cyclenoe – 1D NOE Difference Experiment

NOTE: If you are interested in qualitative or long-range NOE's, you should use the NOESY1D experiment (see NOESY1D handout for experimental details). However, you should be aware of the differences in these experiments. Please read below and see table.

Cyclenoe is a steady-state NOE difference experiment. In a steady-state NOE experiment, a specific resonance (i.e. peak in the spectrum) is saturated by low power RF saturation for a designated time (sattime) and a percent increase (or decrease) in the spectrum relative to a reference spectrum is observed (hence the word 'difference' in the experiment name). During the saturation time, the NOE's will reach an equilibrium value. This is the traditional NOE experiment and the percent NOE determined from this technique will be similar to those reported in early literature.

In contrast, NOESY1D employs a series of pulses and gradients to invert a particular spin, a mixing time to allow the NOE to develop, and a read pulse to obtain a NOE spectrum. Therefore, an equilibrium value for the NOE's is not obtained. NOE's obtained from transient NOE experiments are generally lower in intensity (38% max vs. 50% for small molecules) and their relative intensities not directly comparable to steady-state NOE's. However, setup and execution of NOESY1D is faster and there are essentially no subtraction artifacts, which can be problematic for cyclenoe. The combination of very low artifacts and faster per cycle repetition make the NOESY1D experiment a good choice for routine qualitative NOE determination.

Quantification of the % enhancement, which is a typical value reported for NOE's, is not readily obtained from NOESY1D. Since the target resonance is not saturated with NOESY1D but is inverted, there will be relaxation of that signal during the mixing time. Therefore, setting the inverted peak to 100% as is typical with traditional steady-state NOE's is not appropriate. Further experiments are necessary to obtain reliable quantitative numbers. Therefore, quantification is simpler with cyclenoe.

Comparison of Transient (NOESY1D) vs. Steady-State NOE (cyclenoe).

NOESY1D	cyclenoe
Optimum NOE when mixing time equals ^{1}H T_1's-quicker recycle time and more scans per unit time	Must have saturation time larger than longest ^{1}H T_1's
% NOE is less than and not directly comparable to traditional steady-state techniques. Quantification is difficult	Larger % NOE and better S/N for same number of scans. Quantification is straightforward
Relatively insensitive to calibration errors and instrument instability	Careful calibration is recommended and a stable instrument is necessary
Essentially no subtraction or other artifacts, which allows for measurement of long range NOE's	Subtraction artifacts are common and long range NOE's difficult to detect
Setup is quick and straightforward	Setup is a little more complicated
Can generate NOE build-up curve for distance calculations	Can not generate build-up curves

Preliminary Information:

1. Ideally the sample should contain 10 to 20 mg of the compound dissolved in a solvent with a strong and sharp deuterium signal, such as acetone-*d*6 or DMSO-*d*6 (common solvents such as $CDCl_3$ and C_6D_6 are also acceptable). The sample should be filtered, if necessary, and properly degassed (3 to 5 freeze-pump-thaw cycles are usually required) and sealed in order to obtain the maximum NOE.
2. For long acquisitions (>4 hours), consider running the experiment with VT on at around 30°C to minimize temperature fluctuation.
3. The experiment should be run with the sample NOT spinning to minimize artifacts in the difference spectra.
4. The default **pw90** (90º pulse width) is usually adequate, unless the physical properties of your sample are very different from those of the 0.1% ETB standard. If a new one is determined, remember to change the parameter **pw90** to the newly determined value in part 1 below.
5. The longest T_1 for the sample, excluding TMS and solvent peaks, MUST be determined (see T_1 determination handout for procedure). This value is needed for the setting of the parameter **sattime** in the *cyclenoe* experiment.
6. If your experiment involves the selective irradiation of closely spaced or overlapped signals, you should optimize the saturation conditions, which include **satpwr** and perhaps **satfrq**, **pattern**, and **spacing** as well, in order to avoid frequency spread to the neighboring peaks. If maximum NOE is to be obtained, the minimum **satpwr** for the complete saturation of individual signals as well as the optimal **sattime** should be determined. See instructions on page 10 - 13 of this handout for the optimization of these parameters.

The following topics will be covered in this handout:

I. Collecting a Reference ^1H Spectrum.
II. Setting the Irradiation Frequencies.
III. Setting up the cyclenoe Experiment.
IV. Processing the Data and Measuring the % NOE.

