

# Automation in Solid-State NMR

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## Introduction

Automation reduces the workload of spectroscopists, protects the spectrometer hardware and permits bench chemists and molecular biologists to do routine NMR work without requiring experts for standard analytical work. Solid-state NMR (ssNMR) has become one of the premier analytical methods to study any condensed material that is not a fluid, i.e., low-viscosity liquid. To study such structures by ssNMR spectroscopy, a plethora of established experiments are available for interrogating atomic and molecular interaction Hamiltonians. Many of these experiments are straightforward routine methods where automation reduces the workload. For more complicated experiments, automation enables fast and easy setup and increases reliability. For more complicated experiments, automation enables fast and easy setup and increases reliability.

Significant progress has been made in the hardware technology of the recent spectrometer generation affording highest stability of rf-hardware. From frequency, phase and amplitude stability and low-ns amplitude phase and frequency settling times of linear transmitters, to linear receivers, modern instruments provide a sound basis for reproducible state-of-the-art ssNMR spectroscopy.

Recent advances are sample shuttle systems introduced by Bruker with its sample changer SampleCase™, as well as Jeol with its sample changer, called ASC 30, which utilize the existing liquids sample changers for ssNMR in mixed-use NMR spectrometers, employing a MAS Shuttle for 4mm and 3.2mm MAS rotors.

Here, we present a broader approach to both, full- and semi-automation, for solid state NMR experiments which only requires that the spectrometer is well setup. That means that the values for rf-powers required for standard rf-pulses on all rf-channels for the available ssNMR probes are stored in a global lookup table.

We consider full experiment automation as the process in which a ssNMR CPMAS experiment is executed successfully without the need of manual parameter optimization. This process entails loading the sample into the probe, spinning it up to the desired MAS-frequency, matching and tuning the probe, and executing the experiment. Full automation does not require optimization procedures for regular solid state NMR CPMAS experiments using spin ½ nuclei and MAS frequencies below 20kHz while achieving >90% of the optimal signal to noise.

## Method

For rotating solids, the HH CPMAS matching condition depends on the mechanical sample rotation frequency [3]. The matching condition for the two spinlock fields requires that the difference of these fields is equal to integer multiples of the MAS frequency for zero quantum cross polarization:

$$\frac{\gamma_1}{2n} B_{11} - \frac{\gamma_2}{2n} B_{12} = n f_{rot} \quad (1)$$

With  $n \in \mathbb{N}$ , but practically,  $n = \pm 1, \pm 2, n=0$  is a special condition called second order CP [29]. For double quantum cross polarization, the sum of the rf fields of each participating nucleus equals an integer multiple of the rotation frequency,

$$\frac{\gamma_1}{2n} B_{11} + \frac{\gamma_2}{2n} B_{12} = n f_{rot} \quad (2)$$

with  $n = \pm 1, \pm 2, f_{rot}$  is the MAS frequency, and  $\frac{\gamma_1}{2n} B_{11}$  and  $\frac{\gamma_2}{2n} B_{12}$  are called the spin nutation frequencies, or rf-fields or spinlock-fields of the two nuclei irradiated, with  $\gamma_{1/2}$  the gyromagnetic ratio for  $i$  and  $s$  nuclei, respectively. Practically, for MAS frequencies below 20 kHz, the HH CPMAS condition is determined by the probe specification for the maximum rf-field for the spinlock pulse for a given low  $\gamma$  nucleus, with

$$f_{nut,s} = cnst50 \quad (2a)$$

and therefore, the HH match on <sup>1</sup>H or <sup>19</sup>F is given as

$$f_{nut,i} = cnst50 \mp f_{rot} \quad (2b)$$

for the spins  $i$  and  $s$ . For advanced purposes, the spinlock pulse amplitude is defined relative to the rotation rate  $cnst31$ ,

$$f_{nut,s} = \alpha cnst31 \quad (2c)$$

and accordingly for the abundant spin's spinlock field with  $n=1$  (equation 2),

$$f_{nut,i} = (1 \pm \alpha) cnst31 \quad (2d)$$

RF-fields for amplitude-modulated spinlock pulses are calculated using the integral factor of the amplitude-modulated shape on the  $s$ -nucleus, typically proton, with  $\mp$  for ZQCP and  $-$  for DQCP,

$$f_{nut,i} = (1 \pm \alpha) \frac{cnst31}{s(0)} \quad (3)$$

with  $s(0)$ , the integral factor of the amplitude modulated pulse chosen for the experiment and  $0 < \alpha < 1$ . Protection files in the pulse program ensure that the maximum permitted spinlock field for the low  $\gamma$ -nucleus is not exceeded. A similar scaling factor  $s(1)$  is used for the low- $\gamma$  nucleus.

The proposed concept of ssNMR experiment automation is independent of the vendor spectrometer platform. The concept requires global parameter tables and global scripts. The global parameter tables contain for liquids NMR pulses with various flip angles, durations and the related rf-powers. Solid state NMR requires shifting the focus from pulse widths and spin flip-angles to include *spin-nutation frequencies*,  $f_1$ , with  $f_1 = \frac{\gamma}{2n} B_1$  and  $B_1$  the magnetic field amplitude of the applied rf-field. These nutation frequencies  $f_1$ , also colloquially called *rf-fields* or  $B_1$ -fields, play a key role in solid state NMR. These globally stored parameters relating rf-power and the achieved rf-field for a given nucleus in a specific hardware configuration provide the rf-field parameters to the experiments. The approach provides the flexibility to define decoupling fields, spinlock fields and all other rf-parameters for the specific experiment without the need of cumbersome rf-power optimization when changing experimental elements like decoupling or recoupling sequences or MAS frequencies.

