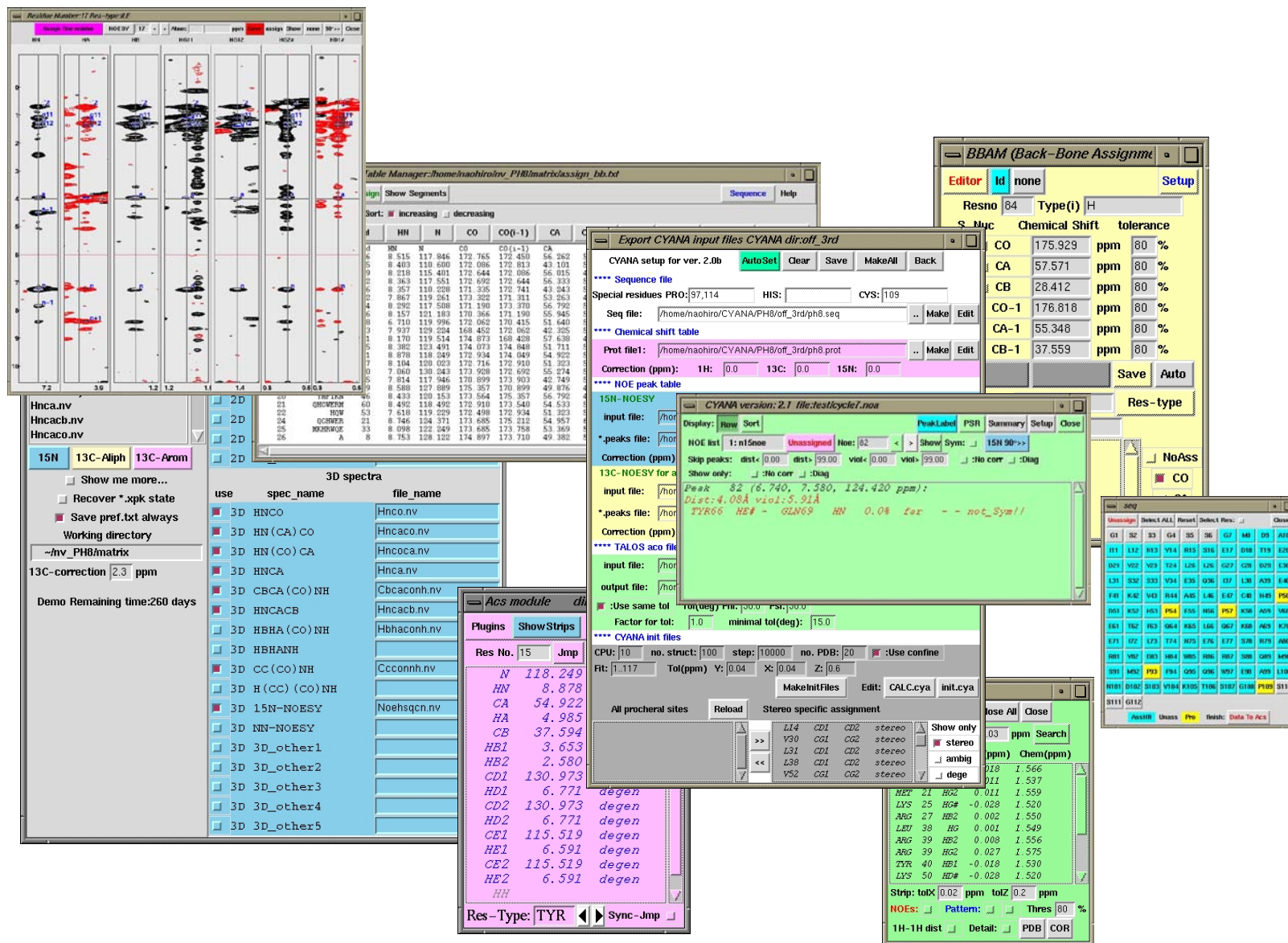


The 52nd Annual Meeting of the Biophysical Society of Japan
Sept. 2014

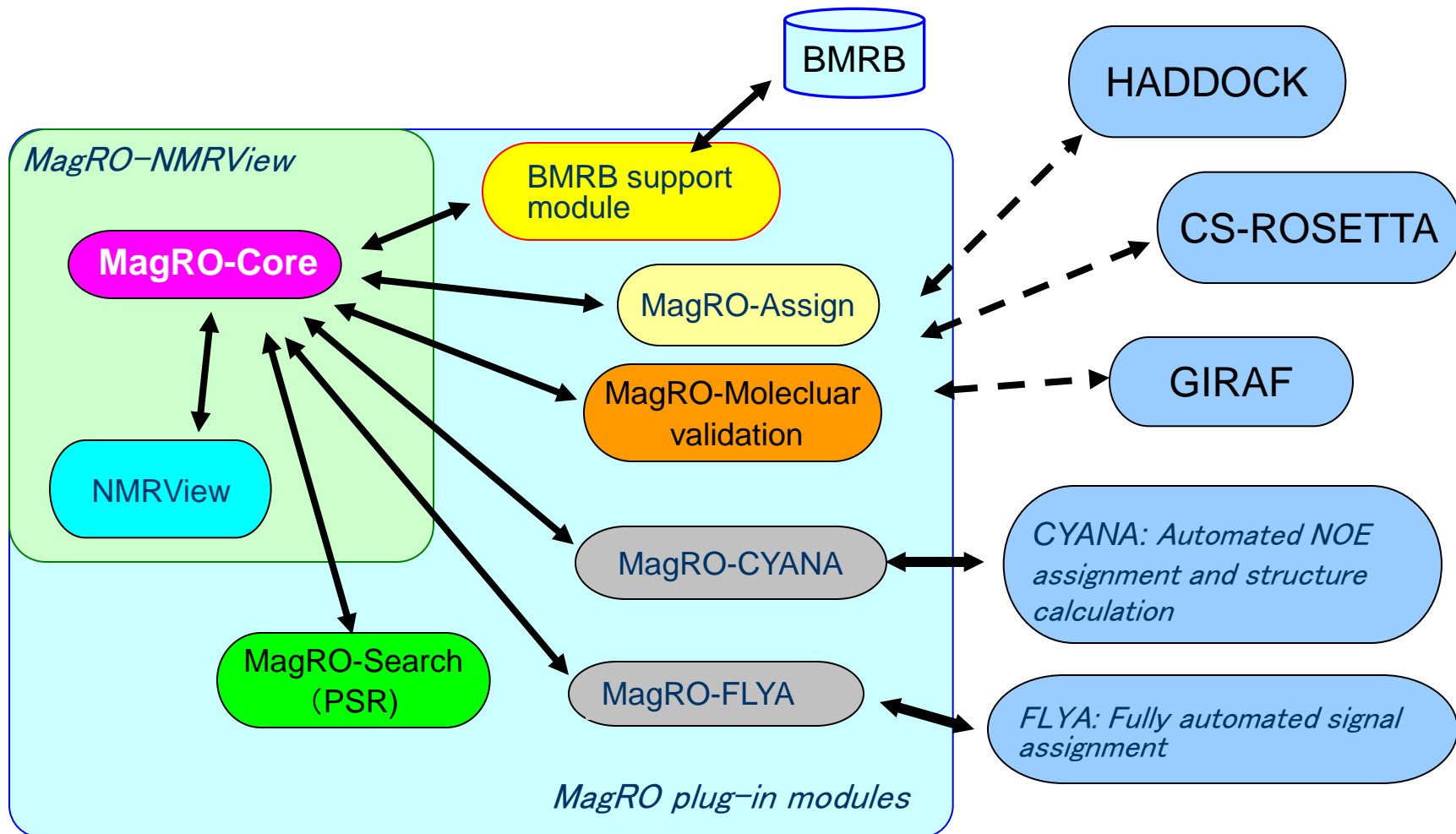
Tools for highly automated NMR analysis and applications using database

