Selective nOe

One huge advantage of selective saturation or excitation is that once you know up-front what resonances are being saturated or excited, you can use the detection for something other than simply asking to see what was excited. Thus, we can use the detection period to learn what protons are close in space to the one whose resonance was saturated. This will include the saturated proton, but it will also include neighbours 5 Å or less away. Thus we gain insight into structure with a simple 1D.

Again, begin from a beautiful 1d with a good choice of sw, tof and tpwr/pw90 (**Page 2**).
Copy these parameters to a different experiment. For example if you are in experiment 1 and plan to execute the nOe in experiment 2, type mp(1,2) then join experiment 2 (jexp2). [1]

In your nOe experiment, type '**cyclenoe**' [2]
In any Acquire page click [Sequence] (**Page 3** [1])
You see the 1D nOe pulse sequence complete with purge pulses [2] at the beginning and then a long string of low-power pulses lasting a total of seconds [3], before the read pulse [4].
In Acquire>Acquisition, many of the parameters called for in the pulse sequence do not appear. This is because this pulse sequence is not enabled under VNMRJ and chempack. We will have to set this up button-free !
Go to Acquire>Overview, where you will see a listing of the VNMR parameters.[3]
This panel tells you what the parameters' names are in the pulse sequence. Each can be assigned a value via the command line. However first we need to determine those values.

In order to choose the frequencies of the line to be saturated and a useful off-resonance control frequency where irradiation should not saturate anything, you need values for satfrq [5] and control [6], respectively. To determine these, go back to the beautiful 1d you have retained in experiment 1 by typing **Jexp1**.

In Acquire>Acquisition click on [DisplaySpectrum] and look at the spectrum and identify a resonance that you would like to use as a starting-point for identifying resonances of protons nearby. For example, I chose the region near 3.8 ppm.

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[1] In my slides you will see that my beautiful 1d is in exp9 and my nOe is being set up in exp 13.
[2] Note that if you use Tools>Browser, go to /vnmr/parlib and select cyclenoe.par , dragging this into your graphical display window will bring in wrong values for the power and pw90 as well as sw and tof. It is better to issue the command.
[3] There is also warning that VNMRJ cannot find a parameter hdof, in the command response line. hdof is the homodecoupler frequency offset. This is no longer used, but persists in this very old pulse sequence.

Zoom in on that region (**Page 4**). Place the cursor right in the middle of split lines, or right on the top of singlets. For the example here, we have a doublet, I put the cursor in the centre at 3.88 ppm.
Check in Acquire>Channels and if necessary change Decouple to H1 [1].
Now type '**sd**' (set decoupler)[4]. The frequency offset of the decoupler jumps to a value corresponding to the frequency in Hz of your line [2]. Write that down (-477.8 in this example) The dof value is also quoted in the response line above the command line [3] as well as the feedback line at the bottom right of the screen [4].
Now measure the splitting of your line by placing one cursor on a downfield component of the multiplet and the right cursor on the next component (**Page 5**). The distance between the two is now provided in the right-most yellow block, but the value is in ppm. To get it in Hz type **axis='h'**. Now we read 10.58 [1] as the splitting. Write this down and return to a ppm axis by typing **axis='p'**.

Now redisplay the full spectrum and move the cursor to a wide resonance-free area (**Page 6**, [1]) and type **sd** again. Now the decoupler offset acquires a new value [2], (which is also reported in the command response area as dof [3]. Write this down too (13.4).

Now return to the experiment containing the cyclenoe parameters (jexp2). (**Page 7**)
Type **satfrq=**-477.8 (the frequency of the resonance to be saturated).
control=13.4 (the frequency where there are no signals, providing a control for the effects of non-specific irradiation)[5]
pattern=2 (for a doublet), for a triplet **pattern=3** (for a singlet **pattern=1**) [1]
spacing=10.58 Set spacing to the Hz separation between adjacent lines in your multiplet. [2]
(To refresh Acquire>Overview you need to move to a different page (such as Acquire>Acquisition) and come back).

Sample values follow for other parameters:

tau = 0.1 (the time spent on each line of a multiplet, in units of s). [3]

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[4] sd will set dof to the value corresponding to the position of the cursor, so long as dn=tn

[5] For the most rigorous application, subtract off-resonance effects on a resonance near the one to be saturated. To do this, note the separation between the target resonance and the bystander (in Hz), move to the other side of the bystander to a position the same distance away (Eg. if the target is 30 Hz down field of a bystander, set control 30 Hz upfield of the bystander.) Thus, off resonance effects will be the same and will cancel out when the control spectrum is subtracted from the saturated one. One will however need to worry about possible saturation of *bonafide* resonances on the far side of the bystander signal.

sattime=4 (the total time spent establishing saturation, choose a value near T1, as a first try). [4]

cycle='y' turns on cycling among lines of a resonance. [5]

mix=0 is a time allowed for saturation transfer after cessation of saturation, it can usually be set to 0. [6]

If **intsub='y'**, the software will automatically subtract the spectra acquired with saturation at the control frequency from the spectra collected upon saturating a line. This difference scan will contain only the resonances affected directly and indirectly by saturation. [7]

In Acquire>Acquisition, click on [DisplaySequence] to be sure that the instrument is using your desired satpwr for the (very long) saturation period. Do not use a value higher than 6).

Array satpwr to identify the power that saturates the target line by 50-75%. Array from –16 to 3. (We found that –4 was good)

Use nt set to some multiple of 32 for best results, when intsub='y'.

Be sure that d1 is $3xT_1$. This in combination with the extensive phase cycling makes for a moderately time-consuming experiment.

Arraying to get a good value for satpwr.

Set **intsub='n'**, but leave **cycle='y'**. This will execute scans for the satfrq, but will not do the control scans. I began by setting satfrq on the isolated line identified by the curson in **Page 8**.

Go to Acquire>acquisition to get the Arrays button (**Page 9**). Click on 'Arrays' [1] to activate the array window on the left. Enter satpwr under 'Parameter Name' [2] and <return>. Now you can enter the number of values in the array [3], the first value [4] and the step size [5]. After you click <return> the software will calculate all the values to be tested [6]. For example: array satpwr, for example over 8 steps from -10 by steps of 2 .

Page 10 shows that I really have saturated the intended line (compare with page 8) . The satpwr dependence on **page 11** shows that a satpwr of 0 is plenty [1] as our large peak is now similar in size to the noise [2]. We can see that this power has not saturated the rest of the spectrum (**page 12**) based on the fact that the nearby resonances that are between the cursors on page 12 are not decreasing in amplitude over the same satpwr range (**page 13**).

Now disable the array by typing **satpwr=0** (our chosen value). Set **intsub='y'**, and **nt=16**. Show Time shows that 16 scans total are being acquired. A good number of

steady-state scans as well as a good number of scans (both ideally multiples of 8) improve the quality of the subtraction being perfomed. This subtraction is not easy because we are subtracting one strong spectrum from another in search of small differences.

