Basic parameters and techniques in 1D NMR – *2017.Fu Chen*

How to get the best out of your NMR measurements

1) **Sample preparation:**
   a. Avoid solvent and/or chemical shift references that give large residual NMR signal of the same nuclei being measured.
   b. Avoid overlapping (temperature can affect the chemical shifts).
   c. Avoid using high viscous solution (temperature and solvent dependent).

2) **For dilute or limited amount of samples:**
   a. Reduce the volume from 0.6ml to 0.4 ml; align the solution to the center of NMR coil.
   b. Extreme case: use small volume NMR tube ~ 150 ul solution.

Signal to noise ratio:

$$S/N \propto n\,\gamma_e \sqrt{\gamma_d^3 B_o^3 t}$$

where n concentration; B field strength; and t time (NS).

γ_e Gyro-magnetic ratio of excitation nucleus.

γ_d Gyro-magnetic ratio of detection nucleus.

3) **Choice of Spectrometer and the detector (probe).**

   a. **AM400 MHz** **BBI probe:** H-1, 1D & 2D

   b. **500MHz** **BBO probe:** Multi-nuclei tunable best for X-nuclei, and 1D H-1.

   c. **600MHz** **Probe varies weekly.**

BBO-auto (default); C-13/H-1 (high temperature); BBI (high H1 sensitivity).

Refer to NMR web page for more details.

Relative Compare	**Routine Spectrometers**	**AM400**	**500**	**600**
H-1 sensitivity(BBI)	0.75	2	1	**2.5 (highest)**
C-13 sensitivity(BB0)	0.40	No available	1	**1.3**

4) Apply suitable Applications (Pulse sequence).

Example: Sensitivity Enhancement Methods:

Use additional available nucleus with higher γ.

- **Use NOE:** C13 with H-1 broad band decoupling. ~ 2 (max) **~~ γ_{h1} / $(2 * \gamma_{c13})$**
- **Use Polarization transfer:** **~~ γ_{h1} / γ_{C13}**

- 1D DEPT: through H-1 polarization transfer.~ Ratio of Gyromagnetic ratios
  - 2-4 for C13 observed, DEPT, with H-1.
  - - 5 for Si-29 DEPT, with H-1.
  - -9 for N-15 DEPT, with H-1

Insensitive Nuclear Detection:

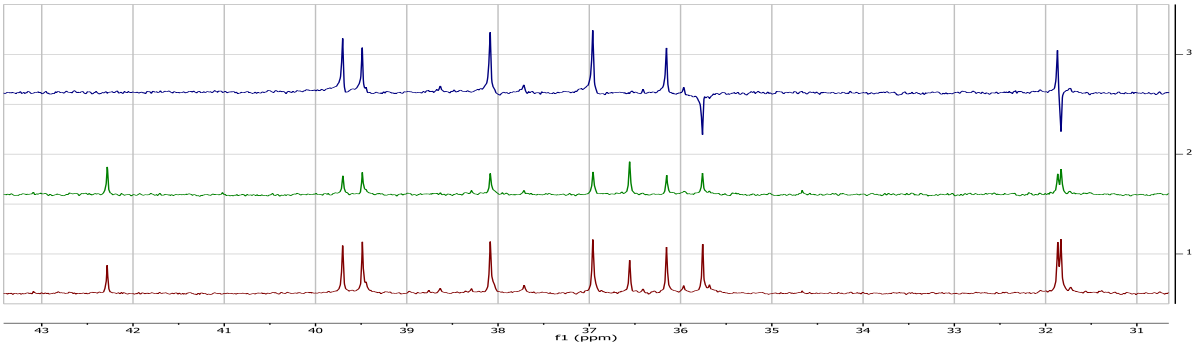
Sensitivity Enhancement Methods: ***for*** insensitive nuclei detection.

Nuclei	Relative sensitivity (Enriched)	Gyro-magnetic ratio 10^7 rad $s^{-1}T^{-1}$
H-1	100	26.75
C-13	1.59	6.73
N-15	0.104	-2.71
P-31	6.63	10.84
Si-29	0.78	-5.31

γ_p = (2.675 222 005±0.000 000 063)×10^8 rad $s^{-1}T^{-1}$.
$\gamma_p/2\pi$ = (42.577 480±0.000 002) MHz/T. This value is called gamma-bar.

