Use of Hermes, the Varian Mercury-300

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Contents

Normal Operation

1. A *Login* screen should be present, grey with a large number indicating the lowest numbered position available on the sample trays.

   If the *Login* screen is not present, click on **Utilities** $\rightarrow$ **Switch Operator**.

2. ***Login*** by typing your username, and your password.

3. Set your sample tube depth using the gold depth gauge.

   ➢ *Use standard NMR tubes, 9" being the maximum length. J-Young tubes can be accommodated by the robot, but only when placed in the back row (tray positions 46-50), and only for tubes that are < 11.5" in total length. Longer tubes, or tubes placed in other tray positions will likely be broken by the robot.*

   ➢ *If your sample is low on solvent(<500μl), make sure to center the sample about the dashed box in the depth gauge (marking the center of the magnetic field)*

   Make certain tray position 0 is empty and do not use position 99 or 100.

   Place your sample into an open tray position, being careful to note the position.

4. ***Click on the tray position.*** Click on the 0 in the upper left corner of the spectrum window, if present, to switch to the tray position window. Click on **Zone 1**, **2**, or **all** as applicable.

5. ***Choose your first experiment,*** by single clicking on it.

   If you make a mistake so serious you are not sure you can correct it, e.g., clicking on the wrong protocol (you selected Carbon, but really want a Proton), you can remove the protocol by simply dragging it into the trashcan (lower left corner).

6. In the **Start → Standard** panel (below the tray position/spectrum window), ***choose your solvent***. This panel is specific to your *sample*, not to the experiment. The information in it will carry over to all the experiments performed on this sample.

    a) ***Enter a sample description*** if you wish (can be left blank); this description will become part of the sample folder name (or *Studyname*), so keep it relatively short and ***do not* use special characters**. Spaces will be replaced with underscores _ .

    b) ***Enter a notebook number and page number*** if you wish (can be left blank). These will also become part of the sample folder name.

    c) The **Find Z0** and **Gradient Shim → 2H Gradient** boxes should be checked.

    d) Check or uncheck the **Plot All** box as desired.

    e) Check to make sure your e-mail address is correct (it will e-mail you when done).

7. ***If you need to change parameters***, double-click on the experiment name in yellow on the left: e.g., [1 min] Proton. The upper window bar will flash with white/black strips. After that stops, changes parameters as needed.

    Common parameter changes:
    nt → larger number (for lower sample concentrations; especially common for 13C 1Ds)
    d1 → longer value, esp. for 13C 1Ds; **d1=4** is often more optimal for observation of quaternary carbons

    Choose Update Time to see a new estimate of the total experiment time.
    Bruker → Varian parameter names (for a bigger listing, see also :
    http://www.chem.wisc.edu/~cic/nmr/Guides/VUG/AMAC_tab.pdf):

    NS → nt   AQ → at   D1 → d1   TD1 → ni

8. If you want to ***do more experiments on this sample***, goto to 5 and repeat.

9. Depending on the combined lengths of your experiments, this sample can be **Submitted** to the **DayQ** (limited to 35 min) or **NightQ** (starts at 7pm; limited to 3h *per user*, i.e., not per sample but all samples combined from one user). Use **Study Options → Update Time**, or double-click the experiment name to update the times after making parameter changes.

10. Note the Studyname, e.g., **fry_brucine_IV21_4_01** indicating:

    user_sampledescription_NotebookPage_traylocation_#use

    The last number after the tray location is the # of times that tray location has been used in the current queue (starting at _01).

11. *If you have more samples*, go to 3 and repeat.

12. *Logout* using **Utilities → Switch Operator**

13. To more explicitly learn how to set up a sample on Hermes, you may check out our interactive web site, which emulates Hermes' screens:
    http://www.chem.wisc.edu/~cic/nmr/DistanceTraining/Training1.htm

14. Your data is located on a new ftp server (ftp:\\apollo.chem.wisc.edu), until it is approximately 10 days old (see point 19 below). This server can be accessed via a web browser (IE, mozilla firefox):

    ftp://apollo.chem.wisc.edu/hermes

    If you use Windows Explorer, the server will show up similar to a disk drive on your computer. See one of the NMR staff for more assistance. The apollo server is mapped to the **R:** drive on all the facility PCs.

15. Copy the data to your PC storage device (USB key, Zip drive, or CD), or use the S:\ directory on facility PCs. Move to the **studyname** folder. To open the data in NUTS, move into the *data* directory, into the *experiment* folder (e.g., Proton_01.fid), and finally double-click on the file *fid*.

