Basic of Biomolecular NMR

(protein NMR)

&

New Software in HFNMRC

(Topspin2.1)

Chi-Fon Chang, Ph.D.

Basic of Biomolecular NMR

(protein NMR)

Why Biomolecular NMR ?

Structure Determination in Atomics Resolution

- Sample in Solution
  - Native-like Condition
- Structure Biology
  - Structural Basis Biological process / questions

Molecular Recognition

- Protein-Protein, Protein-Nucleic Acid, Protein-Ligand

Molecular Dynamics

- Conformational dynamics
- Folding

Why NMR ?

What's N, M, and R?

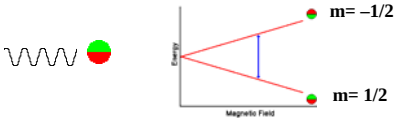
Properties of the Nucleus

- *Nuclear spin : I*
- *Nuclear magnetic moments :* $\mu = \gamma I (h/2\pi)$

Nucleus in a Magnetic Field Bo

- *Precession and the Larmor frequency :* $\upsilon = \omega/2\pi = \gamma Bo/2\pi$
- *Zeeman effect & Boltzmann distribution :* $P_{m=-1/2} / P_{m=+1/2} = e^{-\Delta E/kT}$

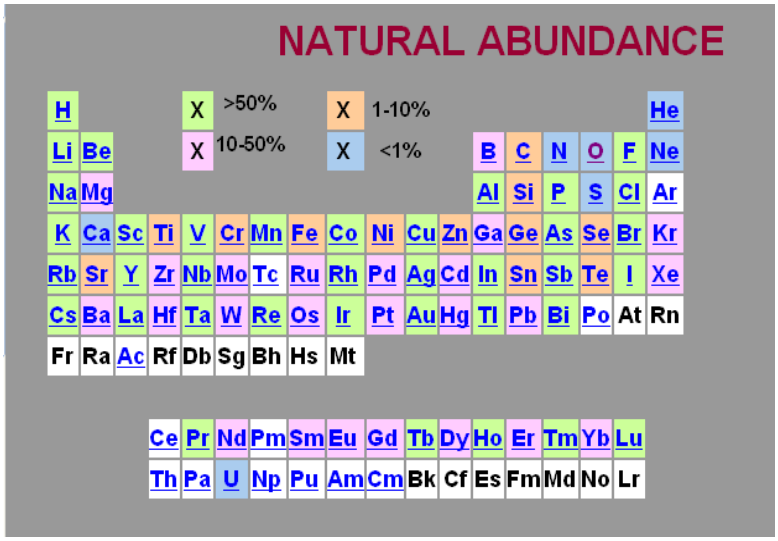
Apply radio frequency at Resonance to measure the nuclear precession frequencies $\omega = \gamma Bo$



I=1/2, m_I= -1/2, 1/2

Resonance Frequency

http://arrhenius.rider.edu/nmr/NMR_tutor/periodic_table/nmr_pt_frameset.html



不同核種 → 1H, 13C, 15N

不同的 gyromagnetic ratio γ → γ(1H)=26.7522, γ(13C)=6.7285, γ(15N)=-2.7126

在相同磁場 →  B0 (ex:14.7 Tesla)

產生/吸收不同共振頻率的電磁波 → ω= γB0

產生不同的NMR 訊號 → At 14.7 Tesla
1H ~ 600MHz
13C ~ 150MHz
15N ~60MHz

相同核種 → 1H

相同的 gyromagnetic ratio γ → γ(1H)=26.7522

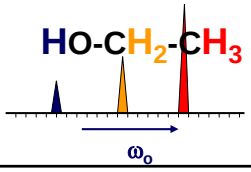
不同化學環境 → HO-CH2-CH3

雖然在相同靜磁場B0
但感受到不同淨磁場(Beff)

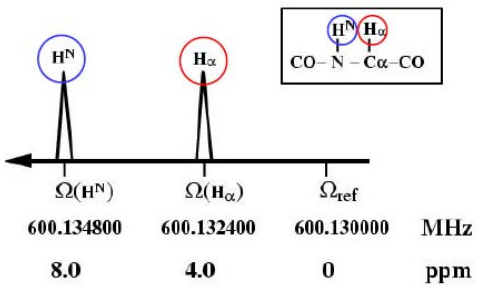
Beff=(B0-Blocal)
=B0(1-σ)
where σ is shielding parameter

產生/吸收不同共振頻率的電磁波 → ω= γBeff

產生不同的NMR 訊號



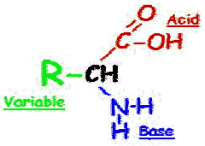
Chemical Shift (frequency vs. ppm)



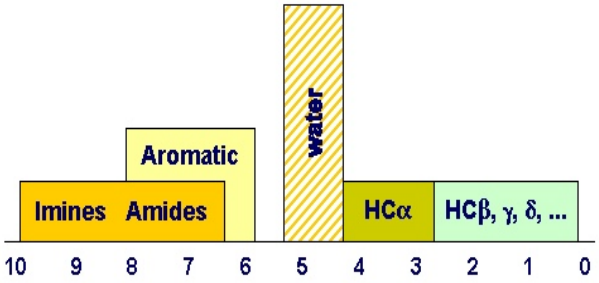
$$\delta(\text{ppm}) = (\Omega - \Omega_{ref})/\omega_0 * 10^6$$

(Copy from http://www.embl.de/nmr/sattler/teaching)

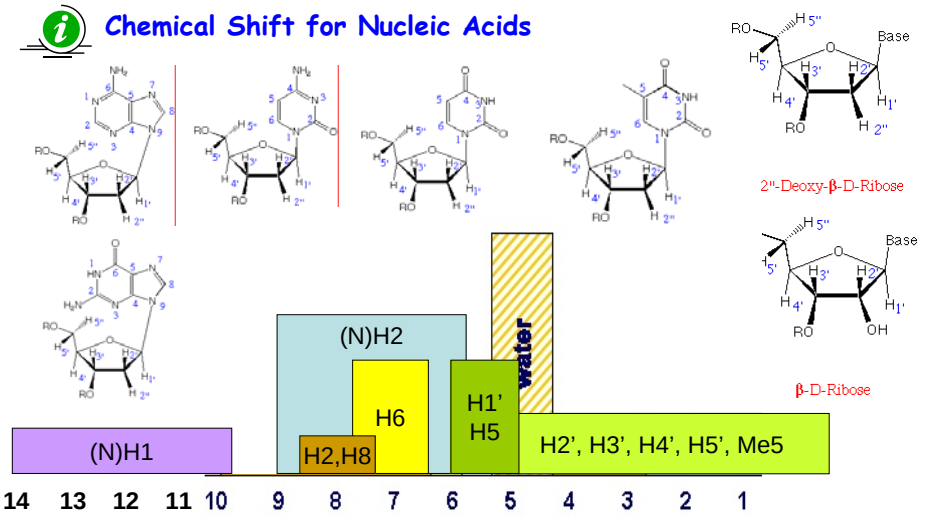
Chemical Shift for Amino Acids



A: Ala, **C**: Cys, **D**: Asp, **E**: Glu, **F**: Phe, **G**: Gly, **H**: His,
I: Ile, **K**: Lys, **L**: Leu, **M**: Met, **N**: Asn, **P**: Pro, **Q**: Gln,
R: Arg, **S**: Ser, **T**: Thr, **V**: Val, **W**: Trp, **Y**: Tyr



Chemical Shift for Nucleic Acids



Imino exchangeable protons resonating at 12-14 ppm are characteristic of imino protons participating in a regular Watson-Crick A.T and G.C base pair. On the other hand, imino resonances at 9-11 ppm are characteristic of imino protons not involved in such Watson-Crick H-bonding.

Steps for doing Biomolecular NMR

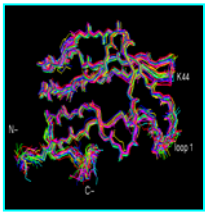
取得樣品 → 取得NMR圖譜 → 分析圖譜結果

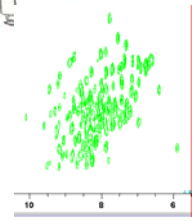
適當的實驗方法

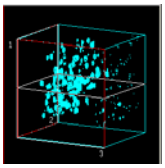
1D, 2D, 3D…..nD

決定生物分子結構

動性、反應機制……







Determination of Protein Structure by NMR

Protein in solution 0.3~0.5ml, ~0.1~1mM

15N and/or 13C , 2H protein

NMR spectroscopy

Useful experiments

Sequence-specific &
Total resonance assignment

Secondary structure

Determining conformational constraints

- NOE: distance restraints
- J-coupling: dihedral angle
- Others: H-bonds……

Calculation of initial structure

Structure refinement

Structure calculation Soft wares

Final 3D Solution NMR Structure

Determination of Protein Structure by NMR

Protein in solution 0.3~0.5ml, ~0.1~1mM

15N and/or 13C , 2H protein

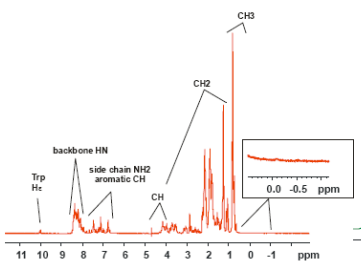
Protein in solution 0.3~0.5ml, ~0.1~1mM

Non-labeled

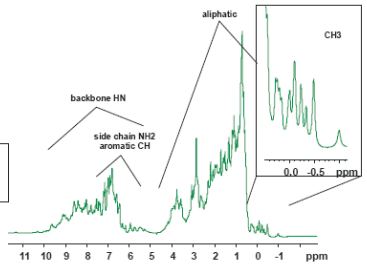
Case 1: Non-labeled protein

Q: Is the protein folded?

Unfolded 20 kDa protein



Folded 20 kDa protein



(Copy from http://www.embl.de/nmr/sattler/teaching)

A: Yes → maybe just non-labeled sample (ex: peptide) or labeled protein

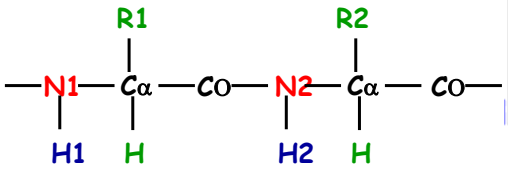
No → adjust sample condition (buffer, pH, temperature……)

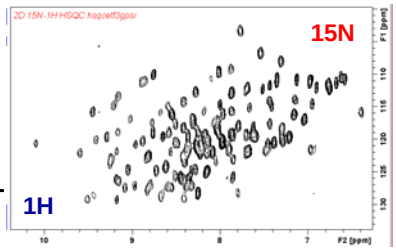
Protein in solution 0.3~0.5ml, ~0.1~1mM

15N labeled

Case 2: 15N-label sample

Q: How's 15N-1H HSQC spectrum?





A: Good → if MW<10KDa, maybe just 15N-labeled

if MW>10KDa, then double labeled

Bad → adjust sample condition (buffer, pH, temperature……)

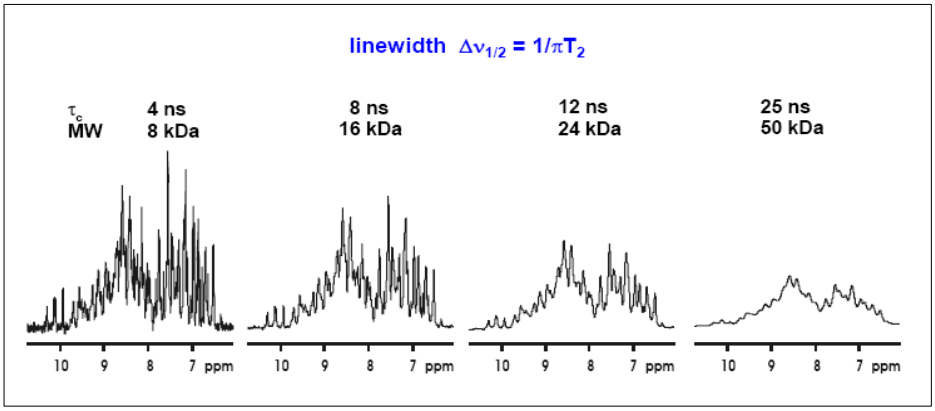
T2 testing (need TROSY or 2H Sample for short T2 sample)

Tips: buffer without 1H-C (ex: phosphate buffer … easy but not necessary good for all system) or using 2H-buffer (ex: 2H-Tris, or 2H-HEPES …. expensive…)

Tips:

•Shorter T2 → Fast decay of NMR signal → broaden line width → poor signal to noise resolution

•Size or shape of the molecule may cause slower tumbling in solution (shorter T2, slower tumbling in solution)



(Copy from http://www.embl.de/nmr/sattler/teaching)

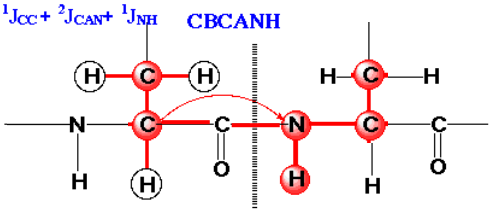
Protein in solution 0.3~0.5ml, ~0.1~1mM

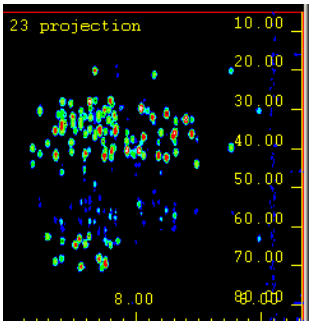
15N/13C labeled

Case 3: 15N/13C double labeled sample

Q: How's single for HNCACB or TR-HNCACB? (or quick test on T2)

$^1J_{CH} + {}^1J_{CC} + {}^1J_{CAN} + {}^1J_{NH}$
$^1J_{CH} + {}^1J_{CC} + {}^2J_{CAN} + {}^1J_{NH}$





A: Good → YES !! Collect more useful data !!