Consequently, experiments can be run without any additional task for the spectroscopist except for adjusting the recycle delay following a 1H T1-experiment. There are two major advantages of the automation package. Firstly, it allows to work automatically through an analytical project, on one sample or with multiple samples if a sample changer is available. Secondly, it eases experiment setup for the hands-on spectroscopist without the need to optimize rf-power parameters. The automation package reduces repetitive work to the bare minimum thereby increasing productivity.

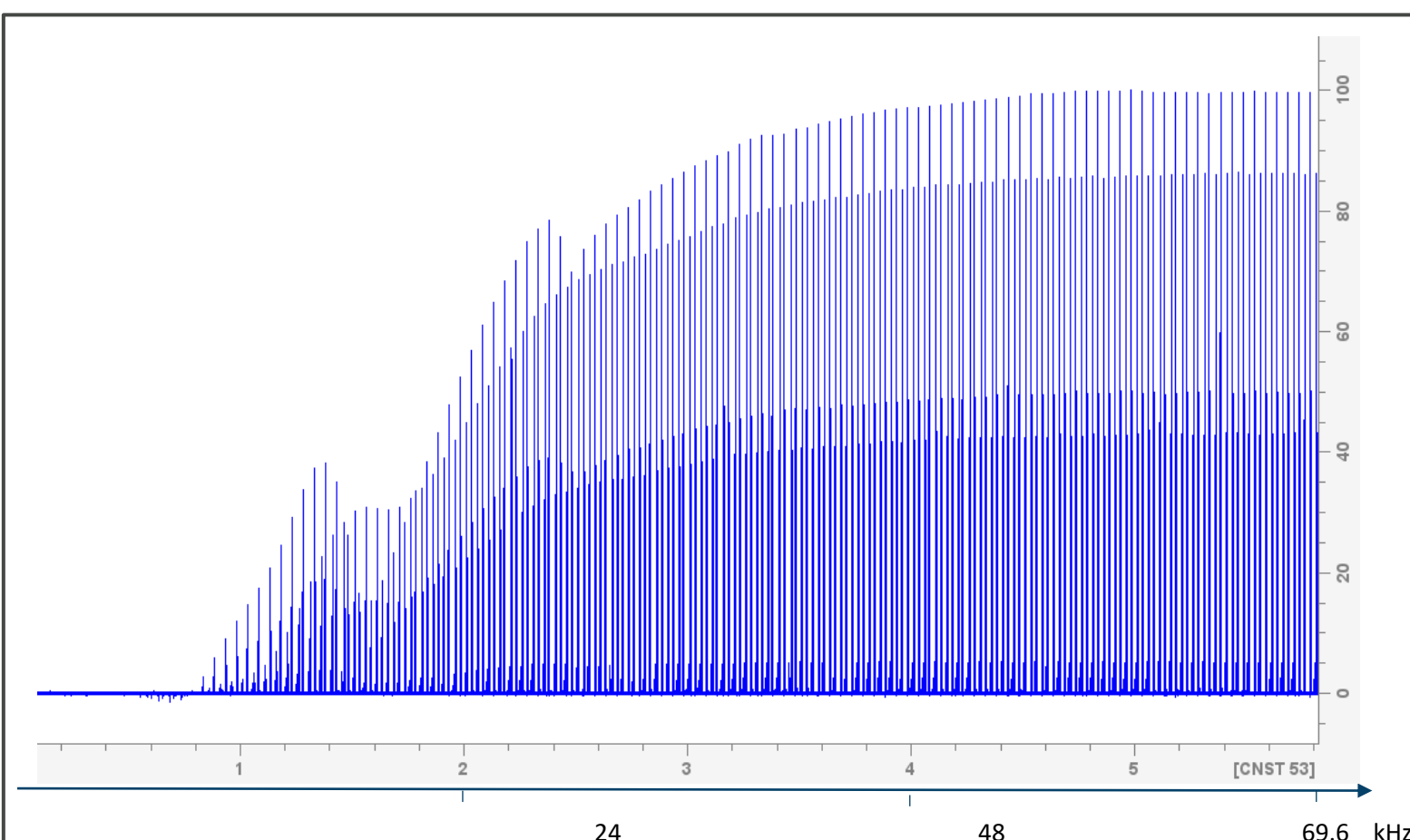


Figure 1 shows the CP enhancement profile for  $\alpha = cnst53$  (lower axis displays the amplitude for the <sup>13</sup>C spinlock field,  $f_{nut,s}$  (equation (2c)) in a g-glycine powder sample, at 12 kHz MAS. The tanhc60 ramp, a hybrid of a 30% amplitude ramp with tangential amplitude modulation, was applied. The incremented parameter,  $\alpha = cnst53$ , changes the relation of the spinlock fields to the rotation rate while the CP condition is maintained, see equation (1, 2c and 2d). The profile includes both g-glycine <sup>13</sup>C resonances. One can clearly see the minima of the CO resonance at  $cnst53=1.6$  and  $cnst53=2.45$  while the C $\alpha$ -resonance increases more continuously for  $cnst53>1$ .

