Summer 2013 Chem 637 – Lab #4

Assignment due at beginning of labs July 23–29.

Use TopSpin on Callisto or Persephone for this HW.

This week you will learn how to setup and acquire 2D experiments, both "routine" and more advanced, using the TopSpin environment. This is a 2 week lab. Routine experiments — cosy and hsqc — will be done the 1st week. More advanced experiments — tocsy, hmbc, noesy and roesy — will be done the 2nd week.

1. Acquire high-quality "routine" 2D data (1st week)

Some types of 2D data can be acquired in a relatively standard fashion: few parameters changes need to be considered. cosy (COSYGPSW) and hsqc (HSQCEDETGPSISP, HSQCETGPSISP) are primary examples, where correlations of ^{1}H to other ^{1}H or ^{13}C provide (often very) useful information. For these experiments, the following parameters should be checked:

d1 → ≥ T_1(longest of interest)
→ default = 2s
→ peptides and proteins can often be run faster, up to **d1**=1s
→ samples in O_2-free atmospheres: check T_1 values to avoid potentially serious artifacts, as relaxation can get significantly longer than "normal"

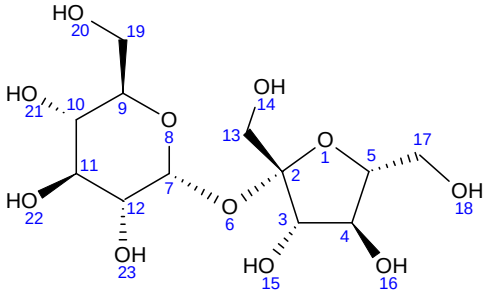
. **AQ[F2] ≤ 0.2 s for hsqc** ; longer values can damage the cryoprobes (DCH and Prodigy) .

TD[F1] → 128 to 512 is typical
limits resolution overall for cosy, and in the ^{13}C dimension [F1] in hsqc
F1 resolution ≈ SW[F1] / TD[F1]
linear prediction can improve the resolution; but TD[F1] remains as the limiting factor

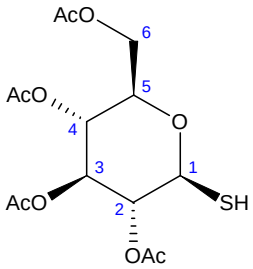
ns → check the pulse sequence listing (toward the end) for minimum **ns** settings;
for hsqc and hmbc, the 1st row should show proton peak intensities; otherwise **ns** should be increased; if the peaks are large, decrease **ns** (but not below the minimum)

(a) Acquire a proton spectrum of a sample of your choice, with knowledge of T_1 values for this sample. You can use the facility samples sucrose in D_2O, or thio-glucose in $CDCl_3$ as alternatives.

sucrose



thio-glucose



(b) Acquire a ^{13}C spectrum (if possible) of the same sample. This step is not required, but can be helpful when 1st working with hsqc and hmbc data. Similar with a dept-135 or -45: useful, but not required.

(c) Setup a cosy spectrum (rpar COSYGPSW) using the minimum **ns** (check in the pulse sequence).

(d) Apply sweepwidth optimization as follows:

