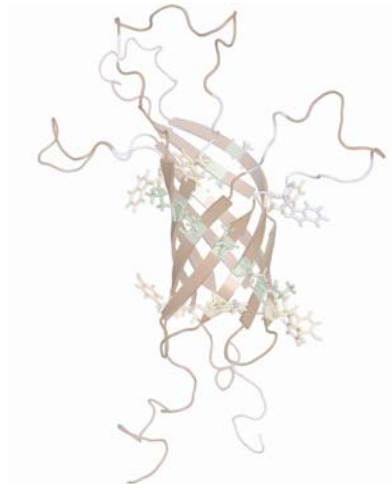


Advanced Practical Course in NMR Techniques for Membrane

Rutherford Appleton Laboratory, Oxfordshire, UK : 11th - 14th May, 2009

Structure determination of porins by solution state NMR: practical aspects

O. Saurel



Outlines

HR NMR of membrane proteins - strategy

*Relaxation – deuteration
Pulse program considerations*

Backbone assignment

Demonstration : nmripe and nmview

Backbone dihedral angle prediction

Demonstration : talos

Methyl-based NOes

Additional restraints

Overview of RDCs and RPE experiments

Structure calculation using CNS

Overview

**Pulse
programs**

Assignment

Talos

**Backbone
NOes**

Me-NOes

**Structure
calculation**



Pulse programs

Assignment

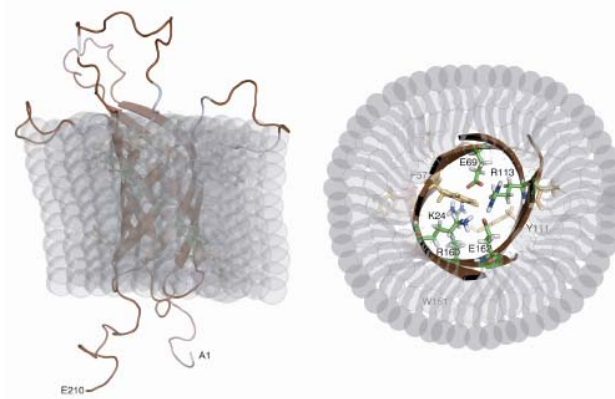
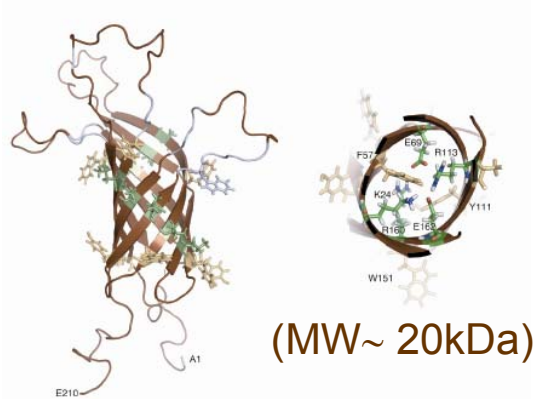
Talos

Backbone NOEs

Me_NOEs

Structure calculation

NMR spectroscopy beyond 25 kDa



Large correlation time
(high molecular weight
complex $\geq 50\text{kDa}$)

Slow molecular tumbling $\gg \gg$ Fast T_2 nuclear spin relaxation

- Dipole-dipole interactions (homo and heteronuclear)
- CSA interactions

Length of primary
sequence



Signal overlap



Selective isotopic labeling

Broad lines

Decrease of efficiency of 3D
NMR experiments used for
resonance assignment

Low spectral resolution, low sensitivity



TROSY and perdeuteration

NMR spectroscopy beyond 25 KDa

In 3D exp. For macromolecules R_2 relaxation resulted from :

^1H - ^1H dipolar interaction (DD)

^1H - ^{15}N and ^1H - ^{13}C dipolar interactions (DD)

^1H , ^{15}N and ^{13}C chemical shift anisotropy (CSA)

TROSY detection scheme Transverse Relaxation-Optimised Spectroscopy

For a 2 spin sys. I and S (spin $1/2$), considering the two R_2 nucleus S transitions :

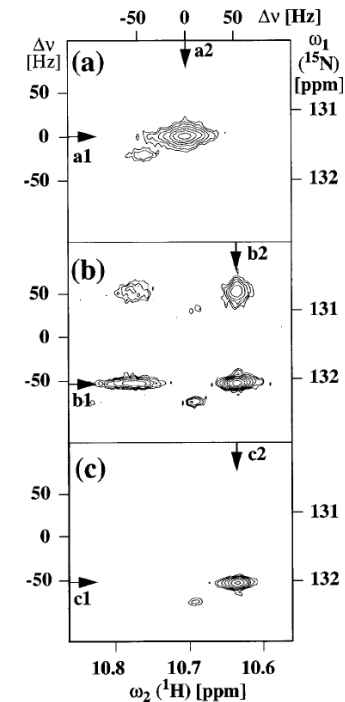
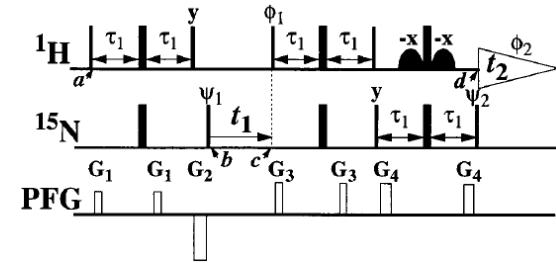
$$R_{1212} = (p - \delta_S)^2 (4J(0) + 3J(\omega_S)) + p^2 (J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)) + 3\delta_I^2 J(\omega_I), \quad [2]$$

$$R_{3434} = (p + \delta_S)^2 (4J(0) + 3J(\omega_S)) + p^2 (J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)) + 3\delta_I^2 J(\omega_I), \quad [3]$$

with $p = \frac{1}{2\sqrt{2}} \gamma_I \gamma_S \hbar / r_{IS}^3$, $\delta_S = \frac{1}{3\sqrt{2}} \gamma_S B_0 \Delta\sigma_S$

HN DD interac.

^{15}N CSA interac.



Compensation of HN DD and ^{15}N CSA interactions
($p - \delta \approx 0 \gg \gg$ optimum 1.1 GHz)

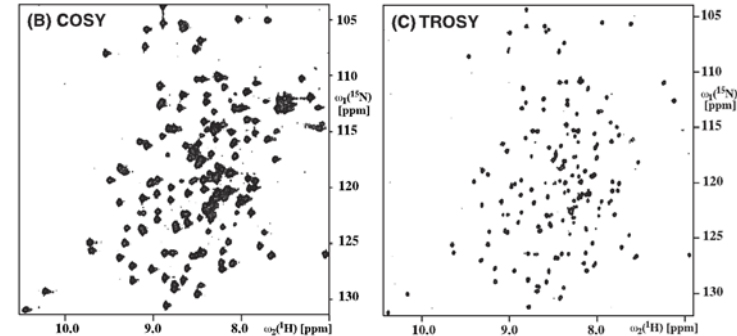
NMR spectroscopy beyond 25 KDa

TROSY detection scheme

Linewidth reduction (at least 40-60%)

Compensation almost independent of molecular size

Main residual T_2 relaxation comes from dipolar coupling with remote protons (side-chain protons) (75% ^{15}N and 95% for HN)



From Fernandez C. et al *FEBS letters* (2001) 173-78

Perdeuteration

$$p = \frac{1}{2\sqrt{2}} \gamma_I \gamma_S \hbar / r_{IS}^3, \quad \delta_S = \frac{1}{3\sqrt{2}} \gamma_S B_0 \Delta \sigma_S$$

^1H - ^1H dipolar interaction (DD)

^1H - ^{15}N and ^1H - ^{13}C dipolar interactions (DD)

^1H , ^{15}N and ^{13}C chemical shift anisotropy (CSA)

>>> improve sensitivity of 3D experiments (HNCA, HN(CA)CO, HNCACB etc..)

Magnetic field strength

^1H - ^1H dipolar interaction (DD)

^1H - ^{15}N and ^1H - ^{13}C dipolar interactions (DD)

^1H , ^{15}N and ^{13}C chemical shift anisotropy (CSA)

