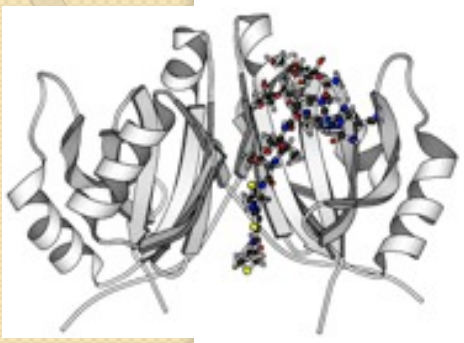
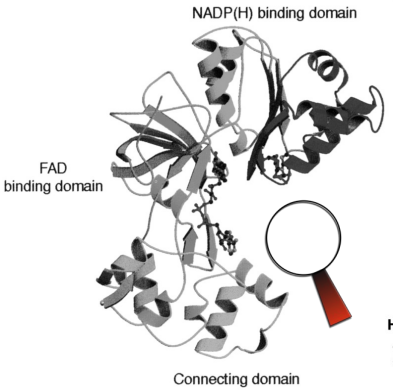
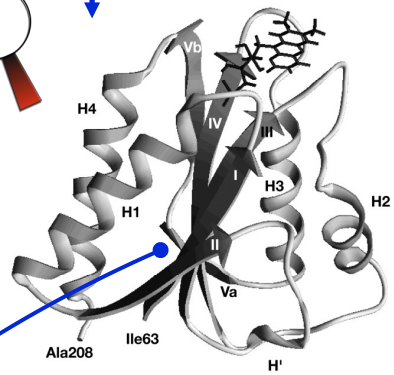
Background

1997 – 2000	2000 – 2003	2003 – 2005	2005 – 2006	2006 – 2011
Licence, maîtrise et DEA Physique	Doctorat de Physique (Université Joseph Fourier Grenoble I)	Post-doctorat (Université d'Utrecht, Pays-Bas)	Post-doctorat (Université de Lille1)	Chargée de Recherche 2ème classe section 16 DR18	Chargée de Recherche 1ére classe section 16 DR13



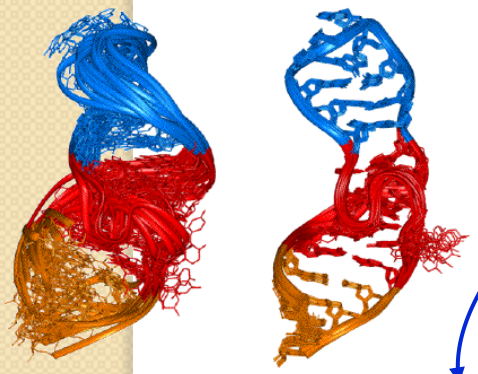


Backbone RDC

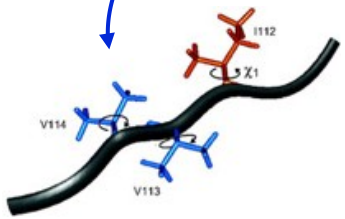


M1 (1999): Relaxation

IBS : B. Brutscher and C.Vanbelle



Side Chains RDC (methyles)



M2 (2000): RDC

IBS : M. Blackledge et JP. Simorre

Coll: A. Pardi, University of Colorado

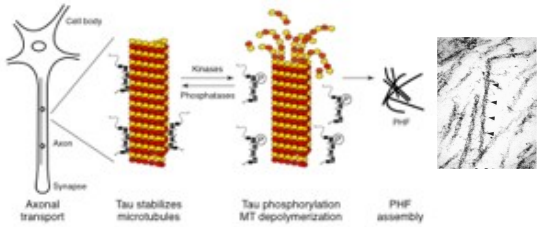
M.E.N.R.T (Sept. 2000 / Déc. 2003)

IBS: B. Bersch et B. Brutscher

Coll: Laboratoire de Chimie et biochimie des Centres Rédox-CEA-Grenoble: J. Covès

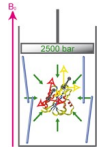
CR2 CNRS (2006): Neuronal Tau protein

UGSF : G. Lippens

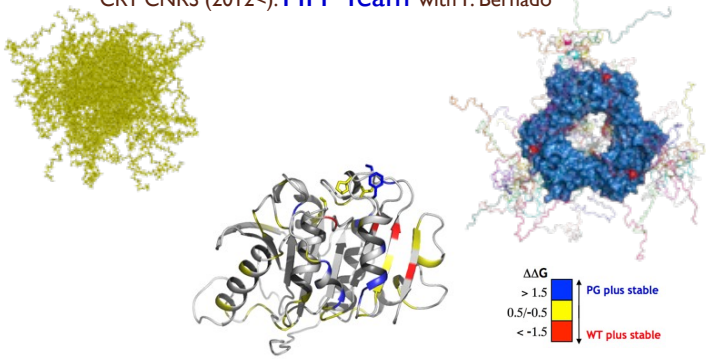


CR1 CNRS (2011): High Pressure and NMR

CBS : C. Royer and C. Roumestand



CR1 CNRS (2012<): HFP Team with P. Bernado



Post-Doc (Jan. 2004 / Août 2005)) : Folding

Utrecht Univ.: N. van Nuland , R. Boelens and A. Bonvin

Collaboration: Genencor International Inc., CA, USA: R. Bott

Contributions de la RMN à la biologie structurale : Approches multi-échelles spatiales et temporelles

CBS

Nathalie SIBILLE

CNRS UMR 5048, CBS

nathalie.sibille@cbs.cnrs.fr

cnrs

Inserm

UNIVERSITÉ DE MONTPELLIER

NMR - Spatial and Time scales are highly intermingled

- "Small" proteins can give very bad spectra
  - and difficult medium size proteins (conformationnal and chemical exchange) (Arf1/Arf6)

- "Large" proteins can give beautiful spectra
  - ^{2}H, ILV $^{13}CH_3$ labeling …
  - Evolution of spectrometers, …
  - Depends on the question … complete structural analysis? HSQC ^{15}N, ^{13}C … 10uM
  - "easy" large proteins (CPR, Proteasome) – IDPs

- Looking at small systems bound to very large systems
  - Small ligands : using the free forms to investigate the bound states (GPCR, drugs, …)
  - Disordered regions remain observable
  - Specific NMR experiments to analyze interactions implying large systems (DEST/CEST)

- Investigating folding/recognition processes
  - Characterize intermediate folding states -> protein physico chemistry, sequence/stability/ dynamics/structure
  - Disorder->order transitions

- Investigating excited states …

  Kay, Wright, Kalodimos

NMR - Spatial and Time scales are highly intermingled

Size vs dynamics ?

Size ⟺ molecular tumbling + local dynamics

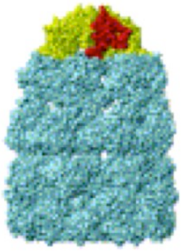
⇕

relaxation processes

⇕

linewidths & S/N

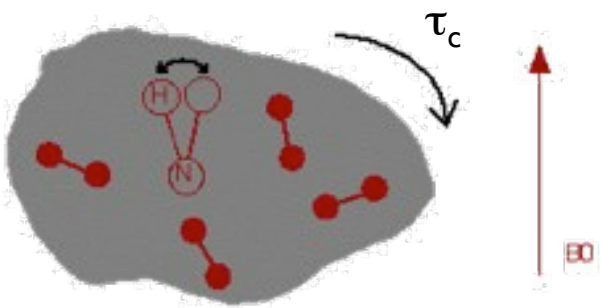
solubilized membrane proteins



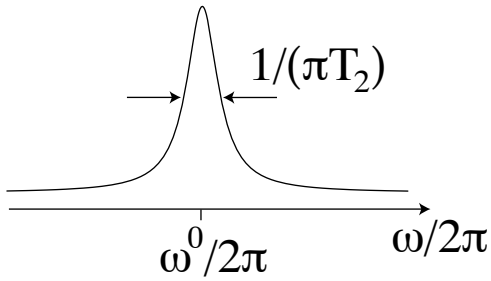
"large" size globular proteins or complexes

"Small" globular Protein / domain

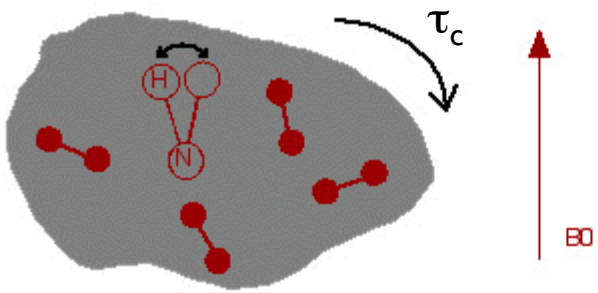
Global correlation time τ_c (size)



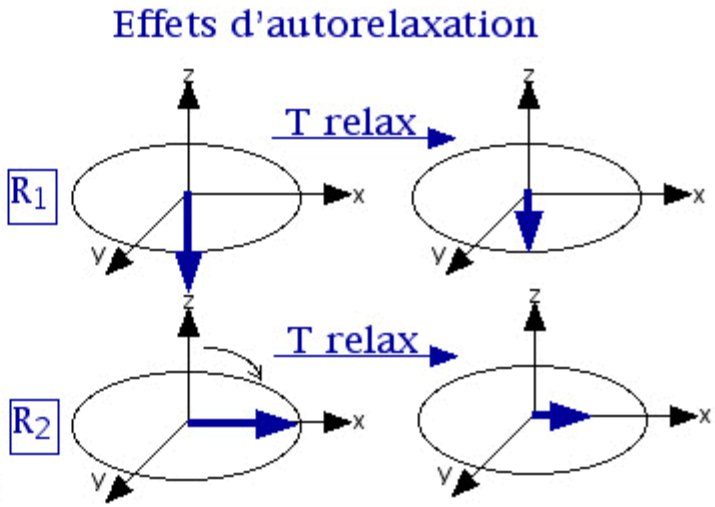
Linewidth



Relaxation times T1 and T2



Modulation temporelle de:
(1) l'interaction dipolaire ^{15}N-^{1}H
(2) l'anisotropie du déplacement chimique.

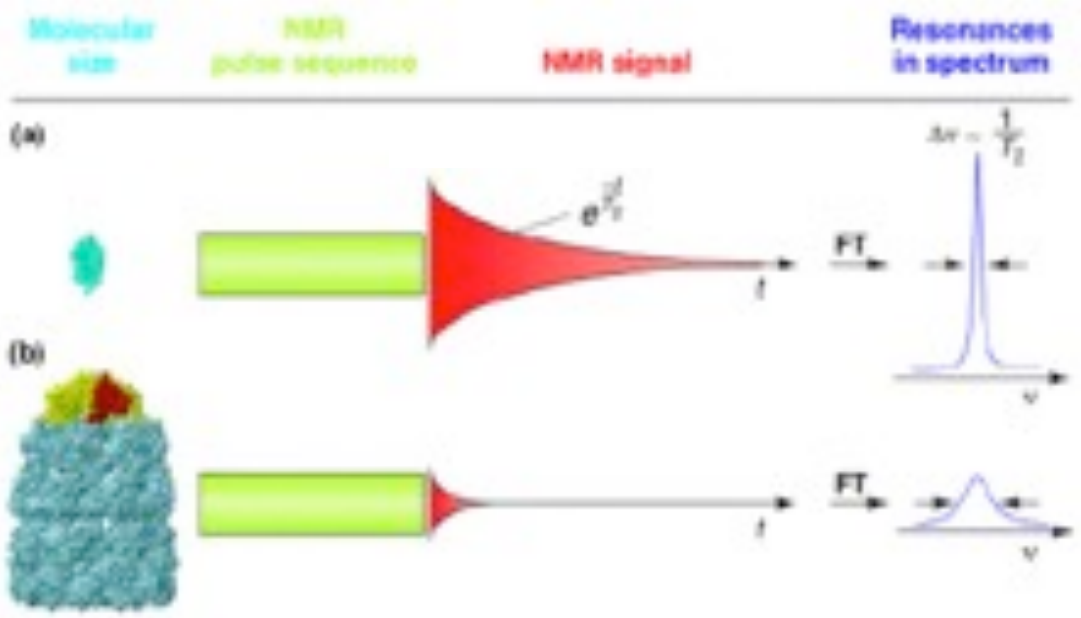


- Longitudinal relaxation time constant T1 characterizes the time it takes to the spin system to return to equilibrium.
- It determines the interscan delay
- T1 depends on the magnetic field strength B0, the type of spin, **the state of neighboring spins**, the size of the molecule, the local dynamics of the system, the temperature, etc.

- Transversal relaxation time constant T2 characterizes the lifetime of the signal.
- It determines the linewidth of the resonances
- T2 depends on the magnetic field strength B0, the type of spin, the environment of the spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

NMR - Spatial and Time scales are highly intermingled

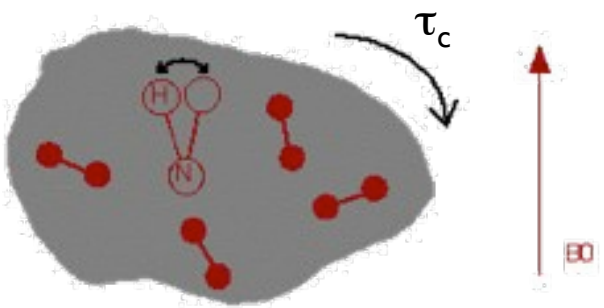
"Small" globular Protein / domain



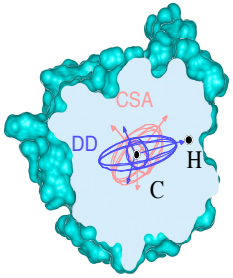
"large" size globular proteins or complexes or solubilized membrane proteins

Global correlation time τ_c (size)

Linewidth

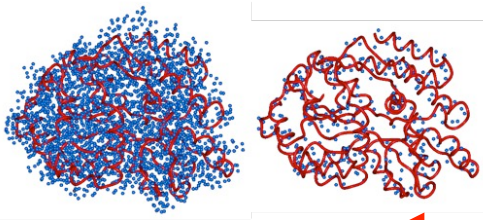


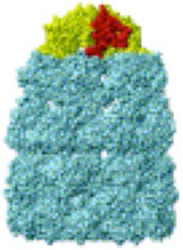
NMR - Spatial and Time scales are highly intermingled



solubilized membrane proteins

TROSY, high fields





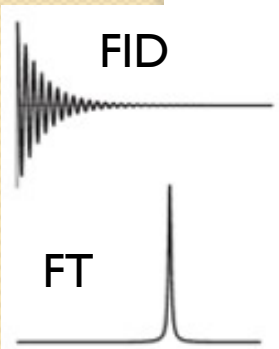
"large" size globular proteins or complexes

"Small" globular Protein / domain

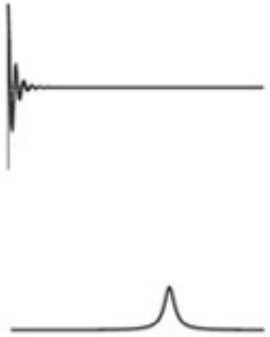
Labelling, Deuteration

Global correlation time τ_c (size)

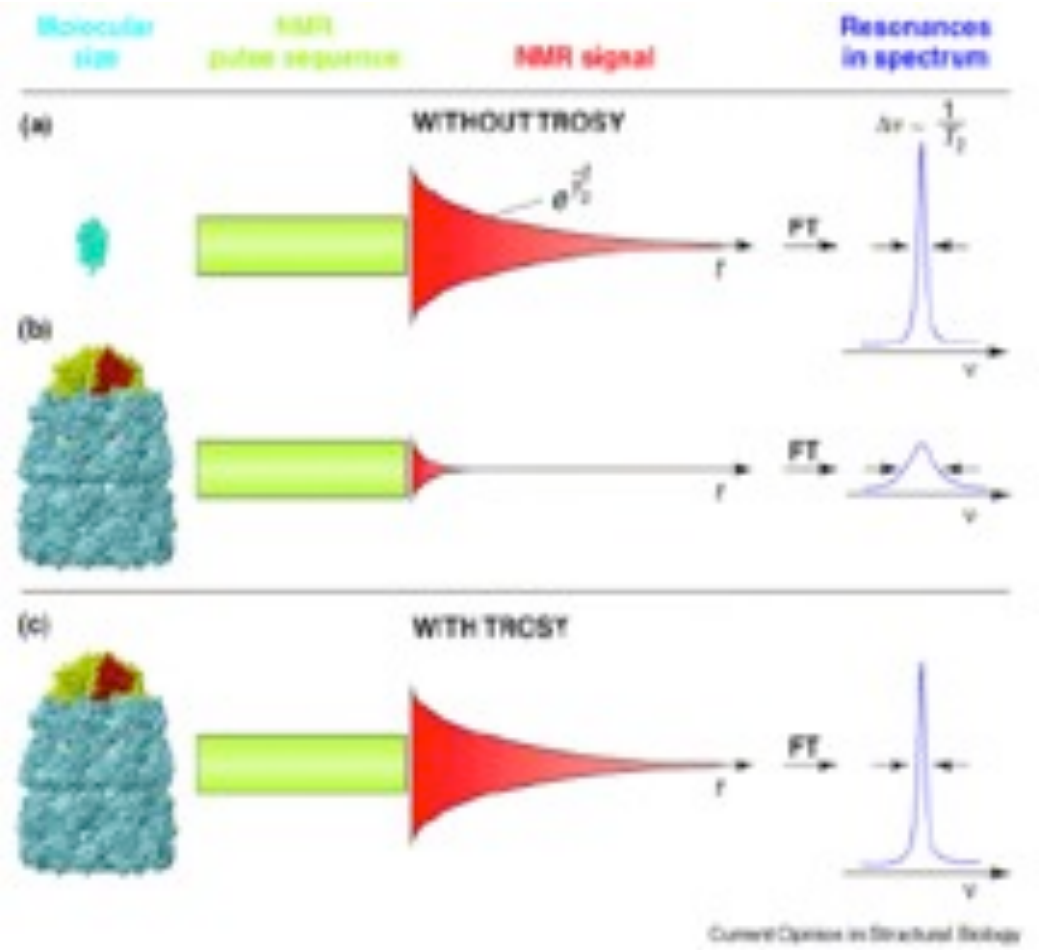
+ Local Rotational fluctuation timescale
+ density of relaxation sources

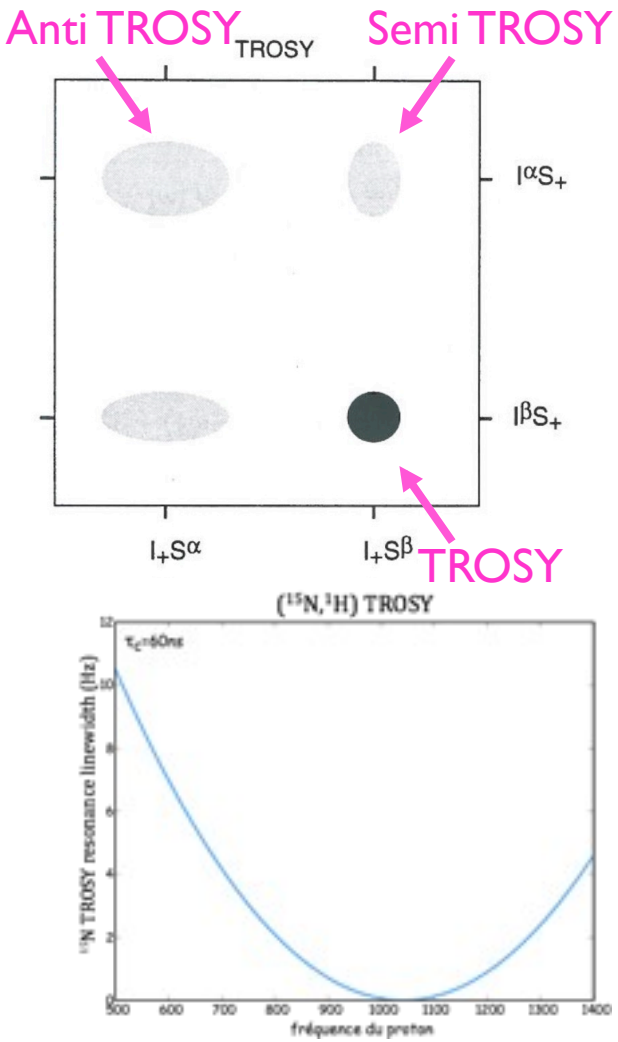


Linewidth



« Size » and lifetime of the signal T2

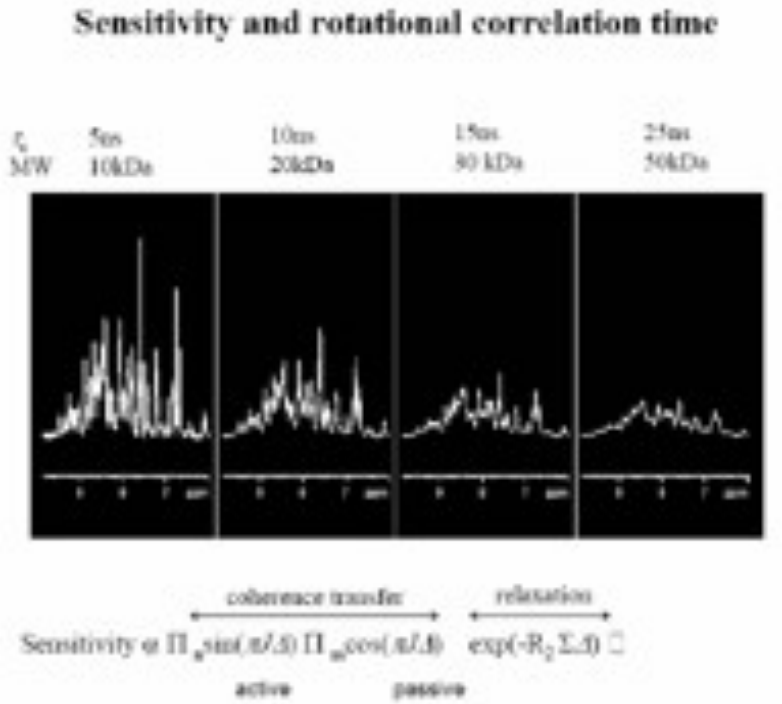


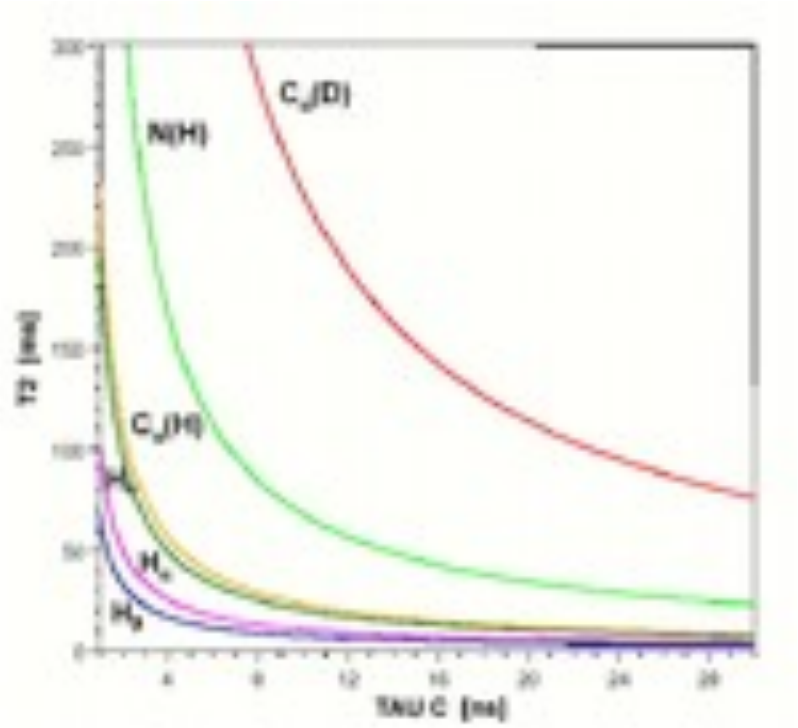


Playing with T2 : TROSY experiments
Transverse Relaxation Optimized SpectroscopY

Pervushin, K. Riek, R., Wider, G. and Wütrich, K(**1997**) Attenuated T2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. Proc. Natl. Acad. Sci. U. S. A. 94, 12366–12371

« Size » and lifetime of the signal T2





- **MW < 5 kDa**

Homonuclear assignment (TOCSY,NOESY)

- **5 kDa < MW < 10 kDa**

Heteronuclear assignment: ^{15}N

- **10 kDa < MW < 20 kDa**

Heteronuclear triple resonance assignment : ^{15}N, ^{13}C

- **20 kDa < MW**

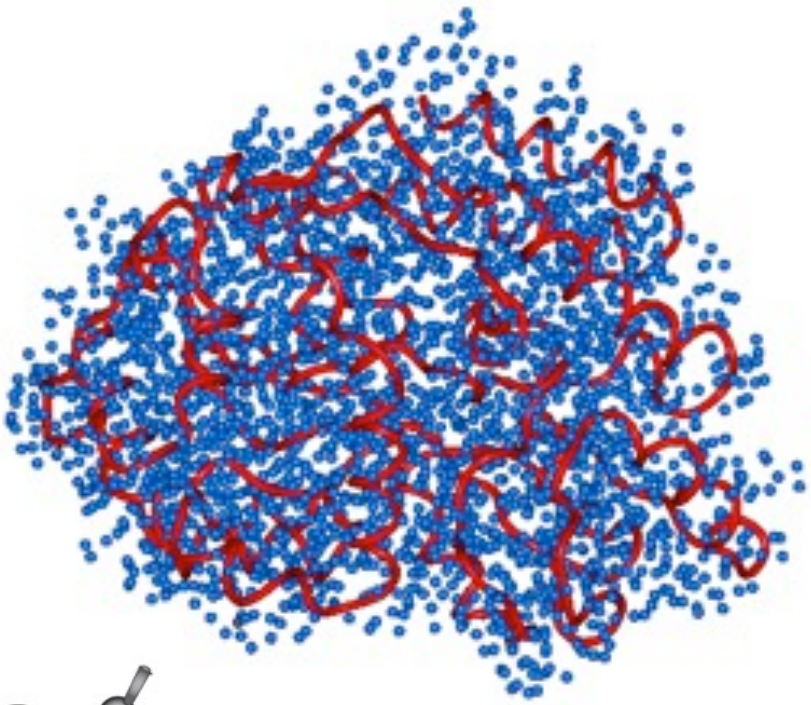
Heteronuclear triple resonance assignment : ^{15}N, ^{13}C, ^{2}H (*E.coli* BL21+++)

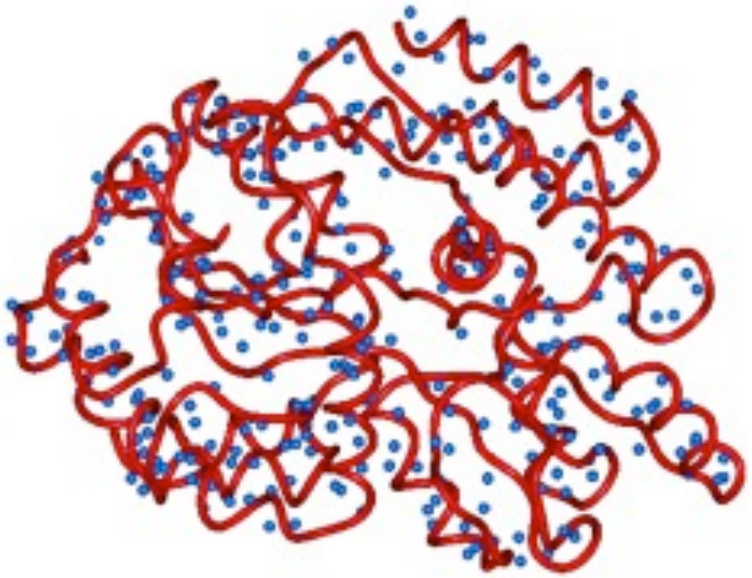
The signal loss is exponential while the size of the molecule increases

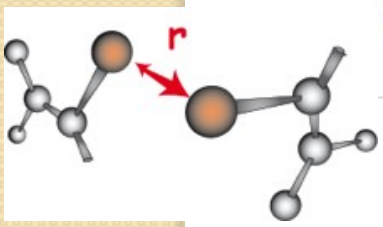
γ_H/γ_D = 6.5…. Relaxation gain !!!!

New restraints in NMR for « large » proteins

- Fully protonated protein
- Deuterated protein (only $^{1}H_{N}$)

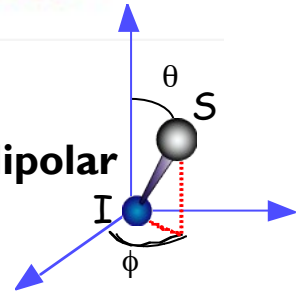


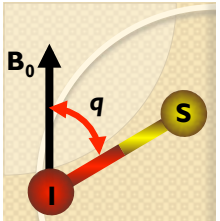




- Classical method : NOE ^{1}H-^{1}H
- Ambigous NOEs restraints

- New approach : residual dipolar couplings (RDC)
- RDC non ambigous

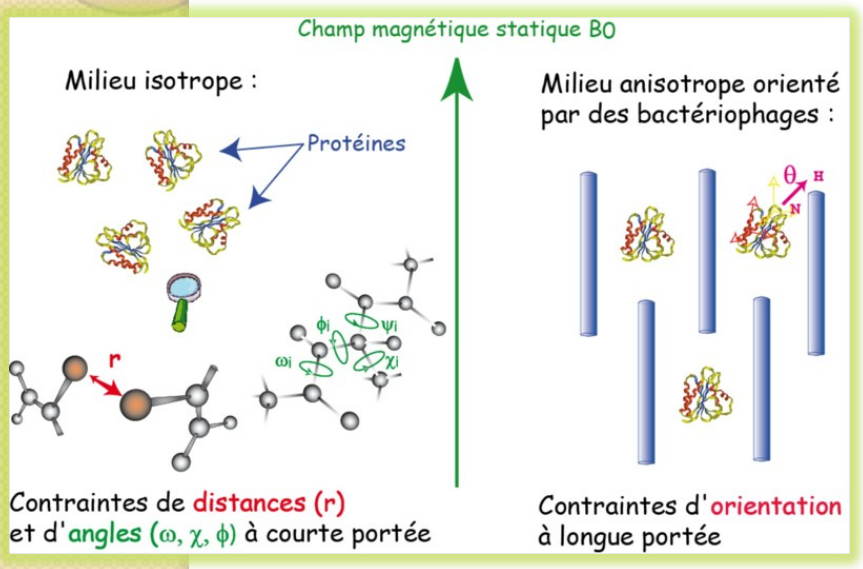




RDCs by NMR

Orientational information in an anisotropic medium

Liquid crystal Media



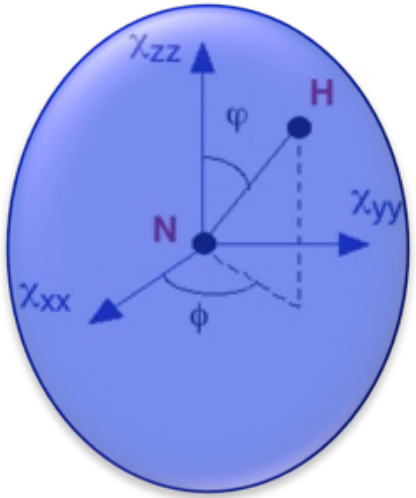
- **Isotropic medium :**
  scalar coupling = J
- **Anisotropic medium :**
  apparent splitting of the doublet = $J + D_{IS}$

=> Dipolar Interactions non averaged to zero

Geometrical dependency of RDCs :

$$D_{IS} = -S\frac{\mu_0\hbar}{8\pi^2}\frac{\gamma_I\gamma_S}{\left\langle r_{IS}^3\right\rangle}\left\{A_a\left(3\cos^2\theta - 1\right) + \frac{3}{2}A_r\sin^2\theta\cos 2\phi\right\}$$

Alignment tensor



Restricted reorientation

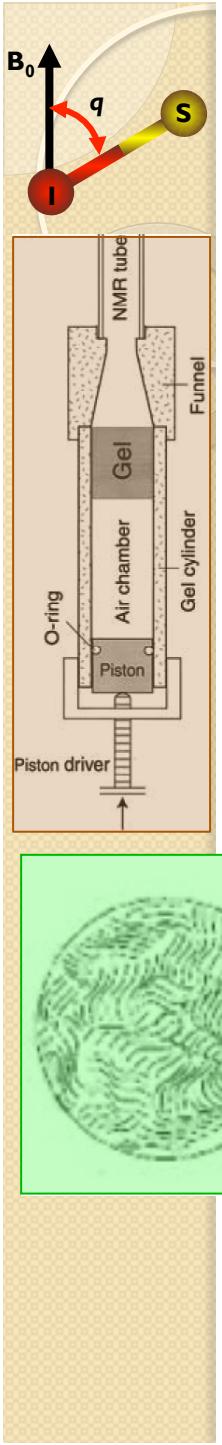
Steric Interactions:

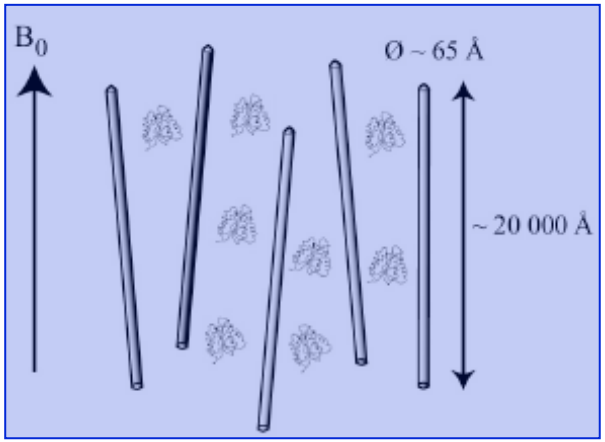
Alcohol mixture, gels, cellulose crystallites