A successful example

The signal treated above was an easy one, because it is isolated in the spectrum, however it did not show any good nOes, as it is apparently also isolated in the molecule. To illustrate nOes, I chose a different resonance, at 1.9 ppm, which is split by 6.47 Hz into a doublet (**Page 15**).

For greater selectivity than we had above, decrease satpwr a little (from 0 to -4) and increase sattime from 2 to 4. The result on **page 16** is a relatively clean saturation of the peak near 2 ppm [1] and a clear nOe of the opposite sign at 3.9 ppm [2]. The saturated signal looks inverted because we are subtracting a spectrum in which that signal is not saturated from a spectrum in which it is (subtract a positive line from an case in which the line is missing). **plotnoesel**

Plots

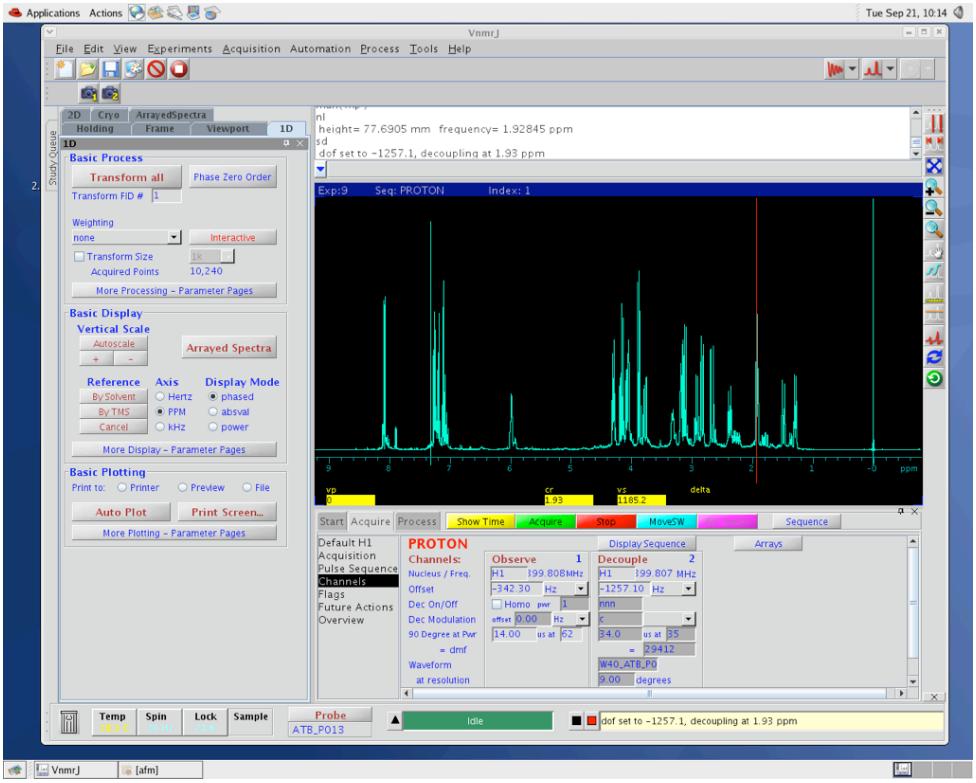
To get an electronic output file, go to the left-hand panel, under the 1D tab, and below 'Basic Plotting' select 'File' and then select the 'Print Screen ....' [2]. In that [3] select File [4] and enter a file name [5]. Make the choices below. My choice of 'mono' [6] produces a black-and-white plot, regardless of the black background, aqua spectrum, red cursor etc. Then click 'Save' [7]. You should get a message confirming that your spectrum has been written as a file [8]. **plot** and **plotnoe**