Optional Optimization Procedures:

A. Optimizing satpwr or satpwrf for Irradiation Selectivity.
B. Partial Saturation for Improved Selectivity.
C. Optimizing satpwr for Complete Saturation.

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Explanation of Types of Commands Found in this Handout:

1. The vnmr software and the UNIX operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (i.e. CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.

   EXAMPLE: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by a hitting the RETURN key.

   EXAMPLE: **su**

   Hitting the RETURN key is assumed for all bold text.

3. Some commands are executed by clicking a mouse button with its pointer on a 'button' found on the screen. The execution of these commands is indicated by a two letter designation (LC {left click}, RC {right click}, or CC {center click}) followed by a word or words in bold that would appear in the 'button'.

   EXAMPLE: LC **Main Menu**

   This means to click the left mouse button with its pointer on the 'button' that says "Main Menu".

4. Some commands are executed by the mouse itself. These commands are indicated by the two-letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.

   EXAMPLE: LC (at 6 ppm)

   This means to click the left mouse button with the mouse cursor at 6 ppm.

5. Parameters are entered by typing the parameter name followed by an equal sign, the value, and a return.

   EXAMPLE: **nt=16** <rtn>

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Step I. Collecting a Reference ^{1}H Spectrum:

NOTE: It is necessary to acquire a reference ^{1}H spectrum now, instead of retrieving a spectrum that you may have acquired at another time. This is to ensure identical conditions for the reference spectrum and the NOE data.

Insert your sample and turn off sample spinning from the VNMR ACQUISTION window.

*In exp1, retrieve an appropriate shim file (if you don't have a personal shim file, type **fixshims**), select standard parameters, and set **spin='n'**.*

*If you are using VT, set it now with **temp** and **su** commands. Be sure to allow ample time for temperature equilibration prior to acquisition.*

Lock and shim as usual. When shimming is complete, set the lock power to just below saturation and reduce lock gain to maintain a lock level between 80 to 90% to maximize field stability and minimize artifacts in the difference spectra.

If necessary, determine 90º pulse following procedures outlined in '90º Pulse Width Determination' handout.

pw90= *your determined value*	skip if you did not determine 90º pulse
pw=pw90	set excitation pulse width to 90°
d1= _____	set relaxation delay to 1 to 2 x T_1(longest)
nt=1	set minimum number of transients to see all signals
ga	start acquisition with autogain
f full aph	display full spectrum to full screen and autophase

Expand around solvent peak and place cursor at top. Then,

nl rl(___p)	set reference to your solvent's chemical shift
f full	display full spectrum

Place the two cursors to include ALL signals plus 2 to 3 ppm of empty baseline on either side of the spectrum, then;

movesw	moves spectral window to cursor positions
gain='y'	turn off autogain and set gain value to that selected by autogain during previous acquisition
nt=___	set *nt* to give a good S/N reference ^{1}H spectrum
lb=0.5	larger than normal line broadening (up to 1) to suppress noise and improve peak definition

ga	start acquisition
ffa	full display and autophase

*Inspect the spectrum carefully (increase the vertical scale and expand around appropriate signals) to make sure that it is phased and referenced correctly. Phase the spectrum manually if necessary. This is important because the phasing parameters **lp** and **rp** will be used later for phasing the NOE difference spectrum.*

*If everything looks good, you will save the data as usual (e.g. **svf**).*

If signals being irradiated are multiplets, expand around them, set the threshold to include ALL lines in the multiplet, then enter:

dpf	make sure that all lines in the multiplet are picked. If not, try **dpf(0)**
plotPH	macro to plot the expanded region with peak picking in Hertz and a scale in ppm

These plots will be used later for calculating and setting the parameters **pattern** and **spacing** in the cyclenoe experiment.

Step II. Setting the Irradiation Frequencies:

NOTE: The irradiation frequencies needed for the NOE experiment are not the ones found in the expanded plots or line listing above, but the decoupler offset frequencies (*dof*) determined below.

Step IIA. SETTING THE CONTROL (OR OFF-RESONANCE) FREQUENCY:

***Choose** a frequency in an 'empty' region of the spectrum. If possible, there should be no peaks with ±1 ppm of this frequency. Place the cursor at the chosen position and type:*

sd	set the off-resonance decoupler offset frequency. Will be used for **control** setting.

