bbNew.xipp: Backbone Assign Study Tutorial

Instructions to create a Backbone Assign Study such as the bbNew.xipp file are in the createSetStudies document under the Backbone Assign Dialog.

This document is a tutorial on making backbone assignments from scratch in Xipp using the bbNew.xipp example that is available for download. In order to follow along using the HPr example data and bbNew.xipp study file you need to install Exmpl_Linux_Backbone.sh or Exmpl_Linux_ALL3D.sh for Linux computers. The file Exmpl_Linux_Backbone.sh has just the backbone assignment data while the file Exmpl_Linux_All3D.sh has all of the processed NMR data for all examples (excluding 4D Noesy).

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.

Select bbNew (HPr) and the click Show to run the bbNew Study.

It will take 1 – 2 minutes the first time bbNew study is run in order to create the extrema files for the CBCA(CO)NH and HNCACB NMR experiments. The extrema file contains all of the local extrema found in each experiment and are used here to locate adjacent spin-systems. These files are not included in the download in order to keep the download file size as small as possible.

Before the Canvas and Table Window are shown you should be prompted with the warning dialog 'File not Found for Backbone Assign Map".

Click OK in order for a default Backbone Assign Map to be created.
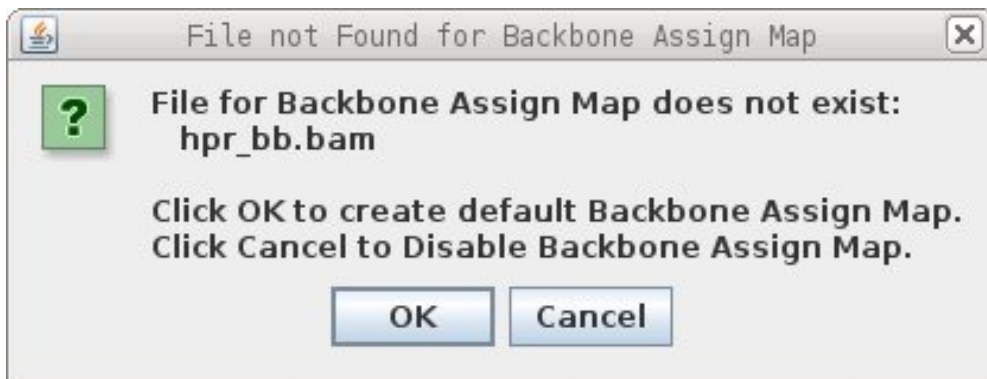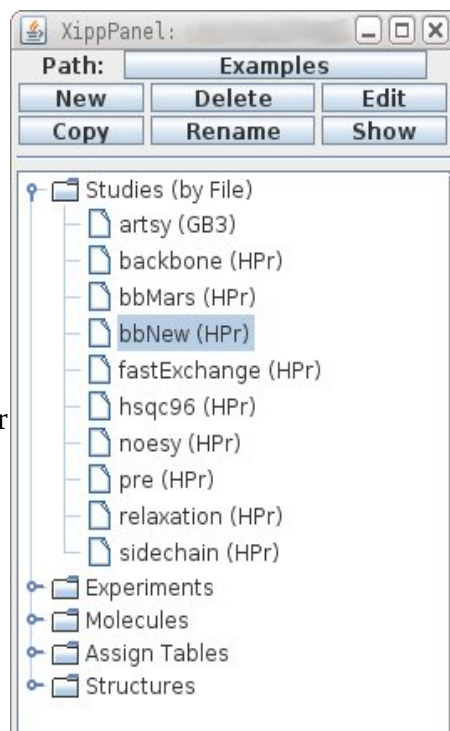
Backbone Assign Steps for HPr

(1) Peak-Pick 15N-HSQC Experiment
- (a) Manually Peak-Pick using mouse in Xipp or start auto peak-pick program CAPP from Xipp and read in CAPP results into Xipp.
- (b) Inspect/Clean-up CAPP Peak-Pick results.

(2) Peak-Pick CBCA(CO)NH
- (a) Manually Peak-Pick using mouse in Xipp or start auto peak-pick program CAPP from Xipp and read in CAPP results into Xipp.
- (b) Inspect/Clean-up CAPP Peak-Pick results.

(3) Peak-Pick HNCACB
- (a) Manually Peak-Pick using mouse in Xipp or start auto peak-pick program CAPP from Xipp and read in CAPP results into Xipp.
- (b) Inspect/Clean-up CAPP Peak-Pick results.
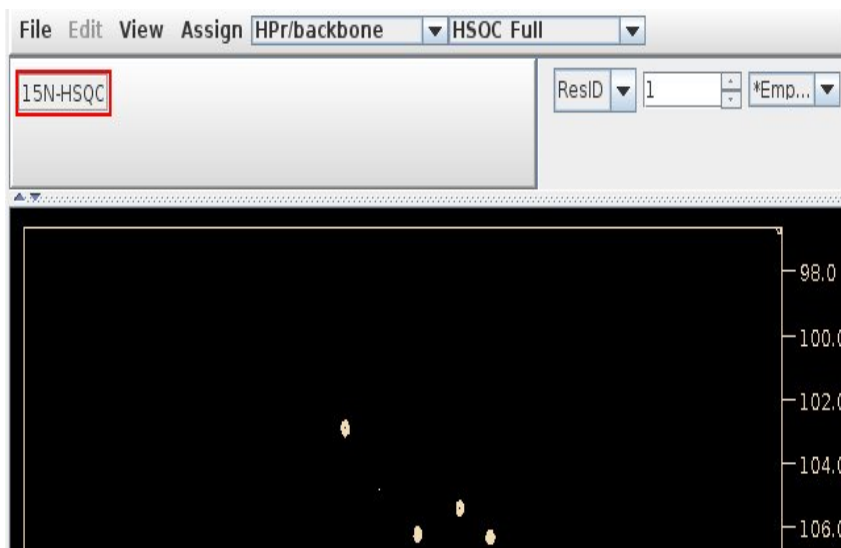
(4) Inspect/Clean-up Spin-Systems on ShiftTable

(5) Initial Assign via MARS program
Create draft assignments using MARS program.

(6) Inspect/Clean-up Assignments using BAMTable and Named PeakPick Table

Initial Setting for Canvas and Table Windows

In the Canvas Window with the mouse over the spectrum enter the Canvas Command **n** to switch to the Name Peak Table.

The Table Window should look something like this.

The top part is tabbed and can show the ShiftTable, ie Assignments or the BAMTable which is the Backbone Assign Map 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.

Select the BAMTable-* tab to show the Backbone Assign Map. The BAMTable shows 1 row for each SS-ID (Spin-System ID). The table is sorted to show all residues in sequence followed by all unassigned SS-ID. Assigned SS-ID are shown on row with their Res-ID. An SS-ID is only shown on 1 row, ie can only be assigned to one Res-ID at a time.

(1) Peak-Pick 15N-HSQC Experiment

This can be done manually by pointing your mouse at every peak and clicking the Left-Mouse button. Each peak should be automatically assigned to an arbitrary Spin System and will show up at the top of the Name PeakPick Table.

This can also be accomplished on Mac or Linux computers by right clicking on the Exp button in the tool bar and selecting Auto Peak-Pick to run the CAPP Auto Peak Picker. For more information see the section for Auto Peak Pick Dialog in the Xipp_Commands document.

Shown below is the top part of the 'CAPP Auto Peak-Pick Dialog'. It is very important to **select** the '**Assign Peaks using Xipp**'. To start CAPP click Ok at the bottom of 'CAPP Auto Peak-Pick Dialog'. When CAPP completes click OK on the 'Capp Auto PeakPick Completed' dialog. If these two steps are not done then the peaks created by CAPP will not be assigned.
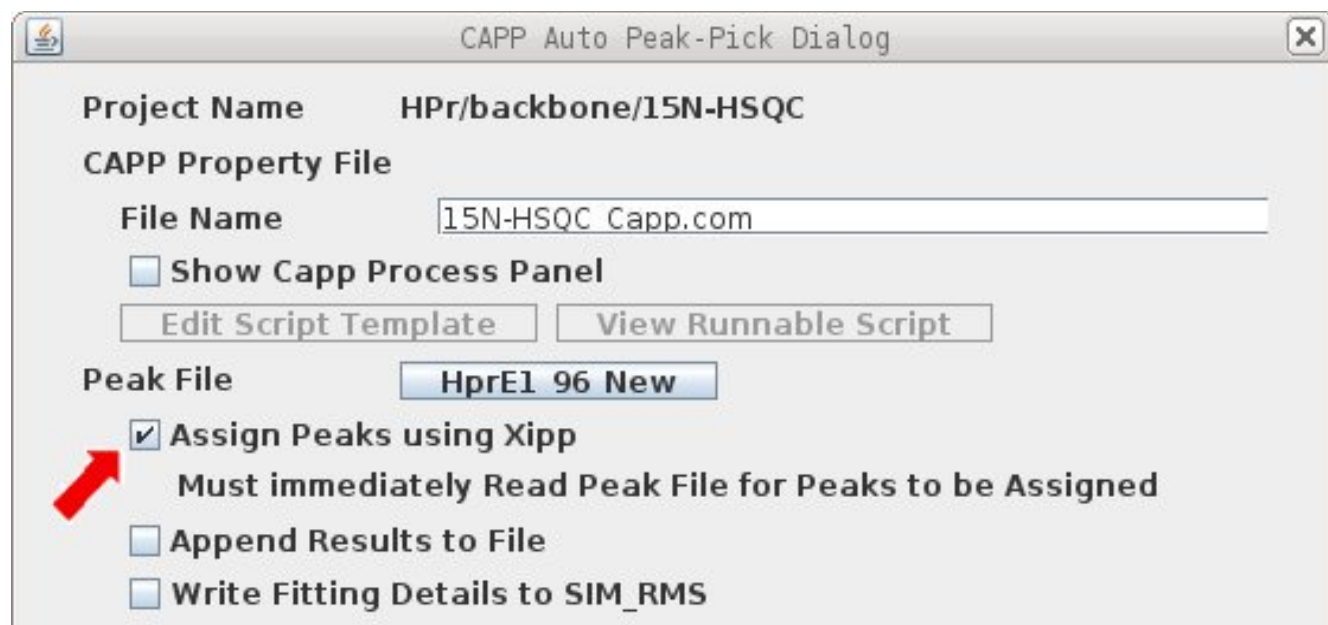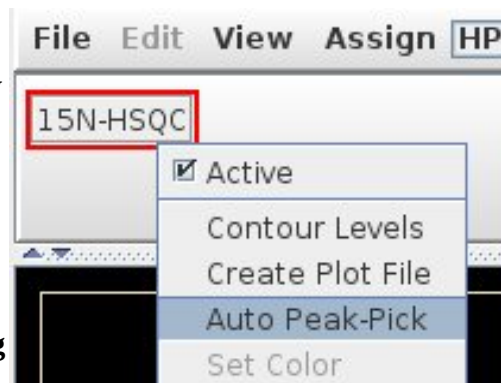
The modal dialog 'Capp Auto Peak-Pick Completed' will show up after CAPP is finished. After Clicking Ok on this dialog the Table Window will get updated, but the Canvas Window will not get updated until the 'f' command is entered in the Canvas Window.
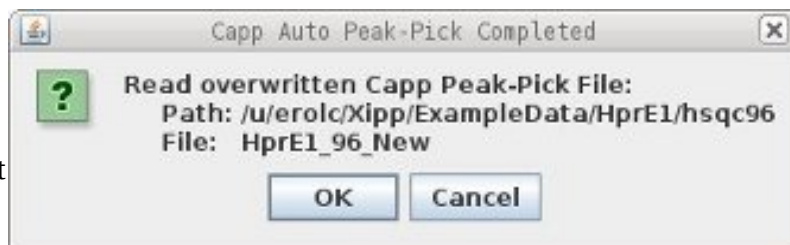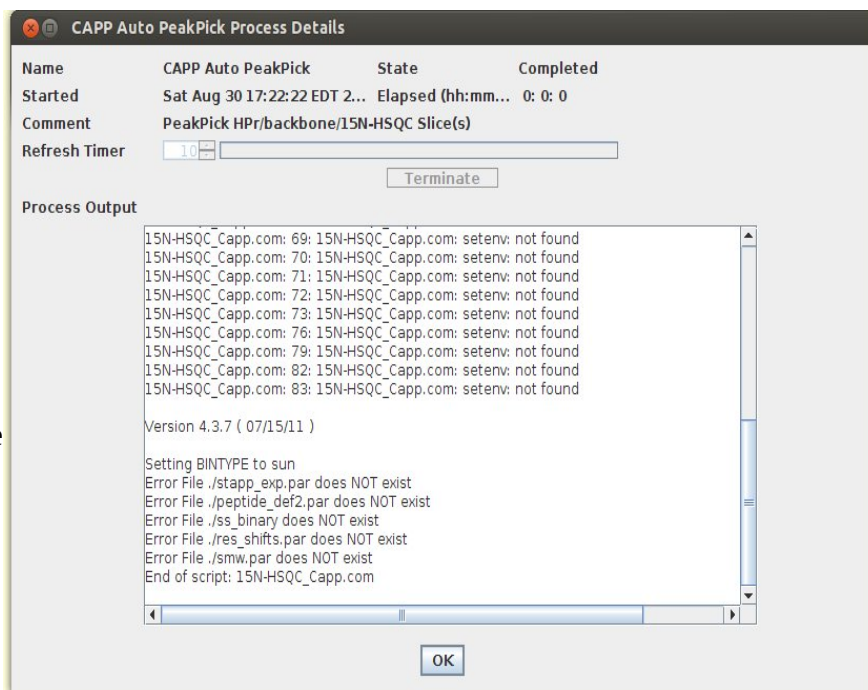
CAPP Requires /bin/csh for setenv

If CAPP completes very quickly and shows the this error messages then you need to install the csh. The shell script that runs CAPP requires the csh command setenv to set properties.

Ensure /bin/csh exists and it is really the csh not a link to tcsh or bash.
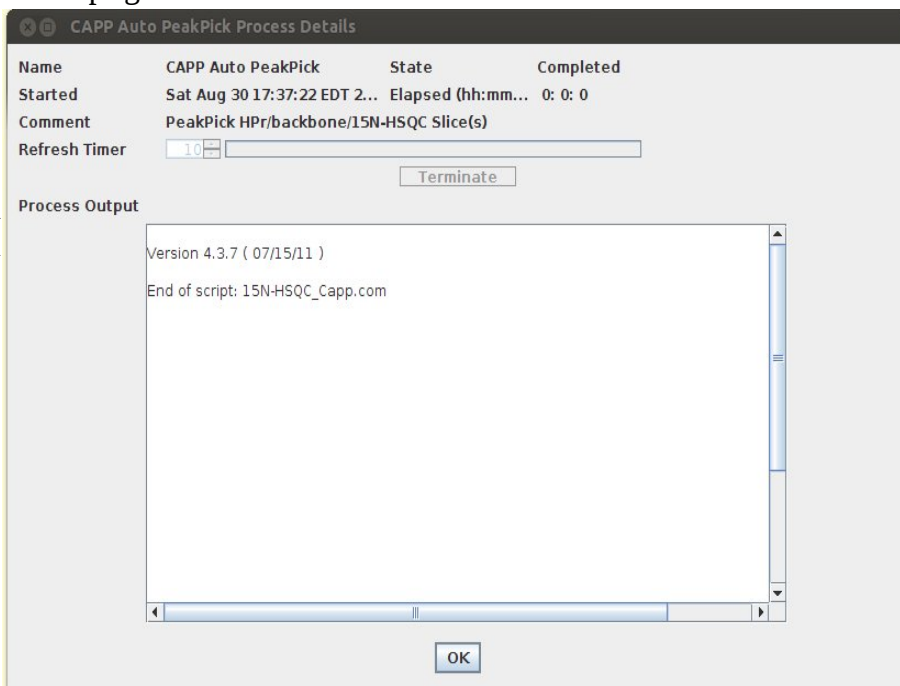
If problems continue then try running the shell script directly from a terminal window to diagnose problem.
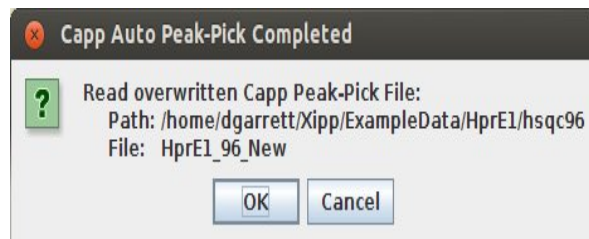
CAPP Auto PeakPick Process Details

Name	CAPP Auto PeakPick	State	Completed
Started	Sat Aug 30 17:22:22 EDT 2...	Elapsed (hh:mm...	0: 0: 0
Comment	PeakPick HPr/backbone/15N-HSQC Slice(s)		
Refresh Timer	10		

Terminate

Process Output

```
15N-HSQC_Capp.com: 69: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 70: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 71: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 72: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 73: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 76: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 79: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 82: 15N-HSQC_Capp.com: setenv: not found
15N-HSQC_Capp.com: 83: 15N-HSQC_Capp.com: setenv: not found

Version 4.3.7 ( 07/15/11 )

Setting BINTYPE to sun
Error File ./stapp_exp.par does NOT exist
Error File ./peptide_def2.par does NOT exist
Error File ./ss_binary does NOT exist
Error File ./res_shifts.par does NOT exist
Error File ./smw.par does NOT exist
End of script: 15N-HSQC_Capp.com
```

OK

To install csh on Ubuntu systems use: sudo apt-get install csh

After installing csh you must completely exit and restart xipp.