Electrostatic Interactions:

Phage (Pf1), purple membranes, bicelles

Alignment Media



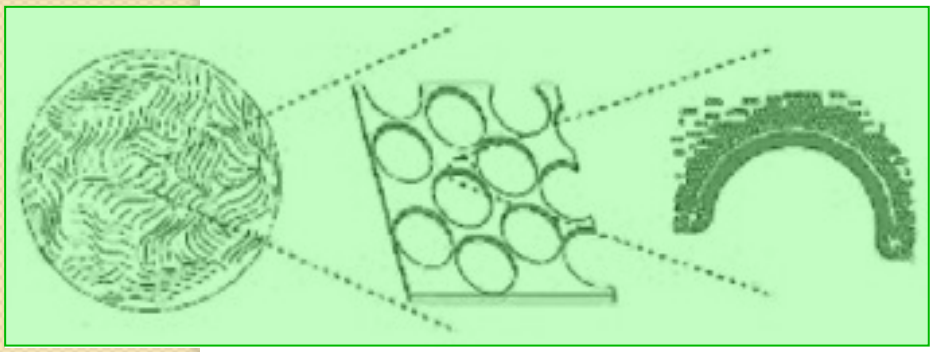


➡ Electrostatic Interactions:

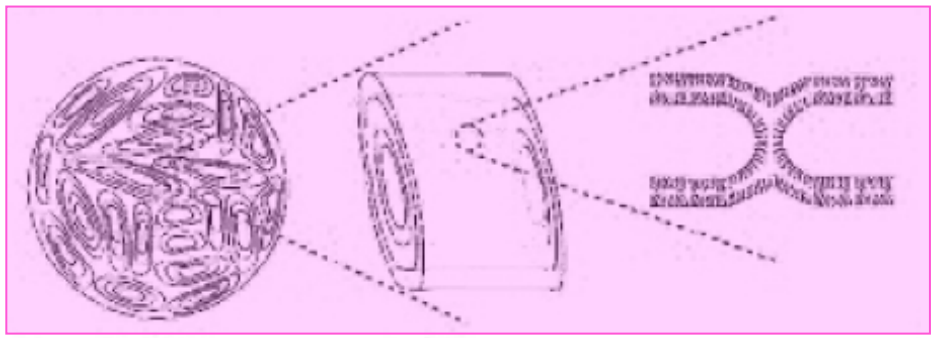
- ✧ **Filamentous phage Pf1** or other rodlike viruses (fd, TMV)
- ✧ DNA nanotubes, crystalline phase G-tetrad DNA
- ✧ **Bicelles** consisting of various charged lipids (addition of CHAPSO, CTAB)

➡ Steric Interactions :

- ✧ **Bicelles** with uncharged lipids (DMPC/DHPC)
- ✧ Compressed or **stretched polyacrylamid Gels**
- ✧ **Lamellar phases** consisting of ether/alcohol mixtures ("Otting media")

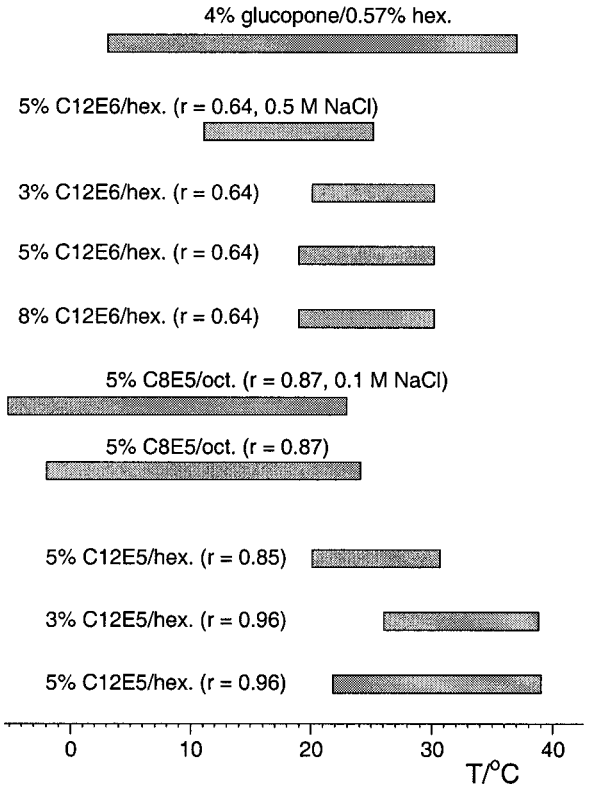


In addition alignment can also be achieved without medium by intrinsic or artificially coupled **paramagnetic groups**

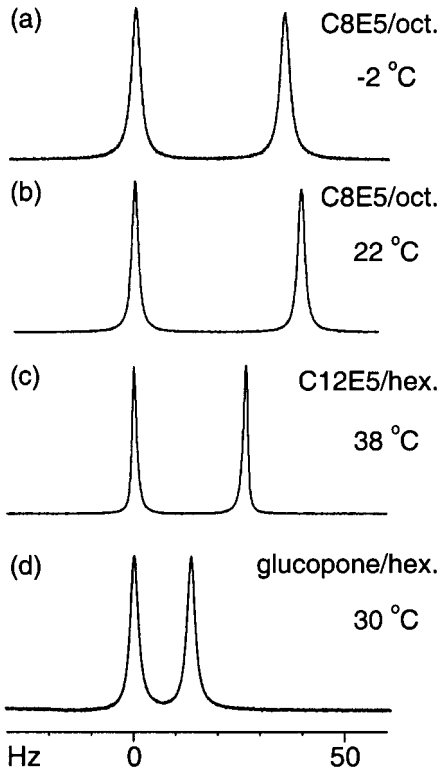


Alignment Degree

Temperature range of Alcohol mixtures



Quadrupolar Splitting

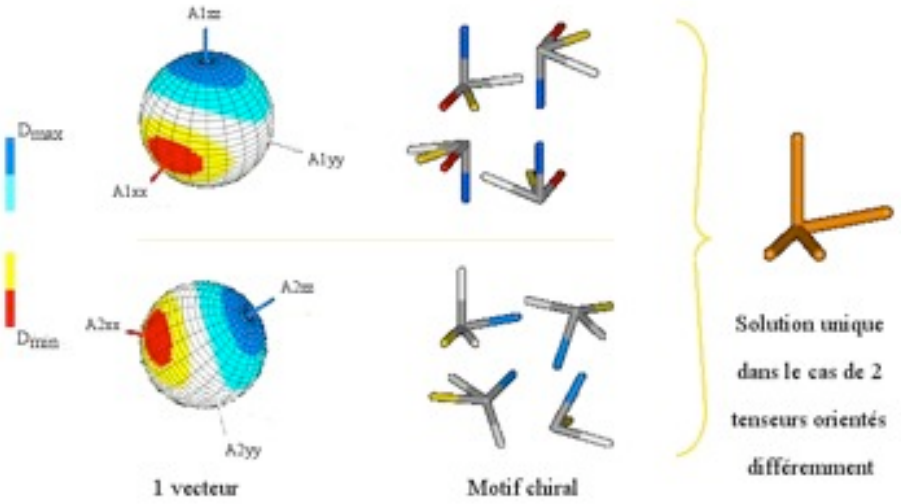


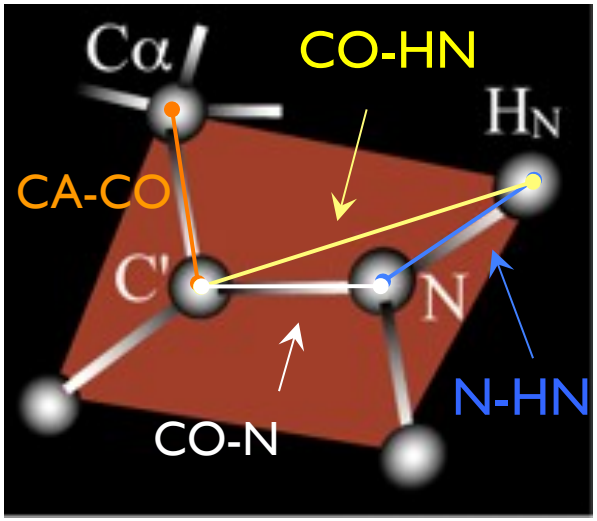
- Quadrupolar splitting (ΔD) of the 5% of D_2O used to lock the spectrometer
- ΔD is proportional to the concentration of the alignment medium and the degree of alignment of the molecule (around 10^{-3}-10^{-4})

Backbone couplings

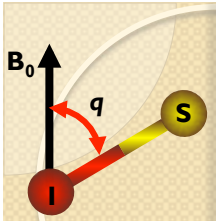
HNCO-type experiments

$^1D_{NH}$, $^2D_{COH}$, $^1D_{COCA}$, $^1D_{CACB}$, $^1D_{CON}$ et $^2D_{CAHN}$



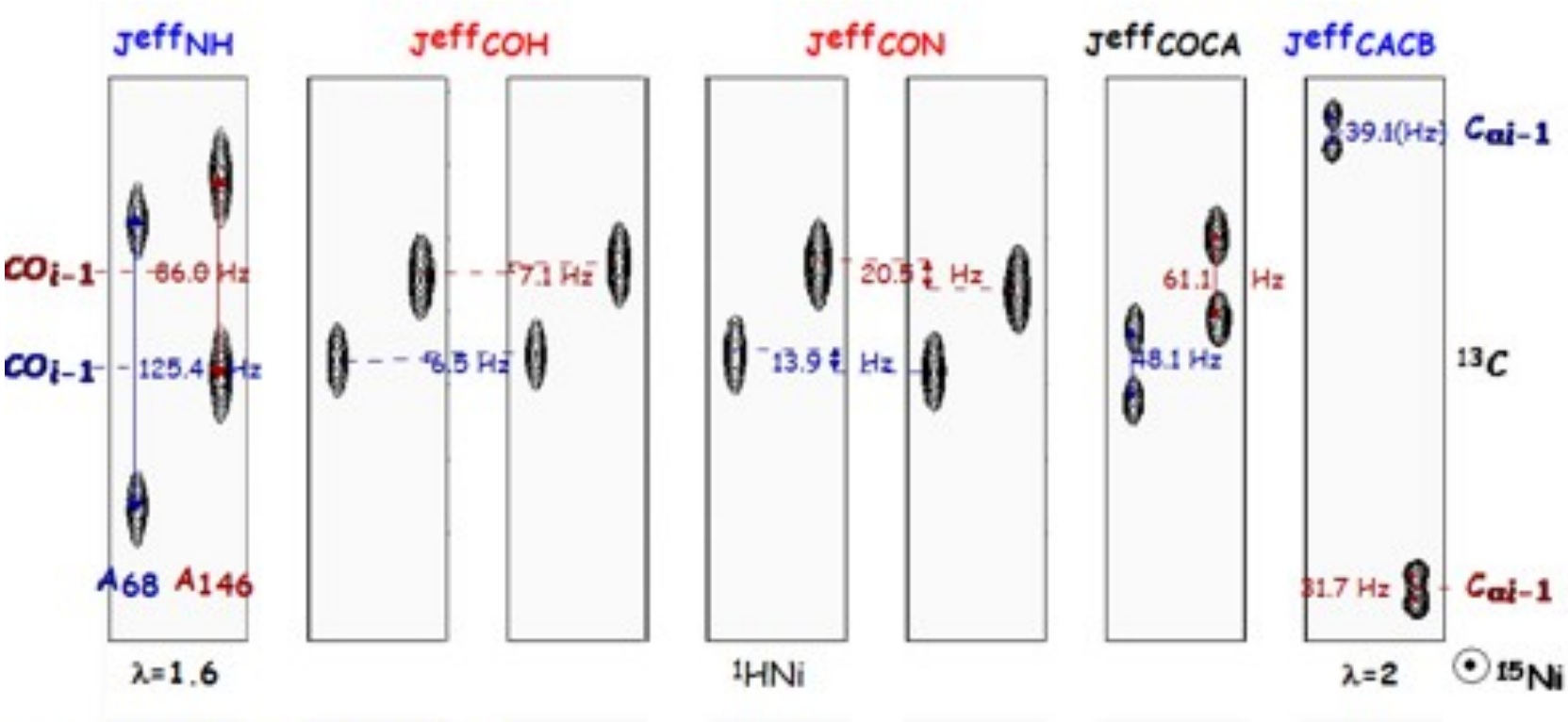


RDC mesuré	Distance internucléaire effective (Å)	Facteur de normalisation par rapport à NH
$^1D_{NH}$	1.04	1
$^2D_{COHN}$	2.05	0.300
$^1D_{C_\alpha CO}$ et $^1D_{C_\alpha C_\beta}$	1.53	0.198
$^1D_{CON}$	1.33	0.120
$^1D_{C_\alpha H_\alpha}$	1.12	2.08
$^1D_{CH3}{}^1$	1.11	0.628



RDCs Measurement

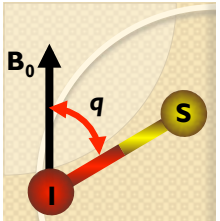
3D HNCO type experiments



Spectroscopie Accordéon

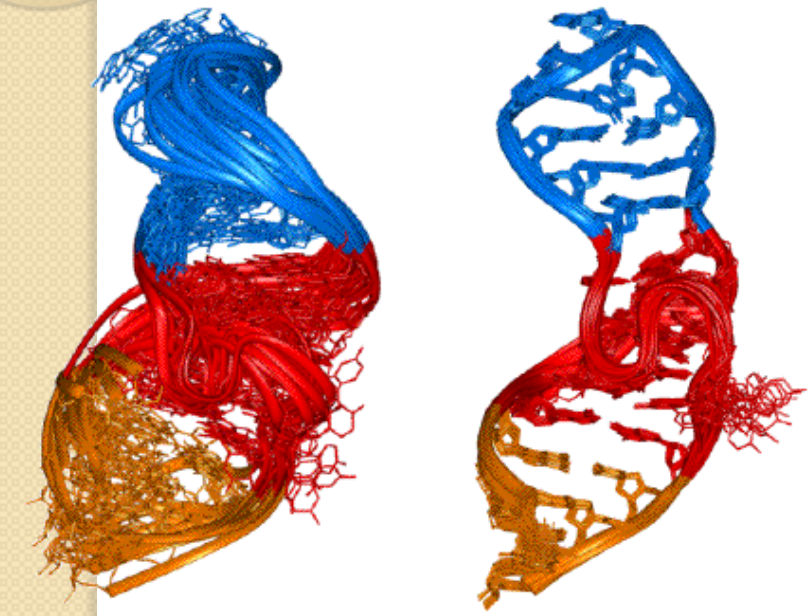
Séparation des deux pics du doublets dans deux spectres en utilisant un filtre DIPSA P Brutscher (2001) *JMR*, **151**, 332.

$$RDC = (J+D)_{anisotrope} - J_{isotrope}$$



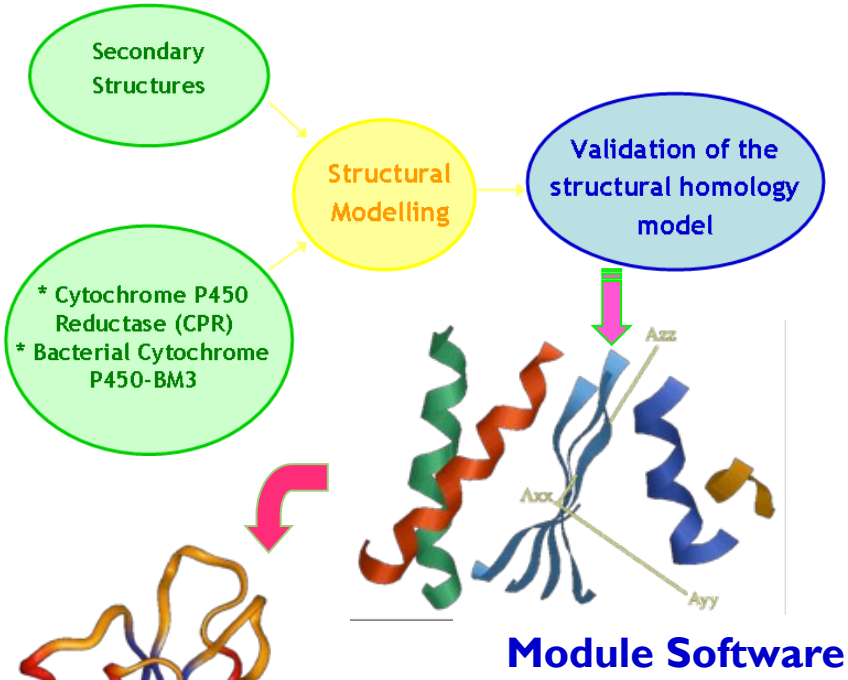
Applications RDCs on Folded Biomolecules

Theophylline-Binding RNA
^{13}C-^{1}H RDCs

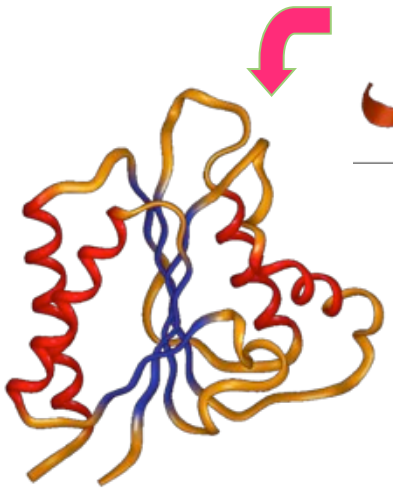


N. Sibille *et al.* J Am Chem Soc 123: 12135-46, 2001

Sulfite Reductase FMN domain
Ca-Co,N-Hn, and Co-HN RDCs



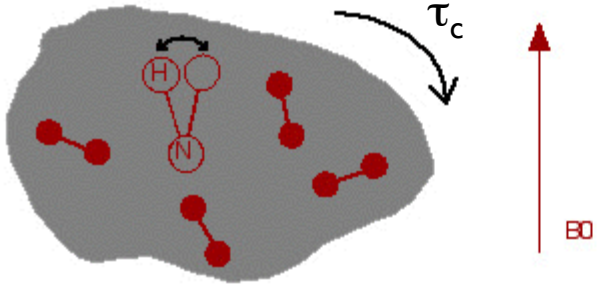
Module Software



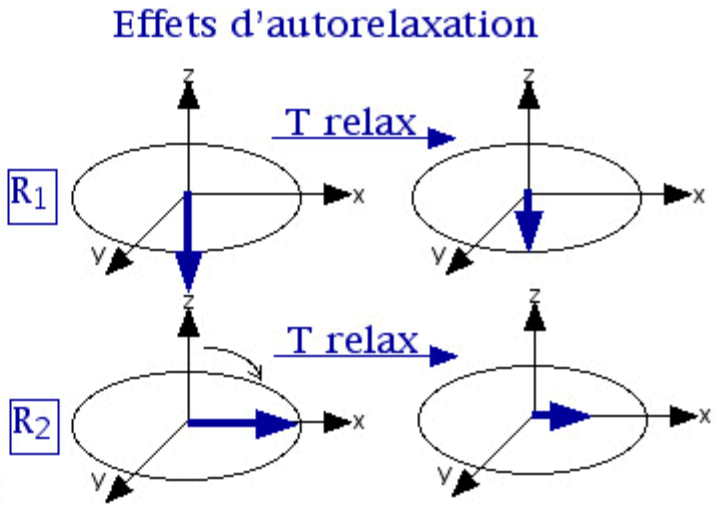
The structural **homology model** was **refined** using the **restrained molecular dynamics program** SCULPTOR

N. Sibille *et al.* Biochemistry, 2002
N. Sibille *et al.* Biochemistry 44:9086-95, 2005

Relaxation times T1 and T2



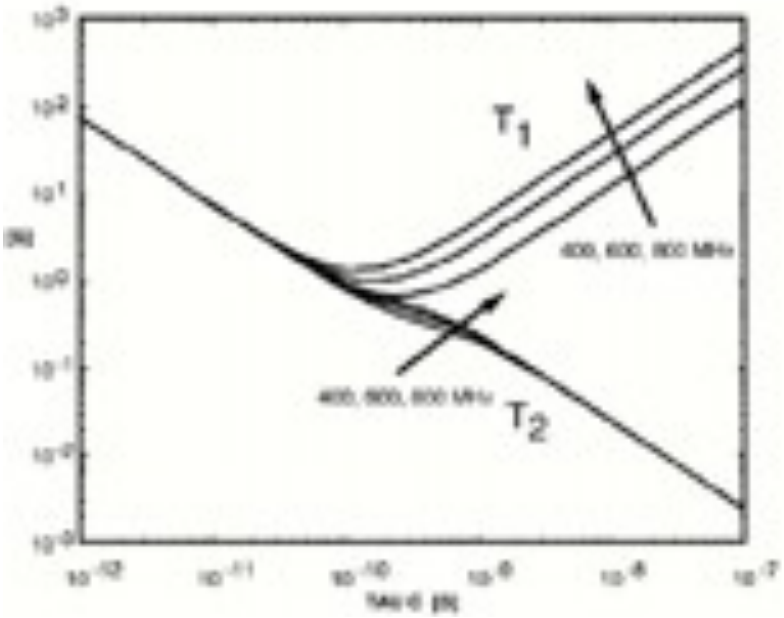
Modulation temporelle de:
(1) l'interaction dipolaire ^{15}N-^{1}H
(2) l'anisotropie du déplacement chimique.



- Longitudinal relaxation time constant T1 characterizes the time it takes to the spin system to return to equilibrium.
- It determines the interscan delay
- T1 depends on the magnetic field strength B0, the type of spin, **the state of neighboring spins**, the size of the molecule, the local dynamics of the system, the temperature, etc.

- Transversal relaxation time constant T2 characterizes the lifetime of the signal.
- It determines the linewidth of the resonances
- T2 depends on the magnetic field strength B0, the type of spin, the environment of the spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

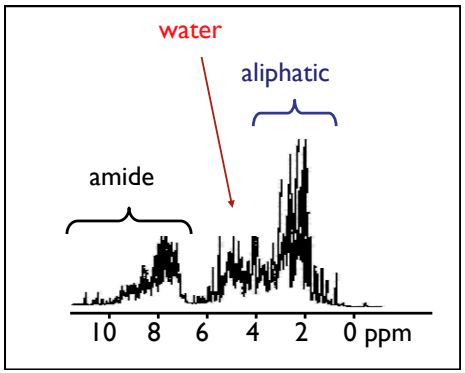
« Size » and lifetime of the signal T1

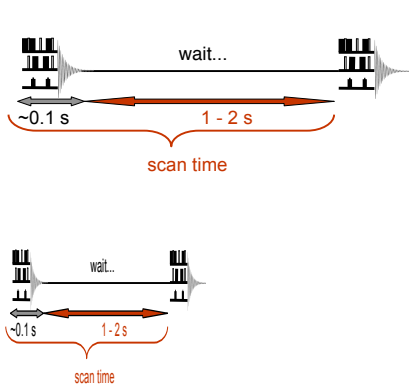


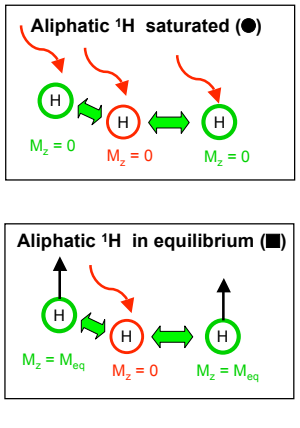
VeryFast : Schanda et al, J. Am. Chem. Soc. (2005) 127, 8014
SOFAST: Schanda et al, J. Biomol. NMR (2005) 33, 199
Hadamar: Schanda et al, J. Magn. Reson. (2006) 178, 334
Het-SoFast: Schanda et al, Magn. Reson. Chem. (2006) 44, 177
Speeding up 3Ds: Schanda et al, J. Am. Chem. Soc. (2006) 128, 9042

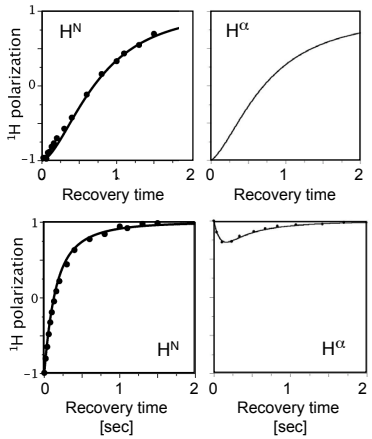
Playing with T1 : BEST experiments (Lescop et al, J. Magn. Reson. (2007) 187, 163)

BEST-type experiments Band-Selective**E**xcitation**S**hort-**T**ransient Experiments



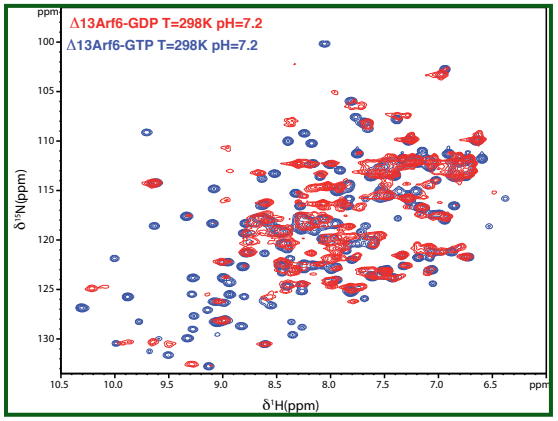




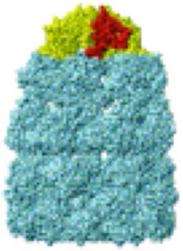


Figures from Brutscher

NMR - Spatial and Time scales are highly intermingled



solubilized membrane proteins



"large" size globular proteins or complexes

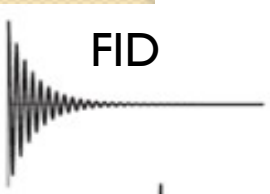
"medium" size complexes

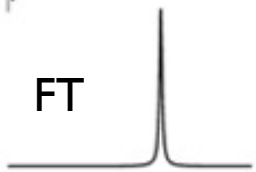
"medium" size globular proteins

"Small" globular Protein / domain

Global correlation time τ_c (size)

Linewidth



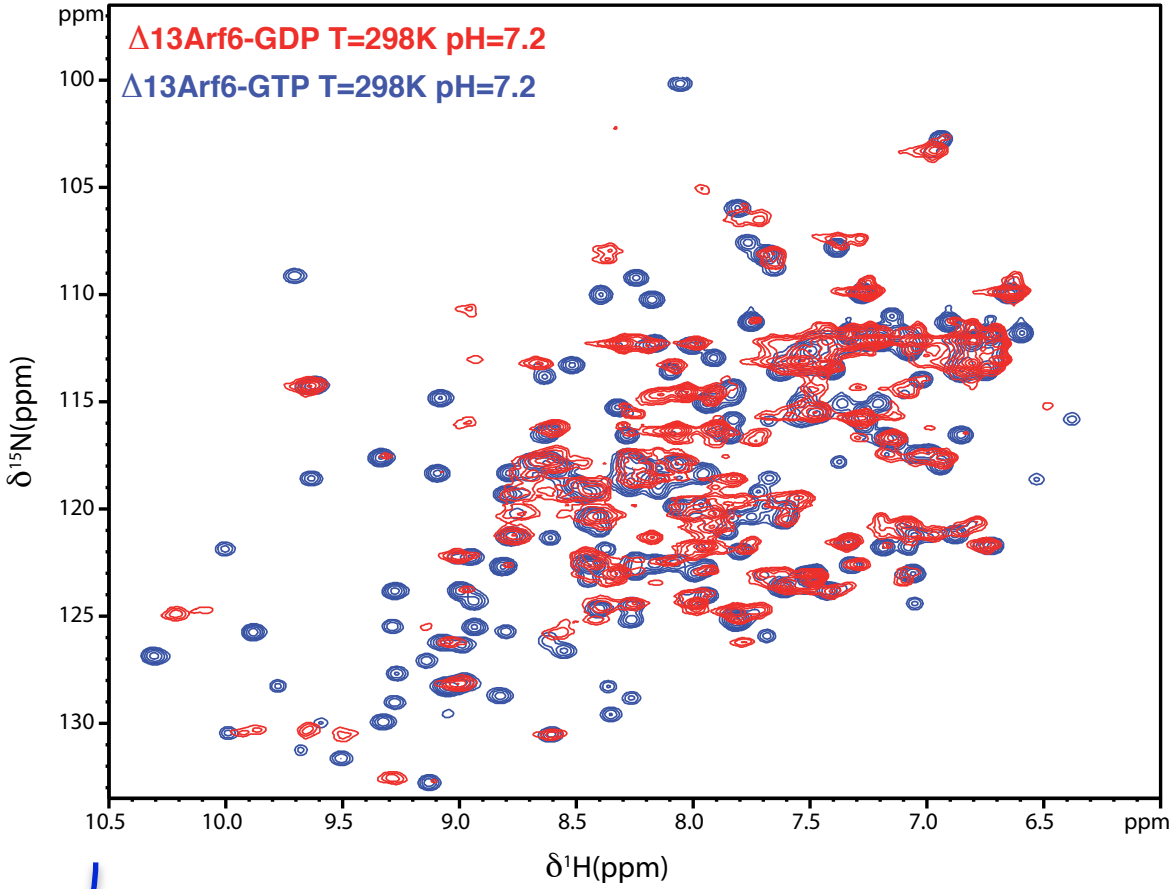


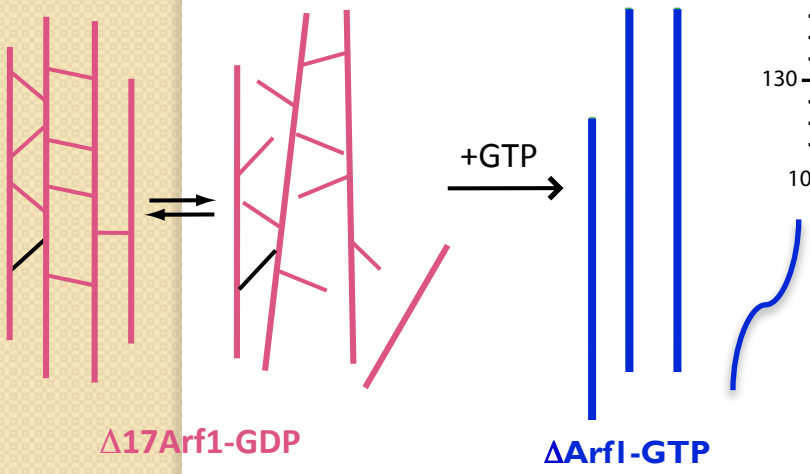
+ Local Rotational fluctuation timescale
+ density of relaxation sources

+ conformational/chemical exchange

NMR - Spatial and Time scales are highly intermingled

Insight into the role of dynamics in the conformational switch of the small GTP-binding protein Arf1
Vanessa Buosi, Jean-Pierre Placial, Jean-Louis Leroy, Jacqueline Cherfils, Éric Guittet and Carine van Heijenoort*
JBC 2010

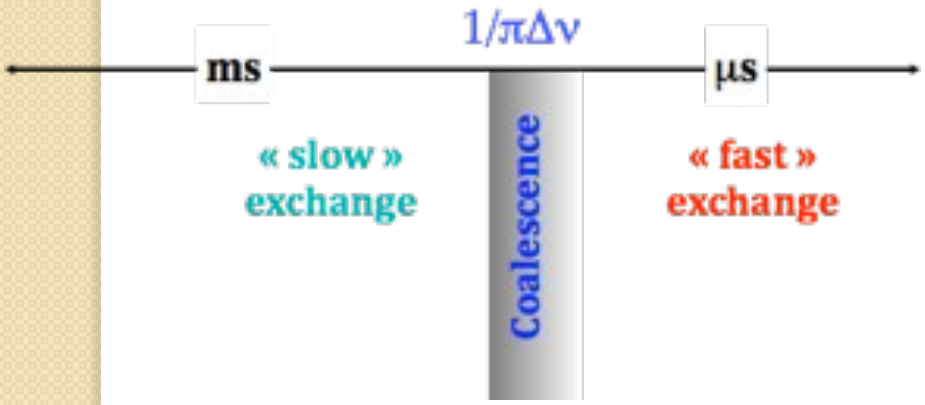


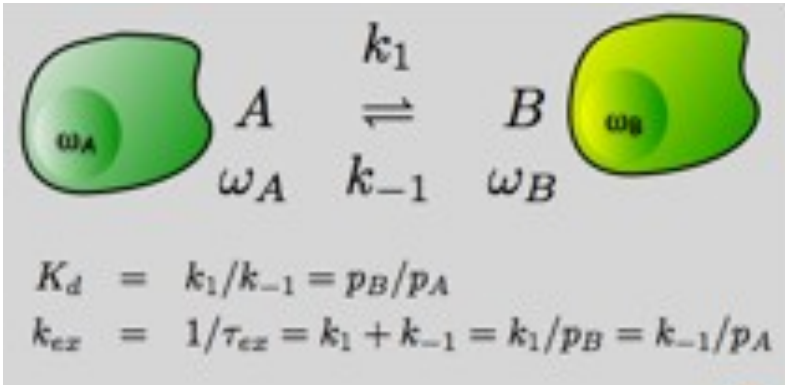


+ conformational/chemical exchange

« Size » and exchange

- Processes that are on the spectral timescale (μ s-ms range) affect the appearance of the spectra
- These processes generally correspond to conformational /chemical exchange
- The effects on spectra depend on the relative values of $k_{ex} = \Delta\omega/2$ ($\tau_{ex} = 2/\Delta\omega$)
- One talk of **slow** or **fast** exchange at the chemical shift timescale



