

An Introduction to CCPNmr Analysis and Solution-NMR Assignment

Vicky Higman

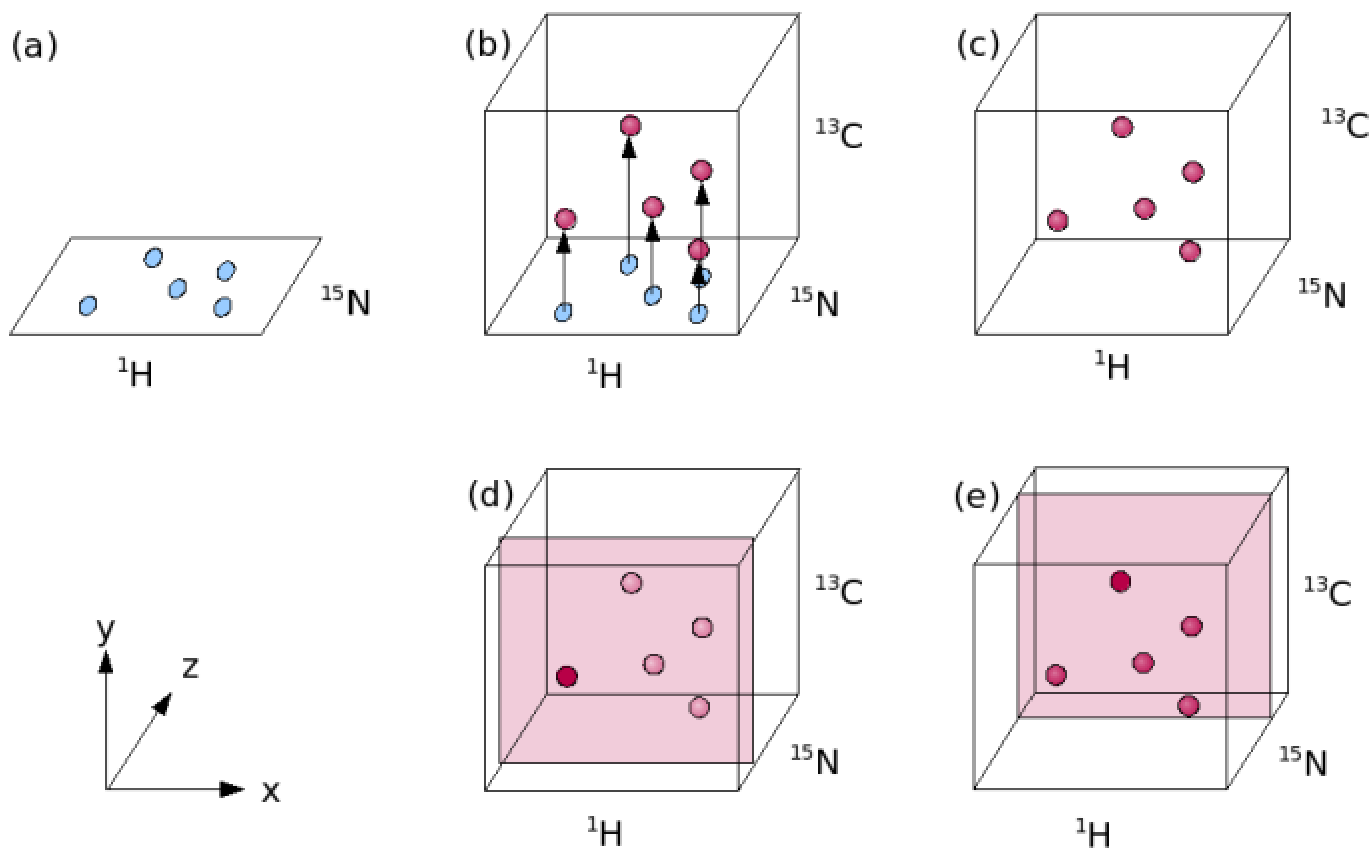
Leibniz-Institut für Molekulare
Pharmakologie, Berlin

www.protein-nmr.org.uk

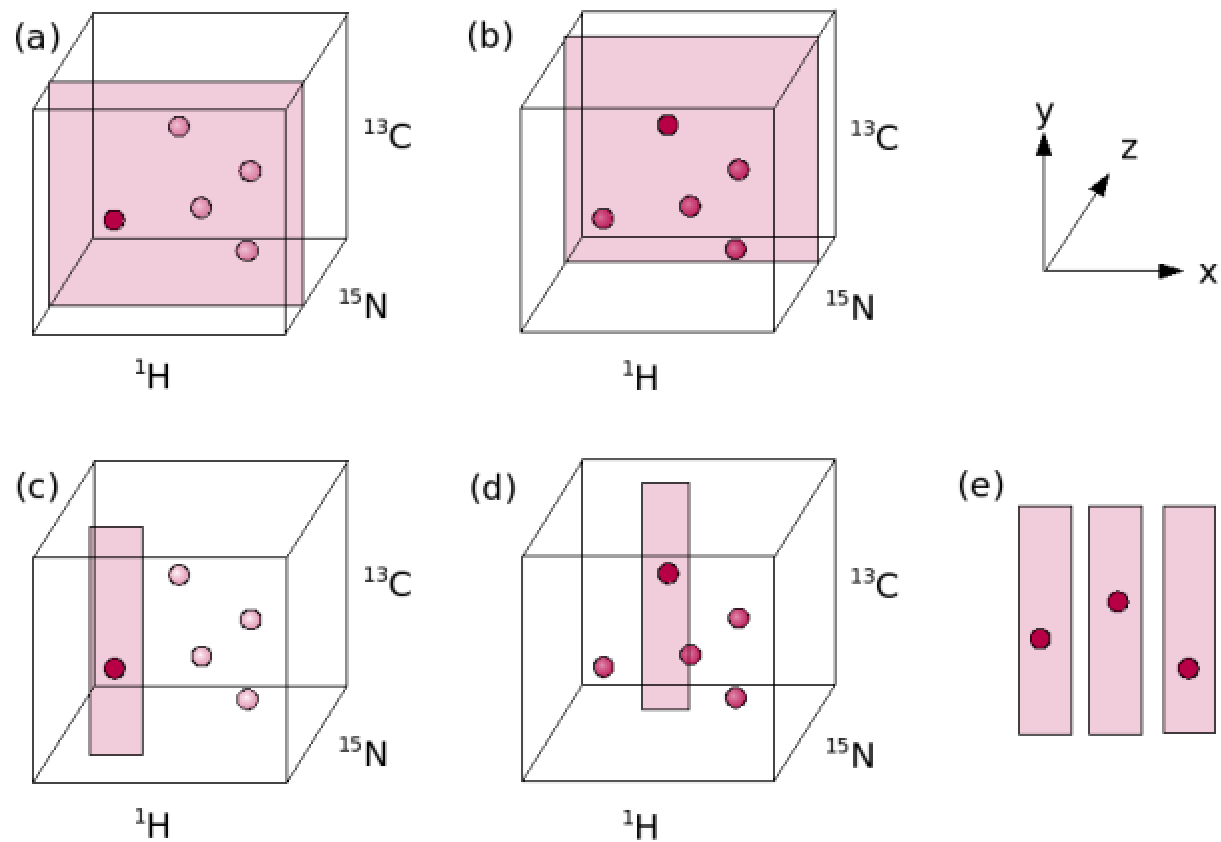
Outline

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

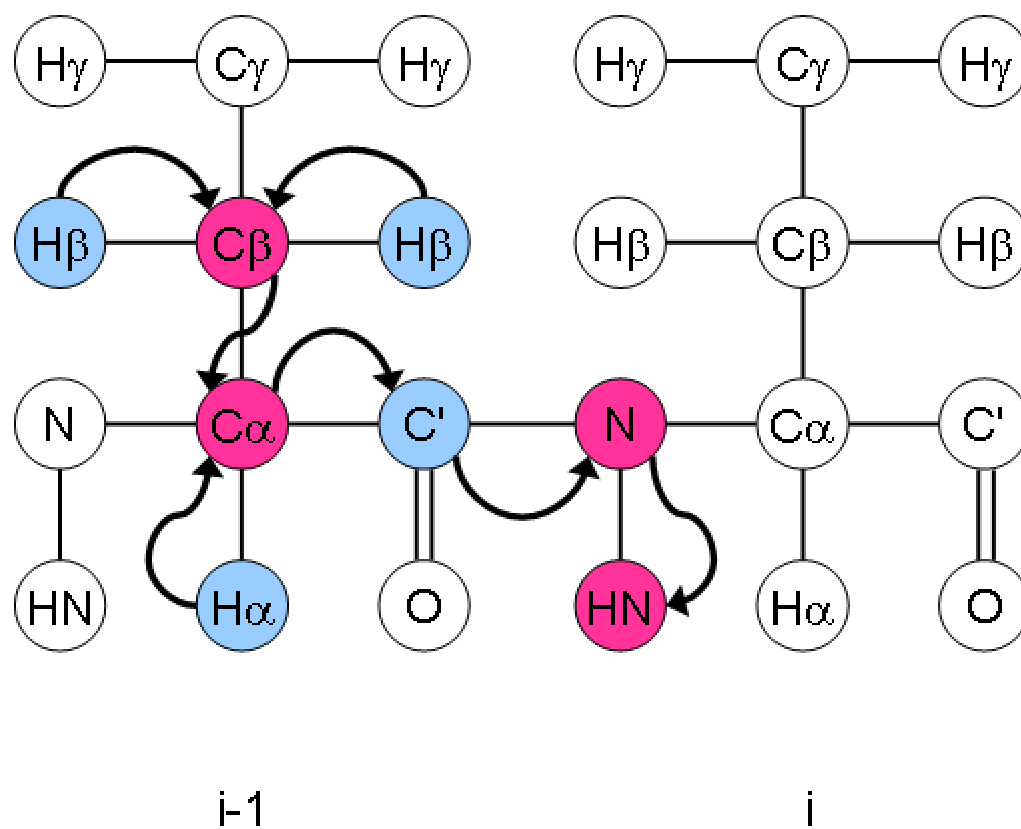
Visualising 3D Spectra



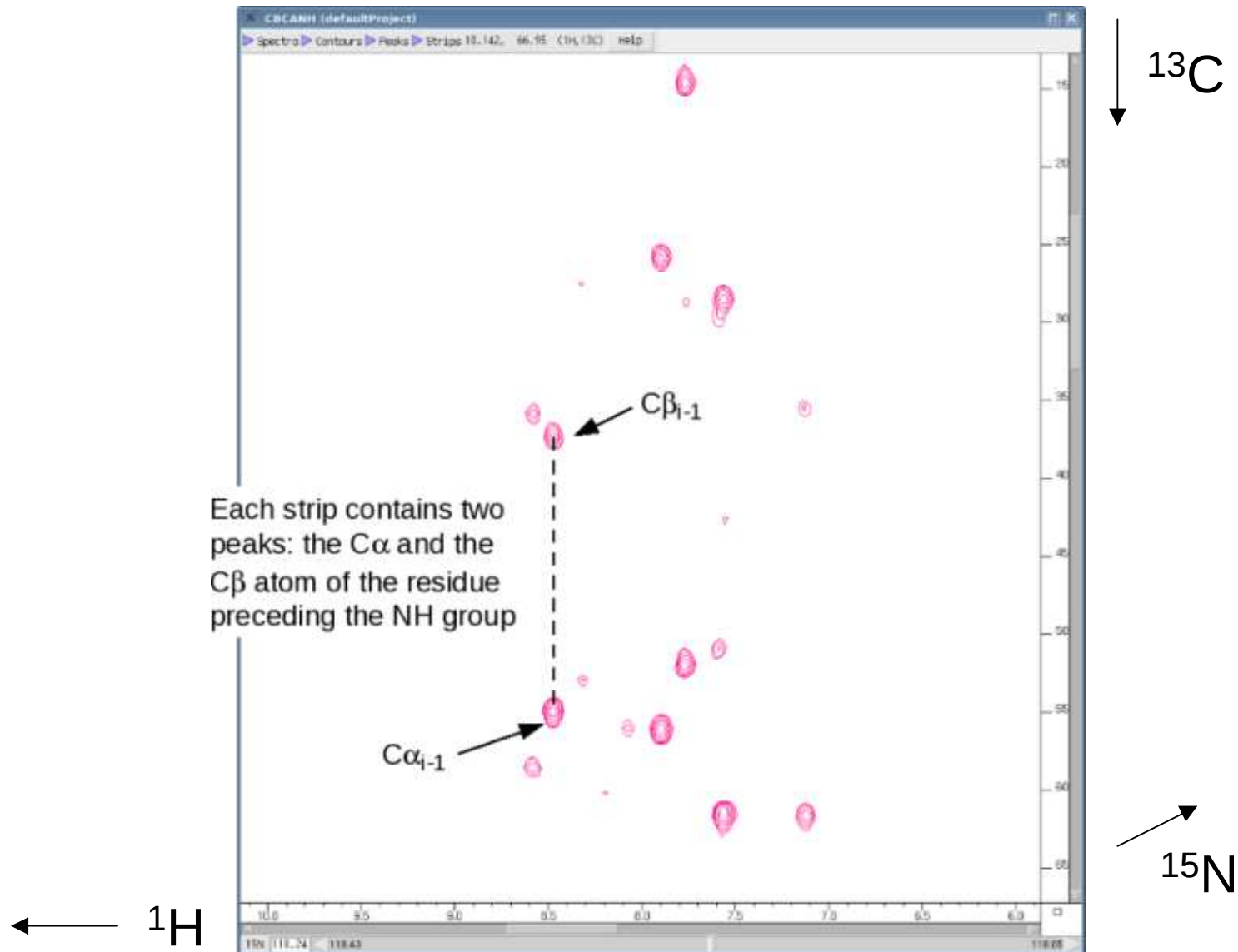
Visualising 3D Spectra



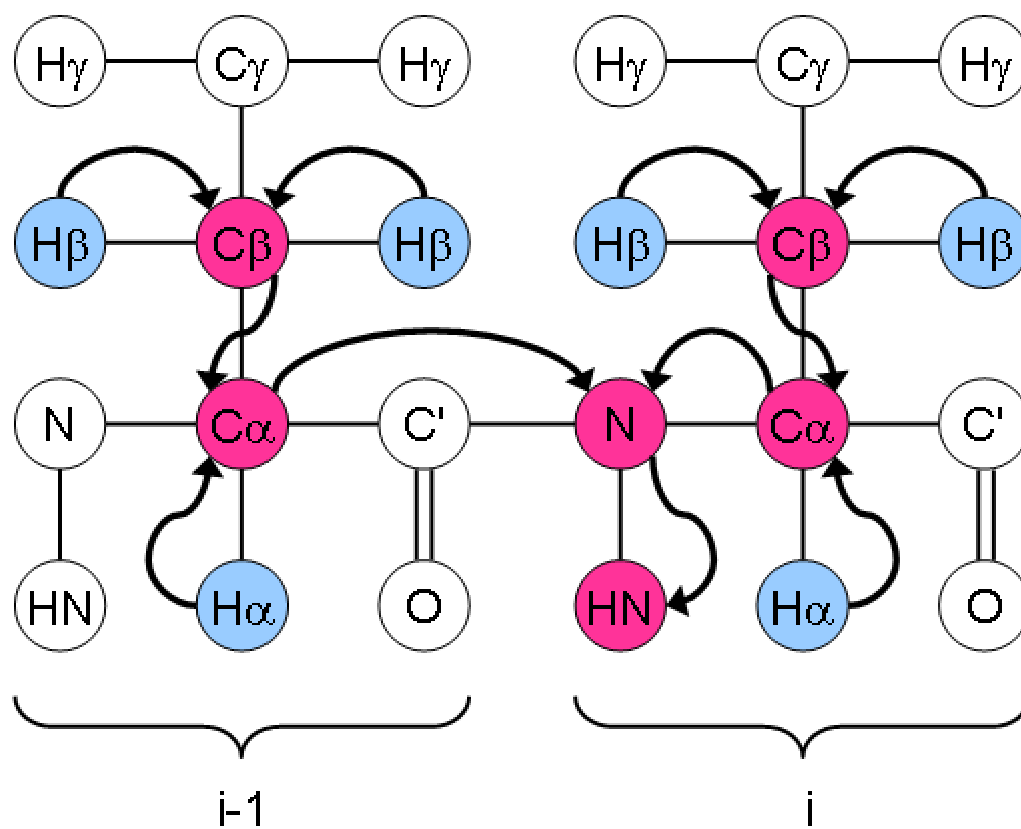
CBCA(CO)NH



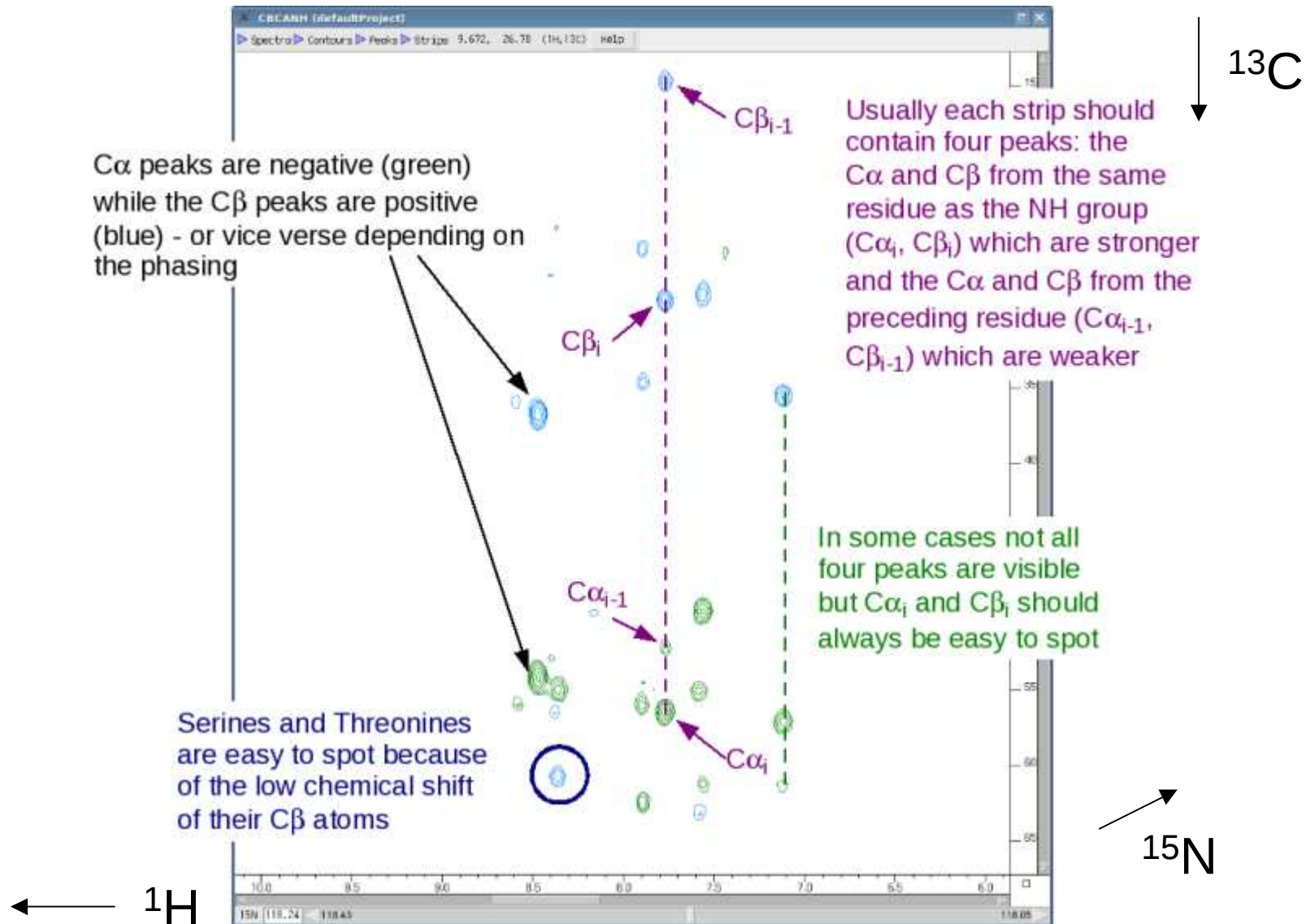
CBCA(CO)NH



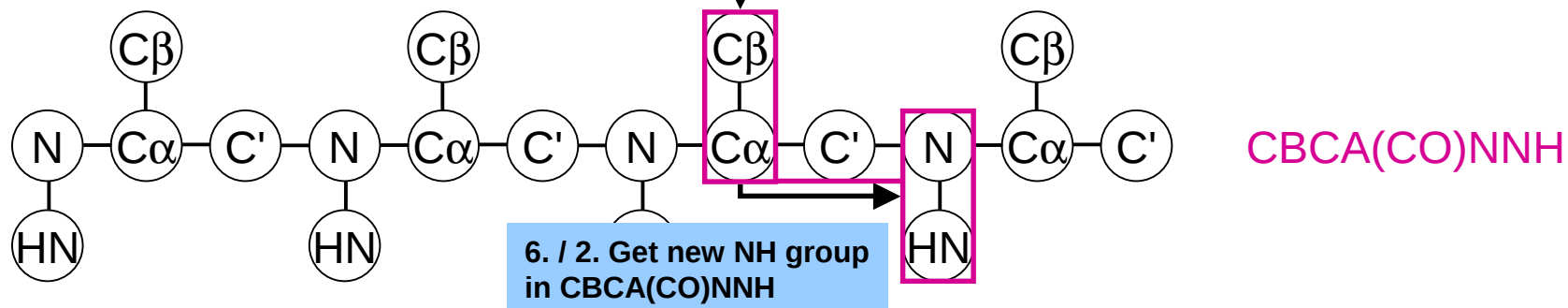
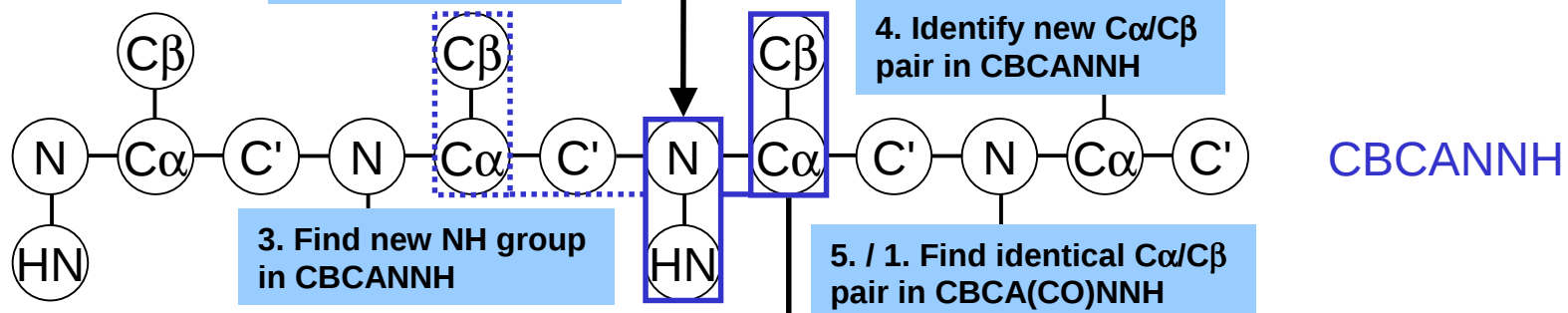
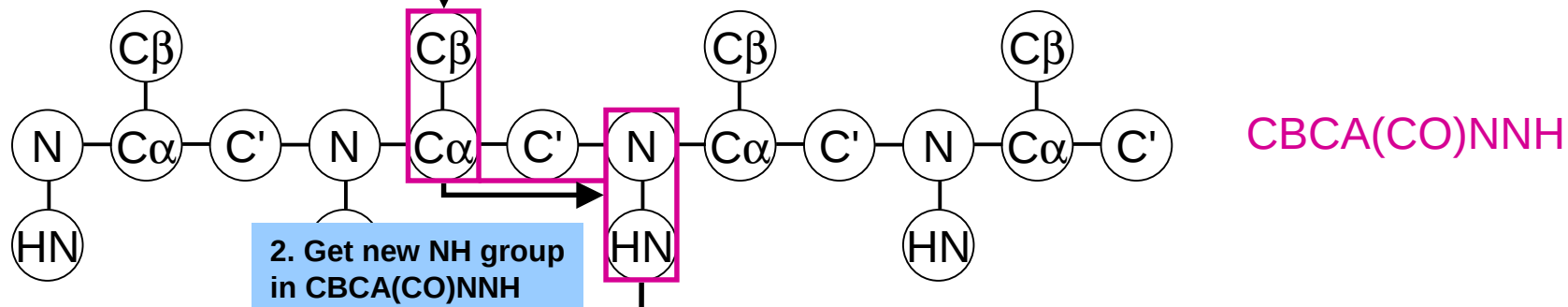
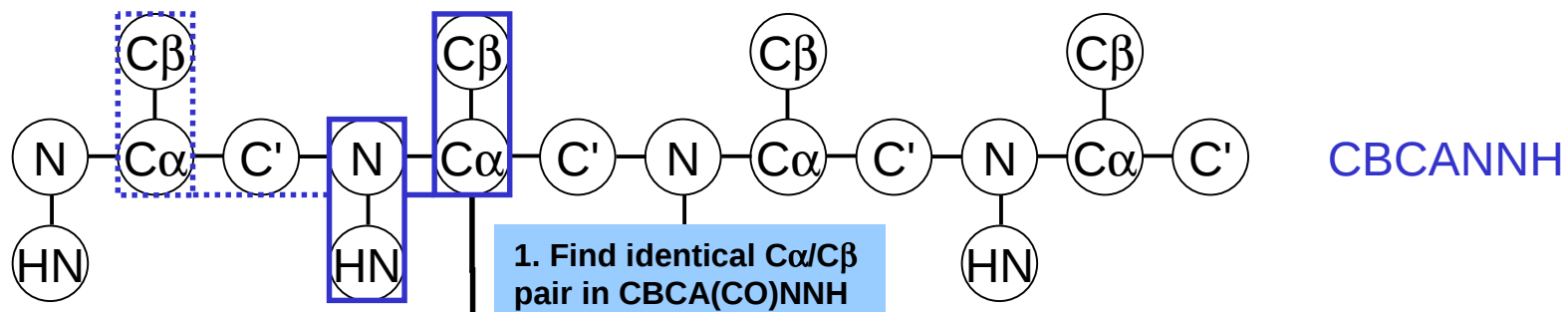
CBCANH



