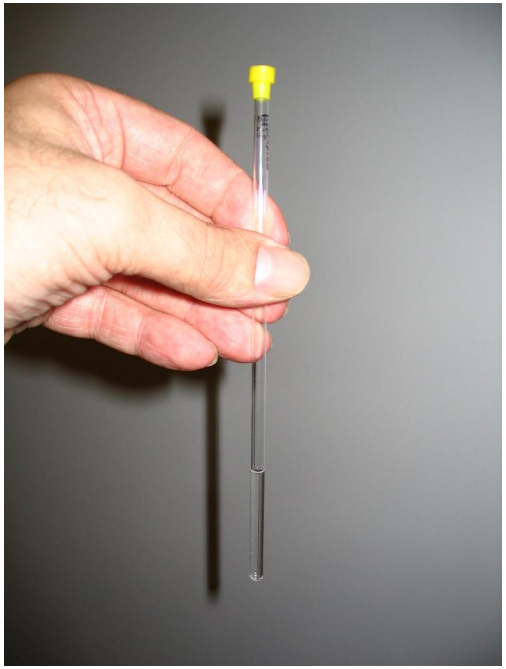
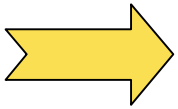
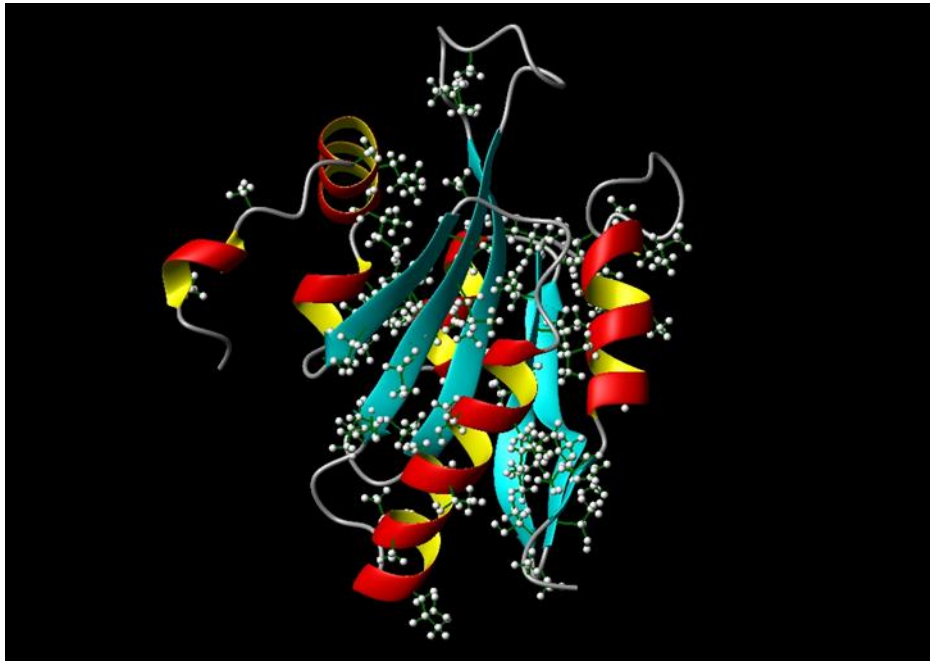






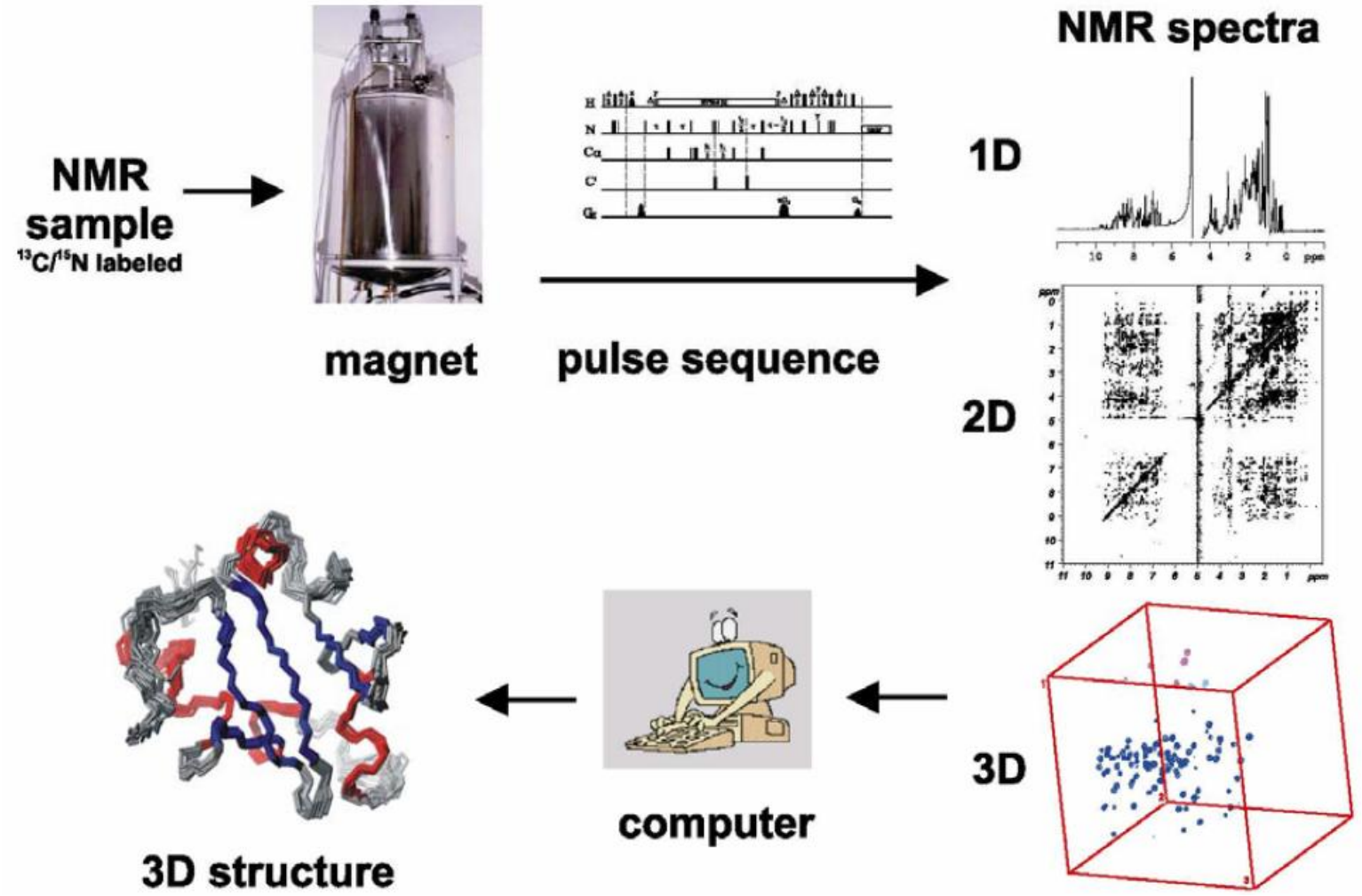


Figure from: http://www.embl.de/nmr/sattler/teaching

Why NMR (instead of X-ray crystallography)

- a great number of macromolecules won't crystallize)
- natural environmant (water)
- ligand binding and inter-molecular interactions
- dynamics and characterisation of mobility
  - conformational changes and their time-scale
  - folding
  - enzyme function (turn-over, kinetics)
- molecular size: NMR < 50 - 100 kDa (900 kDa?)
  crystallography > 200 kDa

  => COMPLEMENTING TECHNIQUES!!
- NMR:about 4015 solved protein structures, crystallography: about. 26100 (autumn 2005)

History of NMR

1946 Bloch, Purcell	First nuclear magnetic resonance
1955 Solomon	NOE (nuclear Overhauser effect)
1966 Ernst, Anderson	Fourier transform NMR
1975 Jeener, Ernst	Two-dimensional NMR
1985 Wüthrich	First solution structure of a small protein from NOE-derived distance restraints

→ NMR is about 25 years younger than X-ray crystallography

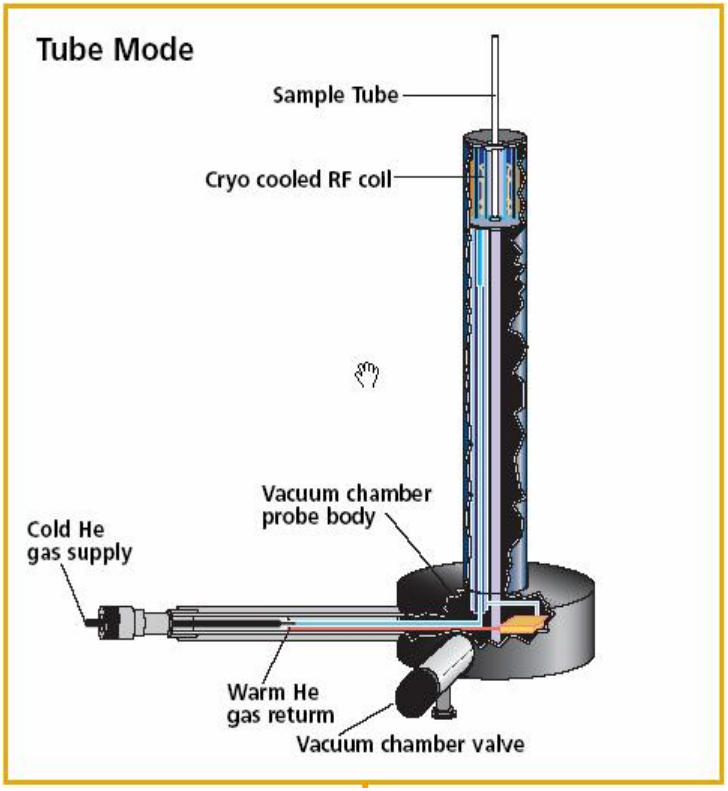
1987/8	3D NMR + ^{13}C, ^{15}N isotope labeling
1996/7	New long-range structural parameters:
	- residual dipolar couplings (also: anisotropic diffusion)
	- cross-correlated relaxation
	TROSY (molecular weight > 100 kDa)
2003	First solid-state NMR structure of a small protein

