NMR for structural biology
Specificities and limitations

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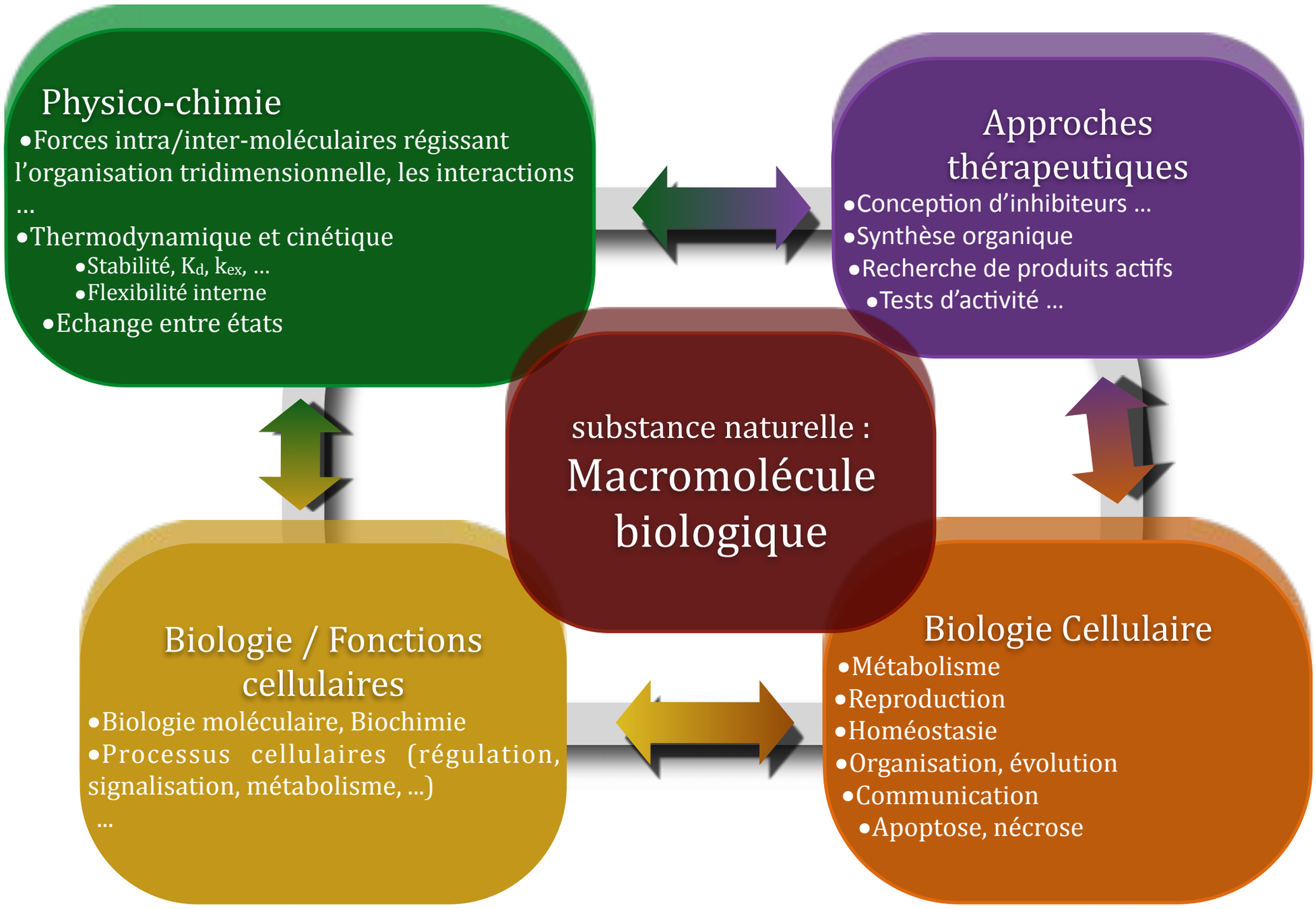
Biologie Structurale

Discipline cherchant à décortiquer, expliquer et prédire les mécanismes à la base du fonctionnement des macromolécules biologiques



Biologie Structurale et RMN

décortiquer, expliquer et prédire les mécanismes à la base du fonctionnement des macromolécules biologiques
RMN : sonde à l'échelle atomique de l'environnement électronique des atomes et de leur variation au cours du temps

Relation Séquence-Structure-Dynamique-Fonction des protéines

Utiliser et développer des méthodes de RMN adaptées

Répondre à une problématique biologique

Attribution automatique basée sur la structure (NOEnet)

Cinétique-thermodynamique-structure des états multiples en échange

La dispersion de relaxation

Caractérisation des états multiples dans les protéines

Petites protéines G Arfs
Domaines d'annexines

Compréhension des processus d'interaction mettant en jeu des protéines intrinsèquement dépliées

Domaines T
Domaine Cter de ErbB2

NMR as a tool for structural biology

✓ 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 (environment, distances, angles)

→ Thermodynamics and kinetic parameters (entropy, ΔG, K_d, pKa, k_{ex} …)

NMR as a tool for structural biology

✓ Questions

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

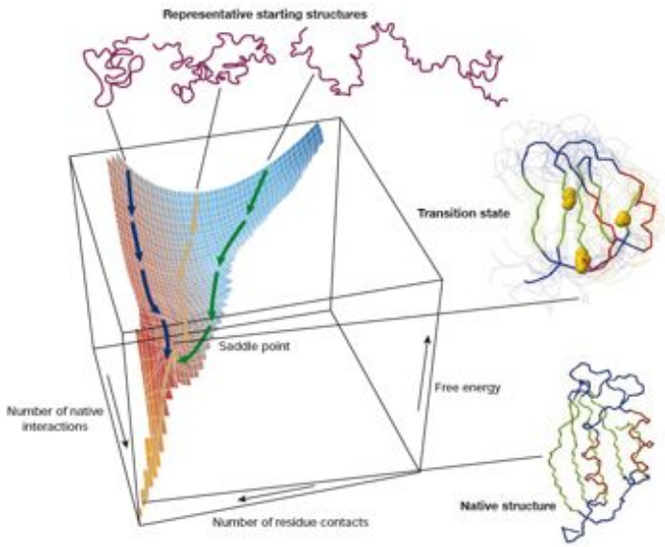
✓ Specificities …

- **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 labeling approaches, importance of the sample preparation.**

A view of protein multiple states



3D structure

« lock & key » induced fit

α ↔ β

conformational switch

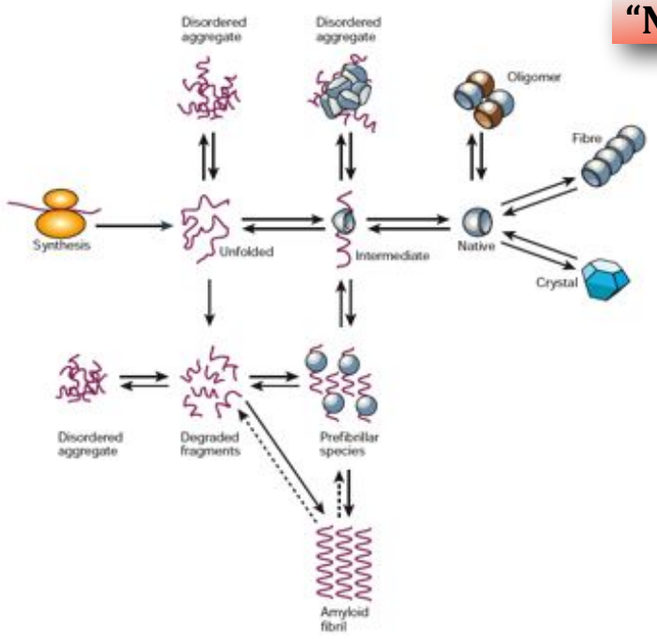
order → disorder

virus/pathogen penetration
membrane insertion
Nucléosome activation

Folding

Sequence

“Non folding”



flexible ensemble

flexible linkers
display of sites
entropic bristles, springs and clocks

disorder → order

molecular recognition
virus/phages assembly
stepping motors

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

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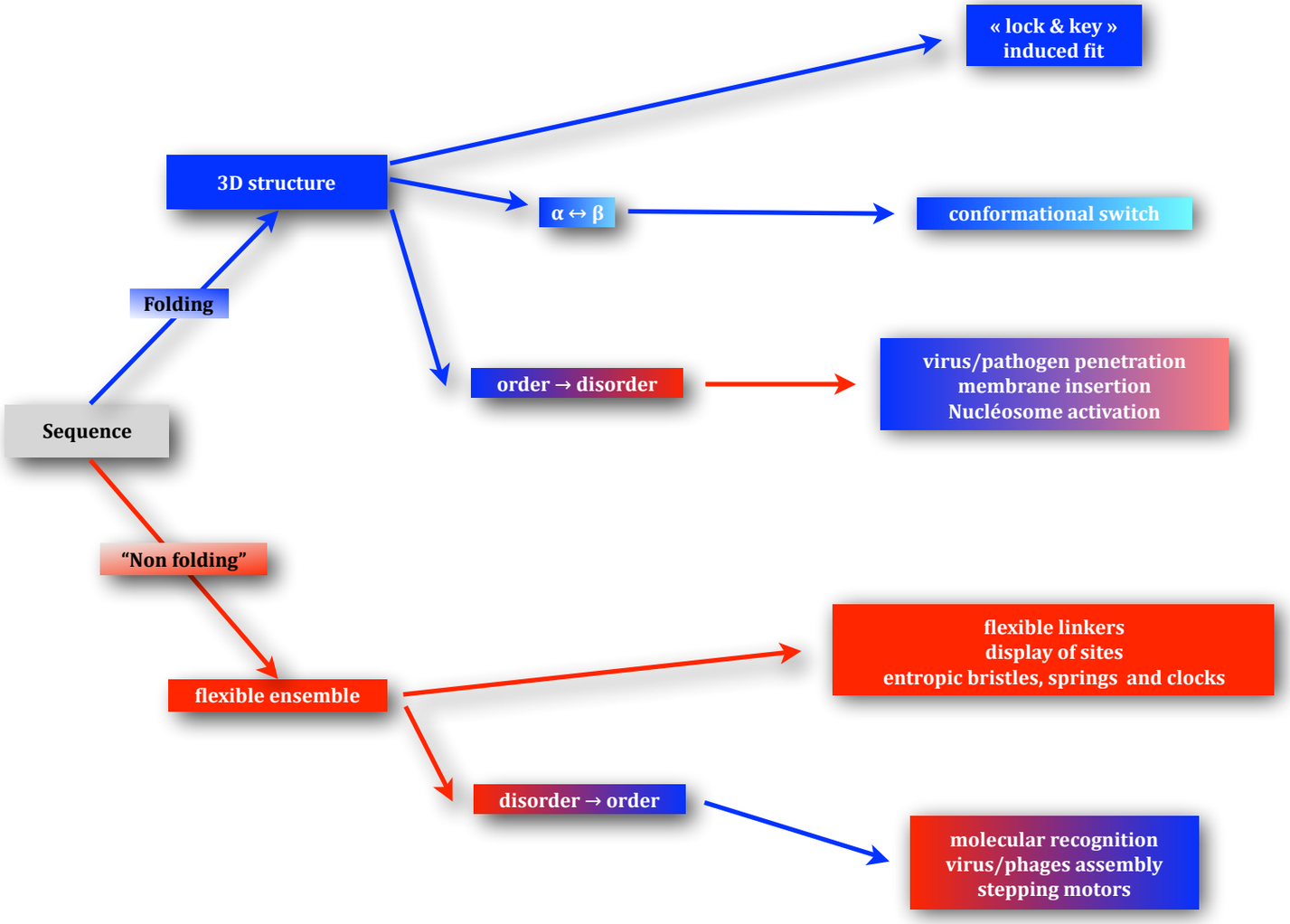
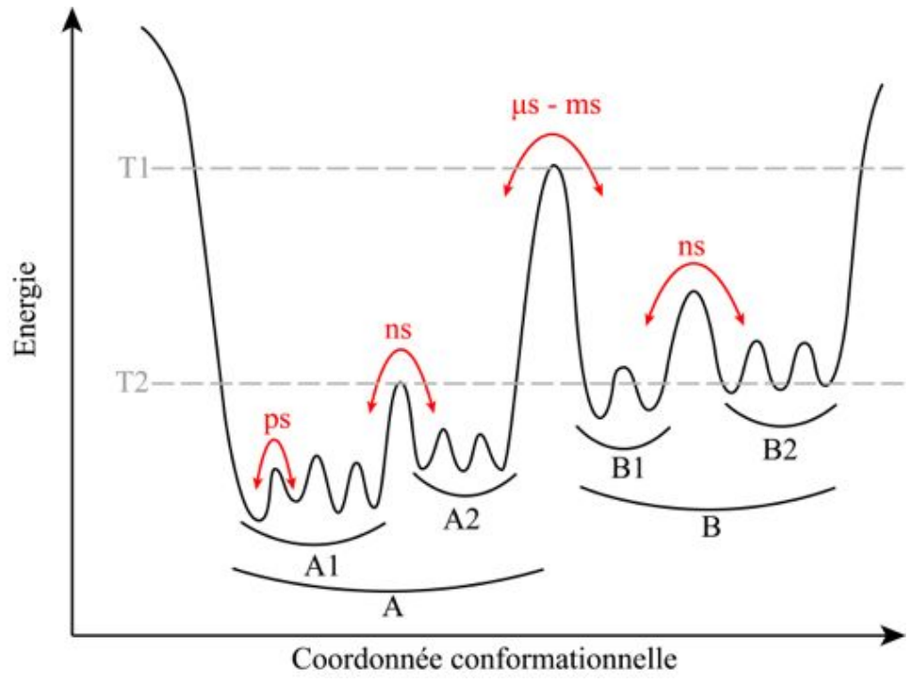
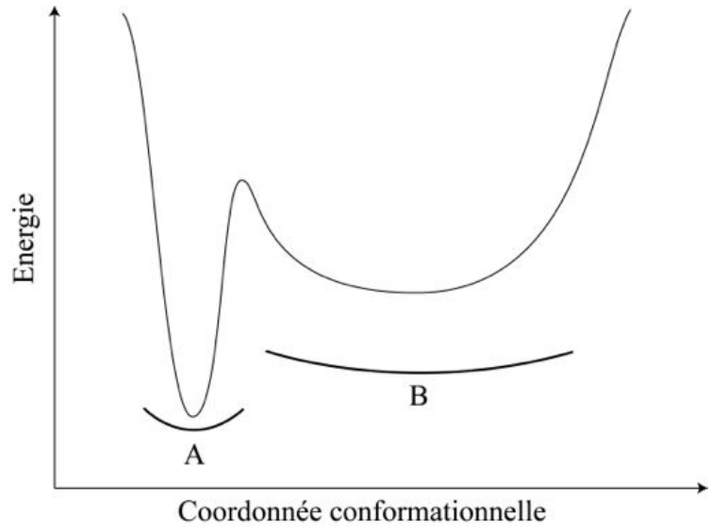


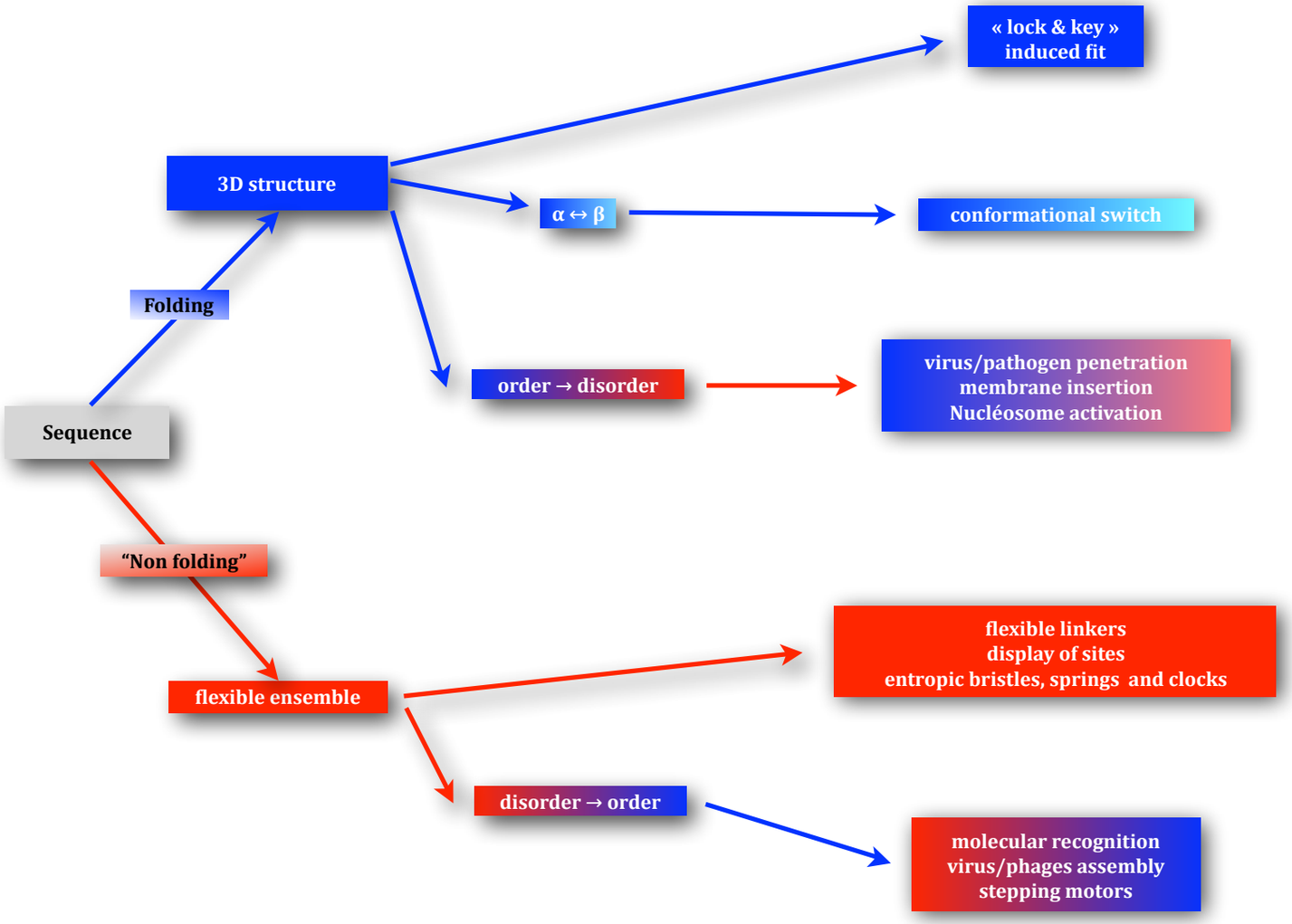


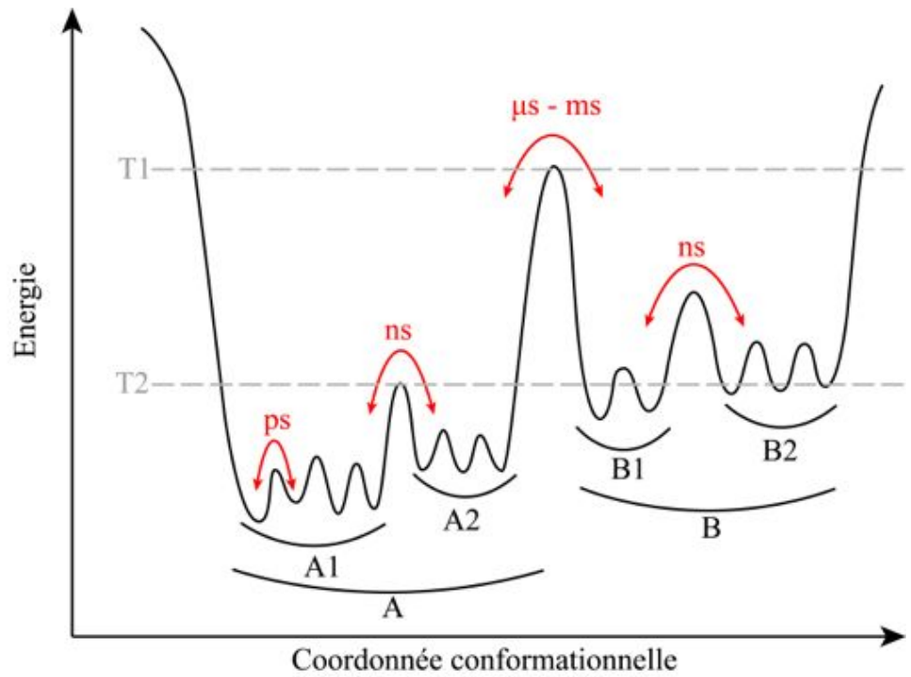


- ✓ Les protéines sont des ensembles thermodynamiques : ensemble d'états conformationnels en échange
- ✓ Différents ensembles d'états peuvent être fonctionnellement différents.
- ✓ L'ensemble le plus peuplé n'est pas forcément l'état de plus basse énergie interne (état A) ni celui de plus grande entropie (état B) mais celui de plus basse enthalpie libre (G=H-TS).
- ✓ La valeur moyenne d'une propriété n'est pas forcément représentative d'une conformation.

