Laboratoire de Biologie et Chimie Structurales

Institut de Chimie des Substances Naturelles

infrastructure de recherche en résonance magnétique nucléaire, très hauts champs

Integrated Structural Biology

Relaxation et phénomènes dynamiques en Résonance Magnétique Nucléaire

Application aux protéines

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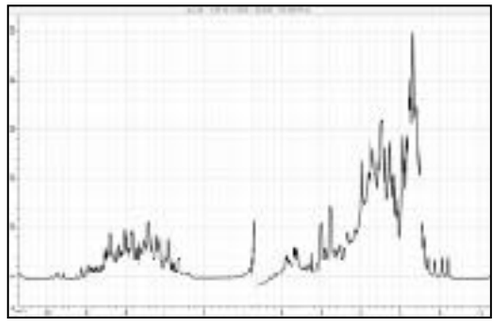
MOTS CLES

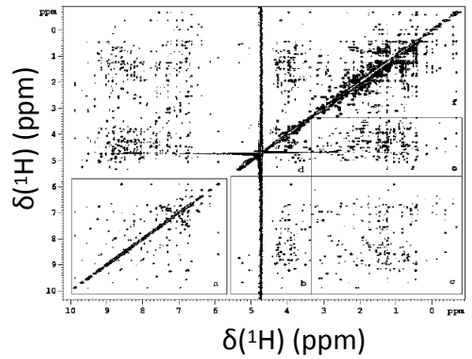
- ➡ Spins, Champs magnétiques
- ➡ Fréquence de résonance
- ➡ Spectre
  - ➡ Déplacement chimique
  - ➡ Largeur de raie
- ➡ Environnement électronique
- ➡ Communication entre spins
  - ➡ Proximité le long des liaisons
  - ➡ Proximité dans l'espace

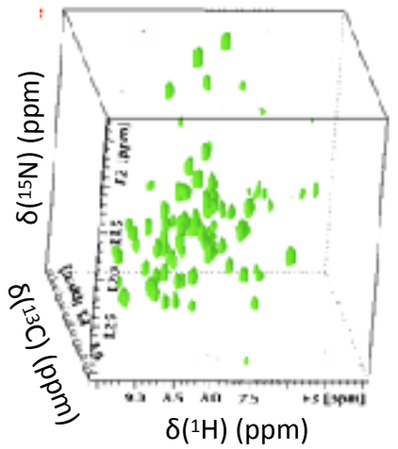
- ➡ Interactions
- ➡ Mouvements, flexibilité
- ➡ Structure

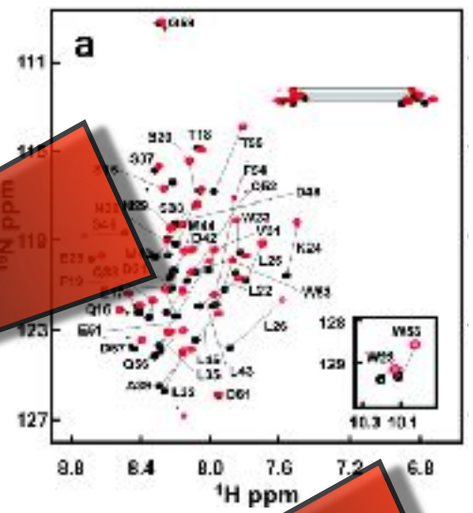
- ➡ Caractériser des interactions protéines/partenaires (sites d'interaction, affinités, cinétique)

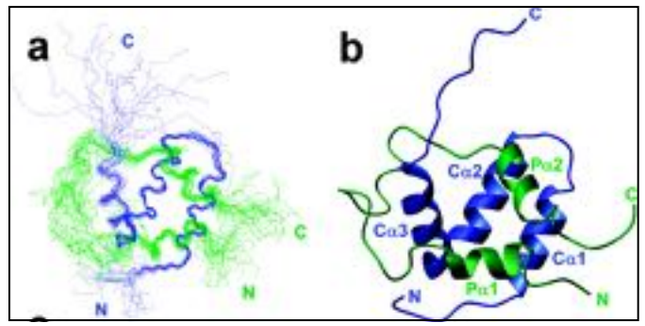
- ➡ Caractériser des états multiples fonctionnels de protéines

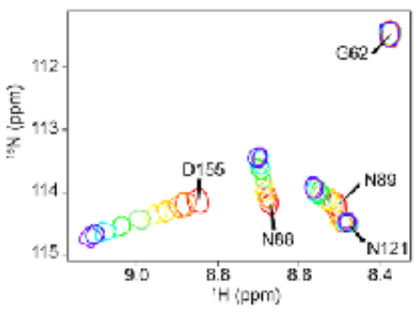


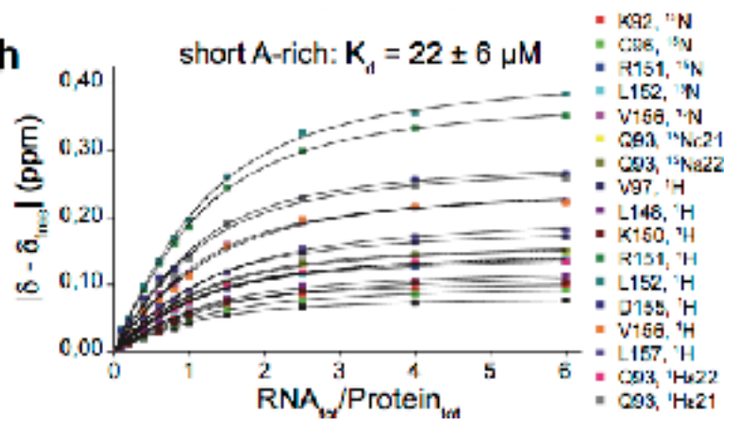


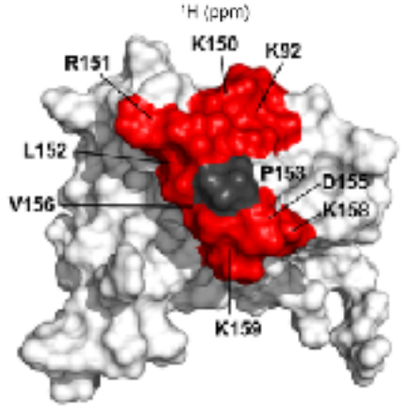




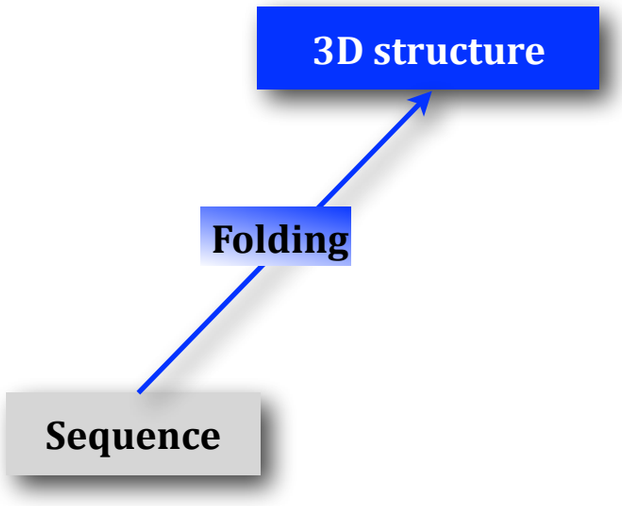


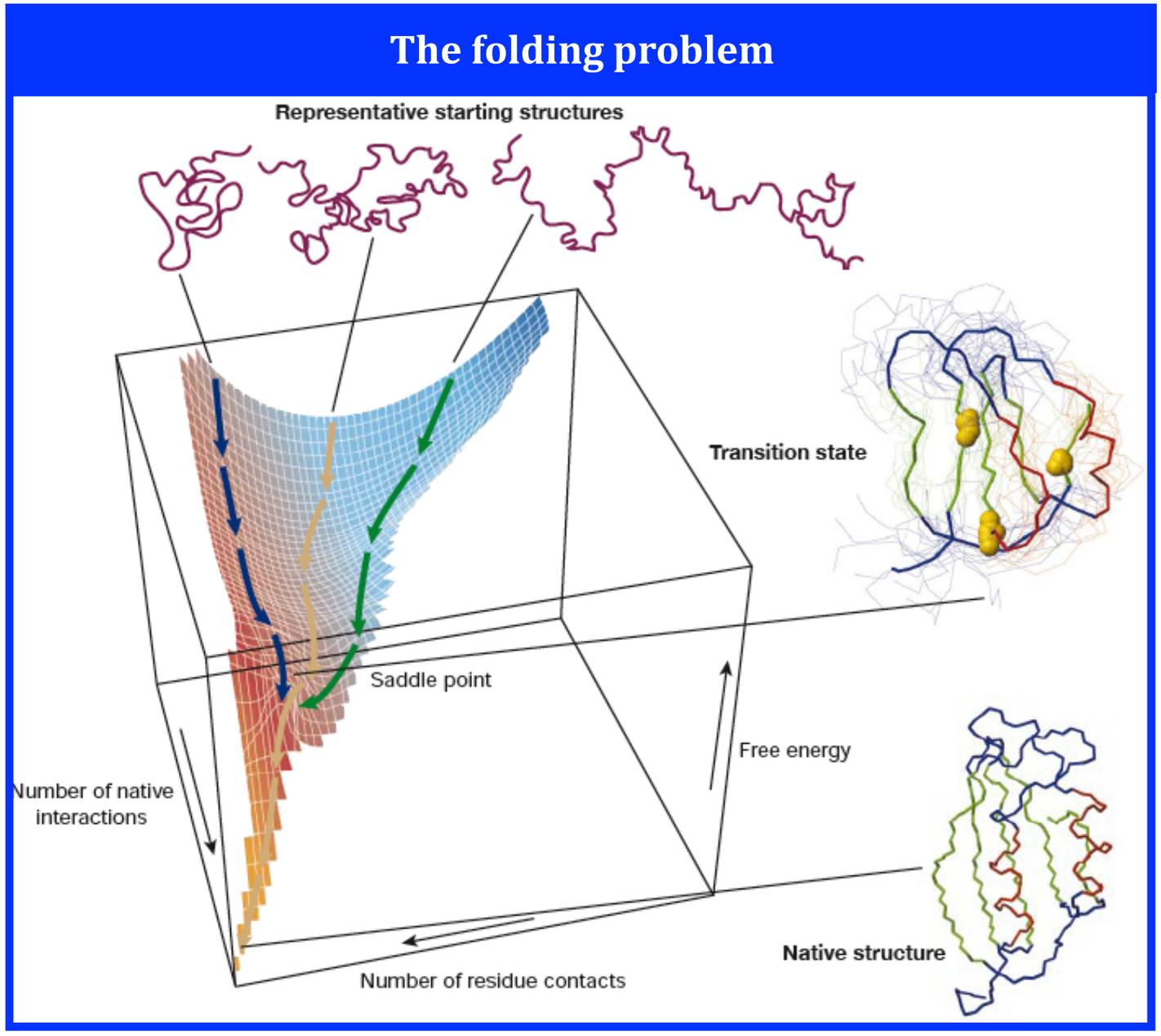






A key question :
explore multiple states of proteins and their plasticity





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

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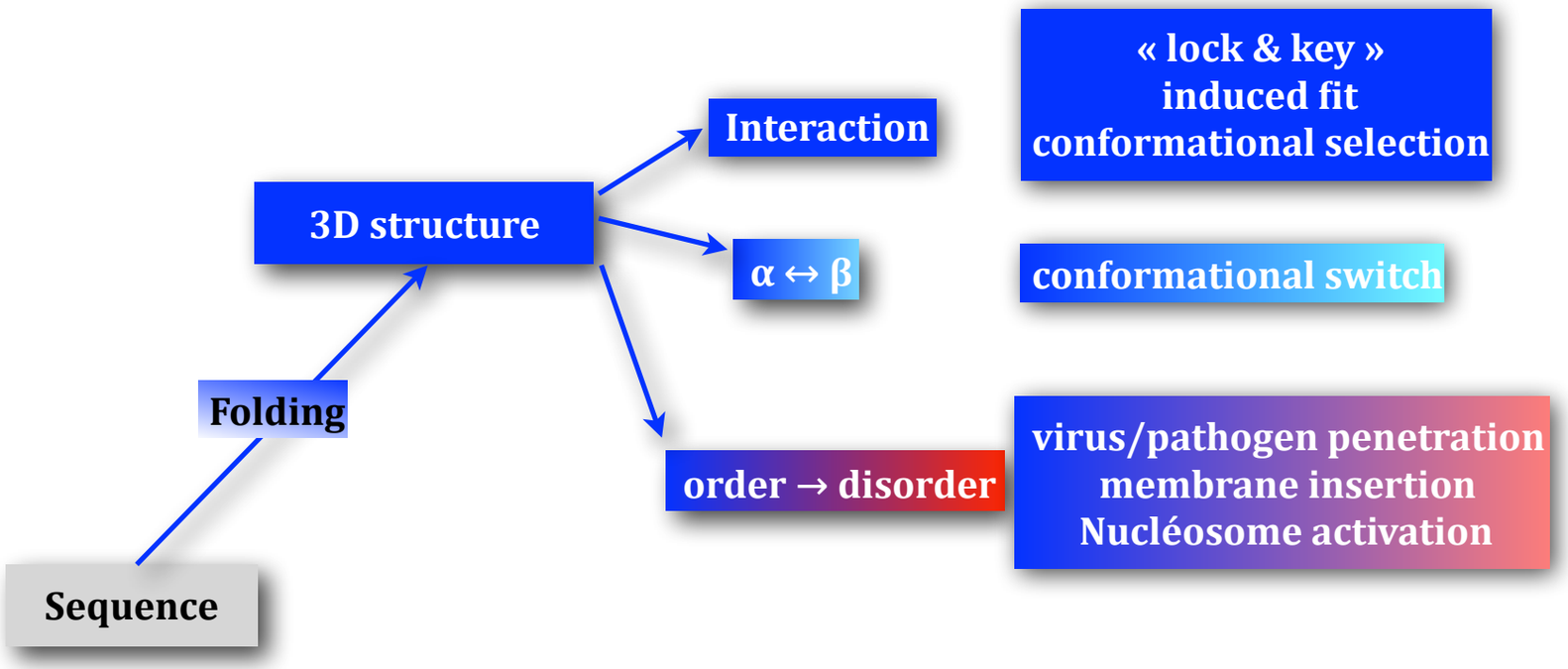

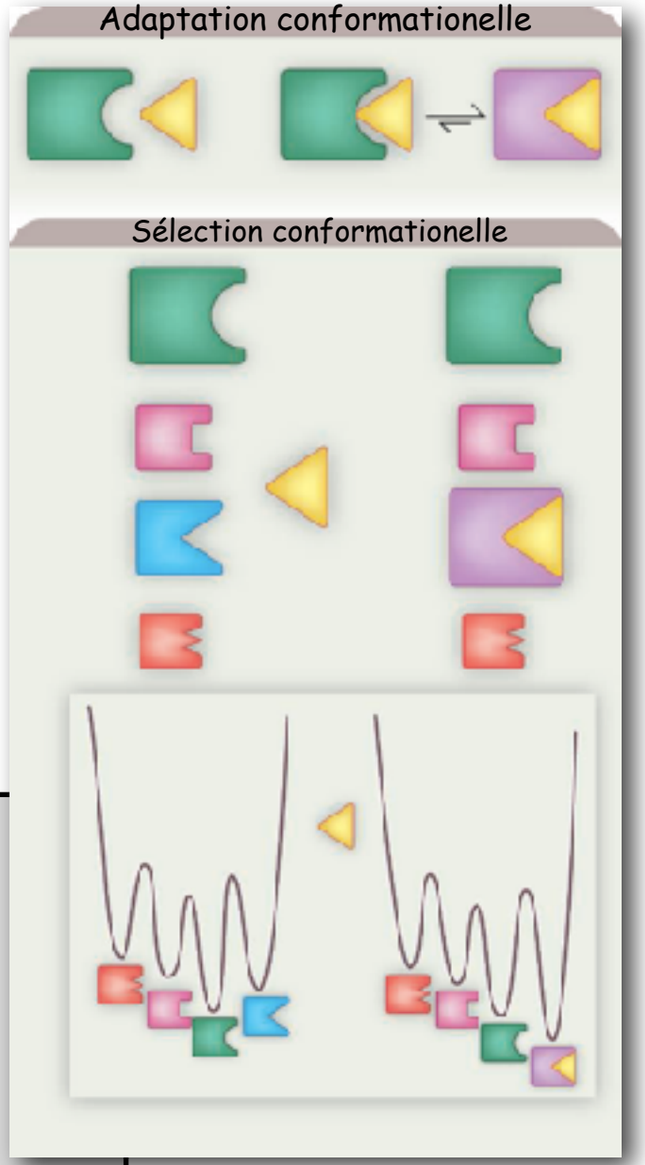

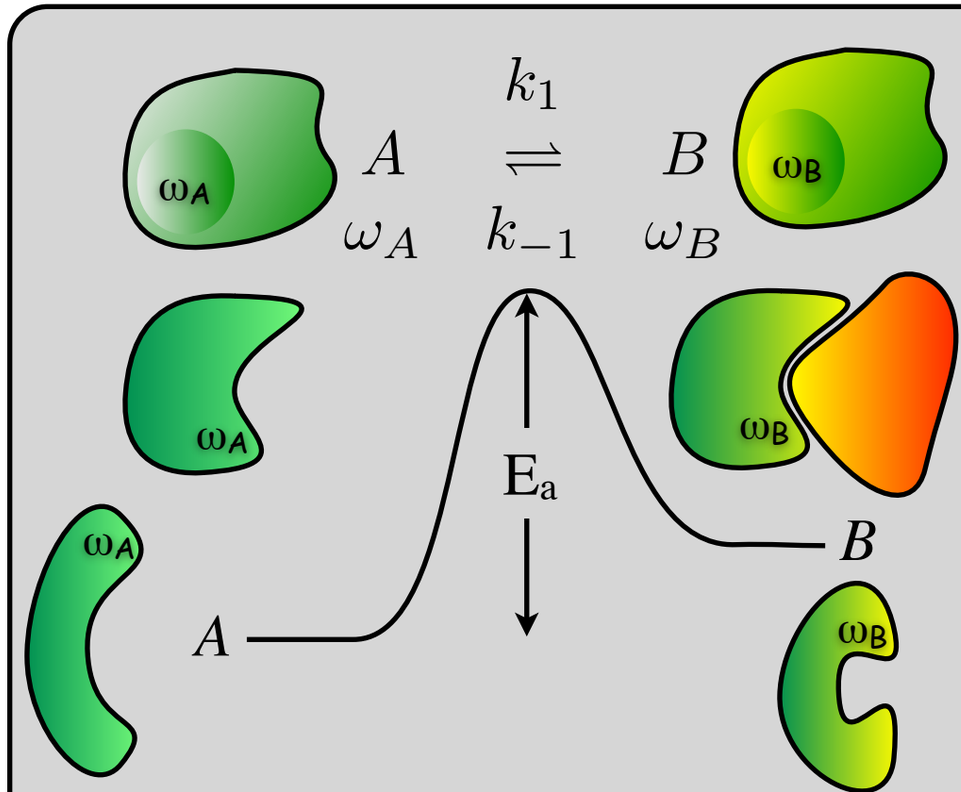

thermodynamic parameters

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

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

kinetic parameters

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

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

structural parameters

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

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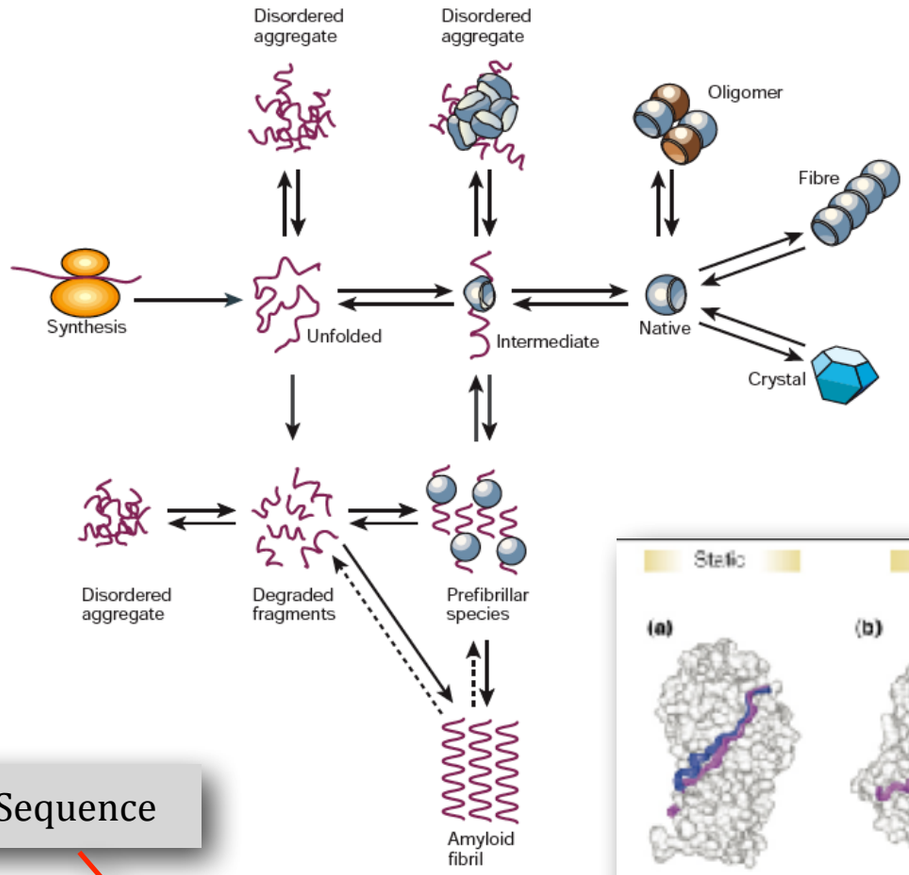
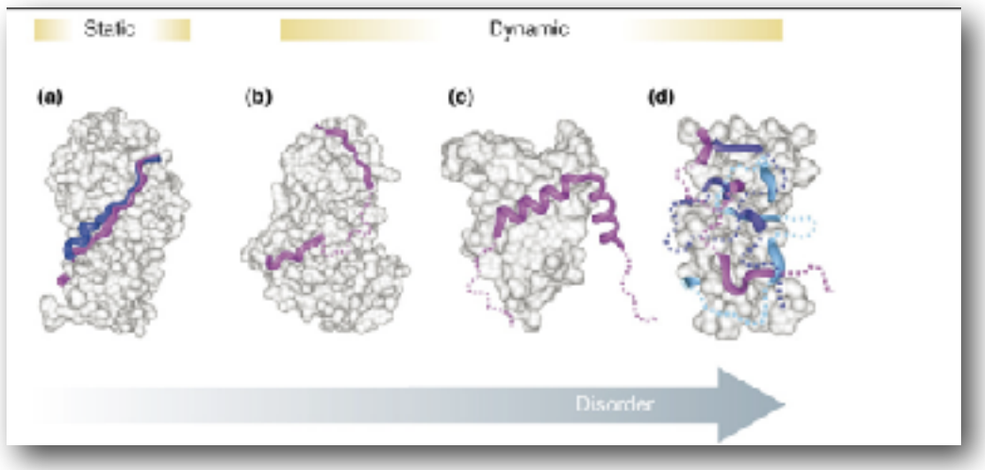
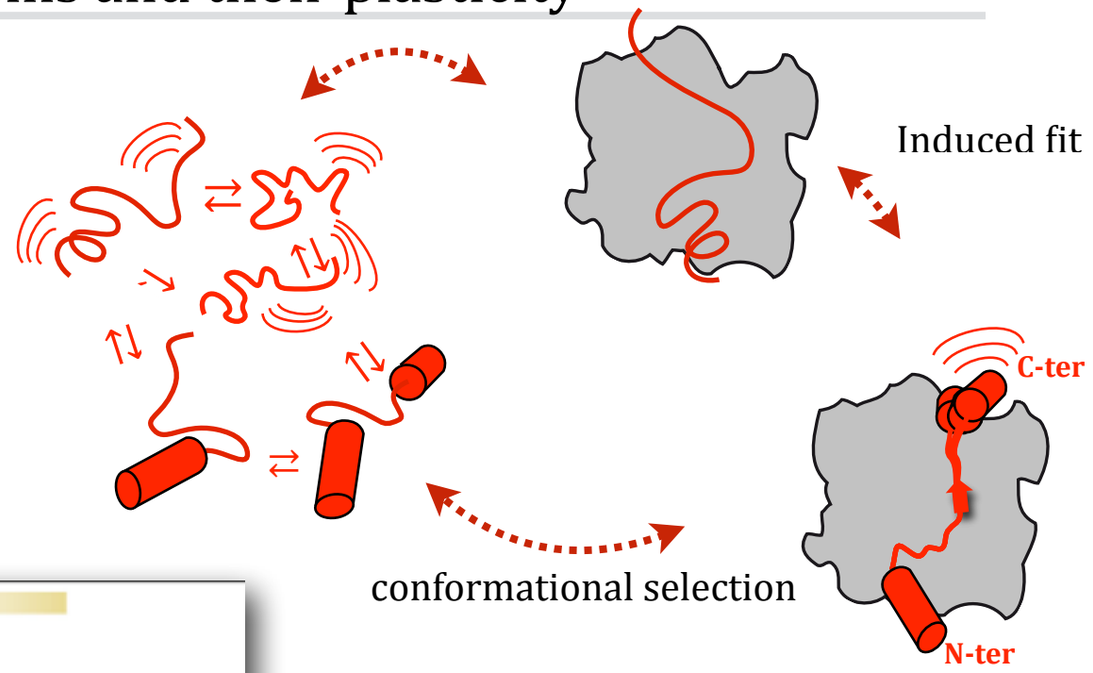






Sequence

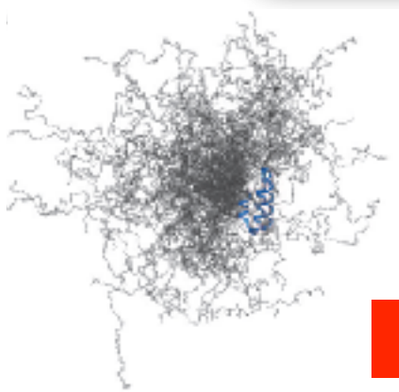
“Non folding”

flexible ensemble

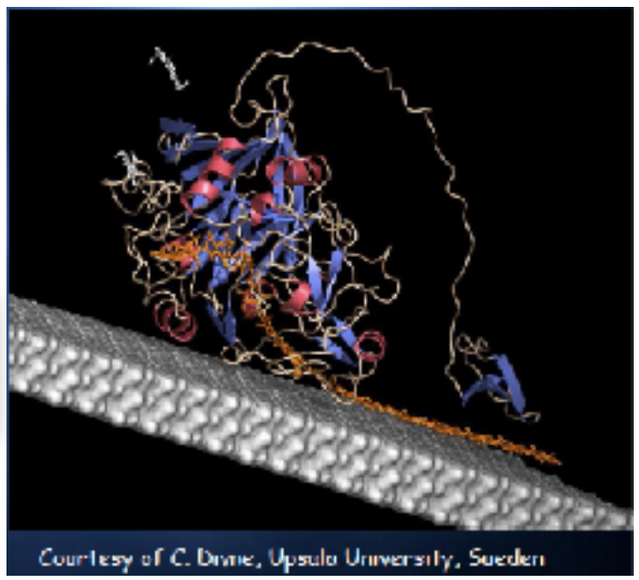
flexible linkers
display of sites
entropic bristles, springs and clocks

disorder → order

molecular recognition
virus/phages assembly
stepping motors

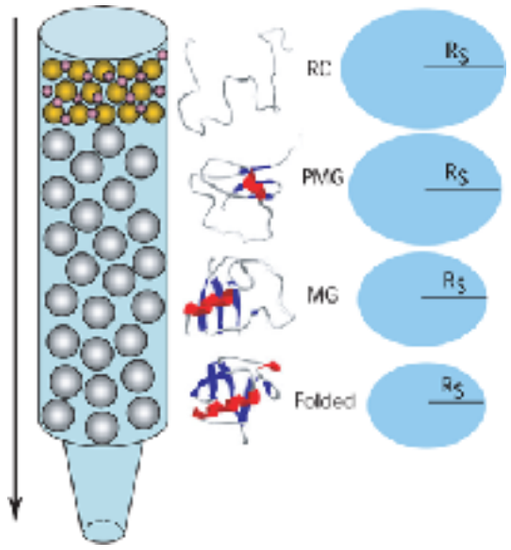


The “non” folding problem



Cellulases

Multiple states of proteins : a continuum from order to disorder



3D structure

Interaction

α ↔ β

order → disorder

Folding

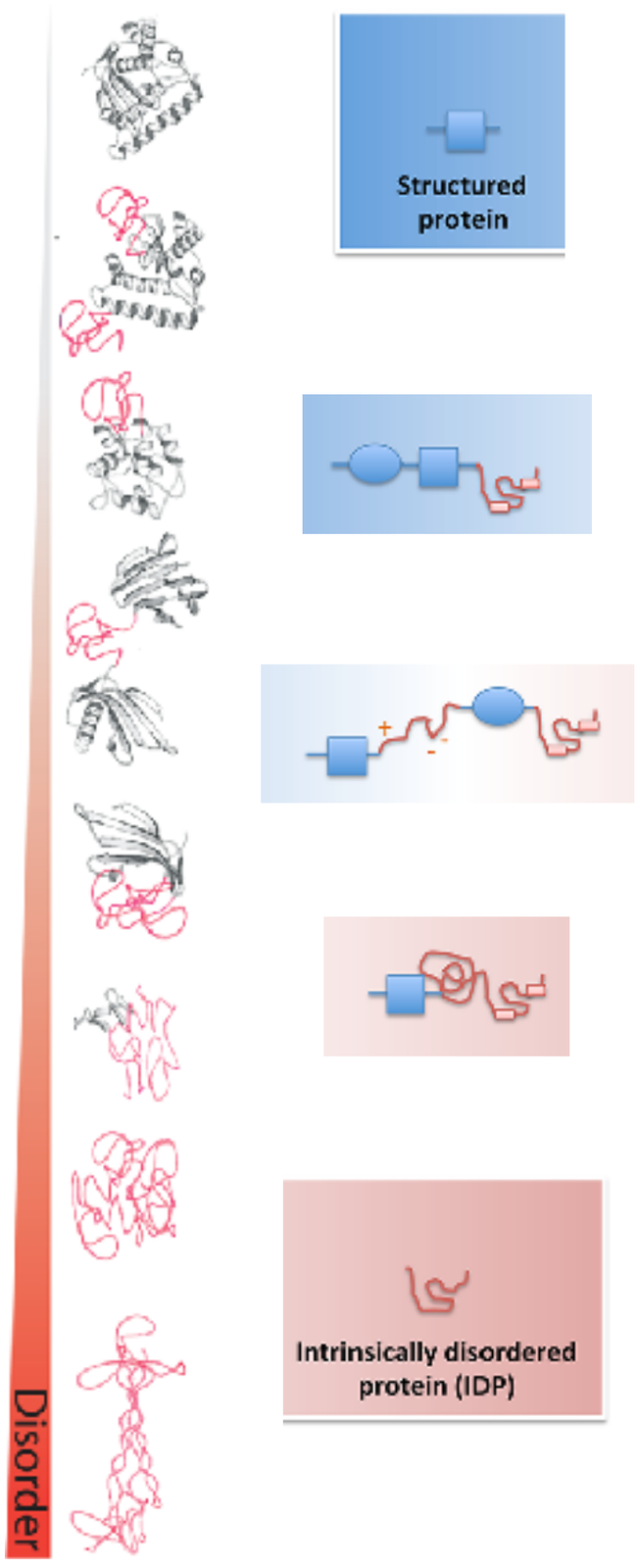
Sequence

"Non folding"