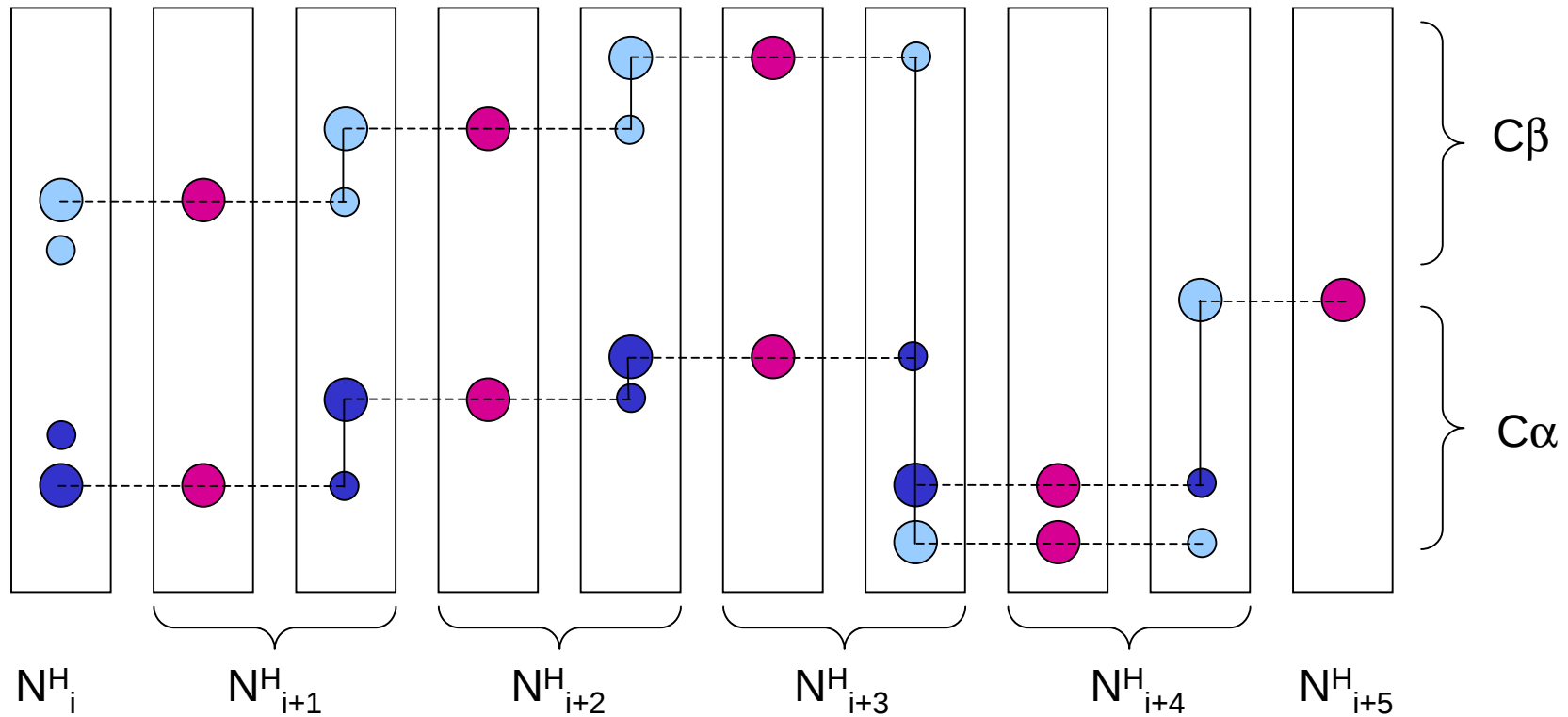
CBCANH



Sequential Assignment



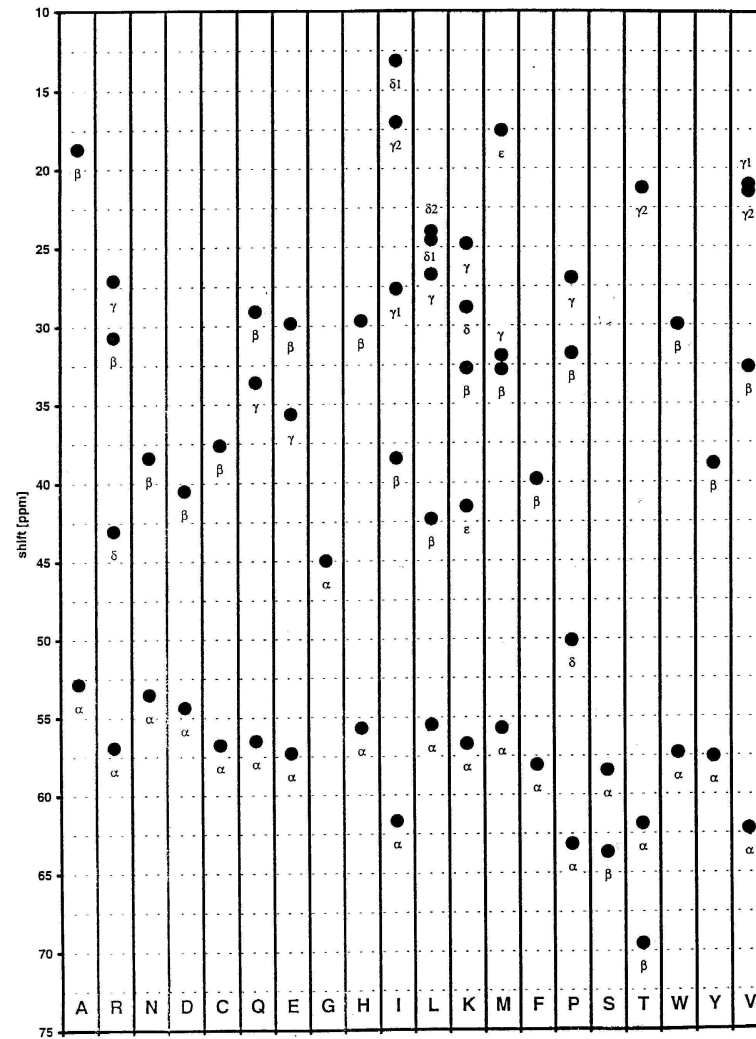
Sequential Assignment

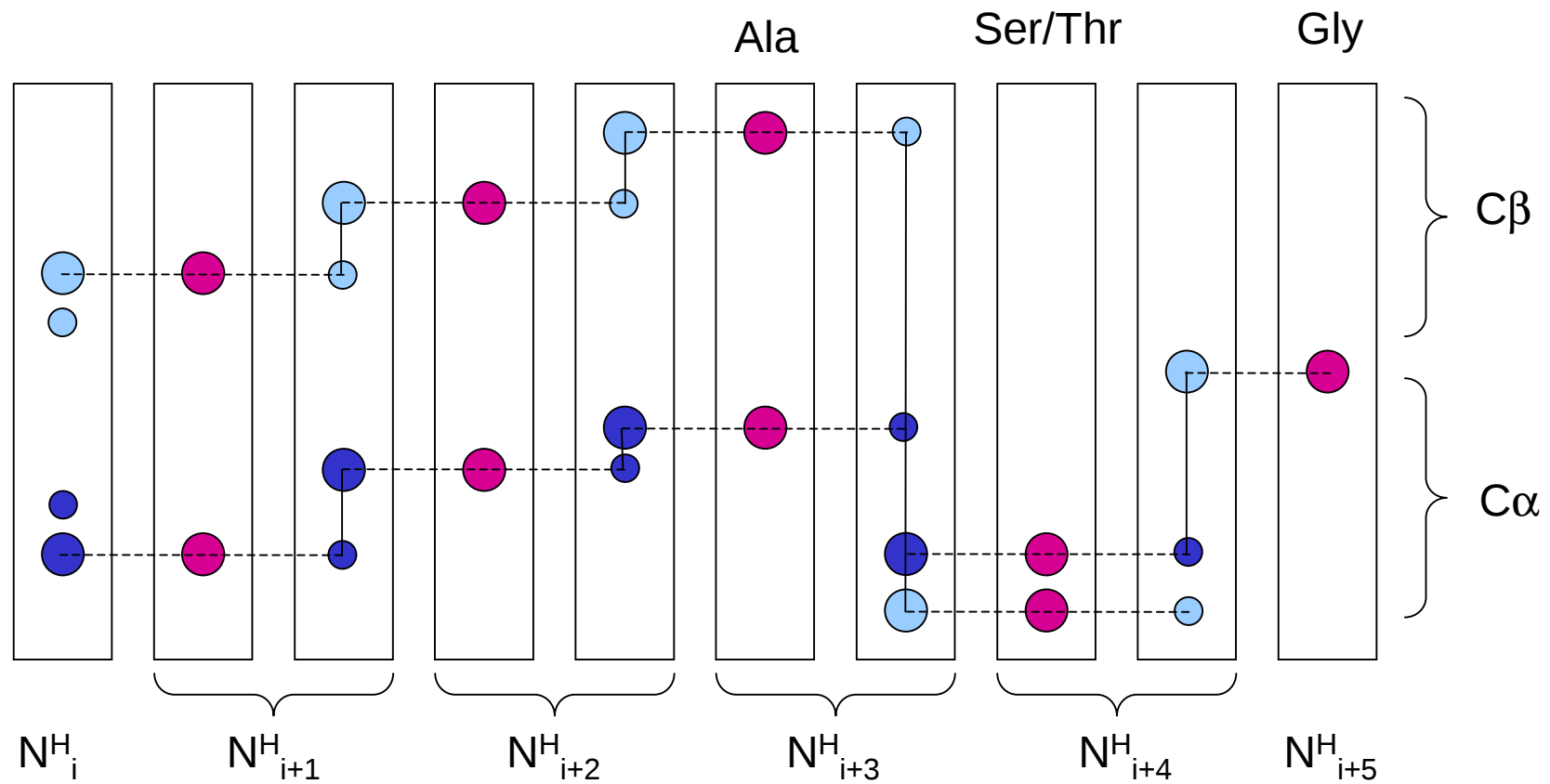


CBCANH

CBCA(CO)NH

Sequence Specific Assignment

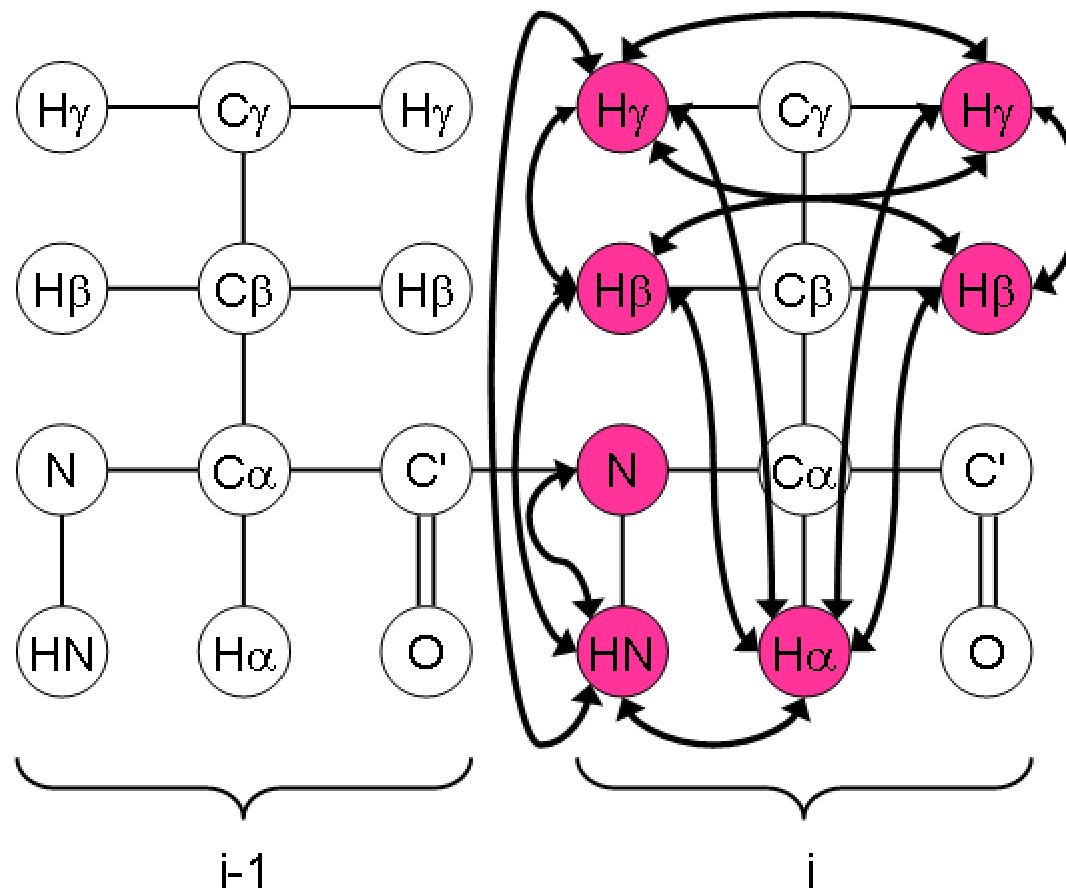




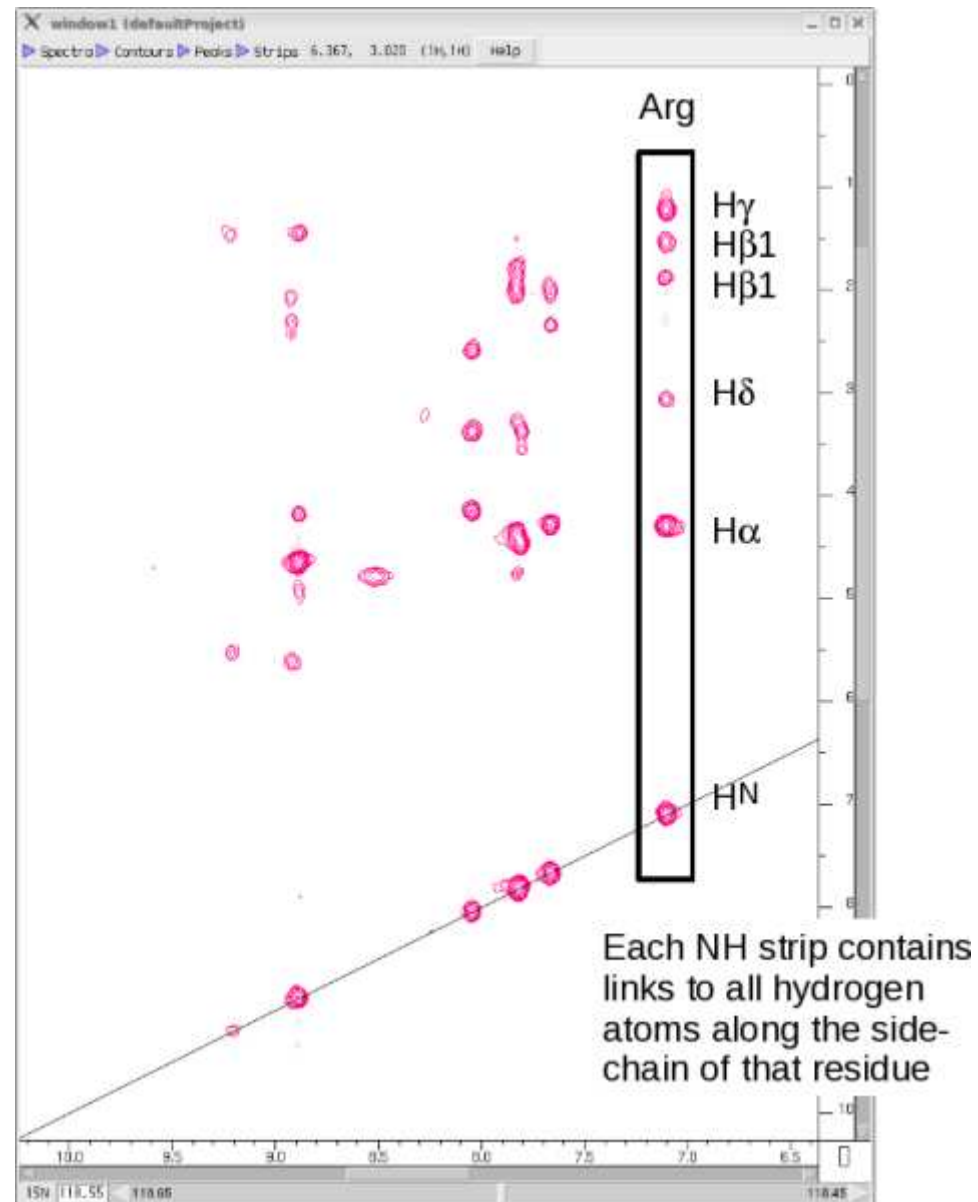
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FWIEASGDFK KSKGPQQIHL KAKAIYEKFI QDTEASGVNL
DFHTKEVITN SITQPTLHSF DAAQSRVYQL MEQDSYTRFL
KSDIYLDLME GRPQRPTNLR RRSRSFTCNE

