



Relaxation et phénomènes dynamiques en Résonance Magnétique Nucléaire

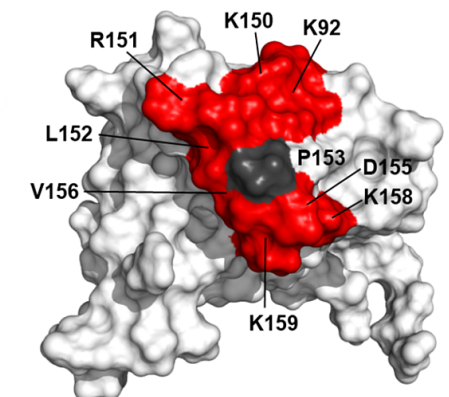
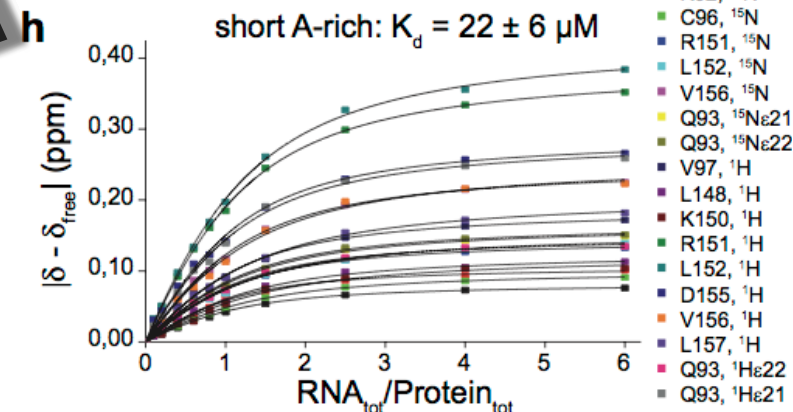
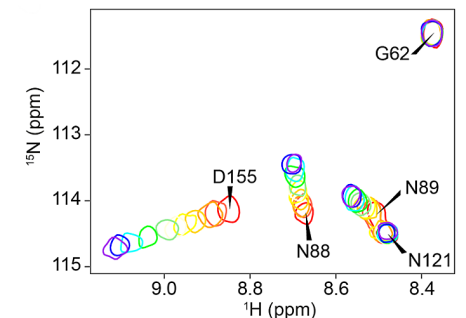
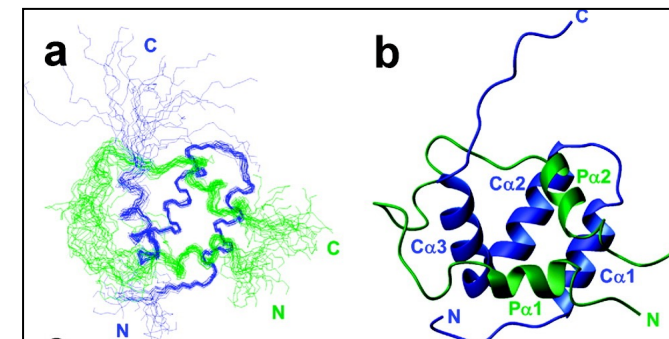
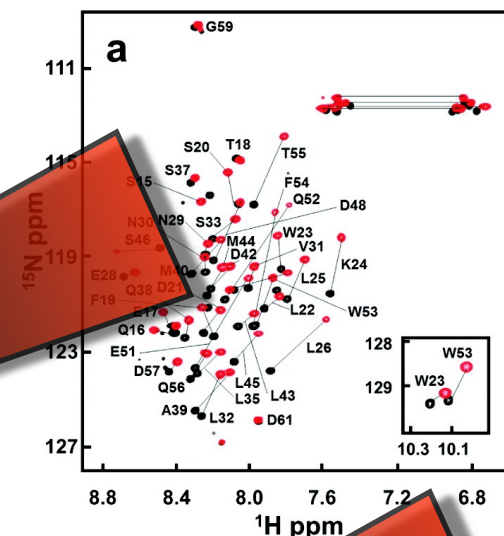
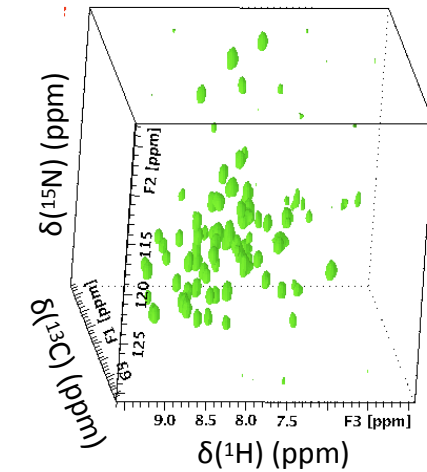
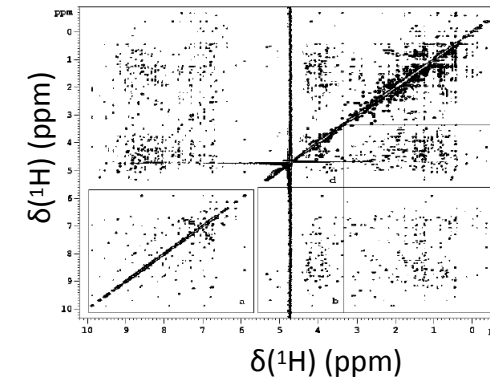
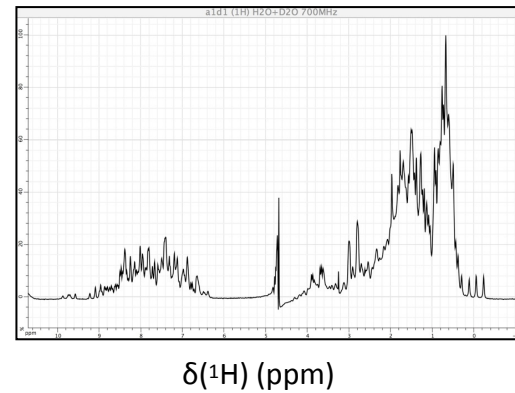
Application aux protéines



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MOTS CLES

- ➔ Spins, Champs magnétiques
- ➔ Fréquence de résonance
- ➔ Spectre
 - ➔ Déplacement chimique
 - ➔ Largeur de raie
- ➔ Environnement électronique
- ➔ Communication entre spins
 - ➔ Proximité le long des liaisons
 - ➔ Proximité dans l'espace

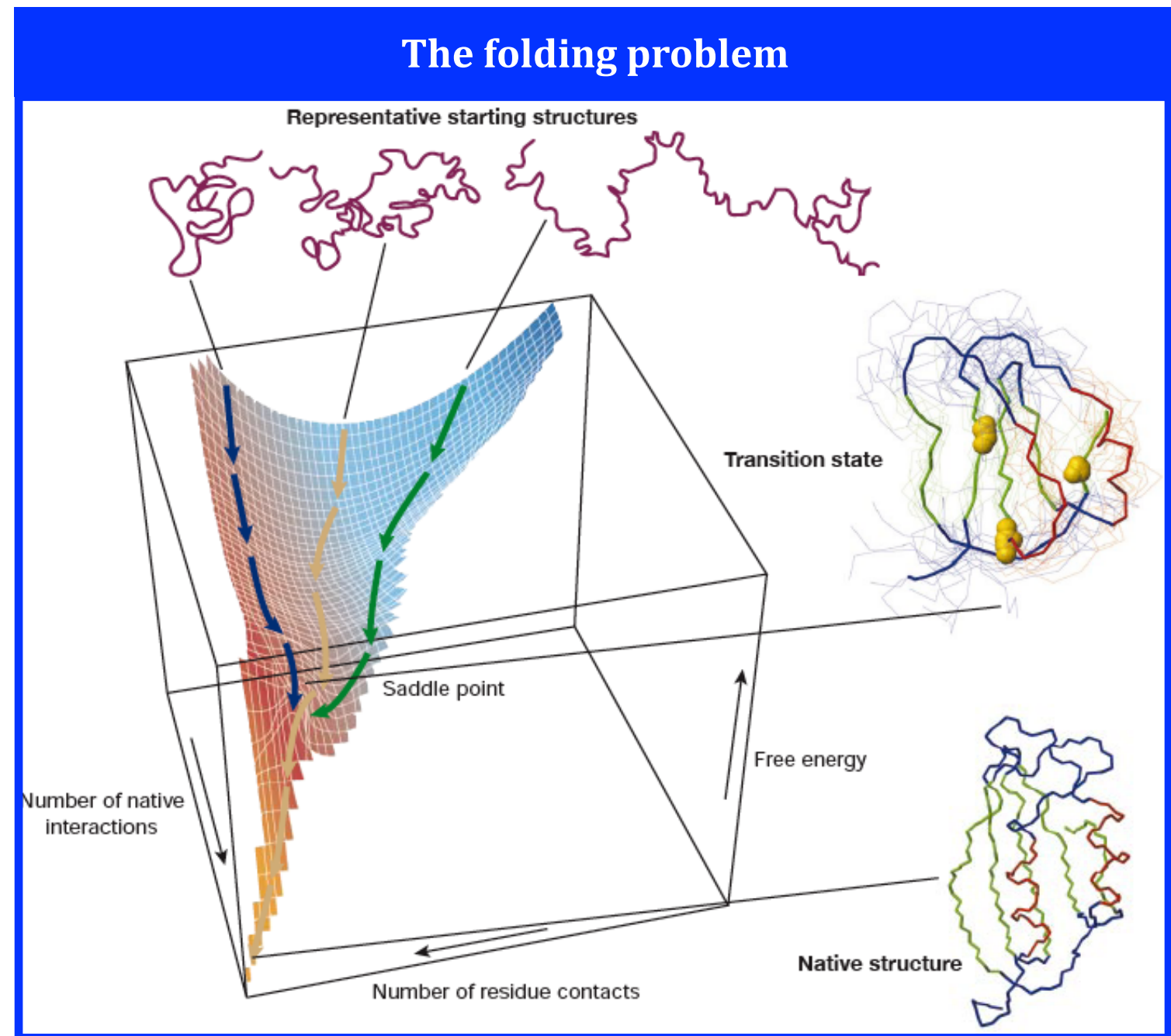
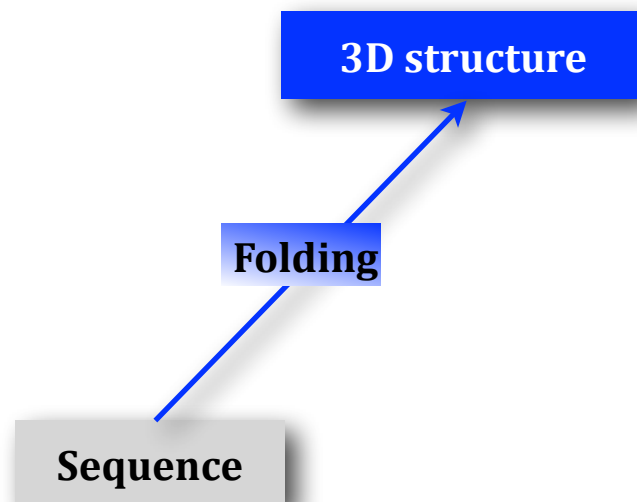


- ➔ Interactions
- ➔ Mouvements, flexibilité
- ➔ Structure

➔ Caractériser des interactions protéines/partenaires (sites d'interaction, affinités, cinétique)

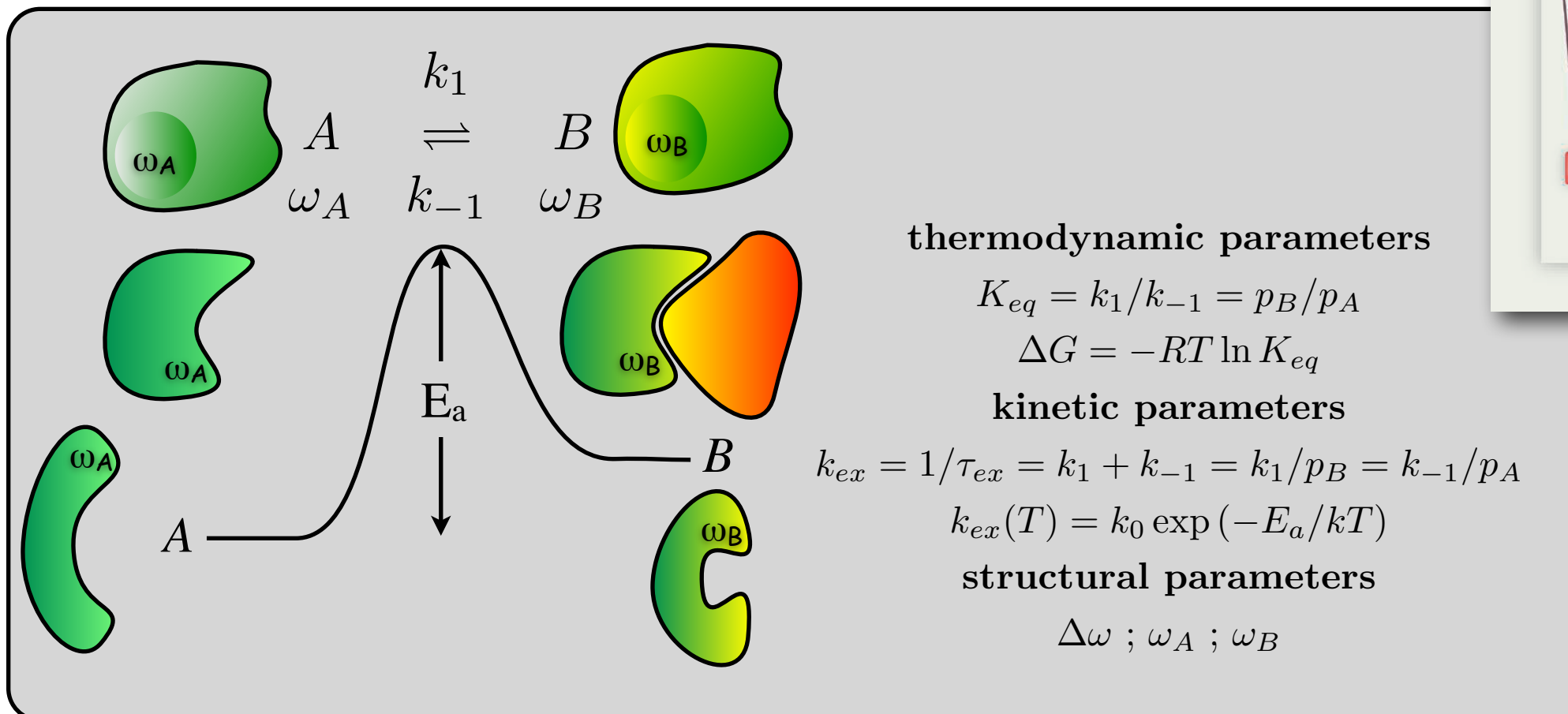
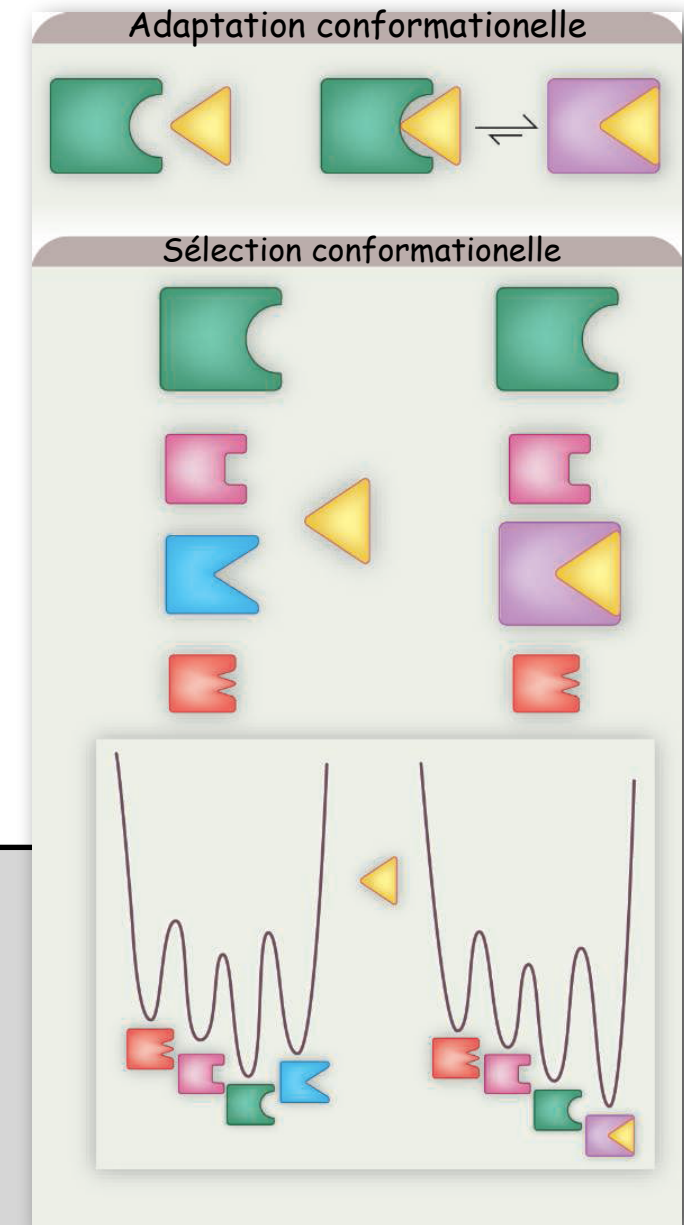
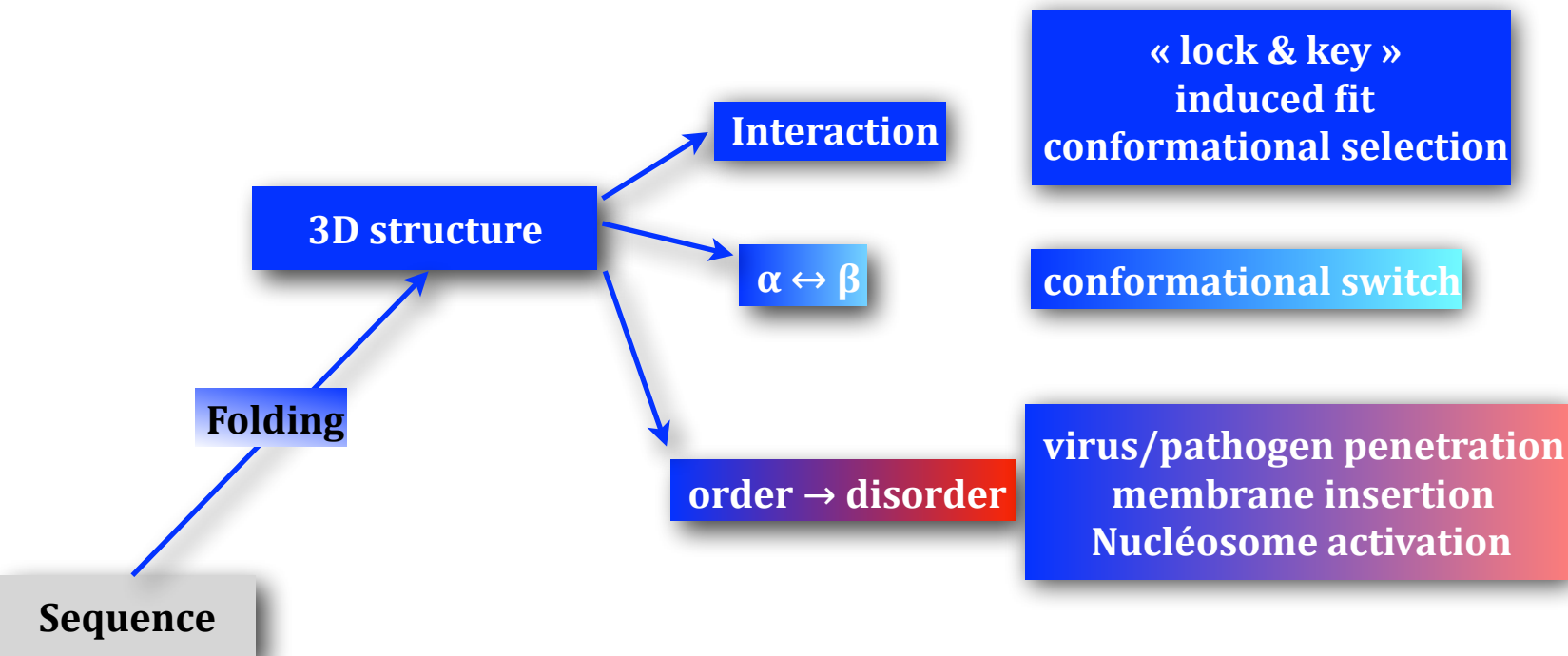
➔ Caractériser des états multiples fonctionnels de protéines

A key question : explore multiple states of proteins and their plasticity

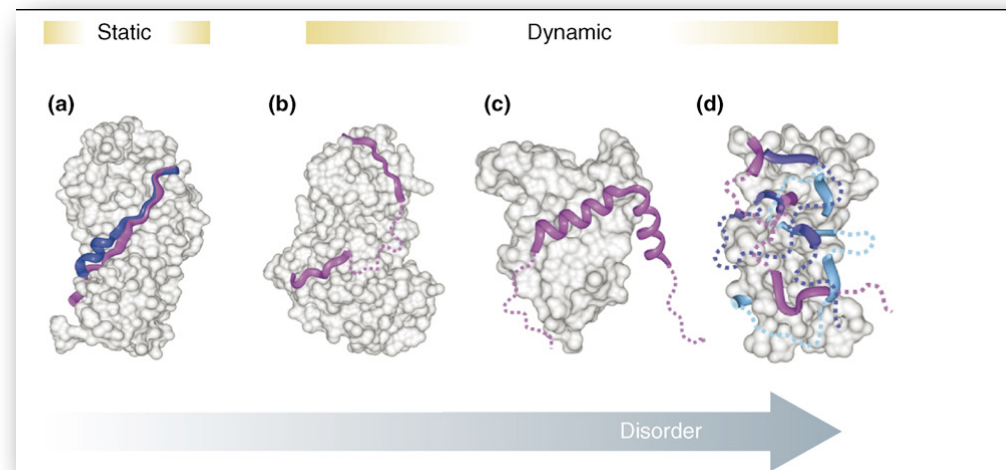


Dunker et al., *Journal of Molecular Graphics and Modelling* **19**, 26–59, 2001
Dobson, C., *Nature* **426**, 18-25, 2003

A key question : explore multiple states of proteins and their plasticity



A key question : explore multiple states of proteins and their plasticity



Sequence

“Non folding”

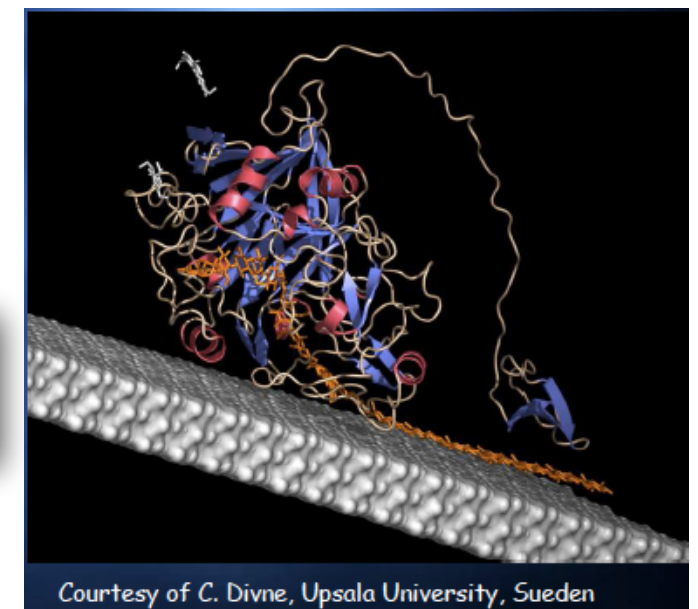
flexible ensemble

flexible linkers
display of sites
entropic bristles, springs and clocks

disorder → order

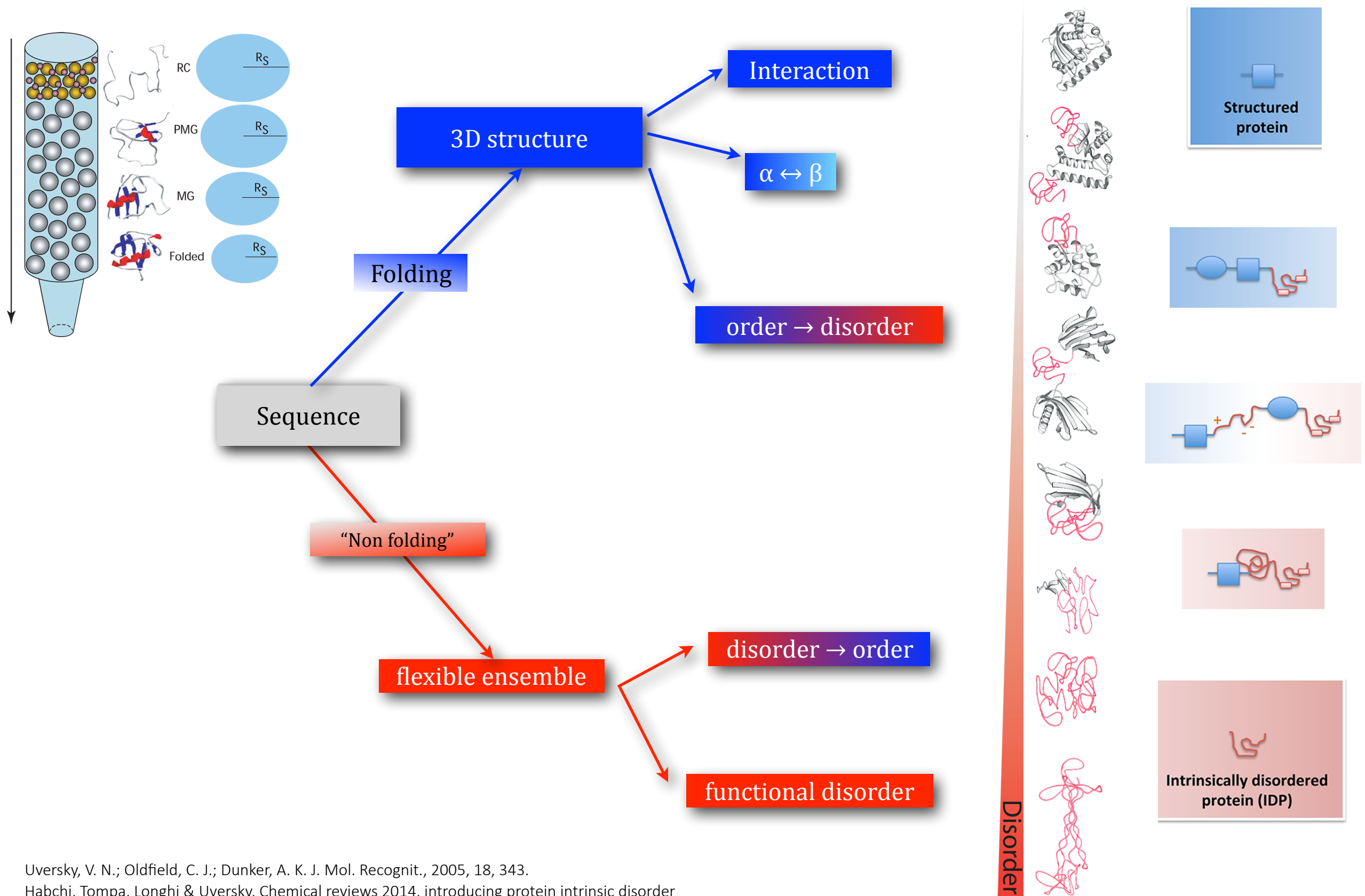
molecular recognition
virus/phages assembly
stepping motors

The “non” folding problem



Cellulases

Multiple states of proteins : a continuum from order to disorder

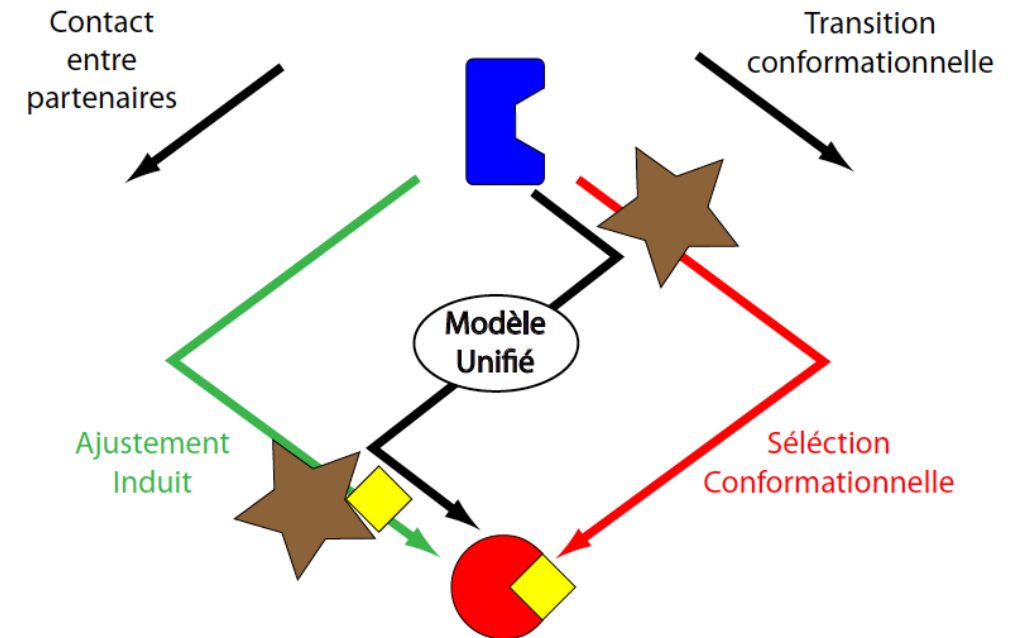
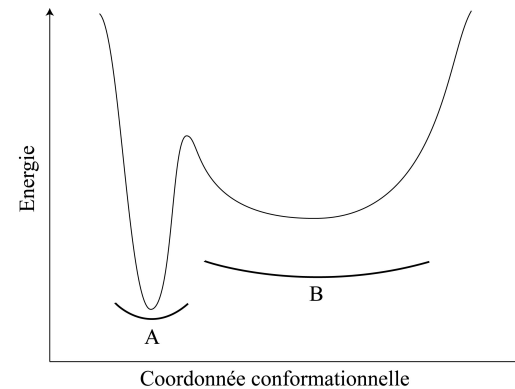
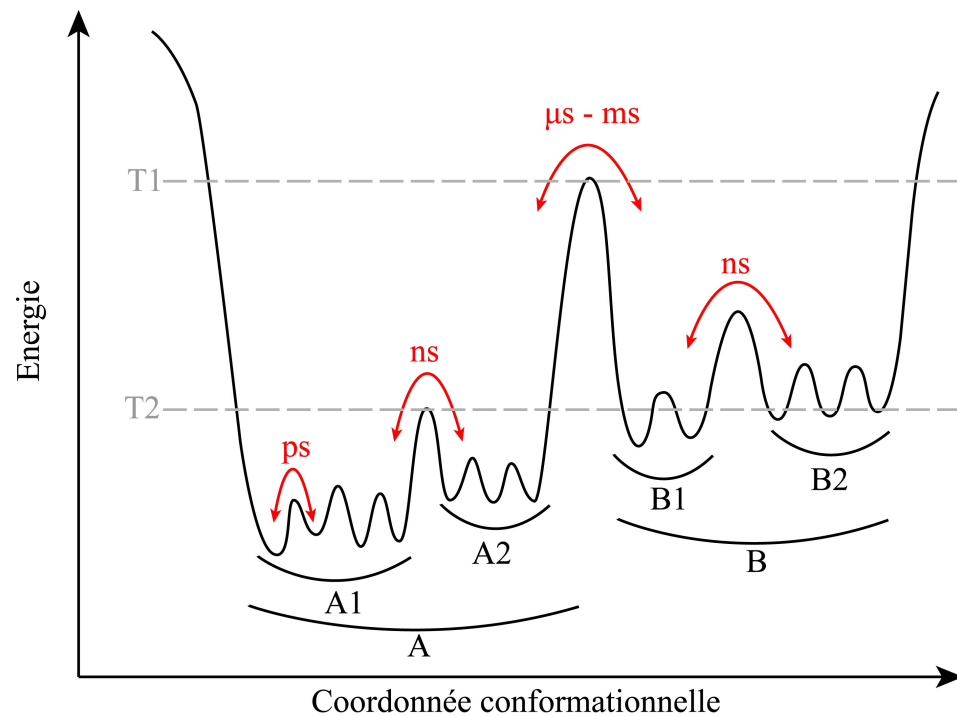


Uversky, V. N.; Oldfield, C. J.; Dunker, A. K. J. Mol. Recognit., 2005, 18, 343.

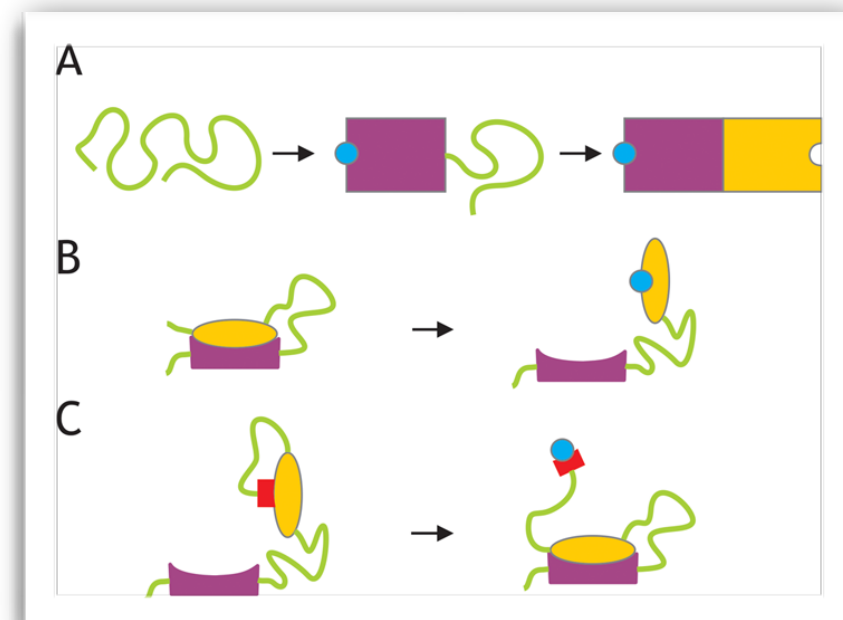
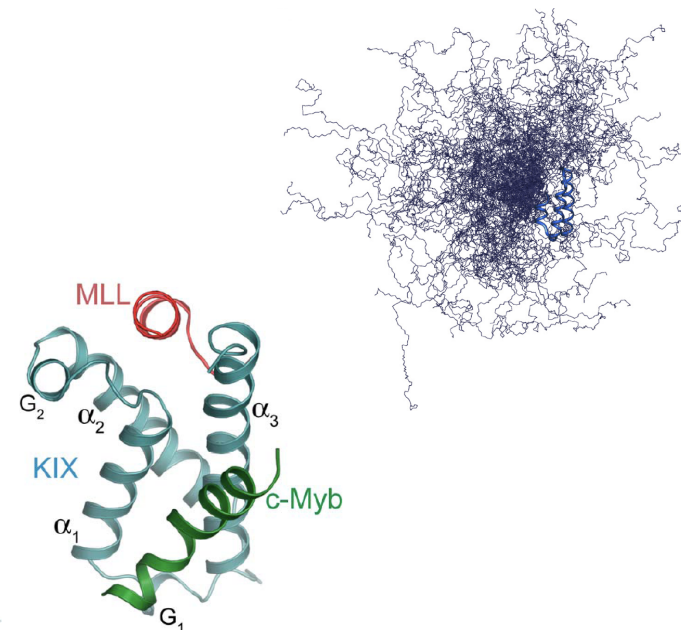
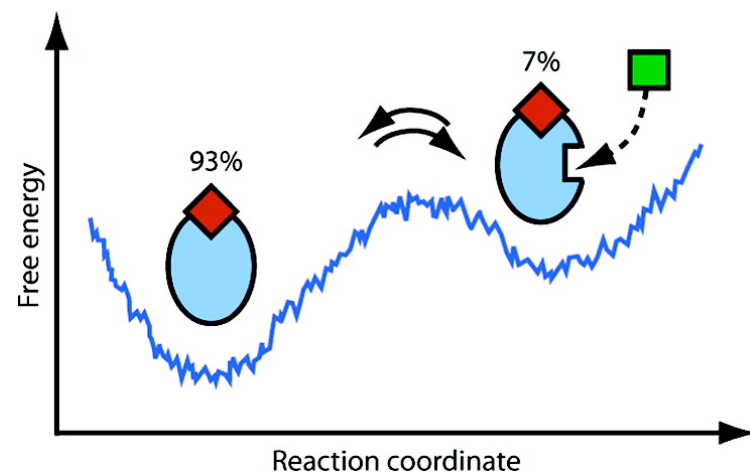
Habchi, Tompa, Longhi & Uversky, Chemical reviews 2014, introducing protein intrinsic disorder

van der Lee et al., Chemical reviews 2014, classification of IDRs and IDPs

A key question : explore multiple states of proteins and their plasticity



- ▶ les protéines sont des ensembles thermodynamiques complexes : multiples états en échange
- ▶ Différents ensembles conformationnels peuvent être fonctionnellement différents.
- ▶ Importance cruciale de caractériser la dynamique et la multiplicité des états d'une protéine le long de son cycle de vie.



Qu'est ce que la RMN des protéines apporte aujourd'hui ?

Les questions spécifiques pour lesquelles la RMN n'est pas la meilleure solution!

► Structure

- "Grosses" protéines / assemblages bien structurés
- Complexes macromoléculaires

Diffraction des RX

Microscopie Electronique

Spectrométrie de masse

► Dynamique

- cinétiques pouvant être suivies par d'autres techniques
- suivi de réactions "rapides" hors équilibre ($< s$)
- "faibles" quantités ($< 10 \mu M$)

Fluorescence, DSC, ...

Stopped-flow

► Interactions

- Paramètres cinétiques et thermodynamiques globaux pour les interactions "fortes"
- Changement "globaux" de structures
- "In cell"

ITC, SPR, ...

DC

DLS, SEC-MALS

SAXS

Fluorescence

Qu'est ce que la RMN des protéines apporte aujourd'hui ?

Les questions spécifiques auxquelles la RMN peut apporter des réponses spécifiques

► Structures

- substances naturelles, petites molécules libres ou liées
- protéines de taille 'raisonnable'
- protéines difficilement cristallisables, membranaires
- partenaire au sein de complexes supramoléculaires
- états multiples en échange
- états peu peuplés

► Dynamique

- flexibilité interne sur une large échelle de temps
 - boucles
 - fragments flexibles ("Intrinsèquement Désordonnés")
 - allostérie
 - mouvements de domaines
- cinétique
 - repliement, interaction

► Interactions

- Changements de conformations/flexibilité au cours de l'interaction
- Interactions transitoires
- Cinétique et thermodynamique des interactions
- Interfaces

► Variation des conditions expérimentales (T, P, concentration en sel, solvant, etc.)

► In cell analysis

Questions

● Qu'est ce que l'on entend par "dynamique" ?

- ⇒ Entropie (thermodynamique), cinétique, trajectoires ...
- ⇒ Protéines : une échelle de temps et des types de mouvements très variable, indispensables à la fonction.

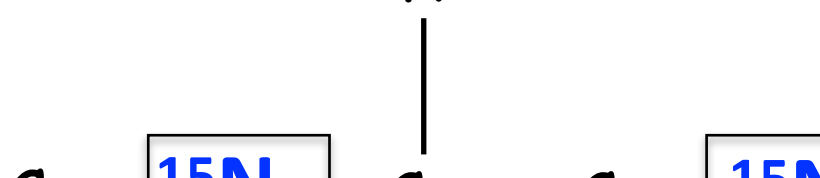
● Pourquoi la RMN est-elle "la" méthode pour l'analyse de la dynamique (des protéines) ?

- ⇒ Méthode physique ⇒ mesure de paramètres qui peuvent dépendre du temps
Connaissance de la dépendance en temps des paramètres ⇒ information sur la dynamique
- ⇒ Les spins "sentent" les mouvements : les paramètres mesurés dépendent très fortement de la dynamique.
 - fluctuations ($\pm$ browniens) par rapport à un champ magnétique (principal ou créé par les autres spins)
 - ▶ relaxation ⇒ constantes de vitesse de relaxation $R_1, R_2, \dots$
 - ▶ densités spectrales des mouvements (décomposition en fréquences)
 - ▶ paramètres des mouvements : temps de corrélations, restrictions spatiales, etc.
 - variation temporelle de l'environnement électronique
 - ▶ lié à une modification conformationnelle ou un échange chimique (i.e. avec le solvant) : populations d'états en échange (différence d'énergie entre états ΔG), constantes de vitesse d'échange, temps de vie des états (barrière d'énergie, k_{ex}), différences de déplacements chimiques (variations structurales)
 - ▶ $\pm$ défocalisation des spins -> essentiellement échos de spin:
 - CPMG (R_2 , dispersion de relaxation) : $R_2 = f(k_{ex}, p_{min}, \Delta\omega, \nu_{CPMG}, N_{echos})$
 - gradients de champ B_0 (diffusion) : $I = f(D, Gdt, t_m)$

● Relations entre paramètres mesurés et paramètres dynamiques?

- ⇒ Hamiltoniens dépendants du temps
- ⇒ équation de Bloch-McConnell, etc.

- When is NMR “the” appropriate technique?
- What does a protein “look like” by NMR?



The diagram illustrates a segment of a protein backbone. A central carbon atom, labeled C_α , is bonded to an R group above it and a hydrogen atom ($^1H_\alpha$) below it. To the left and right of C_α are amide groups. Each amide group consists of a carbonyl carbon (C) double-bonded to an oxygen (O) and single-bonded to a nitrogen (^{15}N). The nitrogen is further single-bonded to a hydrogen atom (1H). The ^{15}N and 1H labels are highlighted in blue and enclosed in boxes. Ellipses ($\dots$) at the ends of the backbone indicate the continuation of the polymer chain. In the top right corner, there is a small molecular model of a protein structure.



- 👉 A local probe of the atoms electronic environment : resonance frequencies.
- 👉 A local probe of the dynamic : shape/width of the resonances

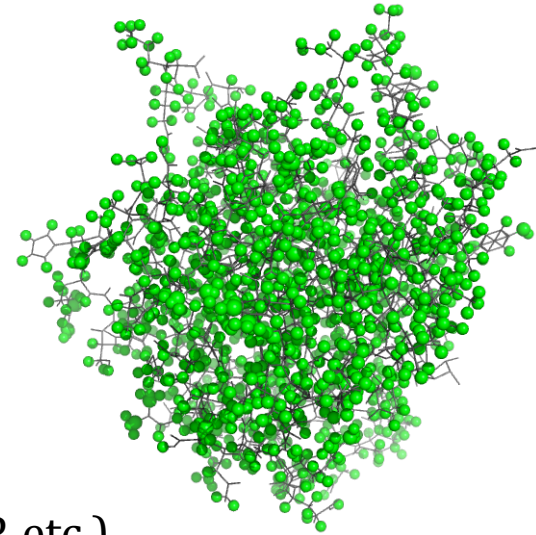
NMR for the study of proteins dynamics

Questions

- When is NMR “the” appropriate technique?
- What does a protein “look like” by NMR?

Specificities

- Local (one peak per observable atom - ^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P , etc.)
- Simultaneous measurement of all the local probes (large number of molecules at equilibrium, measurement of averaged values in space and time)
- Quantitative (volume of peaks proportional to number of observed atoms)
- Highly sensitive to local and global environment, and to the variation of this environment
 - Internal flexibility (100ps-ns)
 - Internal dynamics - exchange between conformations (μs -ms)
 - Buffer, pH, temperature, pressure ...
- ▶ Structural parameters of multiple states (environment, distances, angles)
- ▶ Thermodynamics and kinetic parameters (entropy, ΔG , K_d , pK_a , k_{ex} ...)



Questions

- When is NMR “the” appropriate technique?
- What does a protein “look like” by NMR?

Specificities ...

