Noesy Assign Study Tutorial

This tutorial describes 4 Studies:

1) **noesyNew.xipp** Analysis of 3D ^{13}C-NOESY, 3D ^{15}N-NOESY, 4D ^{13}C,^{13}C-NOESY and 4D ^{15}N,^{13}C-NOESY on labeled HPr bound to unlabeled EIN. This study does not make use of structures so distance filters are not used.

2) **noesy.xipp** Briefly shows same Experiments as in noesyNew.xipp with a single PDB file to show structures being used to aid in NOE assignments.

3) **noesyInter.xipp** Setup and analysis of a pair of 3D ^{13}C edited ^{12}C filtered NOESY experiments on HPr:EIN complex used to get inter-molecular NOEs. This study has two samples of the Hpr:EIN complex. The first sample has HPr ^{13}C-labeled and the second sample has EIN ^{13}C-labeled.

4) **noesyCoEvolve.xipp** Descibes setup and analysis of 3D ^{13}C,^{15}N-Noesy in which both ^{13}C and ^{15}N co-evolve during the same evolution period on AlphaIIB trans-membrane protein. Data provided by Jun Yang and Jun Qin.

In order to follow along this tutorial using the example data and study files you need to install Exmpl_Linux_NOE3D.sh or Exmpl_Linux_ALL3D.sh for Linux computers. The file Exmpl_Linux_NOE3D.sh has only the 3D NOE data while the file Exmpl_Linux_All3D.sh has all of the processed NMR data for all examples excluding the 4D NOEs. The 4D ^{13}C,^{13}C Noesy and 4D ^{15}N,^{13}C Noesy are only in the installation file Exmpl_Linux_NOE4D.sh due to the large size of the 4D experiments.

By default the examples are installed at ~/Xipp/v1/Examples Start xipp from the Examples directory so that all of the example studies are immediately available.

On the XippPanel open the 'Studies (by File)' to show all of the Studies defined by the *.xipp study files in this directory.

To view properties for noesyNew.xipp:
    Select noesyNew (HPr) and click Edit button.
        If the 4D NOEs were not downloaded a warning dialog will be shown that can be ignored since Xipp will show a study with missing experiment data as long as at least one experiment exists.

To display NMR data for noesyNew.xipp:
  Select noesyNew (HPr) and click the Show button.
        It will take 1 – 2 minutes the first time the Noesy study is run in order to create the extrema files for the 3D ^{13}C Noesy and 3D ^{15}N Noesy NMR experiments. The extrema file contains all of the local extrema found in each experiment and are used here to locate 3D symmetry positions. These files are not included in the download in order to keep the download file size as small as possible.
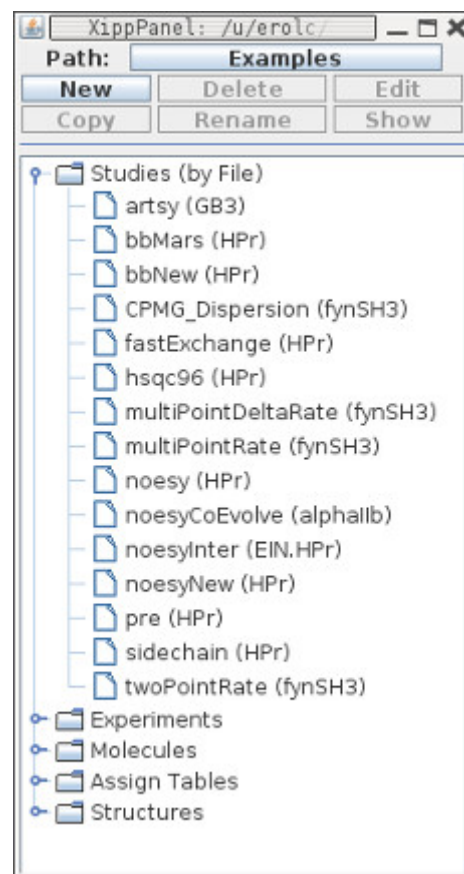
noesyNew.xipp
Edit Study

The noesyNew tutorial will describe features of Xipp useful to analyzing NOESY data prior to initial structure generation. In other words a new structure project and you have no structure or do not want to bias the peak-pick assignments with a preliminary structure. Refer to section on Backbone Assign Study in createSetStudies.pdf for general information about setting properties on Studies using the Linked Group Study Dialog.
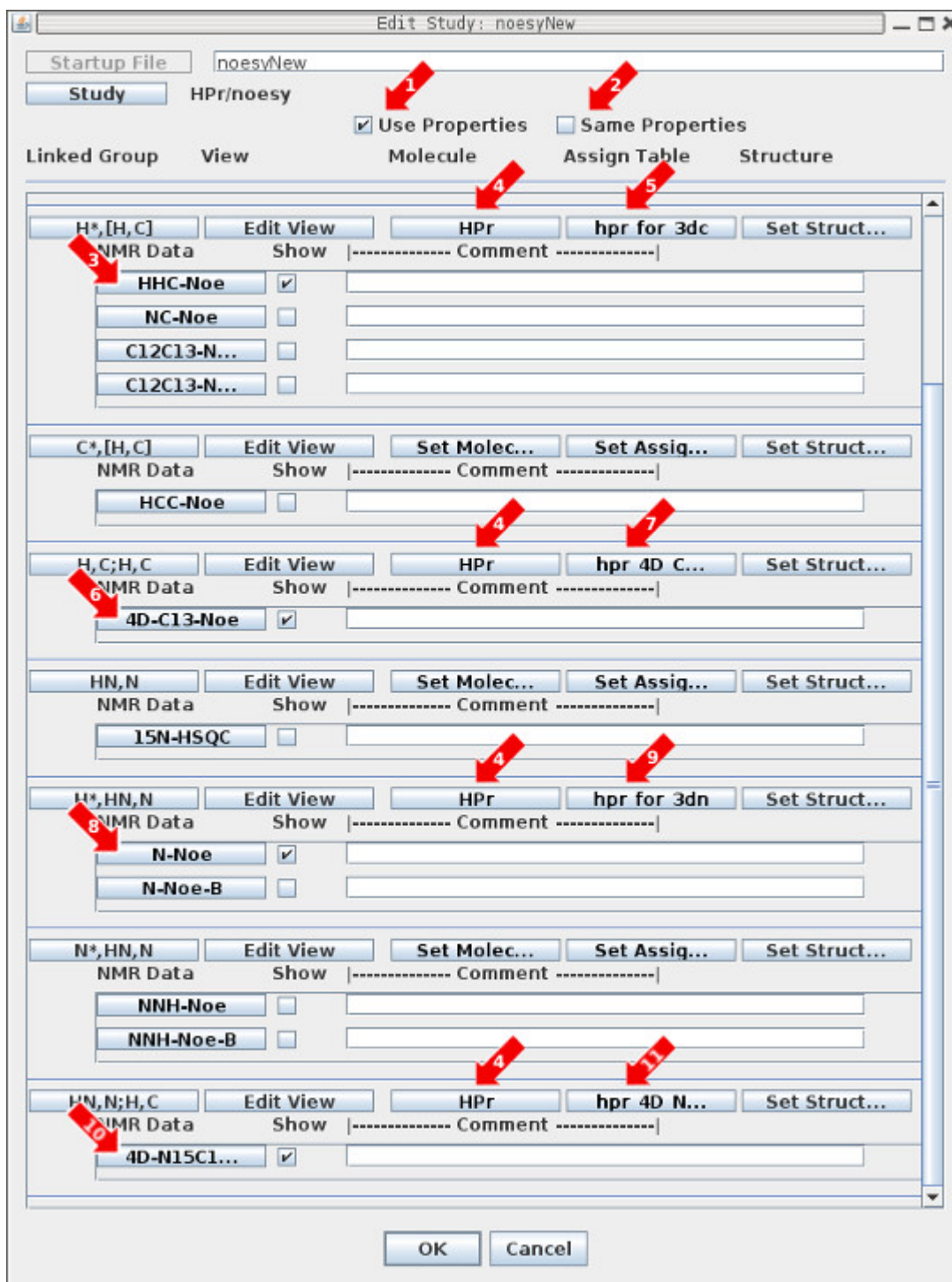
noesyNew.xipp
Edit Study

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1: 'Use Properties' button must be selected in order to make NOE assignments which will use Molecules and Assign Tables. 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.

2: 'Same Properties' has been deselected (ie toggled off) so that each linked group will define its own Molecule and Assign Table. This can be very useful so that the assignment table matches closely the shifts observed in each experiment allowing the error range to be set to match the variation in a single experiment instead of matching the variation between different experiments that may be in different solvents (ie H_2O vs D_2O).

3: Clicking the button labeled HHC-Noe brings up the NMR Data Experiment Dialog for the 3D ^{13}C-Noesy experiment. The Axis Order for the 2 proton Axis must be manually set after selecting the NMR Data File. The 'Use Extrema' should be selected (ie toggled on) so that Extrema can be used to find 3D symmetry peaks.

4: Clicking the button labeled HPr brings up the Molecule Select/Create Dialog that is used to select a previously defined molecule or define a new molecule. Prior to setting the molecule the button is labeled 'Set Molec...'. The name for the molecule must be a unique molecule name in the current study and is used to identify the molecule within Xipp. The names from all molecules in all studies in the current directory are displayed. See Molecule Select/Create Dialog in createSetStudies.pdf for more details. All linked groups have the same molecule name since they have the same sequence.

5: Clicking the button labeled hpr_for 3dc brings up the Assign Table Select/Create Dialog that is used to select a previously defined assignment table or define a new assignment table. Prior to setting the assign table the button is labeled 'Set Assig...'. The assignment table name is not a file name. The name for the assignment table must be a unique assignment table name in the current study and is used to identify the assignment table within Xipp. The names from all assignment tables in all studies in the current directory are displayed. See Assign Table Select/Create Dialog in createSetStudies.pdf for more details. All linked groups are using different assignment tables with different names.

6: Clicking the button labeled 4D-C13-Noe brings up the NMR Data Experiment Dialog for the 4D ^{13}C,^{13}C-Noesy experiment. The Axis Order must be manually set after selecting the NMR Data File. It is best to put H{C2} and C2{H} along the Z and A axis. The carbon and its bonded proton are on Z and A axis so that the 2D plane displayed shows all NOE's from a single proton. The 'Use Extrema' should **not** be selected (ie toggled off) since symmetry is not used in 4D data.

7: Clicking the button labeled hpr_4D_C... brings up the Assign Table Select/Create Dialog for the 4D ^{13}C,^{13}C-Noesy experiment.

8: Clicking the button labeled N-Noe brings up the NMR Data Experiment Dialog for the 3D ^{15}N-Noesy experiment. The Axis Order for the 2 proton Axis must be manually set after selecting the NMR Data File. The 'Use Extrema' should be selected (ie toggled on) so that Extrema can be used to find 3D symmetry peaks for amide to amide NOEs.

9: Clicking the button labeled hpr_for 3dn brings up the Assign Table Select/Create Dialog for the 3D ^{15}N-Noesy experiment.

10: Clicking the button labeled 4D-N15C1... brings up the NMR Data Experiment Dialog for the 4D ^{15}N,^{13}C-Noesy experiment. The Axis Order must be manually set after selecting the NMR Data File. It is best to put HN and N along the Z and A axis. The amide nitrogen and proton are on Z and A axis so that the 2D plane displayed shows all NOE's from a amide proton. The 'Use Extrema' should **not** be selected (ie toggled off) since symmetry is not used in 4D data.

11: Clicking the button labeled hpr_4D_N... brings up the Assign Table Select/Create Dialog for the 4D ^{15}N,^{13}C-Noesy experiment.

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

Note the text on a button or label will be red when the properties that are accessed by that button are not correctly set or inconsistent with other properties. Clicking OK with incorrectly set properties will bring up a Warning Dialog asking if you want to Fix the problem(s) (i.e. re-show the dialog) or Ignore the problem(s) and save the properties with the problems. Incorrect properties may not show correctly and another pop-up window will warn when a Study with incorrect settings is attempted to be shown. Some problems such as an incompletely defined Experiment with the Show check box clicked on will actually Show OK, but the warning message is still displayed.

