# 14. 1D NOE Measurements

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#### I. Discussion

Measurements of NOE's are nontrivial, but the experiment is so powerful as an assignment aid as to make the learning curve more than worthwhile. Sanders & Hunter contains an excellent discussion and many examples of applications of NOE's in their text.

The measurement of NOE's via difference experiments should no longer be attempted, except possibly in some very special circumstances. The NOESY-1D experiment as implemented on the Varian equipment in our laboratory is a significantly (by a lot!) better experiment than NOEDIFF, and NOESY-1D should always be the first choice of experiments. DO NOT ATTEMPT NOEDIFF or NOEMULT unless you have clear reasons for doing so.

General comments about the theory:

- NOE's in general are positive for small molecular weights, and go negative for high molecular weights. The crossover molecular weight in *very* approximate terms is  $MW_c \approx 10^6/v_o$ , where  $v_o$  is the <sup>1</sup>H frequency in MHz. Thus, for compounds of MW = 1000 to 5000, NOE's may be very small or completely absent. The experiments should be tried on the smallest (Phoenix) and largest (Brutus) fields available for this MW range.
- For small *MW* compounds, NOESY experiments are often not successful. 1D NOE experiments can be performed, or 2D ROESY experiments.
- For high *MW* compounds, spin-diffusion can significantly interfere with NOE's; keeping the saturation time (<u>D2</u>) small will help avoid the spin-diffusion problems.
- In general, for small *MW* saturation of the faster relaxing proton(s) will lead to larger NOE's at the close-by proton(s) than the opposite experiment. Saturation of methyls and methylenes is often best in a small molecule. Experimental measurement of the various  $T_I$  values is necessary (see below), and spin geometry can strongly effect interpretations (see next).
- Three spin systems will often give plus-minus NOE signatures (useful for assignments!). E.g., saturating a proton close to a methylene will often give a large positive NOE to the closest proton on the methylene, which then induces a smaller negative NOE on its *gem* partner (see Sanders & Hunter, pgs. 169*ff*).
- Chemical exchange will appear in all types of NOE experiments, usually looking much like a negative NOE. Temperature dependent experiments will separate exchange from NOE phenomena.
- Observation of a true NOE can be confirmed using NOE buildup, where the increase in NOE intensity should track proportionally with length of the saturation pulse (D2 in the experiments discussed here) up to roughly  $T_1$ . See the last chapter of this guide for measurements of  $T_1$ .

#### **General guidelines for performing NOE measurements:**

- Measuring  $T_I$  for the protons of interest is highly recommended. Correct setup and interpretation of NOE experiments relies on reasonable knowledge of  $T_I$  values. See Chap. 11 for  $T_I$  experiments.
- Do not ignore assignments that can be made directly from the  $T_I$  measurements. Saunders and Hunter give a number of examples in their discussion about NOE and relaxation (see Figs. 6.14, 6.15, 6.20, 6.21).

- Saturate both sides of a through-space pair, if at all possible. Make certain, in any case, to saturate the faster relaxing proton in the pair; this should lead to larger, more easily assigned NOE's.
- For determining NOE's for a small number of clearly resolved spectral peaks, use the most straightforward experiment, NOEDIFF.AU, as discussed in section III. For many compounds, NOEMULT.AU is preferred. *Use NOESY-1D first, before trying the significantly inferior NOEDIFF or NOEMULT experiments.*
- For determining NOE's for a small number of closely spaced multiplets, use NOEMULT.AU, as discussed in section IV.
- For complex spectra, or when decoupler "spill-over" into close-by multiplets cannot be avoided, use the 2D NOESY or ROESY experiment.
- Use of a degassed sample is highly recommended for obtaining accurate NOE's. Qualitative stereochemical information can often be obtained by bubbling N<sub>2</sub> gas through the sample just prior to performing the experiment.
- Saturation pulses *must not* spill over into nearby protons, so care should be given to the choice of saturation power, S3, in both NOEDIFF.AU and NOEMULT.AU.
- Uneven saturation of a multiplet must be rigorously avoided, or selective population transfers (SPT) will be observed to the nuclei coupled to the multiplet. The resulting SPT's, which have an appearance as shown below for a simple doublet, can be as large or larger than the desired NOE's, and confuse interpretation. NOEMULT.AU helps avoid SPT's by spreading the decoupler more evenly over the multiplet than the simpler NOEDIFF.AU saturation scheme.



