IV. 2D NMR on the AVANCE Spectrometer

[cgfry: updated 1 Aug 2004]

a) The Philosophy Used to Setup 2D Exps on the AVANCE-360

Bruker's XwinNMR unfortunately does not allow preset parameters, e.g., from a ^1H 1d experiment, to be easily passed to a new experiment. All multidimensional experiments are therefore setup here by a "brute-force" method as follows:

- A parameter set containing a valid set of parameters is read in using rpar .
- Standard probe rf parameters are read in by setting PROSOL to TRUE in eda .
- The sweepwidth, center frequency, and referencing parameters must be set by manually copying them from the appropriate 1d data set into the 2d data set. eda is the best place to perform these changes, as this panel shows the parameters for both dimensions.
- All individually calibrated parameters must be copied to the 2d data set, such as:
  - RG
  - the 90° ^1H pulsewidth (usually p1 @ pl1), and
  - the repetition delay (d1) based on the proton T_1.
- It is recommended that the user open the associated pulse sequence, and carefully check that *all* parameters are set as described in the comments section of the sequence (especially for long experiments). The sequence is best read/opened as follows:
  - Find the pulse sequence named by looking at the first line in either the eda or ased panel.
  - In a unix window, enter jot *filename*↵

b) COSY 2D Experiments

- Setup normally: edc, edhead, edte, lock, shim, wob.
- Acquire a standard 1H 1d dataset. Optimize the sweepwidth and O1 and reacquire.
- Use edc or iexpno to move to a new experiment #, and read in the cosy parameters:
  - on 5mmbbo, use rpar COSYGS.UW all↵
  - for the 10mmbbo, use rpar COSY90SW all↵ (or COSY45SW)
- Copy SW, O1 and RG from the 1H 1d dataset into the 2d.

  SW1 = SW , and
  O2 = O1 .

  Do ***not*** use RGA on a cosy dataset, as the 1st row is lower in intensity than later rows.

- Check that TD1 in eda is sufficiently large to provide the desired J-coupling observation. TD1 = 128 will be sufficient on the 360 in most cases.
- Set d1 ~ T_1 of the longest protons of interest.
- Set NS to 1, 2, 4, or multiples of 8; 1 or 2 should be sufficient unless the sample concentration is very low.
- Start the acquisition, and look at the first row acquisition in the acqi window; protons should be observed. Transform the 1st row if necessary; increase NS if necessary to provide sufficient sensitivity.
- expt will give an estimate of the total time of the experiment.
- For processing, go into edp: SI1 must equal SI for symmetrization.
  - Use xfb and sym to transform and symmetrize the data.
  - Make sure SR = SR1 equals the SR value in the 1H 1d to get the referencing correct in both direct and indirect dimensions.

c) HSQC 2D Experiments

- Setup normally: edc, edhead, edte, lock, shim, wob.
- Acquire a standard 1H 1d dataset. Optimize the SW and O1 and reacquire. Properly reference the spectrum.
- In another experiment, acquire a 1 scan ^{13}C (or ^{31}P, etc.). Optimize SW and O1 based on knowledge of the compound; limit to only 1-bond protonated carbons if only hsqc will be acquired. You might expand to include all quaternary carbons if a long-range hmbc will also be acquired.
- Use edc or iexpno to move to a new experiment #, and read in the hsqc parameters:
  - on 5mmbbo, use rpar ghsqcse.UW all↵
  - for the 10mmbbo, see Charlie for assistance in locating a non-gradient version of the experiment, and learning how to optimize it.
- Copy SW, O1 and SR from the 1H 1d dataset into the 2d for these exact same parameters. SR can be typed in, or found on the edp page.
- Copy SW, O1 and SR from the 13C 1d dataset into SW1, O2 and SR1. SR1 is found on the edp page.
- Check that TD1 in eda is sufficiently large to provide the desired resolution in 13C chemical shift. TD1 = 128 will be sufficient on the 360 in most cases, although where some carbons are close, TD1 up to 512 will be better.
- Set d1 ~ T_1 of the longest protons of interest.
- Set NS to 2, 4, or multiples of 8; 2 should be sufficient unless the sample concentration is low.

- Do ***not*** use RG from the 1H 1d dataset here. Only 1.1% of the protons, those attached to 13C, will be observed.

  Use RGA to properly set RG . [The probe must be properly tuned!]
- Transform the 1st row of data; increase NS if necessary to provide sufficient sensitivity to see some of the 1H directly.
- expt will give an estimate of the total time of the experiment.
- For processing, go into edp: set SI equal to 1/2×TD (no zerofill), and SI1 ≥ TD1 (at least one zerofill; two is common).
  - Use xfb to transform the data.
  - Make sure SR and SR1 are set properly form the 1H and 13C 1d datasets, respectively, to get the referencing correct in both direct and indirect dimensions.

d) HMBC 2D Experiments

- Follow the instructions for HSQC above, but with the following changes:
  - Make certain the 13C 1d SW is large enough to include all quaternary 13C.
  - Use rpar ghmbc.UW all for the 5mmbbo probe. HMBC on the 10mmbbo probe is not recommended; gradients very significantly improve the quality of this experiment.
  - Always calibrated the proton channel 90° pulse width for HMBC.
  - See the pulse sequence listing to insure that the long-range J-coupling parameter is set properly.
  - Always use NS equals 8, or a multiple of 8.