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 the 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 at most 1 – 2 minutes the first time the 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 (all minima and maxima) found in each experiment and may be used 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. The 1 – 2 minutes is how long it takes Xipp to scan all slices to locate and use 3 point parabolic interpolation to calculate the extrema for these 2 experiments.

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.
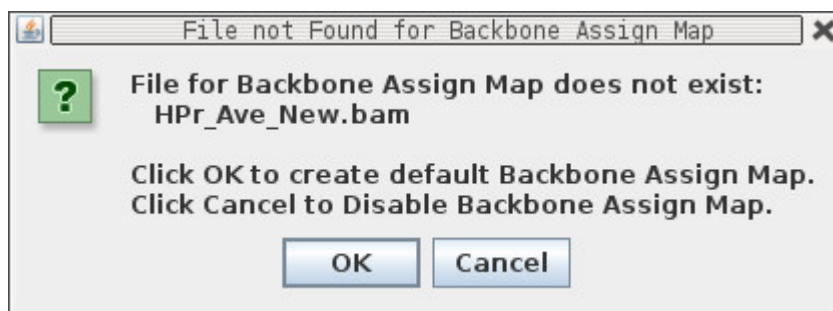
Backbone Assign Steps for HPr

(1) Peak-Pick ^{15}N-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 initial 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 select the HSQC_Full view if it is not already selected by clicking the View Chooser and selecting the view HSQC_Full.
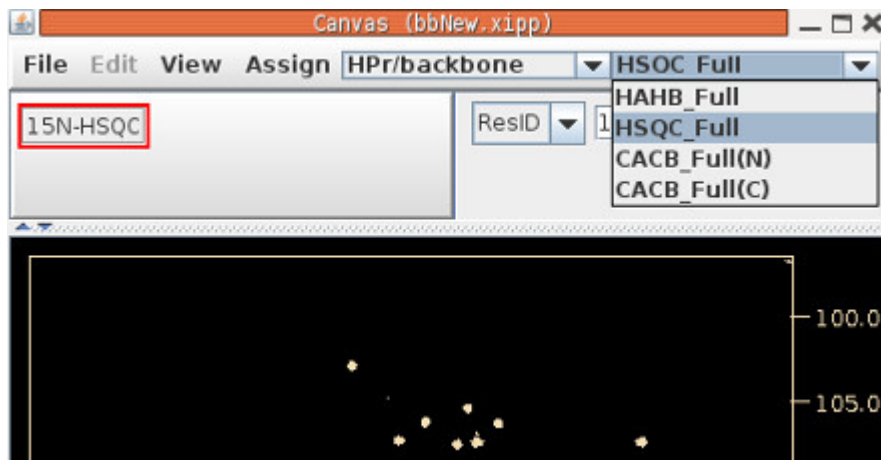
In the Canvas Window with the mouse over the spectrum enter the Canvas Command **n** to switch to the Name Peak Table.

The Table Window should look something like this.
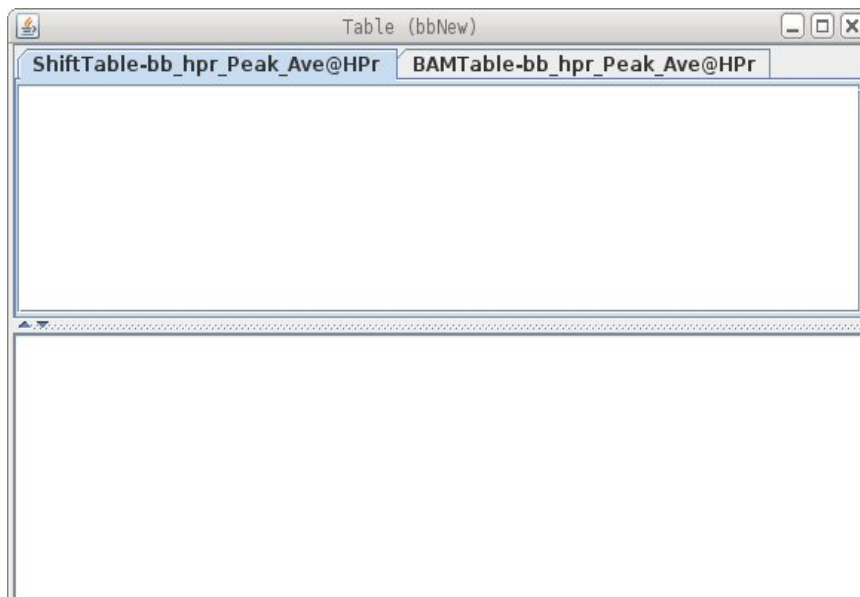
The top part is tabbed and can show the ShiftTable, ie Assignments or the BAMTable which is the Backbone Assign Map Table.

The bottom part always shows information about peaks that have been recently picked unless the divider is moved to the bottom of the window. 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.
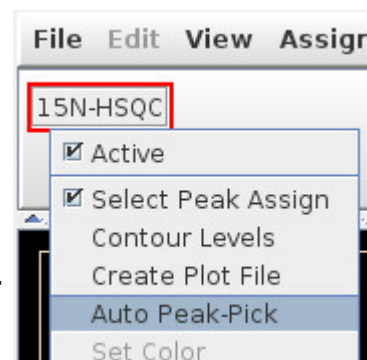
Shown below is the top part of the 'CAPP Auto Peak-Pick Dialog'. It is very important to **select** the '**Assign Peaks using Xipp**'. To start CAPP click Ok at the bottom of 'CAPP Auto Peak-Pick Dialog'. When CAPP completes click OK on the 'Capp Auto PeakPick Completed' dialog. If these two steps are not done then the peaks created by CAPP will not be assigned.
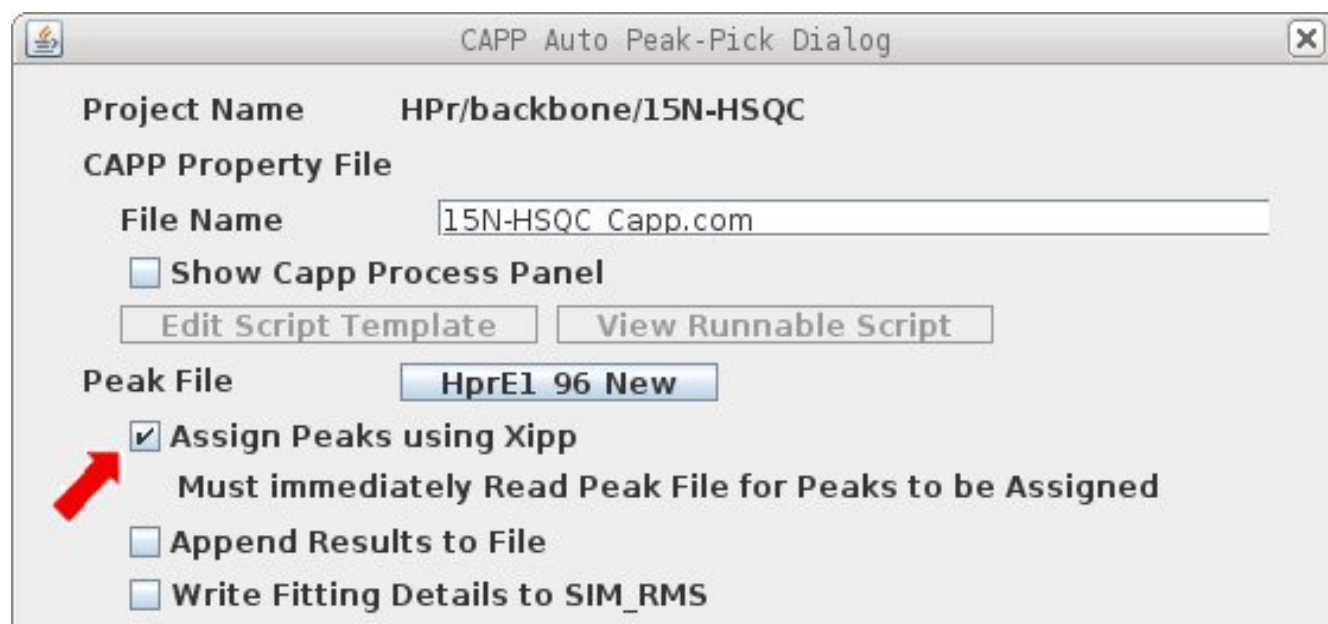
The modal dialog 'Capp Auto Peak-Pick Completed' will show up after CAPP is finished. After Clicking OK on this dialog the Table Window will get updated, but the Canvas Window will not get updated until the 'f' command is entered in the Canvas Window. In order to have Xipp assign the peaks in the CAPP created peak-pick file the file must be read in by clicking OK.
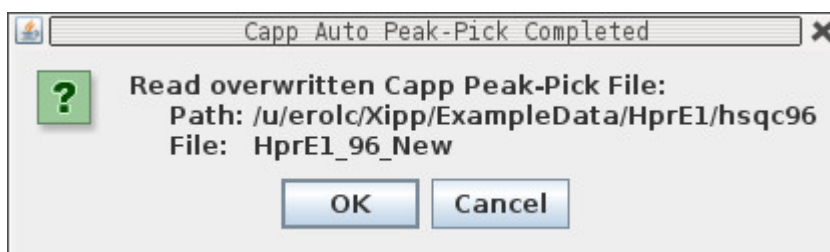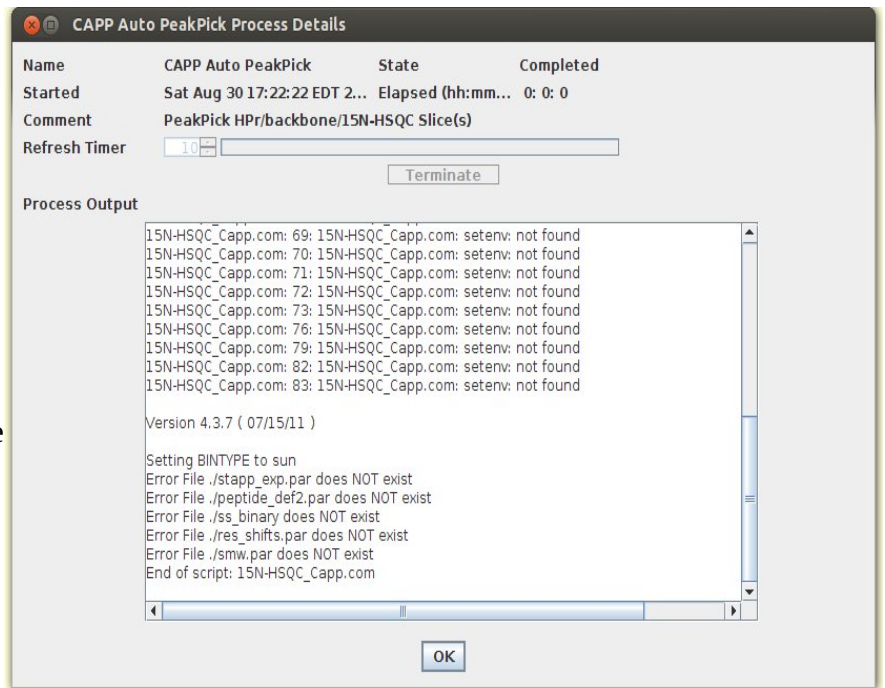
CAPP Requires /bin/csh for setenv

If CAPP completes very quickly and shows the thess 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 csh not a link to tcsh or bash.
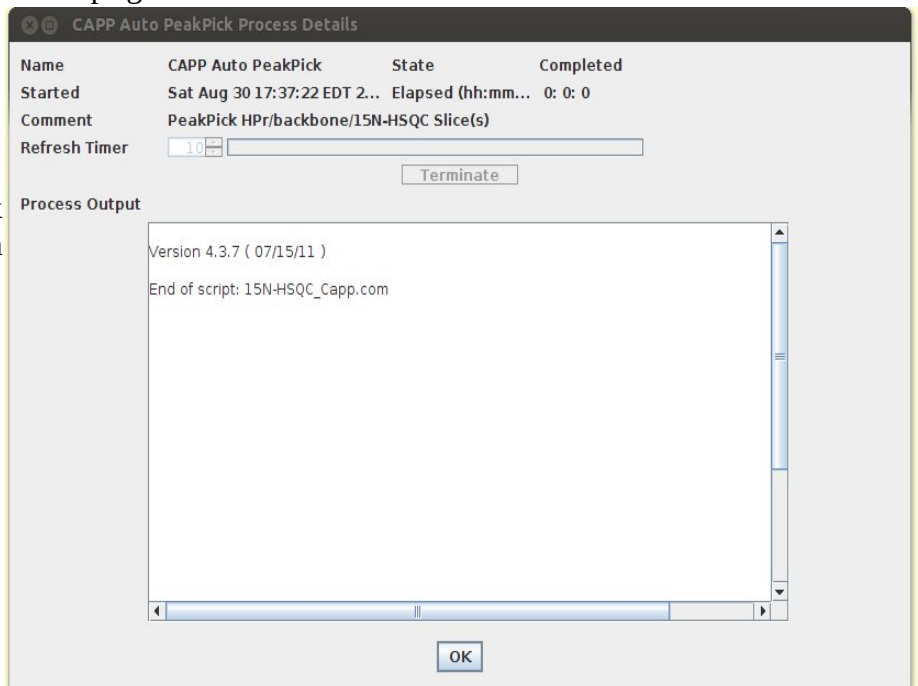
If problems continue then try running the shell script directly from a terminal window to diagnose problem.
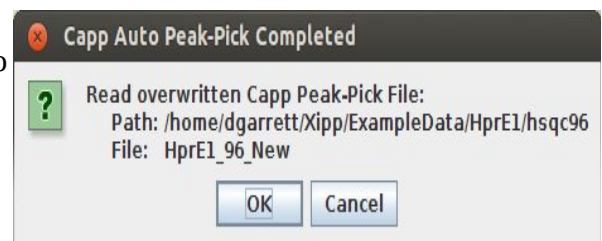
CAPP Auto PeakPick Process Details

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 dialog titled "Capp Auto Peak-Pick Completed" 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 and not read in.