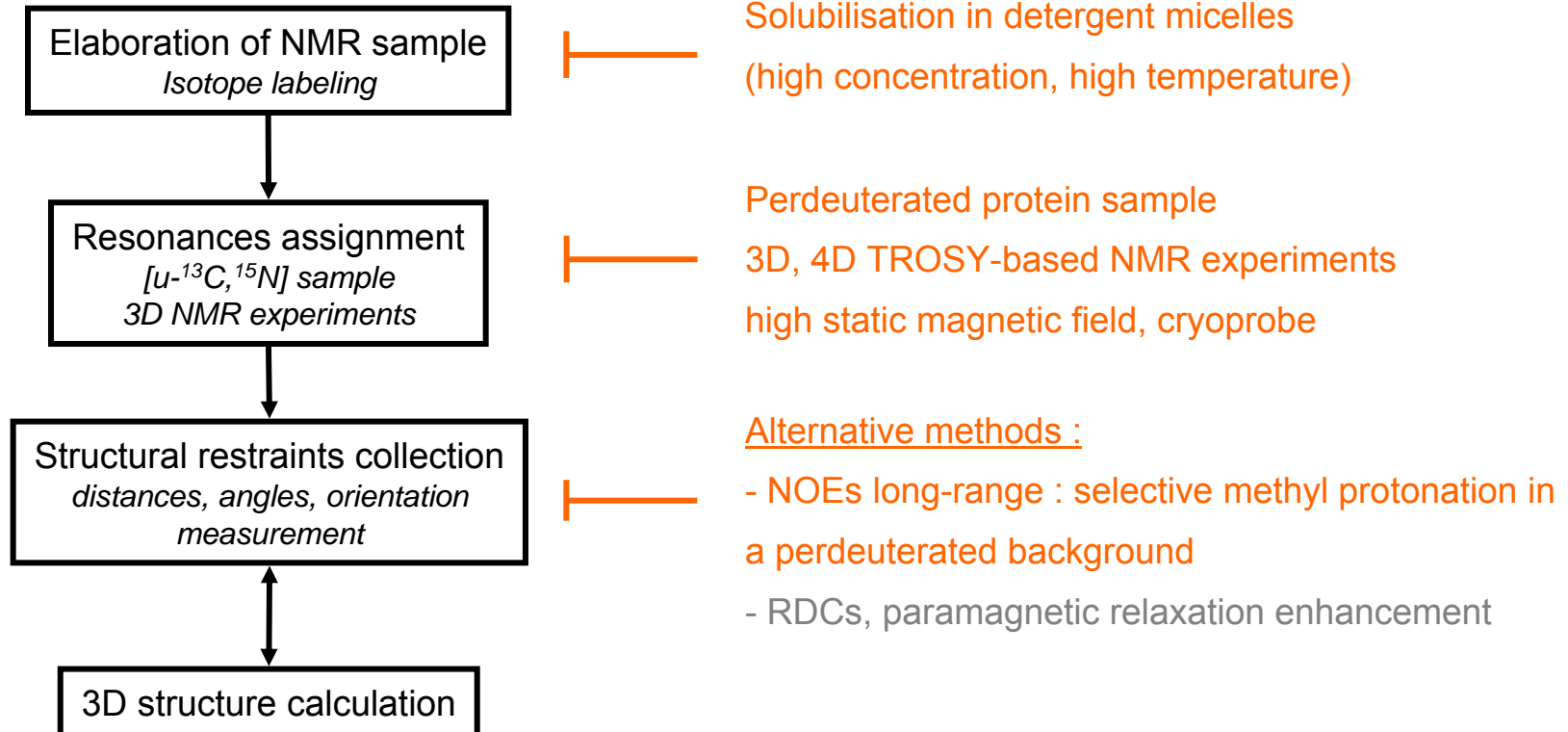
>>> higher for TROSY

>>> 600MHz for experiments with coherence pathway through the carbonyl

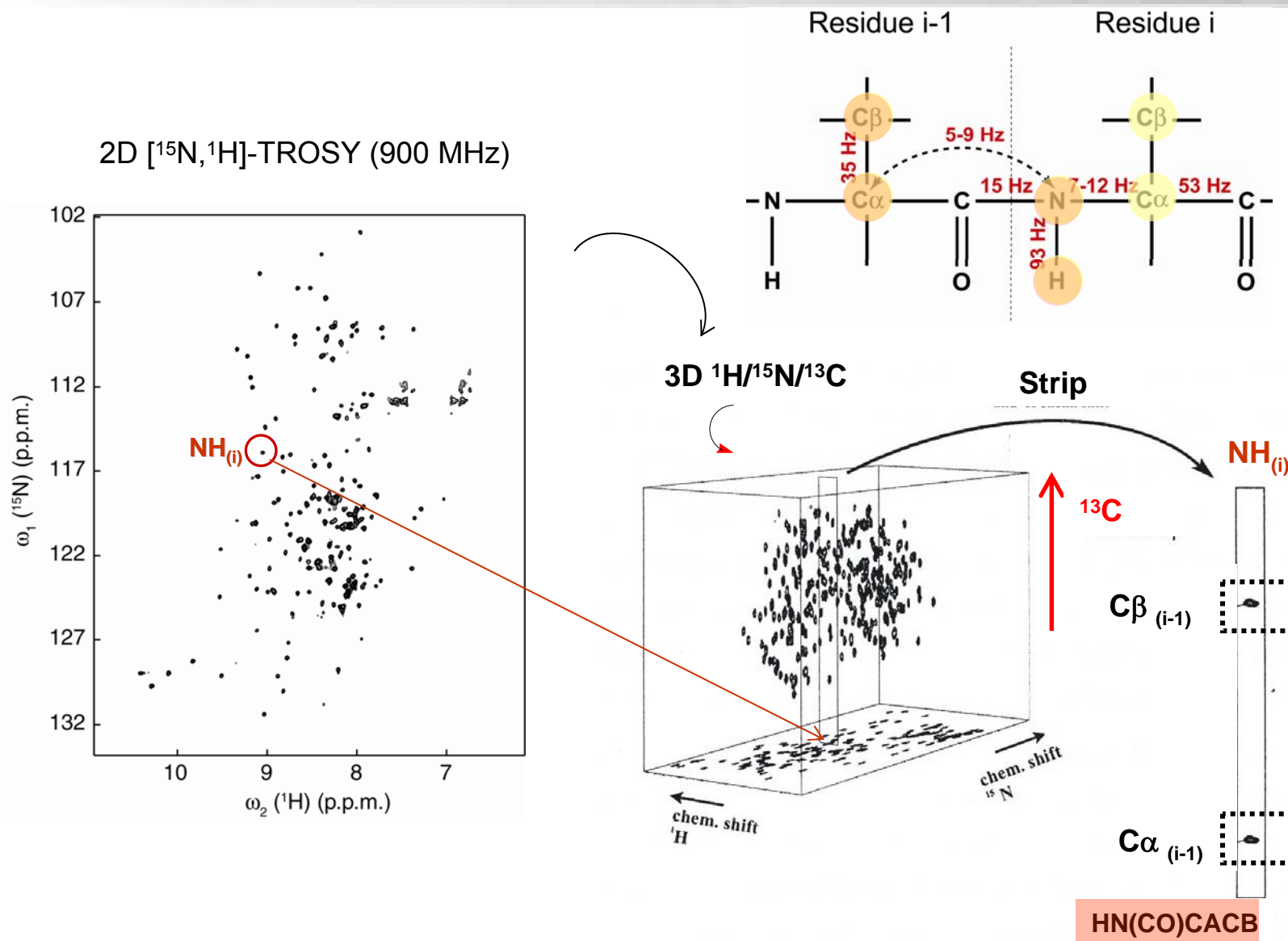
Strategy

Specific considerations for high molecular weight proteins or protein complexes like membrane proteins studied by HR liquid state NMR

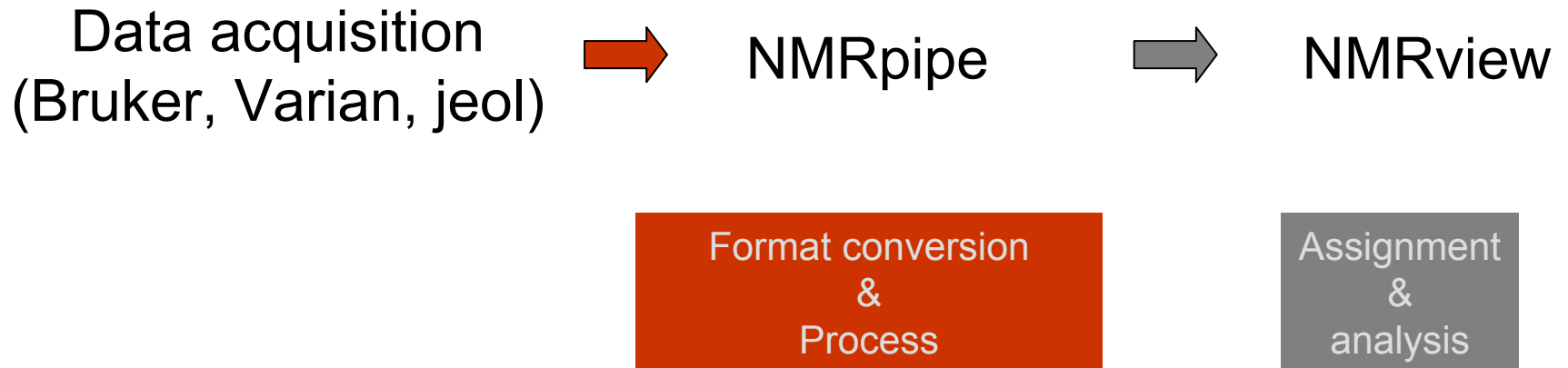
NMR structure determination process



Principle



ASSIGNMENT



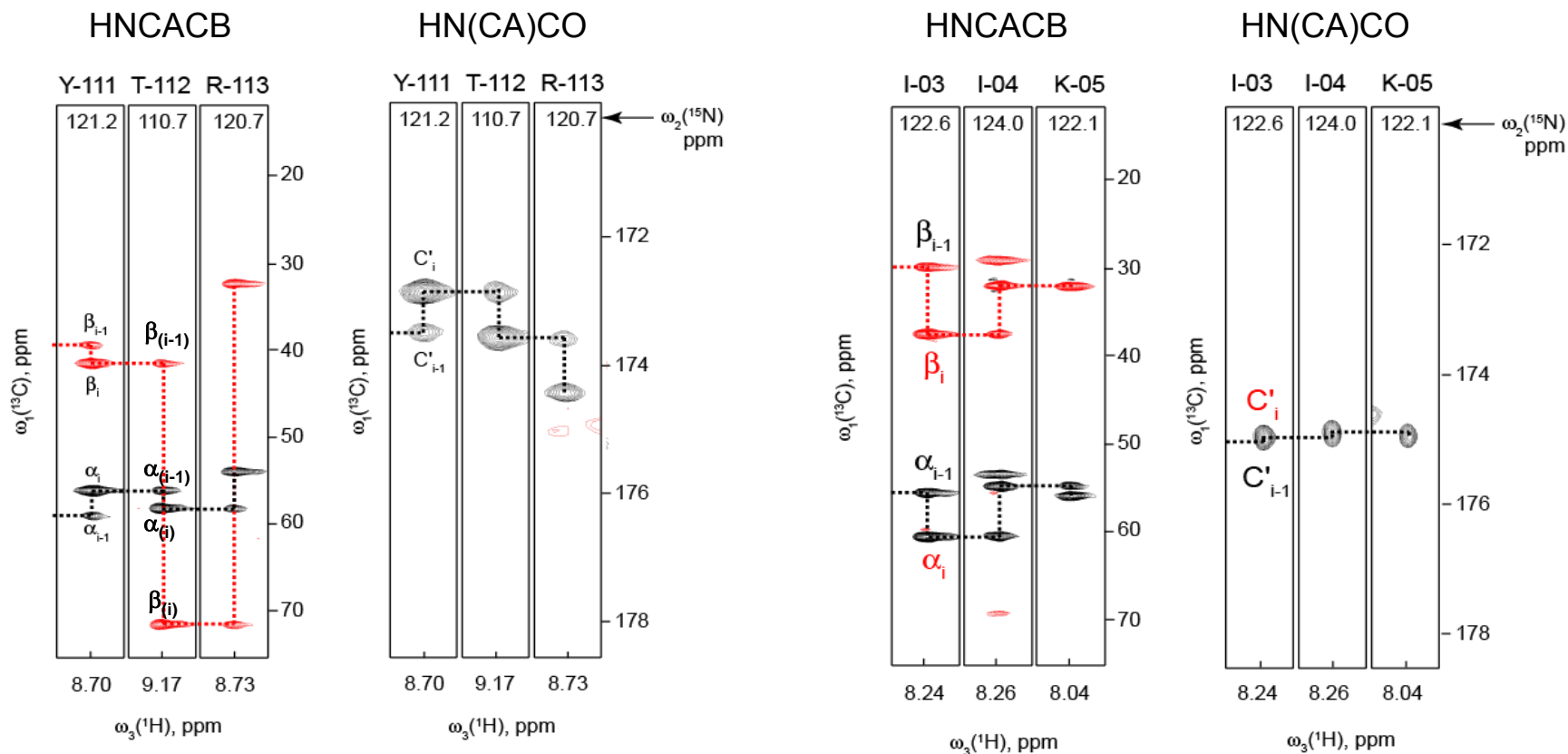
Experiments performed

U-[¹³ C, ¹⁵ N] protein	Seq .Nuclei	U-[¹³ C, ¹⁵ N, ² H] protein High MW proteins	
3D HNCA 3D HN(CO)CA	Ca	3D tr-HNCA 3D-trHN(CO)CA	700MHz 700MHz
3D HNCACB 3D CBCA(CO)NH	Ca,Cb	3D tr-HNCACB 3D-trHN(CO)CACB	900MHz 600MHz
3D tr-HNCO 3D-trHN(Ca)CO	CO	3D tr-HNCO 3D-trHN(Ca)CO	600MHz 600MHz
3D H(CCCO)NH 3D (H)CC(CO)NH	Side chains (Tocsy)	3D tr-(HNCA)CC(CA)NH	800MHz