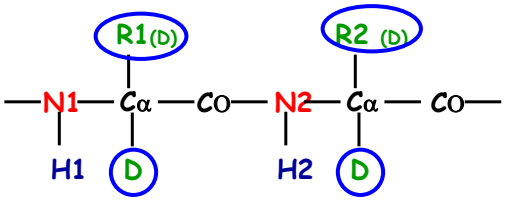
Bad → need 2H labeled?

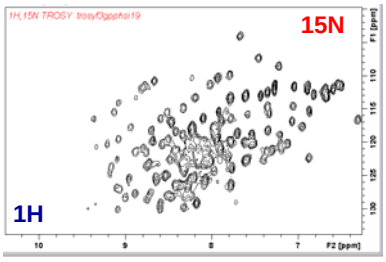
Protein in solution 0.3~0.5ml, ~0.1~1mM

2H/15N labeled

Case 4 : 2H/15N double labeled sample

Q: How's 15N-1H TROSY spectrum?





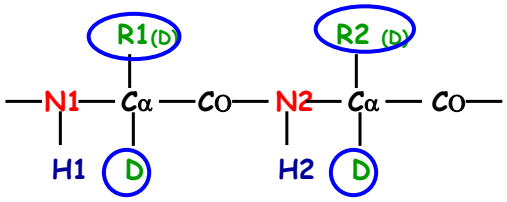
A: Good → YES !! Prepare triple labeled sample.

Bad → adjust sample condition (Can NMR solve the problem?)

Protein in solution 0.3~0.5ml, ~0.1~1mM

2H/15N/13C labeled

Case 5 : 2H/15N/13C triple labeled sample, collect useful NMR data



Tip: Need to pay attention on chemical shift calibration !!

Protein in solution 0.3~0.5ml, ~0.1~1mM

Non-labeled

15N labeled

13C/15N labeled

2H/15N labeled

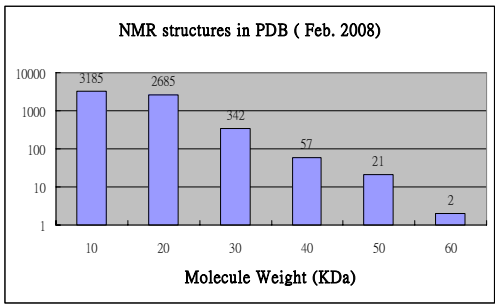
2H/15N/13C labeled

Selected /specific labeled

Protein Size Limitation :

(1) Slow tumbling problem

(2) Signal overlapping problem



瞭解NMR的限制，選擇適合NMR的樣品條件

Determination of Protein Structure by NMR

Protein in solution 0.3~0.5ml, ~0.1~1mM

15N and/or 13C , 2H protein

NMR spectroscopy

Useful experiments

Sequence-specific &
Total resonance assignment

NMR spectroscopy

Structure Based Information

Basic Double and Triple resonance experiments

Backbone assignments

Side-chain assignments

NOE based assignments for structure determination

Molecular Recognition

Filter experiments

"Intermolecular" NOEs

Saturation Transfer Experiments

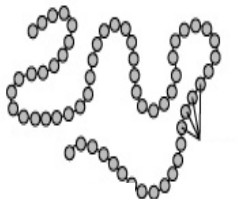
Binding sites interaction

Molecular Dynamics

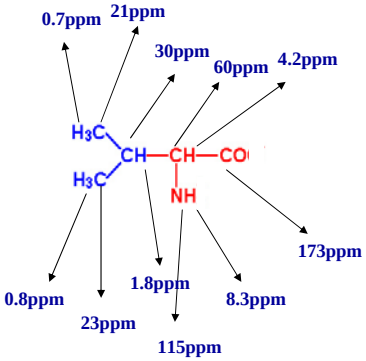
T1, T2 and Hetero Nuclear NOE Experiments

瞭解樣品的特性及研究目標，選擇適合的實驗方法

Sequence-specific and total resonance assignment



Amino Acids Sequence



Given their "NMR ID" for each atom from a specific protein. (Under specific condition, pH, temperature ……)

利用不同實驗方法，取得不同的圖譜訊息，儘量找到所有原子的化學位移

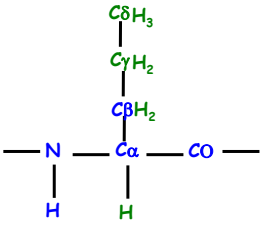
Steps and Experiments for NMR Resonance Assignment

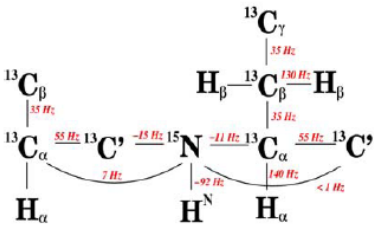
Backbone: Ca, Cb, C', N, NH

HSQC, CBCANH, CBCACONH, HN(CA)CO, HNCO, HN(CO)CA, HNCA

Side chain: all others (especially CHs)

TOCSY-HSQC, HCCCONH, CCCONH, HCCH-TOCSY





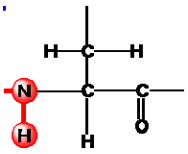
<Step 1 > Backbone Assignment

<Step 1.1 >

Backbone: Ca, Cb, C', N, NH

HSQC, CBCANH, CBCACONH, HN(CA)CO, HNCO, HN(CO)CA, HNCA

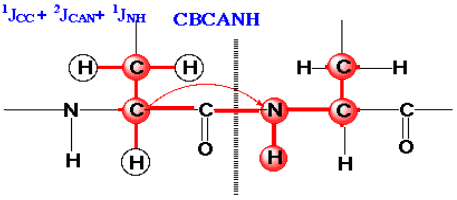
HSQC



$^1J_{CH}$ + $^1J_{CC}$ + $^1J_{CAN}$ + $^1J_{NH}$

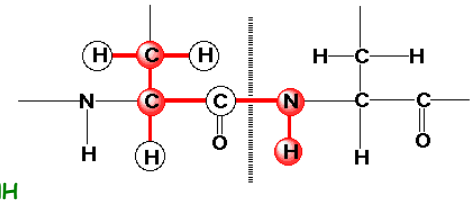
$^1J_{CH}$ + $^1J_{CC}$ + $^2J_{CAN}$ + $^1J_{NH}$

CBCANH



$^1J_{CH}$ + $^1J_{CC}$ + $^1J_{CON}$ + $^1J_{NH}$

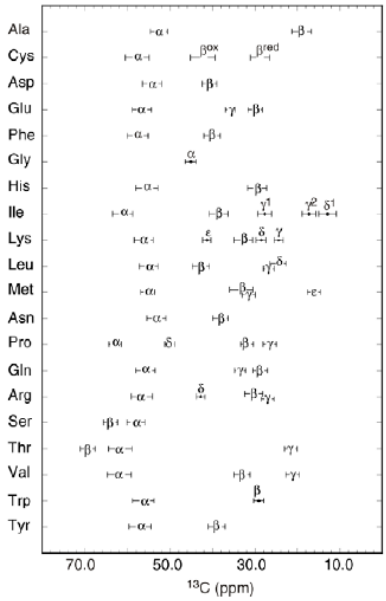
CBCA(CO)NH

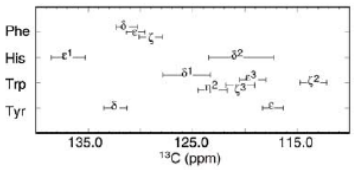


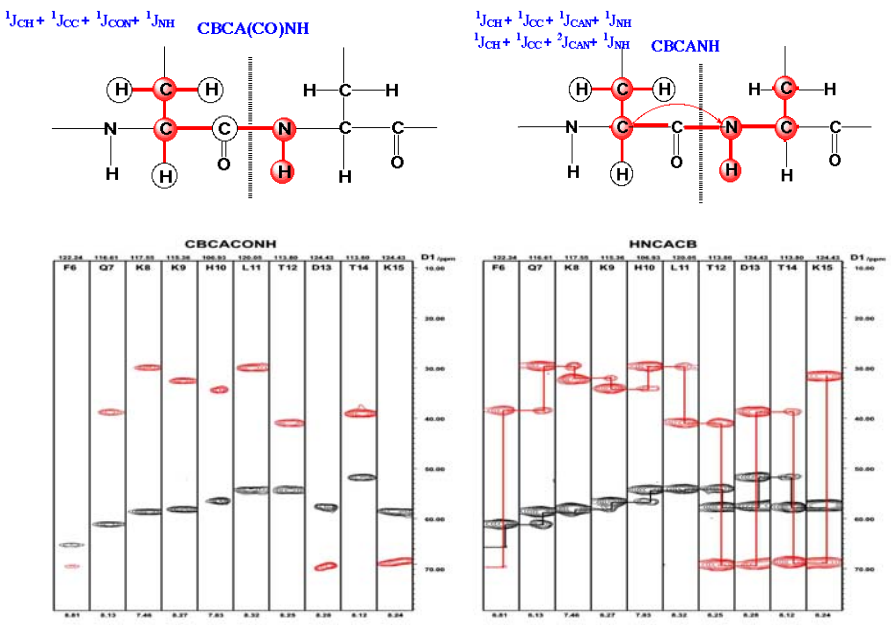
➔Sequential assignment

➔chemical shifts of Ca, Cb, NH

Trick : 13C Chemical shifts are amino acid specific (not for CO)