⇨ Importance fondamentale de caractériser la dynamique et les états multiples des protéines

A view of protein multiple states





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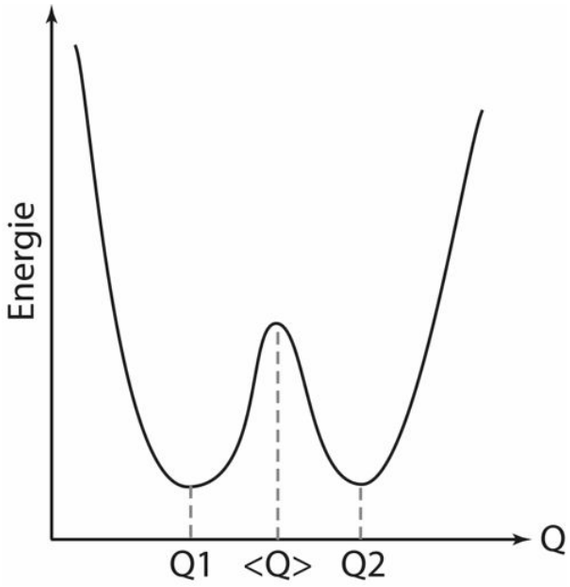
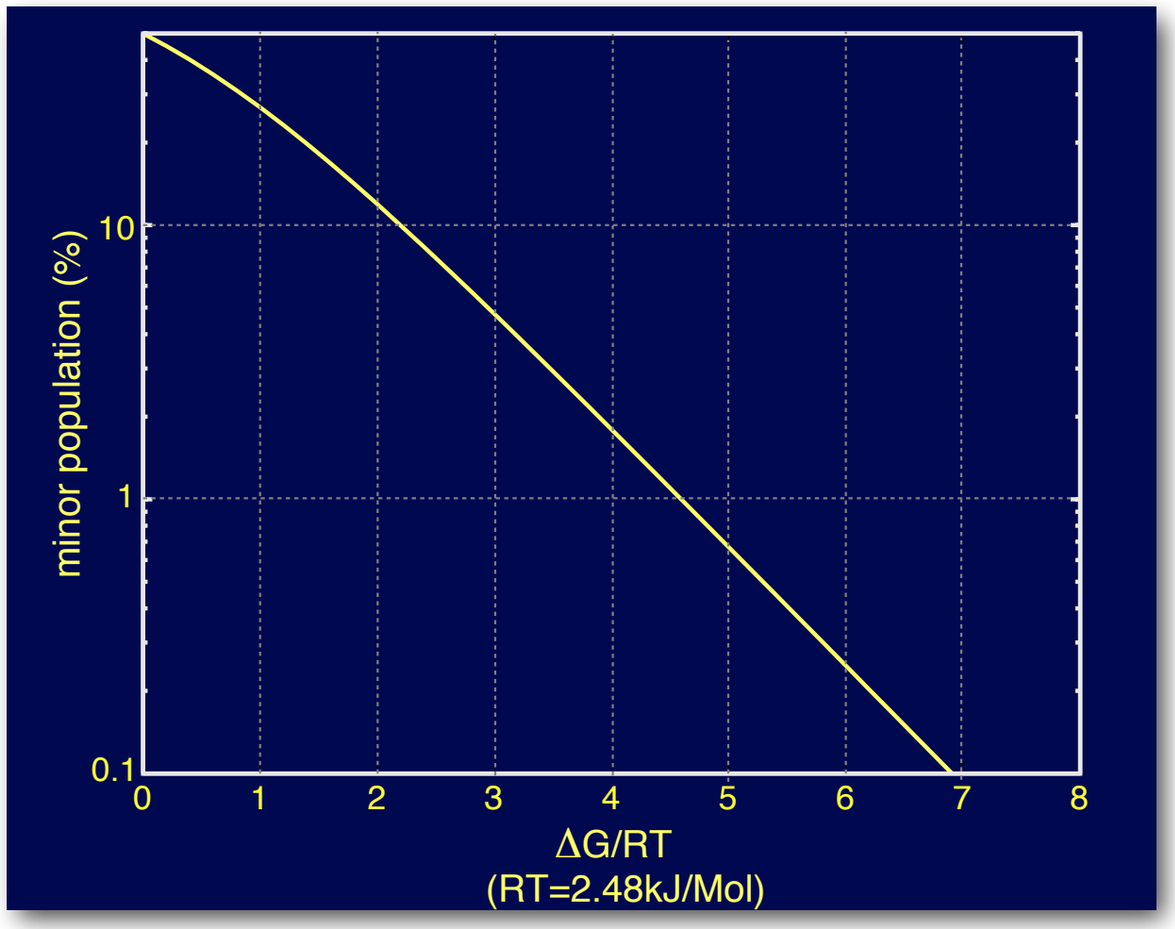


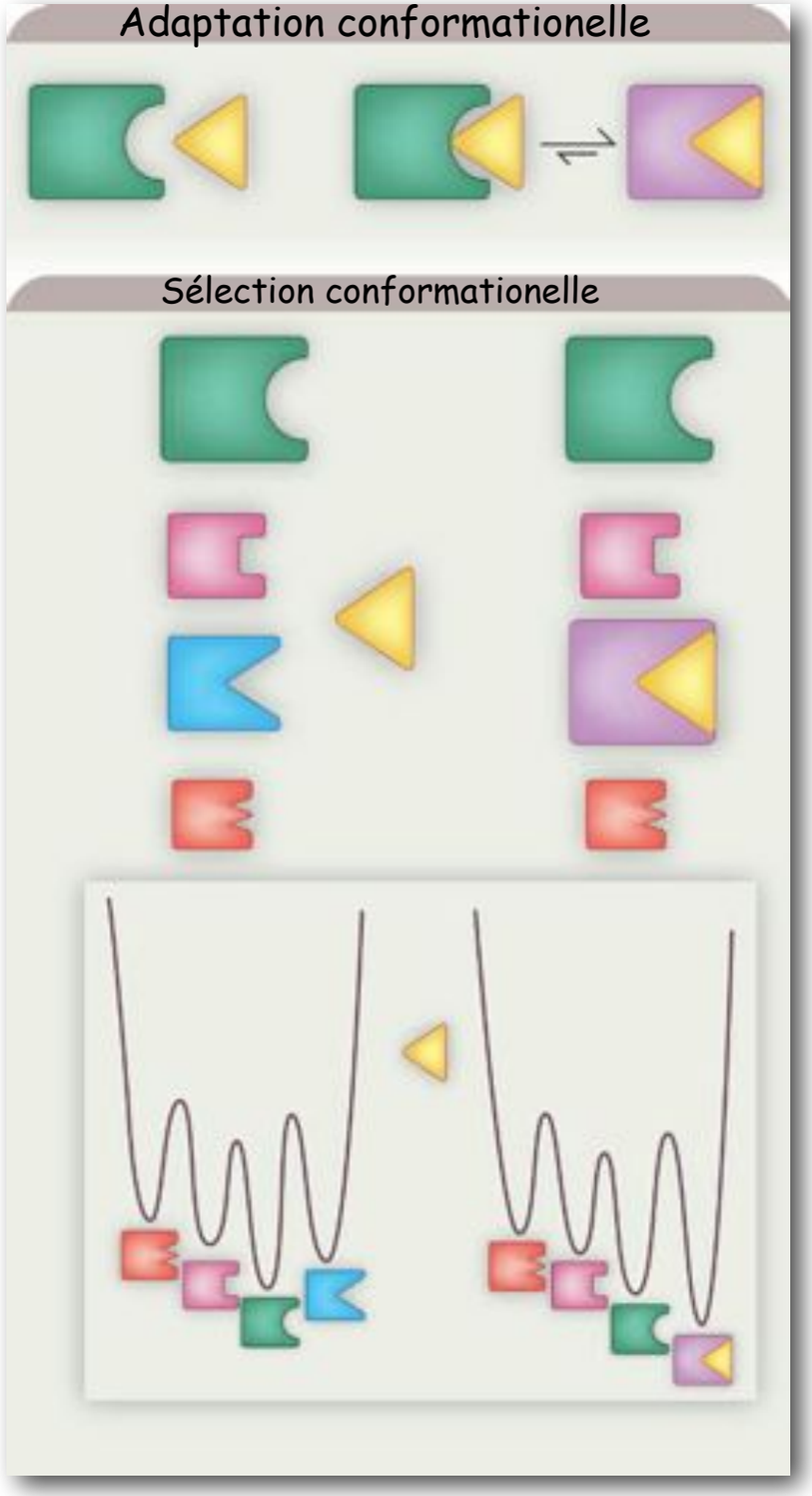
⇨ Importance fondamentale de caractériser la dynamique et les états multiples des protéines

Caractérisation des états faiblement peuplés: une étape importante pour la compréhension des mécanismes d'action des protéines

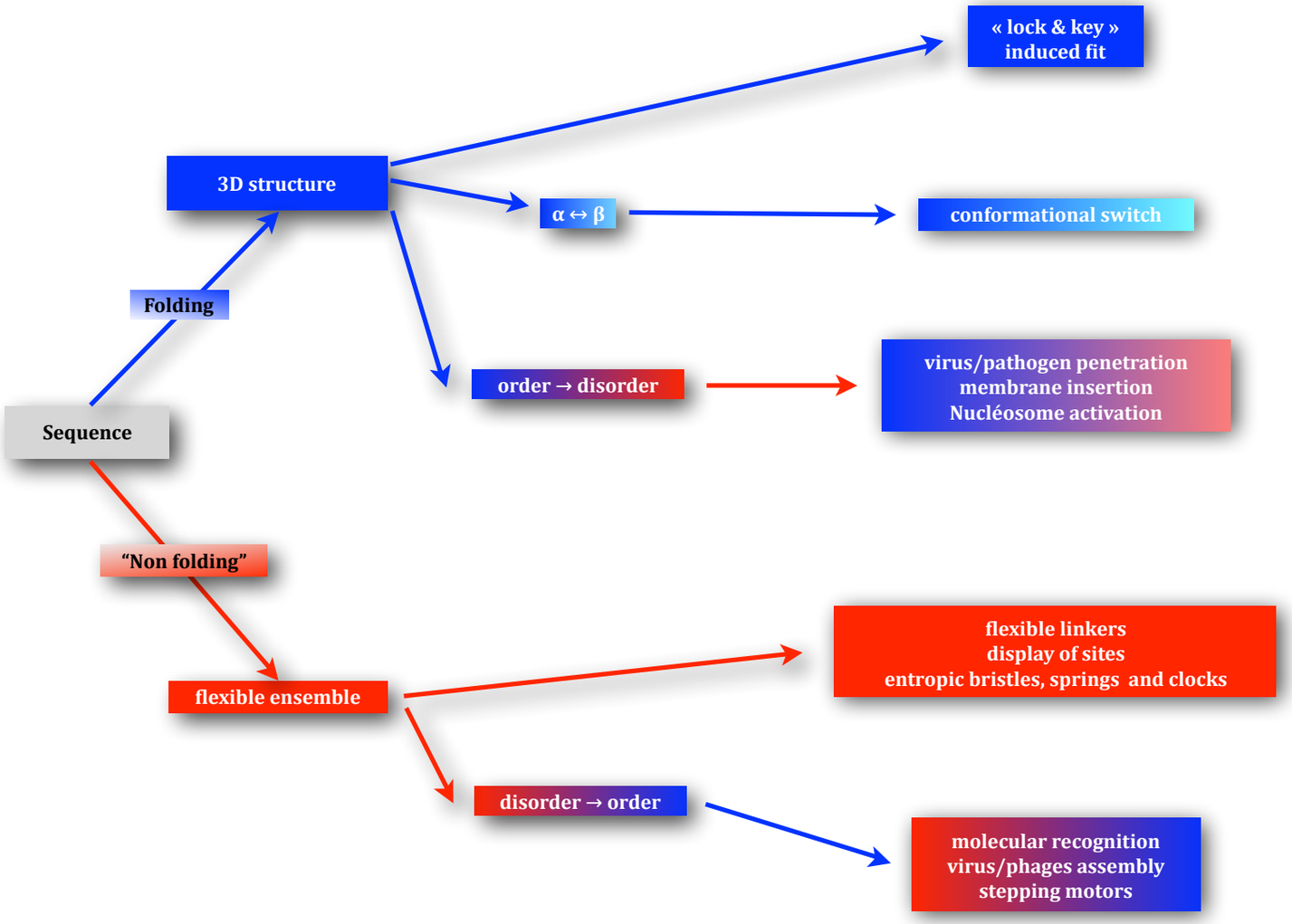
- Protéines : multiple conformations à l'équilibre thermique en solution
  - Cristallographie RX : Sélection d'une conformation lors de la cristallisation
  - RMN : Ensemble conformationel moyenné dans le temps
- Etat "fondamental" > 90%
- Etats de haute énergie, faiblement peuplés : rôles dans la liaison de substrats, dans les cycles catalytiques, dans les processus de repliement (états de transition)

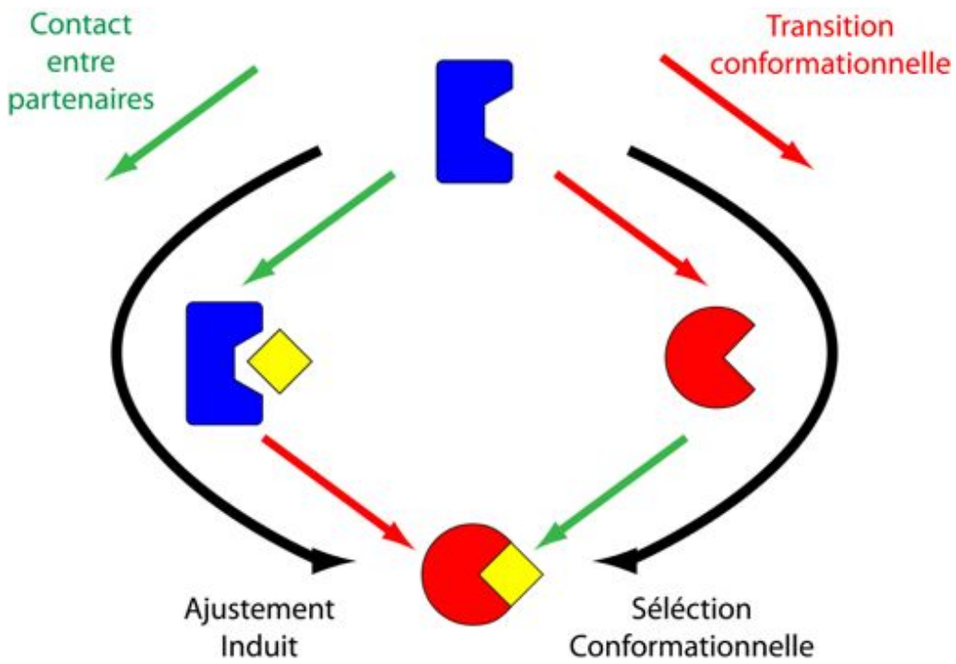
...

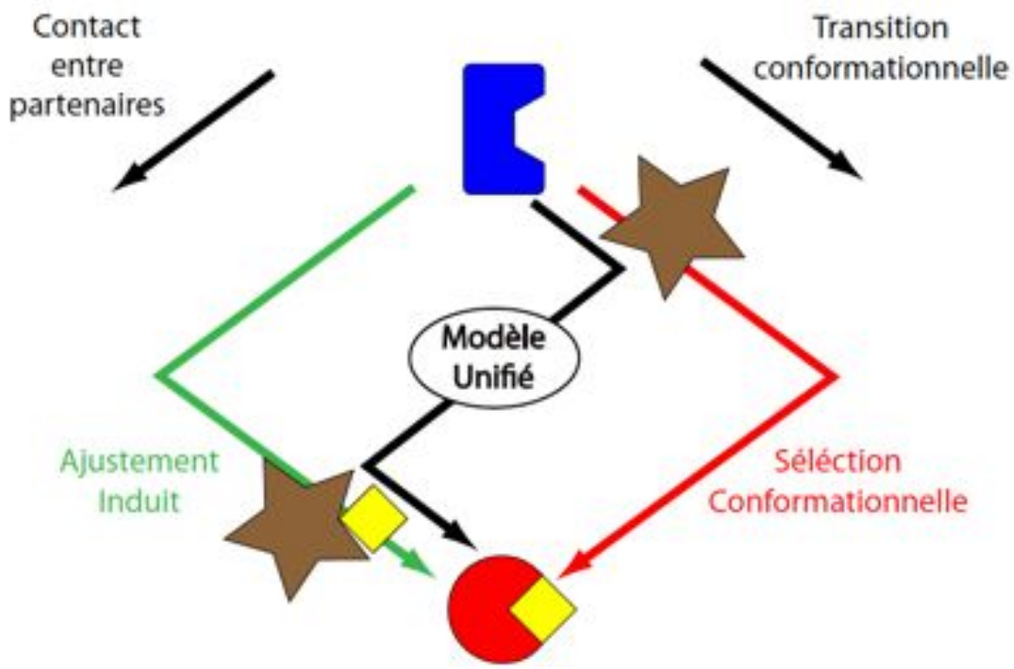




A view of protein multiple states







- Importance fondamentale de caractériser la dynamique et les états multiples des protéines
- Importance fondamentale de caractériser la cinétique de l'échange entre les ensembles d'états conformationnels

Vertessy, B. ; Orosz, F. Bioessays 2011, 33, 30–34.
[Grünberg, R. ; Leckner, J. ; Nilges, M. Structure 2004, 12, 2125–2136.

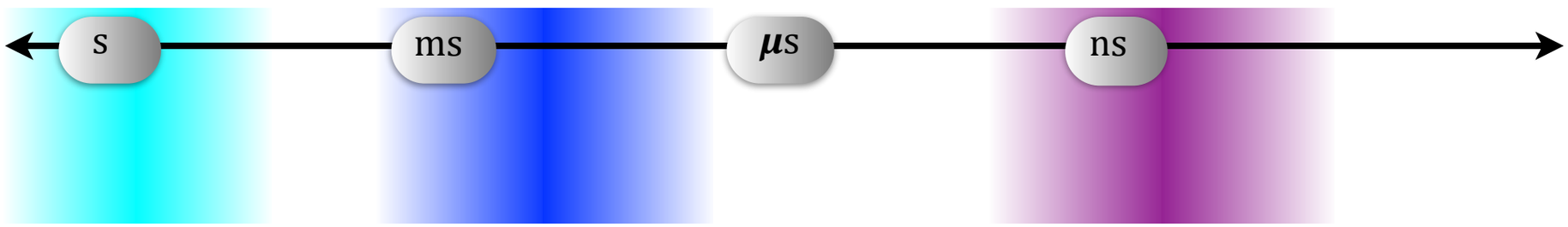
The timescales in NMR
why NMR is sensitive to multiple timescales motions

Molecular motions influence NMR parameters

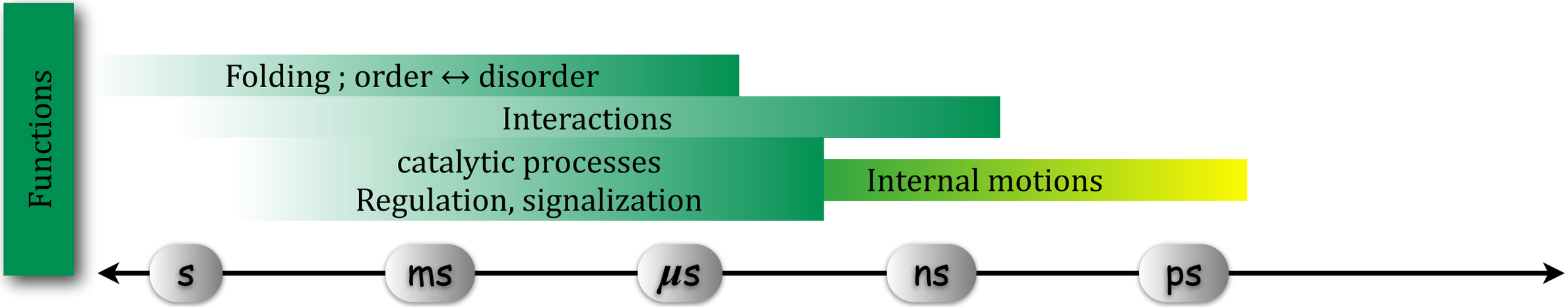
Motions timescales versus NMR timescales ?