Combine additional excitation of near-by nuclei (that have large γ).

- **Via NOE: C13 with H-1 broad band decoupling. ~ 2 (max) ~~ $\gamma_{h1} / (2 * \gamma_{c13})$**
- **Via Polarization transfer: ~~ $\gamma_{h1} / \gamma_{C13}$**
  - 1D DEPT: through H-1 polarization transfer.~ Ratio of Gyromagnetic ratios
    - ~ 2-4 for C13 observed, DEPT, with H-1.
    - ~ - 5 for Si-29 DEPT, with H-1.
    - ~ -9 for N-15 DEPT, with H-1.



Top: DEPT 1hr. Middle C13 no NOE 4 hrs
Bottom trace: Routine C13 with H-1 CPD decoupled 2hrs.

Signal to noise ratio:

$$S/N \propto n\gamma_e\sqrt{\gamma_d^3 B_o^3 t}$$

Frequently used common 1D NMR applications: *Must tune probe and getprosol.*

Application	**UM protocols	Bruker Pulse program	Special Parameters
1D-NOE *Confirmation* *Kinetic*	Aselnoe 1D selective NOE	selnogp	*D1;* *Offset: O1 (Hz)* *D8: mixing time*
1D-TOCSY *Total correlation via J coupling*	Asel-tocsy 1D selective total correlation	selmlgp	*D1:* *Offset: O1(Hz)* *D9: spin locking time (isotropic mixing)*
1D-solvent suppression	Ah1_presat Ah1_watergate Ah1_supress_se Ah1_WET	zgcpqppr zggpw5 zgesqp	*D1;* *Offset : O1(Hz)*

*** Read corresponding title page for help in procedure*

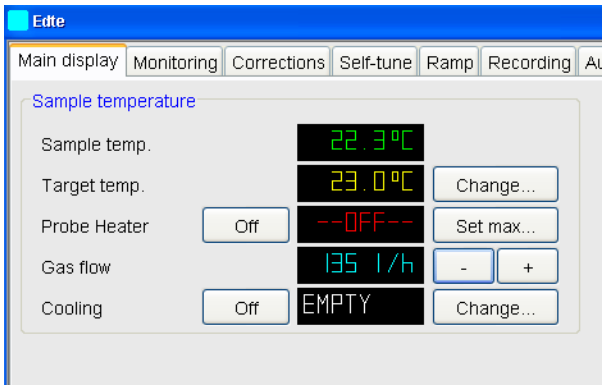
Frequently used 1D X-nuclei detection:

Application	**UM protocols	Bruker Pulse program	Special Parameters
Routine C-13 with H-1 decoupled	Ac13_cpd	zgdc30	**D1** **NS and TD0**
C-13 verse H-1 multiplicity determination	Ac13_dept135 Ac13_dept90	deptsp135 deptsp90	CNT2 (145Hz) One bond Coupling constant
C13 with H-1 inverse gated decoupled for integraton	Ac13_nonoe	zgig45	*D1: relaxation delay* *FIDRES { for accurate integration }*
For other nuclei: P31; Si-29; N-15; etc.	Axx_cpd Axx_dept135 Axx_dept90	deptsp135 deptsp90	D1 SW; o1p and o2p

Logistics

Major critical steps and difference as compared to routine spectrometers.

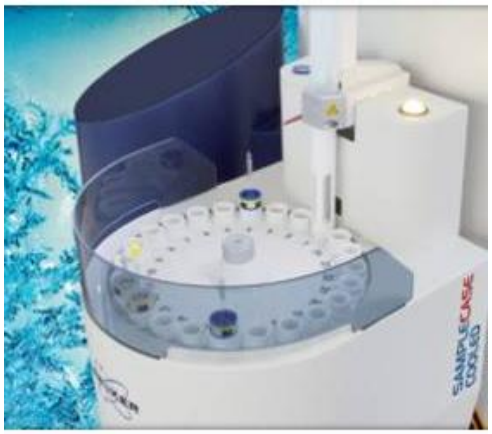
☆ 1. ***Before inserting your sample type "edte" to check temperature.***



For all routine experiments, set as follows:

Probe Heater	"Off"
Gas Flow:	135 l/hr.
Cooling:	"Off".

