

Bruker Avance 400 MHz – Instructions

General Policy:

- 1) All users must pass the training course before they will obtain their own access to the spectrometer.
- 2) A key to Bruker Avance 400 Room is accessible from your supervisor. You **must lock the room** after leaving.
- 3) Samples for measurement must be prepared in user's laboratory.
- 4) Users put their samples into numbered holders on the carousel of the sample changer and submit their experiments to a queue.
- 5) The samples are run on a first-come-first-serve basis with all experiments longer than 20 minutes running at night.
- 6) Night experiments start at 8 p.m. (20:00) and end at 8 a.m. (8:00).
- 7) Users are not allowed to change experiment parameters.
- 8) Status of sample changer (information, if your sample has been already measured) can be viewed on-line using Icon-Web service simply by typing <http://ob-a400.uochb.cas.cz:8015> into your web browser.
- 9) Immediately after the experiments are completed, the data are stored at Bruker Avance 400 workstation in the directory `E:\data\username\nmr` and automatically archived in the directory `I:\MISC\NMR\Data` accessible through the IOCB intranet.
- 10) **Every Thursday morning (till 12:00) is the maintenance time reserved for the NMR Facility staff.**

Before You Start:

- ☛ Your NMR tube **must be at least 17.5 cm** (6.9 inches) in length and **must have a top that is neither broken nor cracked**. If your tube is shorter than 17.5 cm, it will not be grabbed fully by the sample changer and may fall and break on the floor or on the top of the magnet. Tubes with chipped tops are weakened and may be broken by the sample pincers which grab the tube very firmly close to the top. **Any tube shorter than 17.5 cm or with a chipped top submitted to the sample changer will be removed.**
- ☛ Your NMR tube **must not have any label (paper, tape, sticker etc....) attached to it**. This will stop the sample pincher from working properly and may result in sample breakage and equipment damage. **Tubes can be labeled on the glass or the cap with permanent marker**. Any tube with an attached label submitted to the sample changer will be removed.
- ☛ Sample tubes with screw caps or vacuum valves will not be handled by the sample changer. They will cause equipment damage.

- ☛ Approximate amount of middle-size compound (FW ~ 500) advisable for running following experiments:
 - ^1H NMR experiment - about 5 mg
 - ^{31}P NMR experiment - about 5 mg
 - ^{13}C NMR short run experiment - about 30 mg
 - ^{13}C NMR long run experiment - about 10 mg
- ☛ About 600 μl of NMR solvent is appropriate for the right solvent level in NMR tube. Unsuitable solvent level can lead to a bad shimming result and horrible looking spectrum.
- ☛ Never lean or exert any force on the sample changer or magnet. This may cause a magnet quench resulting in many weeks of down time and hundreds of thousands of CZK being spent.

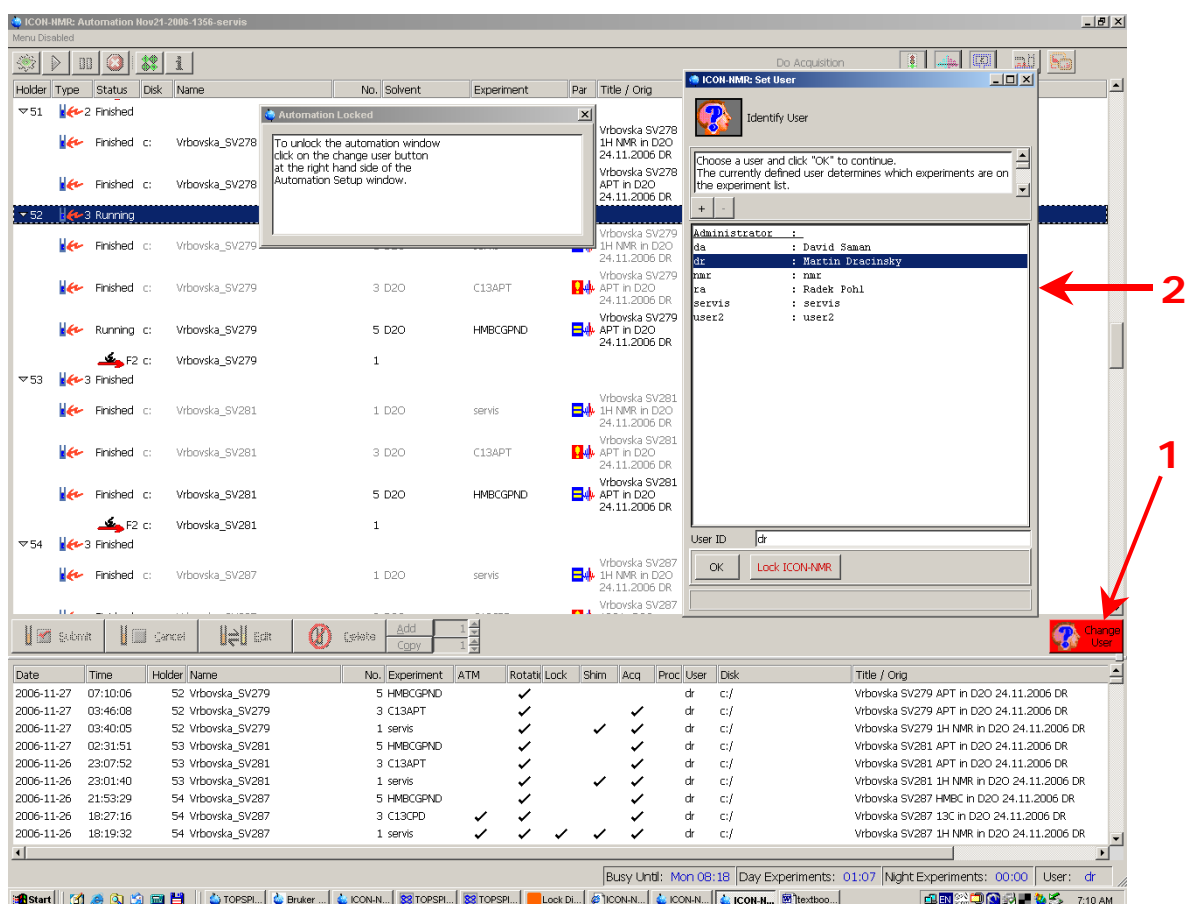
Users are asked to report improper sample tubes to the NMR Facility staff immediately in order to avoid needless down time and equipment damage!