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. The Spin-System ID is arbitrary and should be unique. The specific SS-ID values that you obtain may be different especially if manual peak-picking was performed.
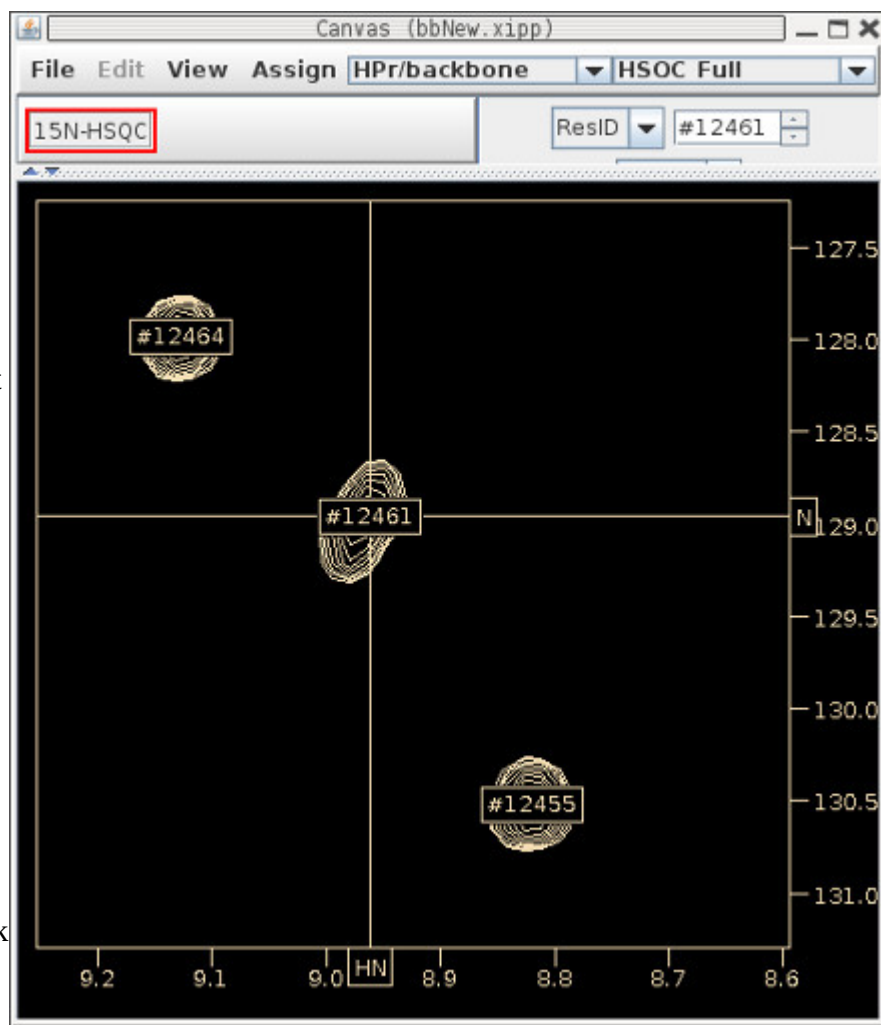
Table (bbNew.xipp)

| ShiftTable-Hpr_Ave@HPr | | BAMTable-Hpr_Ave@HPr | | | |

Residue	Atom-Assignment-Table				
#12455+	**AtomID**	**AtomName**	**Shift**	**RMS**	**Count**
	12456-	N	130.5098	0.0000	1
	12457-	HN	8.8203	0.0000	1
#12458+	**AtomID**	**AtomName**	**Shift**	**RMS**	**Count**
	12459-	N	129.3562	0.0000	1
	12460-	HN	7.3138	0.0000	1
#12461+	**AtomID**	**AtomName**	**Shift**	**RMS**	**Count**
	12462-	N	128.9484	0.0000	1
	12463-	HN	8.9622	0.0000	1
	AtomID	**AtomName**	**Shift**	**RMS**	**Count**

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 Window or clicking View Menu option '**Full [f]**'.
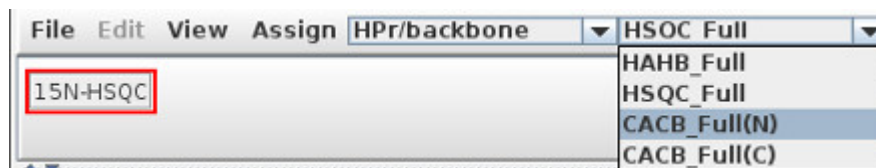
Note CAPP did not assign the peaks. Xipp assigned the CAPP peaks using the same Assignment Rules that are used when manually peak-picking. Each peak with a unique ^{15}N, HN shift was used to define a new Spin-System. Xipp will only automatically create new Spin-Systems for ^{15}N-HSQC and HNCO experiments.

Use the **Space Bar** to toggle the peak labels off and on to look for noise, ridges, artifacts or NH2 peaks. Click the Right Mouse button over the contours of a peak to delete any peak-picks selected by CAPP that arise from noise, ridge, artifact or NH2.

Canvas (bbNew.xipp)

File Edit View Assign HPr/backbone ▼ HSOC Full ▼

15N-HSQC ResID ▼ #12461

#12464
#12461
#12455

127.5
128.0
128.5
129.0
129.5
130.0
130.5
131.0

9.2 9.1 9.0 HN 8.9 8.8 8.7 8.6

(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.
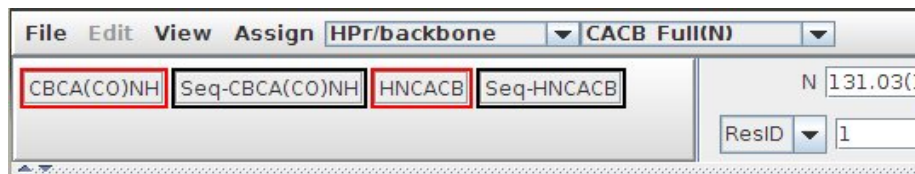
Just like in the HSQC this can be done manually by pointing your mouse at every real peak on every plane and clicking the Left-Mouse button. The ^{15}N, HN for each peak will be compared with the Spin-Systems created from HSQC and the peak will be assigned to the closest Spin-System within an error range and a new ^{13}C assignment will be created. If no Spin-Systems are found within the 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.
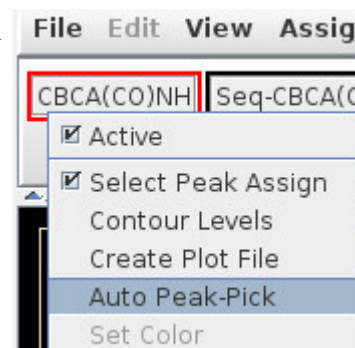
Must select 'Assign Peaks Using Xipp' . All other default settings are OK.

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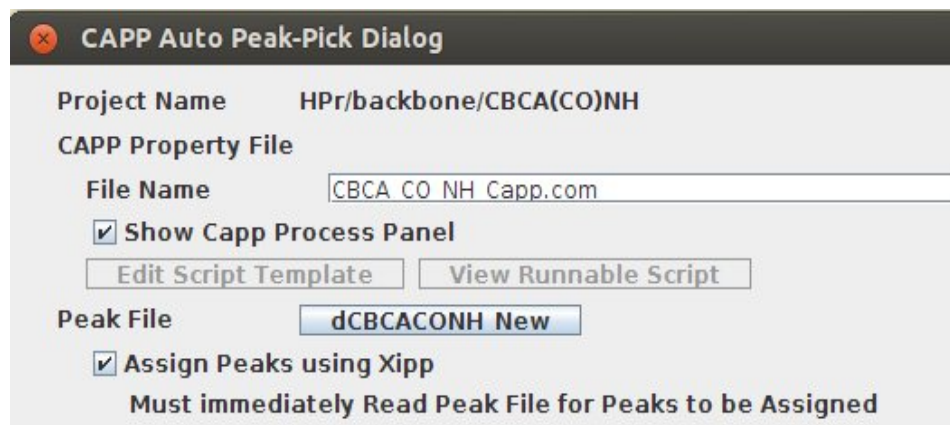
When CAPP completes click **OK** on the modal Capp Auto Peak-Pick Completed window in order for the Peaks to be assigned.
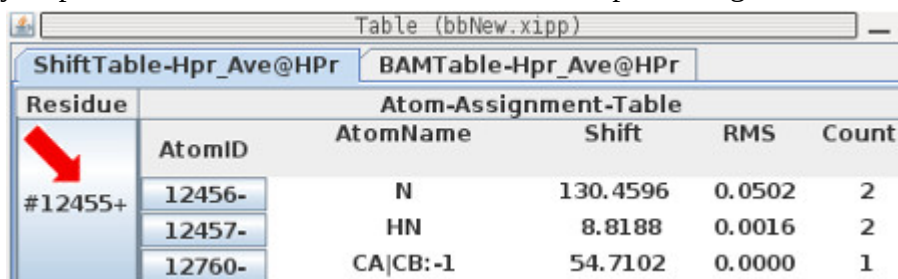
Type **f** in the Canvas Window to show the Xipp Assigned CAPP Peak-Picks. Or click the View Menu option '**Full [f]**' to show the Xipp Assigned CAPP Peak-Picks.

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 other 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 #12455 in this example. After checking #12455 **Click Left-Mouse** on next Spin-System.

Residue	Atom-Assignment-Table			
	AtomID	AtomName	Shift	RMS	Count
#12455+	12456-	N	130.4596	0.0502	2
	12457-	HN	8.8188	0.0016	2
	12760-	CA|CB:-1	54.7102	0.0000	1

The Canvas Window should like this after making the HNCACB inactive and zooming in around the two peaks for #12455. Note the HNCACB should 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. Do **NOT** add the peak-pick at 8.8 by 64.2 PPM. I want to keep the CA:-1 unassigned to show how a single missing assignment affects the HNCACB peak-picking and assignments.
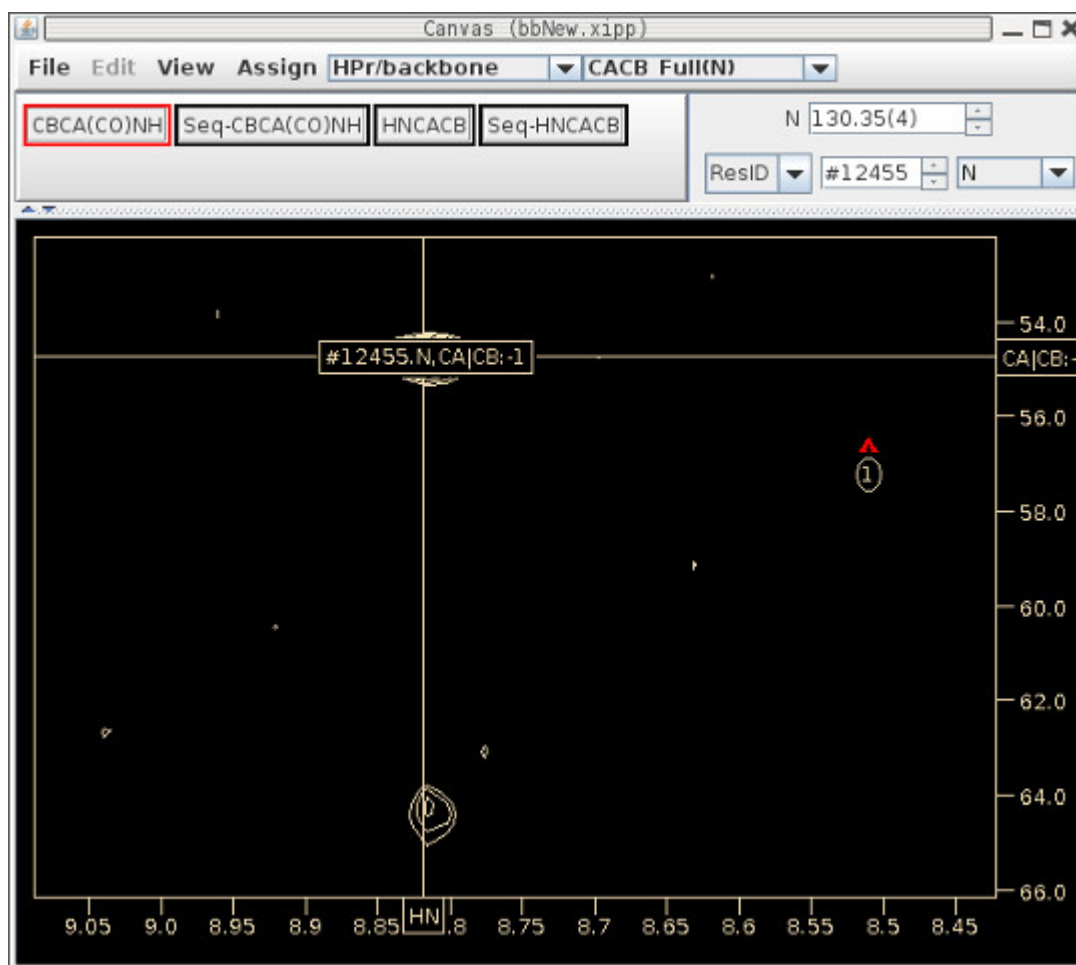
Since this is a tutorial there is no need to inspect all slices or all Spin-Systems. The Spin-System ID #'s shown in the tutorial assume that all slices in CBCA(CO)NH were not inspected/fixed.
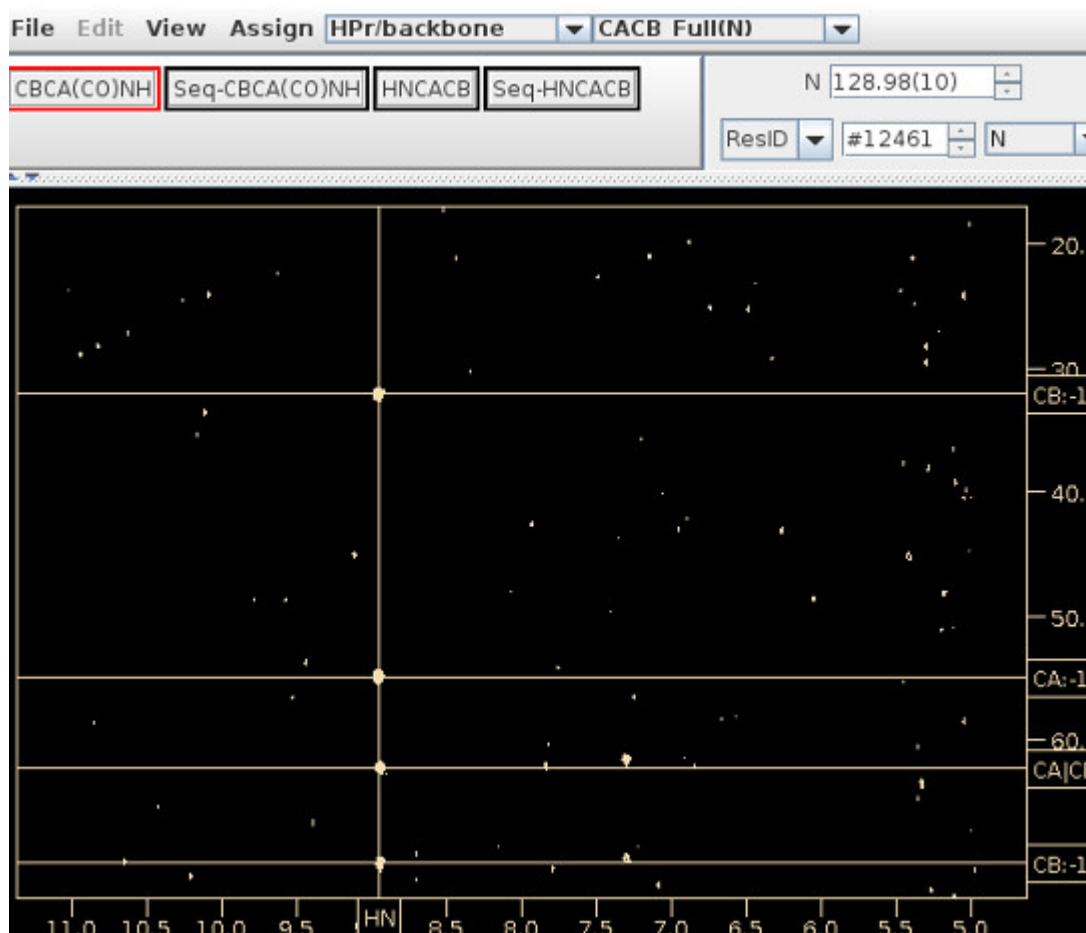
Finding HN, N Degeneracy

The ShiftTable shows that #12461 may have degenerate amides or noise since there are more than two ^{13}C shifts assigned after the CBCA(CO)NH has been assigned when there should be at most two ^{13}C shift assigned (CA:-1 and CB:-1).