This is what the 'CAPP Auto PeakPick Process Details' should look like when CAPP finishes correctly.

CAPP Auto PeakPick Process Details

Name	CAPP Auto PeakPick	State	Completed
Started	Sat Aug 30 17:37:22 EDT 2...	Elapsed (hh:mm...	0: 0: 0
Comment	PeakPick HPr/backbone/15N-HSQC Slice(s)		
Refresh Timer	10		

Terminate

Process Output

```
Version 4.3.7 ( 07/15/11 )

End of script: 15N-HSQC_Capp.com
```

OK

You must click OK to the modal Capp Auto Peak-Pick Completed window in order for the Peaks to be read in and assigned. If you click cancel the Peak-Pick file created by CAPP will stay unassigned.

Capp Auto Peak-Pick Completed

Read overwritten Capp Peak-Pick File:
    Path: /home/dgarrett/Xipp/ExampleData/HprE1/hsqc96
    File: HprE1_96_New

OK   Cancel

The Name Peak Table is automatically updated with newly created Spin-Systems after clicking OK on the 'Capp Auto Peak-Pick Completed' modal dialog.

You must enter the Canvas Command f to update the Canvas Window.

This shows the Canvas Window after reading in Capp Peak-Pick and typing **f** in canvas or clicking View Menu option '**Full [f]**'.

Note CAPP did not assign the peaks. Xipp assigned the CAPP peaks using the same methods that are used when manually peak-picking. Each peak with a unique ^{15}N, HN shift was used to define a new Spin-System. Xipp will only create new Spin-Systems for ^{15}N-HSQC and HNCO experiments.
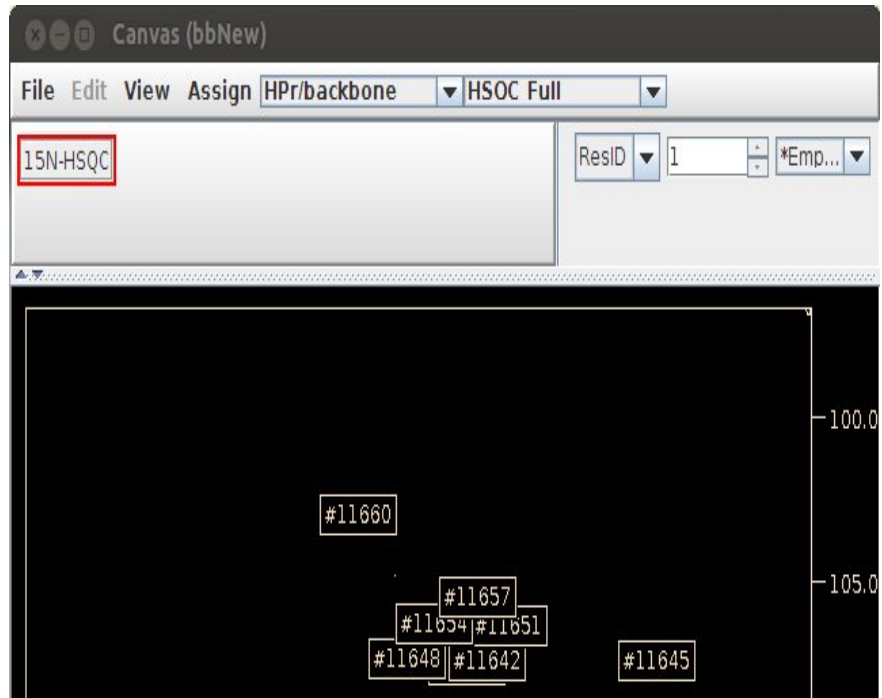
Use the S**pace Bar** to toggle the peak labels off and on and look for noise, ridges, artifacts or NH2 peaks. Use the Right Mouse button to delete any peak-picks selected by CAPP that arise from noise, ridge, artifact or NH2.

(2) Peak-Pick CBCA(CO)NH Experiment

Use the View Chooser to show the CACB_FULL(N) view.
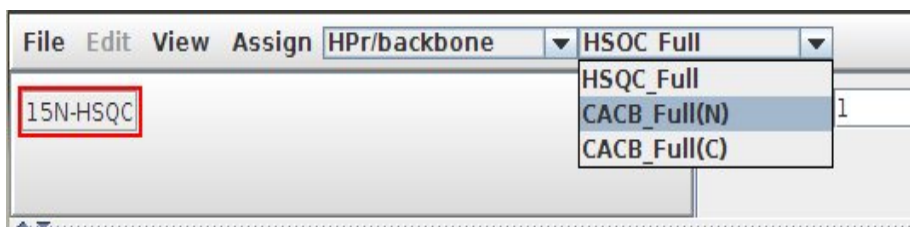
Click the 2 Exp buttons that start with Seq- so that their display is off. The Seq- Exp buttons are used to help find Sequential Spin-Systems.
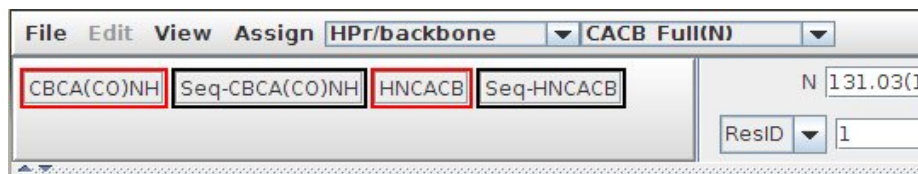
Just like in the HSQC this can be done manually by pointing your mouse at every real peak on every plane and clicking the Left-Mouse button. The ^{15}N, HN for each peak will be compared with the Spin-Systems created from HSQC and the peak will be assigned to the closest Spin-System and a new ^{13}C assignment will be created. If no Spin-Systems are found within error range then the peak is unassigned. You can click on the assignment in the Name-Peak-Pick table to make an assignment.

Peak-Picking can also be accomplished on Mac or Linux computers by right clicking on the Exp button in the tool bar and selecting Auto Peak-Pick to run the CAPP Auto Peak Picker. For more information see the section for Auto Peak Pick Dialog in the Xipp_Commands document.
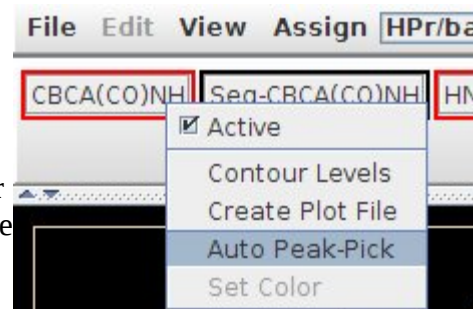
Must select 'Assign Peaks Using Xipp' . All other default settings are OK.

Click OK at bottom of CAPP Auto Peak-Pick Dialog' to run CAPP.
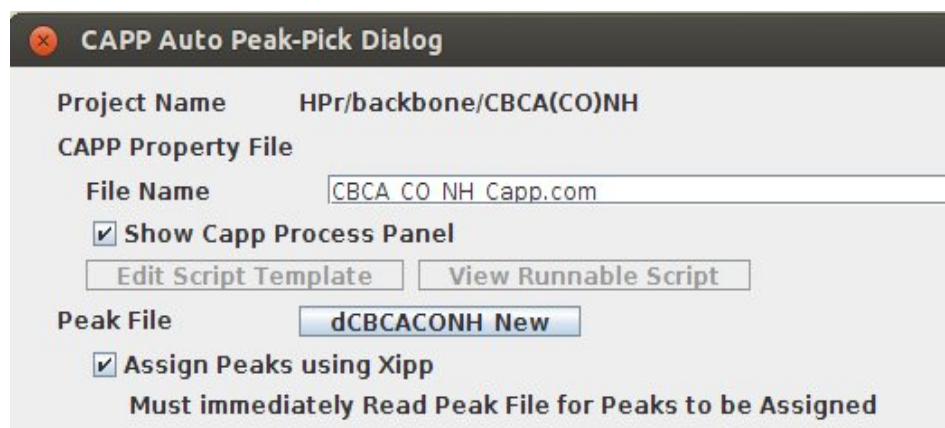
When CAPP completes click **Ok** on the modal Capp Auto Peak-Pick Completed window in order for the Peaks to be assigned.
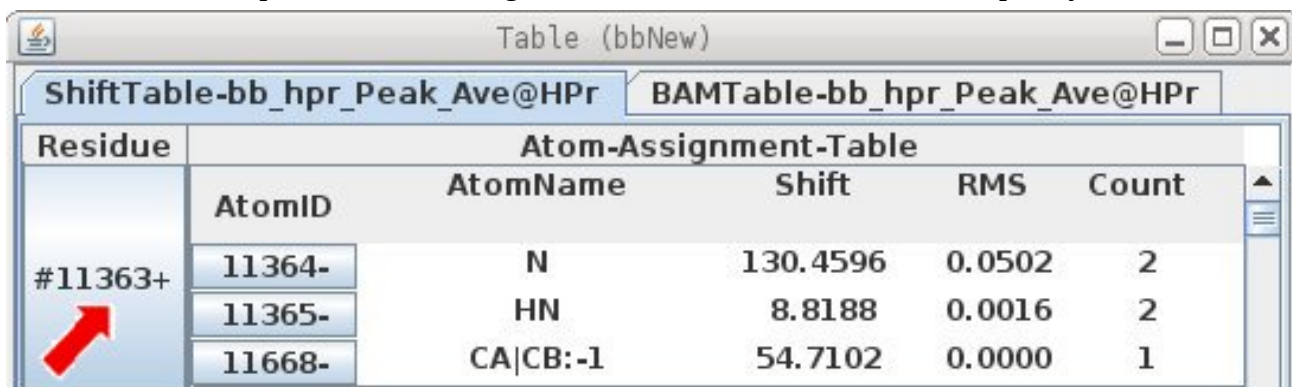
Type **f** in the Canvas Window to show the Xipp Assigned CAPP Peak-Picks. Or click the View Menu option '**Full [f]**' to show the Xipp Assigned CAPP Peak-Picks.

Manually Inspect CAPP Peak-Pick Results

It is essential that all slices or at the very least all spin-systems are visually inspected. Yes, this takes time, but unless the peak-pick table was manually created (ie did not use CAPP or any automated peak-picker) you can not have any confidence in your data unless you look at all of it. This can be done before or after the HNCACB has been Peak-Picked.

To inspect all slices start at slice 1 and scroll to next slice with the **Down-Arrow** key. On each slice use the **Space Bar** to toggle display of peak labels and **Click Middle Mouse** to expand a region for a better view.

To inspect all Spin-Systems start by **Click Left-Mouse** on first Spin-System in Assign Table which is #11363 in this example. After checking #11363 **Click Left-Mouse** on next Spin-System.


The Canvas Window should like this after making the HNCACB inactive. Note the HNCACB may not have been peak-picked at this time.

Look at the peaks along the HN Shift Cursor to see if any peaks have not been picked such as the peak at 8.8, 64.2 PPM. **Click Left-Mouse** at 8.8 by 64.2 to create a new Peak-Pick.

The Peak-Pick with ID 1 that comes from a previous slice is noise and can be deleted by **Click Right-Mouse** over the peak after changing to the previous slice.
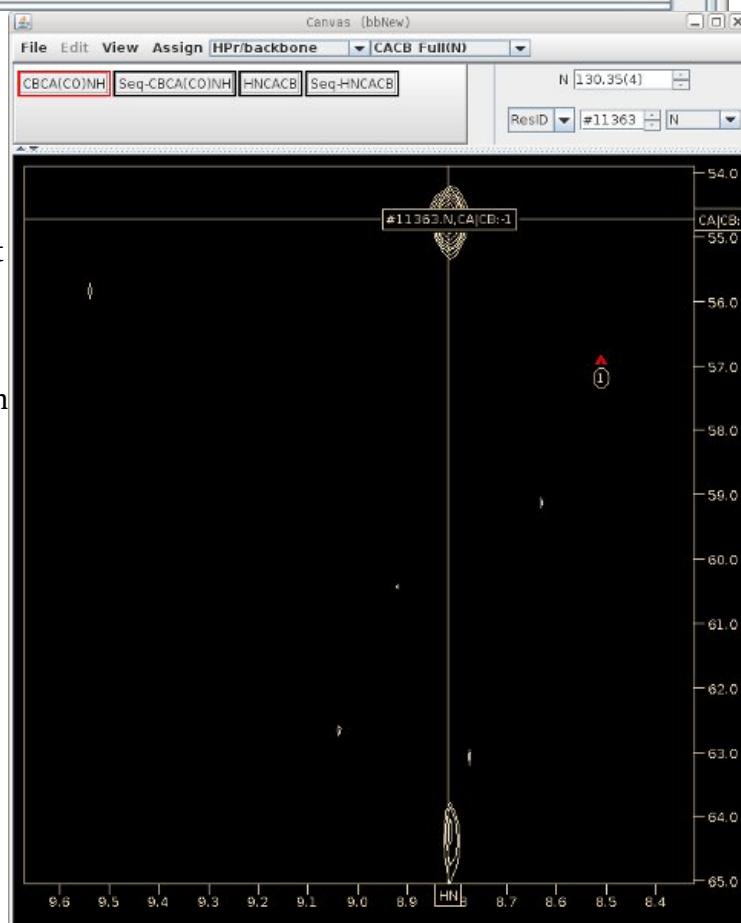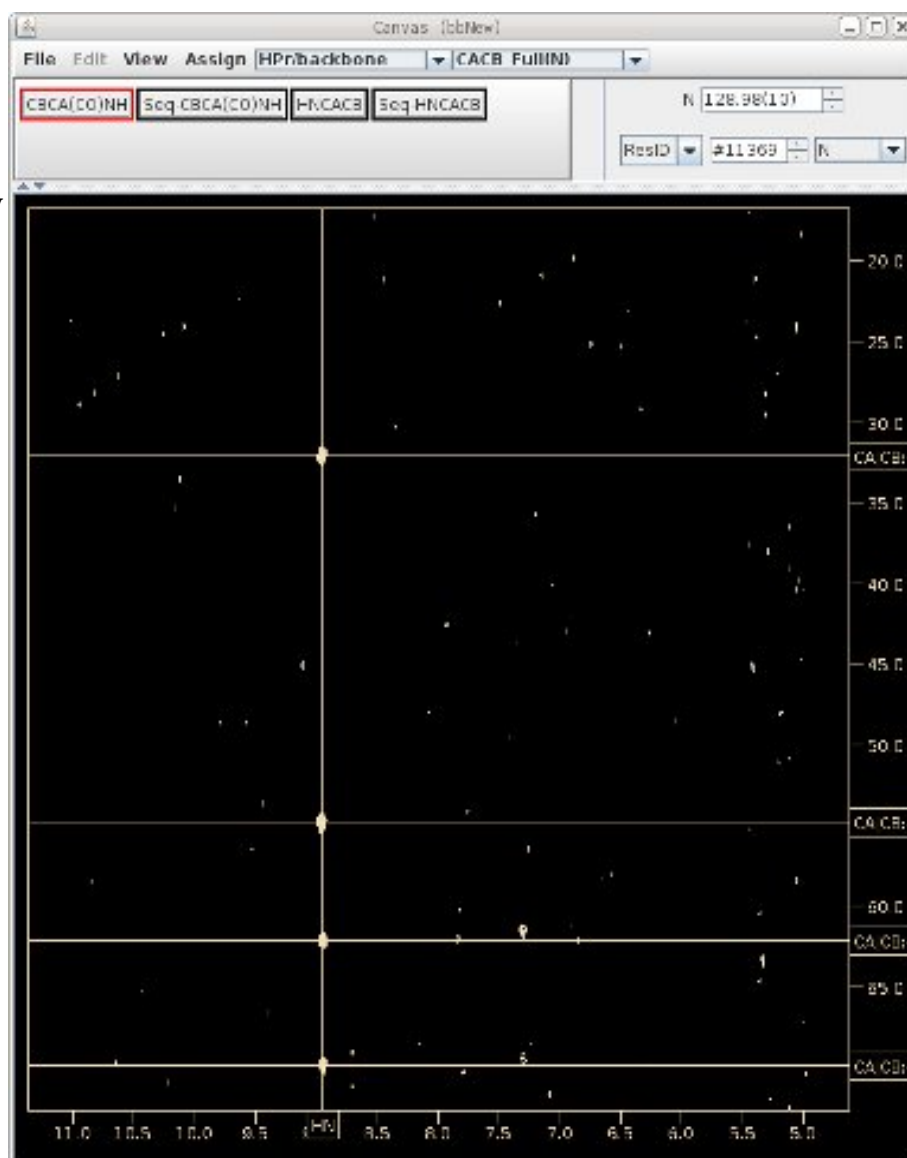
Finding HN, N Degeneracy

The ShiftTable shows that #11369 may have degenerate amides or noise since there are more than two ^{13}C shifts assigned after CBCA(CO)NH when there should be only two ^{13}C shift assigned (CA:-1 and CB:-1).

Table (bbNew)

ShiftTable-bb_hpr_Peak_Ave@HPr | **BAMTable-bb_hpr_Peak_Ave@HPr**

Residue	Atom-Assignment-Table			
12127-	CA:-1	61.4523	0.0000	1
AtomID	AtomName	Shift	RMS	Count
11370-	N	128.8891	0.0946	5
11371-	HN	8.9584	0.0047	5
11671-	CA|CB:-1	32.0497	0.0000	1
11672-	CA|CB:-1	54.7843	0.0000	1
11673-	CA|CB:-1	69.8560	0.0000	1
11674-	CA|CB:-1	62.1193	0.0000	1

(#11369+ is the residue label shown at left of the atom rows)

Res Jump to #11369 (Click Left-Mouse on #1139 button in ShiftTable)

The Canvas Window shows that there are 4 peaks at nearly the same ^{15}N, HN PPM value.