    ➢ If Use GA (File → Open) command in NUTS to import the data, rather than the IM command. GA always works; IM has problems if the folder name gets long.

16. The data can be processed within Vₙₘᵣⱼ by simply double clicking on it, or dragging it into the spectrum window (click on X in upper-left corner of the tray location window if it is present). Please do not do any extensive (more than 1 or 2 mins) looks or processing of data on hermes.

17. The simplest way to see the queue status is to look at your Study Queue window on the left side of the VnmrJ window. It will list everyone's queued experiments along with their experiment times above yours (the one you're currently setting up); NightQueue studies will follow the last DayQueue study. Another way to see the queue status is to check the Status window, which appears behind the VnmrJ panel. (If it's not there, type 'status' in the vnmr command line).

18. Note that the STUDYTIME shown in the **Status** window is the total time for all experiments requested to be run on your sample.

19. Once your data is more than 10 days old, it will be moved to your high-field data folder located on the main NMR server 'babylon6'. This folder is

    /babylon6/hf/*rgroup*/*username*/hermes (accessible from within the NMR facility)

    and is also mirrored onto the apollo snap server at:

> **ftp://apollo.chem.wisc.edu/hf/*rgroup*/*username*/hermes**

You may modify and organize this data/folder however you wish. For instructions on how to do this, see the document "Organizing Hermes' data" located on our website.

Queuing Many Samples Needing Identical Experiments Run on Them

Suppose *fry* has ten samples needing standard Proton and Carbon experiments run on them.
– the carbon experiments need only **nt=32** (samples are concentrated).

1. Put the samples into a <u>*contiguous*</u> section of the tray, e.g., locations 13 through 22.

2. Login and click on Proton, then Carbon to setup the basic experiments. Double-click the carbon parameters, and change **nt=32** (or whatever you desire, but must be n*8).

3. Fill the Study panel normally, with *sample description*, *notebook number*, and *page number*. These will be identical across all samples; only the *location* will change.

4. Be certain you make good notes in your lab notebook about which sample is in which location. You can make these notes in the *Comments* section of the Study panel; these will go into an ascii text file called *text* located in the same folder you find *fid* and *procpar* when working up the data in NUTS, and are therefore always accessible with your data.

5. Now click on the first tray location; in this case, number 13.
Hold down the SHIFT key, and click the last tray location, number 22.
All the tray locations, 13 through 22 will be circled.

6. Submit the samples to the queue as normal, to DayQueue or NightQueue.

That's it! Works fine for 2 to (don't know a limit) samples.

Acquiring NOESY1D Data on Hermes

Hermes performs reasonably well with NOESY1D, but the data quality is greatly improved at 500 or 600 MHz. As for staff assistance if data from Hermes appears to not be sufficient.

a) Submit your sample to acquire a standard ^1H 1d and let it finish.

– If you have no knowledge of the T_1 relaxation properties of your sample, you should run the T_1 experiment as described in the next section: *especially recommended if your sample is in a deoxygenated solvent.*

b) Come back after the ^1H 1d spectrum is acquired, and double-click on it to bring its spectrum up in VNMRj. Transform it into a spectrum by entering **dsxo↵** on the command line.

c) Click on the NOESY1D experiment under the SEL1D tab.

d) Double-click on the NOESY1D parameters, which will re-read in the ^1H 1d (likely with a different expansion).

e) Choose the multiplet of interest by surrounding it with a cursors box (left-drag for cursor on left/high-frequency side, right-drag for 2ⁿᵈ cursor on right/low-frequency side; click on the cursors button to activate if needed):

   – Try to keep the box symmetric about the multiplet.

   – Make the box "close-in" to the multiplet, but not "within" the multiplet.

   – The selective pulse will grab portions of spectrum about 3× the width of the box, i.e., about 1× width on either side of the box.