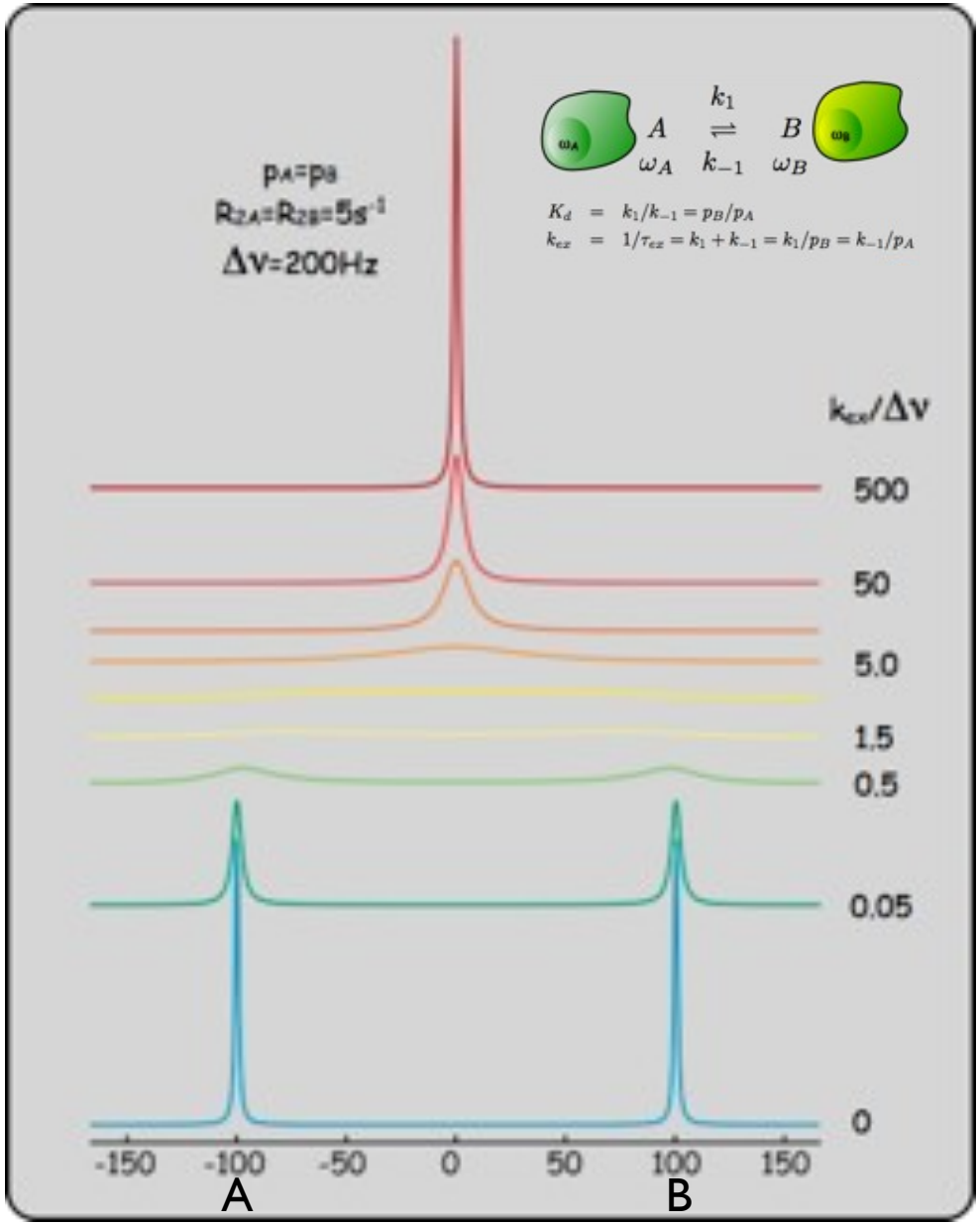


« Size » and exchange

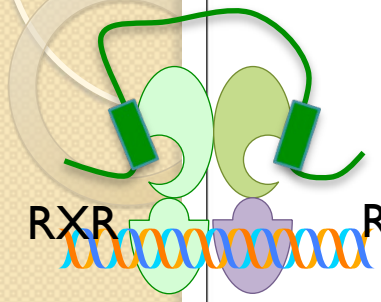
Exchange ⟺ **Broadening**

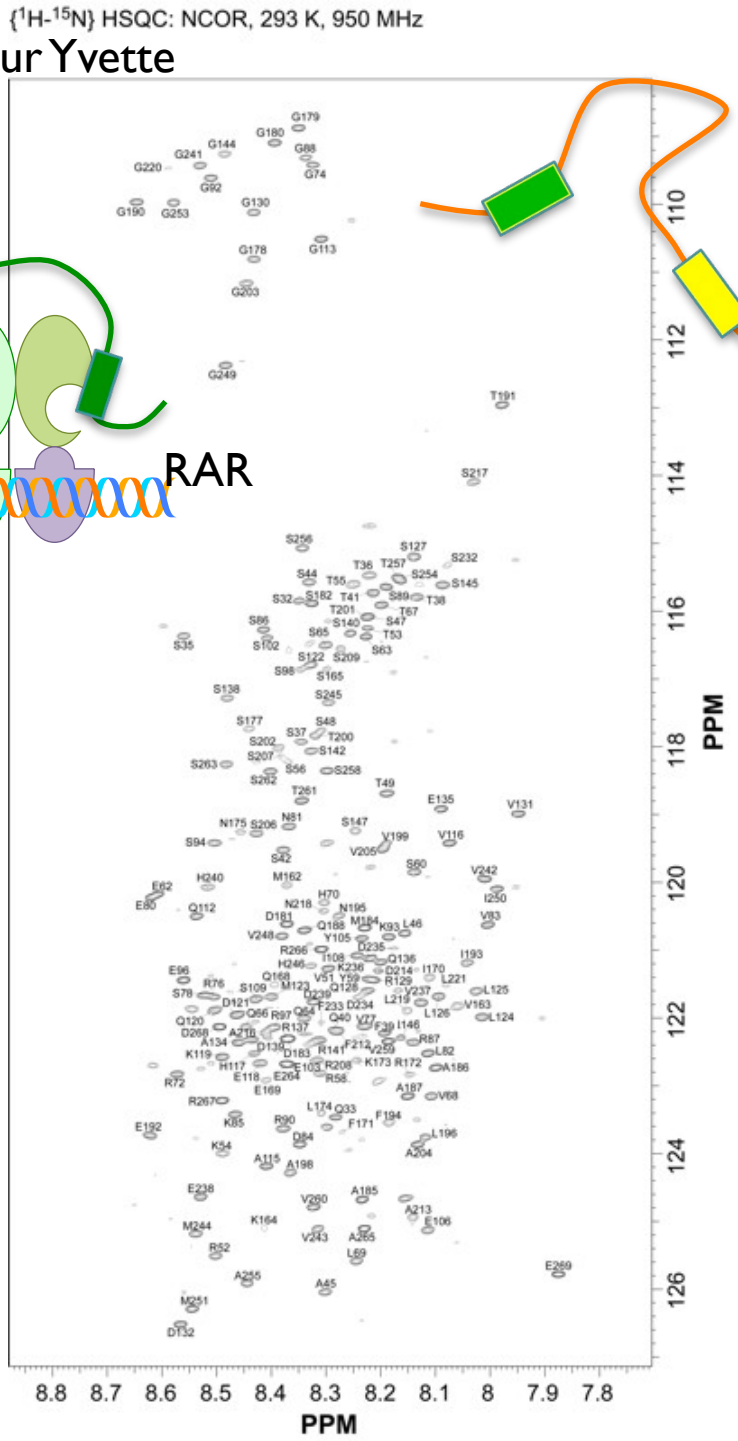
Exchange regime:

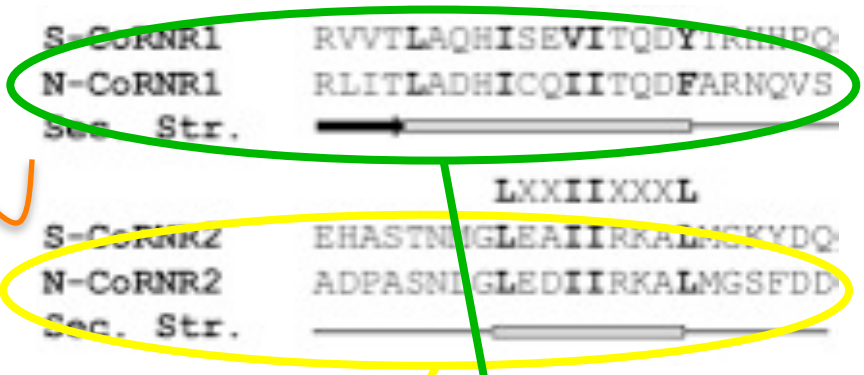
- "Fast" exchange : $\Delta\omega \ll k_{ex}$
  - One peak at $\omega = p_A.\omega_A + p_B.\omega_B$
  - $R_{ex} \propto B_0^2$
- "Intermediate" exchange : $\Delta\omega \simeq k_{ex}$
  - One or several broadened peaks
  - $R_{ex} \propto B_0$
- "Slow" exchange : $\Delta\omega \gg k_{ex}$
  - Several peaks
  - R_{ex} independent of B_0
  - $R_{ex}(B) \rightarrow k_{BA}=p_A.kex$;
  - $R_{ex}(A) \rightarrow k_{AB}=p_B.k_{ex}$

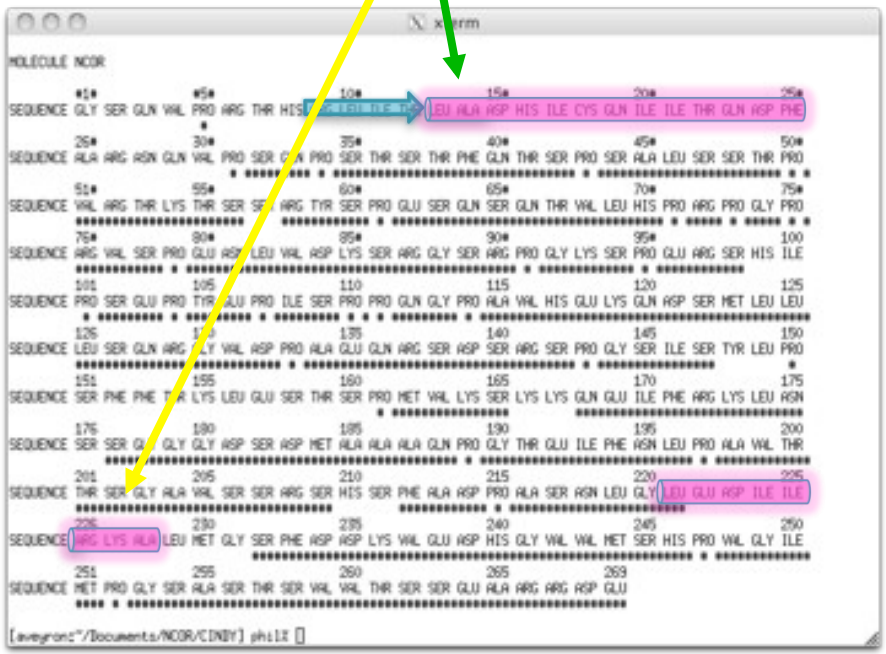


TGIR Gif sur Yvette









210 peaks over 269

Coll. A LeMaire, P Germain, W Bourguet (CBS)

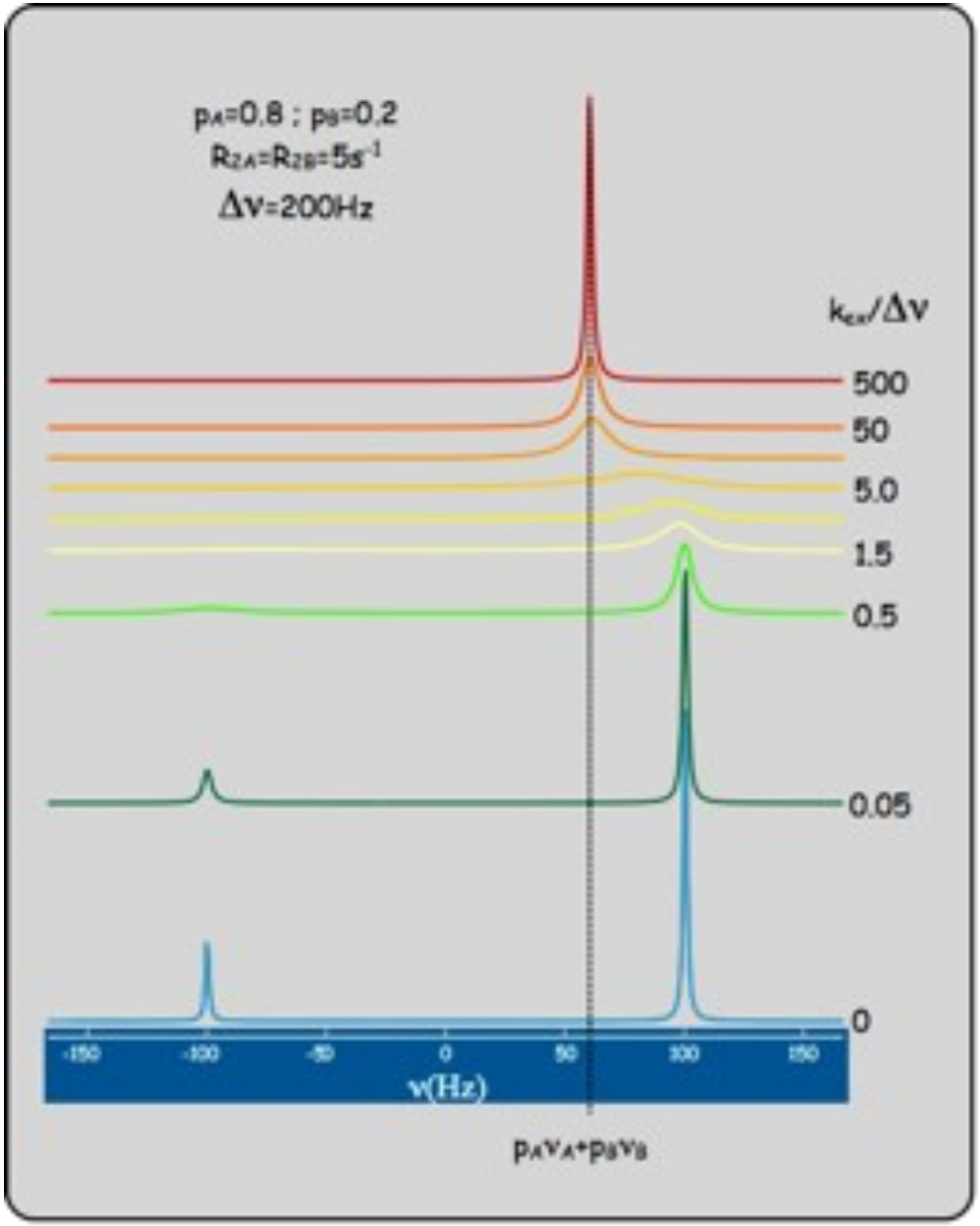
« Size » and exchange

Exchange regime
Case of unequal populations:

- "Fast" exchange : $\Delta\omega \ll k_{ex}$
  - One peak at $\omega = p_A.\omega_A + p_B.\omega_B$
  - $R_{ex} \propto B_0^2$
- "Intermediate" exchange : $\Delta\omega \approx k_{ex}$
  - One or several broadened peaks
  - $R_{ex} \propto B_0$
- "Slow" exchange : $\Delta\omega \gg k_{ex}$
  - Several peaks
  - R_{ex} independent of B_0
  - $R_{ex}(B) \rightarrow k_{BA}=p_A.kex$;
  - $R_{ex}(A) \rightarrow k_{AB}=p_B.k_{ex}$

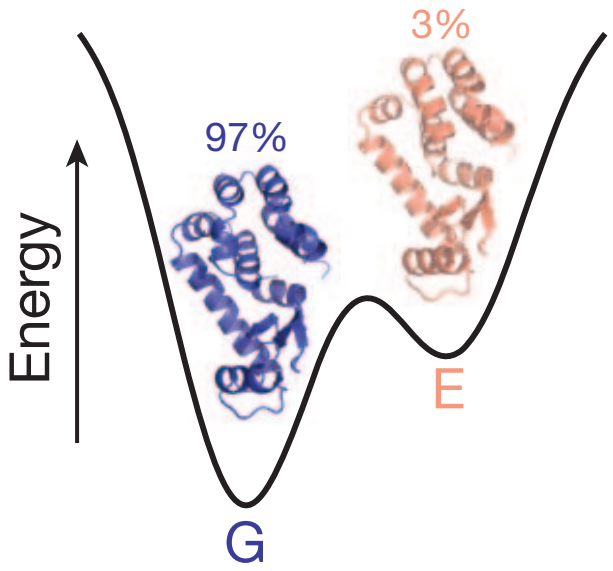
$p_A \gg p_B \Rightarrow R_ex(B) \gg R_{ex}(A)$
☞ The minor peak can be undetectable even in a slow exchange regime.

Experiments to characterize thermodynamics, kinetics and structural parameters of the exchange when only one peaks is observed ?



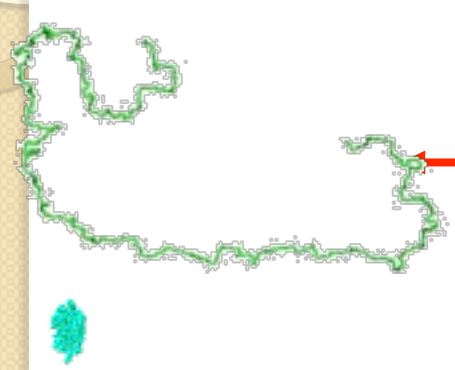
« Size » and exchange

- transiently formed and marginally populated (less than a few per cent of the total number of molecules) cannot be individually characterized by most biophysical tools.
- present the atomic-level model of the 'invisible', excited state obtained using a combined strategy of relaxation-dispersion NMR and CS- Rosetta model building
- rationalizes the observation that T4 lysozyme mutant binding to its hydrophobis ligand occurs only via the ground state



Guillaume Bouvignies, Pramodh Vallurupalli, D. Flemming Hansen, Bruno E. Correia, Oliver Lange, Alaji Bah, Robert M. Vernon, Frederick W. Dahlquist, David Baker & Lewis E. Kay (2011) NATURE 447, 111

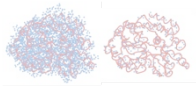
NMR - Spatial and Time scales are highly intermingled



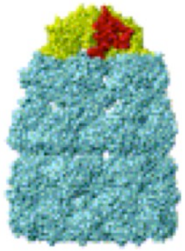
medium size and large IDPs

TROSY, high fields

solubilized membrane proteins



Labelling, Deuteration



"medium" size complexes

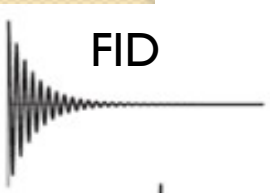
"large" size globular proteins or complexes

"medium" size globular proteins

"Small" globular Protein / domain

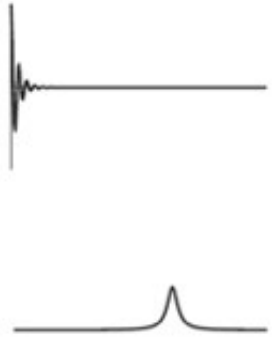
Global correlation time τ_c (size)

Linewidth

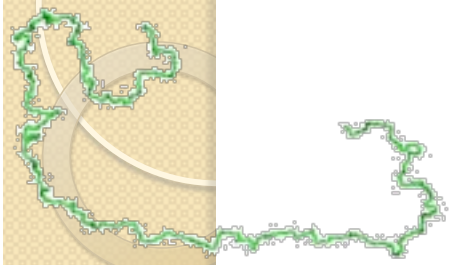


+ Local Rotational fluctuation timescale
+ density of relaxation sources

+ conformational/chemical exchange



« Size » and disorder

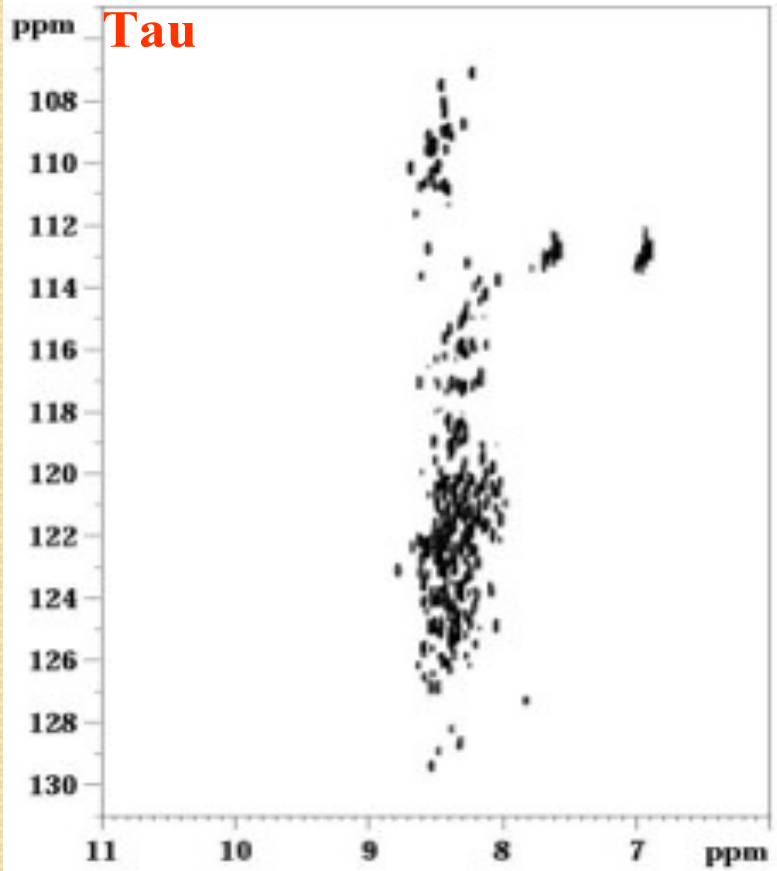


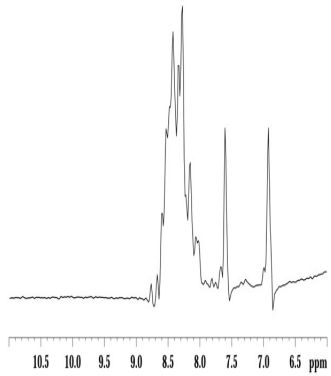
Unfolded protein

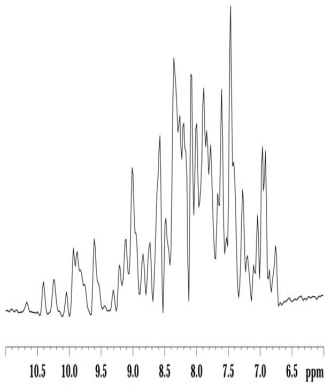
45.9 kDa

Folded protein

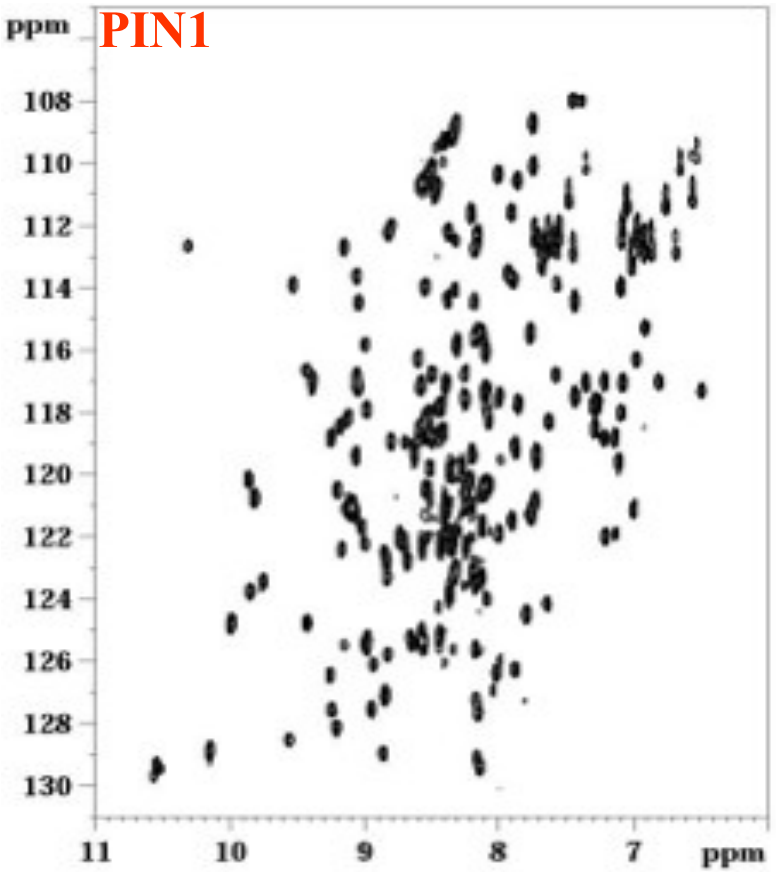
18 kDa







^1H



^1H

What is an IDP ?

- IDPs lack stable tertiary and/or secondary structure
- Very specific amino-acid sequences… Rich in non-structuring residues and depleted in hydrophobic residues
- IDPs are more common in eukaryotes than in bacteria and archaea: Probably linked with their major biological complexity.
- Up to ~30-50% of genome in eukaryotic cells is predicted to code for natively disordered fragments (more that 30 residue fragments)
- Ordered and disordered proteins are associated to distinct biological functions
- Possibility to interact with several partners. Hubs in the interactome
- Involved in cancer, cardiovascular and neurodegenerative diseases

Tau Size : 441 amino acids

---

5 aa = 50% !!!

Reputation : unstructured

Amino Acid composition

	#	%
Gly	49	11.4
Ser	45	10.2
Lys	44	10.0
Pro	43	9.8
Ala	34	7.7

Disorder and Biological Activity

Processes linked with ORDER

keywords
GMP biosynthesis
Amino-acid biosynthesis
Transport
Electron transport
Lipid A biosynthesis
Aromatic hydrocarbons catabolism
Glycolysis
Purine biosynthesis
Pyrimidine biosynthesis
Carbohydrate metabolism
Branched-chain amino acid biosynthesis
Lipopolysaccharide biosynthesis
Sugar transport
Antibiotic resistance
Lipid synthesis
Tricarboxylic acid cycle
Arginine biosynthesis
Ion transport
Rhamnose metabolism
Peptidoglycan synthesis

Both Ordered and Disordered regions are associated with distinct functions

Disordered Proteins complement the functions of ordered protein regions

Processes linked with DISORDER

keywords
Differentiation
Transcription
Transcription regulation
Spermatogenesis
DNA condensation
Cell cycle
mRNA processing
mRNA splicing
Mitosis
Apoptosis
Protein transport
Meiosis
Cell division
Ubl conjugation pathway
Wnt signaling pathway
Neurogenesis
Chromosome partition
Ribosome biogenesis
Chondrogenesis
Growth regulation

- ✧ Up to 80% of proteins involved in cancer are (partly) Unstructured: p53, BRCA1…
- ✧ Unfolded regions are targets for chemical modifications, PPI, and alternative splicing
- ✧ Misfolding, fibrillation… are often linked to disorder: Prions, Aβ, Tau, α-synuclein (?)

Xie et al. *J Prot Res* 2007, 6, 1882.

Historical Background

The « Golden rule » of proteins…

- **SEQUENCE => 3D STRUCTURE => FUNCTION**
- plasticity of proteins around a defined 3D structure

The « lock and key » concept

- **SEQUENCE => ONE 3D STRUCTURE => FUNCTION**
- Fisher (1884) : enzyme and glucoside have to fit to each other to exert a chemical effect
- Mirsky and Pauling (1936) : specific properties of native proteins, uniquely defined conf
- Structures of proteins by X-Ray Cristallography (1960 ->) :

The « induced fit » concept

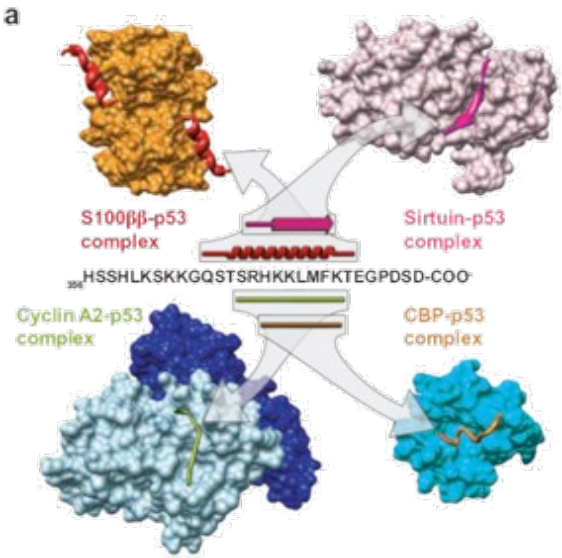
- **SEQUENCE => ONE MEAN 3D STRUCTURE => localized motions => Selection of "the" productive conformation for FUNCTION. Adaptation to a partner**
- Karush (1950) : "configurational adaptibitily"
- Koshland (1958) : "induced fit"
- Plasticity of proteins around a well defined 3D-structure
- Switch between several well defined structures (multi-domain proteins, allosteric motions)

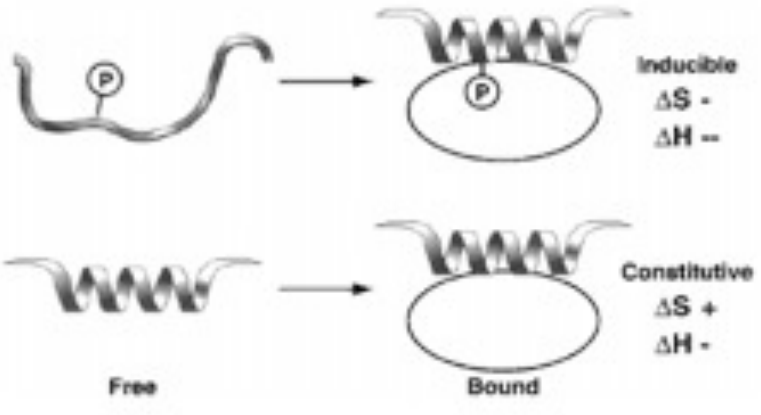
Functional disorder in proteins

- **SEQUENCE => DISORDERED STATE => Folding upon interaction to a target**
- Missing electron density in X Ray structures (1978)
- Flexibility shown by NMR. Relation between motions and NMR spin relaxation (1960)
- Fully unfolded functional proteins revealed by NMR (Kriwacki et al 1996 PNAS)

The Importance of Being Unfolded

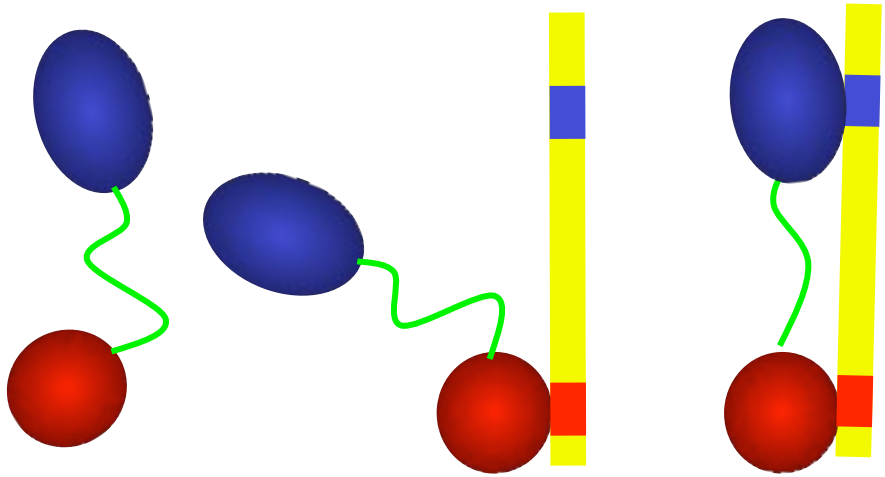
- Increased interaction surface area
- Accessible to post-translational modifications
- Structural adaptability to interact with several targets





- Fine tuning of the thermodynamic properties of the complex

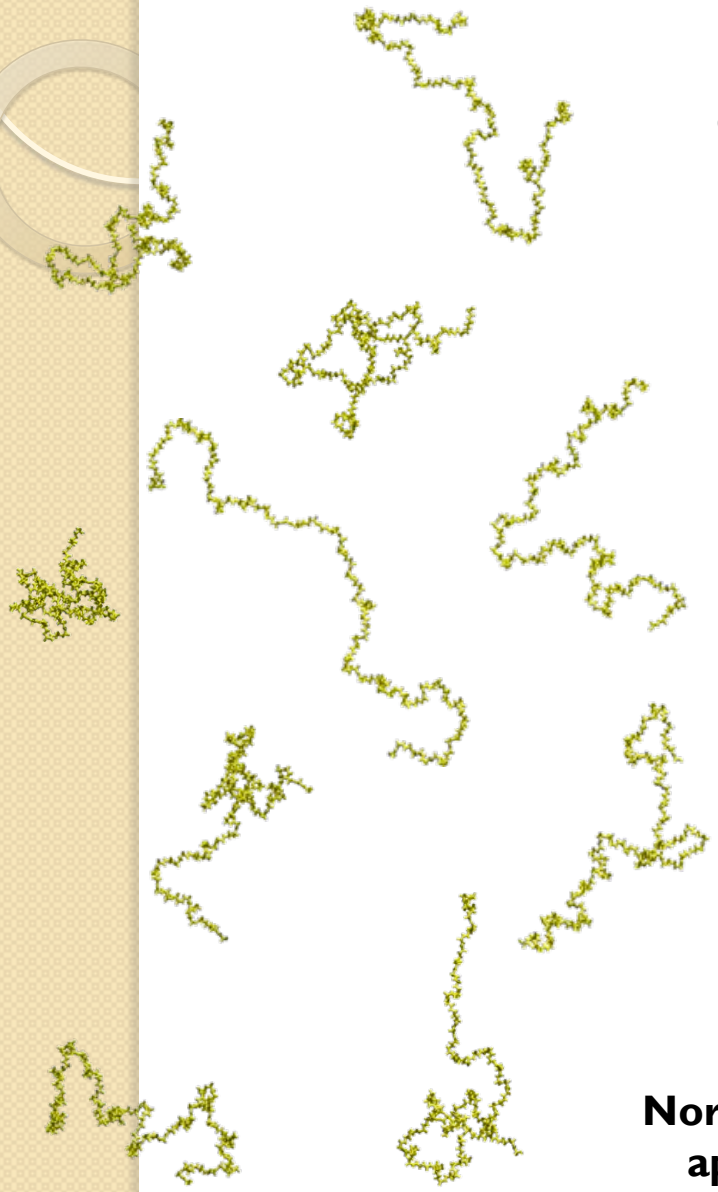
- ID Regions restrict the space sampled by folded domains in multidomain proteins



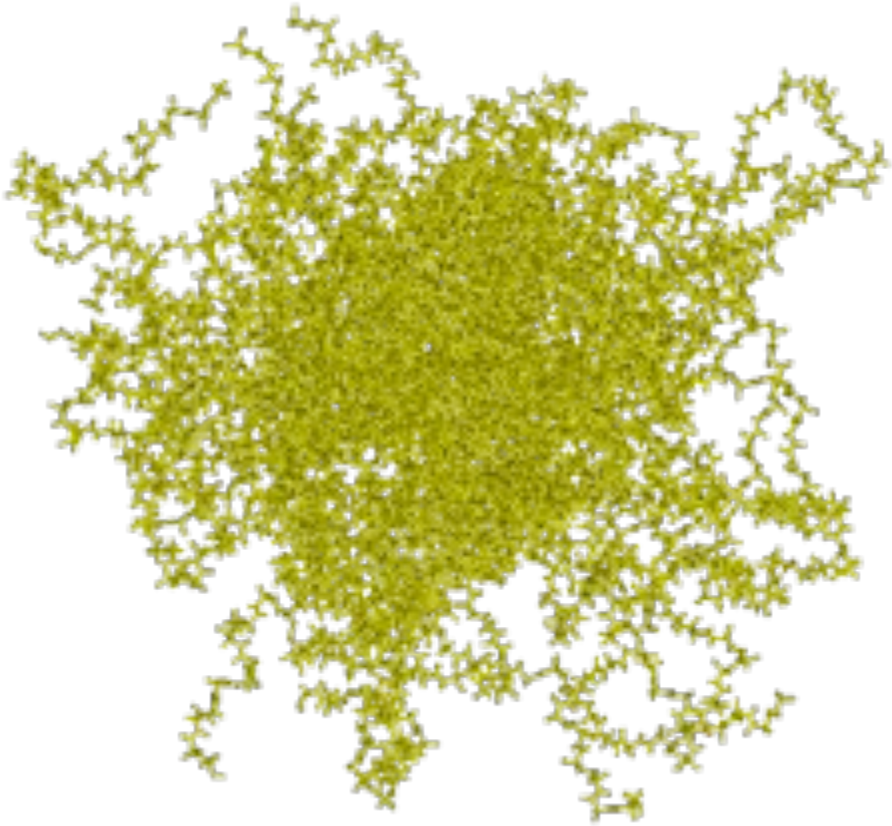
Structural Characterization of IDPs

- Is our protein an IDP ?
- Conformational Nature of the Unfolded state
- Is there some degree of structuration. Where and which kind?
  Linked to recognition events
- Are there long-range contacts in the protein ?
  Size properties of the ensemble. Highly relevant in Multidomain Proteins
- Are there structural changes upon environmental or chemical modifications?
  Effect of Post-translational Modifications
- Time-scale of protein motions
- How the recognition process of the natural partners takes place?