If any changes to properties where made you should click OK to save properties to noesyNew.xipp file. Otherwise click Cancel to exit Study Dialog without saving properties.

noesyNew.xipp
Edit Experiment HHC-NOE
Edit 3D ^{13}C-NOESY

1: 'Max Distance' is the maximum distance when a PDB structure is used with CoordinateDistanceFilter. When no PDB structure is read in this is ignored. Note X-ray structures typically do not have protons which are required in the CoordinateDistanceFilter.

2: 'Base Peak Sign' must be set to match sign of unfolded peaks. Can be set to Unknown if unsure. If set to Unknown then the expected sign of the peak or symmetry peak is not used as an assignment filter.

3: 'Axis Order'. Both proton axis must be explicitly set. H{C}* defines proton axis bonded to C{H}*.

4: 'Use Extrema' should be enabled in order to use 3D symmetry as a filter to assign peaks. The extrema file contains all of the local extrema (maxima and minima) in the entire 3D data above the given 'Level' which defaults to the contour level whenever 'Use Extrema' is toggled on.

noesyNew.xipp
Show Study

Refer to the document XippCommands.pdf for general information about keyboard and mouse commands for Xipp.

Ensure that noesyNew (HPr) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the noesyNewStudy. It will take 1 – 2 minutes the first time the Noesy study is run in order to create the extrema files for the 3D ^{13}C Noesy and 3D ^{15}N Noesy NMR experiments.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

(1) Click right mouse button on button labeled HHC-NOE to bring up the pop-up menu shown and toggle off 'Symmetry Filter' to Turn off 'Symmetry Filter'. The pop-up will disappear after selecting 'Symmetry Filter'

(2) Click left mouse button on button labeled Sym-HHC-Noe to turn off view of symmetry plane. Note Red border indicates that the Experiment or view into an Experiment is active and is the first toggle button on the pop-up menu.

(3) Enter Keyboard command: Alt-J 65 to residue jump A65. You can also type 65 into Jump window and hit enter.

(4) Keyboard command: n
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data

The Table Window should look something like this after clicking n in the canvas window.

The top part is tabbed and shows the 4 ShiftTables. Each NOE experiment has its own Assignment table.

The bottom part always shows information about peaks that have been picked. This is equivalent to the information shown in PIPP's Name mode.
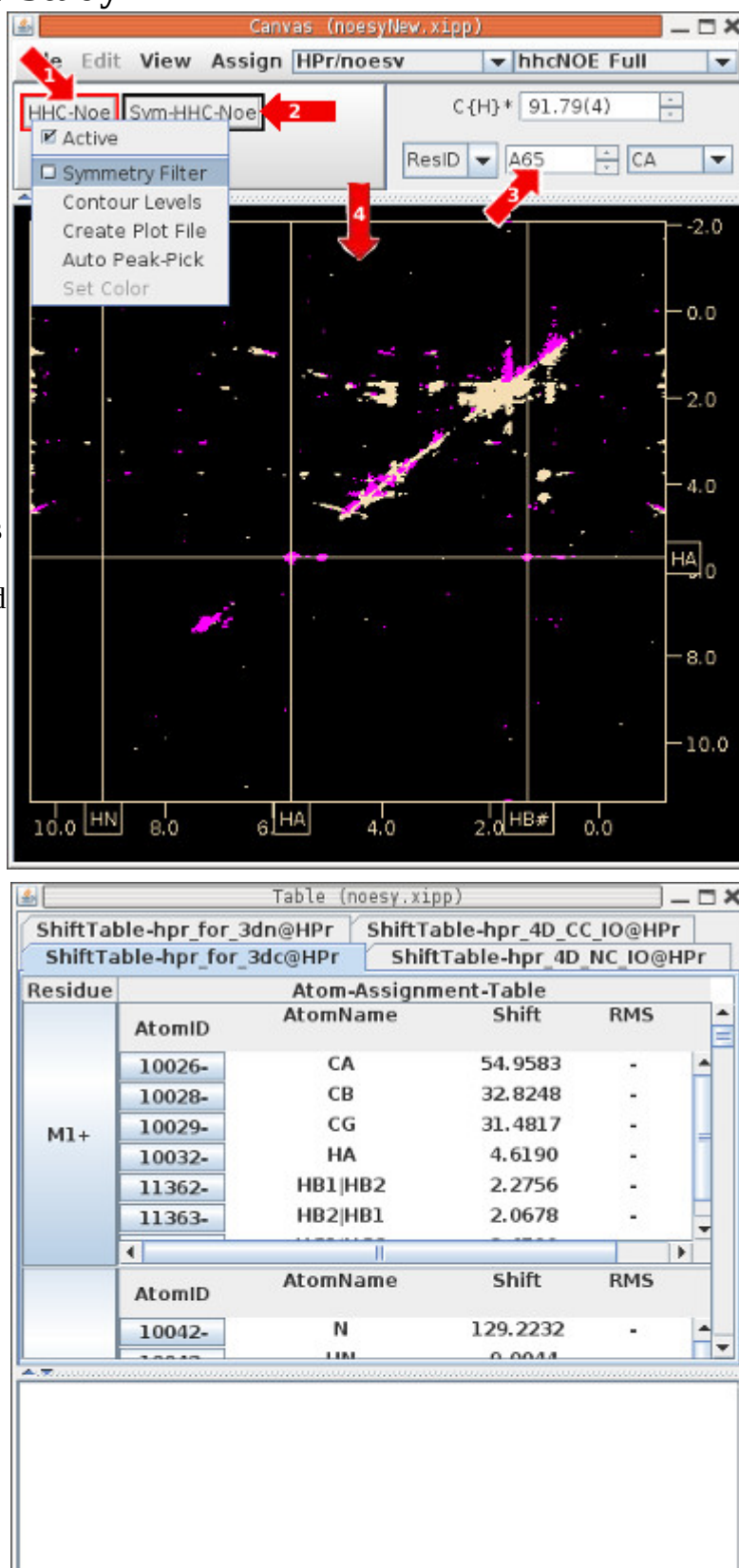
noesyNew (3D ^{13}C Noesy)
Displaying Symmetry

With the mouse pointed just to the right of the diagonal for I65.HA peak double click the middle mouse button to set display region around the peak at 5.3 and 5.7 PPM.

With the mouse pointed at the peak at 5.1 PPM click the left mouse button to create a peak-pick, ie peak-pick it. The Peak-Pick is labeled with the peak-ID since it is currently unassigned.
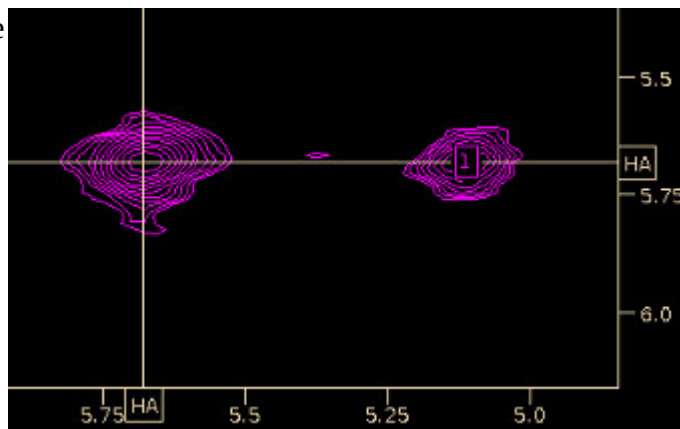
The Table Window for peak-ID 1 shows 2 possible assignments and 6 possible 3D symmetry. Remember the SymmetryFilter was disabled on previous page or A65.CA,HA; S46.HA would be filtered out.

1: Peak-Pick ID number.
2: Current Assignment. *** indicates not assigned.
3: PPM value of peak-pick. Order is arbitrary. Not in axis order.
4: Intensity of peak. This is the height of the tallest data point for peak, ie not volume.
5: Table of Possible Assignments. Selecting a row will assign the peak-pick and update Current Assignment.
6: Table of 3D symmetry peaks (aka Symmetry Table) that are possible 3D symmetry peaks to peak at 5.115, 5.676, 91.78 PPM. Selecting a row will update 2D plane shown in view Sym-HHC-Noe. Usually the most intense extrema is the correct symmetry peak.

The assignment table indicates that the peak at 5.115, 5.676, 91.78 PPM may be an NOE from A65.CA,HA to either I33.HA or S46.HA. The 3D symmetry peak should arise from I33.CA,HA to A65.HA or from S46.CA,HA to A65.HA. The assigned shift for I33.CA is 60.698 which matches (after offsetting for folding (^{13}C SW 20.709PPM)) with the best 3D symmetry peak on slice 68 at 60.656 (81.365-20.709). The next page shows overlaying the symmetry peak with the peak to filter out noise.

To view an overlay of symmetry peak from slice 68 and original peak on slice 4:

      1: On Table Window select desired symmetry peak from Symmetry Table such as slice 68.

      2: On Canvas Window click the left mouse button on button labeled Sym-HHC-Noe to toggle on the symmetry view which will display slice 68 and show the 3D Symmetry boxes in red.


3: The origin of the large red symmetry box is at 5.115, 5.676 PPM which is obtained from peak-ID 1.

4: The expected symmetry location is at the opposite corner at 5.676, 5.115 PPM. The smaller red symmetry box around the expected symmetry location indicates the allowed error in finding the symmetry peak.

By selecting other rows from the Symmetry Table you can easily see that slice 68 has the best symmetry peak.

The green contours are from slice 68 and are only displayed when 2: Sym-HHC-Noe is enabled (has a red border around button). The Sym-HHC-Noe button functions as a toggle to display a separate slice in the 3D ^{13}C Noesy. Right click over Sym-HHC-Noe and select 'Jump to Slice' in the pop-up menu. This will display a non-modal Jump window titled "Select Sym-HHC-Noe Slice" to arbitrarily set the slice for the Sym-HHC-Noe as shown below.
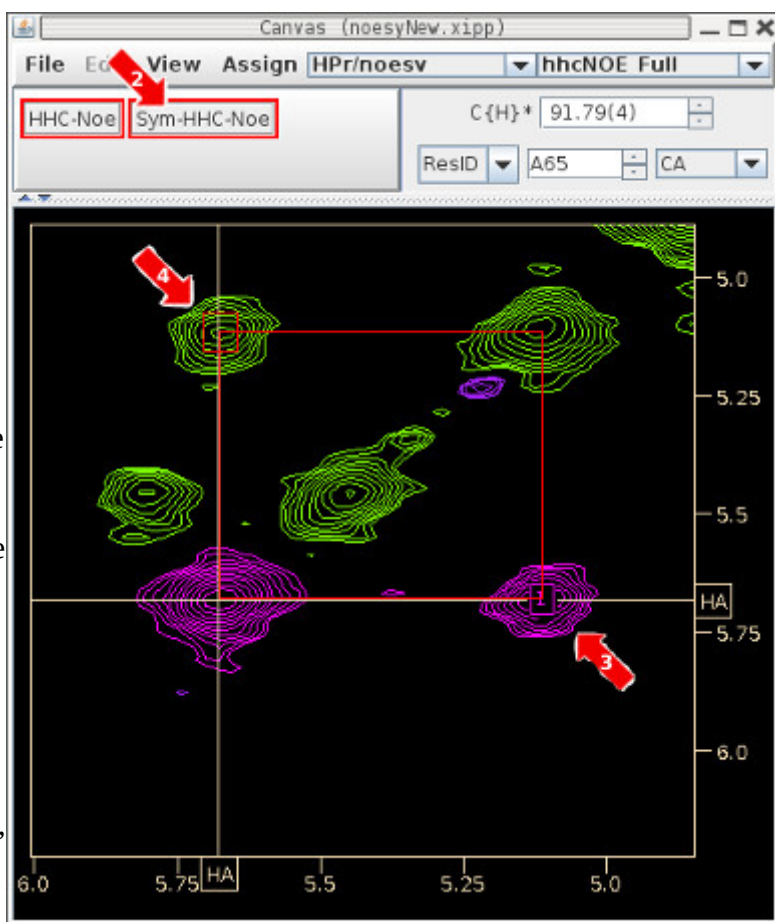
noesyNew (3D ^{13}C Noesy)
Filter Assigns with Symmetry

Displaying 3D Symmetry is very useful to confirm that the selected 3D Symmetry is good, but filtering the possible assignments by 3D symmetry helps to quickly determine which assignments are more likely to be correct. Symmetry Filter will fail when the symmetry peak is a shoulder to another peak and therefore not in the list of extrema. In this situation manually searching for the symmetry by displaying the Jump window for Sym-HHC-Noe as shown on previous page) will help to find the 3D Symmetry.