Table (bbNew.xipp)

ShiftTable-Hpr_Ave@HPr	BAMTable-Hpr_Ave@HPr			
Residue	**Atom-Assignment-Table**			

	AtomID	AtomName	Shift	RMS	Count
	12462-	N	128.8891	0.0946	5
	12463-	HN	8.9584	0.0047	5
#12461+	12765-	CA|CB:-1	62.1193	0.0000	1
	12766-	CB:-1	32.0497	0.0000	1
	12767-	CA:-1	54.7843	0.0000	1
	13257-	CB:-1	69.8560	0.0000	1

Res Jump to #12461 (Click Left-Mouse on #12461 button in ShiftTable)

The Canvas Window below shows that there are 4 good peaks in the CBCA(CO)NH at nearly the same ^{15}N and HN PPM value. At most there should be 2 peaks for a given Spin-System. The space-bar toggles displaying peak-pick labels.

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 labeled N and HN. The position of the Shift Cursors comes from the assignment table which is defined as an average of all assigned peaks.
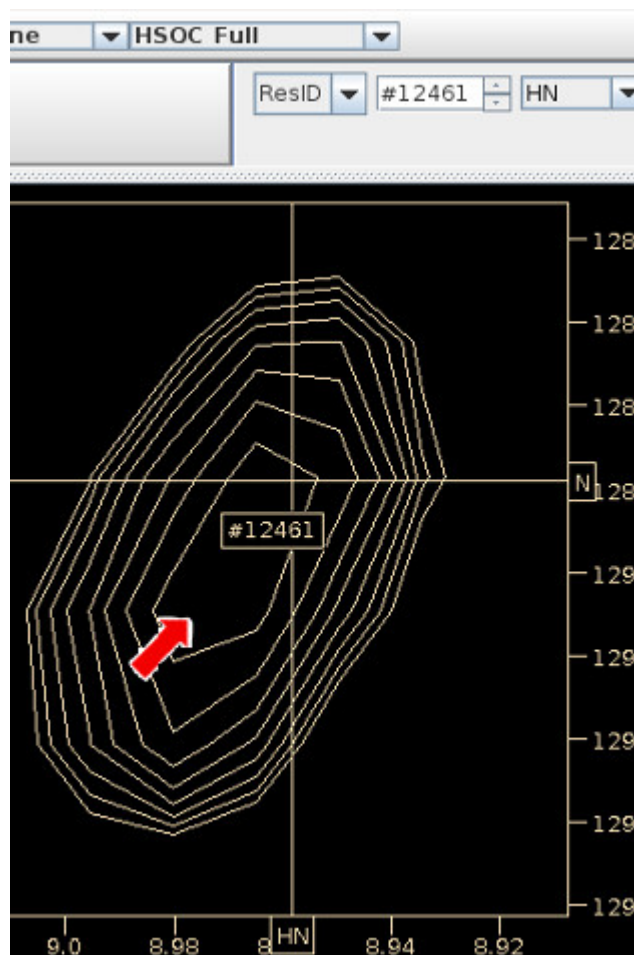
Use the following steps to move the peak-pick:
　　　(1) With the mouse over #12461 click Alt Mouse-Left to copy Peak-Pick to Peak-Buffer,
　　　(2) Position mouse to Shift Cursor intersection,
　　　(3) Click Crtl-Left-Mouse to move Peak-Pick position up and to the right.

Position Mouse over second Spin-System shown by red arrow and Click Crtl-Left-Mouse to create a new Peak-Pick at exactly the current mouse location.

This will create a new Peak-Pick, but will probably not create a new Spin-System ID if the two peak-picks are too close to each other.

If the new peak-pick has the same Spin-System ID #, ie #12461, as the peak-pick that was just moved then this peak-pick must be re-assigned using the 'Assignment Detail Dialog' described below and on the next page.

Click Left-Mouse over recently added peak-pick in Name Peak Table whose button is labeled #12461.N,HN. This will bring up the 'Assignment Detail Dialog' described on the next page. Note the most recently added peak-pick is always at the top of the Name Peak Table and there will be two peak-pick-picks labeled #12461. Click the top peak-pick labeled #12461

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

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

Click the Select button for New Moiety which in this case is ID #13260. Click OK on 'Confirm Peaks Dialog' and then OK on 'Assignment Detail Dialog' after confirming that Spin-System ID for the Assign has been updated with the new Spin-System ID.

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 Segment. The Spin-System ID is the same as the unassigned Moiety ID. The New Moiety Spin-System number that you get may be different than #13260 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 should be different than any existing object ID. You will get #13260 if #12455 CB:-1 at 64.2 PPM is not peak-picked and assigned. You will get a Spin-System ID greater than 13260 if you have already peak-picked and assigned other peaks.
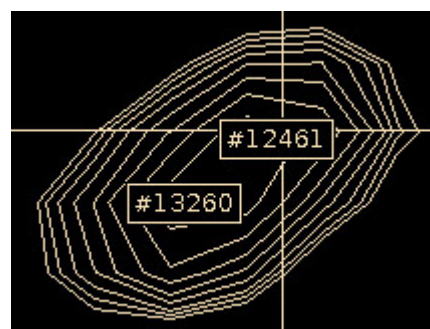

After hitting the space bar twice to update the peak labels each peak should be labeled with a unique Spin-System ID.

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 Spin-Systems #12461 and #13260. 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 HN 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 PPM deviation of HN and N from peaks to #12461 and #13260.
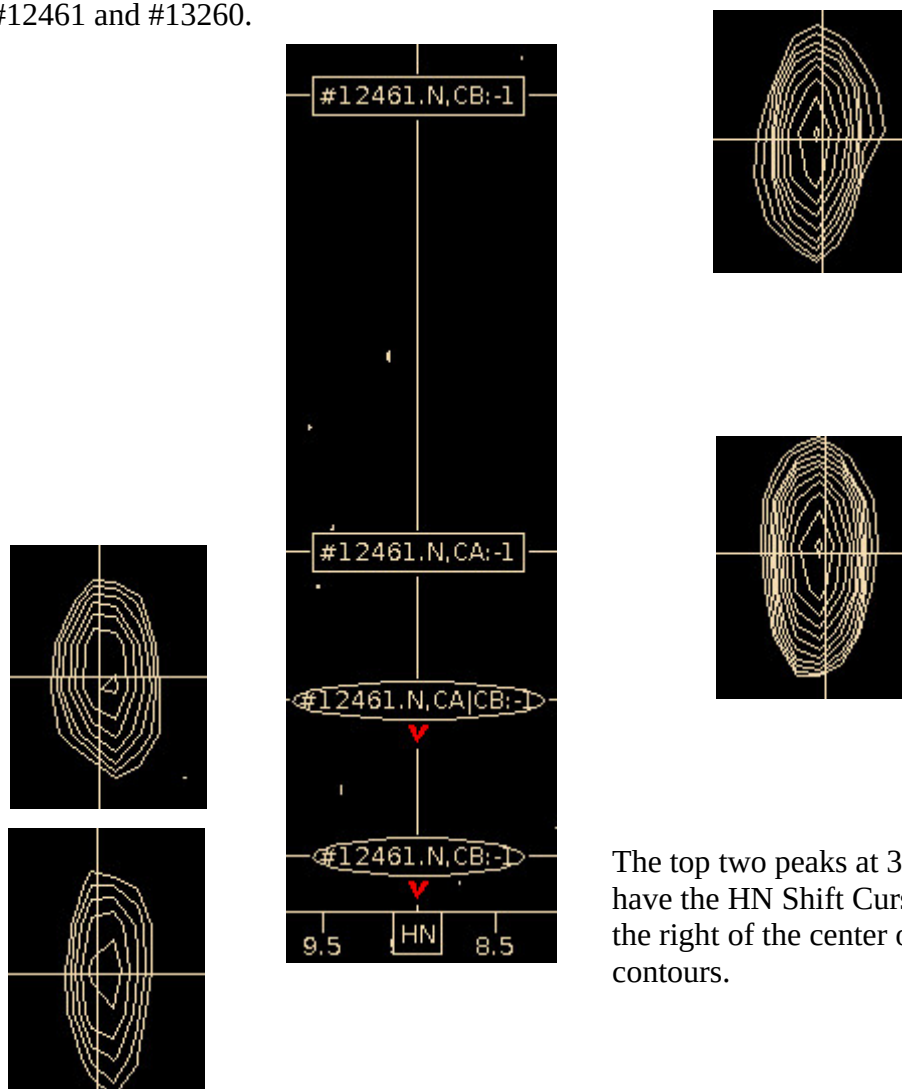
The top two peaks at 32 and 55 PPM have the HN Shift Cursor a little to the right of the center of the contours.

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

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

Fixing HN, N Degeneracy
Using Peak-Pick Tables

Show Peak-Pick Table for View CACB_Full(N):
  Ensure view CACB_Full(N) is selected in View Chooser.
  Use the Canvas Command **n** to show the Peak-Pick Table.

In all of these examples the Spin-System ID # may be slightly different than what is shown here. Always ensure that the ^{15}N and HN PPM values are same between your running of Xipp and what is shown in the examples. In cases where a Crt-Left Click created a Peak-Pick at the mouse position the ^{15}N and HN values should be very close, but will likely be different.

CACB_Full(N) Peak-Pick Table

Exp	PkID	Assign	HN	CACB	N	Intensity
CBCA(CO)NH	6	#12461.N,HN,CB:-1	8.962	32.05	128.98	5.074e+6
CBCA(CO)NH	5	#12461.N,HN,CA:-1	8.962	54.78	128.97	8.676e+6
CBCA(CO)NH	7	#12461.N,HN,CB:-1	8.952	69.86	128.78	3.228e+6
CBCA(CO)NH	8	#12461.N,HN,CA|CB:-1	8.953	62.12	128.77	5.162e+6

The CACB_Full(N) view 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

Exp	PkID	Assign	HN	N	Intensity
15N-HSQC	101	#13260.N,HN	8.978	129.05	1.096e+6
15N-HSQC	11	#12461.N,HN	8.959	128.90	1.122e+6

The HSQC_Full view Peak-Pick Table show that #13260 has the higher HN and higher N PPM value than #12461. Since the peaks at 32.05 and 54.78 have the higher HN and N PPM value they should be assigned to #13260 and the peaks at 62.12 and 69.86 should be assigned to #12461. 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 re-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. It will change the PkID for the Peak-Pick. 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 #13260. The Peak-Pick is assigned and the line gets highlighted as shown below.

Contours	PkID	Assign		HN	CACB...	N	Intensity
	A +-	Assignment-Table					
	S +/-	Seq Strip-Table					
	216	#13260.N,HN,CB:-1		8.962	32.050	128.9...	5.074e+6
	E +	CBCA(CO)NH Assigns (2)		HN	CACB	N	
		#12461.N,HN,CB:-1		-0.0054	-	-0.1234	
		#13260.N,HN,CB:-1		0.0079	0.0000	0.0342	
	S +	Exp	Slice	HN	CACB	N	Intensity	
	9	HNCACB	42	7.857	32.124	121.701	+3.013e+6
		HNCACB	10	8.974	32.104	128.990	-1.187e+6
		HNCACB	24	5.123	32.023	125.732	-7.33e+5

(2) Use Extended Rule to re-assign Peak-Pick on Name Peak-Pick Table without deleting Peak.

This method will preserve the PkID for the Peak-Pick. 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 **Click 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 already show E+ depending on exact position of the new Spin-System that was created. If E+ is already shown then the Extended-Rule

5	#12461.N,HN,CA:-1	8.962	54.784
K +	CBCA(CO)NH Assigns (1)		HN

☑ Table +/-   A:-1   -0.0057
CBCA(CO)NH-Assign ▸ ◉ Known-Rule
S 1   ○ Extended-Rule
Copy Table   ○ New-Rule
Shift errorRange   .855   54.908   10
CACB   0.3   .292   54.894   11
7   7.049   -
HN   0.03

is being used and you do not need to change the Assigner. The only reason to use the Extended-Rule for finding assignments is to list #13620.N,HN.CA|CB:-1 as a possible assignment as shown on the next page.

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	#13260.N,HN,CA:-1		8.962	54.784	128.9...	8.676e+6
	E +	CBCA(CO)NH Assigns (2)			HN	CACB	N
		#12461.N,HN,CA|CB:-1			-0.0076	-	-0.1562
		#13260.N,HN,CA|CB:-1 ⬅			0.0051	-	0.0269
	S +	Exp	Slice	HN	CACB	N	Intensity
	17	HNCACB	104	7.855	54.981	107.623	-1.338e+6
		HNCACB	107	7.855	54.908	106.947	+2.546e+6

To assign the Peak-Pick select the line in the Name Peak-Pick Table for the desired assignment such as #13260. 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 no longer valid.

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

Contours	PkID	Assign		HN	CACB...	N	Intensity
	A +-	Assignment-Table					
	S +/-	Seq Strip-Table					
	5	#13260.N,HN,CA:-1		8.962	54.784	128.9...	8.676e+6
	K +	CBCA(CO)NH Assigns (1)			HN	CACB	N
		#13260.N,HN,CA:-1			0.0051	0.0000	0.0269
	S +	Exp	Slice	HN	CACB	N	Intensity
	17	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

The two peaks at 62 PPM and 70 PPM are left assigned to #12461.
The assignment #12461 CA|CB:-1 at 62.1 PPM will be changed to #12461 CA:-1 using Atom Quick Assign Menu described in the next section.

Below is the CBCA(CO)NH Peak-Pick table after making the changes noted above. PkID 216 for the peak at 32 PPM was PkID 6 that was deleted and then re-added. That is why this PkID is much larger than the other PkIDs. PkIDs are only unique within an Experiment.

Exp	PkID	Assign	HN	CACB	N	Intensity
CBCA(CO)NH	216	#13260.N,HN,CB:-1	8.962	32.05	128.98	5.074e+6
CBCA(CO)NH	5	#13260.N,HN,CA:-1	8.962	54.78	128.97	8.676e+6
CBCA(CO)NH	7	#12461.N,HN,CB:-1	8.952	69.86	128.78	3.228e+6
CBCA(CO)NH	8	#12461.N,HN,CA|CB:-1	8.953	62.12	128.77	5.162e+6

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
#12494+	12495-	N	123.9735	0.0951	2
	12496-	HN	8.1084	0.0010	2
	12822-	CA|CB:-1	46.8029	0.0000	1

Click Left-Mouse on the SS-ID button (#12494 in this example) to residue jump. To confirm that the ^{13}C at 46.8PPM 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 are no other peaks 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 #12494.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 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 12822 for GLY CA assignment to be fixed in ShiftTable and select CA:-1.

The ¹³C 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	RMS	Count
#12494+	12495-	N	123.9735	0.0951	2
	12496-	HN	8.1084	0.0010	2
	13267-	CA:-1	46.8029	0.0000	1

Note the residue that Spin-System #12494 will eventually be assigned to is not a GLY it follows a GLY.

HPr is small (85 residues) 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!

Use the Atom Quick Assign to fix #12461 CA|CB:-1 so that it is #12461 CA:-1

It is easier to fix all GLY CA|CB:-1 from CBCA(CO)NH at the same time as GLY CA|CB from HNCACB are fixed. I recommend waiting until after the HNCACB has been peak-picked and assigned to fix all GLY ambiguous assignments which is described on page 27.

