BARE BONES GUIDE TO gradientHMBC
on the Inova-400&500 (w/ PFG probe installed) VNMRj 3.2

This guide is written assuming proficiency in basic operation of the Varian NMR instrument. You should be experienced in performing basic 1-dimensional NMR experiments before attempting to perform 2D experiments on your own. Please ask for help the first time you perform this, to minimize your frustration.

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Important Consideration before starting:

- You can perform this experiment using either the 4-nucleus (n4n/asw) probe, or one of the indirect-detection (NTR or 3mmID) probes; however, the sensitivity is MUCH better indirect-detect probe. If you need a probe-change on either the 400 or 500, please schedule with Dr. Shoemaker in advance, and reserve an extra 10-20 minutes.

1) Make sure you are working in Exp:1 (type **jexp1<Ret>** and verify that you are working in Exp:1).
2) Acquire a normal ^{1}H NMR spectrum, and save it to be used as a 1D-Trace for the 2D-plots.
3) *Type* **mf(1,2) <space> jexp2 <Enter>** (this means: "move fid" … meaning, "copy" your FID and parameters from Exp:1 to Exp:2, then join Exp:2) – *Note, that you could substitute "3" for "2" in the above line if you have already setup another experiment in Exp:2… i.e., mf(1,3) jexp3 … would copy the 1H NMR located in Exp:1 to Exp:3, and then join Exp:3 for further setup.*
4) Place the red-cursors around the region of interest – DO NOT ZOOM IN. (Note: if you select a region with peaks outside that region, you will have *folded* peaks in your spectrum...this can be OK, or it can be a problem; depending upon where the folded peaks land).
5) Type **movesw** , (or *click* on [MoveSW] in the [Acquire] panel. and this will set the spectral width and offset to match your selected window – ignore the apparent change to the shift scale at this point – go to the next step.
6) Go to the [Experiments] Pull-Down menu, via [*Convert Current Experiment to Do…*], [Indirect Heteronuclear Correlations (Basic)], [gradient HMBC], or [gradient HMBC], or the experiment you wish to perform. gHMBC is a good choice as a "default" HSQC experiment in most cases, but the Adiabatic version sometimes has advantages, especially when already acquiring 4 transients or more anyway due to sensitivity limitations.
   a) From the [Acquire]-Tab/[Defaults]-panel, you can choose the parameters necessary for acquisition:
      i) Enter the desired spectral window for the ^{13}C (indirect) dimension into the appropriate boxes. Remember you need to include the possible shifts of unprotonated carbons like ketone-carbonyls, and/or carboxyl carbonyls.
      ii) Select the number of scans per T1 increment (this is simply the value of "nt", or number of transients), the default (nt=2) may work well for concentrated samples, increase to 4 or 8 for weak samples.
      iii) Select the number of T1 increments: 200 (default) is OK for normal ^{13}C spectral widths. This, combined with linear-prediction in T1, will determine the ^{13}C resolution of your final spectrum. If you need extremely high resolution in 13C, either narrow the spectral window (in step (i) above) or increase the number of T1 increments (this will increase the total acquisition time linearly).
      iv) Multiple-bond Jnxh should match the desired 2,3,or 4-bond $J_{(nCH)}$ coupling. The default of 8 Hz usually works well as an optimal value for vicinal (J3CH) couplings.
7) Type **time** (or *click* on [Show Time]) to see how long the experiment will take (*optional*).
8) *Click* on [Acquire] **(**or type **cpgo**) to start the experiment.
   a) You can process the data while it is acquiring via the [Process][Default] panel, after a minimum of 38 increments have been acquired… you might need to un-check and re-check the Linear Prediction box.
9) If you must stop the acquisition before it is done (i.e., you run out of time), always stop the 2D experiment by typing: **sa('nt')** . This will stop the experiment at the end of the current FID.
10) Save the data using "Save As…", navigate to your Directory in /nmrdata/rgroups/group/user, the same as you would with a 1D spectrum. Verify that the data is saved properly.
11) Type **jexp1** , eject your sample, and re-insert the reference. Lock on the reference as usual.

MestReNova is recommended for Processing, Analysis, and Plotting of the 2D NMR Data. Separate instructions are available for this process. NOTE: These gHMBC experiments are acquired as "Magnitude/Absolute Value" mode in t2/F2, and phase-sensitive mode in t1/F1 dimension. So process as such in MestReNova, and be sure to only do the Magnitude calculation (instead of phasing) in the F2 dimension only, after applying Sine-bell of Sine-squared apodization in the t2 dimension only). Use Cosine-squared or Gaussian apodization in t1, combined with Toeplitz Linear Prediction in t1.