To Filter by Symmetry:
> 1: In Canvas Window Right click button labeled HHC-Noe and select 'Symmetry Filter" to turn on 3D Symmetry Filter.
>
> 2: In Canvas Window peak-pick again peak-pick ID 1 (click left mouse button) over peak currently labeled 1. If nothing happens delete the peak-pick (click right mouse button with mouse over peak) and re-peak-pick it.
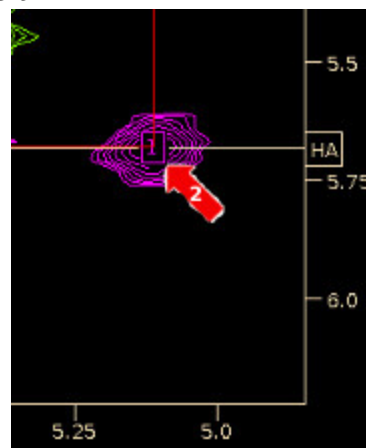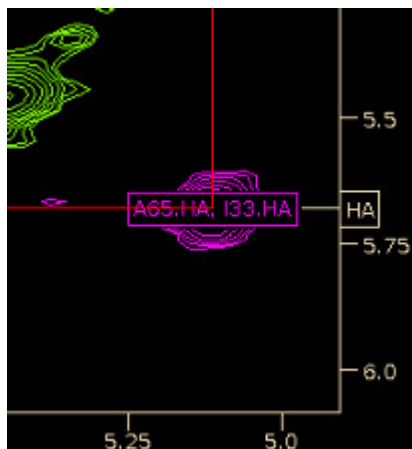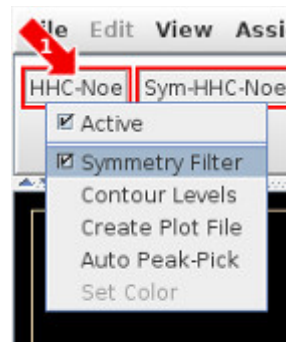
With Symmetry Filter enabled only one possible assignment is found and Xipp automatically assigns the peak to that assignment and updates the Canvas and Table Window. The 3D symmetry shown is the Symmetry for the selected assignment, ie not all possible symmetry positions that was shown previously.

Contours	PkID	Assign	H*|H...	C1{H}	HN|H...	N|C{H...	Intensity
	A +-	Assignment-Table					
	S +/-	Symmetry-Table					
	1	A65.CA,HA; I33.HA	5.115	-	5.676	91.780	-1.248e+6
	K +	HHC-Noe Assigns (1)		H*	-	H{C}*	C{H}*
		A65.CA,HA; I33.HA		0.0005	-	0.0027	0.0161
	S +	Slice	H{C}*	H*	C{H}*	Intensity	
		68	5.117	5.673	81.365	+1.191e+6	

For 3D ^{13}C-Noesy it is usually best to keep the Symmetry Filter enabled and only turn it off when there is a problem such as missing an expected assignment or no assignments are found. The Symmetry Filter can then be disabled and the peak-pick should be deleted and re-added.

noesyNew (3D ^{13}C Noesy)
# Confirm Long Range NOE

In most protein structures there will be multiple long range NOEs arising from different atoms between the same regions. Prior to initial structure determination all long range NOEs should be confirmed by finding other atoms that link the two regions.

To confirm NOE A65.HA – I33.HA:

(1) Set Atom Chooser to CB to view A65.CA,HA NOEs
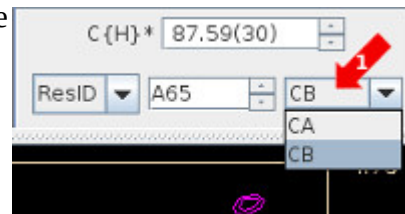
(2) With the mouse pointed just to the right of the cross peak for I65.HA double click the middle mouse button to set display region around the peak at 5.3 and 5.7 PPM.
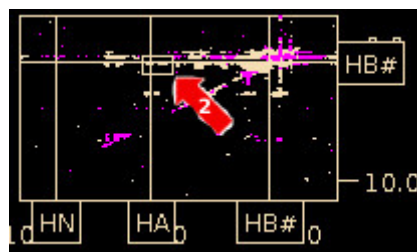
(3) With the mouse pointed at the peak at 5.1 PPM click the left mouse button to create a peak-pick, ie peak-pick it. Since the Symmetry Filter is enabled only one possible assignment is found so the peak is automatically assigned.

(4) In the Symmetry Table select the extrema at slice 68 and ensure that view Sym-HHC-Noe (in Canvas Window) is enabled to display an overlay of slice 68 with the current slice 30.

(5) In the Table Window Peak-Pick ID 2 has a single possible assignment that is automatically selected and assigned to the peak-pick. When there are multiple possible assignments none of the assignments are automatically selected or assigned to the peak.
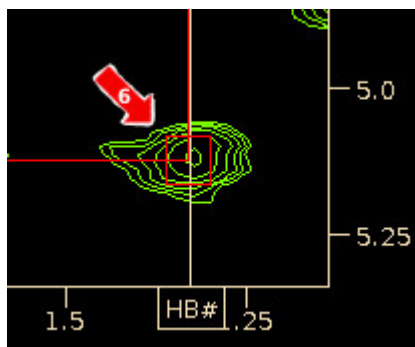
(6) The Symmetry peak on Slice 68 is OK.

noesyNew (3D ^{15}N Noesy)
3D Symmetry
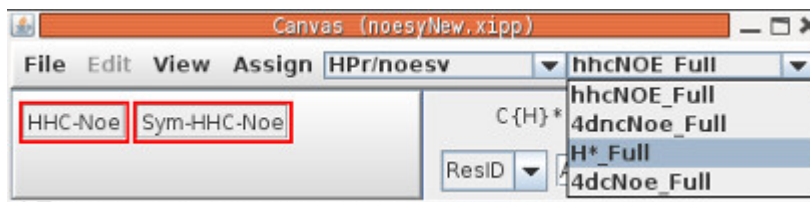
In the Canvas Window click the menu option labeled hhCNOE_Full and select the H*_Full in drop-down menu which is the 3D ^{15}N Noesy experiment.

Note that the slice displayed and Shift Cursors correspond A65 since Residue Jumps are synchronized across all Experiments in the same Study.

Since there are no good HN to HN NOEs from A65.HN to show symmetry we will look at S64.

The Canvas window should look like this after the following changes:
    (1) Click right mouse button on button labeled N-Noe to bring up the pop-up menu shown and toggle off 'Symmetry Filter' to disable 'Symmetry Filter'. The pop-up will disappear after releasing mouse button.

    (2) Click left mouse button on button labeled Sym-N-Noe to turn off view of symmetry plane.

    (3) With mouse over NMR data in Canvas window enter Keyboard command: Alt-Down-Arrow to scroll to previous residue which is S64. You can also type 64 into Jump window and hit enter.

    (4) Zoom into region near S64.HN diagonal peak centered at (9.2,9.2) PPM with zoom size 1 click larger than default so that symmetry peak will show when selected/displayed.

    (5) Click right mouse button on peak at (9.0,9.6) PPM

The Peak-Pick ID is 1 because each experiment has its own separate peak-pick list/file. The peak-pick IDs are not unique between experiments.
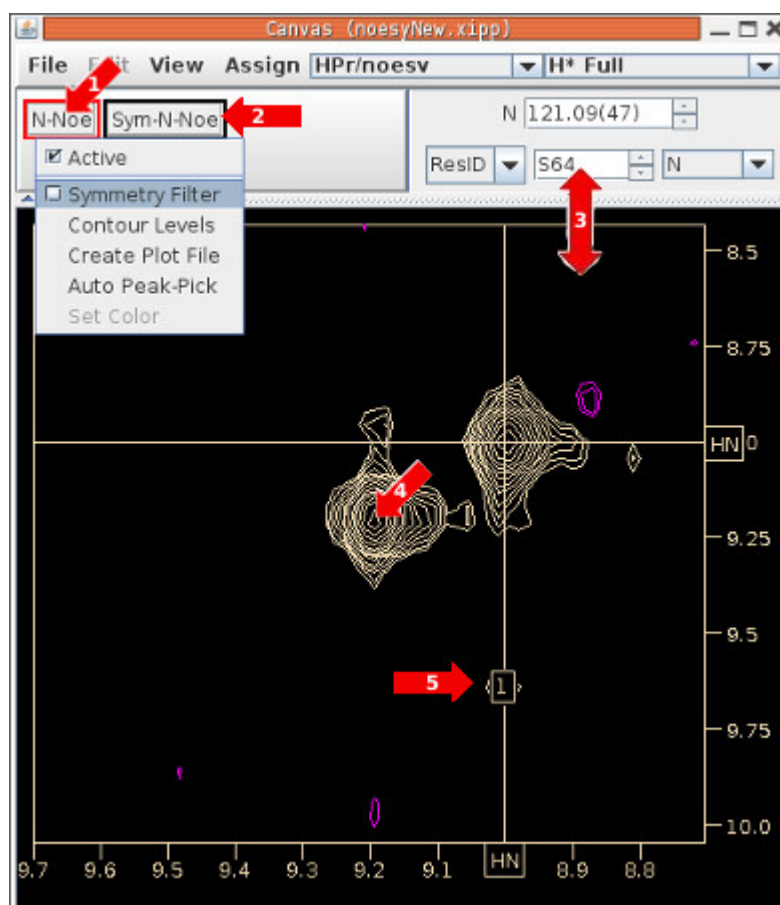
noesyNew (3D ^{15}N Noesy)
3D Symmetry

	PkID	Assign	H*|H...	C1{H}	HN|H...	N|C{H}...	Intensity
Contours	A +-	Assignment-Table					
	S +/-	Symmetry-Table					
	1	S64.N,HN; T34.HN	9.635		-	9.005	121.1...	4.091e+5
	K +	N-Noe Assigns (2)		H*	-	HN	N	
1		S64.N,HN; T34.HN	0.0048	-	-0.0039	-0.0541	
		S64.N,HN; N38.HN	0.0073	-	-0.0039	-0.0541	
	S +	Slice	H*	HN	N	Intensity	
2		31	9.007	9.637	124.689	+5.419e+5	

(1) When there are two or more possible assignments nothing is automatically selected/asigned. Since T34.N is 124.67 PPM and N38.N is 126.93 PPM the peak should be assigned to S64.N,HN; T34.HN by selecting it in the list of possible assignments. You will may have to toggle the overlay display in the Canvas Window with the space-bar to update the peak label.

(2) The possible symmetry peak on slice 31 is not automatically selected. Select the symmetry peak on slice 31 to initialize the Sym-N-Noe view.

(3) Clicking the left mouse button on button labeled Sym-N-Noe to turn on view of symmetry plane the Canvas Window to display the symmetry peak and red symmetry boxes.

When the Sym-N-Noe is view is enabled the destination for the symmetry may be outside the display if the ZoomBox was not enlarged or its position was off. To fix this simply enter the keyboard command f for full display followed by a single click of middle mouse button to initiate a new ZoomBox. Then use the left and right mouse button to adjust to the desired size of the ZoomBox and click the middle mouse button to set the new display range.

