Selective 1D Experiments in TopSpin

A. Preparation for selective 1D (sel1D) experiments:

1. The first issue — true also for 2D experiments — is to ***always(!)*** tune, shim and acquire sel1D experiments without sample spinning: **ro** ↵

   Stop rotation and set the frequency = 0.

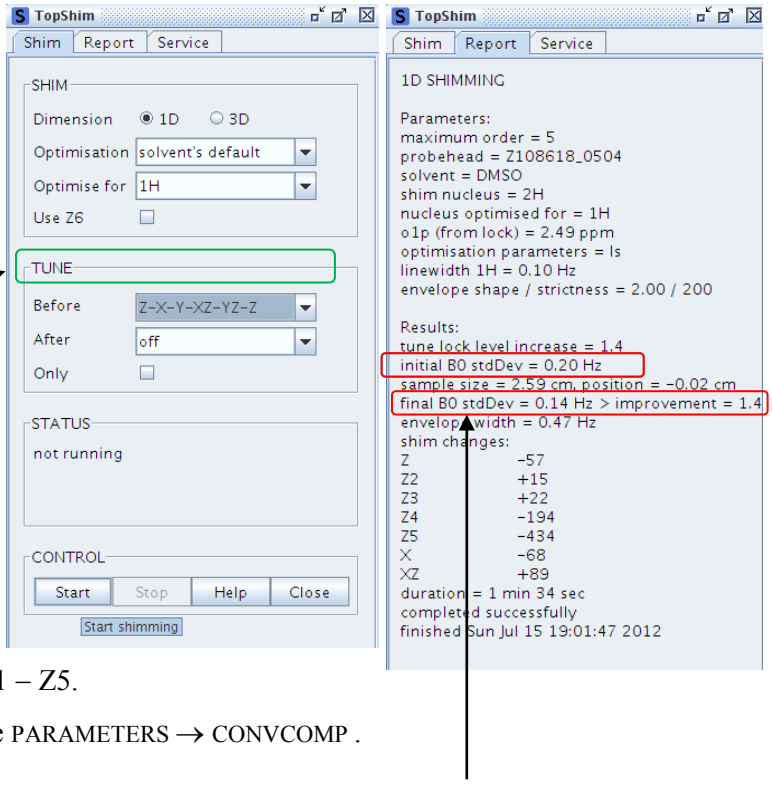
2. **atma** ↵ is fine, but do this with the sample spinning off.

3. **topshim gui** ↵

   select TUNE[1] → After [this works better than Before]:

   This selection will systematically adjust the noted shims in order — doing LOCKPHASE first — to maximize the lock value. This procedure is similar to what used to be called "simplex shimming". A PFG **SHIM** (the *normal* topshim) is run before this, providing high-quality adjustments to Z1 – Z5.

   For DCH and Prodigy probes, *always* include PARAMETERS → CONVCOMP .



4. Click on the REPORT tab and note especially the "initial B0 stdDev" and the "final B0 stdDev" values. The final B0 stdDev should be < 1 Hz. If it is larger, run the full topshim procedure again. If the stdDev stays large — take an NS=1 spectrum to check the actual linewidth — there is likely a problem with your sample: it needs filtering; the tube is scratched (throw it away!); the sample is aggregating (try a lower concentration, or different solvent/buffer); not have enough solvent; etc.

5. Acquire a proton spectrum of your sample.

6. Obtain T_1 estimates for this sample, or use prior knowledge from a set of similar samples. For publishable data, use of T_1 values from your sample to setup noesy1d/roesy1d is strongly recommended.

