

Presaturation, NOE Difference and Homonuclear Decoupling

All three techniques use low-power irradiation of a specific proton resonance in a proton-observe experiment. Presaturation and NOE difference involve irradiation of a signal during the relaxation delay period, either to eliminate an unwanted signal (presaturation) or to observe the enhancement of peak intensity of protons which are close in space to the irradiated proton (NOE difference). In both cases the purpose of the irradiation is to equalize the populations of the two energy levels (spin states). Homonuclear decoupling involves irradiation at a proton peak during the *acquisition* period in order to eliminate a J-coupling to another proton resonance. This is used to identify J-coupling relationships between nuclei. The 2D equivalents of the NOE difference and homonuclear decoupling experiments are known as NOESY and COSY, respectively.

Presaturation of Solvent Resonance. This technique involves irradiating at the precise solvent frequency with a long (~ 1 sec.) low-power signal to saturate (equalize populations) the solvent protons. Then a normal high-power (non-selective) pulse is immediately delivered to excite the solute nuclei and obtain an FID. The solvent protons have no population difference ($M_z = 0$) at the time of the high-power pulse and therefore produce no magnetization in the x-y plane and no signal in the FID.

Insert the sample, lock and shim and acquire a ^1H spectrum using standard parameters (**nt=8**). Place the cursor precisely on the solvent peak and enter **sd** to set the decoupler **dof** value to the solvent peak frequency. Set up the following parameters for presaturation:

dpwr=10	Set decoupler power level (low power)
d1=1.5	Set relaxation delay (presat time period)
dm='yyn'	Decoupler mode: on during relaxation delay only
dmm='c'	Decoupler modulation: continuous wave
homo='y'	Set up for homonuclear irradiation

WARNING: DO NOT SET dpwr TO ANY VALUE GREATER THAN 40!!!! Continuous operation of the decoupler at very high power levels can literally fry the probe, your sample, and the pulse amplifiers. Pulse amplifiers are designed to deliver high power only for short durations of time, on the order of microseconds.

Start the acquisition with **go** as you normally do. Note: samples with large concentrations of non-deuterated solvent such as 90% H_2O must be run on the AM-500 instrument, which has a special water-suppression probe.

NOE difference. The 1D NOE experiment involves recording one spectrum immediately after irradiating a specific peak in the spectrum for several seconds. The irradiated peak has no difference in population between the two energy levels after irradiation, and this perturbation from the thermal equilibrium state propagates through space to perturb the population difference of nearby nuclei. The effect is to enhance the population difference for the

nearby nuclei, which leads to an enhanced signal intensity in the spectrum. A second "control" spectrum is recorded after irradiating a portion of the spectrum with no peaks, and the control spectrum is subtracted from the "irradiated" spectrum. The irradiated peak will show up as a large negative (upside-down) peak in the difference spectrum, and NOE enhancements will show up as weak positive peaks. The power level required to saturate (equalize populations) is less than that required for decoupling. The higher the decoupler power, the less selective the irradiation will be in terms of avoiding the saturation of nuclei which absorb at nearby chemical shifts. In crowded regions of the spectrum, selectivity is very important. Because this experiment involves subtracting two large peaks to obtain a small one, it is very important that all relevant conditions be the same between the two spectra. Temperature in particular must be controlled by using the variable temperature unit.

The variable temperature unit on the Unity is located in the top section of the right-hand half of the console. It has an on-off switch on the left (orange glowing button) and a digital temperature display on the right. For most routine work we leave the temperature unregulated (**temp='n'**) and the sample temperature will be essentially the temperature of the incoming air which flows through the probe and past the sample (21 or 22°C if the air conditioning is working). To operate the VT unit, simply enter the desired temperature (**temp=<temperature in °C>**) followed by **su** (setup). If you do not enter **su**, the VT will not be activated until you start the acquisition. Also, if you join another experiment (**jexpn**) or start over with standard parameters (**setup**) and start an acquisition (for example, to get a quick routine ¹H spectrum), the VT will stop heating unless you set the value of **temp** before starting the acquisition. Always return the VT unit to the unregulated (**temp='n' su**) state and make sure the probe temperature returns to room temperature before exiting VNMR and logging off.