spt-jitt.cdr

- Frequency jitter artifacts, giving dispersive difference line shapes as shown above, can be reduced by increasing the number of scans. NS = 8 should always be used; use NE to increase the total number of scans. Frequency jitters, caused by floor vibrations, and changes in air pressure and temperature will be worse with increasing field, and during daytime hours. Use temperature control if available.
- Use the largest line broadening possible while still preserving chemical information (LB = 1 to 10) to help reduce frequency jitter artifacts.

## II. Critical Parameters for NOEDIFF.AU

D1 = relaxation time ~ 2-4  $T_1$  [want D1+AQ  $\ge$  3  $T_1$ ]

D2 = saturation time ~  $T_1$  for low MW

50 - 200 ms for higher MW (over 5000)

[4 to 5 D2 values from 0 to  $T_1$  seconds will provide buildup curve]

S3 = saturation power ~ 20L to 60L (lower number is higher power; too much power will spill over saturation into nearby proton, *avoid this!*; too low power will give incomplete saturation)

#### III. Setting Up For Well-Resolved Spectra: NOEDIFF.AU

- NOEMULT.AU described in the next section is usually preferred; see comments above.
- Obtain a normal <sup>1</sup>H NMR spectrum. Save using **WR** command.



NOEDIFF.CDR

- In <u>EP</u> subroutine, set the window using **R** <**move cursor**> **R** and <**ctrl**> **O** if you wish to obtain NOE for only that region of the spectrum (use the normal <sup>1</sup>*H* spectrum if folding interferes). Retake the 1d spectrum in this case and save using **WR** command.
- Choose a **control frequency** which is far from all the resonances (i.e. saturation pulse will stay away from all <sup>1</sup>*H* resonances). This frequency will be used to record a reference spectrum. Then, find **all irradiation frequencies** that you want to saturate, i.e. all protons that are close to moiety of interest.
- Select frequencies by:
  - moving the cursor in <u>EP</u> to desired position in the spectrum (for NOEDIFF.AU to middle of multiplet; for NOEMULT to top of multiplet peak)
  - type O2 then L. For the 1st frequency, spectrometer asks for frequency list name. Press <ret> to keep FQLIST.001, or enter any name with .001 as suffix. For rest of frequencies, position the cursor and type O2 then L. Repeat this until you have chosen the control and all the irradiation frequencies.
- <**ret**> to exit <u>EP</u> to Command Interpreter.
- AS NOEDIFF.AU <ret>

; automation setup

#### LO VCLIST.001

- 1 = 3 (the total number of frequencies you chose including control; 3 in this example) <ret>
- 2 = EN (can also type [)

D3 = O.1 (sec)

- O2 FQLIST.001 (or your own freq list name chosen in EP)
  - 1 = (control frequency) <**ret**>
  - **2** = (first irradiation frequency) **<ret>**
  - **3** = (second irradiation frequency) <**ret**>
    - ...and so on, if you have more irradiation frequencies.
  - 4 = END

S3 = 40L (decoupler power; 20L-63L range; the lower the number is, the higher the power) D1 = 2.0 (2-4  $T_1$  for  $^1H$ )

**D2 = 1.0** ( $T_1$  for  $^1H$ )

 $\mathbf{R}\mathbf{D} = \mathbf{0}$ 

 $PW = (same as {}^{1}H job file uses)$  DE = (set automatically by the spectrometer) NS = 8 (use <u>NE</u> to increase number of scans to give desired S/N) DS = 2-4 (number of dummy scans)NE = 1 (number of loops through FL)

- Larger values of <u>NS</u>, <u>DS</u>, and/or <u>NE</u> will increase the S/N ratio and also the total operating time.
- To make a change to VCLIST or FQLIST, enter VC <ret> or FL <ret>, respectively.