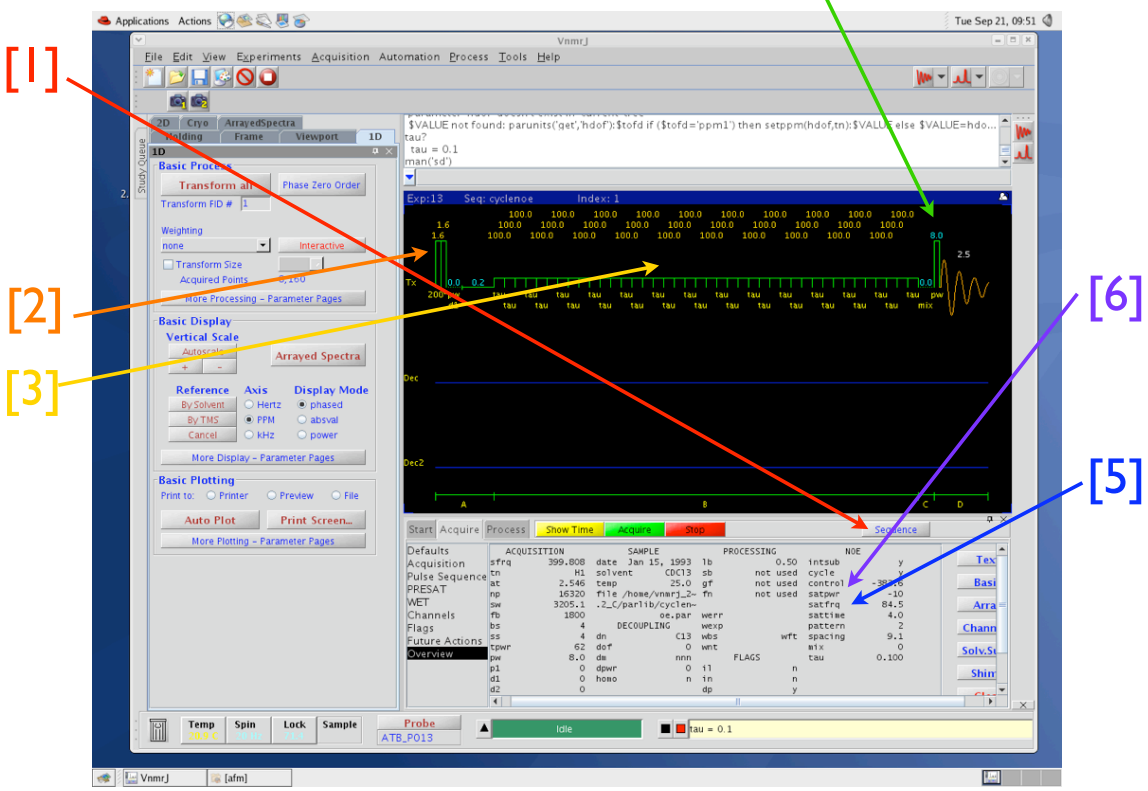
Aside

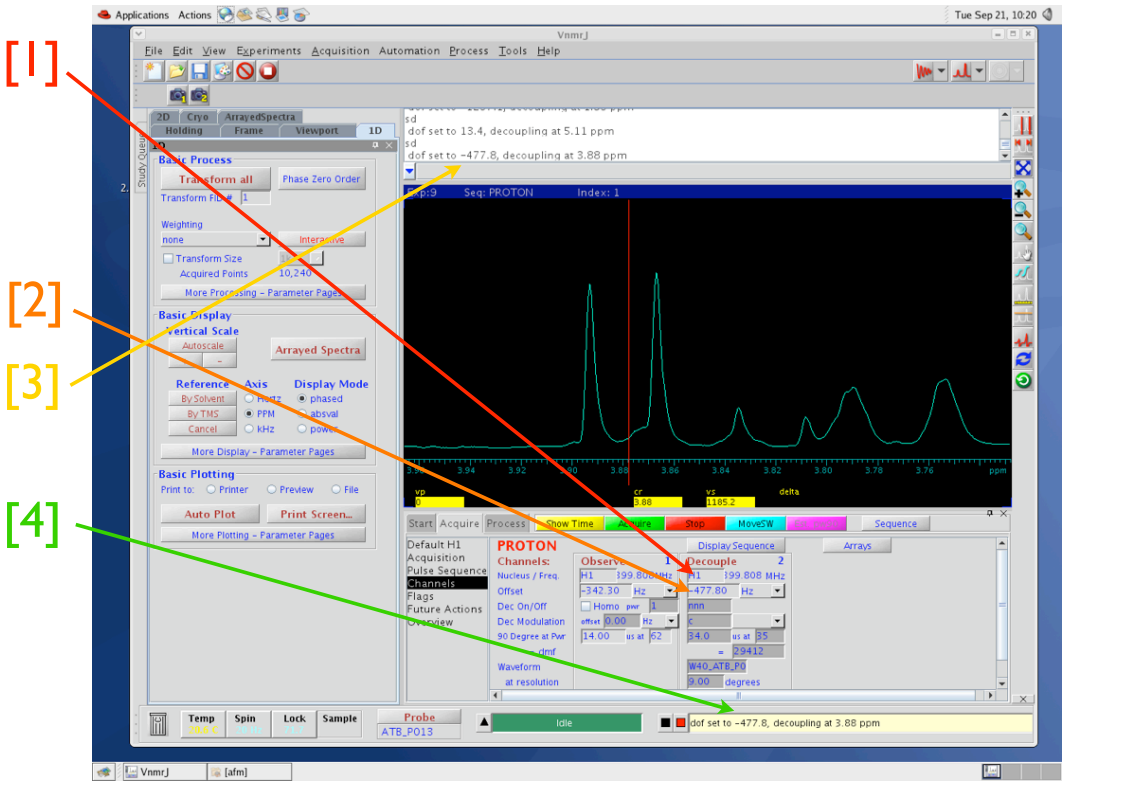
A method whose implementation is similar is selective decoupling. This saturates a single line during observation of the rest of the spectrum, and causes collapse of the splitting of resonances coupled to itself. This method identified through bond couplings.

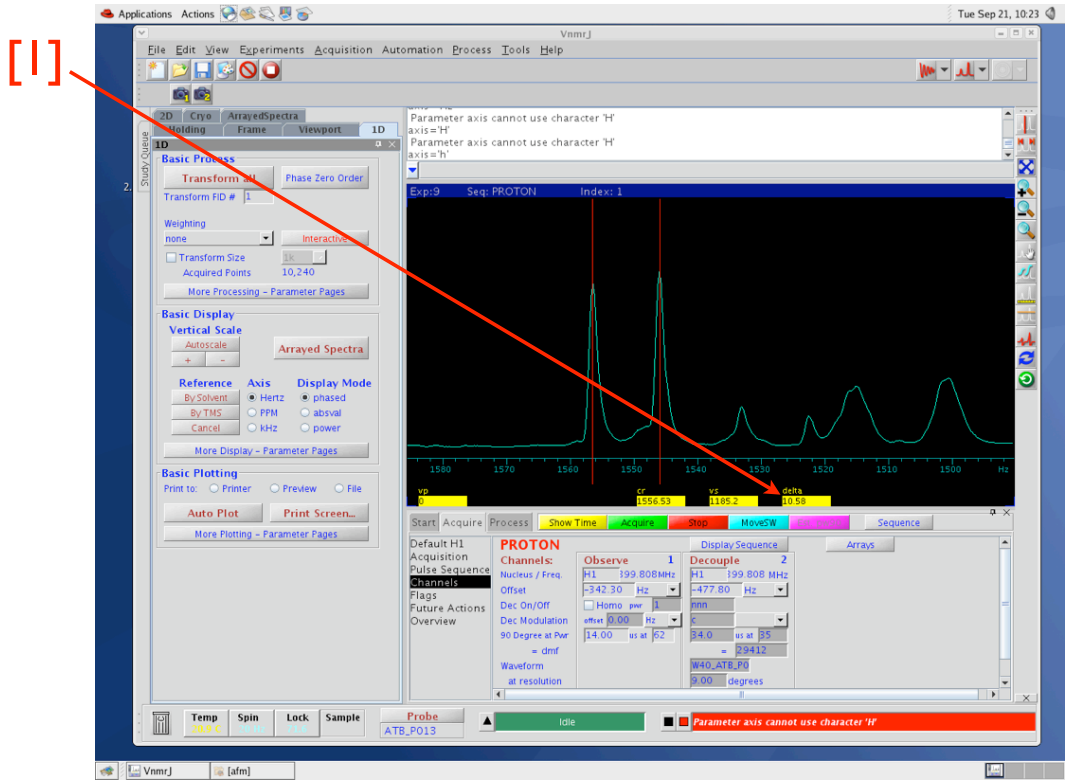
Both experiments should be executed in conjunction with controls to test for effect of the saturating radiation, for example by applying the radiation to an unoccupied spectral region.

Selective 1D nOe
cyclenoe




[1]
[2]
[3]
[4]
[5]
[6]


