Direct Observation of X-Nuclei within TopSpin

updated: 5 Sept 2014 (cgf)

I. Sample Concentrations:

Direct observation of X-nuclei will succeed or fail primarily as a function of sample concentration. Other factors can be very important, especially for low-γ nuclei. For such nuclei, coupling to an abundant spin — ^{1}H, ^{31}P, ^{19}F being the most important — will often determine whether any observation can be made or not. Labeling will often be the only other choice for nuclei such as ^{15}N. [Note the "Direct" component of this discussion. Many nuclei can often be straightforwardly observed via "indirect" detection, usually via 1H-detected 2D experiments.]

Minimum concentrations have not been determined for most nuclei. The following are guides: overnight runs for callisto, Prodigy, and BBFO+ (on Av500); 2 hr runs on Av400

^{13}C nat. abundance: *callisto* ≥ 300 μM, Prodigy ≥ 1 mM; BBFO+ ≥ 3mM Av400 ≥ 15mM

^{19}F : Prodigy ≥ 50μM (w no ^{1}H coupling), BBFO+ ≥ 120 μM, Av400 ≥ 1mM

II. Summary of Commands:

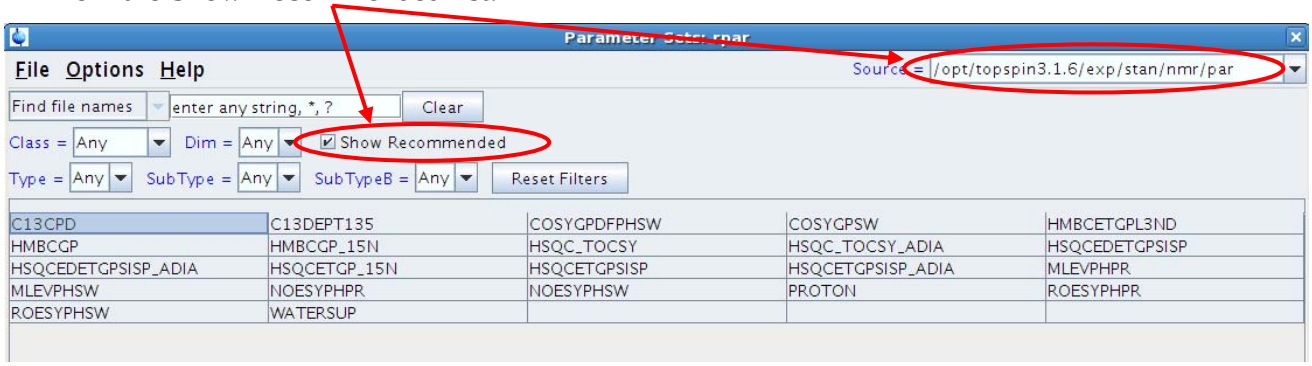
sx #	[callisto: isx #]
new↵ or **rpar**↵	;choose parameter set [follow **rpar**↵ with **getprosol**↵]
atma↵ or **atmm**↵	;be patient, as can take up to 2 mins
topshim gui↵	;add tunexy and/or convcomp
ased↵	;optimize parameters, esp **d1** and **ns**
rga↵	;needed only for ^{1}H, ^{19}F and highly-concentrated ^{31}P
zg↵	; **tr #** and **halt #** are useful
 or **o1p**↵	;adjusts **sw** and **o1p**, and will change **aq** (always check)

→ redo **atma**↵ if **o1p** changes > 50 kHz (check via **o1**)
→ start with **sw** large, and reduce for better resolution (≈ 1/**aq**)

