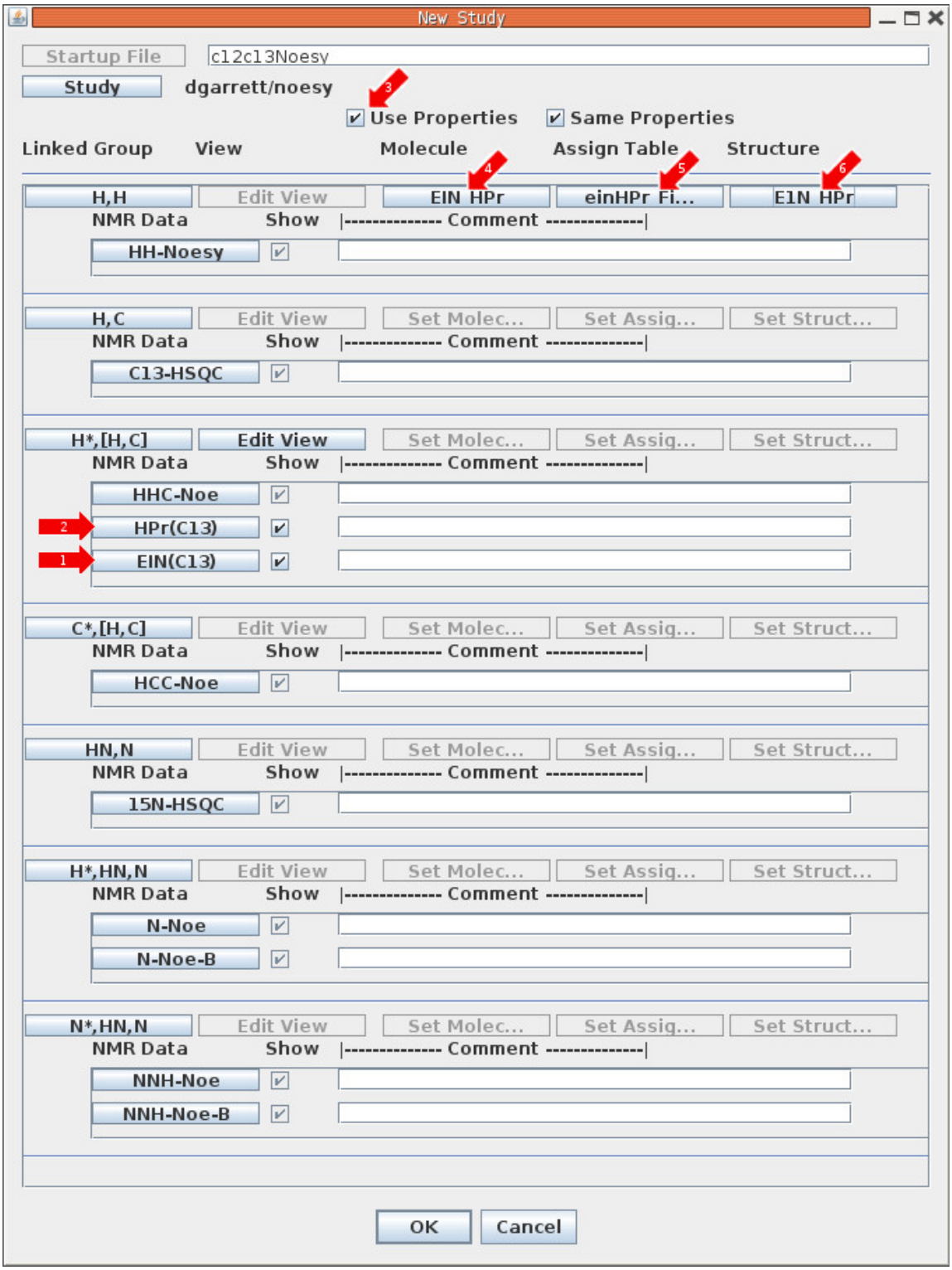
Edit Study: NOESY Assign Dialog
for Intermolecular NOES

This is a rough draft document. I have not created any Examples files.



This is the Noesy Assign Dialog which was briefly described in the createSetStudies document for looking at a 3D ^{13}C Noesy and a 3D ^{15}N Noesy experiments. The Noesy Assign Dialog shown above is created from the XippPanel by clicking “New” and select “Noesy Assign Study” from the 'New Study' dialog. Furthermore this dialog is shown after everything was set. Initially the names of the Experiment buttons to be changed were “C12C13-NOE-A” and “C12C13-NOE-B” with the last part of each name truncated.

1: Click on one of the Experiment buttons that is labeled “C12C13-N...” to bring up the 'Edit Experiment' dialog. See 'Edit Experiment: EIN(C13)' for details on setting properties for sample containing ^{13}C labeled EIN and unlabeled HPr.

2: Click on the other Experiment button that is labeled “C12C13-N...” to bring up the 'Edit Experiment' dialog. See 'Edit Experiment: HPr(C13)' for details on setting properties for sample containing ^{13}C labeled HPr and unlabeled EIN.

3: Click 'Use Properties' button to enable Molecule, AssignTable and Structure properties. The 'Use Properties' button controls whether the study will use Molecules, Assign Tables or Structure. When toggled off none of these properties are used so that when peaks are created the user sets a label for each peak instead of selecting an assignment from a list suggested by xipp.