[1]
[2]
[3]
[4]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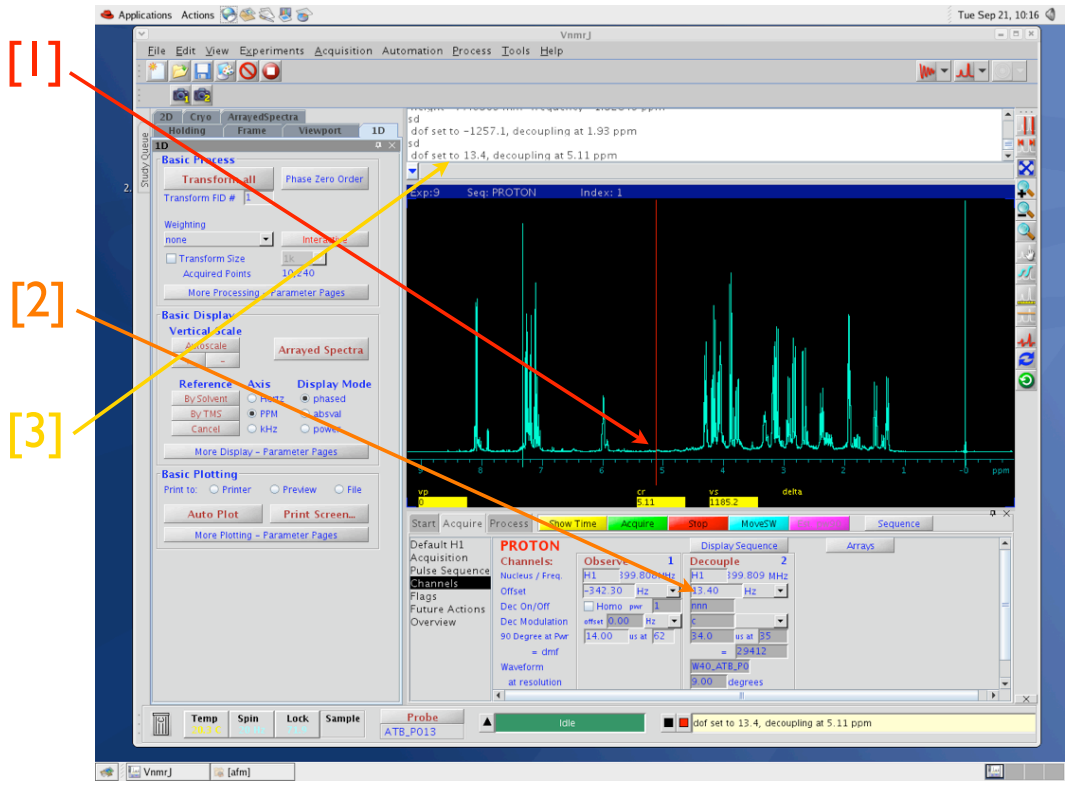
axis='H'
Parameter axis cannot use character 'H'
axis='h'
Exp:9 Seq: PROTON Index: 1
Basic Process
Transform all
Phase Zero Order
Basic Display
Vertical Scale
Autoscale
Arrayed Spectra
Reference
Axis
Display Mode
Basic Plotting
Auto Plot
Print Screen...
PROTON
Channels:
Observe
Decouple
Temp
Spin
Lock
Sample
Probe
ATB_P013
Idle
Parameter axis cannot use character 'H'
VnmrJ
[afm]


[1]
[2]
[3]
Applications Actions
