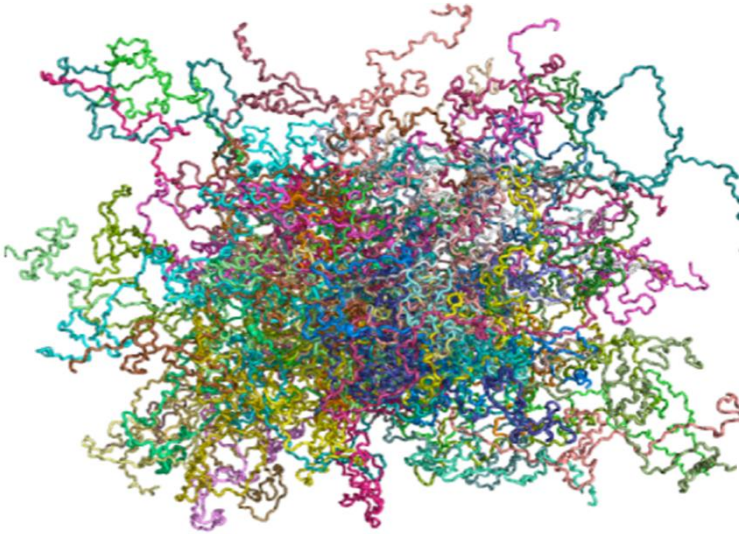
Liquid state NMR spectroscopy of (disordered) proteins



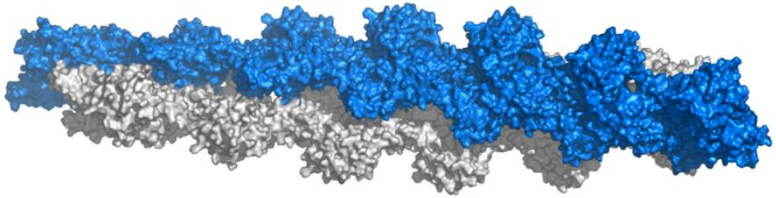
http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1000034

Disordered proteins
Assignment
Conformational ensemble
Interactions

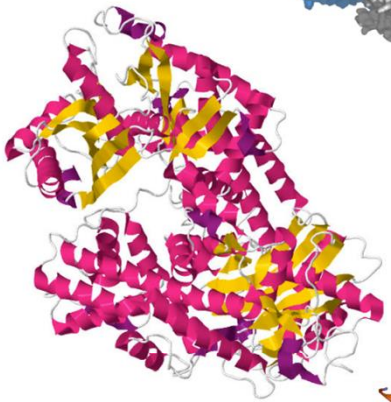
Winter School in Brno, January 7-11 2019

Reminder: Proteins

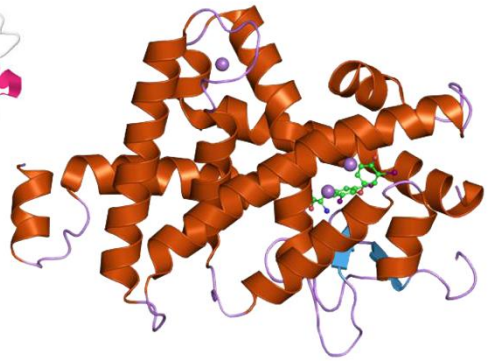
- Biopolymers composed of 20 different amino acids
- Vast range of functions in all domains of life:
  - Catalysis
  - Transport
  - Signalling
  - Structure
  - …



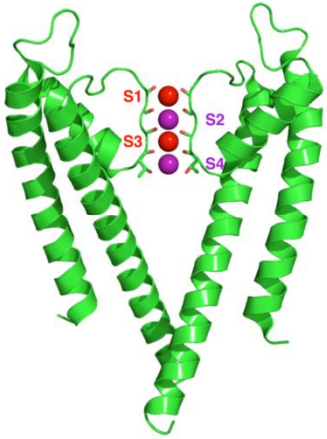
Actin filament



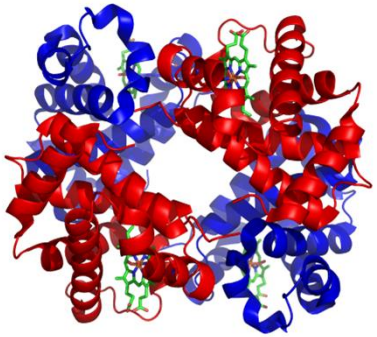
Hexokinase



Thyroid hormone receptor

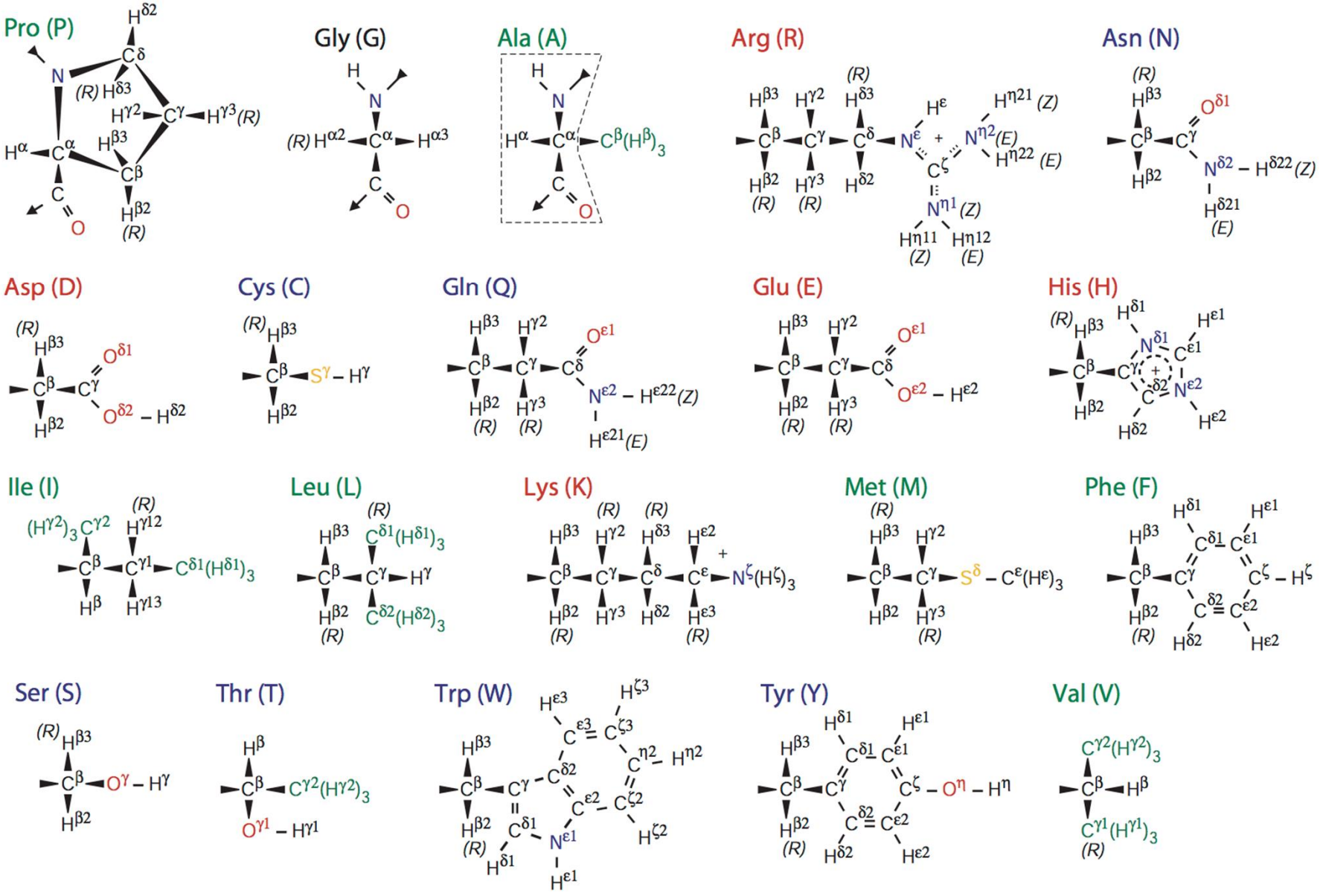


Potassium channel



Haemoglobin

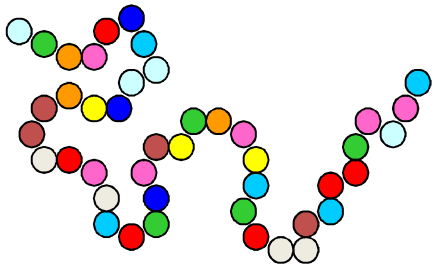
The 20 natural amino acids



Markley et al., Pure & Appl. Chem. 70, 117, 1998

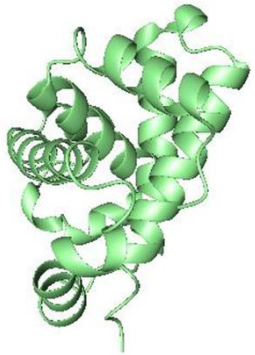
Protein structure

- Primary: sequence

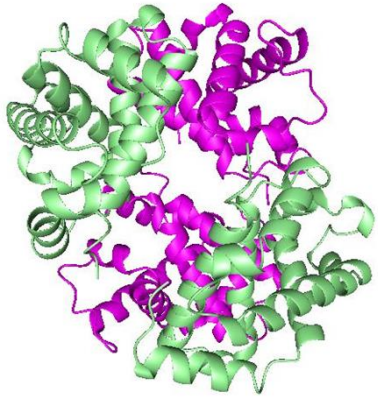


- Secondary: helix, sheet, turn

- Tertiary: domain



- Quaternary: assemblies of domains or proteins



NMR of proteins

- Close-to-native environment: solution, in-cell
- disordered proteins

- "Structure" of IDPs
- Dynamics, conformational changes
- Interactions
- Posttranslational modifications
- …

NMR of proteins: Issues

- Sensitivity
  - mg amounts of protein typically required
- Isotope labeling
  - ^{13}C, ^{15}N, ^{2}H, specific labeling schemesé
- Size
  - slow molecular tumbling / resonance overlap

A project in protein NMR

Typically required:

- Overexpression with isotope labeling
- High B_0 field
- Two- and higher-dimensional spectroscopy

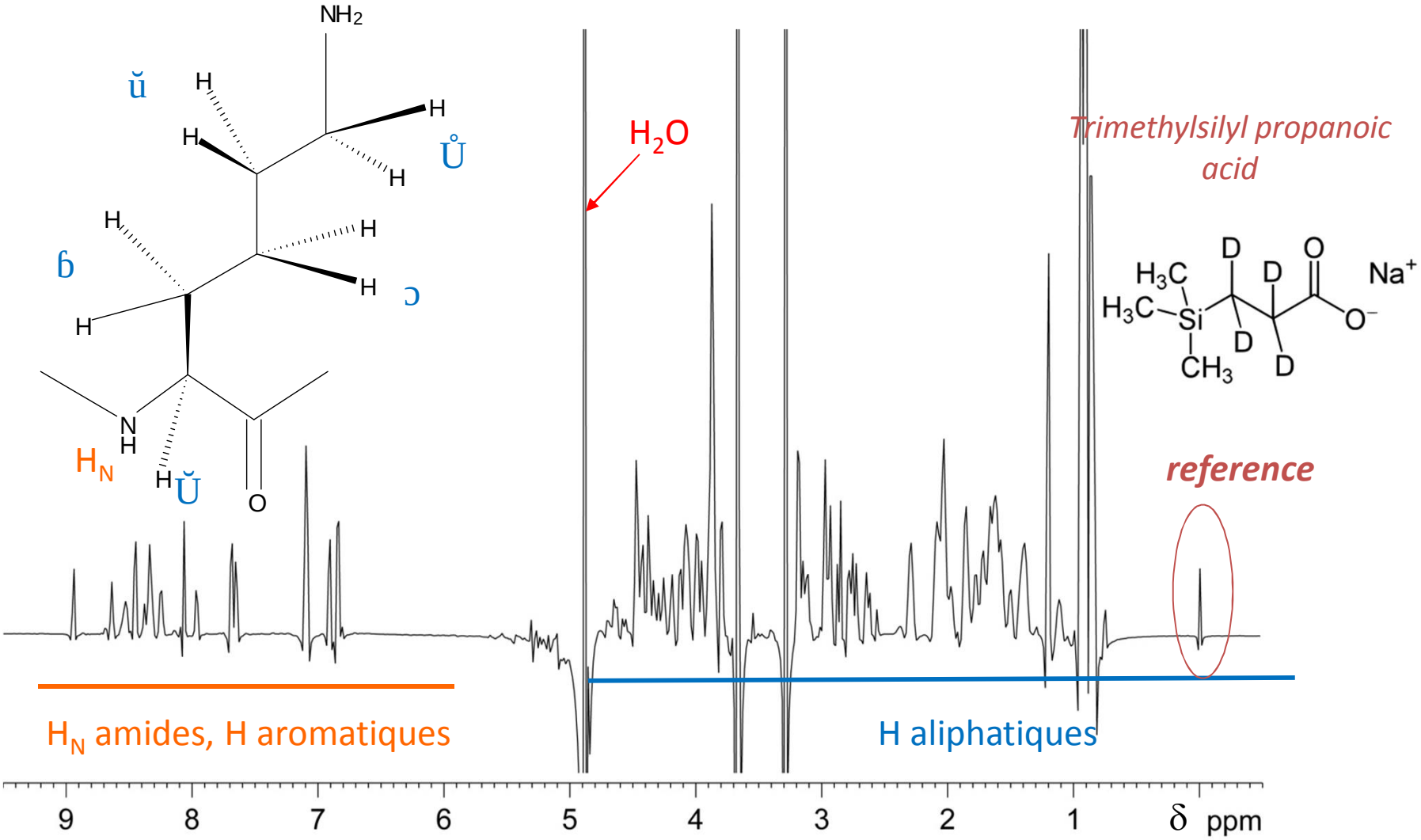
Typical steps:

- Feasibility tests, ñfingerprintò spectra
- Resonance assignment
- Structural information
- Measurement of dynamics, interactions, é

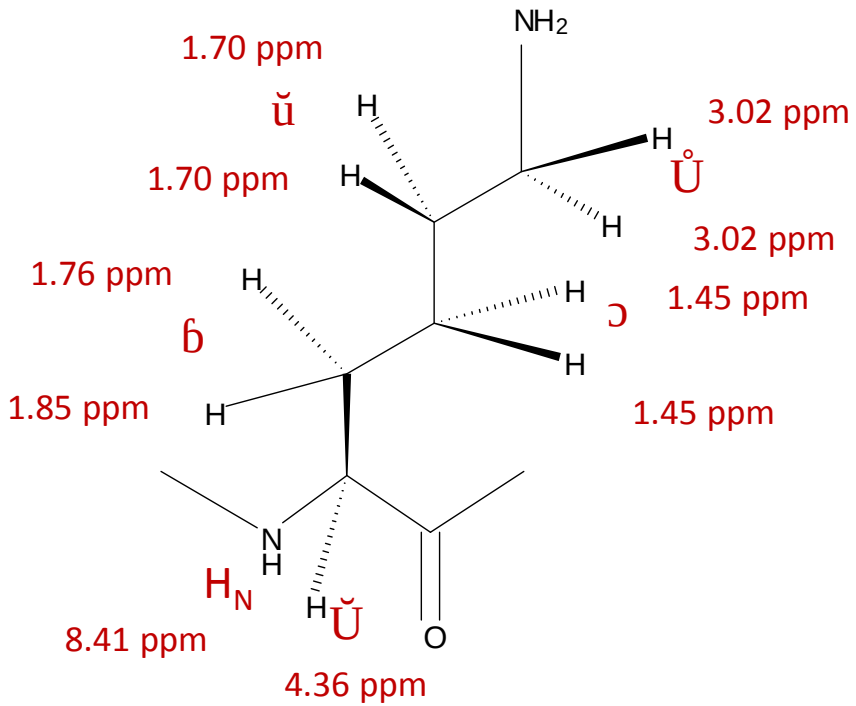
GETTING STARTED:
PROTON NMR

1D NMR

1D proton Spectrum of peptides and proteins



^{1}H Chemical Shifts of amino acids

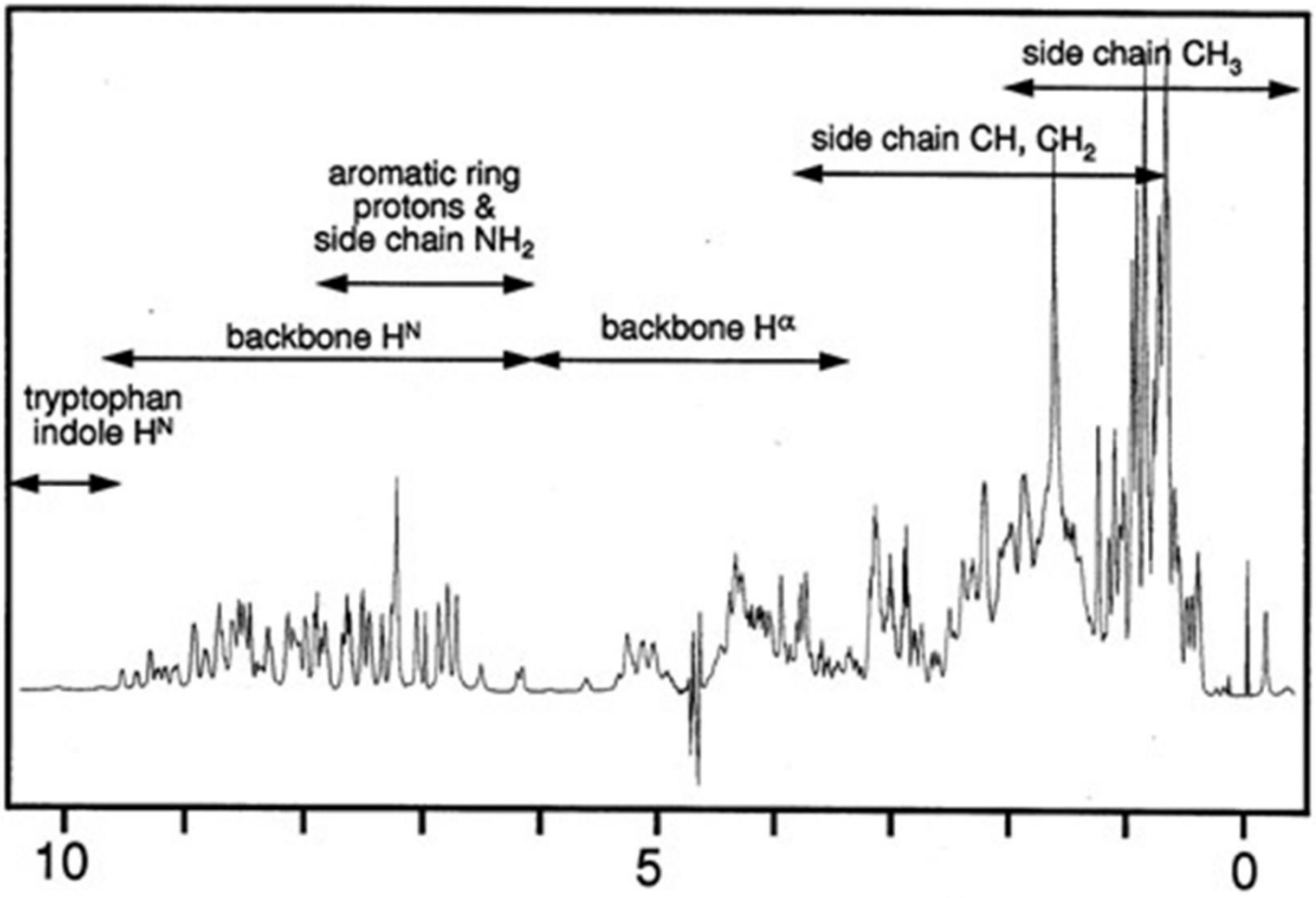


lysine

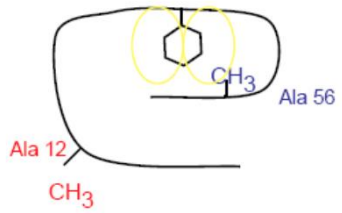
TABLE 2.3. Random Coil ^{1}H Chemical Shifts for the 20 Common Amino Acid Residues[a]

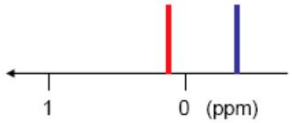
Residue	NH	αH	βH	Others
Gly	8.39	3.97		
Ala	8.25	4.35	1.39	
Val	8.44	4.18	2.13	γCH_3 0.97, 0.94
Ile	8.19	4.23	1.90	γCH_2 1.48, 1.19
				γCH_3 0.95
				δCH_3 0.89
Leu	8.42	4.38	1.65,1.65	γH 1.64
				δCH_3 0.94, 0.90
Pro[b]		4.44	2.28,2.02	γCH_2 2.03, 2.03
				δCH_2 3.68, 3.65
Ser	8.38	4.50	3.88,3.88	
Thr	8.24	4.35	4.22	γCH_3 1.23
Asp	8.41	4.76	2.84,2.75	
Glu	8.37	4.29	2.09,1.97	γCH_2 2.31, 2.28
Lys	8.41	4.36	1.85,1.76	γCH_2 1.45, 1.45
				δCH_2 1.70, 1.70
				ϵCH_2 3.02, 3.02
				ϵNH_3^+ 7.52
Arg	8.27	4.38	1.89,1.79	γCH_2 1.70, 1.70
				δCH_2 3.32, 3.32
				NH 7.17, 6.62
Asn	8.75	4.75	2.83,2.75	γNH_2 7.59, 6.91
Gln	8.41	4.37	2.13,2.01	γCH_2 2.38, 2.38
				δNH_2 6.87, 7.59
Met	8.42	4.52	2.15,2.01	γCH_2 2.64, 2.64
				ϵCH_3 2.13
Cys	8.31	4.69	3.28,2.96	
Trp	8.09	4.70	3.32,3.19	2H 7.24
				4H 7.65
				5H 7.17
				6H 7.24
				7H 7.50
				NH 10.22
Phe	8.23	4.66	3.22,2.99	2,6H 7.30
				3,5H 7.39
				4H 7.34
Tyr	8.18	4.60	3.13,2.92	2,6H 7.15
				3,5H 6.86
His	8.41	4.63	3.26,3.20	2H 8.12
				4H 7.14

^{1}H spectral Dispersion due to protein 3D folding

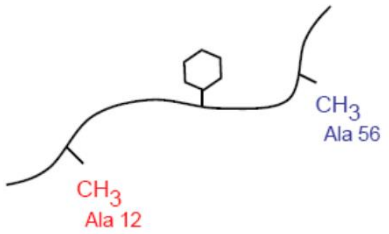


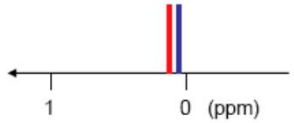
^{1}H Spectral Dispersion by 3D fold of proteins



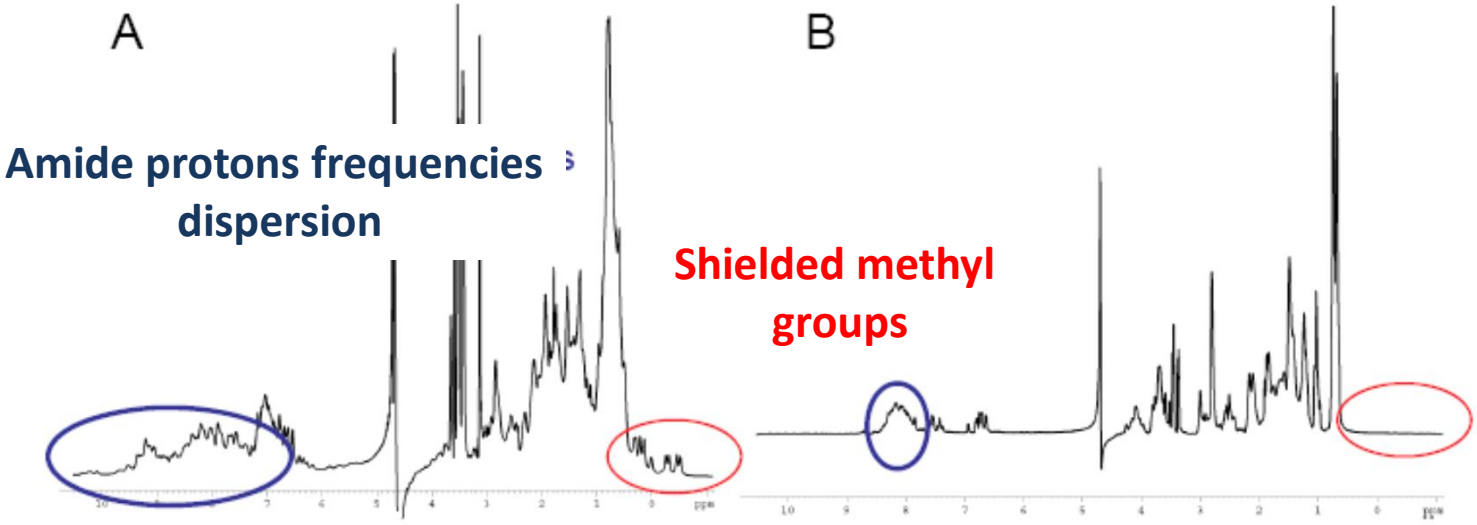


Folded protein





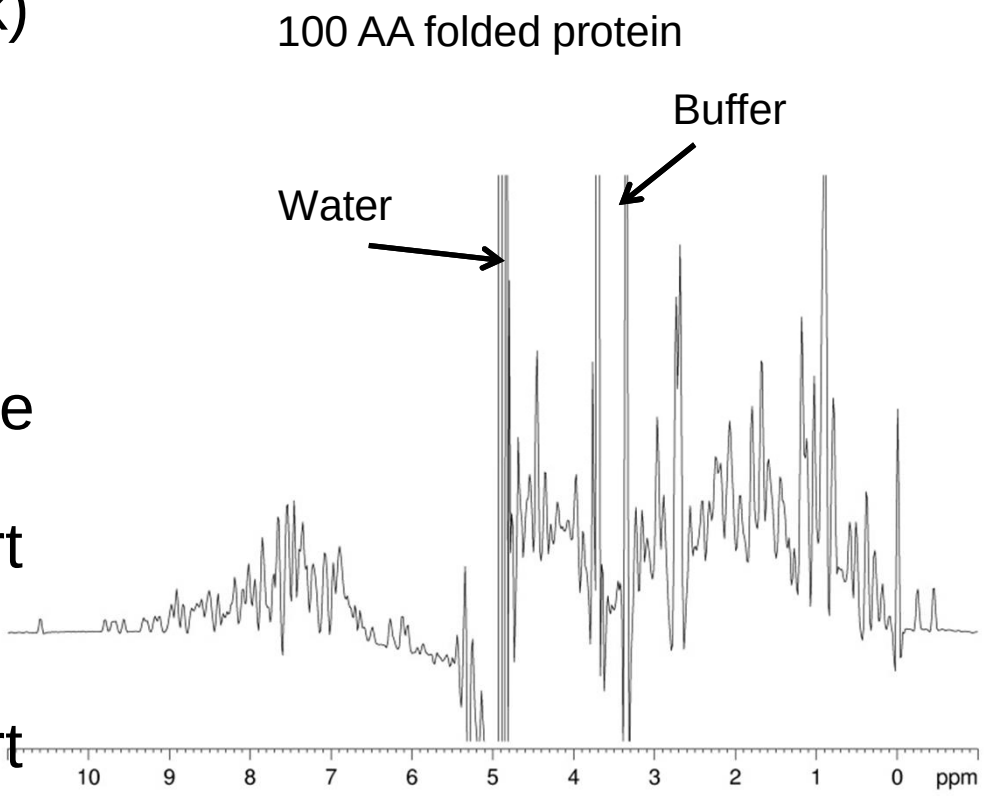
Disordered protein



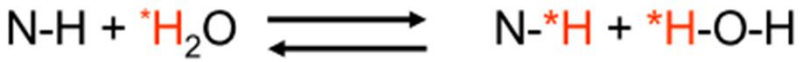
spectre ^{1}H @ 600MHz, 298K 200 residue protein

Specificity of protein NMR (and peptides)

- NMR experiment in H_2O (with 5-10% D_2O for the lock)
- Need water suppression and, ideally, NMR-invisible buffers
- Detected signals are pH dependent (pH<7) because amide N-H protons exchange with water protons
- High Molecular Weight, short T_2 ⇒ fast relaxation (loss of coherence)
- High Molecular Weight, short T_2 ⇒ fast relaxation (loss of coherence)
- Isotopic labeling: ^{15}N, ^{13}C, ^{2}H
- Most applications require higher-dimensional spectra!

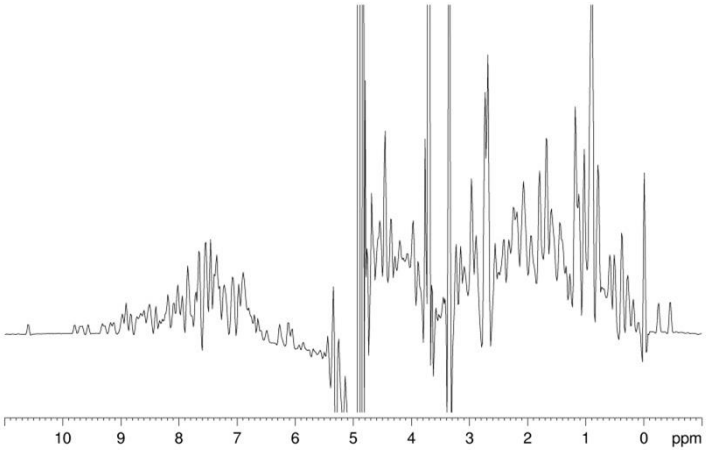


C. Smet-Nocca



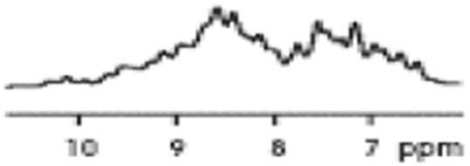
Protein NMR

1D proton spectrum
100 aa protein



1D proton spectrum
500 aa protein

High Molecular Weight, short T_2 ź fast relaxation (loss of coherence)



Resonance broadening ↔ relaxation

→ NMR spectroscopy is « traditionnally » ***limited to 200-250 aa proteins of about 20-25 kDa***

^{1}H NMR Spectrum of proteins

Proteins, ^{1}H spectrum interpretation is impossible :

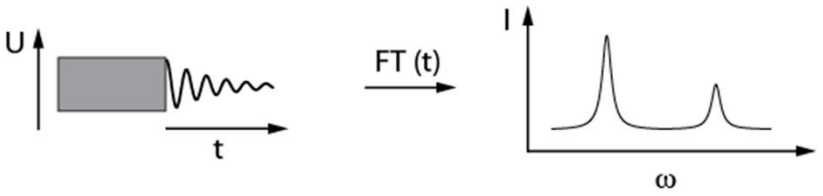
- ❑ Signal number
- ❑ overlap

But usefull to:

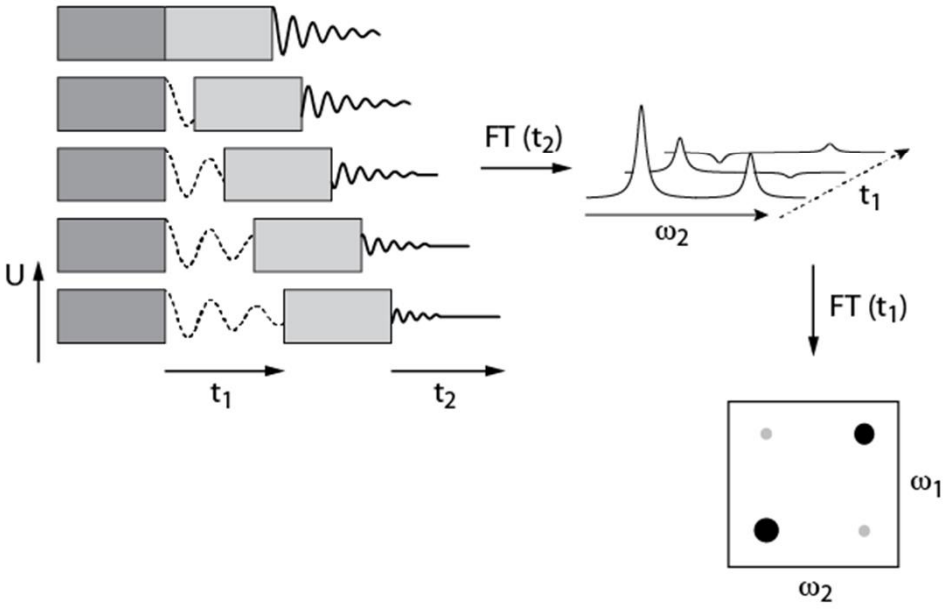
- ❑ Check protein in sample
- ❑ Check protein concentration (by comparison with a protein reference spectrum)
- ❑ Check signal to noise
- ❑ solution contamination (bad dessalting)
- ❑ Globular or disordered
- ❑ calibration of ^{1}H excitation pulse (P1)
- ❑ Check shims

From 1D to 2D

1D



2D

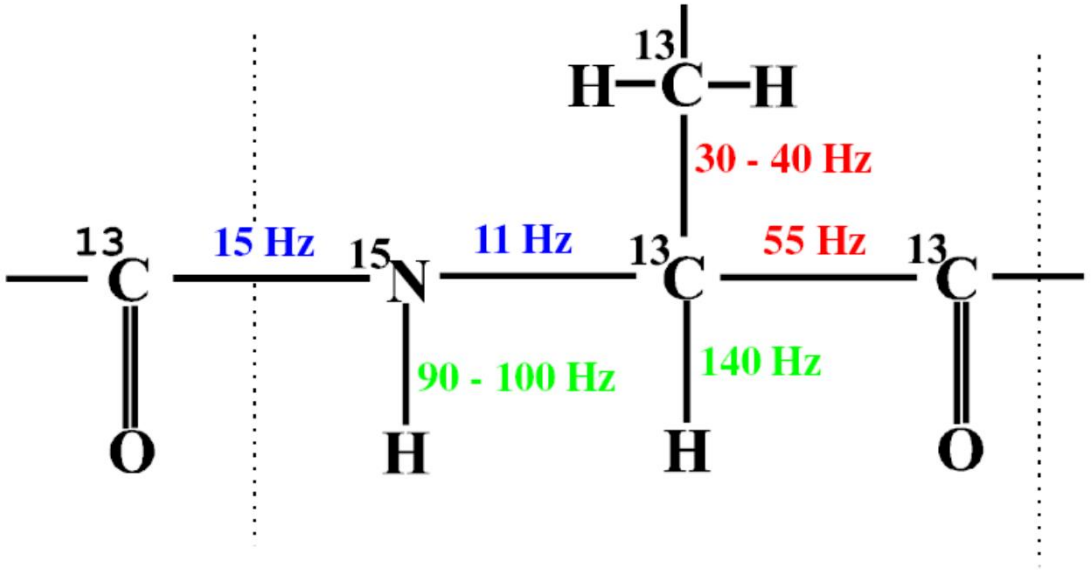


- Additional frequency dimension is indirectly recorded via an incremented delay
- Imprints frequencies of coupled nuclei onto signals of the 1D spectrum

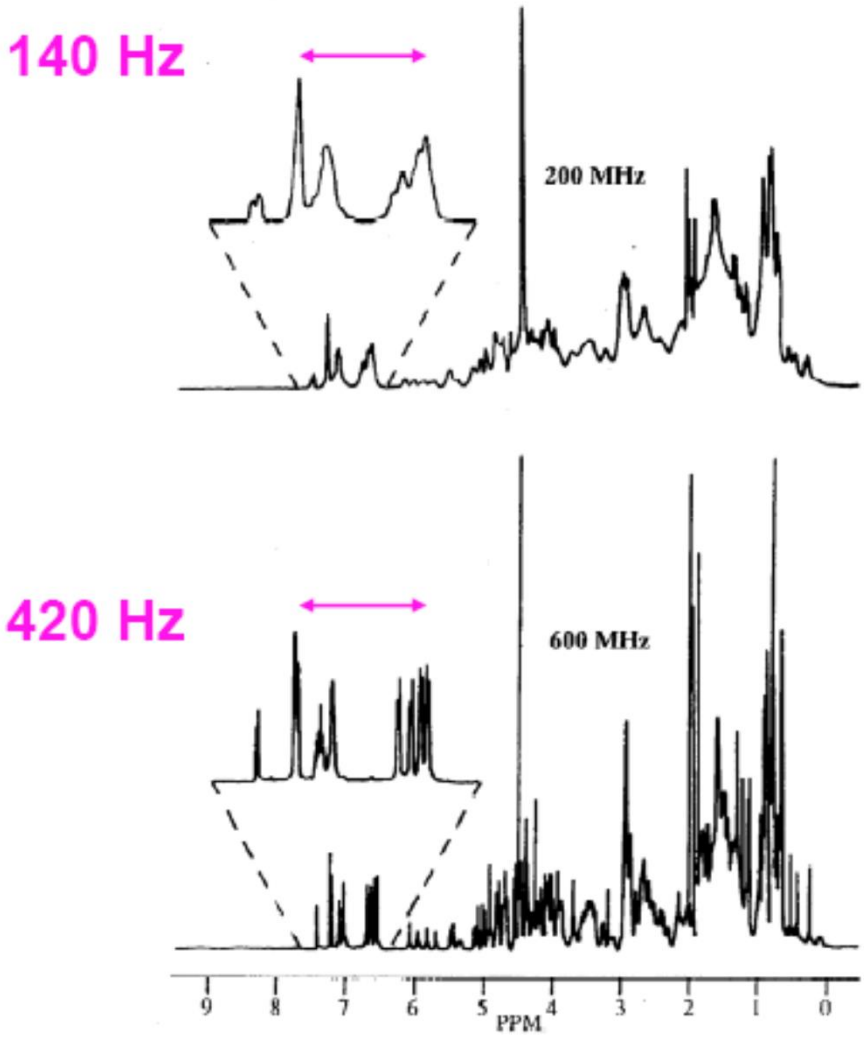
⇒ 2-dimensional Fourier transformation

Scalar coupling (J)

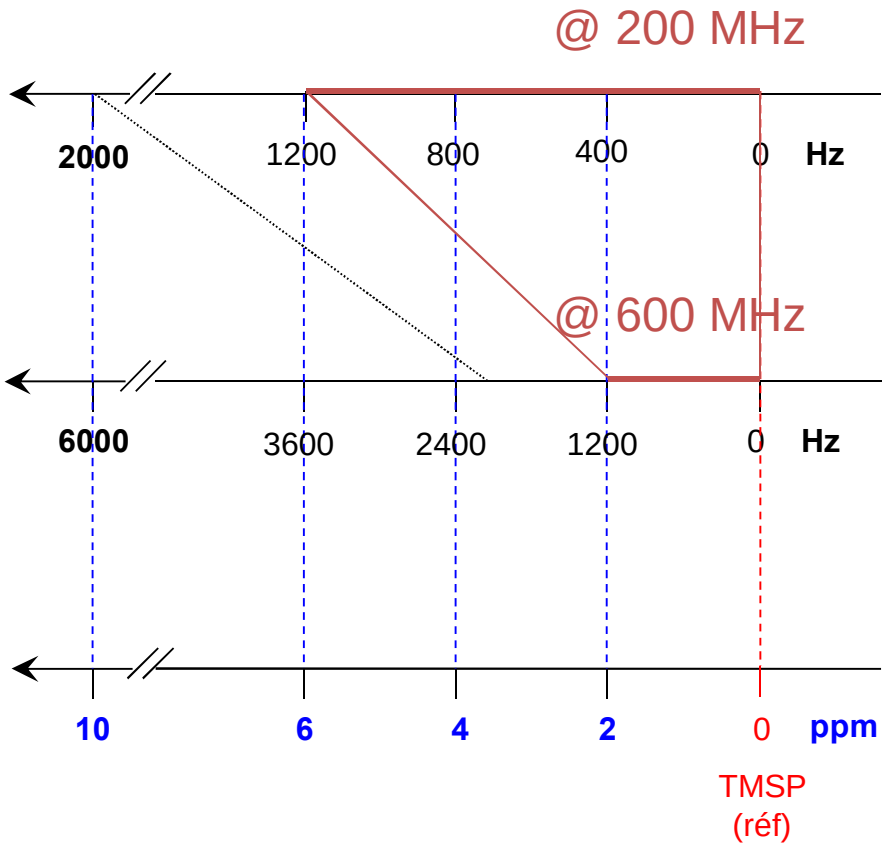
Heteronuclear coupling in polypeptidic chains



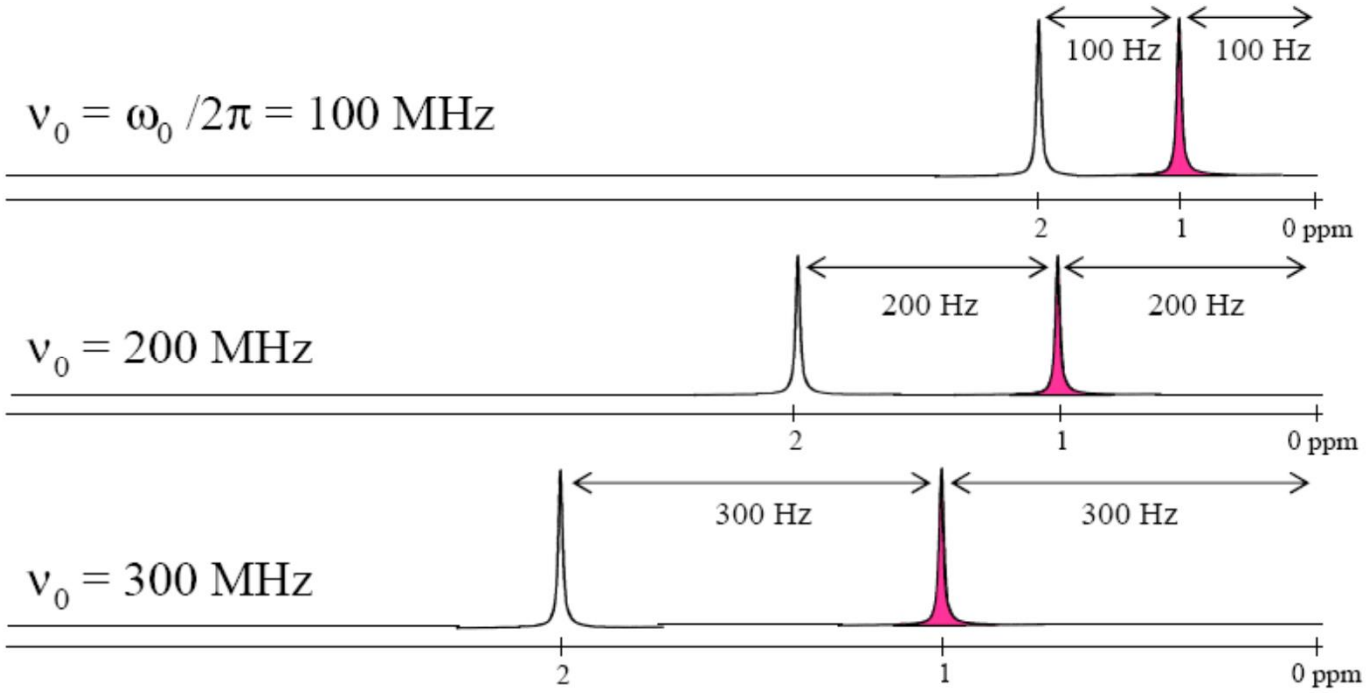
Spectrum Evolution in function of B_0 : spectral resolution



Increase resolution



Spectrum Evolution in function of B_0 : spectral resolution



→ « stretch » of the scale with increase B_0

Solvent

Solvent:

- compound solubilisation/ signal resolution
- lock and shim (total or partial deuteration)

→ but intense signal because [^{1}H of H_2O] = 111 M (1.11 M si 1% H_2O)

→ [proteine] = 10 µM – 1 mM

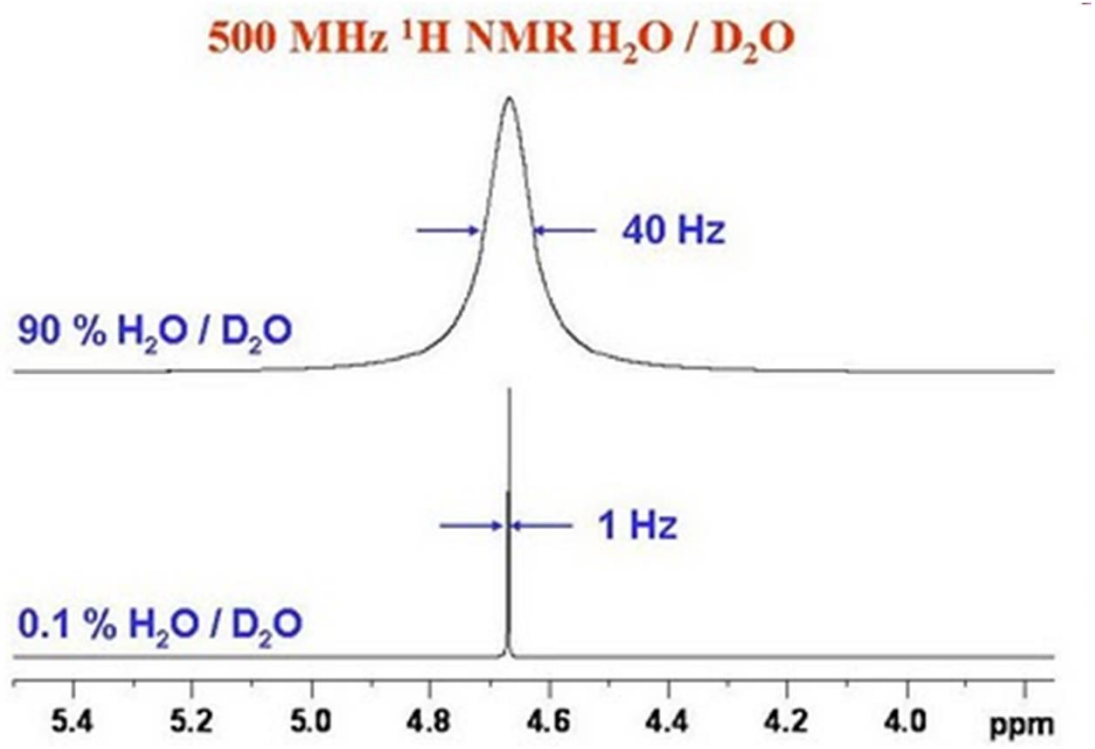
Å eliminate signals from solvent

Å Residual signal from protonated solvant when using deuterated solvent

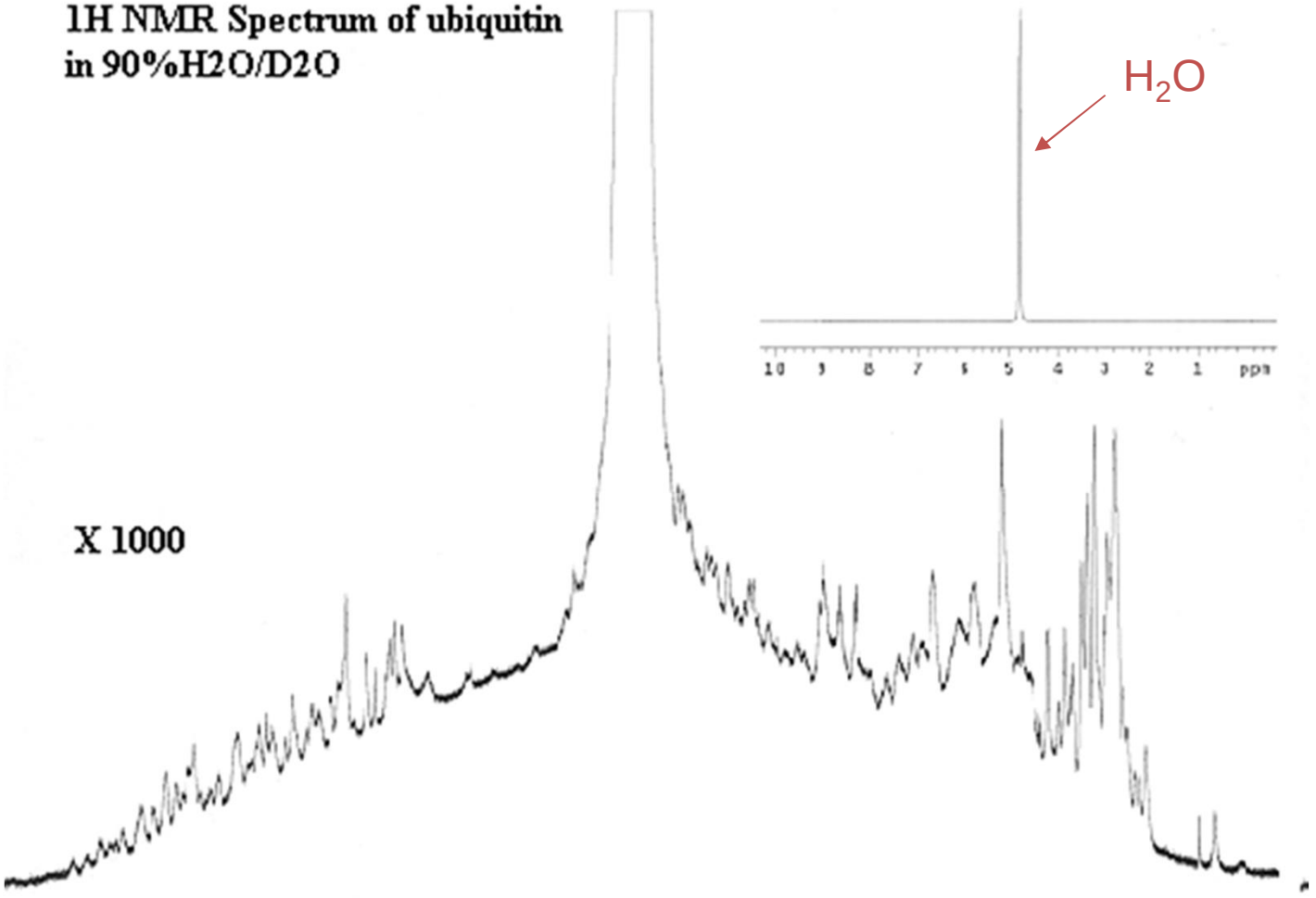
Å Solvent signal from protonated solvent (par ex. H_2O for protein study)