Strip illustration of sequential assignment

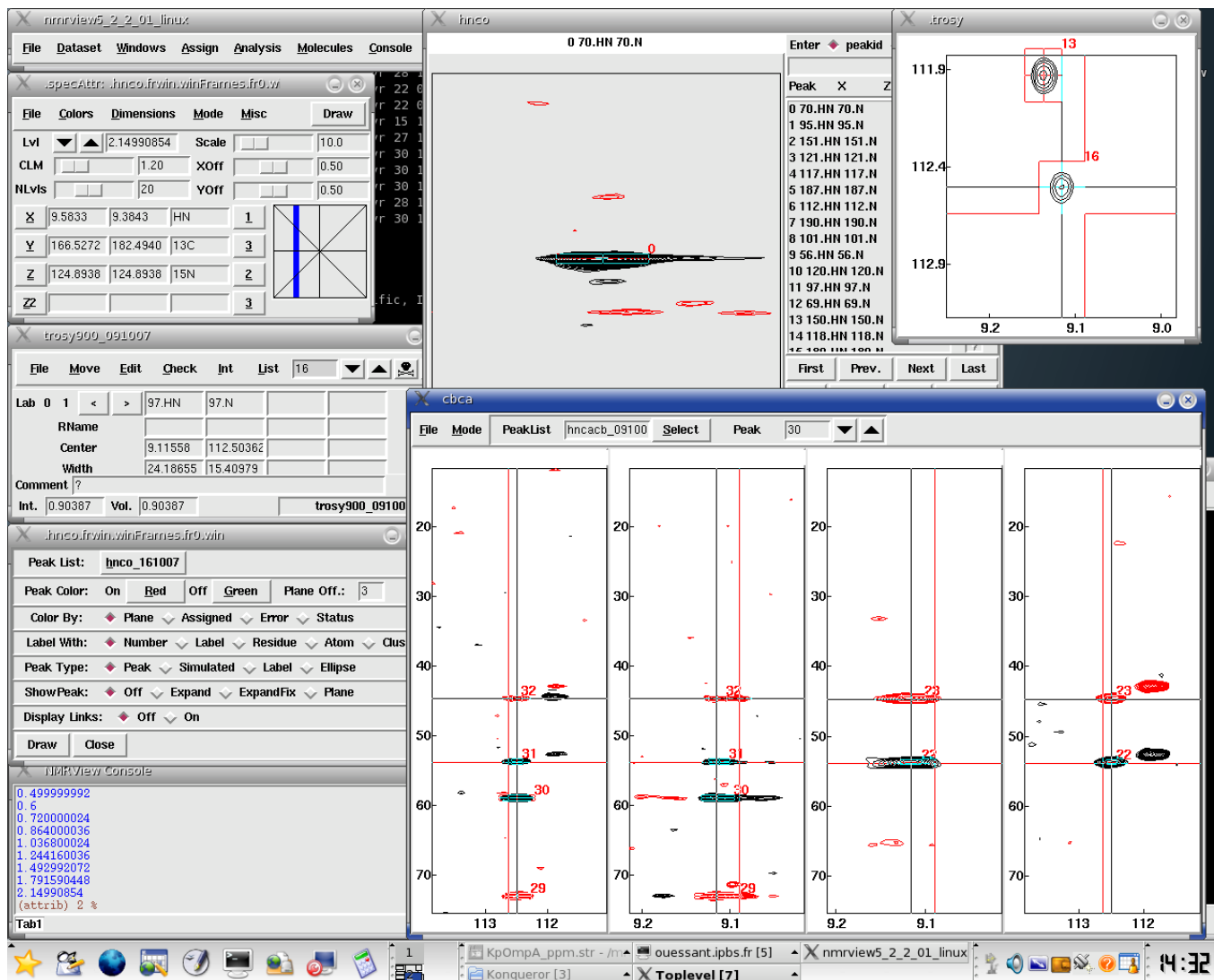
Sequential assignment based on C_a , C_b and C' connections pathways

"Crowded" regions



sequential assignment ... in practice

Demonstration



NMRPipe

Demonstration

The image shows a demonstration of the NMRPipe Conversion Utility. The main window, titled "NMRPipe Conversion Utility Version 97.027.12.55", has the following settings:

- Spectrometer Input: /mnt/rote/data/home/saurel/KpOmpA/attri
- Output Template: /mnt/rote/data/home/saurel/KpOmpA/attri
- Output Script: fid.com
- Input Protocol: Bruker (NIH)
- Output Protocol: NMRPipe
- Dimension Count: 3
- Temperature (K): 313.000
- Digital Oversampling Correction: During Conversion, During Processing

Below the settings, there are fields for "Total Points R+I:", "Valid Points:", "Acquisition Mode:", "Spectral Width Hz:", "Observe Freq MHz:", "Center Position PPM:", and "Axis Label:". There are also "Read Parameters" and "Save Sc" buttons.

A "Conversion Script Text" window is open, showing the following script:

```
#!/bin/csh
bruk2pipe -in /mnt/rote/data/home/saurel/KpOmpA/attrib/3D_trHNCACB/brukdata/ser \
  -bad 0.0 -noaswap -DMX -decim 16 -dspfv3 12 -grpdlly 0 \
  -xN 1536 -yN 200 -zN 184 \
  -xT 768 -yT 100 -zT 92 \
  -xMODE DQD -yMODE Echo-AntiEcho -zMODE Complex \
  -xSW 8992.806 -ySW 3127.932 -zSW 7246.377 \
  -xOBS 900.132 -yOBS 226.347 -zOBS 91.220 \
  -xCAR 4.630 -yCAR 43.584 -zCAR 117.620 \
  -xLAB 1H -yLAB 15N -zLAB 13C \
  -ndim 3 -aq2D States \
  -out /mnt/rote/data/home/saurel/KpOmpA/attrib/3D_trHNCACB/brukdata/fid/test%03d.fid -verb -ov
sleep 5
```

A terminal window shows the execution of the script:

```
tcsh> cd prodata/
tcsh> ll
total 44
drwxr-xr-x 4 saurel grpmilon 4096 avr 10 14:15 ./
drwxr-xr-x 7 saurel grpmilon 4096 avr 28 18:40 ../
-rwxrwx--- 1 saurel grpmilon 1747 jui 1 2008 2Dxyhncacb.com*
-rwxrwx--- 1 saurel grpmilon 1764 jui 1 2008 2Dxzhncacb.com*
-rwxrwx--- 1 saurel grpmilon 1603 jui 1 2008 3Dhncacb.com*
drwxr-xr-x 2 saurel grpmilon 4096 avr 10 14:14 fid/
-rwxrwx--- 1 saurel grpmilon 828 jui 1 2008 fid.com*
-rwxrwx--- 1 saurel grpmilon 756 jui 1 2008 hj_fid.com*
-rwxrwx--- 1 saurel grpmilon 870 jui 1 2008 hj_procx.com*
-rwxrwx--- 1 saurel grpmilon 944 jui 1 2008 hj_procxz.com*
drwxr-xr-x 2 saurel grpmilon 4096 avr 10 14:15 proc/
tcsh>
```

Another terminal window shows the command "tcsh> ksnapshot" and the output:

```
QMultiInputContext::changeInputMethod(): index=0,
slave=xim
```

NMRPipe

Demonstration

Process 2D

```
#!/bin/csh
source /usr/local/rmn_apps/NMRPipe/com/nmrlnit.linux9.com

nmrPipe -in ./test.fid -verb \
#| nmrPipe -fn POLY -time \
| nmrPipe -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5 \
| nmrPipe -fn ZF -size 4092 \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 -26.4 -p1 -84 -di \
| nmrPipe -fn EXT -x1 6.0ppm -xn 10.7ppm -sw \
| nmrPipe -fn YTP -verb \
#| nmrPipe -fn LP -ord 12 -fb -pred 64 \
| nmrPipe -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5 \
| nmrPipe -fn ZF -size 2048 \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 85.4 -p1 0 -di \
| nmrPipe -fn REV \
| nmrPipe -fn POLY -auto \
| nmrPipe -fn TP -verb \
#| nmrPipe -fn POLY -auto \
| nmrPipe -out test.ft2 -ov
```

```
nmrPipe -in test.ft2 | pipe2xyz -nv -out ostrosy900_151007.nv -verb
```

Process 3D

```
# NMRPipe 3D HNCACB processing (xyz) optimise
```

```
#!/bin/csh
source /usr/local/rmn_apps/NMRPipe/com/nmrlnit.linux9.com
set NMR=./
set INP=fid/test
set NAME=proc/test
```

```
xyz2pipe -in $NMR/$INP%03d.fid -x -verb
#| nmrPipe -fn SOL
#| nmrPipe -fn POLY -time
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 0.5
| nmrPipe -fn ZF -auto
| nmrPipe -fn FT -auto
| nmrPipe -fn PS -p0 65 -p1 0 -di
#| nmrPipe -fn POLY -auto
| nmrPipe -fn EXT -x1 6ppm -xn 11ppm -sw
| nmrPipe -fn TP
| nmrPipe -fn LP -ord 16 -pred 28 -fb
| nmrPipe -fn SP -off 0.5 -end 0.99 -pow 2 -c 0.5
| nmrPipe -fn ZF -auto
| nmrPipe -fn FT -auto
| nmrPipe -fn PS -p0 0 -p1 0 -di
#| nmrPipe -fn REV
```

```
| pipe2xyz -out $NAME%03d.dat -y
```