Flexible Proteins, a Challenge for Structural Biology



IDPs explore an astronomical number of conformations that we assume are in fast equilibrium

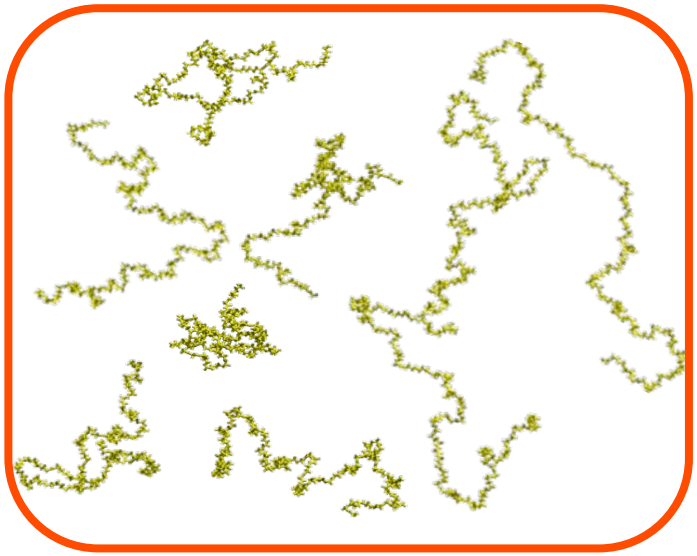


Normally averaged properties are measured. Ensemble approaches have to be used for their interpretation

Flexible Proteins, a Challenge for Structural Biology

AVERAGED

CS, RDCs, J-couplings, NOEs, PREs
SAXS, Hydrodynamic data, EPR



- The ensemble is underdetermined
  - Cross-validation or simplification of the structural model are required

- Structural content of the ensemble depends on the information (experimental data) introduced…
  - Residue-specific data ▸ local conformation
  - Overall data ▸ size and shape

Is our protein an IDP

- Bioinformatics
  Identification of disordered/ordered regions

- Biophysical characterization: CD, FTIR, FRET, hydrodynamics
  Partial Information

- Small-Angle X-ray Scattering (SAXS)/Small-Angle neutron Scattering (SANS)
  Averaged Intensity profiles...
  Qualitative Interpretation of averaged R_g and Kratky Plots

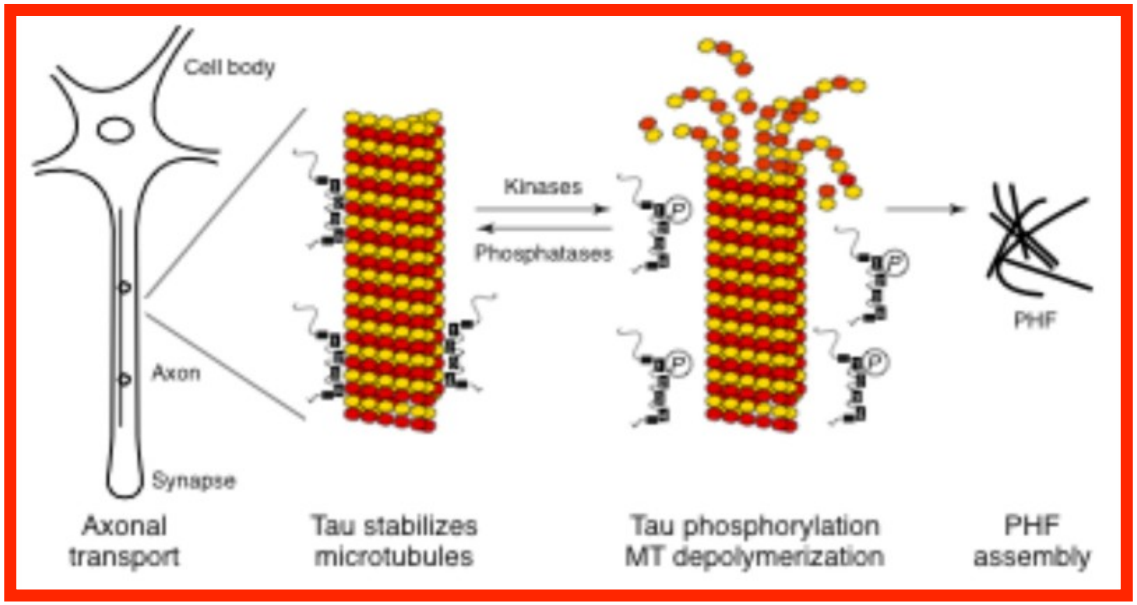
- Nuclear Magnetic Resonance (NMR)
  Ensemble averaged observables: CS, J-Couplings and RDCs
  Dynamic dependent Parameters: Relaxation Rates and PREs

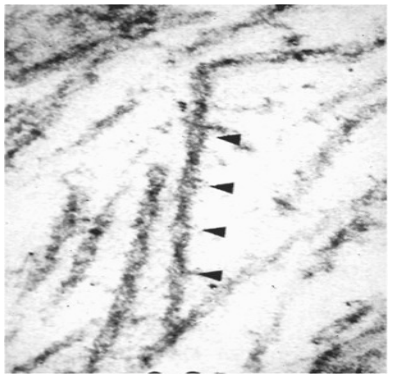
- X-ray Crystallography
  Structure determination in the bound form

Is there some degree of structuration ?

Where and which kind ?

Linked to functional events





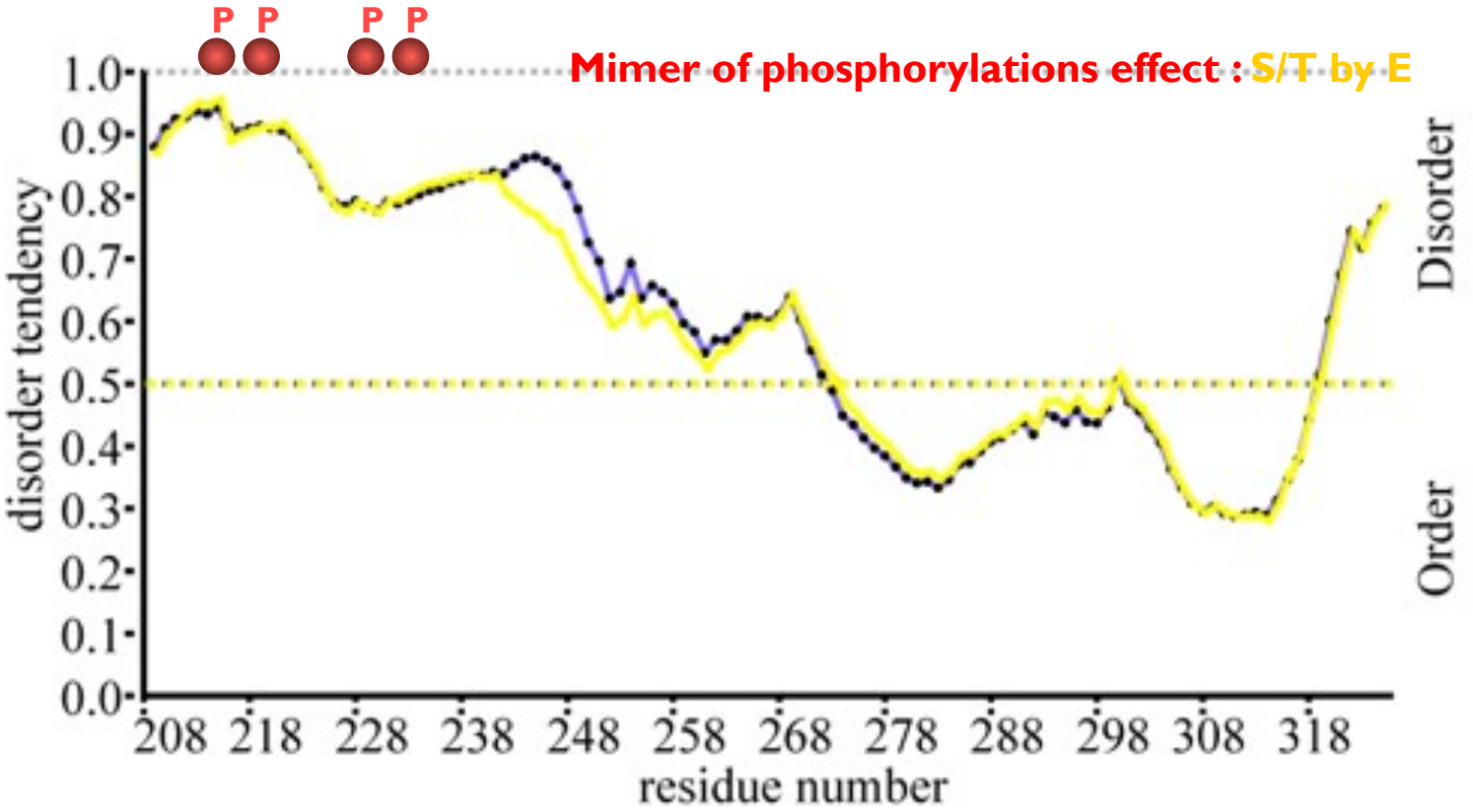
Microscopie Electronique

Neuronal protein involved in tubulin polymerization into microtubules

Tau aggregation ➔ Alzheimer disease

BioInformatics

Predictors of disordered/ordered regions

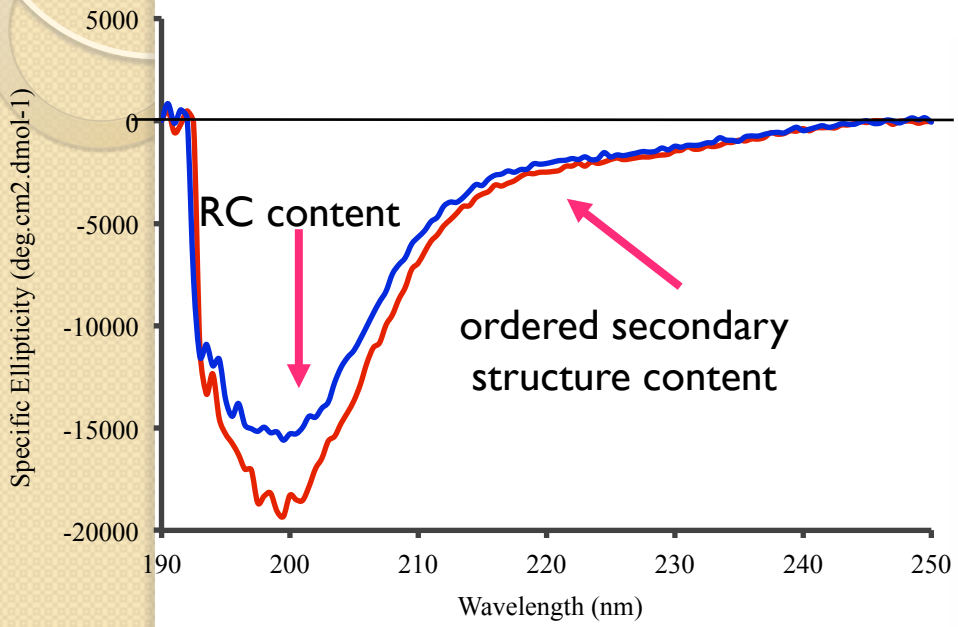


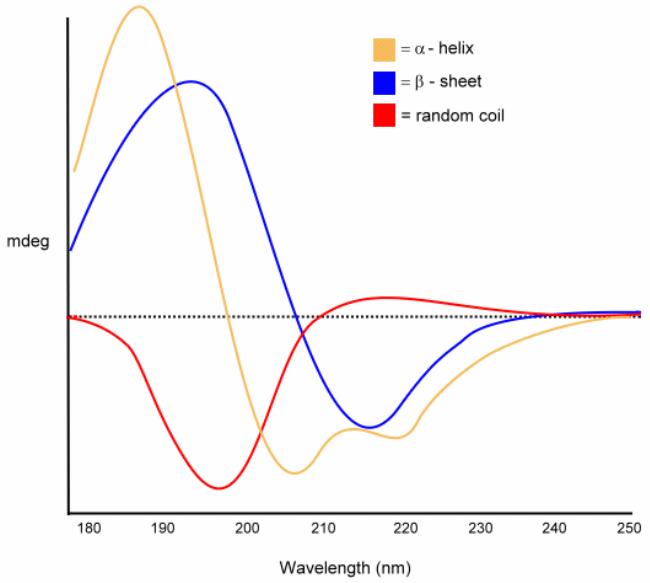
metaPrDOS integrates the results of different prediction methods
seven predictors: PrDOS (Ishida and Kinoshita, 2007), DISOPRED2 (Ward *et al.*, 2004), DisEMBL (Linding *et al.*, 2003), DISPROT (VSL2P) (Peng *et al.*, 2006), DISpro (Cheng *et al.*, 2005), IUpred (Dosztanyi *et al.*, 2005b) and POODLE-S (Shimizu *et al.*, 2007)

"DisProt: the Database of Disordered Proteins" Nucleic Acids Res. 2007 Jan;35 (Database issue):D786-93. Epub 2006 Dec 1.

Circular Dichroism

Partial/Global Information





- random coil polymer : broad minimum at 195-200 nm
- less pronounced minimum at 200 nm suggesting a reduced random coil contribution

↳ Low level of secondary structure

$\theta_{220}/\theta_{200}$

- positive (p -> p*) at 192 nm
- negative (p -> p*) at 209 nm
- negative (n -> p*) at 222 nm

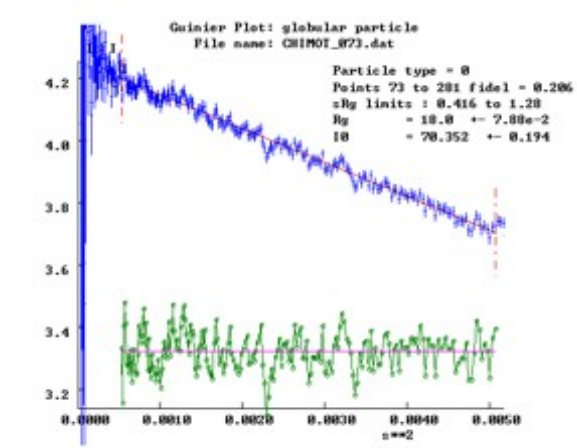
- negative at 218 nm (p -> p*)
- positive at 196 nm (n -> p*)

- positive at 212 nm (p -> p*)
- negative at 195 nm (n -> p*)

SAXS/SANS

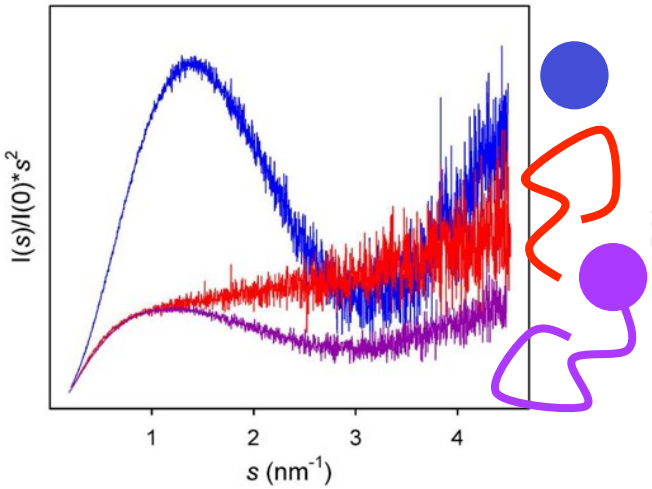
Qualitative interpretation of averaged Intensity Profiles

Guinier



R_g , Molecular Weight

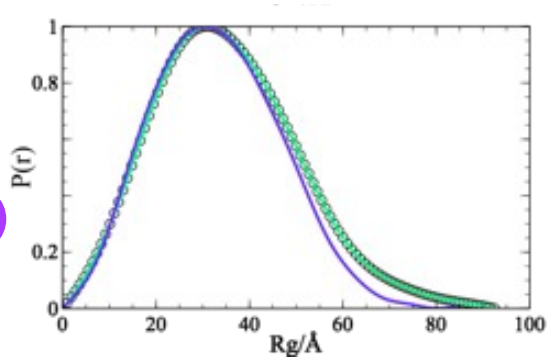
Kratky



Mass Density

- **folded**
- **unfolded**
- **folded and unfolded**

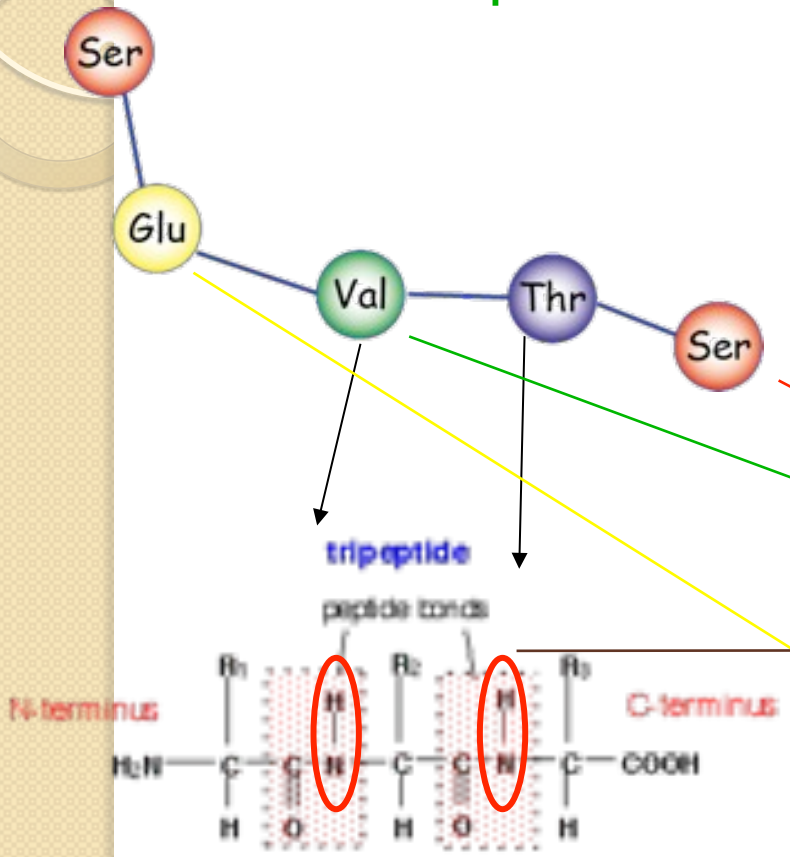
P(r)

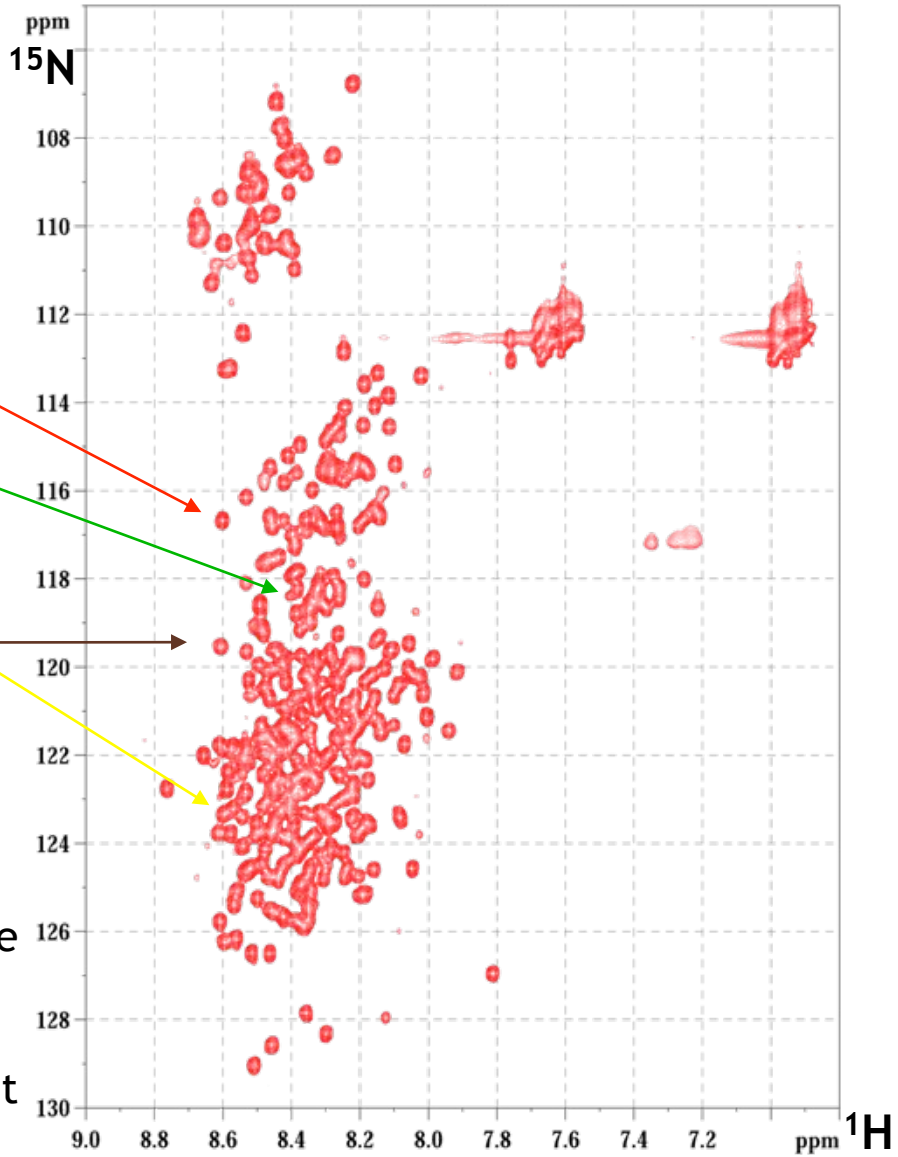


1D-Structure
Maximum Distance

NMR

Neuronal protein Tau of 441 residues = 45.9 kDa

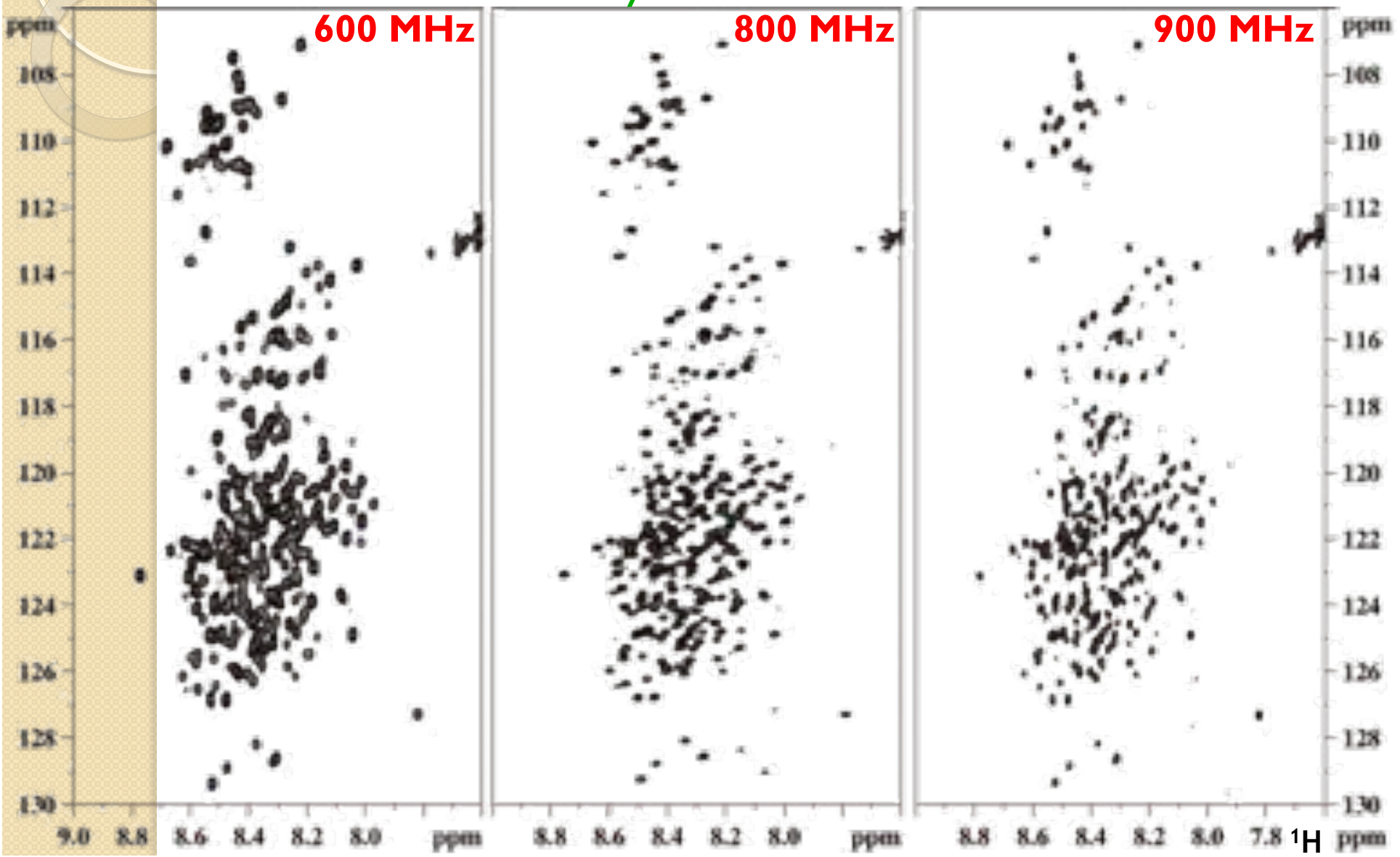




- Each active nucleus can be identified
- Isotopic labelling is necessary but not deuteration

NMR

Sensitivity and Resolution



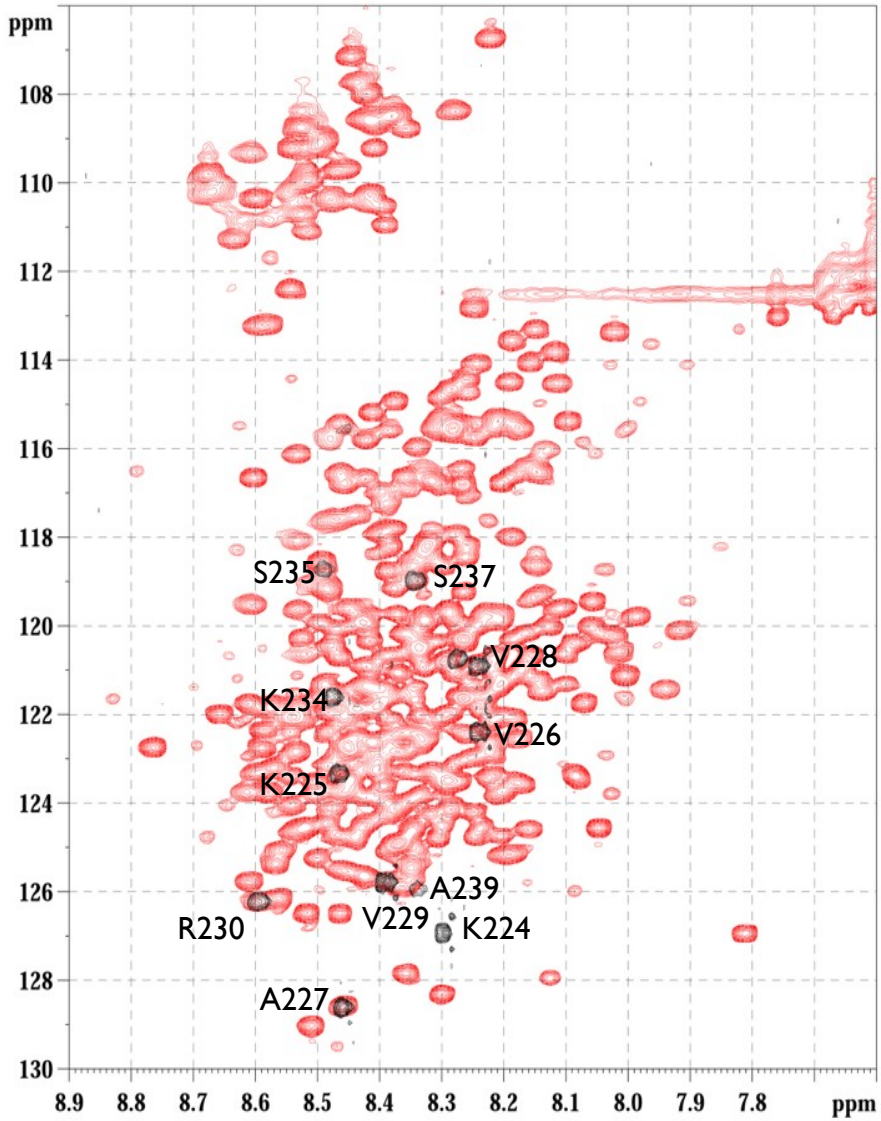
Peptide mapping

Tau protein peptide mapping

T231 peptide :
KKVAVVR TPPKSPSSAK

Non labeled, 5mM in 300ml
Regular TXI probe : 1.5 days
Cryoprobe : 4 hours

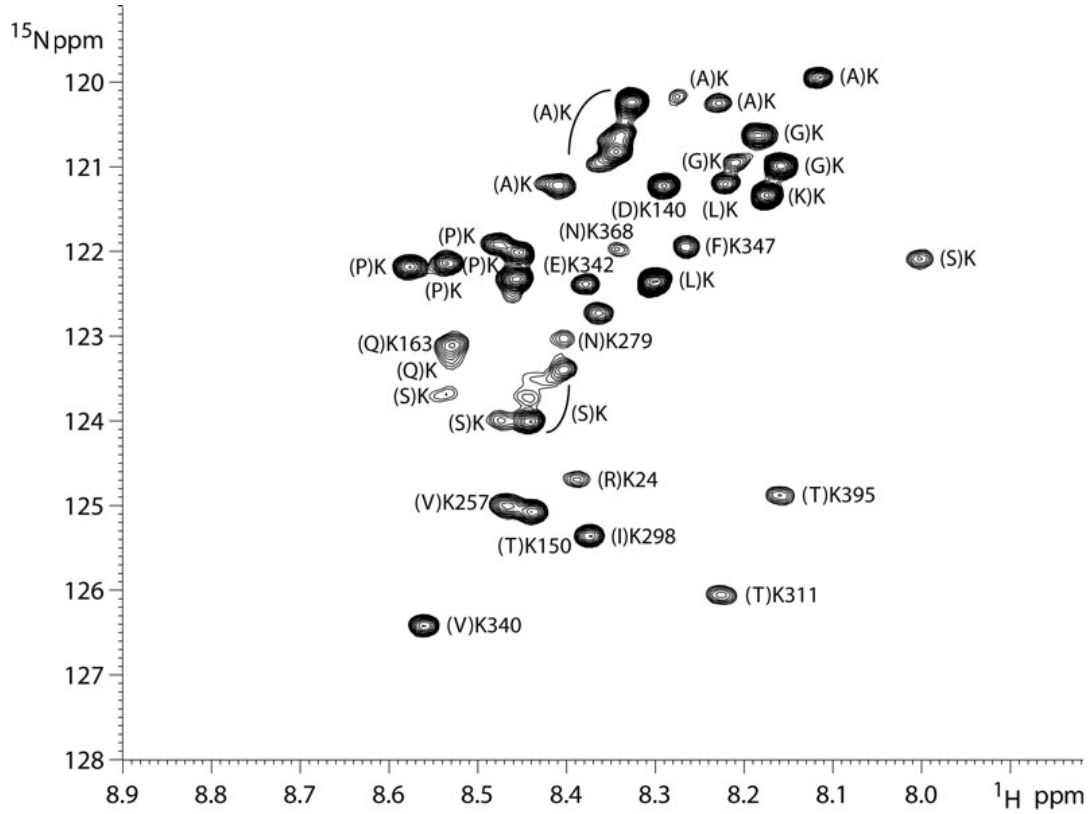
Assign peptide with TOCSY/NOESY



Random coil = samples identical phase space as the aa in small peptide

Specific labeling

Tau protein pairwise assignment ^{15}N Lys



N. Sibille *et al.* Biochemistry 45(41):12560-72, 2006

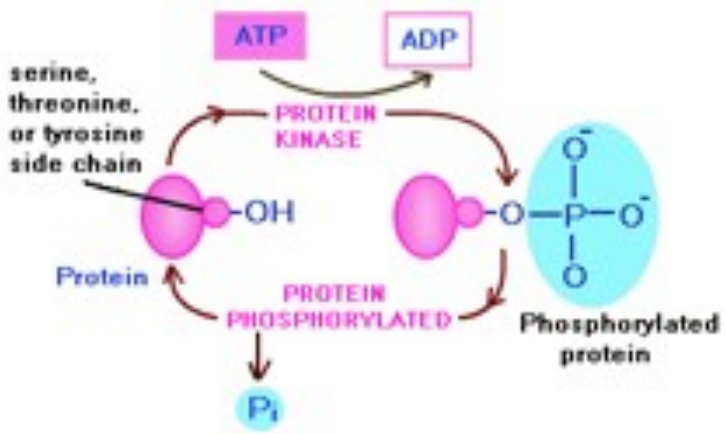
^{1}H-^{15}N-HSQC of the specifically [U-^{13}C, Lys-^{15}N] labeled Tau sample

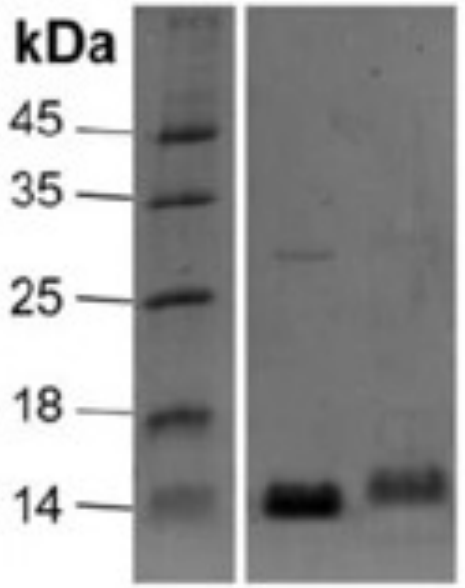
Are there Structural changes upon chemical modifications ?