2. ***Sample handling***:
   a. *400MHz magnet: has standby sample.*
   b. *All High temperature operation (400, 600 and 500MHz) No standby sample (before or after the experiment).*



   c. *600 (default mode): with sample changer is activated (yellow LED light).*

   d. ***"Sx n " Insert sample (n is the position of your sample);***

   e. ***"SX 1 "(retrieve your sample and insert standby sample into magnet)***

☆ 3. *Load the application "**RPAR**" and make sure to type "**getprosol".***

4. ***Tune probe:***
   a. *400 & 500: Manually "WOBB"*
   b. *600: Manually on selective probe, "ATMA" for BBO-auto probe.*

5. ***Lock and Shim*** ("gradshimau " 500 Spectrometer or "topshim" for 400 and 600MHz spectrometers).

For temperature controlled measurements, ask staff for special training. 24 hrs advanced request is required. When your measurements are completed,

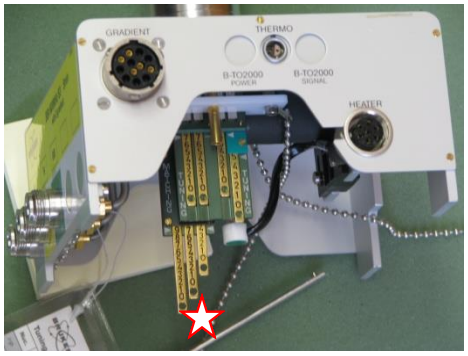
DON'T put any standby sample after you eject your sample from the magnet!

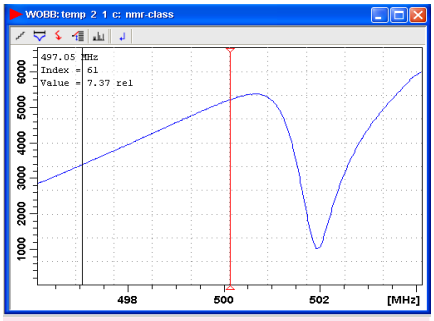
****Essential pre-requisition adjustment:** **Must getprosol before probe tune!**

***** Probe Tuning: for improved sensitivity and transfer precise excitation pulses.**
Caution: Strong magnetic field, remove your credit card etc before approaching the probe.

Command:
Manual: "WOBB" for 400, 500 and 600 without automatic tune.
Automatic: "ATMA" only available for 600 BBO probe.

Manual procedure:





Adjust M and T capacitors such that the dip is centered and shows the maxium absorption.

Figure shown is for X-nuclei tunning paddles.

- Click or type **[WOBB] to start the tune probe software.**
- First-- adjust the capacitor in the flat panels at the bottom of the probe on C13 (Request TA present the first time).

- Use the fine slider (far right) to adjust the WOBB curve:
  - T panel for centering the positioning; M panel for maximize the absorption.
  - Note the T and M adjustments could interact slightly with each other, cycle T and M a couple of times to get the best fit.

- Once X-nuclei adjustment is done, click  in the wobble window to switch to H-1 frequency.

  - Screen will change to H-1 WOBB curve in a few seconds; adjust alternatively the T and the M the capacitors similar conditions as for C13.

- When adjust is complete, click  or type "**halt**".

Further reading: ..\high-sensitive_NMR-training notes\Tune-probe-2011.2.pptx

***** Probe Shimming:**

A: Basic non-gradient Shimming: Monitor Lock level

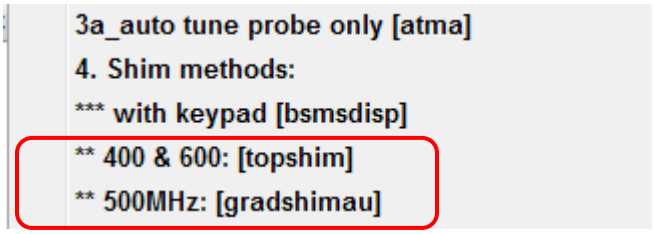
Remarks: 5000MHz and 600MHz have Different keypads layout.