Naohiro Kobayashi
Institute for Protein Research

GUI modules implemented in MagRO-NMRView

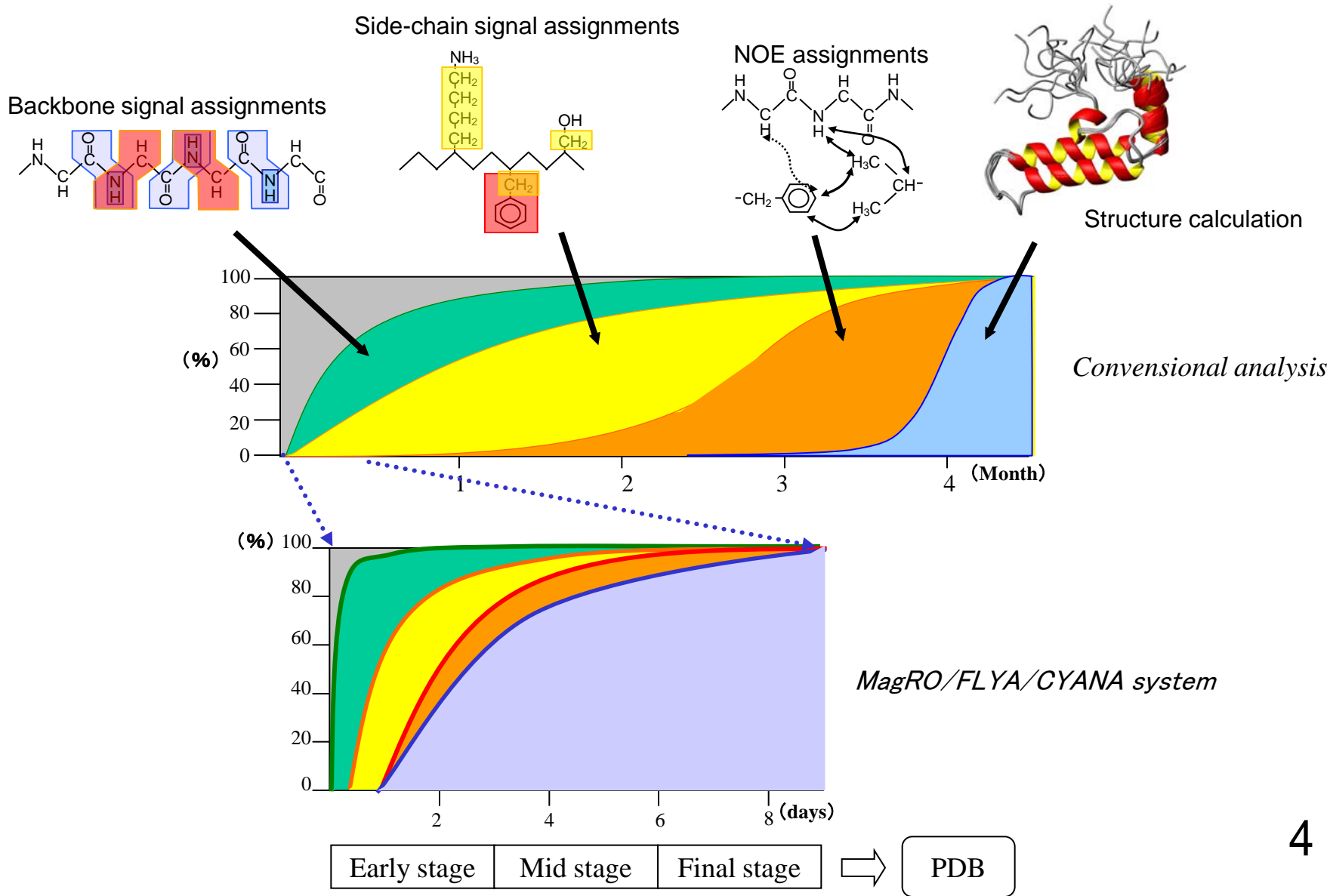


Modules networked in MagRO system



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



GUI module for managing NMR spectra

The image displays three overlapping windows of the MagRO-NMRView software (version ver1.18.14). Each window shows a 'Startup' menu with options: 'setup', 'save', 'start MagRO', and 'exit'. The main interface is divided into 'Working directory' and '2D spectra' sections.

Top Window: Shows a list of files in the working directory and a table of 2D spectra.

use	spec_name	file_name	thres	width	aspect
<input checked="" type="checkbox"/>	2D 15N-HSQC1	nhsqc.nv	0.0315	0.20	10.0
<input type="checkbox"/>	2D 15N-HSQC2				
<input type="checkbox"/>	2D 15N-HSQC3				
<input type="checkbox"/>	2D 15N-HSQC4				
<input type="checkbox"/>	2D 2D-15N-other1				

Middle Window: Shows a different set of 2D spectra.

use	spec_name	file_name	thres	width	aspect
<input checked="" type="checkbox"/>	2D 13C-HSQC1	chsqcct.nv	0.4431	0.20	10.0
<input type="checkbox"/>	2D 13C-HSQC2				
<input type="checkbox"/>	2D 13C-HSQC3				
<input type="checkbox"/>	2D 2D-13Cal-other1				

Bottom Window: Shows a comprehensive view including 2D and 3D spectra.

use	spec_name	file_name	thres	width	aspect
<input checked="" type="checkbox"/>	2D 13C-HSQC1	chsqcct-aro.nv	0.0045	0.20	10.0
<input type="checkbox"/>	2D 13C-HSQC2				
<input type="checkbox"/>	2D 13C-HSQC3				
<input type="checkbox"/>	2D 2D-13Car-other1				
<input type="checkbox"/>	2D 2D-13Car-other2				
<input type="checkbox"/>	2D 2D-13Car-other3				
<input type="checkbox"/>	2D 2D-13Car-other4				

use	spec_name	file_name	thres	width	aspect
<input type="checkbox"/>	3D HCCH-TOCSY				
<input type="checkbox"/>	3D HCCH-COSY				
<input type="checkbox"/>	3D CCH-TOCSY				
<input checked="" type="checkbox"/>	3D 13C-NOESY	cnoesy-aro.nv	0.3024	0.20	10.0
<input type="checkbox"/>	3D CC-NOESY				
<input type="checkbox"/>	3D 2D-13Cal-other1				
<input type="checkbox"/>	3D 2D-13Cal-other2				
<input type="checkbox"/>	3D 2D-13Cal-other3				
<input type="checkbox"/>	3D 2D-13Cal-other4				

Below the 3D spectra table, there is a section for 1H-1H, 15N, 13C-al, and 13C-ar, followed by a '13C-correction' field set to 0.0 ppm.

At the bottom, it states: 'To be expired:653 days remaining'.

Sync-Jump: a function to synchronizedly control NMR spectra

Sync-jump class

^{15}N

^{13}C -ar

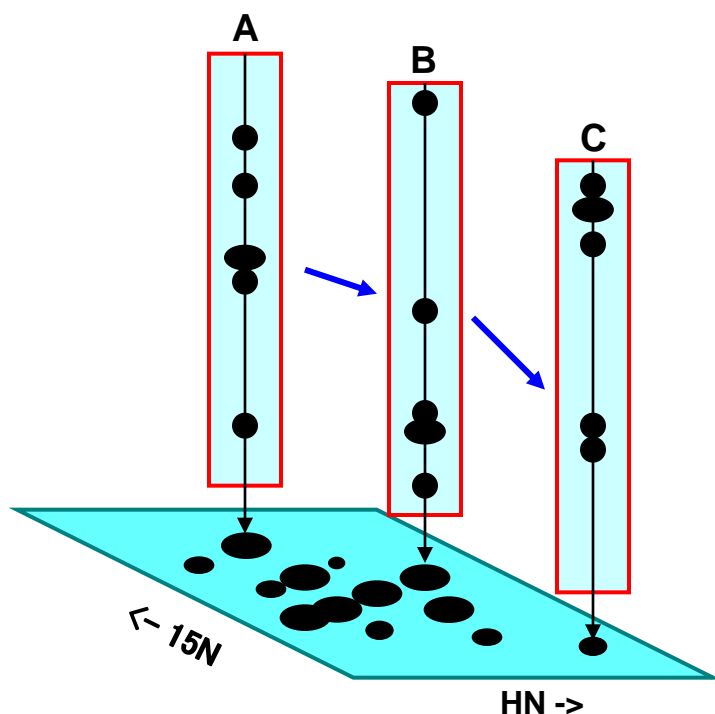
^{13}C -al

Corresponding spectrum types

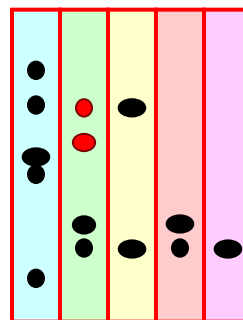
^1H - ^{15}N type

^1H - ^{13}C for aromatic type

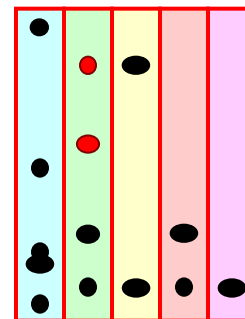
^1H - ^{13}C for all region or aliphatic type



