

Laboration 7

NOE-diff.

KR

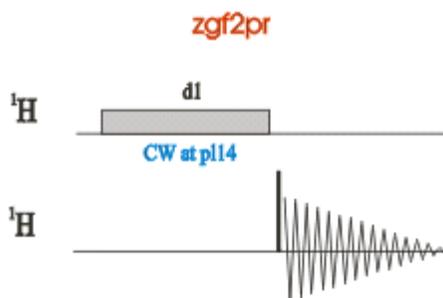
Theory

The **Nuclear Overhauser Effect** is a net change of the signal intensity from one spin due to the relaxation of a saturated spin that is dipole-dipole coupled to the first spin. NOE's develop due to through-space rather than through-bond interactions, and so contain information on the distances between spins.

The rate or efficiency of NOE buildup depends on the rate or efficiency of dipole-dipole relaxation, which itself depends on the strength and frequency of the fluctuating fields. These fluctuating fields depend on the distance between the nuclei involved, the tumbling rate of the molecule, and the characteristics of the nuclei themselves.

In a NOE difference experiment (see pulse program below), a ^1H resonance is selectively reirradiated until saturation is achieved. During the preirradiation period, NOE buildup occurs at other ^1H nuclei close in space. A $\pi/2$ pulse then creates observable magnetization, which is detected during the acquisition period. The experiment is repeated using different preirradiation frequencies, including one which is off-resonance. The latter is used to obtain a reference or control spectrum. The final spectra are displayed as the difference between a spectrum recorded with on-resonance preirradiation and the reference spectrum.

Very small phase or frequency shifts between two spectra will give rise to imperfect signal subtraction. To minimize subtraction artifacts an efficient signal averaging and maximal acceptable line broadening should be used. Other artifacts from temperature instability or magnetic field drift may be minimized by acquiring the preirradiated and the reference data in an interleaved manner.



1D NOE Difference Pulse Sequence

The pulse sequence begins with the recycle delay time **d1**. This is followed by the cw irradiation period of total time **L4*d1**. The final 90° pulse **p1** creates the observable magnetization and is followed by the acquisition period.

Several spectra are acquired during an NOE difference experiment, and for each spectrum a different irradiation frequency is used. For the reference spectrum, the cw pulse is applied off-resonance.

Practical

1. Run a *PROTON* according to "Bruker run manual for 500 MHz NMR", to see that the shimming is acceptable.

Experiment setup

Way one

1. **edc** or **new** and read the parameter set *NOEDIFF*. You will find it when you are in the correct directory. Press the arrow and choose the pathway to Brukers parametersets under Experiment Dirs. /opt/topspin21/exp/stan/nmr/**par** (Bruker)
2. Turn the spinner off, NOE experiments are recorded *without sample spinning*.
3. **getprosol** (get the preset probe and solvent specific parameters from the prosol list)
or if the ^1H 90°-pulse value need to be changed

Set the measured **p1** (^1H 90°-pulse)

getprosol 1H 'p1-value' 'p11-value' (get probe and solvent specific parameters and use your p1 value to calculate other pulses)

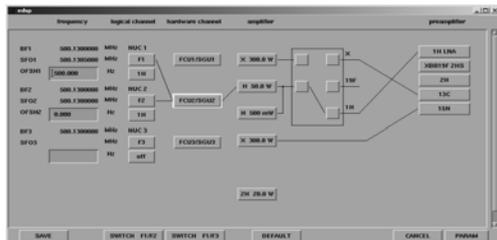
4. in the *AcquPars*,



- a. **o2p** Set it in a blank region
- b. **PL14** The power level and the duration of the irradiation (depends of the required selectivity, **120dB=no power**).

You find the parameter under the  icon

5. Type **edasp**



and click on *default* and then *save*.

6. The expected experimental time is displayed with the **expt** command or 
7. **rga** find the receiver gain
8. **zg** start the acquisition
9. Process the spectrum, **efp**, **apk**, **abs n**
10. Create another identical experiment by typing **wrpa** *exp.no.*, go to created experiment by **re** *exp.no.*
11. Set **o2p** on resonance for the selected resonance you want to irradiate
12. **pl14** start with *80 dB*
13. **zg** start the acquisition
14. **efp**, **abs n** (the spectra should be processed in the same way as the reference spectra, should have the identical phase values)
15. Repeat step 10-14 until the peak has disappeared. Change power **pl14** in steps of two-five.

Processing

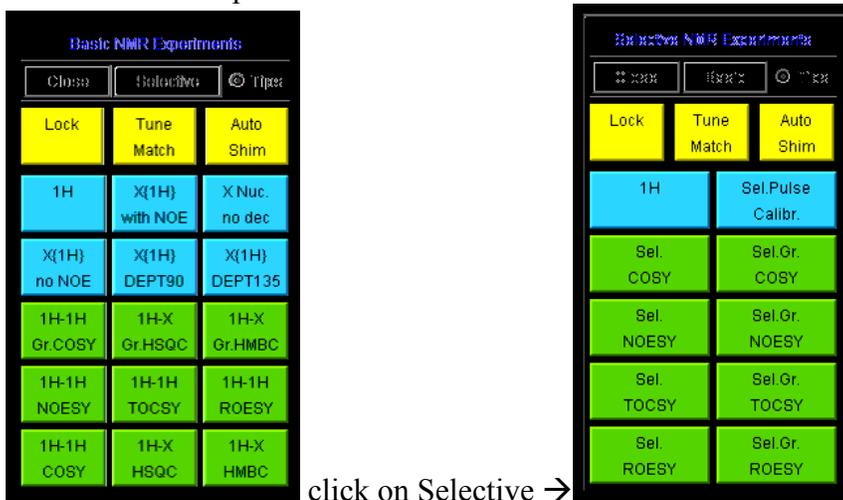
NOE difference spectra can be processed in different ways. Since one wants to observe signal intensity changes of 2% to 10%, one should use an exponential window function Process the two spectra separately using a digitally *identical phase correction*.

16. **lb=1-2 Hz** to minimize artefacts of subtraction.
17. **ef** add a window function and Fourier transform the data.
18. **apk**, perform an automatic phase
19. **abs n** perform a baseline correction
20. In the *ProcPars* section under *Phase correction* can you find the 0th and 1st order phase correction values. Use the same value in all spectra.
21. In multiple display mode  can you subtract the two spectra.
22. Save the *difference spectra* as a new process number.
23. In the difference spectrum, adjust the phase of the large signal to be negative. Evaluate only signals which have correct phase and have therefore not been affected by inadequate spectrometer stability.

Experiment setup

Way two

1. The command **bnmr** opens an icon box from which you can run basic or selective 1D and 2D.
2. Enter selective experiments



click on Selective →

3. Run a ^1H , 
4. Process the ^1H , **efp**, **apk**, **abs n**
5. Integrate the desired regions/peaks to be irradiated 
6. Save region/regions as “reg” file
7. Start the noediff experiment 

Processing

8. **lb=1-2 Hz** to minimize artefacts of subtraction.
9. **ef** add a window function and Fourier transform the data.
10. **apk**, perform an automatic phase
11. **abs n** perform a baseline correction
12. In the difference spectrum, adjust the phase of the large signal to be negative.
Evaluate only signals which have correct phase and have therefore not been affected by inadequate spectrometer stability.