Insert the sample and lock but don't shim. Set up for a standard ¹H acquisition and set **pw** to the 90° pulse width for ¹H at **tpwr=63** (posted on the blackboard; typically around 15 μs). Start the VT heating towards 30°C (**temp=30 su**) and wait until the display consistently reads 30.0. Now you can shim (shims are dependent on temperature). Acquire a simple 1D spectrum (**nt=8**) and transform and phase it. Determine the **dof** values for the peaks you wish to irradiate: For each resonance, place the cursor on the center of the multiplet and enter **sd** (set decoupler), writing down the **dof** value. In addition to the peaks of interest, select a control frequency by placing the cursor in the middle of a noise region and entering **sd**. Set the decoupler offset (**dof**) to an array of these **dof** values, using the control value as the last one. Now set up for the NOE difference experiment:

gain='y'	turn off the autogain and use the gain value already determined
nt=128	number of transients for each dof value
bs=8	block size: number of transients collected at one time, before moving to a new dof value
d1=3	3 seconds of irradiation before pulse and acquisition
il='y'	interleaved acquisition: move to next dof after bs transients
dpwr=4	decoupler power set very low for selectivity
homo='y'	homonuclear decoupling on
dm='yyn'	decoupler mode: decoupler on only during the relaxation delay
dmm='c'	decoupler modulation: continuous wave
pw	set to 90° ¹ H pulse calibrated at tpwr=63

Enter **da** to view the **dof** array; check to be sure that all of the decoupler frequencies have been entered correctly. Enter **time** to see how long the entire acquisition will take. Start the acquisition with **go**. When the acquisition is complete, save the array of FIDs (**svf**). Print out the parameters and the **dof** array (**printer='DeskJet' printon dg da printoff**). When you are finished with the spectrometer, enter **temp='n' su** to disable the VT and make sure that the temperature reading goes down to ambient temperature.

To process the data, enter **wft** to transform all five FIDs in the array. To plot all of the spectra in a vertical stacked plot, enter **ds(1)** to display the first spectrum. Phase correct it and set the reference chemical shift (e.g., TMS = 0.0 ppm). Enter **vsadj** to set the vertical scale (**vs**). Divide **vs** by the number of spectra in the array (number of **dof** values) to allow for n spectra displayed vertically (**vs=vs/n**). Set the horizontal offset to zero (**ho=0**) and the vertical offset to **vs** (**vo=vs**) and display the spectra in absolute intensity mode (**ai dss**). Edit the text file (**textvi**) to give the experiment a title and short description, and plot the spectra (**pl('all') pscale pltext page**). Remember that **pl** alone will only plot the first spectrum of an array. Notice that the irradiated peak in all but the control spectrum (determined by the **dof** value) is reduced in intensity due to saturation (equalization of populations) during the **d1** period. The NOE enhancements will probably be too small to see without subtraction of spectra. The last (top) spectrum is the "control": you may see a "glitch" at the point of irradiation.

To calculate the difference spectra, we want to subtract the control spectrum from each of the NOE spectra. This is done by using experiment 5 as a workspace for addition and subtraction of spectra. To clear (set to zero) any data that is in experiment 5, enter **clradd** (this will destroy data and parameters in experiment 5!). Enter **ds(m)**, where m is the spectrum number, to display the spectrum resulting from irradiation of the m^{th} peak (m^{th} **dof** value in array). Enter **spadd** (add spectrum) to add the data values of spectrum m to the data in experiment 5. Since experiment 5 has just been cleared, this is equivalent to transferring your spectrum to experiment 5. Now display the last (control) spectrum (**ds(n)**, where n is the total number of spectra or **dof** values in the array) and subtract it from the data in experiment 5 (**spsub**). At this point the desired difference spectrum is located in experiment 5. Join experiment 5 (**jexp5**) and display the difference spectrum (**ds**). Adjust the vertical position (**vp**) and the vertical scale (**vs**) to display the baseline at mid-screen (**vp=75**) and to place the top of the largest positive peak near the top of the screen display. Edit the text file (**textvi**) to give the difference spectrum a title and short description, including the chemical shift of the irradiated peak, and plot the difference spectrum (**pl pscale pltext page**). Join the experiment you started in (**jexp#**) and repeat the process (starting with **clradd**) for the next NOE spectrum in the array until you have processed all four.