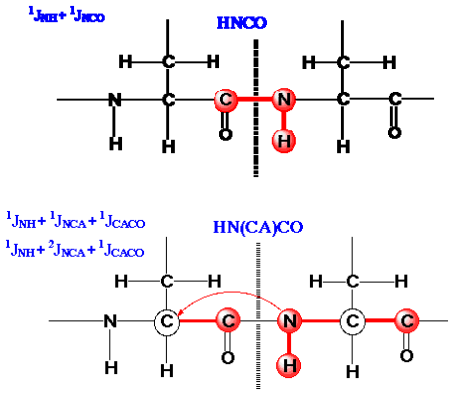




‹Step 1.2 ›

Backbone: Ca, Cb, C', N, NH

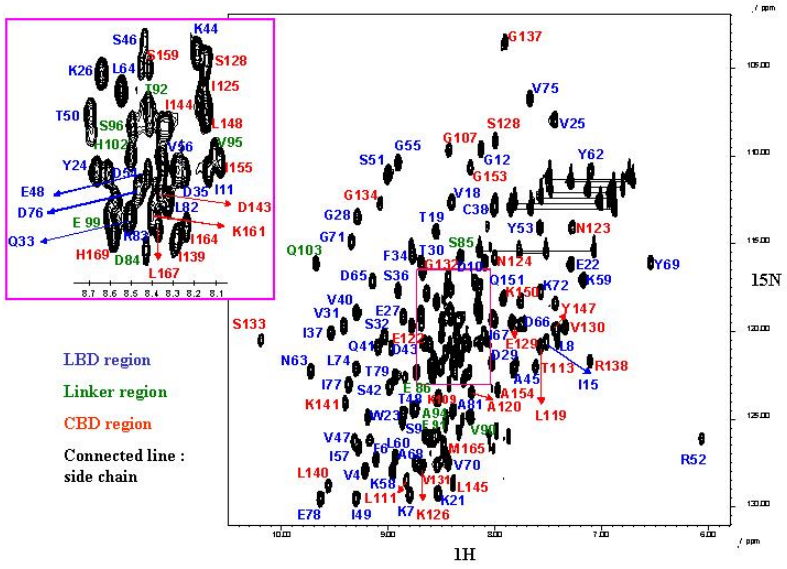
HSQC, CBCANH, CBCACONH, **HN(CA)CO, HNCO,** HN(CO)CA, HNCA



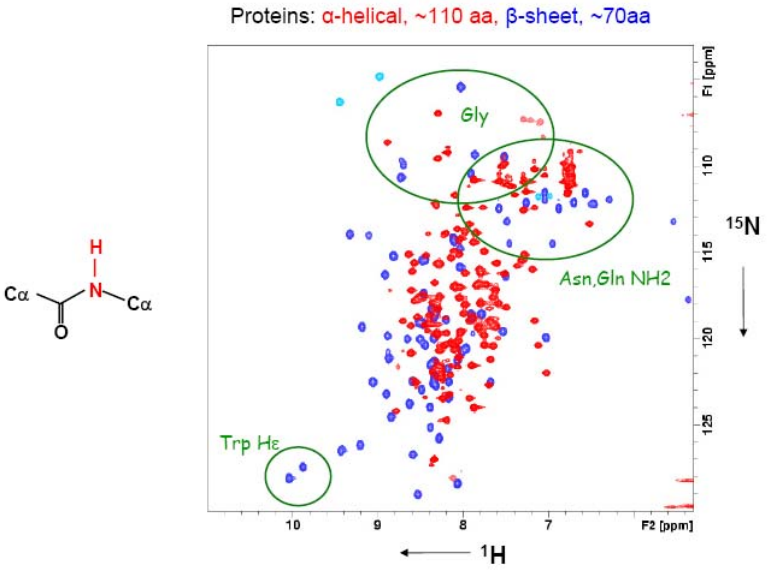
➔confirm the previous sequential assignment

➔adding CO chemical shifts

HSQC with assignment (protein with 175 amino acids)



Tip: The NMR Fingerprint : 2D 1H-15N HSQC spectra



(Copy from http://www.embl.de/nmr/sattler/teaching)

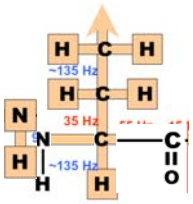
<Step 2> Side Chain assignment

all other atoms (i.e. total assignment, especially H)

1H : TOCSY-HSQC, HCCCONH → based on backbone NH assignment

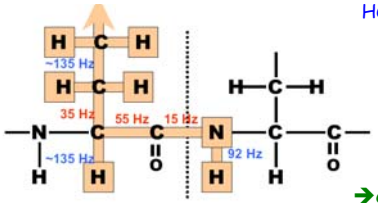
13C: CCCONH → based on backbone NH assignment

1H and 13C : HCCH-TOCSY, HCCH-COSY → based on CH correlations



TOCSY-HSQC : intra residue H

HCCCONH : inter residue H or CCCONH: inter residue C



➔confirm the previous sequential assignment

➔adding side chain H & C chemical shifts

3D Hetero Nuclear

1H-15N-1H

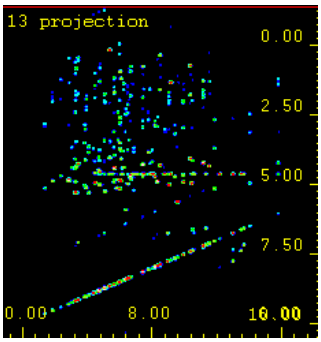
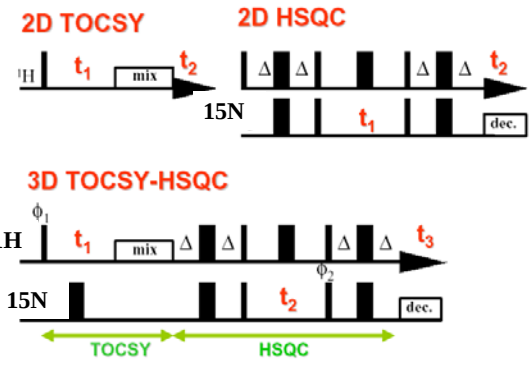
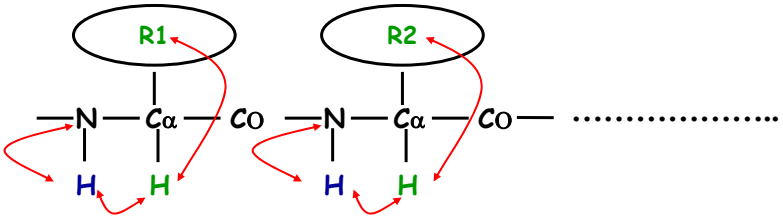
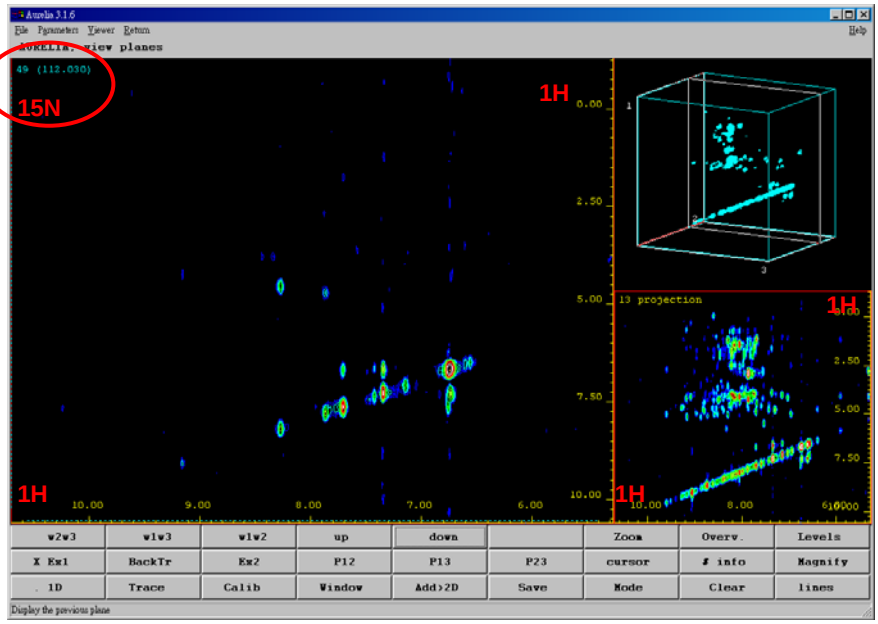


Fig. 1. The 3D pulse sequence.

