10. IS THE WHOLE GREATER THAN THE SUM OF ITS PARTS?

In this tenth and final video of the “lesson 1” demonstration, we will use this sample, which is a mixture of two different amino acids, alanine and glycine, dissolved in $D_2O$. The alanine has a CH quartet, since it's attached to a methyl group, which will be a, . . . [you guessed it], doublet. And the glycine $CH_2$ group will appear as a singlet. Any remaining protons on the amino acids will be undergoing chemical exchange with the aqueous solvent.

After the previous videos in this series, we've already seen what to do, so let's do it!. Sign the log book, check. The program is open, check. Create a new file, experiment number 2, solvent D2O, experiment, 0_1H, title: alanine + glycine – I'll add that to my log file, copy and paste, OK, check.

Let's insert the sample: spinner, depth measure, NMR tube, cap is up, wipe off finger prints, lift-on, leave depth measure here, leave the sample on the air cushion, lift off, red missing light goes to green down. Check.

Let's spin the sample tube – flashing, 2nd button, spin measure, there it goes. Fine, stand-by. Check.

Open the lock display, read the shim file: rsh qnp, lock $D_2O$. Waiting for end of lock - - - deuterium lock is a feedback loop to prevent the field from drifting during the experiment, in four words: lock prevents field drift. Lock finished. Next we'll shim, which will correct for any field inhomogeneities.

Click on Z, turn the dial one direction, is the line going up? No, click on Z to undo changes. Turn the dial the other direction, still the $^2H$ lock signal is not going up, click on Z to undo changes. Check the same thing with $Z^2$ – no increases so I will *not* change the shim current values.

From the keyboard, ased, Here are the acquisition parameters. “zg” is the pulse acquire NMR sequence, td stands for the number of digitization points or time-domain points, ns is 1, ds is zero, sw is 20 ppm, the acquisition time is 2 seconds, let's set the receiver gain for this sample – rga, enter. It tells me down here, start receiver control unit and timing control unit . . . rga, acquisition running, rga finished.

I'm ready for a scout shot – start acquisition. Here's my acquisition window. Here's the fid. Finished. There's our NMR signal. Now I'll click here to run a macro that does ft, apk, etc. How's the shimming?

Here's the alanine methyl doublet . . . the linewidth is about 2 Hz. Here's the exchanging water peak, a residual proton signal from the heavy water, i.e. it belongs to and HOD molecule, with a line width of about 12 Hz, it's much broader due to chemical exchange with the labile protons of the amino acids. So, our $^2H$ $D_2O$ signal in our lock display isn't sensitive enough to get good shimming on these sharper NMR spectral transitions. That's okay, we've learned about going into gs mode in order to shim on the spectrum in real time. Let's set d1 to zero. And choose gs mode – and shim. That's better. Stop.

This is still our old spectrum – gs mode doesn't save a new fid. And even if it did, we would need to reprocess the data to get a new spectrum. Let's do that – acquire fid. Process fid. Here's our new spectrum with better shimming. How long do I estimate for the acquisition time? Maybe ~ 6s.

Let's optimize. I'll expand this region and set sw and o1p according to the limits on the screen using this icon with the red lighting, representing the center of the spectrum, over the blue pond representing the spectral width. I'll close this information window. In the acquisition parameters table, we see the new values for swh and the absolute value in MHz of the center frequency (of both the spectrum and

the transmitter pulse) sfo1. Let's double td to bring the aq to around 6s. And I'll set si to twice td now.

The signal to noise ratio looks decent. Let's acquire 4 shots for twice the SNR. I haven't measured $T_1$, so I don't know what value to use for d1 to set quantitative measurement, but a standard spectrum is good enough. I'll arbitrarily put 2.5 s. Some folks prefer setting up with an Ernst angle, which has some benefits, but you still need to know $T_1$ to ensure quantitative conditions for integration.

This experiment with these parameters will take ___. Start acquisition. There it is. These beats arise from the addition of all the different overlapping frequencies. To make it easy for our eyes to detect the individual frequencies, let's Fourier transform the FID. Ft and apk. And here is our NMR spectrum. I can't use the water peak to calibrate chemical shift on the x-axis. That makes sense since we know that chemical shift depends on the electron density around the proton of interest. In water that value is going to be very sensitive to hydrogen bonding, which in turn depends on temperature, chemical exchange, impurities, solute and solvent concentrations, pH – too many things to know the value accurately. I might want to look up a literature value for the alanine methyl peak, or I could add something like DSS or TSP, which are water soluble corollaries to TMS, and remeasure a new NMR spectrum. I'll use the value of 1.48 ppm for the alanine methyl peak from the SDBS spectral database for organic compounds from Japan's National Institute of Advanced Industrial Science and Technology (AIST), which should give ~3.8 for the methine quartet. And ~ 3.6 for the glycine methylene protons That looks fine. That puts our HOD peak at ____ ppm. Do you know why J-couplings are reported in Hz and not in ppm? Because, unlike chemical shifts, the J-couplings are field independent. So, if I measure this same sample at 500 MHz and go into multiple display mode – this is how the two compare with ppm on the x-axis. And this is how they look if I align them in units of Hz. Good to know . . .

Abs, ppf, that didn't catch all of our peaks. Let's go into peak picking mode. Here we'll capture these. And here we'll manually pick this one due to exchanging labile protons on the amino acids. Save and return. Now integration mode. Let's select all and delete all. Are you sure? OK. I'll click this icon. I move the mouse away and it's green now. That means this mouse sequence that I usually use to expand a region of the spectrum will define an integration region: the arrow on one side of a peak, hold-down the left mouse button and move to the other side and then release. If I click on this icon again, so it's not green, I can use the mouse to expand. I can also use this button to make navigating across the spectrum easier. Let's calibrate the integral of the methyl doublet to be equal to 3. Now save, return, and prnt to print the screen.

How should we finish? We haven't yet covered this! I'm on #10 of the basic instructions. I want to save the data onto another computer. This file opens the local data. This icon opens a remote computer. I'll drag and copy files to another computer in the way this lab has defined normal operating procedure.

Now I'll take the sample out of the magnet. I turn off the lock – the sweep automatically comes on. That's fine. It important that it's off while I measure the FID, but otherwise no harm if it's on. I'll turn off the spin. I'll press lift, so the green button is lit. Here's the sample and the spinner. I'll put these away. And I'll turn off the lift air. Nothing is hissing or blinking. That's good.

On our training time line your next step is to schedule lesson 2. I recommend reading through the theoretical exam questions at the end of the training guide. We've covered all the answers in these videos. Good luck with your experiments!