NOESY1D-(DPFGSE NOE) Experiment for Measurement of Transient NOE’s

NOESY1D is the Varian implementation of the 1D NOE experiment using pulsed field gradients and selective pulses developed by Shaka et al. At the heart of the experiment is a double-pulsed-field-gradient-spin-echo (DPFGSE) pulse sequence that achieves selective spin inversion effectively and cleanly.

It is important to recognize that NOESY1D is a transient NOE experiment, which differs from the traditional steady-state NOE experiment (cyclocoe on Varian). In a steady-state NOE experiment, like cyclocoe, 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. During the saturation time, the NOE’s will reach an equilibrium value. 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 are not directly comparable to steady-state NOE’s.

An example of a steady-state cyclocoe and a NOESY1D experiment are shown below. With the same number of scans, cyclocoe gave 2 to 3 times the S/N. However,
because the cyclenoe experiment requires the saturation time to be greater than the longest $^1H T_1$, the total time to acquire the spectrum was ~70 minutes for cyclenoe and ~11 minutes for NOESY1D. Furthermore, there are essentially no subtraction artifacts in the NOESY1D spectrum while the cyclenoe spectrum has obvious artifacts (e.g. between 7 to 7.5 ppm and around 2 ppm). 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.

Comparison of Transient (NOESY1D) and Steady State NOE (cyclenoe) Experiments.

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

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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>

* * * * * * * *
Running the NOESY1D Experiment

Preliminaries:

- It is recommended to degas your sample prior to running the experiment because dissolved oxygen will decrease the NOE. If necessary, you should also filter the sample.

- **Insert** and **Lock** as usual.

- Shim as usual.

- Since NOE’s are temperature dependent, it is recommended to run the experiment at a slightly elevated temperature. Refer to VT operation handout for adjusting spectrometer temperature.

Creating the Selective Pulses:

```plaintext
jexp1
```

join experiment 1

Setup and run a standard $^1$H experiment.

**(OPTIONAL):** For optimal performance, you can determine your 90° proton pulse, and set $pw90=\text{determined value}$. See the handout for determination of the 90° proton pulse.

When the $^1$H experiment is completed:

```plaintext
mf(1,2)
jexp2
wft
```

move the $^1$H FID from exp1 to exp2

join experiment 2

Fourier transform FID

LC View, Experiment Selector

opens Experiment selector pane

LC (H)NOESY1D

sets up parameters for NOESY1D

Expand around first resonance to be inverted.

In the **Parameter Panel** (If not open, click **View, Parameter Panel**), click **Acquire**, **Defaults**. The display should look like the following:
**LC Box/Cursor**  
Selects Cursor mode

LC on spectrum to the LEFT of the region to be inverted, then RC (Right Click) to the RIGHT of the region to be inverted. Only invert one multiplet at a time. You should have two red cursors on the screen like the image above.

**LC Select**  
selects current region between cursors for selective inversion

Repeat for all desired resonances. When completed,

If, at any point, you make an error, it is easiest to restart from \textbf{mf(1,2)}.

Select \textbf{nt=128 or desired number}  
set number of scans. MUST BE A MULTIPLE OF 8

Select **NOE mixing time**  
set mixing time. mix=500ms is the default value and is optimal for short-range interactions of small to medium-sized molecules. If you are looking for long-range interactions, mixing times comparable to the $^1$H T$_1$’s can be used

Make sure the sample is not spinning as gradient selected experiments like NOESY1D do not work with spinning.

Click the **green Acquire button** in the top middle of the Parameter Panel. **DO NOT** use \textit{ga} or \textit{go}.
Data Manipulation:

lb=1.0 wft  
set line broadening to 1.5 Hz and Fourier transform your data

vp=80 ds  
move the spectrum up and display spectrum

Phase the largest peak (this is the irradiated peak) so that it is negative (i.e. it points down). The small NOE peaks will be opposite phase to the irradiated peak.

dssa  
view all spectra in array

To view individual spectra, type ds(#), where # is the number of the desired spectrum. For example, I decided to do 5 different NOE experiments irradiating at 9, 6, 5, 3, and 1 ppm. I selected the peaks in the order given, which means that typing, ds(1), will give me the spectrum with irradiation at 9 ppm. Typing ds(4) would give me the spectrum with 3 ppm irradiation.

Integration and peak picking are done exactly like $^1$H spectra. Remember that the integrated values are NOT directly comparable to traditional steady-state NOE’s. It is best to use the values qualitatively.

Printing your NOESY1D Spectra:

To print all spectra in the array:

dssa  
look to see if the spectra overlap. If so, type ds(1) and reduce the scale of the spectrum. Type dssa. If necessary, readjust by typing ds(1), adjusting scale, and typing dssa.

pl('all') pscale page  
plots all spectra

To print individual spectra:

ds(spectrum#)  
for example, ds(1)

Phase, peak pick, and integrate as desired.

pl pir pscale pff page  
print spectrum, scale, integrals, and peak frequencies