The Spin-System IDs shown in this tutorial assume that no other GLY are fixed at this time.

ShiftTable Changes for HNCACB Experiment
During Peak-Picking and Assigning

Click Left-Mouse over HNCACB experiment button to activate/show in order to display and manually peak-pick it.

For Spin-System #12494 (Do I really need to tell you to ResJump to #12494 with ^{15}N 123.9 PPM and HN 8.1 PPM if needed?) the HNCACB has 3 peaks. One in the GLY CA PPM range 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 #12494 in the ShiftTable.** If the first peak added is at 53.6 PPM it is assigned CA|CB. After the second peak at 39.75 PPM is picked they are both uniquely assigned to a single atom. If the CB is picked first it will get uniquely assigned to CB since CA shifts do not normally occur at 39.75 PPM in proteins.

	AtomID	AtomName	Shift	RMS	Count
#12494+	12495-	N	123.9704	0.0674	4
	12496-	HN	8.1089	0.0009	4
	13267-	CA:-1	46.8029	0.0000	1
	13269-	CB	39.7530	0.0000	1
	13272-	CA	53.6073	0.0000	1

The Shift, RMS and Count for N and HN have been changing as peaks get assigned into #12494. **Click Left-Mouse** over AtomID 12495 for N to open the PeakPick-Table showing all peaks used in assigning this atom/shift. The + or – after the SS-ID and AtomID numbers indicates whether the sub-table is open (+) or closed (-). By default the sub-table for all SS-ID are open and the sub-table for all AtomID are closed.

Bad assignments can sometimes be caught by looking for large RMS values and then opening the AtomID sub-table to see if one Peak-Pick is an outlier.

	AtomID	AtomName		Shift	RMS	Count
				PeakPick-Table		
		N		123.9704	0.0674	4
		PkID	Exp	PPM	Dev.	
#12494+	12495+	33	CBCA(CO)NH	123.8783	-0.092	
		38	15N-HSQC	124.0686	0.098	
		218	HNCACB	123.9722	0.002	
		219	HNCACB	123.9626	-0.008	
	12496-	HN		8.1089	0.0009	4
	13267-	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.
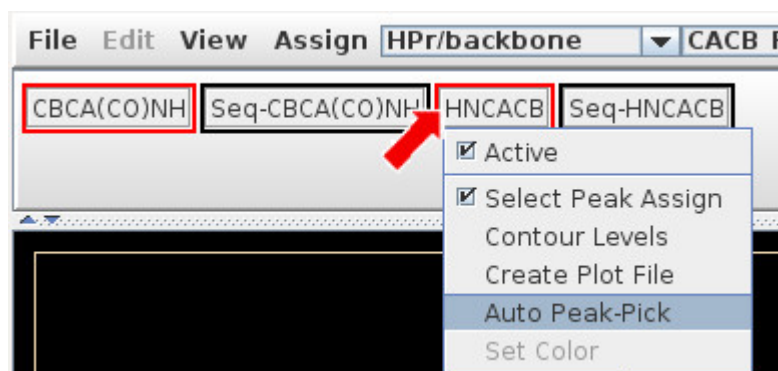
Must select 'Assign Peaks Using Xipp' . All other default settings are OK.

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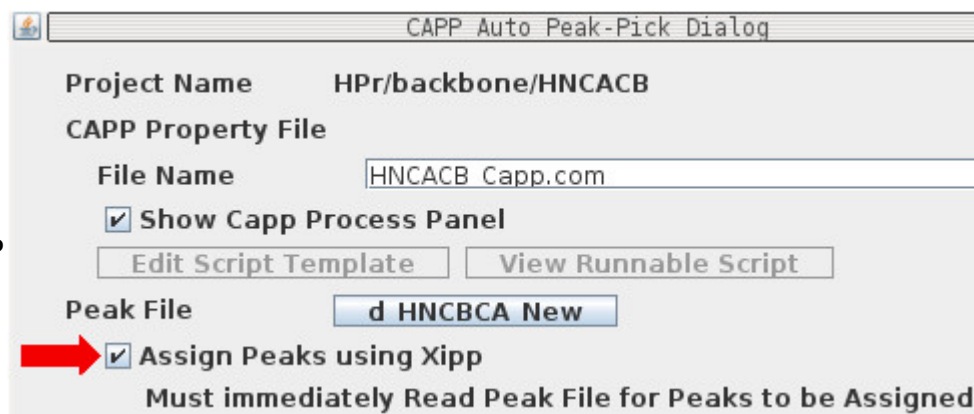
When CAPP completes click **OK** on the modal Capp Auto Peak-Pick Completed window in order for the Peaks to be assigned as they are read in.

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 when making assignments from scratch. 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. In the interest of time this is not done in the tutorial.

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 ^{13}C shifts assigned to specific atoms so that it will be easier to link adjacent Spin-Systems.

Spin-System #12455 has 4 assigned atoms, but some of the atoms are ambiguous on Linux. On a Mac the 4 assigned atoms in #12455 are not ambiguous.

Spin-System #12455 has 4 ^{13}C shifts assigned to it which is good, but the atoms may not be uniquely assigned.

Res-Jump to #12455 by clicking button #12455.

Residue	AtomID	AtomName	Shift
	12456-	N	130.4263
	12457-	HN	8.8216
#12455+	12760-	CA|CB:-1	54.6999
	13287-	CB	41.8481
	13288-	CA	57.6536
	13289-	CA|CB:-1	64.2585
	AtomID	AtomName	Shift
	12459-	N	129.2867
	12460-	HN	7.3131
#12458+	12763-	CB:-1	69.4118

Table (bbNew.xip) — ShiftTable-Hpr_Ave@HPr / BAMTable-Hpr_Ave@HPr

The problem in #12455 is that CAPP on Linux missed the ^{13}C at 64.26 PPM in the CBCA(CO)NH experiment and as mentioned previously 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 some of the assignments for #12455 ambiguous. On a Mac system CAPP picked the peak at 64.26 PPM in the CBCA(CO)NH experiment 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 #12455 HN ShiftCursor immediately and completely resolved the assignments for all shifts in #12455.

Residue	AtomID	AtomName	Shift
	12456-	N	130.4201
	12457-	HN	8.8205
#12455+	13287-	CB	41.8481
	13288-	CA	57.6536
	14366-	CB:-1	64.2299
	14367-	CA:-1	54.6999

ShiftTable-Hpr_Ave@HPr / BAMTable-Hpr_Ave@HPr

Fixing HN, N Degeneracy
in #12461 & #13260

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 ^{15}N PPM values in the HSQC and CBCA(CO)NH.

Care must be taken when extending the assignments to the intra-residue Cα and Cβ atoms observed in the HNCACB experiment since the error in determining an HN or ^{15}N PPM value can be comparable to the difference between the degenerate Spin-Systems.

Shown at the right are two regions from the CBCA(CO)NH and HNCACB overlayed . The white vertical line is the HN ShiftCursor for #12461. The red vertical line is a vertical Cursor manually positioned to the center of the contours for the HNCACB peak in top region labeled #13260.N,CB:-1 (this label is partially obscured by the equivalent label for the CBCA(CO)NH peak). Initially the HNCACB peak at 32.1 PPM may be absent depending on settings in CAPP.

Top region is centered at 32.1 PPM and shows equivalent peaks from CBCA(CO)NH overlayed on HNCACB for shift #13260.CB:-1. Peak-Pick the HNCACB peak at 32.1 PPM if it was missed by CAPP. Xipp easily handles small offsets between experiments as long as the assignment table has the assignment. Xipp had no problem using the Known-Rule in assigning the HNCACB peak at 32.1 PPM because it was already assigned in the CBCA(CO)NH experiment.

The bottom region is centered at 37.6 PPM and shows one peak from the HNCACB for shift #12461.CB. Xipp could not automatically assign the Peak-Pick created by CAPP because there were two possible candidates in the Extended-Rule. Click Mouse-Left over the HNCACB peak at 37.6 PPM and select #12461.N,HN.CB from the Name Peak-Pick Table.

The difference between HN PPM values between #12461 and #13260 is about the same as the change in position for the CB:-1 of #13260 between the CBCA(CO)NH and HNCACB so that it appears when looking at the HNCACB that the peak at 37.6 PPM and comparing it to the CBCA(CO)NH peak at 32.1 PPM that the 37.6 PPM peak should be assigned to #13260 when in reality it should be assigned to #12461.

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 which were derived from ^{15}N-HSQC and CBCA(CO)NH.
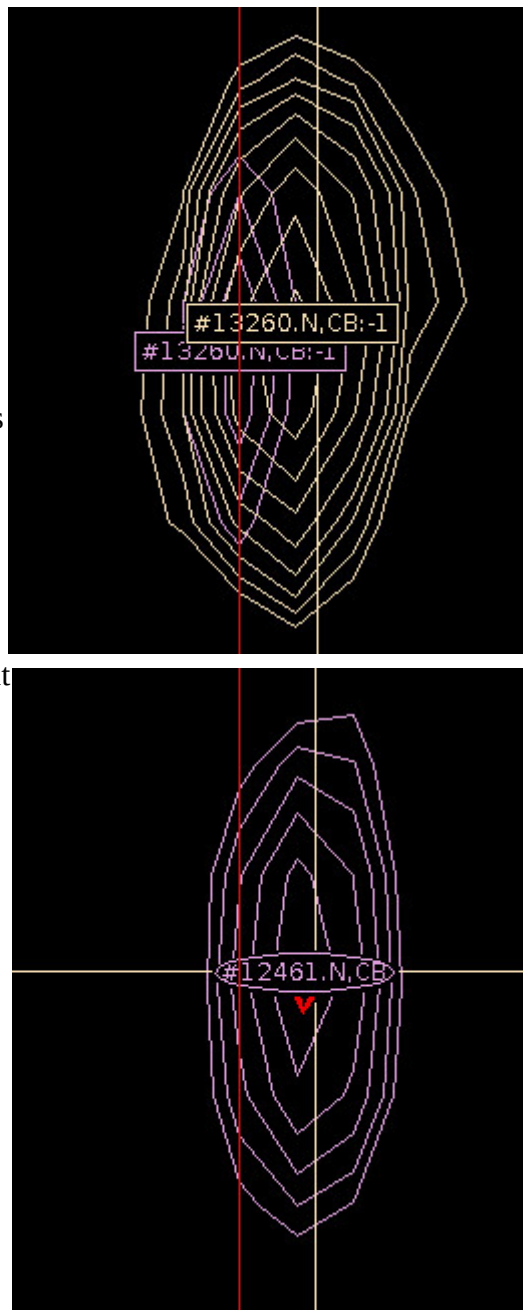
# Fix Possible Wrong Assignment
in #12461

AtomID		AtomName	Shift	RMS	Count
#12461+	12462-	N	128.7910	0.0485	6
	12463-	HN	8.9568	0.0030	6
	12767-	CA:-1	62.0443	0.0750	2
	13257-	CB:-1	69.7757	0.0804	2
	13338-	CB	37.6635	0.0000	1

Spin-System #12461 in the ShiftTable may be missing the CA or CB shift or have it mis-assigned in the HNCACB. The CBCA(CO)NH peak at 62.12 PPM 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 #12461. The HNCACB peak at 60.38 PPM that is Peak-ID 28 in the figure is the true intra-residue CA shift for #12461. The HNCACB peak at 37.7 PPM is the intra-residue CB shift for #12461.

If the HNCACB peak at 61.97 PPM is assigned to #12461.N,CA, it should be re-assigned to #12461.CA:-1 by Clicking Mouse-Left over the peak with the CBCA(CO)NH Exp undisplayed. In the Name Peak-Pick Table select the assignment for #12461.N,HN,CA:-1.
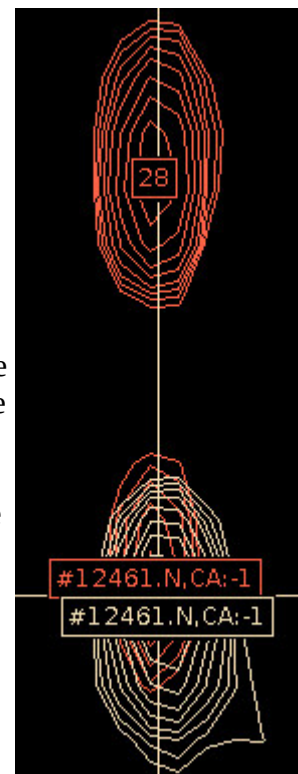
Assign Peak-ID 28 by Clicking Mouse-Left over the HNCACB peak at 60.38 PPM .   In the Name Peak-Pick Table select the assignment for #12461.N,HN,CA|CB for Peak-ID 28.
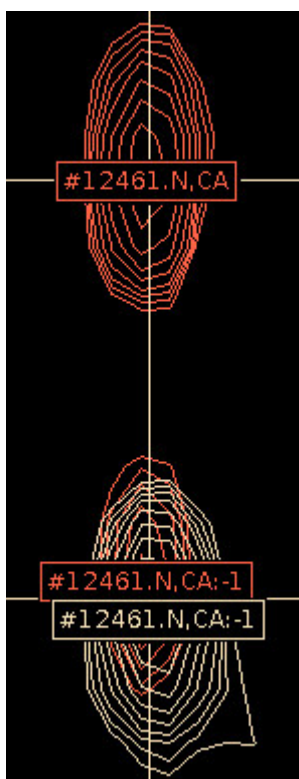
The Canvas Window and ShiftTable shown to the left and below are now correct for #12461.

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 that #12461 was missing the CA assignment is what led to taking a closer look at #12461.

If needed assign Peak-ID 31 (at 37.7 PPM) in HNCACB to #12461 so that #12461 has 4 unique ^{13}C assignments as shown below.

AtomID		AtomName	Shift	RMS	Count
#12461+	12462-	N	128.7901	0.0450	7
	12463-	HN	8.9570	0.0029	7
	12767-	CA:-1	62.0443	0.0750	2
	13257-	CB:-1	69.7757	0.0804	2
	13338-	CB	37.6635	0.0000	1
	14368-	CA	60.3791	0.0000	1

Assign HNCACB Peaks
in #13260

Scroll the ShiftTable down to bottom to see Spin-System #13260 (at 129.0, 8.97 PPM) which was previously created to resolve the degeneracy in #12461.   The Peak-Pick IDs 18 and 24 at 57.2 PPM and 42.1 PPM are the CA and CB intra-residue shifts for #13260 that are missing in the ShiftTable.

	AtomID	AtomName	Shift	RMS	Count
#13260+	14939-	N	128.9978	0.0266	5
	14940-	HN	8.9703	0.0067	5
	14941-	CB:-1	32.0770	0.0273	2
	14942-	CA:-1	54.7622	0.0221	2

Mouse Left-Click peak at 57.2 PPM and select #13260.N,HN,CA|CB from Name Peak-Pick Table if it is not automatically selected.

Mouse Left-Click peak at 42.2 PPM and select #13260.N,HN,CB from Name Peak-Pick Table if it is not automatically selected.

	AtomID	AtomName	Shift	RMS	Count
	14939-	N	129.0026	0.0238	7
	14940-	HN	8.9717	0.0061	7
#13260+	14941-	CB:-1	32.0770	0.0273	2
	14942-	CA:-1	54.7622	0.0221	2
	15469-	CB	42.2667	0.0000	1
	15470-	CA	57.2216	0.0000	1

You will need to scroll down the ShiftTable to see the new CA and CB entries for #13260.

