Manual instruction of MagRO ver. 1.20.x

1-1. Installation (NMRView C-version)

The following 3 files are required for installation of MagRO;

nmrview_5_x_x.tar.gz (NMRView ver5.2.2 or later)
protein.seq (sequence file. 1-letter code)
MagRO_NMRView_v1.18.x.tar.gz

1) copy and extend the compressed NMRView file at somewhere in your home directory;

```
cp /home/naohiro//nmrview_5_x_x.tar.gz ~/
tar xvzf nmrview_5_x_x.tar.gz
```

After that, change the directory name as you want;

```
mv nmrview nv_protein
```

nv_protein/matrix is going to be a working directory.
Place all of the spectrum data under the nv_protein/matrix

2) Next, copy and extend MagRO_NMRView_v1.18.x.tar.gz at somewhere in your home directory.
For example;

```
cp MagRO_NMRView_v1.18.18.tar.gz   ~/bin
cd ~/bin
tar xvzf MagRO_NMRView_v1.18.18.tar.gz
```

Then, move to the MagRO directory,

```
cd   ~/bin/MagRO_NMRView_v1.18.18
```

You will find a text file whose name is run_temp_C.csh. Open the file with text editor, then edit the following line as you have set up for your data;

```
setenv NKDIR /home/naohiro
setenv MGFIR MagRO_NMRView_v1.18.18
setenv NMRVIEW5HOME $NKDIR/nv_protein
```

After that, copy the file to your home directory and change the name of the file as you can see easily what it work for;

```
cp run_temp_C.csh ~/run_protein_C.csh
```

Now you are ready to start MagRO_NMRView.
Type the file name to start the program;

```
./run_protein_C.csh
```

If you find error message, please check the permission of the csh file firstly.

1-1. Installation (NMRView Java-version)

Before the installation of MagRO, you have to complete the installation of NMRViewJ.
The following 2 items are required for installation of MagRO;

nv_protein (Project diretory)
protein.seq (sequence file. 1-letter code)
MagRO_NMRView_v1.18.x.tar.gz

Make diretory "matrix" under the nv_protein directory

```
mkdir nv_protein/matrix
```

nv_protein/matrix is going to be a working directory.
Place all of the spectrum data under the nv_protein/matrix

2) Next, copy and extend MagRO_NMRView_v1.20.x.tar.gz at somewhere in your home directory.
For example;

```
cp MagRO_NMRView_v1.20.18.tar.gz  ~/bin
cd ~/bin
tar xvzf MagRO_NMRView_v1.20.18.tar.gz
```

Then, move to the MagRO directory,

```
cd  ~/bin/MagRO_NMRView_v1.20.18
```

Please find "run_temp_J9.csh" in the MagRO directory.
Open the file with text editor, then edit the following lines as you have set up for your data;

```
setenv MAGDIR      /home/nmruser/MagRO_NMRView_v1.20.18
setenv NMRVIEWJ    /home/nmruser/NMRViewJ
setenv NVDIR       /home/nmruser/nv_protein
```

MAGDIR, NMRVIEWJ and NVDIR specify the MagRO directory, installed NMRViewJ directory and project diretory, respectively.

After that, copy the file to your home directory and change the name of the file so that you can see easily what it is;

```
cp run_temp_J9.csh ~/run_protein_J9.csh
```

Now you are ready to start MagRO_NMRView.
Type the file name to start the program;

```
./run_protein_J9.csh
```

If you find error message, please check the permission of the csh file firstly.

1-2. How to add new project

1) For NMRView C-version, you can easily to add new project by copying and extending nmrview_5_x_x.tar.gz file.

```
cp nmrview_5_x_x.tar.gz ~/
tar xvzf nmrview_5_x_x.tar.gz
mv nmrview_5_x_x nv_protein2
```

For NMRView Java-version, you have to create project directory such as "nv_protein2".

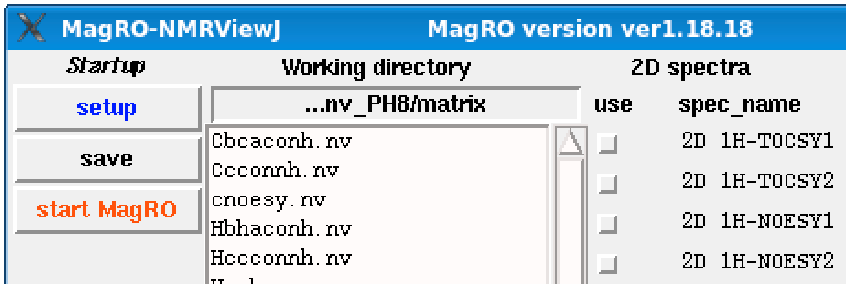
If you don't find matrix directory in the uncompressed directory, you have to create it;

```
mkdir nv_protein2/matrix
```

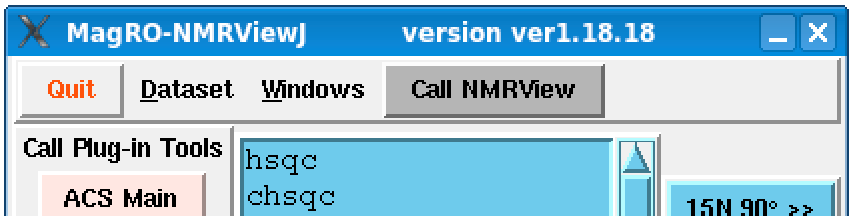
2) Similaly as described previous page, you have to copy and edit the run_**.csh file.

1-3. How to update MagRO

To update MagRO, user can replace with the old directory of MagRO-NMRView with new one.
You can confirm the version of MagRO-NMRView on window header of the startup module;

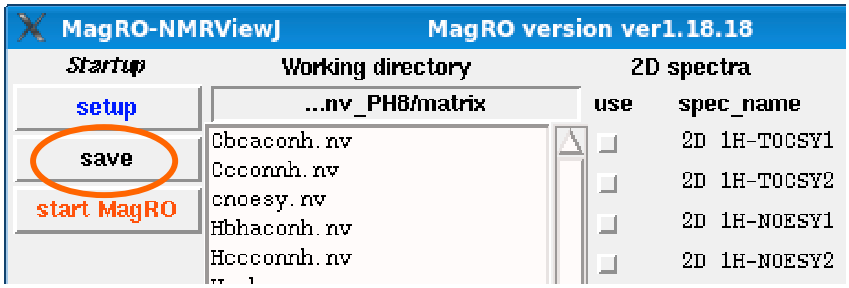


or you can find it on the window header of main module;



1-4. Initial setup of MagRO

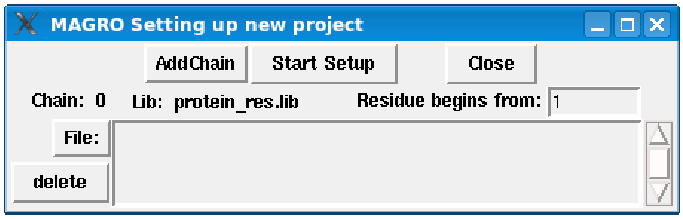
If you start MagRO-NMRView, you will see the follwoing window;



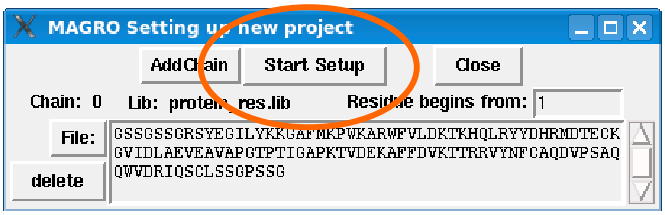
If you have not set any project before, the program will ask you to setup sequence of sample with the following popped up windows;

To load protein sequence file, you have to select library. Click AddChain -> Library: protein_res.lib;

Then the window will change;



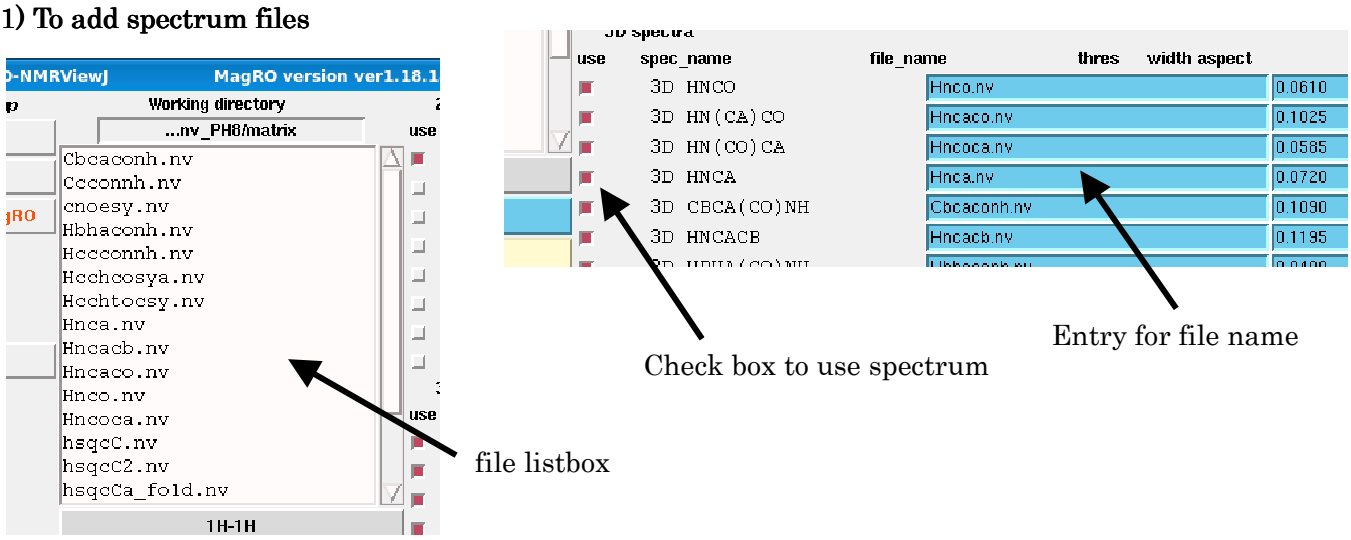
To load protein sequence file, press "File:" button. **The sequence should be described in 1-let code in a text file.** If you load the file, you will see the sequence in the textbox;



Any space, tab and return codes will be ignored. The sequence should not be included non-standard amino acid. To finish the setting up, press "Start setup".

1-5. Detail of the startup window

1) To add spectrum files



The listbox on the left-side of startup window lists spetrum files populated in the working diretory (in the sample case, /home/naohiro/nv_PH8/matrix)

To add the spectrum file to the file name entry, firstly activate one of the target entry, then double-click one of the spectrum file in the listbox.

If you would like to use the added spectrum file, press check-box in the left side of the target entry.

The startup module uses the following names of NMR spectrum;

name of spectra in startup	full name of spectra
2D 15N HSQC	2D ^{1}H-^{15}N HSQC
2D 13C HSQC-all-aliph	2D ^{1}H-^{13}C HSQC for allregion or apliphatic
2D 13C HSQC-aro	2D ^{1}H-^{13}C HSQC for aromatic
HNCO	3D HNCO
HNCACO	3D HN(CA)CO
HNCOCA	3D HN(CO)CA
HNCA	3D HNCA
CBCA(CO)NH	3D CBCA(CO)NH
HNCACB	3D HNCACB
HBHA(CO)NH	3D HBHA(CBCA)(CO)NH
CC(CO)NH	3D C(CC)(CO)NH
H(CCCO)NH	3D H(CC)(CO)NH
15N NOESY	3D ^{1}H-^{15}N HSQC NOESY
13C NOESY	3D ^{1}H-^{13}C HSQC NOESY for all region or apliphatic
13C NOESY arom	3D ^{1}H-^{13}C HSQC NOESY for aromatic
HCCH-COSY	3D H(C)CH-COSY for aliphatic
HCCH-TOCSY	3D H(C)CH-TOCSY for aliphatic
CCH-TOCSY	3D (H)CCH-TOCSY for aliphatic
HCCH-COSY arom	3D H(C)CH-COSY for aromatic
HCCH-TOCSY arom	3D H(C)CH-TOCSY for aromatic

If you do not find spectrum type in the above list, you can use "other1, other2...."

2) Short-cut nmaes for NMR spectra used in MagRO

MagRO uses short-cut names of NMR spectra as shown below. They are used in control UIs of MagRO system.

Short-cut names	Spectrum names
hsqc	2D ^{1}H-^{15}N HSDC
chsqc	2D ^{1}H-^{13}C HSQC for allregion or apliphatic
chsqc-ar	2D ^{1}H-^{13}C HSQC for aromatic
hnco	3D HNCO
hncaco	3D HN(CA)CO
hncoca	3D HN(CO)CA
hnca	3D HNCA
cbcaconh	3D CBCA(CO)NH
hncacb	3D HNCACB
hbhaconh	3D HBHA(CBCA)(CO)NH
hnhahb	3D HNHAHB
ccconh	3D C(CC)(CO)NH
hccconh	3D H(CC)(CO)NH
nnoesy	3D ^{1}H-^{15}N HSQC NOESY
cnoesy	3D ^{1}H-^{13}C HSQC NOESY for allregion or apliphatic
cnoesy-ar	3D ^{1}H-^{13}C HSQC NOESY for aromatic
hcchc	3D H(C)CH-COSY for aliphatic
hccht	3D H(C)CH-TOCSY for aliphatic
ccht	3D (H)CCH-TOCSY for aliphatic
hcchc-ar	3D H(C)CH-COSY for aromatic
hccht-ar	3D H(C)CH-TOCSY for aromatic

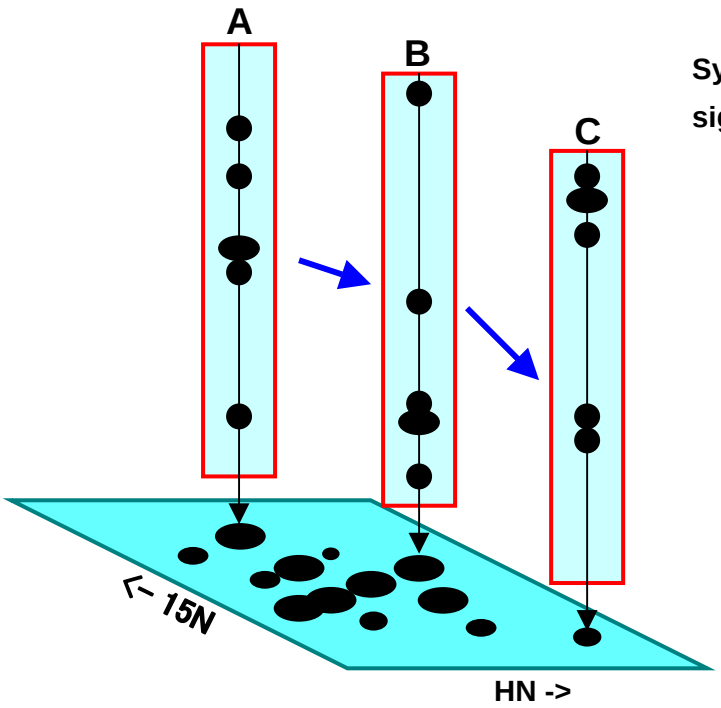
3) "Sync Jump" function

There are three attributes for "SyncJump" fuction in MagRO;

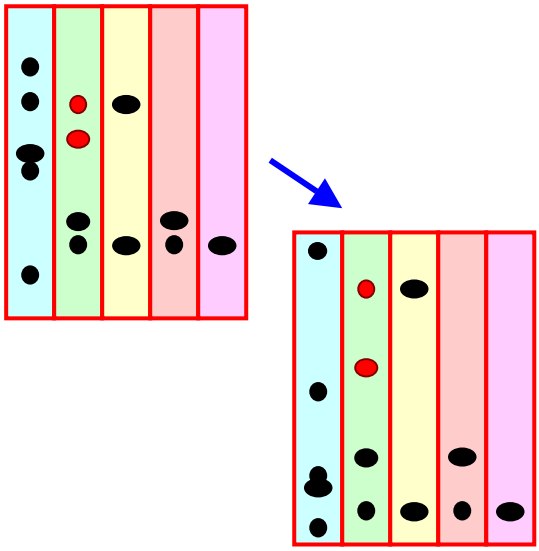
Sync Jump attribute	Corresponding stectrum type
15N	^{1}H-^{15}N
13C-al	^{1}H-^{13}C for all region or aliphatic
13C-ar	^{1}H-^{13}C for all region or aromatic

The spectrum windows whose sync-jump attribute are same can be synchronized by the Sync-Jump command.

For example, if you specify 3D-HNCACB and 3D-^{15}N-edite NOESY with sync-jump attribute 15N, all the spectrum windows will display 2D- spectrum region corresponding to the HN-^{15}N position on HSQC plane. If you change the HN-^{15}N position to the other position (from A to B), all the spetra will dislplay 2D-spectrum strip as synchronizedly moving HN-^{15}N position.

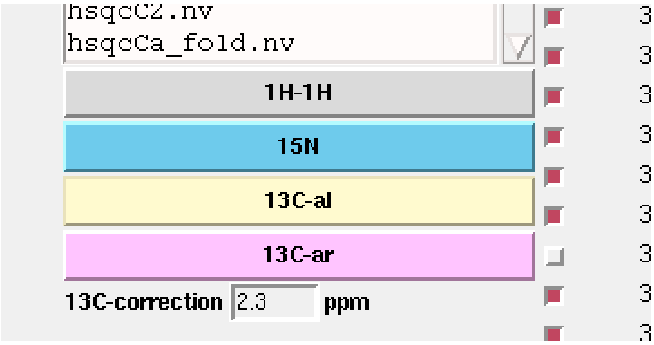


Sync-Jump corresponding to the signal position A on HSQC



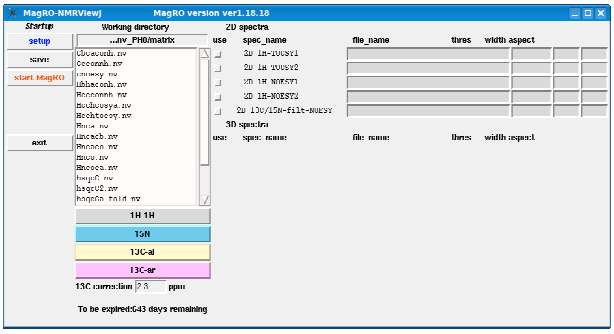
Sync-Jump corresponding to the signal position B on HSQC

If you look at the startp up window, you will find the following buttons;

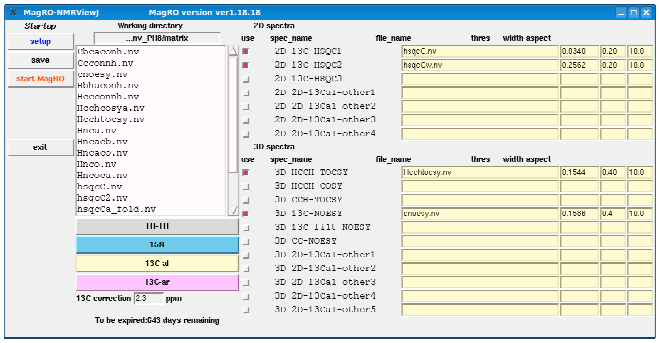


If you press 15N, 13C-al and 13C-ar, you can switch sync-jump attribute of the stratup window.

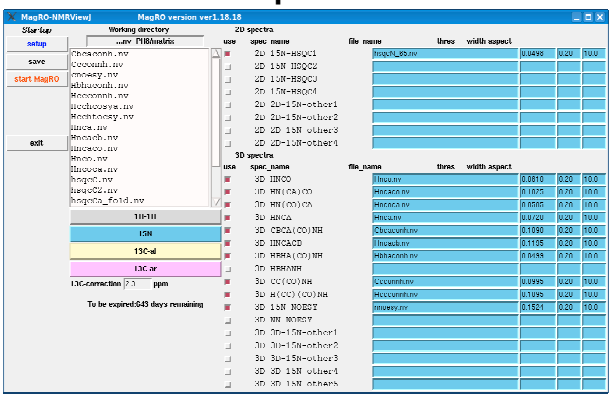
^{1}H-^{1}H spectra



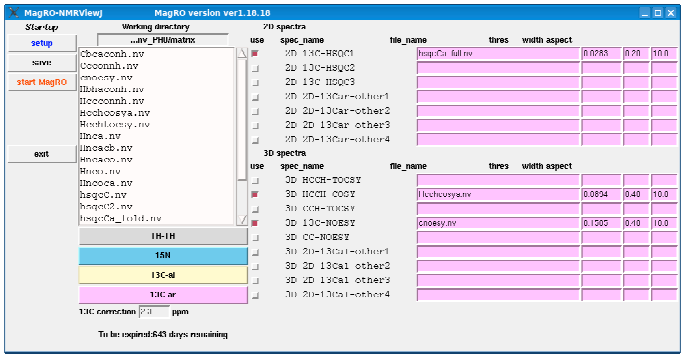
^{13}C-aliphatic spectra



^{15}N spectra

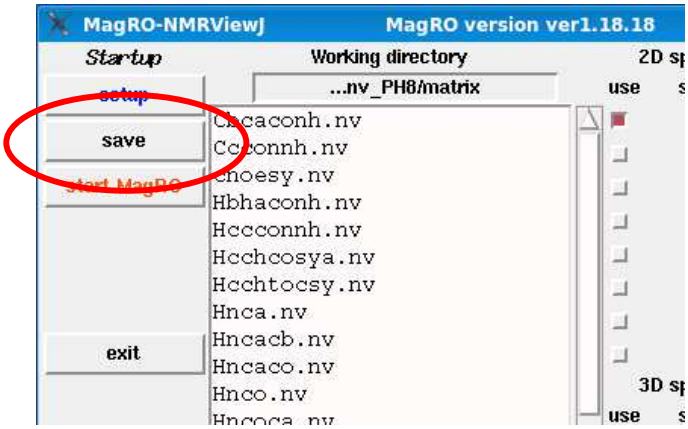


^{13}C-aromatic spectra



4) Detail settings on startup window

You can set threshold, width and aspect of the spectrum strip



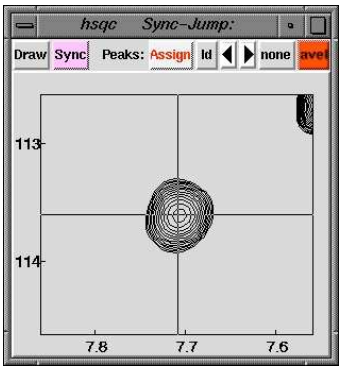
Press "Save" button to save the current settings.

5) Optimizing strip width and aspect ratio for Sync-Jump

You can set width and aspect ratio on the startup window for displaying 2D spectrum strips controlled by Sync-Jump command.

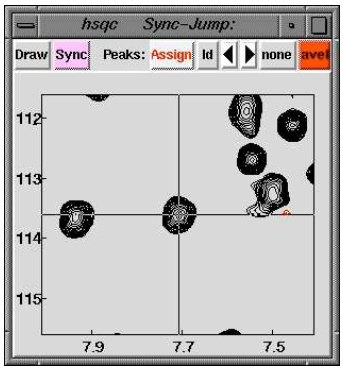
Firstly you should play around the values for width and aspect.

For the 2D- spectrum;



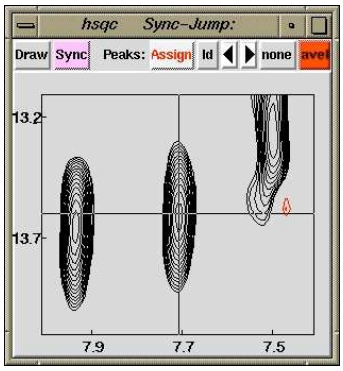
width: 0.5

aspect: 80



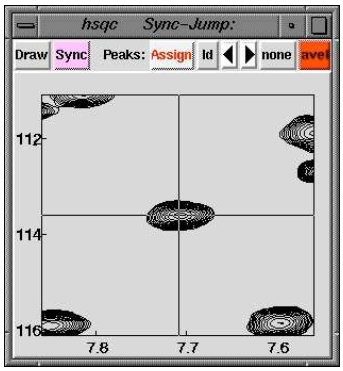
width: 1.0

aspect: 80



width: 1.0

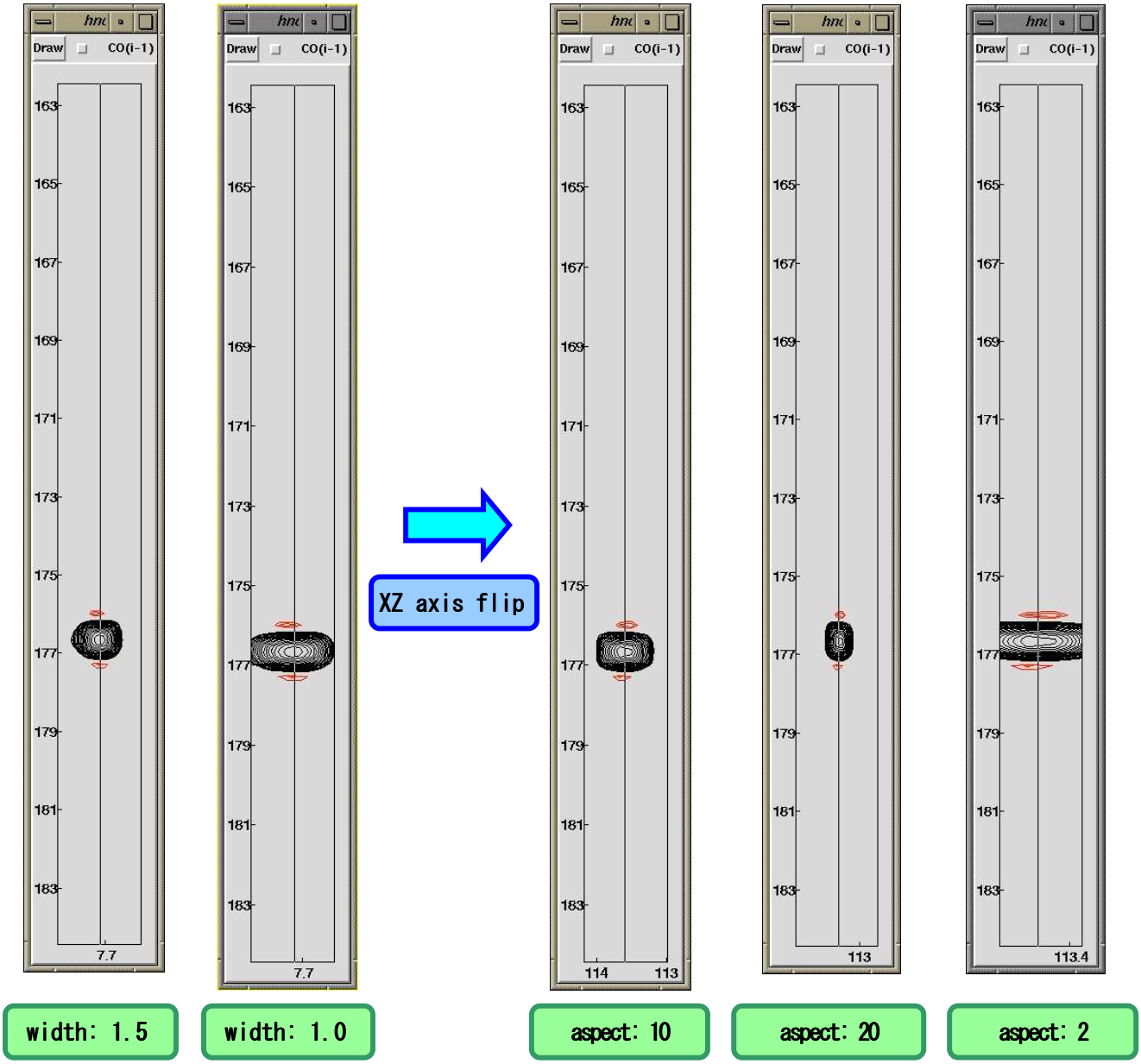
aspect: 20



width: 1.0

aspect: 200

And 2D spectrum strips extracted from 3D spectra, "width" is corresponding to the X-dimension width for the 2D spectrum strip, while "aspect" controls aspect ratio of X- and Z-dimension width.
If you flip the 2D-strip by y-axis by pressing "90° filp" button, you can confirm the aspect ratio is optimized.

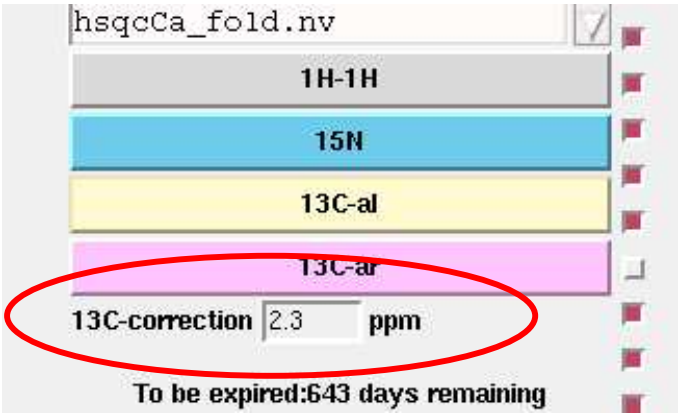


6) Start MagRO

To start MagRO, press "start MagRO" button.

(starting MagRO without pressing "save", the setting will be automatically saved)

7) offset correction of ^{13}C signals



If ^{13}C offsets are systematically shifted for all the spectra used for MagRO analysis, user can specify the offset value correction in the entry.

The offset correction will be applied to the input files for the program such as TALOS and FLYA which are all sensitive to the systematic error in chemical shift table.

1-6. Switching sync jump mode on spectrum window

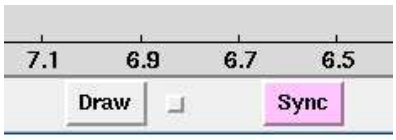
Buttons for switching sync-jump modes on all the spectrum windows used in MagRO.

Normally, there are two modes for the spectrum windows, "Silent" and "Sync-Jump". On the other hands, the 2D-HSQC type spectrum windows have three modes, "Silent", "Sync-Jump" and "Click and Jump".

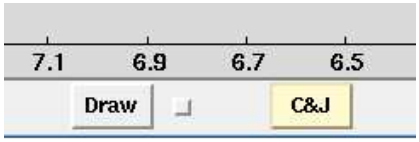
In the "Sync-Jump" mode, 2D spectrum windows and 2D spectrum strips extracted from 3D spectra with the same sync-jump attribute display part of spectrum at same position on 2D HSQC plane.

In the "Click and Jump" mode, 2D spectrum window act as clickable HSQC map. If you click 2D spectrum window, you can move the display region of 2D-spectrum windows and 2D spectrum strips with the same Sync-Jump attribute.

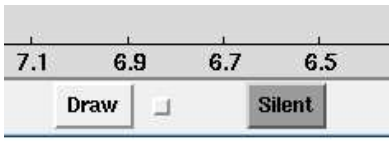
In the "Silent" mode, the spectrum window will be insensitive to any sync-jump action.



Sync-Jump mode



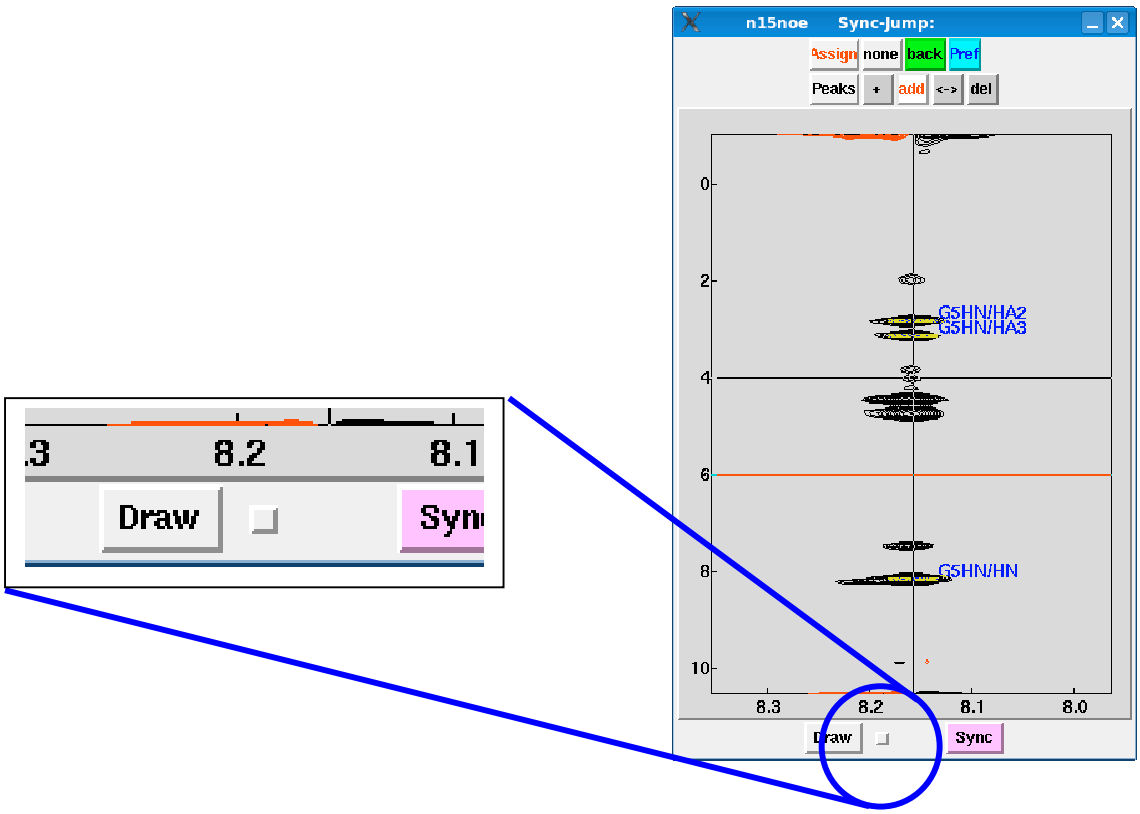
Click and Jump mode



Slient mode

1-7. Fixed mode for XY aspect ratio for 2D spectrum strip

Usually 2D strip displays full region of spectrum on y-axis. To fix aspect ratio for x- and y-dimension, activate check box on the bottom of spectrum window.