Nobel prizes

1944 Physics	Rabi (Columbia)
1952 Physics	Bloch (Stanford), Purcell (Harvard)
1991 Chemistry	Ernst (ETH)
2002 Chemistry	Wüthrich (ETH)
2003 Medicine	Lauterbur (Urbana), Mansfield (Nottingham)

Cold (cryo) probe

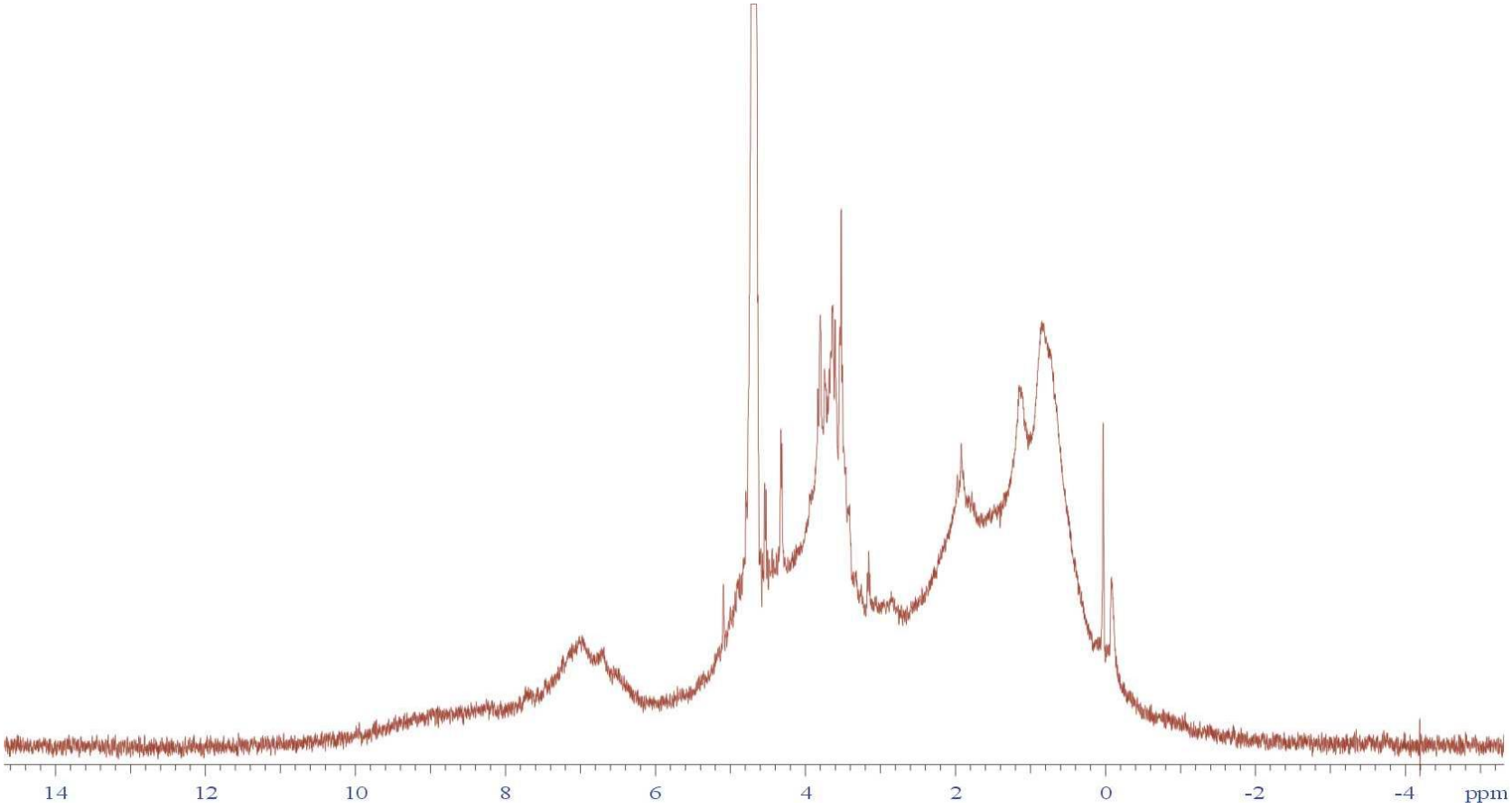
- in a cold probe all the electronics before the preamplifier, including the rf coils are maintained in the temperature of 25 K => reduced thermal noise
=> increased signal to noise ratio
- the sample is not in cold



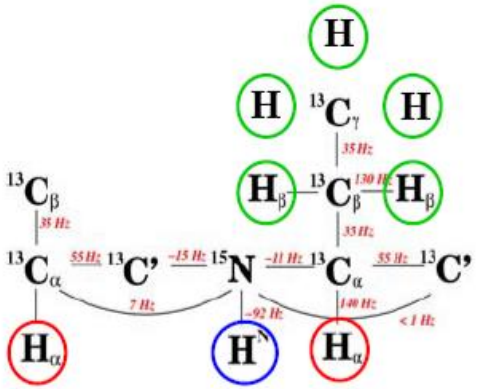
Protein samples

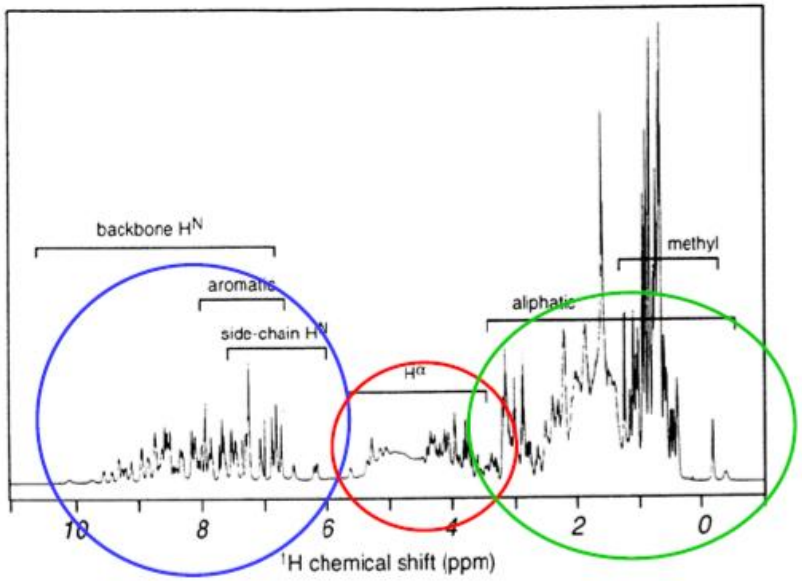
- required concentration 1 mM (1 mg)
  - high concentration => higher aggregation probability => cryo probe
- usually double (triple) labelled: ^{15}N,^{13}C(,^{2}H)
  - produced in bacteria (yeasts)
  - or cell-free production (enables e.g. highly efficient use of labels and position specific labelling)
- buffer
- 90% H_2O / 10% D_2O (amino groups => NH_2 in stead of ND_2)
- 200 µl (Shigemi tube) - 600 µl (normal tube)

1D 1H NMR spectrum of a protein



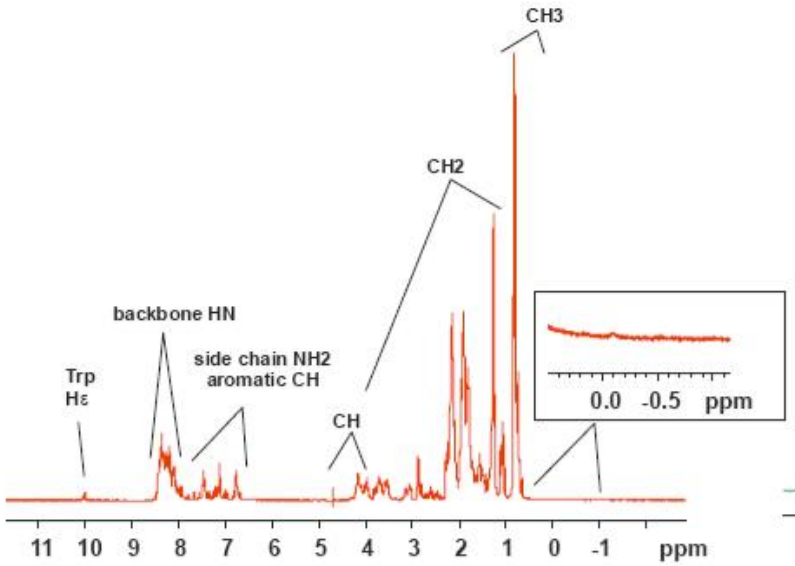
1D NMR spectrum of a protein



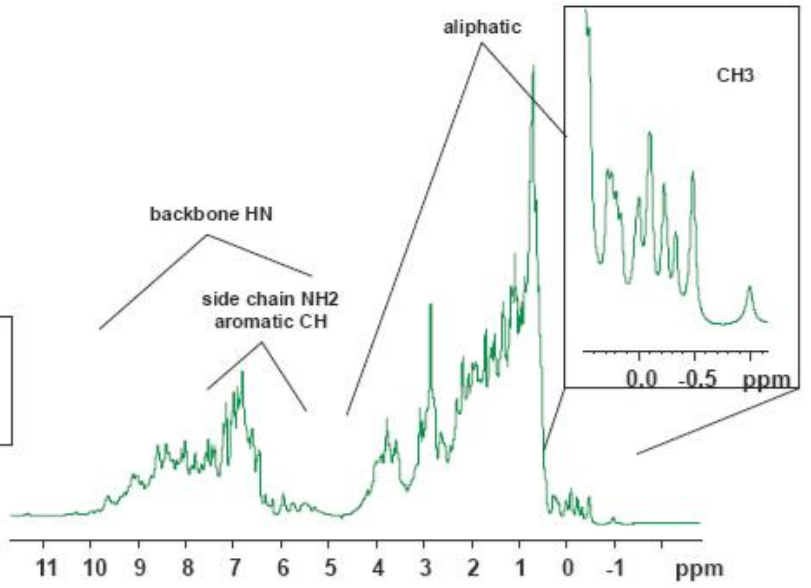


Folded or not?