Fixing HN, N Degeneracy
in HSQC

The HSQC_Full View confirms that there should be two Spin-Systems, but only one has been created.

Note how the peak label is lower and to the left of the Shift Cursors.

Click Alt Mouse-Left to copy Peak-Pick to Peak-Buffer , position mouse to Shift Cursor intersection and Click Crtl-Left-Mouse to move Peak-Pick position up and to the left.

Position Mouse over second Spin-System shown by red arrow and Click Crtl-Left-Mouse to create a new Peak-Pick.
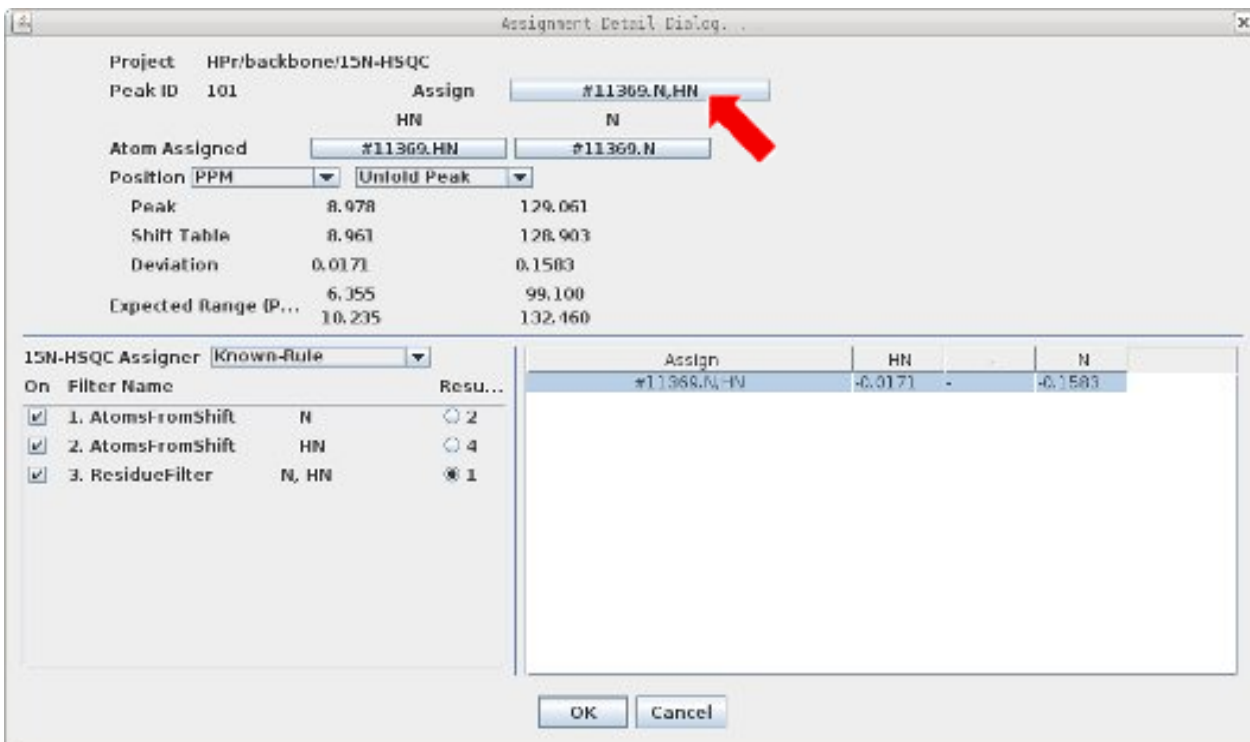
This does not create a new Spin-System ID which is needed because the peaks are close to each other.
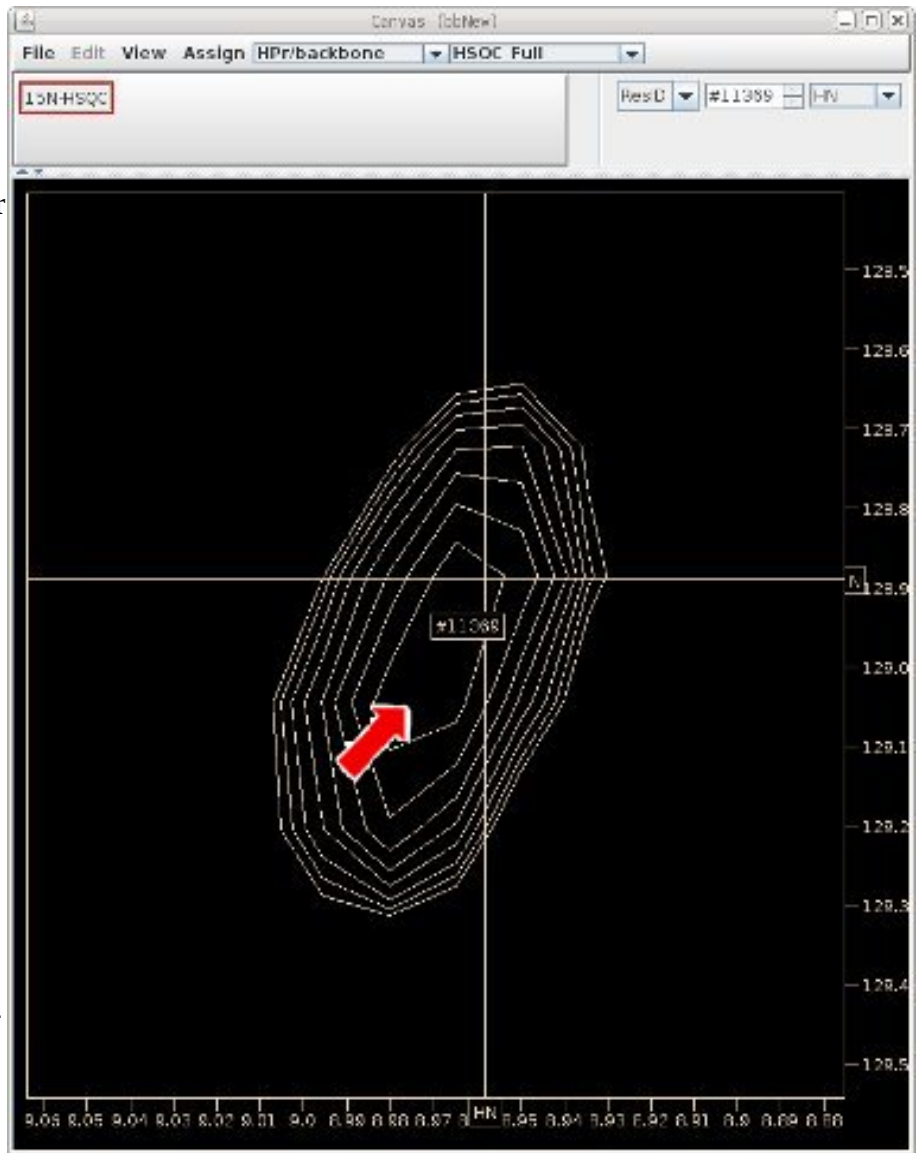
Click Left-Mouse over Name Peak Table on button labeled #11369.N,HN to bring up the 'Assignment Detail Dialog' shown below to modify assignment of Peak-Pick.

Assignment Detail Dialog . . .
to Create a new Spin-System

In Assignment Detail Dialog above click button identified by red arrow to bring up Change Moiety dialog shown at right.

Click Select button for New Moiety which in this case is ID #11721. Click OK on 'Confirm Peaks Dialog' that pops up and then OK on 'Assignment Detail Dialog' after confirming that Spin-System in Assign has been updated with the new Spin-System.

A Spin-System and a Residue are two types of Moiety objects used by Xipp. An unassigned Moiety is a Spin-System. Each molecular object in Xipp has a unique ID, ie Atom, Moiety, Residue and Segemnt. The Spin-System ID is the same as the unassigned Moiety ID. The New Moiety Spin-System number that you get will most likely be different than #11721 and that is the Spin-System number that you should use below as you follow this example. The number generated by Xipp in the 'Change Moiety ' dialog is the next unique unused object ID which is guaranteed to be different than any existing ID.

After clicking space bar twice to update peak labels each peak should be labeled with a unique Spin-System ID.

Fixing HN, N Degeneracy
Using Canvas View CACB_Full(N)

The 4 CBCA(CO)NH peaks in the CACB_Full(N) view need to be correctly assigned to #11369 and #11721. What is most important is that the CA and CB that belong to the same residue get assigned to the same Spin-System.

There are at least 3 ways to match the peaks:
  (1) Zoom in on each peak at 8.9 PPM on the CBCA(CO)NH and see where the Shift Cursor is drawn relative to the peak (use space bar to toggle display of peak labels),
  (2) Compare HN,N shifts in Peak-Pick Table,
  (3) Delete each Peak-Pick and re Peak-Pick and carefully look at deviation of HN and N from peaks to #11369 and #11721.
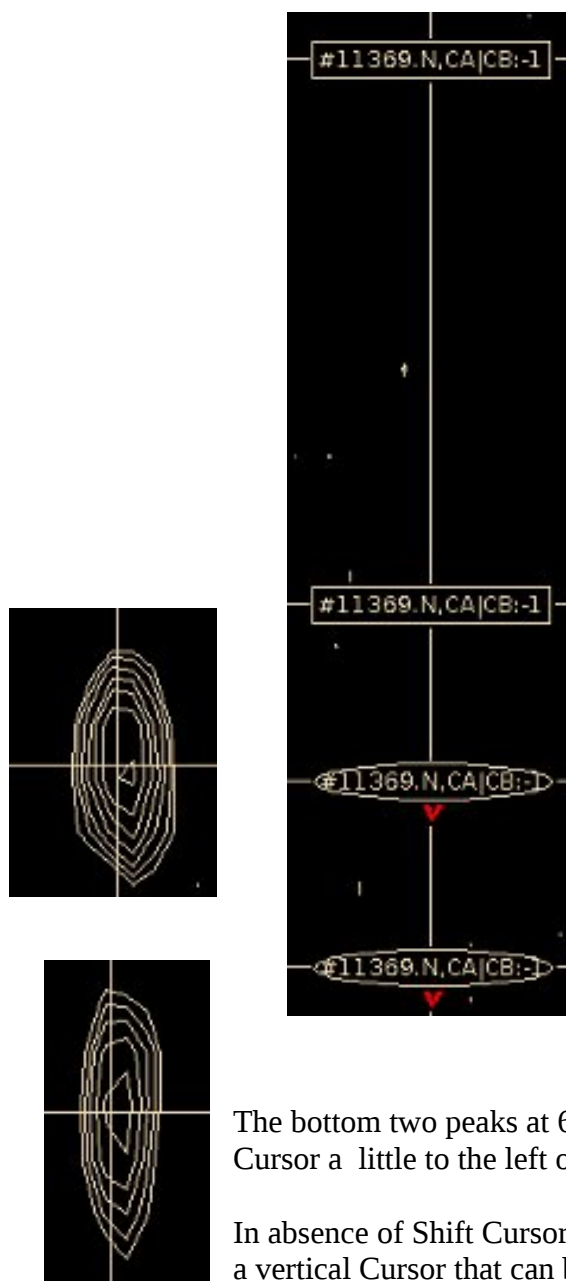
The top two peaks at 32 and 55 PPM have the HN Shift Cursor a little to the right of the center of the contours.

The bottom two peaks at 62 and 70 PPM have the HN Shift Cursor a little to the left of center of contours.

In absence of Shift Cursor use Canvas Command **Crtl-v** to create a vertical Cursor that can be moved to the center of a peak.

Fixing HN, N Degeneracy
Using Peak-Pick Tables

Show Peak-Pick Table for View CACB_Full(N):
  Click View Chooser on Canvas Window and select CACB_Full(N)
  Use the Canvas Command **n** to show the Peak-Pick Table.
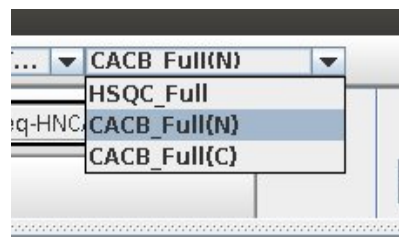
CACB_Full(N) Peak-Pick Table

CBCA(CO)NH	6	#11369.N,HN,CA|CB:-1	8.962	32.05	128.98	5.074e+6
CBCA(CO)NH	5	#11369.N,HN,CA|CB:-1	8.962	54.78	128.97	8.676e+6
CBCA(CO)NH	7	#11369.N,HN,CA|CB:-1	8.952	69.86	128.78	3.228e+6
CBCA(CO)NH	8	#11369.N,HN,CA|CB:-1	8.953	62.12	128.77	5.162e+6

The CACB_Full(N) Peak-Pick Table confirms that the peaks at 32.05 and 54.78 PPM belong together since their HN and N are closest to each other while the peaks at 69.86 and 62.12 PPM belong together since their HN and N are close to each other.

HSQC_Full Peak-Pick Table

15N-HSQC	101	#11721.N,HN	8.973	129.02	1.096e+6
15N-HSQC	11	#11369.N,HN	8.963	128.89	1.122e+6

The HSQC_Full Peak-Pick Table show that #11721 has the higher HN and higher N PPM value than #11369. Since the peaks at 32.05 and 54.78 have the higher HN and N PPM value they should be assigned to #11721 and the peaks at 62.12 and 69.86 should be assigned to #11369. Spin-System IDs are arbitrary. Swapping the assignments for HSQC Peak-Pick ID 11 and 101 should swap which Spin-Systems the CBCA(CO)NH peaks are assigned to but preserve the assignments to the underlying Peak-Pick PPM values.

Fixing HN, N Degeneracy
Re-Assigning Peaks

Two relatively easy ways to re-assign a Peak-Pick:
> (1) Delete and Re-Add Peak-Pick on Canvas Window.
> (2) Use Extended Rule to assign Peak-Pick on Name Peak-Pick Table

(1) Delete and Re-Add Peak-Pick on Canvas Window
This is the simpler method of re-assigning the peak and is the preferred method.
In the Canvas Window **Click Right-Mouse** over the peak at 32 PPM to delete it. The
assignment in the ShiftTable should automatically be deleted when the Peak-Pick is deleted.
In the Canvas Window **Click Left-Mouse** over the peak at 32 PPM to re-add it.
Initially the Peak-Pick is not assigned since there are multiple assignments.
To assign it select the line in the Name Peak-Pick Table for the desired assignment, ie #11721.
The Peak-Pick is assigned and the line gets highlighted as shown below.

(2) Use Extended Rule to assign Peak-Pick on Name Peak-Pick Table
In Canvas Window **Click Left-Mouse** on peak at 54.8 PPM to add it in Name Peak-Pick Table.

In Name Peak-Pick Table C**lick Right-Mouse** over small button labeled K+ with red arrow labeled 1 and select CBCA(CO)NH assigner Extended-Rule as shown at right with red arrow 2.
The button may show E+ depending on exact position of new Spin-System created. If E+ is shown then Extended-Rule is being used and you do not need to change the Assigner.

The button should change its label from K+ to E+ indicating that the assigner is using the Extended-Rule. Click Left-Mouse over E+ button twice to update the assignments shown.

Contours	PkID	Assign		HN	CACB	N	Intensity
	A +-	Assignment-Table					
	S +/-	Seq Strip-Table					
	5	#11721.N,HN,CA|CB:-1		8.962	54.784	128.9...	8.676e+6
	E +	CBCA(CO)NH Assigns (2)		HN	CACB	N	
		#11369.N,HN,CA|CB:-1		-0.0079	-	-0.1603	
		#11721.N,HN,CA|CB:-1		0.0039	-	0.0227	
	S + 17	Exp	Slice	HN	CACB	N	Intensity
		HNCACB	104	7.855	54.981	107.623	-1.338e+6
		HNCACB	107	7.855	54.908	106.947	+2.546e+6
		HNCACB	69	8.292	54.894	115.568	+5.442e+6

To assign the Peak-Pick select the line in the Name Peak-Pick Table for the desired assignment, ie #11721. Unlike Delete and Re-Add the line does not get highlighted because as soon as the Peak-Pick is assigned the assignment table changes and the Extended-Rule assignment is not valid.

Click Right-Mouse over E+ and select Known-Rule to see that it is selected under Known-Rule.

| Contours	PkID	Assign		HN	CACB	N	Intensity
	A +-	Assignment-Table					
	S +/-	Seq Strip-Table					
	5	#11721.N,HN,CA|CB:-1		8.962	54.784	128.9...	8.676e+6
	K+	CBCA(CO)NH Assigns (1)		HN	CACB	N	
		#11721.N,HN,CA:-1		0.0039	0.0000	0.0227	
	S +	Exp	Slice	HN	CACB	N	Intensity

The two peaks at 62 PPM and 70 PPM are not changed and left assigned to #11369.

Fixing GLY CA:-1 Assignments
Using Atom Quick Assign Menu

Scroll down the ShiftTable looking for a Spin-System that has a single ^{13}C assigned between 40 – 50 PPM, ie in the GLY CA range.