flexible ensemble

disorder → order

functional 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

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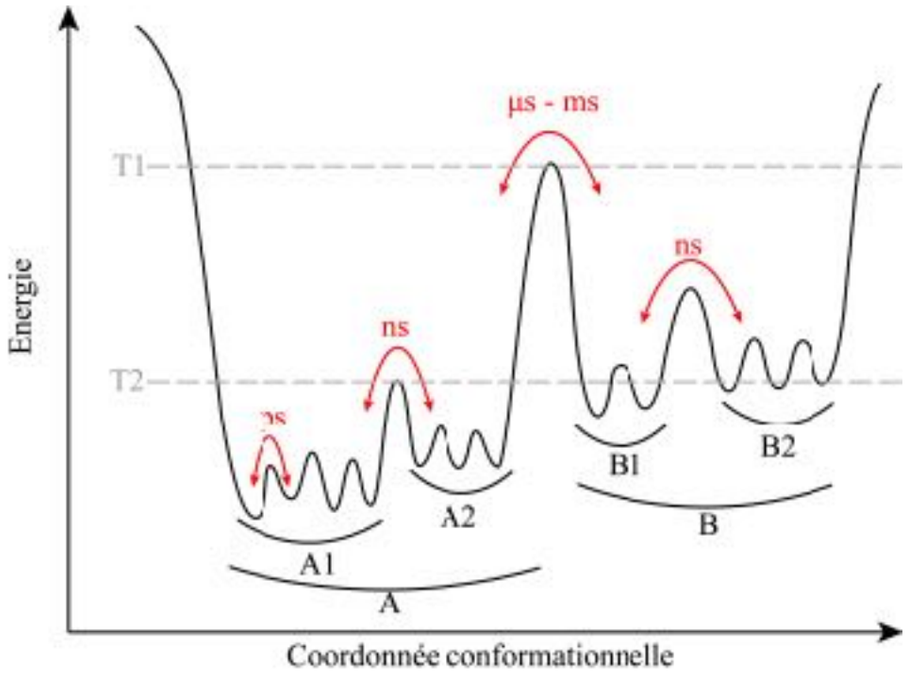
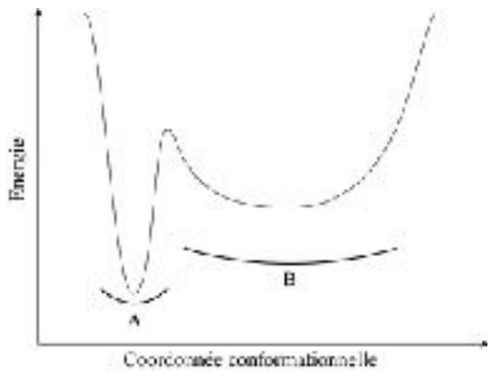
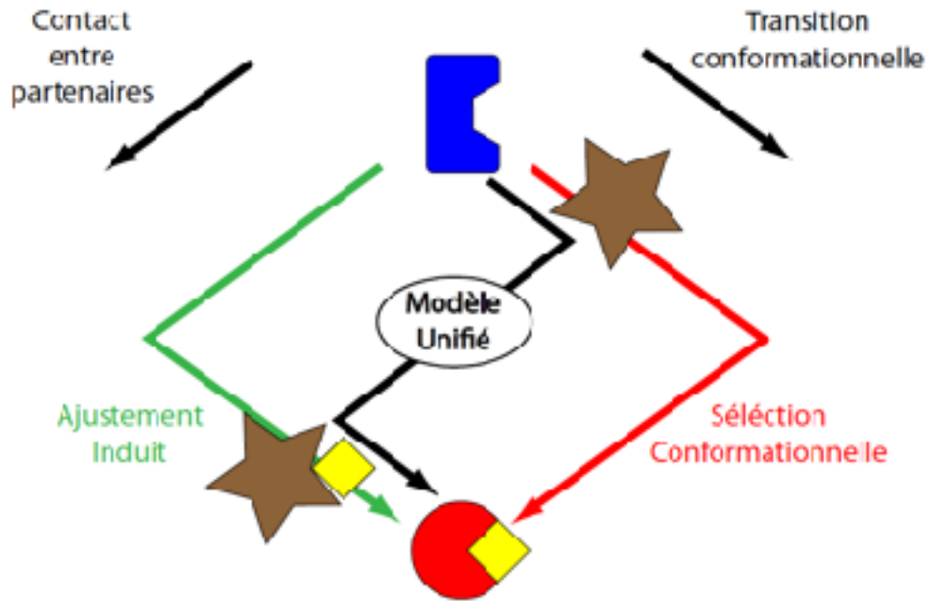
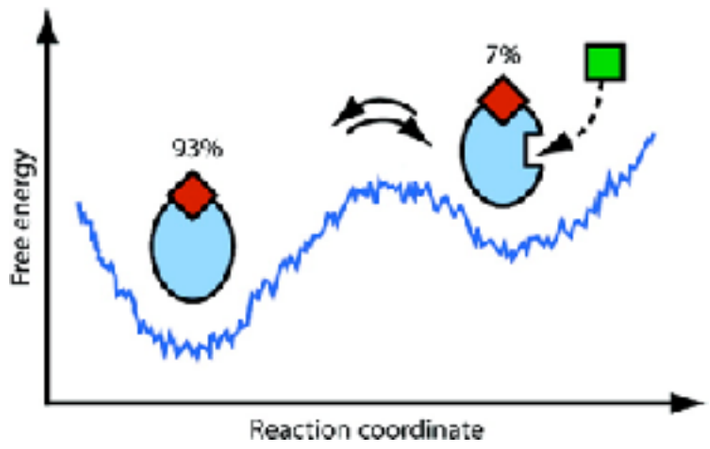
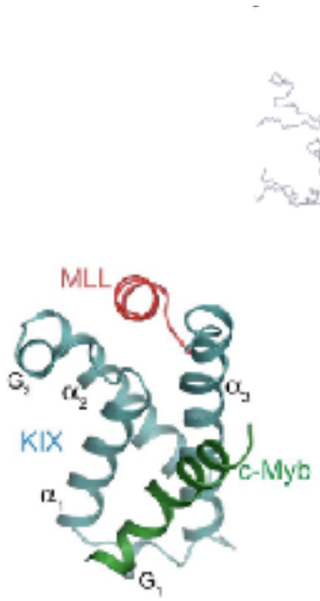


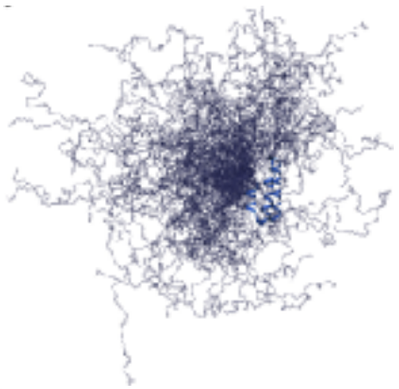


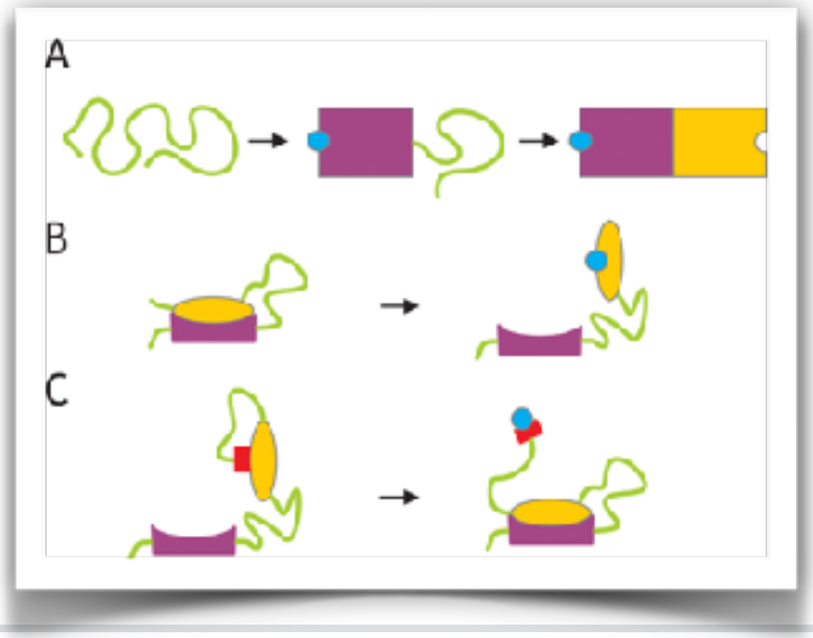


- les protéines sont des ensembles thermodynamiques complexes : multiples états en échange
- Différents ensembles conformationnels peuvent être fonctionnellement différents.
- Importance cruciale de caractériser la dynamique et la multiplicité des états d'une protéine le long de son cycle de vie.









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 (<10µM)

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 ?

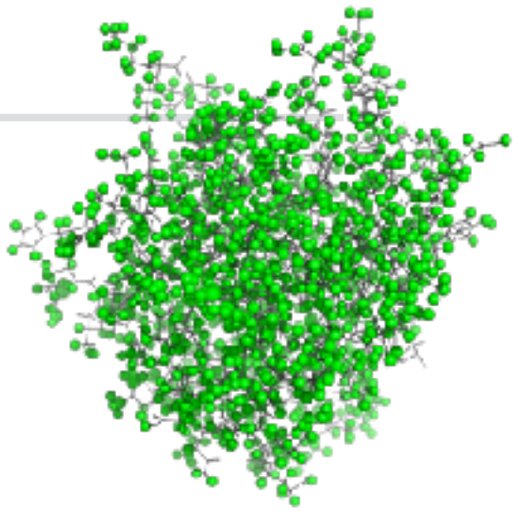
- Les questions spécifiques auxquelles la RMN peut apporter des réponses spécifiques

- 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 (± browniens) par rapport à un champ magnétique (principal ou crée par les autres spins)
      - relaxation ⇒ constantes de vitesse de relaxation R_1, $R_{2, \ldots}$
      - 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 chimique (variations structurales)
      - ± défocalisation des spins -> essentiellement échos de spin:
        - CPMG (R2, dispersion de relaxation) : $R2=f(k_{ex}, p_{min}, \Delta\omega, \nu_{CPMG,} N_{echos})$
        - gradients de champ B0 (diffusion): $I=f(D, Gdt, t_m)$
- Relations entre paramètres mesurés et paramètres dynamiques?
  - ⇨ Hamiltoniens dépendants du temps
  - ⇨ équation de Bloch-Mc Connel, etc.

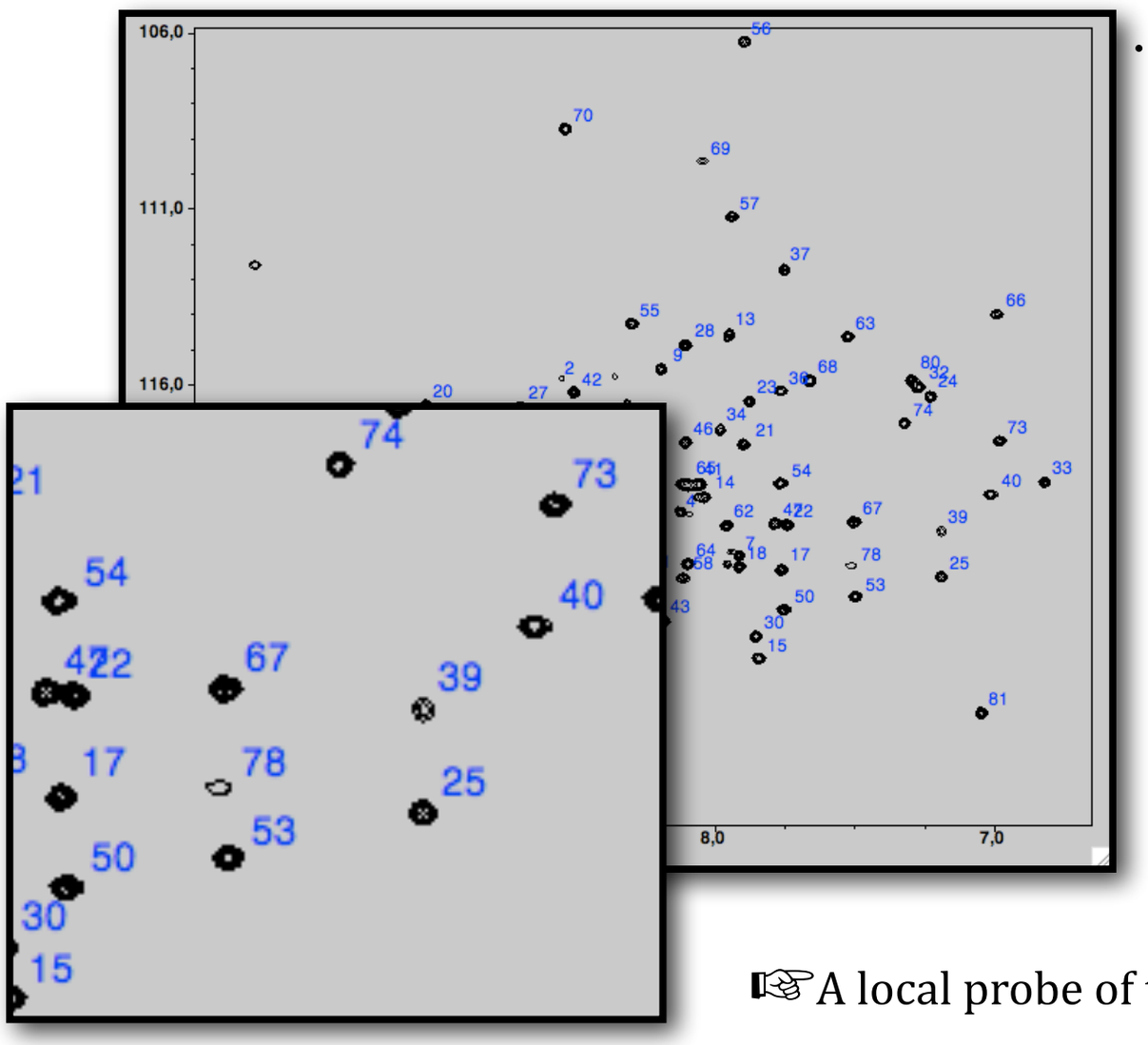
NMR for the study of proteins dynamics

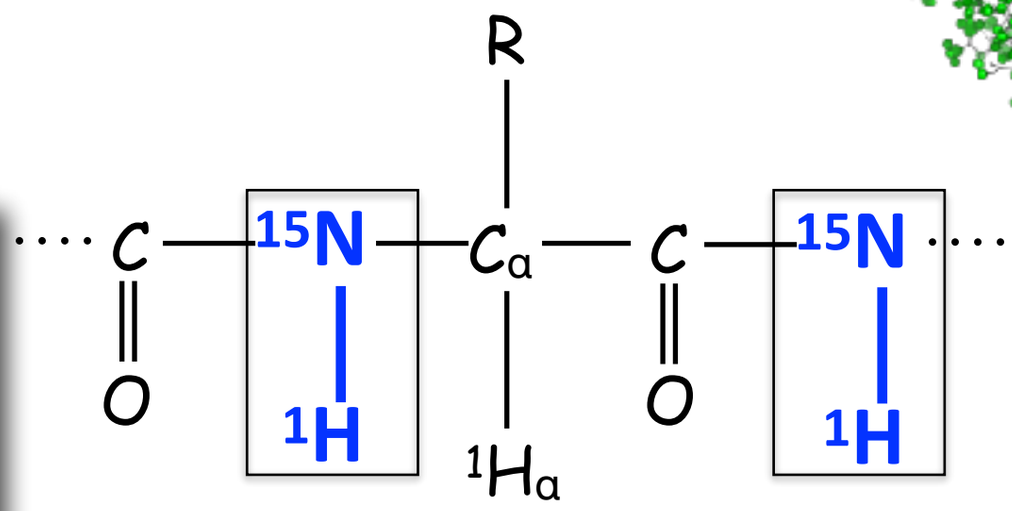


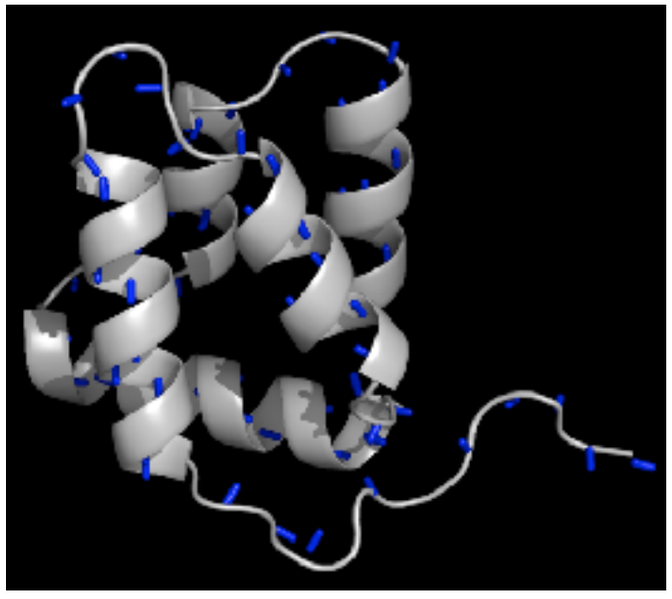
Questions

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

Spectre HSQC: empreinte de la protéine



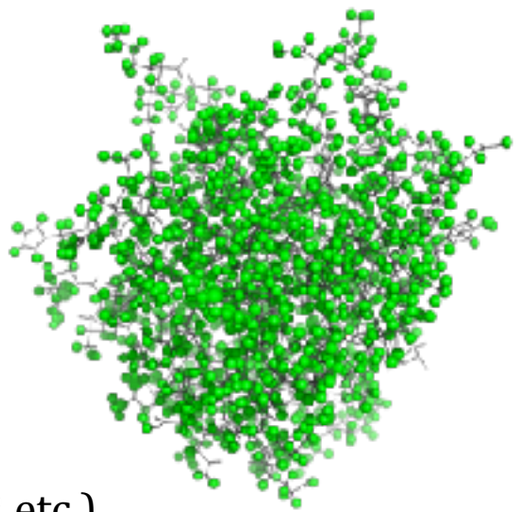




☞A local probe of the atoms electronic environment : resonance frequencies.

☞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 - ^{1}H, ^{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, pKa, k_{ex} …)

NMR for the study of proteins dynamics

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 conformationels

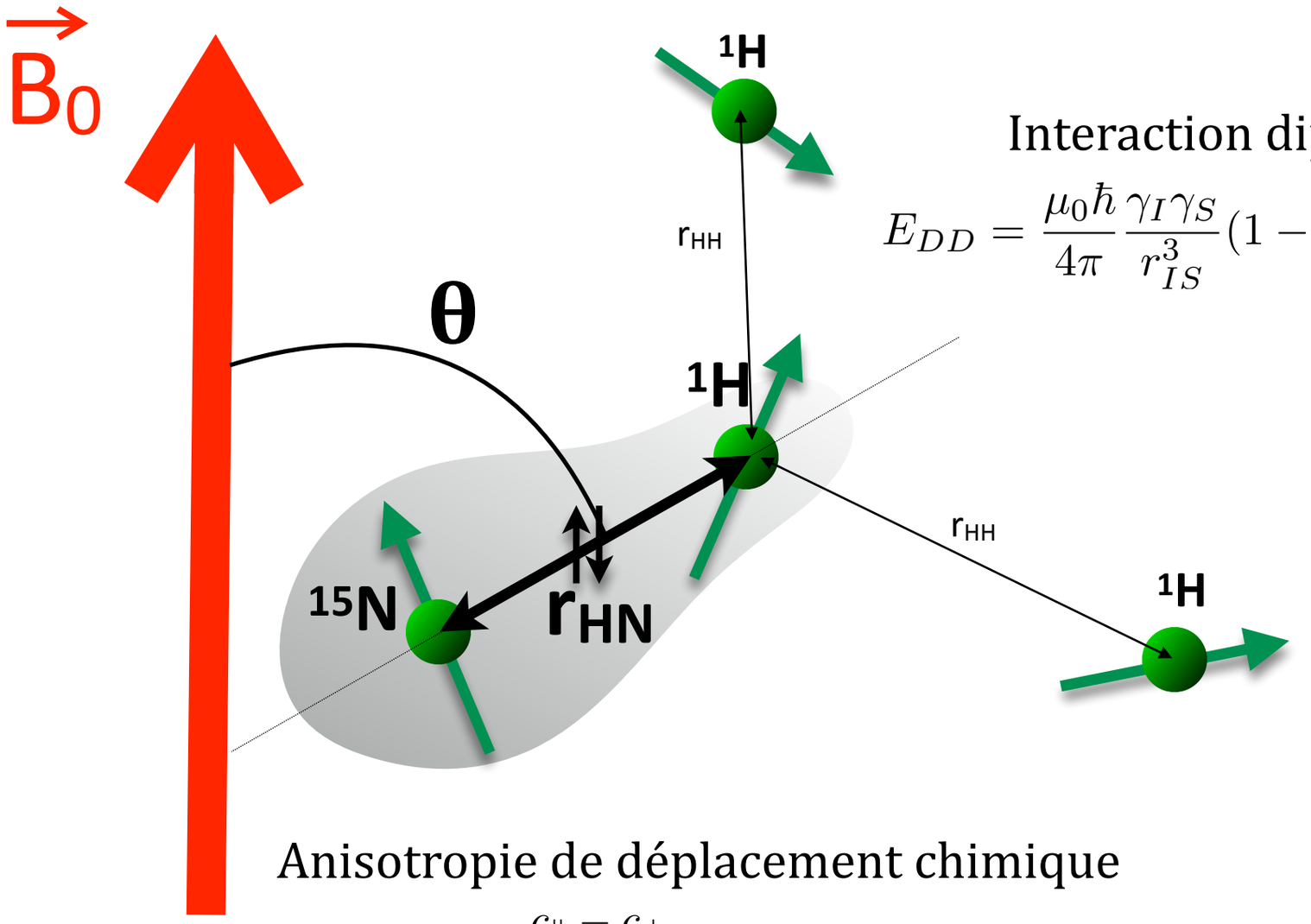
Cinétique et Thermodynamique

INTERACTIONS
SPINS/ELECTRONS/CHAMP

DEPLACEMENT CHIMIQUE
COUPLAGE SCALAIRE
COUPLAGE DIPOLAIRE
ANISOTROPIE de DEPLACEMENT CHIMIQUE

INFORMATIONS
sur la
DYNAMIQUE

- **f(environnement(t), r(t),θ(t))**



Interaction dipolaire

$$E_{DD} = \frac{\mu_0 \hbar}{4\pi} \frac{\gamma_I \gamma_S}{r_{IS}^3} (1 - 3cos^2\theta) m_I m_S$$

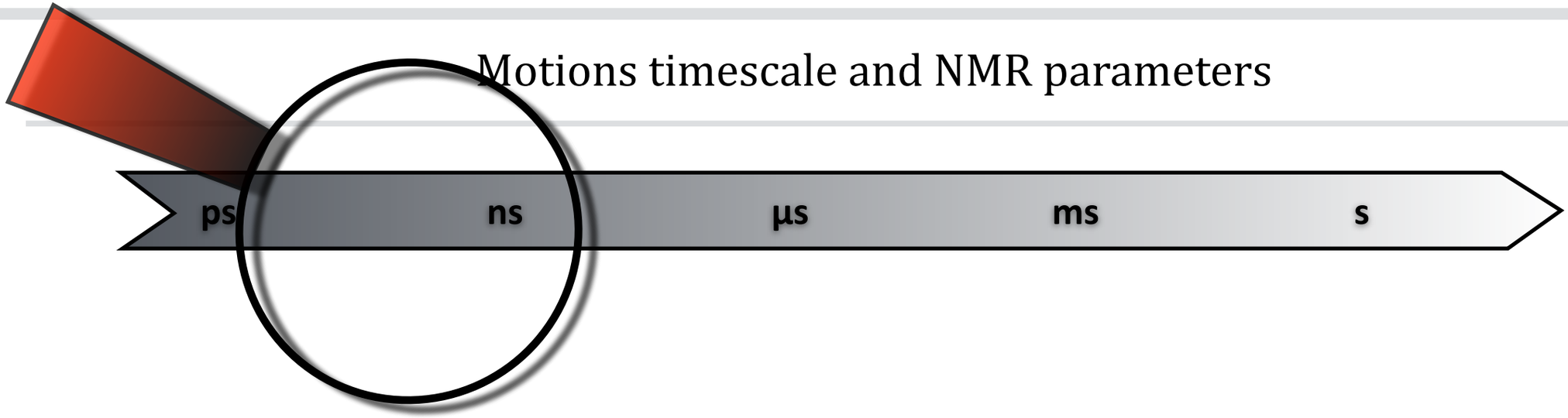
Interaction scalaire

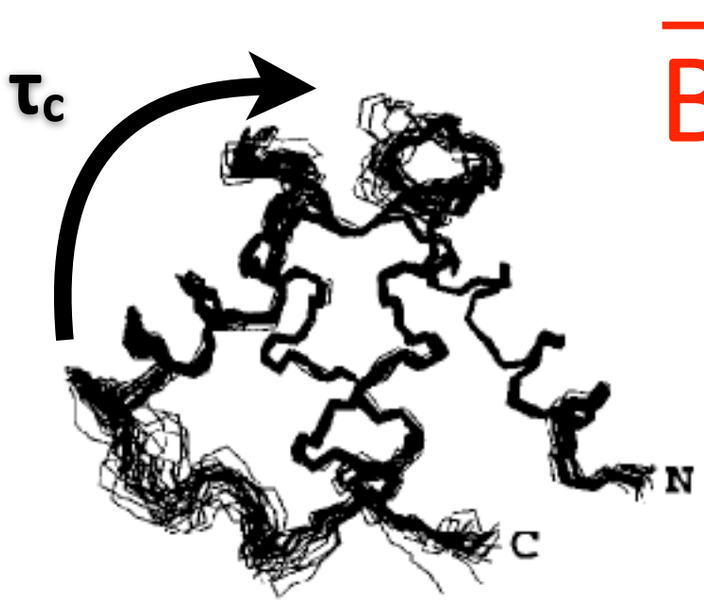
$$E_{CS} = 2\pi \hbar J_{IS} m_I m_S$$

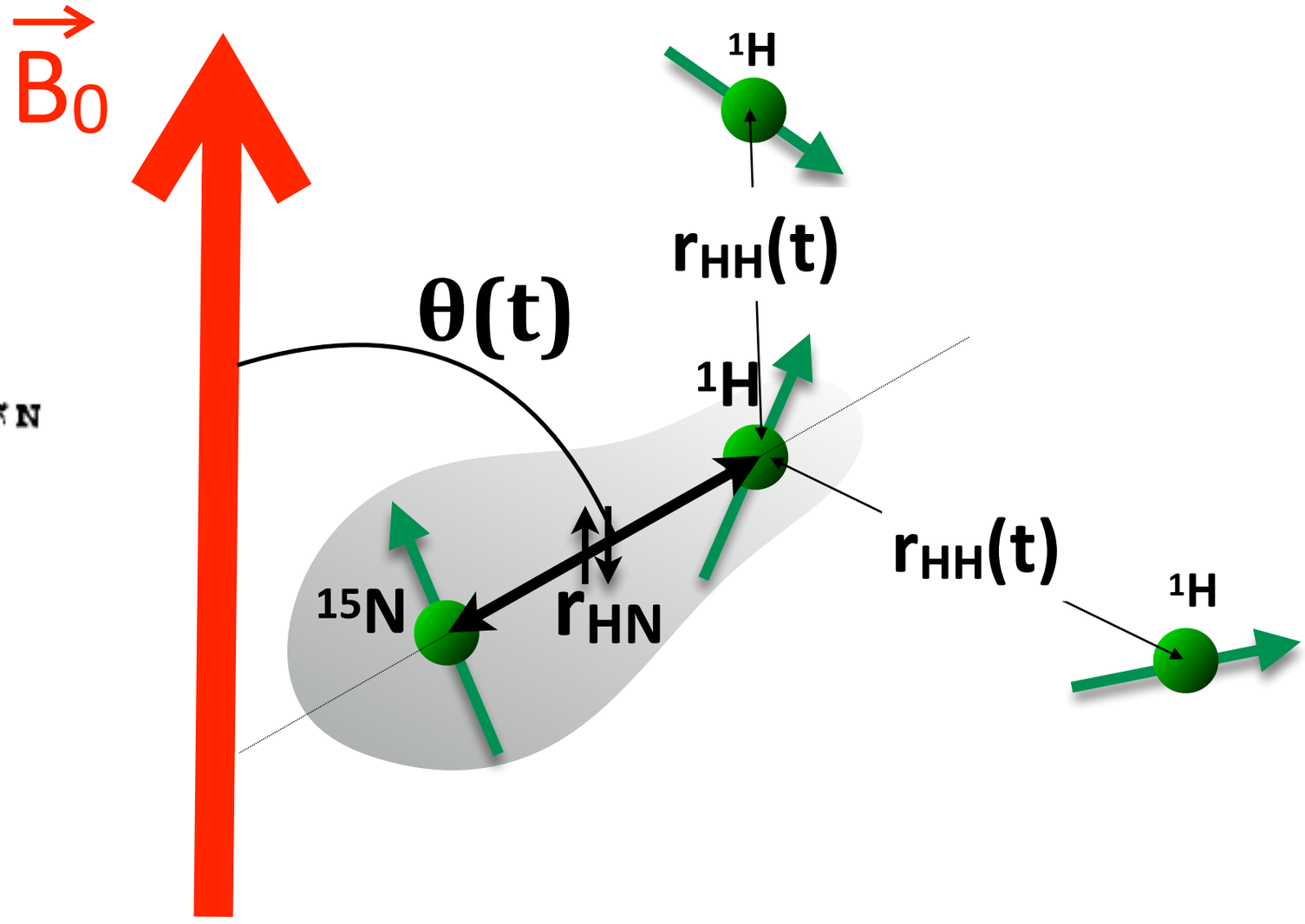
Anisotropie de déplacement chimique

$$E_{CSA} = \hbar \gamma_S \frac{c_\parallel - c_\perp}{3} B_0 (1 - 3cos^2\theta) m_S$$