Improper sample tubes are:

- ⊗ sample tubes shorter than 17.5 cm (6.9 in)
- ⊗ sample tubes with chipped tops
- ⊗ sample tubes with screw caps or vacuum valves
- ⊗ sample tubes with attached labels
- ⊗ sample tubes with inaccurate outer diameter, which does not fit perfectly the plastic spinner hole

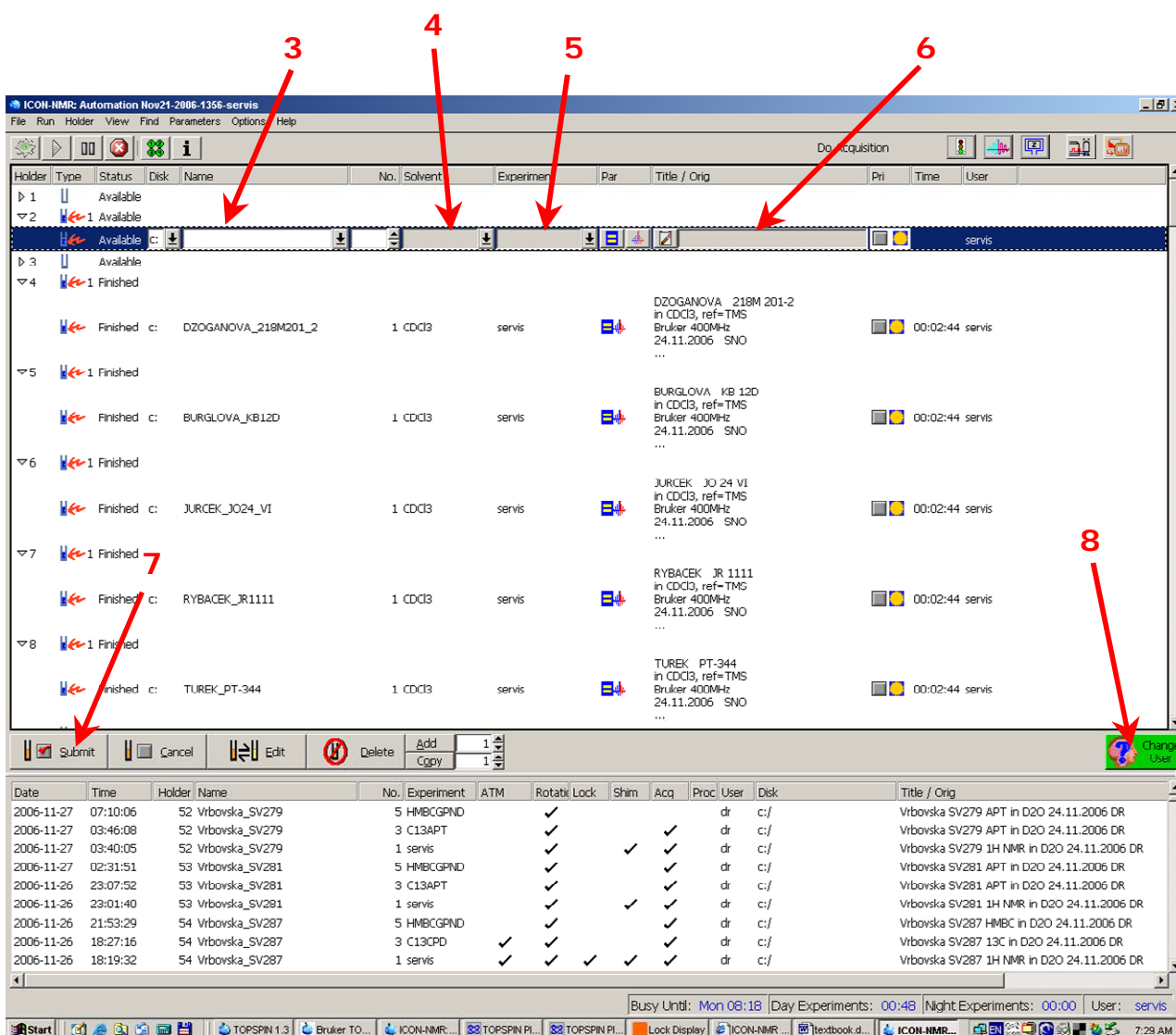
Collecting Data:

1. Check that your tube is in good shape (no chips or cracks), is at least 17.5 cm in length, is free of paper or tape labels and has a standard NMR tube cap.
2. Put your tube in a rotor and set the depth with the sample depth gauge.
3. Clean bottom part of the NMR tube with provided piece of special cloth.
4. Gently place your tube in any available holder on the sample changer. **DO NOT LEAN OR EXERT ANY FORCE ON THE SAMPLE CHANGER.**
5. At the computer in ICON-NMR window, if the login window is not being displayed, click the **"Change User"** button (1) located near the bottom right of the display.
6. Double click on your login name (2).
7. Enter your password.



8. Double click on the holder number in which you placed your sample.
9. Click in the **"Name"** field and give a name for your data set (3). Make sure the name contains letters, numbers, hyphens and underscores only. The name is case sensitive.
10. Click the down arrow to the right of the **"Solvent"** field and click the solvent used to prepare your sample (4).
11. Click the down arrow to the right of the **"Experiment"** field and click the desired NMR experiment (5).