Unfolded 20 kDa protein



Folded 20 kDa protein



Secondary structure from chemical shifts

- intrinsic chemical shifts (depending on amino acid or nucleotide type)

  random coil chemical shifts in proteins (G-G-X-G-G)

- conformational chemical shifts, i.e. ***secondary chemical shift*** Δδ

  difference of actual chemical shift to random coil chemical shift

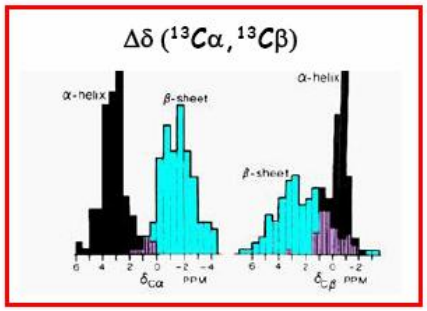
  ➔ secondary structure/backbone conformation from ^{1}H,^{13}C shifts

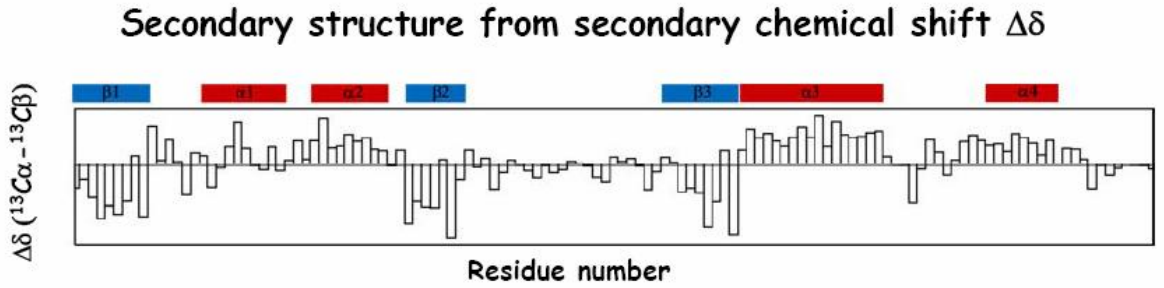
- ***ring-current shifts*** ➔ tertiary structure

- applications (proteins):

  → secondary structure identification: chemical shifts index

  → secondary structure prediction, combined with database (TALOS)





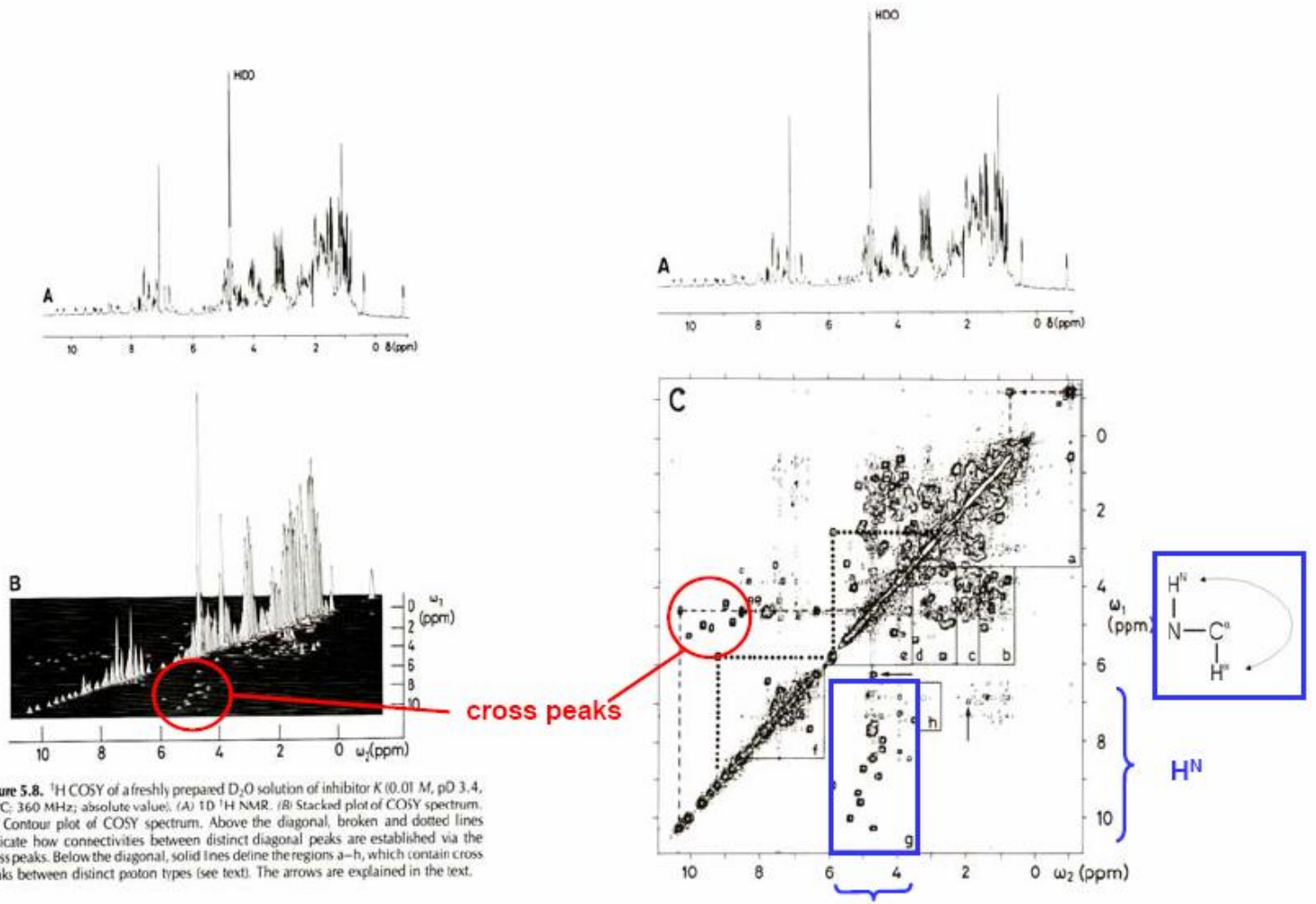
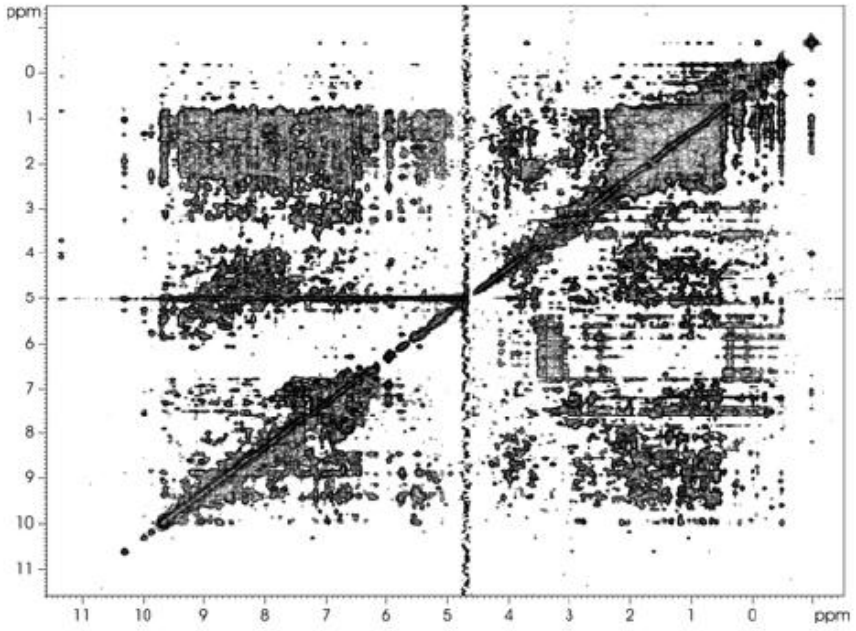


Figure 5.8. ^{1}H COSY of a freshly prepared D_2O solution of inhibitor *K* (0.01 *M*, pD 3.4, 25°C; 360 MHz; absolute value). *(A)* 1D ^{1}H NMR. *(B)* Stacked plot of COSY spectrum. *(C)* Contour plot of COSY spectrum. Above the diagonal, broken and dotted lines indicate how connectivities between distinct diagonal peaks are established via the cross peaks. Below the diagonal, solid lines define the regions a–h, which contain cross peaks between distinct proton types (see text). The arrows are explained in the text.

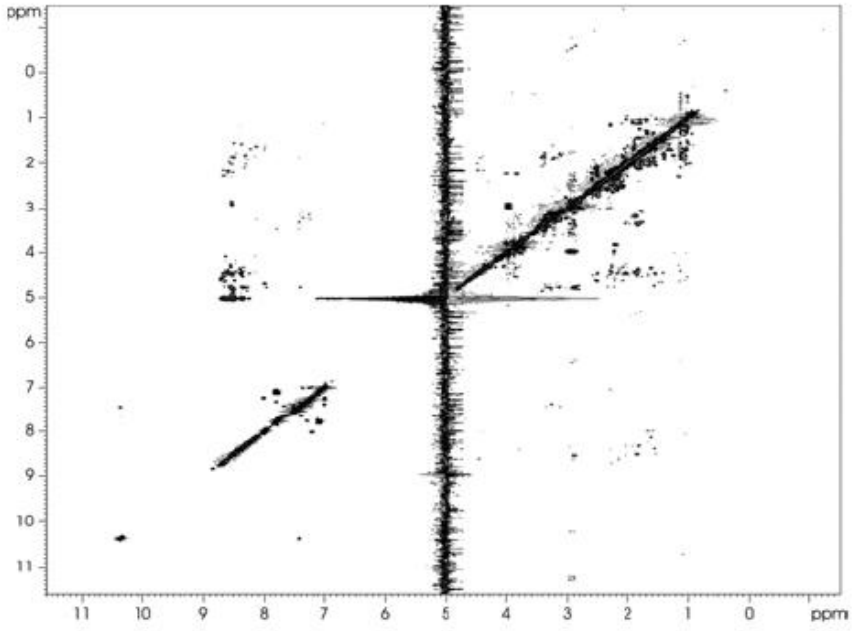
(Wüthrich "NMR of proteins and nucleic acids")