- ▶ **Disordered regions, Intrinsically Disordered Proteins, conformational changes, excited states, allosteric processes, interactions (transient), interfaces (loose/sliding/high throughput), post-translational modifications**
- ▶ **... and possibly/marginally protein structure ...**

Limitations

- NMR is not a sensitive technique ... NMR is sensitive to the size of the molecule ...
 - ▶ **depends on the question (300μM->10μM)**
 - ▶ **depends on the dynamics of the system**
 - ▶ **depends on the physical state (liquid/solid) of the sample.**
- **Requires labelling approaches, importance of the sample preparation.**

Résonance Magnétique Nucléaire

Principes physiques

Spins des noyaux
(moment angulaire
&
moment magnétique)
Champ magnétique
Onde électromagnétique
Spectroscopie
Résonance

Précession

Interactions
spin-champ magnétique
spin-spin
spin-électrons

Relaxation

Paramètres

Fréquence de résonance
déplacement chimique

Communication entre les spins
couplages scalaires
couplages dipolaires

Temps caractéristiques
Constantes de temps de relaxation
T1, T2
nOes
Moyennage des interactions
Fluctuations des interactions

Informations Structures / dynamique

Environnement électronique
Structuration
Structures secondaires
Interactions/Interfaces

Liaisons chimiques
Information angulaire
Distances inter atomiques

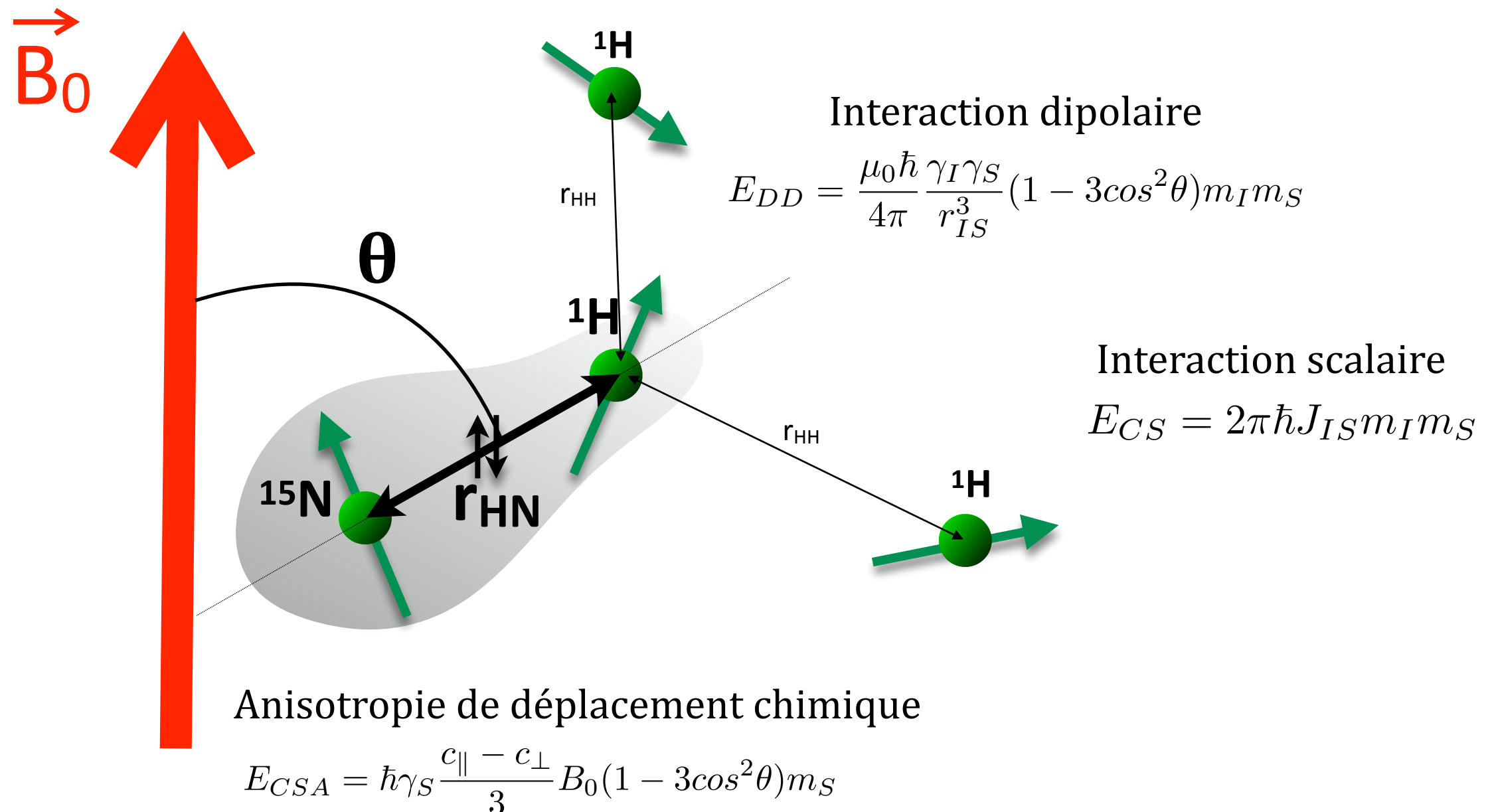
Flexibilité interne
Réorientation moléculaire
Echanges conformationnels
Cinétique et Thermodynamique

INTERACTIONS
SPINS/ELECTRONS/CHAMP

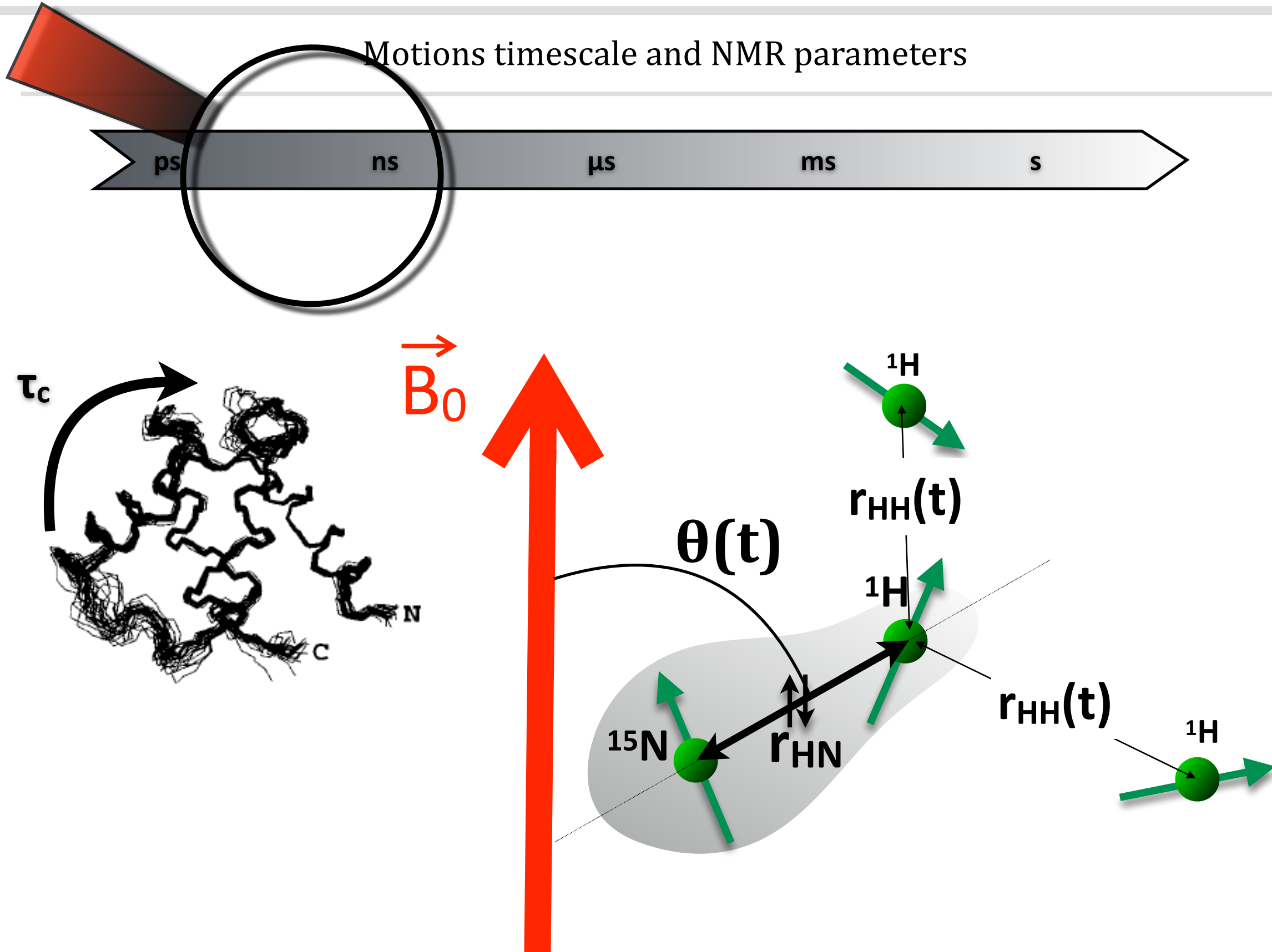
DEPLACEMENT CHIMIQUE
COUPLAGE SCALAIRE
COUPLAGE DIPOLAIRE
ANISOTROPIE de DEPLACEMENT
CHIMIQUE

INFORMATIONS
sur la
DYNAMIQUE

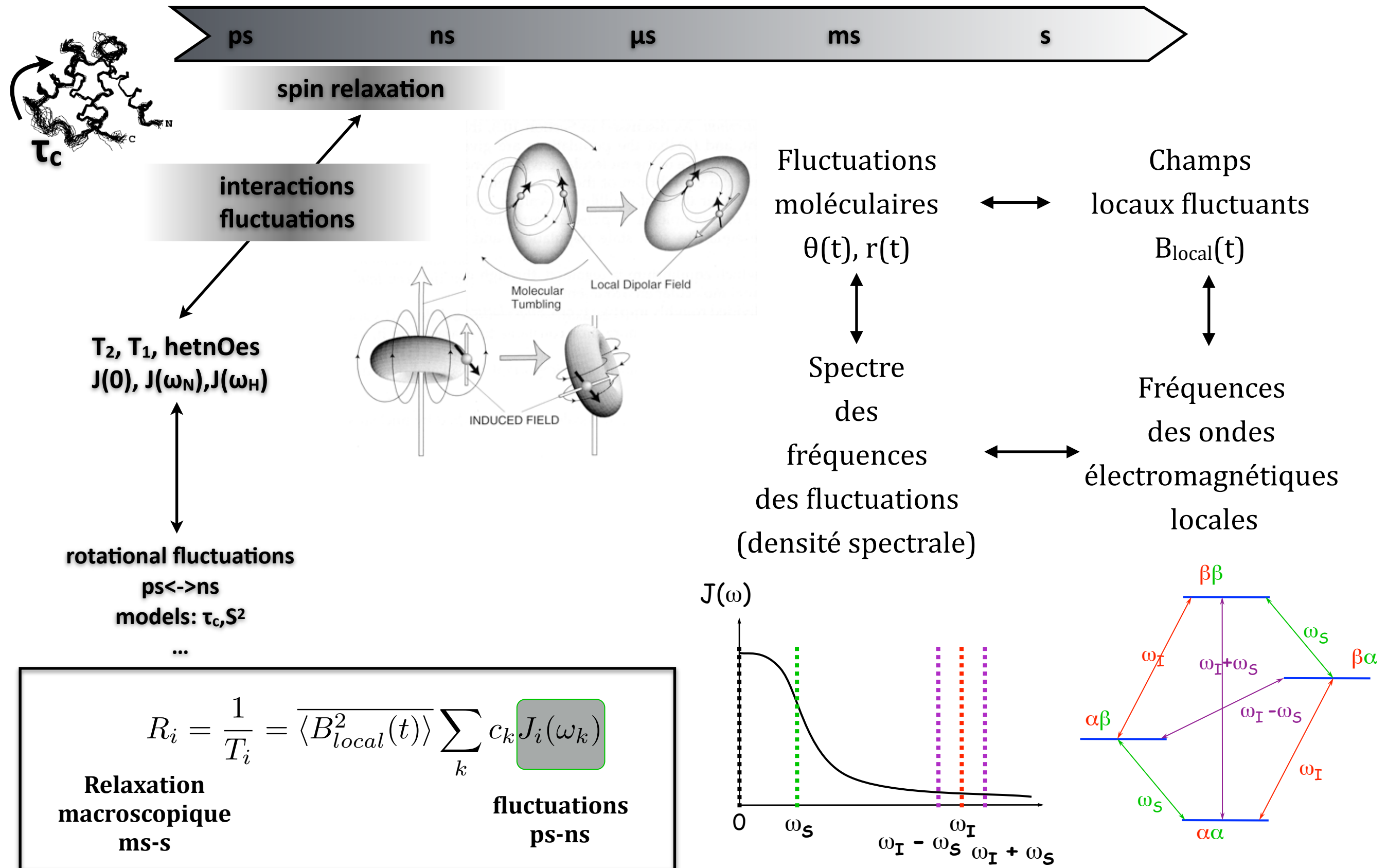
► $f(\text{environnement}(t), r(t), \theta(t))$



Motions timescale and NMR parameters



Larmor timescale:
spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)



Larmor timescale:
spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)



spin relaxation

interactions
fluctuations

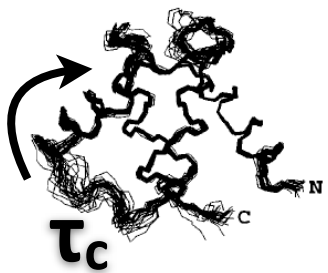
relaxation parameters [T_2 , T_1 , hetnOes]
depend on the spectral distribution of the molecular fluctuations
 $\rightarrow J(0), J(\omega_N), J(\omega_H)$

$$R_i = \frac{1}{T_i} = \overline{\langle B_{local}^2(t) \rangle} \sum_k c_k J_i(\omega_k)$$

rotational fluctuations

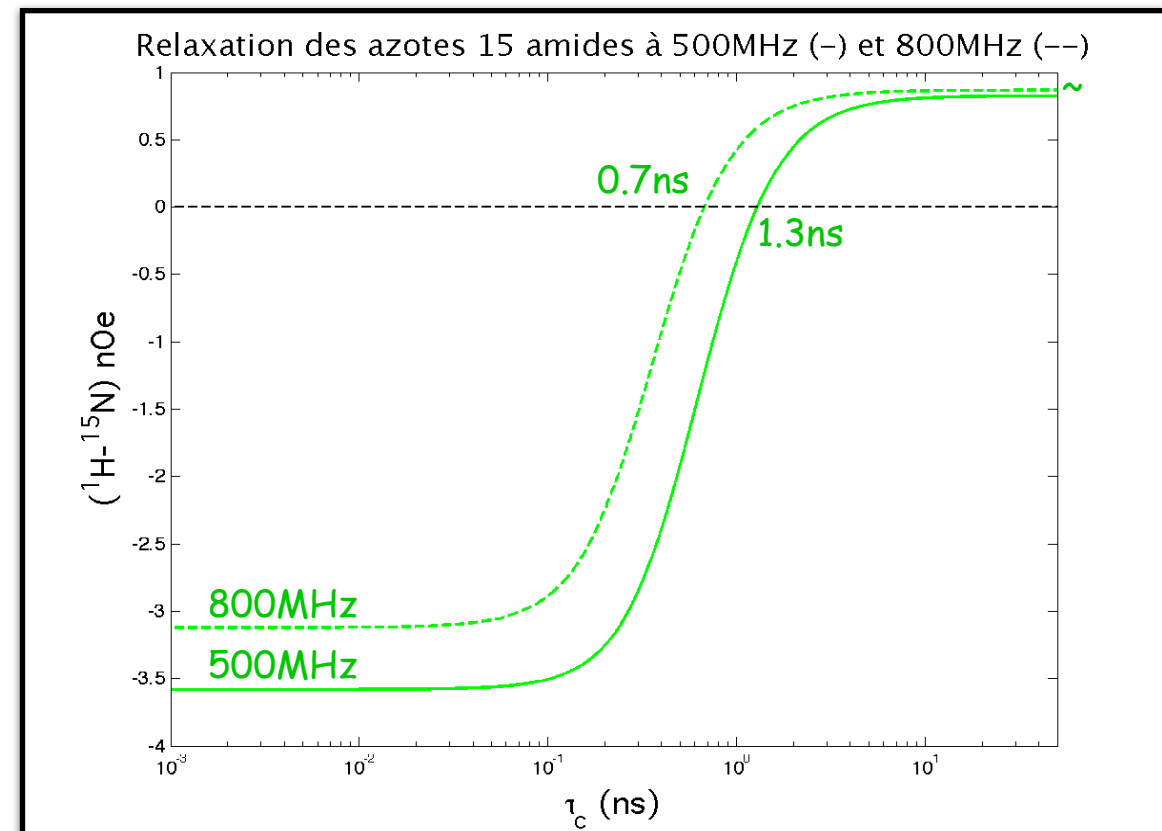
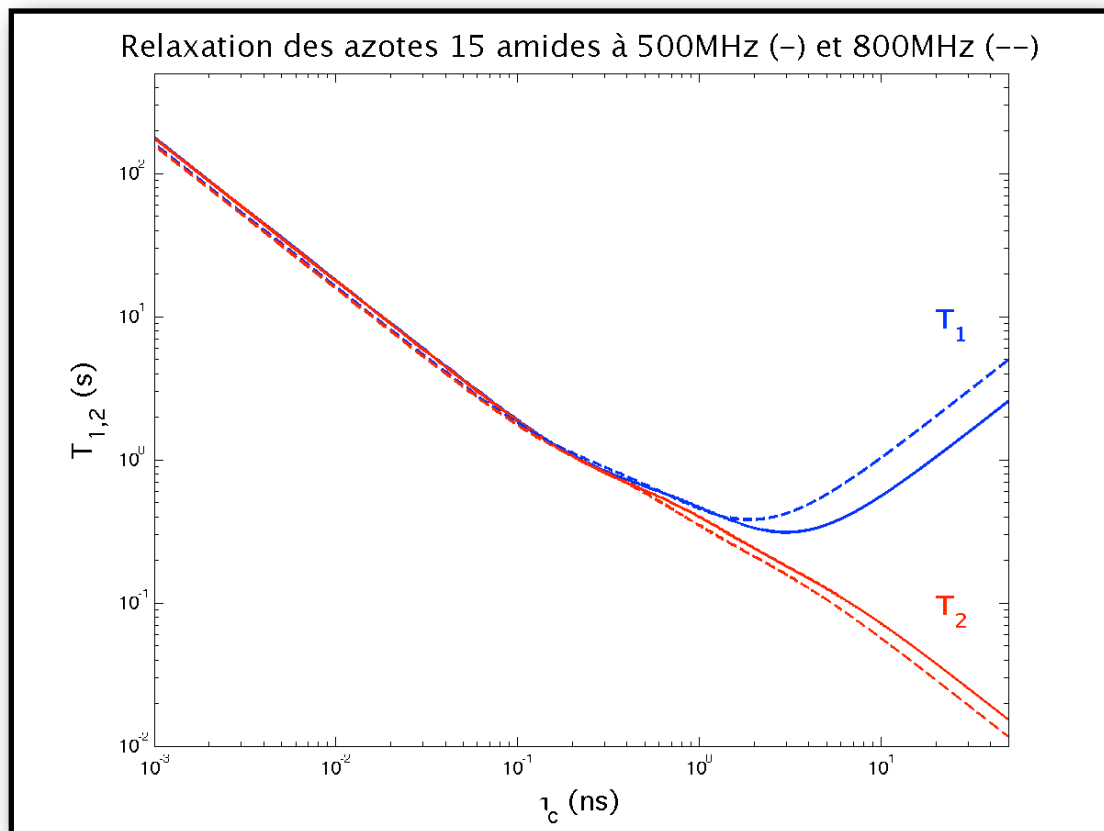
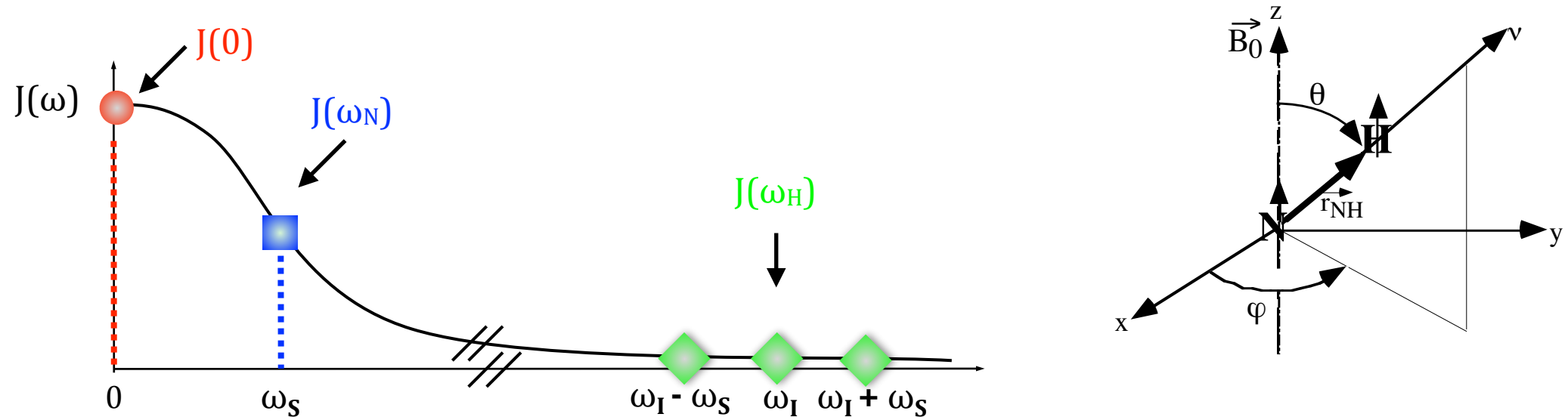
ps $\leftrightarrow$ ns
models: τ_c, S^2

...



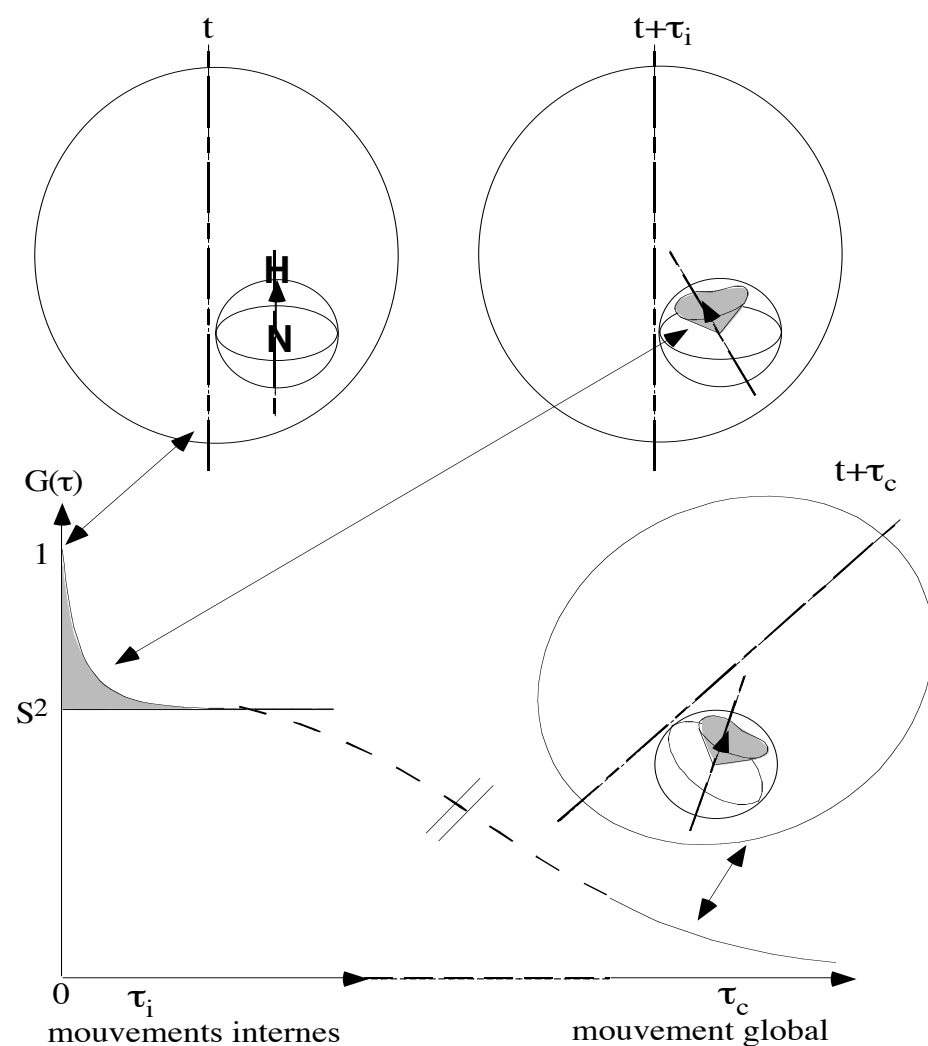
Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)

- ⇒ In practice : measurement of 3 relaxation parameters: R_1 , R_2 , and hetNOE
- ⇒ information on local flexibility of ^1H - ^{15}N bonds in the 100ps-ns range.



Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)

- In practice
- ⇒ measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^1H - ^{15}N bonds in the 100ps-ns range.
 - ⇒ Models to describe the flexibility with motion parameters: global/local correlation time; order parameters (amplitudes)



Lipari & Szabo "model free" approach

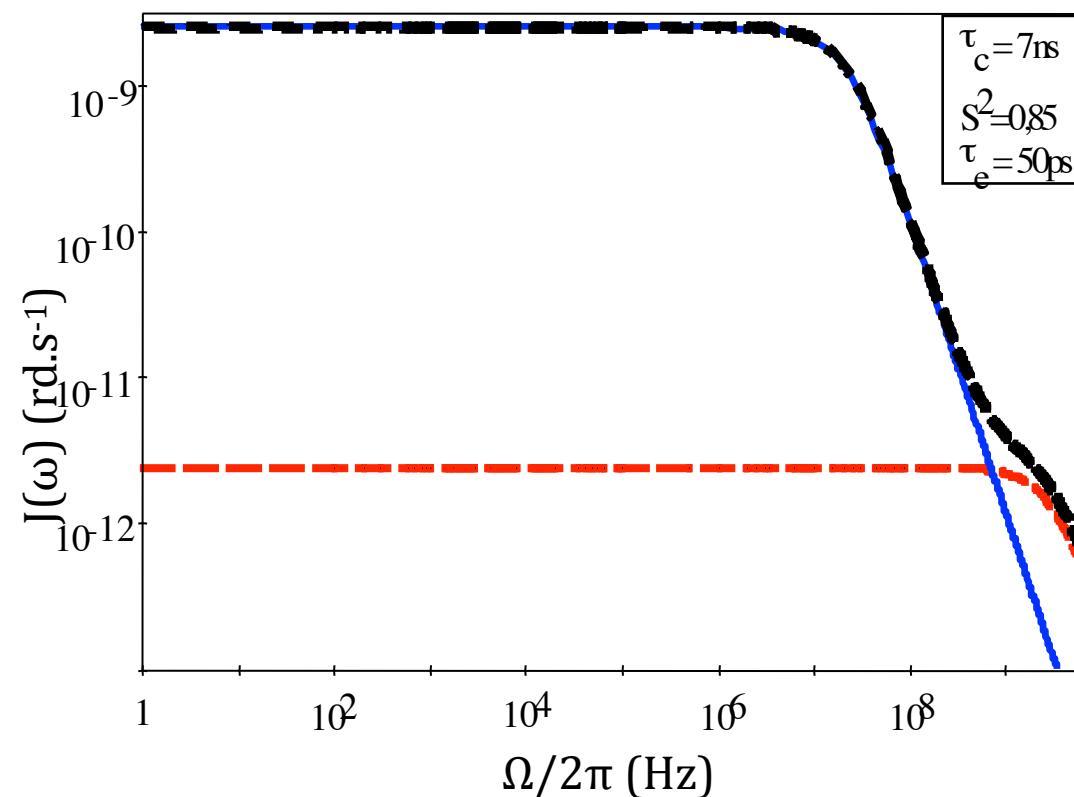
Lipari, G.; Szabo, A. J. Am. Chem. Soc. 1982, 104, 4546–4559

$$G(\tau) = G_{\text{glob}}(\tau)G_{\text{int}}(\tau)$$

$$G_{\text{glob}}(\tau) = \frac{1}{5} e^{-t/\tau_c}$$

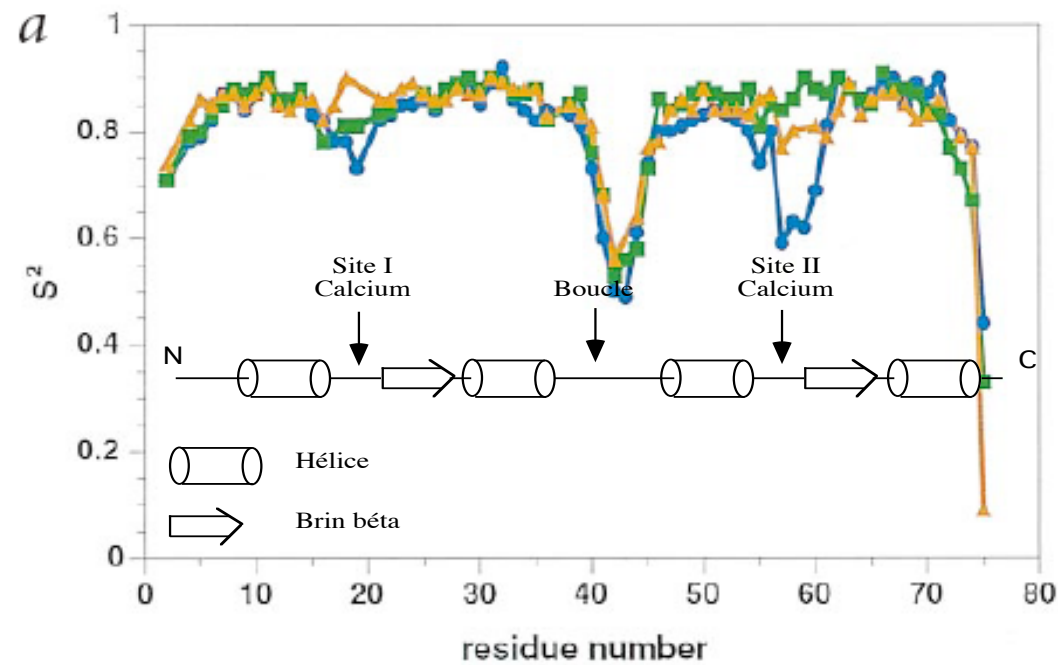
$$G_{\text{local}}(\tau) = S^2 + (1 - S^2) e^{-t/\tau_i}$$

$$J(\omega) = \frac{2}{5} \left[\frac{S^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{(1 - S^2) \tau_e}{1 + \omega^2 \tau_e^2} \right] \quad \tau_e^{-1} = \tau_c^{-1} + \tau_i^{-1}$$



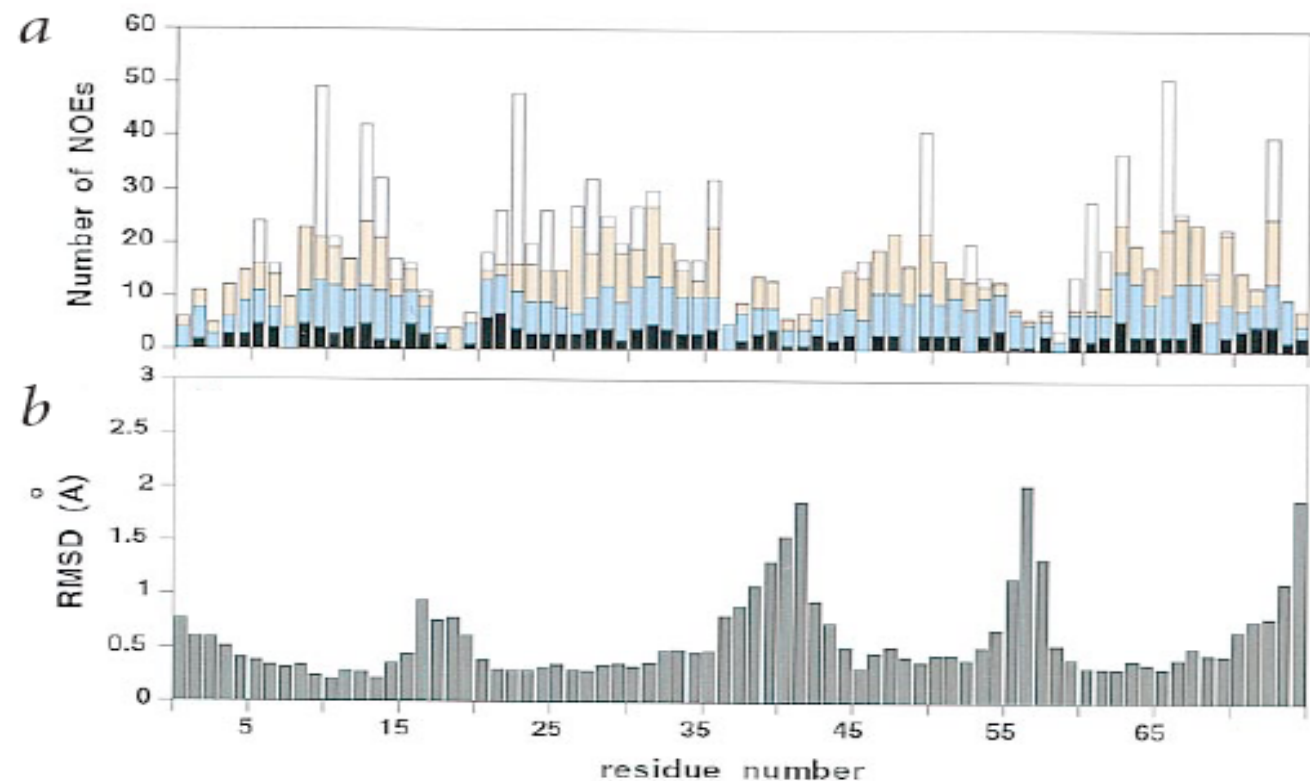
Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)

- $\Rightarrow$ measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^1H - ^{15}N bonds in the 100ps-ns range.
- $\Rightarrow$ local flexibility in structured proteins



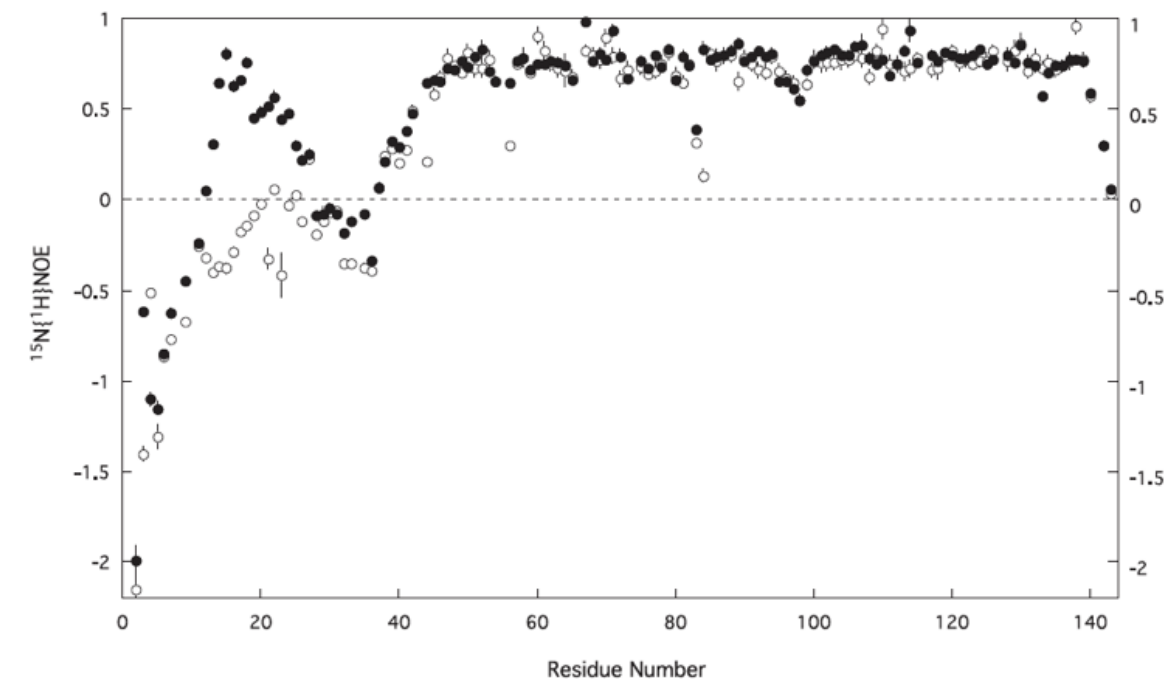
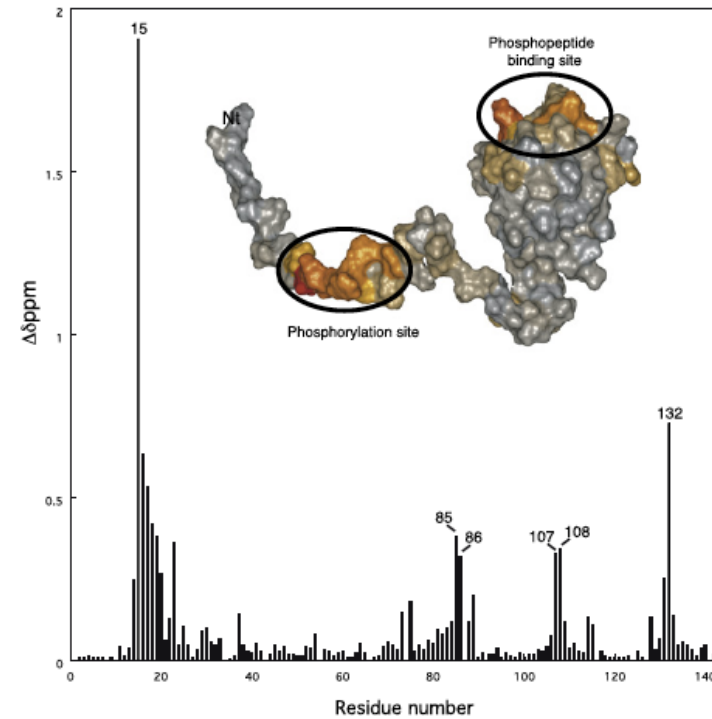
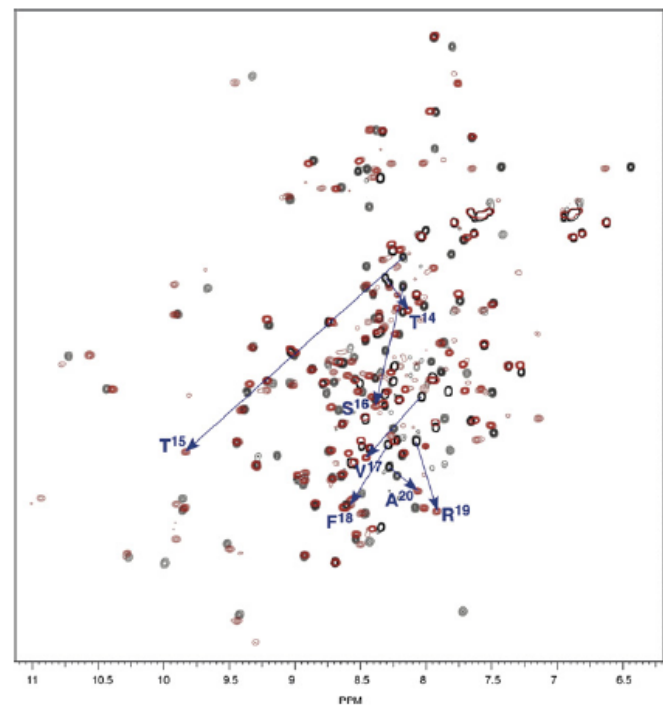
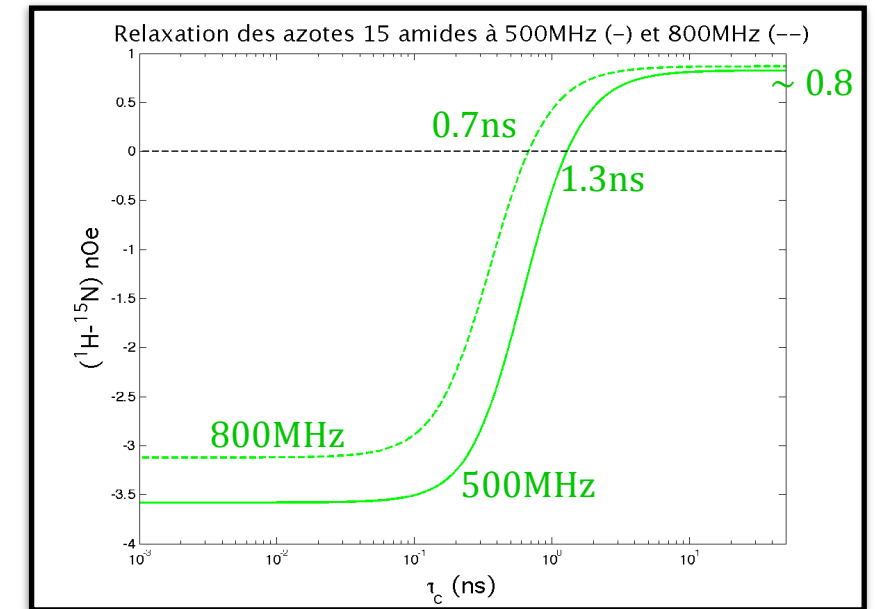
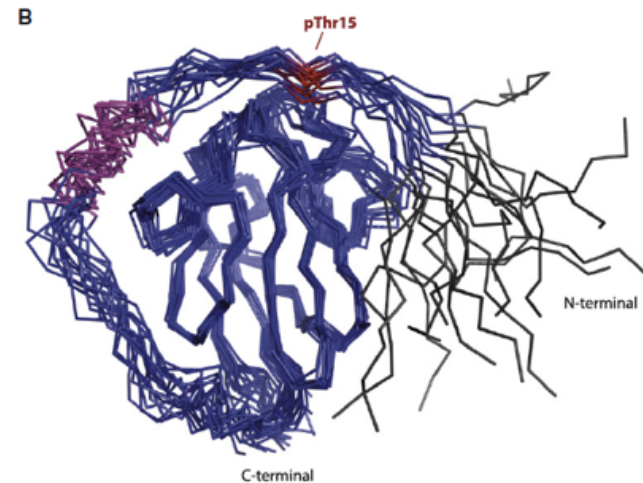
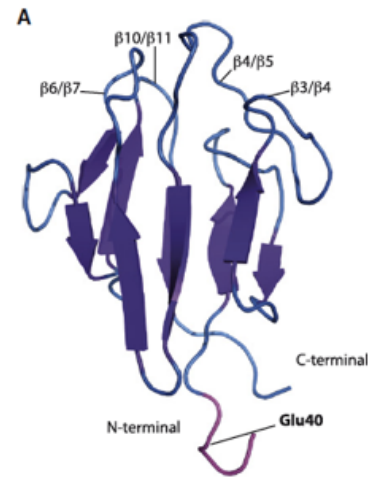
Calbindin D_{9k}
Apo
(Ca₂₊)₁^I-N56A
(Cd₂₊)₁^{II}

Mäler et al., Nat. Struct. Biol. 7, 245 (2000)



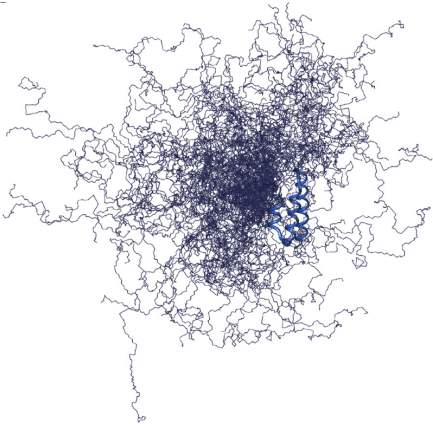
Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)

- ⇒ heteronuclear ($^1\text{H}^{\text{N}} \rightarrow ^{15}\text{N}$) nOes
- ⇒ easy to measure
- ⇒ qualitative information about local flexibility in the 100ps range

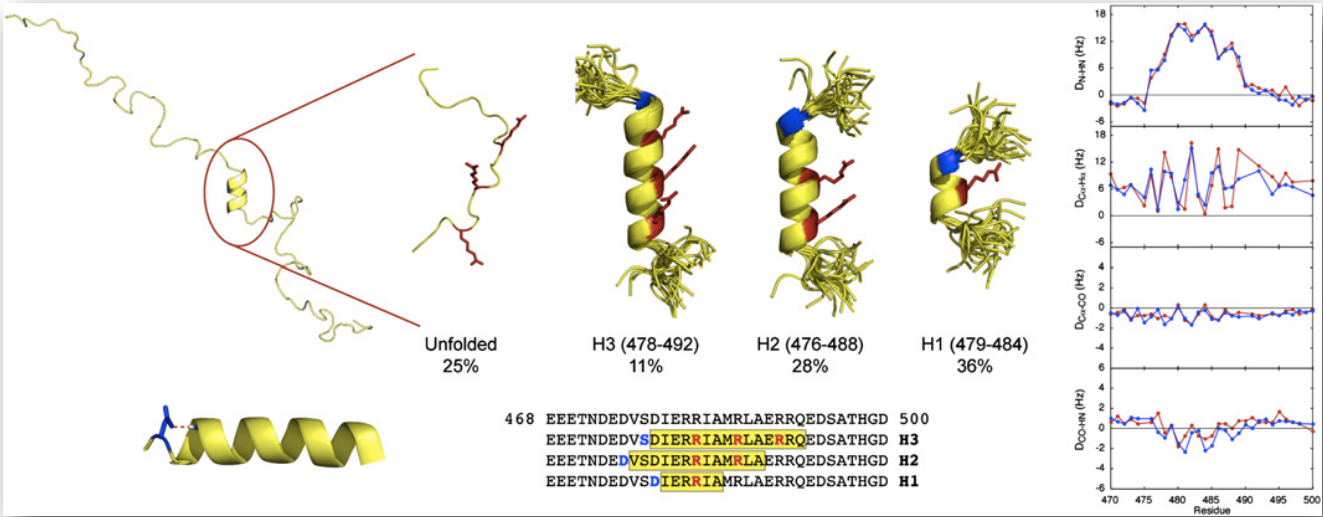
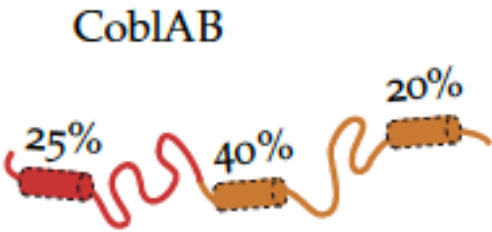
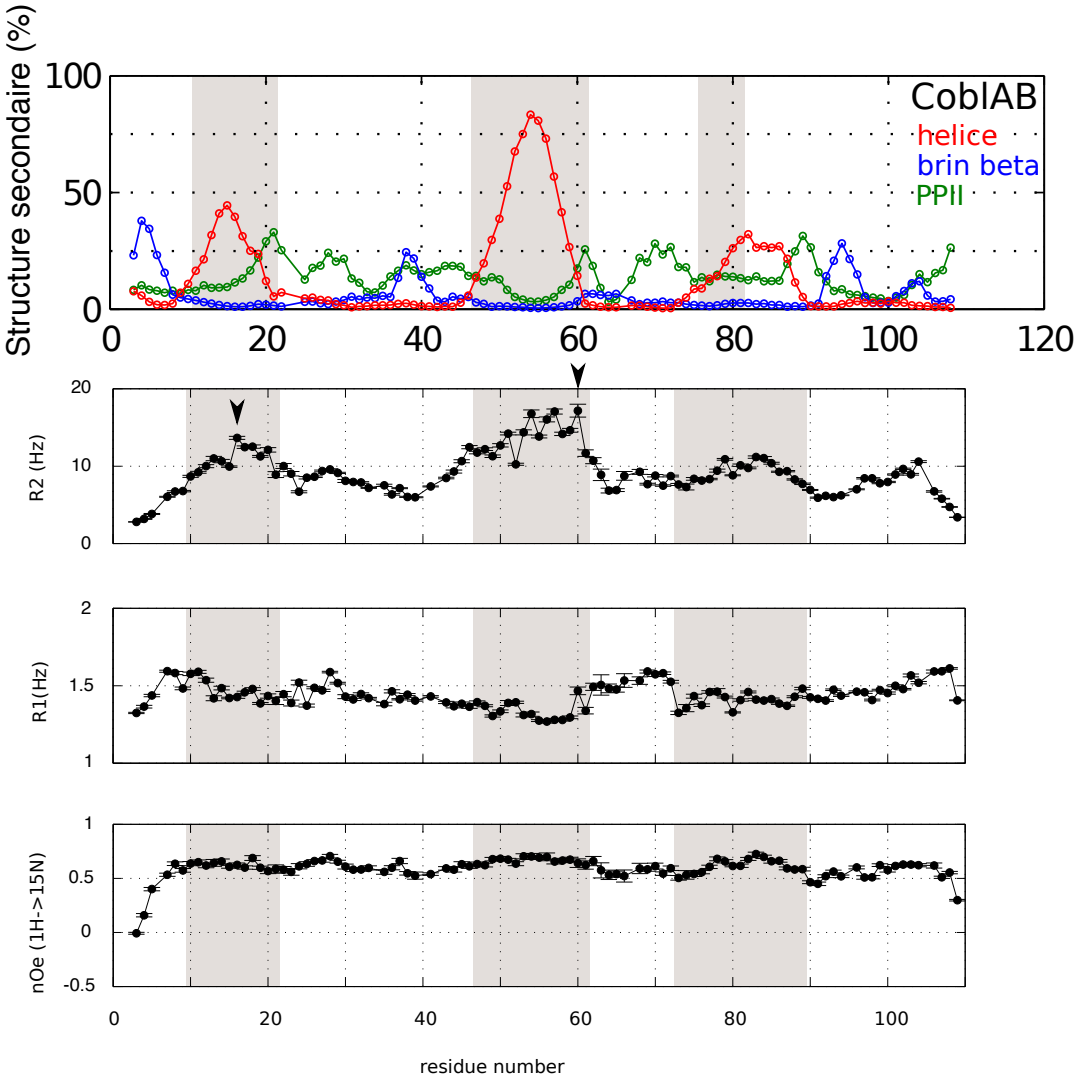


Barthe et al, "Dynamic and Structural Characterization of a Bacterial FHA Protein Reveals a New Autoinhibition Mechanism". Structure 17, 568-578 (2009).