## **IV. Critical Parameters for NOEMULT.AU**

- D1 = relaxation time ~ 2-5  $T_1$
- D2 = saturation time at each O2 ~ 100 ms too long (> 0.1  $T_I$ ) will allow relaxation within multiplets and lead to reappearance of SPT's too short (?) will cause frequency modulations and large artifacts
- $n^2$  = number in second line of VCLIST; must be  $\geq$  largest number of O2 values used for one multiplet; VC2·(D2+D5) = total saturation length ~  $T_1$  for small MW or 50-200 ms for large MW
- S3 = saturation power ~ 30L to 60L (use lower power than in NOEDIFF; too much power will spill over saturation into nearby proton, *avoid this!*; too low power will give incomplete saturation)



NOEMULT.CDR

## V. Setting Up Best-Case Spectra: NOEMULT.AU

Set-up is the same as for NOEDIFF.AU except for the two sets of lists: VCLIST and the FQLIST, and S3.

- VCLIST contains the number of multiplets to be irradiated as in NOEDIFF in the first line, but now should also contain the total number frequencies to be irradiated on a single multiplet in the second line. Suppose you have chosen six O2 points to irradiate over for the most complex multiplet. The second line, containing  $n^2$ , must be  $\geq 6$ ; e.g., if D2 = 0.2 s and  $T_1 = 5$  s, then set  $n^2 = 25$ .
- FQLIST now is a series of lists, FQLIST.001, FQLIST.002, ..., FQLIST.00*n*, where *n* is the number of multiplets to be irradiated plus one (for the control spectrum). *n* is the first number in VCLIST.

Each FQLIST is made by entering **EP** and making the first (always the most complex multiplet), then exiting EP, and re-entering **EP** for the next list (type in the new name FQLIST.002, for example, following the first **O2** and **L**). Repeat entering **EP** until all lists are made.

FQLIST.00*n* should be the single frequency used for the control spectrum (i.e. on flat baseline)

FQLIST.001 *must* be the longest list and read in before starting AU (do this by typing FL).

• S3 should be smaller than used in NOEDIFF, if you have run this first. If you see SPT's, reduce S3.

## **VI. Acquiring NOE Difference Spectra**

• The command **EXPT** does not work; calculate the experiment time manually as:

$$EXPT = (NS + DS) \cdot (D1 + D2 + AQ) \cdot NE \cdot N_{f}$$

where  $N_f$  is the number of frequencies. If *EXPT* is too long, adjust <u>NS</u>, <u>DS</u>, or <u>NE</u> accordingly.

• AU NOEDIFF.AU <ret> or AU NOEMULT.AU <ret> DEFINE FID FILENAME #1: KL (any filename with no extension) <ret>

DEFINE FREQ. LIST FILENAME #2: FQLIST <ret>

• FIDs are now collected and stored automatically into FILENAME #1's with extensions 1 through the total number of frequencies (i.e., KL.1, KL.2, and KL.3).

## **VII. Processing FIDs**

- Work up the control spectrum first as follows:
- **AI** = 1 (absolute intensity); **RE KL.1** (to read FILENAME #1.1); **LB** = 1 (usually set to 1-10 Hz); **EF** (do <u>EM</u> plus <u>FT</u>); **EP**; **P**; phase the spectrum with **C** and **D**-knobs; **M**; **<ret>**.
- Set NE = # freqs (# files). Run AU FT.AU to transform all the data:

DEFINE FID FILENAME #1: KL (filename without extension of FID's) <ret>

DEFINE FID FILENAME #2: KLFT (any filename with no extension for FT'd spectra) <ret>

## VIII.Obtaining NOE Difference Spectrum

## A. via <u>AT</u> (additive Transfer):

- **DC** = -1 (data constant multiplied to 1st spectrum).
- AT KLFT.ref,KLFT.i1/KLDF.i1 (the 1st difference spectrum is stored as KLDF.i1);

AT KLFT.ref,KLFT.i2/KLDF.i2 (the 2nd difference spectrum is stored as KLDF.i2).

• **AT A,B/C** is equivalent to [spectrum A x DC] + spectrum B = spectrum C. The result of this operation is automatically stored into spectrum C.