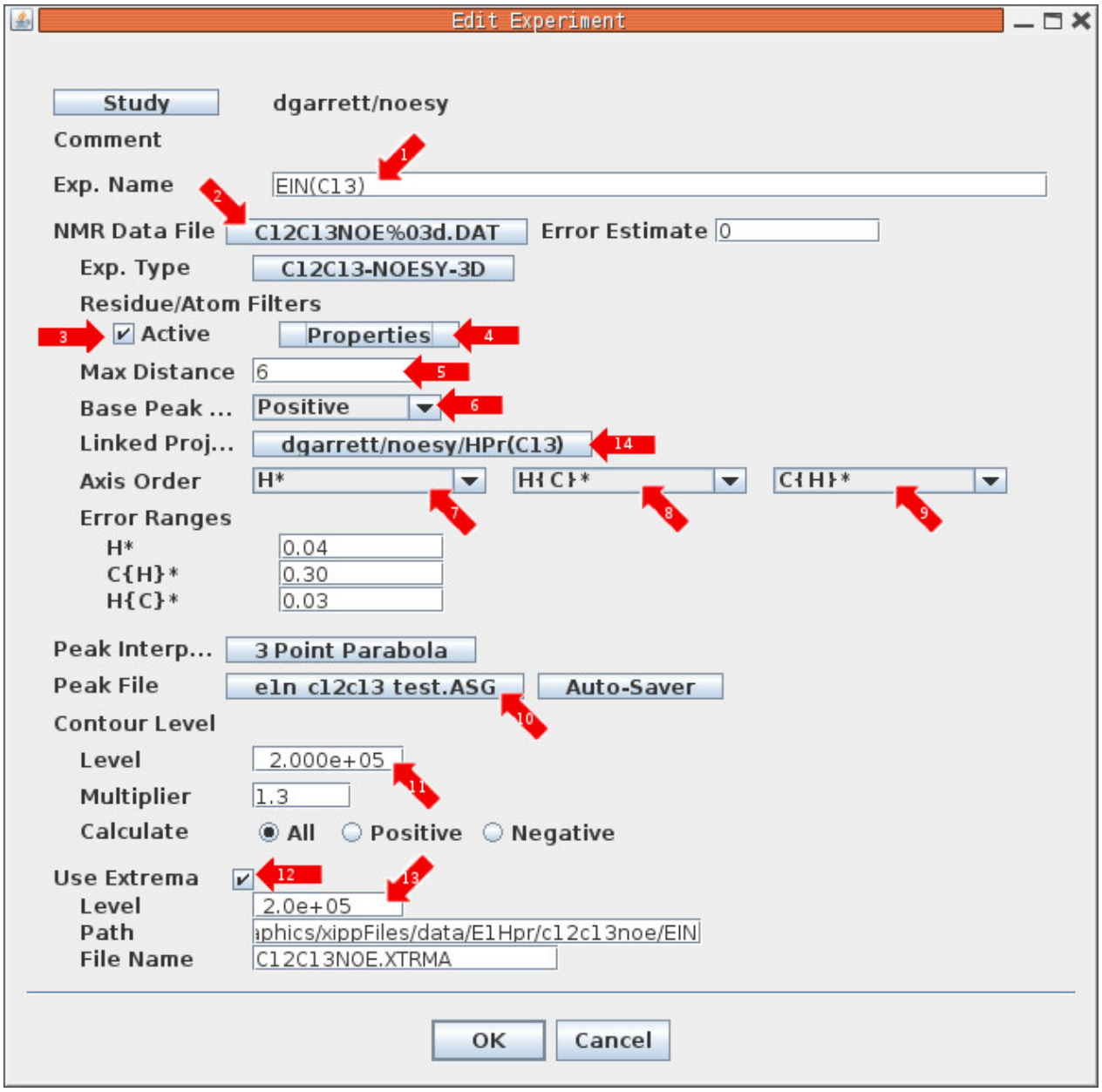
4: Click the 'Set Molec...' to bring up the “Select/Create Molecule” dialog. See section below 'Define Molecule for Intermolecular Noesy' for details on defining a single poly-peptide chain that defines both molecules.

5: Click the 'Set Assign...' to bring up the “Select/Create Assign Table” dialog. See 'Define Assign Table for Intermolecular Noesy' for details on setting the assignment file and what must be in the assignment file.

6: Click the 'Set Struct...' to bring up the “Select/Create Structure” dialog. See 'Define Structure for Intermolecular Noesy' for details on setting the structure file property. Note this can be left blank if there are no preliminary structures.

Clicking OK at the bottom of the New Study Dialog will save all of the properties into the file based on the study name, ie c12c13Noesy.xipp in this example.

Edit Experiment: EIN(C13)



This is the Experiment Dialog created by clicking on the Exp button 1 in the "Edit Study: NOESY Assign Dialog" for the sample that contains ^{13}C labeled EIN complexed with unlabeled HPr, ie carbon atoms are ^{12}C. This dialog is shown after everything was set.

Edit Experiment: EIN(C13) (details)

1: Replace the name of the experiment with the name of one of the proteins that is ^{13}C labeled, ie EIN in this case. The name should not be too long since this will be used as the text on the Exp button in Xipp. If you click OK at the bottom of this dialog and then re-open it this name will be saved and used as project name in other dialogs such as the NMR Data File Chooser(2), Residue/Atom Filters (4) and Peak File Chooser (10). This will make it easier to keep track of which protein sample you are setting.

2: Click on NMR Data File button to bring up the “NMR Data File Chooser” and select the NMRPipe processed file for the protein identified in (1). The “NMR Data File Chooser” is described in the createSetStudies document.

3 & 4: Click the Active Checkbox under the label Residue/Atom Filters to enable the Residue/Atom Filters Property button. Click the Residue/Atom Filters Property button to bring up the “Residue/Atom Filters Dialog” for the EIN(C13) experiment which is described in section ' Residue/Atom Filters Dialog'.

5: The 'Max Distance' defines the initial maximum distance filter to use in assigning a Peak-Pick if one or more structures are defined. If structures are not defined then this has no effect.

6: The 'Base Peak Sign' defines the sign of an unfolded peak. If an unfolded peak is phased to have a positive sign then this should be Positive. The two other options are Negative and Unknown.

