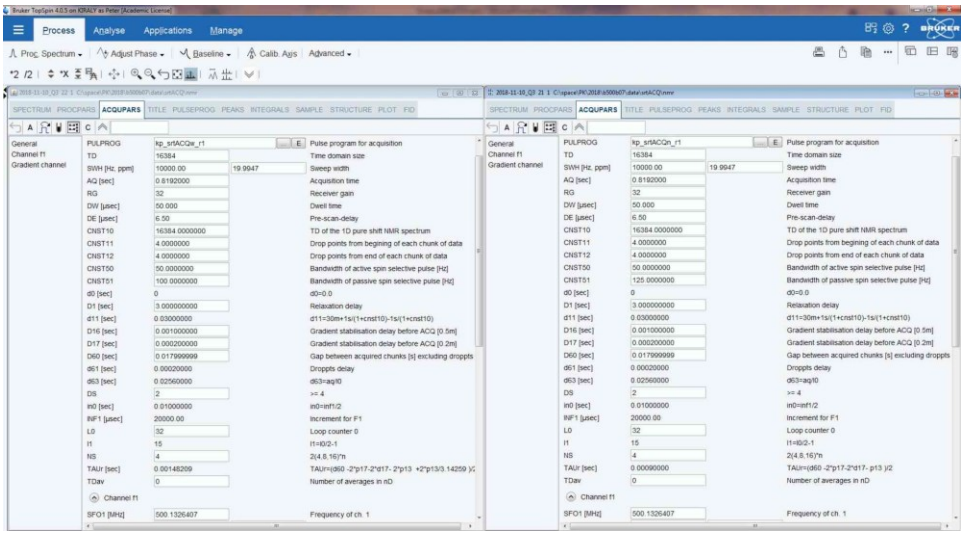

Figure 7 Display of acquisition parameters via ASED. The experiment on the left corresponds to srtACQw, that on the right to srtACQn. The setup au-macro select parameters that may be suitable for most examples, but as generally in pure shift experiments one may need to change experimental parameters to get the best results for a given sample.

Customising the parameters in ASED:

As explained in the caption to Figure 1, the pure shift "FID" is made by interleaving chunks of data acquired in two separate acquisitions, each of L0 chunks. The chunks to be interleaved are of duration 1/SWH (F1), where SWH (F1) is the spectral width in the indirect dimension of a 2D acquisition of just two increments (i.e. TD (F1) = 2). SWH (F1) should be large compared to the maximum value of J, and greater than the width of the widest multiplet to be decoupled. As with other pure shift methods that acquire data in chunks, SWH (F2) must be an integer multiple of SWH (F1). In each increment L0 chunks of data lasting slightly longer than 1/SWH (F1) are acquired, in order to allow some overlap between the data chunks in the two acquisitions. This is so that the first CNST11 and last CNST12 (both even numbers) data points of each chunk can be discarded in the data processing, to minimise problems caused by the digital signal processing. The final pure shift "FID" therefore contains CNST10 = 4 L0 SWH (F2)/ SWH (F1) data points; this will be the value of TD in the experiment where the FID is placed.. Each data chunk that is acquired consists of CNST11 + CNST12 + 2 SWH (F2)/ SWH (F1) data

points, so in each of the two increments acquired TD (F2) = L0 (CNST11 + CNST12 + 2 SWH (F2)/ SWH (F1)) and AQ (F2) = L0 [(CNST11 + CNST12)/(2 SWH (F2))+1/ SWH (F1)), where TD (F2) should be a power of 2 that is at least 256, and L0 should be an integer greater than 4. The delay D60 seconds is equal to 1/SWH (F1) – (CNST11+CNST12)/SWH (F2), and must be sufficient to accommodate the chosen passive spin inversion element (i.e. the value of TAUr calculated by the pulse program should be greater than zero). The value of the parameter CNST10, which determines the TD of the pure shift "FID", can be altered after acquisition if required; it will be a little less than twice the TD (F2) of the raw data. The parameters SWH (F1), CNST11 and CNST12 should be set after the inversion waveform has been calculated, to make sure that D60 is long enough (*i.e*. that the TAUr calculated by the pulse program is greater than zero).

CNST11 and CNST12 must be even integers; if set to too small a value, they will cause the digital signal processing to introduce slight discontinuities at the boundaries between data chunks, which give rise to sharp sidebands at a spacing SWH (F1) in the resultant spectrum.