Three distinct timescales in NMR

- Equilibrium constant time T_1 / Signal lifetime T_2
  - NMR experiment <-> perturbation of spins system
  - Typical timescale for (macro)molecules in solution : 100 ms - s (T_1) / 10ms-s (T_2)
  - Determine the lowest frequency of motions that can be characterized during one NMR experiment.
- Spectral range : $\tau=1/\Delta\nu$
  - Spectrum features : chemical shift range, couplings, …
  - Averaging if the interactions by motions that have higher frequencies
  - Perturbation of spectral appearance by motions/processes occuring around this timescale
- "Larmor" timescale: $\tau=1/\omega_0$
  - Precession frequency of the spins in the magnetic field $B_0=\omega_0/\gamma$
  - Efficiency of spins state transitions during the relaxation processes is determined by the spectral density of molecular motions around these frequencies.



A view of protein motions timescales



Types of motions

- Macroscopic diffusion
- Conformational exchange
- Molecular rotations
- Molecular vibrations

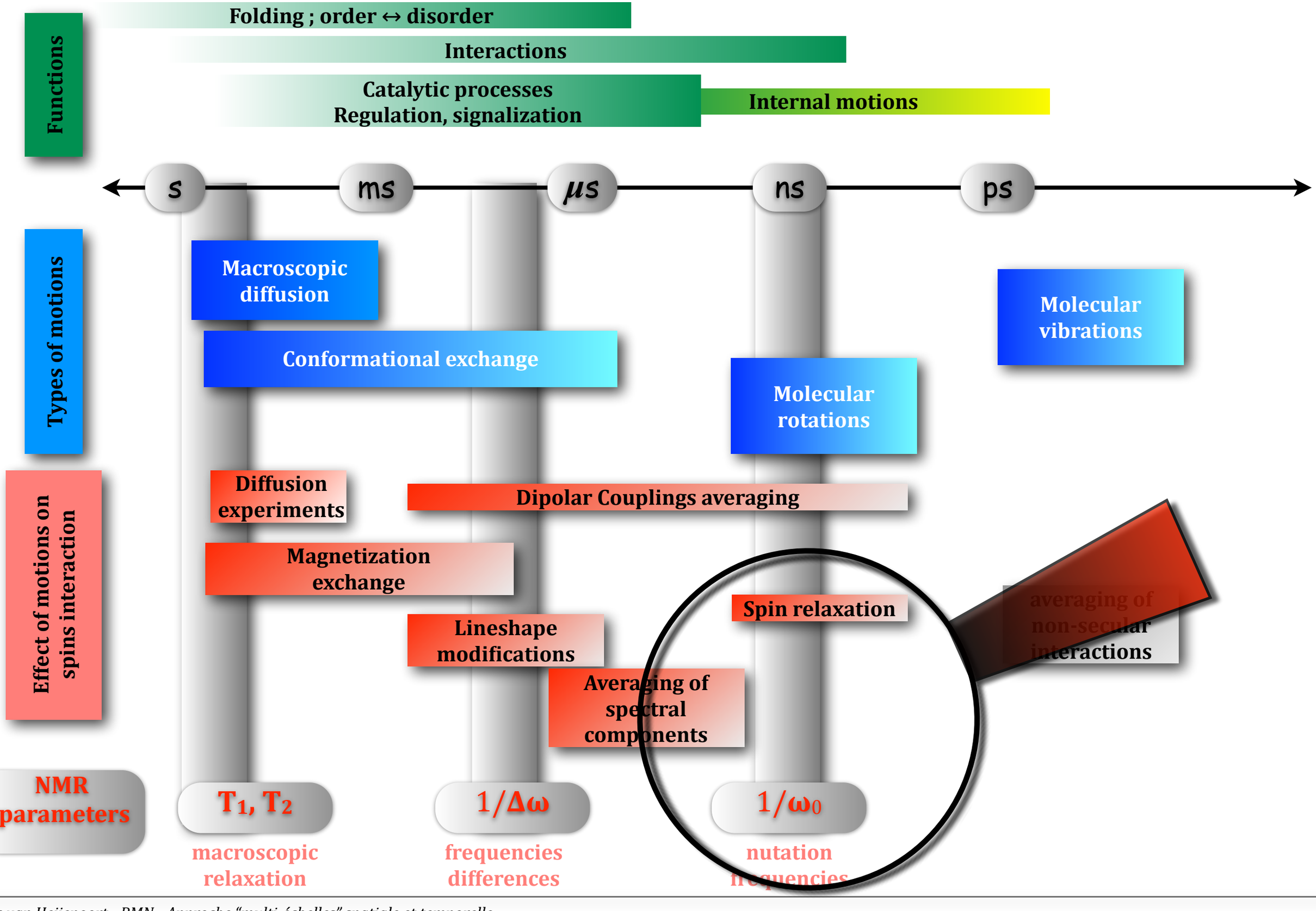
Rotation of aromatic sidechains and of buried sidechains

Allosteric processes

Local unfolding

Elastic vibration of globular domains
Exposed sidechain rotations
Loop motions
Restricted motions of buried sidechains
Secondary structures relative displacements
Global rotation
Relative motions of domains

How motions are « visible » in NMR ?



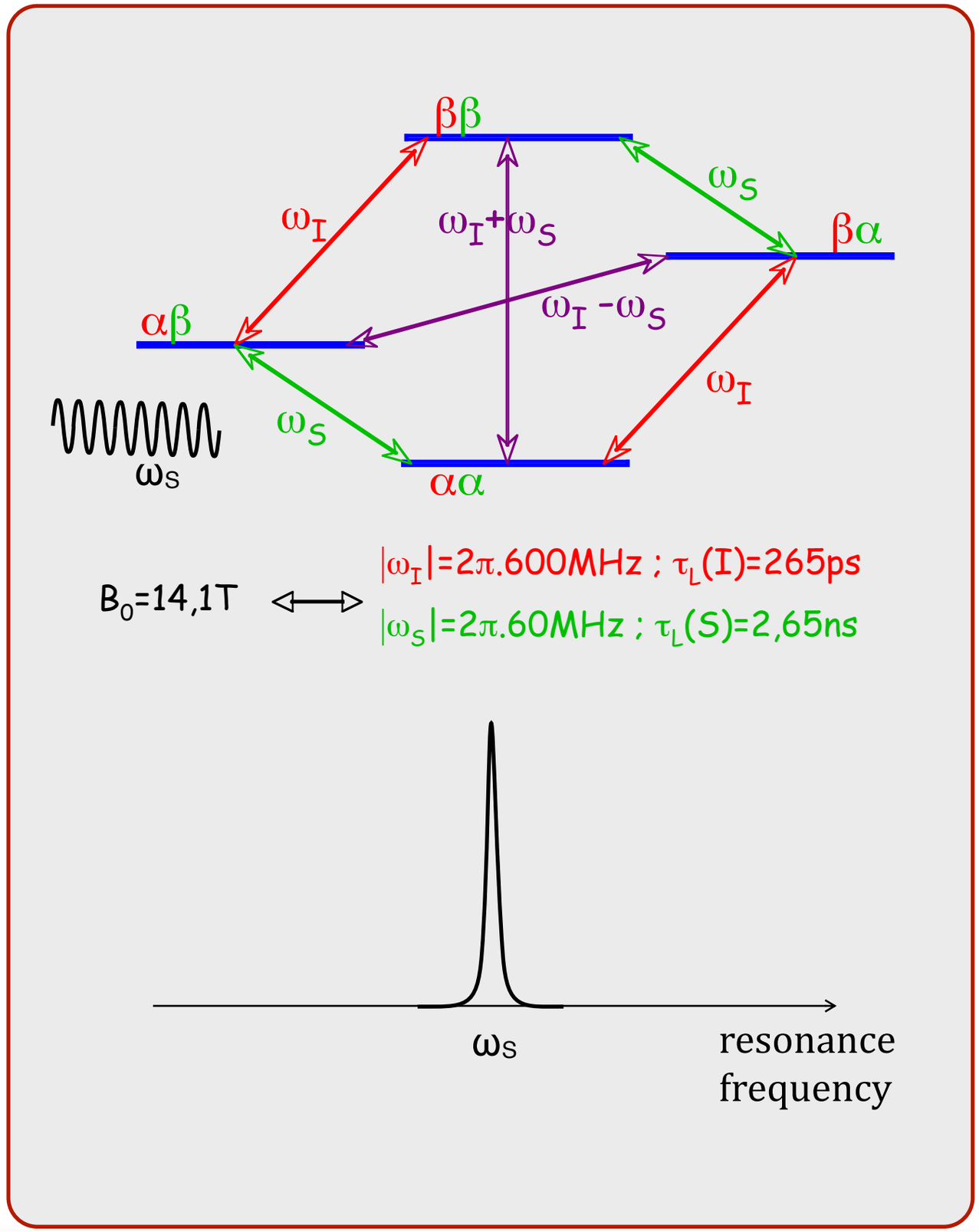
NMR timescales
1. Larmor timescale

- ✓ Resonance frequencies of the spin (i.e. difference of energy between the different observable states of the spin system)

$$|\omega_0| = |\gamma B_0|$$

$$\tau_{Larmor} = \frac{1}{\omega_0}$$

- ✓ Motions in this timescale have no effect on the appearance of the spectra.
- ✓ Motions in this timescale are responsible for the efficiency of the relaxation processes.
- ✓ The relationship between «motions» and relaxation rate constants is indirect.



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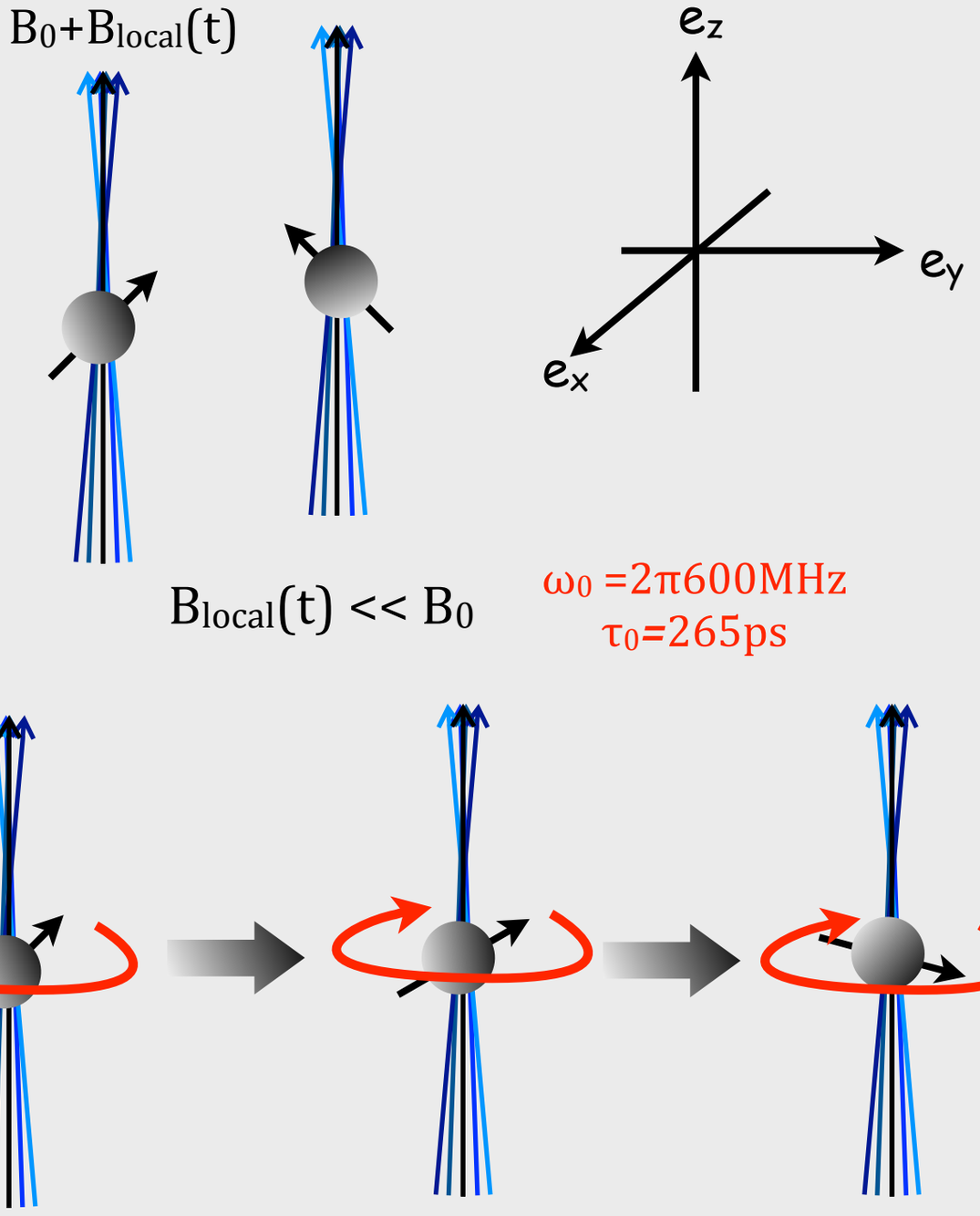
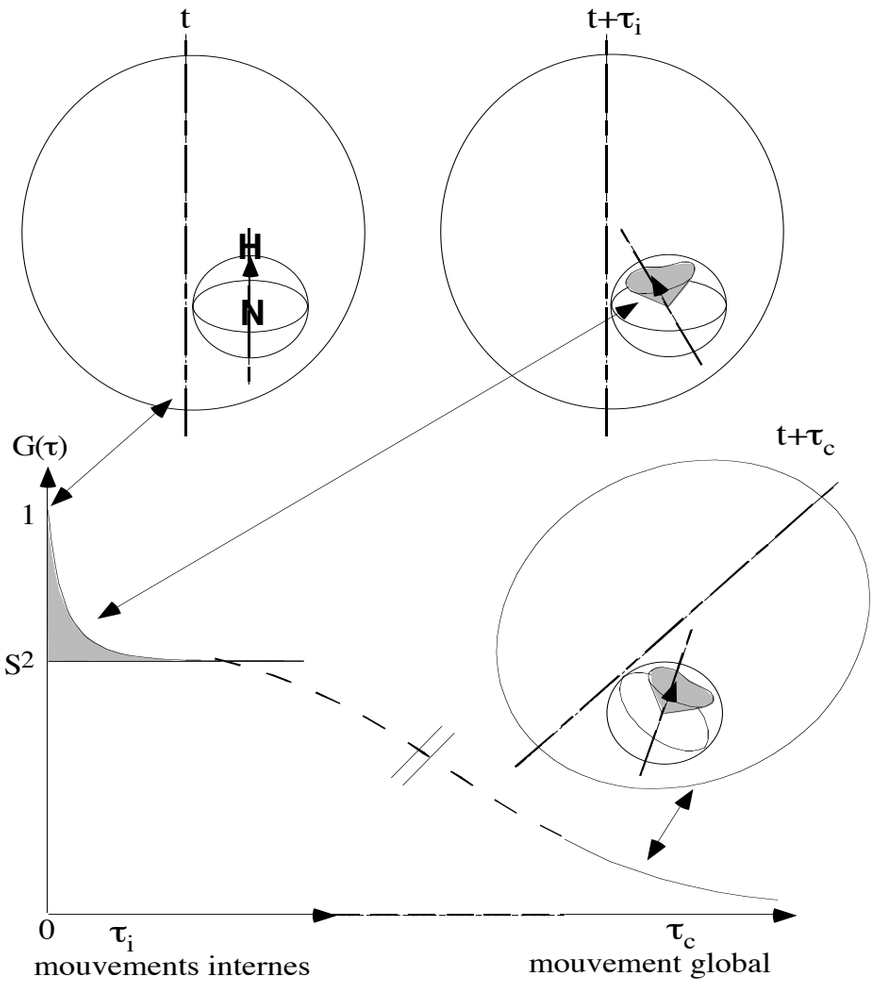
- ✓ $B_{local}(t)$ depends on the local interactions between spins (dipolar interactions, anisotropy of chemical shifts, electrons) whose fluctuations create local electromagnetic waves.



NMR timescales
1. Larmor timescale

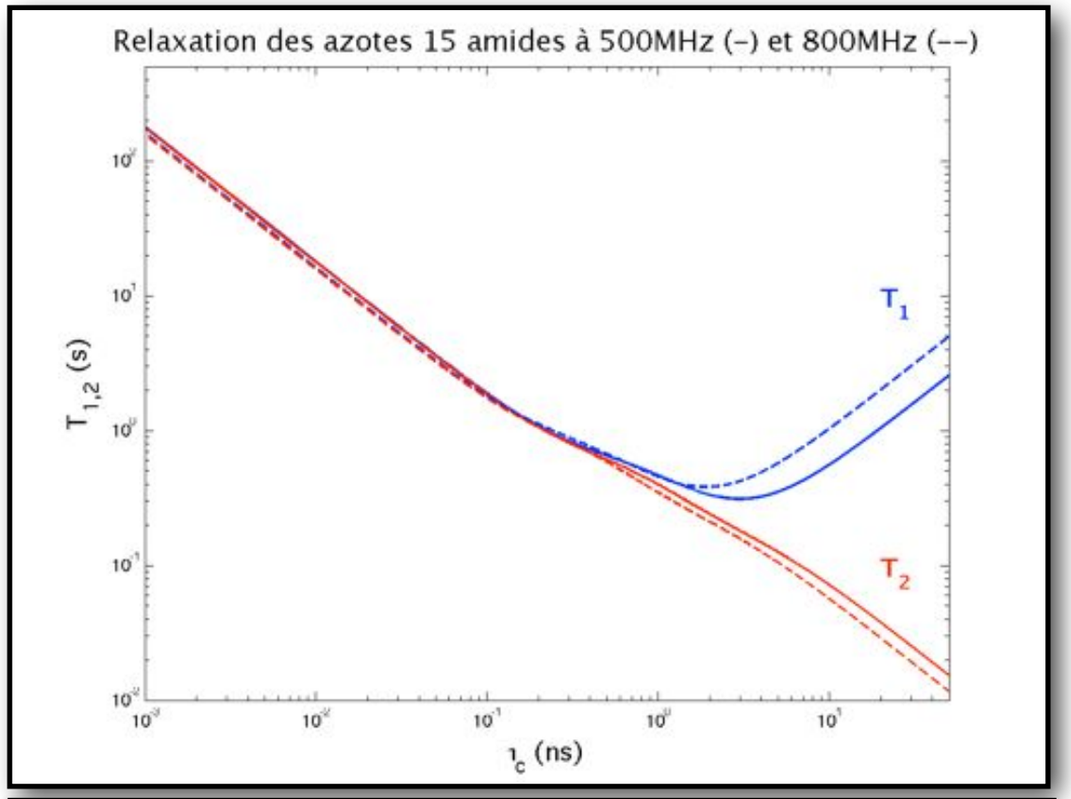
In practice

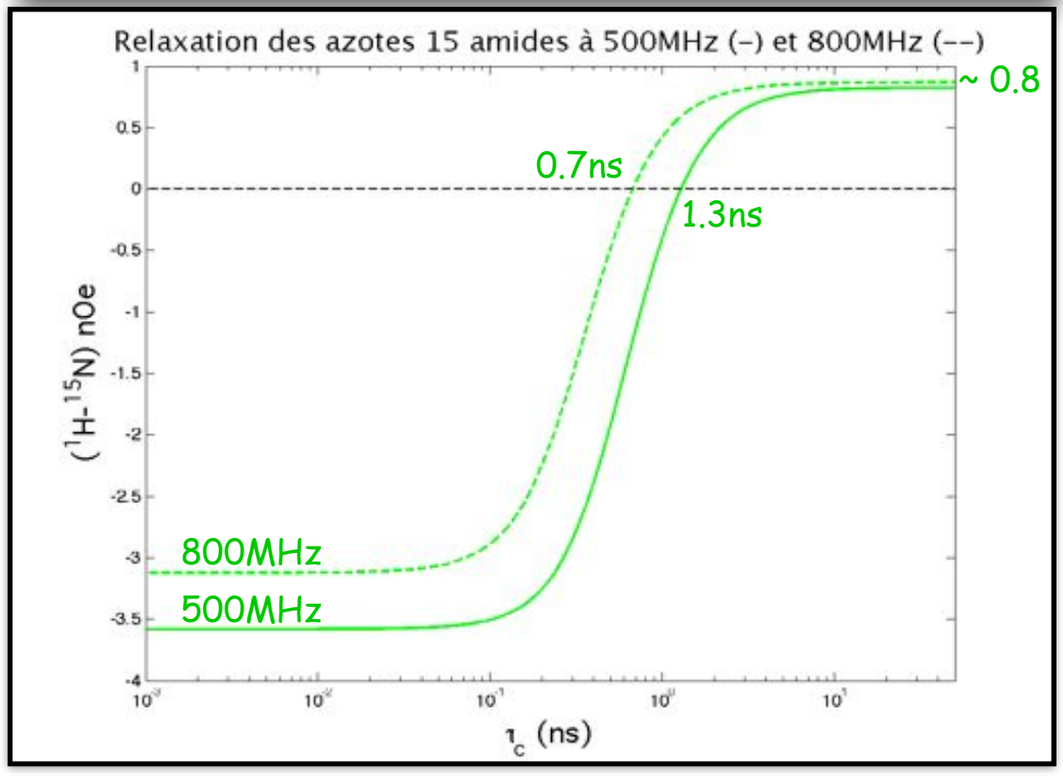
- ✓ Measurement of 3 relaxation parameters, R1, R2, and hetNOE that gives information about the local flexibility of ^{1}H-^{15}N bounds in ps-ns range.
- ✓ Models to extract parameters of motions : global/local correlation times (timescale), order parameters (amplitude), etc.