Water resonance width

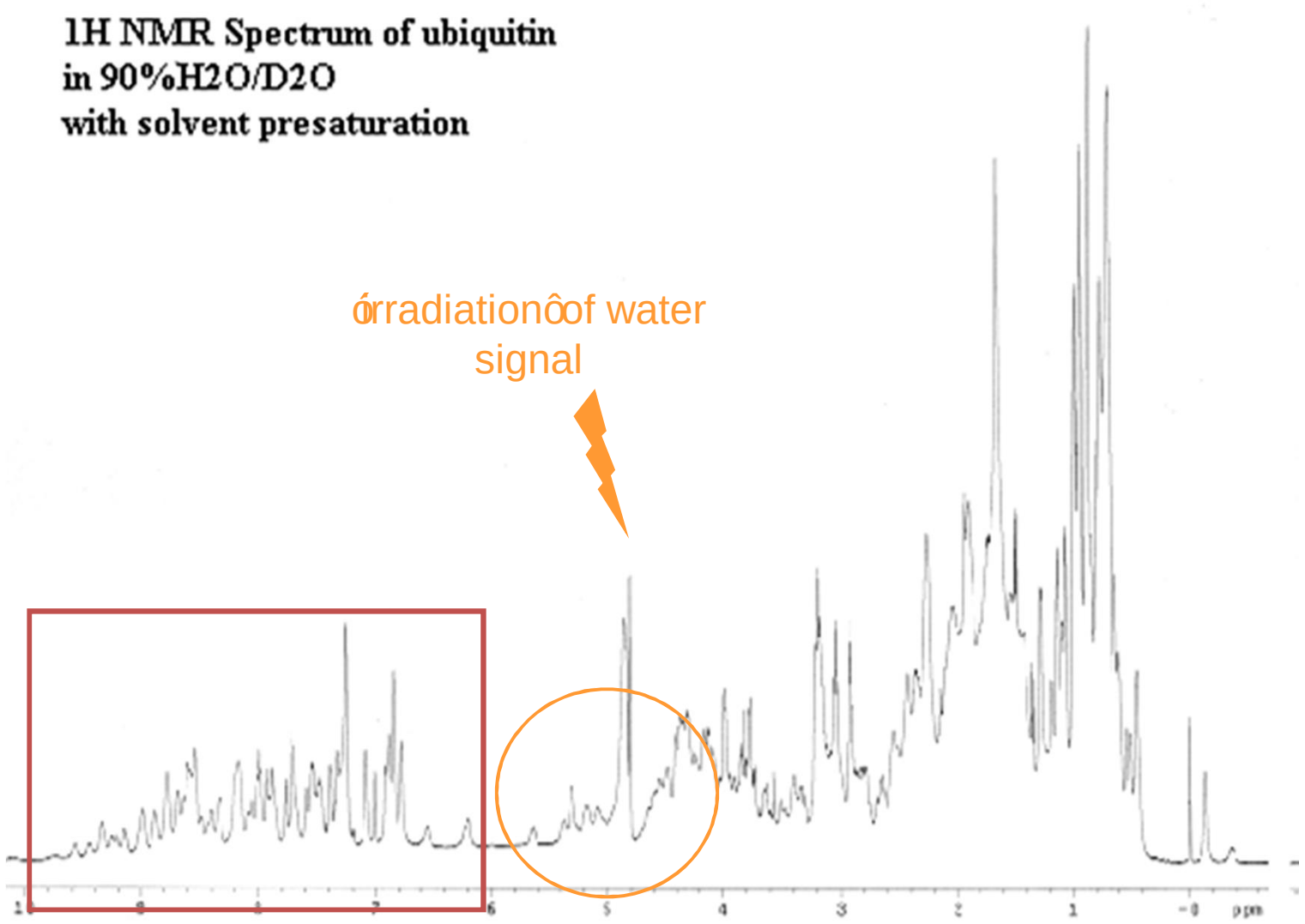
- ***radiation damping* :** The strong magnetisation of the water signal induces currents in the coil of the spectrometer, which generate magnetic field affecting peak width
- depend on water amount, tuning of the probe, B_0, é



NMR spectrum of a model protein, ubiquitin



NMR spectrum of a model protein, ubiquitin



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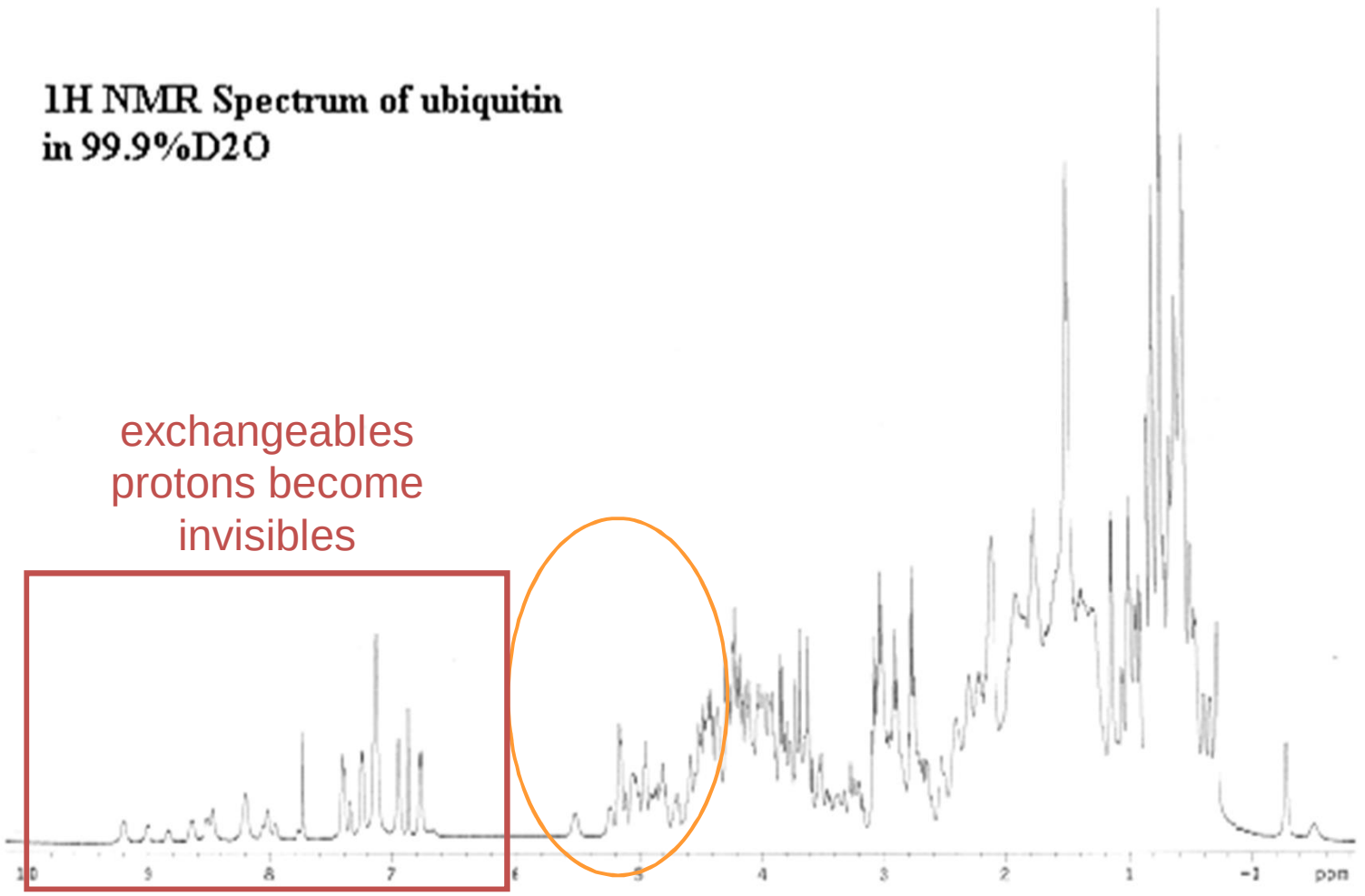
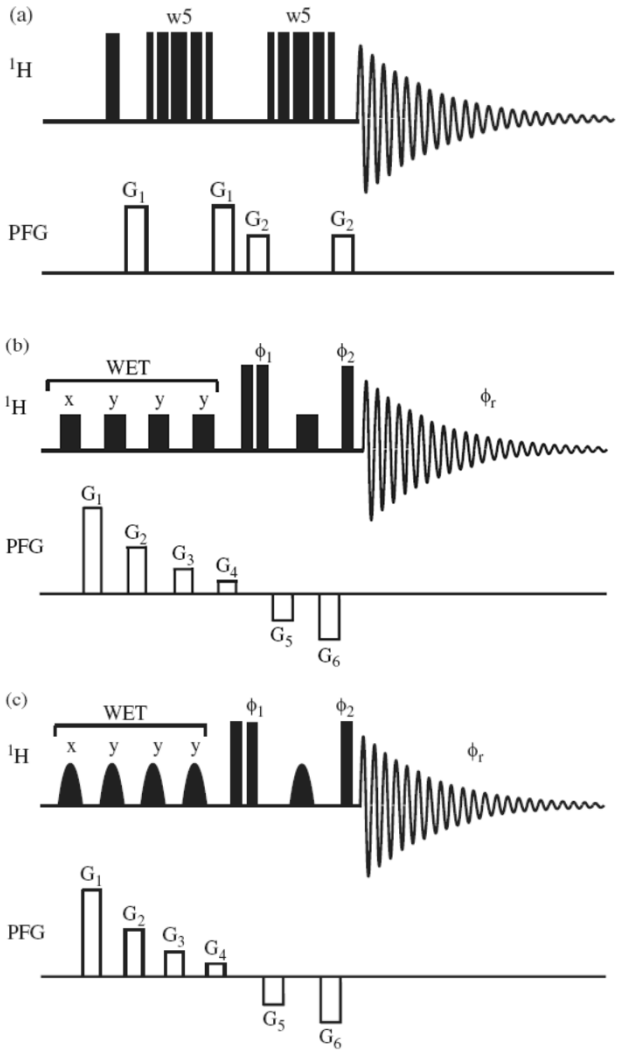
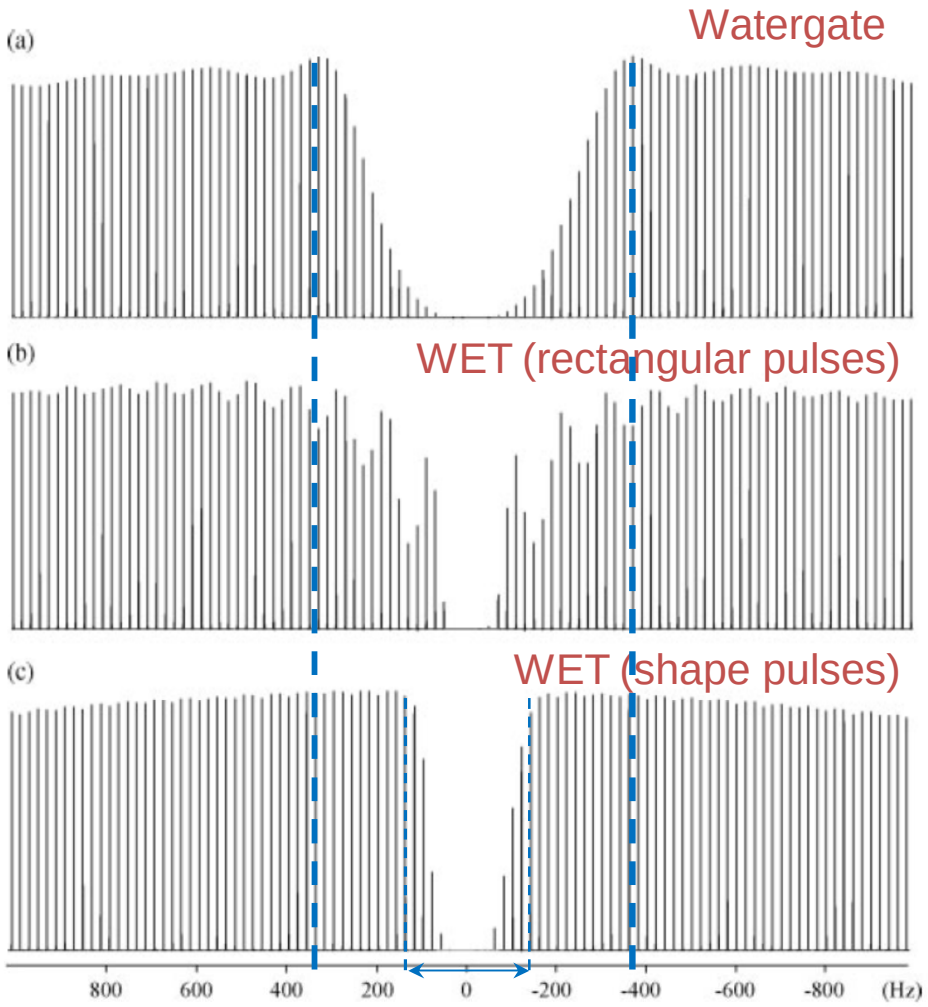


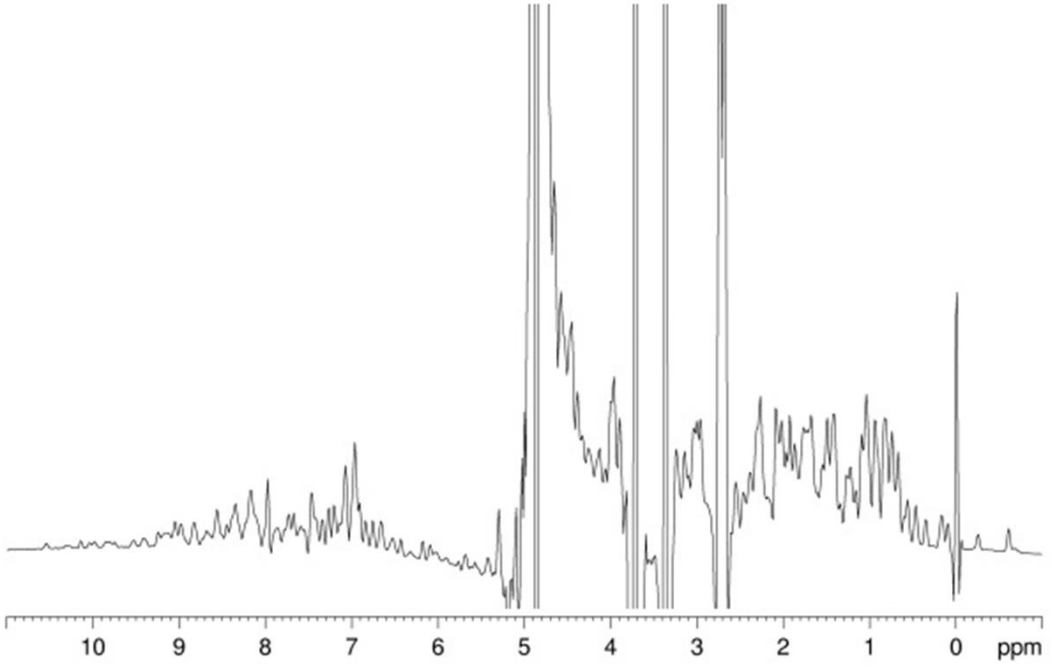
Solvent Signal Suppression





700 Hz soit 1.4 ppm
@ 500 MHz

NMR 1D to NMR 2D



RMN 1D ↔ 1 frequency

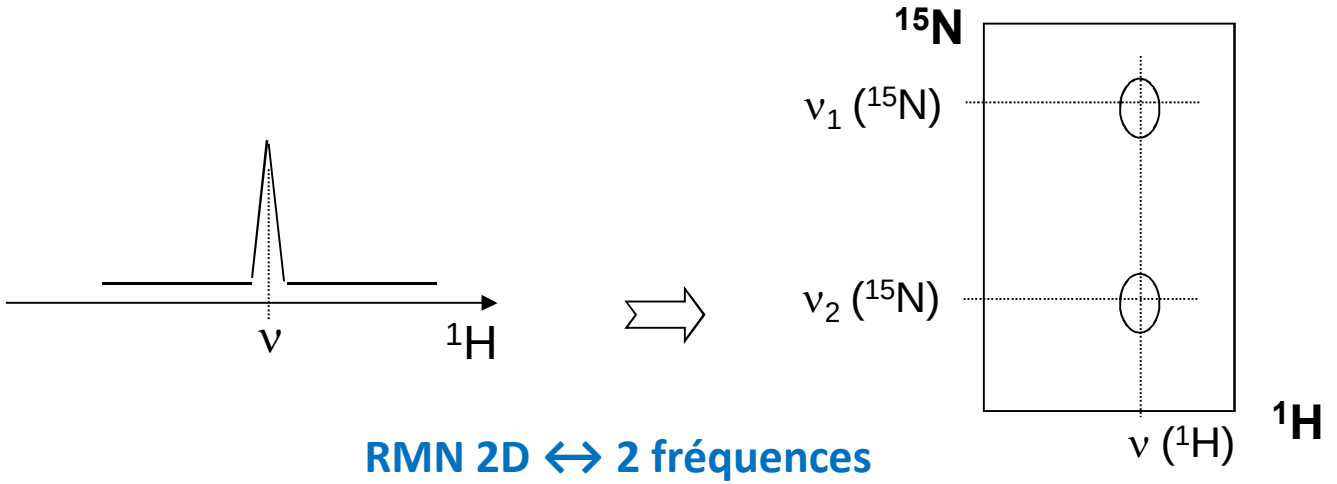
NMR 1D to NMR 2D

- 2D NMR : 2nd dimension = ^{1}H or ^{15}N or ^{13}C
- → isotopic labeling ^{15}N and/or ^{13}C : stable isotopes, non radioactives!
- for example : detection of coupled ^{15}N-^{1}H in proteins(all backbone HN + lateral chain HN)
- two ^{1}H with the same resonance frequency could have different ^{15}N frequencies

% 1H	99,9885
% 2H	0,0115
% 3H	0

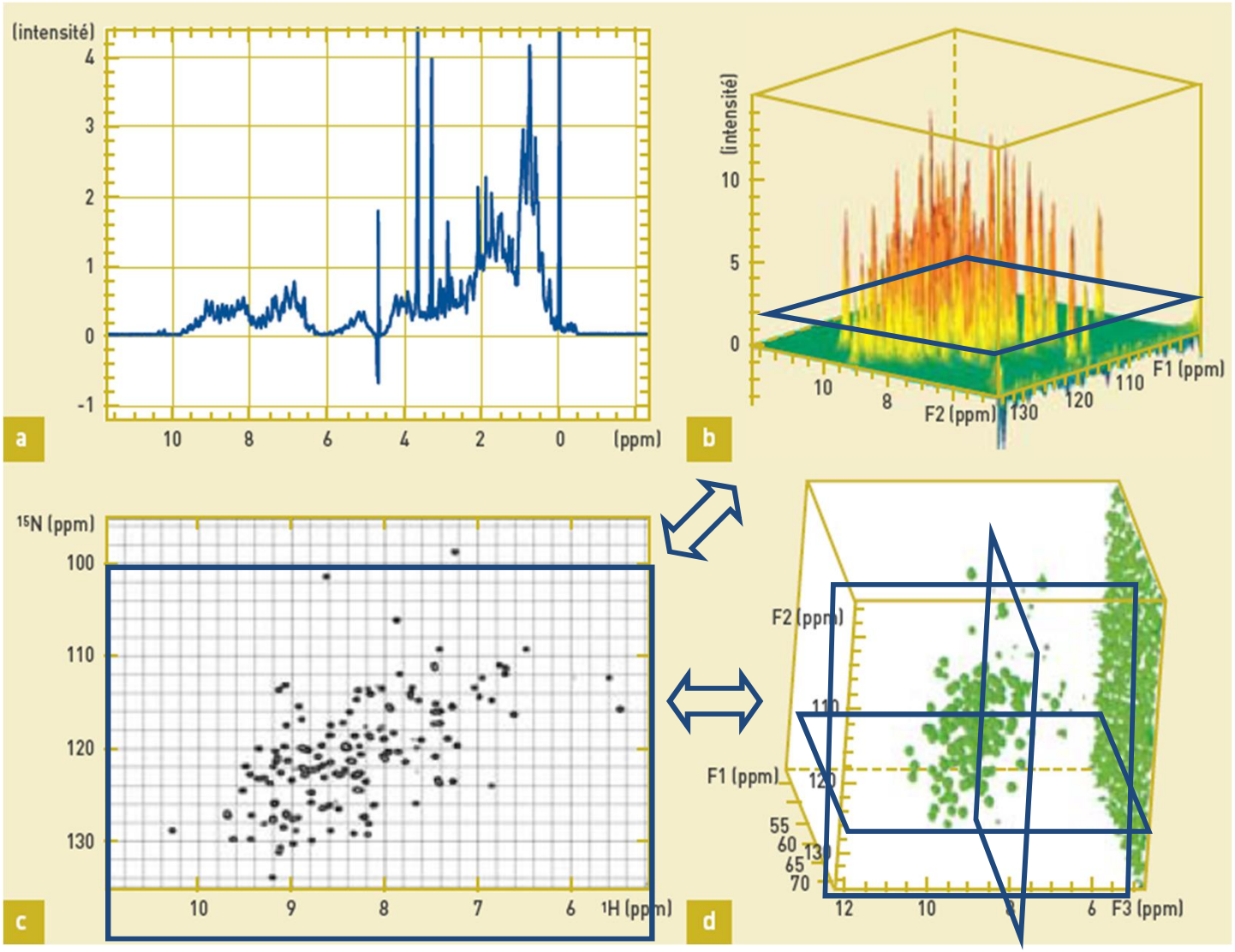
% 12C	98,93
% 13C	1,07
% 14C	0

% 14N	99,632
% 15N	0,368



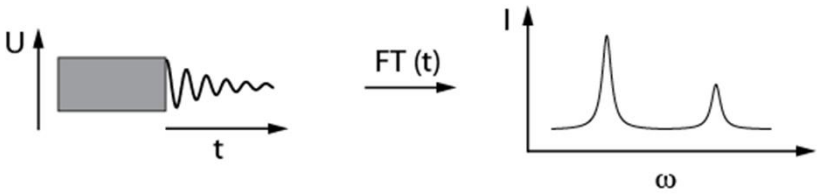
RMN 2D ↔ 2 fréquences

From 1D NMR to multi-dimensional NMR

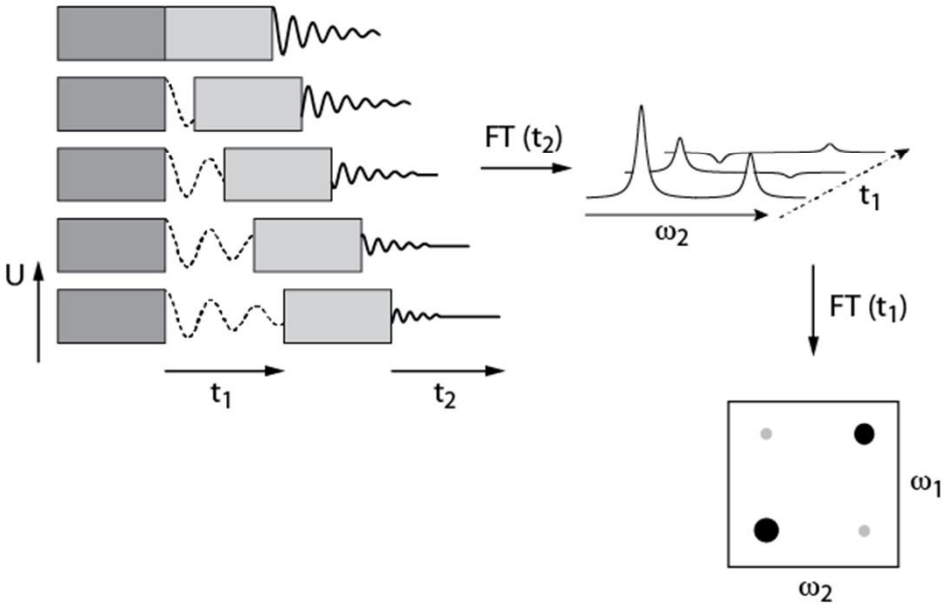


From 1D to 2D

1D



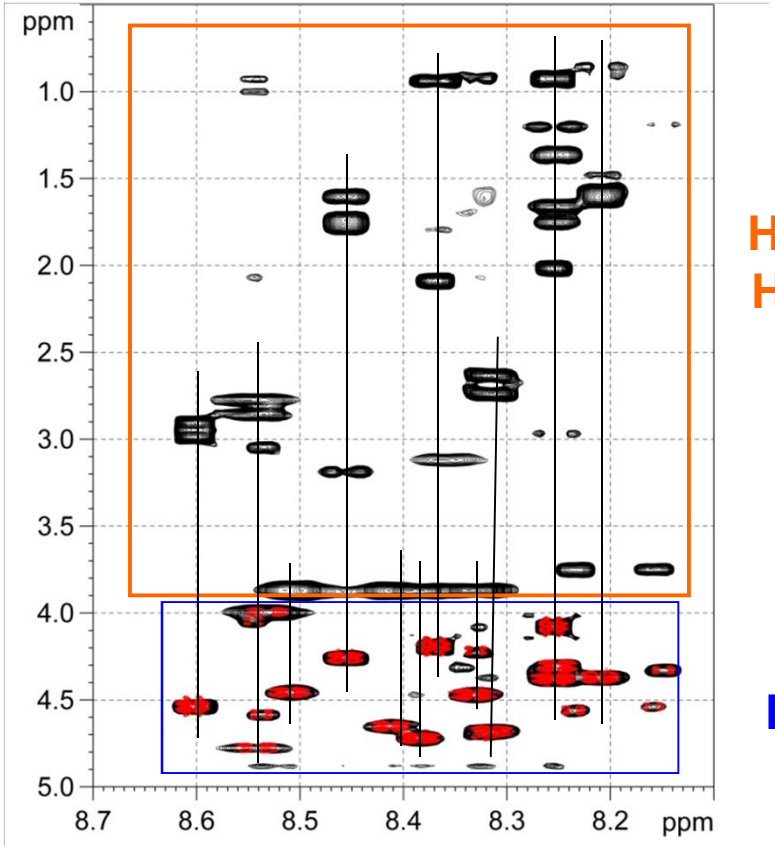
2D



- Additional frequency dimension is indirectly recorded via an incremented delay
- Imprints frequencies of coupled nuclei onto signals of the 1D spectrum

⇒ 2-dimensional Fourier transformation

2D ^{1}H NMR: covalent connections



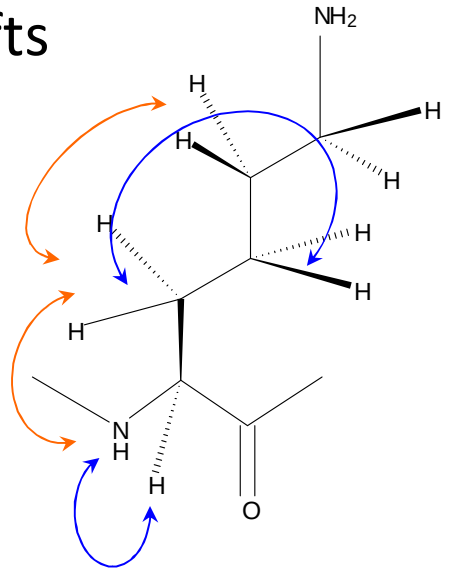
H_N-Hβ,
H_N-Hγ,
…

H_N-Hα

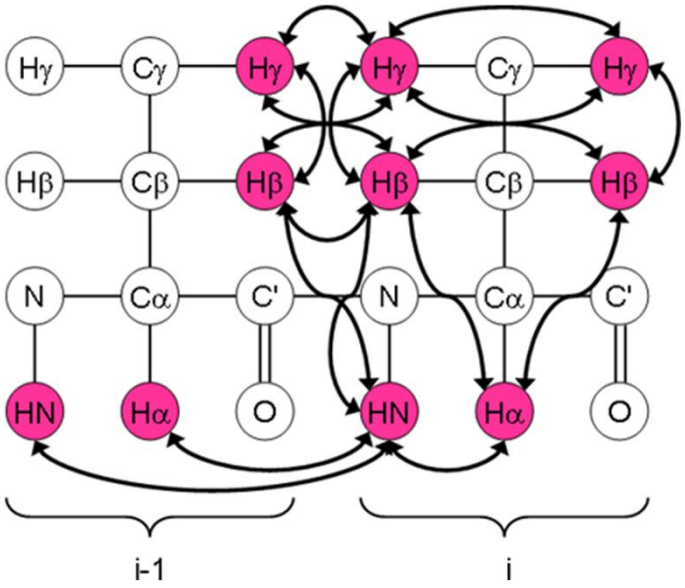
TOCSY
COSY

C. Smet-Nocca

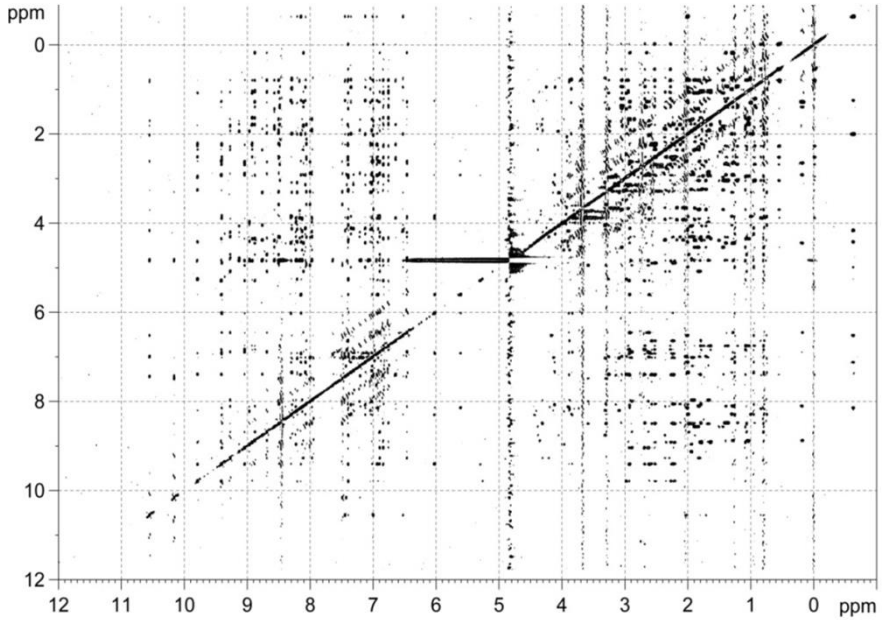
- Through-bond correlations of protons within individual amino acid residues via J coupling: COSY, TOCSY
- Identify residue type via characteristic chemical shifts



2D ^{1}H NMR: through-space connections



http://www.protein-nmr.org.uk/solution-nmr/spectrum-descriptions/h-h-noesy/



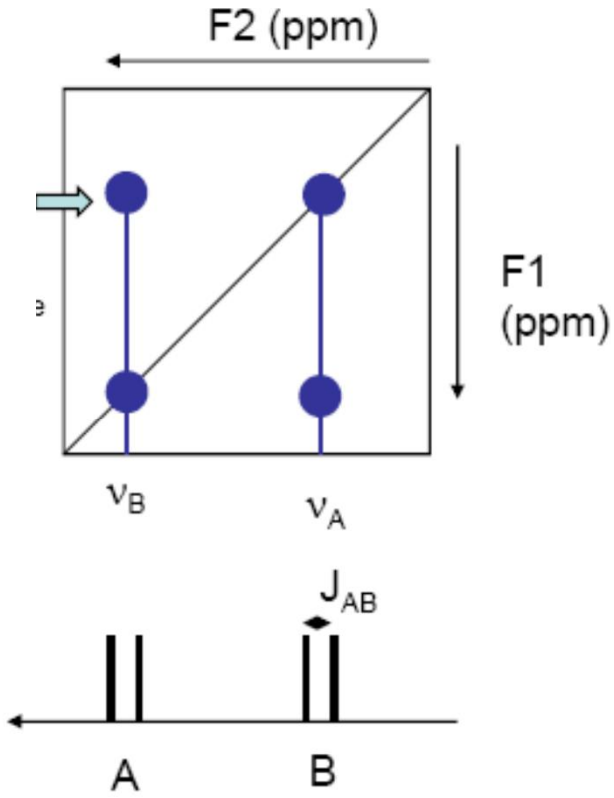
- NOESY: through-space correlations of protons within and between amino acid residues via dipolar coupling (NOE)
- Sequential assignment of ^{1}H resonances
- Structural information

C. Smet-Nocca

Homonuclear correlation spectroscopy

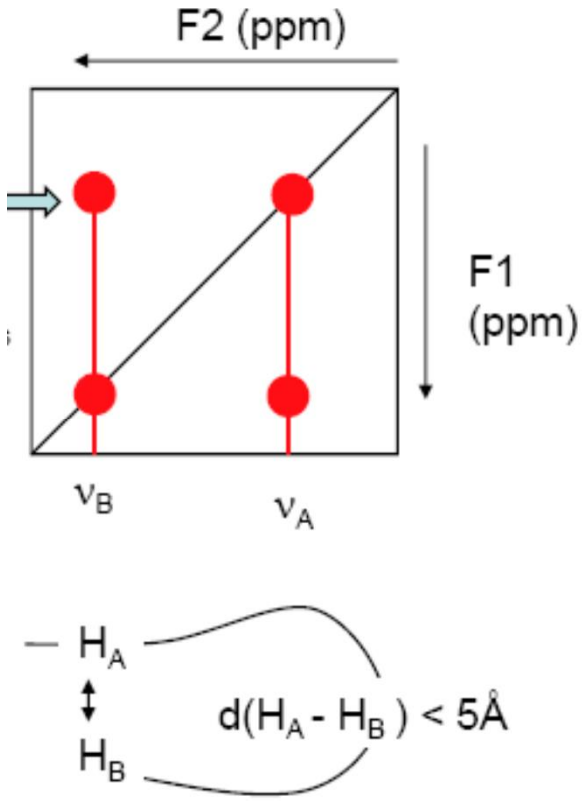
via scalar coupling (COSY, TOCSY)

Correlation peak due to a coupling constant



Dipolar coupling (NOESY, ROESY)

Correlation peak due to proximity (through space) of 2 protons



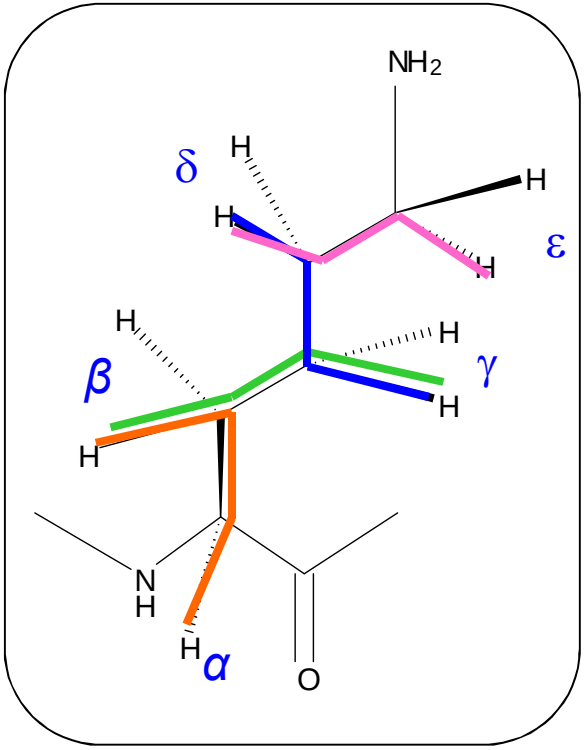
^{1}H-^{1}H COSY et TOCSY

- COSY : homonuclear coupling ^{1}H-^{1}H

^{3}J (3 chemical bonds)

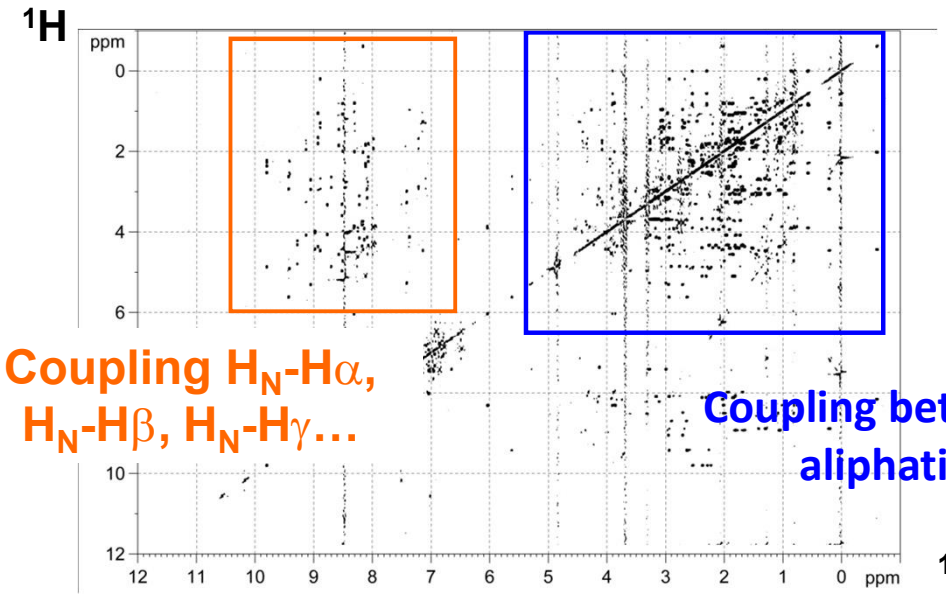
- TOCSY : homonuclear coupling ^{1}H-^{1}H

$^{3}J+ {}^{4}J+ {}^{5}J + {}^{6}J$ (de 3 to 6 chemical bonds)



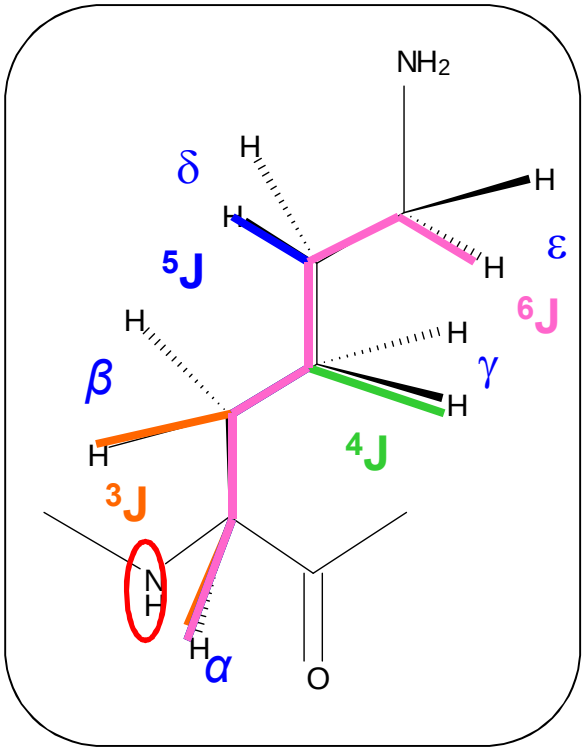
- No isotopic labeling
- Experiments allowing assignment of protons chemical shift values

^1H-^1H COSY et TOCSY



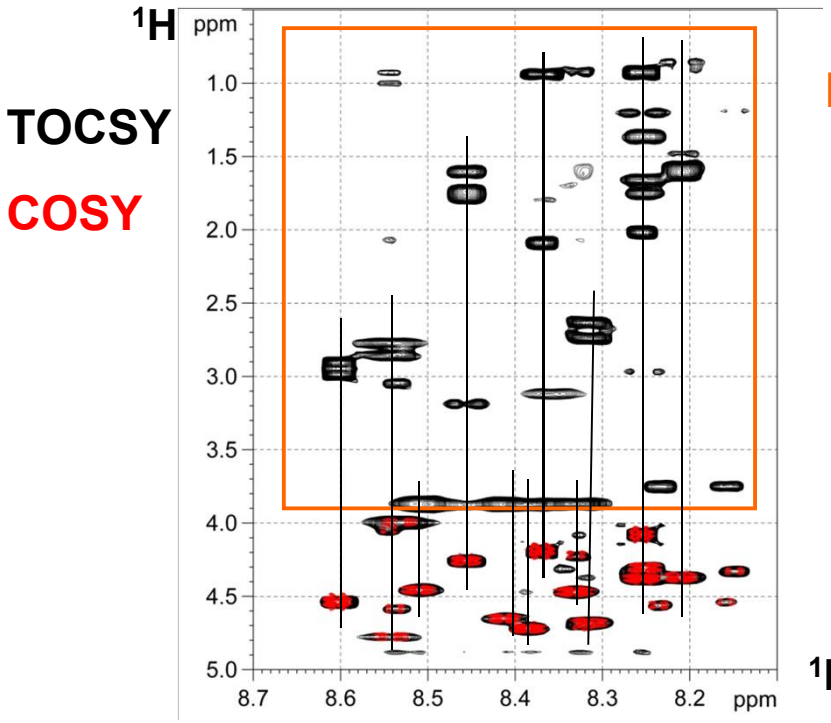
Coupling H_N-Hα, H_N-Hβ, H_N-Hγ…

Coupling between aliphatic protons



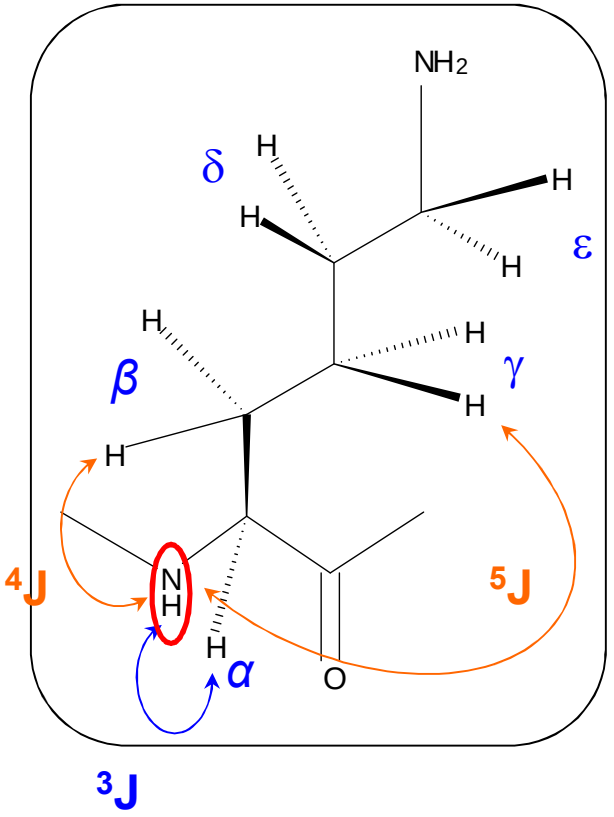
^{1}H-^{1}H COSY and TOCSY

- Chemical shift tables δ H_N, Ha, Hb, Hg, Hd, He,… :
  - identification of the residue type