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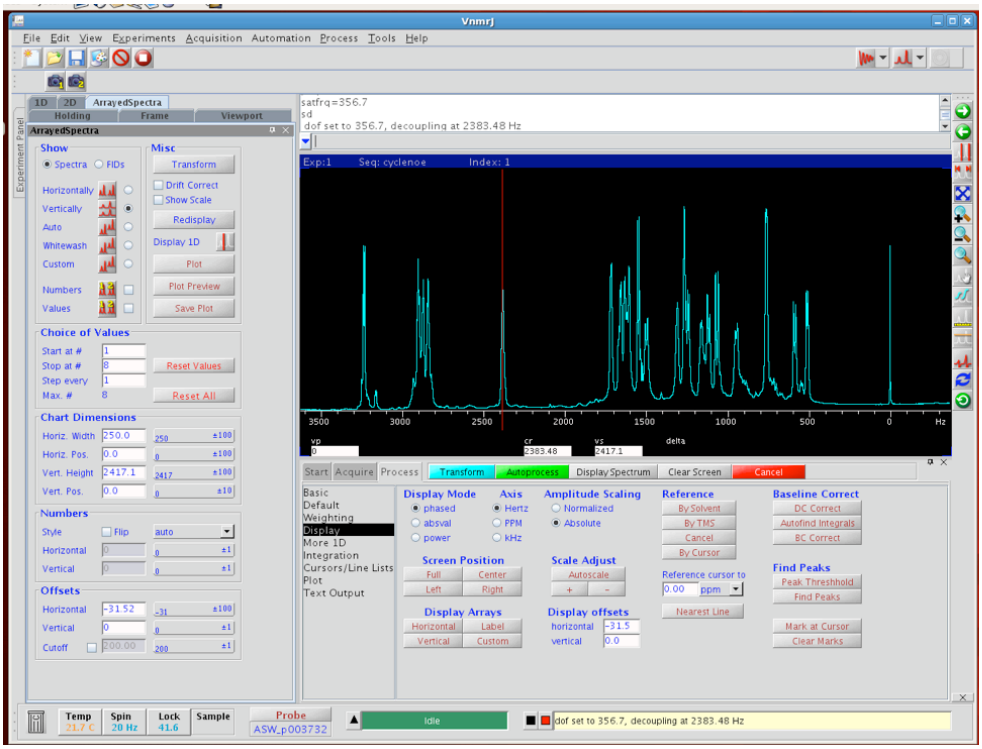
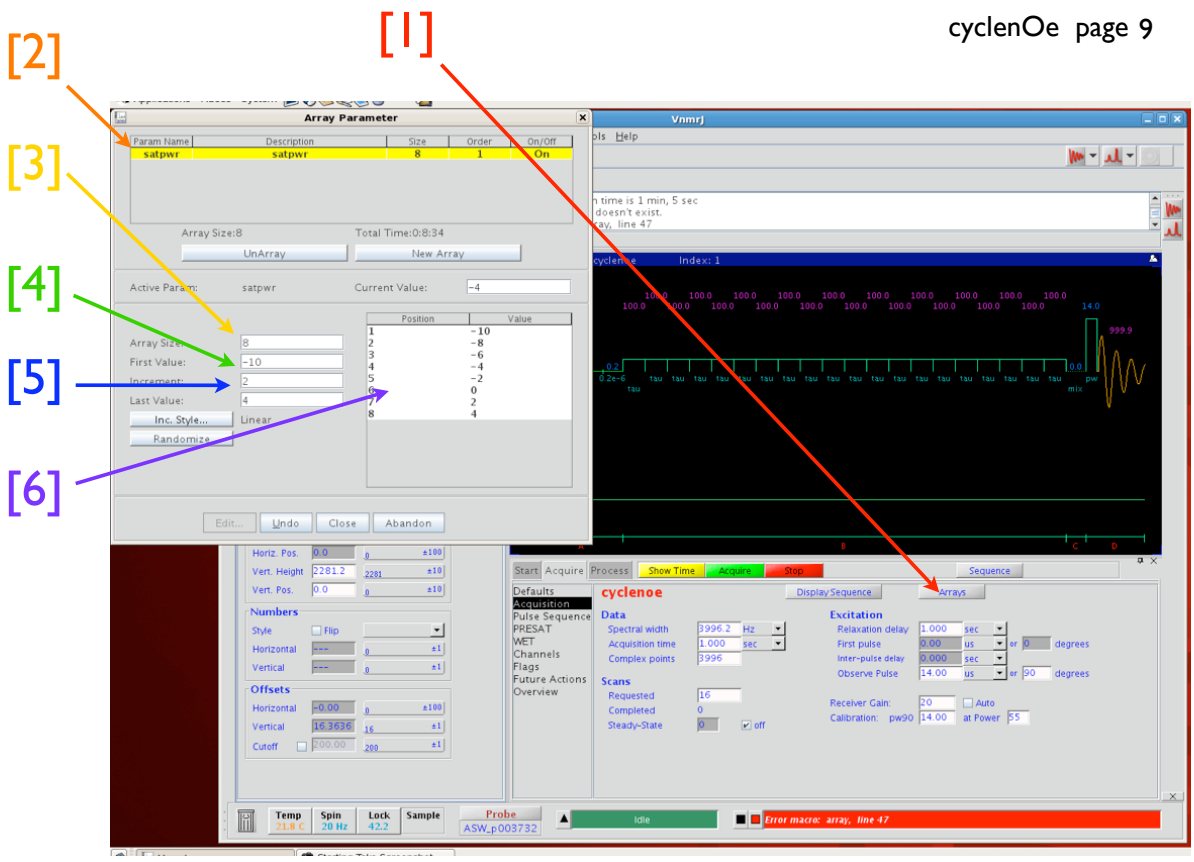
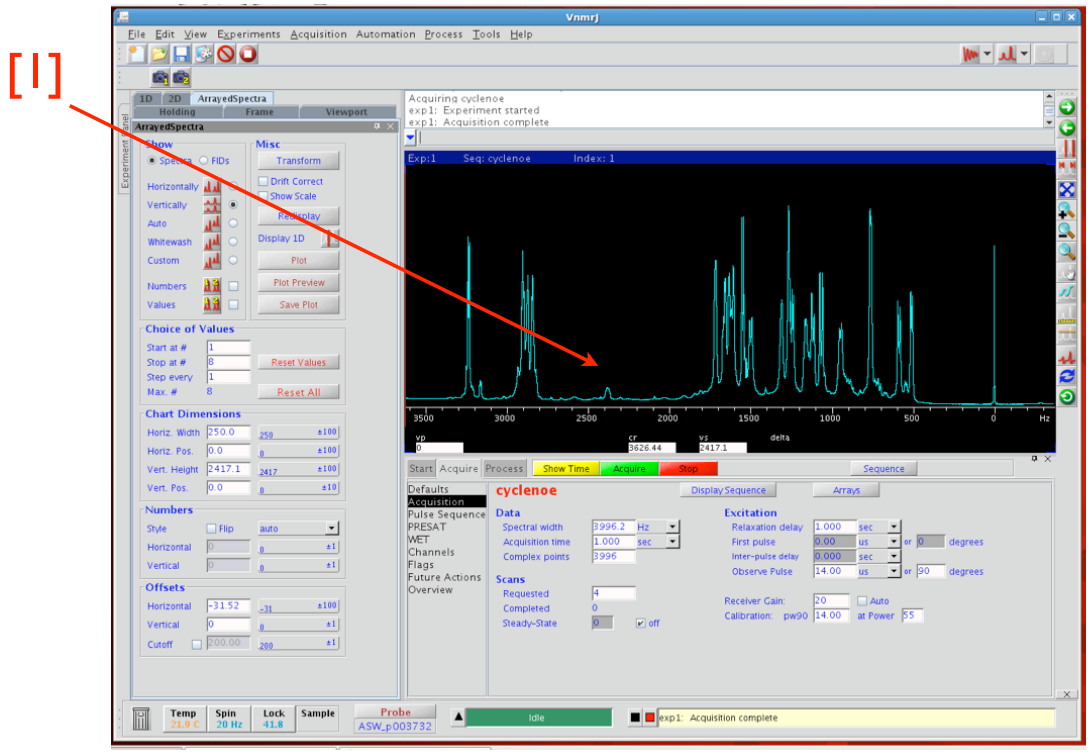
sd
dof set to 13.4, decoupling at 5.11 ppm
Exp:9 Seq: PROTON Index: 1
Basic Process
Transform all
Phase Zero Order
Basic Display
Vertical Scale
Autoscale
Arrayed Spectra
Reference
Axis
Display Mode
Basic Plotting
Auto Plot
Print Screen...
PROTON
Channels:
Observe
Decouple
Temp
Spin
Lock
Sample
Probe
ATB_P013
Idle
dof set to 13.4, decoupling at 5.11 ppm
VnmrJ
[afm]


