The NOE difference

Justin Douglas (justindouglas@ku.edu) – KU NMR Labs v1 4/10/09

Special Note:

This experiment involves setting acquisition parameters that could potentially damage the spectrometer. Do **not** attempt to set up this experiment without first walking through it with Justin or Sarah.

Concept of the 1D NOE difference experiment:

The steady-state NOE experiment is used to assess the spatial proximity of protons in a molecule. One resonance is selectively irradiated at low power for several seconds. The effect of this irradiation is to saturate the target resonance. This means the difference in populations between the spin-up and spin-down is zero for this resonance and there is no NMR signal. However, perturbing the populations of this spin have subtle effects on the population of spins that are close in space (< ~5 Å). This is called the nuclear Overhauser effect (NOE).

To measure the NOE, we acquire (a minimum of) two spectra. The first spectrum will be irradiated at a target resonance. The second spectrum will be irradiated at a frequency far off-resonance (-5 ppm) and is called the reference spectrum. The reference spectrum is subtracted from the irradiated spectrum to generate the difference spectrum. Peaks with positive intensity in the reference spectrum are interpreted as being close in space with the irradiated peak.

How to set up 1D NOE difference experiment in Topspin:

#1) Insert sample into magnet. Lock the spectrometer. Shim Z and Z2. Note that we do not spin the sample during this experiment. Make sure the temperature controller is on. Type edte at the command line and make sure the display looks something like this:
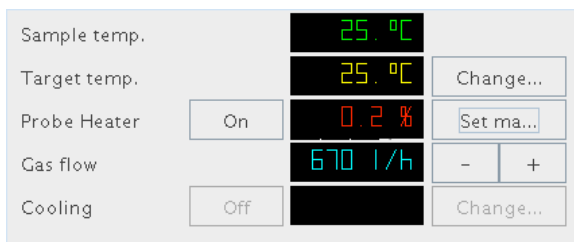

Figure 1. Bruker Temperature control window obtained by typing edte at the command line. Notice the sample temperature equals the target temperature.

#2) Acquire a 1D ^{1}H. In my case I will acquire this specrum in experiment number (experno) 1. Use this opportunity to optimize your offset (e.g. the center of your spectrum) and your sweep width. Estimate the center of your spectrum and the appropriate sweep width to cover all the peaks. Be sure to leave a few ppm on each side to use to set the off-resonance irradiation. In my case I set

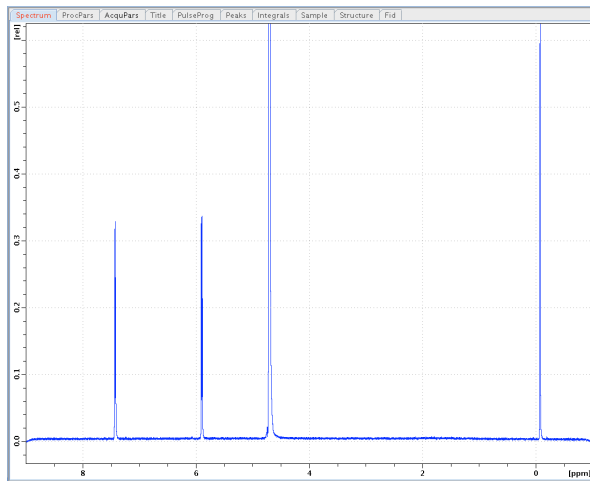o1p to 4 ppm and sw to 10 ppm (see below). Run your 1D 1H again to confirm that you still see all of your peaks.

Figure 2. 1D 1H NMR spectrum of 1 mM cytosine and 150 μM TMS in 99.9% D_2O. The offset and sweep width are set to 4 ppm and 10 ppm, respectively.

#3) Define the irradiated frequencies. To do this we define a frqlist. Zoom in on the peak you want to irradiate and click the ⬛ button. Chose the fq2list. The list name is "noedif.N" where N is an integer (1, 2, 3, etc.) that defines the total number of peaks you will irradiate, plus one for the reference spectrum. In my case, I will only irradiate on peak (at ~5.89 ppm), hence I'll create two lists, noedif.1 and noedif.2. Use the mouse to define the peaks you wish to irradiate and overwrite any existing list. Right click to save. For multiplets, it is best to irradiate each member of the multiplet to avoid Selective Population Transfer (SPT).
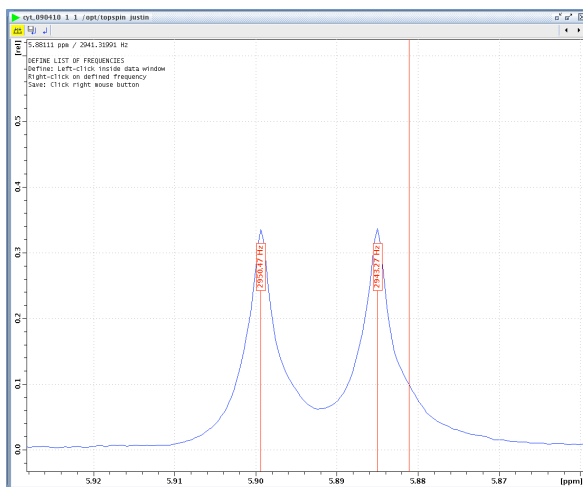