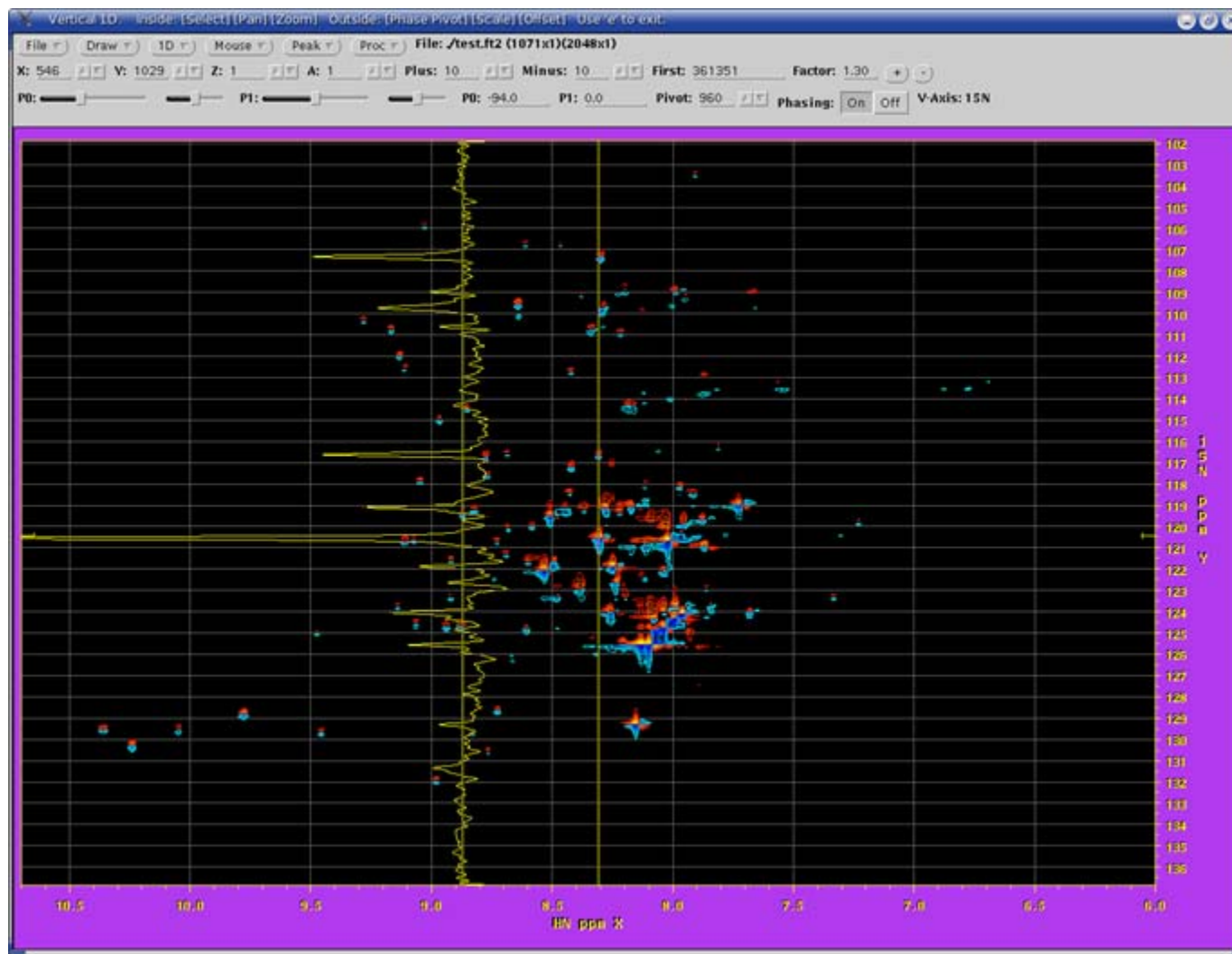
```
xyz2pipe -in $NAME%03d.dat -z -verb
| nmrPipe -fn LP -ord 16 -pred 36 -fb
| nmrPipe -fn SP -off 0.5 -end 0.99 -pow 2 -c 1
| nmrPipe -fn ZF -auto
| nmrPipe -fn FT -auto
| nmrPipe -fn PS -p0 -90 -p1 180 -di
#| nmrPipe -fn REV
#| nmrPipe -fn BASE -nw 3 -nl 0% 2% 60% 100%
#| nmrPipe -fn POLY -auto
| pipe2xyz -out $NAME%03d.ft2 -z -inPlace -ov
```

```
xyz2pipe -in $NAME%03d.ft2 | pipe2xyz -nv -out oshncacb.nv -verb
```

NMRPipe

Demonstration

Phase correction



Pulse programs

Assignment

Talos

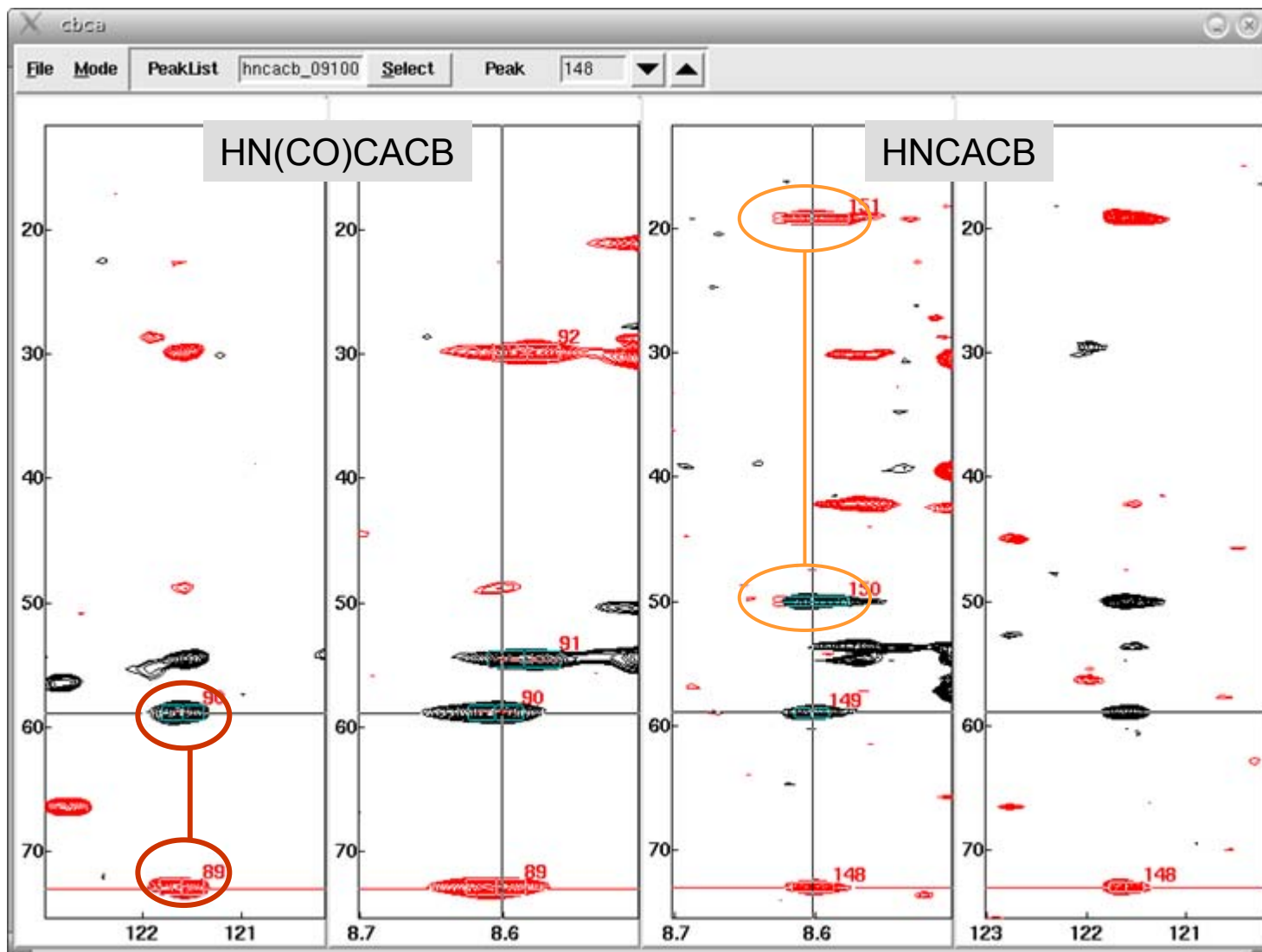
Backbone NOes

Me_NOes

Structure calculation

Assignment with CACB panel of nmrvue

Demonstration



Pulse programs

Assignment

Talos

Backbone NOes

Me_NOes

Structure calculation

Assignment in progress ...

65 YLGFEHGYDW...

95 QLTAKLGYP...T₉₇A₉₈K₉₉L₁₀₀G₁₀₁

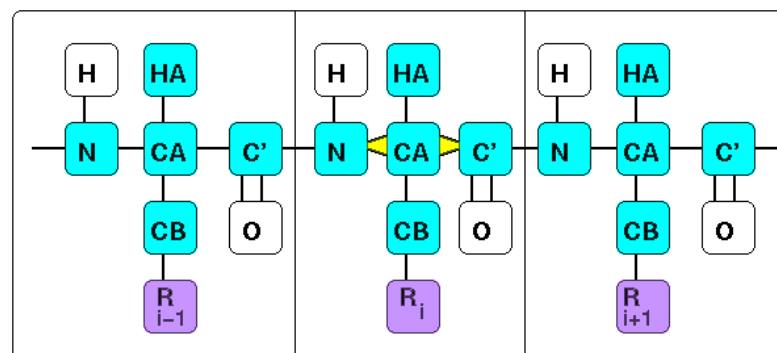
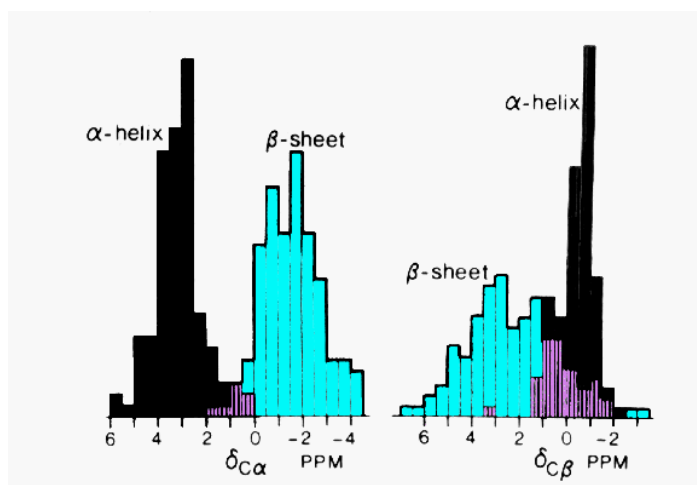
	T ₉₇	A ₉₈	K ₉₉	L ₁₀₀	G ₁₀₁		
HNCACB							
HNi,Ni,Cai	30	150	74	55	22		
Cbi	29	151	78	56	--		
Cai-1	31	149	75	54	21	66 or 97	
Cbi-1	32	148	79	57	23	68 or 99	
HN(CO)CACB							
HNi,Ni,Cai-1	22	90	45	33	15		
Cbi-1	23	89	47	34	16		
tr-HSQC						(Y ₇₂)	(Y ₁₀₂)
HNi,Ni	16	53	29	24	13	27	37
HNCO							
HNi,Ni,C'i-1	11	47	23	18	31	21 or 31	
HN(CA)CO							
HNi,Ni,C'i-1	16	82	38	30	10		
C'i	17	81	39	29	11		

≠ [HN and N] but overlapped on C', Ca and Cb !!!