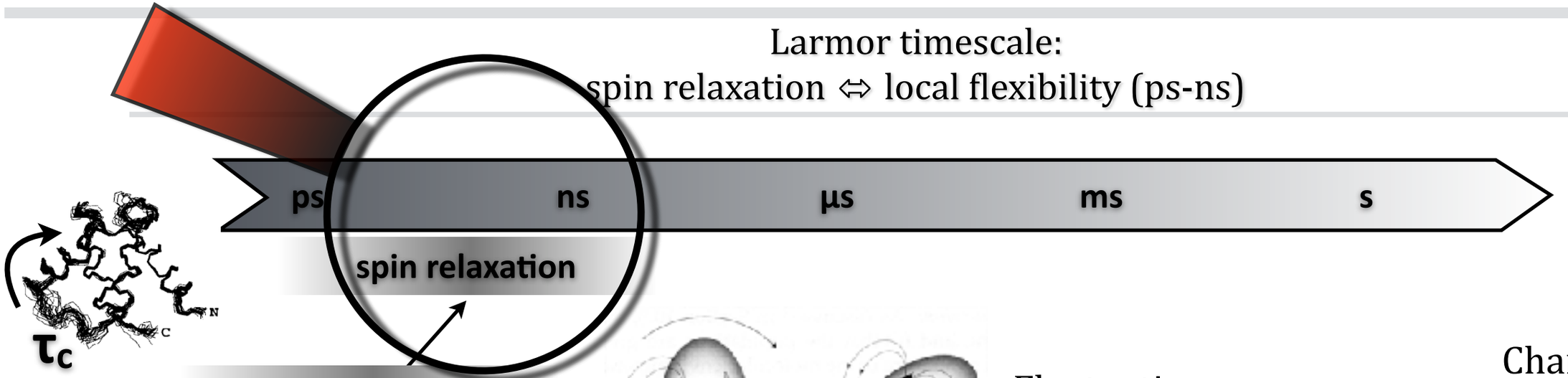
Motions timescale and NMR parameters







Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)



T_2, T_1, hetnOes
$J(0)$, $J(\omega_N)$,$J(\omega_H)$

rotational fluctuations
ps<->ns
models: τ_c,S^2
…

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

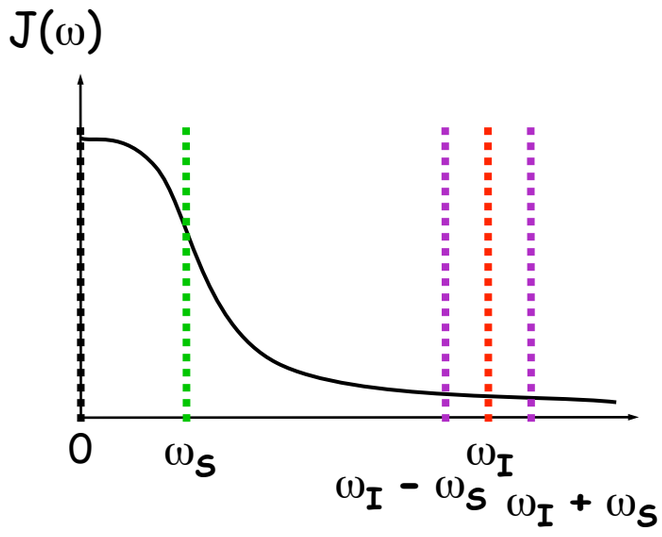
Relaxation macroscopique ms-s

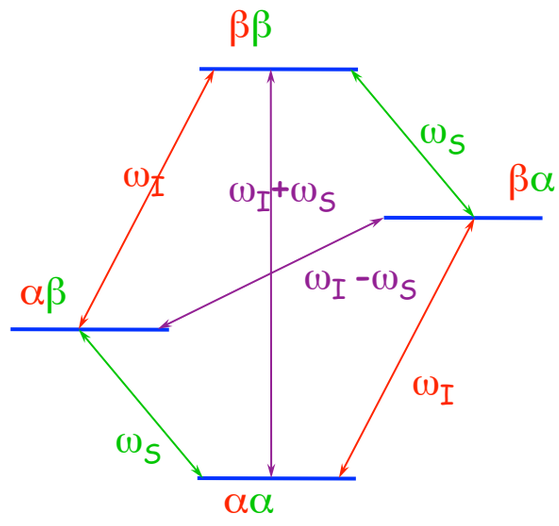
fluctuations ps-ns

Fluctuations moléculaires ⟷ Champs locaux fluctuants $B_{local}(t)$

↕ ↕

Spectre des fréquences des fluctuations (densité spectrale) ⟷ Fréquences des ondes électromagnétiques locales





Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)

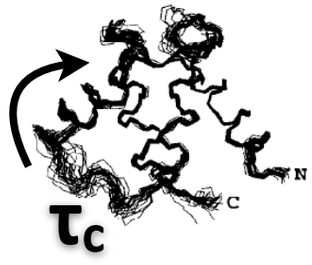
spin relaxation

interactions
fluctuations

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

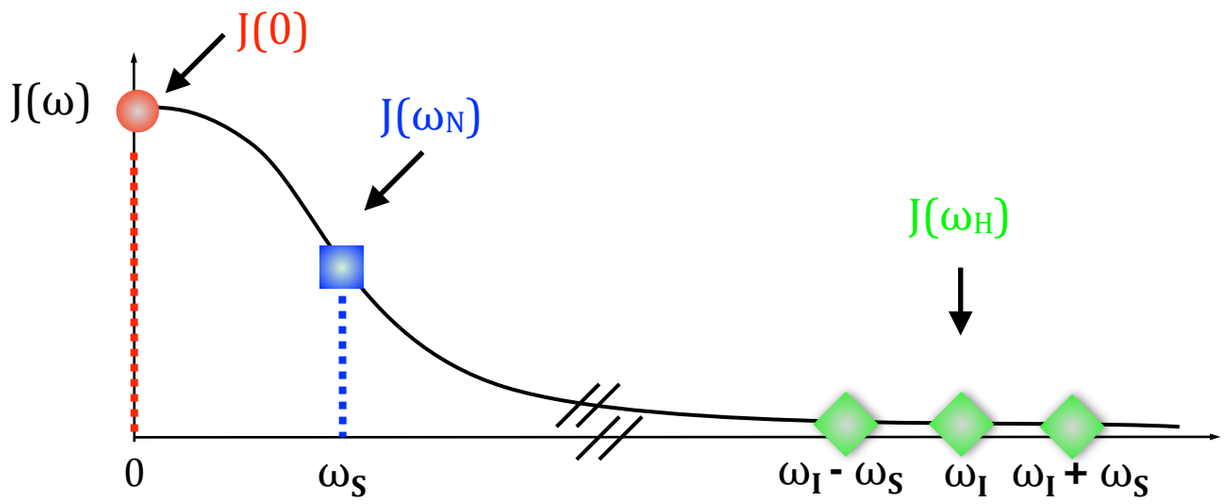
$$R_i = \frac{1}{T_i} = \overline{\langle B^2_{local}(t)\rangle} \sum_k c_k J_i(\omega_k)$$

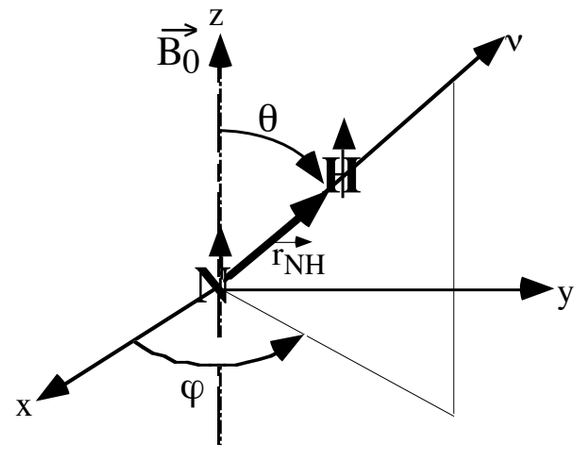
rotational fluctuations
ps<->ns
models: τ_c,S^2
…

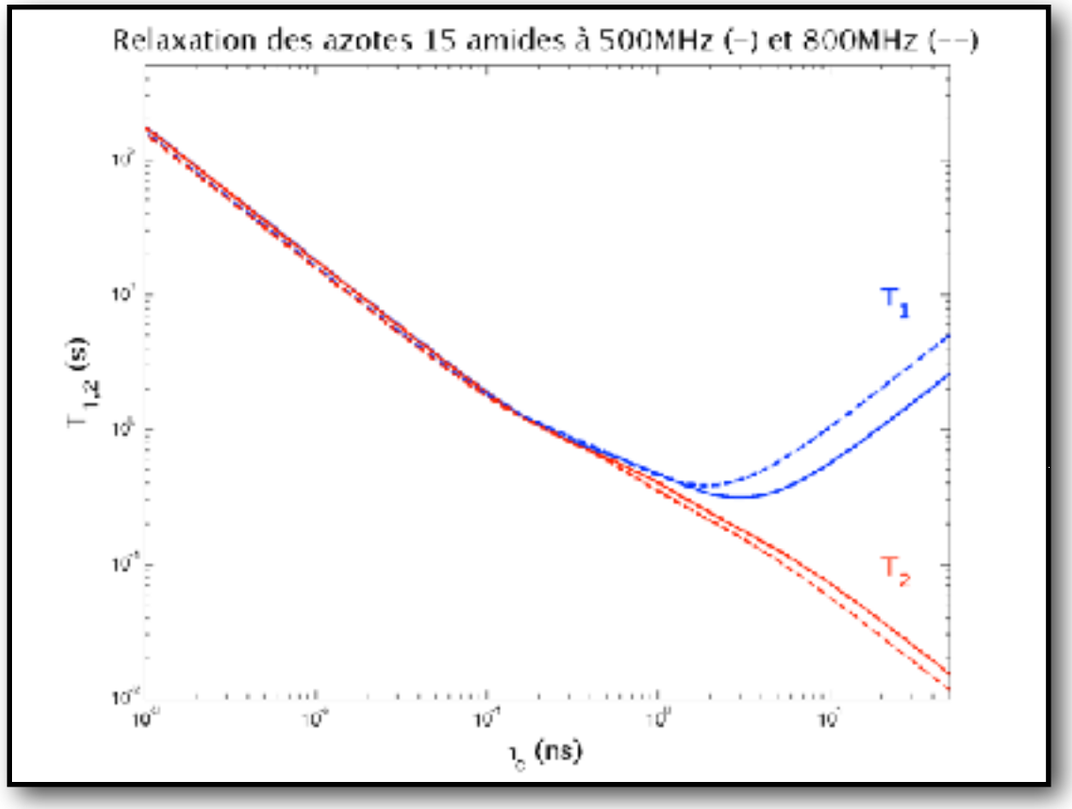


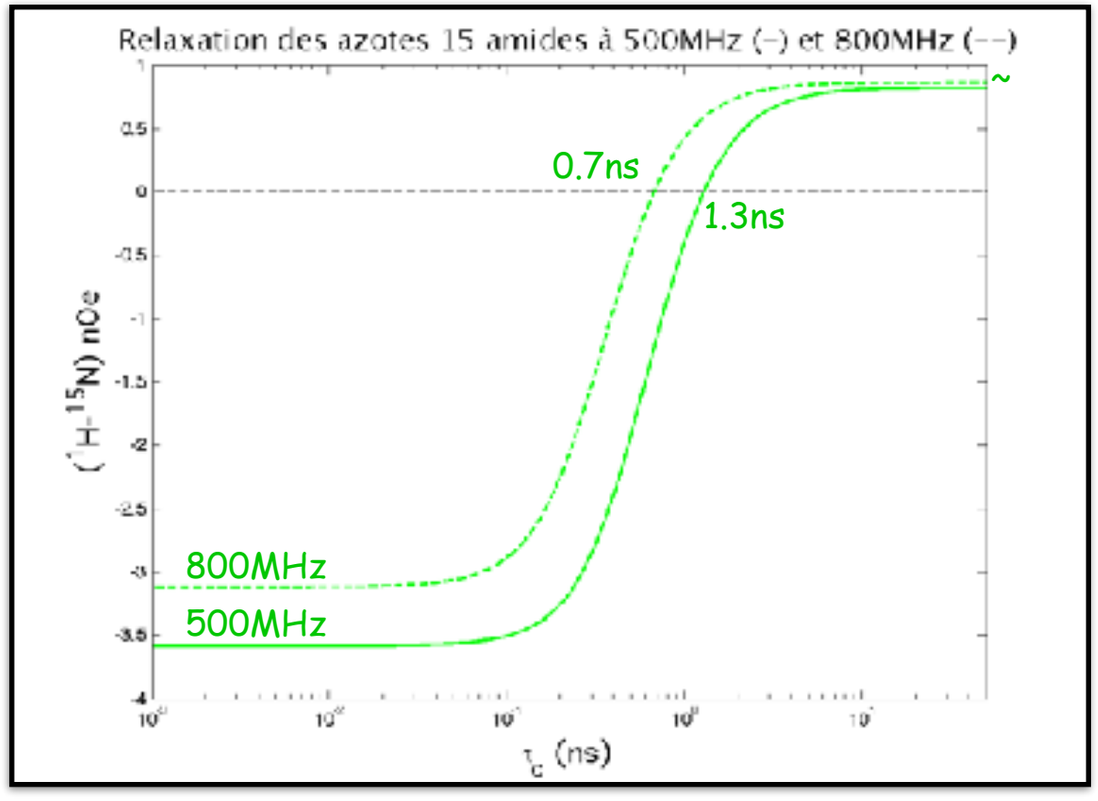
Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)

- ⇨ In practice : measurement of 3 relaxation parameters: R1, R2, and hetNOE
- ⇨ information on local flexibility of ^{1}H-^{15}N bounds in the 100ps-ns range.





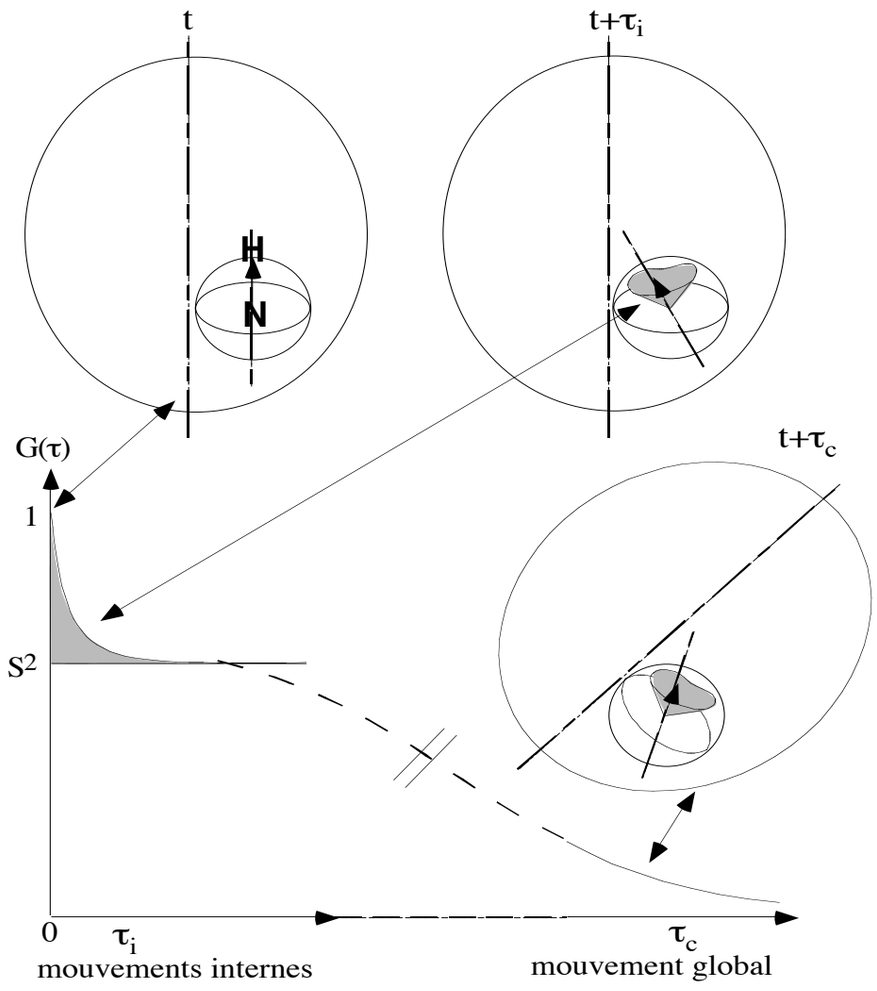




Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)

In practice

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

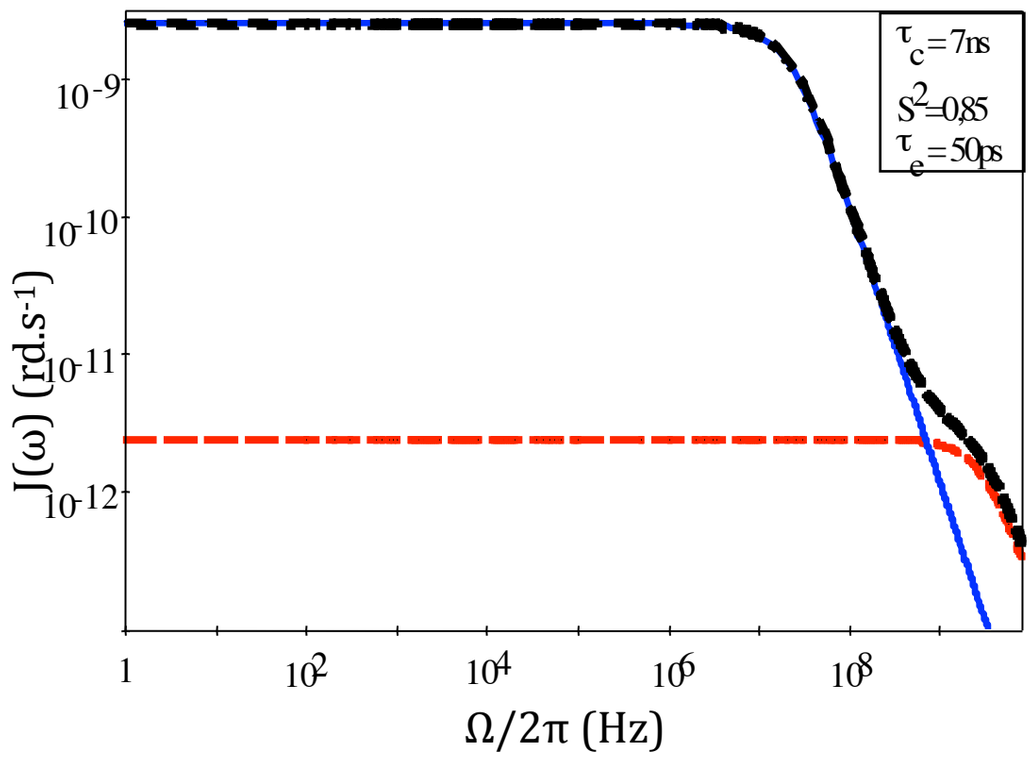


$$G(\tau)=G_{glb}(\tau)G_{int}(\tau)$$

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

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

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



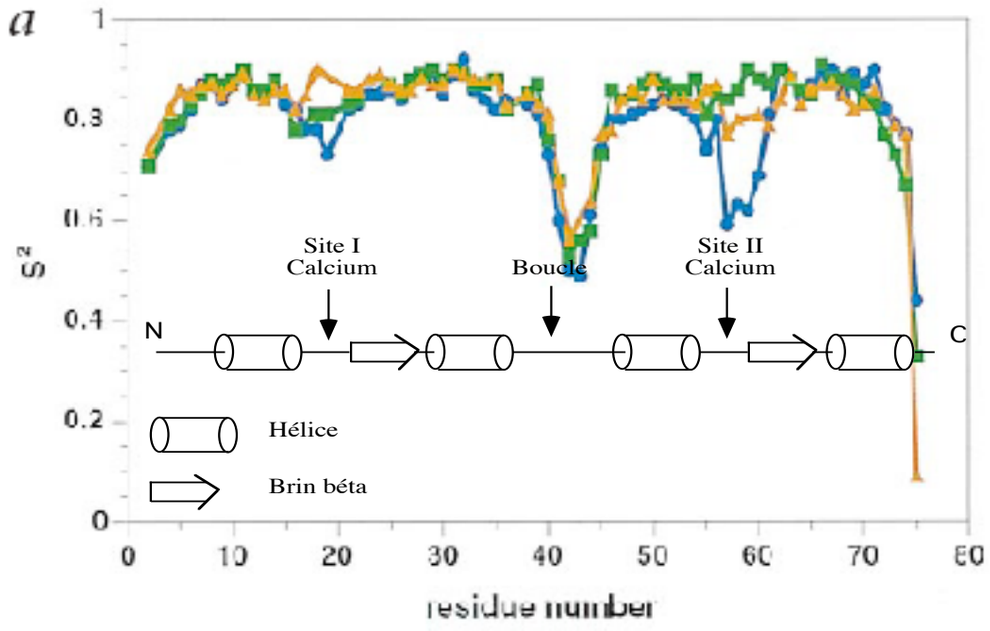
Lipari & Szabo "model free" approach

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

Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)

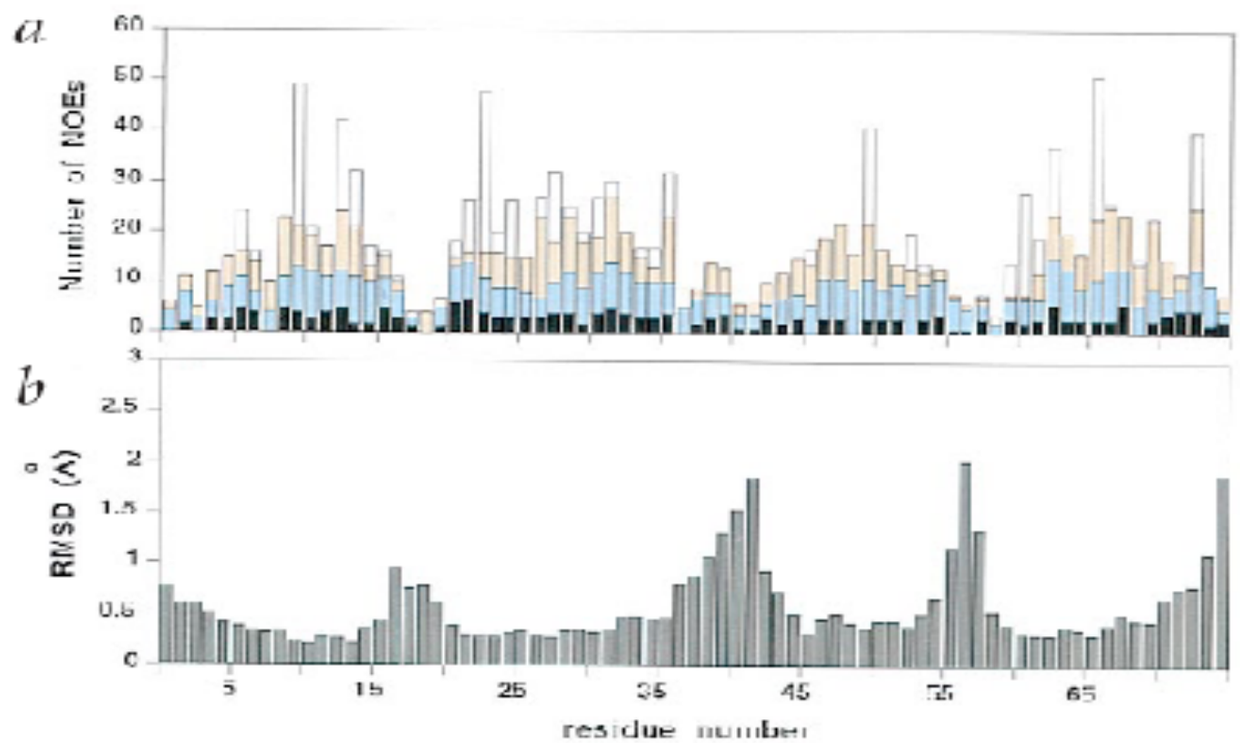
- measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^{1}H-^{15}N bounds in the 100ps-ns range.

- local flexibility in structured proteins



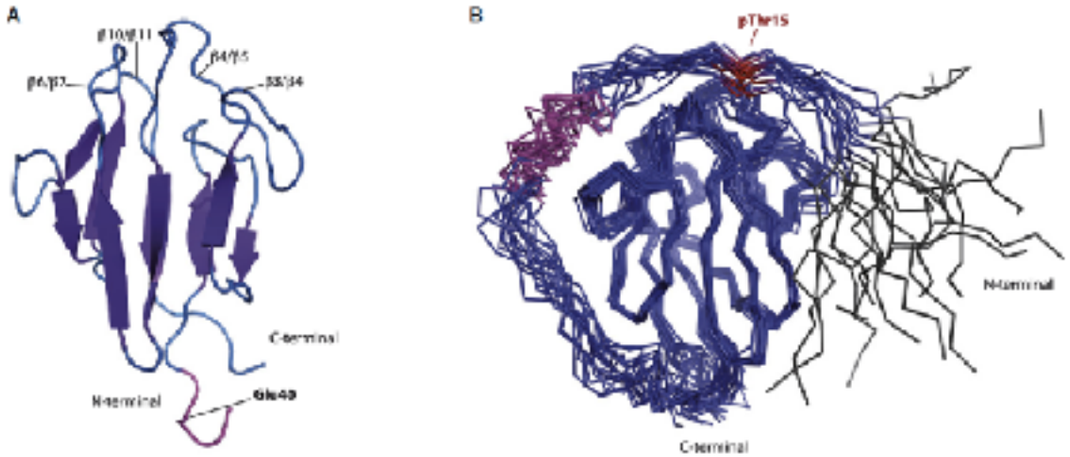
Calbindin D_{9k}
Apo
$(Ca_{2+})_1^{I}$-N56A
$(Cd_{2+})_1^{II}$

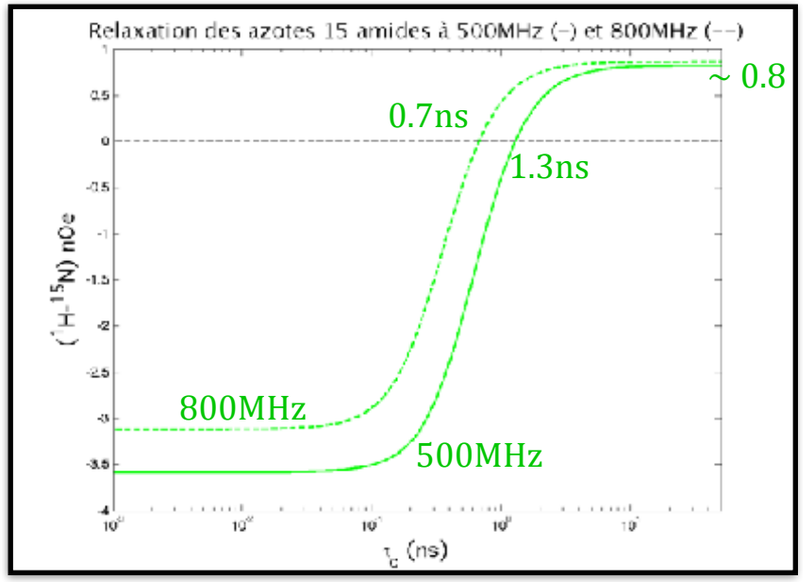
Mäler et al., Nat. Struct. Biol. 7, 245 (2000)

