# 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- $\gamma$  nuclei. For such nuclei, coupling to an abundant spin — <sup>1</sup>H, <sup>31</sup>P, <sup>19</sup>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 <sup>15</sup>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*  $\geq 300~\mu M,$  Prodigy  $\geq 1~mM;$  BBFO+  $\geq 3mM$  Av400  $\geq 15mM$ 

 $^{19}$ F : Prodigy  $\ge 50 \mu$ M (w no  $^{1}$ H coupling), BBFO+  $\ge 120 \mu$ M, Av400  $\ge 1$ mM

### II. Summary of Commands:

sx #	[callisto: isx #]
new니 or rpar니	;choose parameter set [follow <b>rpar</b> , with <b>getprosol</b> ]
atma⊣ or atmm⊣	;be patient, as can take up to 2 mins
topshim gui₊J	;add tunexy and/or convcomp
ased₊J	;optimize parameters, esp <b>d1</b> and <b>ns</b>
rga₊J	;needed only for <sup>1</sup> H, <sup>19</sup> F and highly-concentrated <sup>31</sup> P
zg₊J	; tr # and halt # are useful
<b>∑</b> or <b>01p</b> ₊J	;adjusts <b>sw</b> and <b>o1p</b> , and will change <b>aq</b> (always check)

 $\rightarrow$  redo **atma** if **o1p** changes > 50 kHz (check via **o1**)

 $\rightarrow$  start with sw large, and reduce for better resolution (  $\approx 1/aq$ )

#### **III. General Direct-X Experiment Guide:**

- 1. Acquisition of a <sup>1</sup>H spectrum is always recommended, prior to any X-direct experiment (including <sup>13</sup>C experiments). The <sup>1</sup>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.

<u>نه</u>		Parameter SCICI spar			
<u>F</u> ile <u>O</u> ptions <u>H</u> elp			Source /opt/top	ospin3.1.6/exp/stan/nmr/par	
Find file names 👻 enter ar	ny string, *, ? Clear	•			
Class = Any 💌 Dim =	Any Show Recomme	ended			
Type = Any - SubType =	Any V SubTypeB = Any	Reset Filters			
				1	
C13CPD	C13DEPT135	COSYGPDFPHSW	COSYGPSW	HMBCETGPL3ND	
	LILLING OD A FLI	LIDOD TO DOV			
НМВССР	HMBCGP_15N	HSQC_TOCSY	HSQC_TOCSY_ADIA	HSQCEDETGPSISP	
	HSQCETGP_15N	HSQC_TOCSY HSQCETGPSISP	HSQC_TOCSY_ADIA HSQCETGPSISP_ADIA	HSQCEDETGPSISP MLEVPHPR	
HMBCGP HSQCEDETGPSISP_ADIA MLEVPHSW					

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 <sup>15</sup>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  $\gamma$  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.

