The 52nd Annual Meeting of the Biophysical Society of Japan
Sept. 2014

Tools for highly automated NMR analysis and applications using database

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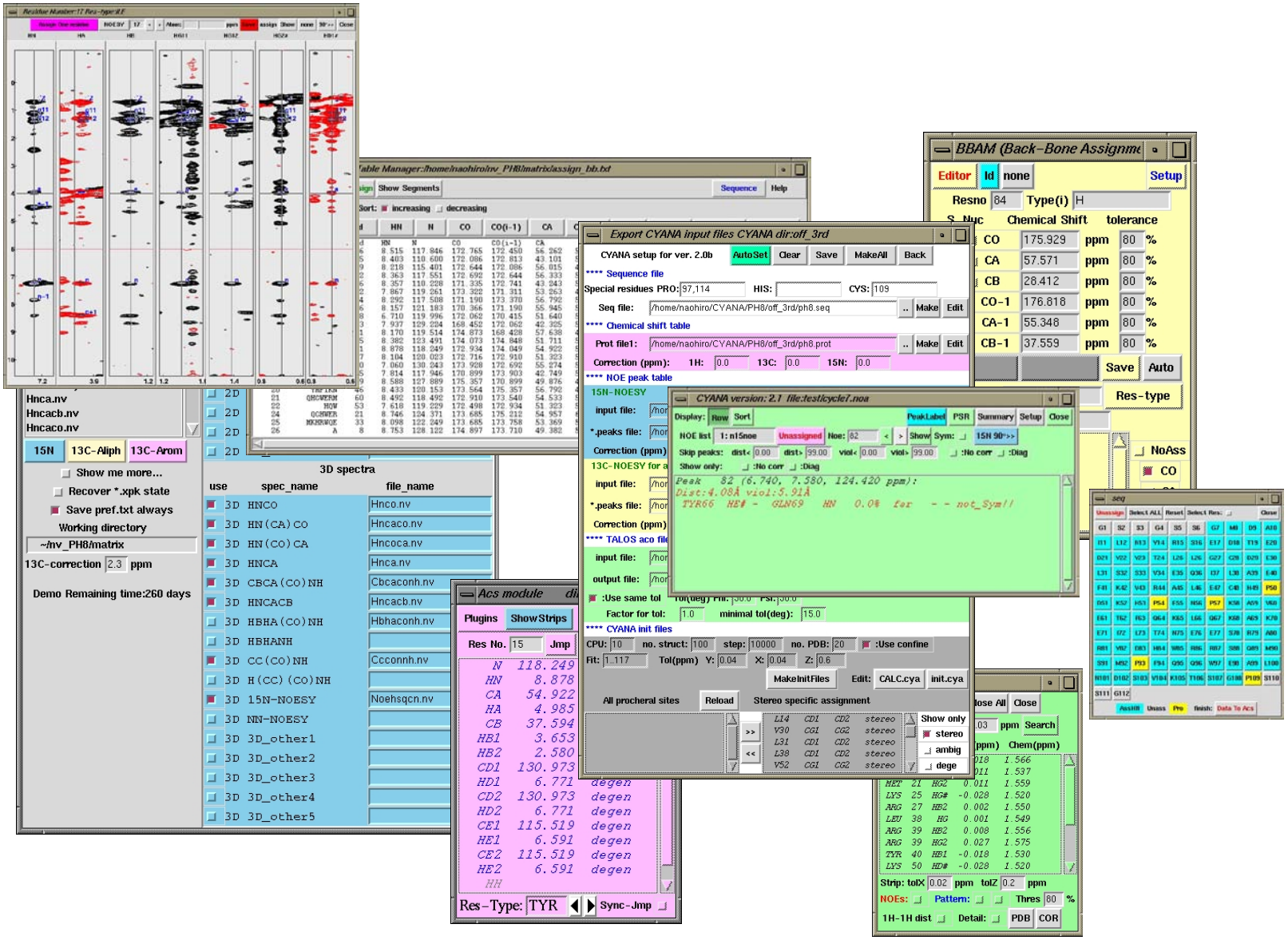
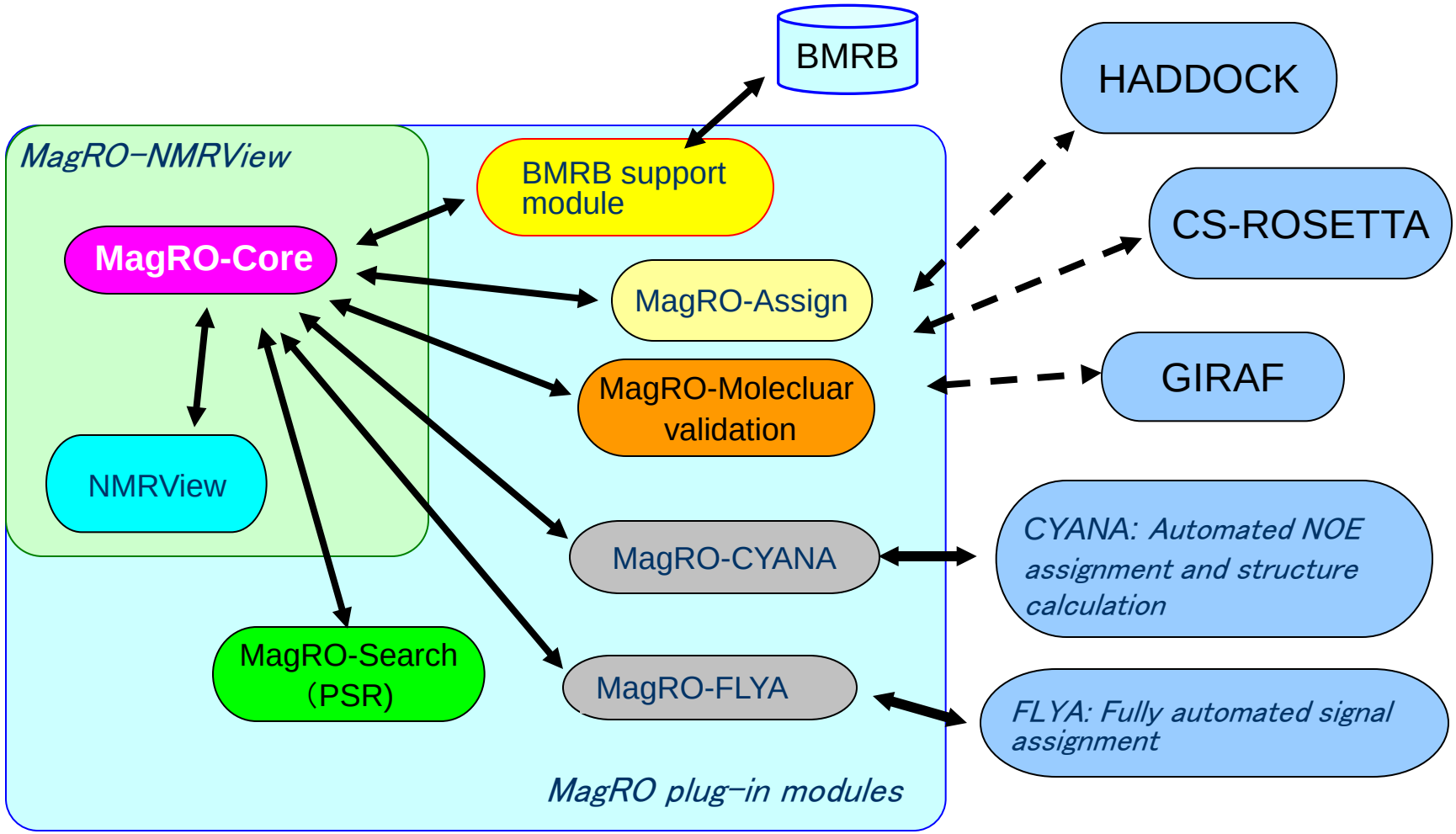
Institute for Protein Research

GUI modules implemented in MagRO-NMRView



Modules networked in MagRO system



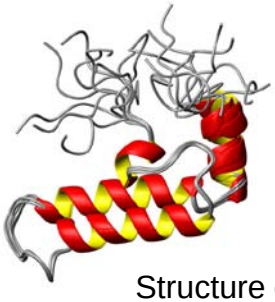
Controlling and managing data having complicated data structure and automated and/or semi-automated function to support NMR analysis