Since Bruker thinks and lives in attenuation, a sign change is required, which changes the above equation for the rf-power change in dB by inverting the fraction with the compared spin nutation frequencies,  $f_{nut,i}$  and  $f_{nut,s}$ :

$$\Delta P_{dB} = 20 \cdot \log_{10} \left( \frac{f_{nut,s}}{f_{nut,i}} \right) \quad (8)$$

With the reference RF-field,  $f_{ref} = \frac{1}{4\pi\rho_{90}}$  and the relation between rf-power in dB to rf-power in W,

$$P_W = 10^{-\frac{\Delta P_{dB}}{10}} \quad (9)$$

Based on these relations,  $P_{rf} = V_{rf}^2 R_{rf}^{-1}$  with the peak-to-peak rf-voltage  $V_{rf}$  and the circuit's impedance  $R_{rf}$  we can replace the voltage by the rf-field amplitude,  $f_{nut,i}$ , created by the NMR probe's rf-coil and calculate the required rf-power for any experimentally desired  $f_{nut,i}$  using a reference rf-power  $P_{ref}$  required for a reference  $B_1$ -field  $f_{ref}$ .

$$P_{rf,i,s} = P_{ref,i,s} \left( \frac{f_{nut,i,s}}{f_{ref,i,s}} \right)^2 \quad (10)$$

Equation (10) is the central equation, used in the new pulse programming for ssNMR experiments, enabling calculations of the rf-power values once the MAS frequency is entered into the parameter set and the reference rf-requirements are known parts of the parameter set. The same equation calculates the rf-powers for any chosen decoupling field within the limits of probe specifications.

The new pulse programs contain comprehensive code to calculate all the required rf-power levels for the desired experimental conditions. For instance, for the CP contact pulses, the rf-power values are calculated such that the HH conditions, equation (1) or (2), are always fulfilled. In its most basic form for full automation use, the spinlock pulse for <sup>1</sup>H follows equations (2b) and equation (10) is written in the pulse program code with a correction term for the <sup>1</sup>H spinlock pulse that can be used for a fine adjustment of the HH contact.

$$P_{rf,i,s} = P_{ref,i,s} \left( \frac{f_{nut,i,s} + \epsilon_{i,s}}{f_{ref,i,s}} \right)^2 \quad (11)$$

Practically, the spectroscopist finds an optimum signal intensity by varying  $\alpha$  in a reasonable range, based on her/his experience and a consecutive optimization of the correction term  $\epsilon_{i,s} = cnst33$ , ideally followed by a final optimization of the best CP condition with  $\alpha$ . This approach is particularly helpful when trying to find a CP condition for half-integer quadrupolar nuclei, which require very low spinlock amplitudes for the half-integer quadrupolar nucleus. The intensity plot as a function of the nutation frequency for an AIP014 sample rotating at 12 kHz MAS is shown in figure 2 below.

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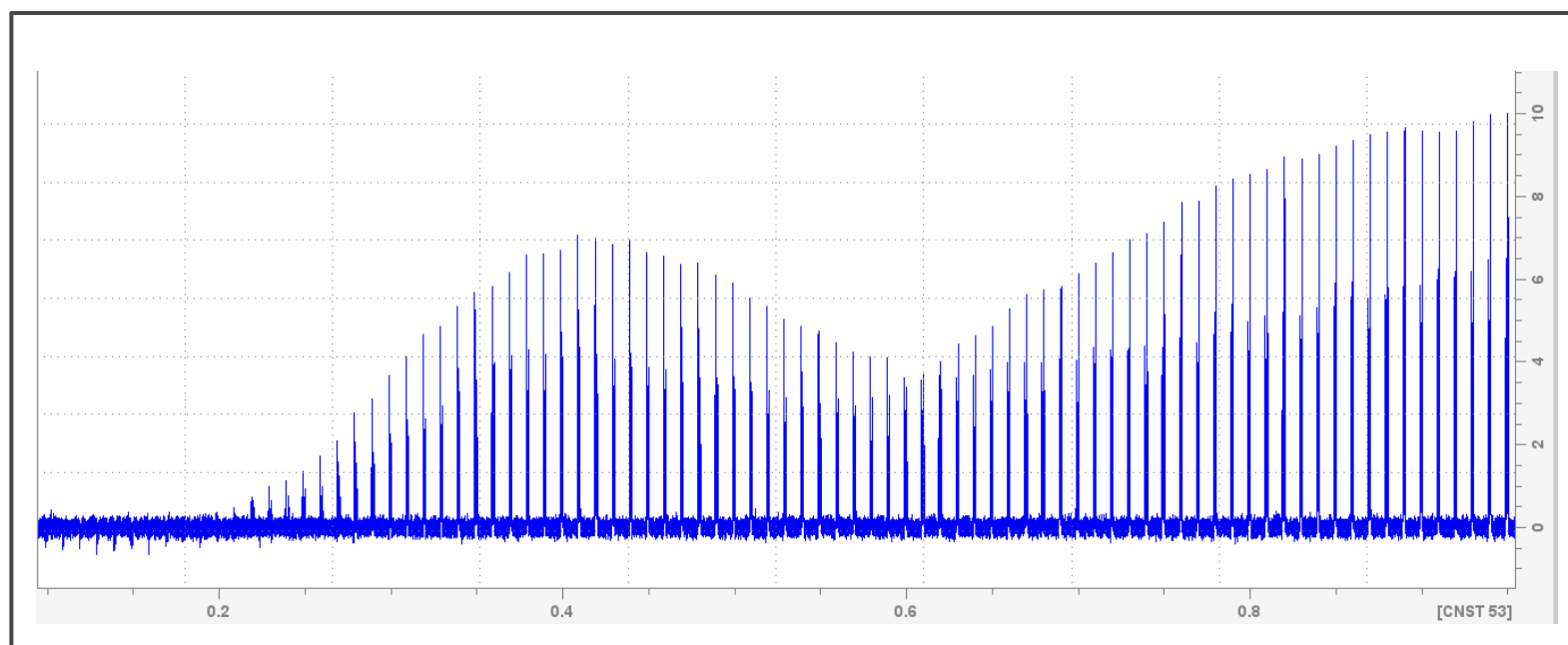