1-8. Conversion of NMRPipe format to NMRCiew format and axis order of spectrum

MagRO-NMRView system can handle only spectrum data in NMRView format.
To convert NMRPipe format into NMRView format, user can use NMRPipe.
Follwoings are the example of macro command for the conversion

```
xyz2pipe -in /home/naohiro/NMRPipe/ft/n15noe_%03d.ft3 -x -verb ¥
| pipe2xyz -out n15noe.nv -nv -ov
```

If you execute the macro, you will see the following output and you will get the converted file;

```
  555   1024   32   18   32     1    31    1 500.13   3257.16     4.80   614.00 H
  256    256   16   16   16    18    15    5 500.13   6756.76     4.80   128.00 HN
  128    128    8   16   16   288     7    9  50.68   1315.79   120.12    64.00 N
```

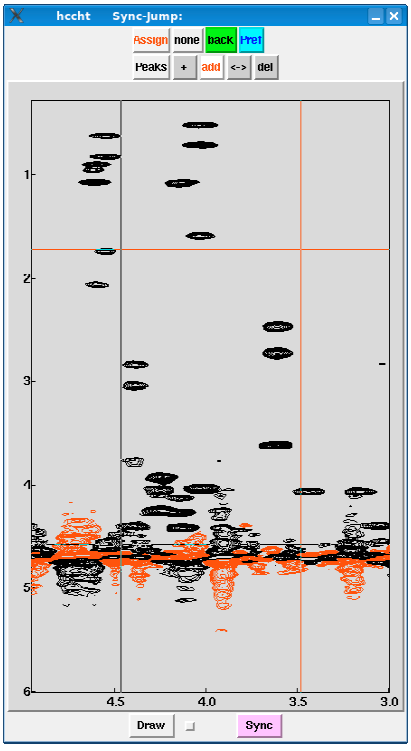
[Importnat] You will have to check axis order from the standard output. Above exmaple showing axis order is H-HN-N

For the analysis with MagRO system, 15N or 13C dimension for 3D spectrum should be set Z-axis.
For example;

Sync-Jump	Spectrum types	Axis order
15N	HNCO, HNCACB, CBCACONH	HN-C-15N
	^{15}N edited NOESY, H(CCCO)NH	HN-H-15N
13C	HCCH-TOCSY, ^{13}C-edited NOESY	HC-H-13C

For all above examples 15N and 13C dimension is set Z-axis and the indirect dimension are set Y-axis.
If you find the converted spetrum data is need to be transposed, you have to execute NMRPipe macro as follwoings;

```
xyz2pipe -in /home/naohiro/NMRPipe/ft/n15noe_%03d.ft3 -x -verb ¥
| pipe2xyz -out n15noe.nv -nv -ov
```

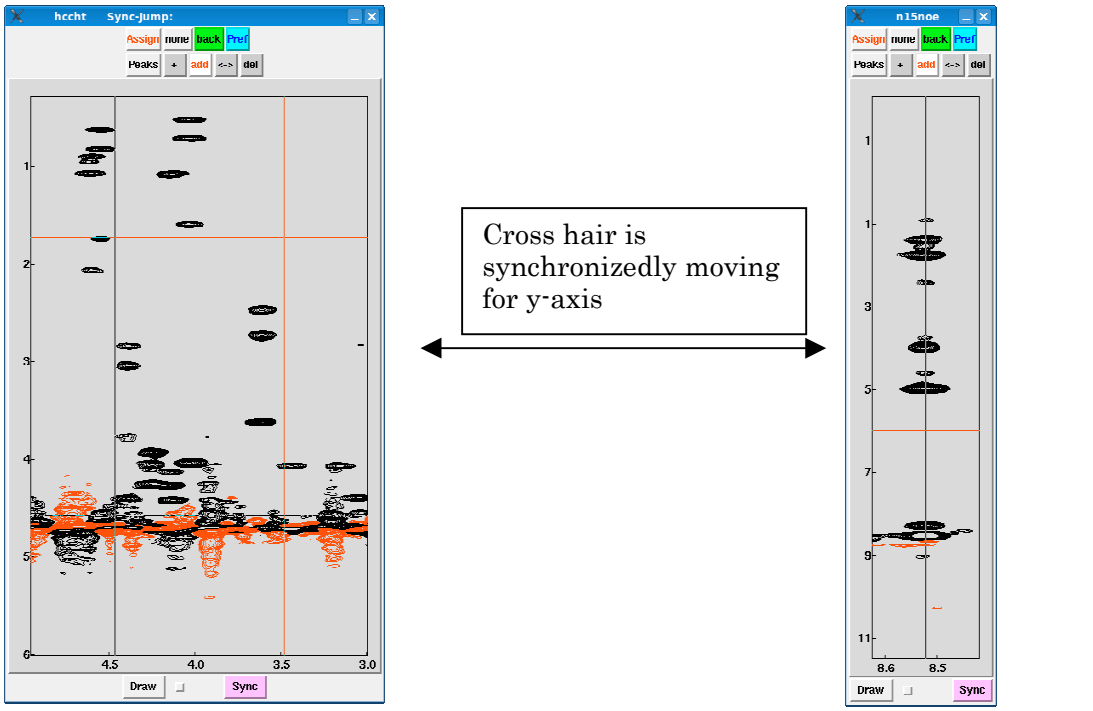


Instead of the above macro example, you can use TP commandto transpose the spectrum data.

[Important] User have to be careful to check axis order of HCCH-TOCSY spectrum.

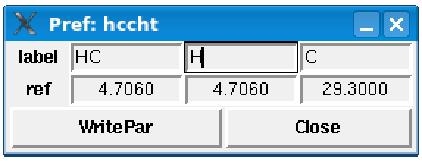
左の図のスペクトルでは X 軸が H、 y 軸が観測核(direct dimension)、Z 軸が 13C 核になっています。
The left figure shows the example of HCCH-TOCSY spectrum.
In this case axis order is set H-HC-13C.
Please note that the dimension for acquisition is set X-axis.

1-9. Axis labeling and sychronizing curosor (cross hair)

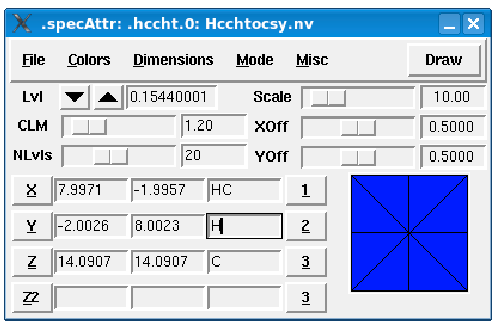


The function of sychronizedly moving cursor (cross-hair) is an important for NMRView.
This is useful for checking signal position using multiple spectrum window by pointing the corss-hair.
To use the function the axis of spectra should be set same axis labeling.

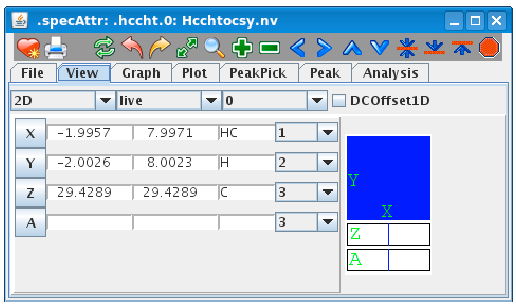
As shown in the above example, The y-axis of 3D HCCH-TOCSY is labeled with "H", while the y-axis of 3D 15N edited NOESY is also labled with "H".
If you find the cross-hair is not moving synchronizedly, you have to check axis labeling for each spectrum on the Pref window. You can open the Pref UI window by pressing the button "Pref" on the header of spectrum. You can change the axis labeling and save the information.



You can temporary set the axis labeling using Attribut window. By mouse-right-button -> attribute -> View to open Attribute window, you can change the axis labeling.



NMRView C-version



NMRView Java-version

1-10. Directories and files used for MagRO system

The project diectories and files included under the matrix directory

matrix ----------|

```
                |
                MagDB
                    protein_0_0_acs.db      Assigned chemical shift table
                    protein_0_0_seq.db      sequence table

                backup                      Backup directory

                xpkfiles                    Peka list (xpk file) direcotry

                CYANA_results               Storing files for CYANA result analysis

                000temp                     directory for temprary files
```

The most importand directory is MagDB. The directory has the file for assigned chemical shift table (*_acs..db) and sequence table file (*_seq.db).

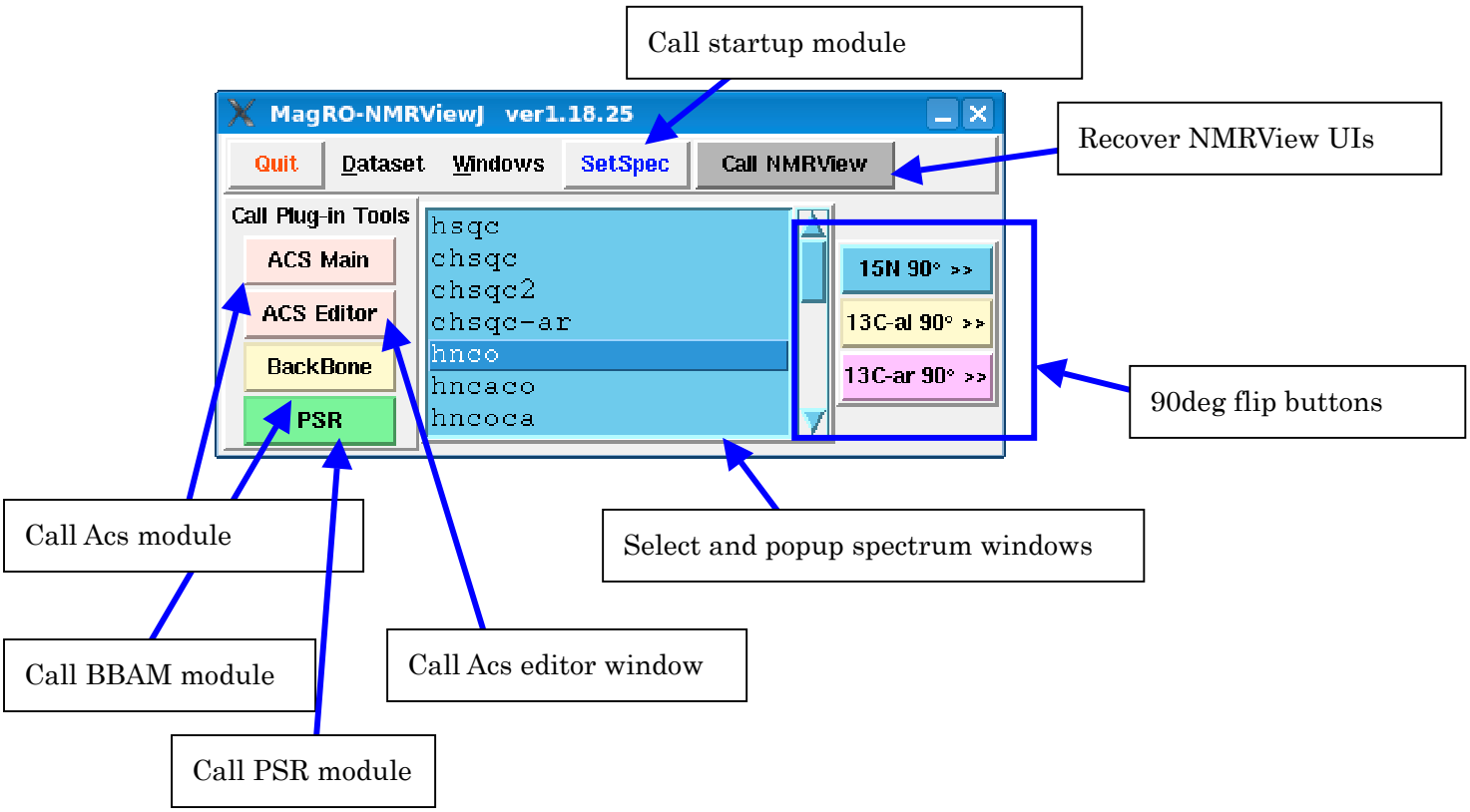
It is strongly recommended that user should take the backup of the directory periodically.

000temp has several temprary files for setting window size and position. The directory can be deleted if you have some problem to display window UIs.

The directory "CYANA_results" includes temporary files which can be recreated automatically. So user can delete them if necessary.

1-11. Detailed instruction of Main window of MagRO

When you start MagRO, you will see a small window UI as shown below;



Quit:	Quit MagRO system
SetSpec:	Display startup module window
Call NMRView:	Recover NMRView UI system
Acs Main:	Popup Acs module
Acs Editor:	Popup Acs Editor module
BackBone:	Popup Backbone Assignment Module (BBAM)
PSR:	Popup Patern Search Robot (PSR)

To popup spectrum window, you can select and double-click one of the spectrum type listed in the listbox placed in the center of module. The short-cut names are used for selection of spectrum as defined in the Startup module (see section 1-5 2) page 1-6).

The 90deg flip buttons are used to flip 2D-spectrum strips for each sync-jump attribute.
The Z-axis of 2D spectrum strips can be inter- changed by clicking the button between the direct dimension (HN or HC) and heavy atom dimension (15N or 13C).

By pressing the button "SetSpec" user can call Startup module to define sync-jump settings of spectrum. Using the module you can add spectrum window which has not yet been loaded.

2-1. Backbone signal assignment

1) required spectra

for the backbone signal assignments, the following spectra are required.

name of spectrum	symbol of spectrum	atom type	Axis order
3D HNCO	hnco	CO(i-1)	HN-C-N
3D HN(CA)CO	hncaco	CO(i)	HN-C-N
3D HN(CO)CA	hncoca	Cα(i-1)	HN-C-N
3D HNCA	hnca	Cα(i)	HN-C-N
3D CBCA(CO)NH	cbcaconh	Cβ(i-1)	HN-C-N
3D HNCACB	hncacb	Cβ(i)	HN-C-N

Above list describes required spectra and its symbolic name which are used in spectrum manager.
In the analysis of backbone signal on MagRO, atom types are important as listed above. For example, the spectrum window, HNCACB is used to get chemical shift value of Cβ signal on residue i-th, but CBCACONH window is used to get the value of Cβ signal on residue (i-1)th.

[Caution] When you prepare 3D-spectrum from FID, Please make sure the order of axis is correctly set, otherwise MagRO can not properly display the spectrum strips. (For example HNCO should have axis order proton[1], carbon[2] and nitrogen[3]).

[Caution] DO NOT USE same axis label. This will cause error when you save peak list into xpk file. Please use different axis label for each dimension;

(Bad) HN C13 C13
(Good) HN C13 C

2) Prepare HN-15N peak list

For the backbone signal analysis, firstly you have to prepare HN-15N peak list.
Select "hnco" in the top window and double-click to popup the HNCO spectrum window.

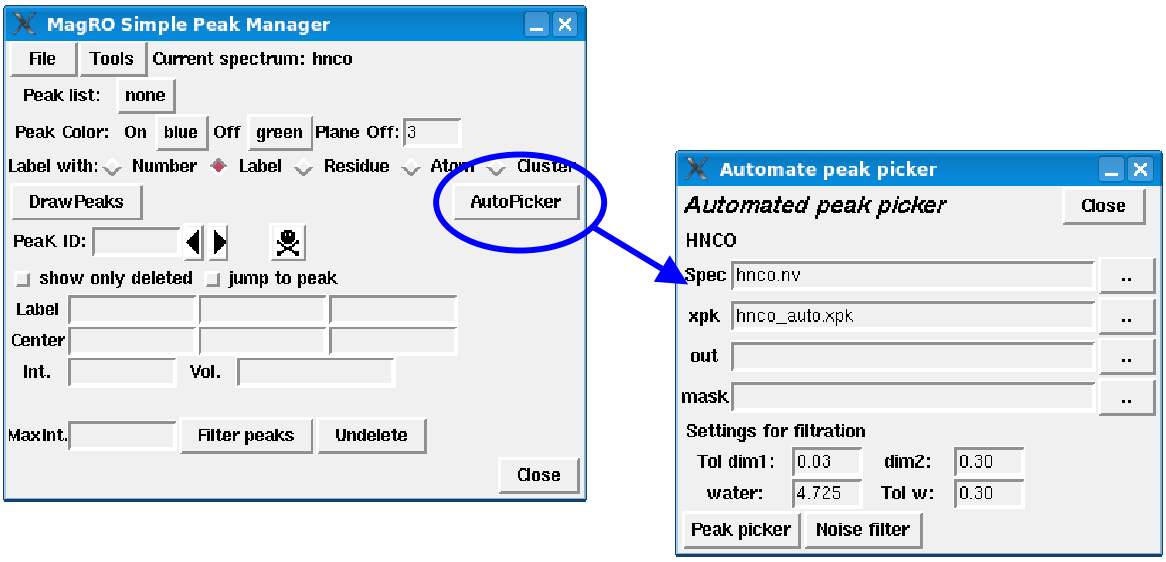
3) optimize spectrum threshold

Touch the HNCO spectrum window to activate it.
Press up- and down-key to move z-dimension and to display several peaks.
Then press R- and F-key to optimize spectrum threshold not to display too much nose.

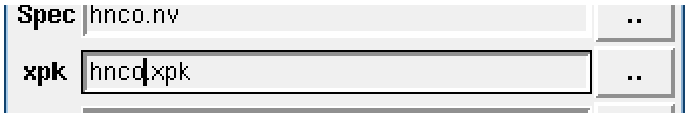
Now you can press "Peaks" button to open "MagRO Simple Peak Manager".

4) Start peak picking

Press "AutoPicker" button in the "MagRO Simple Peak Manager",



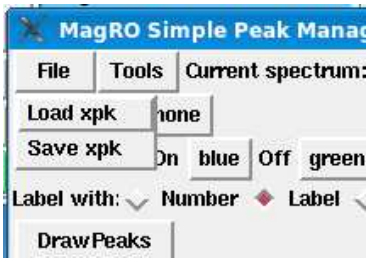
In the Auto peak picker window, chage the name of out put file as hnco.xpk;

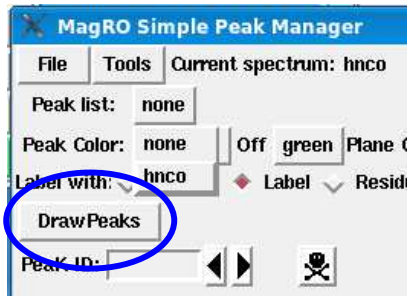


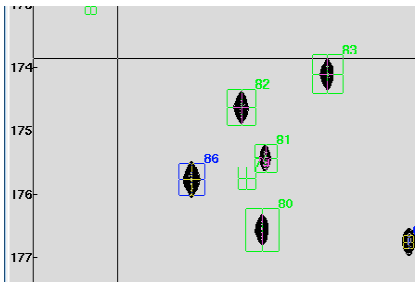
Then press "Peak picker", the automated peak picking will began.

5) Load peak list

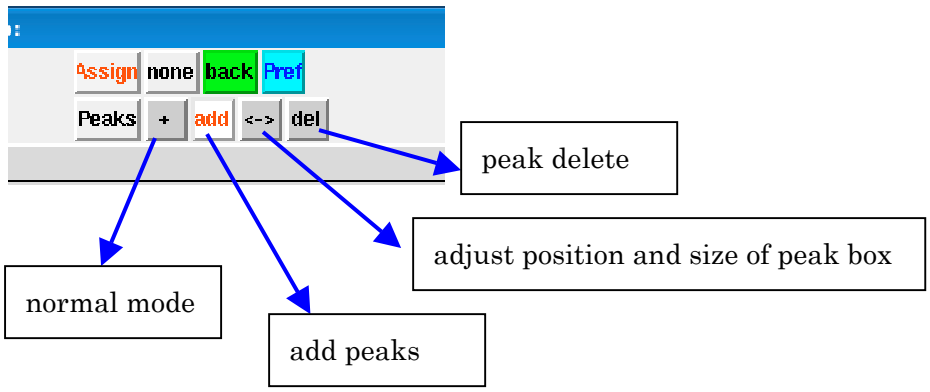
Find the created peak list file. For example nv_demo/matrix/hnco.xpk.
To load the peak list file, press File->Load xpk (see left panel).
Select loaded peak list, hnco (see middle panel).
Press "DrawPeaks" button to display peak boxes (see right panel).





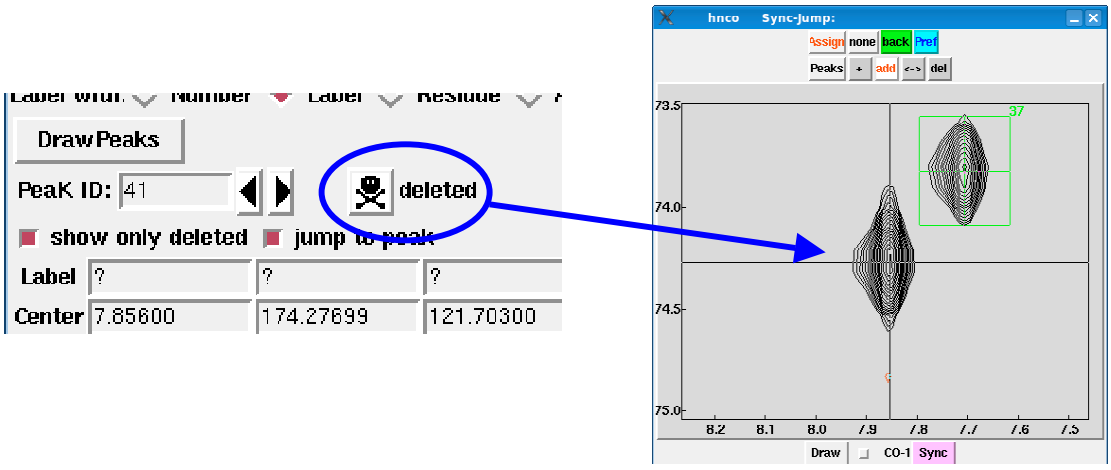


As shown in the right panel, detected peaks just on 2D-plane are indicated bu blue boxes, while the boxes near the 2D-plane are green.



If you look at the header of spectrum window, you will find buttons to switch cusor mode.
As shown in the above figure, there are four buttons; "normal mode", "add peaks", "adjust position and size of peak box" and "peak delete".
[TIPS] press W, A, D and S keys to move the spectrum region up, left, right and down, respectively.
[Caution] There is no undo function for peak delete mode, so please carefully to handle the cursor.

<If you mistakenly deleted peak box...>

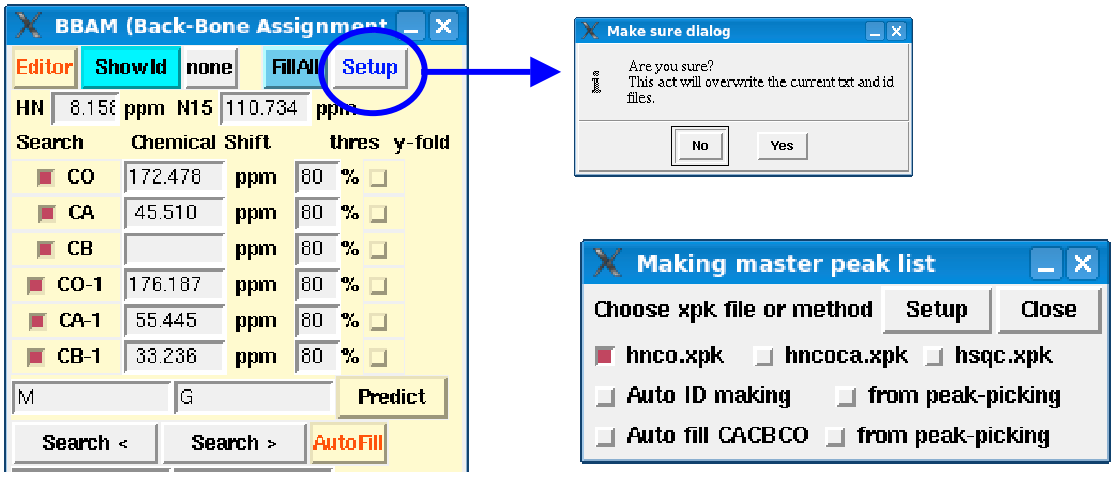


Open the MagRO Simple Peakmanager, select peak list that you would like to edit.
Activate the checkboxes "show only deleted" and "jump to peak" (left panel).
Press < > buttons to move and display peaks.
Find the peak that you have deleted, and press "pirate" button to recovery from the status "deleted".

6) Save edited peak list to xpk file
To save the edited peak list into a xpk file, press "Tools" button
then select "Get-Int,Cmp&Degap,Write".
This action will get intensity of all peaks, compressing peak list (permanently delete peaks), degaping the peka ID numbers and write peak list to xpk file. You will be asked the file name of output file, you should type "hnco.xpk", then overwrite the old xpk file.

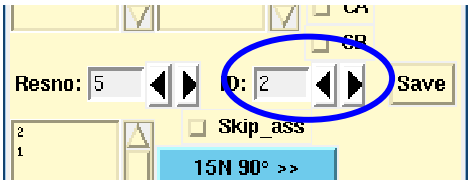
[important] you have to keep saving the peak list in hnco.xpk which will be used for making HN-15N peak list.

7) Create HN-15N peak list table, assign_NN.txt

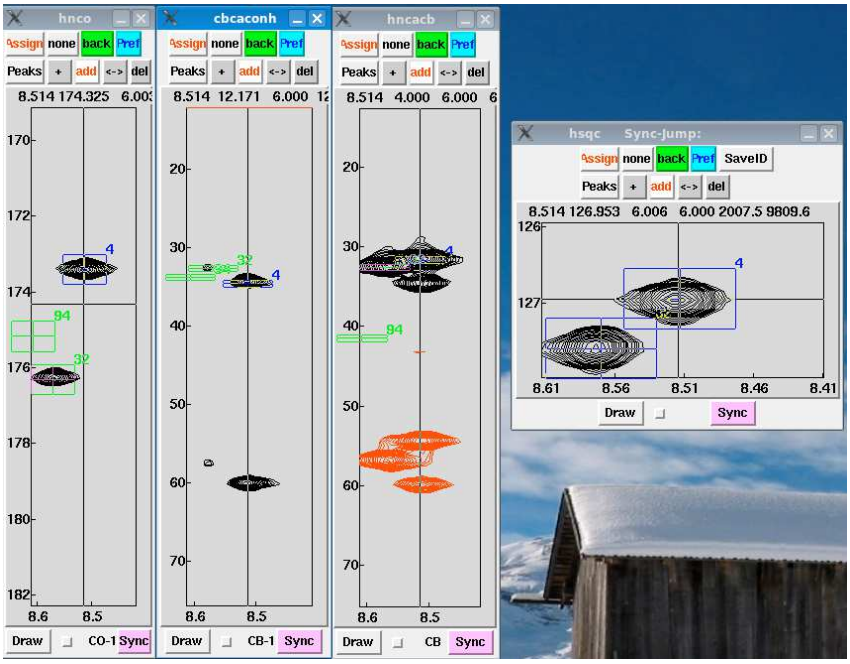


Press "Setup" button on the yellow module (BBAM, Back-Bone Assignment Manager). If you don't find the yellow module, press "Backbone" button on the top window.
You will be asked to make sure what you are going to do, press "Yes".
If you don't see any error message, the setting job has been sucessfully completed.

8) Edit information tored in assign_NN.txt file

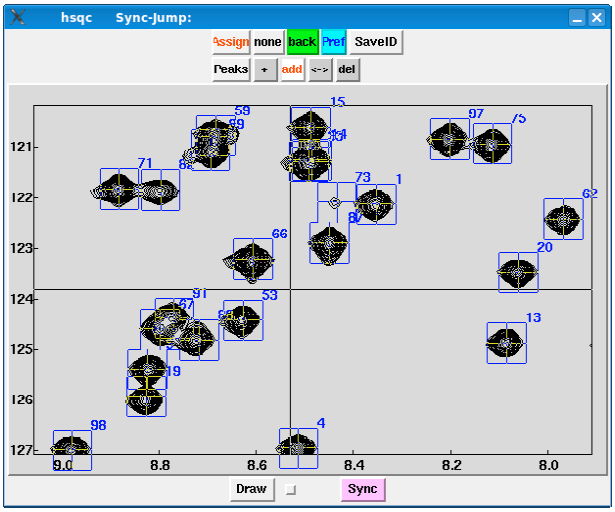


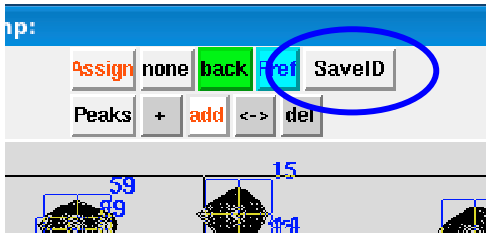
If you press "<" or ">" button, you will see the number of peak ID are incrementing and decrementing. At the same time, spectrum windows with 15N sync-jum attribute are also moving to the corresponding HN-15N position in HSQC projection..



IF you press "ShowID" in the yellow module, blue boxes appear in the spectrum windows. They indicate peak positions expected from assign_NN.txt file.

You can edit assign_NN.txt using a standard text editor.
Peak ID number should not be consecutive, but should not be duplicated. Please use positive number for Peak ID. The number of column should be important. Lacking of column number may cause serious error.



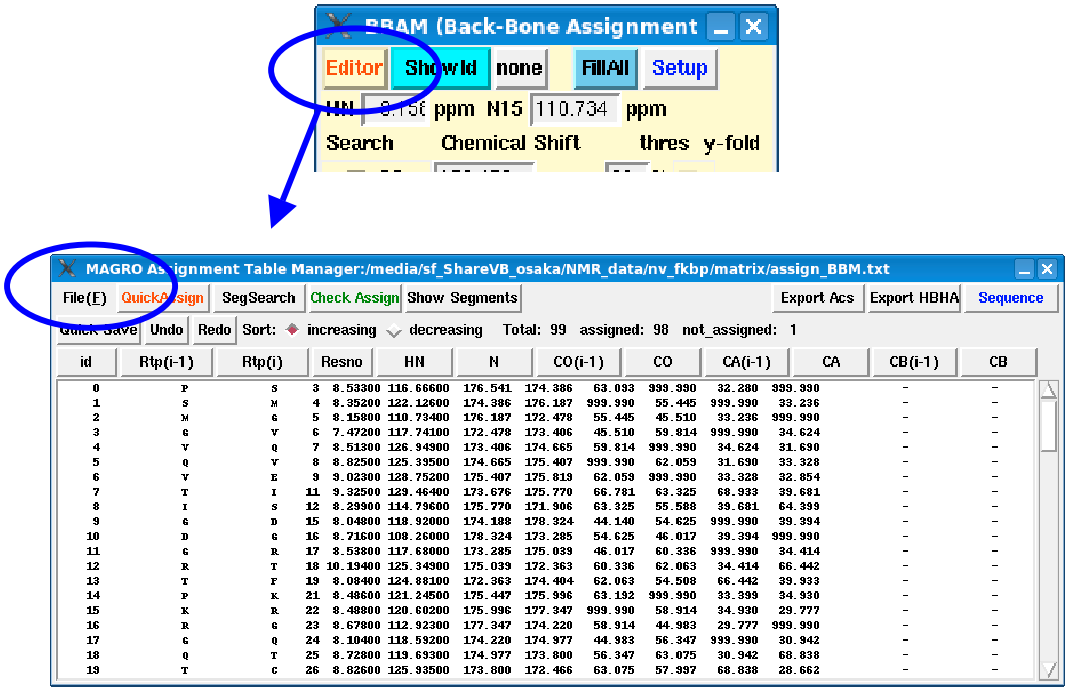


Next thing to do is removing peak ID derived from minor peaks and noise peaks.

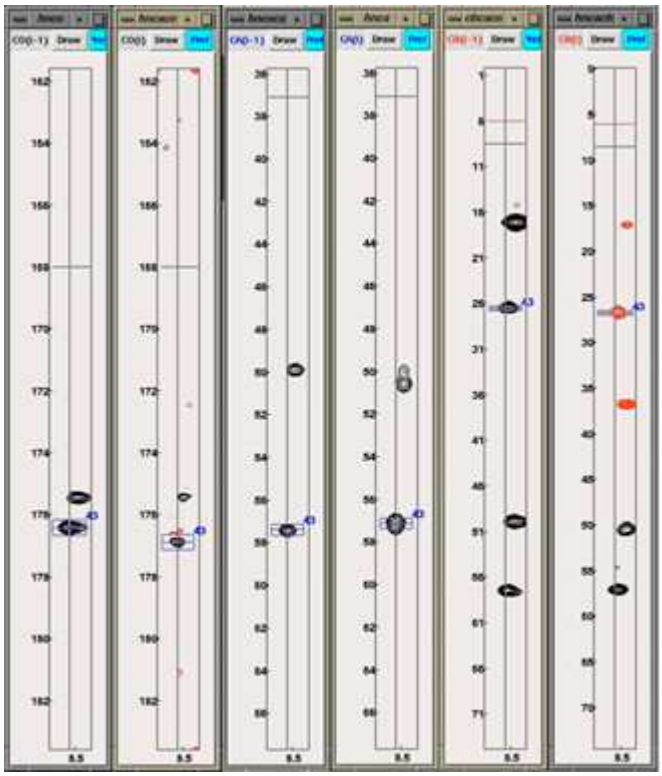
By pressing "ShowId" button on the header of yellow module, blue boxes are displayed on HSQC spectrum window. Then press "del" button on the header of HSQC spectrum window to change the cursor to deletion mode. By clicking blue boxes you can delete the Peak ID derived from noise peaks.
To save the peak ID in assign_NN.txt, press "SaveID" button on the header of HSQC spectrum window.

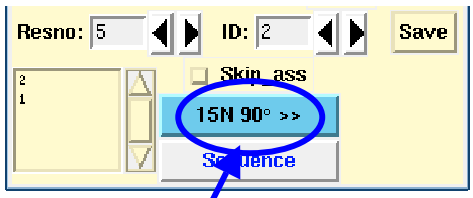
9) Loading assign_NN.txt file

If you check the directory matrix/backup, you will see the backuped file of assign_NN.txt as assign_001.txt, assign_NN.002, assign_NN.003... and so on. Click "Editor" button on the header to open "MagRO Assignment Table Manager", and click File->Open to load the old assign_NN.txt file.



10) Test of sync-jum and fine tuning of spectrum offset



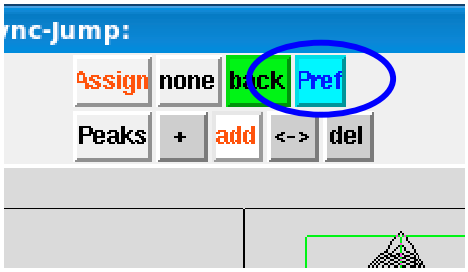


90deg flip ^{15}N Sync-Jump

To test the Sync-Jump function, align the spectrum 2D strips as shown above.
Peass "<" and ">" and "15N 90>>" buttons on yellow module to see what happen.

Next,try to move cursor and cross-hair lines are moving. If the y-axis line does not seem to be moving synchronizedly, check the axis labeing are same.

To edit the axis label, press "Pref" button on the header of spectrum window to open pref-edit UI.
You can optimize spectrum offset for each axis on the pref-edit UI.