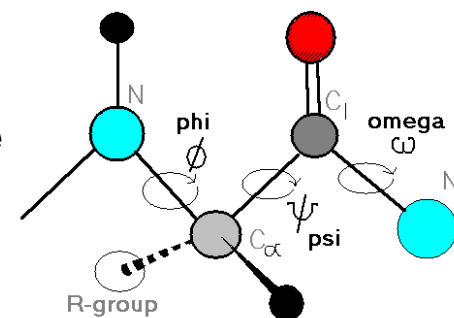
NOESY

TALOS

The TALOS relies on the observation that many kinds of secondary chemical shifts (i.e. differences between chemical shifts and their corresponding random coil values) are highly correlated with aspects of protein secondary structure



TALOS uses secondary shift and sequence information in order to make quantitative predictions for the protein backbone angles phi and psi .



TALOS

input file : KpOmpa.str

Nmrview
« assign panel »

input file : talos-shifts.tbl

`<Talos.tcl -in talos-shifts.tbl>`

Talos.tcl

searches the database
for shifts matches

output file : pred.tab

output folder : pred/res*.tab

`<rama.tcl -in talos-shifts.tbl>`

rama.tcl

used to display and
refine the predictions

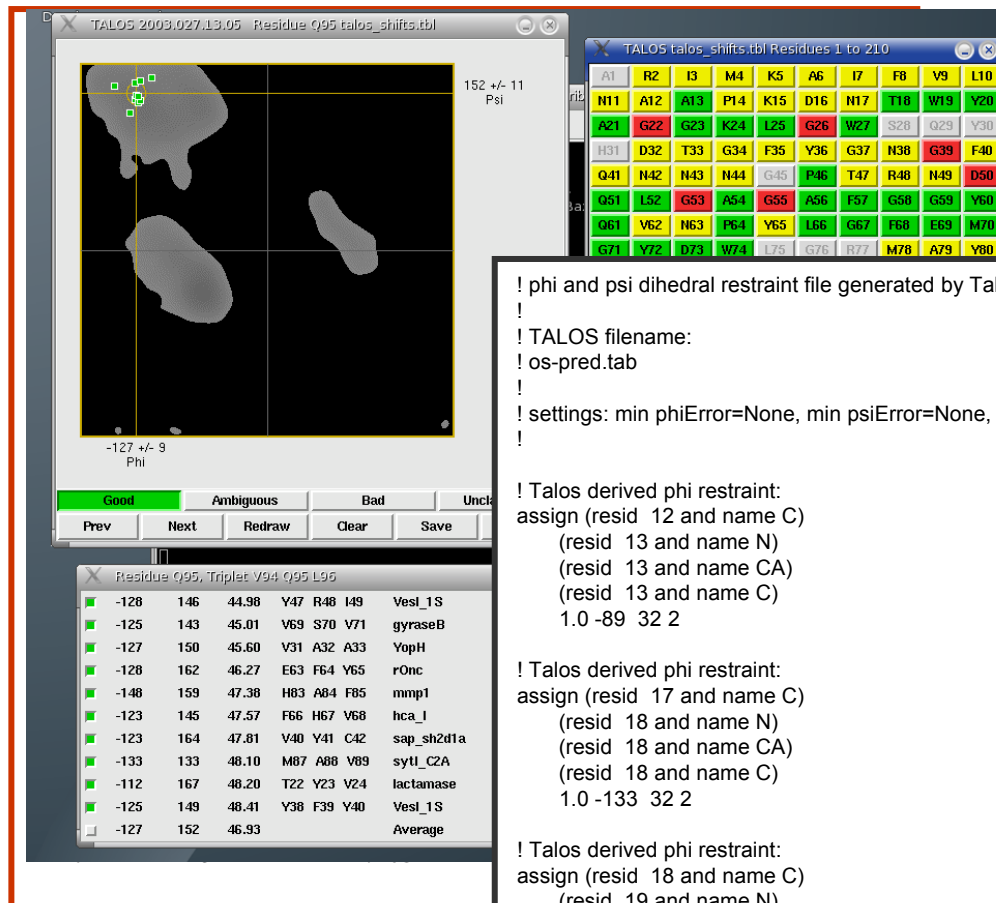
output file : pred.tab

Talos2Aria.py

`<python Talos2Aria.py pred.tab>`

output file : talos_phi_psi.tbl

(torsion angle restraints input file for CNS)



! phi and psi dihedral restraint file generated by Talos2Aria.py

!

! TALOS filename:

! os-pred.tab

!

! settings: min phiError=None, min psiError=None, errorFactor=2.0

!

! Talos derived phi restraint:

assign (resid 12 and name C)

(resid 13 and name N)

(resid 13 and name CA)

(resid 13 and name C)

1.0 -89 32 2

! Talos derived phi restraint:

assign (resid 17 and name C)

(resid 18 and name N)

(resid 18 and name CA)

(resid 18 and name C)

1.0 -133 32 2

! Talos derived phi restraint:

assign (resid 18 and name C)

(resid 19 and name N)

(resid 19 and name CA)

(resid 19 and name C)

1.0 -119 32 2

! Talos derived phi restraint:

assign (resid 19 and name C)

(resid 20 and name N)

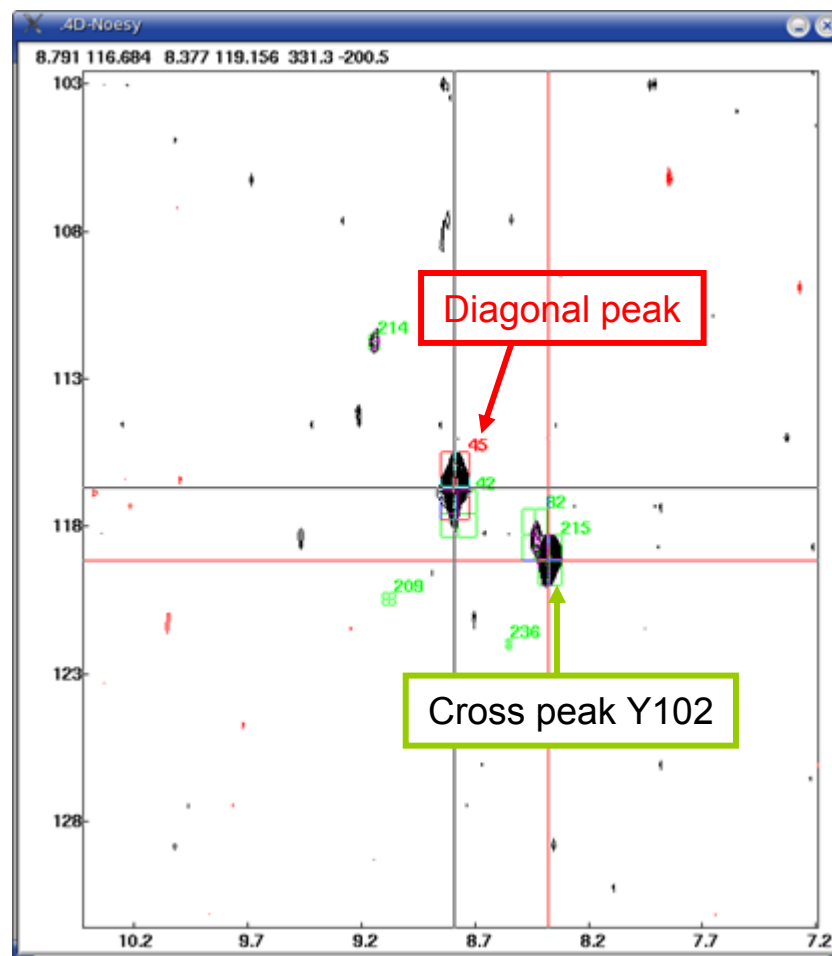
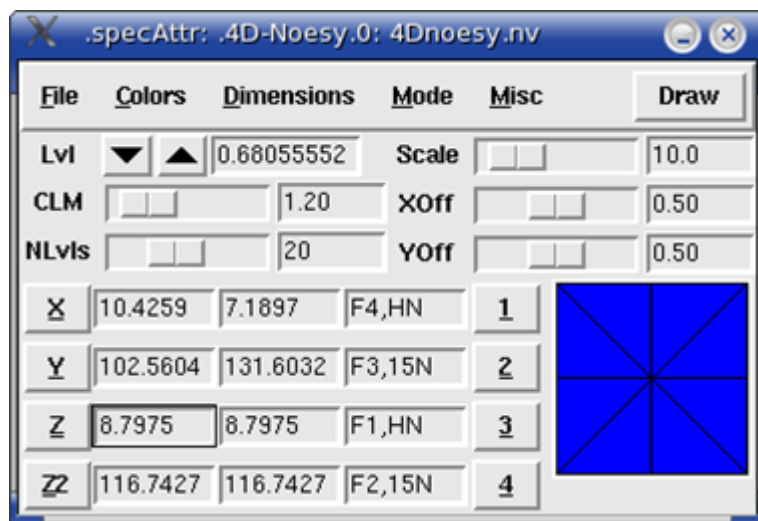
(resid 20 and name CA)

(resid 20 and name C)

1.0 -141 32 2

Backbone NOes

demonstration

4D ^{15}N , ^{15}N separated HMQC-NOESY-trHSQC

Methyl-based NOes

The sparse proton density in perdeuterated proteins leads to relatively few long range NOes (and poorly defined structure)

>>> **protonation of methyl groups of Val, Leu and Ile :**

- Important for hydrophobic cores and to stabilize the molecular fold
- Widely spread in membrane proteins
- Narrow and intense lines due to the fast rotation even in large proteins
- Easy to label with commercial biosynthetic precursors in E. Coli

^1H and ^{13}C Methyl groups assignment

3D (H)C(CCA)NH-TOCSY (800MHz)

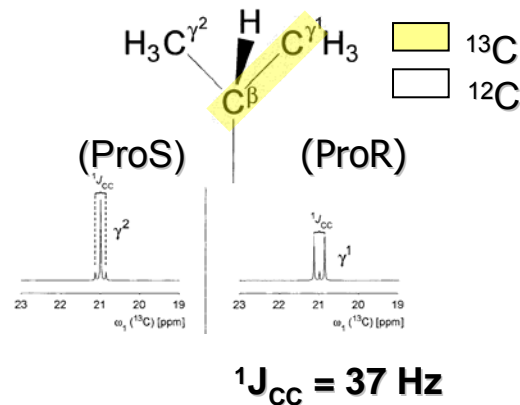
($^{13}\text{C}_{\text{Methyl}}$ with $^{15}\text{N}/\text{HN } i, i+1$)

3D H(CCO)NH-TOCSY (500MHz)

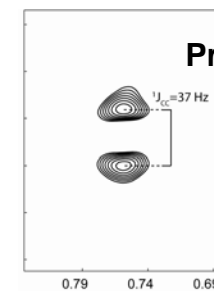
($^1\text{H}_{\text{Methyl}}$ with $^{15}\text{N}/\text{HN } i+1$)