Sync-Jump based on the signal A



Sync-Jump based on the signal B



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

Chemical structure + Sequence

protein_res.lib
protein_atom.lib
protein_atom_amb.lib
protein_atom_topo.lib
protein_0_seq.db

Chemical
structure graph

Assigned chemical shifts
protein_0_0_acs.db

Observable signal info +
Spectrum pulse scheme info

protein_atom_isotope.lib
magn_spin.lib
magn_spec.lib

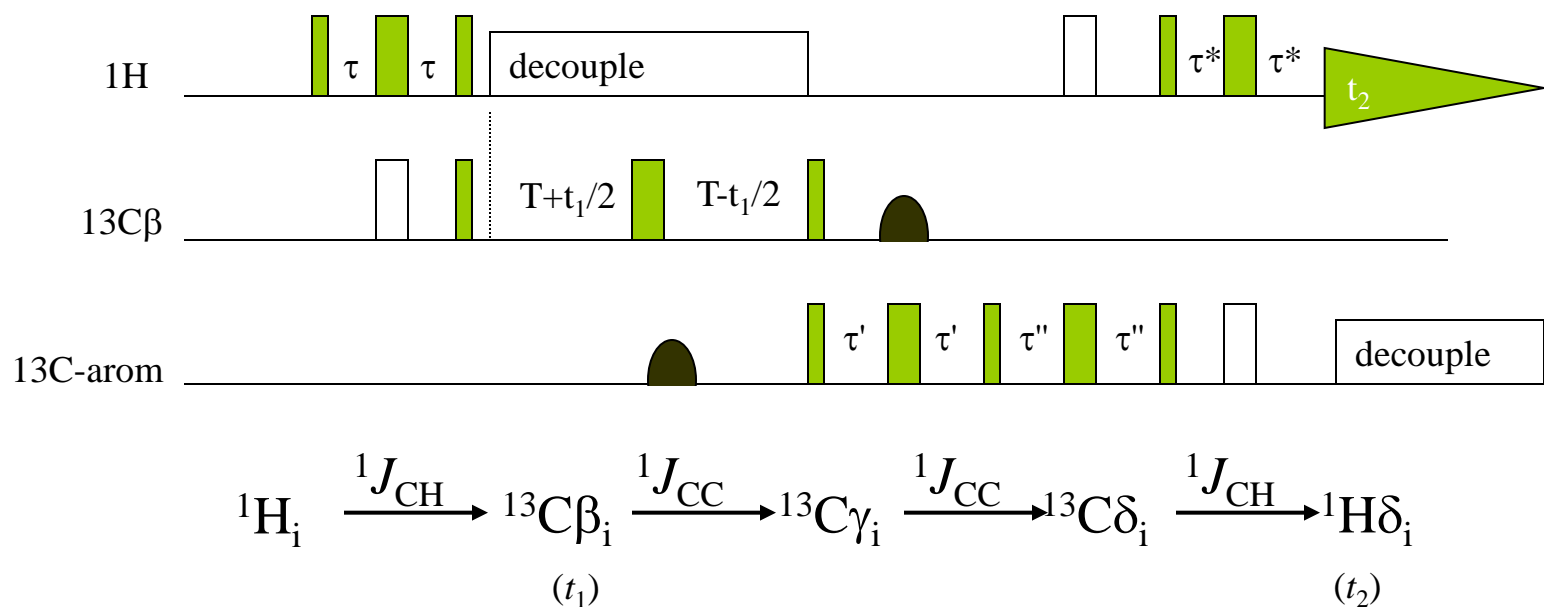
Coherence
transfer graph

Coherence
automata

NMR signal simulation

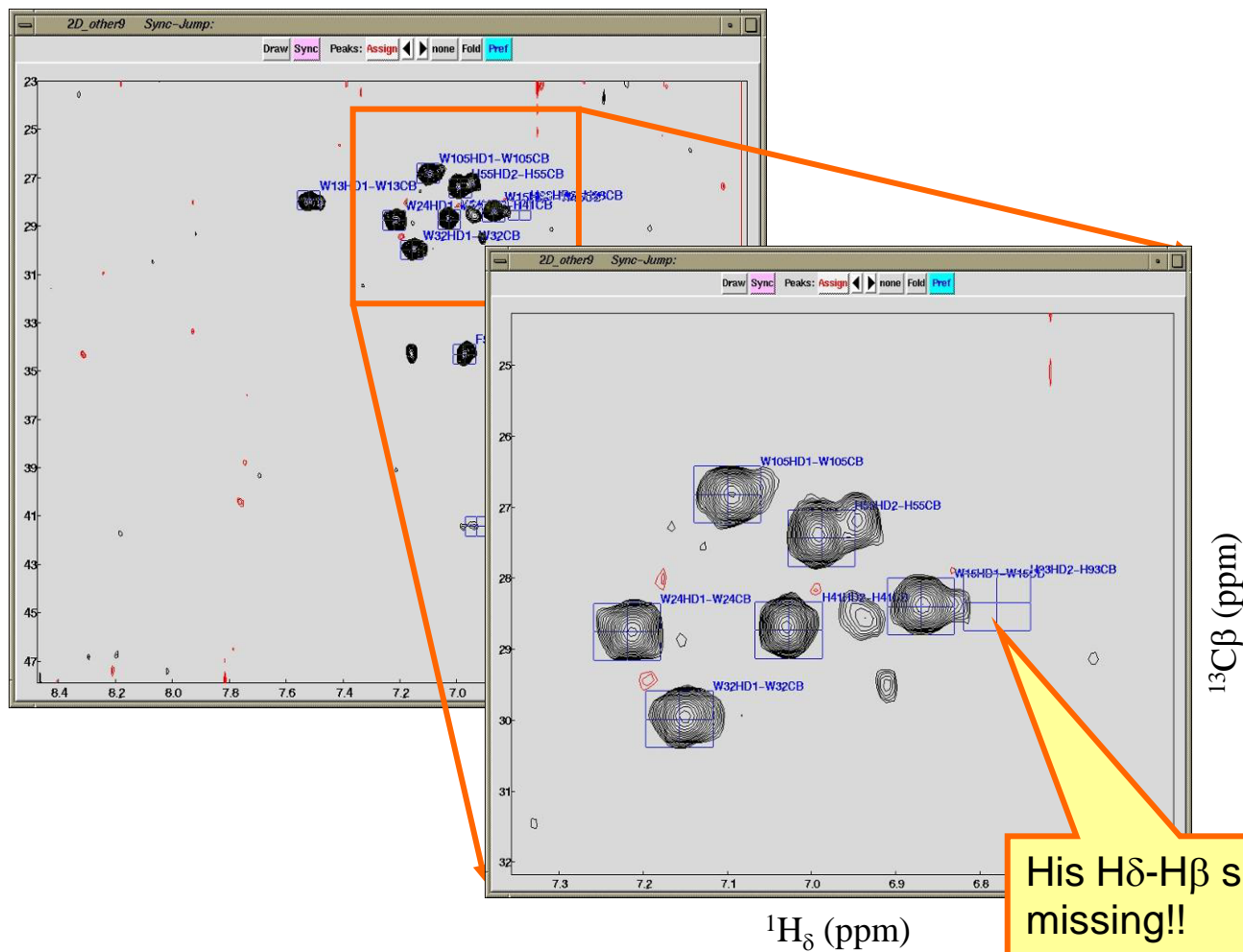
2D – (HB)CB(CGCD)HD spectrum

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

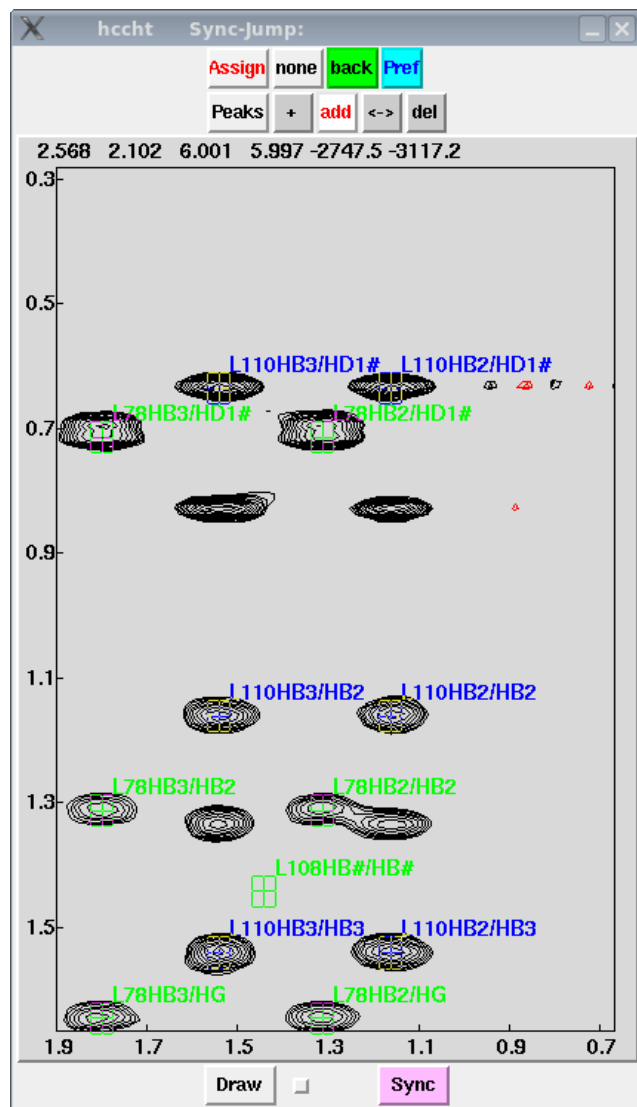


<i>Spectrum_type</i>	<i>step</i>	<i>res1</i>	<i>atom1</i>	<i>res2</i>	<i>atom2</i>	<i>time</i>	<i>seq1</i>	<i>seq2</i>	<i>inten</i>	<i>fold</i>
Spec: hbcbcgcdhd	0	arom	HR	arom	HR	1	0	0	1.0	0
Spec: hbcbcgcdhd	1	arom	HR	arom	CR	0	0	0	1.0	0