7 & 8: Click these Combo boxes to ensure that the Axis order is consistent with the order of the processed NMRPipe data file.. The Axis Order is X, Y and Z. Note H* is the proton that is bonded to ^{12}C and H{C}* is the proton bonded to ^{13}C. In this sample H* are protons from HPr and H{C}* are protons from EIN.

9: This is automatically chosen as C{H}* based on gyromagnetic ratio and spectrometer frequencies. You should not change this unless the NMRPipe header is wrong and C{H}* is actually along a different axis.

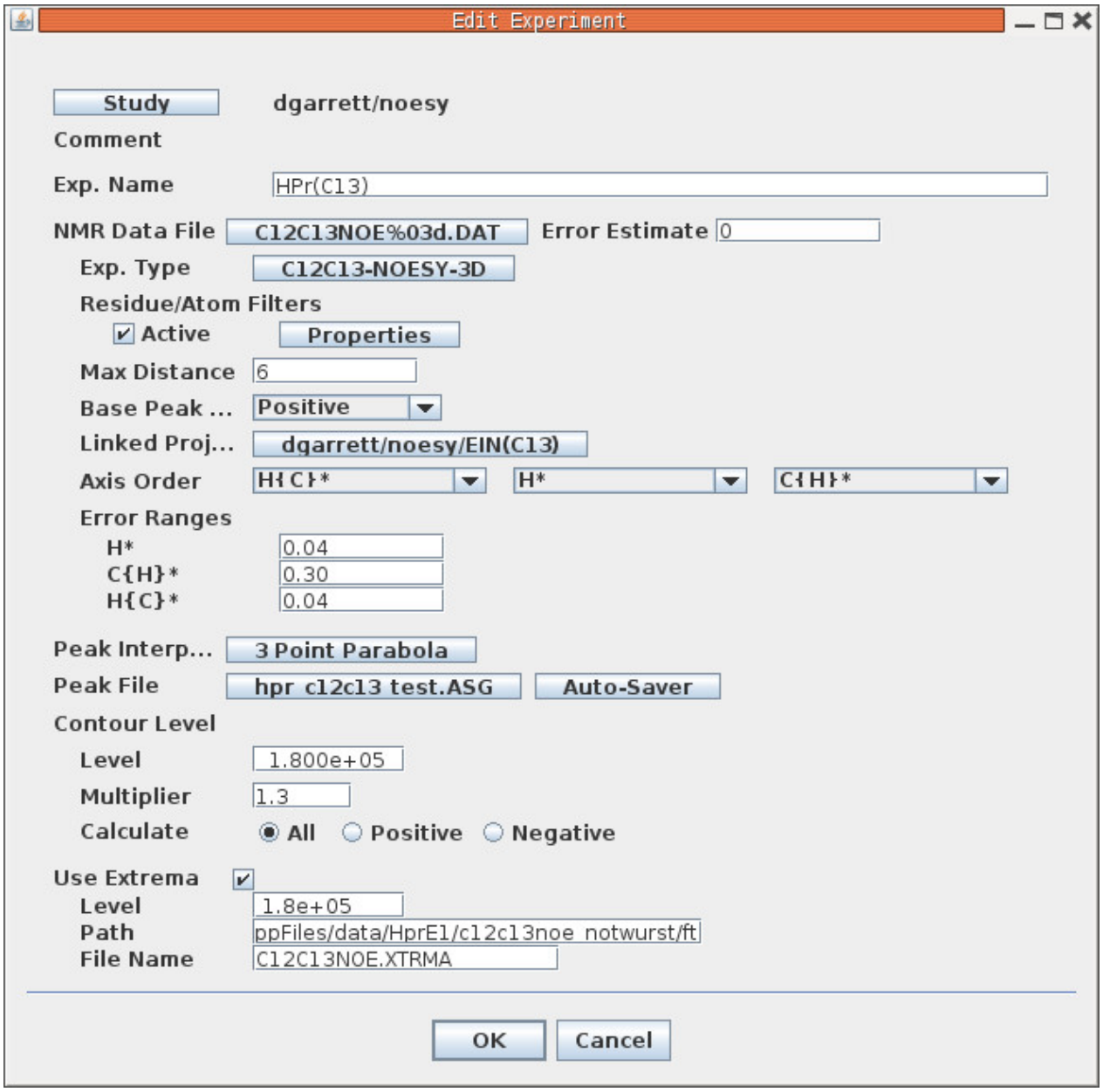
10: Click the Peak File button to bring up the “Peak-Pick File Chooser Dialog” which is described in the createSetStudies document.

11: Set the minimum Contour Level threshold if desired. This can be changed in Xipp, but at this time changes in Xipp are not saved to the *.xipp file.

12 & 13: Extrema are only needed and used in intermolecular Noesy if you have NMR data for two samples that are labeled complementary. In this case the current experiment's sample has ^{13}C labeled EIN and ^{12}C labeled HPr (ie unlabled). The complementary sample described later has ^{12}C labeled EIN and ^{13}C labeled HPr. The extrema are used to find 3D symmetry between the two NMR experiments.