The active spin selectivity depends on a soft 180° pulse defined by the parameters P12, SPNAM12 and SPW12. The selectivity of the passive spin inversion element is defined by the parameters P13, SPNAM13 and SPW13. When using srtACQw, the duration of the passive spin inversion element is P13+P2+P13 because it consists of a soft 90° - hard 180° - soft 90° pulse sandwich. The pulse programs are compatible with using the user-friendly WaveMaker program to create the waveforms and update experiment parameters. The appropriate user-controlled parameters are: 1) CNST50, the bandwidth of the active spin selection RSNOB pulse; 2) CNST51 (for srtACQw), the inverse of the soft 90° pulse duration (i.e. 4 times the RF amplitude of the rectangular soft pulse), which is proportional to the useful null excitation region in the srtACQw experiment; and 3) CNST51 (for srtACQn), the bandwidth of each of the RSNOB pulses applied to the user-defined list of passive spin frequencies. This list should be updated prior to creating the passive spin inversion waveform for a srtACQn experiment. The au-macro kp_setPL_r1 prompts for user input to define the number of passive spins, and the chemical shift of each in ppm.

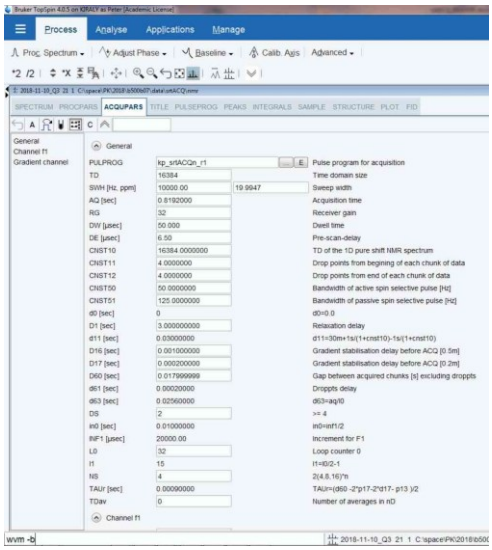

Figure 8 The command `wvm -b` can be used to simulate the excitation profile of the selected passive spin inversion pulse, after creating the shapes with `wvm -q`.

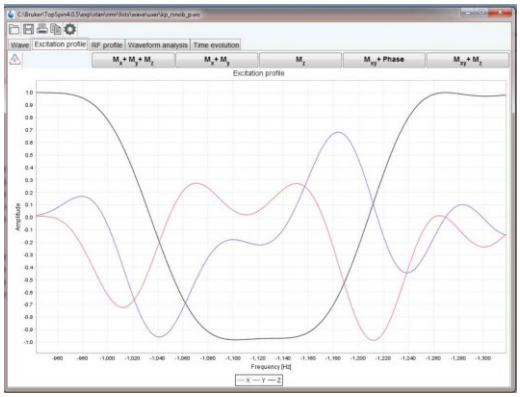

Figure 9 The popup window automatically shows the relevant data for most shapes but sometimes, as here, it fails to select appropriate parameters for the shape. Click on the button just below the Wave tab to manually set the parameters.

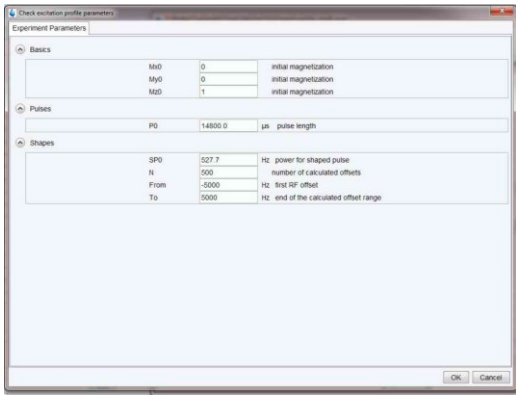

Figure 10 In the next popup window the most important parameters to set are those at the bottom, defining the frequency range to be calculated . Set values for “From” and “To” which cover the relevant proton spectrum. The number of points to calculate (N) can be increased for better digital resolution of the excitation profile (it may be useful to calculate just a smaller region with high resolution). The pulse duration (P0) and power (SP0) should be set automatically, but if not they should be corrected by reading the waveform file content with a suitable text editor. Click OK once all parameters are correct.

