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

CBS

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NMR - Spatial and Time scales are highly intermingled

Size vs dynamics ?

Size ⟺ molecular tumbling + local dynamics

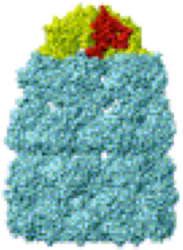
⇕

relaxation processes

⇕

linewidths & S/N

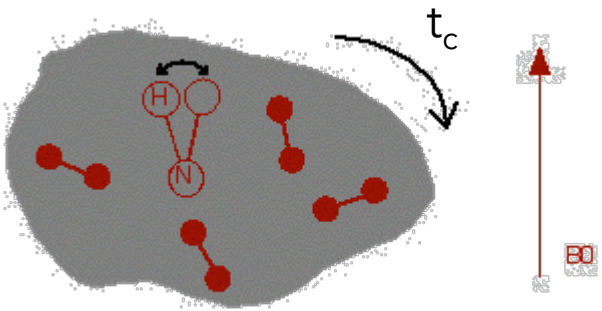
solubilized membrane proteins

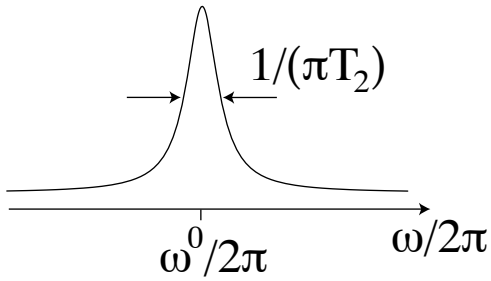


"large" size globular proteins or complexes

"Small" globular Protein / domain

Global correlation time t_c (size) → Linewidth





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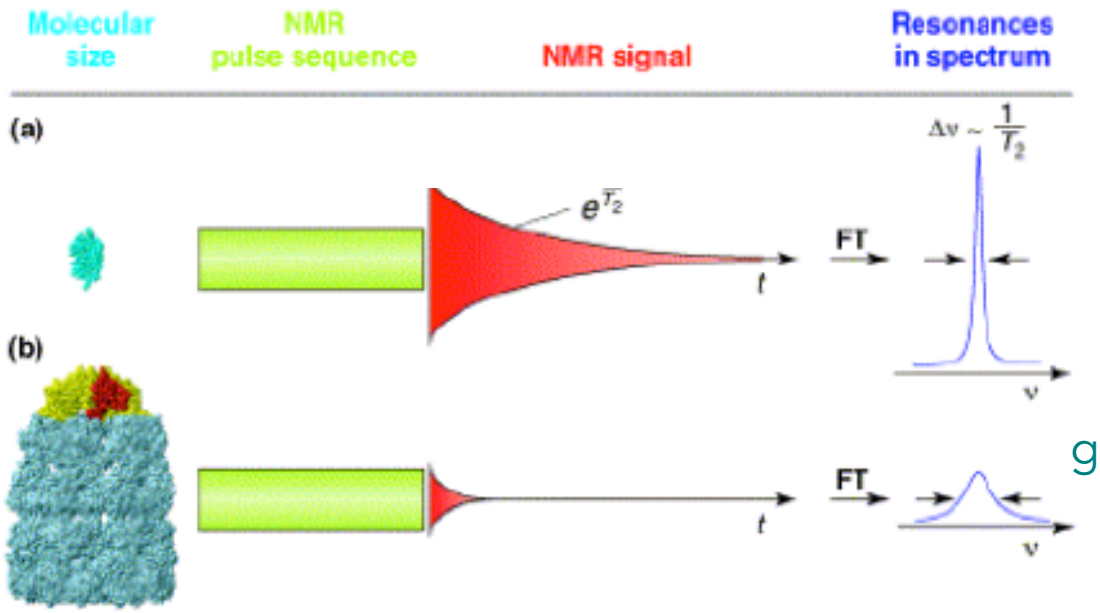
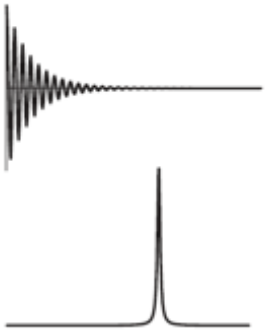


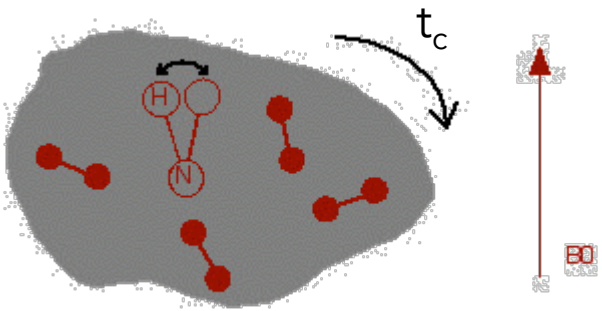
"Small" globular Protein / domain

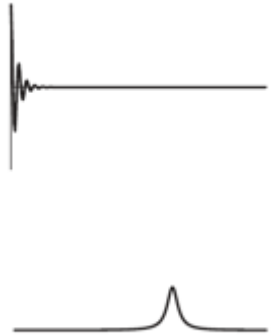
"large" size globular proteins or complexes or solubilized membrane proteins

Global correlation time t_c (size)

Linewidth







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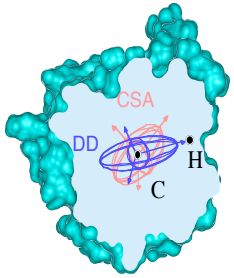
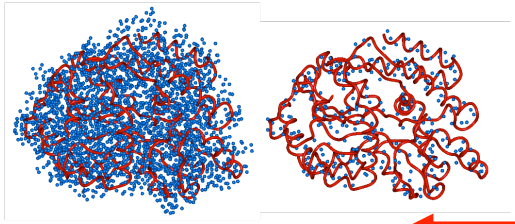
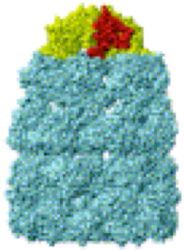


solubilized membrane proteins

TROSY, high fields





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Labelling, Deuteration

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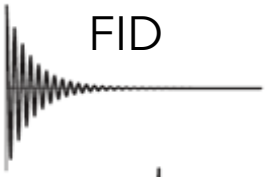
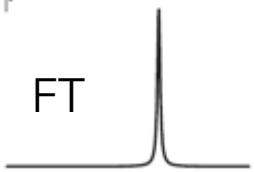
Global correlation time t_c (size)

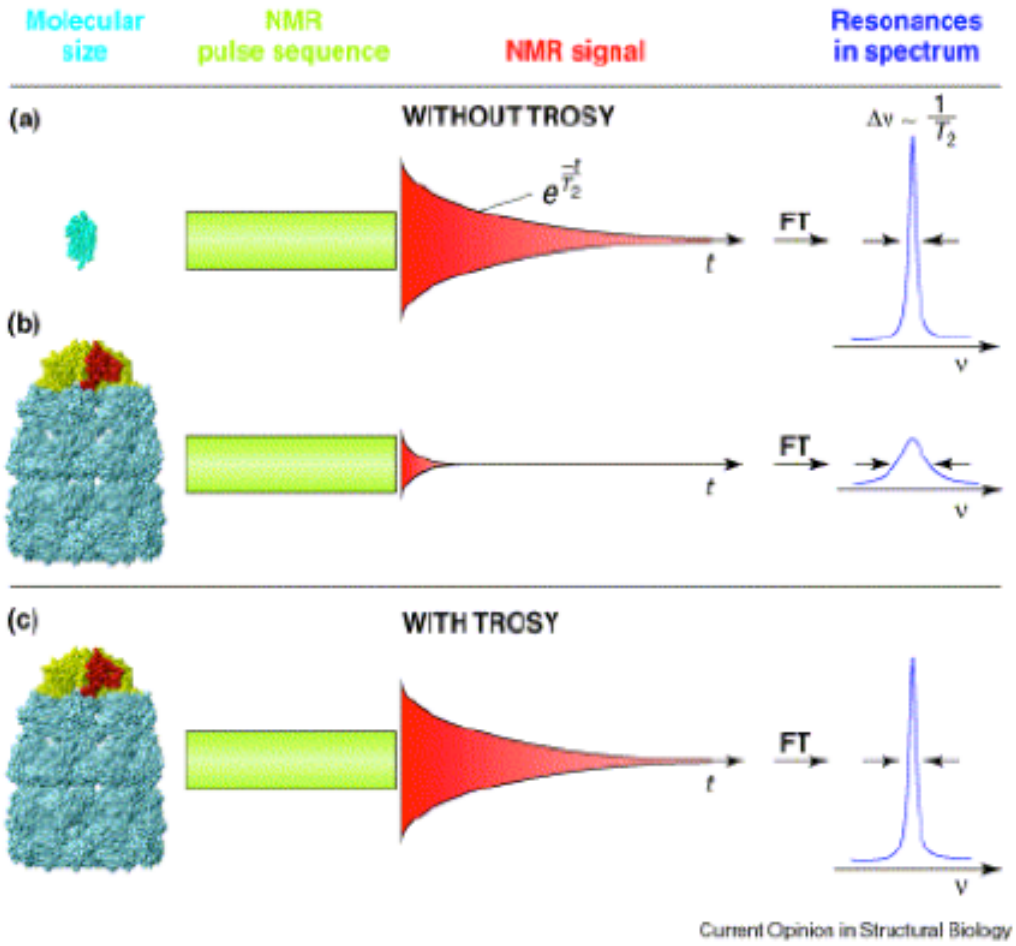
+ Local Rotational fluctuation timescale
+ density of relaxation sources

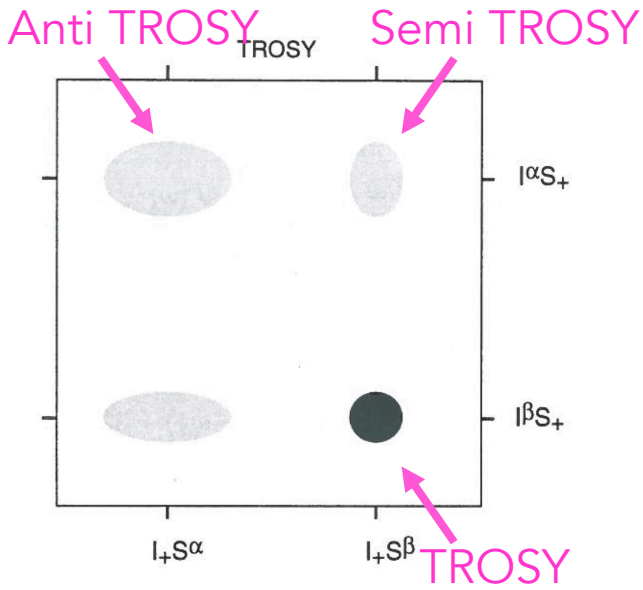


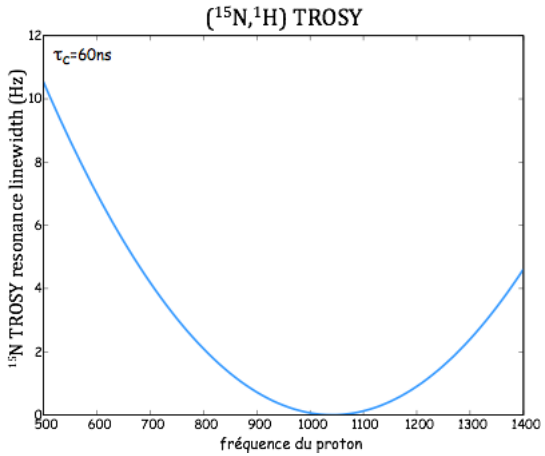


Linewidth

« Size » and lifetime of the signal T2



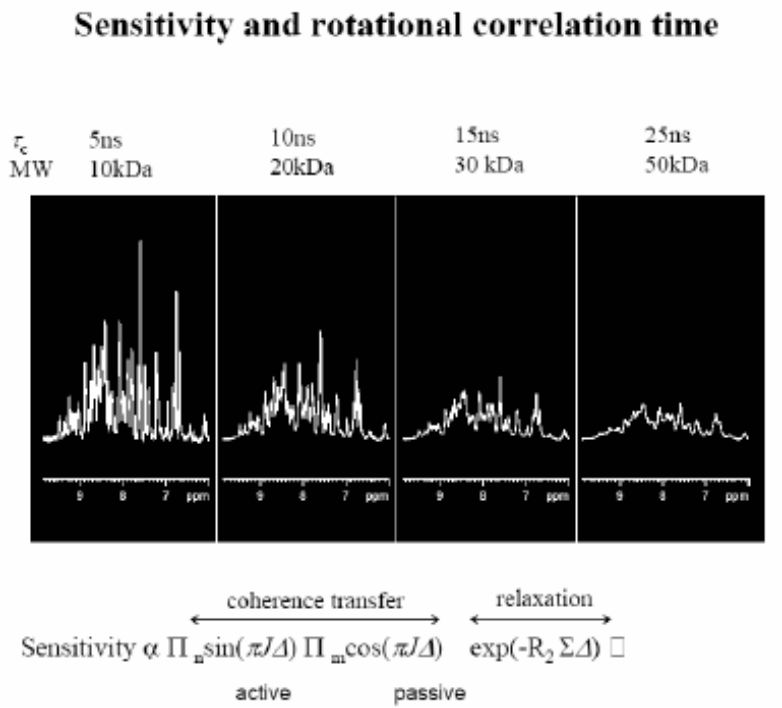


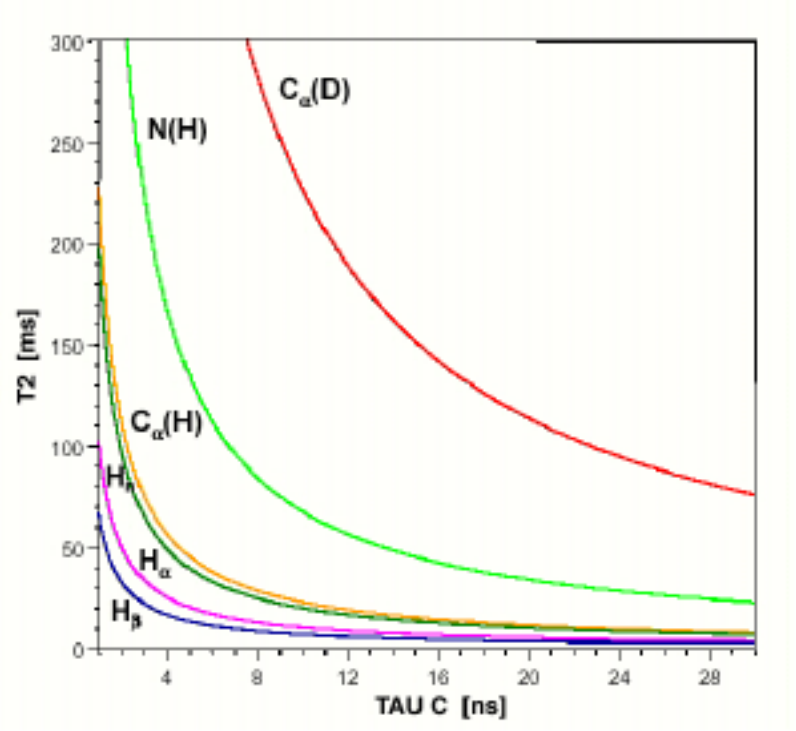


Playing with T2 : TROSY experiments
Transverse Relaxation Optimized SpectroscopY

Pervushin, K. Riek, R., Wider, G. and Wütrich, K(1997) 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+++)

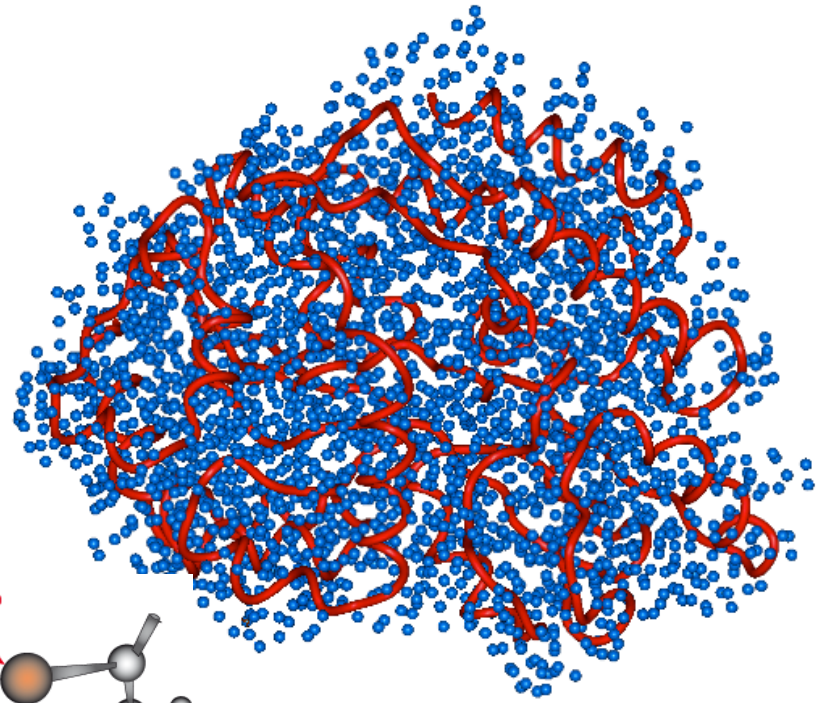
The signal loss is exponential while the size of the molecule increases

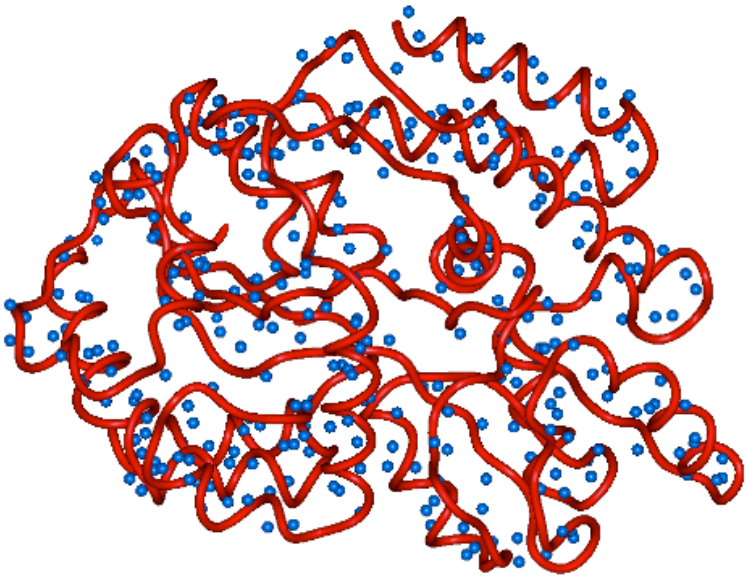
$\gamma_H / \gamma_D = 6.5\ldots$ Relaxation gain !!!!