- For all Z shims: press both the "ONAXIS" and corresponding Z buttons to adjust the value.
- For all non-spinning shims: press both the "Z^0 "and the desirable non-Z buttons to adjust the X, Y etc.

B: Gradient shimming: Spin OFF

600MHz and 400MHz spectrometers:

- Click menu or type "TOPSHIM".
- Command line will echo "topsphim finished", and the lock level re-appears when shimming is done.



500MHz spectrometer:

- Click GRADSHIMAU.
- Different graphic will appear in the spectral window.
- The process will take several minutes, please wait!
- When shimming is completed, the lock level re-appears again.

TIPS: To check for good shimming. Always collect a quick H-1 spectrum. Examine the line shape of any singlet in your spectrum (not a multiplex).

END-Lecture 1.

Essential basic NMR parameters & commands *for optimization:*

Excitation Pulses:

Basic strong Radio Pulse :

P(i) Pulse width; PL(i) ; PLW(i) or PLdb(i) Pulse power level

e.g. Bruker Convention
P1 90 degree pulse for nucleus to be detected.
PL1 the corresponding power to obtain the 90 degree for maximum signal.

Soft pulses :
Shape pulse (e.g. used in Solvent suppression and 1D NOE).
Gradient pulse (e.g. used in most 1D and 2D NMR measurements).

Further reading: http://www2.chem.umd.edu/nmr/umcpnote/nmr-lecture-notes.php
..\high-sensitive_NMR-training notes\Pulse-review-2012.9.pptx

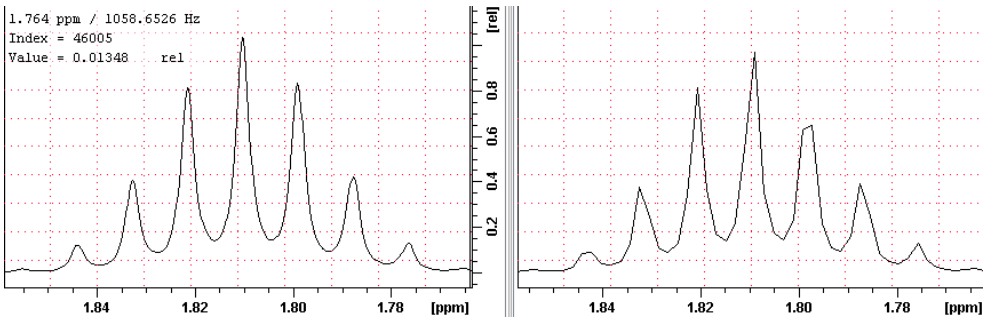
1. **D1(s)**: Relaxation delay in seconds. Typically used to allow long relaxation nuclei to return to thermo-equivalent conditions.
2. **AQ(s)**: Time for collecting the FID; acquisition time in seconds.
3. **DS**: Dummy scan, pre-data collection scans used to establish an equilibrium state of the spins with the same excitation pulse sequence, typically 4 to 16 scans.

Spectral width and digital resolution: **SWH (Hz)** or SW (ppm)

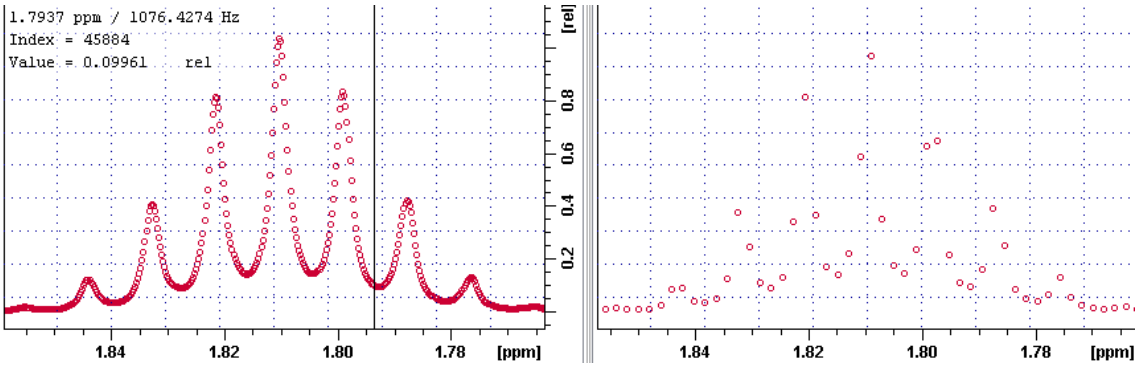
TD (point) : **Size of computer memory to store the FID.**
SI (point) : **Size of memory allocated for Fourier transfer of FID.**