1D NOE difference is probably the most misinterpreted experiment in all of NMR, so be very careful. In interpreting the NOE difference spectra, you will see large dispersive (up/down) peaks at many positions; these are subtraction errors and not NOEs. In J-coupled systems you may also see multiplets with distorted intensity patterns - some components may be upside-down. These result from selective population transfer (SPT) and not NOE. True NOE peaks will show up as positive peaks with normal multiplet structure, just as they would in a 1D spectrum. You can integrate these peaks and compare them to the (negative) integral of the irradiated (upside-down) peak in the difference spectrum to get an actual percent NOE value. Remember also that the *lack* of an NOE is not particularly useful information - there are a number of reasons why protons which are "close" in space may not show a measureable NOE. It is particularly difficult

to observe NOEs in flexible molecules which undergo rapid conformational changes in solution. Rigid ring systems are the most useful systems for using the NOE for stereochemical assignments. The *cis* and *trans* ¹H-¹H distances in a 5-membered ring, for example, are not different enough in most cases to permit a clear assignment of stereochemistry. If you are trying to prove you have a particular stereoisomer, it is best to study both isomers by 1D NOE difference or 2D NOESY and compare the NOE intensities.

Homonuclear decoupling. Acquire a normal 1D ¹H spectrum (temperature need not be controlled) using a 90° pulse width for the ¹H pulse. Transform and phase the spectrum, set the cursor on the each of the ¹H peaks you wish to decouple and enter **sd** to display the **dof** value corresponding to that frequency. Place the cursor on a region of noise and enter **sd** to get the final (control) **dof** value. Write down each **dof** value and the corresponding chemical shift, and enter the **dof** values into a **dof** array (**dof** = <dof₁>, <dof₂>, ..., <dof_n>). Enter **da** to display the array values and check to see that they were entered correctly. Set parameters:

dm='nny'	Turn decoupler on only during acquisition period
dpwr=10	Set decoupler power higher than needed for NOE
nt	Fewer transients required than for NOE
il='n'	No need for interleaved acquisition
d1=1	Shorter relaxation delay than NOE since there is no irradiation during d1
homo='y'	Set up for homonuclear decoupling
dmm='c'	Decoupler modulation: continuous wave
gain='y'	Autogain disabled because experiment is arrayed
pw	Set to 90° pulse width for ¹ H at tpwr=63

Enter **time** to check the time required and **go** to start the acquisition. When acquisition is complete, save the array of FIDs (**svf**) and transform the FIDs to give an array of spectra (**wft**). Print out the parameters and the **dof** array (**printer='DeskJet' printon dg da printoff**).

To plot all of the spectra in a vertical stacked plot, enter **ds(1)** to display the first spectrum. Phase correct it and set the reference chemical shift (e.g., TMS = 0.0 ppm). Enter **vsadj** to set the vertical scale (**vs**). Divide **vs** by the number of spectra in the array (number of **dof** values) to allow for *n* spectra displayed vertically (**vs=vs/n**). Set the horizontal offset to zero (**ho=0**) and the vertical offset to **vs** (**vo=vs**) and display the spectra in absolute intensity mode (**ai dss**). Edit the text file (**textvi**) to give the experiment a title and short description, and plot the spectra (**pl('all') pscale pltext page**). Remember that **pl** alone will only plot the first spectrum of an array. Notice that the irradiated peak in all but the control spectrum is either gone or severely distorted, and a sharp peak (of random phase) is present at the exact frequency of irradiation. For each resolved (non-overlapped) resonance in the spectrum, look for cases among the decoupled spectra where the coupling pattern is simplified (for example, triplet changes to a doublet) in comparison to the control spectrum by the elimination of one J coupling. In some cases the resonance pattern simply becomes "ugly" because decoupling is incomplete and you see a reduction of the coupling constant (J) of interest but it is not completely eliminated (for example, triplet changes to a doublet of doublets). Sometimes a broadened peak becomes sharp, indicating the removal of a small, unresolved long-range coupling as a result of irradiation.