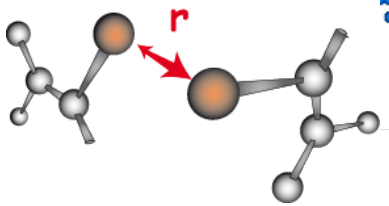
New restraints in NMR for « large » proteins

✧ Fully protonated protein

✧ Deuterated protein (only ^1H_N)





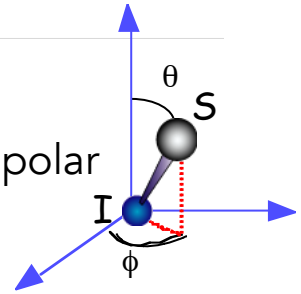


✧ Classical method : NOE ^1H-^1H

✧ Ambigous NOEs restraints

✧ New approach : residual dipolar couplings (RDC)

✧ RDC non ambigous



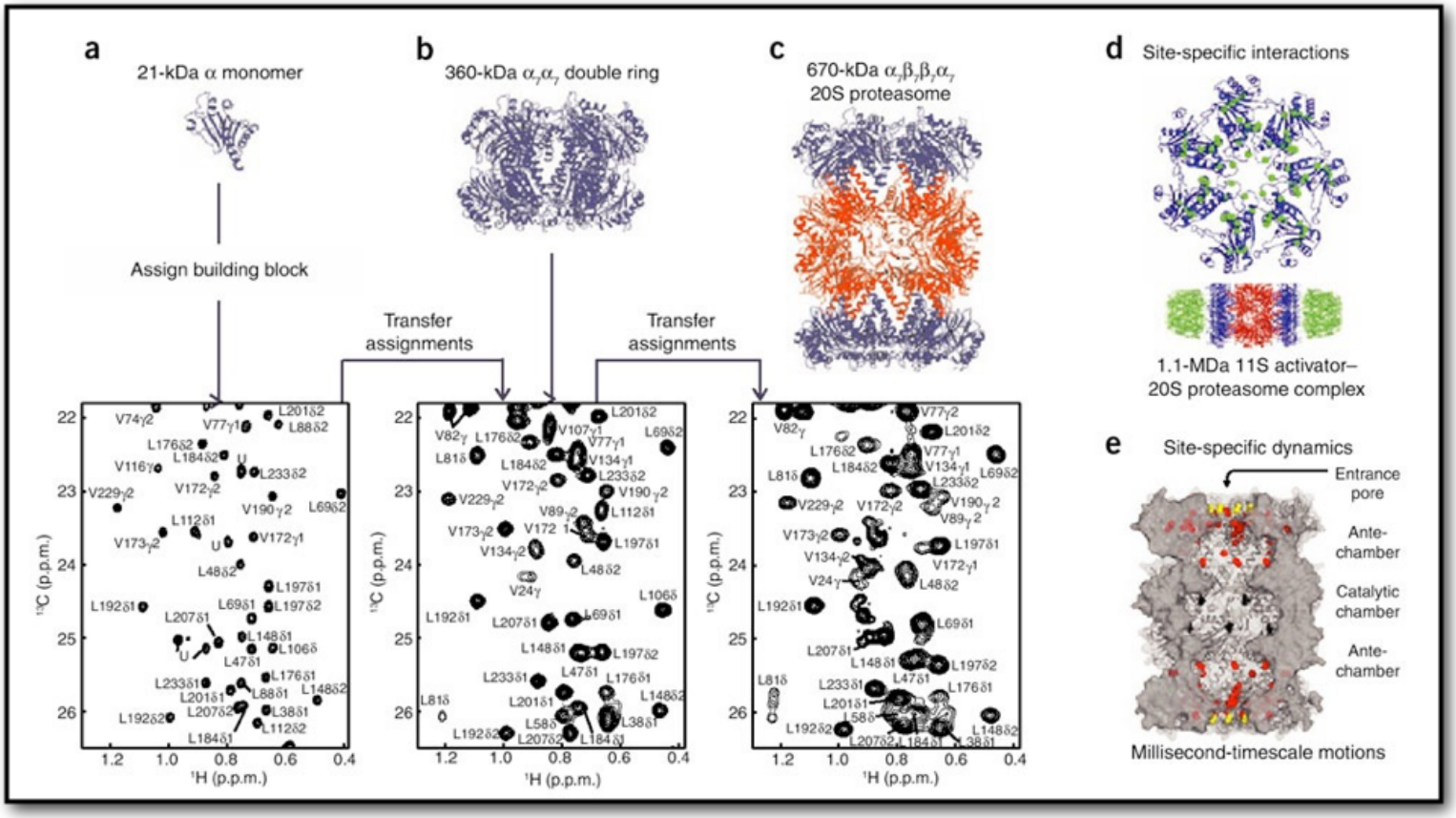
Towards high molecular weight complexes

Special interest of methyls :

- well dispersed throughout the primary structures and localized in hydrophobic core
- favorable transversal relaxation property of CH3 (sensitivity gains)
- high sensitivity due to the presence of 3 equivalent protons

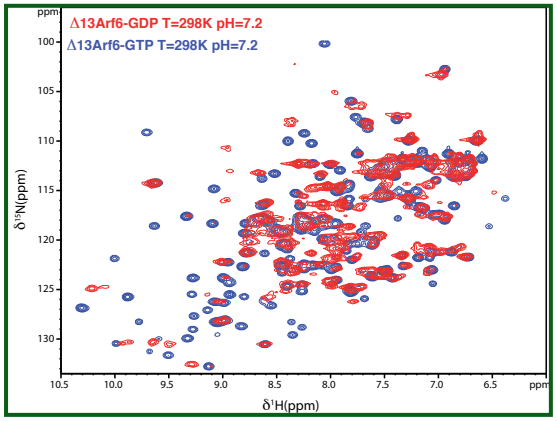
➪ Archaeon proteasome methyl assignment (20S CP, 670kDa)

➪ Methyl groups with concerted millisecond-timescale motions

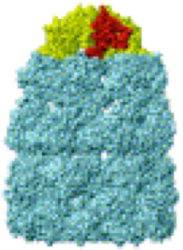


Remco Sprangers, Algirdas Velyvis & Lewis E Kay, Nat. Methods 2007 (Sept), 4, 697

NMR - Spatial and Time scales are highly intermingled



solubilized membrane proteins



"medium" size complexes

"Small" globular Protein / domain

"medium" size globular proteins

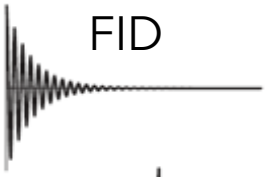
"large" size globular proteins or complexes

Global correlation time t_c (size)

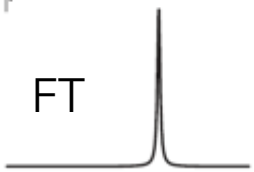
+ Local Rotational fluctuation timescale
+ density of relaxation sources

+ conformational/chemical exchange

FID



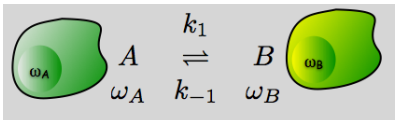
FT

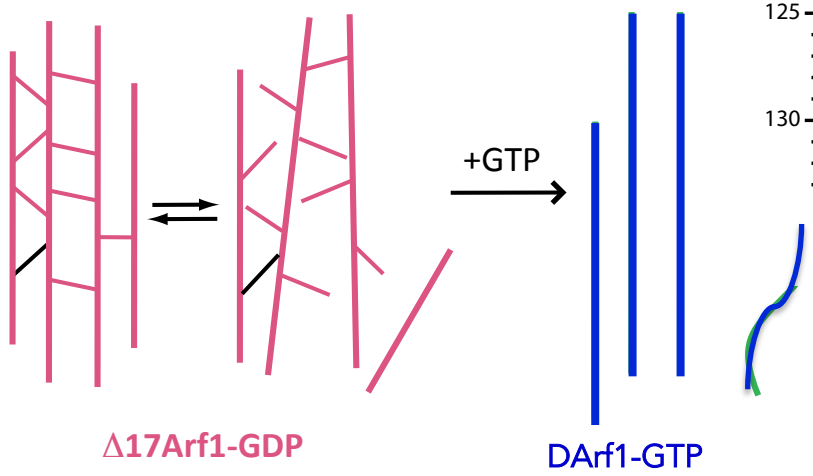


Linewidth

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

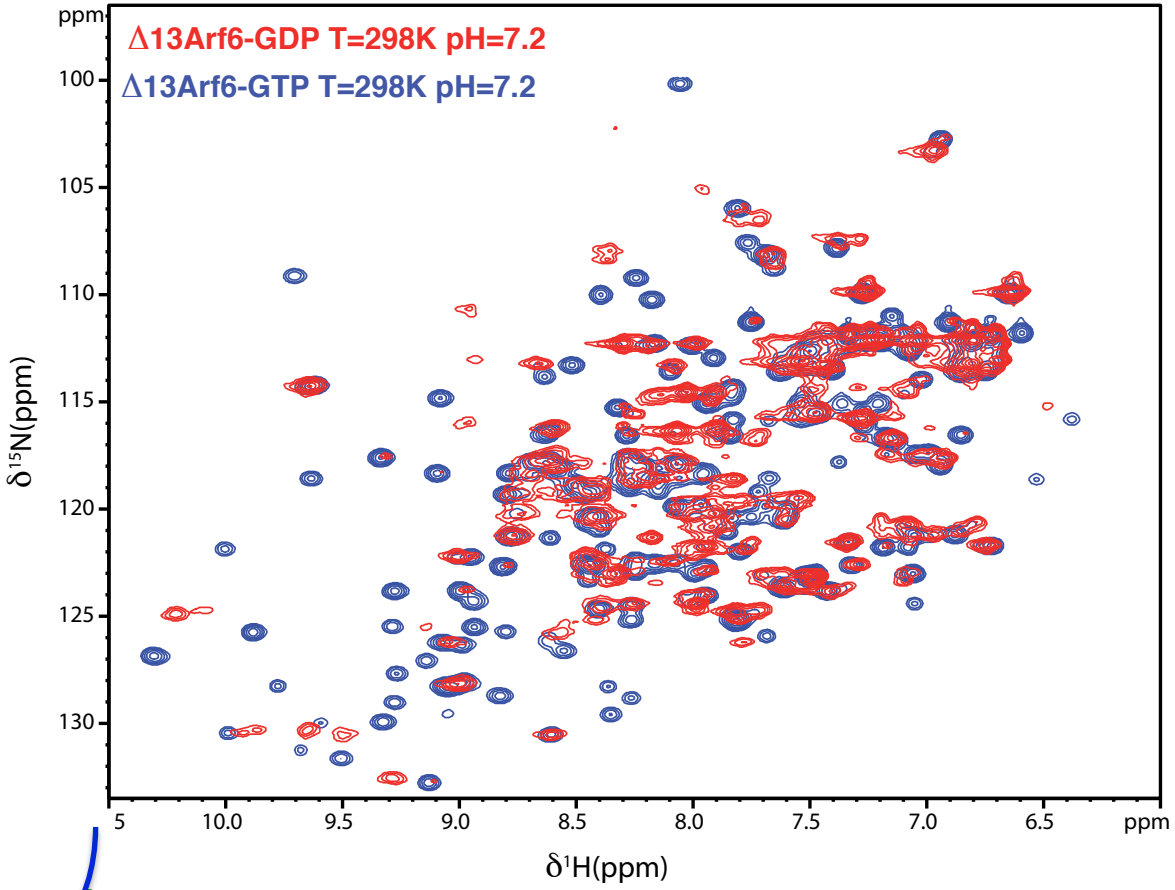


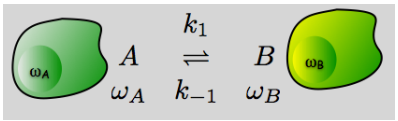


Δ17Arf1-GDP

DArf1-GTP

+ conformational / chemical exchange



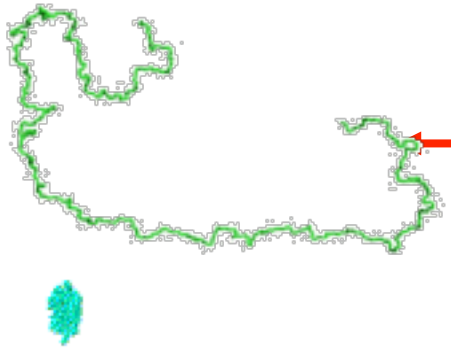


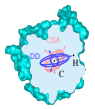
"Slow" exchange

"Intermediate" exchange

"Fast" exchange

NMR - Spatial and Time scales are highly intermingled

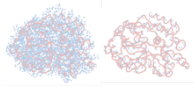


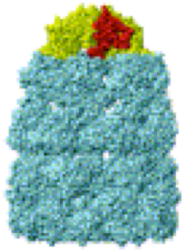


medium size
and large
IDPs

TROSY, high fields

solubilized
membrane proteins





Labelling, Deuteration

"medium" size
complexes

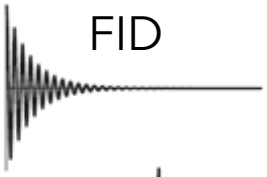
"medium" size
globular proteins

"Small" globular
Protein / domain

"large" size
globular proteins
or complexes

Global correlation time t_c (size)

Linewidth



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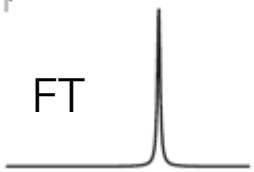

FT

+ Local Rotational fluctuation timescale
+ density of relaxation sources

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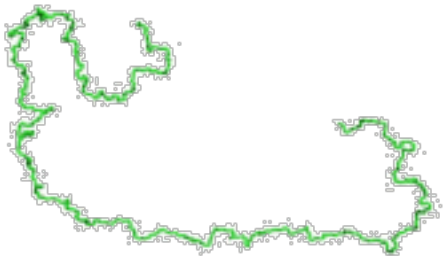
« Size » and disorder

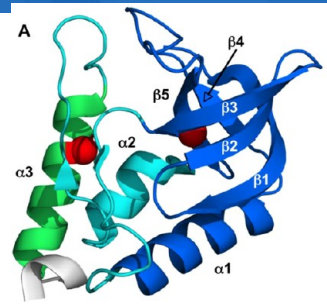


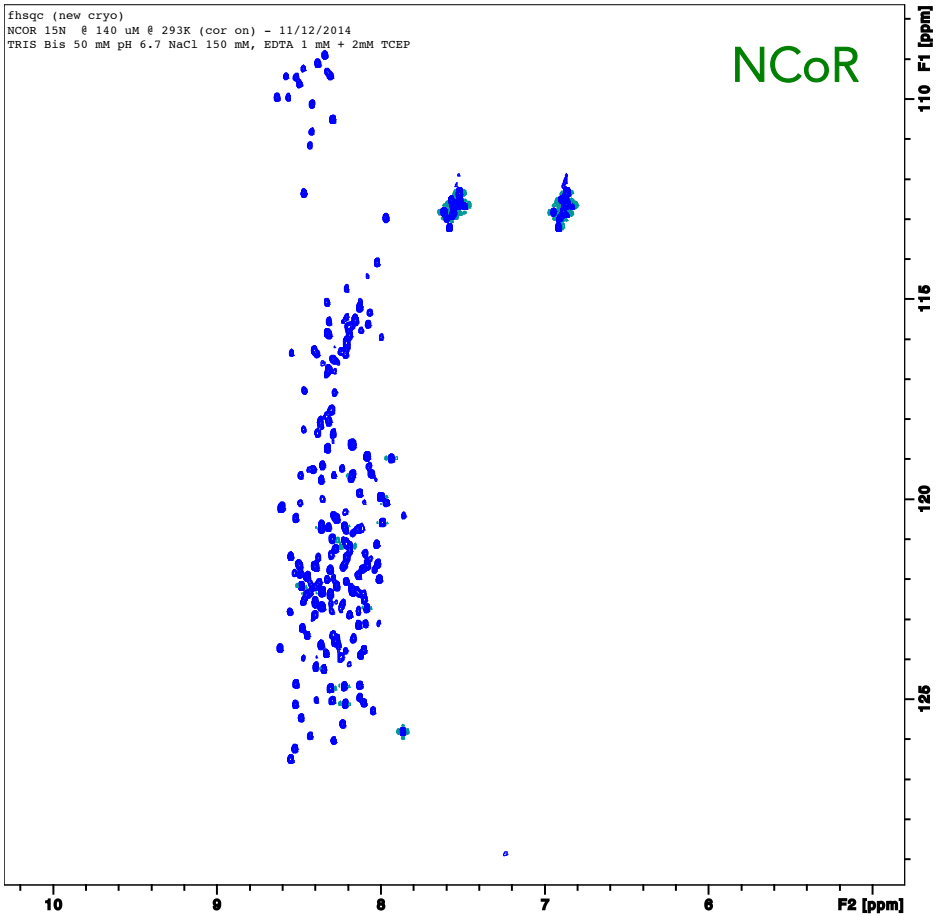
Unfolded protein

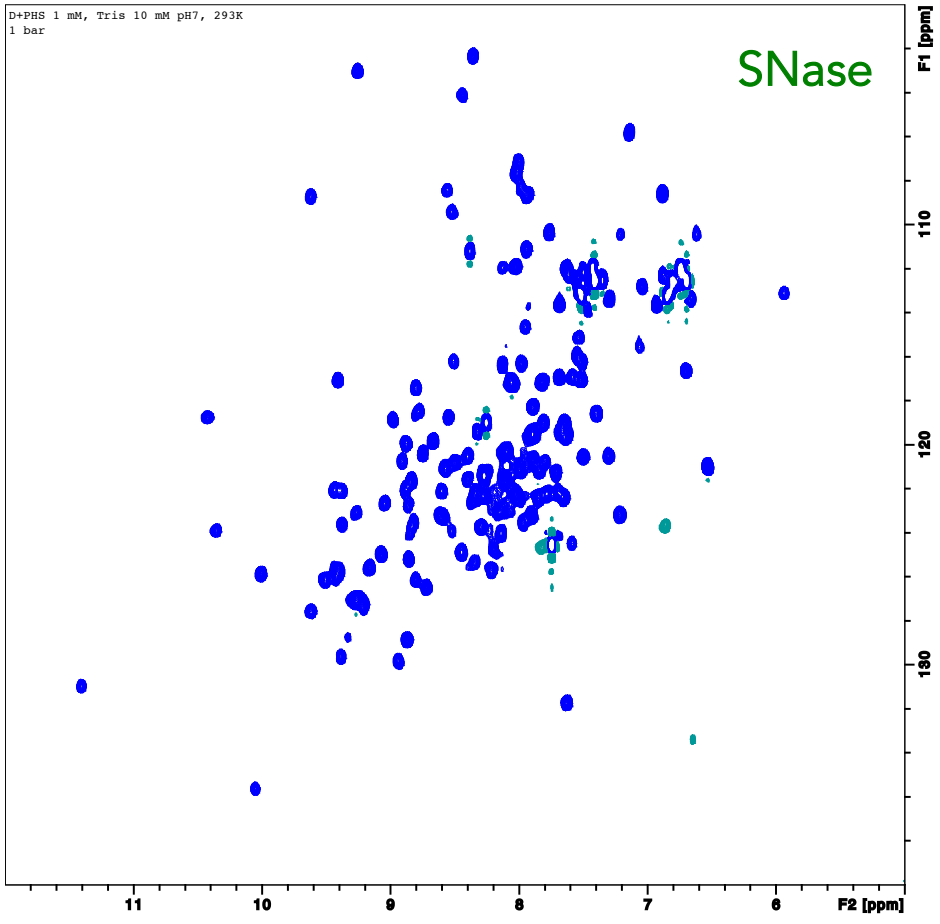
29 kDa

Folded protein

15 kDa





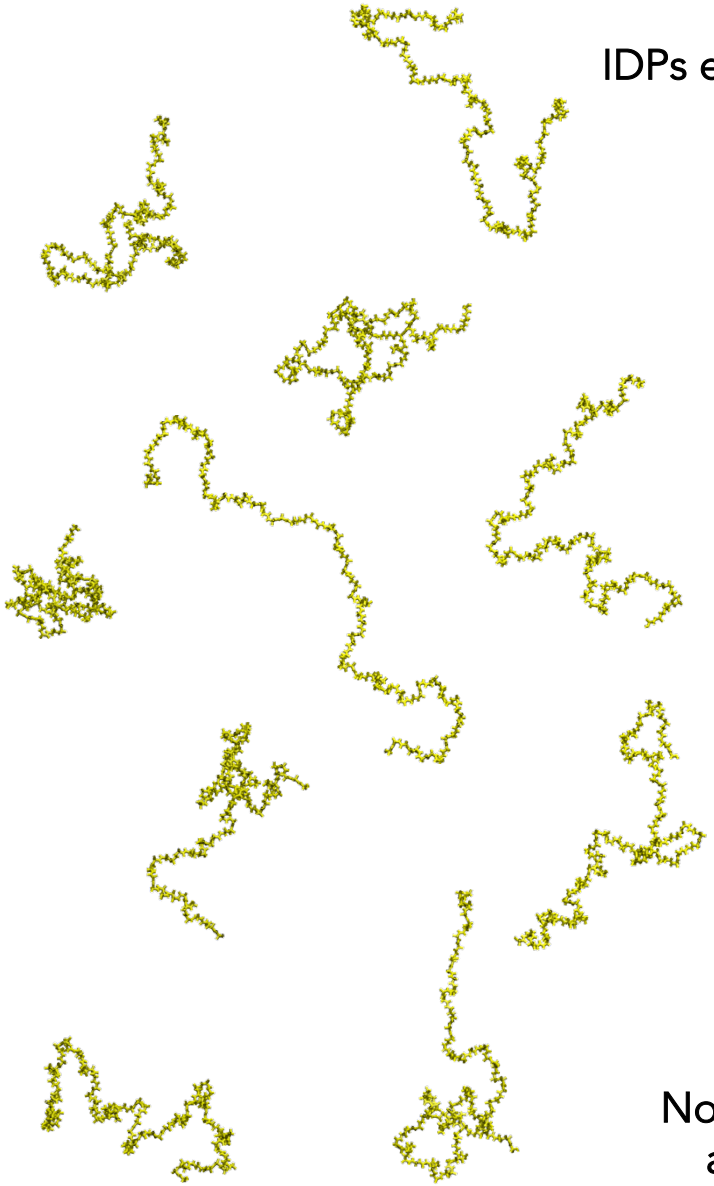


What is an IDP ?