New paradigm for NMR structure analysis using MagRO/FLYA/CYANA system

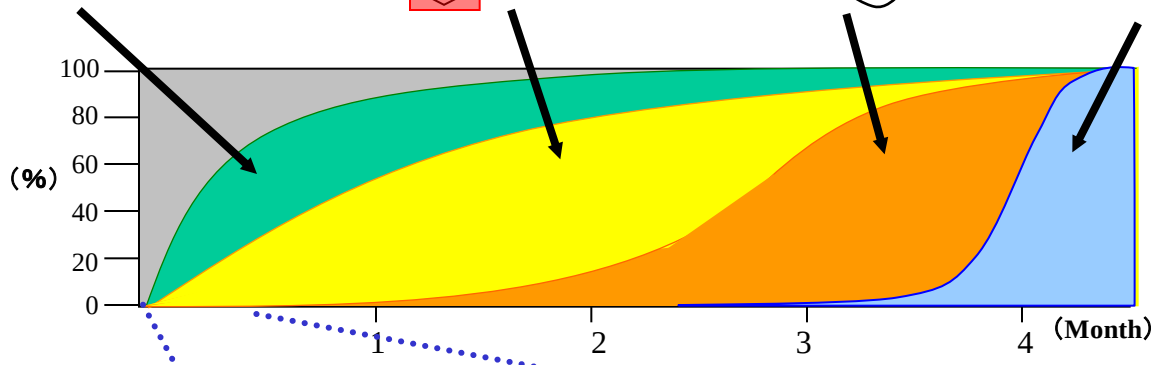
Backbone signal assignments

Side-chain signal assignments

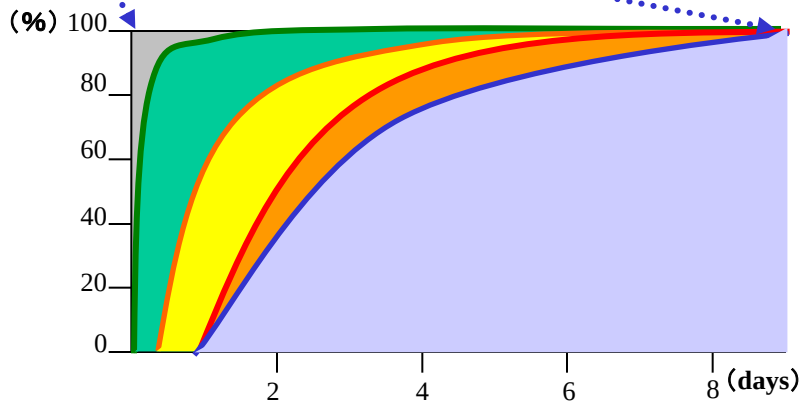
NOE assignments



Structure calculation



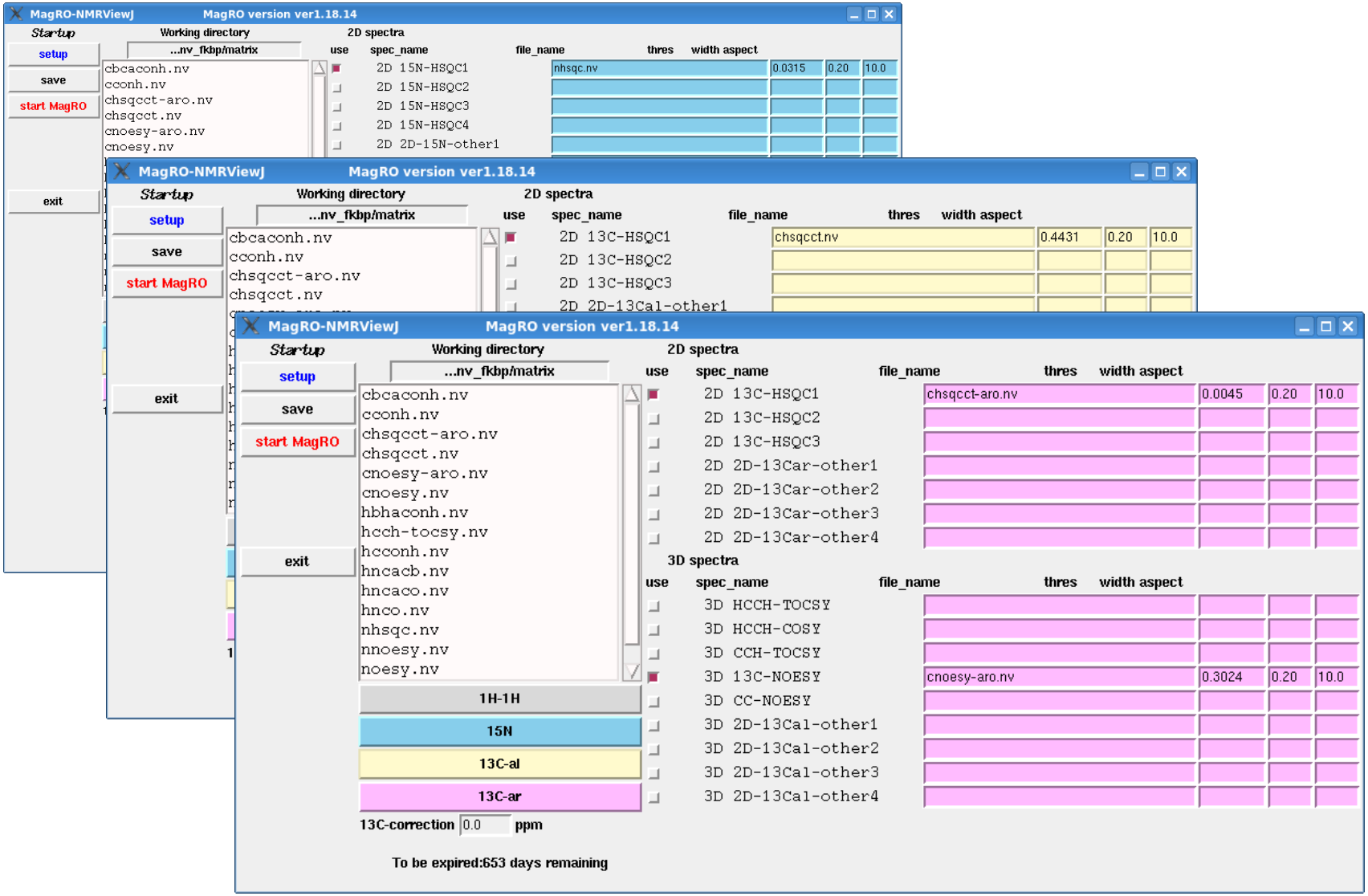
Convensional analysis



MagRO/FLYA/CYANA system

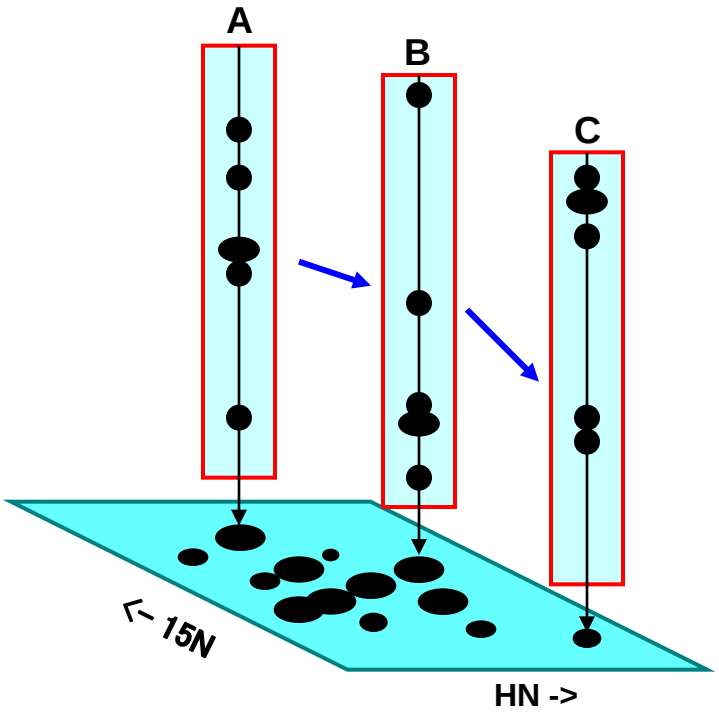
Early stage	Mid stage	Final stage	⇨	PDB

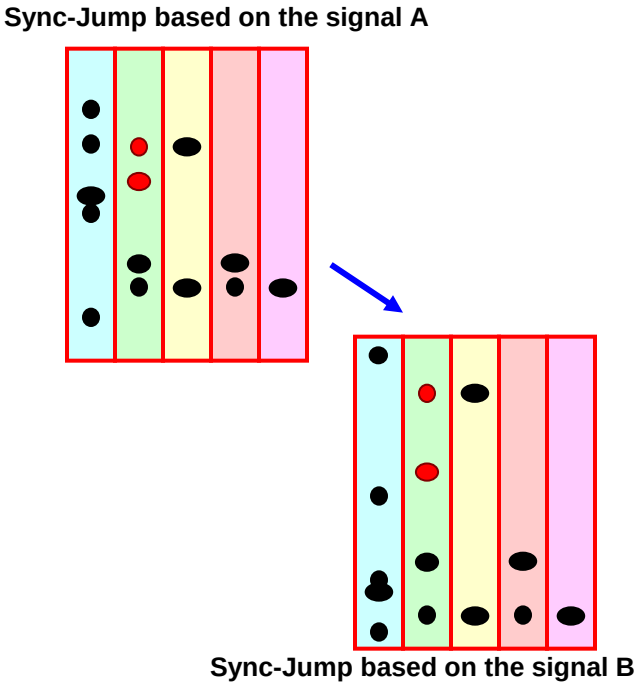
GUI module for managing NMR spectra



Sync-Jump: a function to synchronizedly control NMR spectra

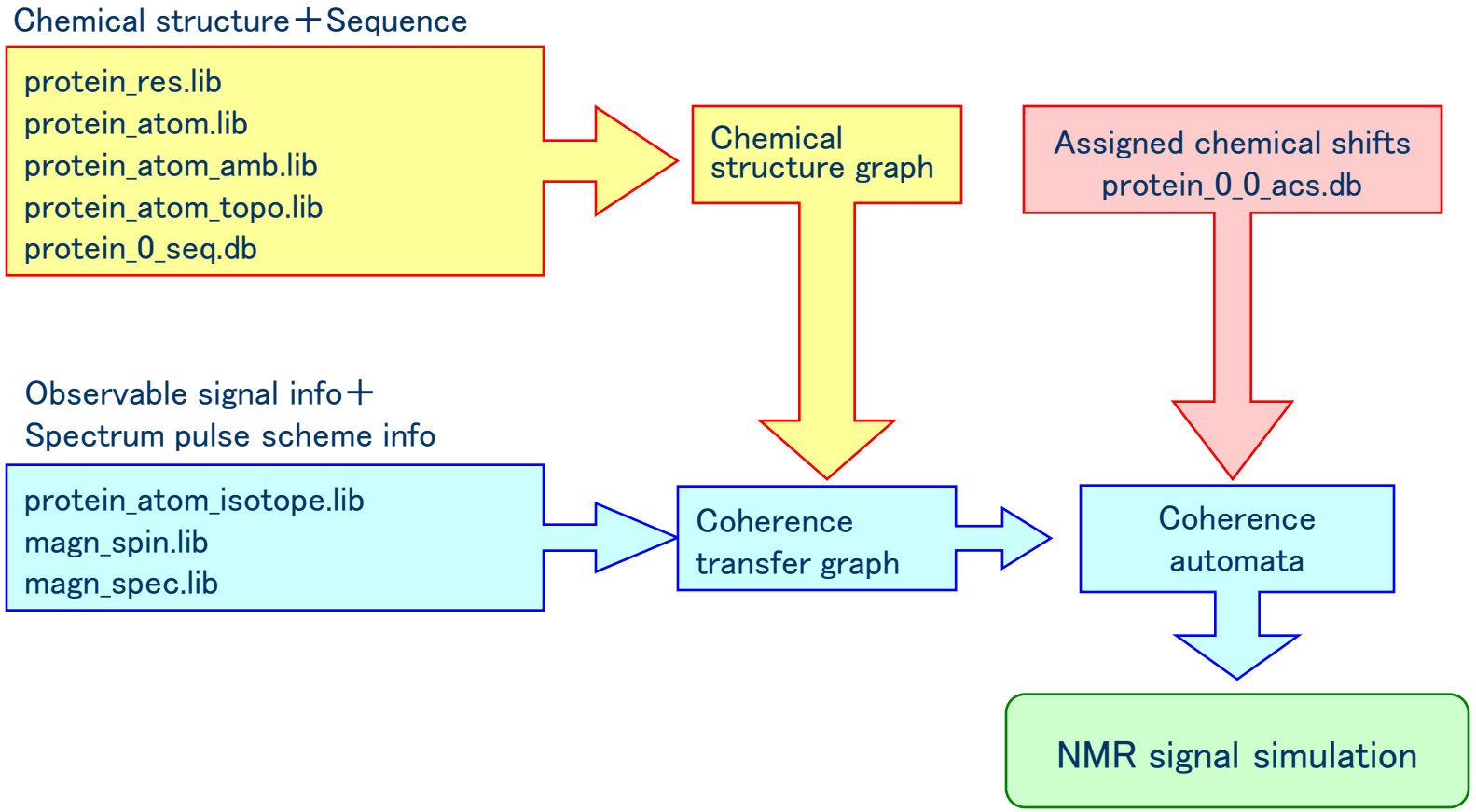
Sync-jump class	***Corresponding spectrum types***
