An Introduction to CCPNmr Analysis and Solution-NMR Assignment

Vicky Higman

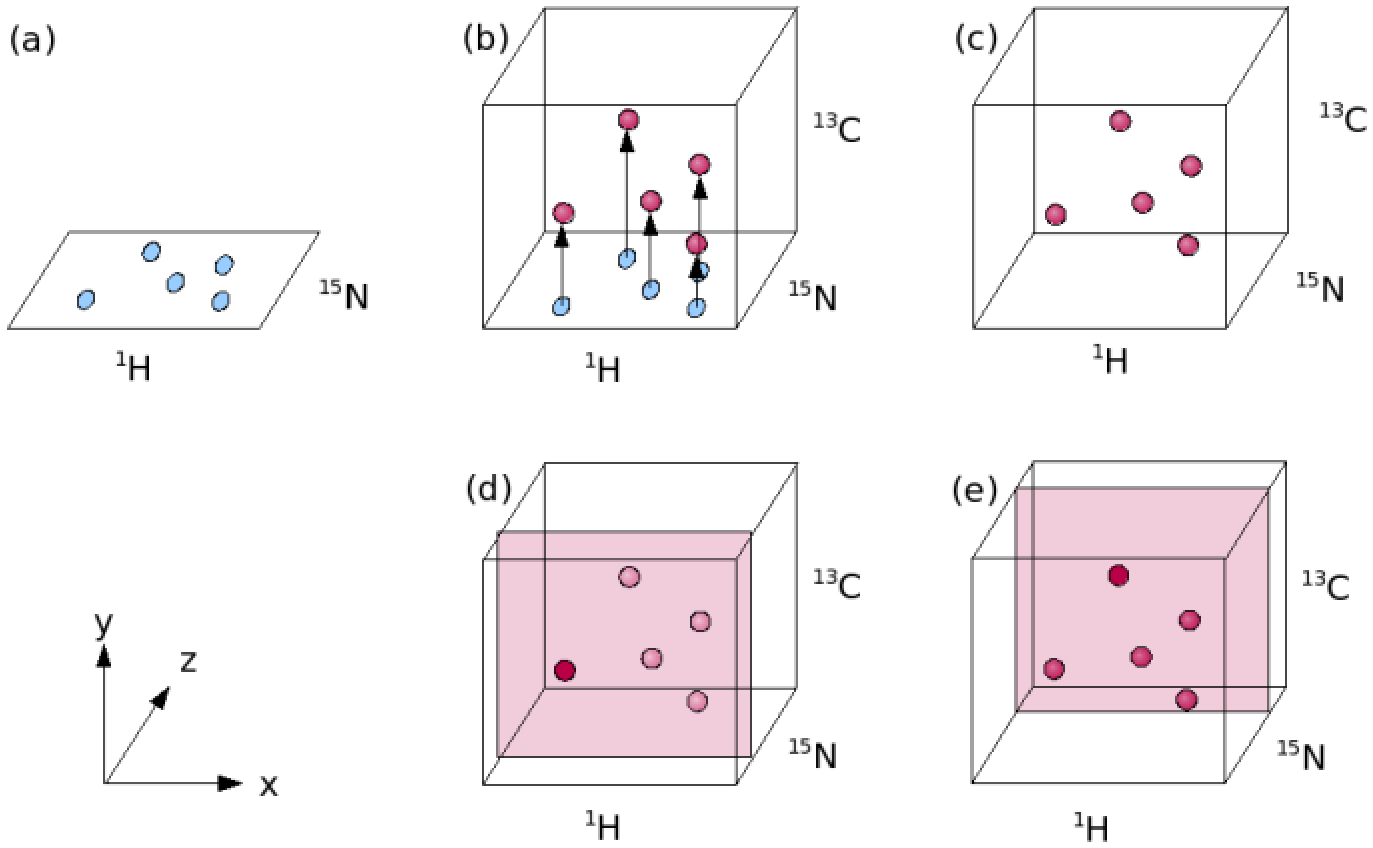
Leibniz-Institut für Moleculare Pharmakologie, Berlin

www.protein-nmr.org.uk

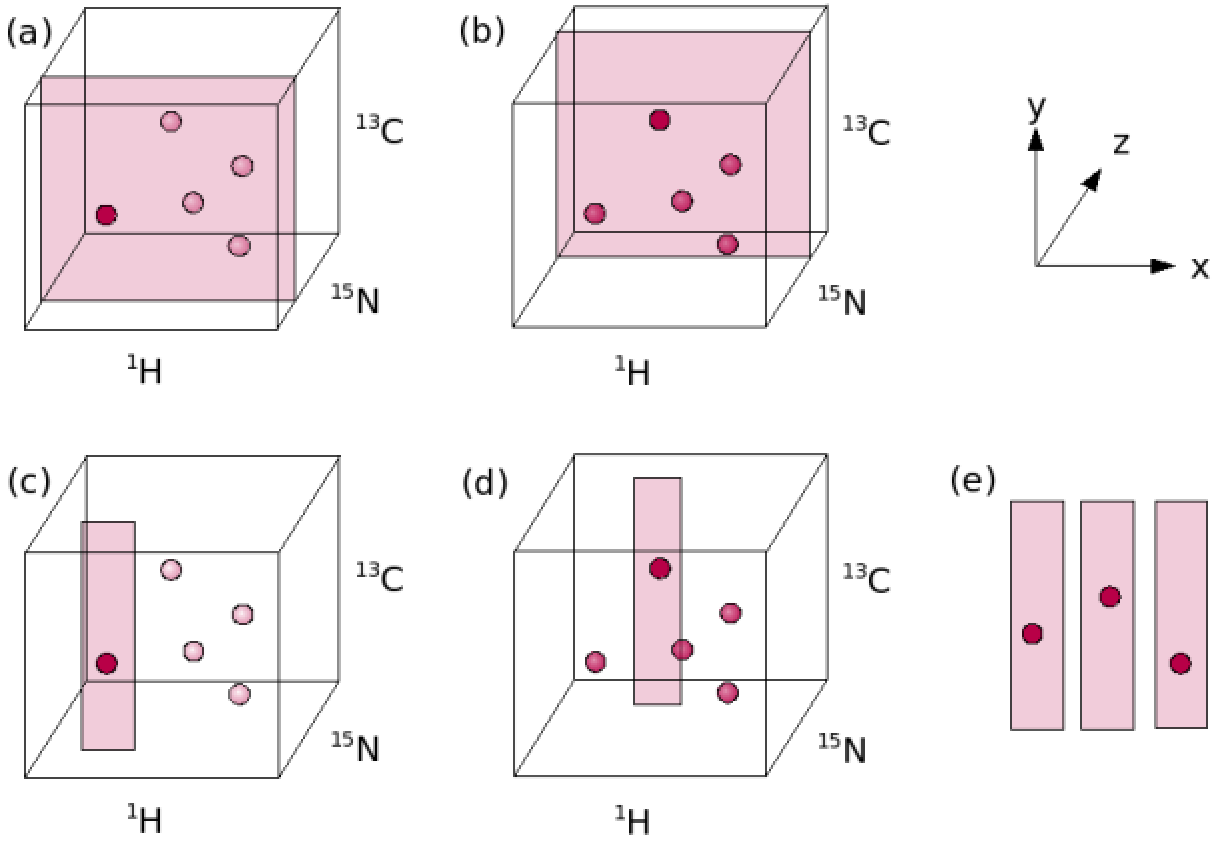
Outline

- Theory – Backbone and side-chain resonance assignment
- CCPNmr Analysis Software
- Practice – Using CCPNmr Analysis to assign a protein

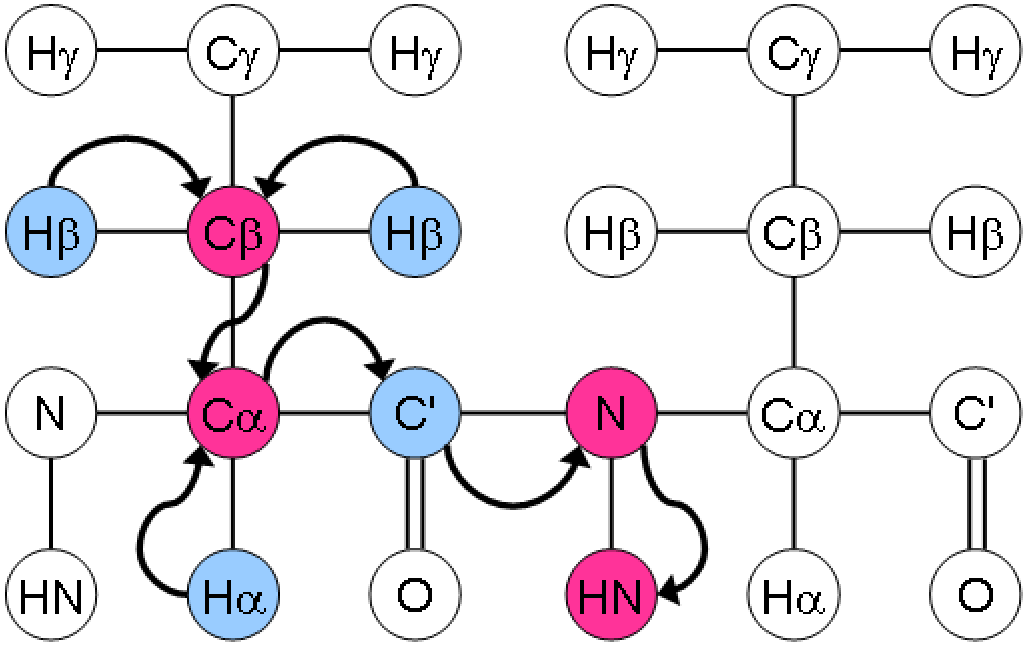
Visualising 3D Spectra



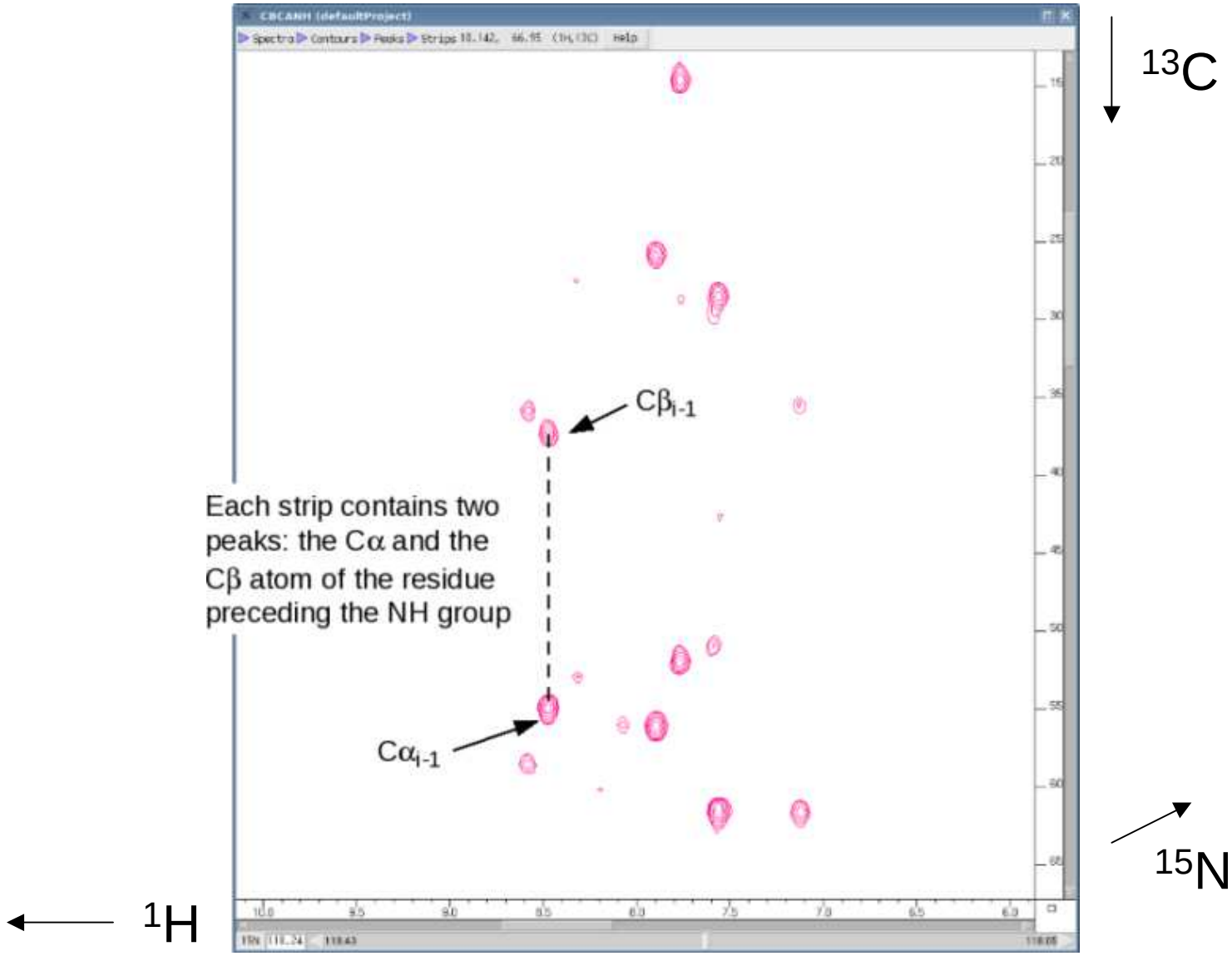
Visualising 3D Spectra



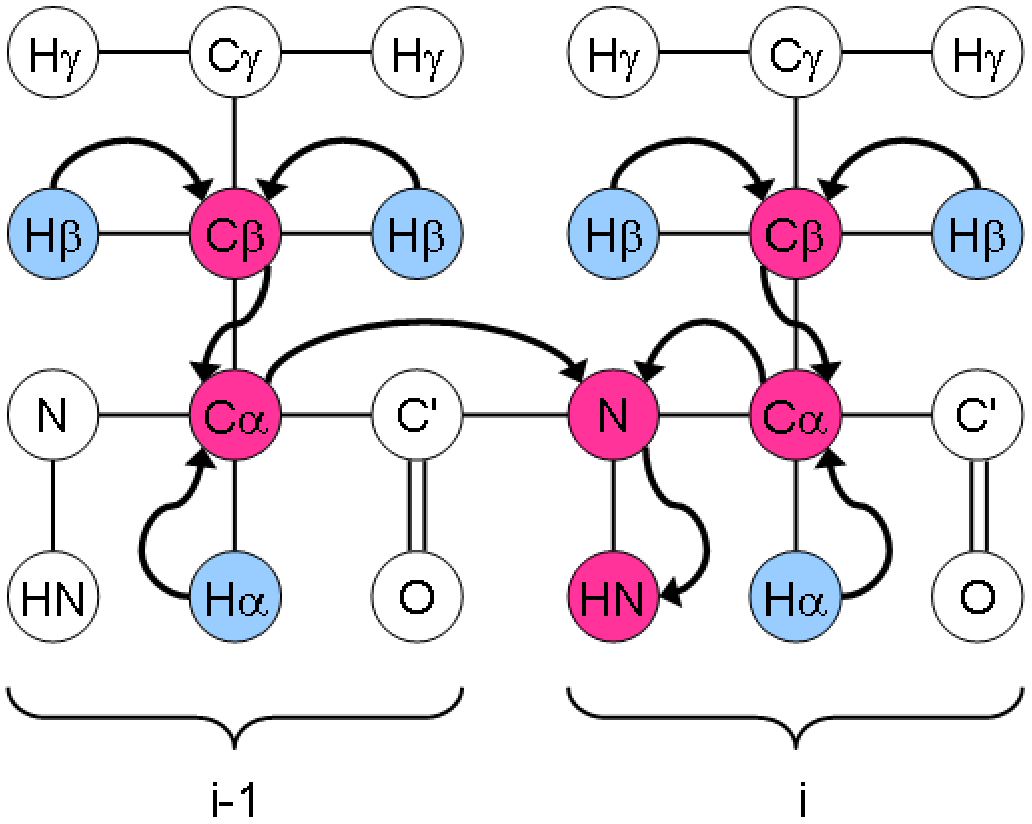
CBCA(CO)NH



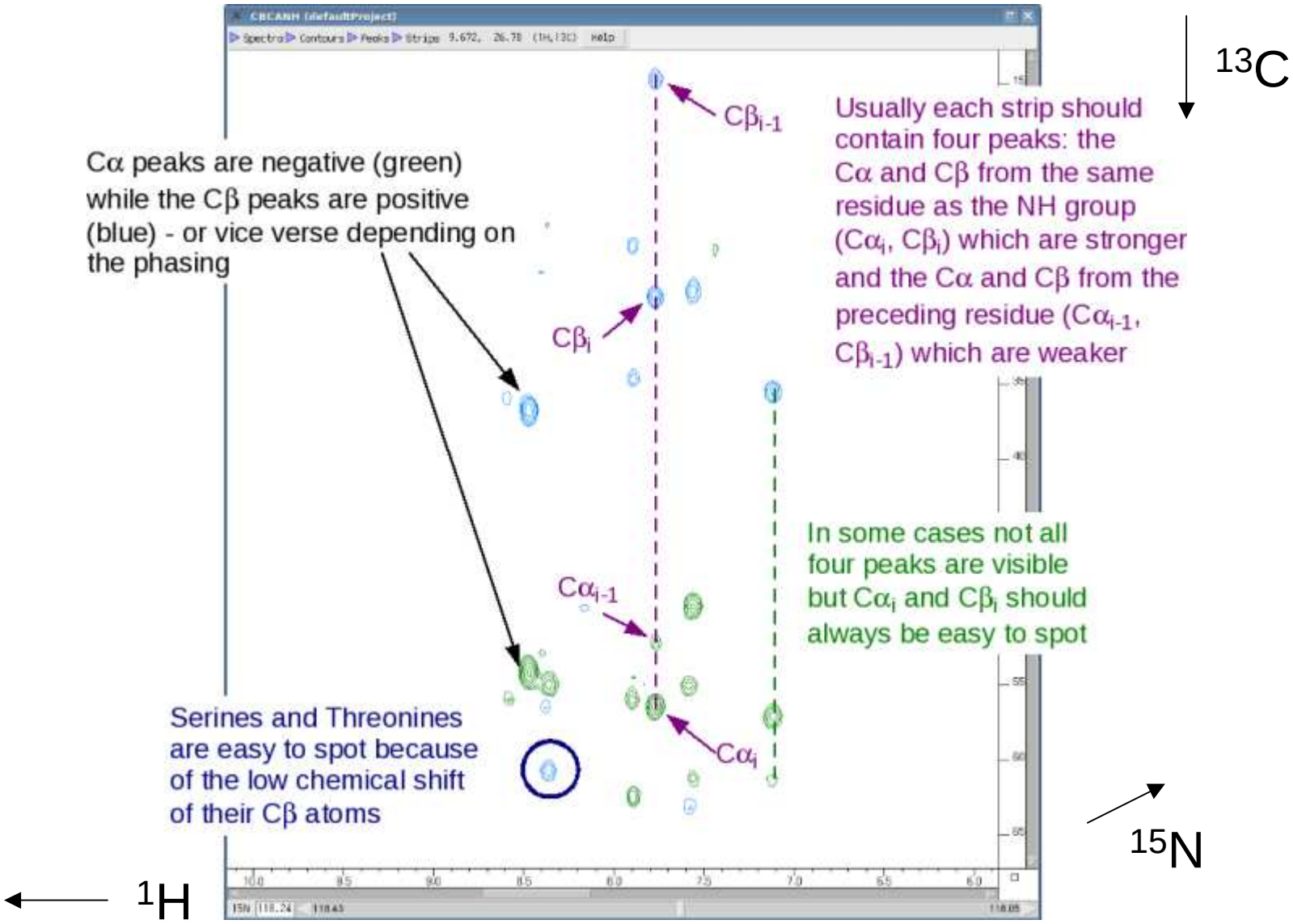
CBCA(CO)NH


^{13}C
$C\beta_{i-1}$
Each strip contains two peaks: the Cα and the Cβ atom of the residue preceding the NH group
$C\alpha_{i-1}$
^{15}N
^{1}H

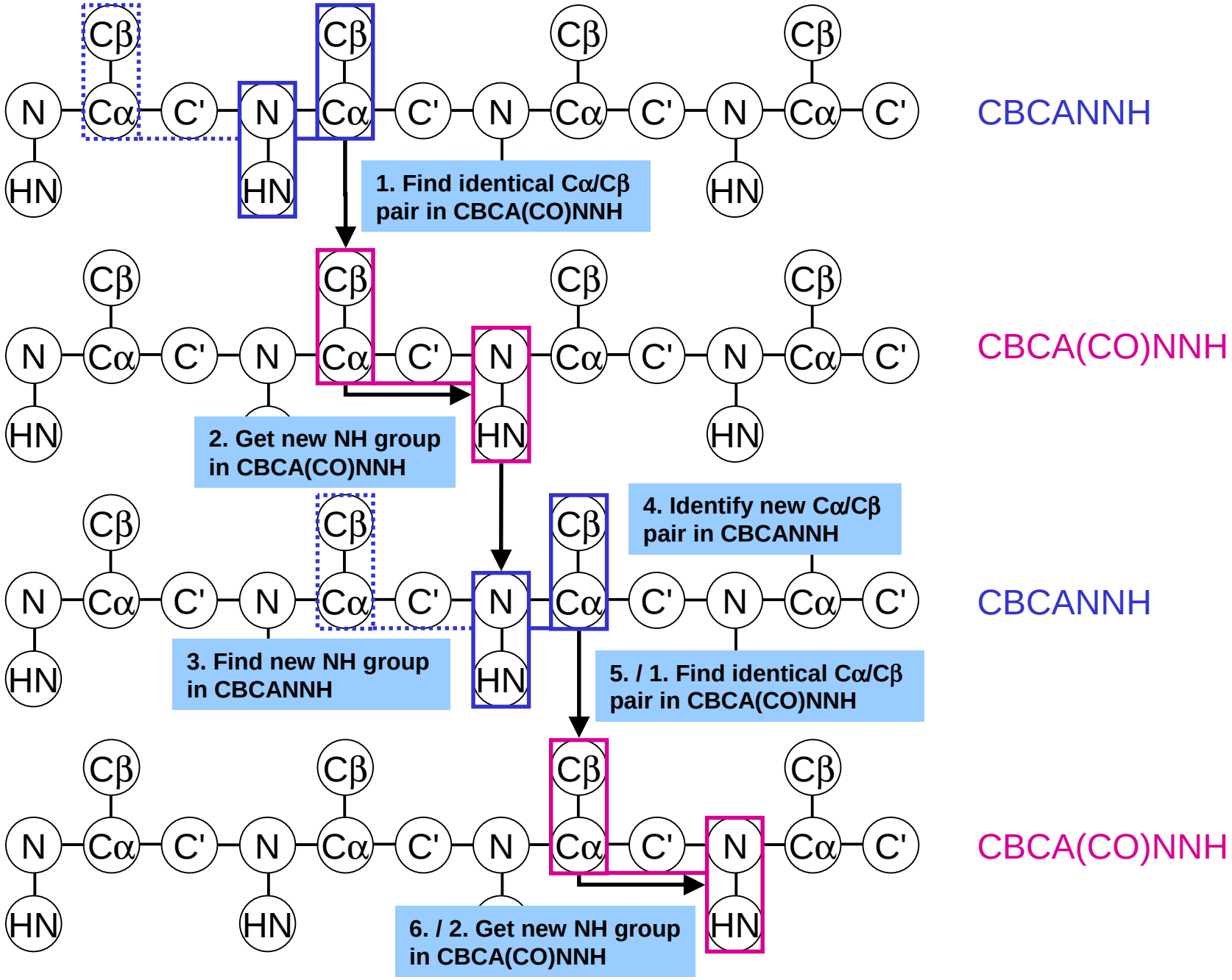
CBCANH



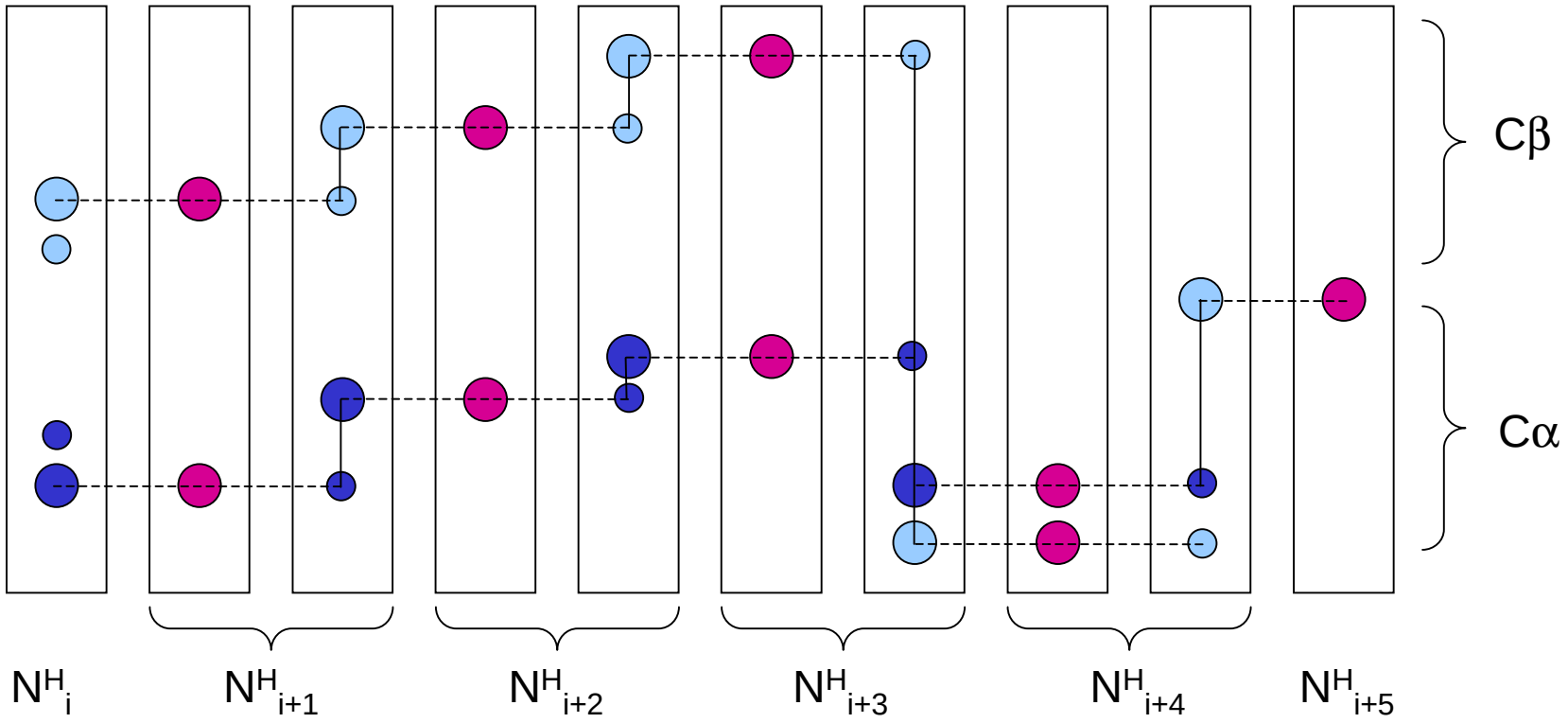
CBCANH


^{13}C
Usually each strip should contain four peaks: the Cα and Cβ from the same residue as the NH group ($C\alpha_i$, $C\beta_i$) which are stronger and the Cα and Cβ from the preceding residue ($C\alpha_{i-1}$, $C\beta_{i-1}$) which are weaker
$C\beta_{i-1}$
Cα peaks are negative (green) while the Cβ peaks are positive (blue) - or vice verse depending on the phasing
$C\beta_i$
In some cases not all four peaks are visible but $C\alpha_i$ and $C\beta_i$ should always be easy to spot
$C\alpha_{i-1}$
Serines and Threonines are easy to spot because of the low chemical shift of their Cβ atoms
$C\alpha_i$
^{15}N
^{1}H

Sequential Assignment


Cβ
Cα
C'
N
HN
CBCANNH
1. Find identical Cα/Cβ pair in CBCA(CO)NNH
CBCA(CO)NNH
2. Get new NH group in CBCA(CO)NNH
4. Identify new Cα/Cβ pair in CBCANNH
CBCANNH
3. Find new NH group in CBCANNH
5. / 1. Find identical Cα/Cβ pair in CBCA(CO)NNH
CBCA(CO)NNH
6. / 2. Get new NH group in CBCA(CO)NNH