	AtomID	AtomName	Shift	RMS	Count
#11402+	11414-	N	123.9735	0.0951	2
	11415-	HN	8.1084	0.0010	2
	11416-	CA|CB:-1	46.8029	0.0000	1

Click Left-Mouse on the SS-ID button (#11402 in this example) to residue jump. To confirm that the ^{13}C belongs to a GLY CA the CACB_Full(N) View for this spin-system is checked to ensure that there is only one peak in the GLY range between 40 – 50 PPM along the HN ShiftCursor. If the peak is weak or the spectrum is crowded it is best to get a vertical trace to ensure that there is no other peak hiding under the threshold.
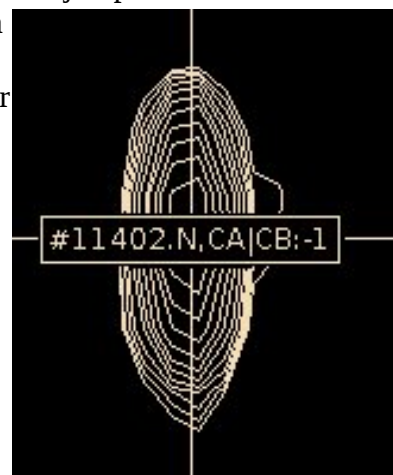
The Xipp assigner methods can not distinguish if a peak is a GLY CA or a Leu CB so without more information the peak is ambiguously assigned to #11402.N,HN,CA|CB:-1

Use Atom Quick Assign Menu on ShiftTable AtomID button to change assignment and re-assign the peak. The Atom Quick Assign Menu only exists when an atom is has an ambiguous assignment such as CA|CB. Once the shift is assigned to a single atom the Atom Quick Assign options are not shown. The ReAssign Shift . . . menu option as described in Xipp_Commands brings up the 'Select Atom Dialog' to assign the Shift to any existing or new atom in absence of Quick Assign menu options.

Click Right-Mouse on button for AtomID 11416 for GLY CA assignment to be fixed in ShiftTable and select CA:-1.

	AtomID	AtomName	Shift
#11402+	11414-	N	123.9735
	11415-	HN	8.1084
	11416-	CA|CB:-1	46.8029
		☐ Table +/-	Shift
	Atom	ReAssign Shift . . .	
#11405+	1141	CA:-1	123.7175
	1141	CB:-1	9.1039
	11419-	CA:-1	55.7861

The ^{13}C assignment in the ShiftTable is immediately updated with the new assignment. The peak label assignment in the Canvas Window is updated only after the screen is refreshed such as hitting the space bar twice. The AtomID number is changed in the ShiftTable to reflect a new Atom object being created for the assignment. As noted previously when the shift is assigned to a single atom such as CA:-1 the Atom Quick Assign options are no longer provided. The vertical bar in the atom name or residue offset is what triggers the creation of the Atom Quick Assign Menu options.

	AtomID	AtomName	Shift
#11402+	11414-	N	123.9735
	11415-	HN	8.1084
	11732-	CA:-1	46.8029

Note Spin-System #11402 is not a GLY it follows a GLY.

HPr is small and has only 6 GLY residues so there should be 5 more GLY to find. None of HPr's GLY come before a PRO, but degeneracy can hide a GLY and it is possible that CAPP missed one when it auto peak-picked the CBCA(CO)NH experiment.

This is why it is very important to look at your data!

ShiftTable Changes for HNCACB Experiment
During Peak-Picking and Assigning

Click Left-Mouse over HNCACB experiment button to activate/show it so we can see it and peak-pick it.

Notice (if you are still on #11402) that the HNCACB has 3 peaks. One at the GLY for the CA:-1 and one above and one below that are only in the HNCACB. Manually peak-pick the 2 peaks that are only in the HNCACB and watch the assignments for #11402 in the ShiftTable. After the first peak is picked it is assigned to CA|CB. After the second peak is picked they are both assigned to a single atom.

	AtomID	AtomName	Shift	RMS	Count
#11402+	11414-	N	123.9704	0.0674	4
	11415-	HN	8.1089	0.0009	4
	11732-	CA:-1	46.8029	0.0000	1
	11737-	CB	39.7530	0.0000	1
	11738-	CA	53.6073	0.0000	1

The Shift, RMS and Count for N and HN have been changing as peaks get assigned into #11402. **Click Left-Mouse** over AtomID 11414 for N to open the PeakPick-Table showing all peaks used in assigning this atom/shift. The + or – after the SS-ID or AtomID number indicates whether a sub-table is open or closed. By default the sub-table for SS-ID are open and the sub-table for AtomID are closed. Bad assignments can sometimes be caught by looking for large RMS and then opening the AtomID sub-table to see if one Peak-Pick is an outlier.

	AtomID	AtomName	Shift	RMS	Count
#11402+		N	123.9704	0.0674	4
		PeakPick-Table			
	11414+	PkID / Exp / PPM / Dev.			
		33 CBCA(CO)NH 123.8783 -0.092			
		223 HNCACB 123.9626 -0.008			
		38 15N-HSQC 124.0686 0.098			
		224 HNCACB 123.9722 0.002			
	11415-	HN	8.1089	0.0009	4
	11732-	CA:-1	46.8029	0.0000	1

(3) Peak-Pick HNCACB Experiment
## Manually or via CAPP

Just like in the HSQC and CBCA(CO)NH experiments this can be done manually or automatically. As it is essential to inspect every slice with peaks after running CAPP you might wonder whether CAPP is saving any time. Since the quality of data vary there is no absolute or right answer. CAPP performs best when peaks are resolved and there is good signal-to-noise. CAPP saves time only when the number of right peaks selected exceed the number of false positives, ie peaks selected that are noise or artifacts. When the parameters for CAPP are well optimized it was shown in JMR Vol 95, pp 214-220 (1991) that the efficiency of CAPP is 78 to 90% where the efficiency is calculated as (correct pick – false positives) / (correct picks + false negatives). On a typical well resolved triple resonance spectra CAPP generally gets around 60% efficiency using the defaults values. Inspecting CAPP results is generally quicker then manually peak-picking since every correct Peak-Pick is looked at but not selected unless there is a problem with the assignment or peak overlap. Missing peaks and false positives will require special action. The positive efficiency of CAPP peak-picking indicates that CAPP will save time in peak-picking for experiments with adequate signal-to-noise with predominantly well resolved peaks.

To run CAPP **Click Right-Mouse** over HNCACB experiment button and select 'Auto Peak-Pick' option.
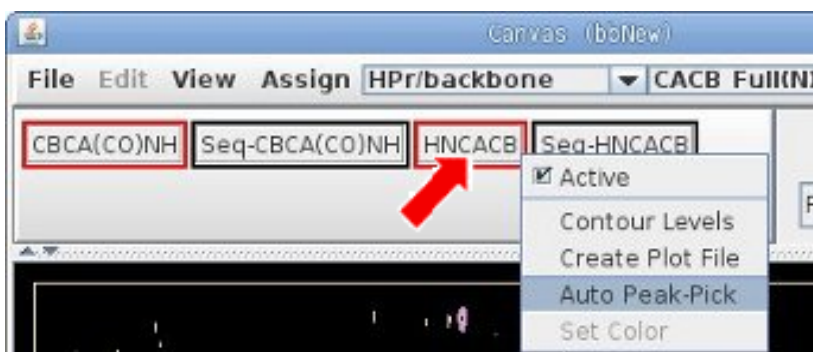
Must select 'Assign Peaks Using Xipp' . All other default settings are OK.

Click OK at bottom of CAPP Auto Peak-Pick Dialog' to run CAPP.
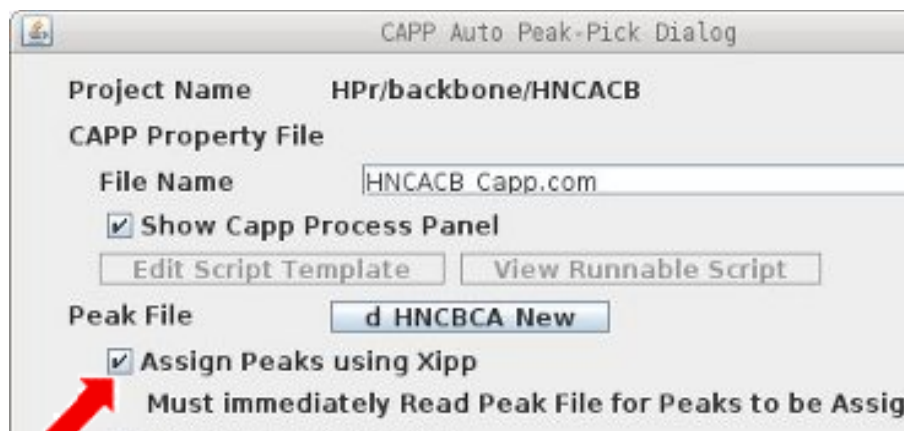
When CAPP completes click **Ok** on the modal Capp Auto Peak-Pick Completed window in order for the Peaks to be assigned as they are read in.

Type **f** in the Canvas Window (or use Menu **View**/'**Full [f]**') to show the Xipp Assigned CAPP Peak-Picks.

(4) Inspect/Clean-up Spin-Systems
on ShiftTable

It is essential that all slices and all spin-systems are visually inspected. Yes, this takes time, but unless the peak-pick table was manually created (ie did not use CAPP or any automated peak-picker) you can not have any confidence in your data unless you look at all of it.

Inspecting the Peak-Pick tables from both CBCA(CO)NH and HNCACB together instead of separately can save time, but for large proteins or complicated spectra (ie significant overlap or missing peaks) it is best to inspect the experiments separately and then together to find missing peaks or overlap.

The main objective at this point is to get all Spin-Systems to have 3 or 4 shifts assigned to specific atoms so that it will be easier to link adjacent Spin-Systems.

Spin-System #11363 has 4 assigned atoms, but the atoms are all ambiguous on Linux. On a MacOS #11363 the 4 assigned atoms is not ambiguous.

Spin-System #11366 is good with exactly 4 shifts assigned to unique atoms.

Jump to #11363 by clicking button #11363.

ShiftTable-bb_hpr_Peak_Ave@HPr		BAMTable-bb_hpr
Residue		Atom-Assignmen
	AtomID	AtomName	Shift
#11363+	11364-	N	130.4263
	11365-	HN	8.8216
	11366-	CA|CB:-1	54.6999
	11765-	CA|CB	41.8481
	11766-	CA|CB	57.6536
	11767-	CA|CB:-1	64.2585
#11366+	AtomID	AtomName	Shift
	11367-	N	129.2867
	11368-	HN	7.3131
	11369-	CA:-1	61.4523
	11370-	CB:-1	69.4118
	12805-	CB	17.9183
	12806-	CA	50.6529

The problem in #11363 is that CAPP on Linux missed the ^{13}C at 64.26 PPM and it was not fixed manually so Xipp did not have enough information to distinguish between (i,i) and (i,i-1) in making assignments which made all the assignments for #11363 ambiguous. On a Mac system CAPP picked the peak at 64.26 PPM so Xipp could easily identify the (i,i-1) assignment.

Adding one Peak-Pick in the CBCA(CO)NH at 64.26 PPM along the #11363 HN ShiftCursor immediately and completely resolved the assignments for all shifts in #11363.

ShiftTable-bb hpr Peak Ave@HPr		BAMTable-bb hpr
Residue		Atom-Assignme
	AtomID	AtomName	Shift
#11363+	11364-	N	130.4201
	11365-	HN	8.8205
	12852-	CB:-1	64.2299
	12853-	CA:-1	54.6999
	12854-	CB	41.8481
	12855-	CA	57.6536

Fixing HN, N Degeneracy
in #11369 & #11721

These Spin-Systems were looked at for the CBCA(CO)NH and the assignments were resolved by looking at the relative order of the HN and N PPM values in the HSQC and CBCA(CO)NH.

Shown at the right are two regions from the CBCA(CO)NH and HNCACB with CBCA(CO)NH on top. The white vertical line is the HN ShiftCursor for #11369. The red vertical line is a vertical Cursor manually positioned to the center of the contours for the HNCACB peak in top region labeled #11721.N,CB:-1 (this label is partially obscured by the equivalent label for the CBCA(CO)NH peak).

Top region is centered at 32 PPM and shows equivalent peaks from CBCA(CO)NH over HNCACB for shift #11721.CB:-1. Xipp easily handles small offsets between experiments as long as the assignment table has the assignment which is true for the peak at 32PPM that was uniquely assign in the CBCA(CO)NH experiment.

The bottom region is centered at 37.5PPM and shows one peak from the HNCACB for shift #11369.CB.

The difference between HN PPM values between #11369 and #11721 is about the same as the change in position for #11721 between the CBCA(CO)NH and HNCACB so that it appears when looking at the HNCACB that the peak at 37.5 PPM and comparing it to the CBCA(CO)NH peak at 32 PPM that the 37.5 PPM peak should be assigned to #11721 when in reality it should be assigned to #11369.

In resolving degeneracy for the HNCACB it is important to remember that when assigning intra-residue, ie CB and CA shifts, to compare relative positions within the HNCACB and not compare absolute positions to the CBCA(CO)NH or with ShiftCursors.
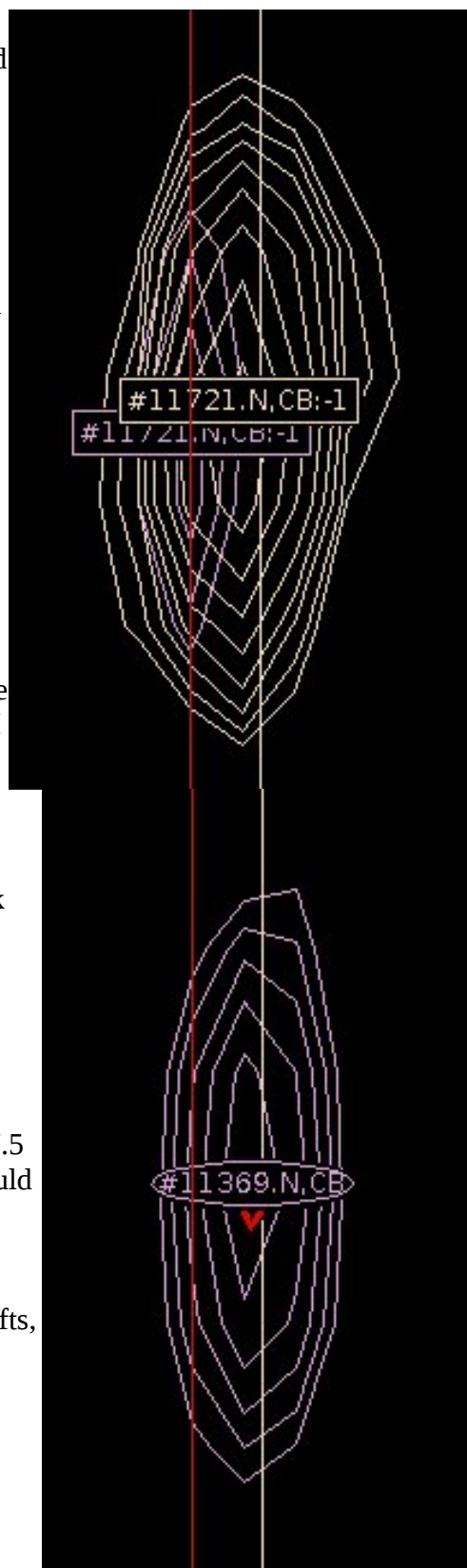
Fix Possible Wrong Assignment
in #11369

	AtomID	AtomName	Shift	RMS	Count
	11371-	N	128.7890	0.0440	6
	11372-	HN	8.9566	0.0029	6
#11369+	12746-	CB:-1	69.7757	0.0804	2
	12747-	CA:-1	62.1193	0.0000	1
	12858-	CB	37.6635	0.0000	1
	12859-	CA	61.9694	0.0000	1

Spin-System #11369 in the ShiftTable looks good, but there may be a wrong assignment in the HNCACB. The CBCA(CO)NH peak at 62.12PPM and the HNCACB peak at 61.97 are for the same atom, but they might be assigned differently depending on manual placement of the ^{15}N-HSQC peak that created #11721. If the HNCACB and CBCA(CO)NH for the peak at 62 PPM have the same assignment then there is no problem and this can be skipped.

If needed this is fixed by Clicking on the HNCACB peak at 61.97PPM that is labeled #11369.N,CA and then in Name Peak-Pick Table selecting the assignment for #11369.N,HN,CA:-1 followed by clicking on the HNCACB peak at 60.38PPM and then in the Name Peak-Pick Table selecting #11369.N,HN,CA
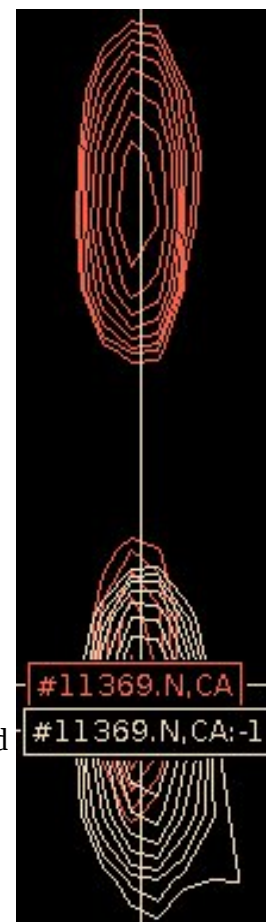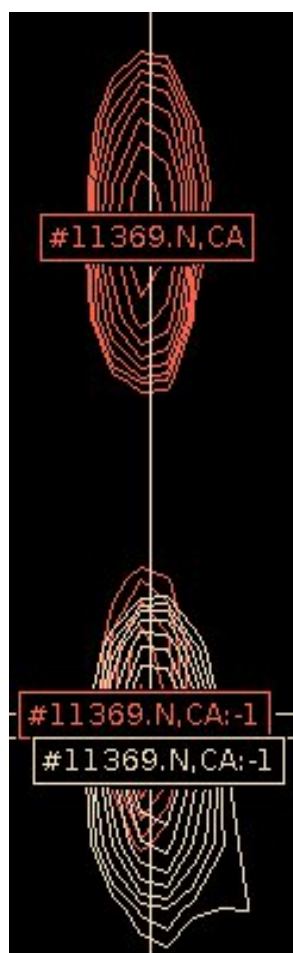
The Canvas Window and ShiftTable are now correct for #11369.

