

Selective irradiation NOEDS (NOE difference spectroscopy) experiment (selNOEi)

(István Pelczar, v.11/01/06)

The *selNOEi* experiment will acquire a set of spectra (in one file) with selected on-irradiations and one (or more) off-irradiation using a train of highly selective Gaussian pulses. The selectivity (width) of irradiation can be changed between 10 Hz and 600 Hz, the default is 60 Hz. Analysis requires subtracting the on- and off-irradiation components. It is currently available on the Varian 400, 500 and 600 NMRs (the 300 does not handle the required selective pulses).

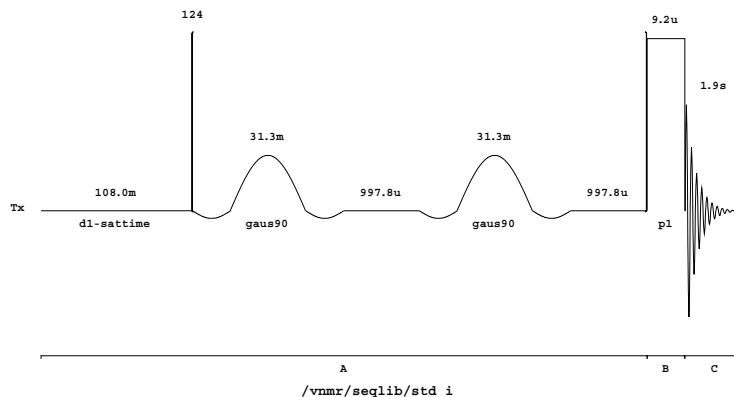
First **run a reference 1D experiment**, and save it. Do not use exp5 for this purpose, as it will be the default site to generate the difference spectra.

Identify the irradiation sites. This is easiest to do by moving the cursor over the selected site and then use the *sd* (select decoupling frequency) command. This will write the Hz position of this site on the command line (upper panel). Make a note of it, and move to the next site, etc.

Calibration of the 90deg pulse on your own sample is strongly advised; use the method looking for the 360deg pulse. The 90deg pulse is expected to be in the regime of 6-15 μ s. You may tune the probe for best sensitivity and shortest 90deg pulse possible, but it is usually not critical. Alternatively an approximate value of 8 μ s can be used. Feed this number into the pw90 storage parameter (*pw90=##*, where ## is the value of the calibrated 90deg pulse).

Receiver gain will be set (cautiously) to 22 by the macro in the next step. If it will be too much and you'll see an "ACD overload" message reduce it further down (*gain=##*, where ## is the desired number – the lower the less amplification you get on the receiver side). If you wish to take advantage of the highest possible receiver gain setting you'll have to run a test experiment first with *ss=2 nt=1, d1=10-at*, and *gain='n'*, then check the result with *gain?*. Set the receiver gain to few units less than the reported value (*gain=##*).

Call the *selNOEi* macro (simply type *selNOEi*), it will set up the default parameters for the NOE difference experiment. The pulse sequence will be popped up onto the screen like this:



Specify the irradiation sites. Feed all the numbers you recorded as irradiation sites and one or more extreme value (-10000 or 10000) for off-irradiation into the *satoff* (saturation offset) parameter separated by commas, no space applied. It may be useful to insert an additional off-irradiation site somewhere in the middle of a list if you have many irradiation sites to target, and using the better one for data processing. This is to better compensate for small instabilities caused by slight heating of the sample due to irradiation. The list may look something like this (where ##-s are your irradiation sites in Hz):

satoff= -10000, ##,##,##,...,10000, ##,##,##

The command *da* (display array) will list these values in the text window to check them.

The default setting will do 32 steady state (blank) scans to reach equilibrium, then overall 128 scans for each irradiation site in 8 scan blocks, stepping through the whole set in a hypercycle for long-time averaging. Irradiation time is set to 8s, overall recycle time is 10s, and the default irradiation width is 60Hz. You can check the estimated overall run-time using the *time* command.

Parameters can be changed interactively if you like:

The selectivity (width) of the irradiation can be changed selecting 10, 20, 30, 60, 120, 300, or 600 Hz, respectively, using an additional small macro *sat##*, where *##* stands for the width in Hz.

The overall number of transients (*nt*) could be another candidate to be changed depending on your sample's concentration and the time available. Use integer multiples of 8.

The saturation time can be set to less than the default 8s (*sattime=##*, where the *##* is the desired time).

If everything is set, start your experiment with *go*.

Processing and analysis:

First of all, make sure you saved your results (use *svf*)!

Process the reference spectrum using the command: *wft(##)* where the *##* is the number of the off-irradiation spectrum in the array. Type *full* to make it full size in the graphics, adjust phase and the width of the visualized spectrum as necessary.

Next generate the difference spectra, one after each. The following string of commands will do it (can be used repeatedly):

```
jexp## clradd df(##) add df(##) sub jexp5 lb=3 wft full vp=50 dc vsadj
```

where the *##*-s designate the experiment you ran the data acquisition in, one of the on-irradiation array elements, and the reference, off-irradiation array element, respectively. The result will behave like any other spectrum, you can take integrals, make plots, etc. You can make the integral of the irradiated signal be 100 units in order to get convenient percentage values for the responses moving the cursor over this integral segment and use the *SetInt* button. It will be taken as -100 if the saturated resonance is negative. If you like, you can save this difference result into a separate file, but it will be easy to re-create it at any time later from the original data.