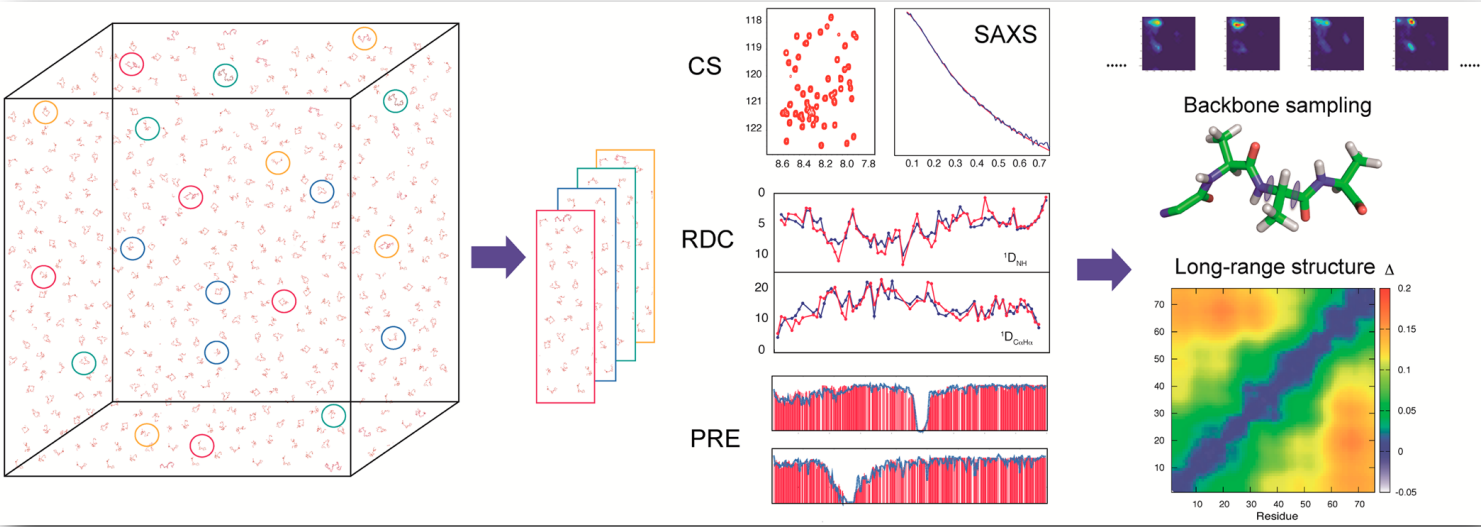
Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)



- Measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^1H - ^{15}N bonds in the 100ps-ns range.
- Local structuration in disordered proteins: secondary structures propensities (+CS, RDCs, PRE, SAXS $\rightarrow$ conformational ensembles)

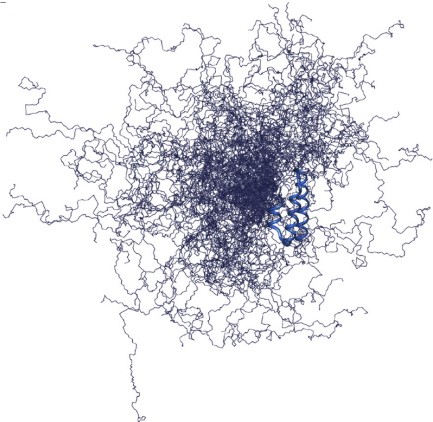


Jensen, M.R et al.,(2008) J. Am. Chem. Soc. 130 ; (2009) structure 17.

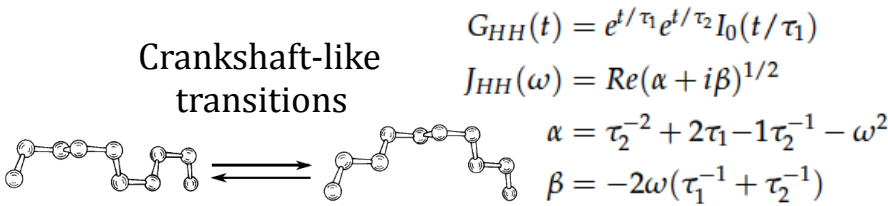


Jensen, Zweckstetter, Huang &Blackledge, Chemical reviews 2014, special issues on IDPs
Exploring Free-Energy Landscapes of Intrinsically Disordered Proteins at Atomic Resolution Using NMR Spectroscopy

Larmor timescale: spin relaxation $\Leftrightarrow$ local flexibility (ps-ns)

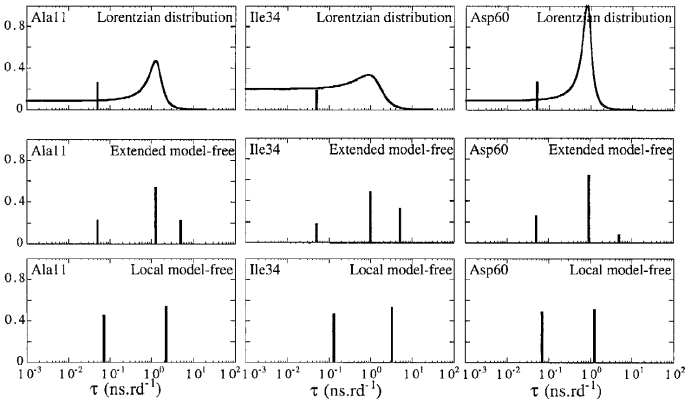


- ⇒ Measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^1H - ^{15}N bounds in the 100ps-ns range
- ⇒ Multiple fields data.
- ⇒ Models for the analysis of relaxation data in disordered proteins

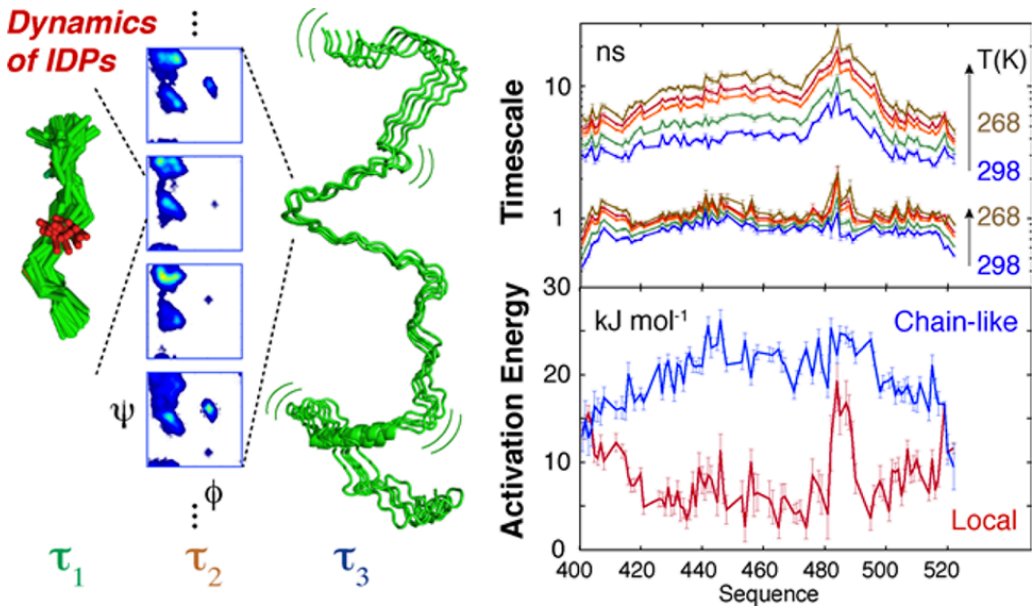


Hall, C. and Helfand, E. Journal of Chemical Physics (1982)
DeJean de la Batie, R., Laupretre, F., and Monnerie, L. Macromolecules (1988).

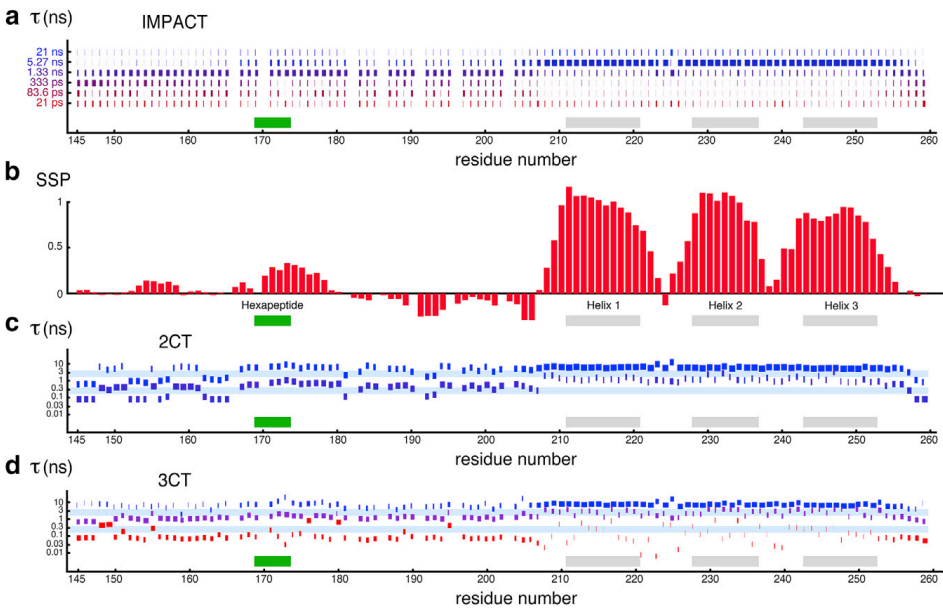
Ochsenbein et al., Protein Science (2002), 11:957–964.
Dynamical characterization of residual and non-native structures in a partially folded protein by ^{15}N NMR relaxation using a model based on a distribution of correlation times



Abyzov et al., J. Am. Chem. Soc. 2016, 138, 6240–6251
Identification of Dynamic Modes in an Intrinsically Disordered Protein Using Temperature-Dependent NMR Relaxation



Khan et al., Biophysical Journal 109(5) 988–999
Distribution of Pico- and Nanosecond Motions in Disordered Proteins from Nuclear Spin Relaxation
IMPACT: Interpretation of motions by a projection onto an array of correlation times



Motions timescale and NMR parameters



spin relaxation

interactions
fluctuations

$$R_i = \frac{1}{T_i} = \overline{\langle B_{local}^2(t) \rangle} \sum_k c_k J_i(\omega_k)$$

T1: -> equilibrium

T2: signal lifetime

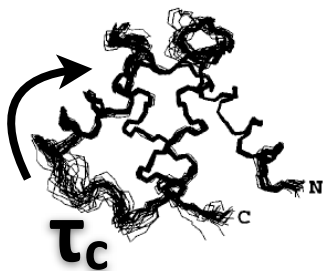
microscopic processes -> macroscopic relaxation parameters [T₂, T₁, hetnOes]
depend on the spectral distribution of the molecular fluctuations
-> J(0), J(ω_N), J(ω_H)

rotational fluctuations

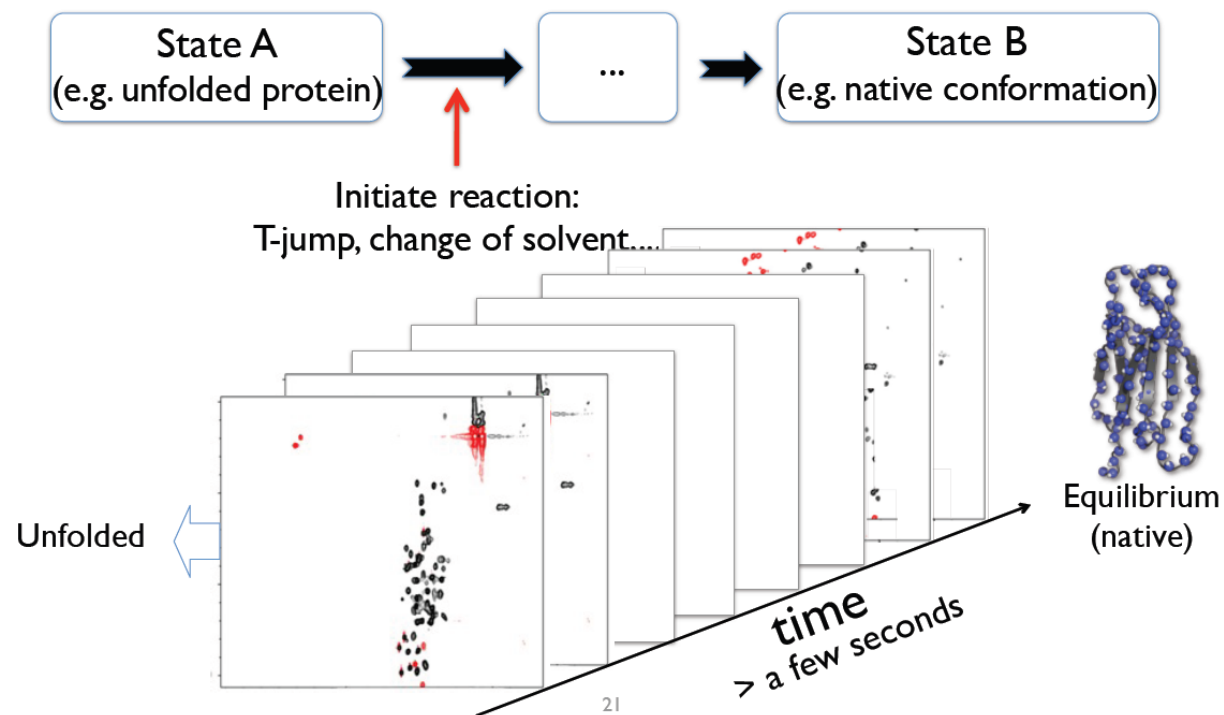
ps<->ns

models: τ_c, S²

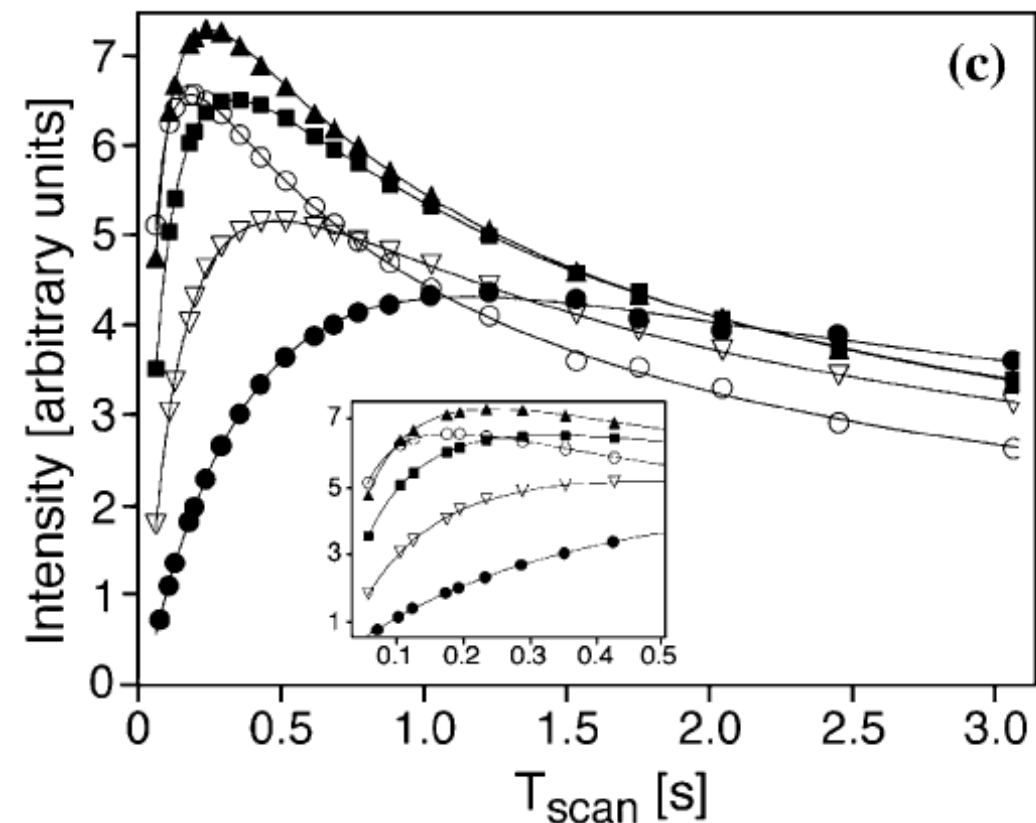
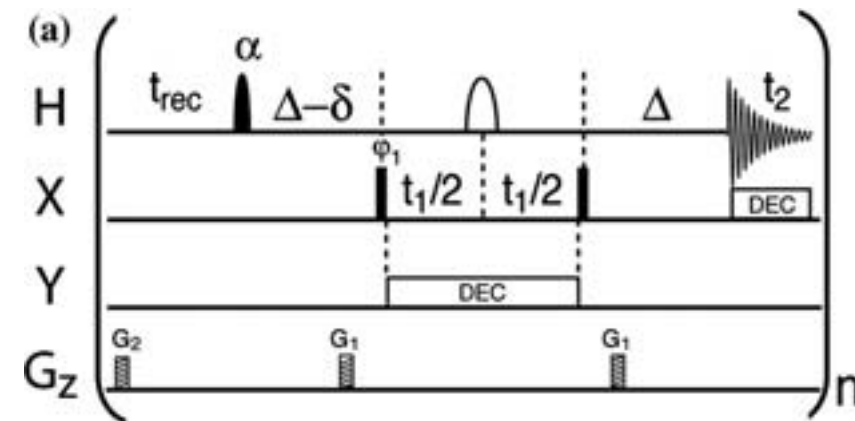
...



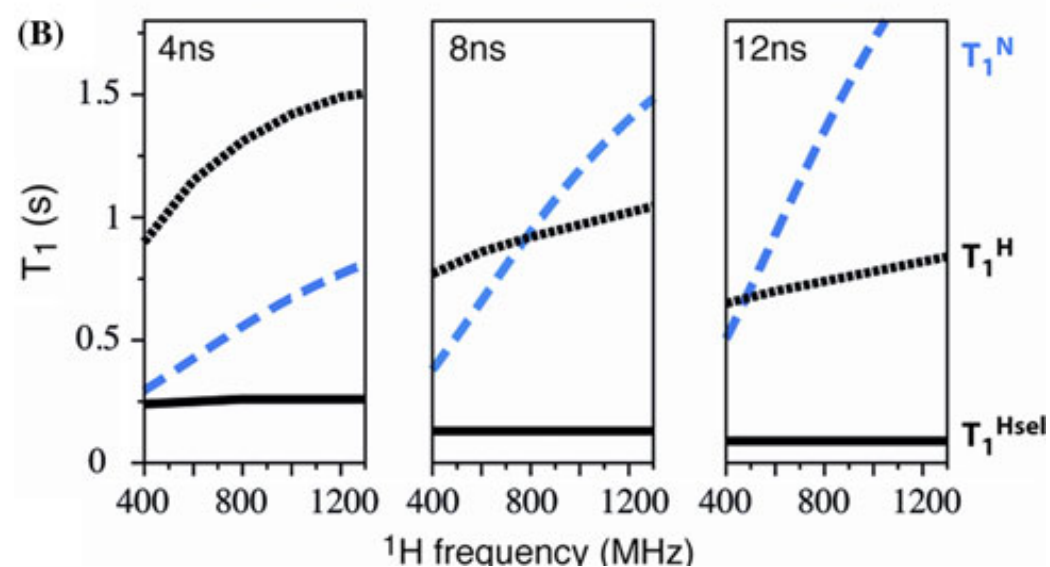
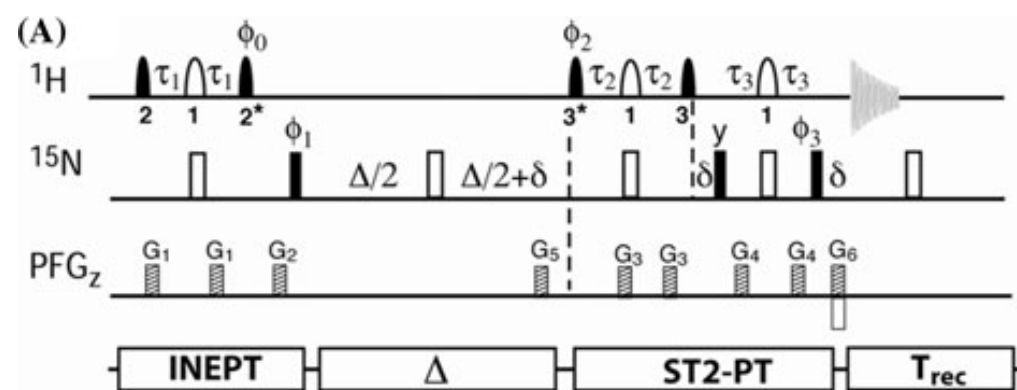
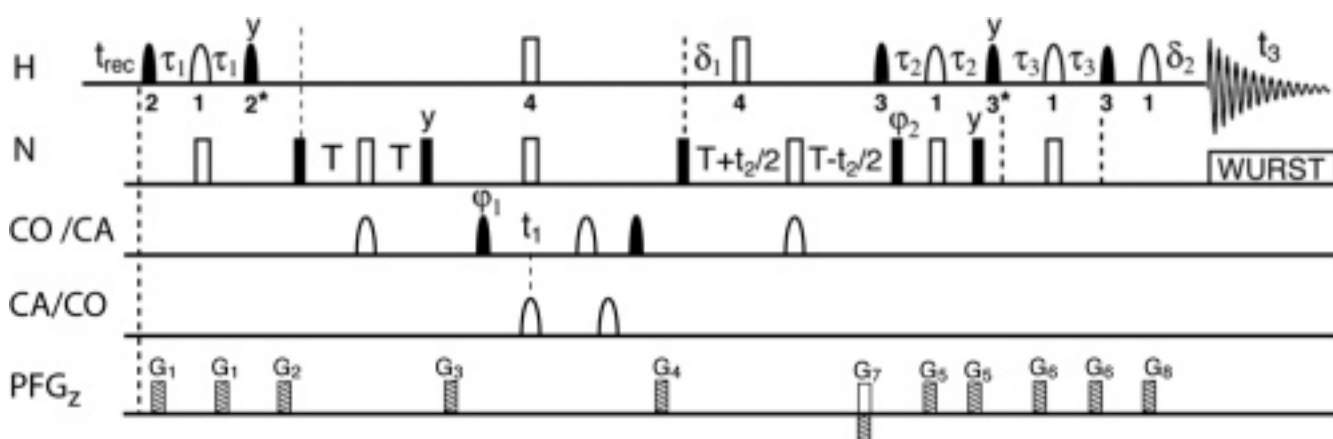
T_1 dépend du champ magnétique B_0 , de la nature des spins, **de l'état des spins voisins**, de la taille de la molécule, de la dynamique locale du système, de la température, etc.



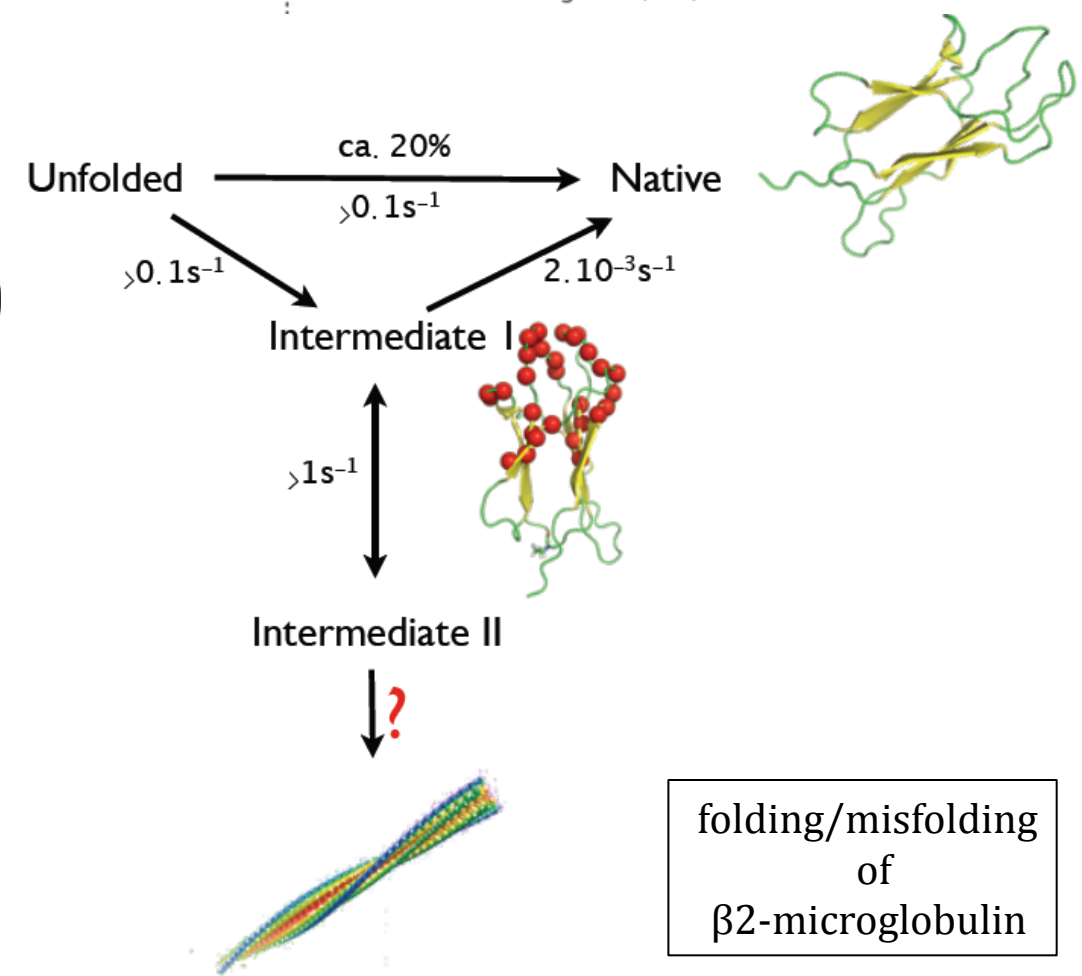
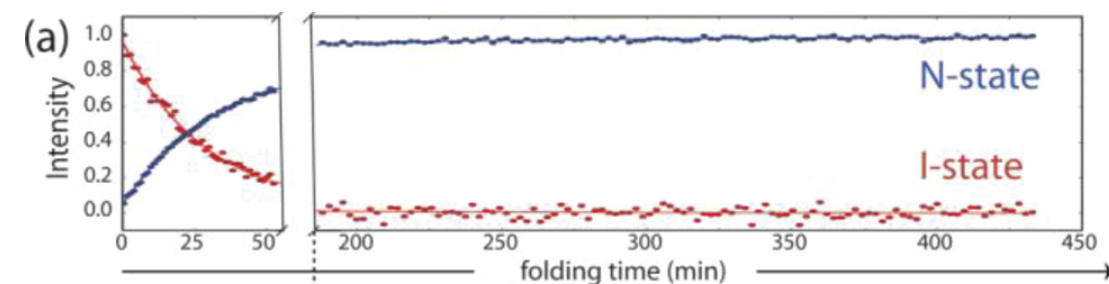
“Jouer” avec T_1 : les expériences FAST/BEST



Schanda et al. J. Biomol. NMR (2005) 33: 199–211



“Jouer” avec T1 : les expériences FAST/BEST

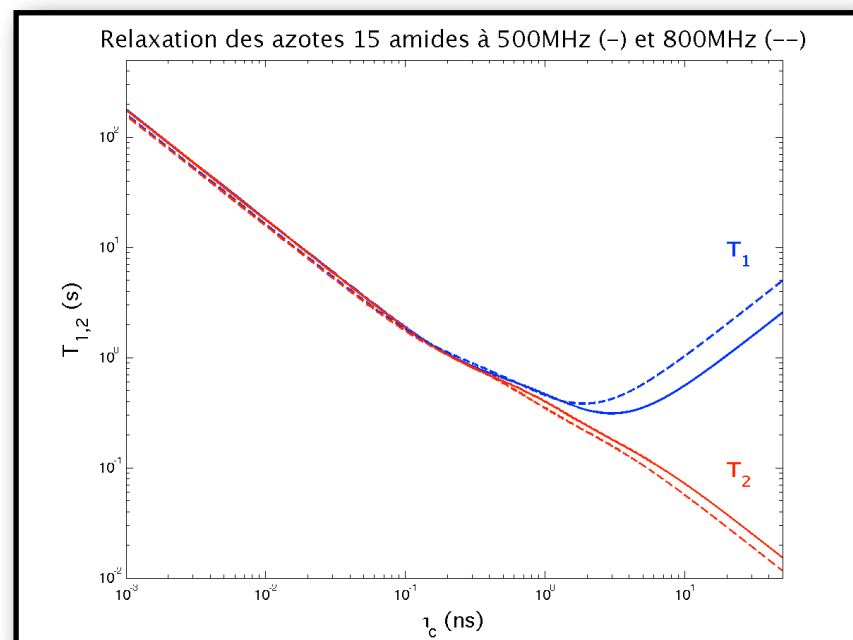


Le temps de vie du signal

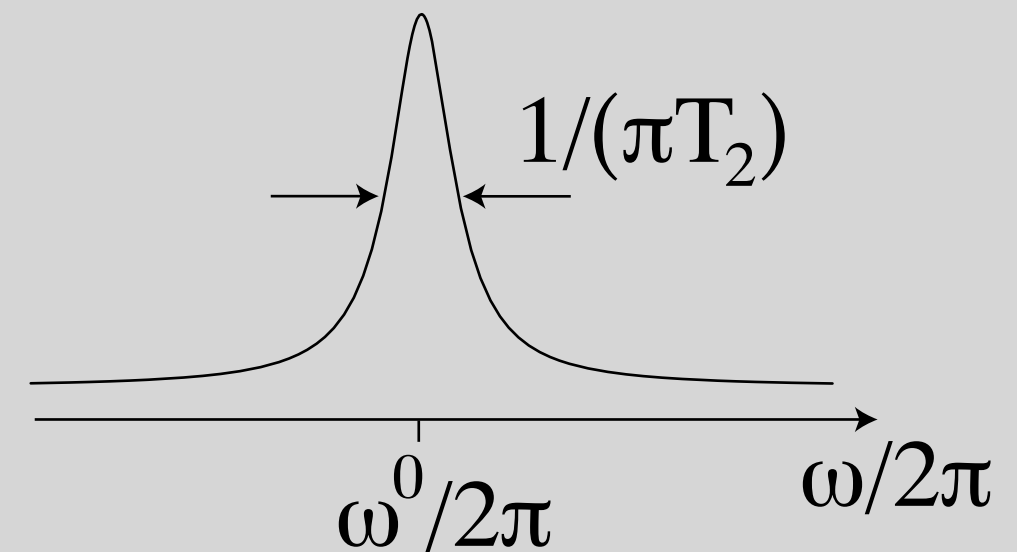
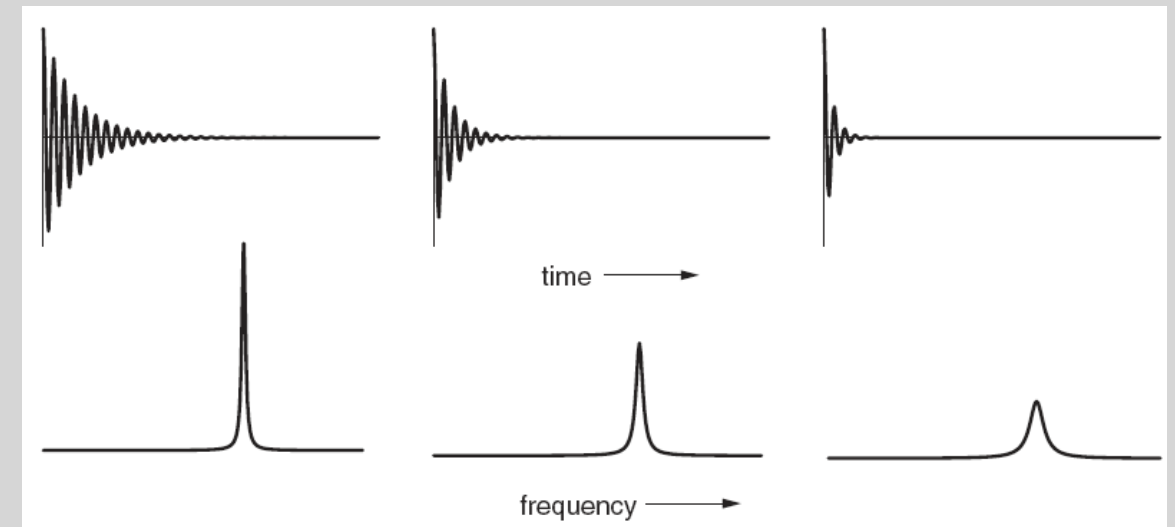
Le temps de relaxation transversale T_2 définit le temps de vie du signal RMN.

Il détermine la largeur des résonances

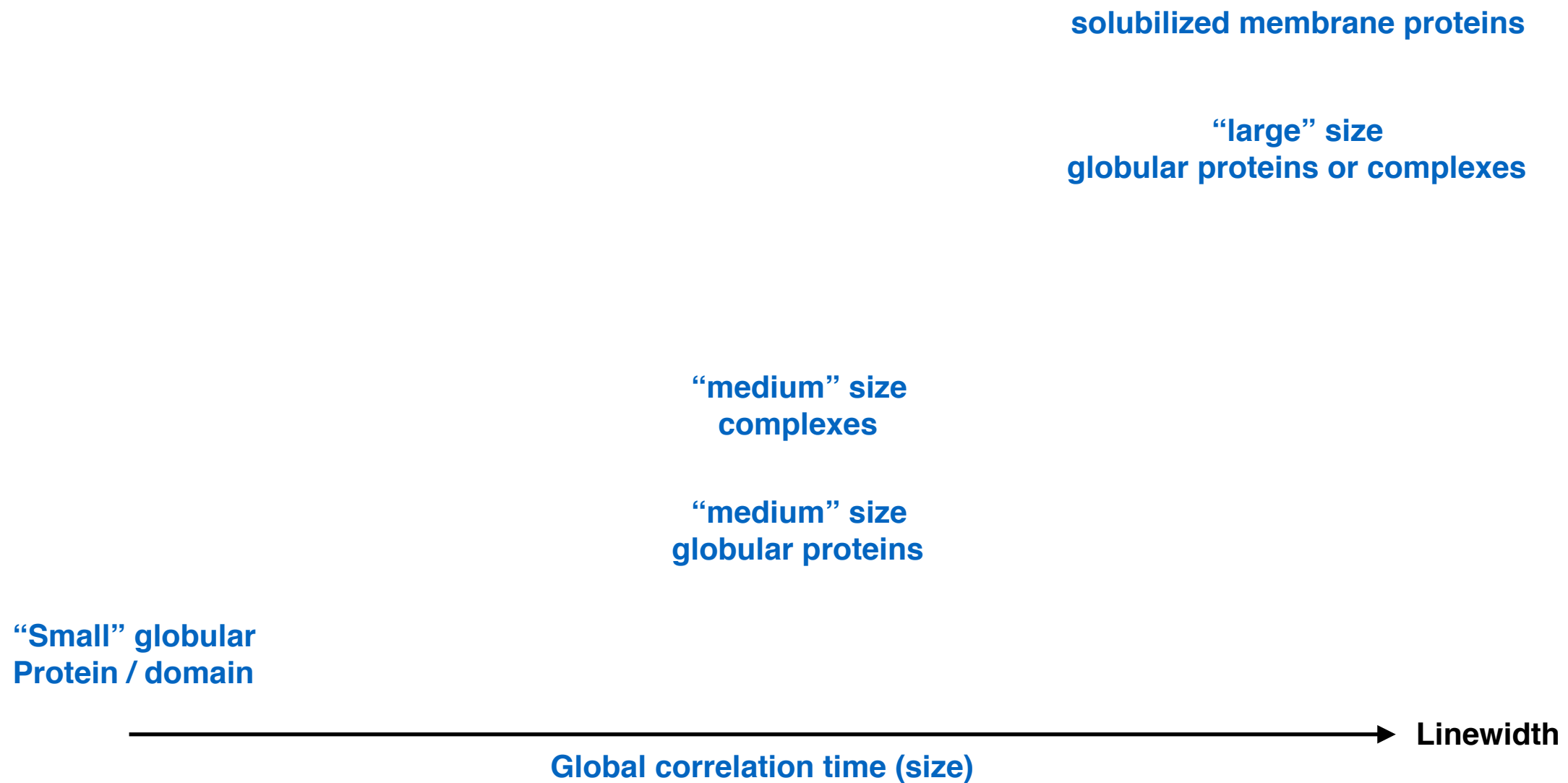
Le temps T_2 dépend du champ magnétique B_0 , de la nature des spins, de la taille des molécules, de la dynamique du système, de la température, etc.



$$M_x(t) = -M_{eq} \sin(\omega_0 t) \exp(-t/T_2)$$



Les échelles temporelles et spatiales sont corrélées



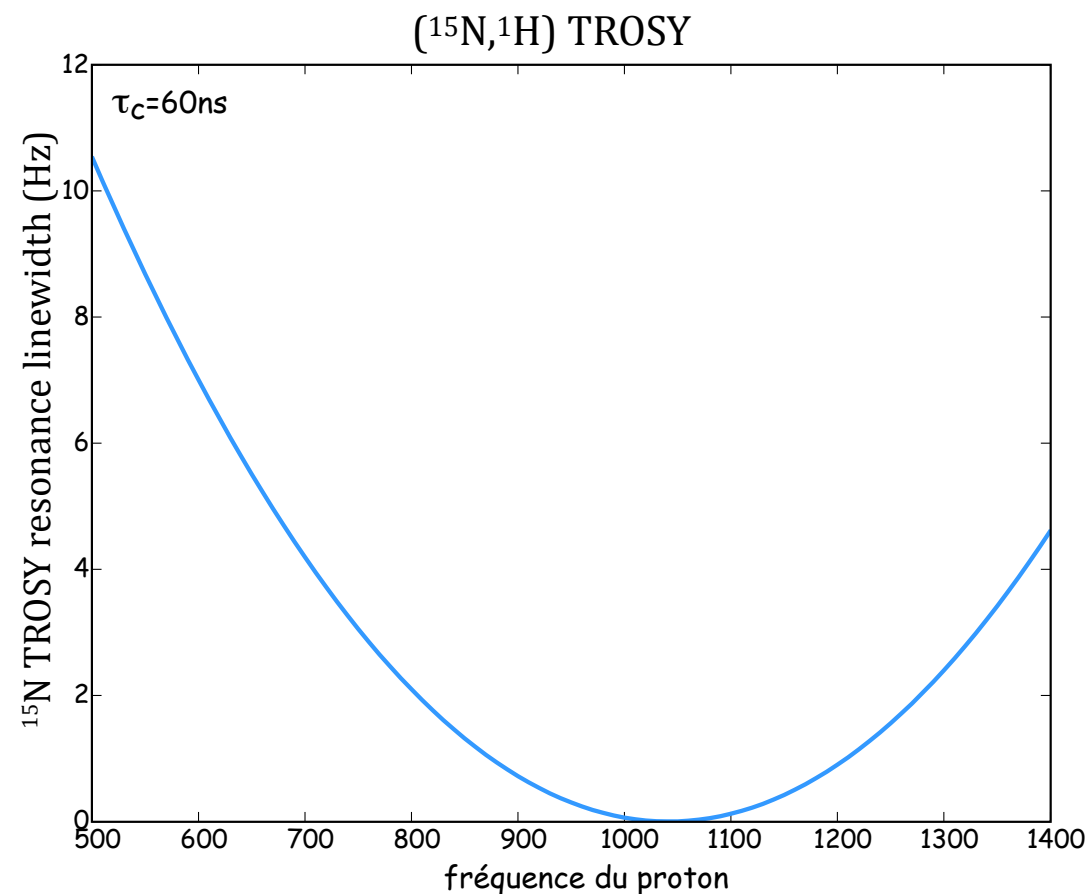
Temps de vie du signal, relaxation transversale & perte de cohérence des spins

Largeur de raie & apparence des spectres

Le temps de relaxation transversale T_2 définit le temps de vie du signal RMN.

Il détermine la largeur des résonances

Le temps T_2 dépend du champ magnétique B_0 , de la nature des spins, de la taille des molécules, de la température, de la dynamique du système, **des sources de relaxation**

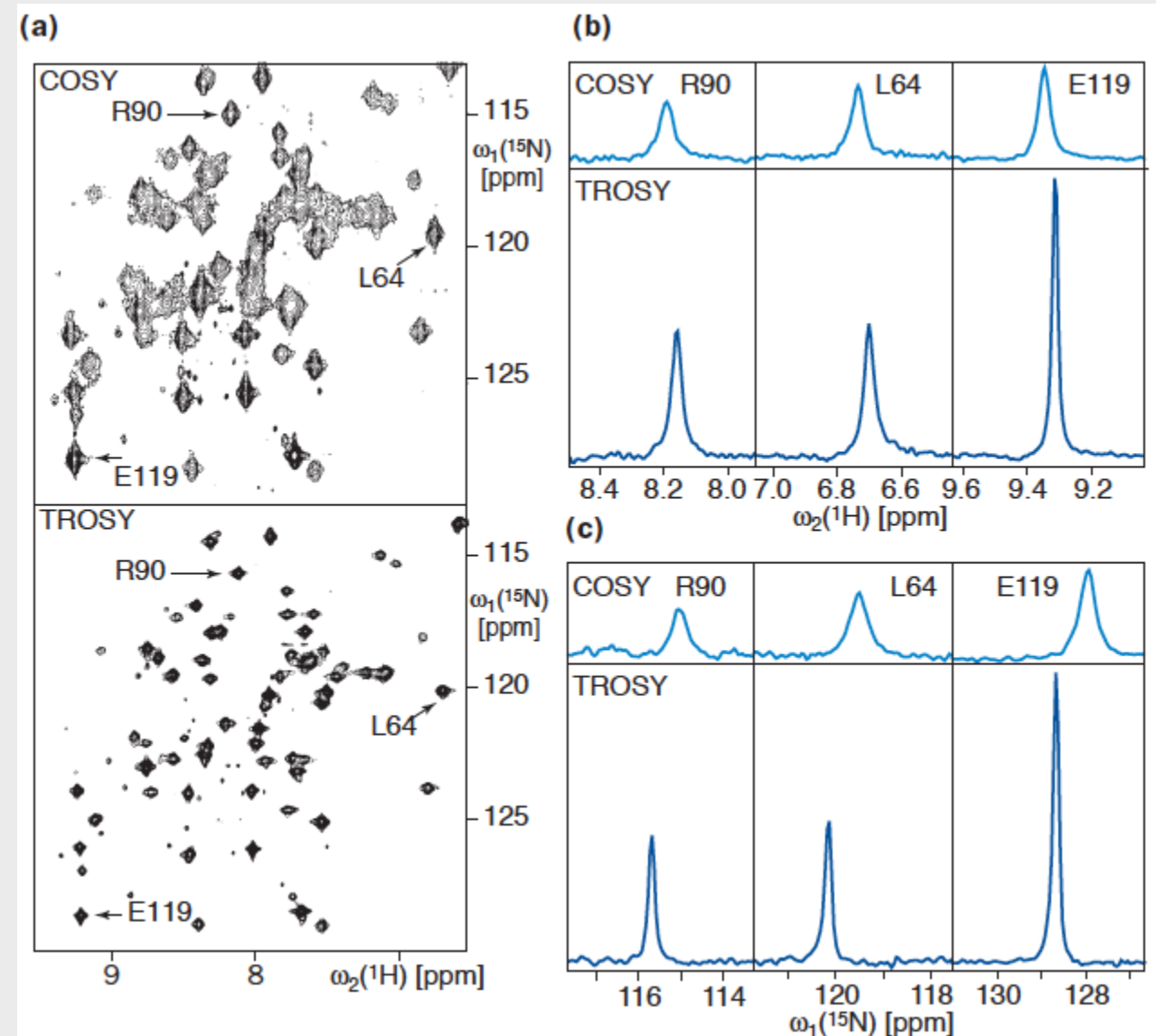


- ⇒ marquages (^2H , ^{15}N , $^1\text{H}^{\text{N}}$)
- ⇒ Très hauts champs

Playing with T_2 : TROSY experiments

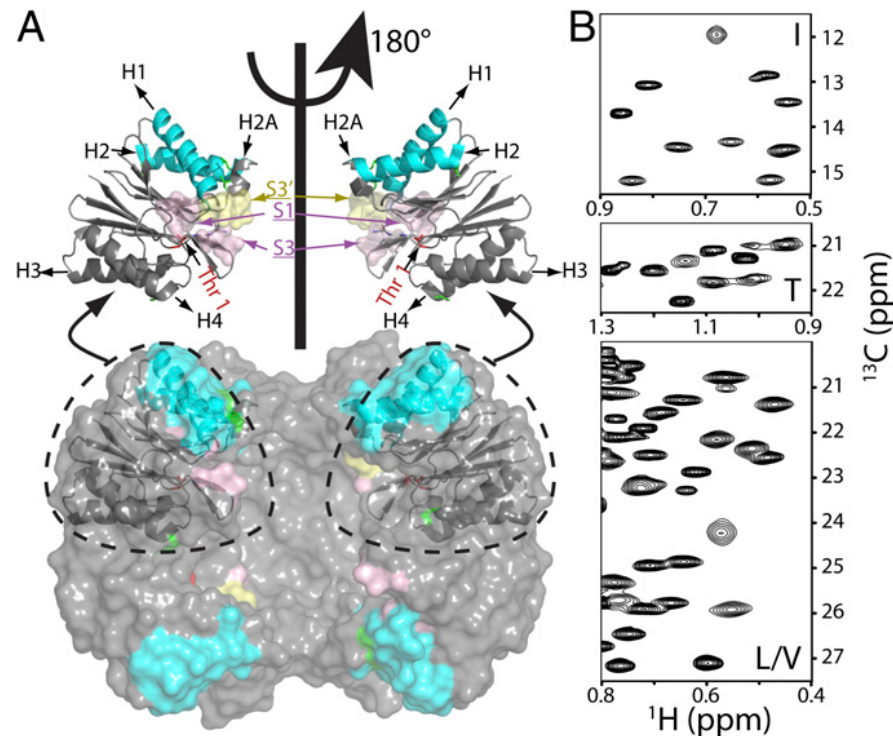
Transverse Relaxation Optimized Spectroscopy

Pervushin, K. Riek, R., Wider, G. and Wütrich, K (1997) Attenuated T_2 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



$^2\text{H}, ^{15}\text{N}$ -labeled 110-kDa octameric protein 7,8-dihydroneopterin aldolase

⇒ Etudes fonctionnelles de gros systèmes



Lichi Shia and Lewis E. Kay (2014), Tracing an allosteric pathway regulating the activity of the HslV protease, PNAS February 11, vol. 111 no. 6

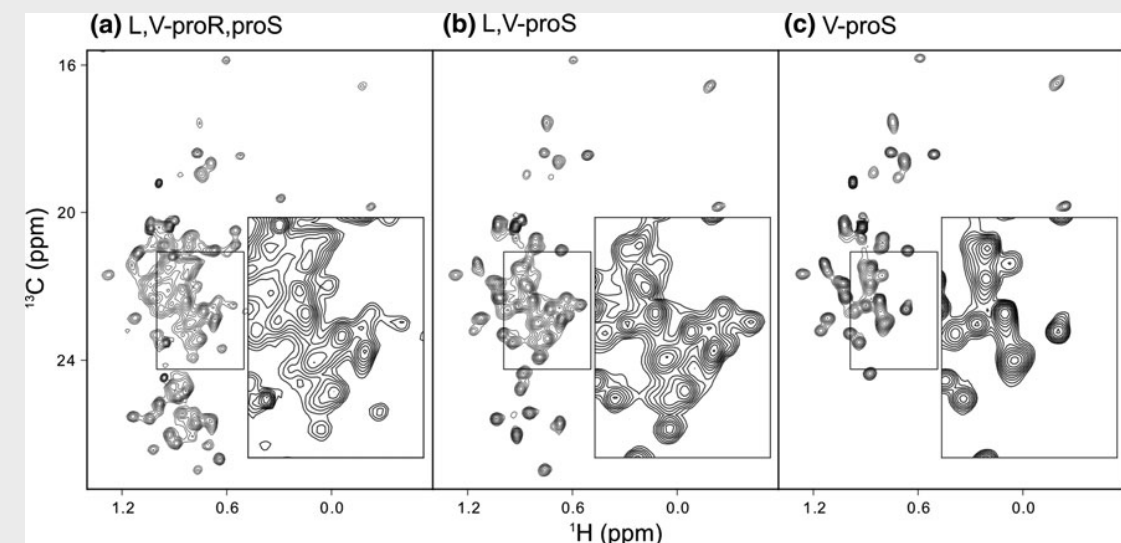
Playing with T2 : TROSY experiments

Transverse Relaxation Optimized Spectroscopy

Vitali Tugarinov, Peter M. Hwang, Jason E. Ollerenshaw, and Lewis E. Kay (2003) Cross-Correlated Relaxation Enhanced ^1H - ^{13}C NMR Spectroscopy of Methyl Groups in Very High Molecular Weight Proteins and Protein Complexes. J. AM. CHEM. SOC. 2003, 125, 10420-10428

TROSY effect that involves cancellation of intra-methyl dipolar relaxation interactions

⇒ ^2H , $^{13}\text{C}^1\text{H}_3$ labeling strategies



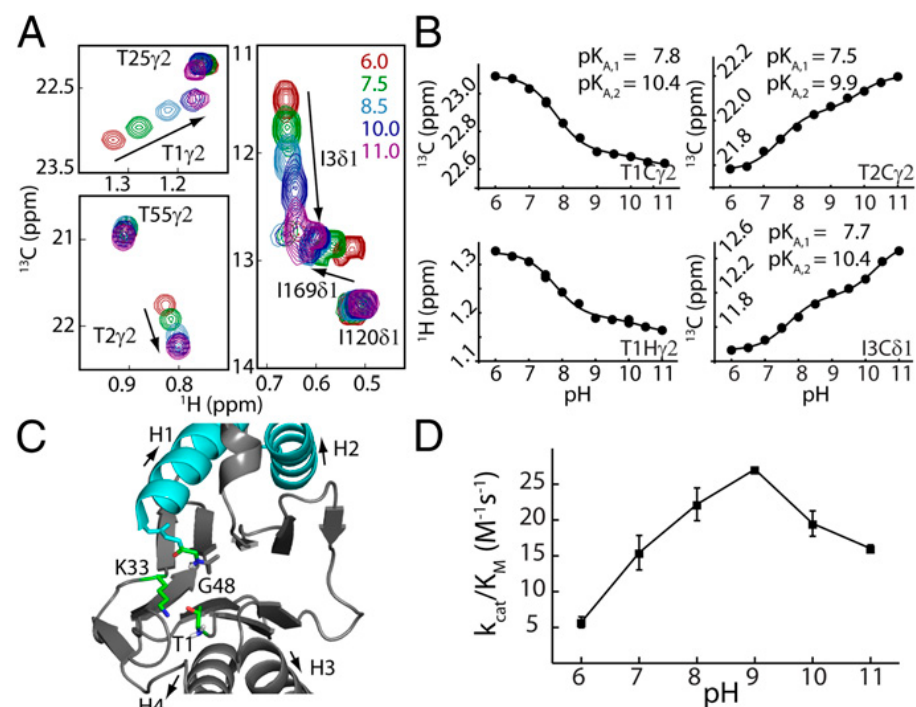
Comparison of HMQC spectra recorded on specifically methyl-labeled TET2 samples (468 kDa).