11) Full-automated making of peak ID table

Followings are steps for making peak ID table in automated manner

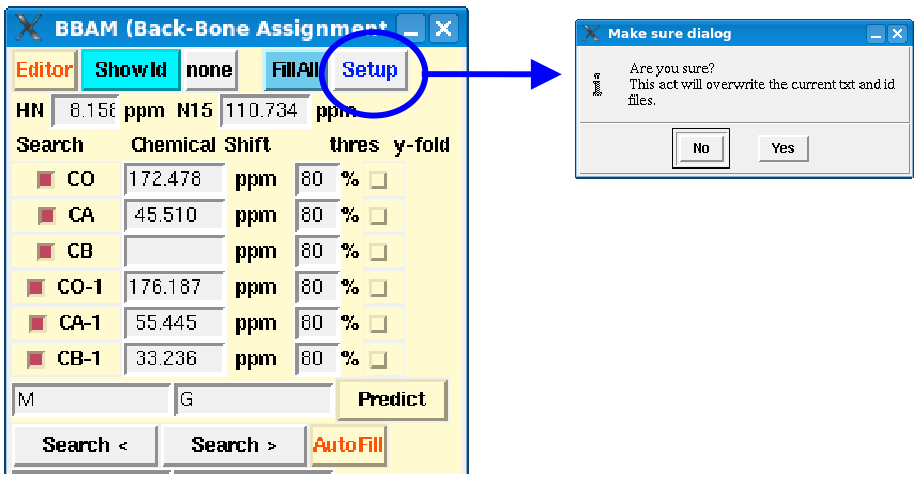
Minimum set of spctraum data

3D-HNCO or 3D-HN(CO)CA

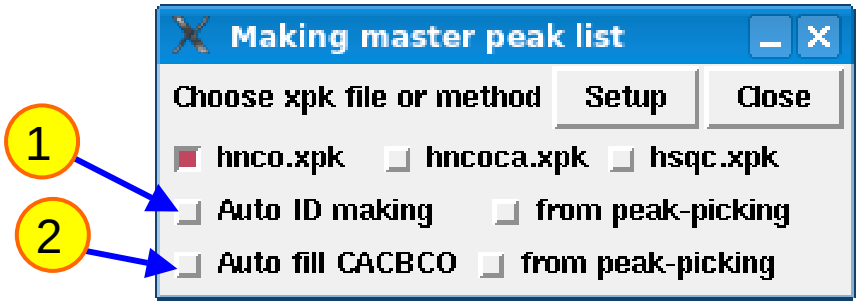
And you may use the follwing data set

3D-HNCO, HN(CA)CO, HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, C(CO)NH

To perform the automated peak ID table making, press "Setup" button on the header of yellow module. Then a dialogue window will popup and ask you if you are sure to do the job.



IF you answer yes, a small window UI will popup;



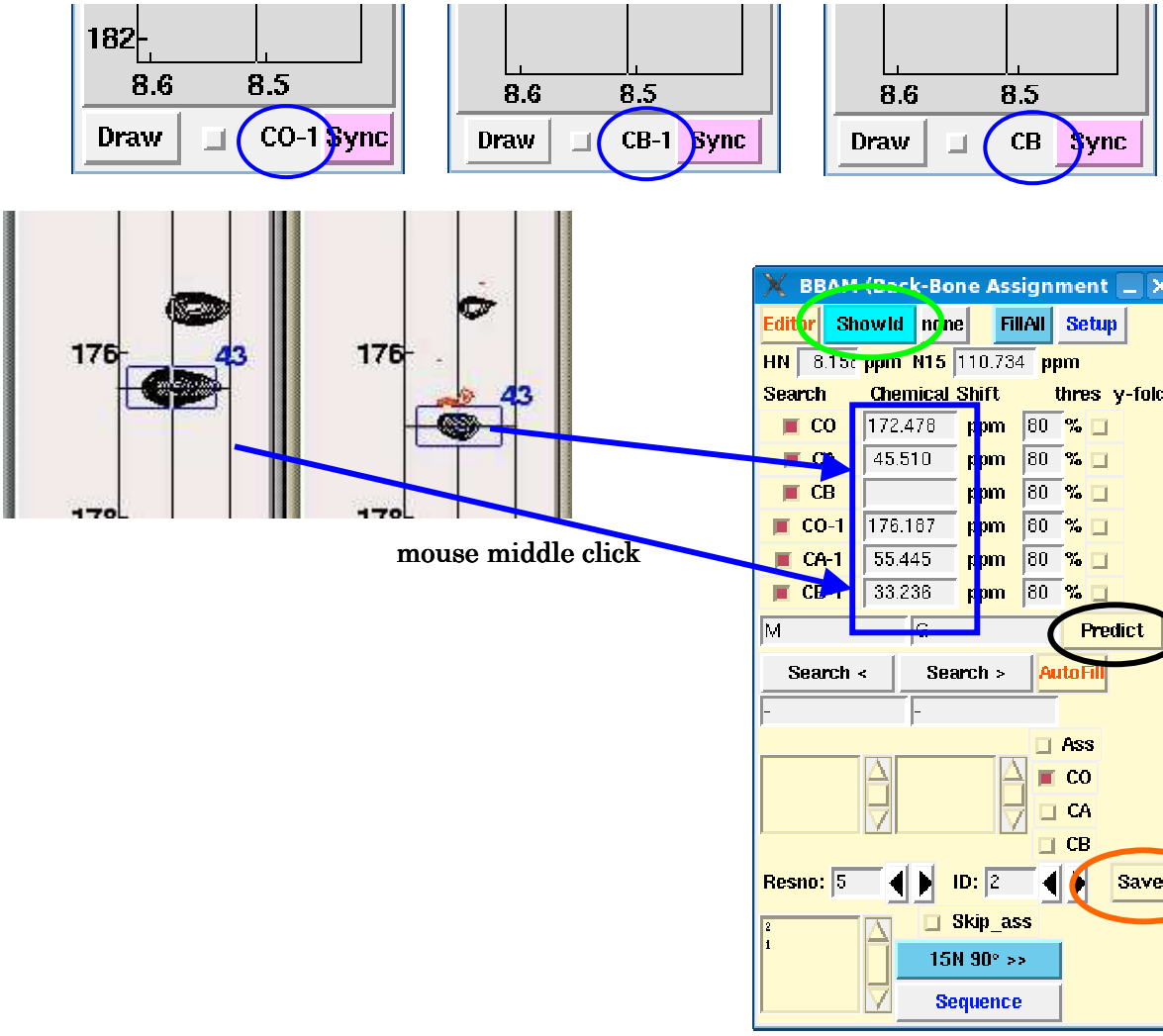
In the window UI, if you select "Auto ID making", the program will create peak ID table including only HN and 15N chemical shift information. If you select "Auto fill CACBCO", the program will create peak ID table including all chemical shift data, HN, 15N, CA(i), CA(i-1), CB(i), CB(i-1), CO(i) and CO(i-1).

Then you press "Setup" button, again the small UI will ask if you are sure to start program. If you answer "Yes", the program will start creating the peak ID table (normally it will take a few mins).

In the middle of yeallow module, there is a button to get CO, CA and CB chemical shifts for each peak ID.

12) Enter chemical shift value to yellow module

For the backbone signal assignment, next thing to do is complete the chemical shift table.
By clicking mouse middle button, user can insert chemical shift value of y-axis on 2D spectrum strip.
Make sure which atom type can be correspond to the spectrum type from the labe on the bottom of spetrum window.



Try to click mouse-middle-buttonon on HNCO, the chemical shift value will be inserted in the entry corresponding to atom type CO(i-1).

Do not forget to save the entered values, by pressing **"Save"** button.

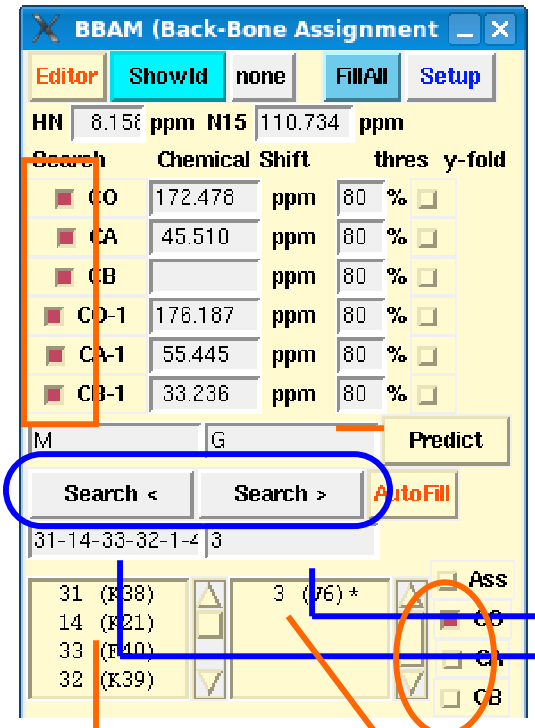
If you fill the entries for atom types, CA and CB, you can predict residue type by pressing **"Predict"**
The predicted amino acid types are displayed in the entry boxes for (i-1) left and (i) right neighboring "Predict" button, showing one-letter code and ordering is heigher probability.

The predicted amino acid type are based on statistic information of chemcal shifts for Ca and Cb derived from BMRB database. If your NMR spectrum is systematically shift, set value of the 13C correction on Startup module.

By pressing **"ShowId"** button, you will confirm the entered chemical shift values with blue boexs on 2D- spectrum strips.

13) Function to search for candidate sequential peak IDs

If you press "Search <" or "Search >" button, based on the entered checmial shift values for Cb, Ca and CO the program will search for peak ID of sequential position (i-1) or (i+1). This function directory uses spectrum intensity for the search, this will be very powerful to find overlapped peaks in crowded region.



The default state, the program will search;

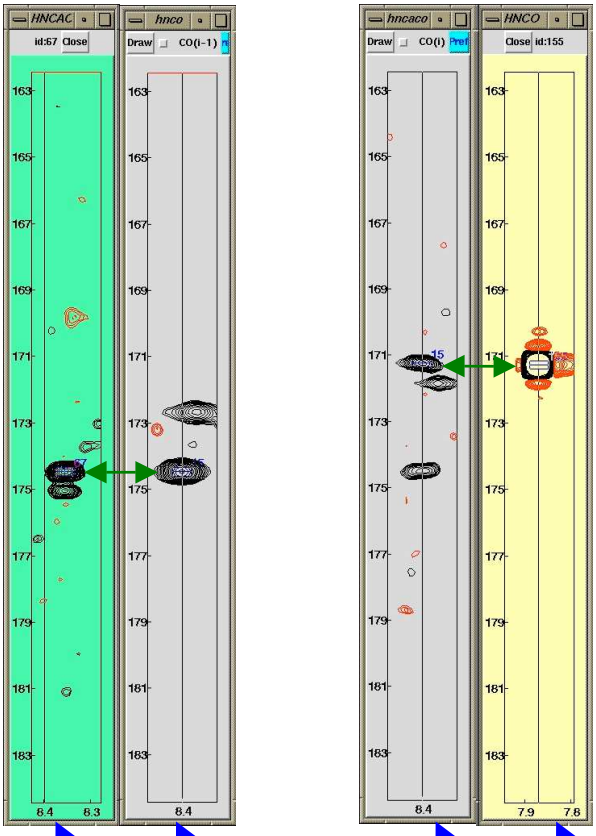
(i-1) Peak ID		(i+1) Peak ID
CO:	HN(CA)CO	HNCO
Cα:	HNCA	HN(CO)CA
Cβ:	HNCACB	CBCA(CO)NH

You can enable or disable atom types for the search with the checkboxes in the left side of yellow module.
The detected Peak IDs will be displayed in the listboxes below the search button in higher probability order.
The most probable Peak ID indicated by bold. If the peak IDs already assigned are displayed with residue type (1-letter code) and residue number in parentheses.

(i+1) search

(i-1) search

double click



If you activate the checkboxes CO, CA and CB on right-side of yellow panel (red ellipse), then double click one of the searched peak IDs, 2D spectrum strips will appear showing corresponding peak ID position.

The left-bottom example showing sequential PeakID 14 of HN(CA)CO which has been searched for sequentila (i-1) residue of peak ID 15.

Similarly, if you double click the searched peak ID (Peak ID 3) for sequential residue (i+1), you will see the popuped 2D strip showing HNCO.

By carefully inspeting the signals in the popuped strips with one in the current 2D strip, you can easily find which peak ID should be assigned sequential residue.

HNCO, PeakID 15

HNCO, PeakID 3

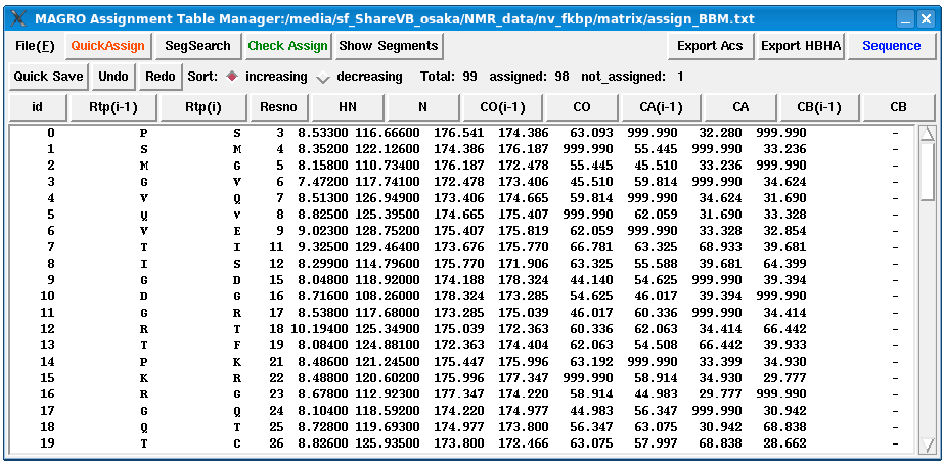
HN(CA)CO, PeakID 14

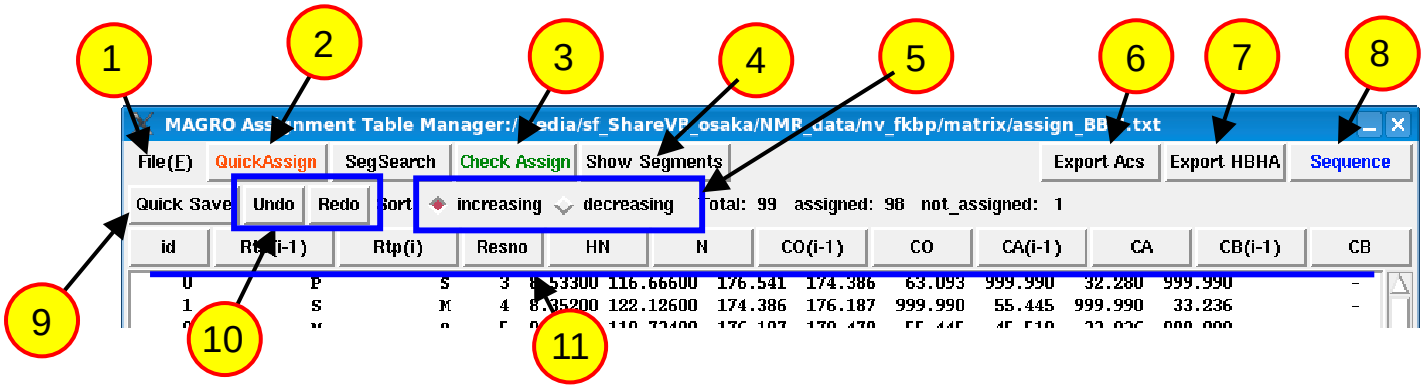
HN(CA)CO, PeakID 15

14) sequential assignments using MAGRO Assignment Table Manager

If you press "Editor" button, you will see the following window.

This editor displays assignment information of backbone signals using the peak ID table, assign_NN.txt file.





1: Manipulation of file

2: Execute "QuickAssign"

3: Execute "CheckAssign" to validate assigned results

4: Execute "Show Seqgment" to confirm and correct sequential assignments using 2D-strip array.

5: Switch increment and decrement for sort function

6: Translation of assigned data to Acs (Assigned Chemical Shifts)

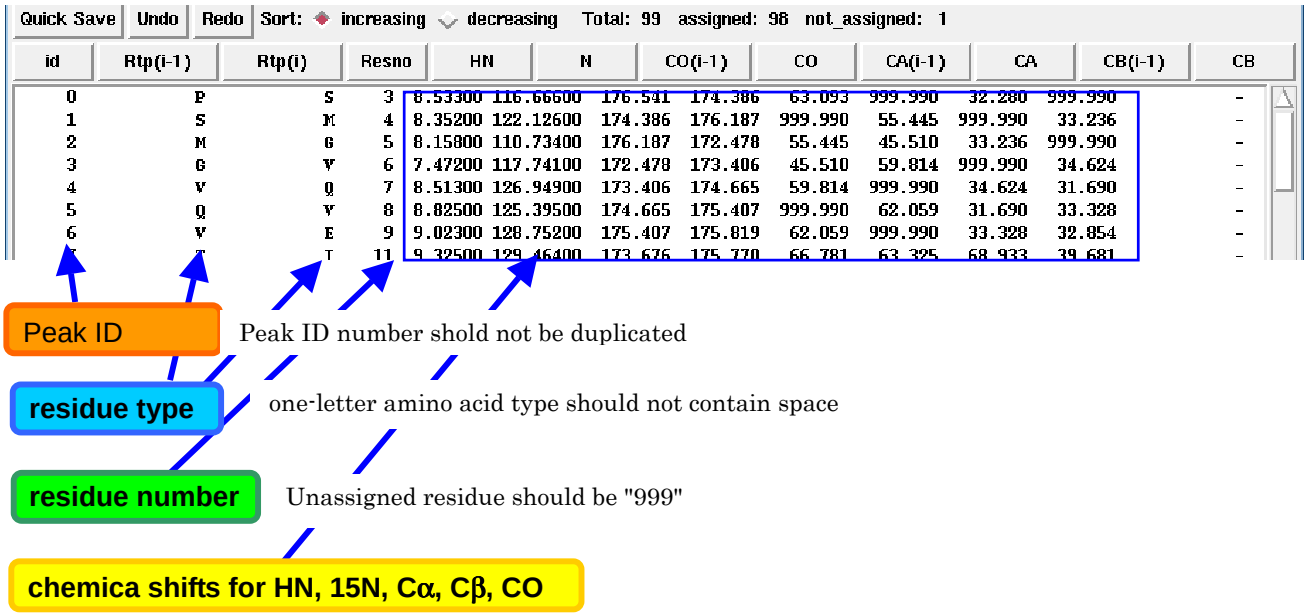
7: Trnaslation of assigned data to Acs and automated assignment of Hα and Hβ signals

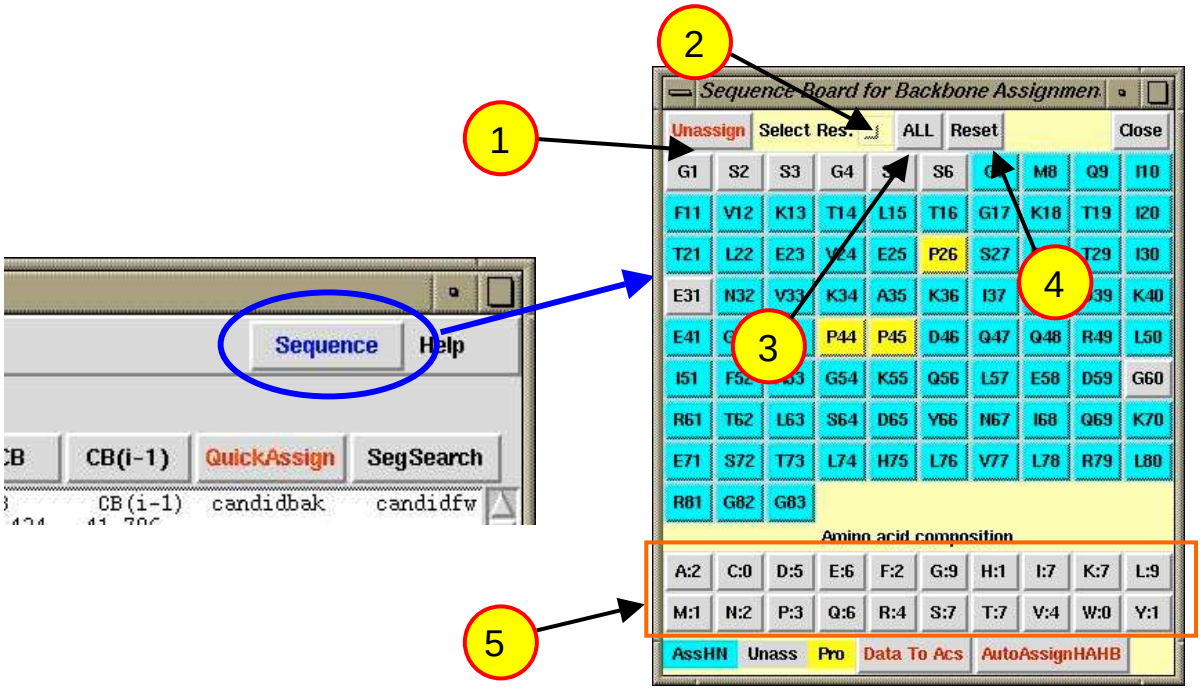
8: Display "Sequence Board" to check assignment completeness, to delete sequential assignments

9: Execute "Quick Save" to save assignment information to assign_NN.txt file.

10: Undo and redo button

11: Sort buttons for residue number, residue type, Peak ID, chemcial shifts





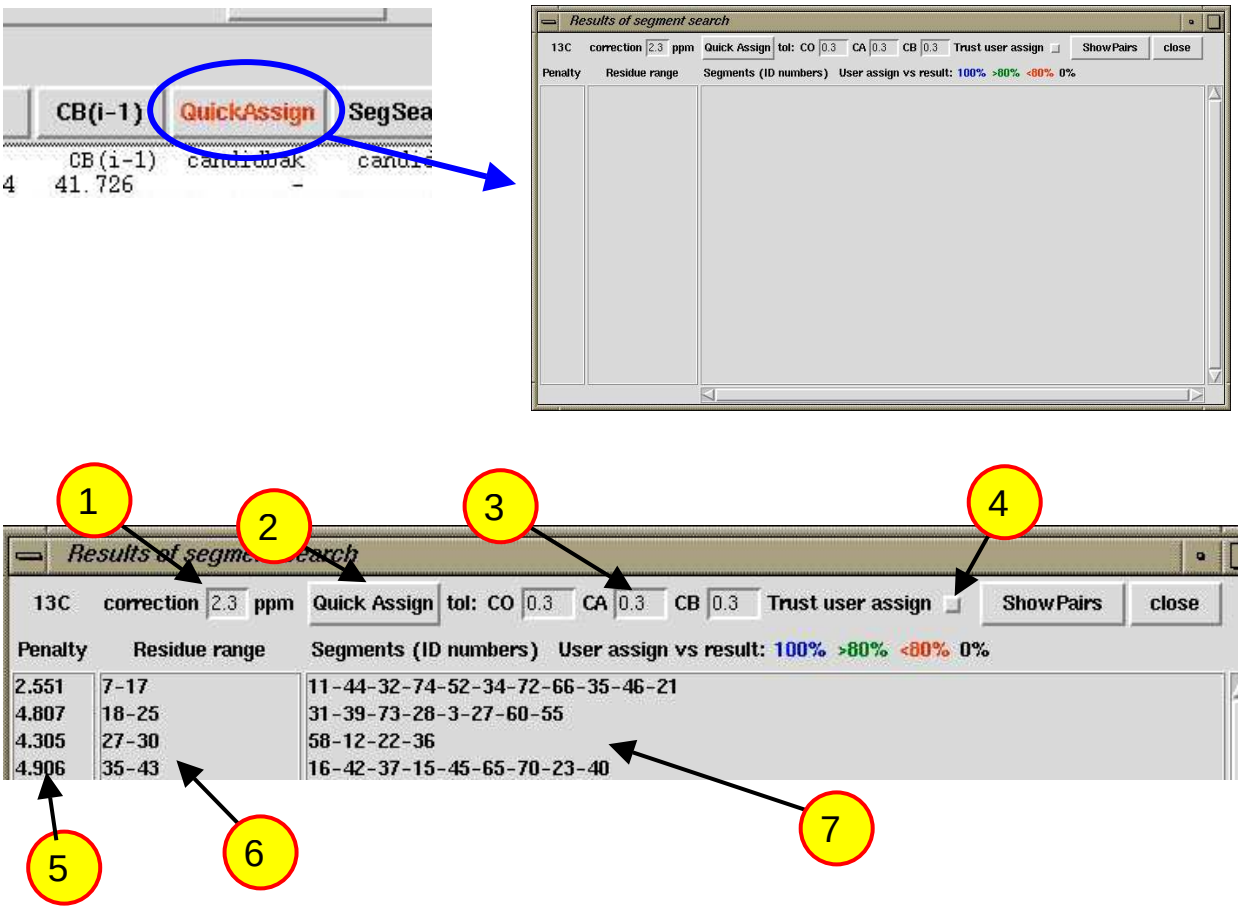
If you press "Sequence" button on the yellow module, you will see a small window as hown above.

1: "Unassigned" Unassign"　User can unassign the residue when "Select Res" is activated.
2: Checkbutton to switch the sequence board to residue selection mode
3: To select all residue, press "All" button
4: Reset button to reset residue selection
5: Amino acid composition buttons. If you press one of them, the sequence button highlighted with the selected amino acid type.

If you activate "Select Res" then press one of the button on the sequence board, the button color will change gray. Then if you press "Unassign" button, the selcted residues will be unassigned in the peak ID Table (residue number willl be 9999). This action will not delete chemical shift information.

15) Automated sequential assignment using "QuickAssign"

If you press "QuickAssign" button on the MagRO Assignment Table Manager, you will see the following window

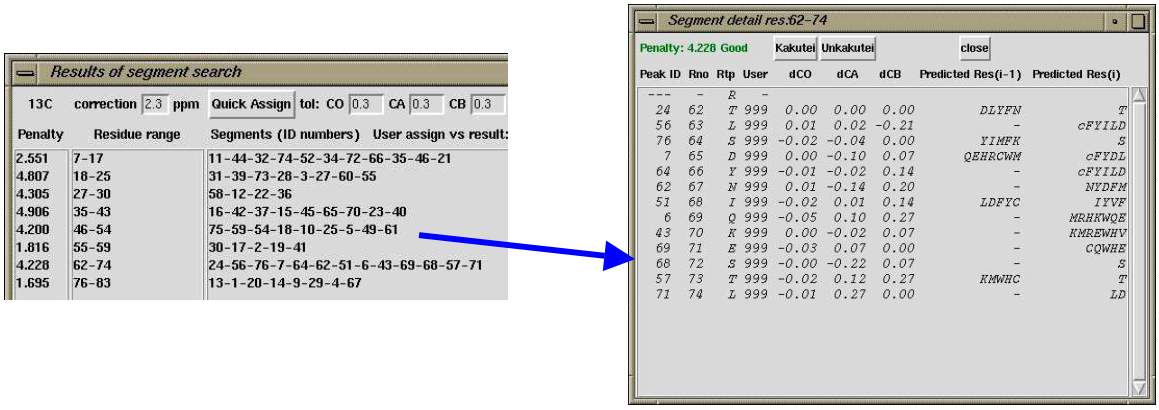


1: correction value of 13C chemical shift. You can add the value for the automated assignment
2: Execute automated sequential assignment by Quick Assign
3: Tolerance values of CO, Ca and Cb chemical shifts for automated assignment
4: fix user defined sequential assignments
5: Penalty value for each segment. For trustful assignment the value should be below 5.0.
6: Begin and end residue number of the segment
7: Peak ID segment obtained from automated sequential assignment. The segment with penalty value higher than 10.0 may not appear.

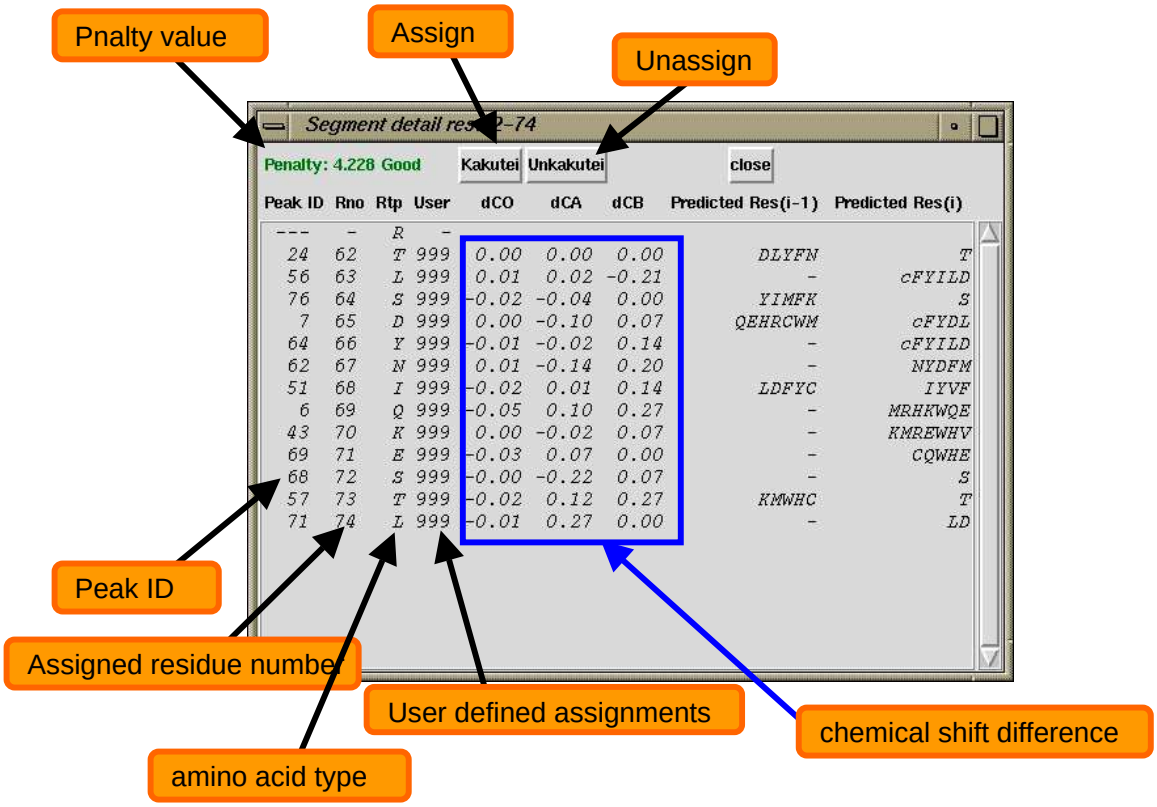
By pressing "QuickAssign" button, the program will perform sequential assignment using peak ID table, assign_NN.txt. The program will connect peak IDs whose CO and CA and CB chemical shifts are smaller than the tolerance value and will generate segments of peak IDs. By checking amino acid types predicted from CA and CB chemical shifts for each segments and comparing with the amino acid sequence, the program will search the best segments. The calculation of the assignments will take a few seconds depending on the size of protein.

[Important] If several blanching segments are found in the results, there might be minor signals in the peak ID table. In this case the minor you have to remove peak IDs derived from minor signal.

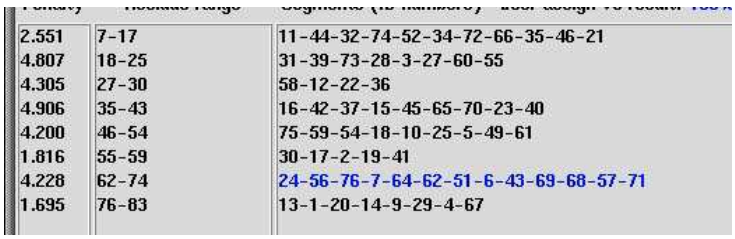
If you double-click one of the segments, you will see the small window;



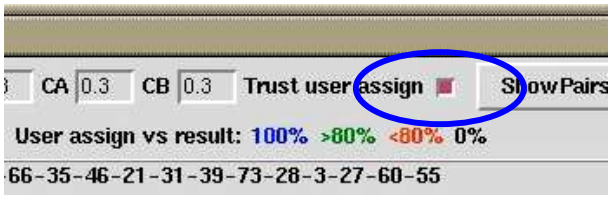
The window display the detinal of the sequential assignments;



If you press "Assign" button, you can fix the sequential assignments then the color of the segment in the QuickAssign module will change to blue.

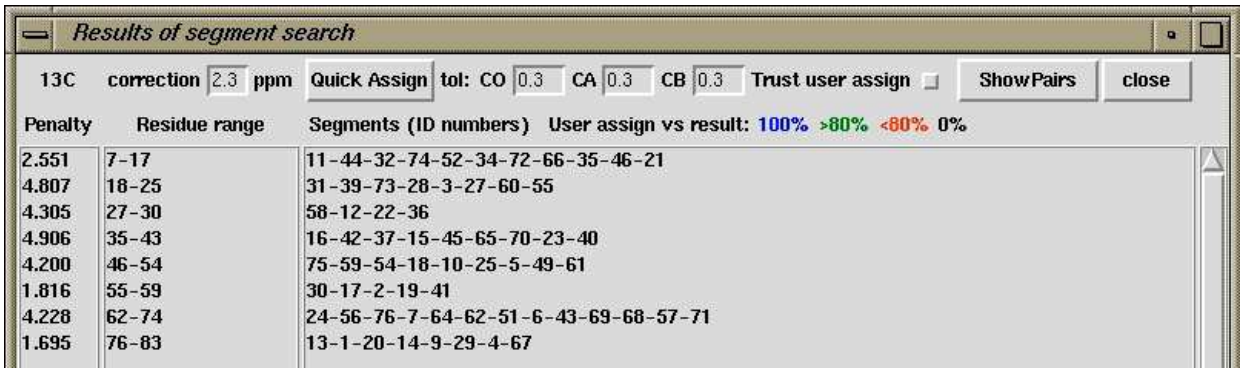


Now you can fix all the assignments for the segments with the penalty value less than 5.0. If you lucky enough, you can complete 60~80% of the sequential assignments

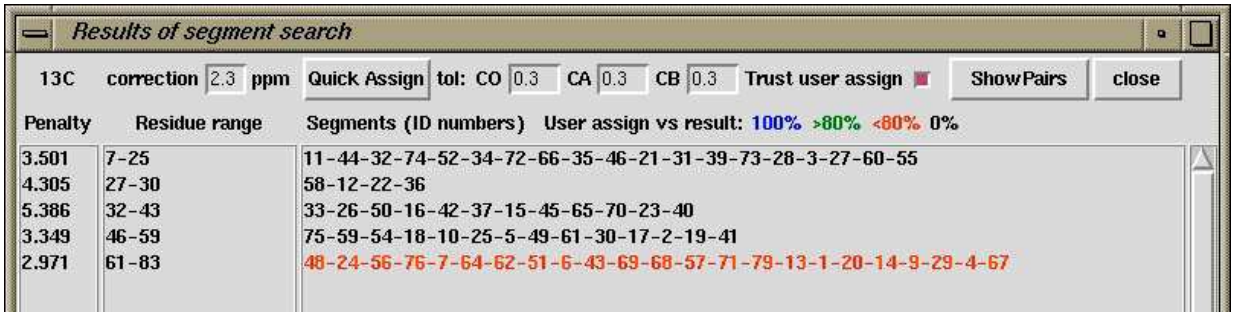


If you would like to perform further assignments, enabling the checkbox "fix user assign" and run the QuickAssign is good idea

If you disable the check button, you only see the following segments,

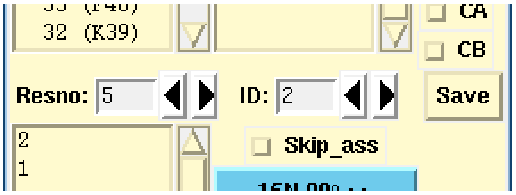


If you activate "fix user assign", now you can see longer segments and additional short segments;



16) manual sequential assignment

Using yellow module, user can assign residue number manually.



Enter assigned residue number in the entry "Resno:" in the middle of yellow module as shown in the left panel. **[Caution] Do not enter duplicate residue number, it will cause serious error.**

17) Unassign assigned residue number

In the peak ID table file, assign_NN.txt, residue number is set to 9999 for unassigned peak IDs.