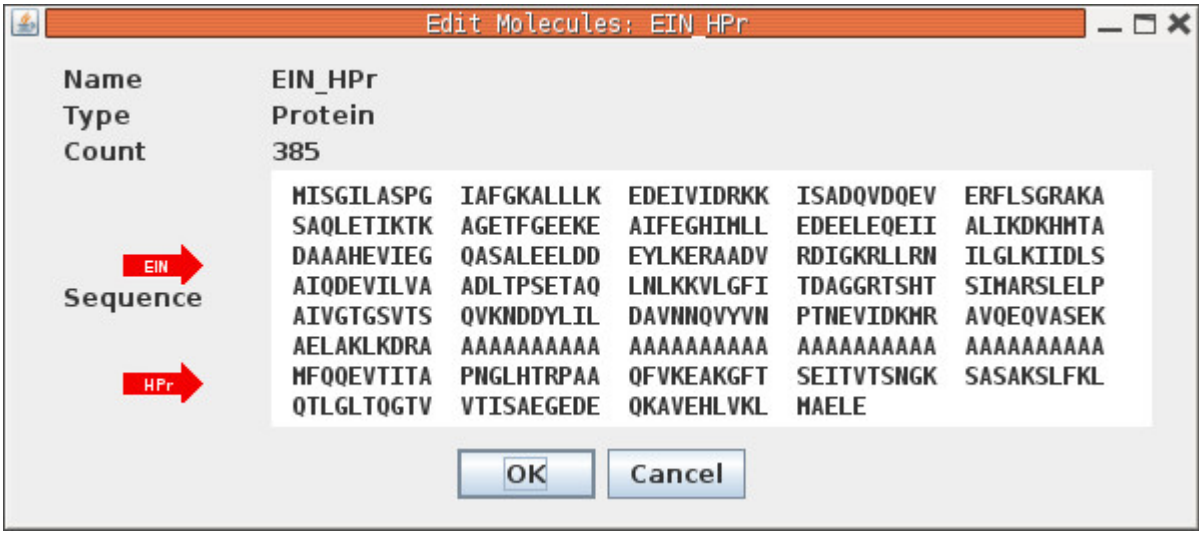
Clicking OK at the bottom of the Edit Experiment Dialog will save all of the properties into the file based on the study name, ie c12c13Noesy.xipp in this example.

Edit Experiment: Hpr(C13)



This is the Experiment Dialog created by clicking on the Exp button 2 in the "Edit Study: NOESY Assign Dialog" for the sample that contains ^{13}C labeled HPr complexed with unlabeled EIN, ie carbon atoms are ^{12}C. This dialog is shown after everything was set. This dialog should be set in an analogous manner as the "Edit Experiment: EIN(C13)" using values appropriate for the ^{13}C labeled HPr NMR experiment. Note that the Axis Order is different for the HPr(C13) sample.

Define Molecule
for Intermolecular Noesy



See createSetStudies section 'Molecule Select/Create Dialog' for detailed instructions on creating a new molecule. This was created as a New Protein Molecule. The protein EIN is 259 residues and is listed from residue M1 to R259. There are then enough spacer ' A' (residue type is not important) so that HPr starts at M301 and ends at E385. For those who have had experience with PIPP this is exactly the same way that PIPP handled protein complexes.

I have looked at introducing segments or samples as done in other software, but the simplicity of identifying the segment by a single residue number makes this approach easy to use.

The Peak-Pick tables and assignment tables must use this same scheme.

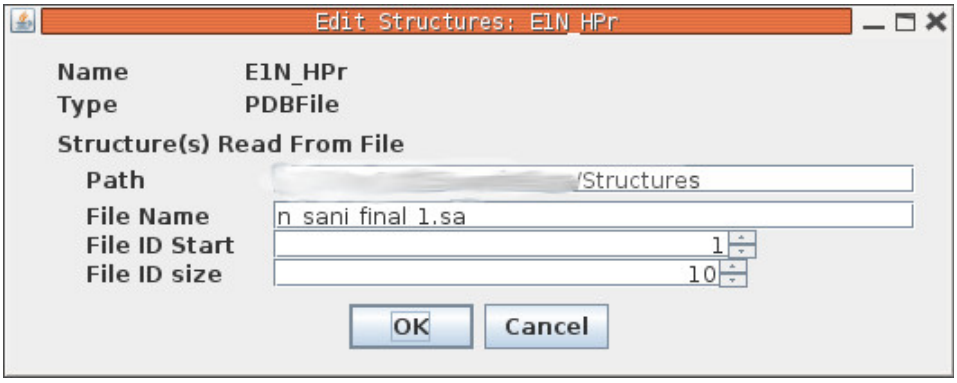
Define Assign Table
for Intermolecular Noesy

See createSetStudies section 'Assign Table Select/Create Dialog' for detailed instructions on creating a new Assign Table. This was created as a New 'Assignmenment File (PIPP V4). As mentioned above the assignments for should use residue M1 to R259. No assignments or anything needs to be included for the spacer ' A' residues. The assignments for HPr starts at M301 and ends at E385. For those who have had experience with PIPP this is exactly the same way that PIPP handled protein complexes. A single assignment table with ^{13}C and ^{1}H shifts can be used for the two NMR experiments EIN(C13) and HPr(C13). The 'Residue/Atom Filters Dialog' described below is used to limit which residues are allowed to be observed along which axis.

The ' Assign Table Select/Create Dialog' for an Assignment File is a simple file chooser. You need to specify the Path and File Name. Do not enable 'Use Backbone Assign Map' as that is only useful for backbone assignments.

Define Structure
for Intermolecular Noesy

There are no detailed instructions on creating a new Structure, but the process is very similar to what was described in createSetStudies sections for 'Molecule Select/Create Dialog' and 'Assign Table Select/Create Dialog'.



This was created as a New PDB Structure File. As mentioned above the atomic positions for EIN should use residue M1 to R259. No atomic positions needs to be included for the spacer ' A' residues. The atomic positions for HPr starts at M301 and ends at E385. For those who have had experience with PIPP this is exactly the same way that PIPP handled protein complexes.

The File ID Start and File ID size are used when the File Name contains C-style formating such as %d or %03d. For example if File Name is final_%d.sa then Xipp would read in 10 files whose names are: final_1.sa, final_2.sa, final_3.sa, final_4.sa, final_5.sa, final_6.sa, final_7.sa, final_8.sa, final_9.sa and final_10.sa If the File Name does not contain a C-style format (as in this case) then the File ID Start and size are ignored and a single file is read in. Sorry there is no way to read in a list of PDB files.