Guillaume Mas • Elodie Crublet • Olivier Hamelin • Pierre Gans • Jérôme Boisbouvier (2013) Specific labeling and assignment strategies of valine methyl groups for NMR studies of high molecular weight proteins. J Biomol NMR 57:251-262

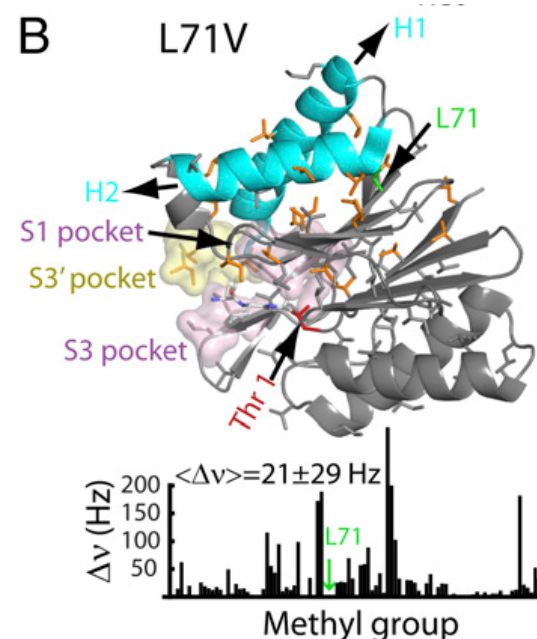
“Jouer” avec T2 : Les expériences $^{13}\text{C}^1\text{H}_3$ Methyl TROSY

⇒ Stratégies d’attribution : mutations + nOes + crystal structures

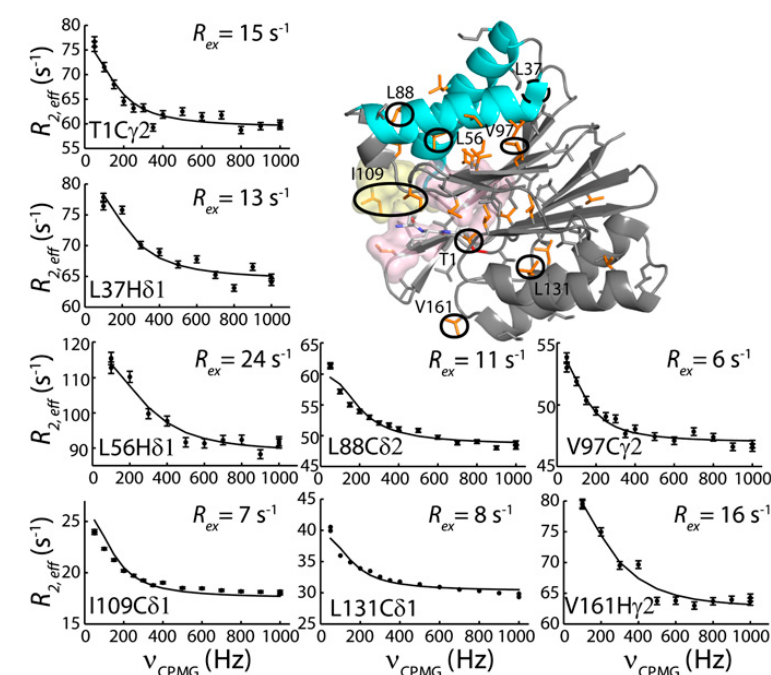
⇒ Etudes fonctionnelles



pKa measurements, catalytic mechanism



mutants, allosteric pathways

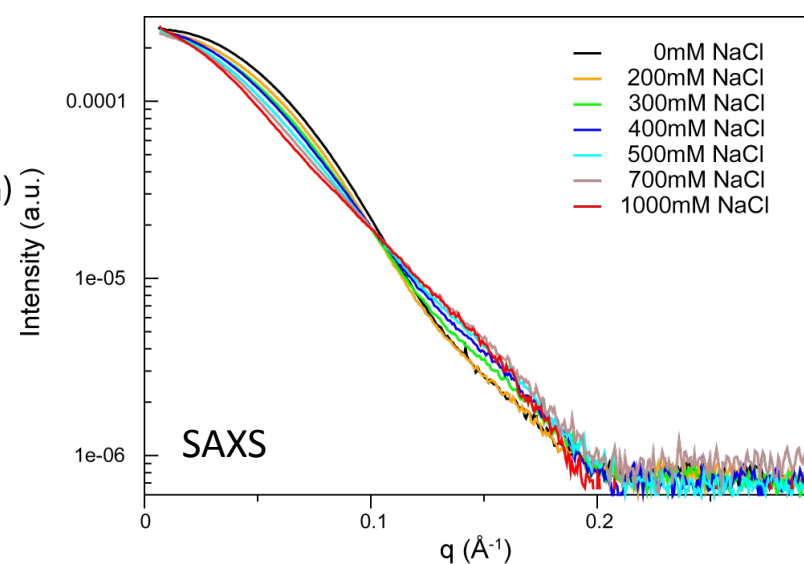
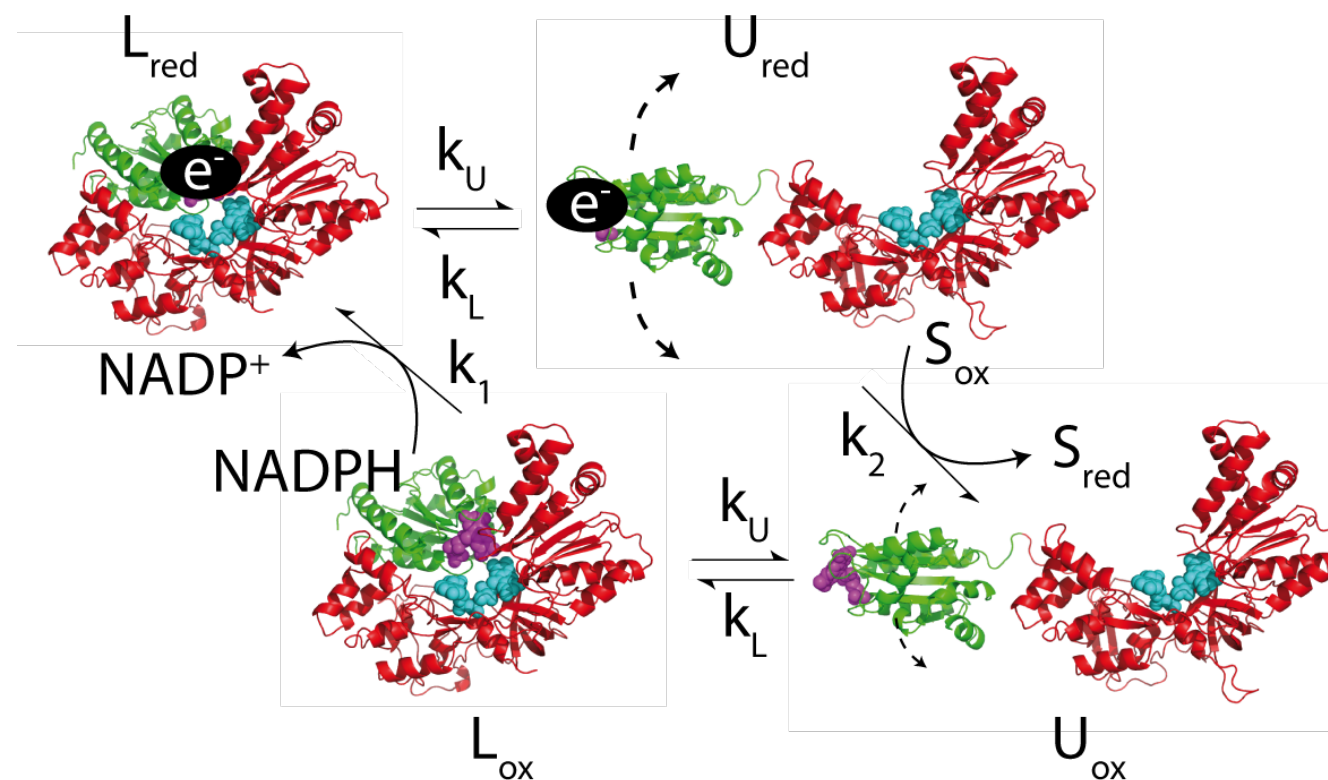
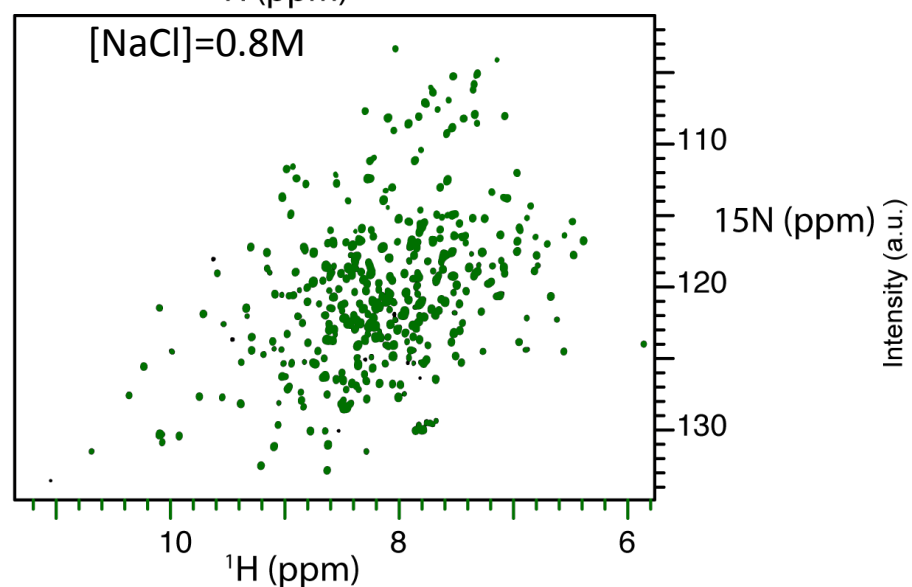
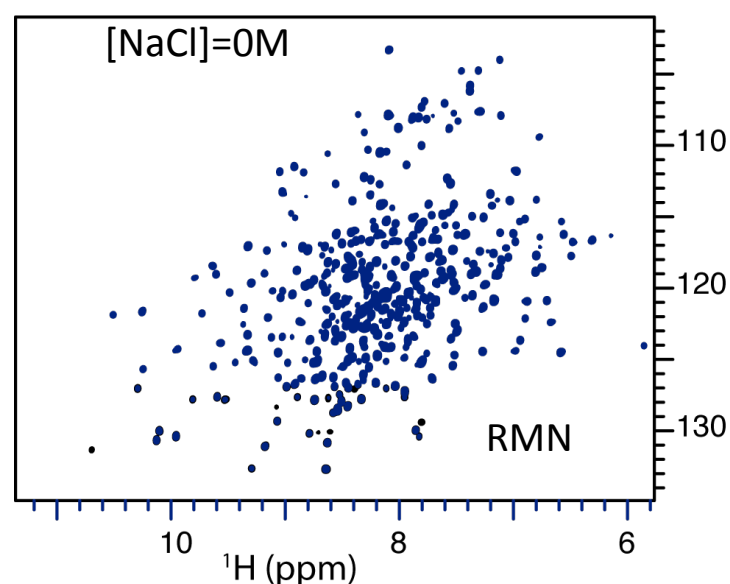


multiple states & flexibility
related to functions

Lichi Shia and Lewis E. Kay (2014) , Tracing an allosteric pathway regulating the activity of the HsIV protease, PNAS | February 11, | vol. 111 | no. 6

⇒ Etudes fonctionnelles de “grosses” protéines multidomaines

Ewen Lescop - Etude de la CPR

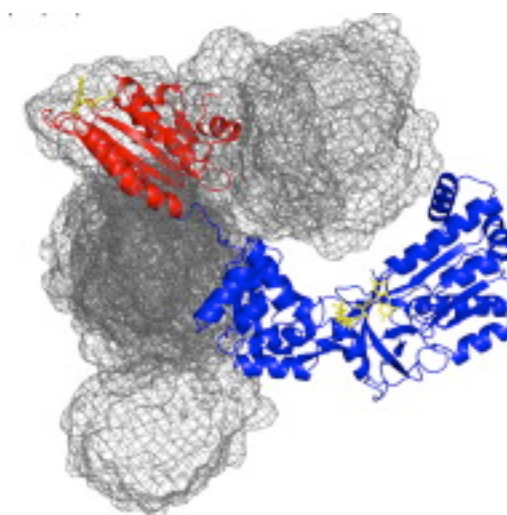
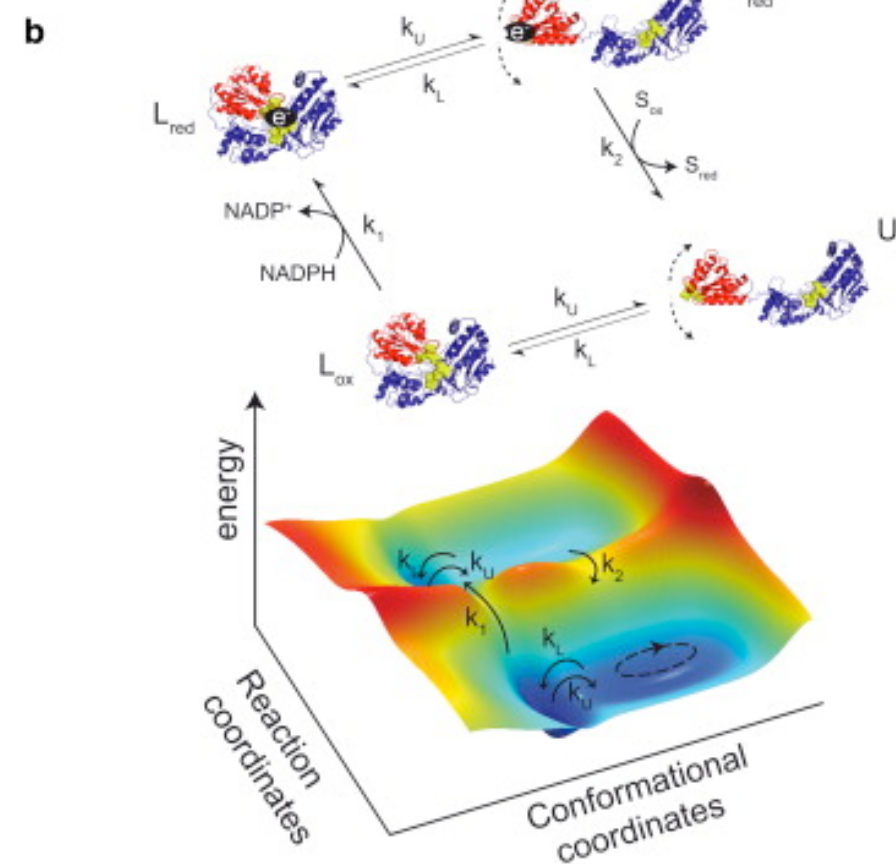
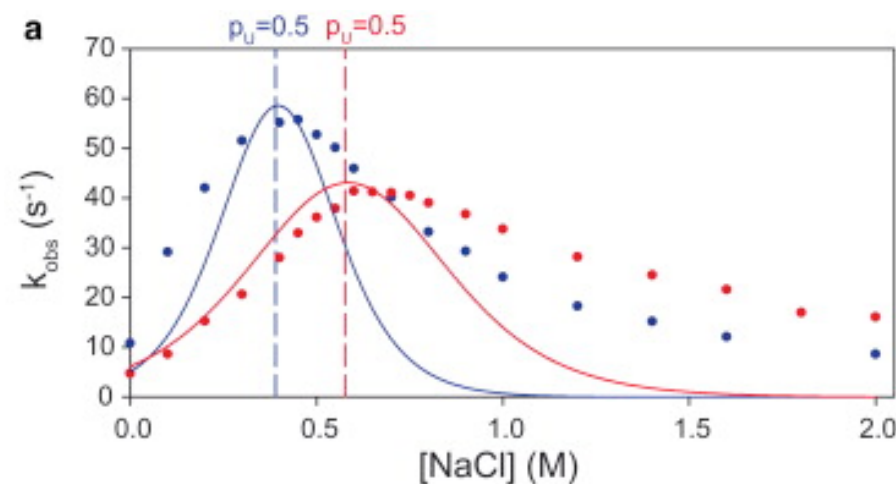
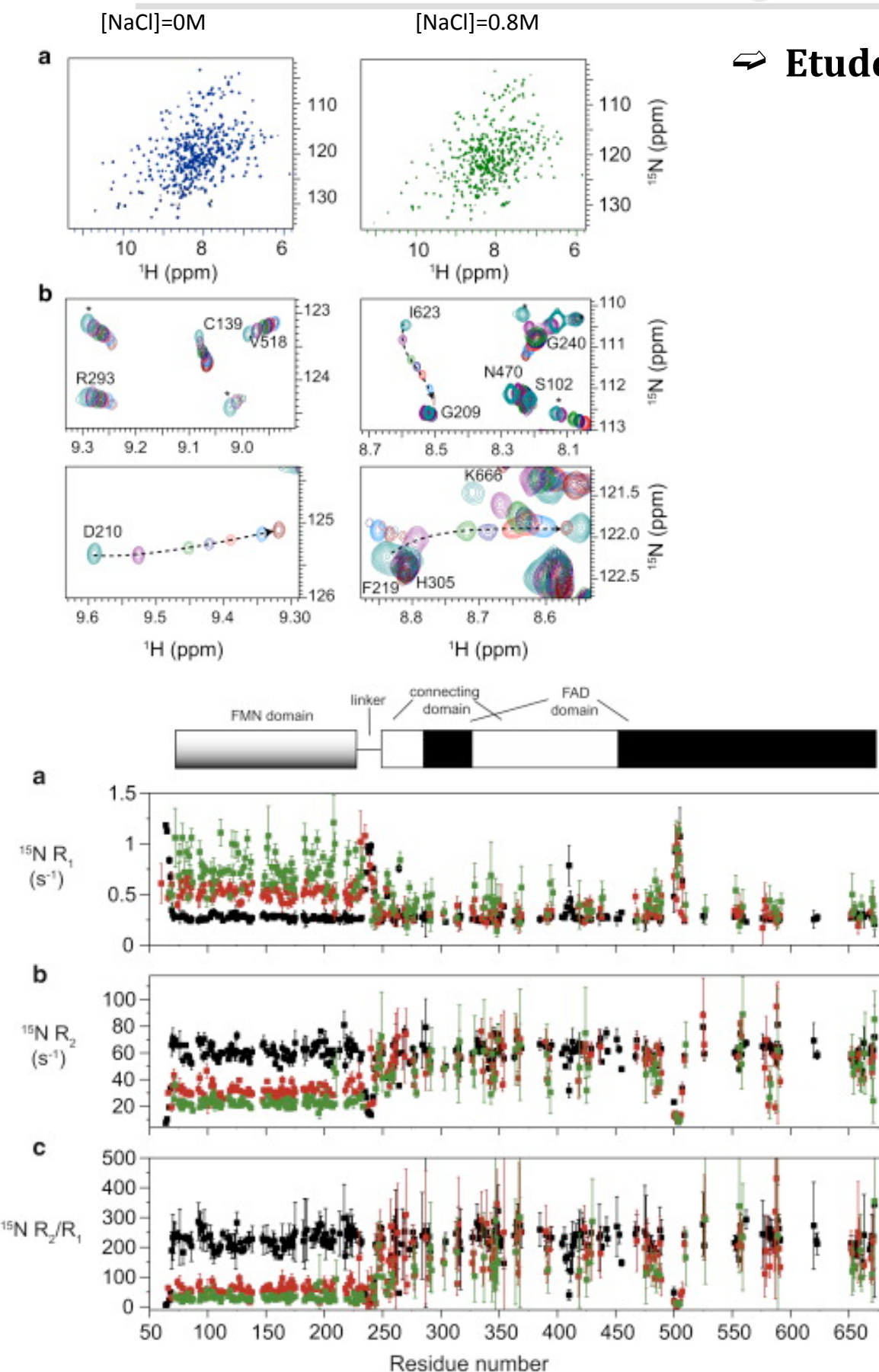


Vincent et al., J Mol Biol. 2012 Jul 20;420(4-5):296-309

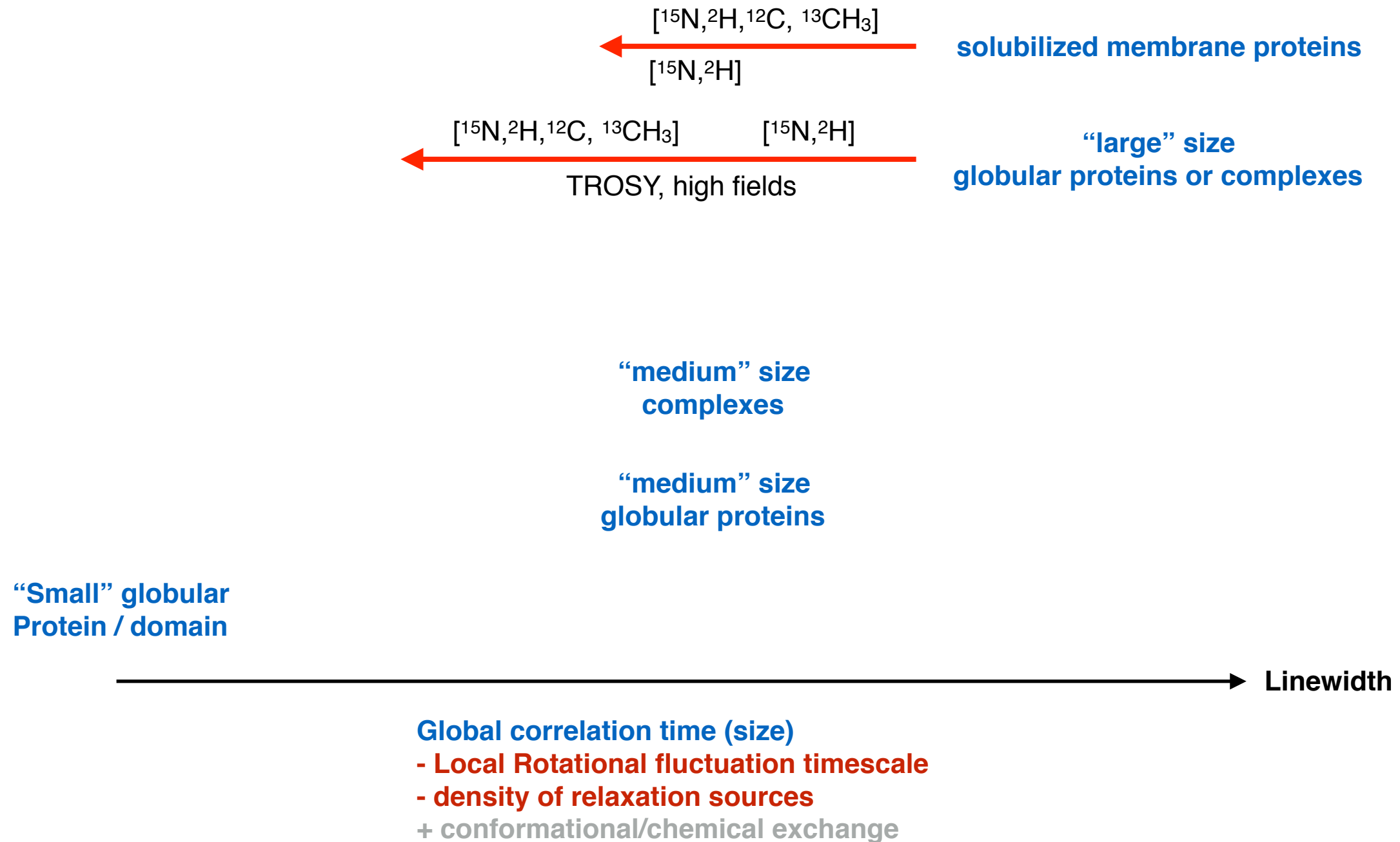
Temps de vie du signal, relaxation transversale & perte de cohérence des spins

Largeur de raie & apparence des spectres

⇒ Etudes fonctionnelles de “grosses” protéines multidomaines

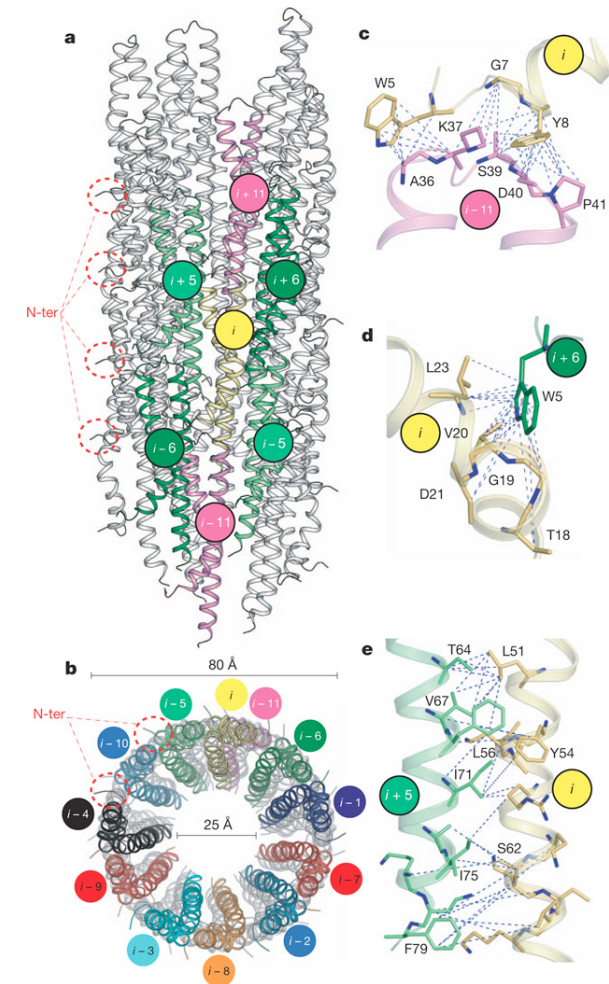
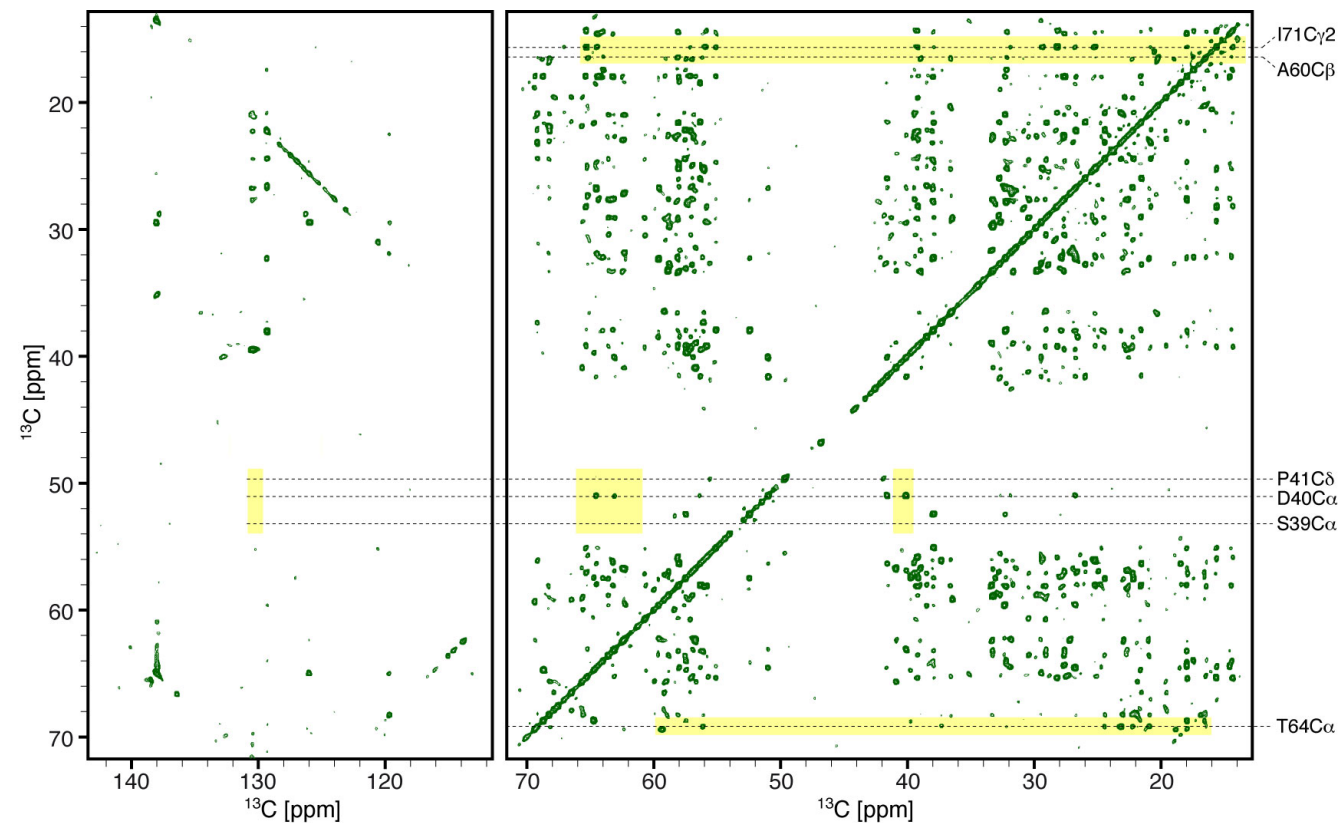


Les échelles temporelles et spatiales sont corrélées



Les échelles temporelles et spatiales sont corrélées

Solid State NMR



Antoine Loquet et al. Atomic model of the type III secretion system needle, Nature 2012

Linewidth

Global correlation time (size)

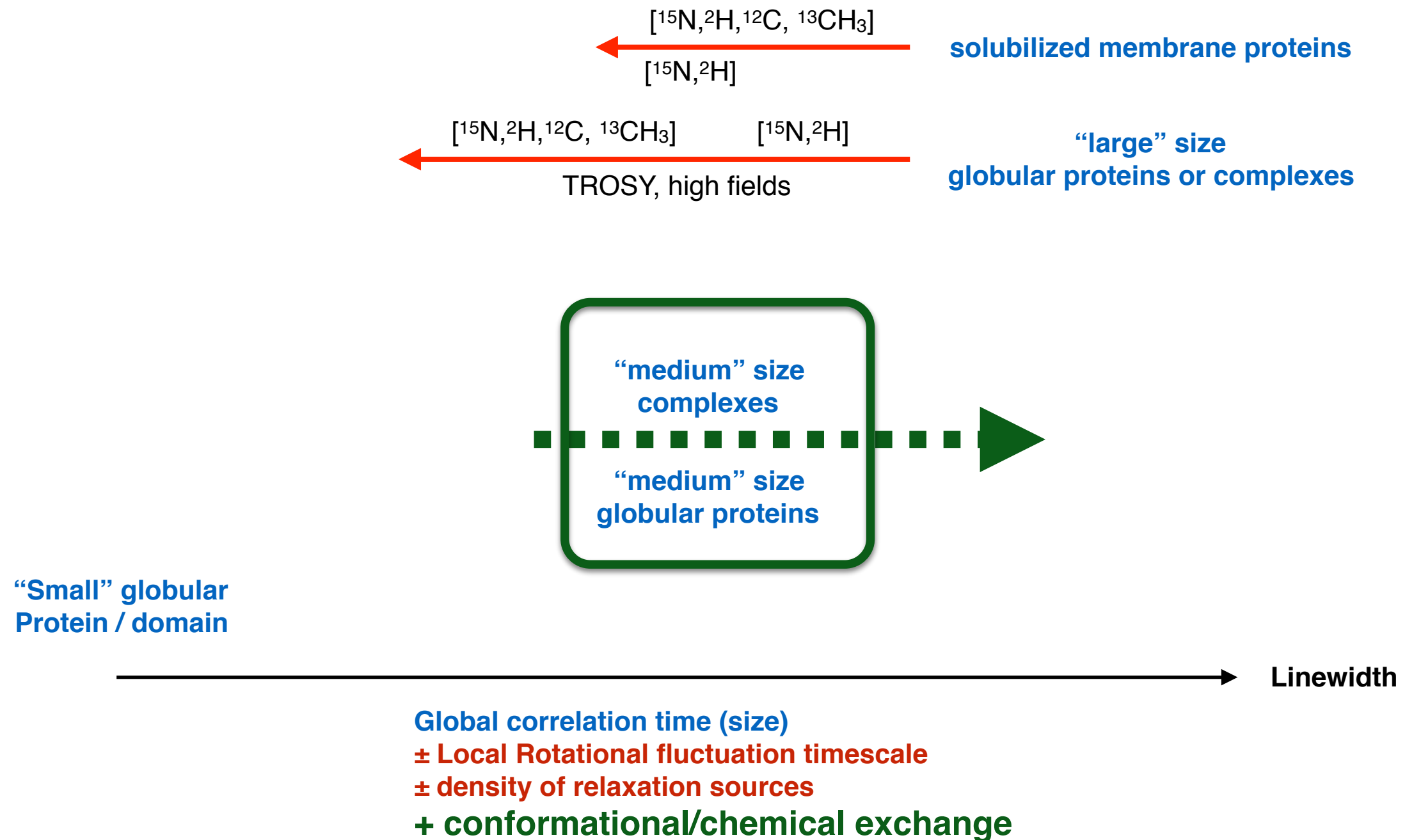
+ Local Rotational fluctuation timescale

+ density of relaxation sources

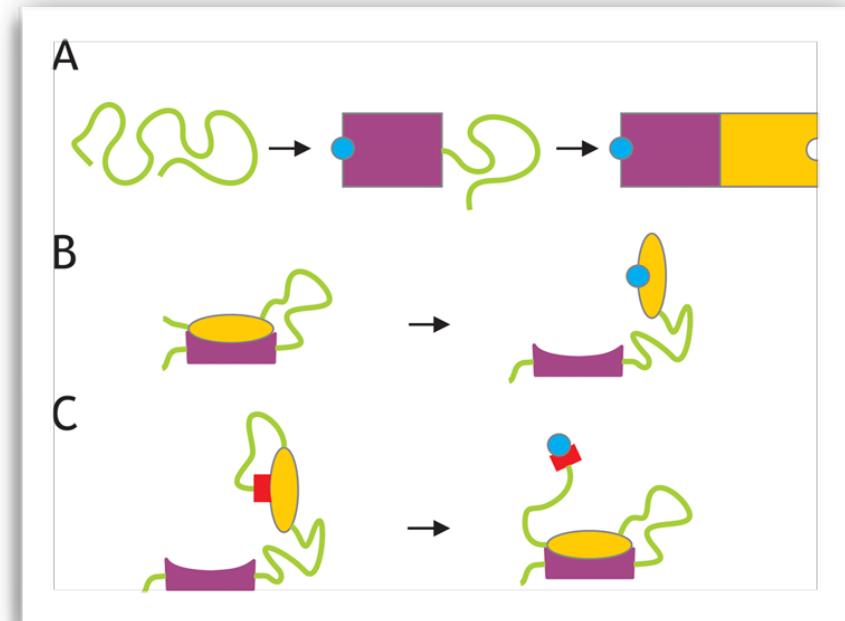
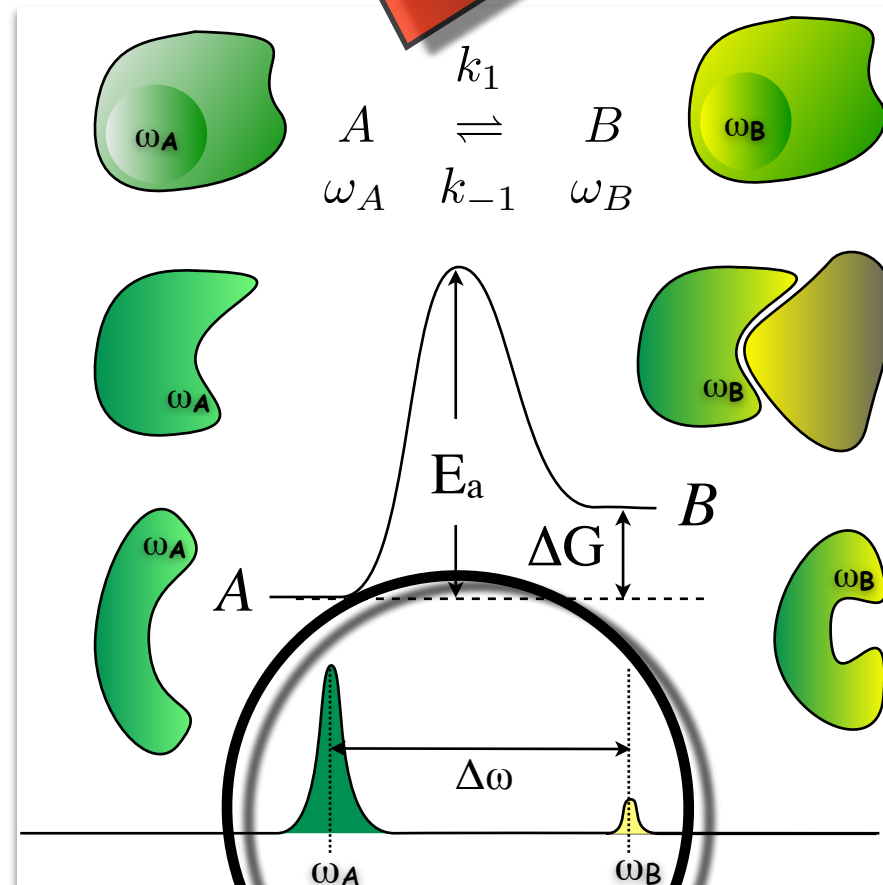
+ conformational/chemical exchange