15N	^{1}H-^{15}N type
13C-ar	^{1}H-^{13}C for aromatic type
13C-al	^{1}H-^{13}C for all region or aliphatic type





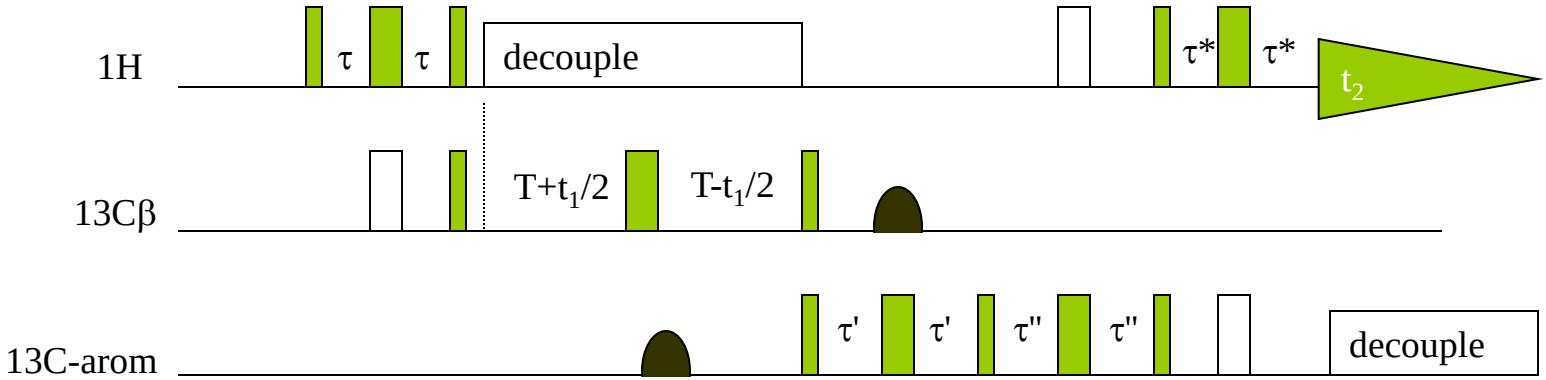
This function allows to display 2D spectrum strips belonging to same sync-jump class.

Signal simulation using finite-state automata with information of chemical structure and coherence transfer



2D – (HB)CB (CGCD)HD spectrum

Yamazaki et al., J. Am. Chem. Soc. 1993

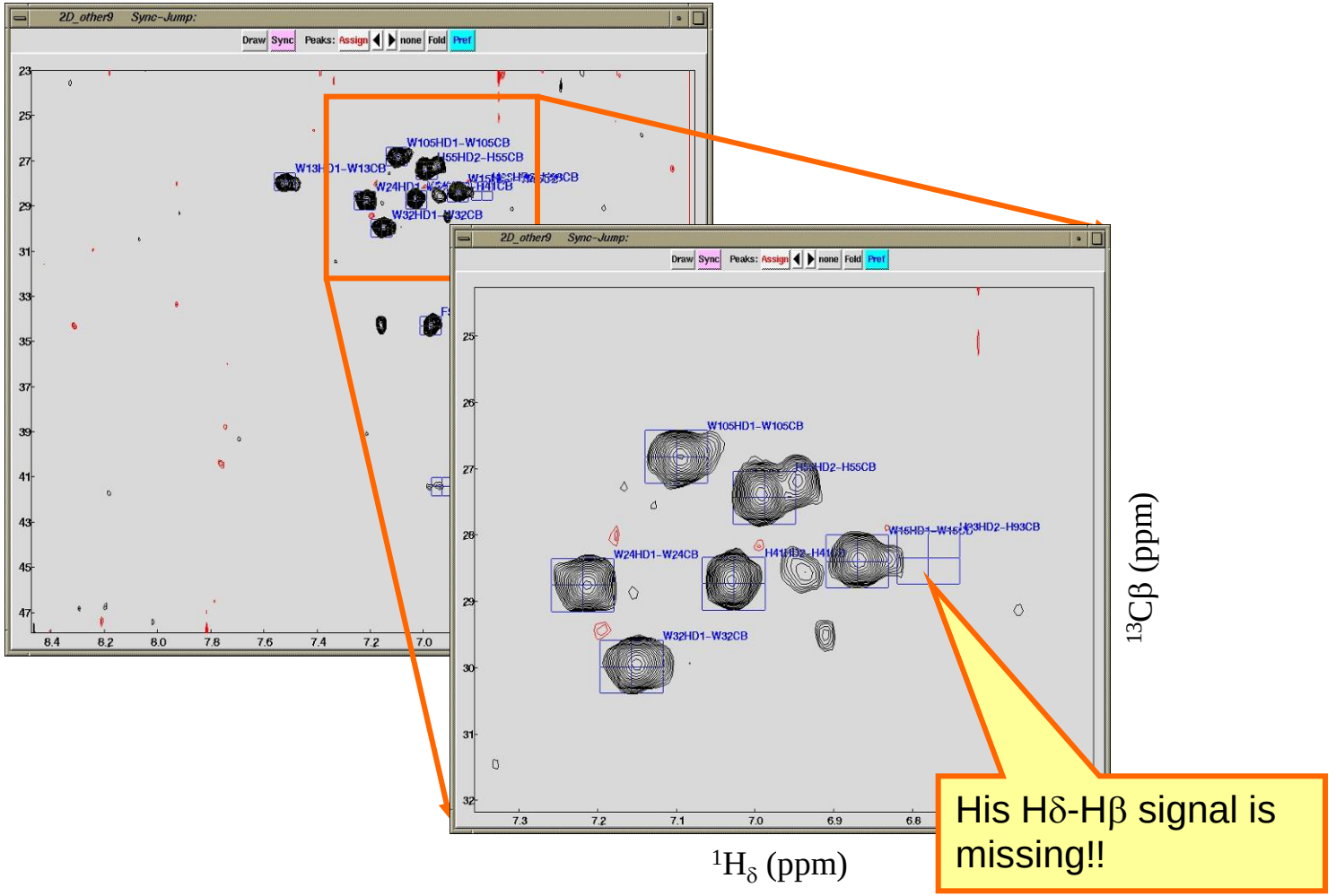


$$^{1}H_i \xrightarrow{^{1}J_{CH}} {}^{13}C\beta_i \xrightarrow{^{1}J_{CC}} {}^{13}C\gamma_i \xrightarrow{^{1}J_{CC}} {}^{13}C\delta_i \xrightarrow{^{1}J_{CH}} {}^{1}H\delta_i$$