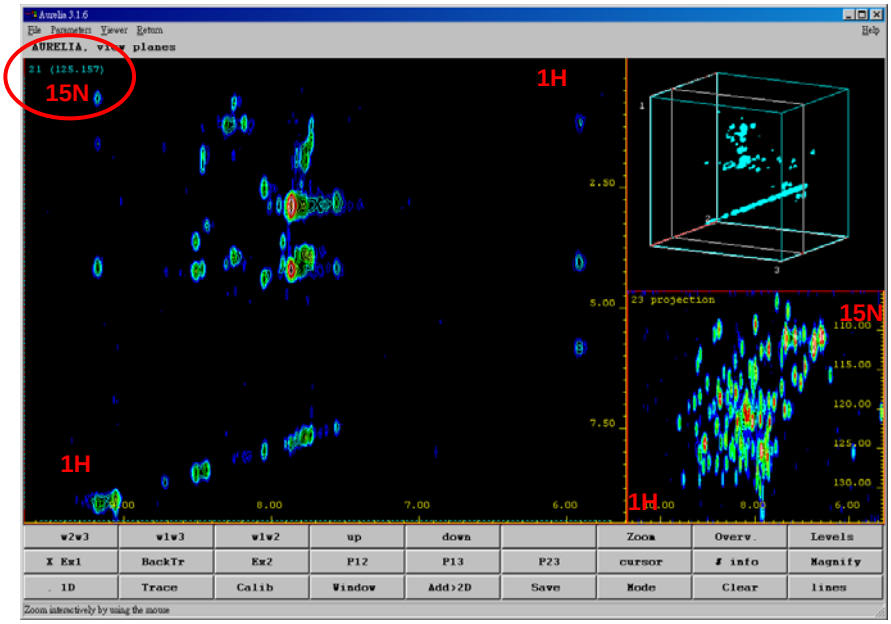




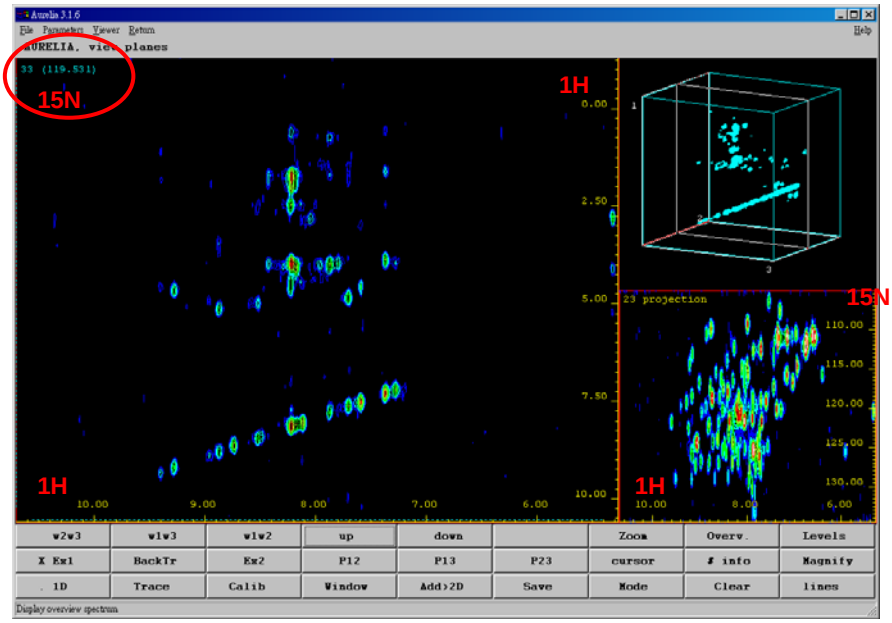
TOCSY-HSQC



TOCSY-HSQC

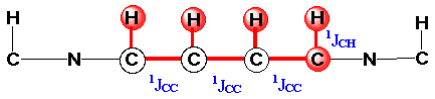


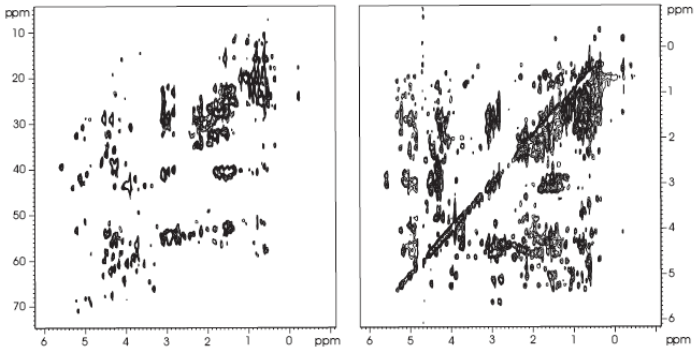
TOCSY-HSQC

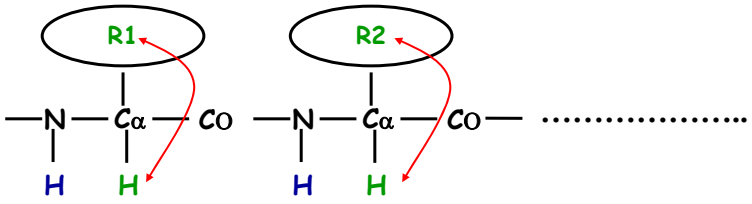


3D Hetero Nuclear

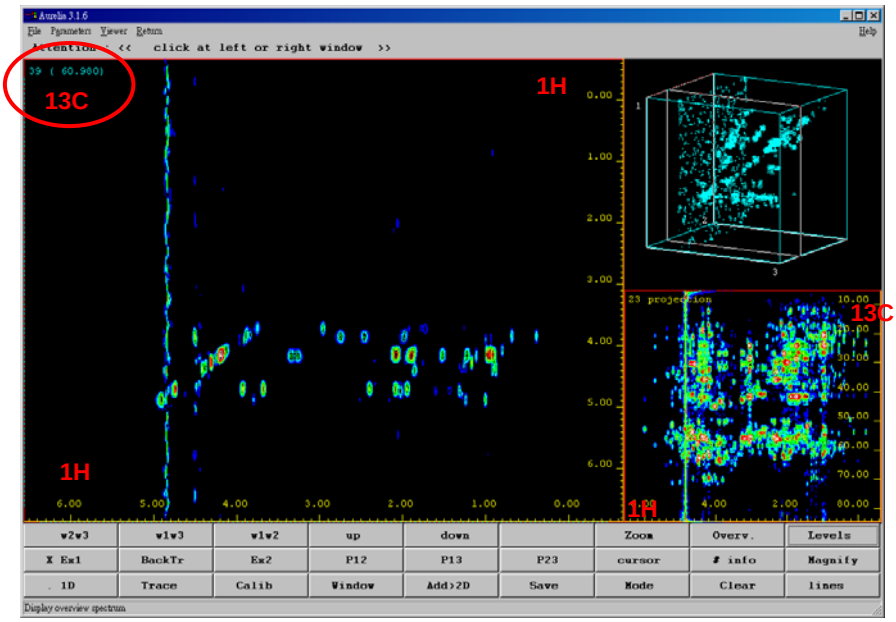
1H-13C-1H



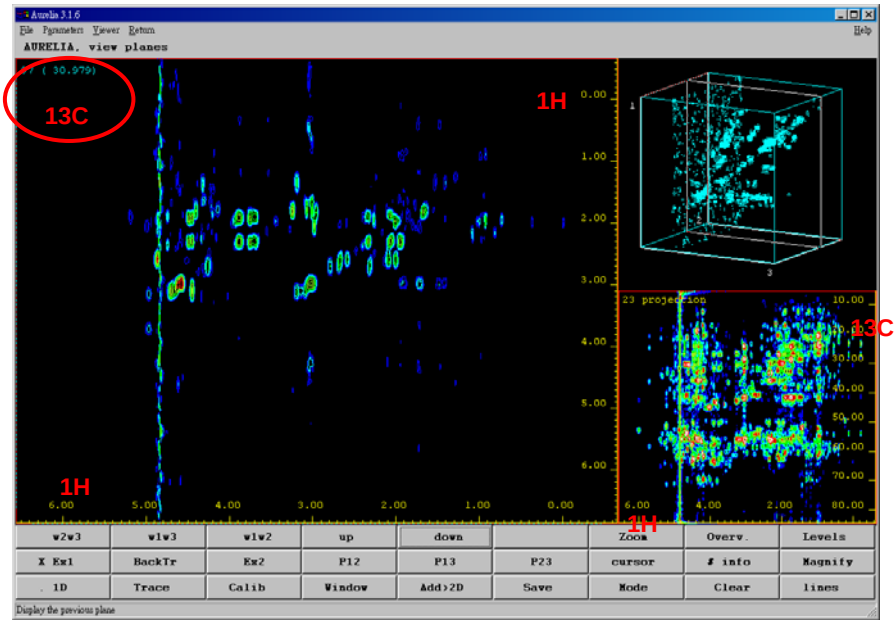




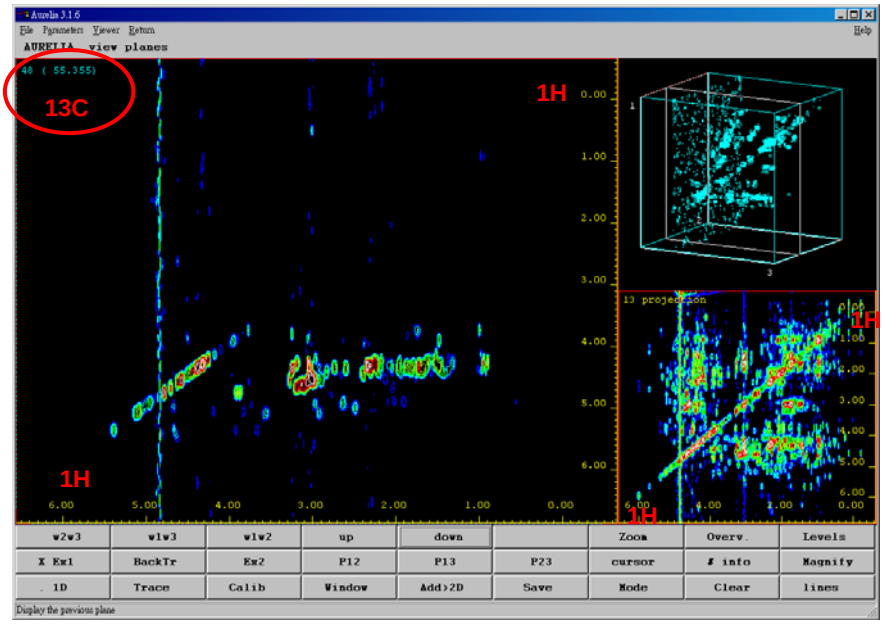
HCCH-TOCSY



HCCH-TOCSY



HCCH-TOCSY



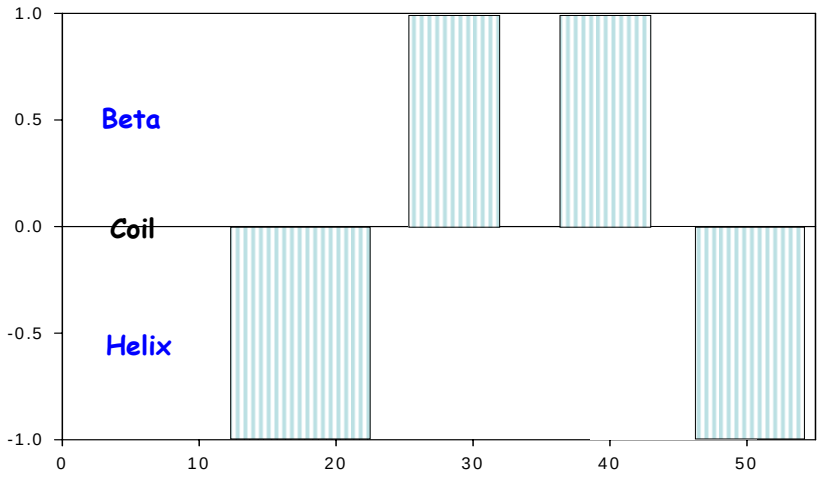
Total Assignment of Residues

AA	N (H)	CO	C^{α}, (Ha)	C^{β} (Hb)	other
G2					
Q3			53.799 (4.522)	(1.909,2.176)	C^{γ}, 32.548(*, *); $N^{\varepsilon 2}$, 110.822 (7.356,6.755)
V4	124.418 (8.674)	175.6	61.663 (4.421)	31.399 (1.899)	$C^{\gamma 1}$, 20.907 (0.844); $C^{\gamma 2}$, 20.985 (0.654)
V5	126.762 (9.148)	174.7	58.928 (4.242)	33.586 (1.72)	$C^{\gamma 1}$, 19.266 (0.531); $C^{\gamma 2}$, 19.11 (0.437)
Q6	123.48 (8.394)	174.7	54.24 (4.958)	28.274 (2.098,1.441)	C^{γ}, 33.017 (2.409 *); $N^{\varepsilon 2}$, 111.76 (7.762,6.76)
F7	126.762 (9.051)	174.7	54.865 (5.033)	38.224 (3.488,2.837)	
K8	128.637 (8.663)	175.1	54.709 (4.195)	32.599 (1.5,1.109)	C^{γ}, 24.579 (1.309,1.121); C^{δ}, 28.017 (0.76 *); C^{ε}, 40.986 (2.914 *)
L9	120.199 (7.372)	175.5	54.006 (4.13)	40.646 (1.238,1.961)	C^{γ}, 25.673 (1.59); $C^{\delta 1}$, 23.329 (0.812); $C^{\delta 2}$, * (0.461)
S10	124.418 (8.48)	171.2	56.975 (4.397)	63.616 (3.773,2.509)	
D11	115.511 (8.028)	176.2	54.553 (4.29)	39.787 (2.655,2.528)	
I12	118.323 (8.157)	175.6	59.475 (4.257)	37.962 (1.858)	$C^{\gamma 1}$, 25.517 (1.223,1.018); $C^{\gamma 2}$, 16.923 (0.76); $C^{\delta 1}$, 12.704 (0.584)

- Chemical shift value for each atom if possible
- Secondary structure information could be extracted (Chemical Shift Index)

Chemical Shift Index (CSI plot)