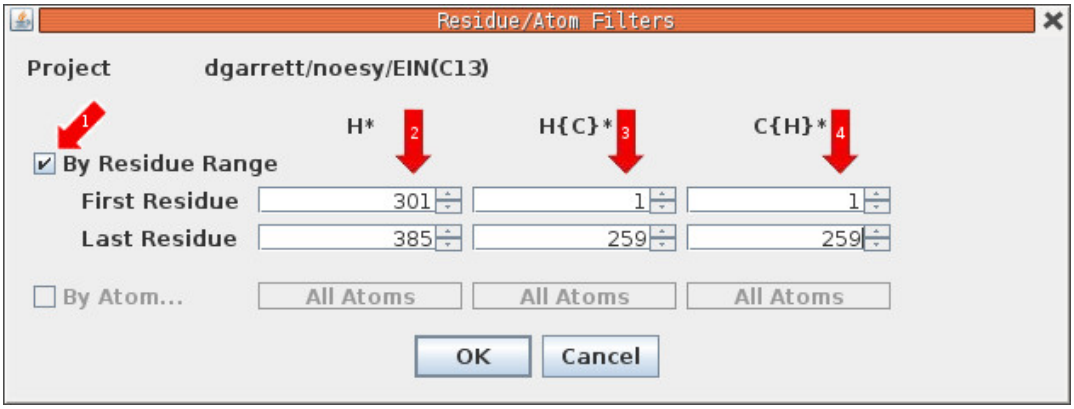
Note the more files read in the longer it takes for Xipp to filter assignments based on inter-proton distances. I suggest using at most 2 to 3 PDB files.

Note the PDB files must have H atomic coordinates. If the PDB file was obtained from a crystal structure you will need to add protons prior to using the file with Xipp. Xipp does NOT add protons.

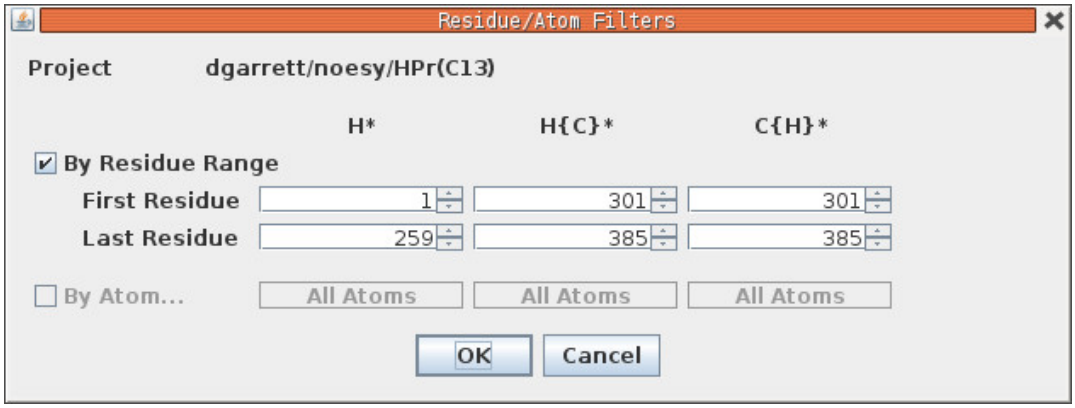
Defining a Structure is optional. Xipp will work OK without identifying preliminary structures. The benefit of using Structures comes at the refinement stage when the Structure is fairly well characterized and additional NOEs are sought from ambiguous NOEs. Once the overall fold is well characterized and is not expected to change, NOEs that are ambiguous based on chemical shift might be easily resolved based on distance as some NOE assignments might involve a distance much larger than an NOE can normally be observed.

Residue/Atom Filters Dialog

EIN(C13)



HPr(C13)



The EIN(C13) Residue/Atom Filter Dialog came from the “Edit Experiment Dialog” for the EIN(C13) sample and the HPr(C13) Residue/Atom Filter Dialog came from the “Edit Experiment Dialog” for the HPr(C13) sample. Both dialogs are shown after everything was set.

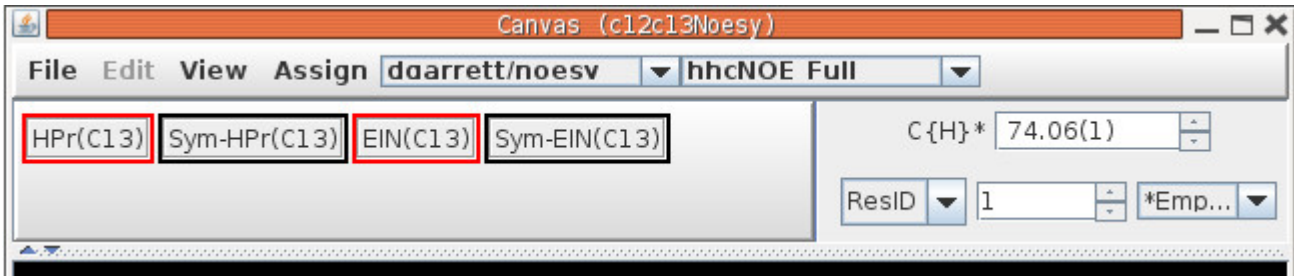
The purpose of these Dialogs is to identify what shifts are observed for each axis using the residue number select a subset of residues. The default value is to allow shifts from all residues for all axis. For C12/C13 intermolecular Noesy spectra the samples are complementary. For the EIN(C13) the H* should come only from HPr (hence residue range 301 – 385) and H{C}* and C{H}* should come from EIN. For the HPr(C13) the opposite is true H* should come from EIN and H{C}* and C{H}* should come from HPr.

The assignment table should have all of the shifts for both proteins ^{1}H and ^{13}C with EIN residues 1 to 259 and HPr residues 301 to 385.