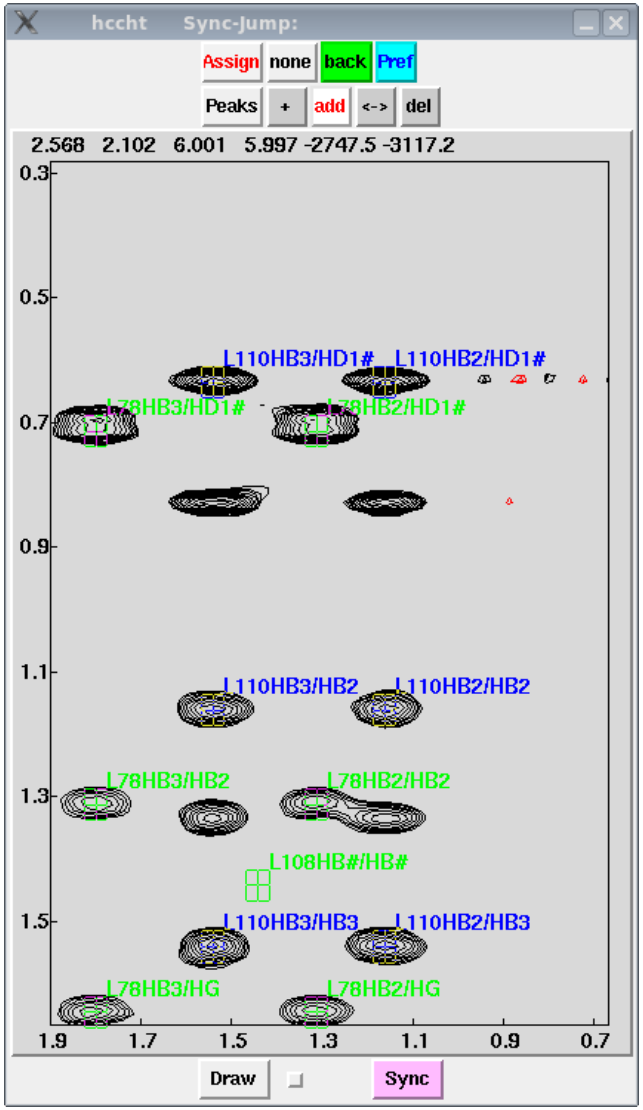
(t_1) (t_2)

Spectrum_type	step	res1	atom1	res2	atom2	time	seq1	seq2	inten	fold
Spec: hbcbcgcdhd	0	arom	HR	arom	HR	1	0	0	1.0	0
Spec: hbcbcgcdhd	1	arom	HR	arom	CR	0	0	0	1.0	0
Spec: hbcbcgcdhd	2	arom	CR	arom	CR	0	0	0	1.0	0
Spec: hbcbcgcdhd	3	arom	CR	all	CL	0	0	0	1.0	0
Spec: hbcbcgcdhd	4	all	CL	all	CL	2	0	0	1.0	1

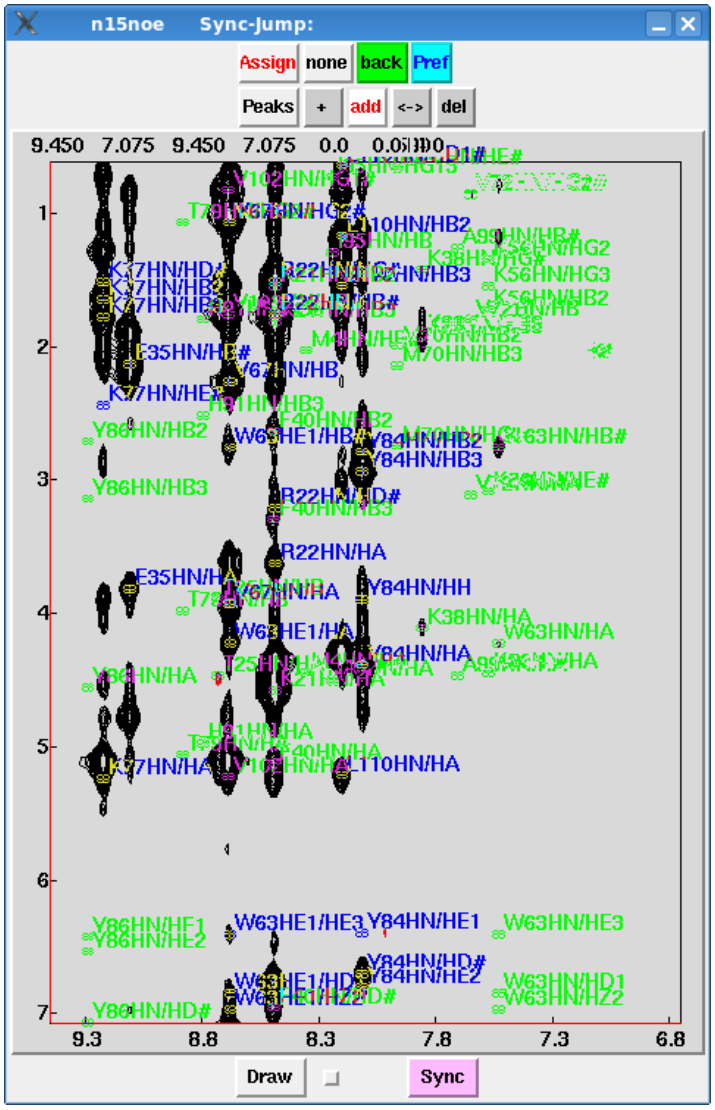
Confirmation of assigned chemical shifts using signal simulation of 2D (HB)CB(CGCD)HD spectrum