Figure 2a: <sup>27</sup>Al(<sup>1</sup>H) CPMAS profile in a AIP014 sample. <sup>27</sup>Al resonance intensity is shown as a function of the CP amplitude relative to the 12 kHz MAS frequency (cnst53) equation (2c). The CP amplitude variation was performed by incrementing cnst53. The maximum cnst53 = 0.9 corresponds to the 10.8 kHz spinlock field. cnst53 was incremented from 0.1 (1.2 kHz) in increments of 0.025 (300 Hz).

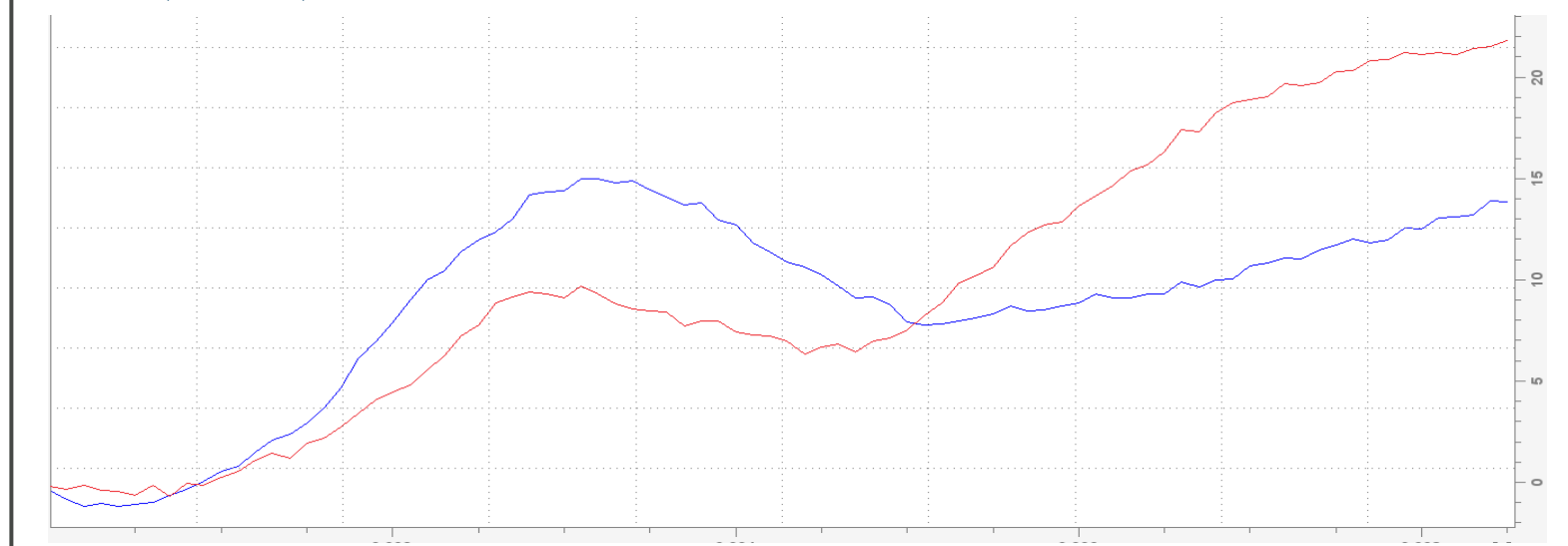


Figure 2b: profiles of the low field <sup>27</sup>Al resonance (blue) and the high field resonance (red).