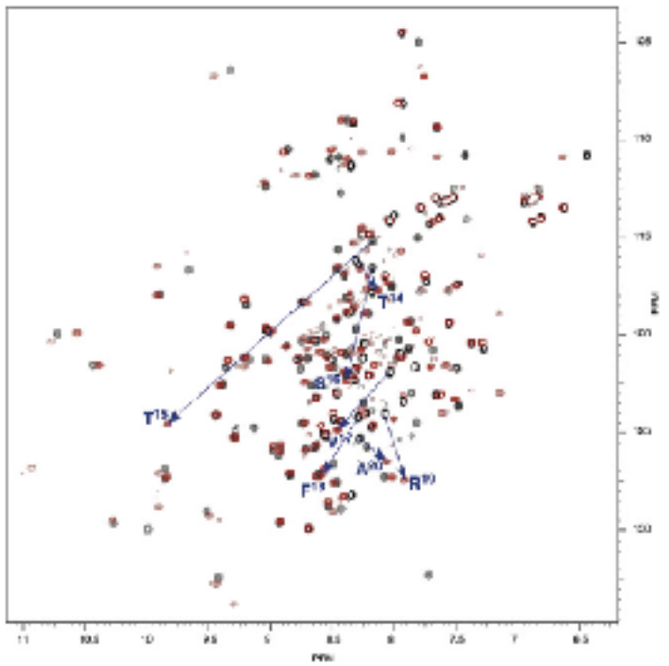


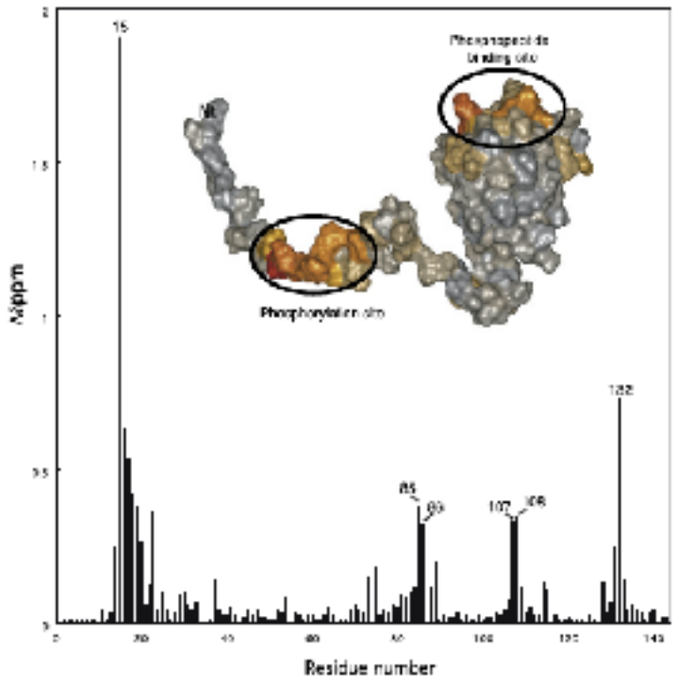
Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)

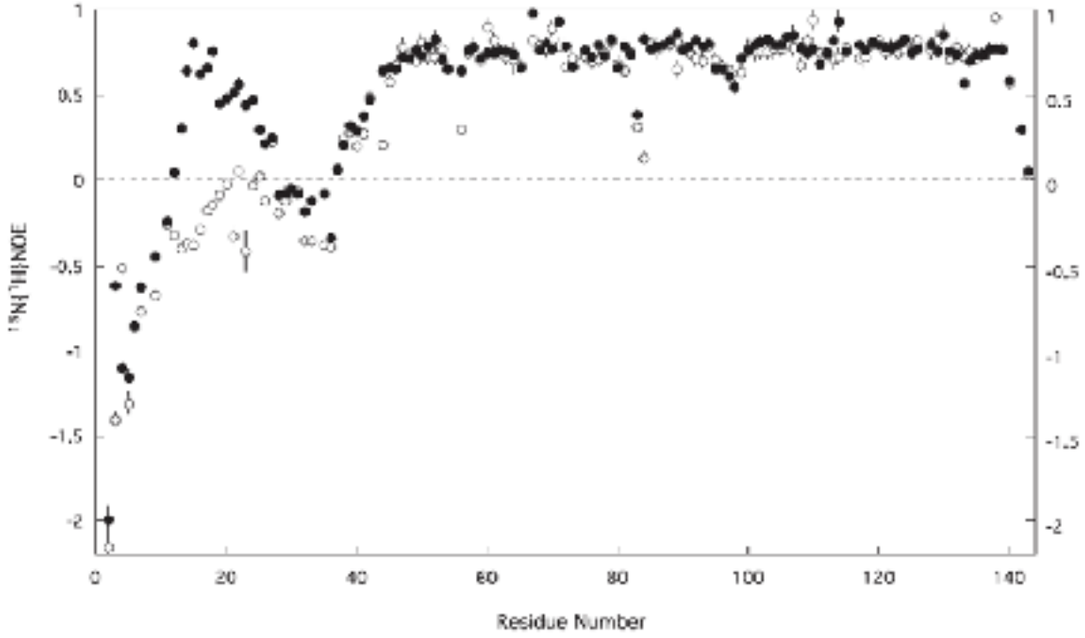
- heteronuclear ($^{1}H^{N}$->^{15}N) nOes
- easy to measure
- qualitative information about local flexibility in the 100ps range





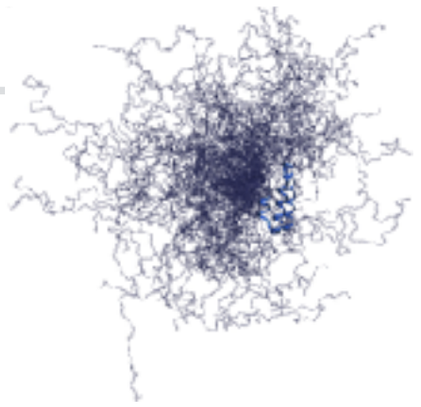




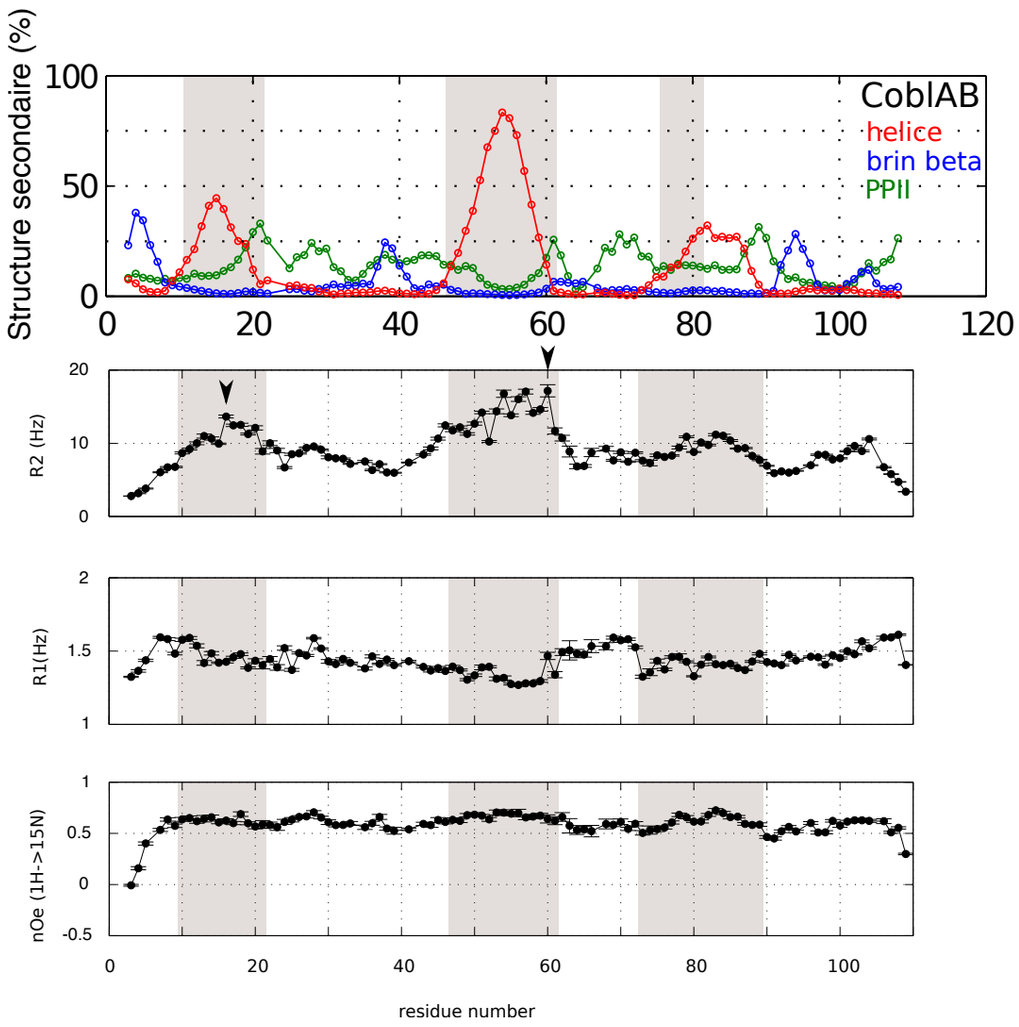


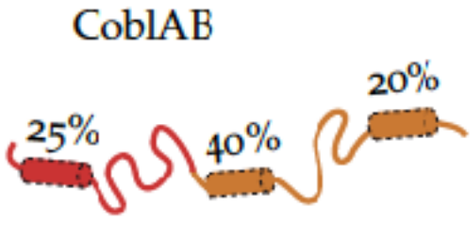
Barthe et al, “Dynamic and Structural Characterization of a Bacterial FHA Protein Reveals a New Autoinhibition Mechanism”. Structure 17, 568-578 (2009).

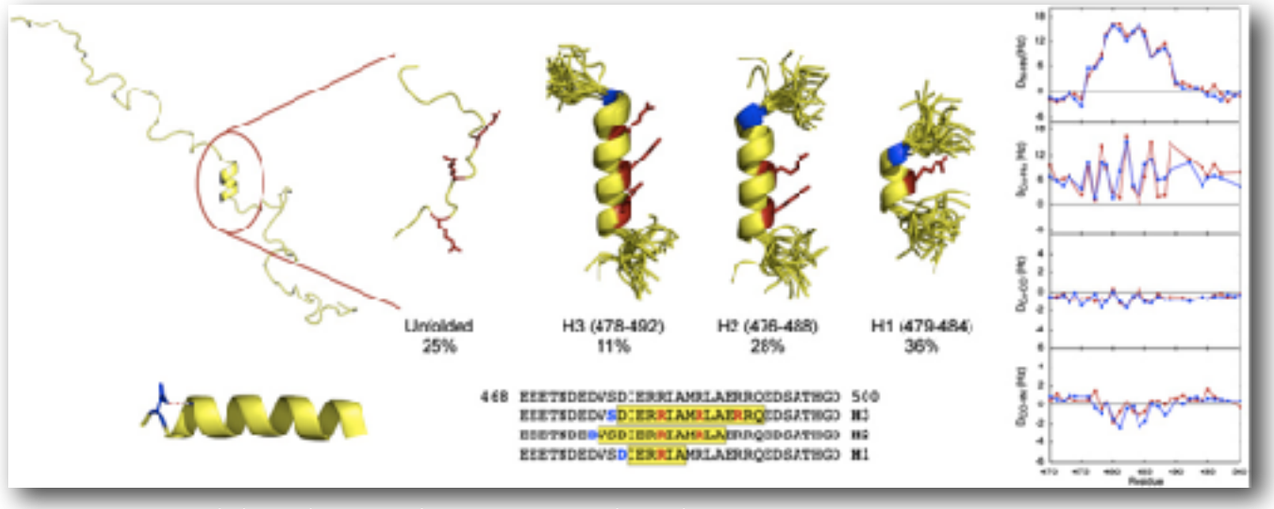
Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)



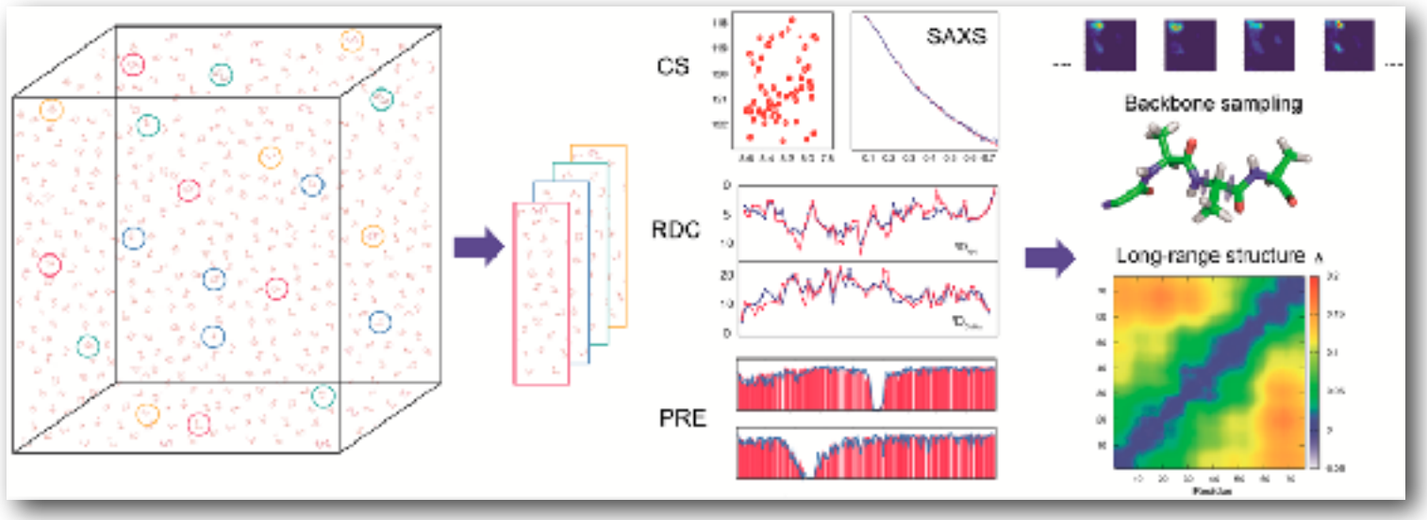
- Measurement of 3 relaxation parameters: R1, R2, and hetNOE: information on local flexibility of ^{1}H-^{15}N bounds in the 100ps-ns range.
- Local structuration in disordered proteins: secondary structures propensities (+CS, RDCs, PRE, SAXS → conformational ensembles)





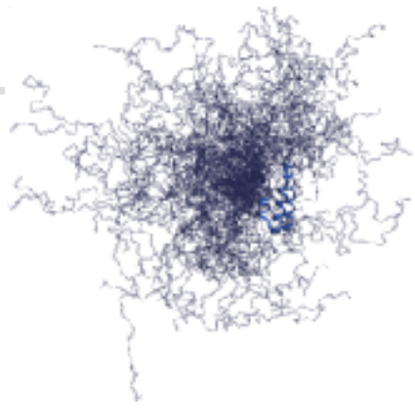


Jensen, M.R et al.,(2008) J. Am. Chem. Soc. 130 ; (2009) structure 17.



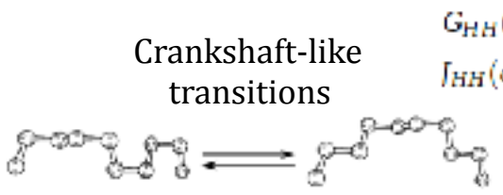
Jensen, Zweckstetter, Huang &Blackledge, Chemical reviews 2014, special issues on IDPs
Exploring Free-Energy Landscapes of Intrinsically Disordered Proteins at Atomic Resolution Using NMR Spectroscopy

Larmor timescale:
spin relaxation ⇔ local flexibility (ps-ns)



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

Crankshaft-like transitions



$$G_{HH}(t) - e^{t/\tau_1} e^{t/\tau_2} J_0(t/\tau_1)$$

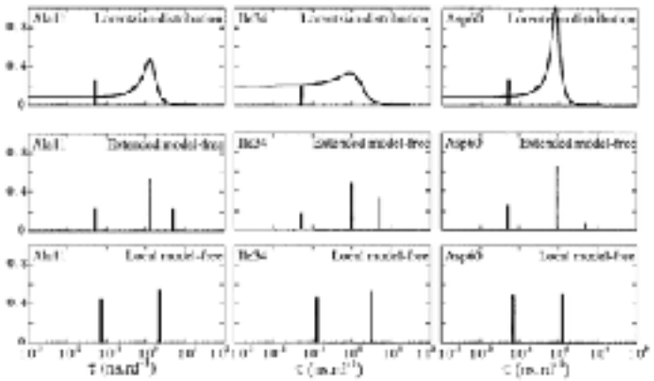
$$J_{HH}(\omega) - Re(\alpha + i\beta)^{1/2}$$

$$\alpha = \tau_2^{\,2} + 2\tau_1 - 1\tau_2^{\,1} - \omega^2$$

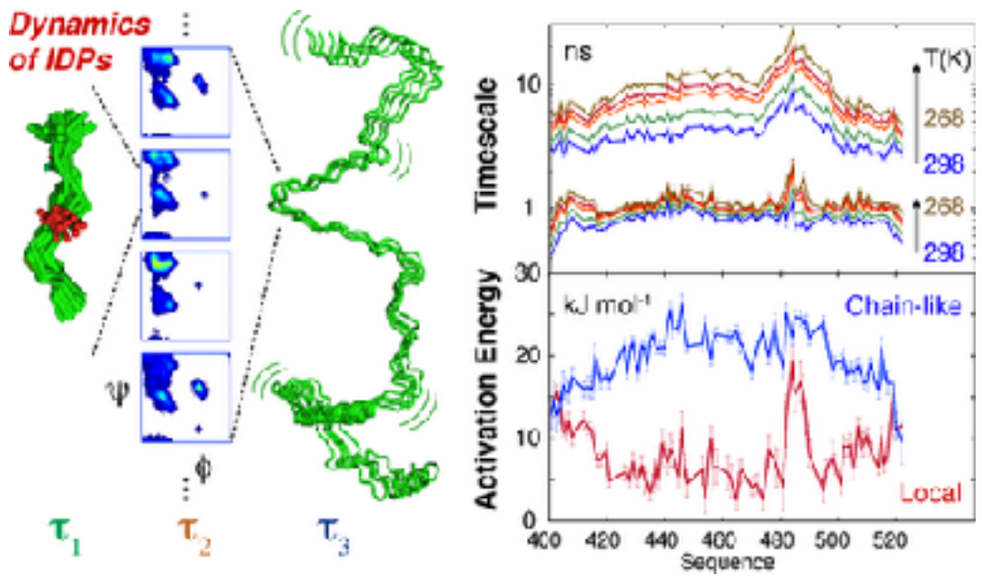
$$\beta - \quad 2\omega(\tau_1^{-1} + \tau_2^{-1})$$

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

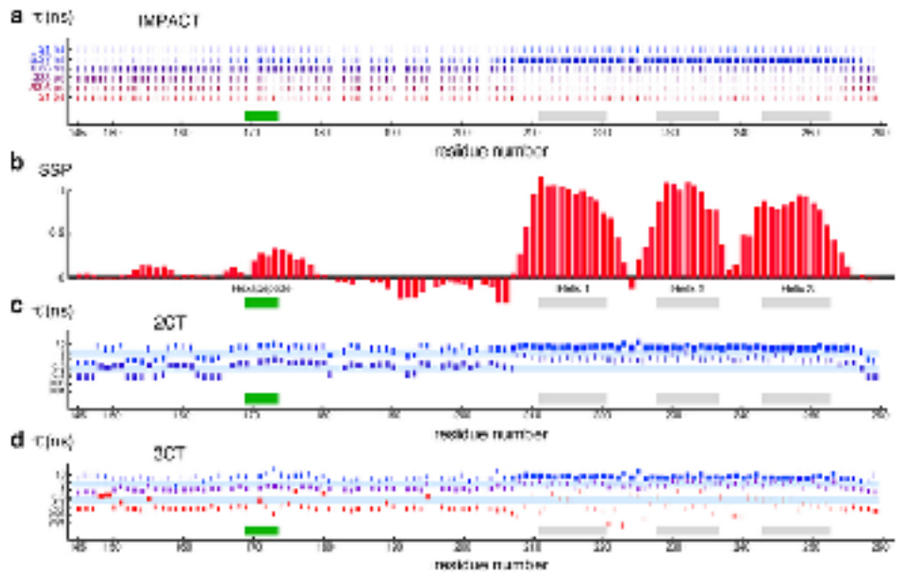
Ochsenbein et al., Protein Science (2002), 11:957–964.
Dynamical characterization of residual and non-native structures in a partially folded protein by 15N NMR relaxation using a model based on a distribution of correlation times



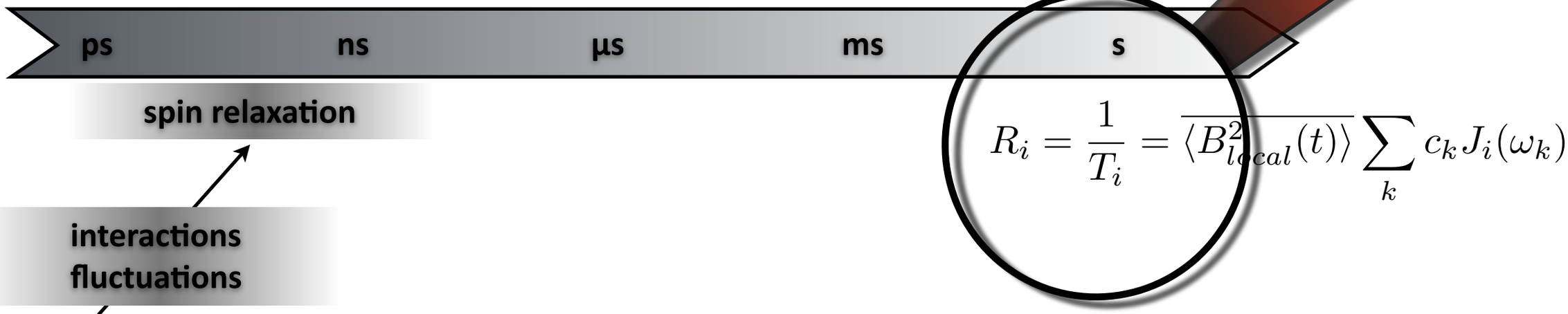
Abyzov et al., J. Am. Chem. Soc. 2016, 138, 6240−6251
Identification of Dynamic Modes in an Intrinsically Disordered Protein Using Temperature-Dependent NMR Relaxation



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

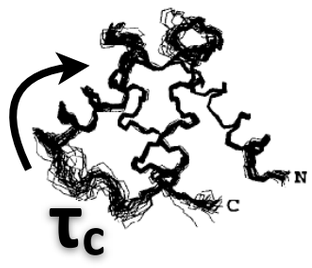


Motions timescale and NMR parameters



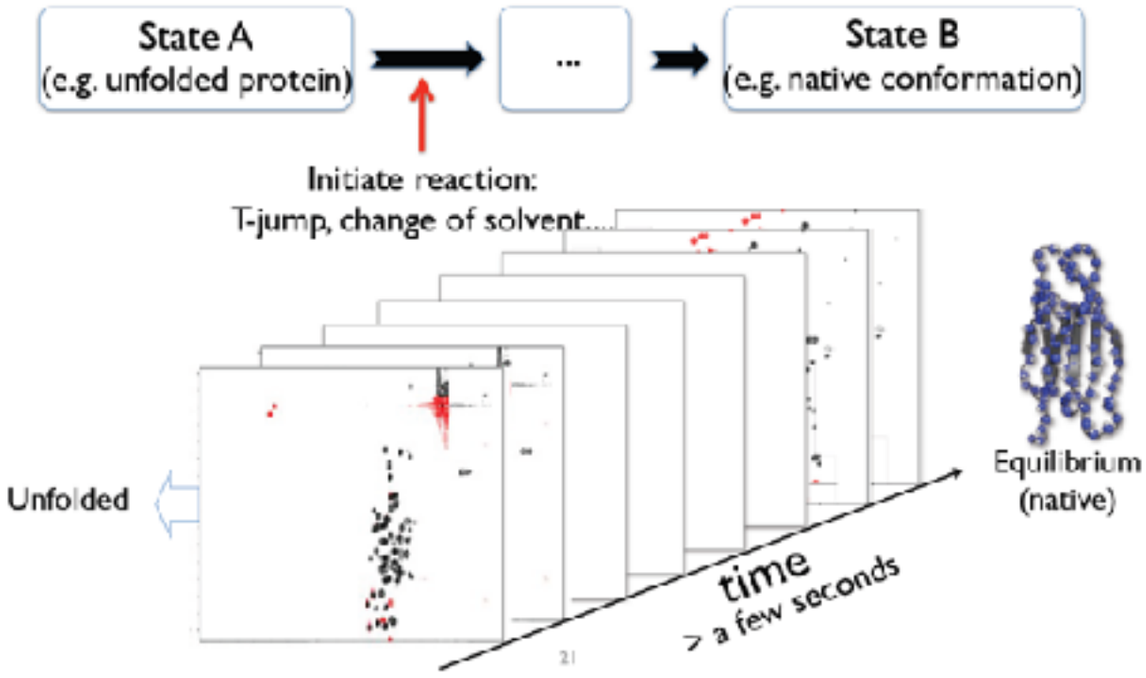
microscopic processes -> macroscopic relaxation parameters [T_2, T_1, hetnOes]
depend on the spectral distribution of the molecular fluctuations
-> J(0), J(ω_N),J(ω_H)

rotational fluctuations
ps<->ns
models: τ_c,S^2
...

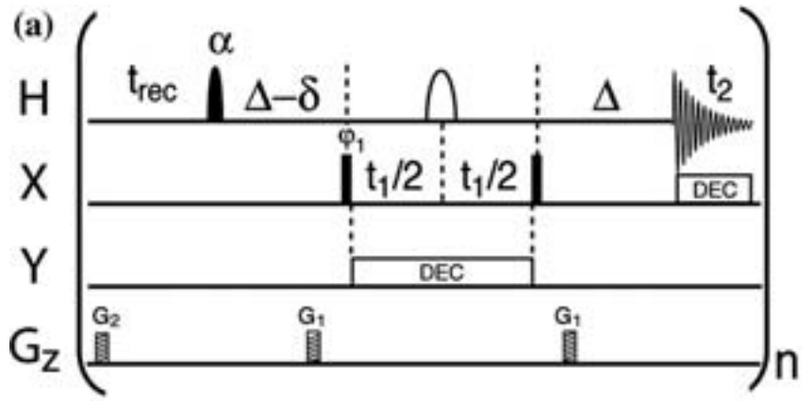


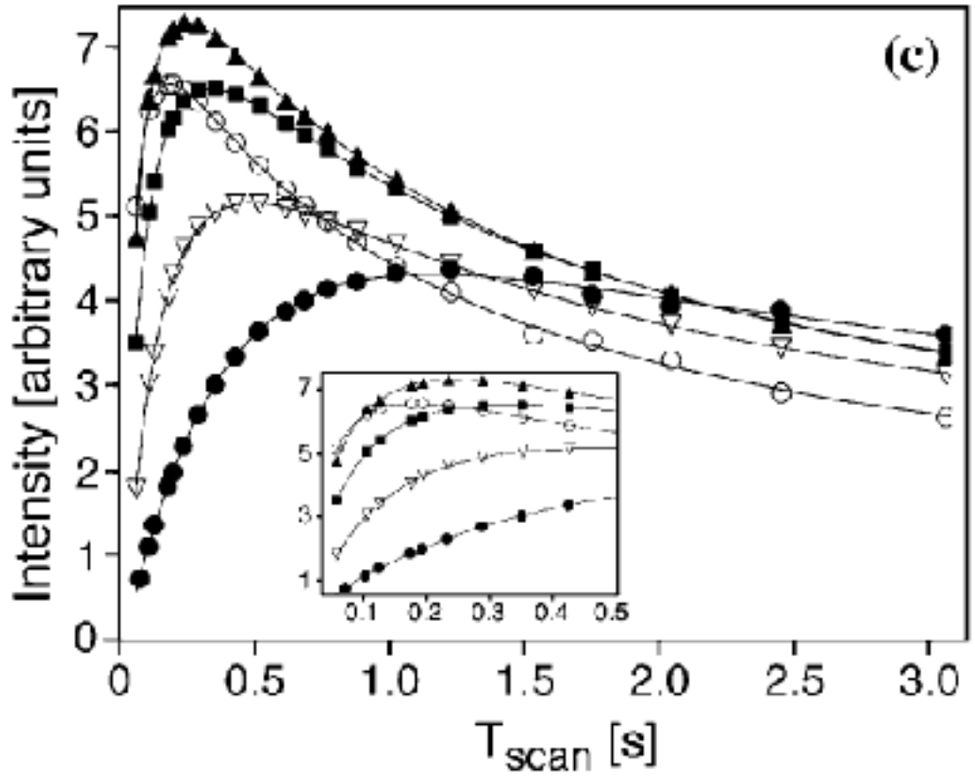
Temps de retour à l'équilibre
Expériences en temps réel : repliement, échanges avec le solvant, interactions

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.