[7]
[5]
[4]
[1]
[2]
[3]
[6]




[1]
[2]
[3]
[4]
[5]
[6]
Array Parameter
Vnmrj
cyclenoe
Index: 1
Array Size:8
Total Time:0:8:34
UnArray
New Array
Active Param:
satpwr
Current Value:
First Value:
Increment:
Last Value:
Inc. Style...
Linear
Randomize
Edit...
Undo
Close
Abandon
Start
Acquire
Process
Show Time
Stop
Sequence
Defaults
Acquisition
Pulse Sequence
PRESAT
WET
Channels
Flags
Future Actions
Overview
Display Sequence
Arrays
Data
Spectral width
Acquisition time
Complex points
Scans
Requested
Completed
Steady-State
Excitation
Relaxation delay
First pulse
Inter-pulse delay
Observe Pulse
Receiver Gain:
Calibration:
Numbers
Offsets
Temp
Spin
Lock
Sample
Probe
ASW_p003732
Idle
Error macro: array, line 47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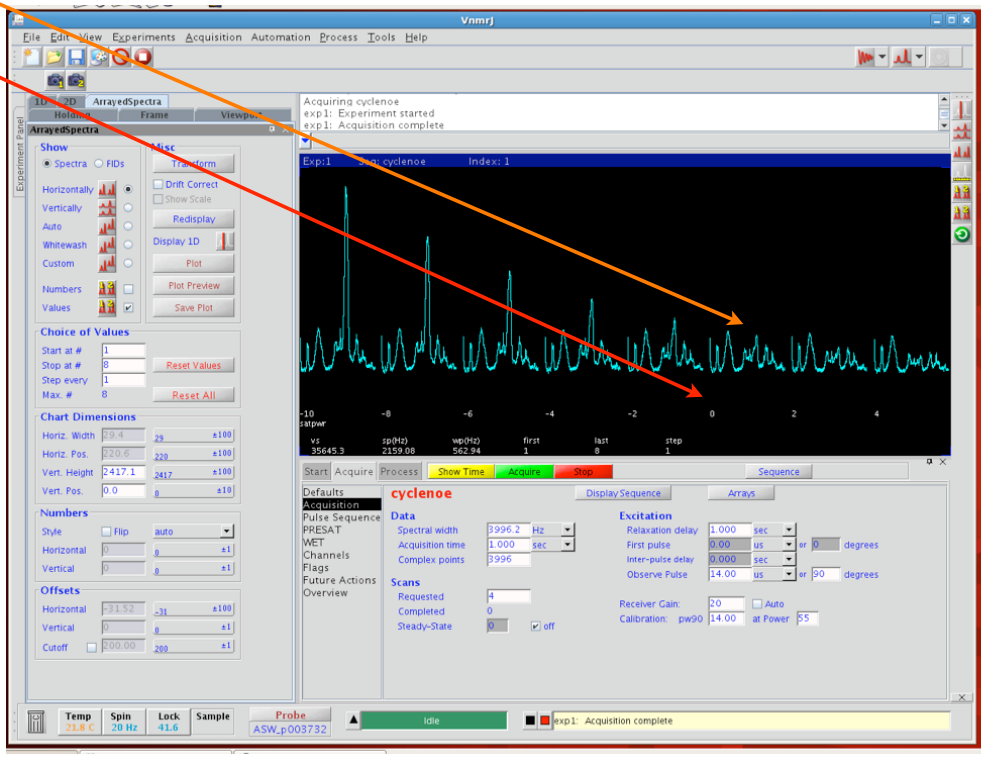
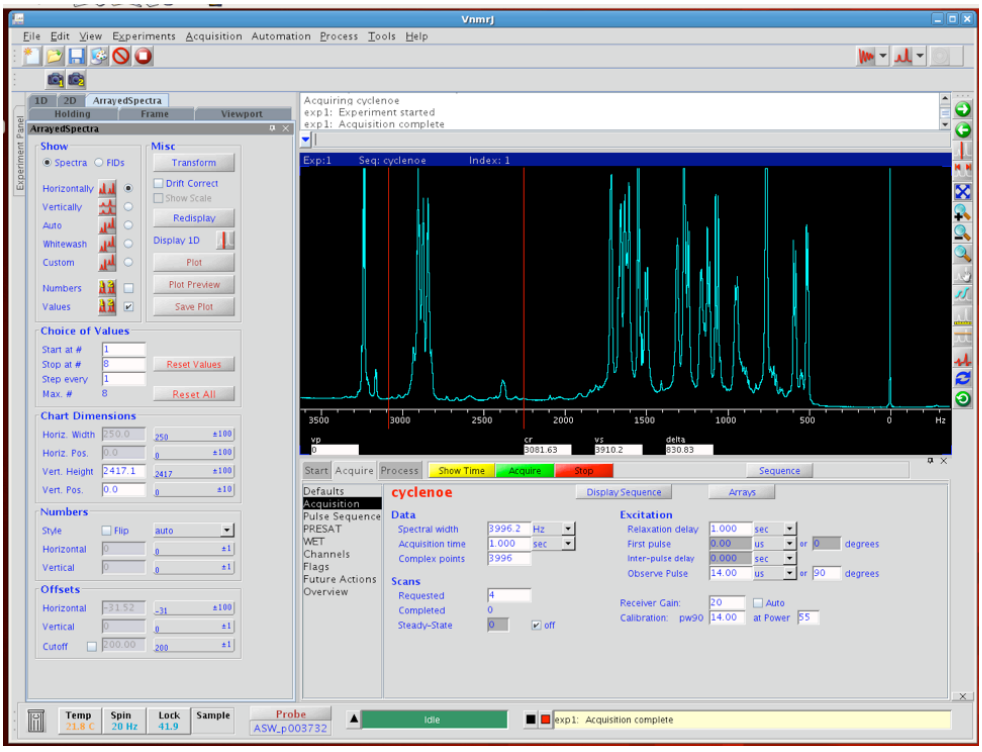
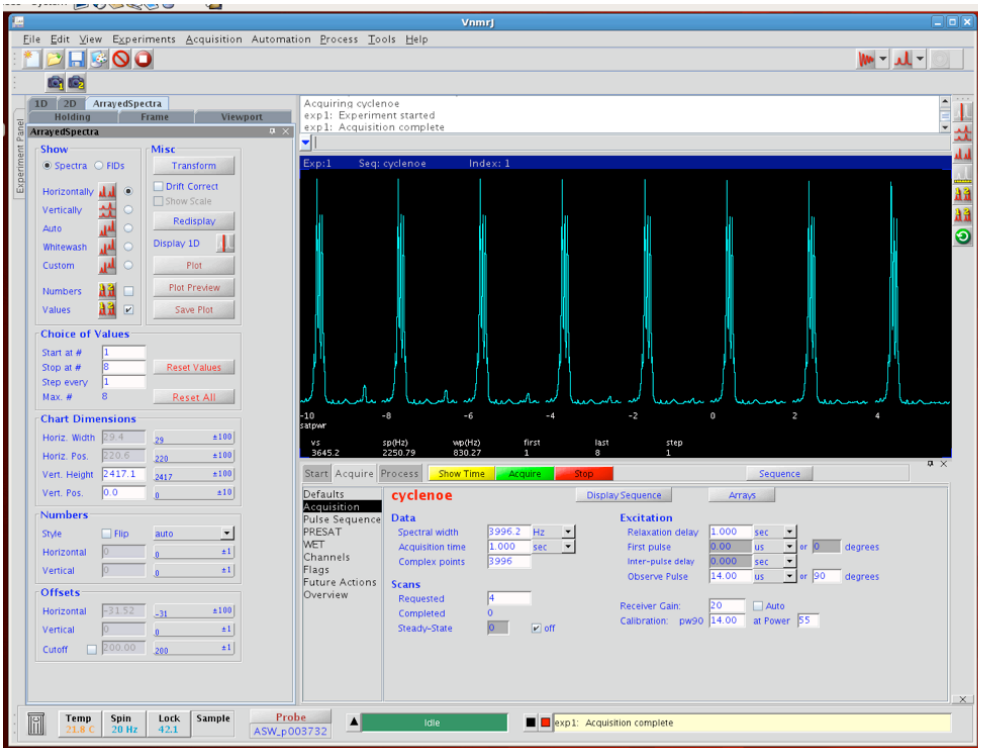
Acquiring cyclenoe
exp1: Experiment started
exp1: Acquisition complete
Exp:1 Seq: cyclenoe Index: 1
Show
Misc
Transform
Choice of Values
Chart Dimensions
Numbers
Offsets
Start
Acquire
Process
Show Time
Stop
Sequence
Defaults
Acquisition
Pulse Sequence
PRESAT
WET
Channels
Flags
Future Actions
Overview
cyclenoe
Display Sequence
Arrays
Data
Scans
Excitation
Temp
Spin
Lock
Sample
Probe
ASW_p003732
Idle
exp1: Acquisition complete

[2]

[1]


Vnmrj
File Edit View Experiments Acquisition Automation Process Tools Help
ArrayedSpectra
Acquiring cyclenoe
exp1: Experiment started
exp1: Acquisition complete
Exp:1 Seq: cyclenoe Index: 1
Start Acquire Process Show Time Acquire Stop Sequence
cyclenoe
Display Sequence
Arrays
Data
Spectral width 3996.2 Hz
Acquisition time 1.000 sec
Complex points 3996
Scans
Requested 4
Completed 0
Steady-State 0 off
Excitation
Relaxation delay 1.000 sec
First pulse 0.00 us or 0 degrees
Inter-pulse delay 0.000 sec
Observe Pulse 14.00 us or 90 degrees
Receiver Gain: 20 Auto
Calibration: pw90 14.00 at Power 55
Temp Spin Lock Sample Probe
Idle
exp1: Acquisition complete