2D NOESY

Folded protein



Unfolded protein



NOEs in structure determination

helix

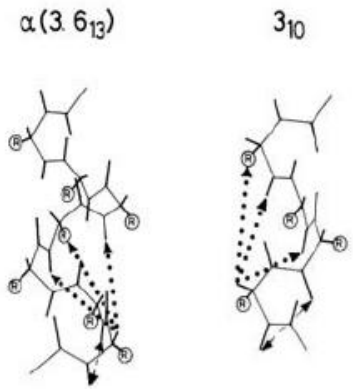


Figure 7.11 Short sequential and medium-range $^1H-^1H$ distances in the α helix and the 3_{10} helix. Broken arrow, d_{NN}. Dotted arrows, $d_{\alpha N}(i,i+3)$, $d_{\alpha\beta}(i,i+3)$, and $d_{\alpha N}(i,i+4)$ [α helix] or $d_{\alpha N}(i,i+2)$ [3_{10} helix].

sheets

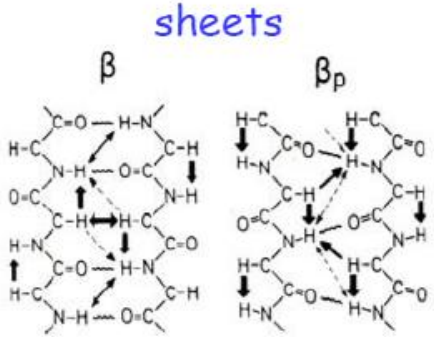


Figure 7.13. Short sequential and long-range backbone $^1H-^1H$ distances in β sheets. Wavy lines indicate interstrand hydrogen bonds. Thick vertical arrows indicate $d_{\alpha N}$. For antiparallel β, short interstrand distances are indicated by thick horizontal arrows $[d_{\alpha\alpha}(i,j)]$, thin solid arrows $[d_{NN}(i,j)]$, and broken arrows $[d_{\alpha N}(i,j)]$. In parallel β, solid arrows indicate $d_{\alpha N}(i,j)$ and broken arrows $d_{NN}(i,j)$ (from Wüthrich et al., 1984a).

turns

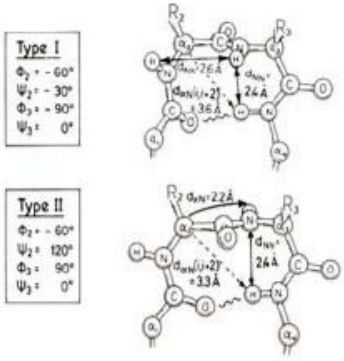
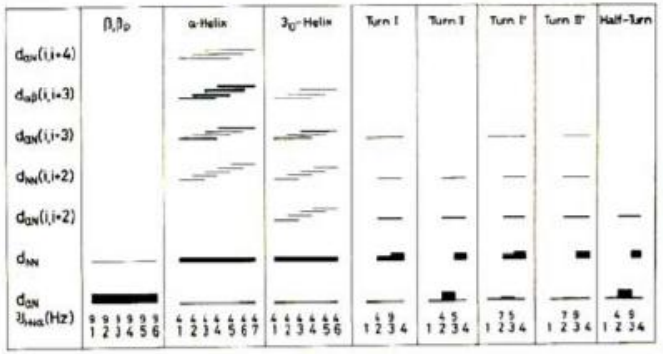


Figure 7.12. Short sequential and medium-range $^1H-^1H$ distances in type I and type II tight turns. The wavy lines indicate hydrogen bonds (from Wüthrich et al., 1984a).

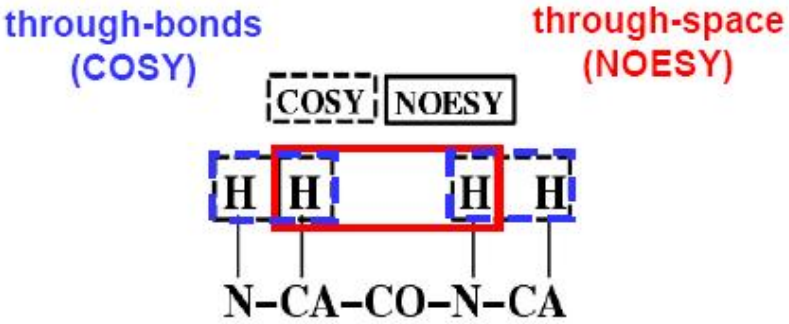


(Wüthrich "NMR of proteins and nucleic acids")

NOE based assignment strategies

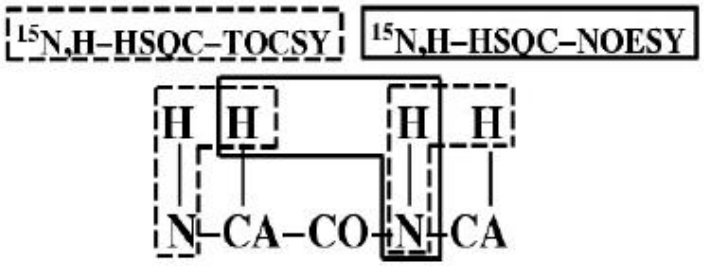
homonuclear

(^{1}H)



heteronuclear

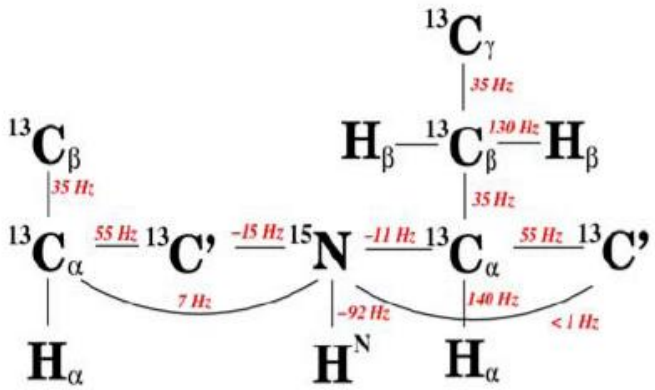
(^{1}H, ^{15}N)



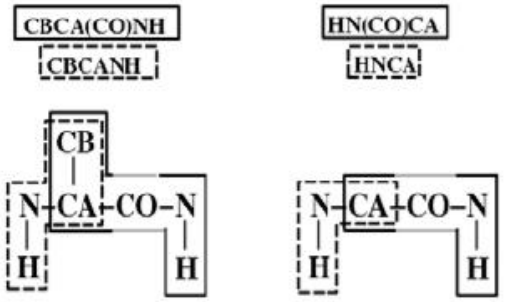
Sattler et al. Prog. NMR Spectrosc. (1999) 34, 93-158.

Scalar coupling based assignment

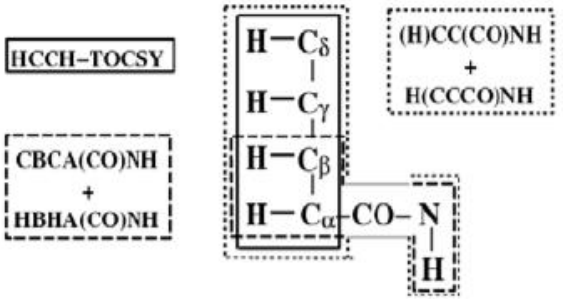
In a uniformly $^{13}C/^{15}N$-labeled protein numerous chemical shifts can be measured and correlated via scalar ^{1}J and ^{2}J-couplings



