EXPERIMENTS TO DO:

1. No deuterium shimming on a sample with protiated solvent
2. Compare different solvent suppression schemes:
   - **No solvent suppression**
   - **Presaturation (presat)**: usually gives the narrowest suppression. However, it reduces the signal amplitude of exchangeable protons. There are a variety of presat pulse sequences.
   - **WATER suppression by GrAdient Tailored Excitation (WATERGATE 3-9-19)**: unlike presat, WATERGATE has no effect on exchangeable protons. It is a gradient echo (single echo) method.
   - **Excitation sculpting:** a solvent suppression method that uses double spin echoes and selective excitation. It produces excellent solvent suppression but some bandwidth around the solvent peak will also be suppressed -- usually ~0.1 ppm on either side.

We will be using the "water suppression" standard sample for this lab. It is 2 mM sucrose, 0.5 mM DSS (with trace NaN_3), in 90% H_2O/10% D_2O. (No, it's not truly a no-deuterium sample, but you can apply the same methods to a completely non-deuterated sample.) For a long experiment, adding 10% deuterated solvent will allow you to use the field-stabilization lock.

No Deuterium NMR

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. As you'll see today, no-D works with a 2 mM 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. Due to radiation damping from the water signal, the difference in this case may be slight. Other solvents such as DMF or THF may demonstrate significantly improved NMR lineshape after gradient shimming on the ^{1}H signal.

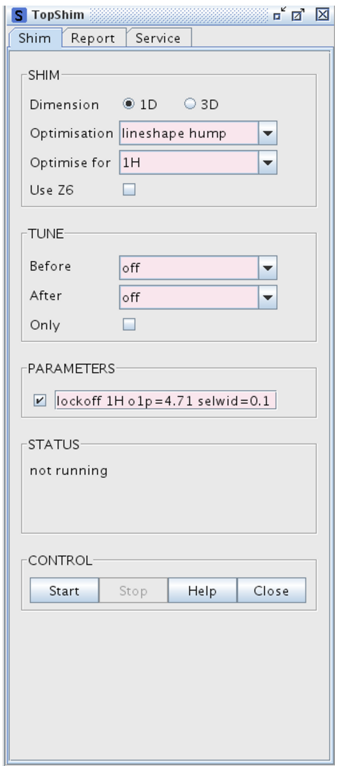

Fig. 1: **topshim gui** for proteo solvents. Under PARAMETERS, check the box and in the pink box, you will need to type **lockoff 1h o1p = X.YZ selwid=0.1**, in which X.YZ is the chemical shift of your solvent.

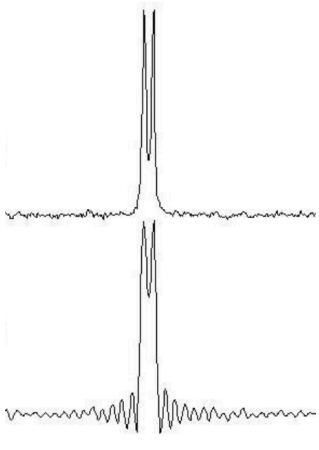

Fig 2: Receiver gain (**rg**) set appropriately (top). Receiver gain (**rg**) set too high results in data clipping, visualized in baseline distortions (bottom).

Note 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.**

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^{o} flip angle compared to a 30^{o} angle). However, sensitivity will reduce as the flip angle is reduced.

Note 3: Given that this sample is 10% deuterated, yes, we could have utilized the ^{2}H signal to lock to and shim on.

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.

<u>Data Acquisition: No Solvent Suppression</u>

1) Since we are fortunate enough to have a 10% D_2O sample, we can lock on to the solvent: **lock h2o+d2o**. Wait for this to finish before moving on. (This keeps the **o1p** the same throughout the rest of the lab).

2) Calibrate your 90 degree pulse. You can use **pulsecal** if you like.

3) However, for the sake of comparison to the rest of our data, please quickly acquire another ^{1}H data set with **ns = 16, td0 = 2, ds = 2**. Set the **o1p = 4.70** (or whatever the center of your water peak is now that the spectrometer is locked), **sw = 12 ppm**, **aq = 1**

second, and **p1** = to your calibrated 90 degree pulse. Acquire your data with **rga;zg** and process as usual.

Data Acquisition: Presaturation of Solvent Signal

1) Create a new experiment with **iexpno**. 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 **ns = 16, td0 = 2, ds = 2**, **sw = 12 ppm**, **aq = 1 second**, 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 with **iexpno**. 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 **ns = 16, td0 = 2, ds = 4**, **sw = 12 ppm**, and **aq = 1 second**.

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 our specific H_2O/D_2O sample. Play around with some other **d19** values—e.g., 100 us, 150us, 200 us, 250 us. What effect does this have?

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 with **iexpno**. 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 **ns = 16, td0 = 2, ds = 4**, **sw = 12 ppm**, and **aq = 1 second**.

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**. 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.**

Data Processing

Plot all of the spectra. You may need to phase by hand (see the TopSpin Manual Phasing handout on Canvas, or use manual phasing in MestReNova).

When you are done in the lab, make sure to log out. Type **CTRL + ALT + BACKSPACE** to log out of the computer.

What to turn in:

- A comparison plot where you stack the different solvent suppression schemes. Zoom in on the small sucrose peaks, but keep the water signal in the spectra. Qualitatively evaluate the differences between them.
- Answers to the following questions:

Questions

1. Why would you chose a particular solvent suppression scheme over another? (Hint: If you have exchangeable protons, which methods are better? What about non-exchanging protons?)
2. In the WATERGATE experiment, what does the effect of changing **d19** have on your spectra?
3. In addition to sucrose, our "water suppression" sample contains some extra substances. What are they and why are they useful?
4. With samples like water, radiation damping can affect broaden NMR linewidths. What is radiation damping and what are some ways you can combat it?
5. The composite pulse presaturation experiment gives improved solvent (water) suppression compared to the standard presaturation experiment. How? (I'm not looking for an explanation about what composite pulses are per se. Hint: What's the difference in excitation profiles of each method?)