Effect of Post-translational Modifications

Even with recombinant kinases, mass spectrometry and immunodetection are not evident for determining the full phosphorylation pattern in a qualitative and quantitative manner.

- MS: highly charged phospho-peptide
- Immunodetection by antibodies is equally limited by the requirement of a comprehensive antibody library against all possible epitopes and by the absence of a fully phosphorylated standard for every combination of sites in order to quantify the level of phosphorylation

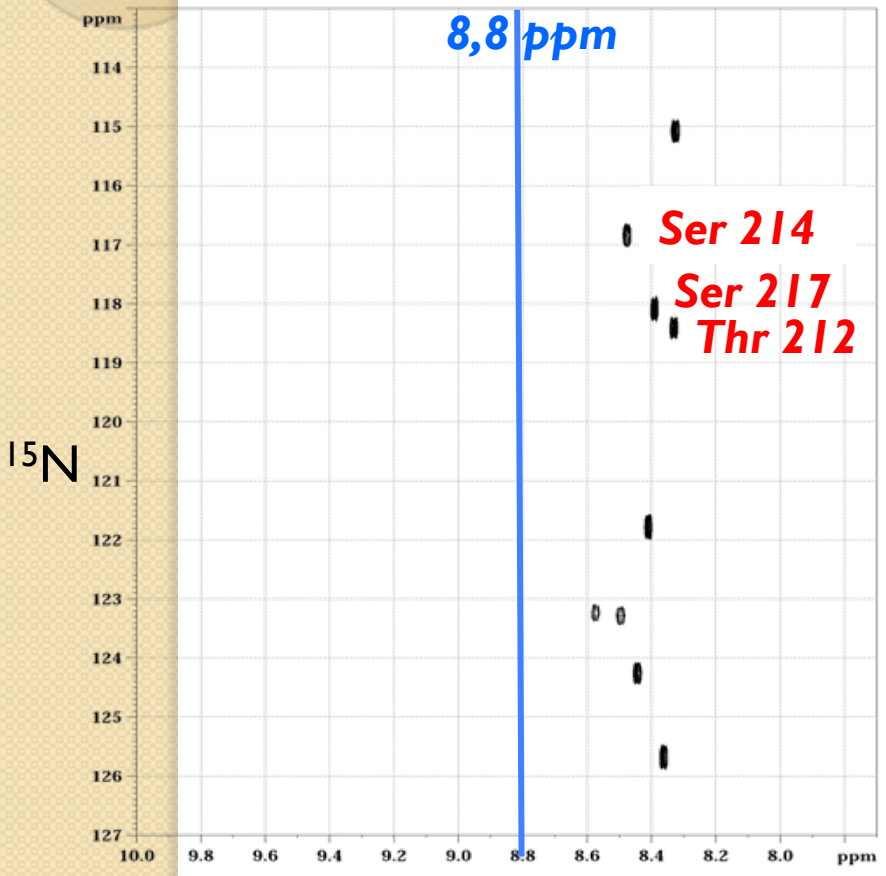




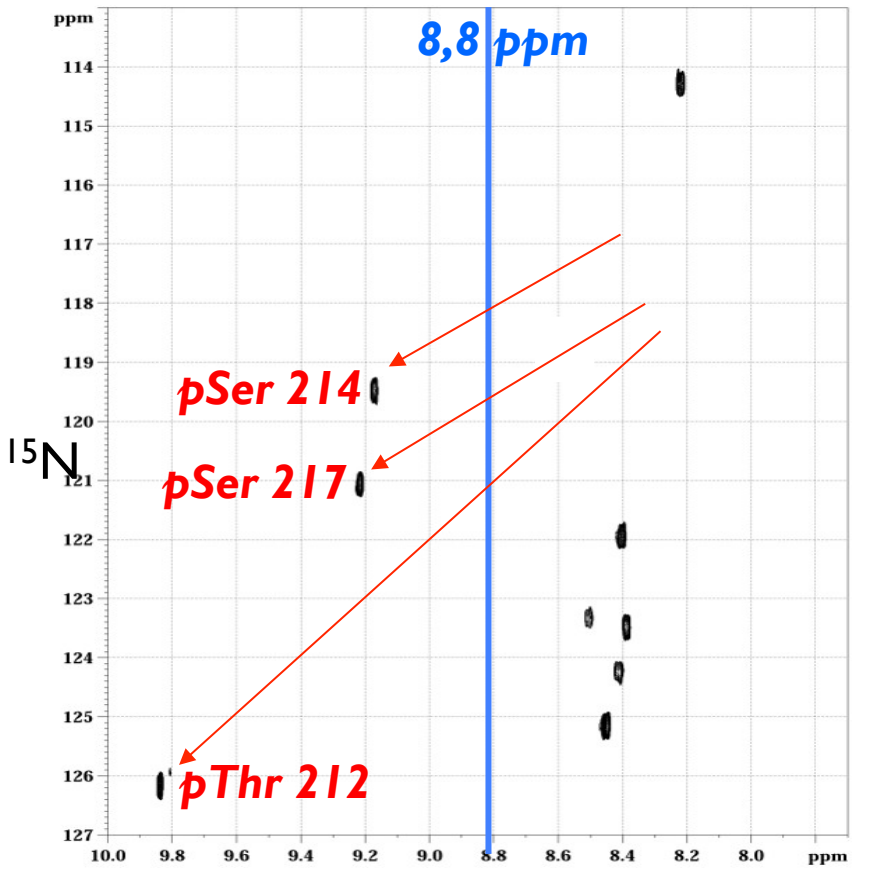
NMR is Qualitative and Quantitative

What sites? and to what extent?

➔ *In vitro* phosphorylation by recombinant kinases: Identification and quantification



SRSRTPSLPSPPTR

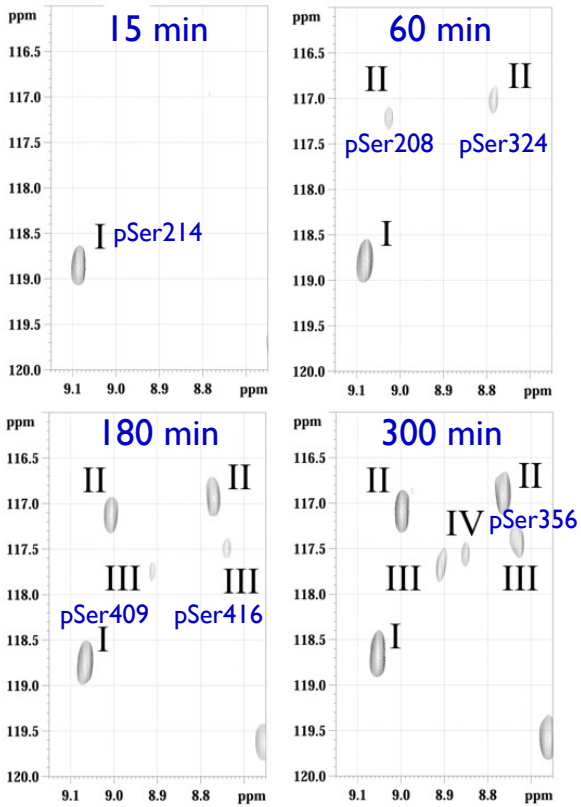


SRSRpTPpSLPpSPPTR

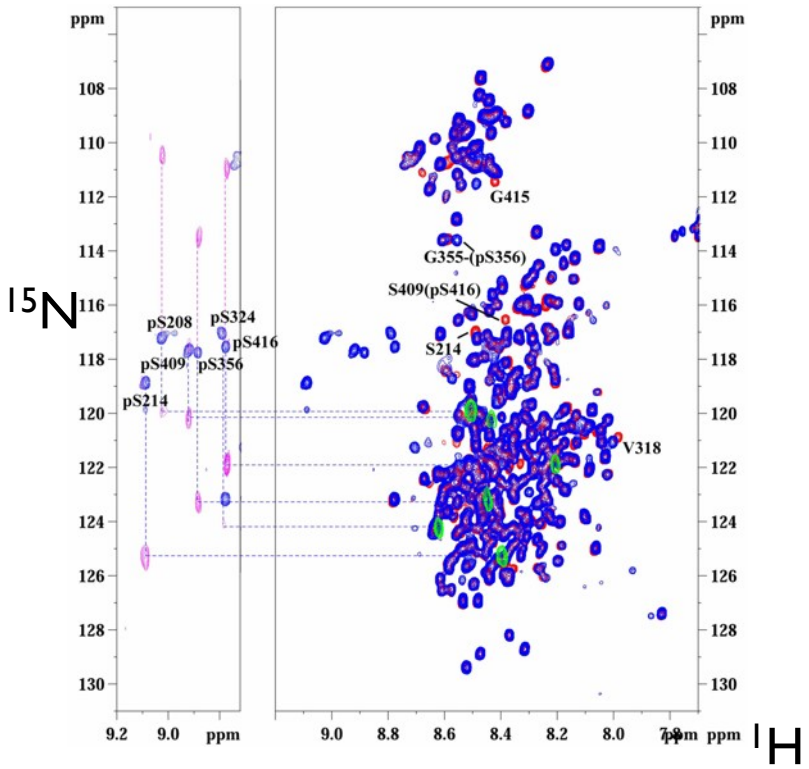
Kinetic of Phosphorylation

cAMP protein dependant kinase PKA

PKA from Prof. Langer/Schwalbe, Frankfurt, Germany



Sequential kinetic

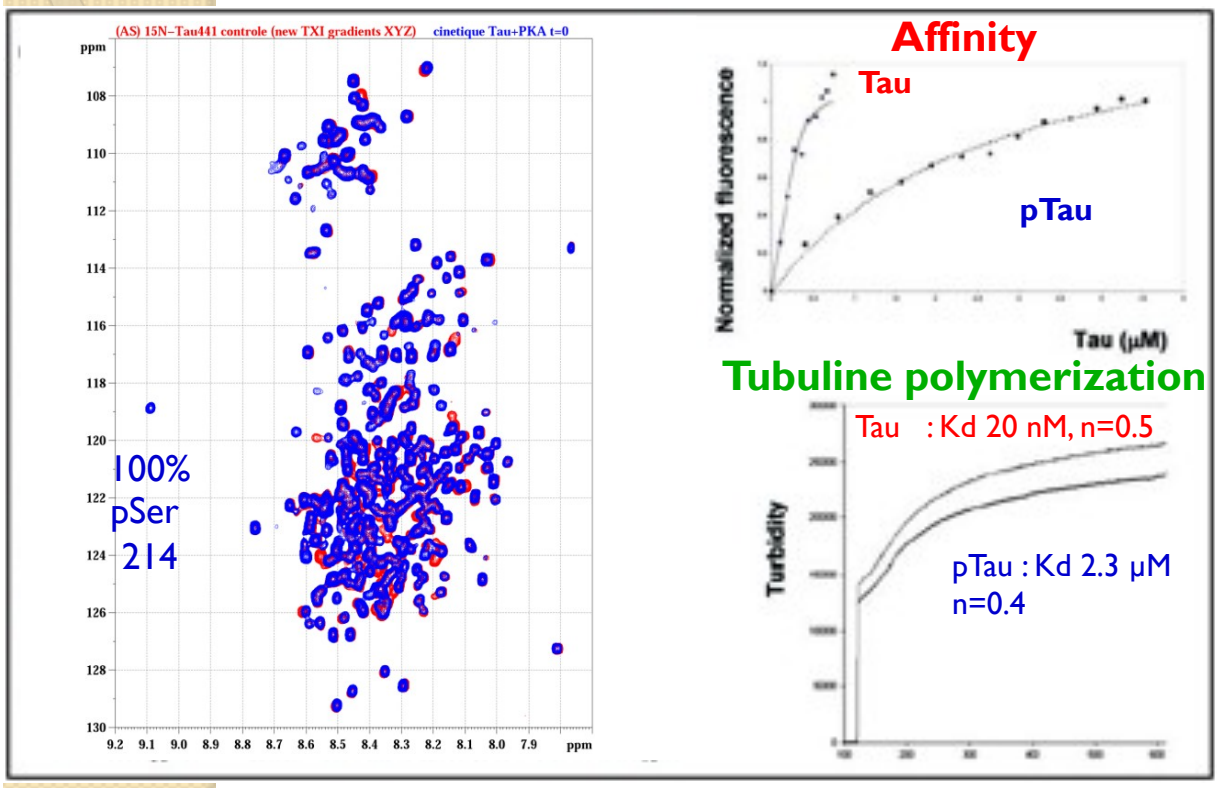


Phosphorylated residues assignment (triple resonance NMR experiments)

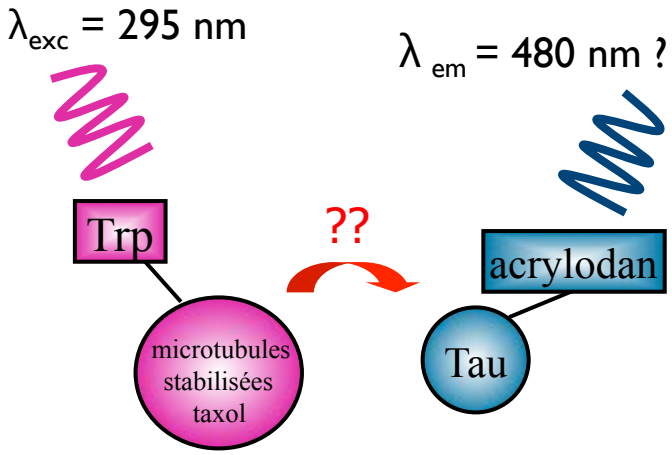
Landrieu I, Lacosse L, Leroy A, Wieruszeski JM, Trivelli X, Sillen A, N. Sibille, Schwalbe H, Saxena K, Langer T, Lippens G. *NMR analysis of a Tau phosphorylation pattern.* J Am Chem Soc 128:3575-83, 2006

Phosphorylation and physiological function

PKA phosphorylation (pSer214): modulation of tau binding to MTs and dynamic of tubulin polymerization



FRET
Tau-acrylodan and W of MTs 1 µM

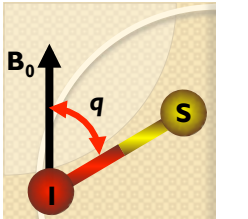


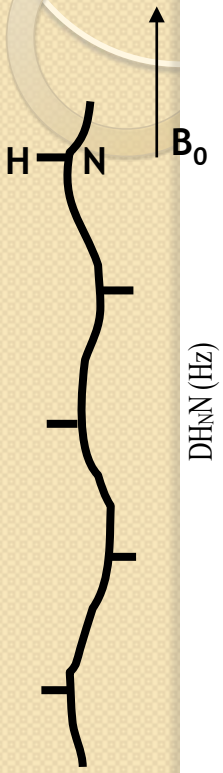
Light Scat. Ex. 350 nm, Em. 363 nm

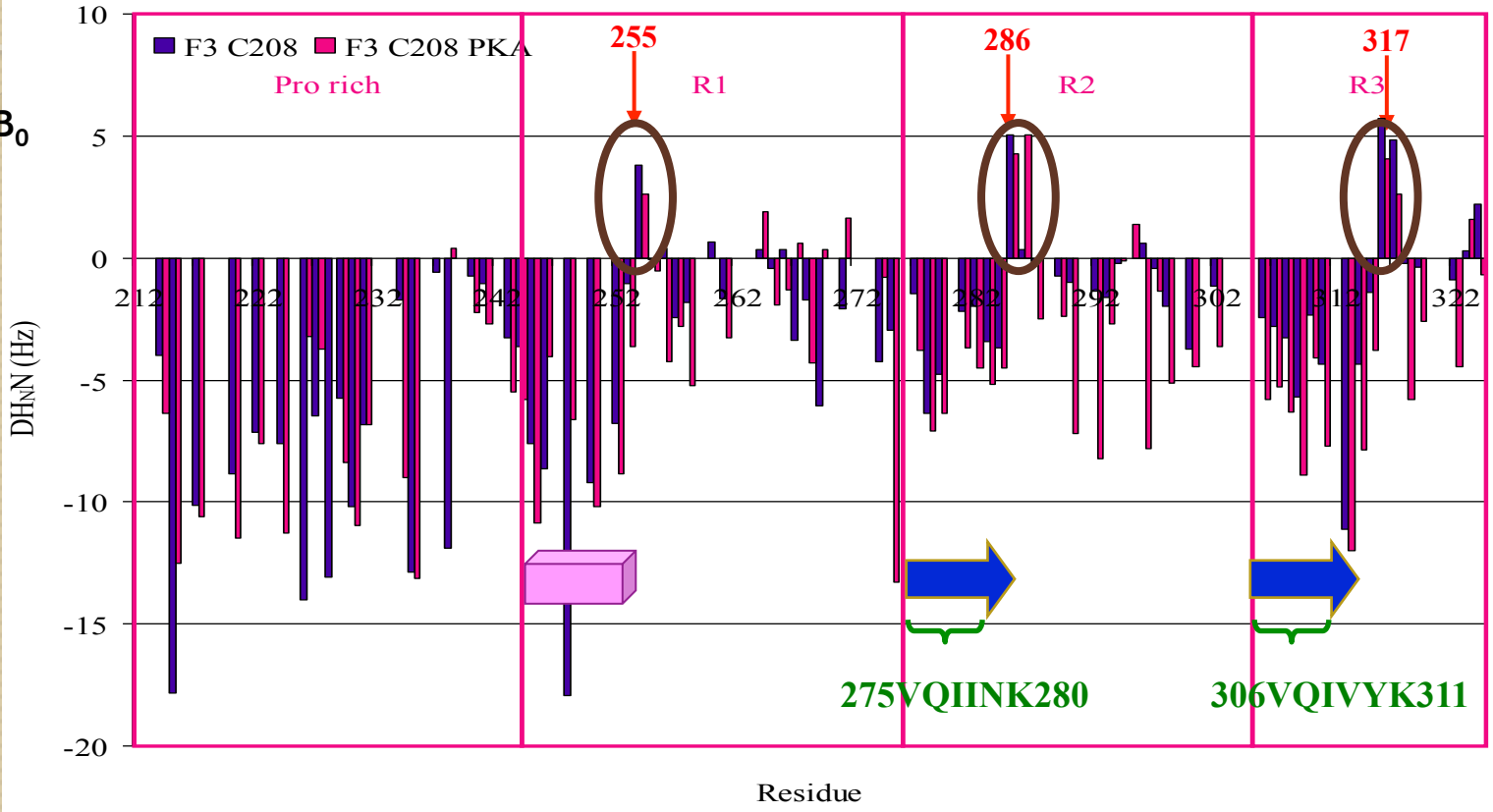
PKA enzymatic reaction of 15 min

G. Lippens, A. Sillen, I. Landrieu, L. Amniai, N. Sibille, P. Barbier, A. Leroy, X. Hanoulle, J.-M. Wieruszeski. Tau Aggregation in Alzheimer's Disease. What Role for Phosphorylation? Prion 1:1, 21-25, 2007

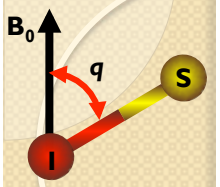
RDC in Disordered States





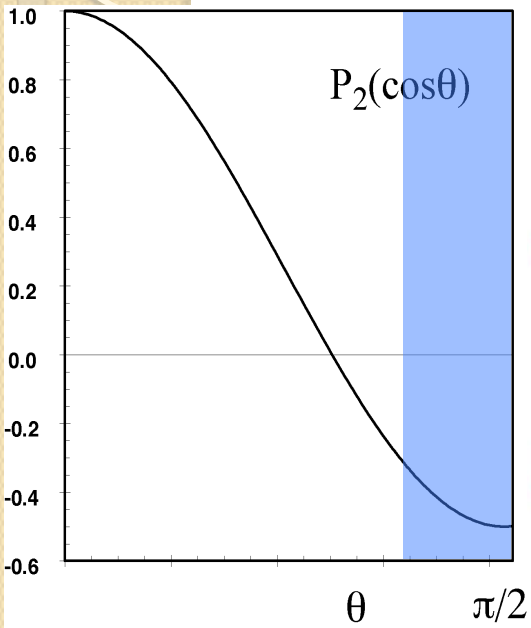


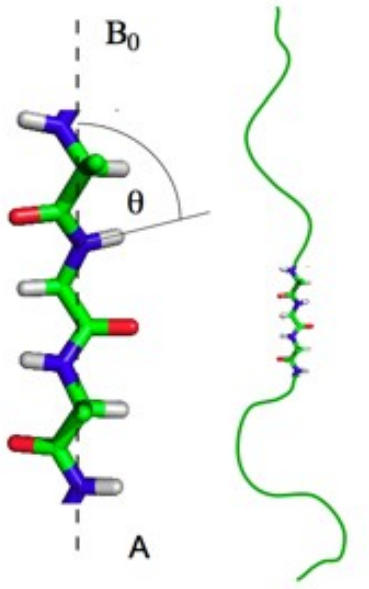
- protéines destructurées ⇒ couplages négatifs le long de la chaîne primaire.
- RDC de plus grande amplitude ⇒ structure plus étendue que le reste de la protéine destructurée
- RDC négatifs ⇒ vecteurs NH ⊥ à l'axe z du tenseur d'alignement (B_0), soit une structure en feuillet b
- changement de signe soudain des RDC ⇒ boucle ou β-turn ou des hélices α (Louhivouri *et al*, 2004)

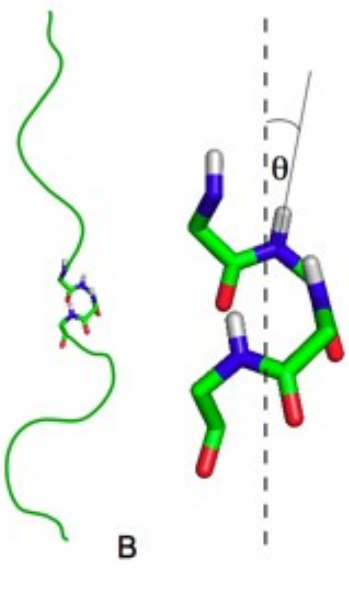


RDC in Disordered States

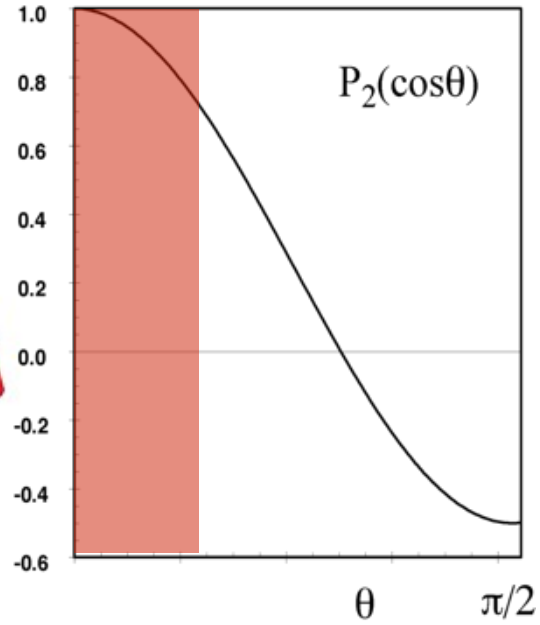
Disordered and Extended Regions

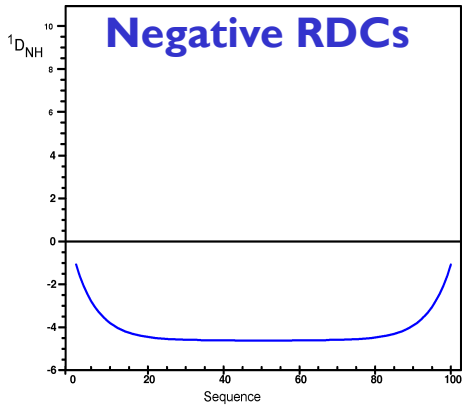


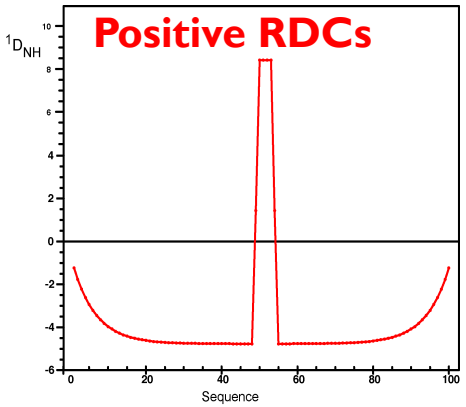




α-Hellical Regions

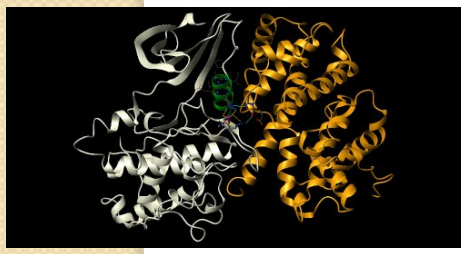


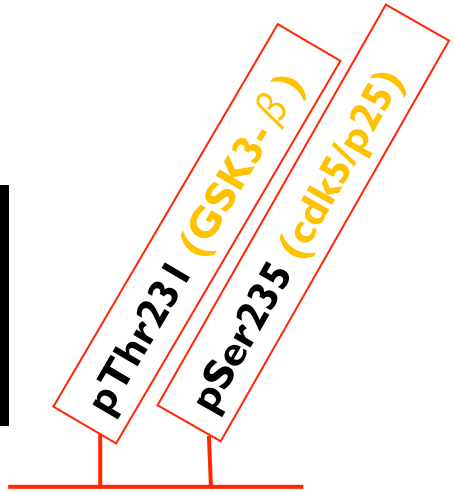




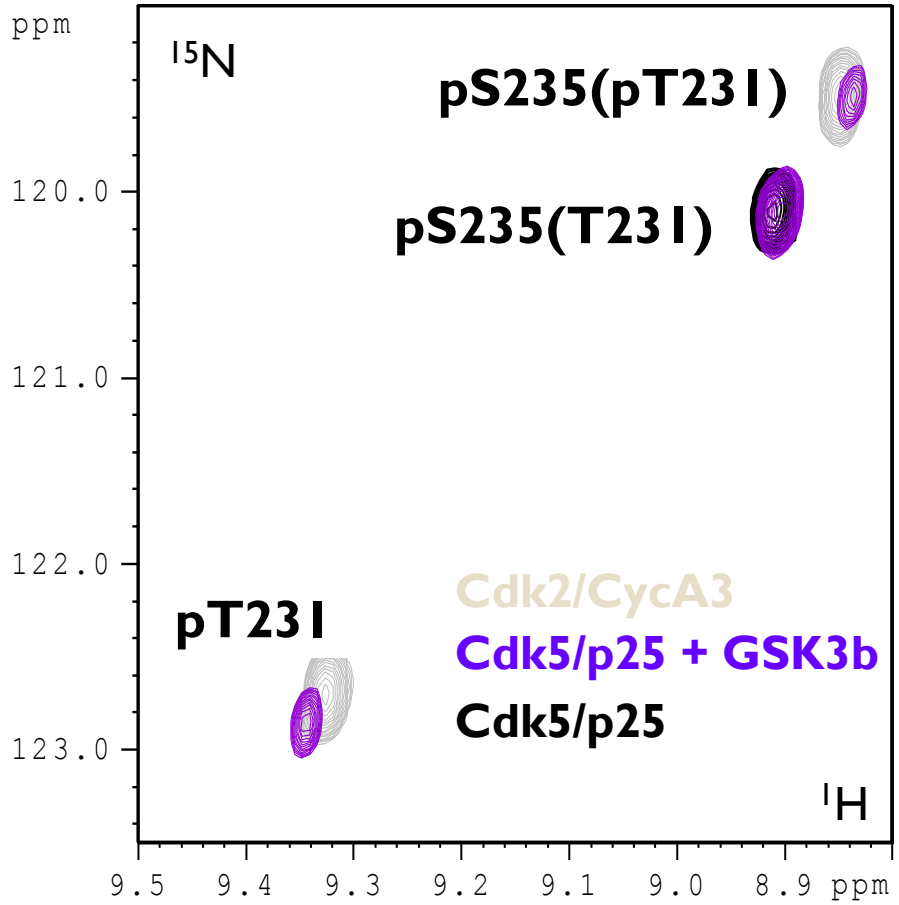
Phosphorylation and priming

Cyclin dependant kinase (CDK2) / cyclin A3 (CycA3)





Sengupta *et al.* 1997



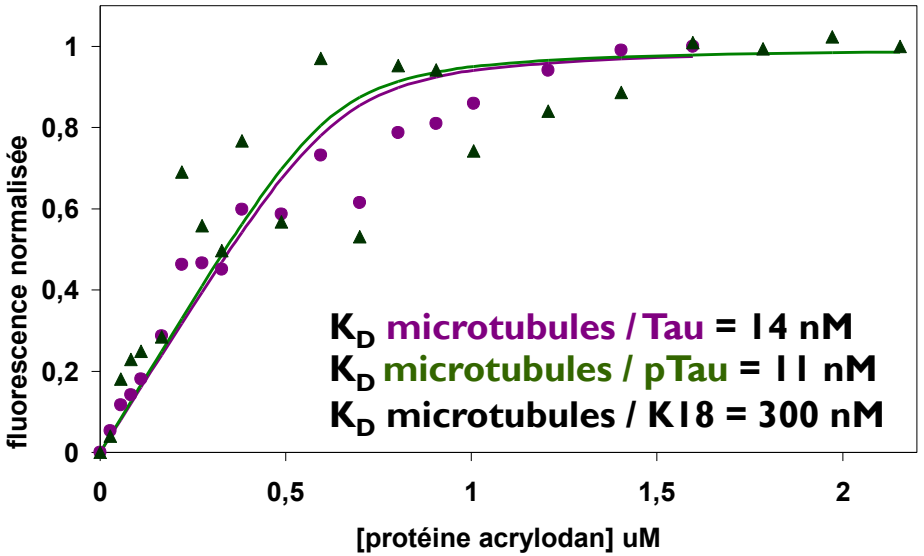
➔ Specific phospho-epitope Alzheimer disease (AT180)

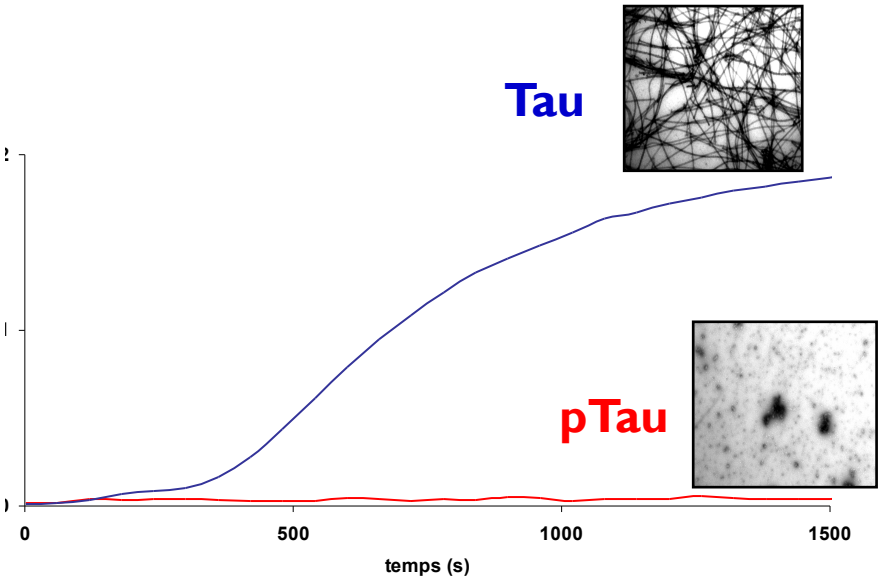
Landrieu I, Leroy A, Smet-Nocca C, Huvent I, Amniai L, Hamdane M, Sibille N, Buée L, Wieruszeski JM, Lippens G. (2010) Nuclear Magnetic Resonance Spectroscopy of the neuronal Tau protein: normal func-tion and implication in Alzheimer's disease. *Mini-review Biochem Soc Trans.* 38(4):1006-11.