For the 3D ^{15}N-NOESY experiment it is best to keep the Symmetry Filter off and only turn it on when clicking on Amide to Amide NOEs. When Xipp starts with Extrema the Symmetry Filter is enabled by default. Clicking on aliphatic NOEs with the Symmetry Filter enabled will always fail since there can be no symmetry. I hope to fix this in a later release.
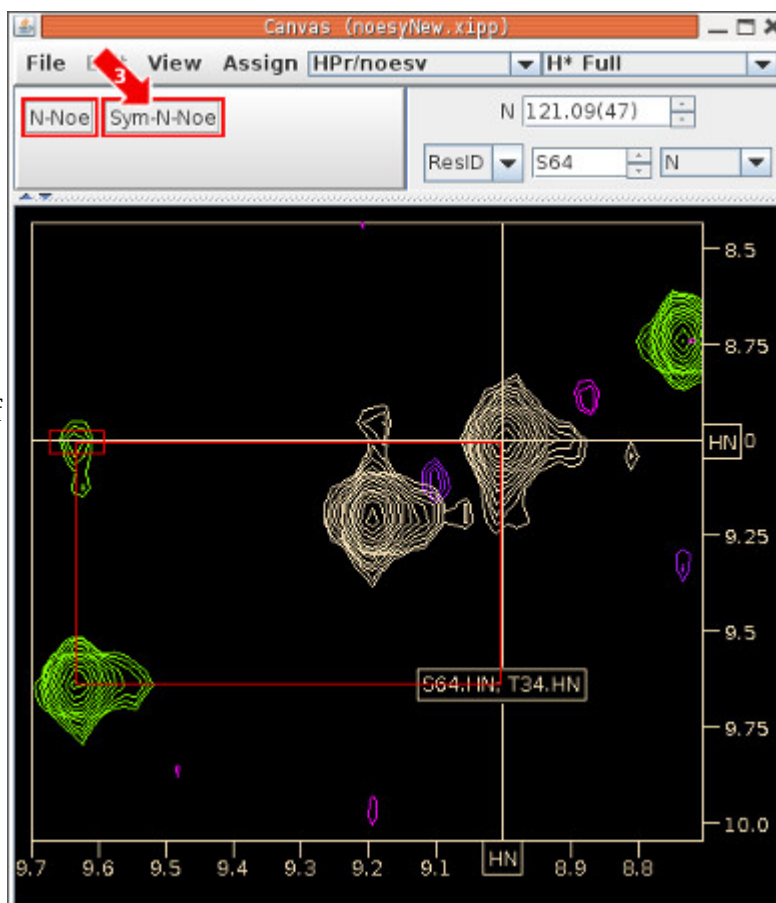
noesyNew (3D [15]N Noesy)
Aliphatic (No Symmetry)

(1) Ensure Symmetry Filter is off.

(2) Zoom to region around 1 PPM centered on S64 HN Shift Cursor.

(3) Create peak-pick at 0.7 PPM that is on the S64 HN Shift Cursor.
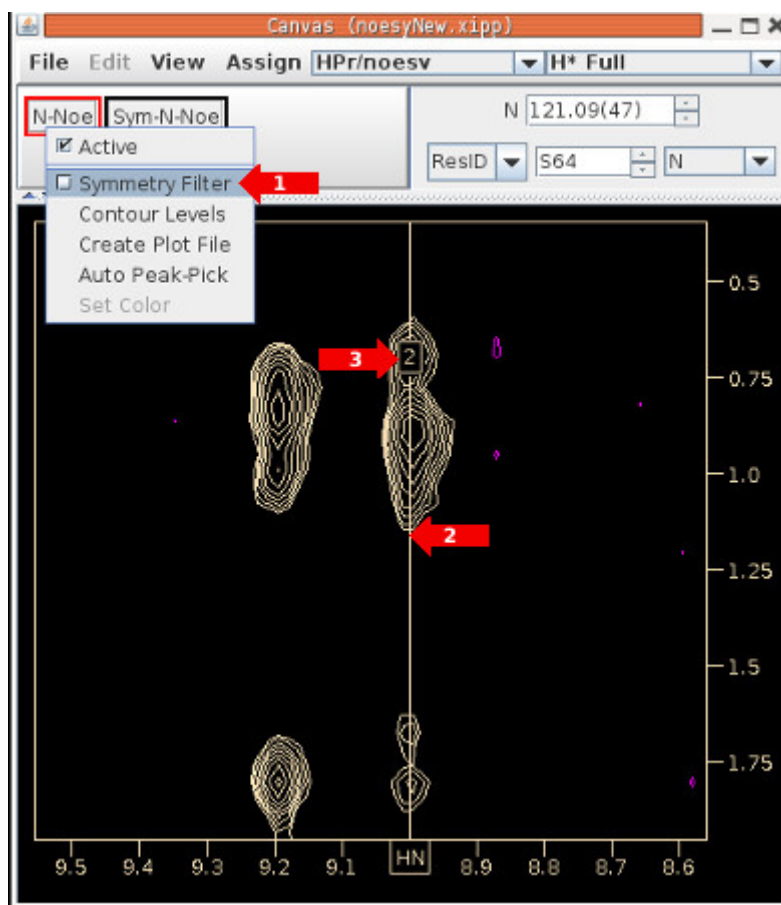
Symmetry can not be used for the aliphatic region since the symmetry peak for the aliphatic proton requires observation of the ^{13}C shift (not available in ^{15}N NOESY experiment) for the carbon bonded to the aliphatic proton. The NC-NOESY experiment described later can determine symmetry for aliphatic protons since it co-evolves ^{15}N and ^{13}C for the third dimension.

There is no way from the 3D ^{15}N-NOESY experiment to determine which NOE is correct. The two most likely assignments are "S64.N,HN; I33.HG2#" and "S64.N,HN; V35.HG2#|HG1#" since we have already made unambiguous assignments between A65 and I33 and also between S64 and T34. At this stage in the NOE assignment it is best to leave the peak unassigned and return to later it after good quality initial structures have been generated by unambiguous assignments.

	PkID	...	H*|H...	C1{H}	HN|H...	N|C{H}...	Intensity
Contours	A +-			Assignment-Table			
	S +/-			Symmetry-Table			
	2	***	0.693	-	9.002	121....	8.213e+5
	K +	N-Noe Assigns (4)	H*	.	HN	N	
		S64.N,HN; I8.HD1#	-0.0151	-	-0.0000	0.0116	
		S64.N,HN; I33.HG2#	-0.0187	-	-0.0000	0.0116	
		S64.N,HN; V35.HG2#|HG1#	-0.0175	-	-0.0000	0.0116	
		S64.N,HN; L55.HD1#|HD2#	0.0247	-	-0.0000	0.0116	
	S +	No Symmetry Found for Peak 2 using All Symmetry					

noesyNew (4D ¹⁵N, ¹³C Noesy)
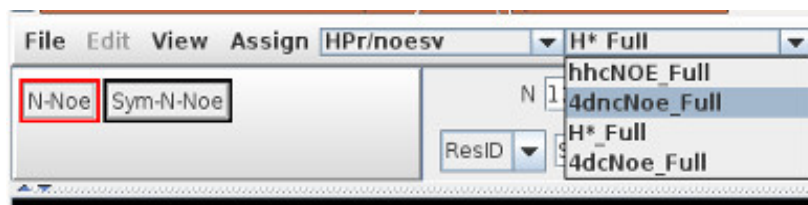
In the Canvas Window click the menu option labeled H*_Full and select the 4dncNoe_Full in drop-down menu which is the 4D ¹⁵N,¹³C Noesy experiment.   The slice displayed and Shift Cursors correspond to S64 and there are two sets of slice choosers to accommodate the added dimension.

(1) Right click the mouse over the peak at the intersection of the S64.CB and S64.HB1|HB2 Shift Cursors at (3.68,68.1) PPM
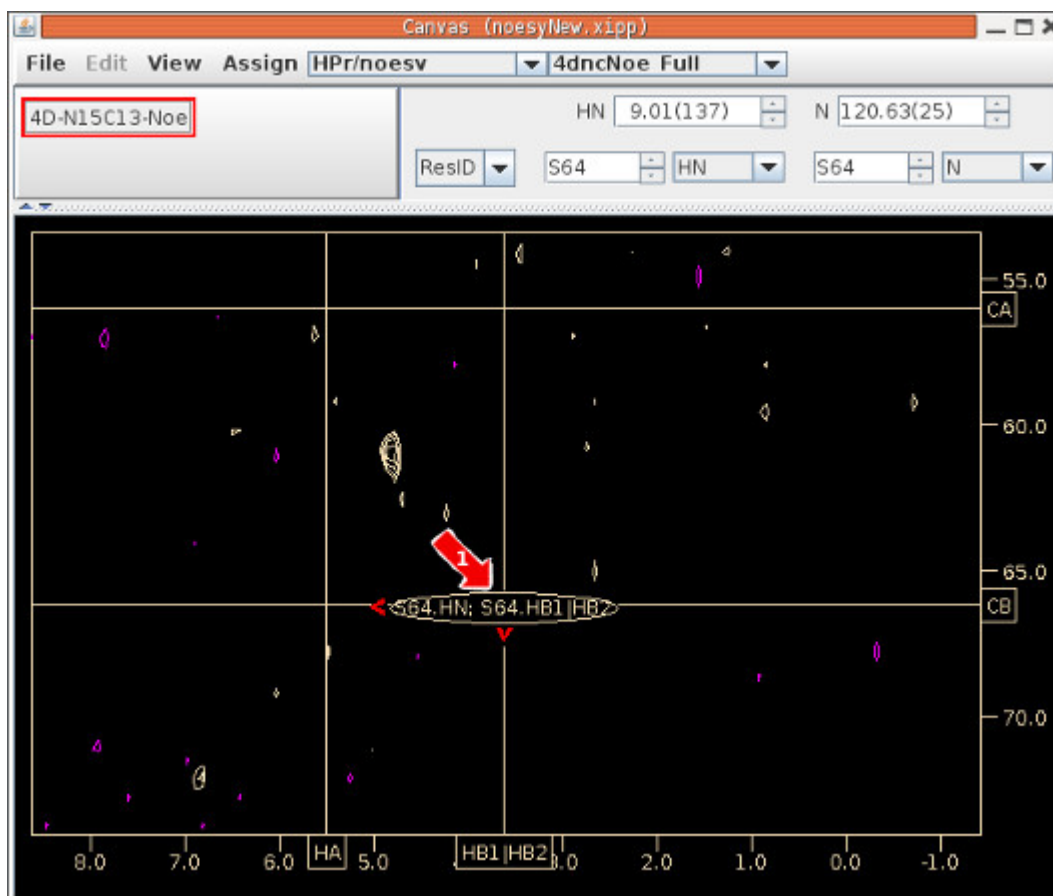
The red annotation around the peak indicates that the top of the peak is on another slice. In this case the top of the peak is one slice down along HN and one slice previous along N. The Down-Arrow key on keyboard will decrease the HN PPM value (ie increase HN slice number) and the Left-Arrow on keyboard will increase the N PPM value (ie decrease N slice number). To scroll to the top of the peak you enter the keyboard arrow that points in the same direction as the red annotation around the peak.

noesyNew (4D ^{15}N, ^{13}C Noesy)

(1) Right click the mouse over the peak at
(4.81,60.9) PPM to create a peak-Pick
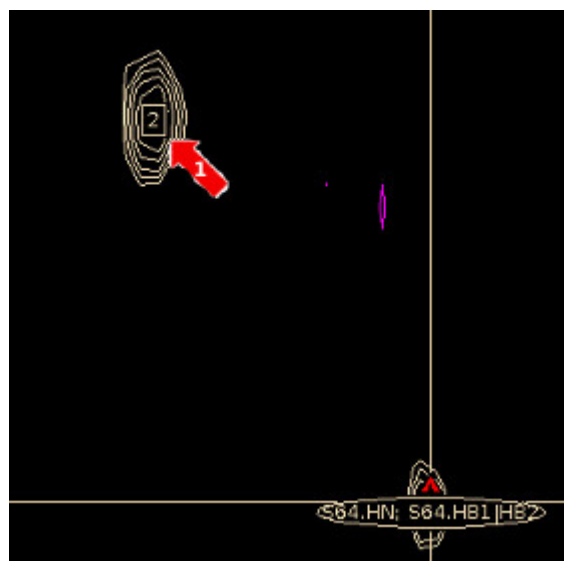
(2) The table of possible assignments has 2 assignments. The most likely NOE assignment is the
sequential assignment S64.N,HN; V35.CA,HA. However care must be taken since thatis not always
true.