7. For quantitative data, re-acquire the proton spectrum using d1 ≥ 3×T_1. E.g., setting up as follows:

   re 1 ↵ ; move to exp#1

   wra 10 ↵ ; copies exp#1 (including data) to exp#10

   re 10 ↵ ; move to exp#10

   d1 T_1(longest)×3 ↵ ; set repetition delay for a quantitative acquisition

   rga ↵ ; readjust receiver gain

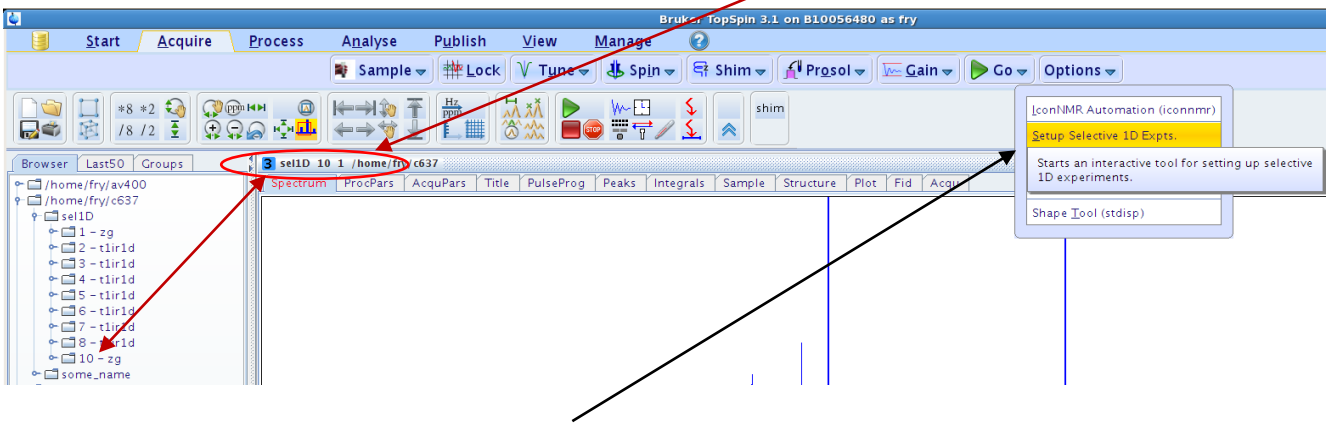
   zg ↵ ; acquire

   PULPROG=zg30 ;in the above is strongly recommended.

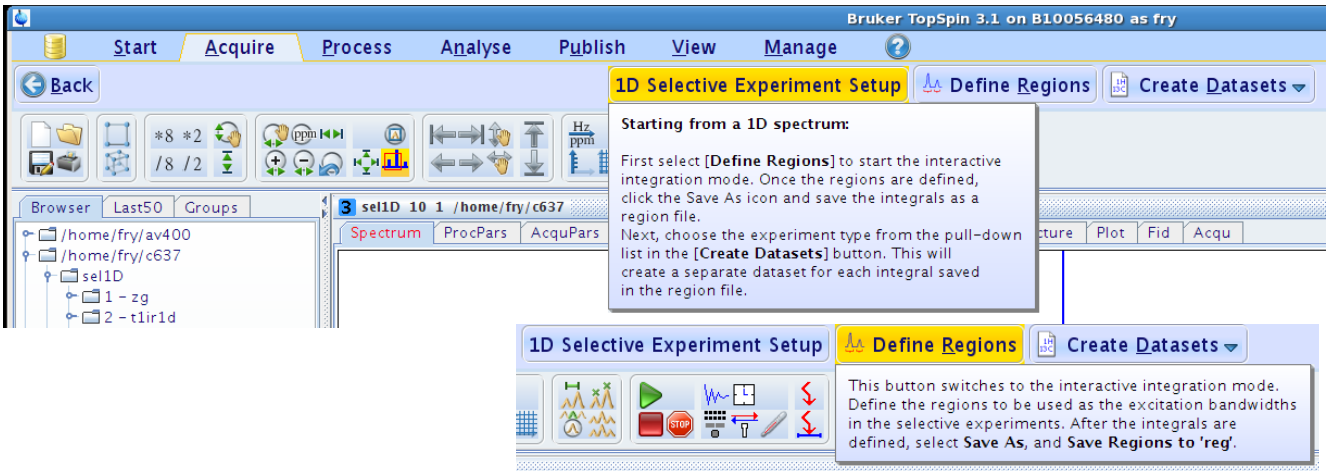
[1] Bruker uses confusing language here. **atma** performs an *electronic* tune and match to the probe's circuitry. This "probe tuning" has nothing to do with TUNE performed in topshim. A better description for the topshim TUNE would be lock-based-shimming (LBS) or something similar. The TUNE language relative to shimming is old: way back when (e.g., on the ACs), lock-based automated adjustment, or "tuning", of the shims was available via commands like TU1.