12. Click on the title icon of the **"Title/Orig"** and fill in your text. Any character is allowed (6).
13. If you want to add an additional experiment to the same sample then click on the **"Add"** button near the bottom of the display. An experiment will be added with the same data set name with an incremented experiment number.
14. Click the holder number to highlight it and then click the **"Submit"** button near the bottom of the display (7). All experiments longer than 20 minutes will be run only at night and all experiments shorter than 20 minutes will be run at any time (day or night).
15. Click the **"Change User"** button to log out (8).



Allowed Experiments:

Name	Description
S_1H	^1H NMR experiment with 32 scans
S_31P_noddec	^{31}P NMR experiment with no decoupling
S_31Pdec	^{31}P NMR experiment with ^1H decoupling
S_13C_short	Short run (~ 20 min) ^{13}C NMR experiment with ^1H decoupling and 256 scans
S_13C_long	Long run (~ 3 hours) ^{13}C NMR experiment with ^1H decoupling and 3072 scans – night only
S_APT_short_normal	Short run (~ 20 min) ^{13}C APT experiment with 256 scans
S_APT_short_purine	Short run (~ 20 min) ^{13}C APT experiment with 256 scans optimized for purine derivatives
S_APT_long_normal	Long run (~ 3 hours) ^{13}C APT experiment with 3072 scans – night only
S_APT_long_purine	Long run (~ 3 hours) ^{13}C APT experiment with 3072 scans optimized for purine derivatives – night only

Accessing and Handling Your Data:

Your data is available to you as soon as it has been acquired in the directory **I:\MISC\NMR\Data** accessible through the IOCB intranet. **Your sample has to be clear out from the sample changer and position in ICON-NMR has to be deleted immediately after experiment completion.** Also check out your folder in the directory **E:\user** and in the directory **I:\MISC\NMR\Data** and delete older data you will not need later. The NMR Facility staff will permanently remove data older than 3 months.

Processing Your Data:

All data will be processed off-line.

Software, which is available for 1D NMR data processing:

- **Mestrec 2** - can be found in **I:\MISC\NMR** and it is free of charge (this version allows 1D processing only). Some of you probably owns commercial version Mestrec 4.9.9.3., which allows both 1D and 2D processing.
- **JEOL Delta** - <http://ai-sup.jeol.com/delta/> (another freeware; registration required)
- **TOPSPIN 1.3** – accessible on workstation in the Bruker Avance 400 room

Processing Your Data Using TOPSPIN 1.3

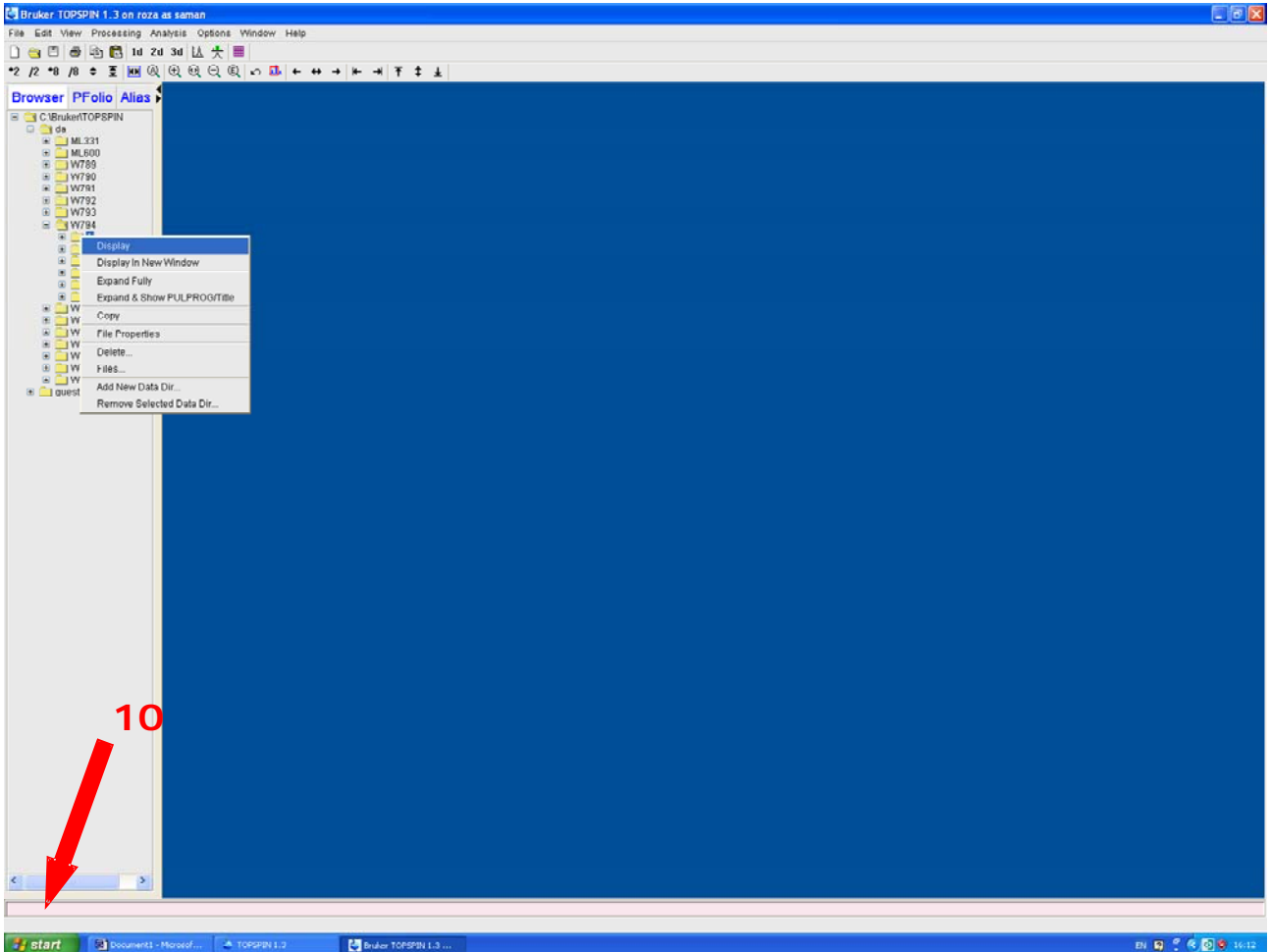
1. Double click on the line in ICON-NMR window with name of your sample (9). This will switch to the Bruker TOPSPIN 1.3 window.