Lipari & Szabo "model free" approach

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





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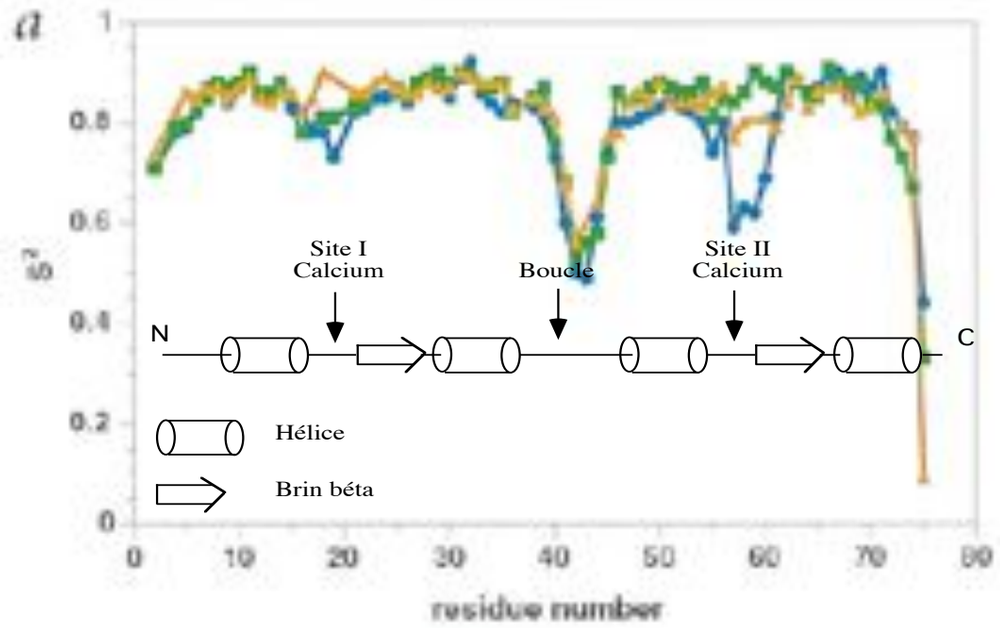
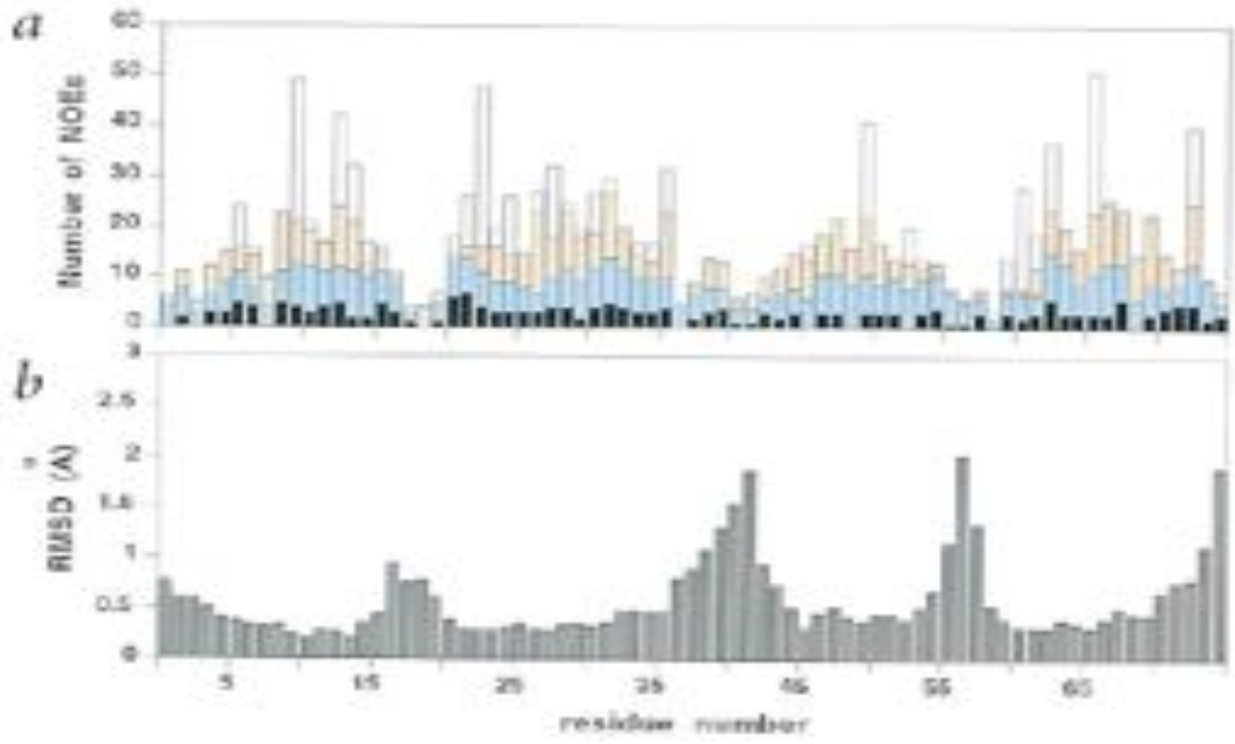


Mäler et al., Nat. Struct. Biol. **7**, 245 (2000)

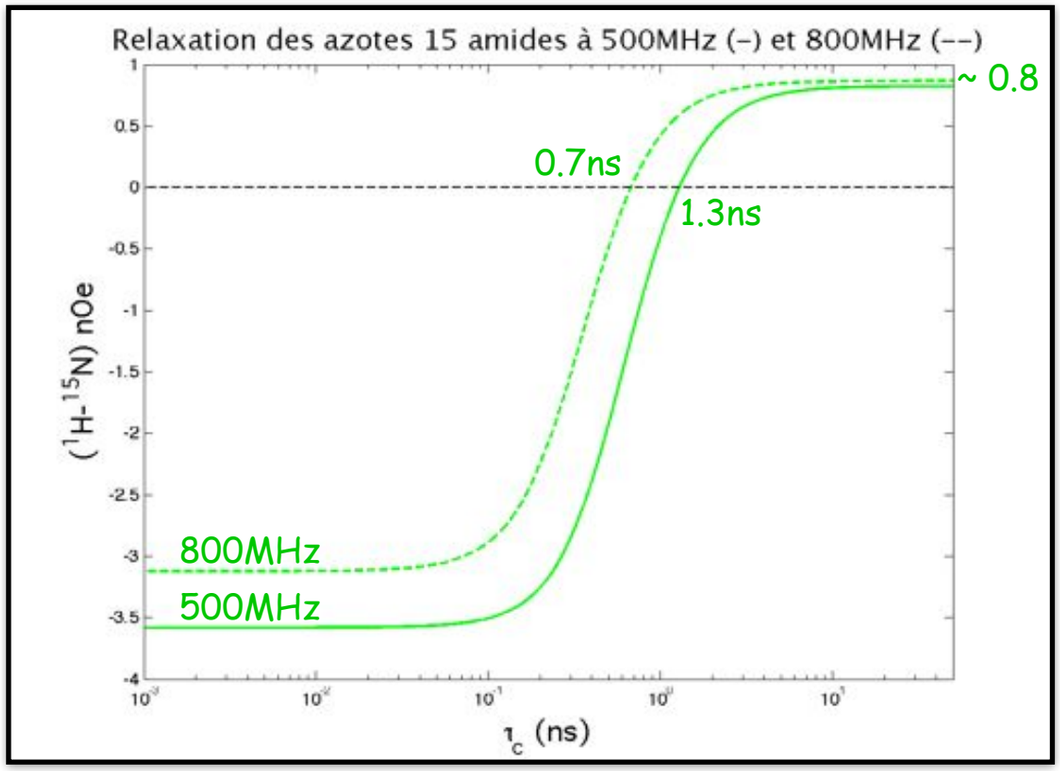
Calbindin D_{9k}
Apo
$(Ca_{2+})_1^{I}$-N56A
$(Cd_{2+})_1^{II}$

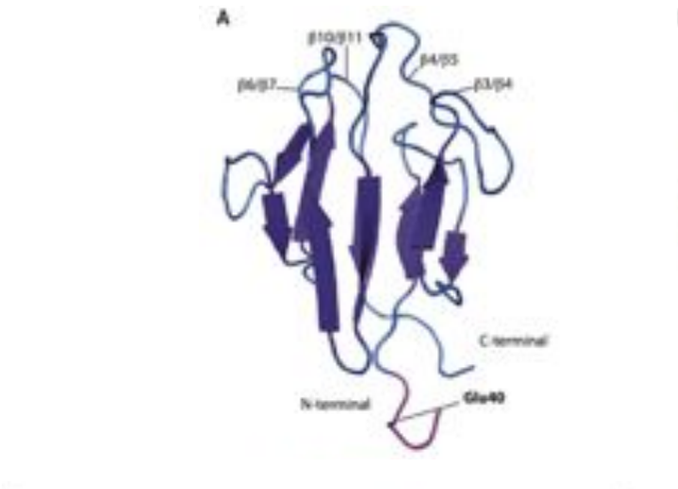


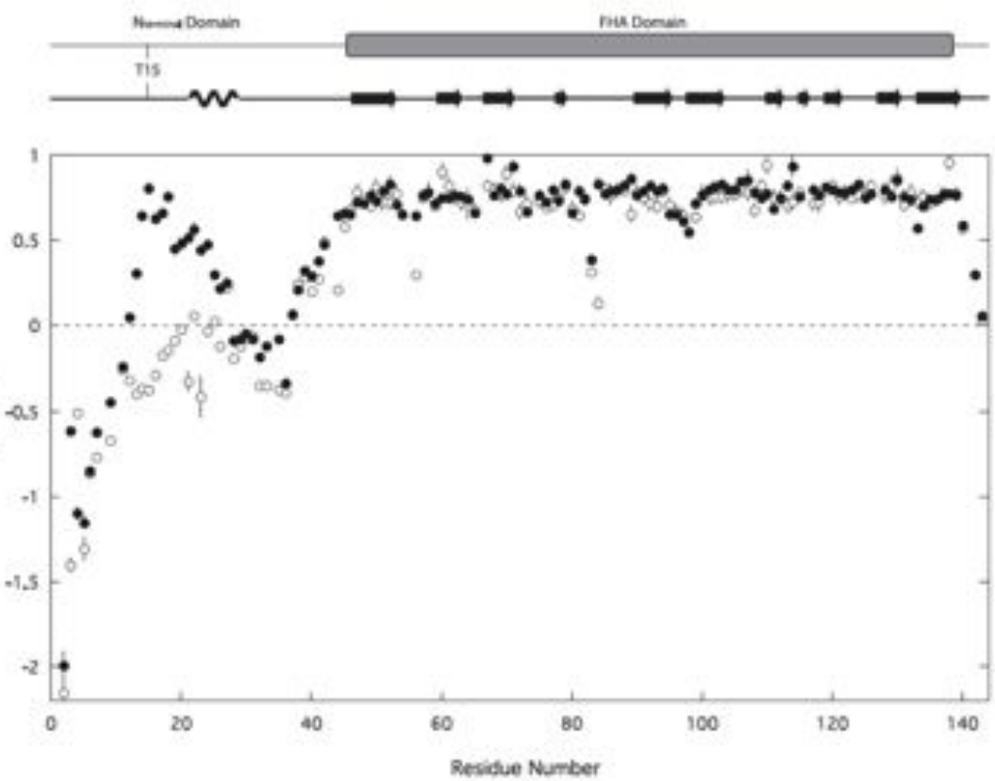
NMR timescales
1. Larmor timescale

In practice

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

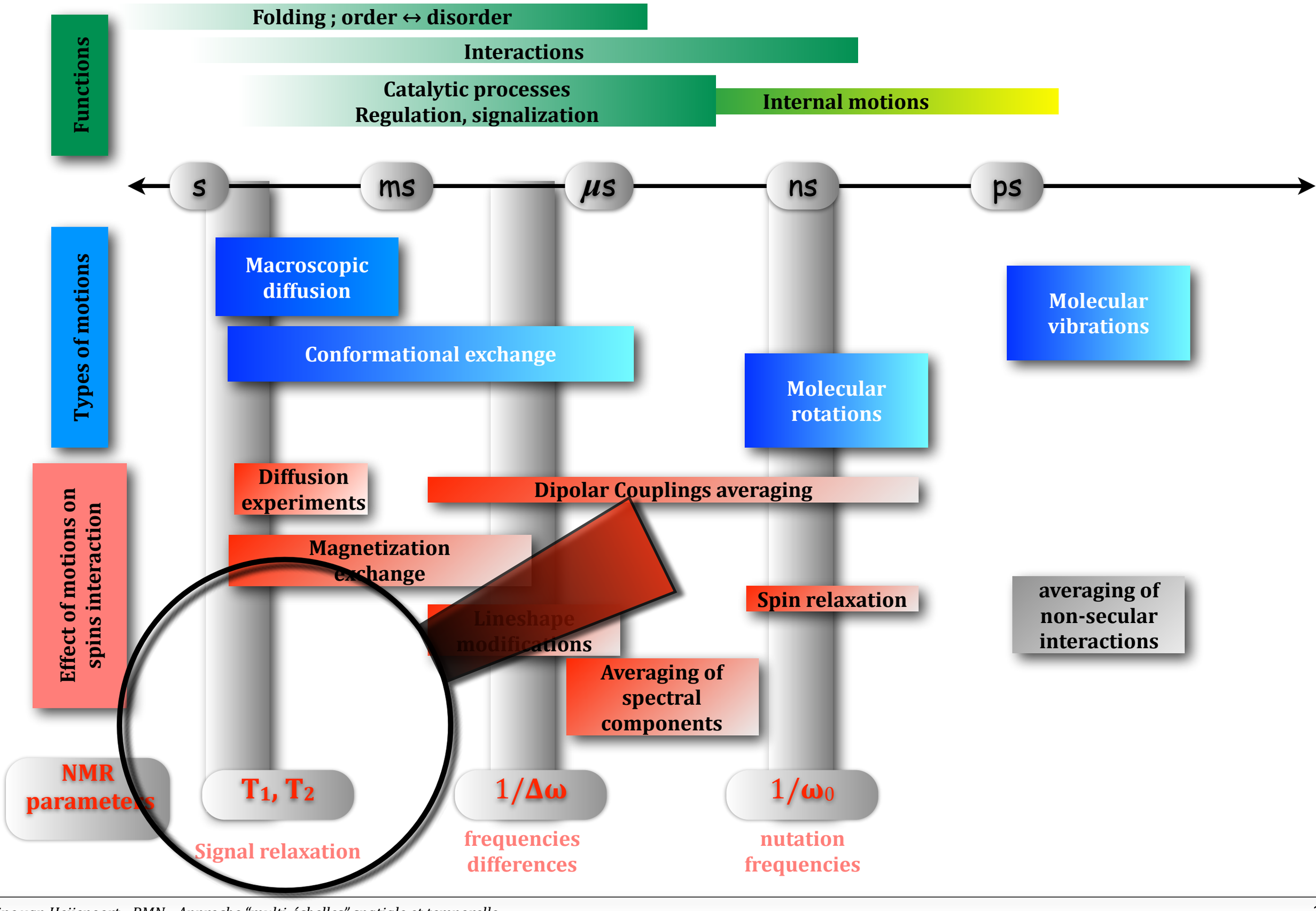






Philippe Barthe, Christian Roumestand, Marc J. Canova, Laurnt Kremer, Corinne Hurand, Virginie Molle and Martin Cohen-Gonsaud, "Dynamic and Structural Characterization of a Bacterial FHA Protein Reveals a New Autoinhibition Mechanism". Structure 17, 568-578 (2009).

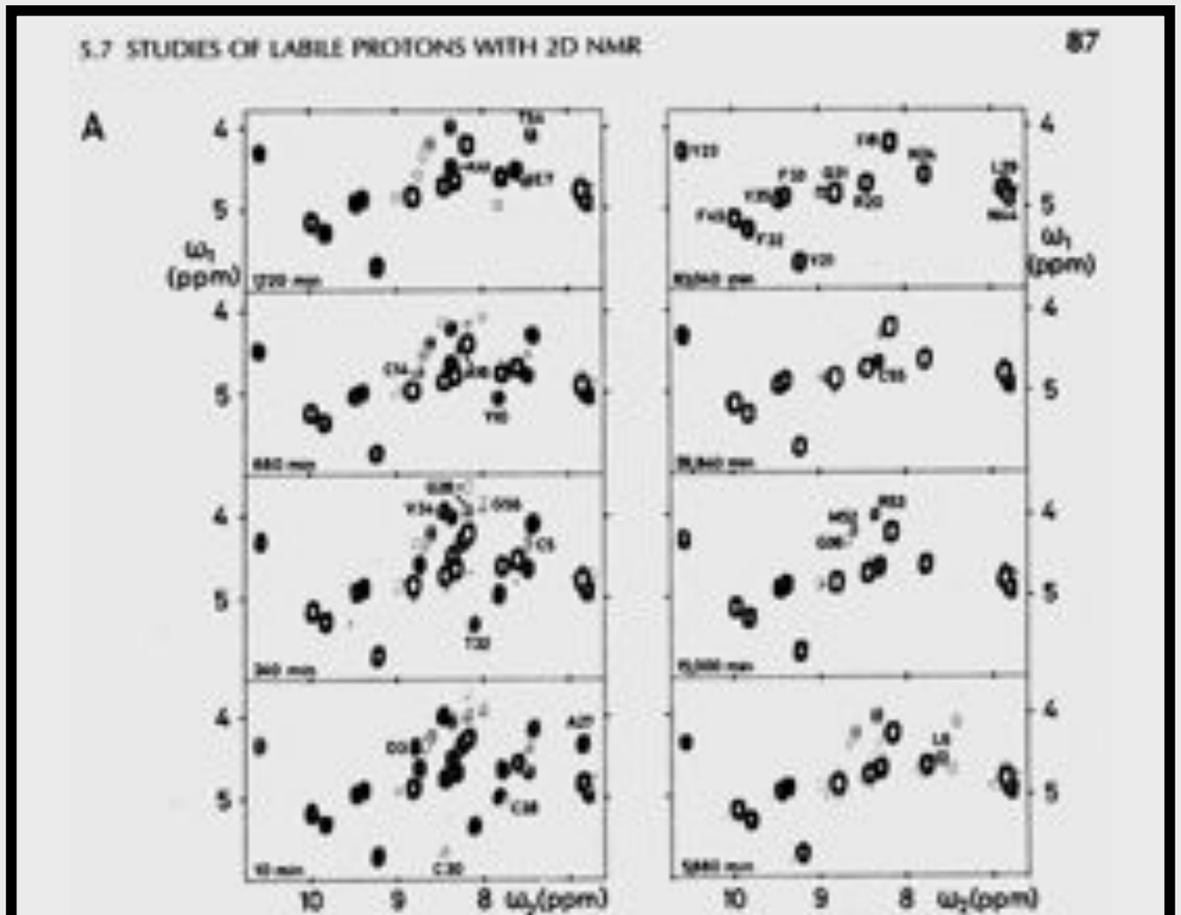
How motions are « visible » in NMR ?



NMR timescales
2. Relaxation times

- ✓ Longitudinal relaxation time constant T_1 characterizes the time it takes to the spin system to return to equilibrium.
- ✓ It determines the delay between two scans
- ✓ Motions slower than T_1 can not be characterized by one NMR scan.
- ✓ NB. T_1 depends on the magnetic field strength B_0, the type of spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

Dynamical processes slower than T_1
- «real time» kinetics
- folding/unfolding
- Interactions, exchange
- H/ D exchange
- ...