B. Creation of selective pulses in the sel1D experiments:

1. Make sure you have a good-quality ^{1}H spectrum in some exp# in the data folder (see above). Stay located in the ^{1}H spectrum (do *not* do an **iexpno** or similar):



2. ACQUIRE → OPTIONS → SETUP SELECTIVE 1D EXPTS. will give the following flowbar:



3. Read through the comments for the buttons. The next thing to do is click on DEFINE REGIONS .

4. DEFINE REGIONS enters Integration mode (identical to PROCESS → INTEGRATE , or **.int** ↵). Place integrals on the peaks/multiplets you want the sel1D experiment(s) to select.

   (a) click off (blue background) to horizontally expand the spectrum

   (b) click on (yellow background) to place integrals (c) click to delete all integrals

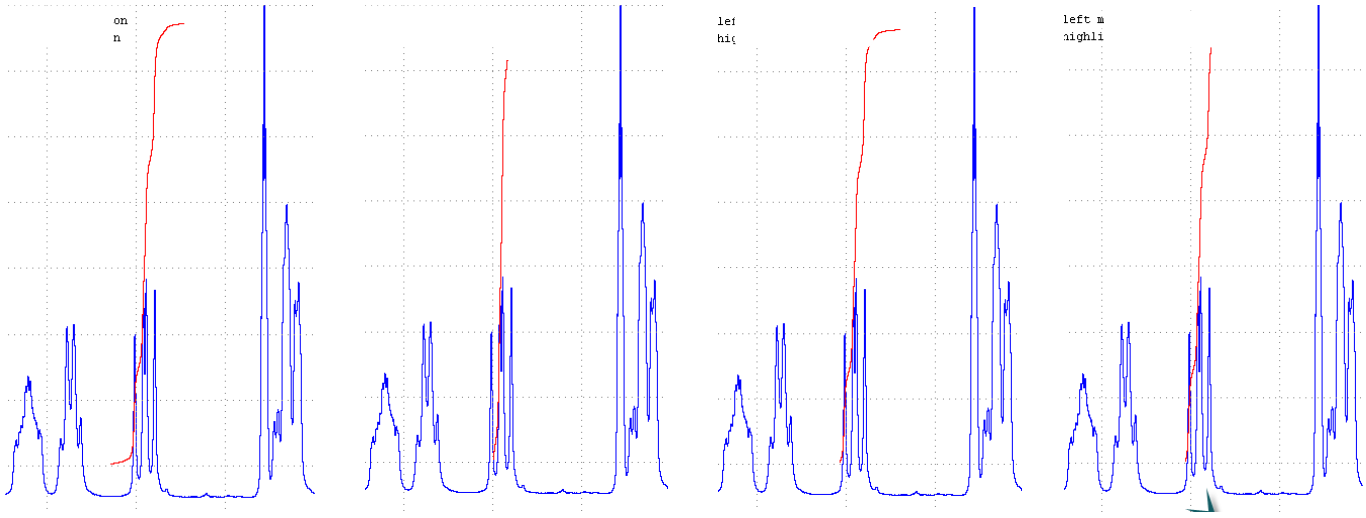
   (d) Proper selection of the integral regions is important to data quality:
      i. The rf pulse will excite a region somewhat wider than the actual integral selection, so keep the end-points close in on the multiplet.
      ii. Do not start or end "inside" the multiplet.
      iii. Typically place the integral symmetrically about the multiplet.

