No Deuterium NMR: Shimming & Solvent Suppression

No deuterium (no-D) NMR is a technique that can be a useful alternative to using expensive deuterated solvents, if you are working with a neat sample, or want to directly monitor a reaction mixture. It is also useful for molecules of biomedical interest such as proteins, which must be studied in water (or 90% H_2O/10% D_2O).

The sample can be shimmed by using a strong ^1H peak (e.g. from a protiated solvent). This shimming can be done manually on the FID or via gradient shimming. The technique works best if you have samples > 10 mM. No-D works OK with a 2 mM sample and a cryoprobe, but ideally you will use a concentration on the order of ~10 or 100 mM for a room temperature probe.

Pros	Cons
Minimizes cost: no need for (expensive) deuterated solvents.	Lock is turned off. Extraneous changes to chemical shifts (e.g., magnetic field drift, temperature change, etc.) will broaden the resonances. You can negate field drift by using 10% deuterated solvent.
Efficient: can transfer reaction mixture in proteo-solvent or neat sample straight to NMR tube and analyze.	Solvent peak(s) will be strong and may overlap with peaks of interest, making it difficult to analyze the sample qualitatively and/or quantitatively, depending on the amount of overlap.
Great for neat samples that are not dissolved in any solvent.	Large solvent signal(s) create a large dynamic range for sample peaks – it's difficult to observe peaks with small intensity especially if the sample is of limited concentration.

1. Gradient Shimming without Deuterium

There are many methods you can use to shim prior to a no-D spectrum. You could 1) use a reference tube of the same volume of deuterated solvent, which is locked and shimmed as usual, and then replaced by the no-D sample; 2) use a capillary insert containing a deuterated sample, which can be locked and shimmed; 3) manual shimming on either the FID or NMR lineshape; or 4) gradient shimming. For the most users, it is easiest to utilize gradient shimming (**topshim**) with some extra options enabled.

Data Acquisition
1) Type **new** to set up a new experiment and read in the proton parameters (**1h.av500** or **1h.av600**). You can pick any solvent you like in the drop-down menu. Tune the probe with **atma** as normal.

2) Instead of **lock**ing to a particular solvent, we will turn off the lock. Type **lock_off** and wait for it to finish. After it finishes, type **ii** and wait for it to finish.

3) Set **ns** = 1 (under AcquPars) and **sr** = 0 (under ProcPars) and acquire a regular proton spectrum: **rga; zg; ef; apk; abs n.** We want sr = 0 so that we have the unshifted ppm value for the solvent peak. Zoom in on the solvent peak and record the value of its chemical shift in ppm.

4) Type **rsh LAST** to read in the most recent shim file. Type **topshim gui** in the command line to start the graphical user interface for TopShim (Fig 1). Click on the check-box for PARAMETERS. (If there is no check-box, go to the Service tab > Additional Preferences > Enable Parameters.

5) In the box for PARAMETERS, enter **lockoff 1h o1p=[solvent_peak_in_ppm] selwid=0.1**. Here, **[solvent_peak_in_ppm]** is the center of the solvent peak that you want to use for shimming. Press Start and wait for shimming to finish.

6) If the software complaints about reducing the echo time, add **rga convcomp** to the line above and re-run.

7) Create a new experiment with **iexpno** and then acquire your data. You can compare the results with the spectrum from Step 4 to see the improvement in NMR lineshape.

Note 1: You can do all of this from the command line as well, without launching the TopShim GUI. Type **topshim (rga convcomp) lockoff 1h o1p=[solvent peak in ppm] selwid=0.1.**