Spec: hbcbcgcdhd	2	arom	CR	arom	CR	0	0	0	1.0	0
Spec: hbcbcgcdhd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

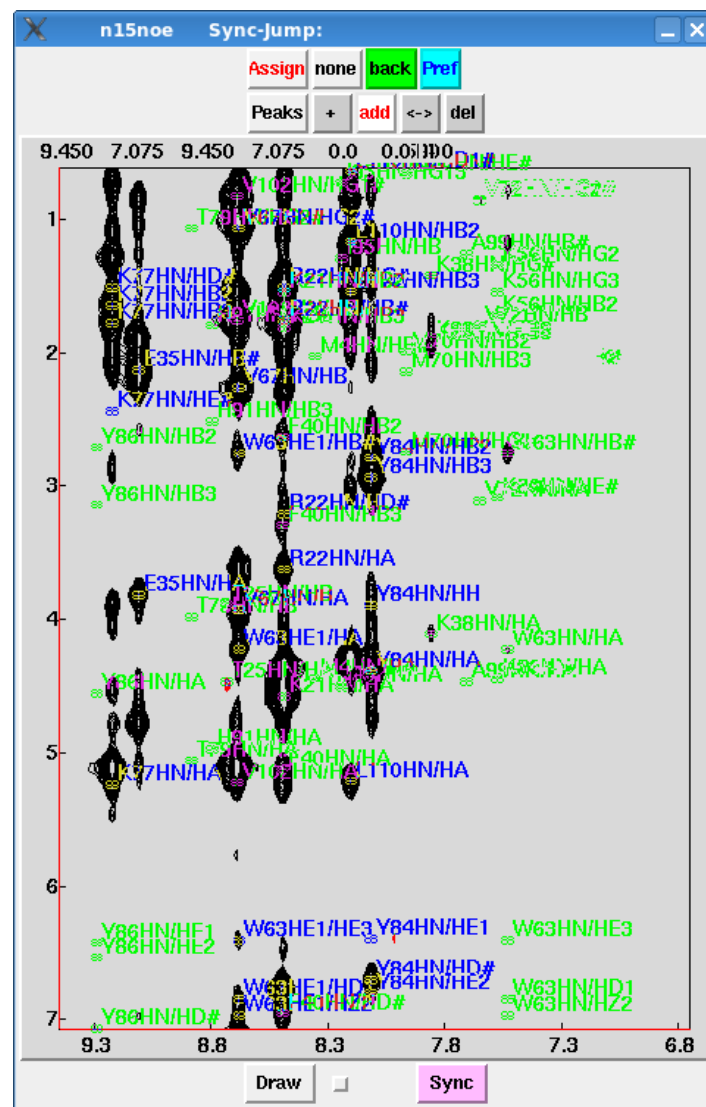
Confirmation of assigned chemical shifts using signal simulation of 2D (HB)CB(CGCD)HD spectrum



Signal simulations of the other spectrum types



3D HCCH-TOCSY



3D ¹⁵N edited NOESY

FLYA GUI module implemented in MagRO-NMRView

MagRO-ACS Path: nv_fkbp

Plugins: Import/Export Tools

Molecule: ID: Export Chemical shift table for BMRB
Quick setup for BMRB deposition

Chain: S Export TALOS+ input file

Residue: Na Export/Import Flya files

Atom: Ty Export CYANA input files
Import CYANA calculation results

* N 121.4
* H 8.1
* CA 60.642
* HA 5.219
* CB 34.409
* HB 1.753
* CG1 20.898 stereo
* HG1# 0.825 degen
* CG2 21.476 stereo
* HG2# 0.896 degen
* C 175.141

FLYA setup module

clear backbone all signals Close

13C-corr: 0.0 ppm CPU: 4

MakeDir Job dir: flya_test ..