The screenshot displays the ICON-NMR Automation software interface. The main window shows a list of experiments with columns for Holder, Type, Status, Disk, Name, No., Solvent, Experiment, Par, Title / Orig, Pri, Time, and User. A red arrow points to the entry 'TUREK_PT-344' in the list. Below the main window is a detailed log table with columns for Date, Time, Holder, Name, No., Experiment, ATM, Rotati, Lock, Shim, Acq, Proc, User, Disk, and Title / Orig. The taskbar at the bottom shows the Start button and several open applications including TOPSPIN 1.3, Bruker TO..., ICON-NMR..., and TOPSPIN PI... The system tray shows the time as 7:29 AM.

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Par	Title / Orig	Pri	Time	User
1	U	Available										
2	U	Available										
3	U	Available										
4	U	Finished	c:	DZOGANOVA_218M201_2	1	CDCl3	servis		DZOGANOVA 218M 201-2 in CDCl3, ref=TMS Bruker 400MHz 24.11.2006 SNO ...		00:02:44	servis
5	U	Finished	c:	BURGLOVA_KB12D	1	CDCl3	servis		BURGLOVA KB 12D in CDCl3, ref=TMS Bruker 400MHz 24.11.2006 SNO ...		00:02:44	servis
6	U	Finished	c:	JURCEK_JO24_VI	1	CDCl3	servis		JURCEK JO 24 VI in CDCl3, ref=TMS Bruker 400MHz 24.11.2006 SNO ...		00:02:44	servis
7	U	Finished	c:	RYBACEK_JR1111	1	CDCl3	servis		RYBACEK JR 1111 in CDCl3, ref=TMS Bruker 400MHz 24.11.2006 SNO ...		00:02:44	servis
8	U	Finished	c:	TUREK_PT-344	1	CDCl3	servis		TUREK PT-344 in CDCl3, ref=TMS Bruker 400MHz 24.11.2006 SNO ...		00:02:44	servis

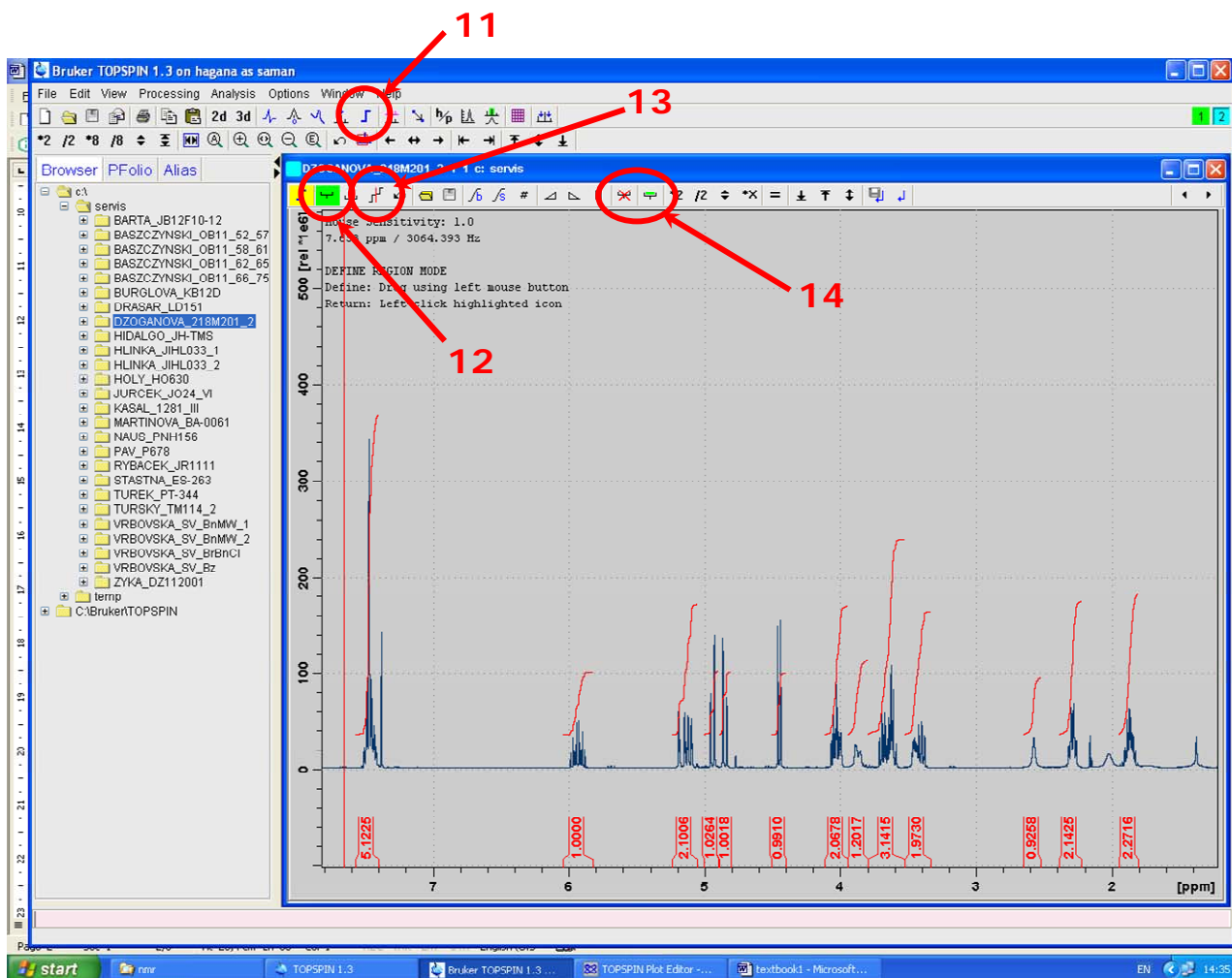
Date	Time	Holder	Name	No.	Experiment	ATM	Rotati	Lock	Shim	Acq	Proc	User	Disk	Title / Orig
2006-11-27	07:10:06	52	Vrbovska_SV279	5	HMBCGPND			✓				dr	c:/	Vrbovska SV279 APT in D2O 24.11.2006 DR
2006-11-27	03:46:08	52	Vrbovska_SV279	3	C13APT			✓				dr	c:/	Vrbovska SV279 APT in D2O 24.11.2006 DR
2006-11-27	03:40:05	52	Vrbovska_SV279	1	servis					✓		dr	c:/	Vrbovska SV279 1H NMR in D2O 24.11.2006 DR
2006-11-27	02:31:51	53	Vrbovska_SV281	5	HMBCGPND			✓				dr	c:/	Vrbovska SV281 APT in D2O 24.11.2006 DR
2006-11-26	23:07:52	53	Vrbovska_SV281	3	C13APT			✓				dr	c:/	Vrbovska SV281 APT in D2O 24.11.2006 DR
2006-11-26	23:01:40	53	Vrbovska_SV281	1	servis					✓		dr	c:/	Vrbovska SV281 1H NMR in D2O 24.11.2006 DR
2006-11-26	21:53:29	54	Vrbovska_SV287	5	HMBCGPND			✓				dr	c:/	Vrbovska SV287 HMBC in D2O 24.11.2006 DR
2006-11-26	18:27:16	54	Vrbovska_SV287	3	C13CPD	✓		✓				dr	c:/	Vrbovska SV287 13C in D2O 24.11.2006 DR
2006-11-26	18:19:32	54	Vrbovska_SV287	1	servis	✓		✓	✓	✓		dr	c:/	Vrbovska SV287 1H NMR in D2O 24.11.2006 DR