Step IIB. SETTING THE ON-RESONANCE FREQUENCIES:

***Place** the cursor at the center of the signal to be irradiated (expand the appropriate areas and adjust the vertical scale to ensure the accuracy of the positioning of the cursor) and type:*

sda	set decoupler offset frequency for the selected signal and add it to the *dof* array

*For each additional signal to be irradiated, **place** the cursor at the center of the signal and enter:*

sda	set *dof* for the selected signal and add it to the array

When done with setting the on-resonance decoupler frequencies, enter:

printon da dg printoff	print the arrayed decoupler offset frequencies. Keep these values handy (see NOTE below).

NOTE: The first frequency on the printed list is the control, or off-resonance, irradiation frequency, and will be entered as the **control** below. The rest of the frequencies are the on-resonance irradiation frequencies, and will be entered as the **satfrq** below.

Step III. Setting up the cyclenoe Experiment:

NOTE: If you need to optimize the irradiation parameters for selectivity and/or complete saturation, you should do it now by following the procedures on page 6 - 7 of this handout. Also, it maybe worthwhile to run one or more preliminary NOE experiments (*i.e.*, with **nt=4** and **il='n'**) first to select the best irradiation points and/or to determine if the experiment is worth running at all.

Setup the cyclenoe experiment in exp2 as follows:

jexp2	(if *exp2* does not exist, create it first with the **cexp(2)** command, then **jexp2**)
mp(1,2)	move parameters from experiment 1 to experiment 2
cyclenoe	macro to set up the cyclenoe experiment 2
nt=____ (multiple of 32 if *intsub='y'*) (multiple of 16 if *intsub='n'*)	typically, *nt* is set to 32, 64, 96, or 128, depending on sample concentration and the size of NOE being measured

*The following are listed under the heading **SPECIAL** in the **dg** parameter group.*

intsub='y' (default setting)	internal interleaved subtraction of data acquired by on-resonance and off-resonance selective saturation

If *intsub='y'*, one difference fid will be stored for each on-resonance irradiation. If *intsub='n'*, data acquired by on-resonance and off-resonance irradiation will be stored separately. Normally, *intsub='y'* should be used because better signal cancellation and more accurate NOE measurements are achieved with internal data subtraction.

cycle='y' (default setting)	frequency cycling

If *cycle='y'*, on-resonance saturation is performed using frequency cycling according to the parameter *satfrq*, *pattern*, and *spacing*, which will be set below. If *cycle='n'*, single frequency irradiation is performed. *cycle='y'* is recommended because frequency-cycling

saturation appears to be more efficient and selective than the single frequency alternative.

control=_____	This is the off-resonance irradiation frequency determined in Step IIA (inactive if *intsub='n'*)

satpwr=_____ (-16 is recommended) selective saturation power

The limits for **satpwr** are 16 to -16, with -16 as the lowest power output and the highest saturation selectivity. Generally the minimum **satpwr** should be used, which is sufficient to achieve >95% saturation for most signals. Using **satpwr** > 0 is totally unnecessary and thus not recommended.

For Inova systems with inverse detection probes only!
satpwrf=_____ (default 4095) selective saturation power fine attenuation

This parameter is needed only on Inova systems equipped with inverse detection probes and only when there are signals near the one being irradiated. Normally, 200 to 300 Hz away is considered far enough for selective irradiation. The broader the linewidths, the further apart they need to be. The range for **satpwrf** is from 0 (min) to 4095 (max, same as not being used) and it is linear (i.e. a setting of 2048 will cut the power by half from the default value of 4095). Typical values for **satpwrf** are 4095, 2048, 1024, and 512 depending on the signal separations and linewidths. **satpwr** should be set to –16 if **satpwrf** is less than 4095.

satfrq=_____	this is the on-resonance saturation frequencies determined in Step IIB

If there is more than one **satfrq**, separate them by commas (,) to set up an array (see example under **array**). For example, three **satfrq**'s would be set by entering ***satfrq=-1000,-560,-200***. If you do array, you must use **array** described later.

sattime=_____	total length of saturation at the specified **satfrq**

Ideally, **sattime** = 5 x T_1(longest). However, if T_1(longest) is too long (e.g., >10 sec), **sattime** may be set to < 5 x T1(longest), but it should be > 3 x T_1(longest), to shorten the experiment time at the expense of lower %NOE's. See note at the end of this handout for a discussion on the effect of **sattime** on the observed %NOE's.

pattern=_____ number of lines in the signal being irradiated: 1 for singlet, 2 for doublet, 3 for triplet, *etc*.