Wüthrich, « NMR of proteins and nucleic acids », Wiley Interscience, 1986

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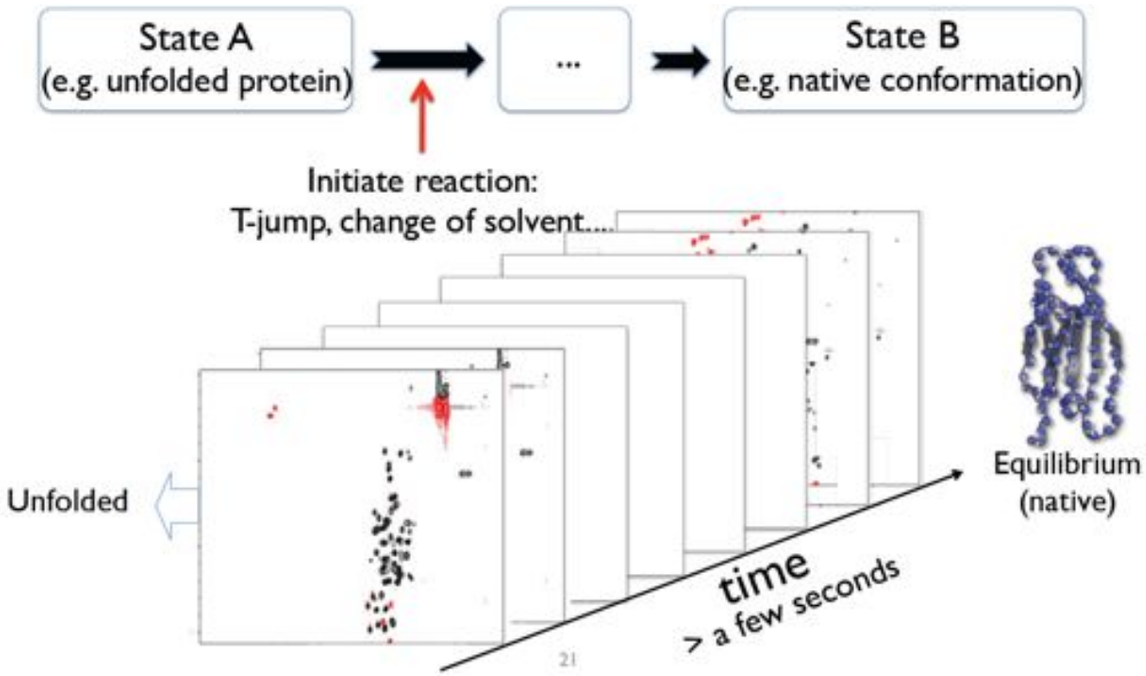
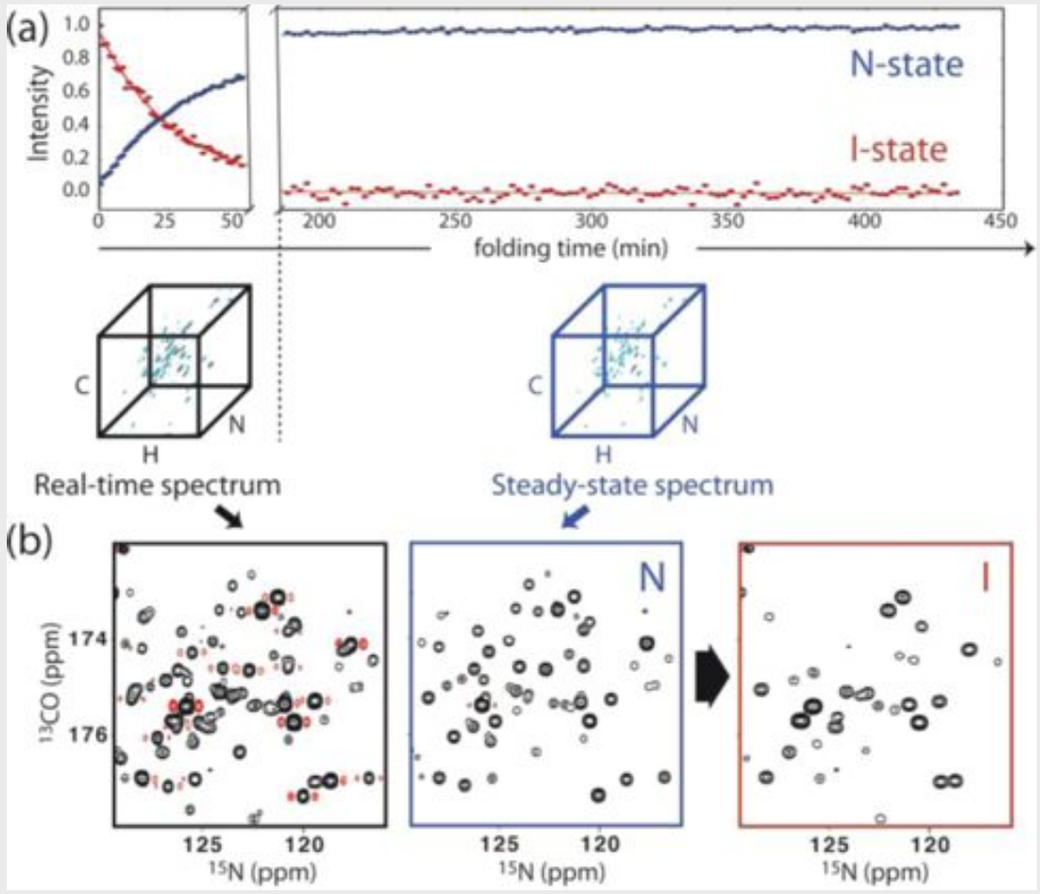
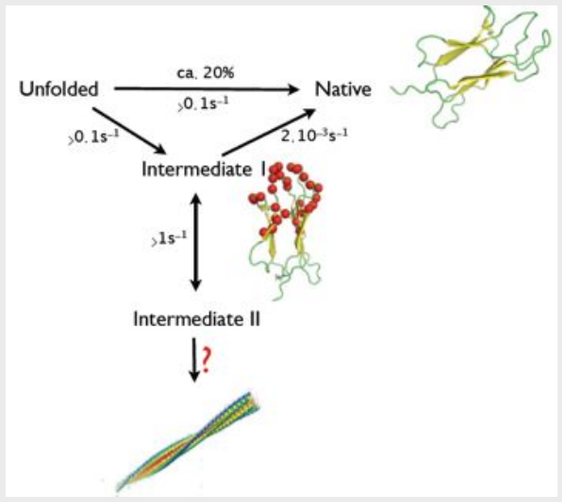


"Playing" with T1 : FAST/BEST experiments





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

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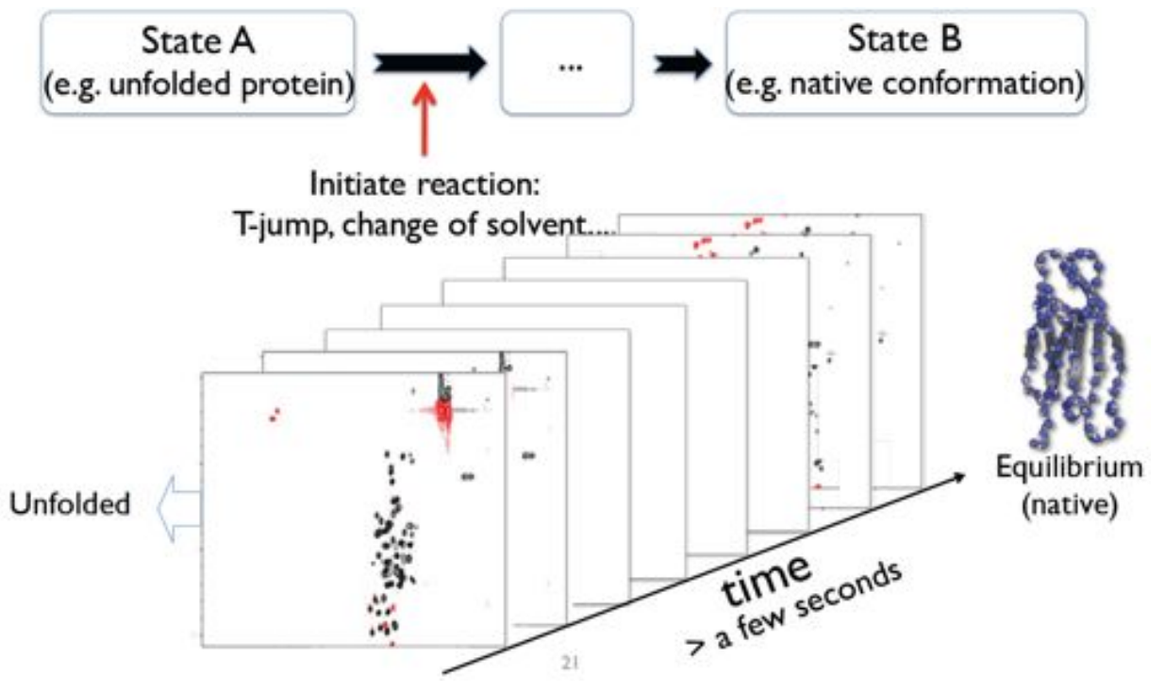

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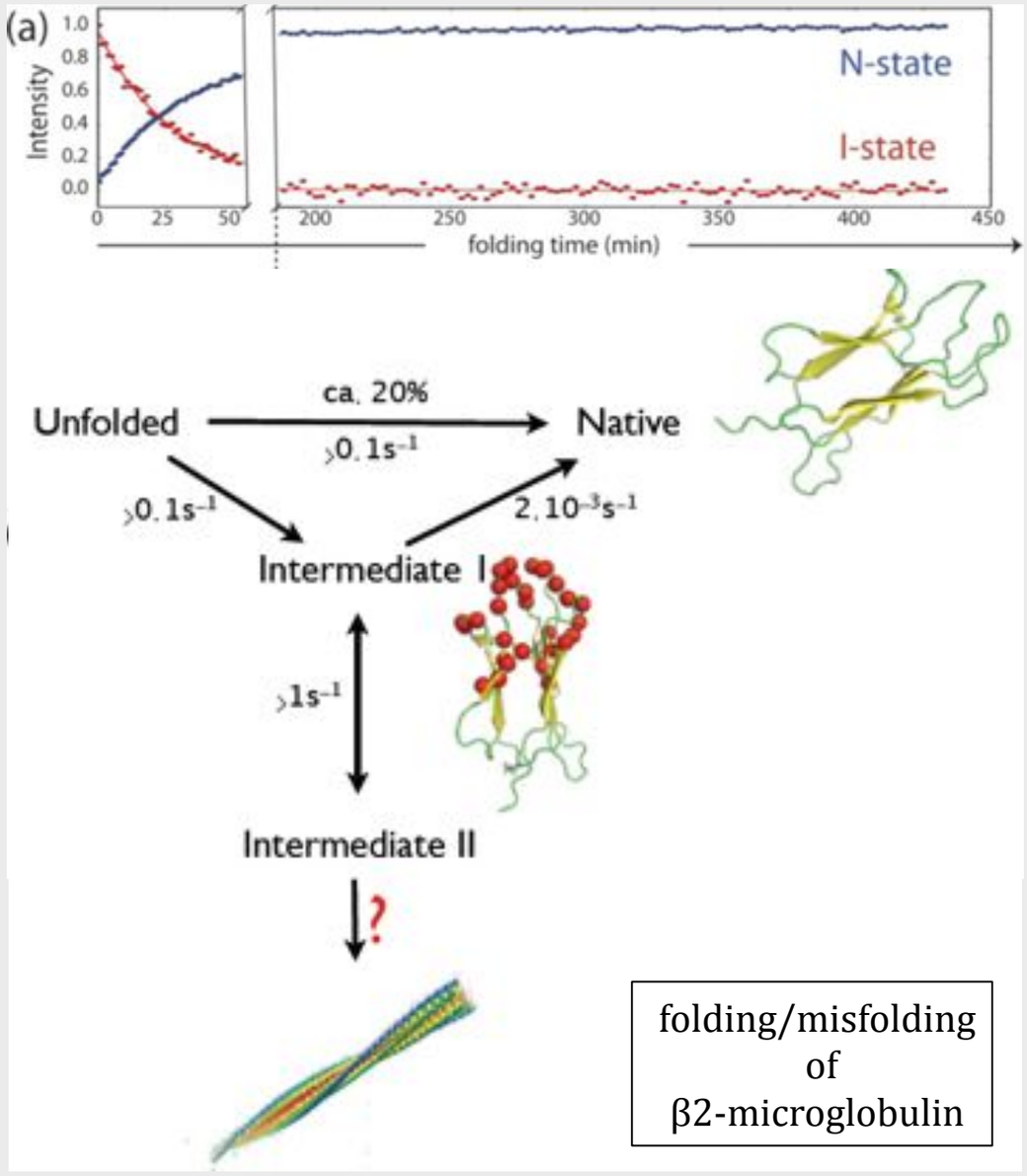
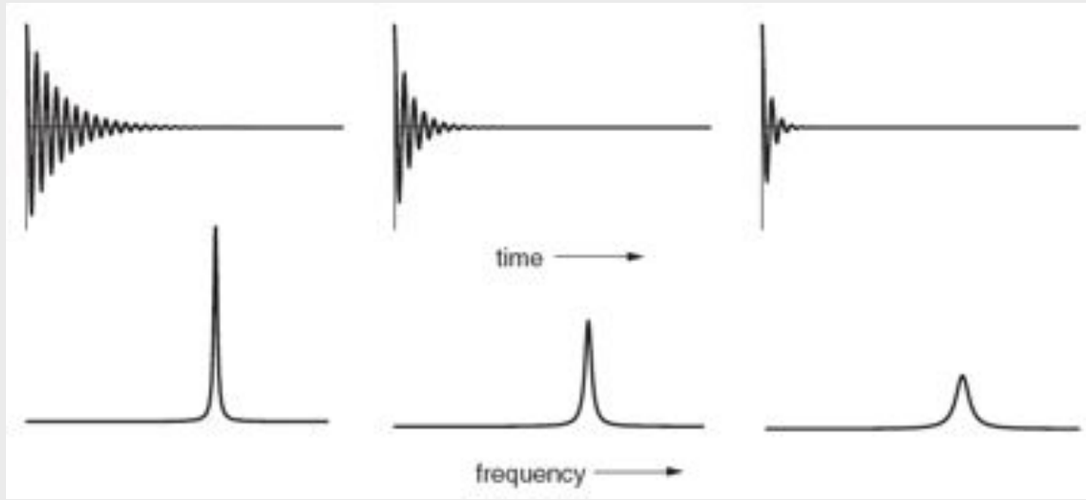


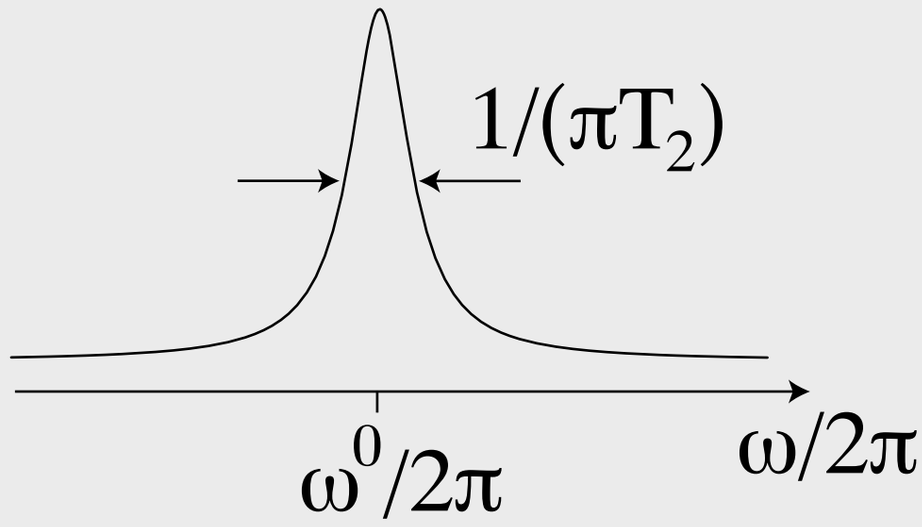
Corazza et al., *J. Biol. Chem.* 2010,Rennella et al. *J. Am. Chem. Soc.* 2012

NMR timescales
2. Relaxation times

- ✓ Transversal relaxation time constant T_2 characterizes the lifetime of the signal.
- ✓ It determines the linewidth of the resonances
- ✓ NB. T_2 depends on the magnetic field strength B_0, the type of spin, the environment of the spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

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





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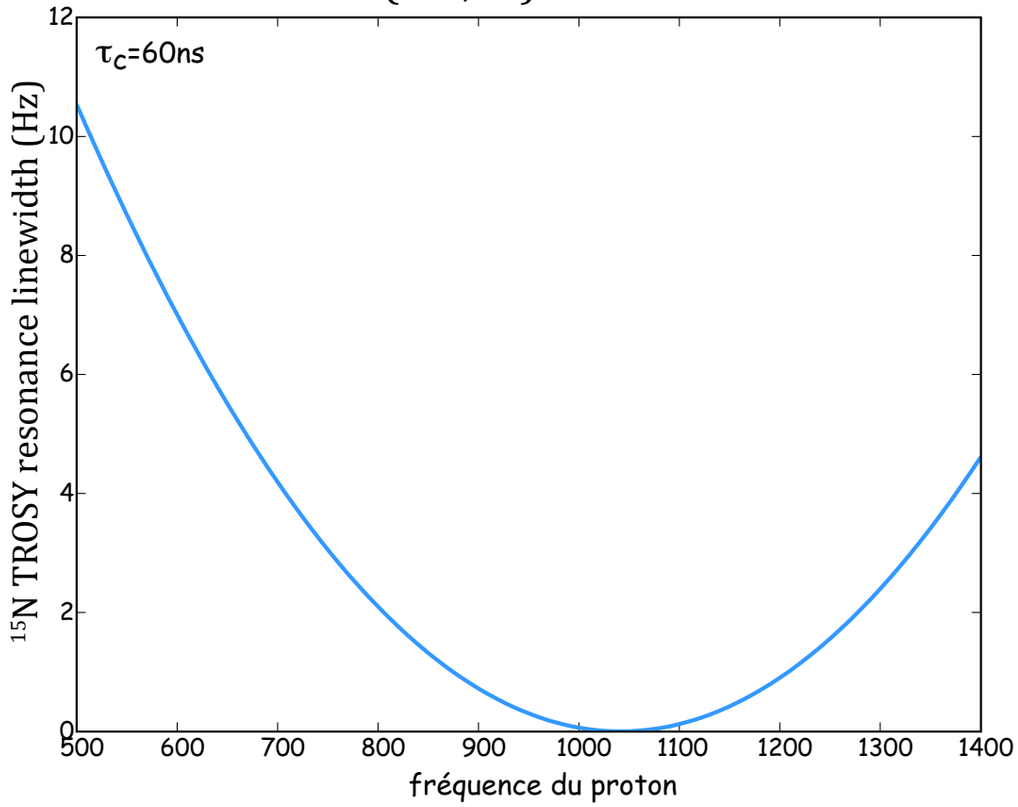
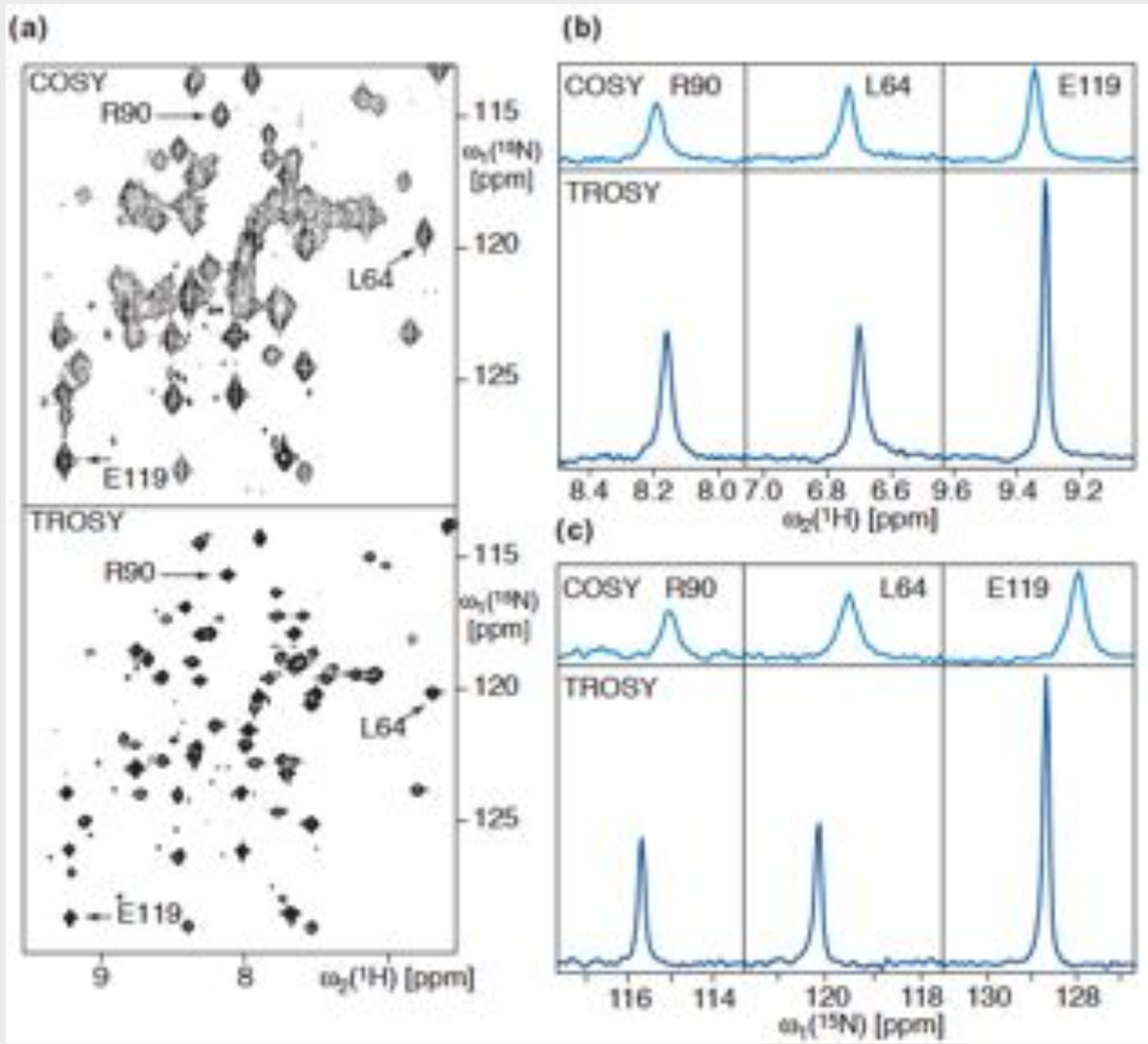
- NB. T_2 depends on the magnetic field strength B_0, the type of spin, the environment of the spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

