







Proton Pulse Calibration

- ▶ 90° pulse is the time to apply RF pulses to rotate the magnetization from the longitudinal direction to the transverse plane.
- NMR is an art of manipulating magnetizations using RF pulses. Knowing precisely how long the magnetization travels to the xy- plane under a specific strength of RF pulse is critical to the success of many NMR experiments.



rotation of magnetization by RF pulse



Proton Pulse Calibration

- ▶ NMR measures transverse magnetization. While applying RF pulse, the magnetization rotates about the RF. As a result, the intensities of NMR signals oscillate with pulse lengths. $I \sim sin(\gamma B_1 t)$, where γB_1 is the field strength of the RF pulse.
- ▶ A 360° pulse generates a null signal. We normally pick the 360° pulse to measure the 90° pulse for two reasons: 1) 360° pulse returns magnetization to equilibrium, 2) rapid changes of intensity around the null point.





Proton Pulse Calibration

 Load proton parameters, acquire a proton spectrum (optimize SW and O1 if needed), phase it properly;

• Change your relaxation delay (d1), set it at least to 5 seconds;

- Change pulse sequence (*pulprog zg*), set *ds* to 0 and *ns* to 1;
- Keep receiver gain constant, change pulse width *p1* to somewhere around 360° (see next slide), acquire data and process it (*efp*);
- Repeat the last step until you find a p1 value which gives a null (or minimal) signal. To get your 90° pulse width, divide it by 4;
- If you notice a significantly different values for 90° pulse width, please report to the NMR facility staff.

Spectrometer	Power Level (dB)	90° pulse length (μs)
AV300	0	11.5
AV400dir	I	17
AV400inv	1	9.5
AV600	3.5	9.2













- Run proton experiment, optimize SW and O1 if needed;
- Type pulprog zg to set your pulse sequence;
- ▶ set d1+AQ=5*(longest T1 of your signals), p1=90° pulse length;
- ▶ set ds=2, ns to a desire number (multiples of 4);
- rga and acquire, process the data, integrate the targeted signals. The integrals should now properly represent the number of the protons contributing to the signals.











































Step-by-Step 1D NOE

- run regular proton experiment, process the data, integrate the targeted signals to be irradiated (singlets or multiplets), integrate signals without baseline (i.e. as close to the signal as possible), save the integral(s) as a reg file. To do so, go to file and choose reg, return and save it.
- type butselnmr, choose sel.gr.noesy experiment. XWINNMR will now create a series of files based on how many signals you wish to irradiate. Answer all questions and click CANCEL at the last dialog box if you intend to change ns (The default is 32 scans) and mixing time (The default is 600ms). The last dialog appears as : "default mixing time d8=0.6 sec OK: starts acquisition CANCEL; creates data sets only"
- Change ns and d8 (mixing time) accordingly on all experiments. The optimal d8 value can be set to the d7 value in the inversion recovery experiment that nulls the signal. rga on each experiment and multizg on the first experiment of the series.
- ► ef to process the data, adjust a negative phase for the irradiated signal manually. For small molecules (<600), you will observe a positive NOE (positive signals); for big molecules (>2K), you will observe a negative NOE (negative signals); chemical exchange will always give a negative NOE.







Step-by-Step for 1D TOCSY

UBC

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run regular proton experiment, process the data, integrate the targeted signals to be irradiated (singlets or multiplets), integrate signals without baseline (i.e. as close to the signal as possible), save the integral(s) as a reg file. To do so, go to file and choose reg, return and save it.

type butselnmr, choose sel.gr.tocsy experiment. XWINNMR will now create a series of files based on how many signals you wish to irradiate. Answer all questions and click CANCEL at the last dialog box if you intend to change ns (The default is 32 scans) and spin locking time (The default is 80ms). The last dialog appears as : "default mixing time d9=80 ms OK: starts acquisition CANCEL: creates data sets only"

Change ns and d9 (mixing time) accordingly on all experiments.rga on each experiment and multizg on the first experiment of the series. If you wish to observe an extended J coupling network, set d9 to 120ms. Prolonged spin locking may generate substantial heating in the probe coil. Make sure your d9 is less than 200ms.

• efp to process the data and phase every signal to positive. It is known that your signals may have some impurities from anti-phase components.







Step-by-Step to Solvent Suppression

 run 1D proton, optimize SW, O1 and reacquire your data. Record your SWH (in Hertz), O1 (in Hertz), the frequencies of your solvent signal on a piece of paper.

 create a new experiment, rpar ubc_SOLSUP, input your recorded SWH (in Hertz), O1 (in Hertz), and set SPOFF1=the frequency of your solvent signal minus O1.

 rga (your receiver gain should be much higher now), set ns to a desired number and zg to acquire.







UBC	Me	Step-by-S casure Diff	Step Guide to fusion Coefficient
•	When you find varying gradier experiment, kee experiments.	a correct p30 (and d it strength spaced b ep it the same for al	20), create a series (10-15) of experiments with etween 2% and 95%. <mark>rga</mark> on the first l your series and <mark>multizg</mark> to start the
•	Process your fin targeted signals process your sp on all spectra.	st spectrum, phase s (we recommend to ectra the same way	it well, baseline correct it and integrate the pick only well resolved signals). multiefp to and multi_integ to perform signal integration
•	You need max g each spectrome these values are convert it to a s experiments.	gradient strength va eter. The following t e given for rectangu inc shape, as you ar	lue to calculate the diffusion coefficients for able supplies you with these values. However lar gradients. You need to multiply 0.63 to e using sinc shaped gradients for diffusion
		AV400inv AV400dir	57.5 Gauss/cm
	, i	AV600	54.9 Gauss/cm