2D CT-HSQC (600MHz) KpOmpA 10% ^{13}C

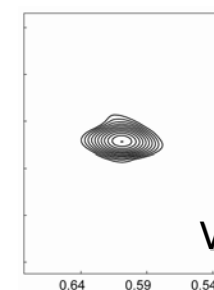
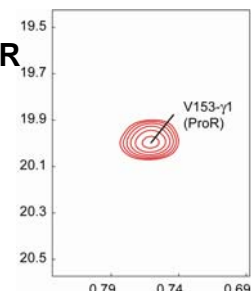
(stereospecific assignment of proS and proR H_{methyl} of V and L residues)



^1H - ^{13}C HSQC

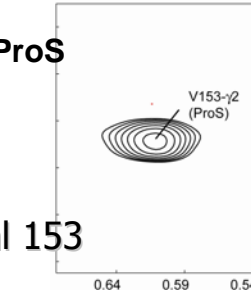


CT-HSQC

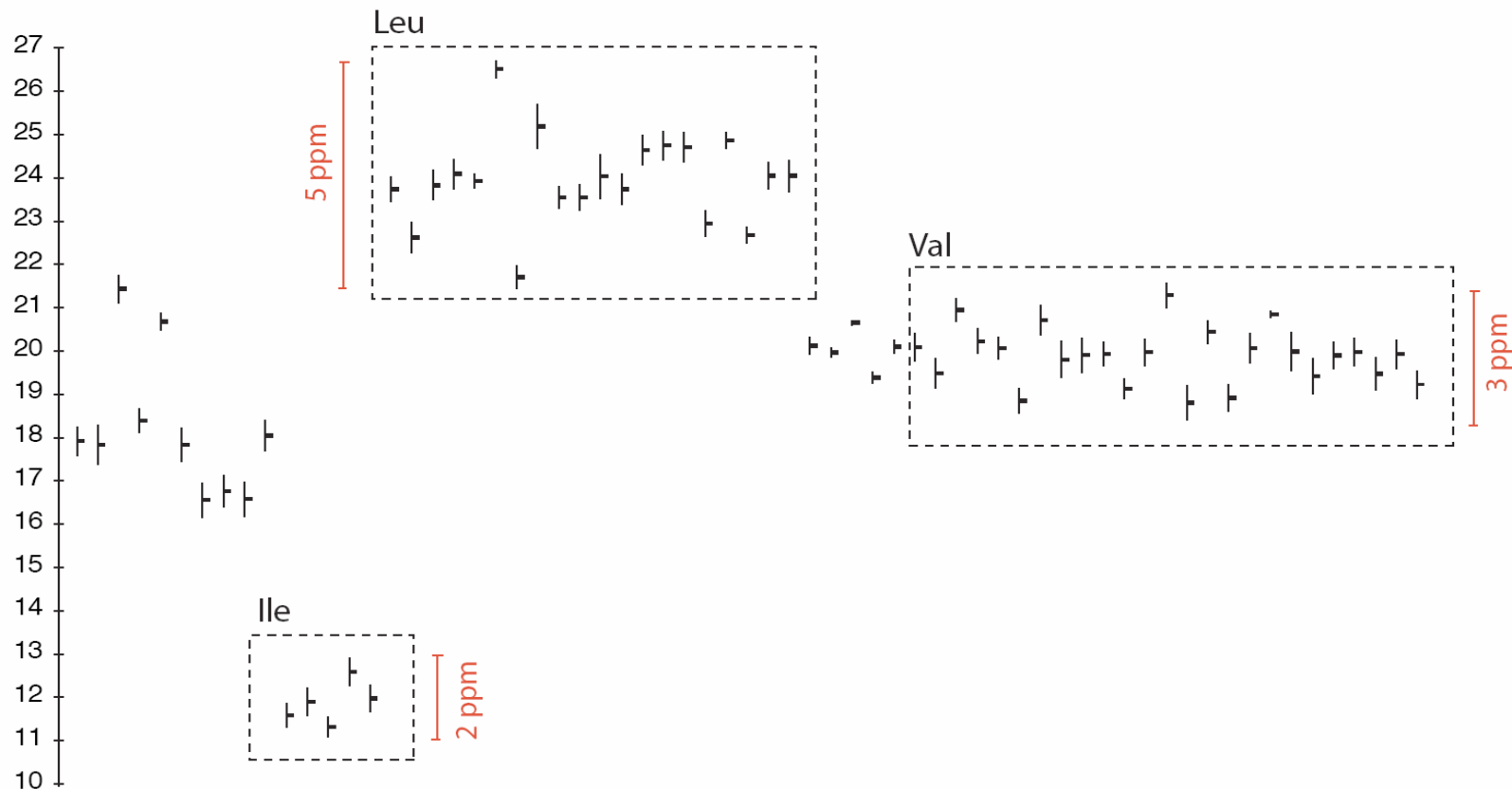


ProS

Val 153

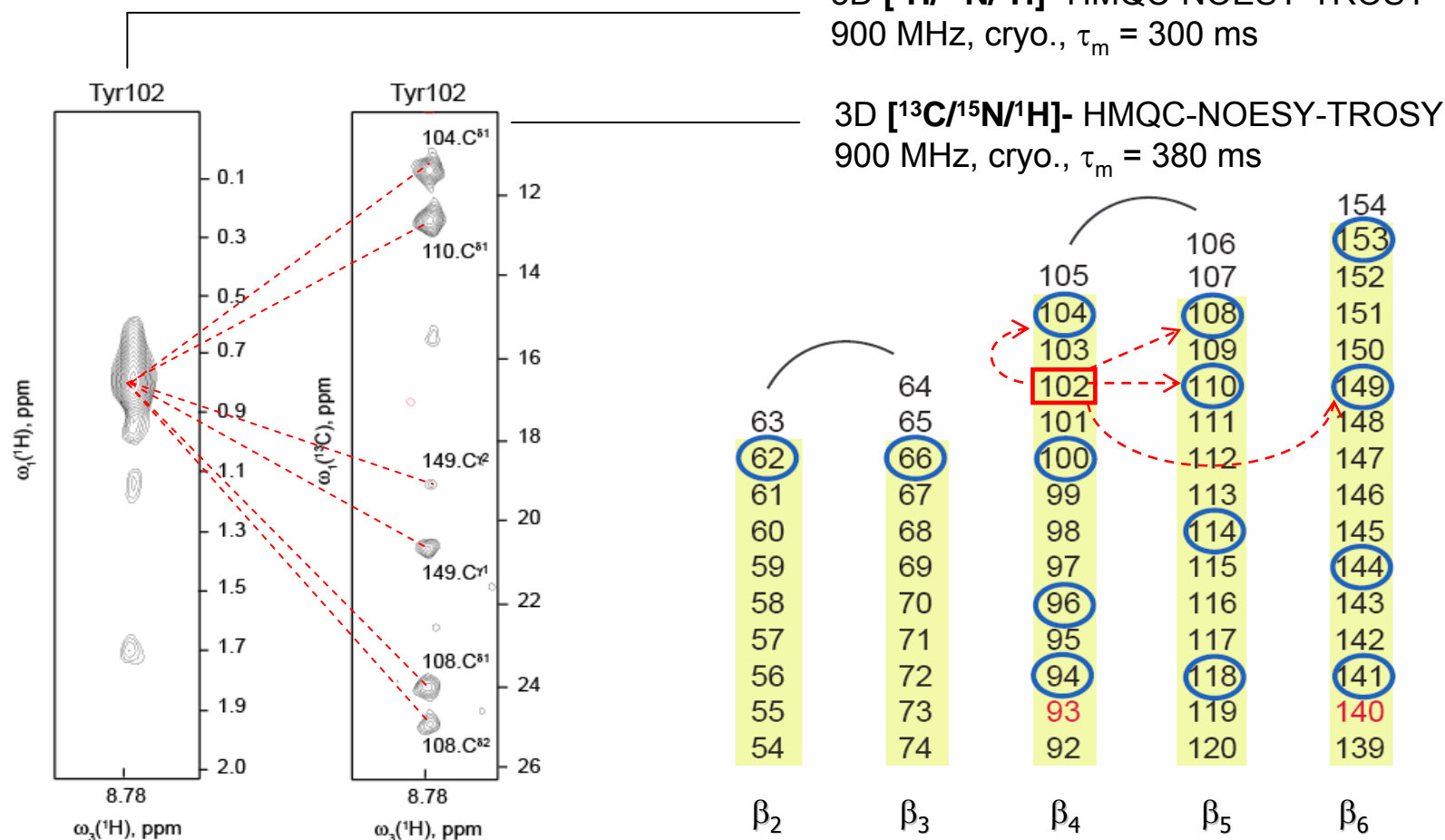


^1H , separated or ^{15}N and ^{13}C separated HMQC-NOESY



^1H , separated or ^{15}N and ^{13}C separated HMQC-NOESY

Contacts NOEs between amide proton of Tyr102 and proximal methyl group



○ Possible contacts

⋯→ Observed NOEs

Conclusion – Experiments performed

Backbone and side-chain assignment

3D tr-HNCO	600MHz
3D-trHN(Ca)CO	600MHz
3D tr-HNCA	700MHz
3D-trHN(CO)CA	700MHz
3D tr-HNCACB	900MHz
3D-trHN(CO)CACB	600MHz

KpOmpA u- ^{2}H , ^{15}N , ^{13}C]**Caliph. assignment**

3D tr-(HNCA)CC(CA)NH	800MHz
----------------------	--------

Methyl groups assignment

3D (H) C (CCA)NH-TOCSY	800MHz
3D H (CCO)NH-TOCSY	500MHz
2D CT-HSQC	600MHz

KpOmpA u- ^{2}H , ^{15}N , ^{13}C], V,L,I δ_1 -CH₃KpOmpA 10%- ^{13}C] and DHPC-d₂₂**NOESY experiments**

4D [15N , 15N separated]- HMQC-NOESY-TROSY	800MHz
3D [1H/15N/1H]- HMQC-NOESY-TROSY	900MHz
3D [13C/15N/1H]- HMQC-NOESY-TROSY	900MHz

KpOmpA u- ^{2}H , ^{15}N , ^{13}C]KpOmpA u- ^{2}H , ^{15}N , ^{13}C] and DHPC-d₂₂

NMR restraints

NMR and refinement statistics for the 20-lowest energy CNS-conformers

Experimental restraints

NOEs HN - HN

NOEs HN - CH₃NOEs CH₃ - CH₃

Hydrogen bonds

Torsion angle restraints

920

173

243

112

128

264

```
assign (resid 18 and name HN) (resid 61 and name HN) 3.3 0.7 0.1
assign (resid 19 and name HN) (resid 192 and name HN) 3.3 0.7 0.1
assign (resid 20 and name HN) (resid 59 and name HN) 3.3 0.7 0.1
assign (resid 21 and name HN) (resid 190 and name HN) 3.3 0.7 0.1
assign (resid 22 and name HN) (resid 57 and name HN) 3.3 0.7 0.1
```

```
assign (resid 20 and name O) (resid 59 and name N) 2.90 0.30 0.50
assign (resid 20 and name O) (resid 59 and name HN) 1.90 0.50 0.10
assign (resid 59 and name O) (resid 20 and name N) 2.90 0.30 0.50
```

```
! Talos derived phi restraint:
assign (resid 1 and name C)
      (resid 2 and name N)
      (resid 2 and name CA)
      (resid 2 and name C)
      1.0 -100 82 2
```