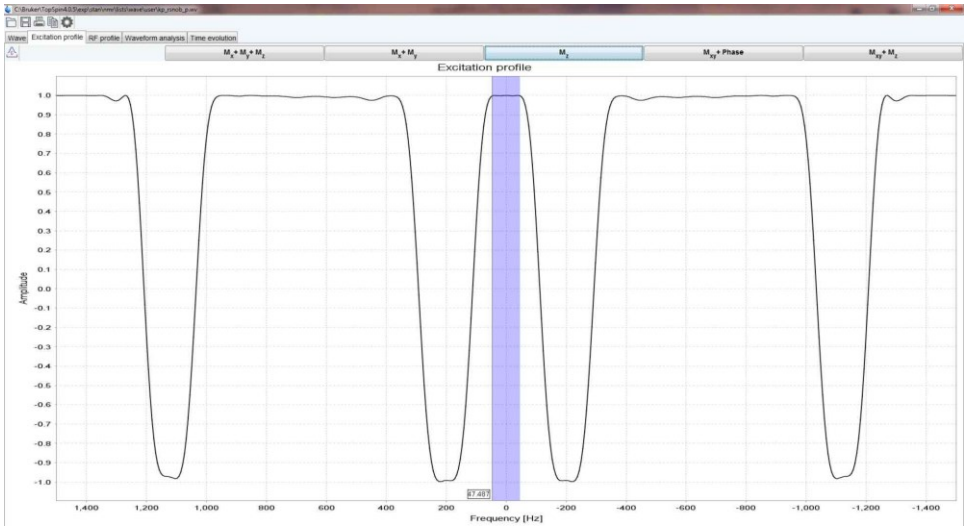

Figure 11 The excitation profile in this example shows four inversion regions, corresponding to the two user-defined chemical shifts and their mirror images with respect to O1P. There has to be a suitable null-excitation region in the middle (highlighted in the figure), to avoid disturbance of the active spin chemical evolution. Likewise, the inversion regions should not overlap; if they do, their bandwidths/positions need to be adjusted.

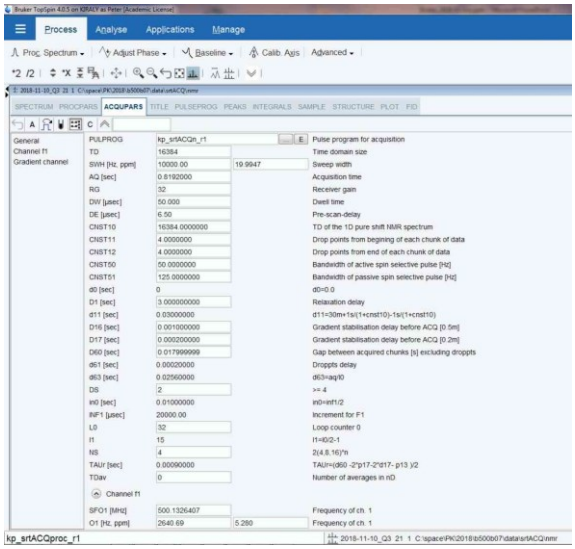

Figure 12 After acquisition of the data has been completed, the data need to be processed using the au-macro `kp_srtACQproc_r1`. The pure shift NMR data will be stored as a conventional 1D experiment in a new experiment (the current number plus 1000).

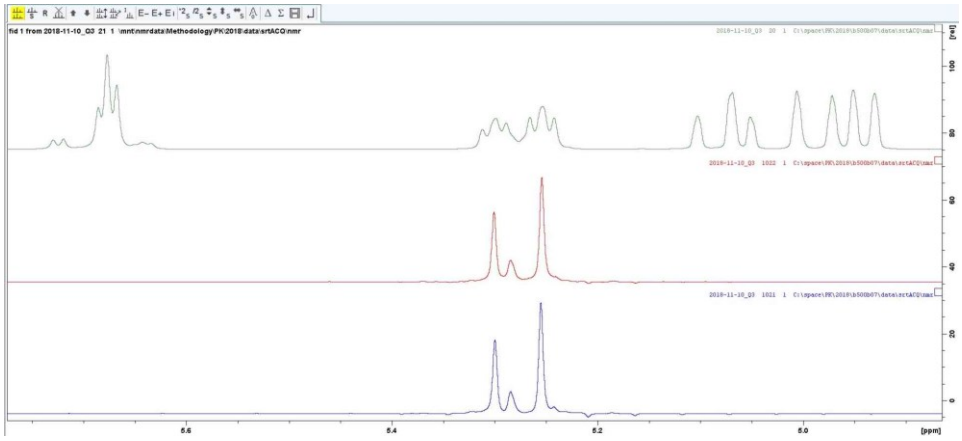

Figure 13 Example showing a conventional proton spectrum (top, green), semi-real-time pure shift spectrum using WATERGATE-like passive spin inversion (middle, red), and semi-real-time pure shift spectrum using multi-frequency RSNOB pulses (bottom, blue). The overlapping signals of the three main component of the mixture are easy to identify in the pure shift spectra. The best resolution has been obtained in the bottom spectrum, in which one can also identify the signal of a minor impurity just next to the most intense peak. The sample is a mixture of quinine (100 mM), quinidine (73 mM), and cinchonidine (13 mM) in dmso-d_6.