Contours	PkID	...		H*|H...	C1{H}	HN|H...	N|C{H}...	Intensity
	A +-		Assignment-Table					
	2	***		4.809	60.875	8.992	120....	6.216e+5
	K +		4D-N15C13-Noe Assigns (2)	H{C1}	C1{H}	HN	N	
			S64.N,HN; V35.CA,HA	-0.0215	-0.0843	0.0271	-0.1454	
	2		S64.N,HN; I63.CA,HA	0.0107	0.0608	0.0271	-0.1454	

noesyNew (4D ^{13}C, ^{13}C Noesy)

In the Canvas Window click the menu option labeled 4dncNoe_Full and select the 4dcNoe_Full in drop-down menu which is the 4D ^{13}C,^{13}C Noesy experiment.   The slice displayed and Shift Cursors correspond to S64 and there are two sets of slice choosers to accommodate the added dimension.
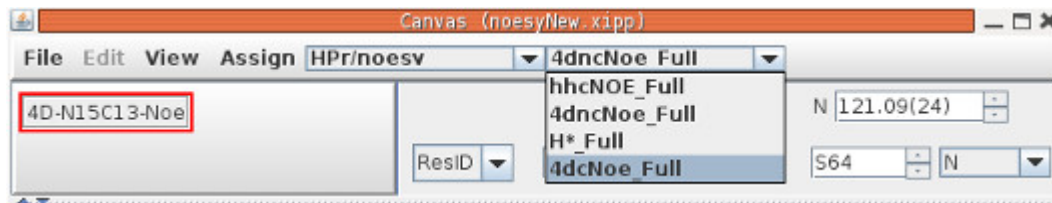
(1) Right click the mouse over the peak at (5.75,53.9) PPM which get automatically assigned since there is a single assignment.
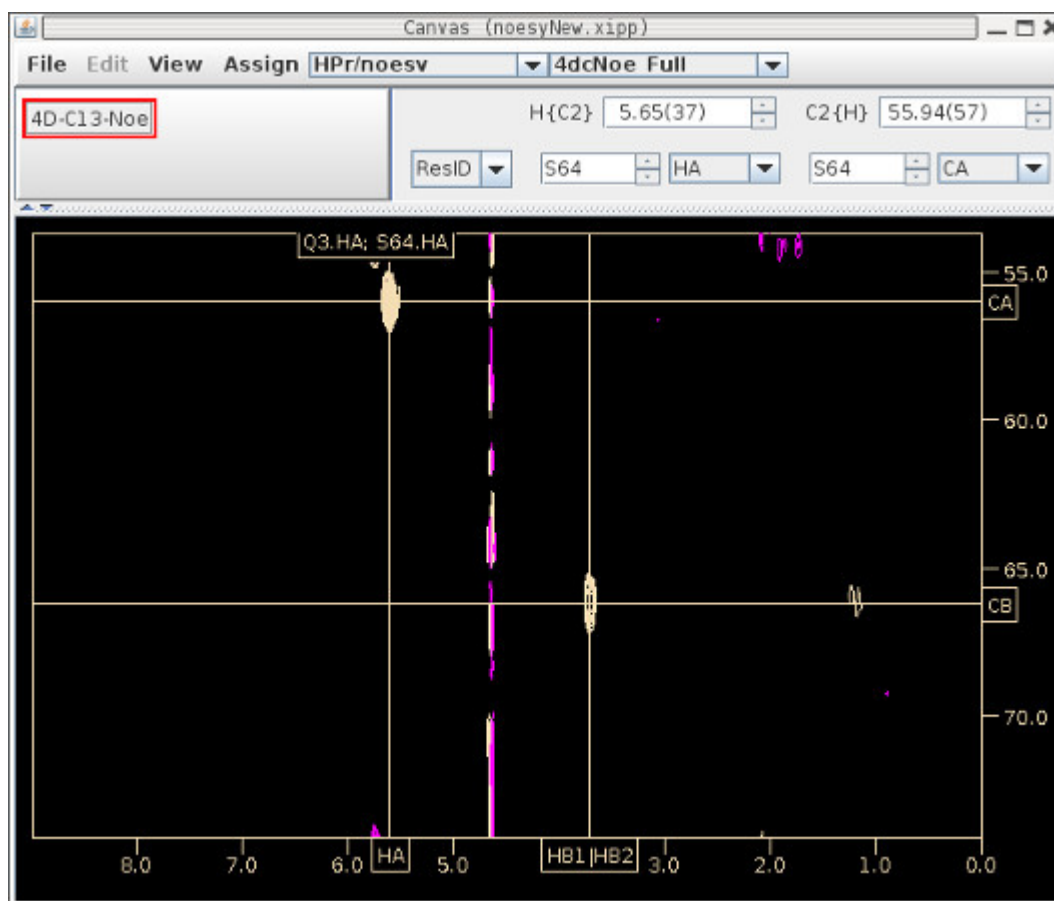
noesyNew (4D ^{13}C, ^{13}C Noesy)

The atom choosers in the two slice chooser are not properly linked. On every residue jump the ^{1}H atom chooser gets set to the first atom in the list. But the ^{13}C atom chooser will keep the same atom as long as the new residue has the same atom as previously selected. Changing either the 1H or the 13C atom chooser will leave the other atom chooser unchanged.

(1) Select CB of 13C atom chooser and the 1H atom chooser stays at HA and there are no good peaks on this slice. **You have to manually change both atom choosers.**
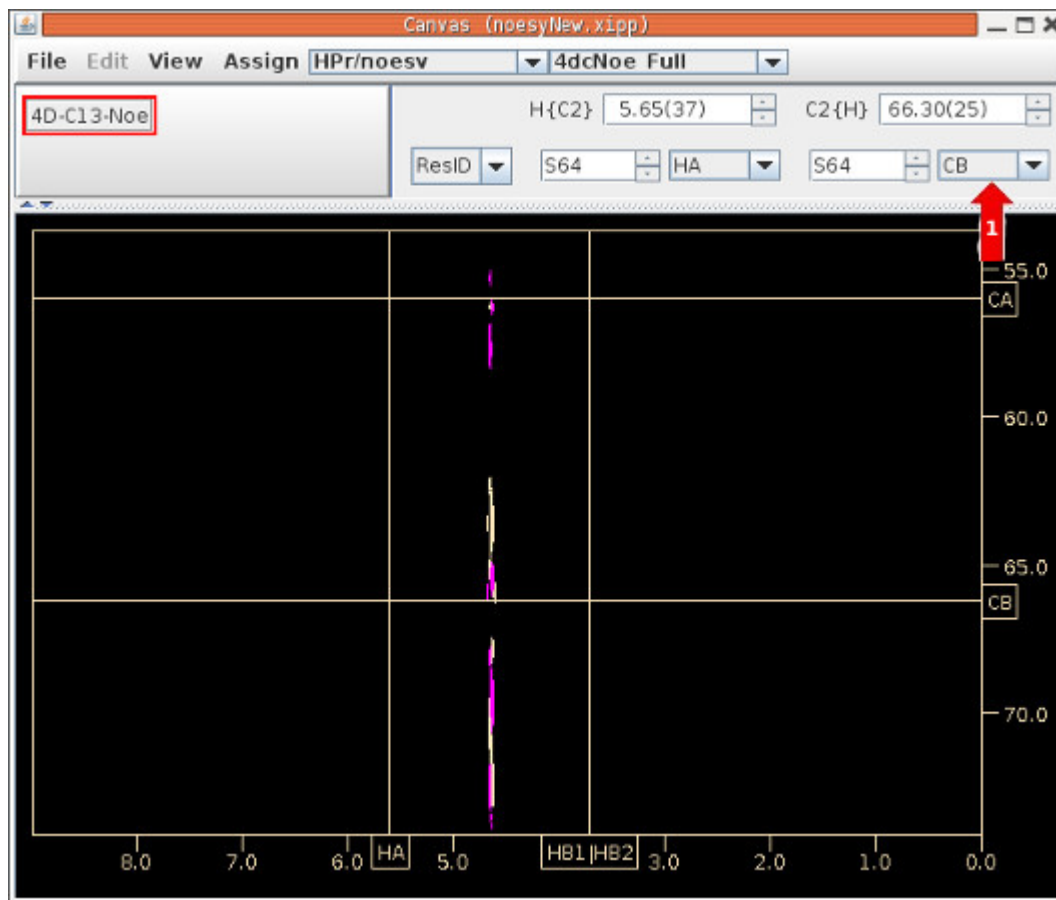
noesy.xipp
Edit Study

The noesy tutorial describes properties specific for using a PDB structure to assist in assigning NOEs. Refer to the noesyNew tutorial for a description of other properties, ie editing experiment properties.

1: Click button 27Feb98 to define location of the PDB structure. Since 'Same Properties' is toggled off every linked group must be set.

noesy.xipp
Edit Structure

After clicking one of the structure buttons labeled 27Feb98 the Select/Create Structure dialog whose top is shown at right. The highlighted entry '27Feb98 (noesy)' is the currently selected structure being used. If nothing Is shown under Structures then you need to click New to define a new structure set. If New is grayed out you will need to first select Structures then click New.
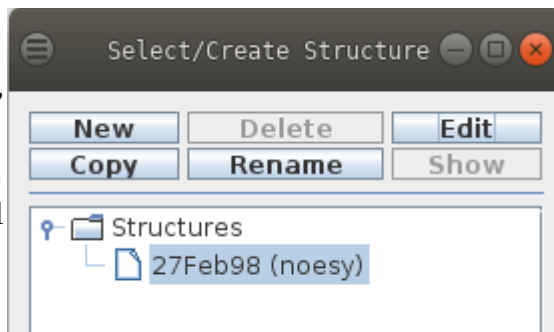
With '27Feb98 (noesy)' selected click the Edit button to display the dialog below titled Edit Structures: 27Feb98.
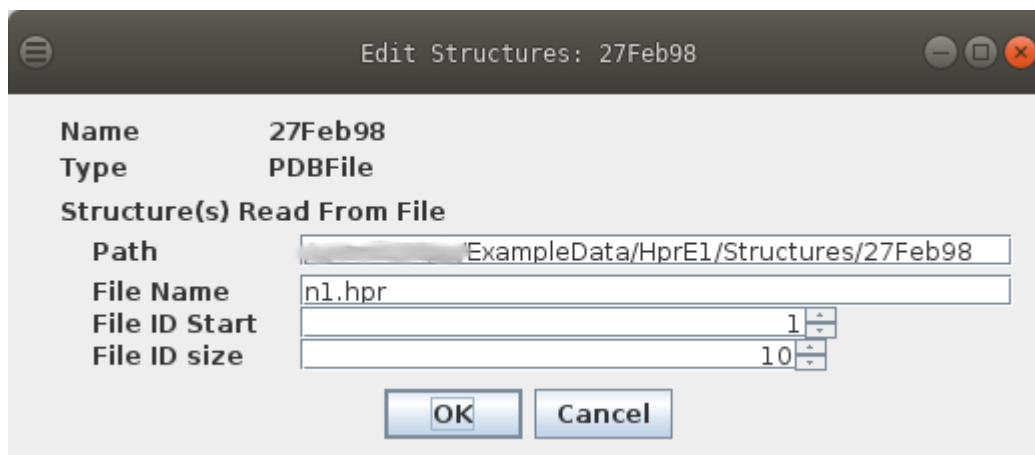
The File ID Start and File ID size are used when the File Name contains C-style formatting such as %d or %03d. For example if File Name is final_%d.sa then Xipp would read in 10 files whose names are: final_1.sa, final_2.sa, final_3.sa, final_4.sa, final_5.sa, final_6.sa, final_7.sa, final_8.sa, final_9.sa 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 at this time. Click OK to save Structure 27Feb98 and then OK at bottom (not shown) of Select/Create Structure dialog.

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 for large proteins. Also the PDB files must have H atomic coordinates. If the PDB file was obtained from a crystal structure you will need to explicitly add protons prior to using the file with Xipp. Xipp does NOT automatically 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 and symmetry might be resolved based on distance.

All other properties are set similarly to study noesyNew. If any changes to properties where made you should click OK to save properties to noesy.xipp file. Otherwise click Cancel to exit Study Dialog without saving properties.

noesy.xipp
Show Study

Ensure that noesy (HPr) Study is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the noesy Study. It will take 1 – 2 minutes the first time the Noesy study is run in order to create the extrema files for the 3D ^{13}C Noesy and 3D ^{15}N Noesy NMR experiments.
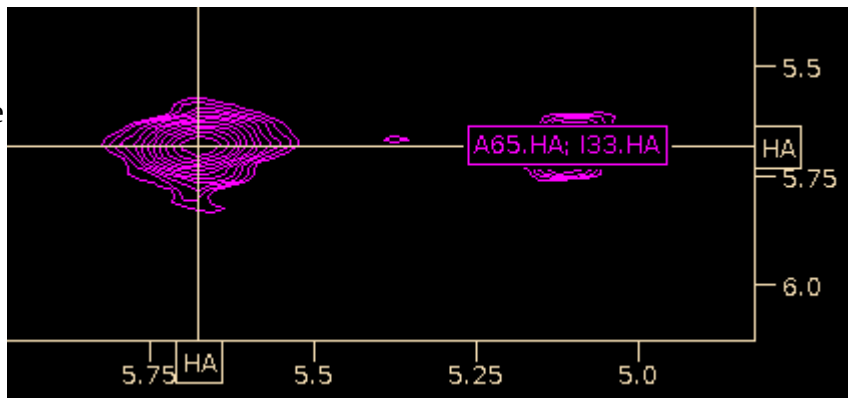
After the Canvas and Table windows are displayed enter the following commands and mouse clicks on the Canvas just like in th enoesyNew study:

(1) Click right mouse button on button labeled HHC-NOE to bring up the pop-up menu shown and toggle off 'Symmetry Filter' to Turn off 'Symmetry Filter'. The pop-up will disappear after selecting 'Symmetry Filter'

(2) Click left mouse button on button labeled Sym-HHC-Noe to turn off view of symmetry plane. Note Red border indicates that the Experiment or view into an Experiment is active and is the first toggle button on the
pop-up menu.