Different steps of structure calculation using CNS

Step. 1 : generating a topology file based on the protein sequence

File containing information about the molecular connectivity (covalent topology)

Script : `generate_seq.inp`

Input file : `<my-sequence>.seq` >>> output file : `<my-sequence>.mtf`

generate_seq.inp

Generate structure file for protein, dna/rna, water, ligands and/or carbohydrate from sequence information only
 modified by Brian Smith (Edinburgh University) to allow protein residue renumbering

Authors: Paul Adams, and Axel Brünger
 Copyright © Yale University

input files	
multiple sequence files of the same type can be defined by duplicating the entries below and incrementing the file number	
protein sequence file	<input type="text" value="my_sequence.seq"/>
	segid <input type="text"/>
	start residue numbering at <input type="text" value="1"/>
nucleic acid sequence file	<input type="text"/>
	segid <input type="text"/>
water sequence file	<input type="text"/>
	segid <input type="text"/>
carbohydrate sequence file	<input type="text"/>
	segid <input type="text"/>
prosthetic group sequence file	<input type="text"/>
	segid <input type="text"/>
ligand sequence file	<input type="text"/>
	segid <input type="text"/>
ion sequence file	<input type="text"/>
	segid <input type="text"/>
output files	
output structure file	<input type="text" value="my-toplogy.mtf"/>
disulphide bonds	

CNS prompt `< generate_seq.inp>generate_seq.out`

Different steps of structure calculation using CNS

Step. 2 : generating initial extended coordinates

Starting structure for restrained molecular dynamic/simulated annealing calculation

Script : `generate_extended.inp`

Input file : `<my-topology>.mtf >>>` output file : `<my-extended-protein>.pdb`

CNS prompt `< generate_extended.inp>generate_extended.out`

generate_extended.inp

Generates an extended strand with ideal geometry for each connected polymer. The molecular structure file must not contain any closed loops except disulfide bonds which are automatically excluded from the generation of the strand conformation.

Authors: Axel T. Brunger

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molecular structure		
structure file(s)	<input type="text" value="my-tology.mtf"/>	
parameter file(s)	<input type="text" value="CNS_TOPPAR:protein-allhdg.par"/>	
	<input type="text"/>	
	<input type="text"/>	
	<input type="text"/>	
input parameters		
maximum number of trials to generate an acceptable structure	<input type="text" value="10"/>	
output files		
output coordinates	<input type="text" value="my_extended prot.mtf"/>	
<input type="button" value="View updated file"/>	<input type="button" value="Save updated file"/>	<input type="button" value="Reset values"/>

Different steps of structure calculation using CNS

Step. 3 (optional): generating initial extended coordinates

Distance geometry with simulated annealing regularization starting from extended strand >> prefolded structure

Script : `dg_sa.inp`

Input files : `<my-topology>.mtf`
`<my_extended-protein>.pdb`
`<NOes-restraints>.tbl`
`<talos-restraint>.tbl`
`<H-bonds>.tbl`

CNS prompt `< dg_sa.inp>dg_sa.out`

`>>> output files : <my-protein_dg>.pdb`
`... then sort according to the lowest energy`
`<my-protein_dg_best>.pdb`

Step. 4 generating initial extended coordinates

Dynamical annealing with NMR restraints starting from extended strands or pre-folded structure

Script : `anneal.inp`

Input files : `<my-topology>.mtf`
`<my-protein_dg_best>.pdb`
`<NOes-restraints>.tbl`
`<talos-restraint>.tbl`
`<H-bonds>.tbl`

CNS prompt `< anneal.inp>anneal.out`

`>>> output files : <my-protein>.pdb`
`... then sort according to the lowest energy`
`<my-best-prot>.pdb`

Different steps of structure calculation using CNS

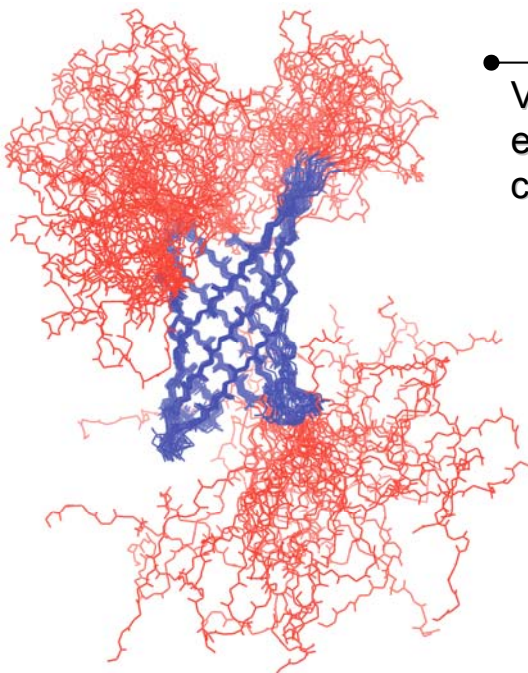
Step. 5 : Determined accepted structures and generate ensemble averageScript : `accept.inp`

Input files : `<my-topology>.mtf`
`<my-best-prot>.pdb`
`<NOes-restraints>.tbl`
`<talos-restraint>.tbl`
`<H-bonds>.tbl`

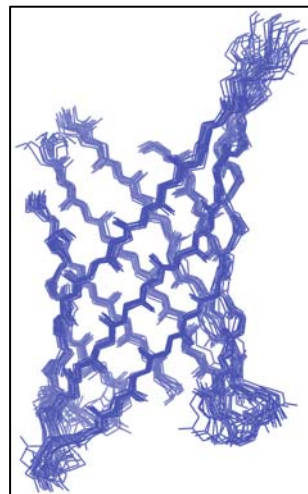
CNS prompt `< accep.inp>accept.out`

`>>>` output files : `<my-protein>.pdb`
... then sort according to the lowest energy
`<my-best-prot-a>.pdb`

Structure calculation using CNS



View of the 20
energy-refined CNS
conformers



Best defined regions
Barrel + periplasmic turns

Table 1. NMR and refinement statistics for KpOmpA/
DHPC

A. NMR distance and dihedral constraints

Total NOE	528
Sequential ($ i-j =1$)	132
Medium range ($ i-j \leq 4$)	93
Long range ($ i-j \geq 5$)	303
Intermolecular	0
Hydrogen bonds	128
Total dihedral angle restraints	264

B. Violations (mean \pm s.d.)

Distance constraints (\AA)	0.0072 \pm 0.0005
Dihedral angle constraints ($^\circ$)	0.0410 \pm 0.0049
Max. dihedral angle ($>2.5^\circ$)	0
Max. distance constraint violation ($>0.1 \text{\AA}$)	0
Deviations from idealized geometry	
Bond lengths (\AA)	0.0009 \pm 0.00002
Bond angles ($^\circ$)	0.2535 \pm 0.0021
Impropers ($^\circ$)	0.1241 \pm 0.0049
PROCHECK Ramachandran plot analysis	
Most favored regions (%)	70.5
Additionally allowed regions (%)	26.7
Generously allowed regions (%)	2.6
Disallowed regions (%)	0.2
Average pairwise r.m.s. deviation (\AA) ^a	
Heavy (\AA)	1.906
Backbone (\AA)	0.543

Additional restraints

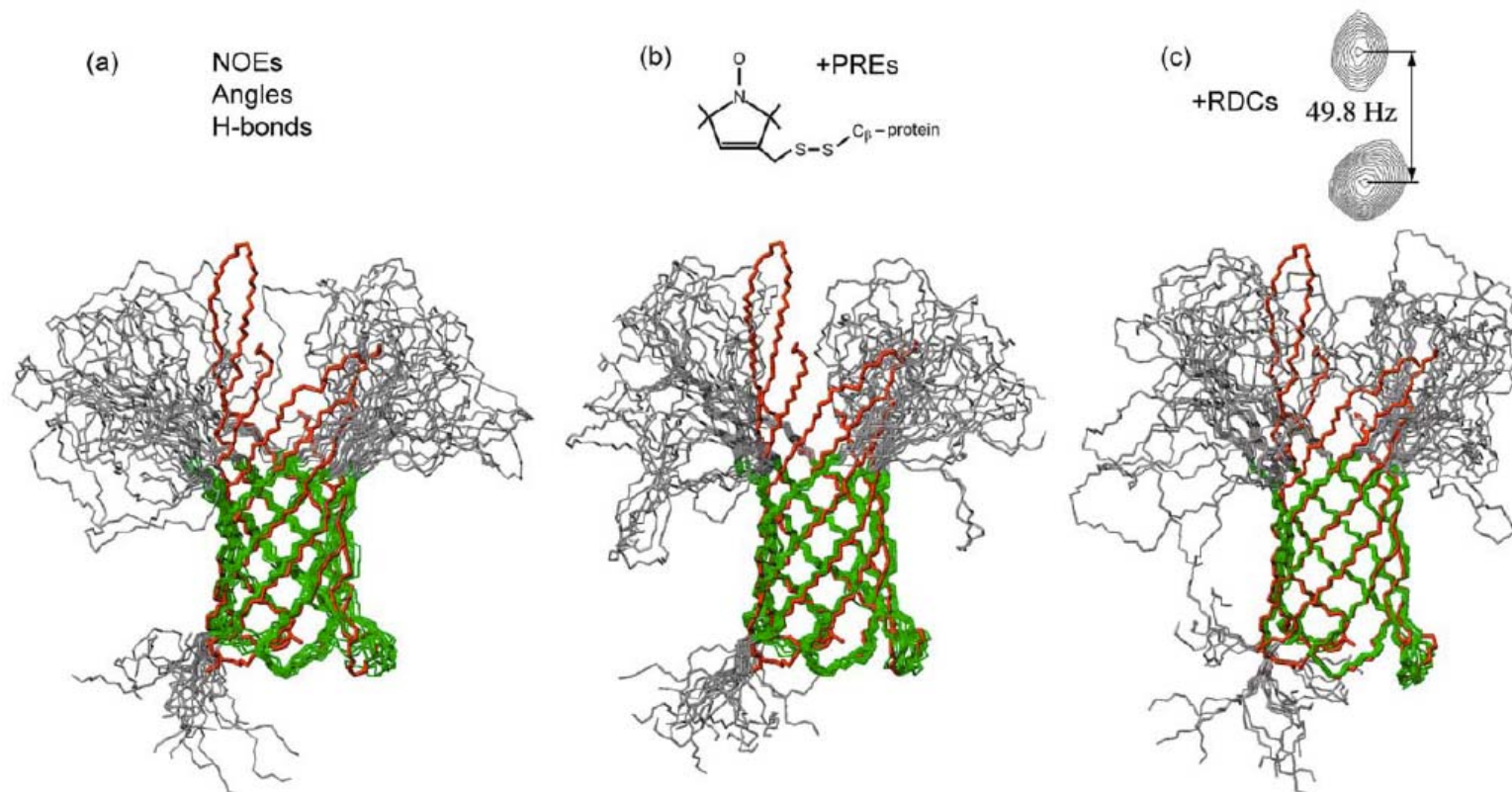


Fig. 2. Superposition of the ten lowest energy OmpA conformers calculated with (a) NOE, (b) NOE plus PRE, and (c) NOE plus RDC restraints, in addition to dihedral angle and H-bond restraints. Structured fragments used for the overlay are colored green. For comparison, the crystal structure (PDB accession code 1QJP [71]) is shown in red. The backbone rms deviations for the precision and accuracy of the green residues in the three ensembles are: (a) 1.25 ± 0.29 and 1.62 ± 0.16 Å, (b) 0.85 ± 0.17 and 1.09 ± 0.12 Å, and (c) 0.62 ± 0.16 and 1.11 ± 0.06 Å. Panels (a) and (b) reproduced with permission from Ref. [38]. The figure in panel (c) was recalculated using the data of Ref. [53], but employing the same criteria for structure calculation as those used to produce the structures shown in panels (a) and (b).