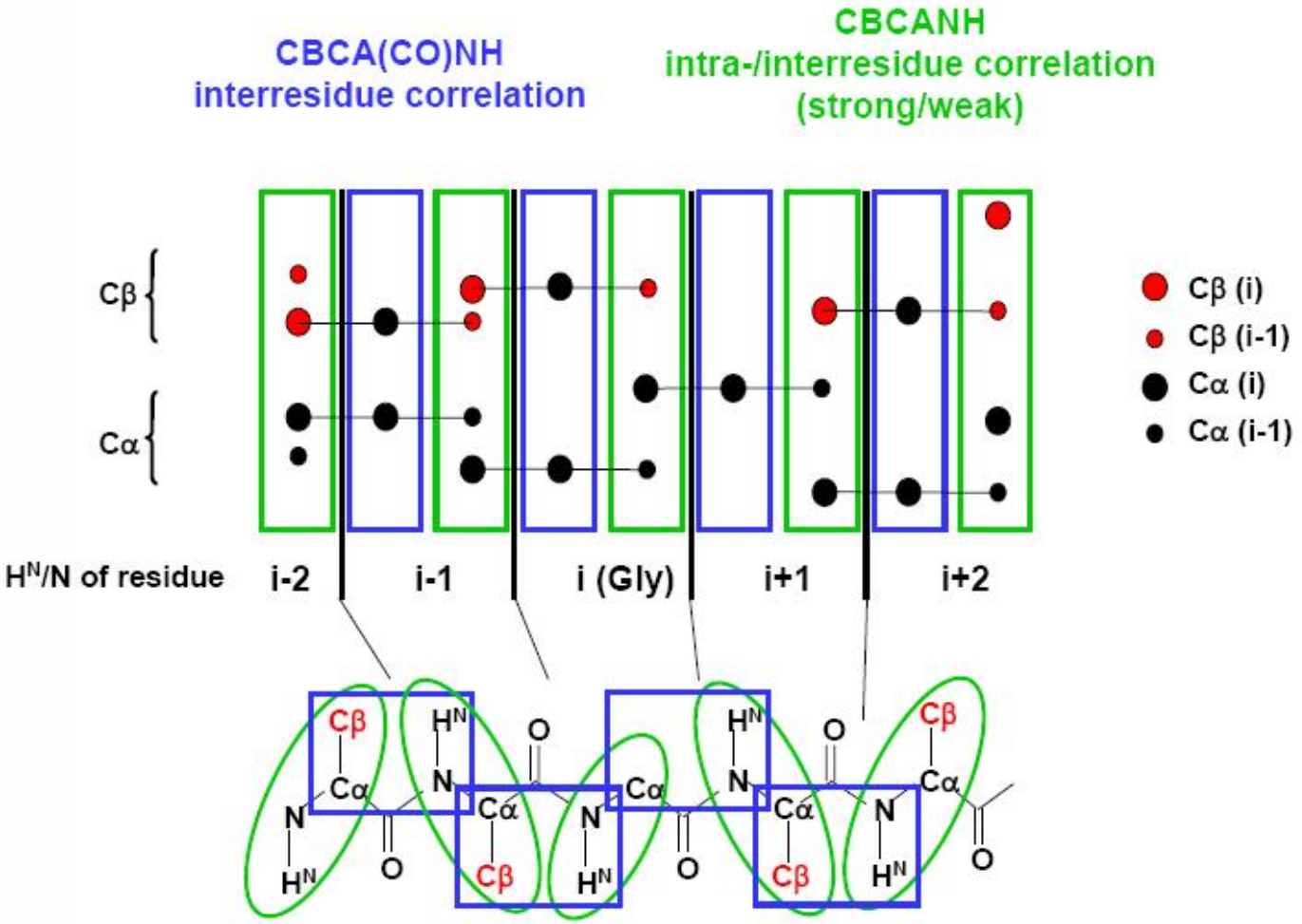
Backbone assignment



Side chain assignment

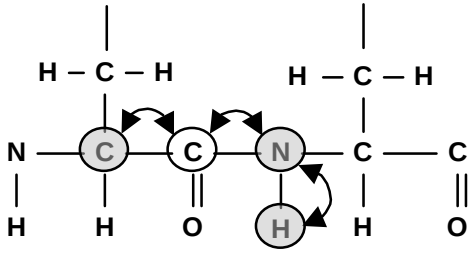


Sattler et al. Prog. NMR Spectrosc. (1999) 34, 93-158.


CBCA(CO)NH
interresidue correlation
CBCANH
intra-/interresidue correlation
(strong/weak)
Cβ
Cα
Cβ (i)
Cβ (i-1)
Cα (i)
Cα (i-1)
H^N/N of residue
i-2
i-1
i (Gly)
i+1
i+2

Back-bone assignment

-experiments needed:
15N-HSQC, HNCA (trosy),
HN(CO)CA, HNCACB,
HN(CO)CACB,HNCO



- recuires double labelled protein (^{13}C and ^{15}N)
- large protein => also ^{2}H labelling needed
- auto-assignment programs => 40-90%

- binding site identification
- conformation
- dynamics

Nuclear Overhauser Effect (NOE): spin interactions through space

Cross peaks are only observed if ^{1}H-^{1}H distance r <5Å

NOE ~ $1/r^6$

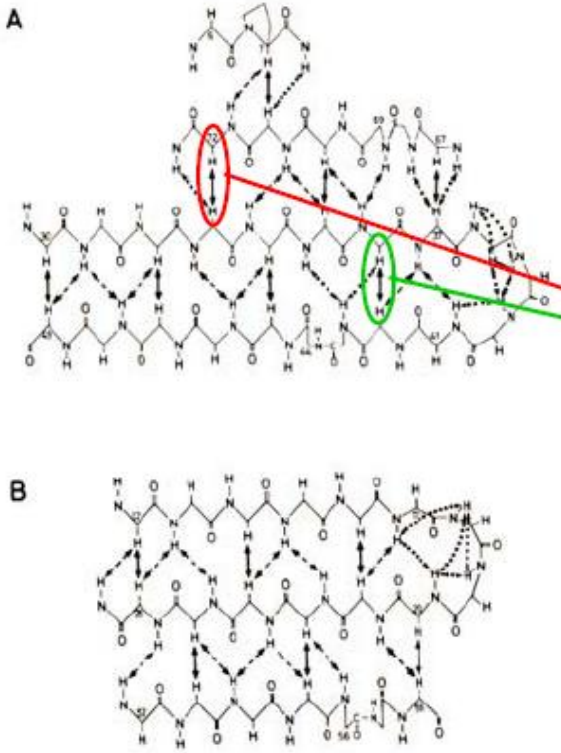


Figure 9.6. Antiparallel β structures in Tendamistat. Interstrand ^{1}H–^{1}H NOE's are indicated by arrows: Solid arrows, $d_{\alpha\alpha}(i,j)$; broken arrows, $d_{\alpha N}(i,j)$ and $d_{NN}(i,j)$ observed in D_2O solution; dotted arrows, $d_{\alpha N}(i,j)$ and $d_{NN}(i,j)$ observed only in H_2O solution (from Kline and Wüthrich, 1985).

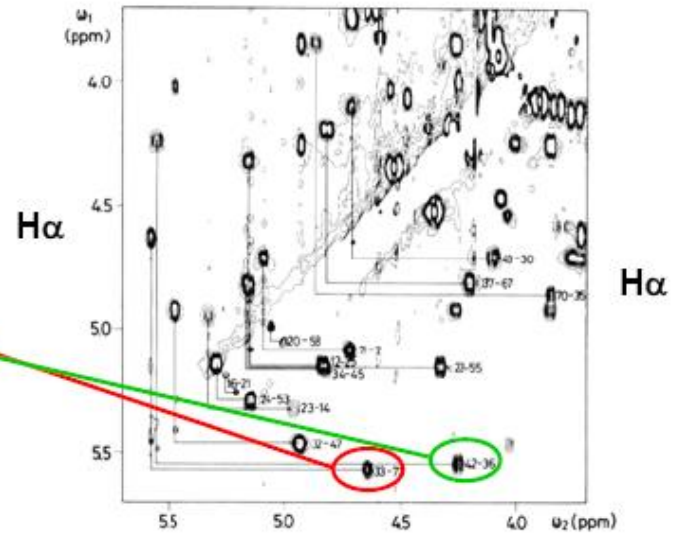


Figure 9.7. Region of the ^{1}H NOESY spectrum of Tendamistat containing the αH–αH cross peaks corresponding to the solid arrows in Figure 9.6 (0.007 *M*, D_2O, pD 3.2, 50°C; 500 MHz; τ_m 200 ms; absorption mode, diagonal suppression; digital resolution 4.5 Hz/point in ω_1, 2.2 Hz/point in ω_2). Corresponding cross peaks above and below the diagonal are connected by lines and identified by the sequence locations of the connected residues (from Kline and Wüthrich, 1985).

(Wüthrich "NMR of proteins and nucleic acids")

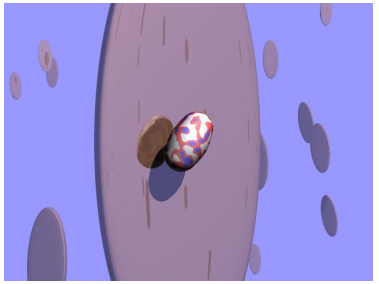
NMR determination of protein 3D structure

- double (triple) labelled protein: ^{15}N, ^{13}C, (^{2}H)
- back-bone (main chain) assignment
  - e.g. autoassign
    - close to the performance of man
    - automatic peak picking (=> +10%)
    - very fast: less than 1 min for a medium sized protein
      - (cf. 2 weeks manually)
- side chain assignment
  - more difficult (crowded spectra)
  - manually at least a month
  - very important (mistakes won't reveal themselves but result in a wrong structure)
  - some attempts for automation, so far very heavy and slow, manual double checking required

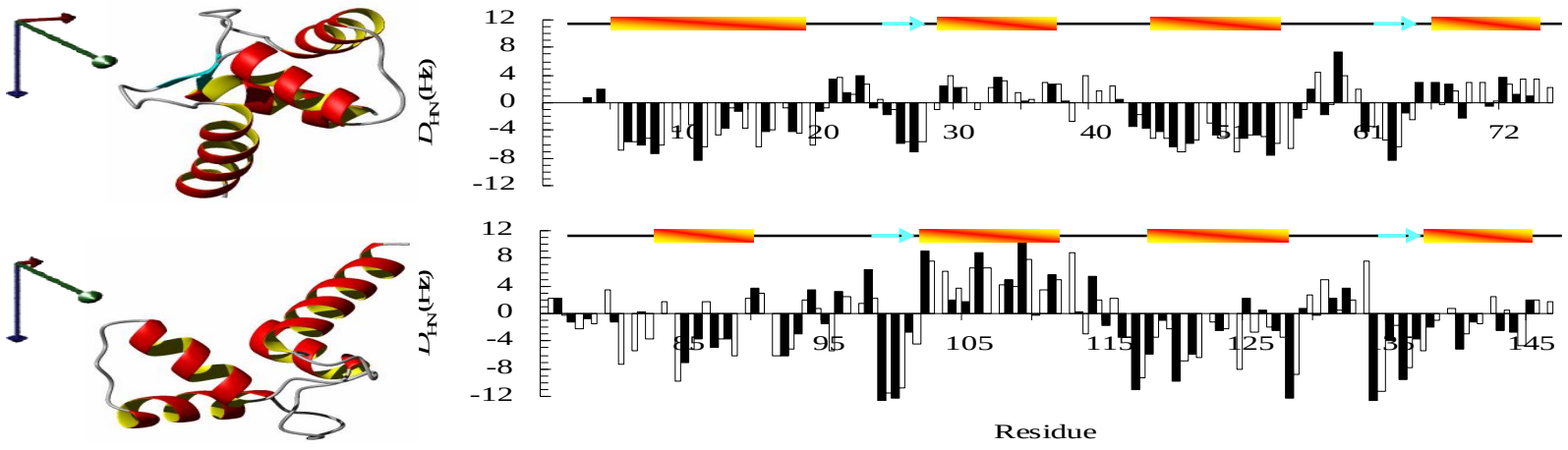
- assignment of the NOEs and structure calculation
  - ^{15}N and ^{13}C NOESY
  - manually 1-12 months
  - e.g. ~2000 signals for a 100 amino acid protein
  - CYANA: ~24 h with one processor (100 aa)
    - can use parallel computing: 15 min. with a 128 CPU cluster
    - uses torsion angle space, simulated annealing
    - repeates the cycle several hundreds of times
    - autoput: the sructure in pdb format
- 3D structure determination of a medium sized (well behaving) protein takes about 2 months