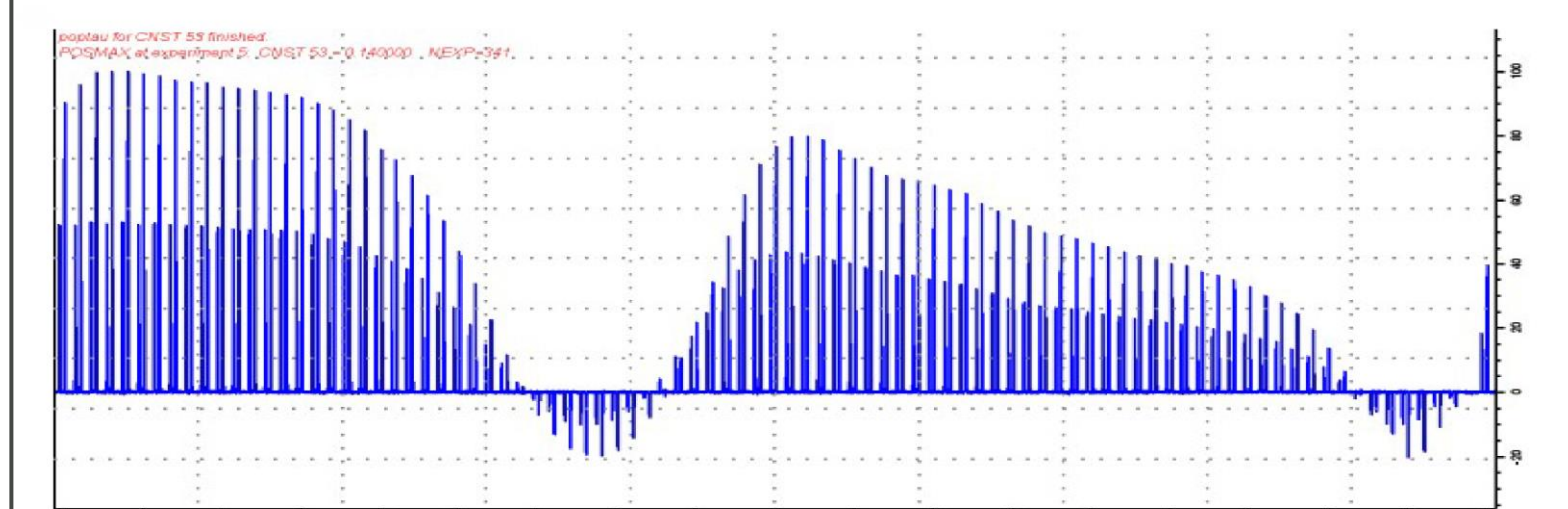


Figure 3 shows DQ CP at 80 kHz MAS with the DQ CP signal inverted such that we see a positive intensity. The experiment is a cross polarization to <sup>19</sup>F from <sup>1</sup>H following equation  $f_{nut,s} = (1 - \alpha) \frac{cnst31}{s(0)}$  with  $\alpha f(X) + (1 - \alpha) f(H) = mf(MAS)$  with  $m=1$ , optimize CP; (cnst53= $\alpha=0.14$ ),  $f_{nut,s} = (1 + cnst53) cnst31$ ;  $f_{nut,i} = cnst53 cnst31$  The optimum is at  $f(^{19}F) = 11.2 \text{ kHz}$ ;  $f(^{1}H) = 68.8 \text{ kHz}$