HA, CA, CB, CO



Determination of Protein Structure by NMR

Protein in solution 0.3~0.5ml, ~0.1~1mM

NMR spectroscopy

Sequence-specific & Total resonance assignment

Determining conformational constraints

Calculation of initial structure

Structure refinement

15N and/or 13C , 2H protein

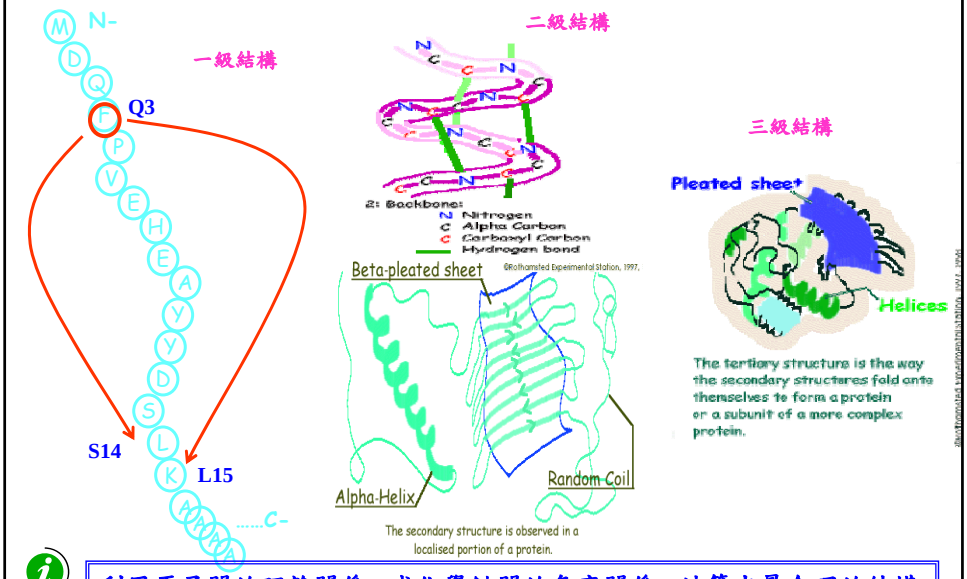
Useful experiments

Secondary structure

·NOE: distance restraints
·J-coupling: dihedral angle
·Others: H-bonds……

Structure calculation Soft wares

Determine conformational constraints



利用原子間的距離關係，或化學鍵間的角度關係，計算出最合理的結構

Steps and Experiments for Conformational Constraints

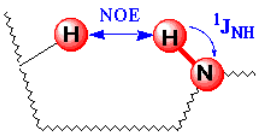
Constraints based on Distance

Nuclear Overhauser Effect (NOE) NOE ~ $<1/r^6>$

Experiments: 2D, 3D, 4D….. NOESY

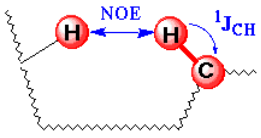
1H-15N

3D 15N-edit NOESY-HSQC



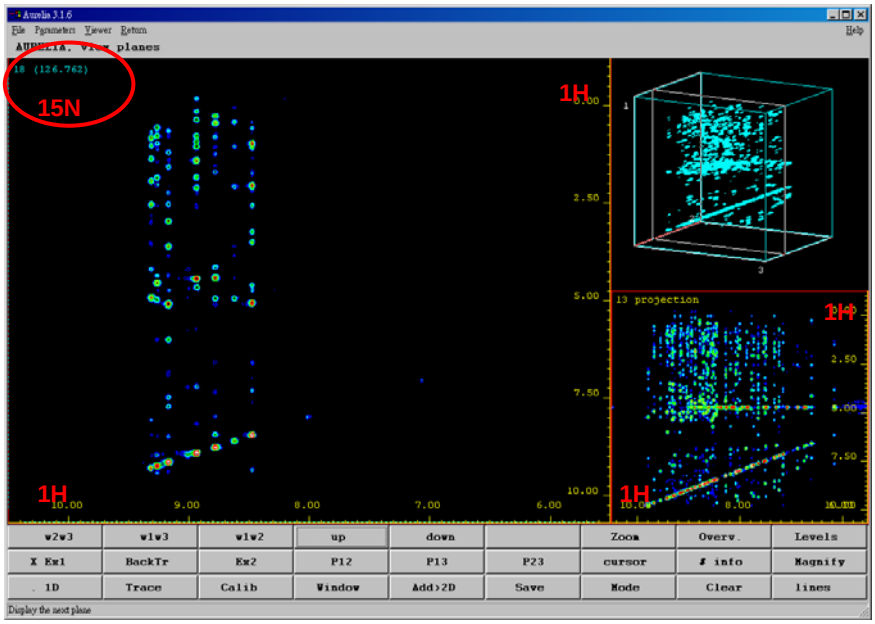
1H-13C

3D 13C-edit NOESY-HSQC

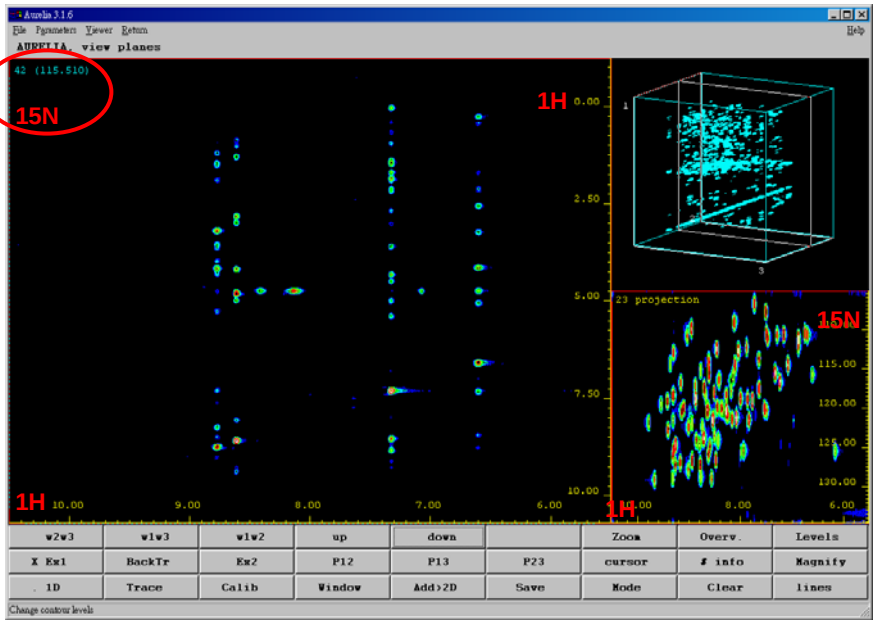


➔Only those two atoms which close in space can contribute a NOE peak

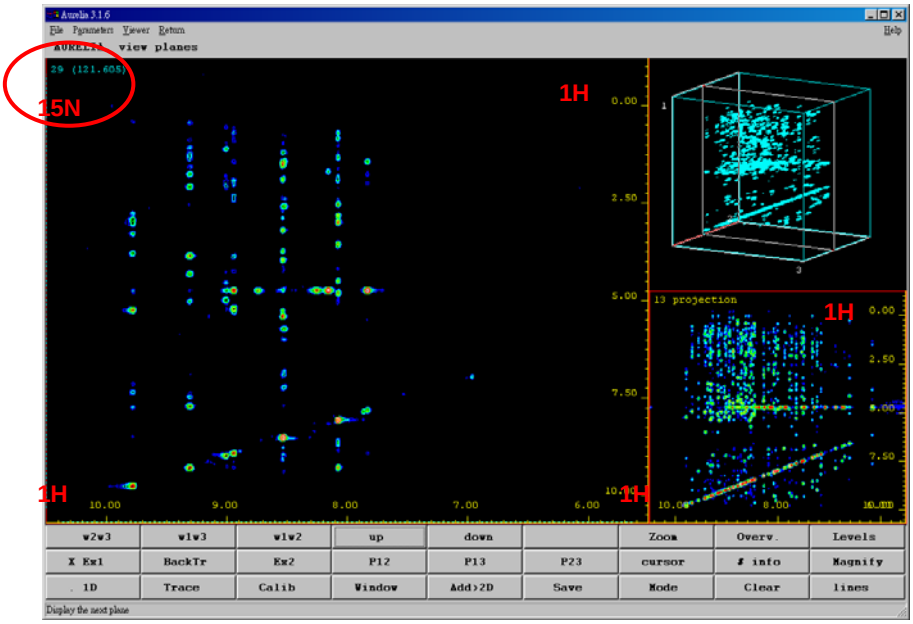
NOESY-HSQC



NOESY-HSQC



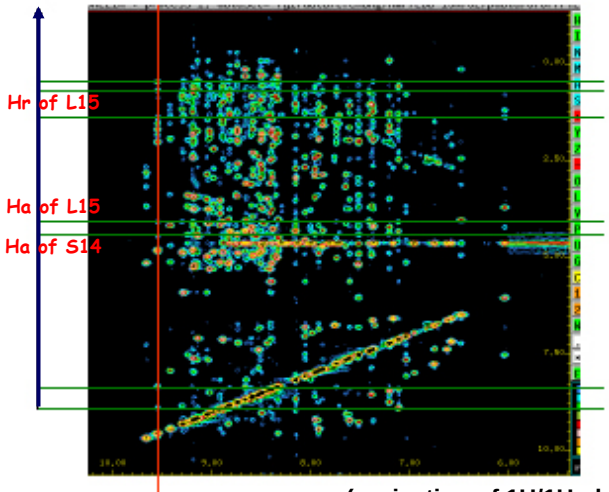
NOESY-HSQC



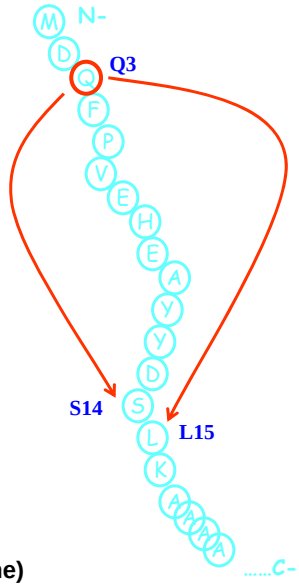
15N-NOESY-HSQC

1H-15N-1H

Looking for H from other residues which close to NH of residue Q3

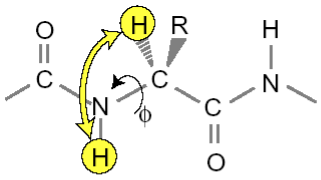


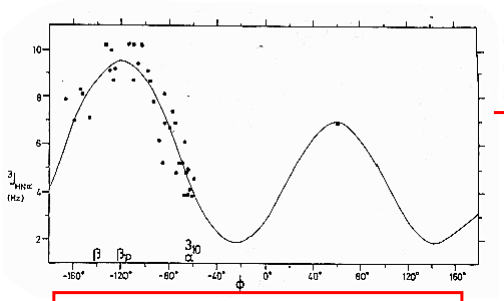
(projection of 1H/1H plane)



Constraints based on Dihedral Angles

- HNHA, HNHB to measure coupling constant → dihedral angles can be derived
- Based on Backbone chemical shifts → dihedral angles can be derived (TALOS)





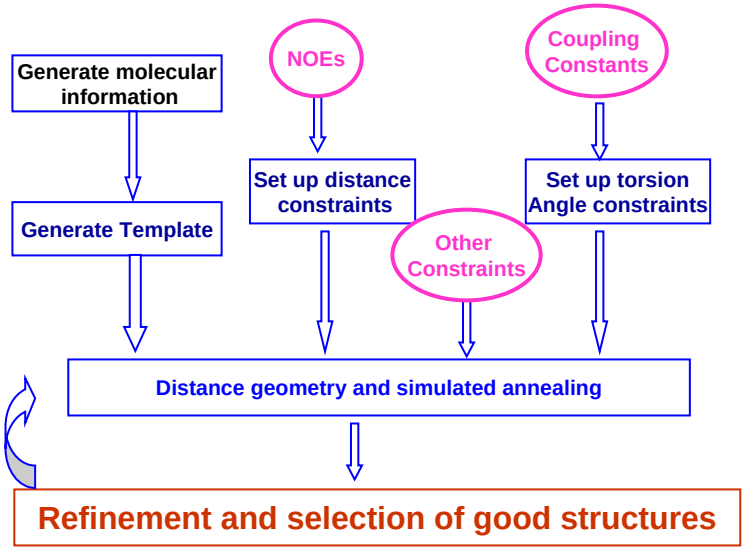
$$J(\theta) = A\cos^2(\theta) + B\cos(\theta) + C$$

	Bond Angle (deg)	
	ϕ	Ψ
β	-139	+135
βp	-119	+113
α-helix	-57	-47
3_{10}-helix	-49	-26
π-helix	-57	-70
Polyproline I	-83	+158
Polyproline II	-78	+149
Polyglycine II	-80	+150

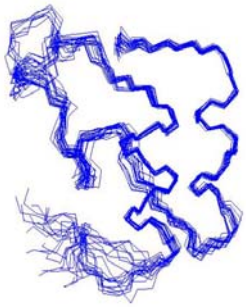
Adapted from G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.* 23, 283-437(1968); IUPAC-IUB Commission on biochemical Nomemclature, *Biochemistry* 9, 3471-3479 (1970).

Calculation of initial structure

Constraints in → Structures out



Final Structure (ex: 20 structures)



r.m.s.d. from average coordinates; ordered residues	
Backbone atoms	0.28
Heavy atoms	0.91
Ramachandran plot statistics	
Most favored regions (%)	76.4
Additional allowed regions (%)	23.6
Generously allowed (%)	0.0
Disallowed regions (%)	0.0

Why Biomolecular NMR ?

Structure Determination in Atomics Resolution

- Sample in Solution
  - Native-like Condition
- Structure Biology
  - Structural Basis Biological process / questions

Molecular Recognition

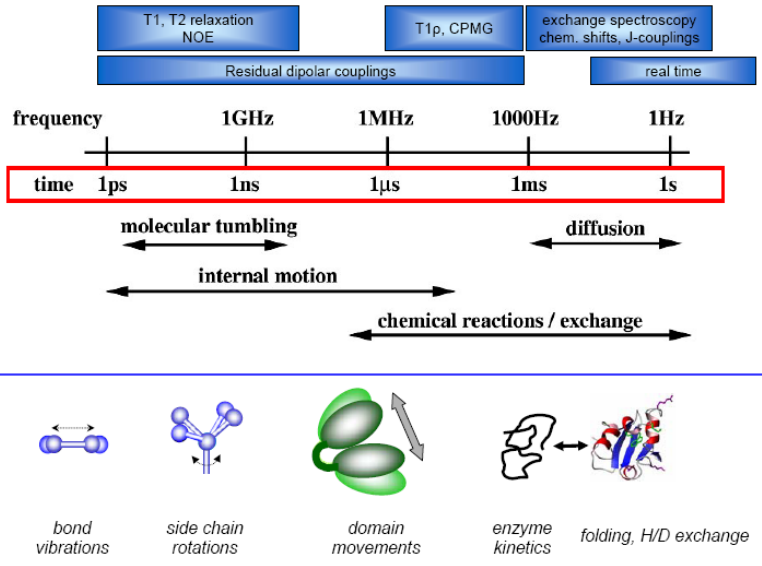
- Protein-Protein, Protein-Nucleic Acid, Protein-Ligand

Molecular Dynamics

- Conformational dynamics
- Folding

Other Applications

NMR time scales and dynamics in biology



(From http://www.embl.de/nmr/sattler/teaching)

Protein Backbone Dynamics by NMR in picosecond to nanosecond (ps-ns)

- Spin-lattice relaxation T_1
- Spin-spin relaxation T_2
- ^1H-^{15}N NOE factor

For each amino acid

$I(t)=I_0\exp(-t/T_{1,2})$

$\langle NOE\rangle=\langle I_{H,sat}/I_{un\text{-}sat}\rangle$

⇩

Relates T_1, T_2, NOE to special density function j(w)