Residual dipolar couplings

Protein alignment for dipolar coupling detection



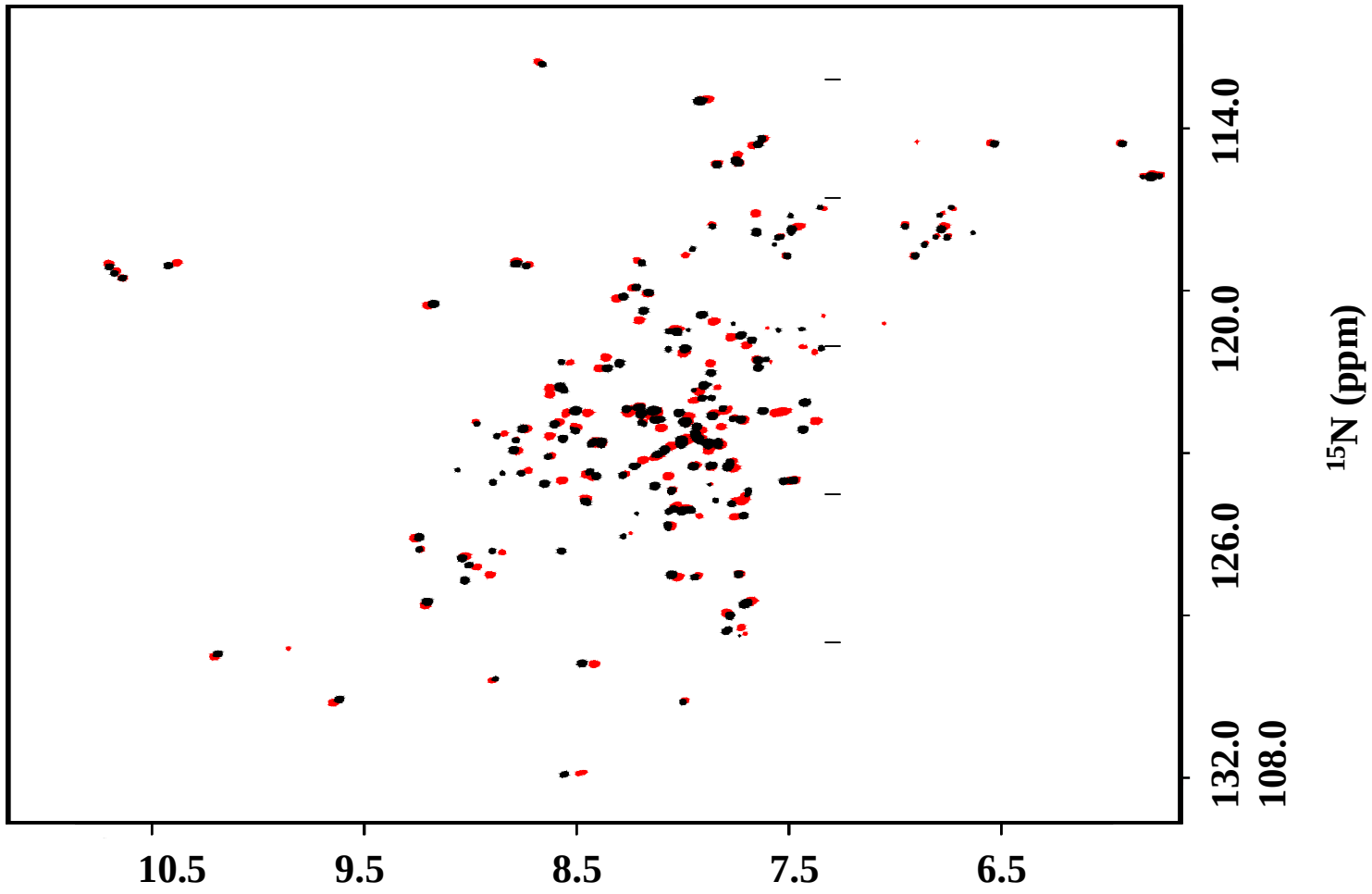
Domain orientations



Protein ligand interactions

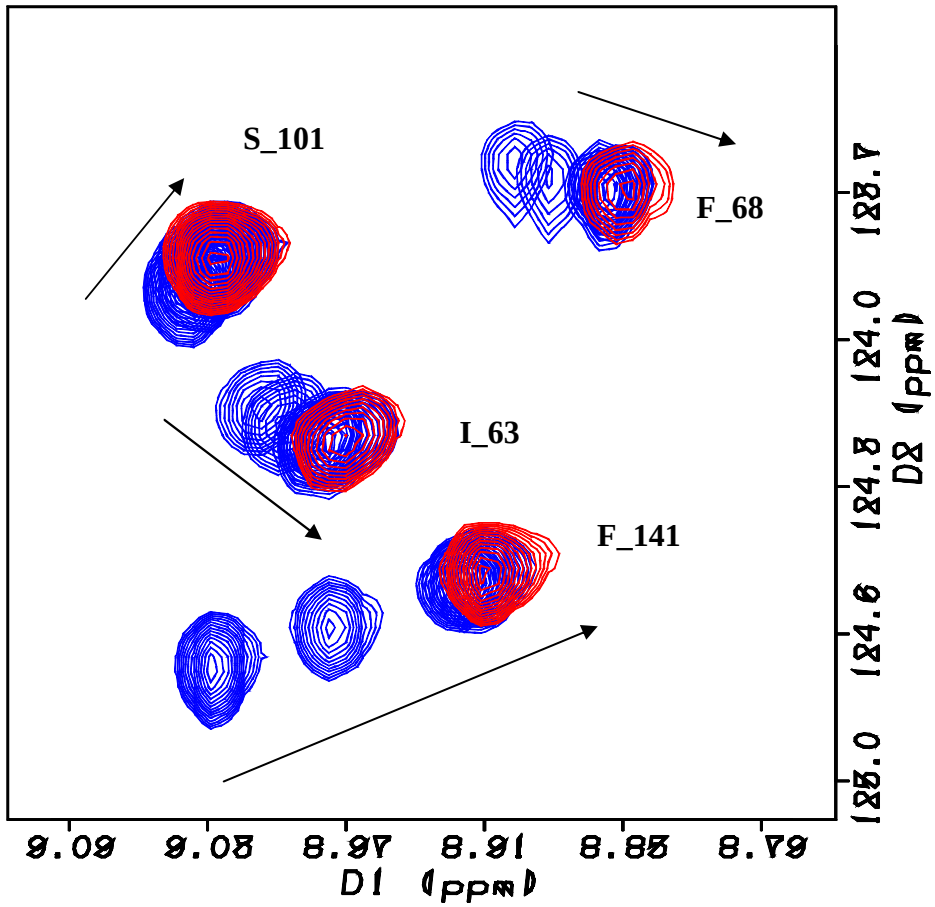
^{1}H-^{15}N-HSQC spectrum

in the absence (black contours) and presence (red contours) of the ligand

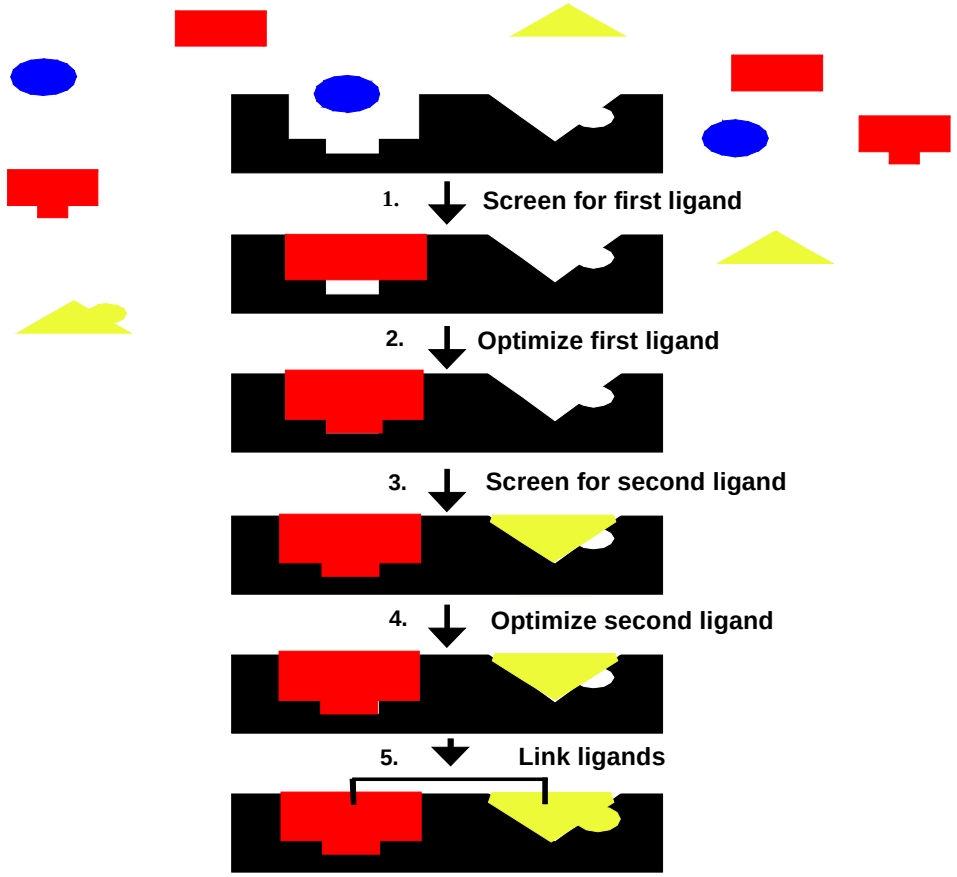


$\Delta\delta(^{1}H/^{15}N) = \Delta\delta(^{1}H) + \Delta\delta(^{15}N/5) > 0.04$ ppm