Signal simulations of the other spectrum types

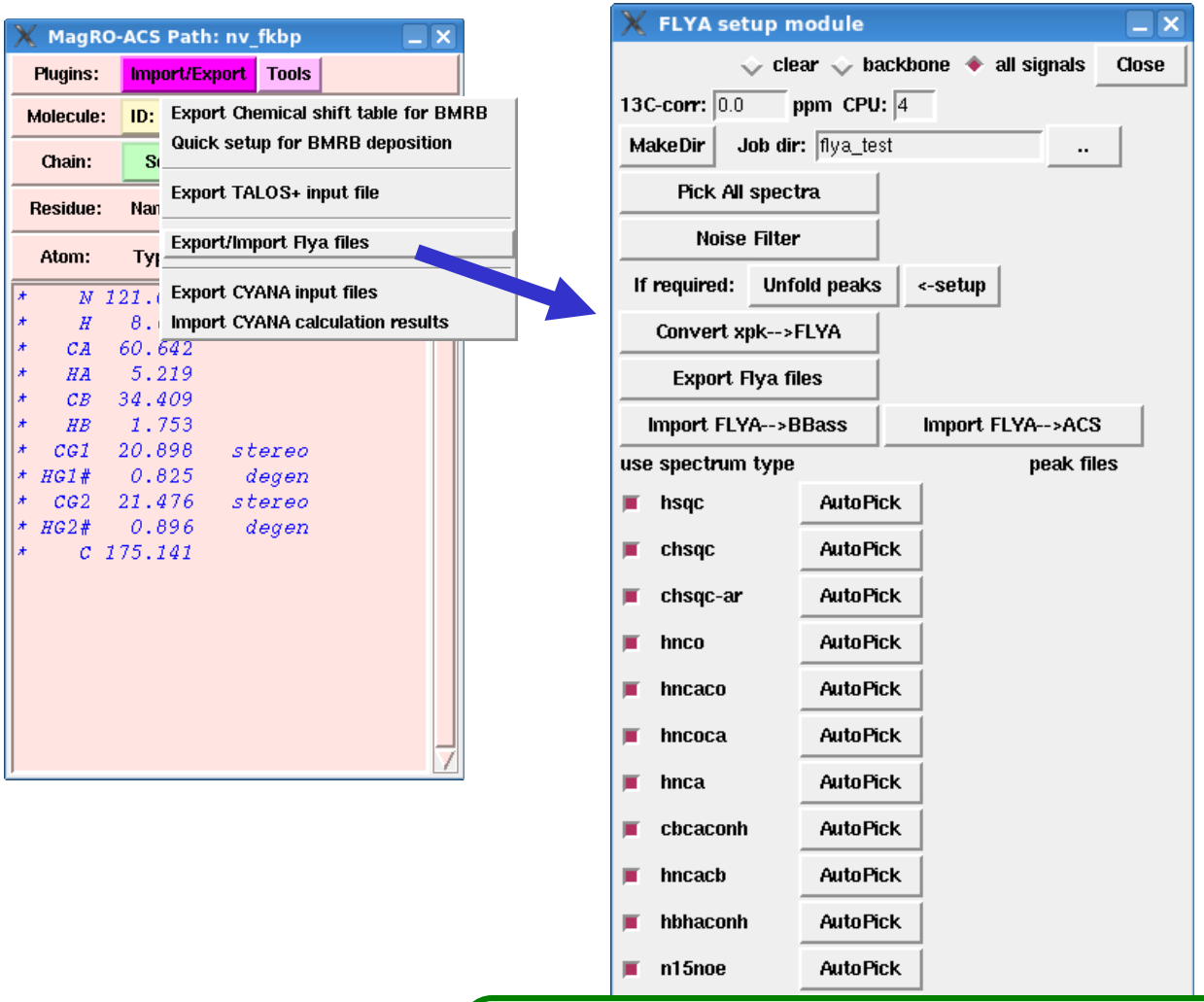


3D HCCH-TOCSY



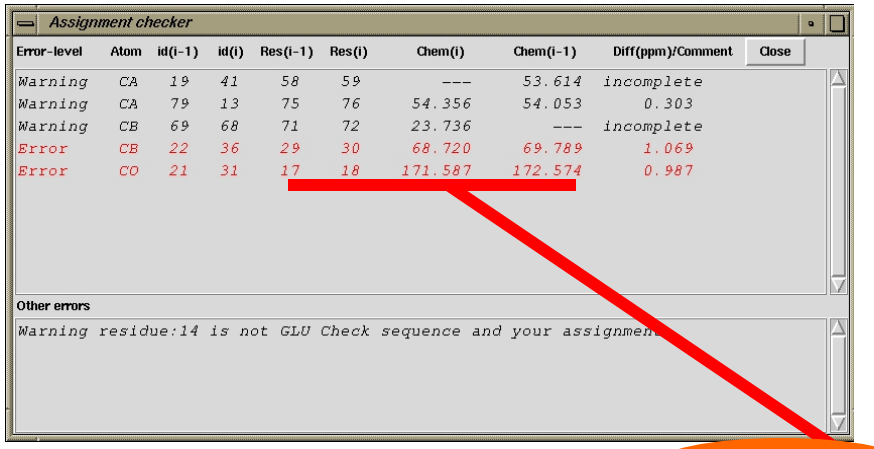
3D ^{15}N edited NOESY

FLYA GUI module implemented in MagRO-NMRView



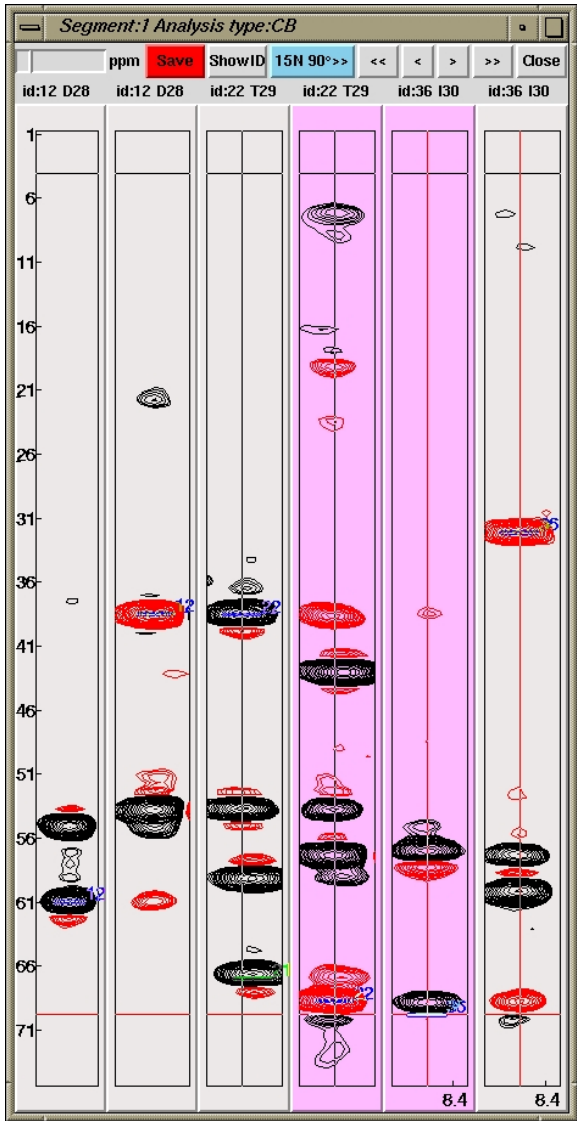
All files required for FLYA calculation can be automatically generated with this module

Confirmation and correction of assigned chemical shifts for backbone signals



Double-click

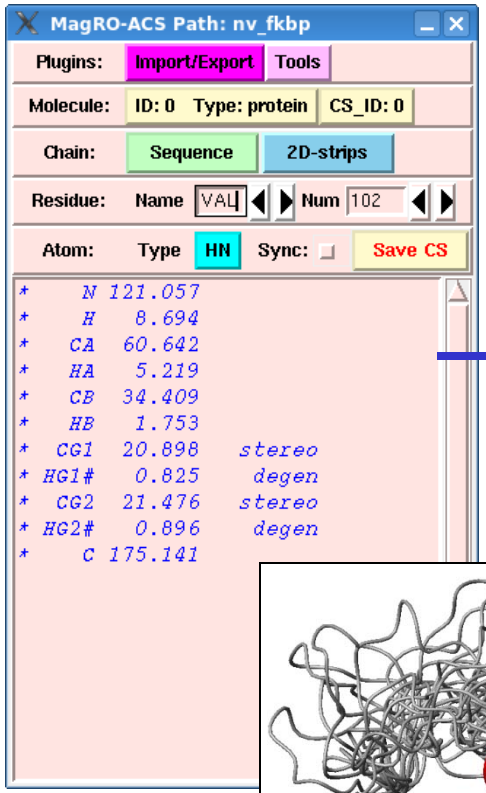
Highlited the problematic assigned signals



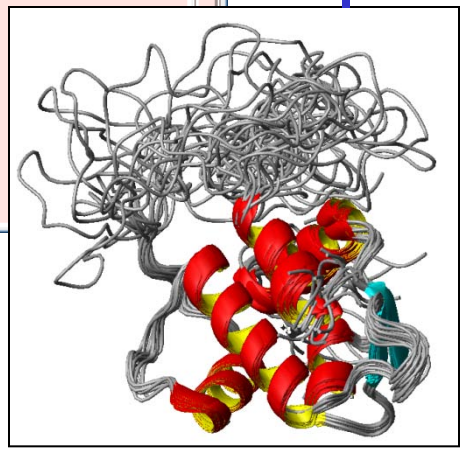
Can be interactively corrected on the GUI module

Confirmation and correction of assigned chemical shifts for side-chain signals

Assigned chemical shift table

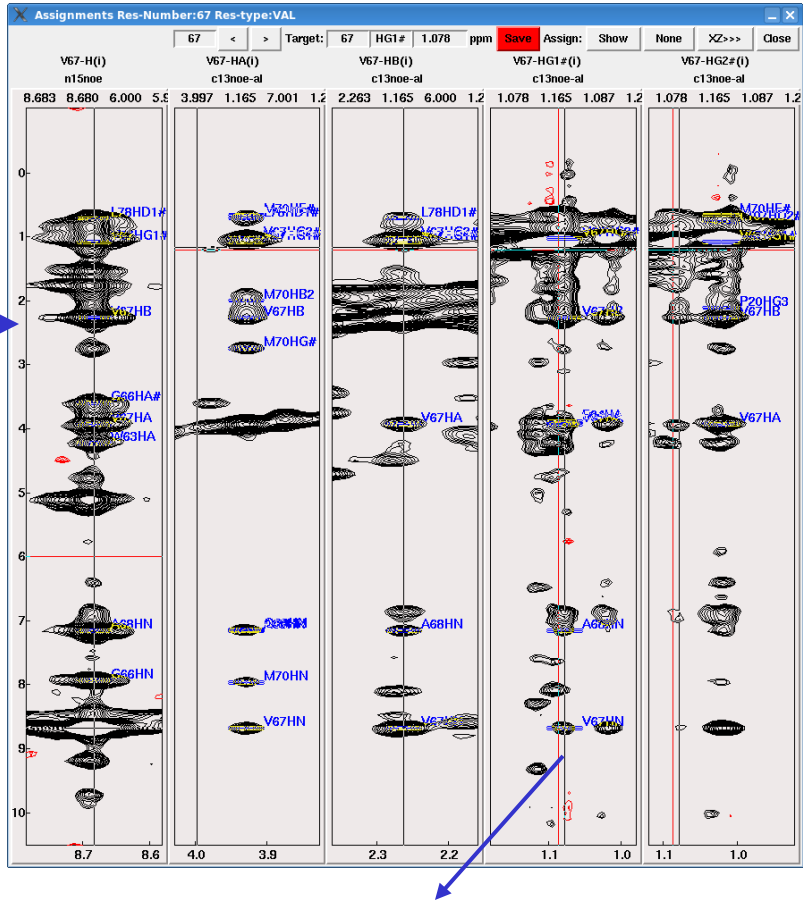


Simulation of NMR signals



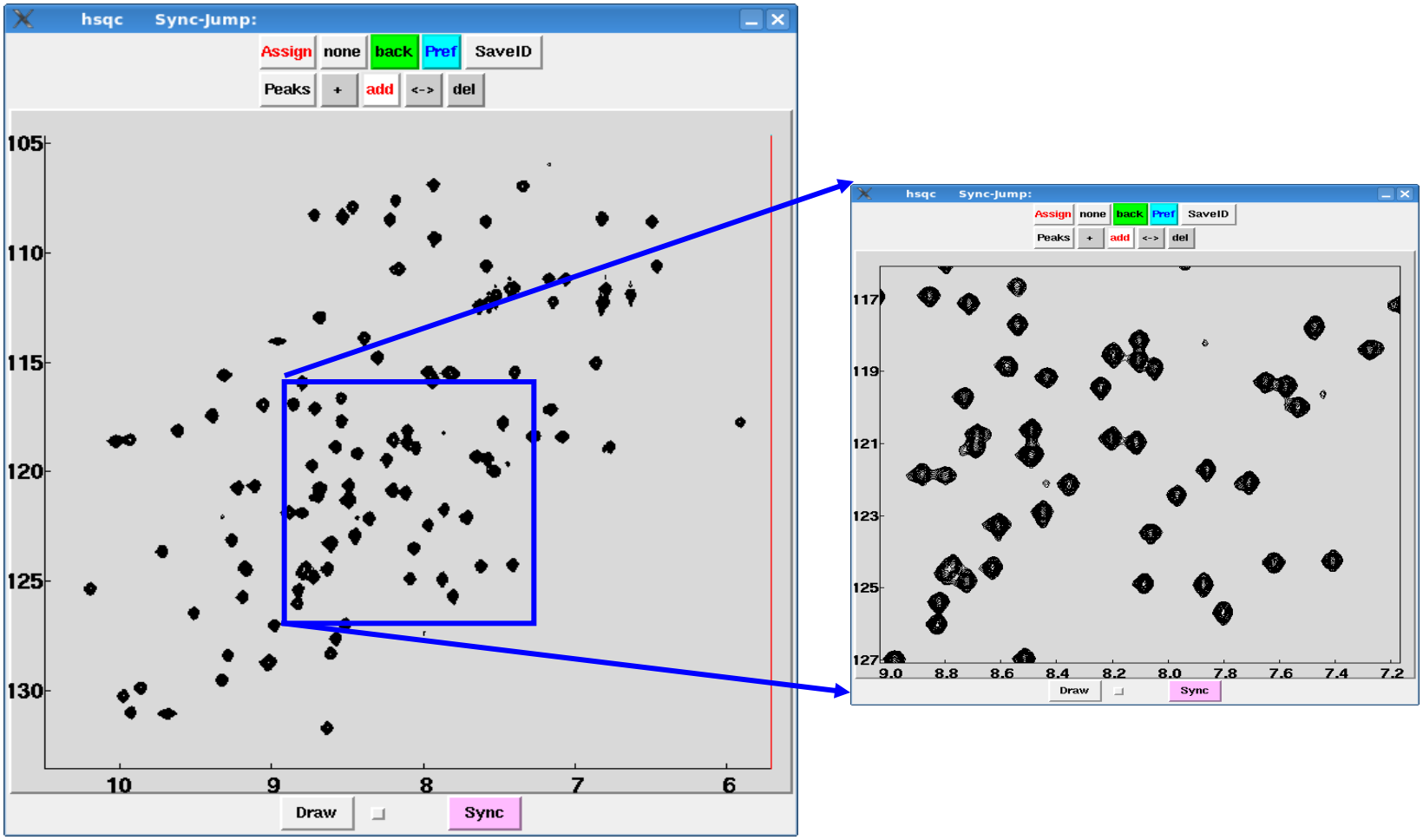
Structure coordinates

^{15}N−edited NOESY,^{13}C−edited NOESY



Interactively correct the wrongly assigned signals

NMR analysis Case1: FKB binding protein (111a.a.)



