## Double-Differencing NOESY-1D Spectra

[updated: 22 Sept 2009]

Zero-quantum (ZQ) and other artifacts can make interpretations of NOESY1D spectra difficult. ZQ artifacts arise from J-coupling, so the problems are most prevalent when proximity of coupled spin pairs is important. The quality of spectra for quantitative distance determinations is also degraded. A double-differencing technique [Hu et al., *JMR* **171** (2004) 201] relies on ZQ and other artifacts not being dependent on the **mix**. A spectrum with **mix=0** will therefore contain the artifacts, which can then be subtracted from the **mix≠0** spectra, greatly reducing ZQ artifacts, and often dramatically improving the quality of the final data.

1. Set up NOESY1D experiments as described in the previous section (sbs_NOESY1D.pdf); do not array selections (select on a single multiplet).

2. Array **mix** in the linear buildup region, starting at ~0.6×$T_1$ and decreasing in **mix** time. Set the *last* value of the mix array to 0. For example, with $T_1^{\text{shortest}}$ = 1 s, setup as:

   *example:* **mix = 0.6,0.5,0.4,0.3,0.2,0.1,0.0**

3. Typically use more **nt** than in previous experiments.

4. After acquisition, process the arrayed data set as normal (e.g., **lb=0.5** to **lb=1**).

5. The next processing step will use exp5. Save any data there, if needed.

6. Go to the NOESY1D dataset, and apply double-differencing using:

   **ddnoe(0,1)**

   The differenced dataset (each arrayed spectrum will have the last spectrum subtracted from it) will be placed into exp5. **dssa** will display the set. Note that the macro turns the array around, such that now:

   *ddnoe dssa in exp5 has:* **mix=0.1,0.2,0.3,0.4,0.5,0.6**

7. The two datasets can be compared using 2-windows setup:

   MAINMENU → MORE → WINDOWS → 2 COLUMNS

   You can move between the two windows by double-left-clicking in the one you want (it will obtain the red frame). Do a **dssa** in the initial NOESY1D dataset, and interrogate **vo**. Click into the other window, do **jexp5** and set **vo** to the same value, then use **dss**.

8. When integrating, set **insref='y' insref=1e-4**. Obtain the integration values for the 3rd spectrum using **ds(3) dli** .