A. Too wide: dwfd peak may be excited.

B. Too narrow: integral is inside multiplet.

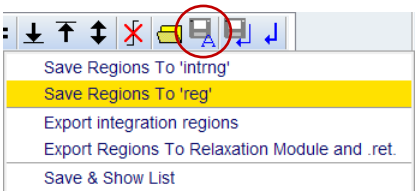
C. Asymmetric: may work OK, but best left sym.

D. Good setup: integral definition at edges of multiplet.



(e) when you have the integrals properly selected, perform a

Save As → Save Regions To 'reg'



(f) click 'Yes' if asked to Save Changes? when exiting (by clicking ↲)

(g) right click on the spectrum and select: **Spectral Display Preferences**

to toggle whether the integrals are shown when not in integration mode.

5. click on: **CREATE DATASETS**

or better, enter in the topspin command line: sel1d↲

and select the experiment you want (fig. on next page). The "gradient" versions of all experiments are strongly recommended, as they provide better artifact suppression using the pulsed-field gradients (PFG).

Use a multiple of 8 scans for all selective-1D experiments except tocsy1d (which can be run with NS=2).

SELCOGP – *selective cosy 1D*

– set **d1 = T_1(longest of interest) × 1-1.5**

– adjust **d4** depending on the J_{HH} desired

– this experiment is relatively new to our facility; its most likely utility is for observing small couplings (which cannot be done with the selective tocsy experiment), or perhaps to measure coupling constants; try **ps** or **mc** following **efp**, which will remove phase distortions

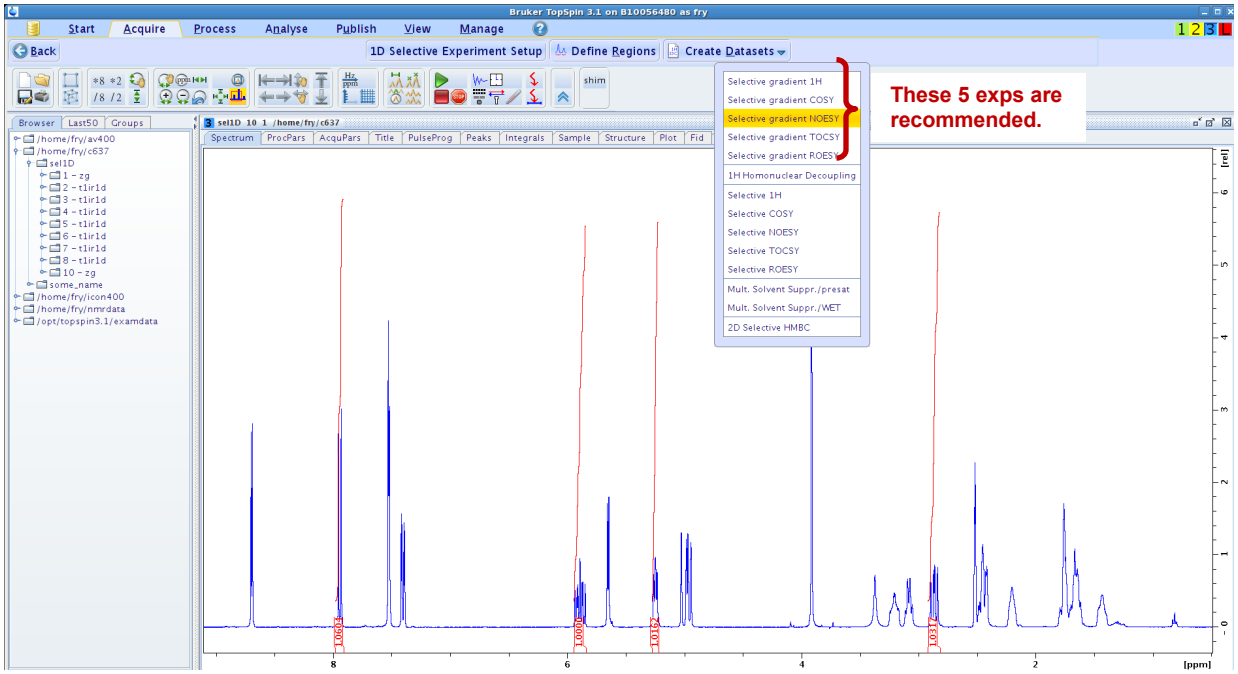
tocsy1d.UW – *selective tocsy 1D*

– set **d1 = T_1(longest of interest) × 1.5**

– usually run multiple mix times (i.e., take multiple spectra; 6 spectra if following the list below):