2. Type *fp* (Fourier transformation for ^1H data) or *efp* (Fourier transformation for ^{13}C data) in the command line **(10)**; *enter*;
apks (automatic phase correction); *enter*;
abs (automatic baseline correction for ^1H data) or *abs13c* (automatic baseline correction for ^{13}C data); *enter*.

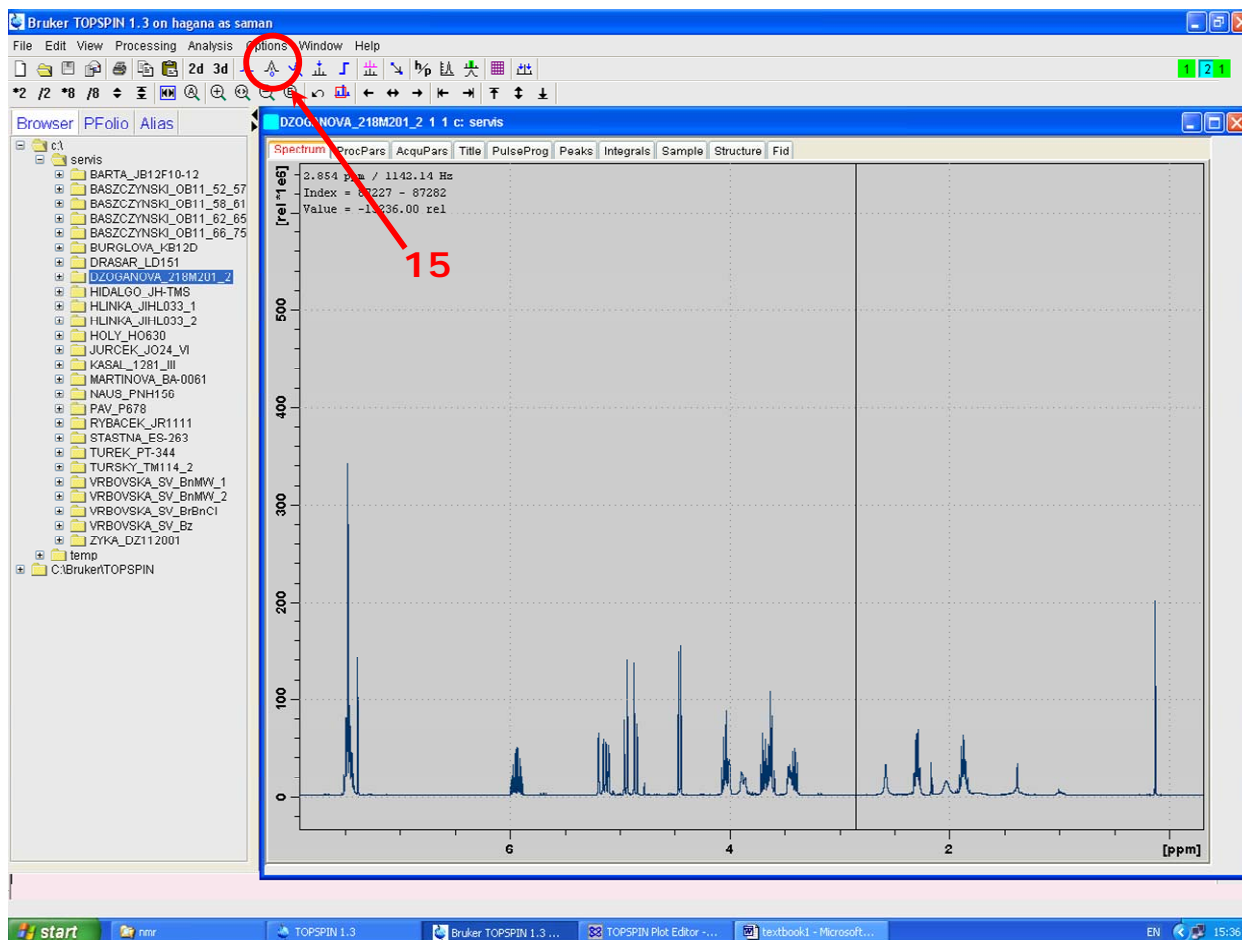


How to Make Integrals and Calibrate My Spectrum?