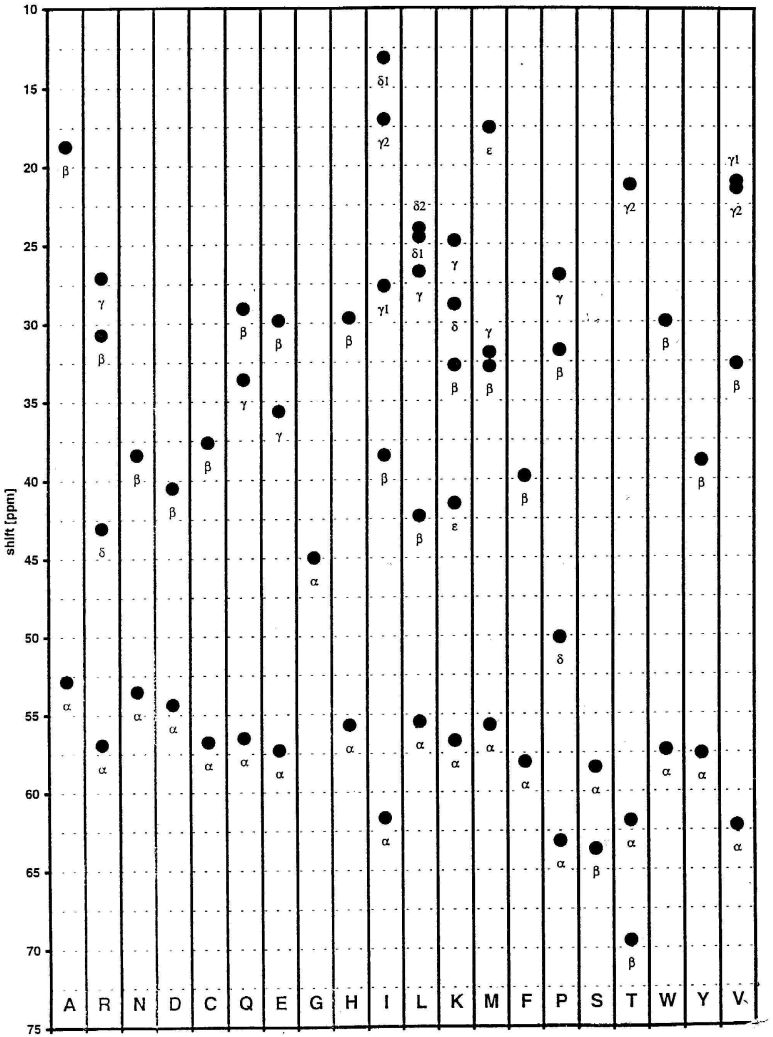
Sequential Assignment

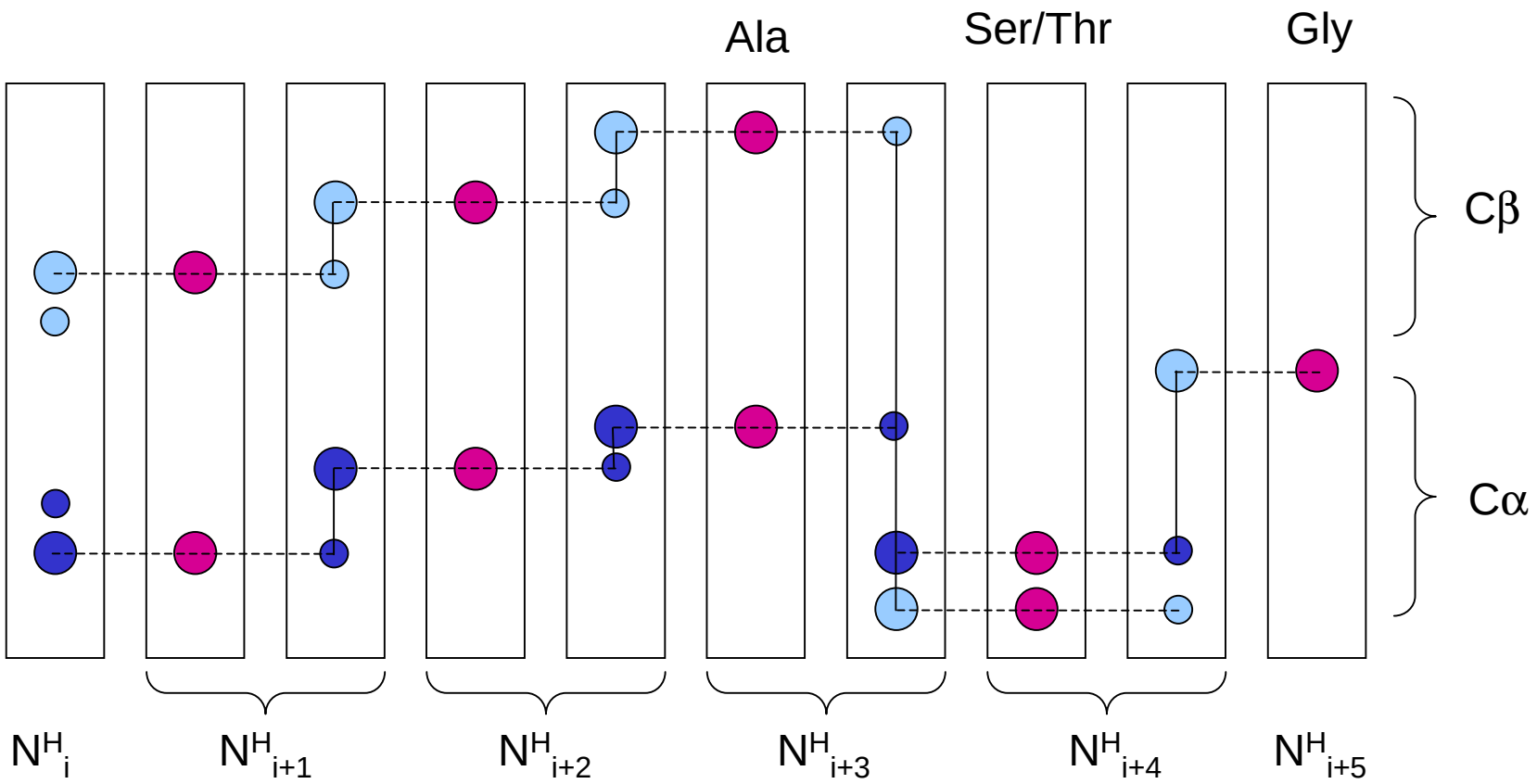


CBCANH

CBCA(CO)NH

Sequence Specific Assignment

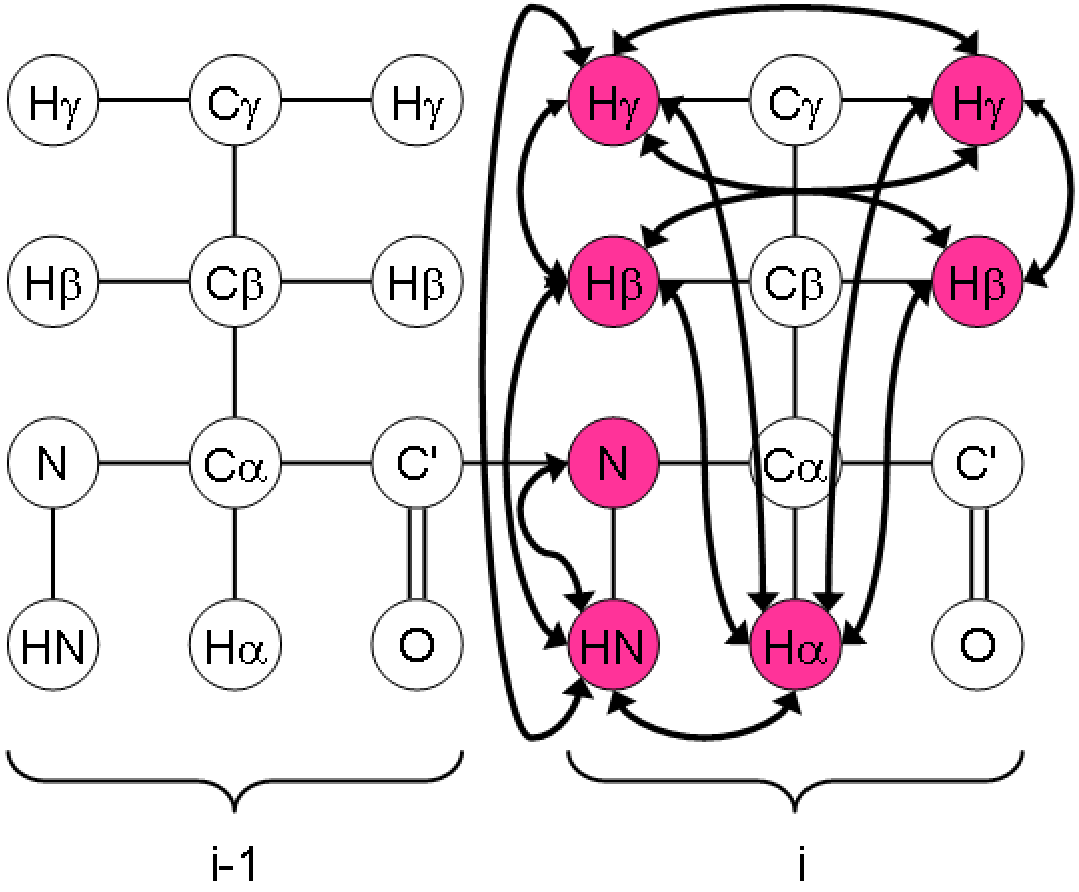




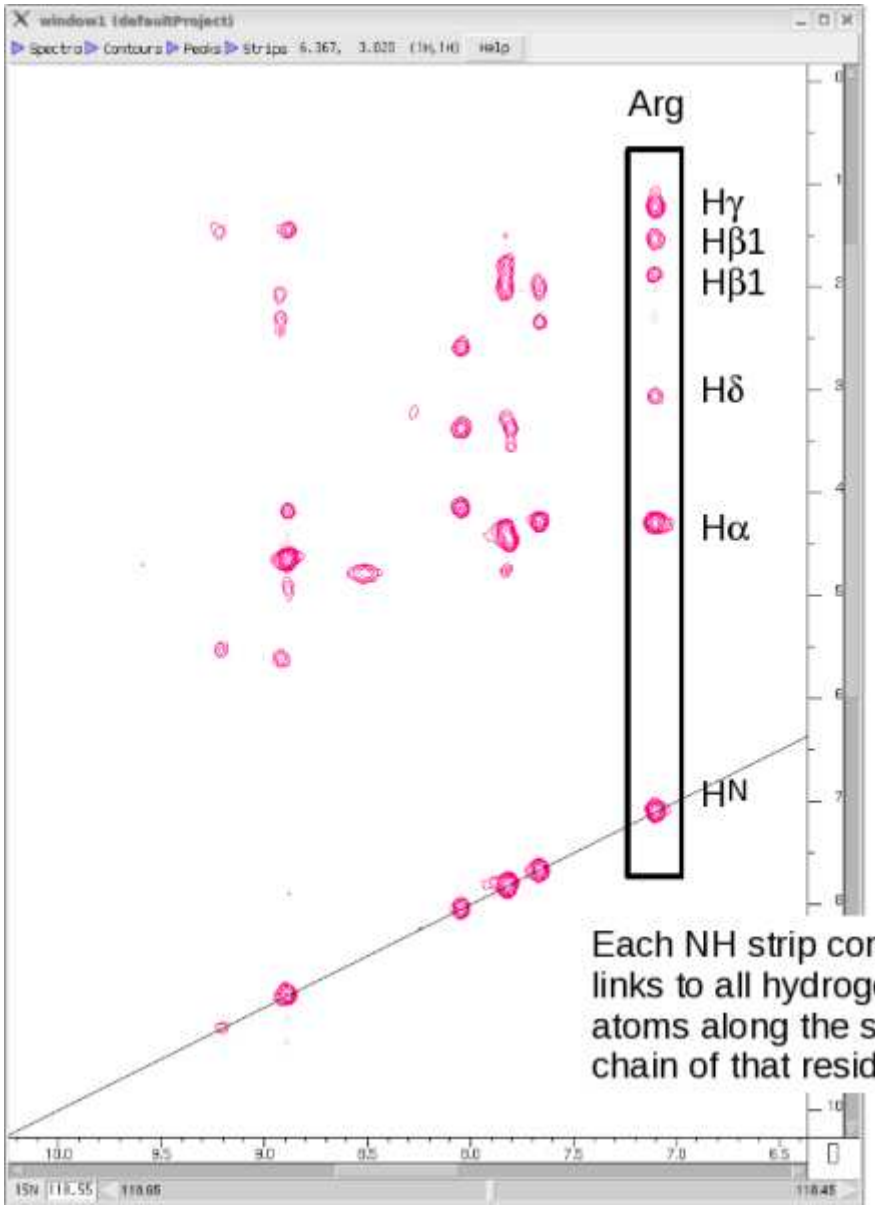
MVSPEEAVKW GESFDKLLSH RDGLEAFTRF LKTEFSEENI
FWIEASGDFK KSKGPQQIHL KAKAIYEKFI QDTEASGVNL
DFHTKEVITN SITQPTLHSF DAAQSRVYQL MEQDSYTRFL
KSDIYLDLME GRPQRPTNLR RRSRSFTCNE

Side-Chain Assignment

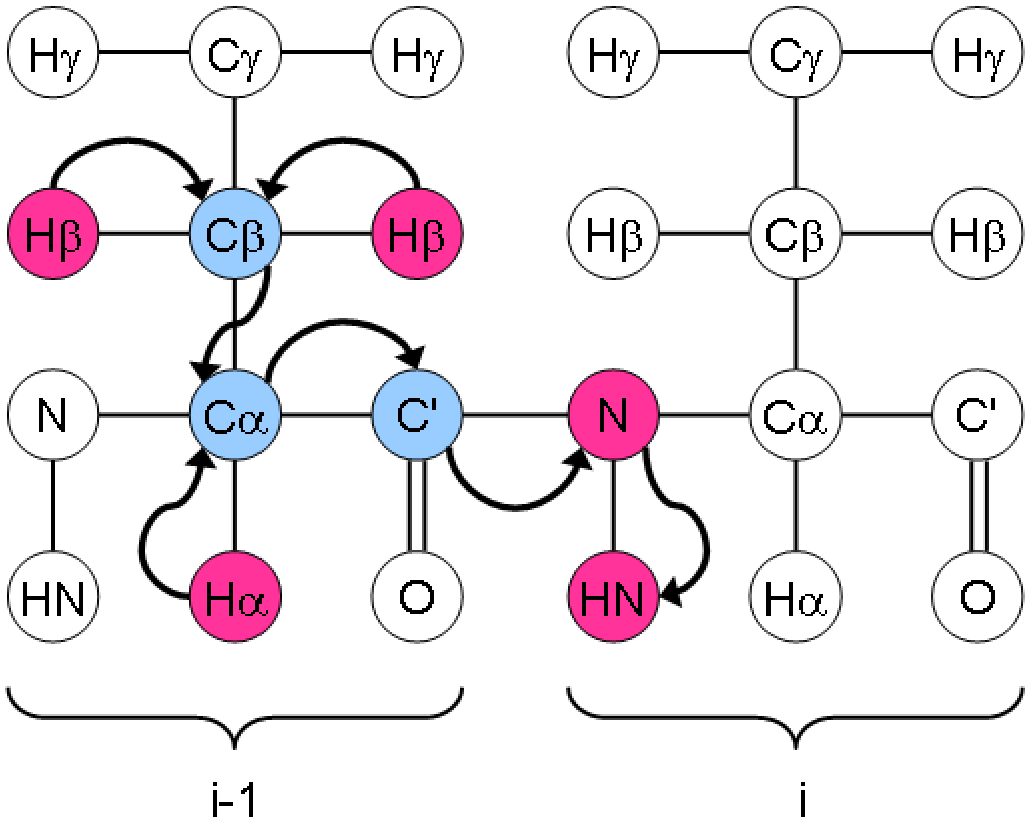
^{15}N-TOCSY



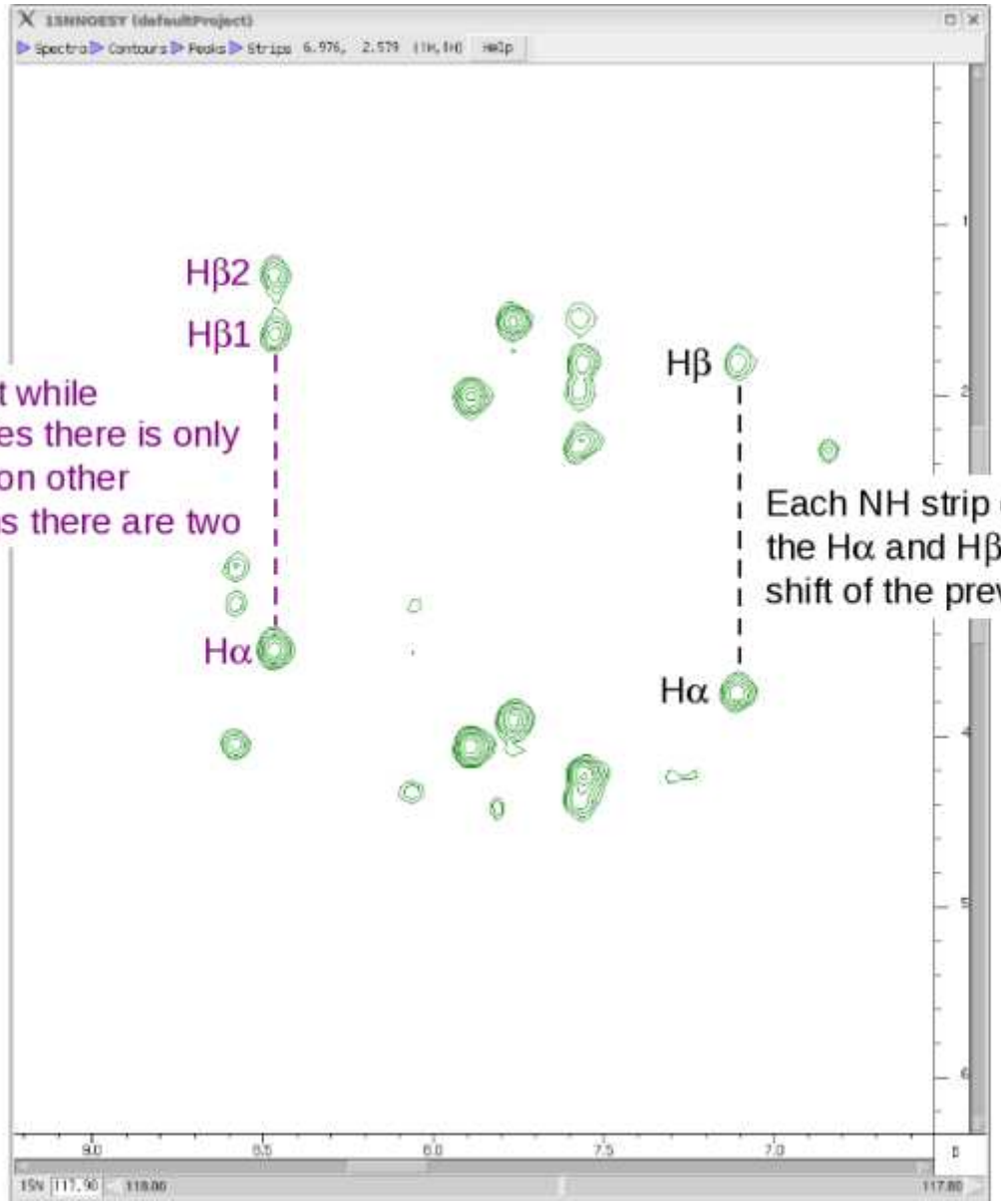
^{15}N-TOCSY


Arg
Hγ
Hβ1
Hβ1
Hδ
Hα
HN
Each NH strip contains links to all hydrogen atoms along the side-chain of that residue

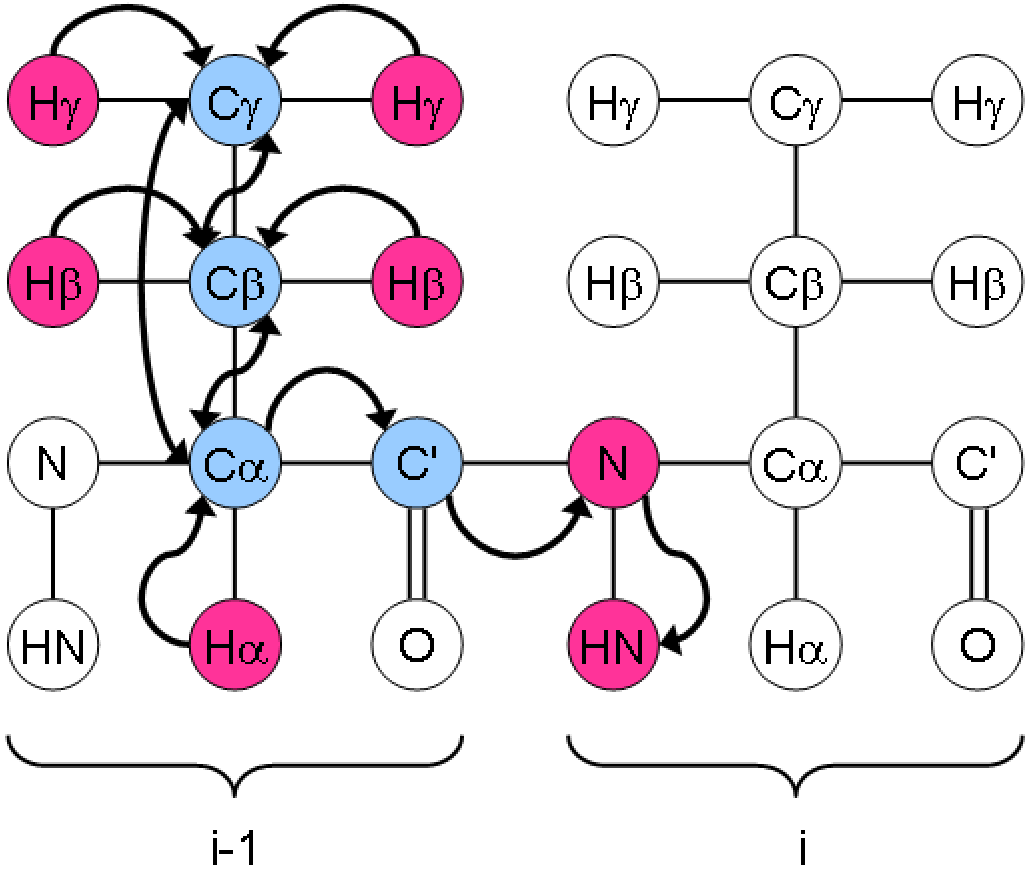
HBHA(CO)NH



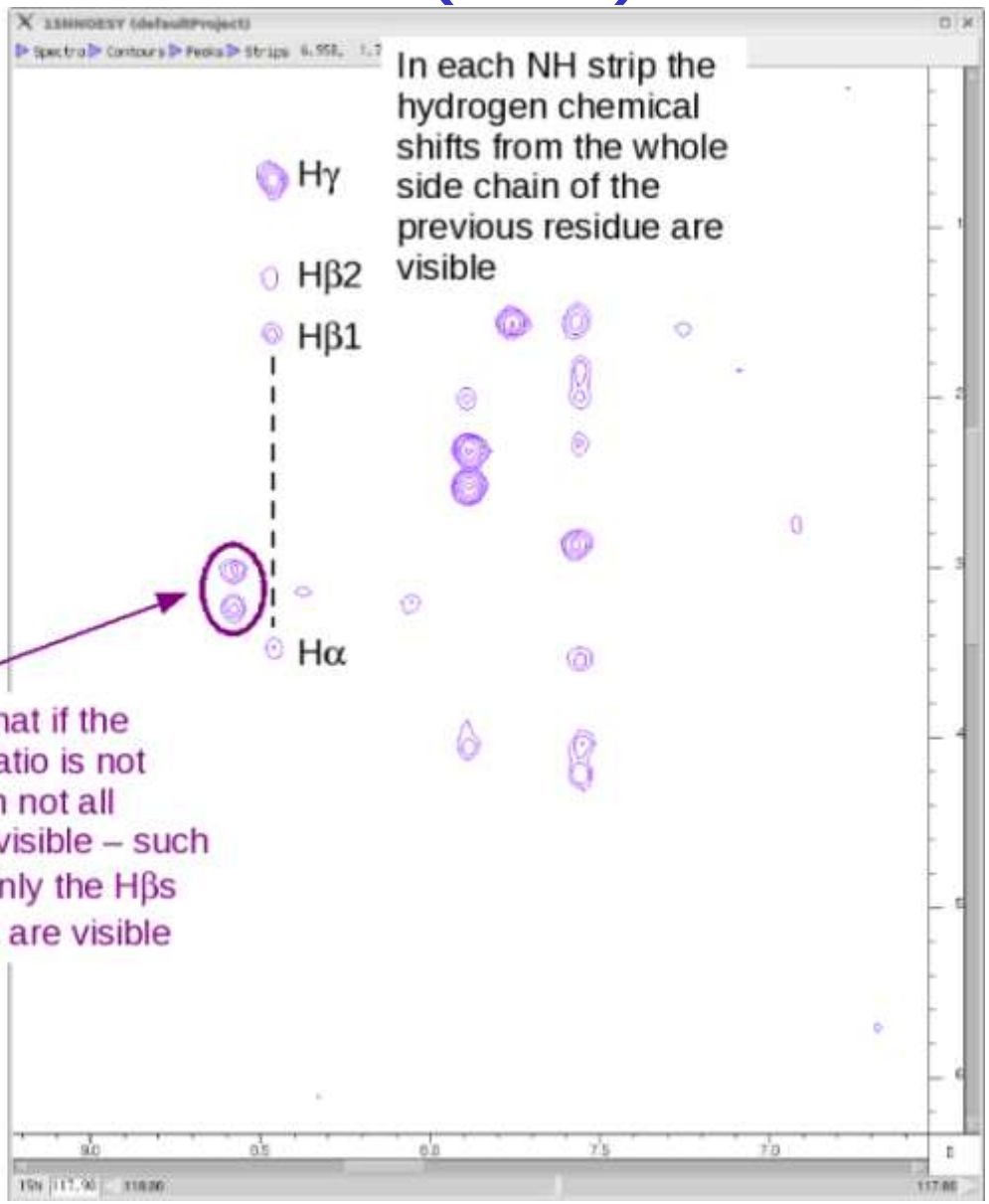
HBHA(CO)NH


Hβ2
Hβ1
Hα
Hβ
Hα
Note that while sometimes there is only one Hβ, on other occasions there are two
Each NH strip contains the Hα and Hβ chemical shift of the previous residue

HCC(CO)NH

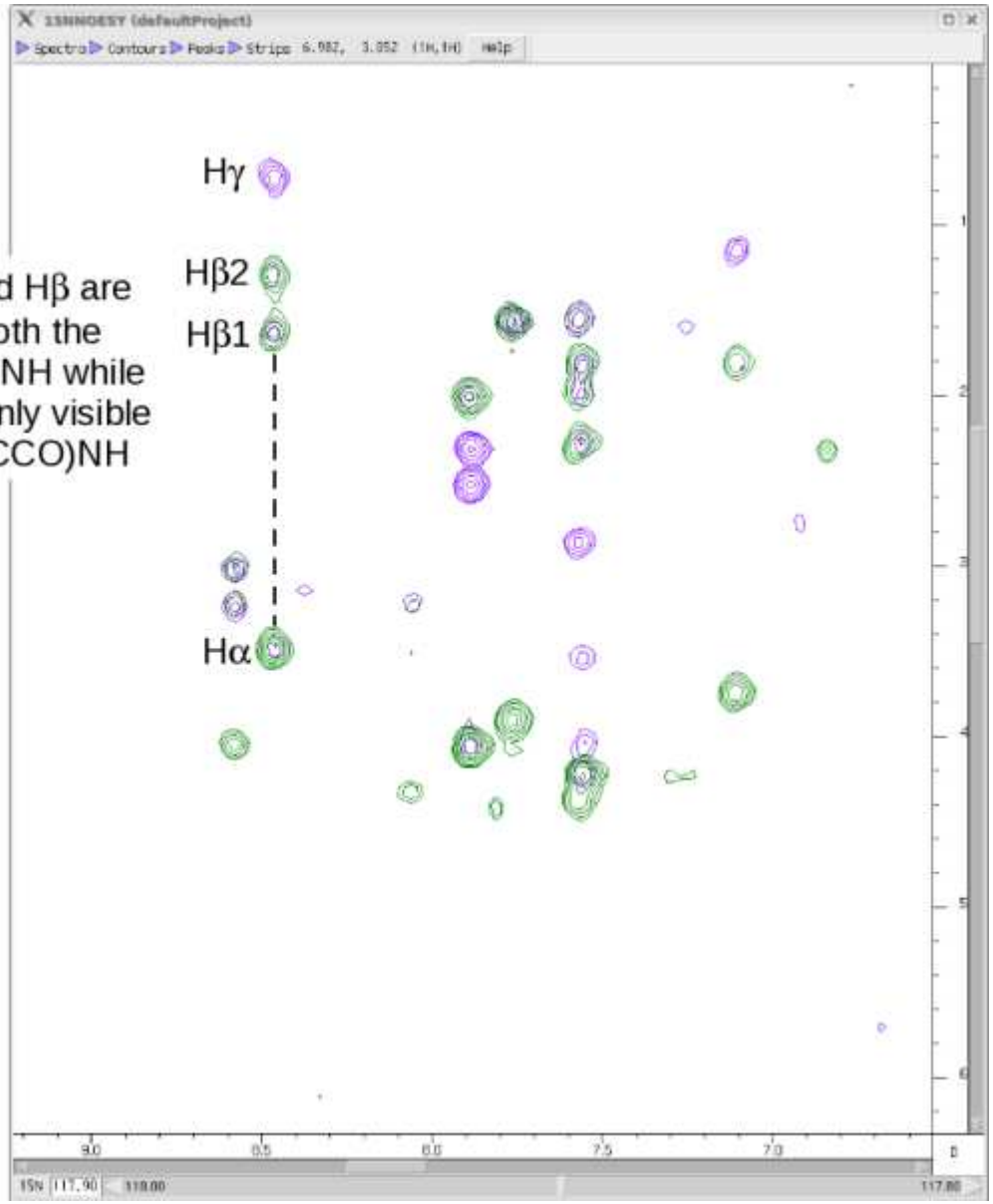


HCC(CO)NH

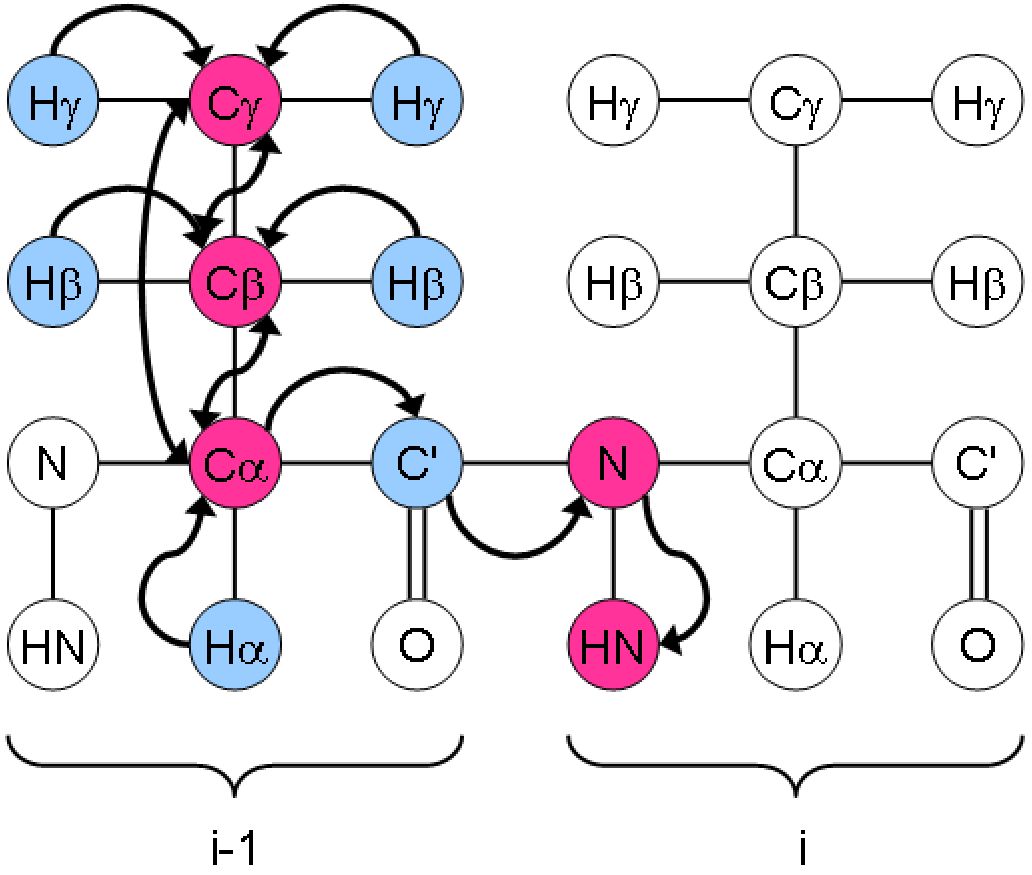

In each NH strip the hydrogen chemical shifts from the whole side chain of the previous residue are visible
Hγ
Hβ2
Hβ1
Hα
Keep in mind that if the signal / noise ratio is not very good, then not all peaks may be visible – such as here were only the Hβs and not the Hα are visible

HCC(CO)NH

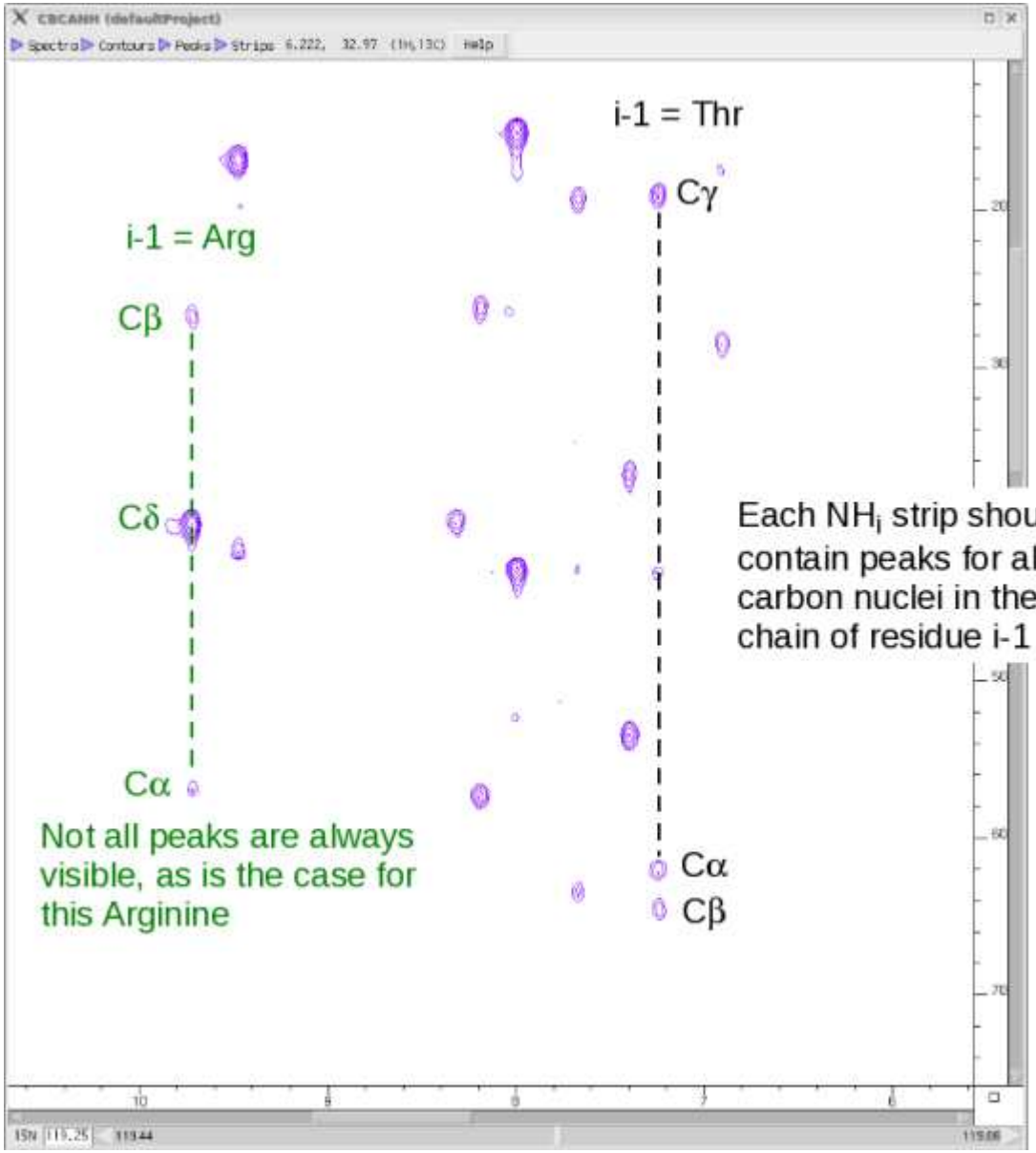
The Hα and Hβ are visible in both the HBHA(CO)NH while the Hg is only visible in the H(CCCO)NH



