BARE BONES GUIDE TO NOE-Difference

(when irradiating several peaks/multiplets in a single experiment)

on the Inova-400&500

Note: there is a separate, simplified, set of instructions if you only wish to irradiate a single peak (or multiplet). These instructions allow you to choose several peaks to be irradiated in a single arrayed experiment.

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Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<**angle brackets**> are used to designate a key to be pressed (i.e. <**Ret**> for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button
MMB will be used to designate the Middle Mouse Button
RMB will be used to designate the Right Mouse Button
Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press <**Return**> afterwards.

Important Considerations before starting:

- This version of *steady*-state NOE replaces the former "noediff" macro. It uses the "cyclenoe" sequency, which saturates multiplet frequencies individually, using lower power. The advantage is that selectivity of presaturation is greatly improved, and artifacts are significantly reduced. The difference *FID* is generated directly, eliminating the need for mathematical subtraction of on/off resonance FIDs, and eliminating about 90% of the usual subtraction errors.
- For optimum results, the sample temperature should be controlled. To set the temperature do the following:
  - If the Temperature Control window is not already running, type **temp**, and the temperature control window should appear. Adjust the slider to the desired temperature (21 degrees C is recommended for ambient), and wait until the sample temperature equilibrates to the desired temperature. The green LED labeled "VT" will flash until the temperature is regulated, and then should illuminate continuously.
  - To disable temperature control, *click* on "Turn Temperature Control Off", and the green LED over "VT" should turn off. If the first click doesn't work, click on it again. Reduce the "Temp" window to an icon, or close it using the Right Mouse Button.

BEGIN HERE:

1) Acquire a normal ^{1}H NMR spectrum, setting **nt=4**.
2) Identifying multiplets for presaturation: Use a piece of scratch paper or your notebook here.
   a) Zoom-in on each multiplet that you wish to irradiate for NOE, one at a time.
      i) Place the cursor exactly at the center of the multiplet, and type **sd**. Note/write down the frequency displayed at the top of the window, as this will be the value for *satfrq* for this peak.
      ii) Carefully measure the coupling constant (or average frequency spacing) of the multiplet:
         (1) Use two cursors (left button/right button) to measure the frequency separation of the peaks in the multiple, *type* " **delta?** ". Write down this value..this will be the value for *spacing* for this peak.
      iii) Write down the number of peaks in this multiplet, as this will be the value for *pattern* for this peak (i.e. 1 is a singlet, 2 is a doublet, 3 is a triplet, 4 is a quartet… choose the one that best fits the overall appearance of the multiplet. If the multiplet is a doublet of triplets, but the major splitting is a doublet, choose "2" for pattern.
   b) Repeat step (2-a) above, for every multiplets that you wish to irradiate. You will be able to "array" the values for irradiation of several multiplets in a single experiment. Simply note the values (*satfrq, spacing, & pattern*) for each signal of interest, and you will use these in steps 7-10 below.
   c) Finally, select an open region of noise (not near any peaks), place the cursor there, and type **sd**. Record this frequency, as it will be used as the *control* parameter (off-resonance presaturation frequency).

3) Type **gain='y'** to turn off autogain (required when arrays are used).
4) Turn OFF the Spinner:
   a) Go into the [Acqi] panel, select [Lock] and turn OFF the spinner.
5) With your 1D spectrum on the screen, type **cyclenoe** to load the standard parameters for NOE difference.

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6) Type **satfrq=#1,#2,#3...etc.**, where #1, #2, #3,...etc. are the values determined for *satfrq* above in step-(2-a-i) above. Numbers should be separated by commas. This creates an array of frequencies for presaturation.
7) Type **spacing=#1,#2,#3...etc.**, where #1, #1, #3,...etc. are the values determined for *spacing* above in step-(2-a-ii) above. Be sure that the order matches/corresponds to the values for "satfrq" above.
8) Type **pattern=#1,#2,#3...etc.**, where #1, #2, #3,...etc. are the values determined for *pattern* above in step-(2-a-iii) above. Again, keep the order the same as "satfrq" and "spacing" above.
9) Type **array='(satfrq, spacing, pattern)'** . (pay attention to the location of single-quotes and parentheses).
10) Type **da**, and if you did everything correclty, "arraydim" should be the total number of multiplets selected for irradiation.

