Selective 1D Experiments on the Bruker NMR

(1D NOESY, 1D TOCSY, etc)   by Monika Ivancic, PhD (December, 2018)

1. Acquire a ^{1}H 1D experiment (uvm.1H1D) (see Bruker Speedy setup guide)
   DO NOT spin the sample! (OR turn spin off after you acquire the ^{1}H 1D)

2. Determine T1 relaxation times for the ^{1}Hs of interest in your sample (see Estimating ^{1}H T1 values on the Bruker)
   Note: this will take some time (20-30 min) OR you may try to guestimate these values and see what the selective data provides

3. For a quantitative ^{1}H 1D, re-acquire the experiment with using D1 > 5* longest T1 of sample (you may do this in expt. no. 2, making sure you change D1)

4. To set up the selective experiment, start in the ^{1}H 1D dataset that you just acquired (standard or quantitative). Under the acquire tab, go to 'options' and choose 'Setup Selective 1D Expts.'

5. This brings you to the following flow bar. Read thru the comments under the buttons.
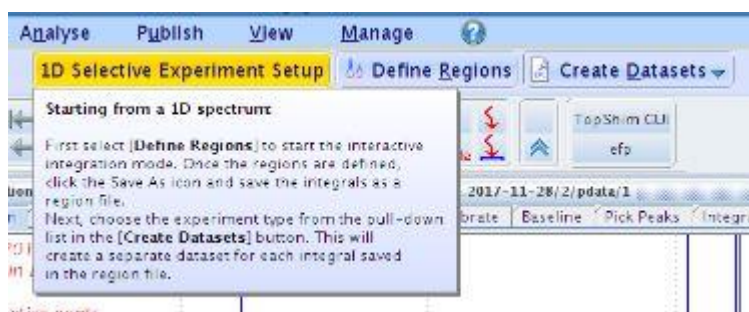

6. Then click on 'Define regions'.

7. This puts you in integration mode (same as using Process -> Integrate), where you will be placing integrals on the peaks that you want to irradiate for the selective 1D.

   a) click ⊥ off (get to blue background) to be able to expand the spectrum

   b) click ⊥ on (get to yellow background) to choose integration regions   c) click ✳ to delete all integrals

   1▪ Acquisition finished: /opt/topspin3.2.6/data/nmrsu/quinine-selective1Ds_2017-11-28/2/pdata/1
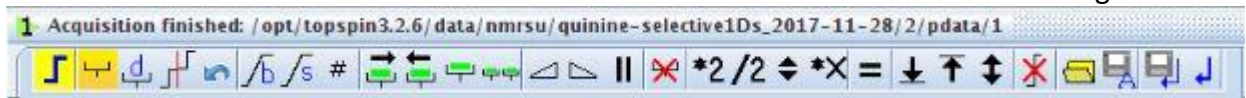
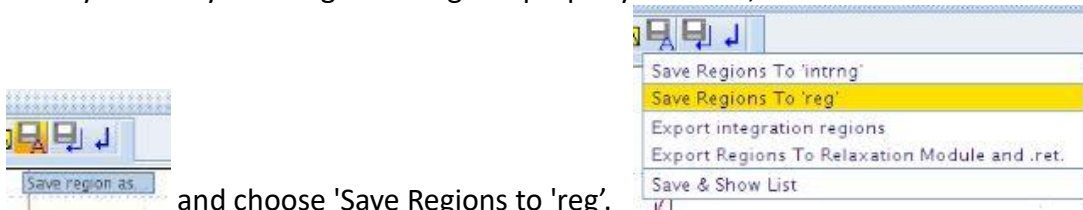   d) proper selection of integration regions is important to data quality:
      i. The rf pulse will excite a region nearly twice as wide as the integration region, so keep endpoints close on the peak/multiplet
      ii. Do not start or end the integration region inside the peak/multiplet
     iii. Place integration region symmetrically about the peak/multiplet