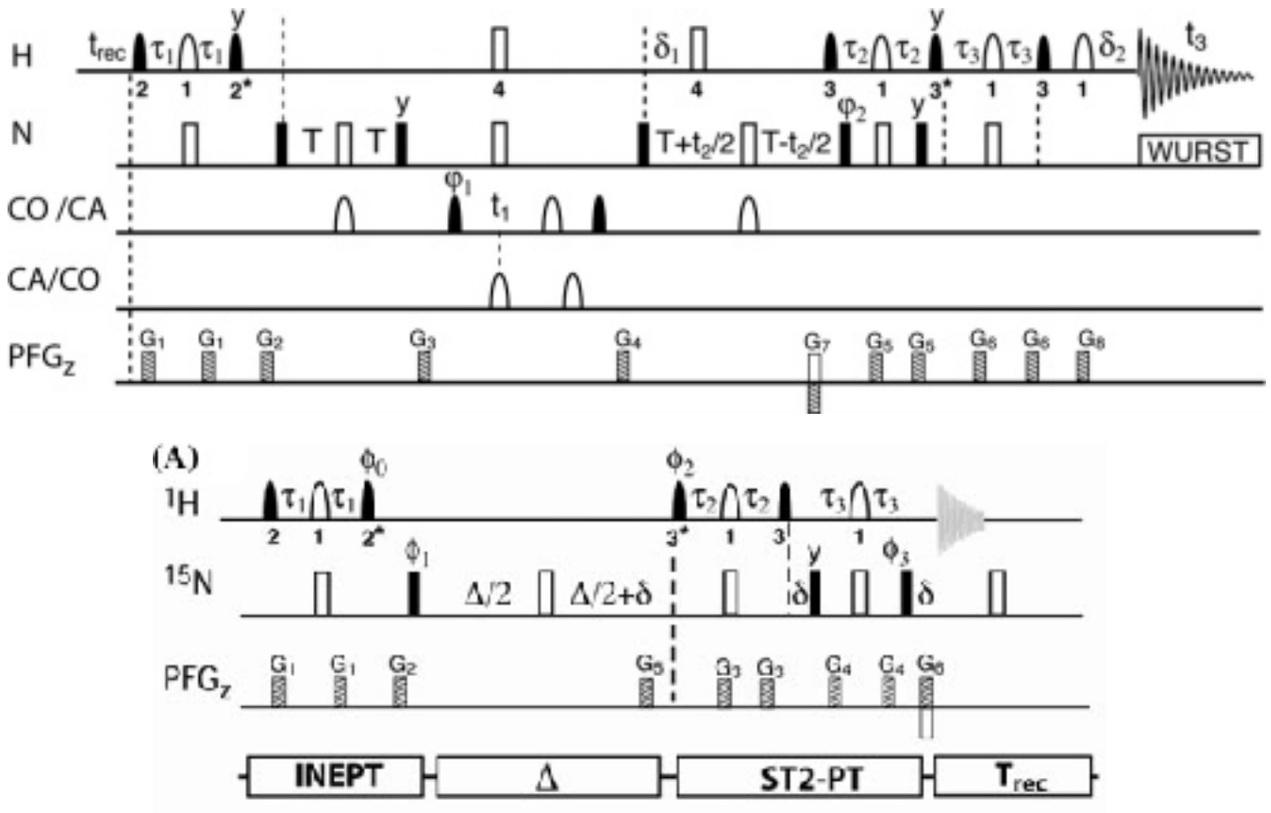
"Jouer" avec T1 : les expériences FAST/BEST

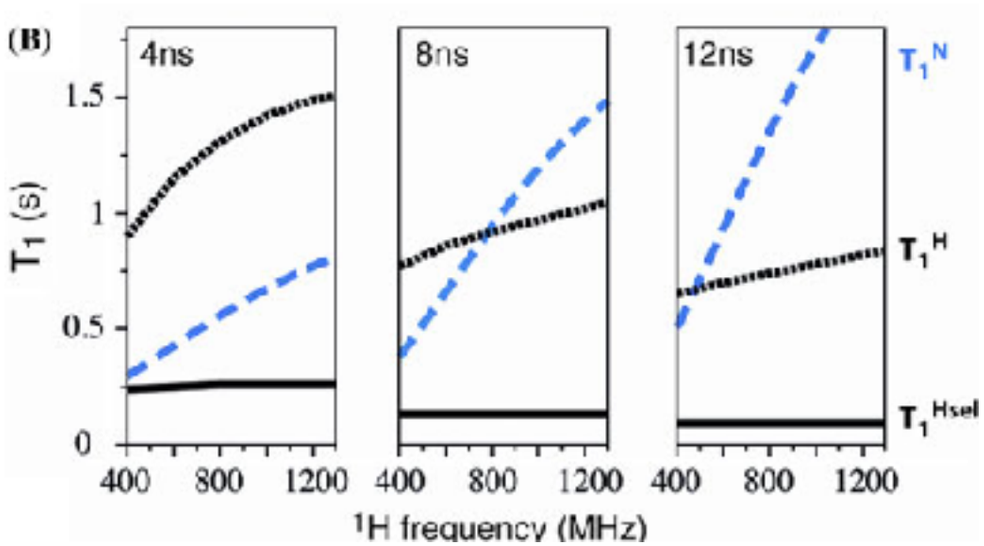




Schanda et al. J. Biomol. NMR (2005) 33: 199–211

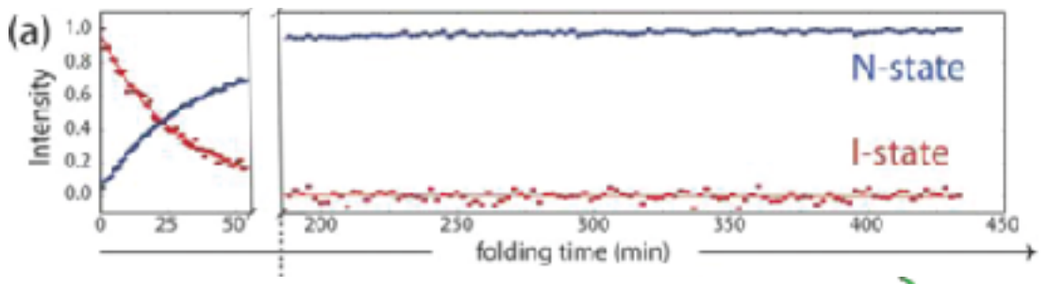
Temps de retour à l'équilibre
Expériences en temps réel : repliement, échanges avec le solvant, interactions

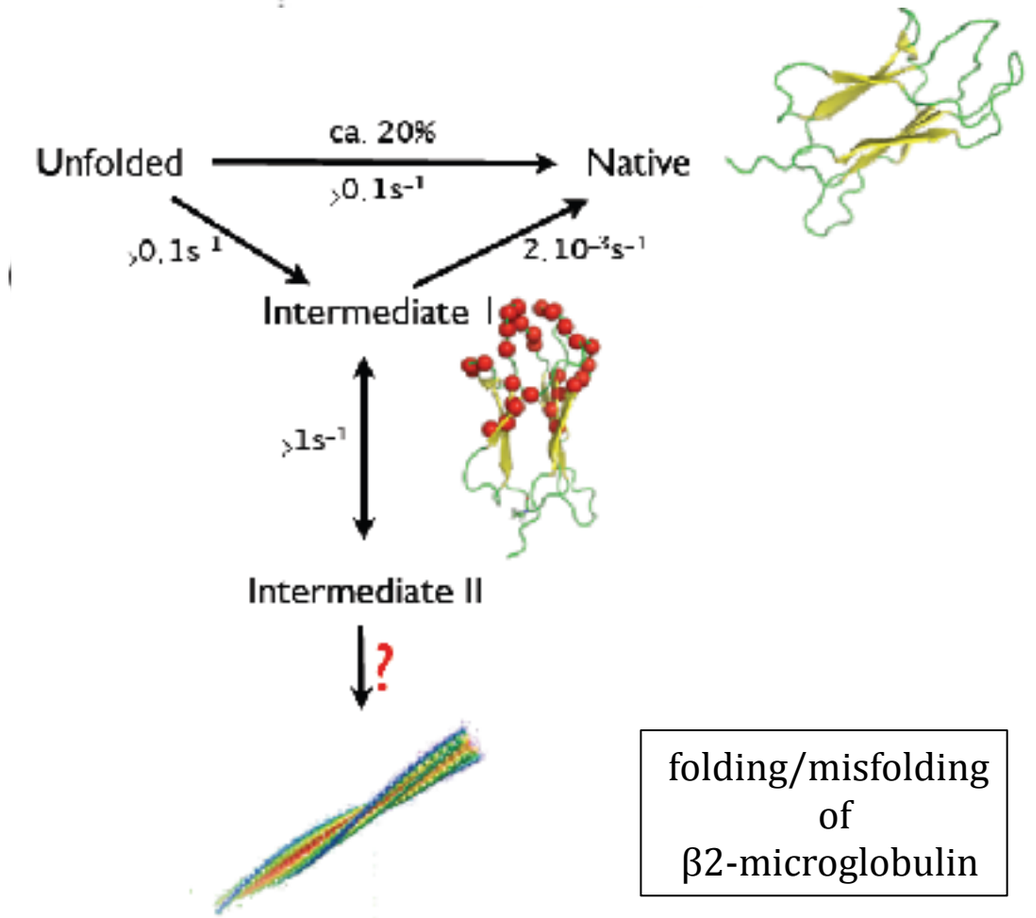




Schanda et al., JACS 2006, Favier & Brutscher, J. Biomol. NMR 2011

"Jouer" avec T1 : les expériences FAST/BEST





folding/misfolding of β2-microglobulin

Corazza et al., J. Biol. Chem. 2010, Rennella et al. J. Am. Chem. Soc. 2012

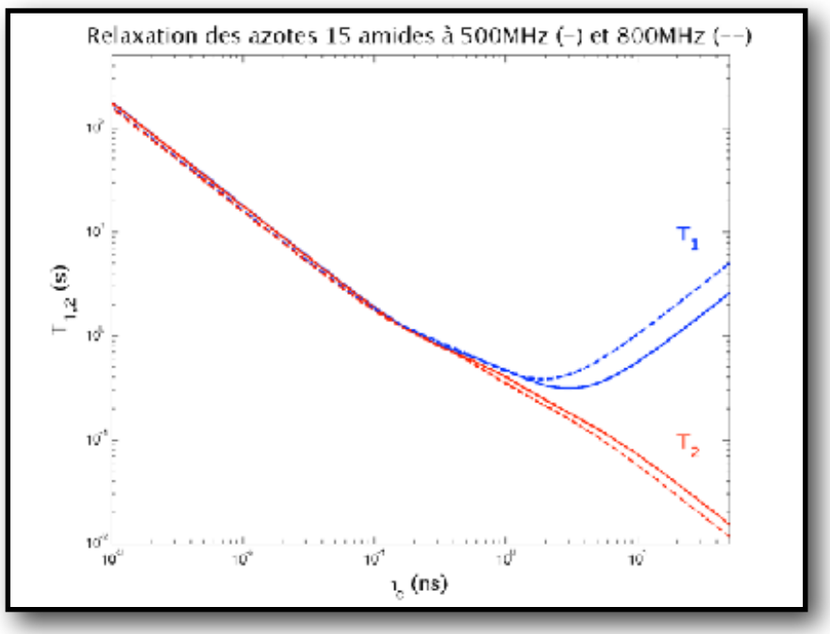
Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

Le temps de vie du signal

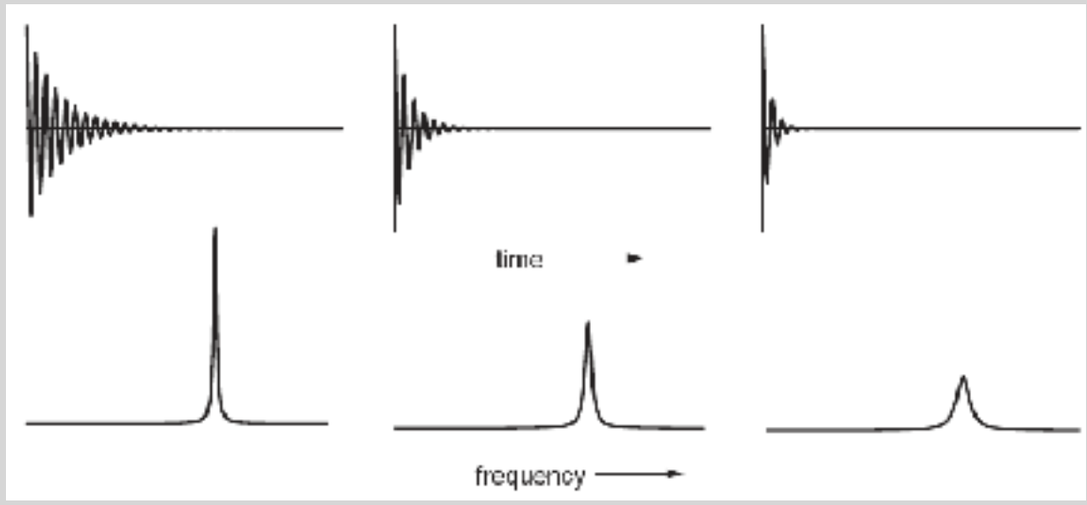
Le temps de relaxation transversale T2 définit le temps de vie du signal RMN.

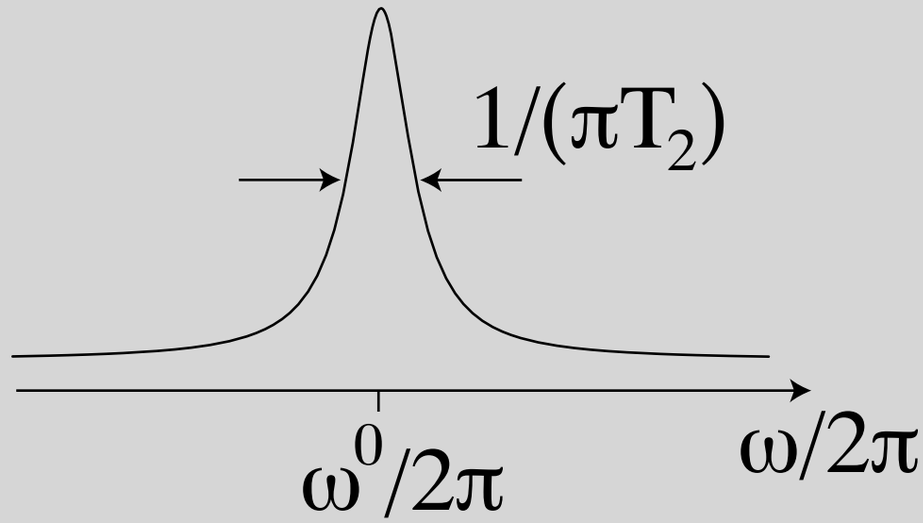
Il détermine la largeur des résonances

Le temps T2 dépend du champ magnétique B0, de la nature des spins, de la taille des molécules, de la dynamique du système, de la température, etc.



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



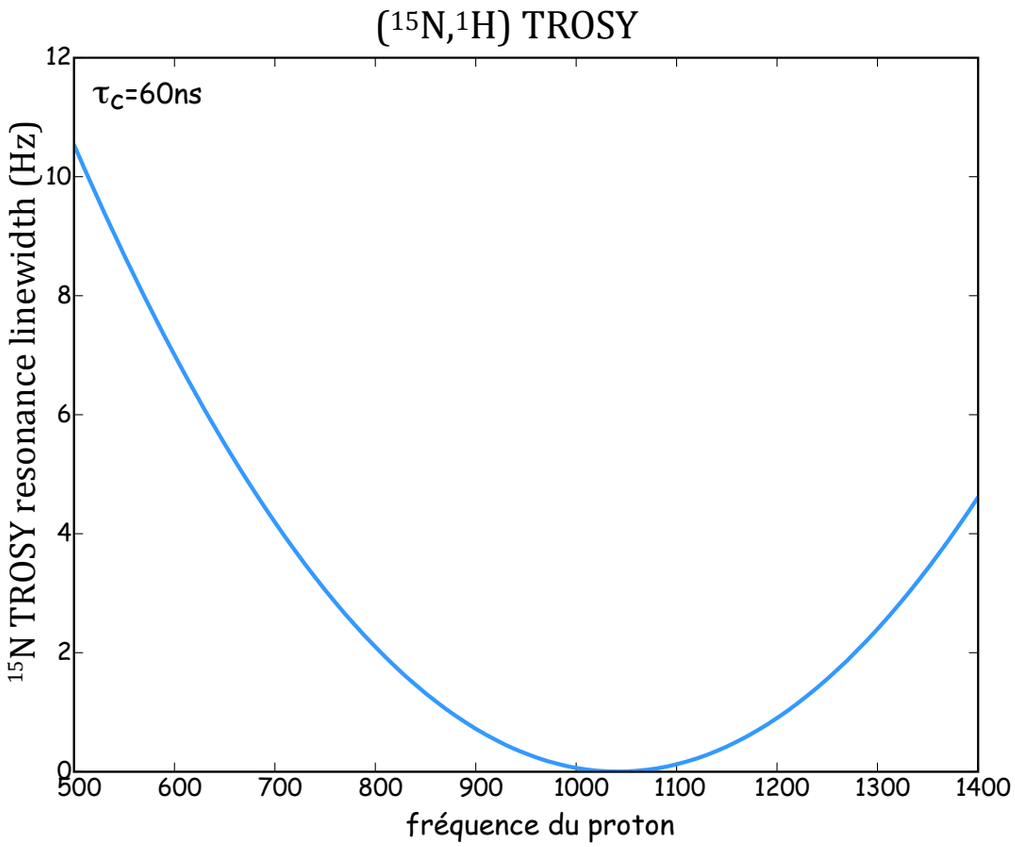


Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

Le temps de relaxation transversale T2 définit le temps de vie du signal RMN.

Il détermine la largeur des résonances

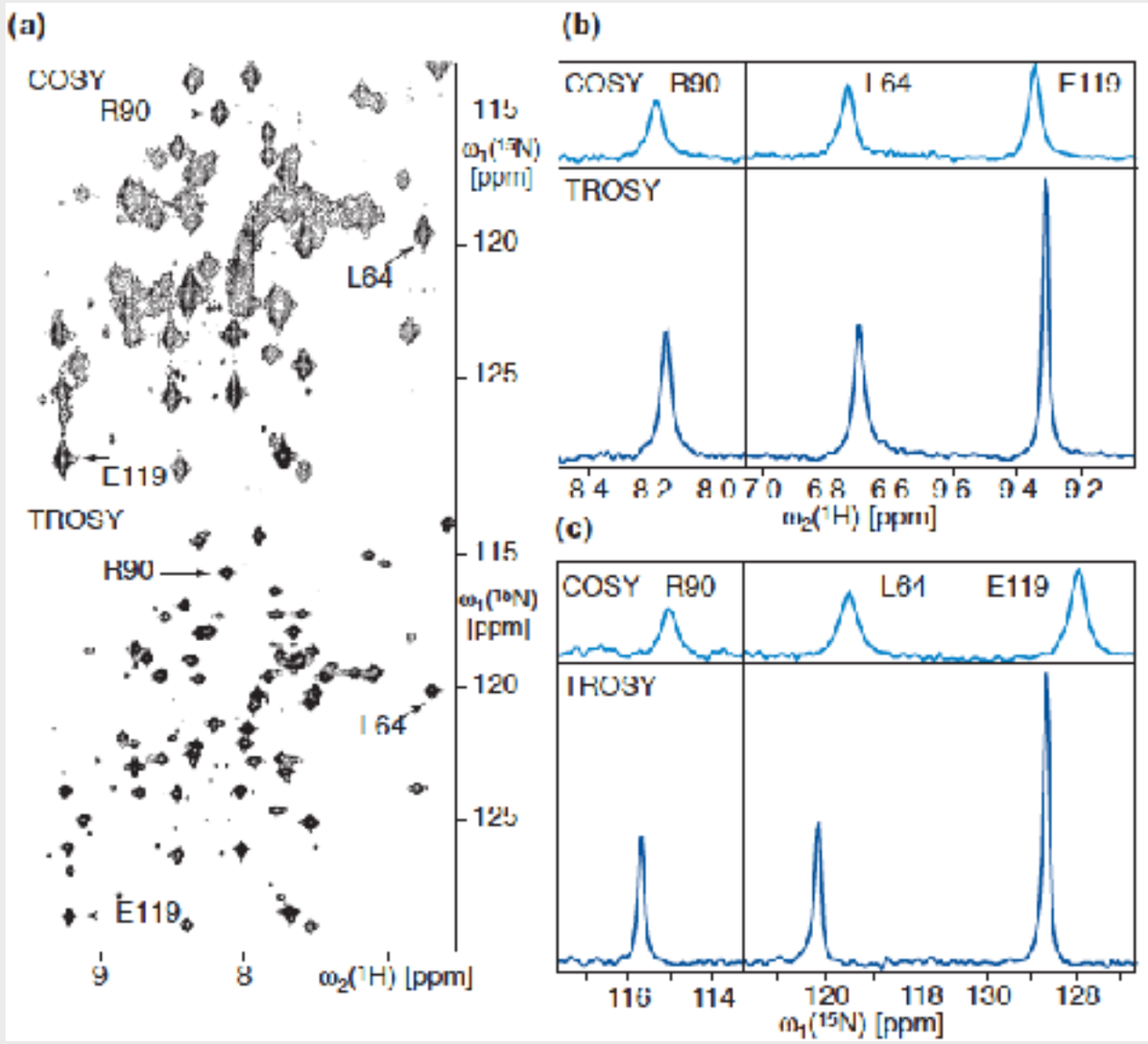
Le temps T2 dépend du champ magnétique B0, de la nature des spins, de la taille des molécules, de la température, de la dynamique du système, **des sources de relaxation**



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

Playing with T2 : TROSY experiments
Transverse Relaxation Optimized SpectroscopY

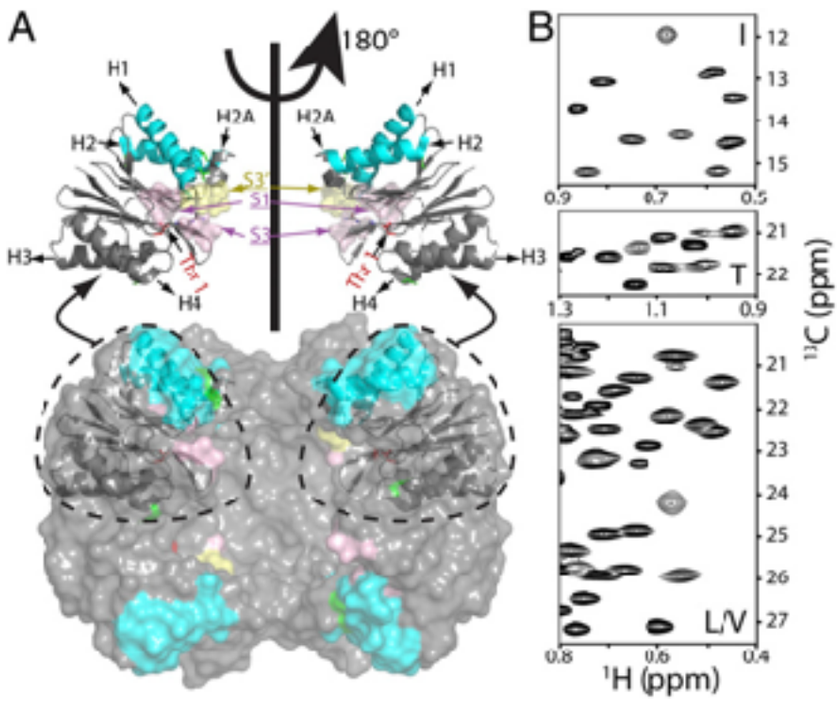
Pervushin, K. Riek, R., Wider, G. and Wütrich, K(1997) Attenuated T2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. Proc. Natl. Acad. Sci. U. S. A. 94, 12366–12371



2H,15N-labeled 110-kDa octameric protein 7,8-dihydroneopterin aldolase

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

➪ Etudes fonctionnelles de gros systèmes



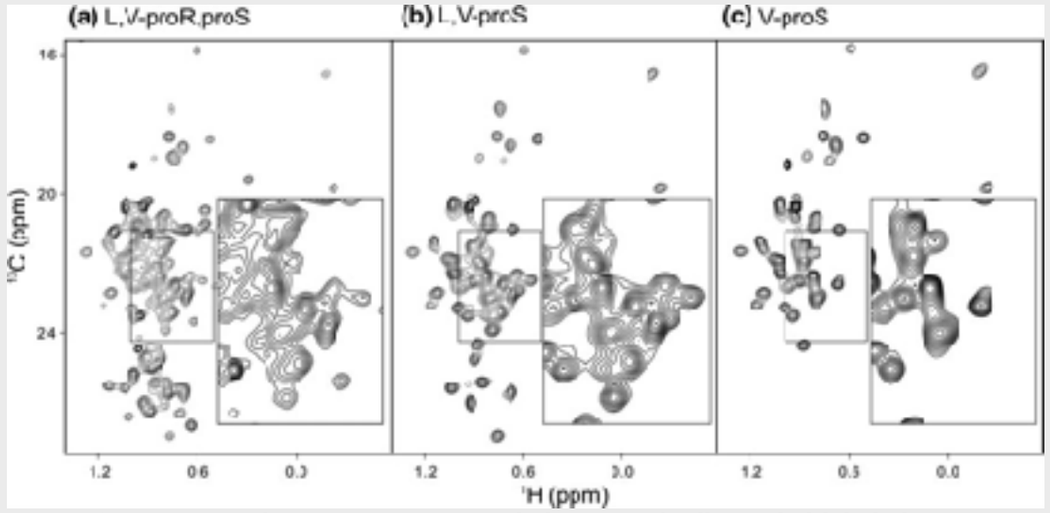
Lichi Shia and Lewis E. Kay (2014) , Tracing an allosteric pathway regulating the activity of the HslV protease, PNAS | February 11, | vol. 111 | no. 6

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 ^{1}H-^{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

➪ ^{2}H, $^{13}C^{1}H_3$ labeling strategies



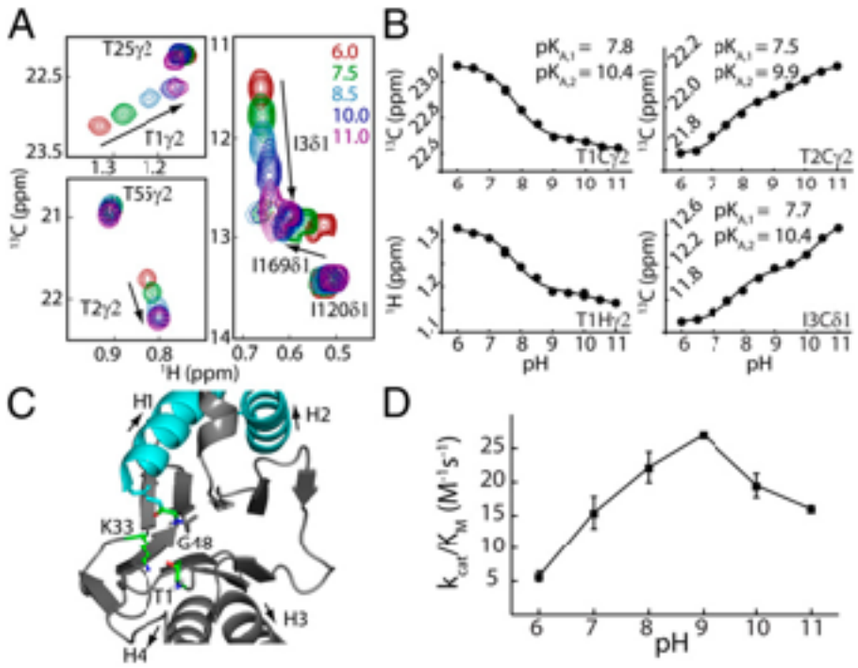
Comparison of HMQC spectra recorded on specifically methyl-labeled TET2 samples (468 kDa).

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

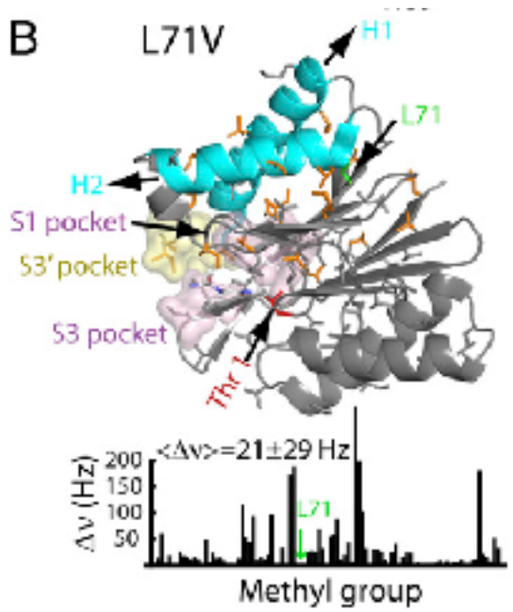
Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

“Jouer” avec T2 : Les expériences $^{13}C^{1}H_3$ Methyl TROSY

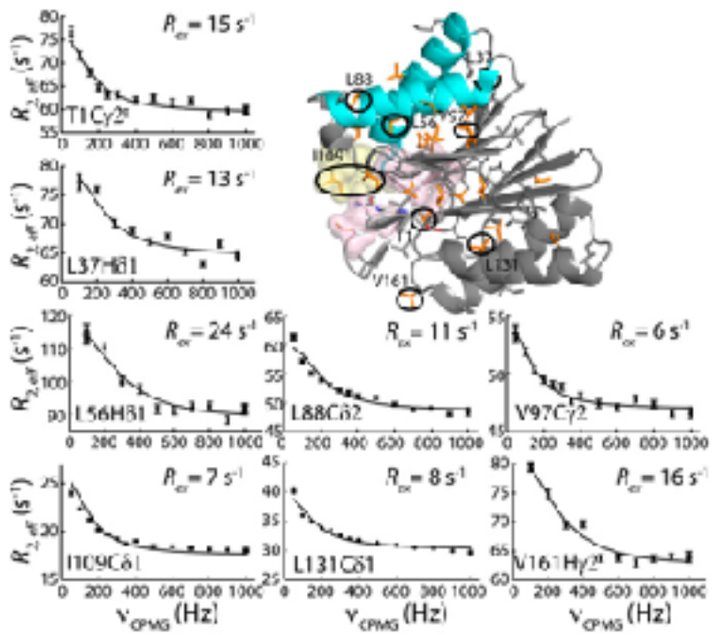
- Stratégies d’attribution : mutations + nOes + crystal structures
- Etudes fonctionnelles



pKa measurements, catalytic mechanism



mutants, allosteric pathways



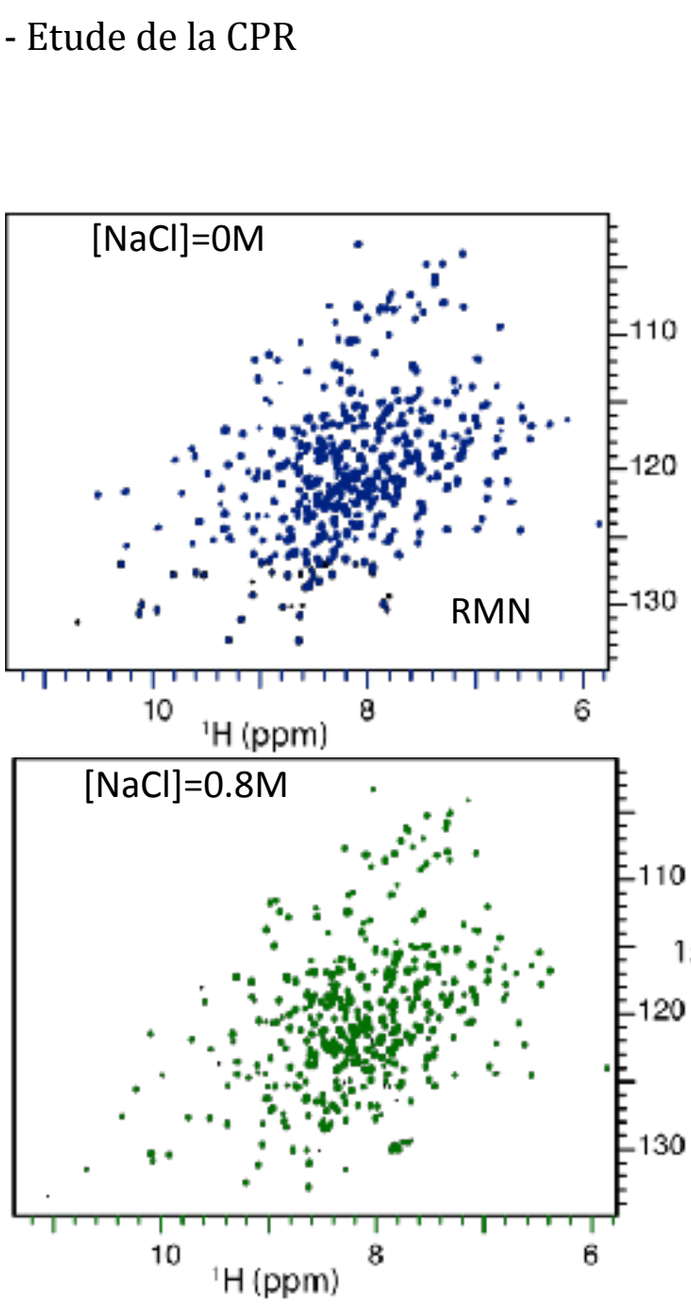
multiple states & flexibility
related to functions