   – The Mercury will not perform as clean a selection as the 500s. For close-in multiplets, use the INOVA-500. The Mercury also has significantly less sensitivity than the 500s, so the 500s are by far better instruments on which to perform NOESY1D experiments. All that said, the Mercury does an adequate job for many samples.

f) Click on SELECT and then PROCEED

   – you can make multiple SELECTions prior to a PROCEED (*not recommended!!*); this will array different multiplets. It is usually easier to run a separate NOESY1D experiment for each other multiplet, as arrayed data are difficult to work with in NUTS.

g) Make certain **d1 = 3 × [longest T1 value for protons of interest]**

h) Make certain **nt ≥ 64** on the Mercury, and keep nt equal to a multiple of eight.

i) Set **mix ~ [0.7× shortest T1 value for protons of interest]**

   – longer mix times can reduce J-coupling artifacts

j) Update the STUDYNAME, select the tray position (click on 0 in the upper-left corner of the spectrum window to switch to the tray display), and then SUBMIT the study.

k) The selected multiplet should show up negative; slight phase adjustments are often needed. On the Mercury, enter

   invert dscale↵   to invert the peaks, and place the scale.

   – Positive peaks that show clean absorptive behavior are *positive NOE's*; these are common for compounds having MW < 1000.

   – Negative peaks that show clean adsorptive behavior are *negative NOE's*; common for compounds having MW > 3000.

   – The in-between MW region, and other protons depending on structure, solvent and temperature, can crossover between positive and negative NOEs, therefore giving no apparent NOE (even when in close proximity). ROESY1D should be used for these protons/compounds, as ROE's are always positive, independent of MW or other sample conditions.

   – Dispersive peaks (going positive and negative, often having an integral ~0) typically are (zero-quantum) artifacts in the experiment. True *NOE*'s will usually show a strong absorptive line shape, although some small dispersive character may be present.

Proton T_1 Measurements on Hermes

a) <u>Obtain the proper gain setting:</u>

Run a Proton 1D experiment first, letting it complete. Come back when it is finished, and double click on it to load the data. Type:

> **gain?↵**

and note the value given in the parens (6 in this case), e.g.:   **gain = Not Used (6)**

The value can also be read from **procpar** (an ASCII text file) in the **Proton_01.fid** folder:

```
gain 1 1 39 0 2 2 1 0 1 64
1 6
```

The last number in the string (6 in this example) is the gain setting. Enter the value found above – 10 (if negative, true in this example, enter 0) for the gain in the T_1 experiment.

b) <u>Setting up the T_1 experiment:</u>

1. In the experiments STD 1D panel click on, or drag to the Study panel, the **PROTON T1** experiment. This will setup a **HT1measure** experiment in the current Study.

2. Enter all normal information into the Study panel as usual (e.g., select the proper SOLVENT).

3. Decide whether to plot the data out (or not) for this experiment by checking (or not) the PLOT ALL DATA check box. ***For this experiment, plotting the data is recommended***, as a useful summary of the T_1 values will be plotted out, as well as a stack plot of the data.

4. Double-click on the yellow parameters line to load in the parameters, and click into the **ACQUIRE** panel.

5. Set **gain = gain[from Proton 1D] – 10**

   gain < 0 is not allowed, so if, e.g., you found 6 from the proton spectrum, enter **gain = 0**.

If you have a "normal" organic compound, everything at this point is likely setup OK, and you can submit the experiment to the DayQueue or NightQueue.

6. If your sample is sealed under an inert gas, or has some other unusual characteristics, you may need to modify the following parameters for the experiment:

   a) In the **ACQUIRE** panel, change the following settings:

   > **T1 mode**   **;** always select **Inversion Recovery**
   >
   > **Min T1**   ; enter the minimum T1 expected in seconds
   >   0.5 is the default, and correct for common organic cmpds
   >   0.3 for proteins/peptides
   >   1 for samples sealed under inert atms
   >
   > **Max T1**   ; enter the maximum T1 expected in seconds
   >   4 is the default, and good for most cmpds
   >   7 to 20 if MW is small and/or sample is sealed under N2 or Ar

Total Exp Time ; enter the approx length for the experiment *in hours*

b) Click on **Array Relaxation Delay d2**. The **Relaxation Delay (d1)** and **Number of Scans (nt)** parameters are updated, as is the **d2** array, which can be viewed by clicking on **Arrays** in the upper right of the **ACQUIRE** panel.

c) Reduce **Total Exp Time** slightly if the time is too long; the automated setup software rounds up or down depending **nt**, **d2** and **d1**.

7. When everything looks OK, submit the Study as normal.

c) <u>**Re-analysis and manually working up and viewing the T_1 results:**</u>

The following is specific to working the data up using the Varian VNMRJ software on Hermes, which has some advantages (it's a lot easier!) over using other software.

1. If you are working on the same day the data was taken, double-click on the **HT1measure** parameters line to read in the data. If you are working at a later time, see Charlie or Monika for assistance with how to find and read the data into the Varian software.