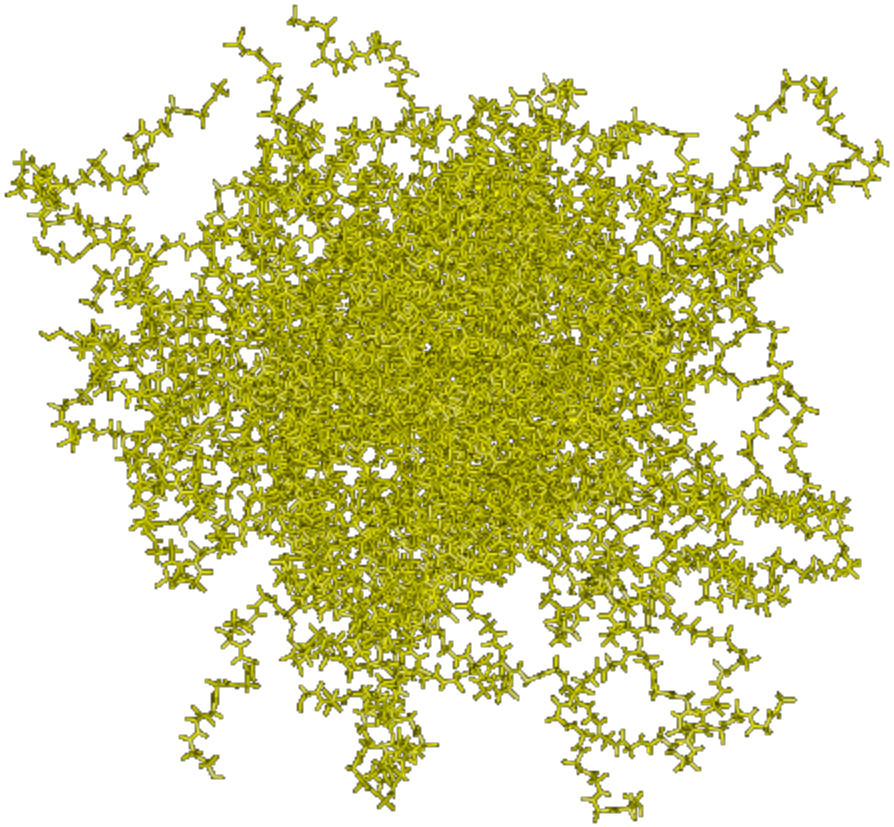
- IDPs lack stable tertiary and/or secondary structure
- IDPs are more common in eukaryotes than in bacteria and archaea: Probably linked with their major biological complexity… Up to ~30-50% of genome
- Very specific amino acid sequences rich in P G R K and E, and depleted on hydrophobic residues
- IDPs are the target of the vast majority of PTM: Phosphorylation, Glycosylation…
- Interactome hubs are enriched in disordered regions
- Partner recognition of IDPs is normally performed through short linear motifs (SLiMs) that are embedded in disordered poorly conserved regions

Chem. Rev. 2014 vol 114

Flexible Proteins, a Challenge for Structural Biology



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

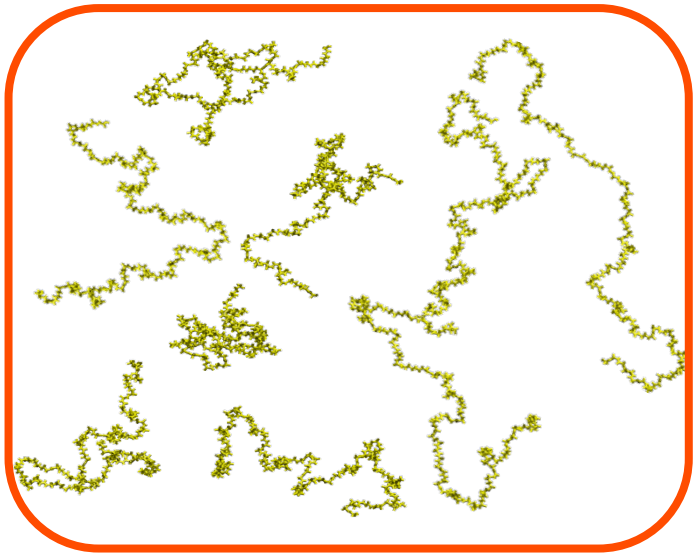


Normally averaged properties are measured. Ensemble approaches have to be used for their interpretation

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

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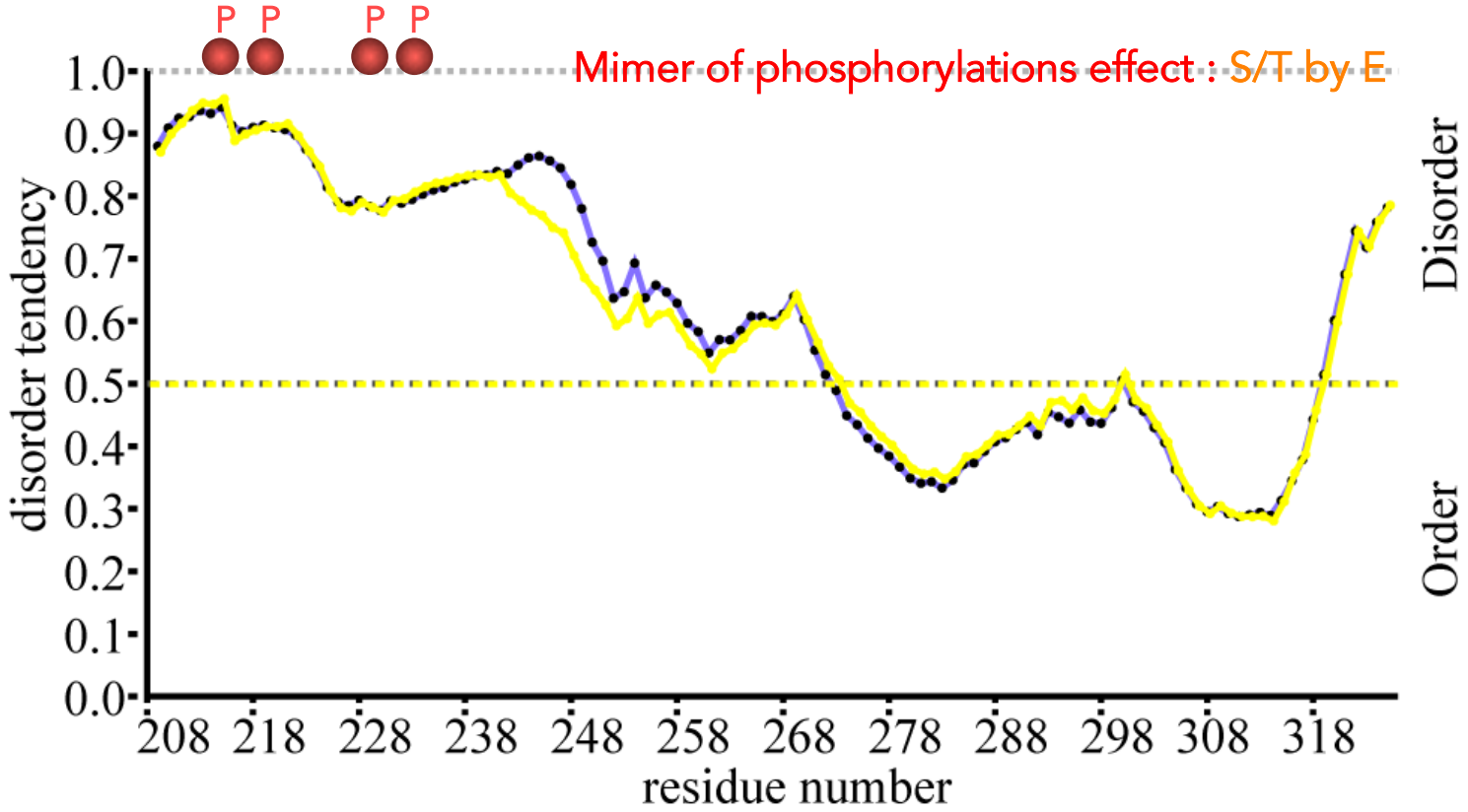
BioInformatics

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.

BioInformatics

- Other predictors of disordered/ordered regions
  - the meta-predictor PONDR-FIT (http://disorder.compbio. iupui.edu/pondr-fit.php) that combines the results of six different methods (PONDR-VLXT, PONDR-VSL2, PONDR-VL3, FoldIndex, IUPred, and TopIDP)
  - PrDOS http://prdos.hgc.jp a structure-based method
  - SPOT-disorder http://sparks-lab.org/server/ SPOT-disorder/ based on a window-based neural network (SPINE-D)
  - DISOPRED3 (http://bioinf.cs.ucl.ac.uk/psipred/) that combines three machine learning models: support vector machine, neural network and nearest neighbor

- Secondary structure predictions
  - PSIPRED v3.3 http://bioinf.cs.ucl.ac.uk/psipred/
  - Jpred http://www.compbio.dundee.ac.uk/jpred4
  - SOPMA https://npsa-prabi.ibcp.fr/NPSA/npsa_sopma.html
  - alpha- turnspredictor ALPHAPRED crdd.osdd.net/raghava/alphapred/
  - PSSpred https://zhanglab.ccmb.med.umich.edu/PSSpred/

- Sequence conservation
  - GREMLIN (Generative Regularized ModeLs of proteINs) software

Is our protein an IDP ?

- Bioinformatics
  Identification of disordered/ordered regions

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

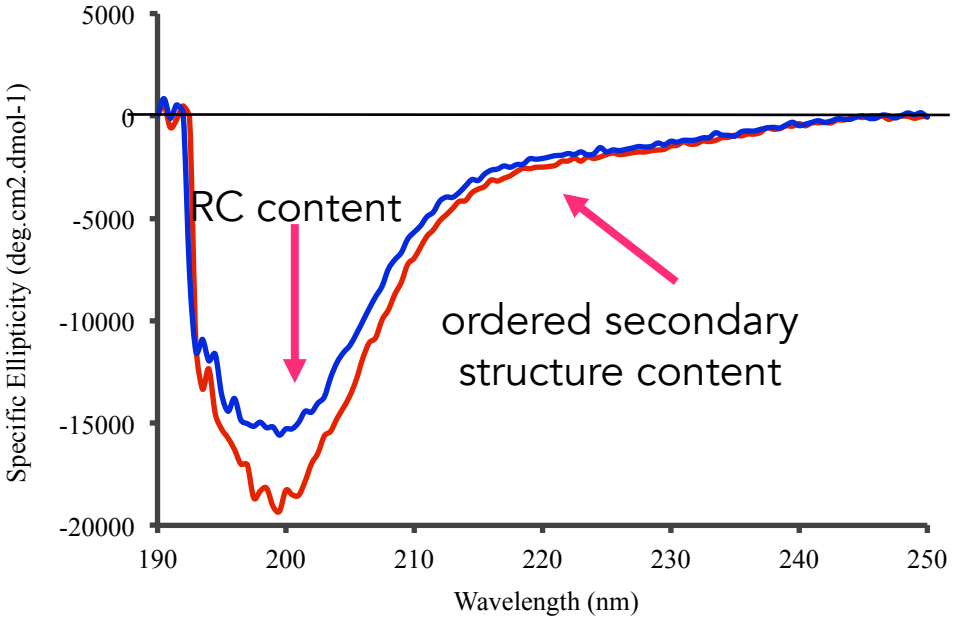
- Small-Angle X-ray Scattering (SAXS)/Small-Angle neutron Scattering (SANS)
  Averaged Intensity profiles...
  Qualitative Interpretation of averaged R_g and Kratky Plots

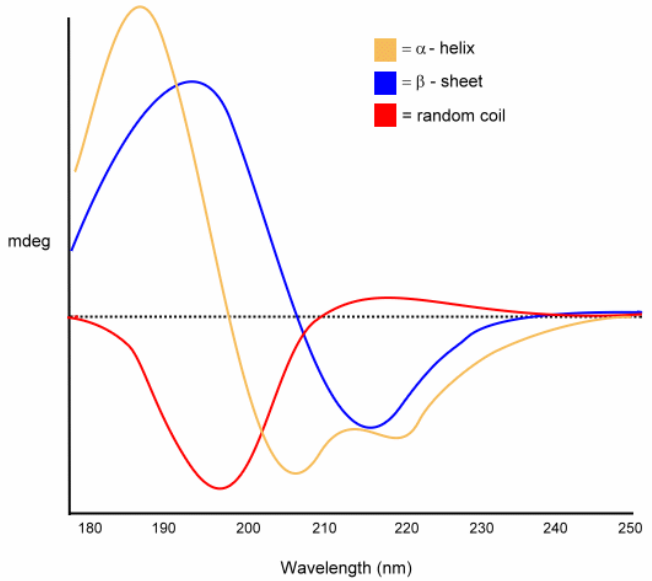
- Nuclear Magnetic Resonance (NMR)
  Ensemble averaged observables: CS, J-Couplings and RDCs
  Dynamic dependent Parameters: Relaxation Rates and PREs

- X-ray Crystallography
  Structure determination in the bound form

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*)

Is our protein an IDP ?

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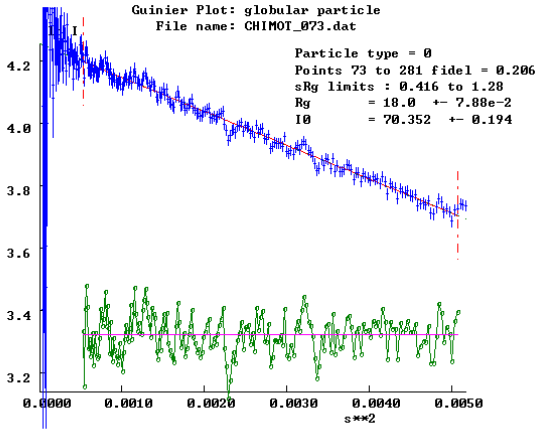
SAXS/SANS

Qualitative interpretation of averaged Intensity Profiles

Guinier

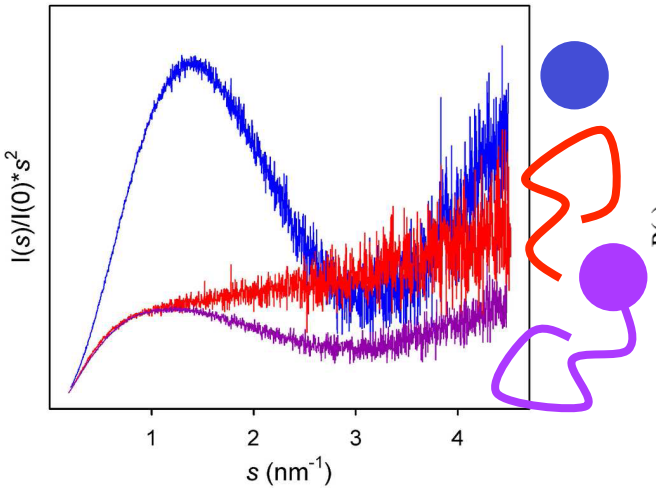


R_g , Molecular Weight

Flory's equation was parametrized for IDPs

$R_g = (2.54 \pm 0.01) \cdot N^{(0.522 \pm 0.01)}$

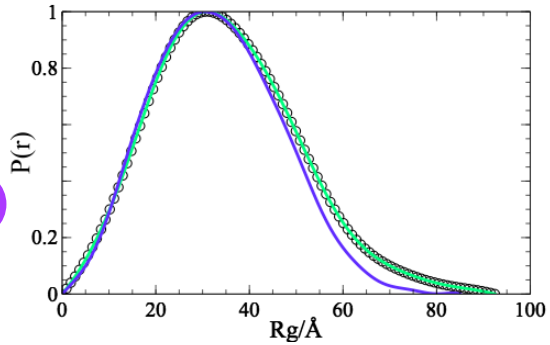
Kratky



Mass Density

- folded
- unfolded
- folded and unfolded

P(r)



1D-Structure
Maximum Distance

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)
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- Nuclear Magnetic Resonance (NMR)
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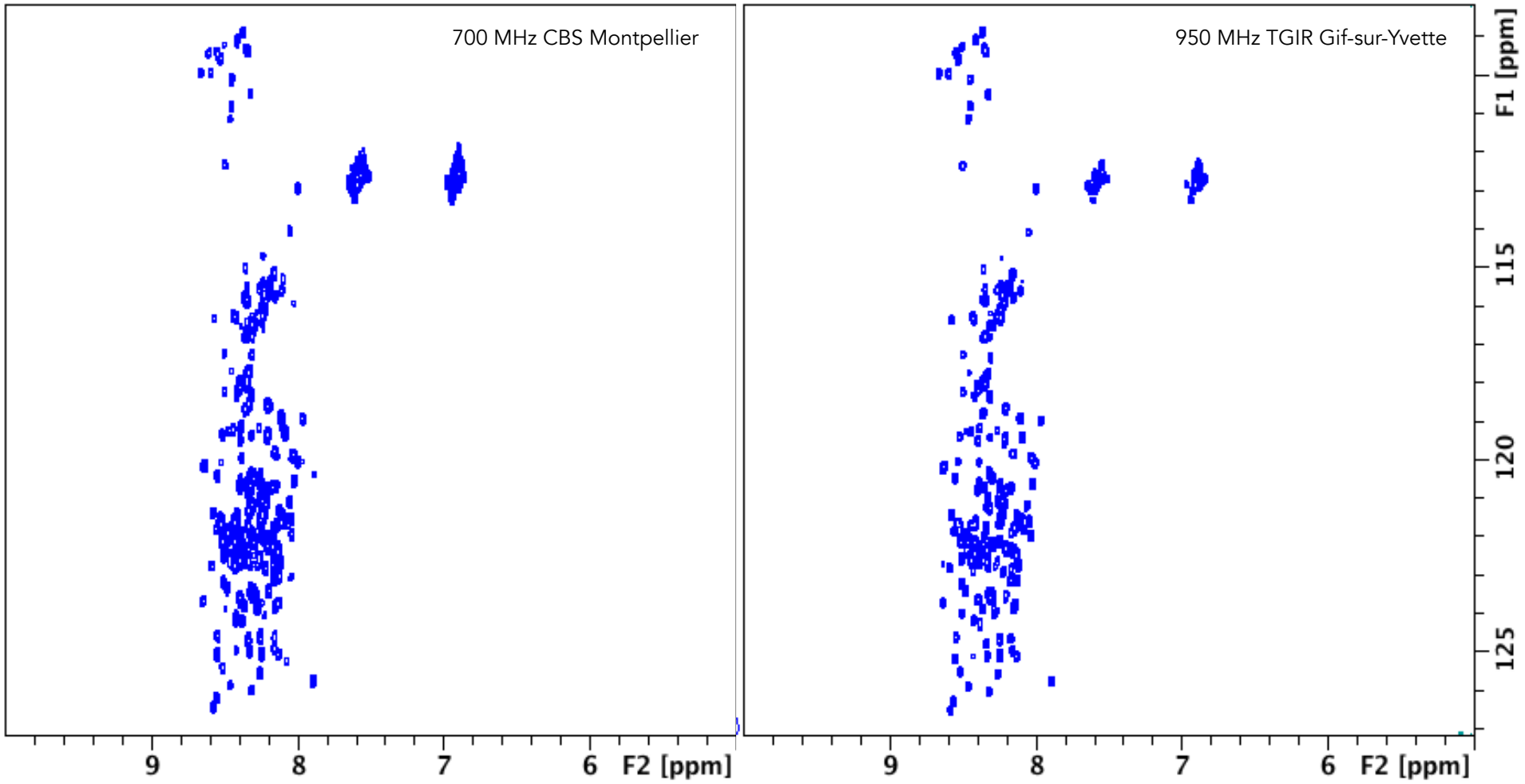
NMR