A region of a HSQC titration series



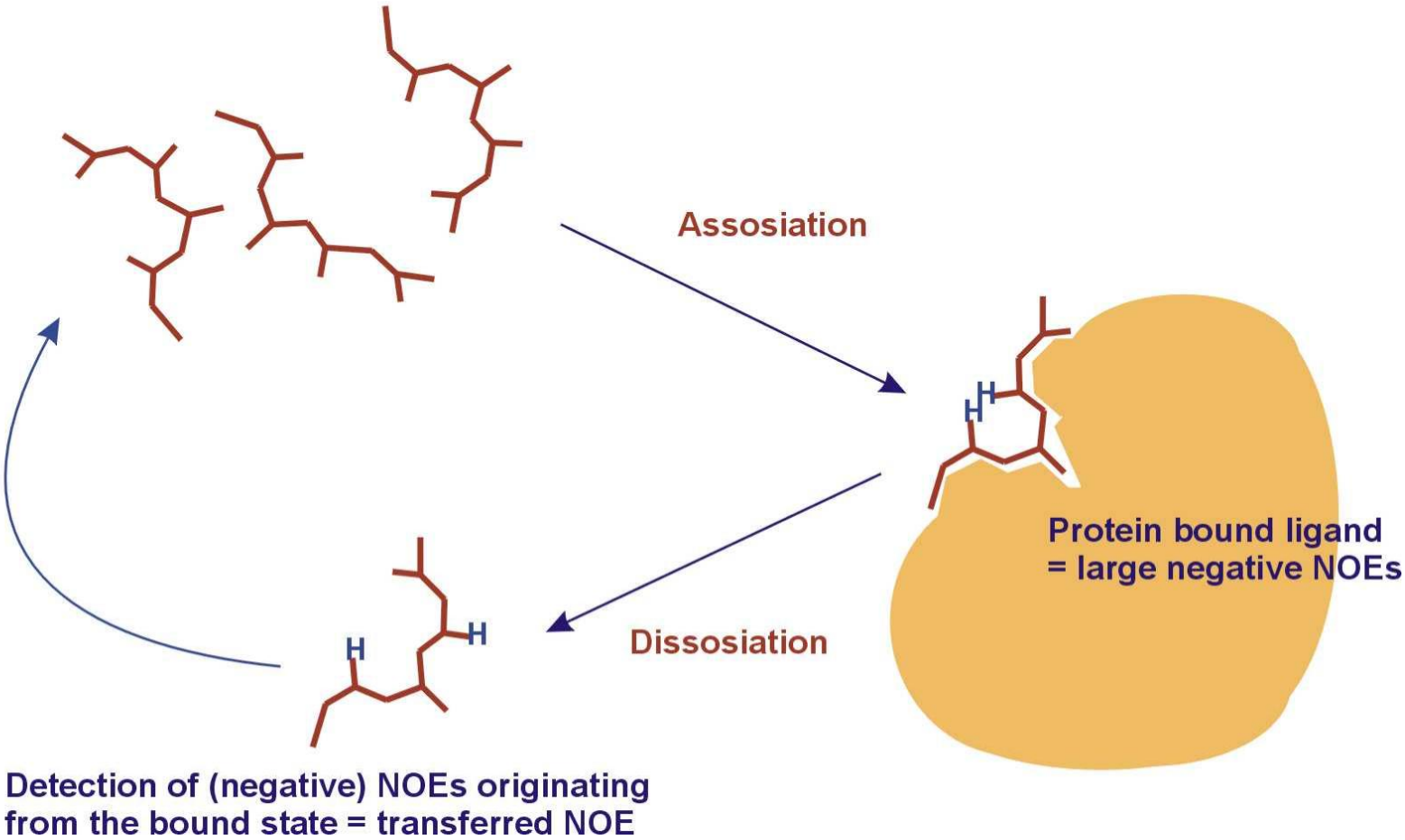
"SAR by NMR™", basic principle



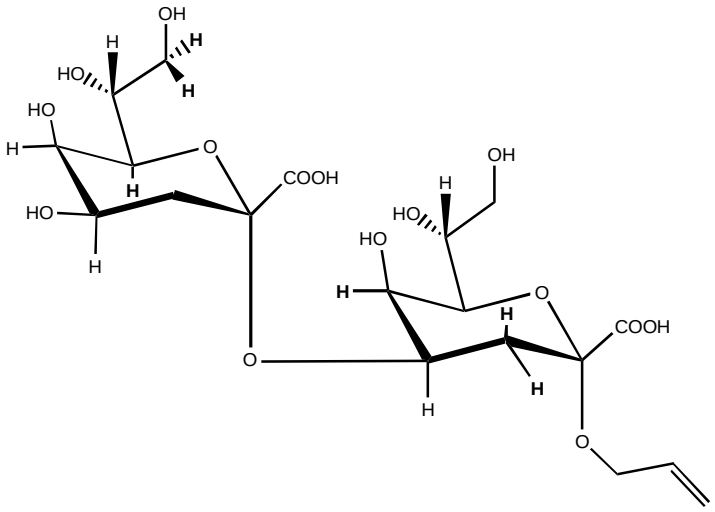
Limitations

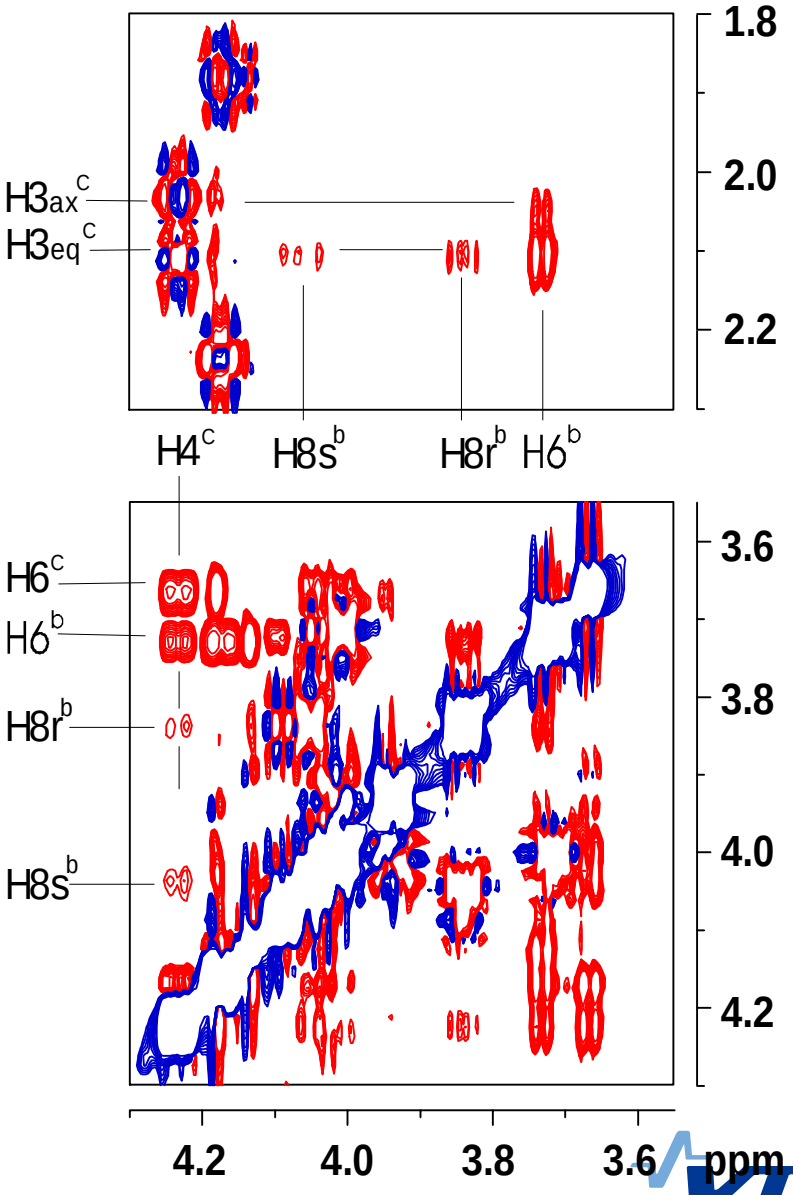
- protein size
- need for labelling (double or triple)
- solubility
- interactions
- "NMR behavior"
- amount protein needed