2. Click into the **PROCESS** panel, and then the **T1 ANALYSIS** tab. The list of buttons available there makes analyzing the data simple to do:

a) You can expand on any section of the spectrum, and the analysis will involve only those protons. The THRESHOLD setting performs the rest of the discrimination of which peaks will be analyzed.

b) The following buttons are most useful for analyzing the data:

DISPLAY LAST SPECTRUM ; perform threshold and expansion settings here

DO T1 ANALYSIS ; runs the automatic fitting and output

DISPLAY FIT SUMMARY ; outputs text listing of T1 values

DISPLAY ALL FITS ; gives graphical display, where outliers might be more easily seen

PRINT FIT SUMMARY ; prints out the text listing of T1 values

3. If you checked the PLOT ALL DATA checkbox [2.b)3 above], then you will get a listing off the printer similar to that shown in Fig. 1.

a) First there is a list of peaks picked, with chemical shift [freq(ppm)] and peak height [intensity]. The listing will run downfield to upfield, so aromatic multiplets first, aliphatics (and likely, as here, TMS) last.

b) Following the peak pick list will be a summary from a T_1 [exponential data] analysis. The index and peak numbers from the two lists correspond 1-to-1. As an example, lines 3 − 6 in Fig 1 are from four peaks of a dd multiplet. The multiplicity can most easily be seen from the T1 listing, where these 4 peaks all have a $T_1 \sim$ 9s.

c) The data for Fig 1 are from a sample of nicotine in $CDCl_3$. All samples will tend to have longer T_1 values for aromatic protons, and shorter values for the aliphatic protons (in general, T_1 decreases as the number of proton neighbors increases). This

particular sample was sealed under vacuum, and the T_1 values are 2 to 3× longer than they would be if the sample was under normal atmosphere.

Deoxygenated samples will have significantly longer ^1H T_1 values than those prepared in standard atmosphere.

d) From the data shown in Fig 1, two important numbers are found: T_1(max) = 9.2 sec, and T_1(min) = 1.5 sec. Note that TMS was ignored (the last rows, 82, in the tables). You should look closely enough at the data to detect and exclude from consideration solvent and impurity peaks. Setting d1 according to long solvent or impurity T_1 values would greatly lengthen experiments, to no profit in data quality.

d) <u>Repetition delays (d1) and *mix* times in other experiments:</u>

T_1 values affect repetition delay settings in other NMR experiments: recommendations are listed in Fig 2. The **mix** time in NOESY1D experiments is also set based on T_1 values, typically to the shortest T_1 of interest.

Fig 1: HT1measure Output

Nicotine (150mg) in $CDCl_3$

```
index    freq(ppm)   intensity
  1      8.33866      909.44
  2      8.33281      939.92
  3      8.28405      573.18
  4      8.27917      615.05
  5      8.26844      623.11
  6      8.26259      617.01
  7      7.5253       322.99
  .
  .
 41      1.9556      6414.33
  .
  .
 80      1.47285      127.42
 81      1.45432       87.63
 82     -0.21825       35.08

Exponential data analysis:
 peak     T1         error
  1      6.934       0.1823
  2      6.965       0.2287
  3      9.213       0.254
  4      9.2         0.1991
  5      9.104       0.2061
  6      9.202       0.2996
  7      5.678       0.1342
  .
  .
 41      1.532       0.0033
  .
  .
 80      2.142       0.0525
 81      2.113       0.0313
 82     12.03        0.568
```

Fig 2: Typical d1 Settings

T_1 **is the longest value found for nuclei of interest.**

Experiment	Recommendation
normal ^1H 1D	$d1 = 1 \times T_1$ (pw=30°)
Cosy	$d1 = 1.5 \times T_1$
Gcosy	$d1 = 1 \times T_1$
Hsqc	$d1 = 1.5 \times T_1$
Ghmbc	$d1 = 1.5 \times T_1$
Tocsy	$d1 = 1.5 \times T_1$
Dqcosy	$d1 = 2 \times T_1$
NOESY, NOESY1D	$d1 = 2$ to $3 \times T_1$
quantitative, kinetics ^1H 1D	$d1 = 5$ to $7 \times T_1{}^*$

* For quantitative work, it is safest to run as recommended above. But this setting is not optimum when *relative* intensities are of interest. In that case, the *difference* or *range* of the T_1 values is the critical factor. See Charlie for more information or help in setting up quantitative experiments.