Pick All spectra

Noise Filter

If required: Unfold peaks <-setup

Convert xpk-->FLYA

Export Flya files

Import FLYA-->BBass Import FLYA-->ACS

use spectrum type peak files

<input checked="" type="checkbox"/> hsqc	AutoPick
<input checked="" type="checkbox"/> chsqc	AutoPick
<input checked="" type="checkbox"/> chsqc-ar	AutoPick
<input checked="" type="checkbox"/> hnco	AutoPick
<input checked="" type="checkbox"/> hncaco	AutoPick
<input checked="" type="checkbox"/> hncoca	AutoPick
<input checked="" type="checkbox"/> hnca	AutoPick
<input checked="" type="checkbox"/> cbcacoh	AutoPick
<input checked="" type="checkbox"/> hncacb	AutoPick
<input checked="" type="checkbox"/> hbhaconh	AutoPick
<input checked="" type="checkbox"/> n15noe	AutoPick

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

Confirmation and correction of assigned chemical shifts for backbone signals

Error-level	Atom	id(i-1)	id(i)	Res(i-1)	Res(i)	Chem(i)	Chem(i-1)	Diff(ppm)/Comment	Close
Warning	CA	19	41	58	59	---	53.614	incomplete	
Warning	CA	79	13	75	76	54.356	54.053	0.303	
Warning	CB	69	68	71	72	23.736	---	incomplete	
Error	CB	22	36	29	30	68.720	69.789	1.069	
Error	CO	21	31	17	18	171.587	172.574	0.987	

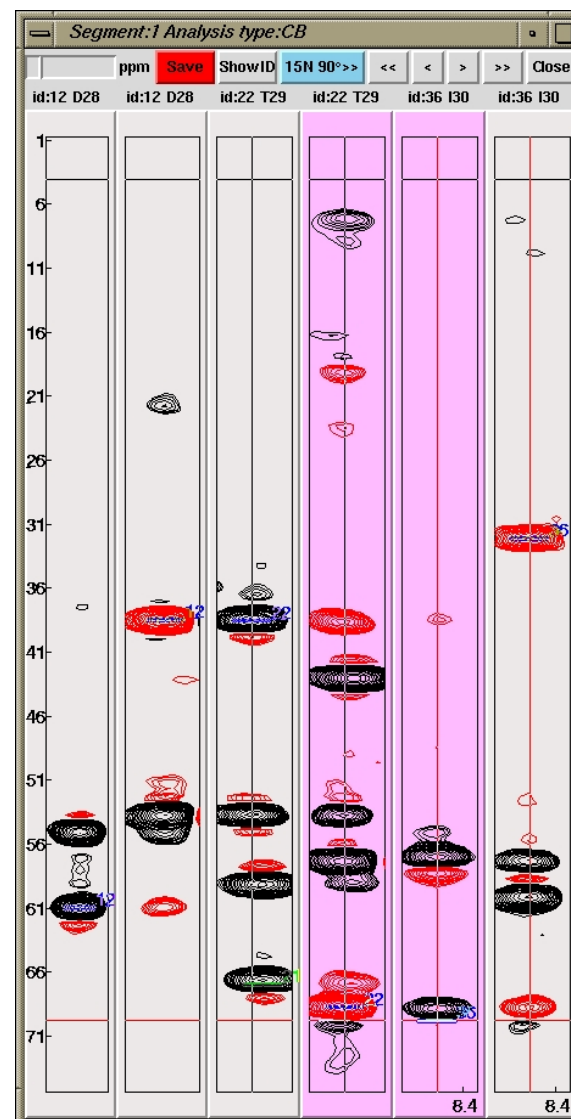
Other errors

Warning residue:14 is not GLU Check sequence and your assignment

Double-click

Highlighted 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

MagRO-ACS Path: nv_fkbp

Plugins: Import/Export Tools

Molecule: ID: 0 Type: protein CS_ID: 0

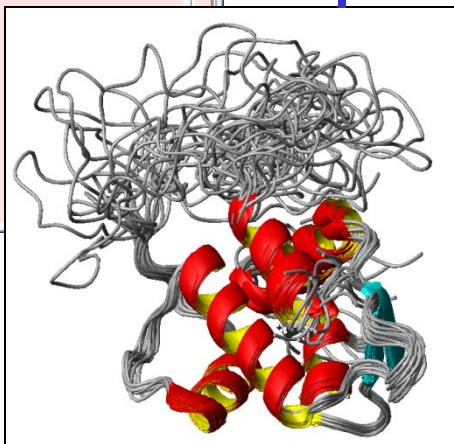
Chain: Sequence 2D-strips

Residue: Name VAL Num 102

Atom: Type HN Sync: Save CS

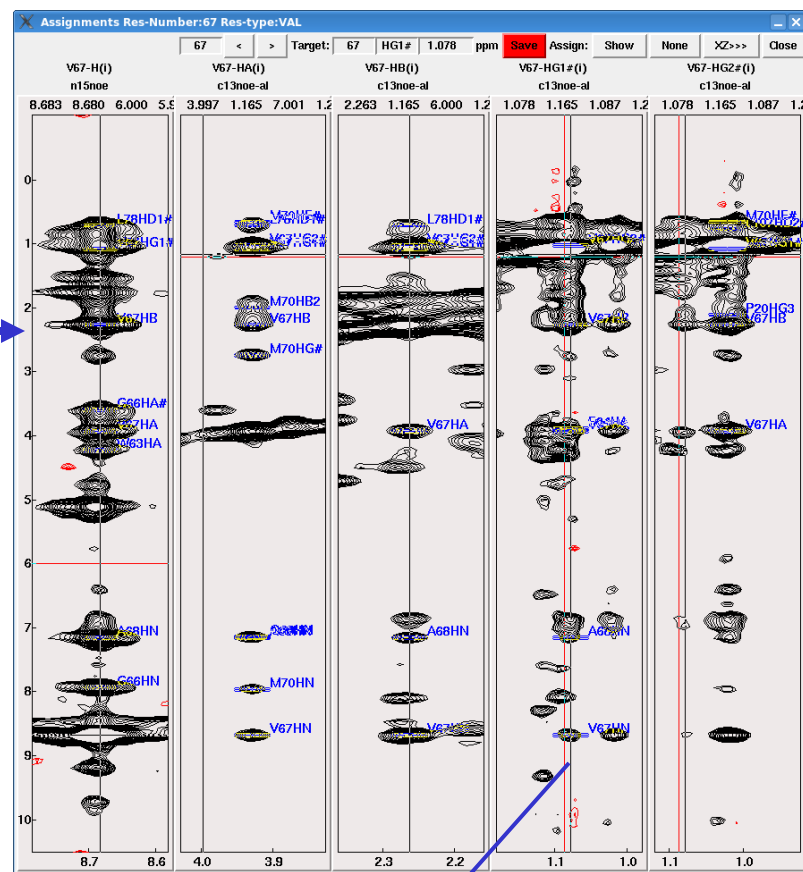
*	N	121.057	
*	H	8.694	
*	CA	60.642	
*	HA	5.219	
*	CB	34.409	
*	HB	1.753	
*	CG1	20.898	stereo
*	HG1#	0.825	degen
*	CG2	21.476	stereo
*	HG2#	0.896	degen
*	C	175.141	