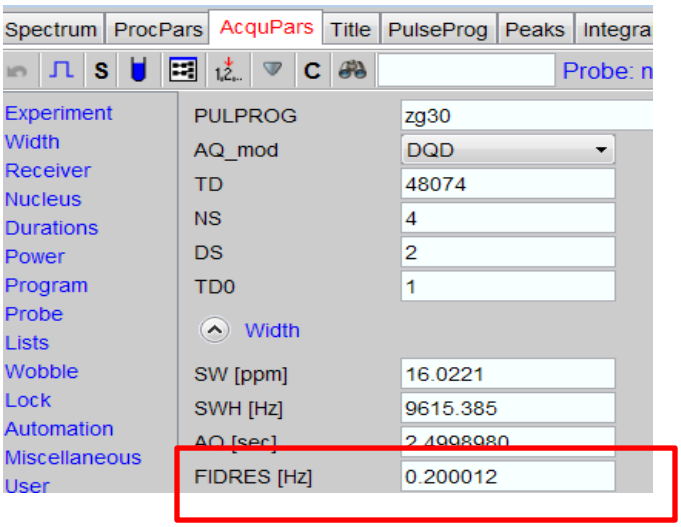
FIDRES (Hz/point): FID or digital resolution. = SW / TD
Spectral resolution; SW / SI

Apparent data video presentation:



Actual Data values:





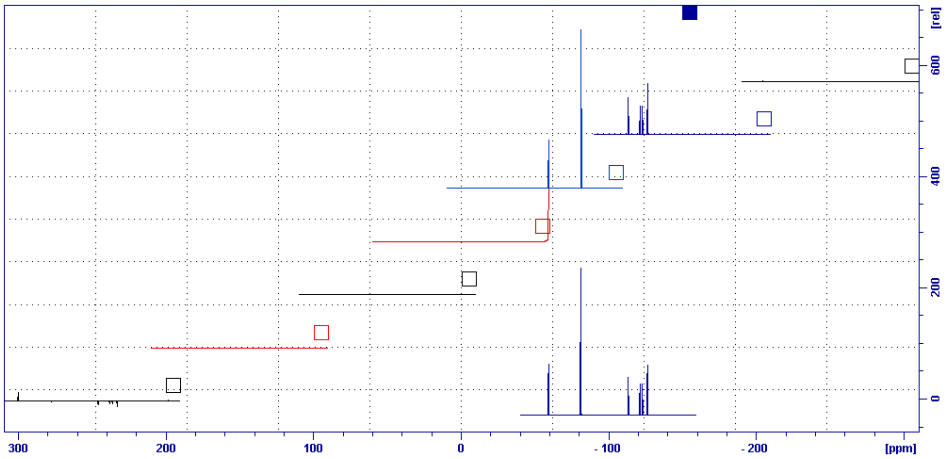
Transmitter offsets:

O1P (ppm) or O1 (Hz): offset of the first transmitter (f1). Center (middle of SW) frequency for the observe nucleus.

O2P (ppm) or O2 (Hz): Offset of the second transmitter (f2).

e.g. For C-13 with H-1 decoupling, O1P is ~ 110ppm, SW 280ppm,
O2P ~ 5 ppm

Example: F-19 signal search (unknown sample). SW 119ppm. O1p from 250ppm to -250ppm.