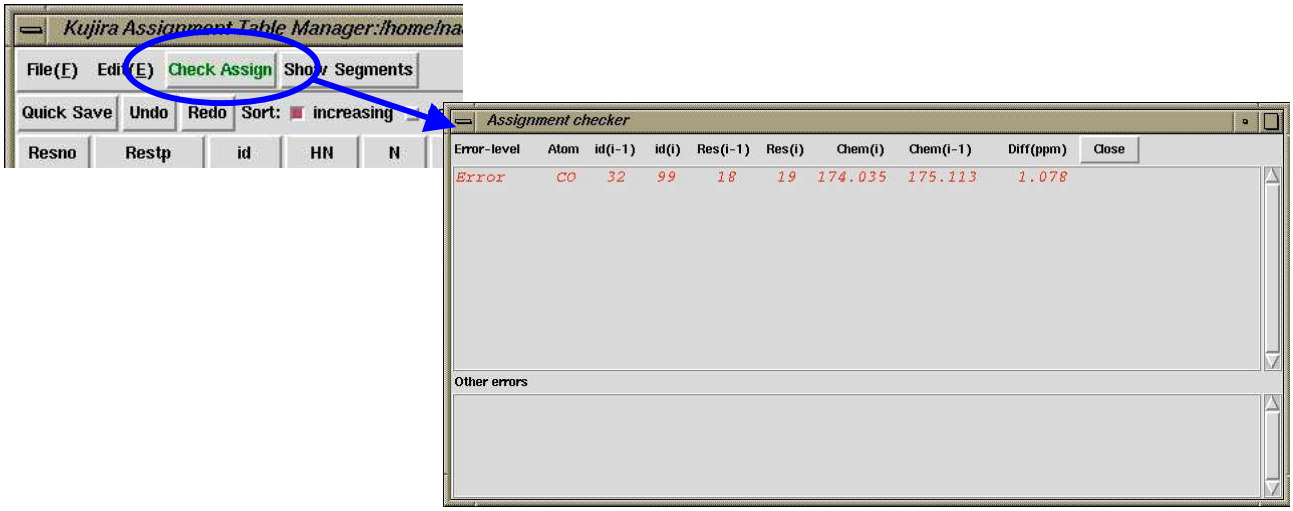
98	Q	S	108	7.935	113.664	176.927	173.545	57.251	58.839	25.865	60.502	- - -
99	S	C	109	7.577	118.443	173.545	999.990	58.839	999.990	60.502	999.990	- - -
100	P	S	115	8.428	116.081	999.990	174.416	60.860	60.111	31.242	60.097	- - -
101	S	S	116	8.5990	116.794	174.416	172.145	60.111	56.059	60.097	61.633	- - -
102	S	S	9999	8.511	117.864	172.465	172.803	55.926	56.231	61.618	61.620	- - -

If you would like to unassign the assigned residue number for a peak ID, open sequence board by clicking "Sequence" button on the yellow module to open BBass Seuqnce Board. Then activate "select res" check button, you can select the residues that you would like to unassign.

The selected buttons are clored by dark gray, then press "Unassign" to unassign the selected residues.

18) Validation and correction of sequential assignments using "CheckAssign"

By pressing "Check Assign" button on the MAGRO Assignment Table Manager, you will see the window UI;



This window tells you if there is some errors and warnings found in the sequential assignments. Typical errors are significant difference between CO, Ca and Cb chemical shifts for assigned residues (i) and (i-1) or (i+1), mismatching residue type predicted from Ca and Cb chemical shifts, and duplication of residue numbers.

The messages for warnings are indicated by black and serious errors are indicated by red.

Large difference between chemical shifts of CO(i) and CO(i-1) (0.3~1.0 ppm)

```
Warning   CO   31   24   61   62   173.342   172.917   0.425
```

Seriously large difference between chemical shifts of CA(i) and CA(i-1) (>1.0ppm)

```
Error     CA   31   24   61   62   54.137    51.917    2.22
```

Duplicated residue numbers are assigned two peak IDs

```
redundant res-number:  61 61  Check the list
```

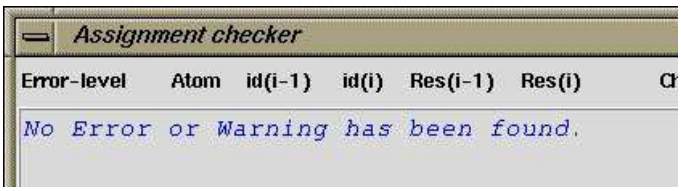
Chemical shift value is missing of CA for sequentially assigned residues

```
Warning   CA   71   79   74   75   51.261    999.990   incomplete
```

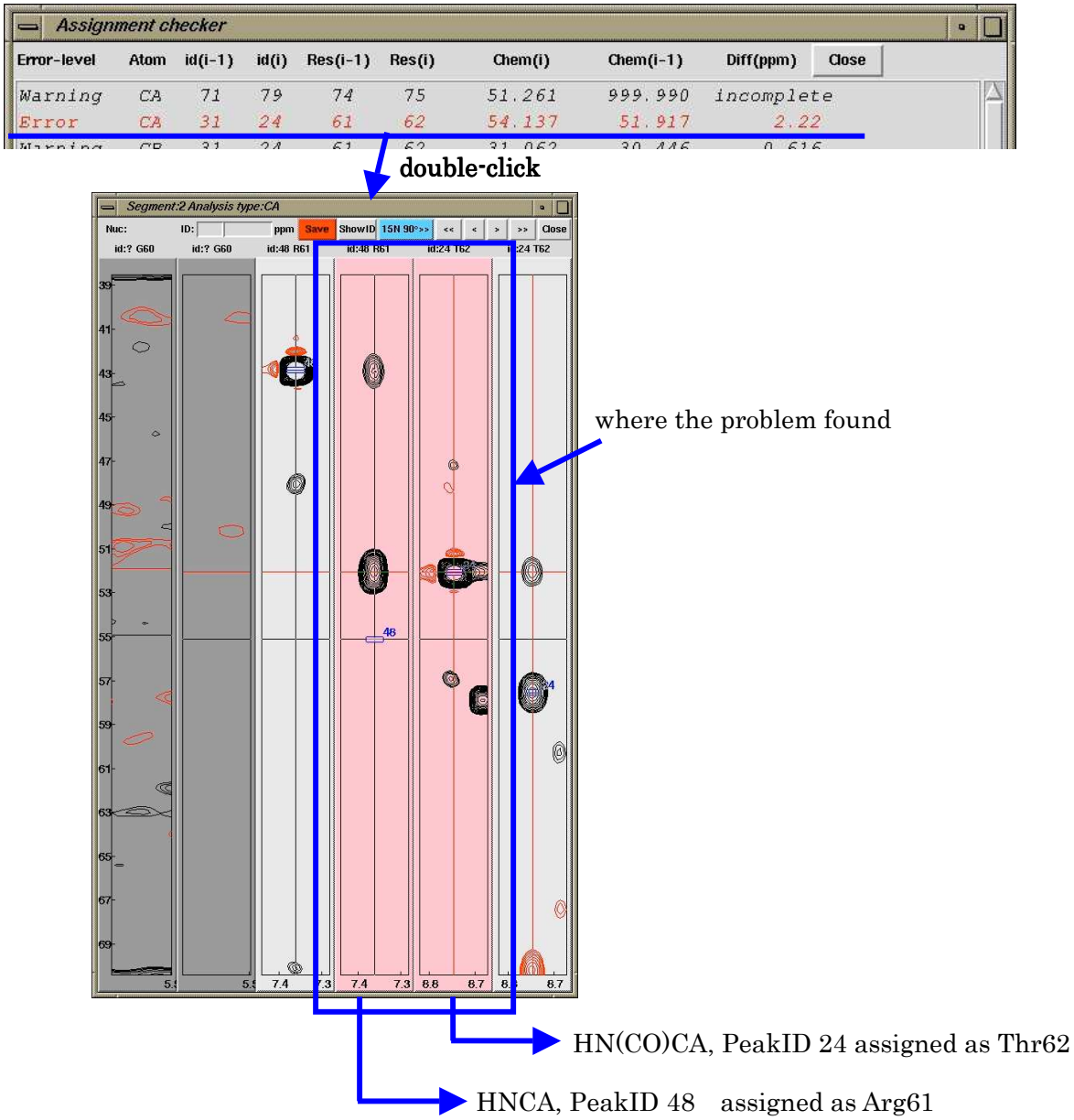
Mismatching amino acid type of assigned residue with sequence

```
Warning residue:22 is not THR Check sequence and your assignment.
```

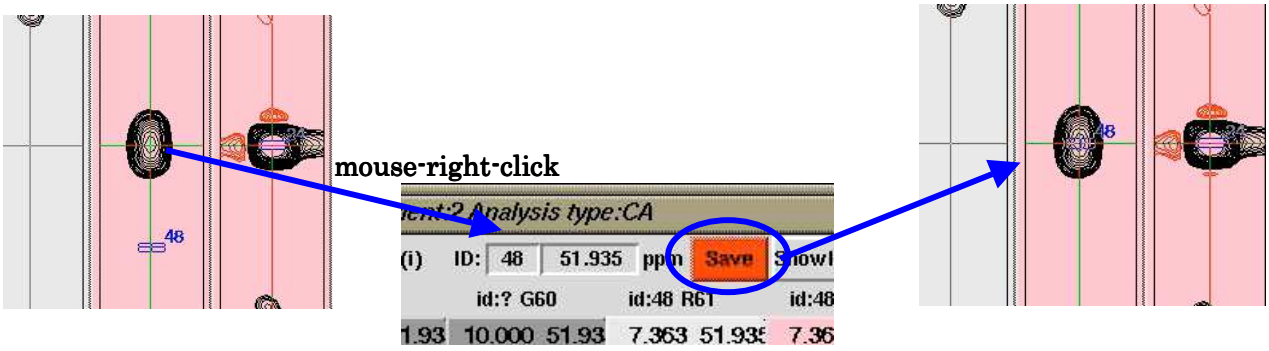
If no error has been found



If you double-click one of the erro or warning message, you will see a window displaying 2D spectrum array;

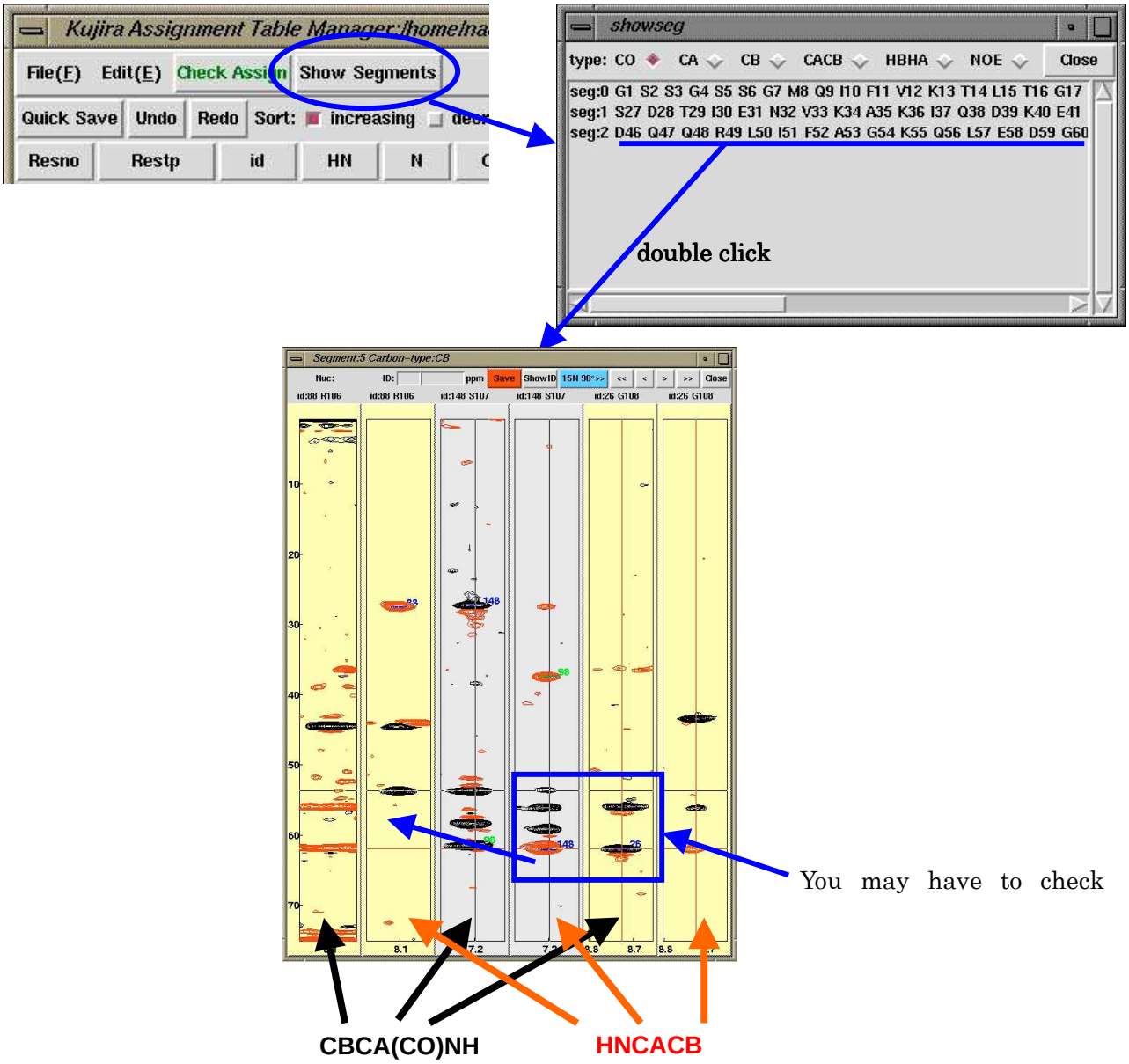


The above example is showing the assigned chemical shifts for CA(Arg61) and CA(Thr62) is significant. The arrayed spectrum strips can help you to recognize which assigned signals is wrong.
By clicking mouse-right-button on the spectrum position of y-axis and pressing "Save" button, you can correct the wrongly assigned chemical shift value directoly.



19) Confirmation of sequential assignments using show segment function

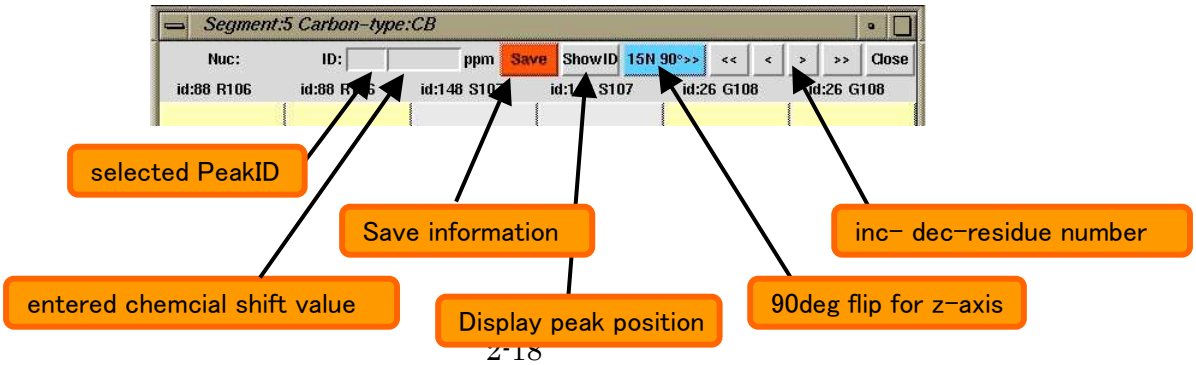
If you press "Show Segments" on the MAGRO Assignment Table Manager, you will see a small window UI listing all possible sequential assignment segments. Select one of the display option among CO, Cα, Cβ, Cα/Cβ, Hα/Hβ, NOE, then double-click one of the segement to show spectrum strip array;



You may have to check

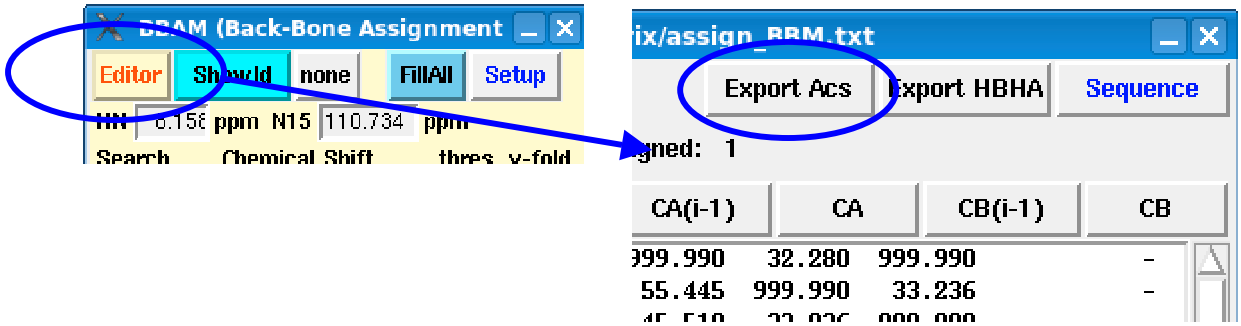
[Important] For each display option, you need to prepare required spectrum windows. For example CA/CB mode requires spectrum windows for CBCA(CO)NH and HNCACB.

[deteil of the header of segment display window]



20) Finishing sequential assignments and data tranportation to Acs module.

If you would like to finish the sequential assignments and to transport the data to Acs module, press "Editor" button on the yellow module to open MAGRO Assignment Manager and press "ExportAcs" button to start the job.



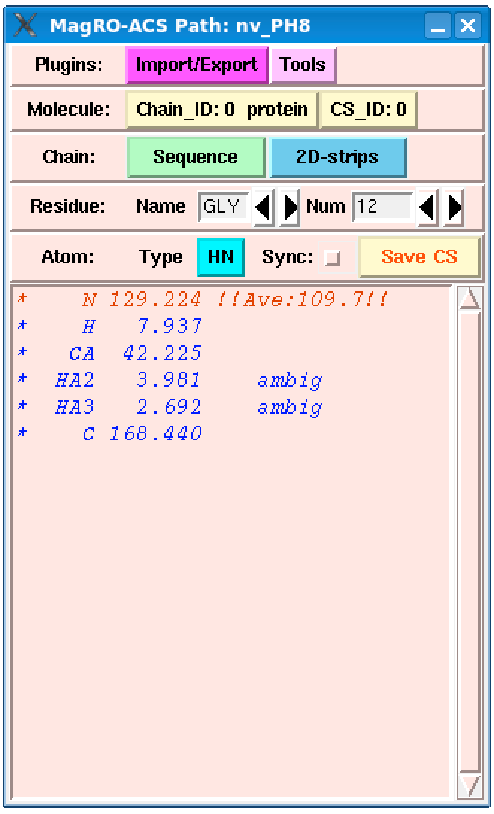
When the data transfer job is completed, you can find a text file "protein_0_0_acs.db" in the directory matrix/MagDB.

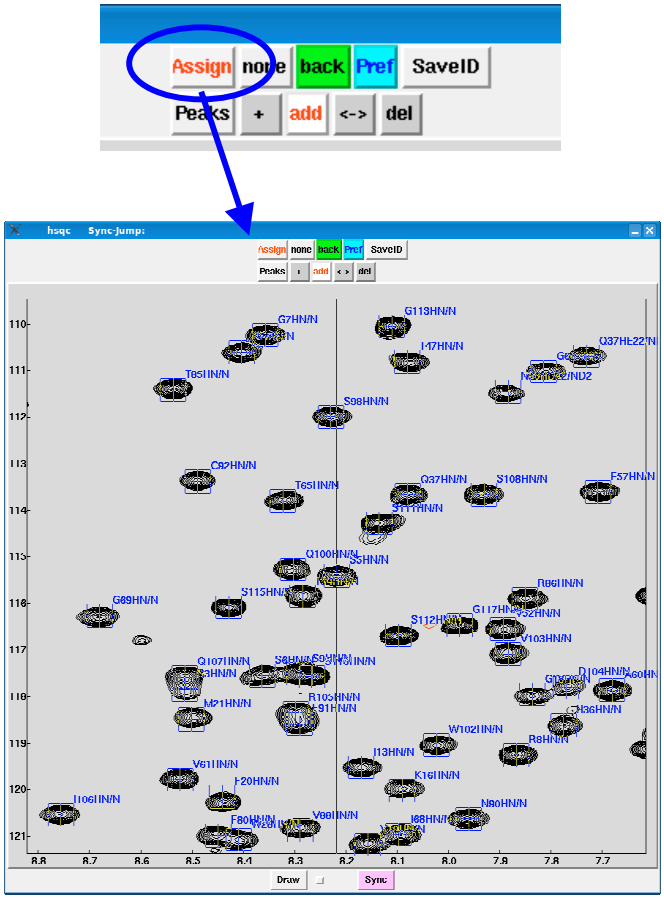
[Caution] This action will overwirte the old assignment data with new one. Please take a backup of MagDB directory before starting the job.

If you have not seen any error message, you will see the magenta module can display assignment information. The below left panel shows the assigned chemical shifts for the residue GLY12.

The job will fill the chemical shift values of HN, CA , CB and CO signals. If the signals for both (i) and (i-1) residues are avilable, the averaged values will be entered.

If you press "Assign" button on the HSQC spectrum, you will see assigned peak positions are displayed by blue boxes in the spectrum window.



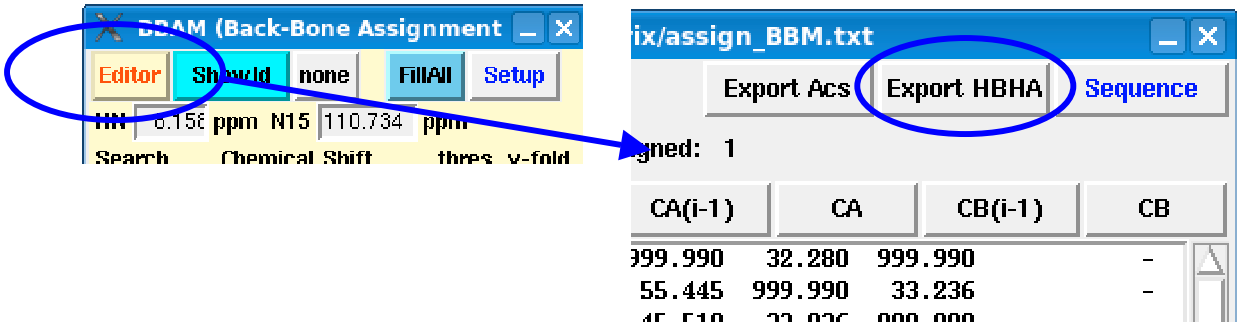


21) Export assigned chemical shifts to Acs with automated assignment of Hα and Hβ signals

In the previous section, you have learned how to export the assigned chemical shifts for backbone signals. This section will describe how to export the data to Acs with automated signal assignments for Hα and Hβ signals.

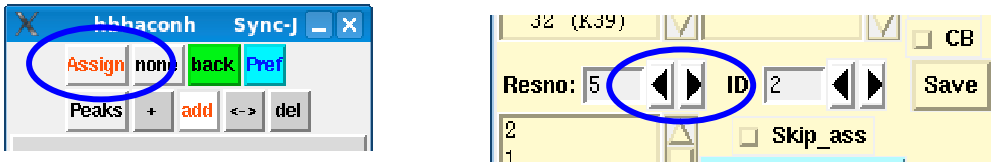
For the exportation and assignment job, the following spectrum data is required;

Spectrum name	Short cut name in MagRO
2D 1H-13C HSQC for aliphatic	chsqc
3D HBHA(CO)NH	hbhaconh



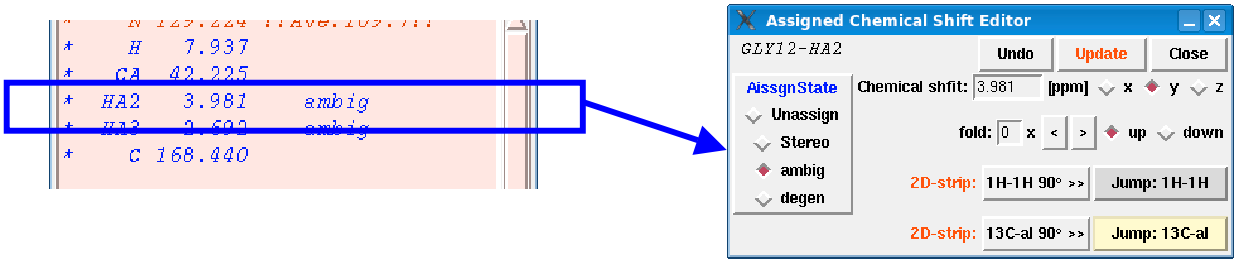
To execute the program, firstly you have to open "MAGRO Assignment Manager" by clicking "Editor" button on the yellow module. Then press "Export HBHA" button. The assignment job takes 10-20sec. Depending on spectrum quality, the accuracy of assignments will be around 70%.

After the exportation and assignment jobs, you can confirm and correct the assigned chemical shifts, for example if you press "Assign" buttons on 2D-^{1}H-^{15}N HSQC, 2D ^{1}H-^{13}C HQSC and HBHA(CO)NH the blue boxes appear on the spectrum windows indicating the assigned signal position. Now you can check the assignments one by one by clicking increment- decrement- residue number button on the yellow module.



[important] In many cases, methylene group such as Hb2 and Hb2 close together in y-axis in HBHA(CO)NH spectrum are assigned as degenerate signal. In such this, you have to separate them and treat as ambiguously assigned signals on Assigned Chemical Shift Editor.

To correct the assignments, firstly you have to set residue number (i) on the MagRO-ACS module and double-click one of the atoms that you would like to edit, "Assigned Chemical Shift Editor" will appear. Secondly, you have to display 2D-spectrum strip of HBHA(CO)NH, then then press "Assign" button on the header of spectrum window. Thirdly, set the residue number (i-1) on the yellow module. Now you can enter chemical shift value to the editor by clicking mouse-middle-button on y-axis of HBHA(CO)NH spectrum window.



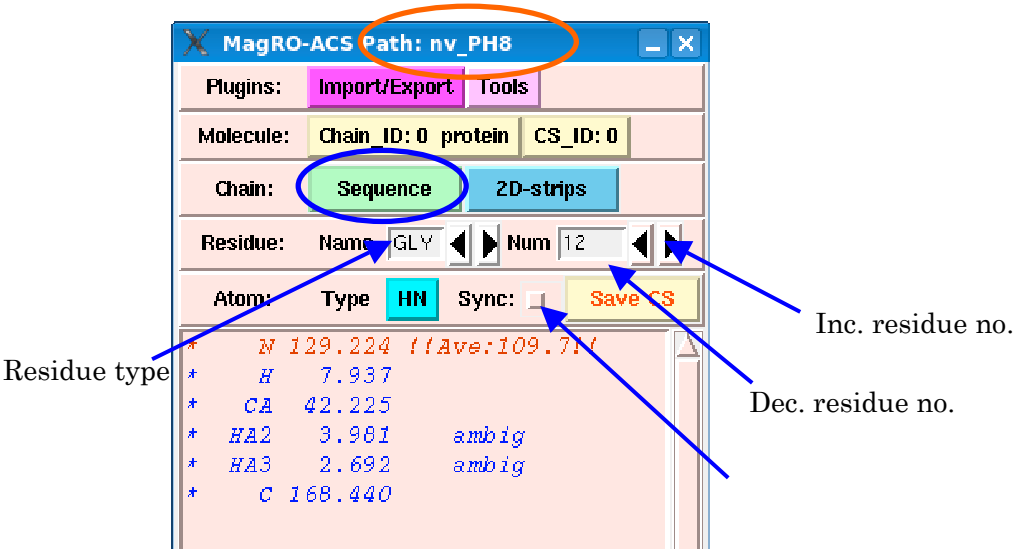
3-1. Assignments of aliphatic side-chain signals

1) required spectra

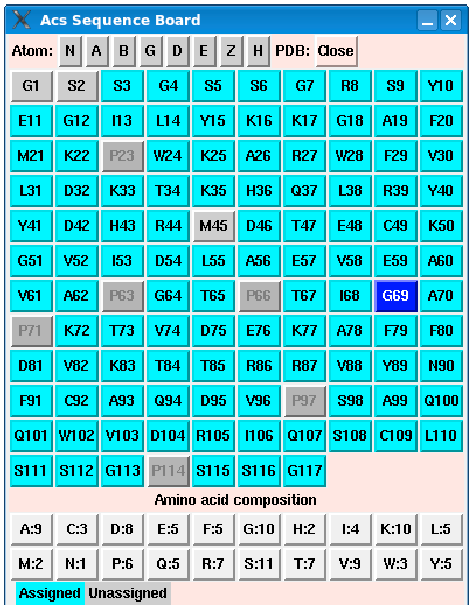
name	axis-order	axis-label
3D HCCH-TOCSY for aliphatic	^{1}H-^{1}H(acq)-^{13}C	HC-H-C
3D HCCH-COSY for aliphatic	^{1}H-^{1}H(acq)-^{13}C	HC-H-C
3D ^{1}H-^{13}C edited-NOESY for aliphatic	^{1}H(acq)-^{1}H-^{13}C	HC-H-C

CC(CO)NH, H(CCO)NH and HCCH-COSY would be helpful but should not be enough. For the side-chain signal assignments, HCCH-TOCSY must be required.
[Important] User has be careful to set axis order and axis labeling. If they are wrongly set, the system may not work properly.

2) How to use MagRO-Acs module

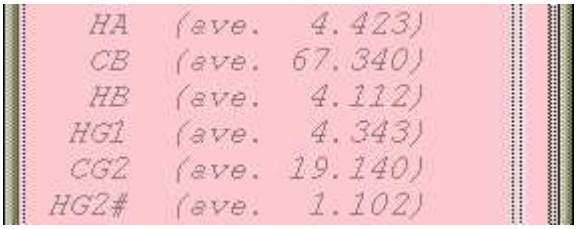


This module is used for managing assigned chemical shift table. You can find the path of working directory as shown in the upper panel indicated with red ellipse.
To move the residue number that you want to display, type residue number in the entry then press return key. You can increment and decrement the residue number by pressing buttons "<" and ">" on the module.
If you can move to the residue number whose residue type can be specified in the "Residue: Name" entry.



If you press sequence button, you will see a small window to display the sequence of your sample as shown in the right panel. In the window, the residues whose signal for H_N and $^{15}N\alpha$ have been assigned are indicated by cyan.

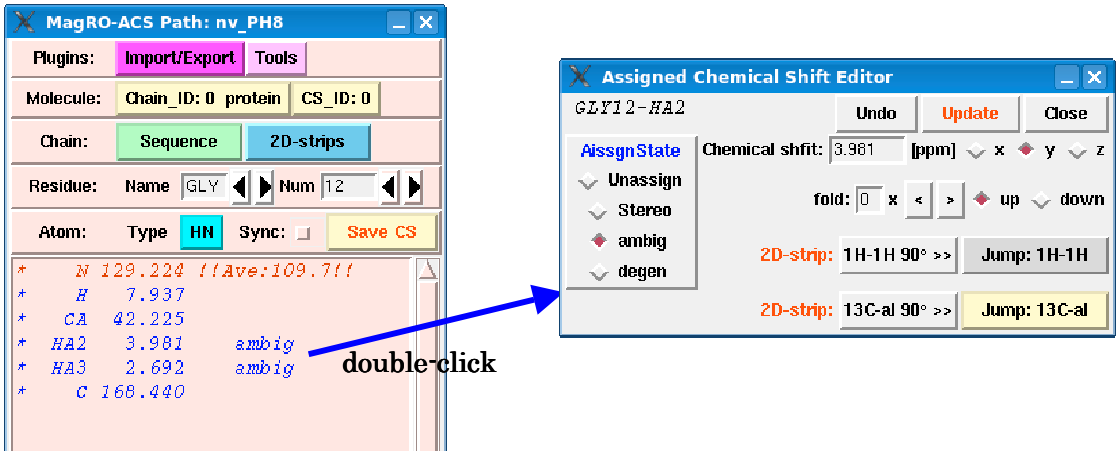
If you press one of the button in the sequence window, you can move to the assignment table of the selected residue number.



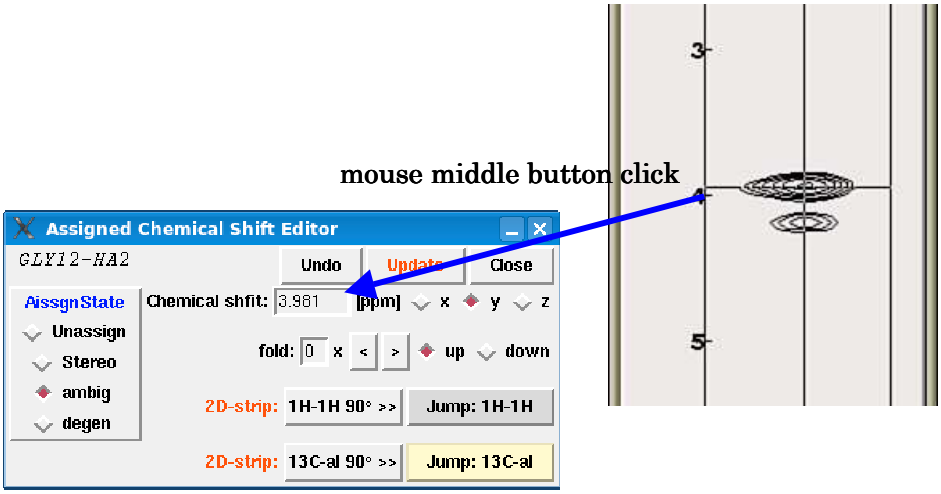
If no signals has not been assigned, the Acs module shows atom name and chemical shifts for statistically averaged values indicated with gray characters as shown in the left panel .

3) How to enter chemical shift value

To enter chemical shift value to the Acs module, firstly you have to open chemical shift editor by double-clicking one of the atom type listed in the module.



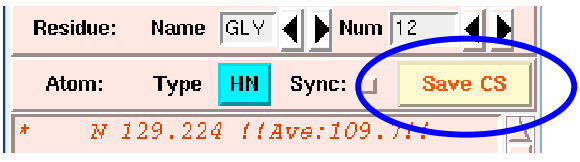
Secondary you have to place y-axis of the cross-hair cursor just on the peak that you would like to get the chemical shift value. Now by middle-button-click on spectrum window, you can enter chemical shift value directly.



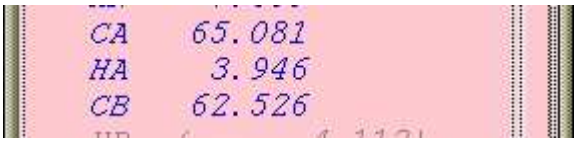
Above example showing how to enter chemical shift value from y-axis of 2D spectrum strip. You can change to x- or z-axis by clicking checkboxes on the editor.

DO NOT FORGET to press "Update" button to update the all information in the MagRO system.
Press "Save CS" button on the Acs module to save and fix the chemical shift information.

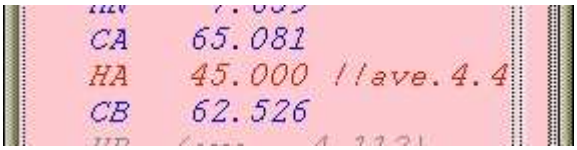
If you successfully finish the assignment job for a certain signal of atom, you will find the atom line in the module turn to be cyan.