   too narrow   too wide   asymmetric   just right integral selection
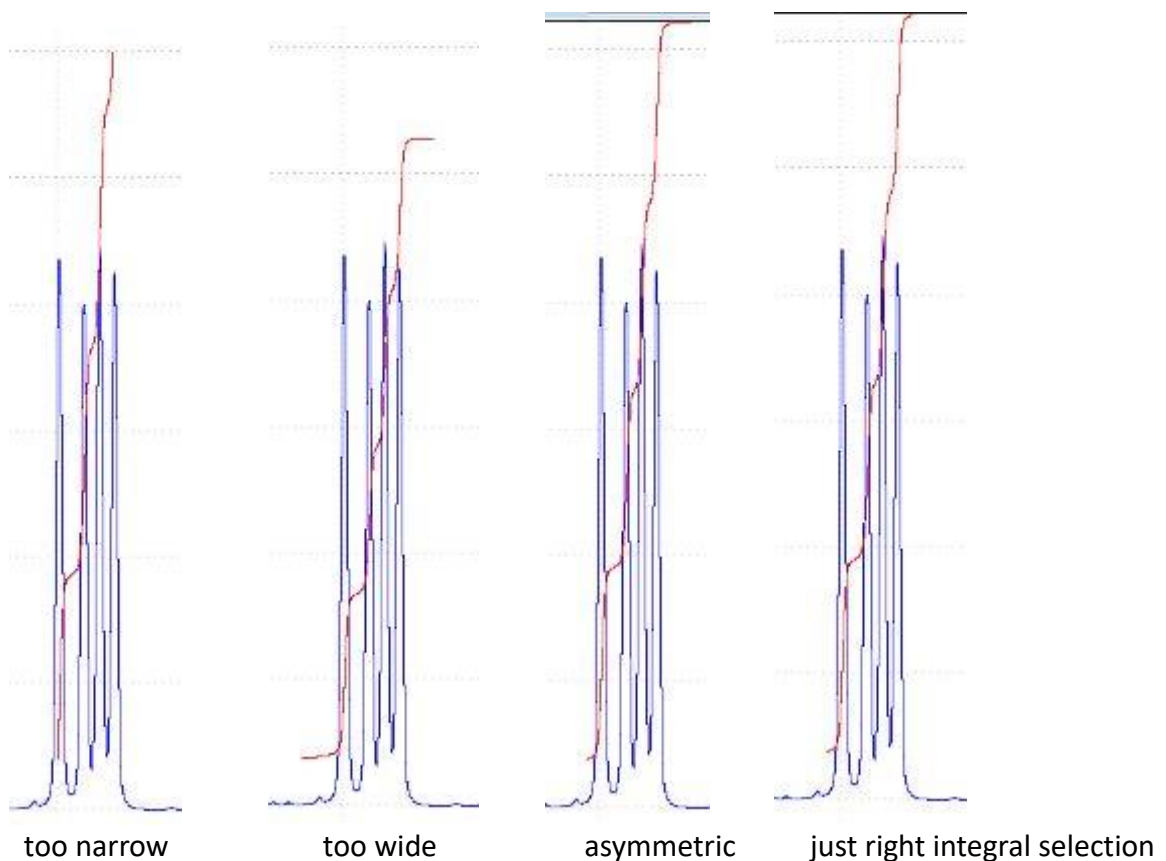
8. Once you have your integration regions properly selected, click on the 'Save As' icon

   Save Regions To 'intrng'
   Save Regions To 'reg'
   Export integration regions
   Export Regions To Relaxation Module and .ret.
   Save & Show List

   and choose 'Save Regions to 'reg'.