<b>é</b>		Parameter Sets: rp	ar	
<u>File</u> Options <u>H</u> elp			Source = /opt	/topspin3.1.6/exp/stan/nmr/par
Find file names Center a	ny string, *, ? Clear			
Class = Any 💌 Dim :	= Any 💌 🗌 Show Recommen	ded		
Type = Any 💌 SubType =	Any V SubTypeB = Any V	Reset Filters		
AL27ND	APSY_HNCA_32	APSY_HNCACB_32	APSY_HNCO_32	APSY_HNCOCA_42
APSY_HNCOCACB_32	APSY_HNCOCANH_62	ASSURE 13C	ASSURE_19F	ASSURE_1H
ASSURE_31P	B HNCACBGP3D	B HNCACBIGP3D	B HNCACOGP3D	B HNCACOGP4D
B_HNCAGP3D	B_HNCAIGP3D	B HNCOCACBGP3D	B_HNCOCACBGP4D	B_HNCOCAGP3D
B_HNCOCAGP4D	B_HNCOGP3D	B_HNCOIGP3D	B_HSQCETF3GPSI	B_TRHNCACBGP3D
B_TRHNCACBIGP3D	B_TRHNCACOGP3D	B_TRHNCAGP3D	B_TRHNCAIGP3D	B_TRHNCOCACBGP3D
B_TRHNCOCAGP3D	B_TRHNCOGP3D	B_TRHNCOIGP3D	B_TROSYETF3GPSI	B_TROSYF3GPPH
B11ZG	BESTPROFILE	C_CACO	C_CACO_IA	C_CACO_S3
C_CAN_IASQ	C_CAN_MQ	C_CAN_MQ.2	C_CANCO_IA3D	C_CANCO_IA3D.2
C_CANCOLIA3D	C_CBCACO_IA3D	C_CBCACO_S33D		C_CBCANCO_IA3D
C_CCCO_IA3D	C_CCCO_S33D	C_CCCON_IA3D	C_CCFLOPSY16	C_CCFLOPSY16_CT
C_CCFLOPSY16_CTIA	C_CCFLOPSY16_IA	C_CCNOESY	C_CCNOESY_CT	C CCNOESY2
C_COCA	C_COCA_IA	C_COCA_MQ	C_COCA_MQ.2	C_CON_IASQ
C_CON_MQ		C_CON_SQ	C_COSY	C_COSY_CT
C_COSY2_CT	C_HACACO_3D	C_HCACO_IA3D	C_HCACO_S33D	C_HCAN_IA3D
C_HCANCO_IA3D		C_HCBCA_IA3D	C_HCBCACO_IA3D	C HCBCACO S33D
C_HCBCAN_IA3D	C_HCCFLOPSY16_3D	C_HNCA_IA3D	C_HNCACO_IA3D	C_HNCACO_S33D
C_HOBCAN_IASD	C_HICCPLOP3118_SD		C13APT	C13CPD
C_HINCO_IASD C13CPD32		C_HNCOCA2_IA3D		
C13DEPT45	C13CPDSN	C13DE45SN	C13DEPT135	C13DEPT135p
	C13DEPT90	C13GD	C13HUMP	C13IG
C13MULT	C13MULT135	C13MULT90	C13MULTCOMP	C13OFF
C13PPTI	C13RESOL	C13SENS	CBCACONHGP3D	CBCACONHGPWG3D
CBCACONHGPWG3D.2	CBCACONHGPWG4D	CBCANHGP3D	CBCANHGPWG3D	CCACONHGP2H3D
CCACONHGP3D	CCACONHGP3D.2	CCANHGP2H3D	CCANHGP3D	CCANHGP3D.2
CCCONHGP2H3D	CCCONHGP3D	CD111ZG	CD113ZG	CL35ZG
CL37ZG	CMCQ_PROTON	CMCQ_WET	CMCse_13C	CMCse_15NHMBCf2
CMCse_15NHSQCf2	CMCse_1H	CMCse_COSY	CMCse_HMBC	CMCse_HSQC
COSY45SW	COSY90SW	COSYCWGPPSQF	COSYCWPHPS	COSYDCPHWT
COSYDFGPPH19	COSYDQFPHSW	COSYGPDFPHSW	COSYGPFIXSW	COSYGPMFSW
COSYGPSW	COSYPHPR	DIPSI2ESFBGPPH	DIPSI2ESGPPH	DIPSI2ETGPSI19
DIPSI2GPPH19	DIPSIHSQCF3GPSI3D		F19	E19CPD
FHSQCCXF3GPPH	FHSQCF3GPPH	GA71ZG	gradshim1d1h	gradshim1d1h_f
gradshim1d2h	gradshim1d2h_f	gradshimdata	gradshimrcb3d	H2OSUPMLEV
H2OSUPNOESY	H2OSUPROESY	HACAHBCOSYGP3D	HACONHGPWG3D	HANHGPWG3D
HBCBCGCDCEHEGP	HBCBCGCDHDGP	HBHACBCANHGPWG4D	HBHACONHGP3D	HBHACONHGPWG3D
HRHACONHCRIMCAD	HRHANHCB3D	HRHANHCRMC3D	HCACOCRED	HEACOCRICED

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 <sup>11</sup>B and <sup>10</sup>B). These parameter sets are kept in a folder specific to the Chemistry NMR Facility at /home/topspin3.1/uwchem/par.

<u>Some experiments must utilize parameter sets provided in the UWChem folder!</u> Any experiment involving <sup>19</sup>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 <sup>19</sup>F-only parameter sets are circled in blue.

۵	Parameter Sets: rpar				
<u>F</u> ile <u>O</u> ptions <u>H</u> elp	)		Source = /home/	topspin3.1/uwchem/par	
Find file names vent	er any string, *, ? Clear				
Class = Any 💌 Dim =	Any - Show Recommended				
Type = Any - SubTyp	e = Any V SubTypeB = Any V	Reset Filters			
				provide a second	
BBFO_109Ag	BBFO_1Hpurge	BBFO_2Hlock	BBFO_2HX_notdone	BBFO_Ag109	
BBFO_B11	BBFO_B11dec	BBFO_Cd113	BBFO_Cs133	BBFO_DOSYcc1d	
BFO_DOSYcc2d	BBFO_F19	BBFO_F19CPD	BBFO_gCOSY_FF	BBFO_gCOSY_PP	
BBFO_HMBC_HP	BBFO_hoesyFH	BBFO_hoesyHF	BBFO_Li7	BBFO_Li7-ig	
BBFO_017	BBFO_017fast	BBFO_PROF19DEC	BBFO_Si29ig	BBFO_W5	
FCH_2Honlock	FCH_CoFdHd	FCH_FobsHdec	FCH_Fonly	FCH_HobsFdec	
notdone	Prodigy_2Hlk	Prodigy_2Hlk-1Hdec	Prodigy_B11dec	Prodigy_B11fast	
Prodigy_F_Pdec	Prodigy_F19	Prodigy_gCOSY_PP	Prodigy_HMBC_HP	Prodigy_HSQC_HP	
Prodigy_Li7dec	Prodigy_Li7nodec	Prodigy_Li7quant	Prodigy_P-Fdec	Prodigy_Si29IG	
	Prodigy_V51	Prodigy_W5	Prodigy_wgroesy	z_Bruk-bkups	

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  $\rightarrow$  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.