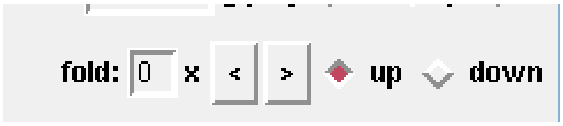
If you would like to unassign the information, select "Unassign" checkbox on the editor, **then press** Update button to update the information. To finalize the operation, don't forget to press "Save CS" button.



It is possible to save the information after several times of pressing update button assignment.
You can retrieve the old assignment state by pressing "undo" button on the editor.



The Acs module has a function to display warning or error message if the assigned chemical shift is largely away from averaged chemical shift value: S.D.*2> warning (orange) and S.D.*3> error (red).



If the signal that you would like to assign is in aliased area of spectrum (State-TPPI mode), you have to calculate the real chemical shift using offset and spectral width. In this case you have to select up or down for the aliased shift and type 1 or 2 in the "fold:" entry. The editor will calculate the real chemical shift automatically. All you have to do is trying up or down and change the value for fold (1 or 2).

If the procheral proton or carbon signal (such as Hβ2 and Hβ3 of Ser, Cγ1 and Cγ2 of Val) have not been assigned stereo-chemically, you have to specify the assignments are "ambiguous". In this case you have to select "ambig" on the editor. If the procheral signals are overlapped, select "degen" which means signals are degenerate.

The information of assigned chemical shifts are saved in the directory;

~/nv_data/matrix/MagDB

You can find chemical shift table file, protein_0_0_acs.db which can be opened by standard text editor. The format is tabular style;

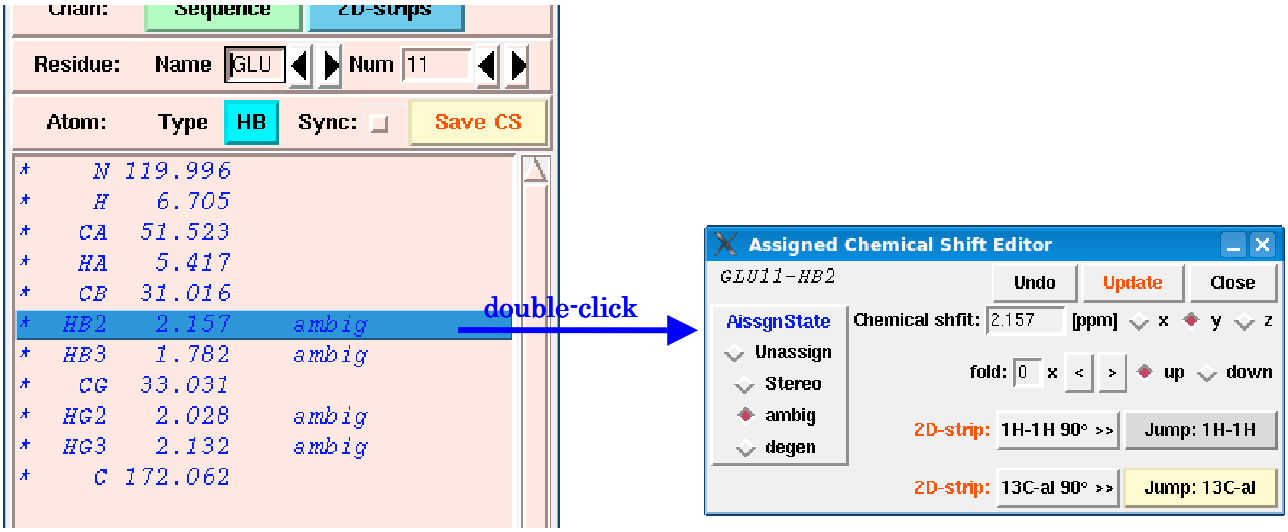
AtomID	Atom	Residue	No	CS value	Ambiguity code
560	NH	GLY	41	8.410	1
561	N	GLY	41	112.500	1
562	CA	GLY	41	43.500	1
563	HA1	GLY	41	4.450	3
564	HA2	GLY	41	4.450	3
565	O	GLY	41	999.990	0
566	C	GLY	41	176.200	1

The chemical shift values for unassigned signals are 999. The ambiguity code indicates the ambiguity of assignment for procheral atoms. The value means 0 for "not assigned, 1 for "assigned", 2 for "ambiguously assigned" and 3 for "signals degenerate".
[important] It would be recommended that user periodically take the backup.

4) Assignments of Hα and Hβ signals using backbone assignment module

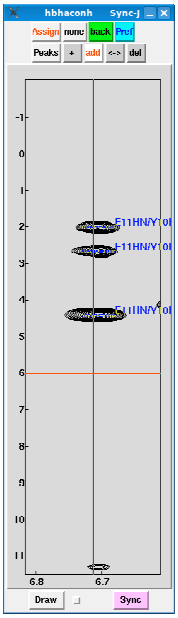
required spectra: HBHACONH, 2D ^{1}H-^{15}N HSQC, 2D ^{1}H-^{13}C HSQC

In most cases you can assign most of all signals using HBHA(CO)NH.
Firstly you have to set residue number (i-1) on Acs module.

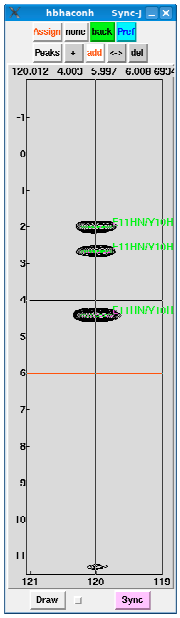


By double-clicking to open editor for the signals Hα or Hβ.
Then set the target residue number using the yellow module to display HB/HA region of 2D spectrum strip of HBHA(CO)NH.

For example, set the y-axis of cross-hair cursor just on Hβ signal and mouse-middle-click to get the chemical shift value. As you don't now which Hβ signal is R- or S-configuration, you have to select "ambig" checkbox on the editor. If the Hβ signals seem to be overlapped, you have to select "degen".



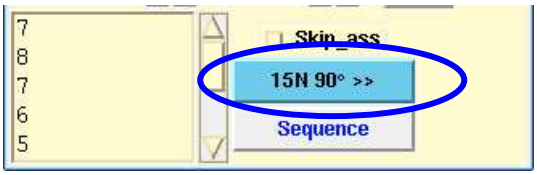
HN-15N



15N-HN

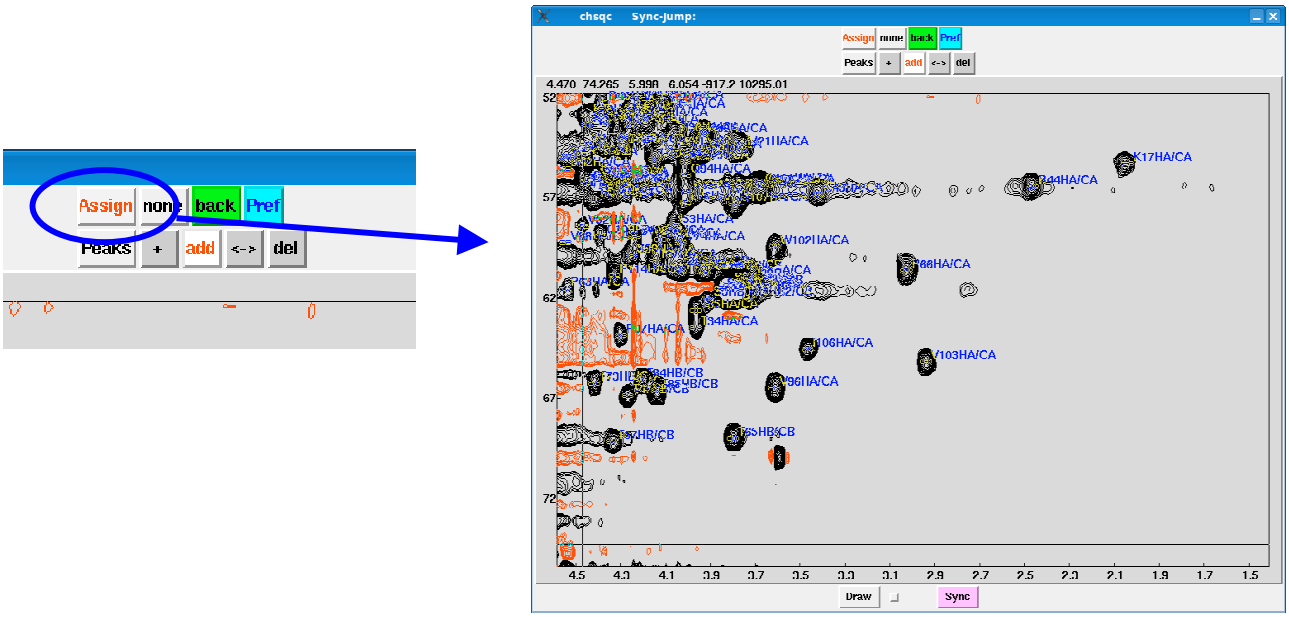
Finally press "Update" button on the editor and "Save CS" button on the Acs module to complete the assignment job.

[Important] If many HN-15N signals are crowded in HSQC plane, try to flip 2D-spectrum strp around X-Z dimension by clicking 15N deg flip button.

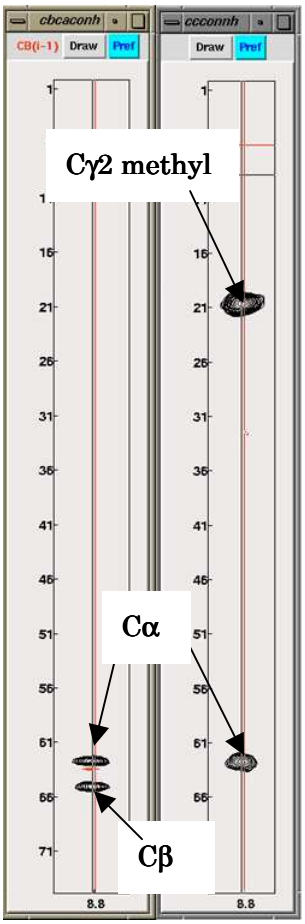


The left panel showing X-Z flipping of 2D epctrum strip of HBHA(CO)NH spectrum.

You can confirm the assigned chemical shifts on spectrum window. Press "Assign" button on the header of 2D 1H-13C HSQC spectrum, the expected signals will be displayed as blue boxes on the window.



5) extension of carbon signal assignment for aliphatic side-chain



CBCACONH CC(CO)NH

required spectra: CC(CO)NH, 2D ^{1}H-^{15}N HSQC, 2D ^{1}H-^{13}C HSQC

Let us assume that we have already finished to assign Cα and Cβ signals. Here we ready to assign Cγ, Cδ and Cε signals using CC(CO)NH.

The left panels show 2D spectrum strips of CBCA(CO)NH and CC(CO)NH for 1H-15N position of Thr at residue (i-1).

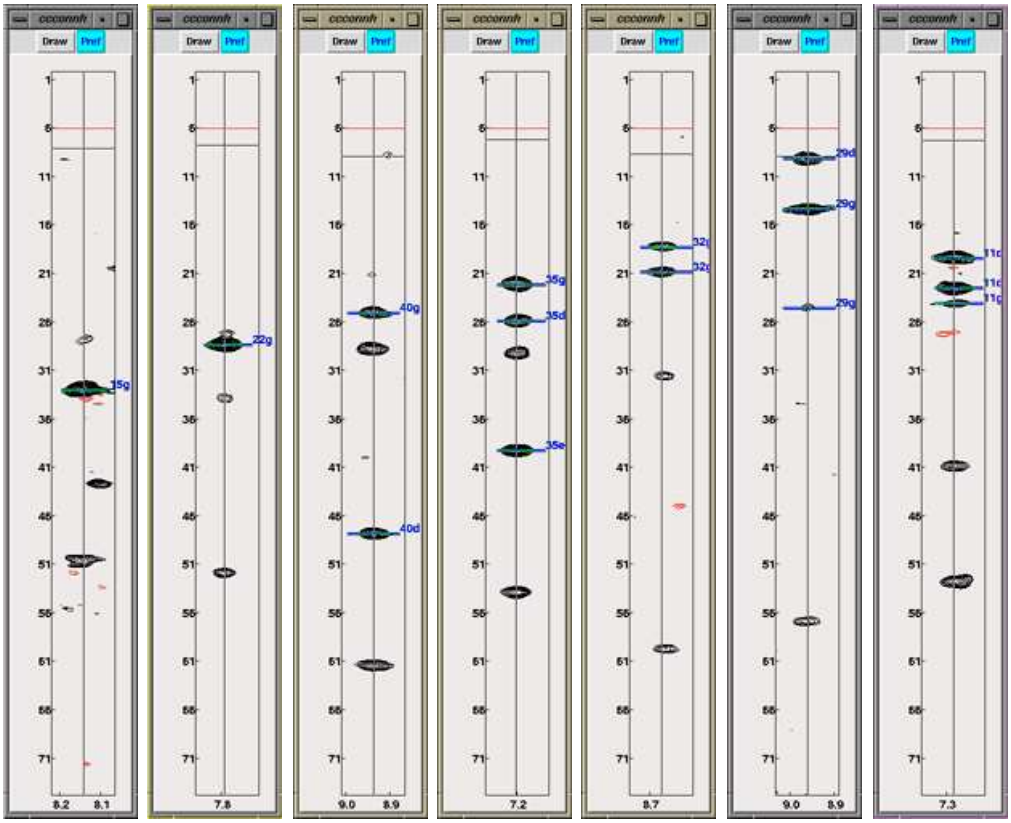
[Important]

1. The peaks observed in CC(CO)NH spectrum are sometimes slightly shifted positions because of sample heating by spin-locking.

2. Some of signals in CC(CO)NH are missing especially for larger protein. Broad signals will be also weak and sometimes missing in the spectrum.

3. Cβ and Cγ signals of Pro residue for cis conformer give largely different chemical shifts from standard value.

The aliphatic side-chain signals for the other residues, Glu, Ile, Lys, Leu, Met, Pro, Gln, Arg and Val can be assigned using standard chemical shift value.



Glu Gln | Met | Pro Arg | Lys | Val | Ile | Leu

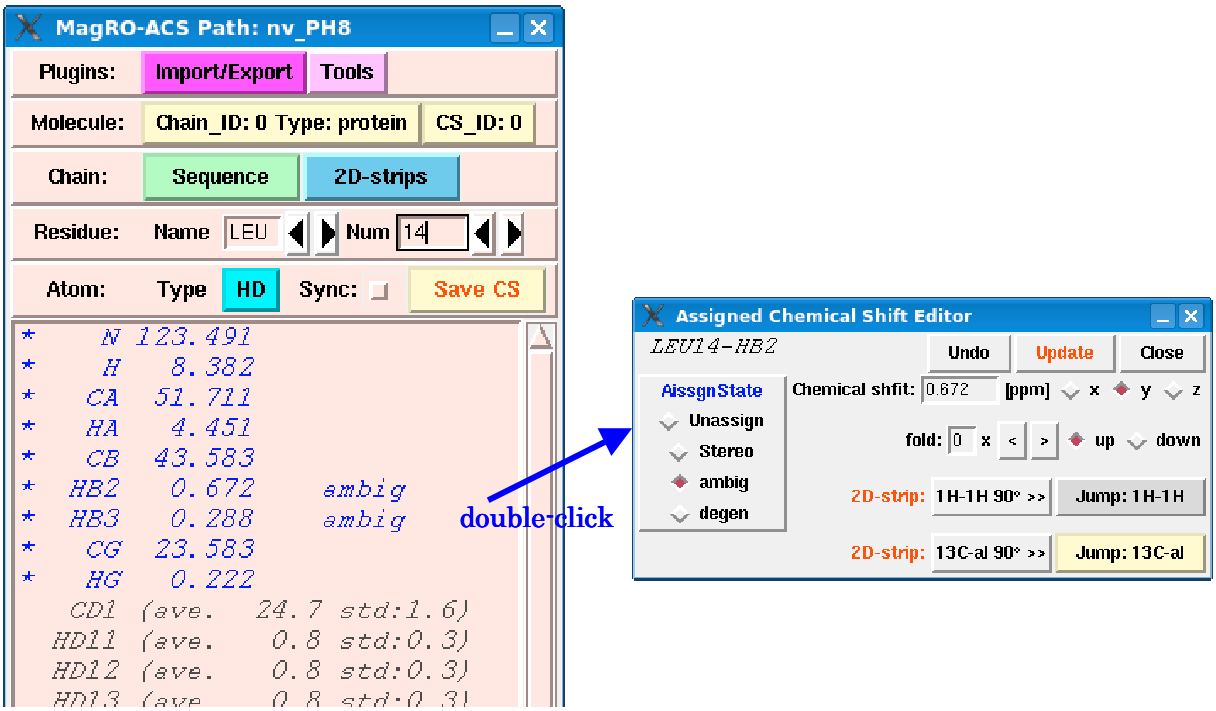
The left panels show typical aliphatic side-chain signals observed in CC(CO)NH spectrum.

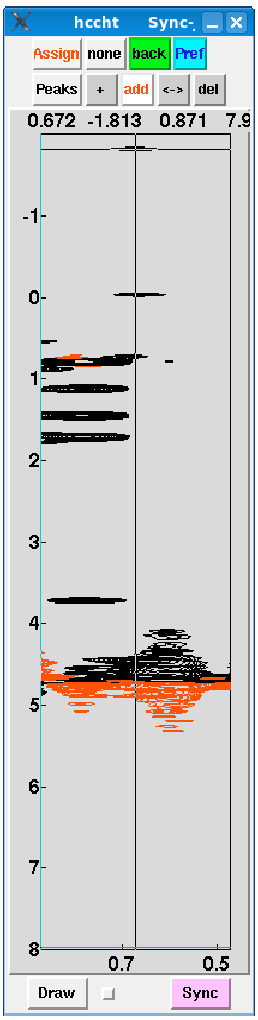
If you press "Assign" button on the spectrum window you can confirm the assigned chemical shifts by visual inspection of blue boxes displayed on expected peak positions.

6) extension of proton signal assignment for aliphatic side-chain

required spectra: HCCH-TOCSY for aliphatic (hccht), 2D ^{1}H-^{13}C HSQC (chsqc)

Here we are going to learn how to extend proton signals of the aliphatic side-chain using HCCH-TCOSY, ^{13}C edited NOESY (CNOESY) and tool implemented in MagRO.
Prior to the assignment works, the signals of Cα, Cβ, Hα, Hβ and Cγ have been already assigned.





Select and show the assignments for the residue Leu14 by typing 14 and press return on magenta module (upper and left panel)

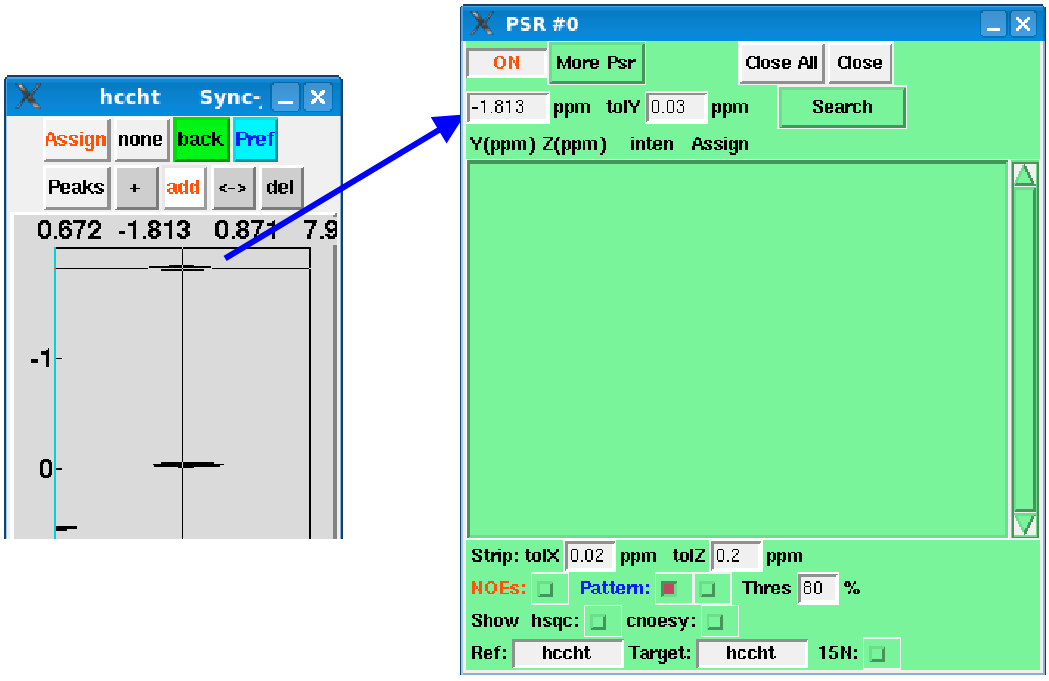
Then double-click HB3 to display assignment editor for the signal Leu14-Hβ (upper and right panel).

Prepare 2D spectrum strip of 3D HCCH-TOCSY for aliphatic (hccht) and click "Jump: 13C-al" button on the editor. Now the 2D spectrum strip displays the region corresponding to the signal of Leu-Hβ-Cβ on 1H-13C HSQC (left panel).

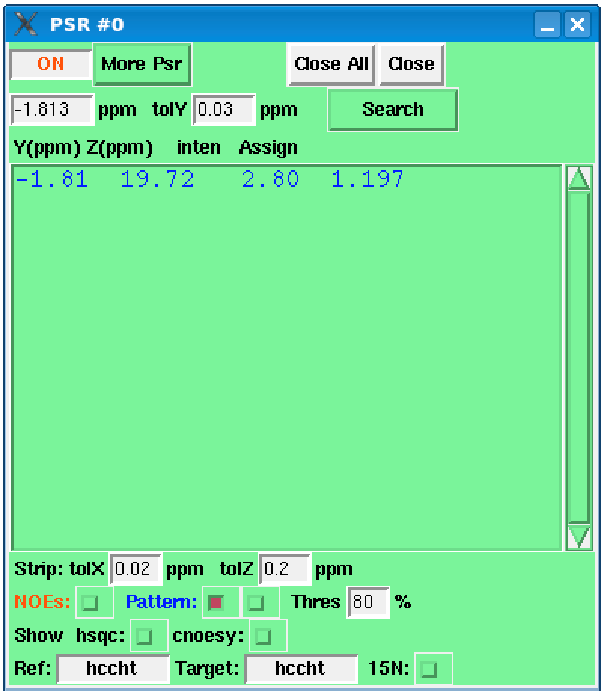
The "PSR" button on the main module to open PSR module.

Now click the check button "Pattern" in the PSR module, the module will change the GUI formation as shown below;

Place the cross-hair cursor just on one of the signals on HCCH-TOCSY. By mouse-middle-button clicking, chemical shift values of the y- and z-axis will be inserted in the PSR module.



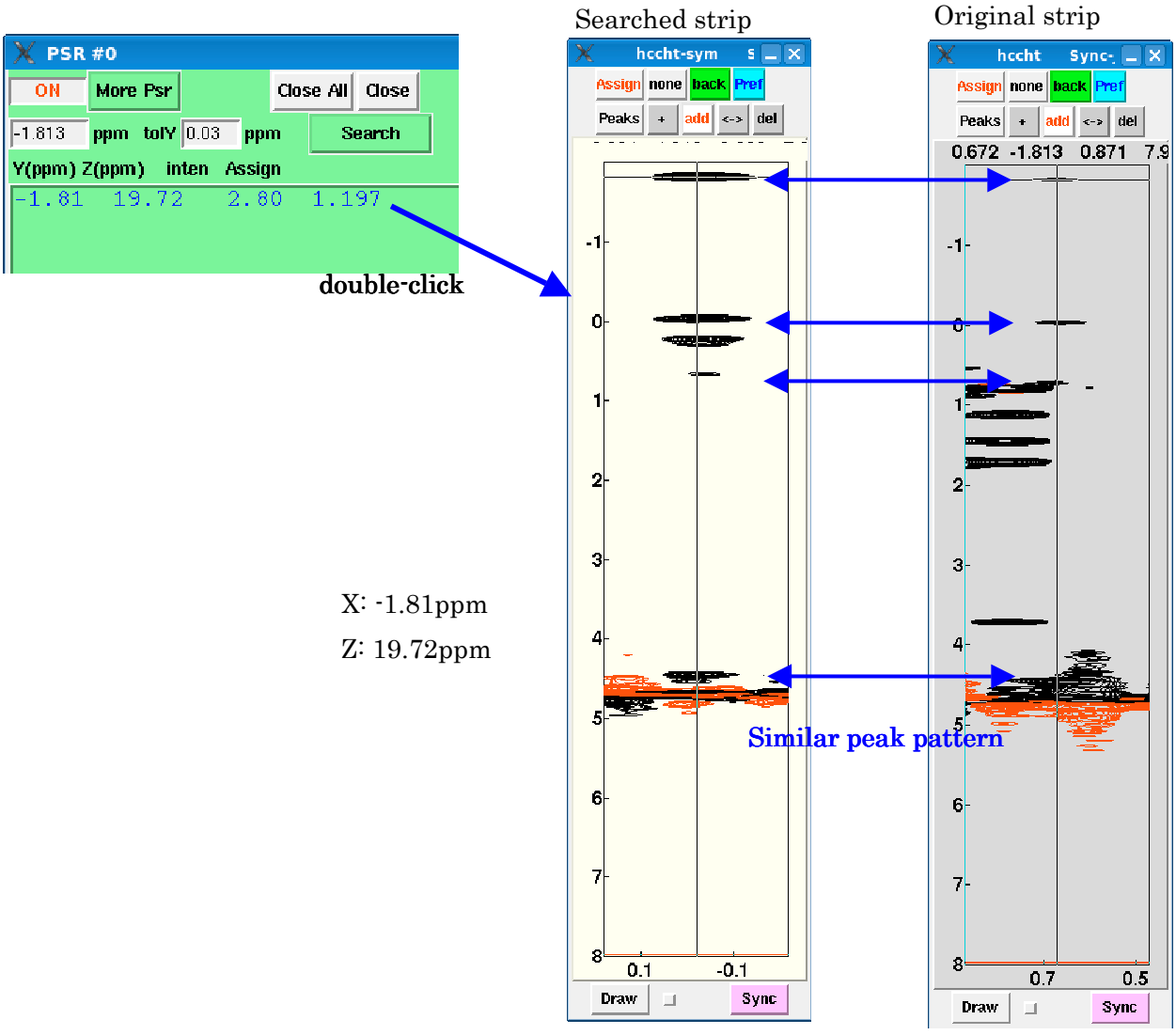
Now you can press "Search" button the PSR module to search for diagonal peaks in HCCH-TOCSY with peaks with the same chemical shifts on x-axis.



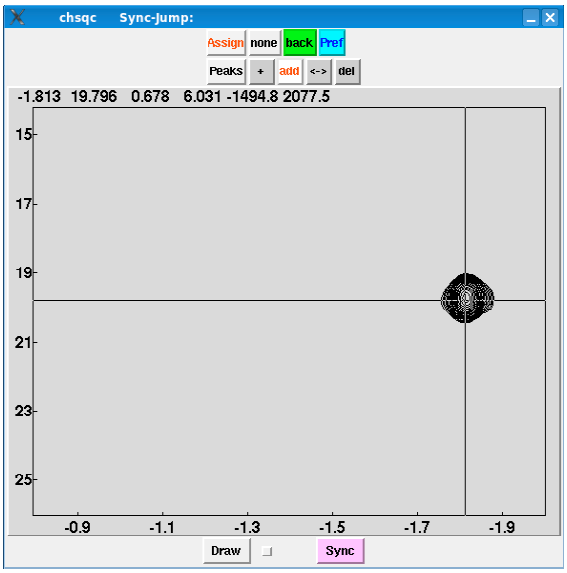
The left panel shows an example searching for diagonal peak (19.72ppm on Z-axis) with the chemical shifts -1.81ppm on X-azis.

The search function can detect diagonal peaks with the 80% of threshold set to the HCCH-TOCSY spectrum window.

If you double-click one of the search results (the left panel example only showing single result) to popup another 2D spectrum strip corresponding to the X-Z position of the search result.

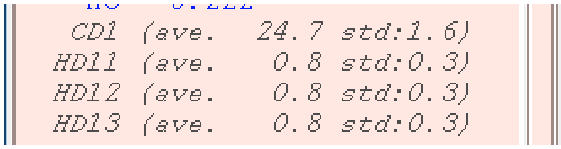


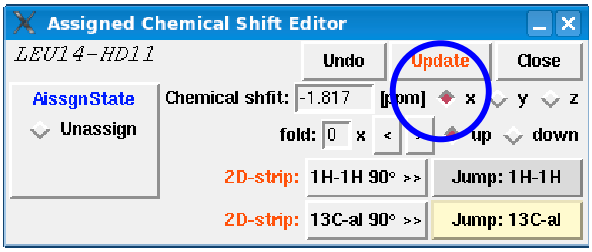
If you find the peak patter is similar in the found 2D spectrum strip, you can assign the signal as side-chain signal of Leu14.



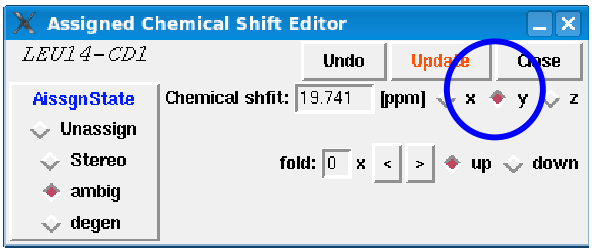
Next, open ^{1}H-^{13}C HSQC spectrum for aliphatic (chsqc), then expand the region near -1.81ppm and 19.71ppm. If you find an unassigned signal near the position, it should be remaining signals, Hγ/Cγ, Hδ1*/Cδ1 or Hδ2*/Cδ2. From the chemical shift value of Y-axis (19.71ppm), the most probable assignment should be Hδ1*/Cδ1.

The standard chemical shift value can be found in Acs module as the values with gray color.





To complete the assignment, double-click the HD11 in the Acs module, then place the cross-hair cursor just on the signal in HSQC and mouse-middle-click to enter the chemical shift value of x-axis (proton). Press "Update" button to update the Acs information, then close the editor.



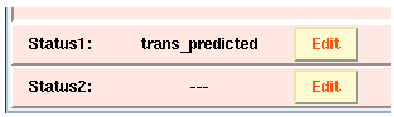
Next thing to do is double-click CD1 in the Acs module to open editor. Click the check-box of y and place the cross-hair cursor just on the signal in HSQC. By mouse-middle-clicking, enter the chemical shift value of y-axis (13C) to the editor. Don't forget to press "Update" to update the Acs information.

If the 13C dimension is folded, the program will automatically predict unfolded chemical shift using offset and spectrum width.
To finish the assignment press "Save CS" button on the Acs module.

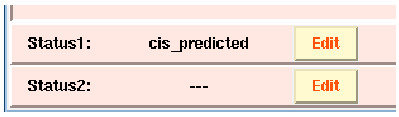
[Important] If the spectrum width has been very narrow, the prediction of folded chemical shift will be failed. In that case, user has to specify the aliasing way (up-file or down-filed) and folding times on the editor widow the try to enter the chemical shift value by mouse-middle-button clicking.

7) How to assign signals of aliphatic side-chain with no HN-15N information
required spectra:HCCH-TOCSY for aliphatic, ^{1}H-^{15}N edited NOESY, ^{1}H-^{13}C edited NOESY,
2D ^{1}H-^{13}C HSQC for aliphatic

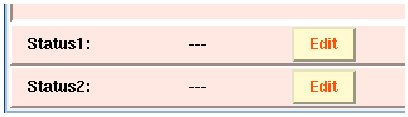
In this section, we will try to assign the signals of aliphatic side-chain if the backbone assignment information for their residues is not available.
The example will be focused on the assigments of Hγ and Hδ signals at Pro66 in the sequence Thr65-Pro66, providing that the assignments of all signals in Thr65 and Hα and Hβ Hα, all carbon signals of Pro66 have been finished.
To identify the Pro configuration, you have to check the status1 in the Acs module. The program will automatically predict the cis/trans configuration of Proline from the chemcal shift difference between Cβ and Cγ signals.



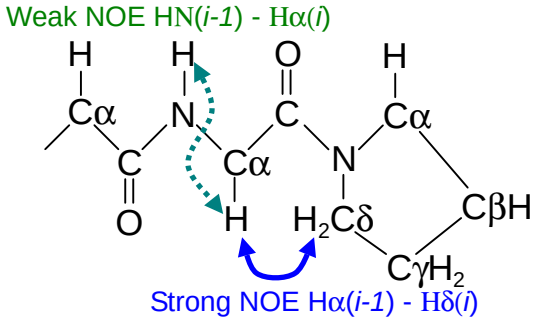
predicte as trans



predicted as cis

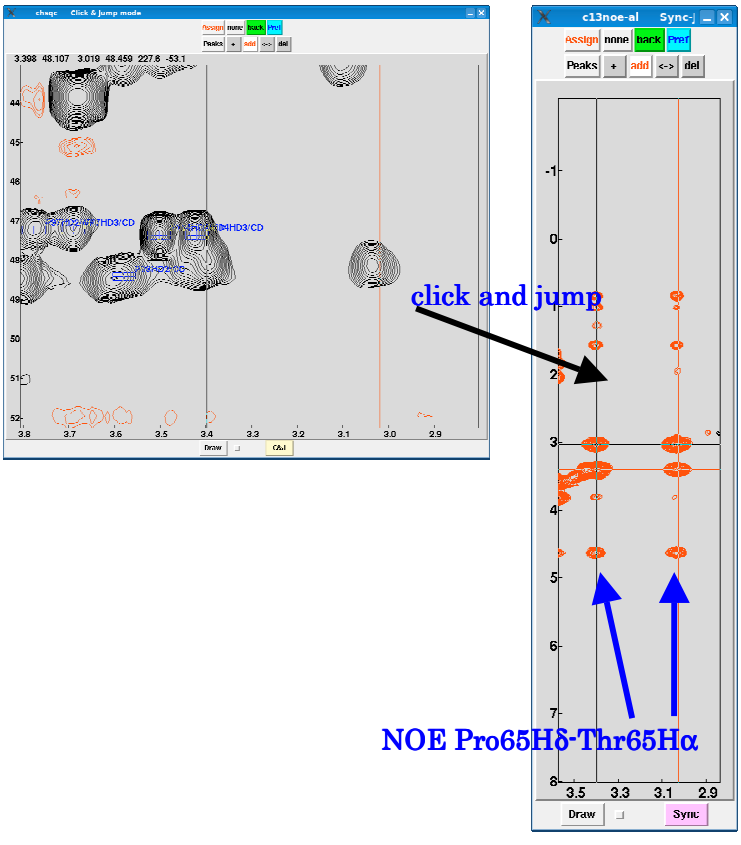


can't say cis/trans



The example of this section Pro66 is trans.

If the Pro isin trans configuration, you can observe strong NOEs between Hα(i-1) and Hδ(i) signals as shown in the left panel. (while Pro is in cis configuration, strong NOEs between Hα(i-1)- Hα(i) signals can be observed).



Now we are going to confirm the configuration and assign Hδ signals.