+ conformational heterogeneity

Les échelles temporelles et spatiales sont corrélées



Motions/processes timescales and NMR parameters



thermodynamic parameters

$$K_{eq} = k_1/k_{-1} = p_B/p_A$$

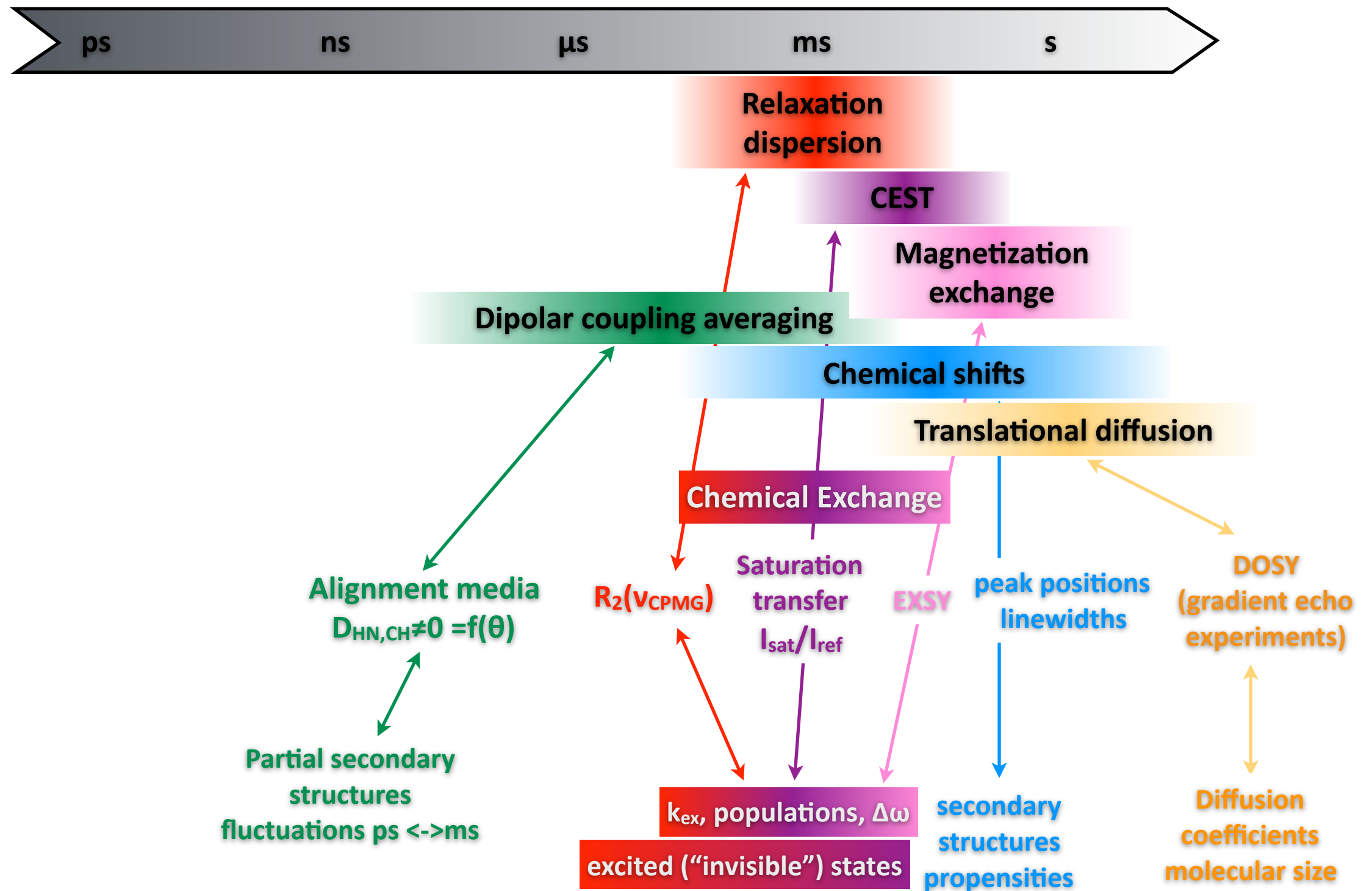
$$\Delta G = -RT \ln K$$

kinetic parameters

$$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$$

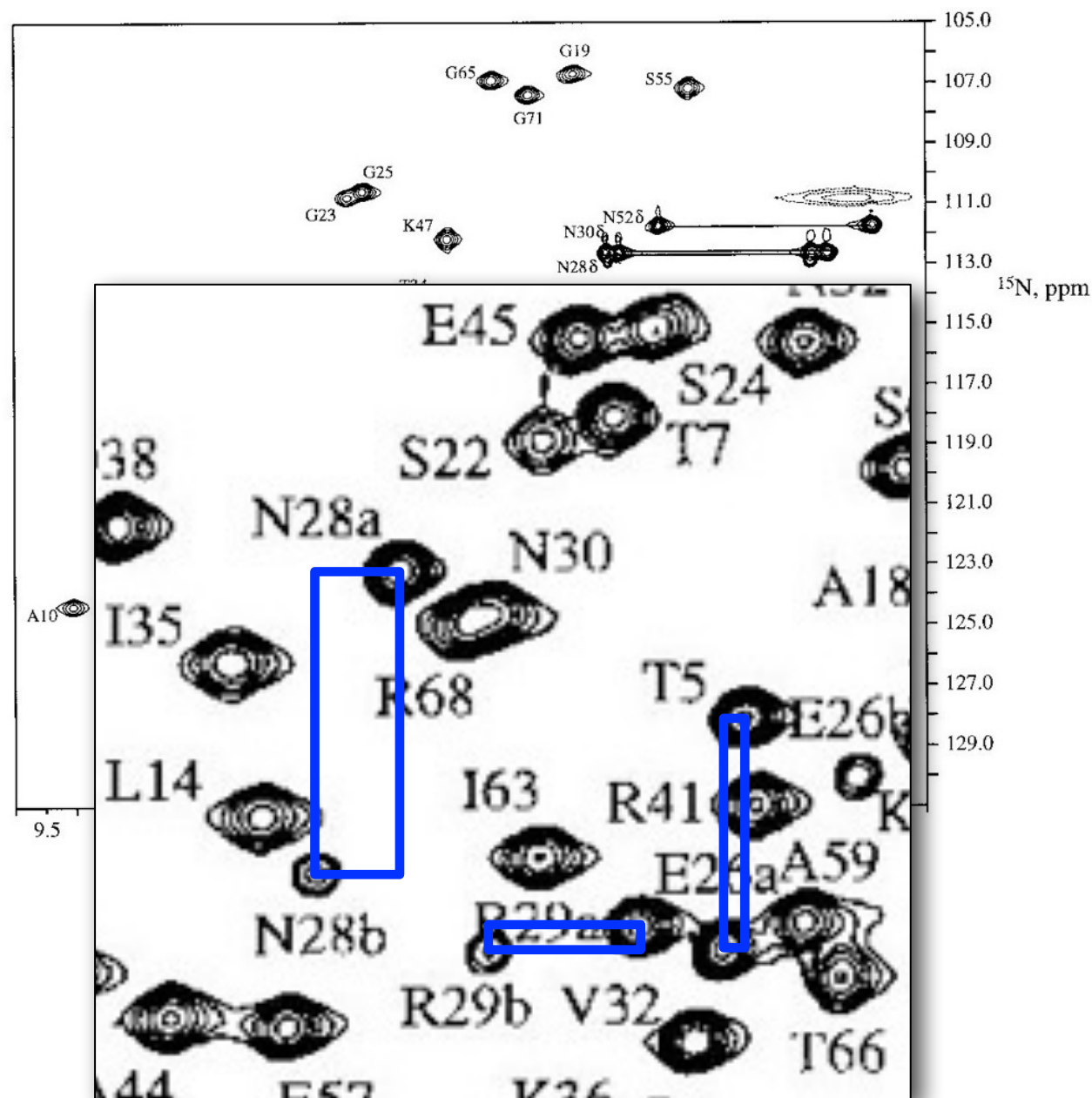
$$k_{ex}(T) = k_0 \exp(-E_a/kT)$$

Motions/processes timescales and NMR parameters



Temps de vie du signal, relaxation transversale & perte de cohérence des spins

Largeur de raie & apparence des spectres

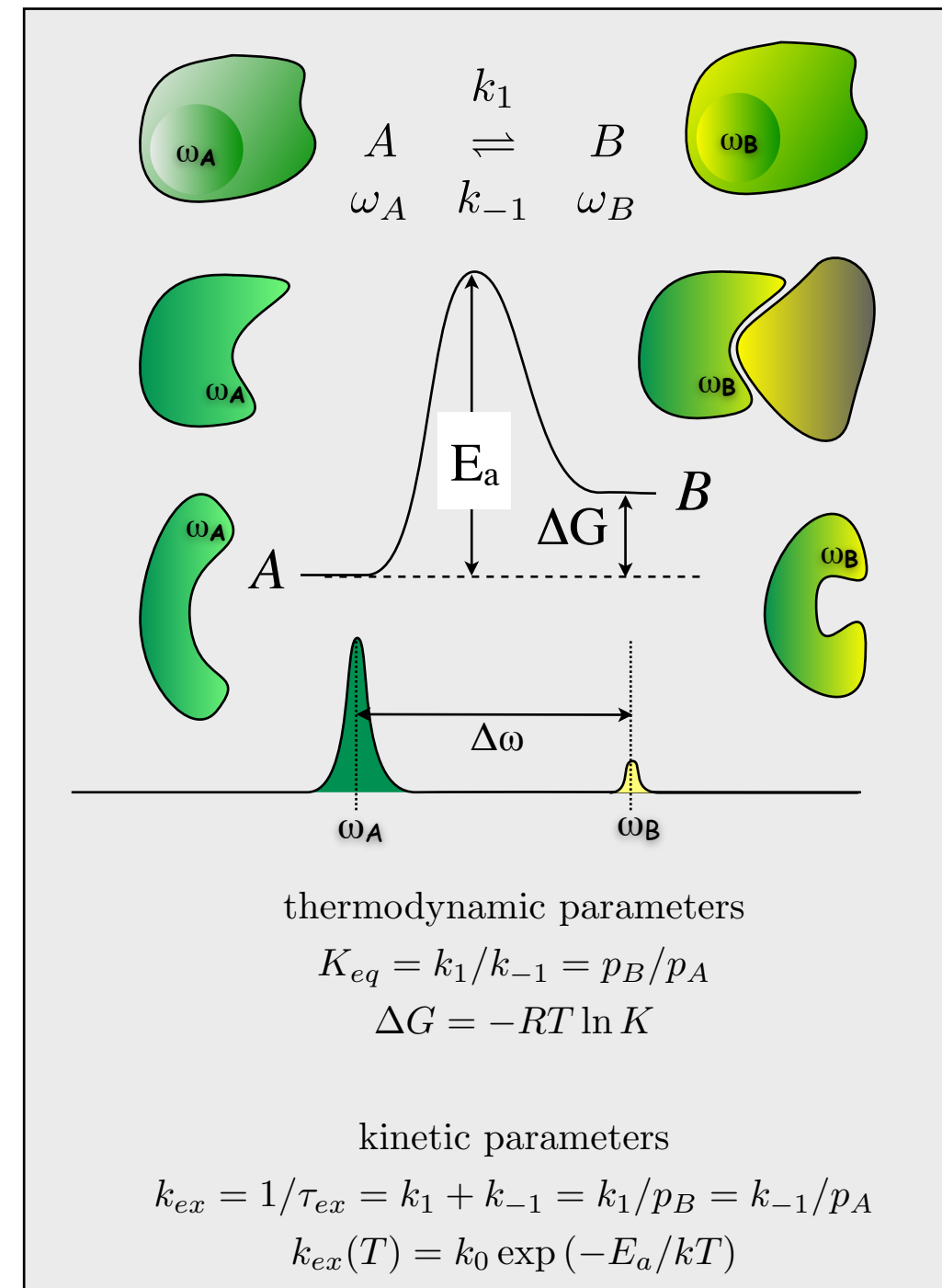


► Two conformations : two peaks

Chemical shifts

Chemical Exchange

peak positions
linewidths





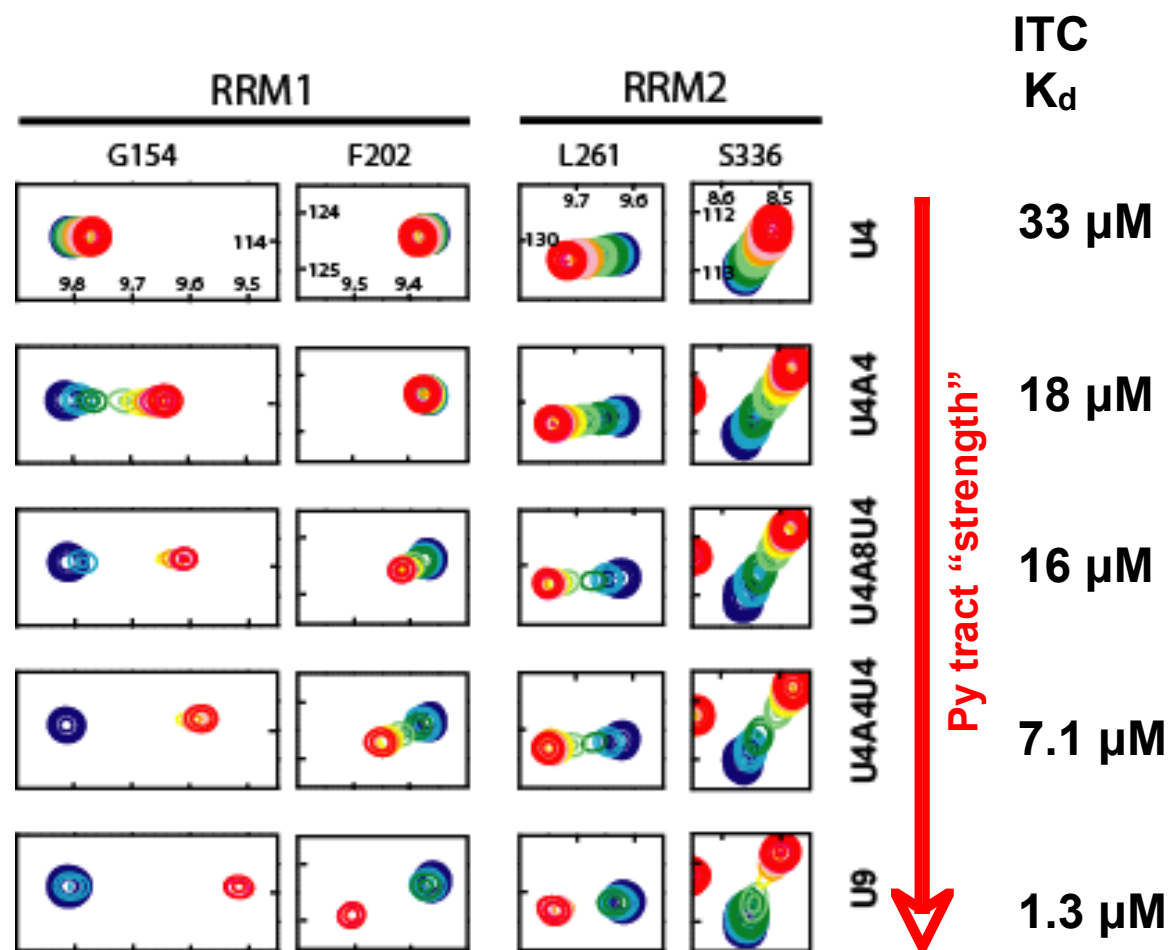
Chemical shifts

Chemical Exchange

peak positions
linewidths

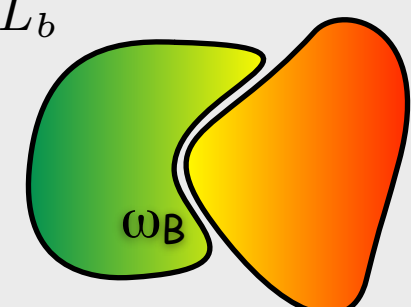
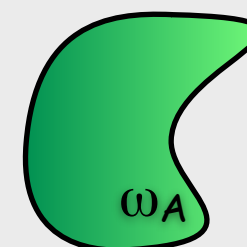
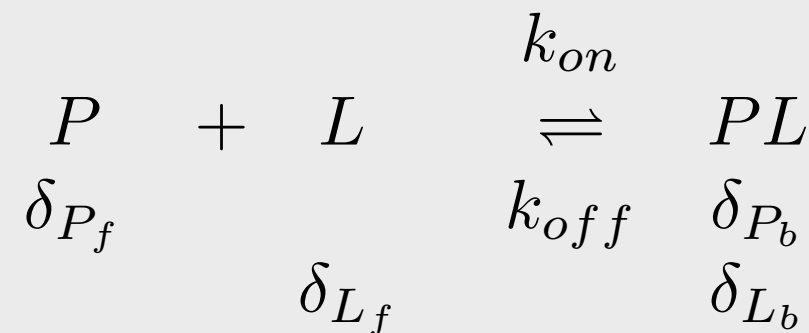
U2AF65 conformation depends on Py tract strength

Exchange regime & RNA binding affinity



► Two conformations: one or two (broad) observable peaks!

Intermolecular processes



$$k_{ex} = k_{on}[P] + k_{off}$$



$$k_{ex} = k_{on}[L] + k_{off}$$

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_{off}}{k_{on}}$$



Le régime d'échange

- ▶ L'effet sur les spectres dépend des valeurs relatives de k_{ex} (τ_{ex}) et de $\Delta\omega/2$ ($2/\Delta\omega$).
- ▶ On parle d'échange **lent**, **intermédiaire** ou **rapide** à l'échelle des déplacements chimiques.

👉 Echange "rapide": $\Delta\omega \ll k_{ex}$

⇒ Un pic $\omega = p_A\omega_A + p_B\omega_B$

⇒ $R_{ex} \propto B_0^2$

👉 Echange "intermédiaire": $\Delta\omega \approx k_{ex}$

⇒ Un ou deux pics élargis

⇒ $R_{ex} \propto B_0$

👉 Echange "lent": $\Delta\omega \gg k_{ex}$

⇒ Deux pics

⇒ R_{ex} indépendant de B_0

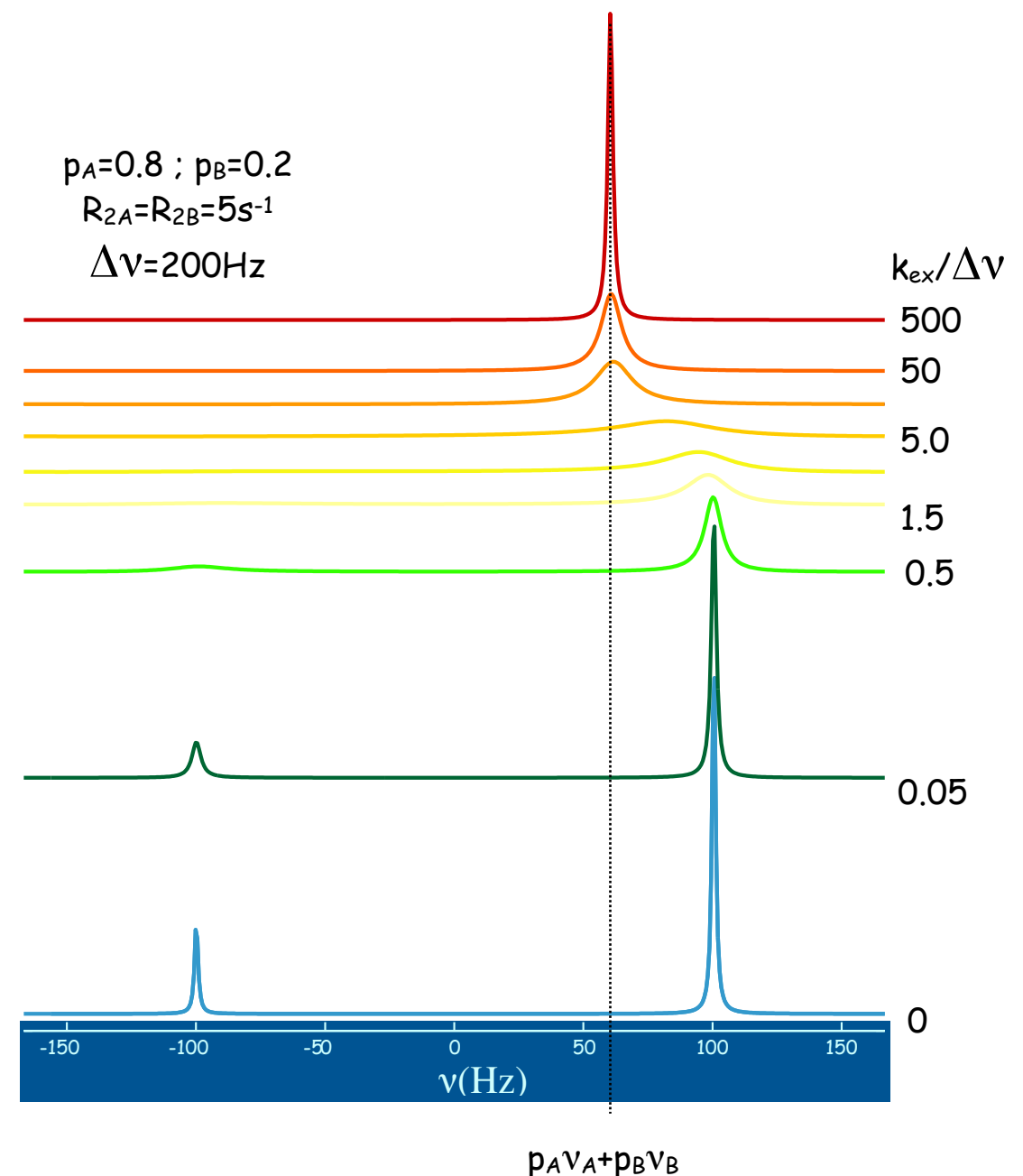
⇒ $R_{ex}^{(B)} \rightarrow k_{BA} = p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB} = p_B k_{ex}$

$p_A \gg p_B \Rightarrow R_{ex}(B) \gg R_{ex}(A)$

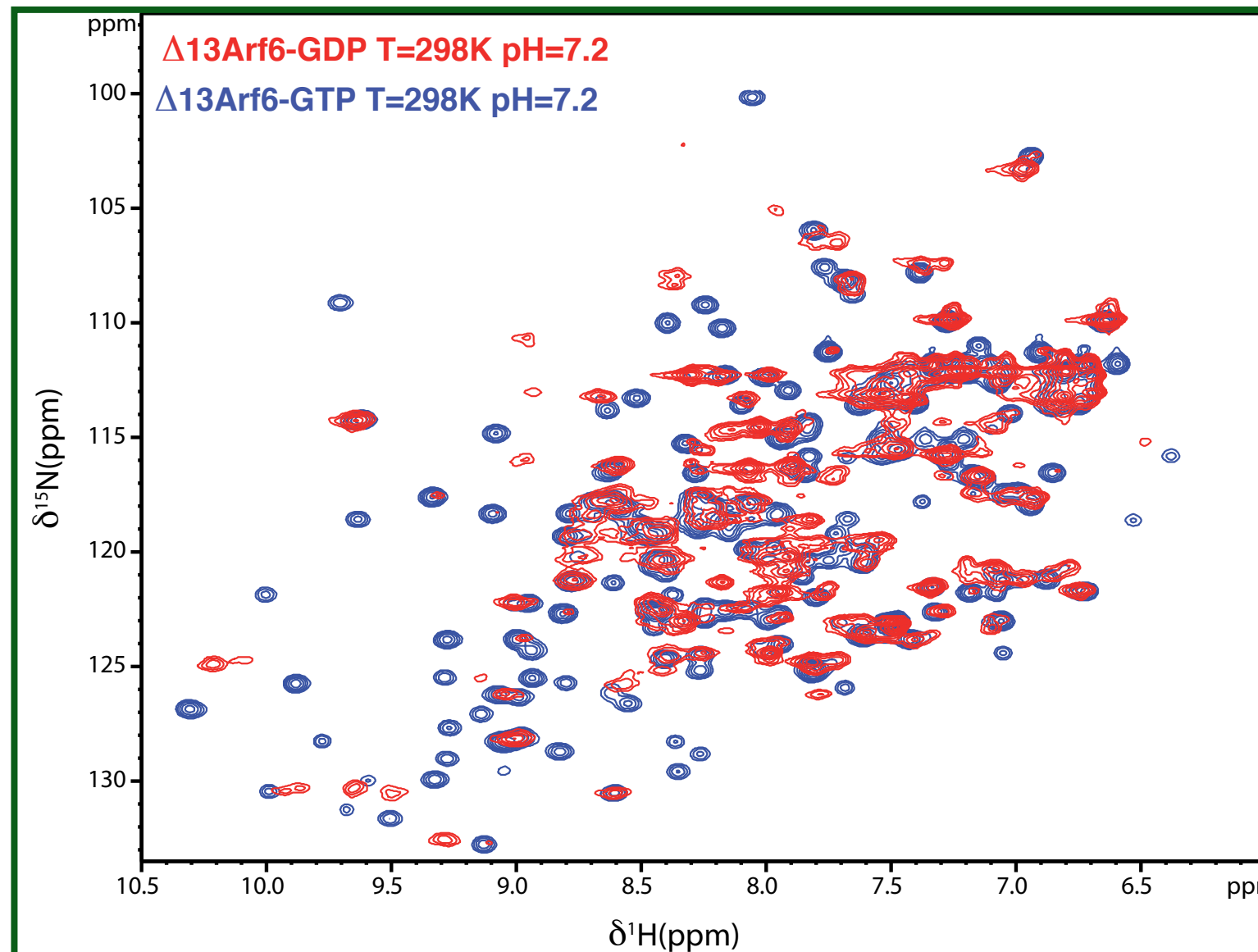
Chemical shifts

Chemical Exchange

peak positions
linewidths



Les échelles temporelles et spatiales sont corrélées



Linewidth

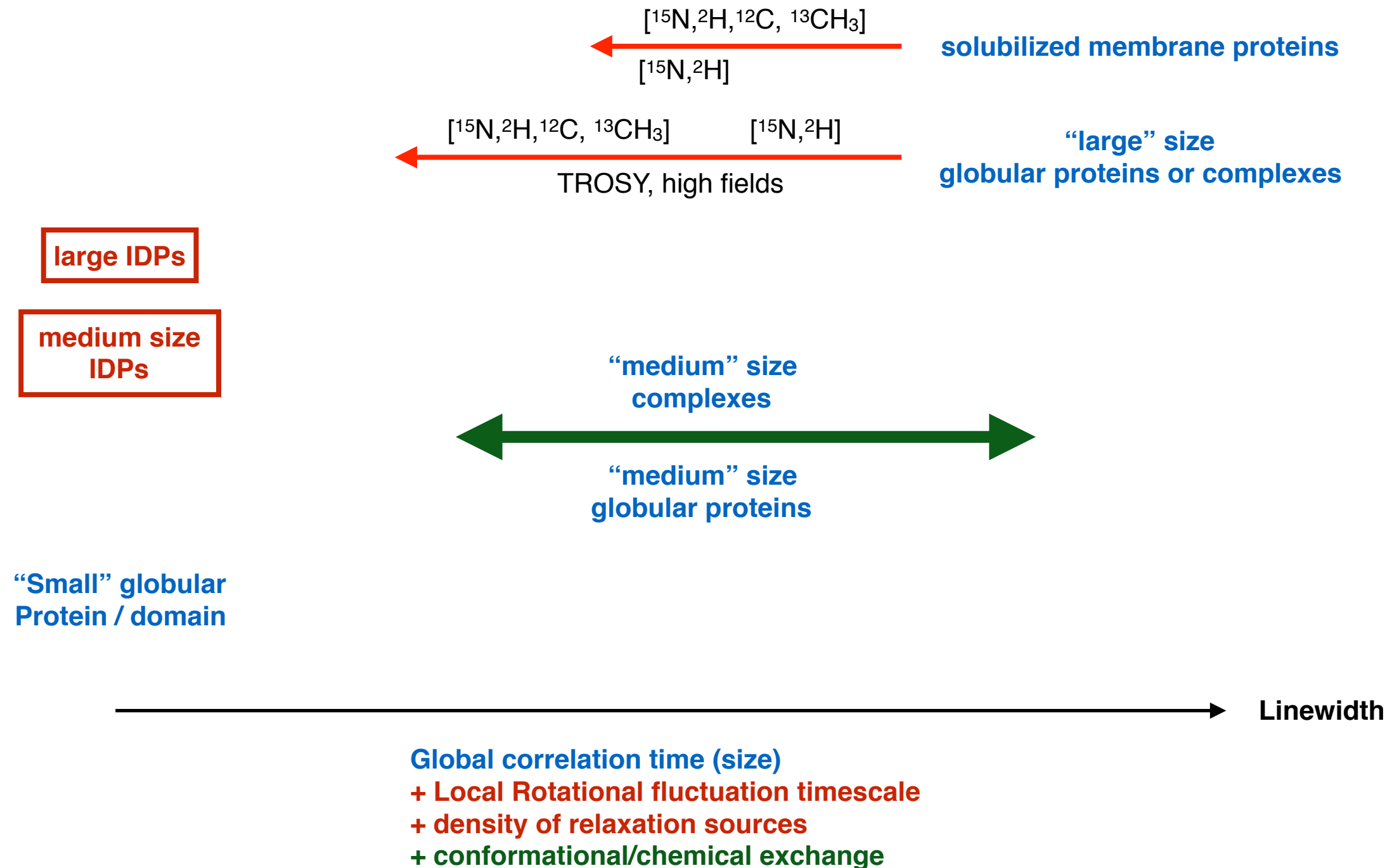
Global correlation time (size)

+ Local Rotational fluctuation timescale

+ density of relaxation sources

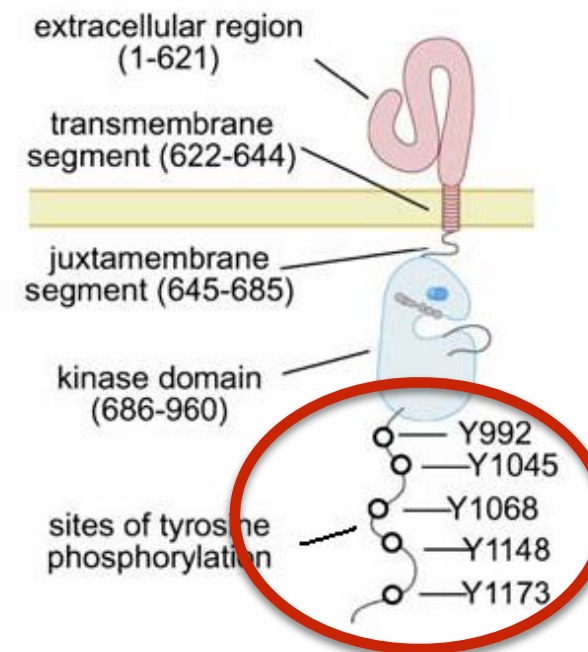
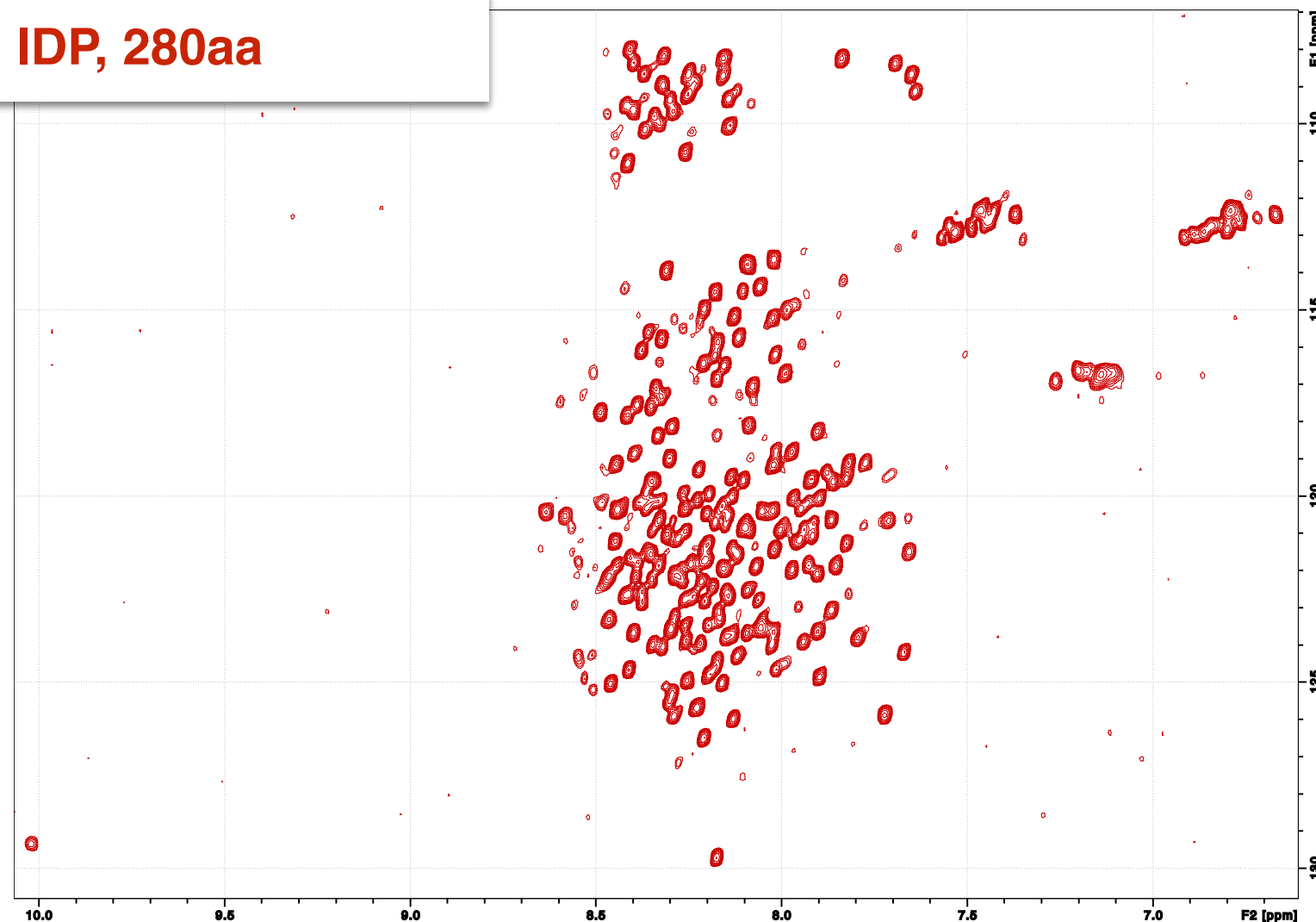
+ conformational/chemical exchange

Les échelles temporelles et spatiales sont corrélées



Les échelles temporelles et spatiales sont corrélées

IDP, 280aa



Linewidth

Global correlation time (size)

+ Local Rotational fluctuation timescale

+ density of relaxation sources

+ conformational/chemical exchange

Caractériser des fragments flexibles “libres” / fragments liés

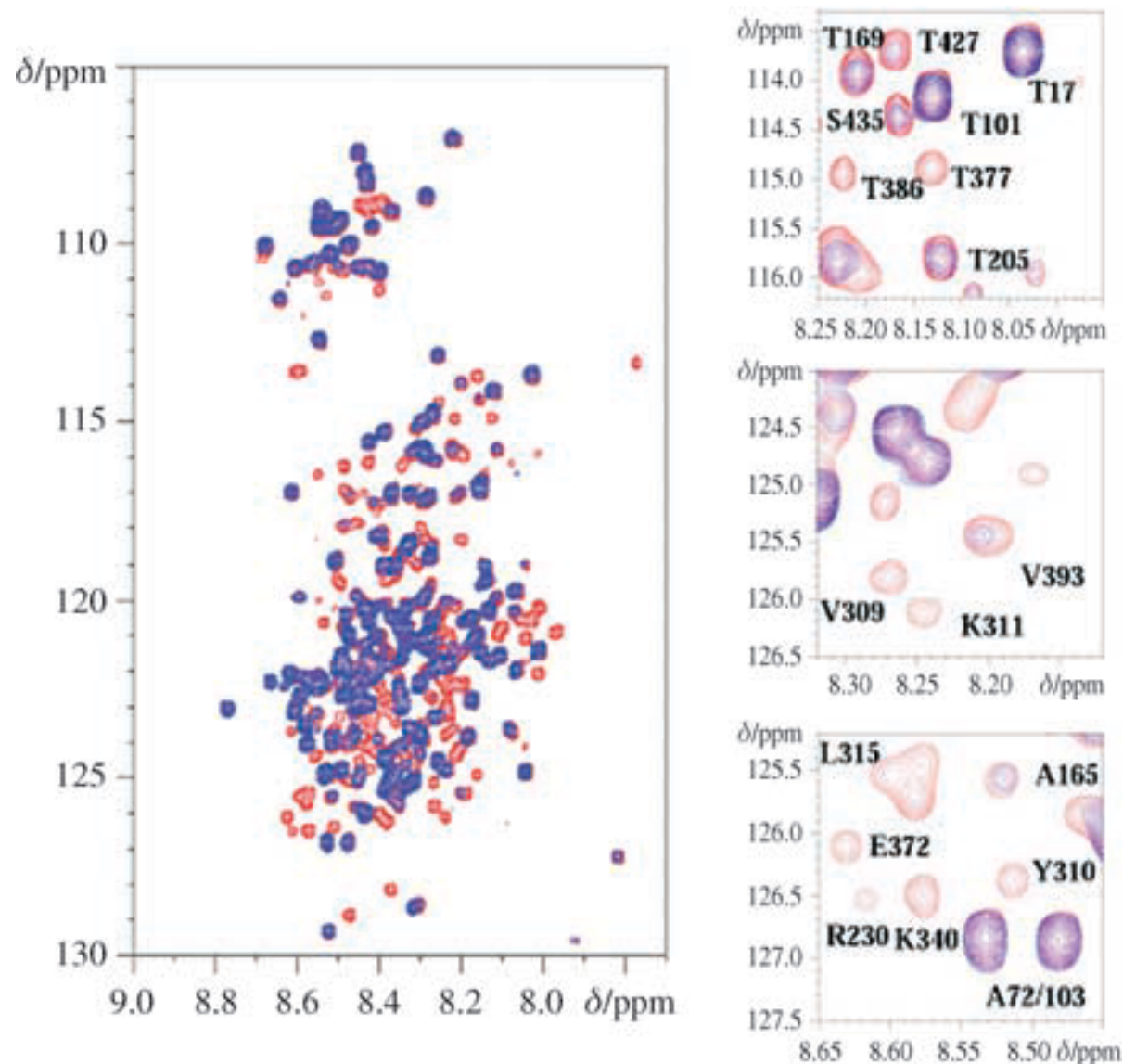


Figure 4. HSQC spectrum (full spectrum (top) and selected annotated zooms (bottom)) of tau free in solution (red) and integrated into mature PHFs (blue).

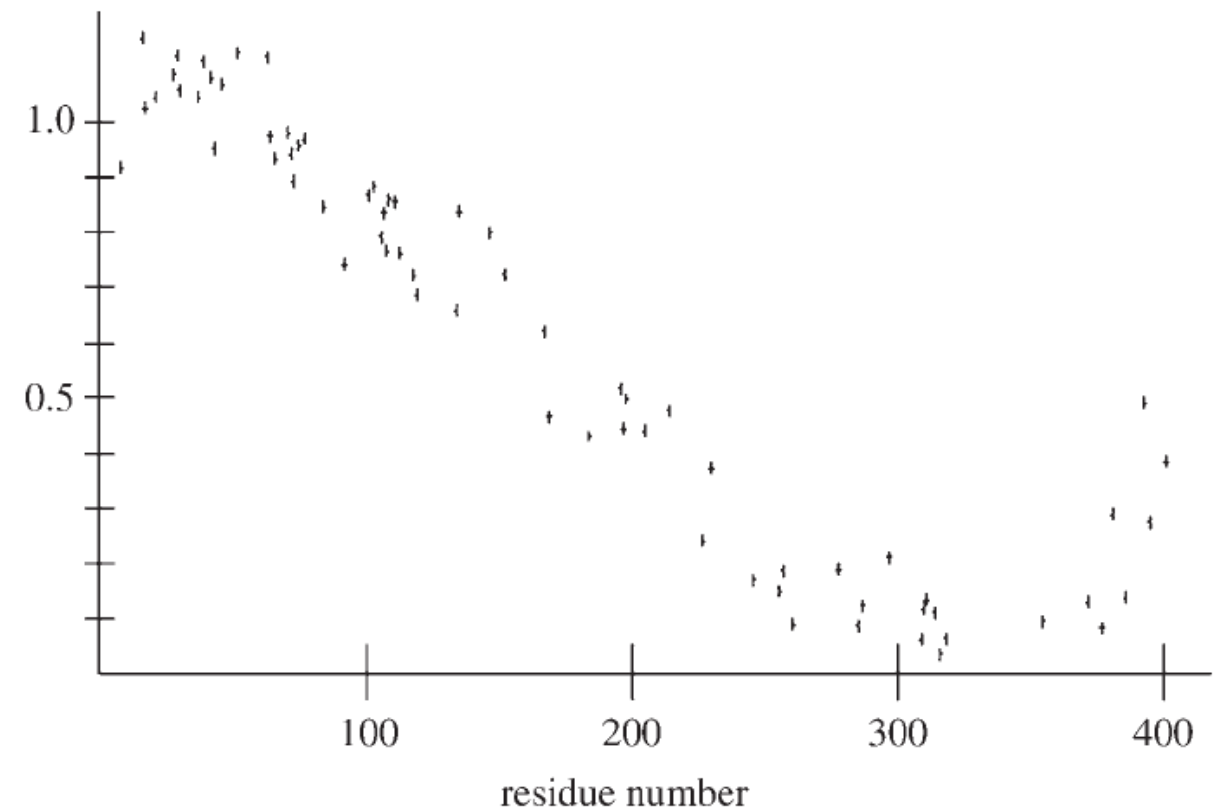
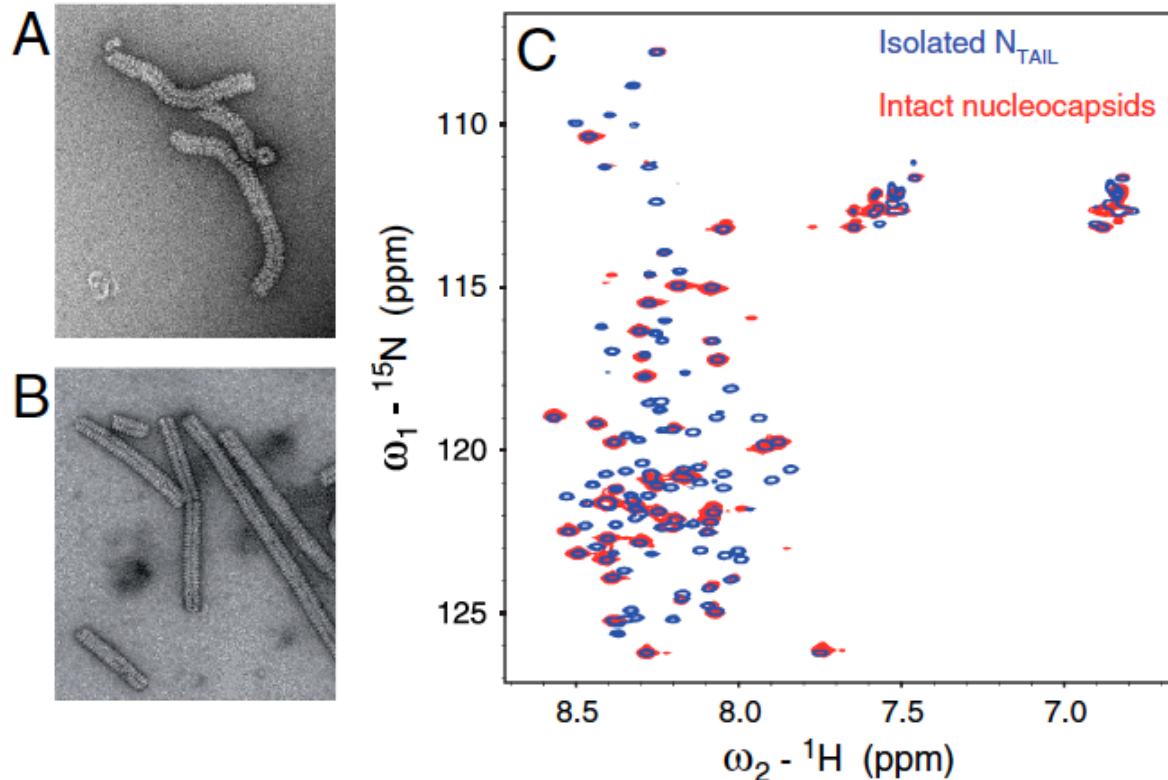
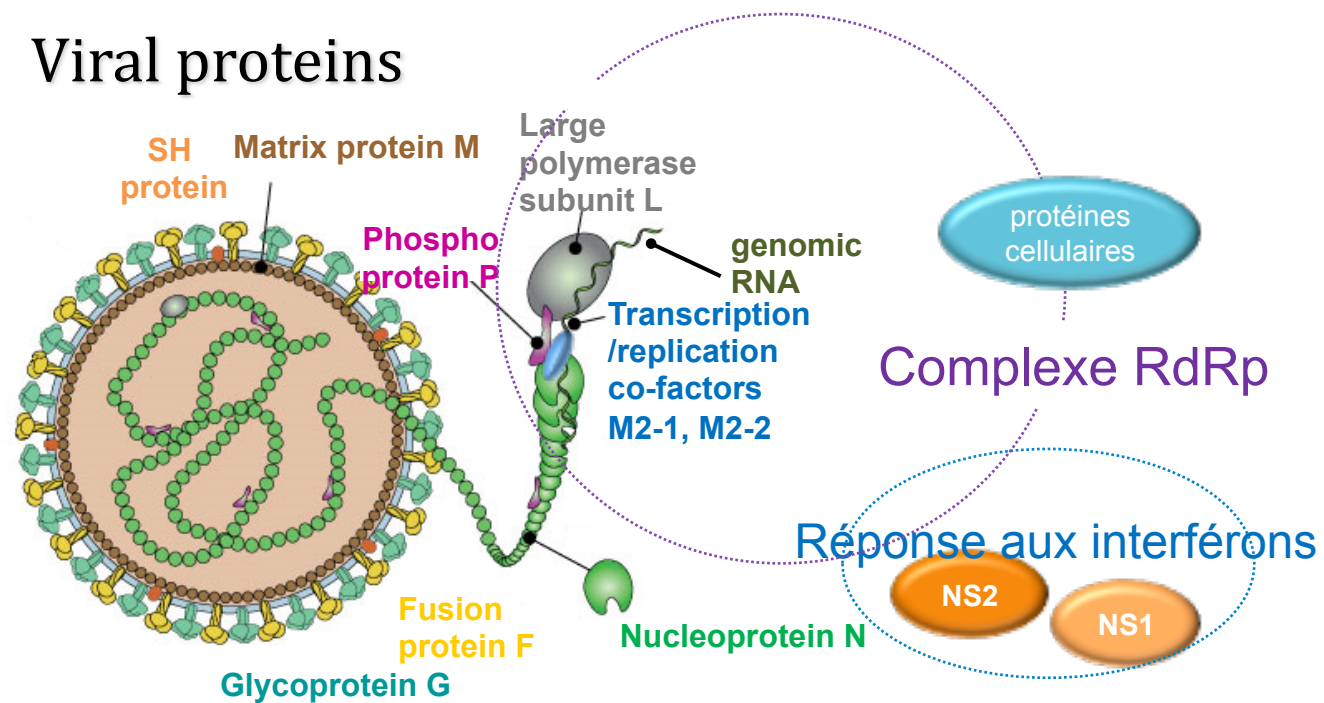


Figure 5. Ratio of the peak intensities in the spectra of free tau and PHF-tau. Only isolated peaks were taken into account.

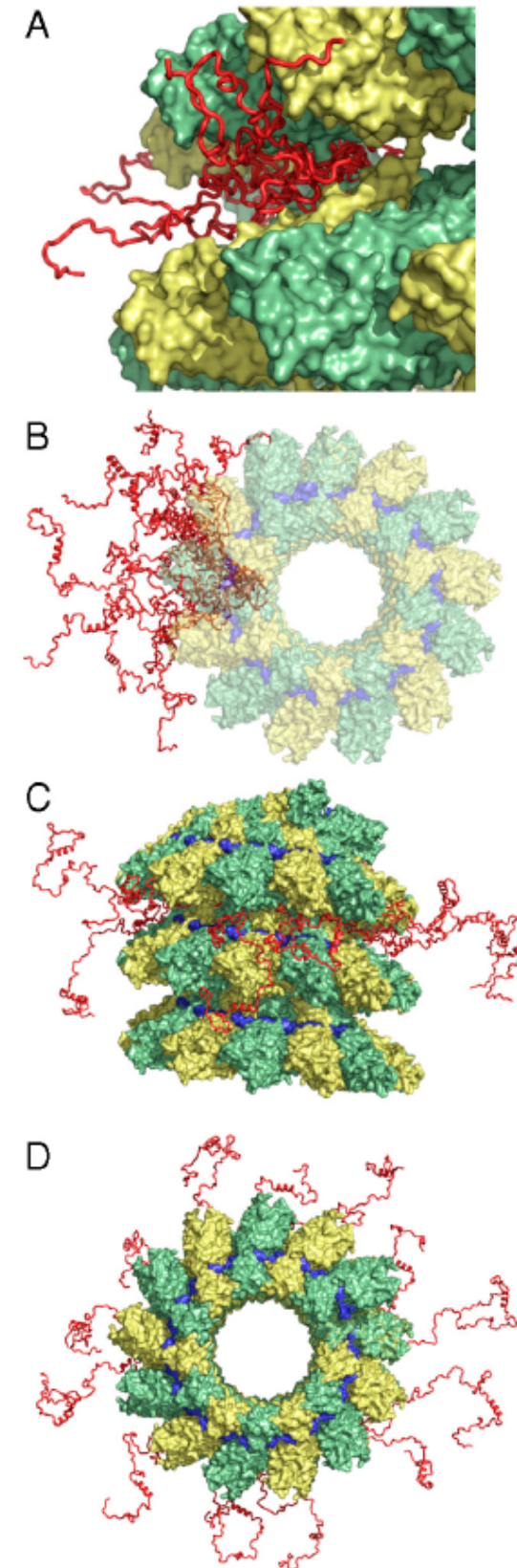
Alain Sillen et al., (2005) ChemBioChem, 6, 1849 – 1856, Regions of Tau Implicated in the Paired Helical Fragment Core as Defined by NMR.