Very good signal separation, no signal overlapping

Steps for MagRO/FLYA/CYANA analysis

Automated peak detection for all spctra

↓

Noise filtering

↓

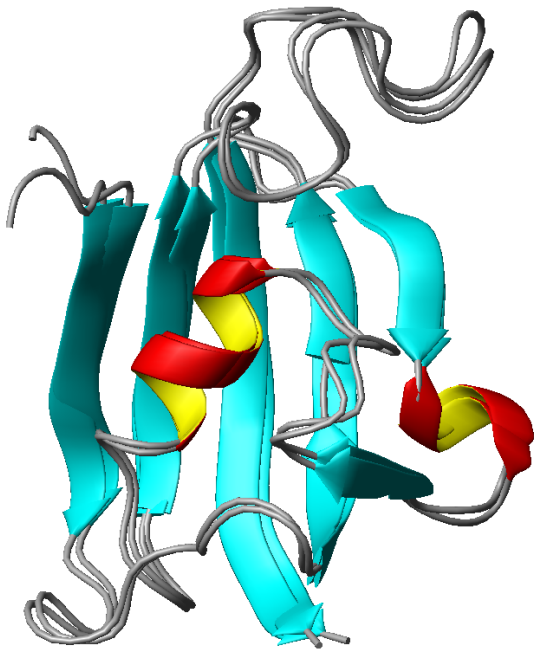
Fully automated signal assignment by FLYA

↓

NOE assignment and structure calculation by CYANA

Summary of results: FLYA and CYANA calculations

^{1}H-^{15}N-HSQC, HNCO, HN(CA)CO, HNCACB, CBCACONH
^{1}H-^{13}C-HSQC, HCCH-TOCSY aliphatic
^{15}N-edited NOESY, ^{13}C-edited NOESY



Automated signal assign by FLYA

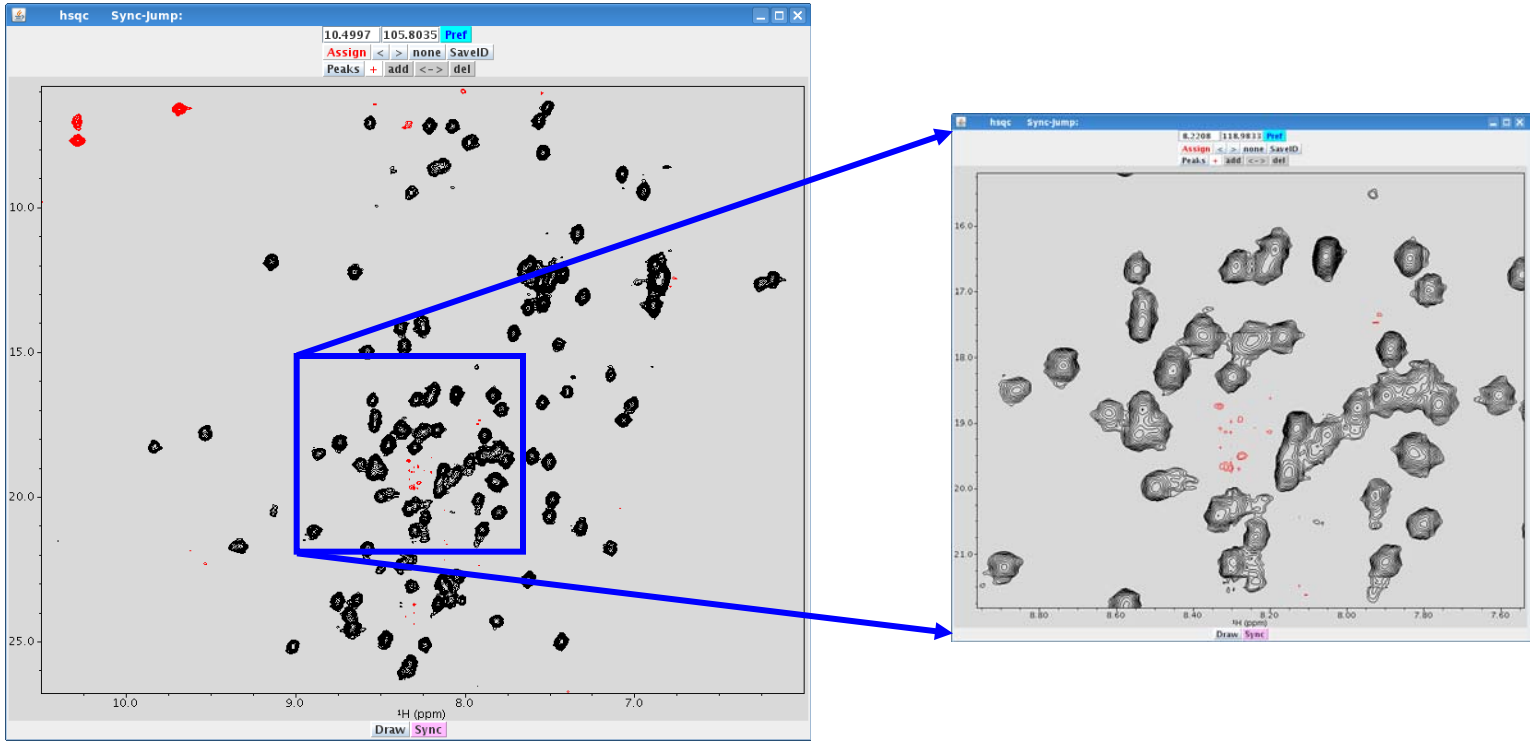
Backbone signal assignments:
Completeness: 99.0% Accuracy: 99.0%
All signal assignments :
Completeness: 94.0% Accuracy: 92.7%

Structure calculation by CYANA

Bacbone atom RMSD between CYANA structure and authentic one: 0.88Å

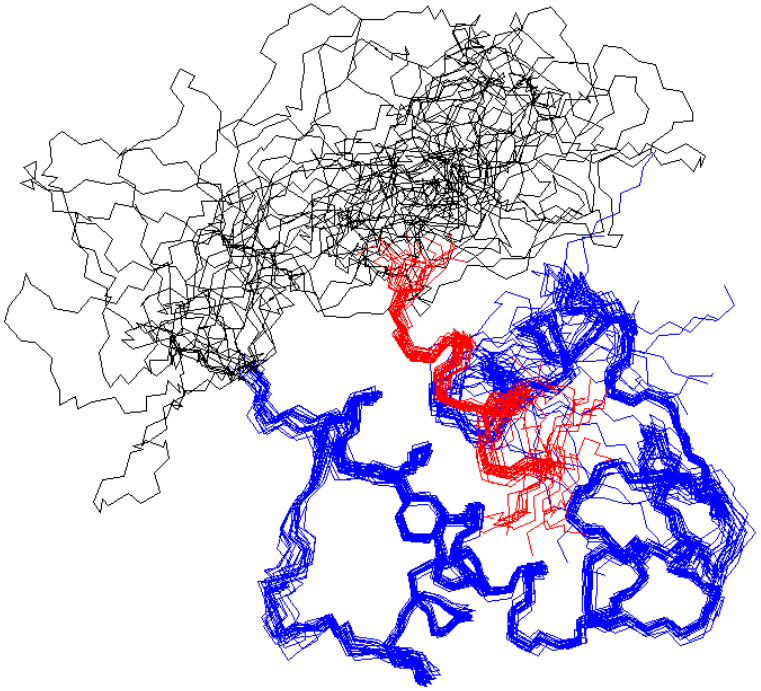
If the spectrum quality is good enough, the signal assignment and structure calculation will be finished in half a day.

NMR analysis Case2: MDM2-peptide fusion protein (131a.a.)

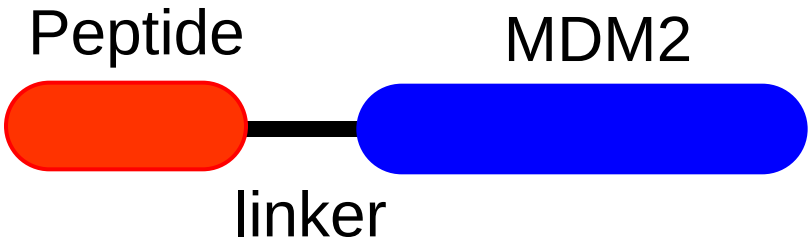


This protein seems to form dimer. Many signals are very broad and overlapping each other. Several signals are missing because of chemical exchange.