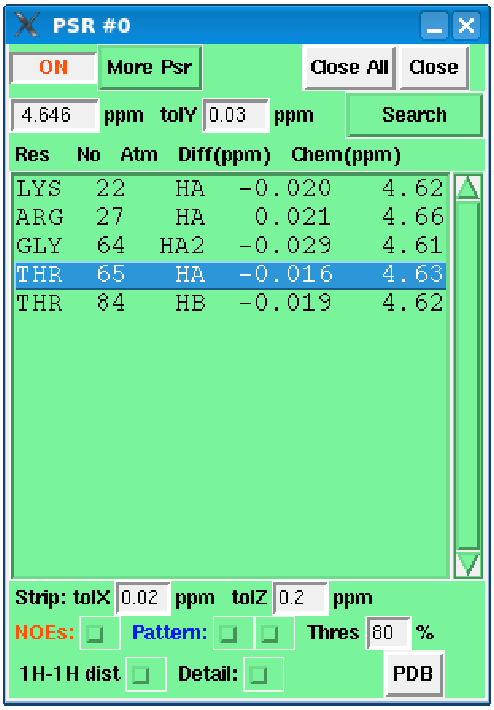
Firstly, press "C&J" button on the bottom of spectrum window of ^{1}H-^{13}C HSQC for aliphatic (chsqc) and expand the region for Hδ-Cδ signals.

Prepare 2D spectrum strip of 13C-edited NOESY, and set cross-hair cursor on one of the signals of Hδ-Cδ signals without assignment.

In the example (left panel) you can find strong NOE peaks around 4.6ppm.

Press "PSR" button on the main window to open PSR module.

Place y-axis of the cross-hair cursor just on the observed strong NOE and press muse-middle-button to enter the chemical shift value in the PSR module.

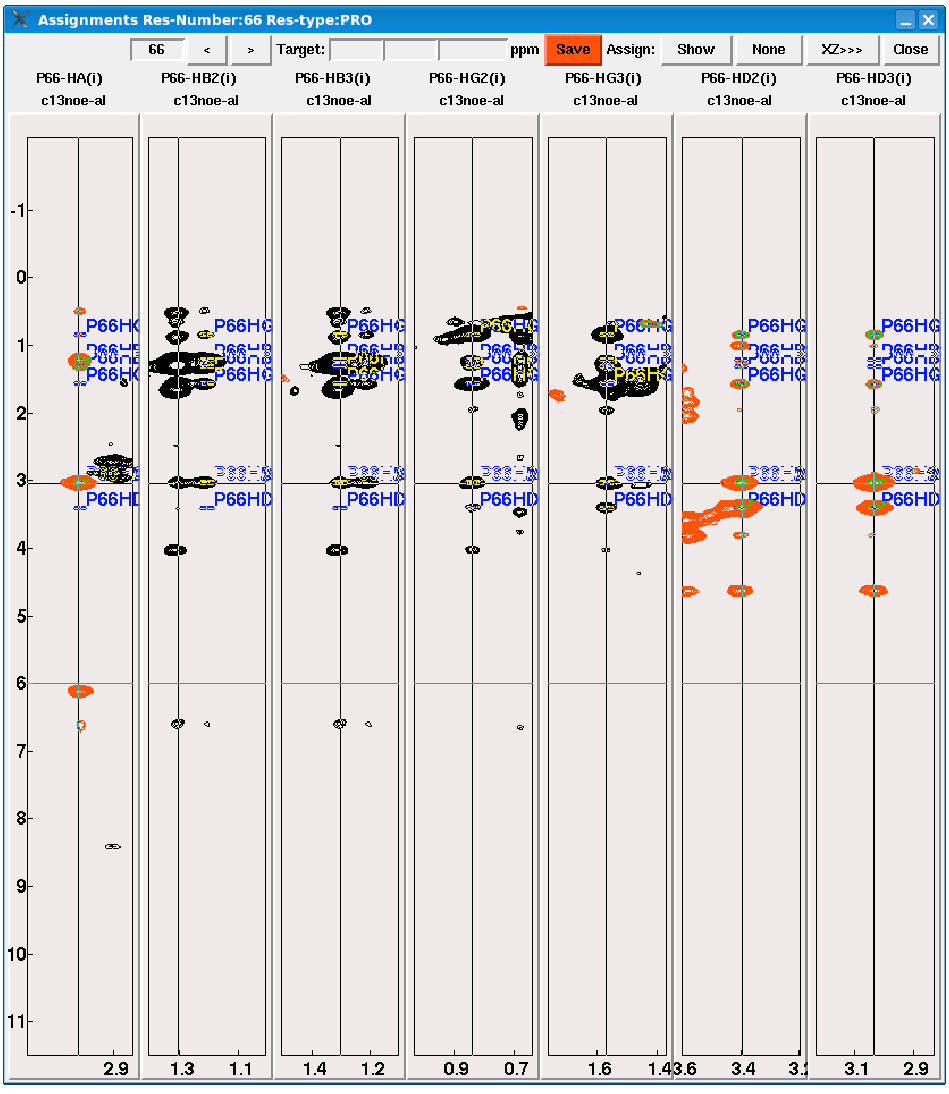


Press "Search" button to search proton chemical shifts in the assigned chemical shift table.

The left panel shows the search results.
The closest peak is Thr65-Hα, indicating the signals on chsqc are considered to be Hδ-Cδ signals in Pro66.

Now you can assign the signals are Hδ-Cδ on the chsqc, input the chemical shift values Hδ and Cδ signals in Acs module similar to the method explained in the previous section.

To confirm the assignment, you can popup spectrum strip array for intra-residual assignments by pressing "2D-strip" button on the Acs module (below panel). Using the spectrum array you can check assignments by inspecting peak patters are similar among the 2D strips displaying X-Z positions saved in assigned chemical shift table.



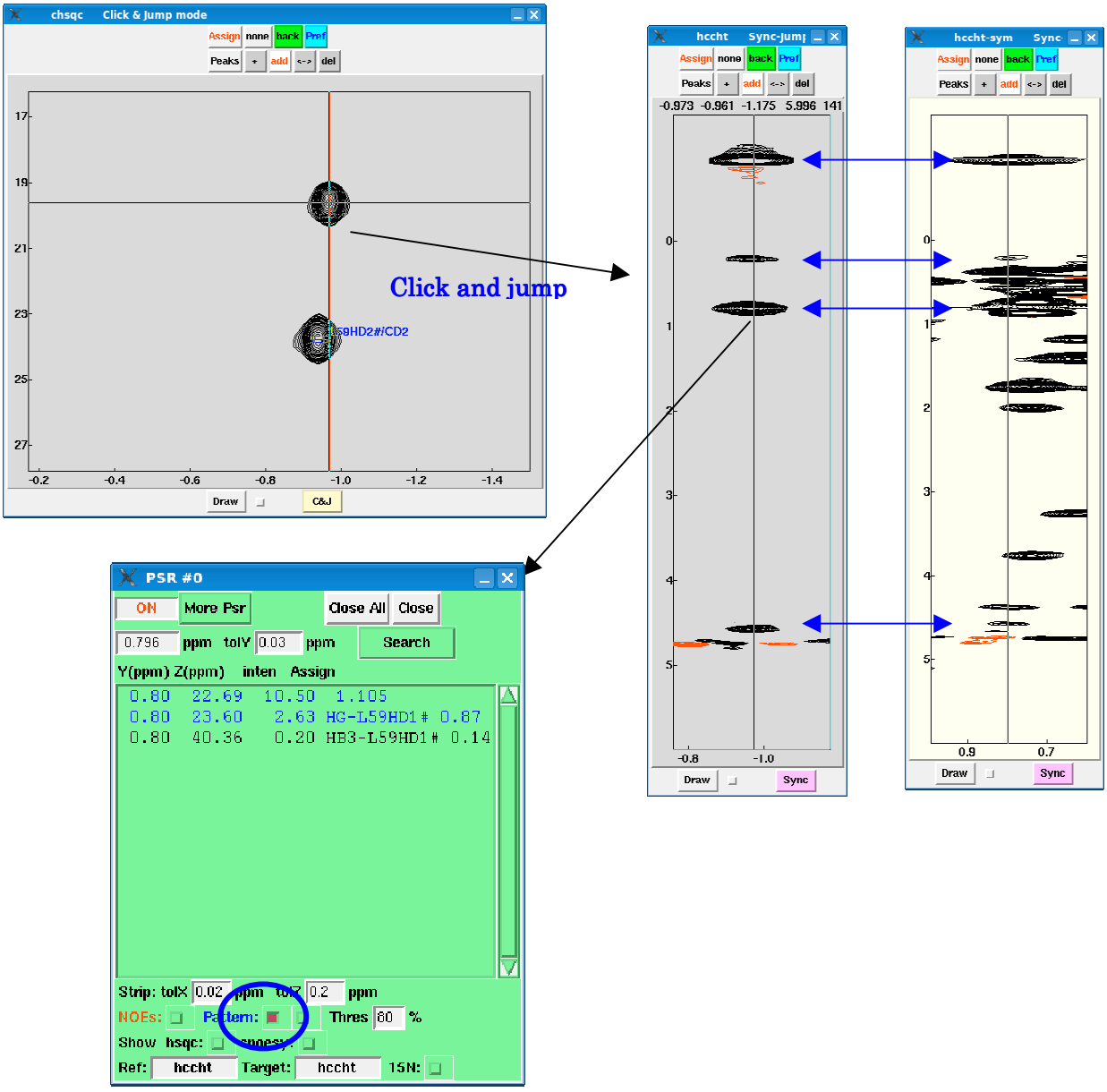
8) How to assign unassigned methyl signals

required spectra: HCCH-TOCSY for aliphatic, 2D ^{1}H-^{13}C HSQC for aliphatic

Firstly open the spectrum window of 2D 1H-13C-HSQC for aliphatic (chsqc), then click the right button in the bottom of spectrum window to switch "C&J" mode.

Then open 3D- HCCH-TOCSY for aliphatic (httht), press "Assign" button in the chsqc. Expand chsqc spectrum window to display methyl signal resuin and search for the signals without assignments.

The below left panel shows a signal without assignment in the methyl signal region, place the cross-hair on the signal and click the window. The C&J function makes hccht display corresponding spectrum region.



Then Open PSR module, press "Pattern" checkbox. Place cross-hair cursor on the larger signal in hccht (0.80ppm), press "Search" button. The program will find most probable diagonal peaks in hccht and list them in the PSR module.

Double-click one of the search results (22.69ppm) to display corresponding 2D spectrum strip in HCCH-TOCSY (right panel). If you find the diagonal peak showing similar peak pattern, the signal should be the side-chain signal of target residue. Try to search other representative signals in the 2D strips using PSR module to identify the residue number of the signal. You can complete the assignments of the methyl signal as described in the previous section.

3-2 How to assign aromatic signals

1) required spectra

Spectrum name	axis order	axis label
2D ^{13}C-HSQC for aromatic	^{1}H(acq)-^{13}C	HC-C
3D ^{1}H-^{13}C HSQC-NOESY for aliphatic	^{1}H(acq)-^{1}H-^{13}C	HC-H-C
3D ^{1}H-^{13}C HSQC-NOESY for aromatic	^{1}H(acq)-^{1}H-^{13}C	HC-H-C

If your ^{13}C edited NOESY measured for all carbon regions (aliphatic and aromatic), you have to specify the spectrum as both 13C-NOESY for aliphatic and aromatic in the startup module.

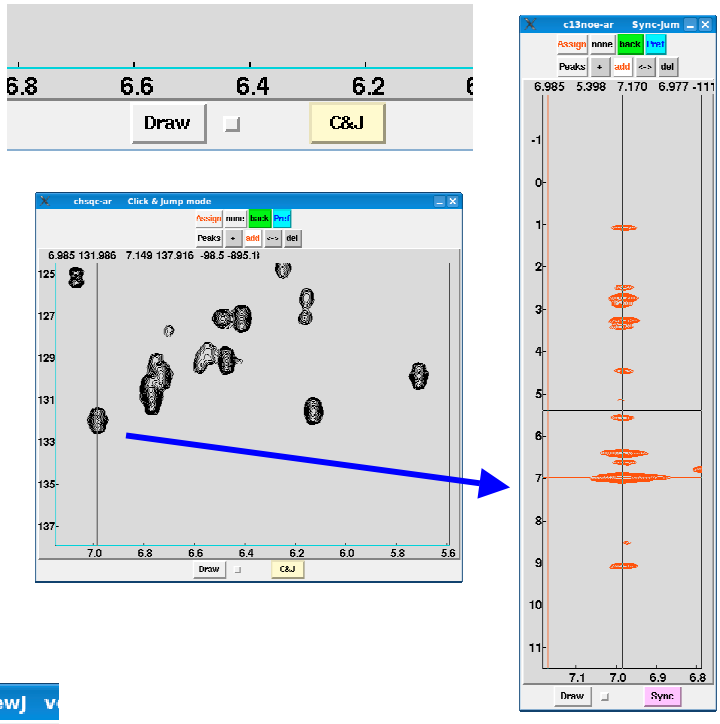
2) assignments of aromatic signals using PSR module

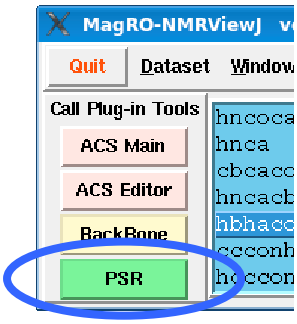
The most important step for the assignments of aromatic signals is finding NOEs between Hβ-H atoms. Prior to this job, the assignments of Hβ-Cβ signals should have been finished.

Firstly, press "Sync" button on the bottom of spectrum window of 2D 1H-13C HSQC for aromatic (chsqc-ar). The button will change to C&J (Click and Jump mode).

Then open the 2D spectrum strp of 13C-edited NOESY for aromatic(c13noe-ar) and expand the region of Hδ-Cδ signal region.

In the chsqc-ar window, then place the cross-hair curser just on the signal that you want to analyze the click mouse-left button. The 2D spectrum strip of c13noe-ar will display corresponding X-Z position as shown in the right panel.

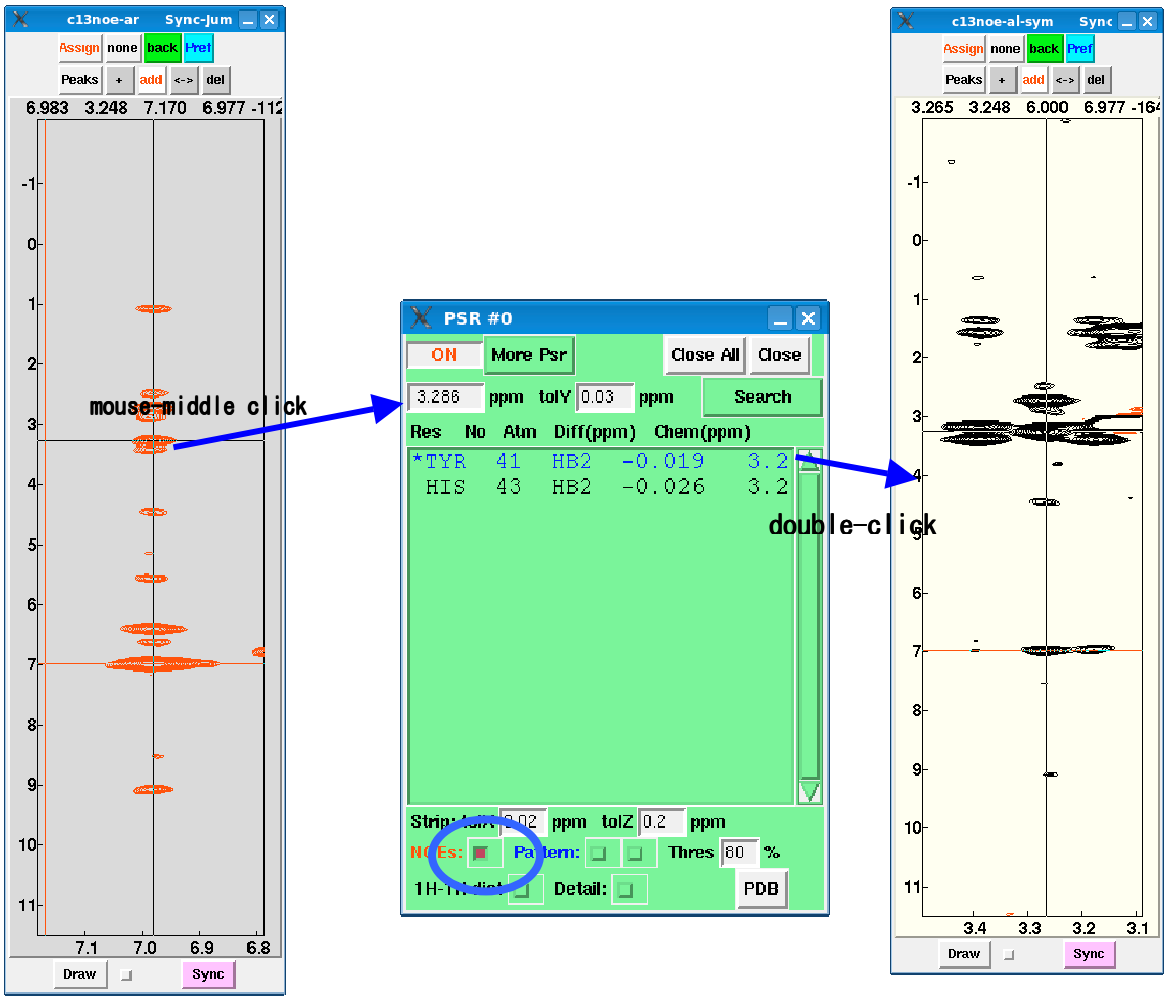




Next, press "PSR" button on the main module to open PSR module.

Click the checkbox "NOEs" (blue eclipse in the below panel) in the PSR module to switch the module to NOE search mode.

The below left panel shows the 2D spectrum strip of 3D ^{13}C-edite NOESY for aromatic (c13noe-ar)displaying the NOEs from target Hδ-Cδ signal. Place cross-hair cursor on one of the strong NOE which might be from Hβ signal and press mouse-middle button to enter the chemcial shift value in the entry of PSR module.



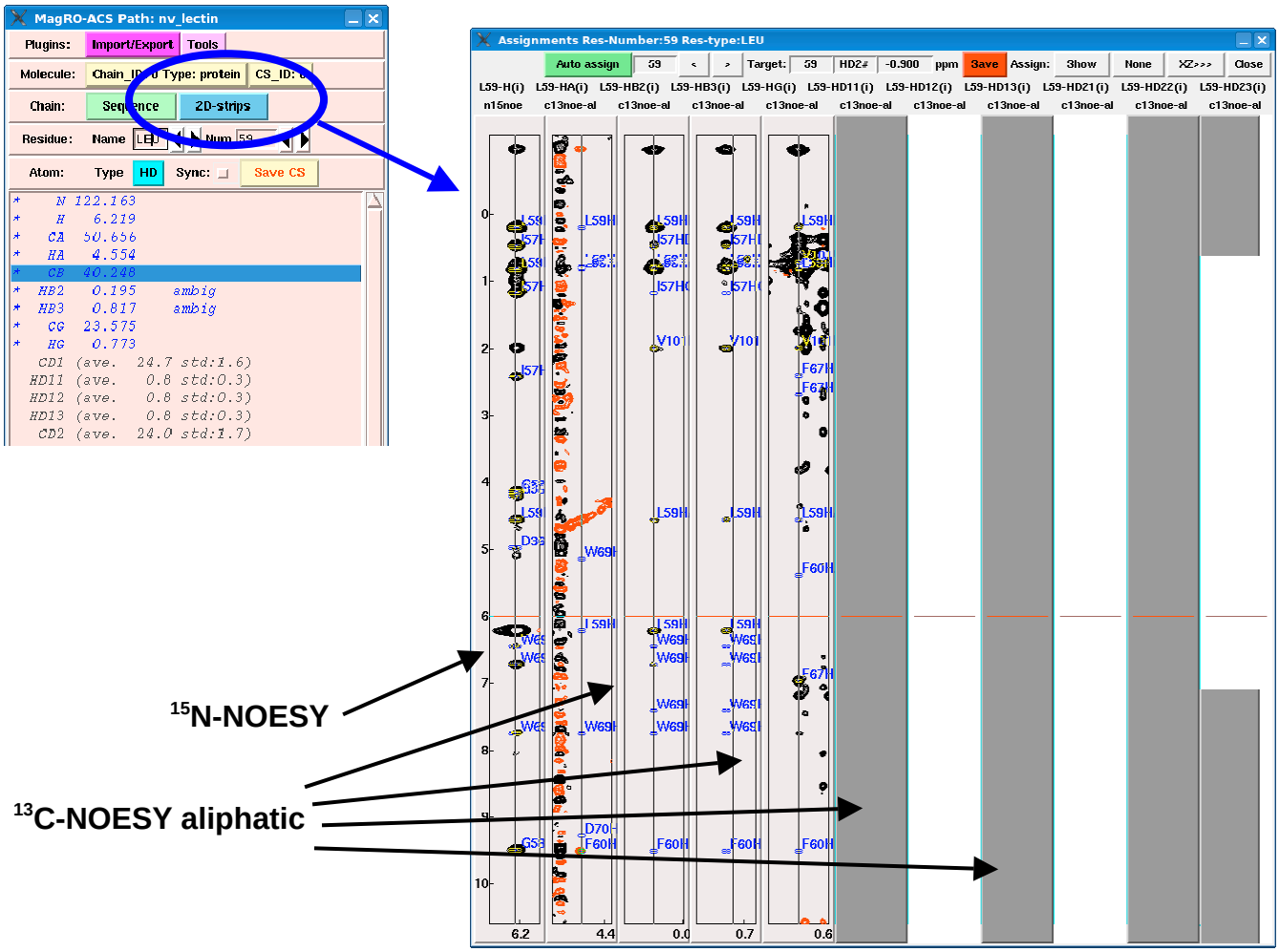
If the checkbox is enabled "NOEs", the program will check the intensity of ^{13}C-edited NOESY on the trnased position. The above example showing the NOEs observed on Tyr41-Hδ-Cδ. The search results on the PSR module are showing the closest proton atoms found in assigned chemical shift table, Tyr41-Hβ2 and His43-Hβ2. On the transposed position of the NOE peak in the left panel, namely the position of 3.286ppm in y-axis on the X-Z position of Tyr-Hβ-Cβ (colored by blue), the signal intensity is detected greater than 80% of the threshold value set for the 13C-edited NOESY.

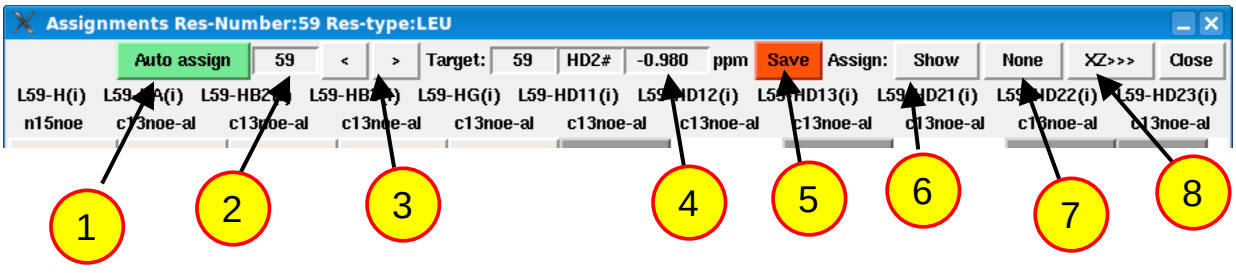
You can confirm the NOE peak pattern in the 2D-spectrum of transposed position by double-clicking the search results listed in PSR module. The 2D-spectrum strip corresponding to the transposed position will appear (right panel).
Using this function, you can assign the aromatic side-chain signals.

3-3 Confirmation of assigned chemical shifts using a function to display 2D spectrum array

Required spectra: ^{15}N-edited NOESY, ^{13}C-edited NOESY

If you press "2D-stripes" button on the Acs module, the window as shown in the right lower panel will popup. In the window, the spectrum 2D regions of X-Z positions corresponding assigned chemical shifts of atoms in selected residue such as HN-^{15}N, Hα-Cα and so on.



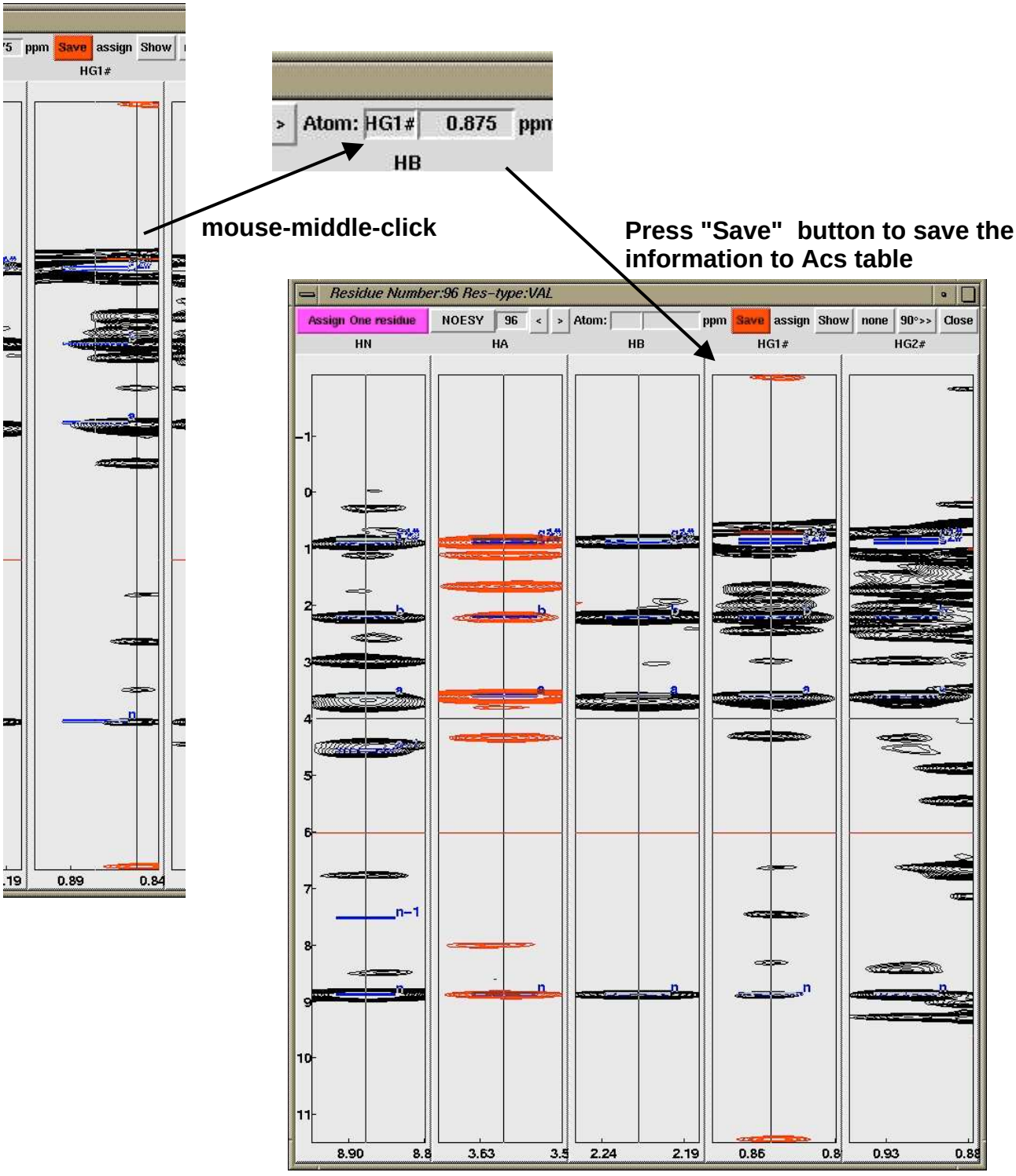


Detail of the header of "2D-sterip array window"

1: Execute automated side-chain signal assignment for the current residue
2: Current residue number, **3:** Inc- and Dec-number of residue ID
4: Chemical shift value **5:** "Save" button to save the edited chemical shifts in Acs table
6: "Show" button to show blue boxes in 2D-strip array
7: "none" button to erase blue boxes in 2D-strip array
8: 90 deg-flip button to flip X-Z dimension of 2D-strips.

Using the 2D-spectrum strip array, you can confirm the assigned chemical shifts with NOE peak patters in ^{15}N- and ^{13}C-edited NOESY. It is easy to find wrongly assigned chemical shift if the NOE peak pattern of one of the 2D strip which is largely different from those observed in the other strips.

If you would like to correct the chemical shifts, place the x-axis of the cross-hair cursor just on the signals that you consider correct position, and click mouse-middle-button. The detected chemical shift value will be displayed in the entry of header of the 2D-strip array window. Then you press "Save" button to save the information in Acs table.

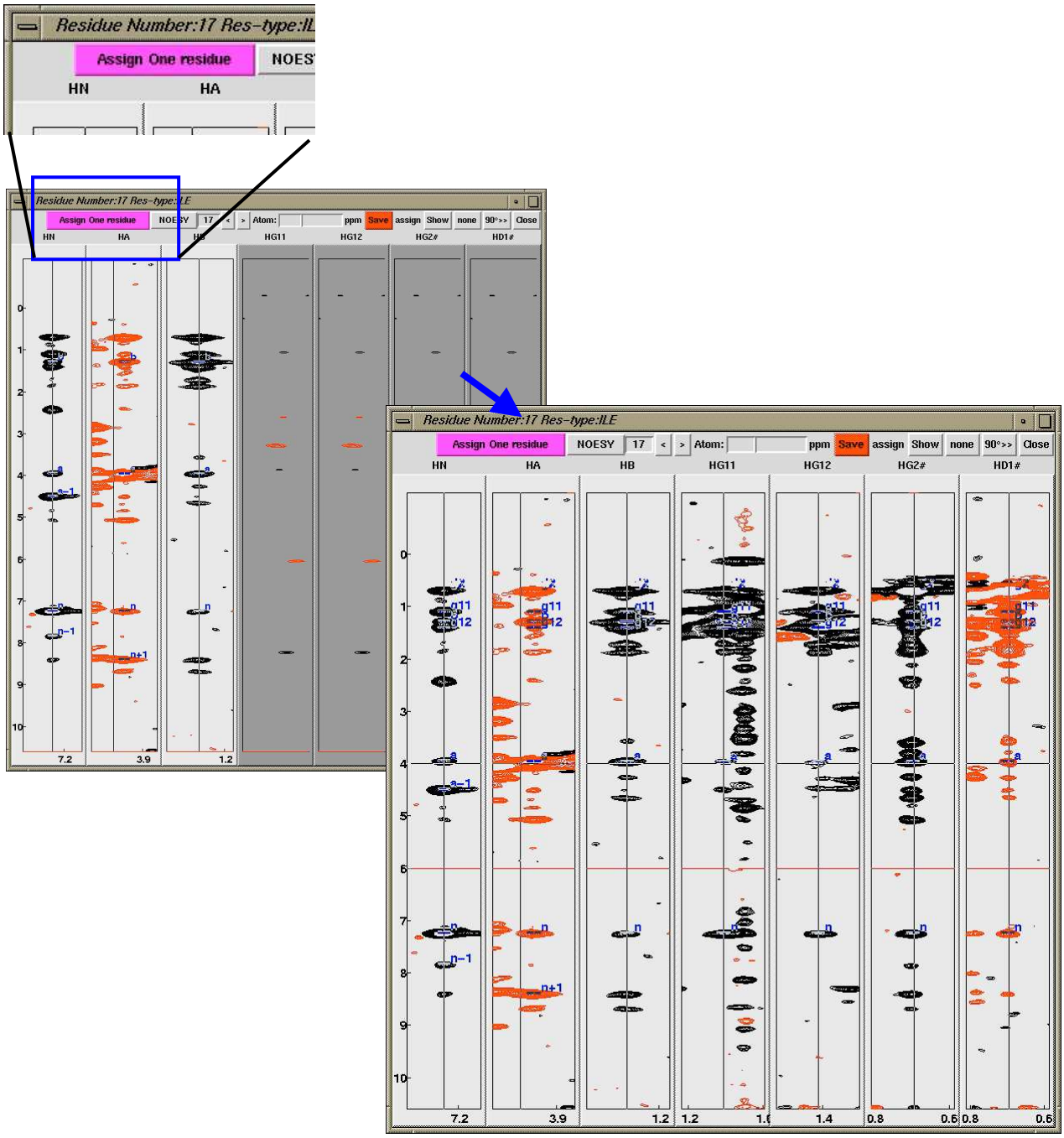


3-4 Fully automated assignment of side-chain aliphatic and aromatic signals

1) for aliphatic and aromatic side-chain signals of selected residue

Requirements: ^{13}C-edited NOESY, HCCH-TOCSY for aliphatic and HCCH-COSY for aromatic
Assigned chemical shifts for Hα-Cα and Hβ-Cβ

Before run the automatic assignments, set the threshold of spectrum windows properly.
Open the 2D-spectrum strip array window and press "Auto assign" button on the header of window. The assignments will take a few seconds. The accuracy and completeness of the automated assignments are around 80~90% and 70~80%, respectively, which are strongly depending on the quality of spectra.



2) for all residues

Please make sure that you have prepare spectrum windows as followings:

Requirements for aliphatic signals:

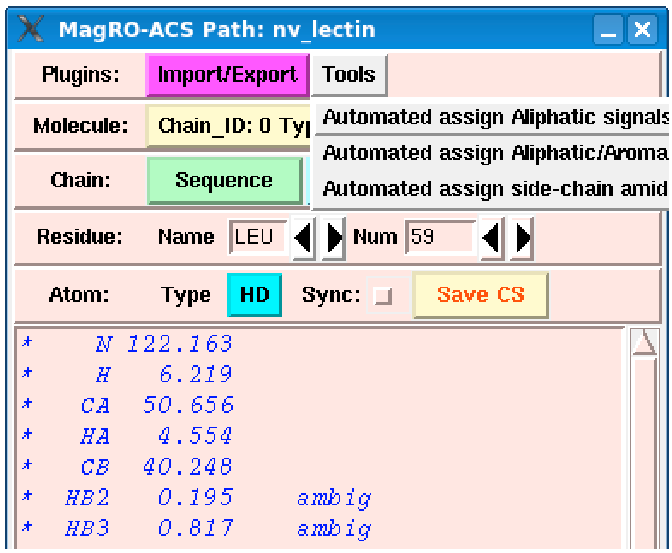
Assigned chemical shifts for Hα-Cα, Hβ-Cβ in Acs module are completed
3D ^{1}H-^{13}C NOESY for aliphatic
3D-HCCH-TOCSY for aliphatic

Requirements for aromatic signals:

Assigned chemical shifts for Hα-Cα, Hβ-Cβ in Acs module are completed
3D ^{1}H-^{13}C NOESY for aromatic (or covering all regions)
3D-HCCH-COSY for aromatic

Before run the automatic assignments, set the threshold of spectrum windows properly.
press "Tools" button on the header of Acs module to open the pull-down menu, then select "Automated assign *". The automated assignment program will begin and assign the side-chain signals one-by-one. The average calculation time should be around 2~3 mins for 100 residue long protein. The accuracy and completeness of the calculation are similar to the function mentioned previous page.