Phosphorylation and pathological role

Phospho-epitope AT180 (pT231, pSer235): ability of Tau to bind MTs and its inability to polymerize tubuline

Résultats thèse Laziza Amniai





FRET of acrylodan-labeled Tau

(Ex. 420 nm, Em. 480 nm
microtubules, 1 µM)

(Preuss *et al.* 1997 ; Goode *et al.* 1997)

Light Scat. Ex. 350 nm, Em. 363 nm

Turbidité de 5.1 µM de tubuline/1.7 µM de Tau ou pTau à 37 °C
Glycérol 3.4 M, NaPi 20 mM, MgCl2 10 mM, EGTA 1 mM, pH 6.48, 0.01 mM GTP

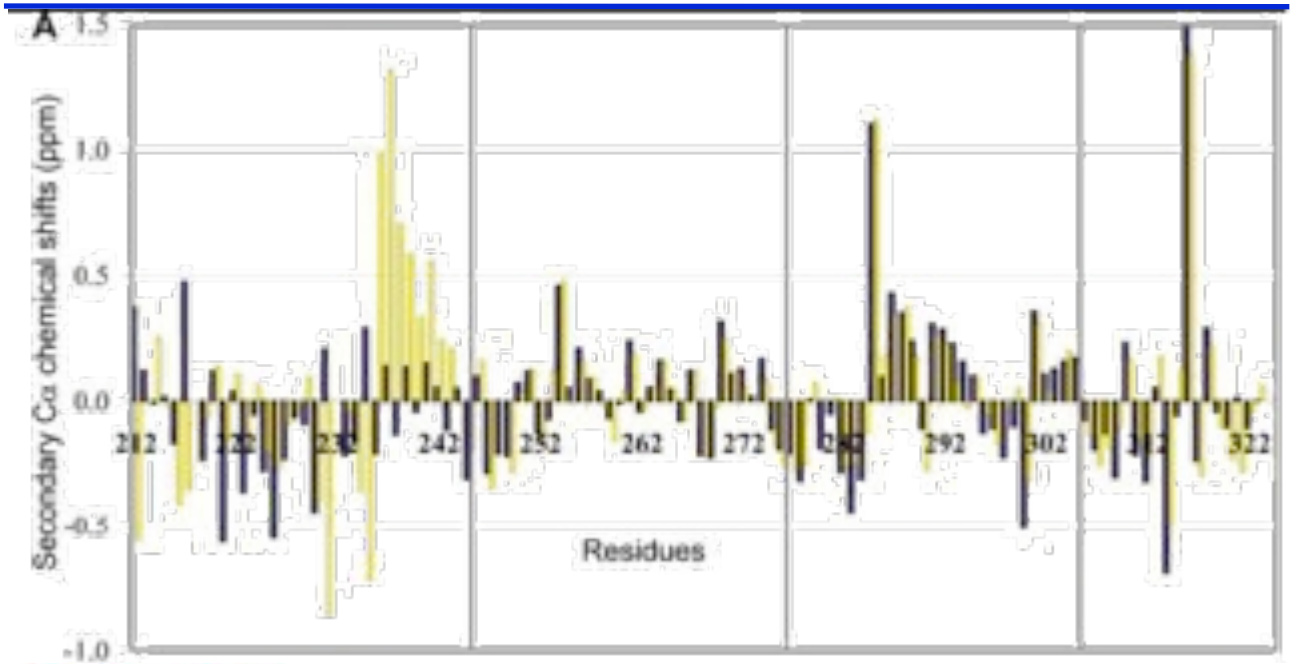
➔ **Are there Structural changes upon chemical modifications ?**

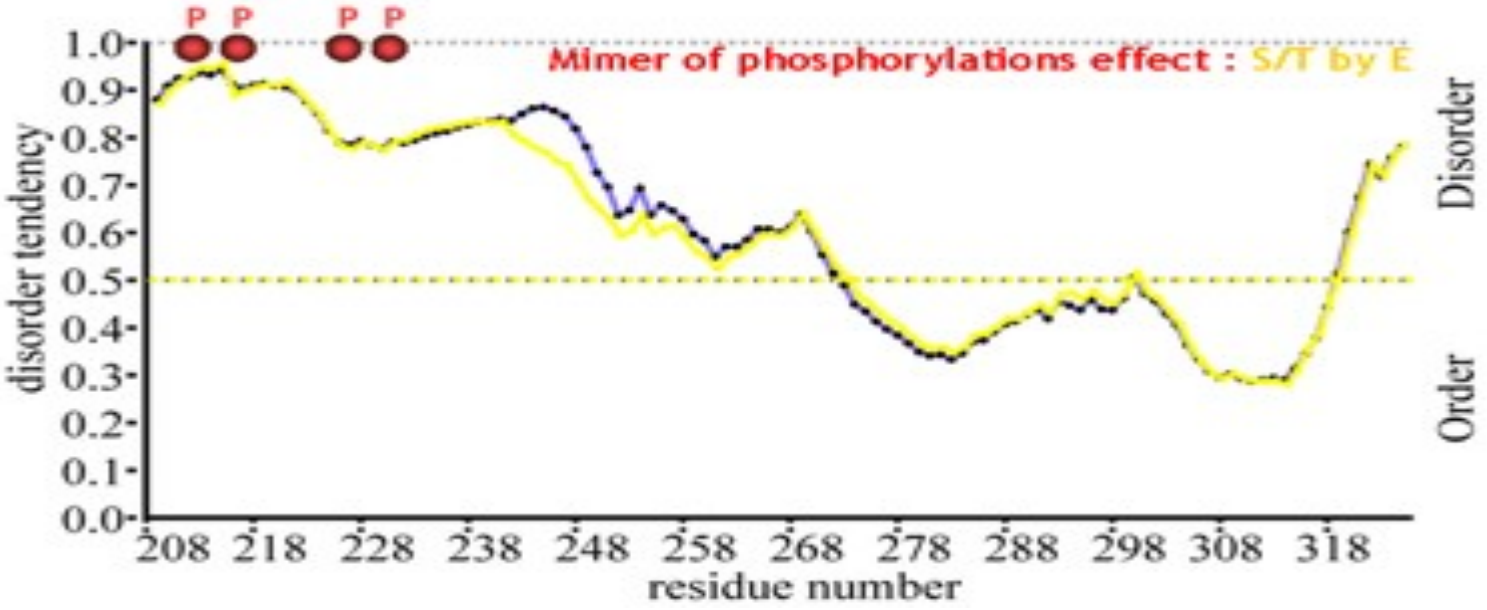
➔ **NMR characterization**

Is there some degree of structuration ?

Linked to recognition events

Here linked to a loss of function



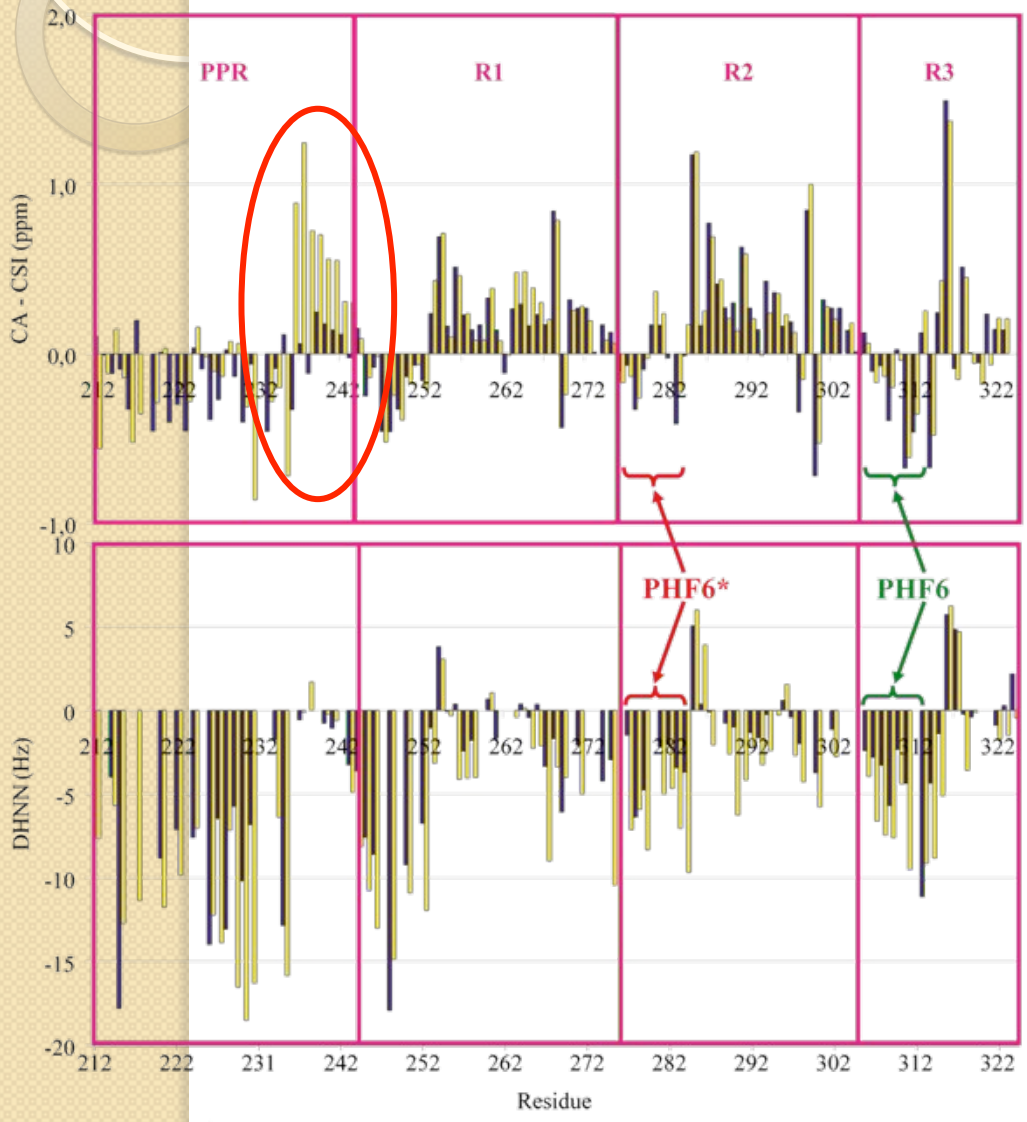


Sibille et al. **Proteins** *(2012)* 80, 454-462

Where and which kind ?

Is there some degree of structuration ?

Local structuration, short range impact

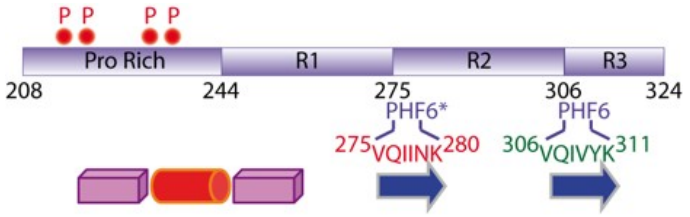


Chemical shift CA

Non Phospho

Phospho

Residual dipolar coupling



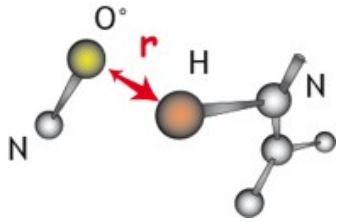
Sibille et al. **Proteins** *(2012)* 80, 454-462

Is there some transient long range structuration ?

Dynamic dependant parameters

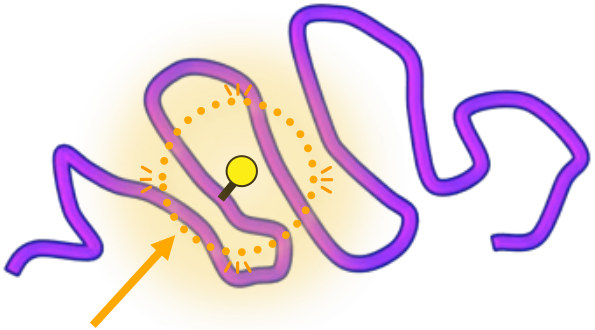
- **Paramagnetic Relaxation Enhancement (PRE)**

$$R_2 \propto \frac{\gamma_S^2 \gamma_e^2}{r^6} f(\tau_c, \tau_s \tau_i)$$



$$\frac{\gamma_e}{\gamma_H} = 658$$

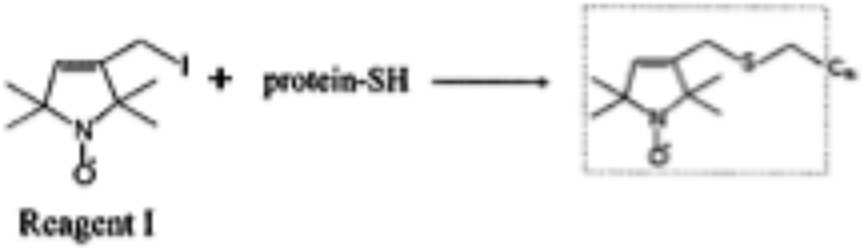
$$PRE \propto \frac{1}{r_{OH}^6}$$



Effective radius ~20 Å

- **Cysteines Proxylation**

- **PROXYL : (1-oxy-2,2,5,5-tetramethyl-D-pyrroline-3-methyl)-iodoacetamine)**

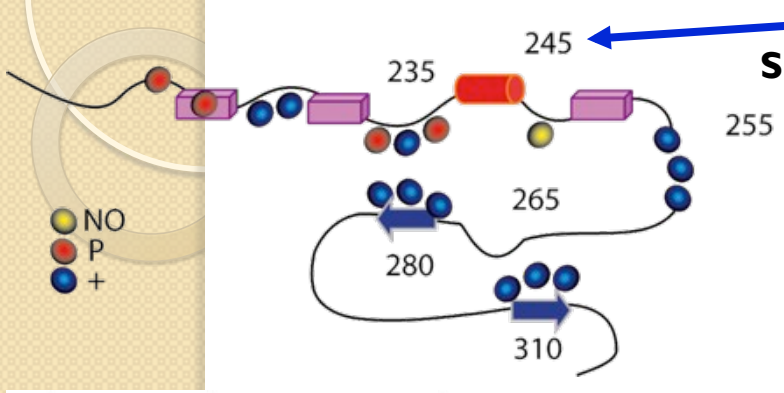


Reagent I

- **NO* (paramagnetic) + VitC = NO (diamagnetic)**

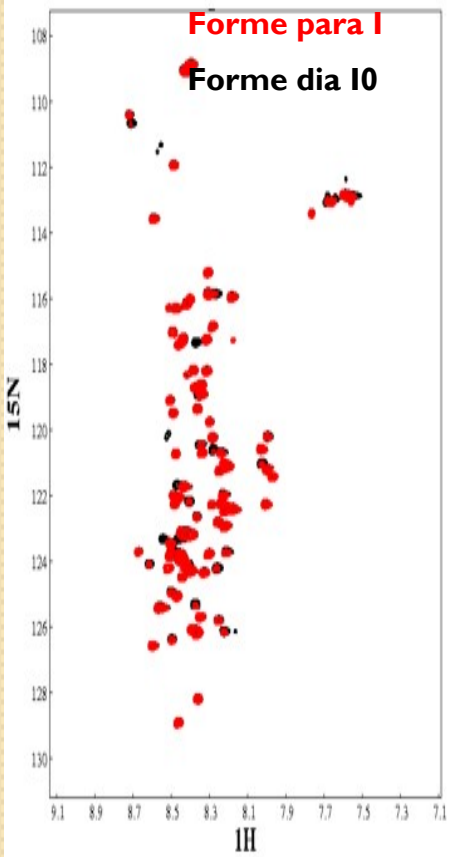
- **Use of a unique sample**

Is there some transient long range structuration ?

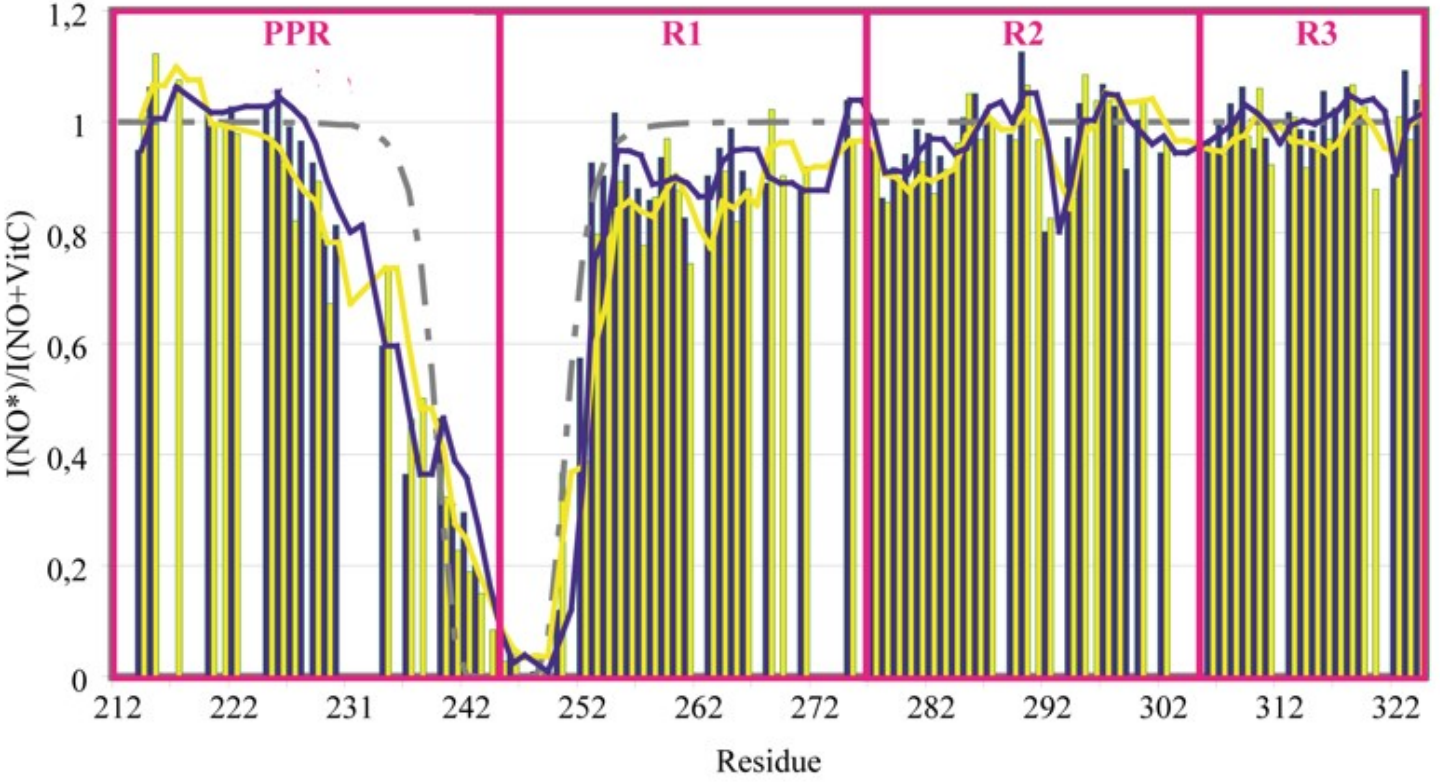


mutant :
S208/T245C/S322

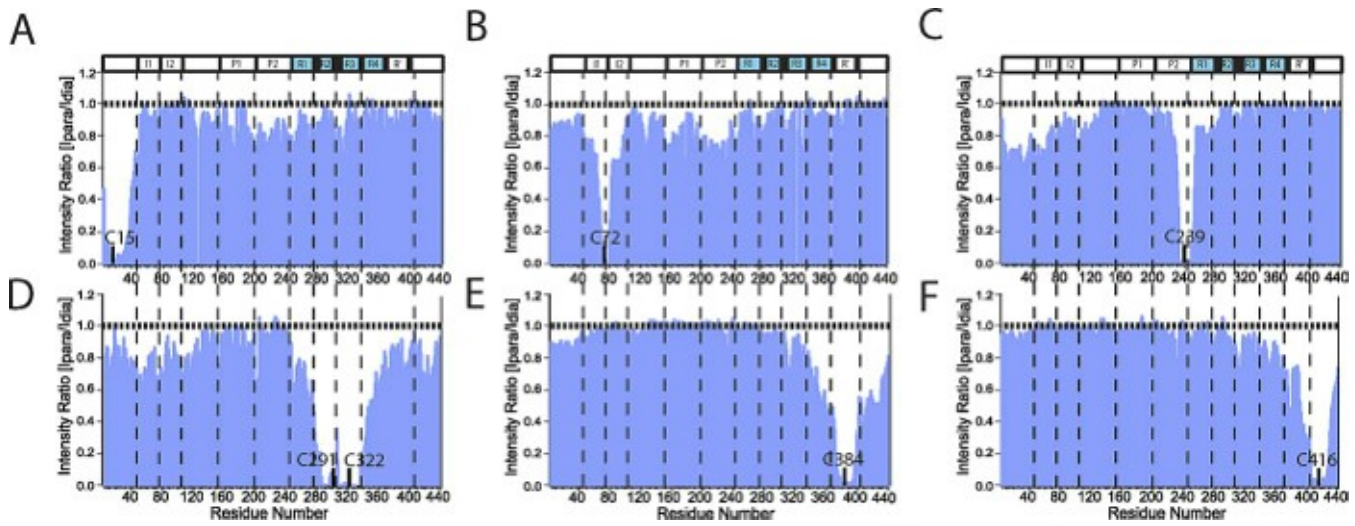
Global Structuration: Long Range Impact



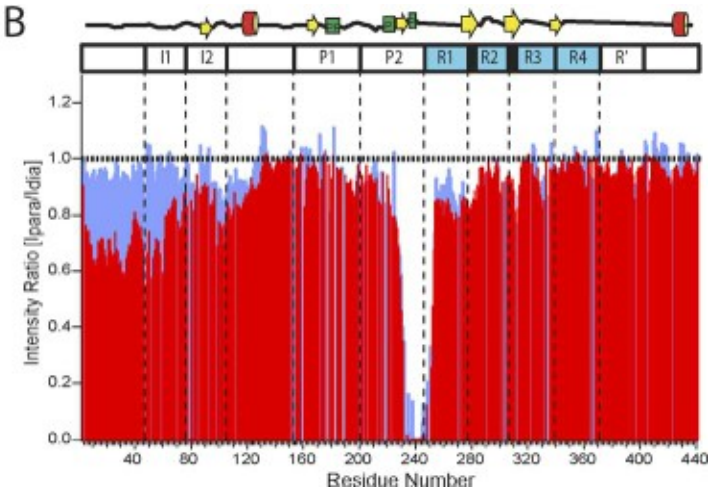
Ratio of Intensity I/I0

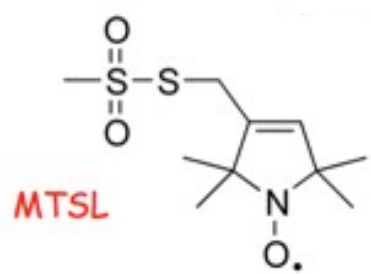


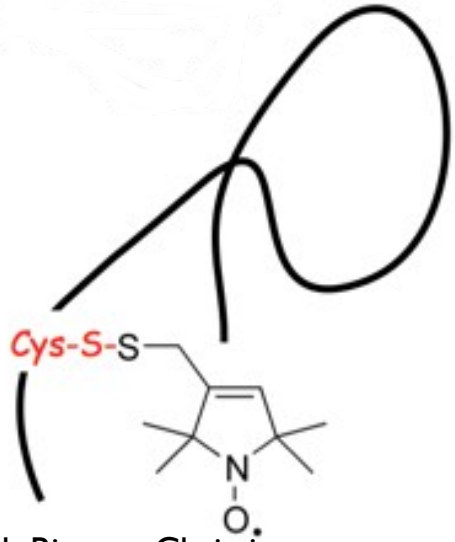
Is there some transient long range structuration ?



Buffer
600 mM
NaCl

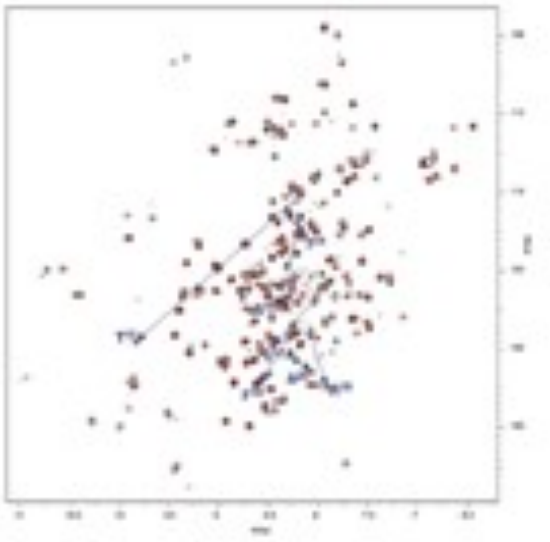


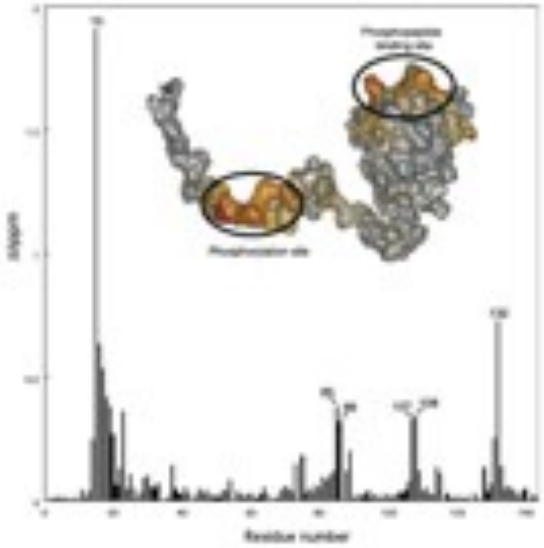


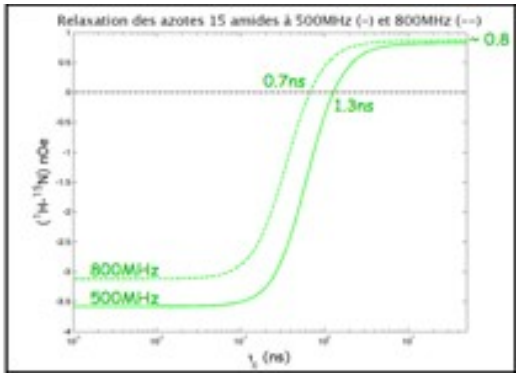


Marco D. Mukrasch, Stefan Bibow, Jegannath Korukottu, Sadasivam Jeganathan, Jacek Biernat, Christian Griesinger, Eckhard Mandelkow, Markus Zweckstetter* (2009) Structural Polymorphism of 441-Residue Tau at Single Residue Resolution
PLoS Biology, 7(2), 399

Phosphorylation and internal dynamic

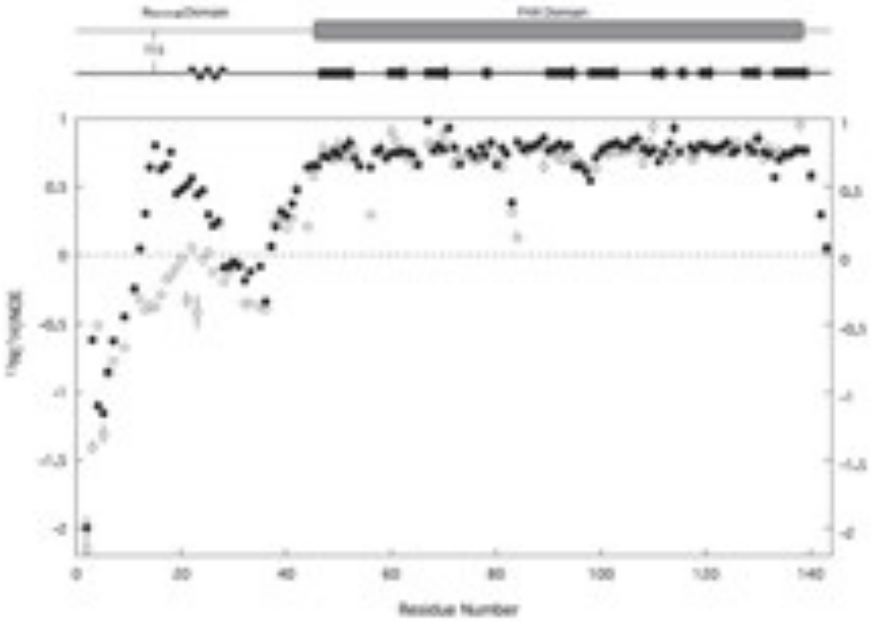






hetNOE (^{1}H->^{15}N) is easy to measure and gives qualitative information about the local flexibility of ^{1}H-^{15}N bounds in 100 ps range.

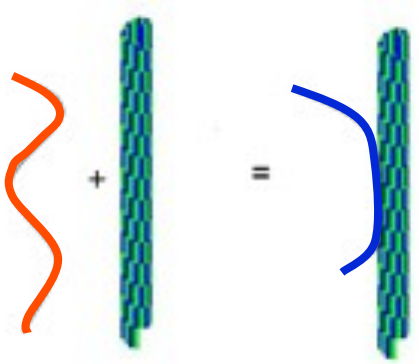
Figure Philippe Barthe



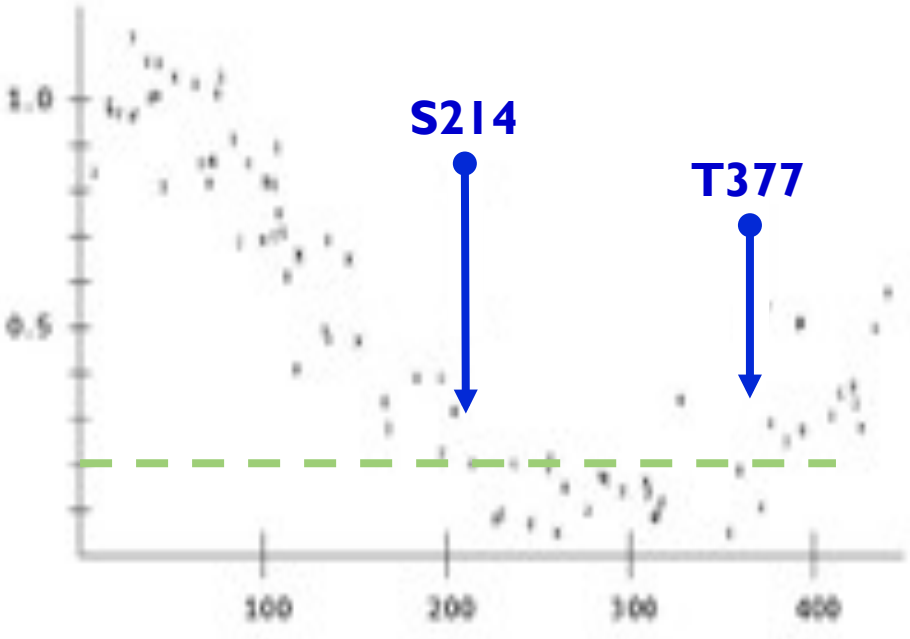
Philippe Barthe, Christian Roumestand, Marc J. Canova, Laurnt Kremer, Corinne Hurand, Virginie Molle and Martin Cohen-Gonsaud, "Dynamic and Structural Characterization of a Bacterial FHA Protein Reveals a New Autoinhibition Mechanism". Structure 17, 568-578 (2009).