Caractériser des fragments flexibles “libres” / fragments liés

Viral proteins



EM
NMR
SAXS



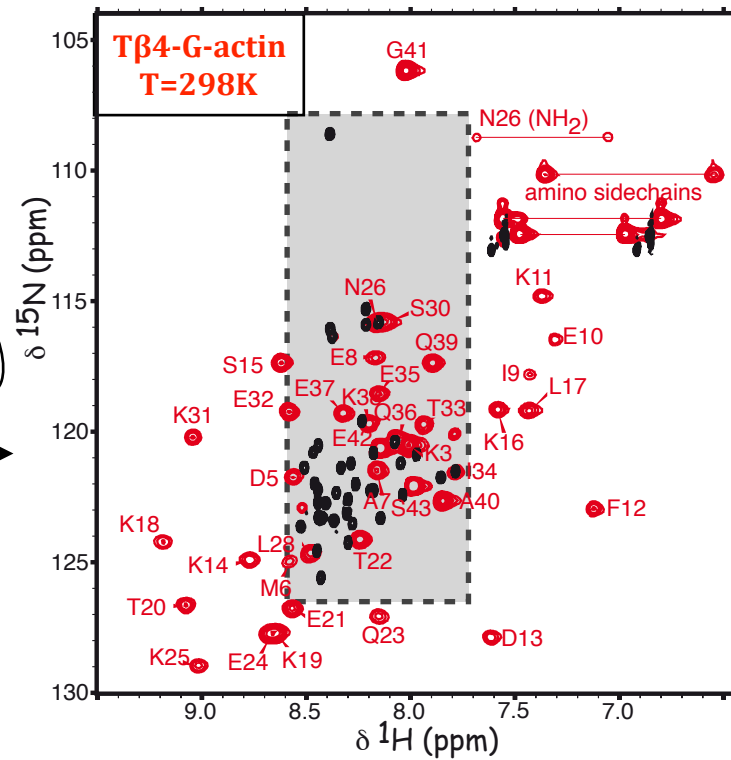
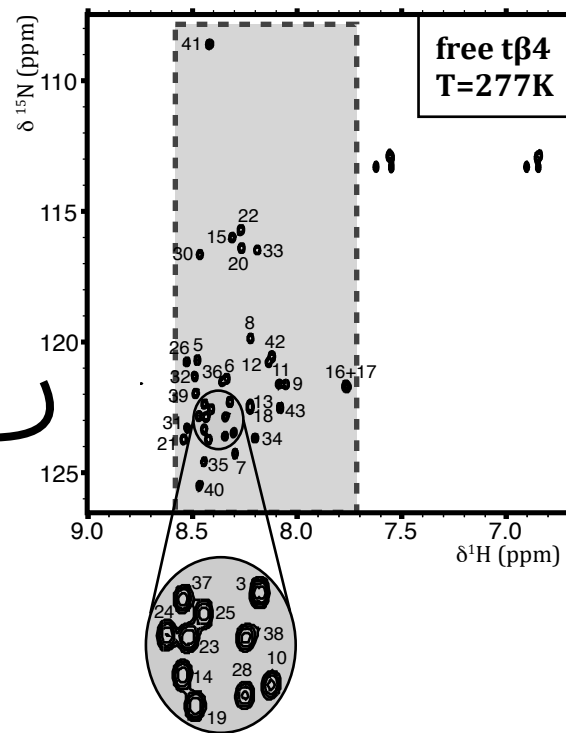
Jensen et al. PNAS 2011

Temps de vie du signal, relaxation transversale & perte de cohérence des spins Largeur de raie & apparence des spectres

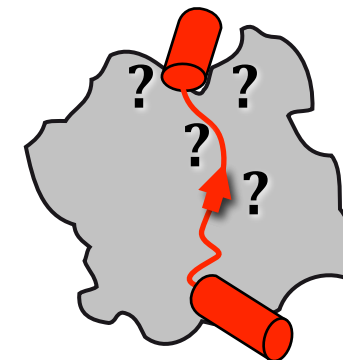
Caractériser des fragments flexibles “libres” / fragments liés

régulation de la polymérisation de l'actine par des domaines T β /WH2

^{15}N - ^1H HSQC SPECTRA

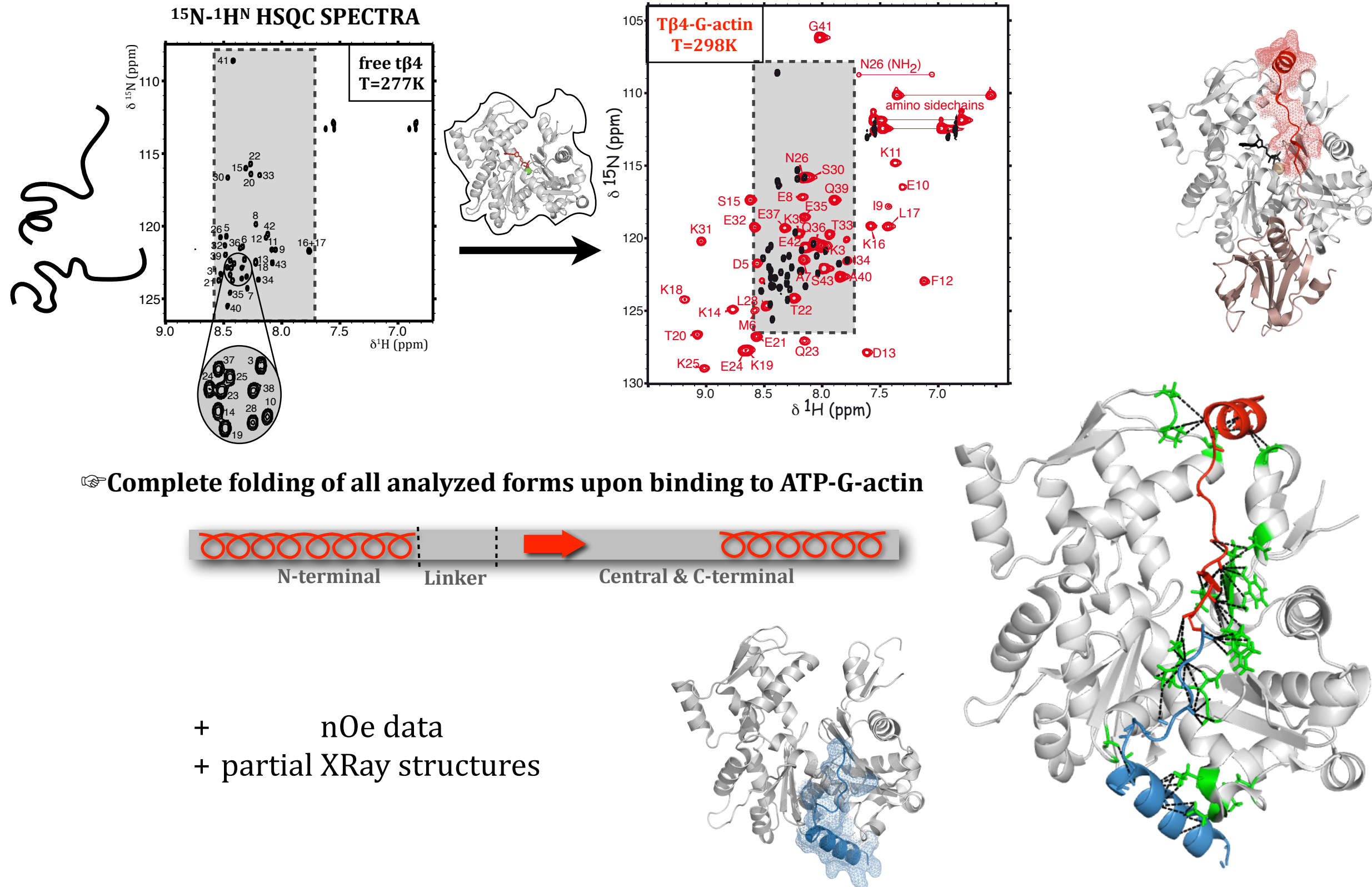


☞ Complete folding of all analyzed forms upon binding to ATP-G-actin



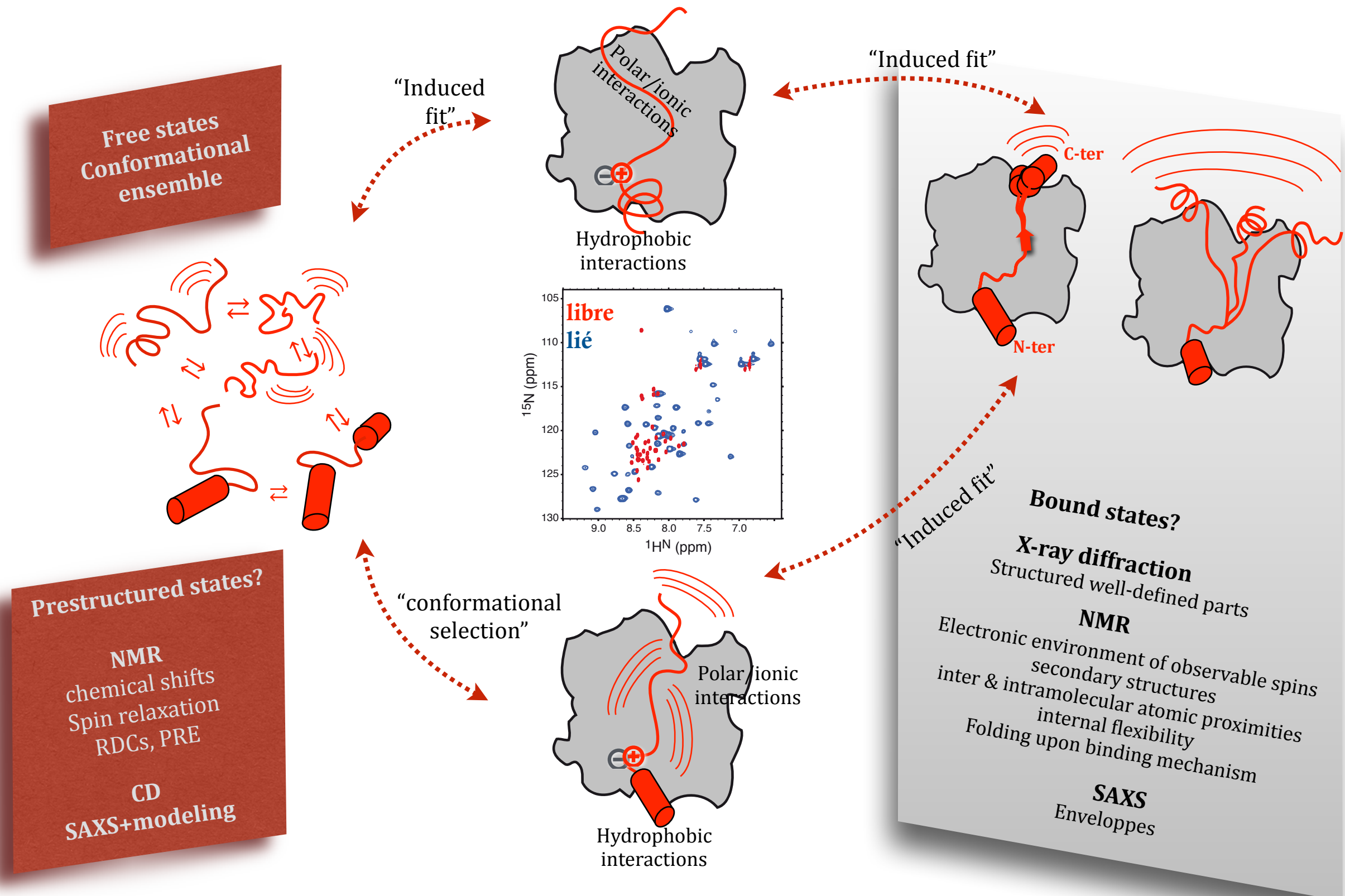
Caractériser des variations de dynamique au cours d'interactions

régulation de la polymérisation de l'actine par des domaines T β /WH2



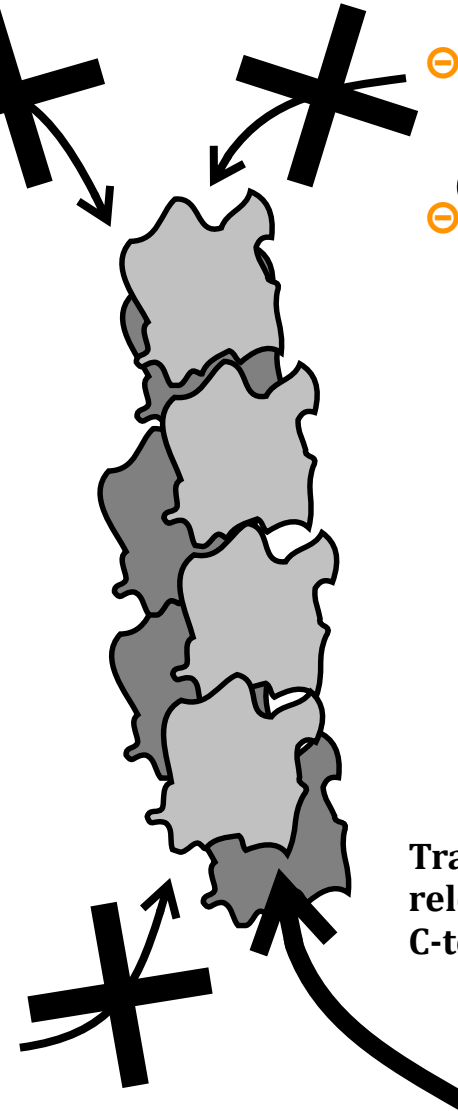
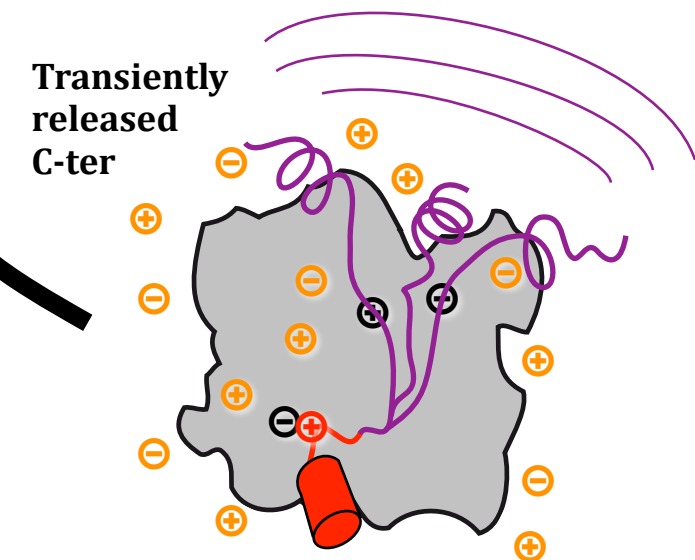
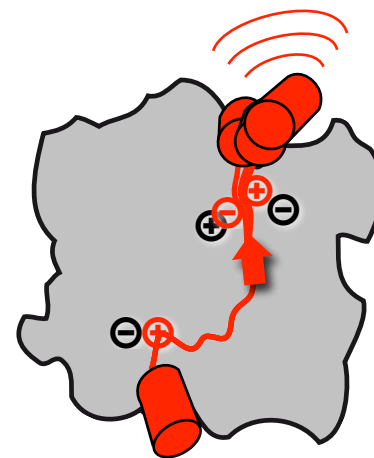
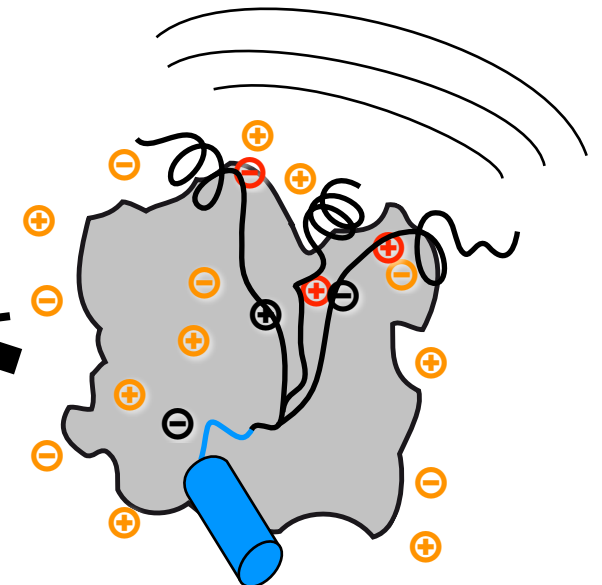
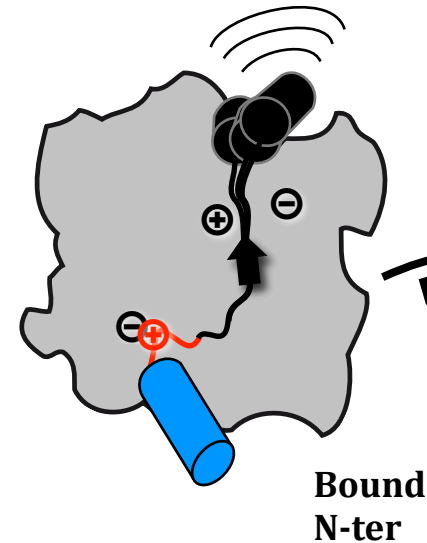
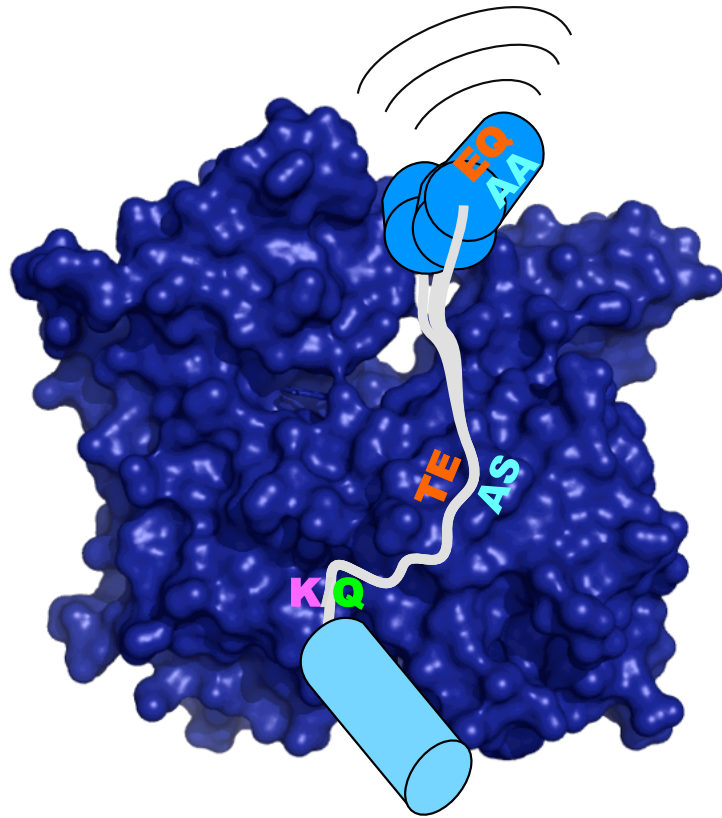
Caractériser des variations de dynamique au cours d'interactions

régulation de la polymérisation de l'actine par des domaines T β /WH2



Caractériser des variations de dynamique au cours d'interactions

régulation de la polymérisation de l'actine par des domaines T β /WH2



- ▶ Interactions électrostatique/polaires modulent la flexibilité locale dans l'état lié
- ▶ Interaction électrostatique clé: contrôle de la flexibilité et de l'ancrage du fragment désordonné sur sa cible
- ▶ Hélice amphipatique/interactions hydrophobes: contrôle de l'ancrage et modulation de l'affinité globale
- ▶ Modulation fine pour obtenir des affinités modérées et des fonctions variées, compatibles avec la dynamique du processus de polymérisation/dépolymérisation de l'actine

Un défi

Polymérisation de l'actine ($60\mu\text{M}$ @ 4°C)

Actine extraite du muscle de lapin

Grande flexibilité des domaines WH2

Flexibilité des états liés

Faible stabilité

Des évolutions majeures

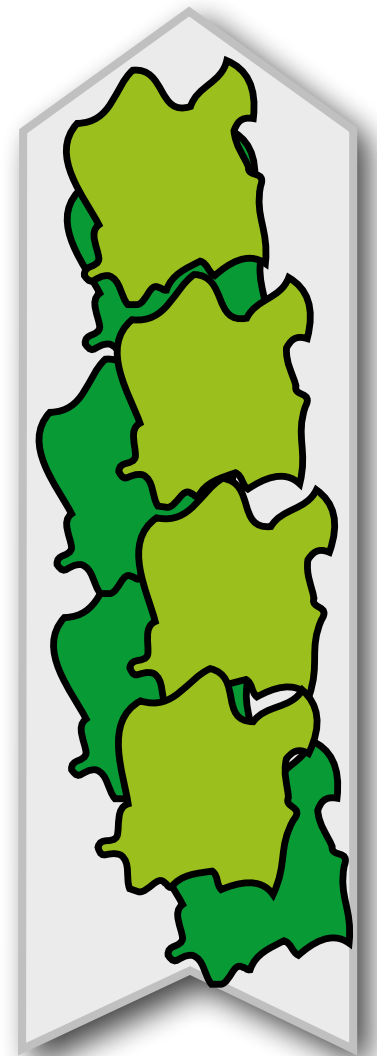
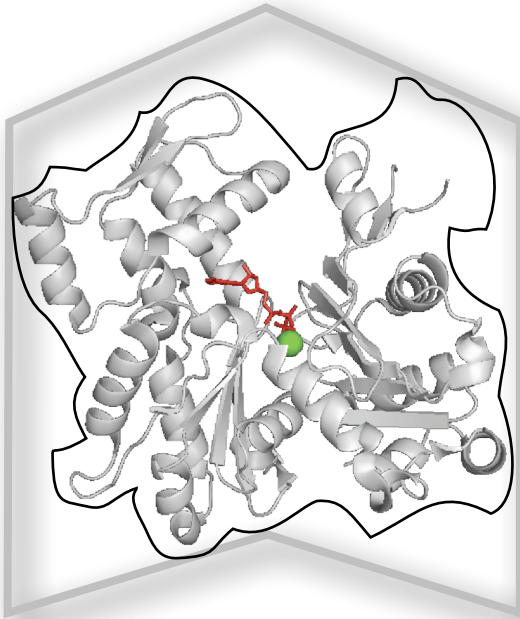
Spectromètres à très hauts champs

Cryosondes

Production d'actine recombinante

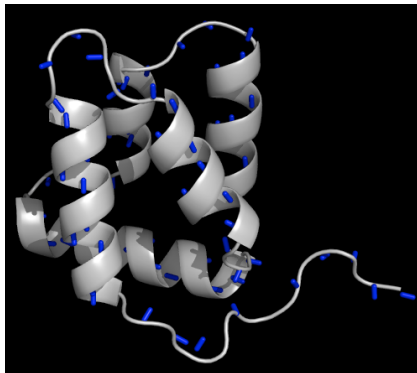
Association de plusieurs techniques

Diffraction EM, RX, RMN, SAXS



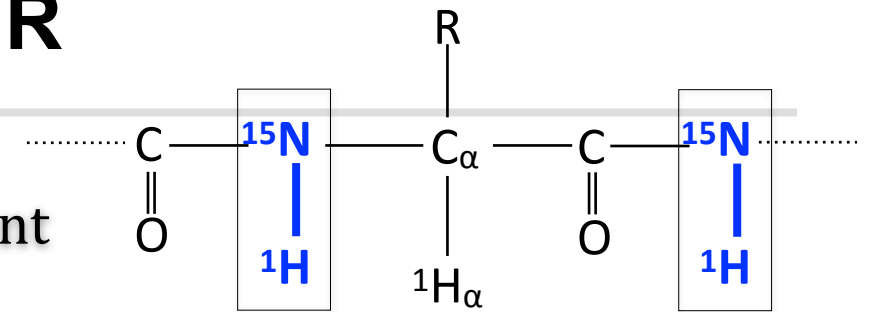
NMR

- **Chemical Shifts**
- **RDCs**
- **Relaxation rates**
- **Spin relaxation**
- **PRE**

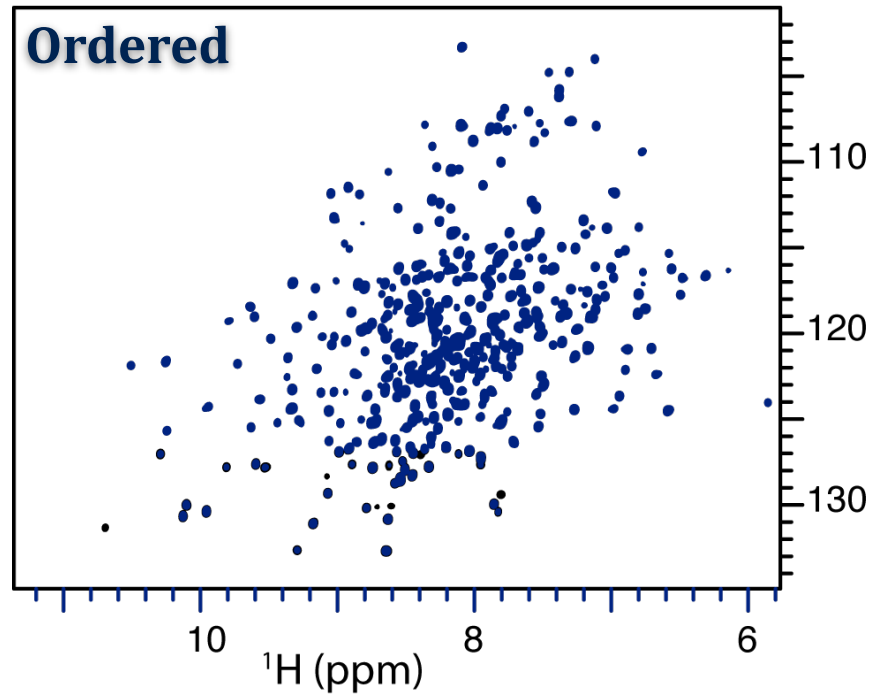


Disorder seen by NMR

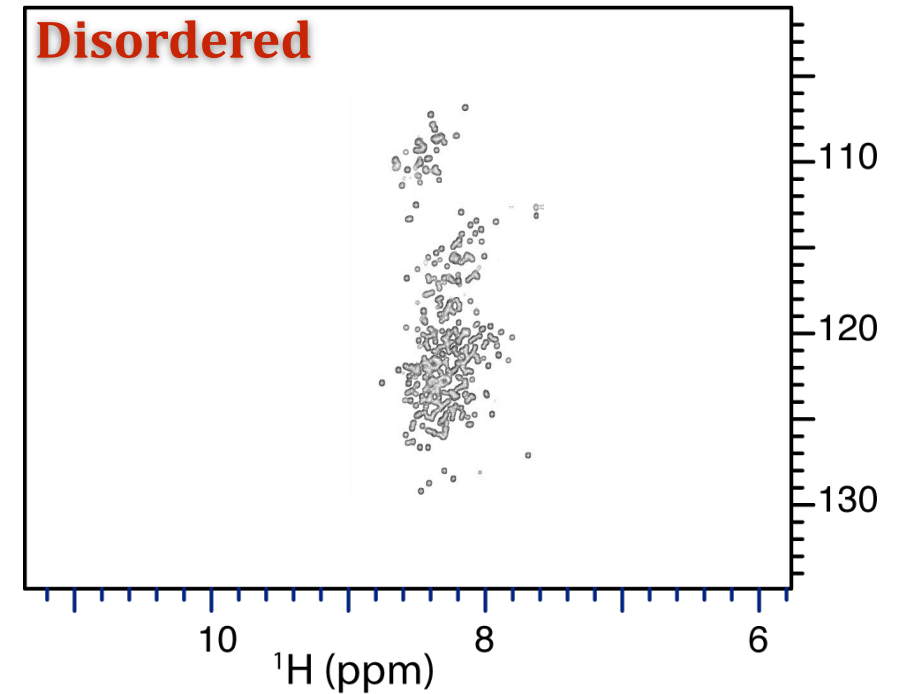
- HSQC spectra $\leftrightarrow$ electronic environment



Ordered

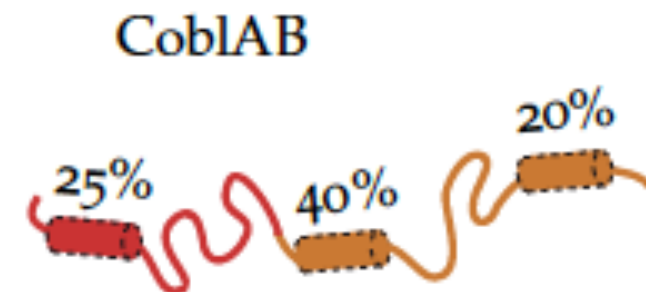
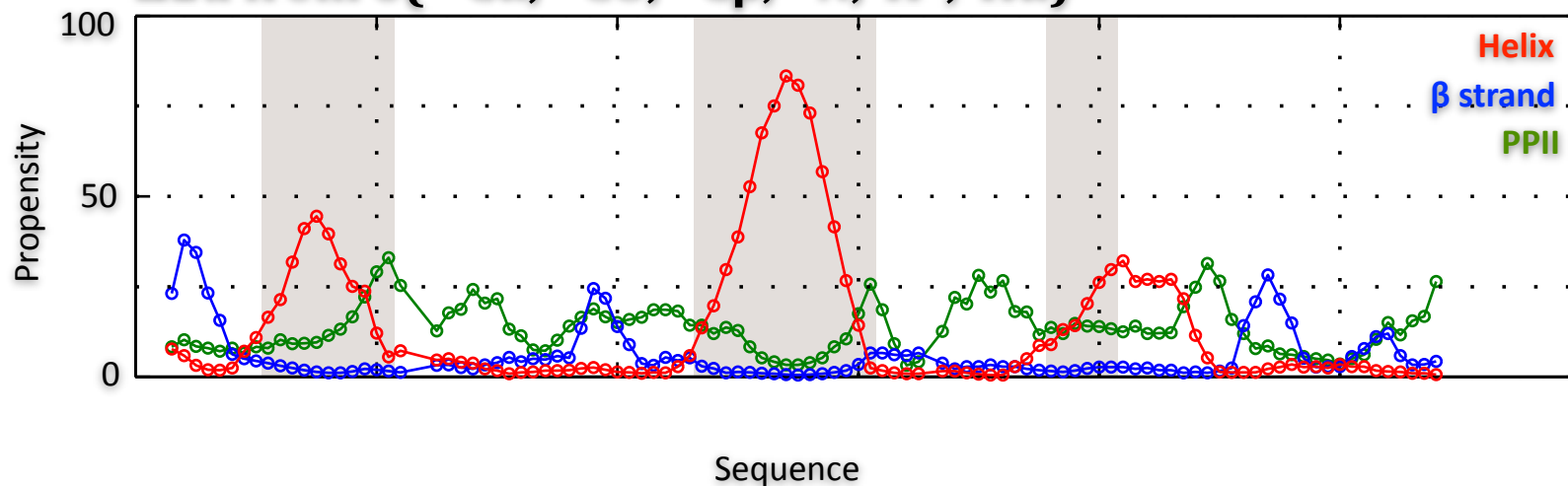


Disordered



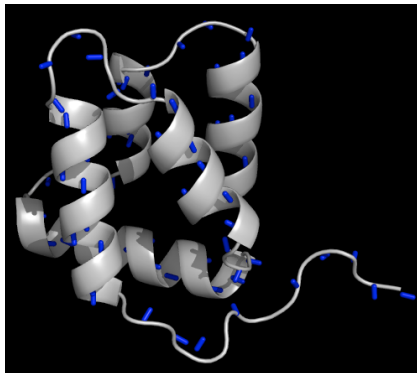
- Secondary shifts : $\delta(\text{measured}) - \delta(\text{random coil}) \leftrightarrow$ Secondary Structure Propensities

$\Delta 2d$ from $\delta(^{13}\text{C}\alpha, ^{13}\text{CO}, ^{13}\text{C}\beta, ^{15}\text{N}, ^1\text{H}^{\text{N}}, ^1\text{H}\alpha)$



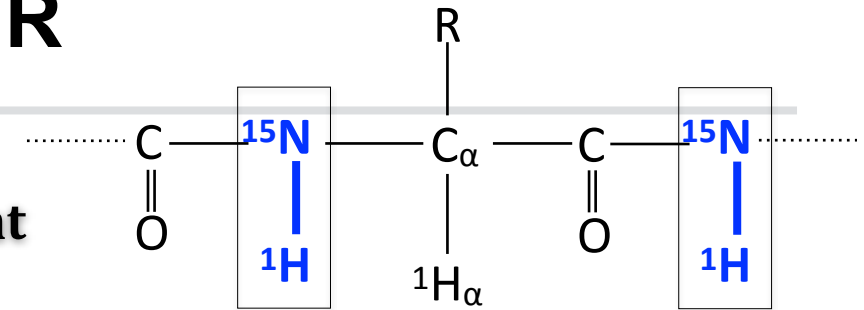
NMR

- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- PRE

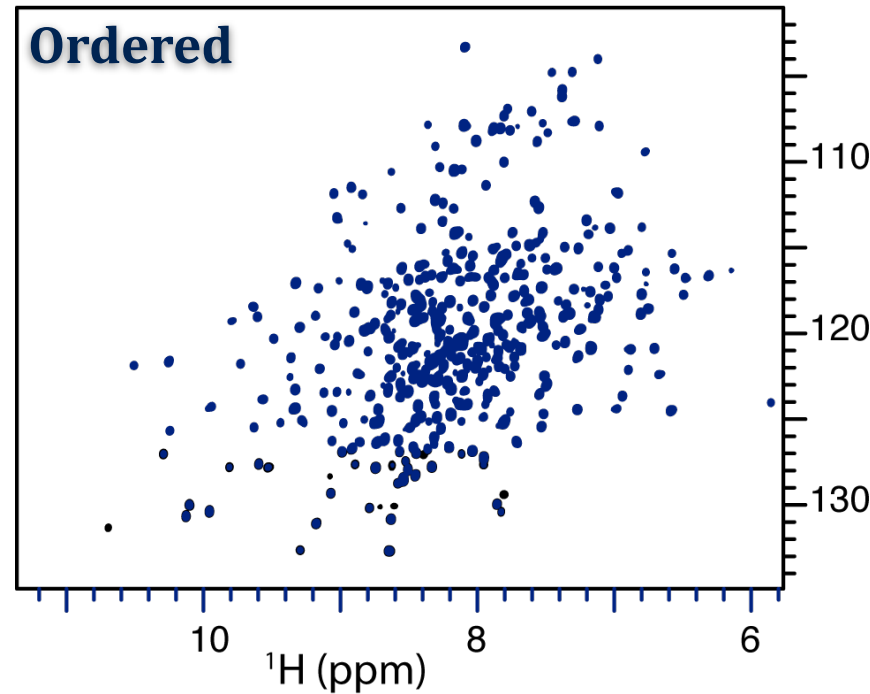


Disorder seen by NMR

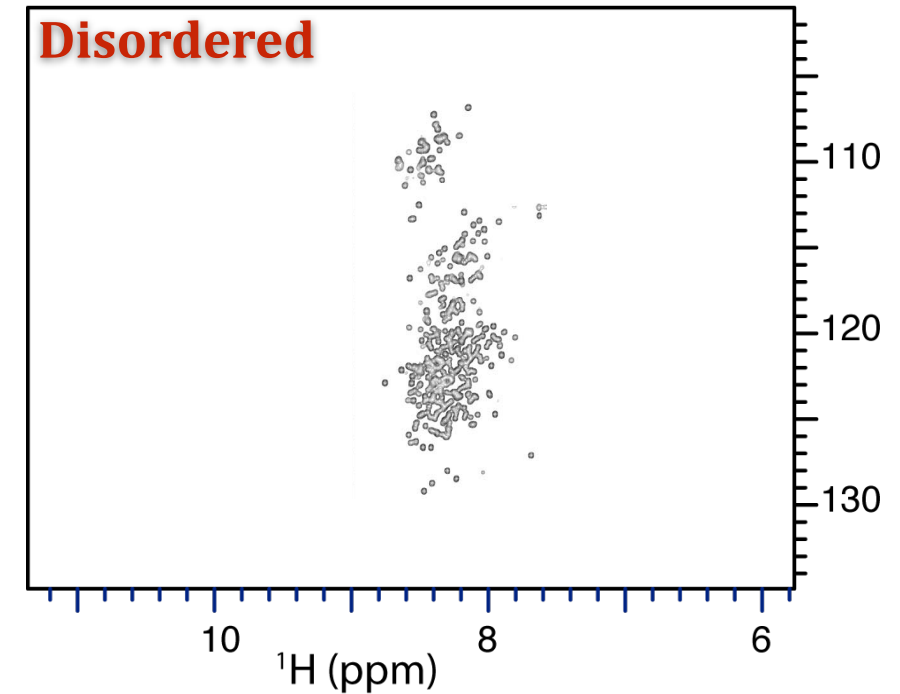
- HSQC spectra <-> electronic environment



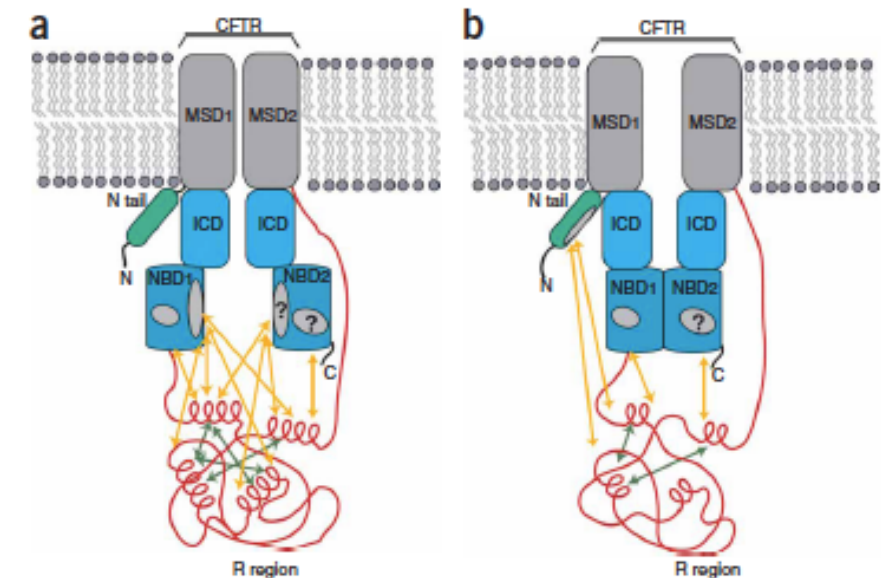
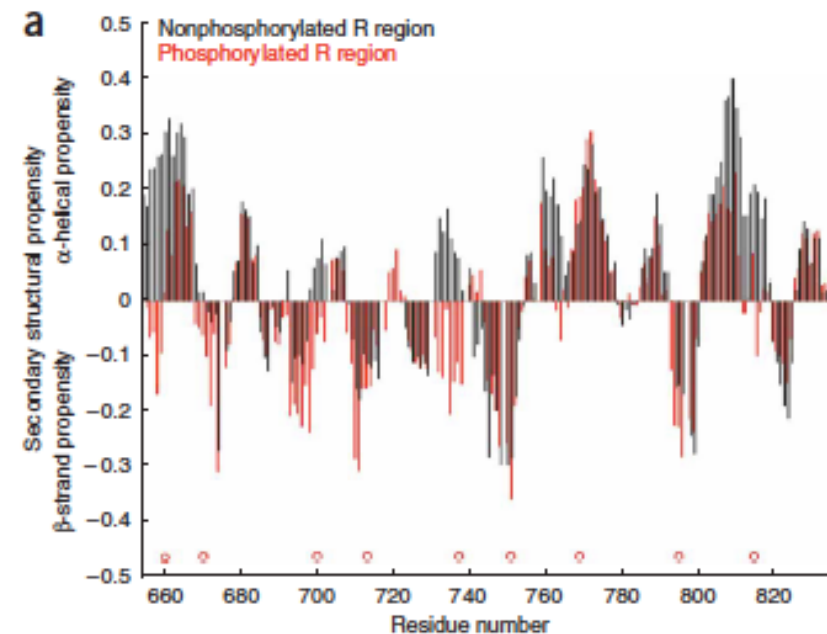
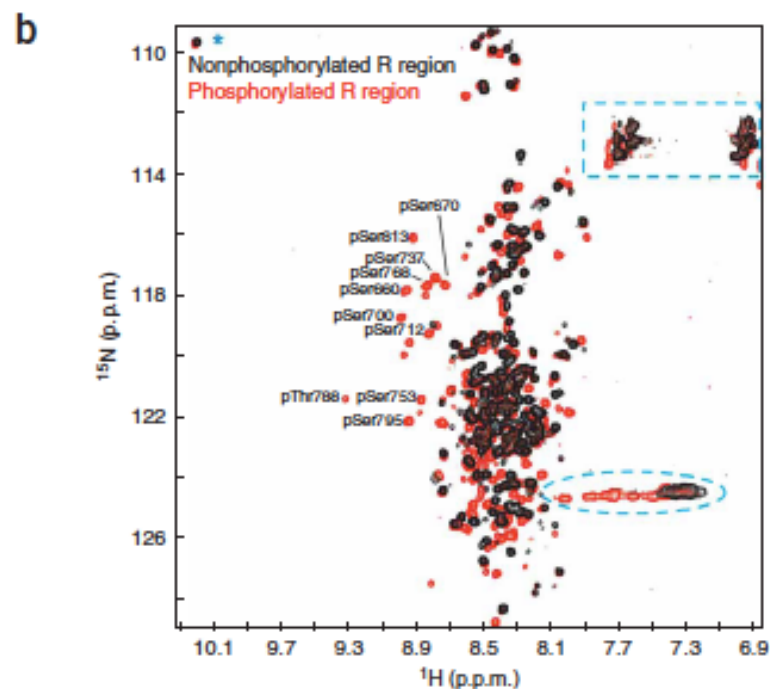
Ordered



Disordered



- Secondary shifts <-> Secondary Structure Propensities



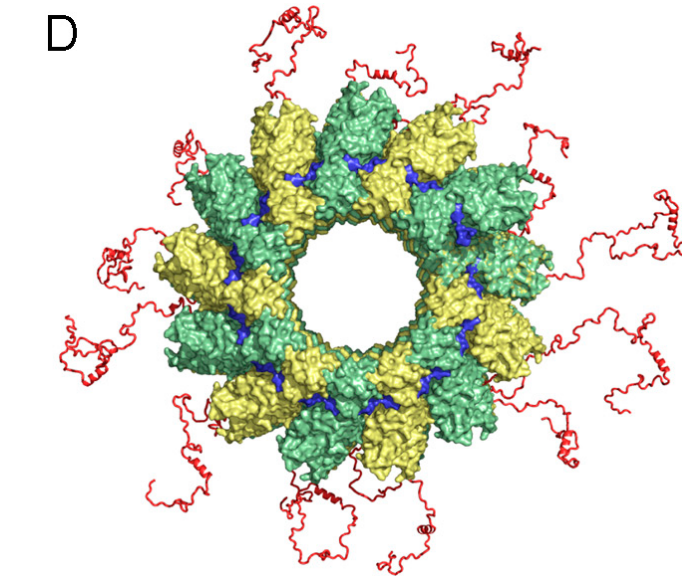
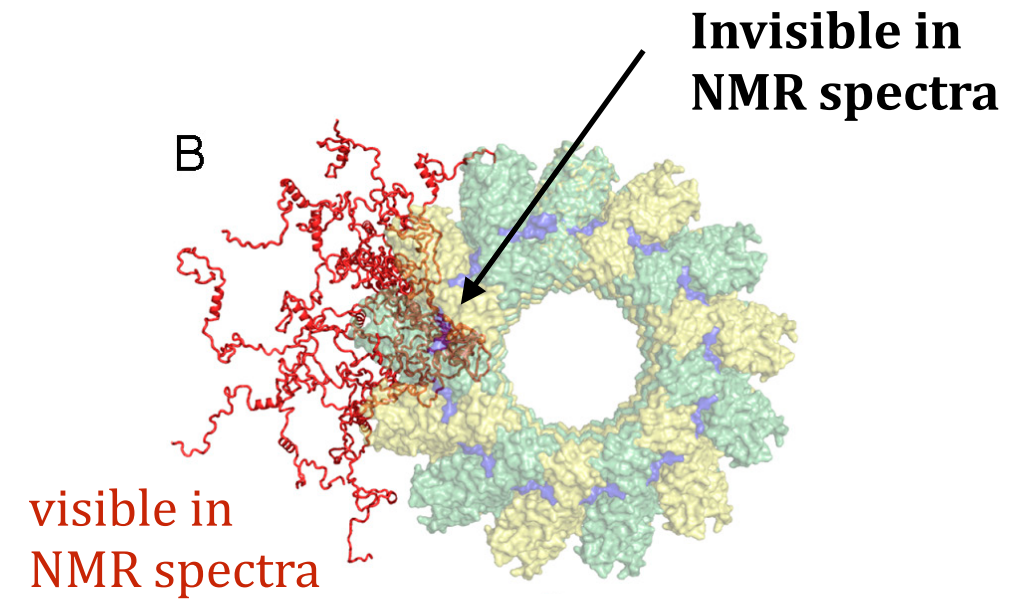
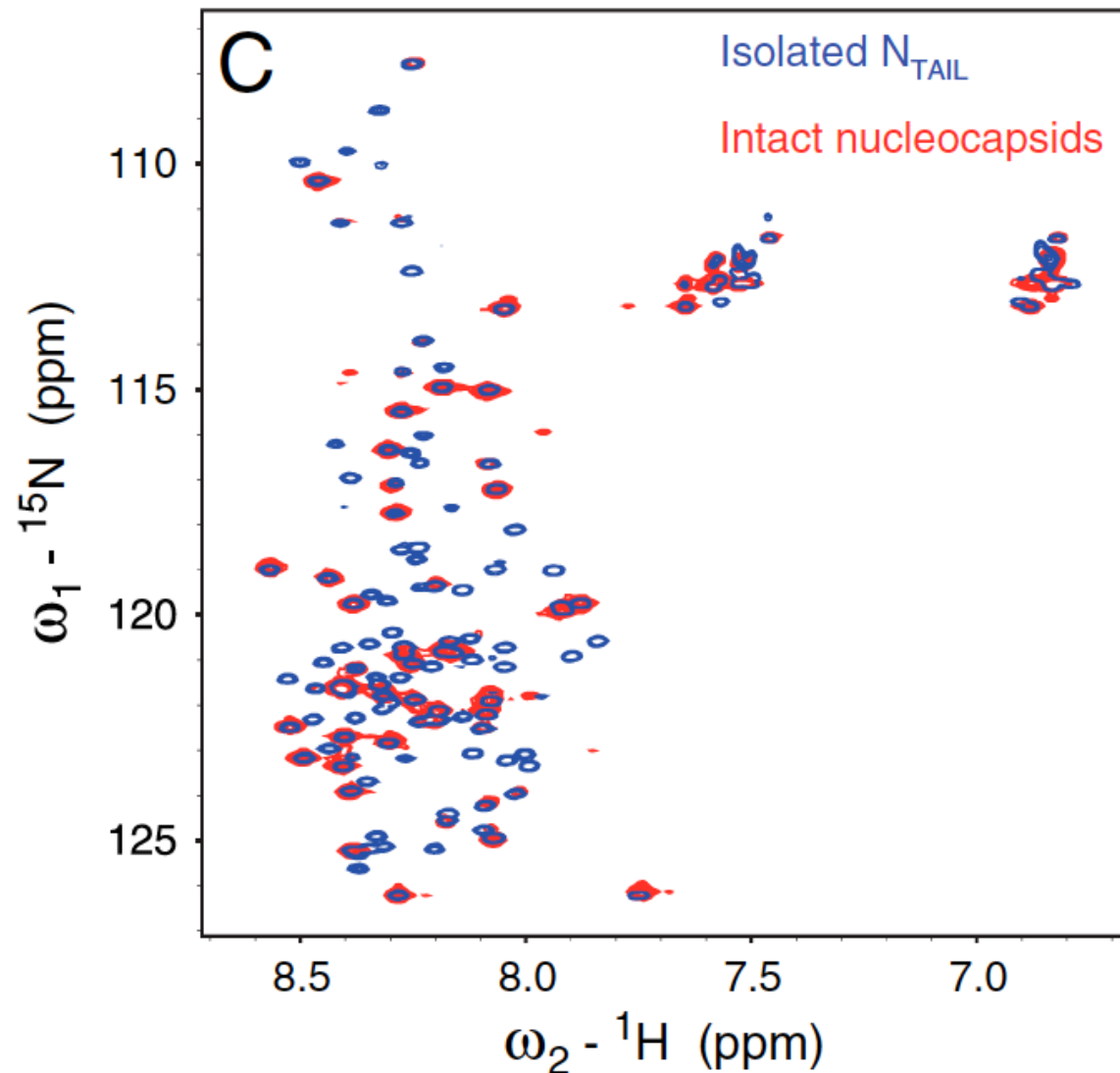
Baker et al 2007 Nat. Struct. Mol. Biol. 14(8), 738