(3) Enter Keyboard command: Alt-J 65
to residue jump A65. You can also type 65 into Jump window and hit enter.

(4) Keyboard command: n
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data

The Canvas and Table Windows should look similar to their appearance in the noesyNew tutorial just after these same commands where issued.

noesy study wit PDB file
CoordinateDistance Filter

With the mouse pointed at the peak at 5.1 PPM click the left mouse button to create a peak-pick, ie peak-pick it. The Peak-Pick is automatically assigned to A65.CA,HA; I33.HA since the CoordinateDistance Filter removed the other possible assignment.
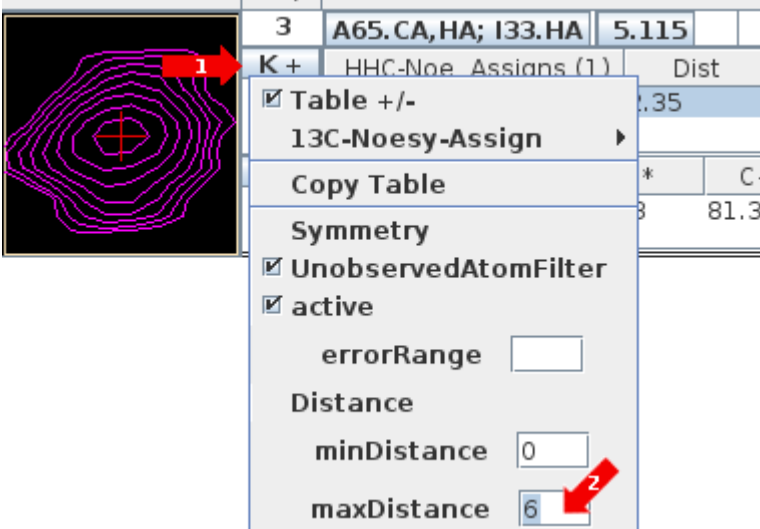

The Table Window for the peak-ID 1 shows two possible assignments and no 3D symmetry.

1: Shows distance between A65.HA and I33.HA from PDB file that was defined in 27Feb98.
2: No symmetry are found because SymmetryFilter and All Symmetry were disabled. Right click over S+ to to toggle All Symmetry on or off to show all symmetry. Note when experiment button "HHC-Noe" Symmetry Filter is off the Symmetry Table will only show All Symmetry or no Symmetry. Must delete the peak and re-add with the experiment button "HHC-Noe" Symmetry Filter enabled in order to see symmetry for a specific assignment after turning off All Symmetry on S+ button. An observant reader will notice that the peak ID number changed to 3 because the peak was deleted and re-picked a couple of times.

Right click the K+ button (1) to bring up a dialog to change max distance (2) as shown at the right. The new max distance will only affect the current peak-pick assignment and the default max distance (currently at 6.0) remains for new peak-picks. There is no way to change the default max distance except via experiment dialog from XippPanel which requires exiting and re-showing the study.

Edit Study: noesyInter Assign Dialog
for Intermolecular NOESY

New Study

Startup File: c12c13Noesy

Study: dgarrett/noesy

☑ Use Properties   ☑ Same Properties

Linked Group	View	Molecule	Assign Table	Structure

| H,H | Edit View | EIN HPr | einHPr Fi... | E1N HPr |

NMR Data   Show |-------------- Comment --------------|

HH-Noesy ☑

| H,C | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

C13-HSQC ☑

| H*,[H,C] | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

HHC-Noe ☑

HPr(C13) ☑

EIN(C13) ☑

| C*,[H,C] | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

HCC-Noe ☑

| HN,N | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

15N-HSQC ☑

| H*,HN,N | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

N-Noe ☑

N-Noe-B ☑

| N*,HN,N | Edit View | Set Molec... | Set Assig... | Set Struct... |

NMR Data   Show |-------------- Comment --------------|

NNH-Noe ☑

NNH-Noe-B ☑

OK   Cancel

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' below.

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 study 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.

14: Linked Project defines the complementary experiment that provides symmetry for this experiment.

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
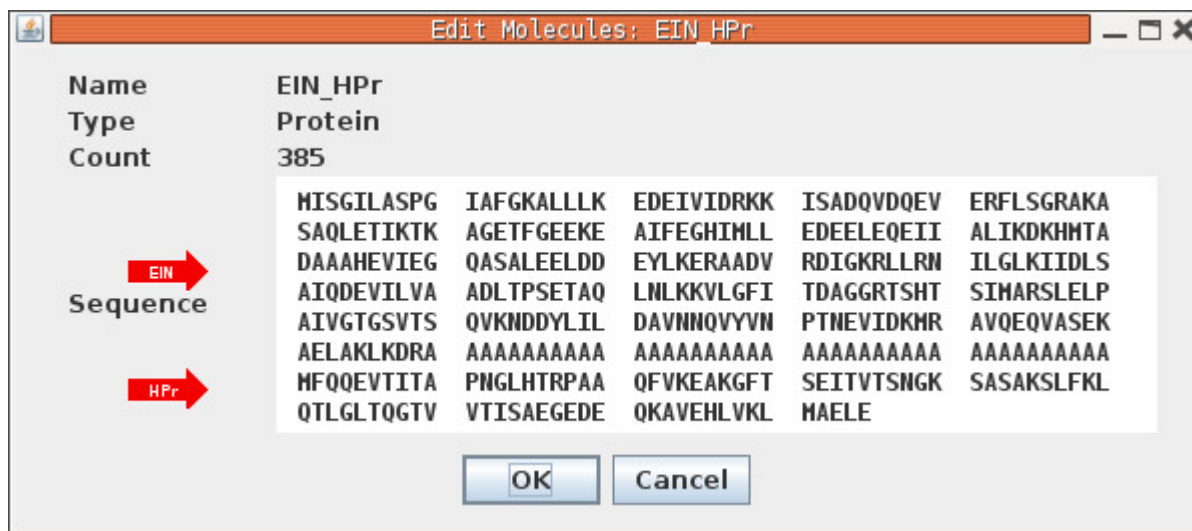
See createSetStudies section 'Molecule Select/Create Dialog' for detailed instructions on creating a new molecule. This was created as a New Protein Molecule. The protein EIN is 259 residues and is listed from residue M1 to R259. There are then enough spacer ' A' (residue type is not important) so that 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 EIN 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

The noesy tutorial describes in greater detail the steps used to define a set of Structures. The description below identifies differences required when analyzing intermolecular Noesy spectra.
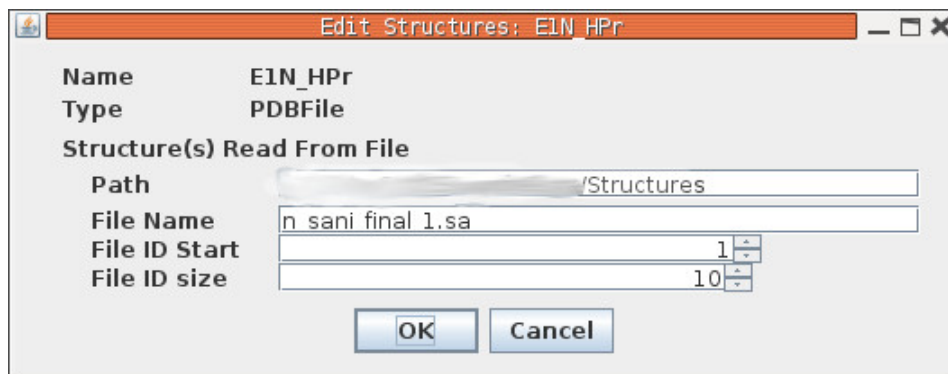
This was initially created as a New PDB Structure File by clicking on "Set Struct . . " button on the New Study dialog panel. As mentioned above the atomic positions for EIN should use residue M1 to R259. No atomic positions needs to be included for the spacer ' A' residues. The atomic positions for 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 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.

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 to 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 on 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.
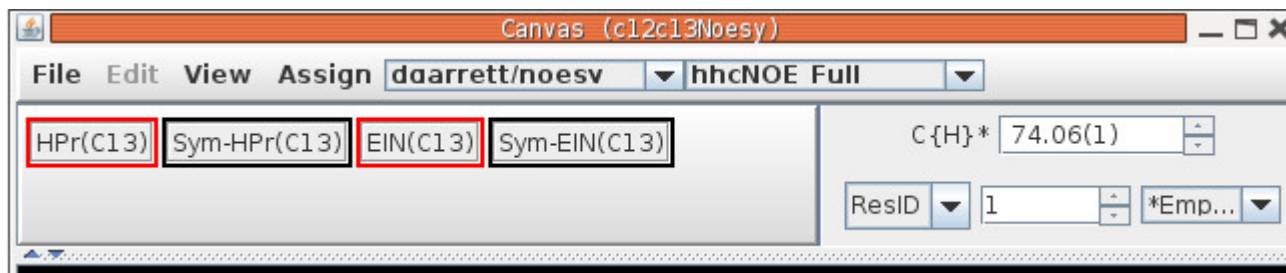
Note that there are 4 NMR buttons based on the 2 Experiments which are displayed in the same way as the '3D Symmetry Tool Bar' used with Sidechain study described in Xipp_Commands (p10). The Sym-HPr(C13) is used to manage a separate view into the HPr(C13) data to overlay Symmetry peaks from HPr(C13). 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:
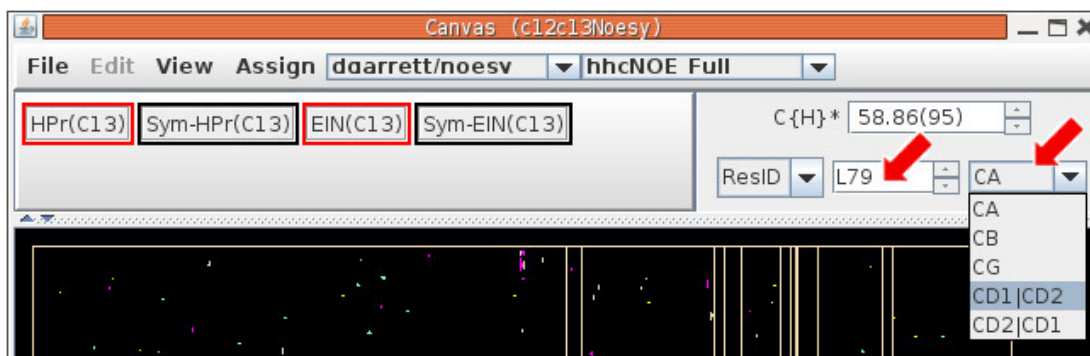
On slice 55 click on the peak at 4.1, 0.82 PPM:

The Name Peak-Pick table in the Table window will show several possible assignments for the peak as well as several possible Symmetry.
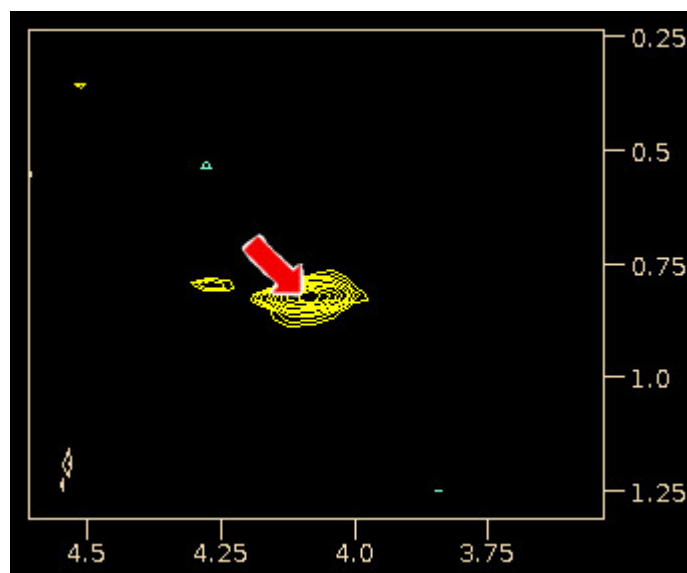
The possible carbon assignments are L79.CD1|CD2 and L115.CD2|CD1 from EIN(C13) which should not be surprising since the peak came from EIN(C13).
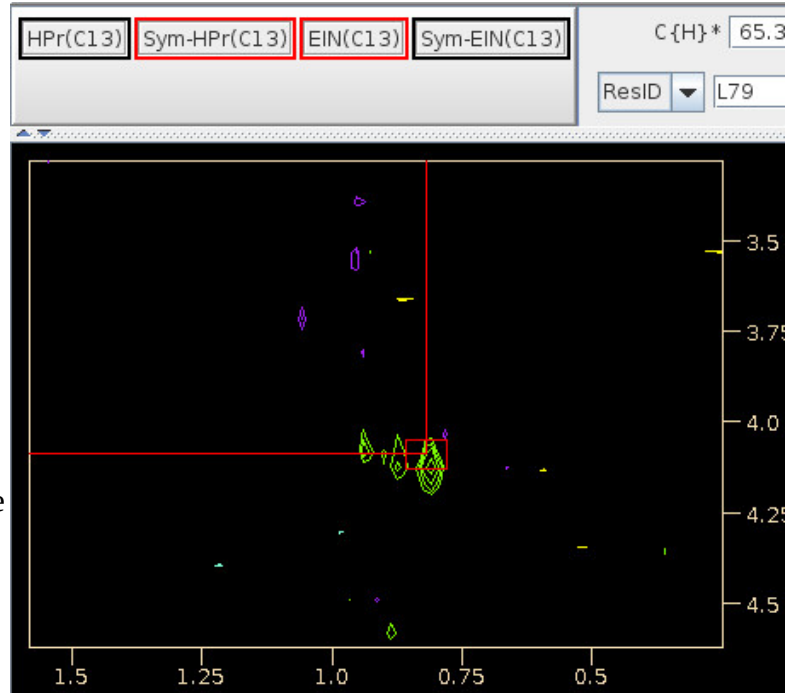
Show Symmetry in c12c13Noesy Study

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 the allowed error.


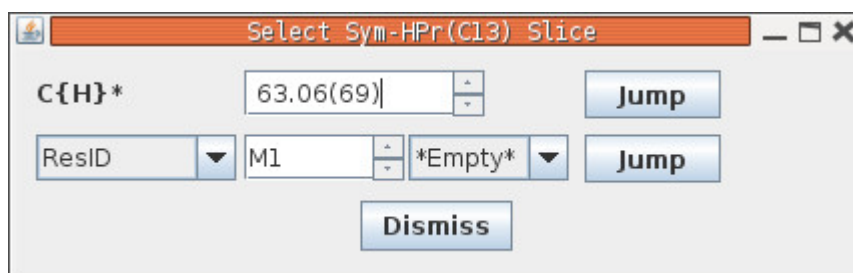On Table Window click on the Symmetry peak on slice 69.
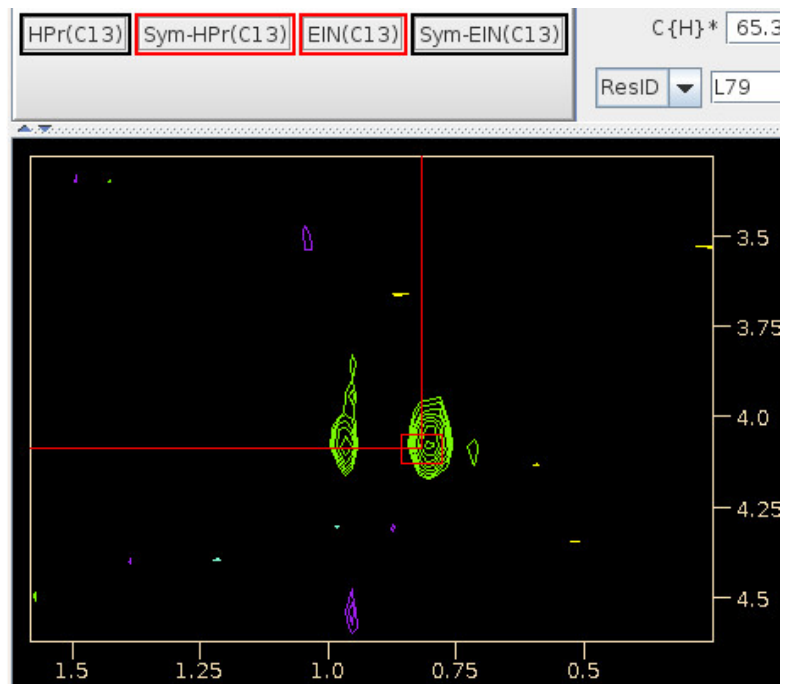
Manually set Symmetry Slice in c12c13Noesy Study

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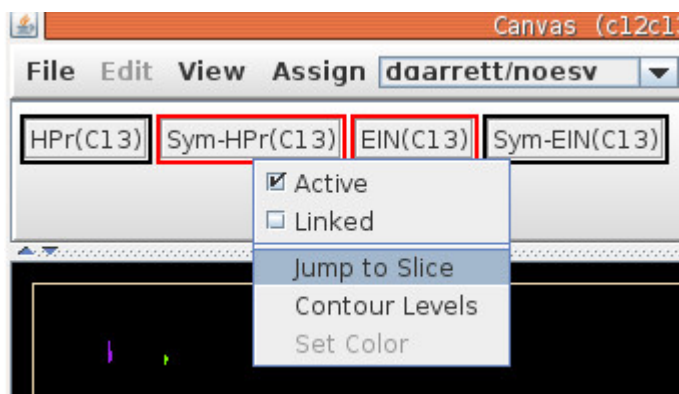
The 'Select Sym-HPr(C13) Slice' dialog is exactly like the one that is fixed at the top of the Canvas window except this only changes the slice of the Sym-HPr(C13) view. This dialog should always be correct unless no slice has been selected. This dialog can remain up while clicking in Table and this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

noesyCoEvolve.xipp
Edit Study

The noesyCoEvolve tutorial describes setting up and analyzing a 3D ^{13}C,^{15}N-Noesy in which both ^{13}C and ^{15}N co-evolve during the same evolution period. Refer to noesyNew for fuller description of analysing NOESY spectra. Refer to section on Backbone Assign Study in createSetStudies.pdf for general information about setting properties on Studies using the Linked Group Study Dialog. Reference for data: Yang, et al. Proc. Natl. Acad. Sci. U.S.A. 106, 17729-17734 (2009)
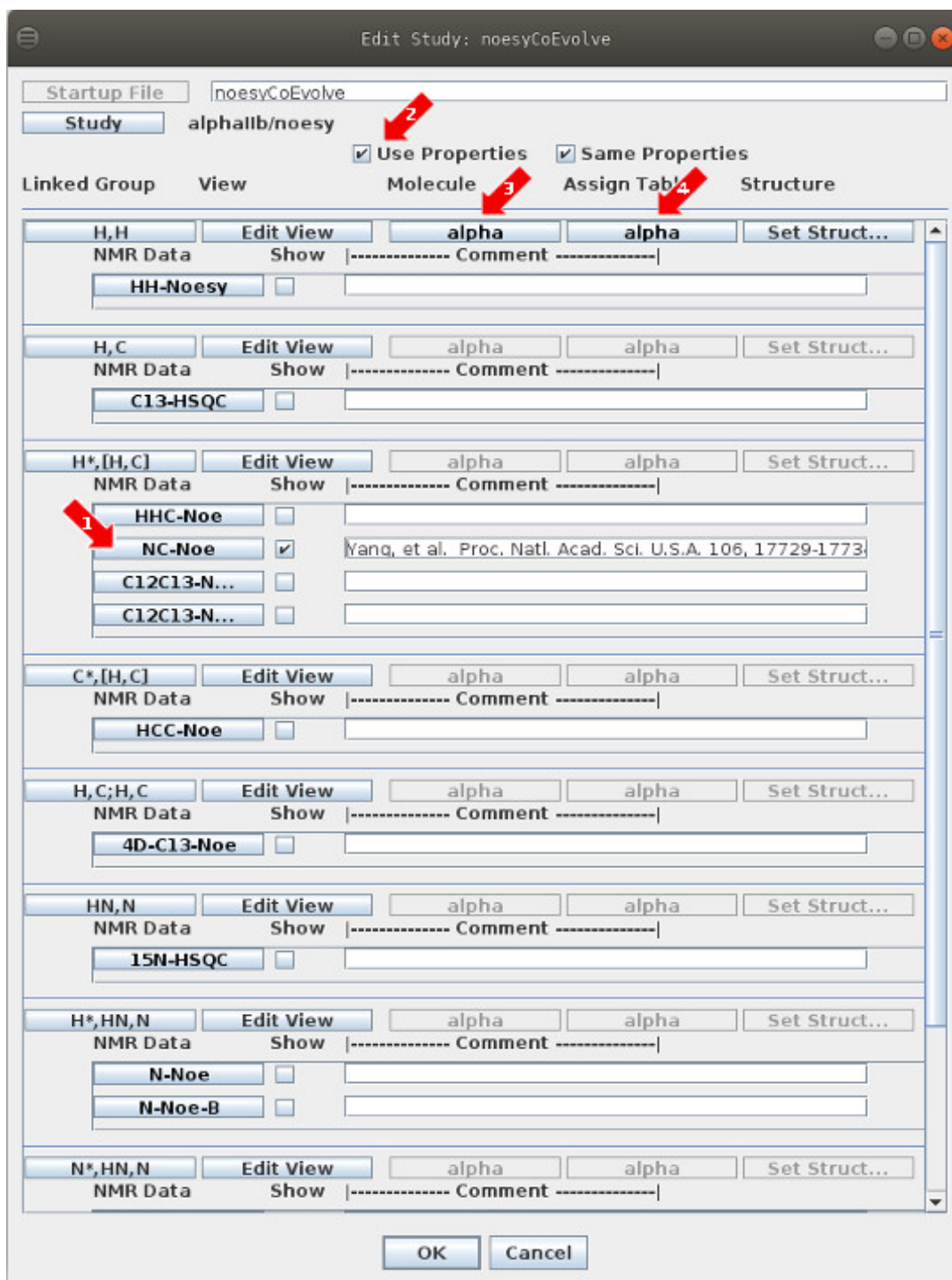
noesyCoEvolve.xipp
Edit Study

This example data and shift table script were provided by Dr. Jun Yang and Dr. Jun Qin. For an example of using the NOESY CoEvolve see: Yang, et al. Proc. Natl. Acad. Sci. U.S.A. 106, 17729-17734 (2009)

1: Clicking the button labeled NC-Noe brings up the NMR Data Experiment Dialog for the 3D ^{13}C,^{15}N-Noesy experiment in which both ^{13}C and ^{15}N co-evolve during the same evolution period. The Axis Order for the 2 proton Axis must be manually set after selecting the NMR Data File. The 'Use Extrema' should be selected (ie toggled on) so that Extrema can be used to find 3D symmetry peaks.

2: 'Use Properties' button must be selected in order to make NOE assignments which will use Molecules and Assign Tables. 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.

3: Clicking the button labeled alpha brings up the Molecule Select/Create Dialog that is used to select a previously defined molecule or define a new molecule. Prior to setting the molecule the button is labeled 'Set Molec...'. The name for the molecule must be a unique molecule name in the current study and is used to identify the molecule within Xipp. The names from all molecules in all studies in the current directory are displayed. See Molecule Select/Create Dialog in createSetStudies.pdf for more details. All linked groups have the same molecule name since they have the same sequence.