In the Canvas Window the peak at 57.2 PPM should now be labeled as #13260.CA.

The Shift-Cursor label giving the horizontal line at 57.2 PPM may not automatically update (ie still shows CA|CB). To update it you can jump to a different Spin-System and jump back or simply Click the Atom-Selector labeled N and re-select N which will update the label. The Atom-Selector menu identifies which Atom to use when multiple Atoms can be observed from a Moiety. For most Amide detected experiments N is the only option.
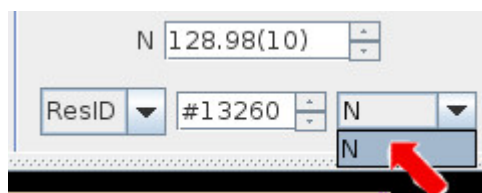
Near Degeneracy Distorts HNCACB
Peak Missed by CAPP

	AtomID	AtomName	Shift	RMS	Count
#12488+	12489-	N	124.3432	0.0288	5
	12490-	HN	9.6350	0.0047	5
	12809-	CB:-1	40.8714	0.0295	2
	12810-	CA:-1	60.4153	0.0000	1
	13498-	CB	71.2255	0.0000	1

The ShiftTable for #12488 shows two inter-residue and one intra-residue assignments.

At right is shown #12488 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 #12488. 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 61.2 PPM were not enough for CAPP to automatically Peak-Pick it.
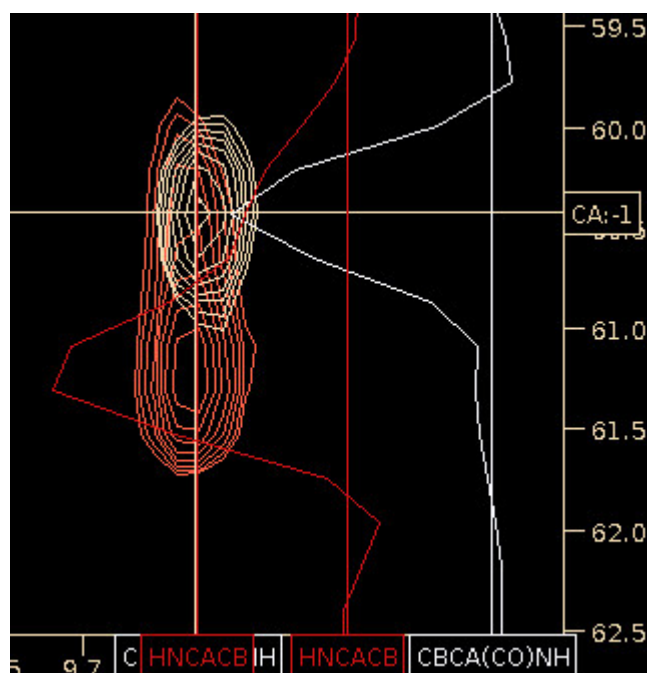
Manually Peak-Picking the intra-residue HNCACB peak at 61.2 PPM resolved the assignments for CA and CB.

	AtomID	AtomName	Shift	RMS	Count
#12488+	12489-	N	124.3389	0.0280	6
	12490-	HN	9.6353	0.0044	6
	12809-	CB:-1	40.8714	0.0295	2
	12810-	CA:-1	60.4153	0.0000	1
	13498-	CB	71.2255	0.0000	1
	14369-	CA	61.2116	0.0000	1

Weak HNCACB Peaks
Missed by CAPP

	AtomID	AtomName	Shift	RMS	Count
#12473+	12474-	N	126.7188	0.0130	3
	12475-	HN	9.6261	0.0063	3
	12781-	CB:-1	65.1901	0.0000	1
	12782-	CA:-1	56.8989	0.0000	1

The ShiftTable for #12473 only shows 2 assignments for CA:-1 and CB:-1 with a count of 1 for each which indicate that nothing was found in the HNCACB.

At right is shown #12473 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 #12473. The HNCACB trace in red shows 2 weak peaks at 37.5 PPM and 54.2 PPM.
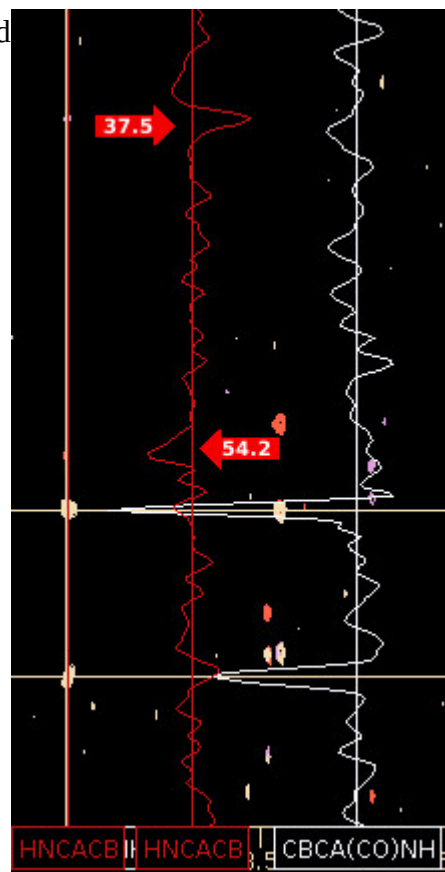
HNCACB Peaks along #12473 ShiftCursor
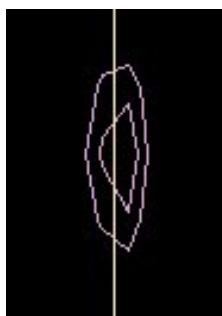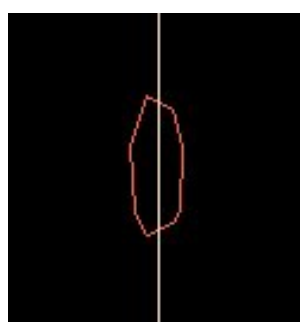
^{13}C at 37.5 PPM ^{13}C at 54.2 PPM

These two HNCAB peaks are very weak and too close to the noise 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 manually Peak-Picking the peaks at 37.5 PPM and 54.2 PPM:

	AtomID	AtomName	Shift	RMS	Count
#12473+	12474-	N	126.6987	0.0265	5
	12475-	HN	9.6262	0.0052	5
	12781-	CB:-1	65.1901	0.0000	1
	12782-	CA:-1	56.8989	0.0000	1
	14372-	CB	37.4830	0.0000	1
	14375-	CA	54.1630	0.0000	1

Very Weak Peaks
Is it Real or Junk?

	AtomID	AtomName	Shift	RMS	Count
#12500+	12501-	N	123.2751	0.0990	2
	12502-	HN	7.7197	0.0034	2
	12831-	CA|CB:-1	62.1650	0.0000	1

The ShiftTable for #12500 only shows only 1 ambiguous assignment from CBCA(CO)NH exp.

At right is shown #12500 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 #12500. The CBCA(CO)NH trace in blue shows that the inter-residue peak at 62.1 PPM and there may be something at 31.7 PPM.

^{15}N HSQC Peak
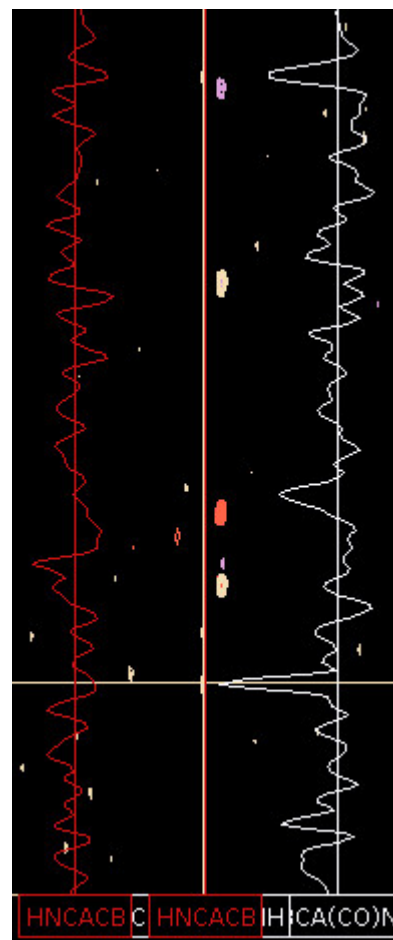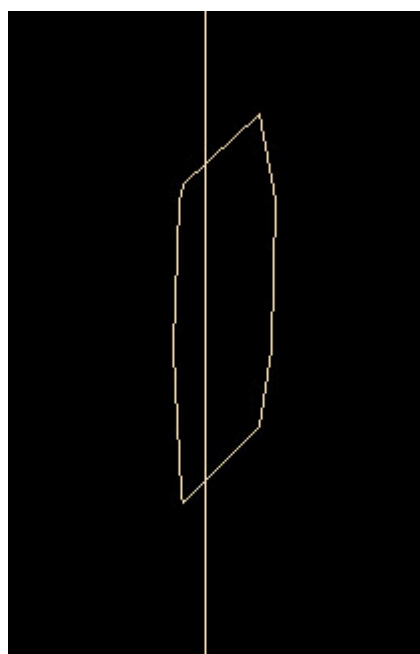
CBCA(CO)NH 31.7 PPM   CBCA(CO)NH 62.1 PPM
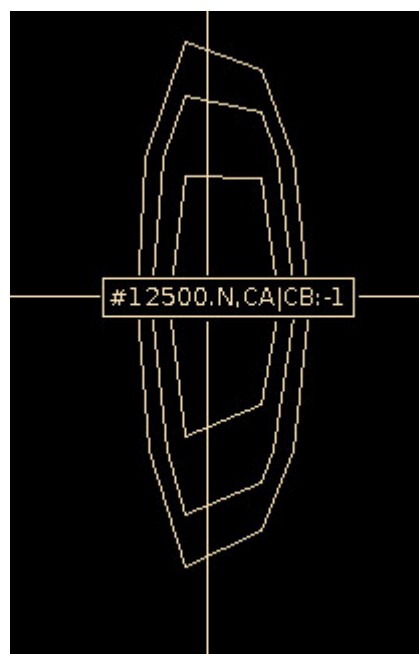
If you believe these are not real peaks, delete the HSQC and CBCA(CO)NH Peak-Picks which will remove #12500 from the ShiftTable.

If you believe that these peaks are real then you should add in the peak at 31.7 PPM. Since the HNCACB shows no peaks it will be difficult to find sequential assignments for this Spin-System.

Fixing GLY CA Assignments
Using Atom Quick Assign Menu

#12647+	AtomID	AtomName	Shift	RMS	Count
	12648-	N	115.2024	0.0296	6
	12649-	HN	9.1617	0.0028	6
	13107-	CB:-1	27.4132	0.0447	2
	13108-	CA:-1	58.3136	0.0216	2
	13867-	CA|CB	44.4994	0.0000	1

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

Click Left-Mouse on the SS-ID button (#12647 in this example) to residue jump. Confirm that the ^{13}C shift 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 are no other peak hiding under the threshold.
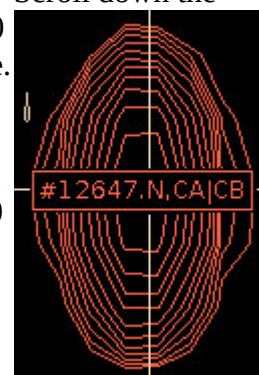
The Xipp assigner methods can not distinguish if a peak is a GLY CA or a LEU CB so without more information the peak is ambiguously assigned to #12647.N,HN,CA|CB.

Use Atom Quick Assign Menu on ShiftTable AtomID button to change assignment which will re-assign the peak. The Atom Quick Assign Menu only exists when an atom 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. To re-assign any shift 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 Atom or to a new atom.

Click Right-Mouse on button for AtomID 13867 for GLY CA assignment to be fixed in ShiftTable and select CA.

To get the best results you should scroll through all Spin-Systems looking for CA|CB and CA|CB:-1 that could be GLY CA or CA:-1 assignments. Do NOT just change all CA| CB to CA or CA|CB:-1 to CA:-1. You must look at the spectrum by doing a ResJump to each Spin-System to be sure that it is a GLY and not missing a CA. In addition to the GLYs in #12494 and #12647 **the tutorial assumes that you used the Quick Assign Menu to fix GLY for #12509, #12608, #12614, #12617, #12740, #12746, #12749 and #12752.** Note #12578 which is degenerate has a GLY CA:-1, but should not be fixed as indicated below. A careful reader will note that only 11 Spin-Systems have been identified as having GLY CA or CA:-1 when there should be 12. The final Spin-System containing a GLY CA:-1 was missed by CAPP in the ^{15}N HSQC and it will be found and fixed at the end of the tutorial.

Is #12536 a GLY?
(^{15}N 121.1 PPM, HN 7.19 PPM)

Spin-System #12536 has only 3 peaks with the now familiar pattern of a GLY CA as shown in Canvas at right., However, the Xipp assigner rules assigned the HNCACB peak at 42.1 PPM as CB instead of CA|CB. Did Xipp make a mistake and the peak at 42.1PPM is really a GLY CA? At first glance it appears that Xipp made an error because there is no good CA peak for #12536. At this point in the assignment Xipp identifies a peak as CA or CB based solely on the peaks ^{13}C shift compared to the expected ranges for CA and CB for all residue types. The CA PPM range is 43.54 – 67.4 which is more about 1.4 PPM outside the expected range of CA. Since 42.1 PPM is within the CB range, but outside the CA range the peak was assigned CB.

The known rule requires that Xipp assign the HNCACB peak at 55.74 PPM to be the CA:-1 since the CBCA(CO)NH peak would have been assigned to CA:-1 when the CBCA(CO)NH was automatically peak-picked and assigned previously. Xipp does not use the relative intensity of peaks to differentiate between (i,i) and (i,i-1) peaks in HNCACB experiments. However, the Peak-Table shown below indicates the the intensity of the HNCACB CA:-1 is stronger (more intense 1.29e7) than the CBCA(CO)NH CA:-1 (9.611e6). The HNCACB experiment is less sensitive for the CA:-1 than it is for the CA peak and for virtually every other Spin-System the intensity of the HNCACB CA:-1 is at least 1.5 less intense than CBCA(CO)NH CA:-1. Xipp can not count on this intensity difference since the HNCACB could have been acquired using a much longer acquisition time making direct comparison between intensities from CBCA(CO)NH and HNCACB difficult.

At the initial stages of backbone assignment it is best to not make any unnecessary assumptions. I suggest leaving the HNCACB peak at 42.1 PPM assigned to CB (ie do not assume that some aromatic group has caused a GLY CA to be shifted upfield). At best you could change this to CA|CB. Also, I recommend not changing the assignment of the HNCACB peak at 55.7 PPM from CA:-1 to CA since that assumes that the CA:-1 and CA of #12536 are degenerate. Again at best you could change this to CA:0|-1 to allow for both possibilities.
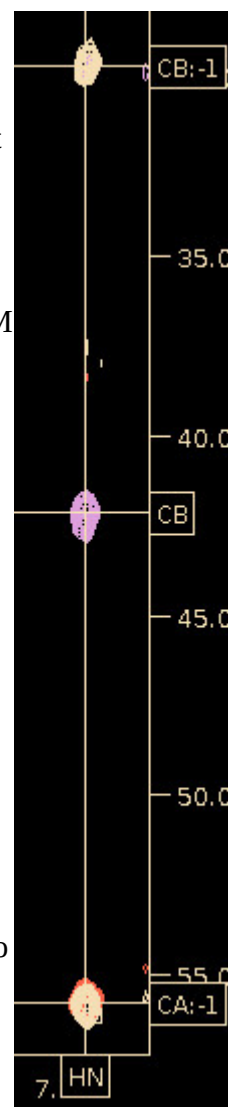
It turns out that #12536 is not a GLY (it's CA is degenerate with CA:-1 at 55.7 PPM). If the assignments are left as is #12536 will be correctly assigned by MARS as L84. This is confirmed with E85 CA:-1 at 55.77 PPM. The point of this page is that you must critically look at all of your data and ensure that the totality of the data makes sense.