If the peak being irradiated is a broad or unresolved signal, set **pattern**=10 and set the **spacing** to cover the bandwidth. If more than one signal is being irradiated and their **patterns** are different, separate them by commas, and make sure that they are in the same order as the corresponding **satfrq** (see example under **array** below).

spacing=_____ spacing in Hz between adjacent lines in the multiplet

This parameter is calculated from the difference between the outer lines of the multiplet (Δ) and the **pattern** determined above: **spacing = Δ/(pattern-1)**. If the signal is broad or unresolved, adjust the signal to full vertical scale, measure the width of the signal at the base, and use it as Δ. If the signal is a singlet, set **spacing=0**. Again, if more than one signal is being irradiated and their **spacing** are different, separate them by commas and make sure that they are in the same order as the corresponding **satfrq** (see example under **array**).

mix=0 (default setting) mixing time

This is a delay between the end of the saturation period and the beginning of the excitation pulse, which is NOT needed for most applications.

tau=_____ time spent on a single irradiation point during the saturation of a signal using frequency cycling

The setting for this parameter is dependent on the length of **sattime**. A rough guideline is provided below:

For	$0 <$ **sattime** ≤ 10	set **tau** = 0.1
	$10 <$ **sattime** ≤ 30	**tau** = 0.2
	$30 <$ **sattime** ≤ 50	**tau** = 0.3

NOTE: If **tau** is too short for a given **sattime**, when you type **ga**, the error message "*Acode overflow, ...*" will appear and the experiment will be aborted. If that happens, increase **tau** in 0.1 sec steps until the system accepts your **ga** command. If **sattime** > 50 sec, consider running the experiment with **sattime** < $5xT_1$(longest)
(see explanations under **sattime** above).

NOTE: Setting of this parameter is necessary *only if more than one parameter is being arrayed. This means that if you are only irradiating one signal, you can skip to the next*

NOTE.

array='(*arrayed parameters separated by commas*)'

This sets up a joint array for all the arrayed parameters, which may include some or all of the following:
satfrq, **pattern**, and **spacing**.

This array will use the first value from each parameter for the first experiment; the second value from each parameter for the second experiment and so on.

da display the **array dim**(ension), make sure that the values and their orders are correct.

Example: if you are irradiating three signals with the following parameters:

satfrq=-1358.7,-1448.5,-1642.4
pattern=2,3,1
spacing=7.3,3.5,0

You should set **array='(satfrq,pattern,spacing)'** which is to make sure that the first signal being irradiated is at -1358.7 with *pattern*=2, and *spacing*=7.3, the second at -1448.5 with *pattern*=3, and *spacing*=3.5, and the third at -1642.4 with *pattern*=1, and *spacing*=0.

If the signals happen to have the same *pattern* (such as when they are all doublets), you should set **pattern=2** and **array='(satfrq,spacing)'**.

The **array dim** is equal to the number of *satfrq's*. In this example, **array dim** = 3, and with **intsub**='y', you will have three difference fid's and spectra at the end of the experiment.

NOTE: The command **time** does not work for this experiment. You should use the following equation to calculate the time for the experiment:

time(min)=[nt*(at+ sattime)*2(array dim)]/60.

Also note that the *Time Remaining* in the Acquisition Status window is fairly accurate at the beginning of the experiment, but becomes too long as the experiment gets closer to completion.

Take a minute to double-check the experiment setup. If everything is OK, start the experiment:

ga start acquisition

Once the experiment is started, it may be aborted by "LC Abort Acq" or by typing **aa** (*no data will be retained)*, such as when you discover that a mistake was made in the setup of

the experiment, or when it has become apparent that the experiment is useless.
If data retention is desired, such as when sufficient signal-to-noise has been obtained or when you have run out of time, you should use the **sa('il')** command which will stop the acquisition at the next complete interleave cycle with retention of the data. In addition, an acquisition stopped with **sa** can be resumed with the **ra** command.

You can examine the data in the middle of the acquisition and in the same experiment that it is acquiring by following the workup procedures described in Step IV below (**wft** would be necessary in this case).