H_N-Hβ, H_N-Hγ…

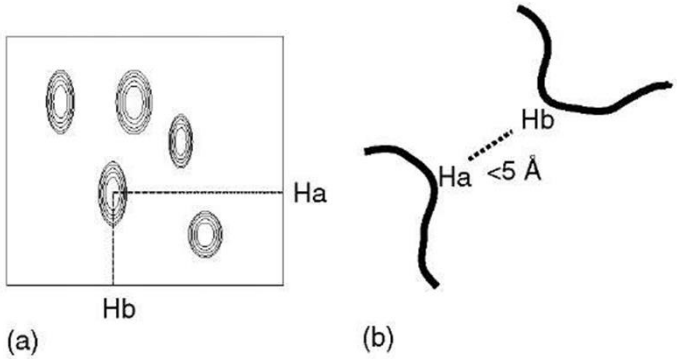
H_N-Hα

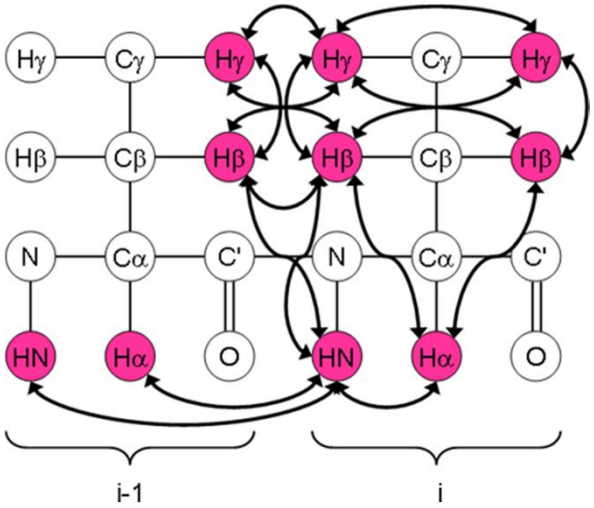


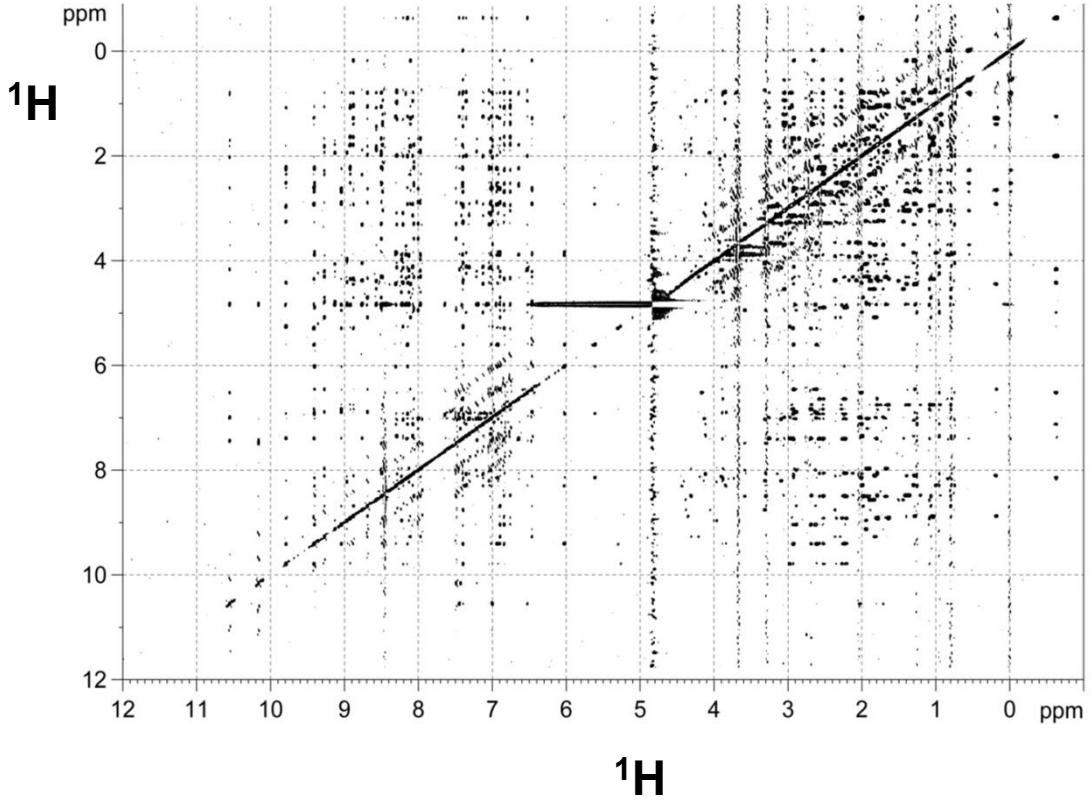
Nuclear Overhauser Effect spectroscopy : dipolar coupling

→No isotopic labeling

→**NOE effect will provide the distance constraints for structure**

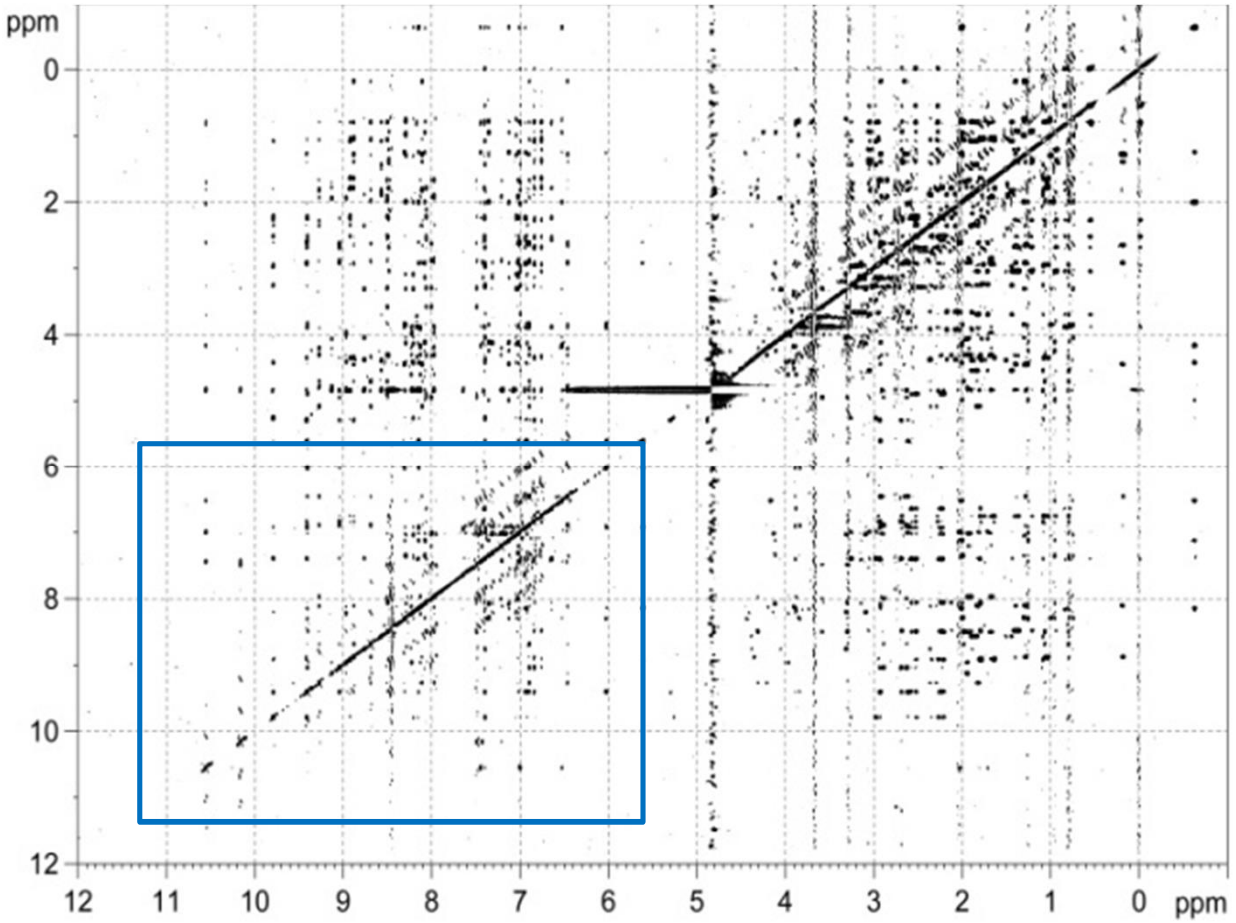






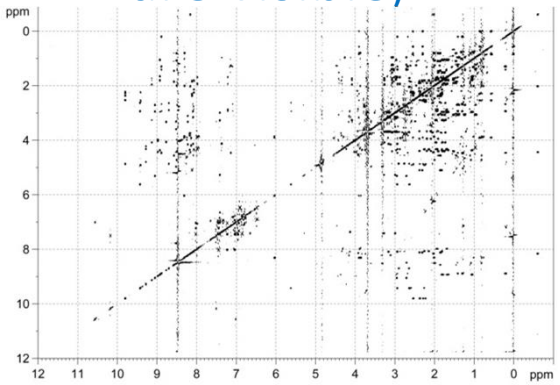
^{1}H-^{1}H NOESY

I_{cross} : proportional to $1/r^6$ with r is the distance between 2 nuclei



H_N-$H_N$coupling ↔ secundary structures

Similar to TOCSY
(some scalar coupling are visible)

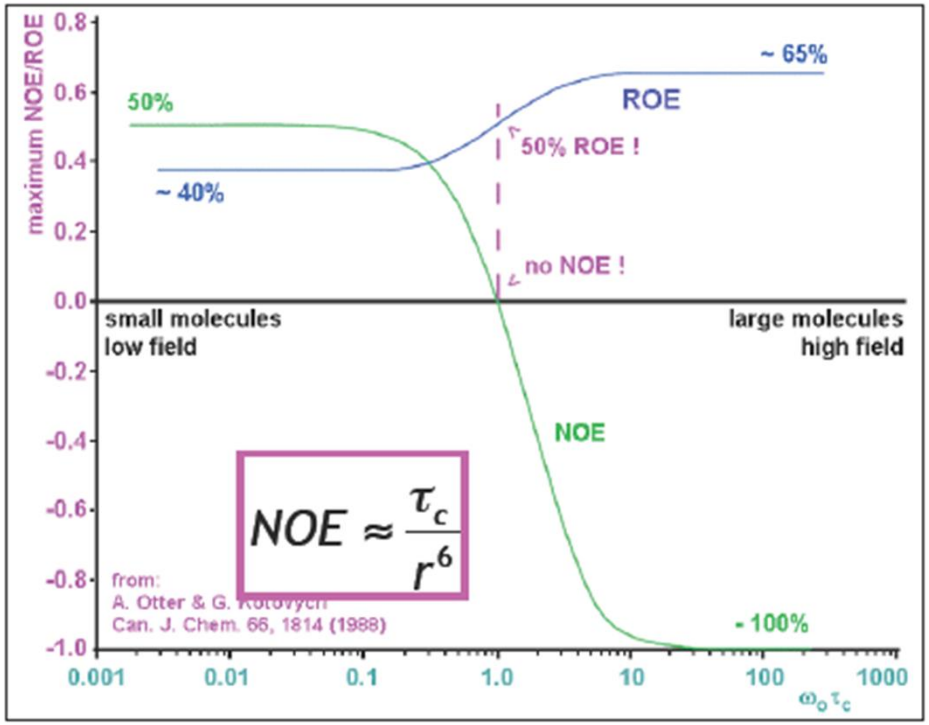


+

dipolar coupling
(through space less than 5 Å apart)

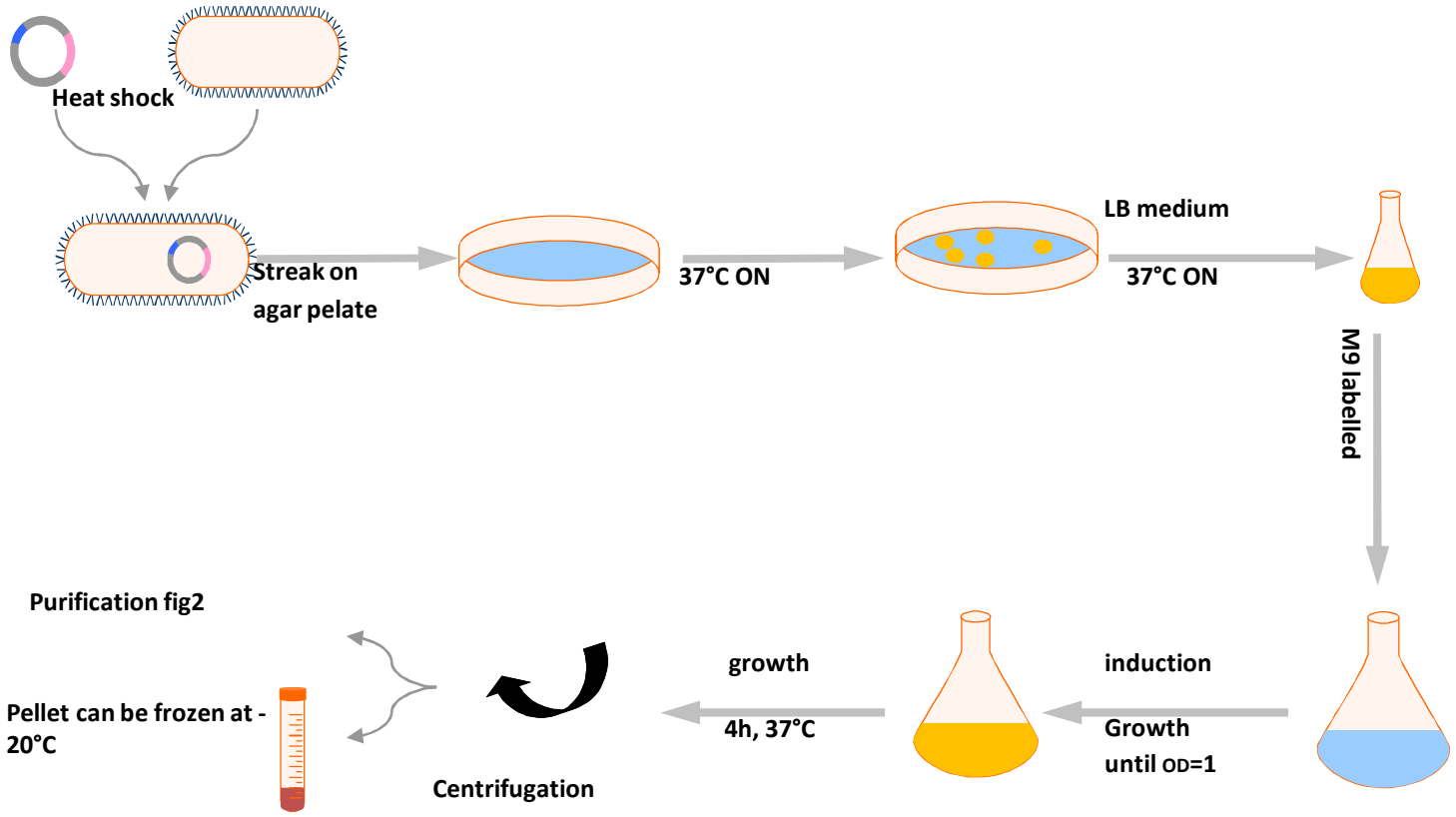
NOESY ou ROESY

- NOESY : not adapted for compound with molecular masses of 800 – 1500 Da (NOE ≈ 0)
- ROESY : less intense signal but ≠0



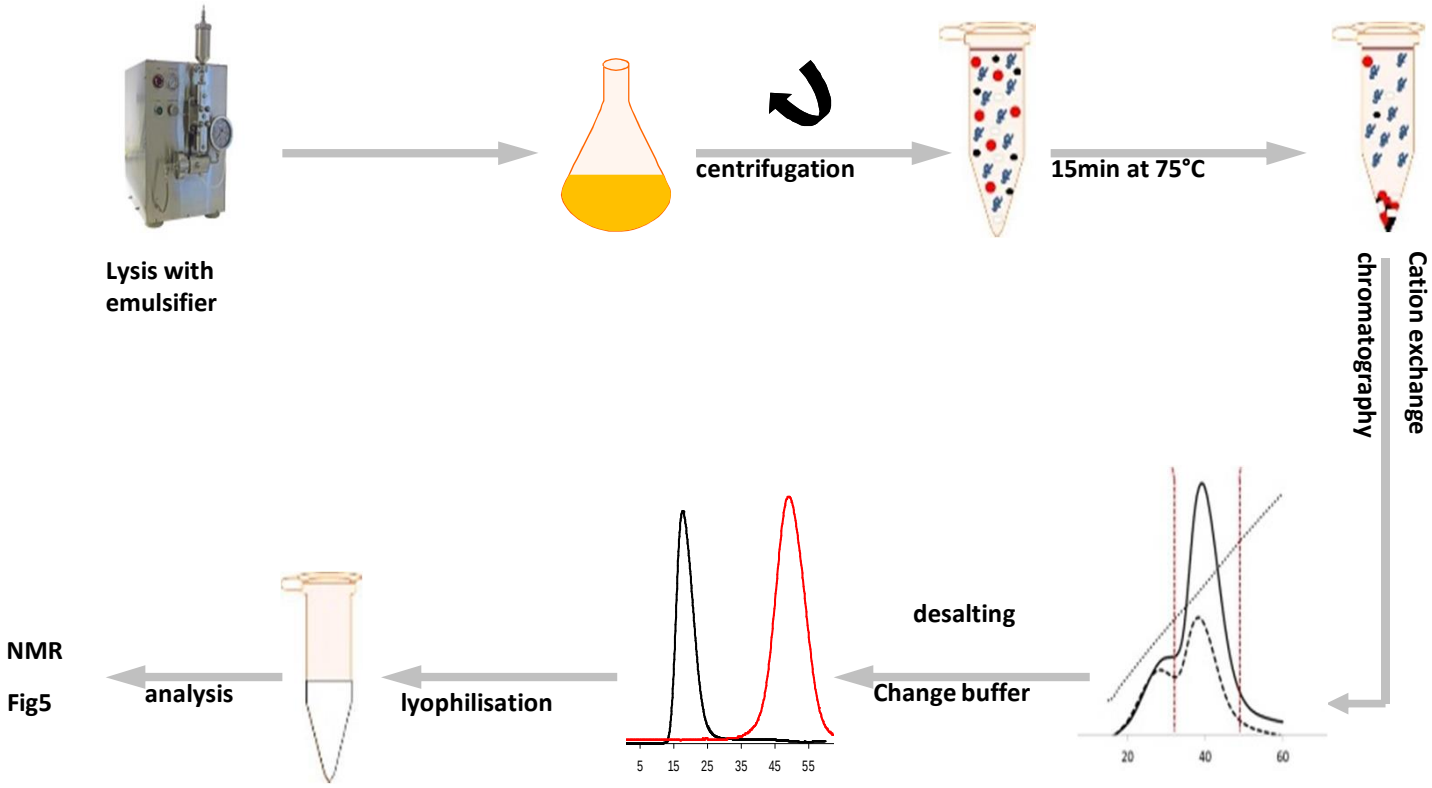
Heteronuclear NMR ï the standard for proteins

Isotopic labeling of proteins



- In most cases, the protein of interest is overexpressed recombinantly in bacteria (E. coli)
- Use minimal medium with $^{15}NH_4Cl$ and ^{13}C-glucose as sole nitrogen and carbon sources

Isotopic labeling of proteins



Å Purification

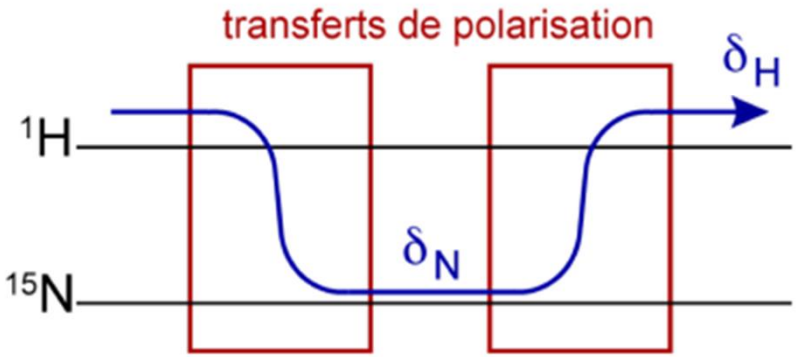
Heteronuclear NMR experiments

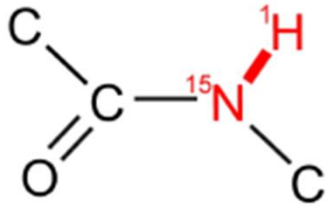
- In most solution NMR experiments, initial excitation and final detection of the signal is still done on ^{1}H due to superior sensitivity
- Rather complex pulse sequences serve to excite desired coherences and suppress unwanted ones

Two-dimension NMR is essential to obtain high-resolution spectra of complex biomolecules

The HSQC experiment: a keystone of biological NMR

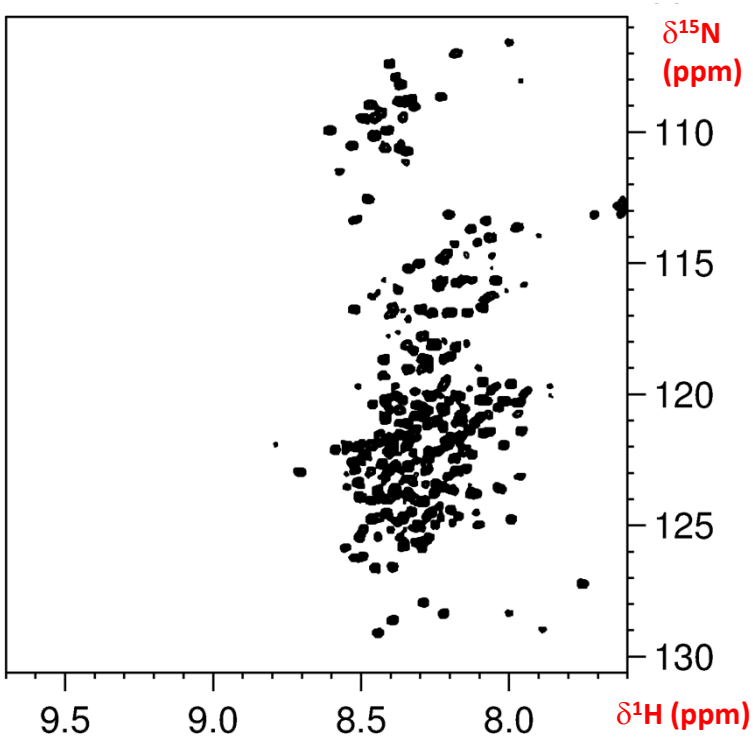
G. Bodenhausen et D. J. Ruben, *Chem. Phys. Lett.* 1980, 69, 185.

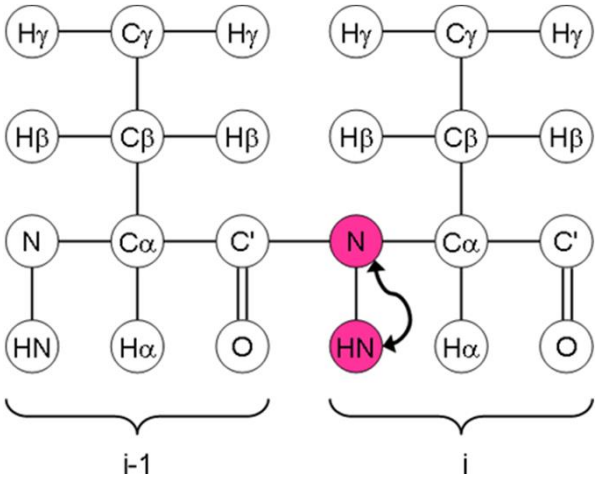




^{15}N-^{1}H HSQC: the fingerprint of a protein

HSQC: **h**eteronuclear **s**ingle **q**uantum **c**oherence





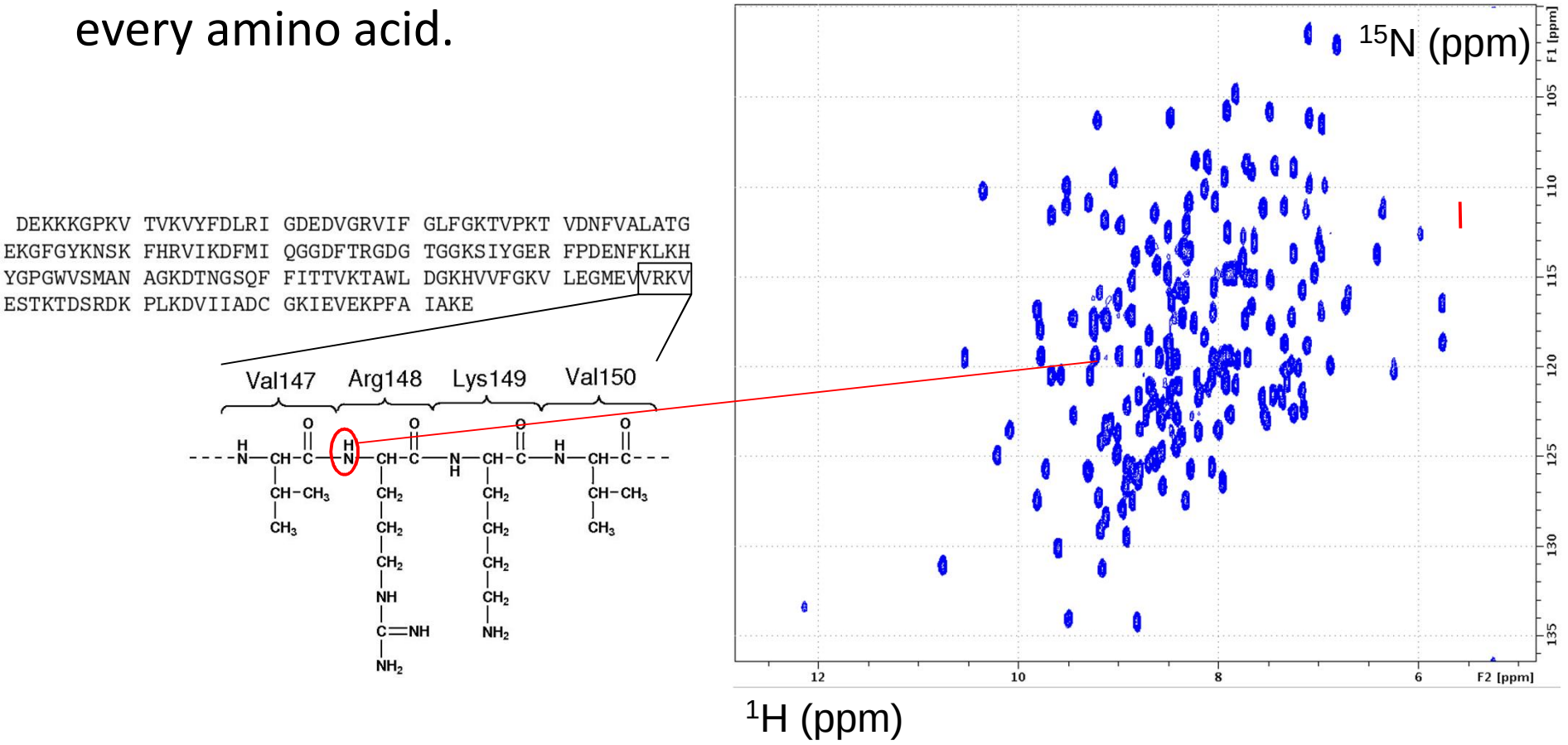
^{15}N isotopic labeling of the protein is required

- One cross-peak for each N-H amide group in the protein (as well as N-H-containing side-chains)
- Check folding and amenability for further study
- Basic experiment for protein NMR in solution

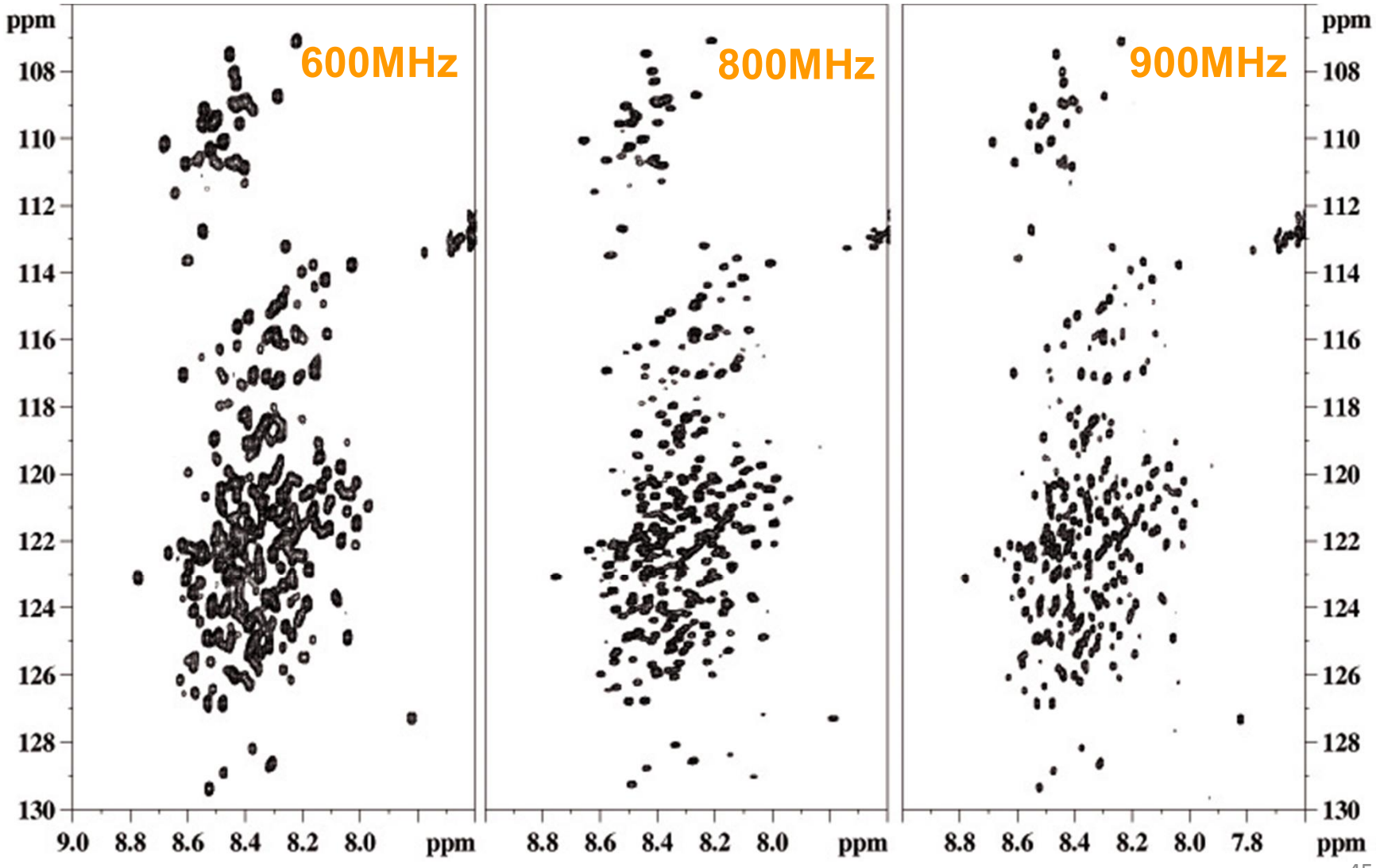
^{15}N-^{1}H HSQC: the fingerprint of a protein

2D proton-nitrogen correlation spectrum

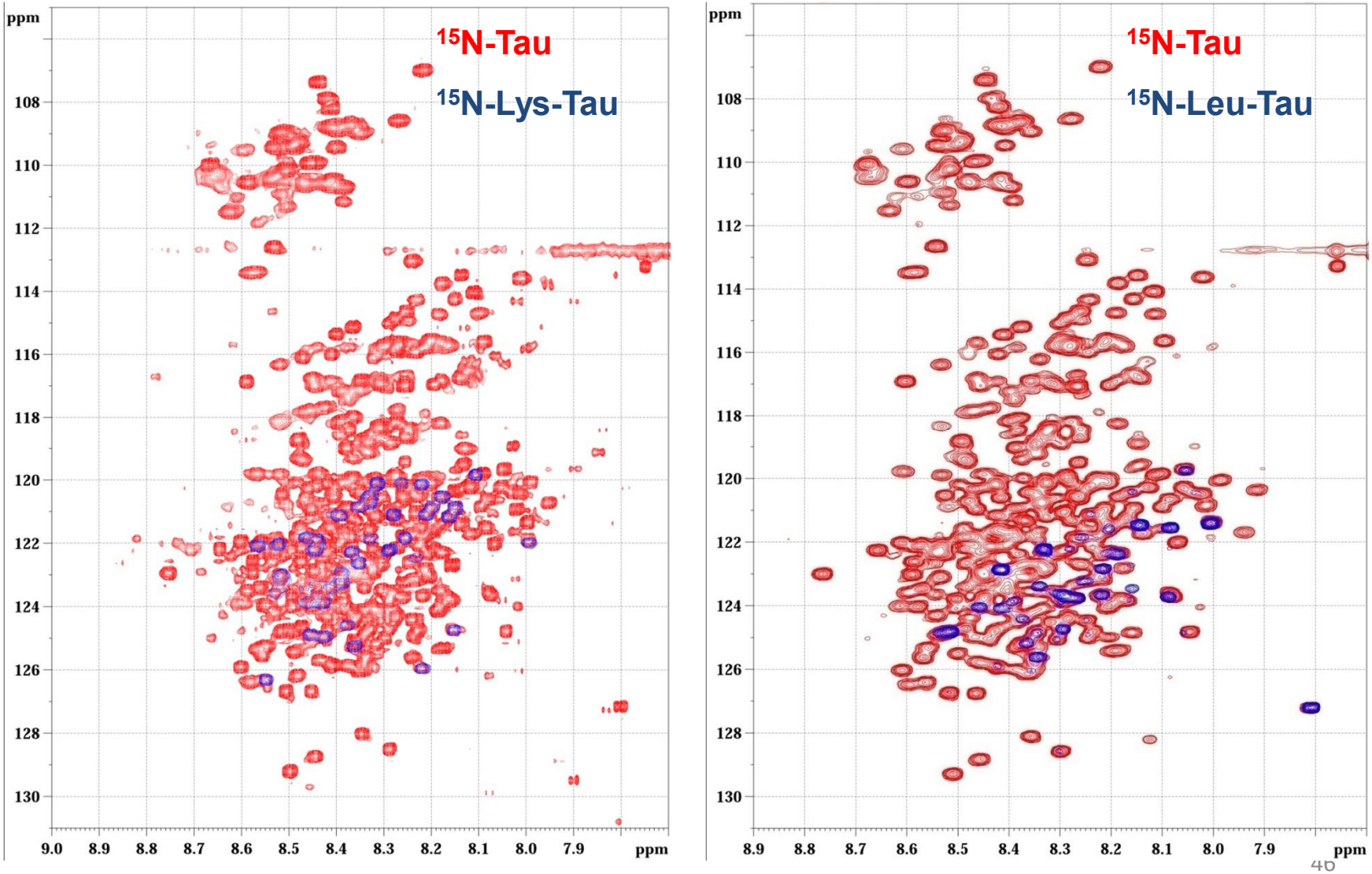
One peak per Amino Acid (excepts prolines) originating from the amide function present in every amino acid.



Resolution: Fields 600, 800, 900 MHz

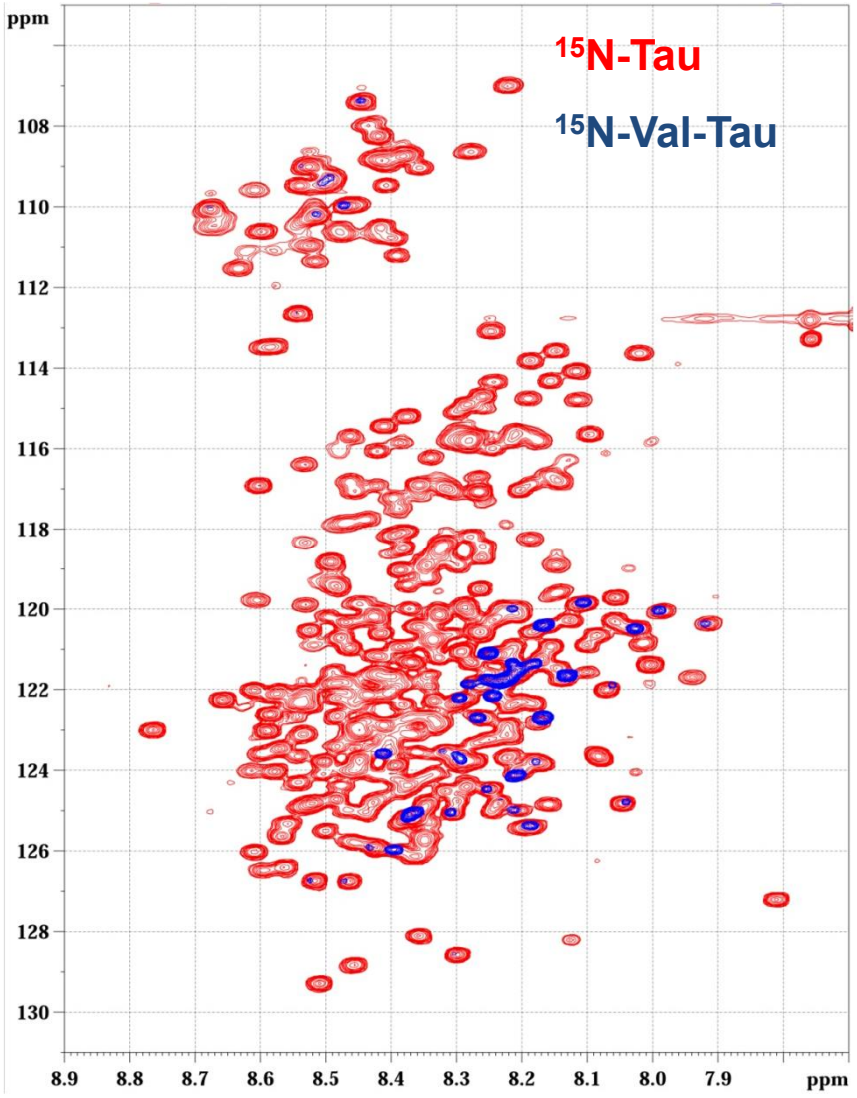


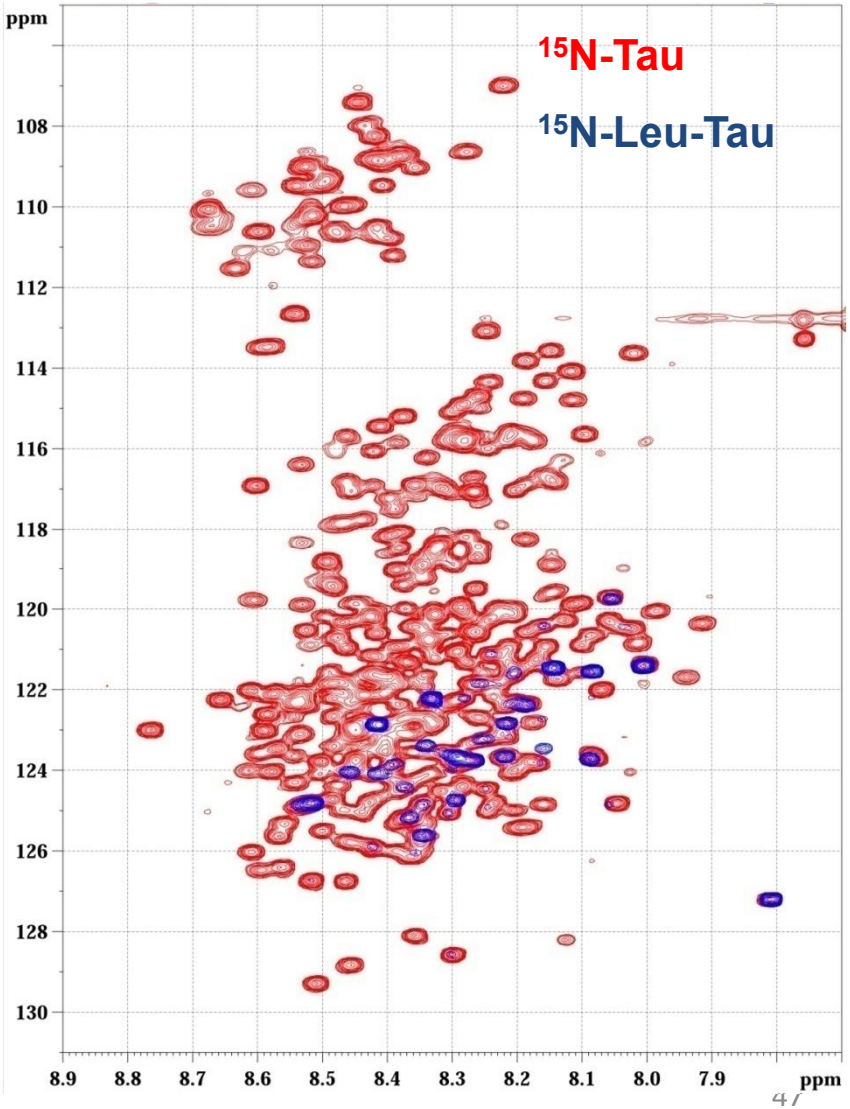
Selective labelling: ^{1}H-$^{15}NHSQC$ sub-spectrum



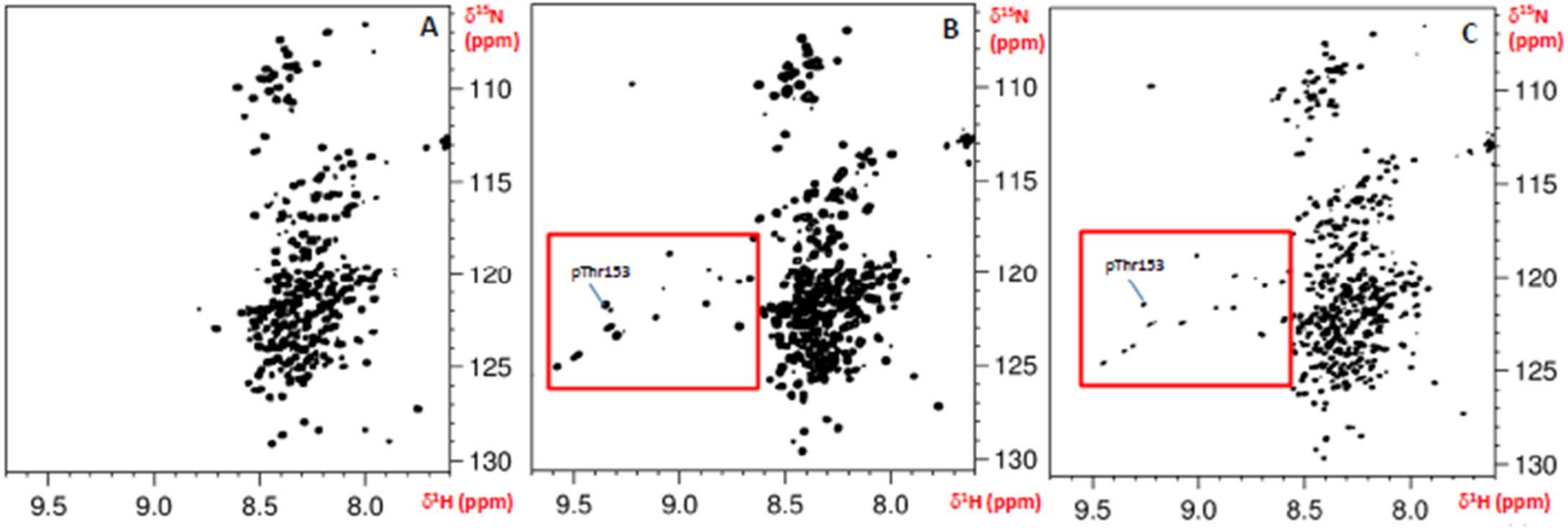
Problem: amino-acid scrambling in bacteria

Selective labelling: ^{1}H-$^{15}NHSQC$ sub-spectrum





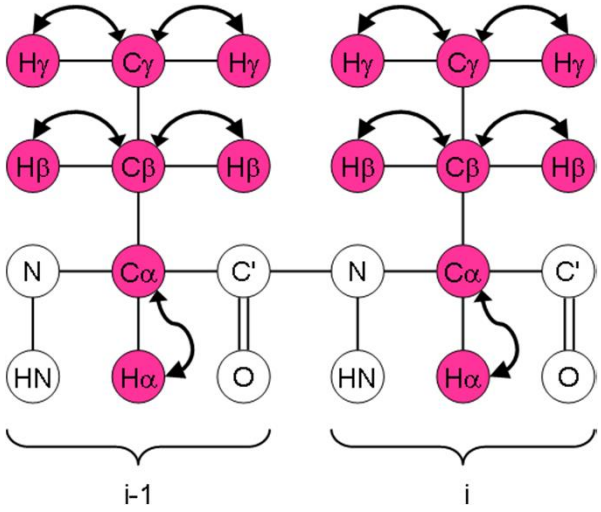
^{1}H-^{15}N HSQC of phosphorylated IDPs

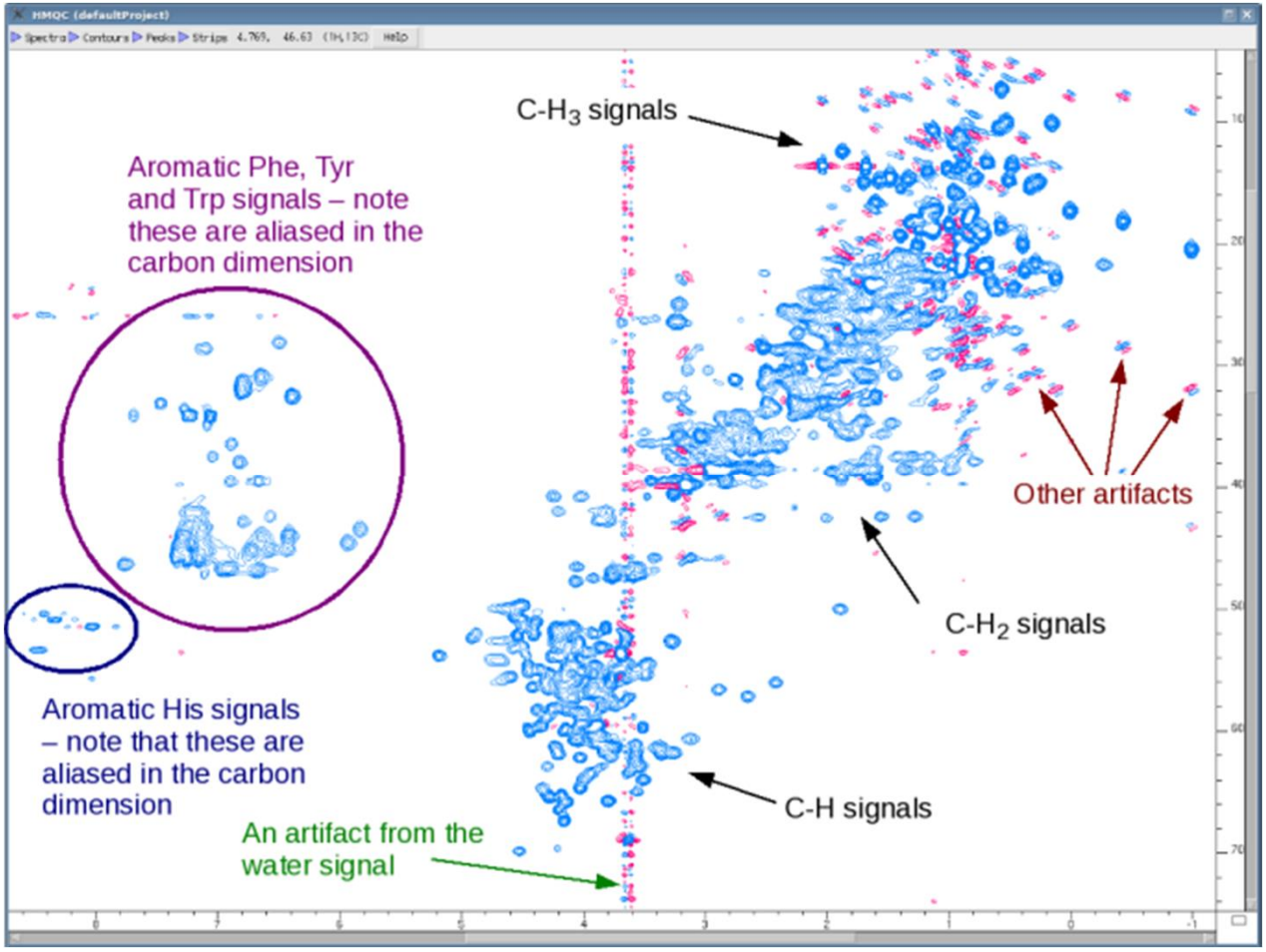


A and **B** at 600 MHz, 2048 and 256 data points at spectral widths of 14 and 25 ppm /^{1}H (F2) and ^{15}N (F1) dimensions,
32 scans were used, and total duration of the acquisition was 2 hr 44 min.