Side-Chain Assignment

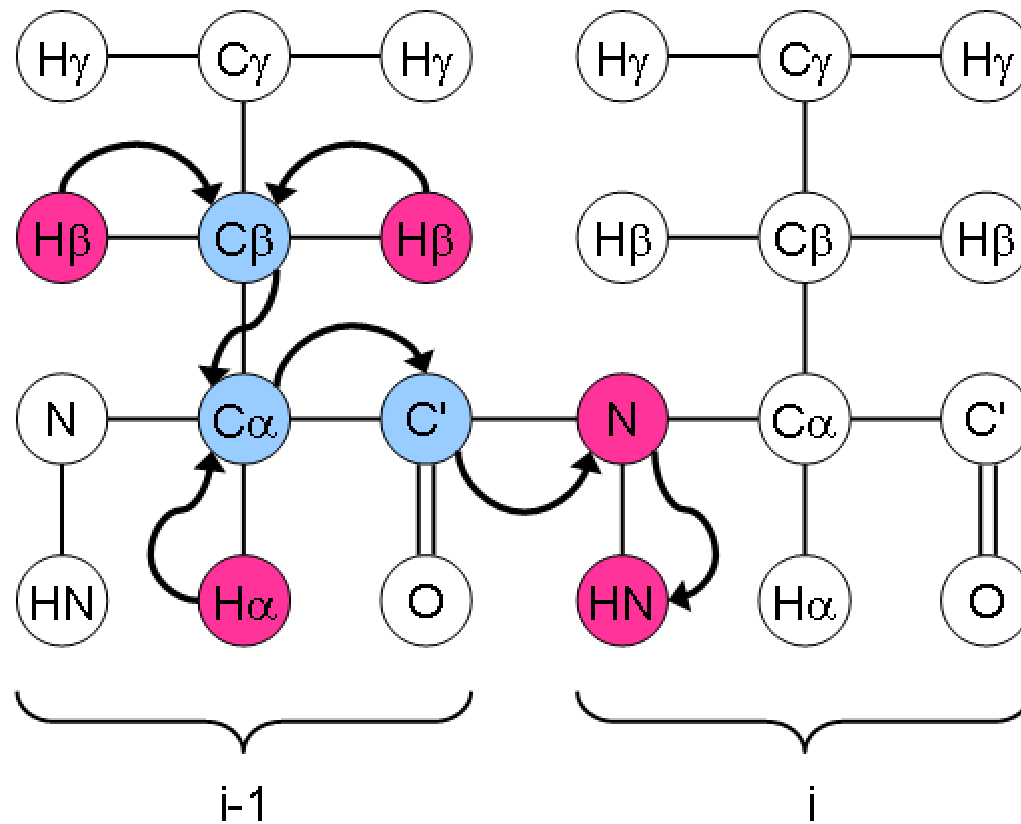
^{15}N -TOCSY



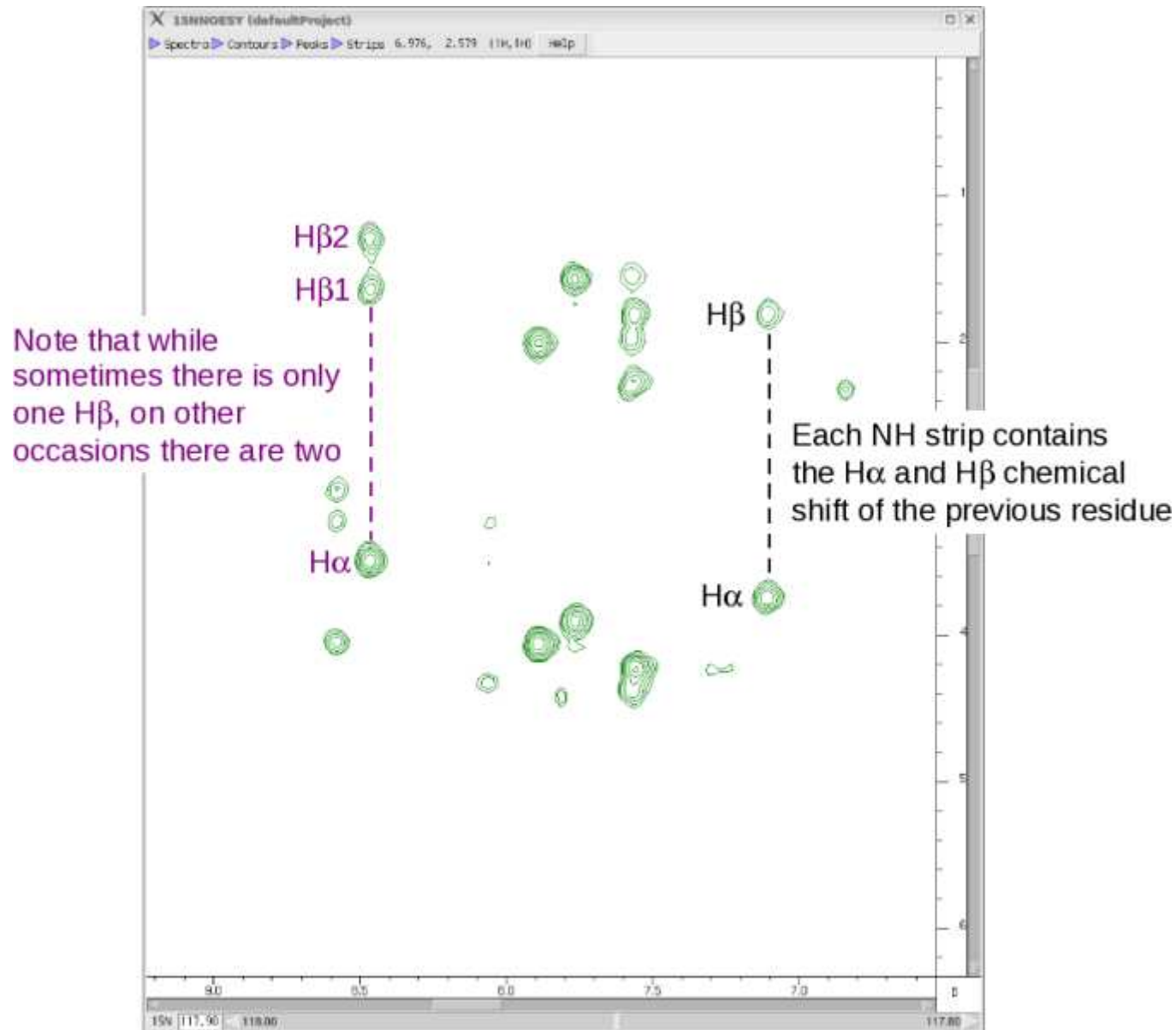
^{15}N -TOCSY

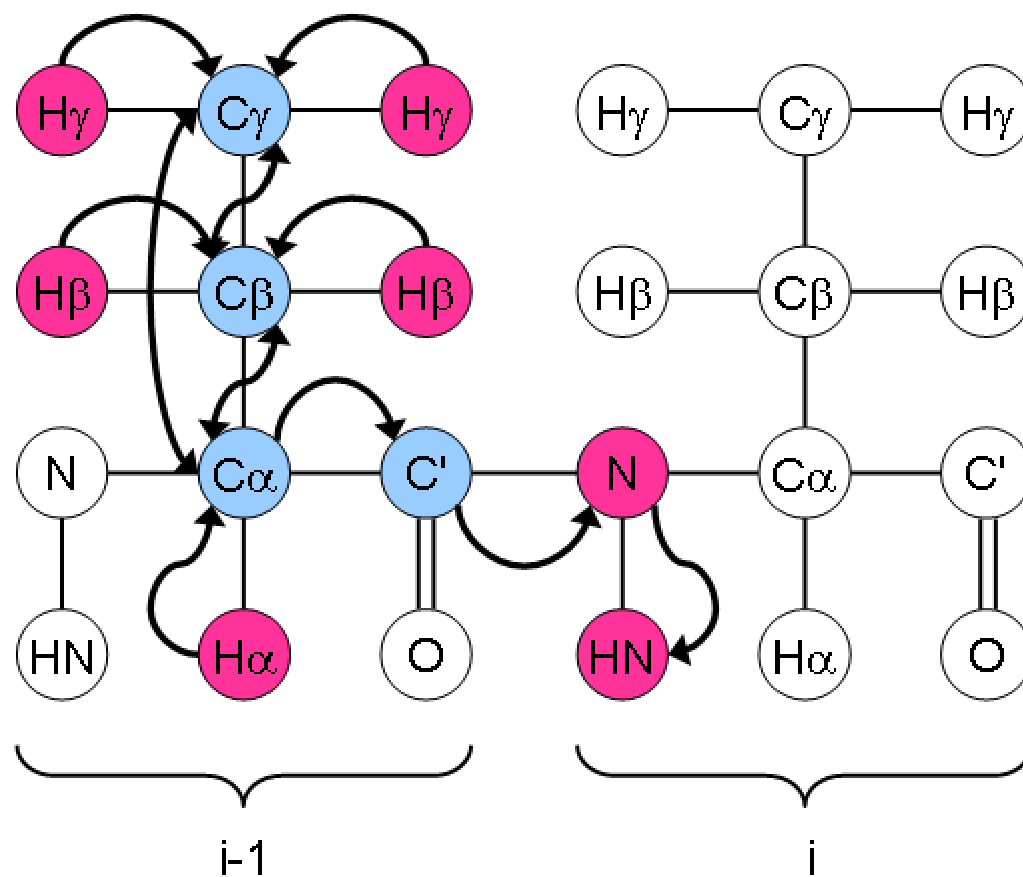


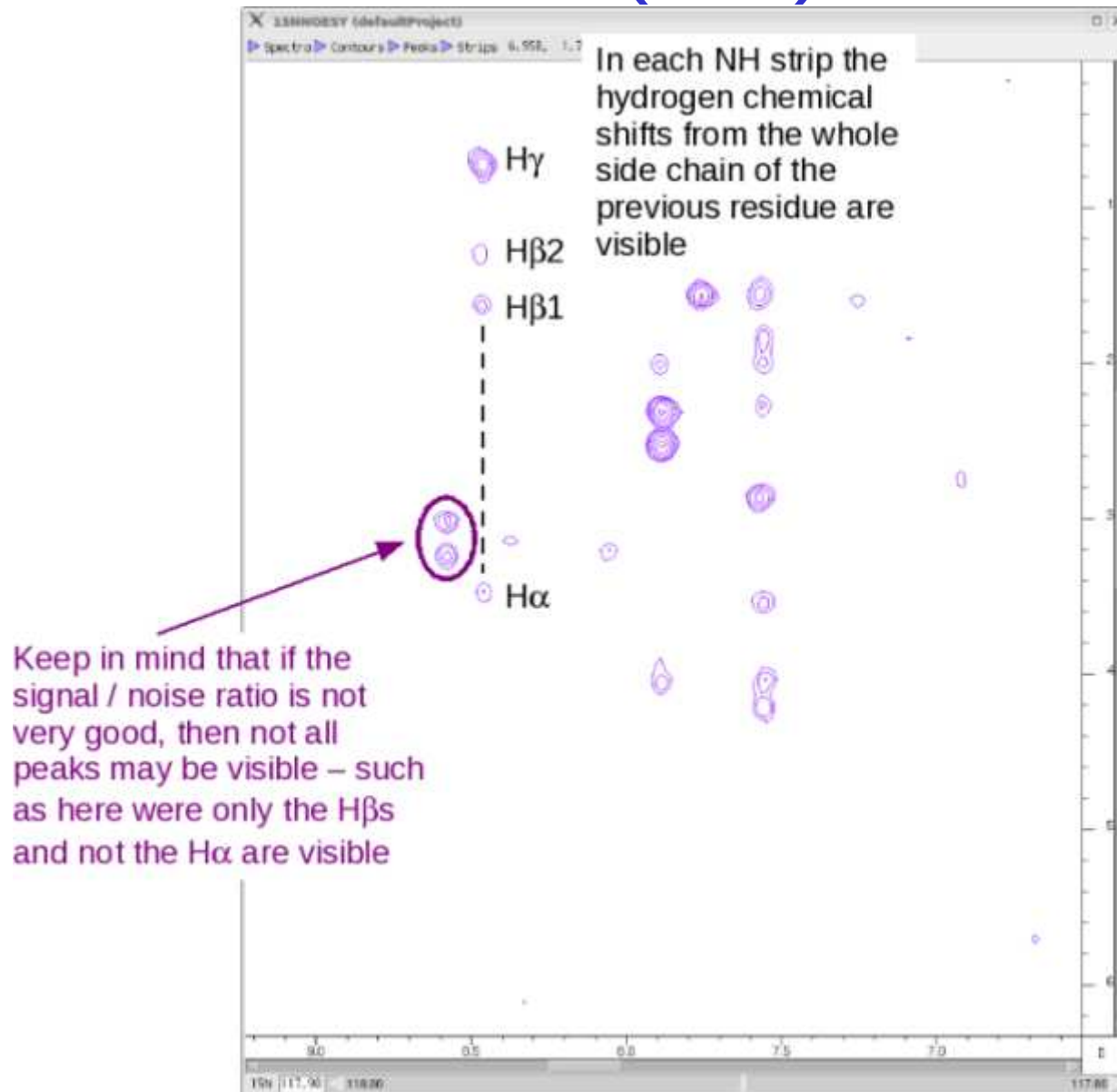
HBHA(CO)NH



HBHA(CO)NH

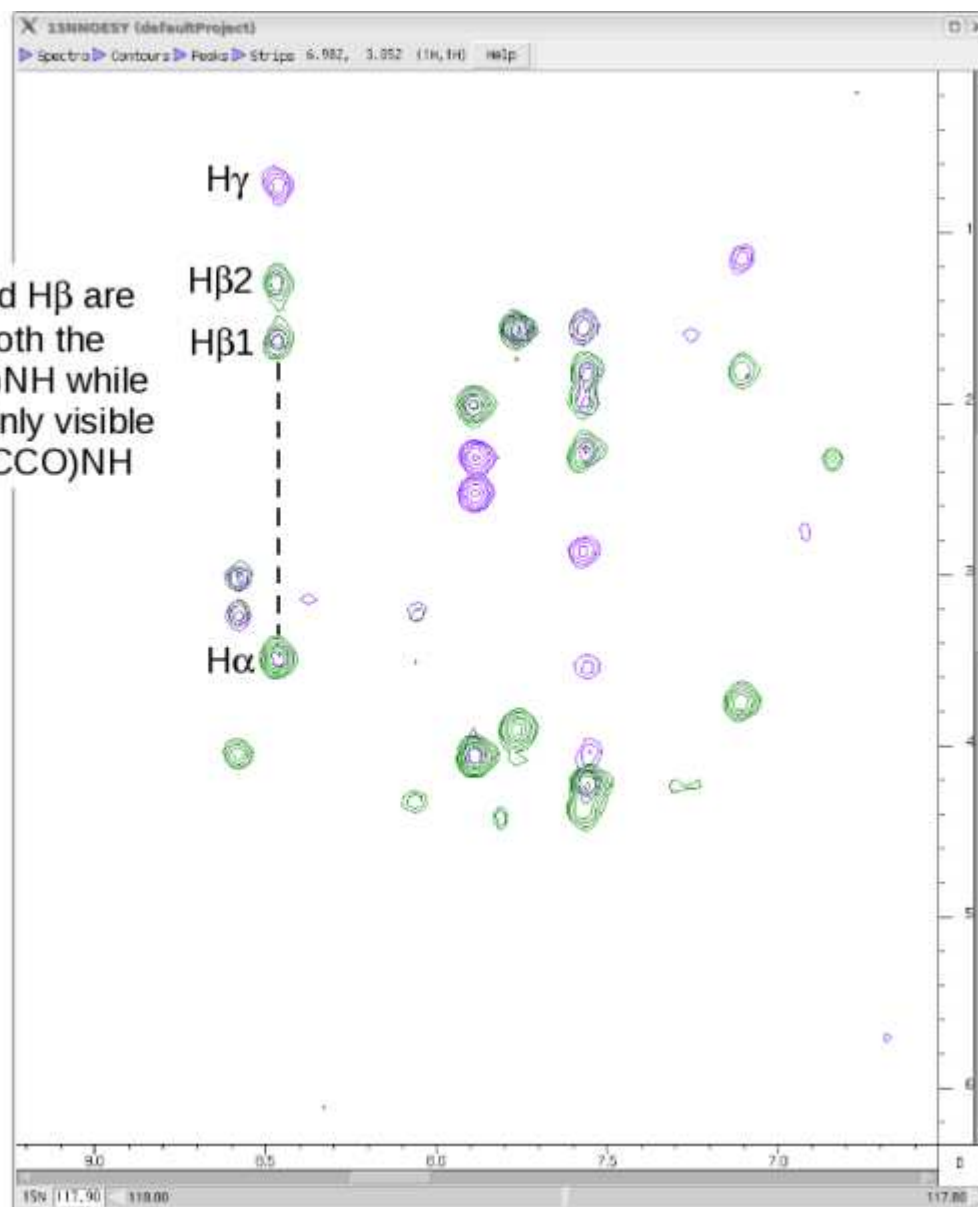


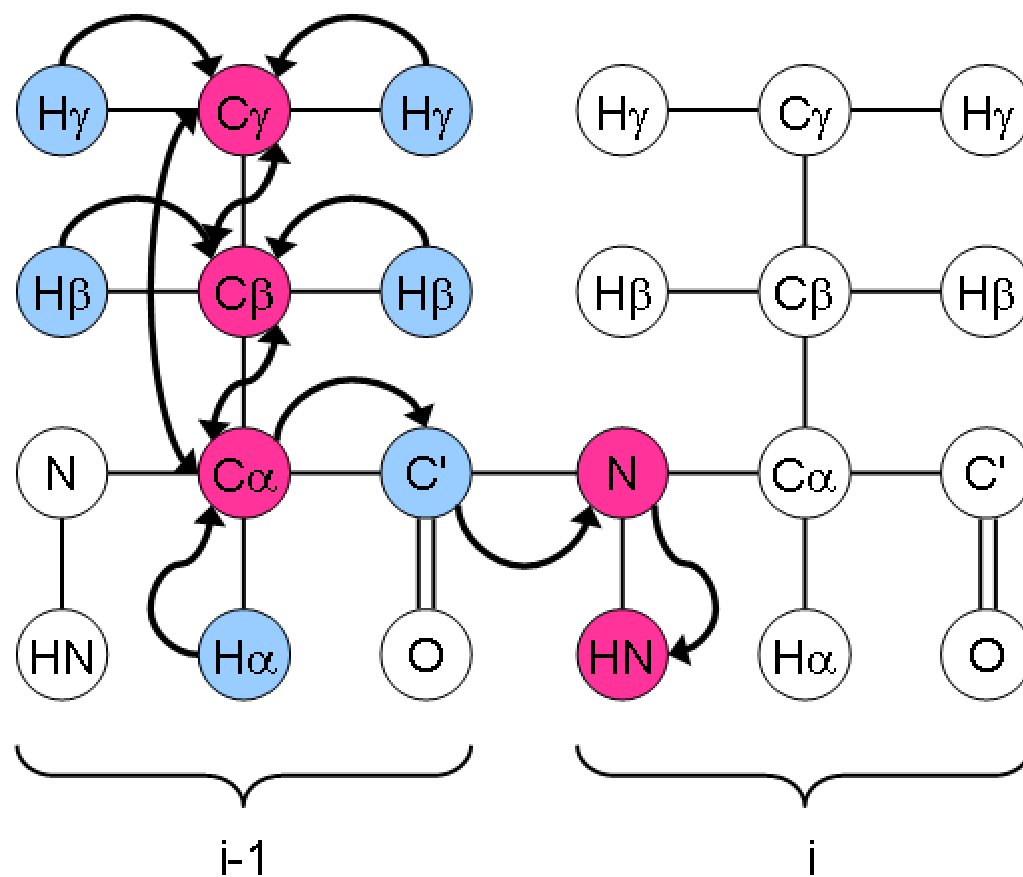




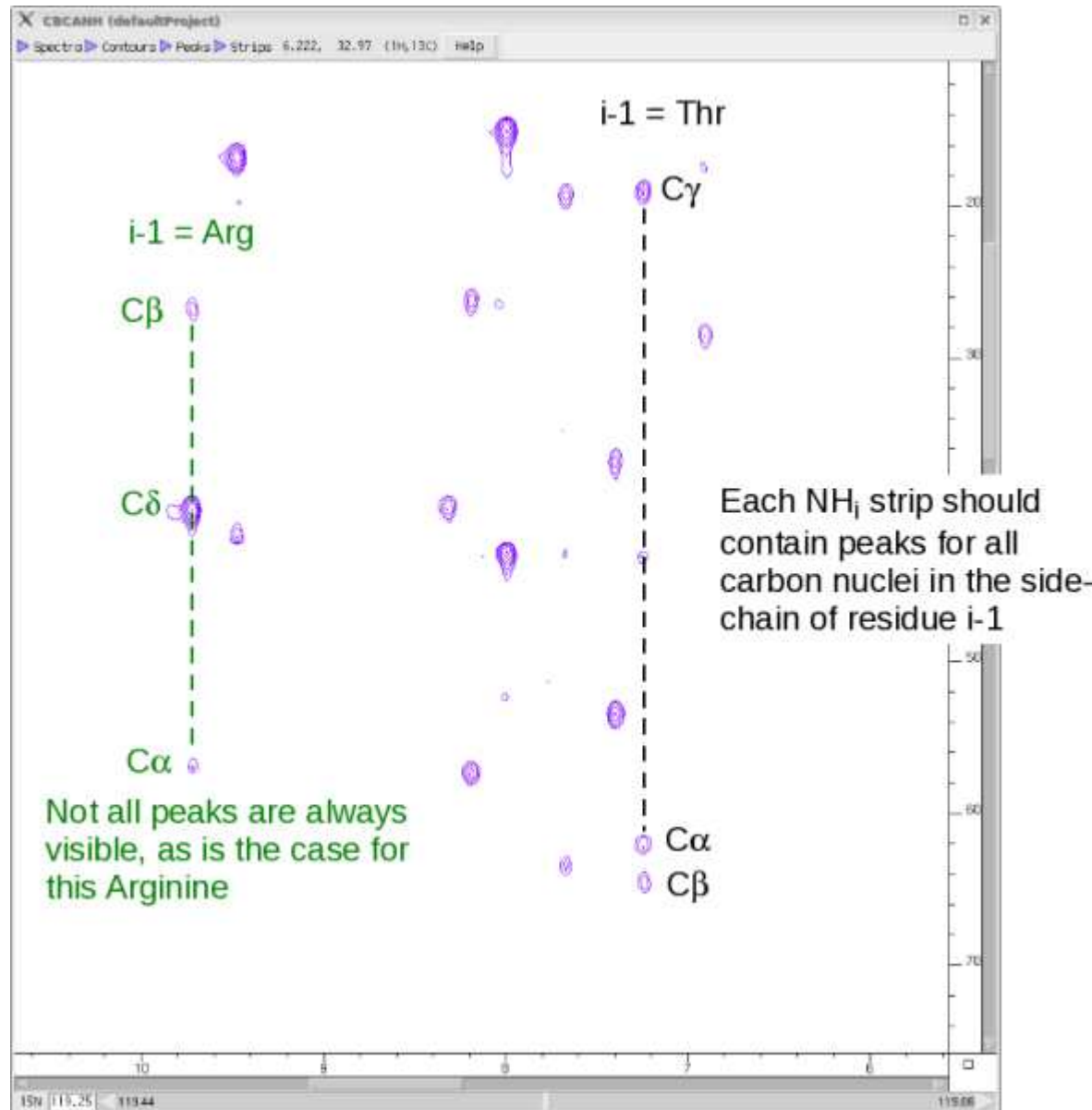
HCC(CO)NH

The H α and H β are visible in both the HBHA(CO)NH while the H γ is only visible in the H(CCCO)NH

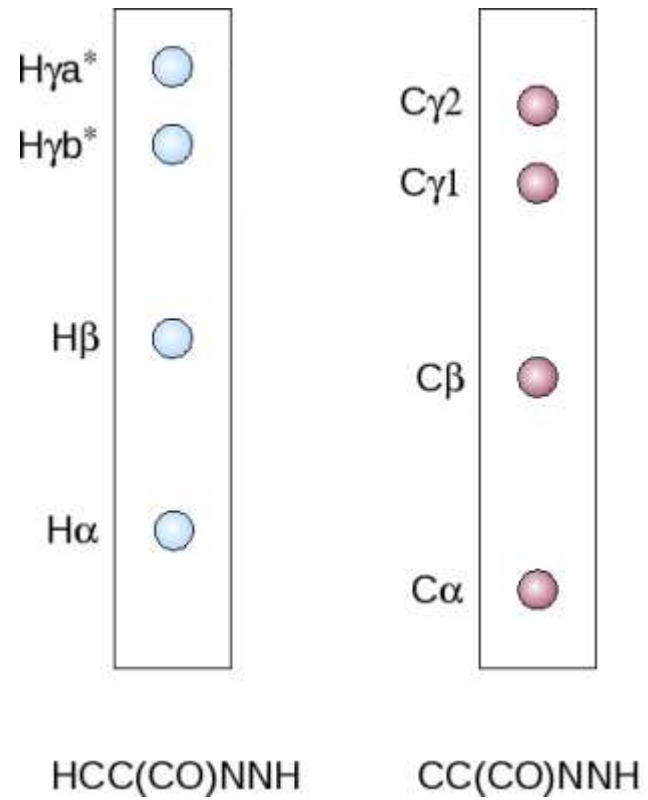




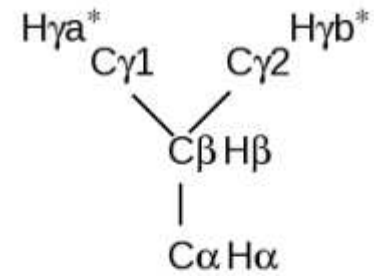
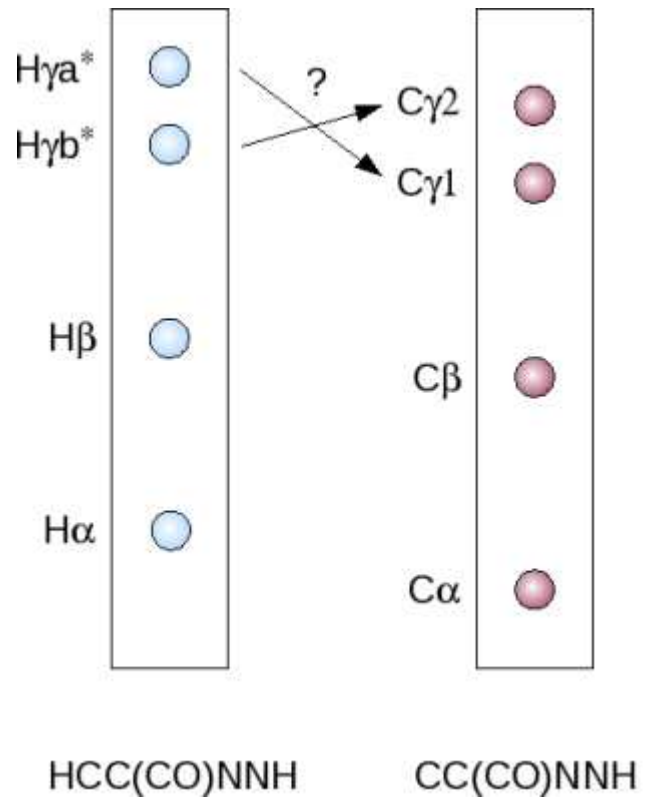
CC(CO)NH



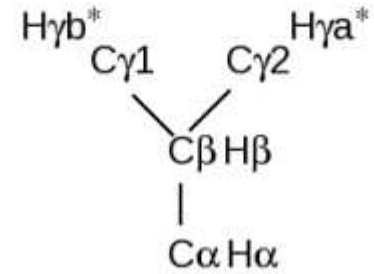
Ambiguities



Ambiguities

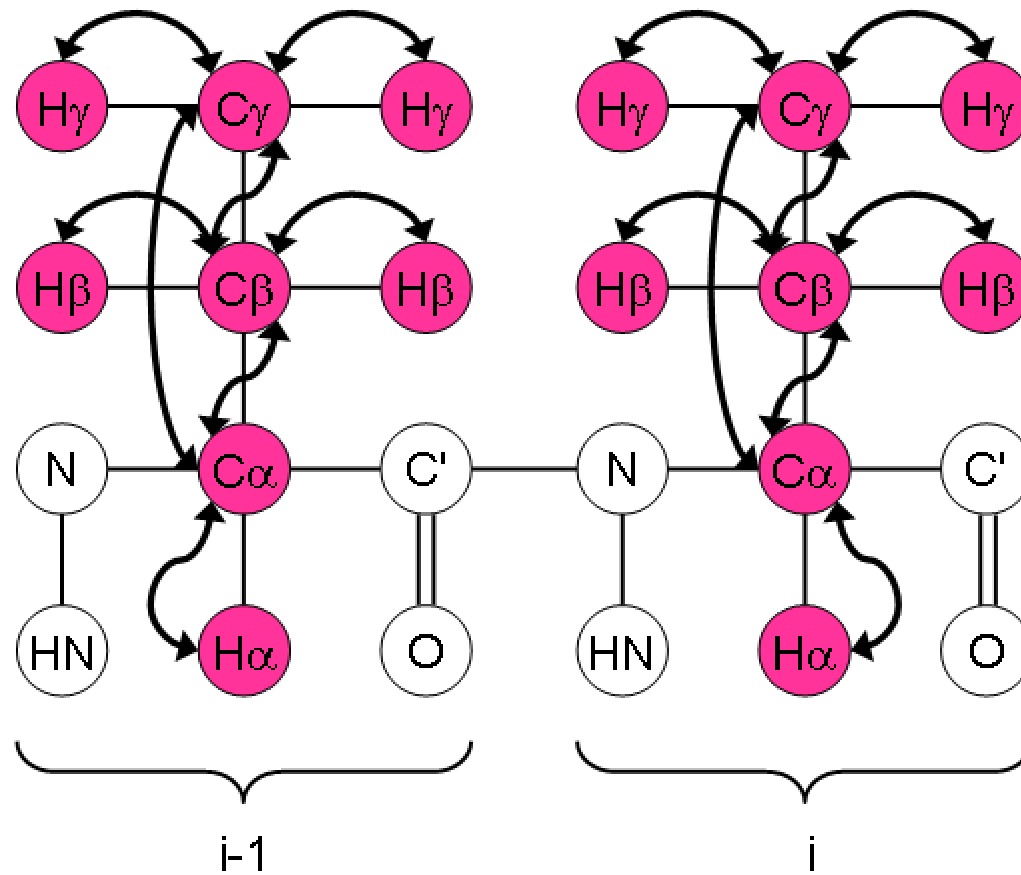


OR



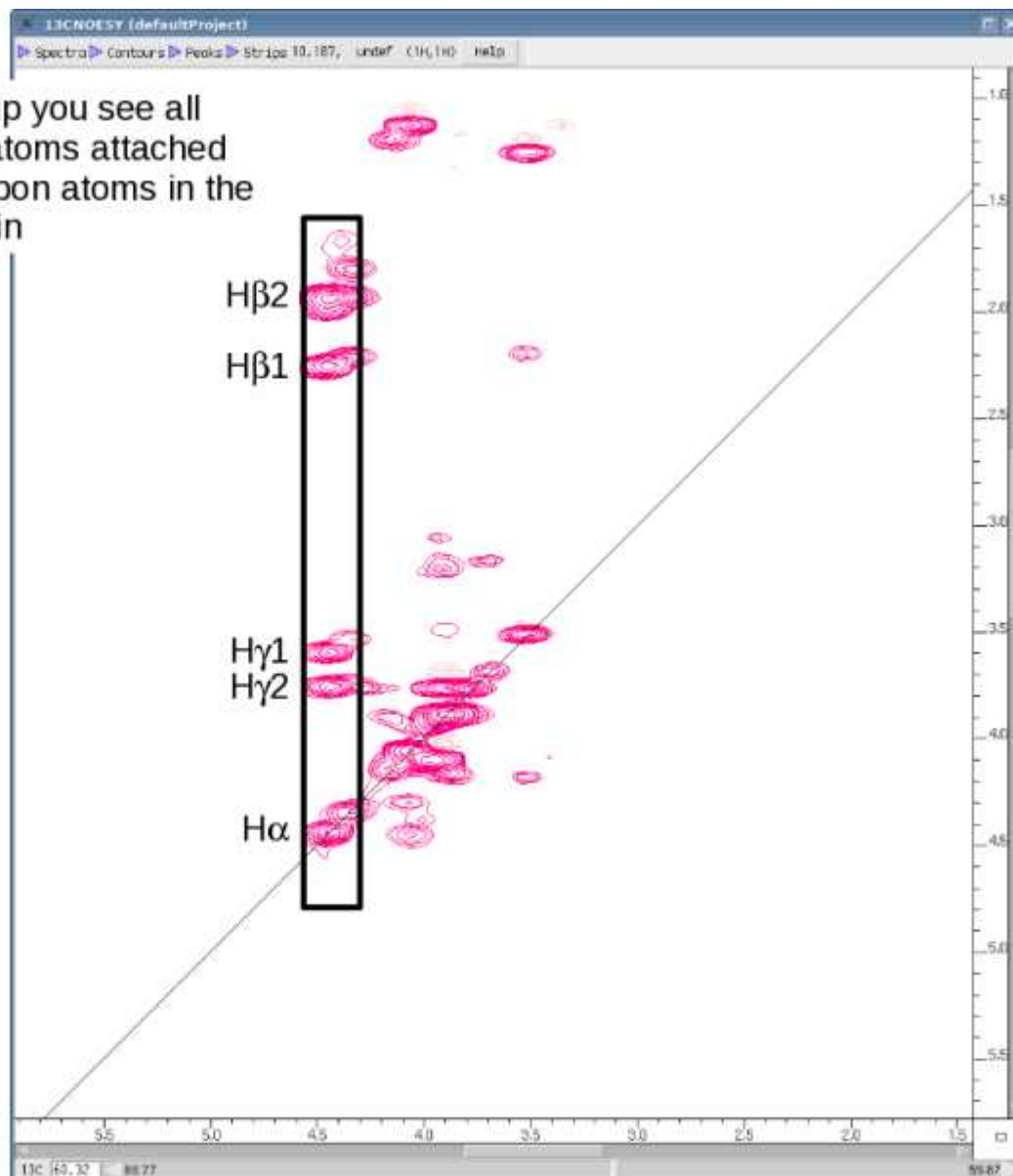
?