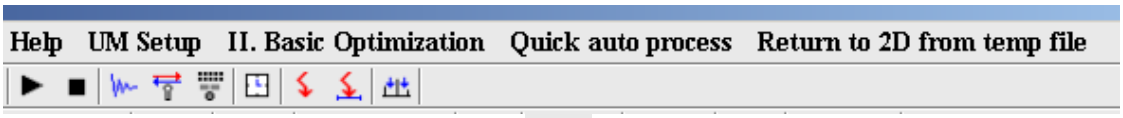
To adjust or modify SW and/or offsets:

1) **Collect a general 1D spectrum using routine settings.**

2) **Expand the spectrum to the desirable width or position of the offset.**

Two options:

- ✓ **Based on your display, type SW and/or o1p and enter the values of SW or offset manually.**
  Or
- ✓ **Click one of the options pending on your desirable changes.**



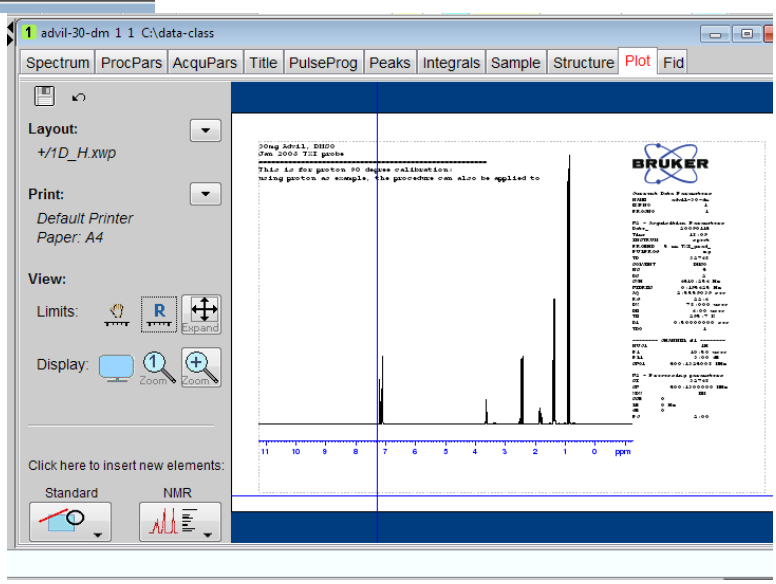
A. **Define/reset new offset**  **ONLY.**

B. **Define/reset BOTH new Sweep width and offset.** 

Both command icons give **O1P** (ppm) or O1 in Hz of the current nucleus of the spectrum on display window. The values can be export to 2D or other applications when the particular nucleus is used.

Plot using TOPSPIN (3.2): Click “Plot” Tab in the top manual bar.

For rough adjustments, use the top icons (except the “e”).

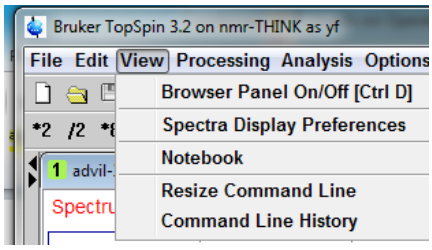


Horizontal and vertical expansions:

- Use buttons in “Limits” row for
  - expansion (rubber band- click drag and release) ,
  - **R** for reset
  - Pan.
- Middle wheel of the mouse for vertical expansion.

-
- For visual inspection, use buttons in “Display” for:
  - Full screen [browser Panel off].
  - Reset or magnify (for visible effect only).

