

## W.M. Keck 800 MHz Salt Tolerance Sample Preparation

**Introduction:** The cold probe for the 800 MHz system (and eventually for the 900 MHz system) is a “salt-tolerant” cold probe. What does that mean to you and to your samples?

In any given NMR experiment the spectrum that you observe is comprised of the detected signal from your sample, plus the noise arising from the sample and detection equipment. Normally in order to achieve a higher signal-to-noise ratio you need to either increase the concentration of your sample (increase signal) or average many acquired spectra together (decrease noise). In the case of the cold probe, the temperature of the detection equipment (20 K) results in dramatically less noise in the resulting spectrum than comparable equipment at room temperature. In this case the signal-to-noise of your spectrum is higher by virtue of the fact that the noise level is actually lower; the signal remains the same in either case. The best results are achieved in cases where the sample itself contributes very little to the spectral noise, and for the 800 MHz system a 4-fold increase in signal-to-noise may be potentially achieved.

Unfortunately, for biological systems the sample itself does contribute a significant amount of noise to the spectrum, and in the case of salty samples this noise is compounded. The result of this is that for samples with a high enough salt concentration (> 250 mM) the gain in signal-to-noise afforded by the cold probe is lost.

It turns out, however, that the noise arising from the sample is confined to a certain region of the sample tube. This is due to the construction of the RF coil that is used for detection. By eliminating the region of the sample that contributes the most noise to the spectrum, some of the sensitivity gains can be recovered. The salt-tolerant cold probe on the 800 MHz system is designed to allow a special tube geometry to be used to achieve the best possible signal-to-noise with salty samples. The optimal tube geometry is a rectangular tube that is 3mm by 6mm wide. This type of tube geometry does present some challenges.

[NaCl] (mM)	5mm	3x6	2, 3mm
0	1544.09	1221.16	1243.26
100	794.65	878.03	762.06
200	561.15	700.46	706.18
400	477.14	528.81	502.50
600	338.74	431.61	429.13

Table 1: Comparison of S/N for 0.2 mM Sucrose solutions with varying salt concentrations. The results are presented for three different tube geometries.

**Discussion:** Three different tube geometries have been evaluated. In each case the sample has been prepared using “standard” protein techniques, i.e. the sample

height is maintained at 15mm using Shigemi style tubes, and solutions are prepared using 10% D<sub>2</sub>O. The three tubes are: standard 5mm Shigemi thin-wall tube (sample volume 240 uL), 3mm x 6mm “S-tube” with Shigemi inserts/plunger (sample volume approx. 160 uL), and two side-by-side 3mm Shigemi tubes (sample volume 147 uL).

It appears that any sensitivity gains one sees from the larger sample volume of the 5mm tube, are almost recovered (Table 1) in the “square” geometry tubes by the time the salt concentration hits 100 mM. For 200 mM NaCl and higher, it appears that the “square” tubes outperform the 5mm tube consistently, and by 600 mM NaCl, the square geometries offer an improvement in sensitivity of about 3%. Also it bears mentioning that while the filling factor for the two side-by-side 3mm Shigemi tubes is somewhat less than the 3x6 S-tube, the sensitivity numbers are fairly consistent between the two. From an experimental standpoint the two 3mm tubes are a reasonable replacement for 3x6mm S-tubes, which is good as the “S-tubes” are especially challenging to use.

Shimming for the tubes presents the biggest challenge. Starting from the shims for the 3x6 lineshape sample it was reasonably easy to shim the two 3mm tubes (about as difficult as the 3x6 tube was to shim). Both the square geometries were more difficult to shim than the 5mm tube, however, and the lineshape was far better for the 5mm tube. In particular it seemed that X1 and XZ shims required adjustment for the “square” tubes, however an increase in the lock level did not necessarily correspond to better lineshape. In the end these shims were adjusted while monitoring the lineshape of the anomeric signal. This practice would be quite difficult with a protein sample.

**Conclusions:** In most cases the sensitivity gains realized by going to a “square” geometry seem like they will be modest at best, and the difficulty in shimming and obtaining effective water suppression may make the point moot in the end. However, if there is a limited sample volume available (~150 uL), using 2 side-by-side 3mm Shigemi tubes will provide much better sensitivity than using a much shorter sample height in a 5mm Shigemi tube.

An additional factor that swings in favor of the “square” geometries is the pulse width. For salty samples the proton pulse width can become quite long (~17 us for 600 mM NaCl). With the “square” tubes, the pulse width is reduced dramatically (~10 us for 600 mM NaCl), which will be desirable in most cases.