## B. via dual display:

- RE KLFT.ref (read reference spectrum); EP; D (dual display); provide a name of irradiation spectrum, e.g., KLFT.i1; S (to subtract reference spectrum from irradiation spectrum); M (to memorize); <ret>; WR KLDF.i1 (to save the resulting difference spectrum into a file named KLDF.i1); repeat until all irradiation spectra are processed.
- Plot difference spectra using <u>DPO</u>, <u>CX</u>, <u>CY</u>, and <u>PX</u> commands as before, or use STACK.AU to for a stack plot.
- *Important:* Set AI = 0 and PO (decoupler power off) before leaving the spectrometer.

#### **IX. Listing of Automation Routines**

#### ; NOEDIFF.AU NOE DIFFERENCE SPECTROSCOPY ; USING ONE FREQ. LIST TO DEFINE A SERIES OF IRRADIATION POINTS ; (ON-RESONANCE) AND ONE CONTROL (OFF-RESONANCE). ; THE INDIVIDUAL FIDS ARE STORED. ; FOR LONG-TERM AVERAGING THE ROUTINE CYCLES THROUGH THE ; FREQ. LIST AND FIDS SEVERAL TIMES. ; ALSO CAN BE USED FOR PSEUDO-INDOR. 1 ZE 2 WR #1 / DEFINE FID ; PREPARE A SET OF ZEROED FILES ON DISK 3 IF #1 4 LO TO 2 TIMES C ; C = NO. OF FIDS TO BE STORED FL #2 / DEFINE FREQ. LIST ; READ IN DESIRED FREQ. LIST 5 RF #1.001 ; RESET FILE EXTENSION TO .001, BEGIN CYCLE 6 RE #1 ; READ CURRENT FID FILE 7 D3 O2 S3 ; SET DEC. FREQ. 02 FROM CURRENT FL LIST ; RELAX. TIME WITH DEC. GATED OFF 8 D1 D0 9 D2 HG ; IRRAD. TIME (CA. T1) USING POWER S3 10 GO = 8 DO; ACQUIRE DATA WITH DEC. OFF, LOOP TO 8 11 WR #1 ; STORE CURRENT ACCUMULATED FID 12 IF #1 ; INCREMENT FID EXTENSION 13 LO TO 6 TIMES C 14 IN = 5 ; LOOP TO 6 FOR EACH FREQ. IN FL LIST ; LOOP FOR ANOTHER CYCLE 14 IN = 5; NE = NUMBER OF CYCLES THROUGH LIST 15 EXIT PROGRAM REQUESTS FILENAME #1 FOR FIDS, #2 FOR FREQ. LIST. ; A FREQ. LIST MUST BE DEFINED WHICH CONTAINS ONE O2 ; ENTRY FOR EACH DESIRED IRRAD. POINT PLUS ONE OFF-RES. CONTROL ; VALUE FOR O2 WHICH SHOULD BE WITHIN THE SW REGION (E.G., AT ONE EDGE OF THE SPECTRUM). THE NUMBER OF FREQ. IN THE LIST MUST BE ; DEFINED BY AN ENTRY IN A 'VC' LIST, WHICH ALSO DEFINES THE ; NUMBER OF FIDS TO BE STORED. ; NS DEFINES THE NO. OF TRANSIENTS PER CYCLE FOR EACH O2 VALUE ; AND SHOULD BE A MULTIPLE OF 8. ; NE DEFINES THE NO. OF CYCLES TO BE MADE THROUGH COMPLETE LIST. ; TOTAL TRANSIENTS PER FID = NE X NS. ; USE 2-4 DUMMY SCANS FOR STEADY-STATE! ; RD = 0; D3 = 0.1 SEC TO SET O2 ; D1 + AQ = 2-4 X T1 FOR TRUNCATED NOE APPLICATIONS WHERE NO SECONDARY OR STEADY-STATE EFFECTS (SPIN-DIFFUSION) ARE DESIRED. D2 = CA. T1 FOR SMALL MOLECULES (EXTREME NARROWING LIMIT) ; D2 = 50-200 MSEC FOR LARGE MOLECULES (CROSS-RELAXATION). S3 DEFINES DEC. POWER TYPICALLY 35-55L DEPENDING ON REQUIRED ; IRRAD. BANDWIDTH.