(^{15}N,^{1}H) TROSY



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

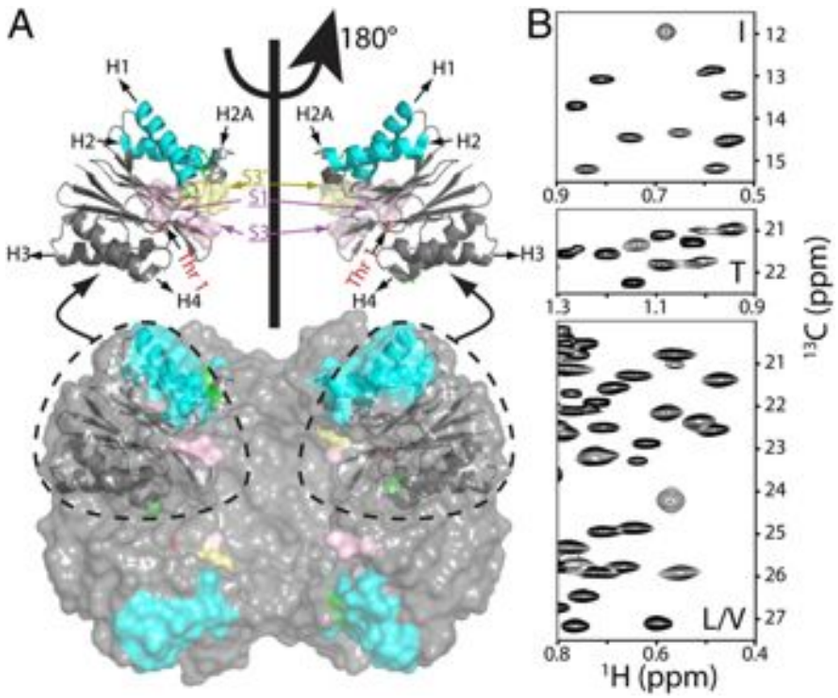


2H,15N-labeled 110-kDa octameric protein 7,8-dihydroneopterin aldolase

NMR timescales
2. Relaxation times

- Transversal relaxation time constant T_2 characterizes the lifetime of the signal.
- It determines the linewidth of the resonances
- NB. T_2 depends on the magnetic field strength B_0, the type of spin, the environment of the spin, the size of the molecule, the local dynamics of the system, the temperature, etc.

➪ functional studies



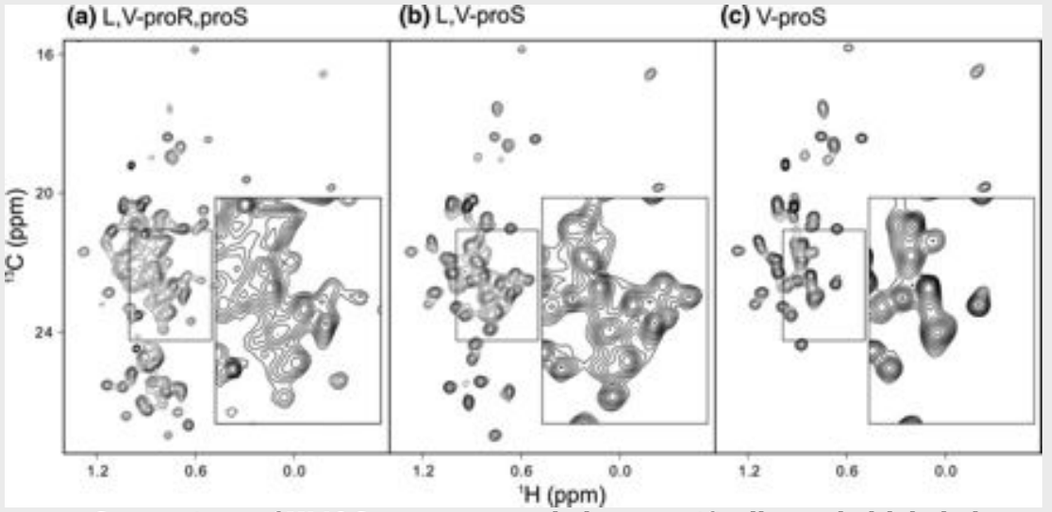
Lichi Shia and Lewis E. Kay (2014) , Tracing an allosteric pathway regulating the activity of the HslV protease, PNAS | February 11, | vol. 111 | no. 6

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

➪ $^{13}C^{1}H_3$ labeling strategies



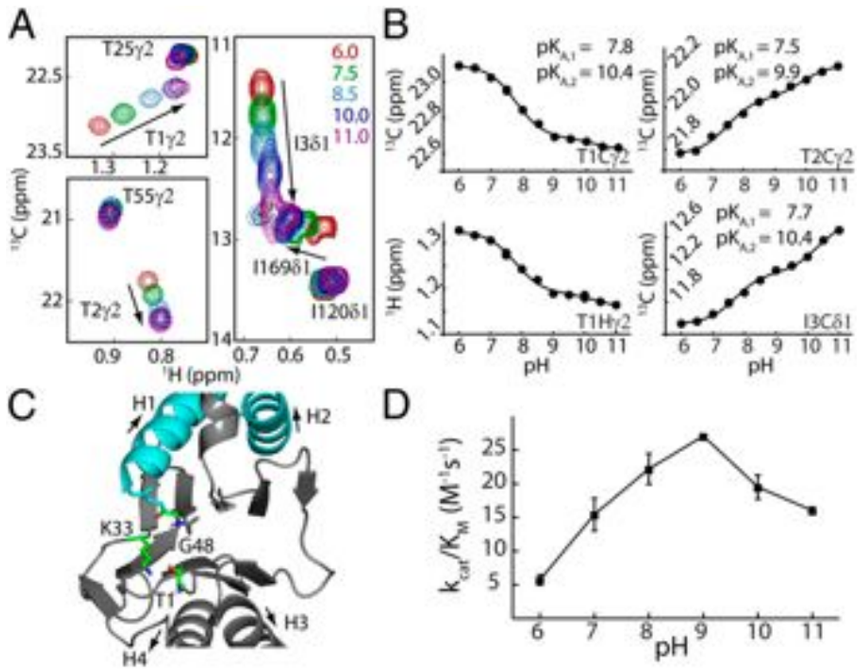
Comparison of HMQC spectra recorded on specifically methyl-labeled TET2 samples (468 kDa).

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

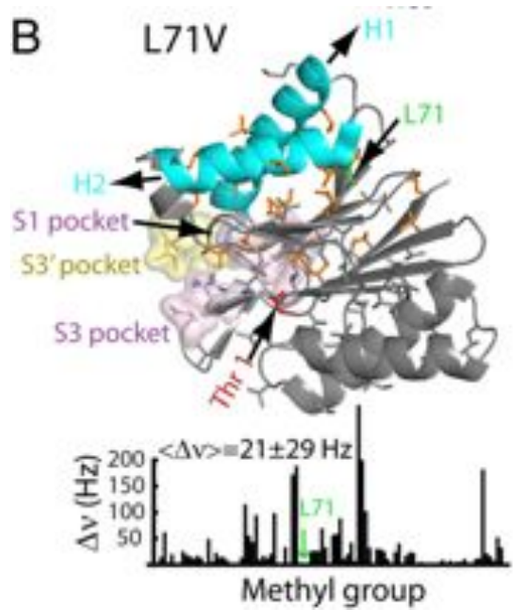
NMR timescales
2. Relaxation times

Playing with T2 : $^{13}C^{1}H_3$ Methyl TROSY experiments

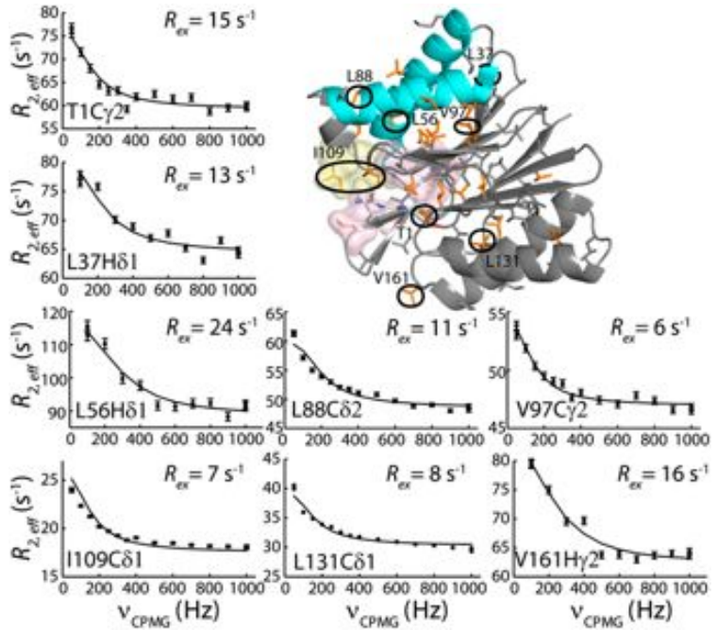
- Assignment strategies : mutations + nOes + cristal structures
- functional studies



pKa measurements, catalytic mechanism



mutants, allosteric pathways

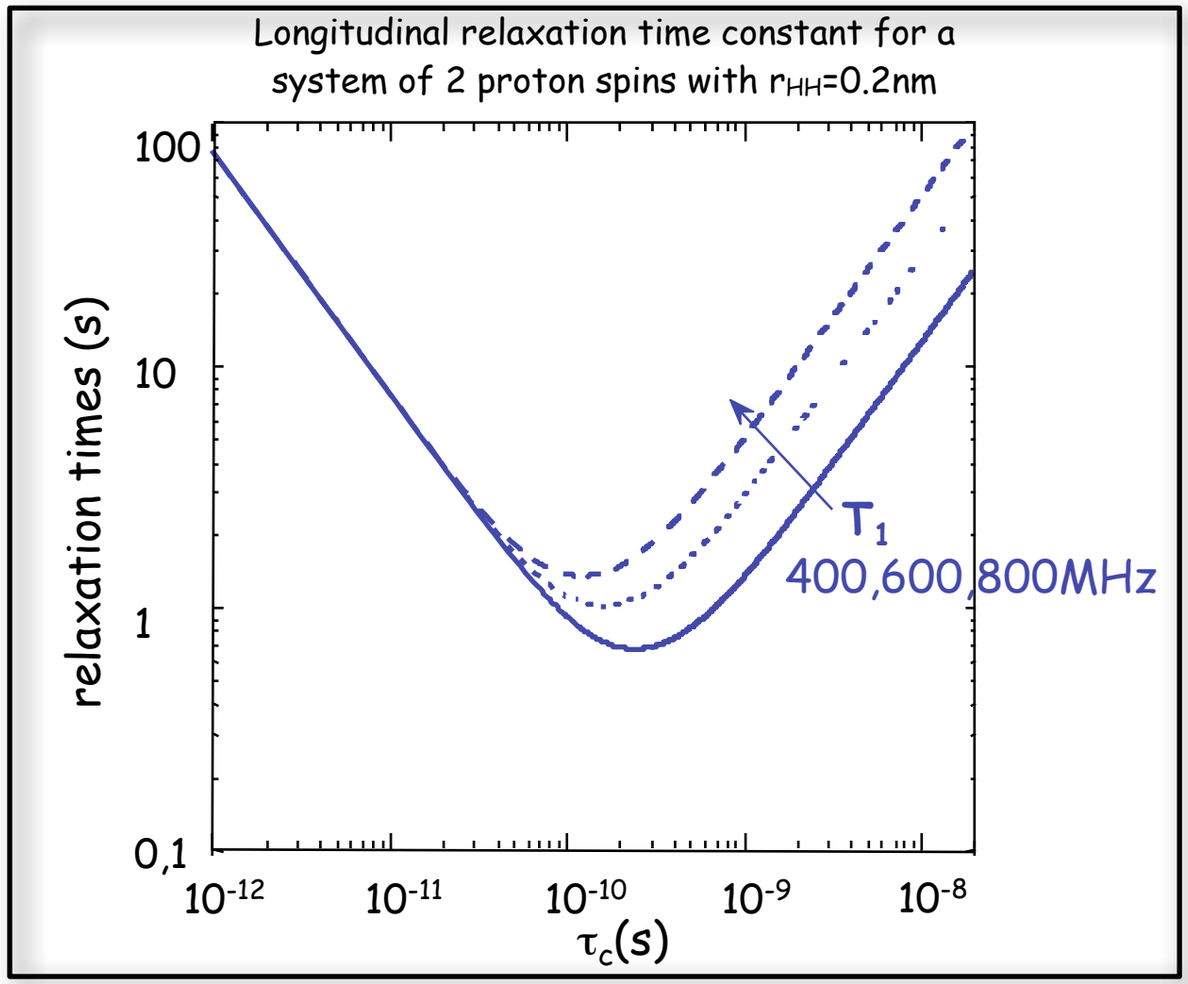


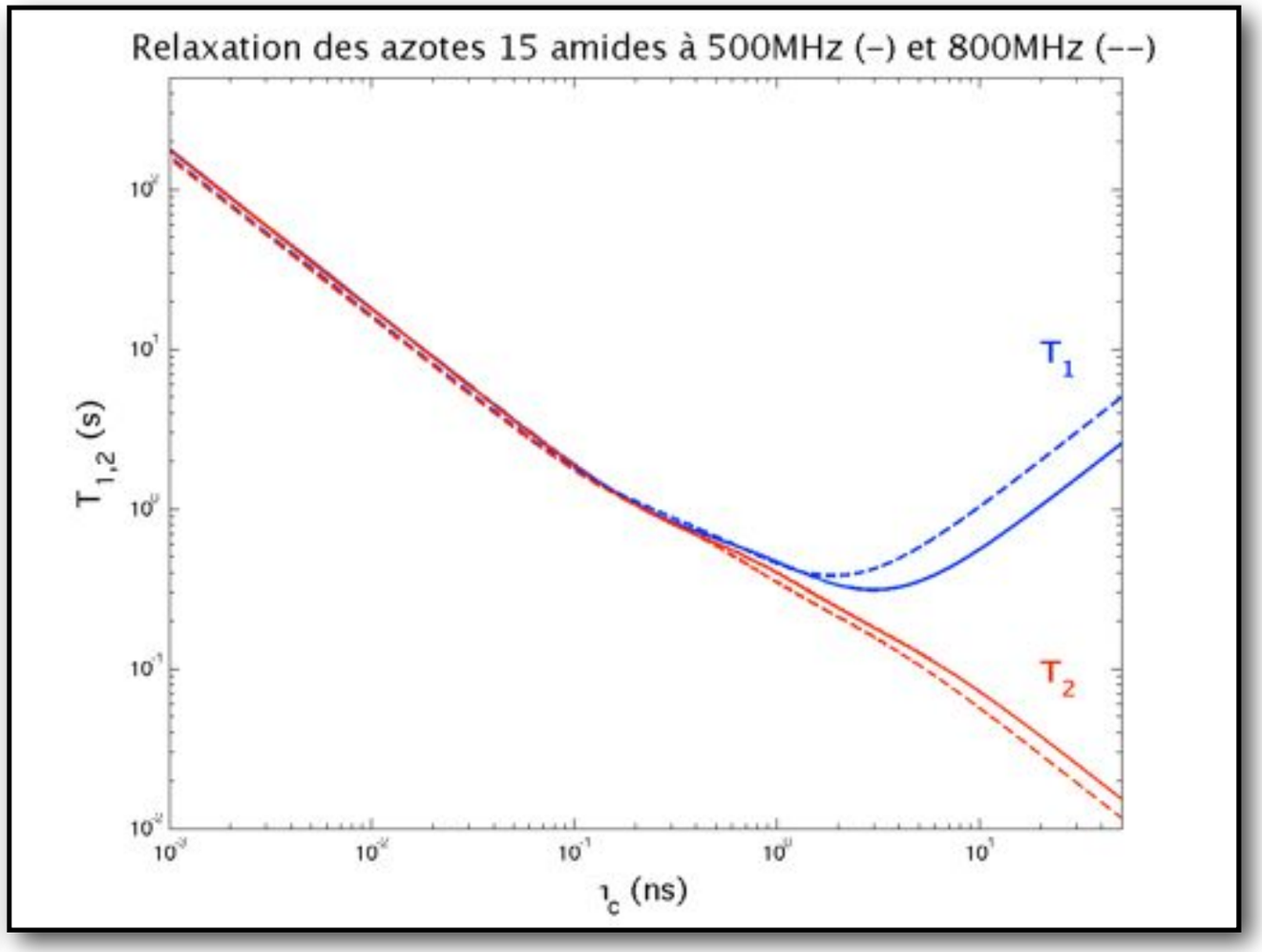
multiple states & flexibility
related to functions

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

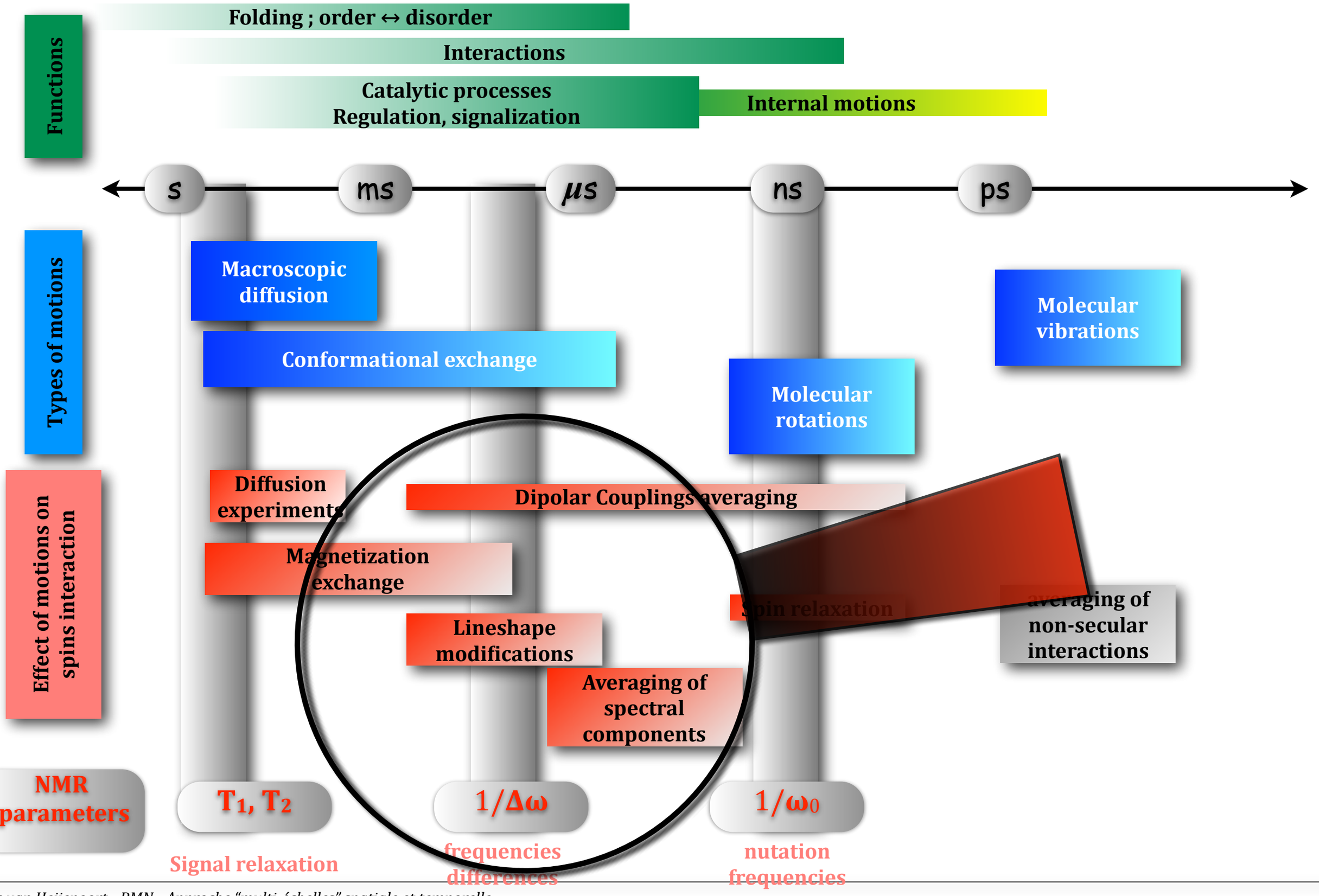
NMR timescales
1. Relaxation times

- ✓ Relaxation contants depends on the magnetic field strength B_0, the type of spin, the size of the molecule, the local dynamics of the system, the environment of the spin, the state of neighbors spins, etc.
- ✓ They can be modified to get faster signal recovery / longer lifetime
  - ☞ analysis of faster processes or unstable systems / large systems.