The only way to find wrong assignments of this kind is to carefully look at the spectrum and the ShiftTable at the same time. Noticing the extra peak at 60.38PPM and seeing that the HNCACB assigned a peak very close to the CA:-1 as the CA is what led to determining that this was a wrong assignment. Had the peak at 60.38PPM been below the contour threshold there would have been no way to determine that the assignment for HNCACB peak at 61.97PPM was wrong.

	AtomID	AtomName	Shift	RMS	Count
	11371-	N	128.7883	0.0407	7
	11372-	HN	8.9569	0.0028	7
#11369+	12746-	CB:-1	69.7757	0.0804	2
	12747-	CA:-1	62.0443	0.0750	2
	12858-	CB	37.6635	0.0000	1
	12859-	CA	60.3791	0.0000	1

Near Degeneracy Distorts HNCACB
Peak Missed by CAPP

	AtomID	AtomName	Shift	RMS	Count
#11396+	11406-	N	124.3432	0.0288	5
	11407-	HN	9.6350	0.0047	5
	11408-	CB:-1	40.8714	0.0295	2
	11409-	CA:-1	60.4153	0.0000	1
	11964-	CA|CB	71.2255	0.0000	1

The ShiftTable for #11396 shows two inter-residue and one intra-residue assignment.

At right is shown #11396 with the HNCACB vertical trace in red and the CBCA(CO)NH vertical trace in light blue. The trace selector is centered on the ShiftCursor for #11396. The HNCACB trace in red shows that the intra-residue peak is distorted by the near degeneracy of the CA:-1 peak. The few contours that were not distorted for the peak at 61.5 PPM were not enough for CAPP to pick it.
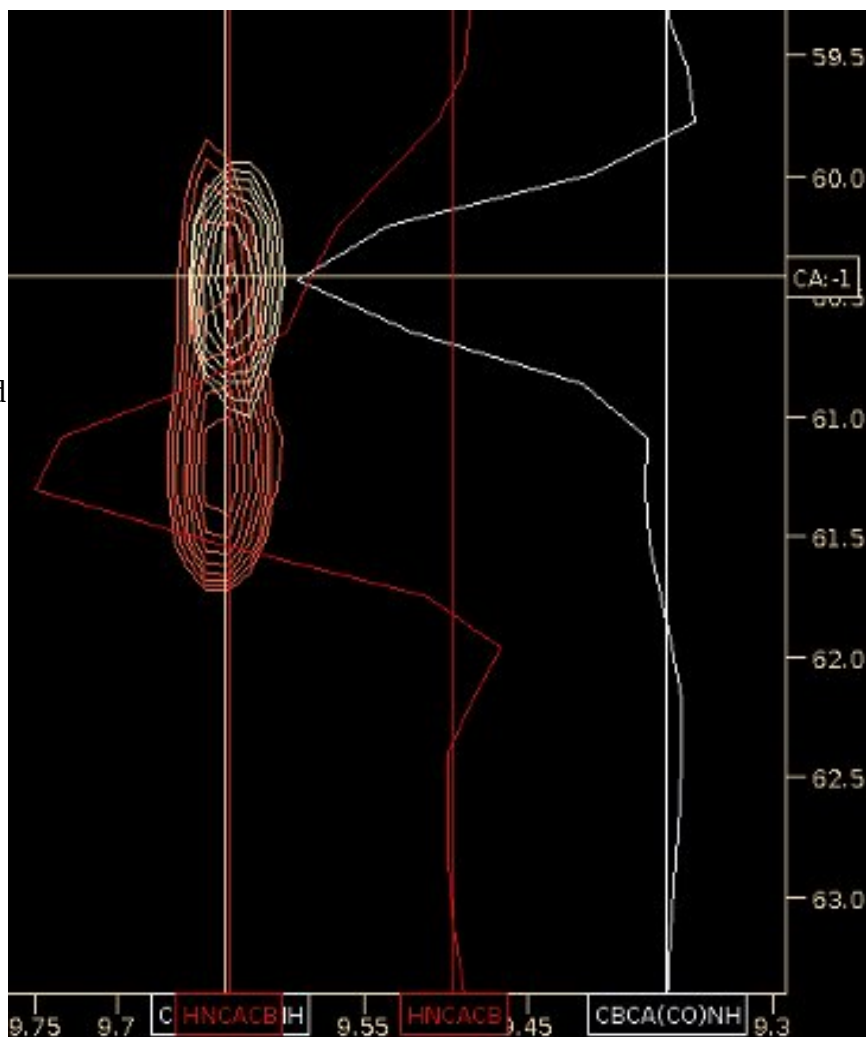
Manually adding the intra-residue peak at 61.2 PPM resolved the assignments for CA and CB.

	AtomID	AtomName	Shift	RMS	Count
#11396+	11406-	N	124.3389	0.0280	6
	11407-	HN	9.6353	0.0044	6
	11408-	CB:-1	40.8714	0.0295	2
	11409-	CA:-1	60.4153	0.0000	1
	12888-	CB	71.2255	0.0000	1
	12889-	CA	61.2116	0.0000	1

Weak HNCACB Peaks
Missed by CAPP

	AtomID	AtomName	Shift	RMS	Count
#11381+	11382-	N	126.7188	0.0130	3
	11383-	HN	9.6261	0.0063	3
	11384-	CA:-1	56.8989	0.0000	1
	11385-	CB:-1	65.1901	0.0000	1

The ShiftTable for #11381 only shows 2 assignments for 1 peak each for CA:-1 and CB:-1 which indicate that nothing was found in the HNCACB.

At right is shown #11381 with the HNCACB vertical trace in red and the CBCA(CO)NH vertical trace in blue. The trace selector is centered on the ShiftCursor for #11381. The HNCACB trace in red shows 2 weak peaks at 37.5 PPM and 54.2 PPM.
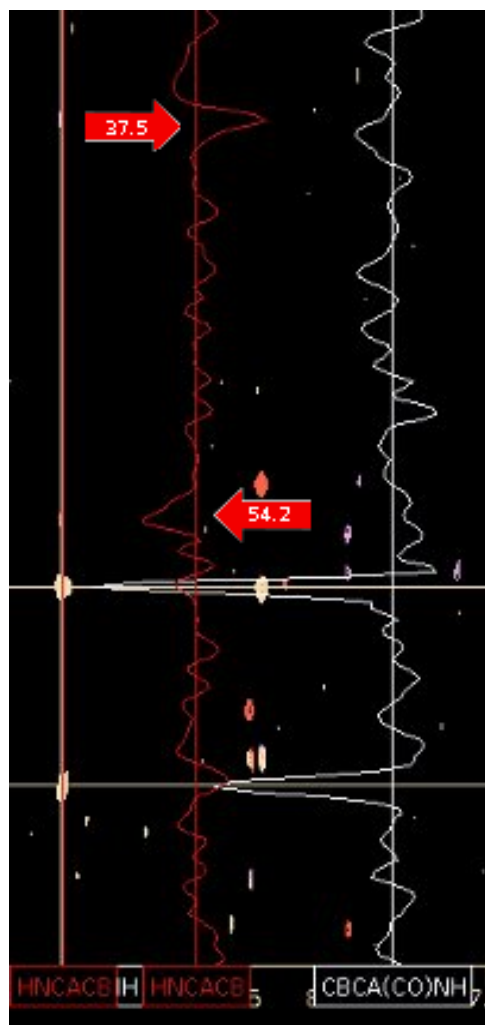
HNCACB Peaks along #11381 ShiftCursor
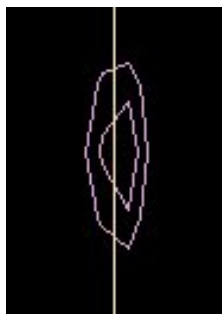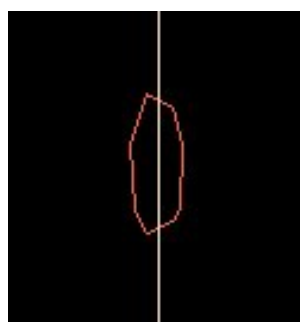
¹³C at 37.5 PPM   ¹³C at 54.2 PPM

These two HNCAB peaks are too week to be picked on their own. The reason for picking them is that the CBCA(CO)NH showed two good peaks and the ^{15}N-HSQC also showed a peak.

After adding peaks at 37.5 PPM and 54.2 PPM:

	AtomID	AtomName	Shift	RMS	Count
#11381+	11382-	N	126.6987	0.0265	5
	11383-	HN	9.6262	0.0052	5
	11384-	CA:-1	56.8989	0.0000	1
	11385-	CB:-1	65.1901	0.0000	1
	12884-	CB	37.4830	0.0000	1
	12885-	CA	54.1630	0.0000	1

Very Weak Peaks
Is it Real or Junk?

	AtomID	AtomName	Shift	RMS	Count
#11408+	11425-	N	123.2751	0.0990	2
	11426-	HN	7.7197	0.0034	2
	11427-	CA|CB:-1	62.1650	0.0000	1

The ShiftTable for #11408 only shows only 1 ambiguous inter-residue assignment.

At right is shown #11408 with the HNCACB vertical trace in red and the CBCA(CO)NH vertical trace in blue. The trace selector is centered on the ShiftCursor for #11408. The CBCA(CO)NH trace in blue shows that the inter-residue peak at 62.1PPM and may be something at 31.7 PPM.

^{15}N HSQC Peak
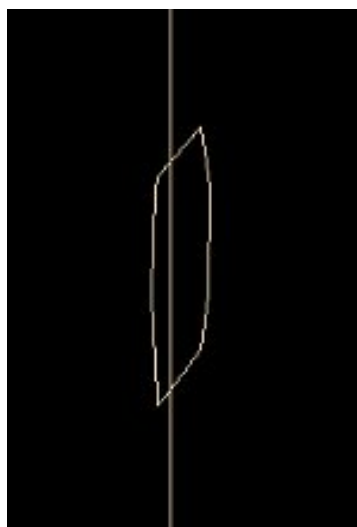
CBCA(CO)NH 31.7 PPM   CBCA(CO)NH 62.1 PPM
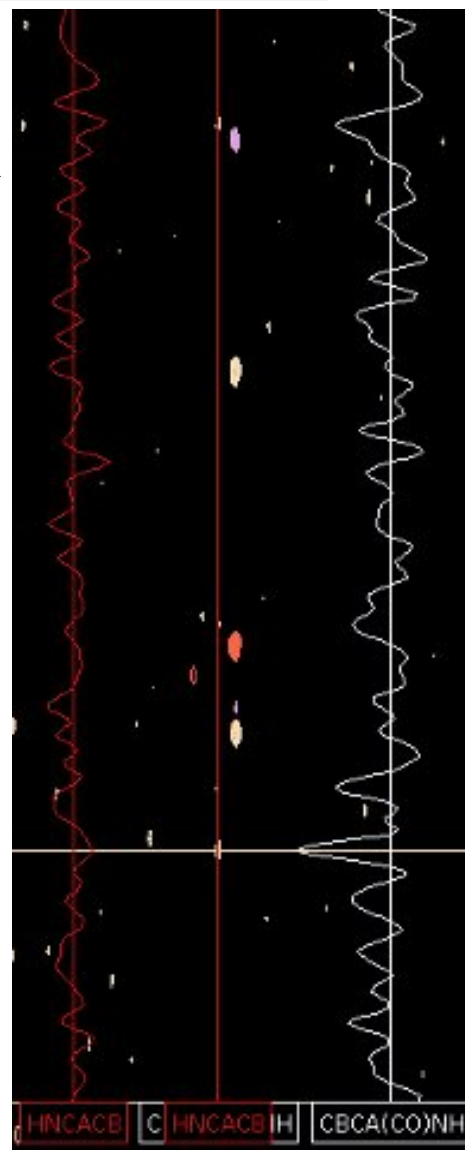
If you believe these are not real peaks, delete the HSQC and CBCA(CO)NH which will remove #11408 from ShiftTable.

If you believe that these peaks are real then you should add in the peak at 31.7PPM.

One problem is that the HNCACB shows no peaks.

Fixing GLY CA Assignments
Using Atom Quick Assign Menu

	AtomID	AtomName	Shift	RMS	Count
#11555+	11595-	N	115.2024	0.0296	6
	11596-	HN	9.1617	0.0028	6
	11597-	CB:-1	27.4132	0.0447	2
	11598-	CA:-1	58.3136	0.0216	2
	12825-	CA|CB	44.4994	0.0000	1

Fixing GLY CA is very similar to Fixing GLY CA:-1 previously described. Scroll down the ShiftTable looking for a Spin-System that has a single intra-residue ^{13}C assigned between 40 – 50 PPM, ie in the GLY CA range.

Click Left-Mouse on the SS-ID button (#11555 in this example) to residue jump. Confirm that the ^{13}C belongs to a GLY CA on the HNCACB experiment and that there is only one peak in the GLY range between 40 – 50 PPM along the HN ShiftCursor. If the peak is weak or the spectrum is crowded it is best to get a vertical trace to ensure that there is no other peak hiding under the threshold.

#11555.N,CA|CB

The Xipp assigner methods can not distinguish if a peak is a GLY CA or a Leu CB so without more information the peak is ambiguously assigned to #11555.N,HN,CA|CB

Use Atom Quick Assign Menu on ShiftTable AtomID button to change assignment and re-assign the peak. The Atom Quick Assign Menu only exists when an atom is has an ambiguous assignment such as CA|CB. Once the shift is assigned to a single atom the Atom Quick Assign options are not shown. Use the ReAssign Shift . . . menu option as described in Xipp_Commands to bring up the 'Select Atom Dialog' to assign the Shift to any existing or new atom.

Click Right-Mouse on button for AtomID 12825 for GLY CA assignment to be fixed in ShiftTable and select CA.

	AtomID	AtomName	Shift
#11555+	11595-	N	115.2030
	11596-	HN	9.1617
	11597-	CB:-1	27.4132
	11598-	CA:-1	58.3136
	12825-	CA|CB	44.4994

		☐ Table +/-	Shift
	AtomID	ReAssign Shift . . .	
	11599-	CA	115.1601
	11600-	CB	7.8207

Check all Spin-Systems
Except #11486

At this point you should visually inspect on the Canvas and the Shift-Table Windows all Spin-Systems and confirm that there are 3 (for Gly) or 4 peaks that are assigned to specific atoms except for Spin-System #11486. As described below Spin-System #11486 will be used to demonstrate resolving difficult ambiguities using backbone assignments.

On the right is a strip from the CACB_Full(N) Canvas view showing the CBCA(CO)NH and HNCACB experiments.

On the left is the ^{15}N HSQC for #11486 which is at 7.32 PPM and 119.3 PPM.

Although the HN and ^{15}N positions are almost completely overlapped for these two Spin-Systems a careful look at each peak on the Canvas and in the Peak-Table (below) shows that the CBCA(CO)NH peaks 98 and 95 belong to the same Spin-System with HNCACB peaks 189 and 191. The second Spin-System has CBCA(CO)NH peak 96 (a Gly) with HNCACB peaks 188, 190 and 192.

Do not create a new Spin-System and do not uniquely assign the peaks for #11486. Leave the peaks as ambiguous assigns to CA|CB and CA|CB:-1. I will use Spin-System #11486 as an example after Mars automatically assigns many peaks to demonstrate how completely degenerate HN, ^{15}N Spin-Systems can be manually resolved using assigned spin-systems.
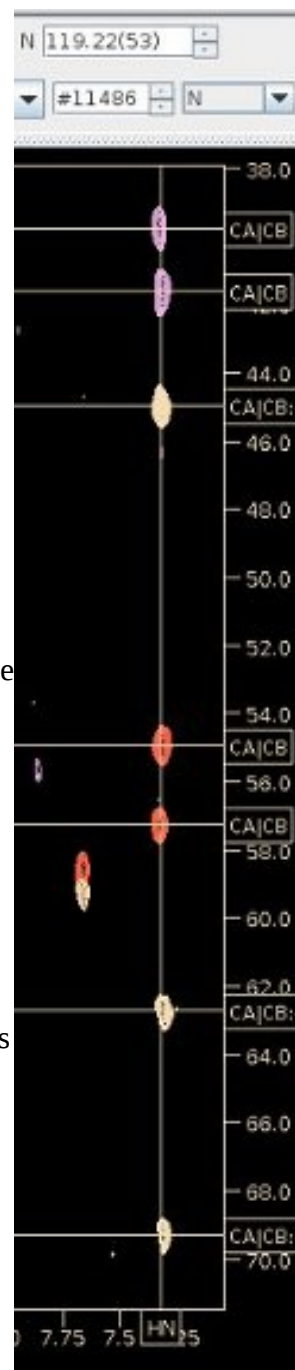
Extracted from Peak-Pick Table showing only #11486 peaks. Xipp does NOT sort peaks by assignment this figure was made by combining disjoint rows into a single figure.