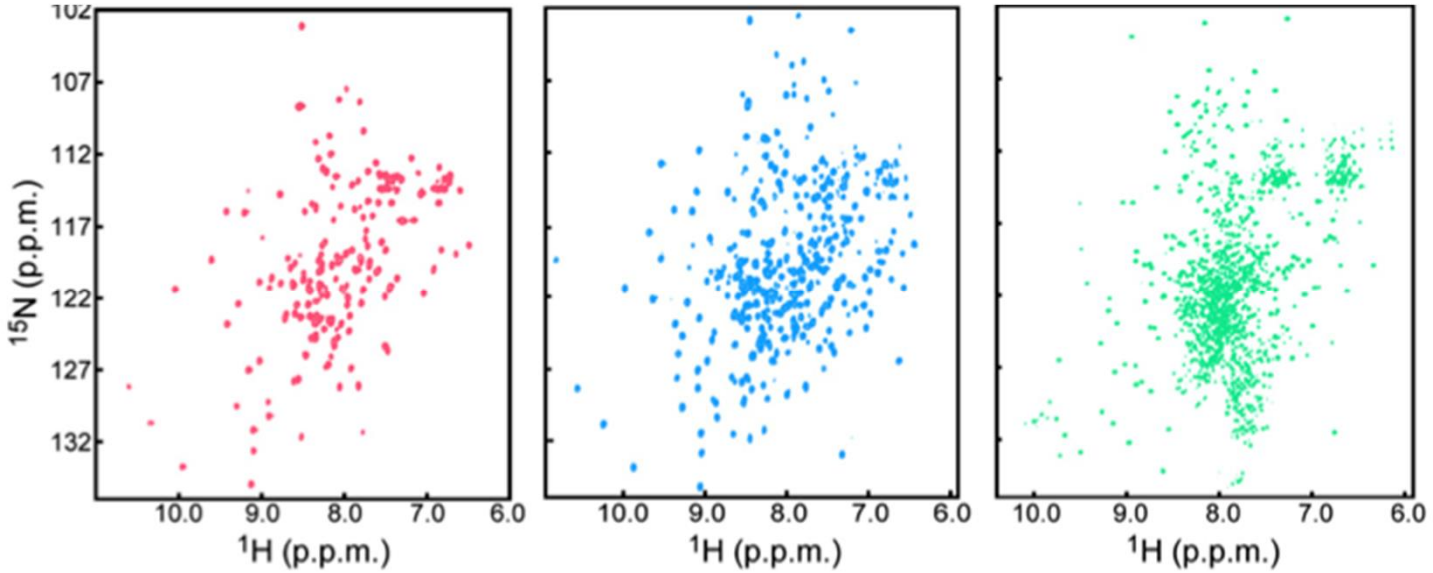
C at 900 MHz, 3072 and 416 data points at spectral widths of 14 and 25 ppm /^{1}H (F2) and ^{15}N (F1) dimensions, 48 scans were used, and total duration of the acquisition was 6 hr 37 min.

^{1}H-^{13}C-HMQC (2D)
Heteronuclear correlation spectroscopy





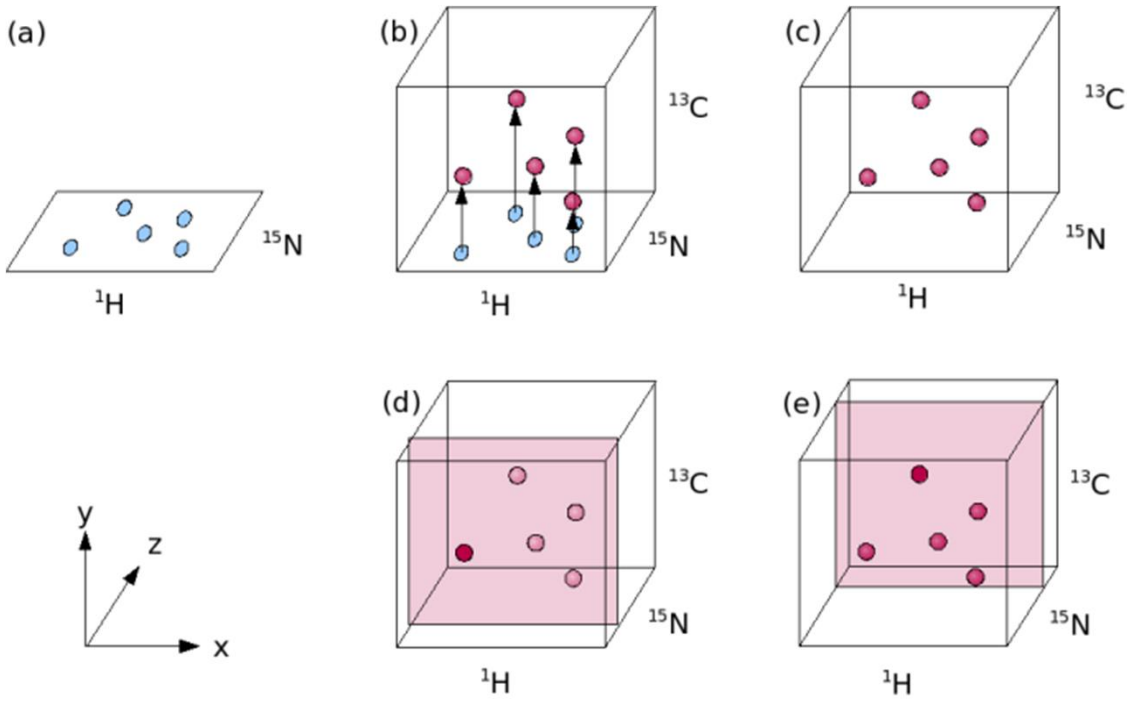
Limits of 2D spectroscopy



Gelis et al., Cell 131, 756, 2007

- Problems of crowding and peak overlap for larger proteins
- Which HSQC peak corresponds to which residue of the protein?

Solution: 3-dimensional spectra

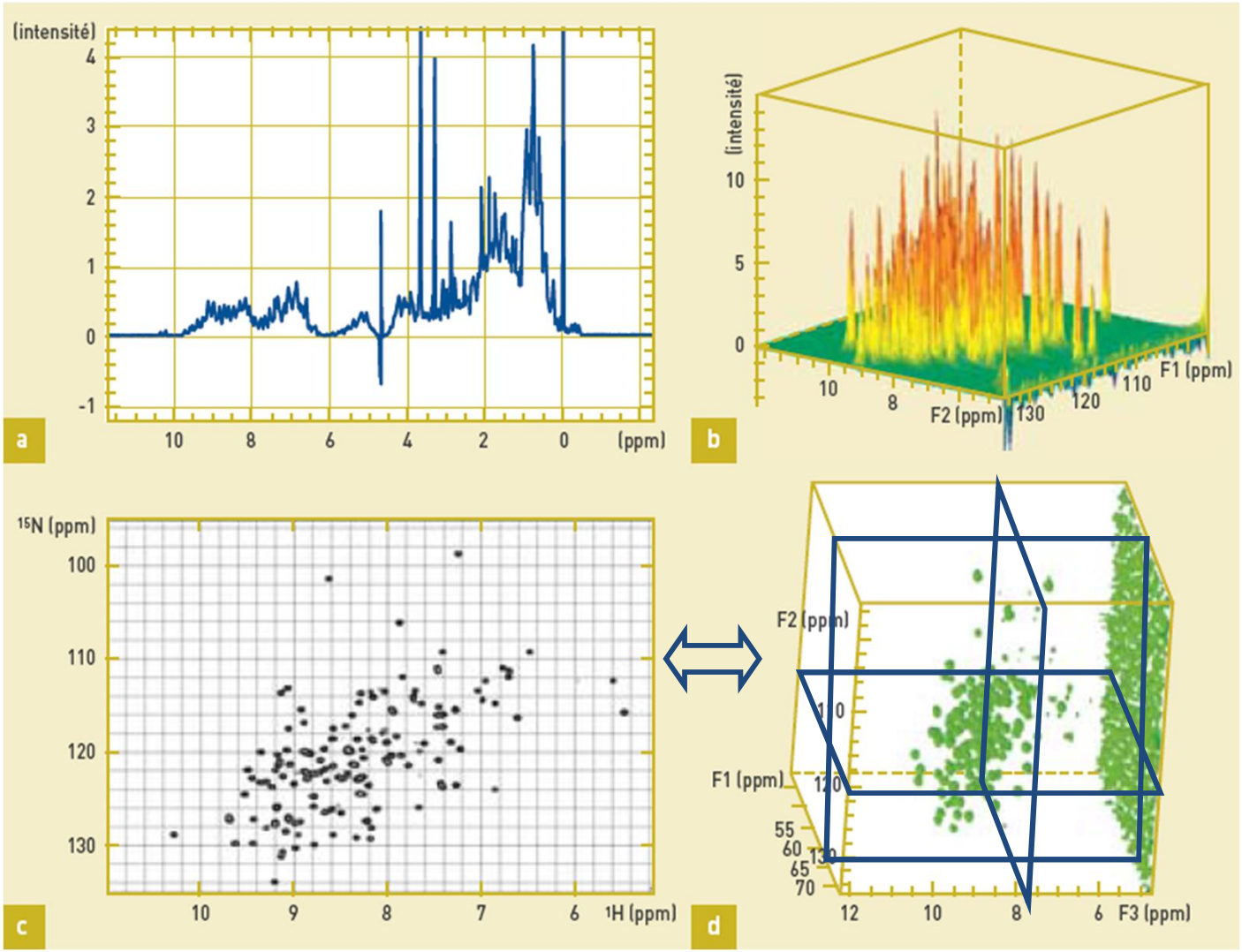


http://www.protein-nmr.org.uk/solution-nmr/assignment-theory/visualising-3d-spectra/

- Resolve ^{15}N-^{1}H HSQC in an additional ^{13}C dimension
- Visualize and analyze as 2-D planes

From 2D NMR to 3D NMR

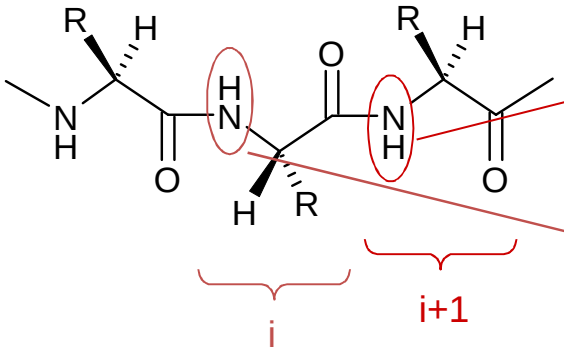
Add a 3rd dimension in frequency

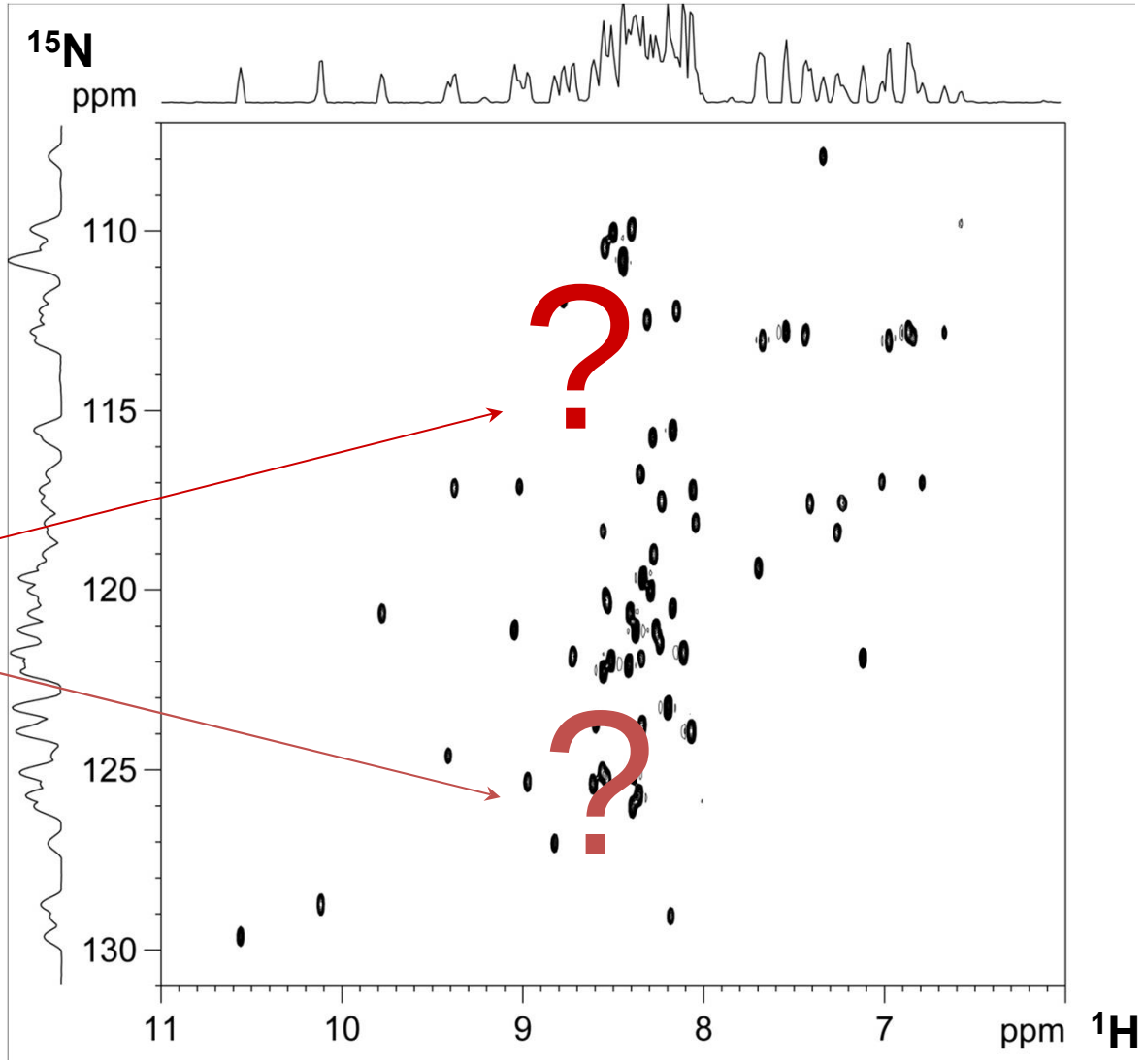


NMR signal assignments

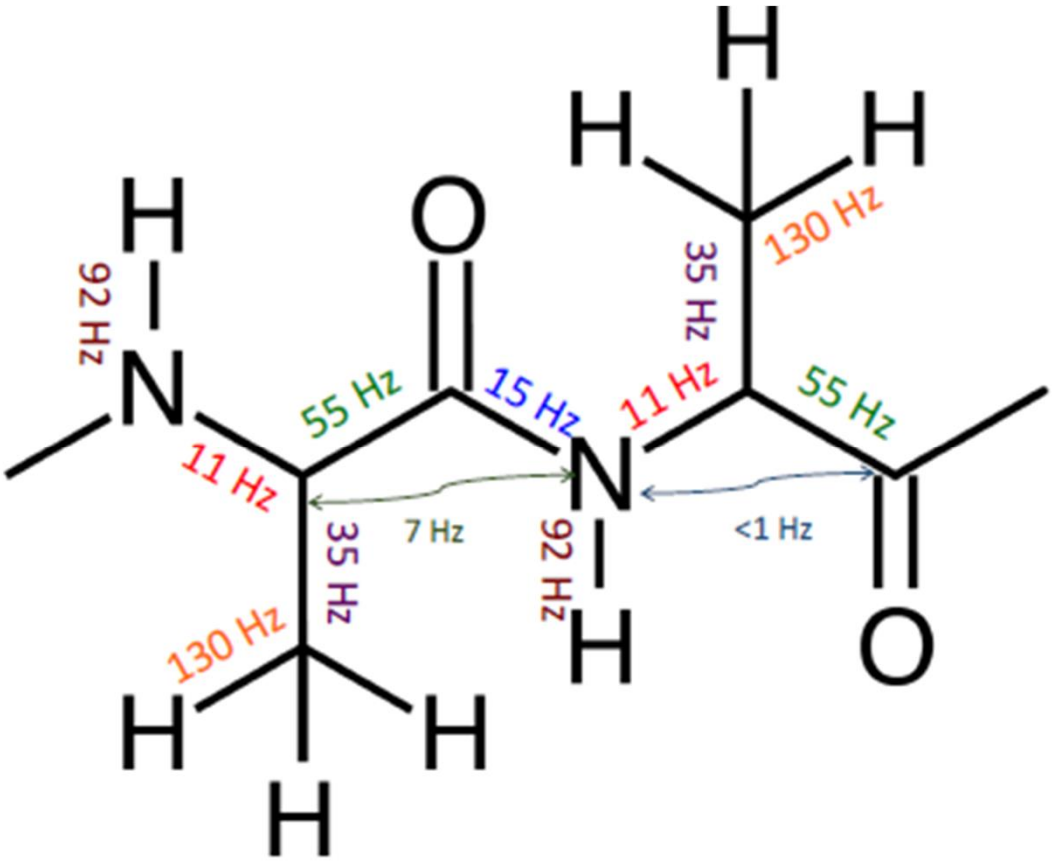
assignment

= link a signal to an atom





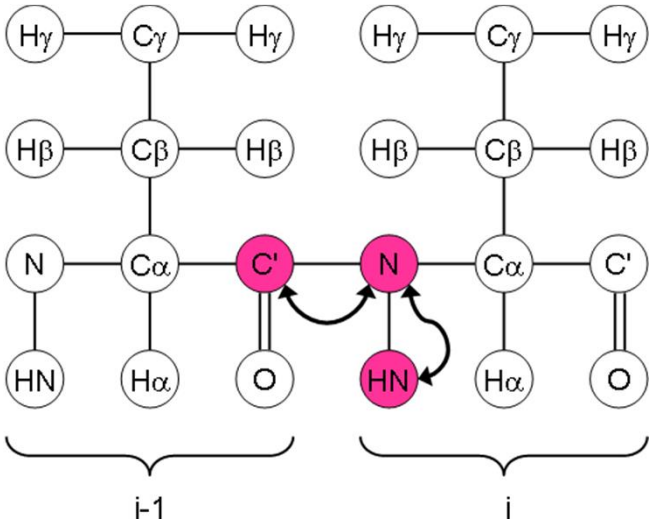
Assignment strategy

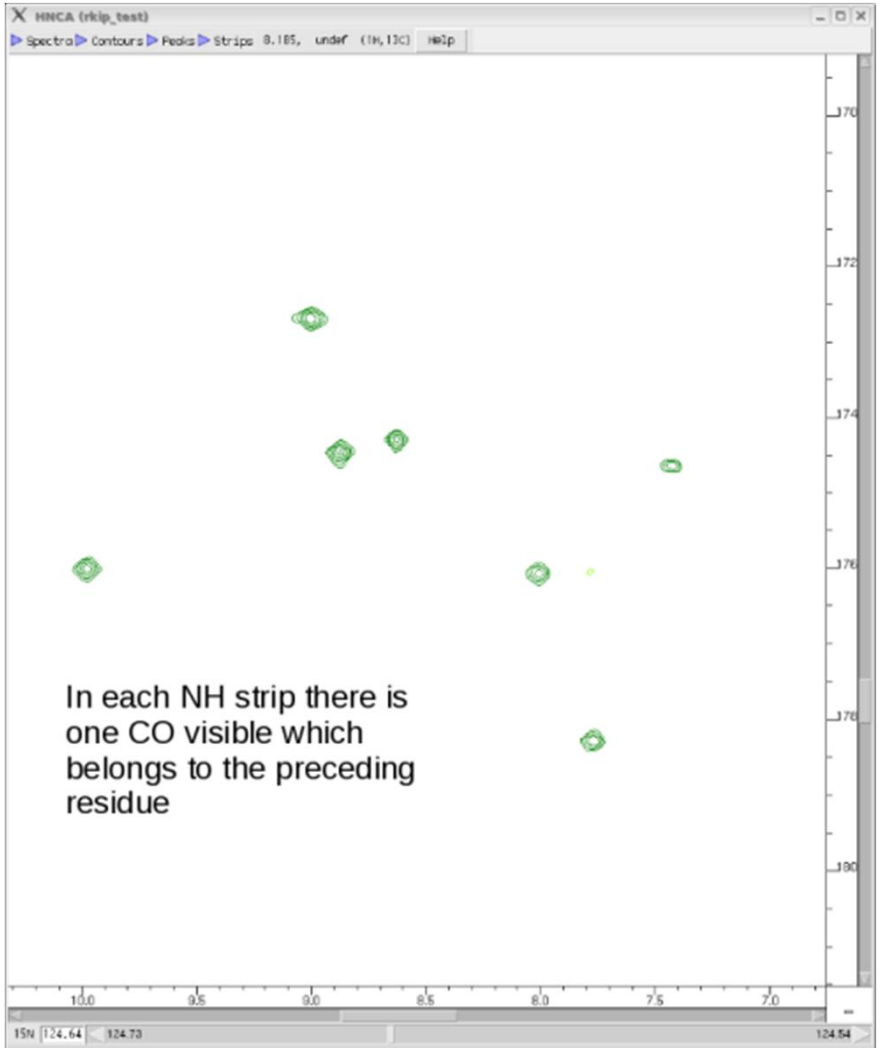


The topology defined by scalar coupling networks on the protein backbone is used to design the assignment strategy

HNCO (3D)

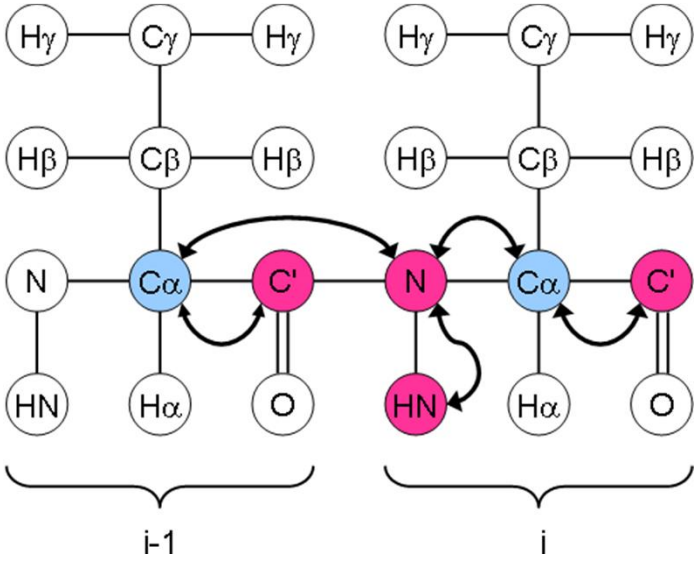
- Need ^{15}N and ^{13}C isotopic labeling
- Experiment to connect H_N from residue i with ^{13}C carbonyl (CO) from residue i-1

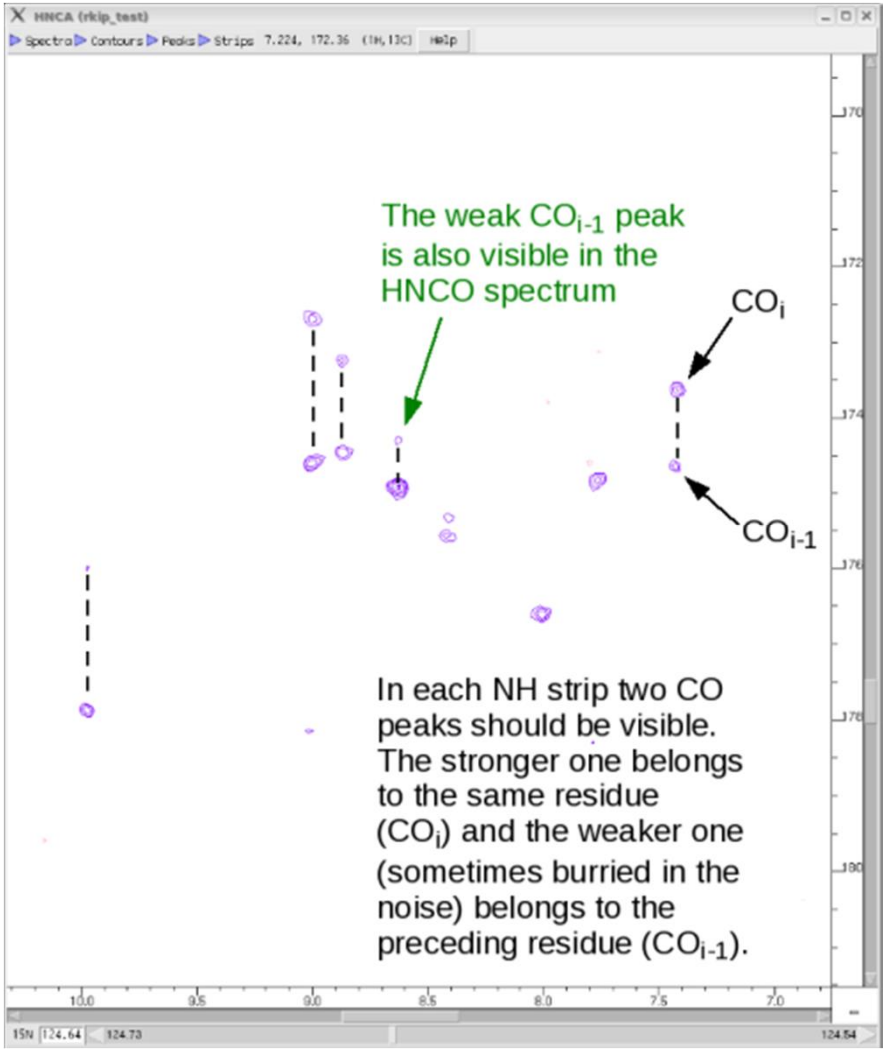




HN(CA)CO (3D)

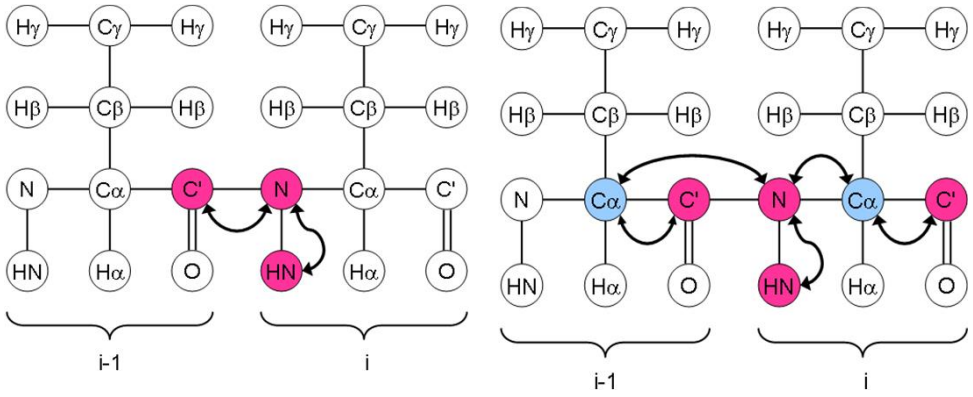
- Need ^{15}N and ^{13}C isotopic labeling
- Experiment to connect H_N from residue i with ^{13}C carbonyl (CO) from residues i and i-1

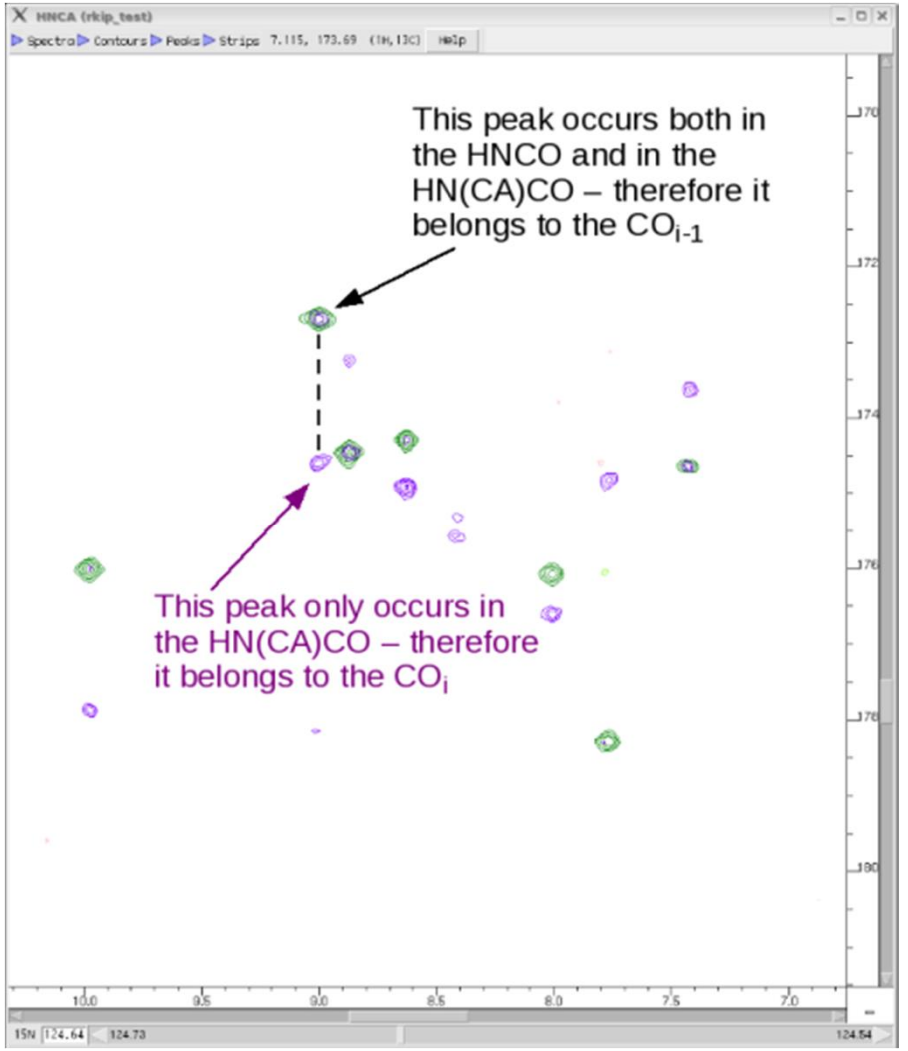




HN(CA)CO et HNCO (3D)

- Need ^{15}N and ^{13}C isotopic labeling
- Experiment to connect H_N from residue i with ^{13}C carbonyl (CO) from residues i and i-1

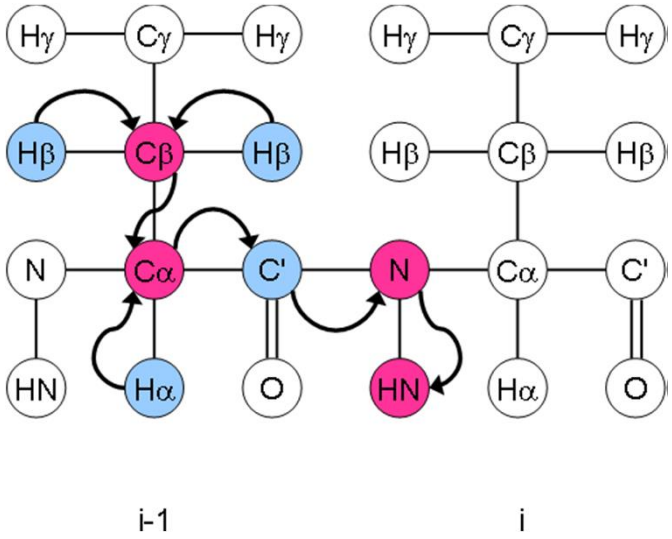


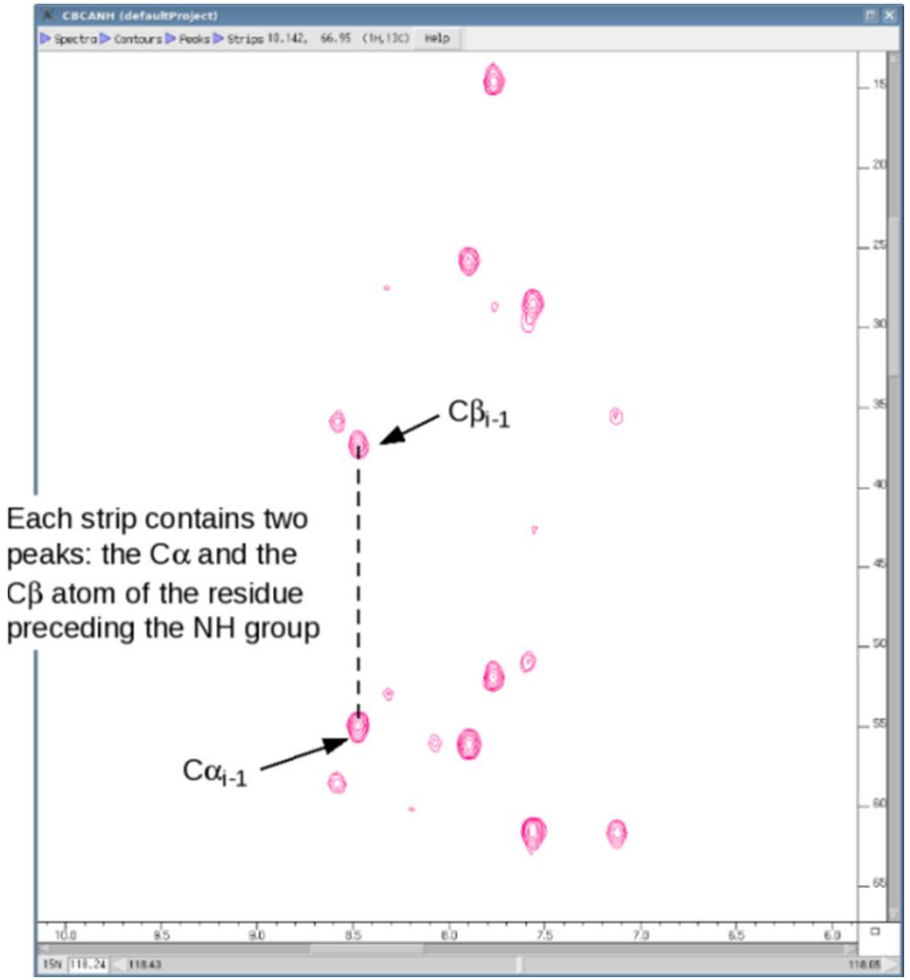


HN(CO)CACB (3D)

→Need ^{15}N and ^{13}C isotopic labeling

→Experiment to connect H_N from residue i with ^{13}Ca and ^{13}Cb from residues i-1

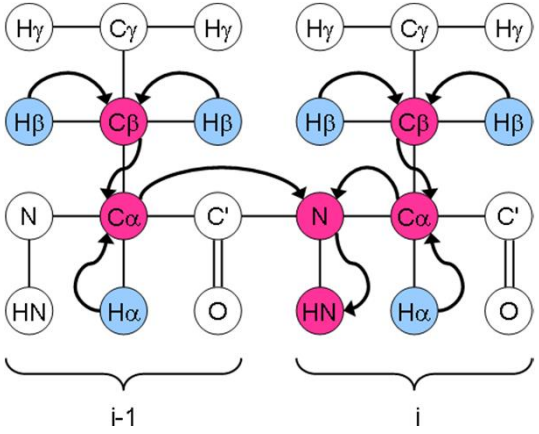


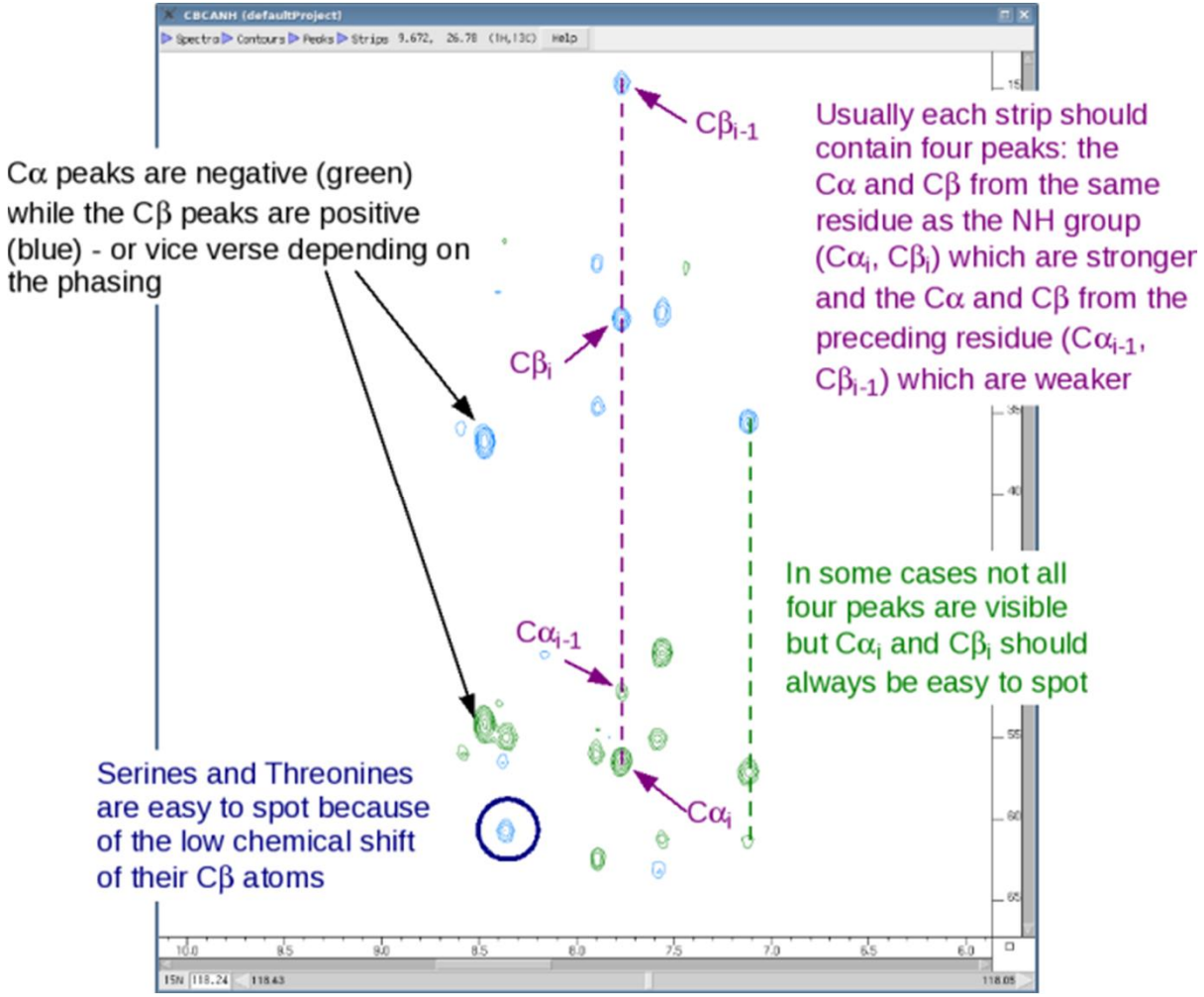


HNCACB (3D)

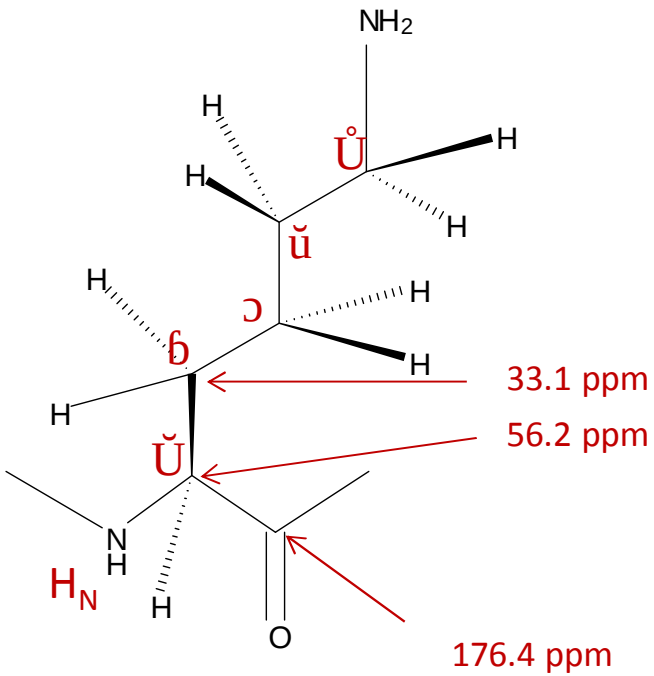
→Need ^{15}N and ^{13}C isotopic labeling

→Experiment to connect H_N from residue i with ^{13}Ca and ^{13}Cb from residues i and i-1





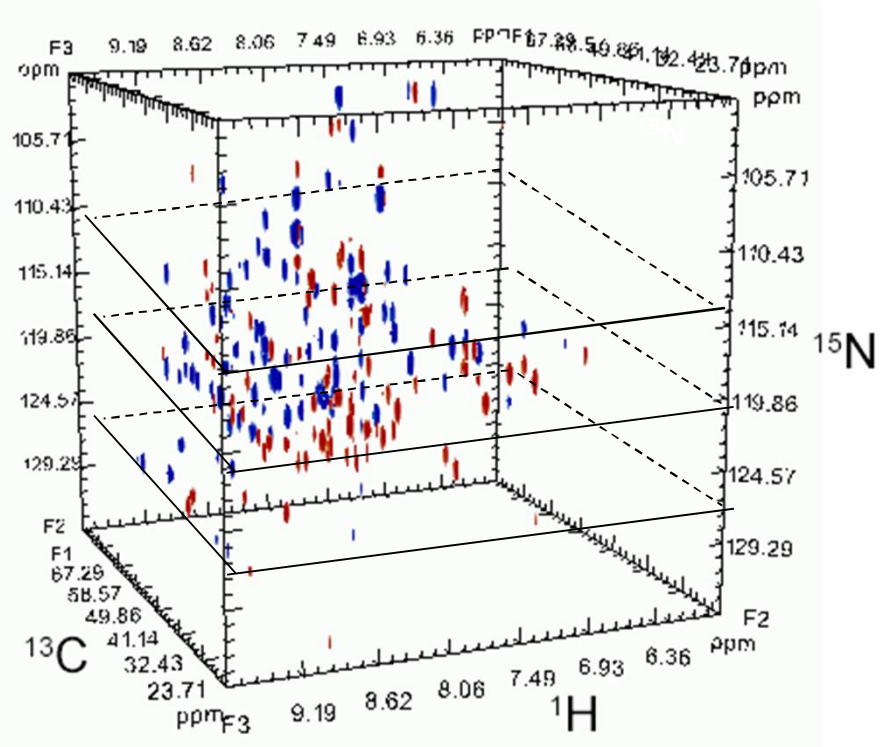
^{13}C Chemical shift values of amino acid residues

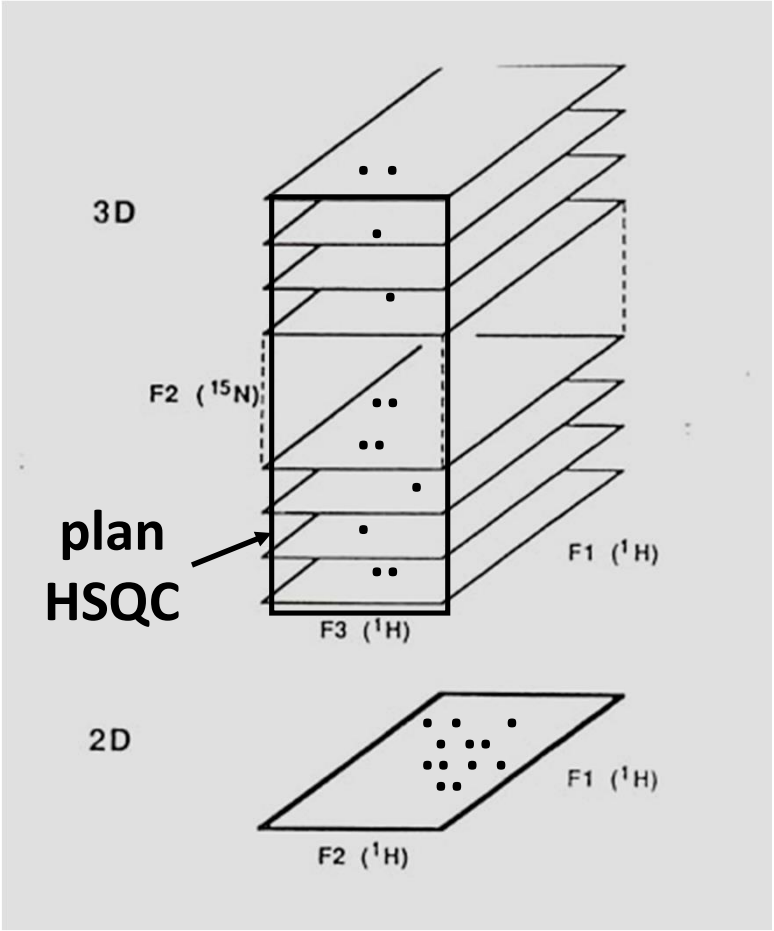


lysine

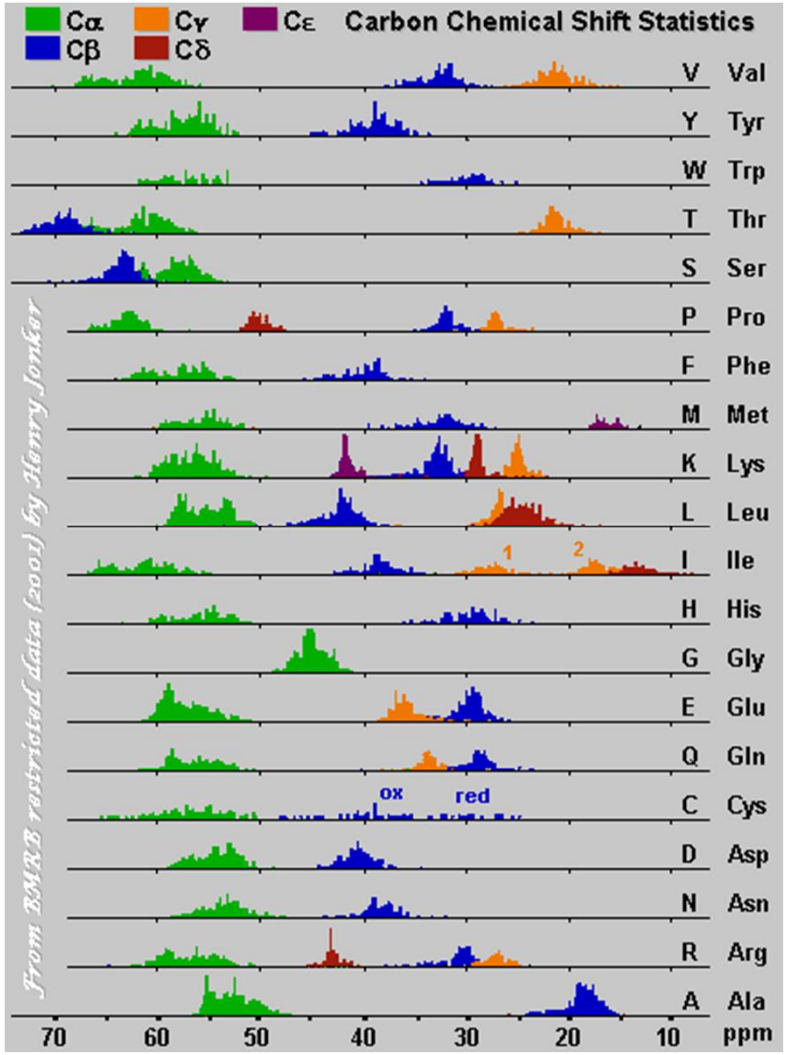
	Cα	Cβ	CO	Hn	N
A	52,5	19,1	177,8	8,35	125,0
C	55,4	41,1	174,6	8,54	118,7
D	54,2	41,1	176,3	8,56	119,1
E	56,6	29,9	176,6	8,40	120,2
F	57,7	39,6	175,8	8,31	120,7
G	45,1		174,9	8,41	107,5
H	55	29	174,1	8,56	118,1
I	61,1	38,8	176,4	8,17	120,4
K	56,2	33,1	176,4	8,36	121,6
L	55,1	42,4	177,6	8,28	122,4
M	55,4	32,9	176,3	8,42	120,3
N	53,1	38,9	175,2	8,51	119,0
P	63,3	32,1	177,3		
Q	55,7	29,4	176	8,44	120,5
R	56	30,9	176,3	8,39	121,2
S	58,3	63,8	174,6	8,43	115,5
T	61,8	69,8	174,70	8,25	112,0
V	62,2	32,9	176,3	8,16	119,3
W	57,5	29,6	176,10	8,22	122,1
Y	57,9	38,8	175,9	8,26	120,9

Principles of sequential assignment of protein with 3D spectra





Identifying residue types

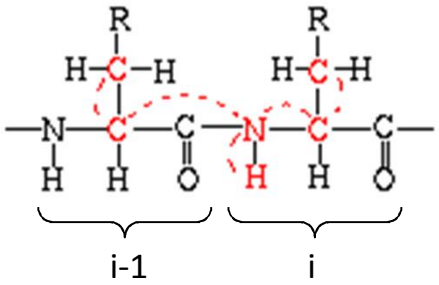


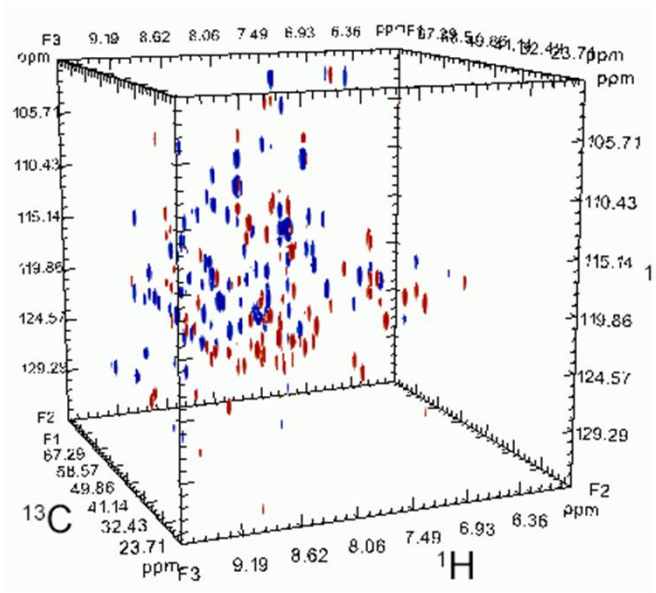
- Different amino acids have characteristic ^{13}C chemical shifts
- map NMR data onto the protein sequence
- if the data correspond to a sequence of amino acids that is unique in the protein, these residues have been sequentially assigned.