Simulation of
NMR signals



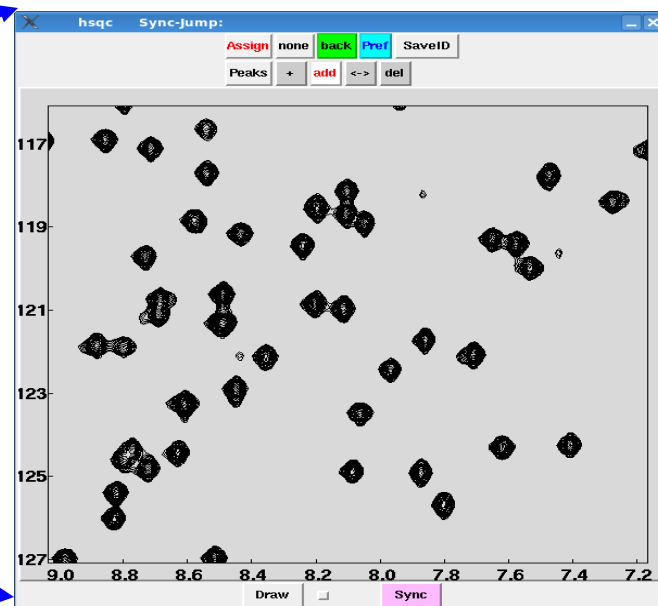
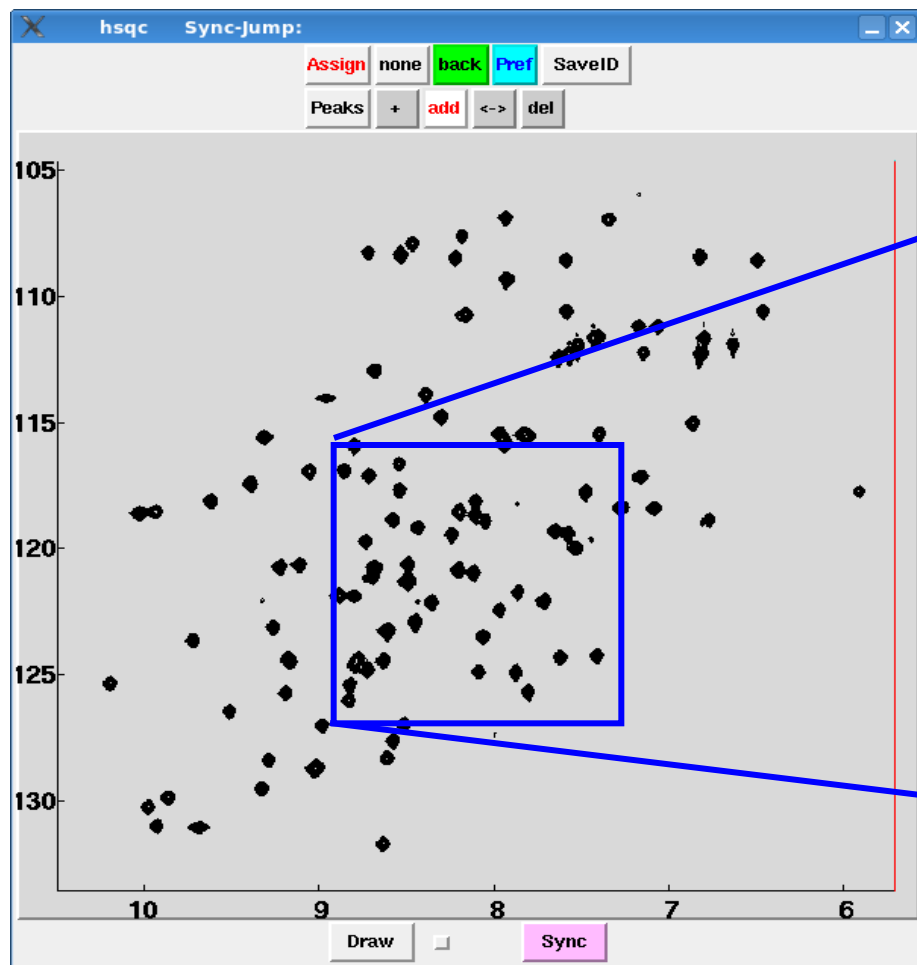
Structure coordinates

^{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 spectra



Noise filtering



Fully automated signal assignment by FLYA



NOE assignment and structure
calculation by CYANA

Summary of results: FLYA and CYANA calculations

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

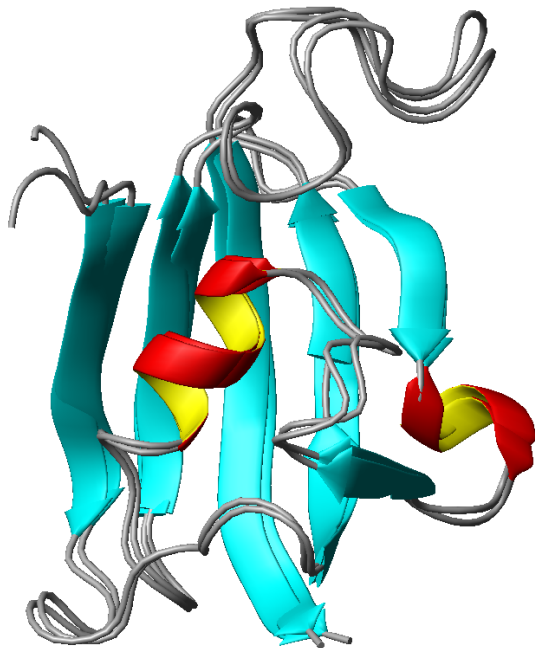
Automated signal assign by FLYA

Backbone signal assignments:

Completeness: 99.0% Accuracy: 99.0%

All signal assignments :