The final recommendation is to use standard 5mm Shigemi tubes for most samples on the cold probe. For routine HSQC experiments, and for samples that may be unstable, the gains that one might achieve from using a different tube will not be enough to justify the difficulty. However, if the sample conditions have been well optimized, and employ high salt concentrations (> 250 mM NaCl) it will be beneficial to use two 3mm Shigemi tubes instead of the 5 mm tube (instructions for using these tubes are below).

**Using side-by-side 3mm Shigemi tubes:** If you do decide to use the dual-tube sample spinner for your sample here are some directions for preparing the sample and setting up the experiments.

1. Sample preparation: you will need much less sample for the two 3 mm tubes because the total volume of the tubes is less than that of a 5 mm Shigemi tube. However there will be some sample lost to the fact that you are filling two tubes rather than one. Most likely 200 uL will be enough to fill both tubes, load 100 uL into each and adjust the sample height using the plungers. You want the two tubes to be as symmetrical as possible, so make sure that the plungers are set to the same place, and that the height of the sample within each tube is the same (15-16 mm ideally). This can be done in the lab, and once the sample heights are normalized parafilm can be used to make sure nothing moves around during transport.

2. Sample positioning: the dual-tube spinner is located at the 800 MHz lab on the bookshelf. It consists of two 3mm holes that are very close to each other, with two rubber o-rings on the top. The o-rings press against the side of each tube and hold it in place. It can be tricky to get the tube into the hole as it is a tight fit. Push a little against the o-ring and it should slide into the hole with little resistance. If you are pushing hard, something is likely wrong. Once you have the tube in the hole it should slide fairly easily into position. Slide one tube about halfway into the spinner before inserting the second tube. Once both tubes are in, use the sample gauge to ensure that both tubes are in the same position and that the sample region is within the dotted box. Occasionally the bottom part of the Shigemi tubes are not identical, so you may need to adjust one tube manually to ensure that the sample regions for each tube are lined up. Once the tubes are loaded, you can insert the sample spinner into the probe as usual, and fine-tune the sample position using the gradient profile/map. As the gradient shimming is done using the z-gradient, the profile for the two tubes should look identical to that for a 5 mm Shigemi tube.

3. Shimming: Initially make sure you are starting from a shimset that was optimized on a 3x6 geometry tube. This is best obtained by loading a lineshape shimset such as:

`~garmstro/vnmrsys/data/tests800/ColdProbe/Lineshape_3x6tube-031209.fid`

This should ensure that the X and Y shims are approximately correct as you will not be able to optimize these for your sample. The Z-shims may be optimized as you would for a 5 mm tube, first by hand for Z1 to Z3, and then using gradient shimming. Make sure you make a good shimmap of your sample region, and shim Z1 to Z4 first. If your sample height is between 15 and 16 mm it is unlikely you will be able to shim Z5 or Z6 using gradient shimming. Using this procedure should help you to achieve reasonable lineshape/water suppression for most protein samples. Make sure that you DO NOT adjust the X and Y shims! An increase in the lock level resulting from changes to the X and Y shims does not necessarily result in an improvement in the lineshape! If you have DSS in your sample, you may be able to tweak up the X and Y shims using an iterative procedure. First take a good high-resolution spectrum of

your DSS peak. Then make small adjustments to X1 or Y1 and reacquire the spectrum. If the resolution improves, continue adjusting in the same direction until you achieve the optimal lineshape for the DSS peak. Most likely you will only be able to adjust X1/XZ and Y1/YZ using this method.

4. Experimental Setup: The main difference in setup using the 3 mm Shigemi tubes is the pulse width, it should be substantially shorter than what you would have obtained with a 5 mm tube. The other potential issue is the water suppression, which may be worse for these tubes than the 5 mm tubes. Since it is much more difficult to obtain a good shim/lineshape for these tubes than for 5 mm tubes, the water resonance will be broader and more difficult to suppress. It may take more optimization of gradients and soft pulses in experimental setup than you are used to.

5. Final Notes: Setting up these tubes is a bit of an art, and you will certainly improve as you get a feel for the differences between these tubes and standard 5 mm tubes. The first time you use them make sure that you give yourself plenty of time to get up and running as they will likely require numerous adjustments. Also, you may want to load the 3x6 lineshape sample and play with the shimming on that first to make sure you are starting from a good initial set. If you take the time to optimize everything you will certainly be happy with the results, but the tradeoff between time and sensitivity gains must be considered before making the decision to use these tubes.