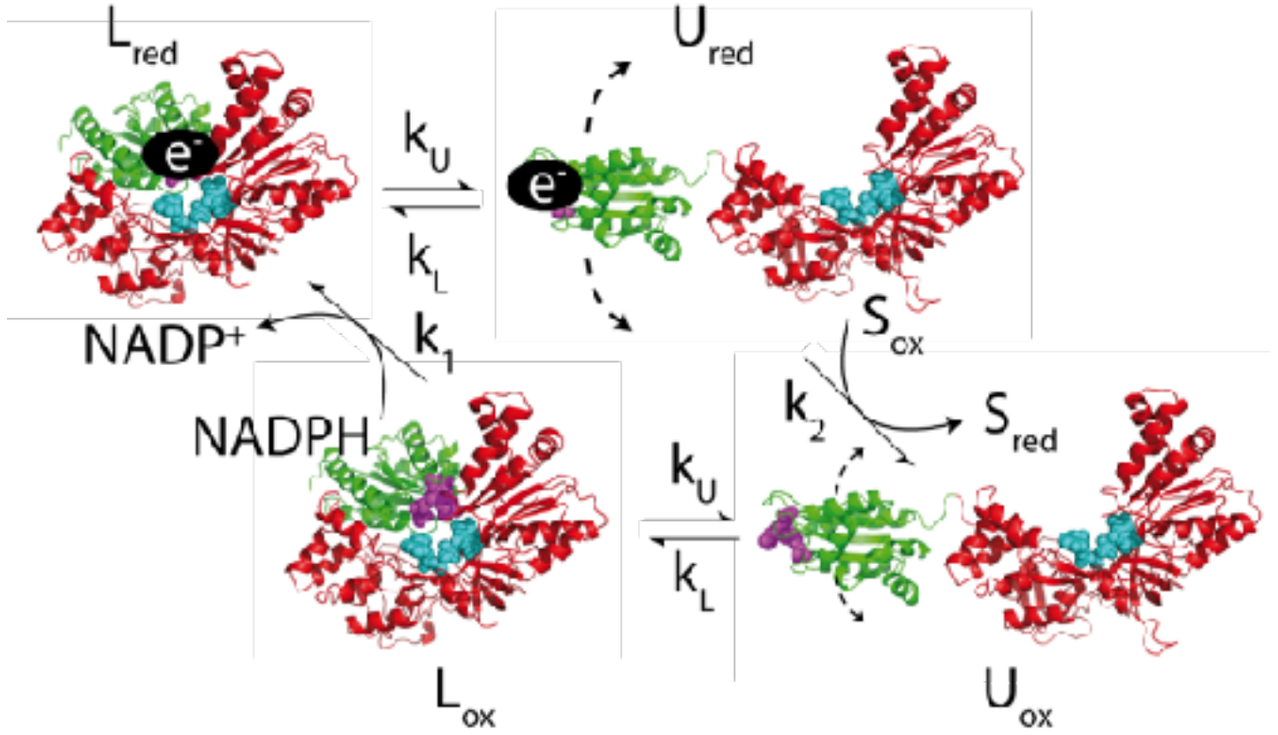
Lichi Shia and Lewis E. Kay (2014) , Tracing an allosteric pathway regulating the activity of the HslV protease, PNAS | February 11, | vol. 111 | no. 6

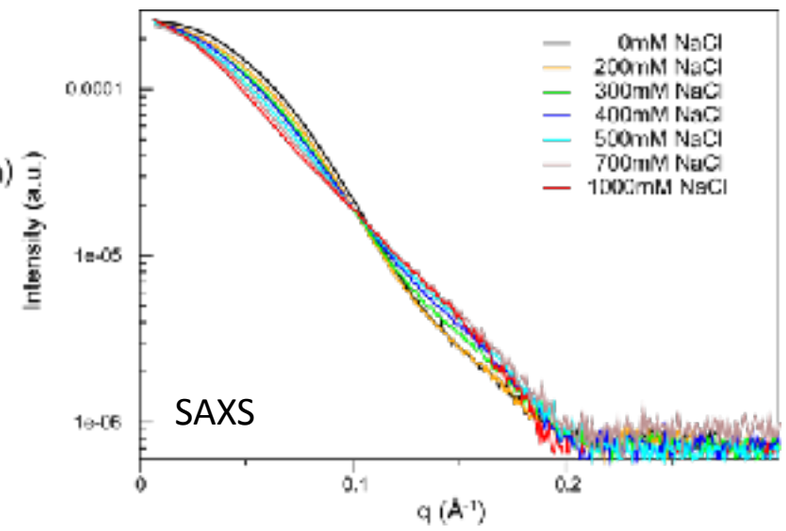
Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

⇨ Etudes fonctionnelles de “grosses” protéines multidomaines

Ewen Lescop - Etude de la CPR



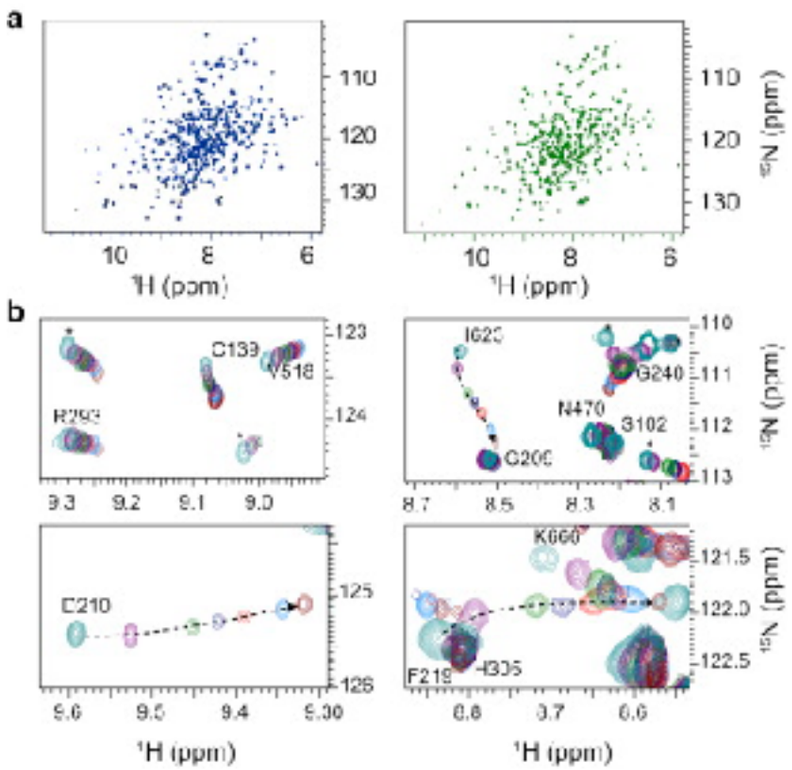


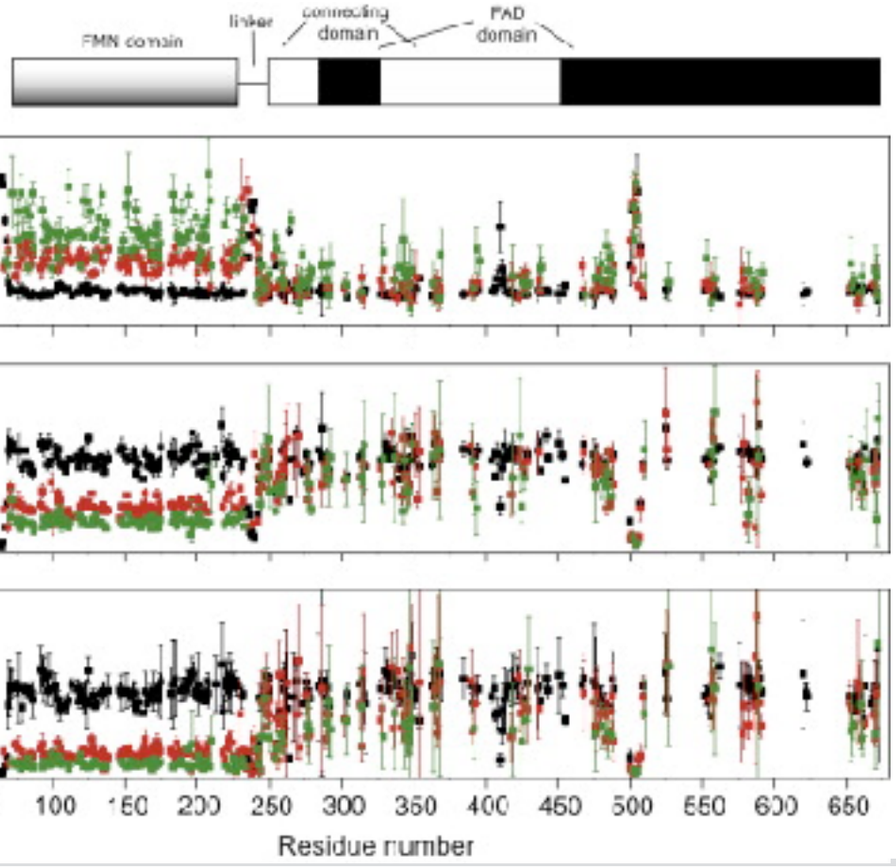


Vincent et al., J Mol Biol. 2012 Jul 20;420(4-5):296-309

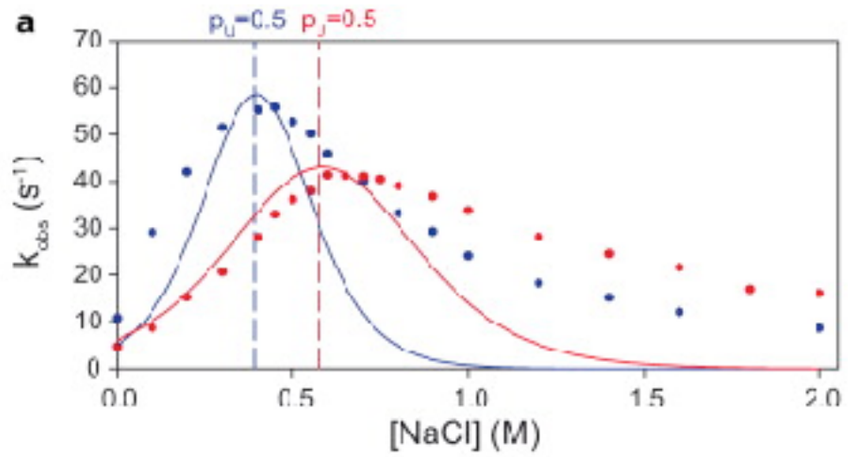
Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

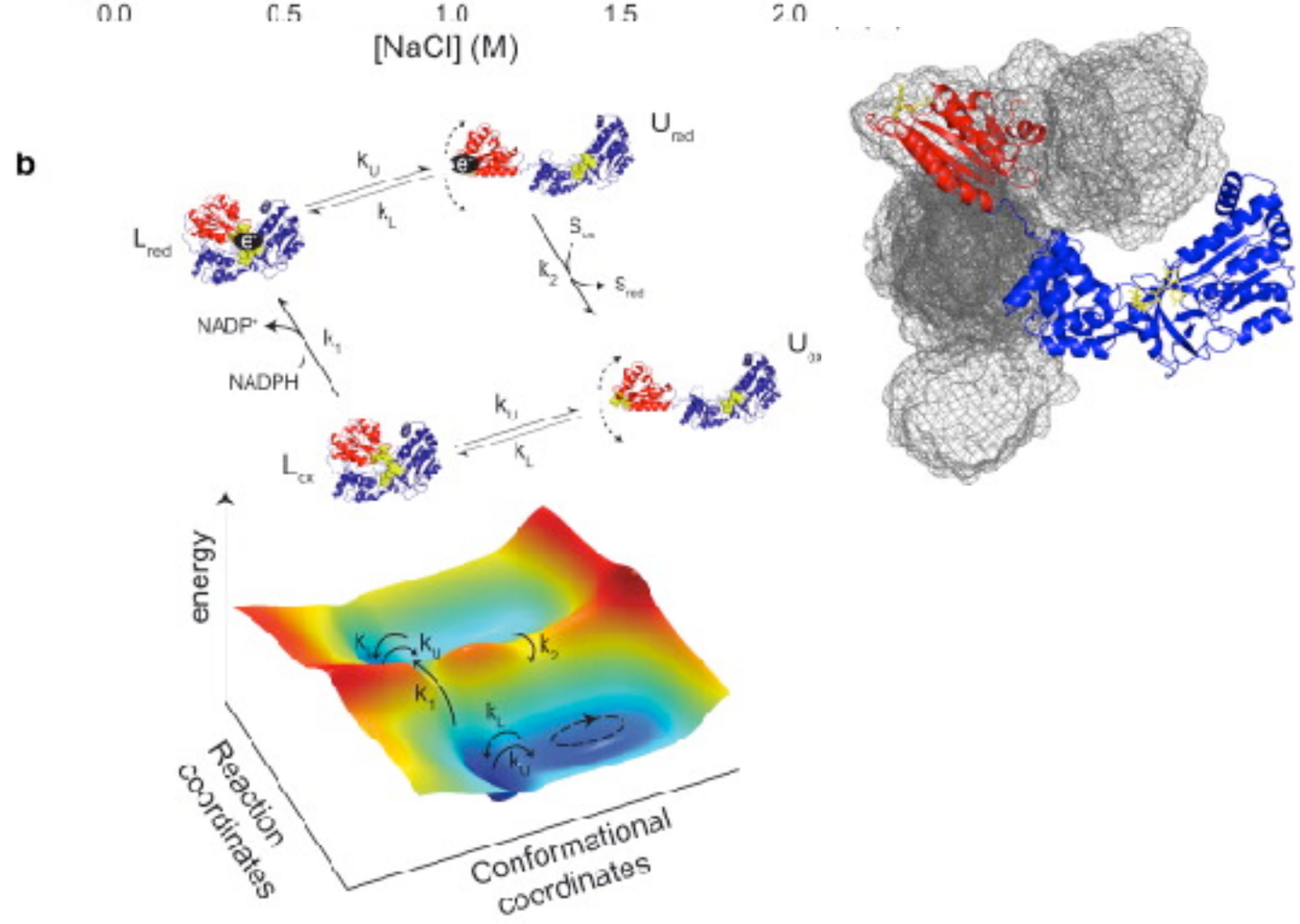
[NaCl]=0M [NaCl]=0.8M





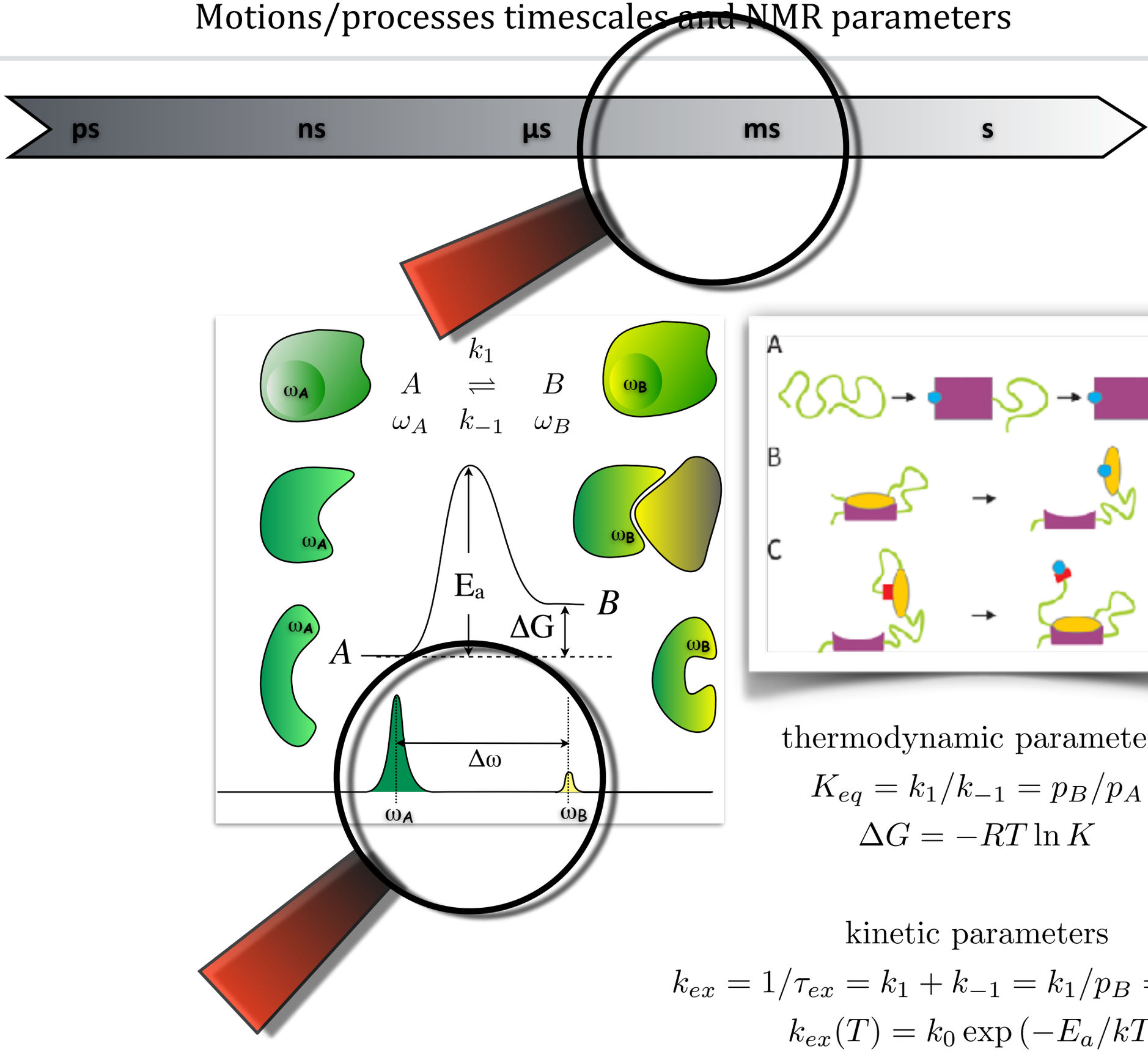
⇨ Etudes fonctionnelles de “grosses” protéines multidomaines





Frances et al., Biophys. J. 2015, Vol. 108, 1527-36

Motions/processes timescales and NMR parameters



thermodynamic parameters

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

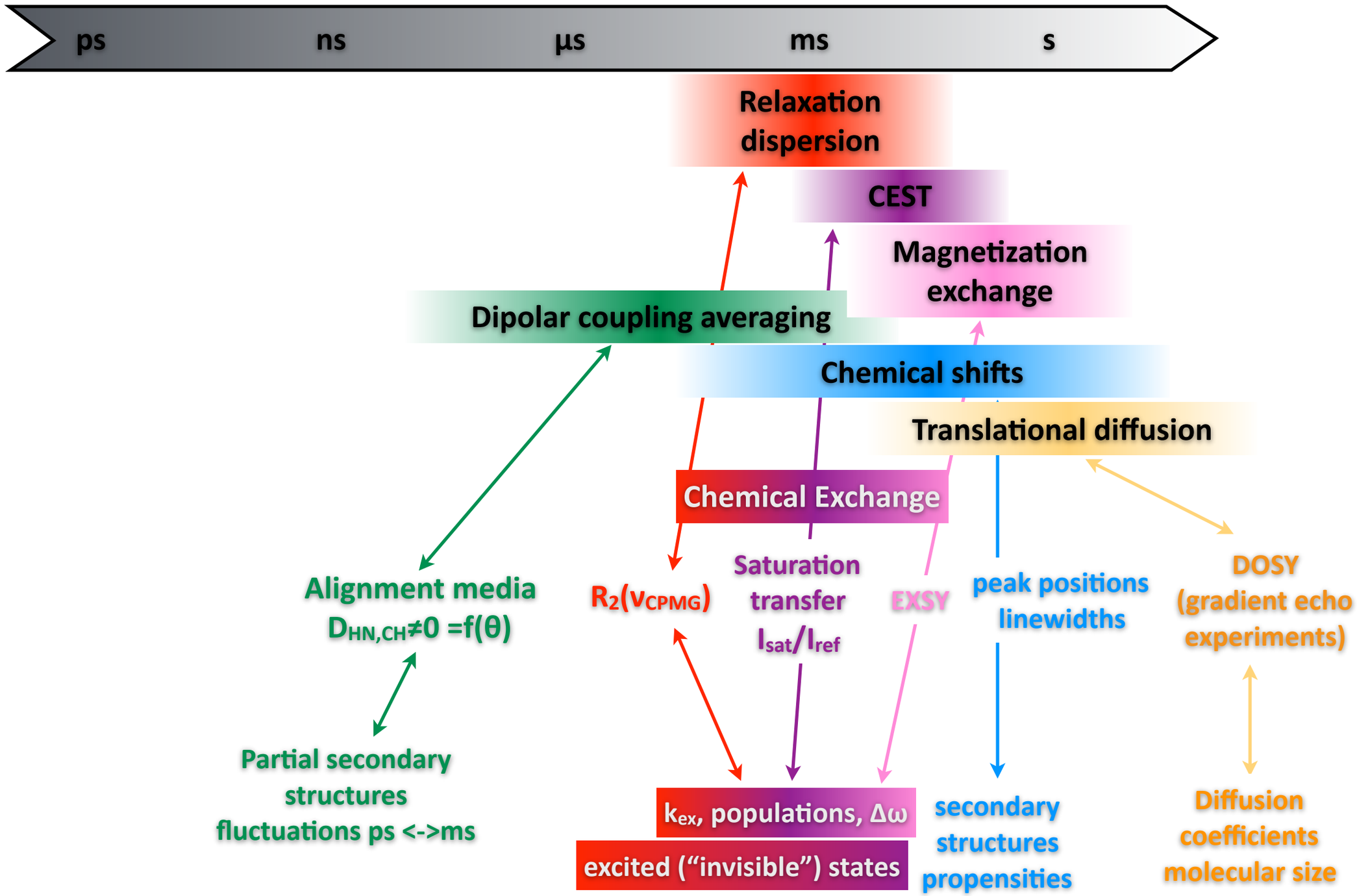
$$\Delta G = -RT \ln K$$

kinetic parameters

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

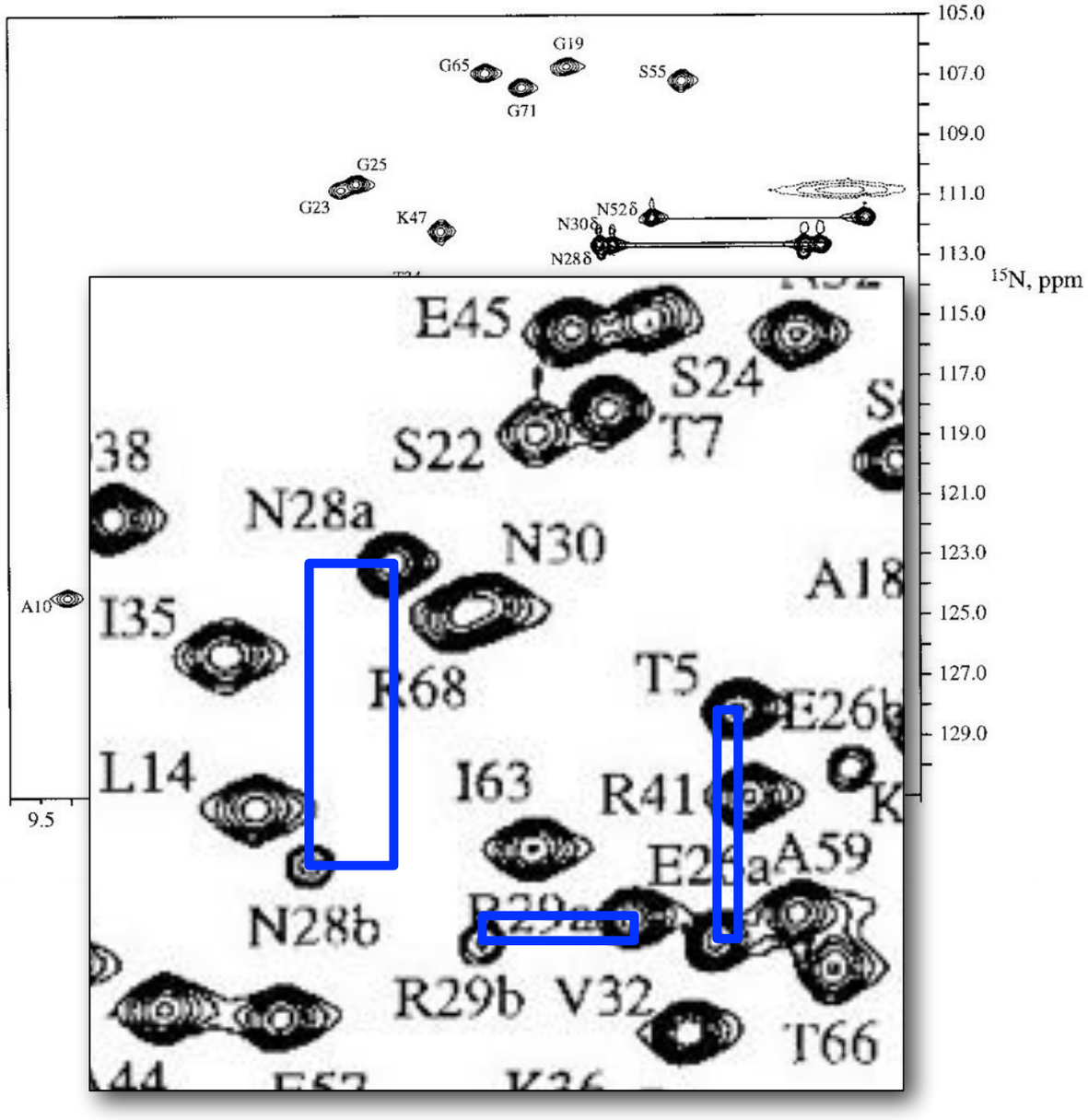
$$k_{ex}(T) = k_0 \exp(-E_a/kT)$$

Motions/processes timescales and NMR parameters



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

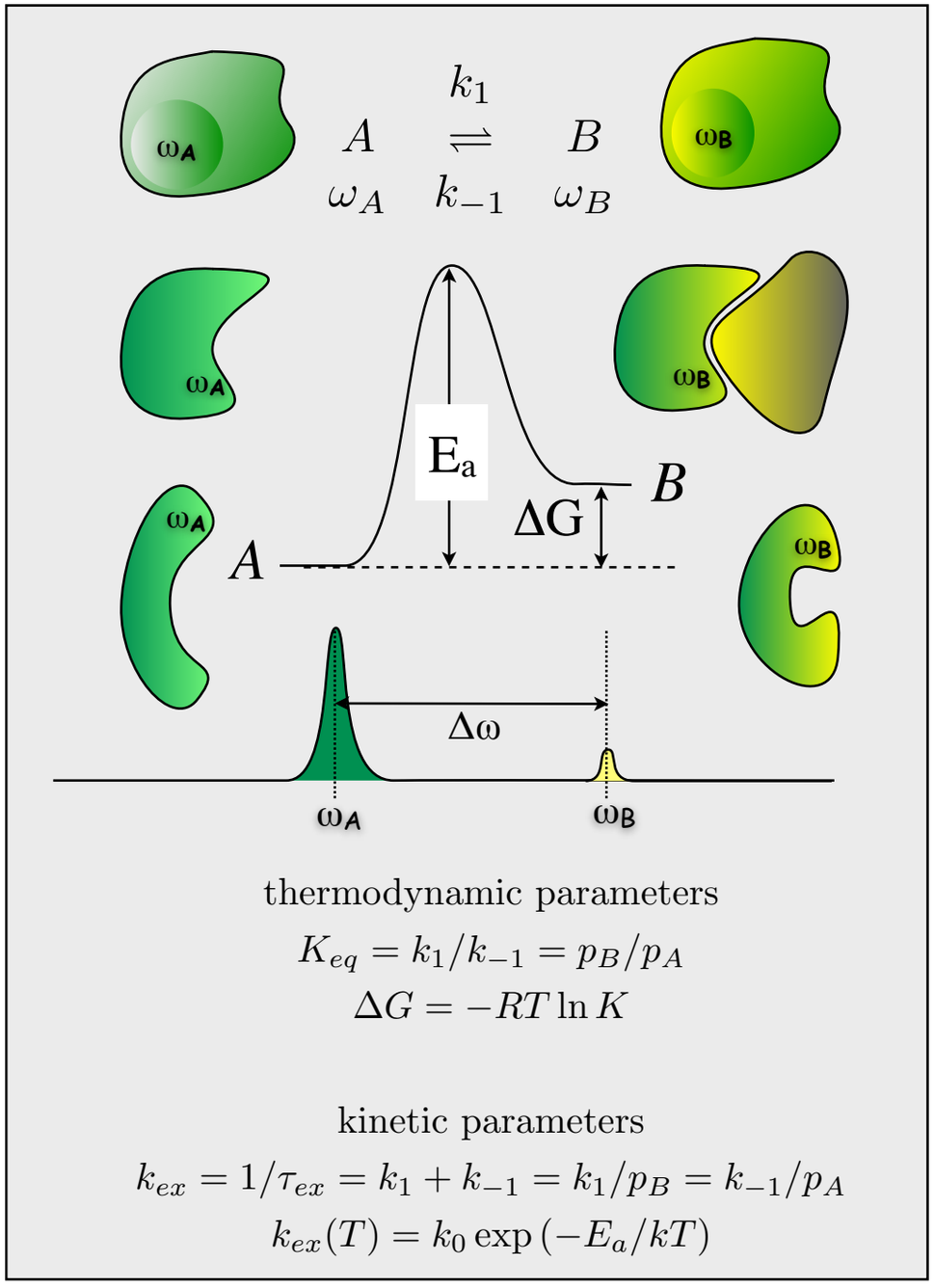
ps **ns** **µs** **ms** **s**



- **Two conformations : two peaks**

Chemical shifts

Chemical Exchange peak positions linewidths



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

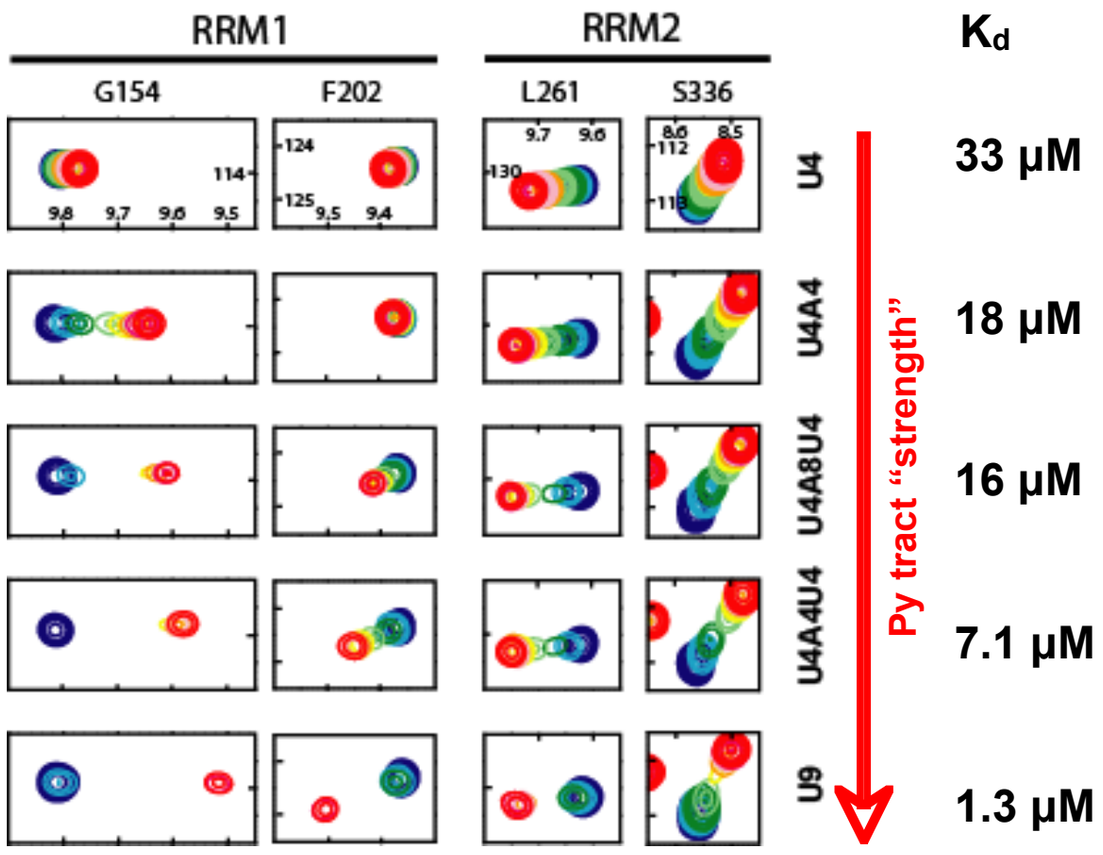
Temps de vie du signal, relaxation transversale & perte de cohérence des spins
Largeur de raie & apparence des spectres