HCCH-TOCSY

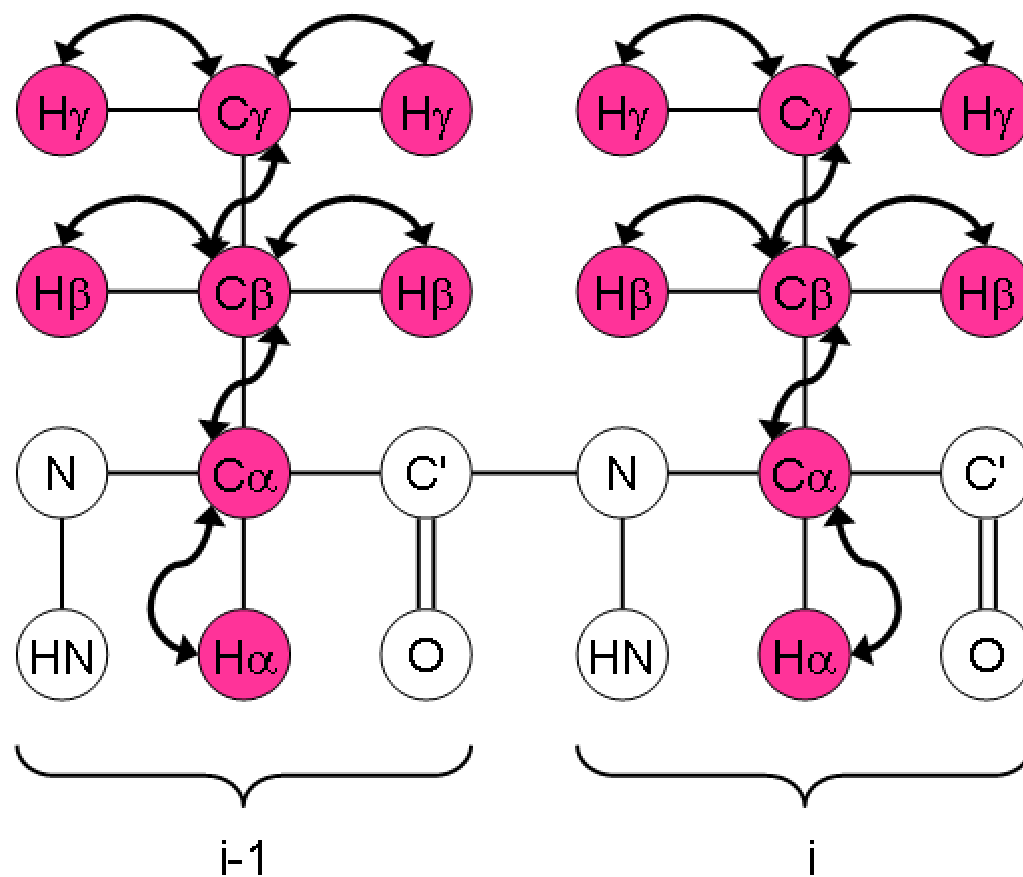


HCCH-TOCSY

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

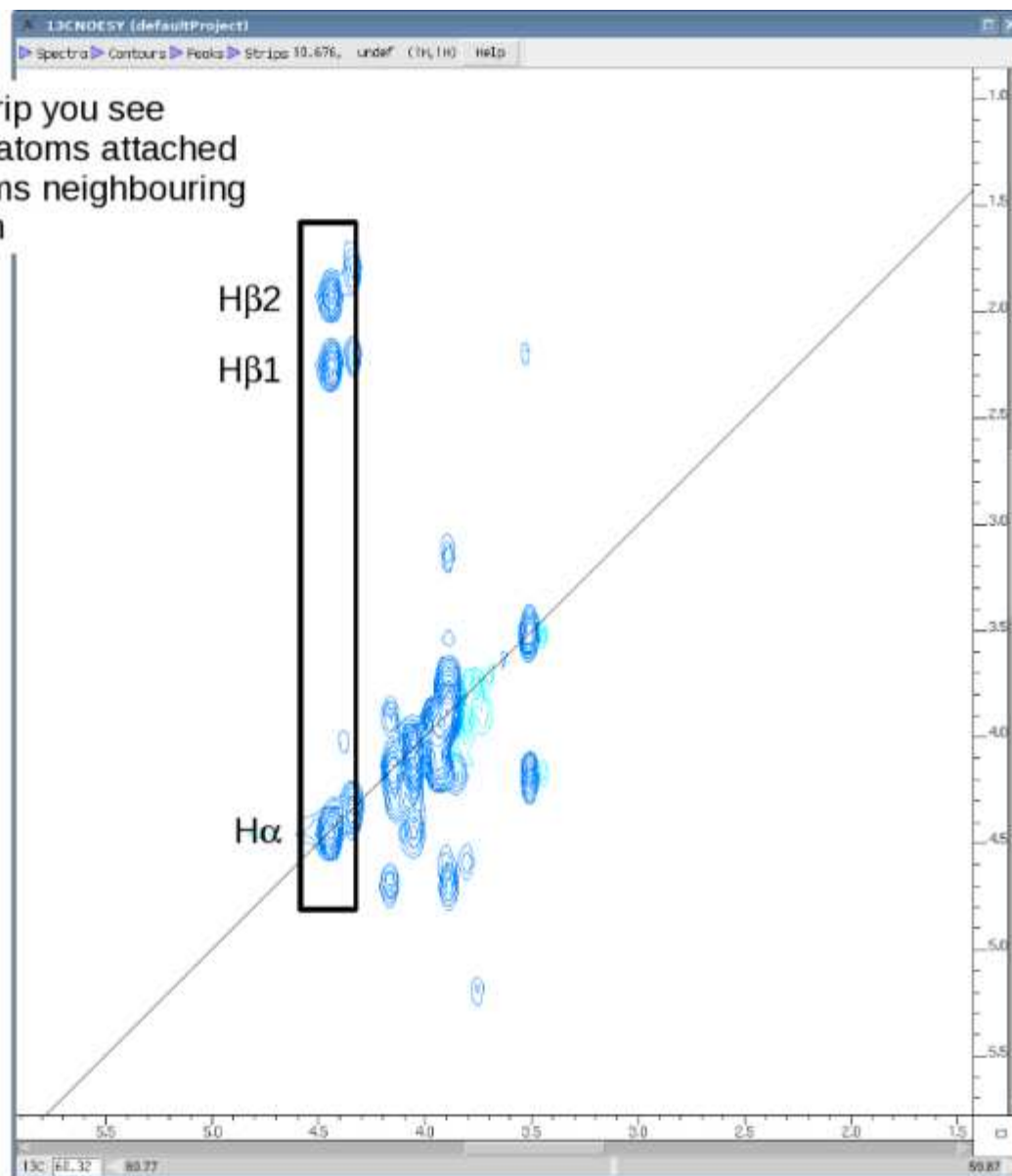


HCCH-COSY

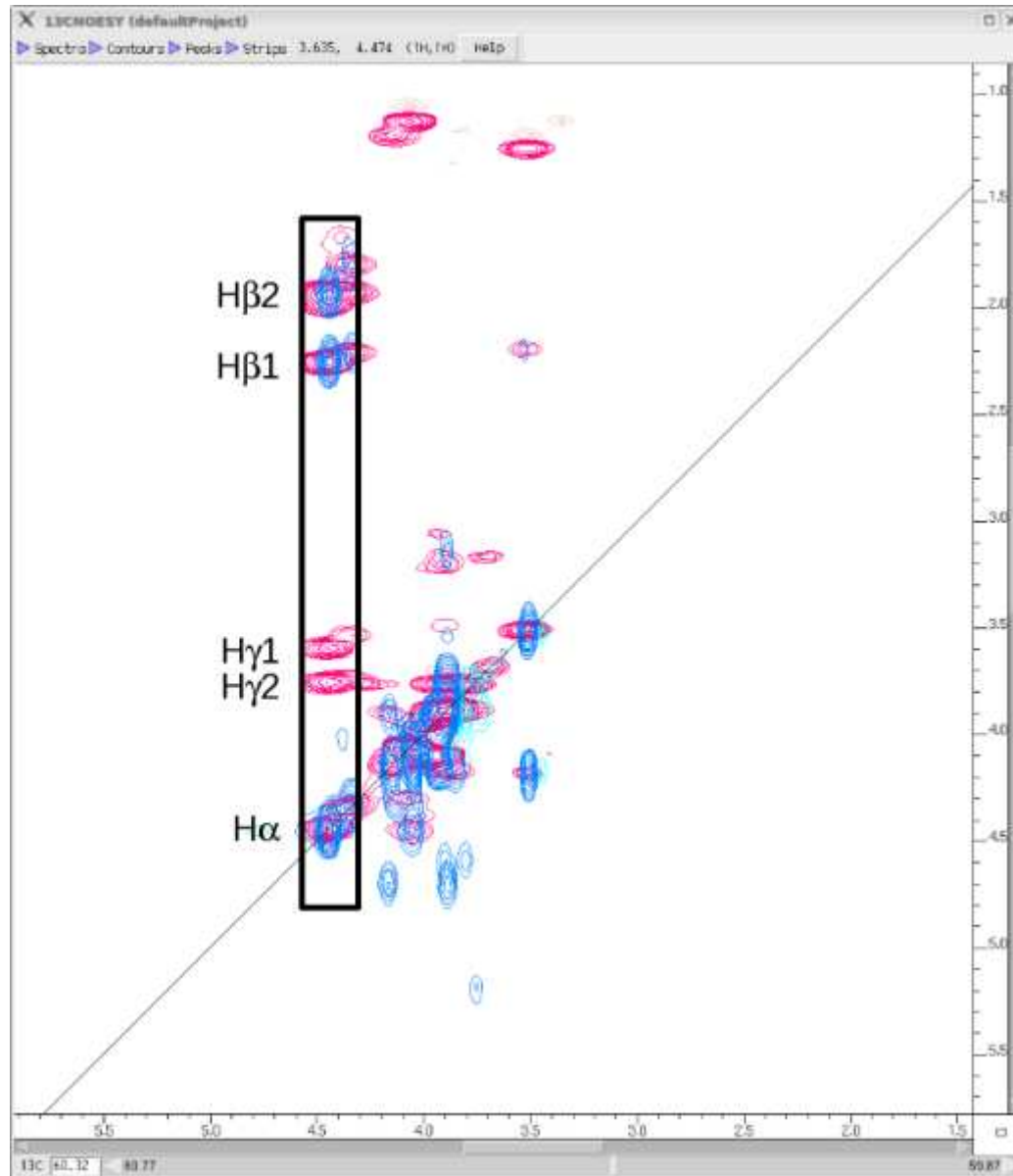


HCCH-COSY

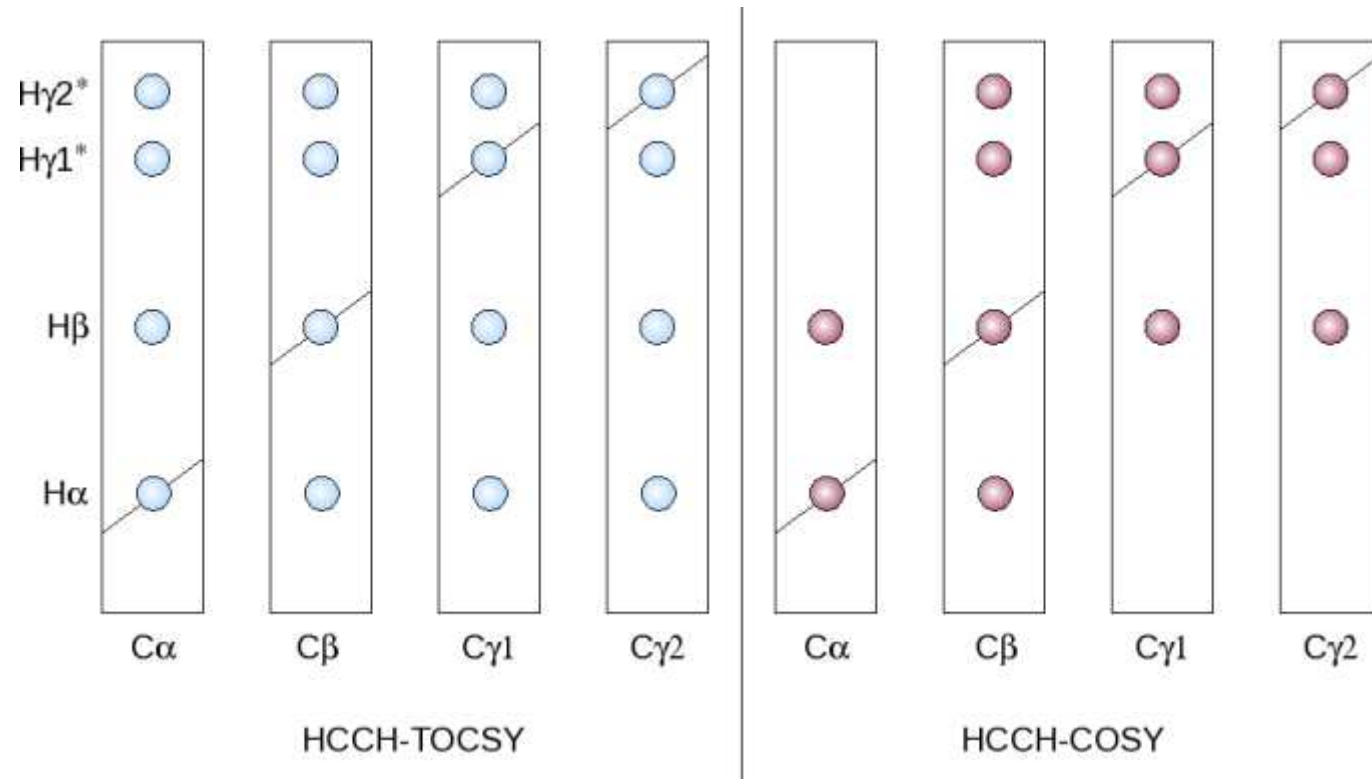
In each CH strip you see
the hydrogen atoms attached
to carbon atoms neighbouring
the CH carbon



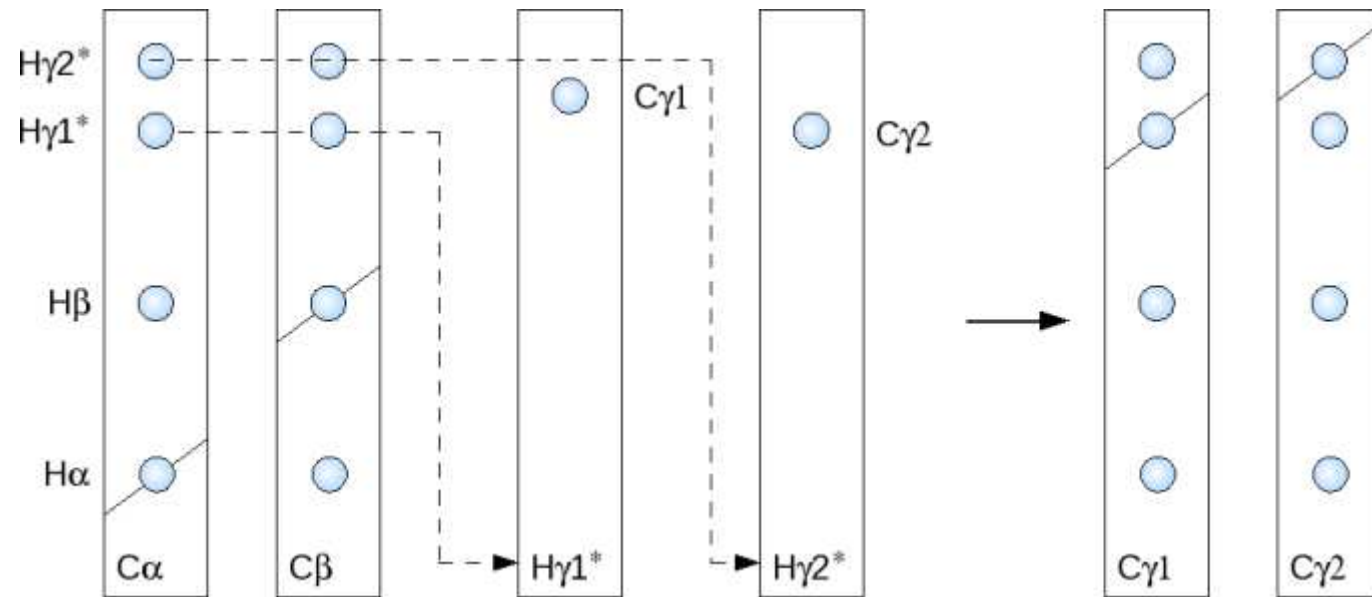
HCCH-COSY



Side-chain Assignment



Side-chain Assignment



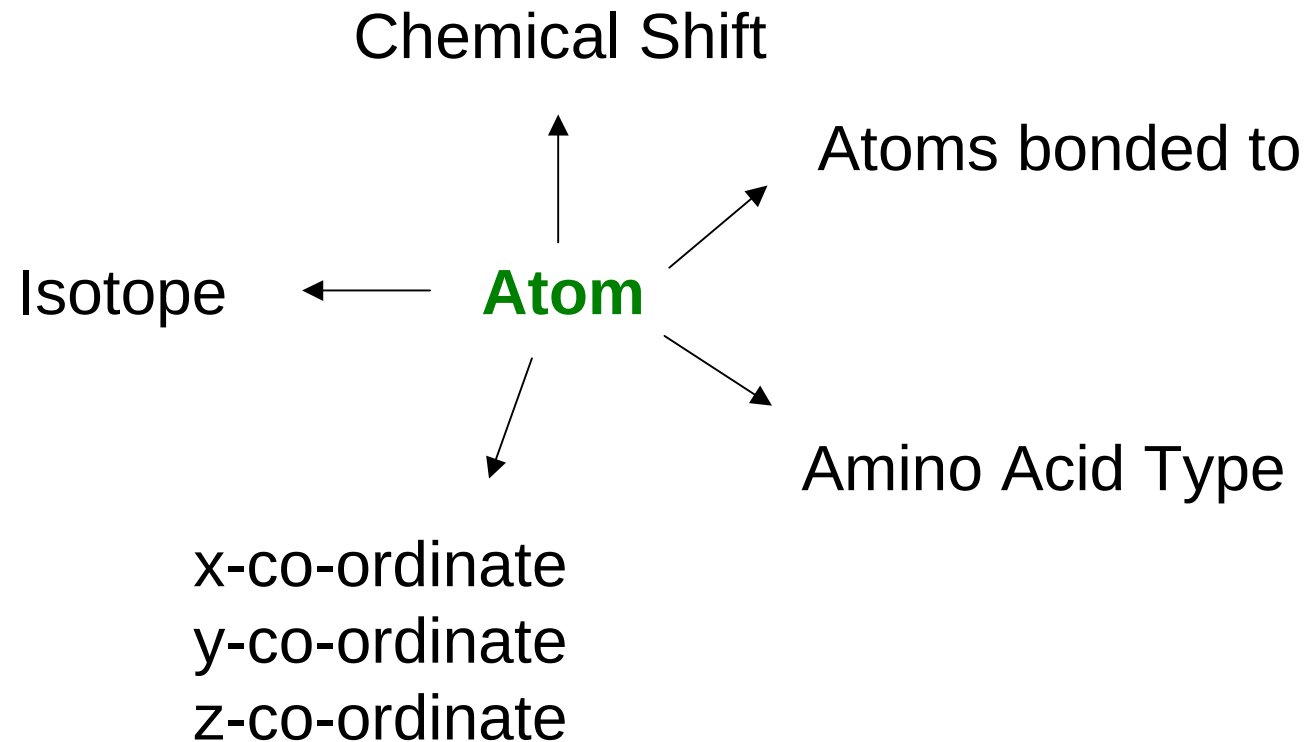
Outline

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

CCPN

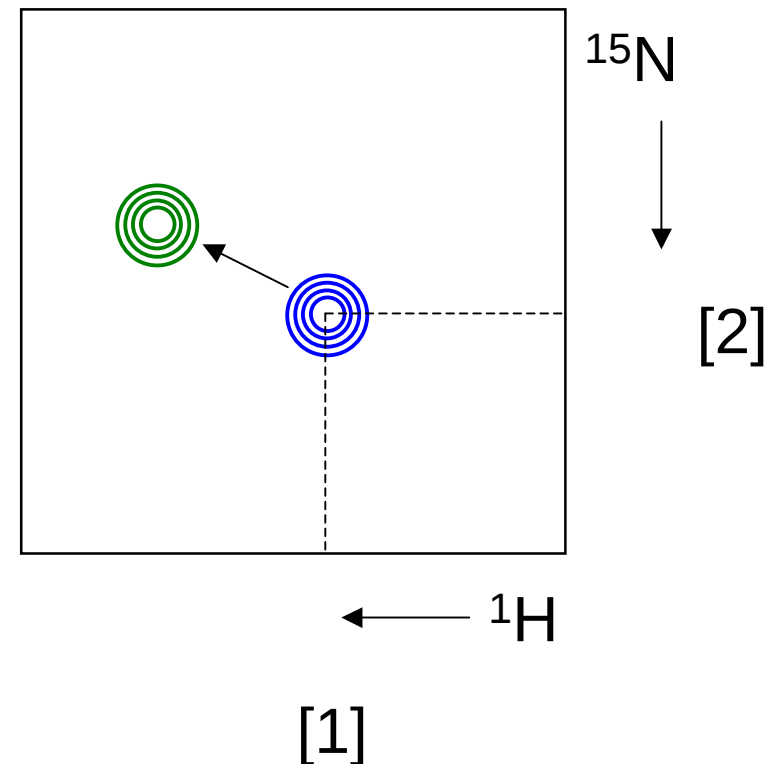
- Collaborative Computing Project for NMR
- CCPNmr Analysis – Assignment Software
- Format Converter
- EXTEND-NMR – interface for numerous different NMR software packages

CCPN Data Model

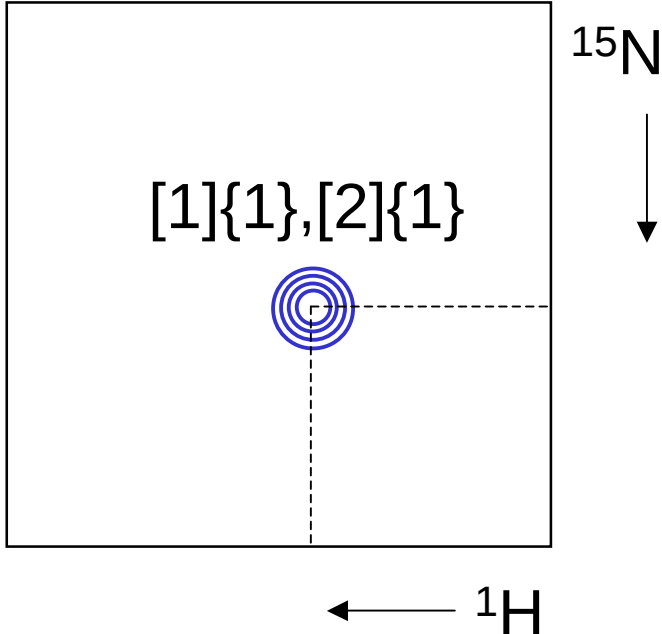
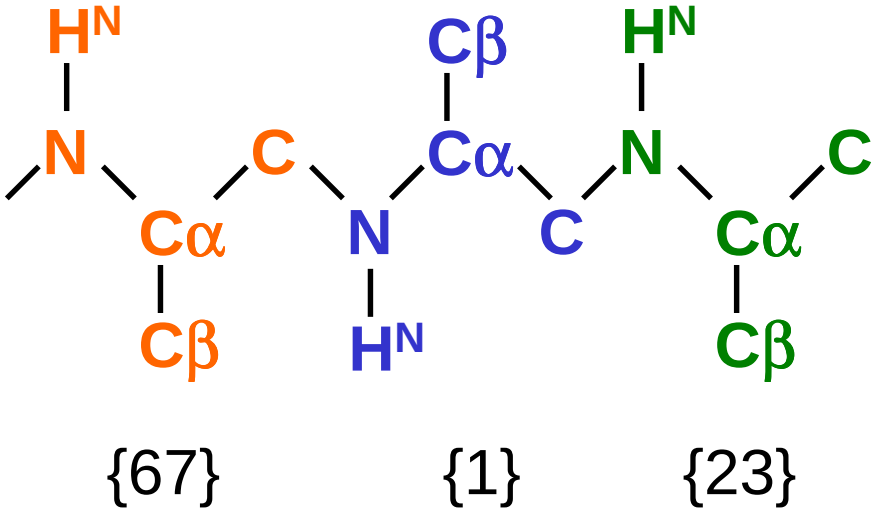


Resonance

- don't know which two atoms belong to the signal
 - cannot define it by the chemical shifts
 - do know that the ^1H and ^{15}N are directly bonded
- use **Resonance** as a placeholder



Spin Systems

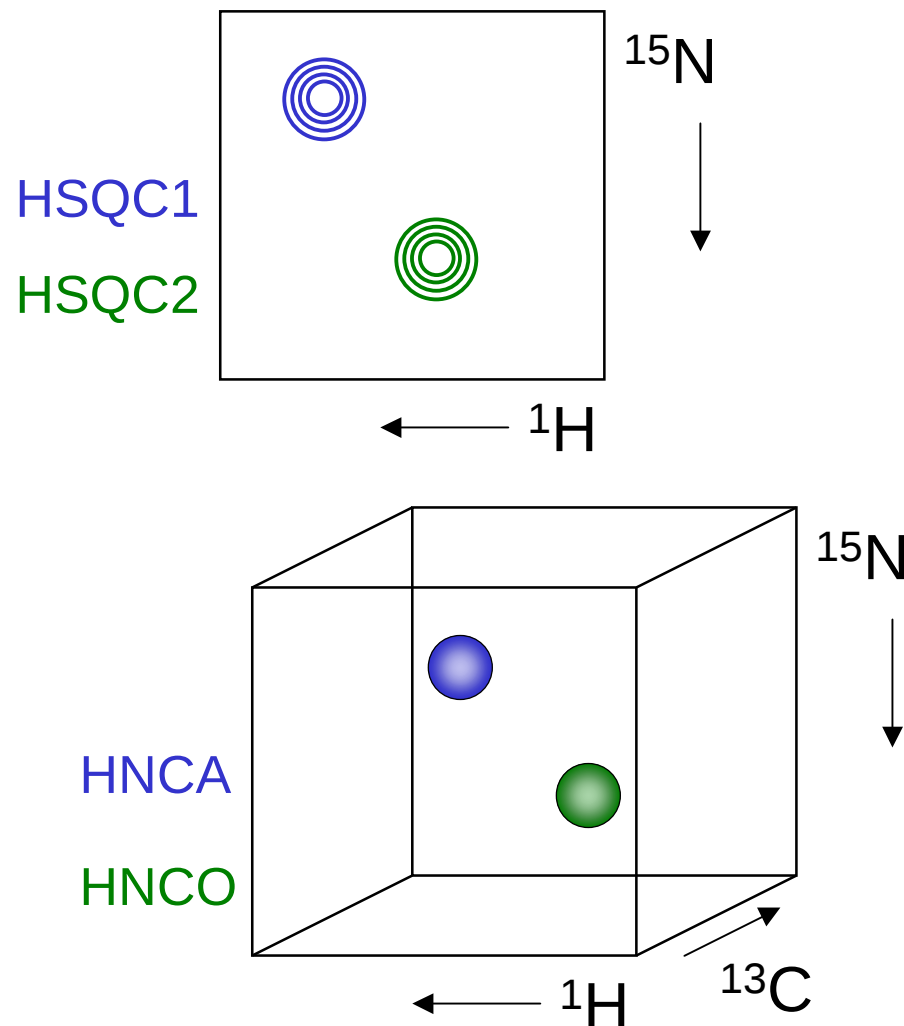


[Resonance] {Spin System}

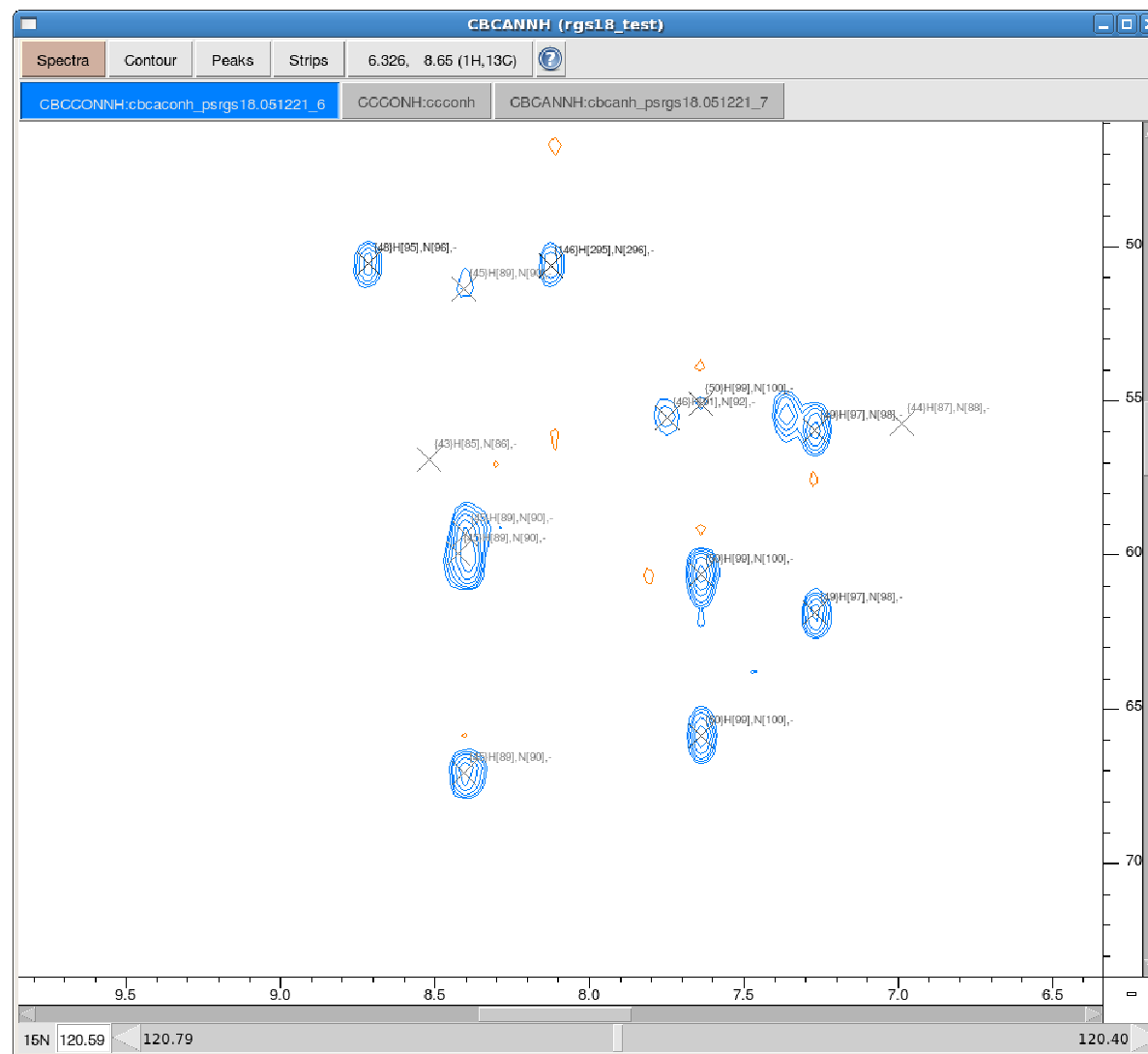
Windows

Each window

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



Windows



Windows

Windows : New Window (rgs18_test) [X]

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 ▼

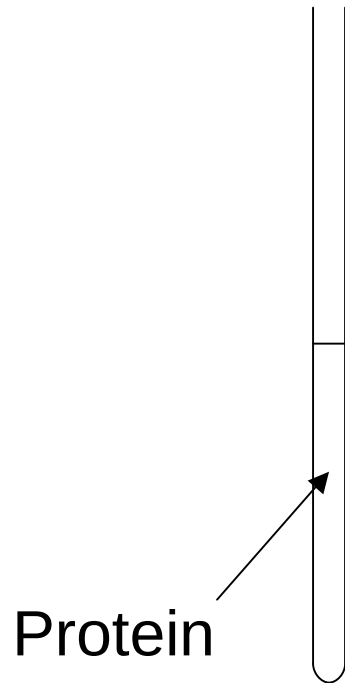
Viewed Spectra

Spectrum	Visible?	In Toolbar?	Peak Lists
HSQC:hsqc_psrgrs18.051221_3	No	Yes	1
CBCCONNH:cbcaconh_psrgrs18.051221_6	No	Yes	1
CBCANNH:cbcanh_psrgrs18.051221_7	No	Yes	1
CCCONH:ccconh	No	Yes	1

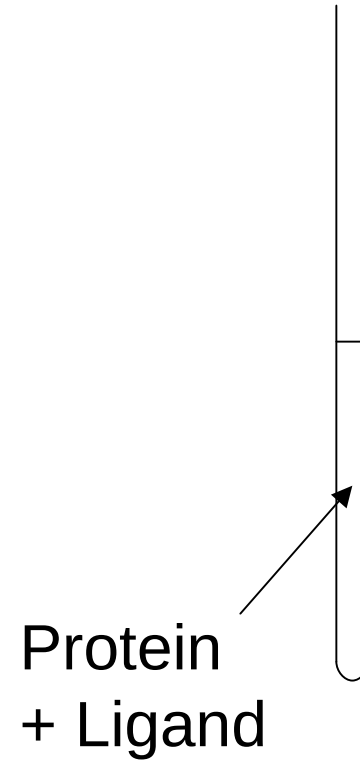
Set Displayed Set Hidden Set Absent

Create [?] [X]

Molecular System



Molecular System 1



Molecular System 2

Molecular System

Molecules : Molecule Setup (rgs18_test)

Chains | Mol Systems | Sequences | Template Molecules | Links | Add Sequence | Small Compounds

Mol System	Chain Code	Molecule Template	Residues	Chain Fragments	Molecular Types	Details
MS1	A	RGS18	151	1	protein	SMVSPEEAVK...

Mol System for new chain: <New> ▾ Template for new chain: RGS18 ▾

Show Seq	Delete Chain	Copy Chain	Add Sequence	Edit Molecule Templates	Make Chain From Template
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Chain "A" Fragments

#	Mol Type	Residues	Start Seq Number	Linear Polymer?	Sequence
1	protein	151	1	Yes	Ser Met Val Ser Pro Glu Glu Ala Val Lys...