III. General Direct-X Experiment Guide:

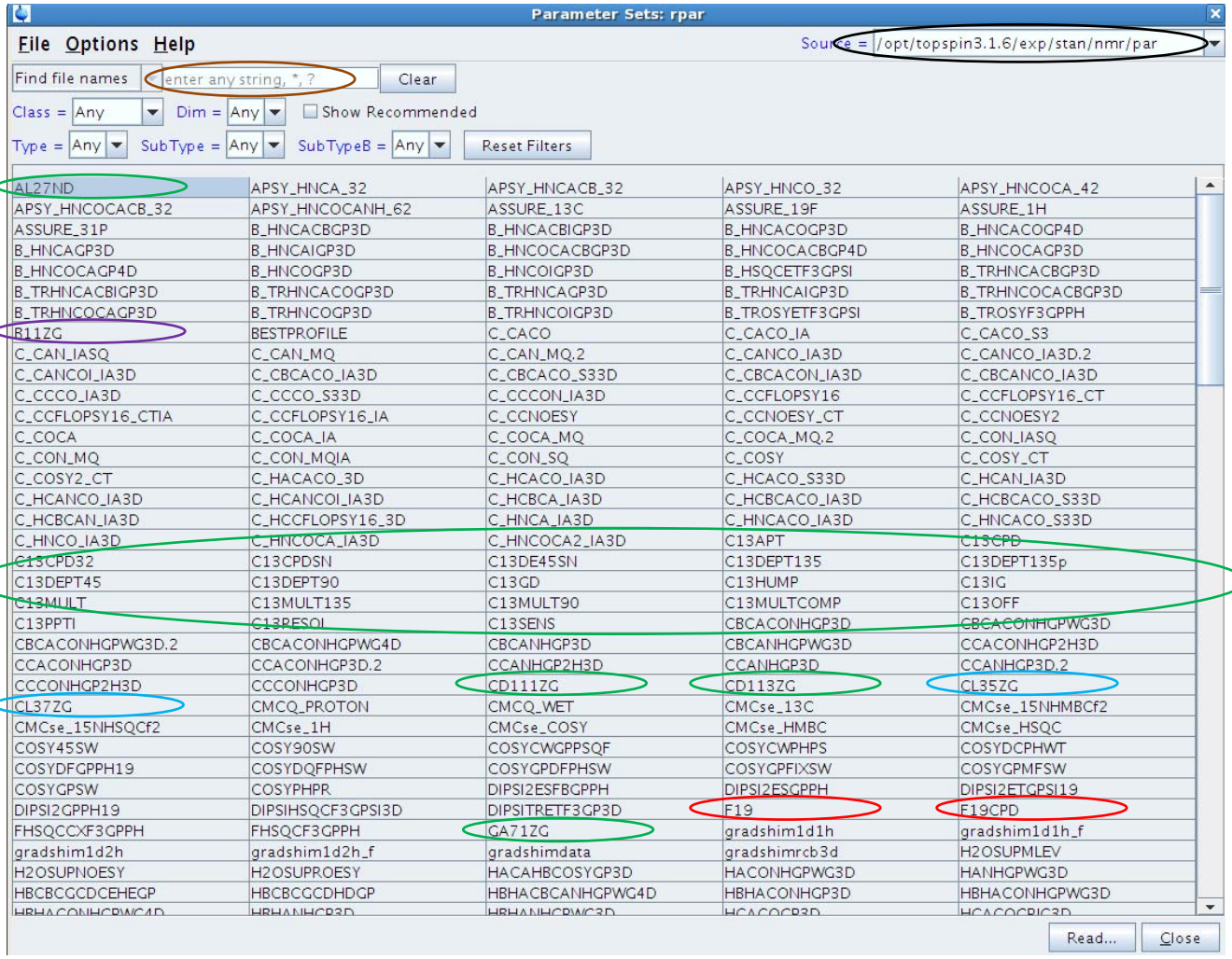
1. Acquisition of a ^{1}H spectrum is always recommended, prior to any X-direct experiment (including ^{13}C experiments). The ^{1}H spectrum will confirm shim quality, and can be used to check the referencing of the X experiment through the use of the **xref** au routine.

2. **new**↵ or **rpar**↵ and choose the proper parameter set. A few experiments can be found from the Show Recommended list.



- Most parameter lists are found with the "Show Recommended" unchecked, with a portion of list shown on the next page. This list can be reduced using the "enter any string, *, ?" search box. For direct-X experiments, these are always listed as nucleus_symbol-isotope_number. Thus, enter **N15**↵ to display direct–detection ^{15}N parameter sets.