Completeness: 94.0% Accuracy: 92.7%

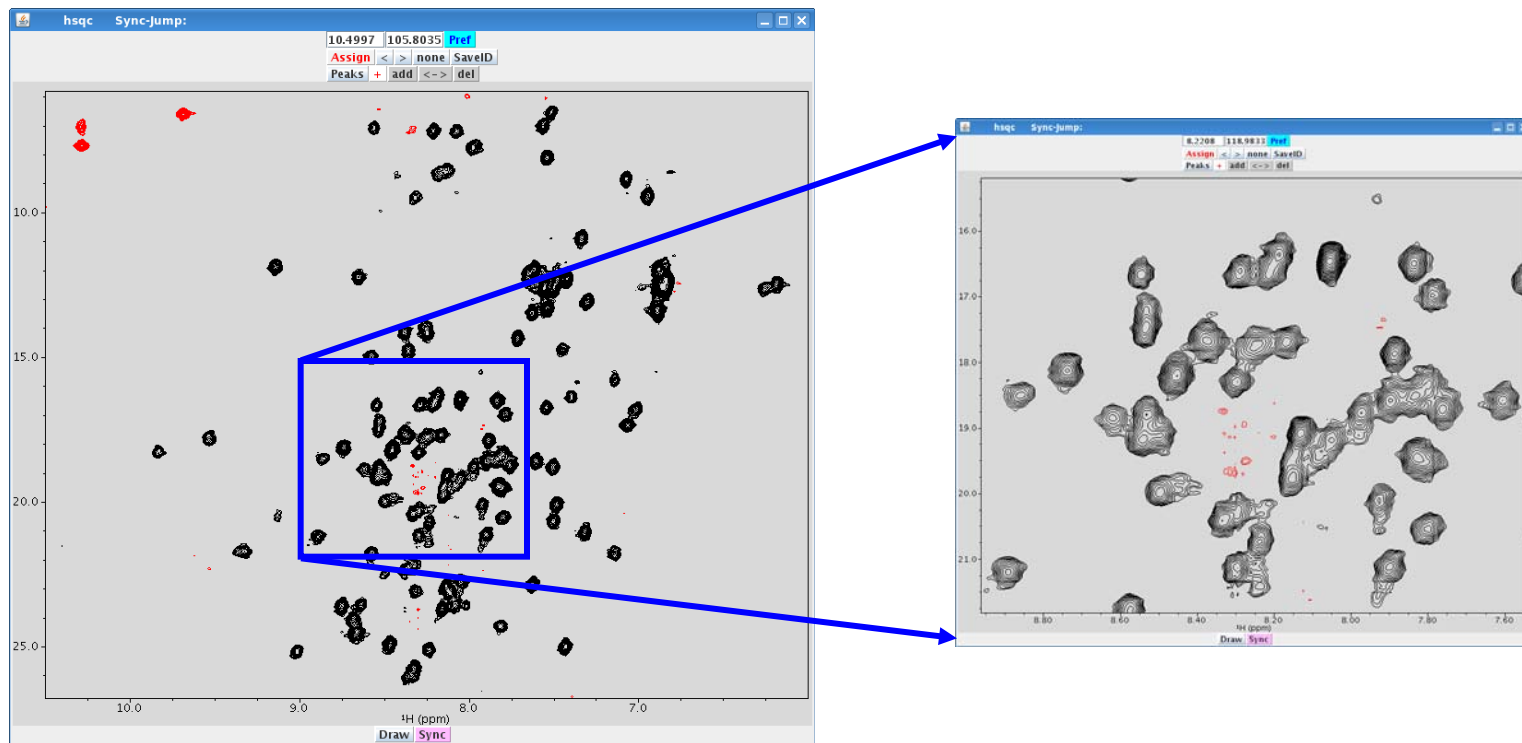


Structure calculation by CYANA

Backbone atom RMSD between CYANA structure and authentic one: **0.88 Å**

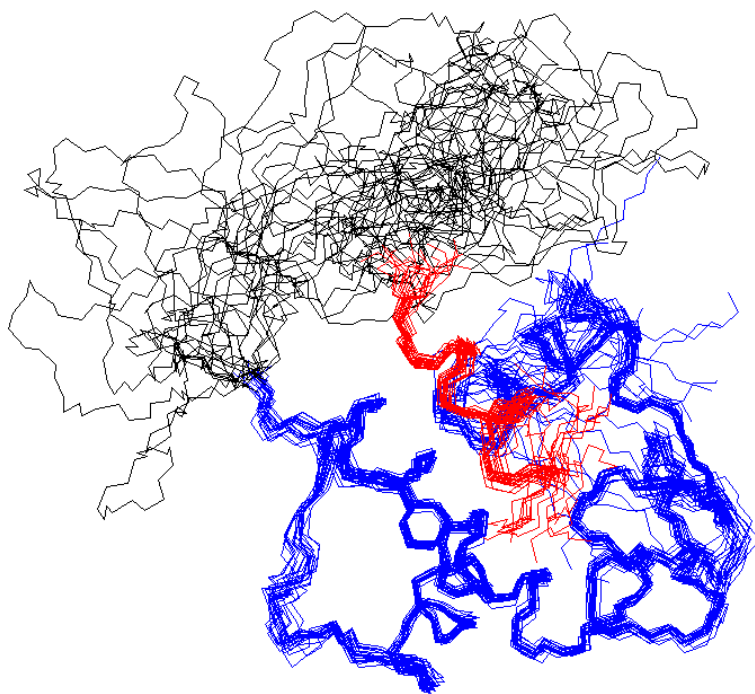
If the spectrum quality is good enough, the signal assignment and structure calculation will be finished in half a day.

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

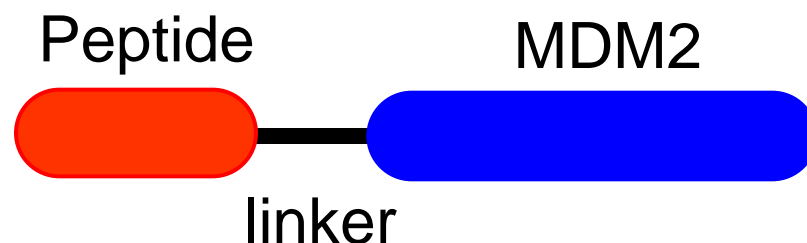


This protein seems to form dimer. Many signals are very broad and overlapping each other. Several signals are missing because of chemical exchange.

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 structure model using Giraf and HADDOCK

Similar steps carried out
for the case1

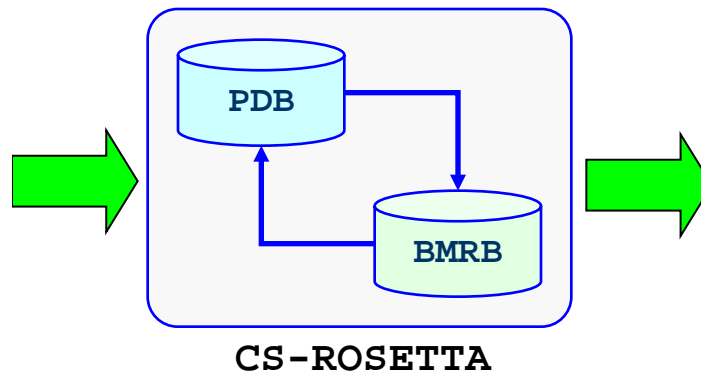
NOE assignment and structure calculation by CYANA

Modeling of helix peptide using CS-ROSETTA

Assigned chemical shifts

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

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



Structure decoys
of peptide

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: Similarity Search for Ligand Binding Sites - Mozilla Firefox

ファイル(E) 編集(E) 表示(V) 履歴(S) ブックマーク(B) ツール(T) ヘルプ(H)

ipr.pdbj.org/giraf/

PDBj GIRAF (beta 4)

Similarity Search for Ligand Binding Sites at Atomic Resolution [Help]

Given a query protein structure, GIRAF searches for ligand binding sites in the PDB that are structurally similar to substructures of the query. As a query, you can specify a PDB ID or upload your own PDB-formatted file. For more information, please refer to the [help](#) page.

Note: This service is currently under development. Submitted jobs may be terminated prematurely at any time.

GIRAF query upload

Interface type

- ☒ nonpolymer binding
- ☐ DNA and RNA binding
- ☐ all types of ligands (nonpolymer, DNA, RNA, peptides, and others).
- ☐ PPI (protein-protein interfaces).
- ☐ all (ligands + PPI).

Input PDB ID:

or upload a PDB file: 参照...

Chain IDs (optional): (comma-separated multiple IDs [e.g., "A,B"] or "all" are allowed.)

Limit target PDB entries (optional): (comma-separated multiple IDs [e.g., "101m,1a00"] or "all" are allowed.)

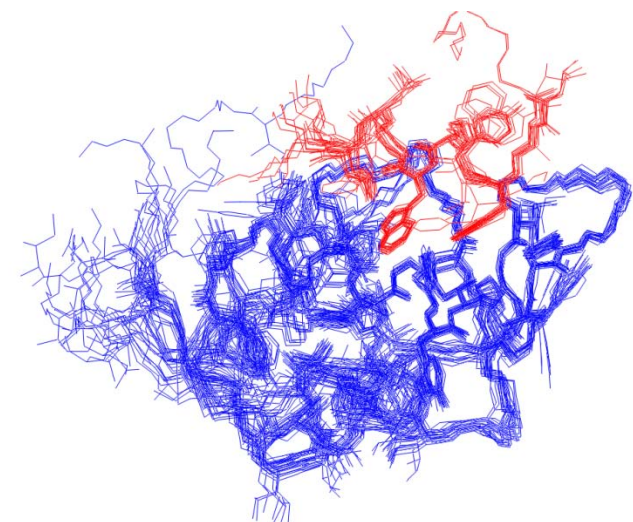
Number of displayed results (optional): 100

Your email address (optional):

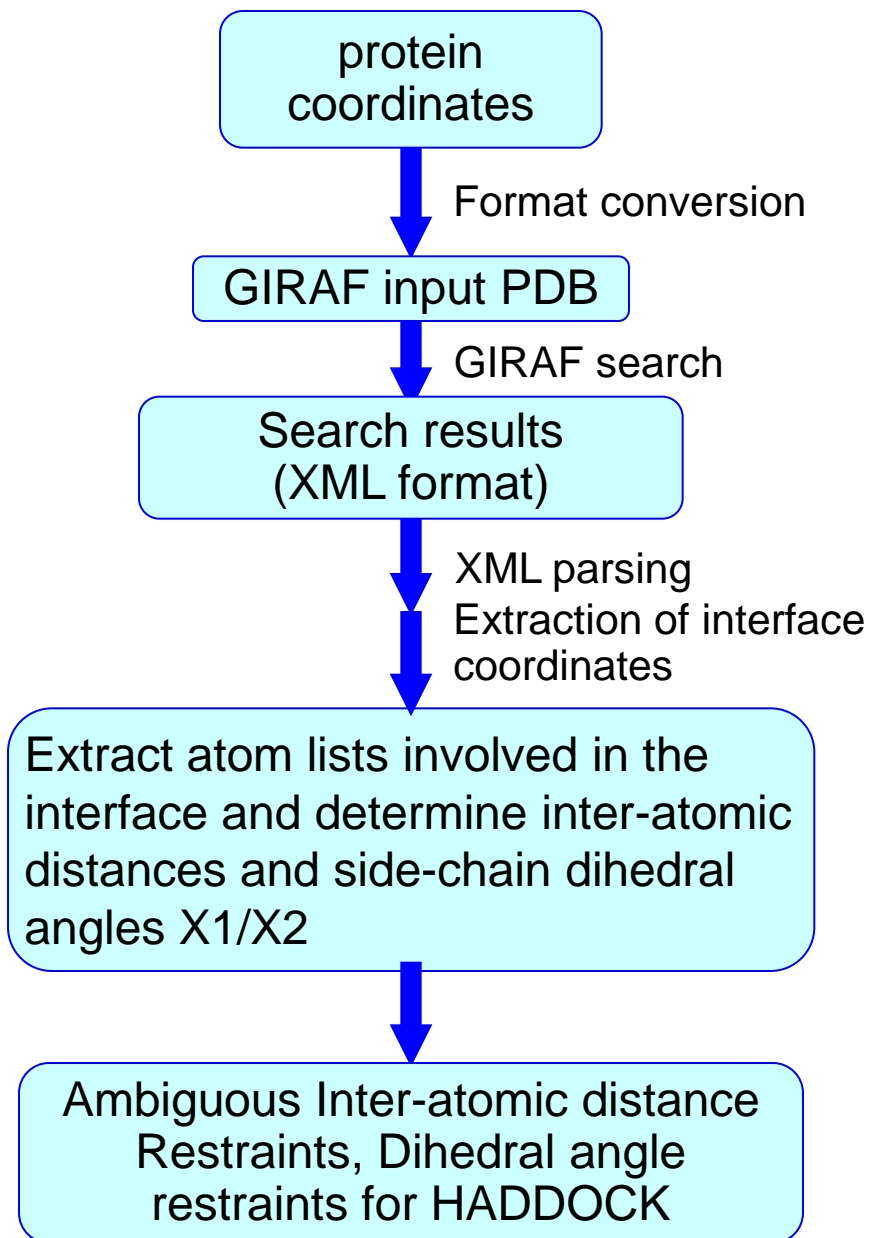
DB version: 2013-07-27: 718863 interfaces