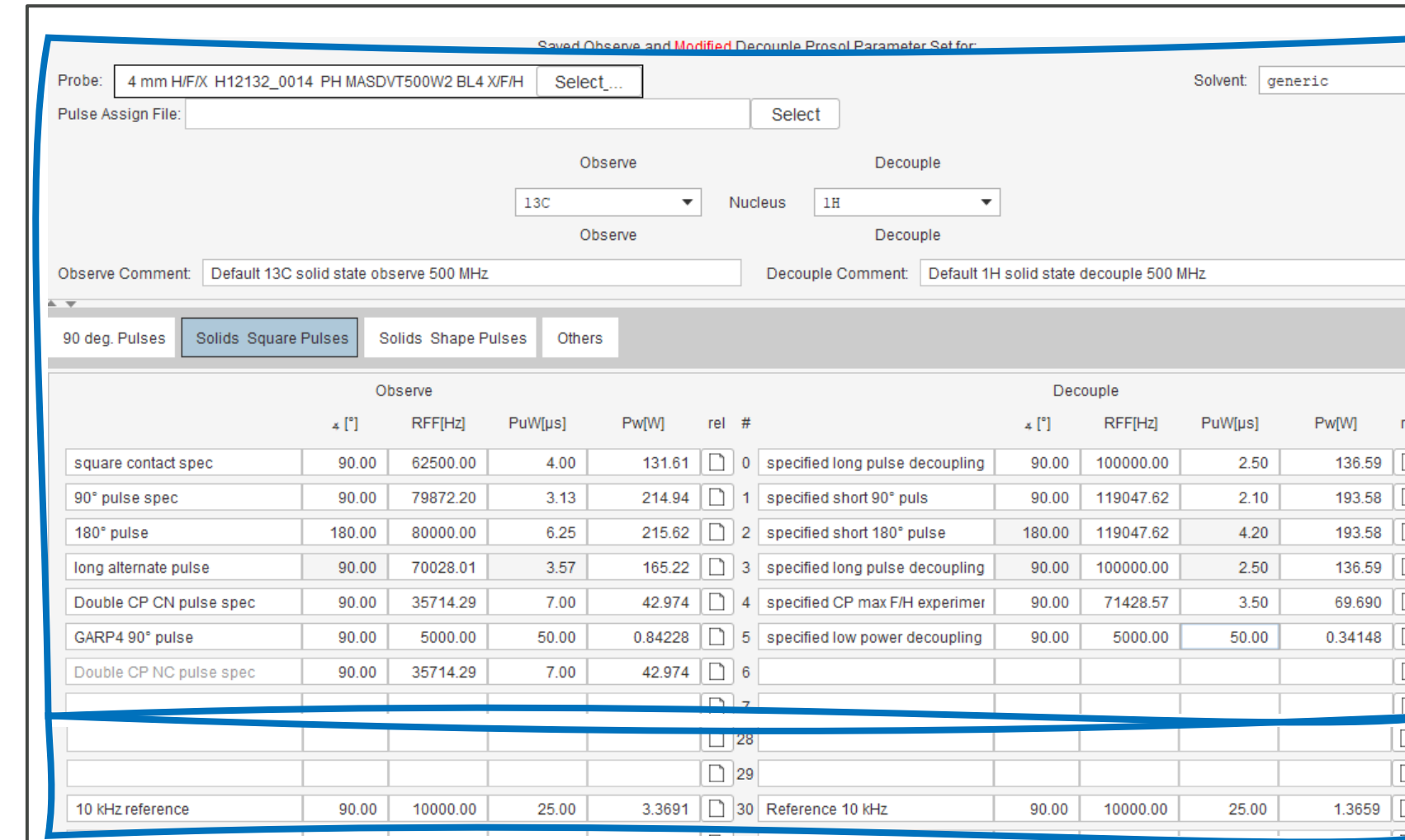


Figure 4 shows a picture of the PROSOL table with its entries and the reference pulses in row 30. The spinlock field cnst50 is in row 0 of the 13C observe channel and the decoupling field cnst52 in row three of the right column. The right column refers to the decoupler channel and the left column contains the parameters for the observe channel. The figure shows the PROSOL table as it is implemented in TopSpin4.2. for a regular 4mm HFx CPMAS WB probe of a 500MHz spectrometer.

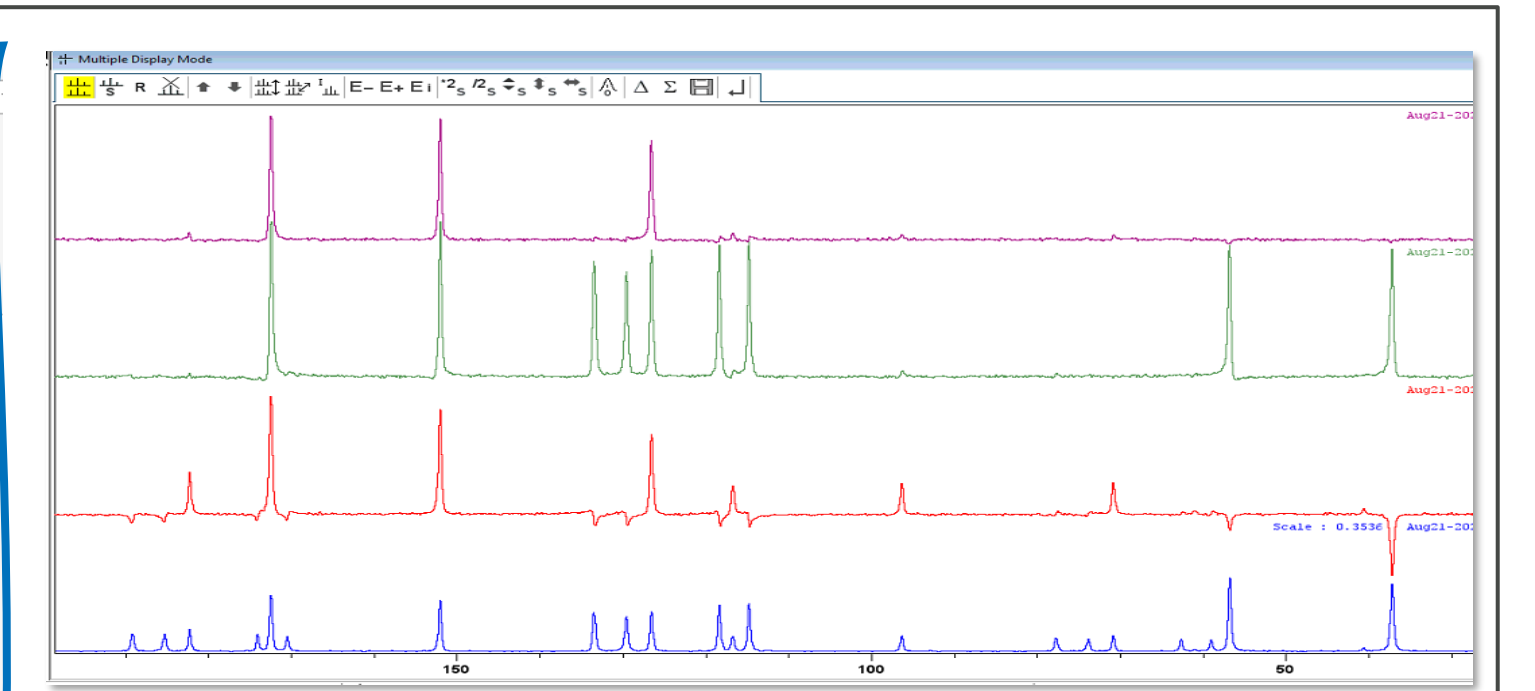


Figure 5 <sup>13</sup>C CPMAS project on tyrosine with an automation run at 7 kHz MAS using ICONNMR CPMAS (blue), CPPI multiplicity editing (red) CPTOSS (green) and TOSS NQS (purple)