How motions are « visible » in NMR ?



NMR timescales
3. «Spectral» or «chemical shift» timescale

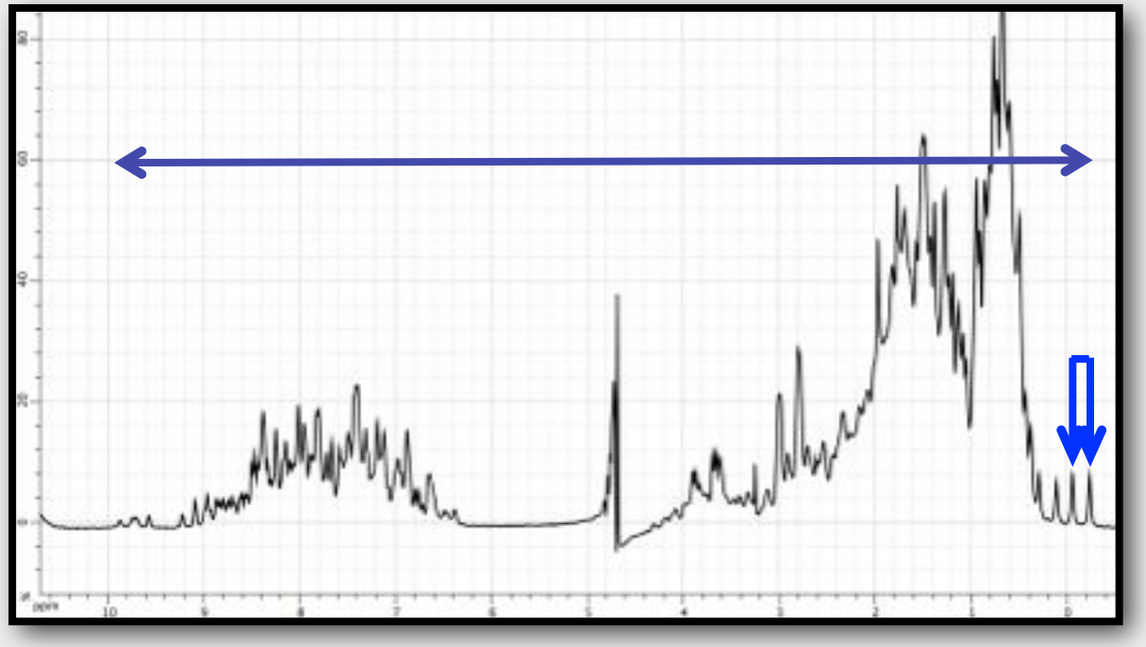
- ✓ Largest observable difference of resonance frequency (spectral width)

- ✓ Smallest observable difference of resonance frequency (resolution)

$$\Delta\nu(Hz) = \Delta\delta(ppm) * 10^{-6} * \frac{\gamma B_0}{2\pi}$$

$$\tau_{spect} = \frac{1}{\pi\Delta\nu(Hz)}$$

- ✓ Δν depends on the magnetic field strength B_0, the type of the spins.
- ✓ Different nuclei or same nuclei in different environments
- ✓ Motions slower than Δν have no effect on the appearance of the spectra.
- ✓ Δν depends on the nature of the interactions between the spins.



$\Delta\omega$=13ppm — B_0=14.1T → $\Delta\nu$=7800Hz

B_0=22.3T → $\Delta\nu$=12350Hz

τ_{spect}~100µs

$\Delta\omega$=0,2ppm — B_0=14.1T → $\Delta\nu$=120Hz

B_0=22.3T → $\Delta\nu$=190Hz

τ_{spect}~5-10ms

NMR timescales
3. «Spectral» or «chemical shift» timescale

- ✓ Largest observable difference of resonance frequency (spectral width)

- ✓ Smallest observable difference of resonance frequency (resolution)

$$\Delta\nu(Hz) = \Delta\delta(ppm) * 10^{-6} * \frac{\gamma B_0}{2\pi}$$

$$\tau_{spect} = \frac{1}{\pi\Delta\nu(Hz)}$$

- ✓ Δν depends on the magnetic field strength B_0, the type of the spins.
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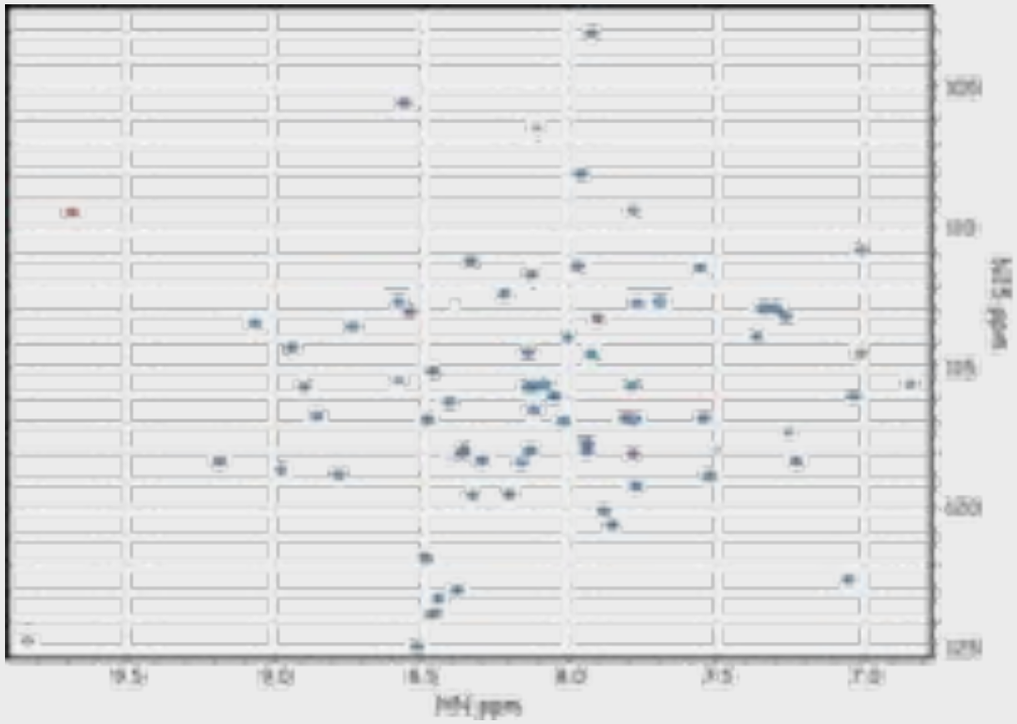


$\Delta\omega(^{15}N)$=25ppm $\xrightarrow{B_0=14,1T}$ Δν~1500Hz, τ_{spect}~0,7ms

$\Delta\omega(^{1}H)$=3,5ppm $\xrightarrow{B_0=14,1T}$ Δν~2000Hz, τ_{spect}~0,5ms

NMR timescales
3. «Spectral» or «chemical shift» timescale

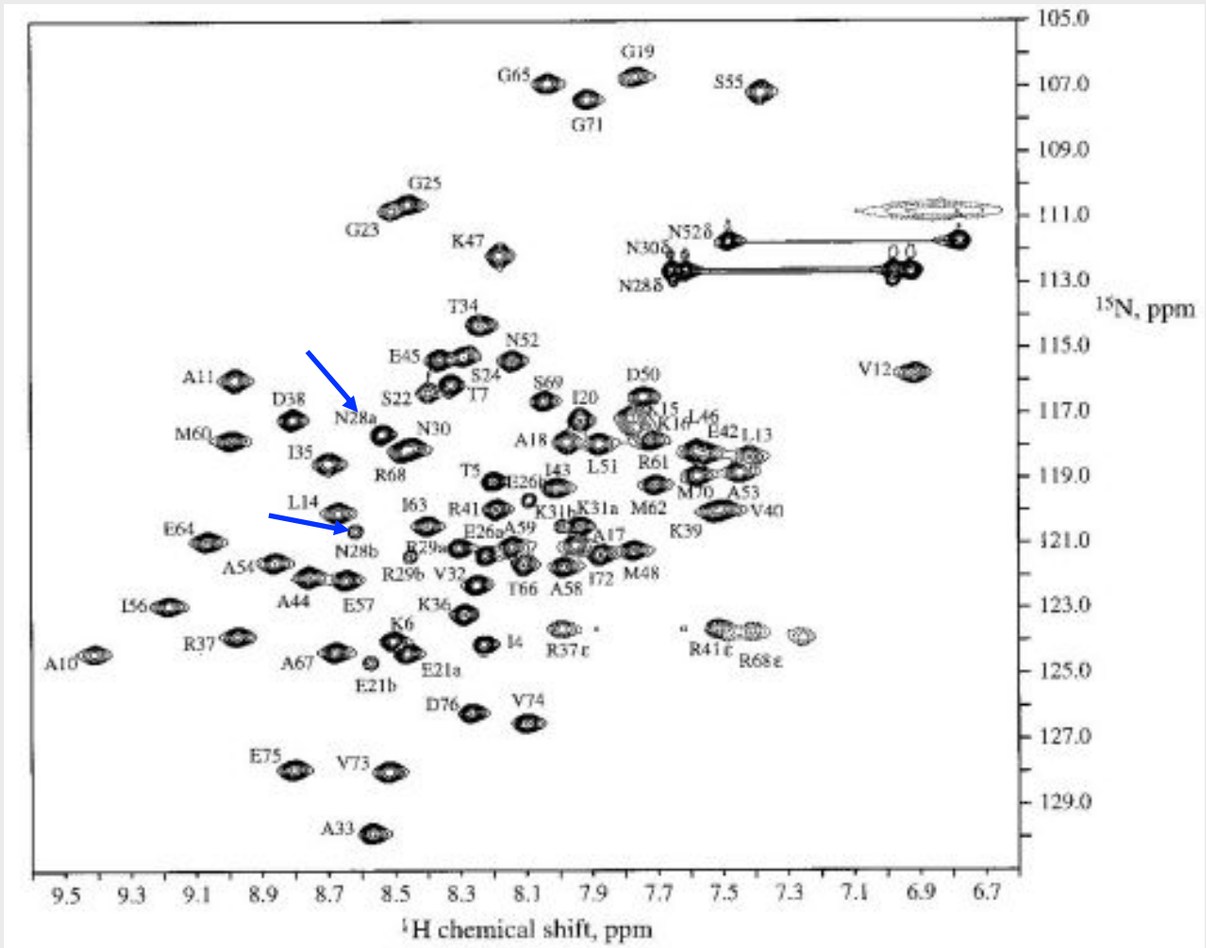
- ✓ Largest observable difference of resonance frequency (spectral width)

- ✓ Smallest observable difference of resonance frequency (resolution)

$$\Delta\nu(Hz) = \Delta\delta(ppm) * 10^{-6} * \frac{\gamma B_0}{2\pi}$$

$$\tau_{spect} = \frac{1}{\pi\Delta\nu(Hz)}$$

- ✓ Motions slower than Δν have no effect on the appearance of the spectra.
- ✓ Δν depends on the magnetic field strength B_0, the type of the spins.
- ✓ Different nuclei or same nuclei in different environments
- ✓ Δν depends on the nature of the interactions between the spins.

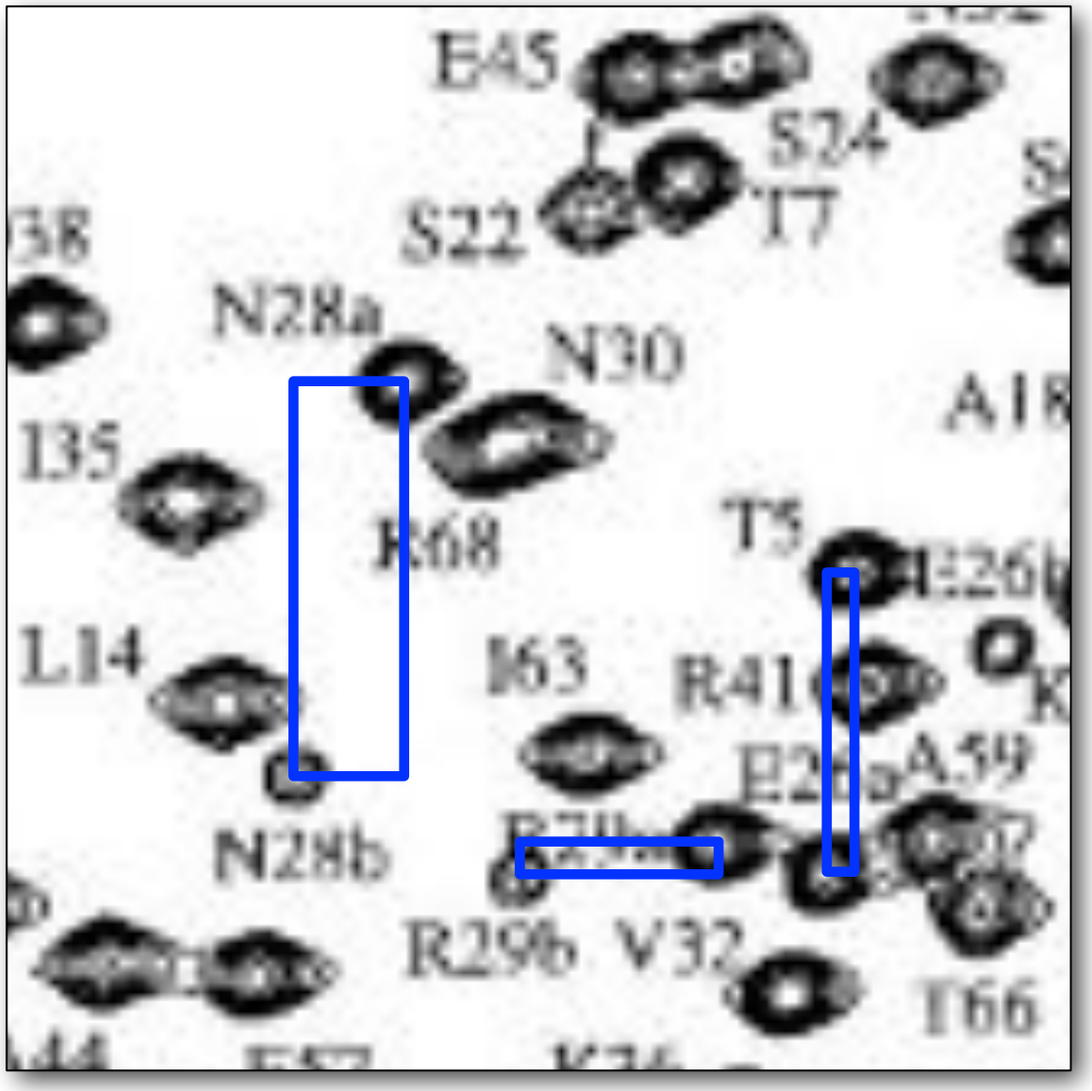


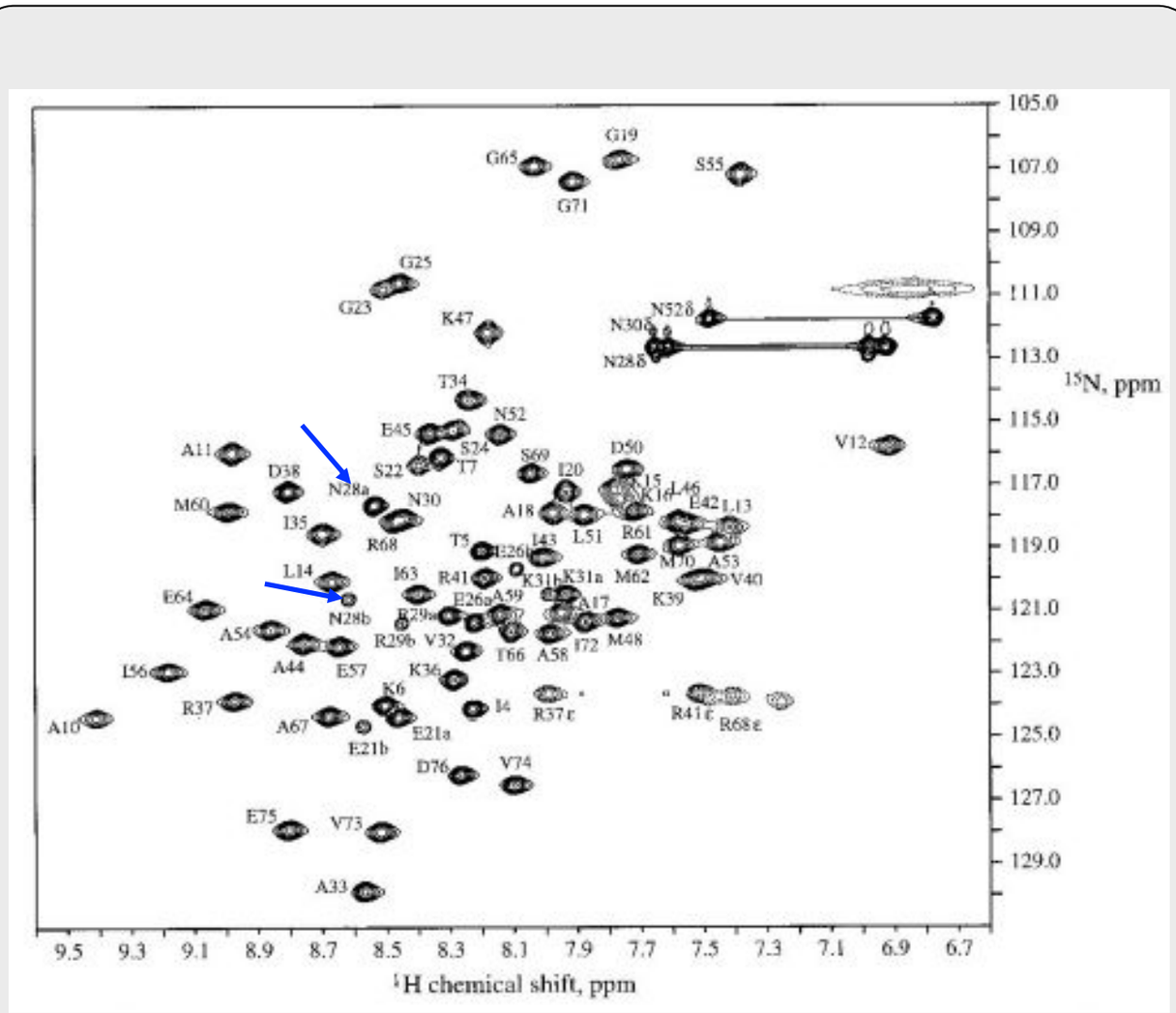
$\Delta\omega(^{15}N)$=1,5ppm ⟷ B_0=14,1T → Δν~100Hz, τ_{spect}~10ms