Exp	PkID	Assign	HN	CACB	N	Intensity
HNCACB	153	#12536.N,HN,CA:-1	7.187	55.74	121.15	1.29e+7
HNCACB	156	#12536.N,HN,CB	7.187	42.13	121.14	-7.878e+6
HNCACB	158	#12536.N,HN,CB:-1	7.185	29.76	121.10	-1.164e+6
CBCA(CO)NH	64	#12536.N,HN,CA:-1	7.185	55.78	121.12	9.611e+6
CBCA(CO)NH	65	#12536.N,HN,CB:-1	7.185	29.67	121.11	7.258e+6

Check all Spin-Systems
Except #12578

To get the best possible results prior to running MARS you should visually inspect on the Canvas and the Shift-Table Windows all Spin-Systems and confirm that there are 3 peaks (for GLY) or 4 peaks that are assigned to specific atoms. This is absolutely necessary for publication quality work, but for the sake of time the tutorial will not do this. If you do check all Spin-Systems skip Spin-System #12578 since it will be used after running MARS 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 #12578 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 CA:-1) with HNCACB peaks 188, 190 and 192.

Do not create a new Spin-System and do not uniquely assign the peaks for #12578. Leave the peaks as ambiguous assigns to CA|CB and CA|CB:-1. I will use Spin-System #12578 on page 43 titled "Finding/Fixing #12578" as an example after Mars automatically assigns many peaks to demonstrate how a fully degenerate HN, ^{15}N Spin-Systems can be manually resolved using assigned spin-systems.

The Table shown below is extracted from Peak-Pick Table showing only #12578 peaks. Xipp does NOT sort peaks by assignment this figure was made by zooming the display (Middle Mouse) to a narrow vertical strip containing only the peaks from #12578 with Crt-v with Zoom-Box displayed.
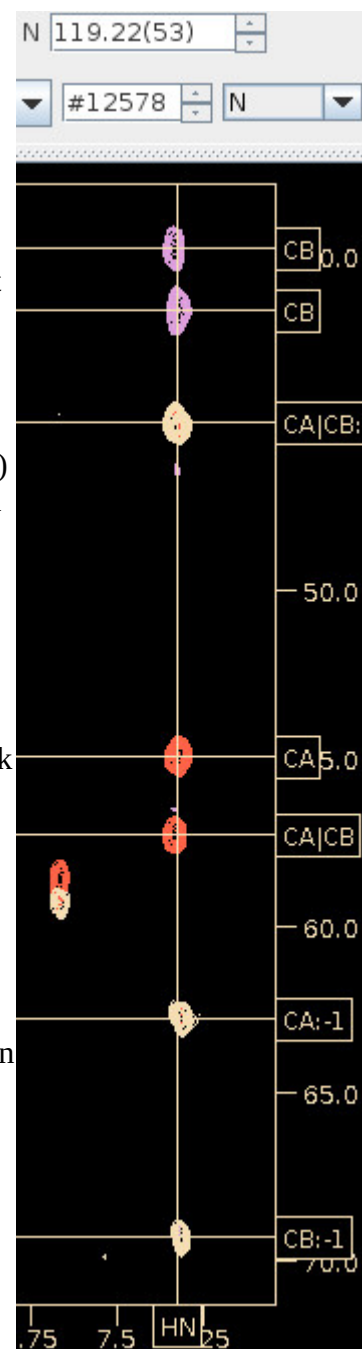
Exp	PkID	Assign	HN	CACB	N	Intensity
HNCACB	191	#12578.N,HN,CB	7.326	41.61	119.30	-5.608e+6
HNCACB	189	#12578.N,HN,CA	7.326	54.93	119.30	7.744e+6
HNCACB	188	#12578.N,HN,CA|CB	7.337	57.22	119.17	6.03e+6
HNCACB	192	#12578.N,HN,CB	7.338	39.75	119.16	-3.297e+6
HNCACB	190	#12578.N,HN,CA|CB:-1	7.338	44.94	119.13	1.863e+6
CBCA(CO)NH	98	#12578.N,HN,CA:-1	7.317	62.72	119.31	4.44e+6
CBCA(CO)NH	95	#12578.N,HN,CB:-1	7.317	69.25	119.30	2.938e+6
CBCA(CO)NH	96	#12578.N,HN,CA|CB:-1	7.329	44.97	119.15	1.002e+7

(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

Only on Linux systems can you open the MARS dialog Panel from Assign Menu to run MARS.
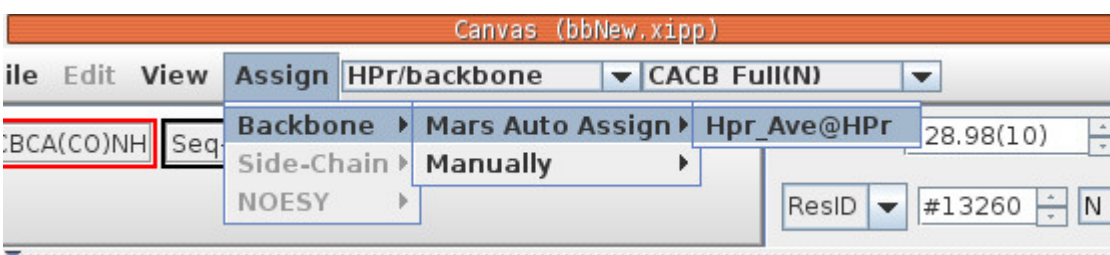
The MARS Dialog is shown on the next page

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.

The bbNew study and bbMars study will give the same SS-IDs as long as you followed the tutorial exactly and did not do any extra or skip any manual peak-picking or assignments and you exit and re-start the bbNew study after running MARS. When Xipp starts the 'maxUniqueID' property is always increased since Atom Object IDs are not saved in any file and as Xipp creates the Atom Objects for Atoms in unassigned Spin-Systems the 'maxUniqueID' property gets artificially increased every time Xipp is re-started. Although the SS-IDs are arbitrary they make an excellent identifier in the tutorial.

MARS Dialog

Backbone Auto-Assign via Mars . . .	✖

Mars Reference

Y.-S. Jung and M. Zweckstetter J. Biol NMR, 30, 11-23 (2004)
  Mars - robust automatic backbone assignment of proteins.

☑ **Create Backbone Assign Map (*.BAM) from Mars Results**

  ☑ Create Fix Assign File from Previous Backbone Assign Map Results

Path	_____/Xipp/ExampleData/HprE1/Asgnmts
BAM File	HPr_Ave_New.bam

Mars Text Files

In Directory	mars
Input File	HPr_mars.inp
Shift File	HPr_mars.shifts
Fix Assign File	NO
Manual Pred. S...	NO
Sequence File	HPr_mars.fasta
Shell Script	HPr_mars.com

  ☑ **Show Mars Process Panel**

Edit Pseudo Residues	Edit Input Template	View Input File

Max # Frag [5]

☐ **Sample Fully Deuterated**

☐ **Protein Disordered**

Atom Connectivity Table

Atom Type	Count	Prev (i,i-1) Use	Thresh	Intra (i,i) Count	Count	Next (i,i+1) Use	Thresh
CO	0	☐	0.4	0			
CA	69	☑	0.4	57			
CB	86	☑	0.4	75			
HA	0	☐	0.04	0			
HN	0	☐	0.04	101	0	☐	0.04
N	0	☐	0.4	101	0	☐	0.4

PSI PRED

  ☑ **Create Dummy PSIPRED File**

File Name	HPr_mars.psipred

OK	Cancel

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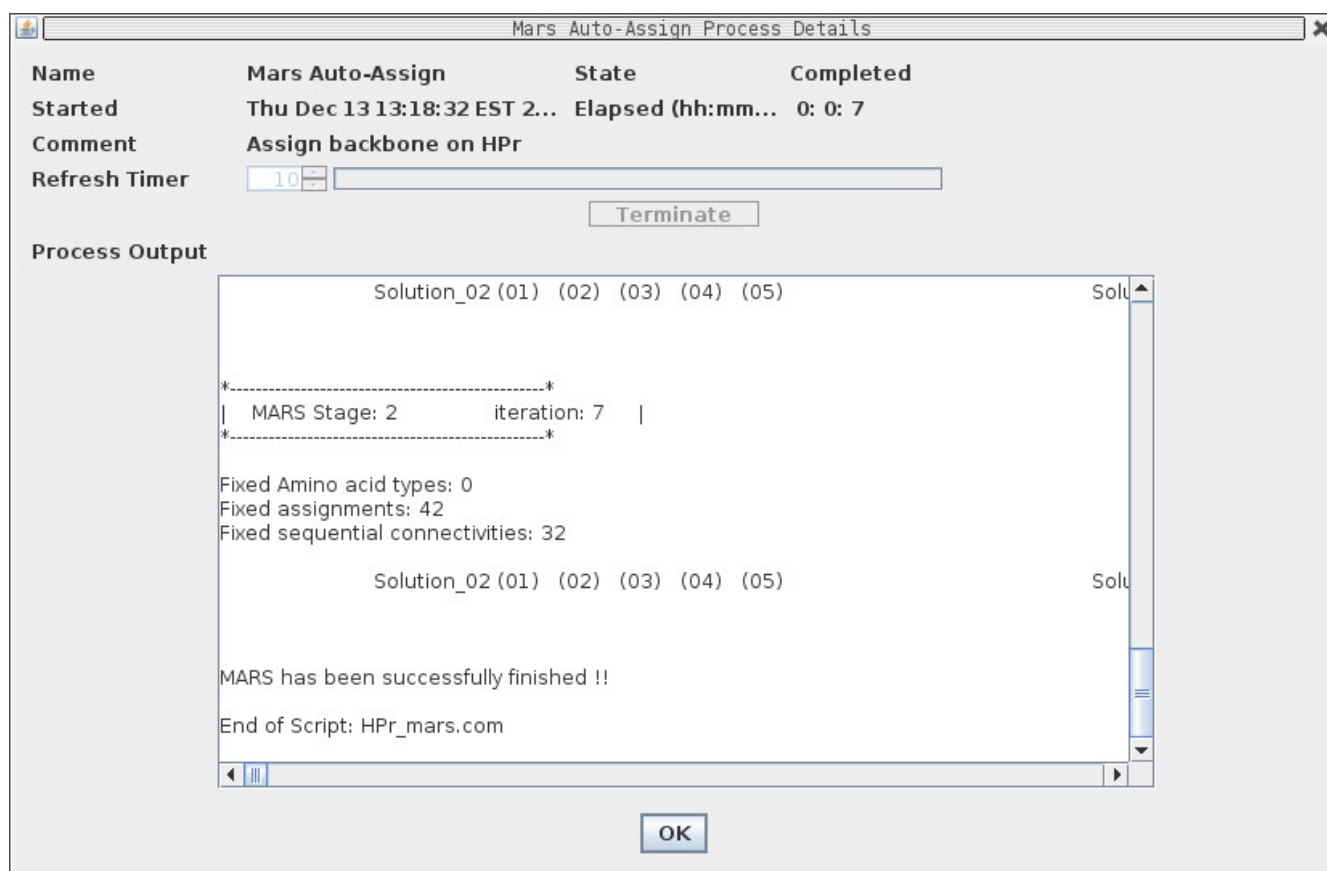
MARS is fast for small and moderate sized proteins such as HPr (85 residues), but it is non-linear with respect to the size of the protein and can take several hours for a 300 residue protein.

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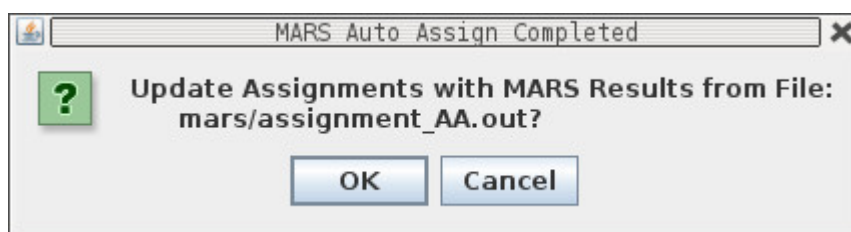
The file mars/assignment_AA.out is read in to assign Spin-Systems to specific residues. As each Spin-System is assigned the Peak-Pick assignments for each newly assigned atom is updated as well as the ShiftTable. In addition the BAMTable is updated to reflect the new assignments so that each Spin-System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

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

The Spin-System IDs from bbNew and bbMars may be different going forward. The SS-IDs in the tutorial will be those obtained from bbNew after running Mars and then exiting Xipp, re-starting Xipp and showing bbNew. On non Linux systems restart Xipp 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 the shifts in each Spin-System were correctly assigned. The results here should match the bbMars Study and are the results of minimal cleaning/fixing up of the Spin-Systems as described in tutorial.

	ShiftTable-Hpr_Ave@HPr	BAMTable-Hpr Ave@HPr				
Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
M1		-	-	-	-	-	-
F2	#13260	129.00	8.9714	54.76	57.22	32.08	42.27
Q3	#12506	122.90	7.6149	57.27	53.68	42.26	32.54
Q4	#12575	119.46	8.5156	53.71	55.42	32.43	33.61
E5	#12518	122.18	8.2183	55.44	54.71	33.62	31.84
V6	#12548	120.91	9.1955	54.69	60.15	31.80	34.43
T7	#12521	121.84	8.0870	60.18	61.57	34.43	69.02
I8	#12476	126.07	8.6216	61.60	-	69.12	36.41
T9	#12566	119.93	8.6276	61.43	-	36.34	69.22
A10	#12458	129.29	7.3131	61.45	50.65	69.41	17.92
R11		-	-	-	-	-	-

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 or unassigned. The BAMTable is divided in two groups with all assigned Spin-Systems shown 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, pale orange indicating 2 minor problems and red indicating more problems.

The Res-ID and SS-ID are ButtonPopup objects with default action to ResJump to the residue or Spin-System defined by the button. In order to keep the CA:-1 and CB:-1 in the same Spin-System when they are 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 to a specific residue. Therefore the PPM value in the BAMTable may be different than in the ShiftTable.

The quality of the assignments obtained by MARS depends on the quality of the assigned PeakPicks. At this point in the tutorial here should be 38 green assignments in the BAMTable which includes fixing 10 GLY CA, resolving the degeneracy at #12461 (by creating #13260) and assigning missing HNCACB peaks for #12473. However, if bbNew is run from scratch using CAPP to peak-pick with Xipp assigning peaks for ^{15}N-HSQC, CBCA(CO)NH and HNCACB followed by running MARS with no other manual fixes then only 25 green assignments are shown in the BAMTable. There are 82 assignable amides in HPr (2 PRO). The few simple fixes in tutorial allowed 46% of HPr Amides to be assigned compared to 30% that are assigned using only automated peak-picking/assigning.