Experiment Types

Experiments : Edit Experiments (rgs18_test)

Experiments Experiment Types Experimental Details Shift References NMR Instruments

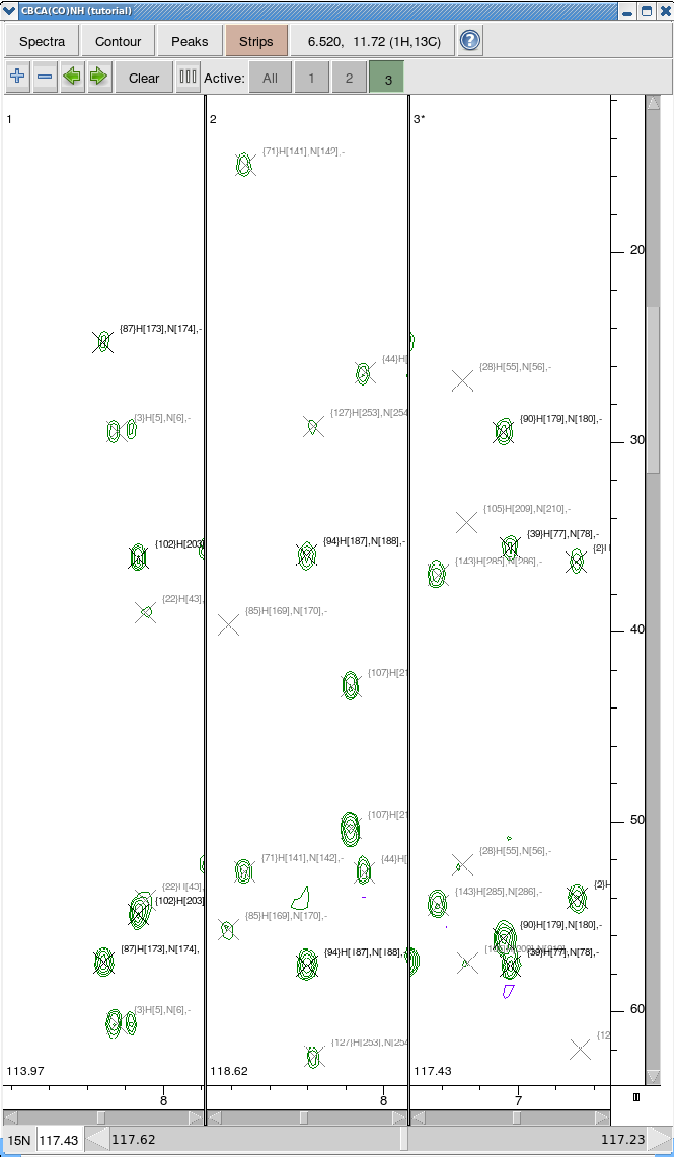
#	Name	Category	Type Synonym	Full Type
1	HSQC	through-bond	15N HSQC/HMQC	H[N]
2	CBCCONNH	through-bond	HNCOCA/CB	H[N[co[{CA caC}]]]
3	CBCANNH	through-bond	HNCA/CB	H[N[{CA ca[Ca]}]]]
4	HBHACONH	through-bond	HBCB/HACACONNH	H[ca cca]coNH
5	HCCCONH	through-bond	HCCONH	HccoNH
6	CCCONH	through-bond	HCCONH	hCcoNH

Propagate Experiment Type Edit Experiment Prototypes

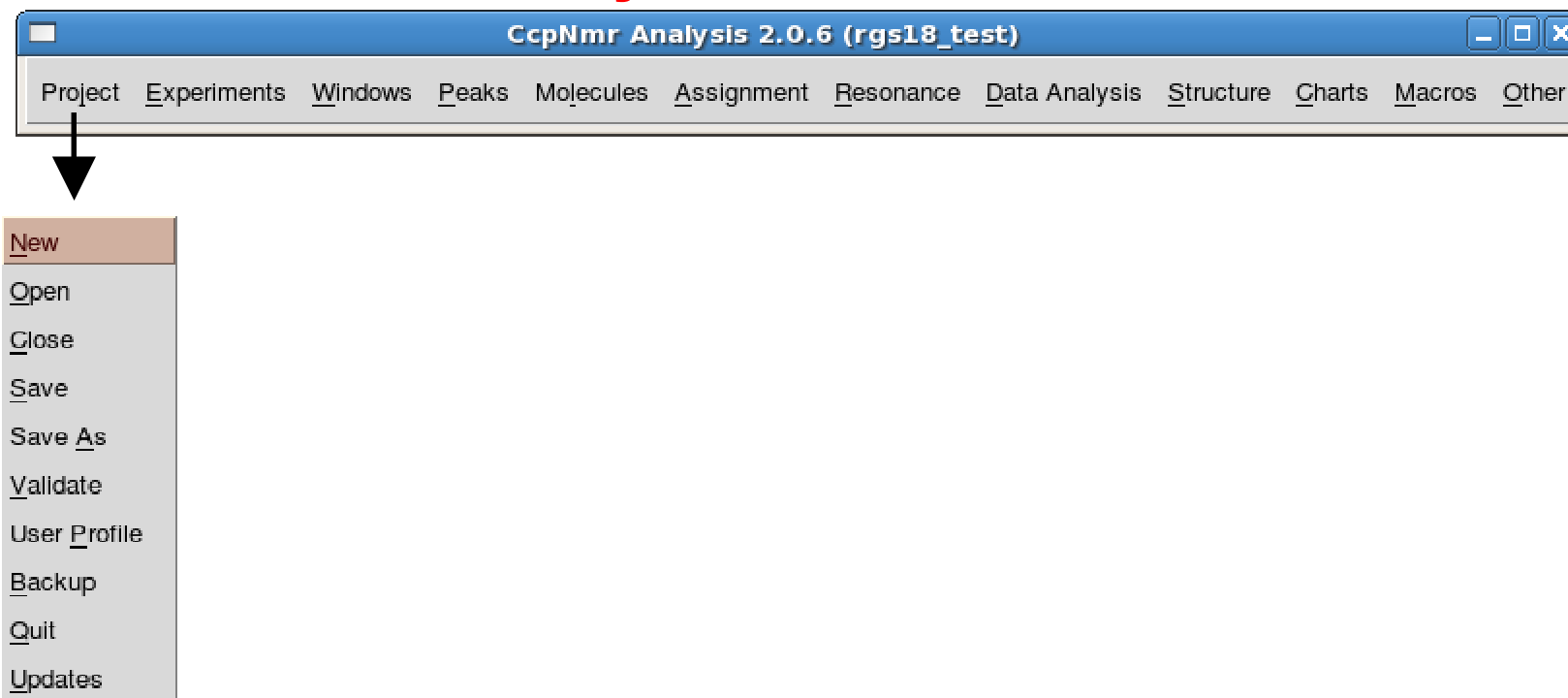
Reference Dimensions

Exp Dim	Ref Exp Dim (.Ref)	Isotope	Ref Measurement

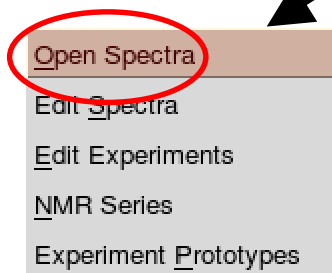
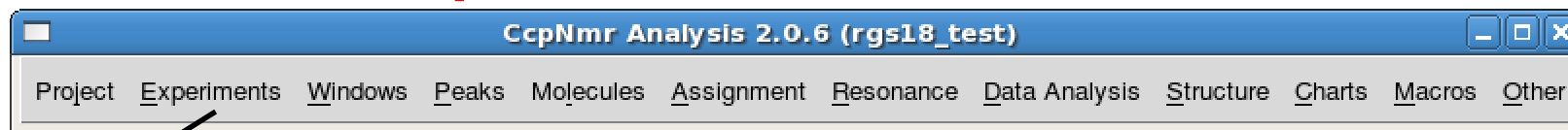
Strips



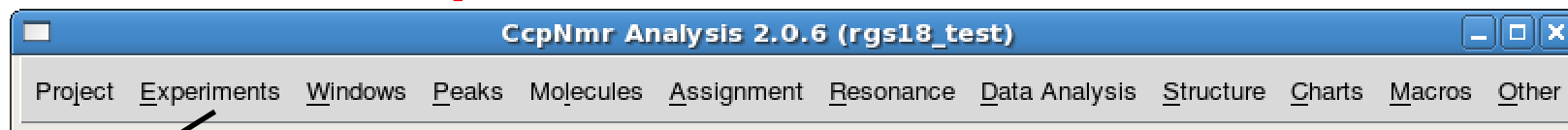
Project Menu



Experiments Menu



Experiments Menu

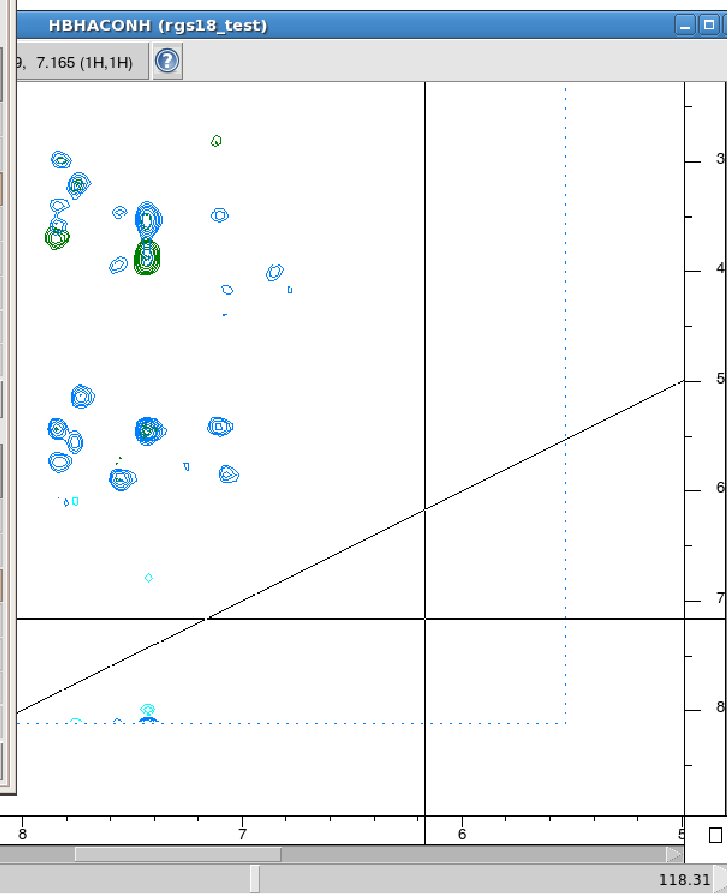
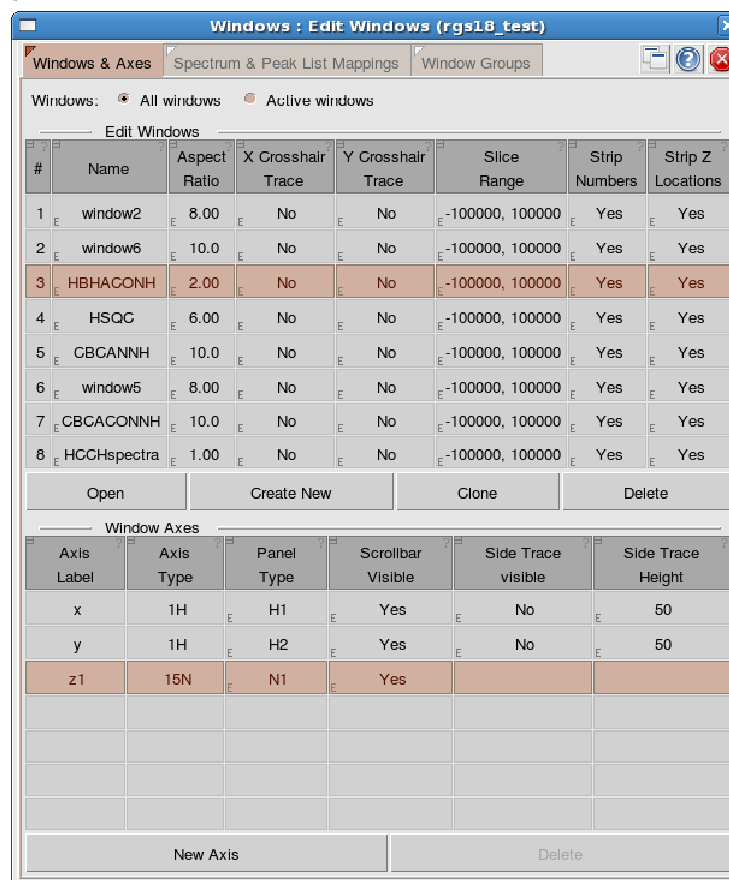
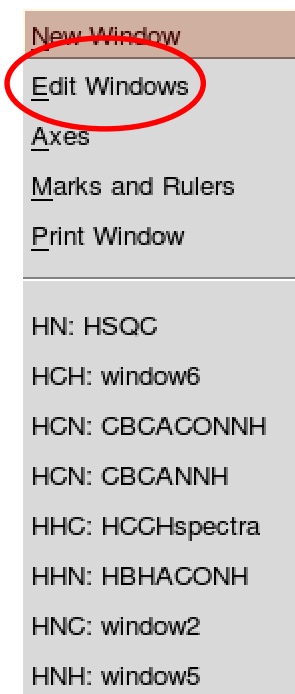
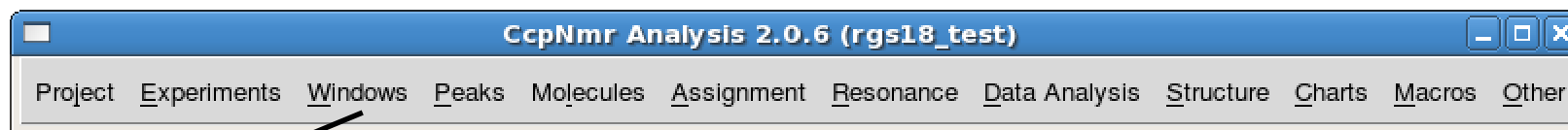


Experiments : Edit Spectra (rgs18_test)

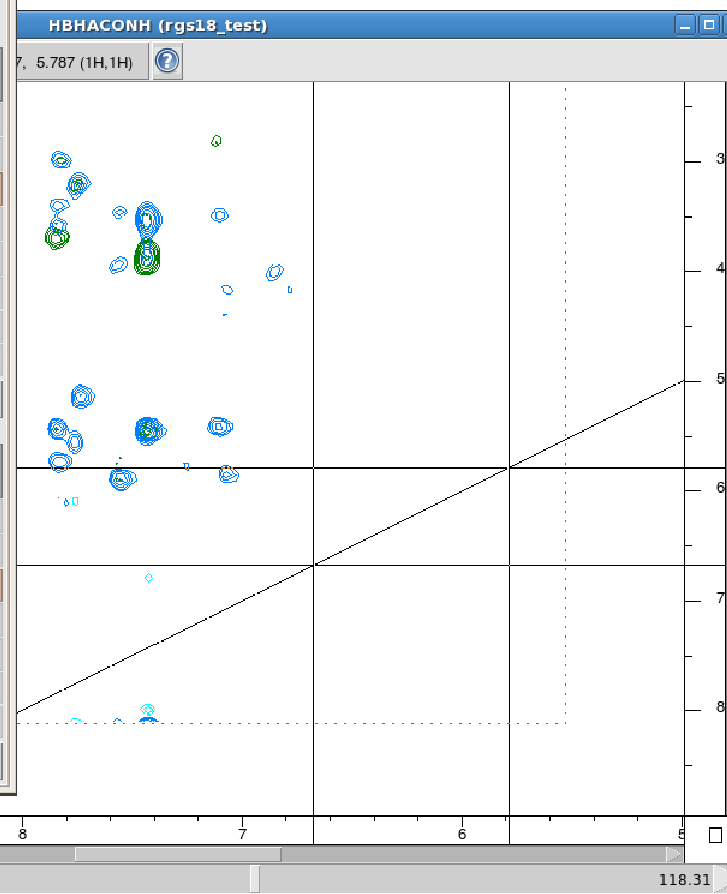
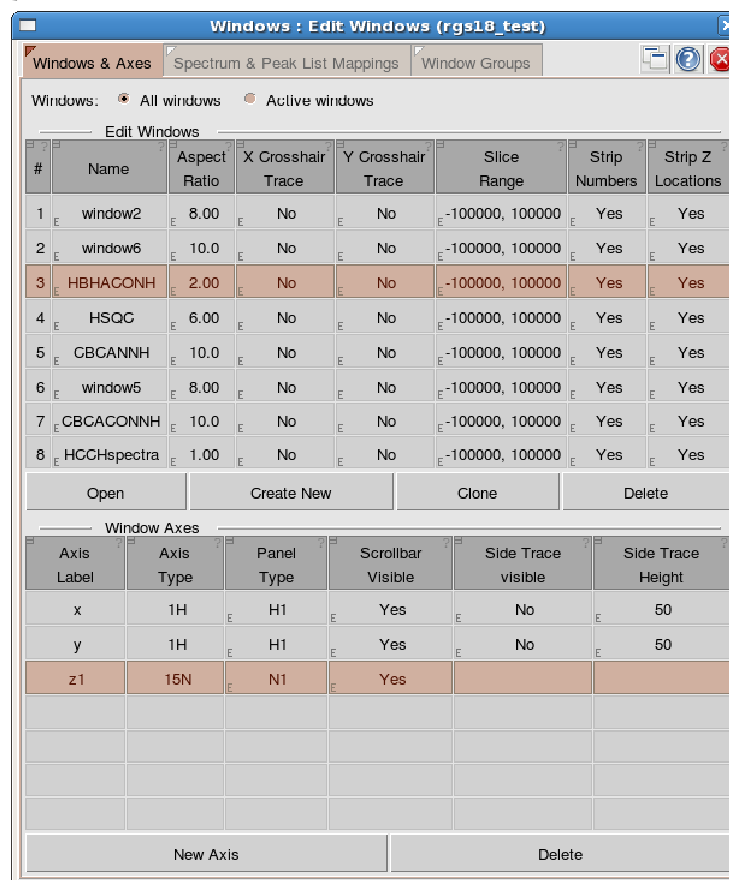
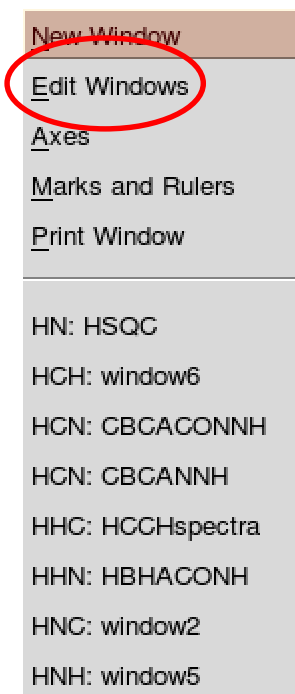
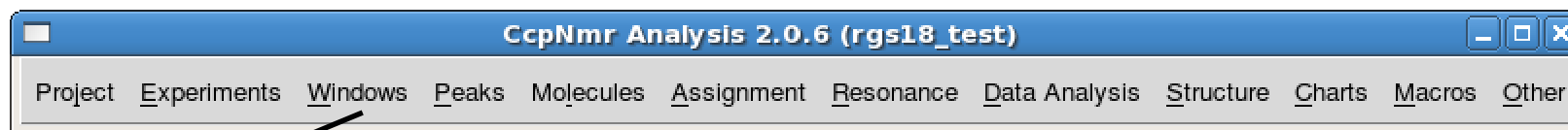
#	Spectrum	Positive colors	Negative colors	Slice Color	Box Visible?	Shortcut	Rank	Positive Contours	Negative Contours	Try Precalc Contours	Peak Font	Peak Pointer?
1	HSQC:hsqc_psrsgs18.051221_3	teal	magenta	#000080	Yes	F1	0	150...[8]...5766	-150...[8]...-5766	No	Helvetica 10	Yes
2	CBCGONNH:cbcanh_psrsgs18.051221_6	skyblue	orange	#000080	Yes	F2	0	300...[8]...11533	-300...[8]...-11533	No	Helvetica 10	Yes
3	CBCANNH:cbcanh_psrsgs18.051221_7	green	mauve	#000080	Yes	F3	0	200...[8]...7689	-200...[8]...-7689	No	Helvetica 10	Yes
4	HBHACONH:hbcacoh	skyblue	cyan	#000080	Yes	F4	0	300...[8]...11533	-300...[8]...-11533	No	Helvetica 10	Yes
5	HCGGONNH:hccconh	green	chartreuse	#000080	Yes	F5	0	300...[8]...11533	-300...[8]...-11533	No	Helvetica 10	Yes
6	CGGONH: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:hccchcosy	green	chartreuse	#000080	Yes	F8	0	70.0...[8]...2691	-70.0...[8]...-2691	No	Helvetica 10	Yes

Contour Levels Contour Files Set Ranks From Order

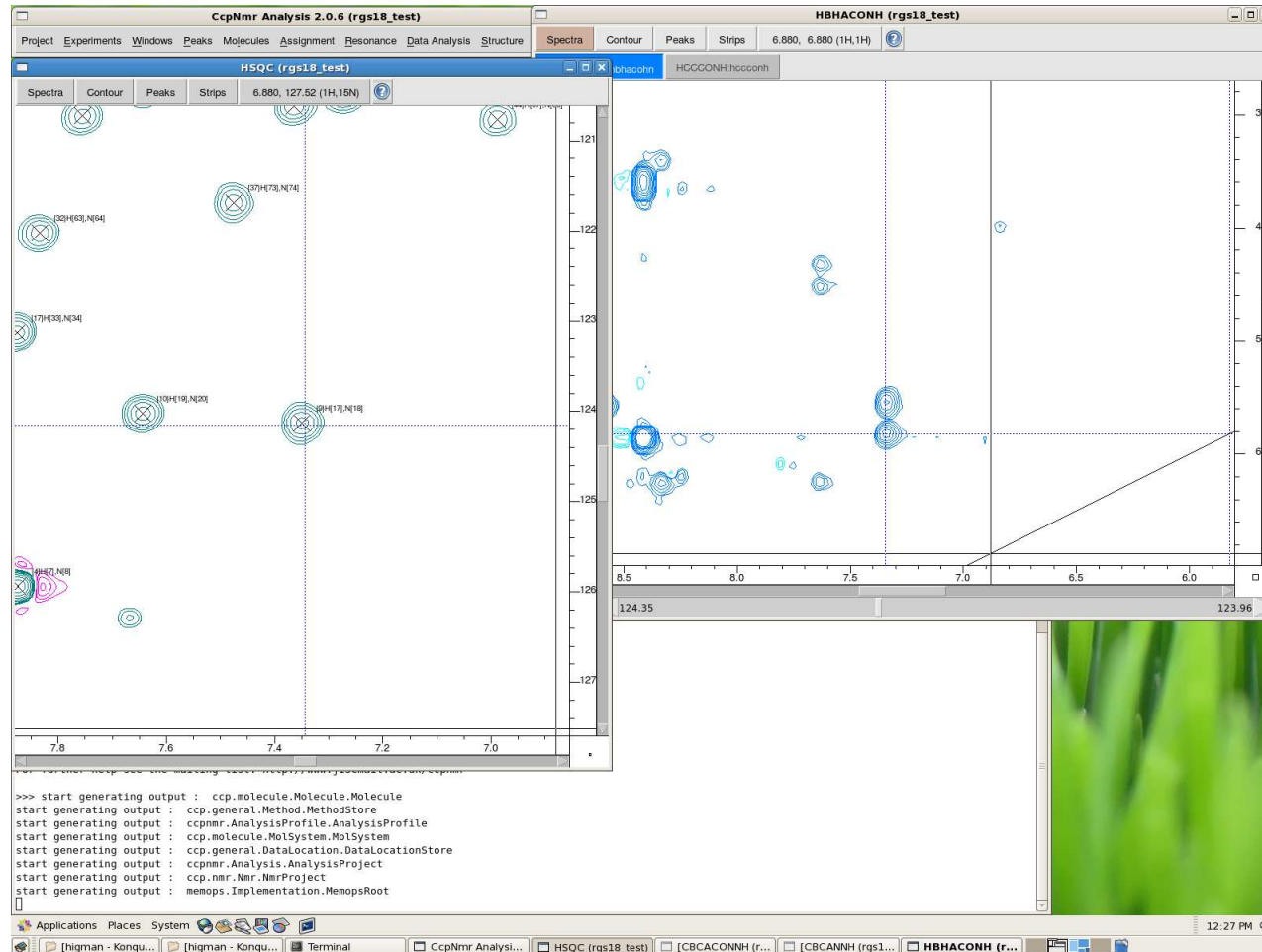
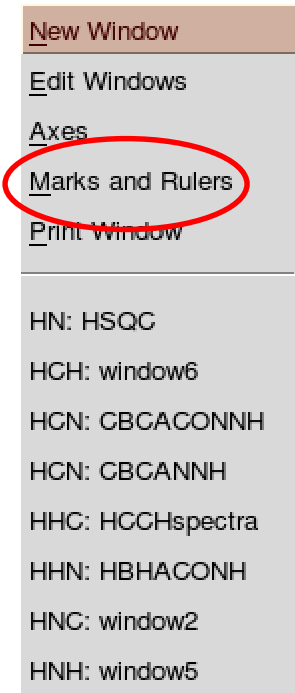
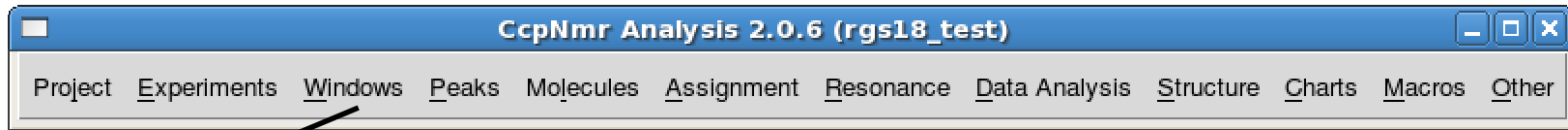
Windows Menu