Model Free Analysis

$1/T_1=(d^2/4)[j(\omega_H-\omega_N)+3\,j(\omega_N)+6\,j(\omega_H+\omega_N)]+c^2\,j(\omega_N)$

$1/T_2=(d^2/8)[4\,j(0)+j(\omega_H-\omega_N)+3j(\omega_N)+6\,j(\omega_H)+6j(\omega_H+\omega_N)]+(c^2/6)[4j(0)+3\,j(\omega_N)]+R_{ex}$

$NOE=1+(d^2/4R_1)(\gamma_H/\gamma_N)+[6\,j(\omega_H+\omega_N)-j(\omega_H-\omega_N)]$

(Clore et al., 1990)

Reduced Spectral Density Mapping

$\sigma_{NH}=R_1(NOE-1)(\gamma_H/\gamma_N)$

$J(0.87\omega_H)=4\,\sigma_{NH}/(5d^2)$

$J(\omega_N)=(4R_1-5\,\sigma_{NH})/(3d^2+4c^2)$

$J(0)=(6R_2-3R_1-2.72\,\sigma_{NH})/(3d^2+4c^2)$

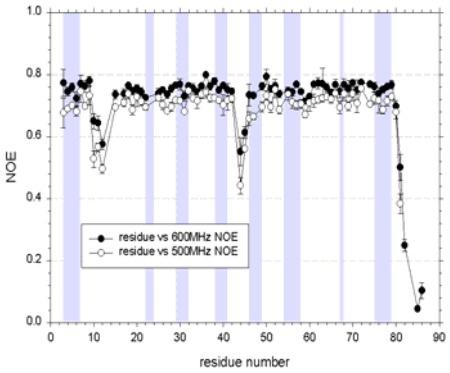
(Farrow et al, 1995)

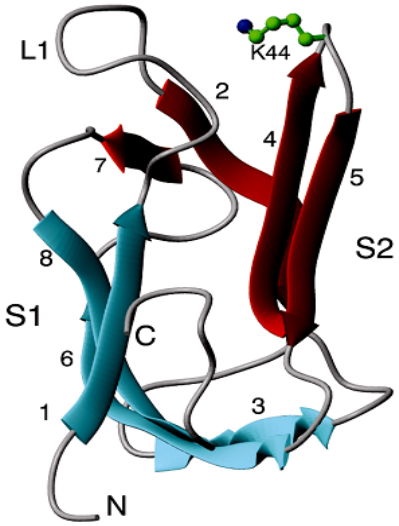
From $j(\omega)$ to S, τ and R_{ex}

- Order parameter, S^2: Degree of internal motion
- Correlation time, τ: Rate of molecule motion
- Exchange rate, R_{ex}: Conformational exchange

- $J(0)$, $J(\omega_N)$ decrease: Greater internal motion
- $J(0.87\omega_H)$ increase: Greater internal motion
- Significantly increased J(0): Chemical exchange

Internal motion from Backbone dynamics data

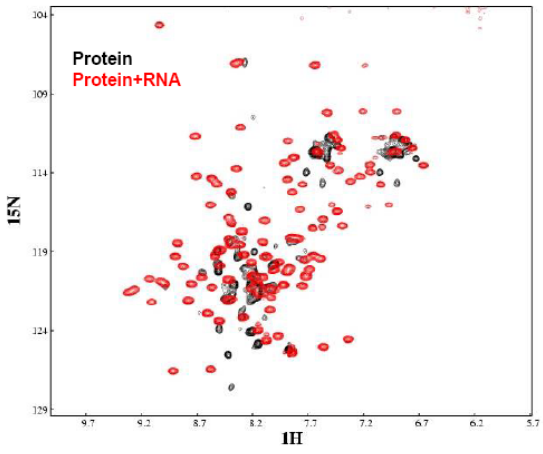




Other Applications

Folding of a protein upon ligand binding monitored by NMR fingerprint spectra

NMR spectrum of a novel RNA binding domain
when bound to an RNA oligonucleotide

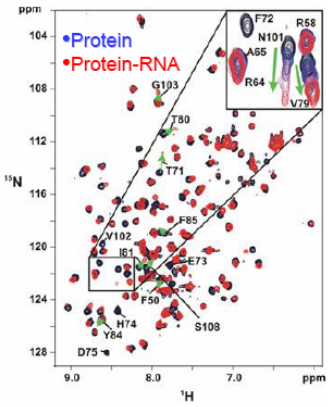


(From http://www.embl.de/nmr/sattler/teaching)

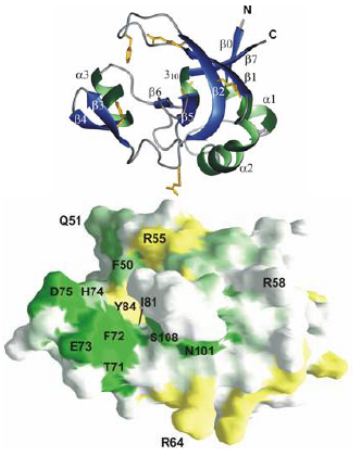
Other Applications

The NMR „band shift" and binding site mapping

Chemical shift perturbation upon ligand binding



Mapping of the ligand binding site onto the structure

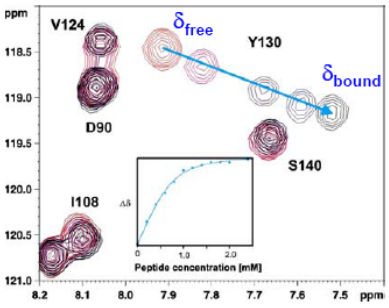


(From http://www.embl.de/nmr/sattler/teaching)

Other Applications

Characterizing binding equilibria using NMR titrations
(fast exchange)

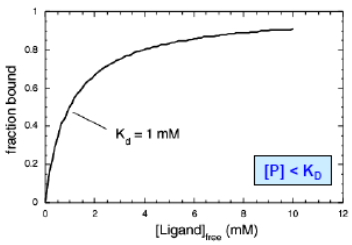
Binding in fast exchange on the NMR chemical shift time scale



Fraction bound

[PL] ~ $\Delta\delta = \delta_{obs} - \delta_{free} = f([L_{tot}])$

Equilibrium Binding Constants from the Langmuir Isotherm



$$P + L \leftrightarrow PL \qquad K_d = \frac{[P]\,[L]_{free}}{[PL]}$$

(From http://www.embl.de/nmr/sattler/teaching)

Biomolecular NMR in HFNMRC

Useful Experiments as Standard Parameter Sets

Structure Determination in Atomics Resolution

- Basic Double and Triple resonance experiments
- Fast Data Acquisitions : PR_NMR and APSY

Molecular Recognition

- Filter experiments
- Saturation Transfer Experiments

Molecular Dynamics

- 1H-15N T1
- 1H-15N T2
- Hetero Nuclear NOE

Software for Data Analysis

NMR data processing software

XWINNMR/Topspin *(process NMR data on IRIX 6.X , Linux, Windows)*

nmrPipe *(process NMR data on IRIX6.X & Linux)*

NMR data analysis software

AURELIA *(analyze NMR data on IRIX 6.X , Linux, Windows)*

nmrDraw *(analyze NMR data on IRIX 6.X & Linux)*

nmrView *(analyze NMR data on IRIX 6.X & Linux)*

Sparky *(analyze NMR data on Linux, Windows)*

CARA (analyze NMR data on Windows)

NMR prediction software

CSI Chemical Shift Index *(making consensus plot on IRIX)*

TALOS *(dihedral angles prediction on IRIX & Linux)*

Structure calculation program

XPLOR *(structure calculation on IRIX)*

CNS *(structure calculation on IRIX & Linux)*

ARIA *(auto NOE assign and structure calculation on IRIX & Linux)*

CYANA *(auto NOE assign and structure calculation on IRIX & Linux)*

Structure display & analysis software

DISCOVERY STUDIO (on Linux and PC) 

MOLMOL (on IRIX & Linux)

PROCHECK *(structure analysis on IRIX)*

GRASP *(on IRIX)*

Others

MODELFREE (Dynamic analysis on Euler)

REDCAT (RDC analysis on Linux)

DYNAMO (RDC analysis on Linux)

New Software in HFNMRC

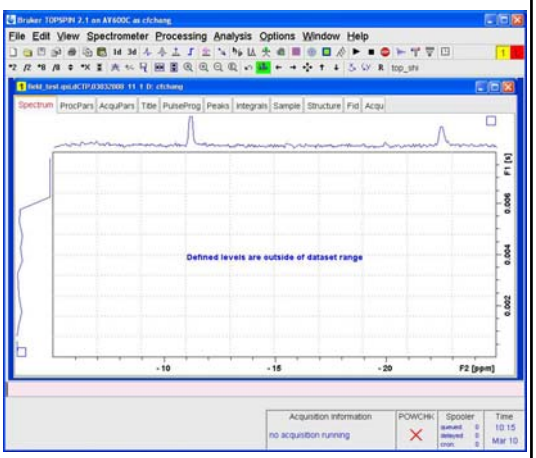
(Topspin2.1)

Spectrometer support - TopSpin

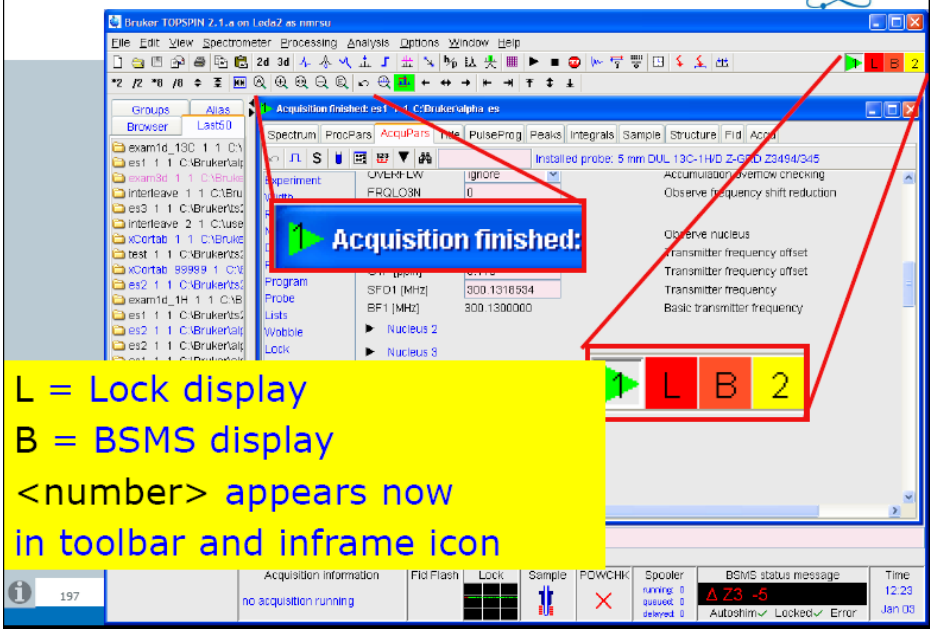
	Avance III	Avance II	Avance I	D*X
TopSpin 2.1	✓	✓	✓	-
TopSpin 2.0	✓	✓	✓	-
TopSpin 1.3	-	✓	✓	✓
TopSpin 1.2	-	-	✓	-
TopSpin 1.1	-	-	-	-

Note: Please use Topspin2.1 for AV600_CHEM. Other version might cause unexpected problem.

