MESTRELAB RESEARCH
NMR Solutions

Instructions by R. Shoemaker

1. 2D-NMR Processing: General Overview.
    a. Drag-and-Drop into MNova to get very basic, un-optimized processing
    b. Optimizing your 2D processing
        i. Select Working Dimension (F2 then F1)
        ii. Use Zero Filling and LP tool
            1. We normally Zero-Fill only in F2.
            2. Linear Prediction is used in the F1 dimension (increase # points by 2x or 4x depending on data).
        iii. Apodization
            1. Defaults usually work well, but you can play around if you want to. See what works.
            2. Always use Sine Bell or Sine Square for Magnitude-Mode (not phase-sensitive) data. In MNova, this is Sine Bell or Sine Square with [0.00] in the phase-shift box.
            3. For Phase-Sensitive Data, you should use a 90-degree shifted (90.0 in the box) for Sine Bell or Sine Square, or use Gaussian with an appropriate amount of line-broadening (5-20 Hz for F2, and 10-20 Hz for F1).
        iv. Adding 1D ^{1}H and/or ^{13}C spectral traces on the sides…. Any 1D of the same sample will do, even if acquired on a different instrument at a different frequency.
            1. Turn on "Traces" display.
            2. Select pull-down via the ▼in the corner of the icon.
                a. Choose "Setup"… and select the 1D file for display along-side the 2D spectrum. Click the Green Check to use the selected 1D spectrum along the horizontal and/or vertical axis.
        v. Display Modes: bitmap vs. contour – You can click the pull-down menu by clicking the ▼in the corner of the 2D display icon, and choose from Bitmap, Contour, Stacked, and Whitewashed Stacked plot modes.
            1. Bitmap is faster, but not as attractive or useful as contour plot mode.
            2. Optimized contour plot (with a lot of levels and optimized spacing) gives the best overall appearance… but screen refresh rate is slower (when you zoom-in/ zoom-out a lot).
        vi. Customizing the display (double-click) the display.
            1. choose number of contours (circles), and scaling (>1.0 but < 2.0)
            2. Add a frame and grid-lines to the spectrum in the "Axis" sub-window. Gridlines are useful to see which peaks are aligned in the 2D spectrum (from a paper plot). Gridlines are not necessary on the computer because you can use the cursor to line-up the signals.
            3. You can change colors and create your own color-combinations… just experiment with it.
    c. Saving Processing-template files for future use.
        i. Under the Processing pull-down menu, select "Processing Template…".
            1. You can save all processing done on this data for future use.
            2. You can open a previously-saved processing-template, and thereby process many 2D datasets using the same processing parameters.
        ii. Linear-prediction needs to be re-calculated if you use a different value for "ni" in your acquisition of the 2D data.
            1. You can change the processing details in the "Full Processing" pull-down menu, but the numerical values for linear prediction need to match the data.
            2. If you usually acquire your 2D data using the same parameters, you can use the same processing template over and over again.

d. 2D-gCOSY (Absolute Value)
   i. Use "Zhu-Bax" Linear Prediction to minimize artifacts in all Magnitude Mode experiments.
   ii. Sine-Bell (unshifted) apodization usually works fine. Sometimes using Sine Squared in F2 and Sine Bell in F1 gives better results. Make sure the phase box is 0.00 because a 90-degree shifted sine-bell or sine-square is actually a Cosine (or Cos-squared) function.
   iii. You can symmetrize the data via the Processing pull-down, select Symmetrize > and check "COSY like". Even if you don't intend to use this (you can always UNDO), it's useful to identify artifacts that are not symmetrical about the diagonal.

e. NOESY or ROESY (Phase Sensitive)
   i. Use default ("Toplitz") Linear Prediction for phase-sensitive Data
   ii. Sinesq(90-degree.. Cosine-square) or Gaussian apodization usually best (can add Gaussian to Cosine Squared to get cleaner lineshapes with fewer artifacts).
      1. If you see "streaks/lines" along F1 or F2, try checking (or un-checking) the box for multiplying the first point by 0.5. For example, if you see these streaks in F1, and if this box is not checked, click on it to check it. If it already checked, try un-checking it… use whichever makes the streaks go away.
   iii. Phasing NOESY/ROESY… try "automatic" using "whitening" method
      1. Manual phasing is straightforward… select F1 or F2 dimension, cursor defines the pivot-point. Left mouse button to phase at the pivot, Right mouse button to phase away from the pivot point.
      2. For NOESY/ROESY (w/ small molecules) the cross peaks will have the opposite phase vs. the diagonal peaks. Normally, we phase the diagonal peaks to be negative, so the cross-peaks are positive, but it doesn't really matter.
      3. Once everything is as good as you can get it, you should try automatic baseline correction (Berstein Polynomials) as the final step in processing the NOESY.

f. gHSQC (Phase Sensitive)
   i. Use default ("Toplitz") Linear Prediction for phase-sensitive Data. Decrease the number of *coefficients* to 8, since each proton should have only 1 ^{13}C attached.
   ii. Sinesq(90-degree) or Gaussian apodization, or both.
   iii. Phasing 2D Data… try "automatic" using "whitening" method
      1. Manual phasing is straightforward… slect F1 or F2 dimension, cursor defines the pivot-point. (See NOESY/ROESY above).
      2. For Edited HSQC, CH/CH3 will have the same phase, CH2s will have the opposite phase (like in a DEPT-135 ^{13}C spectrum).

g. gHMBC (Absolute Value)
   i. Process pretty-much like gCOSY (except no symmetrize because gHMBC is not symmetric along F2 vs. F1). this is a Magnitude Mode experiment.
      1. "Zhu-Bax" Linear Prediction works best in t1/F1 (^{13}C dimension), try to increase the number of points in t1/F1 by 4x, but if the results aren't satisfactory, try using Linear Predicting to only 2x the acquired points. You should use the maximum number of coefficients allowed in the software for HMBC.
      2. Like COSY, use Sine Bell or Sine Squared in both F2 & F1.

h. gHMBC(ps) (Phase-Sensitive in t1/f1, Absolute-Value in t2/f2). This is the way the newest gHMBC and gHMBCad experiments are acquired in VNMRj.
    i. Use default ("Toplitz") Linear Prediction for phase-sensitive Data in the indirect (t1) dimension.
    ii. "t2" Apodization: Sine-Bell (unshifted) apodization usually works fine in the direct (t2) dimension. Sometimes using Sine Squared in F2 and Sine Bell in F1 gives better results. Make sure the phase box is 0.00 because a 90-degree shifted sine-bell or sine-square is actually a Cosine (or Cos-squared) function.
    iii. "t1" Apodizaiton: Sinesq(90-degree.. Cosine-square) or Gaussian apodization usually best (can add Gaussian to Cosine Squared to get cleaner lineshapes with fewer artifacts).
        1. If you see "streaks/lines" along F1 (or un-checking) the box for multiplying the first point by 0.5. For example, if you see these streaks in F1, and if this box is not checked, click on it to check it. If it already checked, try un-checking it… use whichever makes the streaks go away.
    iv. This will not be phased, but the "Magnitude Calculation" must be done in the f2 dimension (the dimension in which the sine-bell/sine-squared apodization was applied).