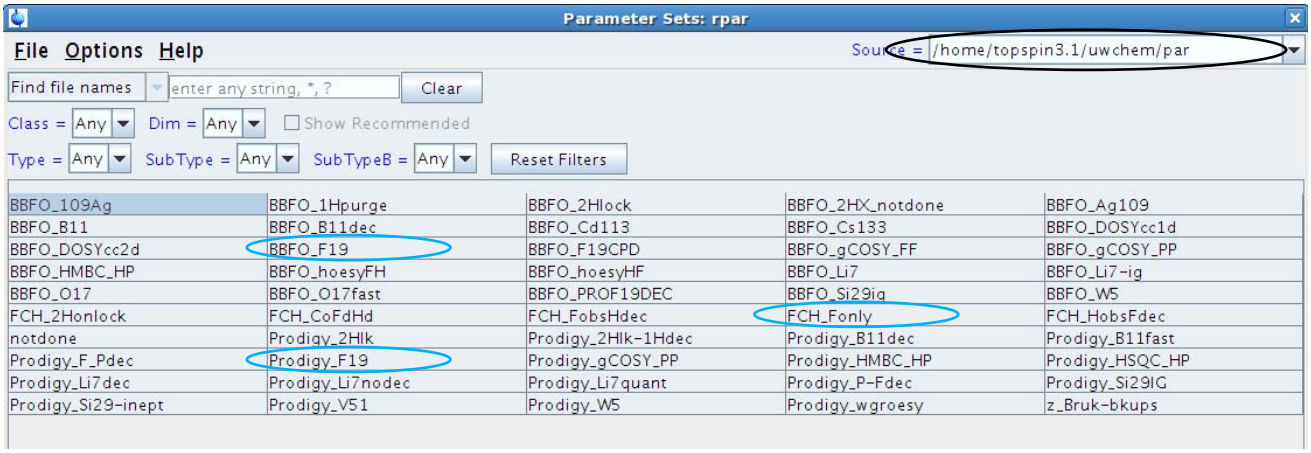
Bruker-supplied parameter sets. Direct-X parameter sets are circled: **green** circles indicate parameters sets OK for BBFO and Prodigy probes; **blue** circles show nuclei accessible only on the BBFO+ probe (^{35}Cl is too low in γ for the Prodigy probe, falling below ^{15}N); **red** circles show parameter sets that should never be used, and should be gotten from the UWChem folder.



- Some parameter sets, such as F19, must be modified to match the specific hardware setup on the spectrometer. Or the parameter set may simply have more optimal parameters than Bruker provides (e.g., for ^{11}B and ^{10}B). These parameter sets are kept in a folder specific to the Chemistry NMR Facility at /**home/topspin3.1/uwchem/par**.

  Some experiments must utilize parameter sets provided in the UWChem folder! Any experiment involving ^{19}F on Persephone falls in this class of experiments. It is therefore important to check the list of experiments available in that folder, and always use that set if it is available. Note the set of parameters shown in the screen capture below: it is up-to-date as of 23 June 2013.

UWChem-supplied parameter sets from Persephone. Note the three sets of parameters are all probe specific: BBFO, FCH, and Prodigy. Always use only those sets matching the probe currently installed (the on-line calendar has the schedule). Three ^{19}F-only parameter sets are circled in **blue**.

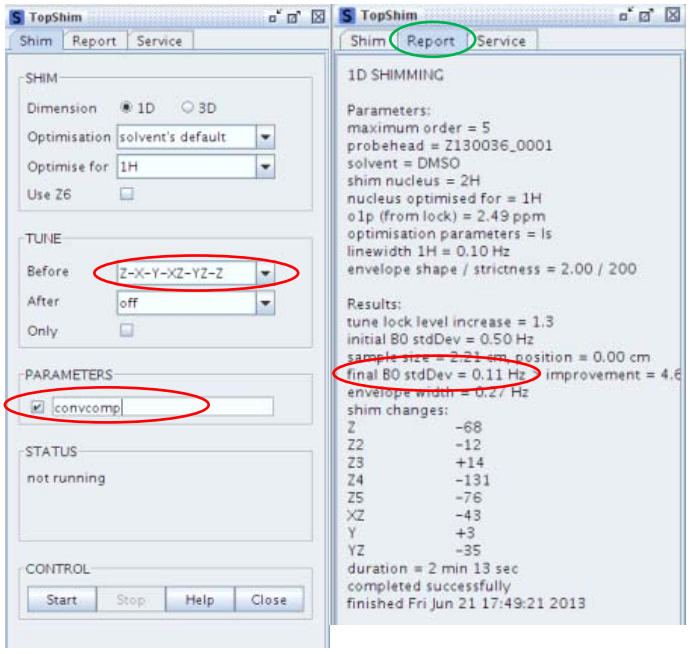


3. Tune the probe as normal, using **atma**↵ . If this fails for some reason, use **atmm**↵ . *Be patient!* The routine accesses capacitor rods that make mechanical changes to the probe. In the Prodigy, the rods pass through vacuum and cryogenic areas; it can only go so fast. Impatience can lead to a stuck rod, and potentially to probe damage.