Exp	PkID	Assign	HN	CACB	N	Intensity
HNCACB	191	#11486.N,HN,CA|CB	7.326	41.61	119.30	-5.608e+6
HNCACB	192	#11486.N,HN,CA|CB	7.338	39.75	119.16	-3.297e+6
HNCACB	190	#11486.N,HN,CA|CB:-1	7.338	44.94	119.13	1.863e+6
CBCA(CO)NH	96	#11486.N,HN,CA|CB:-1	7.329	44.97	119.15	1.002e+7
HNCACB	189	#11486.N,HN,CA|CB	7.326	54.93	119.30	7.744e+6
HNCACB	188	#11486.N,HN,CA|CB	7.337	57.22	119.17	6.03e+6
CBCA(CO)NH	98	#11486.N,HN,CA|CB:-1	7.317	62.72	119.31	4.44e+6
CBCA(CO)NH	95	#11486.N,HN,CA|CB:-1	7.317	69.25	119.30	2.938e+6

(5) Initial Assignments via MARS program

The automated backbone assignment program MARS is an external program developed by Y.-S Jung and M. Zweckstetter, J. Biol NMR, 30, 11-23 (2004). At this time MARS only runs on Linux computers and older Mac computers. For more information about MARS see:
>   http://www.mpibpc.mpg.de/groups/zweckstetter/_links/software_mars.htm

On Linux systems only open MARS dialog Panel from Assign Menu.
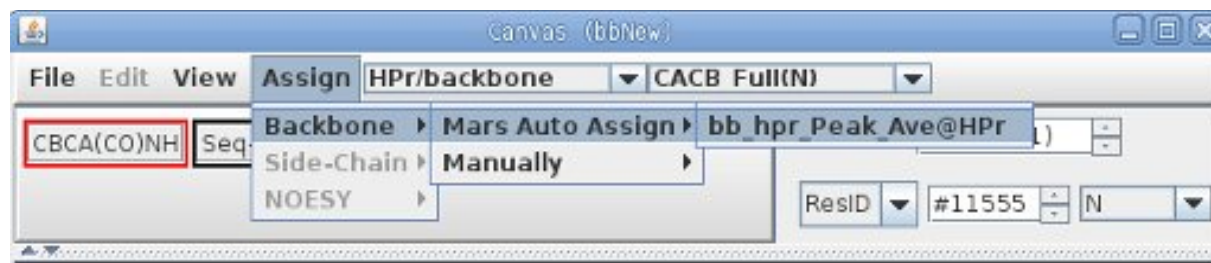
The MARS Dialog is shown on the next page

At the present time the only automated backbone assignment program used by Xipp is MARS and MARS only runs on Linux computers and PowerPC Macs. The study bbMars can be used on non Linux systems to continue the tutorial for users that can not run MARS. On non Linux systems enter the Canvas Command **x** to exit bbNew study and exit XippPanel. Start xipp again and show the bbMars study which picks up the tutorial after running MARS.

MARS Dialog

The default properties for MARS are OK for most situations. Click OK at the bottom of this dialog to start MARS which will immediately bring up the MARS Auto-Assign Process Details.

MARS Auto-Assign Process Details
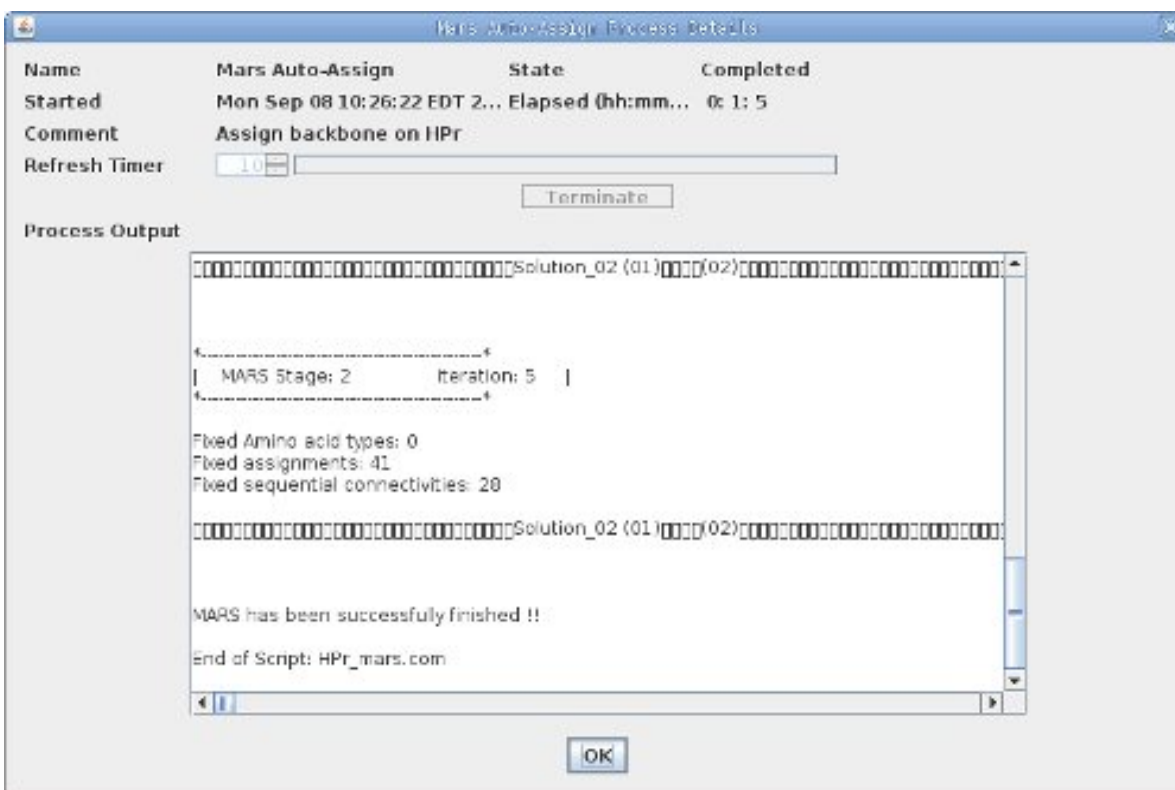
MARS is fast for small and moderate sized proteins such as HPr (85 residues), but it is non-linear with respect to the size of the protein and can take several hours for a 300 residue protein.

Click OK on the MARS Auto Assign Completed dialog to read in the MARS results and update the assignments.
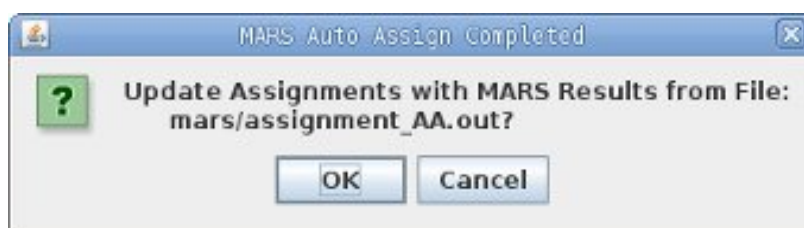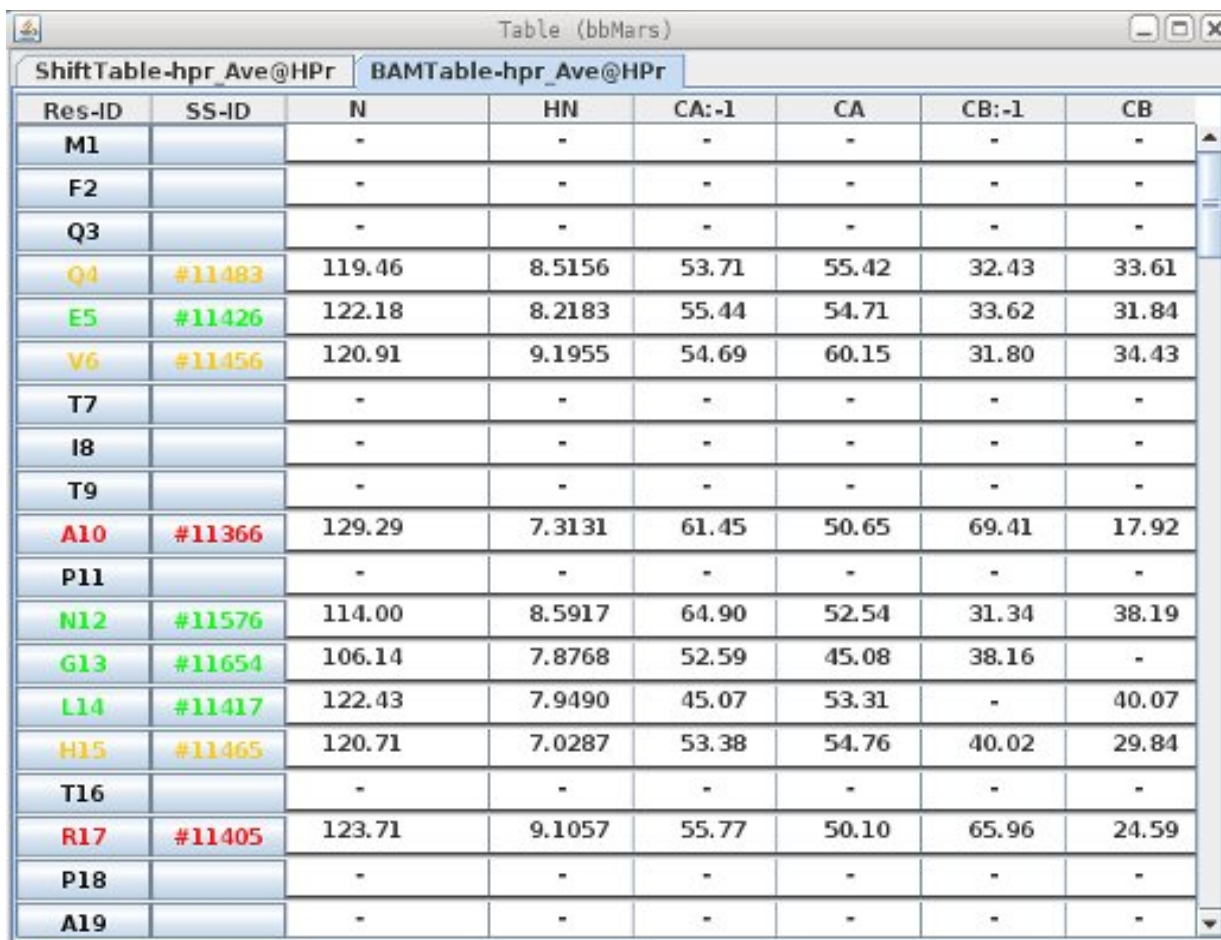
The file mars/assignment_AA.out is read in to assign Spin-Systems to specific residues. As each Spin-System is assigned the Peak-Pick assignments for each newly assigned atom is updated as well as the ShiftTable. In addition the BAMTable is updated to reflect the new assignments so that each Spin-System ID appears on a single line.

The assignments created by MARS are tentative and must be verified manually to ensure that they are correct.

On non Linux systems enter the Canvas Command x to exit bbNew study and exit XippPanel. Start xipp again and show the bbMars study which picks up the tutorial after running MARS.

(6) Inspect/Clean-up Assignments using BAMTable

On non Linux systems show the bbMars study which picks up the tutorial after running MARS. Since the bbNew and bbMars study use some of the same files you must completely exit bbNew and XippPanel before showing bbMars. The results from Mars strongly depends on how well each Spin-System was correctly assigned. The results here are from the bbMars Study and are the results of minimal cleaning/fixing up of the Spin-Systems.

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
M1		.	.	.	.	.	.
F2		.	.	.	.	.	.
Q3		.	.	.	.	.	.
Q4	#11483	119.46	8.5156	53.71	55.42	32.43	33.61
E5	#11426	122.18	8.2183	55.44	54.71	33.62	31.84
V6	#11456	120.91	9.1955	54.69	60.15	31.80	34.43
T7		.	.	.	.	.	.
I8		.	.	.	.	.	.
T9		.	.	.	.	.	.
A10	#11366	129.29	7.3131	61.45	50.65	69.41	17.92
P11		.	.	.	.	.	.
N12	#11576	114.00	8.5917	64.90	52.54	31.34	38.19
G13	#11654	106.14	7.8768	52.59	45.08	38.16	.
L14	#11417	122.43	7.9490	45.07	53.31	.	40.07
H15	#11465	120.71	7.0287	53.38	54.76	40.02	29.84
T16		.	.	.	.	.	.
R17	#11405	123.71	9.1057	55.77	50.10	65.96	24.59
P18		.	.	.	.	.	.
A19		.	.	.	.	.	.

(Table window title: Table (bbMars); Tabs: ShiftTable-hpr_Ave@HPr, BAMTable-hpr_Ave@HPr)

Each row in the BAMTable is an amide defined Spin-System that is either assigned (on a line with a Res-ID) or unassigned. A Spin-System can only be shown on one line and therefore can only be assigned to one residue at a time. The BAMTable is divided in two groups so that all assigned Spin-Systems are shown first at the top of the list sorted by Res-ID followed by all unassigned Spin-Systems sorted by SS-ID. The color of the text on the Res-ID and SS-ID indicate the quality of the assignment with black indicating that it is unassigned, green indicating at most 1 minor problem, yellow indicating 2 minor problems and red indicating major problems.

The Res-ID and SS-ID are ButtonPopup objects with default action to ResJump to residue or Spin-System defined by the button. In order to keep the CA:-1 and CB:-1 together when a Spin-System is assigned there is a separate internal hidden assignment table used by the BAMTable which keeps all Spin-Systems unassigned so that :-1 assigns are not automatically merged into the preceding Res-ID as is done for the Shift-Table when an SS-ID is assigned.

Click Right-Mouse on Res-ID Button

The menu options with the Res-ID and SS-ID buttons form the basis for manual backbone assignment. **Click Right Mouse** over Res-ID button for the menu options for that Res-ID such as T16:

> **Jump** (Default Action)
> Res Jump to this Res-ID (T16).
> **Unassign Residue**
> Unassigns this residue.
> Not available since T16 unassigned
> **Undo**
> Undo most recent assign/unassign to this Res-ID.
> Not avaialbe since T16 has not been assigned.
> **Assign Residue**
> Brings up modal dialog to assign T16.
> > **# Columns Matched**
> > Where # is a number between 1 to 4. Shows in sub-menu all Spin-Systems that have # columns that match between this Spin-System and the preceding and next Spin-Systems.

The sub-menu options under the Columns Matched menus are all the Spin-Systems that could be assigned to this resID, ie T16, based on comparing values in columns CA, CA:-1, CB and CB:-1 of potential matches with the previous and/or next assigned spin-Systems. These menu options are grouped based on the number of column matches found. The '4 Columns Matched' menu group is the largest number of matches possible with CA, CA:-1, CB, CB:-1 columns and indicates the all pairs of matches were found. The matches found under Columns Matched are dynamically determined just before the menu pops up based on all Spin-Systems. There is only 1 possibility under '2 Columns Matched' which is #11594 and given the assignment for H15 is the most likely assignment for T16.

The '1 Columns Matched' for T16 shows all of the Spin-Systems that have exactly 1 column matching.

The matching Spin-System is displayed in ( ) and if it is currently assigned the assignment is shown in the first column. If unassigned then – is shown in the first column and it is written in black. The last column is the RMS deviation of the matched columns for this Spin-System. Columns not in the match are not averaged. The color of the text is the same color as shown on the BAM-Table for the current assignment: black indicates unassigned, green is a good assignment, yellow has minor problems and red has major problems. The color indicates the current assignment NOT the new assignment so if there are no unassigned Spin-Systems and there are two possible assigned Spin-Systems you should always choose the red assignment initially since that assignment has major problems in its current assignment.

Click Right-Mouse on SS-ID Button

 Click Right Mouse over SS-ID button for the menu options available for that SS-ID such as #11465 (assigned to H15):

> **Jump** (Default Action)
>> Res Jump to this Spin-System (#11465).
>> If SS-ID is assigned jumps to Res-ID (H15).
>
> **Find Prev SS-ID**
>> Shows in sub-menu all possible Spin-Systems that can be previous to this Spin-System sorted by columns matched and RMS. Selecting a sub-menu option sets the Canvas and Name-Peak Table to show both Spin-Systems. No Assigns are made. See Find Prev/Next SS-ID below for more details.
>
> **Find Next SS-ID**
>> Shows in sub-menu all possible Spin-Systems that can follow this Spin-System (be next SS-ID) sorted by columns matched and RMS. Selecting a sub-menu option sets the Canvas and Name-Peak Table to show both Spin-Systems. No Assigns are made. See Find Prev/Next SS-ID below for more details.
>
> **Unassign SS-ID**
>> Unassigns this Spin-System from Res-ID.
>
> **Undo**
>> Undo most recent assign/unassign to this SS-ID.
>> Not available since #11465 has not been assigned or unassigned.
>
> **Prev ResTypes**
>> Shows in sub-menu all possible residue types that can be previous to this Spin-System based on CA:-1 and CB:-1 shift values. These menu option do not change anything.
>
> **Cur ResTypes**
>> Shows in sub-menu all possible residue types of this Spin-System based on CA and CB shift values. These menu option do not change anything
>
> **Delete #11465**
>> Will ask for confirmation to delete Spin-System which will remove assignments. This will unassign the peaks assigned to this SS-ID. This is unavailable for #11465 since you can not delete Spin-Systems that are assigned to a Res-ID. There is no Undo for the Delete action.
>
> **Assign Moiety**
>> Brings up modal dialog to assign Spin-System #11465.
>
>> **# Columns Matched**
>>> Where # is a number between 1 to 4. Shows in a sub-menu all Spin-Systems that have # columns that match between this Spin-System and the preceding and next Spin-Systems.