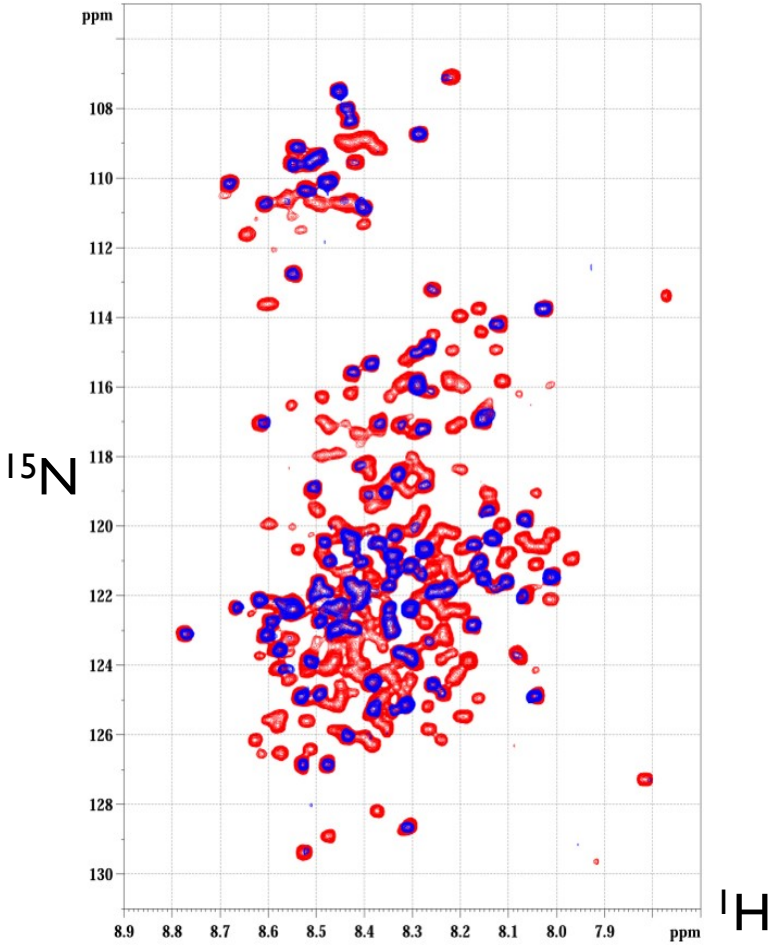
To X-Ray Crystallography

Structure determination of the bound form



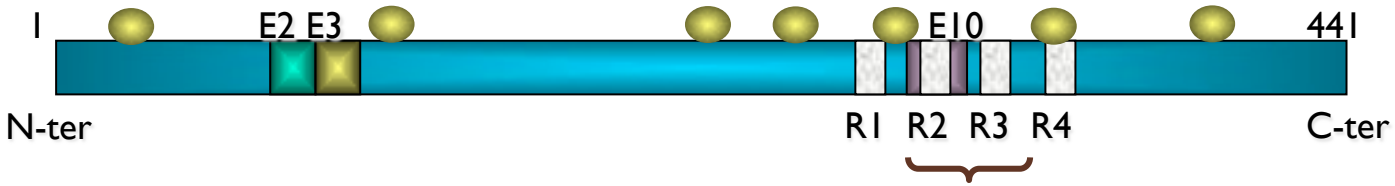
Delimitate the bound region to the partner



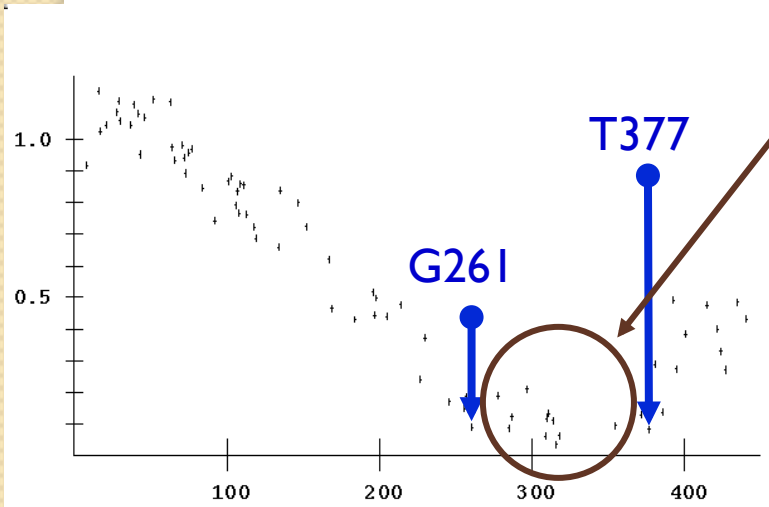


NMR spectra of free form (54 kDa) (5mM) and bound form (TeraDalton) (20mM)

Aggregation



- Localisation des régions intégrées dans le corps des fibres PHF

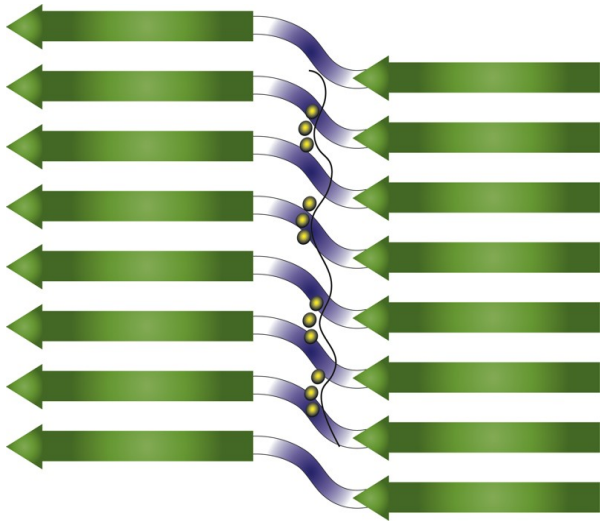


Rapport des intensités des spectres HSQC de Tau libre en solution et de Tau intégré dans les fibres PHF

Sillen et al., Chembiochem 2005

- Modèle

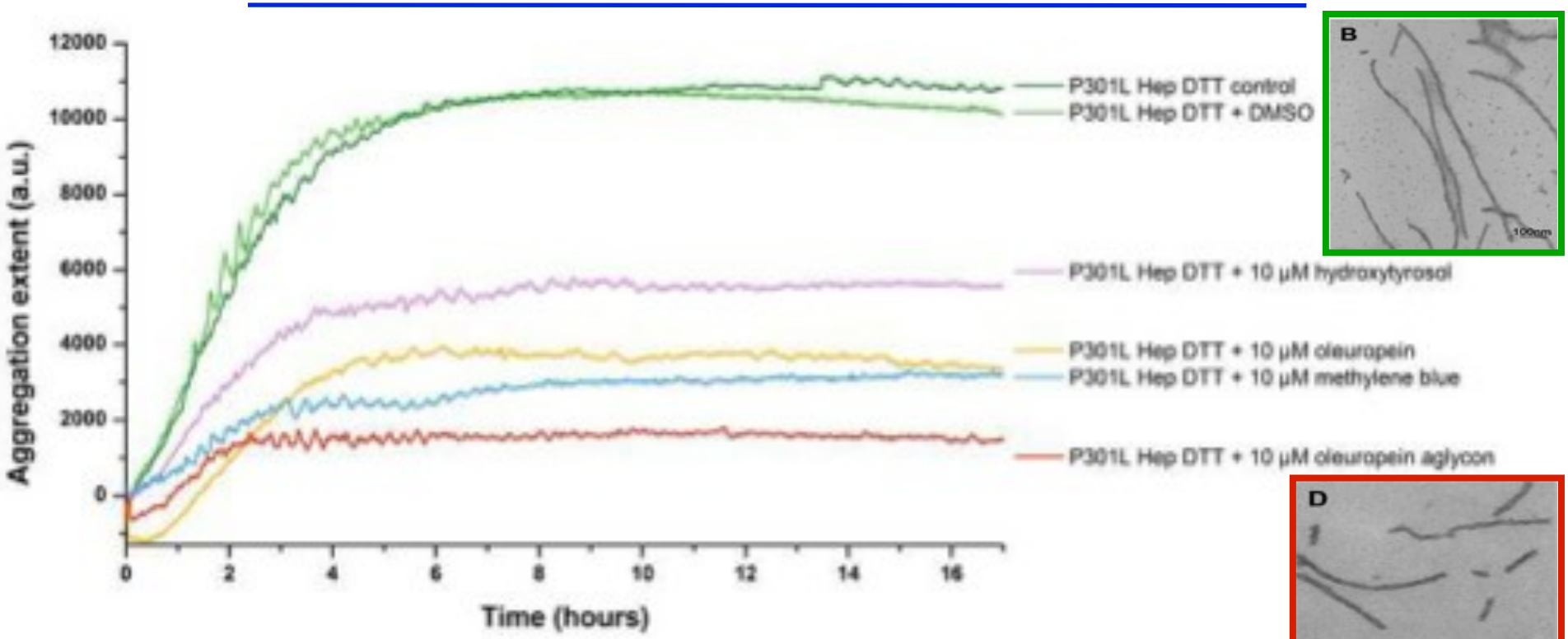
Margittai et Langen, PNAS, 2004



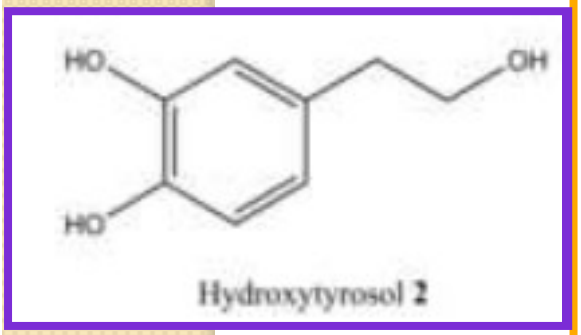
$_{274}$V Q I I N K K L D L S N V$_{286}$

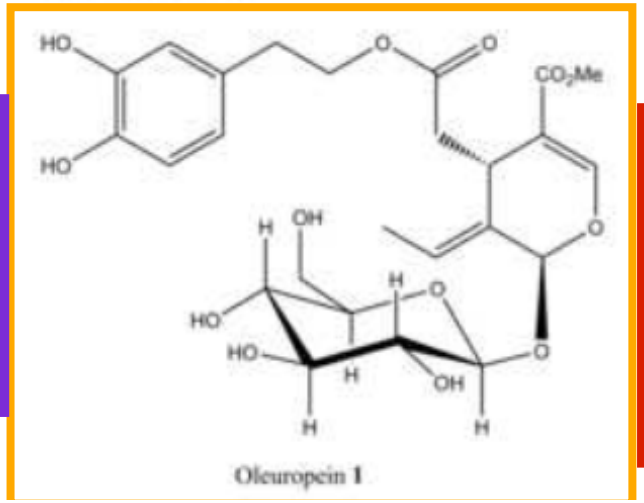
N. Sibille *et al.* Biochemistry 45(41):12560-72, 2006

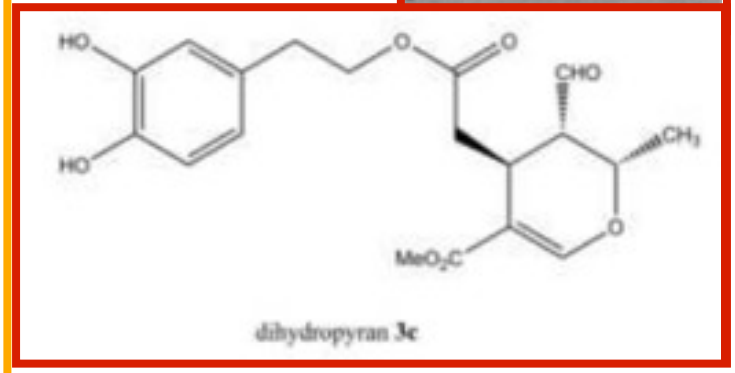
Aggregation



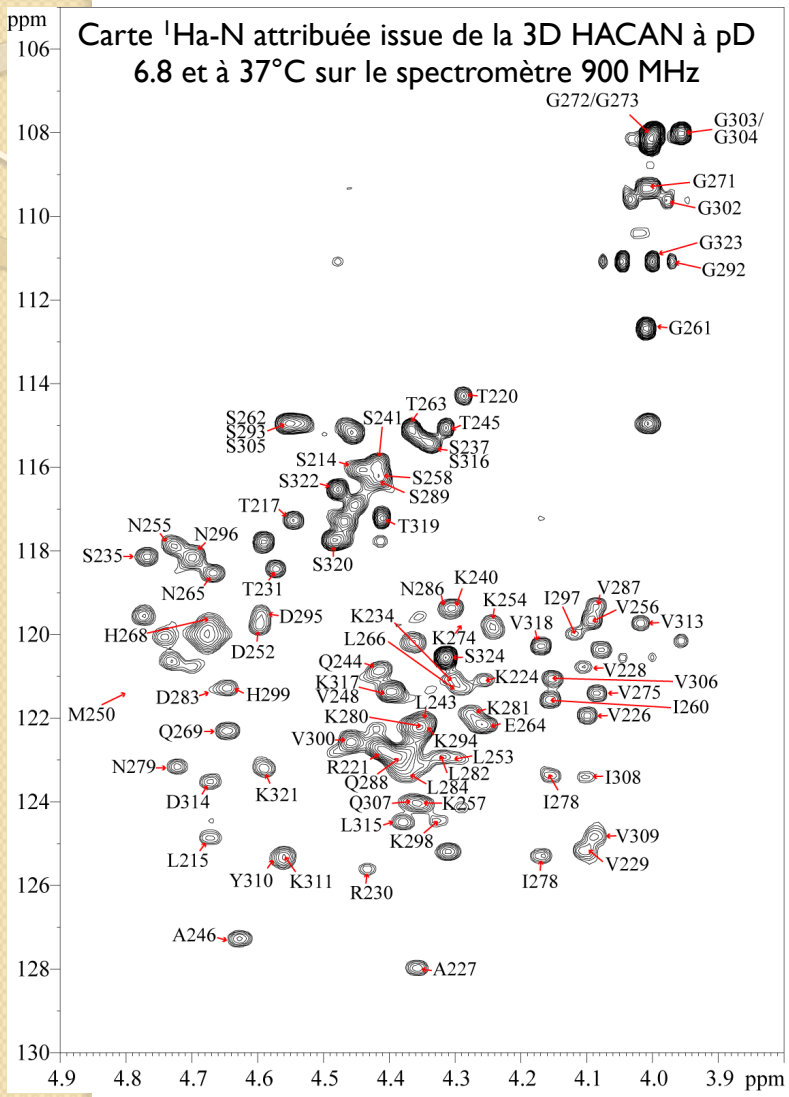
Résultats thèse Anthony Daccache

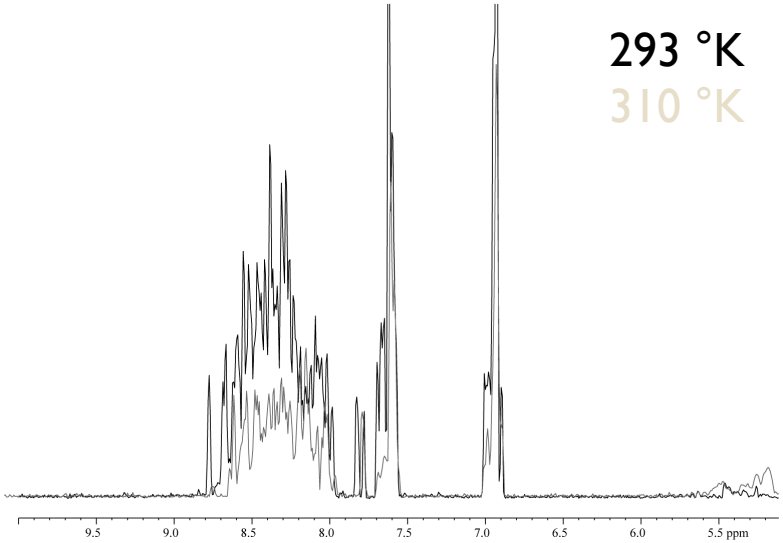






Aggregation and drug design





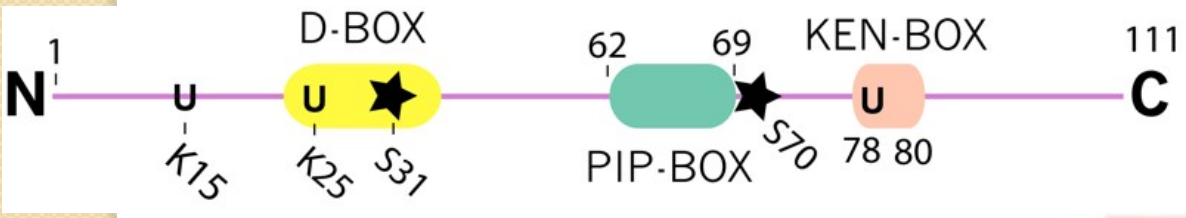
➔ NMR experiments adapted for physiological condition of Tau aggregation

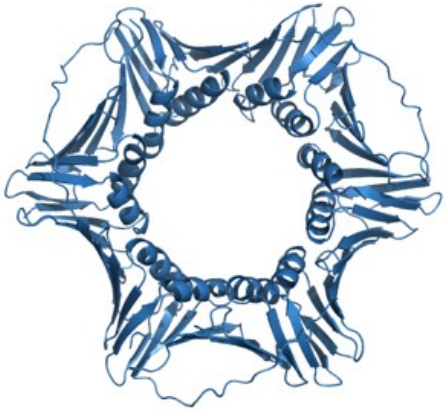
➔ *in vitro* aggregation kinetic with anti aggregative drugs

HA-N experiments for the backbone assignment of intrinsically disordered neuronal Tau proteins at physiological temperature

p15^{PAF} a PCNA-Binding Protein

- ✓ a 12 kDa nuclear protein that acts as a regulator of DNA repair
- ✓ It binds PCNA (DNA clamp) through its conserved PIP-BOX
- ✓ is over-expressed in several types of human cancer
- ✓ Targeted for degradation by the ubiquitin ligase APC through its conserved KEN-BOX.





PCNA

PIP Box

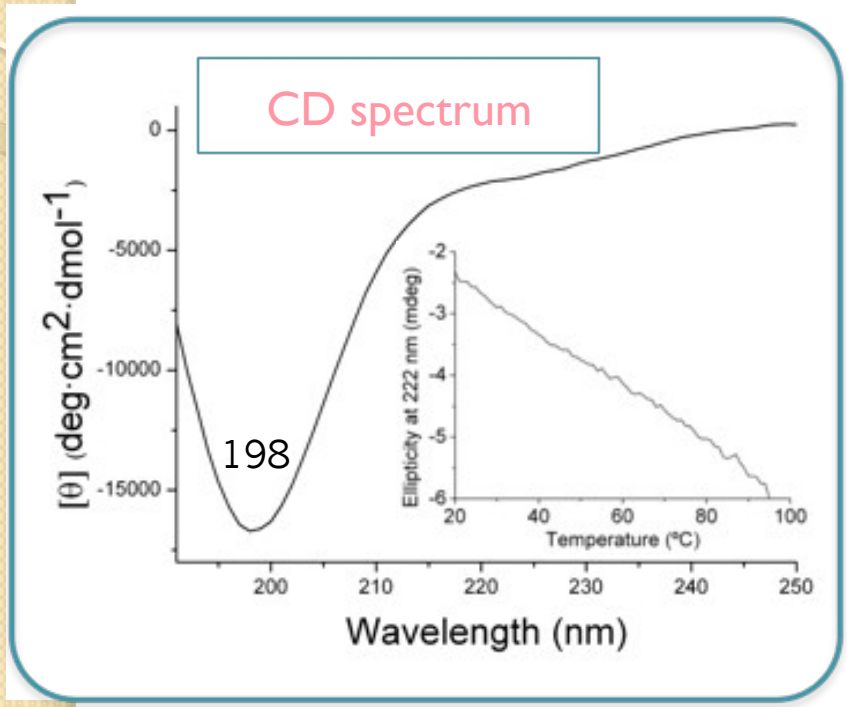
Mouse	QKGIGEFFR-LS
Human	QKGIGEFFR-LS
Bovine	QKGIGEFFS-LS
Xenopus	QKGIGDFFGSPS

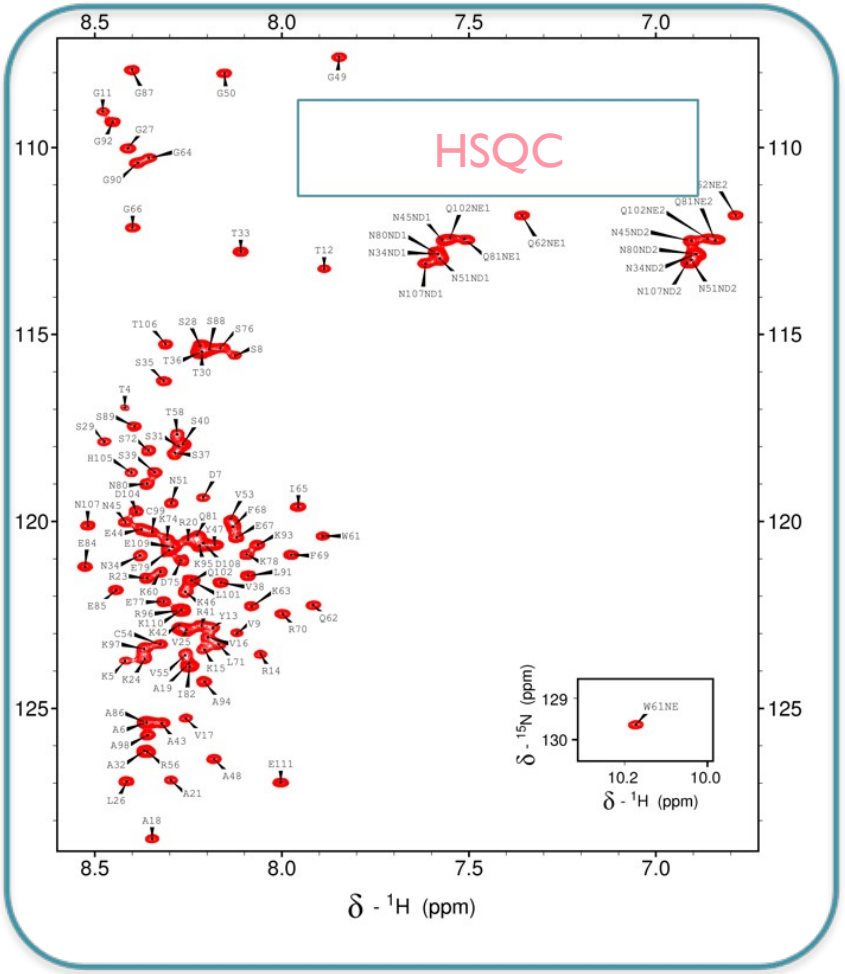
KEN Box

Mouse	SKKENQAP
Human	SEKENQIP
Bovine	SEKENRIP
Xenopus	PEKENRIP

Col. Francisco Blanco (CIC-BioGune-Bilbao)

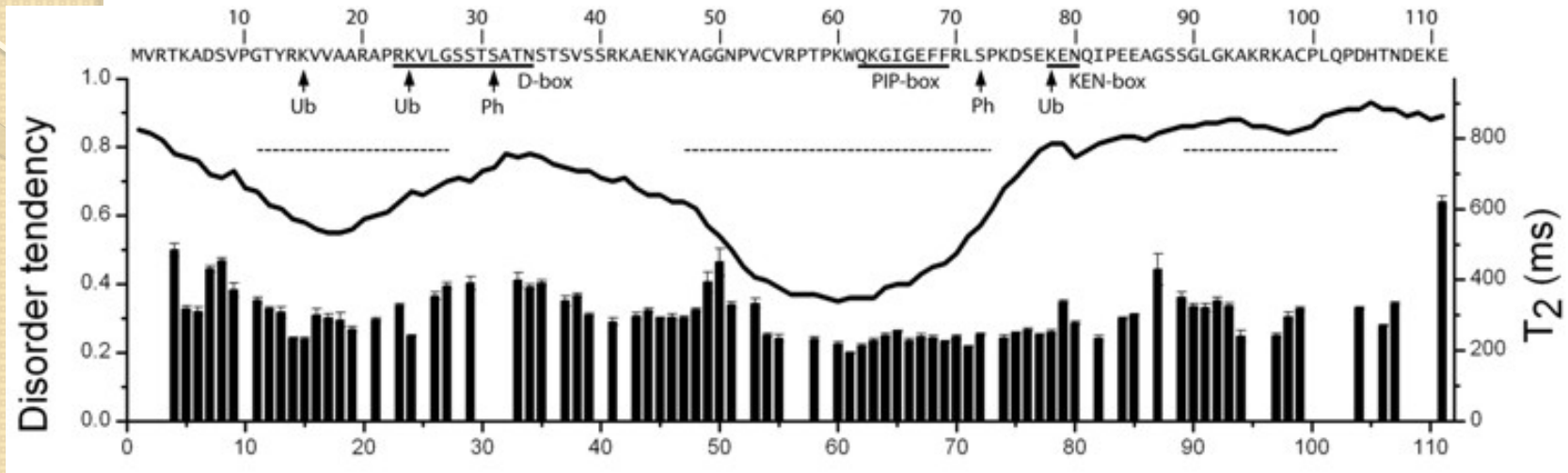
p15^{PAF} is an IDP





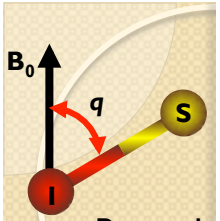
The **far-UV CD** profile and backbone amide ^{1}H chemical shifts as seen in the **^{1}H-^{15}N HSQC** spectrum are consistent with a predominantly **Random Coil** protein.

p15^{PAF} is an IDP … but not everywhere

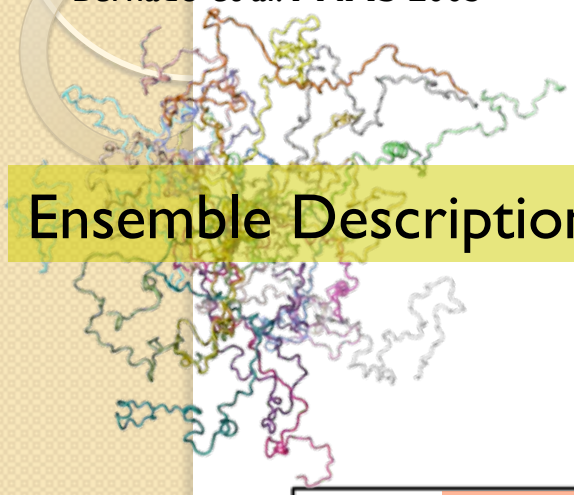


- ✓ Disorder prediction is consistent with p15 being largely unstructured, with the exception of residues of the highly conserved PIP-box motif.
- ✓ p15 shows several relatively short T_2 values, that correspond to sequences with reduced predicted disorder.

Transient structuration monitored by RDCs



Bernado & Blackledge **Nature** 2010
Bernado et al. **PNAS** 2005



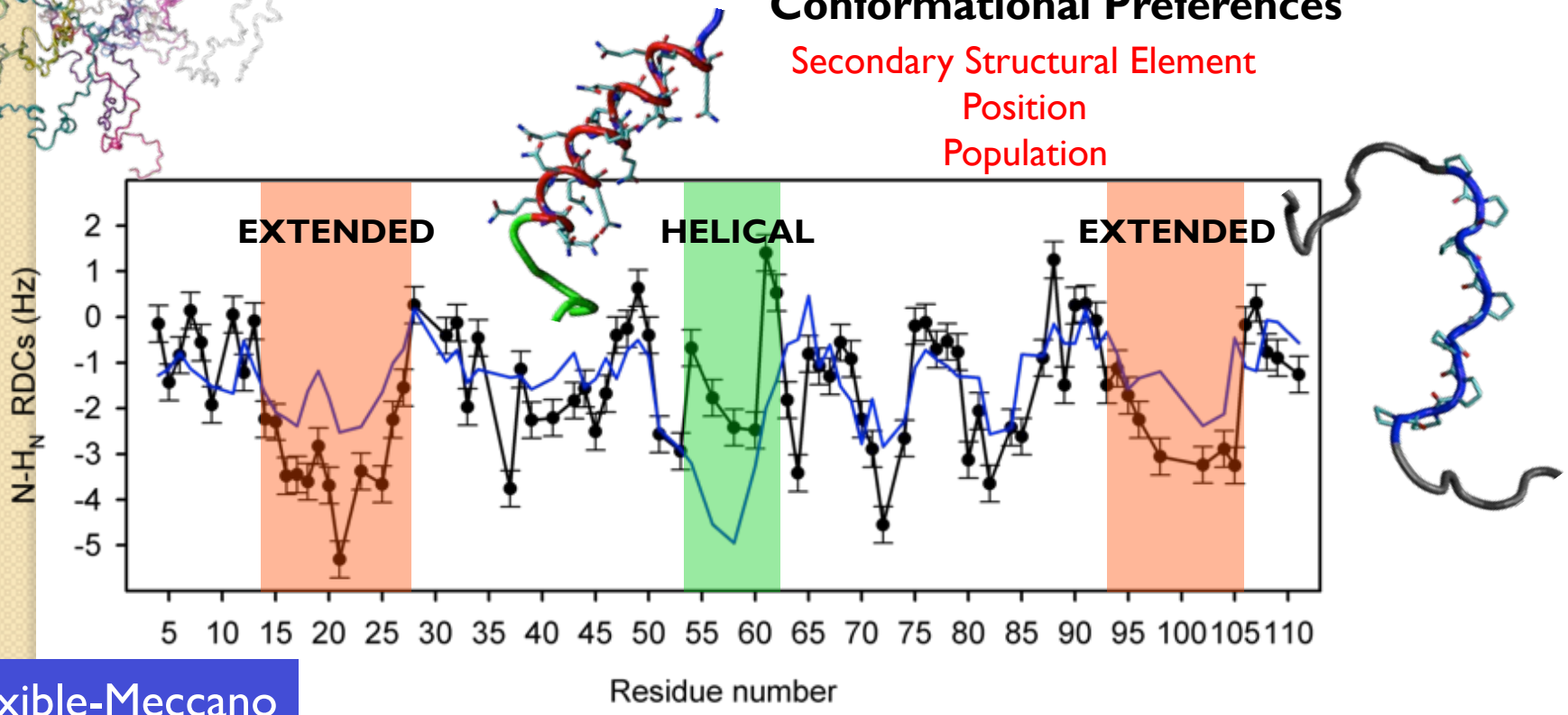
Ensemble Description

Disordered but, according to the RDCs, it presents several sites with partial structuration

FM Random Coil model helps in identifying the nature of the structural elements observed