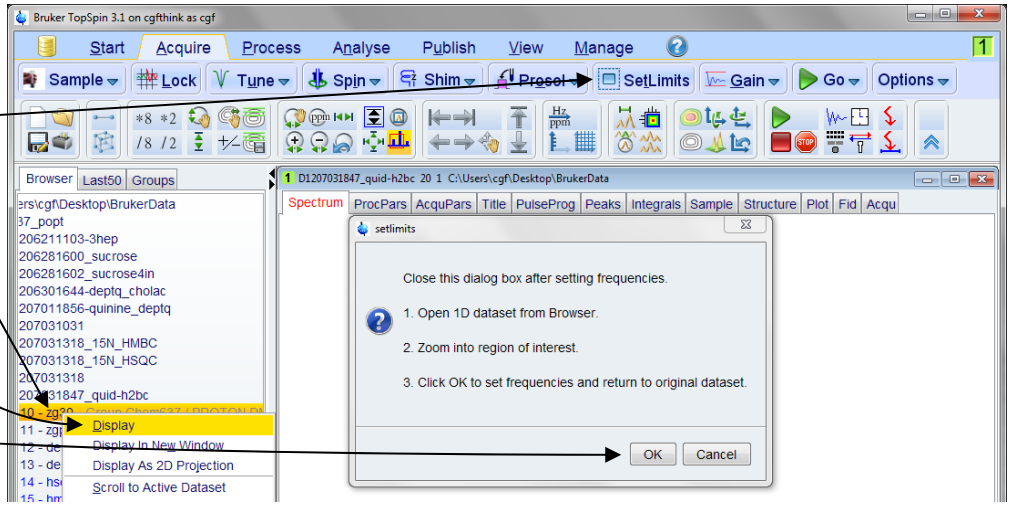
i) click SETLIMITS

ii) right-click on the Proton spectrum

iii) click Display

iv) expand the spectrum (see below)

v) click OK



(e) For COSY, both dimensions are set.

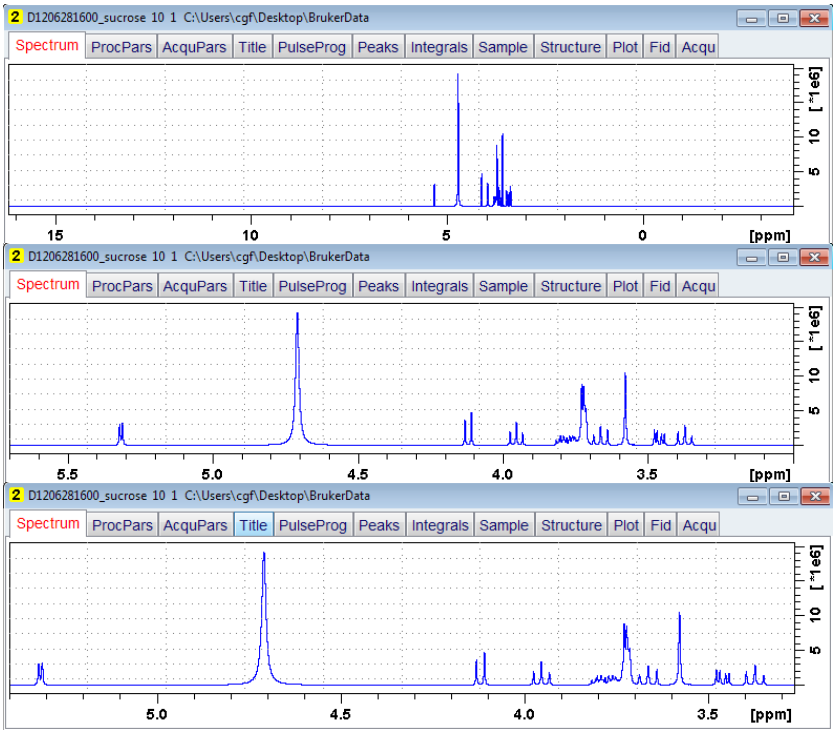
(f) Check **ns** **d1** **TD[F1]** . Set **rg** the same as in the ^{1}H 1D spectrum.

Q1: Suggest why TD[F2] ≤ 8×TD[F1] is a condition usual met with homonuclear COSY spectra.

(g) Acquire a COSY spectrum. Process in TopSpin using **xfb** ↵ and **sym** ↵. Plot and turn in.

^{1}H expansions (β and γ) for sucrose.

α. Full 1D spectrum of sucrose.