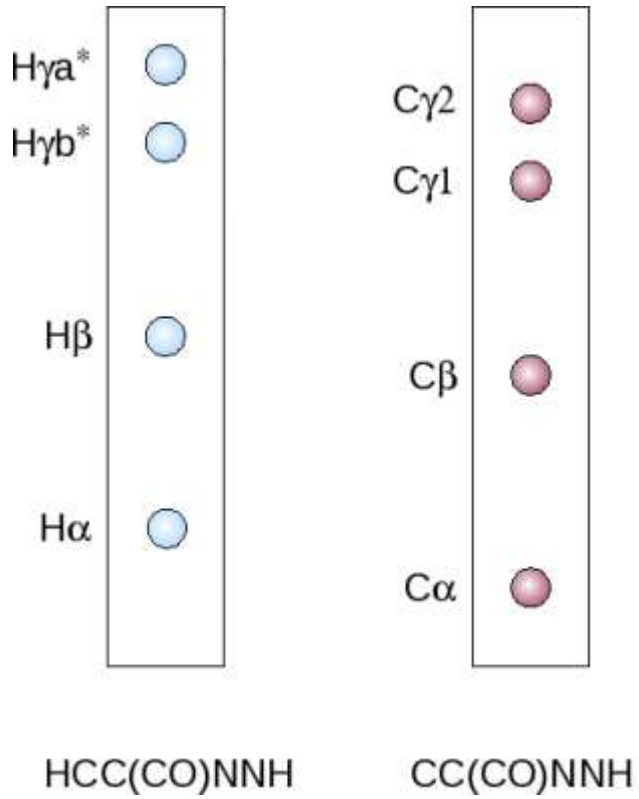
CC(CO)NH



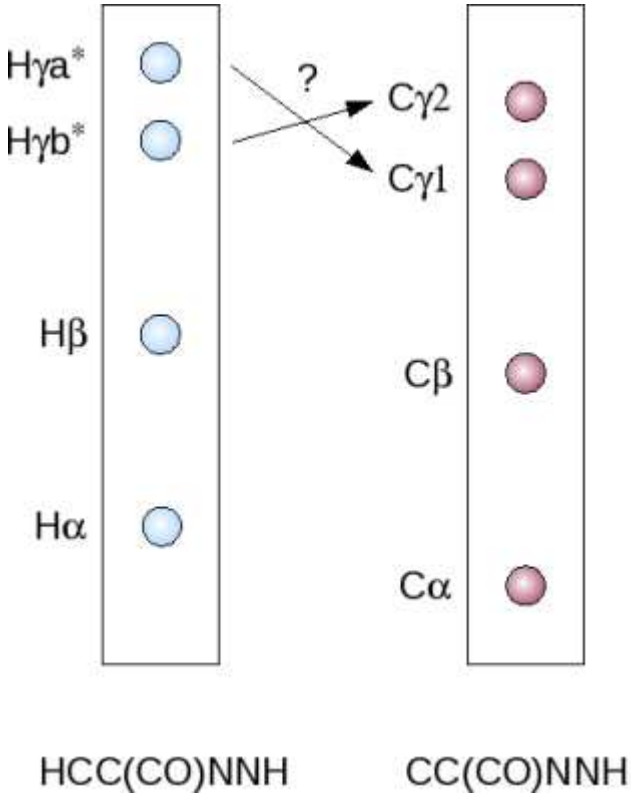
CC(CO)NH

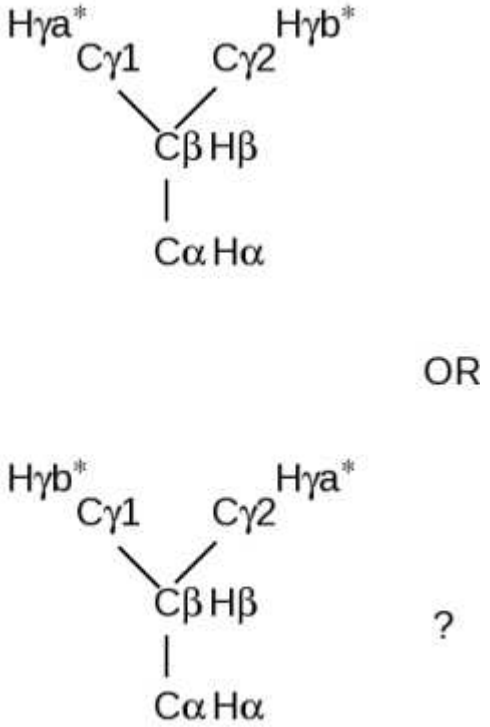

i-1 = Thr
Cγ
i-1 = Arg
Cβ
Cδ
Cα
Not all peaks are always visible, as is the case for this Arginine
Cα
Cβ
Each NHi strip should contain peaks for all carbon nuclei in the side-chain of residue i-1

Ambiguities

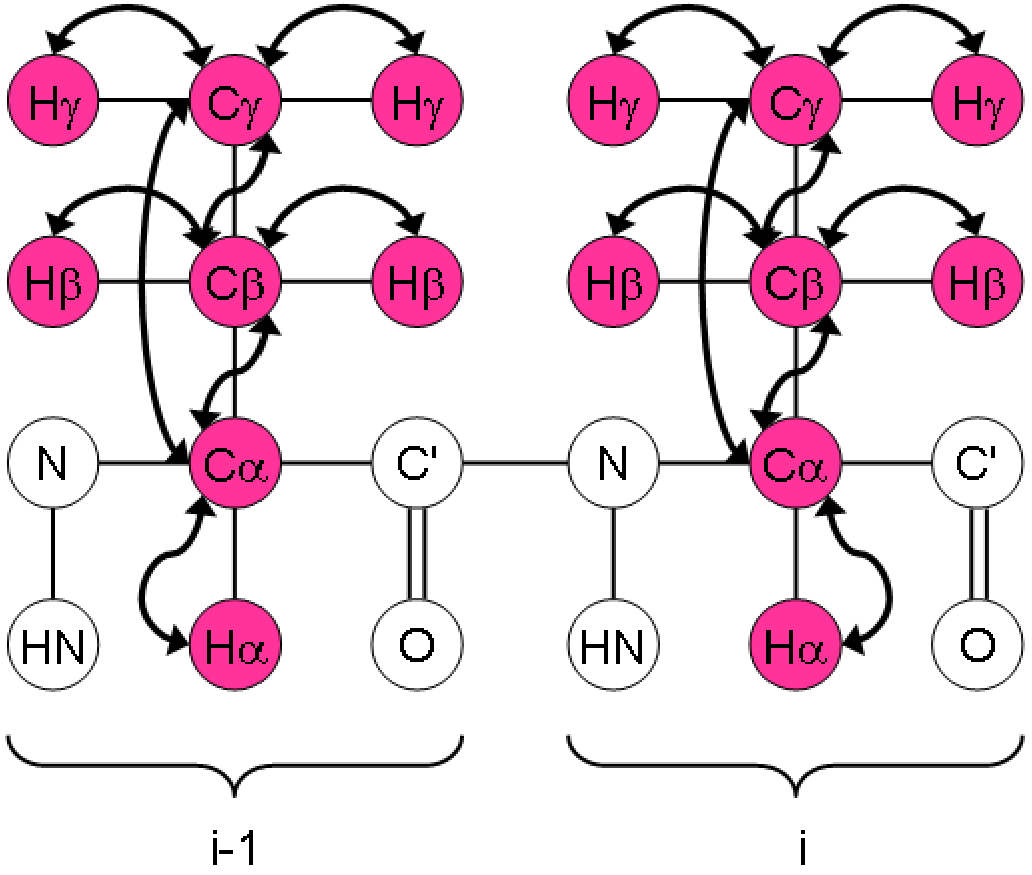


Ambiguities



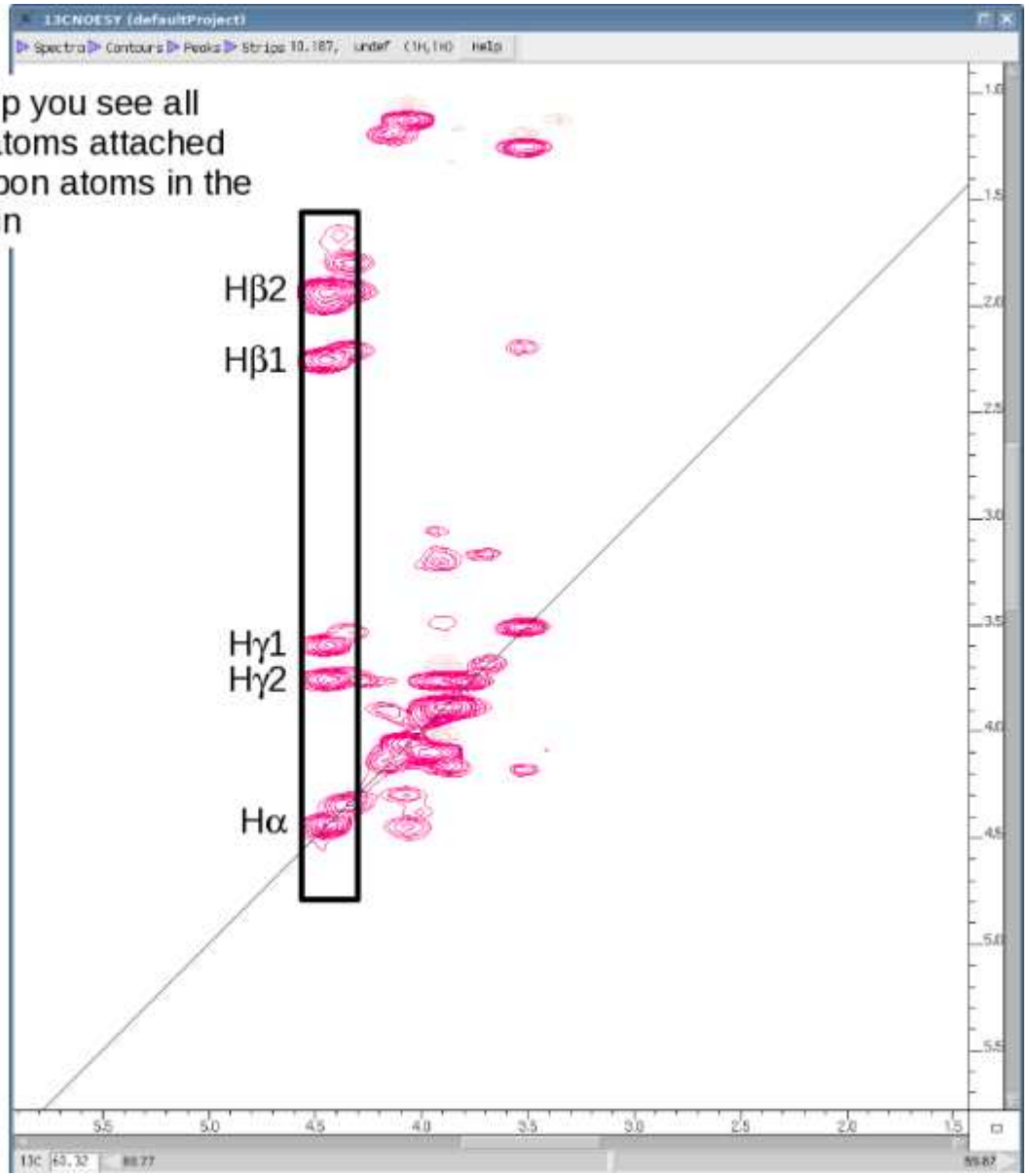


HCCH-TOCSY

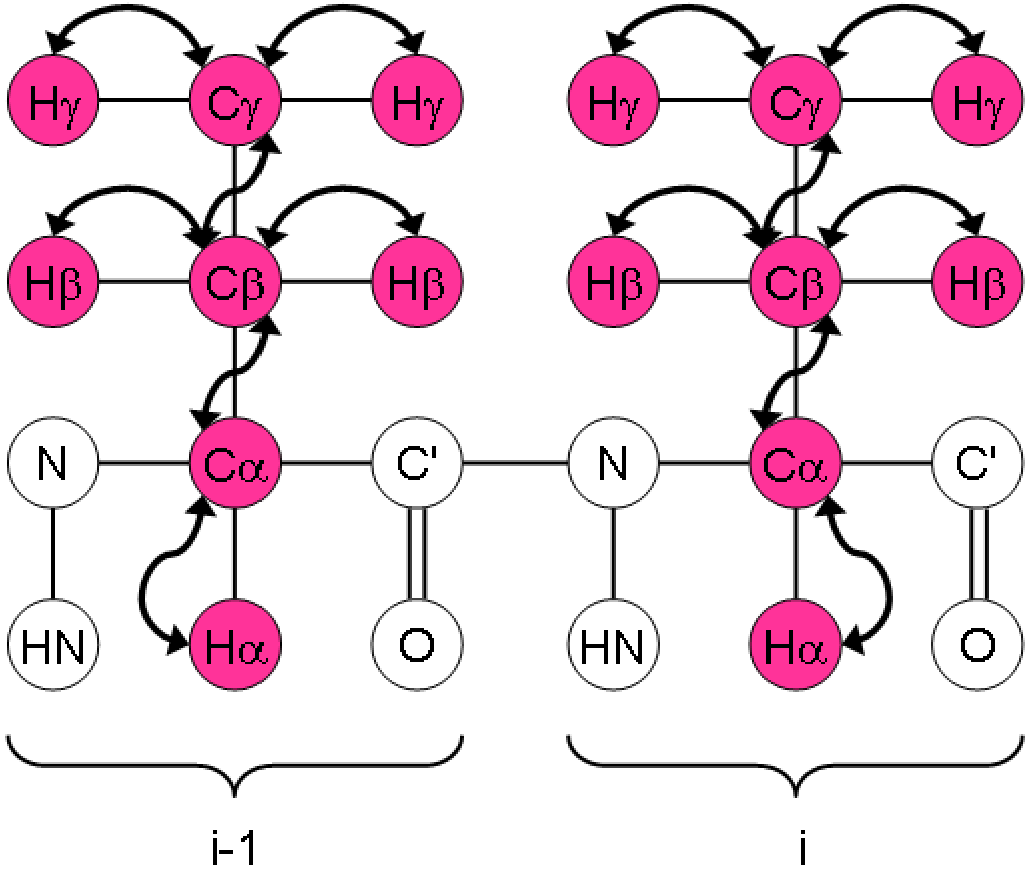


HCCH-TOCSY

In each CH strip you see all the hydrogen atoms attached to all other carbon atoms in the same side chain

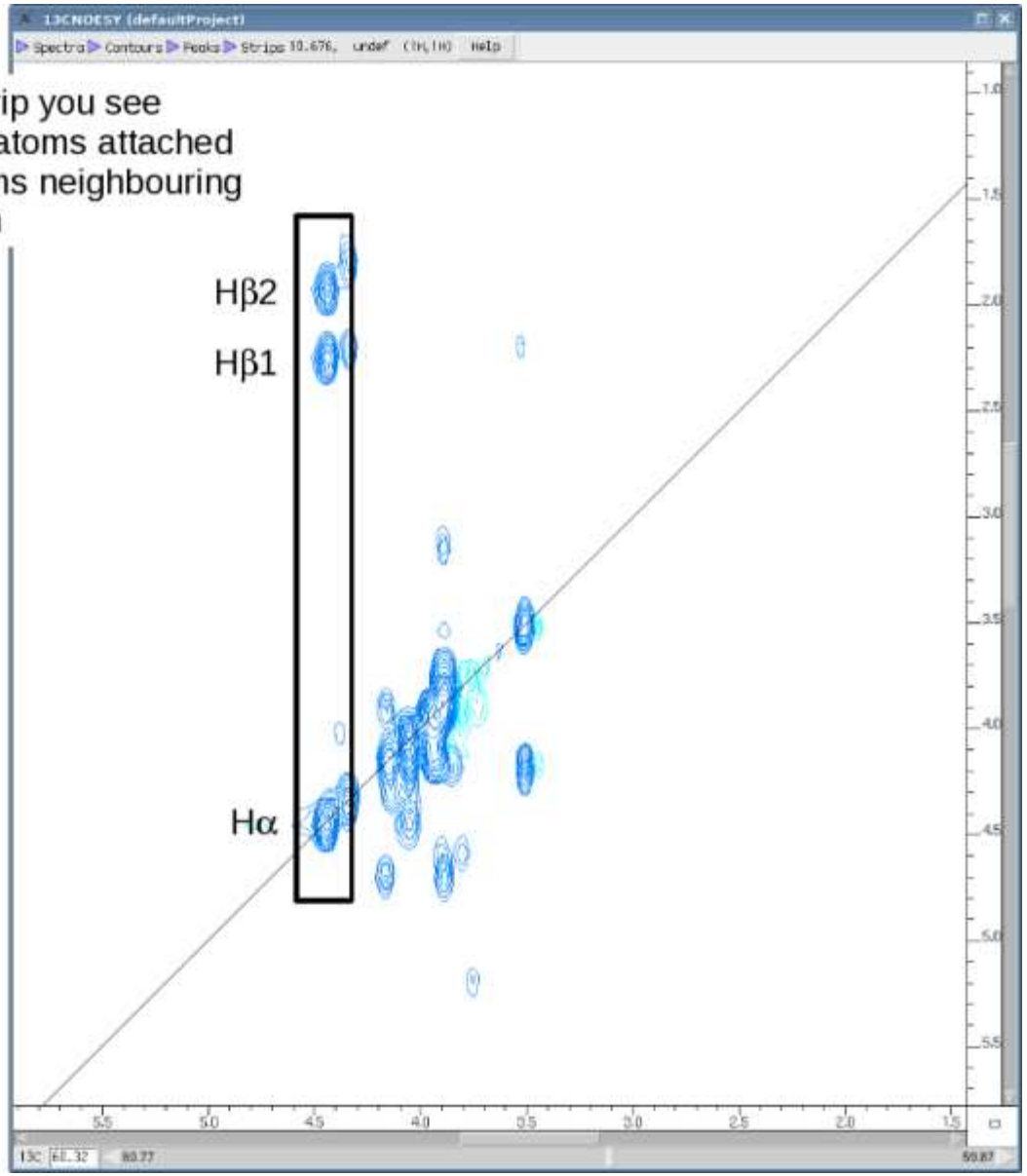


HCCH-COSY

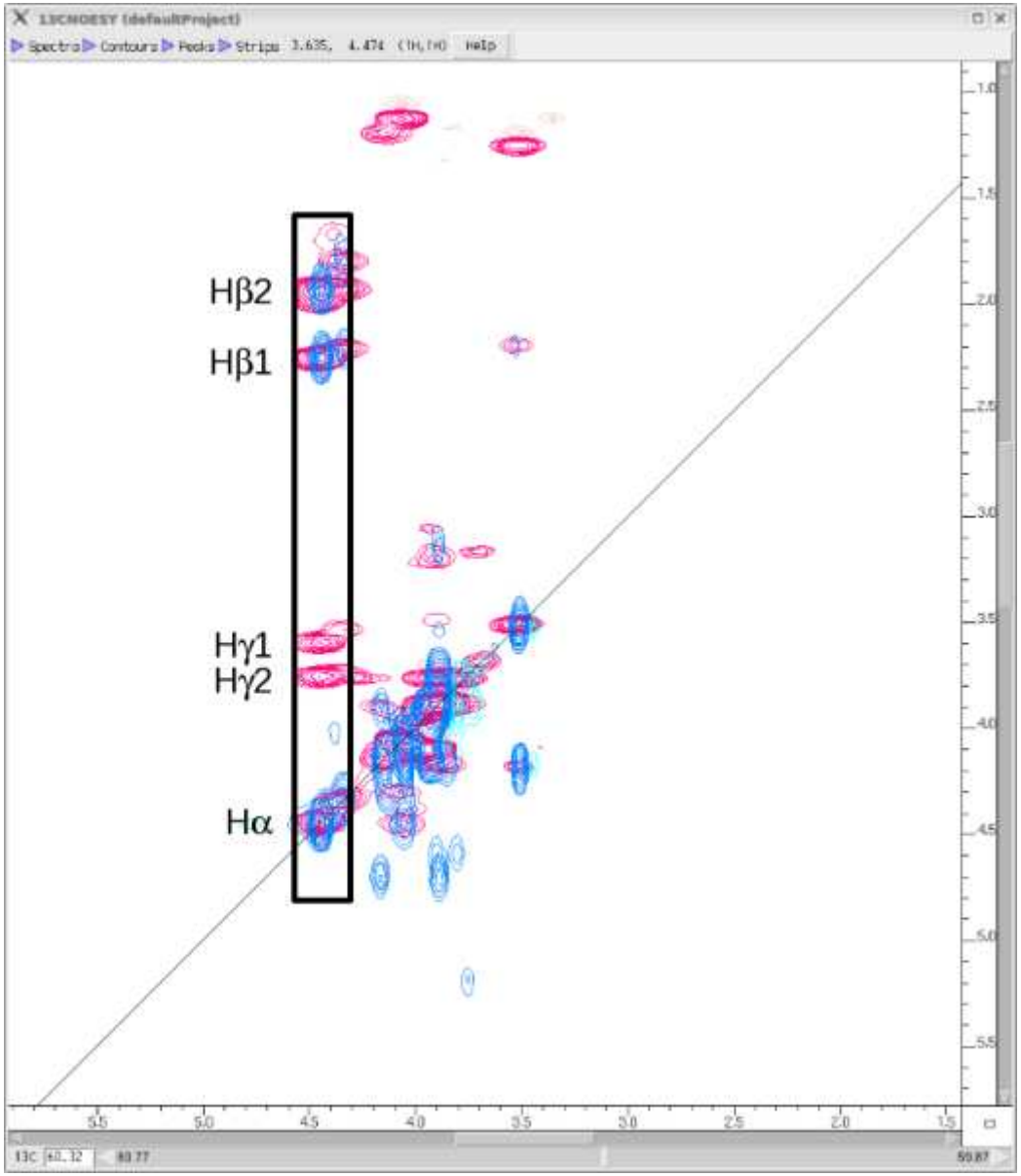


HCCH-COSY

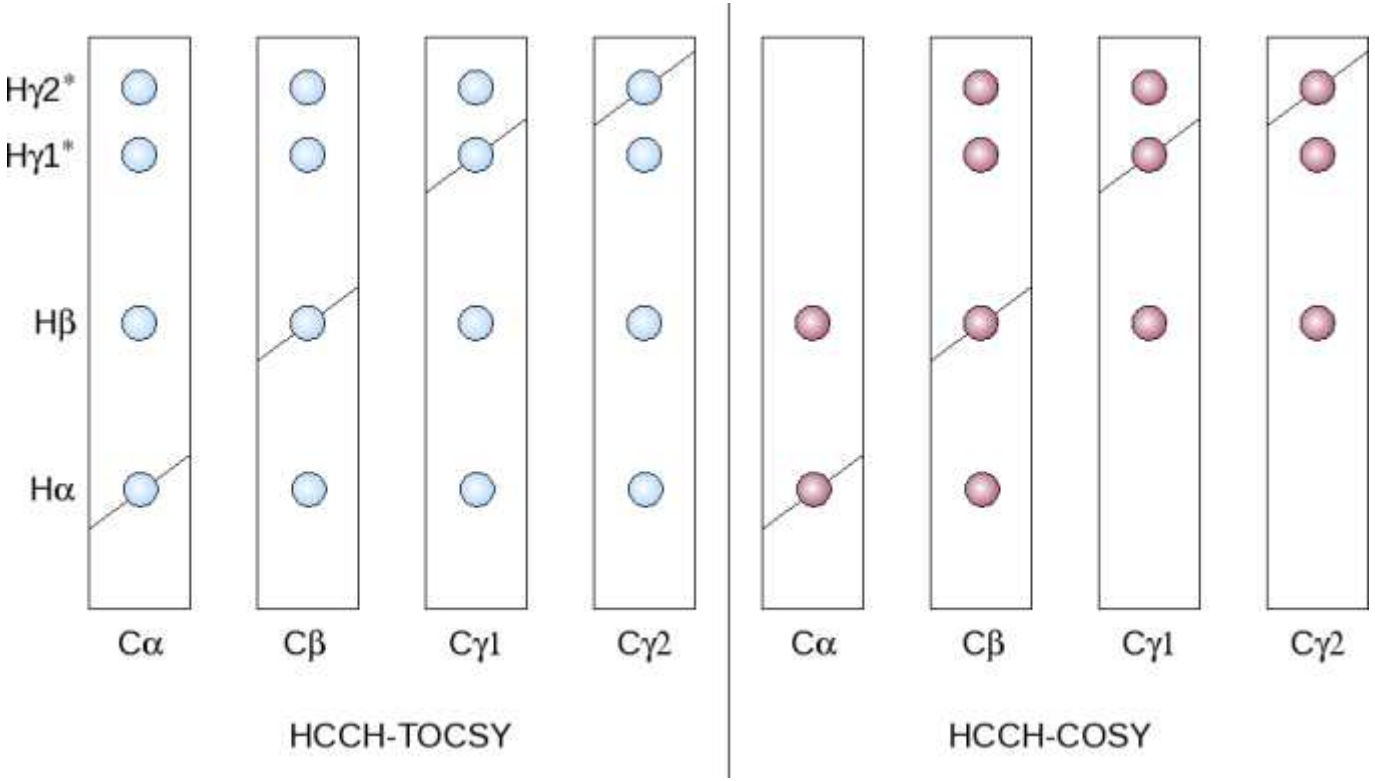
In each CH strip you see
the hydrogen atoms attached
to carbon atoms neighbouring
the CH carbon



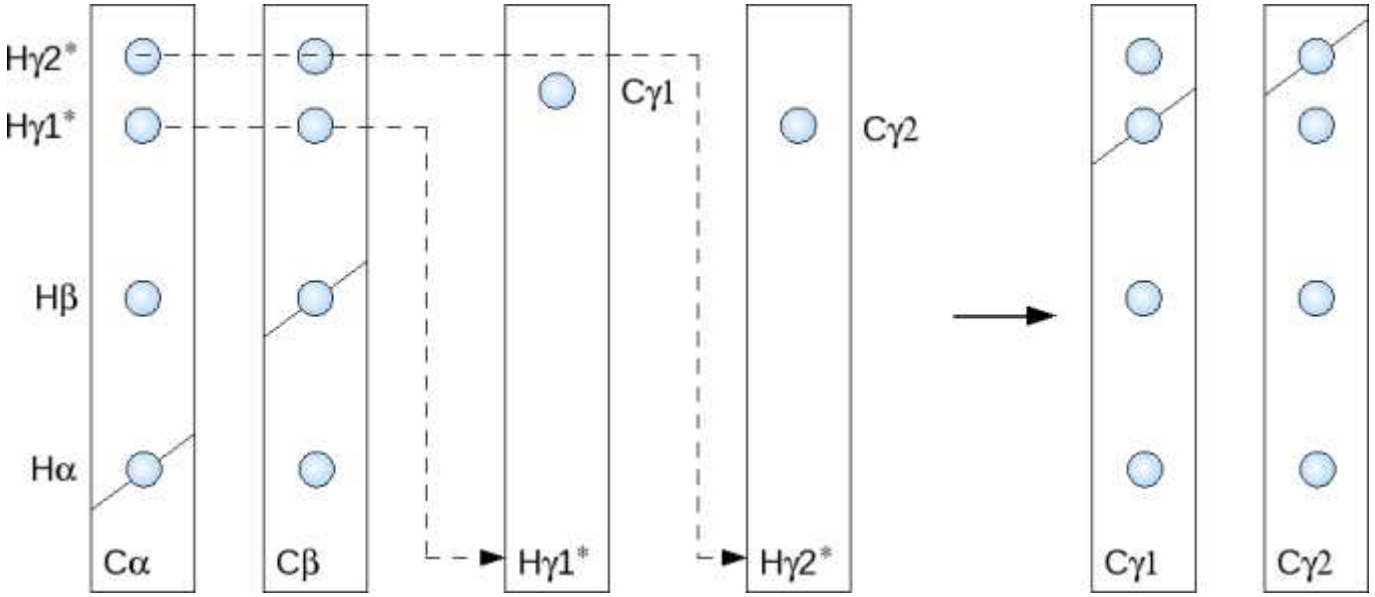
HCCH-COSY



Side-chain Assignment



Side-chain Assignment



Outline

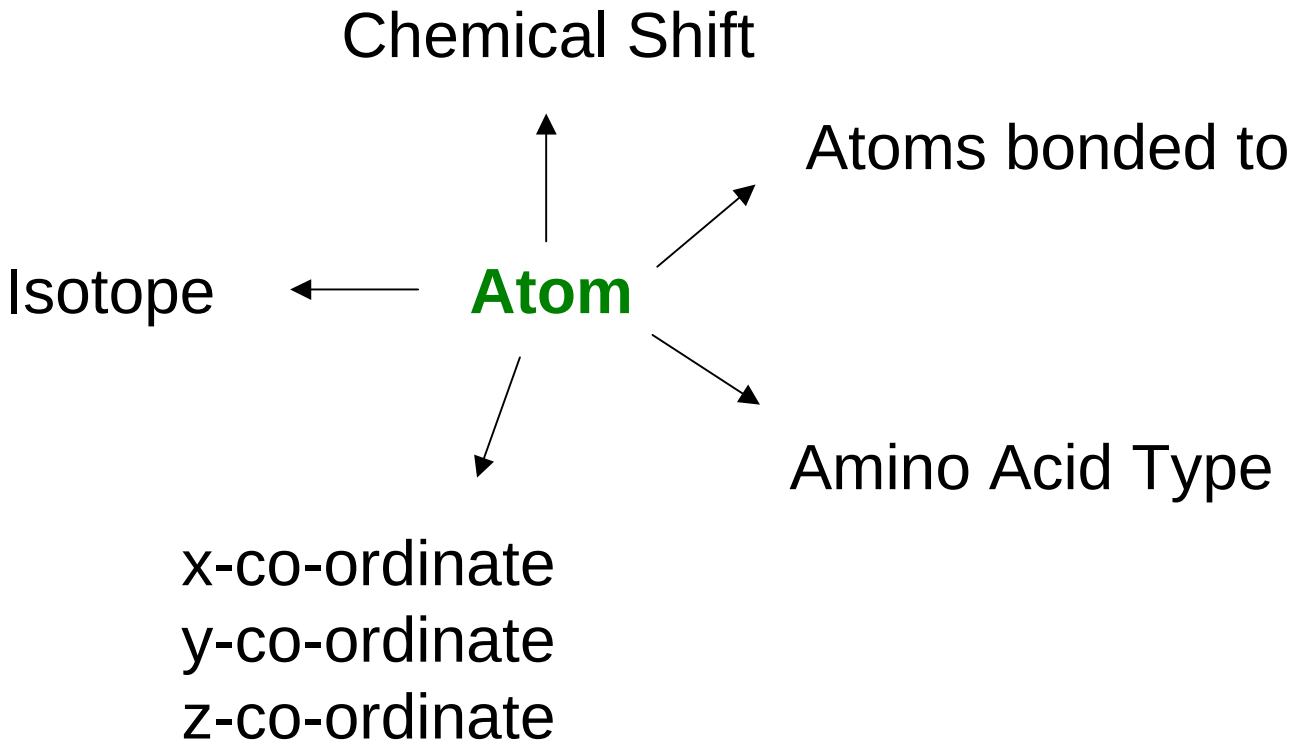
- Theory – Backbone and side-chain resonance assignment
- CCPNmr Analysis Software
- Practice – Using CCPNmr Analysis to assign a protein

CCPN

- Collaborative Computing Project for NMR

- CCPNmr Analysis – Assignment Software
- Format Converter
- EXTEND-NMR – interface for numerous different NMR software packages