Vnmrj
File Edit View Experiments Acquisition Automation Process Tools Help
ArrayedSpectra
Acquiring cyclenoe
exp1: Experiment started
exp1: Acquisition complete
Exp:1 Seq: cyclenoe Index: 1
Start Acquire Process Show Time Acquire Stop Sequence
cyclenoe
Display Sequence
Arrays
Data
Spectral width 3996.2 Hz
Acquisition time 1.000 sec
Complex points 3996
Scans
Requested 4
Completed 0
Steady-State 0 off
Excitation
Relaxation delay 1.000 sec
First pulse 0.00 us or 0 degrees
Inter-pulse delay 0.000 sec
Observe Pulse 14.00 us or 90 degrees
Receiver Gain: 20 Auto
Calibration: pw90 14.00 at Power 55
Temp Spin Lock Sample Probe
Idle
exp1: Acquisition complete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ArrayedSpectra
Show
Misc
Choice of Values
Chart Dimensions
Numbers
Offsets
Acquiring cyclenoe
exp1: Experiment started
exp1: Acquisition complete
Exp:1 Seq: cyclenoe Index: 1
cyclenoe
Data
Excitation
Scans
Defaults
Acquisition
Pulse Sequence
PRESAT
WET
Channels
Flags
Future Actions
Overview
Temp
Spin
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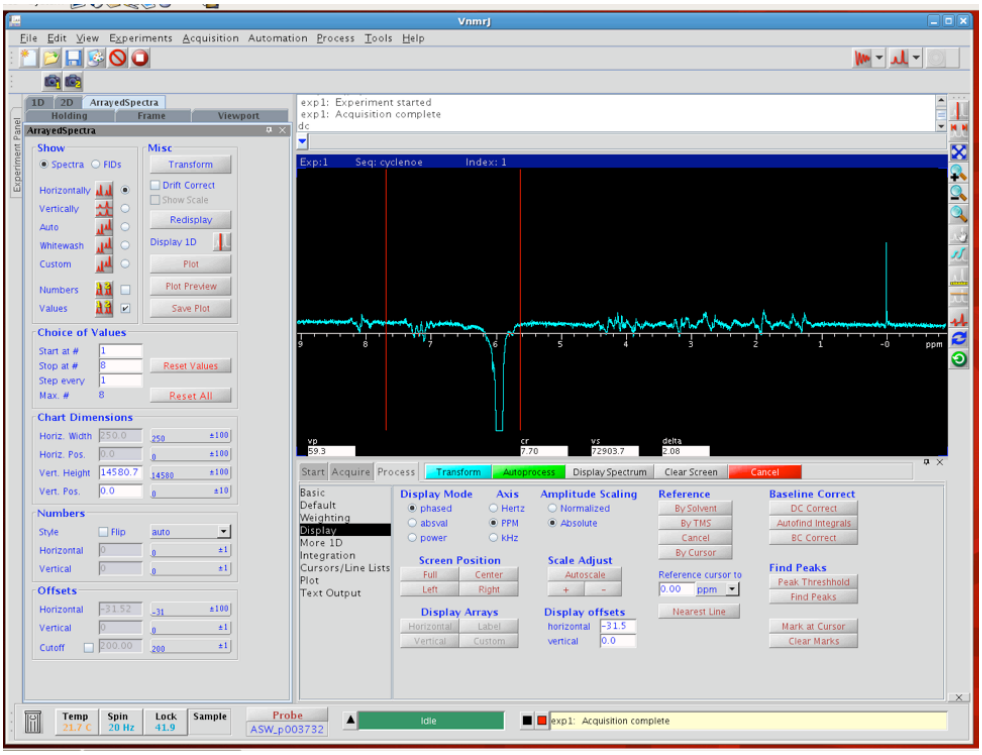
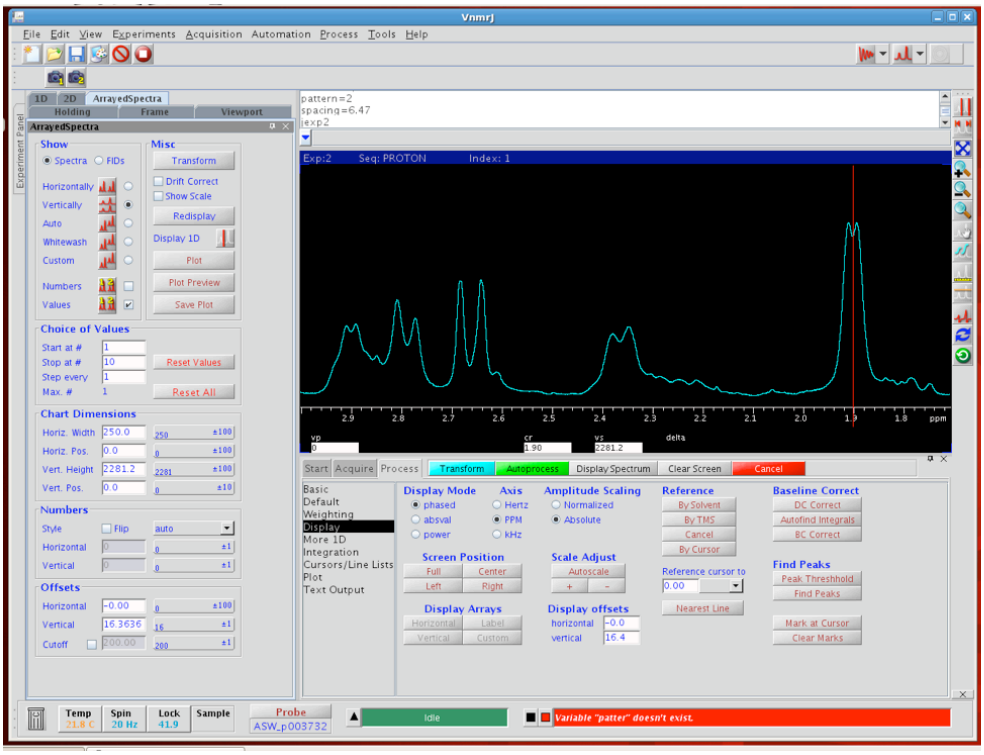
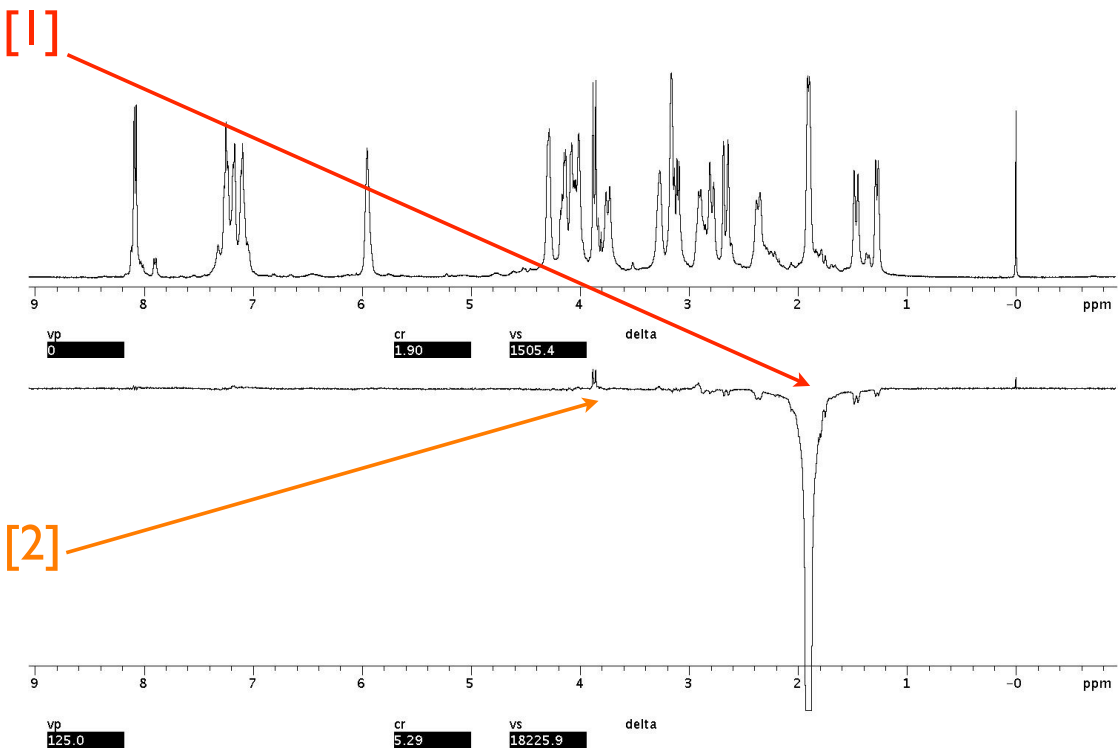
Sample
Probe
ASW_p003732
Idle
exp1: Acquisition complete