The sub-menu options under the Columns Matched menus are all the Res-IDs that #11465 could be assigned to, based on comparing values in columns CA, CA:-1, CB and CB:-1 of potential matches with the previous and/or next assigned Spin-Systems. These menu options are grouped based on the number of column matches found and sorted by RMS of matched columns within each group.

SS-ID Button: Find Prev/Next SS-ID

The Find Prev SS-ID and Find Next SS-ID menu options are grouped under Jump because they involve a ResJump and do not make any assignments. The purpose of these menu options is to show experimental data on the Canvas and Name Peak-Pick Table to help make sequential assignments. The description below focuses on the Find Prev SS-ID menu options. The Find Next SS-ID menu options are similar except that it is looking for the next SS-ID and the Seq-CBCA(CO)NH is used.

After Clicking the Find Prev SS-ID menu and selecting option "L14 (#11417) 0.0614" three changes are made to the Canvas and Table Windows that mimic what you would do manually in order to look for the previous SS-ID:

(1) In Canvas ResJump to H15 since #11465 is assigned to H15,
(2) In Name PeakPick Table add Peak-Picks defining H15.CA:-1 and H15.CB:-1 Shifts.
(3) In Canvas set Seq-HNCACB slice to #11417 ^{15}N and add PPM Cursors at #11417 HN, CA and CB Shift values.

The Name PeakPick Table gets updated with two peaks from the CBCA(CO)NH experiment for H15.CA:-1 and H15.CB:-1 as if these peaks were manually picked.

The Exp Button Seq-HNCAB manages a second view into the HNCACB whose ^{15}N slice is set to L14 N shift by the Find Prev SS-ID menu option. In addition the Seq-HNCACB has three PPM Cursors drawn in red at the PPM shift for L14 HN, CA and CB (not shown in figure). **Left Click Mouse** over the Seq-HNCACB experiment button toggles on/off this second view which includes the red PPM Cursors.
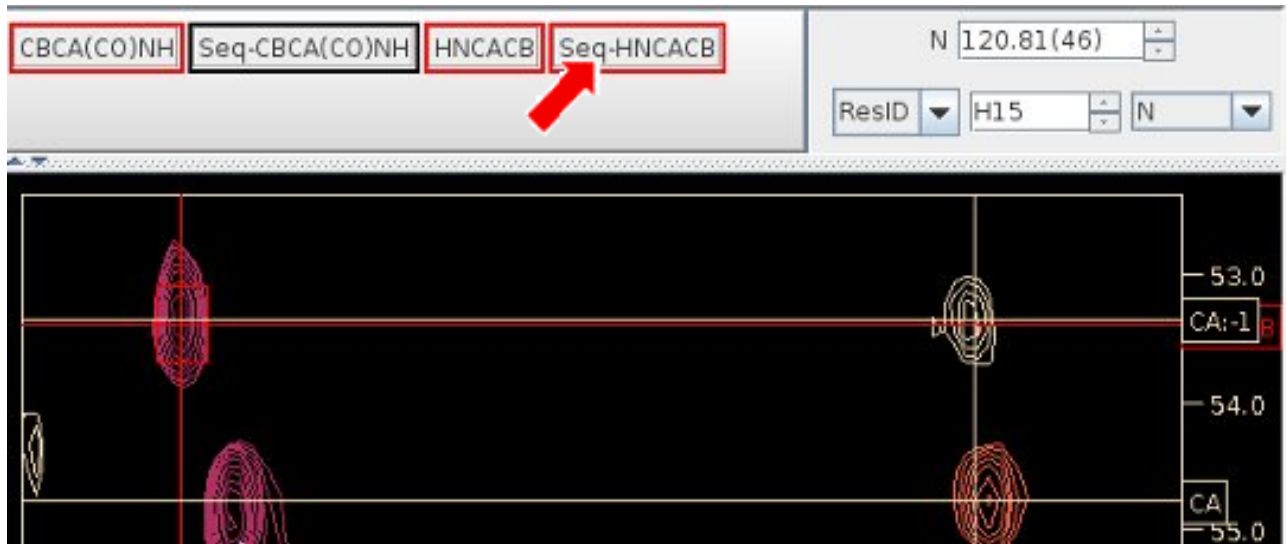
Right-Clicking the Mouse over the Seq-HNCACB Button brings up the Seq-Exp Menu:

- **Active**: Toggle Active state of Exp.
- **Linked**: Toggles linked state of Exp. When Linked is selected changes to slice of Seq-HNCACB will also change slice for Seq-CBCA(CO)NH
- **Jump to Slice**: Displays non-modal Frequency/Residue Chooser dialog for this Seq-HNCACB view.
- **Contour Levels:** Displays modal dialog to change contour levels.
- **Clear PPM Cursors**: PPM Cursors identify potential sequential residues on the Seq-HNCACB view. This option clears all PPM cursors from this Seq-HNCACB view. It erases the red lines.
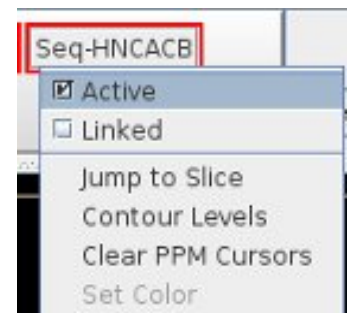
The Frequency/Residue Chooser dialog which is created by the Jump to Slice menu option allows explicit/manually changing the Seq-HNCACB ^{15}N slice by slice number, PPM value or by ResID or SS-ID in exactly the same way that the main Canvas selects the Slice. This window is non-modal so you can keep it open while interacting with Canvas and Table Window. Changes to Seq-HNCACB from Find Prev SS-ID menu options will update the slice and PPM shown here but not the ResID.
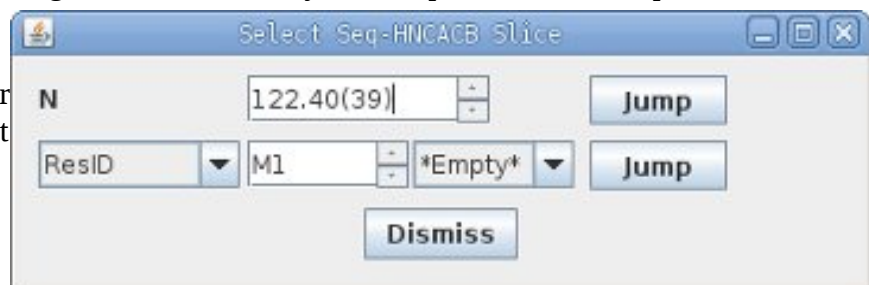
The Seq-HNCACB slice position and PPM Cursors are also updated when the S+ table entries are selected in the Name PeakPick Table. The S+ table entries show all of the possible sequential strips using the single ^{13}C shift of the peak and the Extrema for the HNCACB experiment. Since this uses a single ^{13}C shift instead of two that are used by Find Prev SS-ID this has the potential to find a lot of possible matches. By default all matches start with Require Confirmation enabled which requires that any match be confirmed by having another ^{13}C along the found strip to match a ^{13}C from an Extrema along strip that was peak-picked. For instance if the CA peak was used to find a sequential strip then this confirms the strip by finding another peak in common such as the CB. For H15.CA:-1 when Required Confirmation is disabled there are 24 possible sequential matches found instead of 9 that are found when it is enabled.

Select the top line in S+ for the CA:-1 assign and check to see if it looks good. If not then with the Mouse still in the S+ table click the Down-Arrow key to select the second line and see if that is good. Only one ^{13}C PPM Cursor is shown at a time from the S+ table which corresponds to the Extrema on the S+ table most recently selected. You can select a line from the CA:-1 and CB:-1 S+ tables at the same time, but only the last line selected will have a PPM Cursor shown on the Canvas.

Contours	PkID A +- S +/-	Assign		HN	CACB	N	Intensity
			Assignment-Table				
			Seq Strip-Table				
	80	H15.N,HN,CA:-1		7.039	53.381	120.6...	1.827e+6
	K +	CBCA(CO)NH Assigns (1)			HN	CACB	N
		H15.N,HN,CA:-1			-0.0098	-0.0371	0.0577

	S + 9	Exp	Slice	HN	CACB	N	Intensity
		HNCACB	42	7.857	53.659	121.643	-1.039e+6
		HNCACB	32	8.109	53.607	123.972	+5.176e+6
		HNCACB	90	7.240	53.566	110.835	+1.321e+6

	81	H15.N,HN,CB:-1		7.028	40.025	120.6...	2.23e+6
	K +	CBCA(CO)NH Assigns (1)			HN	CACB	N
		H15.N,HN,CB:-1			0.0007	0.0225	0.0804

	S + 12	Exp	Slice	HN	CACB	N	Intensity
		HNCACB	39	7.950	40.070	122.435	-5.119e+6
		HNCACB	85	5.328	40.053	112.041	-7.827e+5
		HNCACB	57	7.945	39.966	118.316	-2.928e+6

SS-ID Button: Unassign SS-ID & Undo Unassign

The Unassign SS-ID menu option works in exactly the same way as the Res-ID Button Unassign Res-ID by unassigning the Res-ID and SS-ID and updating the menu options for Res-ID and SS-ID to include 'Undo Unassign H15 - #11465'

The 'Undo Unassign H15 - #11465' is shown on H15 Button and #11465 button and reverses the Unassignment so that H15 and #11465 are assigned again. Each Button, Res-ID and SS-ID, can have at most 1 Undo.

SS-ID Button: Prev ResTypes & Cur ResTypes

The Prev ResTypes and Cur ResTypes show in a drop-down list the possible residue types for the previous SS-ID and the current SS-ID respectively based on CA and CB shift values. Selecting a specific ResType from the drop-down list does nothing. The list is information only to help make sequential assignments.

SS-ID Button: # Columns Matched

The menu options shown under the Columns Matched menus are all the Res-IDS that this SS-ID could be assigned to. All Spin-Systems that match are shown, but unassigned SS-ID options (have a – for Res-ID such as #11594) are grayed out since it does not make sense to assign a Spin-System to an unassigned Spin-System. The matches are made by comparing values in columns CA, CA:-1, CB and CB:-1 from #11465 to potential matches from all Spin-Systems. These menu options are grouped based on the number of column matches found. The highest number of Columns Matched is 4 which is only possible if the Spin-System being tested is matched between two assigned Res-IDs and indicates that all pairs of matches were found. The matches found under Columns Matched are dynamically determined just before the menu pops up based on all Spin-Systems.

The matching Res-ID is shown in the first column before the ( ). If the SS-ID is unassigned then – is shown in first column and the menu option can not be selected. The last column is the RMS deviation of the matched columns for this Spin-System. Columns not in the match are not included in the RMS. The color of the text is the same color as shown on the BAM-Table for the current assignment: gray indicates unassigned, green is a good assignment, yellow has minor problems and red has major problems. The color indicates the current assignment NOT the new assignment so if there are no unassigned Res-IDs and there are two possible assigned Res-IDs you should always choose the red assignment initially since that assignment has more problems.

Selecting a menu option under the Columns Matched changes the assignment of this SS-ID (#11465) to the Res-ID defined in the menu option. If this SS-ID and/or the Res-ID selected is assigned a confirmation dialog will appear describing the changes to be made. Thw Warning dialog at right is what would be shown if #11465 would be re-assigned from H15 to T56. The Undo Assign T56 – #11465 reverses these 3 steps.
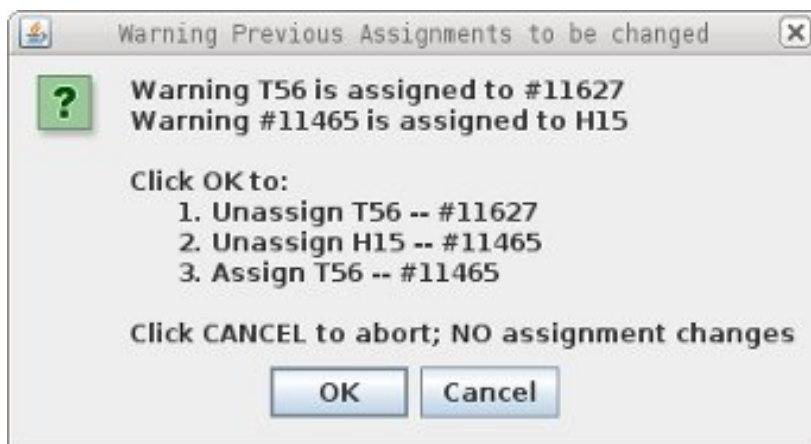
Columns Matched: CA|CB Affects Results

The menu options shown under the Columns Matched menus are different between SS-ID button and RES-ID button when ambiguous assignments such as CA|CB and CA|CB:-1 exist in the assignment table. These ambiguous assignments can arise from GLY CA, missing peaks or degenerate ^{15}N, HN Spin-Systems such as #11486.

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
	#11486	119.24	7.3280	-	-	-	-

Starting from SS-ID button #11486 there are no Prev SS-ID or Next SS-ID and Columns Matched are not found because all of the assignments in #11486 are ambiguous. The SS-ID match starts with the unique assigns for the Spin-System under question and since there are no unique assigns for #11486 nothing is found.

Starting from Res-ID buttons F29 or L53 the Spin-System #11486 shows up under 3 Columns Matched and 4 Columns Matched respectively. The Res-ID match starts with the unique assigns for the previous and next residue which for F29 and L53 are complete. The starting assignments must be unique to be used, but the found Spin-Systems can have ambiguous assignments.

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
G28	#11657	105.32	7.4987	58.57	45.00	32.03	-
F29		-	-	-	-	-	-
T30	#11510	118.44	10.8954	57.26	63.74	39.72	68.95

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
T52	#11651	106.31	7.2356	57.77	62.61	28.05	69.12
L53		-	-	-	-	-	-
G54	#11648	107.18	8.0907	54.90	46.81	41.60	-

Finding/Fixing #11486

There are no special tools to find that #11486 should be assigned to F29 and L53.
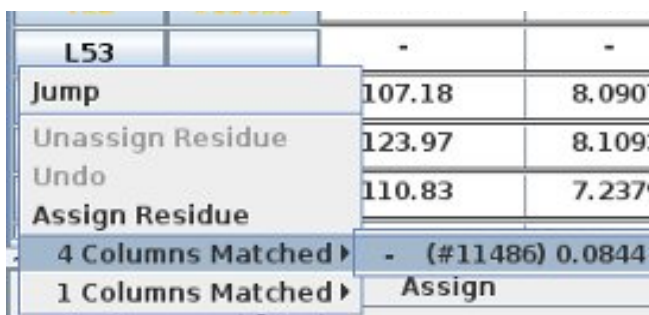
There are two ways to handle an SS-ID that has two Spin-Systems such as #11486:
>   (1) Split up #11486 assignments into two unassigned Spin-Systems, but any mistakes and searching from F29 and L53 will give mixed results,
>   (2) Leave #11486 with ambiguous assignments from two Spin-Systems until the SS-ID button menu options 'Find Next SS-ID' or 'Find Prev SS-ID' matches #11486.

If the assignments can be easily separated then option (1) is best which was described previously for the CBCA(CO)NH with #11369 and #11721. However, if there is any problem separating the assignments then option (2) is best since it keeps all the information together to easily extend existing assignments.
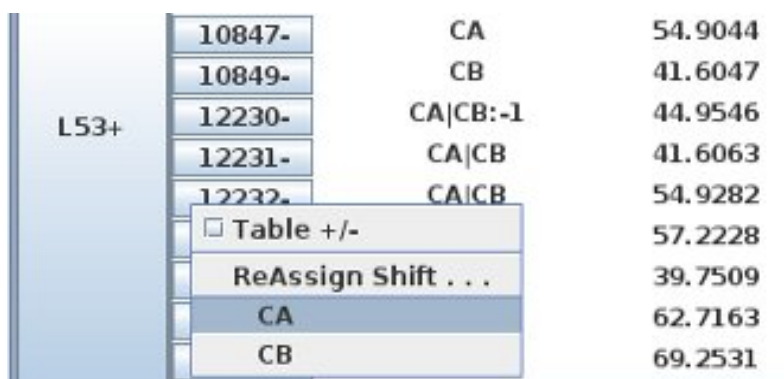
Click Right Mouse on L53 in the BAMTable and select under '4 Columns Matched' the menu option with #11486 to assign all of the atoms in #11486 to L53.

On the ShiftTable use the QuickAssign menu option from AtomID button to merge the same shift values from ambiguous assignments (CA|CB) to specific assignments with the same Shift value. The four Shifts to be QuickAssigned ar shown with a red arrow on the next page. For example the CA|CB at 54.93 PPM should be QuickAssigned to CA since L53 has CA at 54.90 PPM.

The QuickAssign menu option will change the PeakPick assignment as well as averaging the peak position in the ShiftTable and BAMTable shift value.

QuickAssign Ambiguous Assigns to Specific Assigns in L53 and T52

Before using QuickAssign on 4 assignments.