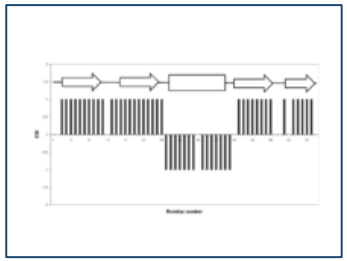
Sensitivity and Resolution

700 MHz NCoR (269 aa) 950 MHz



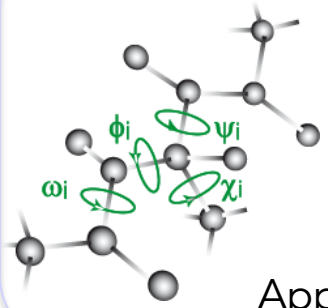
NMR is rich in structural information

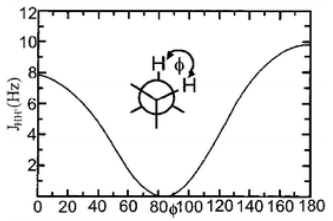
Chemical Shifts

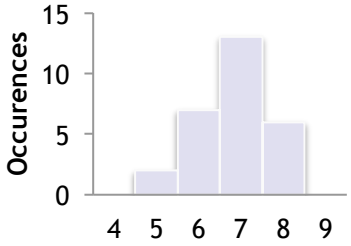


Secondary structure elements

J-Couplings

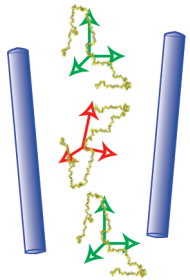


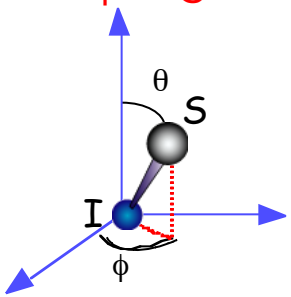




Approximate torsion angles

Residual Dipolar Couplings

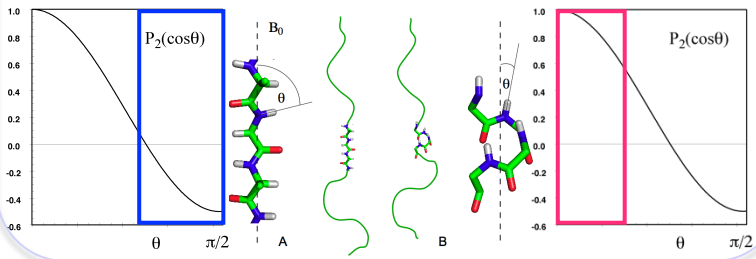




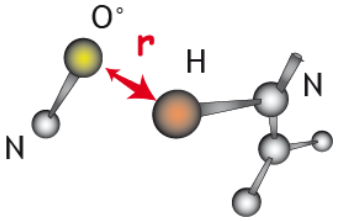
Relative Bond orientations

Extended Regions

α-Hellical Regions

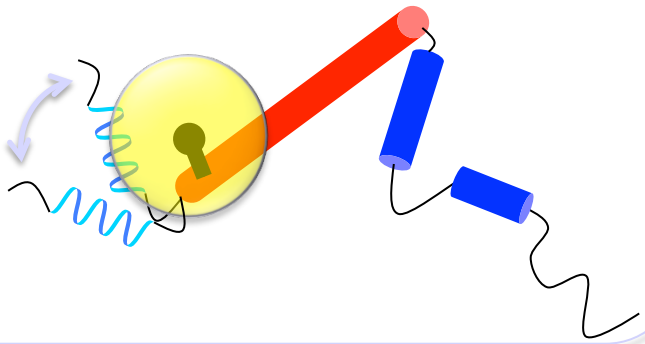


Paramagnetic Relaxation Enhancement



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

Transient long-range contacts



Chemical Shift Index

- Classical database (1994)

  Wishart et al J Biomol NMR 5 (1995) 67-81 :
  Based on Ac-GGX(A/P)GG-NH2 peptides database
  Proline correction factor on preceding residues of ~ 2 ppm on Ca

- Schwarzinger database (2001)

  "Sequence-Dependent Correction of Random Coil NMR Chemical Shifts" by S. Schwarzinger, et al. J. Am. Chem. Soc. 2001, 123, 2970-2978

  Ac-G-G-X-G-G-NH2

  Not representative for the other residue types & in 8 M urea pH 2.3

- Kjaergaard database (2011)

  Random coil chemical shifts for intrinsically disordered proteins: Effects of temperature and pH. Kjaergaard, et al. (2011) J. Biomol. NMR 49(2):139-49

  Ac-QQXQQ-NH2

  Sample temperature (Celsius) and pH corrections, using GGXGG-based neighbor correction for glycines & corrections for perdeuterated protein

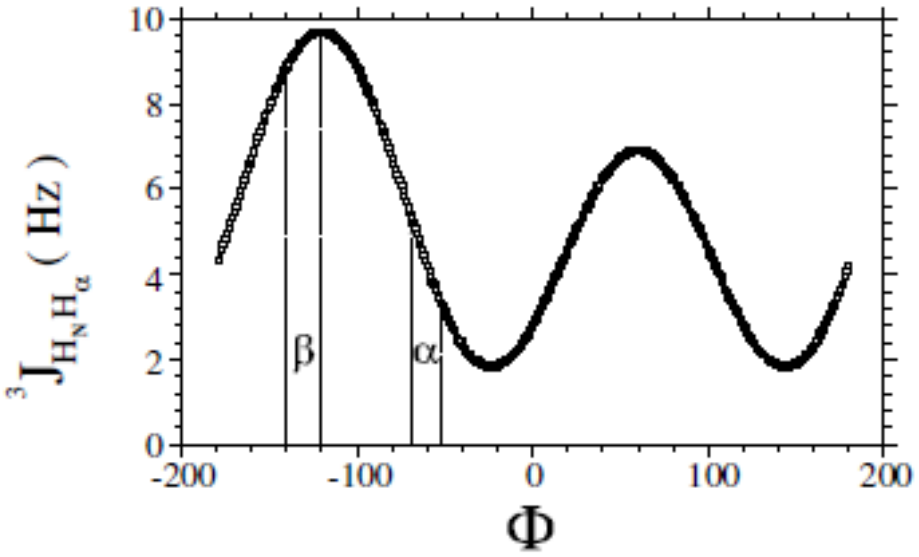
- Tamiola database : ncIDP (2010)

  neighbor corrected Intrinsically Disordered Protein Library calculated according to Tamiola, Acar, Mulder (2009) tables

  IDPs, (POTENCI > 100 IDPs)

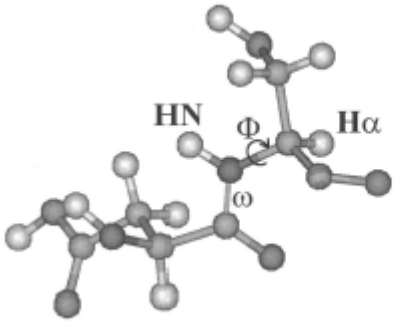
Scalar couplings

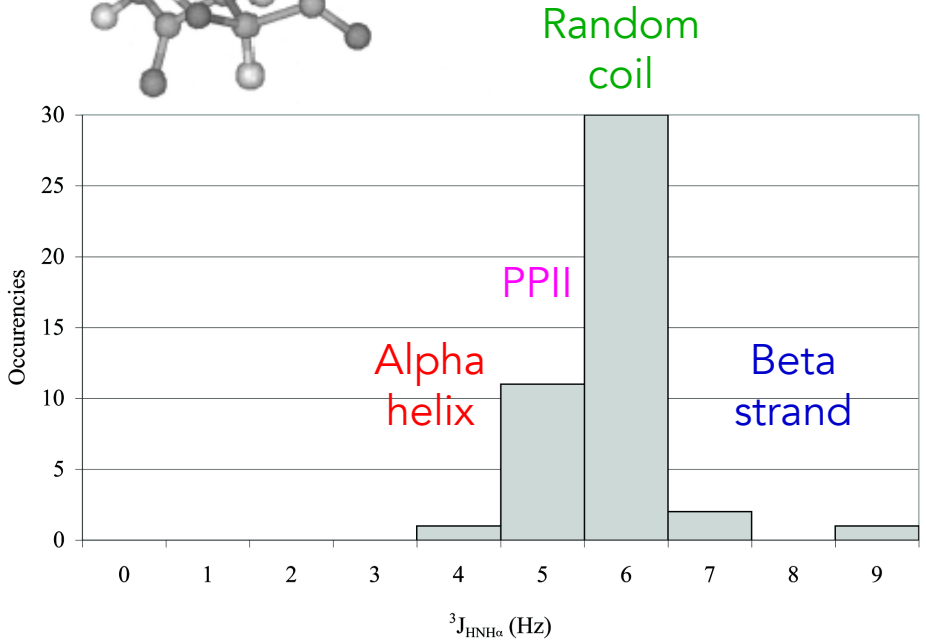
Karplus Law and Φ



$$^3J_{H_NH_\alpha} = 6.4\cos^2\theta - 1.4\cos\theta + 1.9\ \text{Hz}$$

$$\theta = |\Phi - 60|$$





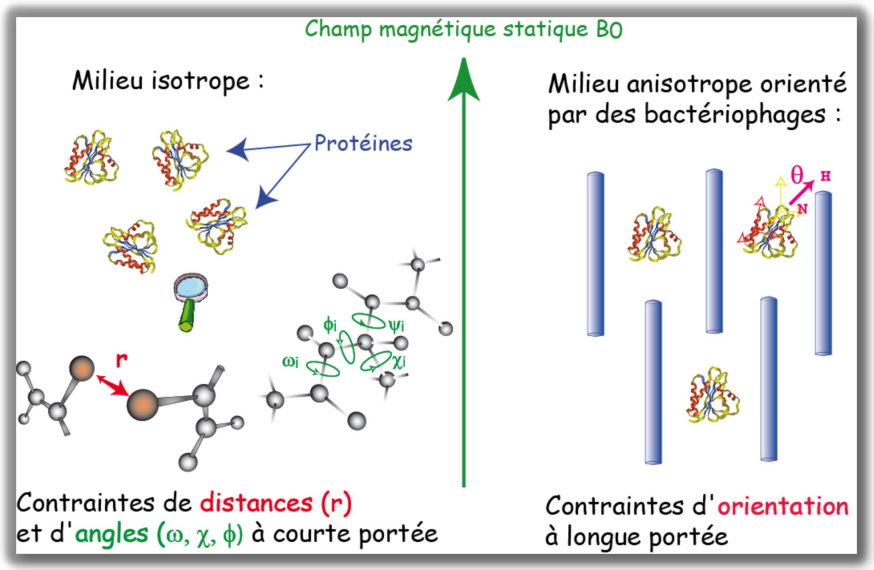
- $^3J_{HNHA}$ Random Coil Values : Schwarzinger j biomol nmr 18 (2000) 43-48
- $^3J_{HNHA}$ librairy : https://spin.niddk.nih.gov/bax/nmrserver/rc_3Jhnha/

Shen, Y, Roche, J, Grishaev, A, Bax, A (2018) Prediction of nearest neighbor effects on backbone torsion angles and NMR scalar coupling constants in disordered proteins. Protein Sci 27(1):146-158

Residual Dipolar Couplings

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

Steric Interactions:

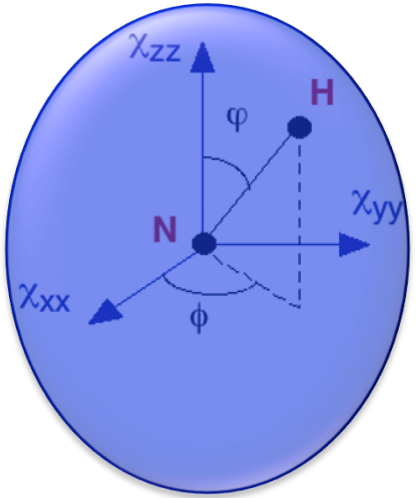
Alcohol mixture, gels, cellulose crystallites

Electrostatic Interactions:

Phage (Pf1), purple membranes, bicelles

Tjandra, N.; Bax, A. Science, 1997, 278, 1111-1114.

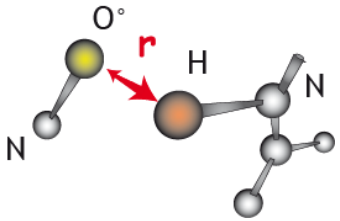
Restricted reorientation



Paramagnetic Relaxation Enhancement

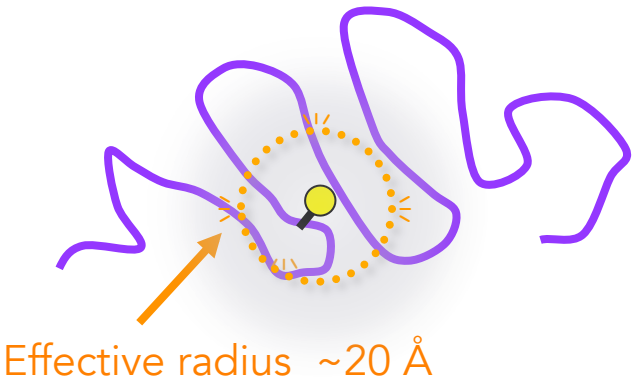
Dynamic dependant parameters

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



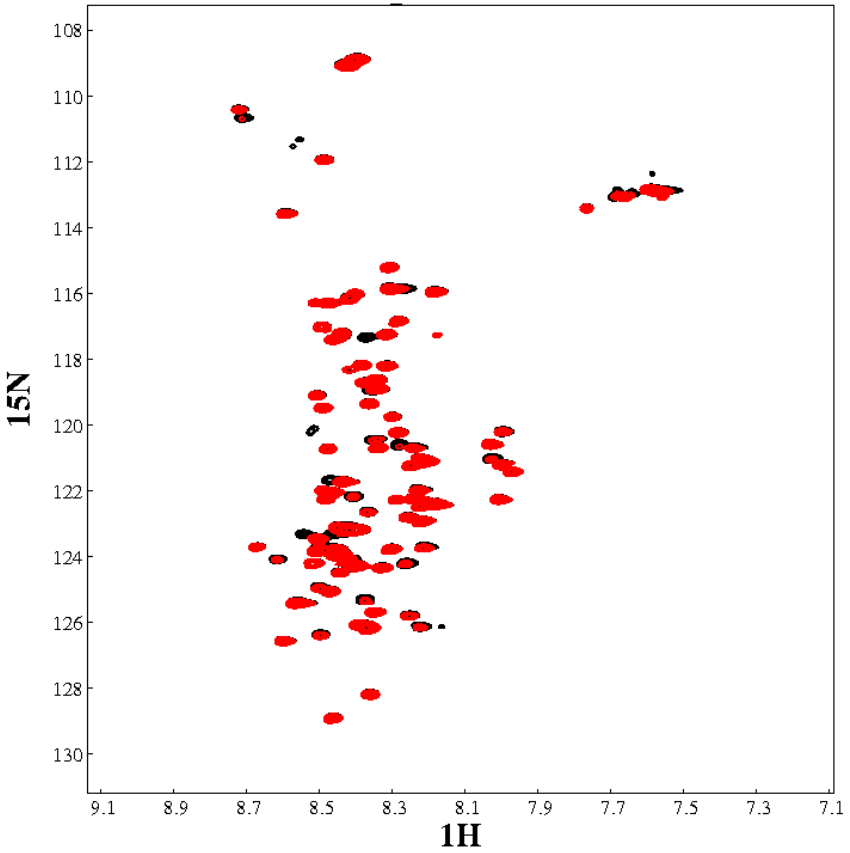
$$\frac{\gamma_e}{\gamma_H} = 658$$

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



Forme para I : NO(C291) 200 uM

Forme dia I0: + 5mM de vitamine C



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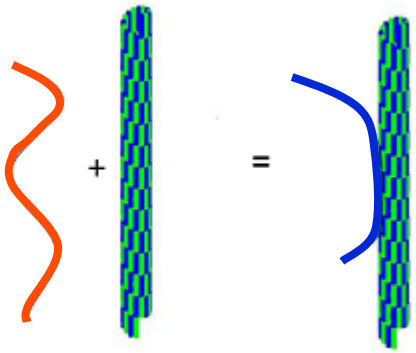
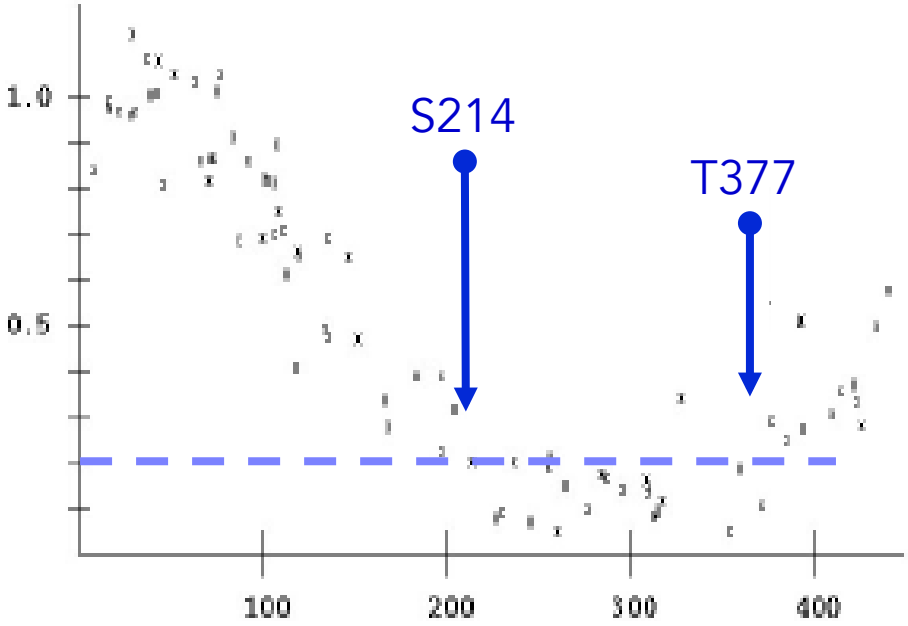
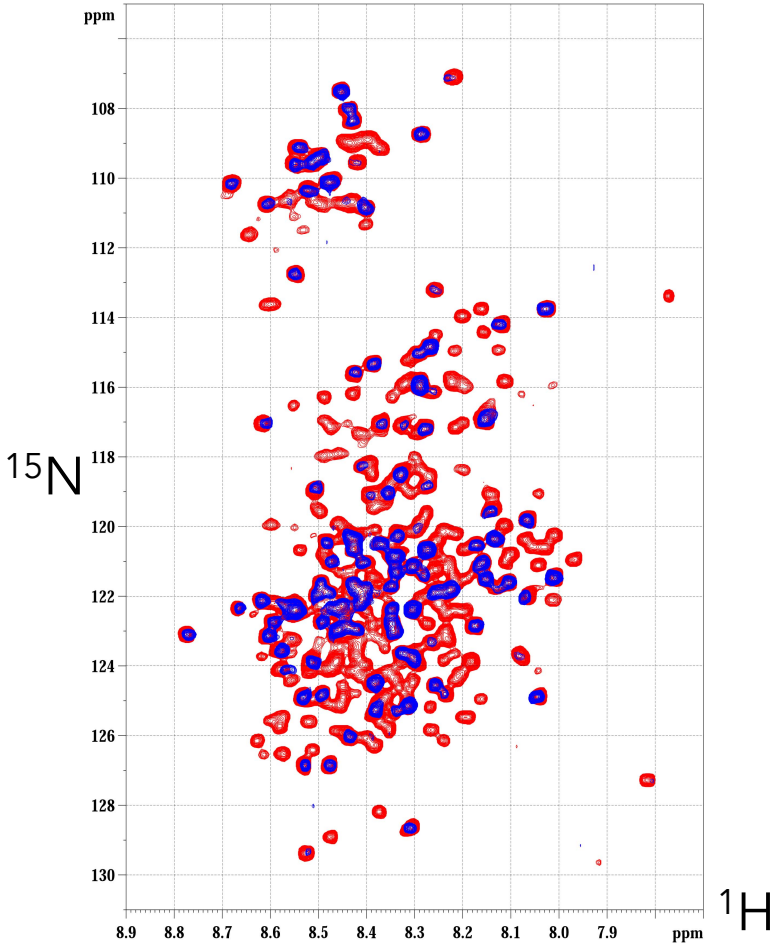
To X Ray cristallography