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 available since T16 has not been assigned.
>
> **Assign Residue**
>> Brings up a simple modal dialog to manually assign T16 to a Moiety/Spin-System. You need to know the Spin-System ID before bringing up modal dialog. Dialog will reject Object IDs that do not belong to a Spin-System, ie Atom Object-IDs and Residue Object-IDs are not accepted and will result in dialog being re-shown. Sorry no error message given.
>>
>> **# Columns Matched**
>>> Where # is a number between 1 to 4. Shows in sub-menu all Spin-Systems that have # columns that match between found Spin-System in sub-menu and existing preceding and/or next assigned Spin-Systems. The # Columns matched is not shown when the adjacent (previous/next) residues are unassigned or no Spin-Systems are found.

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 PPM values in columns CA, CA:-1, CB and CB:-1 with 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 #12686 and given the current assignments for H15 and R17 #12686 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 ID is displayed in ( ) and if it is currently assigned the assignment precedes it. 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 that are not matched are not used to calculate the RMS. 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, pale orange 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 that are found and there are two possible assigned Spin-Systems you should consider first 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 #12557 (assigned to H15):

- **Jump** (Default Action)
  Res Jump to this Spin-System (#12557).
  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 #12557 has not been assigned or unassigned through menus.
- **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 options 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 #12557**
  Will ask for confirmation to delete Spin-System which will remove Peak-Pick assignments. This will unassign the peaks assigned to this SS-ID. This is unavailable for #12557 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 a simple modal dialog to manually assign Spin-System #12557 to a residue. Dialog will reject ResID values outside of sequence by graying out Select button used to select value.
  - **# Columns Matched**
    Where # is a number between 1 to 4. Shows in a sub-menu all residue/Spin-Systems pairs 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 #125576 could be assigned to, based on comparing PPM 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 Next SS-ID menu options. The Find Prev SS-ID menu options are similar except that it is looking for the previous SS-ID and the Seq-HNCACB is used.
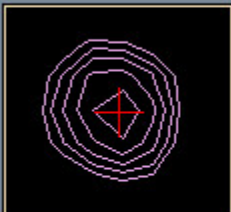
After Clicking the #12577 Find Next SS-ID menu and selecting option "- (#12686) 0.0565" three changes are made to the Canvas and Table Windows that mimic what you would do manually in order to look for the next SS-ID:

(1) In Canvas ResJump to H15 since #12577 is assigned to H15,
(2) In Name Peak-Pick Table add Peak-Picks defining H15.N, H15.CA:-1, H15.CA, H15.CB-1 and H15.CB Shifts.
(3) In Canvas set view Seq-CBCA(CO)NH slice to #12686 ^{15}N and add red PPM Cursors at #12686 HN, CA and CB Shift values.

The Name PeakPick Table gets updated with peaks from the CBCA(CO)NH and HNCACB experiments as if they were manually picked. Shown below are the peaks for the H15.CA and H15.CB shifts.

The Exp Button Seq-CBCA(CO)NH manages a second view into the CBCA(CO)NH whose ^{15}N slice is set to the ^{15}N shift of #12686 since it was selected in the Find Next SS-ID menu option. In addition the Seq-CBCA(CO)NH view has three PPM Cursors drawn in red at the PPM shift for #12686 HN, CA and CB (not shown in figure) assignments. **Left Click Mouse** over the Seq-HNCACB experiment button toggles on/off this second view which includes the red PPM Cursors.
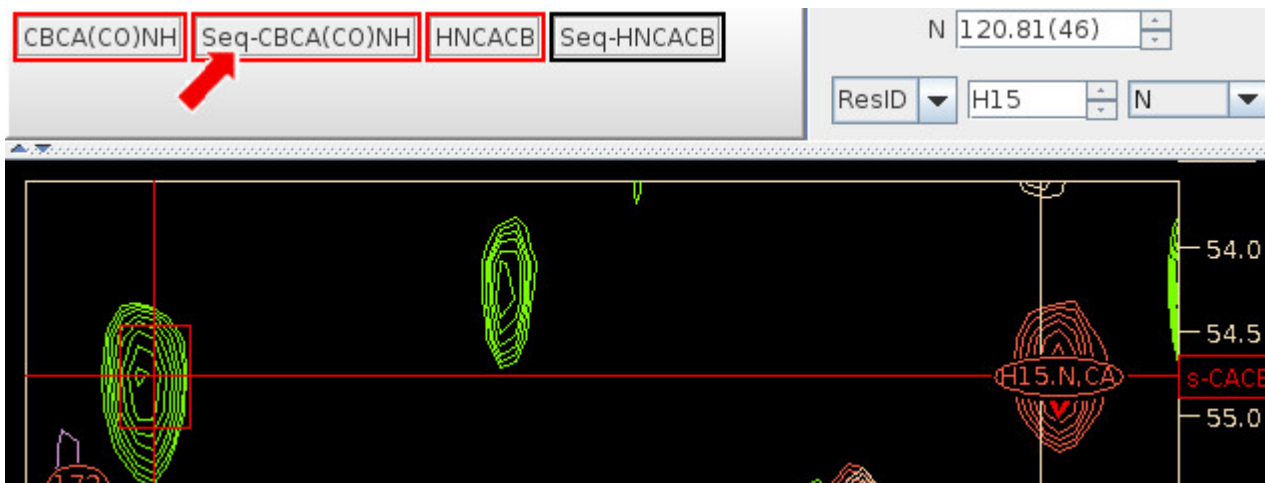

Right-Clicking the Mouse over the Seq-CBCA(CO)NH Button brings up the Seq-Exp Menu:

- **Active**: Toggle Active state of Exp.
- **Linked**: Toggles linked state of Exp. When Linked is selected for both SEQ-* Exps changes to slice of Seq-CBCA(CO)NH will also change slice for Seq-HNCACB
- **Jump to Slice**: Displays non-modal Frequency/Residue Chooser dialog for this Seq-CBCA(CO)NH view.
- **Contour Levels:** Displays modal dialog to change contour levels.
- **Clear PPM Cursors**: PPM Cursors identify potential sequential residues on the Seq-CBCA(CO)NH view. This option clears all PPM cursors from this Seq-CBCA(CO)NH view. It erases the red lines.
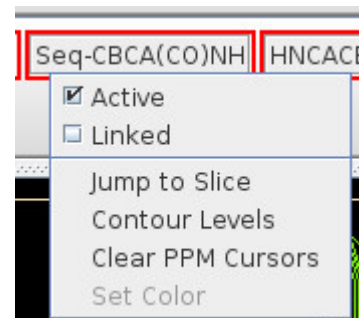
The Frequency/Residue Chooser dialog which is created by the Jump to Slice menu option allows explicit/ manually changing the Seq-CBCA(CO)NH ^{15}N slice by slice number, PPM value or by ResID or SS-ID in exactly the same way that the main Canvas selects the Slice.
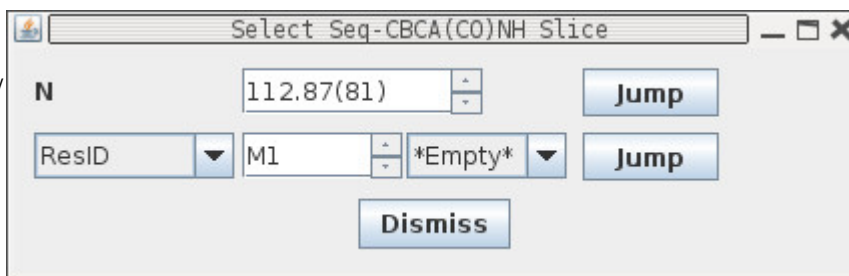
This window is non-modal so you can keep it open while interacting with Canvas and Table Window. Changes to Seq-CBCA(CO)NH from Find Prev SS-ID menu options will update the slice and PPM values shown here but will not the ResID value shown here.

The Seq-CBCA(CO)NH 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 CBCA(CO)NH experiment. Since this uses a single ^{13}C shift instead of two that are used by Find Next 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 the strip that was peak-picked. For instance if the CA:-1 peak was used to find a sequential strip then this confirms the strip by finding another peak in common such as the CB:-1. For H15.CA when Required Confirmation is disabled there are 227 possible sequential matches found instead of 32 that are found when it is enabled.

Select the top line in S+ for the CA 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 and CB S+ tables at the same time, but only the last line selected will have a PPM Cursor shown on the Canvas.

PkID	Assign		HN	CACB...	N	Intensity
A +-	Assignment-Table					
S +/-	Seq Strip-Table					
167	H15.N,HN,CB		7.015	29.841	120.736	-2.038e+6
K +	HNCACB: No Assignments Found for Peak 167					

S + 11	Exp	Slice	HN	CACB	N	Intensity	
	CBCA(CO)NH	115	5.040	30.064	105.115	+6.286e+5	
	CBCA(CO)NH	42	5.945	30.050	121.713	+6.533e+5	
	CBCA(CO)NH	44	5.118	30.043	121.276	+8.171e+5	

170	H15.N,HN,CA		7.014	54.761	120.690	3.168e+6
K +	HNCACB: No Assignments Found for Peak 170					

S + 12	Exp	Slice	HN	CACB	N	Intensity	
	CBCA(CO)NH	44	5.090	55.048	121.265	+6.22e+5	
	CBCA(CO)NH	104	5.984	55.042	107.626	+6.166e+5	
	CBCA(CO)NH	115	5.046	54.966	105.239	+6.033e+5	

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 - #12557' when Spin-System is unassigned via the menu.

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 Cα and Cβ shift values. Selecting a specific ResType from the drop-down list does nothing. The list is provided only to help make sequential assignments, ie confirm that Cα and Cβ shift values are consistent with assigned residue type.

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 #12686) 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 #12557 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 Residue of the match (if SS-ID is assigned) is shown in the first column before the ( ). If the SS-ID is unassigned then – is shown in first column and the menu option is grayed out indicating it can not be selected. The SS-ID is shown between ( ) if Residue is assigned or (-) is shown. The last column is the RMS deviation of the matched columns for this Spin-System. Columns not used 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: black and gray indicates unassigned, green is a good assignment, pale orange 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 consider first 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 (#12577) to the Res-ID defined in the menu option. If this SS-ID and/or the Res-ID selected is already assigned a confirmation dialog will appear describing the changes to be made. The Warning dialog at right is what would be shown if #12577 would be re-assigned from H15 to T56. The menu option "Undo Assign T56 – #12557" is added to the T56 Res-ID and 12557 SS-ID buttons to reverses these 3 steps. Note no Undo is added to the menu for H15 Res-ID and SS-ID #12719 buttons.
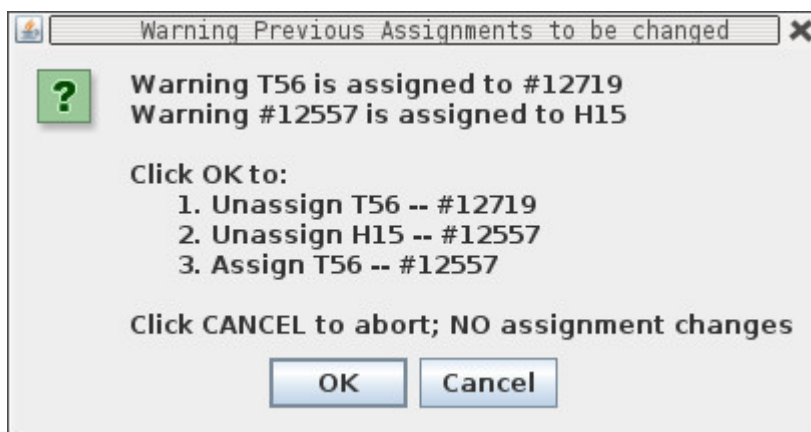
Ambiguous Assignments CA|CB
Affects Proposed Assignments

When all ambiguous assignments (CA|CB and CA|CB:-1) are resolved for #12578 by creating a new Spin-System and the right set of peaks are moved to the new Spin-System MARS is able to assign #12578 (or the newly created Spin-System) to L53. The right set of peaks are the peaks that belong together in a specific residue which can be determined for #12578 because the HN and ^{15}N are different enough to be resolved based on visual and relative position of HN and ^{15}N shift values as shown on pages 10-15 and 21 under the headings Fixing HN, N Degeneracy.

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
L53	#12578	119.24	7.3280	62.72	54.93	69.25	-

As the difference between pairs of degenerate residues HN and ^{15}N shifts gets smaller it becomes nearly impossible to resolve the degeneracy and the assignments of all ^{13}C atoms will be ambiguous. If MARS is run with ambiguous assignments for all ^{13}C atoms in the Spin-System then the Spin-System can not be assigned as shown below for the degenerate Spin-System #12578 after unassigning L53 and making all ^{13}C assignments ambiguous.

Res-ID	SS-ID	N	HN	CA:-1	CA	CB:-1	CB
	#12578	119.24	7.3280	-	-	-	-

In this situation starting from SS-ID button #12578 the menu options for 'Find Prev SS-ID' and 'Find Next SS-ID' are empty and 'Columns Matched' does not exist because all of the ^{13}C assignments in #12578 are ambiguous. The SS-ID match starts with the unique atomic assignments for the Spin-System under question and since there are no unique assigns for #12578 nothing is found. A careful reader will notice the menu option 'Undo Unassign L53 - #12578' which indicates that I explicitly unassigned #12578 from L53. I also modified all of the ^{13}C assignments so that they were ambiguous for this demonstration.

Note Xipp attempts to assign each peak on its own using expected shift ranges for atoms and only in a few circumstances looks at other assignments in the Spin-System. For this reason the order in which the peaks are assigned affects whether a peak is assigned CA|CB or just CA. All peaks in CA shift range are initially assigned to CA|CB, but when the first peak in a CB range is found then the original peak assignment gets changed from CA|CB to CA. As more peaks in the CA range are found they all get assigned to CA|CB. **You can not depend on Xipp to automatically figure out CA and CB when there are degenerate ^{15}N, HN Spin-Systems.**

Re-Assigning #12578 ^{13}C Atoms
for Ambiguous Assignments

In BAM-Table Unassign L53 so that only the assignments from #12578 will get re-assigned. The CA:-1 and CB:-1 from G54 (#12470) should not be re-assigned since there is no ambiguity in their assignments.

In the ShiftTable for each unique ^{13}C atom assignment in #12578 click Mouse-Left button on AtomID button and select 'ReAssign Shift . . .' to bring up the Select Atom Dialog shown below.

Be careful to only select an Atom that is not already assigned to a different Shift. The upper panel with check box 'Select Existing Atoms' shows all of the current Atoms in this Moiety whether assigned or not. The atom CA|CB:-1 (14925) is assigned to #12578.CA|CB:-1 at 44.95 PPM. If #12578.CA:-1 (14791) at 62.72 PPM is re-assigned to CA|CB:-1 (14925) then the two Shifts will be averaged together with a large RMS for the Atom in the ShiftTable. The 5 ^{13}C AtomIDs in #12578 that need to be re-assigned are: 14791, 14794, 14797, 14800, 148935. These are the AtomID shown in second column.

The lower panel with check box 'Create New Atom in Moiety' is used to create new Atoms. You must explicitly click Atom names such as CA and CB. The single offset of -1 was set when dialog created.

The top line of the Select Atom Dialog indicates the change that will occur when OK is clicked.