4: Clicking the button labeled alpha brings up the Assign Table Select/Create Dialog that is used to select a previously defined assignment table or define a new assignment table. Prior to setting the assign table the button is labeled 'Set Assig...'. The assignment table name is not a file name. The name for the assignment table must be a unique assignment table name in the current study and is used to identify the assignment table within Xipp. The names from all assignment tables in all studies in the current directory are displayed. See Assign Table Select/Create Dialog in createSetStudies.pdf for more details. The assignment table must be modified so that ^{15}N shifts are converted to their observed locations within the ^{13}C sweep-width. The GAWK script tblMod5_N2C.gawk written by Jun Yang in the directory ~/Xipp/ExampleData/AlphaIIB/Asgnmts can be used to convert the ^{15}N shifts of a regular assignment table.

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

Note the text on a button or label will be red when the properties that are accessed by that button are not correctly set or inconsistent with other properties. Clicking OK with incorrectly set properties will bring up a Warning Dialog asking if you want to Fix the problem(s) (i.e. re-show the dialog) or Ignore the problem(s) and save the properties with the problems. Incorrect properties may not show correctly and another pop-up window will warn when a Study with incorrect settings is attempted to be shown. Some problems such as an incompletely defined Experiment with the Show check box clicked on will actually Show OK, but the warning message is still displayed.

noesyCoEvolve.xipp
Edit Experiment NC-NOE
Edit 3D ^{13}C,^{15}N-NOESY

1: 'Max Distance' is the maximum distance when a PDB structure is used with CoordinateDistanceFilter.

2: 'Base Peak Sign' must be set to match sign of unfolded peaks.

3 & 4: 'Axis Order'. Both proton axis must be explicitly set. H{C}* defines proton axis bonded to C{H}*. PPM values for HN are listed along H{C}* axis and N PPM values are listed along C{H}* axis.

5: 'Assign Offset' defines a PPM value added to the observed peak position to match the chemical shifts in an assignment table. Note this does NOT change the value of peak position in the peak pick table. However if the assignment table is defined as Average Assigned Peaks then the assignment table chemical will be offset by the amount entered here. All Edit Experiment Dialogs have this, but I have not updated all of the figures.

4: 'Use Extrema' should be enabled in order to use 3D symmetry as a filter to assign peaks as in noesyNew tutorial.

noesyCoEvolve.xipp
Show Study

Refer to the document XippCommands.pdf for general information about keyboard and mouse commands for Xipp.

Show the noesyCoEvolve (alphaIIb) study by selecting it (ie highlighted) on the XippPanel followed by clicking the Show button. It will take 1 – 2 minutes the first time the Noesy study is run in order to create the extrema files for the 3D ^{13}C,^{15}N Noesy NMR experiment.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

(1) Click right mouse button on button labeled NC-NOE to bring up the pop-up menu shown and toggle off 'Symmetry Filter' to Turn off 'Symmetry Filter'. The pop-up will disappear after selecting 'Symmetry Filter'

(2) Click left mouse button on button labeled Sym-NC-Noe to turn off view of symmetry plane. Note Red border indicates that the Experiment or view into an Experiment is active and is the first toggle button on the pop-up menu.

(3) Enter Keyboard command: Alt-J 18 to residue jump G18. You can also type 18 into Jump window and hit enter.

(4) Keyboard command: n To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data

The Table Window should look something like this after clicking n in the canvas window and scrolling down to G18.

The top part shows the ShiftTable with ^{15}N shifts converted to equivalent ^{13}C positions by the script tblMod5_N2C.gawk written by Jun Yang described above. Note G18 ^{15}N shift is converted to 17.292 PPM to be in ^{13}C SW.

The bottom part shows information about peaks that have been picked. This is equivalent to the information shown in PIPP's Name mode.
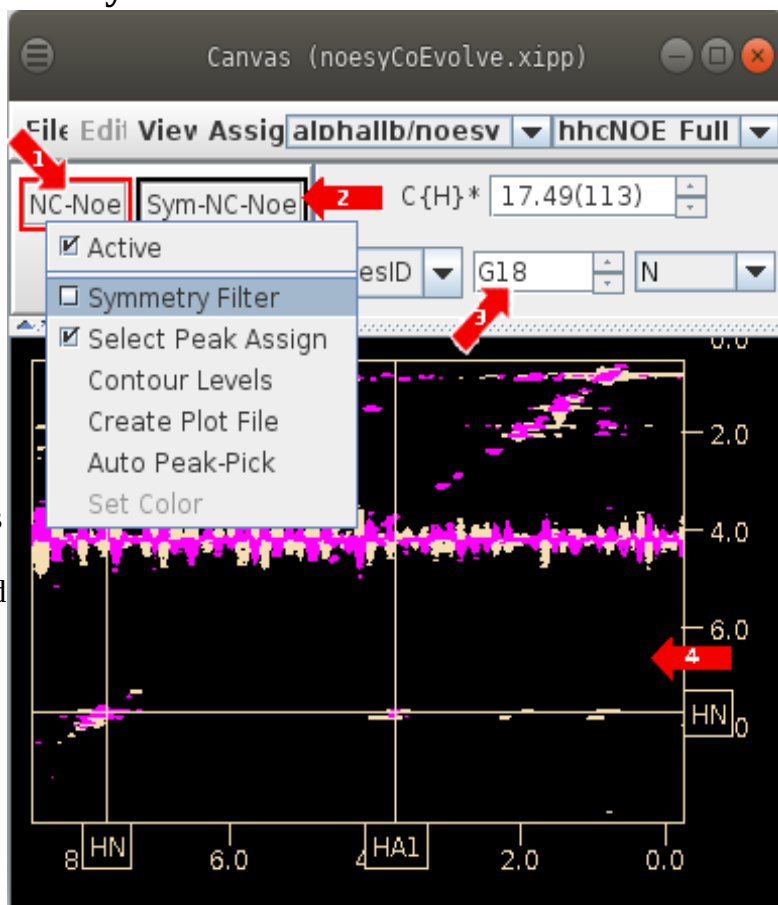
noesyCoEvolve (3D ^{13}C Noesy)
Displaying Symmetry

Peak-picked on Slice 113

With the mouse pointed at the peak at 7.71 and 1.92 PPM click the left mouse button to create a peak-pick, ie peak-pick it. The Peak-Pick is labeled with the peak-ID since it is currently unassigned. The border of the peak is an oval and has a red v underneath it to indicate that the peak has a greater intensity on the next slice.
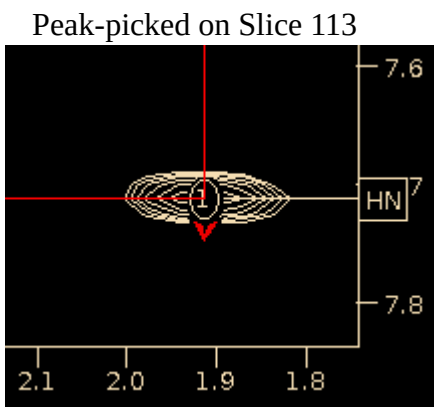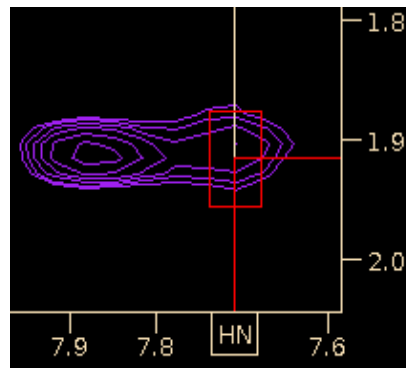
The Name-Peak-Pick in the Table Window for peak-ID 1 displayed below shows 11 possible assignments (only 5 are visible) and 2 possible 3D symmetry at slice 87 and 85. Remember the SymmetryFilter was disabled on previous page.

Enable Sym-NC-Noe view on Canvas Window to check which symmetry slice 87 or 85 is best.

Symmetry Slice 87 @ 31.5 PPM
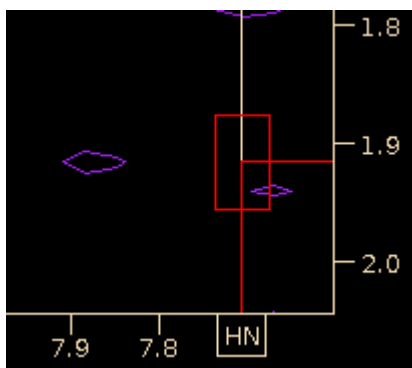
Symmetry Slice 85 @ 30.6 PPM

The Symmetry at slice 85 (30.6 PPM) is the better option and matches V17.CB at 31.34 PPM.

|	PkID	...		H{C}*	H*	C{H}*	Intensity
Contours	A +-		Assignment-Table				
	S +/-		Symmetry-Table				
	1	***		7.710	1.915	17.235	1.968e+7
	K +	NC-Noe Assigns (11)	H{C}*	H*	C{H}*		
		G18.N,HN; M3.HB2	0.0008	0.0212	0.0568		
		G18.N,HN; E6.HB2	0.0008	0.0052	0.0568		
		G18.N,HN; E7.HB2	0.0008	-0.0148	0.0568		
		G18.N,HN; V17.HB	0.0008	-0.0028	0.0568		
		G18.N.HN; I31.HB1	0.0008	-0.0398	0.0568		
	S +	Slice	H{C}*	H*	C{H}*	Intensity	
		87	1.938	7.676	30.609	-4.391e+6	
		85	1.906	7.712	31.487	-8.811e+6	

noesyCoEvolve (3D ^{13}C Noesy)
Filter Assigns with Symmetry

Displaying 3D Symmetry is very useful to confirm that the selected 3D Symmetry is good, but filtering the possible assignments by 3D symmetry helps to quickly determine which assignments are more likely to be correct.

To Filter by Symmetry:

1: In Canvas Window Right click button labeled NC-Noe and select 'Symmetry Filter" to turn on 3D Symmetry Filter.

2: In Canvas Window peak-pick again peak-pick ID 1 (click left mouse button) over peak currently labeled 1. If nothing happens delete the peak-pick (click right mouse button with mouse over peak) and re-peak-pick it. Peak-pick ID 1 should automatically be assigned to G18.N,HN; V17.HB since the Symmetry Filter removed all other possible assignments.
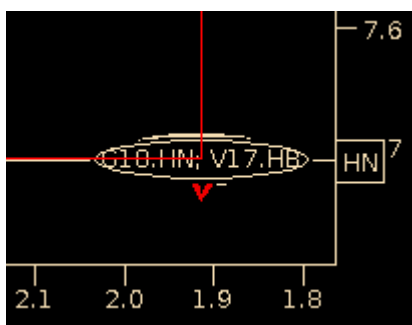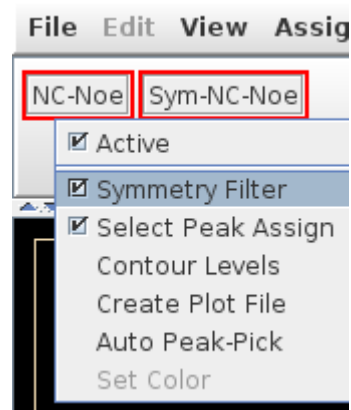
With Symmetry Filter enabled only one possible assignment is found and Xipp automatically assigns the peak to that assignment and updates the Canvas and Table Window. The 3D symmetry shown in the Symmetry Table is Symmetry for the selected assignment, ie not all possible symmetry positions that was shown previously.

	PkID	Assign	H{C}*	H*	C{H}*	Intensity
Contours	A +-	Assignment-Table				
	S +/-	Symmetry-Table				
	1	G18.N,HN; V17.HB	7.710	1.915	17.235	1.968e+7
	K +	NC-Noe Assigns (1)	H{C}*	H*	C{H}*	
		G18.N,HN; V17.HB	0.0008	-0.0028	0.0568	
	S +	Slice	H{C}*	H*	C{H}*	Intensity
		85	1.906	7.712	31.487	-8.811e+6

Warning the Symmetry Filter will fail when the symmetry peak is a shoulder to another peak (false negative) and therefore not in the list of extrema or when noise exceeding threshold is within the error (false positive) for a possible assignment that is incorrect. You should always confirm that the extrema for the selected assignment is good or you might miss the correct assignment. You can manually search for the symmetry by displaying the Jump window for Sym-NC-Noe (as shown previously) to find the the true 3D Symmetry when it is a shoulder. When manually looking for symmetry start by looking at the possible assignments that are obtained when SymmetryFilter is turned off.