Structure determination of the bound form



Delimitate the bound region to the partner

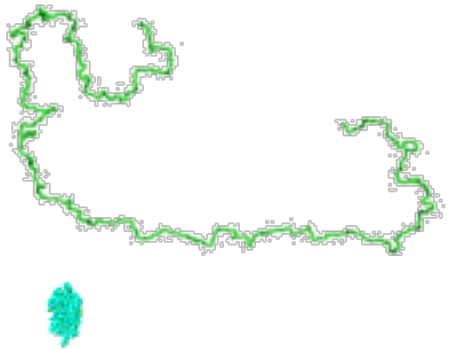


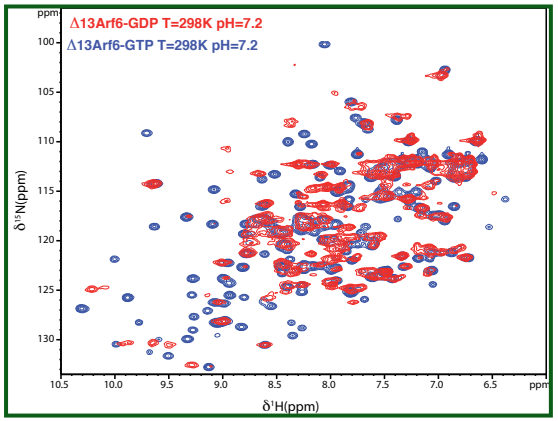


NMR spectra of free form (54 kDa) (5mM) and bound form (TeraDalton) (20mM)

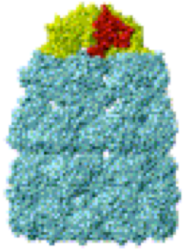
NMR - Spatial and Time scales are highly intermingled

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

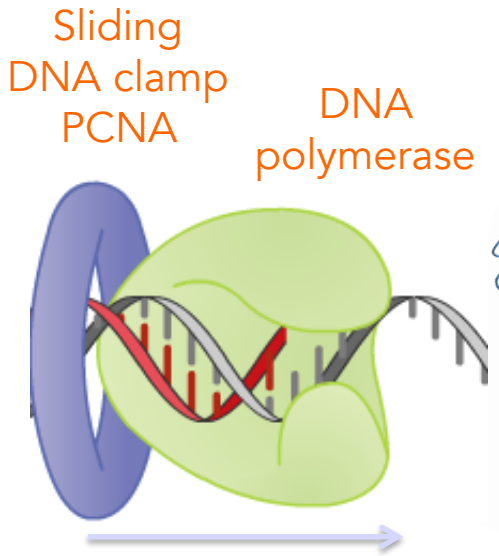
Global correlation time t_c (size)

Linewidth

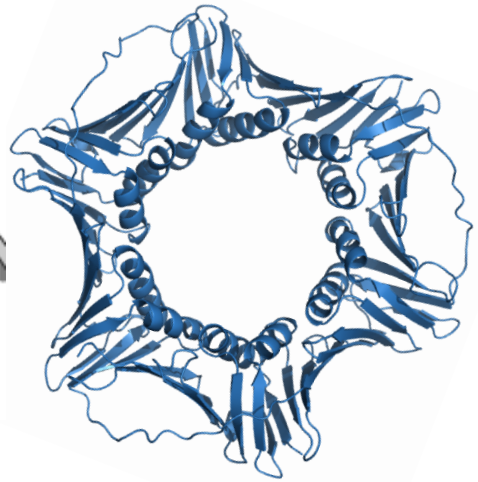
+ Local Rotational fluctuation timescale
+ density of relaxation sources

+ conformational/chemical exchange

p15^{PAF} a PCNA-Binding Protein

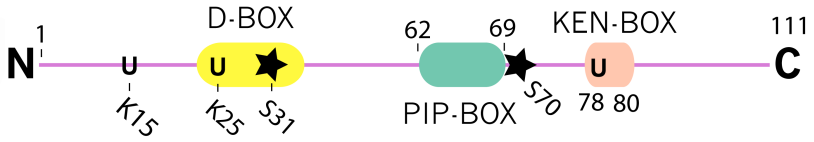


PCNA



- ✓ PCNA is a trimeric ring that can accommodate a DNA duplex through its central channel
- ✓ PCNA acts as a processivity factor for DNA polymerase ε

P15

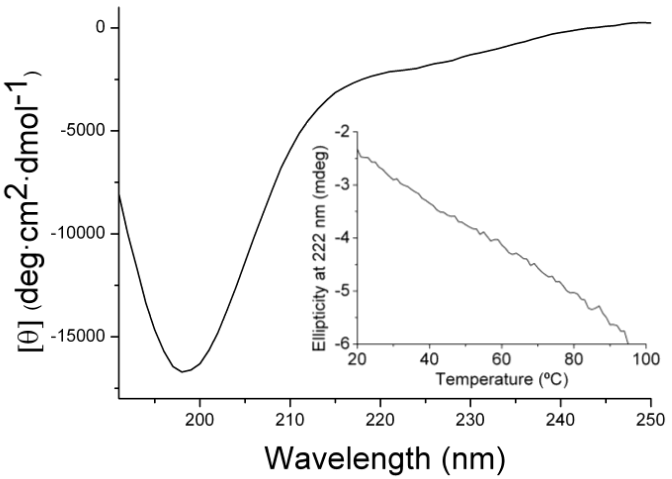


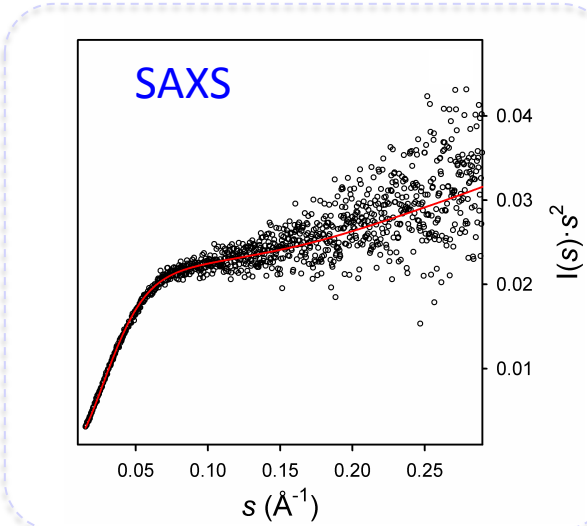
- ✓ P15 acts as a regulator of DNA repair
- ✓ is over-expressed in several types of human cancer
- ✓ It binds PCNA through its PIP-BOX
- ✓ Targeted for degradation by the ubiquitin ligase APC through its conserved KEN-BOX.

Col. Francisco Blanco (CIC-BioGune-Bilbao)

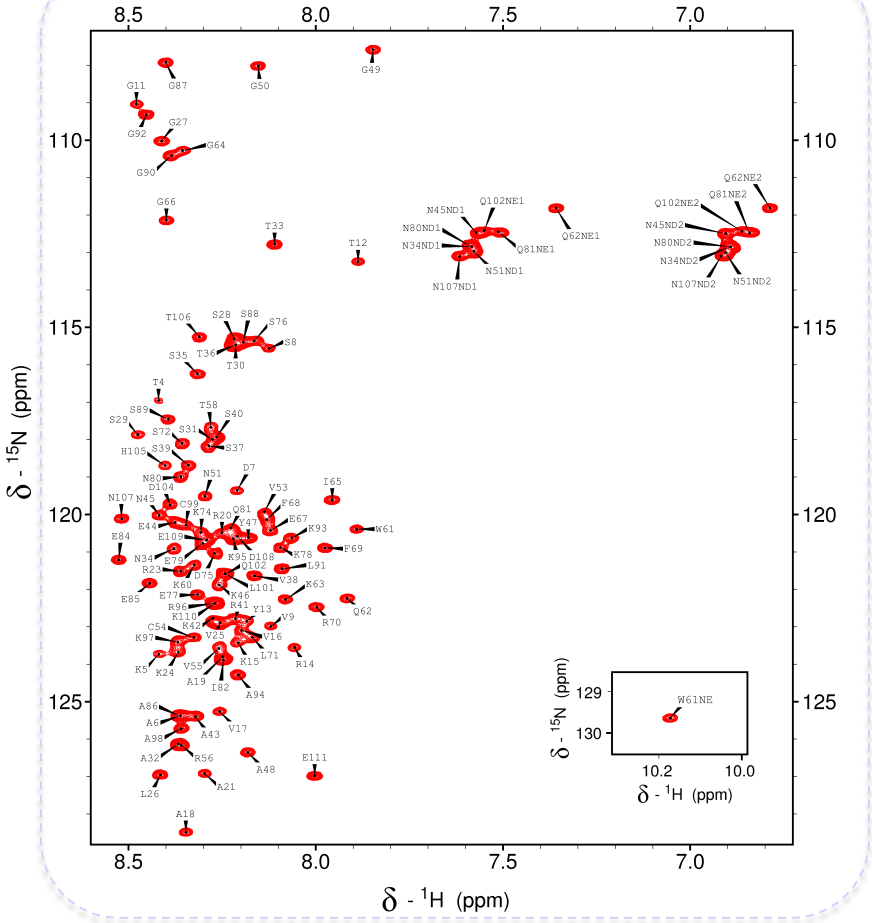
p15^{PAF} is an IDP …

CD



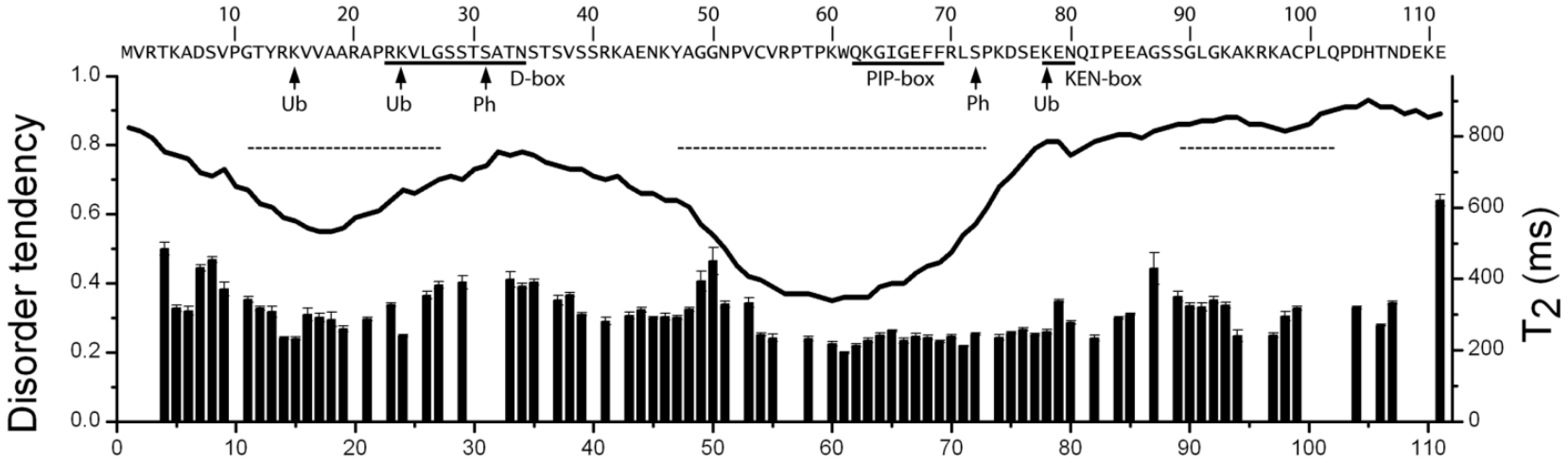


NMR

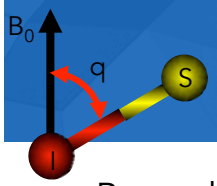


De Biasio *et al. Biophys J.* 2014

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

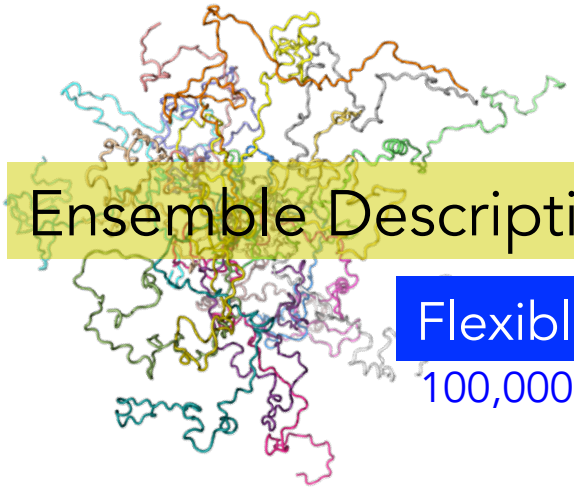


- ✓ Disorder prediction is consistent with p15 being largely unstructured, with the exception of residues of the highly conserved PIP-box motif.
- ✓ p15 shows several relatively short T_2 values, that correspond to sequences with reduced predicted disorder.



Transient structuration monitored by RDCs

Bernado et al. PNAS 2005



Ensemble Description

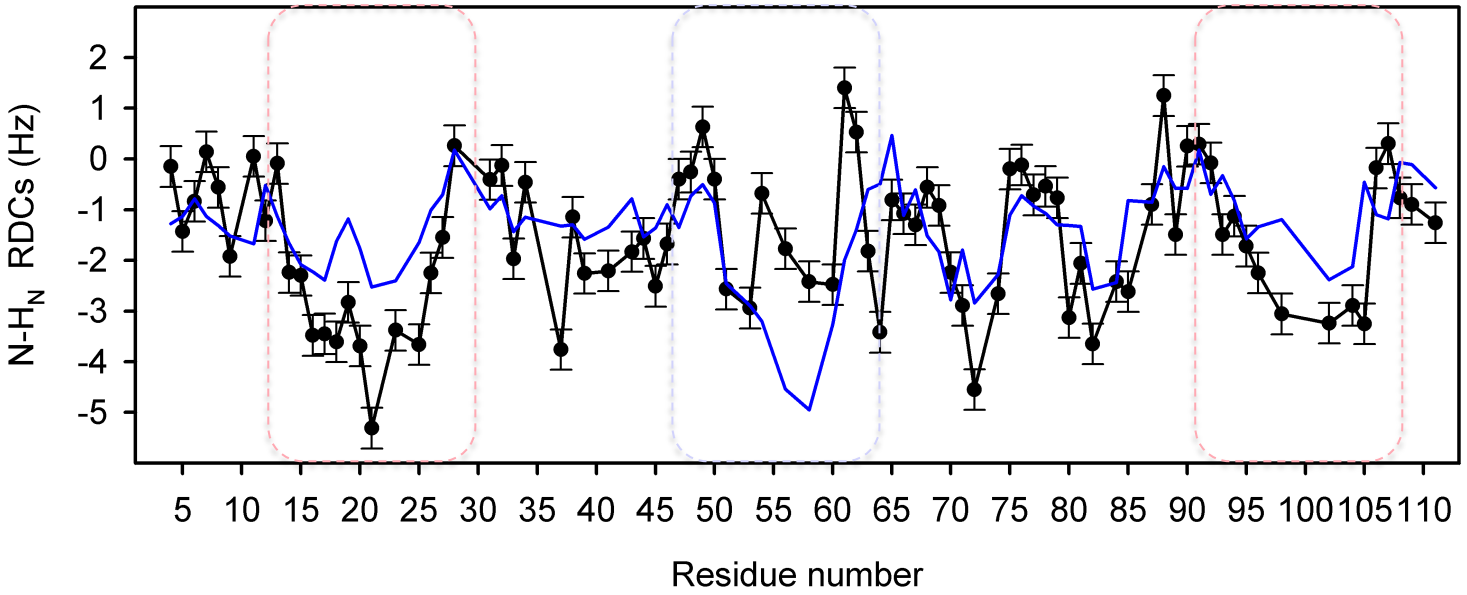
Flexible-Meccano

100,000 RC structures

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

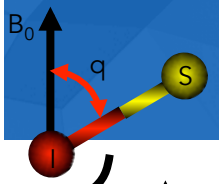
Secondary Structural Element :
Position and Population

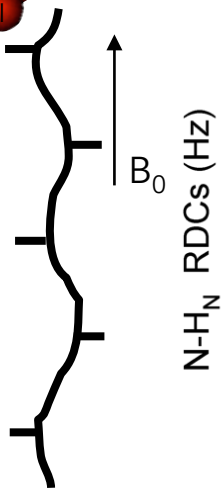


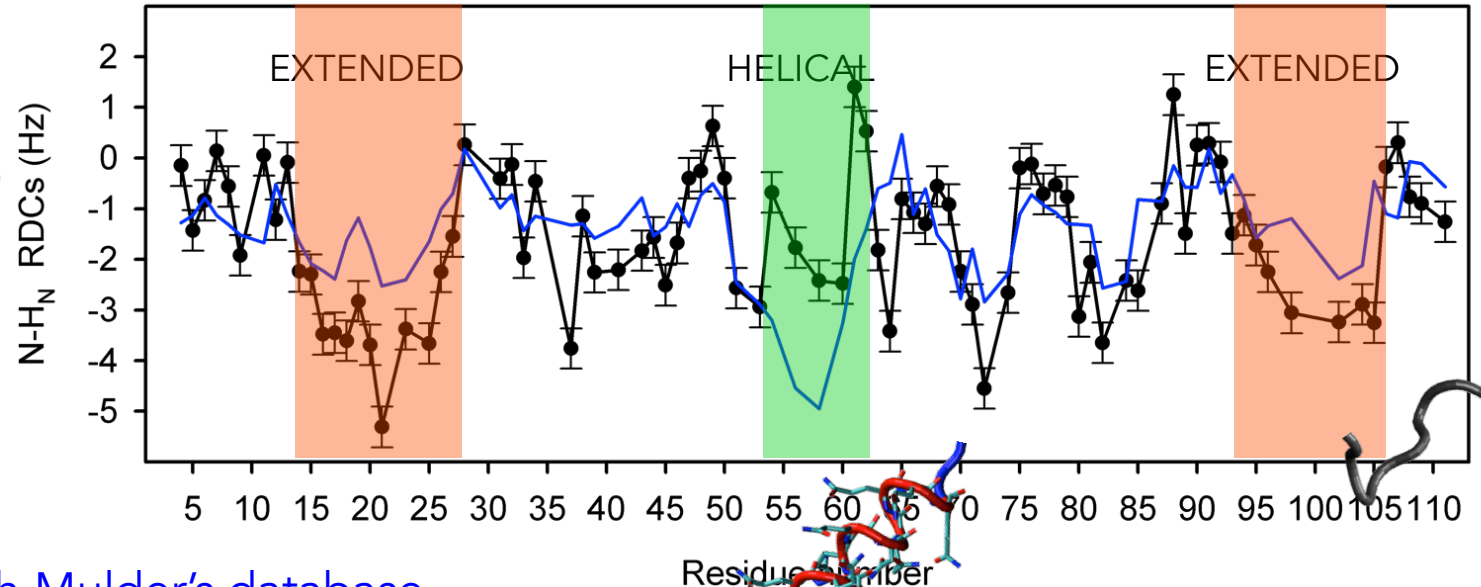
RC RDC
Exp RDC

The RC model cannot fully reproduce the measured RDCs

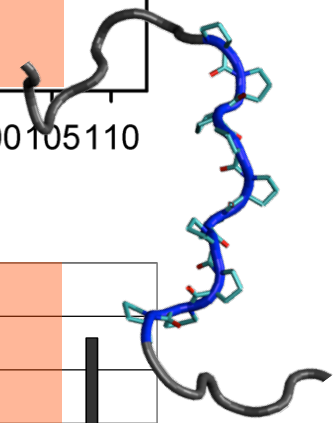
Transient structuration monitored by RDCs



