Lineshape Fitting using nLinLS in nmrPipe

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Introduction

There are many applications that require comparing peak volumes or intensities in multiple HSQC-type spectra. Unfortunately, it is not easy to make reliable, precise and accurate measurements of peak volumes using many popular software packages. This guide provides step-by-step instructions for measuring peak volume using a line shape fitting function in nmrPipe. This method can be extended to pseudo-3D experiments (for example relaxation dispersion data sets).

Let me state explicitly that I am an enthusiastic user of nmrPipe, but I am not affiliated with this software in any way. In other words, if Frank says that everything I say here is crap, I'd advise you to listen to Frank! Also, let me acknowledge that other popular software packages may have similar capabilities. If the developers of these packages claim that their software is easier or more intuitive then I'd suggest using it. I have written this guide as a service to the community. I'm just trying to pass on my experiences.

For general information about nmrPipe, please see

http://spin.niddk.nih.gov/NMRPipe/

For more information about peak picking in nmrPipe please see

http://spin.niddk.nih.gov/NMRPipe/doc2new/#EDITING%20A%20PEAK%20TABLE%20FOR%20SPECTRAL%20QUANTIFICATION%20PURPOSES

#1) Process 2D HSQC data using nmrPipe.

An example script is below. I personally like to use lorentzian-to-gaussian conversion, although nlinLS will fit to both Lorentzian and Gaussian line shapes (see below).

```
#!/bin/csh

var2pipe -in ./fid \
 -noaswap  \
  -xN              2048  -yN               512  \
  -xT              1024  -yT               256  \
  -xMODE        Complex  -yMODE      Rance-Kay  \
  -xSW        12001.200  -ySW         2431.130  \
  -xOBS         599.736  -yOBS          60.778  \
  -xCAR           4.706  -yCAR         117.840  \
  -xLAB              HN  -yLAB             N15  \
  -ndim               2  -aq2D          States  \
  -out ./test.fid -verb -ov

nmrPipe -in test.fid \
#| nmrPipe  -fn SOL \
#| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 2 -c 0.5    \
| nmrPipe -fn EM -lb 2.5 -c 0.5 \
| nmrPipe -fn GM -g1 2.5 -g2 3.125 \
```

```
| nmrPipe  -fn ZF -auto                                  \
| nmrPipe  -fn FT -auto                                  \
| nmrPipe  -fn PS -p0 51 -p1 0.00 -di -verb          \
| nmrPipe  -fn EXT -left -sw \
| nmrPipe  -fn TP                                        \
#| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 2 -c 1.0    \
| nmrPipe  -fn EM -lb 5 -c 1.0 \
| nmrPipe  -fn GM -g1 5 -g2 6.25 \
| nmrPipe  -fn ZF -auto                                  \
| nmrPipe  -fn FT -auto                                  \
| nmrPipe  -fn PS -p0 -90 -p1 180 -di -verb          \
   -ov -out test.ft2

rm -f test.fid
mv -f test.ft2 spec.ft2
```

#2) Pick peaks in nmrDraw.

Adjust contours using plus and minus buttons in upper right of nmrDraw to avoid picking noise peaks. Also, use 2D zoom (in Mouse pull-down menu or command "z") to set field-of-view in the window to avoid residual water peak, which we do not want to pick. Pick peaks automatically using Peak detection (in Peak pull-down menu or command "k").

#3) Manually assign peak list in nmrDraw.

See

http://spin.niddk.nih.gov/NMRPipe/doc2new/#EDITING%20A%20PEAK%20TABLE%20FOR%20SPECTRAL%20QUANTIFICATION%20PURPOSES

for more complete instructions.

In my opinion, assignment in nmrDraw is less intuitive than in other software, such as Sparky or CCPN Analysis, however, with a little practice you'll get the hang of it. In the Peak Detection pop up, choose for the Labels the Variable "ASS" (for assignment). Click the edit button to enter the edit mode. Note the top row of the main nmrDraw window alerts you that the Peak Editing mode is now active. In the spectrum, use the middle mouse button ("Modify Peak") to select a peak. Now you are in the Text Entry mode. Type "cntl+U" to erase the current assignment ("None") and enter your assignment. Be sure to save your work often by clicking the save button in the Peak Detection pop up.

Sometime you have to zoom in and out and enter and exit the Peak Editing mode. It can seem confusing at first, but it gets easier with practice. I'll note here that your peak list is saved (as "test.tab" by default) as an ascii file that can be manipulated by excel, if you prefer to work that way.

#4) Assign the CLUSTIDs.

This is probably the most important step. When nmrPipe does lineshape fitting, it will fit all peaks with the same CLUSTID values simultaneously. Hence, CLUSTID is very important for partially overlapping peaks. Try to set to the same CLUSTID all clusters of overlapping peaks. Likewise, peaks that do not overlap

should have different CLUSTIDs. In my experience, the CLUSTIDs must be set iteratively. Do your best for the first round and go to the next step. At the end, we'll examine our fitting results. If the fit is poor, then go back and adjust CLUSTID.

You adjust CLUSTID in the exact same way you assigned the spectra in nmrDraw. In the Peak Detection pop up, choose for the Labels the Variable "CLUSTID". Click the edit button to enter the edit mode. Note the top row of the main nmrDraw window alerts you that the Peak Editing mode is now active. In the spectrum, use the middle mouse button ("Modify Peak") to select a peak. Now you are in the Text Entry mode. Type "cntl+U" to erase the current CLUSTID and enter the new value. These numbers are arbitrary, so you can enter "1000" for the first cluster, if you like. Be sure to save your work often by clicking the save button in the Peak Detection pop up.

#5) Prepare and run for lineshape fitting.

We will use the tcl program "autoFit.tcl" to create and run the nlinLS fitting script. For more information on autoFit.tcl, type the following on a command line:

```
> autoFit.tcl –help
```

You will see all available options for this script from this result. Some of the most important for our case are specName (name of the input spectrum, test.ft2 by default), inTab (name of input peak table, test.tab by default) and modX and modY (model function for x and y dimension, respectively, GAUSS1D by default). Obviously if your spectrum or table are saved with different names or if you did not do a lorentzian-to-gaussian apodization, you will need to change the defaults. I'll use the command.

```
> autoFit.tcl -specName spec.ft2
```

For a 2D HSQC, this script only takes a minute or so to run on my Mac laptop. The results are the following files: autoFit.com, axt.tab, diff.ft2, nlin.tab and sim.ft2. For our purposes, the latter three files are the most important. The files sim.ft2 and diff.ft2 are nmrPipe files containing the spectra for a simulation based on the fitting and the difference between the simulation and data. I cannot recommend strongly enough that you look at these files ***very carefully***. I open them in CCPN Analysis, ensure the contours are set equally and look very closely for poorly simulated regions. It is these regions that usually do not have proper CLUSTID values.

Assuming sim.ft2 and diff.ft2 conform to expectations, you can the peak table nlin.tab to get peak volumes. I find it helpful to use the tcl script getTabCol to extract assignment and volume columns.

```
> getTabCol.tcl -var ASS VOL > test2.tab
```