Detail of the MDM2-peptide fusion protein



Model protein which mimics a complex of MDM2 protein with a short peptide



The NMR analysis of this fusion protein is considered to be difficult, as very poor sensitivity have been found for the spectra using ^{13}C-spinlock such as CC(CO)NH, HCCH-TOCSY.

Steps for MagRO/FLYA/CYANA analysis

Assigned chemical shifts from **BMRB**
Coordinate file of MDM2 homologue from **PDB**

Backbone signal assignments

Peptide and MDM2 model structure by CS-ROSETTA, Modeller

Complex strucrture model using Giraf and HADDOCK

Similar steps carried out for the case1

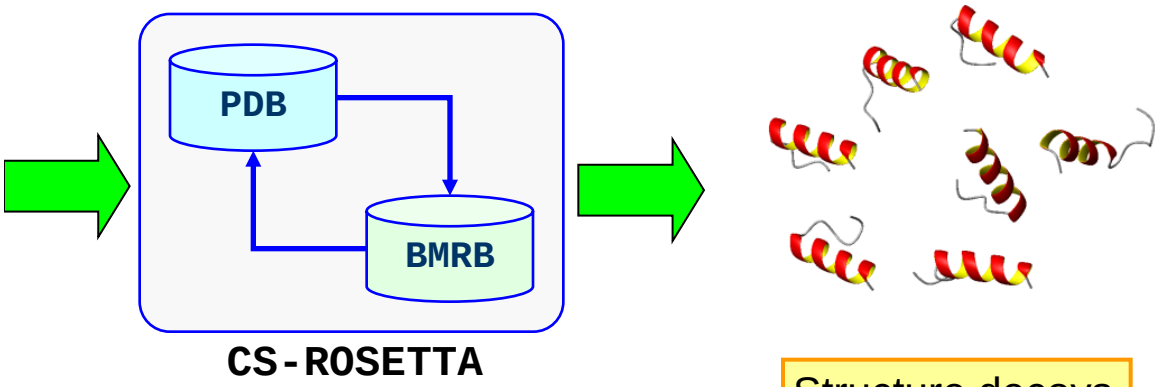
NOE assignment and structure calculation by CYANA

Modeling of helix peptide using CS-ROSETTA

Assigned chemical shifts

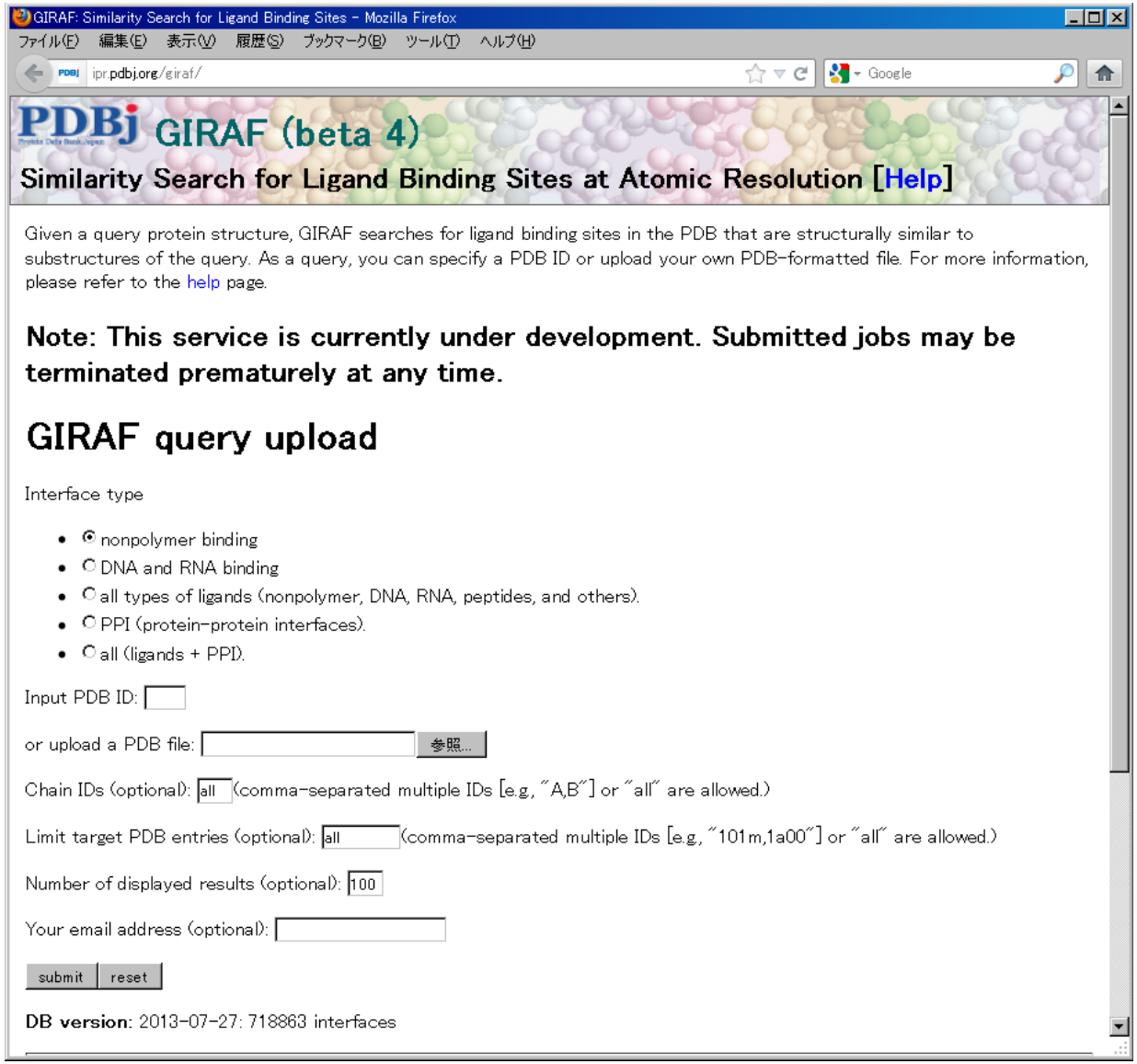
$^{1}H^{N}$, ^{15}N, ^{13}CO, $^{13}C\alpha$ +$^{13}C\beta$

```
 2   Q   CA    55.080
 2   Q   CB    30.760
 2   Q   HN     8.900
 2   Q   C    175.920
 2   Q   HA     5.249
 2   Q   N    123.220
         ....
72   R   HN     8.580
72   R   HA     4.301
72   R   N    123.420
72   R   C    175.350
72   R   CA    55.610
72   R   CB    31.350
```



CS-ROSETTA can generate model helix structure decoys using assigned chemical shifts for backbone NMR signals by searching for structure fragments with similar chemical shifts in database.

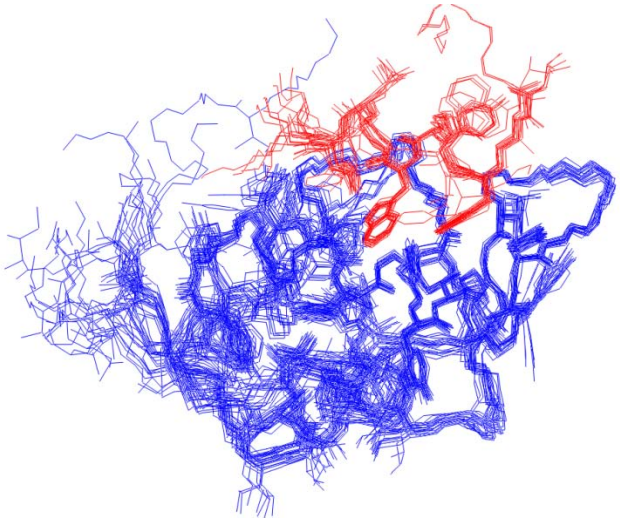
GIRAF: search for interface of peptide/protein complex in database