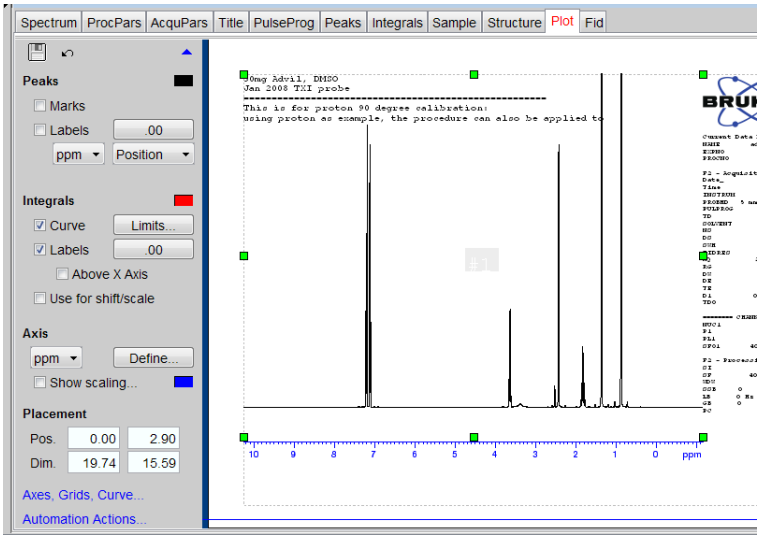
- Click View at the tope menu bar and turn the browser Panel on again.



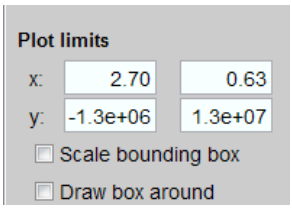
For adding additional plot features:

Put the mouse on the spectrum and left click:

Select the new options by checking the related buttons. e.g. Peaks

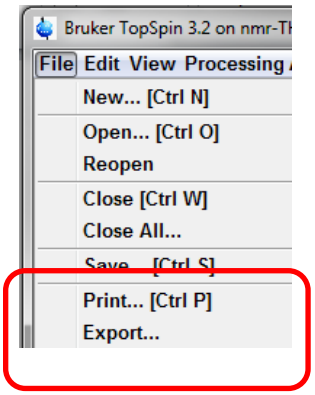


Click **Curve** to define the exact ppm horizontal expansions.



To obtain a hard copy plot or export to pdf file:

Click File in the TOP software main menu bar and click to select the appropriate icon.



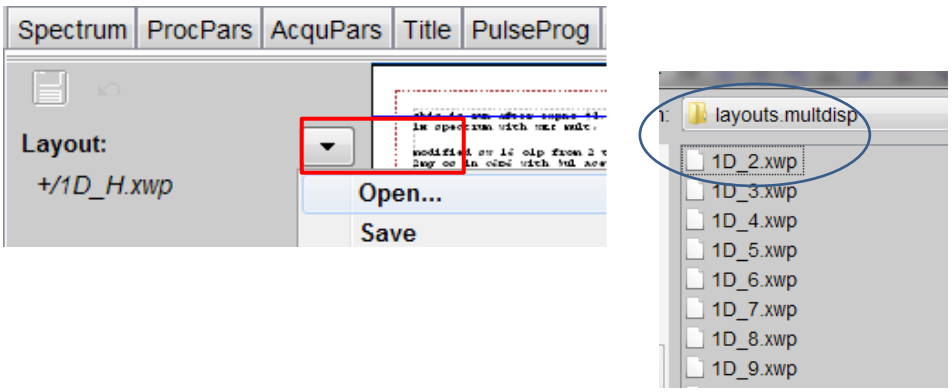
Stack multiple plots with spectra individually adjustable.

1) Click  multiple display icon to define all the spectra to be used in stack plot, using plot for two spectra in this example.

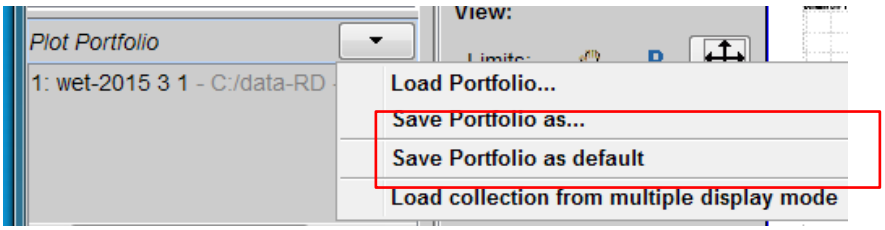
2) Exit the multidisplay window.

3) Click "Layout"

Then, open and select the **multidisplay** folder, then select "1D_2.xwp" in this example.



4) To import all the spectra defined from step 1, click Plot Portfolio; then select "Load collection…".



5) Stack plot is shown and each spectrum can be modified independently.