Fig. 1: **topshim gui** for proteo solvents. Under PARAMETERS, check the box and in the pink box, you will need to type **lockoff 1h o1p = X.YZ selwid=0.1**, in which X.YZ is the chemical shift of your solvent**.**
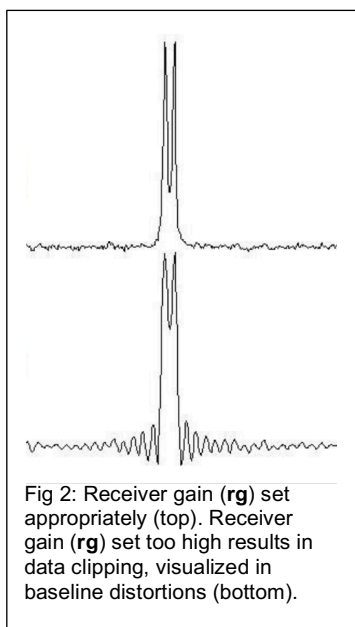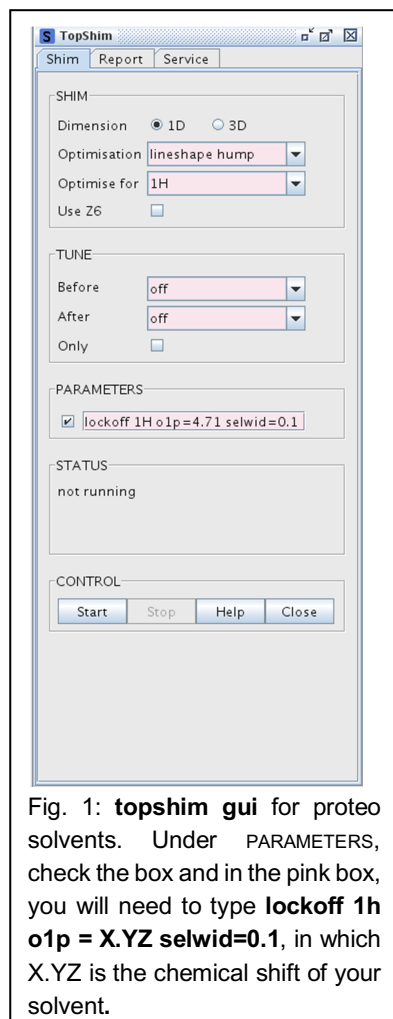
Fig 2: Receiver gain (**rg**) set appropriately (top). Receiver gain (**rg**) set too high results in data clipping, visualized in baseline distortions (bottom).

Note 2: Between the cryoprobe's signal enhancement and the very concentrated proton signal, the NMR spectrometers often cannot get the receiver gain (**rg**) low enough to prevent clipping the FID (Fig 2). You can manually set **rg** = 1 and/or change the pulse sequence from zg30 to zg10 (a 10° flip angle compared to a 30° angle). However, sensitivity will reduce as the flip angle is reduced.

2. Solvent Suppression for ^{1}H NMR

There are a number of solvent suppression methods used in NMR. The technical details can get quite extensive; briefly speaking there are three types, divided into when the suppression occurs (before, during, and after the main part of the pulse sequence). Some example methods are listed below.

Pre	During Main Pulse Sequence	Post
Presaturation	Jump-and-return	Flip-back
WET (Water suppression Enhanced through T_1 effects)	WATERGATE (both spin echo and stimulated echo versions)	Excitation sculpting

The pre-suppression methods saturate the solvent resonance prior to acquiring data. The second type return the solvent magnetization back to +Z axis leaving all solute magnetization is in the transverse (observable) plane for acquisition. The post-suppression methods use often selective pulses (which require PFGs) combined with transverse signal cancellation. Each method has its own pros and cons and the "optimal" solvent suppression scheme will depend on your system of interest. Today we'll study one of each type and compare them all. As an aside: solvent suppression schemes are often combined with other experiments, for example 2D-NMR.

If you have a 90% H_2O/10% D_2O sample, you can **lock h2o+d2o** and **topshim** as normal! You do not have to turn off the lock or use the topshim with the "lockoff" option.

Data Acquisition: Presaturation of Solvent Signal
1) Create a new experiment. Read in the presaturation parameters: **rpar zgpr.av500** or **zgpr.av600**. (Change **o1p** to your solvent resonance if it's not centered at 4.7 ppm.) Set your acquisition parameters properly in ased (ns, td0, sw, aq, etc.). Make sure that **ds = 2** and **p1** = to your calibrated 90 degree pulse.