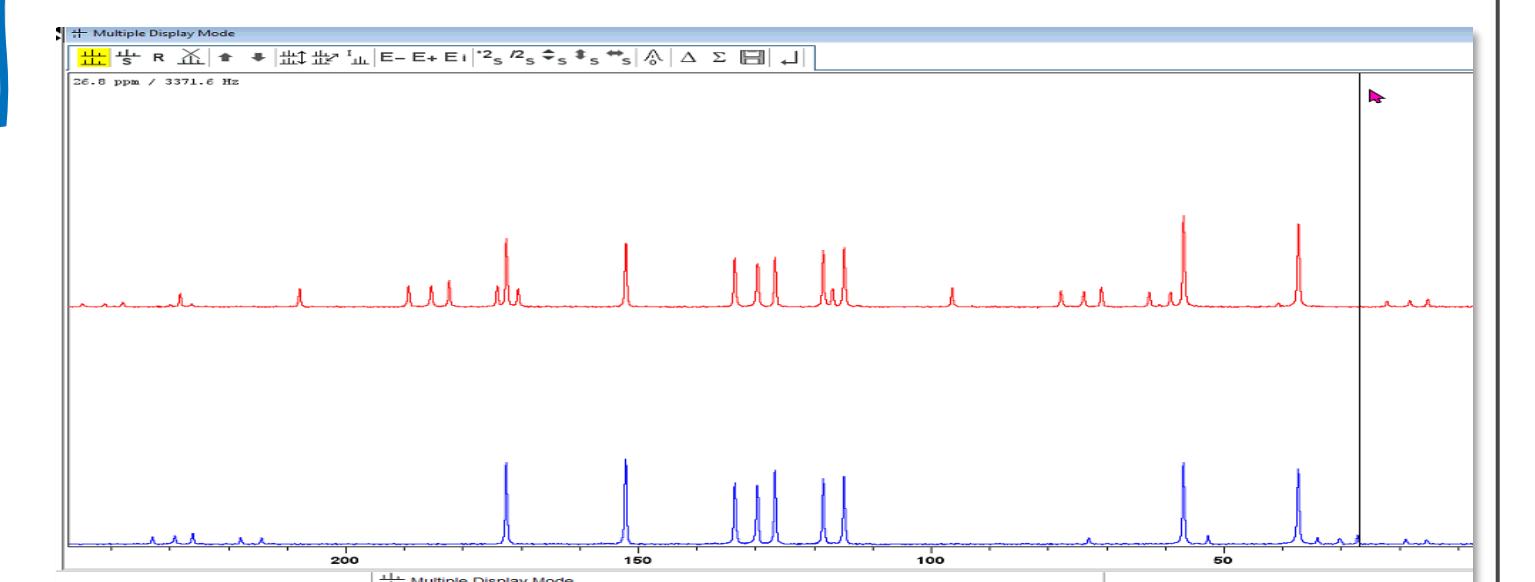


Figure 5 <sup>13</sup>C CPMAS project on tyrosine with an automation run and two MAS rates, 7 kHz (red) and 12.5 kHz (blue), using full automation on a 4 mm WB probe 500MHz using ICONNMR with TopSpin 4.1.

## Implementation

Key of the newly implemented method is its focus on complete experimental freedom, like cross polarization, MAS dependent recoupling sequence elements by providing rf-parameters fulfilling these known interdependent rf-conditions by design. CP spinlock fields require fulfilling HH matching conditions on all involved nuclei which depend on the samples MAS frequency. Based on the rf-power of a reference frequency as described in Equation (11), the pulse program calculates and sets the necessary rf-power parameters. The parameter  $cnst50$  is the specified maximum spinlock field of the heteronucleus under question and is used for automatically setting CP conditions for the probe employed for the experiment. The variable 'cnst52' is used for the specified decoupling field for that same NMR probe, in equation (3) is called, *intefacn* with the index  $n$  relating to the index of the shape file, *spnmr*, for the amplitude modulated pulse, *spnmr* with the associated rf-power parameters *spn*. The rf-power parameter array is available in dB or Watt as *spdbn* or *spwn*, respectively, with the index  $n$  ranging from 0 to 63. In the pulse program, equation (2b) for the high frequency channel reads:

$$"fieldH\_Hz=cnst50+cnst31"$$

For the low gamma spinlock pulse we have, fulfilling the HH condition from equations (2a) and (2b):

$$"fieldX\_Hz=cnst50"$$

For fast MAS or low power CP experiments, with the flag  $-DfastMAS$  as required for CP with half-integer quadrupolar nuclei, the spinlock fields are calculated following equation (2c and 2d) with a more flexible parameter based HH-condition (see equation 13 below), constituting the semi-automated part of experimentation, where some user initiative is required for experiment setup with  $cnst53=\alpha$  from equation (2b).

$$f_{nut,s} = (1 + cnst53) cnst31; \text{ and}$$

$$f_{nut,i} = cnst53 cnst31$$

These relations following equations (1), and (2b) are reflected in a pulse program as:

$$\#elfast fastMAS$$

$$"cnst54=1+cnst53"$$

$$"fieldX\_Hz=(cnst53)*cnst31"$$

$$"fieldH\_Hz=cnst54*cnst31"$$

For the double quantum CP with the flag  $-DfastMASDQ$ , eq. (2) and (2b) become:

$$f_{nut,s} = (1 - cnst53) cnst31; \text{ and}$$

$$f_{nut,i} = cnst53 cnst31$$

With the implementation in a pulse program as:

$$\#ifdef fastMASDQ$$

$$"cnst54=abs(trunc((cnst53)+1-cnst53))"$$

$$"fieldX\_Hz=(cnst53)*cnst31"$$

$$"fieldH\_Hz=cnst54*cnst31"$$

And finally following equation (11) and adding the frequency correction terms

$$"fieldH\_corr=fieldH\_Hz+cnst33"$$

$$"fieldX\_corr=fieldX\_Hz+cnst34"$$

We have:

$$"spw0=plw30*pow((fieldH\_corr/(10000*Intefacn0)),2)"$$

$$"spw1=plw31*pow((fieldX\_corr/(10000*Intefacn1)),2)"$$

Similarly, equation (11) for the decoupling power is:

$$"plw12=plw30*pow(((cnst52+cnst32)/(10000)),2)"$$

With the frequency correction term  $cnst32$  for a fine adjustment of the decoupling rf-power if desired to do so, typical values range between -5000 $cnst32$ ≤5000. The decoupling pulse width is:

$$"pcpd2=1000000/(2*cnst52)"$$

The probe's specified decoupling is entered into the PROSOL table (Figure 4) in line 0 and 3 on the right column. Using the option  $-Dlpedc$ , a low power decoupling option is available where  $cnst52=0.25*cnst31$  which is a low power decoupling with a decoupling field of ¼ of the MAS frequency [14].

The method for calculating rotation frequency dependent pulse parameters can be easily extended to all types of sequences where the rf-amplitude and pulse width depend on the MAS frequency. The following pulse program code lines show the implementation for the PC7 [1], the SPCs [2] [3] [4], the SUPER [5], the ROCSA [6] [28], related R and C type CSA recoupling sequences [12] [13] [14], and the CORO [7] mixing sequence.

$$"plw11=plw31*pow(((7*cnst31)/(10000)),2)" \quad \text{for PC7;}$$

$$"plw11=plw31*pow(((10/3)*cnst31/(10000)),2)" \quad \text{for SPC5;}$$

$$"plw11=plw31*pow(((12.12)*cnst31/(10000)),2)" \quad \text{for SUPER;}$$

$$"plw11=plw31*pow(((4.28)*cnst31/(10000)),2)" \quad \text{for ROCSA;}$$

and for CORO mixing the two rf-power levels

$$"plw14=plw30*pow((cnst31/(10000)),2)" \text{ and}$$

$$"plw15=plw30*pow((cnst31/(20000)),2)".$$

The content of the lookup table, called PROSOL table, see figure 4, is relayed to the parameter set through a "relations file" called *solids\_ICON* in TopSpin 4.2.0 and 4.3. The relations file for the reference pulses looks as follows:

```
PLW[30]=SQPL[30];2 # spinlock field 10 kHz reference, F2
PLW[31]=SQPL[30];1 # spinlock field 10 kHz reference, F1
PLW[32]=SQPL[31];2 # decoupling field 10 kHz reference, F2
PLW[33]=SQPL[31];3 # decoupling field 10 kHz reference, F3
# Shapes
SH[0]=SH[0];2 # contact pulse, F2
SH[1]=SH[3];1 # contact pulse, F1
SH[2]=SH[1];2 # contact pulse, F2
# cnst (e.g. for RF field calculations)
CNST[50]=SQRF[0]*1.0.1 # RF field strength, F1
CNST[51]=SQRF[2]*1.0.2 # RF field strength, F2
CNST[52]=SQRF[3]*1.0.2 # RF field strength, F2
CNST[61]=SQRF[2]*1.0.3 # RF field strength, F3
```

## Benefits

The time-honored SOP of liquid state NMR experimentation of

*rfpar*: for reading parameter sets for the desired experiment,

*getprosol*: for loading all required experiment parameters, rf-power, default wave-forms,

*tune and match*;

followed by automatically reading the MAS controller, and adding the MAS frequency to the experiment parameters before automatic acquisition start has become available for solid-state NMR for executing any basic CP, CP base editing experiments, and T<sub>1</sub>-experiments for low gamma nuclei.

A proton T<sub>1</sub> experiment can be conducted for optimized recycle delay settings prior to the CP experiments as well as an optional <sup>1</sup>H T<sub>1rho</sub> experiment for setting the contact pulse width.

Parameter sets exist in TopSpin 4.1.4 and higher for <sup>31</sup>P, <sup>13</sup>C, <sup>29</sup>Si, <sup>15</sup>N, and <sup>19</sup>F double channel experiments as well as <sup>19</sup>F/<sup>1</sup>H/<sup>13</sup>C triple resonance experiments for using full automation.

All these experiments can be run in full automation using the automation program *IconNMR* with uncompromised high sensitivity.

Extending the new strategy employing both rf-powers fulfilling the HH match, the HH-matching rf-powers can be varied in concert relative to the MAS rate for finding the optimum condition for half integer quadrupole nuclei or high rotation frequency MAS experiments. This saves valuable time setting up experiments. Typically, such conditions require grid searches. The new method permits one set of optimization of few steps between 0 and 1, saving more than a factor of 10 experiment time.

Similarly, triple resonance experiments in structural biology like NcA NcAcX or NCO etc are setup with this method in 1/10<sup>th</sup> of the time it typically takes for prepare such projects.

Experiment		<sup>1</sup> H field	X field	Y field	total N
CP	new	N1	N2	N3	N1*N2 or N2*N3 for each N=0
fast MAS	new	0	0	0	0
fast MAS	traditional	10	0	0	10
fast MAS	new	10	1	1	10
fast MAS	traditional	10	10	10	100
Decoupling	new	0			10