Chemical shifts

Chemical Exchange peak positions linewidths

U2AF65 conformation depends on Py tract strength

Exchange regime & RNA binding affinity



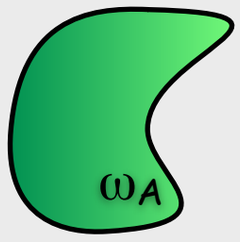
- **Two conformations: one or two (broad) observable peaks!**

(Michael Sattler personal communication)

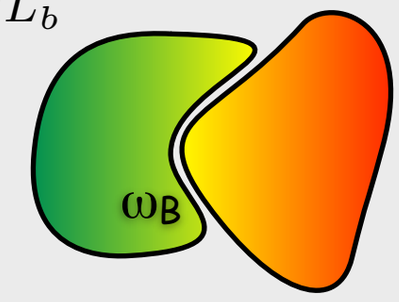
Intermolecular processes

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

δ_{P_f} δ_{L_f} δ_{P_b} δ_{L_b}



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



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

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

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

Chemical shifts

Chemical Exchange peak positions linewidths

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" : $\Delta\omega \simeq k_{ex}$**

⇨ **Un ou deux pics élargis**

⇨ $R_{ex} \propto B_0$

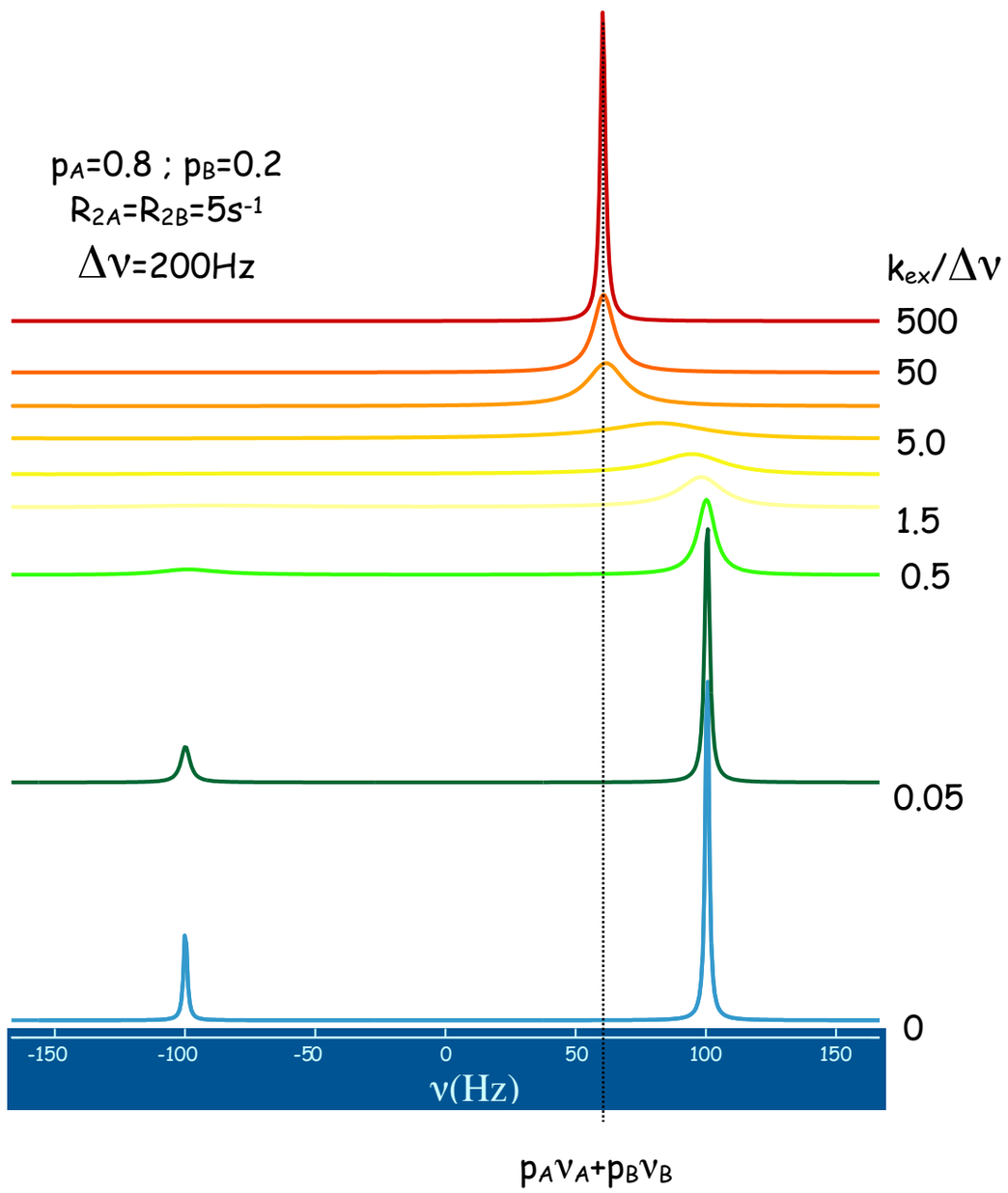
☞ Echange "lent" : $\Delta\omega \gg k_{ex}$

⇨ Deux pics

⇨ R_{ex} indépendant de B_0

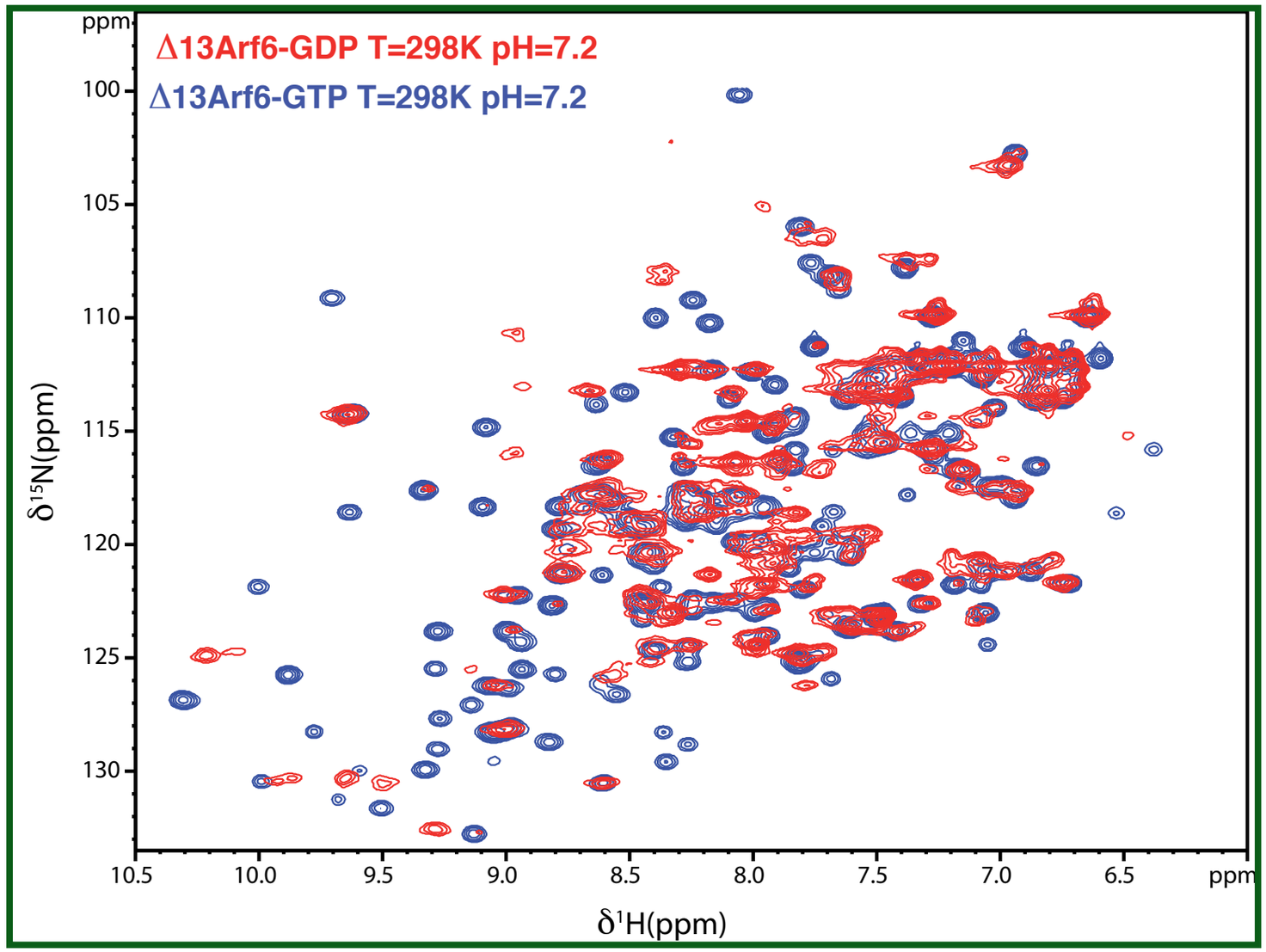
⇨ $R_{ex}^{(B)} \rightarrow k_{BA}=p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_B k_{ex}$

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



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

Les échelles temporelles et spatiales sont corrélées



Linewidth

Global correlation time (size)
+ Local Rotational fluctuation timescale
+ density of relaxation sources
+ conformational/chemical exchange

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 connait pas le nombre de sites en échange

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

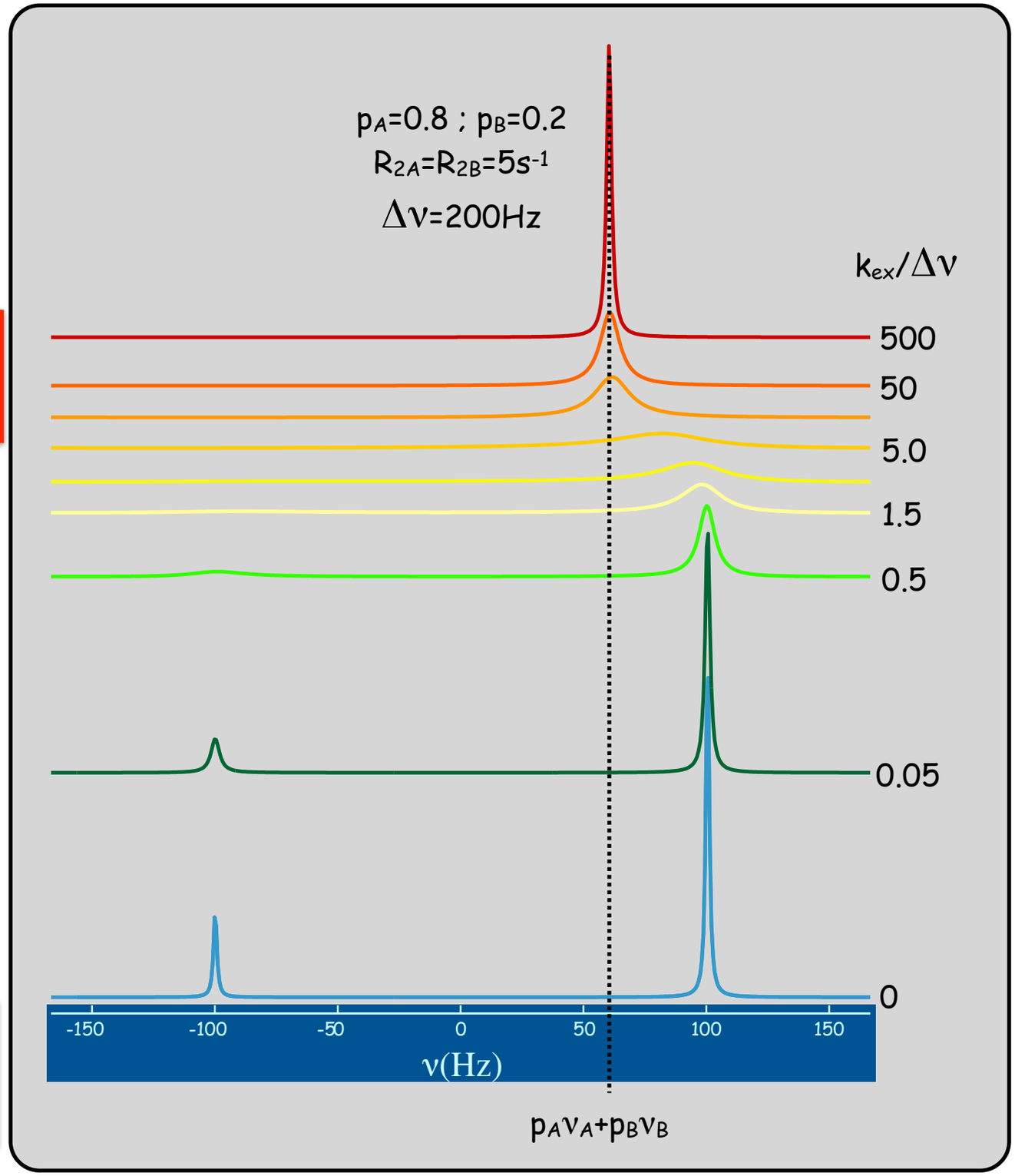
- Un ou deux pics élargis
- $R_{ex} \propto B_0$

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

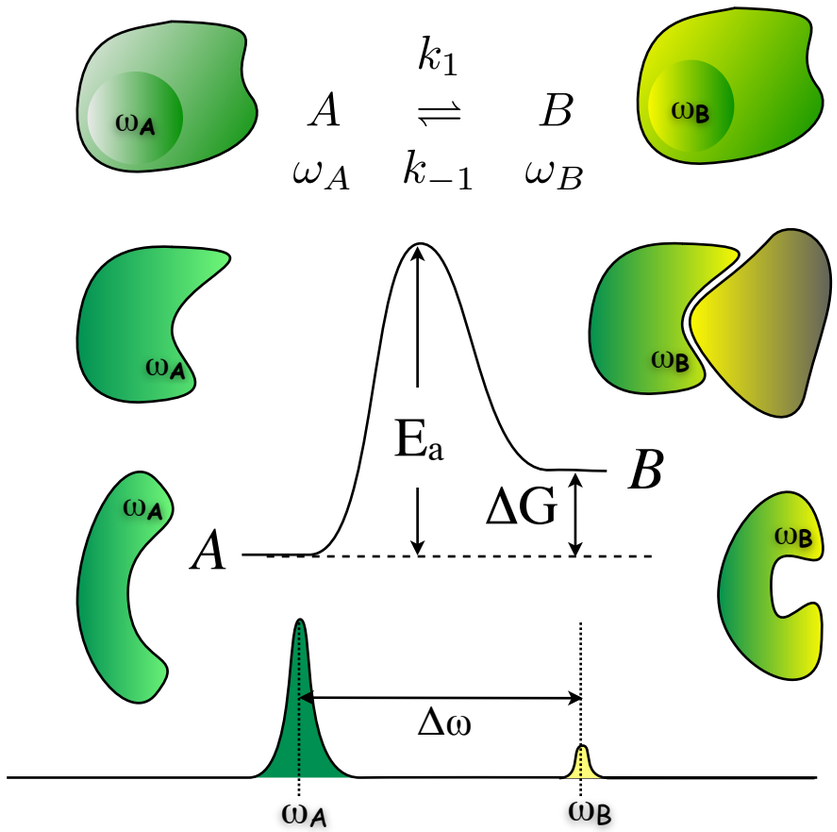
- Deux pics
- R_{ex} indépendant de B_0
- $R_{ex}^{(B)} \rightarrow k_{BA}=p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_B k_{ex}$

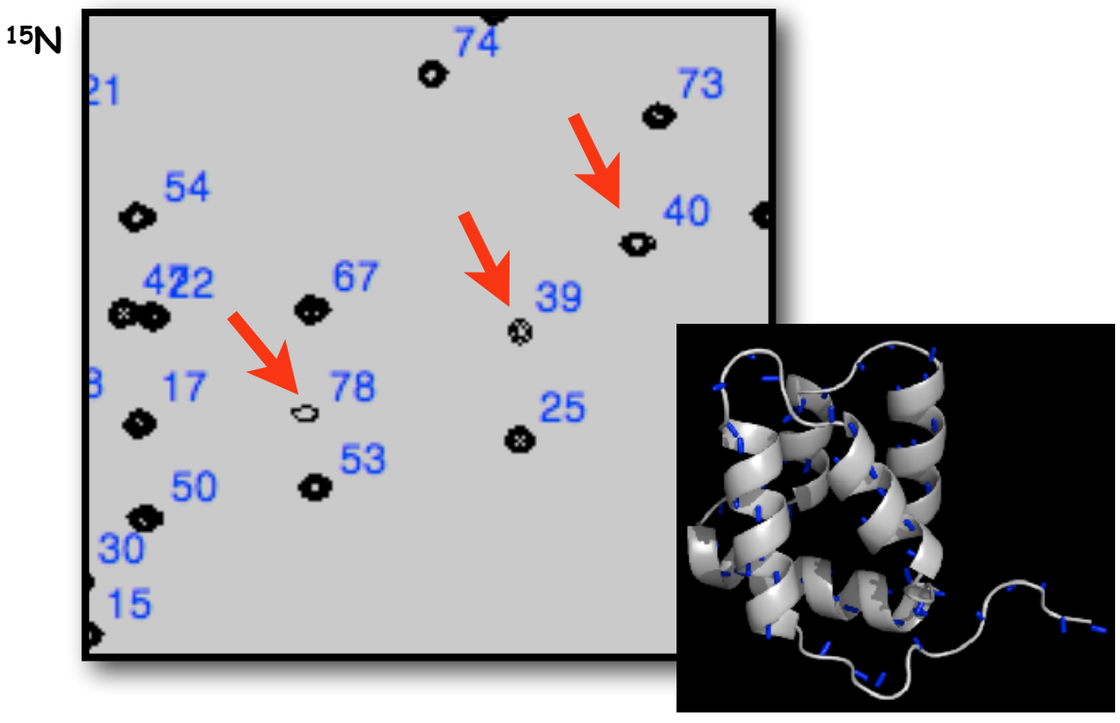
$$p_A \gg p_B \Rightarrow R_{ex}(B) \gg R_{ex}(A)$$

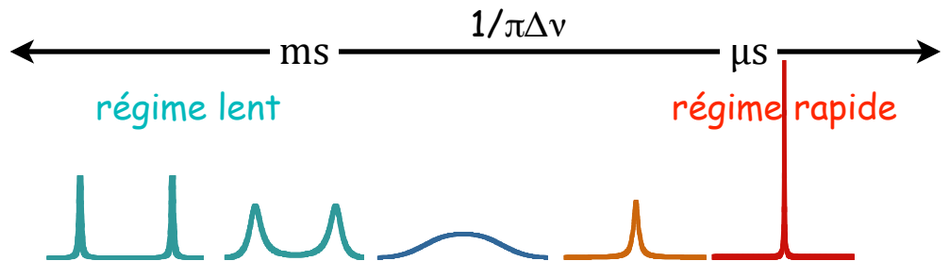
☞ Le pic minoritaire peut devenir indétectable même en régime d'échange "lent"



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







échange d'aimantation longitudinale

valeurs moyennes

forme de raie

Dispersion de relaxation
$R_2^{app}=R_2+R_{ex}$

Une seule résonance apparente, plusieurs états en échange

↓

paramètres thermodynamiques

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

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

paramètres cinétiques

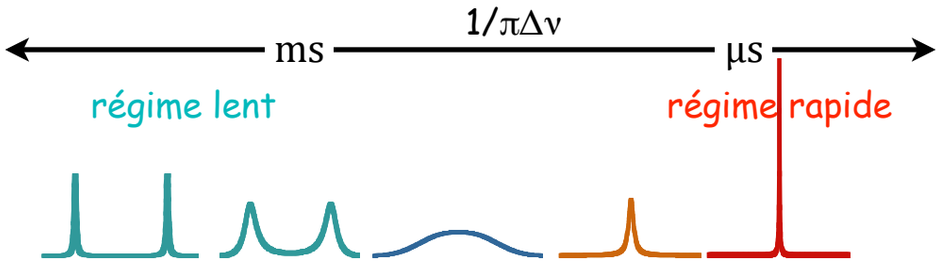
$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$

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

paramètres structuraux

$\Delta\omega$; ω_A ; ω_B

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



échange d'aimantation longitudinale

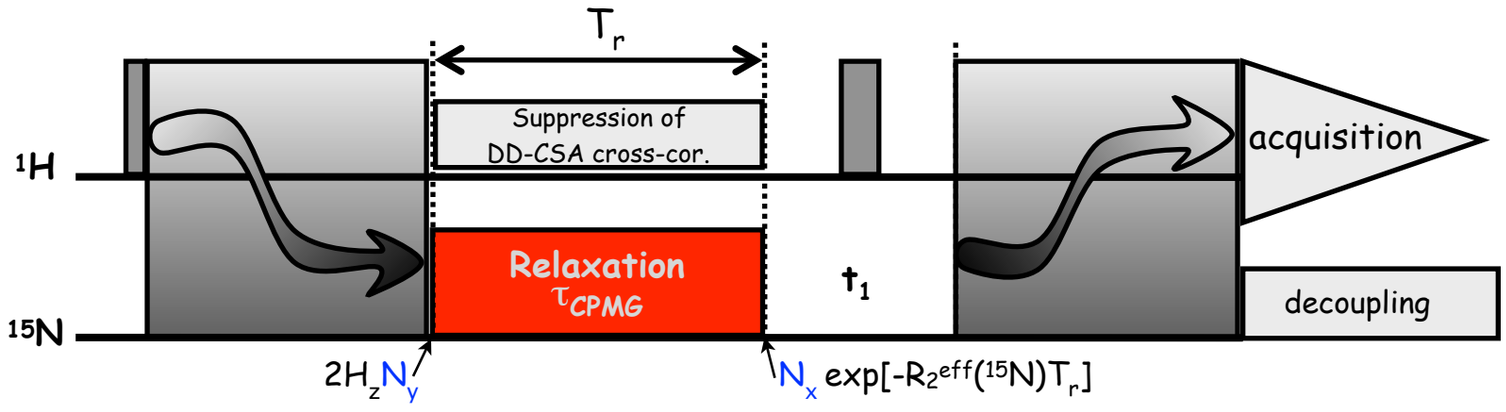
valeurs moyennes

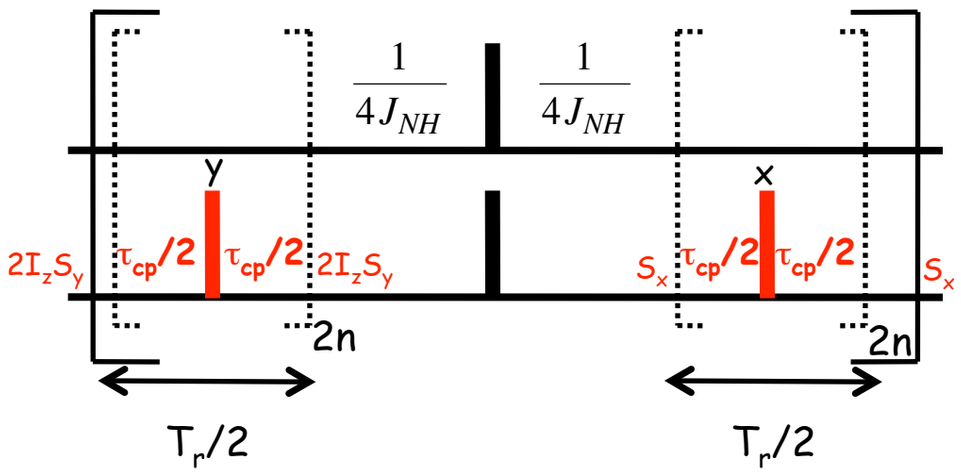
forme de raie

Dispersion de relaxation
$R_2^{eff}=R_2+R_{ex}$

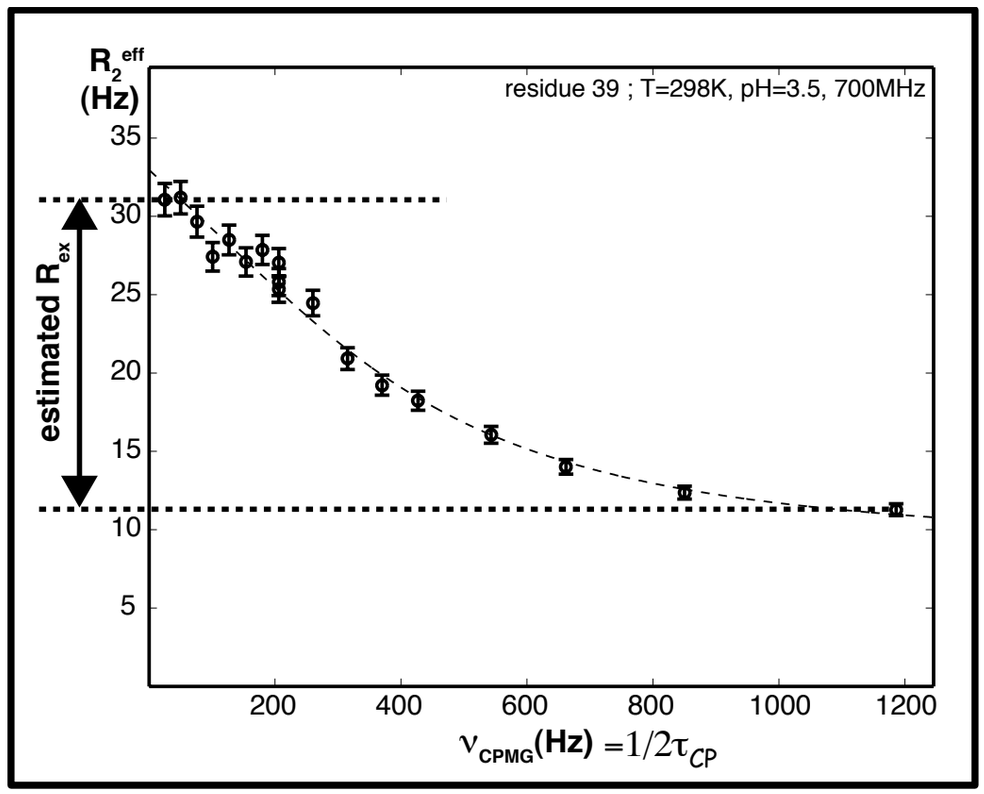
Une seule résonance apparente, plusieurs états en échange