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

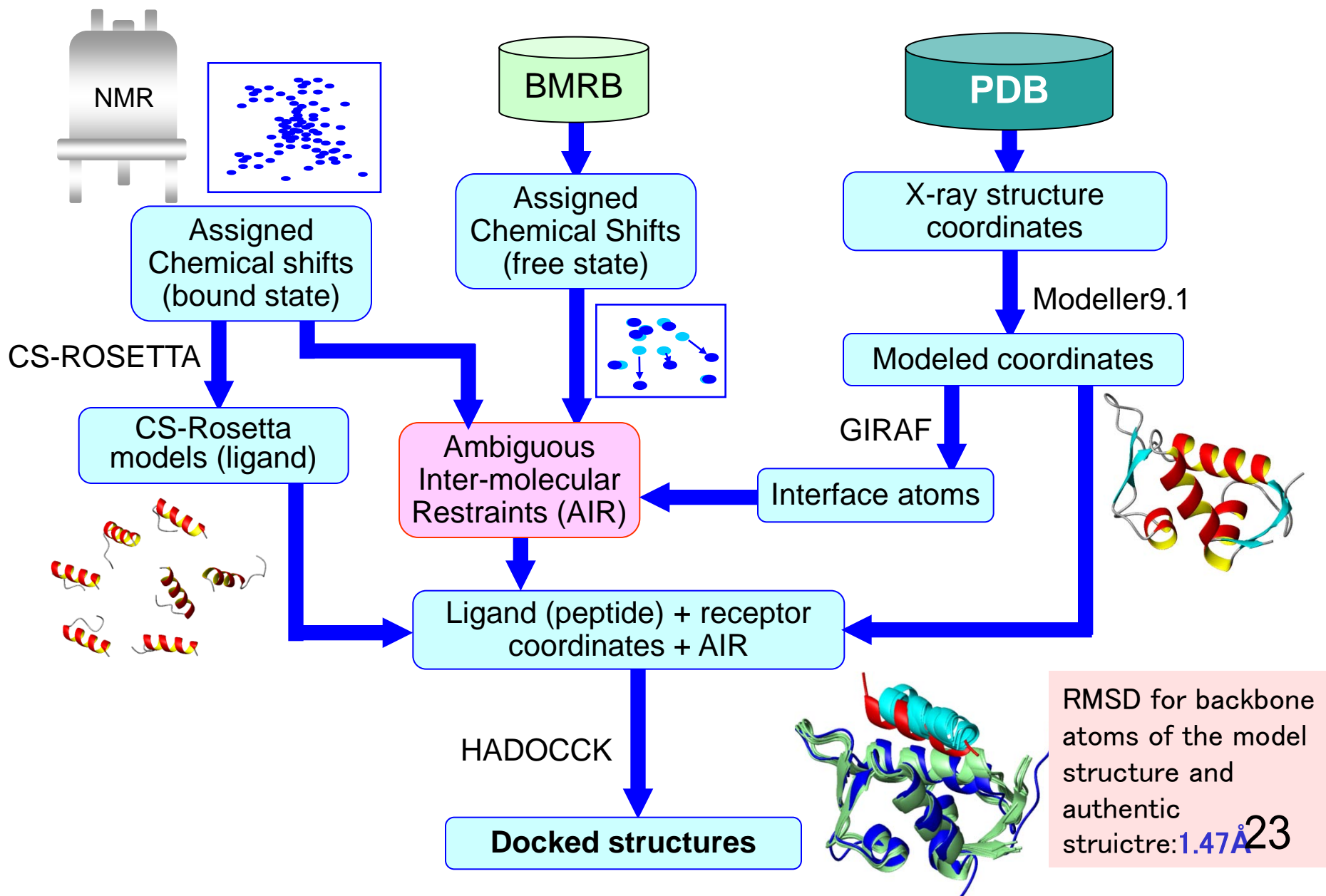
Analysis of results searched by Giraf



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



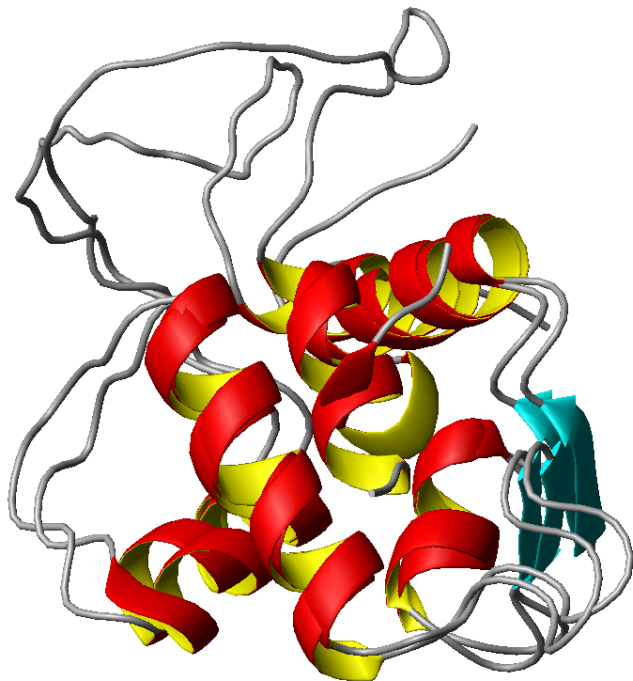
Pipelines to generate model structure of MDM2-peptide complex



Summary of results: FLYA and CYANA calculations

^1H - ^{15}N -HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA,
HNCACB, CBCACONH, ^1H - ^{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"

The screenshot shows the PDBj-BMRB portal site. In the 'Contents' section, the 'NMR Tool BOX' link is highlighted with a blue box. An arrow points from this link to the 'NMRToolBox' page. The 'NMRToolBox' page lists the following tools:

- 1. MagRO-NMRView**
Support tools for NMR data analysis working with NMRView(C-version)
Old version of KUIRA can be available from [RIKEN](#)
(The version working with NMRViewJ8 is in preparation)
Kobayashi et al., 2007 J. Biomol NMR
Kobayashi et al., 2012 J. Biomol NMR
- 2. SPARTA validation tool**
A command line based validation tool for NMR structure
Kobayashi et al., 2012 J. Biomol NMR
- 3. BMRB deposition support plug-ins for MagRO (upon request)**
Useful tools for deposition of NMR data to BioMagResBank, make_macro, Add_inf and Ed_BMRB
A command line based validation tool for NMR structure
Kobayashi et al., 2012 J. Biomol NMR
- 4. MagRO-Sparky (coming soon)**
Support tools for NMR data analysis working with Sparky
- 5. MagRO-Assign (under construction)**

To get other NMR tools

- **NMRView:** Demo version is available from OneMoon Scientific
<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 introducing
MagRO/FLYA/CYANA system

naohiro@protein.osaka-u.ac.jp

Google search: BMRB osaka