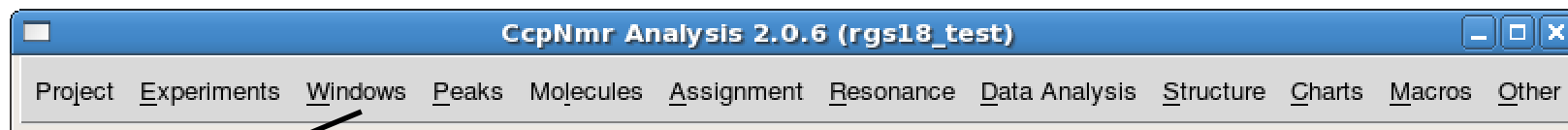
Windows Menu



Windows Menu



Windows Menu



New Window

Edit Windows

Axes

Marks and Rulers

Print Window

HN: HSQC

HCH: window6

HCH: CBCACONNH

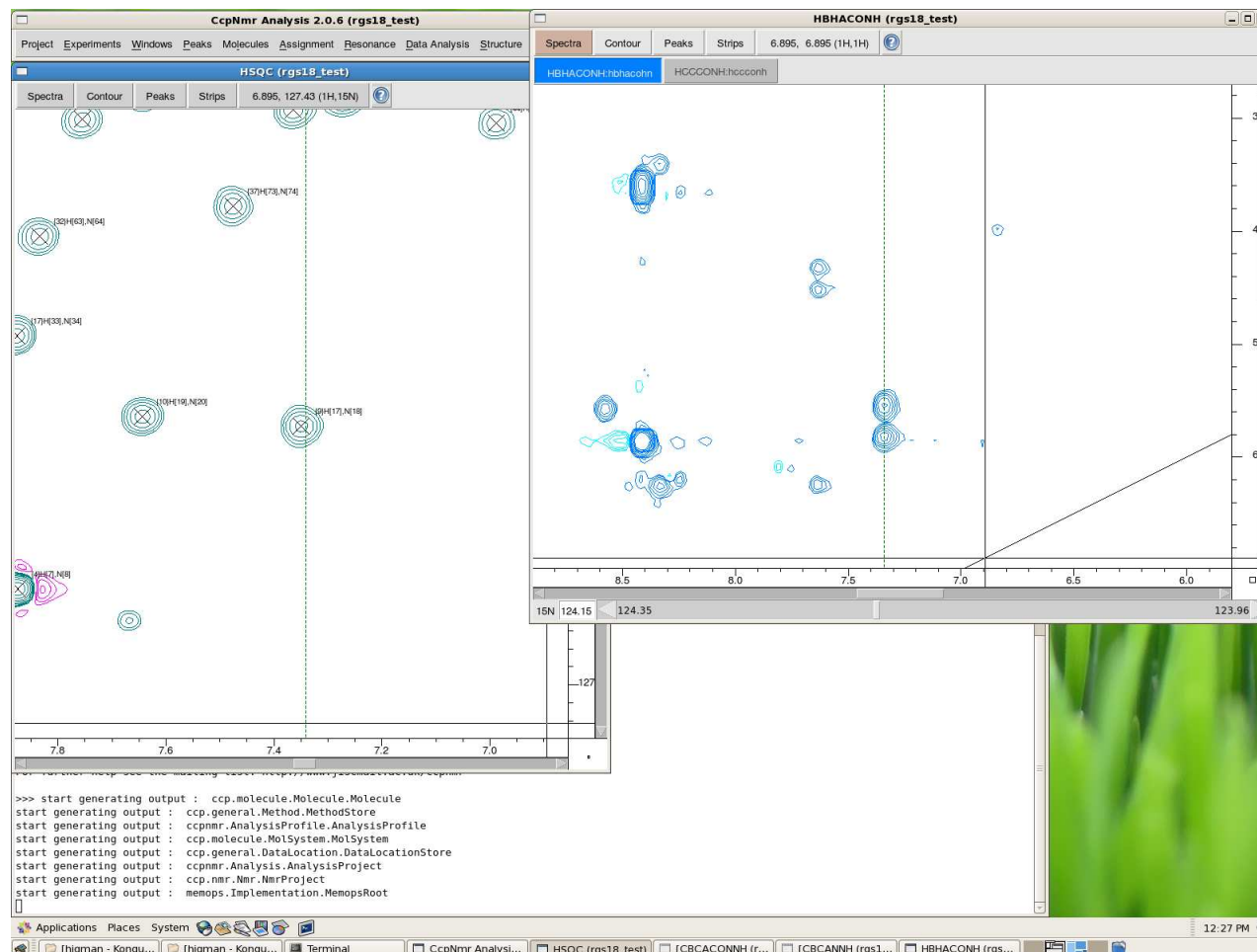
HCH: CBCANNH

HHC: HCGHspectra

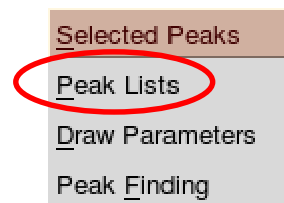
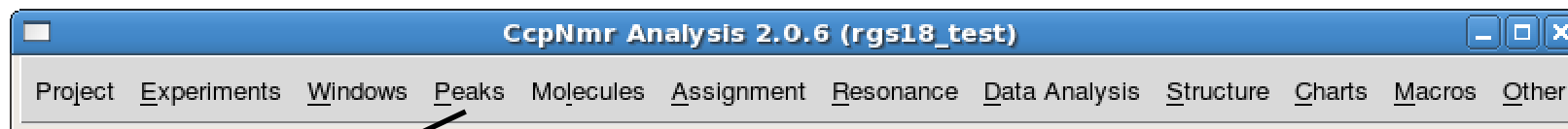
HHN: HBHACONH

HNC: window2

HNH: window5



Peaks Menu



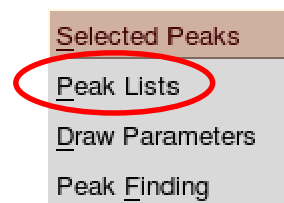
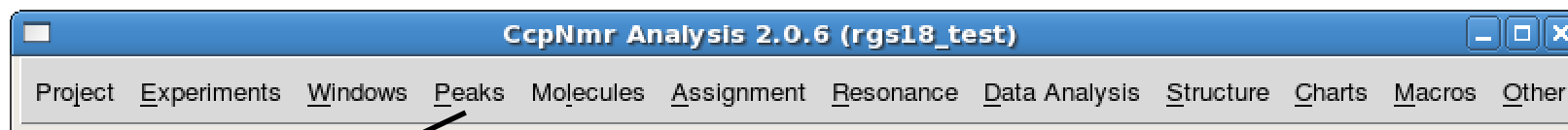
Peaks : Peak Lists (rgs18_test)

Peak Lists Peak Table Synthetic Lists

Experiment	Spectrum	List	Active?	Color	Symbol	No. Peaks	% Assigned	Details
HSQC	hsqc_psrgrs18.051221_3	1	Yes	#000000	x	167	100.0	Default list
HSQC	hsqc_psrgrs18.051221_3	2	No	#000000	x	0	0.0	New list
CBGCONNH	cbcaconh_psrgrs18.051221_6	1	Yes	#000000	x	234	66.7	Default list
CBGANNH	cbcanh_psrgrs18.051221_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



Peaks : Peak Lists (rgs18_test)

Peak Lists Peak Table Synthetic Lists

Spectrum: HSQC:hsqc_psrgrs18.051221_3 Peak List: 1 Make Strips Find Peak Window: window2 Mark Fo

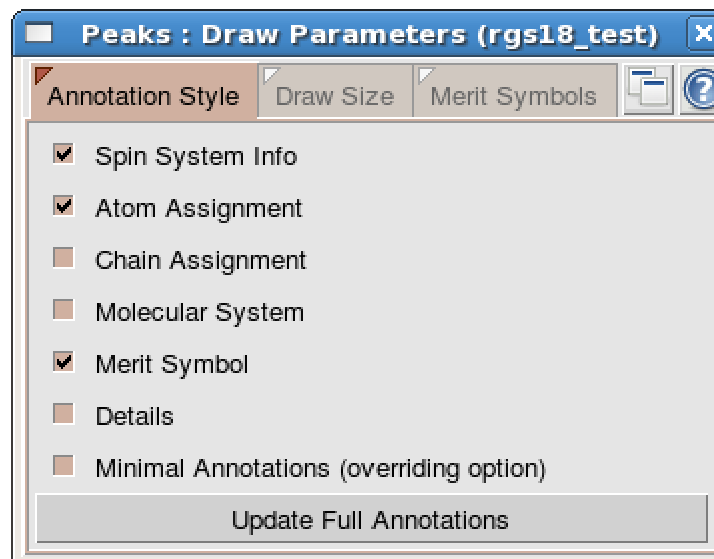
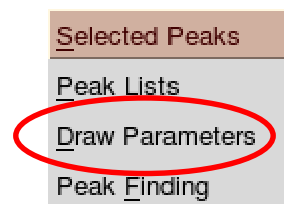
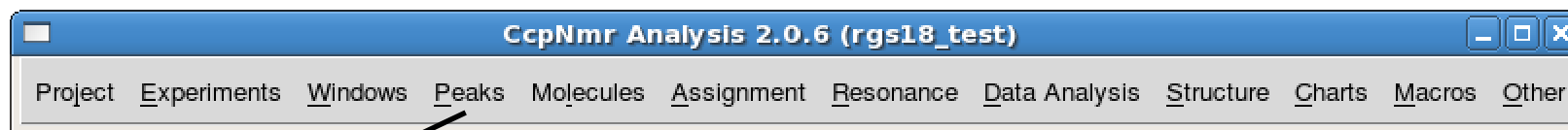
Status: Any Structure: <None> Strip locations Goto position <None> Mark S

#	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	126.412	{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		

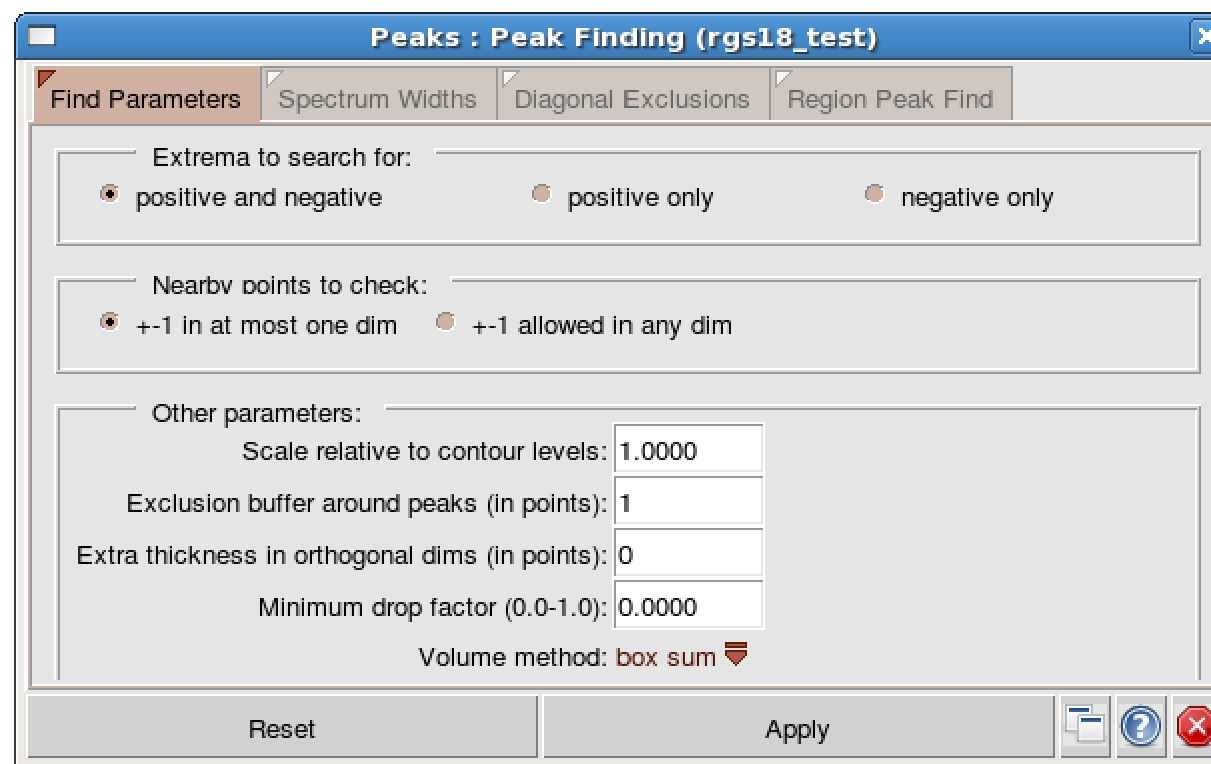
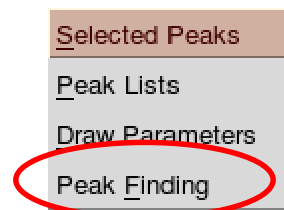
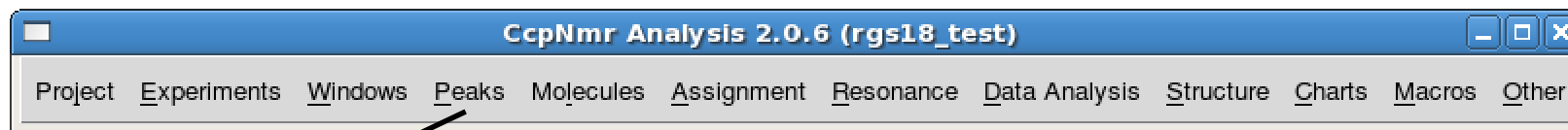
Add Edit Unalias Delete Assign Deassign

Deassign Dim Recalc Intensities Show On Structure Propagate Assign

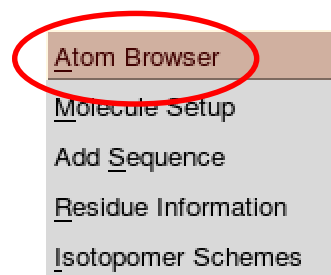
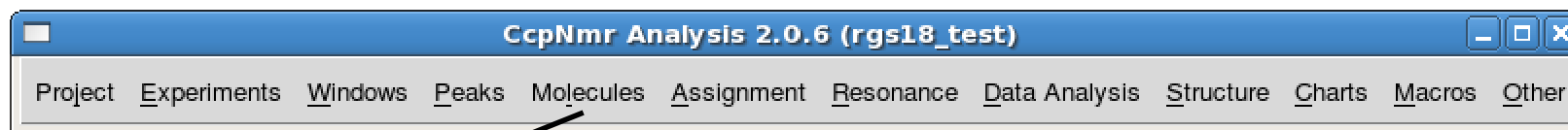
Peaks Menu



Peaks Menu



Molecules Menu



Molecules: Atom Browser (rgs18_test)

Atom Table Options

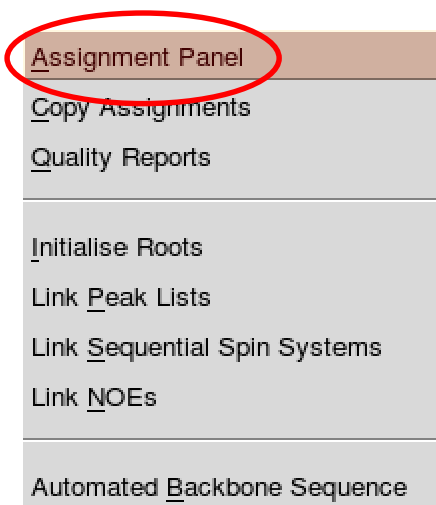
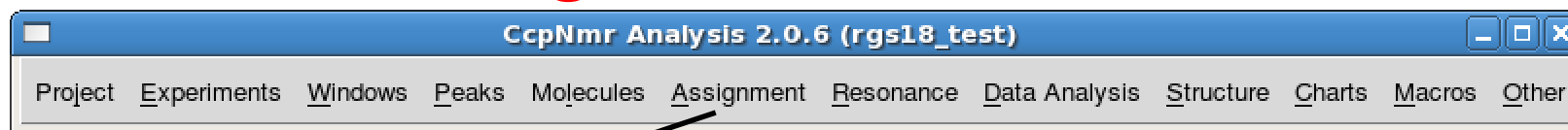
Chain: MS1:A(protein) Residue: <All> Nucleotides: Interlaced

Elements: C H N O S

#	Residue	H*	Ha	Hβa	Hβb	Hy	C	Cα	Cβ		
1	Ser	H*	Ha	Hβa	Hβb	Hy	C	Cα	Cβ		
2	Met	H	Ha	Hβa	Hβb	Hya	Hyb	Hε*	C	Cα	Cβ
3	Val	H	Ha	Hβ	Hya*	Hyb*	C	Cα	Cβ	Cya	Cyb
4	Ser	H	Ha	Hβa	Hβb	Hy	C	Cα	Cβ		
5	Pro	Ha	Hβa	Hβb	Hya	Hyb	Hδa	Hδb	C	Cα	Cβ
6	Glu	H	Ha	Hβa	Hβb	Hya	Hyb	C	Cα	Cβ	Cγ
7	Glu	H	Ha	Hβa	Hβb	Hya	Hyb	C	Cα	Cβ	Cγ
8	Ala	H	Ha	Hβ*	C	Cα	Cβ				
9	Val	H	Ha	Hβ	Hya*	Hyb*	C	Cα	Cβ	Cya	Cyb
10	Lys	H	Ha	Hβa	Hβb	Hya	Hyb	Hδa	Hδb	Hεa	Hεb
11	Trp	H	Ha	Hβa	Hβb	Hδ1	Hε1	Hε3	Hζ2	Hζ3	Hη2

Set ring flip equivalency Remove atom assignments Show peaks

Assignment Menu



Assignment : Assignment Panel (rgs18_test)

Peak: HSQC:hsqc_psrgrs18.051221_3:1:68 Shift List:1 onance: Structure: <None>

Assignment possibilities

F1 1H 8.3137	#	Name	Delta	Shift	SD	Dist	?
{56}H[111]	111	{56}H[111]	0.000	8.314	0.009		
	69	{35}H[69]	0.001	8.315	0.000		
<New>	239	{120}H[239]	0.003	8.311	0.006		

F2 15N 119.7920	#	Name	Delta	Shift	SD	Dist	?
{56}N[112]	110	{55}N[110]	0.035	119.757	0.089		
	114	{57}N[114]	0.048	119.744	0.025		
<New>	112	{56}N[112]	0.049	119.742	0.055		

Options

Aliased Possible ☐ Restrict Mol System ☒ Intra-residue ☐

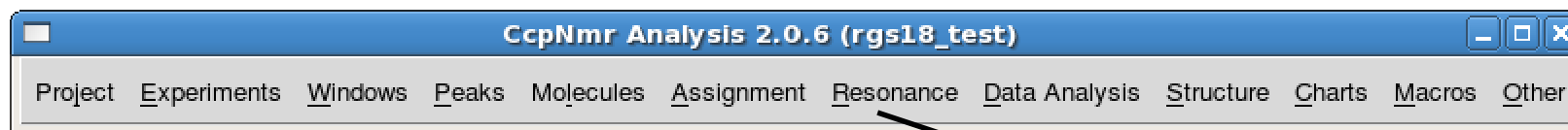
Correlated Dims ☐ Double Tolerances ☐

Labelling Scheme: - Min Isotope Fraction: 0.10000

Assign {56}N[112] Set Atom Type Deassign {56}N[112] Clear contrib Merge F2 Resonances

Set Same Spin System Show On Structure Show Peaks ? X

Resonance Menu



Resonance : Resonance Table (rgs18_test)

Selection
ShiftList: ShiftList 1 ▼ Isotope: Any ▼ Status: Any ▼ Chain: <Any> ▼ CcpCode: <Any> ▼

Navigation & Marks
Strip Selected Goto Position Window: HSQC ▼ Mark Selected Clear Marks

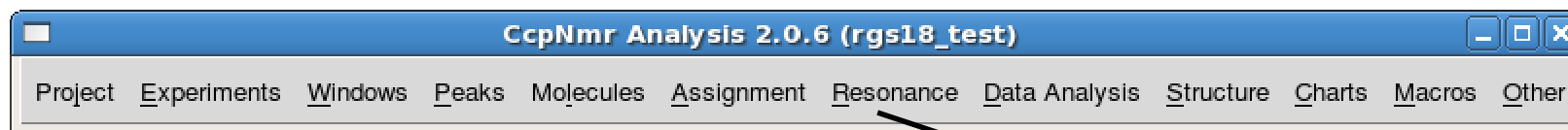
#	Shift	SD	Assign Name	Residue	Isotope	Other Name	Total Peaks	Shift List Peaks	Spin System #	?
325	56.360	0	[325]		13C		1	1		
1	10.600	0	H[1]	{1}	1H		1	1	1	
2	130.129	0	N[2]	{1}	15N		1	1	1	
4	128.445	0.023	N[4]	{2}	15N		3	3	2	
3	8.915	1.822e-03	H[3]	{2}	1H		3	3	2	
5	8.954	0	H[5]	{3}	1H		1	1	3	
6	127.521	0	N[6]	{3}	15N		1	1	3	
7	7.866	5.316e-03	H[7]	{4}	1H		29	29	4	
8	125.889	0.045	N[8]	{4}	15N		29	29	4	
9	10.519	7.241e-03	H[9]	{5}	1H		5	5	5	
10	125.525	0.039	N[10]	{5}	15N		5	5	5	
11	8.334	0	H[11]	{6}	1H		1	1	6	
12	124.532	0	N[12]	{6}	15N		1	1	6	
13	8.579	0	H[13]	{7}	1H		1	1	7	
14	124.490	0	N[14]	{7}	15N		1	1	7	
15	8.418	8.561e-03	H[15]	{8}	1H		27	27	8	
16	124.292	0.058	N[16]	{8}	15N		27	27	8	

Deassign Assign Set Atom Type Add to Spin System Remove from Spin System Swap Prochirals Ambiguate Prochirals

Delete Delete Orphans Merge Show Total Peaks Show Shift List Peaks

Resonance Table
Spin Systems
Spin System Types
Reference Chemical Shifts

Resonance Menu



Resonance : Spin Systems (rgs18_test)

Status: Any Shift List: 1

Display Strips Display Cells Window: window2

Assignments Seq. Links Shifts Details

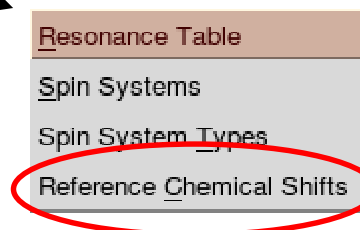
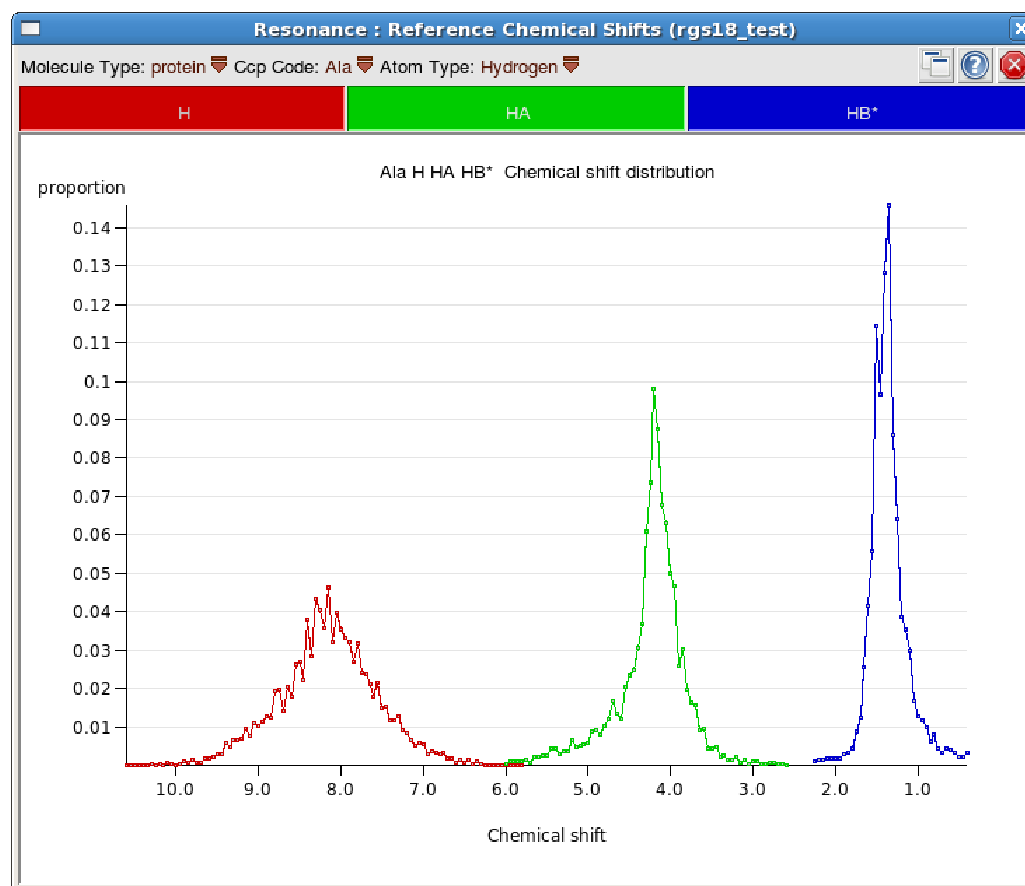
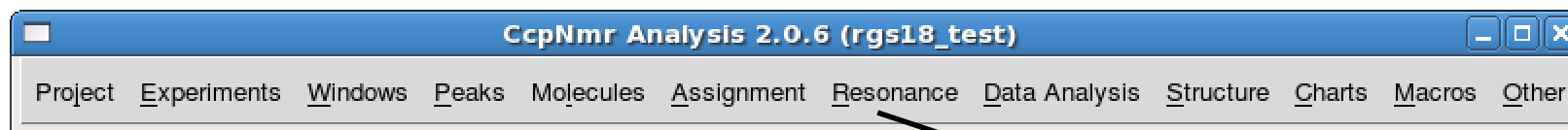
#	Chain	Residue	Resonances	Num Peaks	?
1		E	H[1] N[2]	1	
2		E	H[3] N[4]	3	
3		E	H[5] N[6]	1	
4		E	H[7] N[8]	29	
5		E	H[9] N[10]	5	
6		E	H[11] N[12]	1	
7		E	H[13] N[14]	1	
8		E	H[15] N[16]	27	
9		E	H[17] N[18]	1	
10		E	H[19] N[20]	8	
11		E	H[21] N[22]	6	
12		E	H[23] N[24]	5	
13		E	H[25] N[26]	21	
14		E	H[27] N[28]	4	