Step 1: Starting topspin2.1
Login into PC
Double click on “topspin2.1” icon
(The topspin2.1 window will pop out)



Window numbering



New on Shimming

- Topsim

➢**Topshim (regular tube)**

➢**Topshim shigemi (shigemi tube)**

New on Data Acquisition

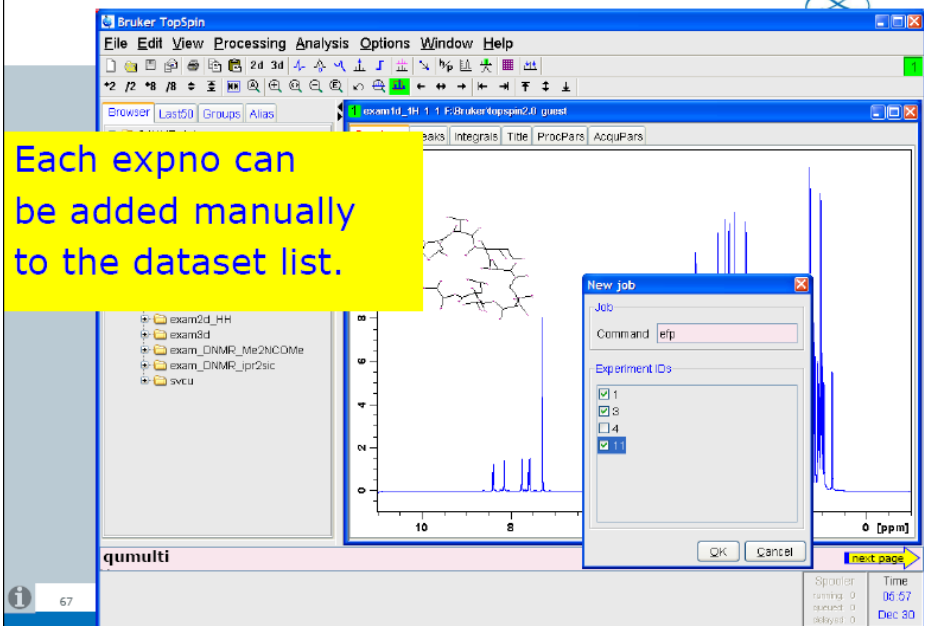
•Spooler

Command spooler

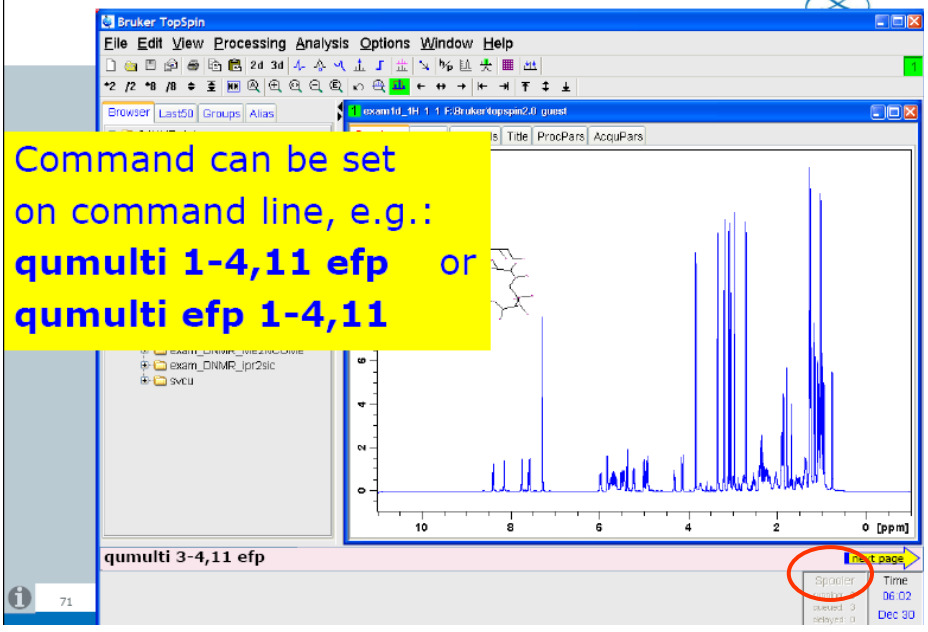
It is now possible to define the experiment number on which a command should be queued:

qumulti atmulti

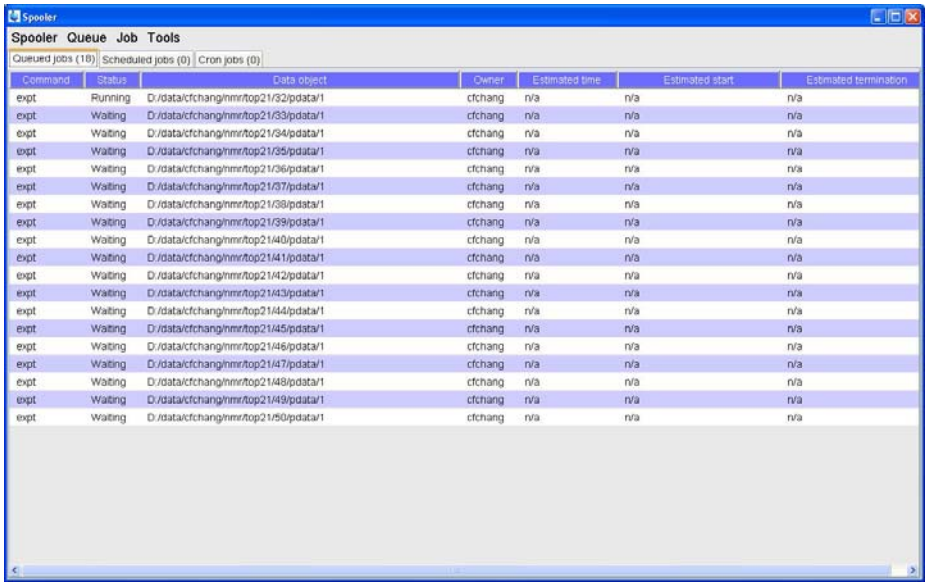
qumulti

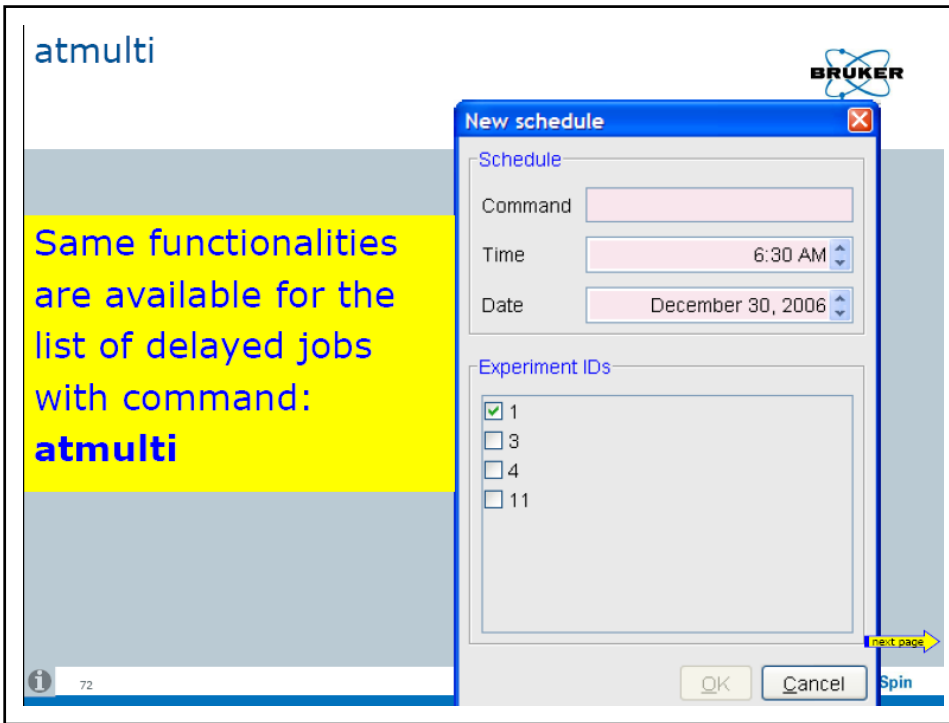


qumulti



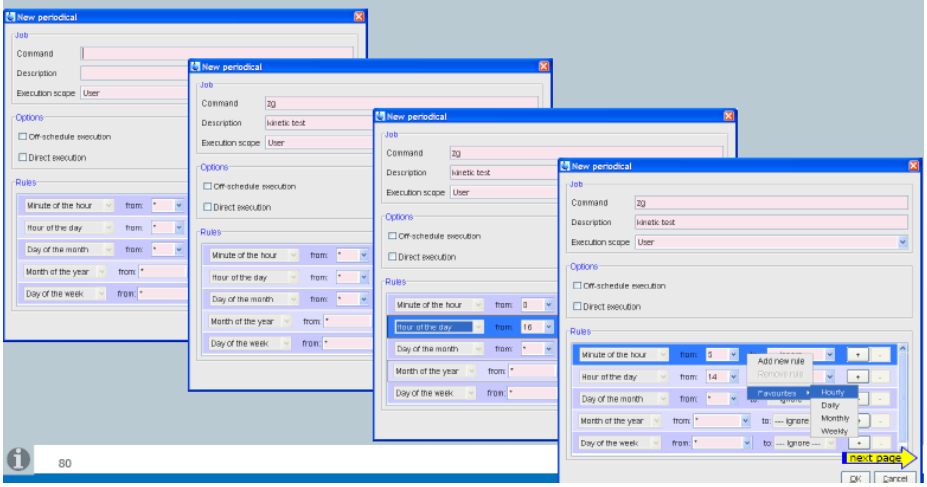
(click on Spooler → you can check or modify the running status)





New command: **cron**

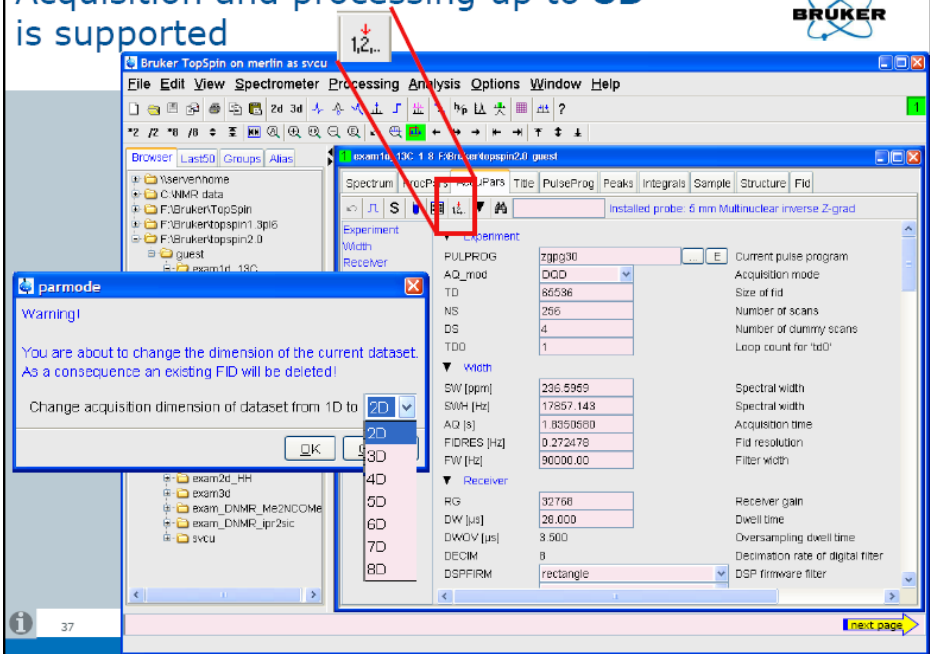
cron allows executing a TopSpin command periodically at a specific time/date:



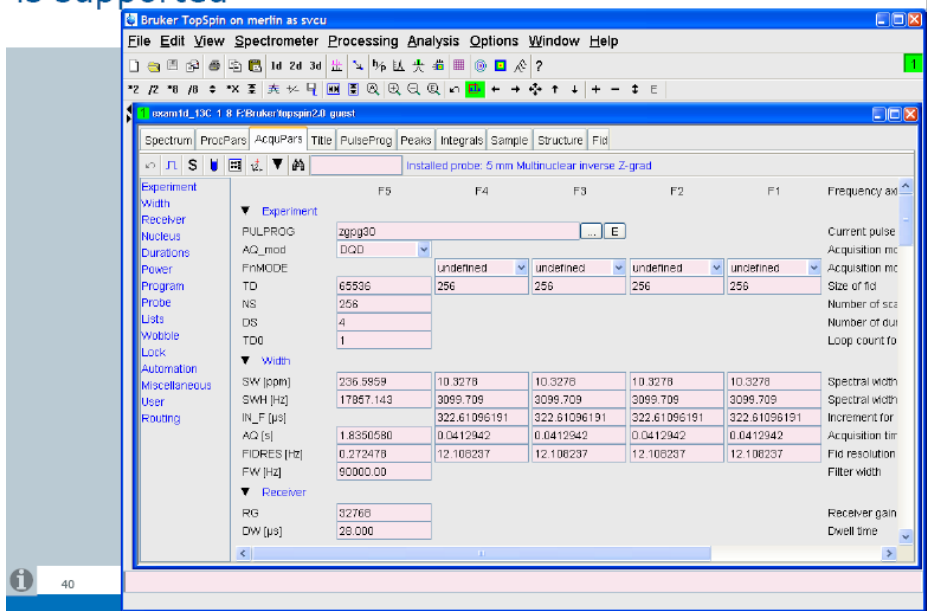
New Experiments

- Up to 8D
- APSY

Acquisition and processing up to **8D** is supported



Acquisition and processing up to **8D** is supported



Acquisition and processing up to **8D** is supported

TopSpin 2.1 now provides examples for 4D experiments (HSQC-NOESY-HSQC):

- 4D parameter sets (ending with 4D)

and

- 4D pulse programs (ending with 4d).