expérience de dispersion de relaxation



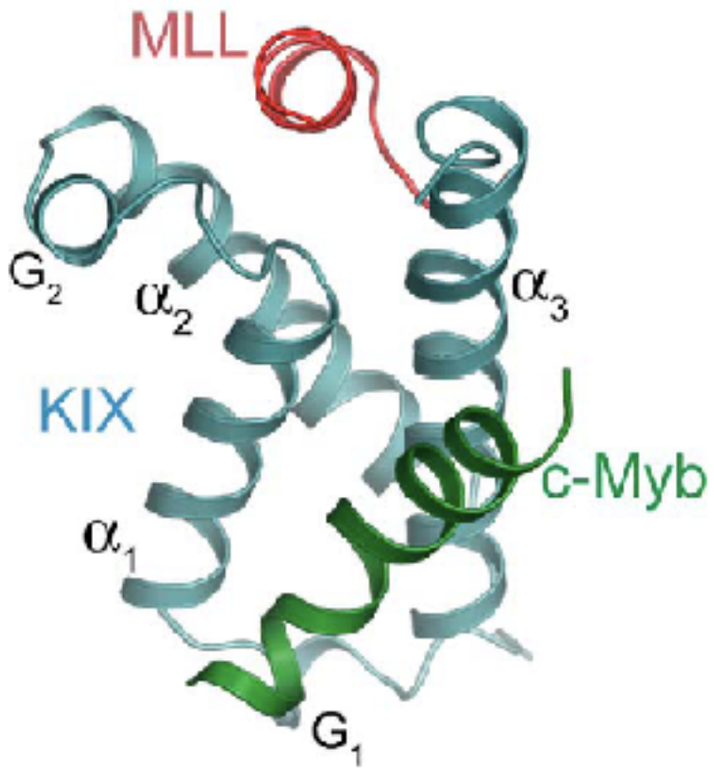


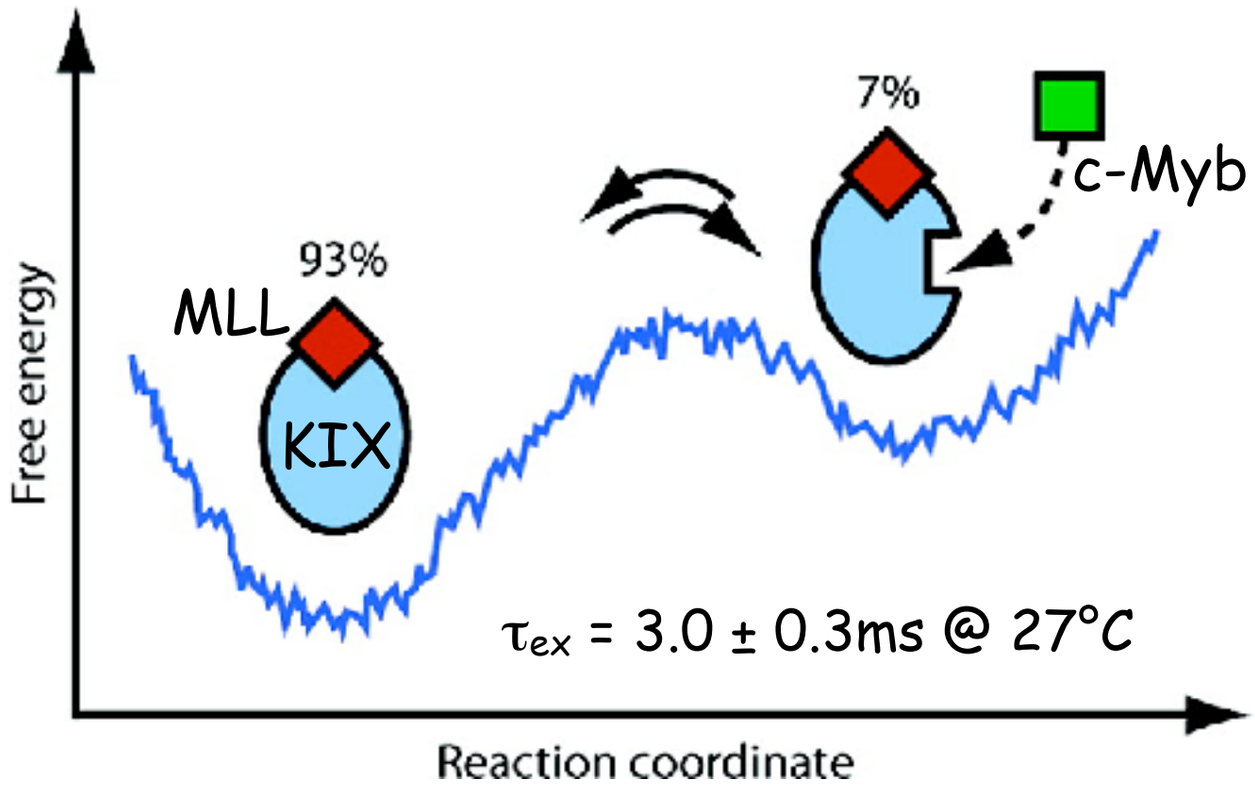
$$R_2^{eff} = R_2^0 + R_{ex}(p_B, k_{ex}, \delta\omega, \tau_{CP}, 2N_{echo})$$



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

Sélection conformationelle / allostérie





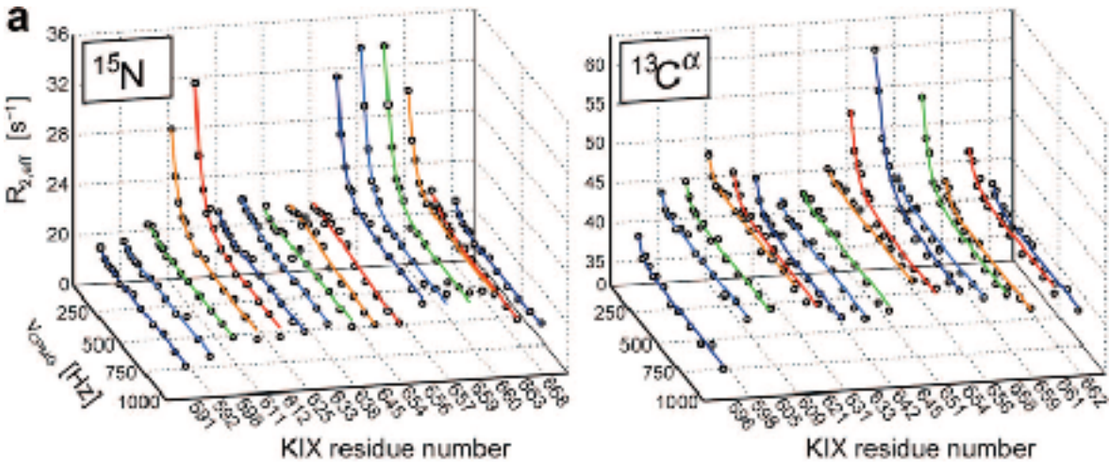
➪ La liaison du domaine d'activation du facteur de transcription MLL à KIX induit une redistribution des populations relatives des conformères de KIX en faveur de l'état dans lequel le site de liaison de c-Myb (pKID) est préformé.

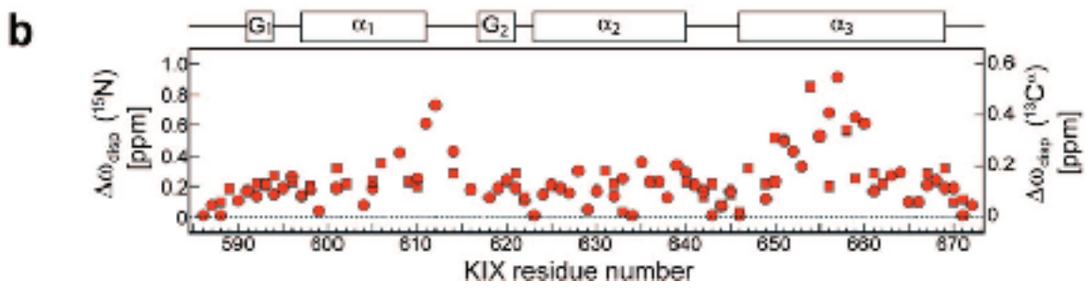
➪ Cet état minoritaire n'est pas détectable dans la forme libre de KIX

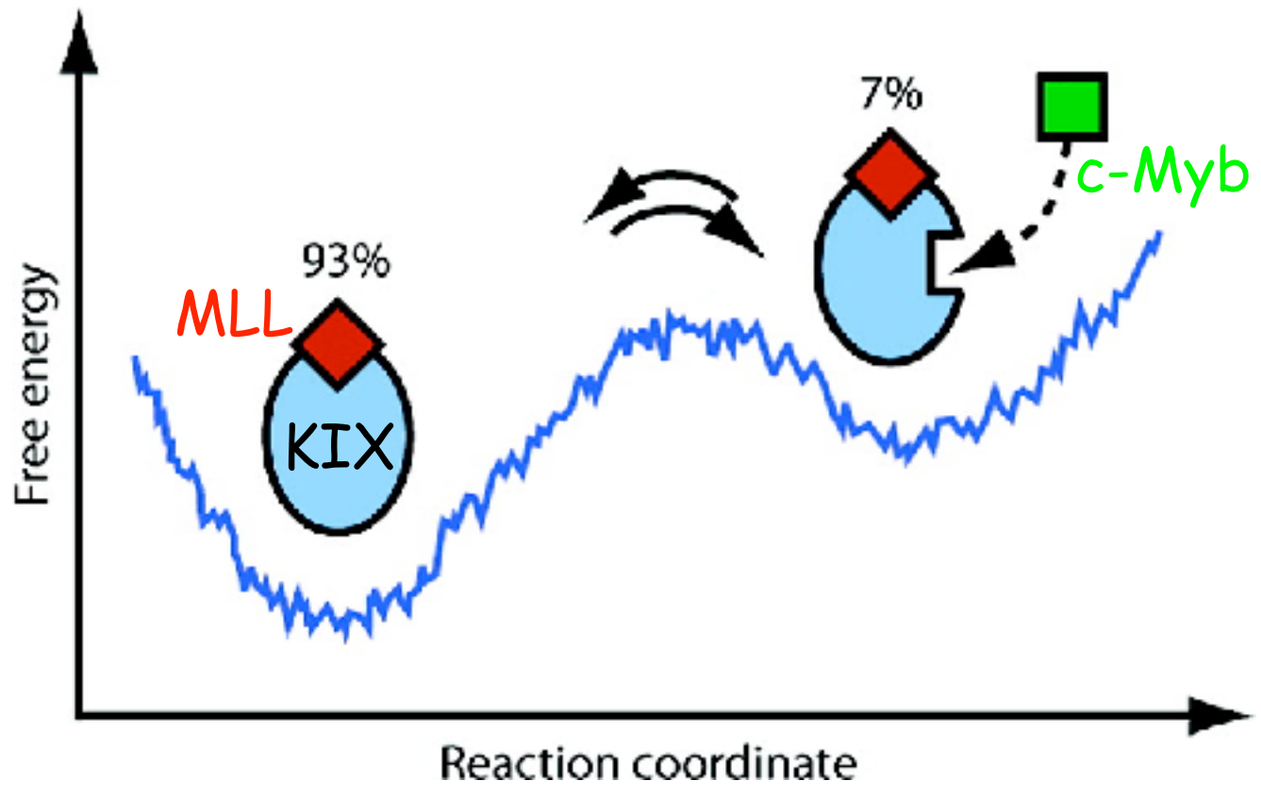
Brüschweiler et al, J. Am. Chem. Soc. (2009) 131, 3063–3068

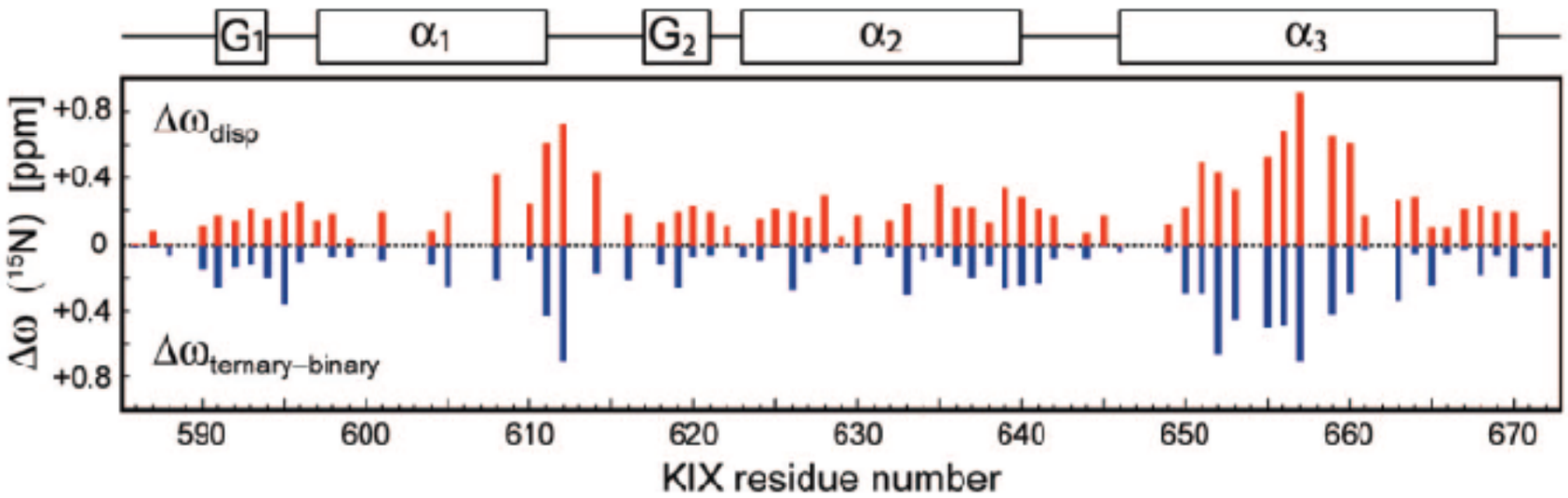
Caractériser des échanges conformationnels entre états
Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

Sélection conformationelle / allostérie



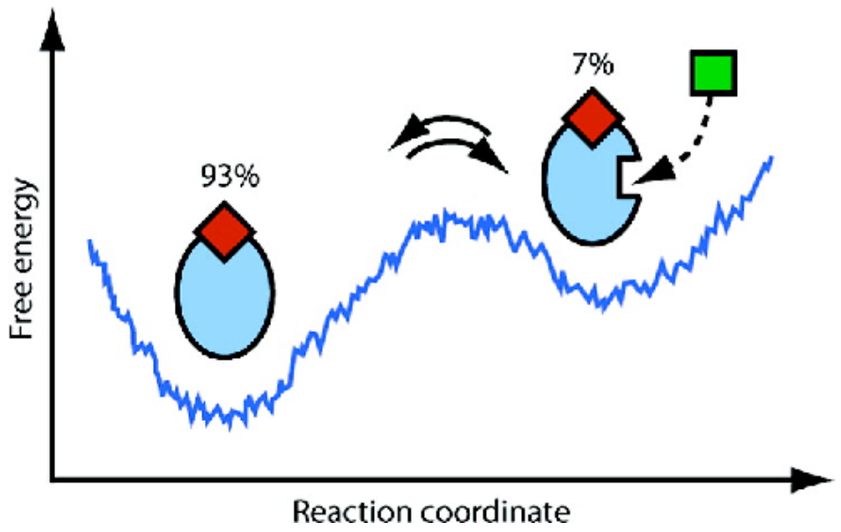


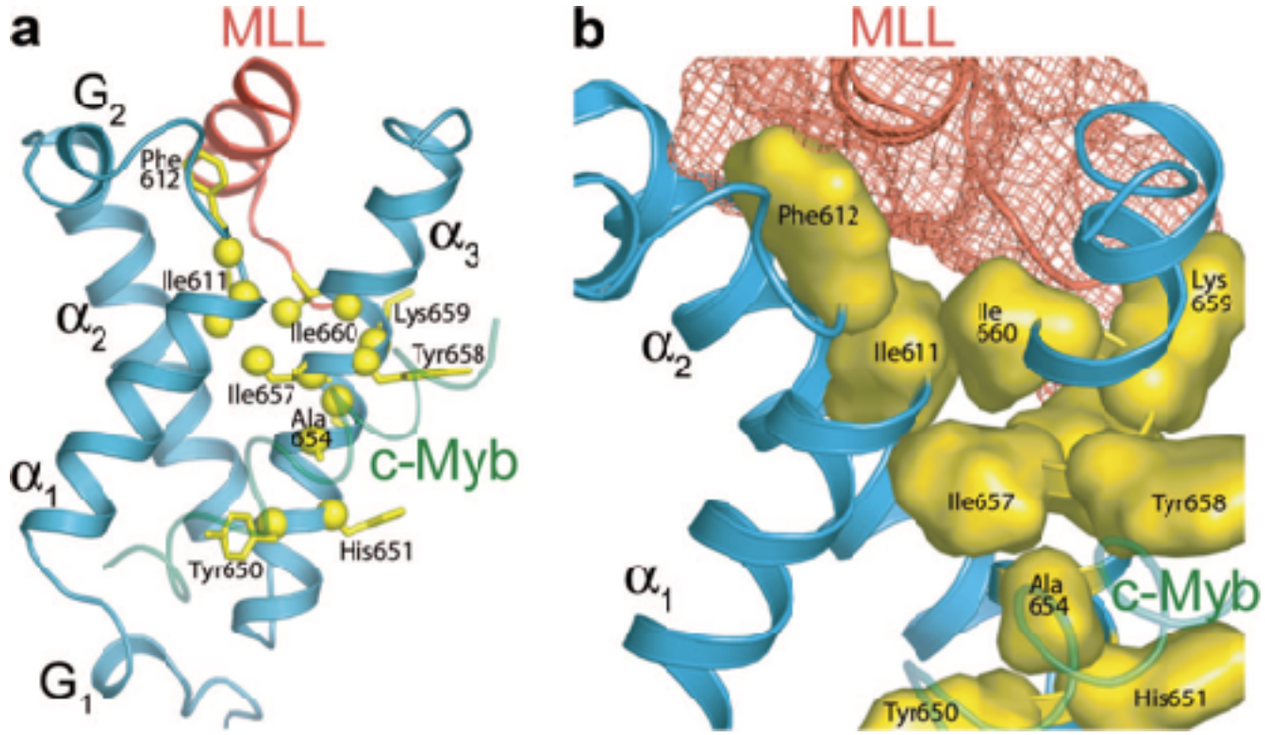




Caractériser des échanges conformationnels entre états
Cas d'un seul pic observé - Dispersion de Relaxation (CPMG)

Sélection conformationelle / allostérie

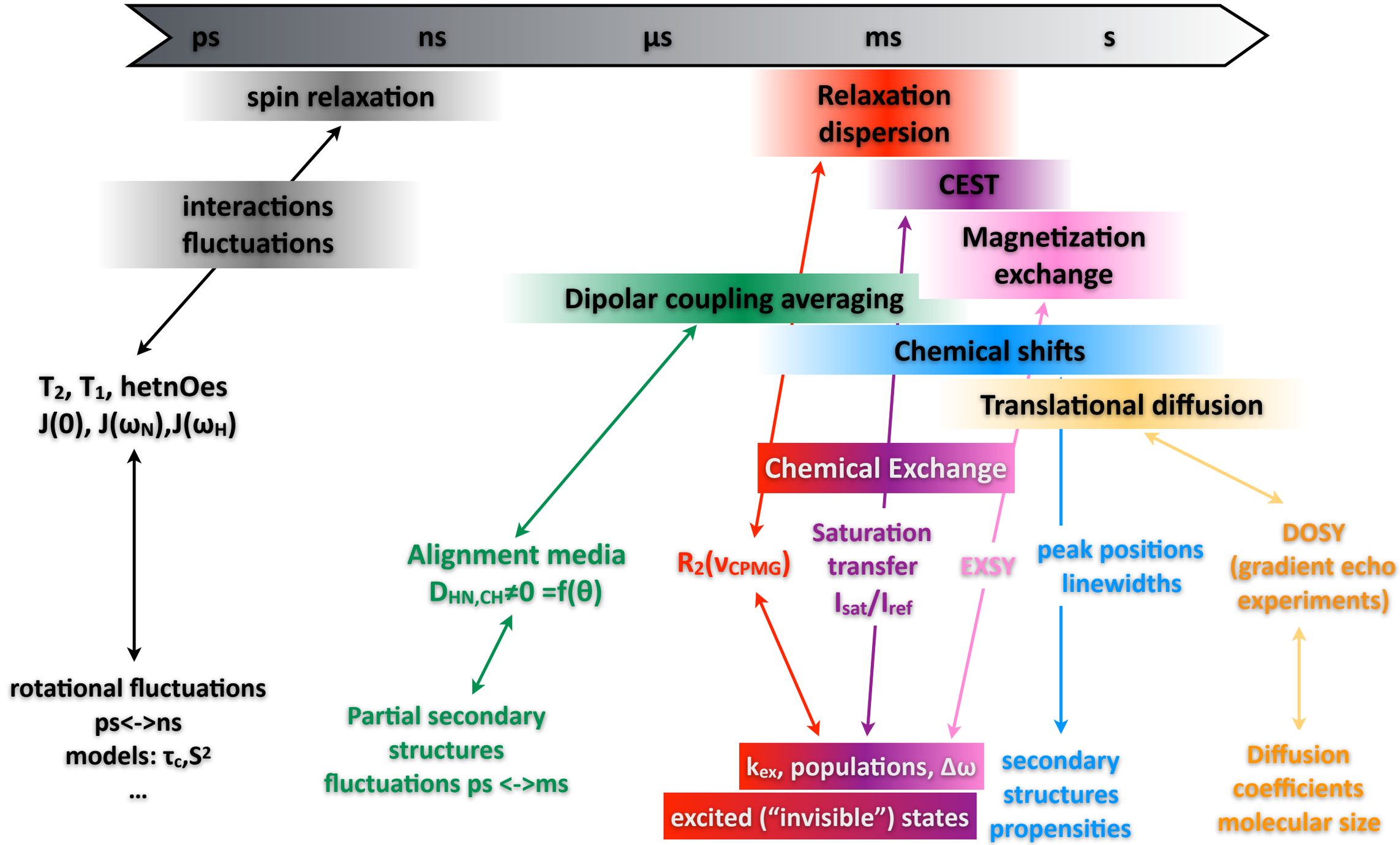




➪ Les chaînes latérales des isoleucines participent à la formation d'un réseau d'interactions qui constitue le «chemin» à travers lequel se transmet l'information allostérique.

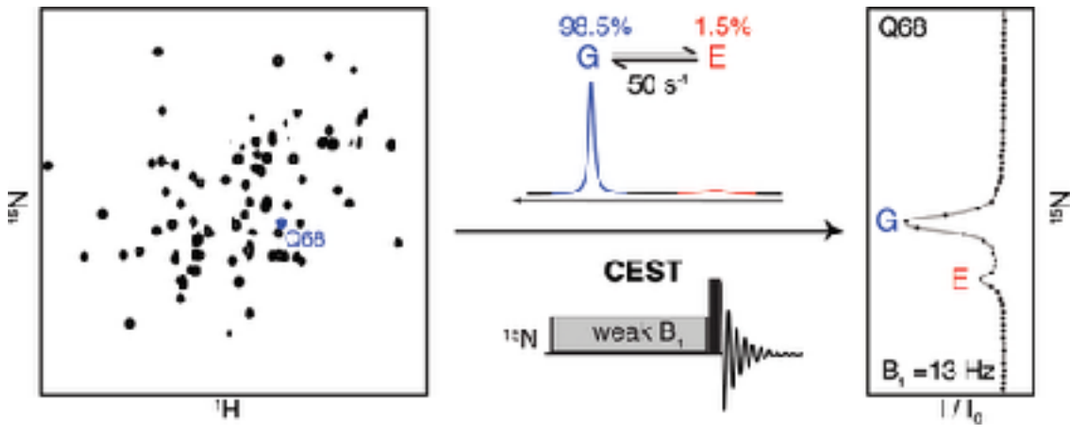
➪ L'analyse de la transition allostérique du domaine KIX de CBP fournit une description à l'échelle atomique du mécanisme de transmission coopérative d'information entre deux facteurs de transcription.

Echelle de temps des mouvements et paramètres RMN



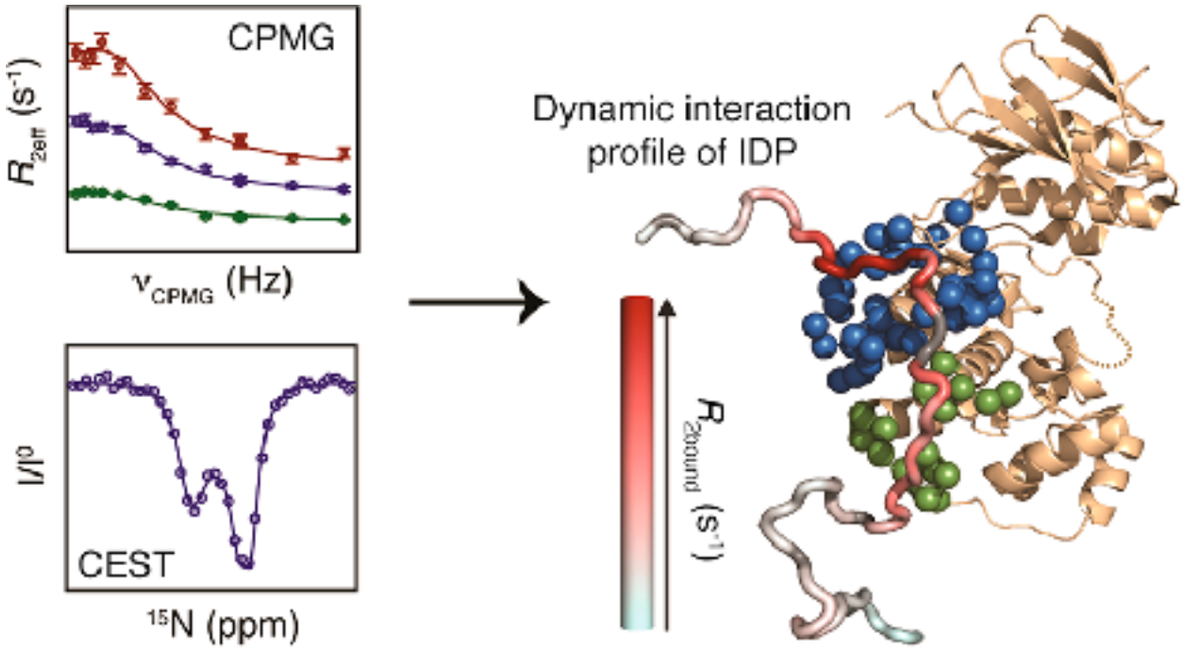
Caractériser des échanges conformationnels entre états
Cas d'un seul pic observé: Dispersion de Relaxation (CPMG) / CEST

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

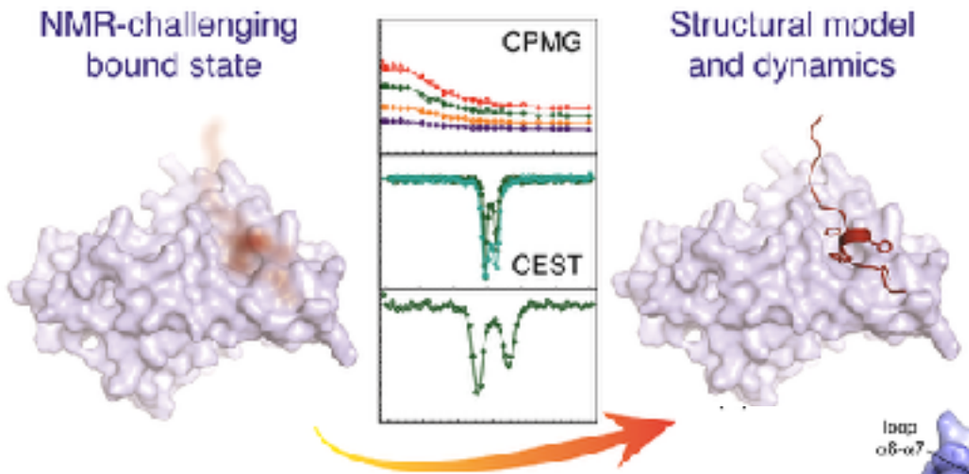


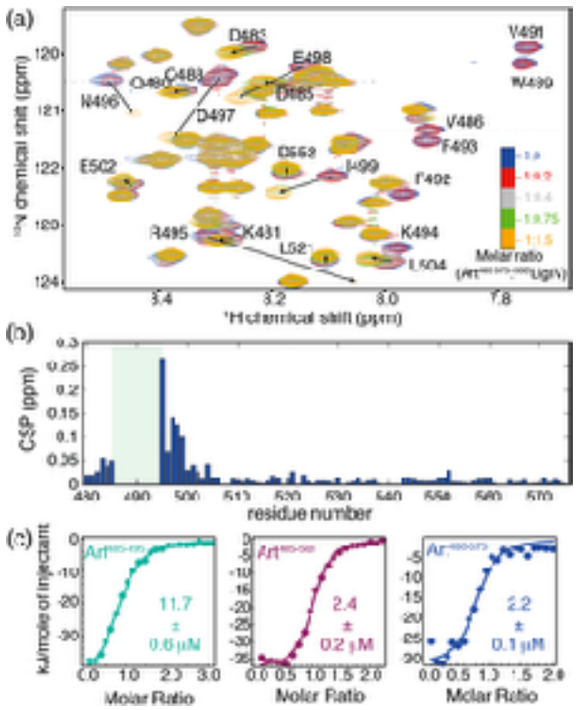
Vallurupalli, Bouvignie & Kay, *J. Am. Chem. Soc.* 2012, 134, 8148–8161

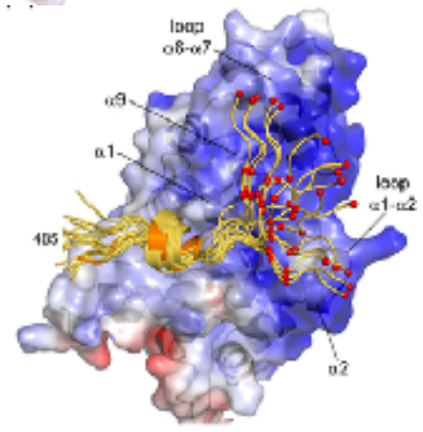
Interaction between the mitogen-activated protein kinase (MAPK) p38α and the intrinsically disordered regulatory domain of the MAPK kinase MKK4. Our study demonstrates that MKK4 employs a subtle combination of interaction modes in order to bind to p38α , leading to a complex displaying significantly 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







kon = (3.00 10^7) ± (0.01 10^7) $s^{-1}M^{-1}$
koff = kon· Kd = 66.1 ± 0.2 s^{-1}
Kd = 2.2 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