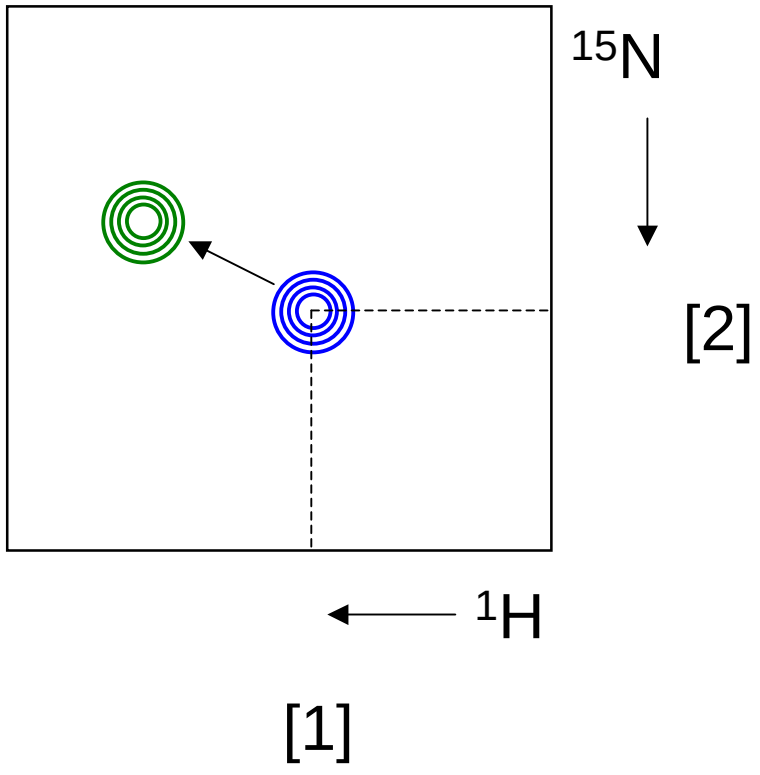
CCPN Data Model



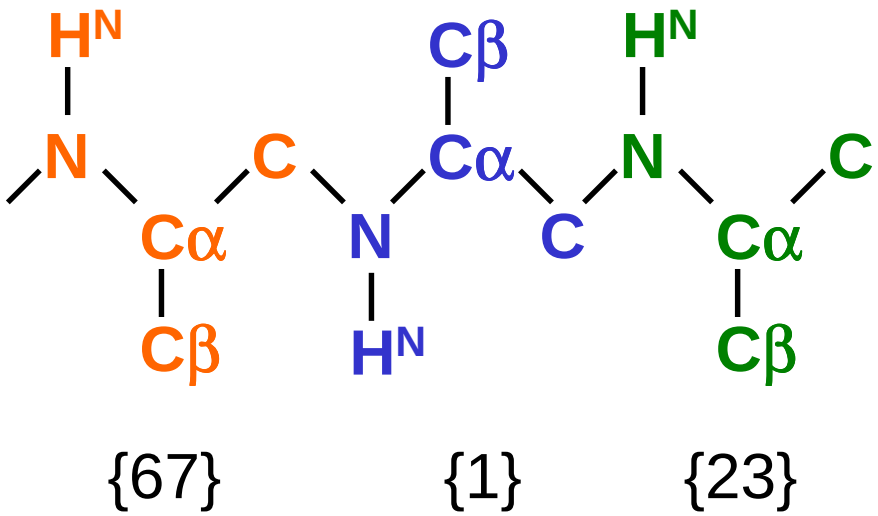
Resonance

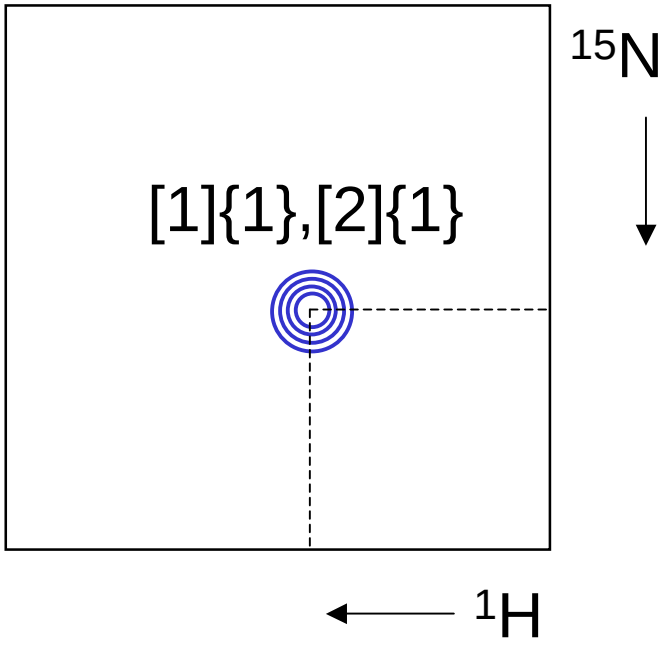
- don’t know which two atoms belong to the signal
- cannot define it by the chemical shifts
- do know that the ^{1}H and ^{15}N are directly bonded

→ use Resonance as a placeholder



Spin Systems



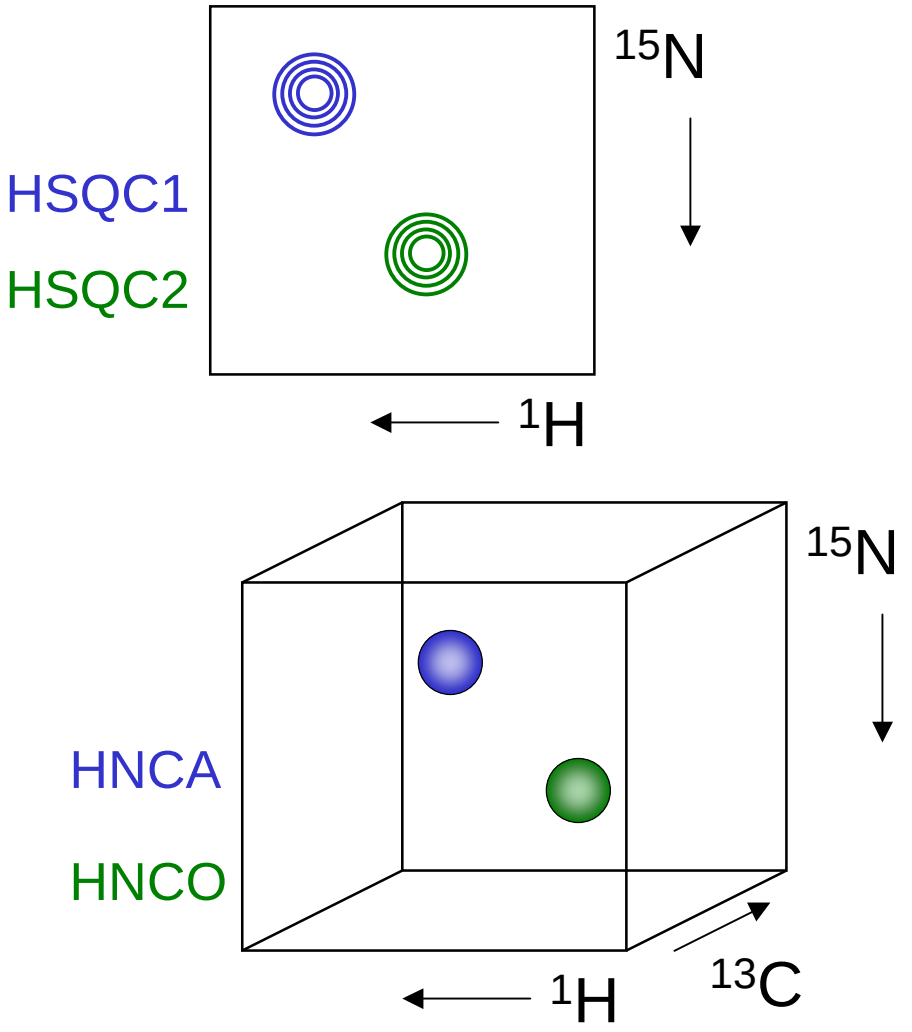


[Resonance**]** **{**Spin System**}**

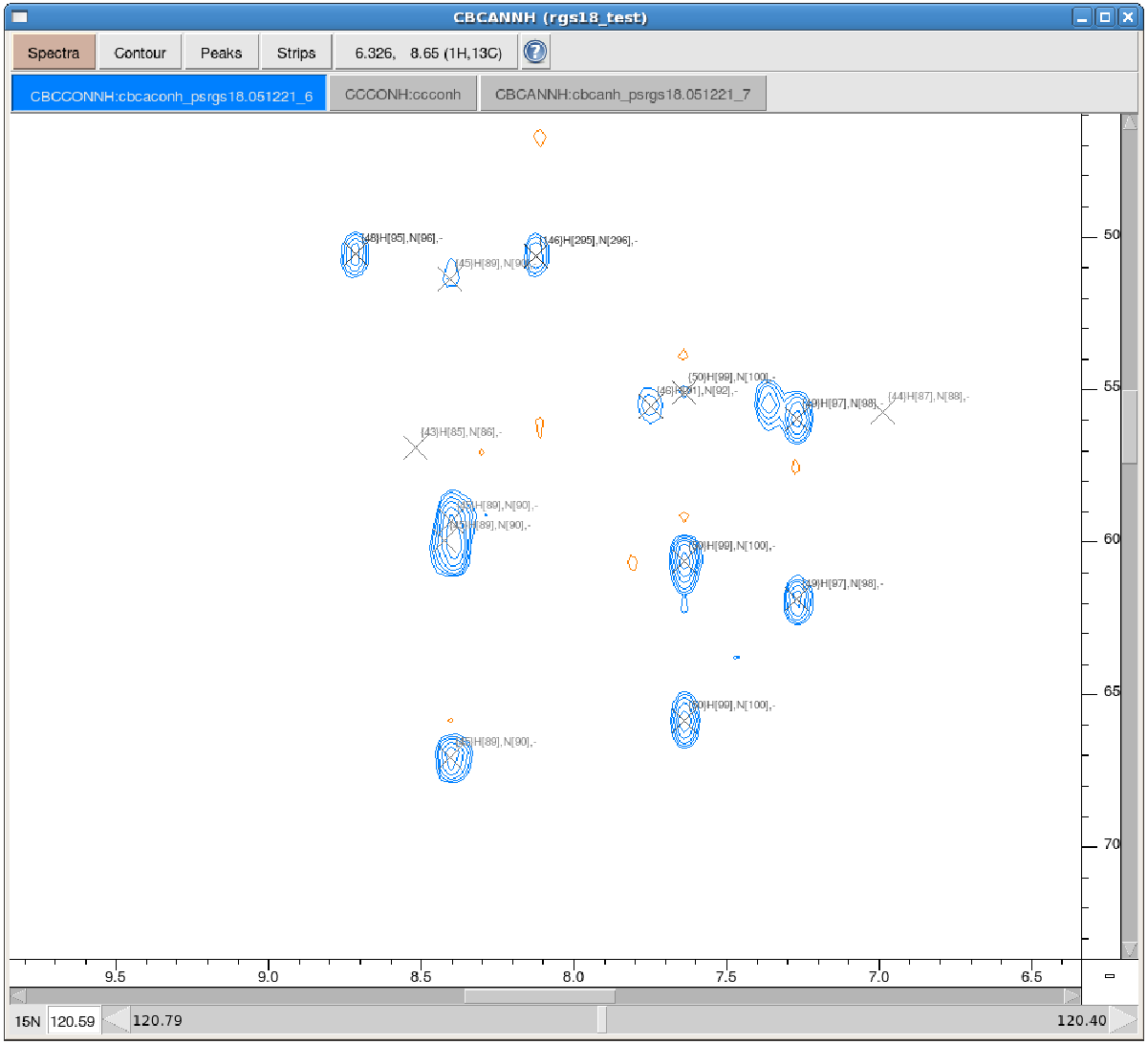
Windows

Each window

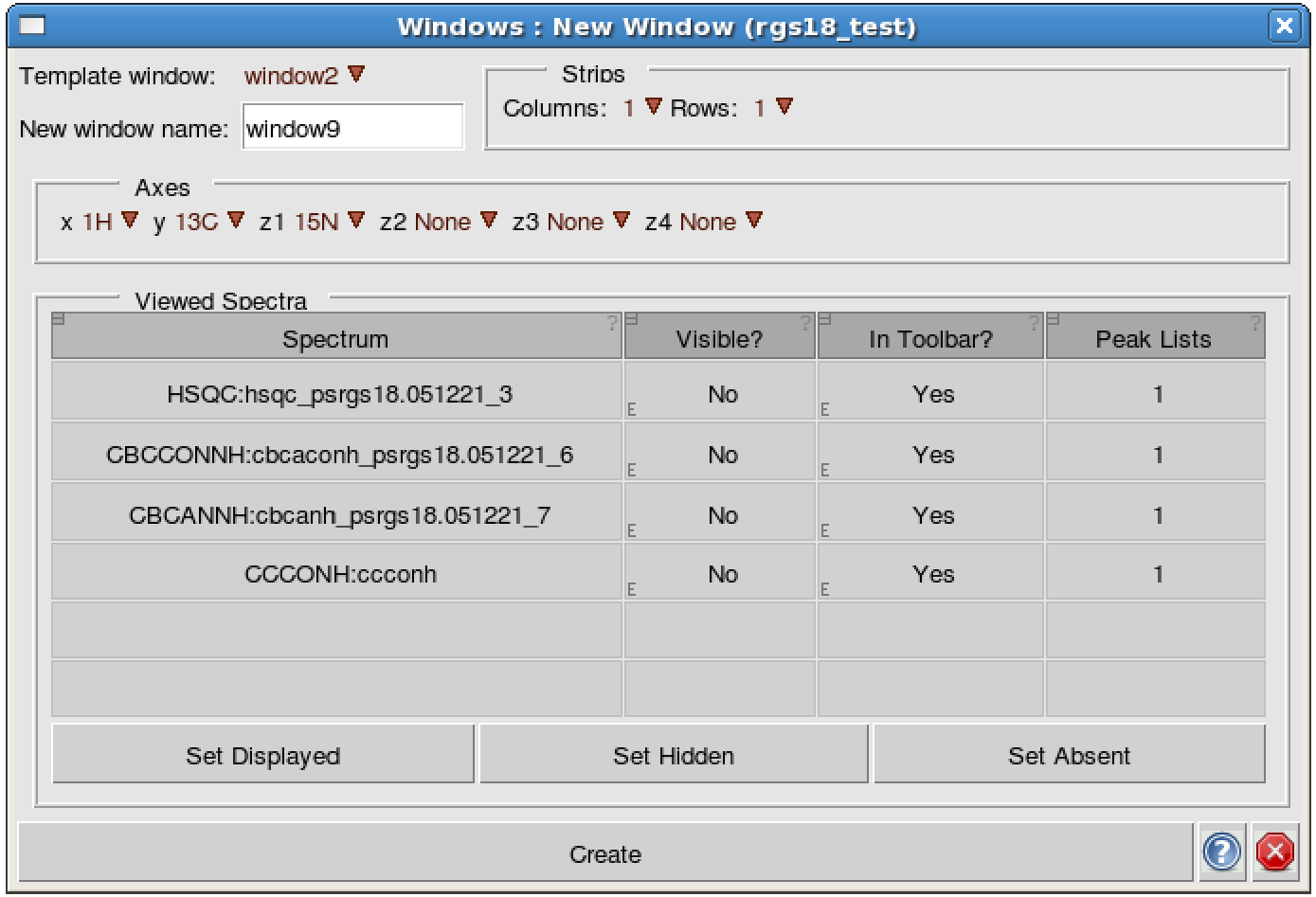
- has a defined set of isotope axes
- can show any spectrum which has those axes



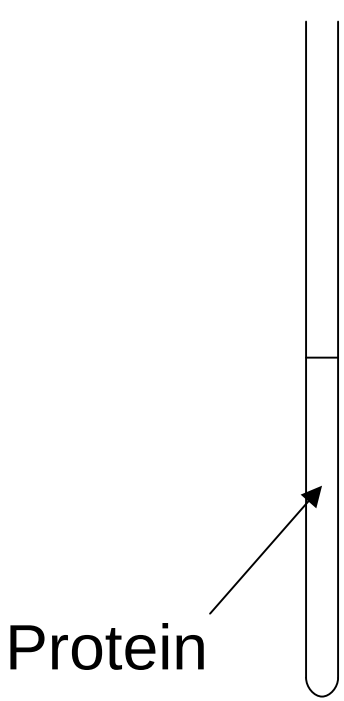
Windows


CBCANNH (rgs18_test)
Spectra
Contour
Peaks
Strips
6.326, 8.65 (1H,13C)
CBCCONNH:cbcaconh_psrgs18.051221_6
CCCONH:ccconh
CBCANNH:cbcanh_psrgs18.051221_7
50
55
60
65
70
9.5
9.0
8.5
8.0
7.5
7.0
6.5
15N
120.59
120.79
120.40

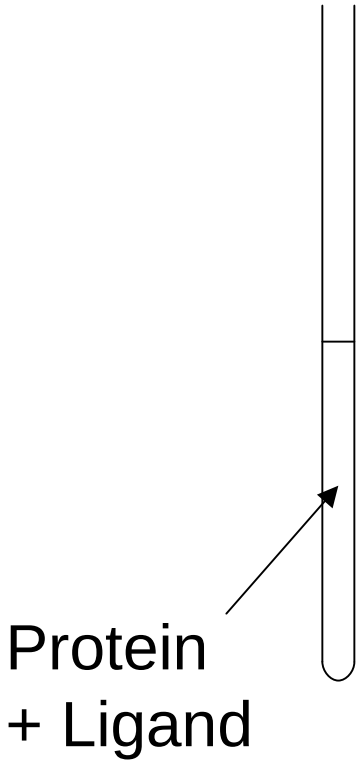
Windows


Windows : New Window (rgs18_test)
Template window: window2
New window name: window9
Strips
Columns: 1 Rows: 1
Axes
x 1H y 13C z1 15N z2 None z3 None z4 None
Viewed Spectra
Spectrum
Visible?
In Toolbar?
Peak Lists
HSQC:hsqc_psrgs18.051221_3 No Yes 1
CBCCONNH:cbcaconh_psrgs18.051221_6 No Yes 1
CBCANNH:cbcanh_psrgs18.051221_7 No Yes 1
CCCONH:ccconh No Yes 1
Set Displayed
Set Hidden
Set Absent
Create

Molecular System

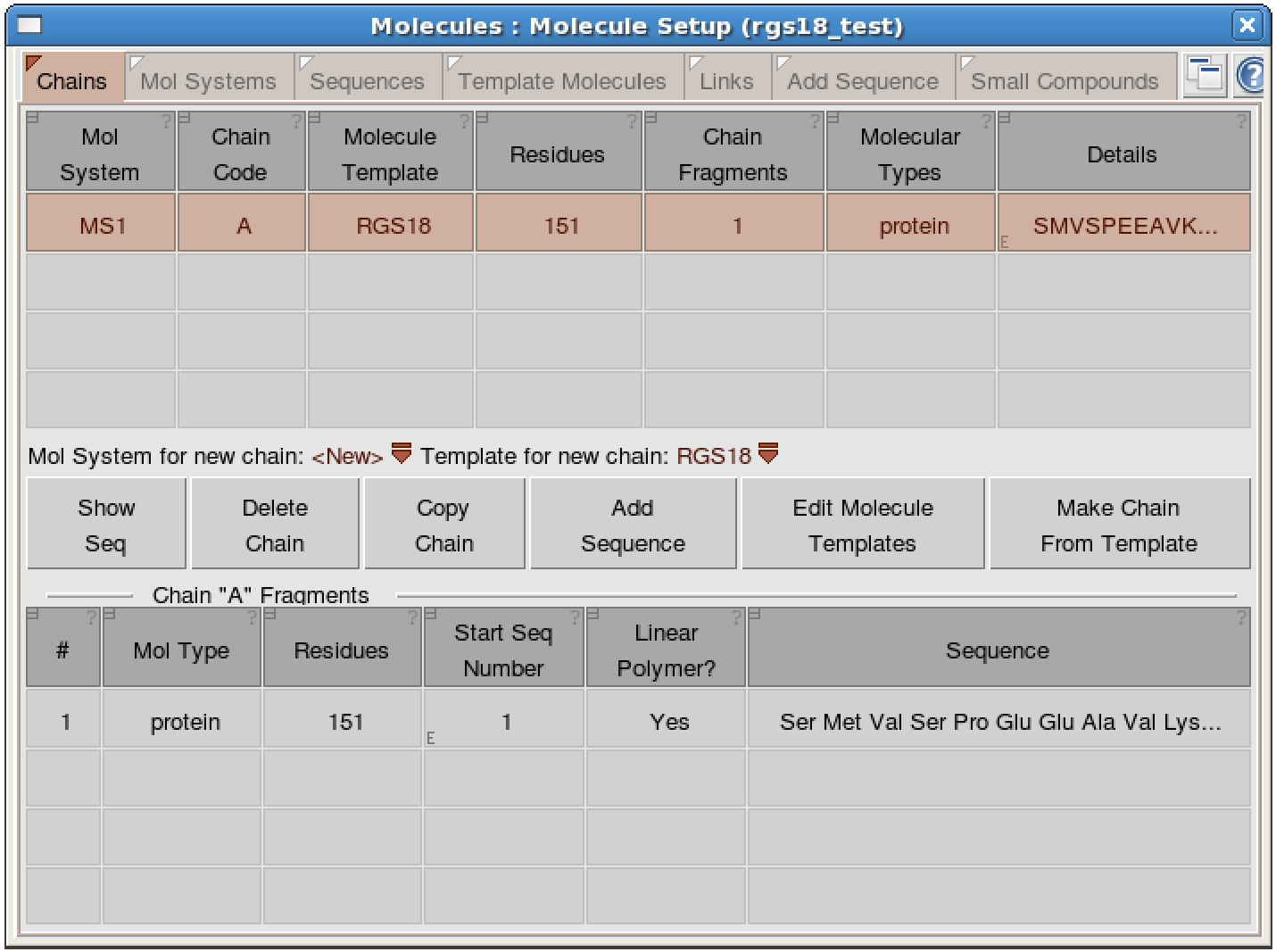


Molecular System 1

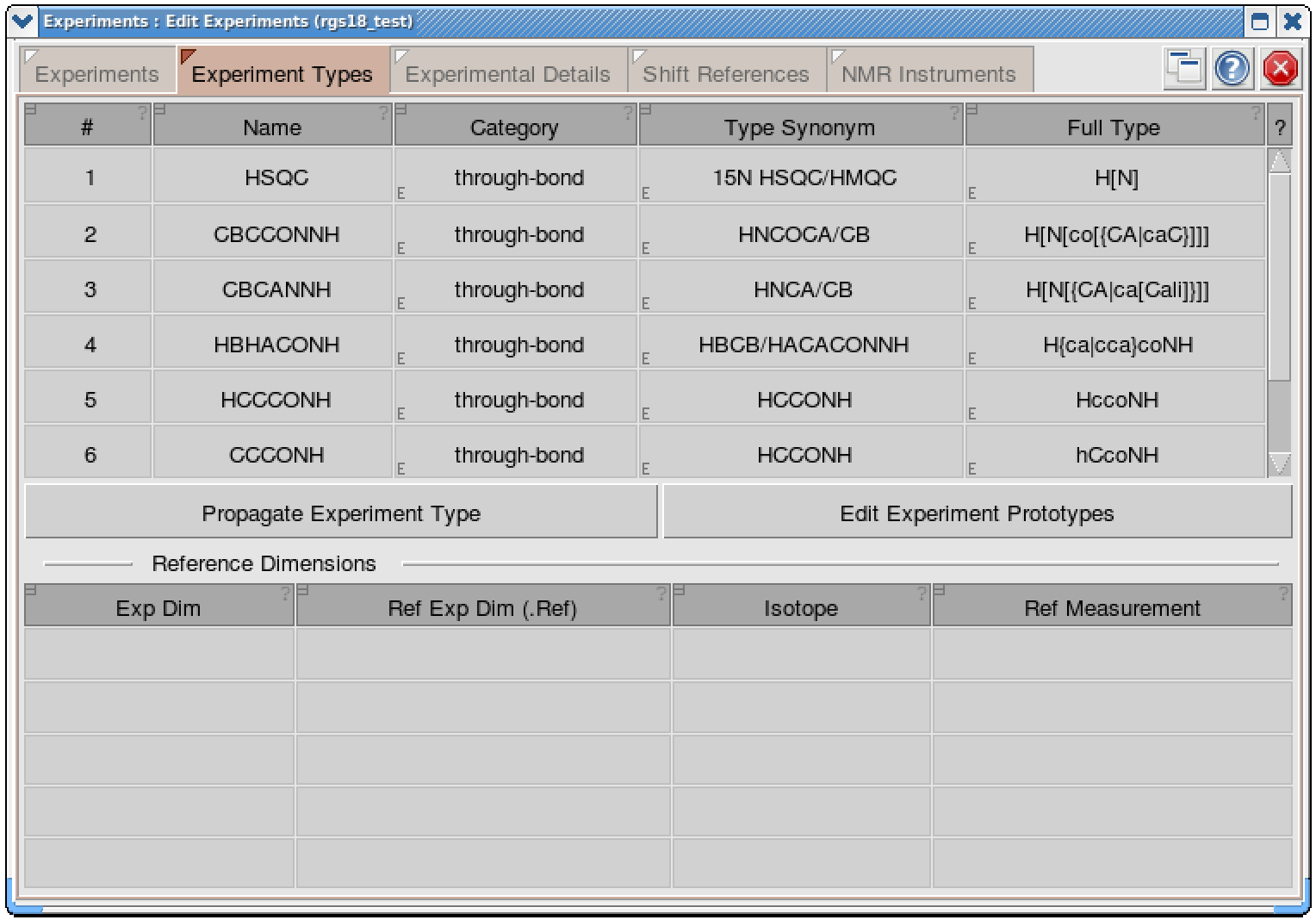


Molecular System 2

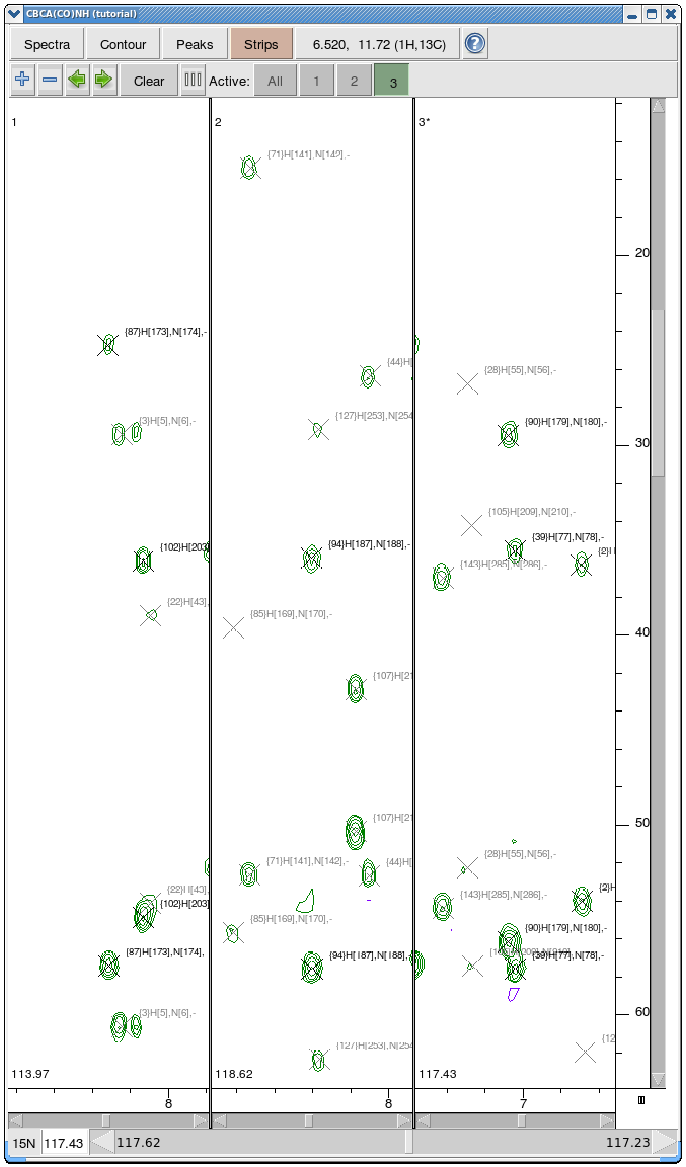
Molecular System



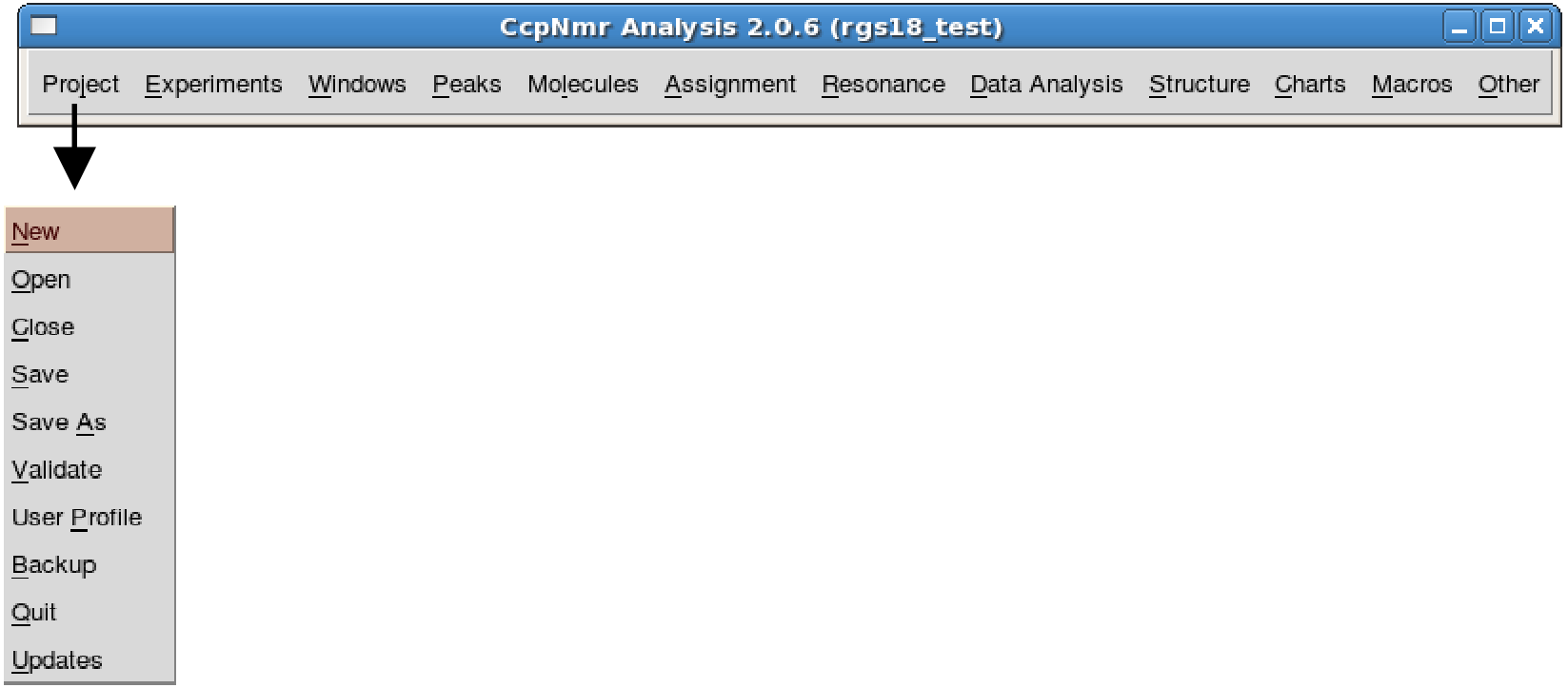
Experiment Types



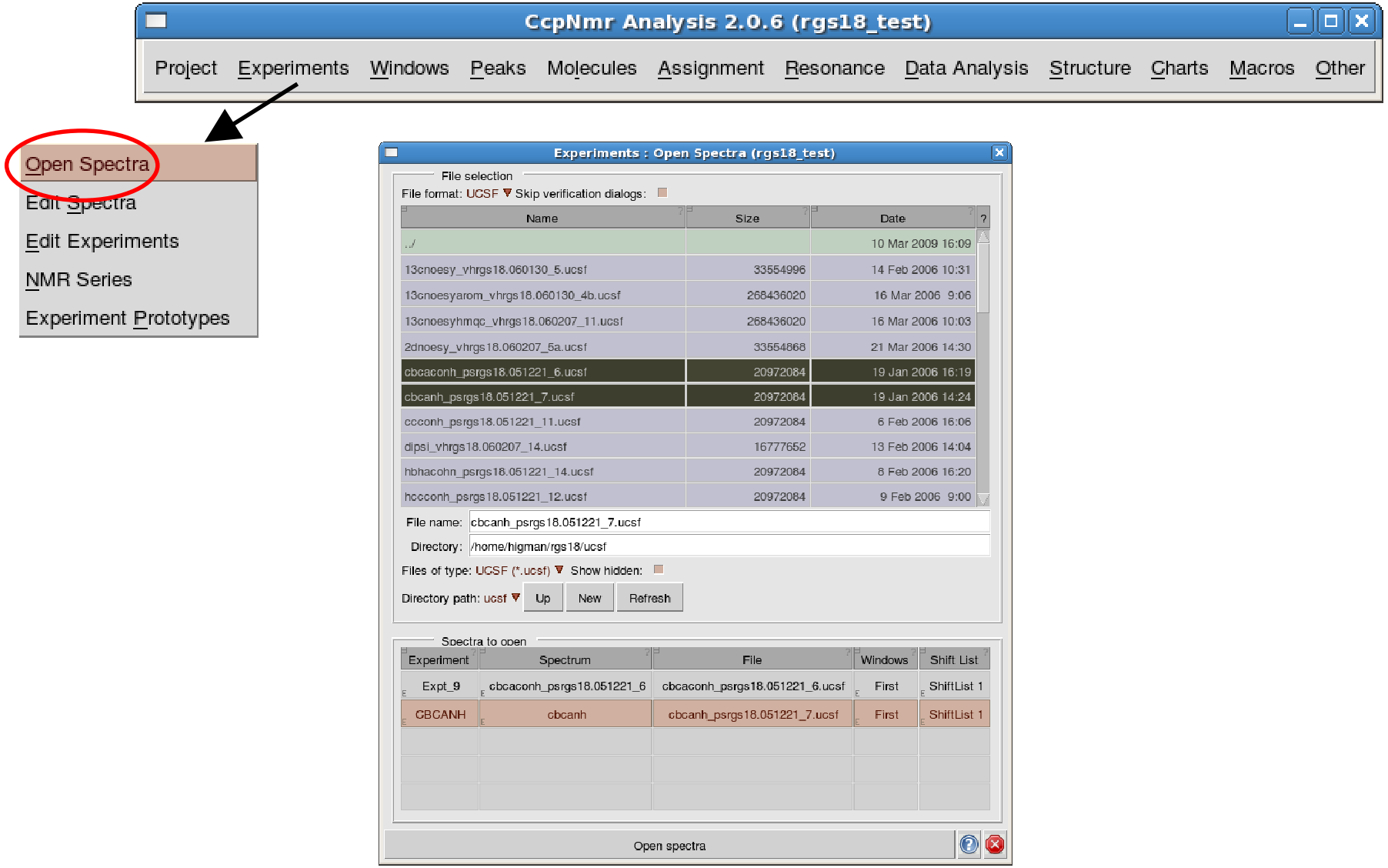
Strips


CBCA(CO)NH (tutorial)
Spectra
Contour
Peaks
Strips
6.520, 11.72 (1H, 13C)
Clear
Active:
All
1
2
3
1
2
3*
113.97
118.62
117.43
15N
117.43
117.62
117.23