[Important] While running the calculation, you can not manipulate MagRO.



11. CYANA setting module

1) Files for CYANA calculation

The module for creating CYANA calculation input files is now available from the magenta module (ACS). The settings are mostly automated, user will be released from the annoying works such as conversion of file format, checking version dependency, and so on.

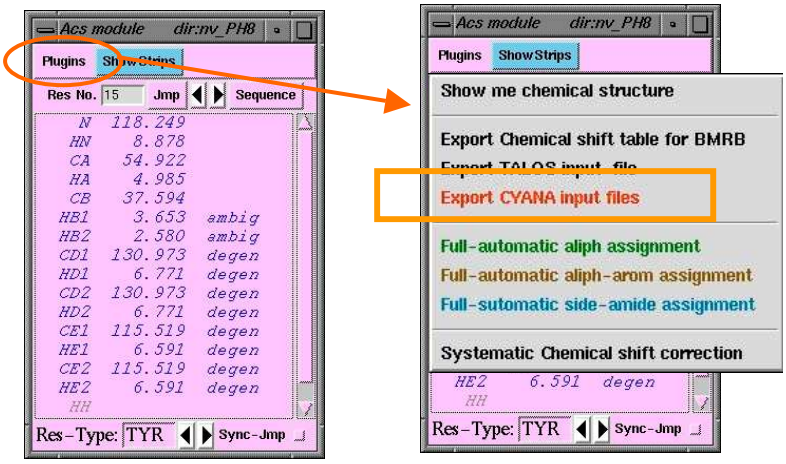
<Requirements>
At least one NOE peak table, 3D-NOESY and ACS directory

<out-put files>

CALC.cya, int.cya	defines CYANA calculation parameters such as constrain file names, violation tolerances, library names, stereo chemistry settings, etc.
*.seq	sequence file. Can be specified cis-Pro, Cystine and t-His **(library should support them).**
*.prot	chemical shift table (now support only one *.prot file).
talos.aco	phi, psi angle constraints derived from TALOS prediction **(pred.tab required)**
*.peaks	supports ^{1}H-^{1}H-^{15}N and ^{1}H-^{1}H-^{13}C xpk files.

2) Quick start CYANA input maker

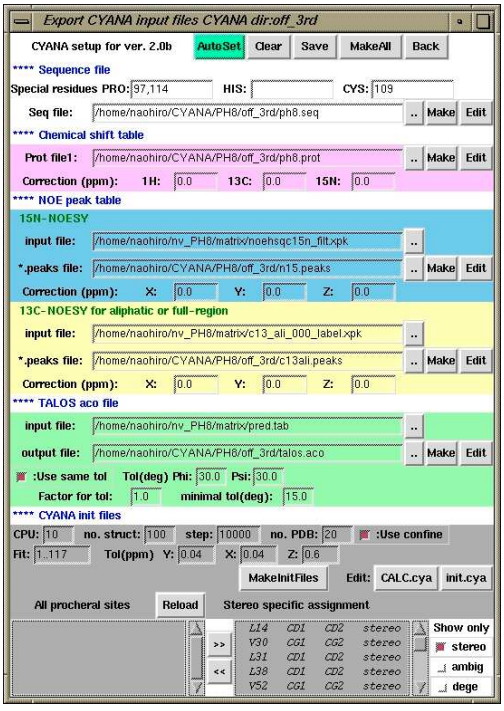
If you click the button "Plugins" on the left-top of the magenta module, you will see a pull-down menus;,



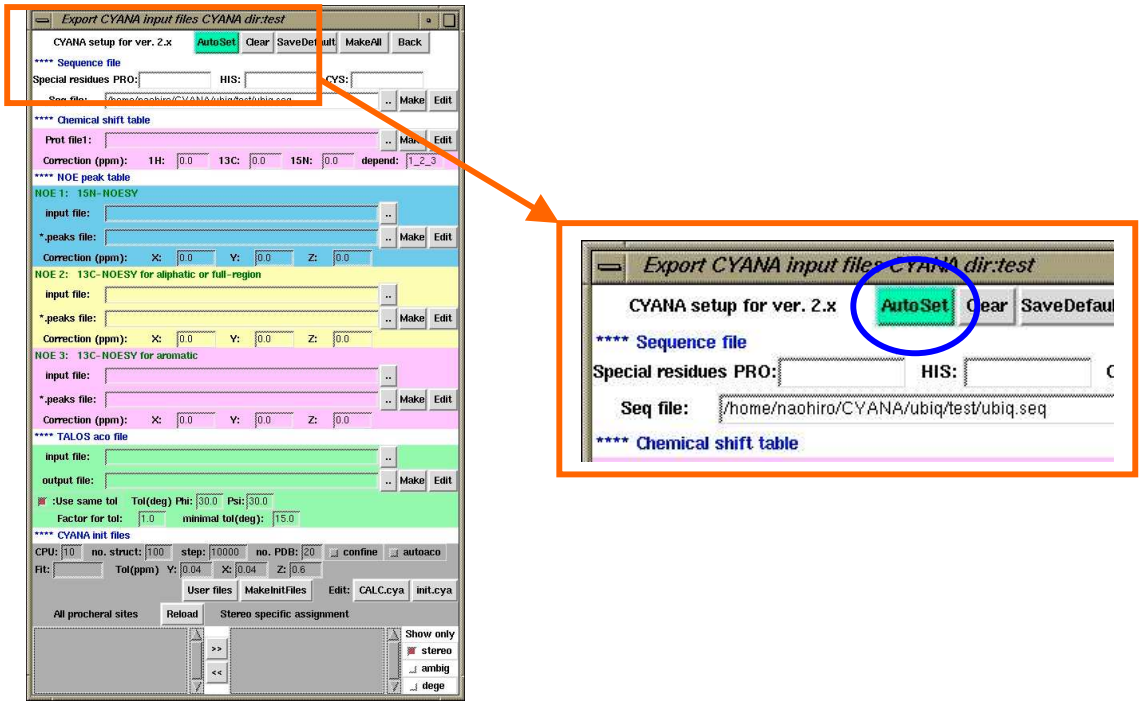
Select "Export CYANA input files" among the menus to open startup window;

Type the sample name and the path name for CYANA calculation and select version of CYANA as shown above panel.

Then press "Next" button to open the main-setting window



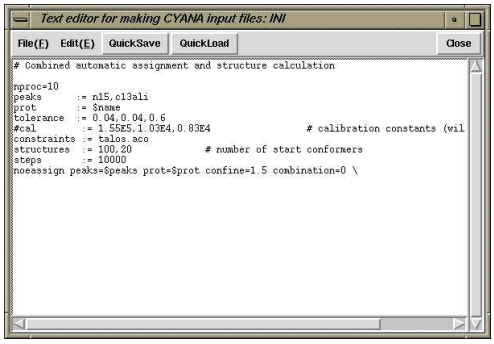
The initial state of the setting entries are empty. **If you don't know whato to do**, please try to press "AutoSet" button, the automated setting program will fill the entries.



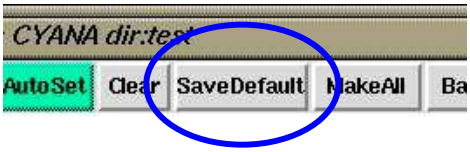
If the settings seem to be OK, then press "MakeAll" button (blue circle) to make all input_files.

You can also make each input_file by pressing "Make" button (green circle) on each file setting section.

And if you would like to edit the created file, click "Edit" button to open the text file editor



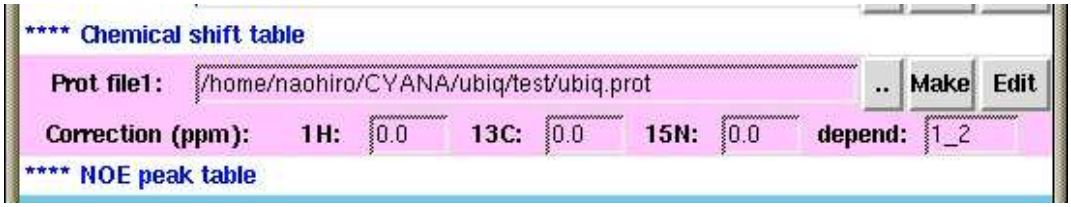
By pressing the "SaveDefault" button, you can save the current setting as default for the next calculations ,



3) Details of the input file sections

A) Chemical shift table:

For the chemical shift table file, *.prot, the GUI stuffs are;



The chemical shift values are loaded from the ACS files, matrix/ACS/acs.*. The ^{15}N and ^{13}C chemical shift values for the signals on the folded region of 3D-NOESY spectrum are calculated using the NOESY spectrum parameters if the spectrum dependencies are defined in the entry "depend:". The example shown above indicates, ubiq.prot file <--> NOE:1 and NOE:2.

B) NOE peak table:

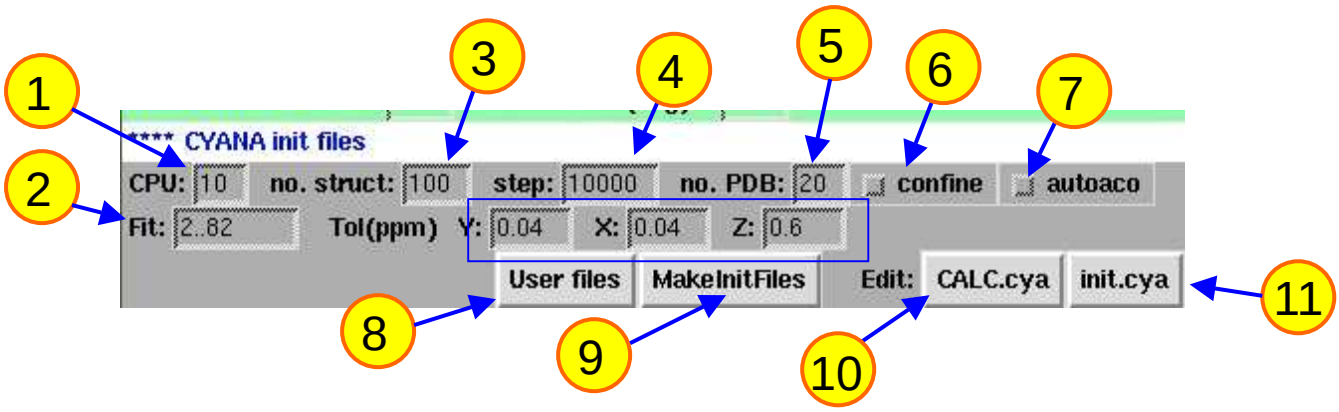
For the NOE peak table file, *.prot, the GUI stuffs are;

The most recently edited xpk file is automatically found and typed in the "input file" entry. User has to define the name and path of the output-file in the "*.peaks file" entry Currently user can specify three NOE peaks files, ^{1}H-^{1}H-^{15}N NOESY, ^{1}H-^{1}H-^{13}C NOESY for aliphatic and aromatic regions.

C) CALC.cya and init.cya files:

The GUI stuffs for making CALC.cya and init.cya files,are found on the bttom gray zone of the module as shown below.

For the settings for calculation;



1: number of CPU for the CYANA calculations. [default 10]

2: residue numbers for fitting calculated structures. [default begin-residue-1 - end-residue]

3: number of structures for calculation [default 100]

4: number of steps for calculation [default 10000]

5: number of selected structures [default 20]

6: confine mode distance constrain [default disable]

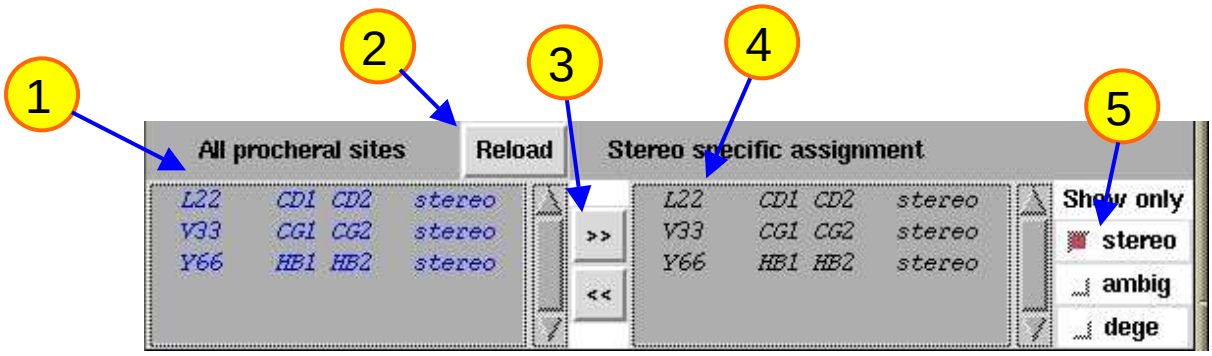
7: automated aco file making [default disable]

8: call child module for setting user defined files for CYANA calculations

9: button to make CALC.cya and init.cya files

10 & 11: buttons to call for text-editor windows

And for the stereo chemistry settings;



1: left listbox to show the procheral atoms found in the chemical shift table, ACS. The atoms colored by blue indicate the stereo-checmistry is defined to include CYANA calculations

2: reload the stereo-chemical atoms from ACS

3: add and delete the elements listed in the right-side listbox

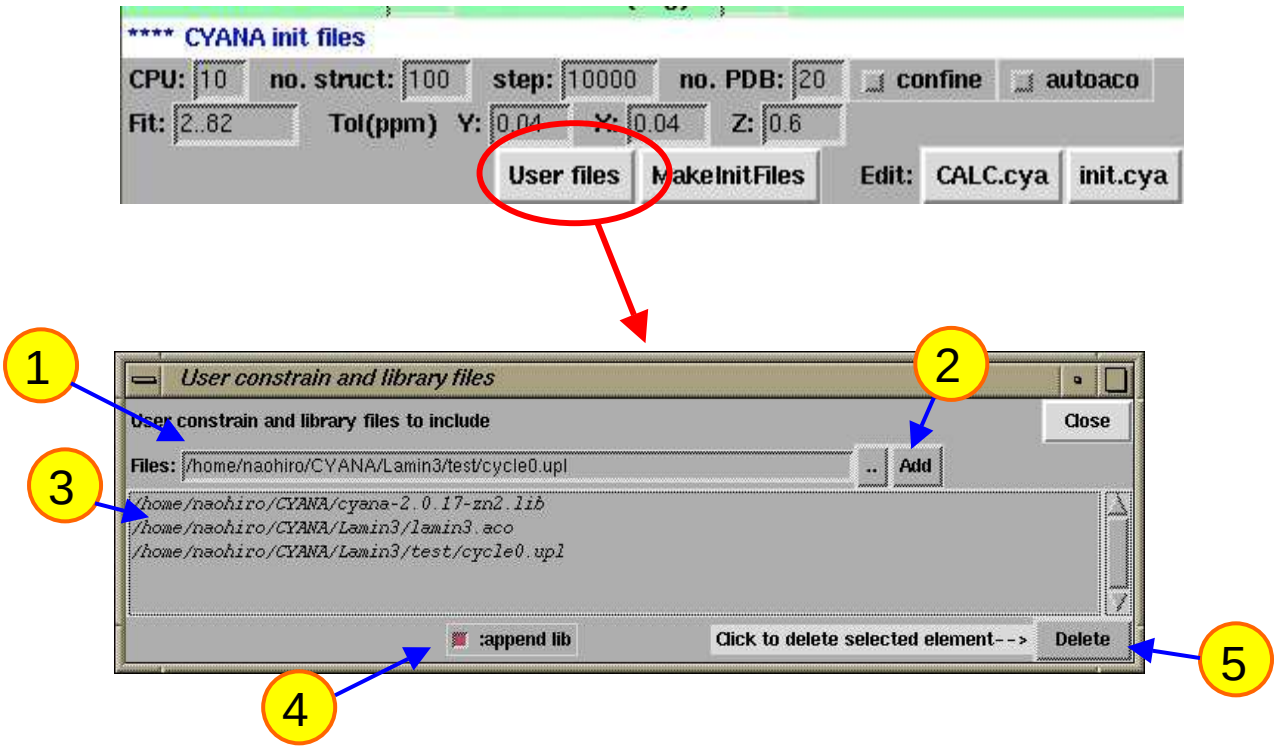
4: right listbox to define the stereo-specific assigned atoms used for CYANA calculations

5: If you select "stereo", only stereo-specifically assigned atoms are loaded when you reload ACS. If you select "ambig", the atoms whose stereo-chemistry is ambiguous are loaded. If you select "dege", all procheral atoms are loaded.

4) If you want to use custom files that you have made such as *.upl, *.lol, *.aco and *.lib files.....

User is allowed to specify the files for additional constrain files and library files. If you click the button on the section for setting CYANA input files (red circle), you will see a child window. You can define the file name and path in the listbox for the following files;

*.upl	file for upper limit distance constraints
*.lol	file for lower limit distance constraints
*.aco	file for dihedral angle constraints
*.lib	library file for atom coordinate



.

1: entry to define the path name of the target file
2: add button used to add the defined path name to below listbox
3: listbox showing files going to be used for CYANA calculation
4: append-lib checkbox. If it is checked, the listed library file will be appended to the standard library file for CYANA calculation.
5: delete button to delete selected item in the listbox

If you click to execute "MakeInitFiles" button or "MakeAll" button, the CALC.cya and int.cya files are created with these informations.

12. Setup CYANA result analysis module

1) Setup of module

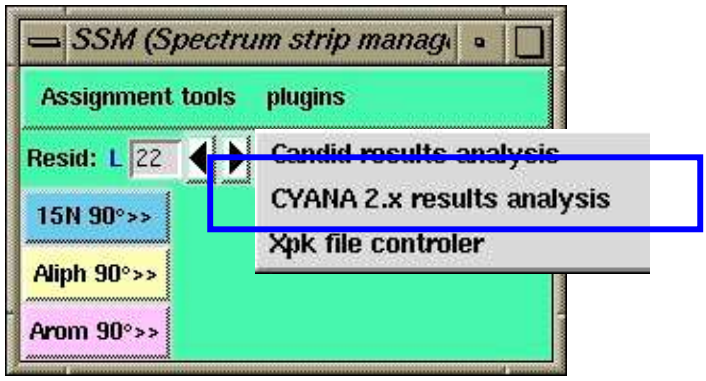
The "CYANA result analysis module" finally supports the official version of CYANA, 2.x.

<Requirements>

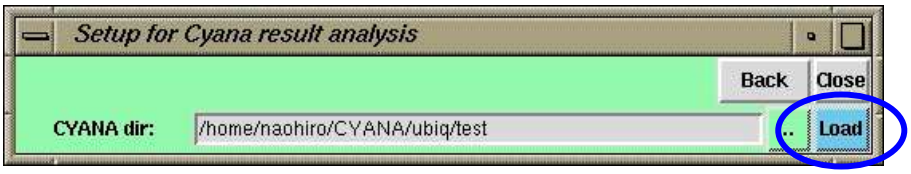
CYANA input files and output files;
CALC.cya and **init.cya** files
cycle*.noa and **cycle*.upl** files
Spectrum data files, *.nv, for 3D ^{1}H-^{1}H-^{15}N NOESY, 3D ^{1}H-^{1}H-^{13}C NOESY

An example of the CYANA result analysis shown in this page has been performed with the following data sets;

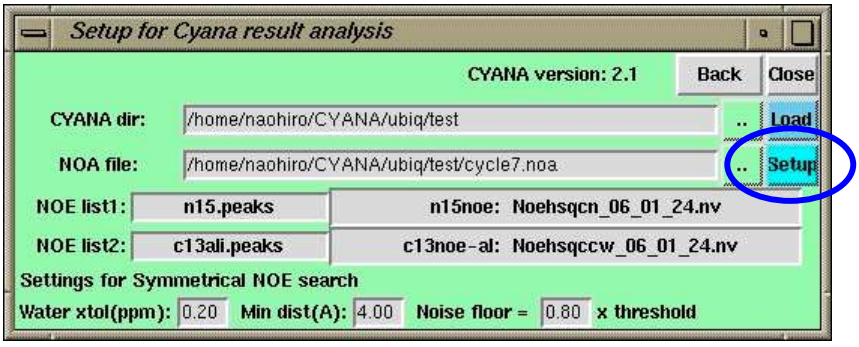
Sample:	ubiquitin
NOESY:	3D ^{15}N edited NOESY, 3D ^{13}C edited NOESY for aliphatic and aromatic
NOE peak table:	n15.peaks and c13noe.peaks obtained from the NOESY spectra
Chemical shift table:	ubiq.prot, refined with the NOESY spectra
CYANA:	official release ver 2.1
target NOA:	final.noa



To start the setting up the CYANA result analysis, click the "plugins" button on the green module



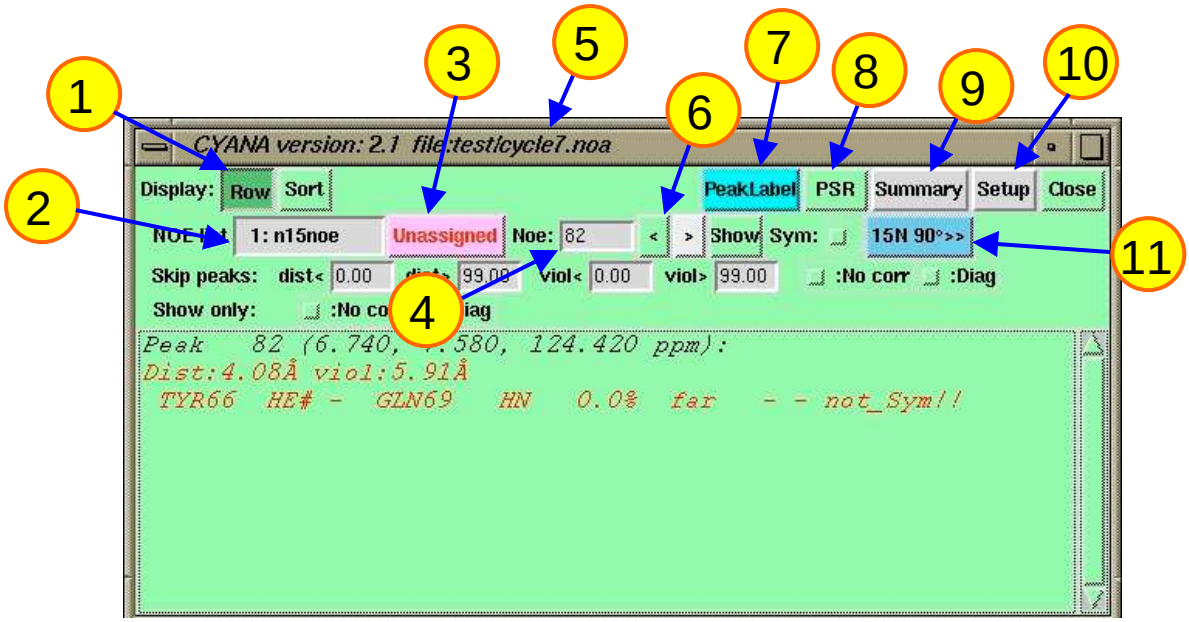
(SSM) to open the pull-down menus.



Select "CYANA 2.x results analysis". Then you will see an initial setup window
Type the path name of the CYANA calculations, then press "Load" button to load the information.
The window will change to show additional GUI items;
Press "Setup" to finish the setting up jobs.

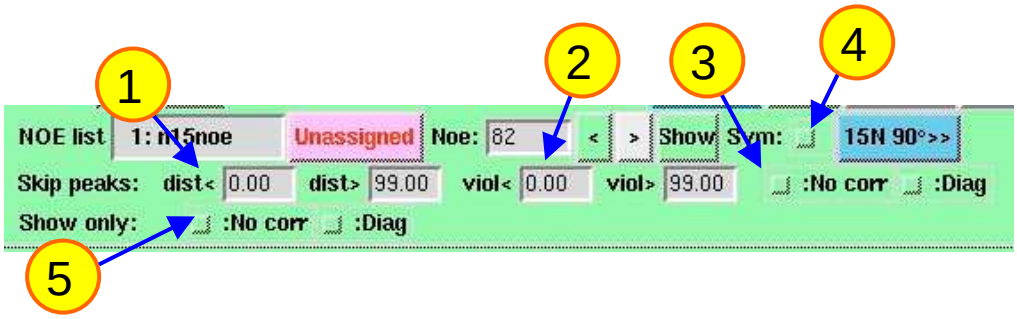
2) Functions of the main window (Row mode)

Representing the NOE assignment results carried out by CYANA for each NOE peaks.



1: Button to switch the window between "Row" and "Sort" modes.
2: Pulldown button to switch NOE peak lists
3: Button to switch between "Assigned" and "Unassigned" NOE peak analysis modes
4: Entry to set NOE peak ID
5: Window header displays version of CYANA and the current path name of *.noa file
6: Increment and decrement buttons for NOE PeakID number
7: Button to open setting window for peak labeling
8: Button to open PSR module
9: Button to open Summary window
10: Button to open main-setting up window.
11: Button to execute 90deg flip of the NOESY spectrum strip

And options to select PeakIDs are;

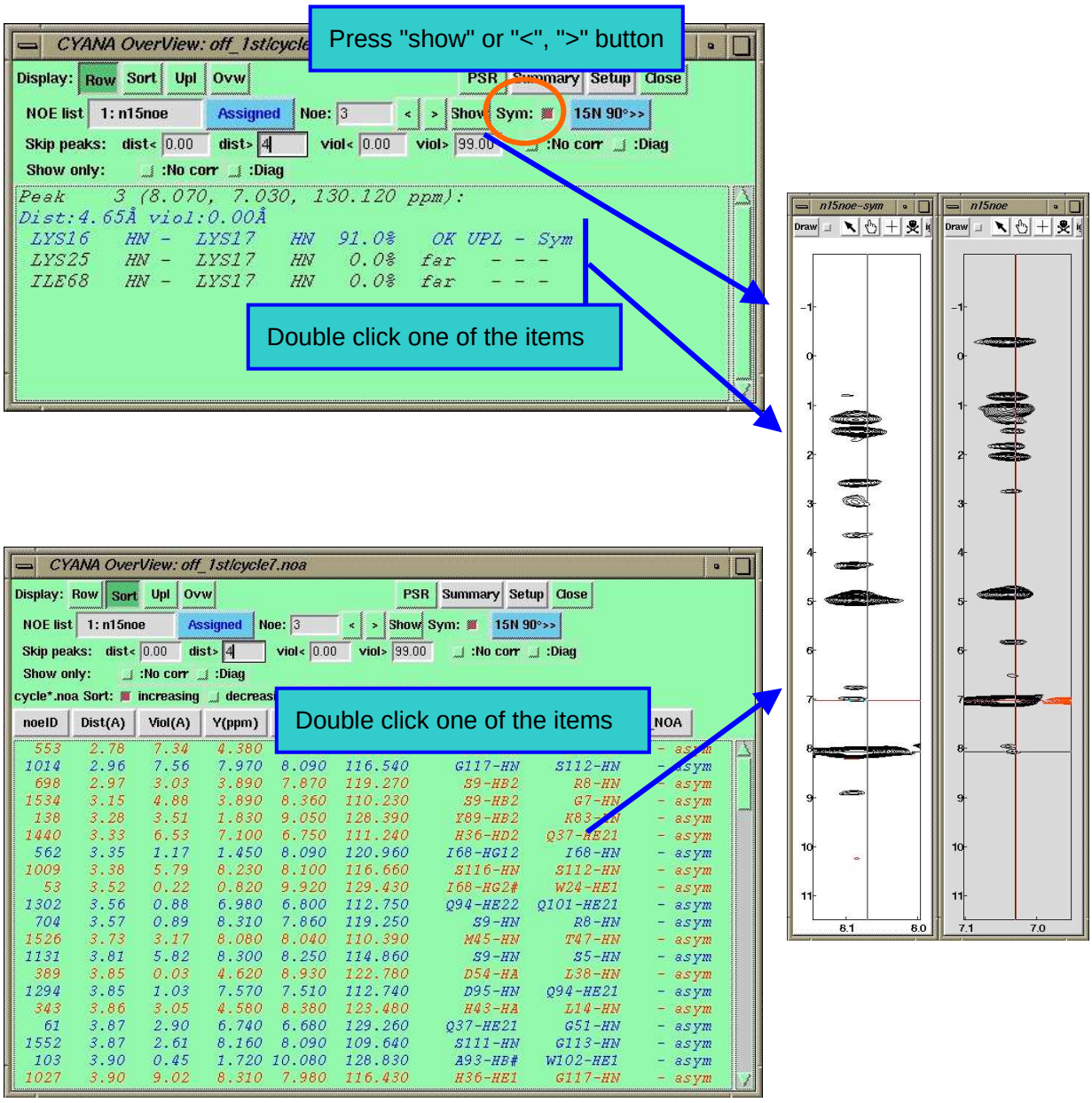


1: options to skip NOE peaks within the defined distance range.
2: options to skip NOE peaks within the defined violation range
3: option to skip peaks of "No corresponding signal" and diagonal peaks
4: switch to enable the **"twin strips display"** to check NOE assignment symmetry
5: option to exclusively show "No corresponding signal" and diagonal peaks

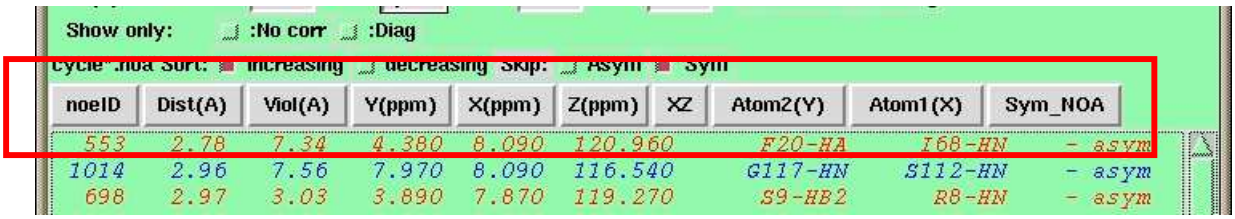
3) The Sync-Jump from CYANA analysis module

The module can provide "Sync-Jump" command to control NOESY spectrum strip corresponding to the selected NOE peakID. By pressing "Show" button, the target 3D-NOESY spectrum get jumped to the peak positions "<" or ">" button increments and decrements peakID number as well as jumps the target NOESY spectrum. If checked the option "Sym" (red circle), the module turns to "twin strips display" mode. The "Sync-Jump" command displays the spectrum strip corresponding to the transposed peak position of the NOE assignment. The function is useful to confirm the **symmetry** of the NOE assignment achieved by CYANA.

Symmetry means: if you see one NOE peak between the atoms Trp43-Hb and Leu-Hg, and you are displaying at 2D strip for the position of Leu-Hg/Cg. Now you are looking at the NOE pathway Trp43-Hb<-> Leu-Hg/Cg . To confirm the symmetry of the NOE pathway, you would like to display 2D strip for Trp43-Hb/Cb and whether the NOE peak exists on the 2D strip. The function in the module can detect the spectrum intensity on the symmetrical position of assigned NOE and can display the corresponding 2D spectrum strip.



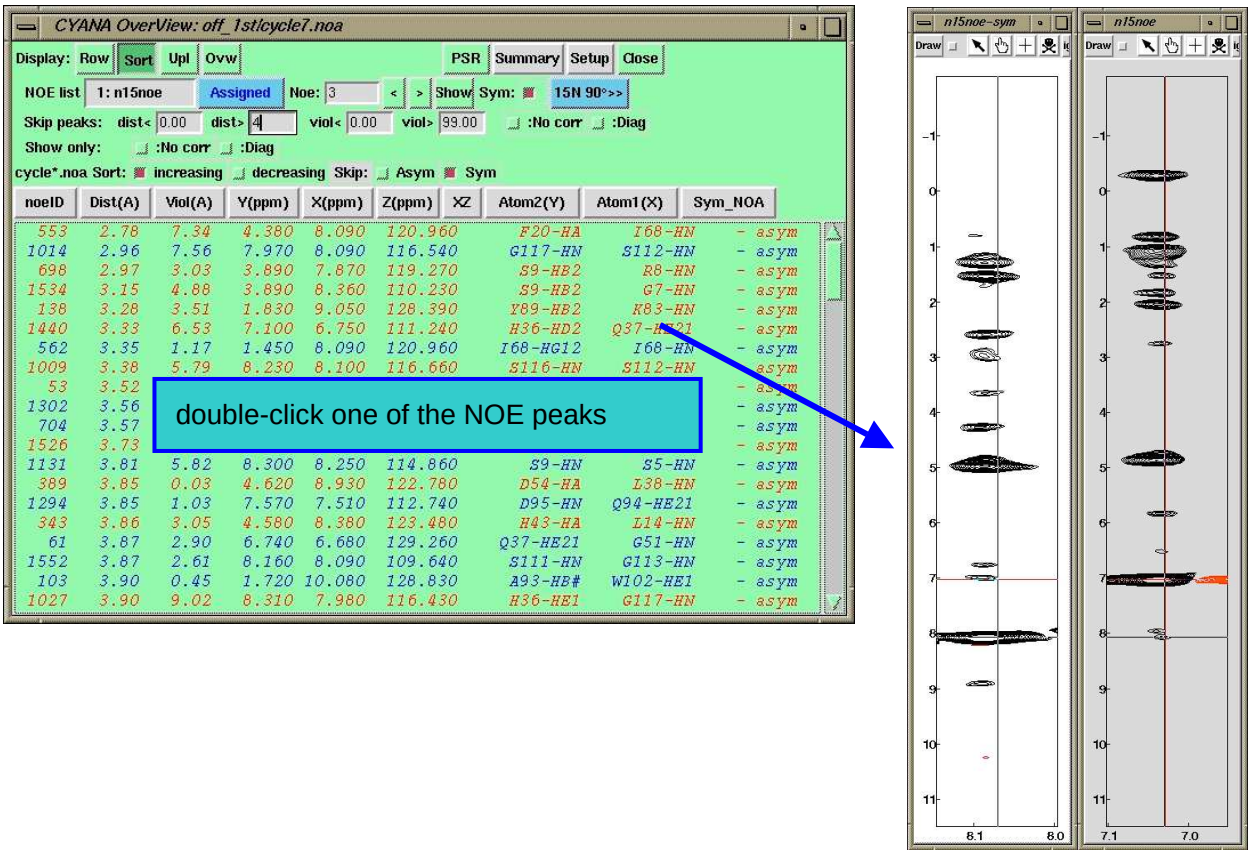
4) Functions of the main window (sort mode)



By pressing "Sort" button on the header of main window, you can switch to "Sort mode" from "Row mode". In this mode, all of the NOE peaks are listed in the window for the selected peak lists. The most probable assignment for each NOE peak will be shown. Using the buttons shown with red rectangle, you can sort the NOE peaks in the listbox.