d9 = 0 ; checks the selection (recommended: always acquire this a spectrum with **d9**=0)
d9 = 15ms ; observe protons 2- to 3- bonds away
d9 = 30ms ; primarily 2- to 3- bond correlation with small relays to next shell
d9 = 40-60ms ; 1 to 2 relays
d9 = 80ms ; common value used to observe 2 to 3 relay shells
d9 = 120ms ; common maximum value, usually showing all protons in a spin system

d9 = 200ms ; maximum value, please do not use longer



noesy1d.UW **–*selective noesy 1D***

- set **d1 = T_1(longest of interest) × 2.5-5**
- start with **d8** = 0.6×T_1(fastest of interest)
- obtain a build-up curve (e.g., three experiments with **d8** = 0.1,0.2,0.3s) to confirm the NOE
- It is often useful/important to obtain a **d8** = 0 spectrum to confirm the selection is clean. It is also sometimes needed for exchange (EXSY) experiments to get **d8** very small. The problem is that the standard sequence cannot go this short. Change the pulse sequence (PULPROG) as follows if short mix (d8) times are needed:

noesy1d.UW	**d8** ≥ 62ms
noesy1d_fast.UW	12 ms ≤ **d8** < 62 ms
noesy1d_veryfast.UW	0 ≤ **d8** < 12 ms

- The rf hardware on the 360 cannot produce modern pulse shapes. Setup experiments using the parameter set: **H1_noesy1d-dante.UW**. The following pulse sequences are available that work well; the first is called in by the parameter set (the 2nd enable short **d8** mixing times, down to 0 s). See the comments within the pulse sequences themselves for more information:

  noesy1ddante60.UW
  noesy1dd60_fast.UW

- Zero-quantum (ZQ) artifacts are common for protons that are J-coupled; use integrals to help measure differences between ZQ and NOE; ZQ artifacts have little mix-time dependence, so subtracting a mix/d8=0 spectrum from the others provides double-differencing that might be useful
- For small MW, an NOE will give a positive integral (opposite the selected peak), whereas for high molecular weight the NOE will switch to negative. NOEs will *crossover* when the MW ~ 1000-3000; in this region roesy1d is recommended, as NOEs may be zero or too small to detect.
- ZQ artifacts are relatively independent of mix time, whereas NOE will build-up

roesy1d.UW **–*selective roesy 1D***

- set **d1 = T_1(longest of interest) × 2.5-5**

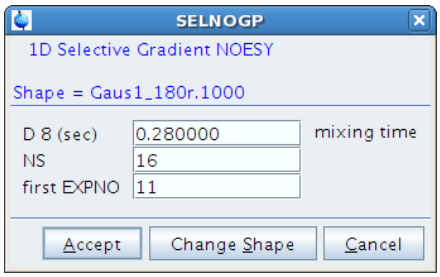
– start with **p15** = 150ms (and only after trying noesy1d.UW)

– vary **p15** = 50ms to 0.6 × T_1(longest of interest); linear buildups will occur as with noesy1d

– exchange will occur as with noesy1d, but since *roe*'s are always positive, and exchange is always negative, the separation here will be less ambiguous in the crossover and high HW regions

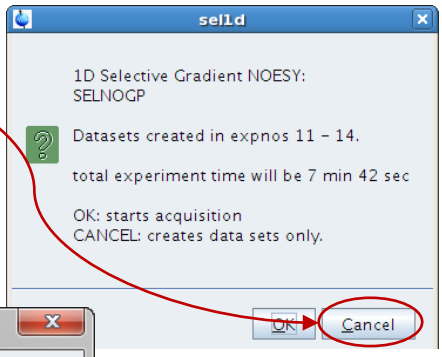
6. After the experiment selection, a parameter box will open allowing modification of mix time and **NS**. See recommended ranges of the mix times and minimum **NS** in the table on the last page.