   Note: **atma**↵ may take up to 2 min to complete. Wait for the task to complete: this involves tuning X, and then ^{1}H, for X{^{1}H} experiments. Typing in TopSpin during **atma** or **atmm** may freeze the software (close TopSpin, wait 5 s, re-open TopSpin will usually solve this).

4. Shim as normal, using **topshim gui**↵ . Addition of **convcomp** is important on the cryoprobes for any volatile solvent (all common solvents other than D_2O and DMSO). Look at the Report panel, and wait until it finishes before switching windows or typing anything into TopSpin.

   Rerun topshim if the "final Bo stdDev" > 1Hz. Try TUNE → Before on XYs as shown, as that may help. Filtering or centrifuging the sample may be important to obtaining good quality shims.



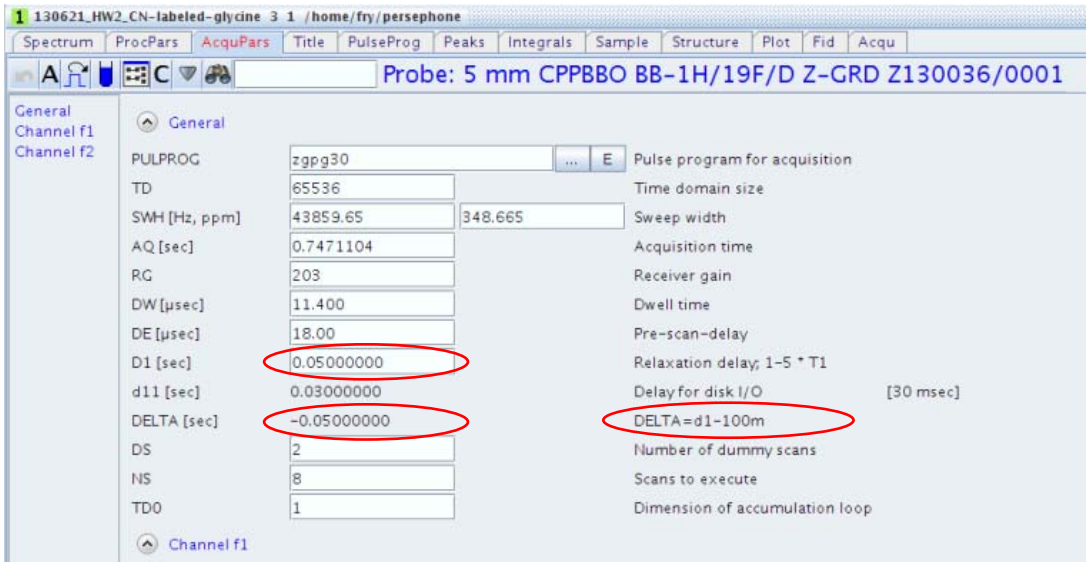
5. **ased**↵ and adjust parameters as necessary; the most important are usually **d1** and **ns**.

   Note: Quantitation is often nontrivial for X-nuclei. See section III for more information.

6. As always on Bruker spectrometers, important information is contained in the pulse sequence listing. Clicking on the PulseProg tab will provide a listing. Particularly useful is the bottom comments section of the listing, which provides parameters comments, such as minimum **ns** cycles for any sequence. **halt #**↵ on a minimum **ns** cycle will assist in avoiding artifacts.

7. Note also that some time delays may be calculated, such as DELTA in the example shown below. These delays must not be set to negative values. TopSpin will warn about such occurrences, but only after a **zg**, and will then lock up the acquisition (yes, this definitely should be fixed by Bruker…). When this happens, exit TopSpin completely, restart it, then:

   i) fix the parameter (in zgpg30, **d1** > 100m)
   ii) **ii restart**↵



8. **rga**↵ only needs to be run on ^{1}H, ^{19}F, or perhaps for ^{31}P on very concentrated samples (or labeled samples). Won't hurt anything to run **rga**, however, which usually will end up at 203.

9. Background signals can be reduced using longer **de** values, if no other broad signals are observed in the sample. The tradeoff is more 1st-order phase correction required as **de** increases, which causes baseline rolling. ^{19}F is the most common nuclei where such problems occur, but the larger **de** could also be used for ^{29}Si and ^{11}B. See staff for more information.

10. Acquire as normal with **zg**↵. Observe during the run with **tr**↵ or **tr #**↵ to transfer at scan #. **halt #**↵ is recommended where # is a multiple of the minimum **ns** phase cycle (usually 8).