9. Exit integration mode by clicking ⏎ , and click 'yes' to save changes.

10. Click on 'Create Datasets' and choose the experiment that you desire.
    <u>Note</u>: the gradient versions of all experiments are recommended, since they provide better artifact supression using PFGs (pulsed field gradients)
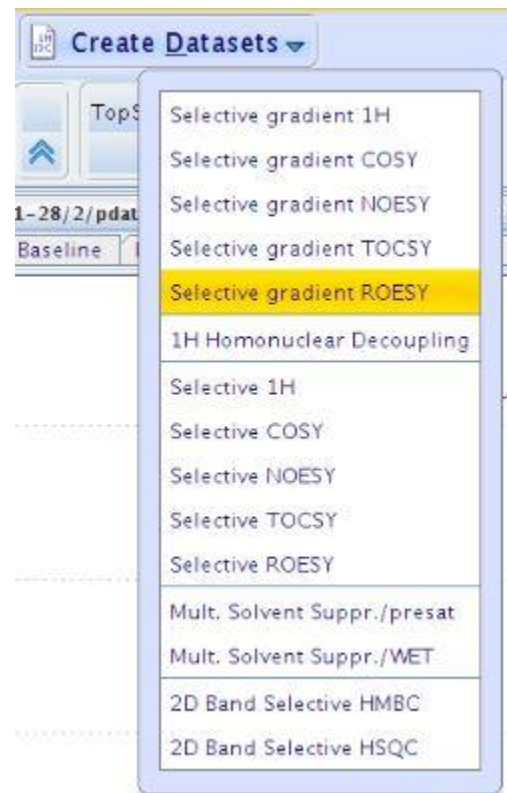

<u>Selective gradient COSY</u>
♦set D1 = 1-1.5 * longest T1 (of ^1H of interest)
♦adjust D4 depending on the J_{HH} desired, typically D4 = $1/(4*J_{HH})$
♦typically folks just acquire a 2D COSY dataset instead of this one

<u>Selective gradient NOESY</u>
♦set D1 = 2.5-5 * longest T1 (of ^1H of interest)
♦start with mix time D8 = 0.6 * fastest T1 (of ^1H of interest)
♦you may obtain a buildup curve to confirm NOE (D8 = 0.2, 0.4, 0.6 sec)
♦ZQ (zero quantum) artifacts are common for 1Hs that are J-coupled; ZQ are independent of mix time, while NOEs will build up; subtracting a D8=0 spectrum from the others may be useful, since it provides double-differencing
♦for small mw, an NOE will give a positive peak (opposite selected peak), while for larger mw, NOE will give a negative peak (same sign as selected peak). NOE will cross over for mw in range of 1000-3000, so for these molecules a ROESY is recommended, as it has no cross over range.

<u>Selective gradient ROESY</u>
♦set D1 = 2.5-5 * longest T1 (of ^1H of interest)
♦start with P15 = 150ms (mix time; though make sure to try the selective NOESY first!)
♦vary P15 = 50ms to 0.6 * longest T1 (of ^1H of interest); linear buildups will occur
♦exchange will occur as with NOESY, but since ROEs are always positive, and exchange is always negative, the separation will be less ambiguous in the crossover and high mw regions
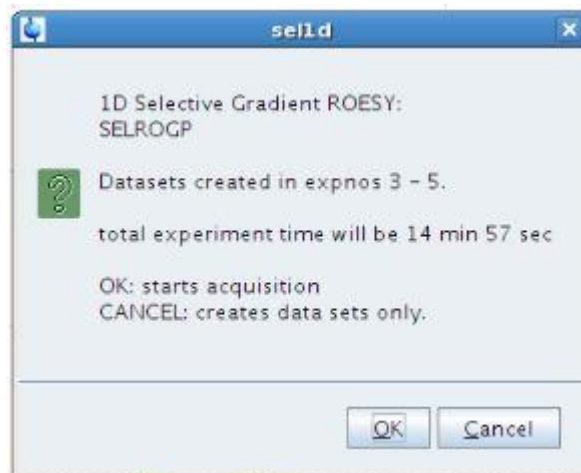
<u>Selective gradient TOCSY</u>
♦set D1 = 1.5 * longest T1 (of ^1H of interest)
♦D9 is the TOCSY mixing time and can range from 0ms to 200ms max; with **80ms** being

the most common (2-3 relay shells); 15-30ms observe ^{1}Hs 2- to 3- bonds away; 40-60ms observe 1-2 relays; 120ms max value, showing all ^{1}Hs in a spin system