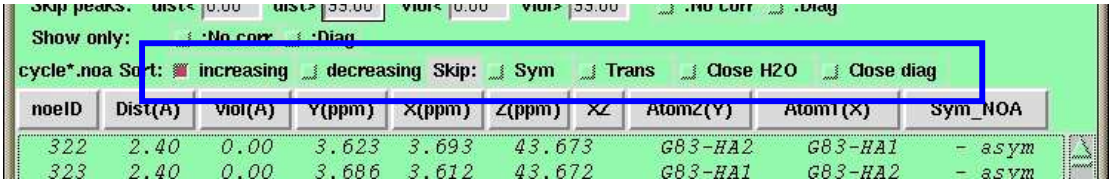
noeID	NOE peak ID
Dist(A)	estimated 1H-1H distance (Å)
Voil(A)	distance violation (Å)
Y(ppm)	Y-axis chemical shift (ppm)
X(ppm)	X-axis chemical shift (ppm)
Z(ppm)	YZaxis chemical shift (ppm)
XZ	Sort for XZ-plane
Atom2(Y)	Atom assigned with Y-axis chemical shift. Sorted alphabetical
Atom1(X)	Atom assigned with X-axis chemical shift. Sorted alphabetical
Sym_NOA	If the NOE symmetrically assigned or not

In this mode, if you double-click one of the NOE peak in the listbox, 2D spectrum strip for the corresponding NOESY spectrum displaying XZ-region corresponding to the XY-chemical shifts of selected NOE peak. Similar to the Row mode, if the "sym" checkbutton is enabled, 2D spectrum strip corresponding symmetrical position will appear.



5) The most powerful function of the module: Sort&Skip searching for problematic NOEs

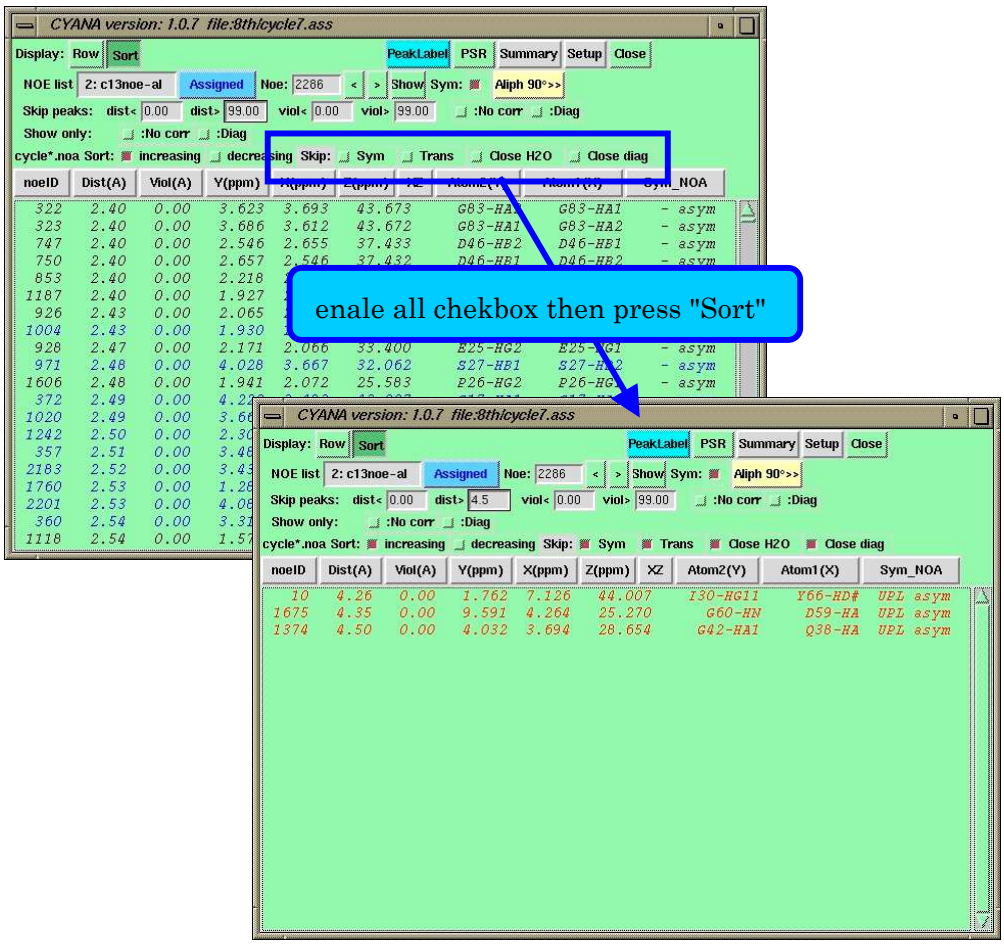
The function that mentioned in this chapter must be powerful in the final refinement stage of NMR structure determination. The most of the NOE peaks assigned by CYANA will be used as distance constraints in structure calculations. If the intensity of such the NOE peak is not weak, and its assignment symmetry has not been confirmed, the peak and assignments should be considered to be spurious. If you try to find the spurious NOE assignments in the cycle7.noa file, it will be a tremendously tedious works.
In the "Sort" of the module, you can find several checkboxes in the module as shown below;



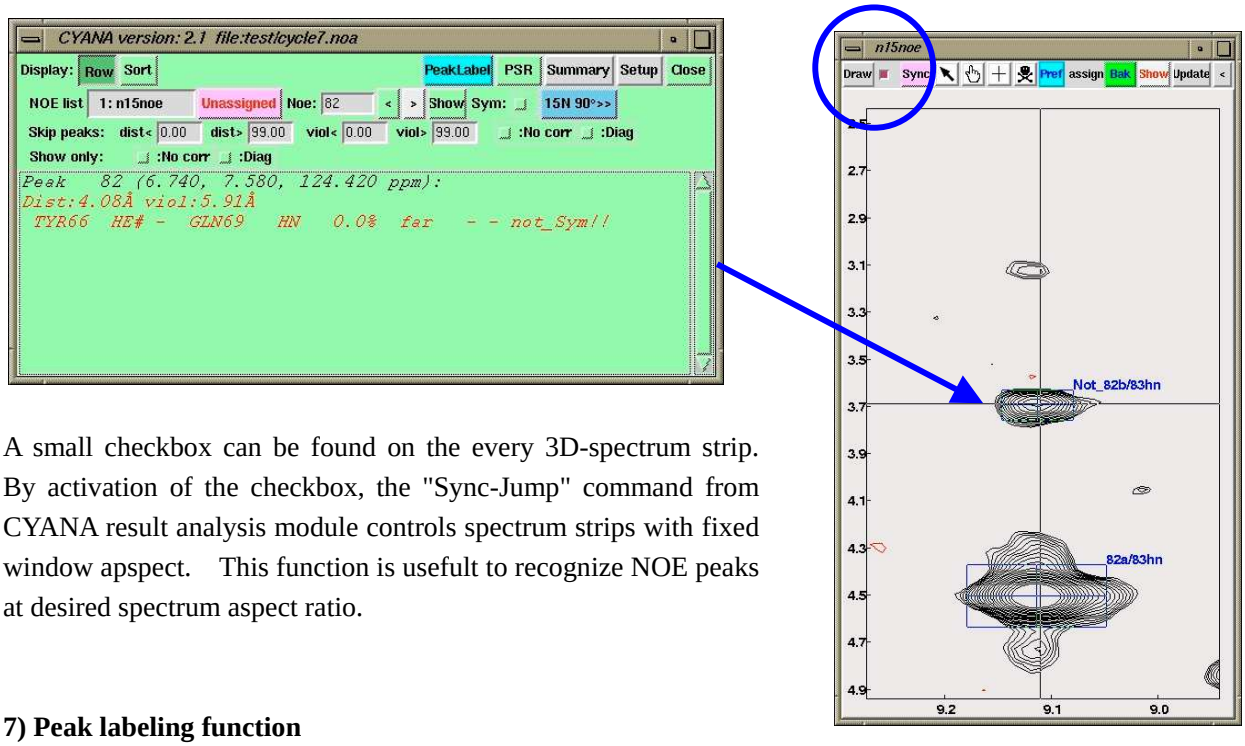
The detailed functions of these checkboxes are as follows;

increasing:	Sort increasing way
decreasing:	Sort decreasing way
Sym:	Skip NOEs having symmetrically assigned NOE
Trans:	Skip NOEs having intensity on transposed position
close H2O:	Skip peaks close to H2O signal
close diag:	Skip peaks close to diagonal peaks

The below panels show more than 2000 peaks which has been assigned by CYANAderived from 13C-edited NOESY. By enabling the checkboxes, the NOE peaks with trustful assignments are all filtered and only the spurious NOEs are listed in the module. The inspection of the three spurious NOEs actually disclosed misassignments in assigned chemical shift table.



6) Sync-Jump of spectrum strip with fixed region.

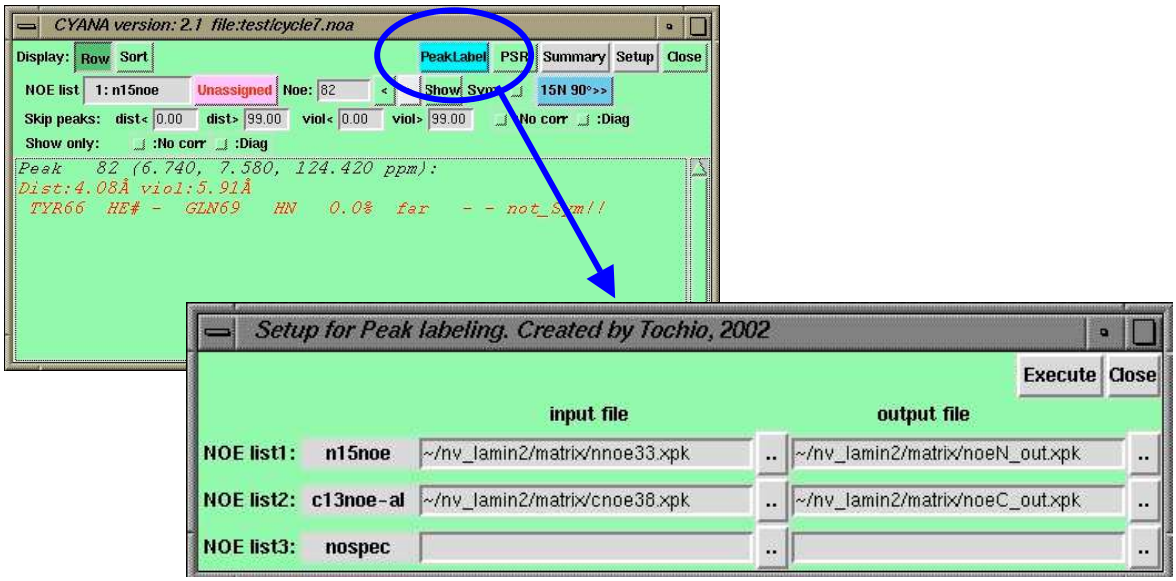


A small checkbox can be found on the every 3D-spectrum strip. By activation of the checkbox, the "Sync-Jump" command from CYANA result analysis module controls spectrum strips with fixed window apspect. This function is usefult to recognize NOE peaks at desired spectrum aspect ratio.

7) Peak labeling function

If user press "Peak label" button, the setting window will appear.

To run the program, user has to define input and output xpk file names, and then press "Execute" button to finish the labeling job.



The peak labeling job finishes in 1-2sec for each xpk-file (including ~200 peaks).

The following are how the labeling codes;

Diagonal peak	Xdiag
No corresponding signal	X0/0
Not assigned, Ala32-Ha <-> Thr55-Hg#	X32a/55g#
Assigned, Met103-Hg# <-> Gln29HN	103g#/29n
Assigned intra-residual, Phe67-HB2<->Phe67-HD#	b1/67d#

12. FLYA setup module

1) required files for FLYA calculation

FLYA setup module will help you to generate files required for FLYA calculation. FLYA is a function implemented in CYANA which can be used for fully automated signal assignment and structure calculation.

Recommended spectrum data sets for backbone signal assignments;
2D 1H-15N HSQC, 3D-HNCO, 3D-HN(CA)CO, 3D-HNCACB, 3D-CBCA(CO)NH
If you have 3D-HNCA and 3D-HN(CO)CA spectra, it would be better idea to include them.

For assignments of all signals including backbone and side-chain atoms;
3D-HCCH-TOCSY for aliphatic, 3D-HCCH-TOCSY for aromatic
3D-^{15}N edited NOESY, 3D-^{13}C edited NOESY for aliphatic and aromatic
If 3D-HBHA(CO)NH, 3D-CC(CO)NH and 3D-H(CCCO)NH are available, it would be better idea to include them.

FLYA calculation requires spectrum data sets with enough data points. It is strongly recommended that you shall make double the data points for in-direct dimension and ^{13}C/^{15}N dimension using zero-filling, linear prediction and non-linear sampling techniques.. The followings are the successful examples of spectrum data sets;

3D HCCH-TOCSY	X: 2048	Y:256	Z:256
^{15}N-edited NOESY	X: 1024	Y: 512	Z:256
^{13}C edited NOESY	X: 2048	Y:512	Z:256

For the FLYA calculation, the following files are required;

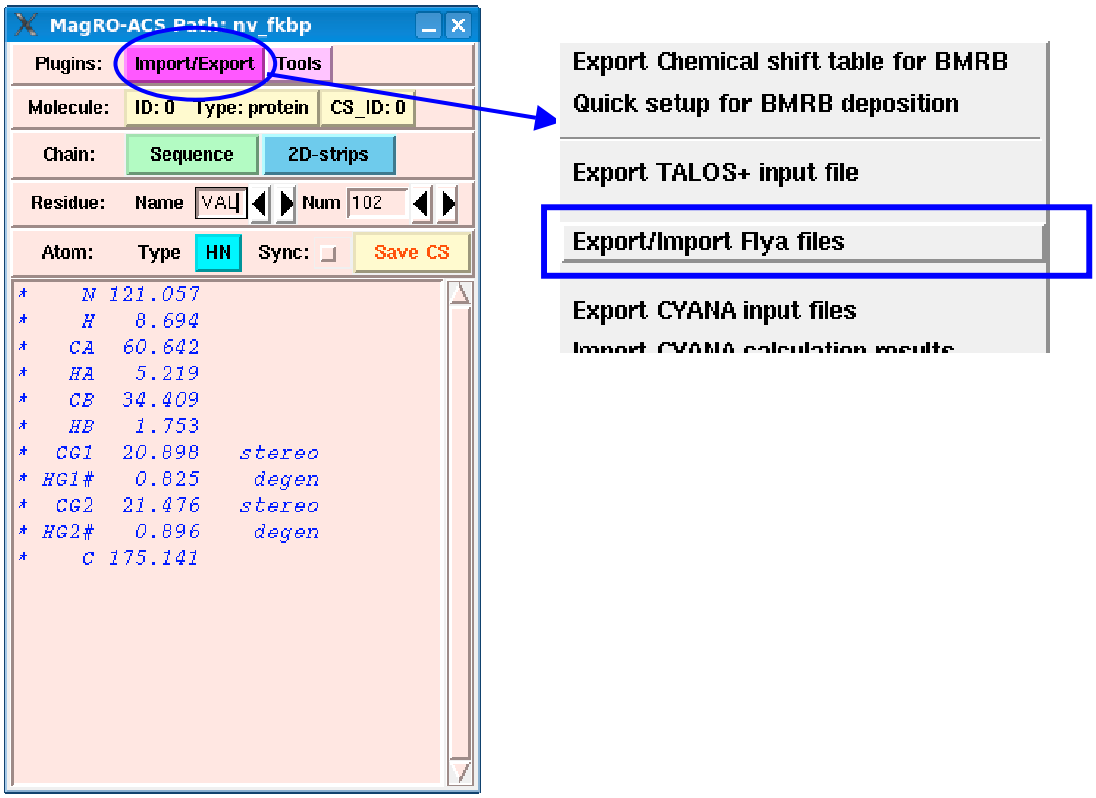
RUNFLYA.cya, int.cya	Setting files for FLYA calculation.
*.seq	Sequence file
*.peaks	Peak table file

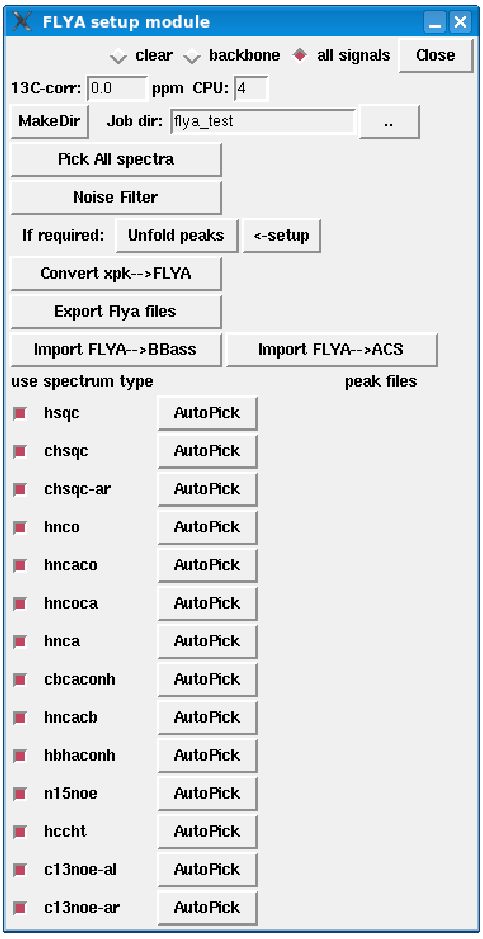
[Important]
The accuracy of the automated assignments strongly depends on spectrum quality. If you find that the peaks HCCH-TOCSY and NOESY spectra are significantly overlapped and missing, you will have to reconsider the plan of signal assignments.
If the assignments are moderately difficult by manual operation, and if the correctly modeled structure is available, you can run FLYA calculation with the structure and in some case you can get correct assignments.

[Warning]
Basically, using spectrum included folded peaks (peaks are detected in folded positions) may decrease accuracy of automated assignments. However, if you have ^{13}C-HSQC for full region (constant time would be better), the program in MagRO can recover the folded peaks in HCCH-TOCSY and ^{13}C-edited NOESY in unfolded position.

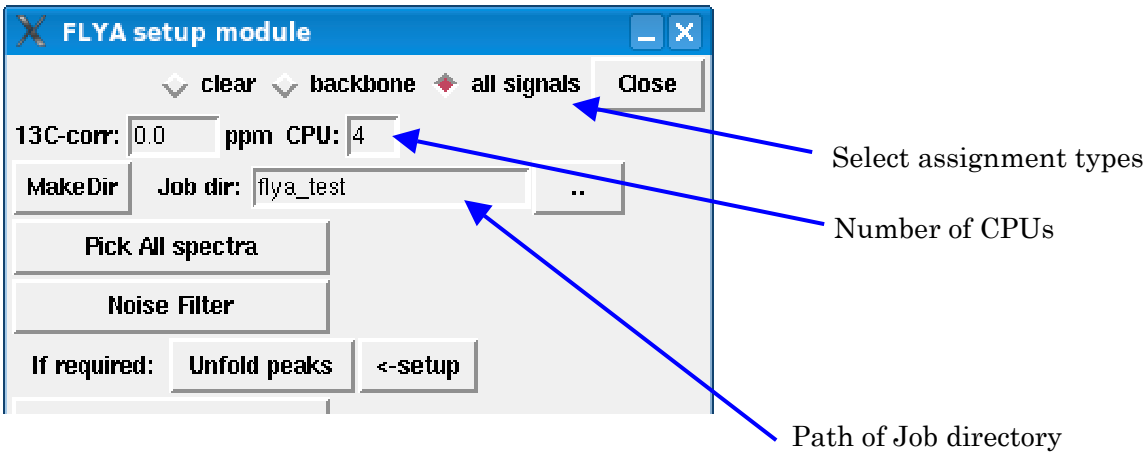
2) Lets start FLYA calculation

Press "Import/Export" button on the Acs module, then select "Export/Import Flya files"



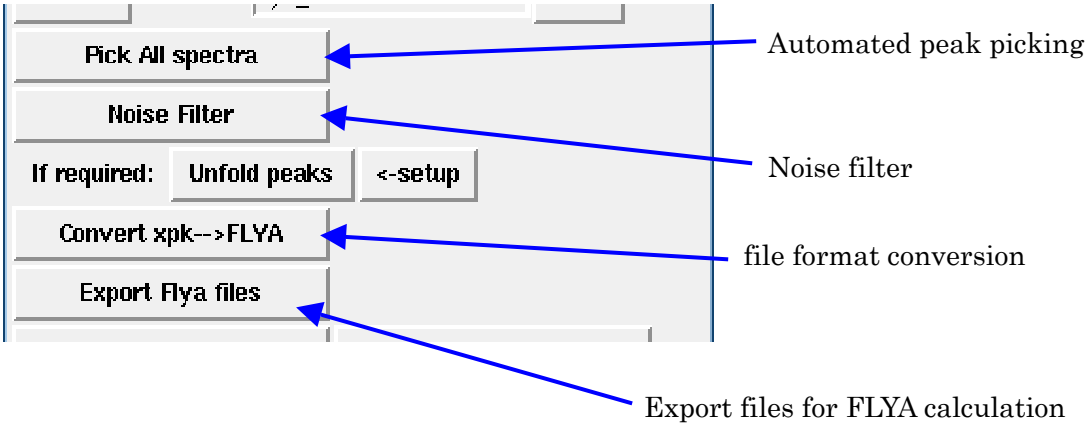


Then you will see the setting up window as shown in the left panel.



a) Firstly, you have to choice type of assignments, backbone signal only or all signals.

b) Secondary, set the directory path for FLYA calculation. Directly type the path or get the directory by pressing ".." button. If you would like to create the directory, press "MakeDir" button.



c) Press "Pick All spectra" button to start automated peak picking for all spectra.
[Important] Before do this job, you have to set spectrum threshold properly for each spectrum window.

d) You can refold peaks in folded position by pressing "Unfold peaks" button.
[Important] To use this function, you need to prepare spectrum windows for 2D ^{13}C-HSQC for aliphatic and aromatic region (chsqc and chsqc-ar) which have been acquired without spectrum folding in ^{13}C dimension.

e) Press "Noise Filter" button to start noise filtration for all peak tables.
Noise filter function will use peak table derived from 2D ^{15}N-HSQC and ^{13}C-HSQC for aliphatic and aromatic as mask table.

f) To covert the peak table file for FLYA calculation, press "Convert xpk-->FLYA" button.

3) Detail of the files created by FLYA setup module

RUNFLYA.cya,	A macro file for FLYA calculation. You can describe spectrum names, structure file name
init.cya	Settings for FLYA calculations such as CPU number, sequence file name, library file name, etc.
protein.seq	Sequence of target protein. Should be described by three letter codes
***_auto.xpk	Peak table identified by auto peak picking
***_filt.xpk	Peak table filtered by Noise filter command
***_unfold.xpk	Peak table with unfolded peaks
***.peaks	peak table in XEASY format

For the conversion of xpk format to XEASY format, program will select the xpk file by the priority order; ***_refine > ***_unfold > ***_filt > ***_auto

[Important] If you edit xpk file, you should overwrite ***_refine.xpk.
For example, hncacb-al_refine.xpk.

4) Execute FLYA calculation

You can start FLYA calculation on the job directory using following command;

/opt/cyana3.95/cyana RUNFLYA

The calculation time using 4CPUs is 5~10min for backbone signals and 60min for all signals.

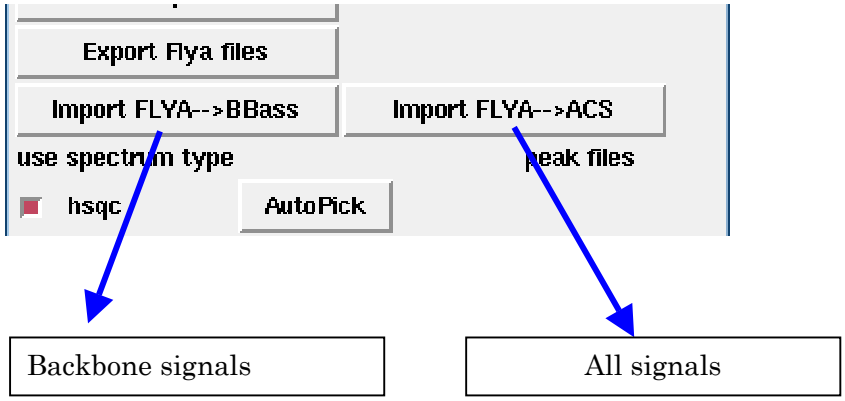
5) Import results of FLYA calculation

After the FLYA calculation you will find an assigned chemical shift table as flya.tab.

```
Total number of shift values: 32057
Cutoff for extent           : 16.00

Atom  Residue      Ref    Shift     Dev  Extent  inside   inref
N     ARG    3          116.353           20.0    55.7     0.0
H     ARG    3            8.181           20.0    54.3     0.0
CA    ARG    3           59.572           20.0    99.8     0.0  strong
HA    ARG    3            3.795           20.0    99.7     0.0  strong
CB    ARG    3           29.085           20.0    99.7     0.0  strong
```

In this table the most important parameter is "inside" on the 5th column. The value estimate the frequency of the assigned chemical shift value in the calculation. The FLYA module will pick up the assigned chemical shifts with the value above 80% (labeled with strong) as they are trustful enough.



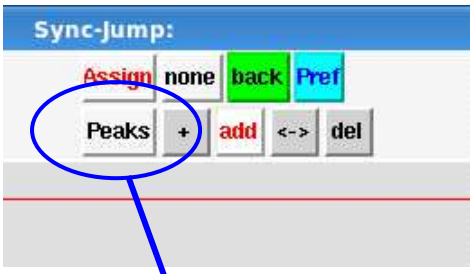
To import the results of FLYA calculation for backbone signals, press "Import FLYA->BBass" button and for all signals press "Import FLYA-->ACS" button.

After the import, the program will overwrite the assigned chemical shifts in assign_NN.txt file for backbone signals and ACS table MagDB/protein_0_0_acs.db file for all signals, respectively.

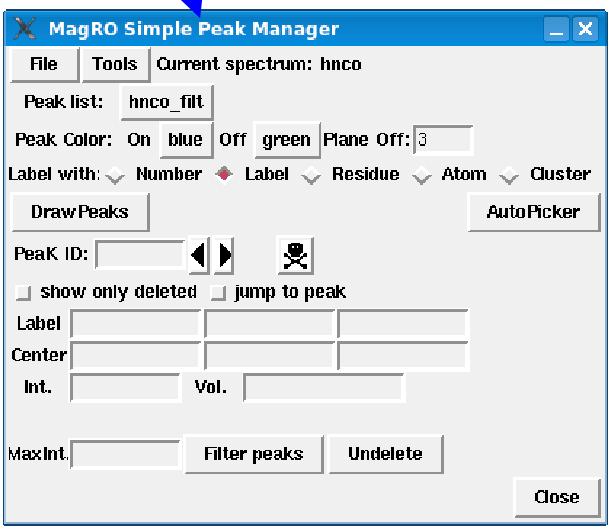
[Important] The assigned chemical shifts in assign_NN.txt and MagDB/protein_0__acs.db will be overwritten with imported values. Please make sure to take backup of the files before do this job.

11) Handling peak list

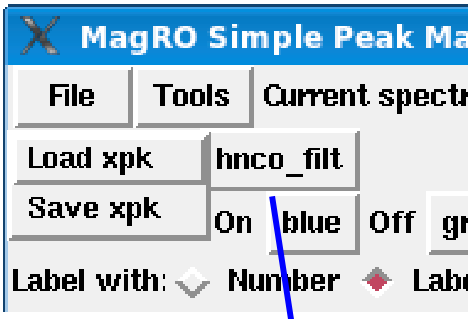
a) howto load peak list



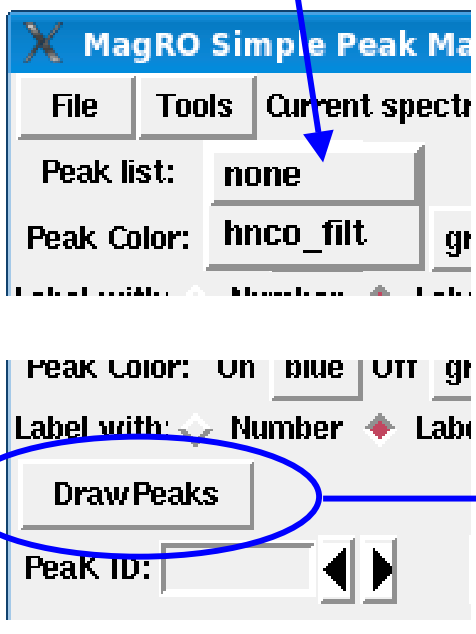
MagRO has a original peak list manager.
You can find "Peaks" button for each spectrum window as swhon in the left panel.

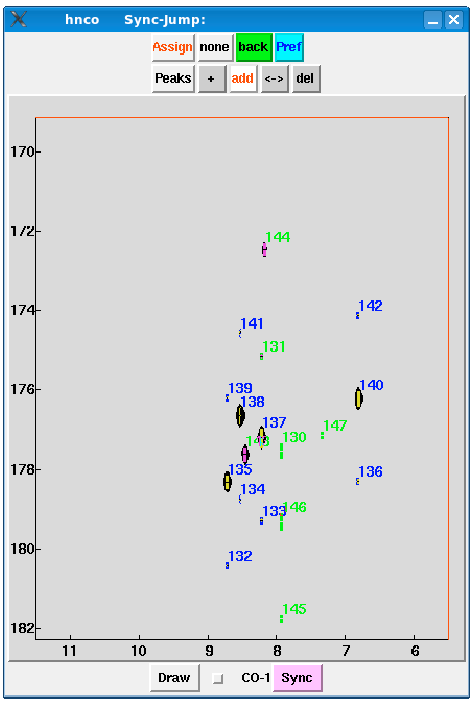


The left pane shows Peak manager of 3D HNCO spectrum.
[Important] If you open a peak module for a spectrum window, they are linked each other. Please confirm which spectrum is linked to the peak module.

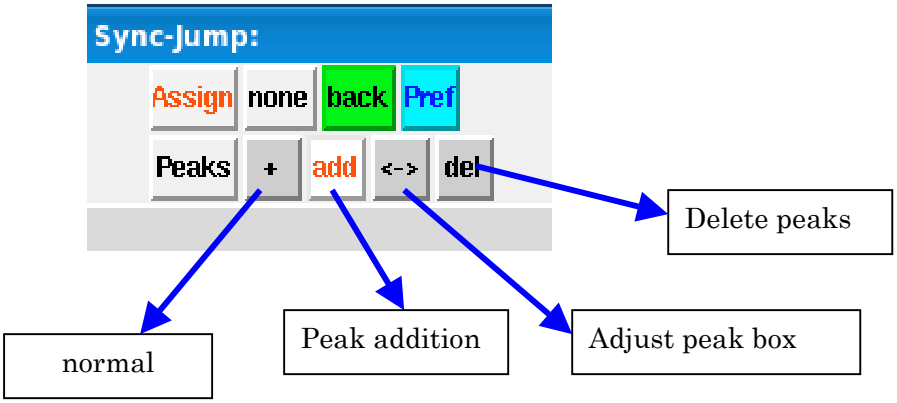


If you want load xpk file, press "File" button on the module, then select "Load xpk". Now you can load xpk file that you want to load.
To display peak boxes on the spectrum window, press button just right side of "Peak list:", then select peak list name that you have loaded. Press "Draw Peaks" button to display peak boxes on spectrum window.





2) How to change cursor mode (add, delete and adjust peaks)



User can change the mode of cross-hair cursor by pressing the buttons on the header of spectrum window as shown above.

[Important] To edit the peak list using above buttons, user has to set peak list that has been previously generated. If you wish to create peak list, just work up as described below.

[Appendix] To create peak list

NMRView C version

Click the mouse-right-button just on the spectrum window to open menu then select "peak". Write name of the peak list in the entry of "List Name:" Click "pick" button to create peak list and to start automated peak picking.

NMRView Java version

Click the mouse-right-button just on the spectrum window to open menu, then selct "Attribute". Select "PeakPick" tab and write name of the peak list in the entry of "List". Click the "Pick" button to start automated peak picking.

3) How to delete and adjust peak box.

Please be careful to delete peak boxes. The deleted peaks disappear immediately but actually no deleted permanently from the peak list. You can recover the deleted peak anytime if you want (see the naext page).

In the adjustment mode, you can move the position of peak boxes and change their size manually.

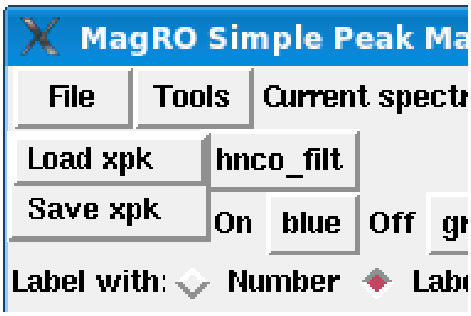
NMRView C version

By clicking just on the peak box and dragging it, you can move the peak box. At the same time, by clicking mouse-middle-button and dragging the peak box, you can change the size of peak box.

NMRView Java version

Click the peak box with the mouse-left-button just on the peak box. The peak box will turn to be yellow. Click the moues-left-button near the center of peak box you can move the peak box. if you click the mouse-left-button near the edge of the peak box you can change the size of peak box.

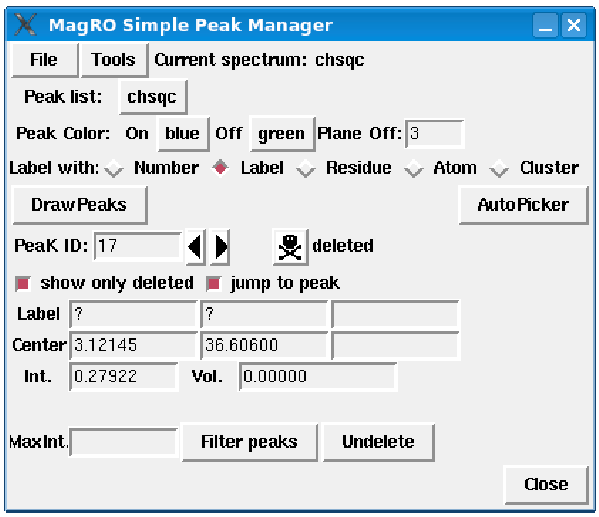
3) How to save peak list



Firstly, make sure the current peak list has been selected in the "Simple Peak Manager".
In the "Simple Peak Manager", press "File -> Save xpk", you can save the peak list as *.xpk file.

If you want to remove the "deleted" peaks permanently from the peak list, Press Tools-> Get-Int,Cmp&Degap,Write. This command will compress the peak list and degap the numbering of peaks.

4) How to recover "deleted" peaks



Firstly you have to set peak list that you want to manage now in the "Peak list:".
Enable the checkboxes "show only deleted" and "jump to peak". Press "<" and ">" buttons to increment and decrement the number peak ID and find the deleted peak that you want to recover. If you find the target peak labeled with "deleted" just neigbour the pirate button, press the pirate button. The deleted peak will be recovered and peak box will appear in the spectrum window.

7-1. Trouble shootings

1) I can't see some window because the window position might be out of screen.

If you change the screen size of desktop, some window can be found anywhere. In this case, try to delete the 000temp directory and restart MagRO.

matrix/000temp

2) I can't open CYANA result analysis module

Some error will cause to fail to open CYANA result analysis module properly.
Please try to delete CYANA_results directory and try to open the module again.

matrix/CYANA_results

3) I can't type anything in entries of MagRO system

If MagRO system seems to be working without any error message, sometime you can not type anything in the entries implemented in modules of MagRO system. The conflict of input method (especially Japanese input method) with NMRView Cversion has been reported long time ago. Please disable any input method on your desktop manager.