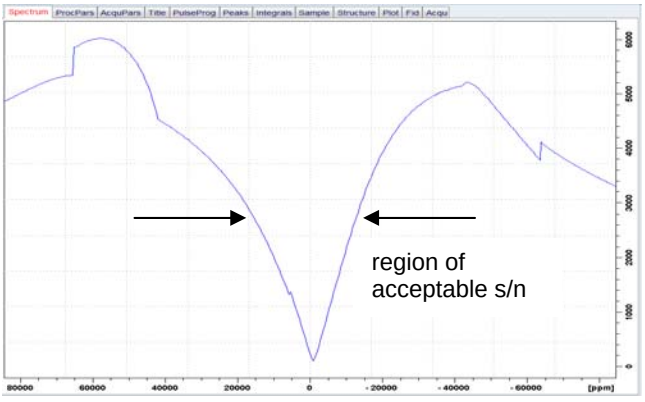
11. A significant issue in many X-direct experiments is **ns** and **d1** settings when observation is not quickly made. A standard sample at a known (high) concentration that can be easily observed is the best method for working through such issues.

11. Adjust the sweepwidth **sw** and spectrum center **o1p** as needed. Bruker digital filters will exclude observation of any signals outside the spectral window.

    sw ≤ 2000000

    can be used, but the probe usually can observe signals only within a 200 kHz bandwidth (bw) about **o1p**. An example of a ^{13}C tune is to the right, where an approx bw is indicated.

    If **o1p** is changed by > 50 kHz, the probe should be retuned (e.g., with **atma**↵).



Reacquire overlapping spectral regions to cover the complete span of chemical shifts for any detection of a novel compound. See cgf for more information and assistance.

IV. Other comments:

1. Obtainable and digital resolution are often issues with X-spectra, especially when sweepwidths get large, or are changed to access different chemical shift regions. The following guidelines will help:

   Always check **aq** after any change in **sw**.

   obtainable resolution ~ 1/**aq** (1)

   Eq (1) is not precisely correct, but the dependence is right. Long **aq** acquiring a lot of noise will not assist with resolution. But if one hopes to resolve peaks that are separated by 1Hz, then **aq** ≥ 1 sec is recommended.

   digital resolution = **sw** / **si** where **si ≤ 1-2×td** (2)

   For **si**: 1 (one full zero-fill) if td = 2^n, but can be almost 2 if td = 2^n+1. In any event:

   si = 2×td is a good setting.

2. Matched filters are ok for X-spectra: **lb** = 1/**aq** . For **aq>1**, usually start with **lb**=1 , but reduce if helps with resolution.

3. Quantitation: Usually, acquire with default parameters (usually with a faster guess for $\mathbf{d1_{fast}}$) until a good s/n is obtained. Integrate(deconvolute) the peak(s) of interest.

   Acquire another spectrum with $\mathbf{d1_{new} \geq d1_{fast} \times 2}$.

   If the integral doesn't change, or an important ratio doesn't change, likely all is OK. But if it does change, repeat with yet a longer **d1**. Repeat until the integral/ratio doesn't change. This is a repetition rate method for estimating T_1 values.

4. ^{13}C DEPT, or INEPT, or APT. Take your choice, they're all OK.

   When the J-value is know, INEPT has the best sensitivity. The ineptrd pulse sequence is preferred; good pulsewidth calibrations are important: see cgf for more info.

5. Indirect detection via ^1H (or possibly ^{31}P) is often superior to the point of being the only option. This is true for ^{15}N at natural abundance, and for most nuclei that resonate at frequencies below ^{15}N.

6. Referencing in TopSpin is typically very good. The software automatically references the spectrum to the assumed position of the ^2H lock signal (e.g., $CDCl_3$ at 7.2 ppm). A very strong recommendation, even so, is to *always* keep a ^1H spectrum taken in the same conditions (same sample, same temp, etc) with the X spectrum. The automation routine **xref** in TopSpin, or the equivalent routine in MNova, can then be used to check the referencing of the spectrum. In TopSpin:

   a) While in the X-spectrum experiment, type **xref**↵

   b) enter the filename+expno+procno of the referenced ^1H spectrum

   That's all there is to it. With the ^1H spectrum, you can go back at any time (e.g., many years from now when you've moved elsewhere) and check a ^{31}P or ^{19}F (etc.) reference. This could avoid contention over published data, as one example, without having to remake the sample.