T52+	AtomID	AtomName	Shift	RMS	Count
	10830-	N	106.3054	0.0203	5
	10831-	HN	7.2356	0.0021	5
	10832-	CA	62.6130	0.0000	1
	10834-	CB	69.1218	0.0000	1

L53+	AtomID	AtomName	Shift	RMS	Count
	10845-	N	119.2424	0.0805	9
	10846-	HN	7.3280	0.0080	9
	10847-	CA	54.9044	0.0411	2
	10849-	CB	41.6047	0.0342	2
	12230-	CA|CB:-1	44.9546	0.0114	2
	12231-	CA|CB **CB**	41.6063	0.0000	1
	12232-	CA|CB **CA**	54.9282	0.0000	1
	12233-	CA|CB	57.2228	0.0000	1
	12234-	CA|CB	39.7509	0.0000	1
	12235-	CA|CB:-1 **CA:-1**	62.7163	0.0000	1
	12236-	CA|CB:-1 **CB:-1**	69.2531	0.0000	1

Use QuickAssign menu option to merge: CA|CB at 41.6 to CB, CA|CB at 54.92 to CA, CA|CB:-1 at 62.72 to CA:-1, and CA|CB:-1 to CB:-1 to get the following ShiftTable.

After using QuickAssign

T52+	AtomID	AtomName	Shift	RMS	Count
	10830-	N	106.3054	0.0203	5
	10831-	HN	7.2356	0.0021	5
	10832-	CA	62.6647	0.0517	2
	10834-	CB	69.1874	0.0656	2

L53-	AtomID	AtomName	Shift	RMS	Count
	10845-	N	119.2424	0.0805	9
	10846-	HN	7.3280	0.0080	9
	10847-	CA	54.9123	0.0354	3
	10849-	CB	41.6052	0.0280	3
	12230-	CA|CB:-1	44.9546	0.0114	2
	12233-	CA|CB	57.2228	0.0000	1
	12234-	CA|CB	39.7509	0.0000	1

The ambiguous assignments that remain at shift values 44.95, 57.22 and 39.75 PPM belong to a different Spin-System. Do not use QuickAssign on these since that would merge different assignments with PPM values that are very different. If QuickAssign is accidentally used the easiest solution is to delete the PeakPick and re-PeakPick and assign to an ambiguous assignment in L53 or to a new Spin-System.

Create new Moiety for Spin-System
and Assign leftovers from L53

The ^{15}N-HSQC peak at right for L53 (#11486) does not show two peaks so there is no benefit to create a second peak in the HSQC.

There are two ways to create a new Moiety and move one of the assignments to the new Moiety/Spin-System. Both methods rely on first **Click Left-Mouse** the CBCA(CO)NH peak at 45 PPM that is assigned L53.CA|CB:-1 to add the peak into the Name PeakPick Table.

The Name PeakPick Table should have PeakID 96 near the top.

1: The easy method to create a new Moiety starts by **Click Right-Mouse** over button K+ and then under CBCA(CO)NH-Assign menu select New-Rule.

The K+ should change to N+ and there should be one un-selected assignment for the peak using a new Moiety ID. If there is no assignment then double click the N+ button with the **Left Mouse** button.

Selecting (ie **Click Left-Mouse**) the new assignment shown (#12234.N,HN,CA|CB:-1) will create Moiety/Spin-System #12234 and assign the Peak to it. Below shows the Name PeakPick Table after selecting #12234. Your SS-ID number will likely be different than #12234.

Move Last two HNCACB assigns to #12234

Move the shifts for the remaining leftover HNCACB L53 peaks that are at 39.8 and 57.2 PPM to #12234. **Click Left-Mouse** on the peaks in the Canvas so that the Name PeakPick Table is updated. Then for both peaks change from Known-Rule to Extended-Rule and select the #12234 assignment. The Name PeakPick Table should look like this when done. The S table has been minimized for both peaks, that is why it is S-.
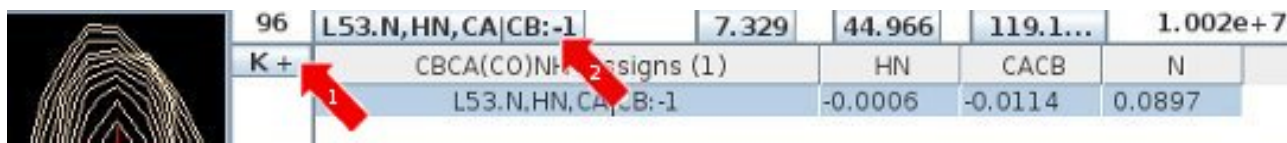
Use QuickAssign on ShiftTable for #12234 ambiguous assign CA|CB:-1 to change it to a CA:-1 since the previous residue is likely a Gly.

#12234 on the BAMTable below shows that F29 is the best match for this Spin-System.
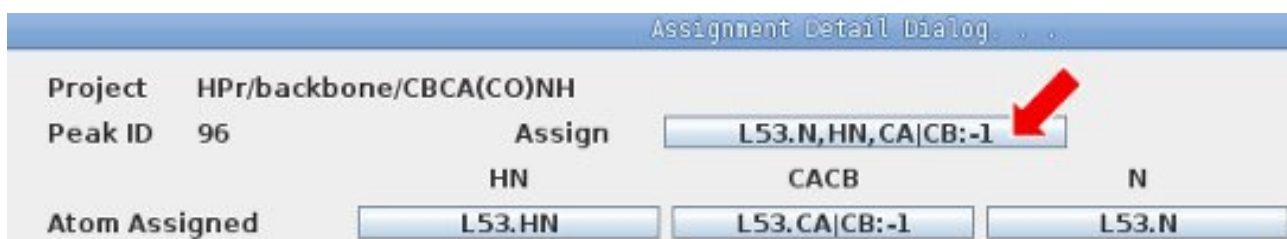
Second Method to create a new Moiety

2: The second method is more difficult because it involves more steps, but it is a much more general way to change peak assignments. This method starts by **Click Left Mouse** over the peak assignment button (red arrow with 2) labeled L53.N,HN,CA|CB:-1 to bring up the Assignment Detail Dialog. The PeakPick assignments and ShiftTable were reverted back to their previous values from before creating #12234 by restoring from a Jar backup made before #11486 was assigned to L53. See section on Recovery Files in Xipp_Commands concerning Jar recovery files.

On the Assignment Detail Dialog above click on the Assign button L53.N,HN,CA|CB:-1 to bring up the Change Residue L53 dialog shown at right and click Select for New Moiety #12267. The New Moiety number you get is arbitrary. What is important is that it is a unique Spin-System ID.
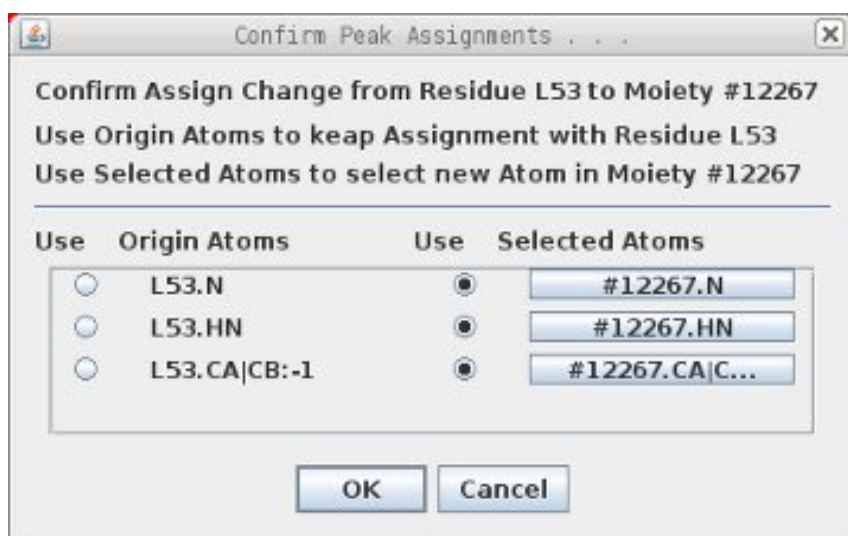
After clicking Select for New Moiety #12267 the Confirm Peak Assignments Dialog at right is shown.

Always check the dialog before clicking OK since if there are any problems moving assignments to a new Moiety such as attempting to move a CB assignment into a Gly the Problem Atoms dialog is shown before the Confirm Peak dialog which will identify which assignments can not be moved.

After clicking OK from on the Confirm Peak Assignments the Assignment Detail Dialog is updated as shown below to use #12267.
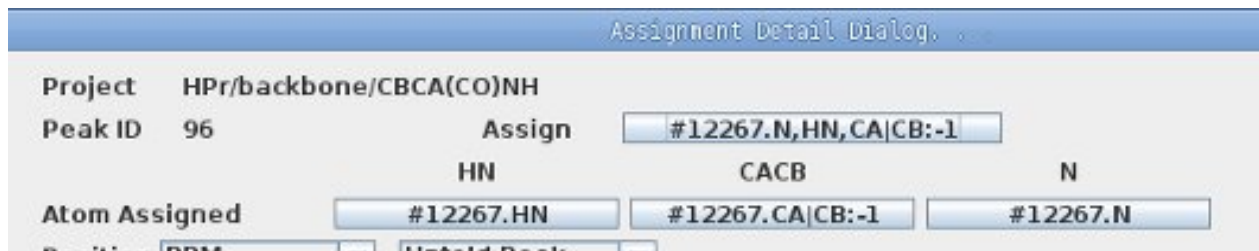
Clicking OK at the bottom (not shown) of the Assignment Detail Dialog changes the PeakPick assignment, the ShiftTable and BAMTable, but the Canvas is not updated until the space bar is hit twice.

At this point a new Spin-System (#12267 in this case) exists with HN and ^{15}N assignments and the steps in the previous section named "Move Last two HNCACB assigns to #12234" can be easily adapted to move the peaks at 39.8 and 57.2 PPM to this newly created Spin-System. In the BAM-Table the "3 Columns Matched" on the SS-ID button for this assignment (#12267) can be used to assign it to F29 which was also shown on previously.

Using Name PeakPick S+ Table

The menus available from the BAMTable are very useful for extending assignments. But they use assigned PeakPicks and there are situations where nothing is proposed because a Peak is not assigned. In these situations the S+ Table shown on next page from the Name PeakPick Table can be helpful.

N38	#11381	126.70	9.6262	56.90	54.16	65.19	37.48
G39	#11660	102.82	8.5147	54.19	45.31	37.59	-
K40		-	-	-	-	-	-
S41	#11552	115.57	8.2902	54.91	56.94	34.28	66.67
A42	#11411	123.23	9.0614	56.96	50.68	66.62	23.03

Take a look at K40 on the BAMTable and note that there are no good possible assignments from G39 or S41. The best assignment would be re-assigning #11486 from L53 to K40.

Click Right Mouse on #11552 (SS-ID for S41) and select L53 (#11586) under the Find Prev SS-ID menu option.

This will do 3 things:
- In Canvas ResJump to S41
- In Name PeakPick Table populate the table with S41.CA:-1 and S41.CB:-1 peaks from CBCA(CO)NH and HNCACB
- In Canvas set Seq-HNCACB slice to L53 ^{15}N and add PPM Cursors at L53 HN, CA and CB Shift values.

In Canvas window have only the CBCA(CO)NH and Seq-HNCACB experiments active.

The vertical line in off-white is at the S41.HN shift and the red vertical line is the L53 HN shift. The S41 CA:-1 is consistent with L53.CA, but S41 CB:-1 is not observed on the L53 HN line. Since #11486 peaks assign well to L53 using different ^{13}C values #11486 is not a good candidate to be re-assigned to K40.

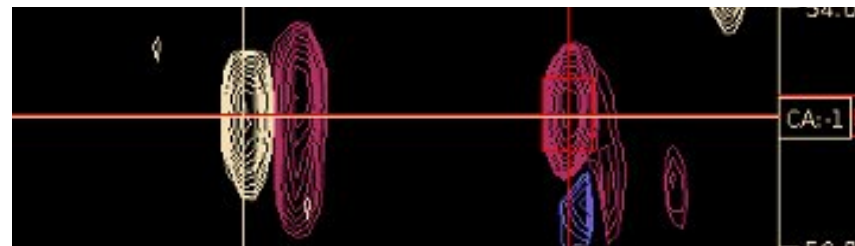| Contours	PkID	Assign		HN	CACB	N	Intens
	A +-	Assignment-Table					
	S +/-	Seq Strip-Table					
	281	S41.N,HN,CA:-1		8.292	54.894	115.5...	5.442e
	K +	HNCACB Assigns (1)		HN	CACB	N	
		S41.N,HN,CA:-1		-0.0016	0.0149	0.0064	
	S +	Exp	Slice	HN	CACB	N	Intensity
	22	HNCACB	6	5.080	55.164	129.842	-7.236e+5
		HNCACB	3	7.697	55.146	116.935	-6.04e+5
		HNCACB		7.752	55.103	110.547	+7.257e+5

In the Name PeakPick Table select the top line in the S+ table for assignment S41.N,HN,CA:-1 in the HNCACB experiment. The S+ table lists all of the possible sequential assignments based on the ^{13}C position of this peak and all of the Extrema in the HNCACB experiment. The red line in the Canvas window should move to 5.08 PPM and the slice for the Seq-HNCACB should change to 6. With the mouse over the S+ table use the Down-Arrow Key on the keyboard to select the next entry in the S+ which should update the Seq-HNCACB. Continue hitting the Down-Arrow key and looking for a match between the S41 CA:-1 and CB:-1 and good peaks (ie not noise) on the red line. After moving down 7 times the selected line should be at slice 39.

HNCACB	107	7.855	54.900	100.947	+2.540e+6
HNCACB	39	7.890	54.898	122.332	+1.134e+7

S41.HN   7.89 PPM
   On Slice 39

S41.CB:-1
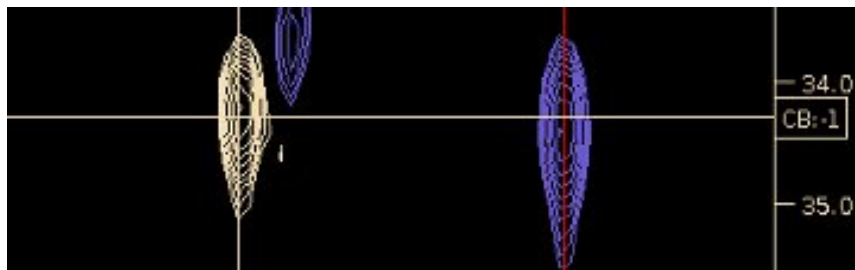
S41.CA:-1

The S+ table uses extrema to find sequential matches and as such it might be necessary to start from the CB:-1 of S41 instead of the CA:-1 in order to find a match if the extrema for the CA is not resolved (such as a shoulder peak). In addition **Click Left Mouse** over S+ toggles Require Confirmation so that more sequential matches can be found.

Confirming Identity of Slice 39, 7.89 PPM

The CBCA(CO)NH and HNCB experiments at the right show three unassigned peaks along 7.89 PPM on slice 39 at 34.4, 45.3 and 54.9 PPM. The 34.4 and 54.9 PPM match with the S41 CB:-1 and CA:-1 and the 45.3 PPM matches nicely with the G39 CA.
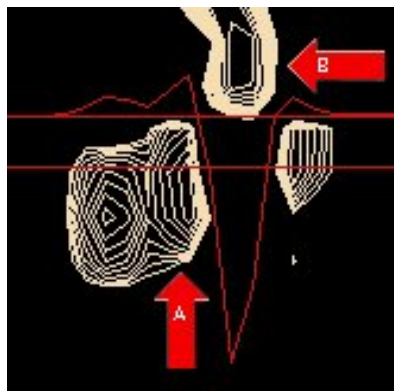
The corresponding peak in the ^{15}N HSQC shown at right labeled A is distorted by an intense peak labeled B whose resolution enhancement artifact is 5 times more intense than the peak at A. The trace displayed shows the relative intensity of the artifact and the peak at A.

Since CAPP did not PeakPick peak A in the ^{15}N HSQC there was no Spin-System created to be assigned to in the CBCA(CO)NH or HNCACB experiments.
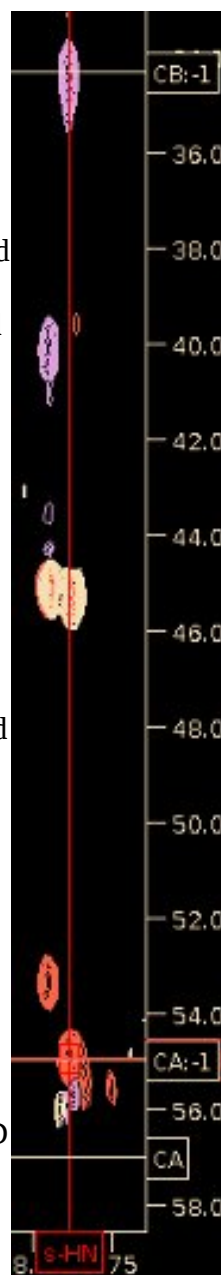
Although CAPP and XIPP do not automatically create a new Spin-System in the CBCA(CO)NH and HNCACB experiments we can easily manually do this in XIPP. First jump to slice 39 (**j 39**) and then **Click Left-Mouse** over peak at 45.3 PPM to add that peak into Name PeakPick table and then select the New-Rule assignment #12251 as shown below.

Selecting #12251 (or whatever arbitrary SS-ID you get) creates a new Moiety/Spin-System, assigns the peak to it, adds 3 new shifts to ShiftTable and BAMTable. **Click Left-Mouse** over the peaks at 54.9 and 34.4 PPM and select the same Spin-System ID as created for peak at 45.3 PPM, ie #12251.

After using QuickAssign in ShiftTable to change the CA|CB:-1 to CA:-1 the BAMTable at right has a 3 Column Match from #12251 to K40 and K40 has a 3 Column Match to #12251.