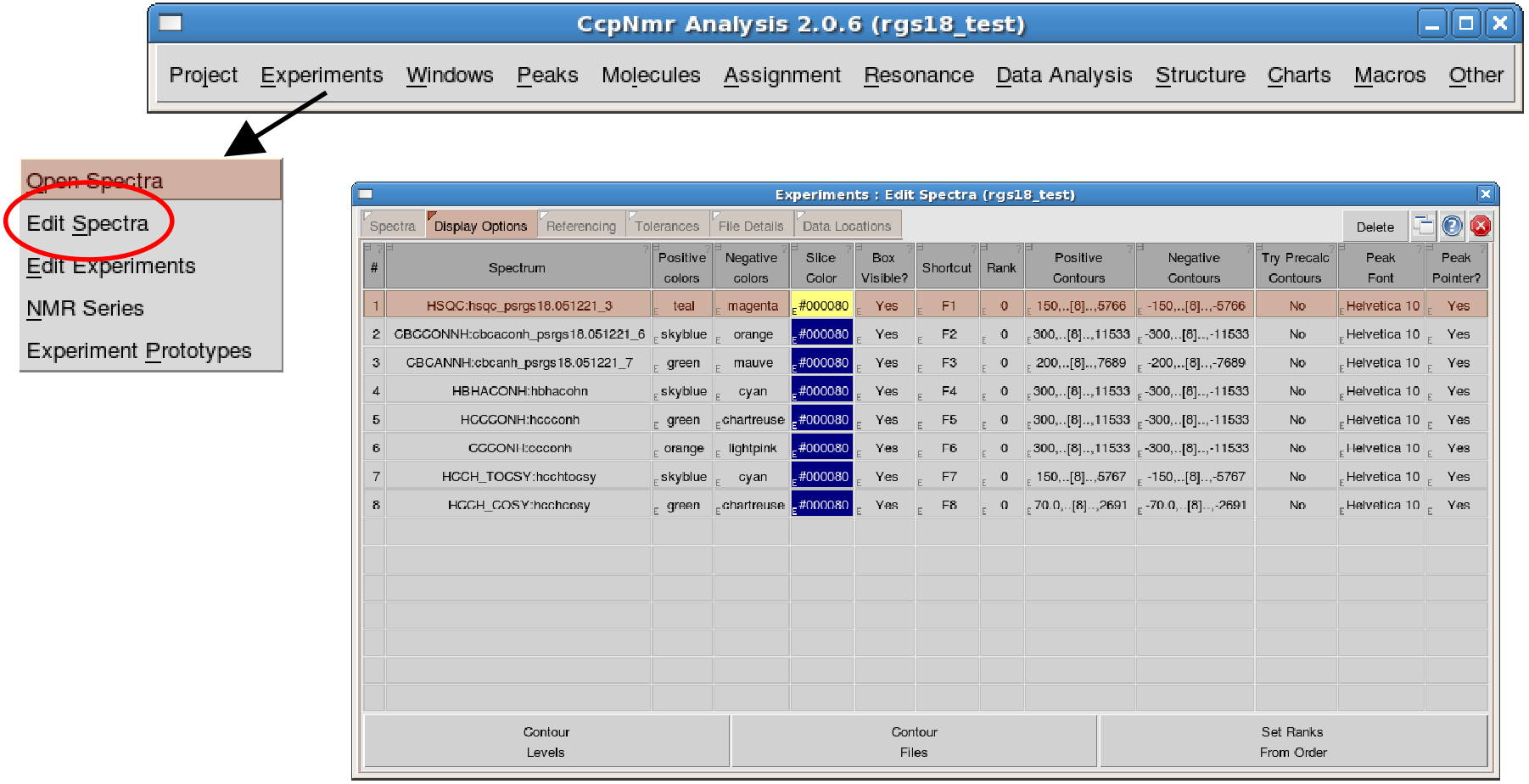
Project Menu



Experiments Menu

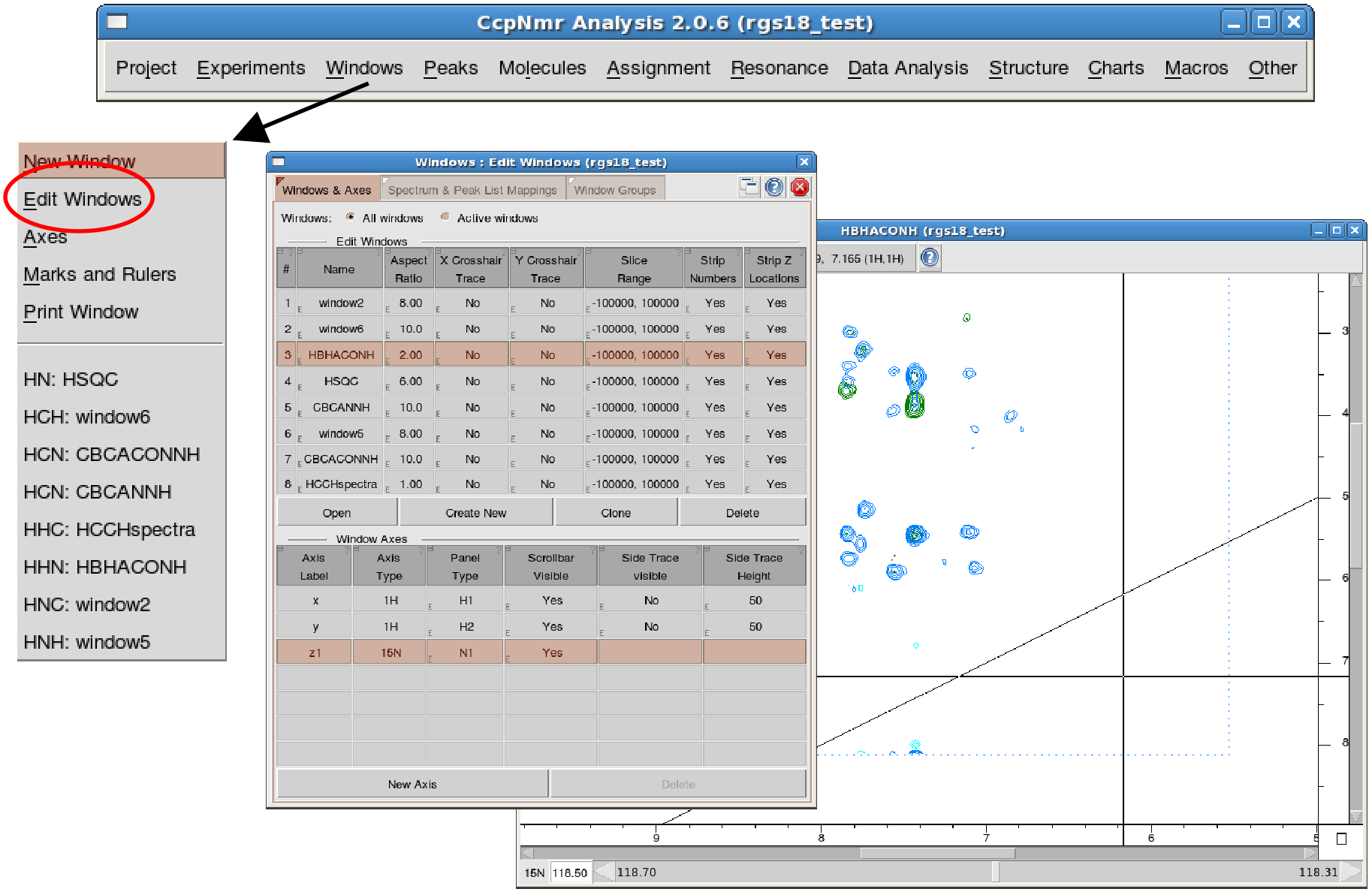


Experiments Menu

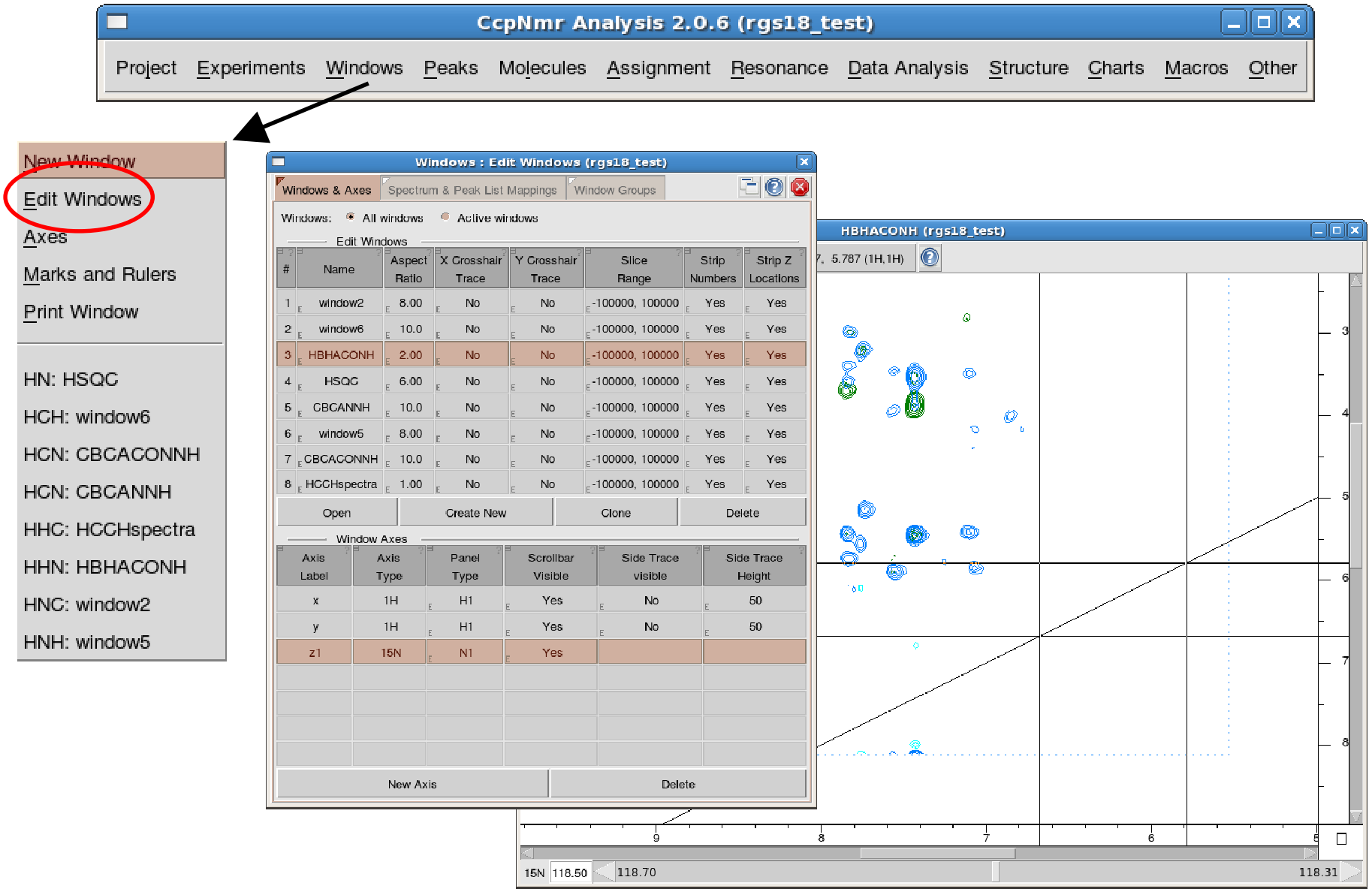


#	Spectrum	Positive colors	Negative colors	Slice Color	Box Visible?	Shortcut	Rank	Positive Contours	Negative Contours	Try Precalc Contours	Peak Font	Peak Pointer?
1	HSQC:hsqc_psrgs18.051221_3	teal	magenta	#000080	Yes	F1	0	150,..[8]..,5766	-150,..[8]..,-5766	No	Helvetica 10	Yes
2	CBCGCONNH:cbcaconh_psrgs18.051221_6	skyblue	orange	#000080	Yes	F2	0	300,..[8]..,11533	-300,..[8]..,-11533	No	Helvetica 10	Yes
3	CBCANNH:cbcanh_psrgs18.051221_7	green	mauve	#000080	Yes	F3	0	200,..[8]..,7689	-200,..[8]..,-7689	No	Helvetica 10	Yes
4	HBHACONH:hbhacohn	skyblue	cyan	#000080	Yes	F4	0	300,..[8]..,11533	-300,..[8]..,-11533	No	Helvetica 10	Yes
5	HCCGCONH:hccconh	green	chartreuse	#000080	Yes	F5	0	300,..[8]..,11533	-300,..[8]..,-11533	No	Helvetica 10	Yes
6	CCCONH:cccconh	orange	lightpink	#000080	Yes	F6	0	300,..[8]..,11533	-300,..[8]..,-11533	No	Helvetica 10	Yes
7	HCCH_TOCSY:hcchtocsy	skyblue	cyan	#000080	Yes	F7	0	150,..[8]..,5767	-150,..[8]..,-5767	No	Helvetica 10	Yes
8	HCCH_COSY:hcchcosy	green	chartreuse	#000080	Yes	F8	0	70.0,..[8]..,2691	-70.0,..[8]..,-2691	No	Helvetica 10	Yes

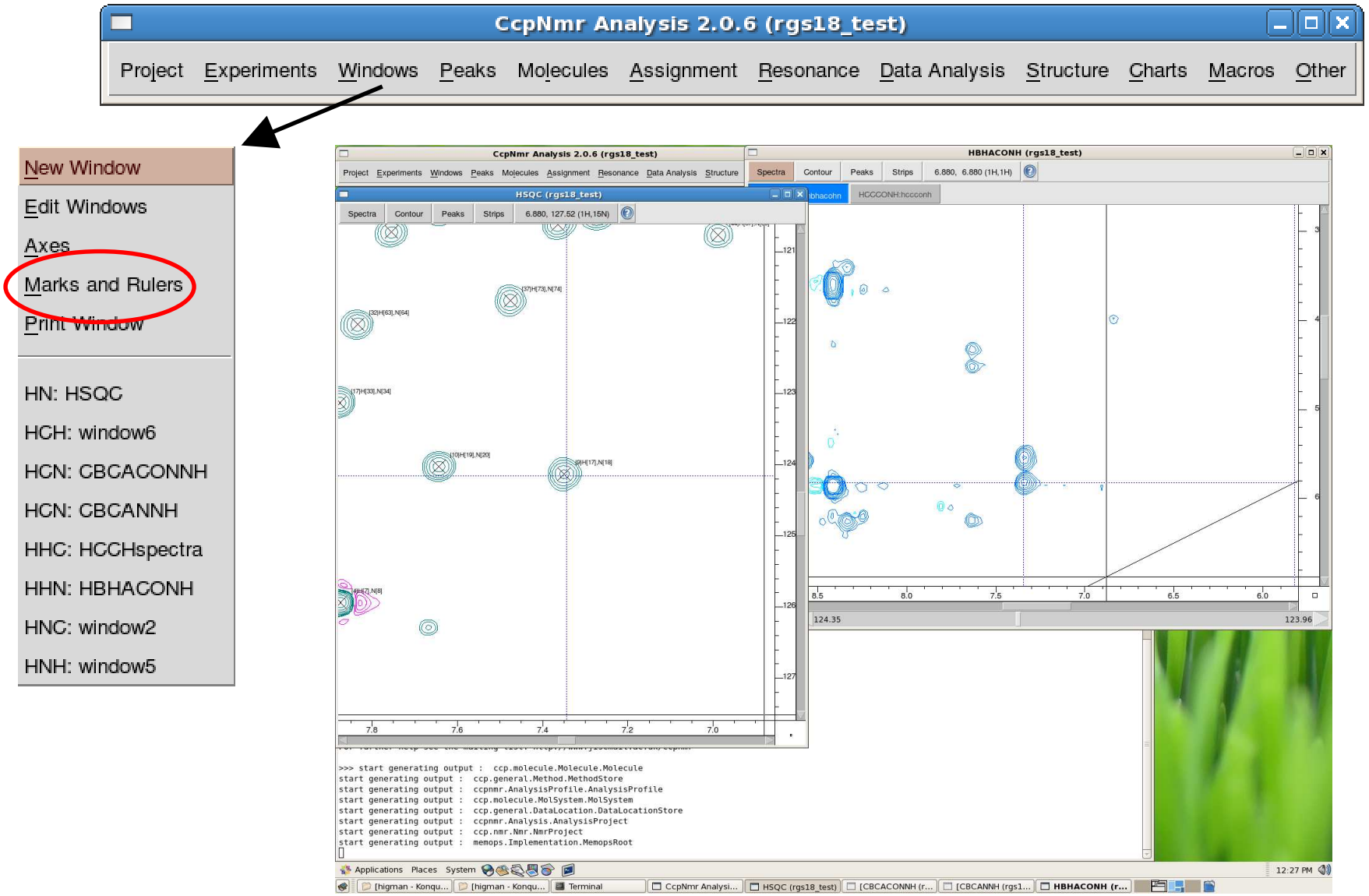
Windows Menu



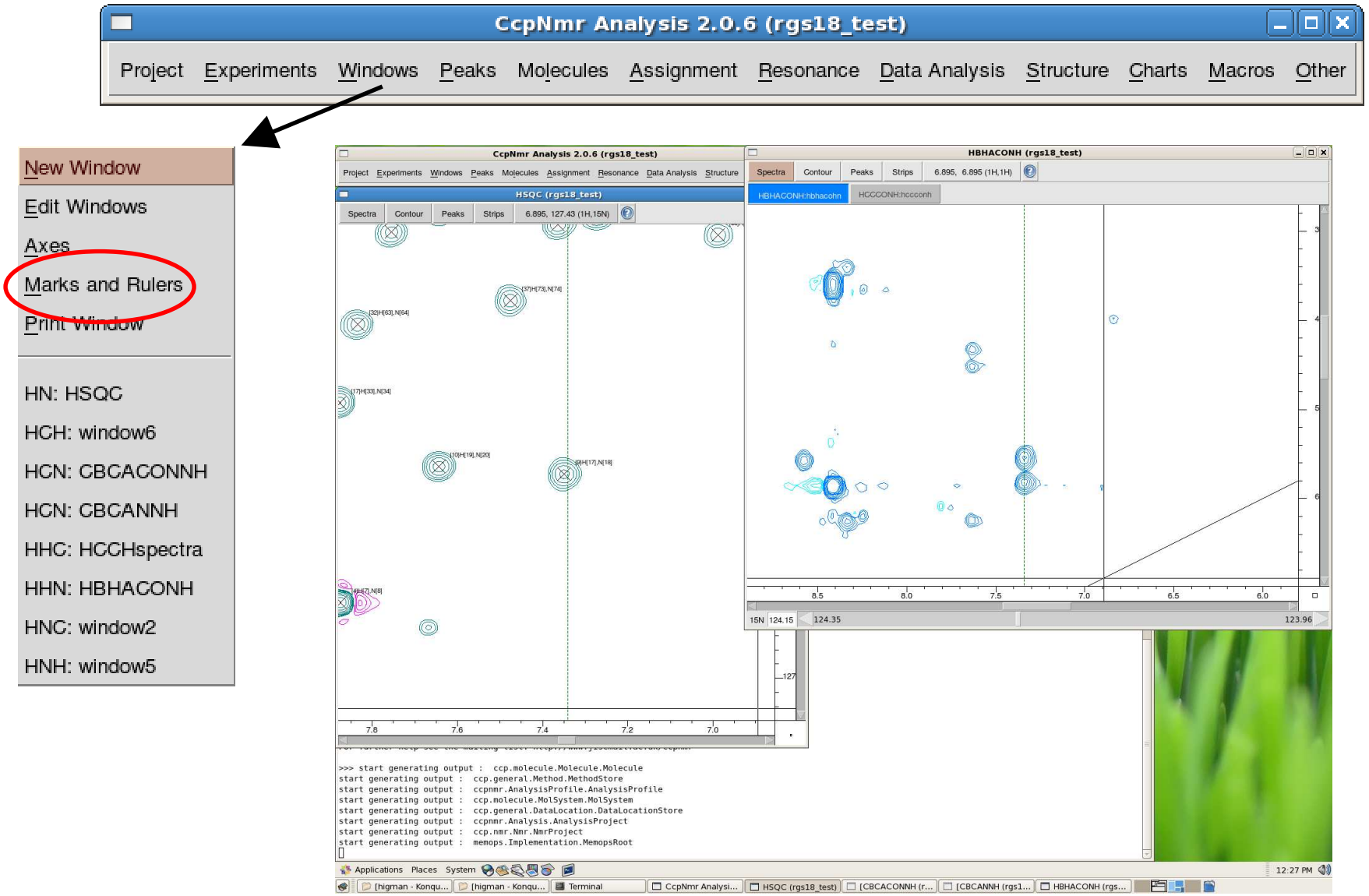
Windows Menu



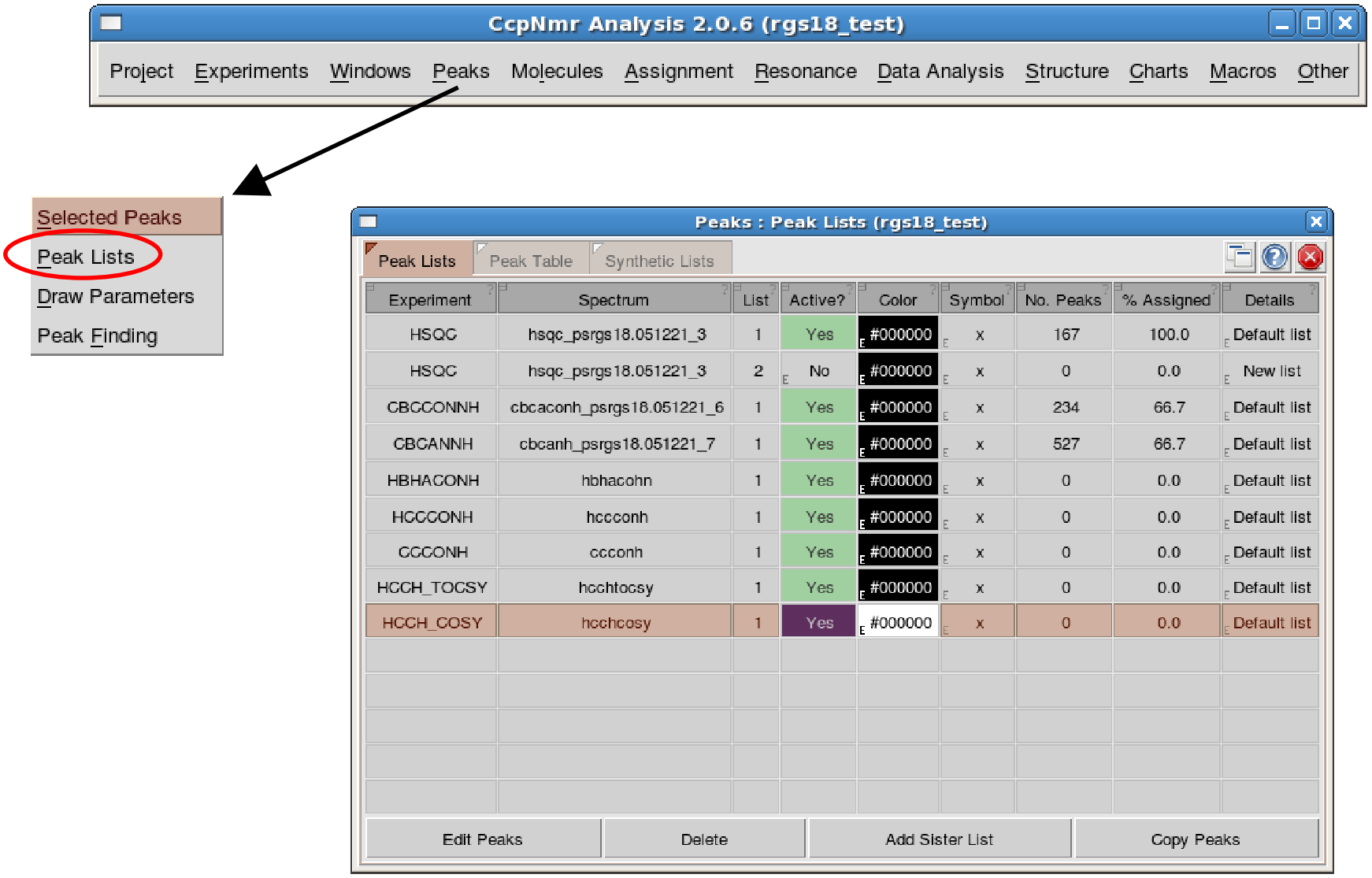
Windows Menu



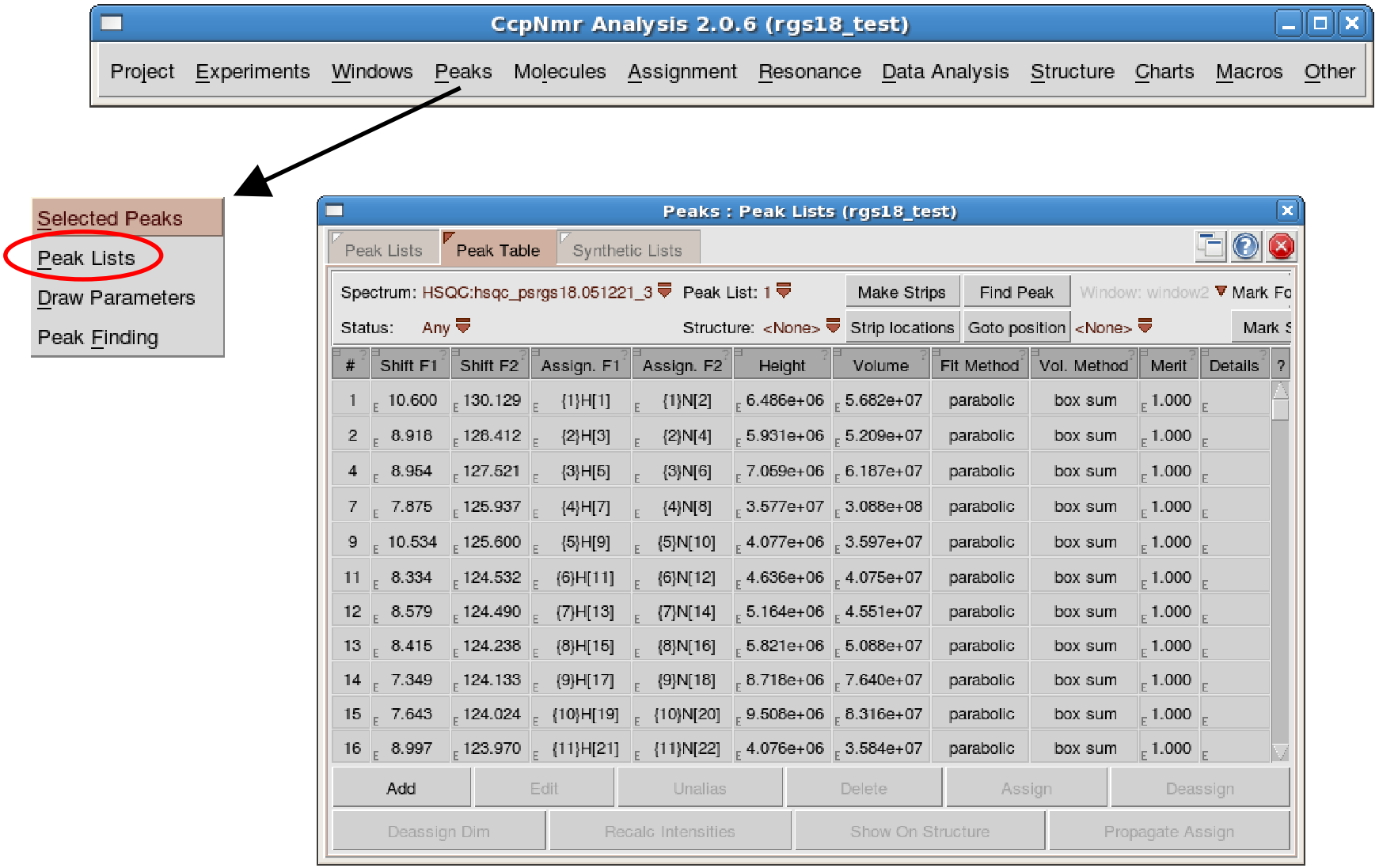
Windows Menu



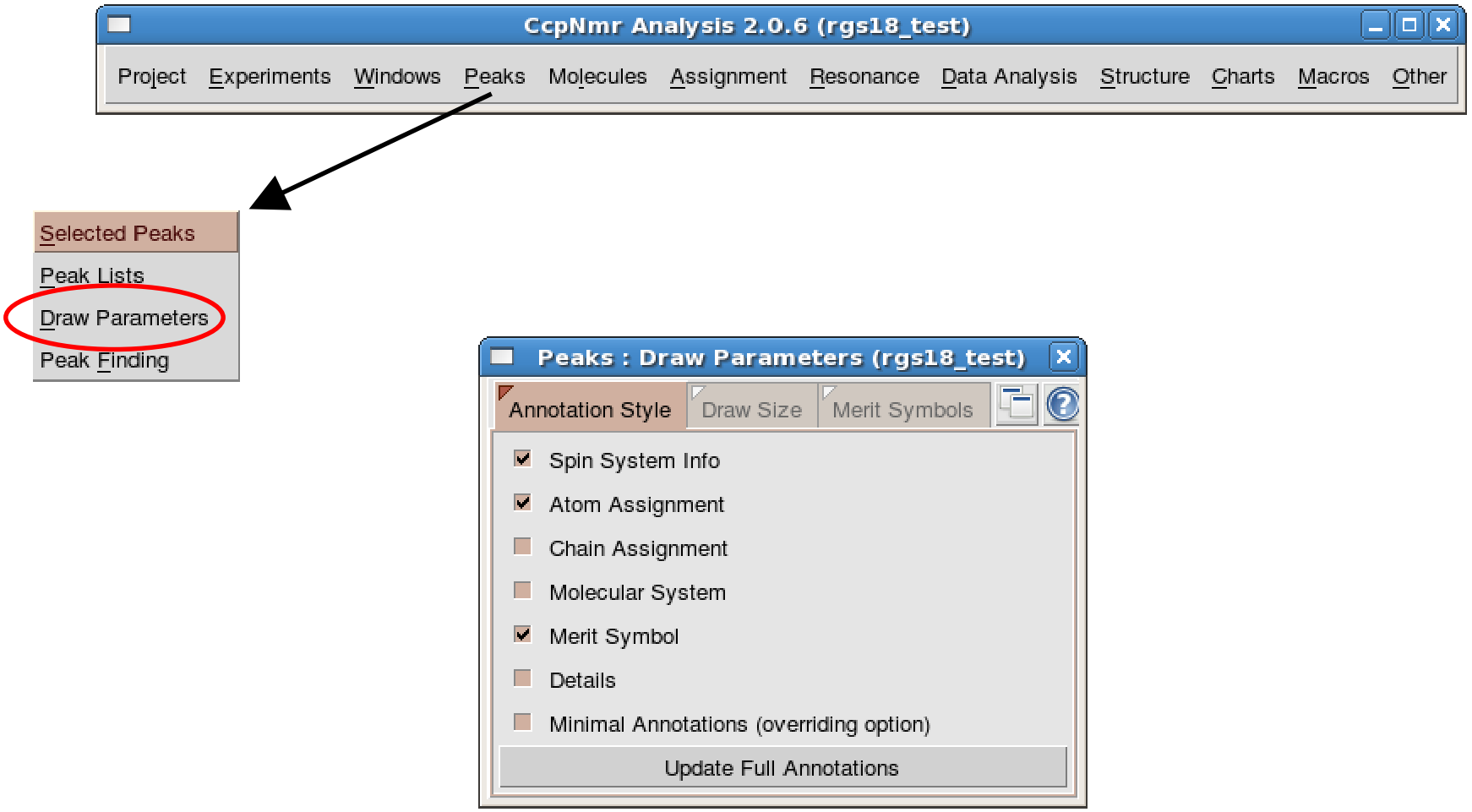
Peaks Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project Experiments Windows Peaks Molecules Assignment Resonance Data Analysis Structure Charts Macros Other
Selected Peaks
Peak Lists
Draw Parameters
Peak Finding
Peaks : Peak Lists (rgs18_test)
Peak Lists
Peak Table
Synthetic Lists
Experiment | Spectrum | List | Active? | Color | Symbol | No. Peaks | % Assigned | Details
HSQC | hsqc_psrgs18.051221_3 | 1 | Yes | #000000 | x | 167 | 100.0 | Default list
HSQC | hsqc_psrgs18.051221_3 | 2 | No | #000000 | x | 0 | 0.0 | New list
CBCGCONNH | cbcaconh_psrgs18.051221_6 | 1 | Yes | #000000 | x | 234 | 66.7 | Default list
CBCANNH | cbcanh_psrgs18.051221_7 | 1 | Yes | #000000 | x | 527 | 66.7 | Default list
HBHACONH | hbhacohn | 1 | Yes | #000000 | x | 0 | 0.0 | Default list
HCCCONH | hccconh | 1 | Yes | #000000 | x | 0 | 0.0 | Default list
CCCONH | ccconh | 1 | Yes | #000000 | x | 0 | 0.0 | Default list
HCCH_TOCSY | hcchtocsy | 1 | Yes | #000000 | x | 0 | 0.0 | Default list
HCCH_COSY | hcchcosy | 1 | Yes | #000000 | x | 0 | 0.0 | Default list
Edit Peaks
Delete
Add Sister List
Copy Peaks