APSY

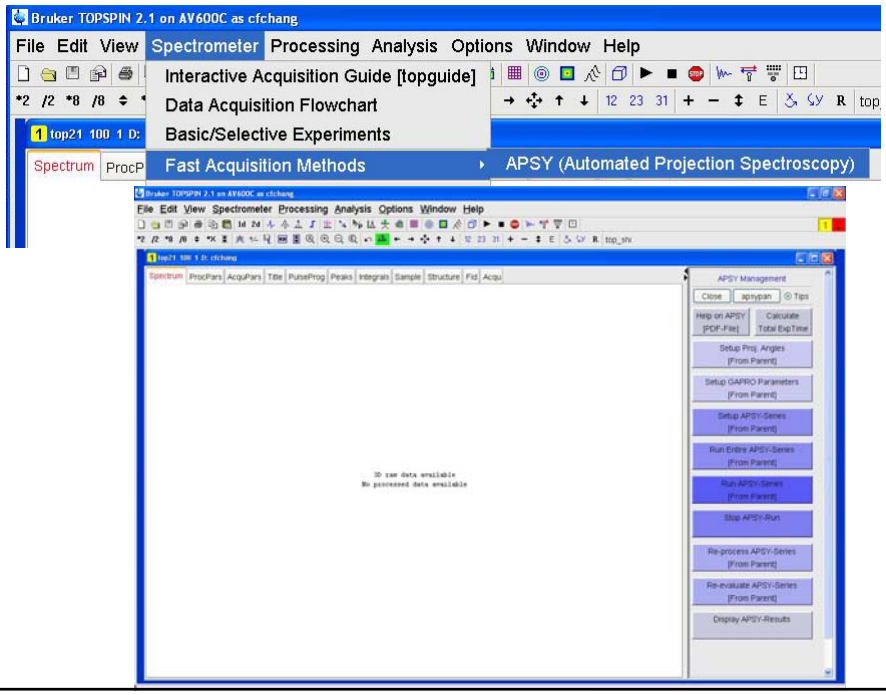
S. Hiller, F. Fiorito, K. Wüthrich and G. Wider, Proc. Nat. Acad. Sci. USA 102, 10876-10881 (2005). Automated projection spectroscopy (APSY).

TopSpin 2.1 supports the fast NMR method APSY (Automated Projection Spectroscopy)

APSY can be started under:

Spectrometer → Fast Acquisition Methods

APSY requires a separate license. A free demo license can be ordered anytime under: www.bruker-biospin.com

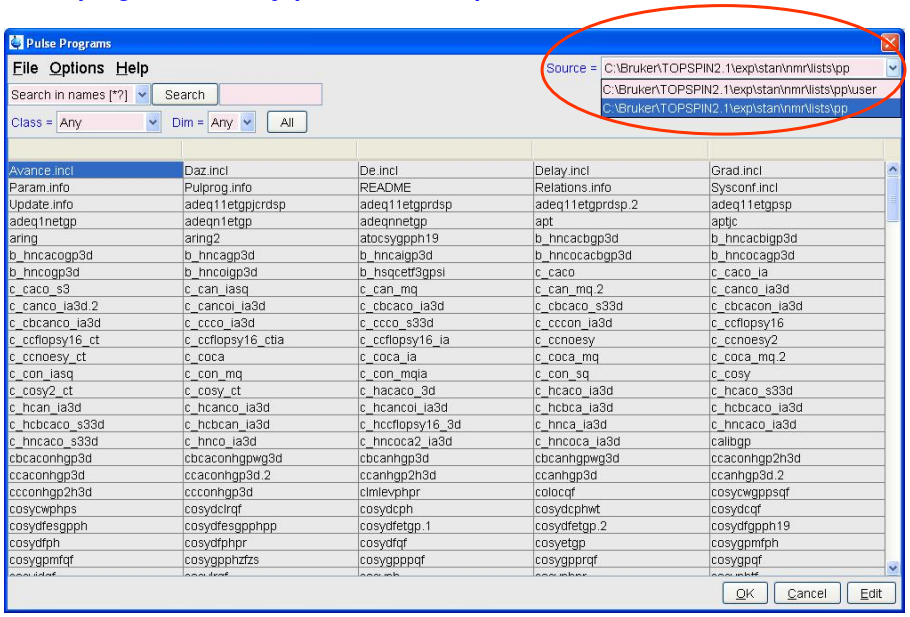


New on pp & par directory

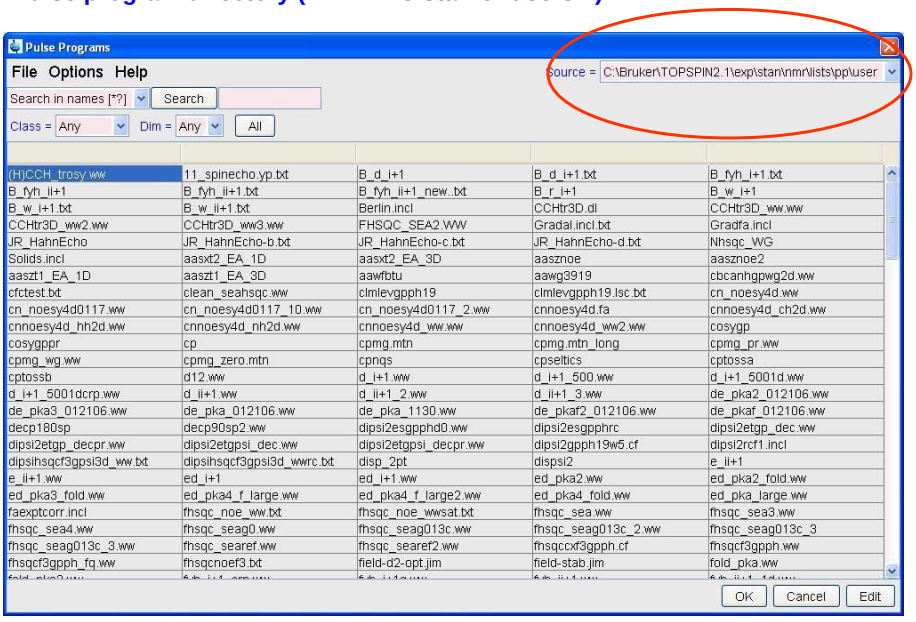
•Pulse program

•Parameter Set

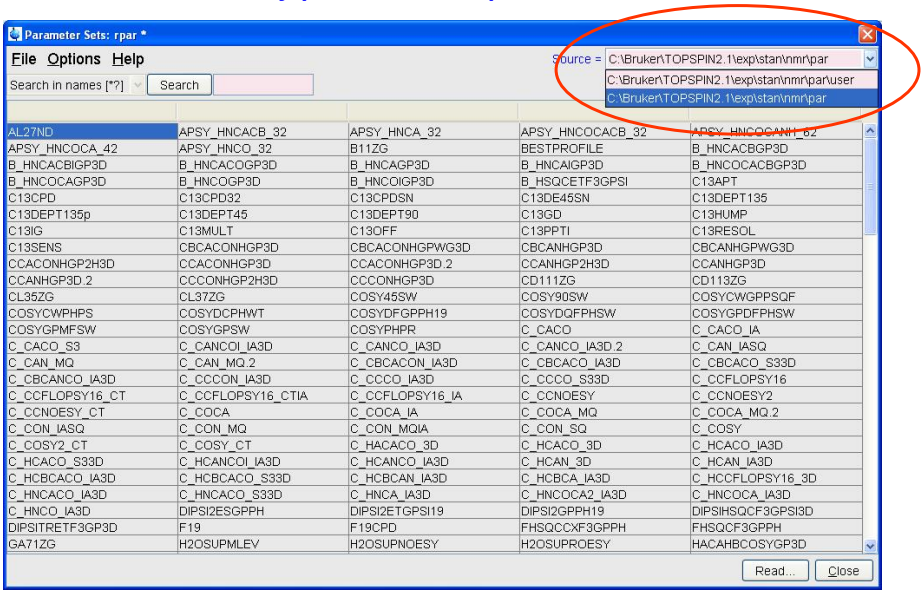
Pulse program directory (Bruker default)



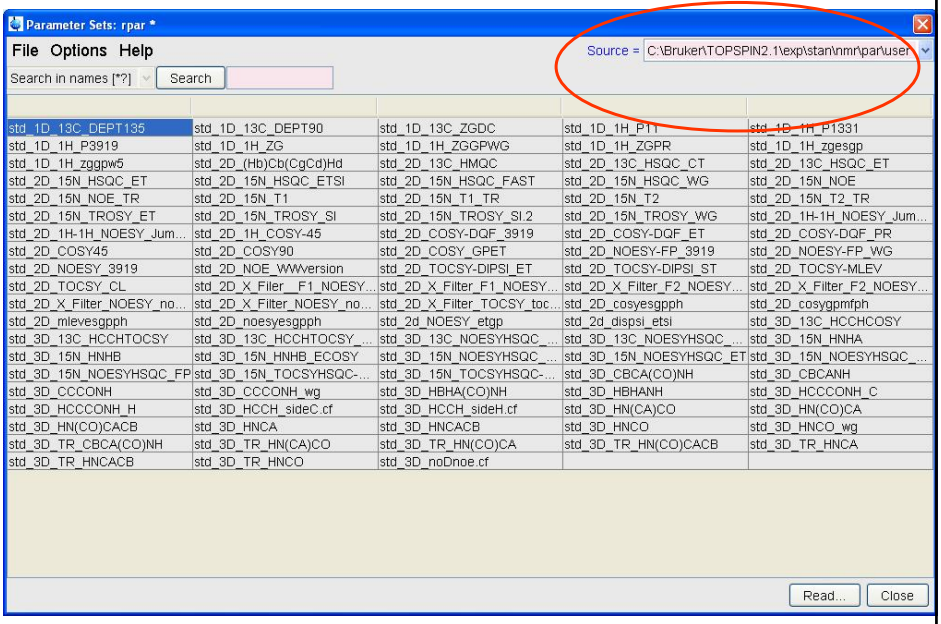
Pulse program directory (HFNMRC staff or users)



Parameter Set directory (Bruker default)



Parameter Set directory (HFNMRC standard)



New Pulseprogram @ Topspin2.1

;Update.info
;avance-version (07/10/18)
****
differences to 07/10/04
_____

added rd_hnca_32 reduced dimensionality (APSY)
rd_hncacb_32
rd_hncocacb_32
rd_hncocanh_62

****
differences to 07/09/14
_____

rd_hnco_32 reduced dimensionality (APSY)
rd_hncoca_42

****
differences to 07/07/16

added hsqcnoesyhsqcncgp4d 4D HSQC-NOESY-HSQC
hsqcnoesyhsqcccgp4d
hsqcnoesyhsqccngp4d
hsqcnoesyhsqcnngp4d

MUSIC (amino acid selective experiments)
music_cm_3d
music_cm_3d_2
music_de_3d
music_de_3d_2
music_fhyw_3d
music_fhyw_3d_2
music_gly_3d
music_gly_3d_2
music_ile_3d
music_ile_3d_2
music_kr_3d
music_kr_3d_2
music_lavia_3d
music_lavia_3d_2
music_pro_1_3d
music_pro_1_3d.2
music_pro_2_3d
music_pro_2_3d.2
music_qn_3d
music_qn_3d_2
music_ser_3d
music_ser_3d_2
music_tavi_3d
music_tavi_3d_2
music_trpe_2d

New on Data Processing /Analysis

Automatic peak picking 2D/3D

TopSpin < 2.1

TopSpin picks as many peaks
as defined in **PPMPNUM**

TopSpin 2.1

TopSpin picks all peaks
- the most intensive ones will be displayed
- as many as defined in **PPMPNUM**

New feature for backbone assignment

TopSpin includes the AutoLink backbone assignment algorithm.
This software analyses the peak information available on a given set of protein spectra and calculates a backbone assignment.