The ShiftTable for #12578 should look like the figure below after all shifts have been re-assigned to ambiguous atoms. Note that all ^{13}C atom names are ambiguous (are CA|CB or CA|CB:-1) and the RMS is reasonable. The RMS can be high if you combined shifts with very different PPM values into the same Atom. The only way to fix this is to change the assignment of the Peak-Pick by clicking the Peak-Pick in the Canvas window and then changing the assignment.

	AtomID	AtomName	Shift	RMS	Count
	14795-	N	119.2424	0.0805	9
	14796-	HN	7.3280	0.0080	9
	14925-	CA|CB:-1	44.9546	0.0114	2
#12578+	14931-	CA|CB	57.2228	0.0000	1
	14941-	CA|CB:-1	62.7163	0.0000	1
	14942-	CA|CB:-1	69.2531	0.0000	1
	14943-	CA|CB	54.9282	0.0000	1
	14944-	CA|CB	39.7509	0.0000	1
	14945-	CA|CB	41.6063	0.0000	1

The BAMTable for F29, L53 and #12578 should look like this:

F29	#12533	121.40	6.8695	-	-	-	21.75
L53		-	-	-	-	-	-
	#12578	119.24	7.3280	-	-	-	-

Note F29 is mis-assigned to #12533 and L53 and #12578 are unassigned. The CA:-1, CA, CB:-1 and CB columns are empty for #12578 because all the assignments are ambiguous.

There are no special tools to find that the peaks in #12578 should be assigned to F29 and L53.

Manually looking for residue assignments for fully ambiguous Spin-Systems such as #12578 should be done after all other Spin-Systems are assigned to simplify the search.

Finding/Fixing
Ambiguous Assignments #12578

.

There are two ways to handle an SS-ID that has two Spin-Systems such as #12578:
>   (1) Split up #12578 assignments into two unassigned Spin-Systems, but any mistakes such as using F29.CA:-1 with L53.CB:-1 will cause problems and will give poor results,
>   (2) Leave #12578 with ambiguous assignments from two Spin-Systems until the SS-ID button menu options 'Find Next SS-ID' or 'Find Prev SS-ID' from another Spin-System finds a match to some of the ambiguous assignments in #12578.

If the assignments can be easily separated then option (1) is best which was described previously for the CBCA(CO)NH with #12461 and #13260.

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.

The search routines in Xipp allow finding an adjacent Spin-System that has ambiguous assignments, ie CA|CB. The 'Find Next SS-ID' for #12749 (from G28) and 'Find Prev SS-ID' for #12602 (T30) both indicate that #12578 is a good match.

Since #12578 is also a good match for Find Next SS-ID from #12743 (T52) and Find Prev SS-ID from #12740 (G54) there are 4 places in the sequence that can be used to pull out which ^{13}C shifts belong together in the same Spin-System.

ResJump to #12533 (F29) to convince yourself that it is not a good Spin-System and then Unassign F29.   It is better to create a new Spin-System and populate it with the peaks for F29 than assigning #12578 to F29. **Do NOT assign #12578 to F29.**

G28	#12749	105.32	7.4987	58.57	45.00	32.03	-
F29		-	-	-	-	-	-
T30	#12602	118.44	10.8954	57.26	63.74	39.72	68.95

ResJump to #12578. The peaks at 45.0, 57.26 and 39.72 PPM belong to the Spin-System that will be assigned to F29. We know this because G28.CA is 45.0, and T30.CA:-1 is 57.26 and T30.CB:-1 is 39.72 PPM. Click Mouse-Left over peak at 45.0 PPM in CBCA(CO)NH which will be the first peak added to a new Spin-System. In the NamePeak Table click on the assignment button to bring up the Assignment Detail Dialog shown on next page.

| 96	#12578.N,HN,CA|CB:-1	7.329	44.966
K +	CBCA(CO)NH Assigns (1)		HN
	#12578.N,HN,CA|CB:-1		-0.0006

Click the top button labeled #12578.N,HN,CA|CB:-1 to bring up the "Change Moiety #12578 to Selected" dialog and click Select on the row that begins 'New Moiety' as shown below.
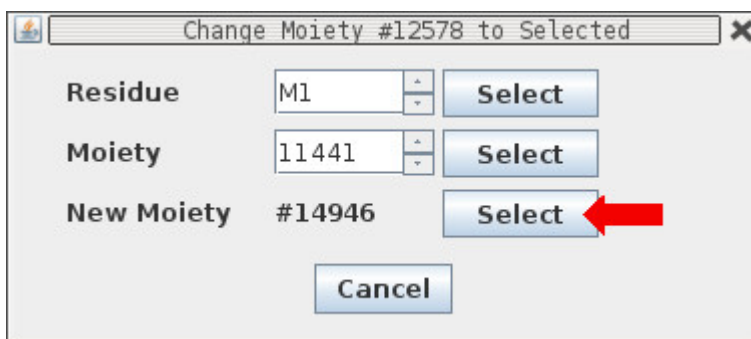
This will bring up a window confirming that all assignments for this Peak-Pick will be changed to the new Spin-System, for example #14946 in this tutorial. Click OK on the Confirm Peak Assignments. The Assignment Detail Dialog should be updated to show #14946. Click OK on the Assignment Detail Dialog to update the Peak-Pick assignments and ShiftTable. If you click Cancel here then nothing changes.

Scroll down to the bottom of the ShiftTable and ResJump to recently created Spin-System (ie #14946). Use Atom Quick Assign to change #14946.CA|CB:-1 to #14946.CA:-1 since this is G28.CA.

Click on the peak at 39.75 PPM and in Name-Peak Table switch to Extended-Rule and select #14946.N,HN,CB from the list of possible assignments.
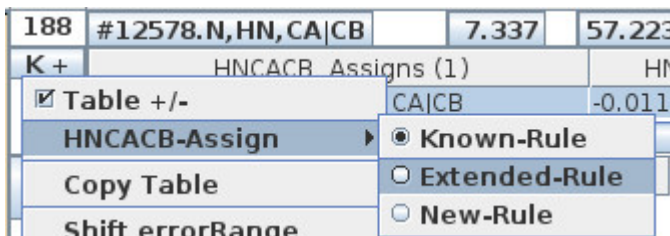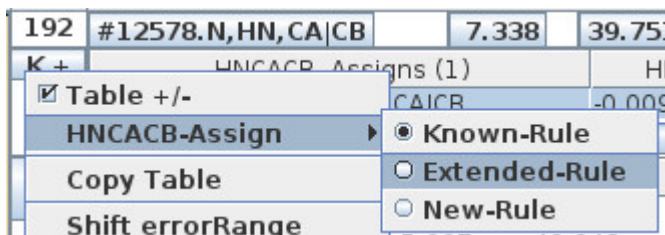
Click on the peak at 57.26 PPM and in Name-Peak Table switch to Extended-Rule and select #14946.N,HN,CA|CB from the list of possible assignments.

Use BamTable F29 button to select #14946 from '3 Columns Matched'.

Re-Assign HNCACB peak at 45 PPM to #14946 and use Atom Quick Assign for remaining peaks in #12578.
Similarly L53 can be assigned to #12578.

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	#12473	126.70	9.6262	56.90	54.16	65.19	37.48
G39	#12752	102.82	8.5147	54.19	45.31	37.59	-
K40	#12656	114.95	8.1719	-	56.34	-	-
S41	#12644	115.57	8.2902	54.91	56.94	34.28	66.67

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 #12578 from L53 to K40.

Click Right Mouse on #12644 (SS-ID for S41) and select L53 (#12578) 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 adds PPM Cursors at L53 HN, CA and CB Shift values.

In Canvas window select only the CBCA(CO)NH and Seq-HNCACB experiments to be 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 the #12578 peaks assign well to L53 using different ^{13}C values #12578 is not a good candidate to be re-assigned to K40.

S41.CB:-1

S41.CA:-1
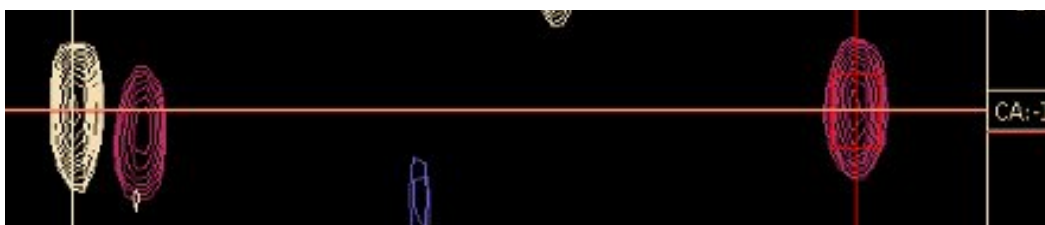
	PkID	Assign		HN	CACB...	N	Intensity
Contours	A +-		Assignment-Table				
	S +/-		Seq Strip-Table				
	281	S41.N,HN,CA:-1		8.292	54.894	115....	5.442e+6
	K +	HNCACB Assigns (1)			HN	CACB	N
		S41.N,HN,CA:-1			-0.0016	0.0149	0.0064
	S +	Exp	Slice	HN	CACB	N	Intensity
	22	HNCACB	6	5.080	55.164	129.842	-7.236e+5
		HNCACB	63	7.697	55.146	116.935	-6.04e+5
		HNCACB	91	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 using good peaks (ie not noise) on the red line. After moving down 7 times the selected line should be at slice 39.

HNCACB	39	7.890	54.898	122.332	+1.134e+7

S41.HN   7.89 PPM
CBCA(CO)NH   Seq-HNCACB (Slice 39)

S41.CB:-1

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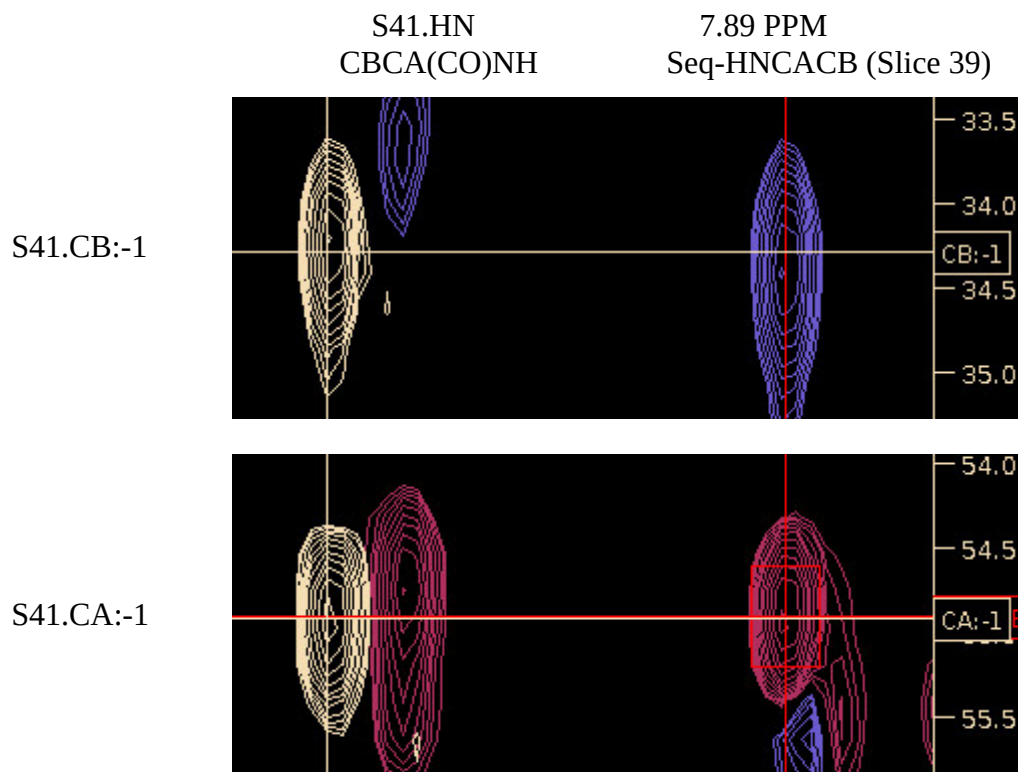
The S+ table uses extrema (all local maxima and minima) to find sequential matches from a single PeakPick and as such it might be necessary to start from the CB:-1 of S41 instead of the CA:-1 in order to find a match if the extrema for the CA is not resolved (such as a shoulder peak). In addition **Click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

On the next page the identity of the peaks on Slice 39 ^{15}N 122.3 PPM and HN 7.89 PPM are confirmed.

Confirming Identity of Slice 39, (122.3 PPM) 7.89 PPM

The CBCA(CO)NH and HNCACB 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.
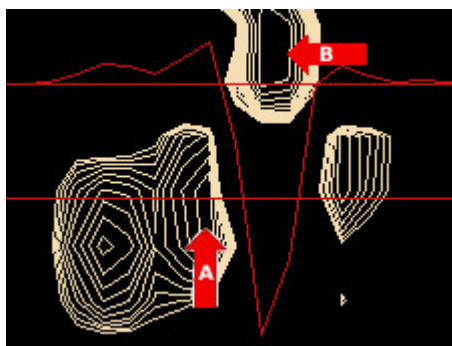
The corresponding peak in the ^{15}N HSQC shown at left labeled A is distorted by an intense peak labeled B whose resolution enhancement artifact is 5 times more intense than the peak at A. The trace displayed shows the relative intensity of the artifact and the peak at A.

Since CAPP did not PeakPick the peak at A in the ^{15}N HSQC no Spin-System was created in the assignment table and the peaks in the CBCA(CO)NH and HNCACB experiments could not to be assigned.

Although Xipp does not automatically create a new Spin-System when reading in CAPP created Peak-Picks from the CBCA(CO)NH and HNCACB experiments we can manually create a new Spin-System. First jump to slice 39 (**j 39**) and then in the CBCA(CO)NH experiment only **Click Left-Mouse** over peak at 45.3 PPM to add that peak into Name PeakPick table and then select the New-Rule assignment such as #14964 as shown below.

Selecting #14964 (or whatever arbitrary SS-ID you get) creates a new Moiety/Spin-System, assigns the peak to it and adds 3 new shifts to ShiftTable and BAMTable. ResJump to #14964 and in HNCACB c**lick Left-Mouse** over the peaks at 54.9, 45.3 and 34.4 PPM and select the same Spin-System ID as created for peak at 45.3 PPM, ie #14964.

53	#14964.N,HN,CA|CB:-1	7.880	45.298	122.273	1.496e+7
N +	CBCA(CO)NH Assigns (1)		HN	CACB	N
	#14964.N,HN,CA|CB:-1		0.0000	-45.0000	-122.0000

Use QuickAssign in ShiftTable to change the #14964 CA|CB:-1 to CA:-1. The BAMTable entries for #14964 and K40 BAMTable show 3 Columns Matched from #14964 and K40.

#14964	122.31	7.8879	45.30

Jump
  Find Prev SS-ID ▶
  Find Next SS-ID ▶
Unassign SS-ID
Undo
Prev ResTypes ▶
Cur ResTypes ▶
Delete #14964
Assign Moiety
  3 Columns Matched ▶ K40 (#12656) 0.0582

K40	#12656	114.95	8.1719
Jump		5.57	8.2902
Unassign Residue		23.23	9.0614
Undo		3.98	8.3642
Assign Residue			
3 Columns Matched ▶		-	(#14964) 0.058
2 Columns Matched ▶		07.45	7.5902