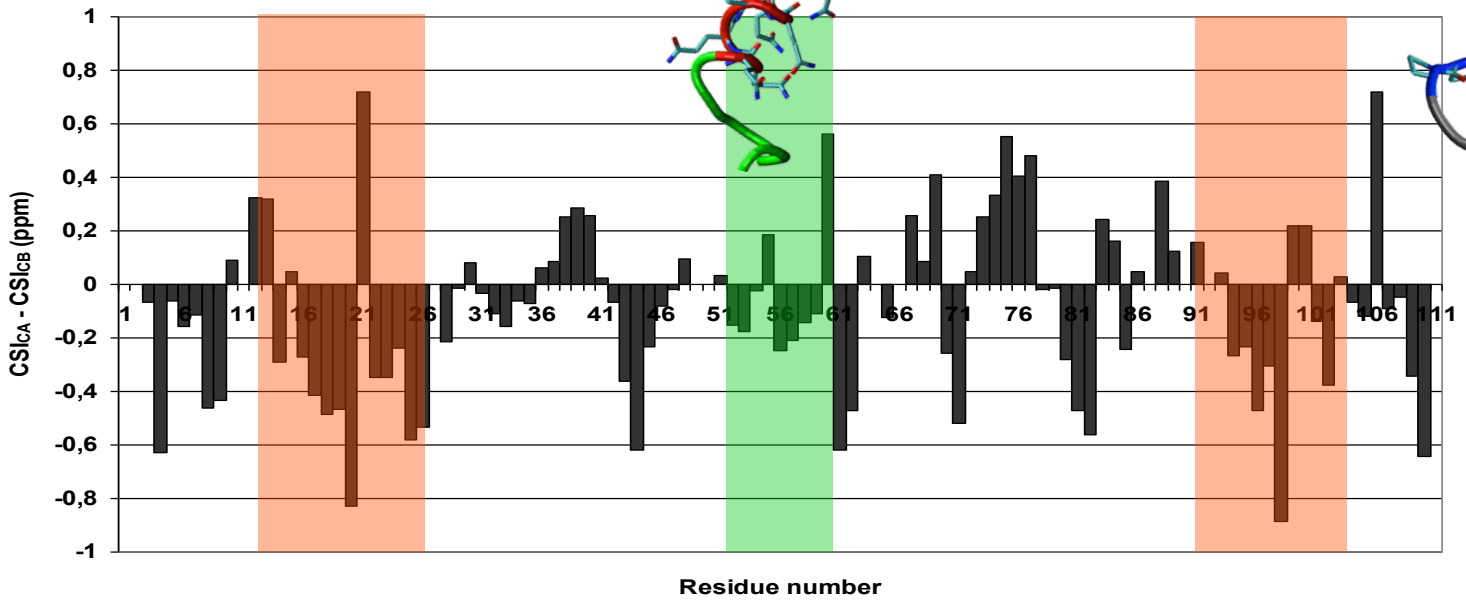




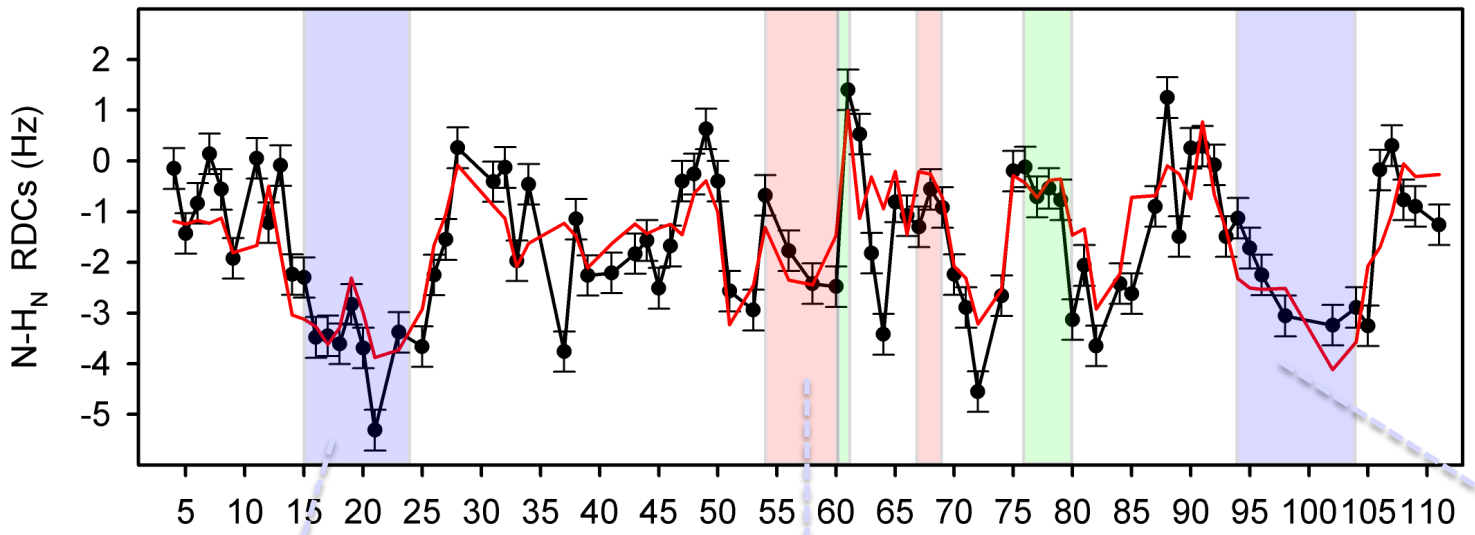
RC RDC
Exp RDC



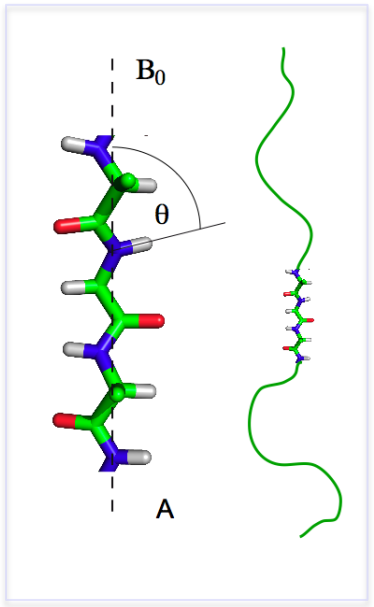
CSI with Mulder's database



Structural Elements in p15



FM RDC
Exp RDC



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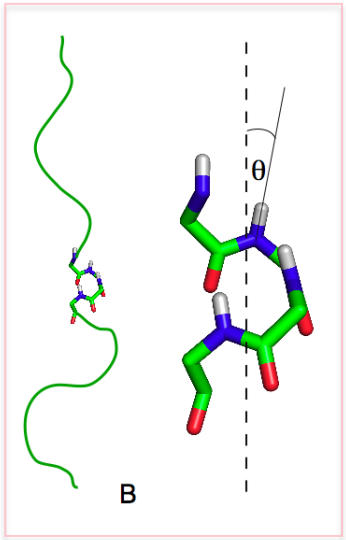

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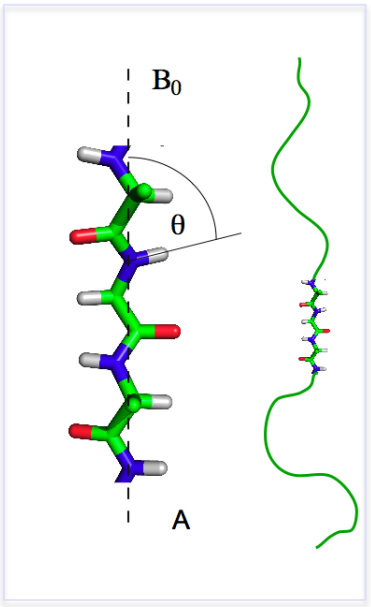
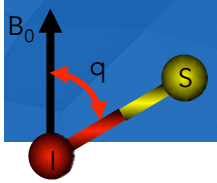
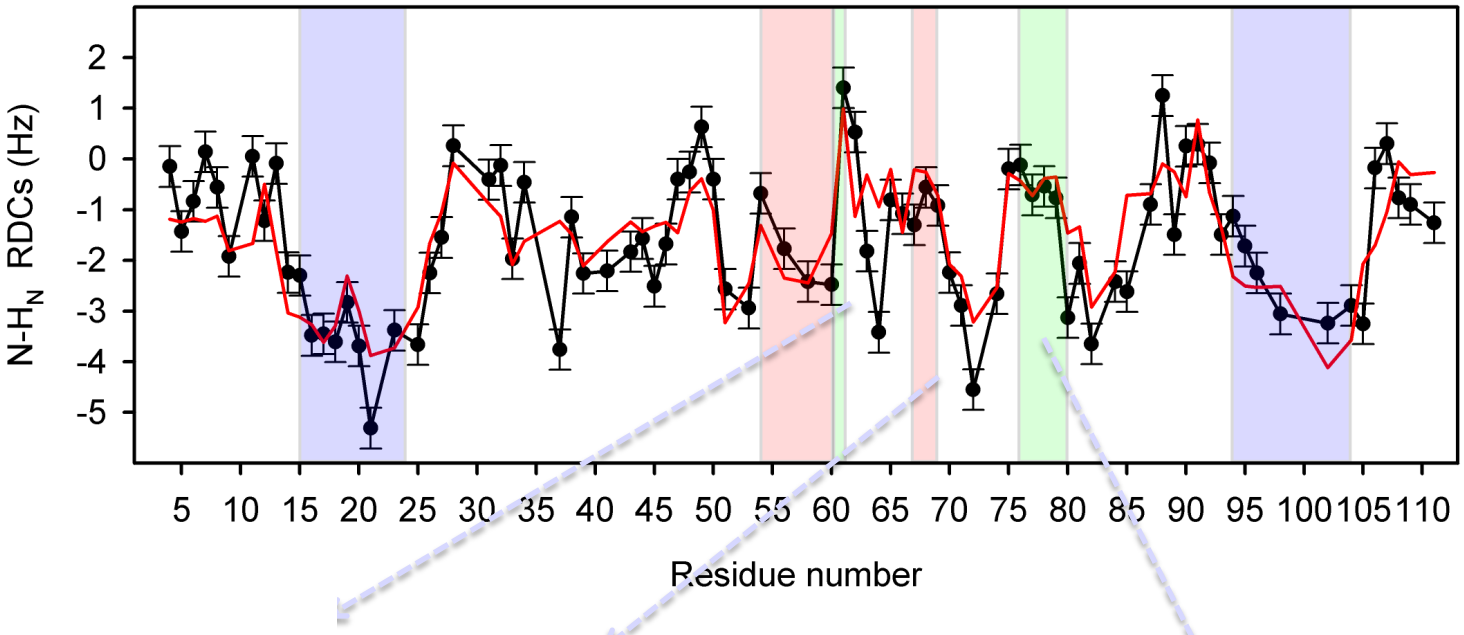


8%

100,000 structures



Structural Optimization



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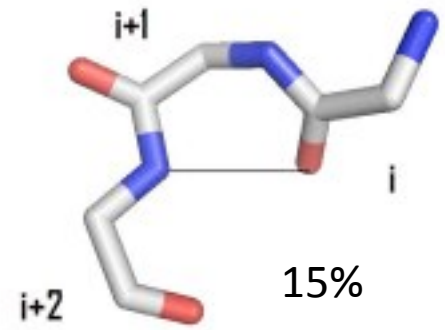

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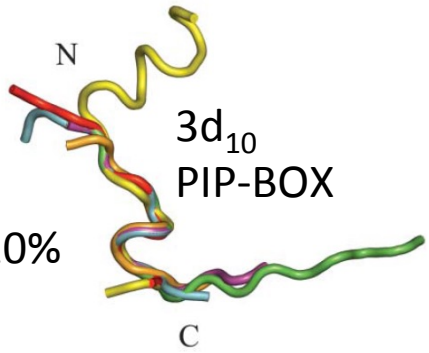
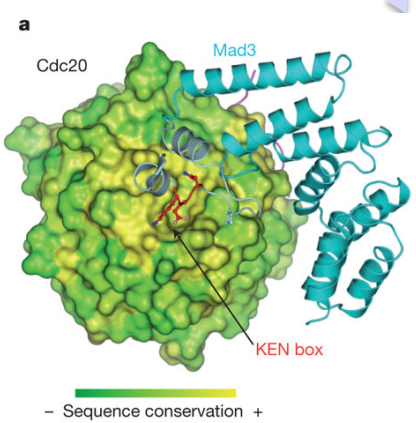
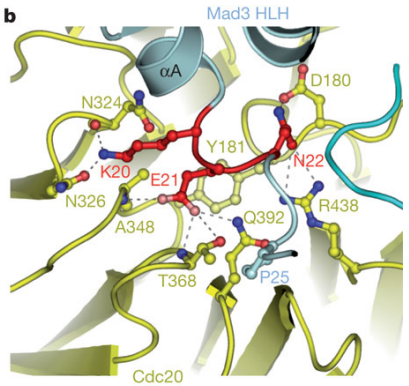


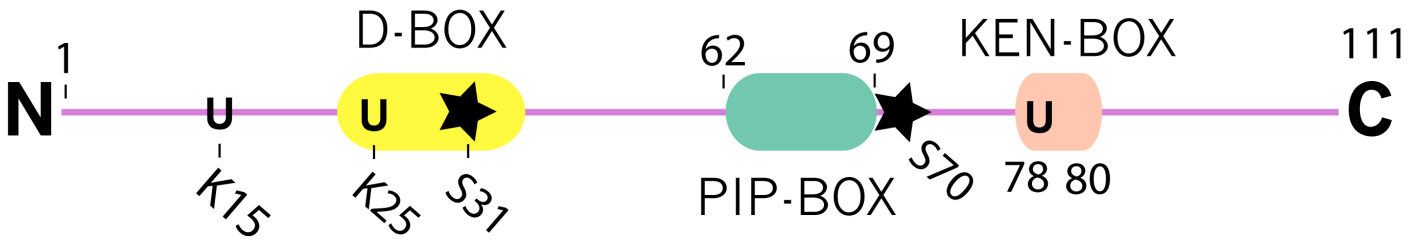
10%

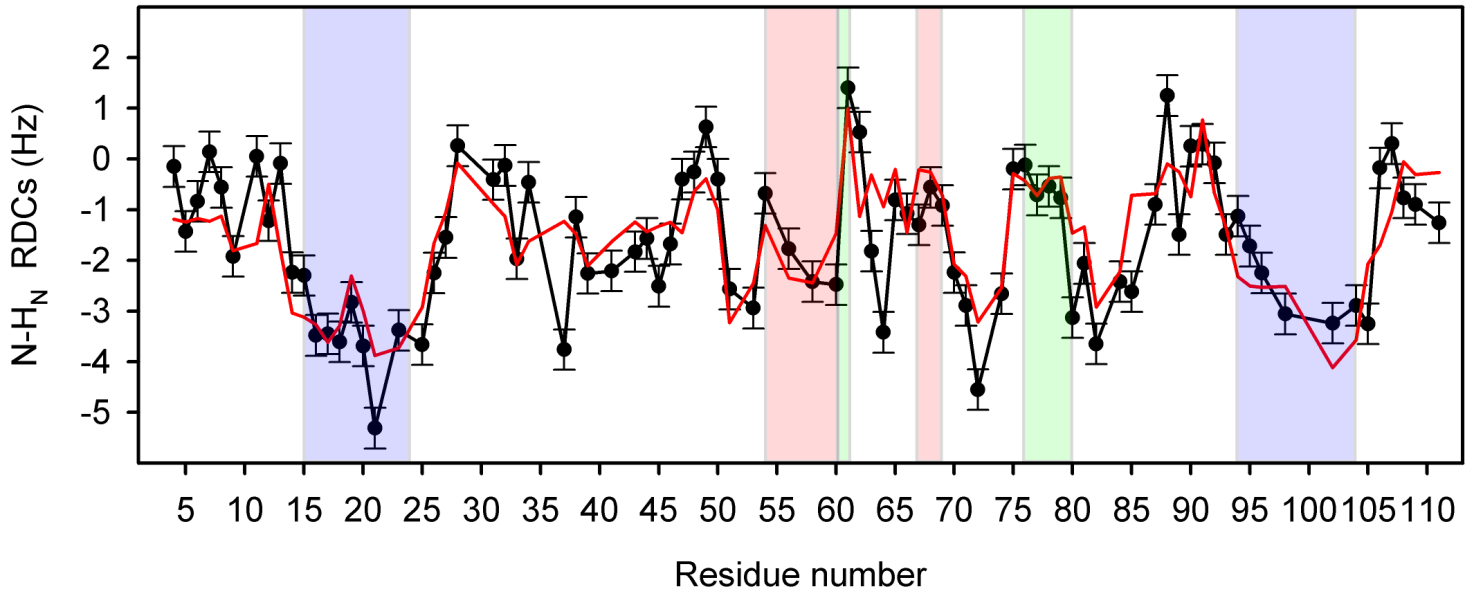
$3d_{10}$
PIP-BOX





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

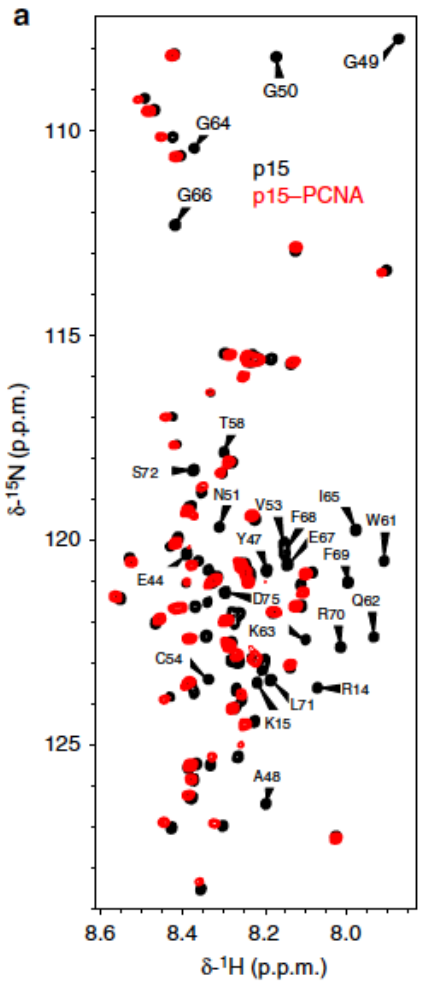


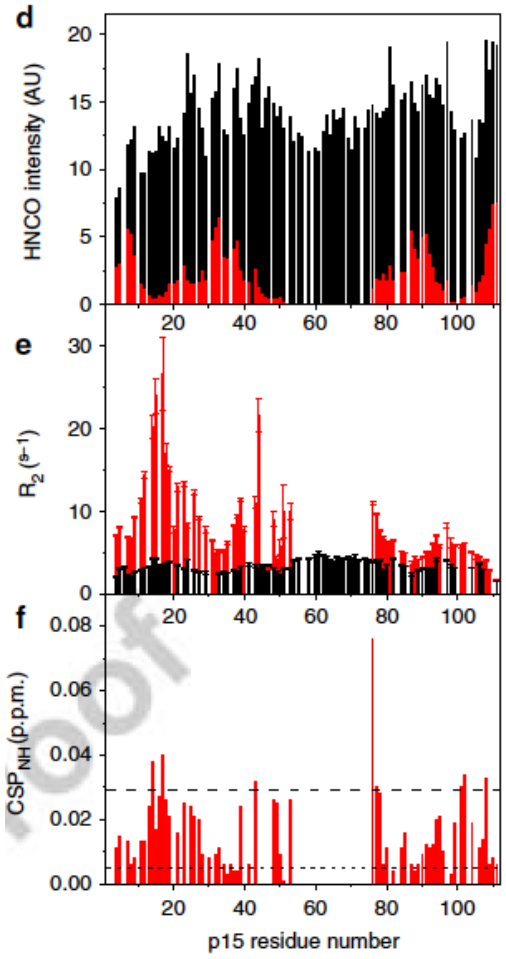


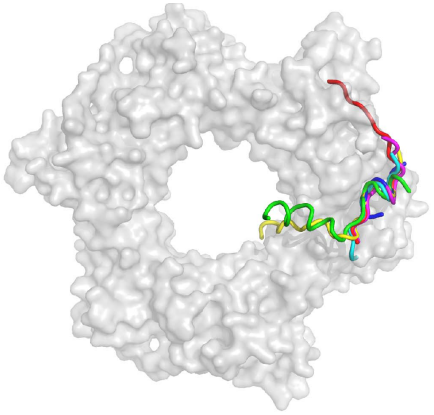
De Biasio A, Ibáñez de Opakua A, Cordeiro TN, Villate M, Merino N, Sibille N, Lelli M, Diercks T, Bernadó P, Blanco FJ. p15PAF is an intrinsically disordered protein with nonrandom structural preferences at sites of interaction with other proteins. Biophys J. 2014 Feb 18;106(4):865-74