Figure 3. Definition of the frqlist. Both peaks of the double at ~5.89 ppm have been selected to minimize Selective Population Transfer (SPT).

You can create as many frqlists as you like (although this will add time to the experiment) and there can be as many peaks in a frqlist as you like. I recommend one frqlist for each multiplet you wish to irradiate. Remember to make one frqlist for the reference spectrum by picking a frequency far from any peaks.

#4) Create a new experiment by entering edc at the command line. In my case the new experiment will be experno 2. Set the following parameters (note that we do this in lieu of typing getprosol at the command line):

Parameter	Value	Comments
pulprog	noemul	
TD	16k	
ns	8	This number can be increase but ns must be a multiple of 8.
ds	4	
pl1	-5 dB	High power level for 1H channel. Double check in 1D 1H experiment.
pl14	70 dB	Power level for NOE saturation pulse. Will be optimized.
d1	30 ms	Keep in mind that the default for a delay in seconds, so enter 0.030.
d11	30 ms	
d12	20 μs	
d13	3 μs	
d20	50 ms	
l4	50	
fq2list	noedif.1	

#5) Setup the F2 channel. Type edasp at the command line. Be sure that NUC2 has been set to 1H and the logical channels are routed properly (see below).
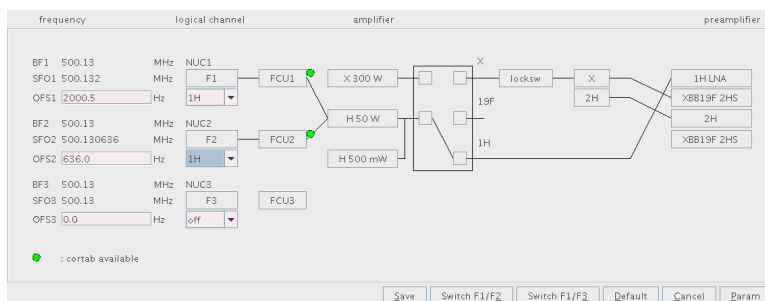

Figure 4. Spectrometer configuration window obtained by typing edasp at the command line.

#6) Optimize the irradiation power and duration. Start experno 2 (the experiment just set up) by typing zg at the command line. The experiment will run for ~ 1 minutes with ns=8. Process and phase the spectrum. Compare this spectrum

with the 1D ^{1}H. Ideally the target resonance is completely saturated by the selective irradiation and the other signals are unaffected.
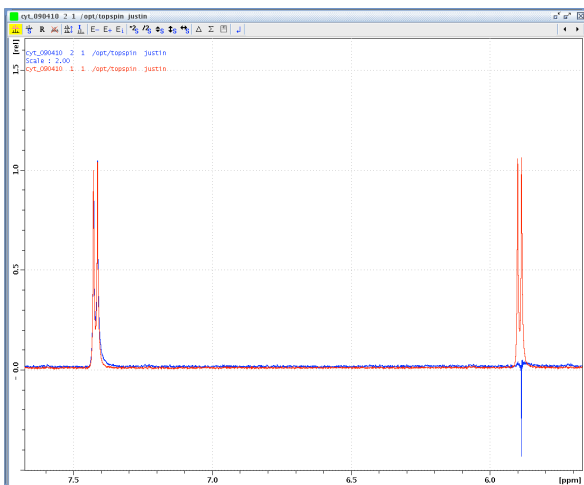

Figure 5. Comparison of 1D ^{1}H and noemul experiment with suppression of peak at ~5.89 ppm. Notice that the intensity of the noemul spectrum has been increased by two. This is to account for twice as many scans being run in the 1D ^{1}H as the noemul experiment.