Assign Residue Assign Type Deassign Seq. Deassign All New Spin System

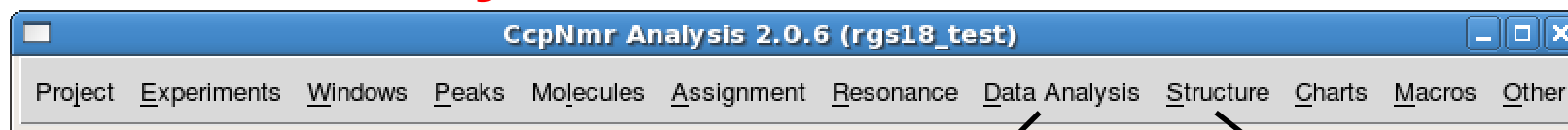
Merge Delete Show Peaks Predict Type

Resonance Table
Spin Systems
Spin System Types
Reference Chemical Shifts

Resonance Menu



Data Analysis / Structure Menus



Measurement Lists

Rates Analysis

Shift Differences

Follow Shift Changes

3J H-H α Coupling

Heteronuclear NOE

Make Distance Restraints

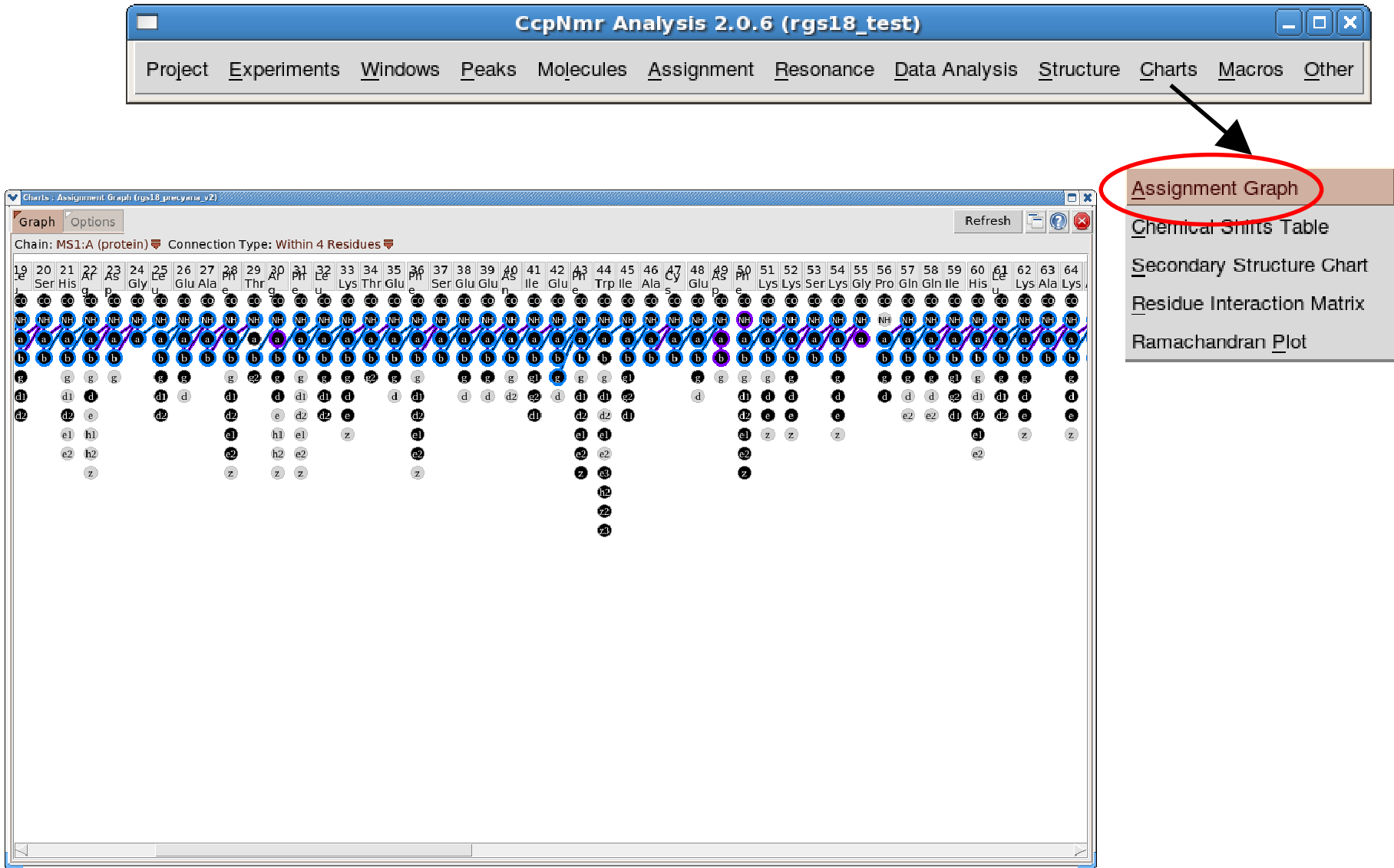
Restraints and Violations

Structure Viewer

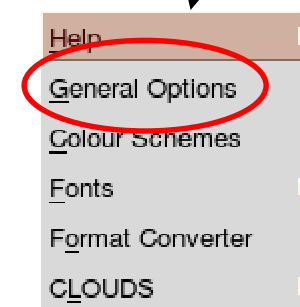
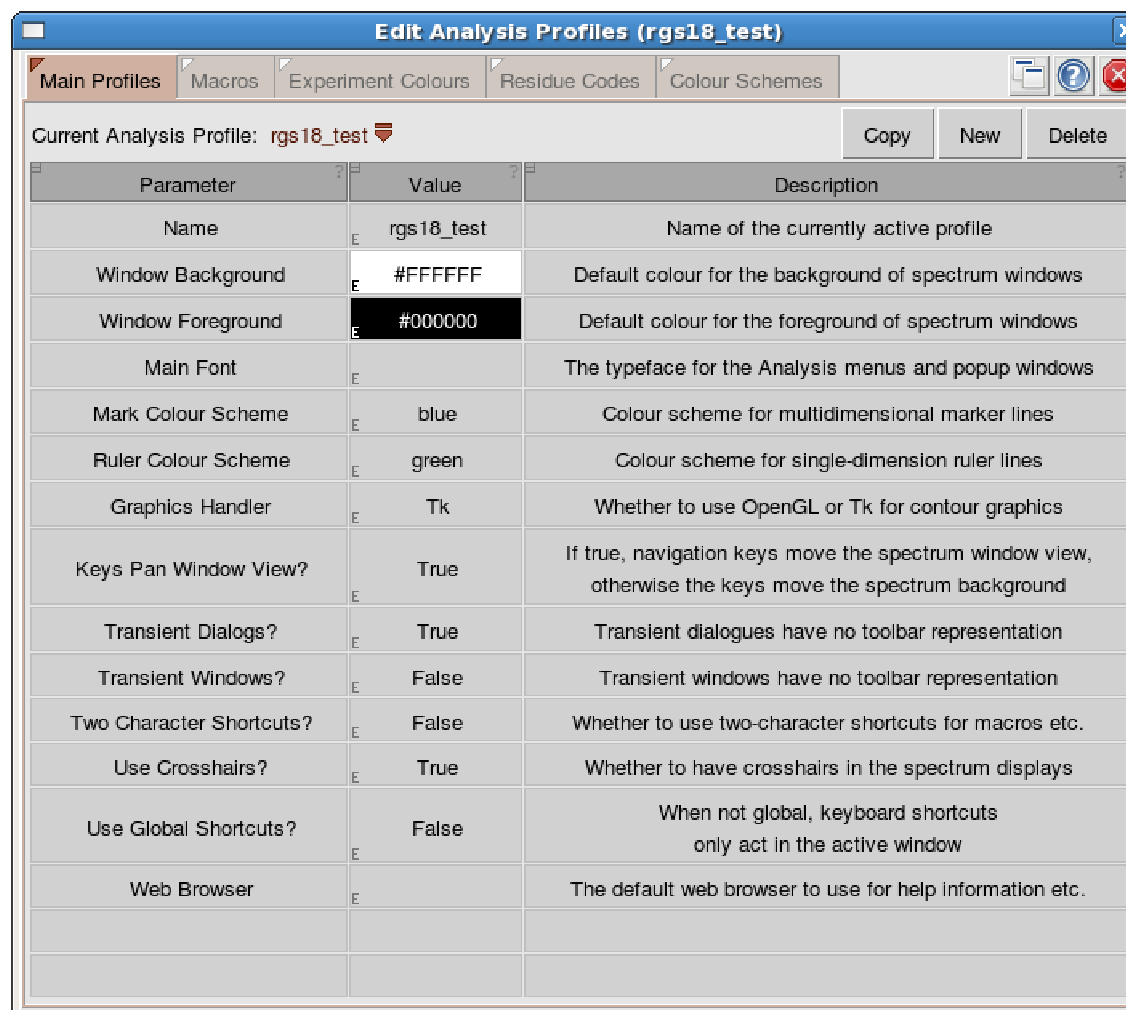
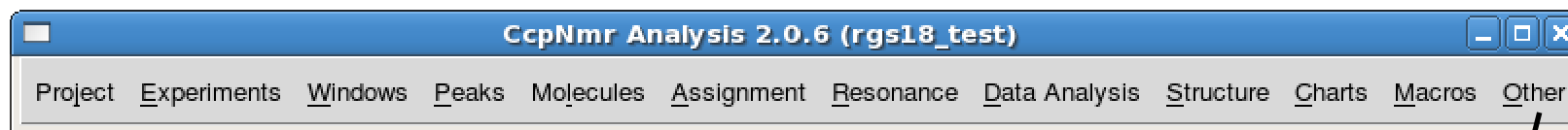
Structure Ensembles

DANGLE (Predict Dihedrals)

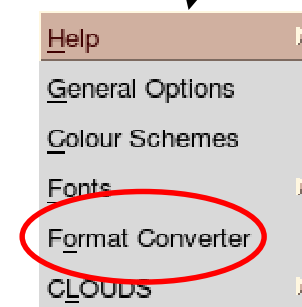
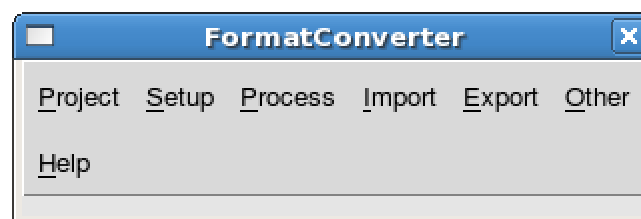
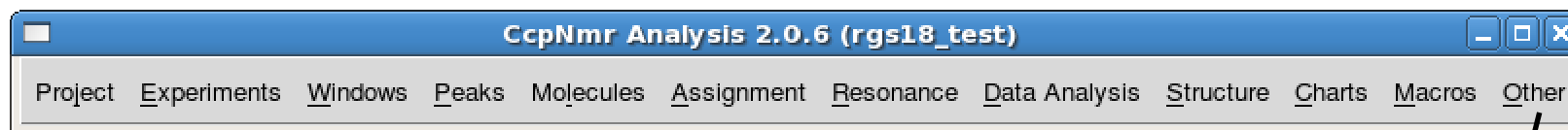
Charts Menu



Other Menu



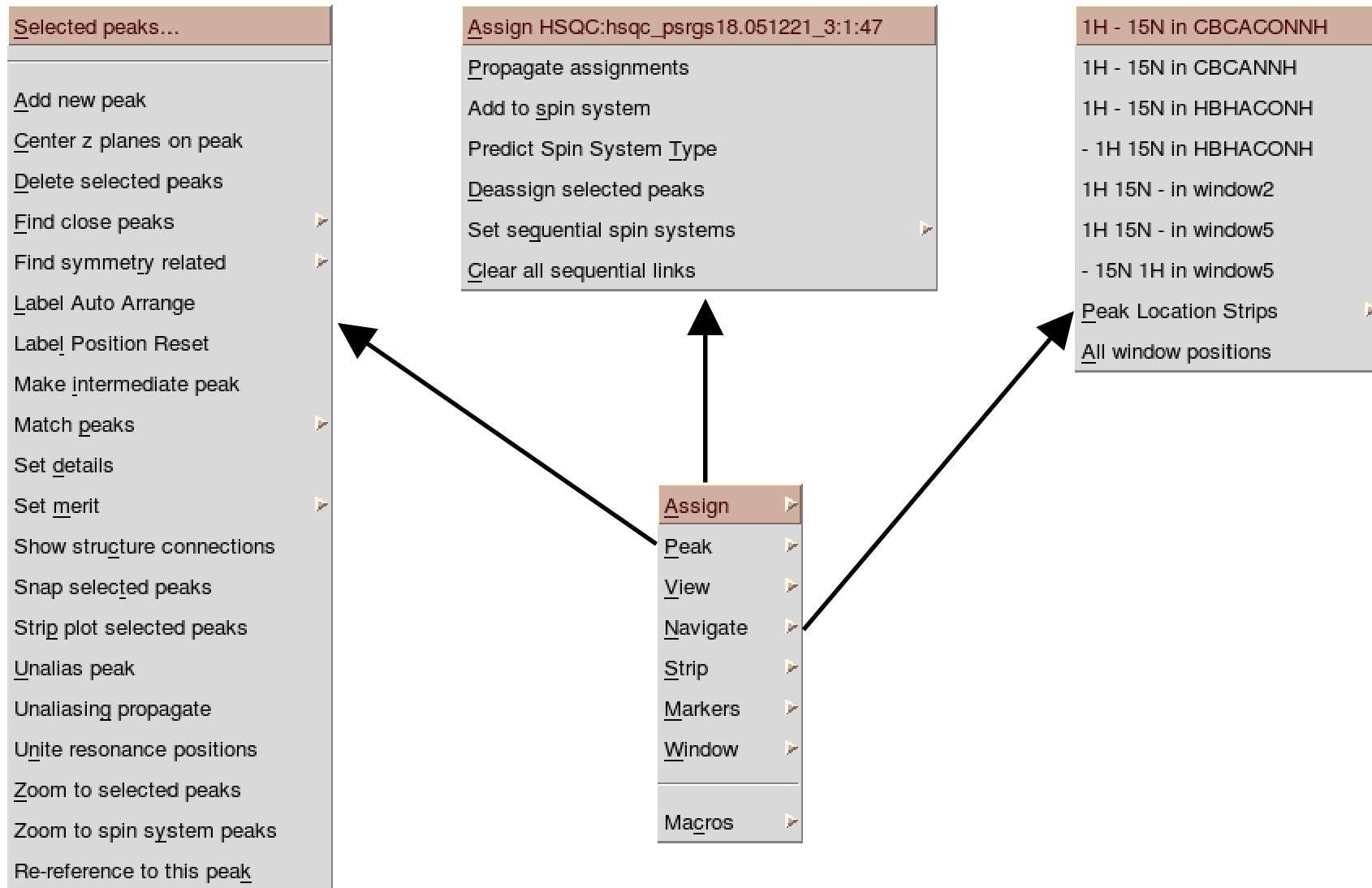
Other Menu



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

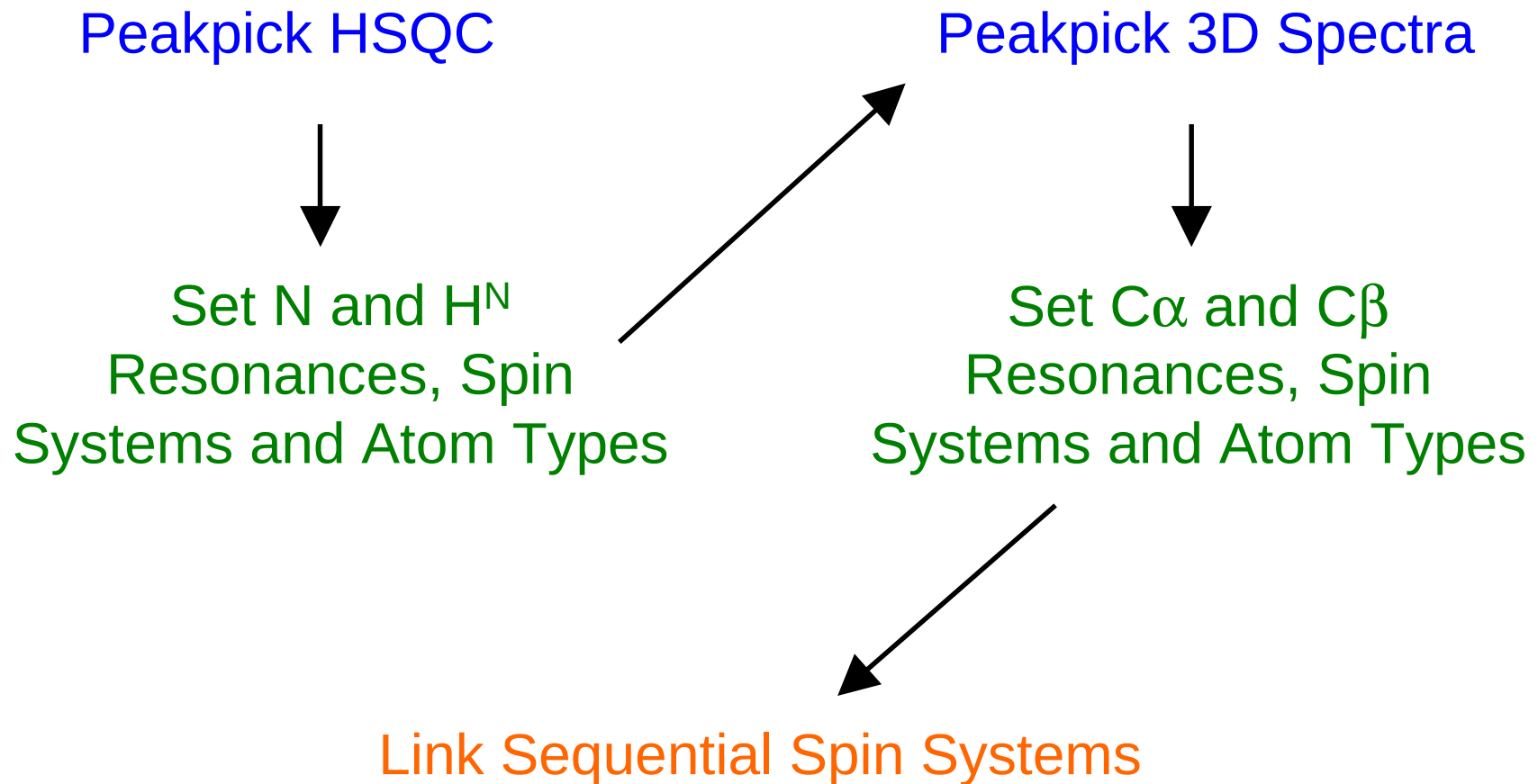
Right Mouse Menu



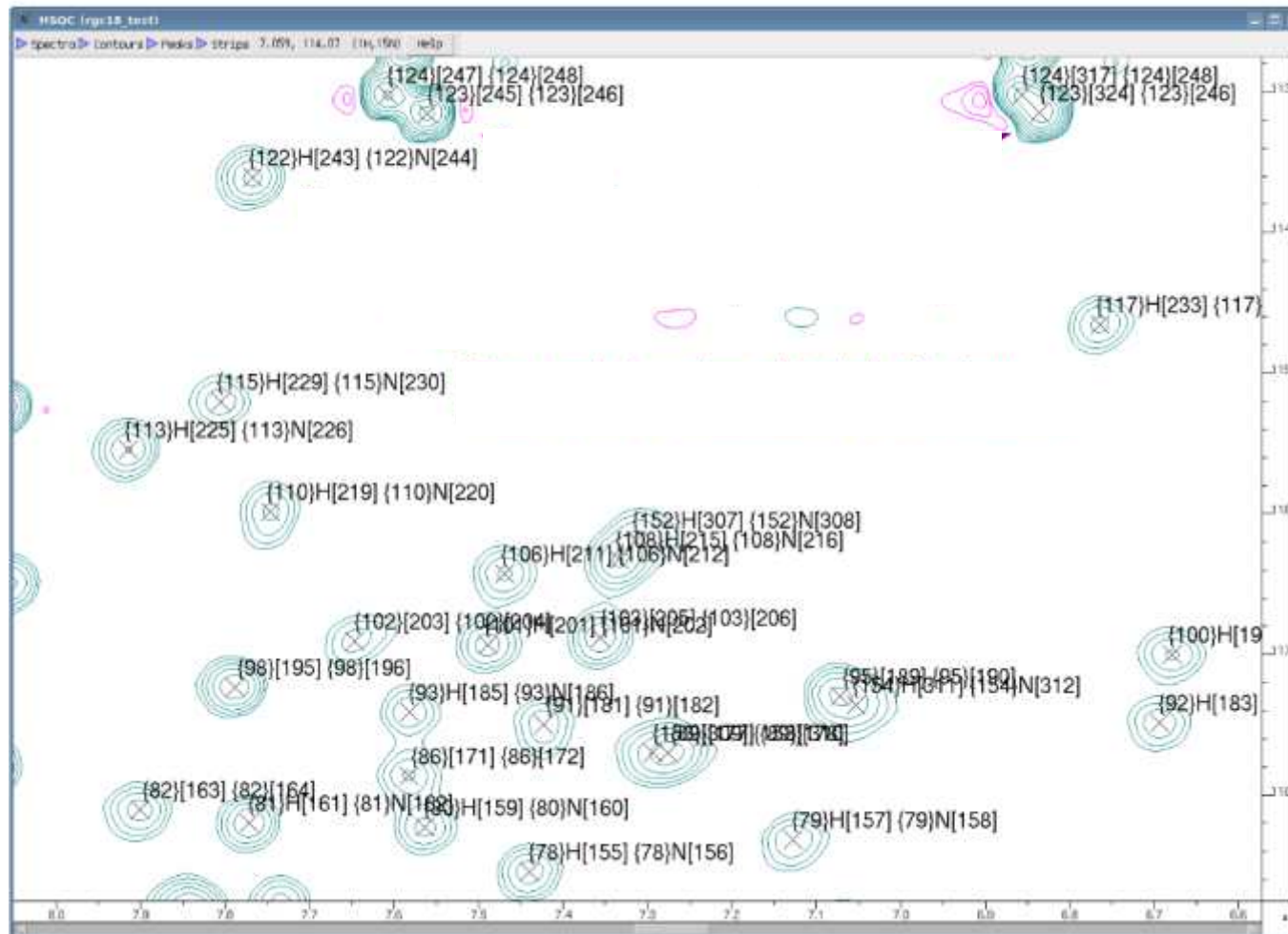
Outline

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

Backbone Assignment

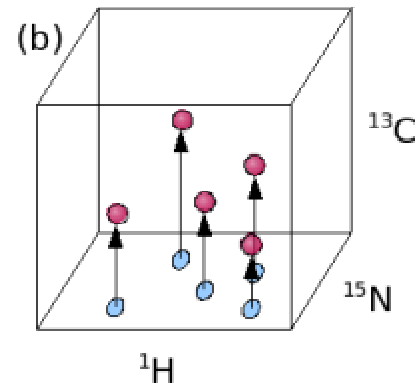
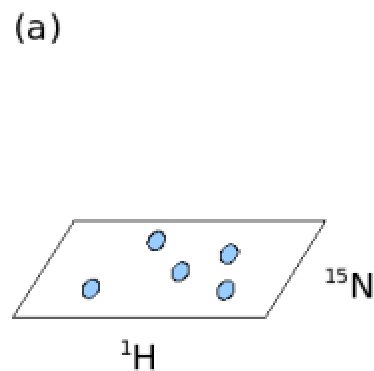


Initialising the HSQC

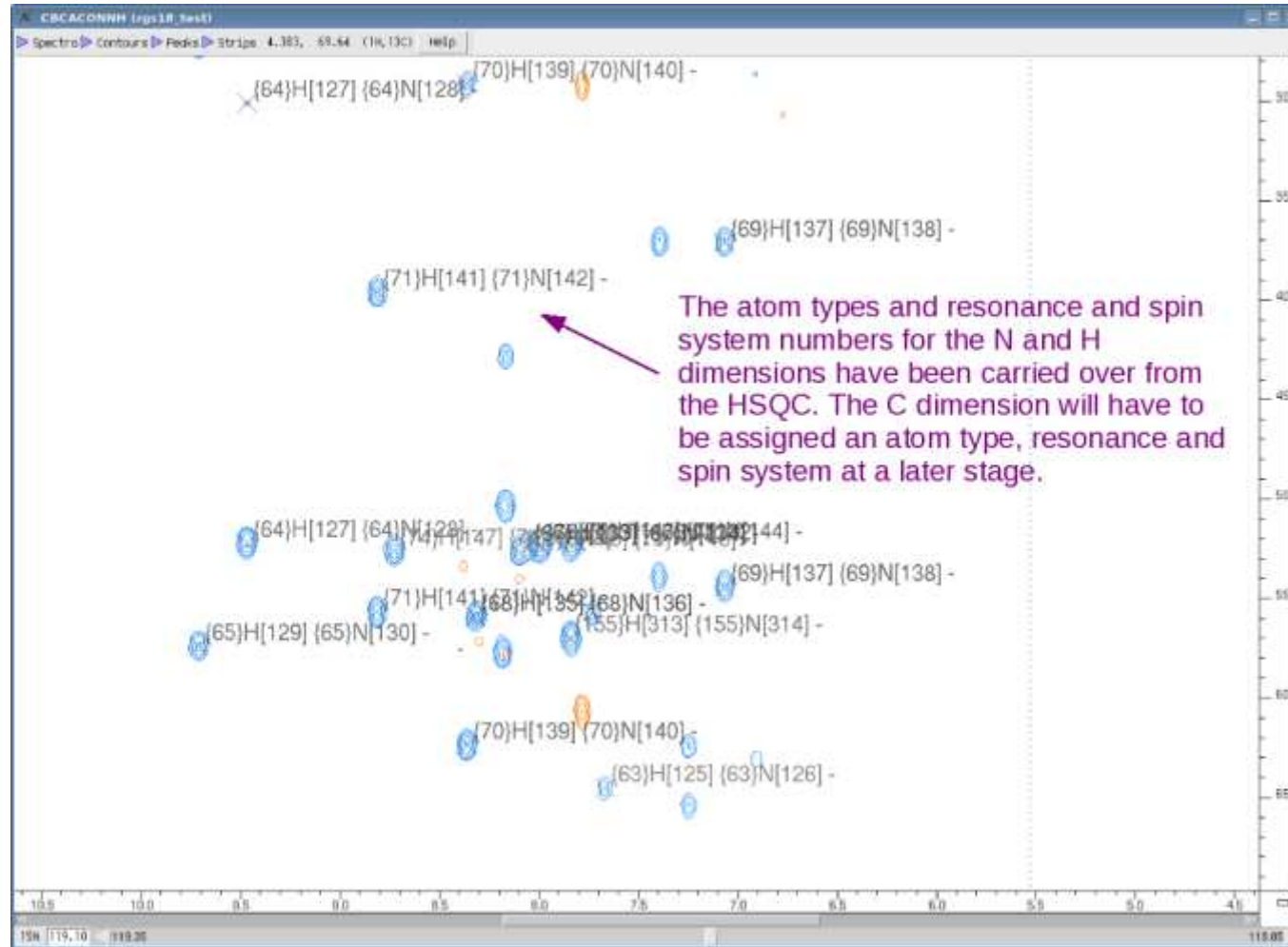


Peakpicking the 3D Spectra

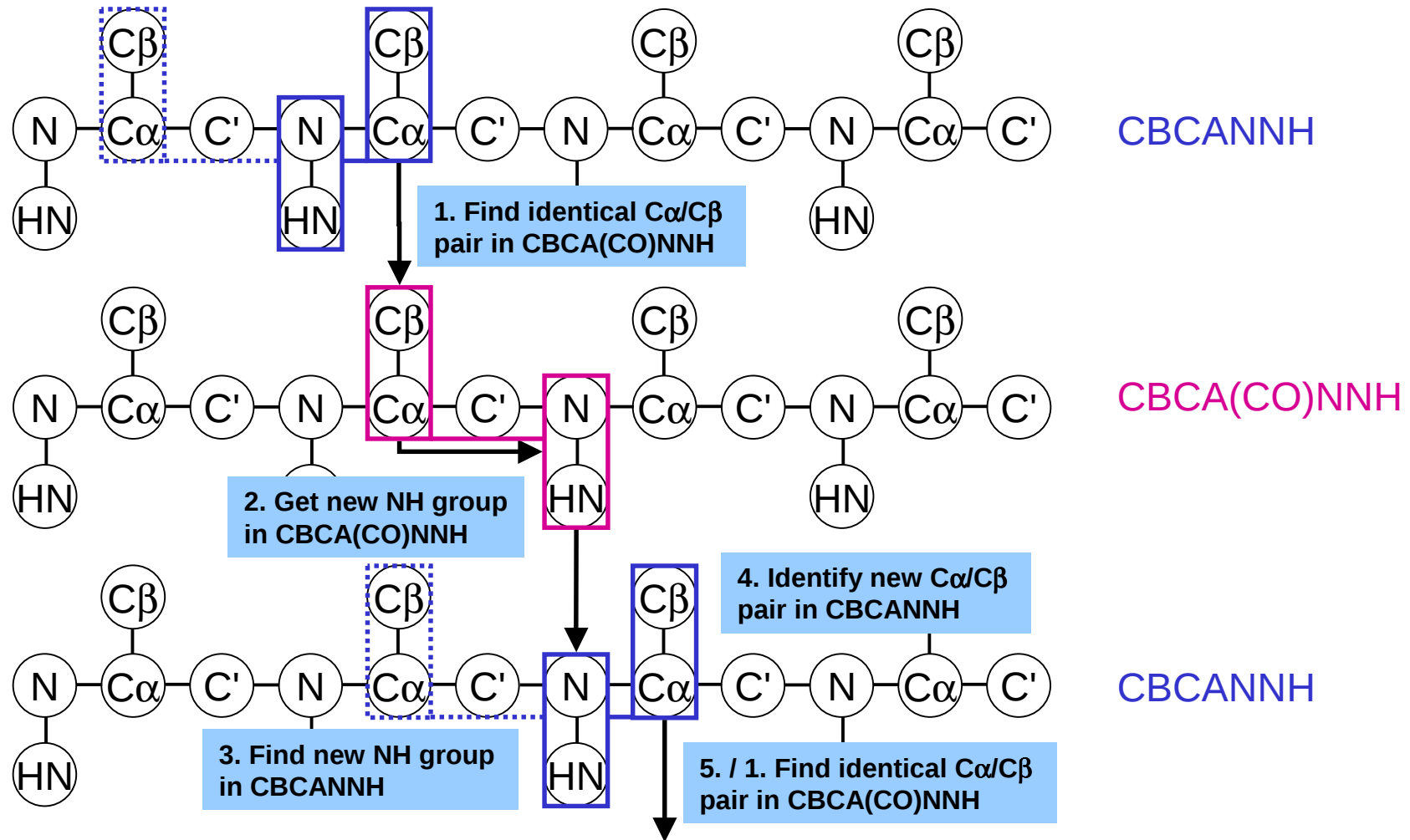
- Assignment → Link Peak Lists
 - Root spectrum
 - 3D Spectrum
 - Tolerances