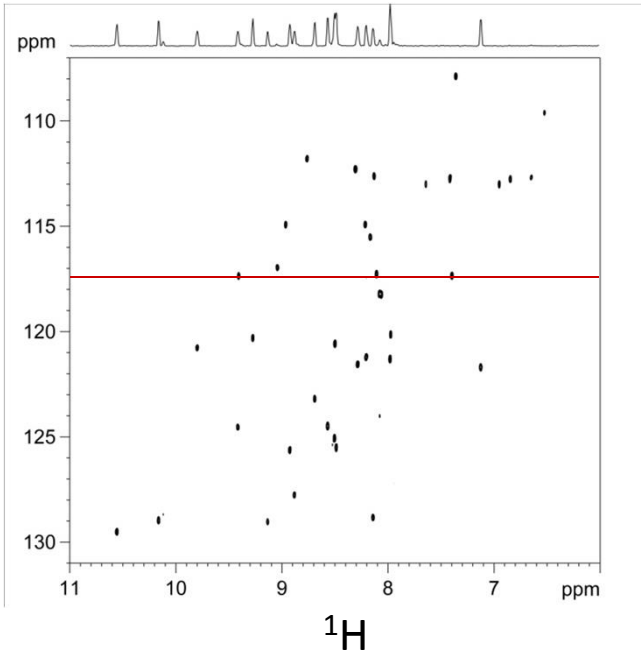
http://www.nmr.chem.uu.nl/~abonvin/tutorials/Assignment-Data/assignment.html

Principles of sequential assignment of protein with 3D spectra

HNCACB

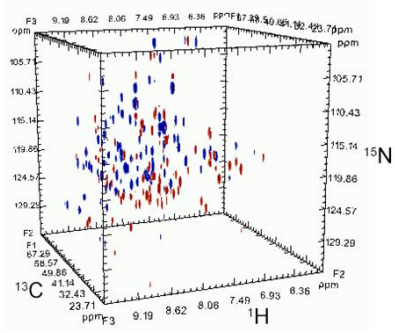




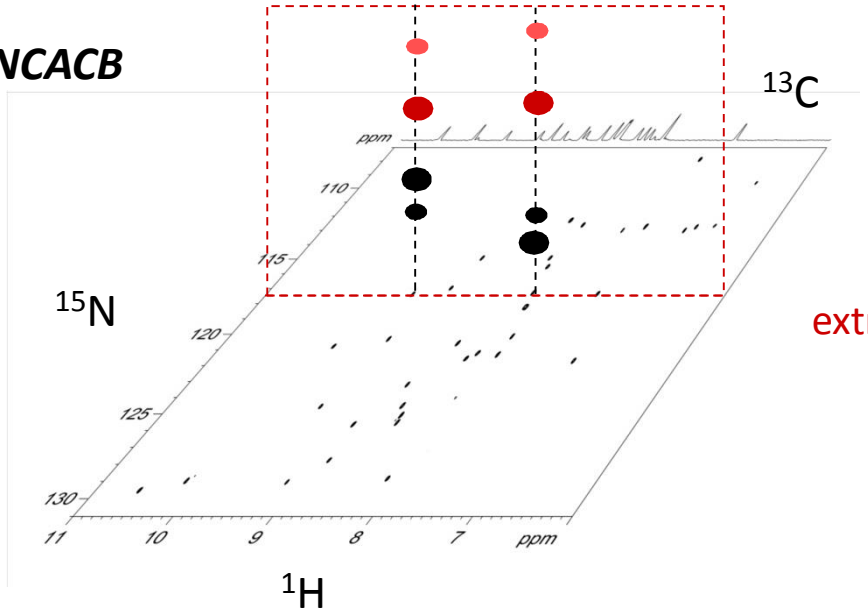


Plane extraction
^{13}C-^{1}H

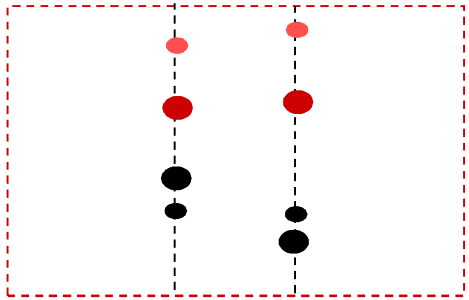
Principles of sequential assignment of protein with 3D spectra



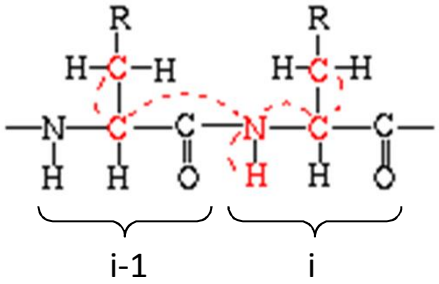
HNCACB



Plane extraction ^{13}C-^{1}H

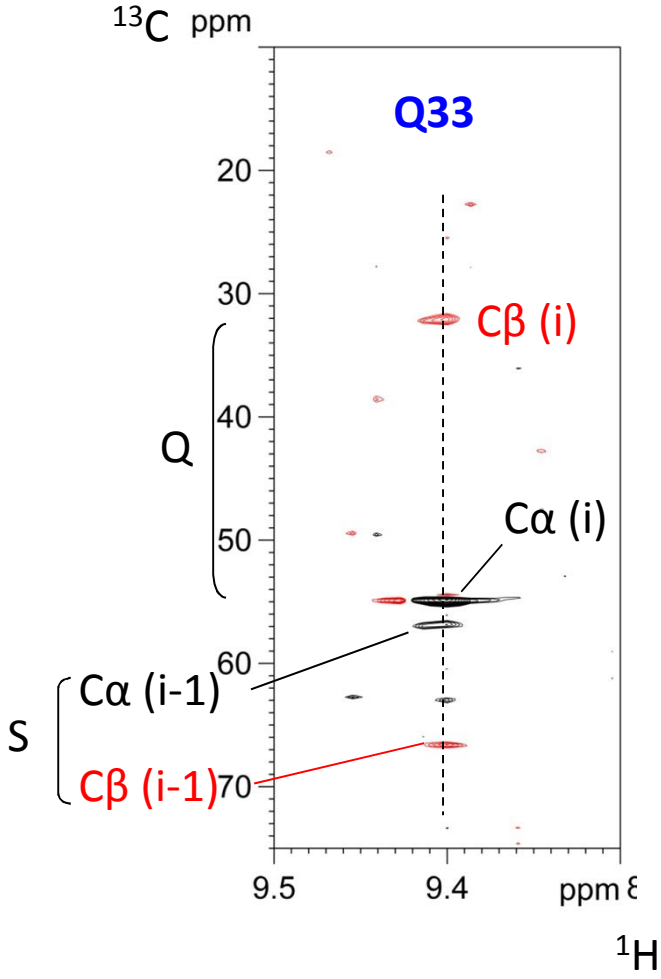


HNCACB



- Intense signals: Cα ●, Cβ ● residue i
- Weaker signals : Cα ●, Cβ ● residue i-1

Principles of sequential assignment of protein with 3D spectra



(Cα i, Cβ i) → i = Gln (Q)

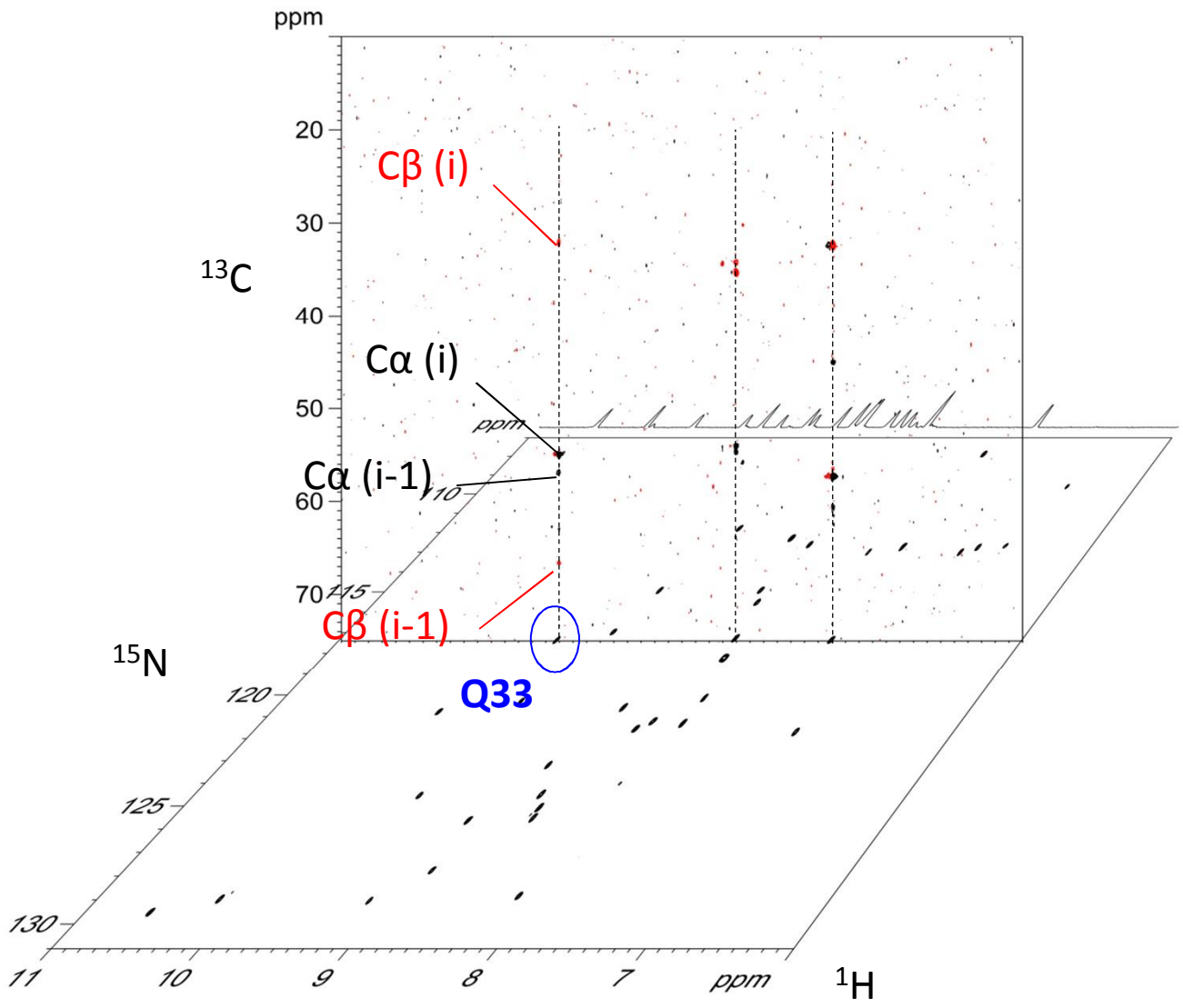
(Cα i-1, Cβ i-1) → i-1 = Ser (S)

→ pair Ser-Gln (SQ)

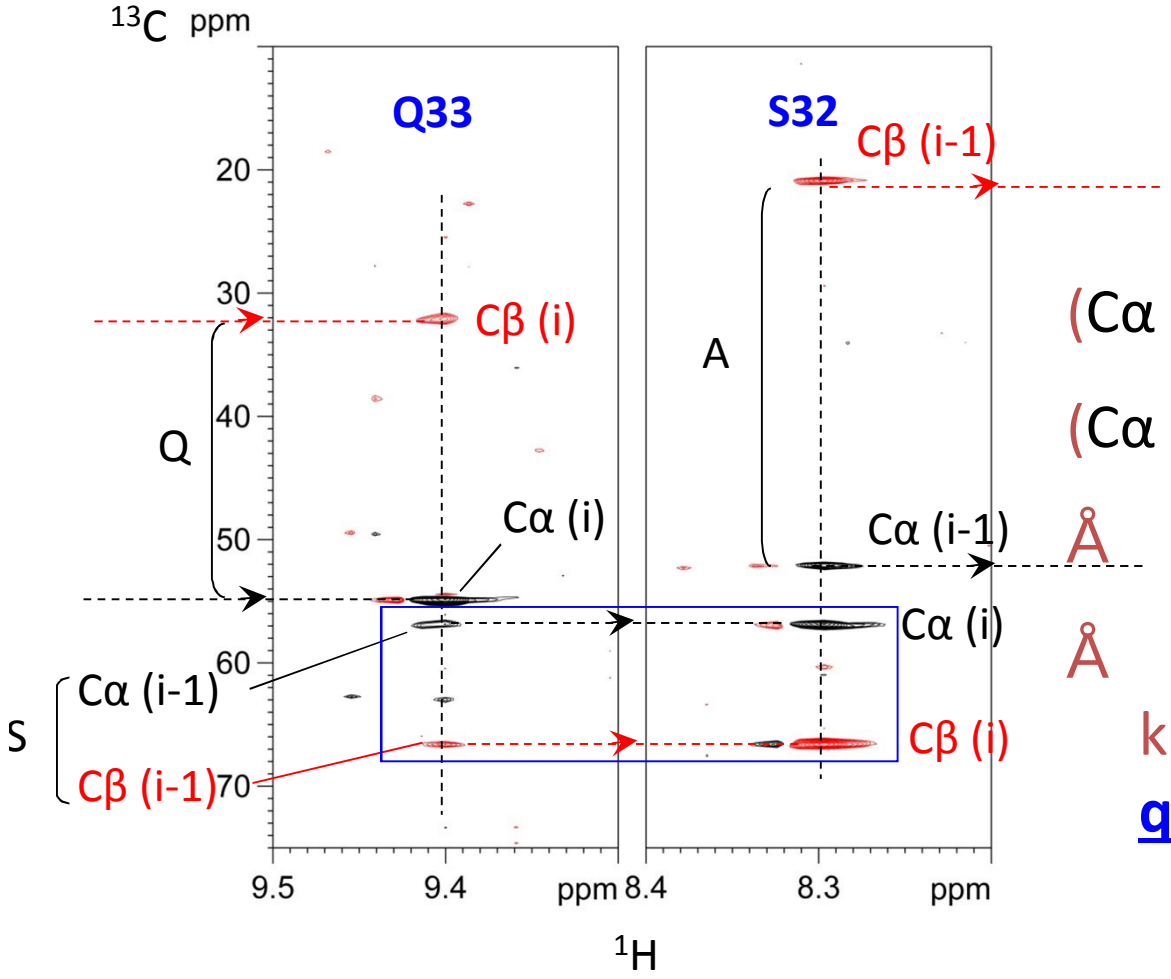
→ Protein sequence

klppgwekrmsrssgrvyyfnhitna**sq**[33]
werpsgns

Principles of sequential assignment of protein with 3D spectra



Principles of sequential assignment of protein with 3D spectra



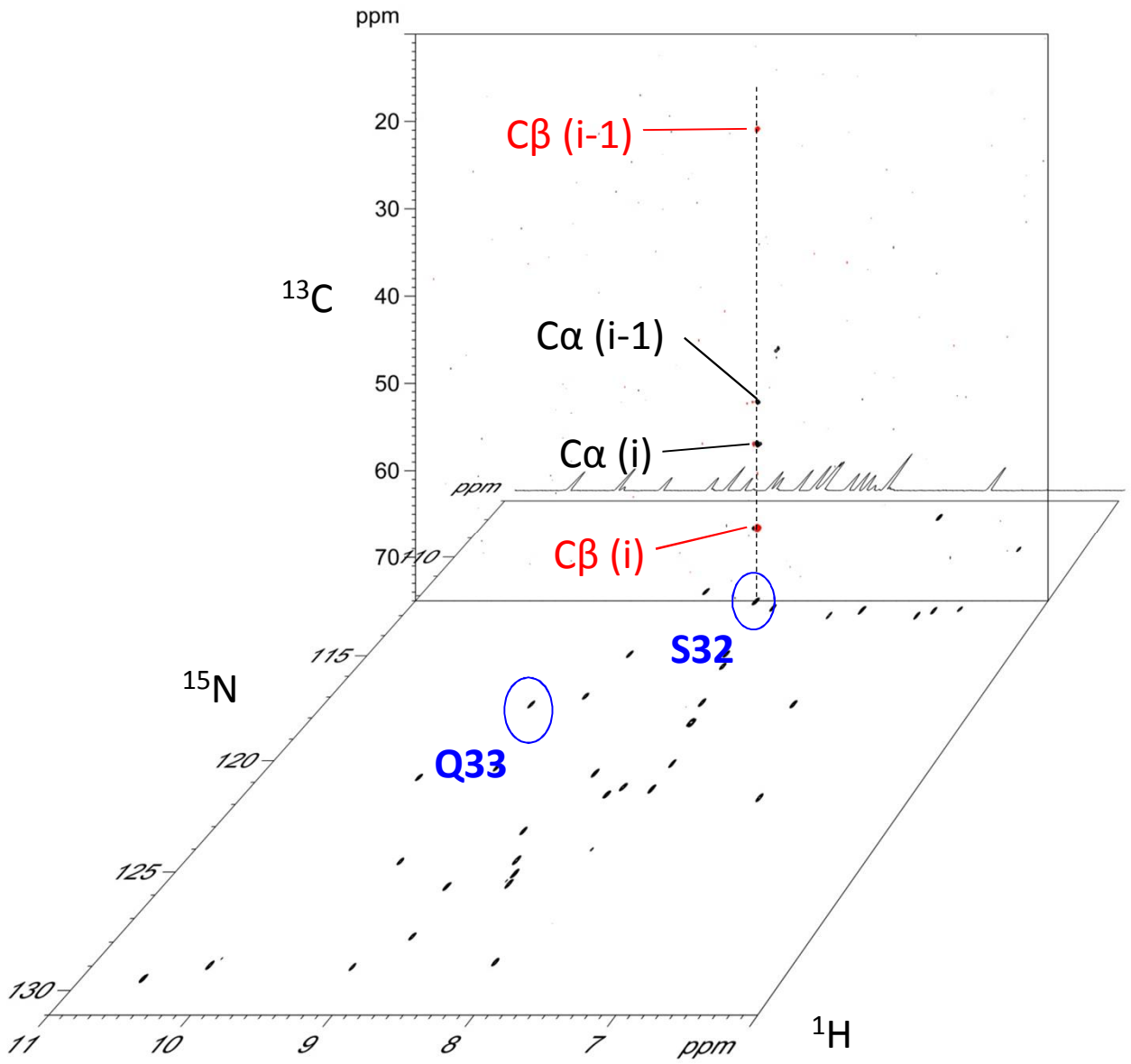
(Cα i, Cβ i) → i = Gln (Q)

(Cα i-1, Cβ i-1) → i-1 = Ser (S)

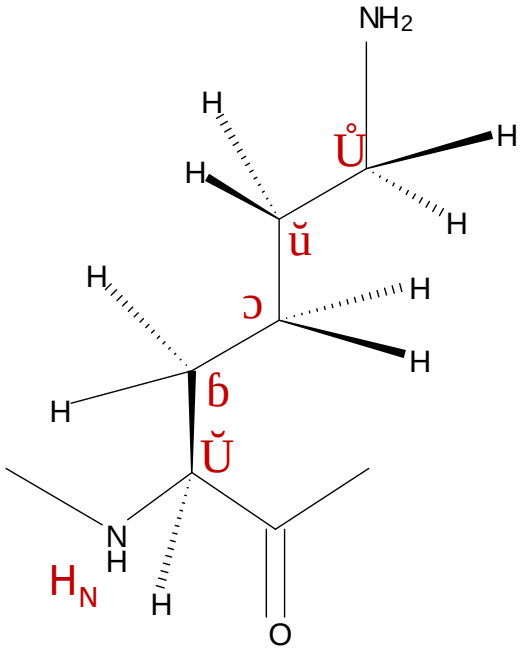
Å pair Ser-Gln (SQ)

Å protein sequence:
klppgwekrmsrssgrvyyfnhitna**s**
q^{33}werpsgns

Principles of sequential assignment of protein with 3D spectra



^{13}C chemical shift values of amino acid residues



lysine

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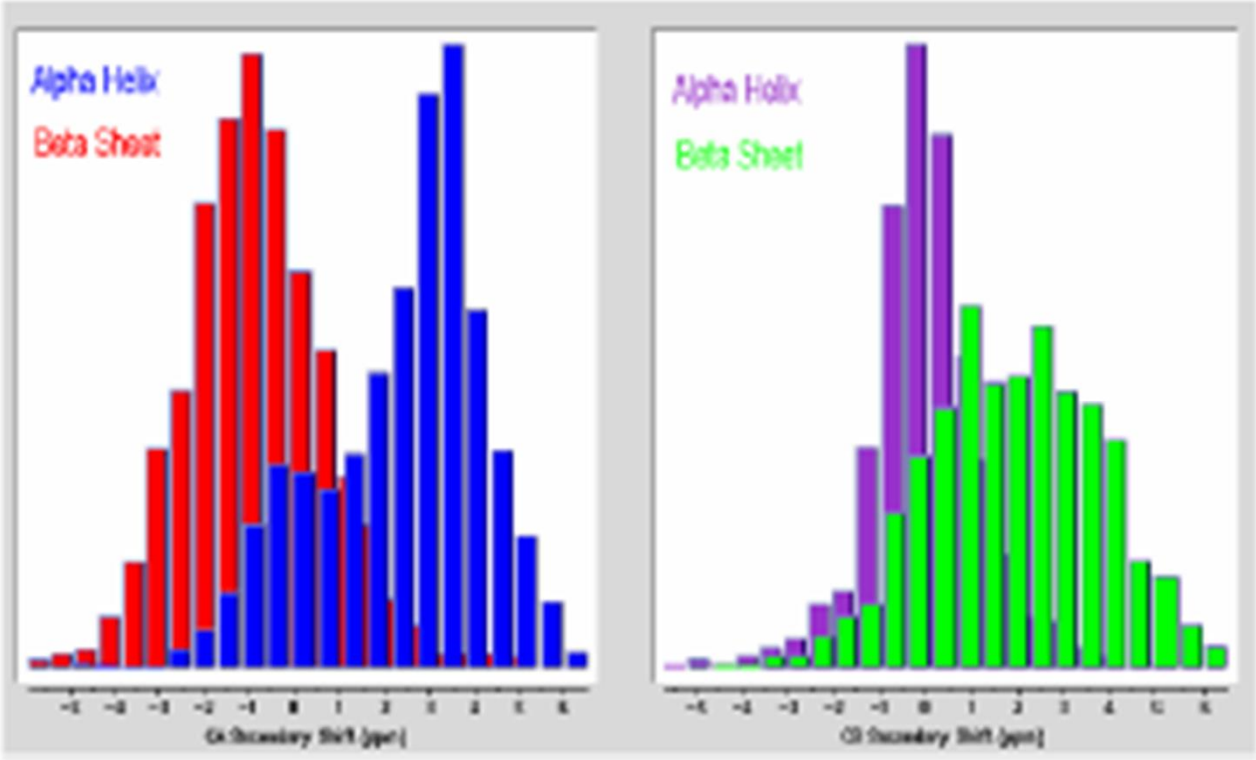
W	57,5	29,6	176,10	8,22	122,1
Y	57,9	38,8	175,9	8,26	120,9

CO, CA and CB chemical shifts depend on the secundary structure

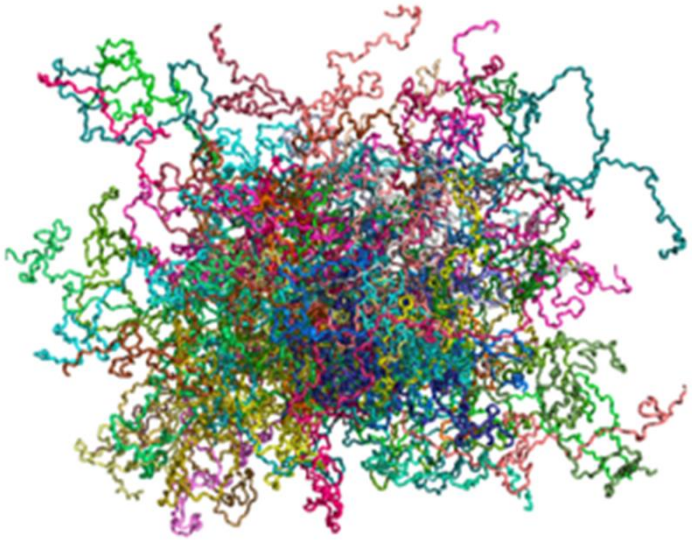
Chemical shifts: first raw information from the assignment



Tau in Alzheimer Disease

NMR APPLICATION IN BIOLOGY: INTRINSICALLY DISORDERED PROTEINS

Intrinsically disordered proteins (IDPs)

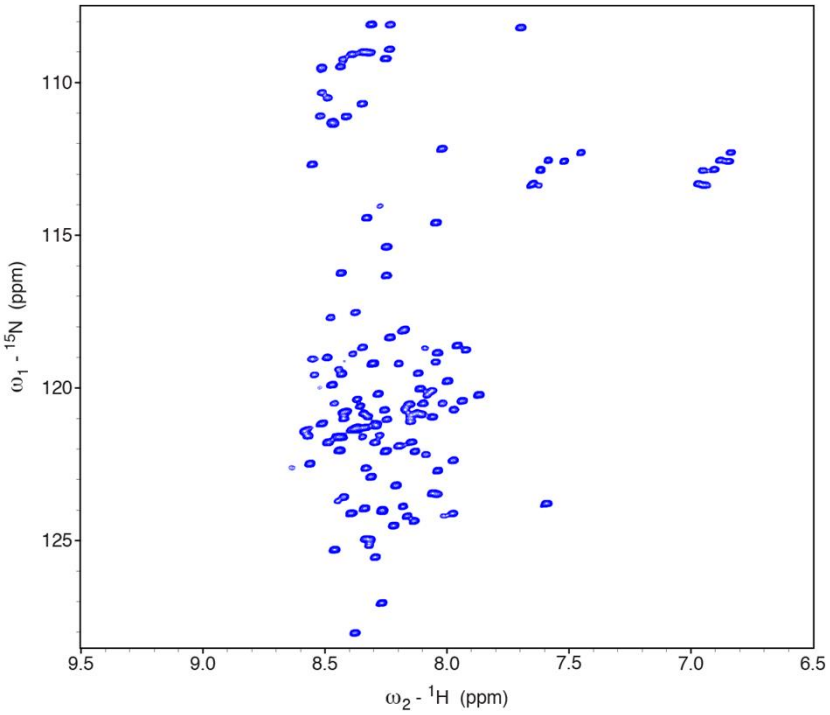


- Proteins or protein regions can be functional even in the absence of a fixed structure
- Often encountered in signaling, scaffolding, cell-cycle regulation, …

- IDPs play crucial roles in several important diseases (Alzheimer's, Parkinson, cancer)

⇒ NMR is the perfect tool to study this class of proteins

Studying IDPs by NMR



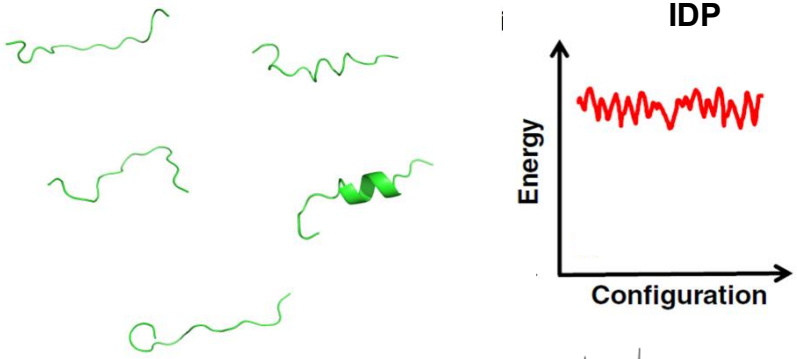
- IDPs are characterized by a narrow amide ^{1}H chemical shift range
- Fast exchange between the many different conformations populated by the protein

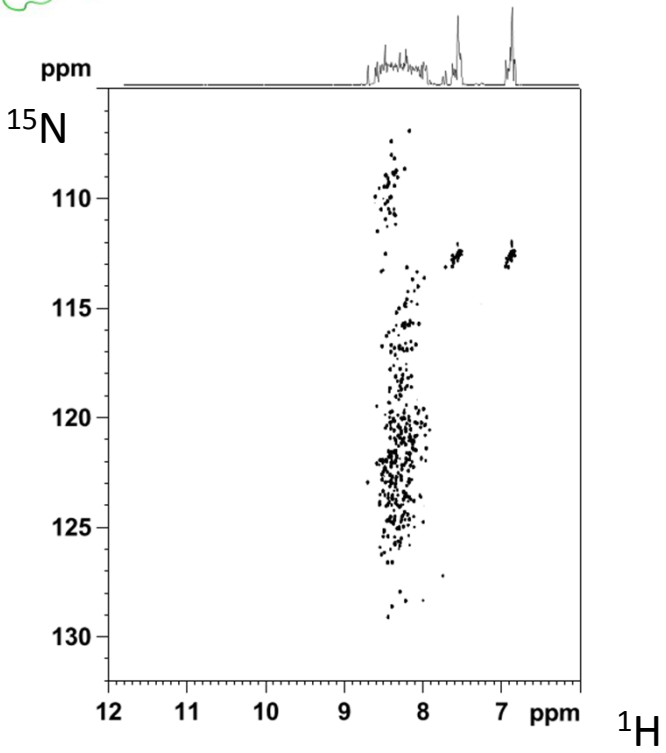
⇒ one peak per residue observed

⇒ Use different NMR techniques (chemical shifts, relaxation, RDCs, PREs,…) to characterize conformational sampling, binding interactions, …

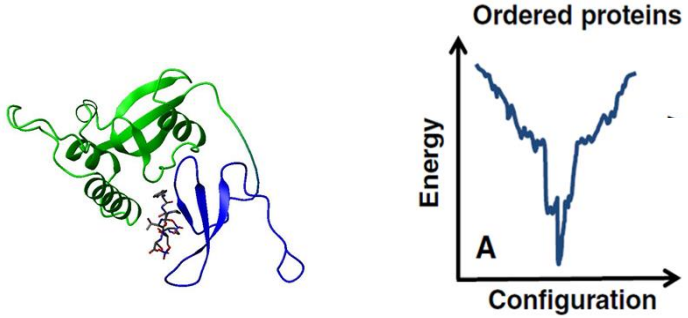
Tau is an intrinsically disordered protein (IDP)

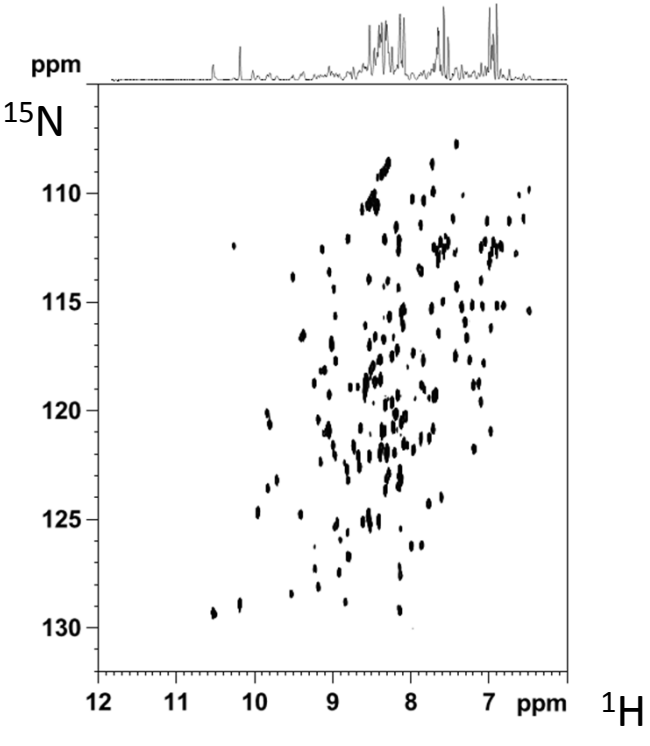
IDP (Tau)





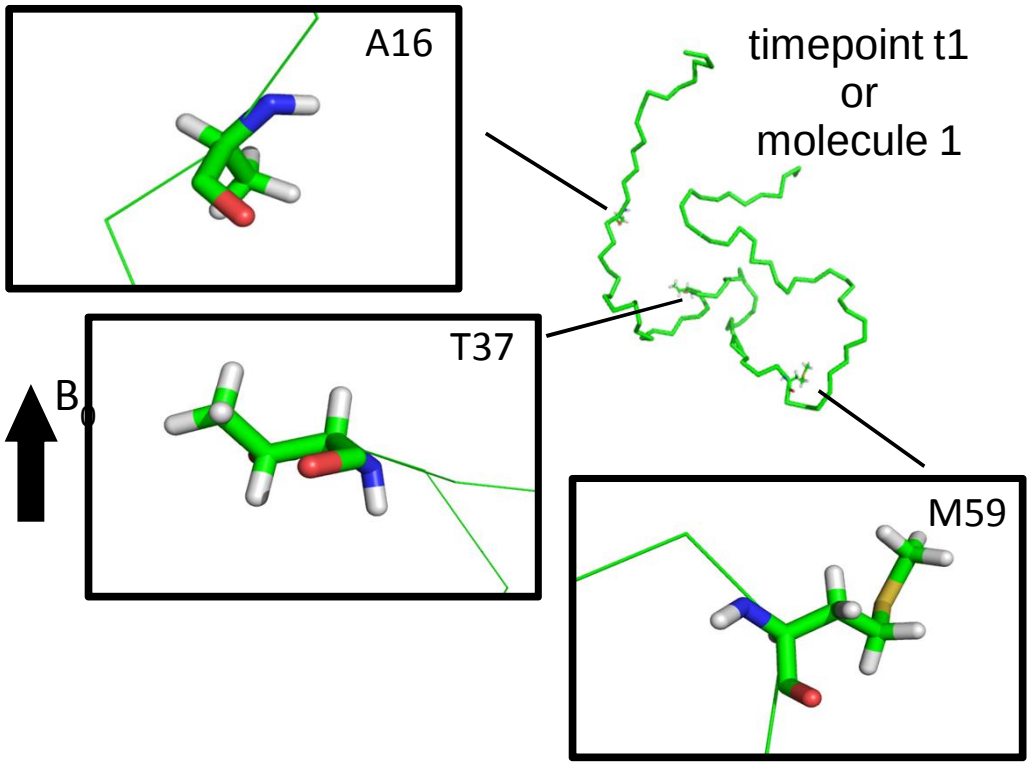
Globular protein (Pin1)

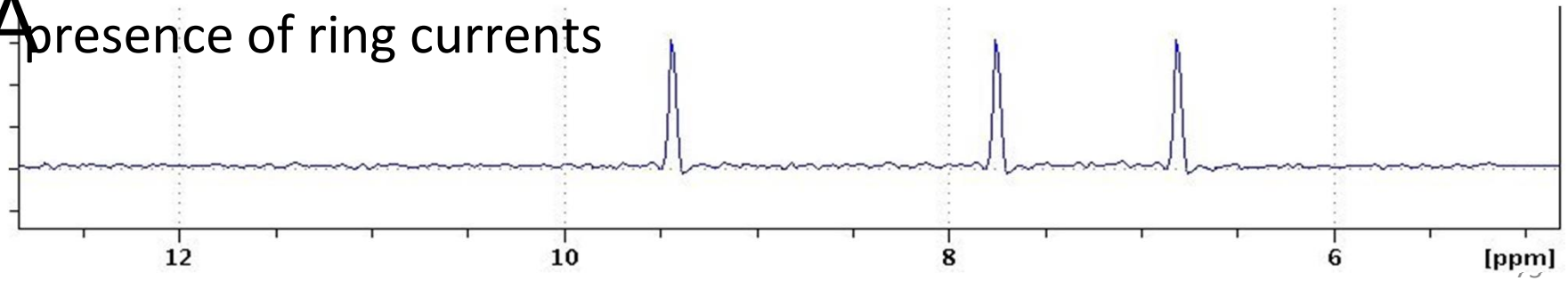




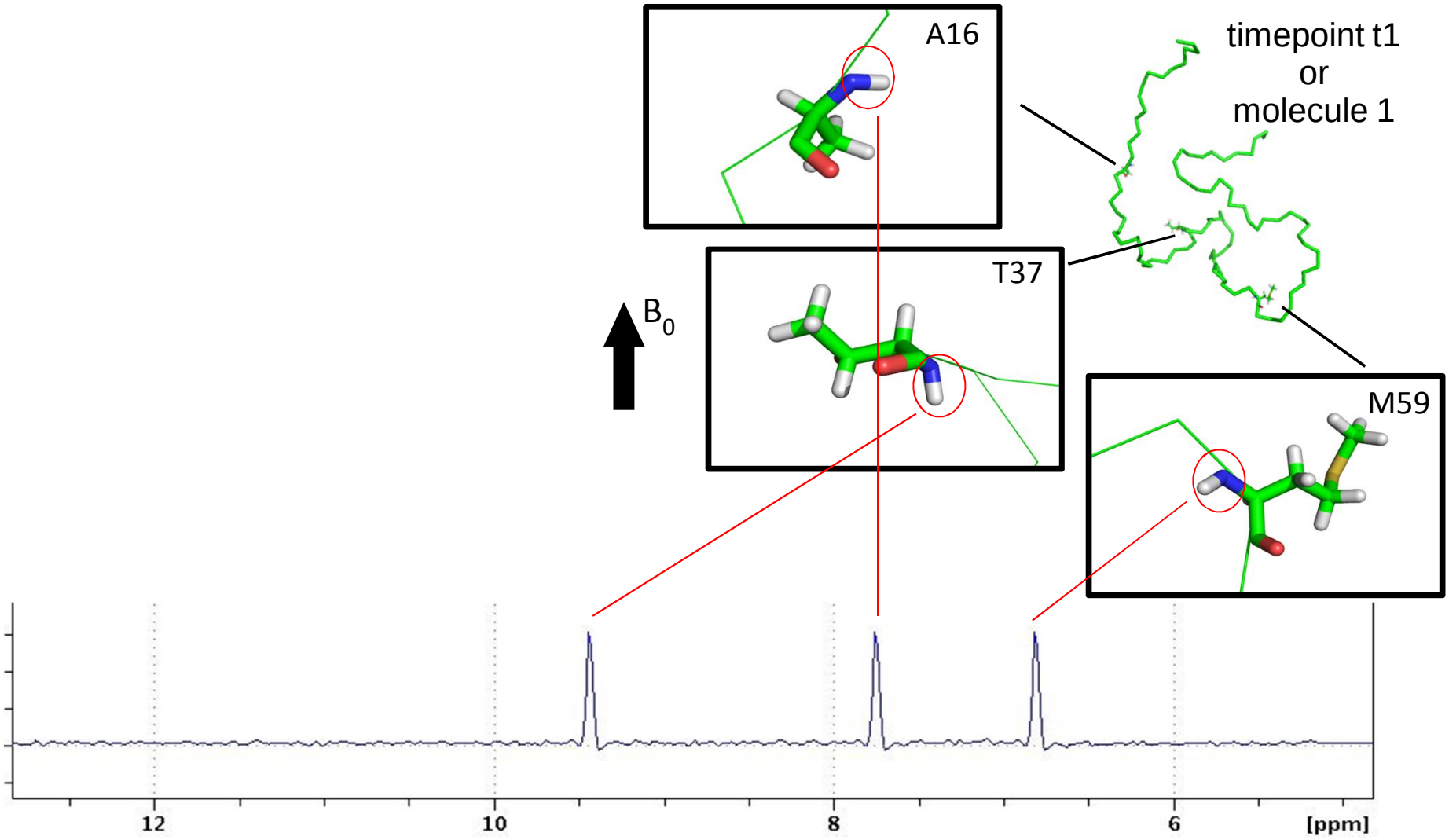
Chemical Shifts of IDPs

- The chemical shifts (coordinates of a peak) are determined by several factors:
  - nature of the sidechain of the residue and neighbouring residues
  - presence of hydrogen bonds
  - local relative geometry of the chemical bonds (torsion angles)
  - presence of ring currents