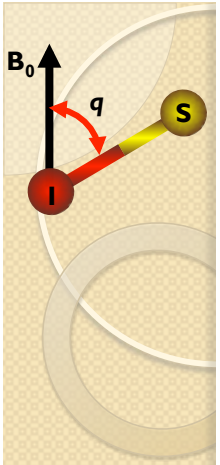
Conformational Preferences

Secondary Structural Element
Position
Population

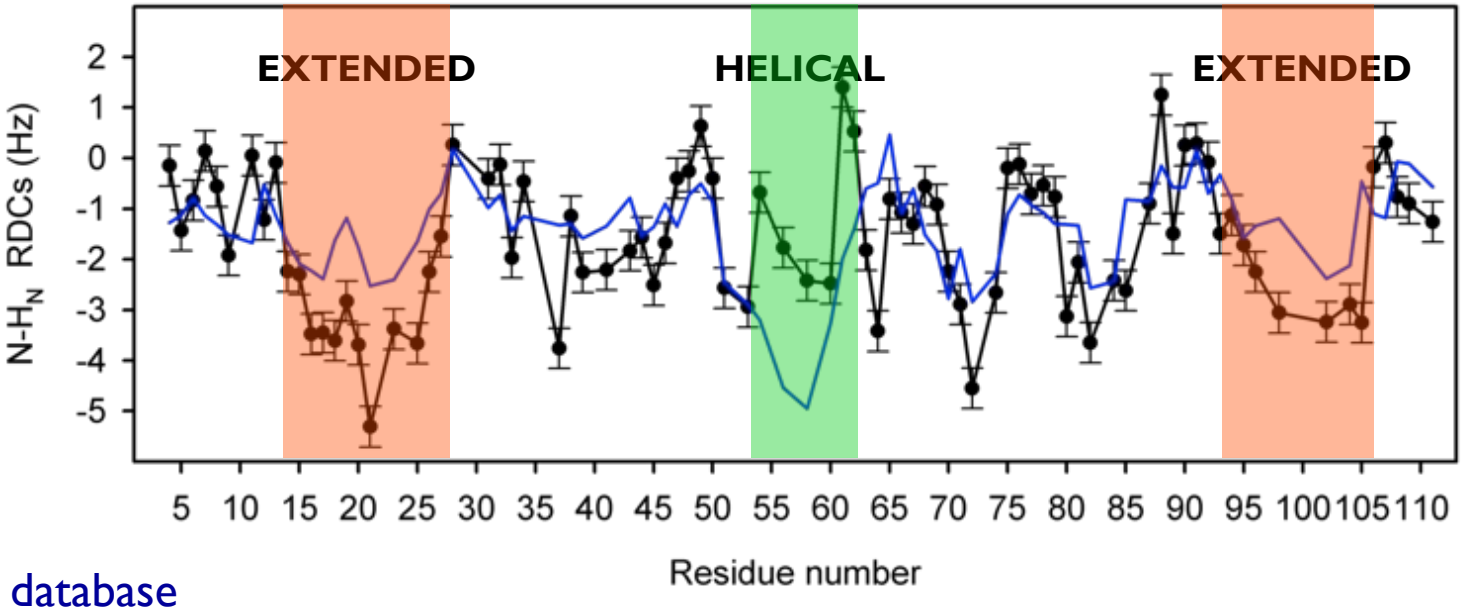


Flexible-Meccano

100,000 RC structures with FM

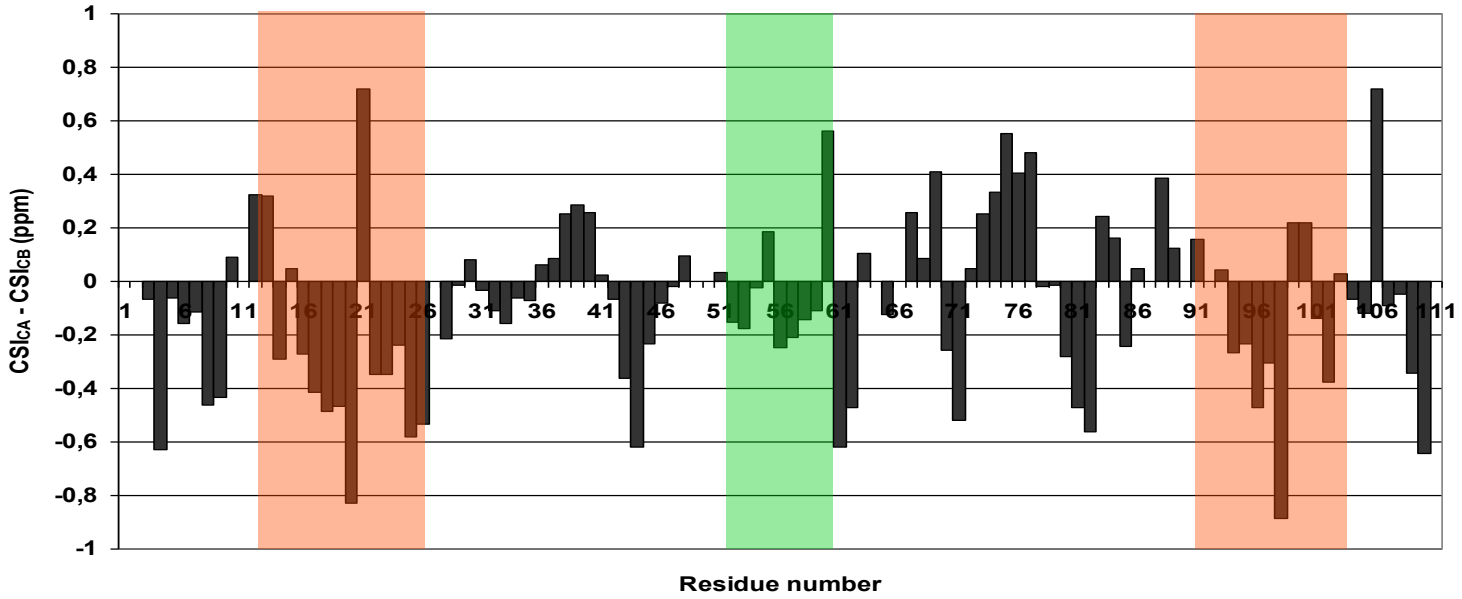


Transient structuration monitored by RDCs

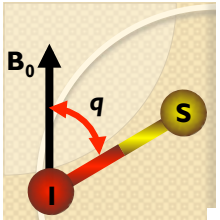


RC RDC
Exp RDC

Mulder's database



CSI

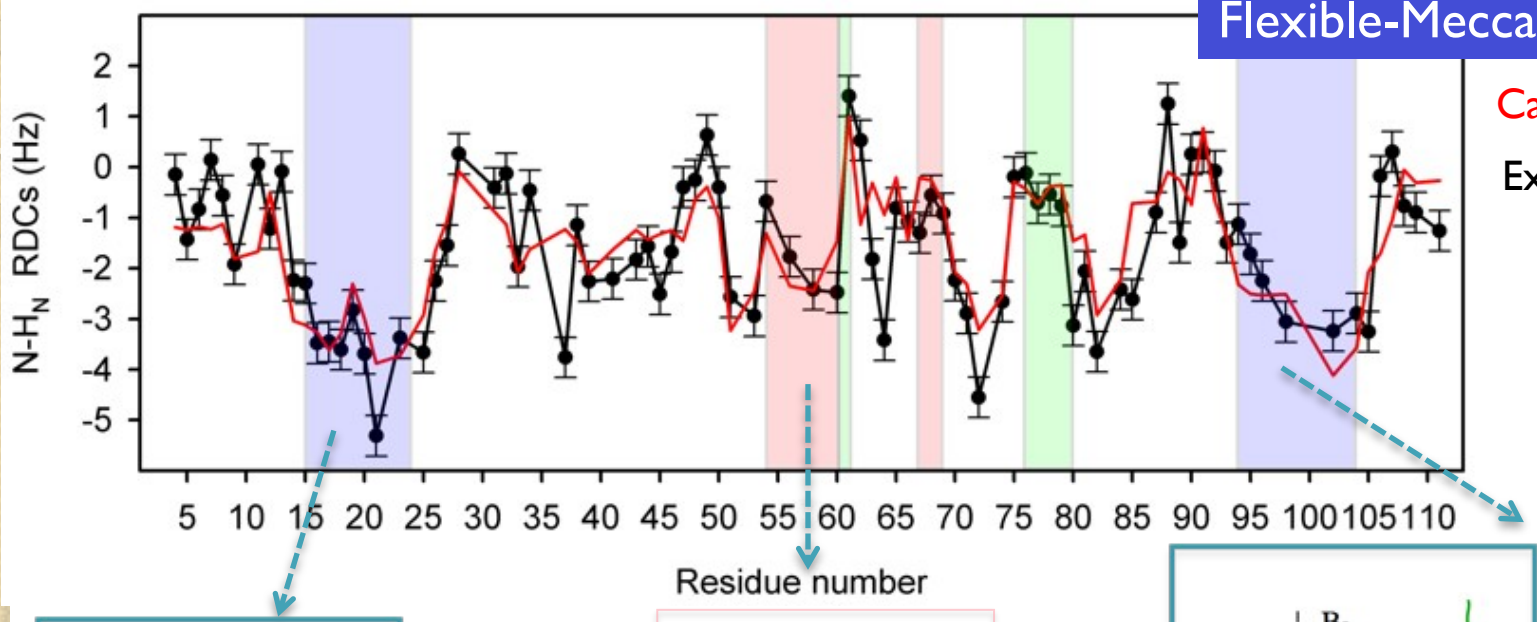


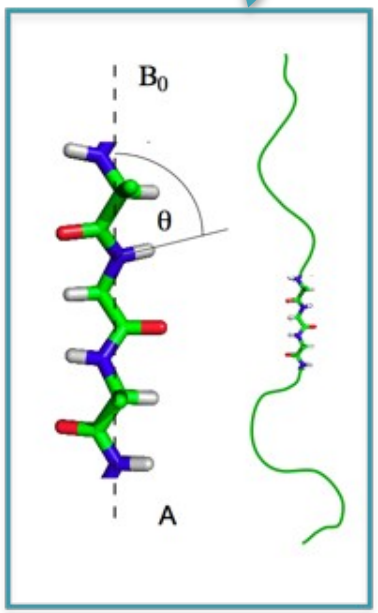
Structural Optimization

Flexible-Meccano

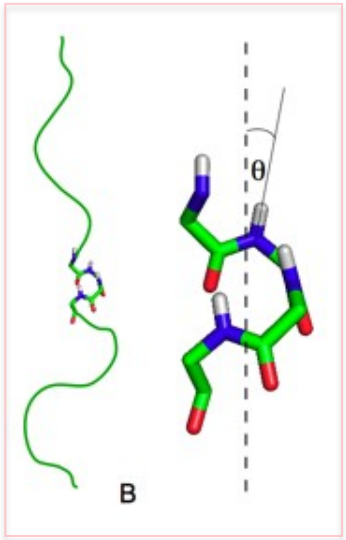
Calc RDC

Exp RDC

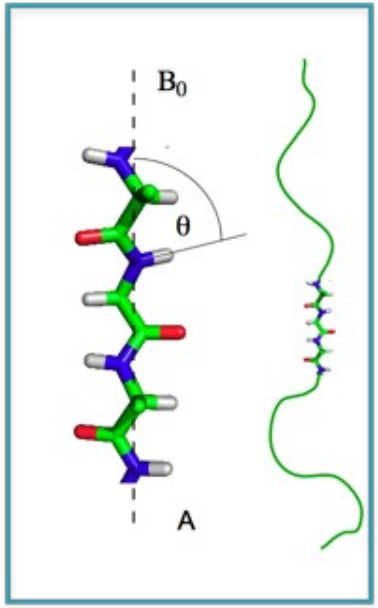




10%



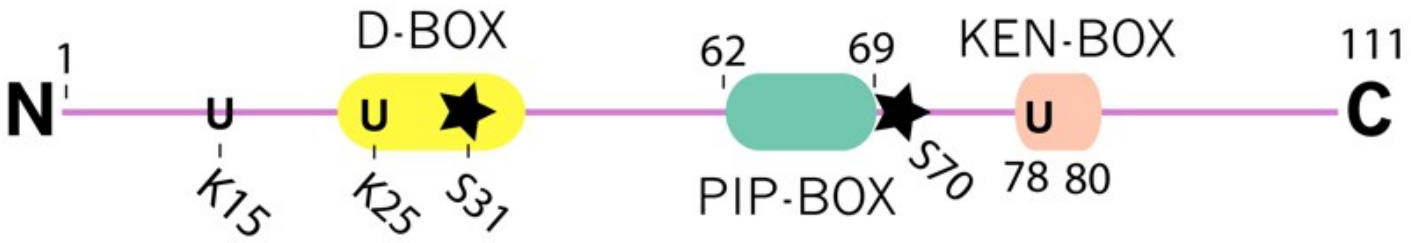
5%

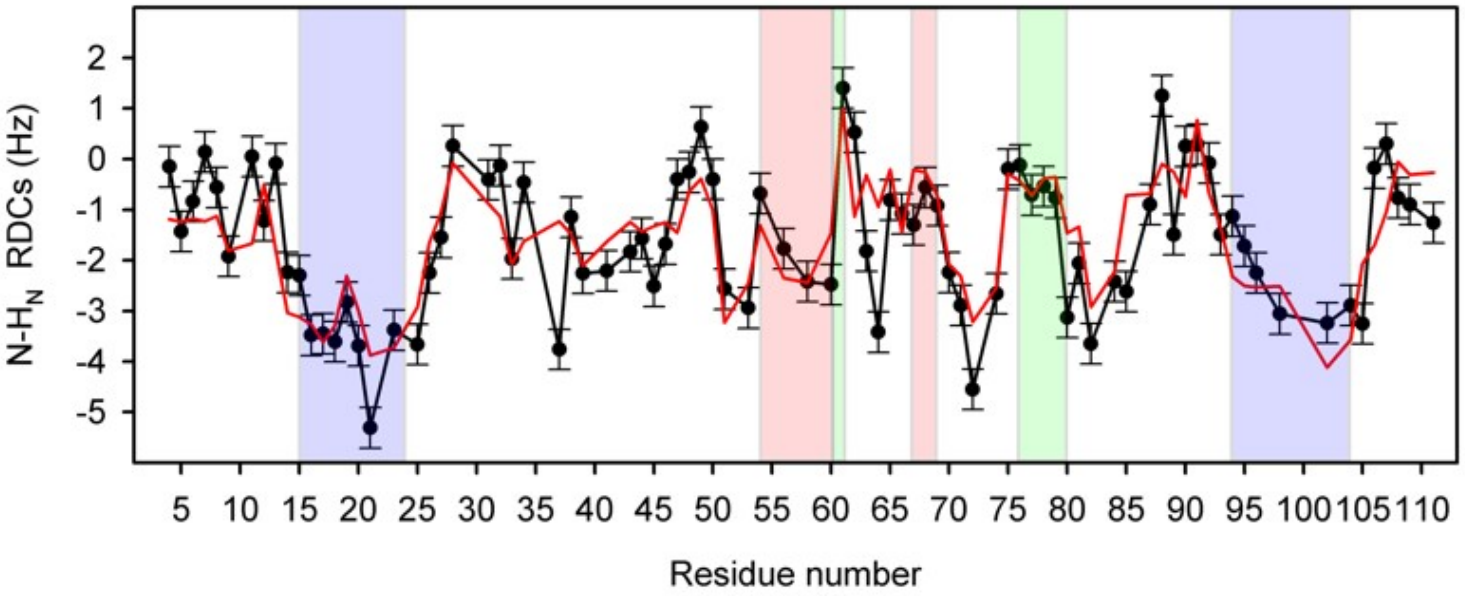


8%

100,000 structures with FM

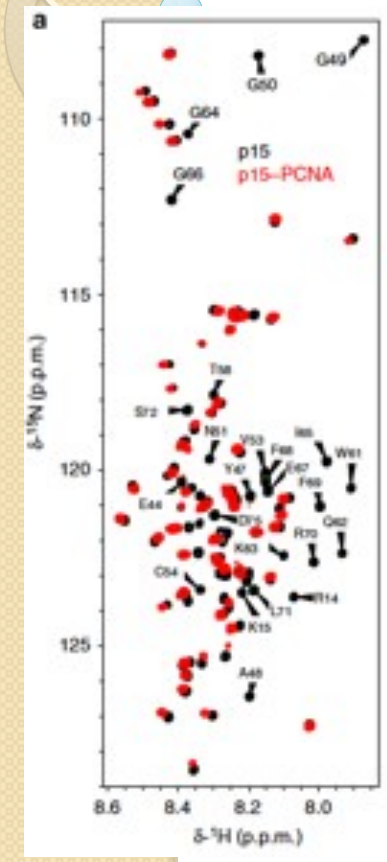
p15^{PAF} is an IDP with partial Structuration at Interaction Sites

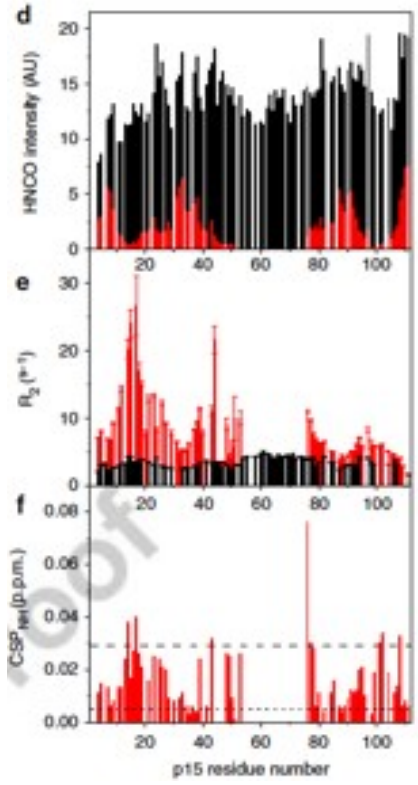


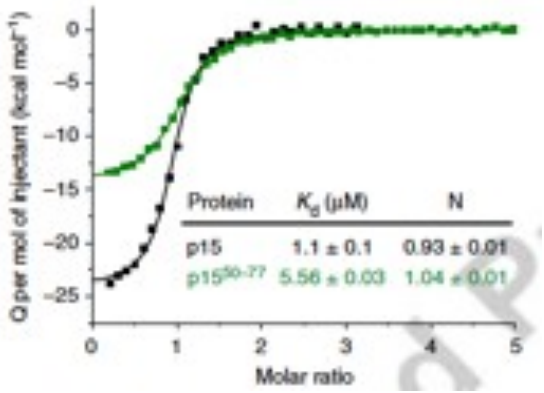


De Biasio A, Ibáñez de Opakua A, Cordeiro TN, Villate M, Merino N, Sibille N, Lelli M, Diercks T, Bernadó P, Blanco FJ. p15PAF is an intrinsically disordered protein with nonrandom structural preferences at sites of interaction with other proteins. Biophys J. 2014 Feb 18;106(4):865-74

Interaction of p15^{PAF} with PCNA

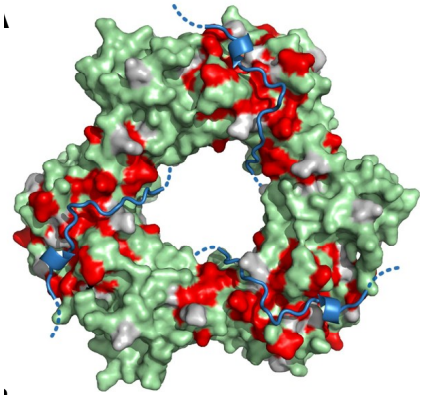




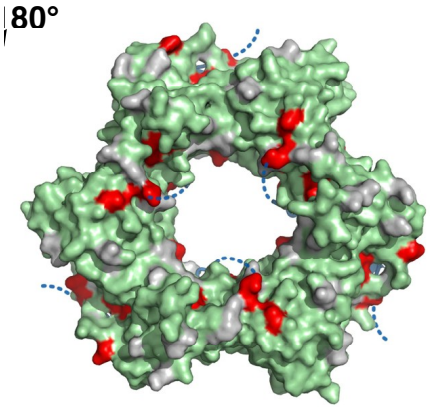


✓ Mapping of p15 binding on PCNA by NMR

PCNA front-side

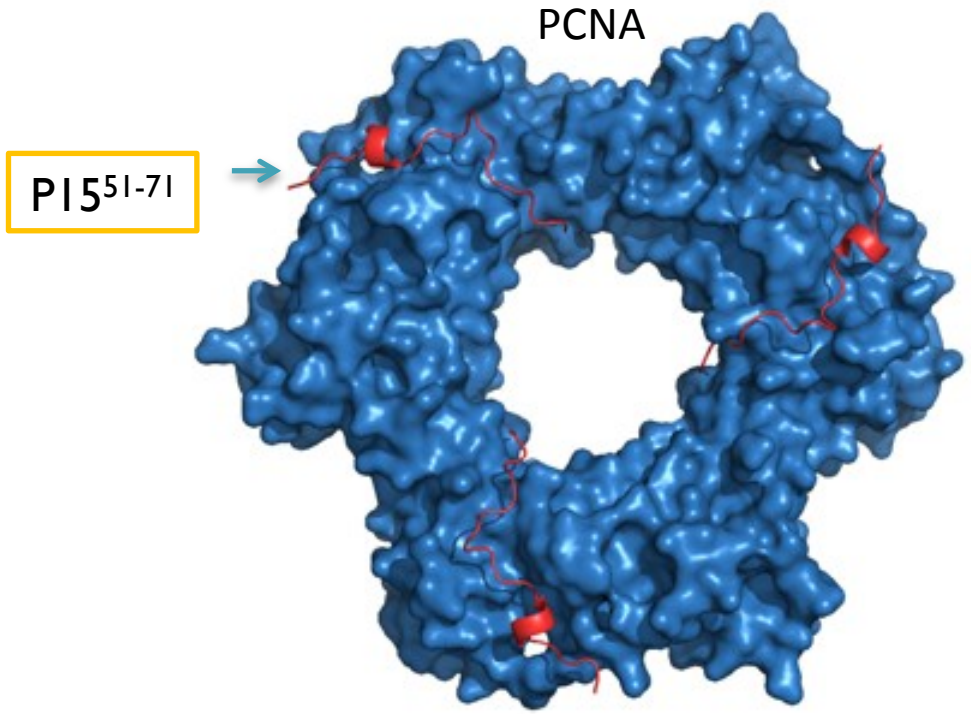


PCNA back-side



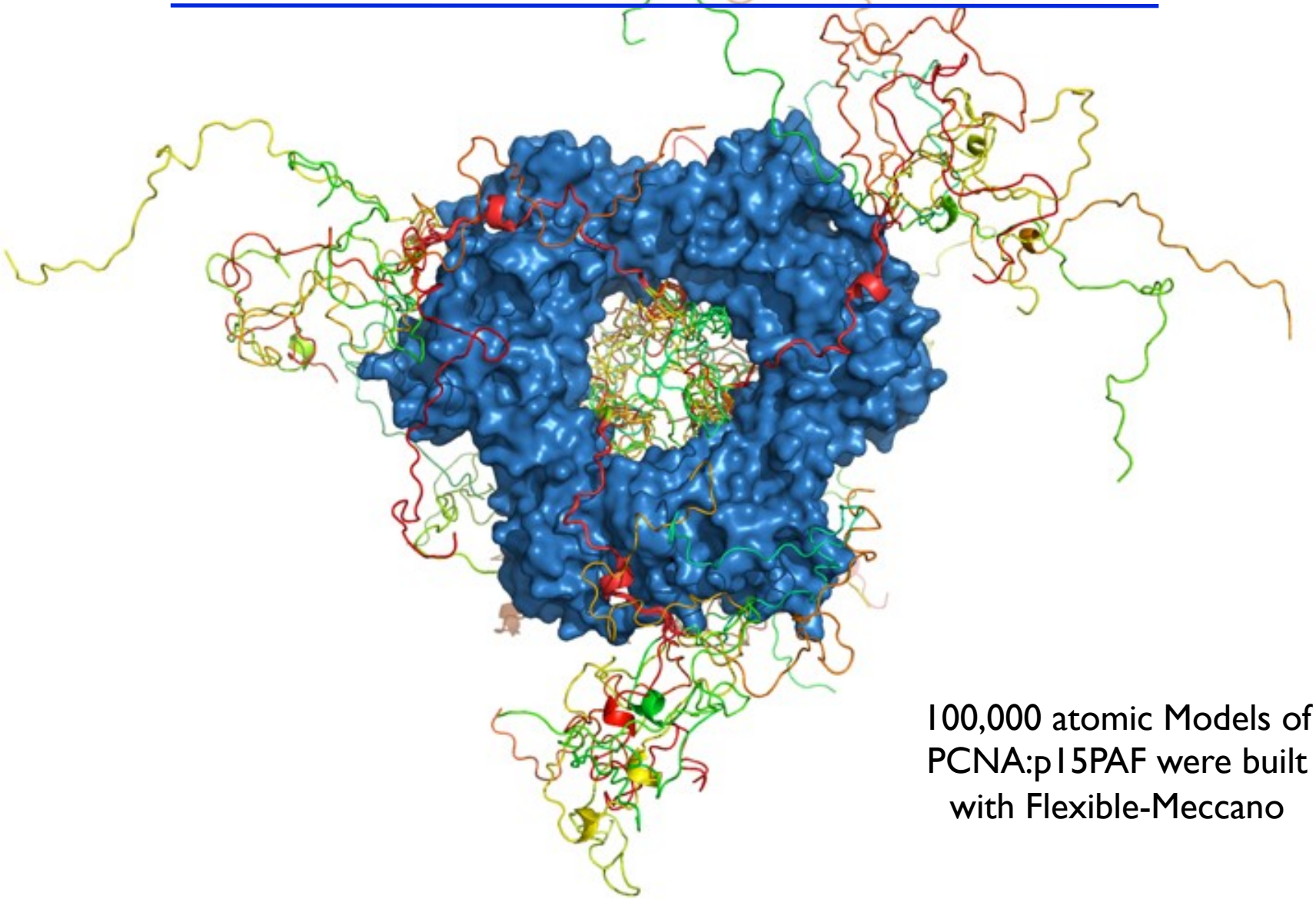
De Biasio *et al. Nature Comm.* 2015

Crystallographic Structure of p15^{PAF} with PCNA



PDB:4D2G

A 3D Model of the PCNA:p15^{PAF} Complex



100,000 atomic Models of PCNA:p15PAF were built with Flexible-Meccano

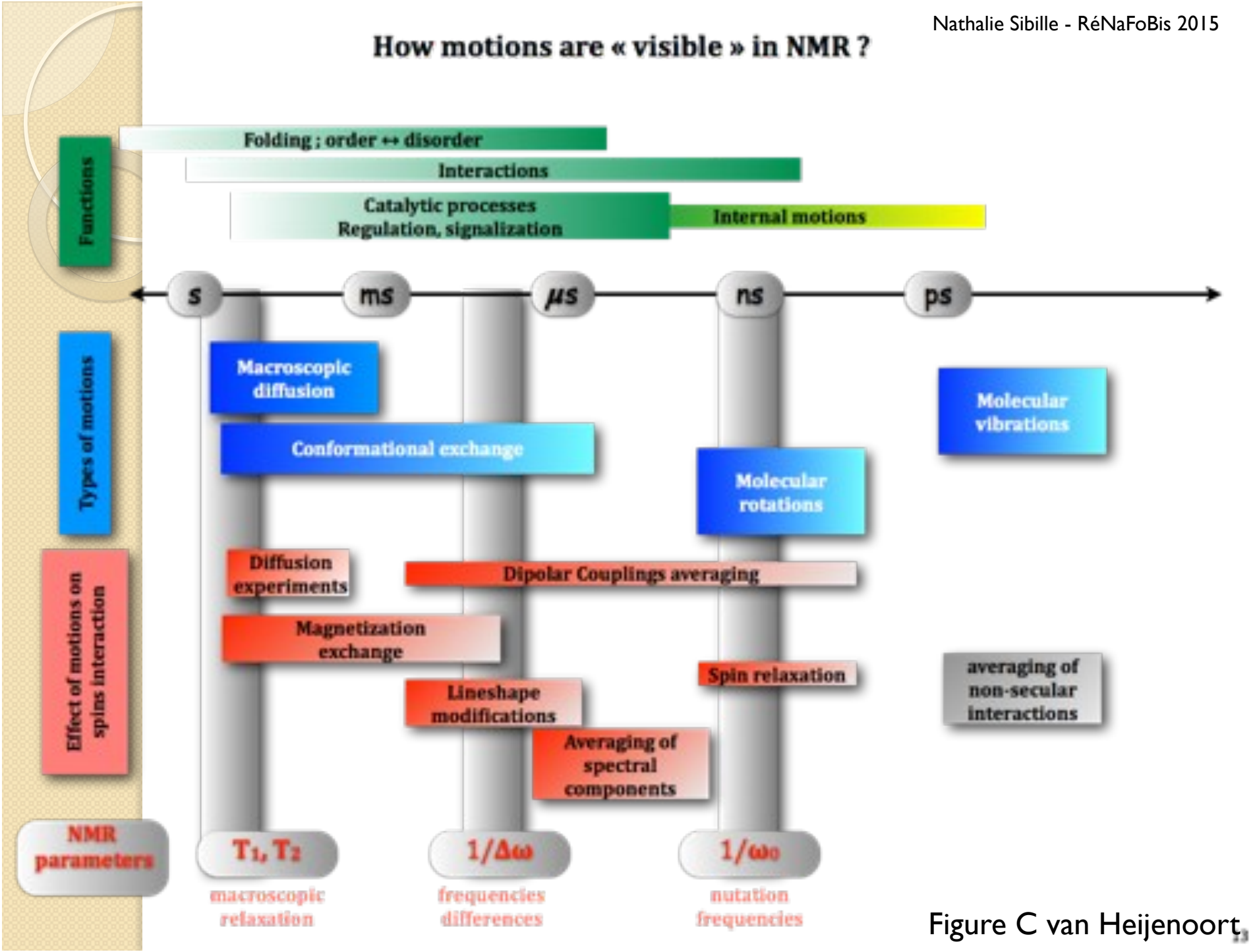
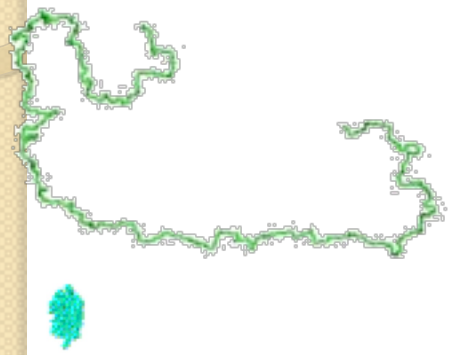
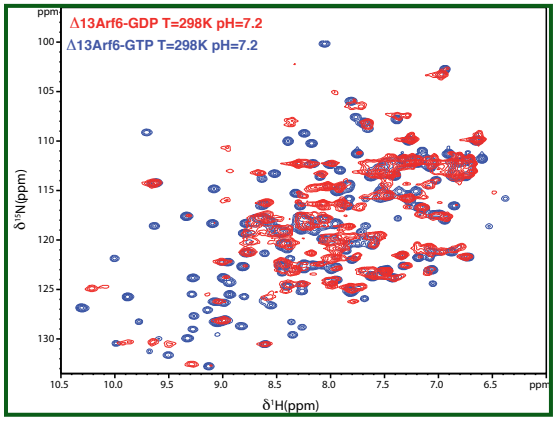


Figure C van Heijenoort

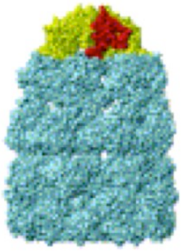
NMR - Spatial and Time scales are highly intermingled

medium size and large IDPs





solubilized membrane proteins



"Small" globular Protein / domain

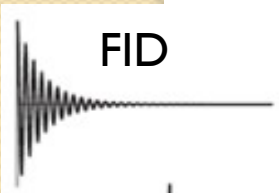
"medium" size complexes

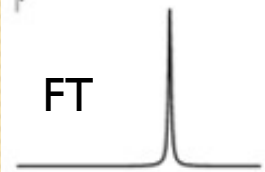
"medium" size globular proteins

"large" size globular proteins or complexes

Global correlation time τ_c (size)

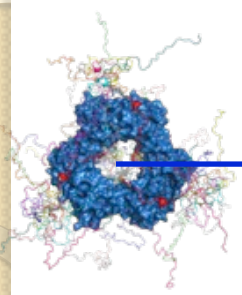
Linewidth



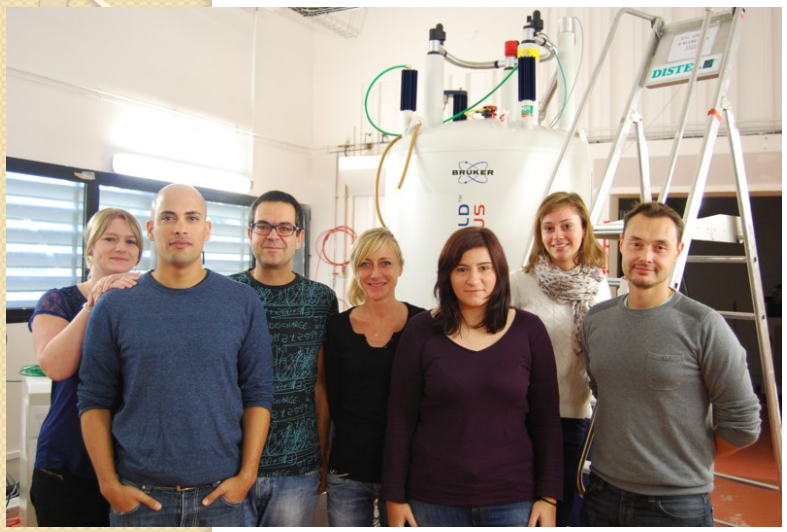


+ Local Rotational fluctuation timescale
+ density of relaxation sources

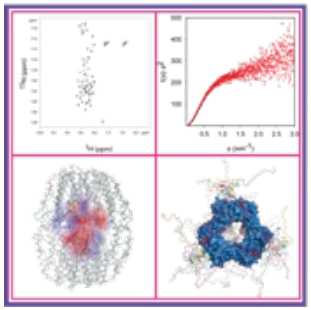
+ conformational/chemical exchange



Highly Flexible Proteins Team



Members of the Team:
- Pau Bernadó (CR1 – INSERM) - SAXS
- Nathalie Sibille (CR1 – CNRS) - NMR
- Tiago Neto Cordeiro (Postdoc) - SAXS
- Fátima Herranz-Trillo (PhD student) – SAXS
- Frédéric Allemand (IR – CNRS) - Molecular Biology
- Aurélie Fournet (Technician) - Biochemistry



Collaborators at the CBS:
-Albane LeMaire (NR)
-William Bourguet (NR)
-Pierre Germain (NR)

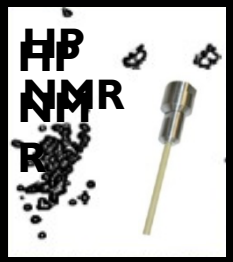
TGIR:
- ICSN Gif sur Yvette
- Carine Van Heijenoort
- Ewen Lescop

Collaborators at the CBS :
- Christian Roumestand (HP)
- Cathy Royer
- Philippe Barthe (NCoR)

Department of Structural Biology

Team 1

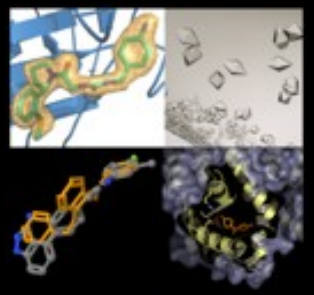
Mtb Structural Biology



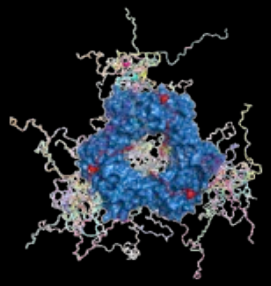
Shared Publications

Structure
Acta D
J Bact
J Biomol NMR
…

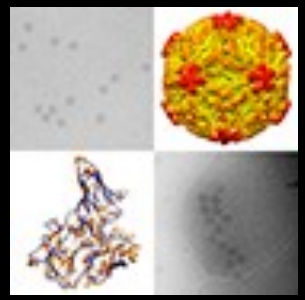
Team 5



Team 2 (2012)



Team 3



Team 4 (2014)

FRISBI Integrated Structural Biology

Platform for Integrated Structural Biology

BiCampus Montpellier

GT-BIO Montpellier 2012

French Meeting GTBIO 2012

NMR steps of a Structural Biology Study