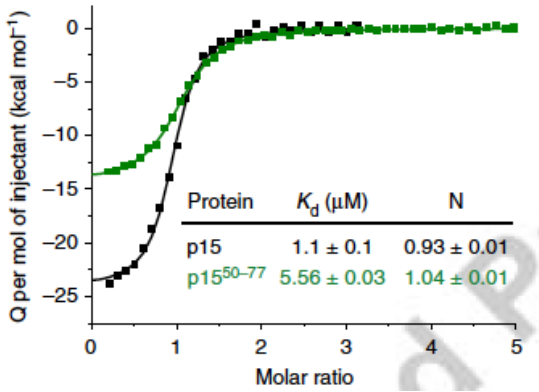
Interaction of p15^{PAF} with PCNA

- ✓ Mapping of PCNA binding on p15 (12 kDa) by NMR



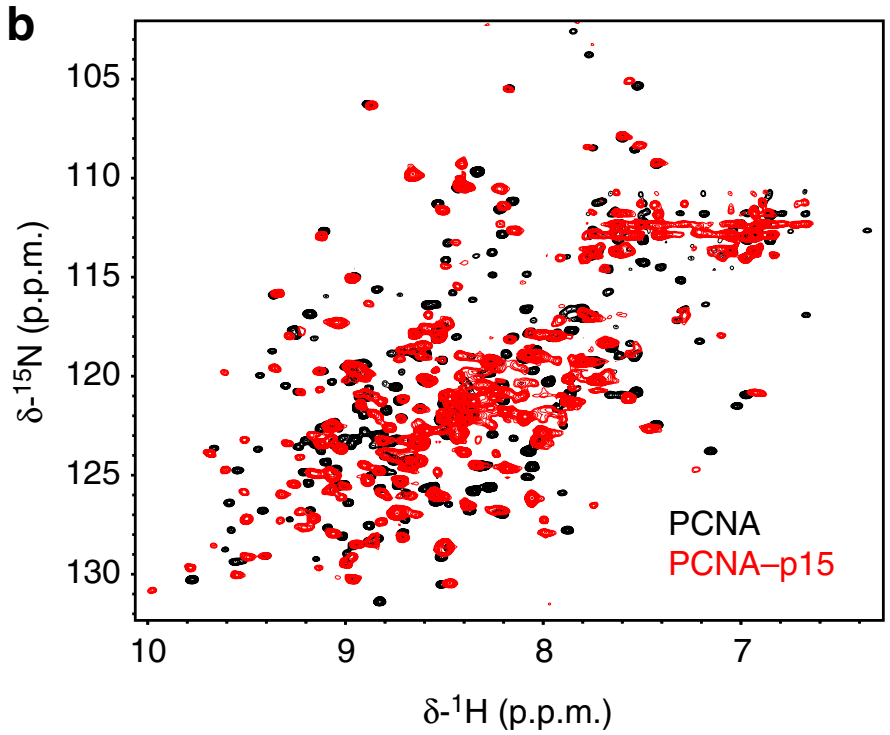




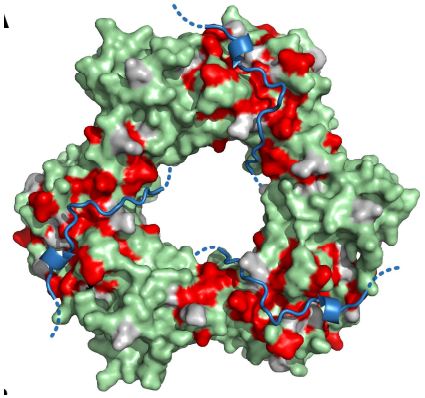


Interaction of p15^{PAF} on PCNA

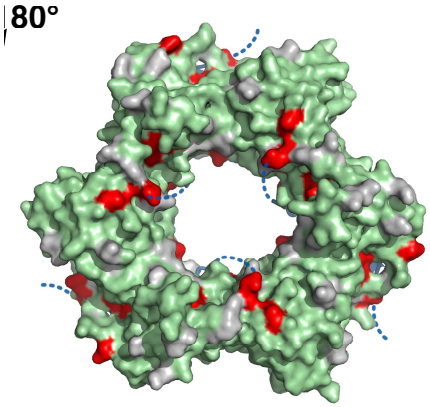
✓ Mapping of p15 binding on PCNA (86 kDa, $^{15}N/^{2}H$) by NMR



PCNA front-side

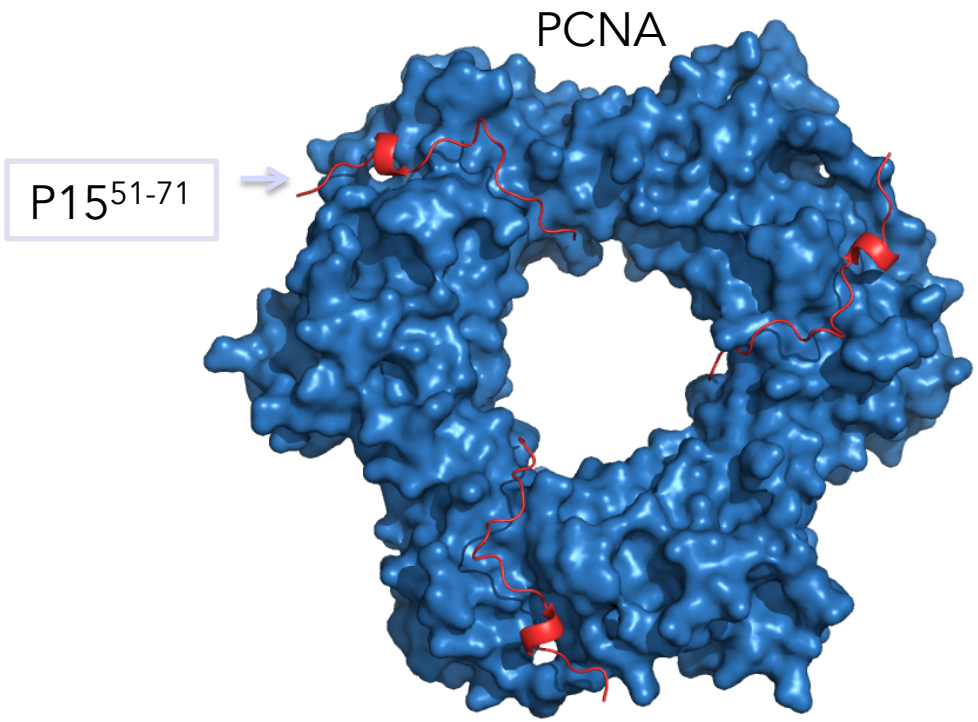


PCNA back-side



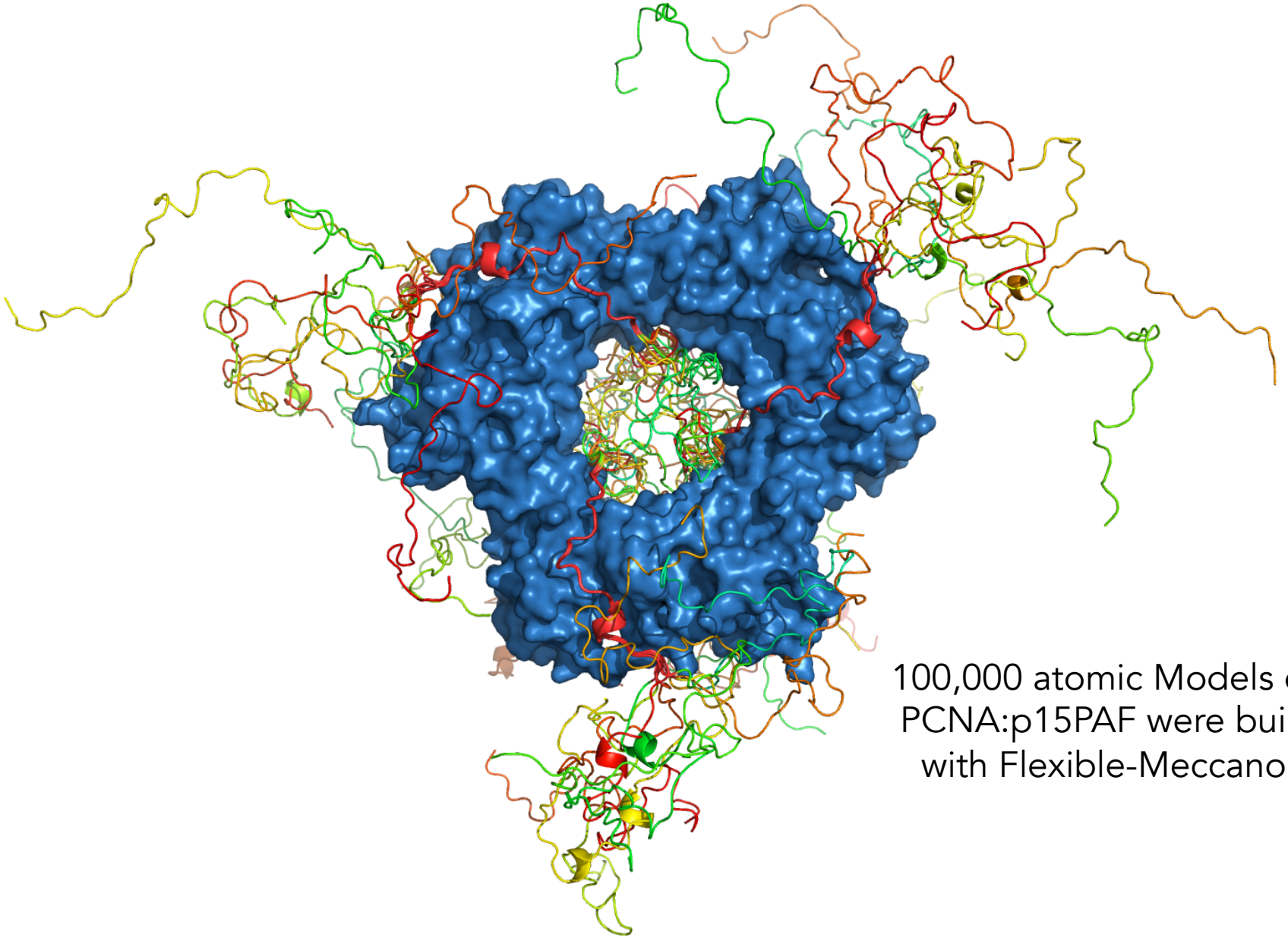
De Biasio et al. Nature Comm. 2015

Crystallographic Structure of p15^{PAF} with PCNA



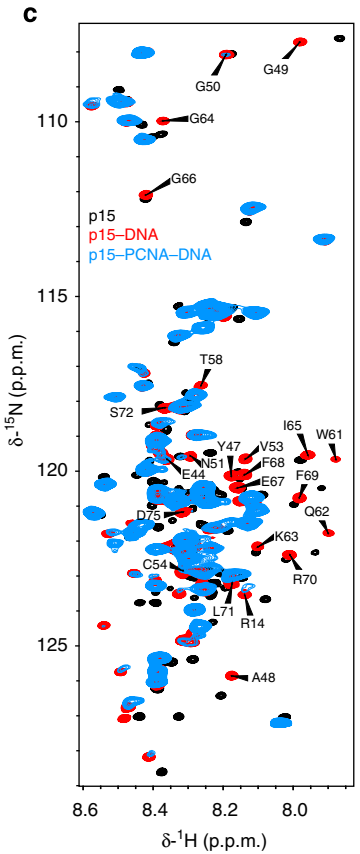
PDB:4D2G

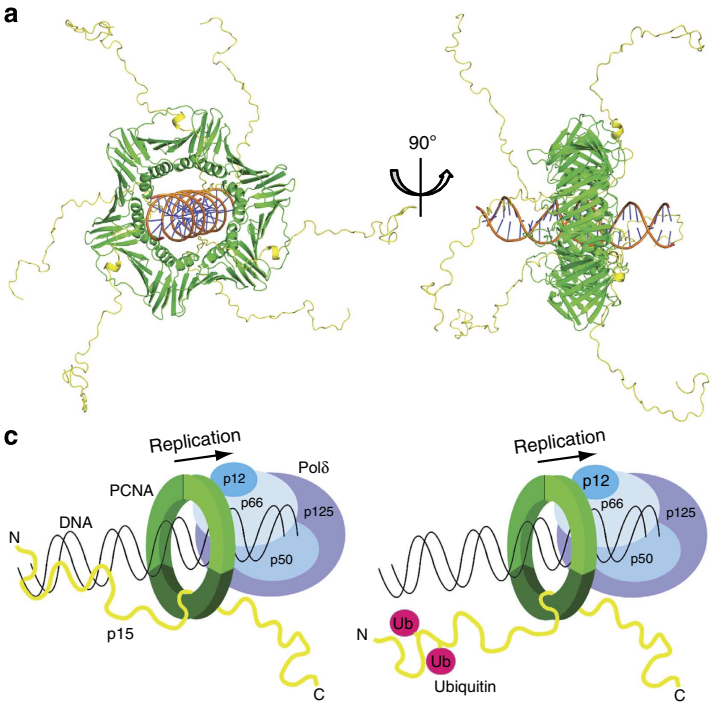
A 3D Model of the PCNA:p15^{PAF} Complex

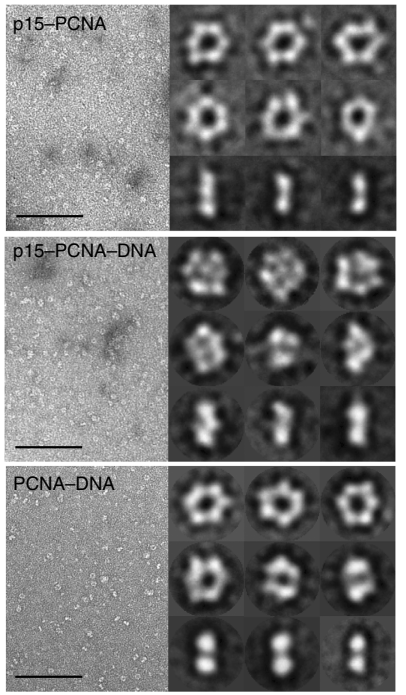


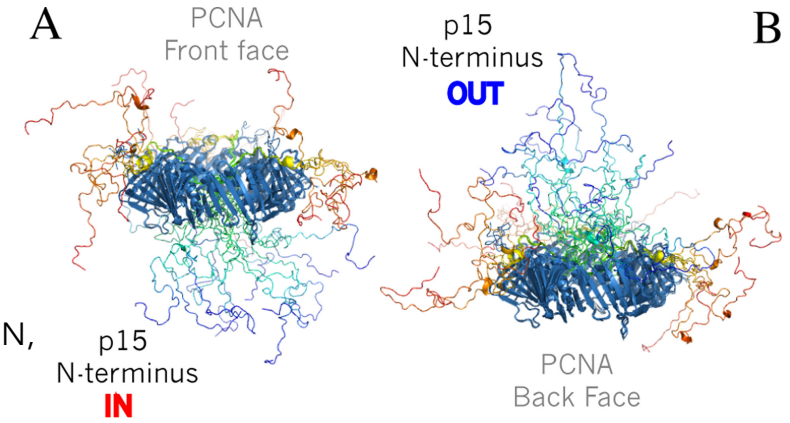
100,000 atomic Models of PCNA:p15PAF were built with Flexible-Meccano

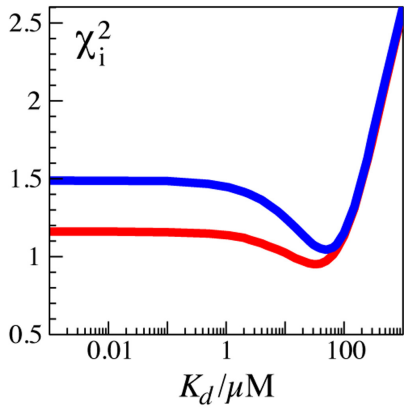
Electron Microscopy









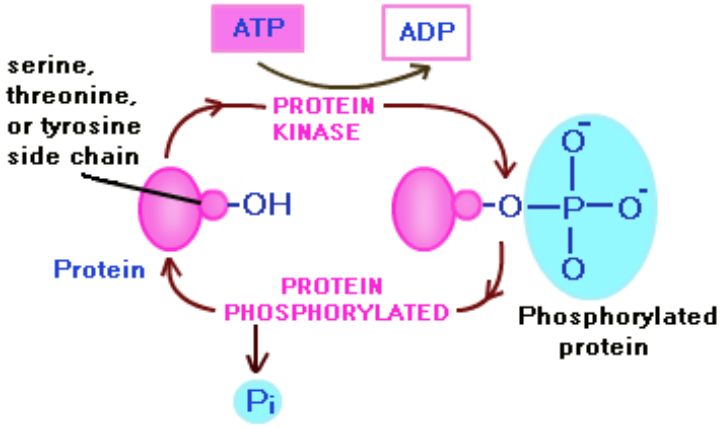


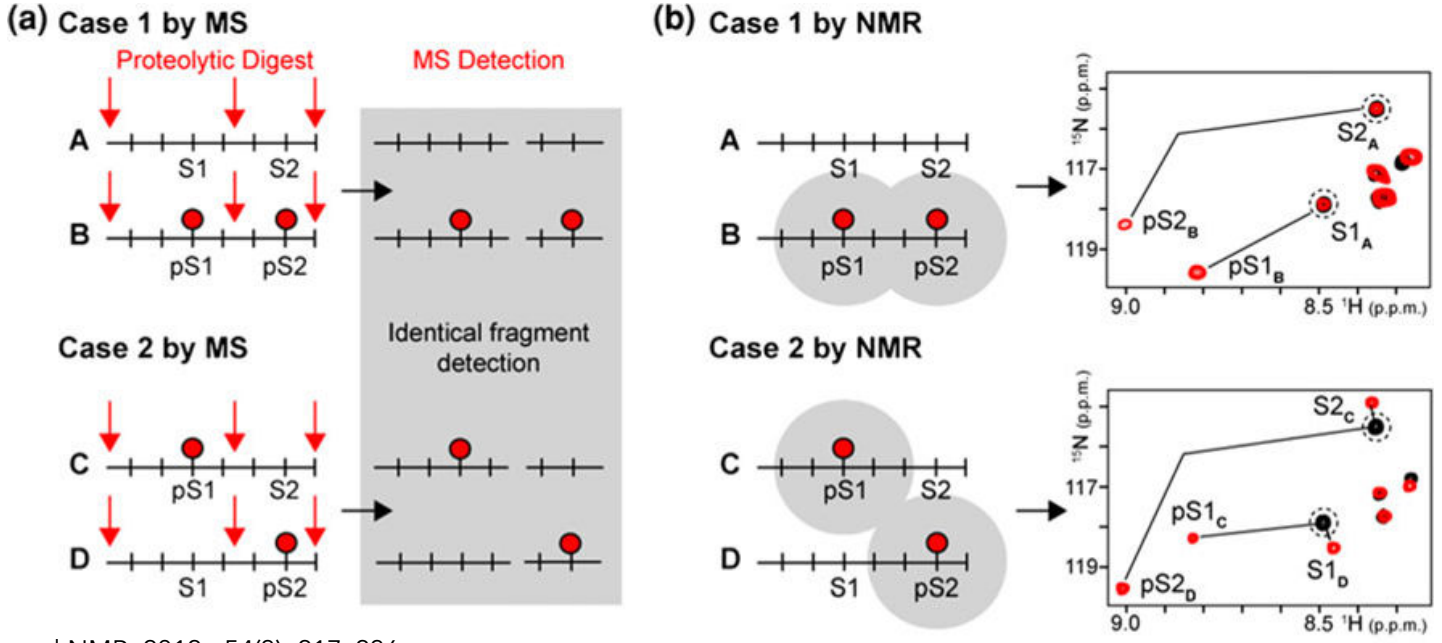
Cordeiro TN, Chen PC, De Biasio A, Sibille N, Blanco FJ, Hub JS, Crehuet R, Bernadó P. (2017) Nucleic Acids Res. 45(3):1501-1515.