*When the experiment is finished, **save** the NOE data as usual (i.e. **svf('filename')**). This is important, as you may need to reprocess the data several times before obtaining satisfactory results.*

Step IV. Processing the Data and Measuring the % NOE:

IMPORTANT: No autophasing (*i.e.*, **aph**) should be done on the difference spectrum due to the presence of the intense inverted irradiated signal. Usually, if the reference spectrum in Step I has been processed properly (*i.e.*, phased and referenced), the difference spectrum should already be in phase and correctly referenced. If the phase is still off, adjust it manually on the inverted signal first, then on another signal as far from it as possible.

wft (needed only if reprocessing data)	weighted Fourier transformation
f full	full display, needed before applying **dc**
vp=_____ (*e.g.*, vp=100)	set vertical position of spectrum so that both positive and negative peaks can be displayed/plotted properly
LC **Integration**	enter integration routine
LC **Part Int**	enter integration subroutine with integral blanking
cz cdc dc	clear previous integral reset points, cancel previous drift correction, and apply new drift correction

Processing the Data *(*cont.*)*

Set integral zero points for all the signals observed (positive or negative, but not dispersive) *as well as for the inverted irradiated signal using **Resets**. Then enter:*

dli	display list of integrals

Find a positive number in the integral list. Note which peak this positive integral belongs to, place the cursor on that peak, and then enter:

LC **Normalize**	set integral normalization factor

***Enter** # determined by the following equation:*

$$\frac{100}{|\textit{saturated peak integral \#}|} \times \textit{positive peak integral \# from above}$$

dli	display list of normalized integrals to ensure that the saturated peak is now set to –100. Repeat process if necessary.
LC 2:No Integral	turn off integral display to simplify spectrum
pl pir pscale page	plot difference spectrum with integrals
printon da dli dg dg1 dgs printoff	print array dim, integral table, and parameter groups

Optional Optimization Procedures:

Optimization for Selectivity and/or Complete Saturation

Selectivity *vs.* Complete Saturation: Selectivity should always be the first and upmost concern in the optimization of parameters for the cyclenoe experiment, with the degree of saturation as a secondary, but still important, consideration. The saturation selectivity of a signal is affected by its distance to the neighboring signals, its irradiation bandwidth, and the parameter **satpwr**. For best results, **satpwr** should be optimized for each signal being saturated in the cyclenoe experiment, for too much power may cause frequency spread, thus lower selectivity, while too little power will result in incomplete saturation, hence lower observed NOE's.

A. Optimizing satpwr or satpwrf for Saturation Seclectivity:

After you have completed Step I and Step II above, enter:

jexp# (#=2, 3, or 4)	join exp2, 3 or 4. {If exp# does not exist, create it first with **cexp(#)**, then enter **jexp(#)**}
mp(1,#)	move parameters from exp1 to exp#
cyclenoe	macro to set up the cyclenoe experiment
nt=4 (or a multiple of 4)	minimum *nt* for a reasonable S/N ^{1}H spectrum
bs='n'	no data storage during acquisition
il='n'	no interleaving
control= _____	enter the off-resonance, or control, frequency determined in Step IIA. This was the value set by the command *sd*.
satpwr=0,-4,-8,-12,-16	array *satpwr*

Optimizing satpwr (*cont.*)

OR for Inova systems with inverse detection probes

satpwrf=4096,2048,1024	array *satpwrf*

satfrq= _____	enter ONE on-resonance frequency from Step IIB
sattime= _____	set to $5xT_1$(longest)
pattern= _____	number of lines in the multiplet
spacing= _____	spacing=*(difference of outer lines in Hz)/(pattern-1)*
tau= _____	setting depends on *sattime*, see page 8 for guidelines
ga	start acquisition

When done, a set of difference spectra will be obtained (the number of spectra in the set depends on the number of elements in the array of **satpwr**).

*Expand around the irradiated signal (including the neighboring peaks) and adjust **vp** (e.g., vp=60 to 100). Enter **ai** and adjust the vertical scale with the mouse (CC), leaving room for the rest of the spectra. Then enter:*

dssa	stacked display the spectra vertically
pl('all') pscale page (optional)	stacked plot the spectra as displayed
printon da dg dg1 dgs printoff (optional)	print array table and parameter groups

*Select the maximum **satpwr** that does not cause the saturation of neighboring signals. Be aware that you may not be able to achieve complete saturation with this **satpwr** setting.*

The degree of saturation at the selected **satpwr** can be checked using the same procedure, but with **intsub='n'**.