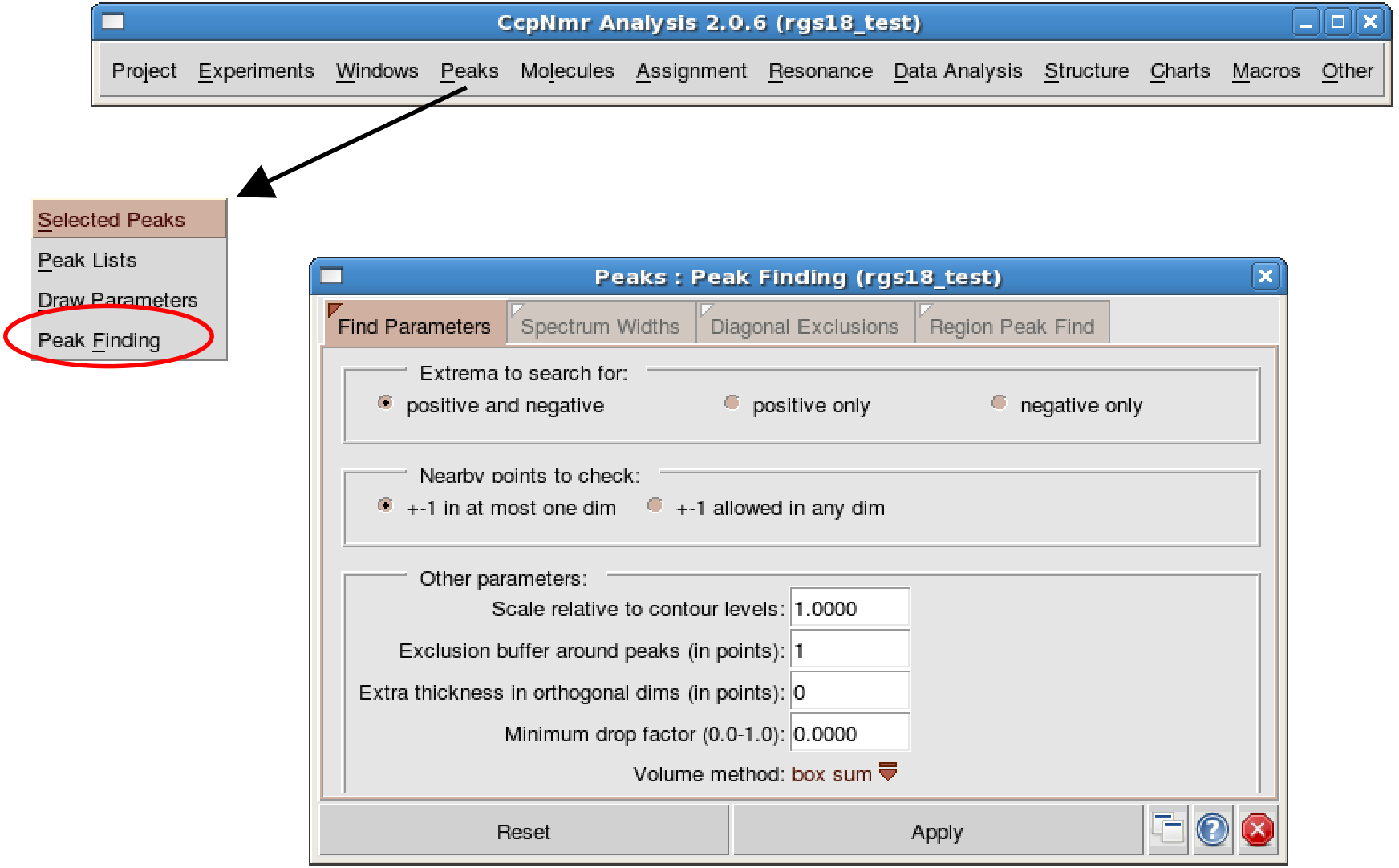
Peaks Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project Experiments Windows Peaks Molecules Assignment Resonance Data Analysis Structure Charts Macros Other
Selected Peaks
Peak Lists
Draw Parameters
Peak Finding
Peaks : Peak Lists (rgs18_test)
Peak Lists
Peak Table
Synthetic Lists
Spectrum: HSQC:hsqc_psrgs18.051221_3
Peak List: 1
Make Strips
Find Peak
Window: window2
Mark Fo
Status: Any
Structure: <None>
Strip locations
Goto position
<None>
Mark S
Shift F1 Shift F2 Assign. F1 Assign. F2 Height Volume Fit Method Vol. Method Merit Details ?
1 10.600 130.129 {1}H[1] {1}N[2] 6.486e+06 5.682e+07 parabolic box sum 1.000
2 8.918 128.412 {2}H[3] {2}N[4] 5.931e+06 5.209e+07 parabolic box sum 1.000
4 8.954 127.521 {3}H[5] {3}N[6] 7.059e+06 6.187e+07 parabolic box sum 1.000
7 7.875 125.937 {4}H[7] {4}N[8] 3.577e+07 3.088e+08 parabolic box sum 1.000
9 10.534 125.600 {5}H[9] {5}N[10] 4.077e+06 3.597e+07 parabolic box sum 1.000
11 8.334 124.532 {6}H[11] {6}N[12] 4.636e+06 4.075e+07 parabolic box sum 1.000
12 8.579 124.490 {7}H[13] {7}N[14] 5.164e+06 4.551e+07 parabolic box sum 1.000
13 8.415 124.238 {8}H[15] {8}N[16] 5.821e+06 5.088e+07 parabolic box sum 1.000
14 7.349 124.133 {9}H[17] {9}N[18] 8.718e+06 7.640e+07 parabolic box sum 1.000
15 7.643 124.024 {10}H[19] {10}N[20] 9.508e+06 8.316e+07 parabolic box sum 1.000
16 8.997 123.970 {11}H[21] {11}N[22] 4.076e+06 3.584e+07 parabolic box sum 1.000
Add
Edit
Unalias
Delete
Assign
Deassign
Deassign Dim
Recalc Intensities
Show On Structure
Propagate Assign

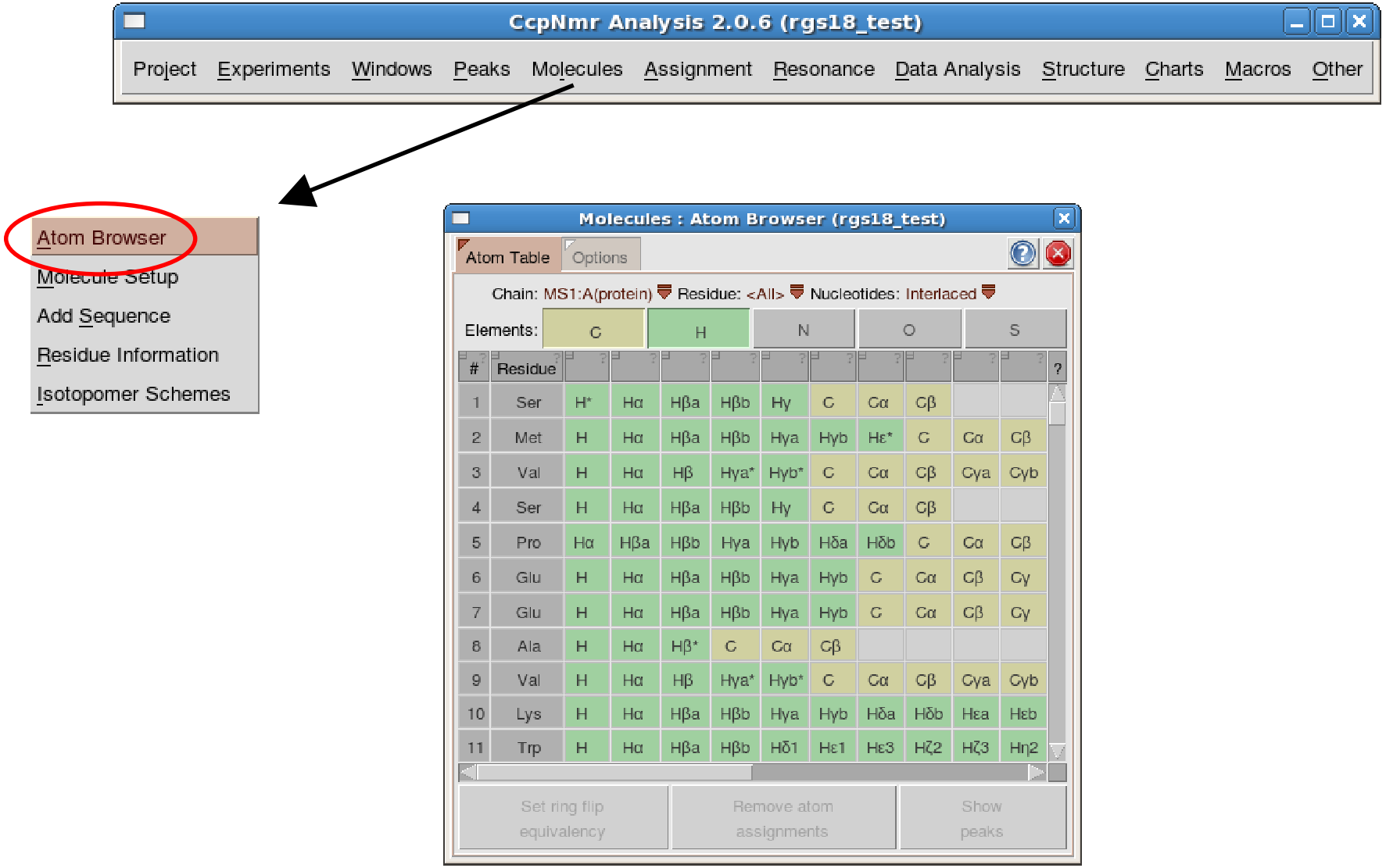
Peaks Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project Experiments Windows Peaks Molecules Assignment Resonance Data Analysis Structure Charts Macros Other
Selected Peaks
Peak Lists
Draw Parameters
Peak Finding
Peaks : Draw Parameters (rgs18_test)
Annotation Style
Draw Size
Merit Symbols
Spin System Info
Atom Assignment
Chain Assignment
Molecular System
Merit Symbol
Details
Minimal Annotations (overriding option)
Update Full Annotations

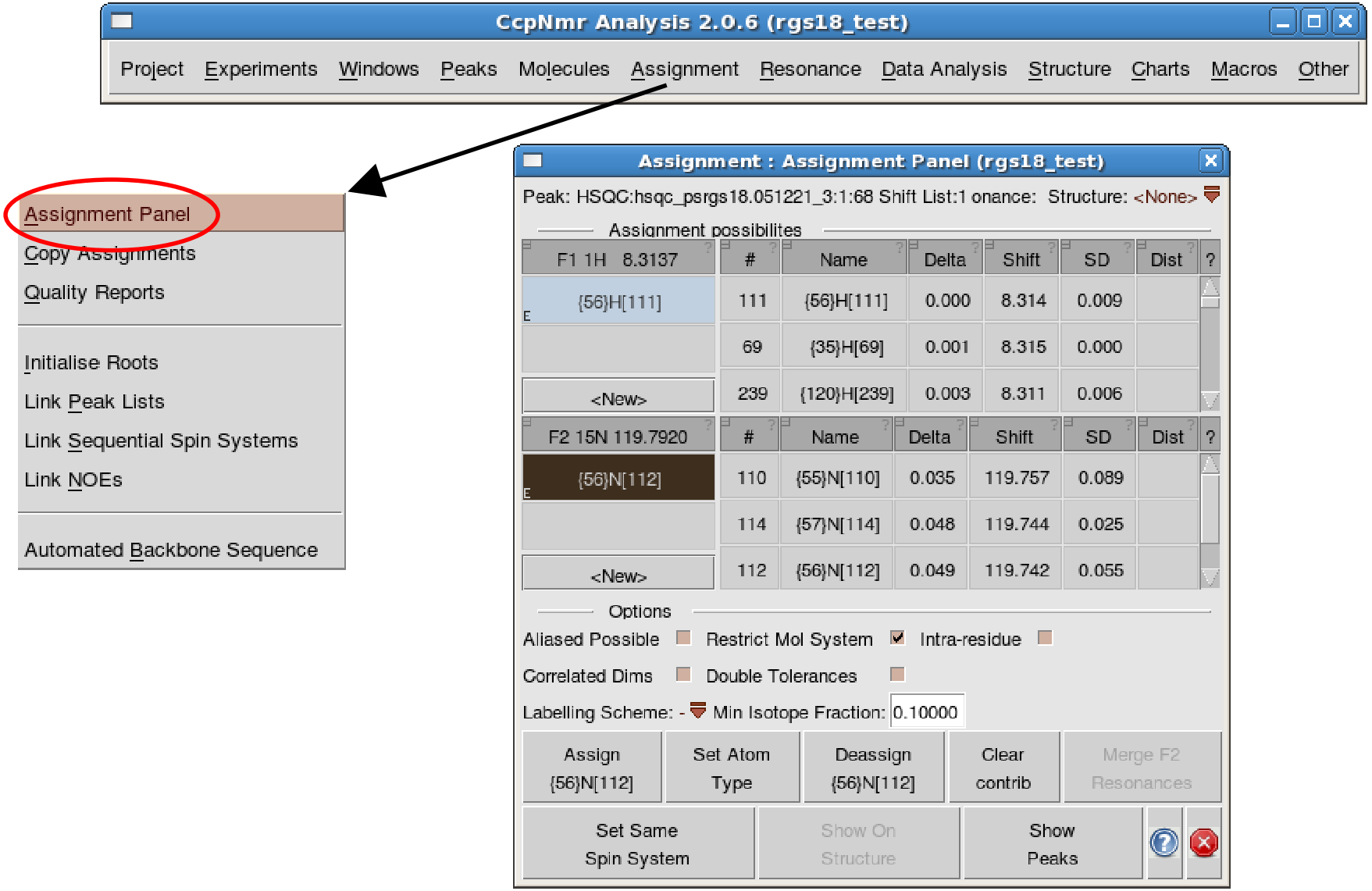
Peaks Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project Experiments Windows Peaks Molecules Assignment Resonance Data Analysis Structure Charts Macros Other
Selected Peaks
Peak Lists
Draw Parameters
Peak Finding
Peaks : Peak Finding (rgs18_test)
Find Parameters
Spectrum Widths
Diagonal Exclusions
Region Peak Find
Extrema to search for:
positive and negative
positive only
negative only
Nearby points to check:
+-1 in at most one dim
+-1 allowed in any dim
Other parameters:
Scale relative to contour levels: 1.0000
Exclusion buffer around peaks (in points): 1
Extra thickness in orthogonal dims (in points): 0
Minimum drop factor (0.0-1.0): 0.0000
Volume method: box sum
Reset
Apply