There are two contingencies we need to deal with. Either the target signal is not sufficiently suppressed or the irradiation is not sufficiently selective and other signals near the target resonance are saturated. If the target signal is not sufficiently suppressed you can decrease pl14 (smaller pl14 means higher power), however **do not decrease** pl14 less than 60 dB. Too high of a power could seriously damage the probe and you will be held accountable. Alternatively you can increase l4 (be sure to increase pl14 by at least 2 dB). l4*d20 should equal the T1 of the proton you are irradiating. If you know that you T1 is very long you can increase l4. If the irradiation is not sufficiently selective you can increase pl14 (lower power) and/or increase l4.
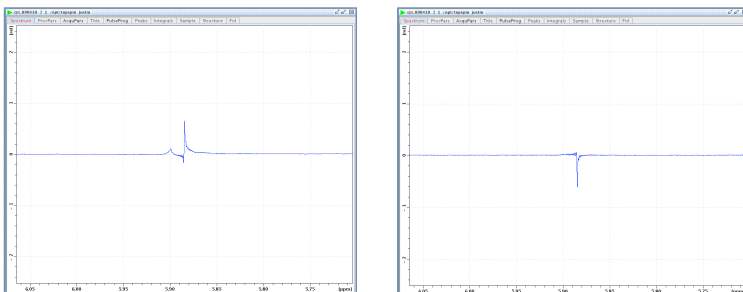

Figure 6. Suppression of the peak at ~5.89 ppm in the noemul pulse sequence. (Left) pl14 is set to 70 dB. (Right) pl14 is set to 62 dB. The suppression is not complete for either power. l4 can be increased to maximize suppression. In practice 62 dB can be used.

You should test with each frqlist you made by changing the parameter fq2list to noedif.N, where N = 1, 2, 3, etc. The off-resonance irradiation should be identical to the 1D 1H spectrum.

#7) Run the experiment. To estimate how long the experiment is type expt. Multiply this number times the number of frequency lists (in my case two) times the number of average cycles, which will be set in the xau program. We normally set these to 10, but it can be set to longer if you like. In my case the experiment

will run for approximately 20 minutes (1 min x 2 x 10). Type xau noemult at the command line to start the experiment.

Base name of all frequency lists:	noediff	
# of frequency lists:	2	(at least 2)
# of cycles through each list	50	This equals l4
# of average cycles:	10	This can be increased.

#8) Process the data. Navigate to the experiment in which you started the xau program. In my case it is experno 2 (type re 2 1 in the command line). Apply window function and FT using the command ef. Phase the spectrum. To apply identical processing to all spectra use the xau program multiefp by typing xau multiefp at the command line.

Enter the first experno to process:	2
Enter the number of experiments:	2

The "first experno to process" will be the experiment that we processed and phases correctly (in my case experno 2).

#9) Create the NOE difference spectrum. The NOE difference spectrum is created by subtracting the reference spectrum from the irradiated spectrum.

Click the ⬛ button. Drag the reference spectrum into the main window. In my case the reference spectrum is in experno 3. To figure out which experno your reference spectrum is in add the experno where the experiment was started (in my case 2) and the number of experiments minus one. Click on the delta to subtract the spectra. Click on the ⬛ button and save the result in procno 999. Exit the dual multiple spectrum menu and navigate to procno 999. Keep in mind the effect can be very modest (see below).
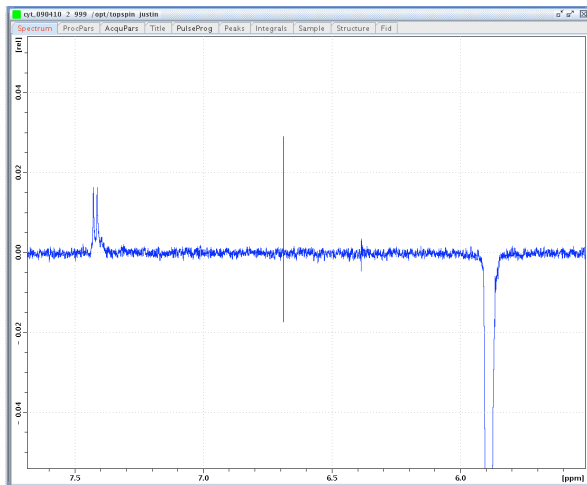

Figure 7. 1D NOE difference spectrum of 1 mM cytosine and 150 μM TMS in 99.9% D_2O. The peak at ~5.89 was irradiated and has negative intensity in the difference spectrum. The peak at ~7.4 ppm shows small positive intensity indicating that it is within 5 Å of the irradiated peak.