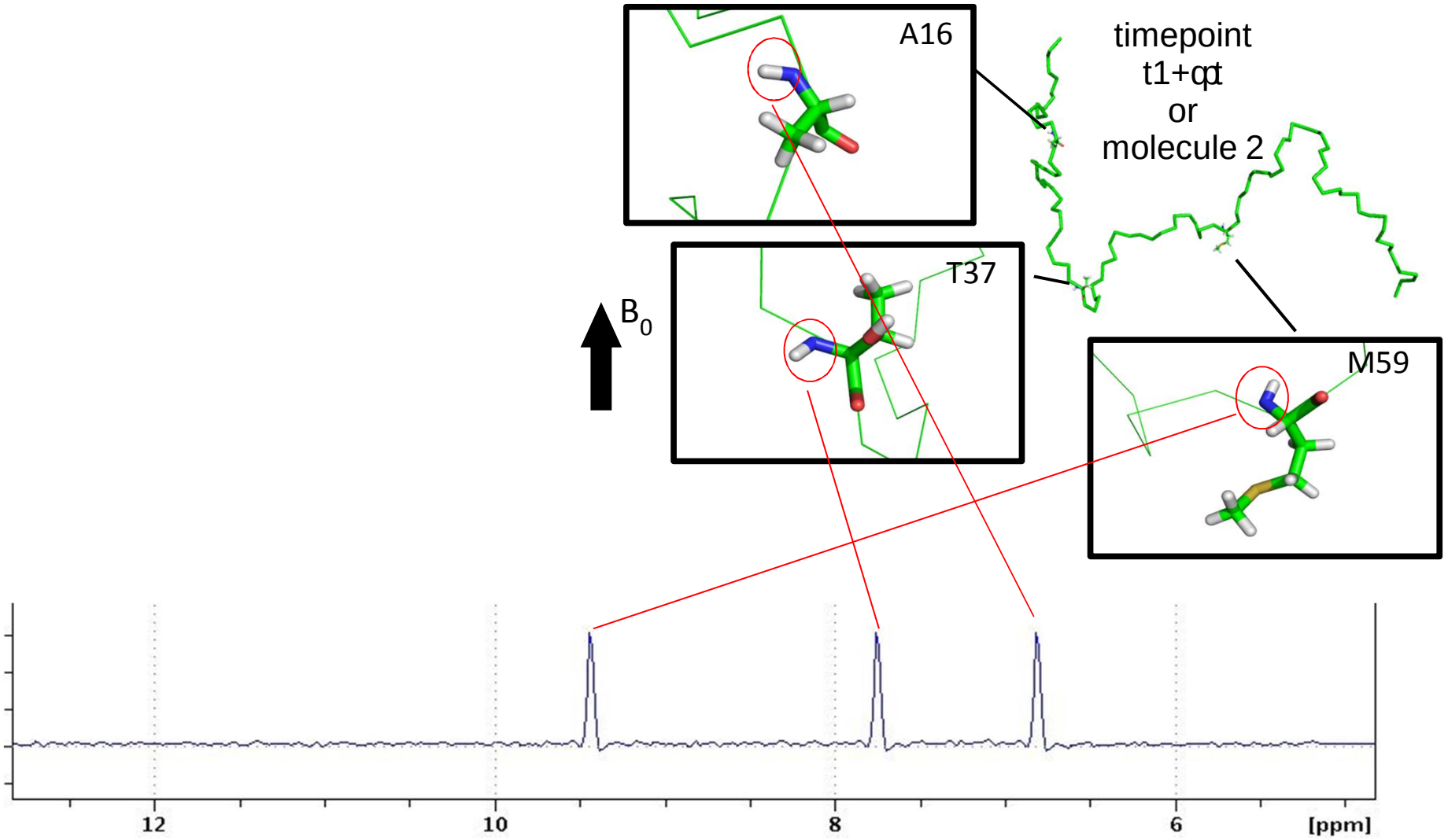




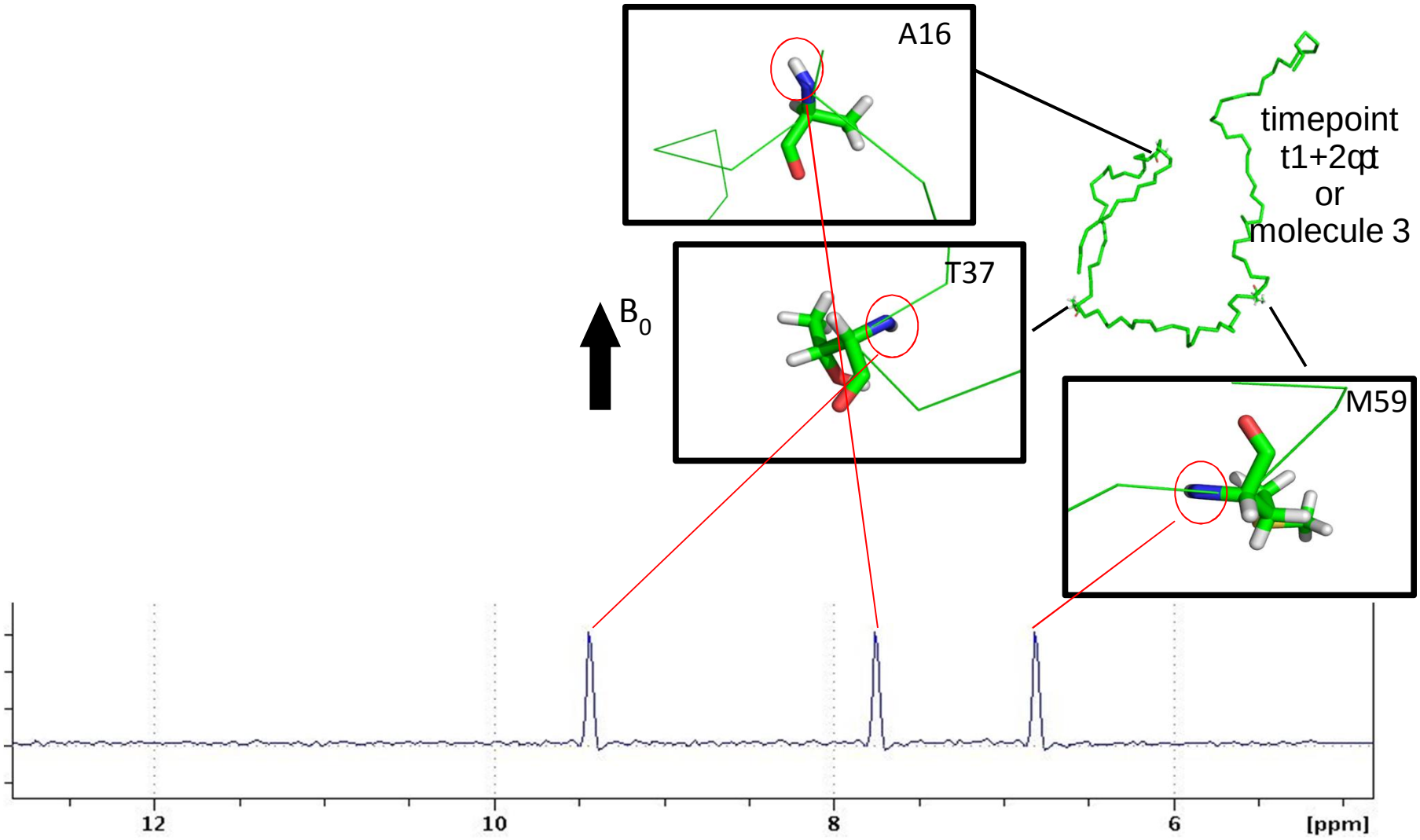
Chemical Shifts of IDPs



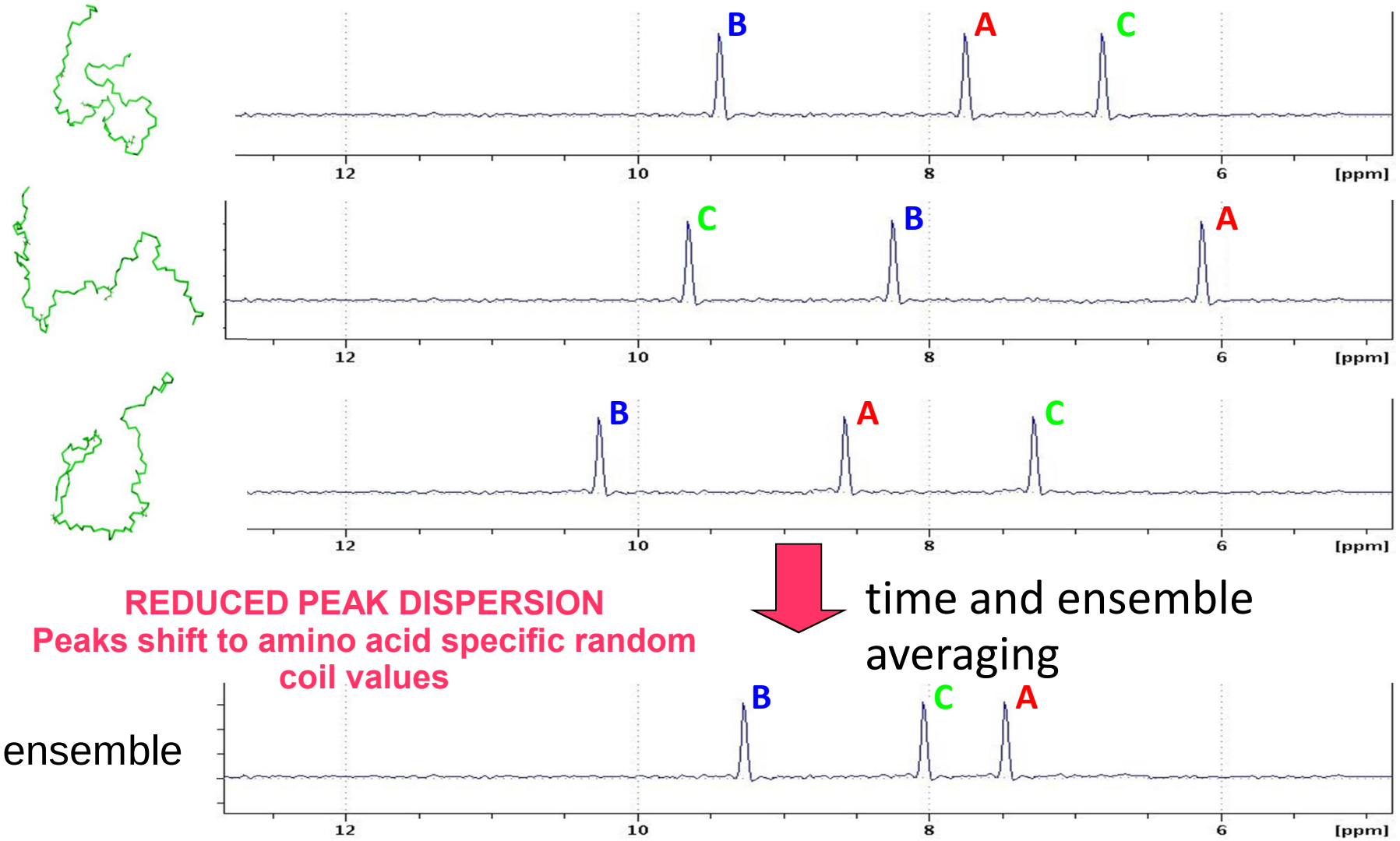
Chemical Shifts of IDPs



Chemical Shifts of IDPs



Chemical Shifts of IDPs : average of conformational sampling

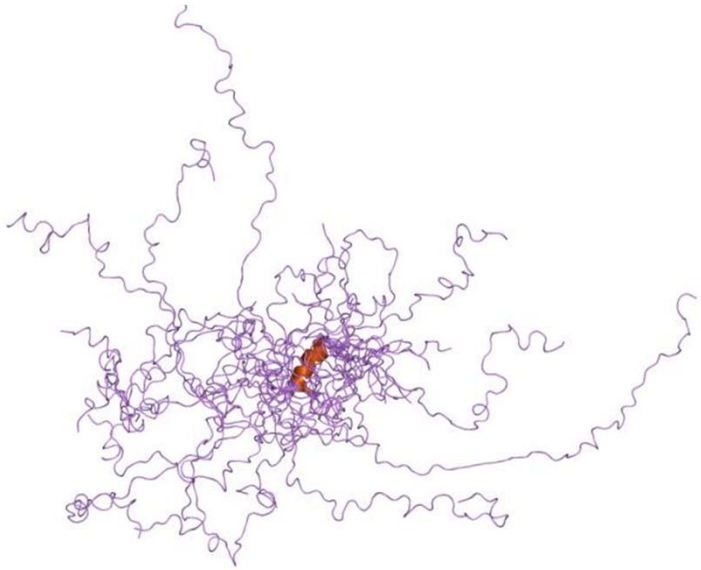


REDUCED PEAK DISPERSION
Peaks shift to amino acid specific random coil values

ensemble

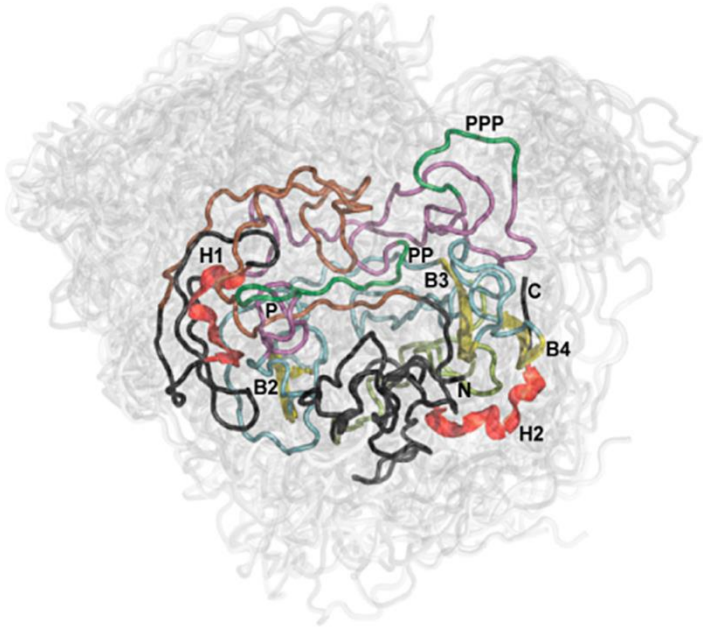
→ IDPs « Structures »

Intrinsically disordered proteins

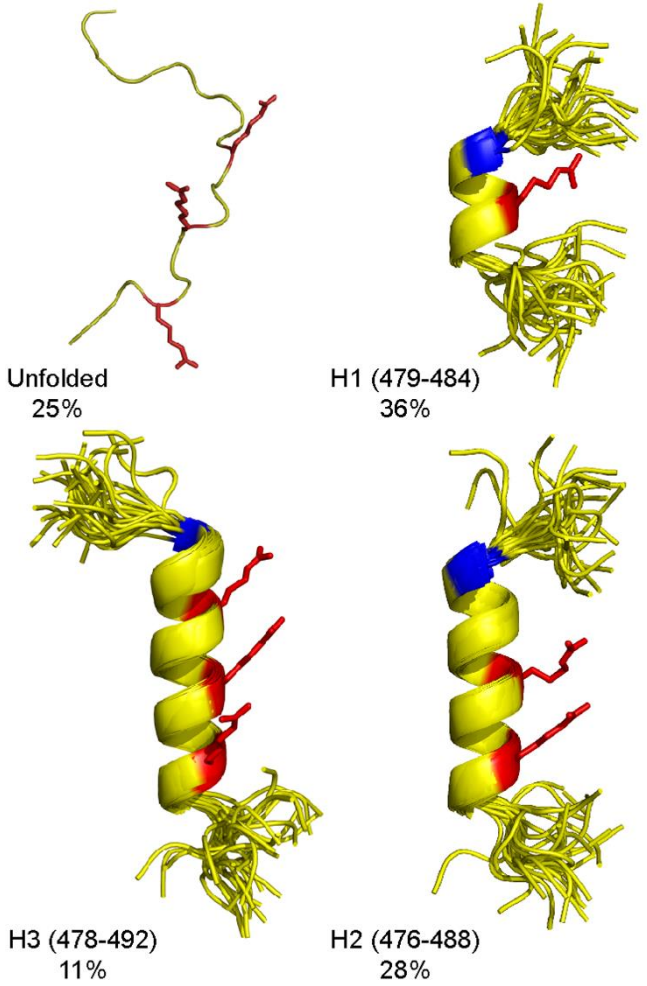


Conformational ensemble

A word of caution



IDPs: not completely random

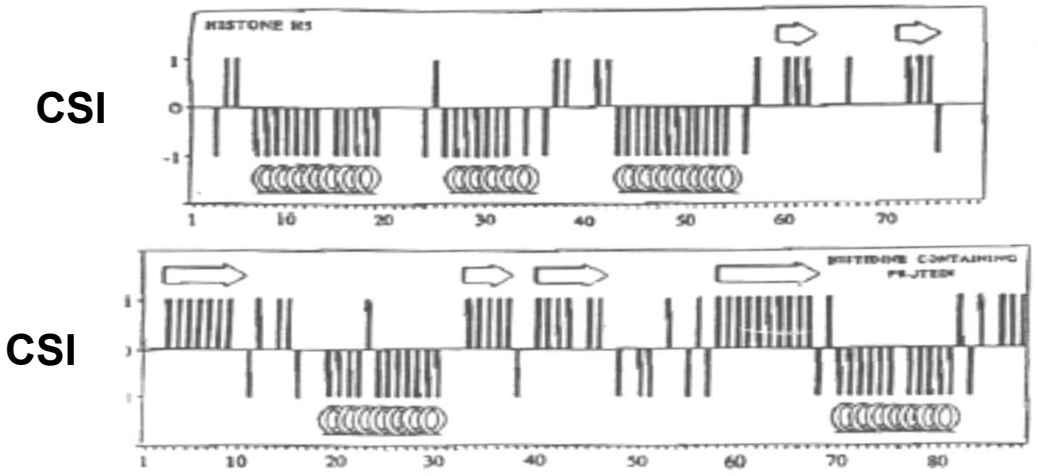


- Binding sites in IDPs can sample conformations similar to the bound state already without their binding partner
- Pre-configuration for binding
- Conformational ensemble characterized in detail by NMR

Identification of secondary structure propensity

→ difference of value of Cα, Cβ ou CO compared to value of Cα, Cβ ou CO 'random coil', respectively

- si ΔCα <0, ΔCβ >0, ΔCO <0 → β sheet
- si ΔCα >0, ΔCβ <0, ΔCO >0 → α helix

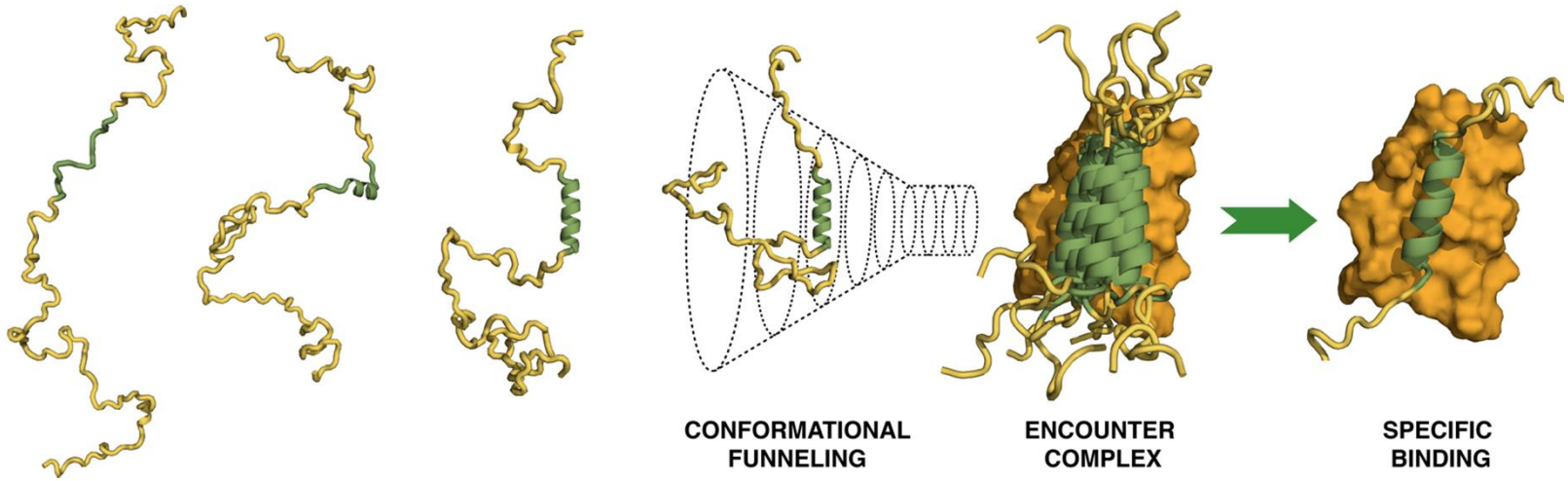


$$\delta(H\alpha)_{obs} - \delta(H\alpha)_{rc} < -0,2\,ppm \Rightarrow CSI = -1$$

$$\delta(H\alpha)_{obs} - \delta(H\alpha)_{rc} > 0,2\,ppm \Rightarrow CSI = 1$$

$$\left|\delta(H\alpha)_{obs} - \delta(H\alpha)_{rc}\right| < 0,2\,ppm \Rightarrow CSI = 0$$

IDPs: characterize binding mechanisms



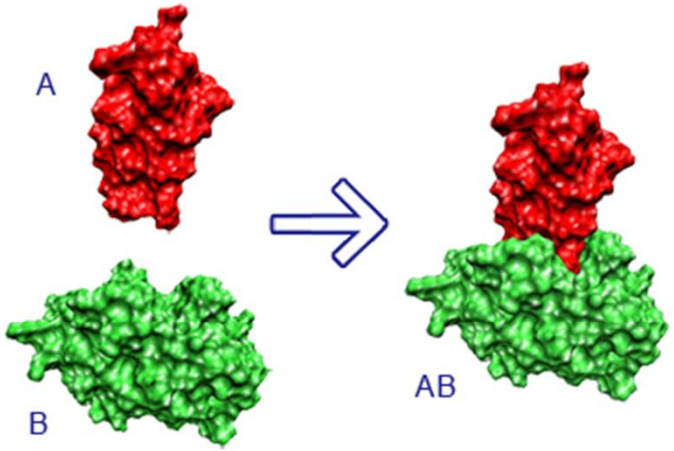
- Relaxation dispersion NMR measurements allow to characterize binding pathways of IDPs
- Binding of pre-configured conformations, formation of nonspecific encounter complexes

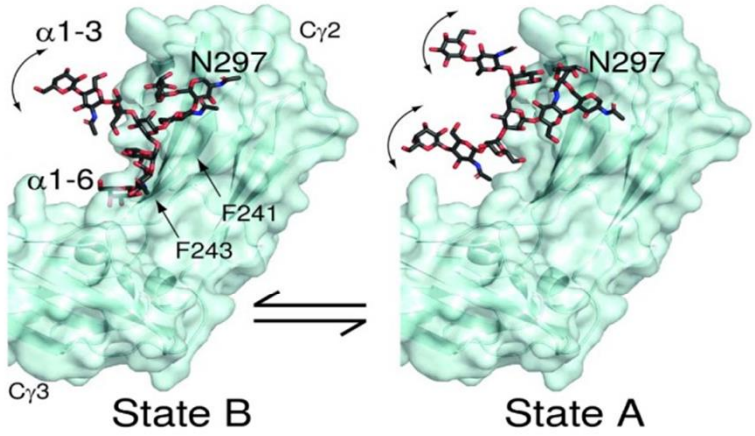
protein-protein and protein ligand Interactions

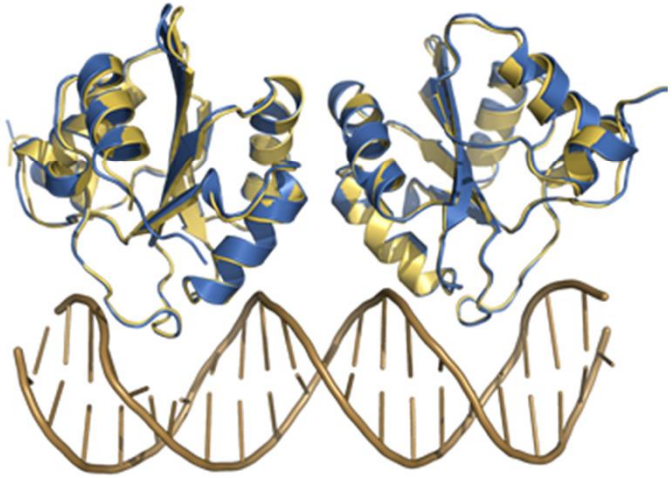
APPLICATION

Biomolecular interaction characterization

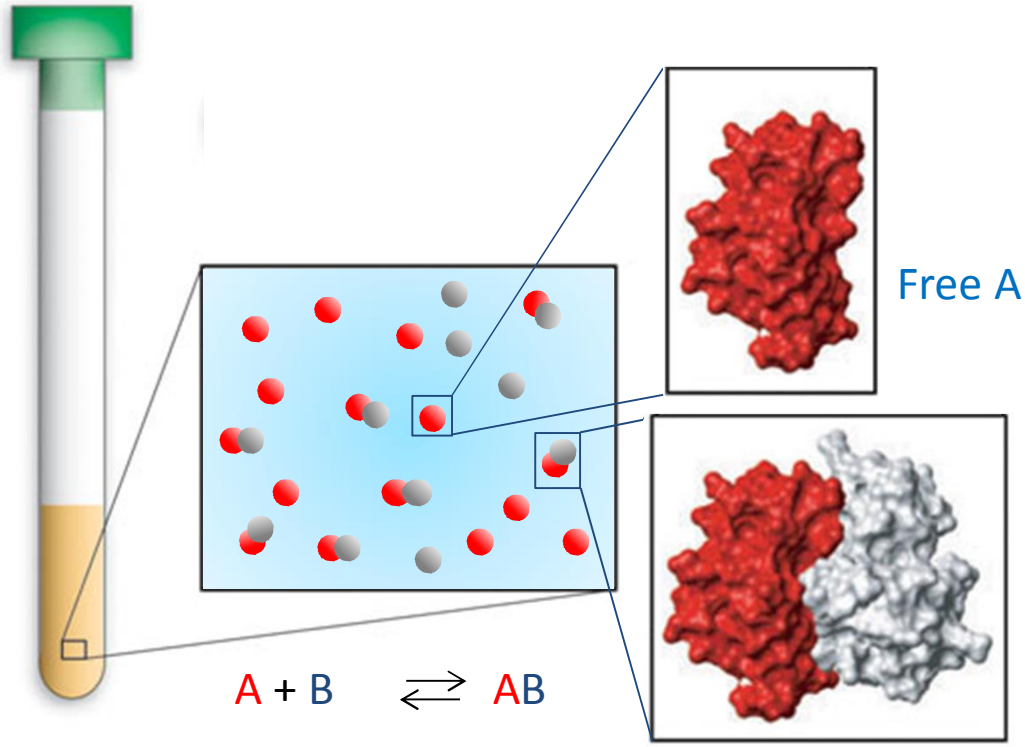
protein-protein, protein-DNA, proteine-glycan ou protein-ligand



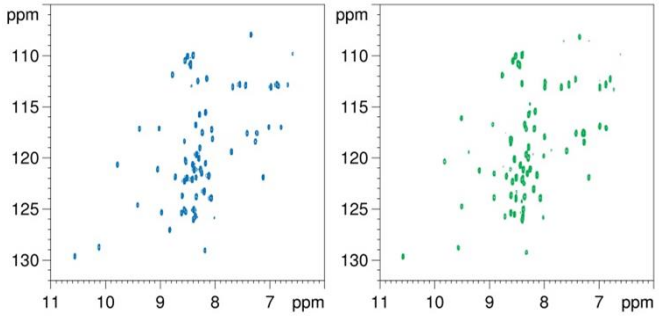




NMR exchange measurements



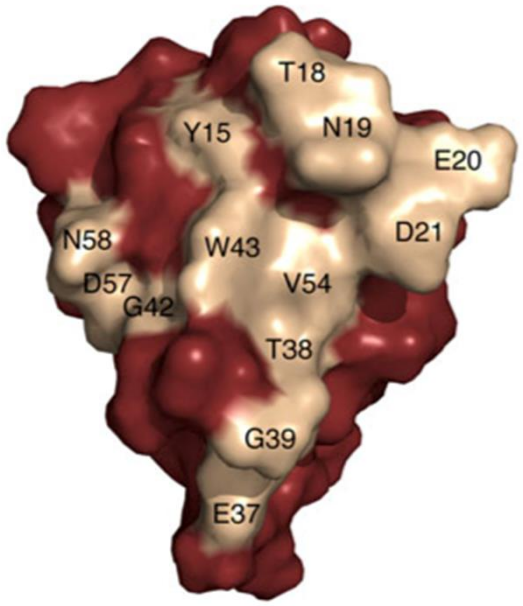
A free ⇄ A bound



→ A free and A bound are different

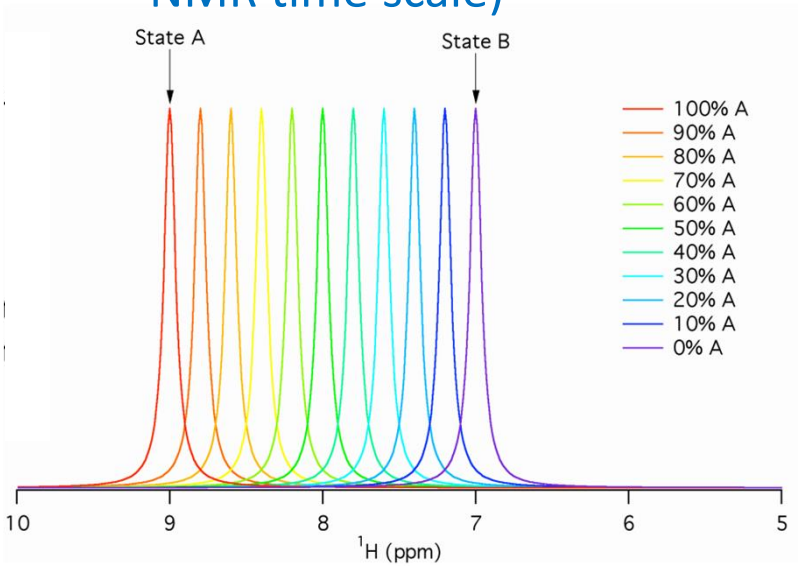
Titrations: K_D from NMR

- Some restrictions apply: concentration range, requirement for peaks shifting in fast exchange
- Even if these conditions are not met, the binding interface can usually still be mapped from shift changes / peak broadening



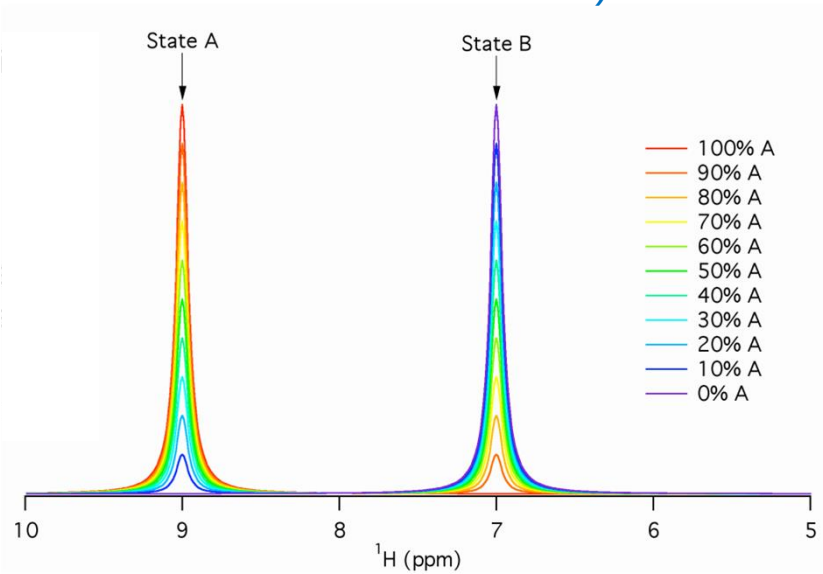
Two exchange regime: fast vs. slow

Fast exchange (on the NMR time scale)



- 1 peak at the « weighted » average of A and B
- The peak will shift between frequency from state A to frequency of state B as the state population varied

Slow exchange (on the NMR time scale)



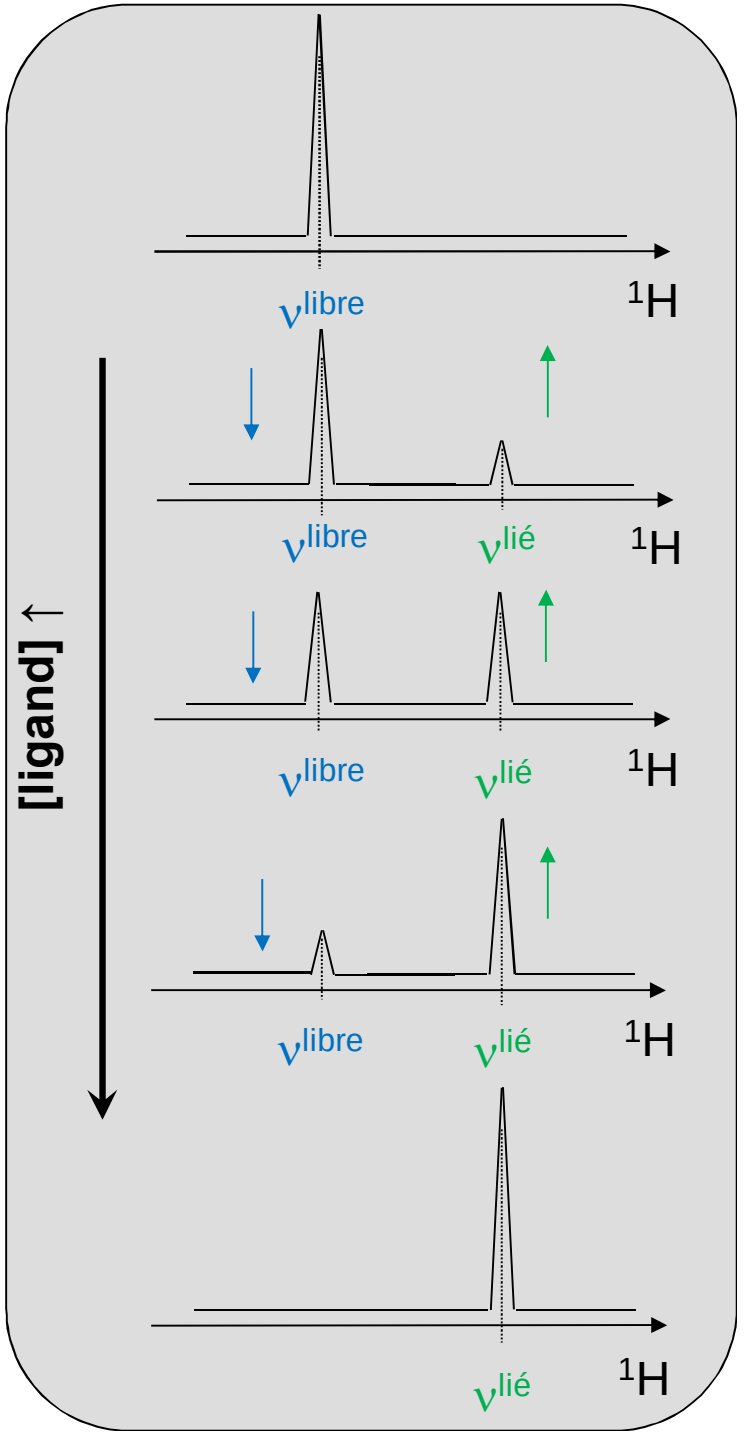
- 2 peaks corresponding to state A and state B, which relative intensities reflects the fraction of population in each state
- Peaks do not shift, rather their intensities are modified as the state populations varied

K_D by NMR

Slow exchange

→ Strong complex (low KD)

- The chemical shift value of an atom is sensitive to its chemical environment
- Protein(A) at constant concentration in the presence of protein (B) at increasing concentration : NMR signal of(A) will change according to the amount of added (B), meaning depending on the amount of(AB)complex that is formed

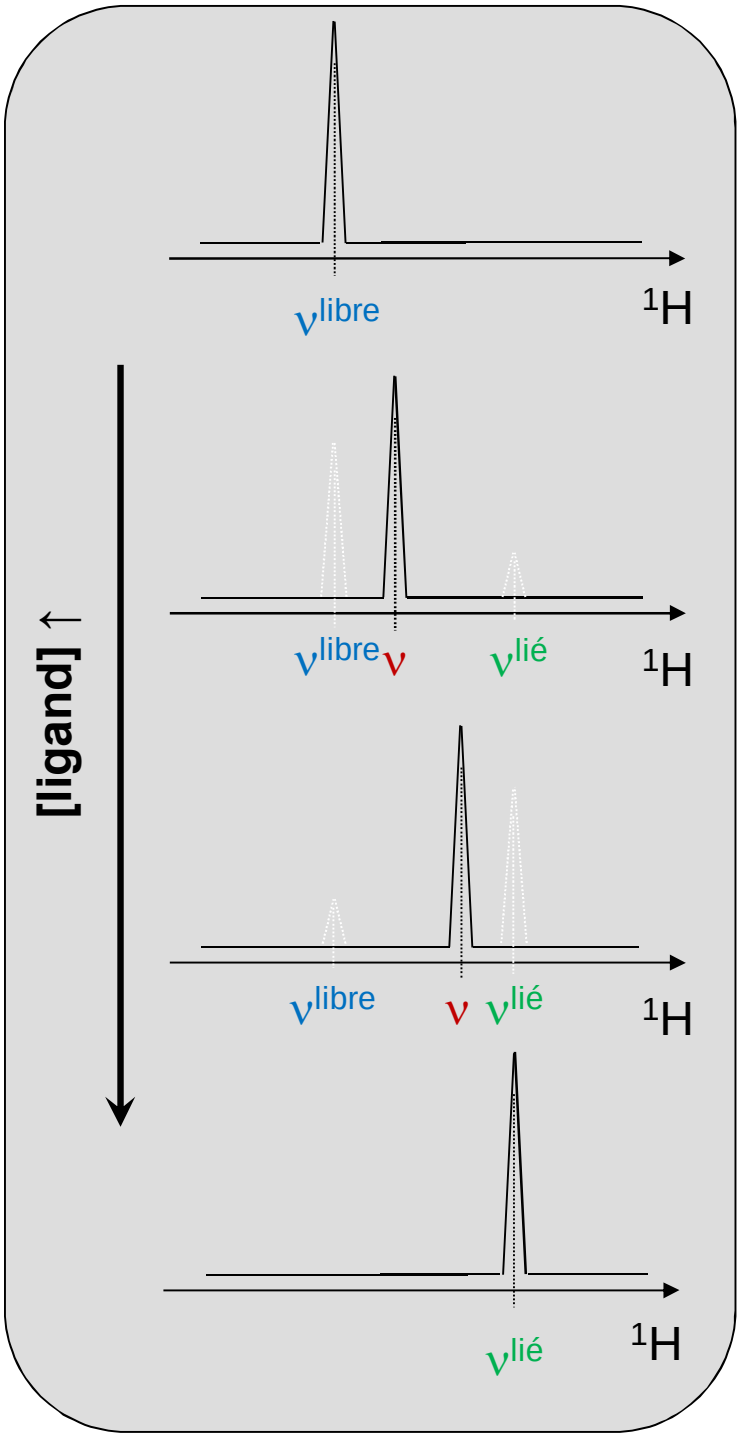


K_D by NMR

Fast exchange

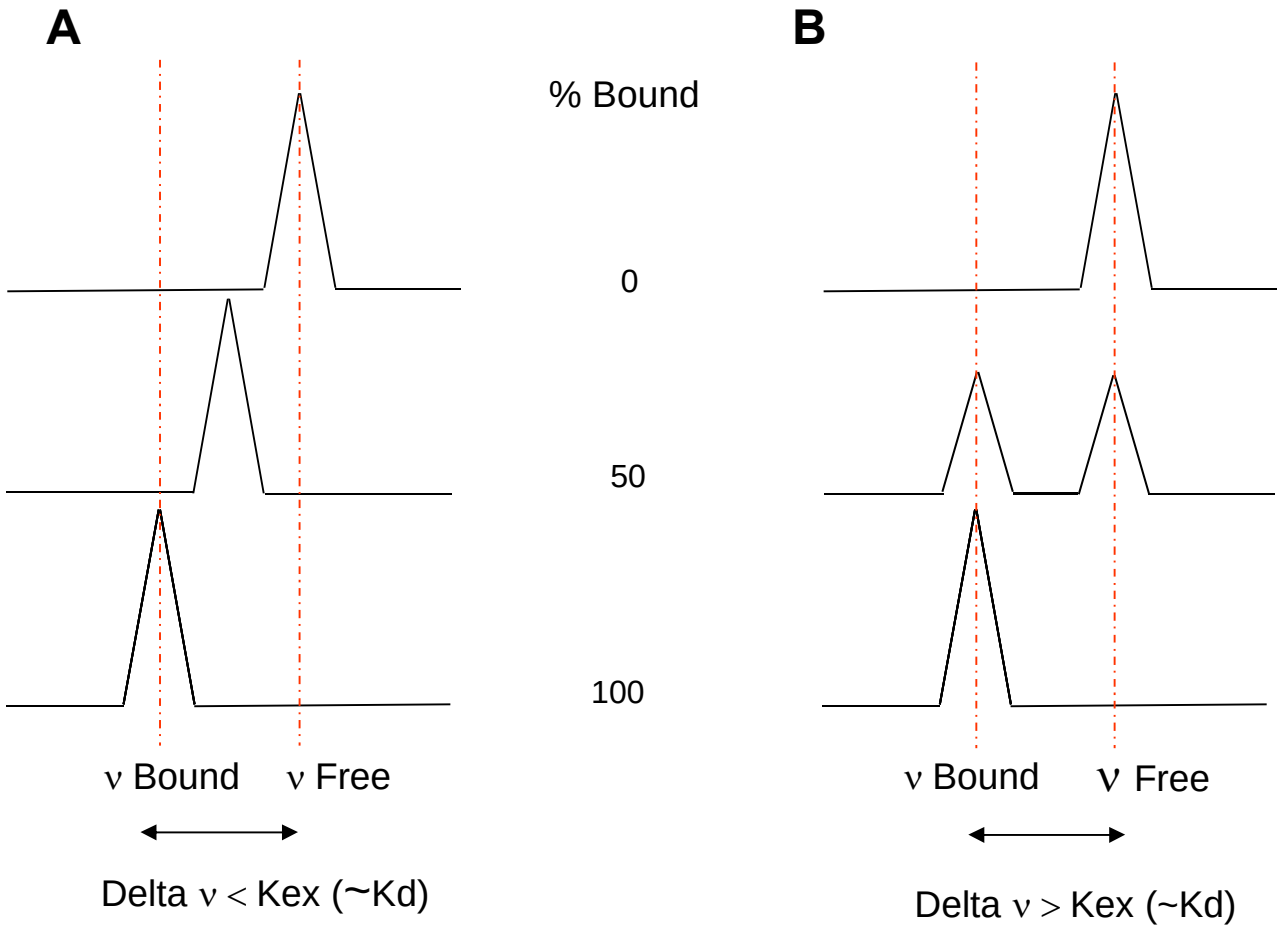
→ Weak complex

- The chemical shift value of an atom is sensitive to its chemical environment
- Protein(A) at constant concentration in the presence of protein (B) at increasing concentration : NMR signal of (A) will change according to the amount of added (B), meaning depending on the amount of (AB) complex that is formed



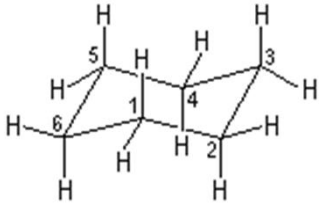
Two exchange regime: fast vs. slow

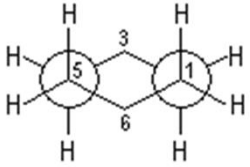
On the NMR time scale



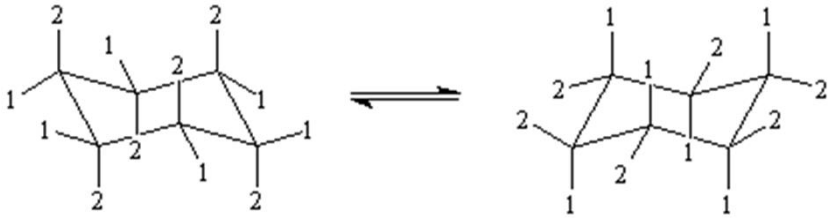
Temperature influence on the exchange

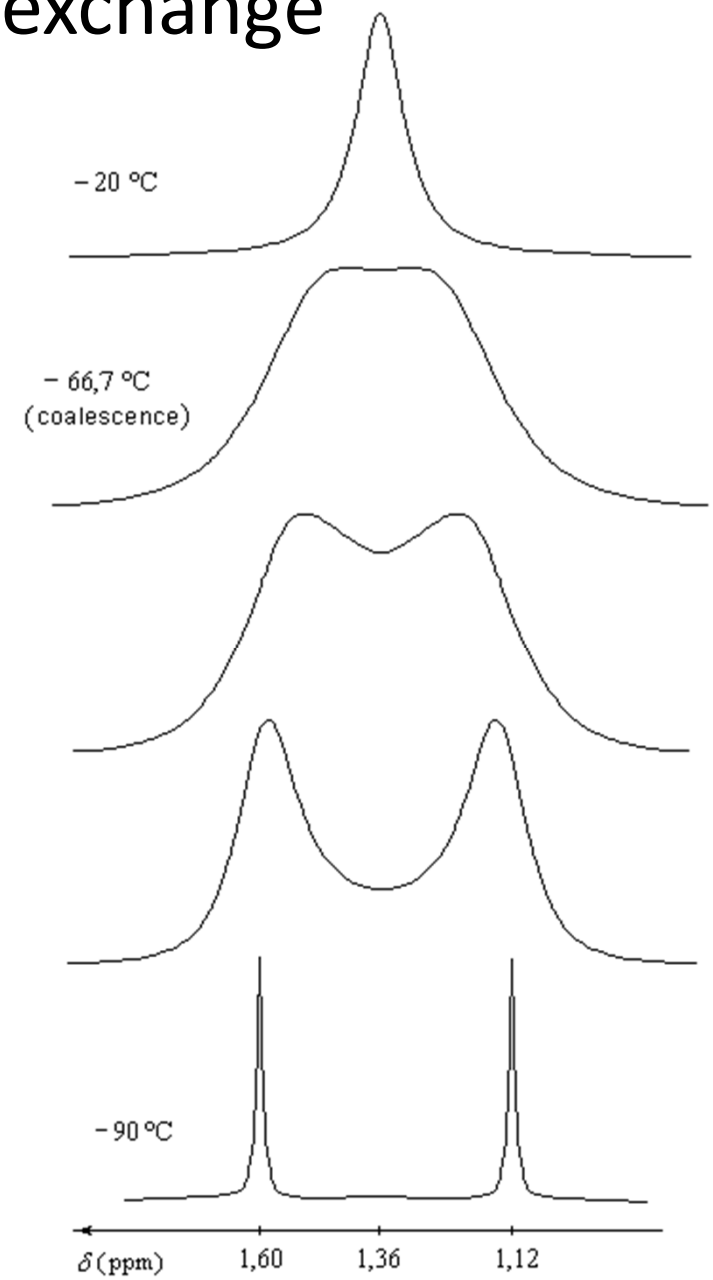
Example of cyclohexane



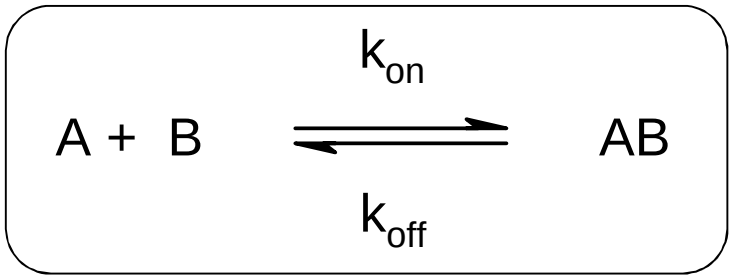


Conformationnel exchange:





Characterization of a protein-protein interface: definition of the dissociation constant for a complex



$$K_D = \frac{[A].[B]}{[AB]}$$

[A], [B] and [AB] are the concentrations of A, B and AB complex at equilibrium.

Concentrations at equilibrium are described as

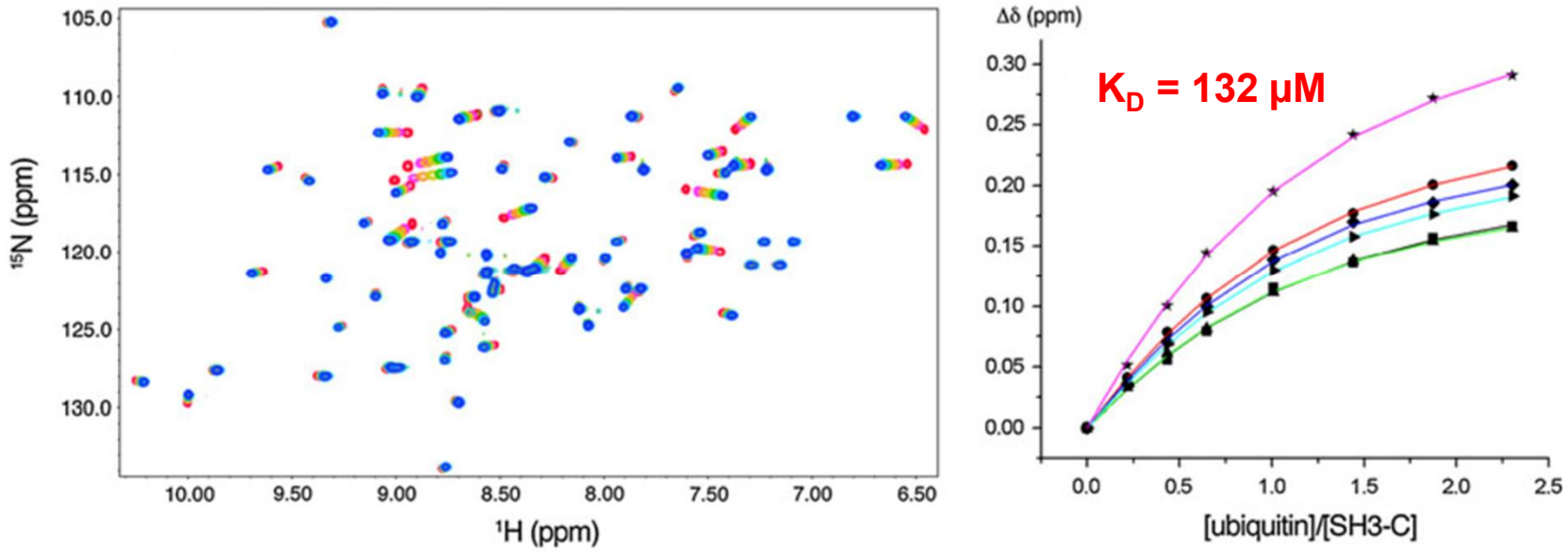
$$[A]_0 = [A] + [AB]$$
$$[B]_0 = [B] + [AB]$$

with $[A]_0$ and $[B]_0$ the initial concentrations of A and B.