Click the icon for integration first (11), you can see a new design of main Topspin window. Activate the tool for integral definition (12). With click left mouse button on the start of region, drag mouse and release the mouse button on the end of the region. The integral appears. To “cut” integrals into several parts use icon (13) in combination with left mouse button for zero points. In case you want to delete all integrals, activate all defined integral regions (14) and delete it. You can also calibrate the integrals. Clicking with right button under the defined integral region you can adjust number of equivalent atoms (see one proton signal at 5.92 ppm in our example).

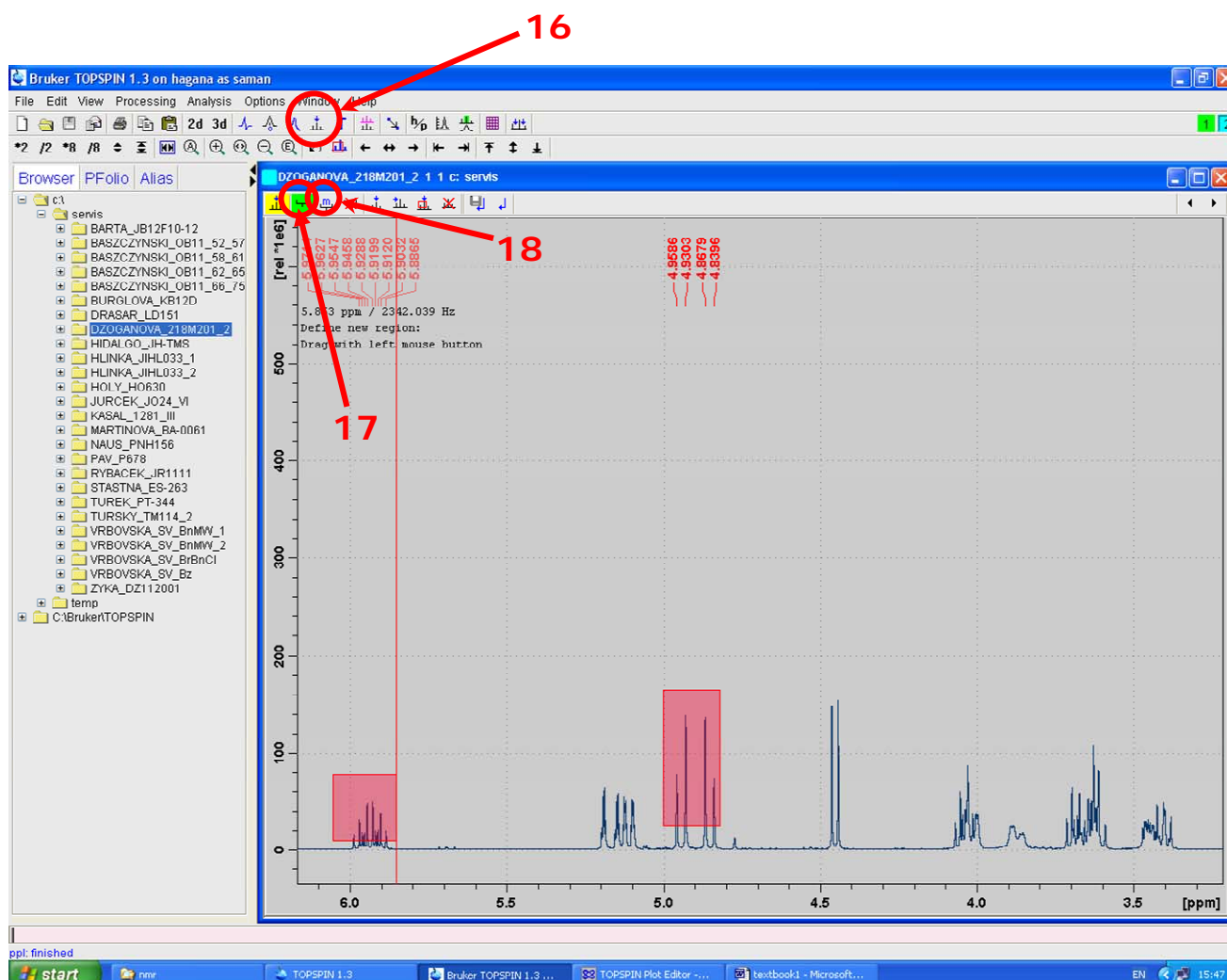


For calibration (setting of chemical shift of known signal in the spectrum) first activate the calibration tool (15). Clicking the left mouse you define the point and chemical shift of that point in the dialog box afterwards.



How to Make Peak Picking?

Activate the peak picking mode first with icon (16). You can see an other window design.



Activate the tool for peak picking definition (17). Click left mouse and drag rectangular areas to define threshold (minimum intensity) and frequency ranges. You can also modify these characteristics by (18).