11. After choosing your experiment, a GUI will pop up with inputs for mix time (D8 in 1D NOE), NS and starting experiment number. See recommended ranges of mix times and minimum NS in table on last page. Click 'Accept' to save parameters.
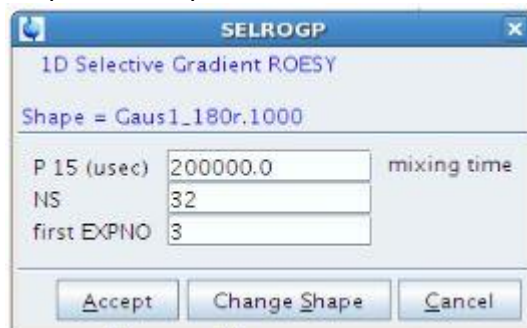
12. The 'sel1d' GUI comes up with an explanation. You may click 'OK' to start the acquisition, however you may not change any other parameter after this point (if you feel OK about the parameters you chose, go ahead and start the experiments).
Clicking 'cancel' is recommended. This will still create the new datasets using the parameters you specified in the previous GUI.

13. If you hit 'cancel', you may now go into the different experiments (**re 2**: gets you to exp. 2; re 3: to exp. 3, etc.) and change parameters: D1 = (1.5-5) * T1

14. Set mix times (D4, D8, D9) following suggestions in the table. For the Selective gradient NOESY and ROESY, you may chose different mix times, depending on the 1H of interest.

15. Don't cheat on experiment times! In 1D NOESY and 1D ROESY, very small NOEs can be observed (down to 0.1% or smaller). Taking data for 10 minutes for each experiment is common; letting it run for a couple of hours (or even overnight) is not unusual. The rule of thumb is that longer datasets provide better data!

16. After you have checked parameters in all of the set up experiments (one for each peak you selected), you may use the 'multizg' command to start the acquisition process. Start in the 1st

selective experiment that was created (typically exp. 2; '**re 2**' will get you there). Then type 'multizg'. A GUI will appear asking for the number of experiments. Enter the number of peaks that you chose, which should equal the number of created experiments.

17. Once you click 'OK' the experiment you are in (ie. 2) will start acquiring and the rest of them will be queued up. The 'Acquisition status' indicator at the bottom will let you know what experiment number is being acquired and how much time is left to completion.

18. You can look at each experiment as they finish acquiring. The selected peak is typically phased negative, while all other peaks most of the time appear positive (see table on last page for exceptions). Once all experiments are finished acquiring, you may stack them up in TopSpin using the icon and selecting and dragging each one from the browser on the left side. Or you may process them and stack them in MNova.

Selective 1D sequences	Description & advice	PARAMETER SET/pulse sequence	D1	mix
selective COSY-1D	protons 2- to 3-bonds from selected mulitplet give anti-phase peaks; d4=large (≤ T1; for small couplings) can be used; coupling will transfer through heterobond	SELCOGP/selcogp	1.5-3 x T1 (long-est)	d4 = 1/4 J (HH) ns = 8 x n
selective NOESY-1D	protons within 5Å produce NOEs; selected peak negative in phase, other peaks are positive for small mw, negative for large mw; exchange will produce negative peaks; ac-quire a mix time series, then plot build-up curve to con-firm NOE	SELNOGP/selnogp	2.5-5 x T1 (long-est)	d8 = 0.1 - 1 x T1 (fast-est) ns = 8 x n
selective ROESY-1D	protons within 5Å produce NOEs; selected peak negative in phase, other peaks are positive for independent of mw; acquire a mix time series, plot build-up curve to confirm ROE	SELROGP/selrogp	2.5-5 x T1 (long-est)	pl5 = 0.1 - 1 x T1 (fast-est) pl5 > 500000 μs is not allowed ns = 8 x n
selective TOCSY-1D	protons 2- to 6- 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	1.5-5 x T1 (long-est)	d9 = 15 - 200 ms d9 > 200ms is not al-lowed ns = 2 x n