The 'By Atom' checkbox can not be selected because I need to write the code that supports filtering based atom types such as methyls and or residue specific atom types. This will eventually be used to tell Xipp that your sample has only protons for select atoms, ie per-deuteration except valine methyls. This is not yet supported.

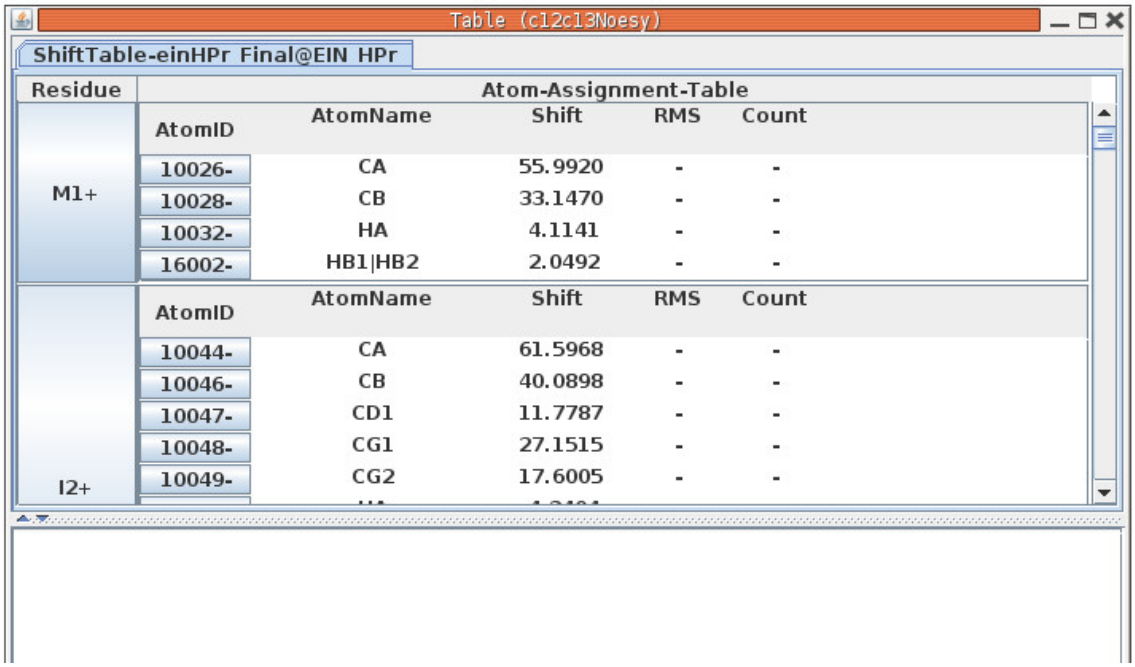
Show c12c13Noesy Study
Intermolecular Noesy Analysis

See Xipp_Commands document for detailed list of Xipp commands. Two windows should be shown: Canvas and Table. This description highlights what is unique/useful in the Intermolecular Noesy Study.



Note that there are 4 NMR buttons based on the 2 Experiments which are displayed in the same way as the '3D Symmetry Tool Bar' used with Sidechain study described in Xipp_Commands (p10). The Sym-HPr(C13) is used to manage a separate view into the HPr(C13) data to overlay Symmetry peaks from HPr(C13). Similarly the Sym-EIN(C13) is used to manage a separate view into the EIN(C13) data to overlay Symmetry peaks from EIN(C13). Toggle off the Sym-HPr(C13) and Sym-EIN(C13) NMR buttons.

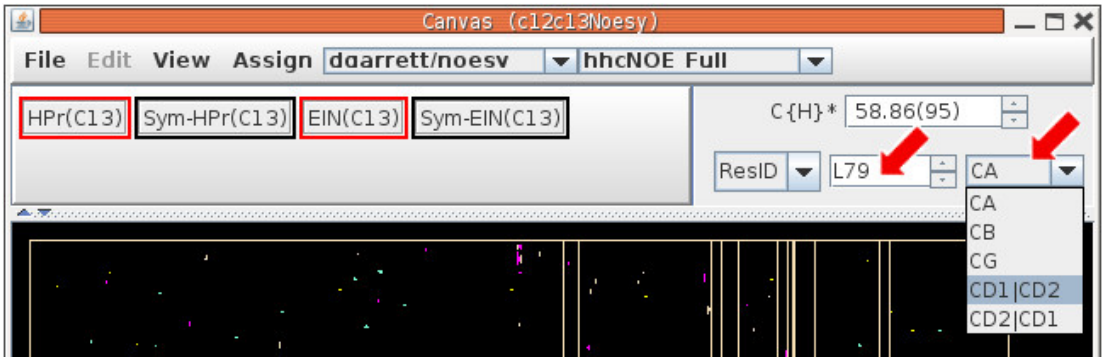
In the Canvas window over the contours enter the Xipp command 'n' (just the letter n no quotes) to toggle the Table window so that the Name/Assign Peak Table is shown:



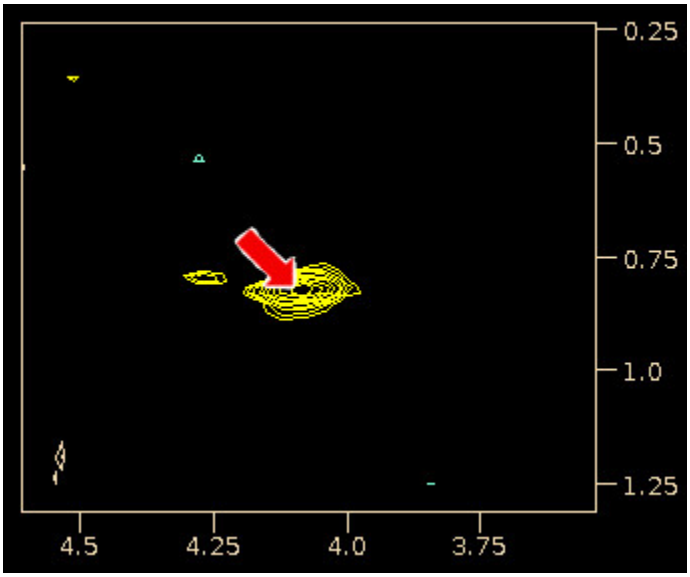
In the Table window when the Name/Assign Peak Table is shown the window is split with all assignments shown on top and the Name Peak-Pick table on the bottom. The Name Peak-Pick table shows details about each peak that is clicked and includes symmetry.