Concentration of complex at equilibrium described as (from K_D expression)

$$[AB] = \frac{1}{2}([A]_0 + [B]_0 + K_D - \sqrt{([A]_0 + [B]_0 + K_D)^2 - 4[A]_0[B]_0})$$

Titrations: K_D from NMR

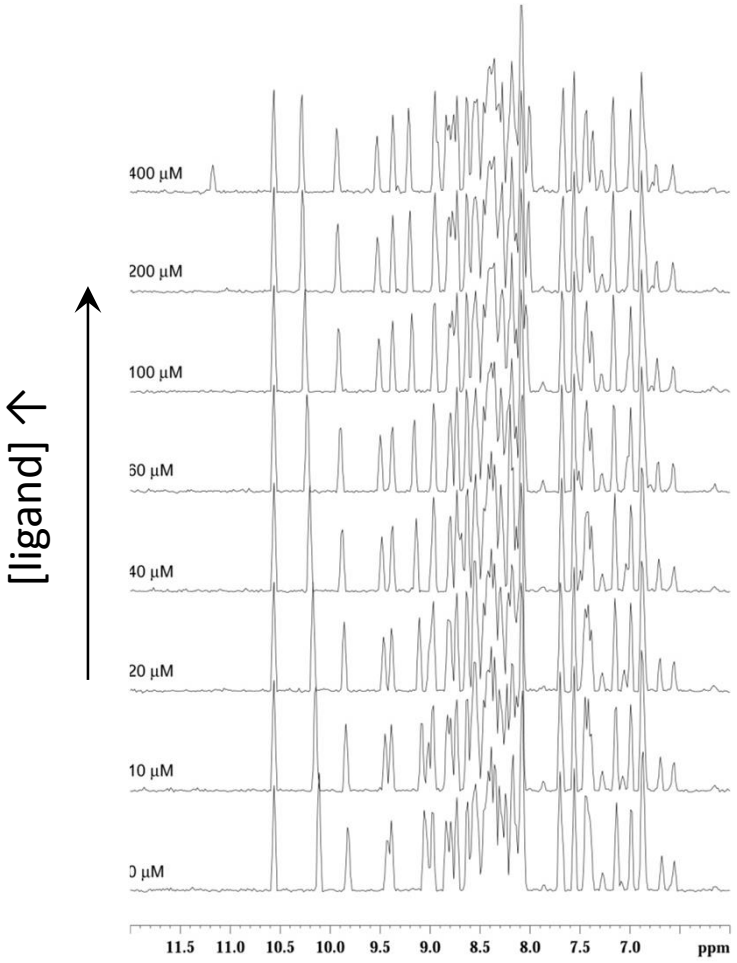


Ringkjøbing Jensen et al., Eur Biophys J 40, 1371, 2011

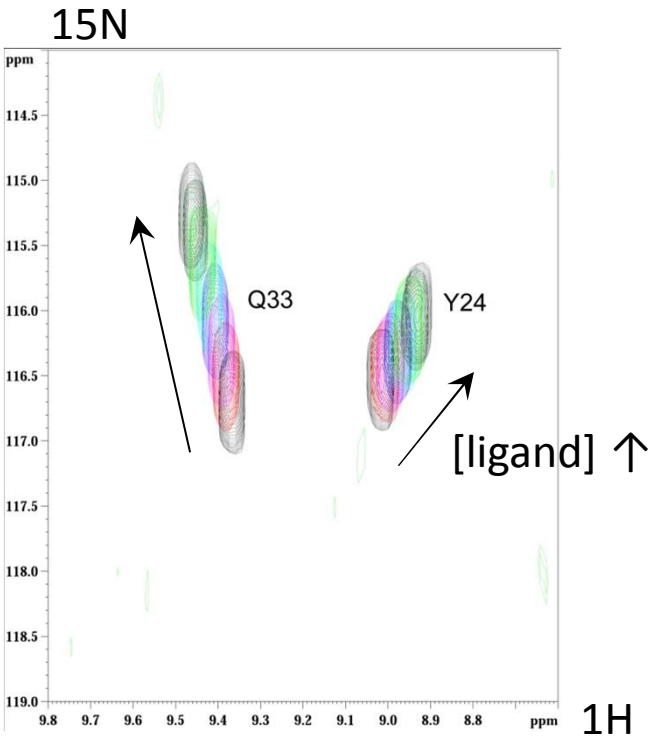
- Add unlabeled partner to isotope-labeled protein in increasing concentrations, record HSQCs
- Determine K_D from peak shifts

K_D by RMN

Chemical shift perturbation of a ^{15}N-labelled protein with increasing amount of unlabelled ligand



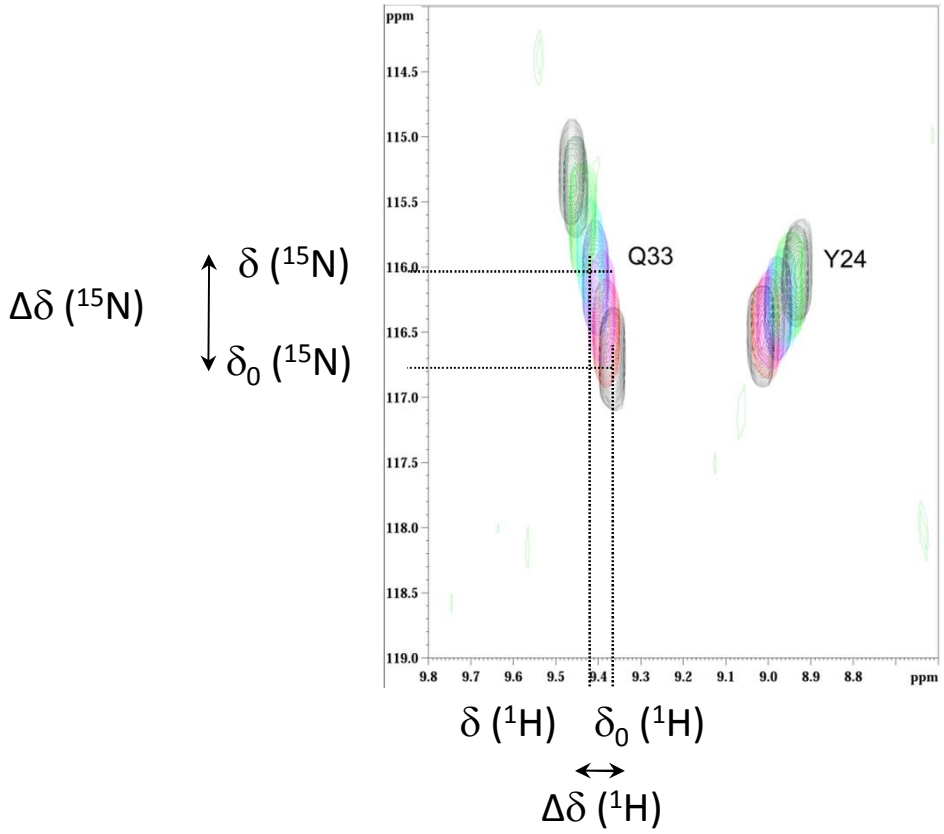
2D : ^{15}N-^{1}H HSQC



K_D by RMN

Chemical shift perturbation of a ^{15}N-labelled protein with increasing amount of unlabelled ligand

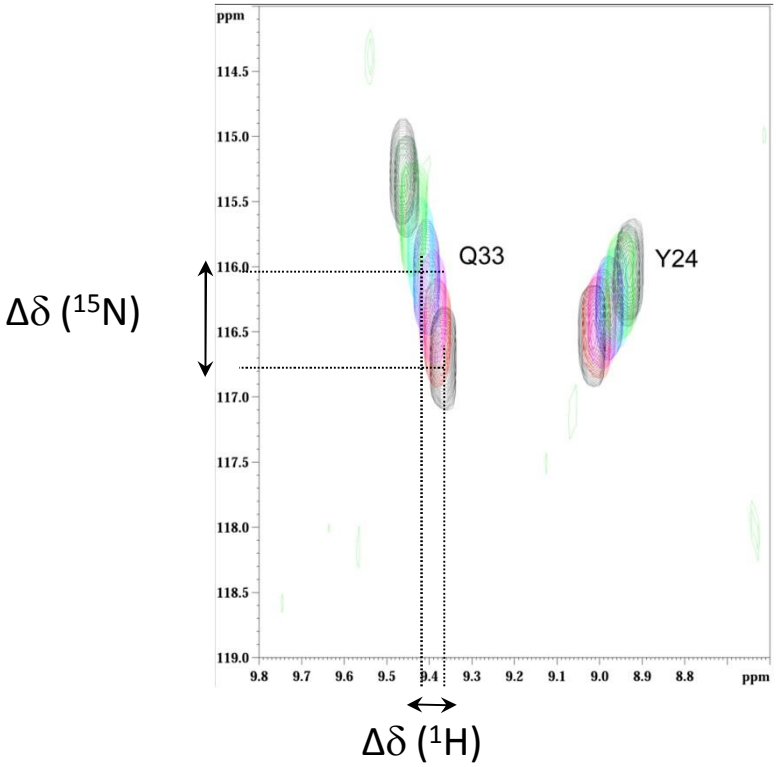
$$\Delta\delta = \delta - \delta_0 = \sqrt{[\Delta\delta\ (^1H)]^2 + 0.2\ [\Delta\delta\ (^{15}N)]^2}$$

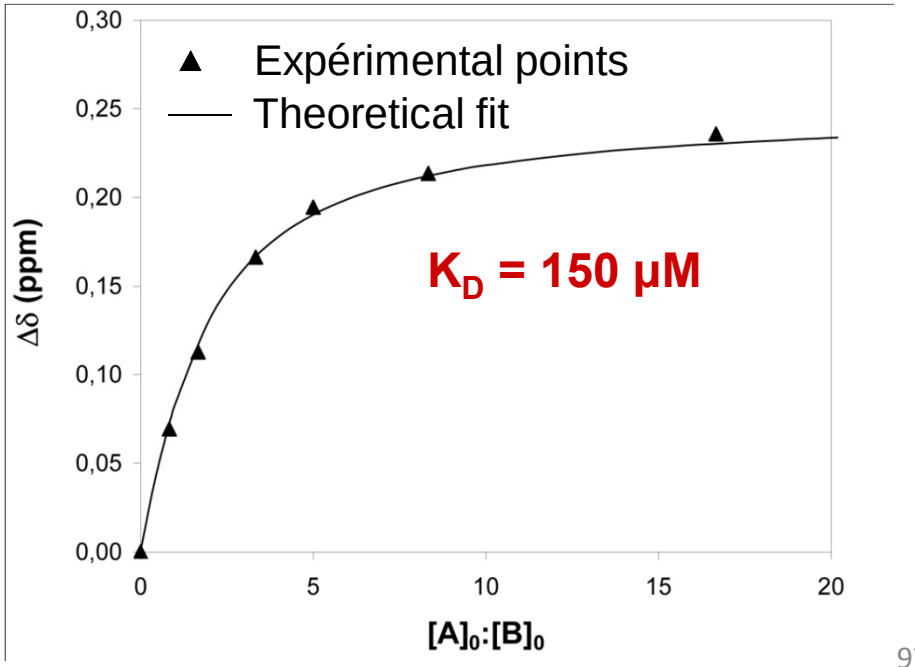


K_D by RMN

Chemical shift perturbation of a ^{15}N-labelled protein with increasing amount of unlabelled ligand → determination of dissociation constant (K_D)

$$\Delta\delta = \frac{\Delta\delta_{max}}{2}([A]_0 + [B]_0 + K_D - \sqrt{([A]_0 + [B]_0 + K_D)^2 - 4[A]_0[B]_0})$$



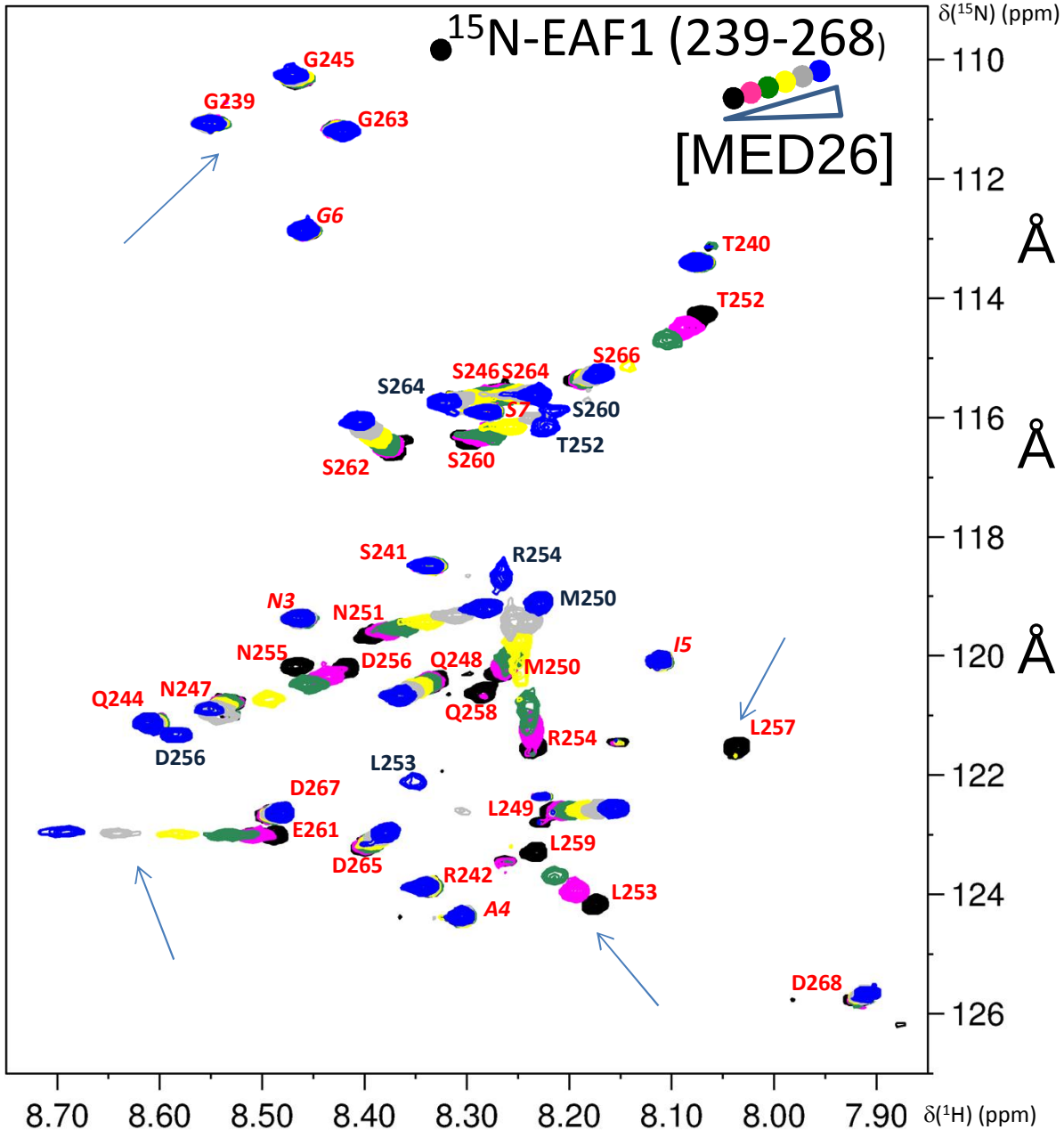


Folding upon binding

Example of the interaction of TAF7 (TBP-associated factor 7) or EAF1 (Eleven-nineteen Lysine-rich in Leukemia-Associated Factor 1) with Med26 N-Terminal domain (NTD) :

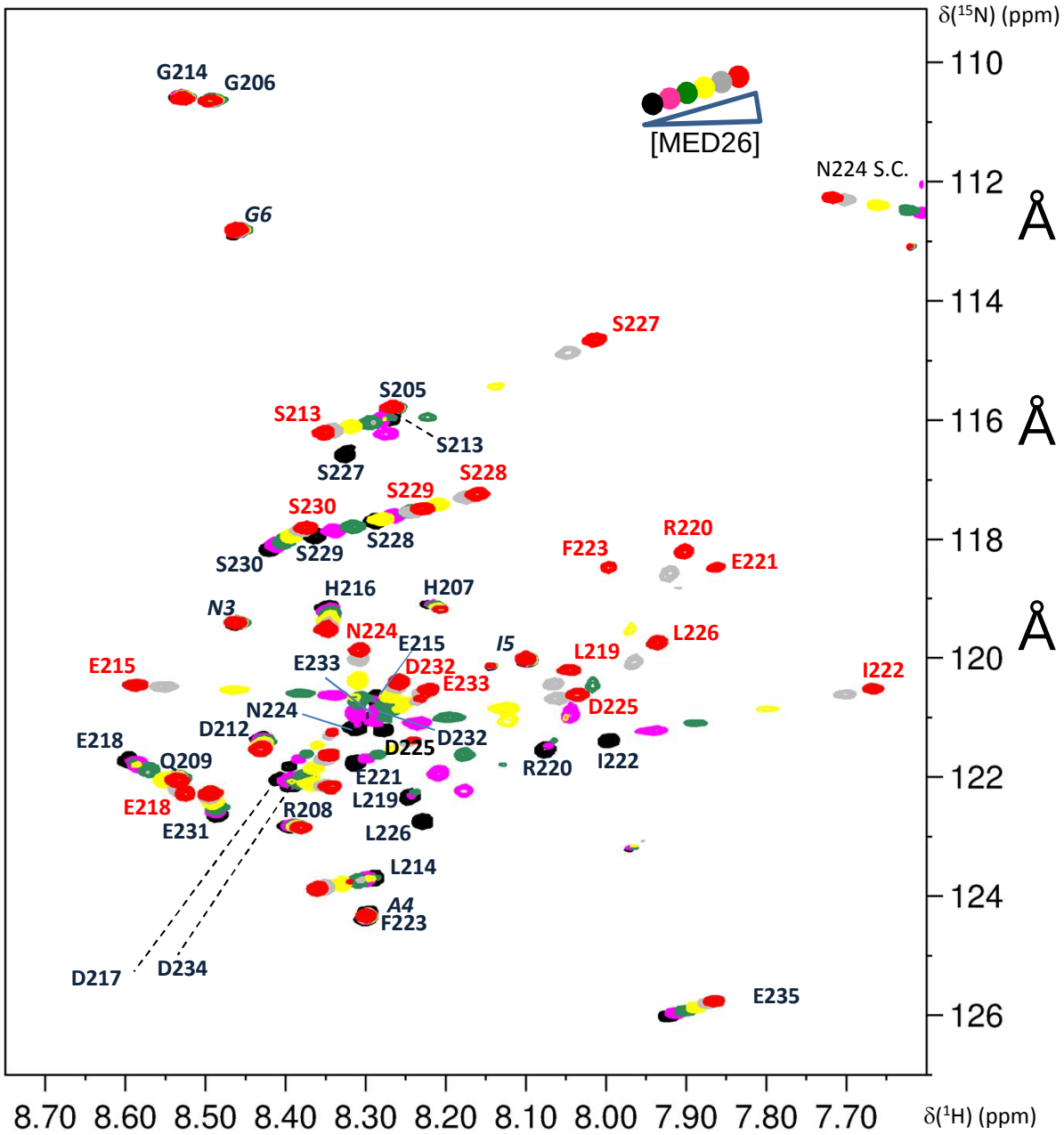
Switch from Initiation to elongation of transcription

Titration of ^{15}N-EAF peptide with unlabelled MED26



- Å Need assignment of the bound peptide (3D)
- Å Definition of the binding region
- Å Kd estimation

Titration of ^{15}N-TAF7 peptide with unlabelled MED26



^{15}N-TAF7 (205-235)

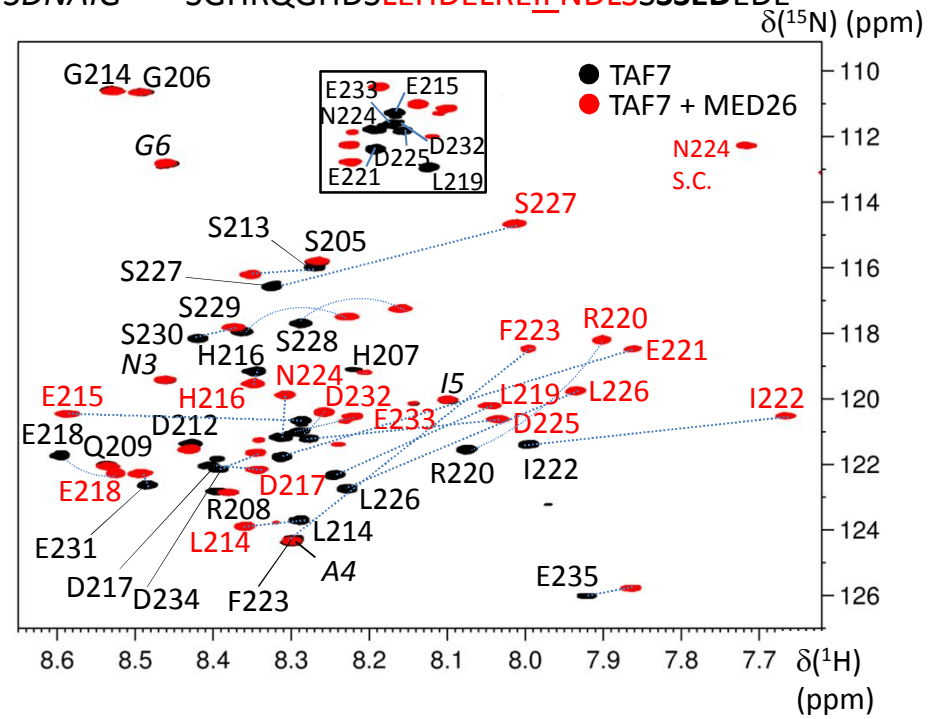
Å Need assignment of the bound peptide (3D)

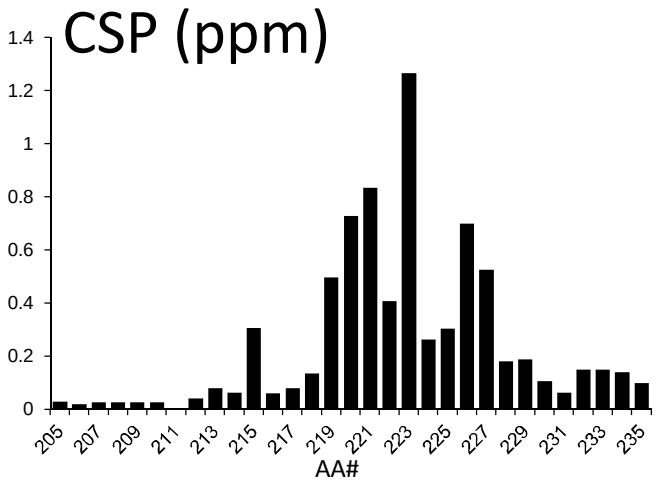
Å Definition of the binding region

Å Kd estimation

Folding of ^{15}N-TAF7 peptide upon binding MED26

^{1}*SDNAIG*^{6}-^{205}SGHRQGHDSLEHDELREIFNDLSS**SSED**EDE^{235}

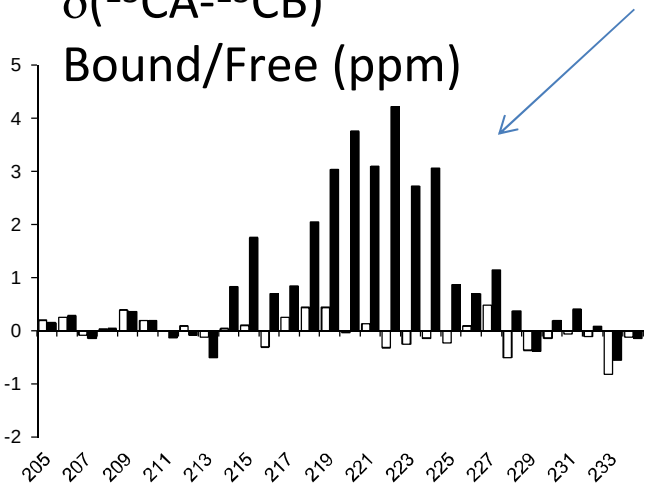




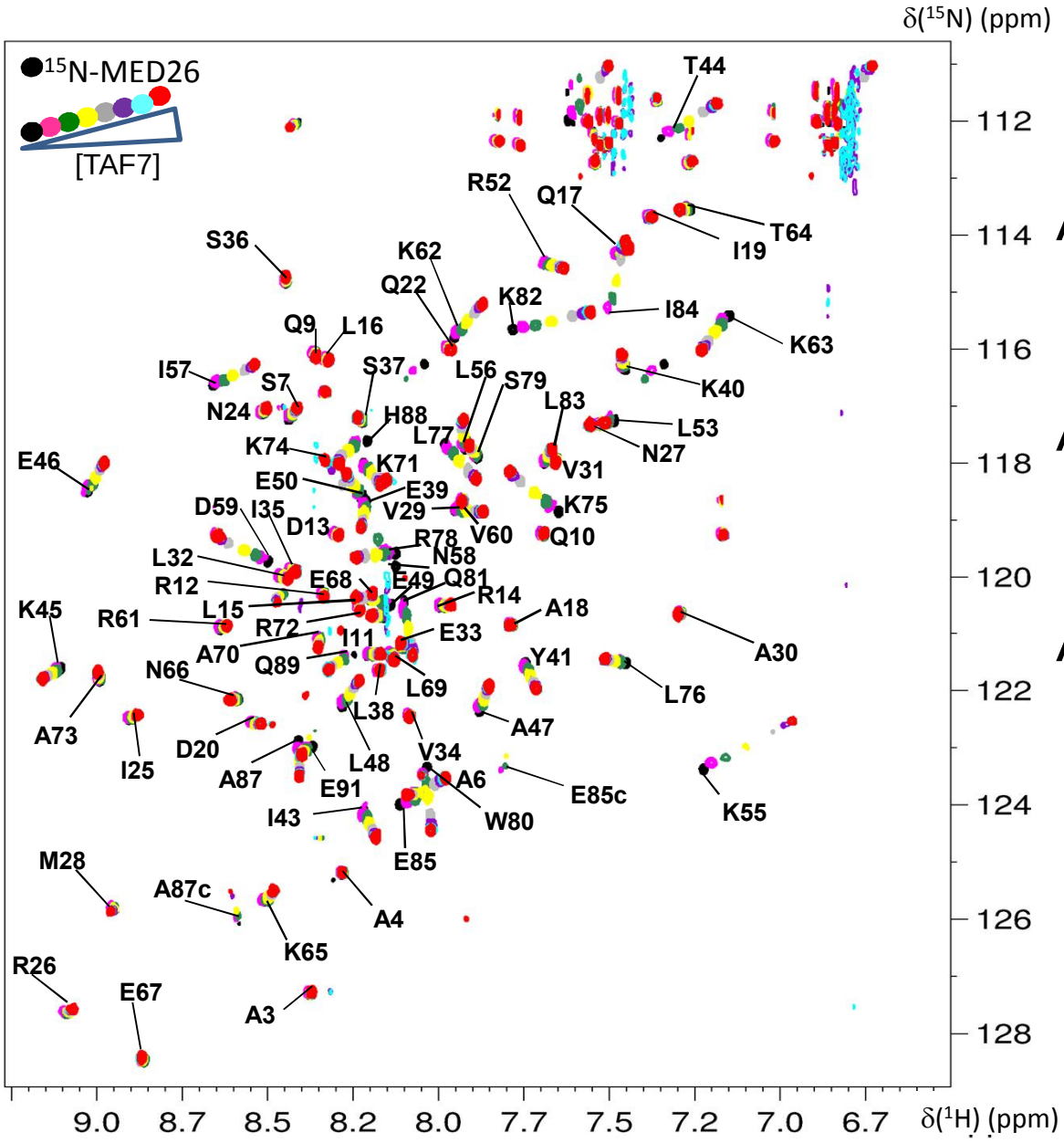
^{15}N-TAF7 (205-235)

- Use assignment of the free/bound peptide to define 2D structure
- Definition of the binding region

Helix formation



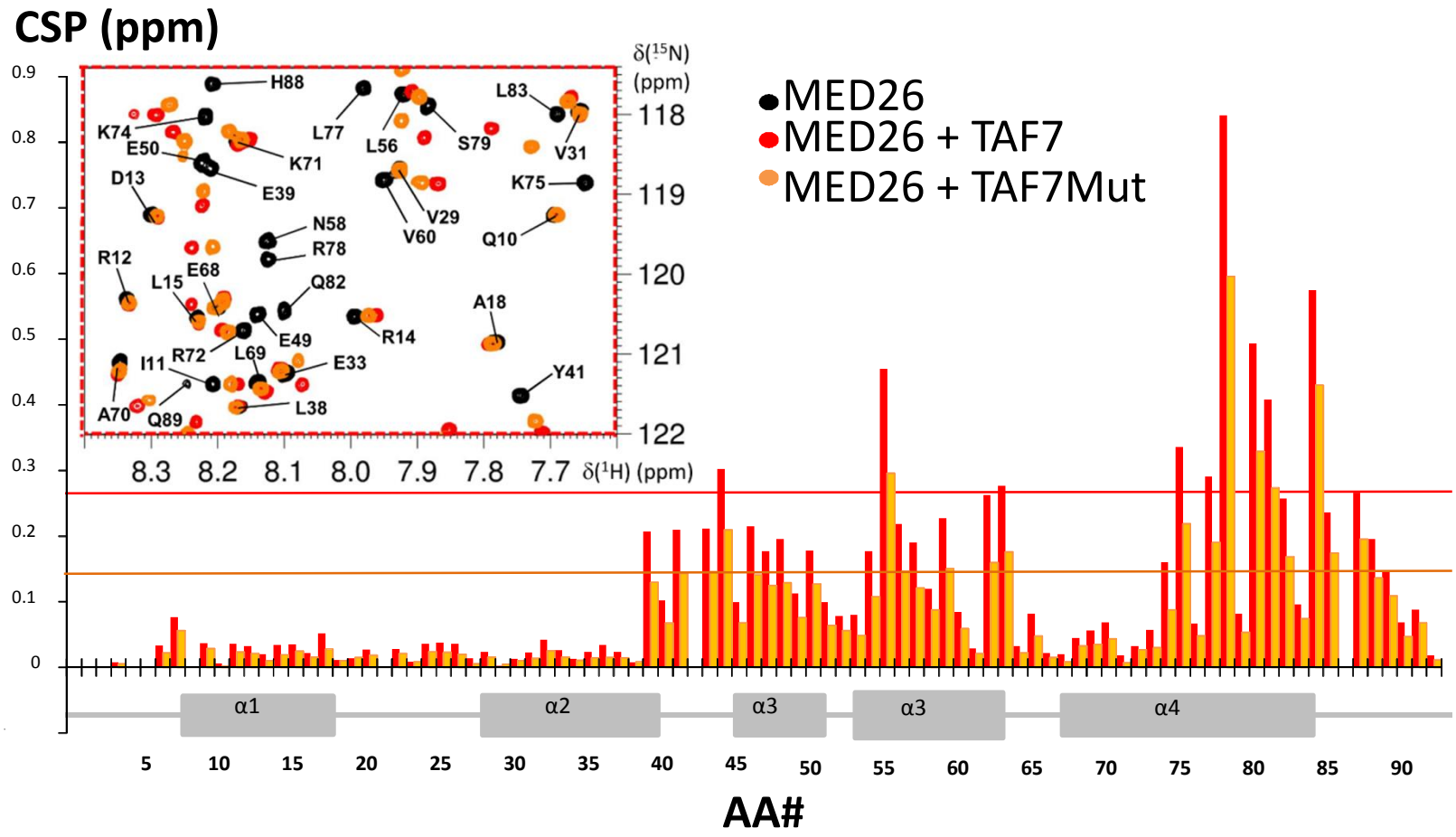
Titration of ^{15}N-MED26 with unlabelled TAF7 peptide



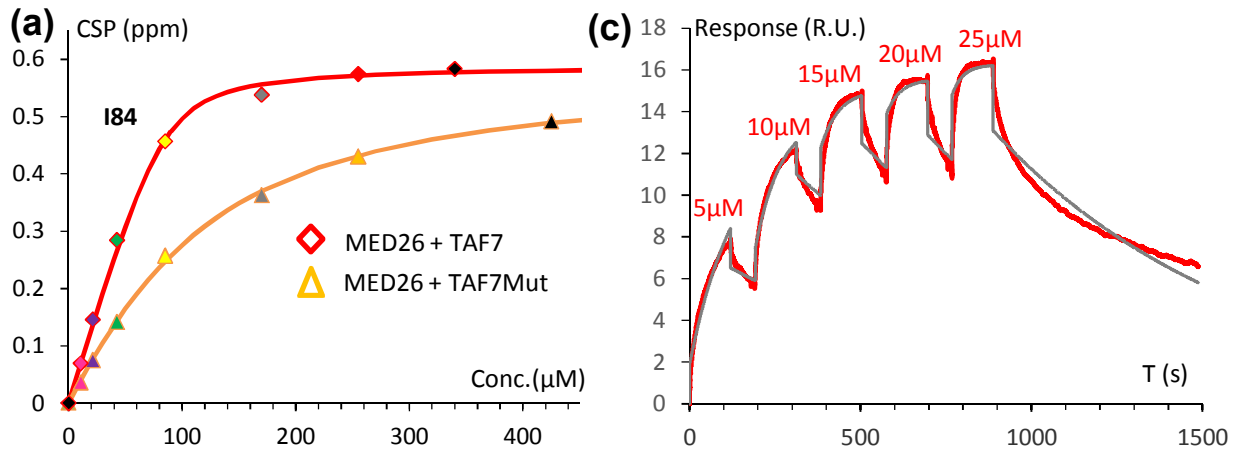
- Need assignment of the bound MED26 (3D)
- Definition of the binding region
- Kd estimation

Titration of ^{15}N-MED26 with unlabelled TAF7 peptide

Definition of the binding region



Titration of ^{15}N-MED26 with unlabelled TAF7 peptide



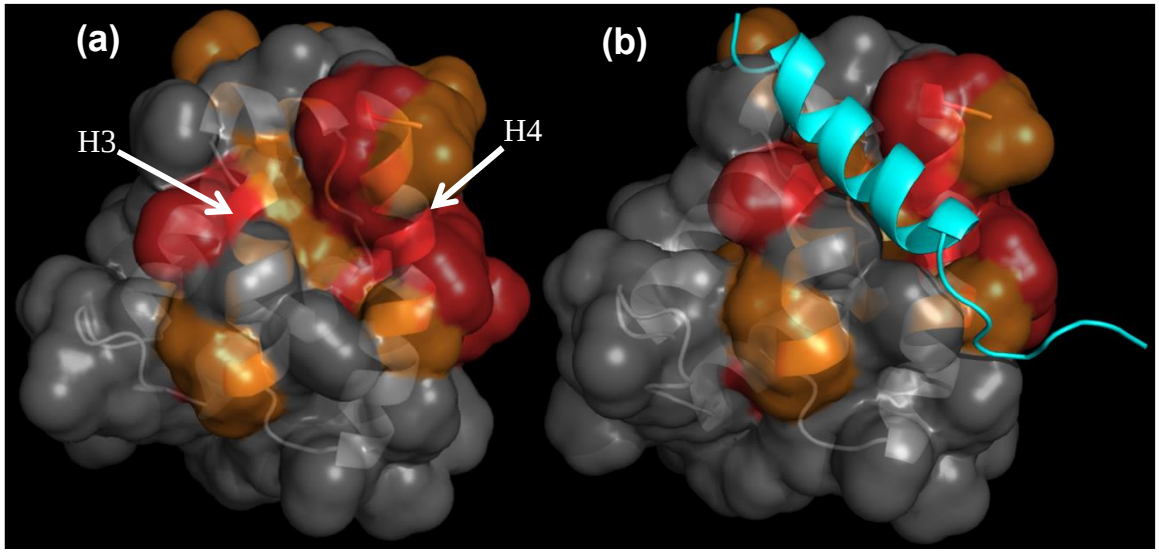
NMR

SPR

^{15}N-MED26 interaction with TAF7 peptide

Color coded CSP reported on MED26 surface

TAF7 calculated dihedral angle estimated from CS with TALOS and NOEs

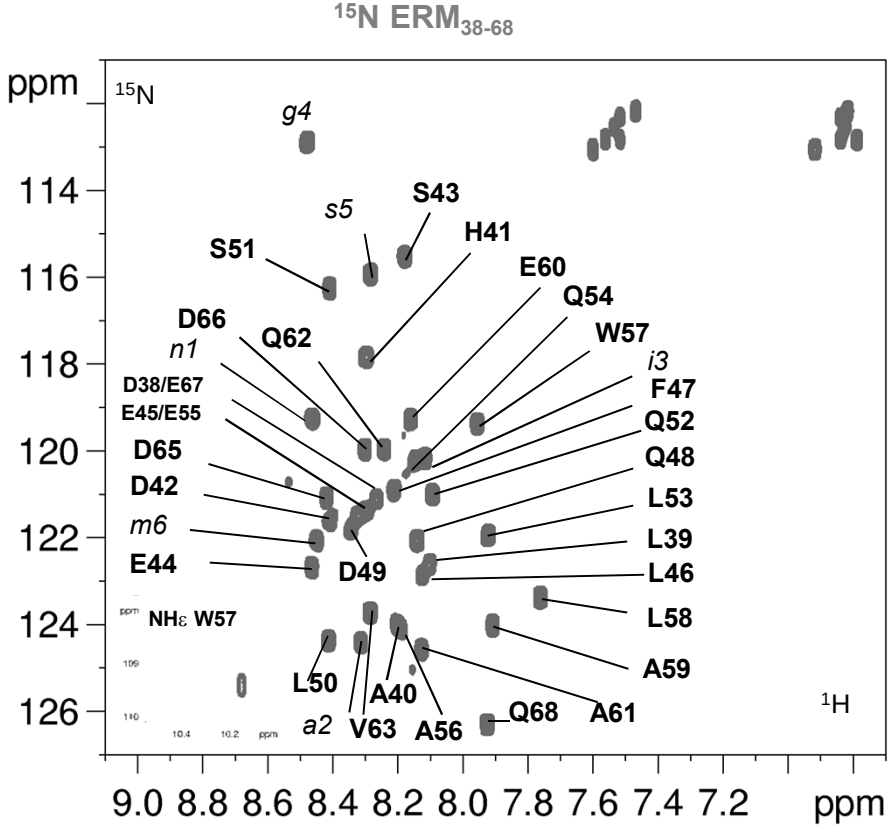


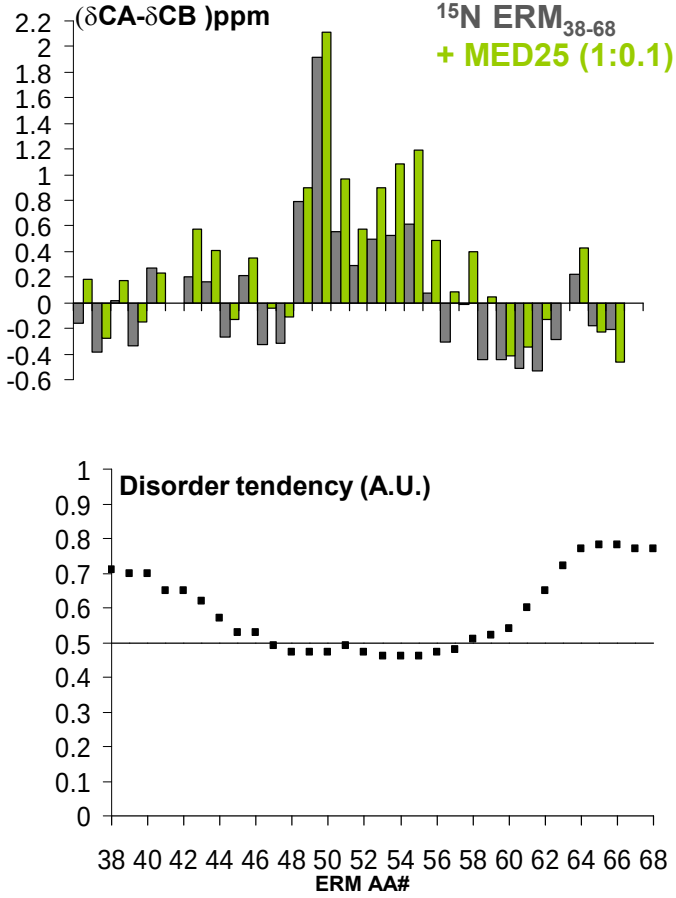
Haddock docking model of the complex based on a few intermoldecular NOEs and CSP data

Folding upon binding, fuzzy complexes

Example of the interaction of the TAD domain of ERM transcription factor with Med25 ACID domain

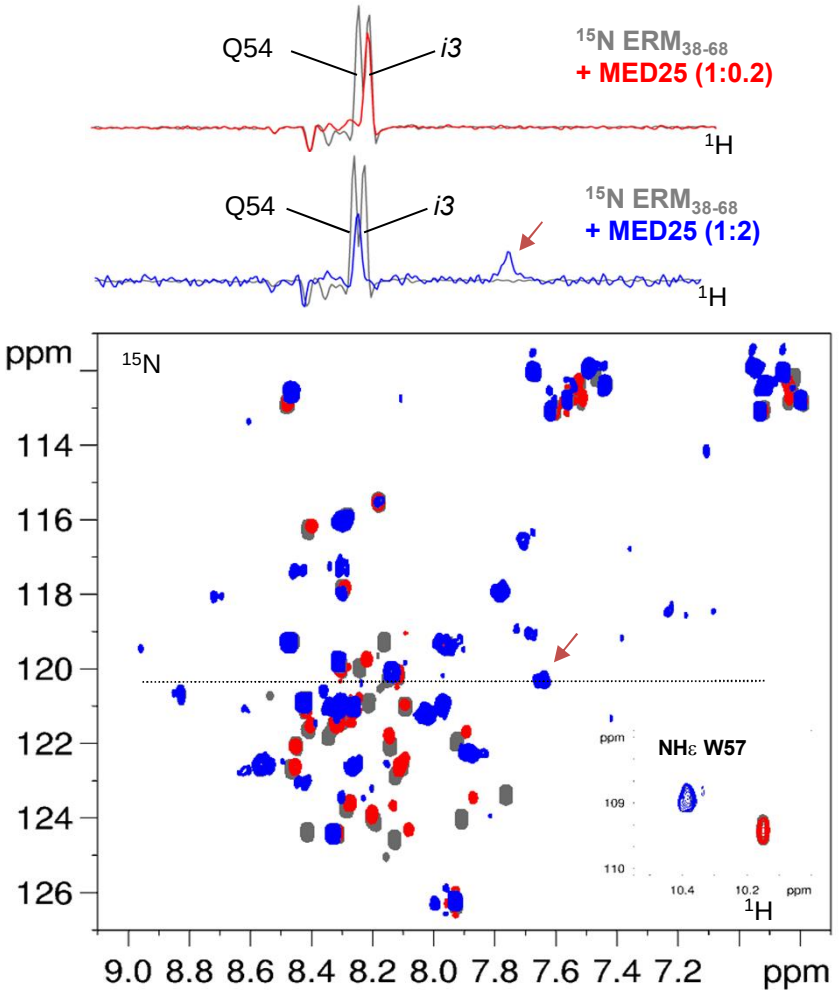
ERM peptide is disordered, with some helical tendency

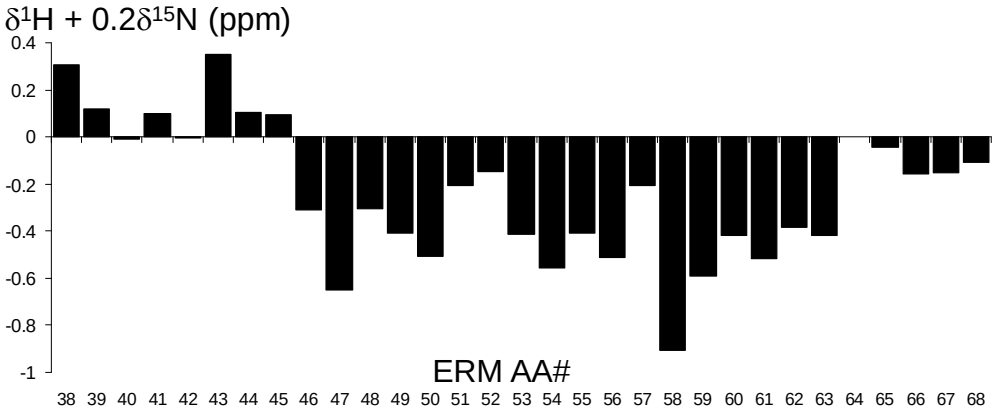




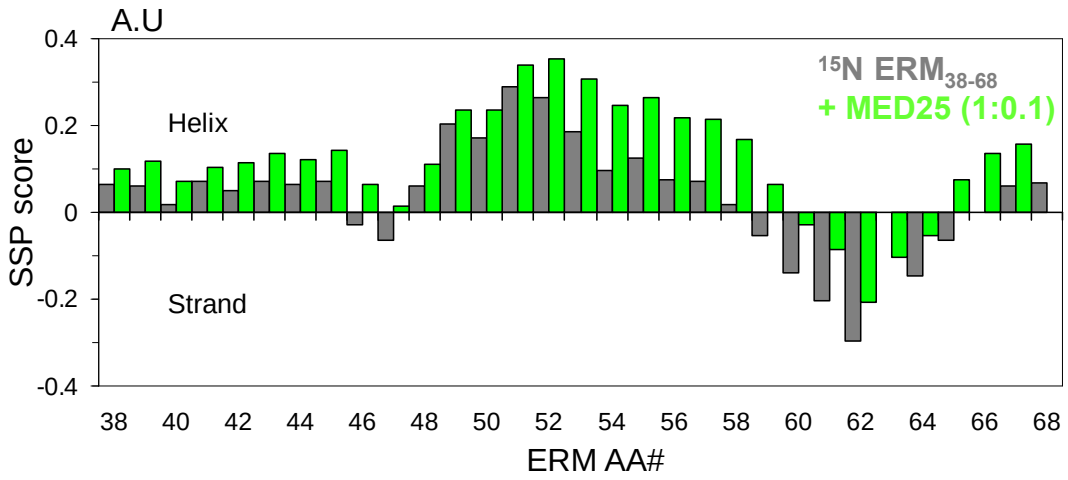
$_{1}nai_{3}\ gsm_{6}$ **38**DLAHDSEEL**F47**QDLSQLQEA**W57**LAEAQVPDDEQ**68**

Mapping of ERM interaction sequence

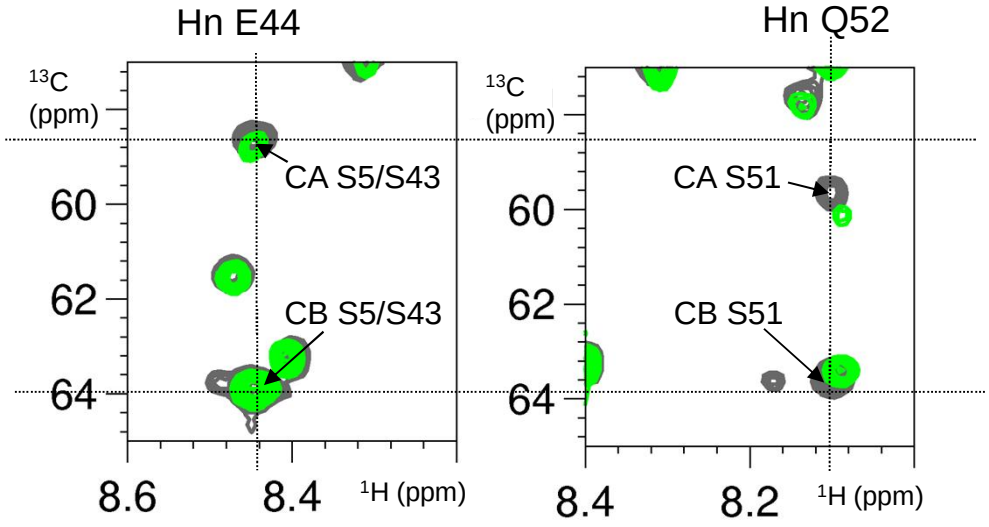




ERM folding upon MED25 ACID binding



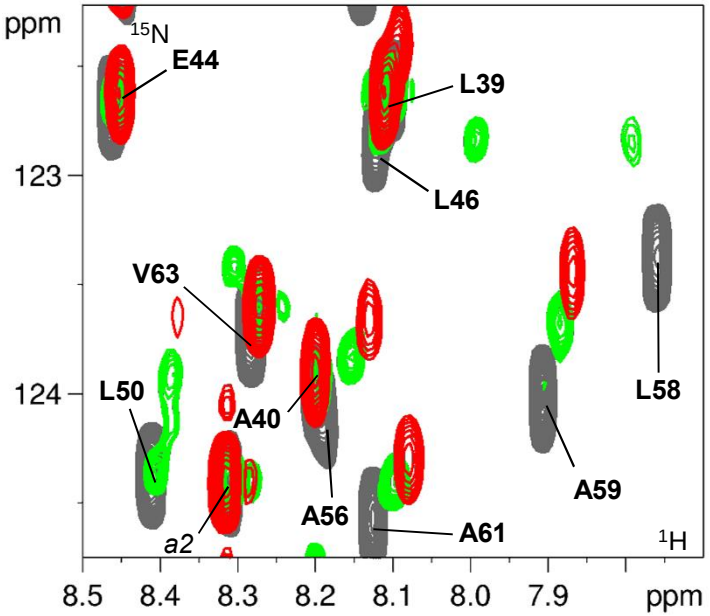
Secundary structure propensity scores: free/bound

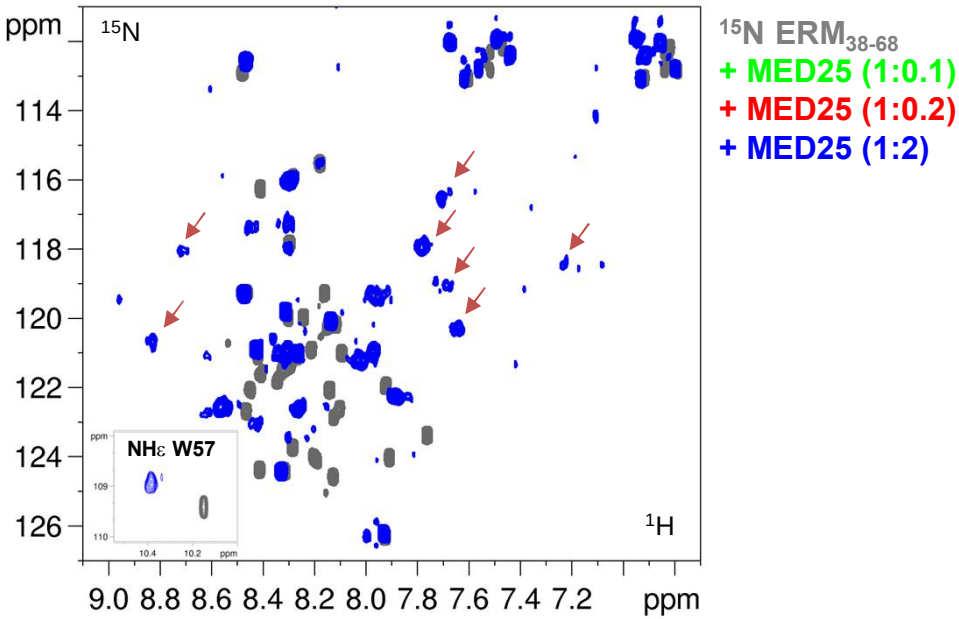


Inconsistent fast and slow exchange regimes

Fast exchange at low stoechiometry

Kd by ITC 0.6µM ????