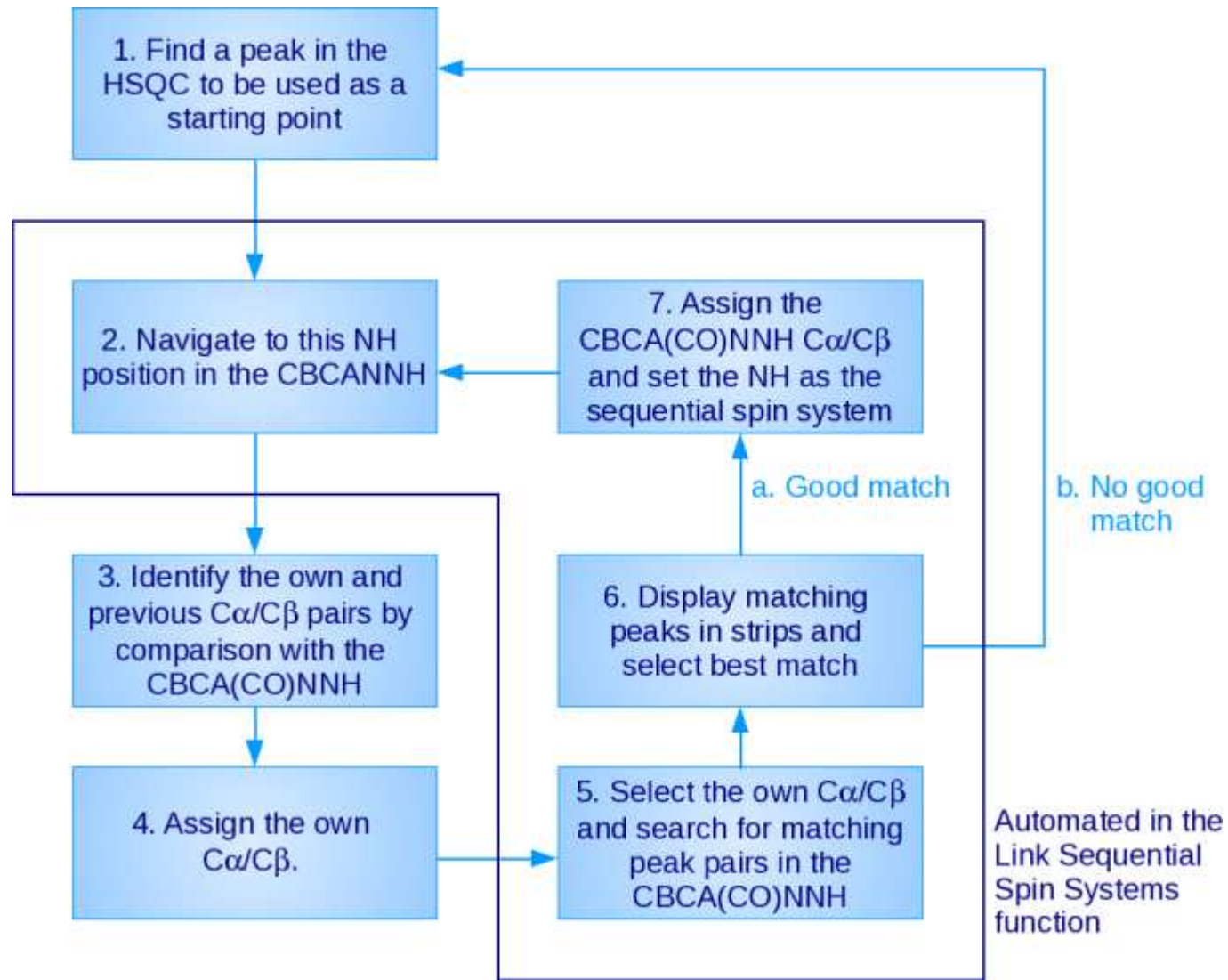
Peakpicking the 3D Spectra



Linking Sequential Spin Systems



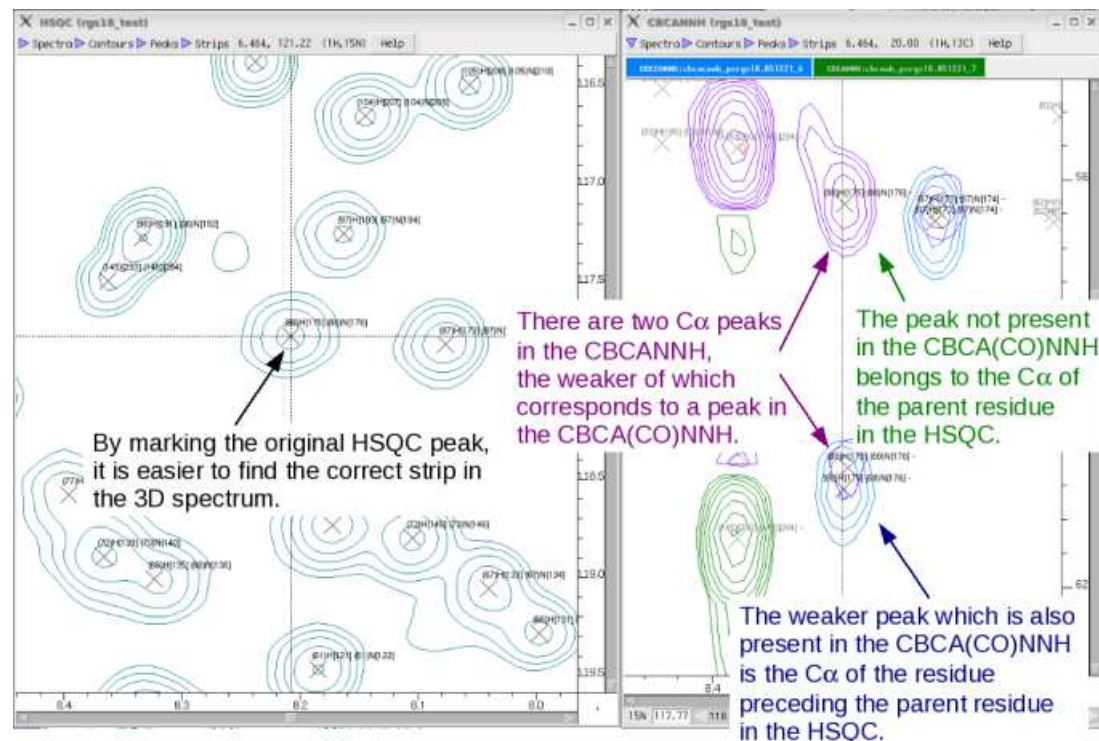
Linking Sequential Spin Systems



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 $C\alpha/C\beta$ by comparison with the CBCA(CO)NH

Assign	
Peak	
View	
Navigate	1H - 15N in CBCACONNH
Strip	1H - 15N in CBCANNH
Markers	1H - 15N in HBHACONH
Window	- 1H 15N in HBHACONH
	1H 15N - in window2
	1H 15N - in window5
	- 15N 1H in window5
	Peak Location Strips
	All window positions



Linking Sequential Spin Systems

4. Assign own C α /C β

1. Create a new resonance

2. Select the resonance and set it to be part of the same spin system as the H and N above

3. Select the resonance and set the atom type which will bring up the Browse Atoms panel

4. Toggle the Carbon atoms on and then select any C α

The screenshot displays two windows from an NMR assignment software. The 'Edit Assignment' window on the left shows a table of assigned peaks. The 'Browse atoms' window on the right shows a list of atoms for various residues.

Edit Assignment (rgs18_test)

Peak: CBCANH:cbcanh_psrps16.051221_7:1:382 Shift List: Structure: <No>

Assignment possibilities

F1 1H	8.2044	#	Name	Delta	Shift	SD	Dist
(88)H[175]		175	(88)H[175]	0.001	8.204	0.005	
<New>							
F2 15N	117.7326	#	Name	Delta	Shift	SD	Dist
(88)N[176]		176	(88)N[176]	0.014	117.746	0.017	
<New>							
F3 13C	56.3589	#	Name	Delta	Shift	SD	Dist
[325]		325	[325]	0.000	56.360	0.000	
<New>							

Options

Aliased possible ☒ Restrict Mol System ☒ Intra-residue ☐
Correlated Dims ☒ Double tolerances ☐

Assign [325] Set Atom Type De-assign [325] Clear contrib Merge F3 Resonances

Set Same Spin System

Browse atoms (rgs18_test)

Molecule: MSHM(protein) Residue: All Shift List: <Ignore> Elements: C H N O S Nucleotides: Interlocked

#	Residue	C	Ca	Cb	Cg	Ce
1	Ser	C	Ca	Cb		
2	Met	C	Ca	Cb	Cg	Ce
3	Val	C	Ca	Cb	Cga	Cgb
4	Ser	C	Ca	Cb		
5	Pro	C	Ca	Cb	Cg	Cd
6	Glu	C	Ca	Cb	Cg	Cd
7	Glu	C	Ca	Cb	Cg	Cd
8	Ala	C	Ca	Cb		
9	Val	C	Ca	Cb	Cga	Cgb
10	Lys	C	Ca	Cb	Cg	Cd
11	Trp	C	Ca	Cb	Cg	Cd
12	Gly	C	Ca	Cb		
13	Glu	C	Ca	Cb	Cg	Cd
14	Ser	C	Ca	Cb		
15	Phe	C	Ca	Cb	Cg	Cd

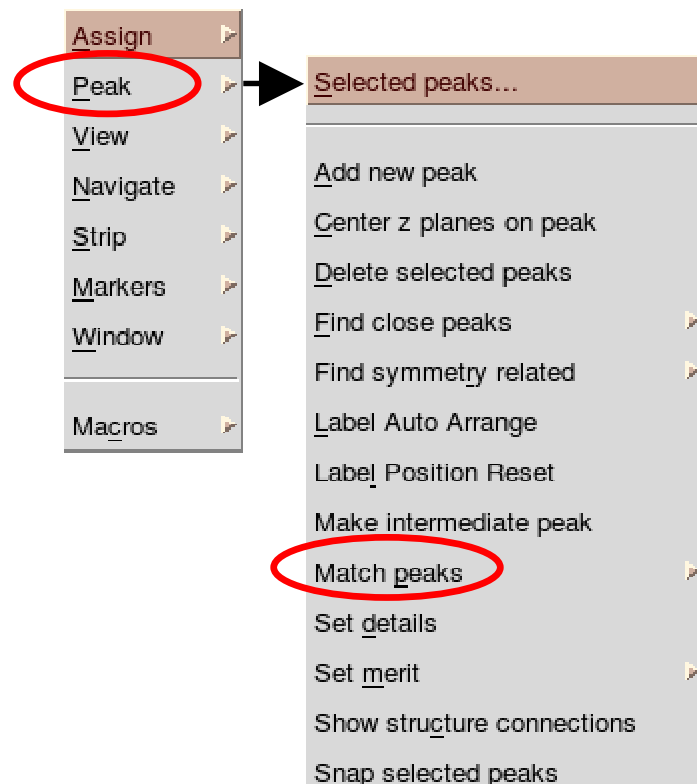
Options Shown

Prochirals: ☒ Non-Stereospecific ☐ Stereospecific ☐ Ambiguous
Assignment: ☒ Any ☐ Assigned ☐ Unassigned

Set ring flip equivalency Remove atom assignments Show peaks Close Help

Linking Sequential Spin Systems

5. Select own $C\alpha/C\beta$ and search for matching $C\alpha/C\beta$ 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

Matching score Number of peaks which match the target peaks Matching spin systems that have been found

1. Select the spin systems that you want to inspect visually

2. Select the window in which you want to view the spin systems

3. Click 'Display groups in strips'

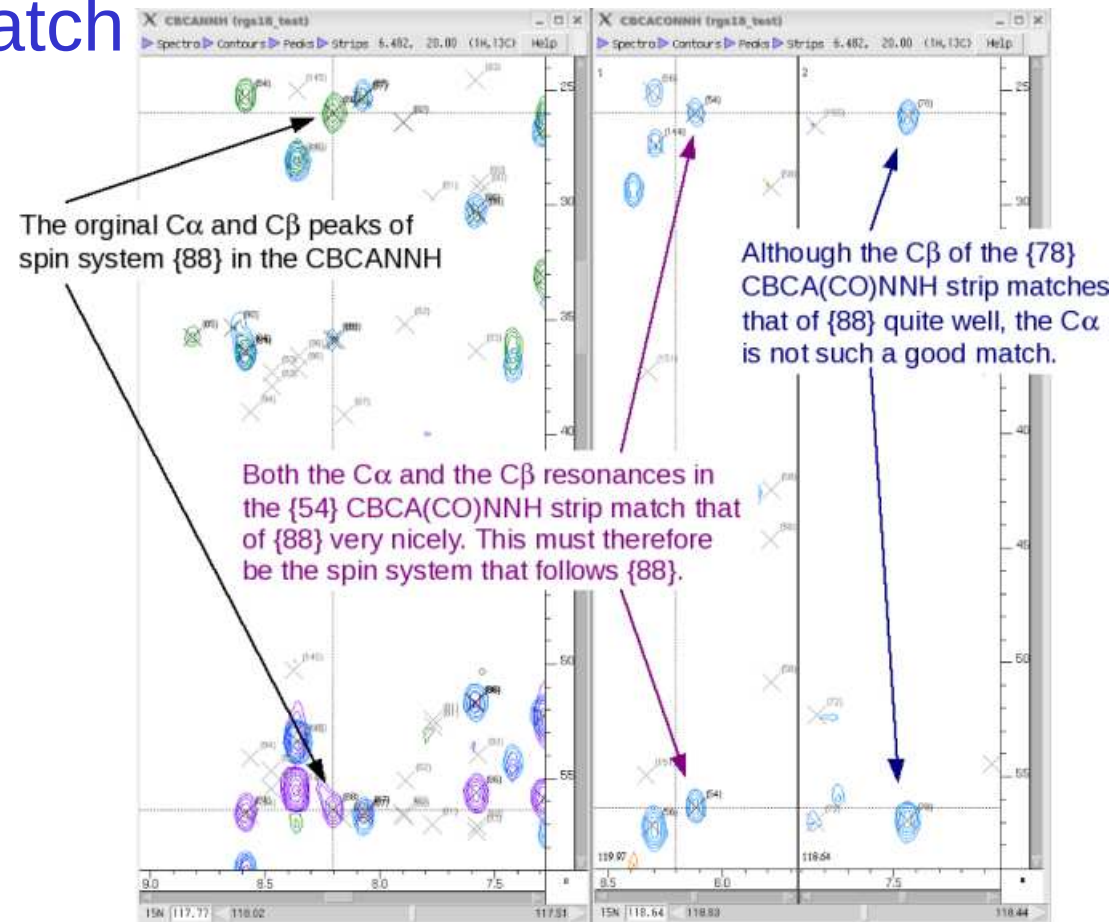
Rank	Score	Num peaks	Root Assignment	F1 position	F2 position
1	0.063645	2	{54}H[107] {54}N[108]	8.115562	119.966981
3	0.003878	1	{78}H[155] {78}N[156]	7.438021	118.530253

Remove groups Show rulers Remove rulers Display groups in strips Target window: 1H 13C 15N in CBCACONNH ▾

Close Help

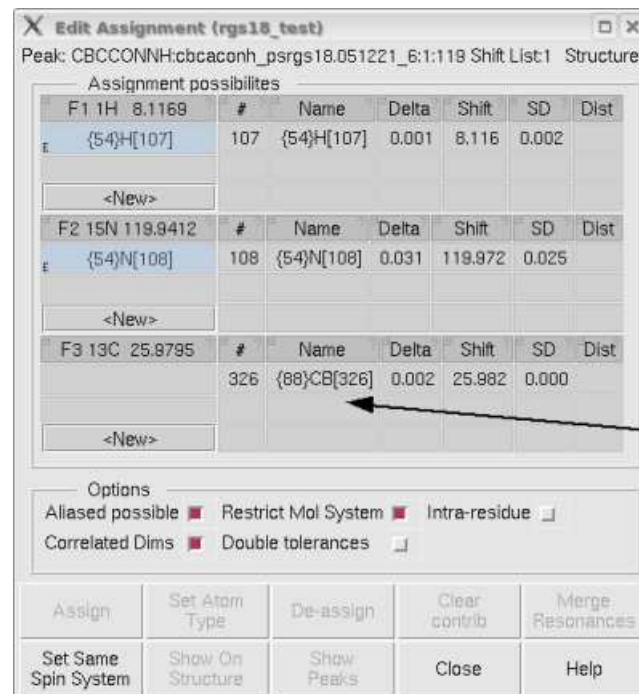
Linking Sequential Spin Systems

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

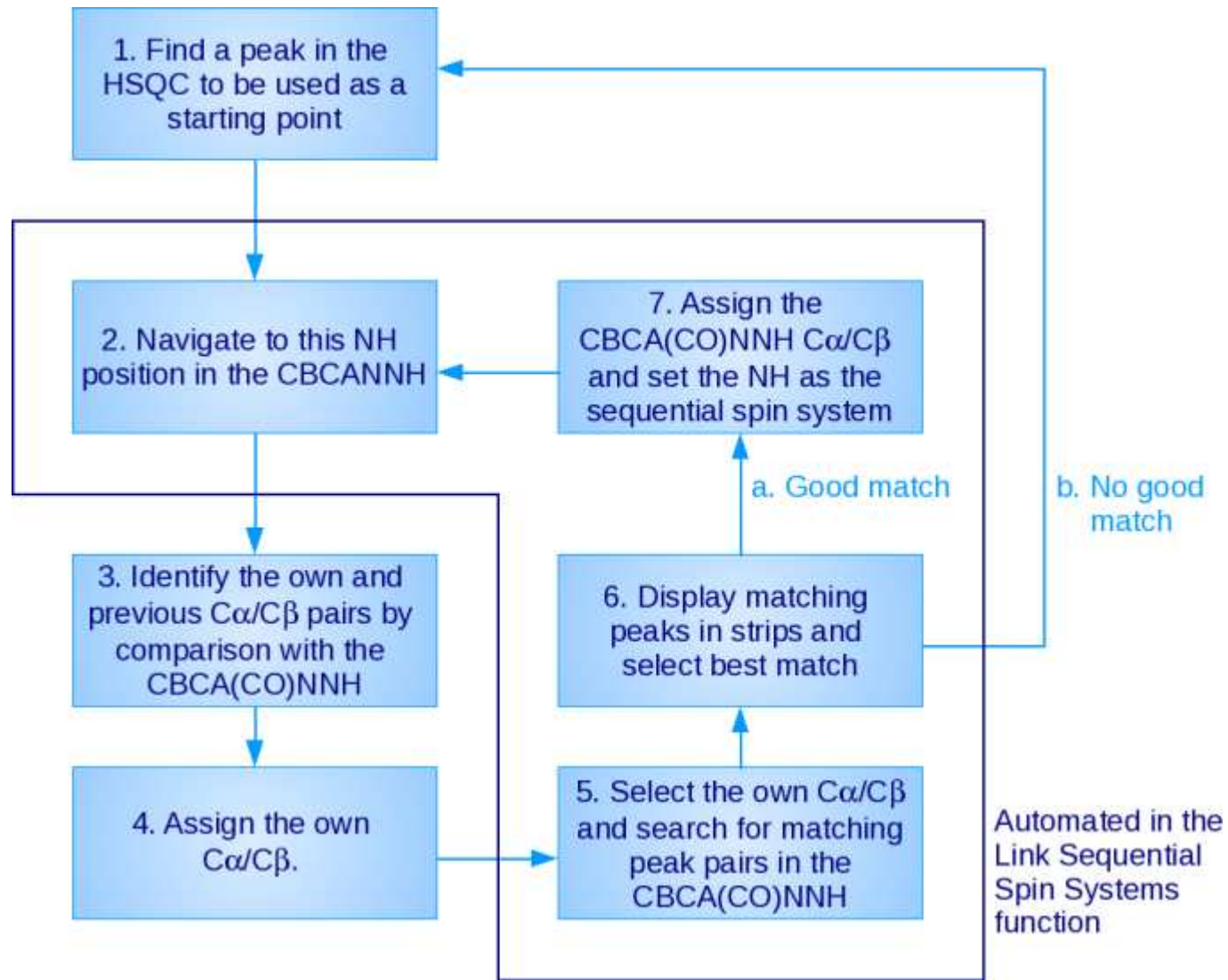


Select this resonance (the C β of spin system {88}) by clicking on it and it will then appear on the left hand side so that the peak then has the assignment Hi-Ni-C β_{i-1} .

Linking Sequential Spin Systems

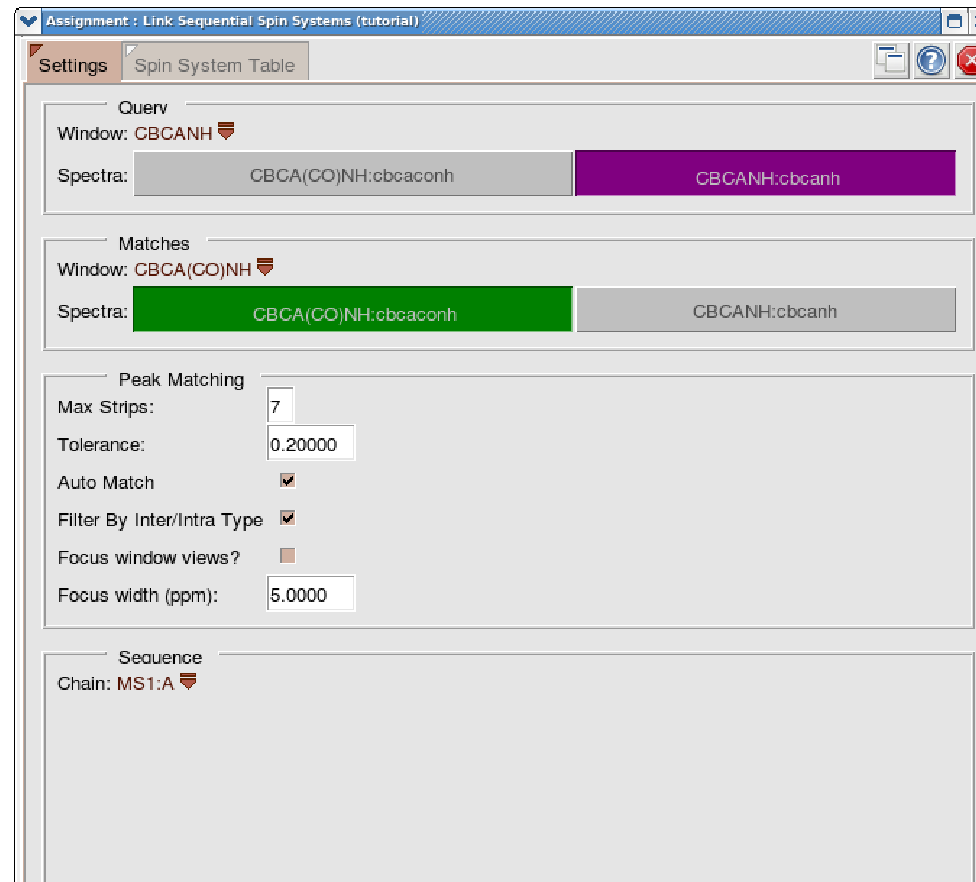
7. (b) No Good Match: Start Again...

Linking Sequential Spin Systems



Semi-automatic Linking

- Assignment → Link Sequential Spin Systems



Semi-automatic Linking

Assignment: Link Sequential Spin Systems (tutorial)

Settings Spin System Table

Spin Systems

#	Name	I-1	I+1	I+0	Seq. Segment	Position
1	{1}				0:000	x=7.747,z1=116.154
2	{2}				61:000	x=8.691,z1=117.487
3	{3}				72:000	x=8.243,z1=114.347
4	{4}				83:000	x=8.636,z1=122.128
5	{5}				94:000	x=9.467,z1=119.373
6	{6}				106:000	x=8.362,z1=117.601
8	{8}				127:000	x=7.352,z1=124.180
9	{9}				138:000	x=8.475,z1=118.168
10	{10}				1:000	x=8.075,z1=117.836

Goto Next Goto Prev Goto I-1 Goto I+1

Clear I-1 Links Clear I+1 Links Clear all Links Show Peaks Show Resonances

Matched Peak Positions

Rank	Score	Spin System	I-1	I+1	Num Peaks
1	0.043118	{87}	?	?	2
2	0.039848	{94}	?	?	1
3	0.033038	{39}	?	?	1
4	0.009507	{89}	?	?	1
5	0.007349	{79}	?	?	1
6	0.006794	{43}	?	?	1
7	0.004412	{28} Overlaps:{105}	?	?	2
7	0.004412	{105} Overlaps:{28}	?	?	2

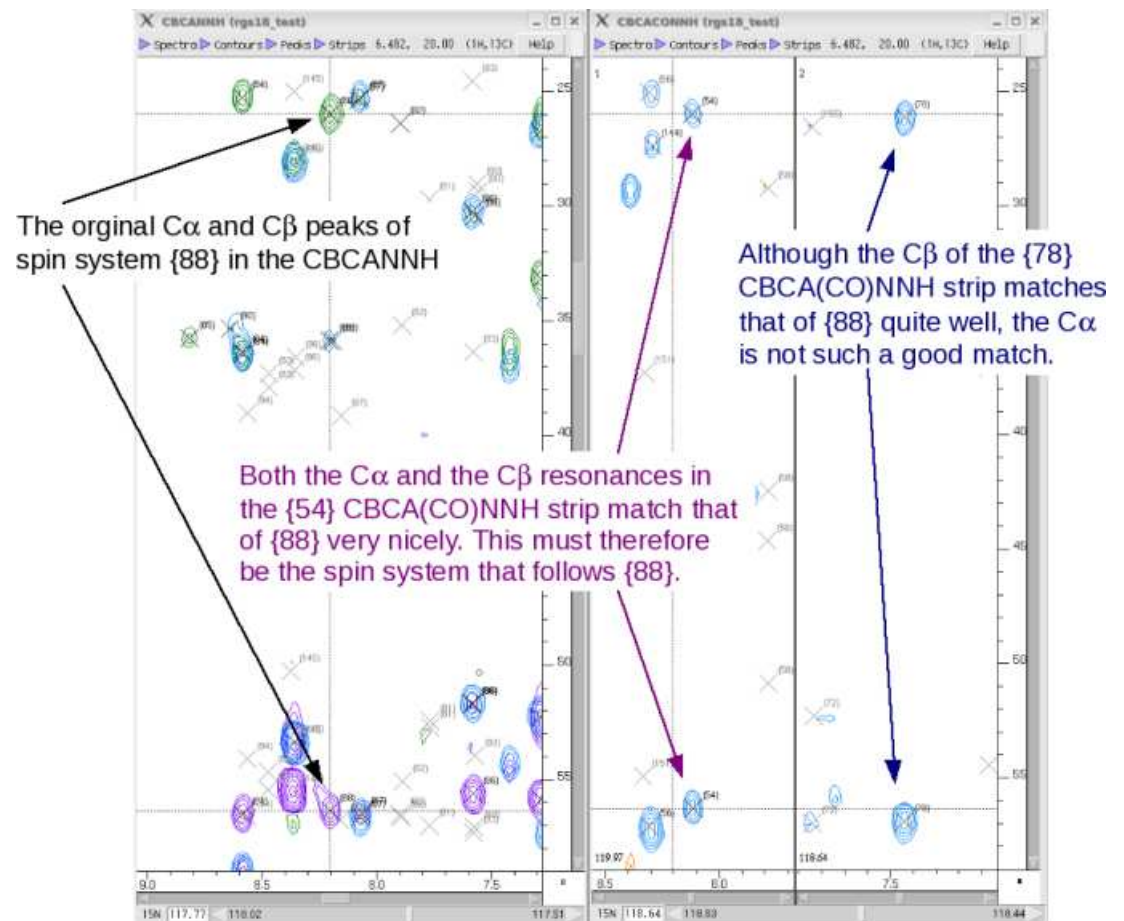
Find Matches Discard Selected Set Seq Link Clear Rulers

Sequence Locations Residue Types

Rank	Score	I-2	I-1	I	I+1	I+2	Residue Types
1	3.627	34Thr	35Glu	36Phe	37Ser	38C	Tyr 7.2 Ser 11.0
2	3.431	11Trp	12Gly	13Glu	14Ser	15F	Phe 7.1 Thr 8.9
3	3.328	17Lys	18Leu	19Leu	20Ser	21H	Ile 6.5 Gly 8.1
4	3.324	97Thr	98Leu	99His	100Ser	101F	Cys 6.3 Asn 7.3
5	3.319	1Ser	2Met	3Val	4Ser	5P	Glu 6.1 His 6.0

Assign Selected

Tyr 5.0 Cys 5.0



That's It

Happy Assigning...