Getting Symmetry in c12c13Noesy Study

Jump to residue L79.CD1|CD2 with Canvas command Alt-j followed by entering 79 and hitting Enter key. This will jump to L79.CA and the atom chooser is used to CD1|CD2:

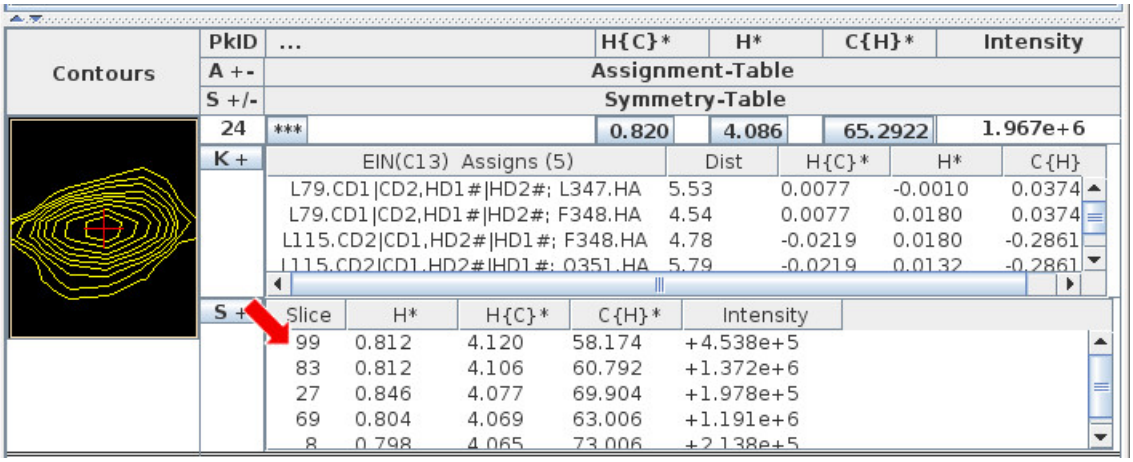


On slice 55 click on the peak at 4.1, 0.82 PPM:



The Name Peak-Pick table in the Table window will show several possible assignments for the peak as well as several possible Symmetry.

The possible carbon assignments are L79.CD1|CD2 and L115.CD2|CD1 from EIN(C13) which should not be surprising since the peak came from EIN(C13).

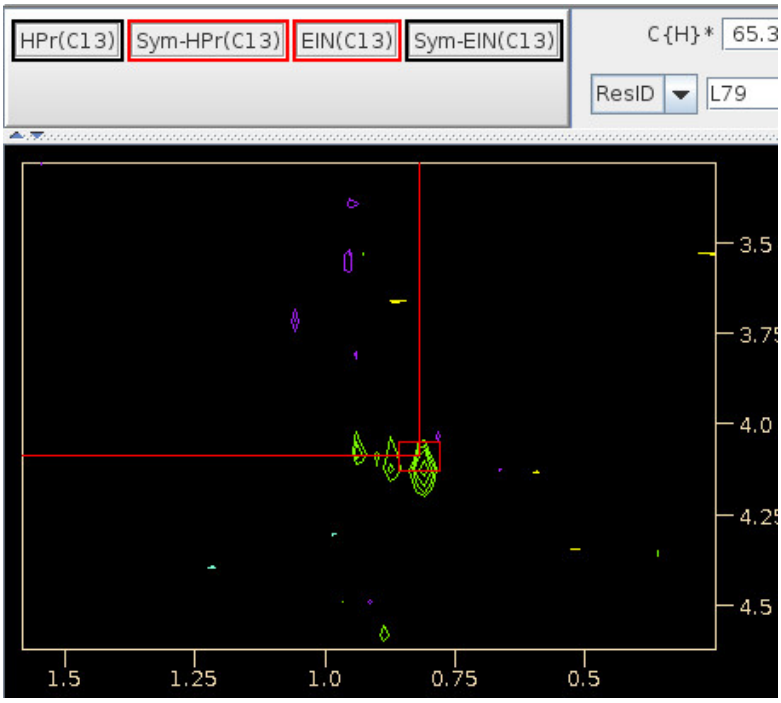


Show Symmetry in c12c13Noesy Study

On the Canvas Window toggle on the Sym-HPr(C13) NMR button. If you never toggled it off then you need to toggle it off at least once and then toggle on.