Additional restraints

Residual Dipolar Couplings (RDCs)

Dipolar coupling :

$$D_{HN} = D_a \left[(3 \cos^2 \theta - 1) + \frac{3}{2} R \sin^2 \theta \cos 2\phi \right]$$

$$D_a = -(\mu_0 h / 16 \pi^3) \gamma_H \gamma_N \langle r^{-3} \rangle S \cdot A_a \quad D_a : \text{amplitude of the alignment tensor}$$

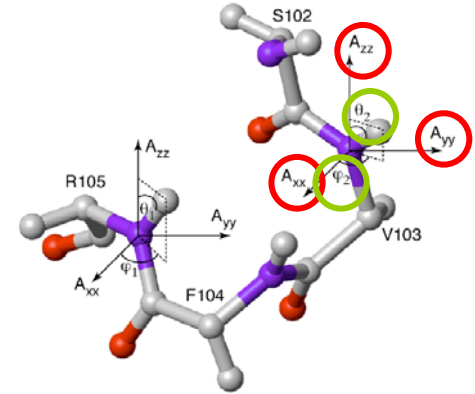
A_a : axial component of the alignment tensor $A_a = A_{zz} - (A_{xx} + A_{yy})/2$

S : order parameter

R : Rhombicity

$$R = (A_{xx} - A_{yy}) / A_{zz}$$

θ et ϕ : polar angles of **HN dipolar coupling** with respect to the **alignment tensor** (A_{zz} , A_{xx} , A_{yy})



RDCs >>> orientational restraints of internuclei vectors (HN, C'Ca, CaN ...)

Alignment media :

- Bicelles
- virus (TMV, phage tails)
- ternary mixture (polyethyleneglycol/hexanol/water)
- Purple membranes
- **Polyacrylamide gel >>>> membrane proteins**

Additional restraints

Residual Dipolar Couplings (RDCs)

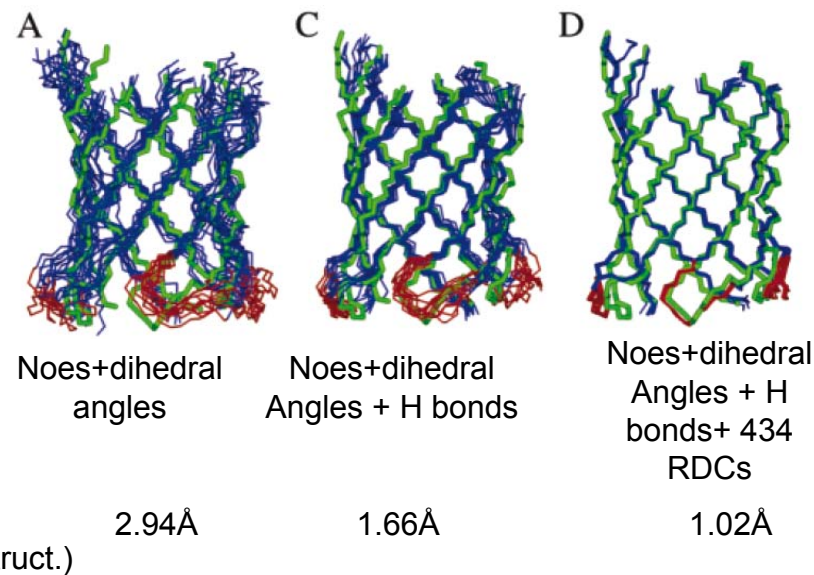
Polyacrylamides gels (3-4%) doped with electrostatic charges :

- copolymer gel 50+M
- copolymer gel 50-S

For each condition : $^1D_{HN}$, $^1D_{C'Ca}$ and $^1D_{NC}$ were recorded >>> a total of 434 RDCs

Table 1. Structural Statistics for the Final OmpA Structure

	no. of restraints	rms dev ^a
distance restraints (Å)	90	0.008 ± 0.001
hydrogen bond restraints ^b (Å)	68	0.009 ± 0.001
dihedral angles ^c (deg)	142	0.067 ± 0.035
RDC (Hz)		
50-S $^1J_{HN}$	74	1.39 ± 0.019
50-S $^1J_{NC}$	72	0.30 ± 0.009
50-S $^1J_{C'Ca}$	73	0.83 ± 0.003
50+M $^1J_{HN}$	71	1.65 ± 0.056
50+M $^1J_{NC}$	74	0.37 ± 0.015
50+M $^1J_{C'Ca}$	70	0.84 ± 0.001
covalent geometry		
bond lengths (Å)		0.0008 ± 0.00002
bond angles (deg)		0.295 ± 0.004
impropers (deg)		0.166 ± 0.006
structure ensemble ^d (Å)		
backbone		0.48 ± 0.08
heavy atoms		1.86 ± 0.18



Substantial improvement of the β -barrel core

Better definition of periplasmic turns (where NOes and dihedral angle are sparse)

Additional restraints

Paramagnetic Relaxation enhancement (PRE)

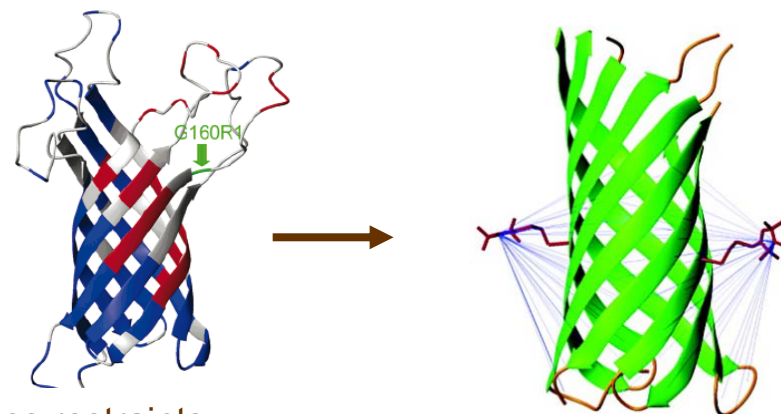
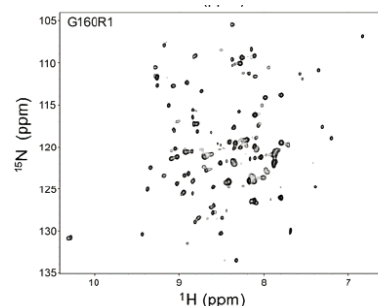
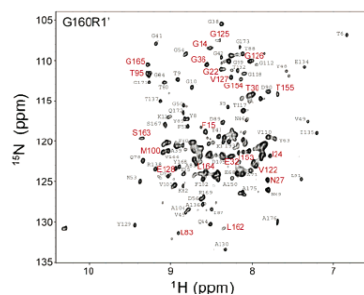
The rate of transverse relaxation rate R_2 is enhanced by paramagnetic spin label

Modified equa. Solomon-Bloembergen :

$$r = \left[\frac{K}{R_2^{\text{SP}}} \left(4\tau_c + \frac{3\tau_c}{1 + \omega_h^2 \tau_c^2} \right) \right]^{1/6} \quad \frac{I_{\text{para}}}{I_{\text{dia}}} = \frac{R_2 \exp(-R_2^{\text{SP}} t)}{R_2 + R_2^{\text{SP}}}$$

>> long-range (15-25 Å) distance restraints of protons to spin label

Spin label : 1-oxy-2,2,5,5-tetramethyl- η^3 - pyrroline-3-methyl-methanethiosulfonate (MTSSL)



11 labeled sites (punctual mutations) >>> 320 RPE distance restraints
>>> Enhancement of the resolution on the transmembrane core and turns

Rmsd
(cristal struct.)

Noes + dihedral angles

Noes + H. bonds

(-) PRE

1.63 Å

1.62 Å

(+) PRE

1.24 Å

1.25 Å

THANKS ..

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Group members :

Prof. A. Milon

Dr. V. Réat

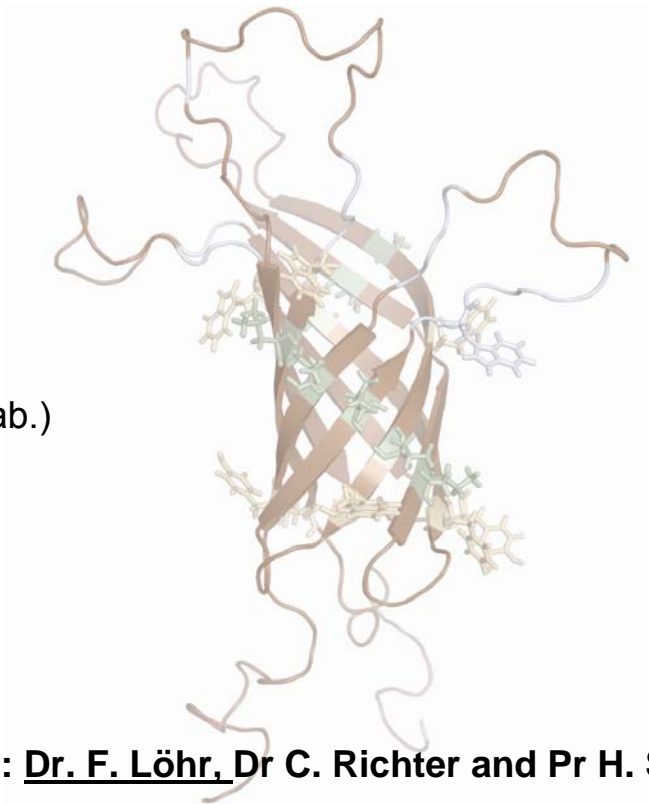
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Dr. P. Demange

Bruker Biopsin

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