2) Acquire your data with **rga;zg** and process as usual.

3) There's an improved version of the 1D water presaturation pulse sequence that utilizes *composite pulses* [Malcolm H Levitt & Ray Freeman, *J. Magn Reson.*, **33**, 473-476 (1979)]. It only takes ~ 2 minutes to run, so we'll also run this one just for a comparison. Type **iexpno** to create another experiment, and change the **pulprog** to **zgcppr** [Ad Bax, *J. Magn Reson.*, **65**, 142-145 (1985)]. Acquire your data with **rga;zg** and process as usual.

Note: The parameter for the presaturation power is **plw9**: the larger the attenuation (in dB), the weaker the rf power applied (in Watts). For increased solvent suppression, you could decrease the attenuation in dB (thus increasing the microwave power in Watts applied). **WHATEVER YOU DO, IT IS IMPORTANT TO KEEP plw9 < 0.3 mW (= 0.0003 W) — OTHERWISE YOU CAN COOK THE PROBE WITH MICROWAVES!**

Data Acquisition: WATERGATE 3-9-19 [M. Piotto, V. Saudek & V. Sklenar *J. Biomol. NMR* **2**, 661-666(1992) andV. Sklenar, M. Piotto, R. Leppik & V. Saudek, *J. Magn. Reson. A*, **10**, 241-245(1993)].
1) Create a new experiment. Read in the WATERGATE parameters: **rpar watergate3919.av500** or **watergate3919.av600**. (Change **o1p** to your solvent resonance if it's not centered at 4.7 ppm.) Set your acquisition parameters properly in ased (ns, td0, sw, aq, etc.). Make sure that **ds = 4** and **p1** = to your calibrated 90 degree pulse.

2) You will also need to set **p0** = **p1** = **p27** = your calibrated 90 degree pulse.

3) The WATERGATE 3-9-19 sequence has a special delay **d19** [s] = $1/(2\Delta\nu_{max})$, where $2\Delta\nu_{max}$ [Hz] = the distance to next null from o1p. Narrowest bandwidth (large d19) may attenuate very upfield and very downfield peaks, but also give a very narrow solvent notch. The default value of **d19** is set to 250 us; this works well enough for the standard

"water suppression" H_2O/D_2O sample. You may have to change this value for your sample.

4) Acquire your data with **rga;zg** and process as usual.

Data Acquisition: Excitation Sculpting [T.-L. Hwang & A.J. Shaka, *J. Magn Reson. A*, **112**, 275-279 (1995)].
1) Create a new experiment. Read in the excitation sculpting parameters: **rpar zgesgp.av500** or **zgesgp.av600**. (Change **o1p** to your solvent resonance if it's not centered at 4.7 ppm.) Set your acquisition parameters properly in ased (ns, td0, sw, aq, etc.). Make sure that **ds = 4** and **p1** = to your calibrated 90 degree pulse.

2) Excitation sculpting uses a selective pulse **SPNAM1** with a duration **d12**. Set **p12** = 2000 us, and **SPNAM1** = "Gaus1_180r.1000".

3) Run the command **edprosol**. This will open a new window as a pop-up. Set the 1H PW to your 90 degree calibrated pulse, e.g., if pulsecal returned 11.54 us, put that in the box. Hit the Calculator button.

4) Change the tab to **Shape Pulse (**or **HR Shape Pulse)**. For the "Select. inversion/refocussing" box (or "Select. inversion"), change the filename to "Gaus1_180r.1000". Enter 2000 into the PuW [us] box and hit the enter key. Note the power level in the A [dB] box, it ought to be ~ 17 dB on the AV-600 and ~ 19 dB on the AV-500.

5) Exit the edprosol window *without saving*. In order to do this, *UN*check all of the boxes and say OK.

6) Back in the main **ased** window, set **SPW1 [dB] (SPdB1**) to the power level obtained in step 4 above.

7) Run the experiment as usual with **rga;zg;ef;apk;abs n.**