NMR

- Chemical Shifts
- RDCs
- **Relaxation rates**
- Spin relaxation
- PRE

Disorder seen by NMR

- Relaxation rates $\leftrightarrow$ line widths



- **Combined NMR, EM**

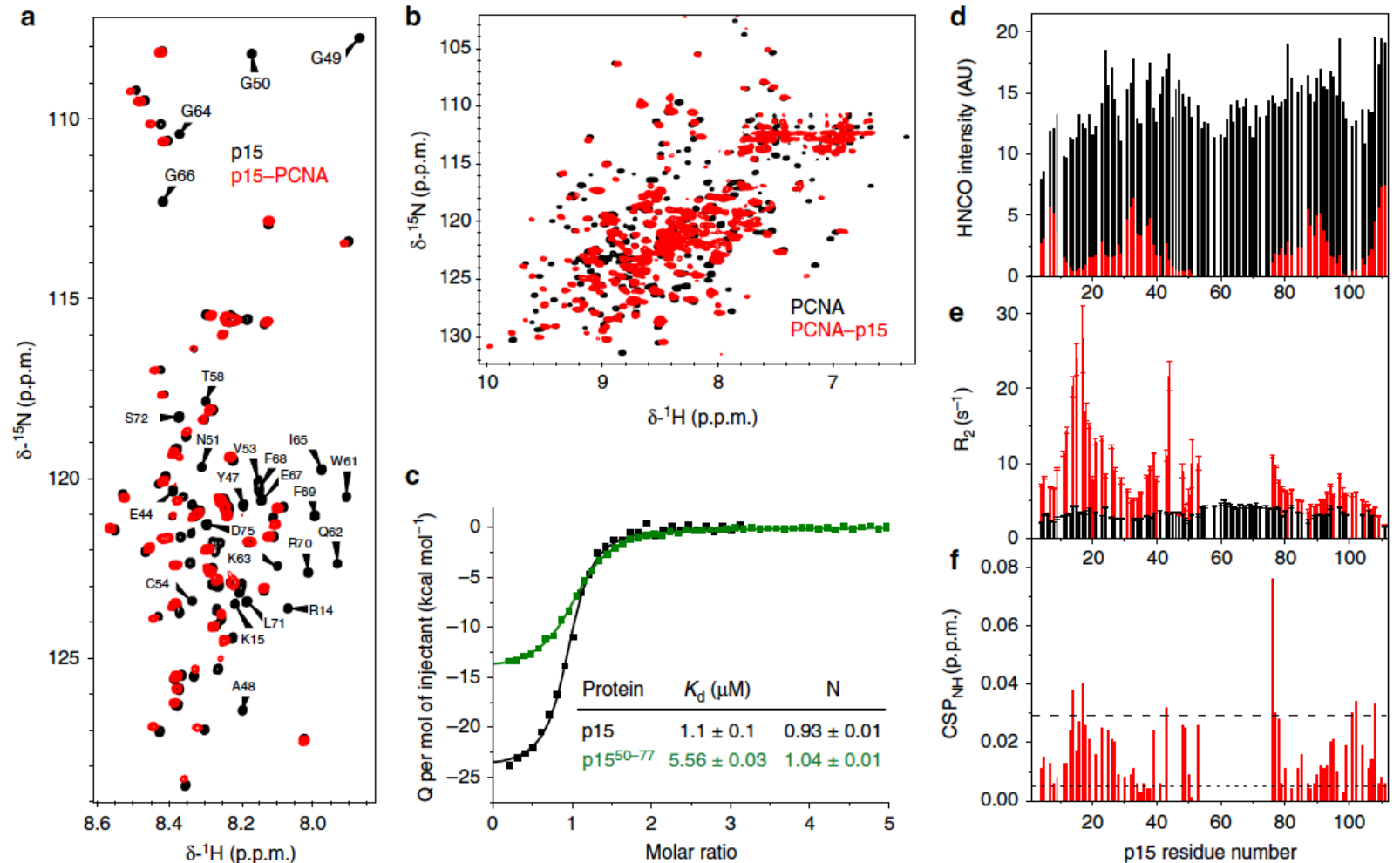
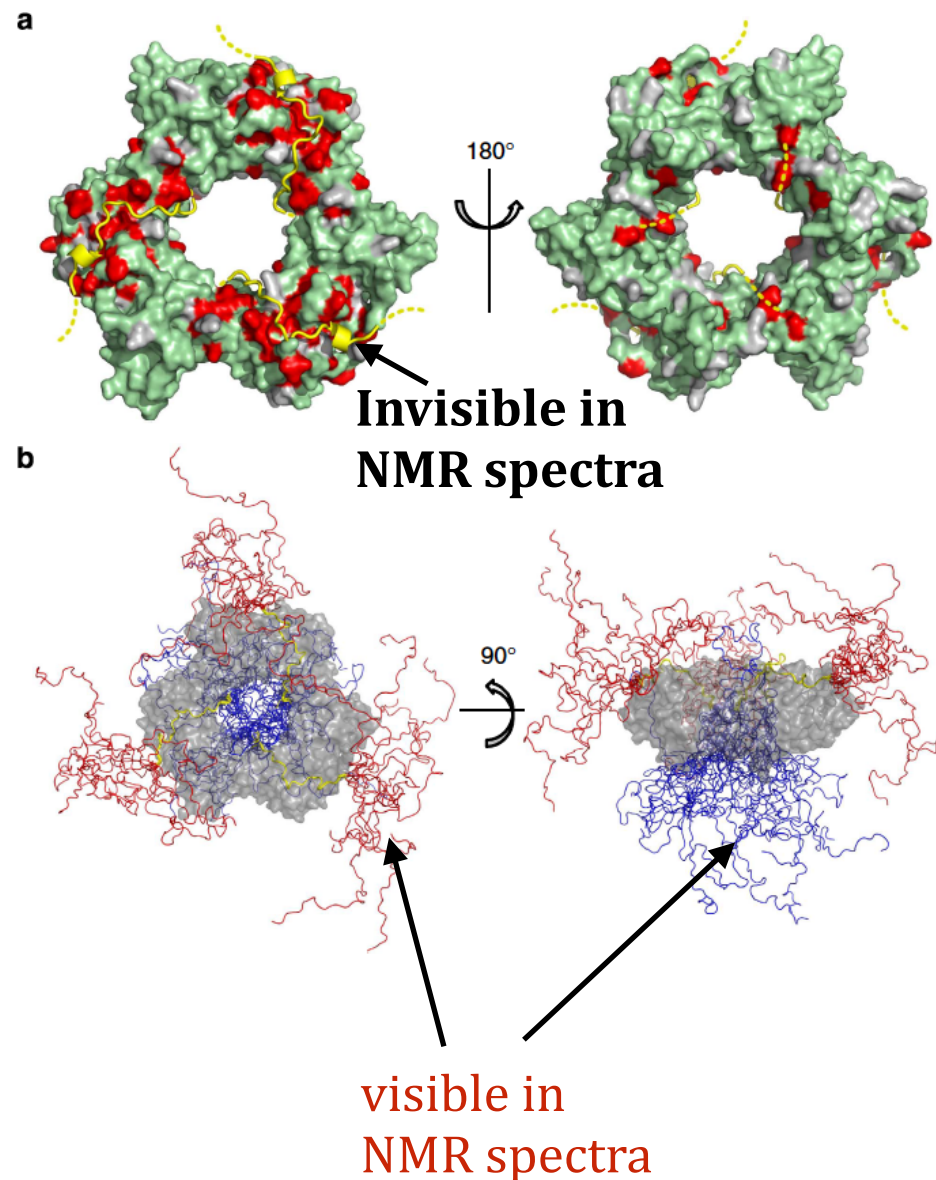
Jensen, M.R et al.,(2011) PNAS 108,24

NMR

- Chemical Shifts
- RDCs
- **Relaxation rates**
- Spin relaxation
- PRE

Disorder seen by NMR

- Relaxation rates $\leftrightarrow$ line widths

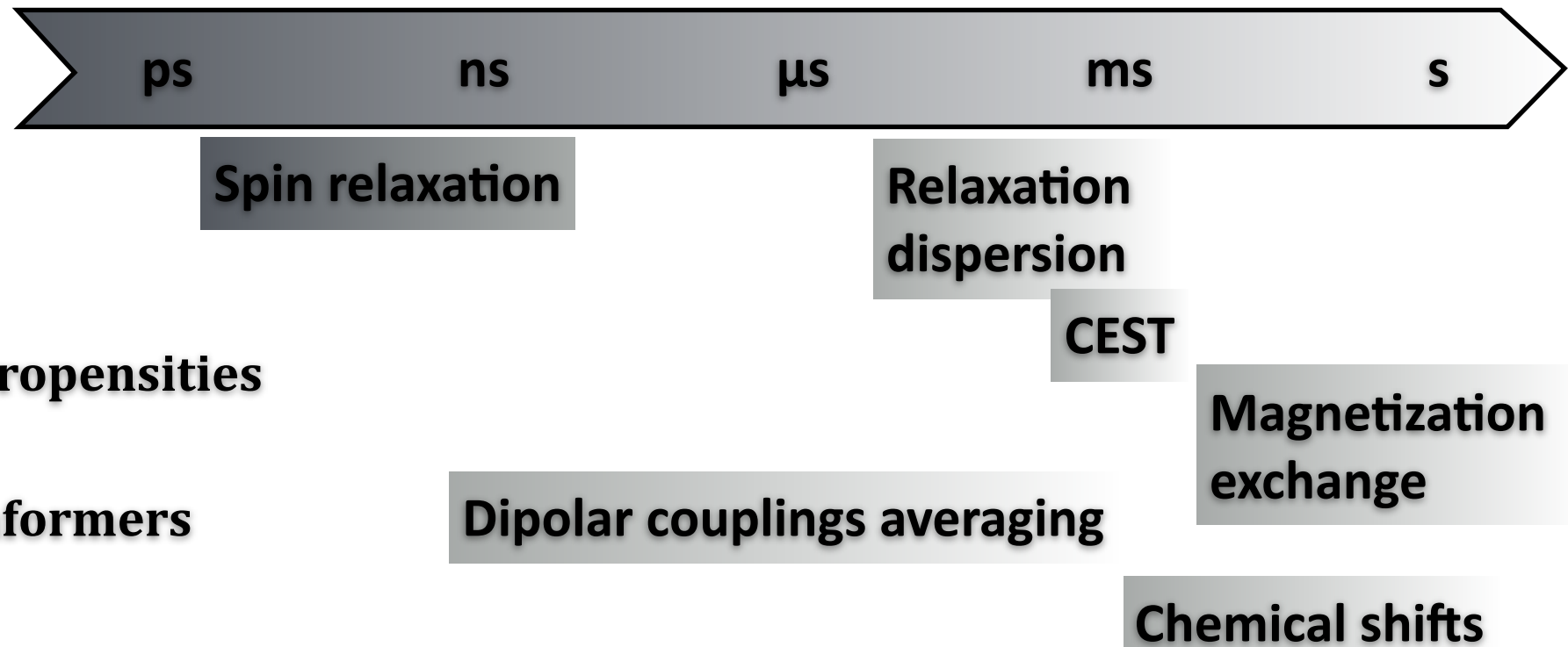


- Combined NMR, XRay, EM, ITC

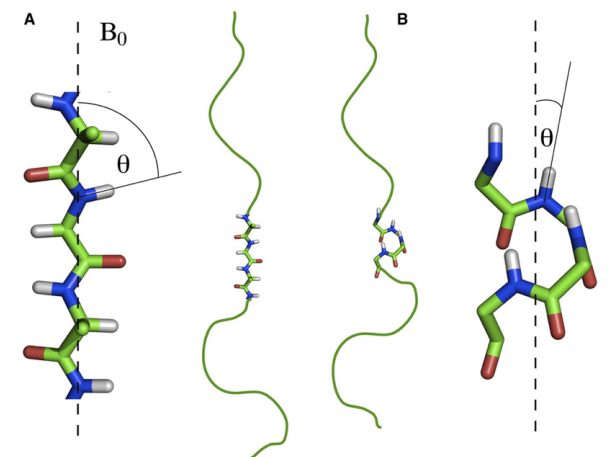
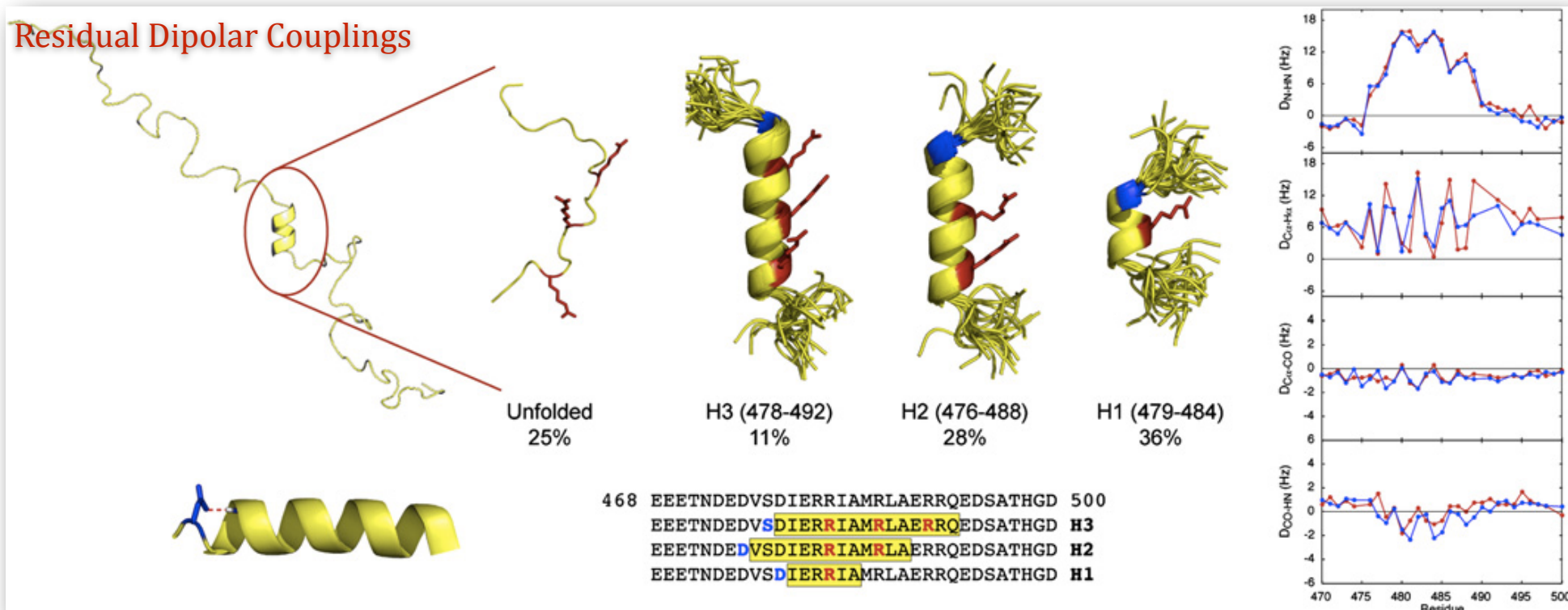
NMR

- Chemical Shifts
- **RDCs**
- **Relaxation rates**
- **Spin relaxation**
- PRE

Disorder seen by NMR



- Secondary Structure Propensities
- Restriction of motion
- Exchange between conformers
- ➔ Different timescales



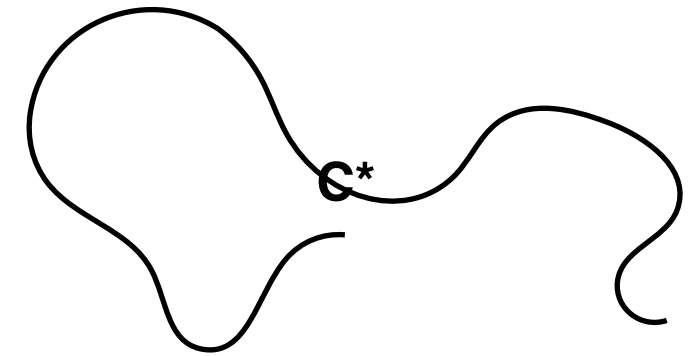
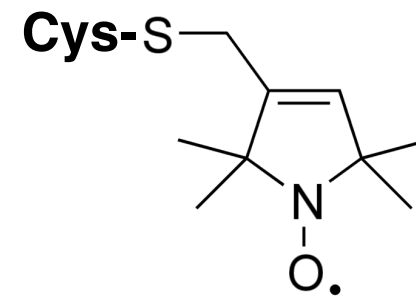
Jensen, M.R et al.,(2008)
 J. Am. Chem. Soc. 130 ; (2009) structure 17.

NMR

- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- **PRE**

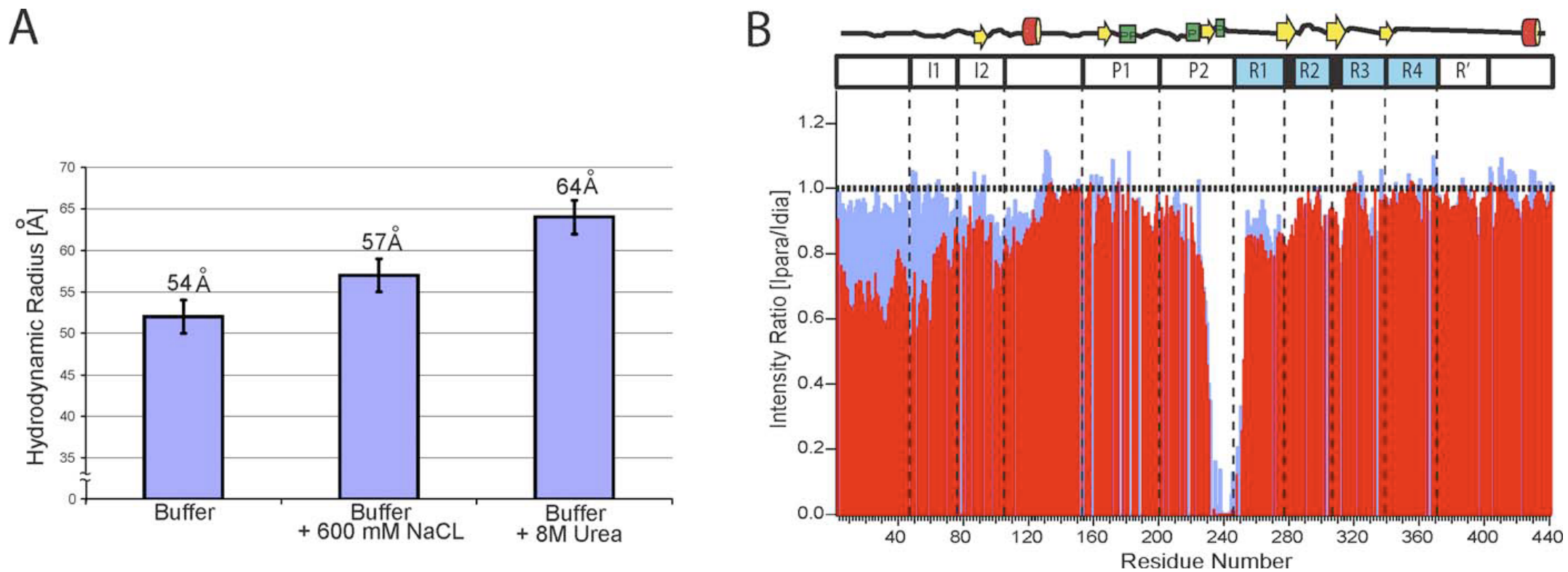
Disorder seen by NMR

➔ Long range contacts



Paramagnetic Relaxation Enhancement

➔ contact => intensity decrease



Influence of Ionic Strength and Urea on Intramolecular Long-Range Interactions

(A) Hydrodynamic radius values of htau40 in buffer (99.9 % D₂O, 50 mM phosphate buffer [pH 6.9]), upon addition of 600 mM NaCl and in the presence of 8 M urea.

(B) PRE profiles of amide protons in spin-labelled htau40 in buffer (red bars) and in the presence of 600 mM NaCl (blue bars). The single-cysteine mutant A239C was labelled with MTSL. Intensity ratios were averaged over a three-residue window.

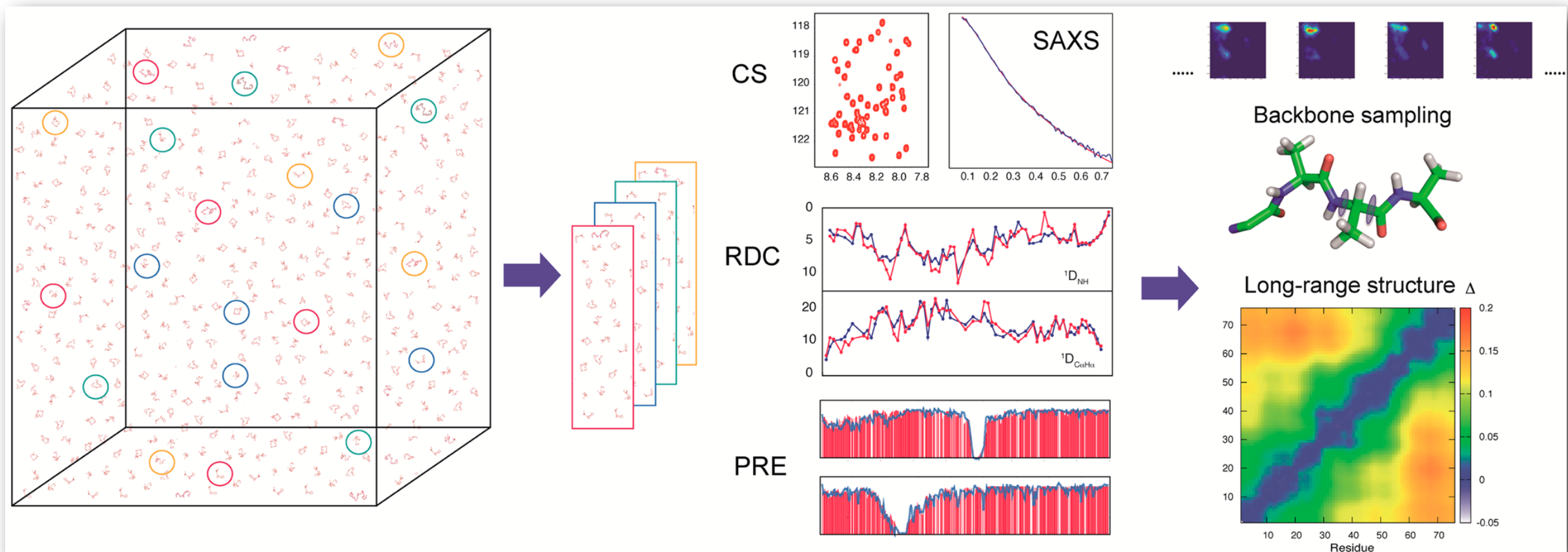
Structural Polymorphism of 441-Residue Tau at Single Residue Resolution.
Mukrasch et al., PloS Biol. 2009, Vol 7, Issue 2

NMR

- Chemical Shifts
- RDCs
- Relaxation rates
- Spin relaxation
- PRE

Disorder seen by NMR

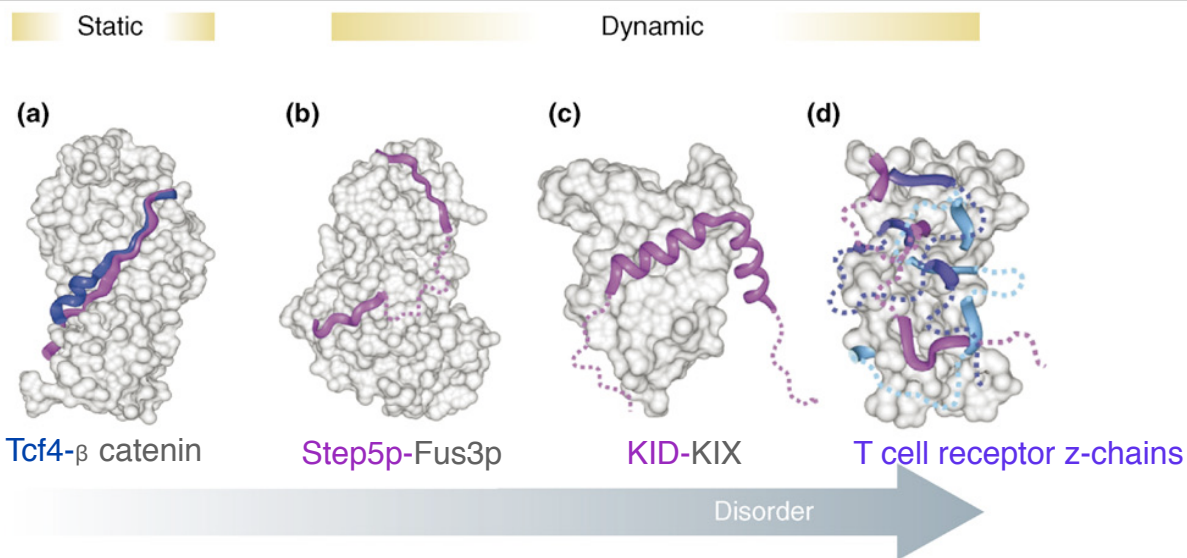
- ✓ Combination of NMR methods
 - ➔ Disordered segments
 - ➔ Secondary structure propensities
 - ➔ Timescales of conformers exchange
 - ➔ Long range contacts
- ✓ Combination of techniques
 - ➔ Back-Calculation of parameters
 - ➔ Modeling of conformers ensembles



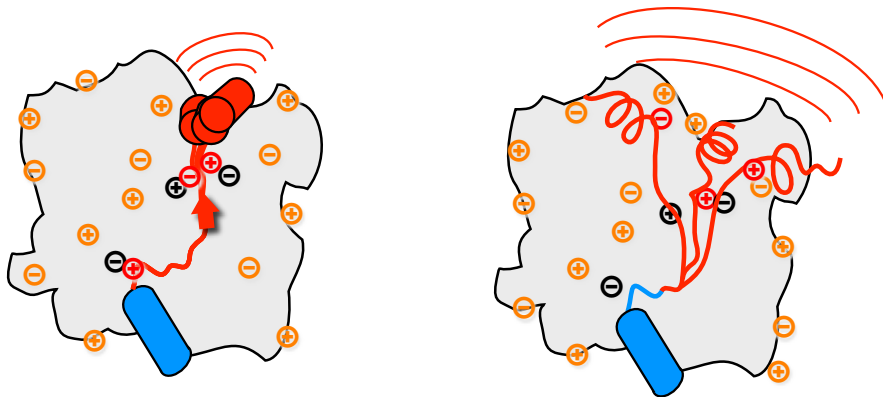
Jensen, Zweckstetter, Huang & Blackledge, Chemical reviews 2014, special issues on IDPs
Exploring Free-Energy Landscapes of Intrinsically Disordered Proteins at Atomic Resolution Using NMR Spectroscopy

Caractériser des variations de dynamique au cours d'interactions et au sein de complexes

polymorphism & structural disorder in protein-protein interactions involving IDPs



Tompa & Fuxreiter, TIBS 2007



Deville et al., FEBS Letters 590 (2016) 3690-99

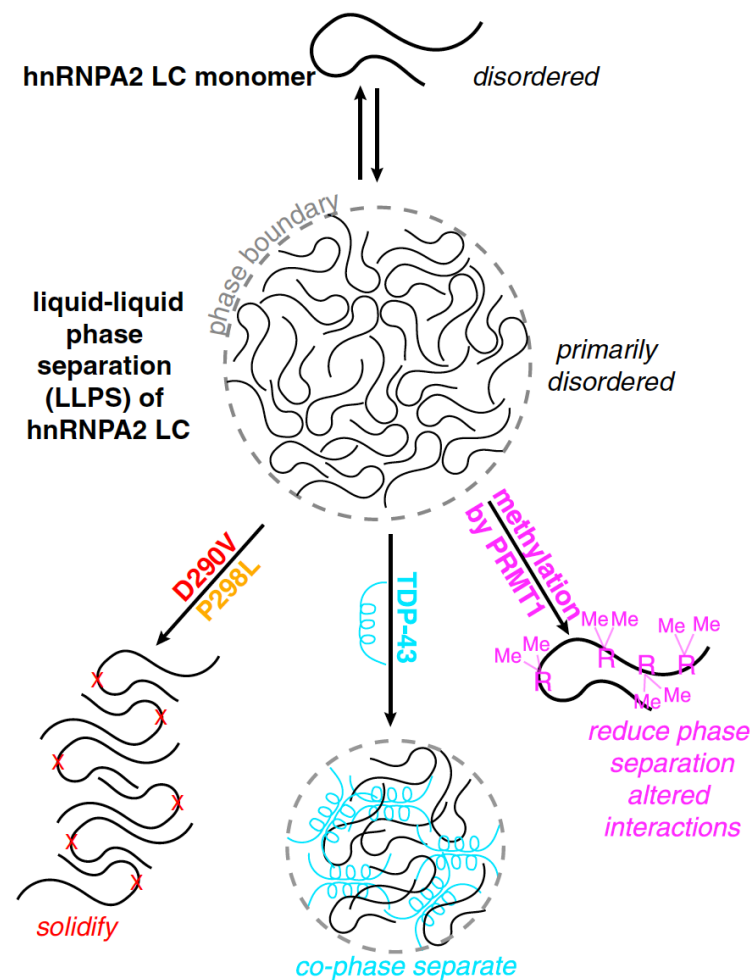
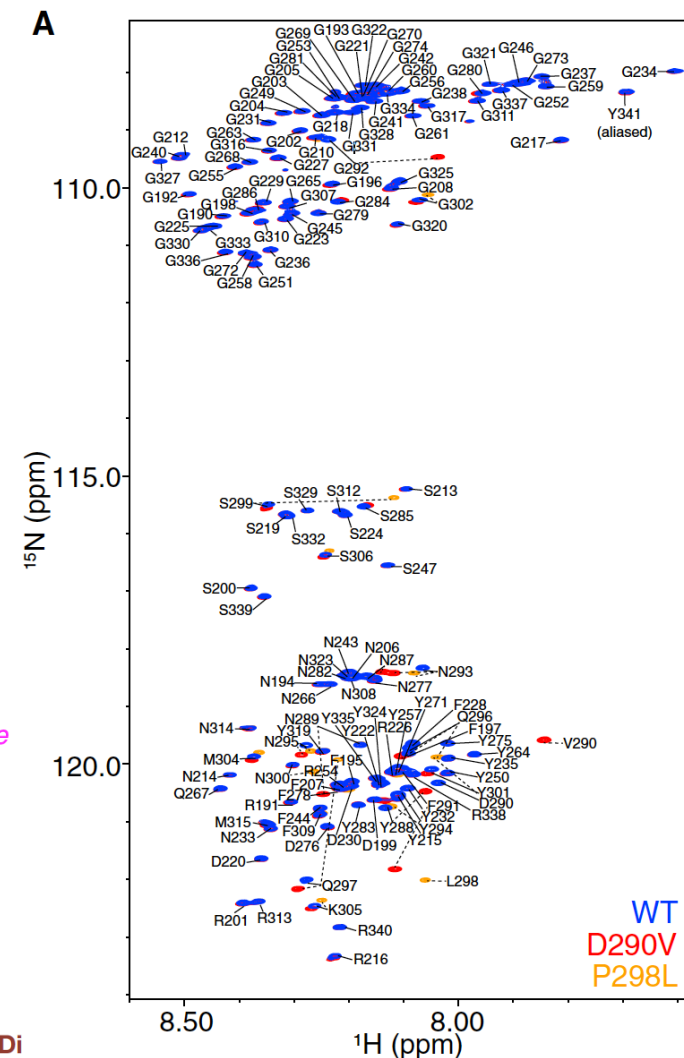


Figure 7. Model: hnRNPA2 Phase Separation Is Modulated by Disease Mutations, TDP-43 Interaction, and Posttranslational Modifications

Ryan et al., Mechanistic View of hnRNPA2 Low-Complexity Domain Structure, Interactions, and Phase Separation Altered by Mutation and Arginine Methylation, Molecular Cell (2018)



Caractériser des variations de dynamique au cours d'interactions et au sein de complexes

polymorphism & structural disorder in protein-protein interactions involving IDPs

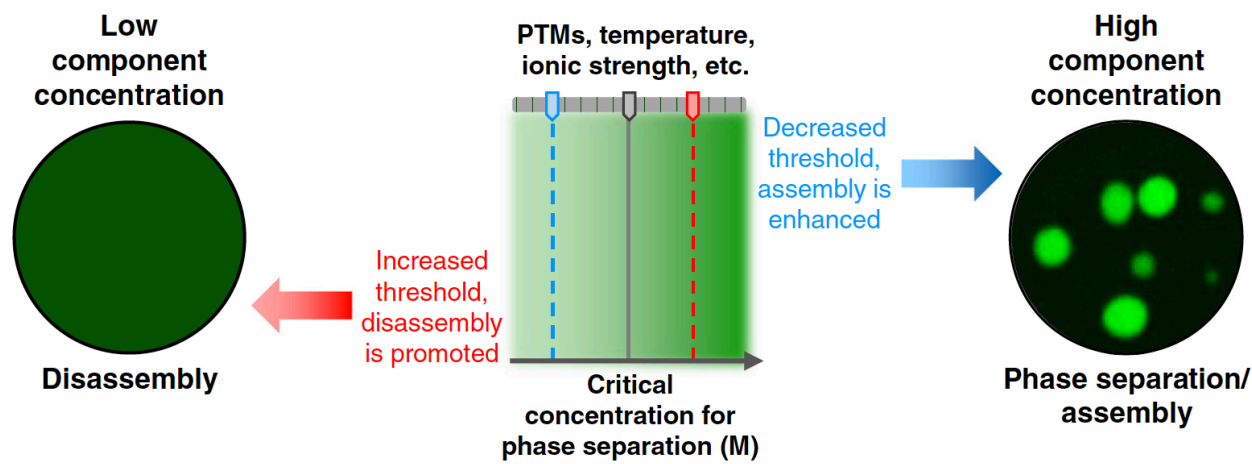


Fig. 1 Macromolecular condensation mediates the formation of membrane-less organelles. Membrane-less organelles are dynamic structures formed via a polymer-condensation-like, concentration-dependent phase separation mechanism. The critical concentration threshold (*grey line*) for phase separation can be tuned within a range of concentrations (*shaded green box*) through physico-chemical alterations to the system (*i.e.*, posttranslational modifications to domains and/or motifs that alter the affinity of their interactions, changes in temperature, altered ionic strength, etc.). These changes can drive phase separation and assembly of membrane-less organelles, or their disassembly

Mitrea and Kriwacki, Cell Communication and Signaling 2016; 14:1

Phase separation in biology; functional organization of a higher order

Burke KA, Janke AM, Rhine CL, Fawzi NL. Molecular Cell 2015; 60, 231–41.

Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II.

Ryan et al., Molecular Cell 2018; 69, 1–15

Mechanistic View of hnRNPA2 Low-Complexity Domain Structure, Interactions, and Phase Separation Altered by Mutation and Arginine Methylation

Brady et al. Proc. Natl. Acad. Sci. USA 2017; 114, E8194–E8203.

Structural and hydrodynamic properties of an intrinsically disordered region of a germ cell-specific protein on phase separation.

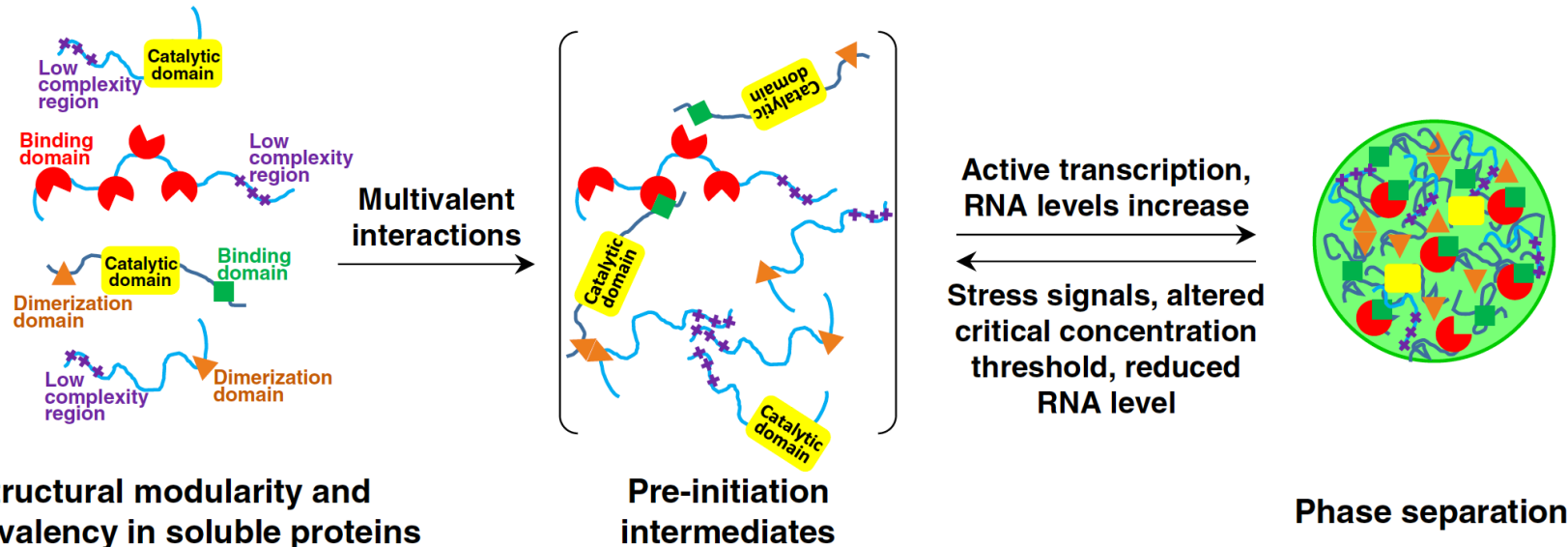
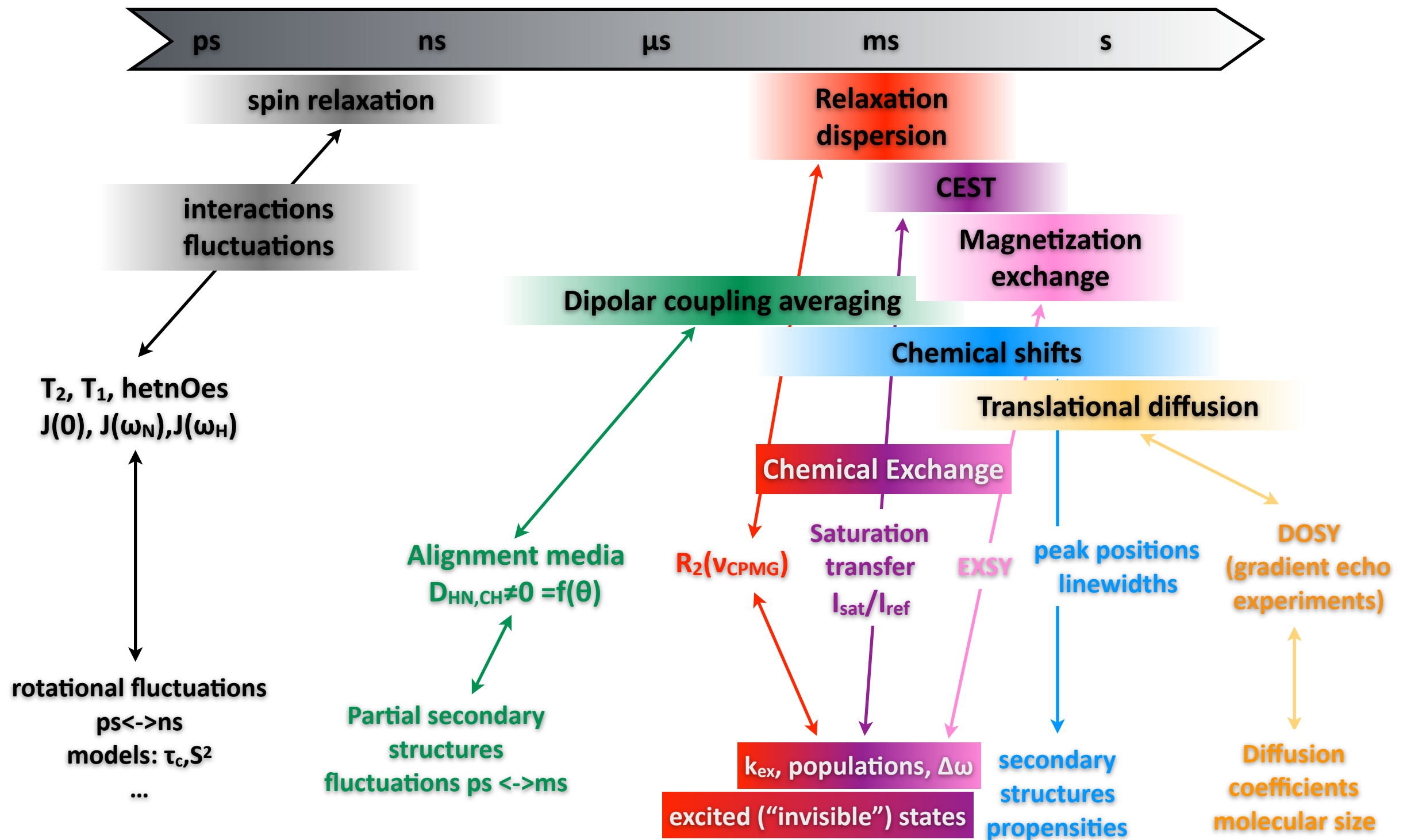


Fig. 2 Molecular basis for membrane-less organelles assembly. The proteins enriched within the matrices of membrane-less organelles commonly exhibit multiple modules that create multivalency, including folded binding domains (*red*) and low complexity regions (*purple*). Valency is often amplified by domains that enable homo-, or hetero-oligomerization (*orange*). Interactions between proteins containing different combinations of these interaction modules provide a framework for building a heterogeneous, infinitely expandable network within membrane-less organelles. Formation of this type of network drives phase separation when the critical concentration threshold is reached. For many of the examples discussed herein, active RNA transcription is needed for membrane-less organelle assembly. We hypothesize that expression of RNA in excess of a critical concentration threshold is needed to nucleate interactions with specific, multi-modular proteins, and for nucleating formation of membrane-less organelles. Stress signals can alter the multivalent interactions that drive phase separation and lead to partial or complete disassembly of the organelle

Echelle de temps des mouvements et paramètres RMN



Caractériser des échanges conformationnels entre états

Régimes d'échange

👉 Echange "rapide": $\Delta\omega \ll k_{ex}$

⇒ Un pic $\omega = p_A\omega_A + p_B\omega_B$

⇒ $R_{ex} \propto B_0^2$

👉 On ne connaît pas le nombre de sites en échange

👉 Echange "intermédiaire": $\Delta\omega \simeq k_{ex}$

⇒ Un ou deux pics élargis

⇒ $R_{ex} \propto B_0$

👉 Echange "lent": $\Delta\omega \gg k_{ex}$

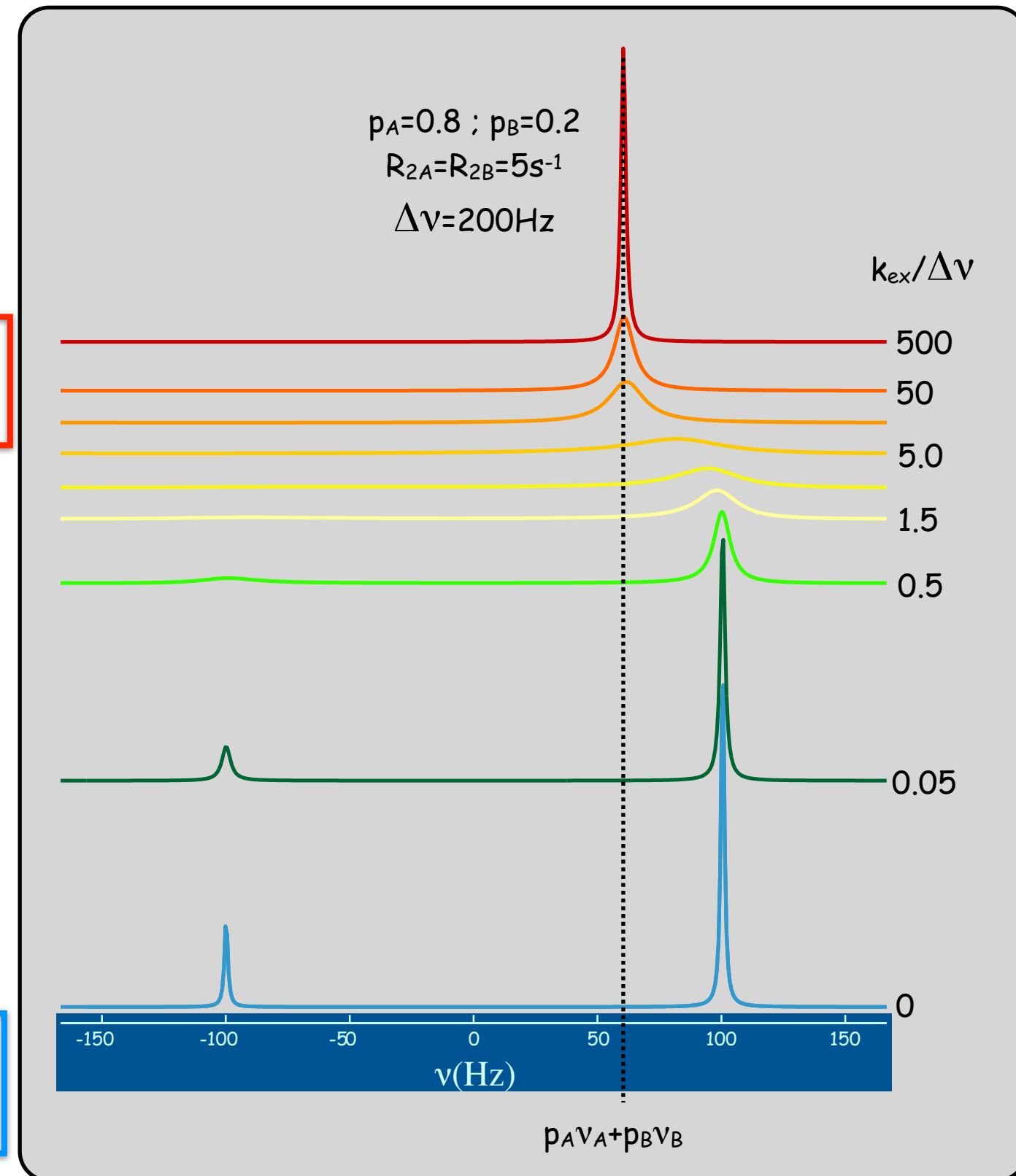
⇒ Deux pics

⇒ R_{ex} indépendant de B_0

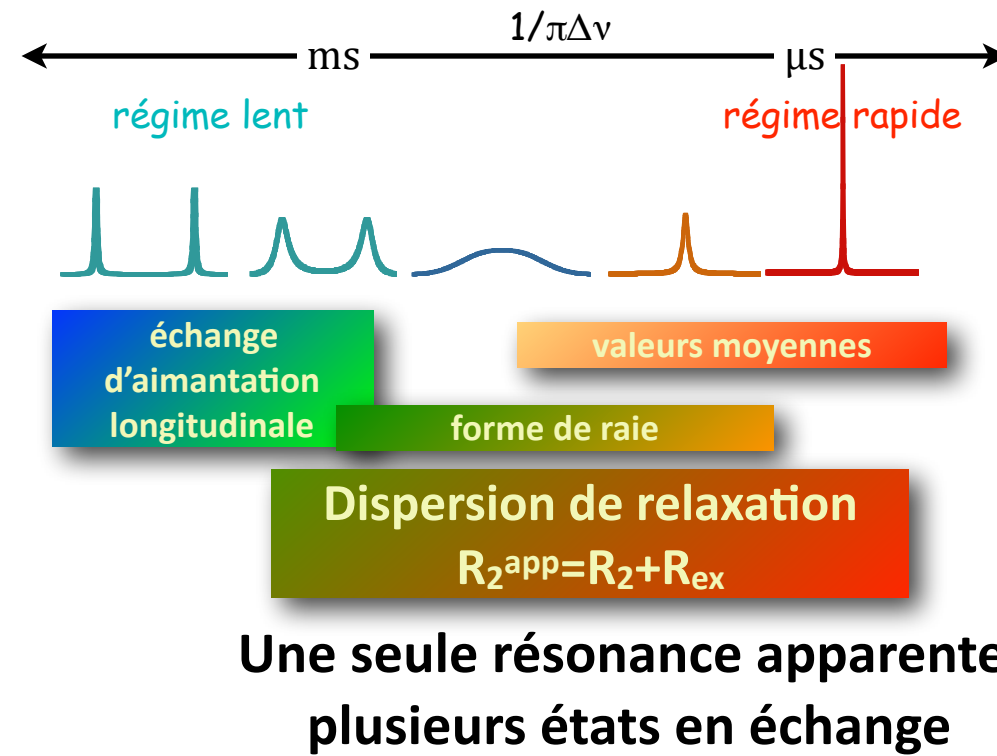
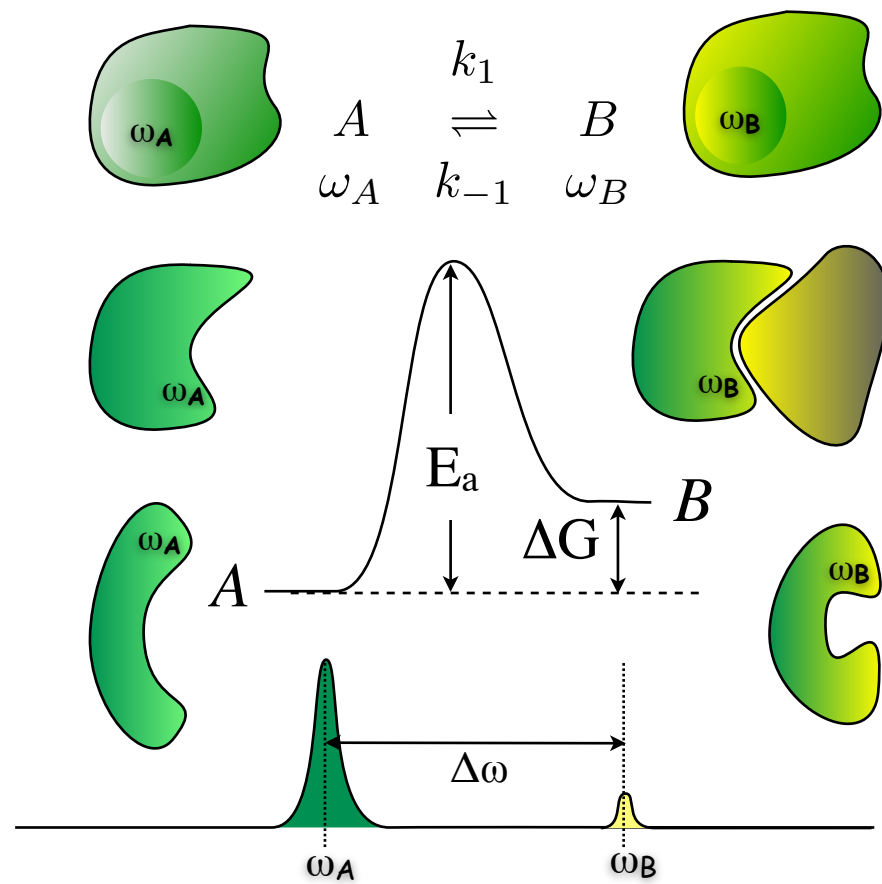
⇒ $R_{ex}^{(B)} \rightarrow k_{BA} = p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB} = p_B k_{ex}$

$$p_A \gg p_B \Rightarrow R_{ex}(B) \gg R_{ex}(A)$$

👉 Le pic minoritaire peut devenir indétectable même en régime d'échange "lent"