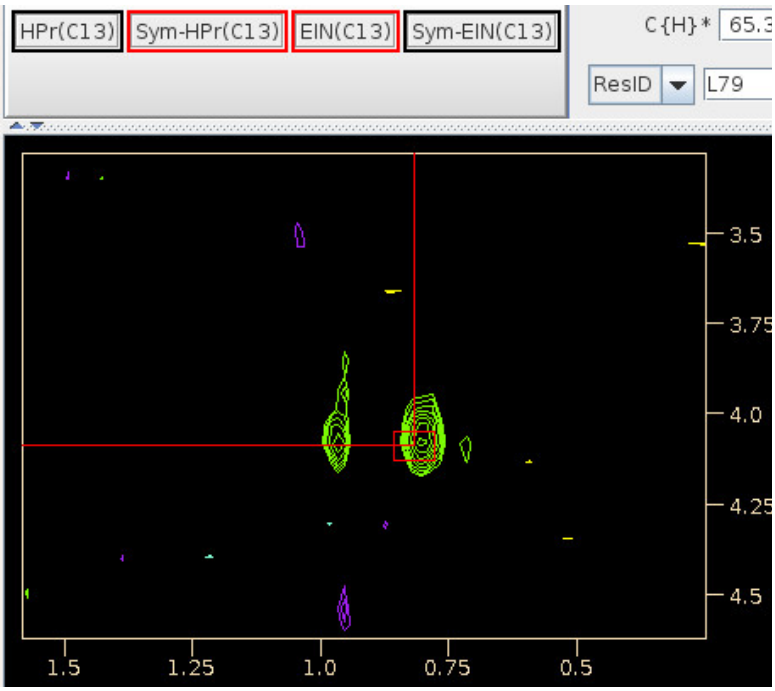
On the Table Window click on the Symmetry peak on slice 99 which is pointed to by red Arrow.

Symmetry from Slice 99 from HPr(C13)



The large red rectangle connects the original peak from EIN(C13) to the symmetry peak on HPr(C13) viewed on Sym-HPr(C13) with the small red rectangle around the symmetry peak. The size of the small red rectangle is allowed error.

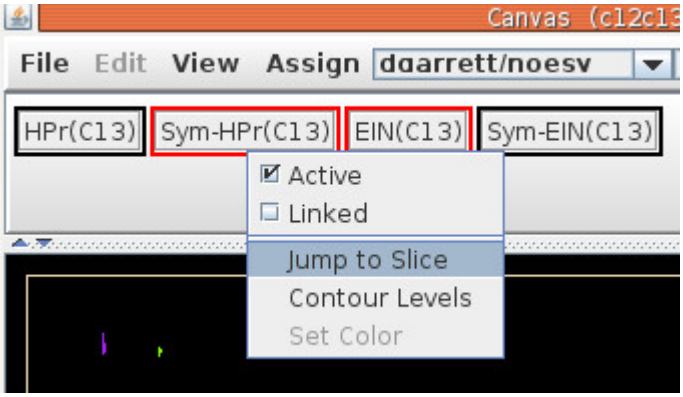
On Table Window click on the Symmetry peak on slice 69.



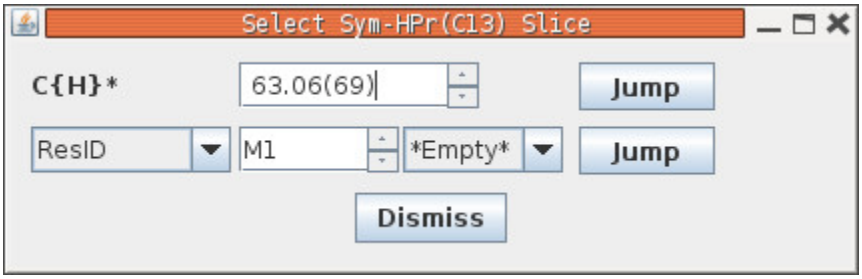
Manually set Symmetry Slice in c12c13Noesy Study

Sometimes the Symmetry list shown on the Name Peak-Pick table does not show any good symmetry peaks, ie all possibilities look like noise.

Right click over the Sym-HPr(C13) and select the menu option 'Jump to Slice' to bring up the non-modal 'Select Sym-HPr(C13) Slice' dialog.

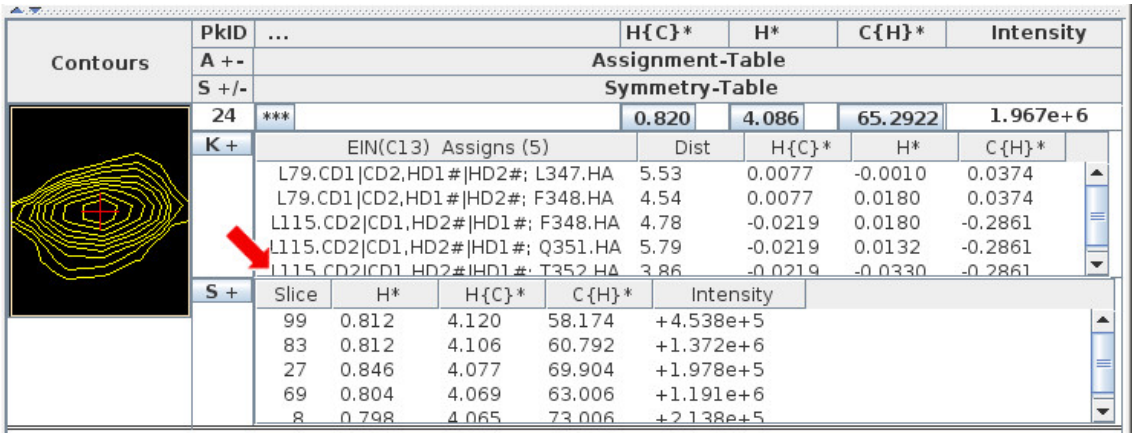


The 'Select Sym-HPr(C13) Slice' dialog is exactly like the one that is fixed at the top of the Canvas window except this only changes the slice of the Sym-HPr(C13) view. This dialog should always be correct unless no slice has been selected. This dialog can remain up while clicking in Table and this dialog will show current slice and PPM value of the Sym-HPr(C13) view.



Select Assignment from Name Peak-Pick Table

On the Table Window click on the assignment for L115.CD2|CD1,HD2#|HD1#; T352.HA:



Immediately after clicking L115.CD2|CD1,HD2#|HD1#; T352.HA the assignment changes to this and the Symmetry list shows only the symmetry that is consistent with T352.CA where previously it had shown symmetry consistent with all of the possible assigns. Use left click on button labeled S+ to toggle on/off 'All Symmetry' which when on will show all possible symmetry in the Symmetry list.