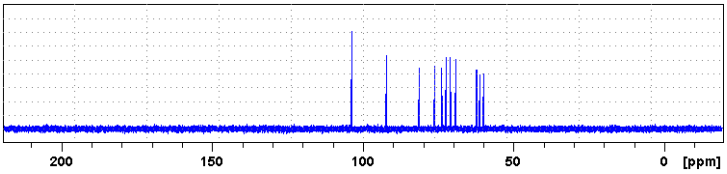
β. A good expansion, especially for the acquisition dimension, F2, as larger SW[F2] costs little. For the F1 (indirect) dimension, the expansion could be a bit tighter (but not by much), improving the F1 resolution. *Leave ~10% of the spectrum on each edge of the spectrum.*

γ. Too tight of an expansion. Proton multiplets at 5.4 and 3.35 ppm are too close to the edges, and will cause a variety of problems in the resulting 2D spectrum.

(h) For HSQC (rpar HSQCEDETGPSISP or HSQCETGPSISP) or HMBC (rpar HMBCETGPL3ND), perform step (d) for the ^{1}H dimension [F2].

(i) Repeat step (d) for the ^{13}C dimension [F1], but now select the ^{13}C or dept spectrum in part ii).

If the sample concentration is too low to acquire a ^{13}C spectrum, the sweepwidth can be set manually. Take sucrose as an example, with its ^{13}C spectrum as shown. It is important to keep in mind that a ^{13}C spectrum is not needed to setup the range of ^{13}C chemical shifts for HSQC or HMBC experiments. For sucrose, reasonable estimates (staying conservative) would be:

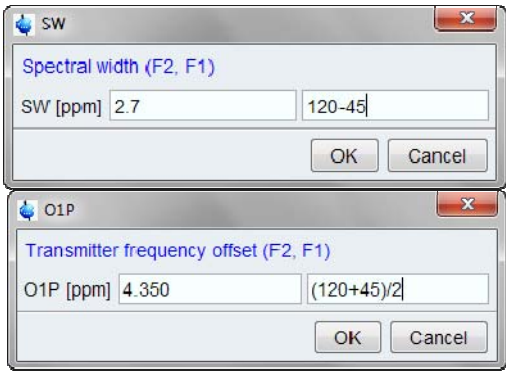


$$45 \text{ ppm} \leq \delta \leq 120 \text{ ppm}.$$

To manually setup the spectra window:

→ type **sw** ↵ in F1 enter: 120-45

→ type **o1p** ↵ in F1 enter: (120+45)/2



(j) Both dimensions are now set. Check **ns** **d1** **TD[F1]** .

Q2: Show a calculation for the resolution in the ^{13}C dimension? Give the answer in Hz/pt and ppm/pt.

(k) Use **rga** ↵ with HSQC and HMBC spectra.

(l) Acquire an HSQC spectrum. Plot and turn in.

2. Acquire a portion of two high-quality "non-routine" 2D spectra (2nd week)

2D data other than standard cosy and hsqc should only be acquired. Choose two types from hmbc, tocsy, noesy and roesy; note the parameter sets listed in the table at the end of this HW. Check and modify as needed the following parameters:

d1 – set according to the "T_1 Abusability" table given in the online guide "Pulse width calibrations and T1 estimates in TopSpin"

ns – make certain **ns** is set to minimum value or a multiple of that value

TD[F1] – resolution must be set according to information required

i) some cosy-types involve J-evolution sufficient to observe small J-couplings; here we need see an unambiguous crosspeak (having sufficient intensity), and often do not need to resolve the coupling (which might not be possible in any event)

ii) when J-couplings need to be measured in a 2D, resolution is critical; see Claridge Fig 5.50 and surrounding discussions involving anti-phase cancellation effects for J measurements

iii) constant-time experiments may be required, especially for labeled compounds

mix – a variety of different parameters are involved; see notes in following table

➜ **proton rf pulse lengths** – a pw90 ≡ p1 calibration may be needed, and a correction can be made:

Suppose you found:

p1 = 15.4

plw1 = –10.3

The power ***must*** be read off properly:

→ typing **plw1** will not give the correct value!! rather

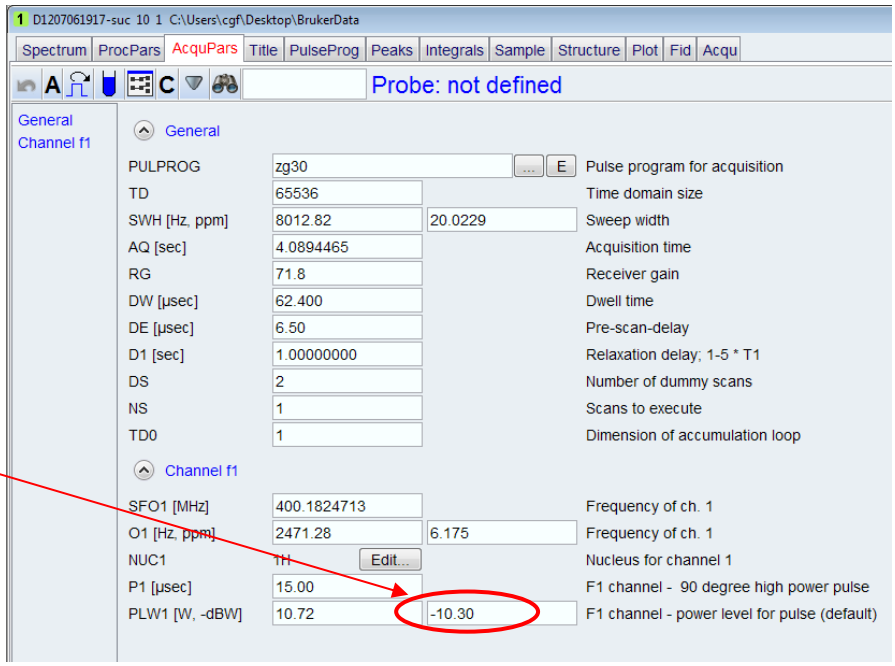
→ type **ased** ↵ or click in ACQUPARS

→ read the value in the 2nd box

Now use the following command:

getprosol 1H 15.4 –10.3 ↵

which updates all ^{1}H rf pulses.



(a) Choose one advanced experiment from the following table: noesy, roesy, tocsy, cosydqf

(b) Read in the parameters for the experiment, and adjust the four parameters listed above: **ns d1 TD[F1] mix** (e.g., = **d8** in noesy types).

(c) Perform the **getprosol** correction if pw90/p1 is > 5% different than the default value.

(d) Do an **expt** or click to estimate the experiment time.

(e) Acquire at least 8 rows, but stop after 5 min (unless you need the spectrum for research purposes, and have sufficient time scheduled).

(f) Plot at least the 1st row and turn in.

3. Brief processing tips for 2D spectra in TopSpin

I (cgf) don't like the **PROCESS → PROC. SPECTRUM** option available in TopSpin's flowbar. No doubt, this antipathy has much to do with my lack of knowledge about the **proc2d** au routine that it runs. But in general, automated processing of 2D spectra must be balanced: ease-of-use seems useful, but knowledge about what is done is usually imperative. Improper processing often leads to significant issues with 2D data.