S TopShim 🛛 🖉 🖾	S TopShim 🛛 🖉 🖾
Shim Report Service	Shim Report Service
SHIM Dimension  1D  3D Optimisation solvent's default  Optimise for 1H  Use 26	1D SHIMMING Parameters: maximum order = 5 probehead = 2130036_0001 solvent = DMSO shim nucleus = 2H nucleus optimised for = 1H
TUINE Before Z-X-Y-XZ-YZ-Z T After off T	olp (from lock) = 2.49 ppm optimisation parameters = Is linewidth 1H = 0.10 Hz envelope shape / strictness = 2.00 / 200 Results: tune lock level increase = 1.3 initial 80 stdDev = 0.50 Hz
PARAMETERS	sample size = 2:21 em. position = 0.00 cm final 80 stdDev = 0.11 Hz envelope width = 0.27 Hz shim changes:
-STATUS not running	Z -68 Z2 -12 Z3 +14 Z4 -131 Z5 -76 XZ -43 Y +3
CONTROL Start Stop Help Close	YZ +3 YZ -35 duration = 2 min 13 sec completed successfully finished Fri Jun 21 17:49:21 2013

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 #,J 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**₊J

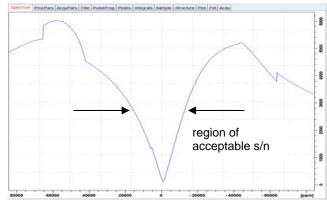
Spectrum	ProcPars AcquPar	s Title PulseProg	Peaks Integral	s San	nple Structure Plot Fid A	:qu	
AR	🖽 C 🔍 🦓	Prot	oe: 5 mm CF	PBBC	BB-1H/19F/D Z-GRE	Z130036/0001	
General Channel f1 Channel f2	S General						
	PULPROG	zgpg30 E		Pulse program for acquisition			
	TD	65536			Time domain size		
	SWH [Hz, ppm]	43859.65	348.665		Sweep width		
	AQ [sec]	0.7471104			Acquisition time		
	RG	203			Receiver gain		
	DW [µsec]	11.400			Dwell time		
	DE [µsec]	18.00			Pre-scan-delay		
	D1 [sec]	0.05000000	$\supset$		Relaxation delay; 1-5 * T1		
	d11 [sec]	0.03000000			Delay for disk I/O	[30 msec]	
	DELTA [sec]	-0.05000000	$\overline{}$	<	DELTA=d1-100m		
	DS	2			Number of dummy scans		
	NS	8			Scans to execute		
	TDO	1			Dimension of accumulation loop		

- 8. **rga**, J only needs to be run on <sup>1</sup>H, <sup>19</sup>F, or perhaps for <sup>31</sup>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 1<sup>st</sup>-order phase correction required as **de** increases, which causes baseline rolling. <sup>19</sup>F is the most common nuclei where such problems occur, but the larger **de** could also be used for <sup>29</sup>Si and <sup>11</sup>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.

#### $sw \leq 2000000$

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 
$$\mathbf{aq}$$
 after any change in  $\mathbf{sw}$ .obtainable resolution ~  $1/\mathbf{aq}$ (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  $\mathbf{aq} \ge 1$  sec is recommended.

digital resolution = 
$$\mathbf{sw} / \mathbf{si}$$
 where  $\mathbf{si} \le 1-2 \times \mathbf{td}$  (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:

$$si = 2 \times td$$
 is a good setting.

- 2. Matched filters are ok for X-spectra:  $\mathbf{lb} = 1/\mathbf{aq}$ . For  $\mathbf{aq>1}$ , usually start with  $\mathbf{lb=1}$ , but reduce if helps with resolution.
- 3. Quantitation: Usually, acquire with default parameters (usually with a faster guess for  $d1_{fast}$ ) until a good s/n is obtained. Integrate(deconvolute) the peak(s) of interest.

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

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. <sup>13</sup>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 <sup>1</sup>H (or possibly <sup>31</sup>P) is often superior to the point of being the only option. This is true for <sup>15</sup>N at natural abundance, and for most nuclei that resonate at frequencies below <sup>15</sup>N.
- 6. Referencing in TopSpin is typically very good. The software automatically references the spectrum to the assumed position of the <sup>2</sup>H lock signal (e.g., CDCl<sub>3</sub> at 7.2 ppm). A very strong recommendation, even so, is to *always* keep a <sup>1</sup>H 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  $\mathfrak{S}$ , can then be used to check the referencing of the spectrum. In TopSpin:
  - a) While in the X-spectrum experiment, type **xref**, J
  - b) enter the filename+expno+procno of the referenced <sup>1</sup>H spectrum

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