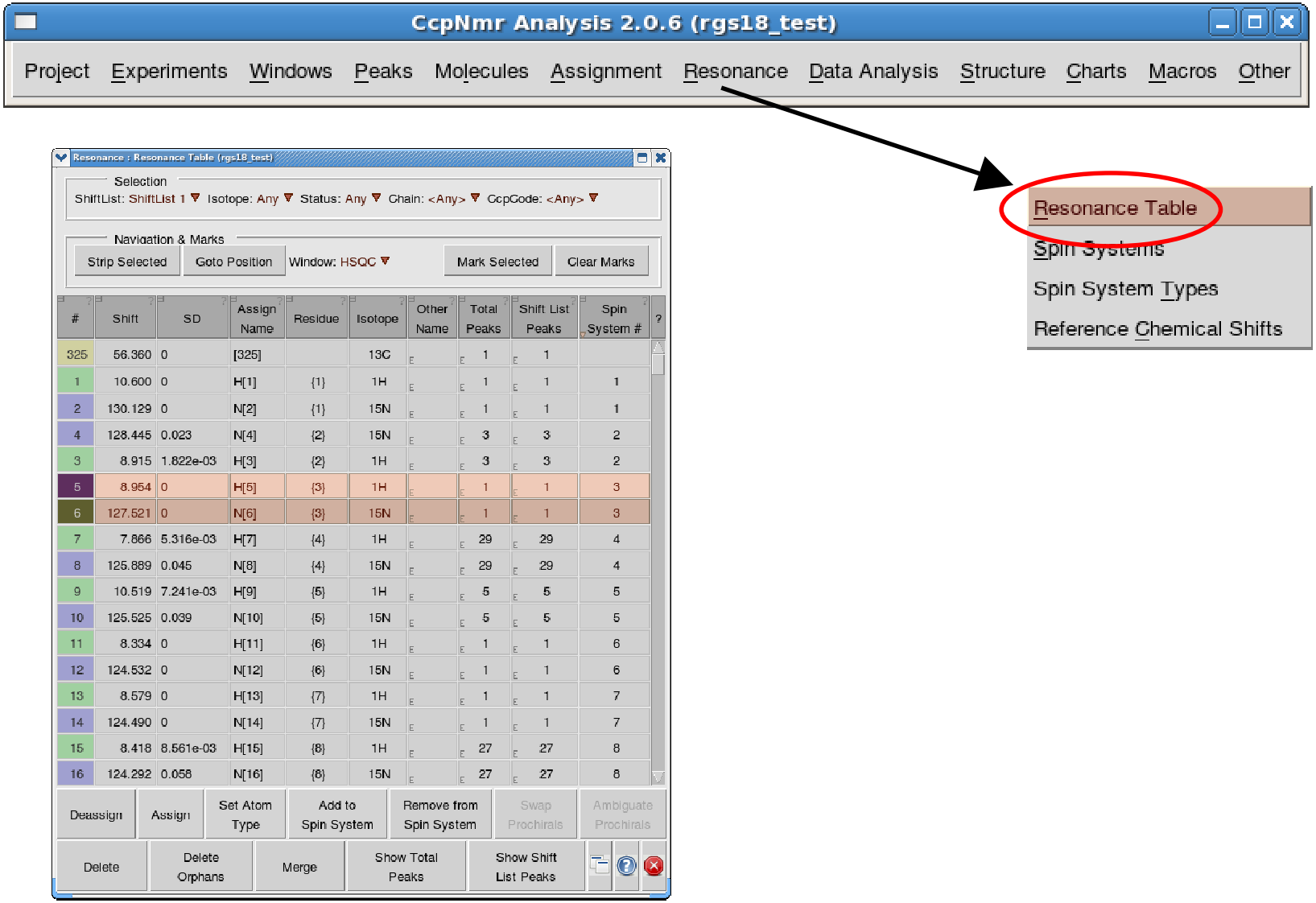
Molecules Menu



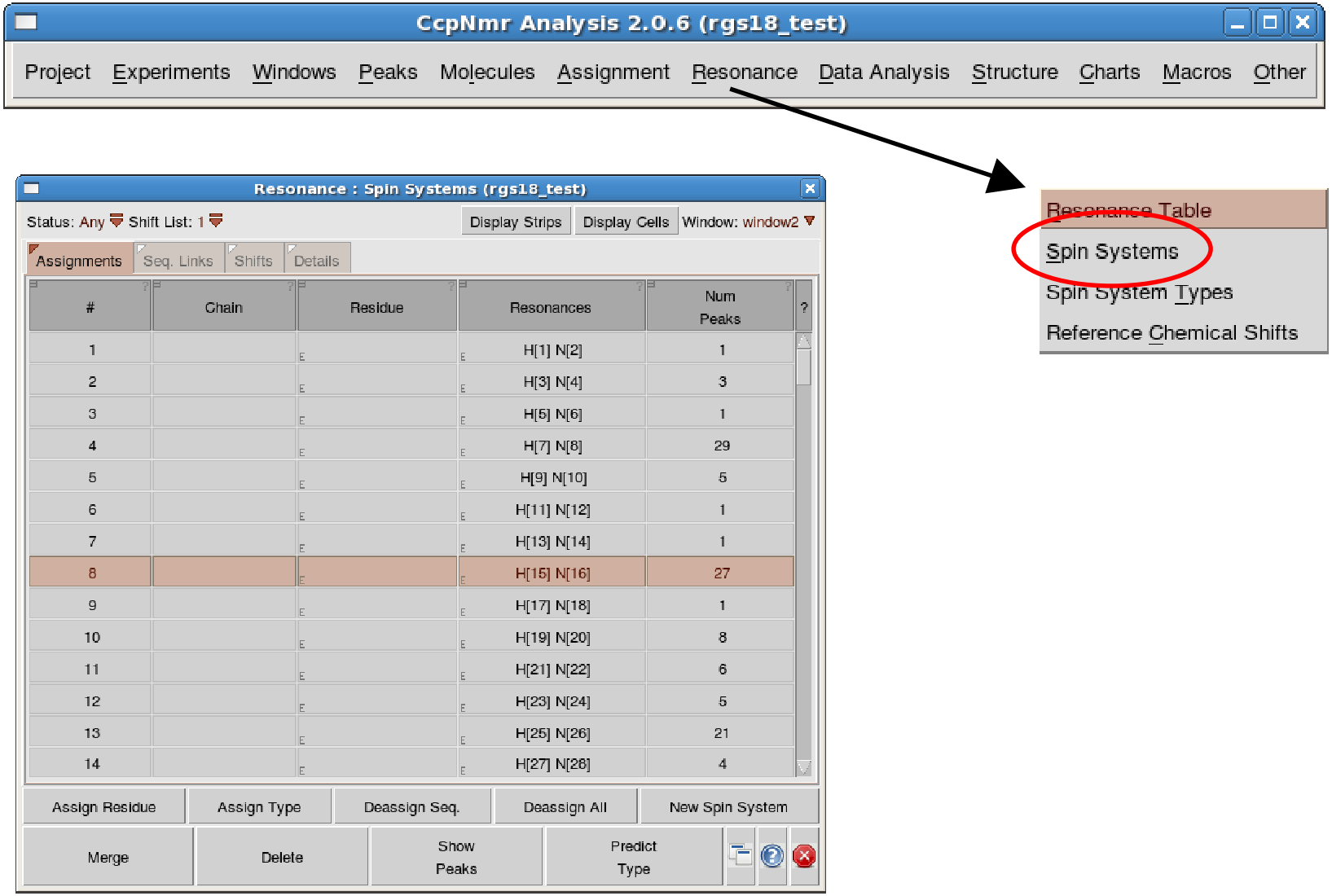
Assignment Menu



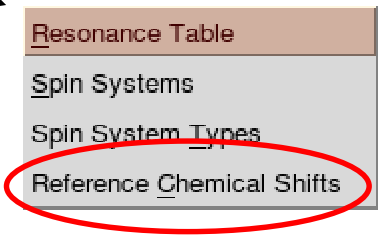
Resonance Menu

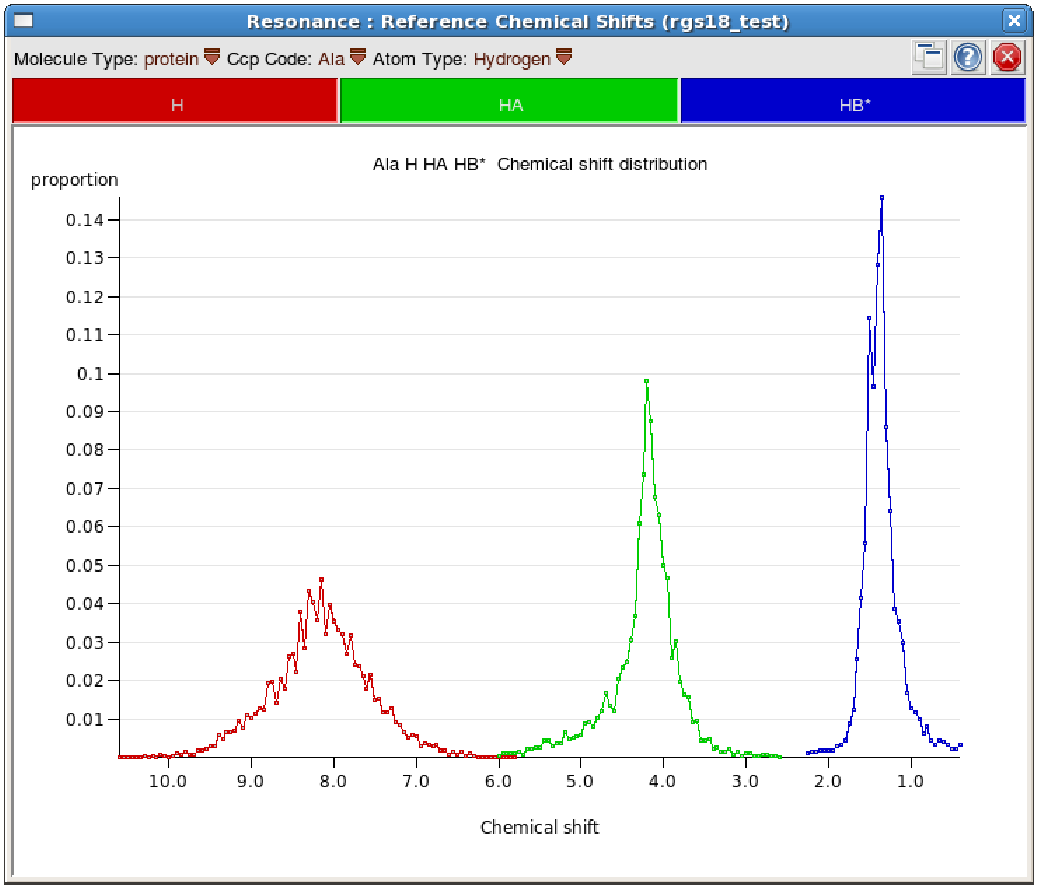


Resonance Menu

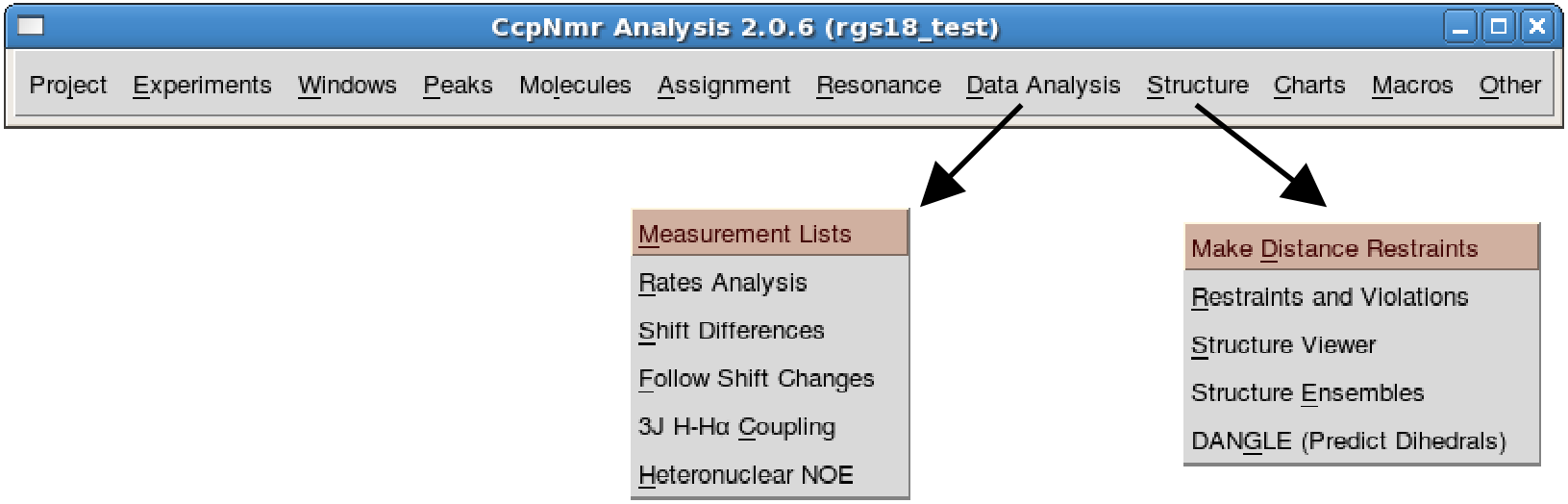


Resonance Menu

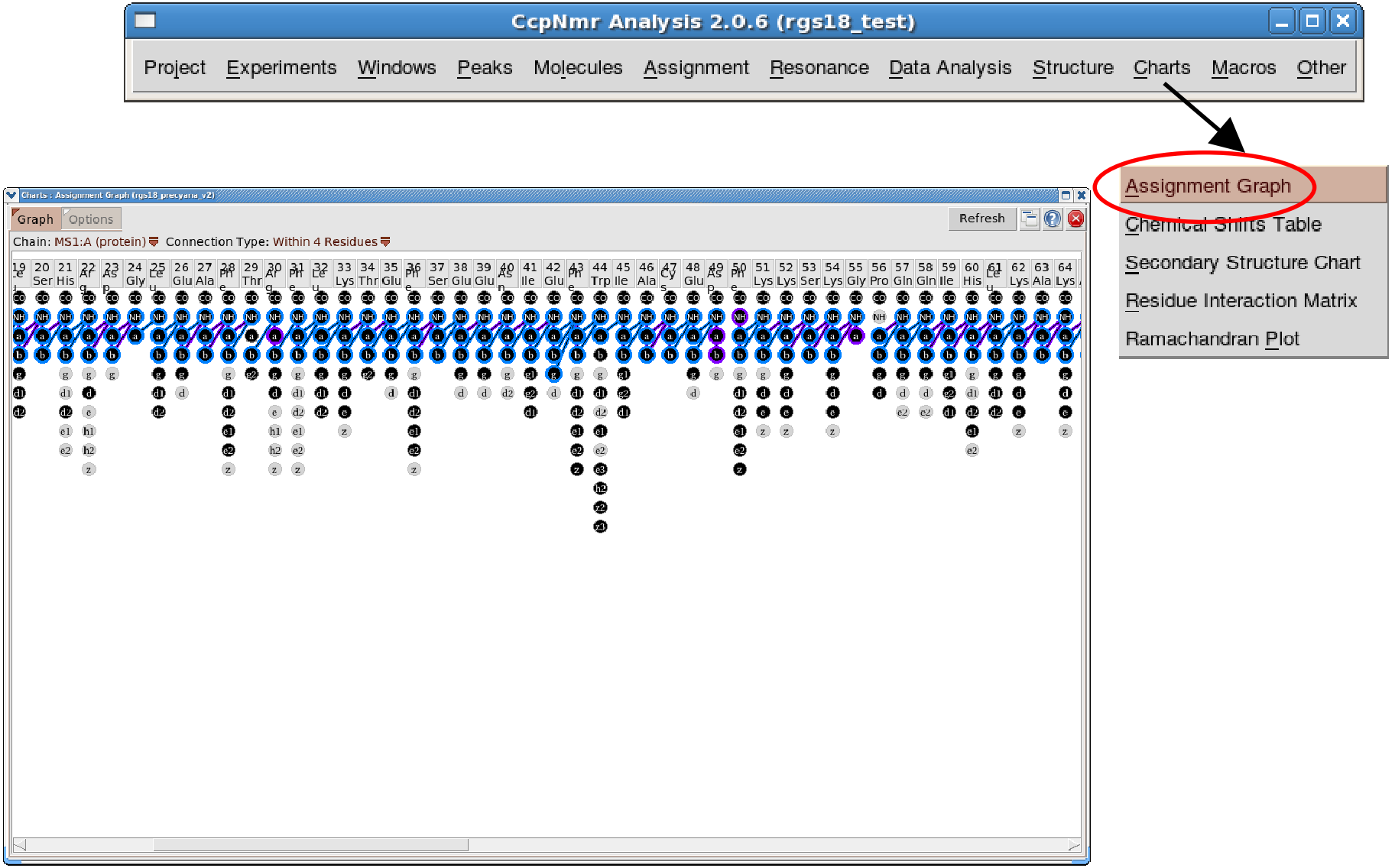




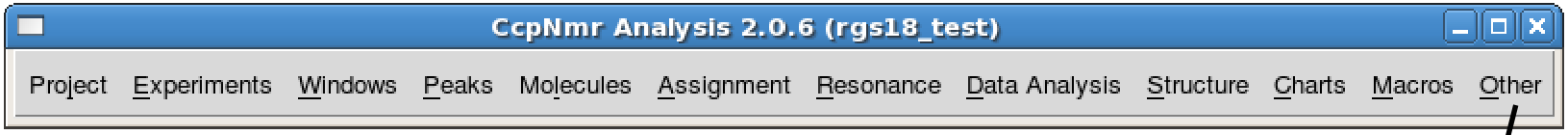
Data Analysis / Structure Menus

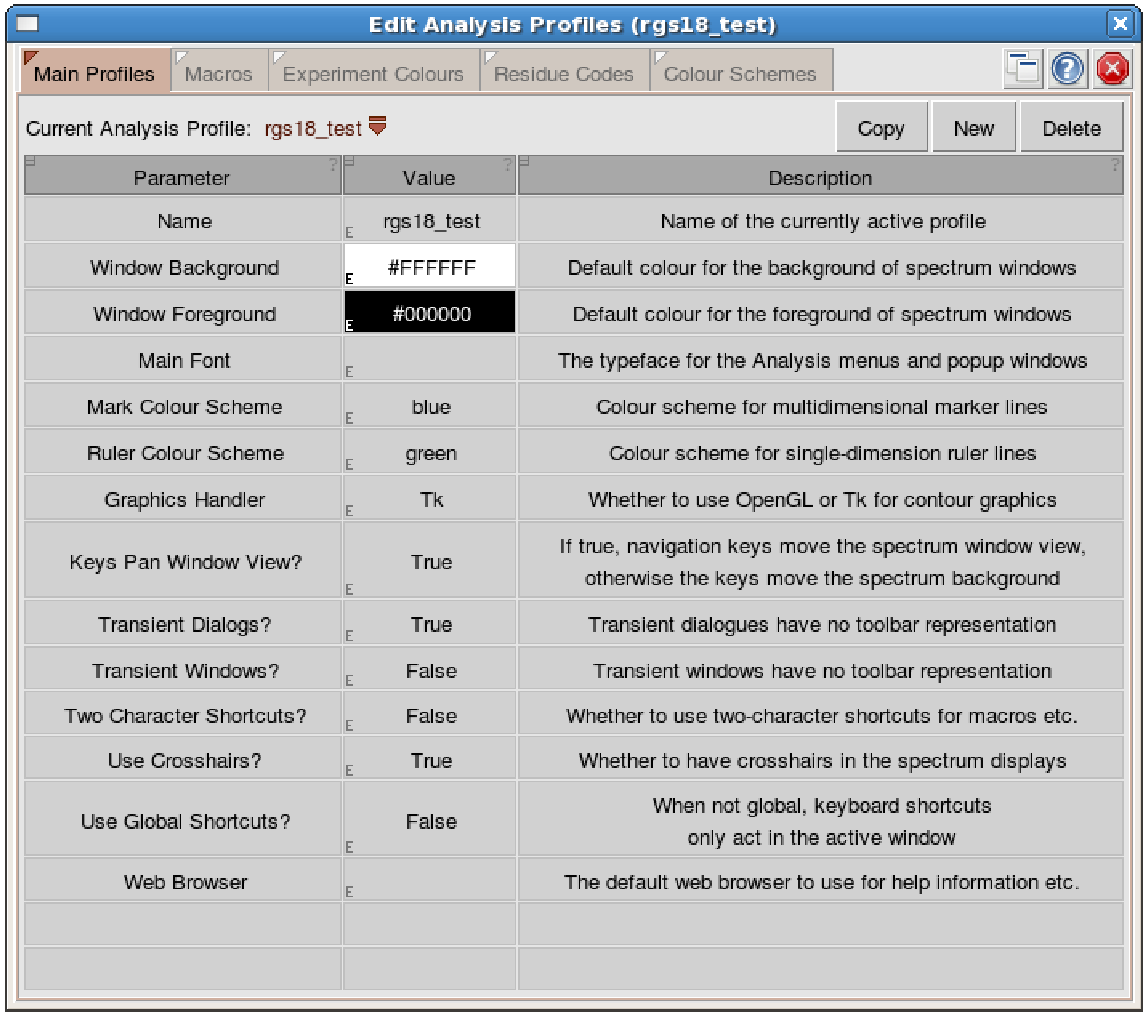


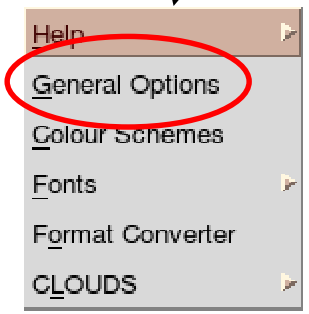
Charts Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project
Experiments
Windows
Peaks
Molecules
Assignment
Resonance
Data Analysis
Structure
Charts
Macros
Other
Assignment Graph
Chemical Shifts Table
Secondary Structure Chart
Residue Interaction Matrix
Ramachandran Plot
Graph
Options
Refresh
Chain: MS1:A (protein)
Connection Type: Within 4 Residues

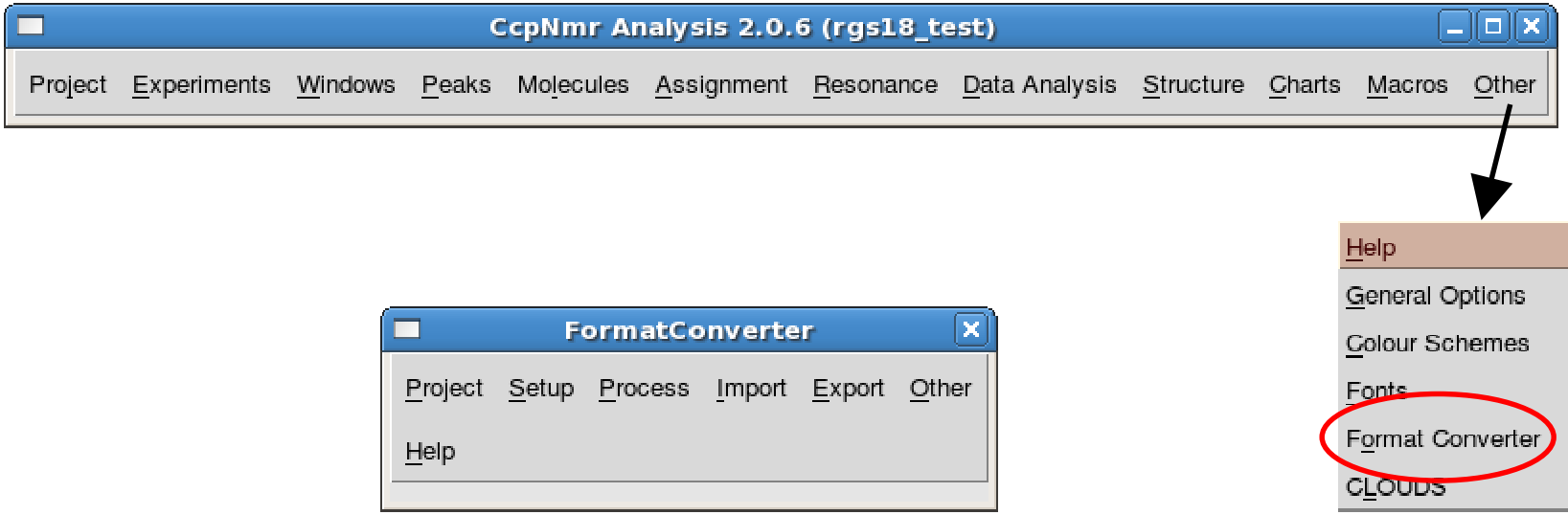
Other Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project
Experiments
Windows
Peaks
Molecules
Assignment
Resonance
Data Analysis
Structure
Charts
Macros
Other


Edit Analysis Profiles (rgs18_test)
Main Profiles
Macros
Experiment Colours
Residue Codes
Colour Schemes
Current Analysis Profile: rgs18_test
Copy
New
Delete
Parameter
Value
Description
Name
rgs18_test
Name of the currently active profile
Window Background
#FFFFFF
Default colour for the background of spectrum windows
Window Foreground
#000000
Default colour for the foreground of spectrum windows
Main Font
The typeface for the Analysis menus and popup windows
Mark Colour Scheme
blue
Colour scheme for multidimensional marker lines
Ruler Colour Scheme
green
Colour scheme for single-dimension ruler lines
Graphics Handler
Tk
Whether to use OpenGL or Tk for contour graphics
Keys Pan Window View?
True
If true, navigation keys move the spectrum window view, otherwise the keys move the spectrum background
Transient Dialogs?
True
Transient dialogues have no toolbar representation
Transient Windows?
False
Transient windows have no toolbar representation
Two Character Shortcuts?
False
Whether to use two-character shortcuts for macros etc.
Use Crosshairs?
True
Whether to have crosshairs in the spectrum displays
Use Global Shortcuts?
False
When not global, keyboard shortcuts only act in the active window
Web Browser
The default web browser to use for help information etc.


Help
General Options
Colour Schemes
Fonts
Format Converter
CLOUDS

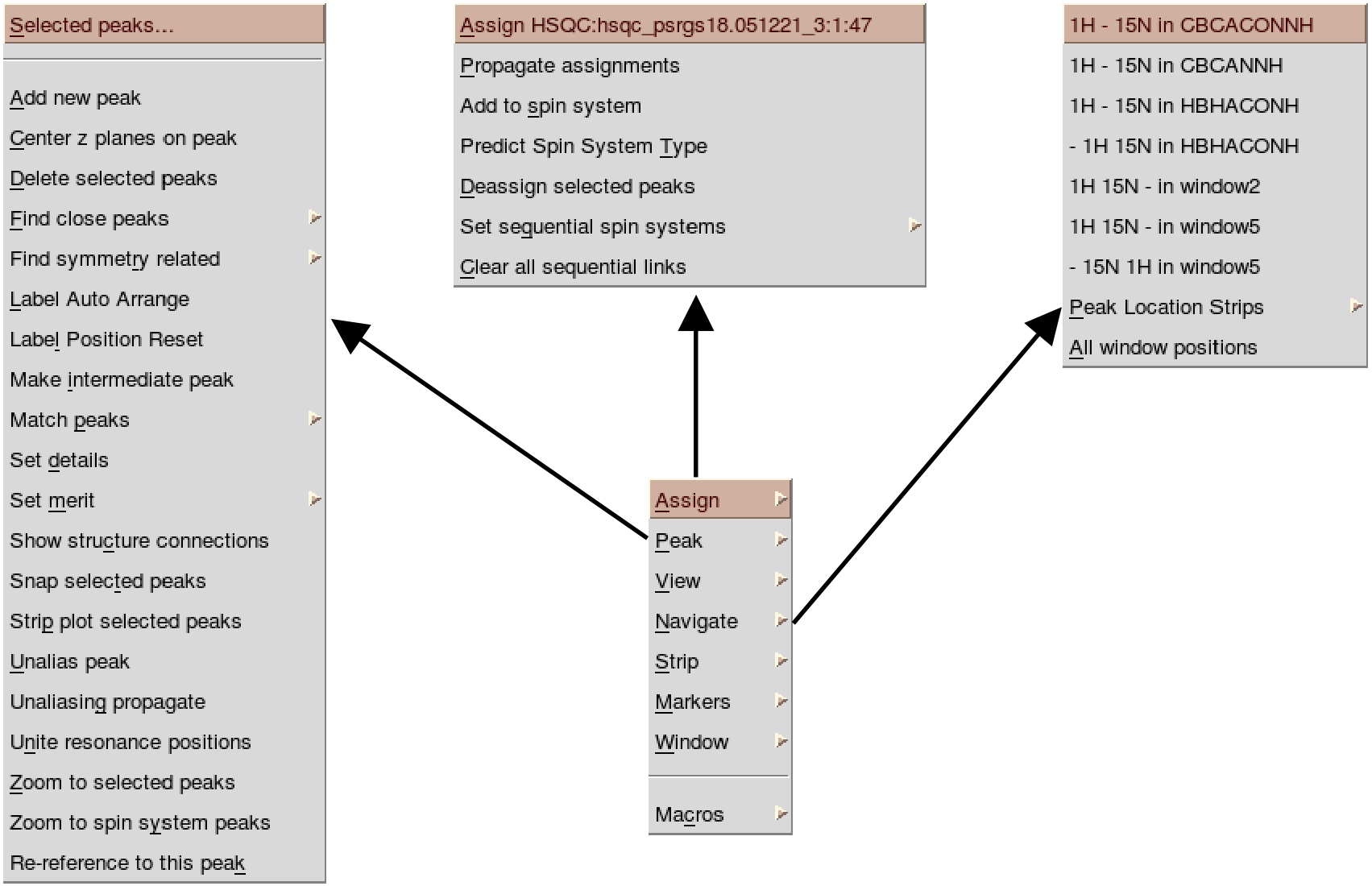
Other Menu


CcpNmr Analysis 2.0.6 (rgs18_test)
Project Experiments Windows Peaks Molecules Assignment Resonance Data Analysis Structure Charts Macros Other
Help
General Options
Colour Schemes
Fonts
Format Converter
CLOUDS
FormatConverter
Project Setup Process Import Export Other
Help

Mouse Usage

Left-click + Shift (+ drag)	add to the selection
Left-click + Shift + Ctrl + drag	pick peaks
Middle-click + drag	move the spectrum around
Middle-click + Shift + drag up/down	zoom in/out
Middle-click + Ctrl + drag	zoom in on dragged region
Middle-click + drag edge of z-scroll bar	in/decrease plane thickness
Right-click	menu with various options
Mouse-wheel	zoom in or out
Mouse-wheel + Ctrl	move through z-planes

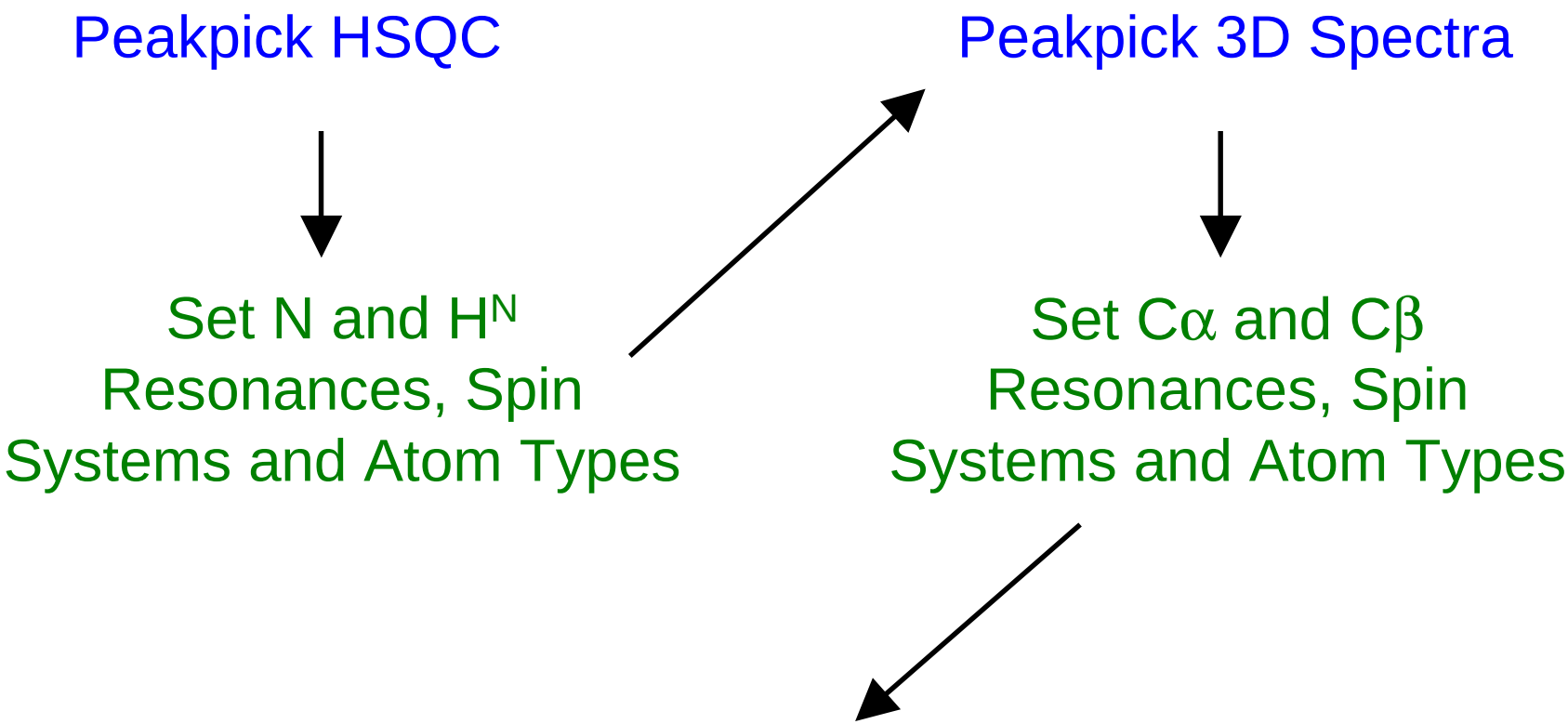
Right Mouse Menu



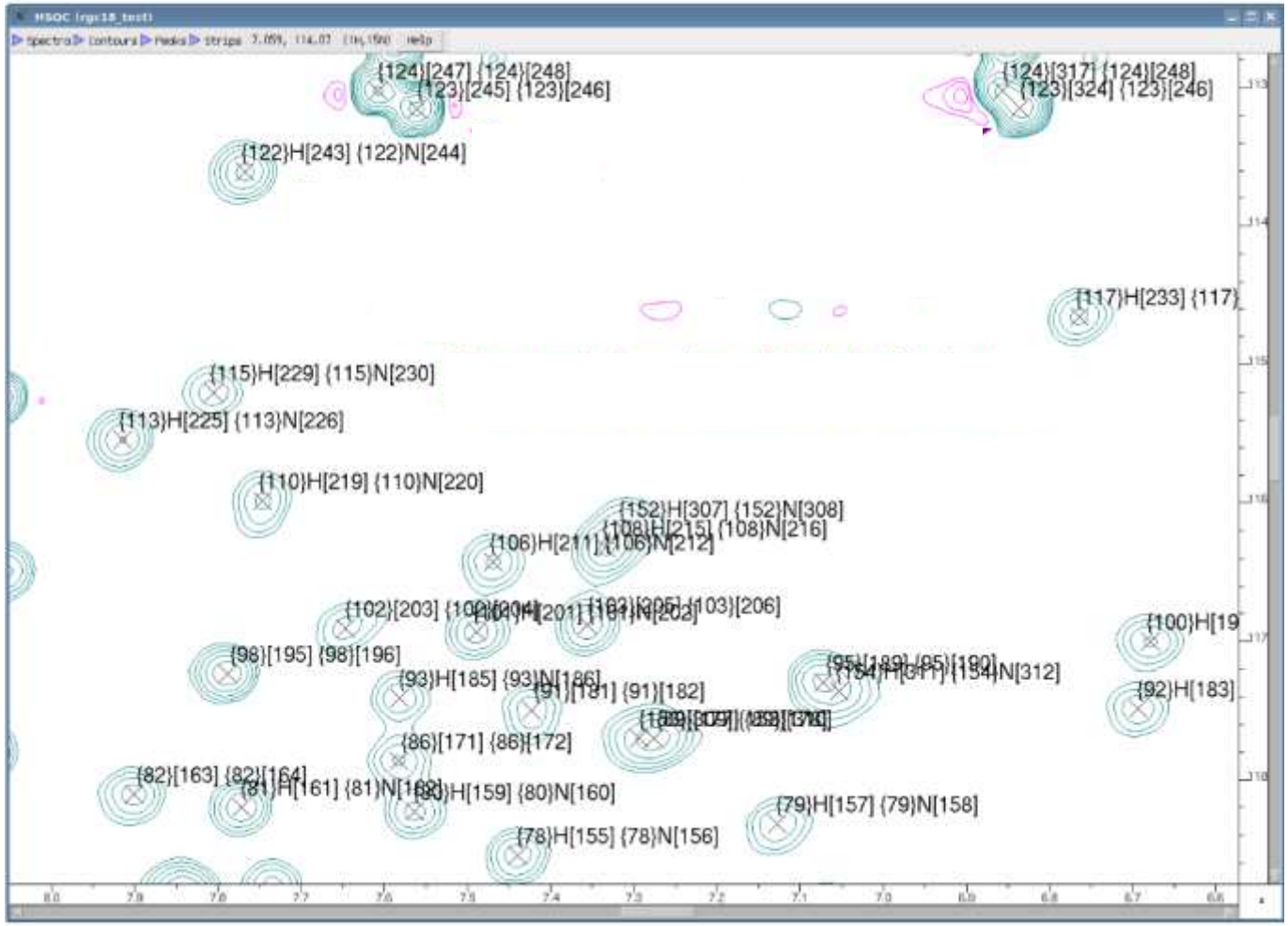
Outline

- Theory – Backbone and side-chain resonance assignment
- CCPNmr Analysis Software
- Practice – Using CCPNmr Analysis to assign a protein

Backbone Assignment

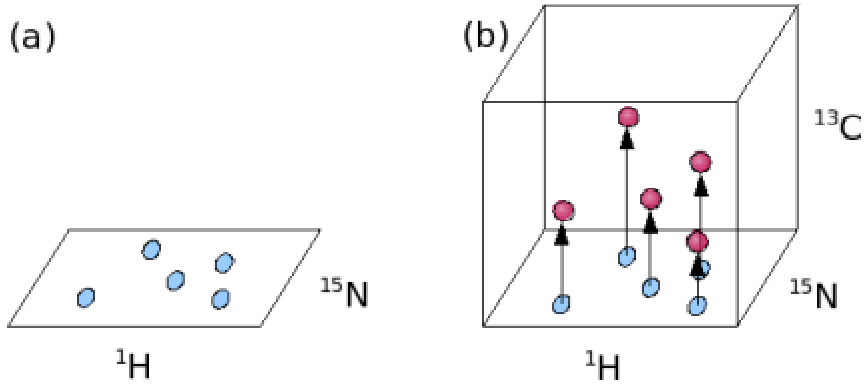


Initialising the HSQC

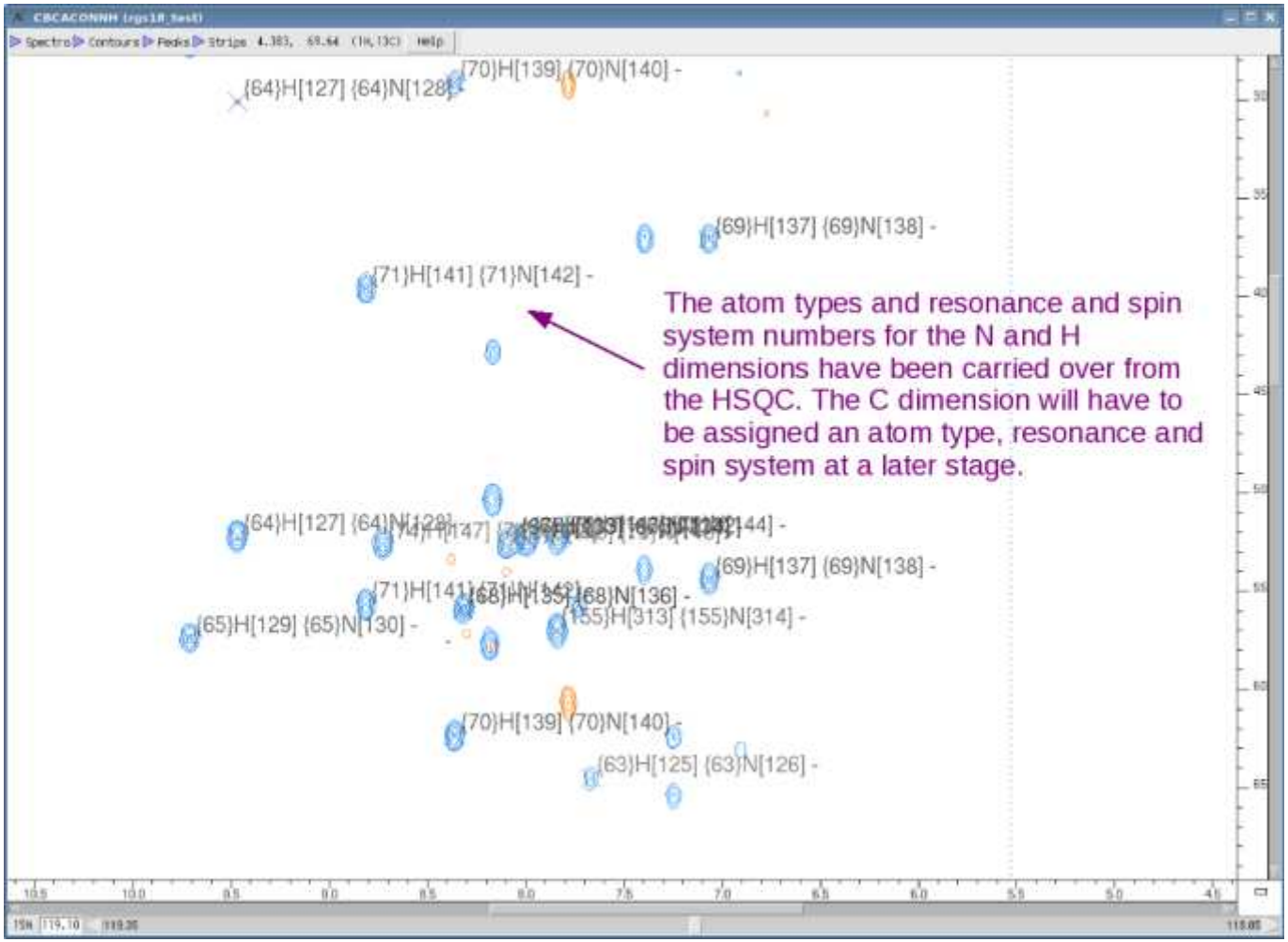


Peakpicking the 3D Spectra

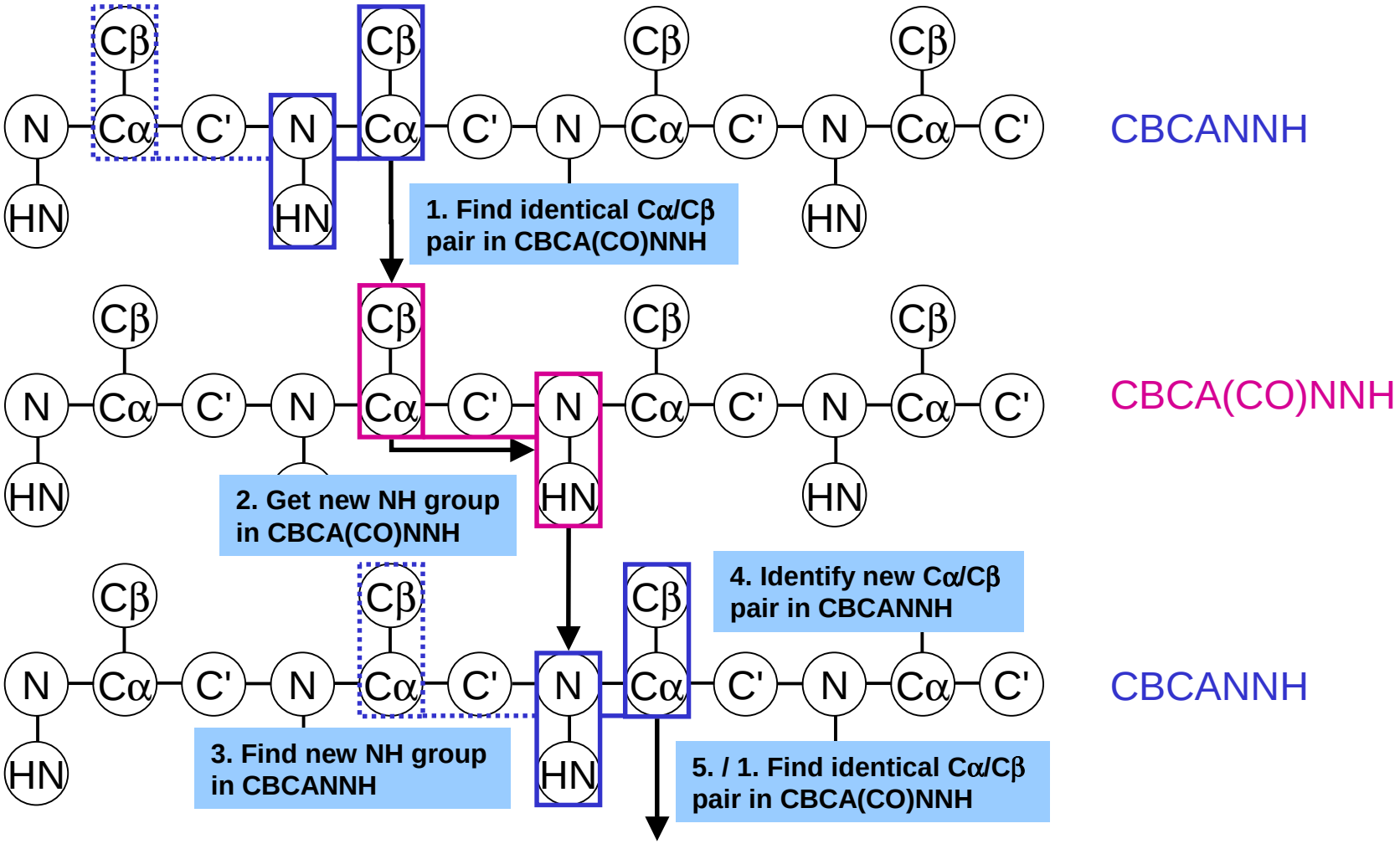
- Assignment → Link Peak Lists
  - Root spectrum
  - 3D Spectrum
  - Tolerances