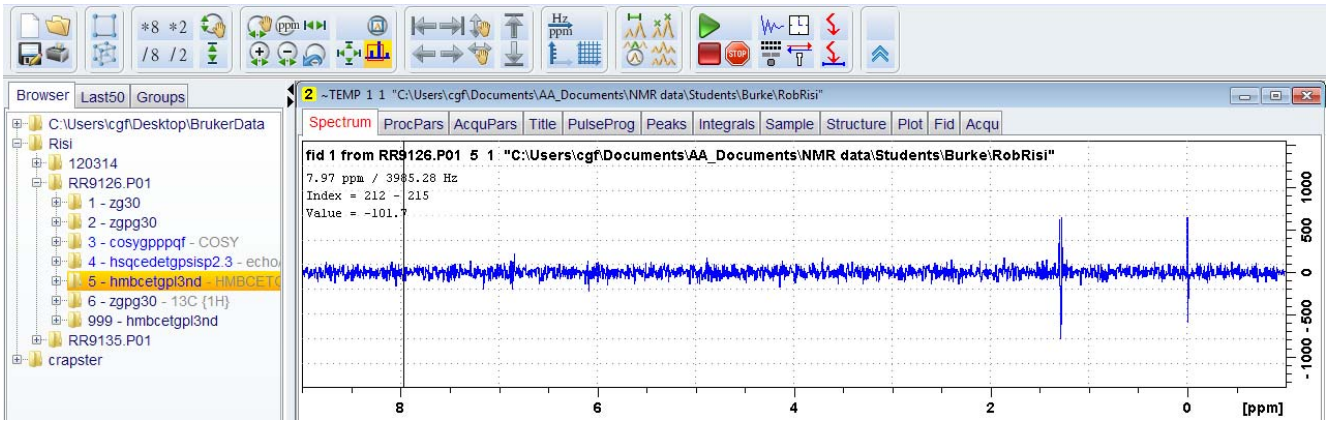
(a) <u>General processing commands:</u>

xfb ; performs apodization (see next section), then transforms data in both dimensions

rser # ; reads row # from the 2D serial file, and puts it in ~TEMP location; the data can now be **efp** 'd, and some proton peaks should be observed (see Fig 3(a) below); if not, **ns** may be too small; use to return to the 2D dataset

xf2m ; some data need to be displayed in mixed-phase mode, such as **hmbcetgpl3nd**; see end comments in the pulse sequence to find out if this command is needed

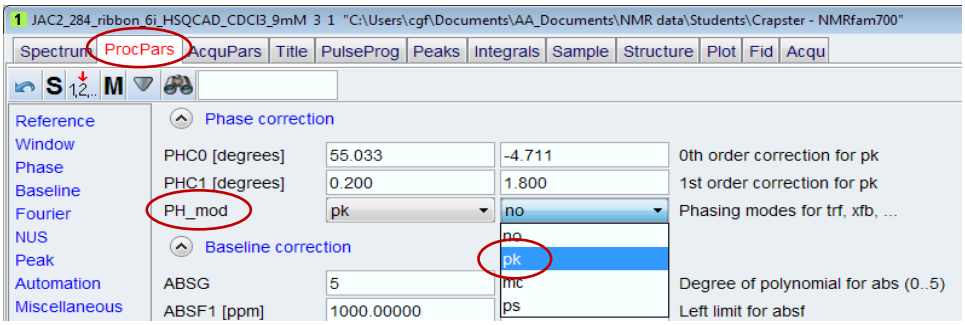
Fig 3(a): **rser 1; efp** from an hmbc at close-to-insufficient **ns** (some protons are observed, but others are not). This method works well for checking **ns** in hsqc/hmbc type experiments. Other methods are needed to check cosy types.



(b) Phasing 2D spectra:

This is simple: click PROCESS → ADJUST PHASE (or type **.ph** ↵). Right-click on peaks and select ADD.

What is not so simple is that many parameter sets have **PH_mod** set to **no** in the F1 dimension. This must be changed to **pk** for correcting phase in that dimension (along the columns).



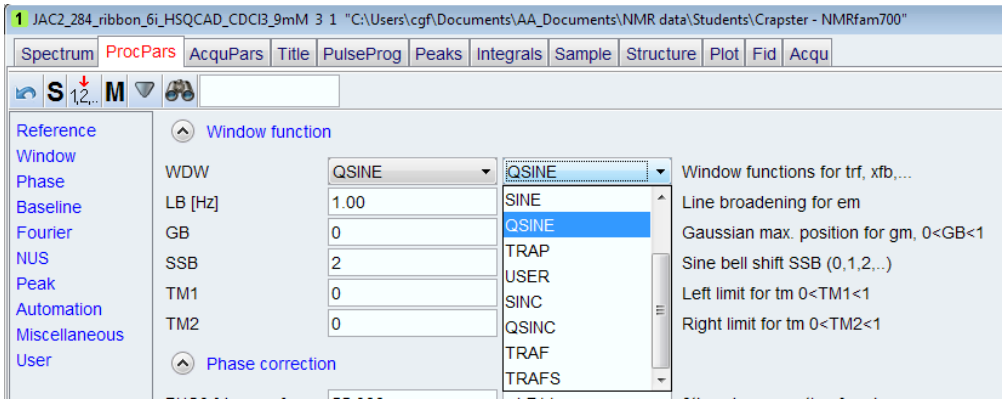
(c) 2D Apodization:

SINE SSB=0 – sine curve from 0 to 180° matching TD: =0 at 0°/pts=1, =1 at 90°/pts=TD/2; =0 at 180°/pts=TD: provide resolution-enhancement, but with some loss (often considerable) in sensitivity

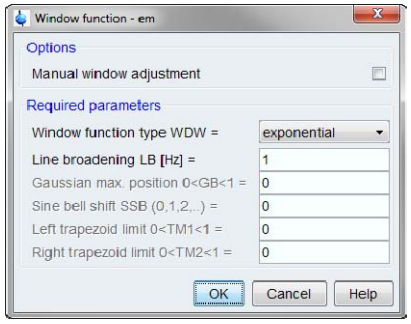
SSB=2 – cosine curve from 0° to 90° matching TD: =1 at 0°/pts=1, =0 at 90°/pts=TD

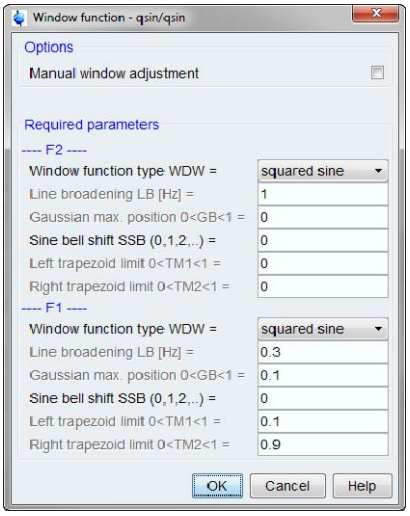
QSINE SSB=0 – sinebell-squared: square of the above curve; provides more resolution-enhancement with additional loss in sensitivity; standard apodization for cosy

SSB=2 – cosine-squared: square of cosine curve; provide excellent retention of signal at the beginning of the fid with a good taper to zero at the end; standard apodization for hsqc



The function is selected either directly from the PROCPARS panel (see above), or by entering **wm** ↵. The dialog box differs depending on whether you are in 1D or 2D mode. You can try the manual window adjustment (works only in 1D mode), which will bring up a new window after clicking OK. Bruker's HELP is reasonably good.





[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	2 to 3 × T_1(loi)	see pulse sequence notes to change to TQF (also 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 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 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 selcogp	1.5 to 3 × T_1(loi)	d4 = 1/4 J(HH)
selective NOESY-1D	protons within 5Å produce NOEs; phase selected peak negative, then other peaks are positive for small MW, negative for large MW; excexhange will produce negative peaks; acquire a mix time series, plot build-up curve to confirm NOE	SELNOGP selnogp	2 to 5 × T_1(loi)	d8 = 0.1 to 1 × T_1(foi)
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 selrogp	2 to 5 × T_1(loi)	p15 = 0.1 to 0.5 × T_1(foi) careful with duty cycle!
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 selmlgp	1.5 to 5 × T_1(loi)	d9 = 15 to 150 ms careful with duty cycle!

[a] **loi** ≡ longest of interest
[b] **foi** ≡ fastest of interest
[c] these parameter sets are located in the **par/user** folder (all others are in **par**, in **/opt/topspin3.1/exp/stan/nmr/**)
[d] these pulse sequences are located in the **pp/user** folder (all others are in **pp**, in **/opt/topspin3.1/exp/stan/nmr/lists/**)