Caractériser des échanges conformationnels entre états Cas d'un seul pic observé



paramètres thermodynamiques

$$K_{eq} = k_1/k_{-1} = p_B/p_A$$

$$\Delta G = -RT \ln K_{eq}$$

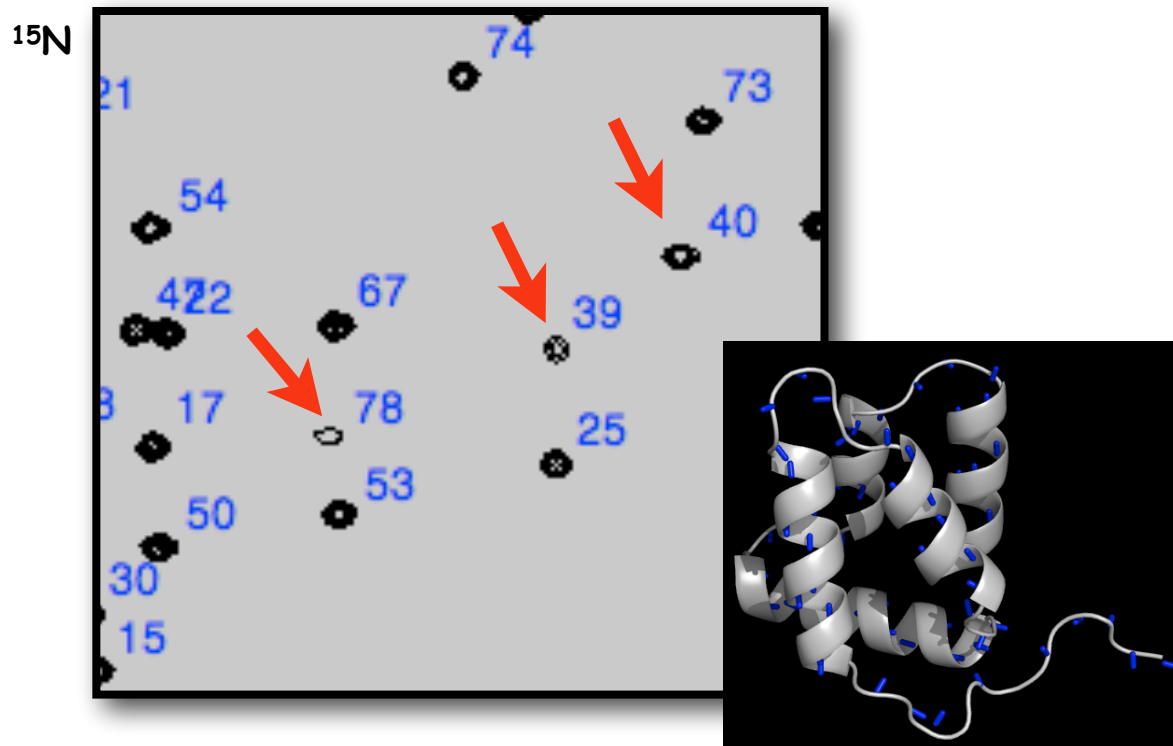
paramètres cinétiques

$$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$$

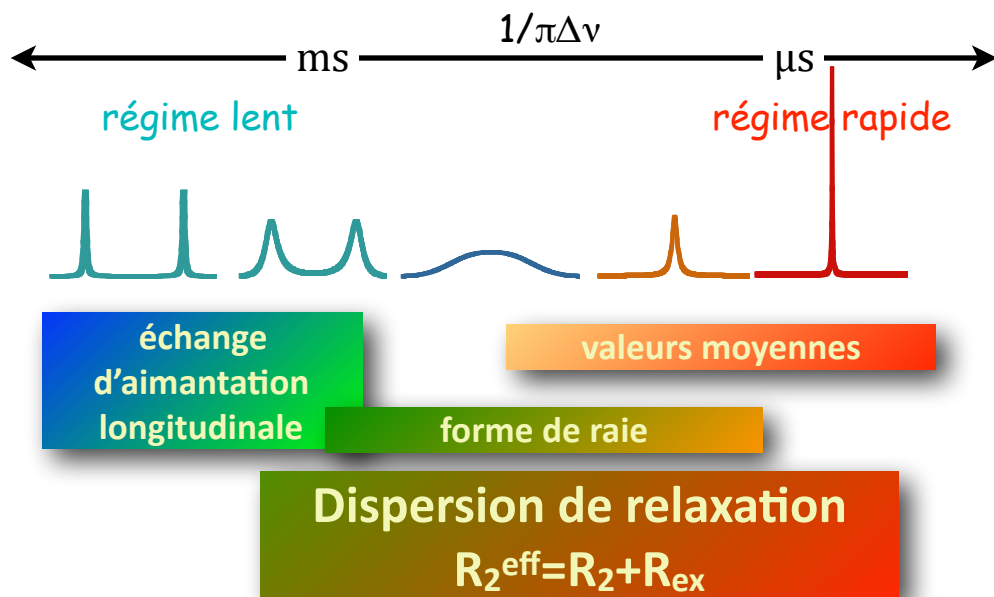
$$k_{ex}(T) = k_0 \exp(-E_a/kT)$$

paramètres structuraux

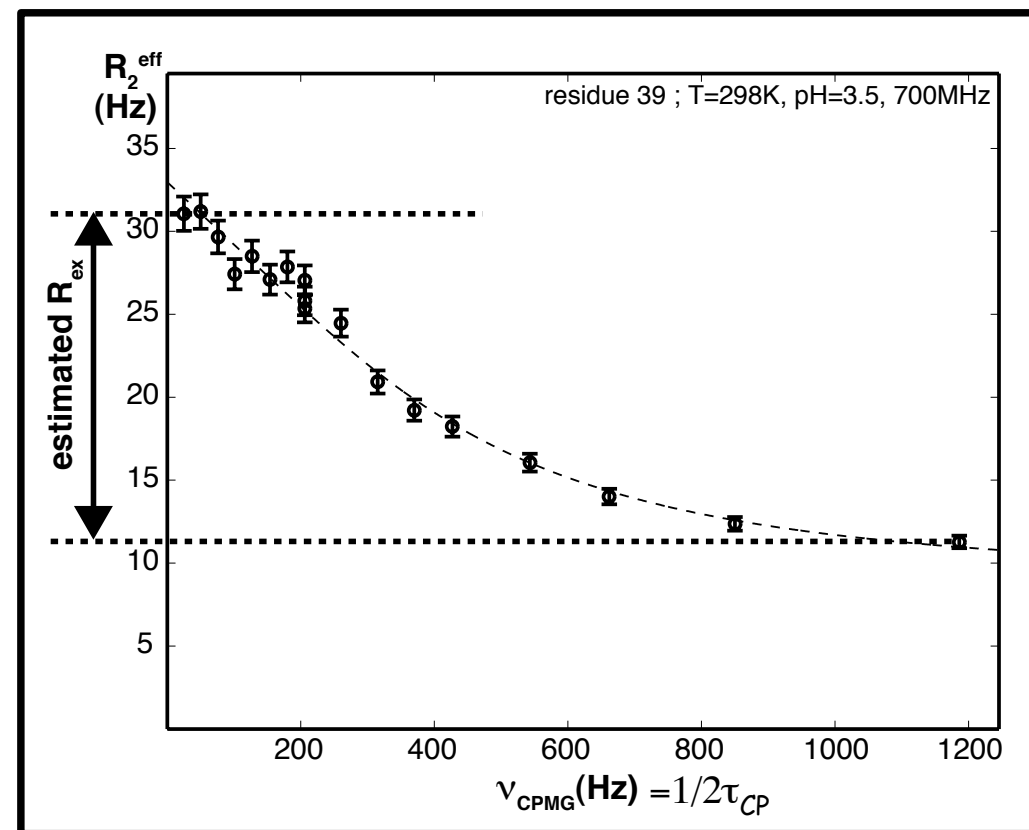
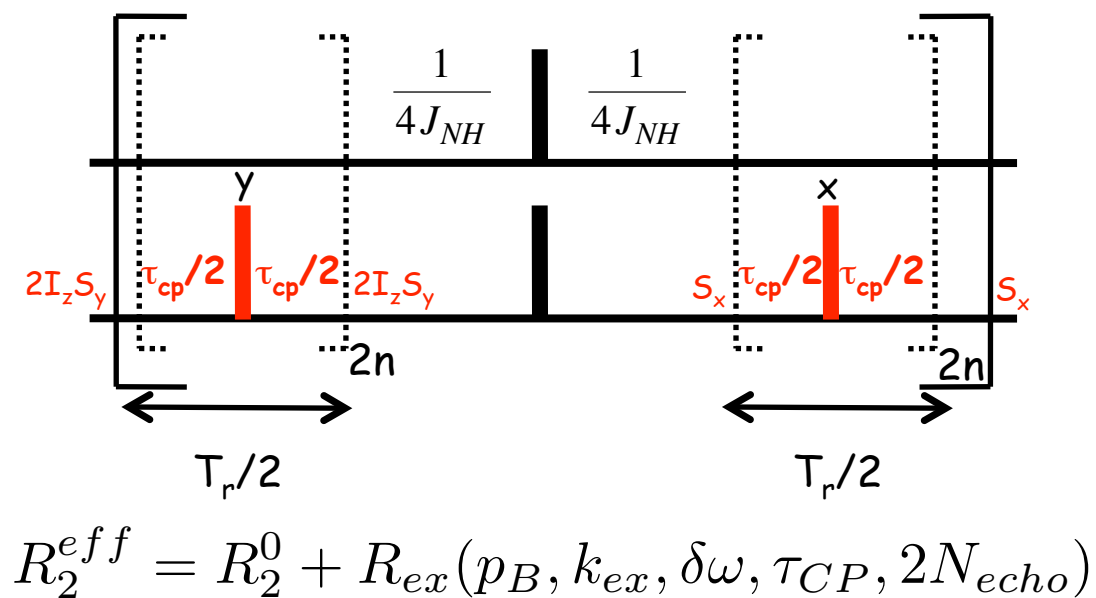
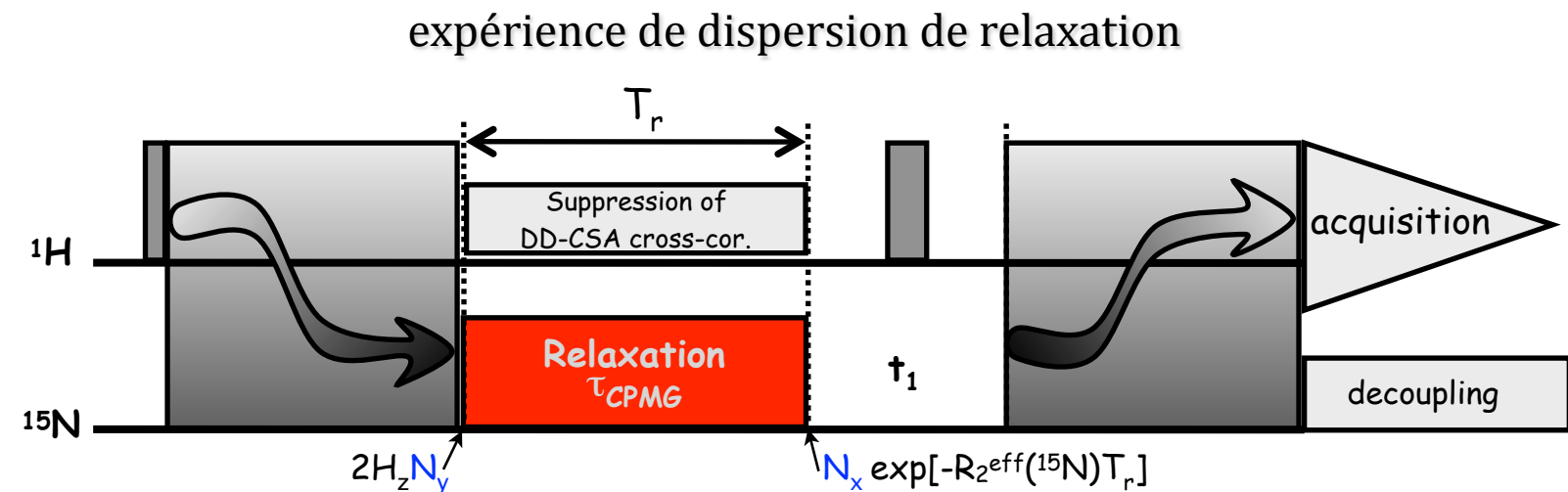
$$\Delta\omega ; \omega_A ; \omega_B$$



Caractériser des échanges conformationnels entre états Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

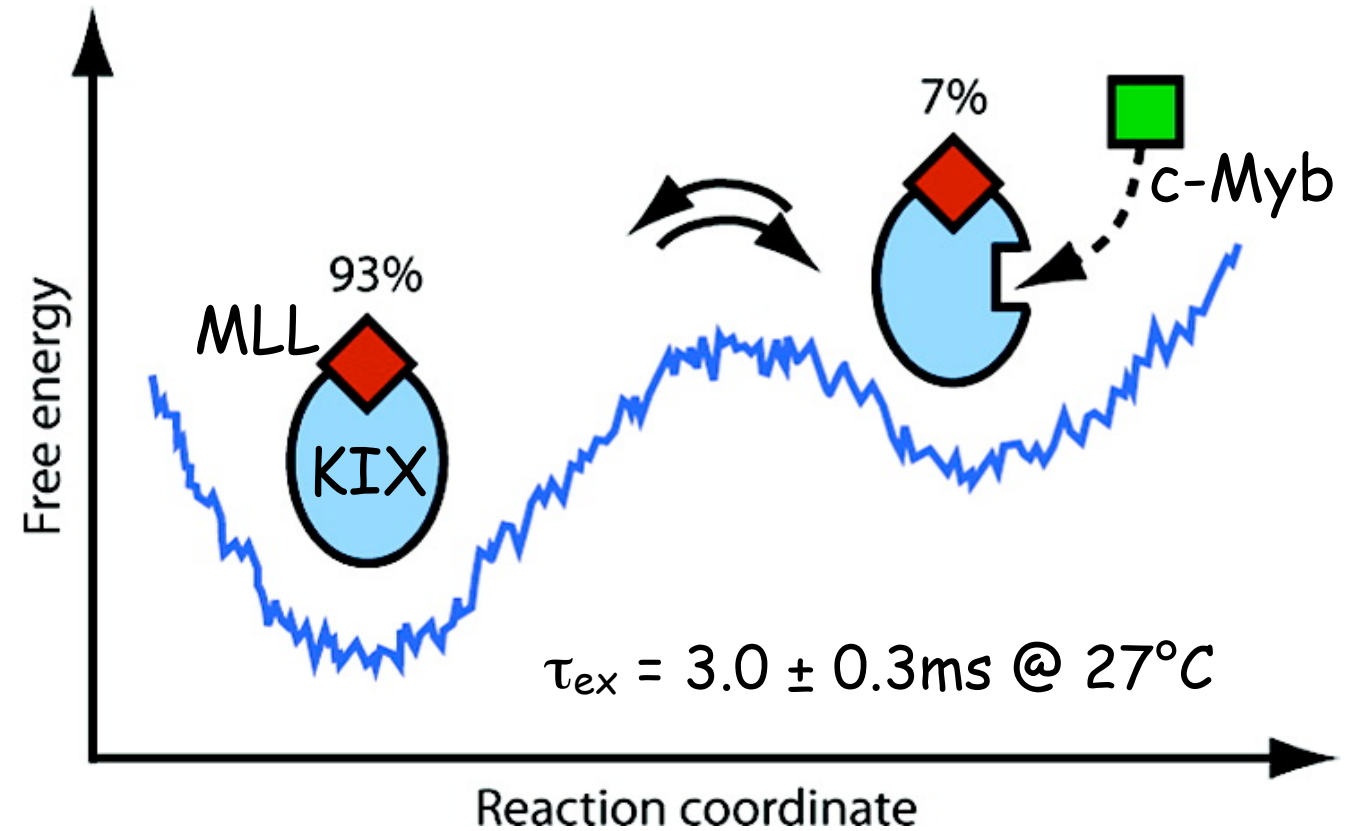
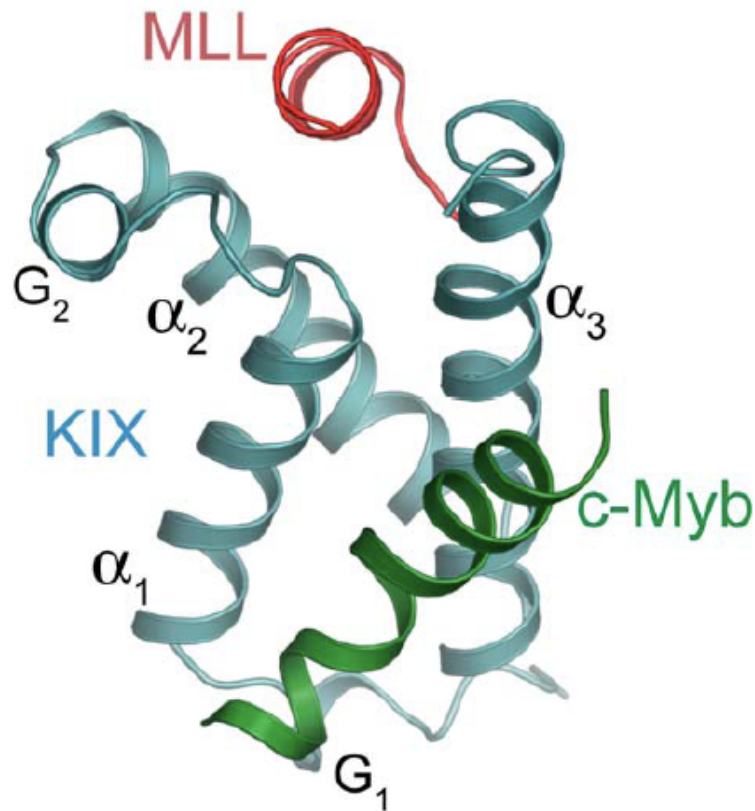


Une seule résonance apparente,
plusieurs états en échange



Caractériser des échanges conformationnels entre états Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

Sélection conformationnelle / allostérie

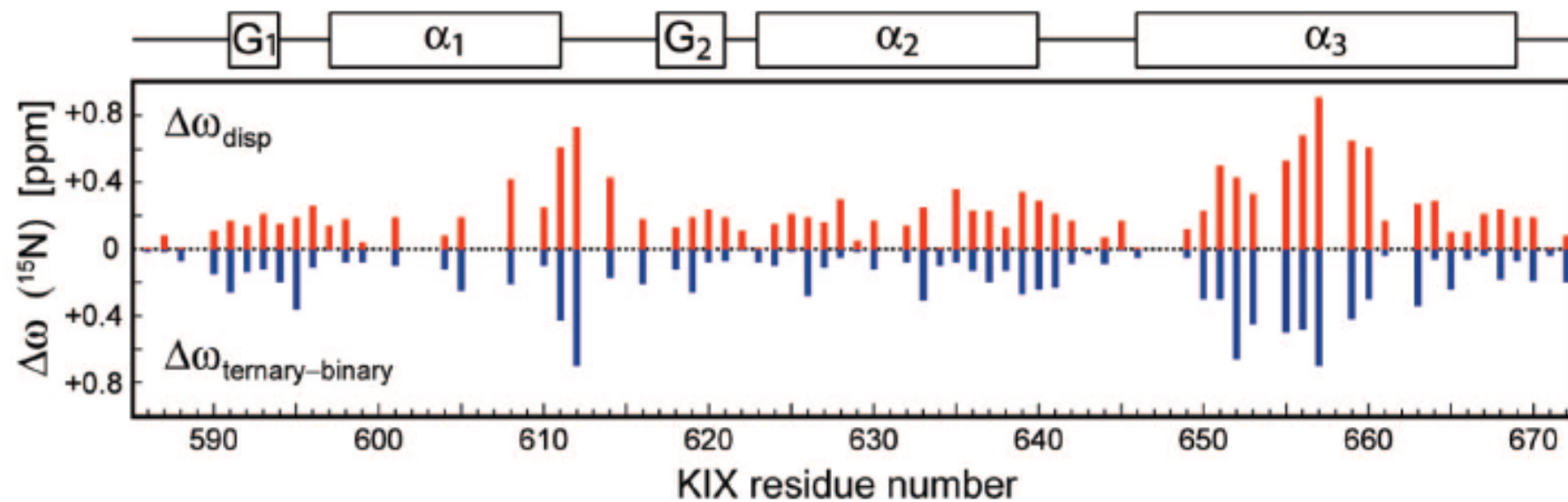
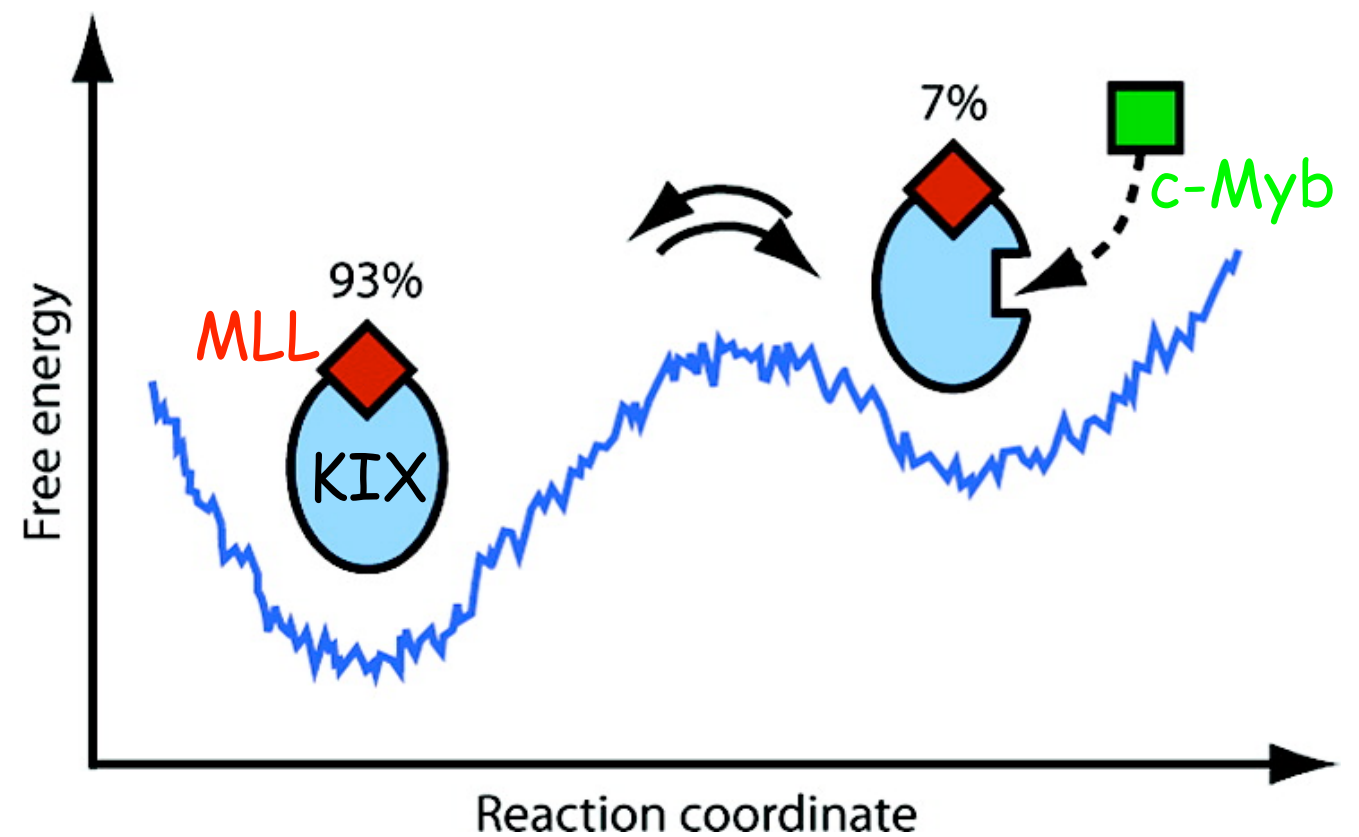
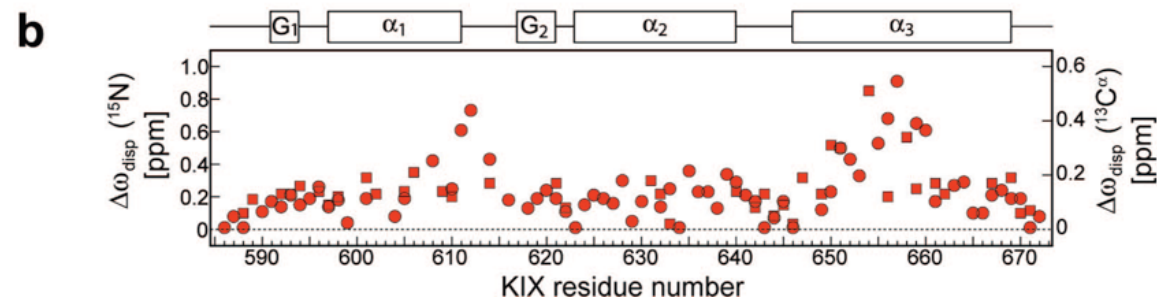
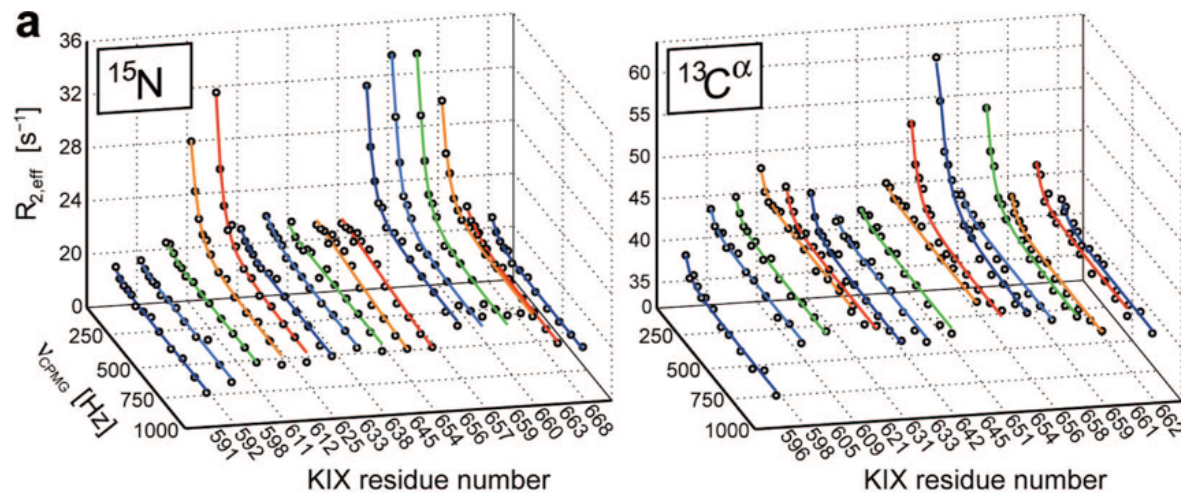


⇒ La liaison du domaine d'activation du facteur de transcription MLL à KIX induit une redistribution des populations relatives des conformères de KIX en faveur de l'état dans lequel le site de liaison de c-Myb (pKID) est préformé.

⇒ Cet état minoritaire n'est pas détectable dans la forme libre de KIX

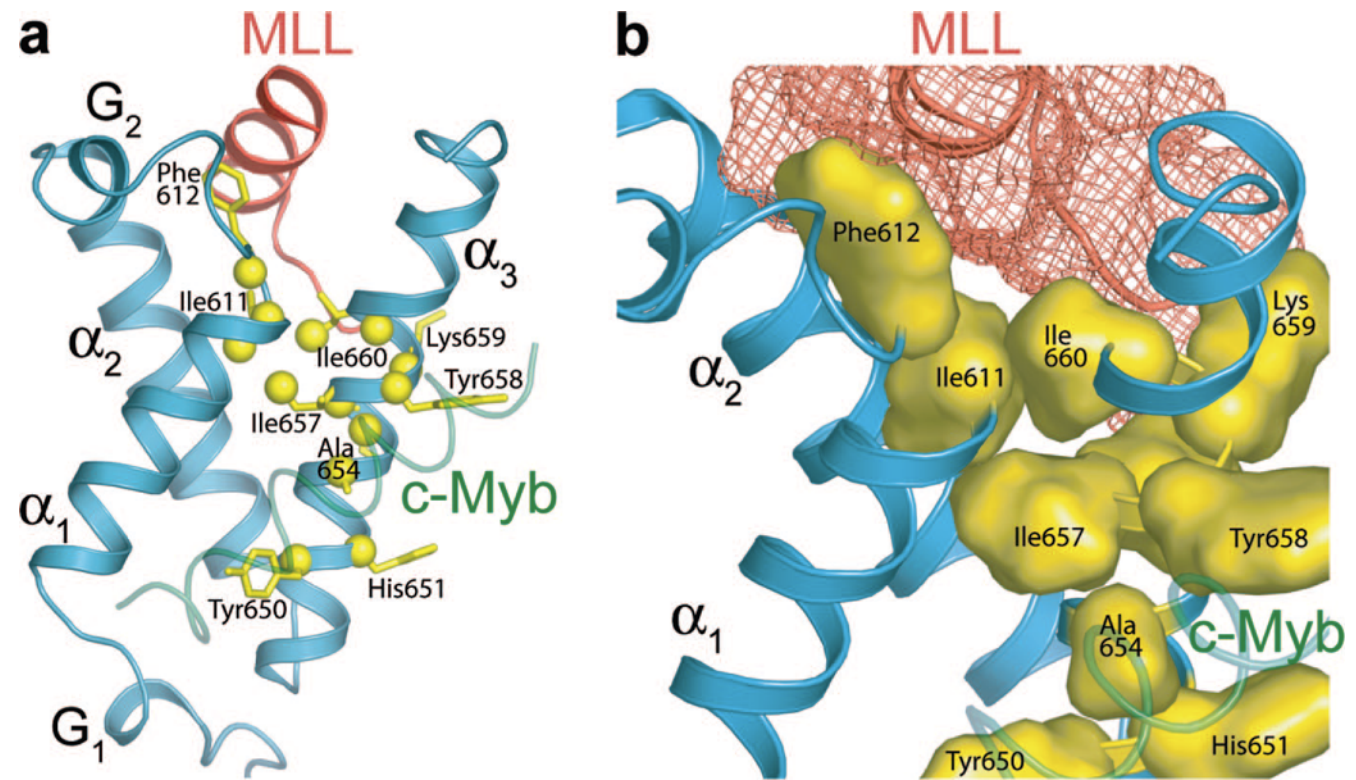
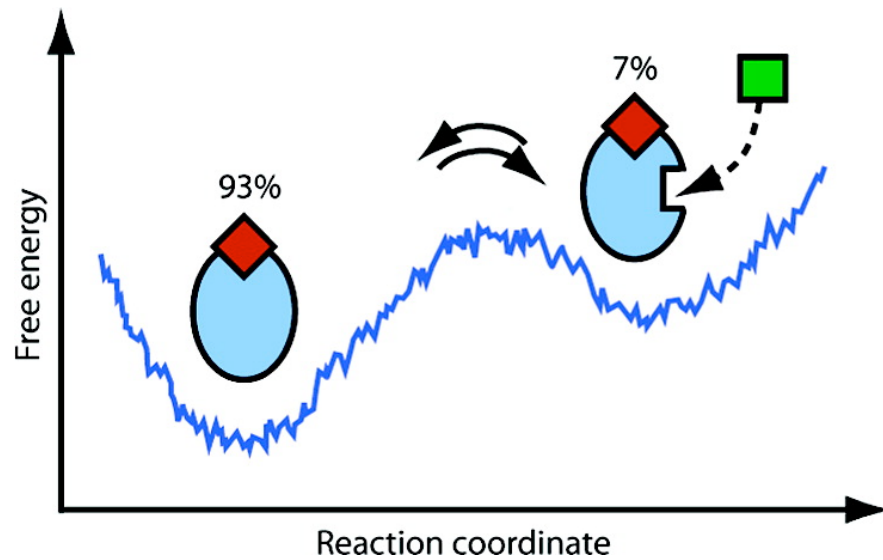
Caractériser des échanges conformationnels entre états Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

Sélection conformationnelle / allostérie



Caractériser des échanges conformationnels entre états Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

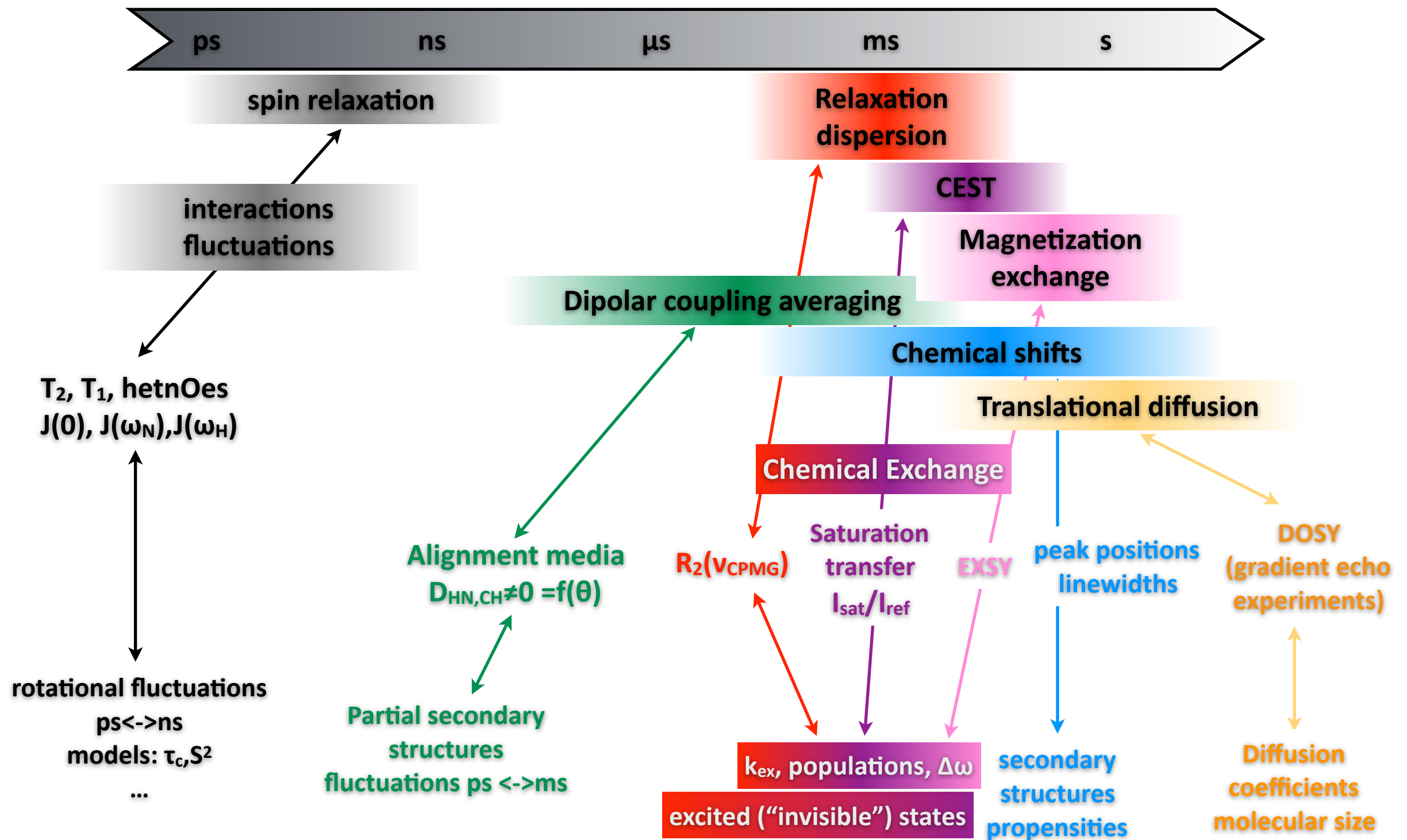
Sélection conformationnelle / allostérie



⇒ Les chaînes latérales des isoleucines participent à la formation d'un réseau d'interactions qui constitue le «chemin» à travers lequel se transmet l'information allostérique.

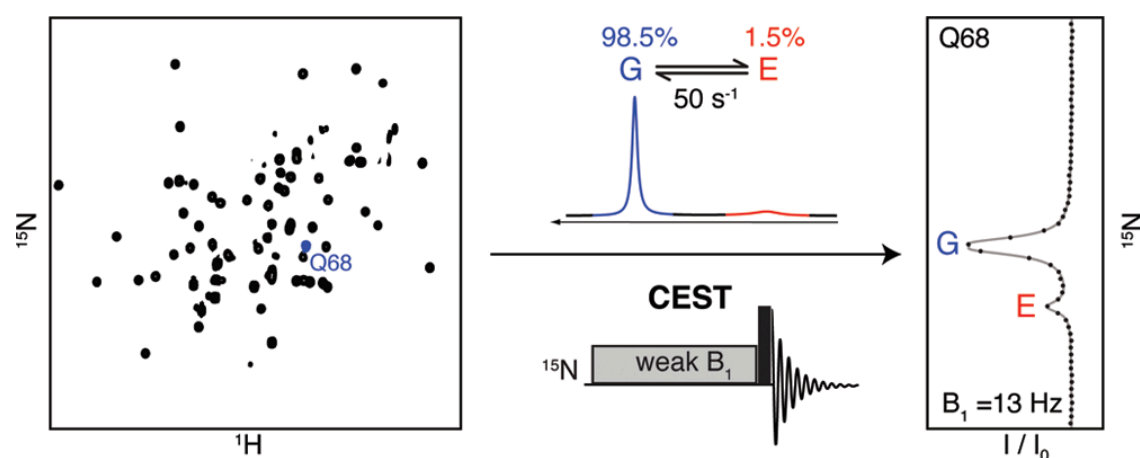
⇒ L'analyse de la transition allostérique du domaine KIX de CBP fournit une description à l'échelle atomique du mécanisme de transmission coopérative d'information entre deux facteurs de transcription.

Echelle de temps des mouvements et paramètres RMN



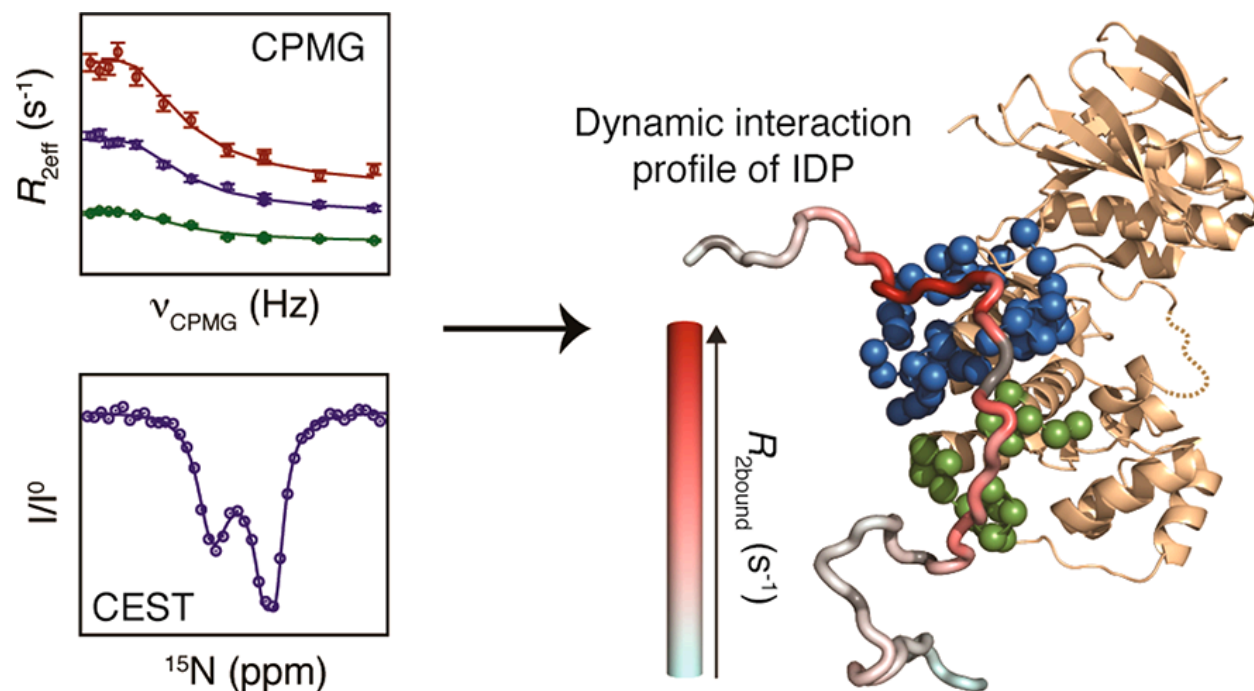
Caractériser des échanges conformationnels entre états Cas d'un seul pic observé: Dispersion de Relaxation (CPMG) / CEST

Caractérisation d'un état lié à partir d'un complexe minoritaire

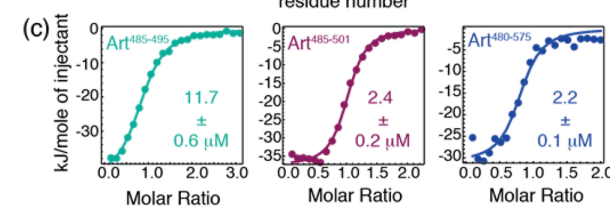
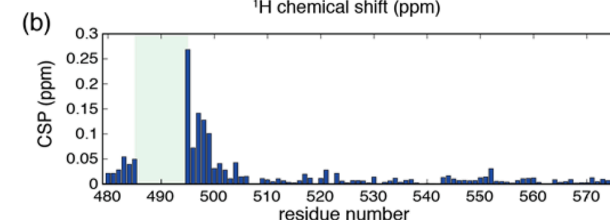
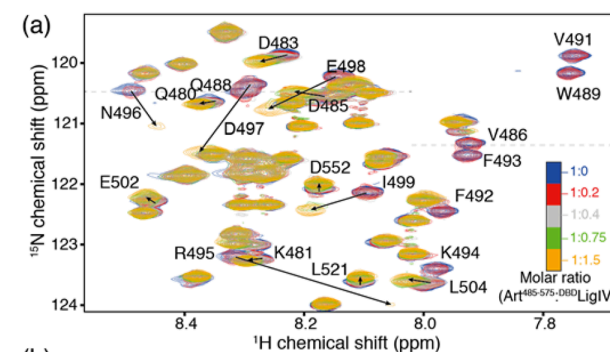
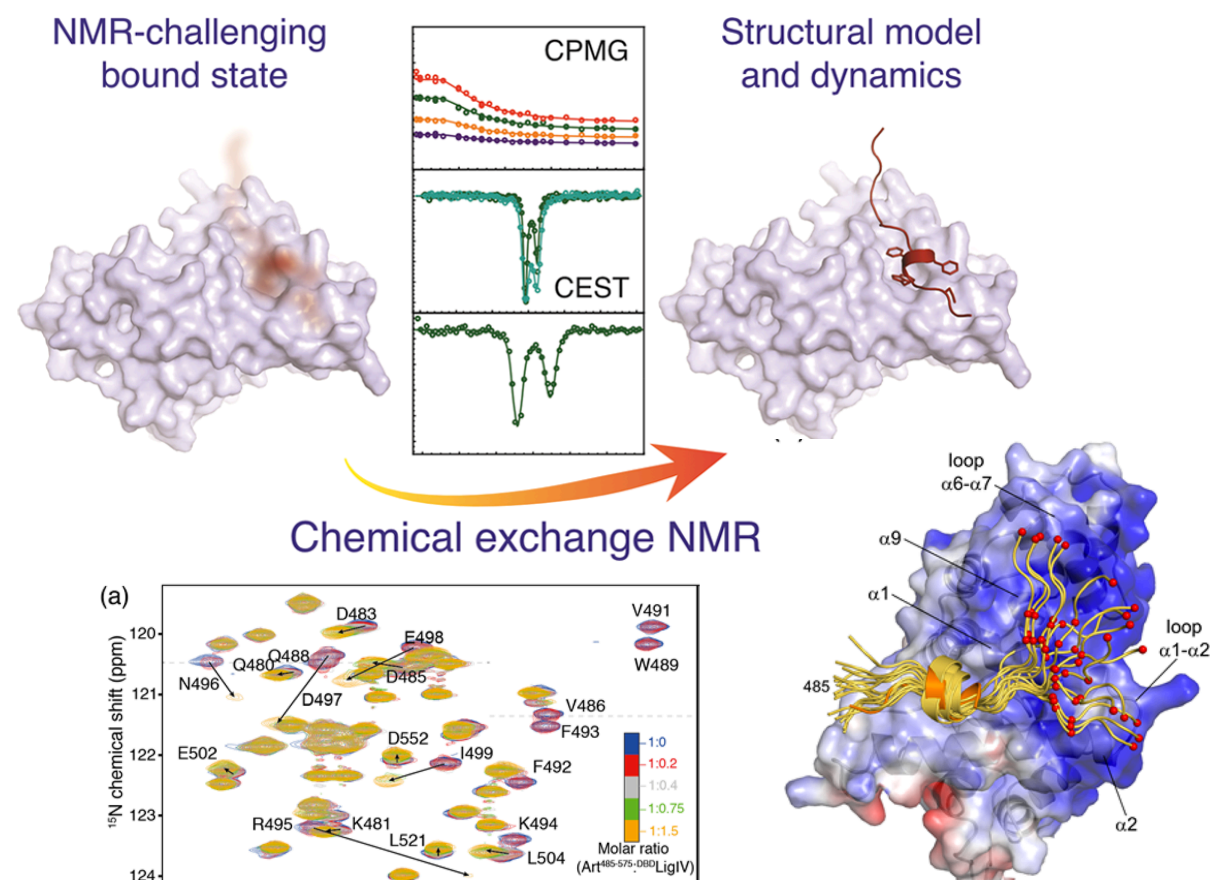


Vallurupalli, Bouvignie & Kay, *J. Am. Chem. Soc.* 2012, 134, 8148–8161

Interaction between the mitogen-activated protein kinase (MAPK) p38 α and the intrinsically disordered regulatory domain of the MAPK kinase MKK4. Our study demonstrates that MKK4 employs a subtle combination of interaction modes in order to bind to p38 α , leading to a complex displaying significantly different dynamics across the bound regions



Delaforges et al., *J. Am. Chem. Soc.* 2018, 140, 1148–1158,
Deciphering the Dynamic Interaction Profile of an Intrinsically Disordered Protein
by NMR Exchange Spectroscopy

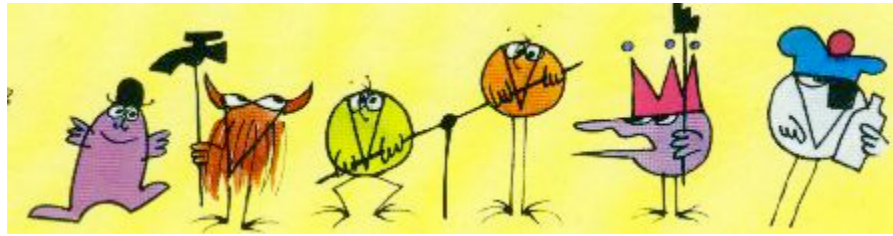


$k_{on} = (3.00 \cdot 10^7) \pm (0.01 \cdot 10^7) \text{ s}^{-1}\text{M}^{-1}$
 $k_{off} = k_{on} \cdot K_d = 66.1 \pm 0.2 \text{ s}^{-1}$
 $K_d = 2.2 \text{ μM (ITC)}$

Charlier et al., *J. Am. Chem. Soc.* 2017, 139, 12219–12227
Structure and Dynamics of an Intrinsically Disordered Protein Region
That Partially Folds upon Binding by Chemical-Exchange NMR



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