Transferred NOEs represent the bound state of the ligand

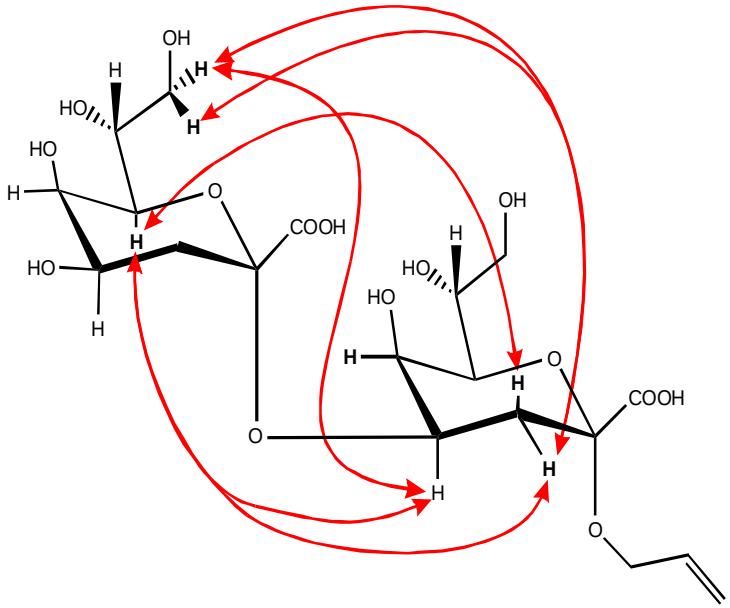


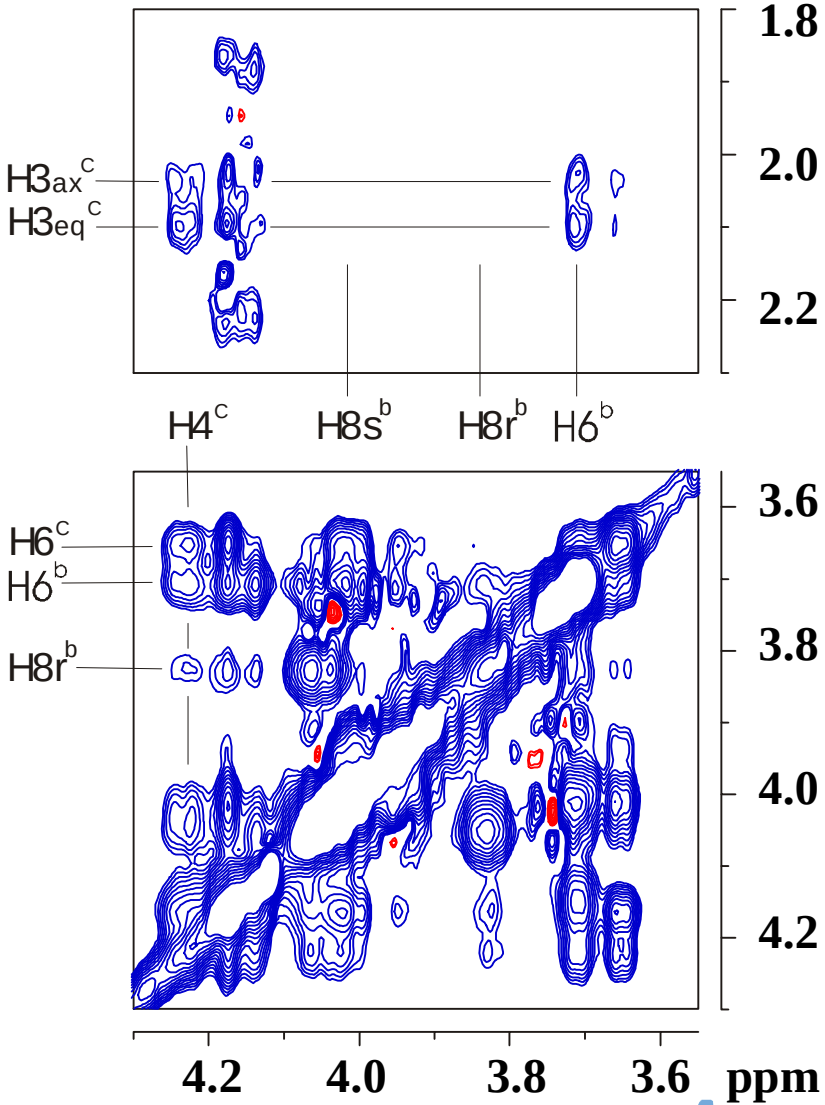
NOESY spectrum of Kdo2-4Kdo-allyl in D_2O



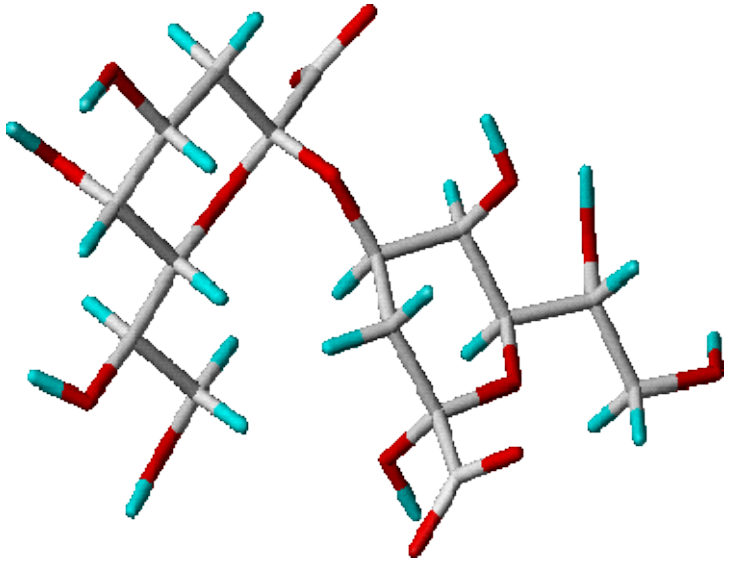


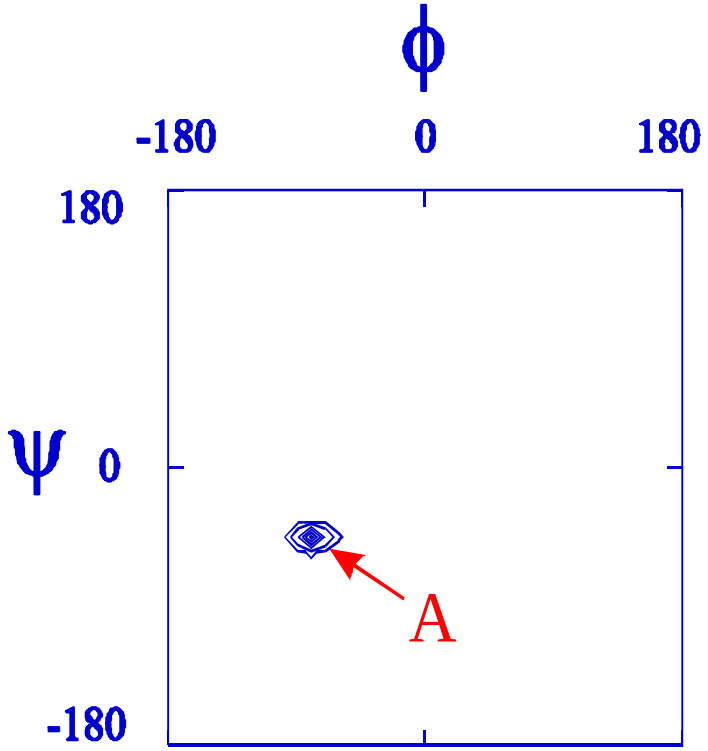
Tr-NOESY spectrum of S25-39 / Kdo2-4Kdo-allyl complex

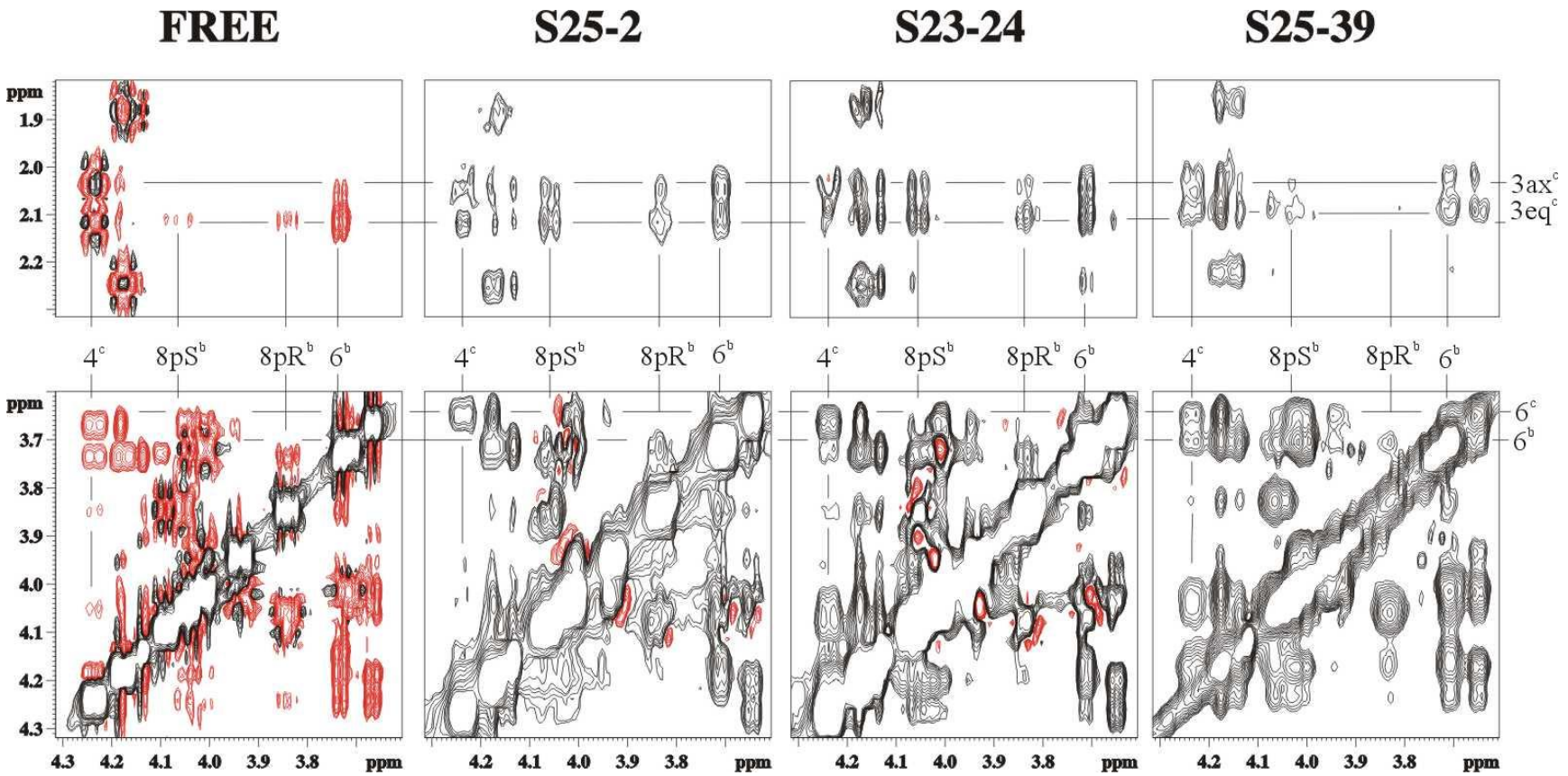




S25-39 bound conformation of α-Kdo-(2→4)-α-Kdo-(2→O)-allyl






FREE
S25-2
S23-24
S25-39
ppm
1.9
2.0
2.1
2.2
3ax^c
3eq^c
4^c
8pS^b
8pR^b
6^b
ppm
3.7
3.8
3.9
4.0
4.1
4.2
4.3
6^c
6^b
4.3 4.2 4.1 4.0 3.9 3.8 ppm

The major binding epitopes of Kdoα2-4Kdo (A) and Kdoα2-8Kdo (B)