Phosphorylation

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.



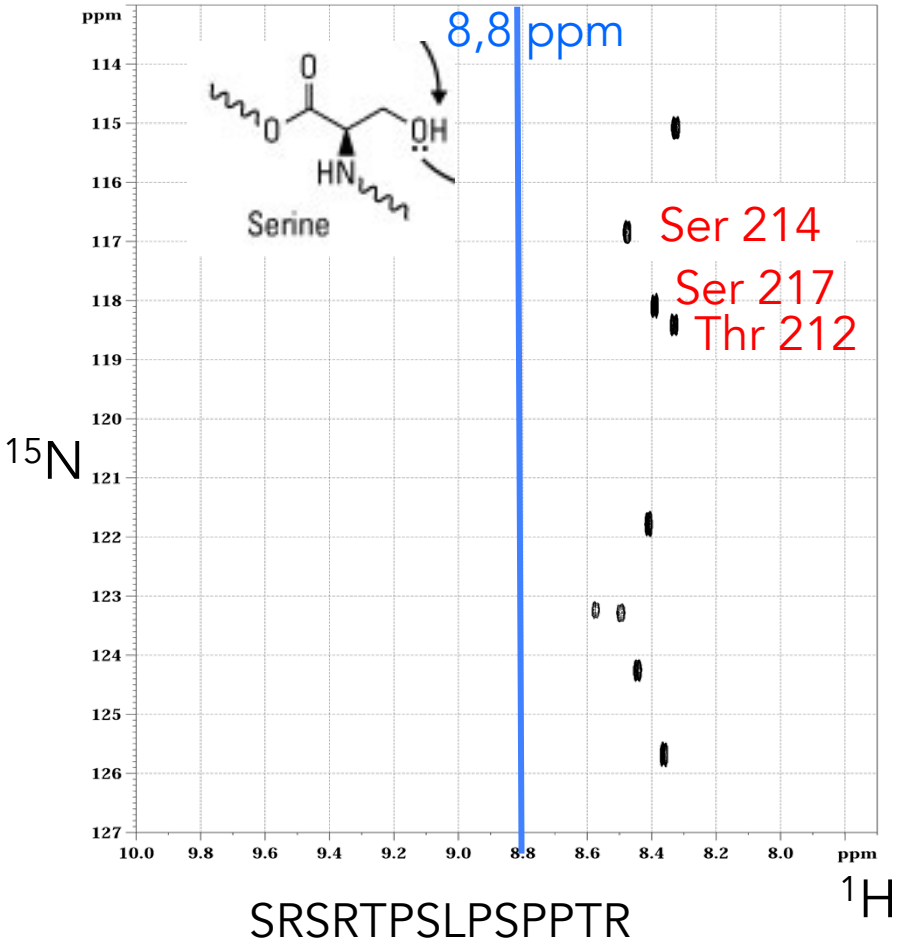


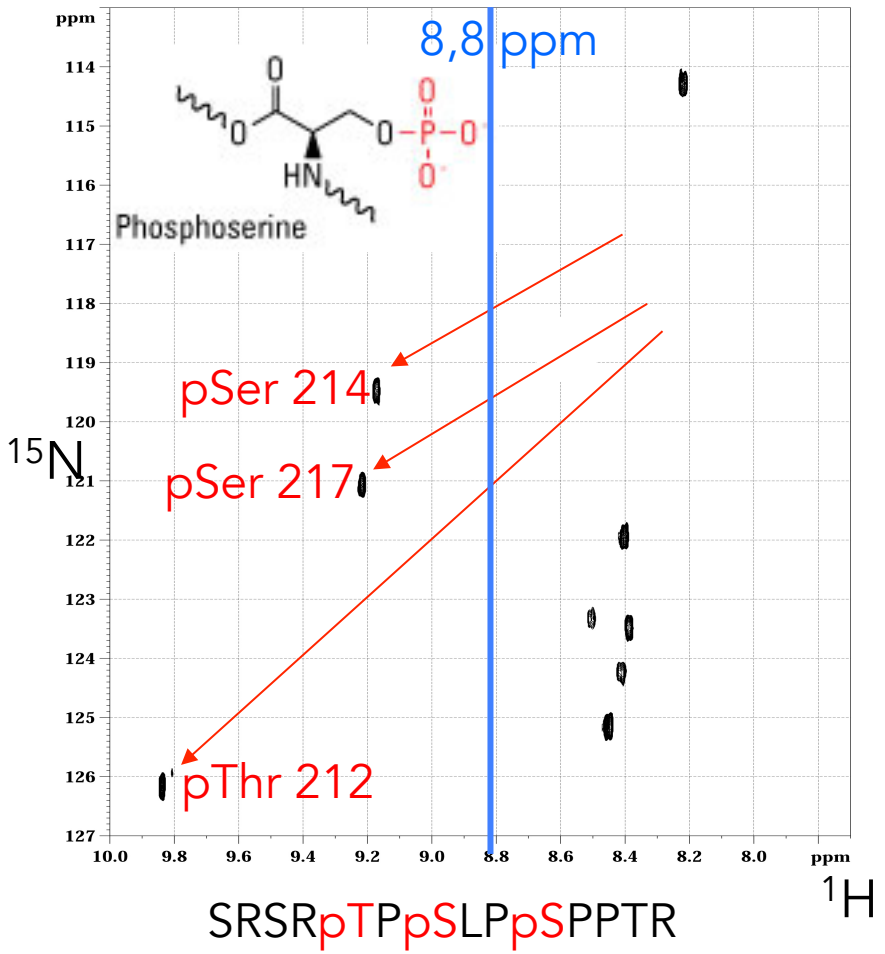
Theillet et al. J Biomol NMR. 2012 , 54(3): 217–236

NMR is Qualitative and Quantitative

What sites? and to what extent?

➔ In vitro phosphorylation by recombinant kinases: Identification and quantification

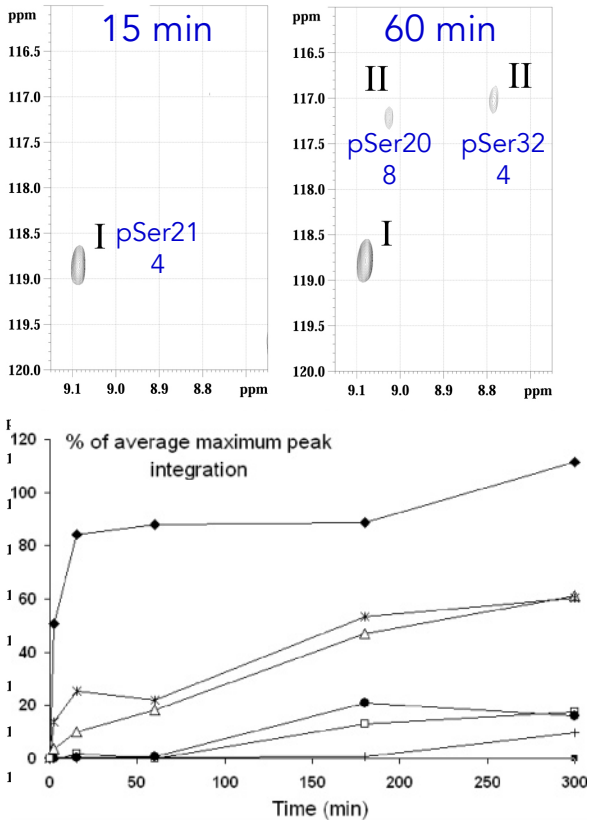




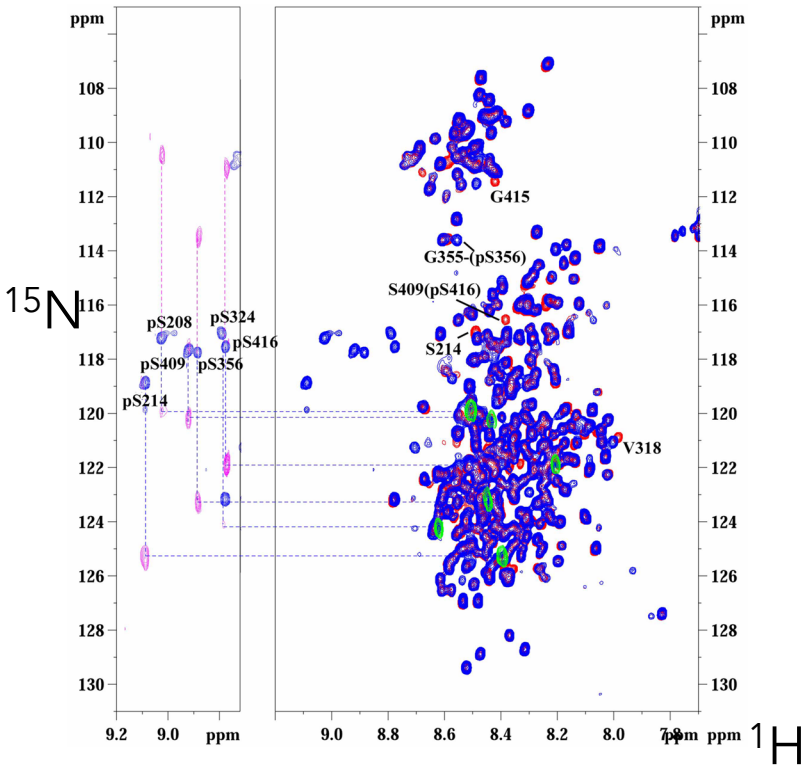
Kinetic of Phosphorylation

cAMP protein dependant kinase PKA

PKA from Prof. Langer/Schwalbe, Frankfurt, Germany



Sequential kinetic



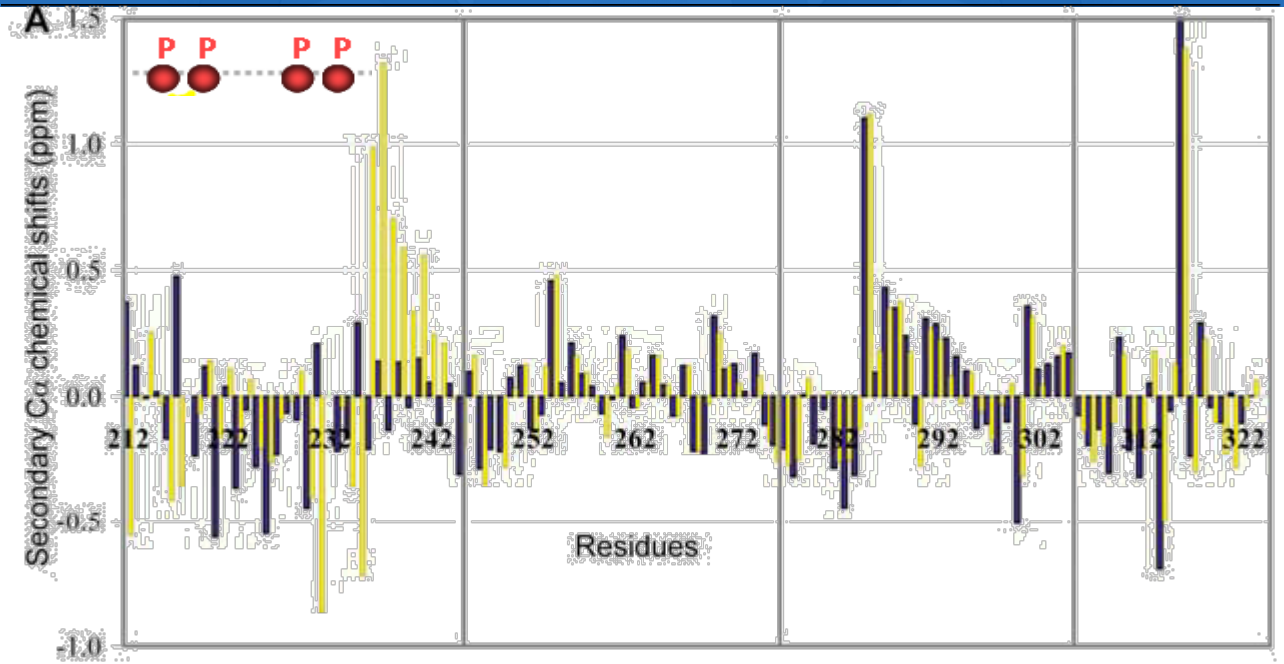
Phosphorylated residues assignment
(triple resonance NMR experiments)

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

Is there some degree of structuration ?

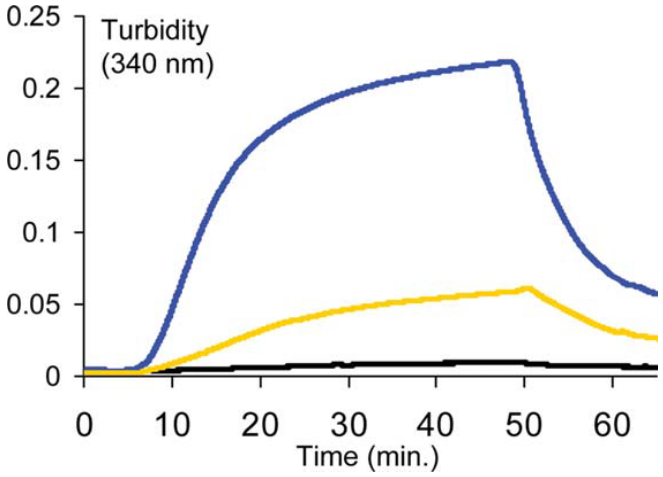
CDK2/CycA3 kinase on Thr231 (AT180 epitope)
Thr235
Thr212
Thr217

Linked to recognition events



Here linked to a loss of function

Tubulin polymerization assay



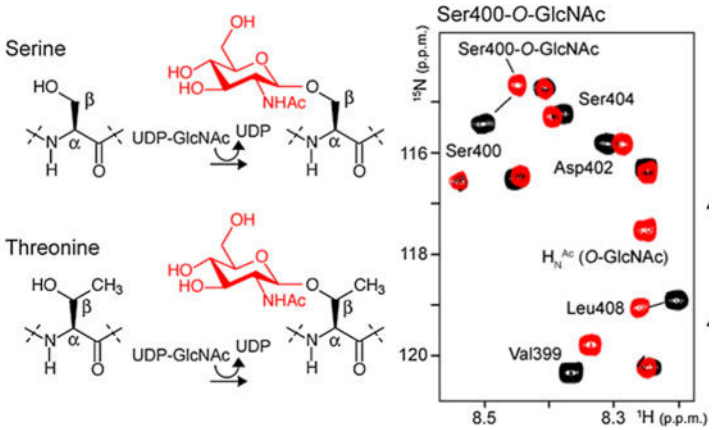
Sibille et al. Proteins (2012) 80, 454-462

Where and which kind ?

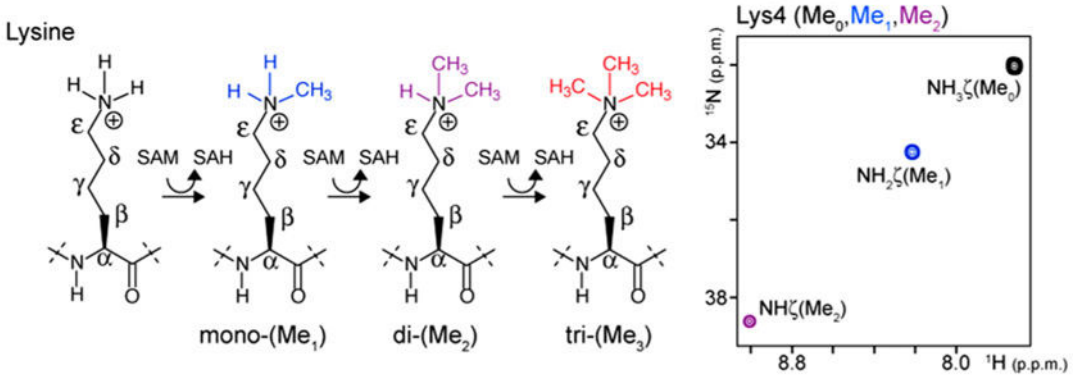
Other Post-translational Modification

Cell signaling, post-translational protein modifications and NMR spectroscopy
Francois-Xavier Theillet et al. J Biomol NMR. 2012 November ; 54(3): 217–236.

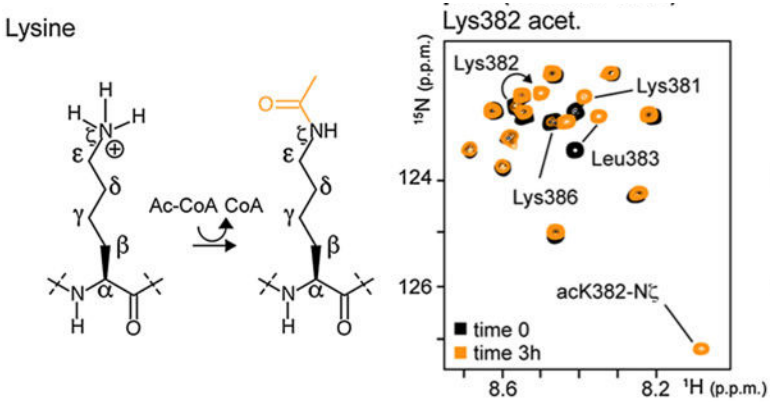
Serine, threonine glycosylation



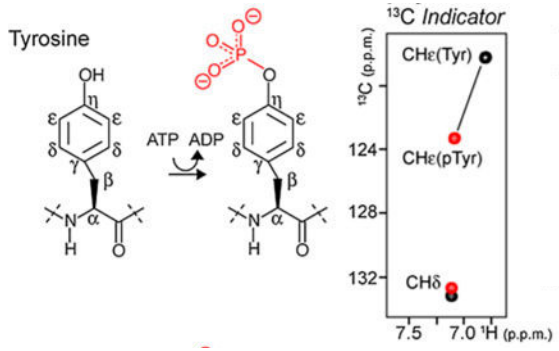
Lysine and arginine alkylation



Lysine acylation



Serine, threonine, tyrosine and histidine phosphorylation



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Zhanna Santybayeva

Thanks for your attention

Questions