; NOEMULT.AU : NOE DIFFERENCE SPECTROSCOPY USING A SERIES OF FREO. LISTS TO DEFINE ; MULTIPLE IRRADIATION POINTS FOR EACH ON-RESONANCE SITE AND ONE CONTROL ; (OFF-RESONANCE). THE INDIVIDUAL FIDS ARE STORED FOR LONG-TERM AVERAGING. ; THE ROUTINE CYCLES THROUGH THE FREQ. LIST AND FIDS SEVERAL TIMES. ; THIS TECHNIQUE ALLOWS USE OF LOWER POWER AND AVOIDS INDOR EFFECTS. ; D.NEUHAUS, J.MAGN.RES. 53, 109 (1983) ; M.KINNS & J.K.M.SANDERS, J.MAGN.RES. 56, 518 (1984) 1 ZE 2 WR #1 /DEFINE FID ; PREPARE A SET OF ZEROED FILES ON DISK 3 IF #1 4 LO TO 2 TIMES C ;C= NO. OF FIDS TO BE STORED ;RESET FILE EXTENSION TO .001, BEGIN CYCLE 5 RF #1.001 RF #2.001 /DEFINE FREQ. LIST ;READ CURRENT FID FILE 6 RE #1 ;READ CURRENT FREQ. LIST FL #2 ;SELECT SECOND 'C' FROM LIST VC VC 7 D3 O2 S3 ;SET DEC. FREQ. O2 FROM CURRENT FL LIST ;RELAX. TIME WITH DEC. GATED OFF ;TIME TO SET O2 VALUE (5 MSEC) 8 D1 D0 9 D5 HG O2 D2 LO TO 9 TIMES C ; IRRAD. C\*(D2+D5) SEC 10 GO=8 DO ;ACQUIRE DATA WITH DEC. OFF, LOOP TO 8 11 WR #1 ;STORE CURRENT ACCUMULATED FID 12 IF #1 ; INCREMENT FID EXTENSION ; INCREMENT FREQ. LIST EXTENSION IF #2 ;SELECT FIRST 'C' IN LIST VC 13 LO TO 6 TIMES C ;LOOP TO 6 FOR EACH FREQ. IN FL LIST ;LOOP FOR ANOTHER CYCLE 14 IN=5 ;NE=NUMBER OF CYCLES THROUGH LIST 15 EXTT ; PROGRAM REQUESTS FILENAME #1 FOR FIDS, #2 FOR FL LISTS

;A FREQ. LIST MUST BE DEFINED WHICH CONTAINS THE O2 VALUES FOR EACH IRRAD. ;POINT IN A MULTIPLET. THE LAST LIST CONTAINS ONE OFF-RES. CONTROL VALUE FOR O2 ;WHICH SHOULD BE WITHIN THE SW REGION (E.G. AT ONE EDGE OF THE SPECTRUM). THE ;NUMBER OF DIFFERENT LISTS MUST BE DEFINED BY THE FIRST ENTRY IN A 'VC' LIST, THE ;SECOND ENTRY DEFINES THE NUMBER OF LOOPS FOR IRRADIATION.

;NB: LONGEST FL LIST SHOULD BE THE FIRST ONE AND IN MEMORY BEFORE STARTING AU!!

;NS DEFINES THE NO. OF TRANSIENTS PER CYCLE FOR EACH FID ; AND SHOULD BE A MULTIPLE OF 8. ;NE DEFINES THE NO. OF CYCLES TO BE MADE THROUGH COMPLETE SET ; OF LISTS. TOTAL TRANSIENTS PER FID=NE\*NS ;USE 2-4 DUMMY SCANS FOR STEADY-STATE! ;RD=0 ;D3 = 0.1 SEC TO SET 02 ;D1+AQ = 2-4\*T1 FOR TRUNCATED NOE APPLICATIONS WHERE NO SECONDARY ; OR STEADY-STATE EFFECTS (SPIN-DIFFUSION) ARE DESIRED. ;SET D2+D5 AND VC COUNTER FOR 'LO TO 9' TO GIVE TOTAL DESIRED ; IRRAD. TIME, WHEREBY MINIMUM VALUE FOR D5 IS CA. 5 MSEC.

;S3 DEFINES DEC. POWER TYPICALLY 40-60L DEPENDING ON REQUIRED IRRAD. BANDWIDTH.