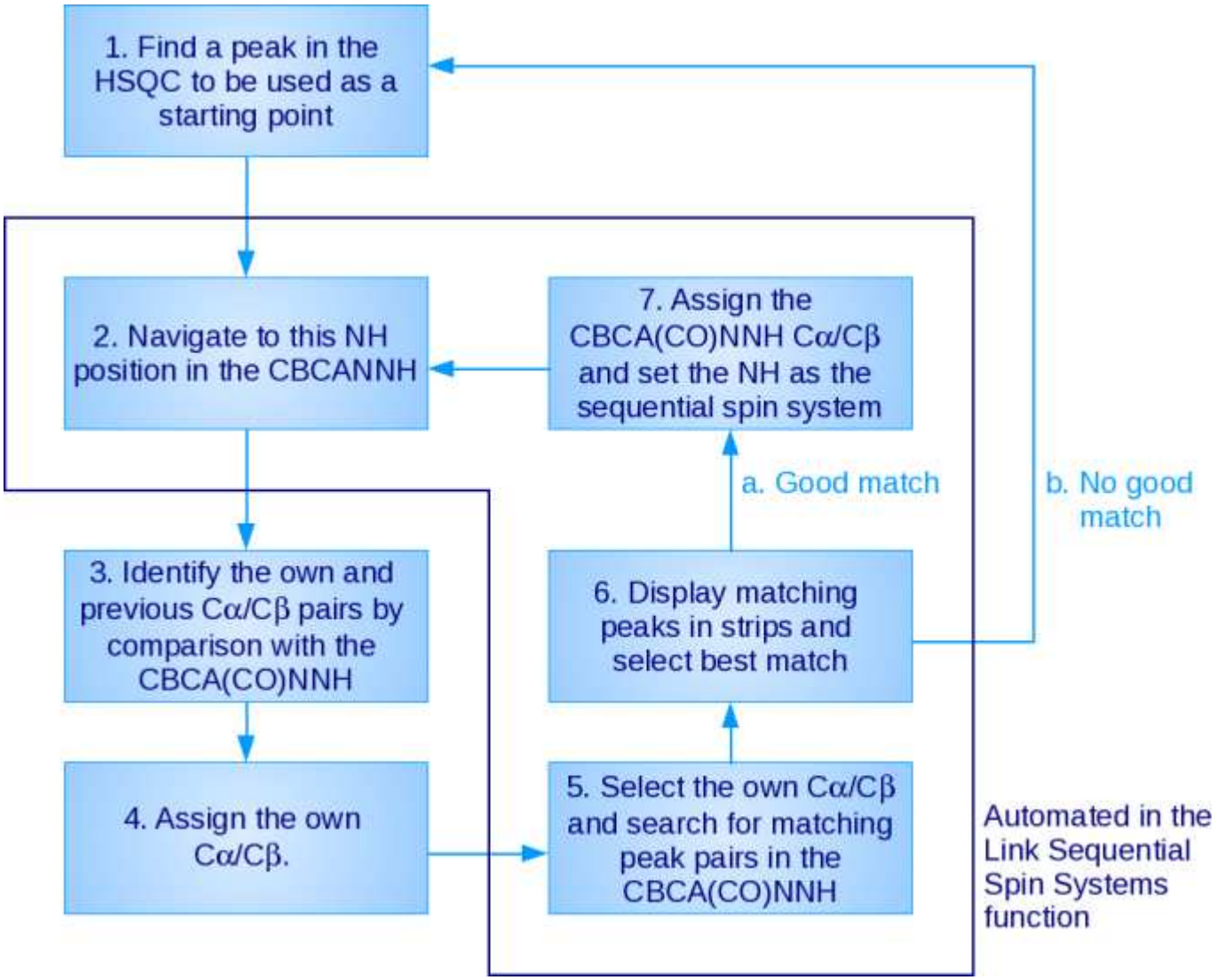
Peakpicking the 3D Spectra



Linking Sequential Spin Systems

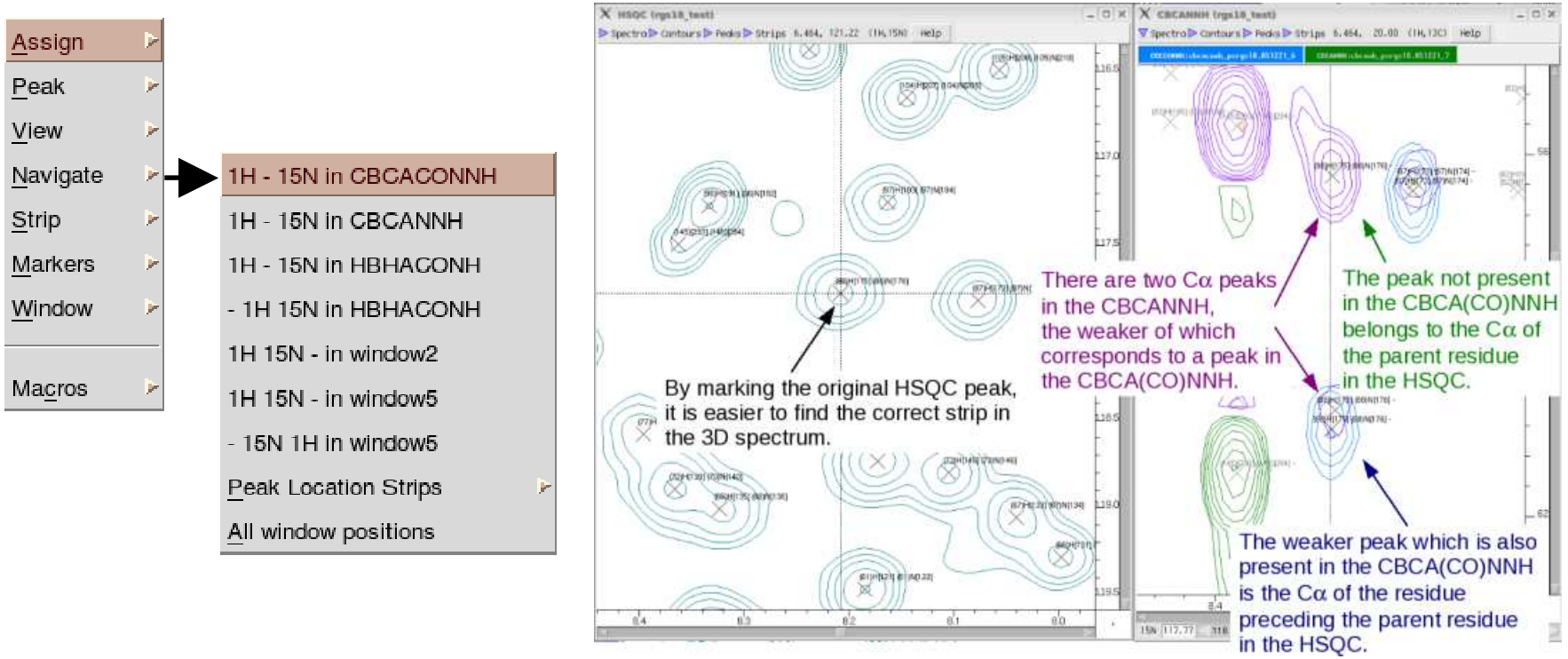


Linking Sequential Spin Systems



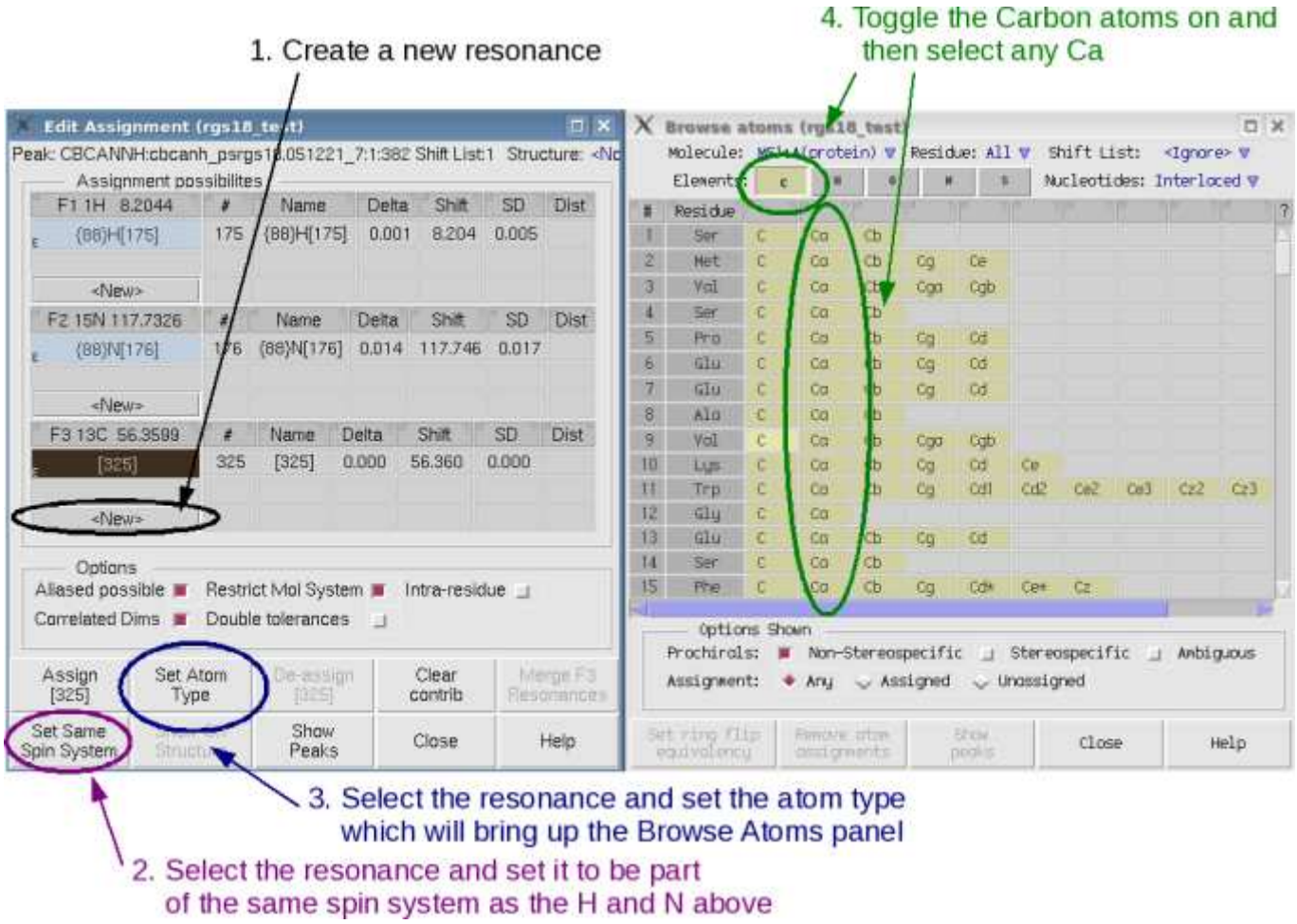
Linking Sequential Spin Systems

1. Find peak in HSQC as starting point
2. Navigate to this NH position in the CBCANH
3. Identify own and previous Cα/Cβ by comparison with the CBCA(CO)NH



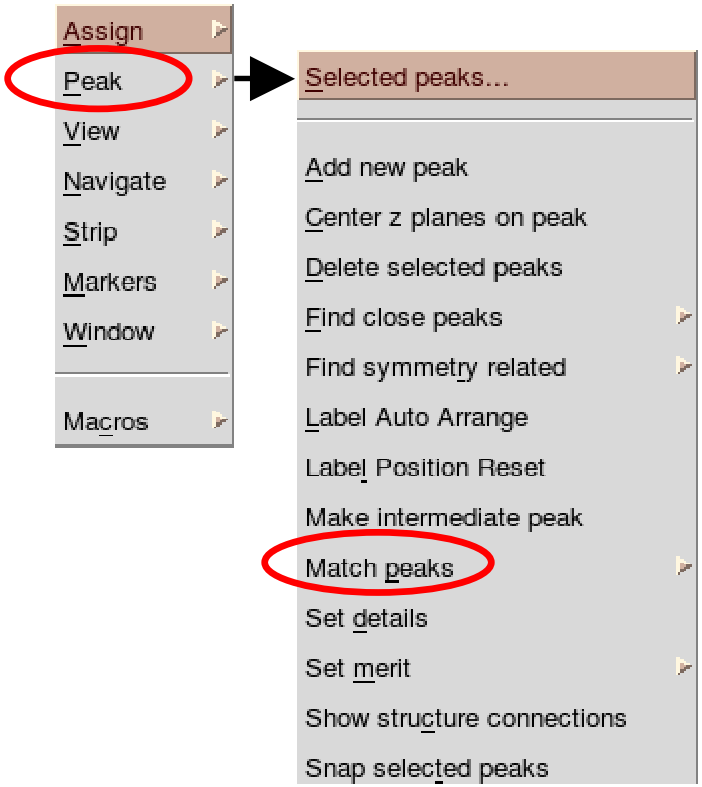
Linking Sequential Spin Systems

4. Assign own Cα/Cβ



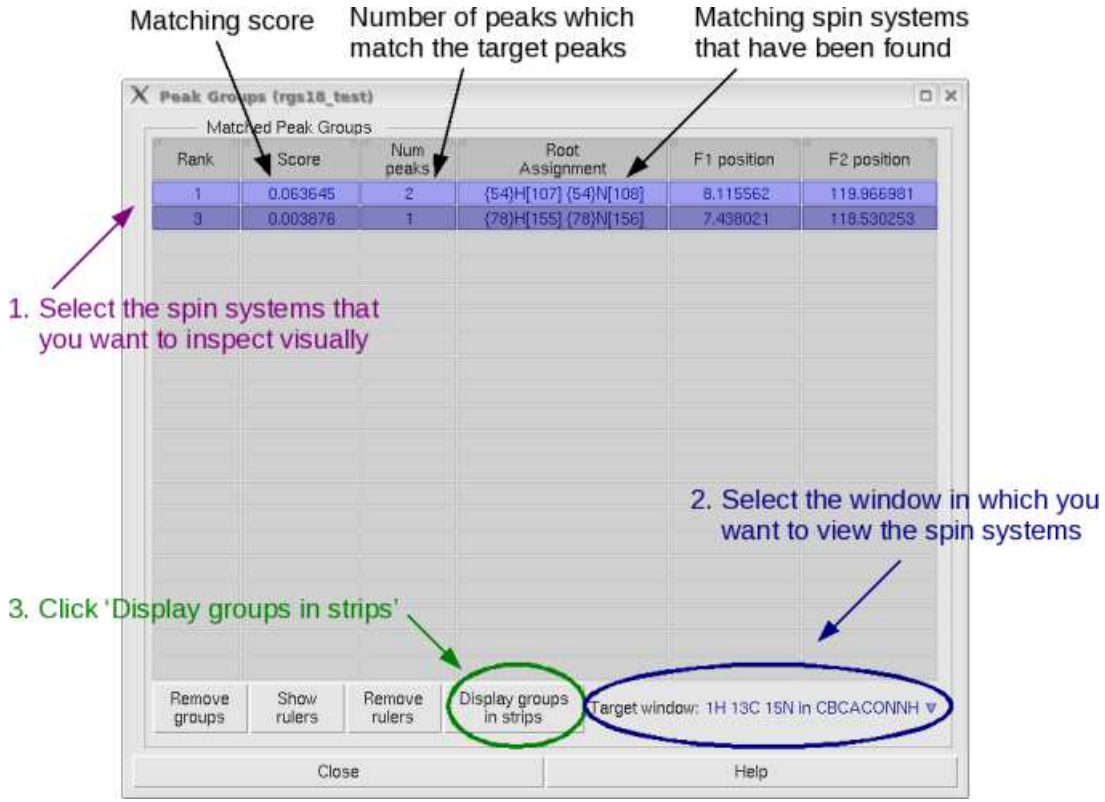
Linking Sequential Spin Systems

5. Select own Cα/Cβ and search for matching Cα/Cβ peak pairs in CBCA(CO)NH
   - Right click → Peak → Match Peaks → In CBCA(CO)NH → F3



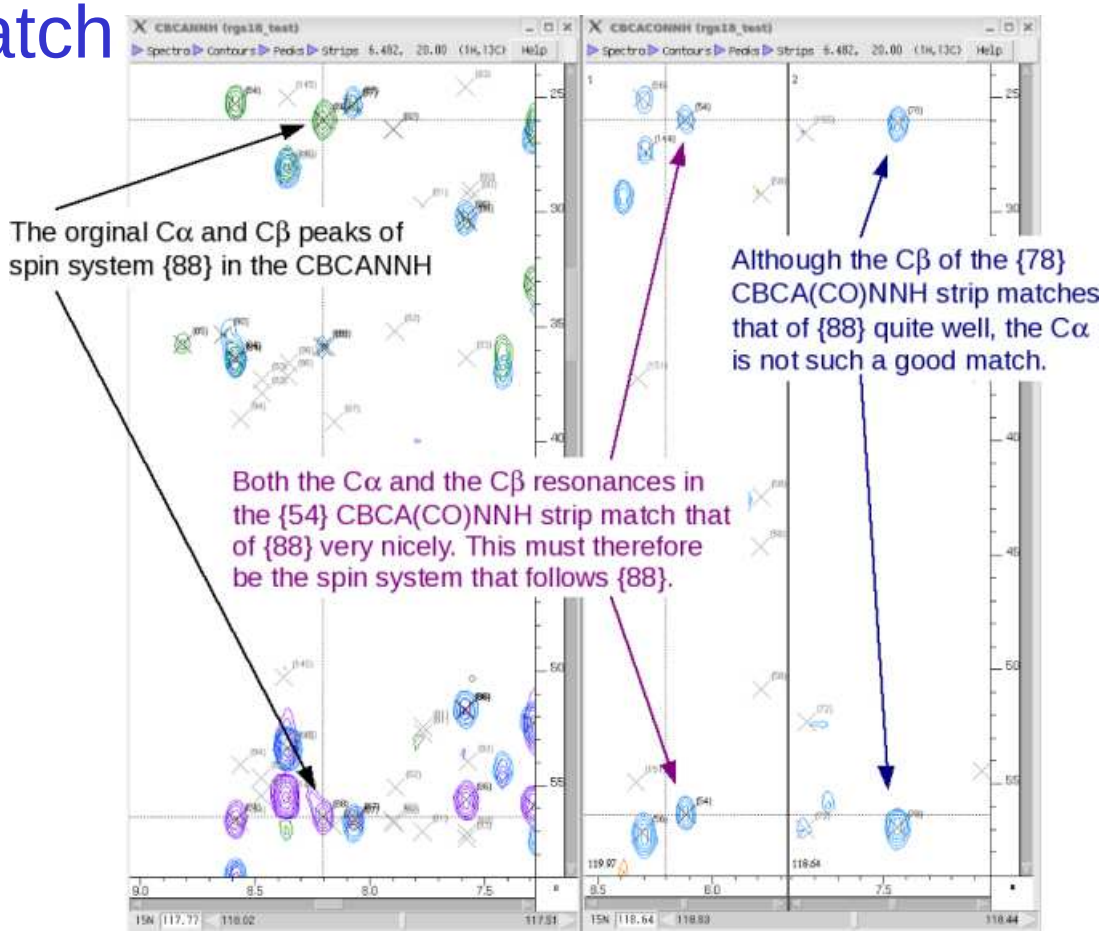
Linking Sequential Spin Systems

6. Display matching peaks in strips and select best match



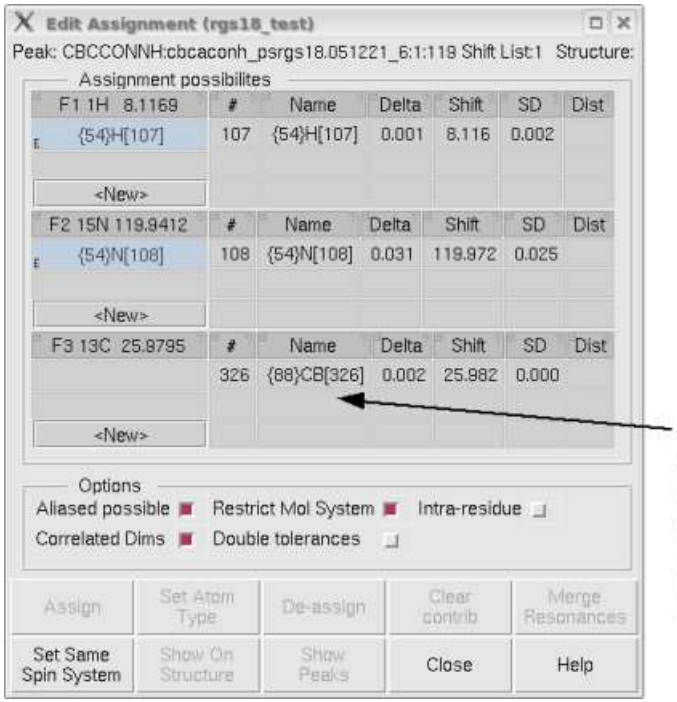
Linking Sequential Spin Systems

6. Display matching peaks in strips and select best match



Linking Sequential Spin Systems

7. (a) Good Match: Assign the CBCA(CO)NH and set the NH as sequential spin system
   - Right click → Assign → Set Sequential Spin Systems → F1 0, F2 0, F3 -1

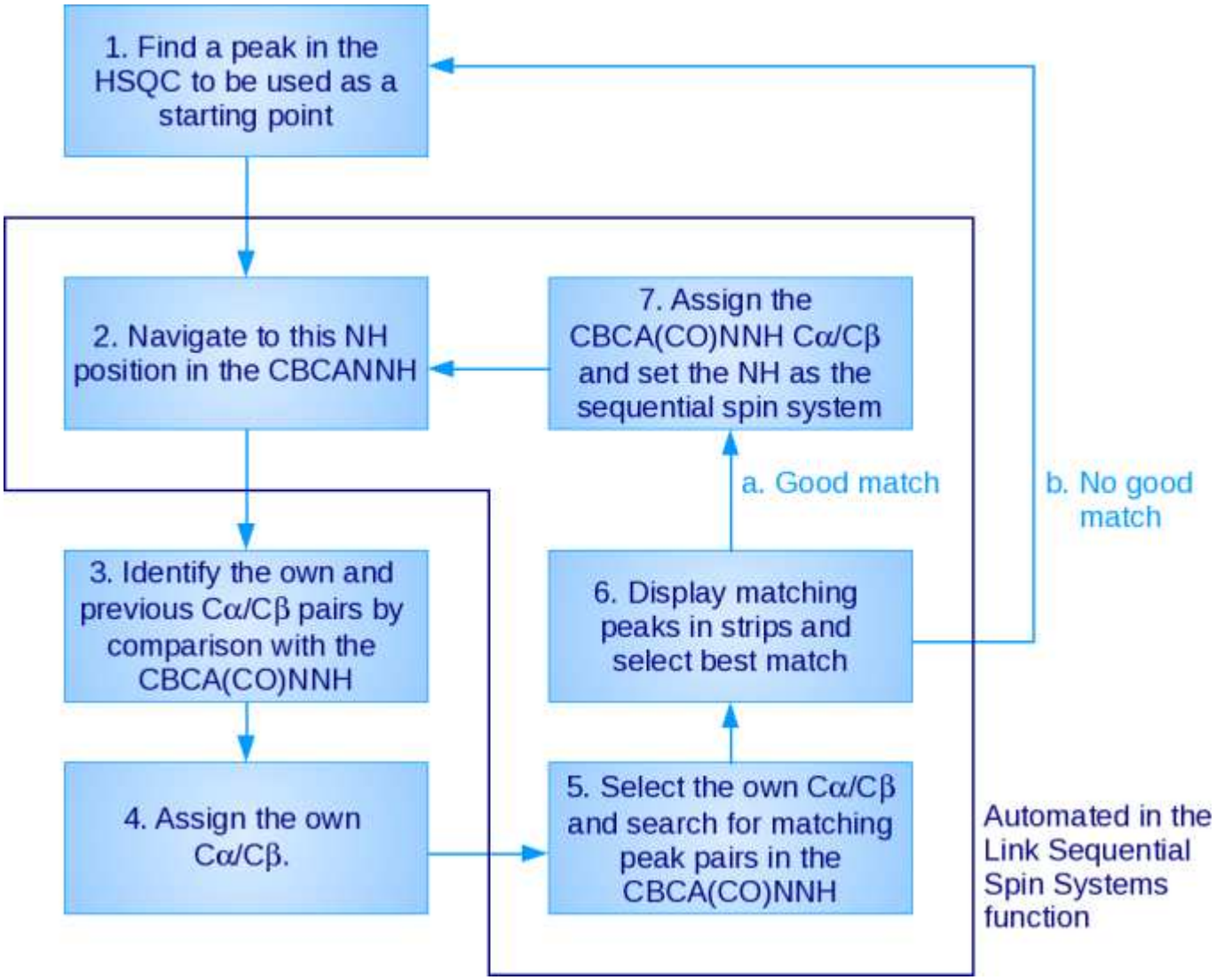


Select this resonance (the Cβ of spin system {88}) by clicking on it and it will then appear on the left hand side so that the peak then has the assignment H_i-N_i-$C\beta_{i-1}$.

Linking Sequential Spin Systems

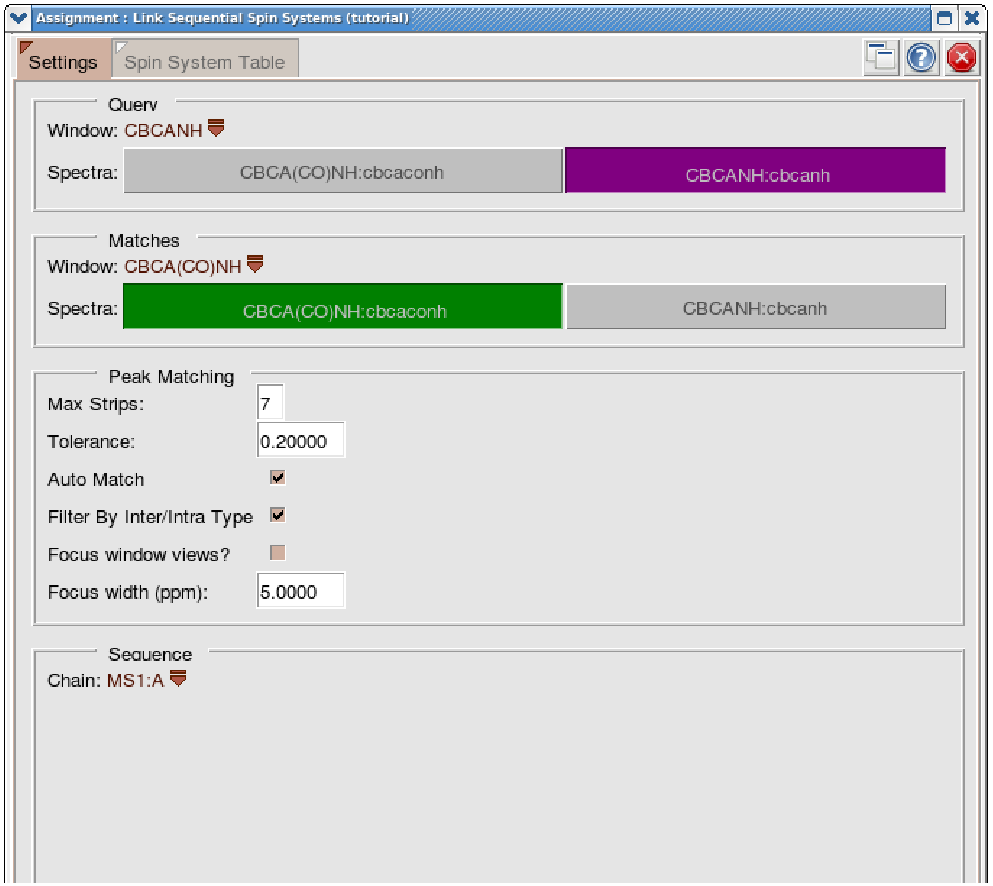
7. (b) No Good Match: Start Again…

Linking Sequential Spin Systems

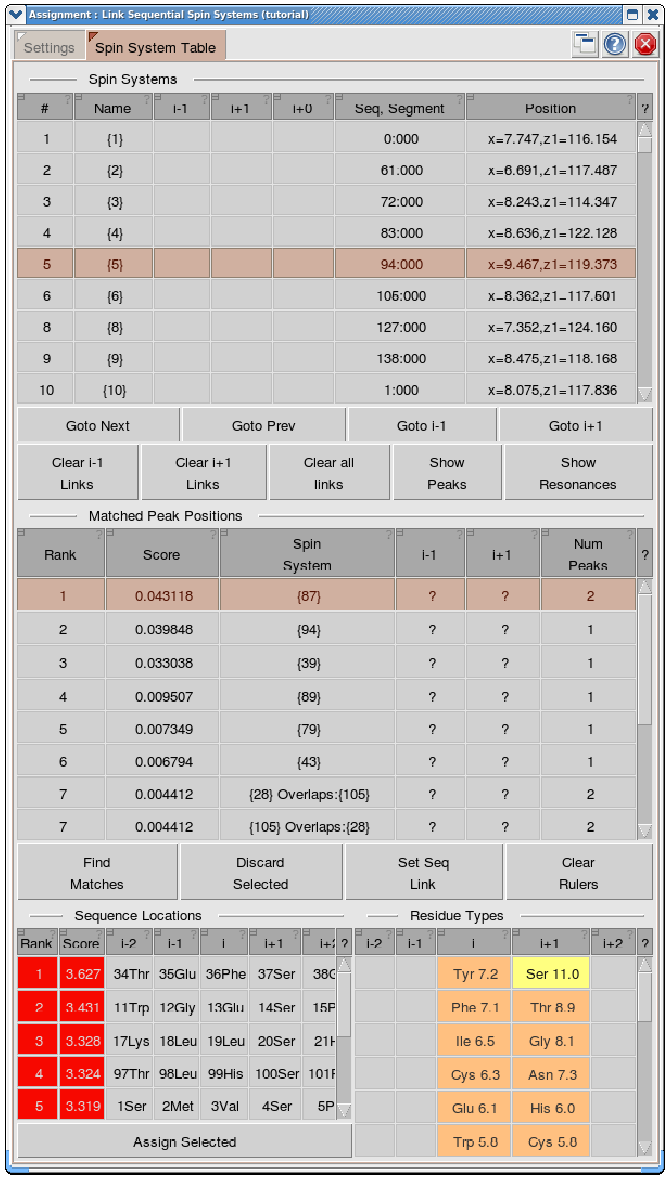


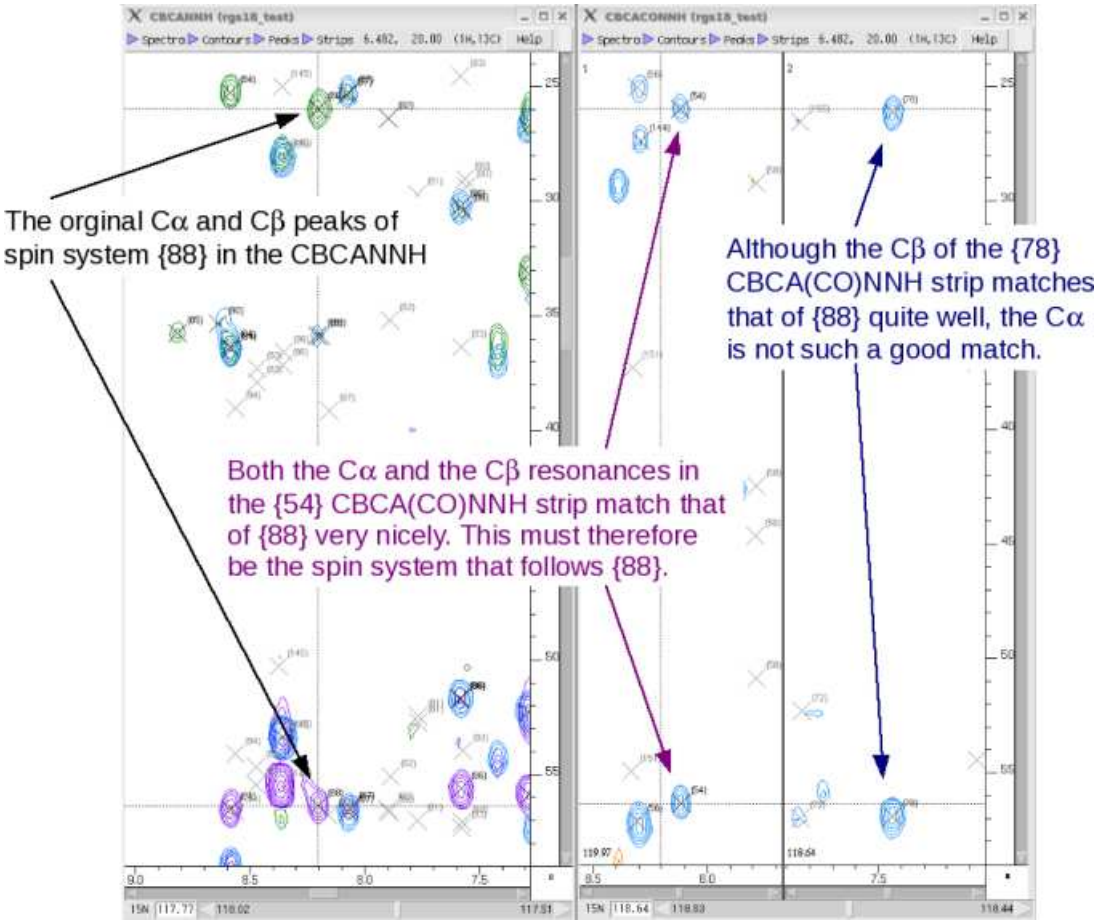
Semi-automatic Linking

- Assignment → Link Sequential Spin Systems



Semi-automatic Linking





That´s It

Happy Assigning…