11) Note: You may enter only a single value each for "satfrq", "spacing", and "pattern", to check NOE for a single resonance. In this case, there is no need to perform steps 9 and 10 because no arrays will be created.

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12) Type **control=#**, where # is the frequency value determined for *control* in step-(2-c) above.
13) Type **satpwr?**, and note the value. It should be "-14". If the individual lines in your multiplet (being irradiated) are unusually broad, you might reduce this to -10 ("satpwr= -10"); however, you shouldn't need to change this parameter in most cases. satpwr=-16 is as low as the power can go.
14) Other parameters that you might want to check (type **dg** to display parameters):
   a) sattime=4
   b) d1=1, bs=8
   c) ss=4 (using 8 might minimize artifacts due to incomplete T1 relaxation)
   d) intsub = 'y'
   e) cycle = 'y'
   f) il='y'
15) Set NT (# transients):
   a) Type **nt=32** (or more). The more transients you perform, the better the results will be.
16) Type **time** to see how long the experiment will take.
17) Type **go** to start the experiment. (don't do "ga" or it will do an FFT after each block of 8 scans).
18) Type **lb=0.5** to minimize difference artifacts (try lb=1.0 for an even better result)
19) When completed, the resulting spectra will contain the difference NOE data, with the selected signal (presaturated signal) being negative. If you selected multiple (arrayed) values for *satfrq, spacing, & pattern*, you will have an array of spectra when done.
   a) Type **ds(1)** to see the spectrum for the first irradiated peak. You may process and plot this spectrum normally
   b) Type **ds(2)** to see the spectrum for the second irradiated peak, and so forth.
20) Type **svf** , followed by your filename, to save the data. If you selected multiple irradiation frequencies, all spectra will be saved in a single "fid" file.

21) Quitting:
   a) Type **jexp1** , eject your sample, and re-insert the reference.
   b) Be sure to turn the spinner back on in **[Acqi]**.
   c) Lock and shim on the reference, and sign-out in the logbook.

Processing, Analyzing, and Plotting the NOE Difference spectrum:

1) After loading the file containing the NOE spectrum (or spectra), type **wft** to process all FIDs in the dataset.
2) Display each spectrum by typing **ds(1)**, **ds(2)**, ..etc.
3) Each individual spectrum may be processed, phased, and plotted using the normal VNMR commands.
4) Use manual phasing, to phase the irradiated peak to be perfectly negative, and the residual peaks due to NOE enhancement positive.
5) Integrate the peaks using the normal procedure, making sure to include the integral region that includes the negative (irradiated) peak.
6) Since the irradiated peak will be negative, you might wish to increase "vp", to move the baseline up on the screen; however, the shift axis (*dscale*) will cross the negative peak. To prevent this, you can increase "vp", and specify that the scale be drawn at the baseline.
   a) Type **vp=60 ds**
   b) Type **dscale(12)**
   c) When plotting, the commands would be: **pl pscale(12) page**.

7) Analyzing/Quantitating the %NOE.
   a) This can be done by using the irradiated peak as an internal standard, but you should phase the selected/irradiated peak "up", and the NOE peaks will be negative. By integrating the selected peak, and setting the integral value for that region ([Set Int]) to 100 (for a CH proton), or 200 (for a CH_2 signal), the %NOE can be read directly by the integral values of the negative peaks resulting from NOE enhancement.
      i) Example 1: If a CH_3 is irradiated, and there is an NOE to a nearby CH_2 signal, you would set the integral value (using [Set Int]) to 300.0 for the selected peak, and measure the integral value for the negative (residual) CH_2 signal. Since there are 2 equivalent protons for the CH_2, you would divide the integral value by 2 for %NOE. (i.e. if the measured integral for the CH_2 were -30.0, after normalizing the positive CH_3 integral to 300.0, the reported NOE enhancement would be 15%).
      ii) Example 2: If a CH_2 multiplet were irradiated, and an NOE enhancement were observed to a nearby CH resonance, you would set the integral value for the selected peak to 200.0, and read the integrated intensity of the residual, negative signal from the CH proton. Since this is a single proton, the displayed integrated intensity is a direct measure of the %NOE enhancement.

-R.Shoemaker