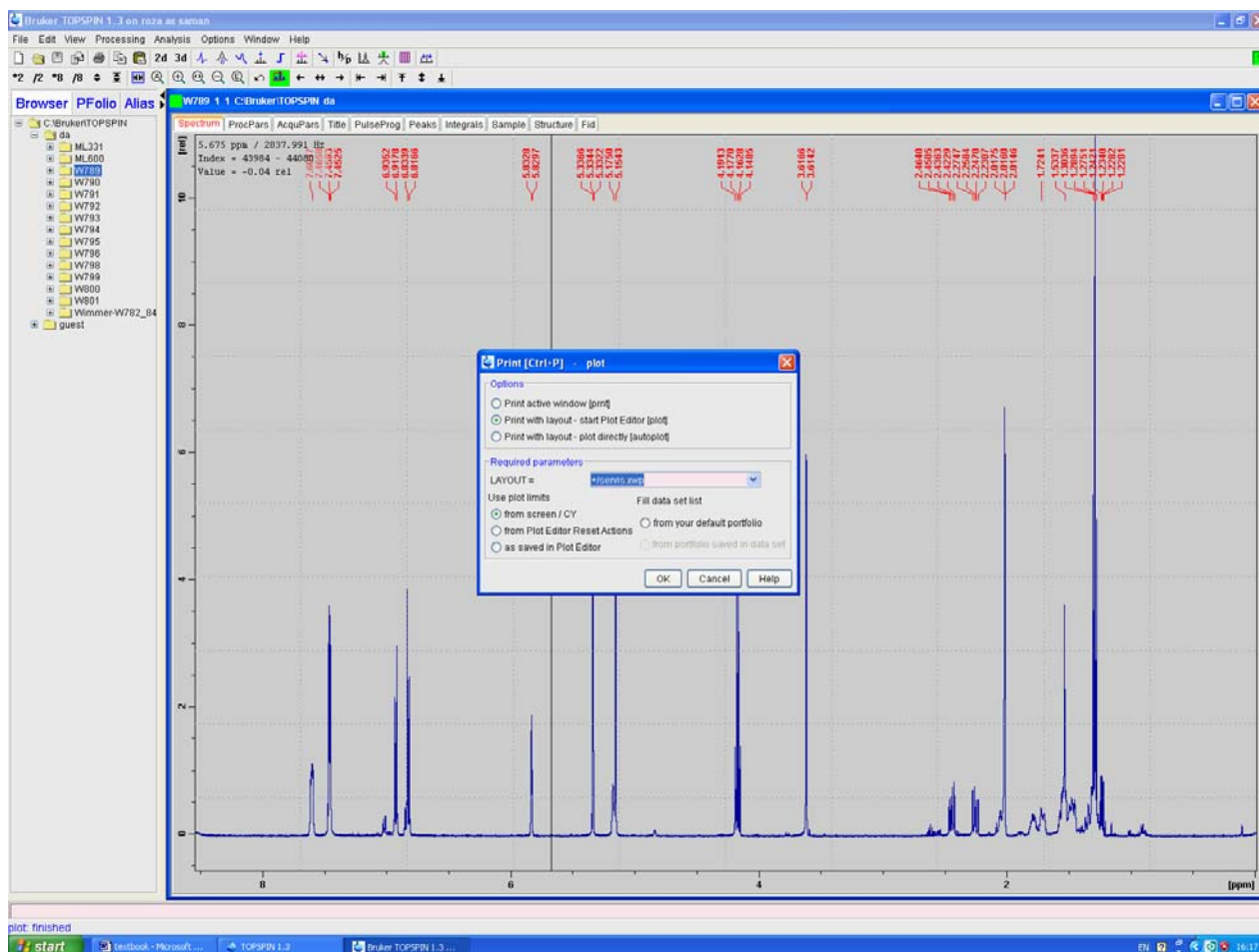
Plotting of Your Spectrum

Generally, you have got two option of plotting your spectra.

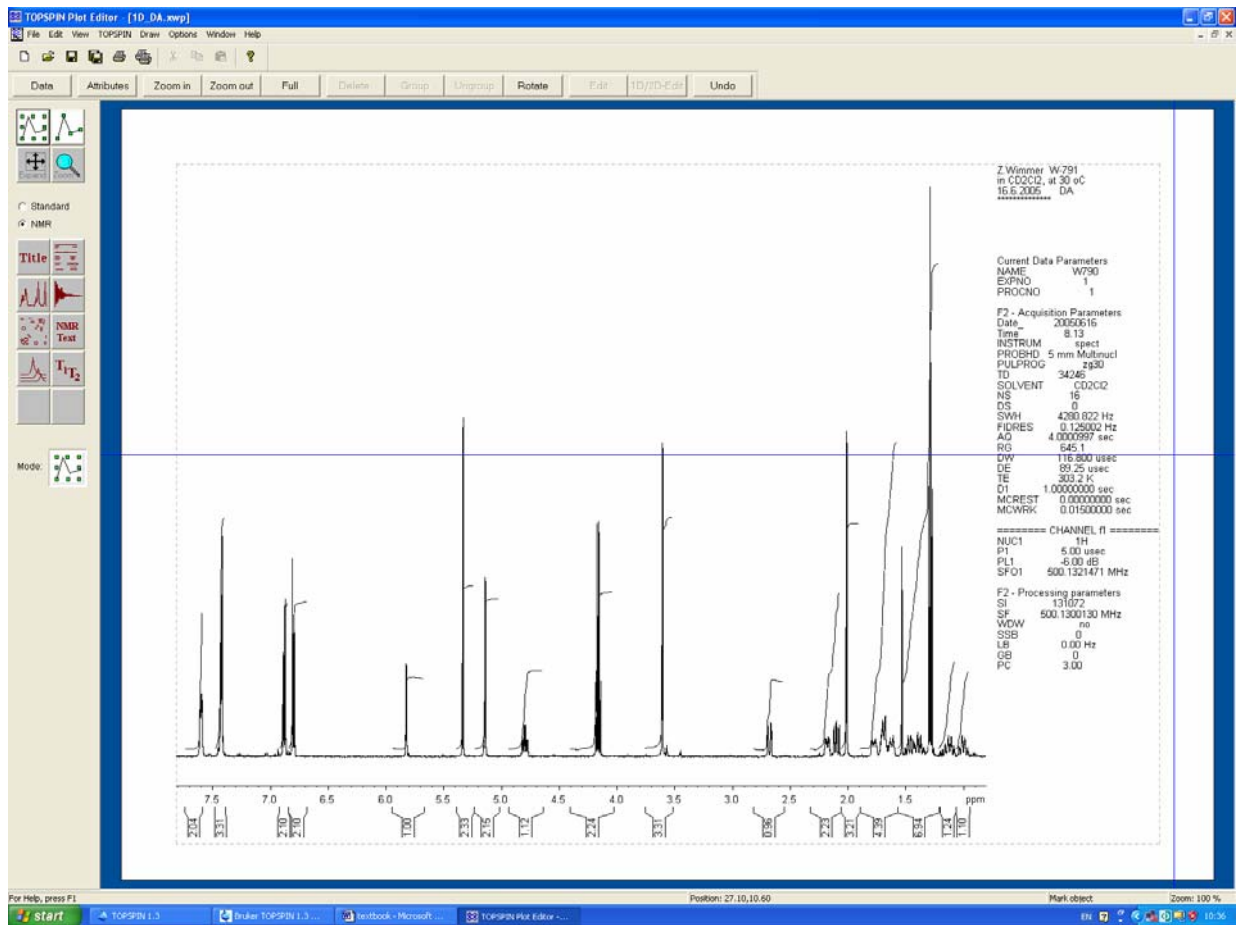
1. Type *prnt* in the command line. Your printout will be the same picture you can see on the screen in TOPSPIN 1.3 window.
2. Second option for fancy looking pictures is using of TOPSPIN Plot Editor.