B. Partial Saturation for Improved Selectivity:

If you are having selectivity problem even with **satpwr** set to -16, you should consider using the partial saturation method, in which only part of the signal is being irradiated. For example, for a 12-line multiplet, you may set the **satfrq**, **pattern**, and **spacing** so that only 6 lines on one side of multiplet will be irradiated. This will have the equivalent effect of increasing signal separation and reducing irradiation bandwidth. As a result, higher selectivity is achieved, but at the expense of the degree of saturation and hence the size of the NOE's observed.

*The setup for this experiment is the same as that in part A above until the parameter **satpwr**:*

satpwr=-16	use the lowest *satpwr*
satfrq=-279.0,-283.8	array *satfrq* for *one signal at a time*

In this case, the first frequency corresponds to setting the decoupler at the center of the signal and irradiating the whole signal; the second frequency corresponds to setting the decoupler so that only one half of the signal will be irradiated (treat that half of the signal

as if it were the whole signal and determine the parameter *satfrq*, *pattern*, and *spacing* for it.)

sattime= _____	set to $5xT_1$(longest)
pattern=12,6	array *pattern*
spacing=1.63,1.68	array *spacing*
tau= _____	setting depends on sattime, see page 8 for guidelines
array='(satfrq,pattern,spacing)'	set up a joint array of these three parameters
ga	start acquisition

When done, a set of difference spectra will be obtained. Process the data as described in part A above, then select a set of **satfrq**, **pattern**, and **spacing** that gives the desired selectivity. If more than one set meets the selectivity requirement, select the one that gives the most complete saturation. The degree of saturation with the selected **satfrq**, **pattern**, and **spacing** can be checked using the above procedure with **intsub='n'** and **array=''** (two single quotation marks).

C. Optimizing satpwr for Complete Saturation:

When selectivity is not a problem and maximum NOE or quantitative analysis is required, **satpwr** should be optimized to achieve complete saturation of the signal. This is usually NOT necessary for routine applications due to the efficiency of the frequency-cycling irradiation (**cycle='y'**), even with **satpwr=-16**.

The setup for this experiment is the same as that in part A above except for the parameter **intsub**:

intsub='n'	no internal interleaved data subtraction

NOTE: The parameter **control** is inactive when *intsub='n'*, so you don't need to set it. Since no control data is being collected, **time(min)=[nt*(at+ sattime)*(array dim)]/60**. Also note that the *Time Remaining* in the Acquisition Status window works rather well for experiments with *intsub='n'*.

When done, a set of on-resonance saturated spectra (not difference spectra!) will be obtained.

For the first spectrum, integrate the irradiated signal along with a couple of single-proton signals as the reference (preferably the reference signals should not have NOE's with the irradiated signal).

Normalize the integrals (set **ins**=***integral of reference signal/100****, then* **dli***) and plot the spectrum with* **pl pir pscale page** *and/or print the integral table with* **printon dli dg printoff**.

For the rest of the spectra, just enter **ds(#) ipart ins=1 dli**, *and* **ins==*integral of reference signal/100*, dli**, *and plot and/or print the results.*

ADDITIONAL NOTES:

1. Another use of the NOE difference experiment is to fish out buried signals, whose patterns and coupling constants may otherwise be impossible to determine.
2. The length of **sattime** does not seem to have any appreciable effect on the frequency selectivity or the degree of saturation in the *cyclenoe* experiment. However, as mentioned earlier, it does have a significant effect on the size of the observed NOE's. There is a direct correlation between the observed %NOE and the ratio between **sattime** and the T_1 of the observed signal. Different signals will have different correlation curves: some reach the maximum at 5 - 6xT_1 while others peak at 10 - 12xT_1; some may level off after reaching the maximum while others actually drop once they are over the hill.
3. Sometimes, it may be necessary to array **sattime** in order to determine the optimal ratio between **sattime** and T_1(observed signal) for achieving the maximum NOE. Optimization of **sattime** can be done by following the same procedure as described in part A above, except that **sattime** is arrayed instead of **satpwr**.

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