Vnmrj
File Edit View Experiments Acquisition Automation Process Tools Help
ArrayedSpectra
exp1: Experiment started
exp1: Acquisition complete
Exp:1 Seq: cyclenoe Index: 1
Display Mode
Axis
Amplitude Scaling
Reference
Baseline Correct
Screen Position
Scale Adjust
Find Peaks
Display Arrays
Display offsets
Temp
Spin
Lock
Sample
Probe
ASW_p003732
Idle
exp1: Acquisition complete


Vnmrj
File Edit View Experiments Acquisition Automation Process Tools Help
ArrayedSpectra
Show
Misc
Choice of Values
Chart Dimensions
Numbers
Offsets
Exp:2 Seq: PROTON Index: 1
Start Acquire Process Transform Autoprocess Display Spectrum Clear Screen Cancel
Display Mode
Axis
Amplitude Scaling
Reference
Baseline Correct
Screen Position
Scale Adjust
Find Peaks
Display Arrays
Display offsets
Temp Spin Lock Sample Probe
Idle
Variable "patter" doesn't exist.


[1]
[2]
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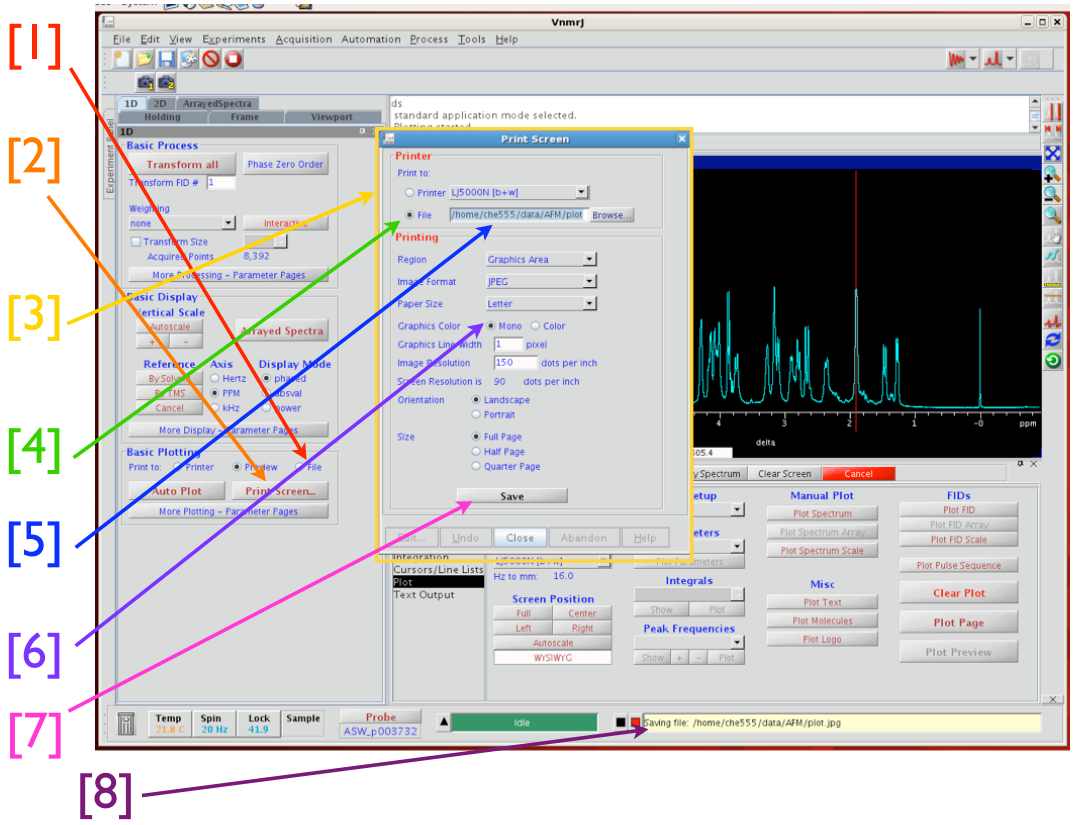
vp 0
cr 1.90
vs 1505.4
delta
vp 125.0
cr 5.29
vs 18225.9
delta


[1]
[2]
[3]
[4]
[5]
[6]
[7]
[8]
VnmrJ
File Edit View Experiments Acquisition Automation Process Tools Help
Print Screen
Printer
Printing
Save
Close
Abandon
Basic Process
Transform all
Phase Zero Order
Basic Display
Arrayed Spectra
Basic Plotting
Auto Plot
Print Screen...
Manual Plot
FIDs
Clear Plot
Plot Page
Plot Preview
Temp
Spin
Lock
Sample
Probe
Idle