Plotting Using TOPSPIN Plot Editor

Be in TOPSPIN (you can see your spectrum on the screen) active with **CTRL+p** the output section of the software. Now choose "Print with layout - start Plot editor" - in LAYOUT box should be "+/servis.xwp".

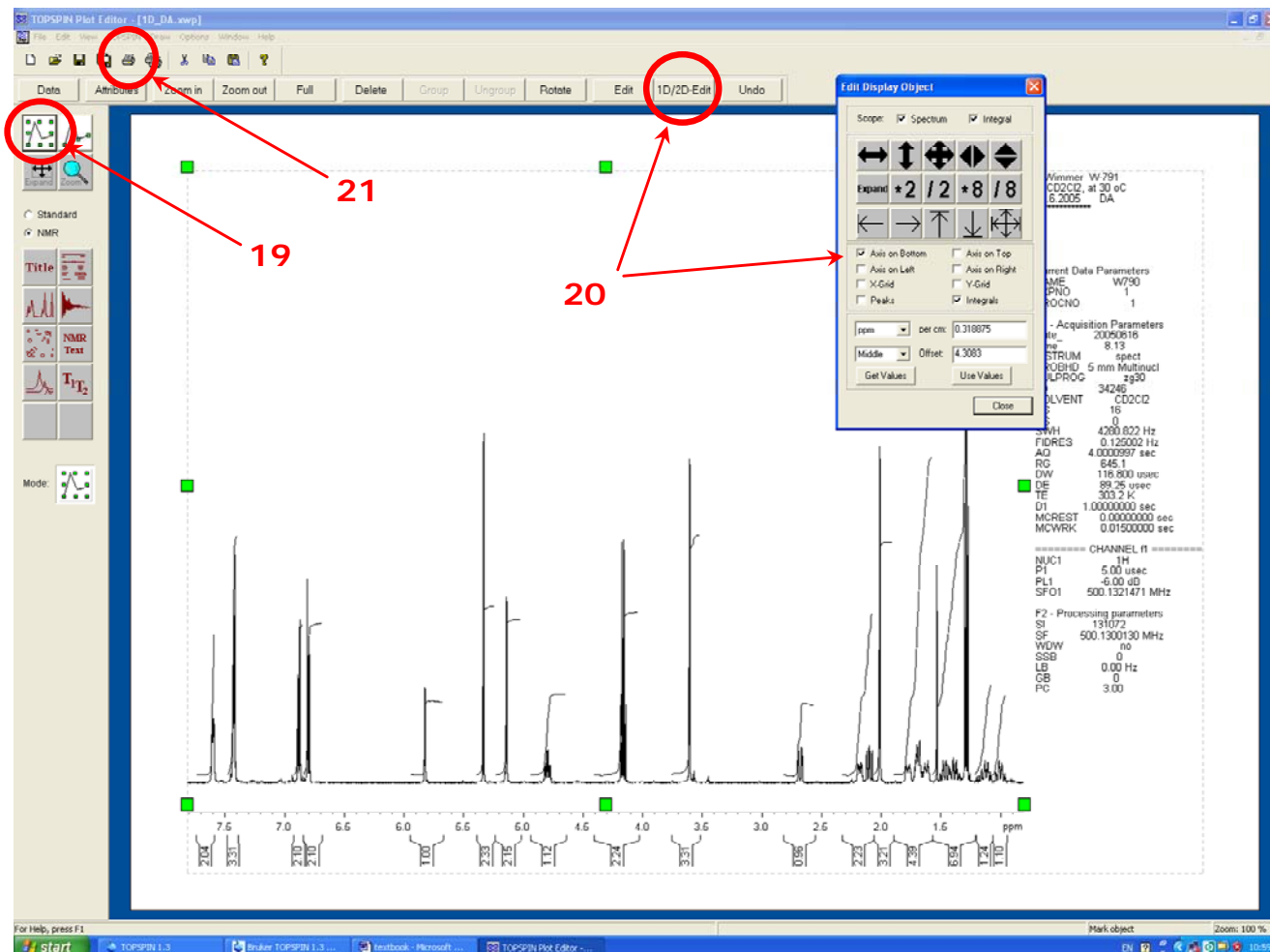


After confirmation with OK the TOPSPIN Plot Editor starts.



To modify the basic characteristics of plot you have to activate the spectrum with (19) and activate the tool for spectrum manipulation (20). This tool is more or less self instructive and we recommend to you just use it.

When spectrum is as you like to see it, activate the plotting and choose the plotter or pdf file – (21). You can make also your expansions of spectra by copy and paste of activated spectrum.



Are You in Troubles?

(follow this priority list)

- consult this remarks
- consult remarks of your colleagues
- consult the manuals presented on Intranet (I:\MISC\NMR\MANUALS_BRUKER)
- consult staff of NMR department

IT'S STRICTLY FORBIDDEN TO MAKE ANY HARDWARE OR SOFTWARE OPERATION ON NMR SPECTROMETER EXCEPT FOR THESE LISTED IN THIS GUIDE!!!