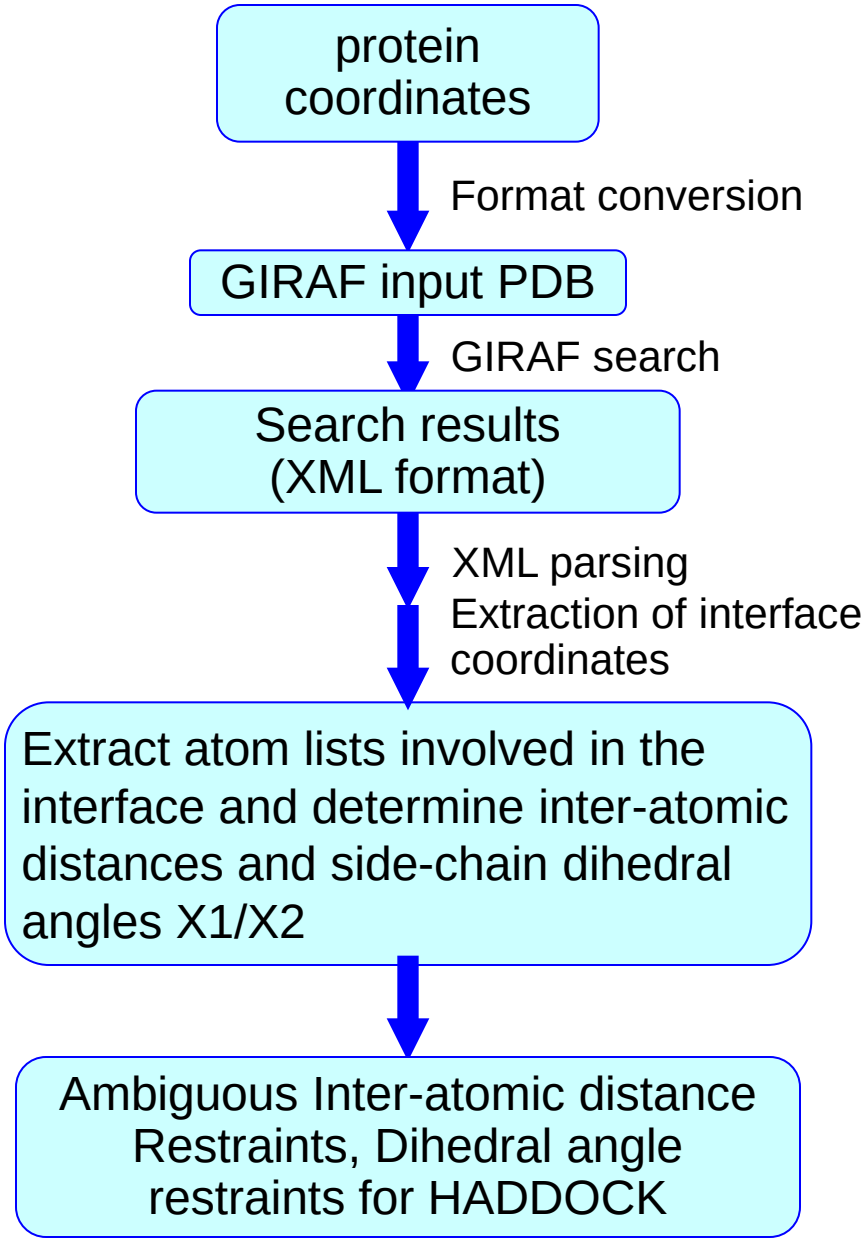
Kinjo, A. et al., Biophysics 2012

Giraf server can search and provide interface of peptide/protein complex

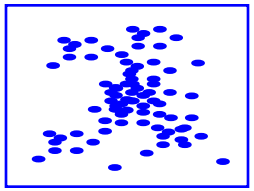
Analysis of results searched by Giraf



Overlay of the 30 structures of peptide/MDM2 complex searched by Giraf



Pipelines to generate model structure of MDM2-peptide complex



BMRB

PDB

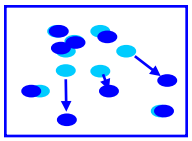
Assigned Chemical shifts (bound state)

Assigned Chemical Shifts (free state)

X-ray structure coordinates

Modeller9.1

CS-ROSETTA



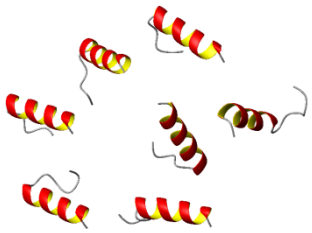
Modeled coordinates

CS-Rosetta models (ligand)

Ambiguous Inter-molecular Restraints (AIR)

GIRAF

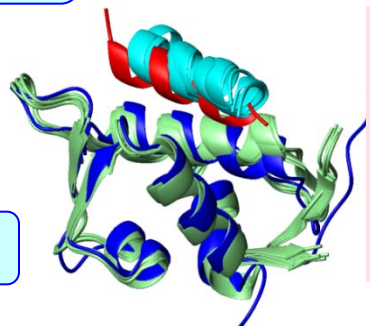
Interface atoms



Ligand (peptide) + receptor coordinates + AIR

HADOCCK

Docked structures

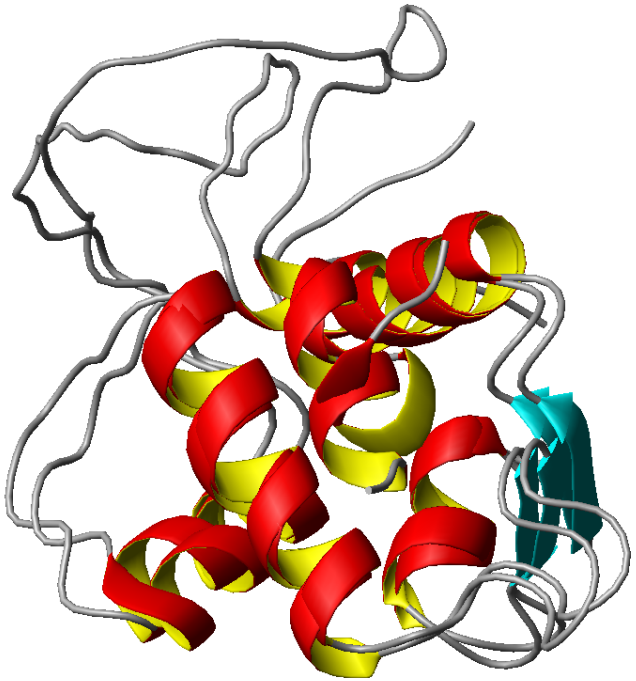


RMSD for backbone atoms of the model structure and authentic struictre:1.47Å

Summary of results: FLYA and CYANA calculations

^{1}H-^{15}N-HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, CBCACONH,^{1}H-^{13}C-HSQC
^{15}N-edited NOESY, ^{13}C-edited NOESY
+ HADDOCK model structure

No TOCSY type spctrum



Automated signal assign by FLYA

Backbone signal assignments:
Completeness: 90.2% Accuracy: 95.0%
All signal assignments :
Completeness: 80.0% Accuracy: 94.2%

Structure calculation by CYANA

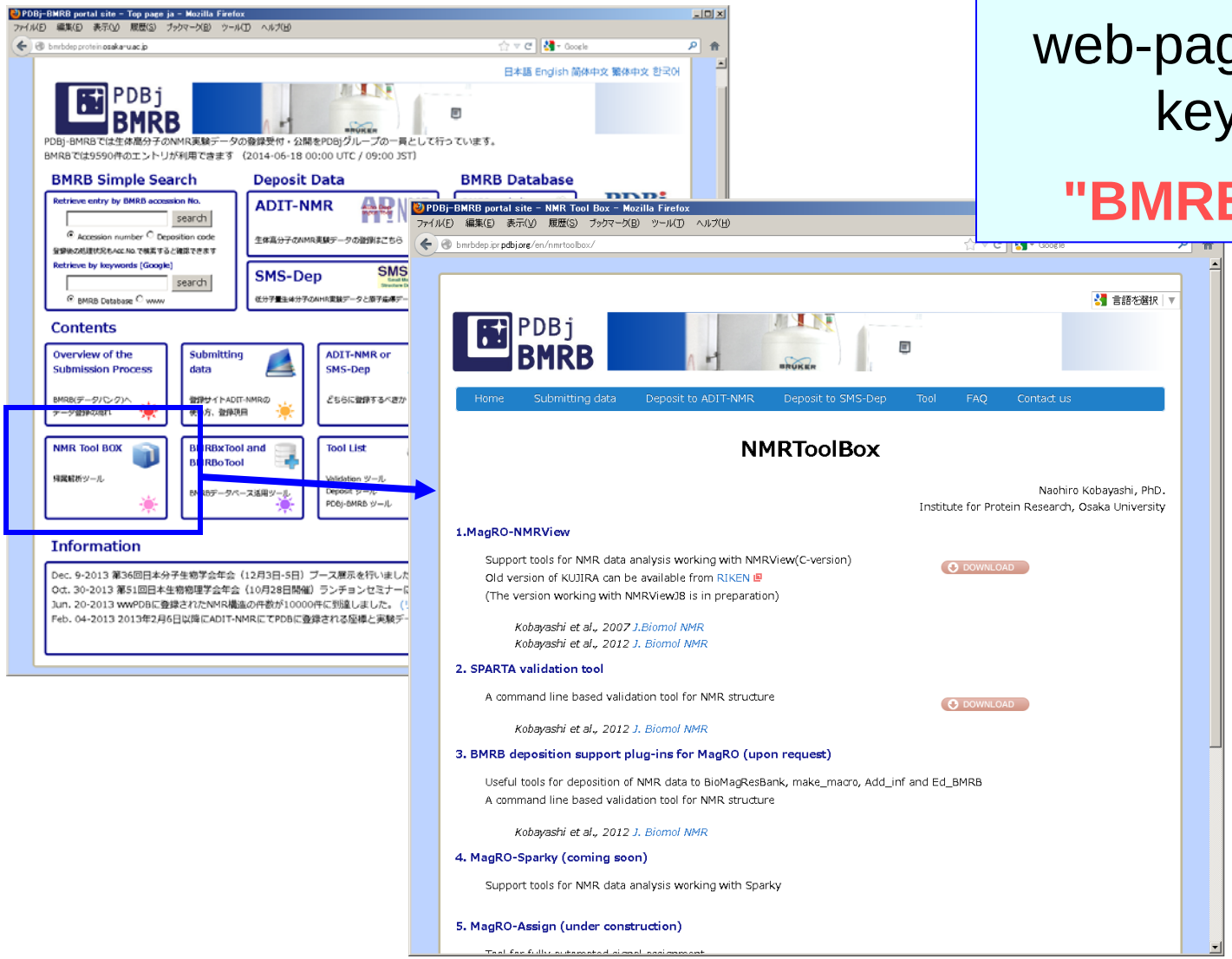
Bacbone atom RMSD between CYANA structure and authentic one: 1.13Å

If a good model structure is available, it is possible to perform highly accurate assignments and structure determination for difficult sample

How to get MagRO-NMRView: access to NMRToolBox

Please search for the web-page with the keyword

"BMRB osaka"



To get other NMR tools

- NMRView: Demo version is available from OneMoon Sceintific
  http://www.onemoonscientific.com/

- CYANA: You can purchase it from LA-Systems
  http://www.las.jp/

- FLYA: Please send a request email to Prof. Guntert when you get the license of CYANA from LA-systems
  http://www.cyana.org/wiki/index.php/Main_Page
  http://www.bpc.uni-frankfurt.de/guentert/wiki/index.php/Main_Page

Please do not hesitate to ask us for introdusing MagRO/FLYA/CYANA system

naohiro@protein.osaka-u.ac.jp

Google search: BMRB osaka