   Click on **ACCEPT** after changing parameters.



7. By clicking on **OK** or **CANCEL** the experiments, one for each integral, will be created. **CANCEL** is recommended.

   The shaped pulses are generated using the calibrated 90° pulsewidth (**p1**) typically read automatically by **getprosol** .

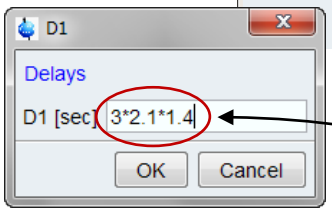


C. Final modifications, acquisition and processing:

1. In each experiment, set (see table below)

   d1 = 1.5 to 5 × T_1

   Note that you can use math in Topspin, as show in the example on the right.



example using **t1ir1d**:
d1 = 3×*nulltime*×1.4

2. Set mix times (**d4, d8, d9**) following suggestions as given in the table below.

3. Don't be chintzy with experiment times. In *noesy1d* and *roesy1d* experiments, very small NOEs can be observed (down to 0.1% or smaller). Taking data for 10 min for each experiment is common; letting it run for a couple hours (to a full overnight) would not be at all unusual. As always, longer data sets provide better data.

4. For ***noesy1d*** and ***roesy1d*** data, use **lb = 1** (resolution is typically not of most interest in this data).

   For **noe/roe** data, build-up curves are a standard method of confirming the true identity of the effect. For small molecules, ZQ artifacts (out-of-phase/dispersive components) are troublesome between coupled multiplets; double-differencing can assist (see on-line notes, or staff for help). For large molecules, spin-diffusion can relay NOEs, but these will produce time delayed non-linear build-ups.

   The ratio of the integral of the multiplet-of-interest to the integral of the selected multiplet, both normalized by # protons, is typically reported in percent along with the mix time (**d8**) and the repetition delay: **d1+aq+d8**. For roesy1d data, also report the spinlock strength in kHz.

5. For ***cosy1d*** data, try **ps** or **mc** (after **efp**) to remove phase distortions.

6. For ***tocsy1d*** data, some mix times (**d9**) will "work" better than others if in-phase multiplets are desired. In particular, magnetization transfer will generate non-absorptive features, but at some mix times, pure absorptive (good in-phase) multiplets will be observed.

   For ***tocsy1d*** data, always acquire a **d9**[mix] = 0 and a range of other mix times. Report the mix time and repetition times **d1+aq**, and the spinlock strength in kHz.

revised: 26 Mar 2019 (cgf)

[Note: Many of the following will not remain the recommended parameter sets; look for updated guides regularly]