Encounter complex

Mapping of interaction surfaces

Wild type ERM sequence

F/W-mutated ERM sequence

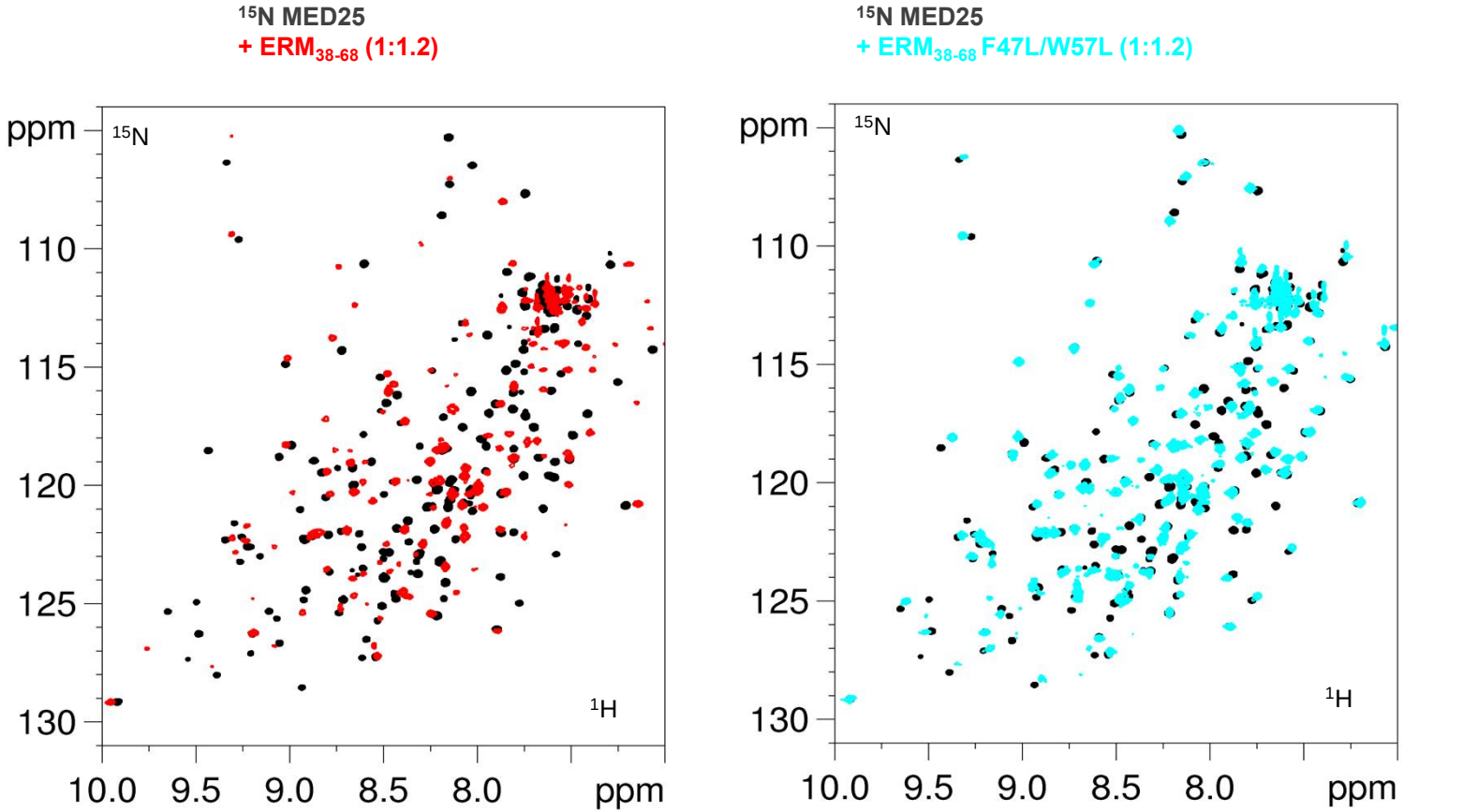
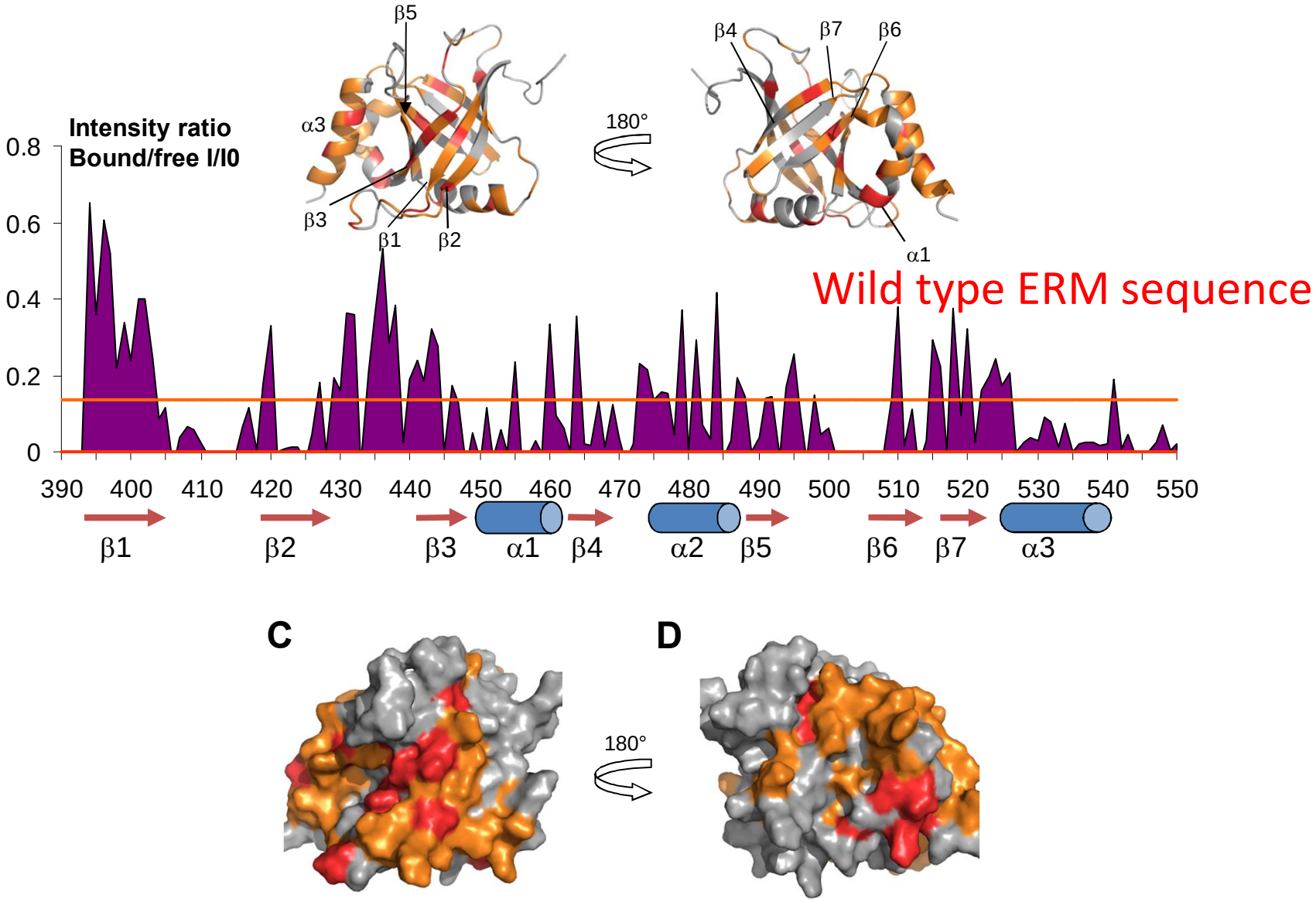


Figure 4

Mapping of interaction surfaces



Mapping of interaction surfaces

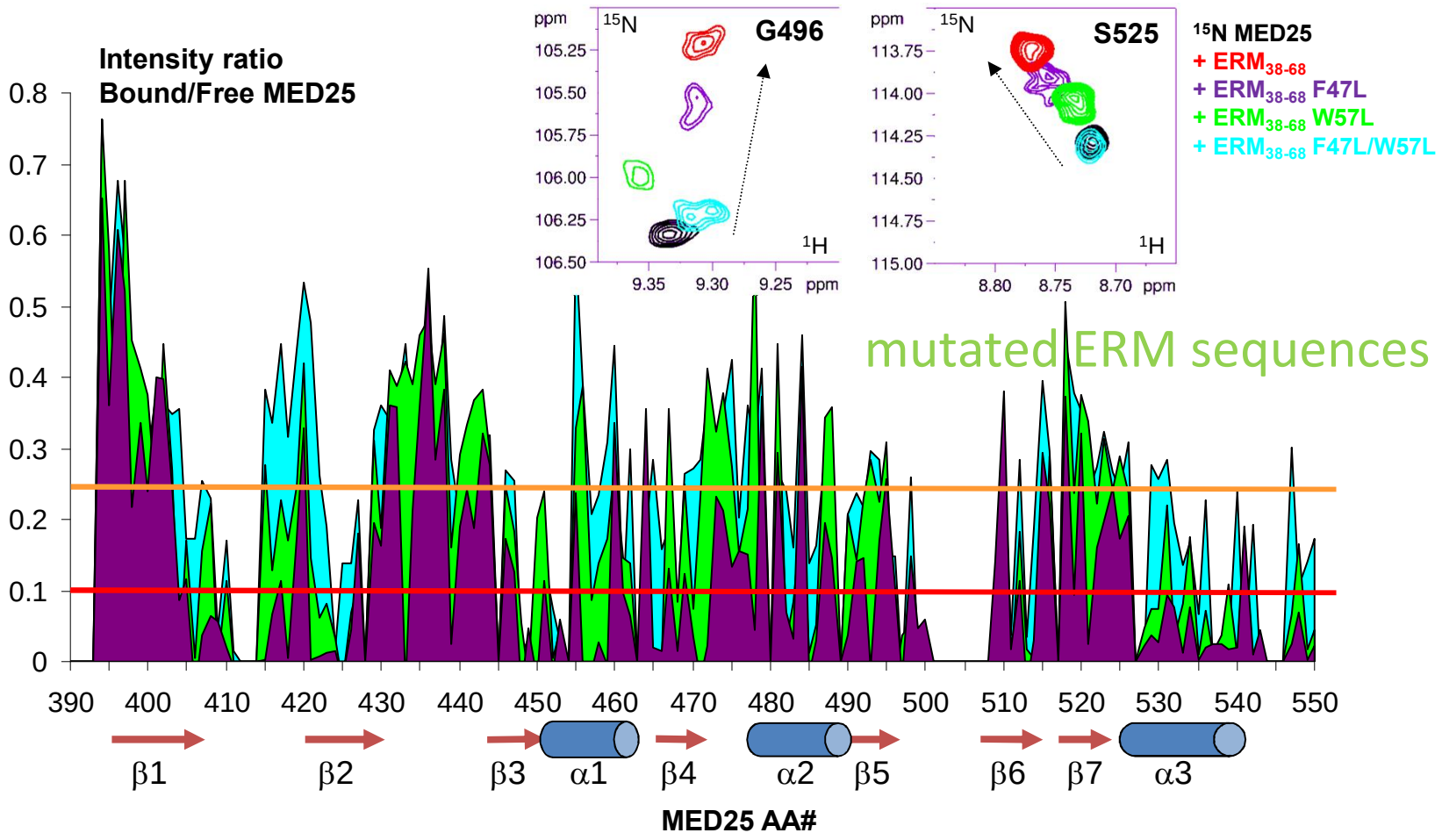
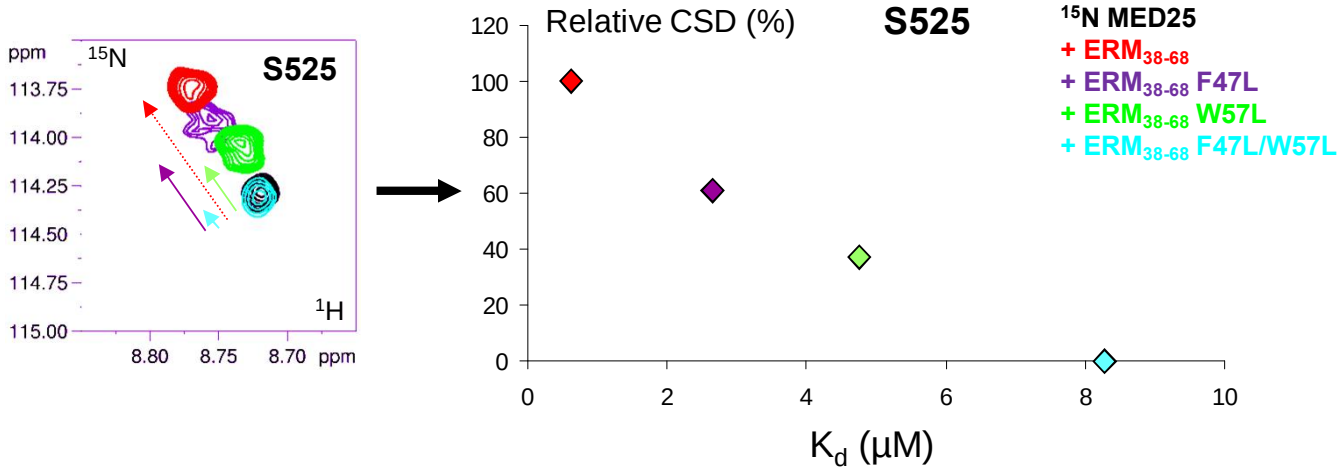
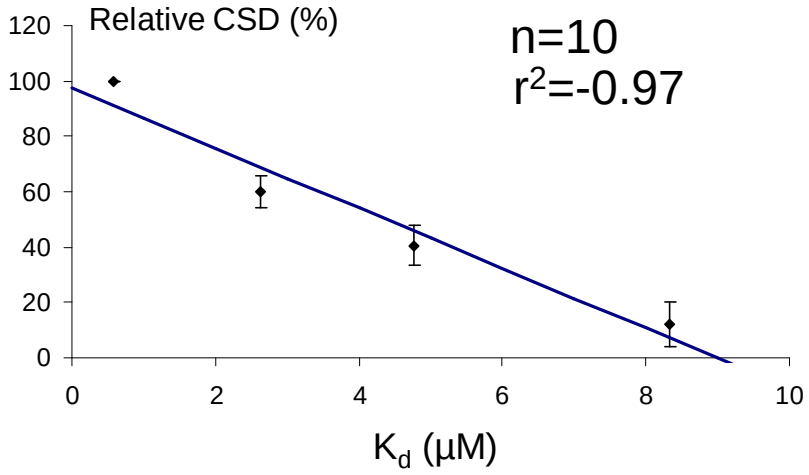


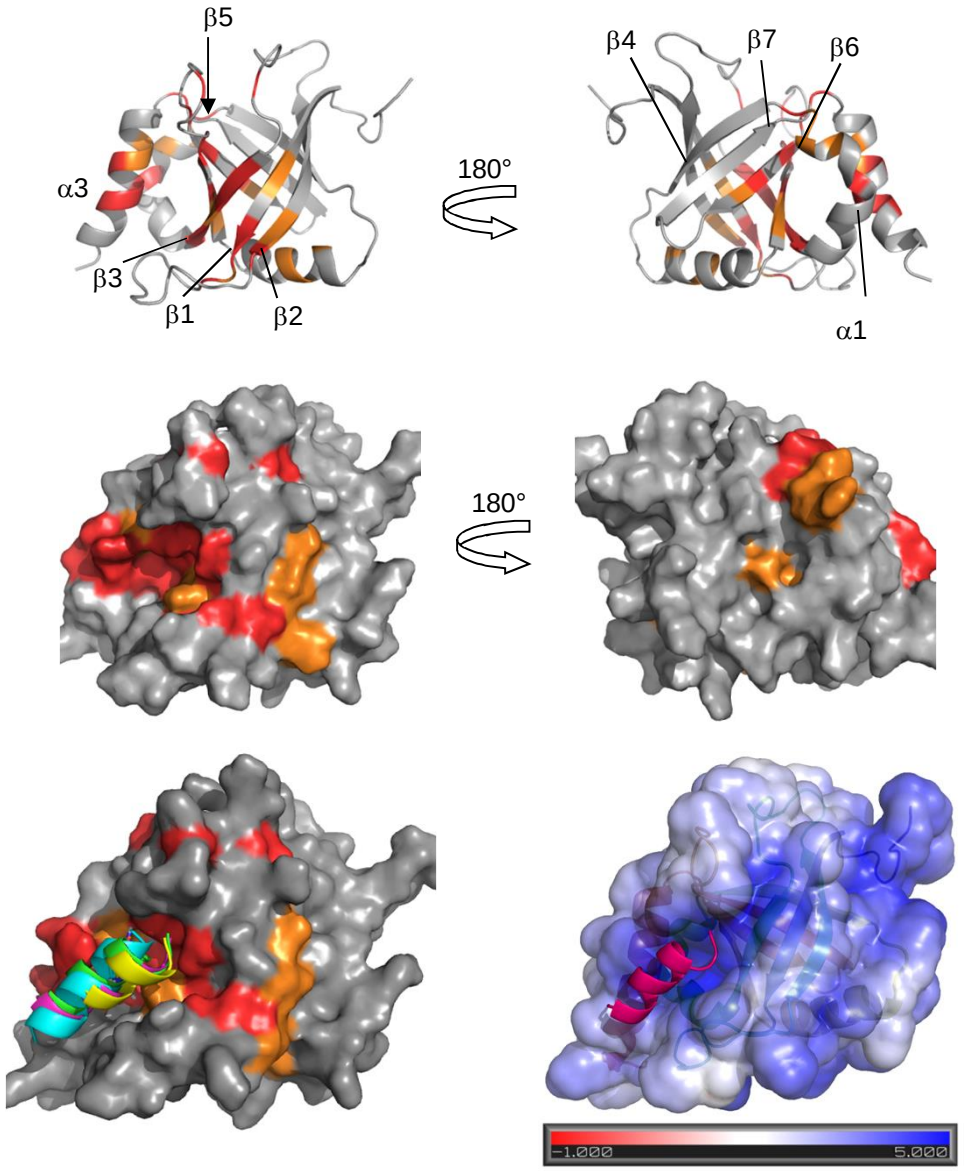
Figure 5

Exchange NMR: sampling bound/free conformations





Haddock model of the complex MED25 ACID/ERM TAD

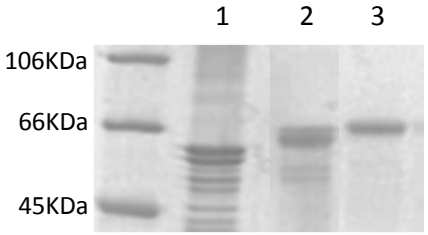


Post-translational modifications

Application

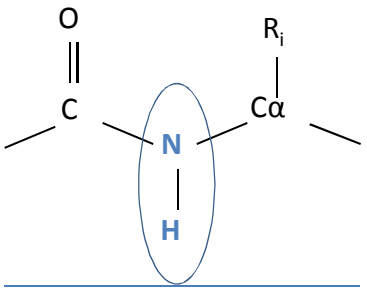
Identification strategies of the PTMs
Example of Tau phosphorylation
and acetylation

Phosphorylation

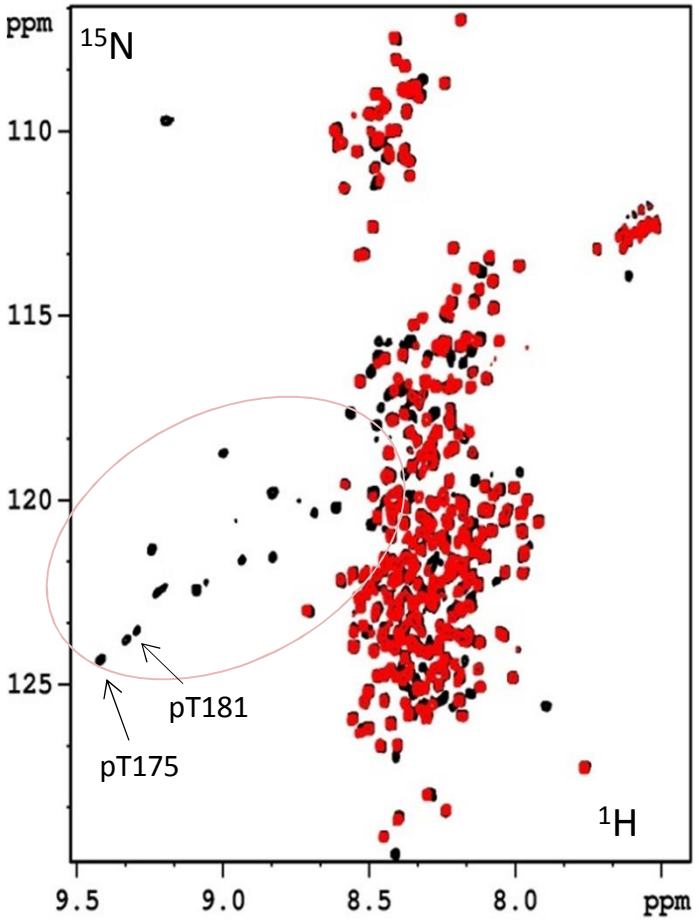


1. Tau control
2. pTau by ERK2
3. pTau by rat brain extract

Description of HSQC spectrum

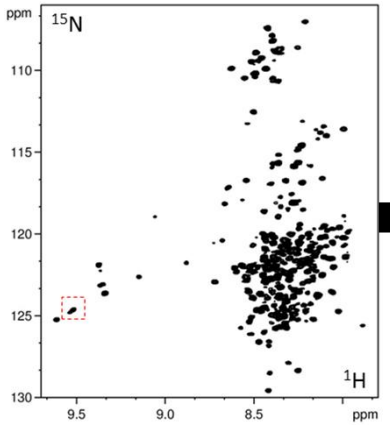


Residue i

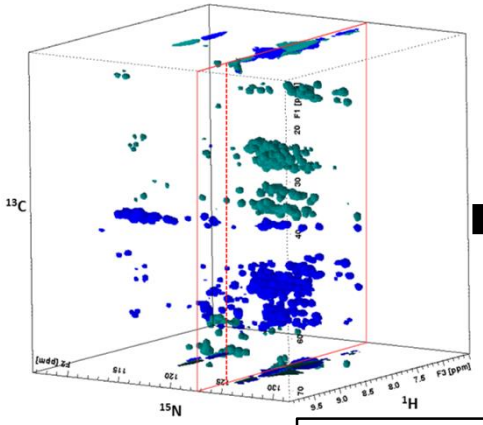


Phosphorylation: strategy of identification

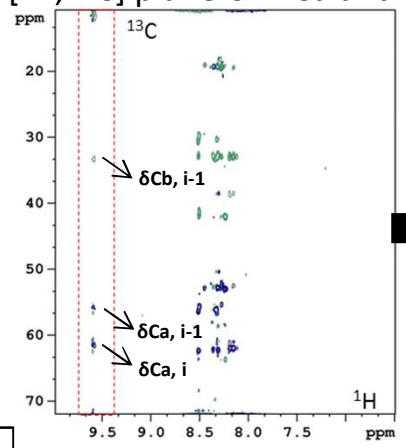
[H, ^{15}N] HSQC of Tau441



[H,^{15}N,^{13}C] HNCACB of ^{15}N,^{13}C Tau441



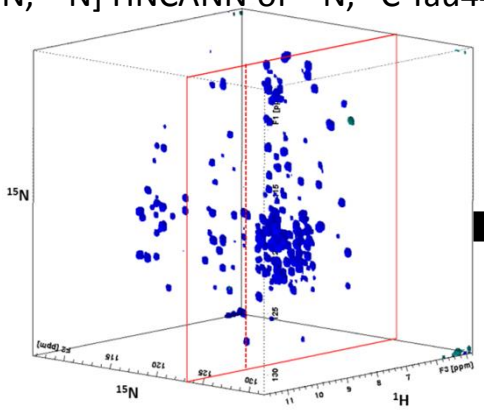
[^{1}H, ^{13}C] plane of ^{13}Ca and ^{13}Cb



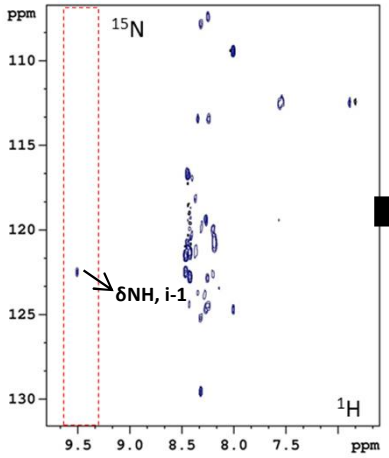
Identification of 'X-pS/pT-P' motif

In case of two identical 'X-pS/pT-P' motifs

[H, ^{15}N, ^{15}N] HNCANN of ^{15}N,^{13}C Tau441



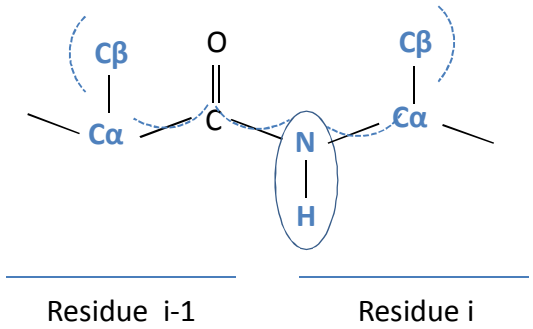
[H, ^{15}N] plane of ^{15}NH

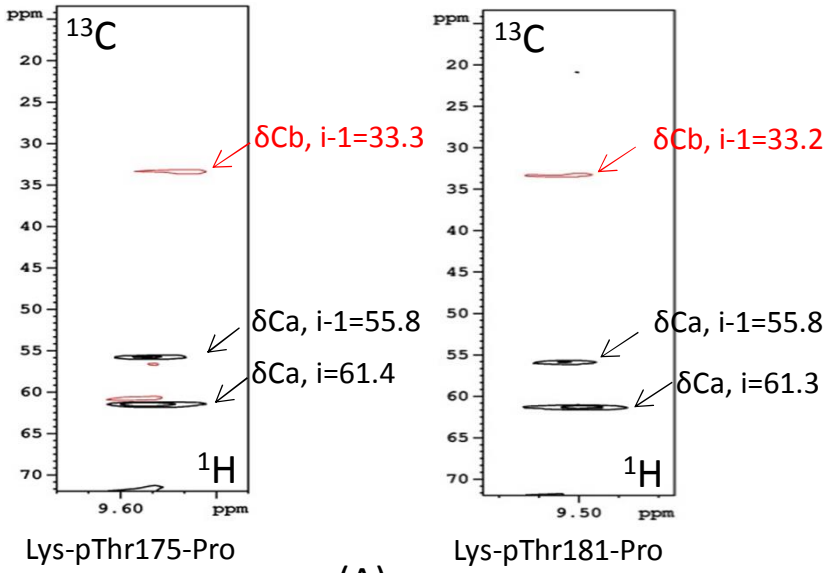


Identification of 'X-pS/pT-P' motif

Strategy of identification: K-pT175, K-pT181

Description of HNCACB spectrum





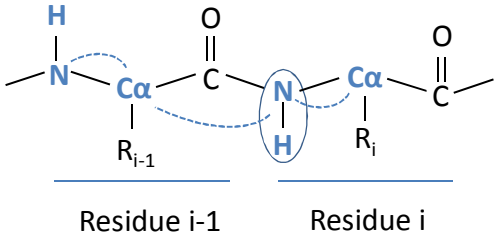
9 pT-P motifs of Tau

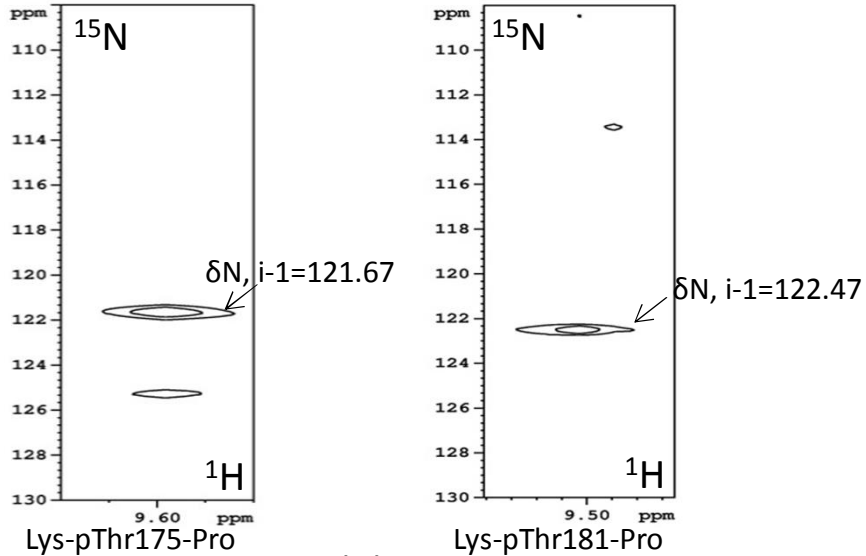
2 K-pT-P motifs of Tau

(A)

^{13}C-CB resonances outside the spectrum window (74 ppm)

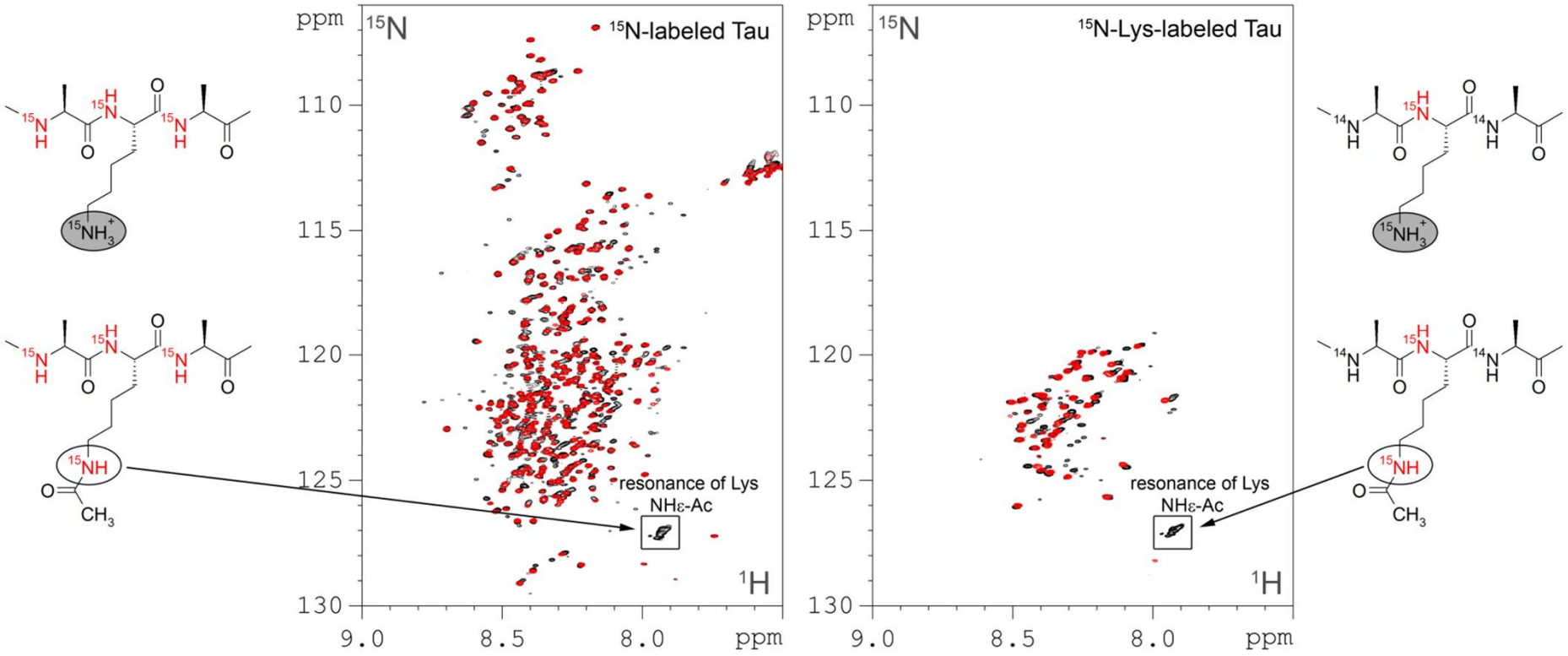
Description of HNCANN spectrum



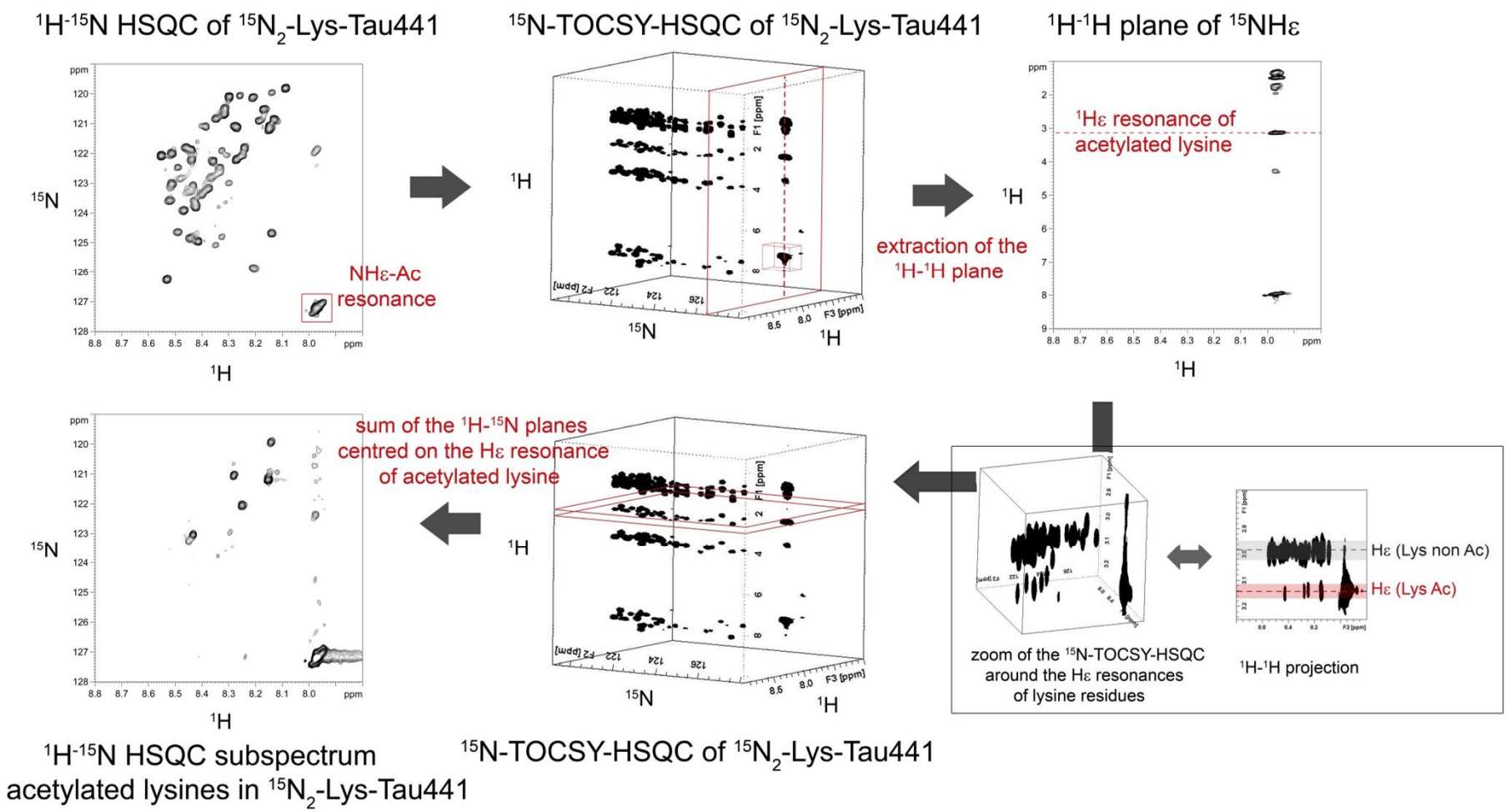


(B)

Acetylation

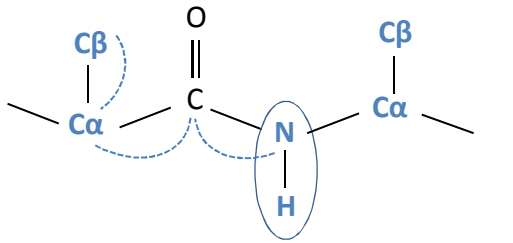


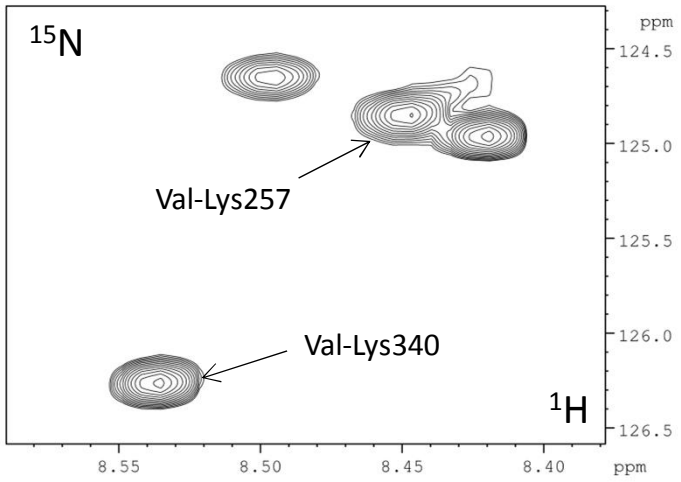
Acetylation: identification strategy



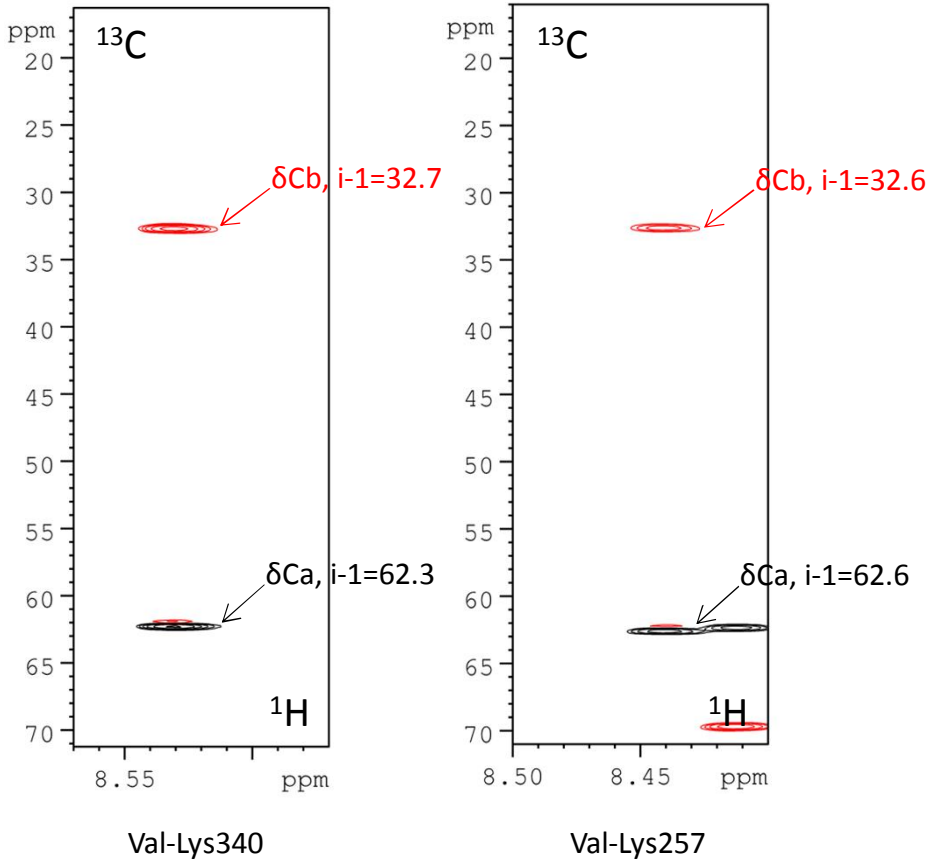
Acetylation: identification strategy

Description of HN(CO)CACB spectrum





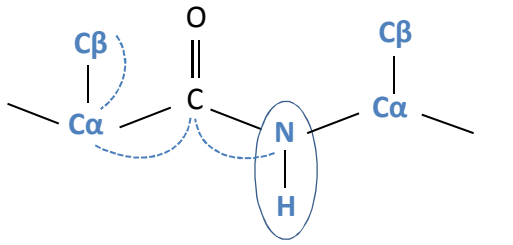
(A)



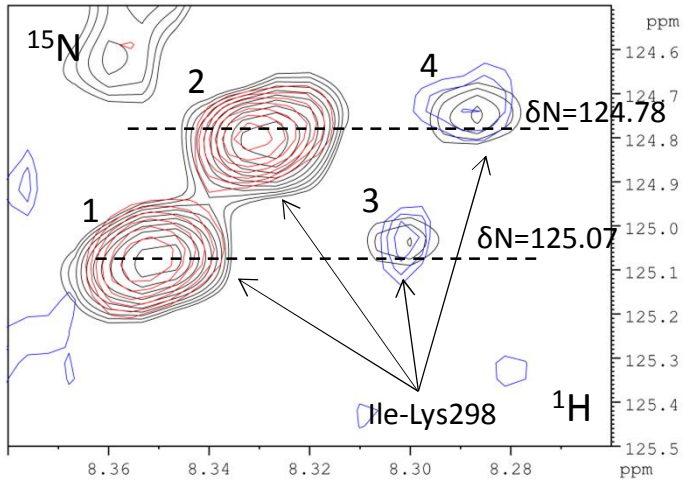
(B)

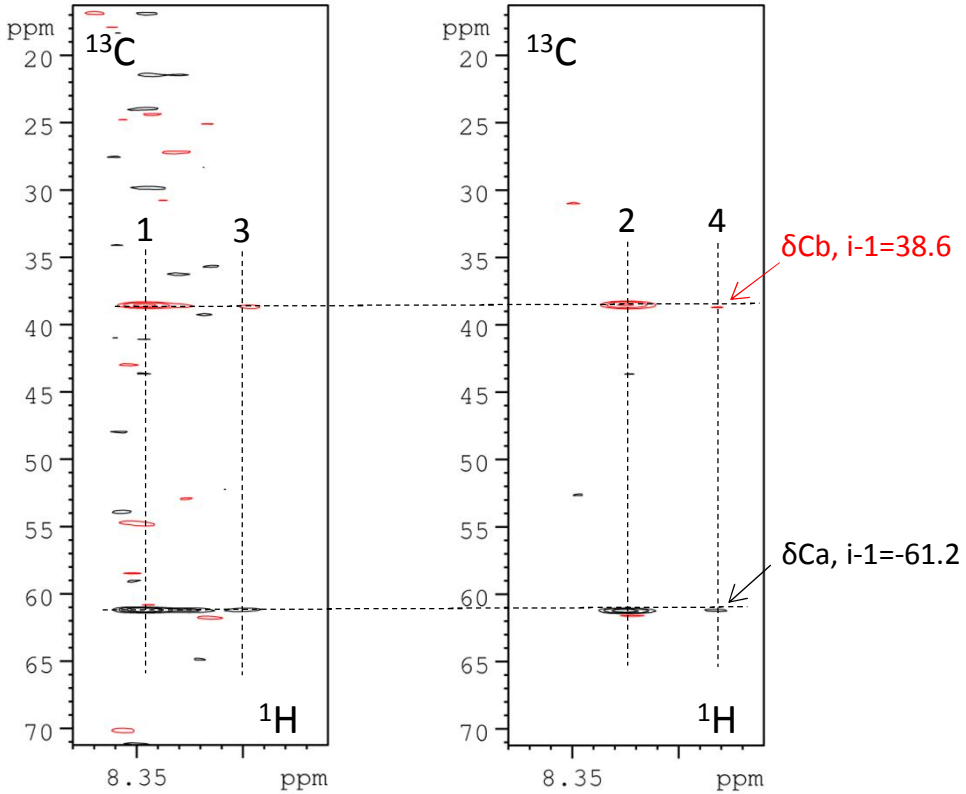
Acetylation: identification strategy

Description of HN(CO)CACB spectrum



Residue i-1 Residue i= lysine





Ile-Lys298
δN=125.07

Ile-Lys298
δN=124.78

1 Ile-Lys motif in Tau sequence

The splitting of each of non-acetylated and acetylated K298 resonance is due to the proximity of another acetylation site in the Tau sequence which has been identified as the ‘i-4’ residue

A few references:

Protocols

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Landrieu I, Verger A, Baert JL, Rucktooa P, Cantrelle FX, Dewitte F, Ferreira E, Lens Z, Villeret V, Monté D. Nucleic Acids Res. 2015 Aug 18;43(14):7110-21. doi: 10.1093/nar/gkv650.

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PPI/folding upon binding

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