STANDARD 2D SEQUENCES	**Description**	**PARAMETER SET pulse sequence**	**d1[a]**	**mix[b]**
standard (magnitude-mode) COSY "*routine*"	^{1}H-^{1}H correlations; usually just 2- to 3-bond couplings	COSYGPSW cosygplrqf	1 to 1.5 × T_1(loi)	–
long-range COSY	confirm ^{1}H-^{1}H correlations w small (0.5 to 3 Hz, 2- to 5-bond) couplings	cosylr.UW[c] cosygplrqf[d]	1 to 1.5 × T_1(loi)	d4 = 50-200 ms [long-range J-evolution delay]
double-quantum filtered COSY	strong singlets (including solvent peaks) via double-quantum filtering (DQF), and enables measurement of ^{1}H-^{1}H coupling constants; note special setup requirements in pp (?for rg?)	COSYGPDFPHSW cosygpmfphpp	1.5 to 3 × T_1(loi)	see pulse sequence notes to change to TPF (Triple Quantum Filtering), which removes doublets
TOCSY	^{1}H-^{1}H correlations based on couplings; 2-3 datasets differing by mix time are often acquired to observe "relayed" couplings	MLEVPHSW mlevphpp	1.5 to 5 × T_1(loi)	d9 = 15 to 150 ms careful with duty cycle!
standard multiplicity-edited HSQC "*routine*"	^{1}H-^{13}C 1-bond correlations, –CH_2– inverted (dept-135 analog)	HSQCEDETGPSISP hsqcedetgpsisp2.3	1.5 to 2 × T_1(loi)	cnst2 = J(CH) = 145 Hz
standard non-edited HSQC "*routine*"	^{1}H-^{13}C 1-bond correlations, all peaks positive (dept-45 analog)	HSQCETGPSISP[c] hsqcetgpsisp2.2[d]	1.5 to 2 × T_1(loi)	cnst2 = J(CH) = 145 Hz
coupled HSQC	^{1}H-^{13}C 1-bond correlations with coupling	HSQCETNDGPSISP[c] hsqcetgpsisp2.2nd[d]	1.5 to 2 × T_1(loi)	cnst2 = J(CH) = 145 Hz
standard HMBC "*routine*"	^{1}H-^{13}C n-bond correlations, 2- and 3-bond (usually), with 3-fold 1-bond filter; often acquire 2nd set with smaller cnst13	HMBCETGPL3ND hmbcetgpl3nd	1.5 to 2 × T_1(loi)	cnst2 = J(CH) = 145 Hz cnst13=Jn(CH) = 10 Hz
NOESY	^{1}H-^{1}H correlations based on proximity (also for exchange)	NOESYGP noesygpphpp	2.5 to 5 × T_1(loi)	d8 = 0.1 to 1 × T_1(foi)
ROESY	^{1}H-^{1}H correlations based on proximity; for intermediate MW	ROESYPHPR roesyphpr.2	2.5 to 5 × T_1(loi)	p15 = 0.1 to 0.5 × T_1(foi) careful with duty cycle!
SELECTIVE 1D SEQUENCES				
selective COSY-1D	protons 2- to 6-bonds from selected multiplet give antiphase peaks; d4=large (≤ T_1; for small couplings) can be used; coupling will transfer through heterobonds	SELCOGP new: cosy1d.UW[c] selcogp; cosy1d.UW[d]	1.5 to 3 × T_1(loi)	d4 = 1/4 J(HH) ns = 8×i
selective NOESY-1D	protons within 5Å produce NOEs; phase selected peak negative, then other peaks are positive for small MW, negative for large MW; exchange will produce negative peaks; acquire a mix time series, plot build-up curve to confirm NOE	SELNOGP new: **noesy1d.UW**[c] selnogp; noesy1d.UW[d]	2.5 to 5 × T_1(loi)	d8 = 0.1 to 1 × T_1(foi) ns = 8×i
selective ROESY-1D	protons within 5Å produce ROEs; phase selected peak negative, all other peaks are positive independent of MW; acquire a mix time series, plot build-up curve to confirm ROE	SELROGP new: **roesy1d.UW**[c] selrogp; roesy1d.UW[d]	2.5 to 5 × T_1(loi)	p15 = 0.1 to 0.5 × T_1(foi) p15 > 500000 µs is not allowed ns = 8×i
selective TOCSY-1D	protons 2- to 3-bonds from selected multiplet give in-phase peaks; only couplings ≥ 3 Hz transfer; couplings will *not* go through heterobonds; use d9 series to see coupling "relays"	SELMLGP new: **tocsy1dzq.UW**[c] selmlgp;tocsy1dzq.UW	1.5 to 5 × T_1(loi)	d9 = 15 to 200 ms d9 > 200ms not allowed ns = 2×i

[a]**loi** ≡ longest of interest [b]**foi** ≡ fastest of interest

[c]these parameter sets are located in the **/home/topspin3.1/uwchem/par** folder (all others are in **/opt/topspin3.1/exp/stan/nmr/par**)

[d] these pulse sequences are located in the **/home/topspin3.1/uwchem/pp** folder (all others are in **/opt/topspin3.1/exp/stan/nmr/lists/pp**)