$\Delta\omega(^{1}H)$=0,5ppm ⟷ B_0=14,1T → Δν~300Hz, τ_{spect}~3ms

Same nucleus ; two different conformations

NMR timescales
3. «Spectral» or «chemical shift» timescale





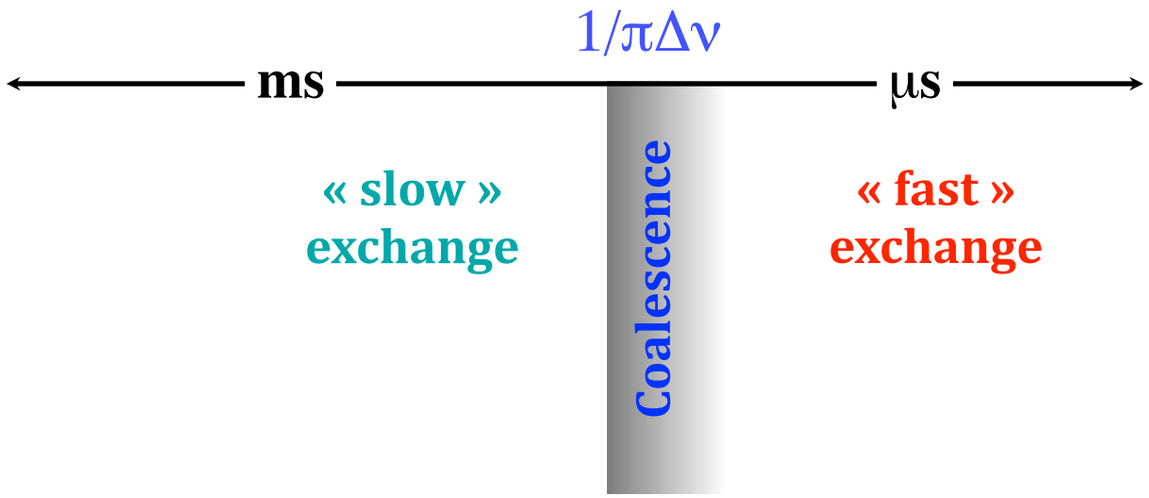
$\Delta\omega(^{15}N)$=1,5ppm $\xleftrightarrow{B_0=14,1T}$ $\Delta\nu\sim100$Hz, $\tau_{spect}\sim10$ms

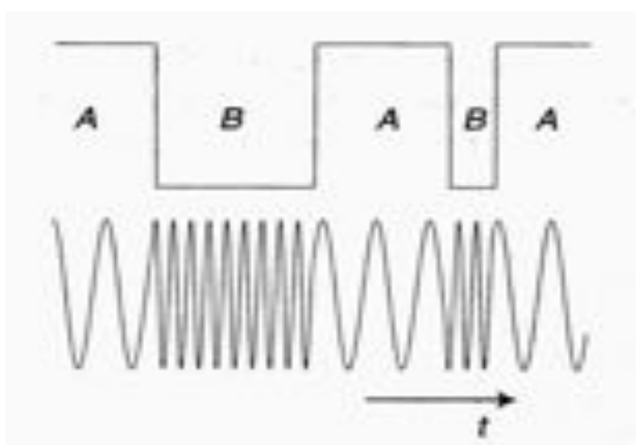
$\Delta\omega(^{1}H)$=0,5ppm $\xleftrightarrow{B_0=14,1T}$ $\Delta\nu\sim300$Hz, $\tau_{spect}\sim3$ms

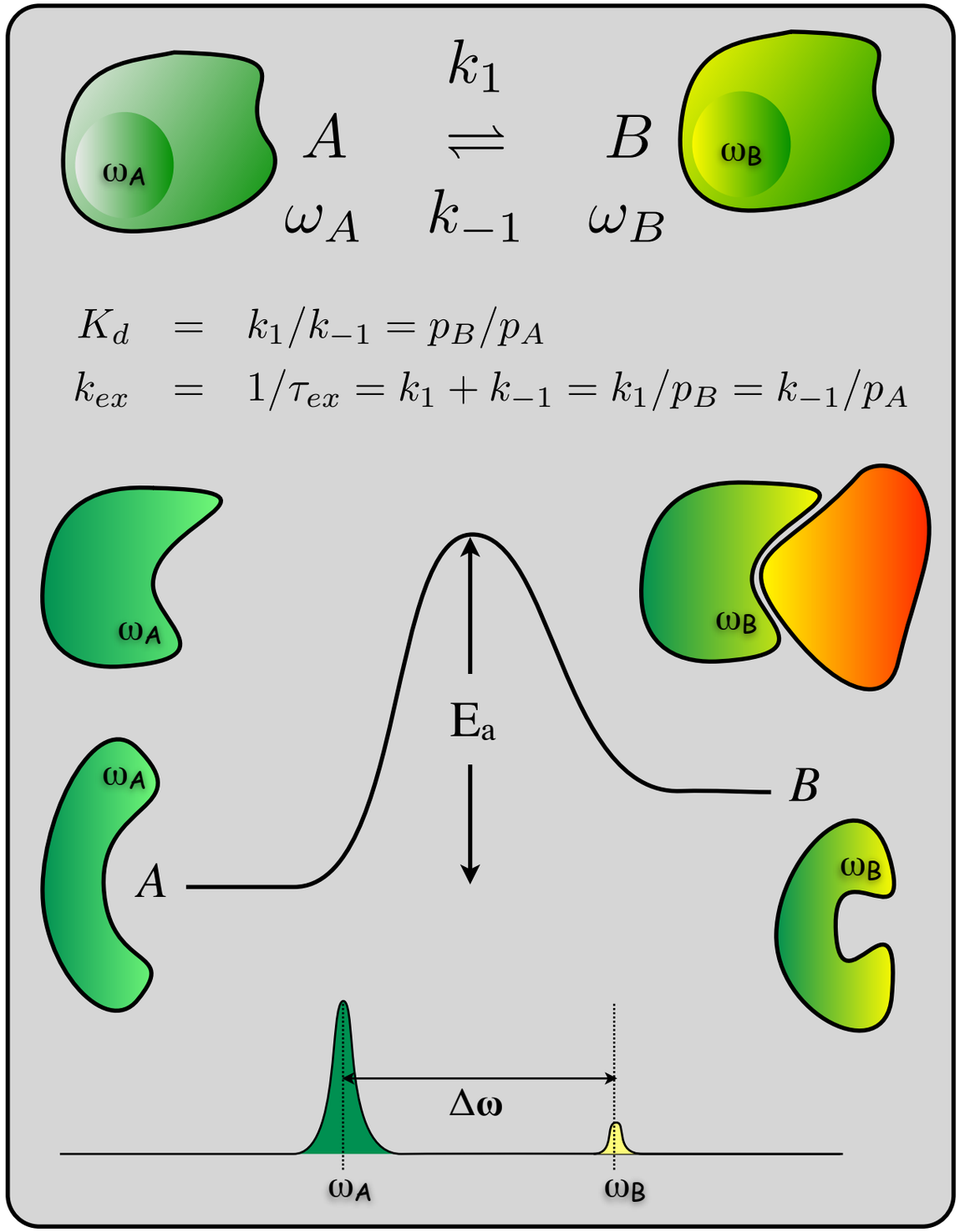
Same nucleus ; two different conformations

NMR timescales
3. «Spectral» or «chemical shift» timescale

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



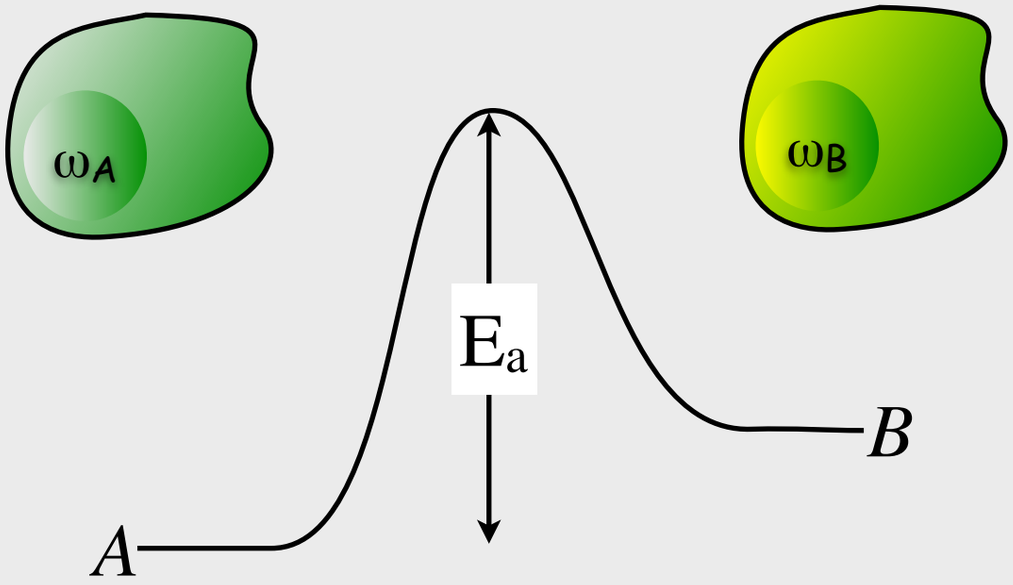




NMR timescales
3. «Spectral» or «chemical shift» timescale

Intramolecular vs Intermolecular processes

$$\begin{array}{ccc} A & \overset{k_1}{\rightleftharpoons} & B \\ \omega_A & k_{-1} & \omega_B \end{array}$$



thermodynamic parameters

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

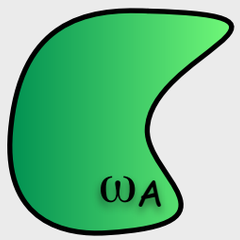
$$\Delta G = -RT \ln K$$

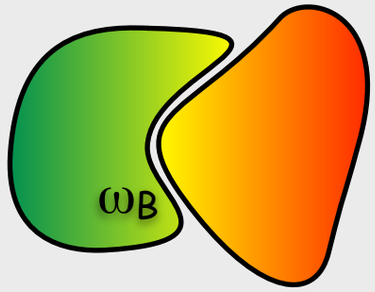
kinetic parameters

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

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

$$\begin{array}{ccccc} P & + & L & \overset{k_{on}}{\rightleftharpoons} & PL \\ \delta_{P_f} & & & k_{off} & \delta_{P_b} \\ & & \delta_{L_f} & & \delta_{L_b} \end{array}$$





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

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

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

NMR timescales
3. «Spectral» or «chemical shift» timescale

Exchange <-> broadening

Exchange regime

☞ "Fast" exchange : $\Delta\omega \ll k_{ex}$

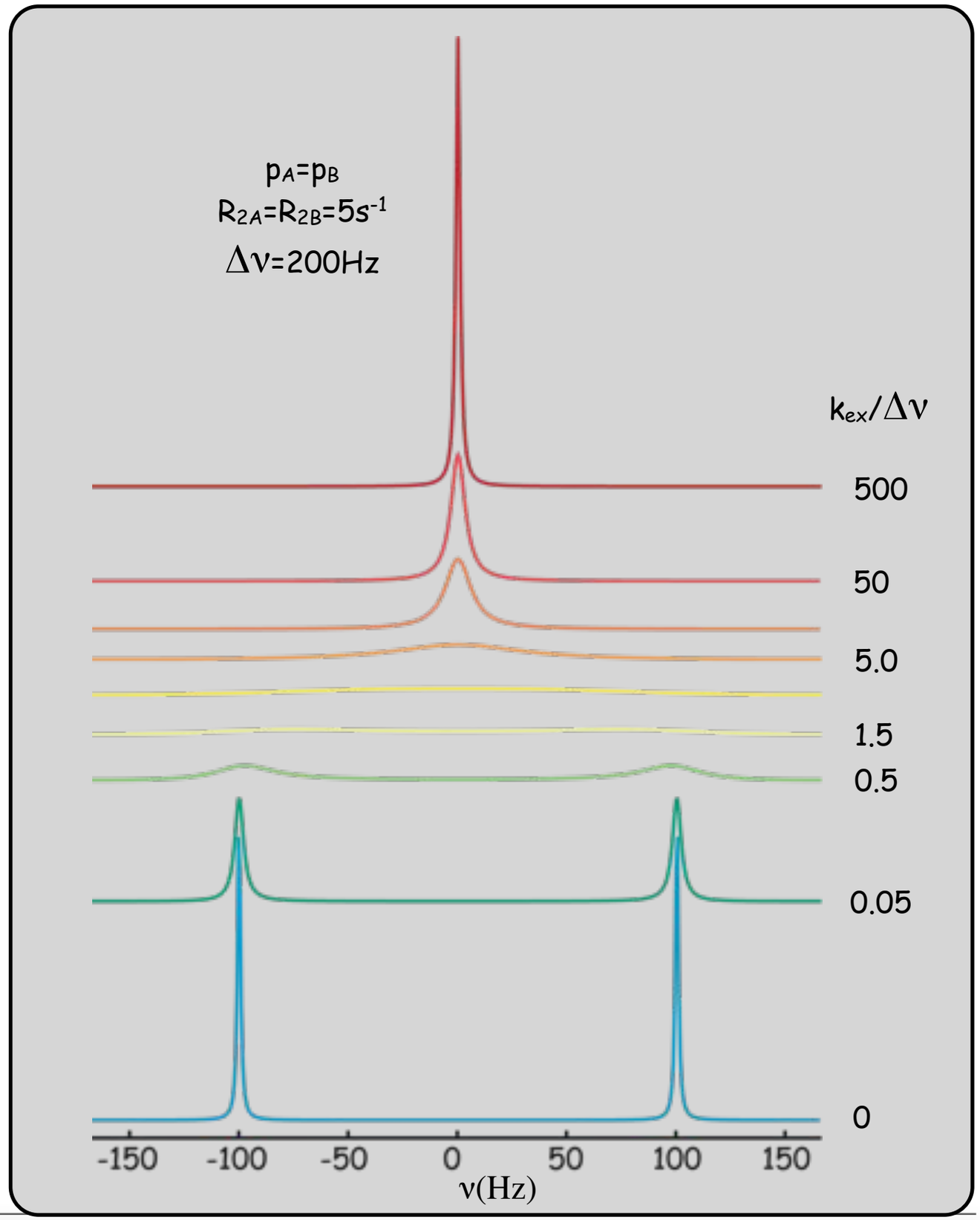
- ⇨ One peak at $\omega = p_A\omega_A + p_B\omega_B$
- ⇨ $R_{ex} \propto B_0^2$

☞ "intermediate" exchange : $\Delta\omega \simeq k_{ex}$

- ⇨ One or several broadened peaks
- ⇨ $R_{ex} \propto B_0$

☞ "slow" exchange : $\Delta\omega \gg k_{ex}$

- ⇨ Several peaks
- ⇨ R_{ex} independent of B_0
- ⇨ $R_{ex}^{(B)} \rightarrow k_{BA}=p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_B k_{ex}$



NMR - Spatial and Time scales are highly intermingled.

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ / $[^{15}N,^{2}H]$ — solubilized membrane proteins

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ $[^{15}N,^{2}H]$ — TROSY, high fields — "large" size globular proteins or complexes

"medium" size complexes

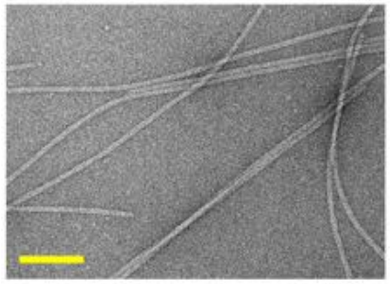
"medium" size globular proteins

"Small" globular Protein / domain

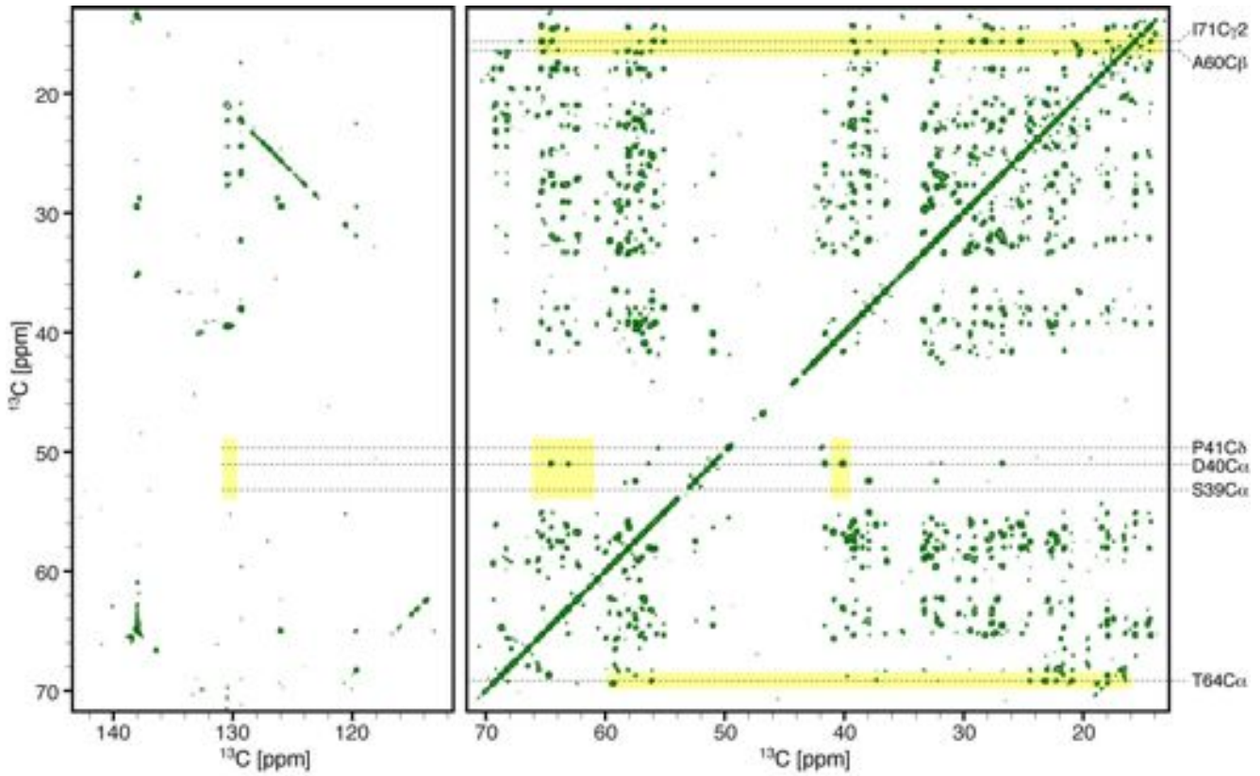
Linewidth

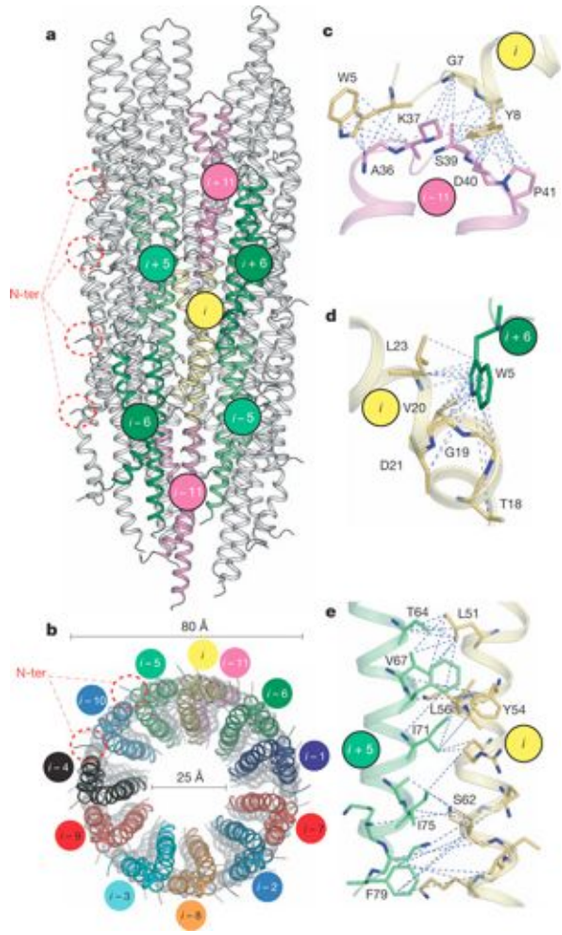
Global correlation time (size)
+ Local Rotational fluctuation timescale
+ density of relaxation sources
+ conformational/chemical exchange

NMR - Spatial and Time scales are highly intermingled.



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

NMR - Spatial and Time scales are highly intermingled.

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ ← $[^{15}N,^{2}H]$ — solubilized membrane proteins

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ $[^{15}N,^{2}H]$ ← TROSY, high fields — "large" size globular proteins or complexes

Very variable linewidths

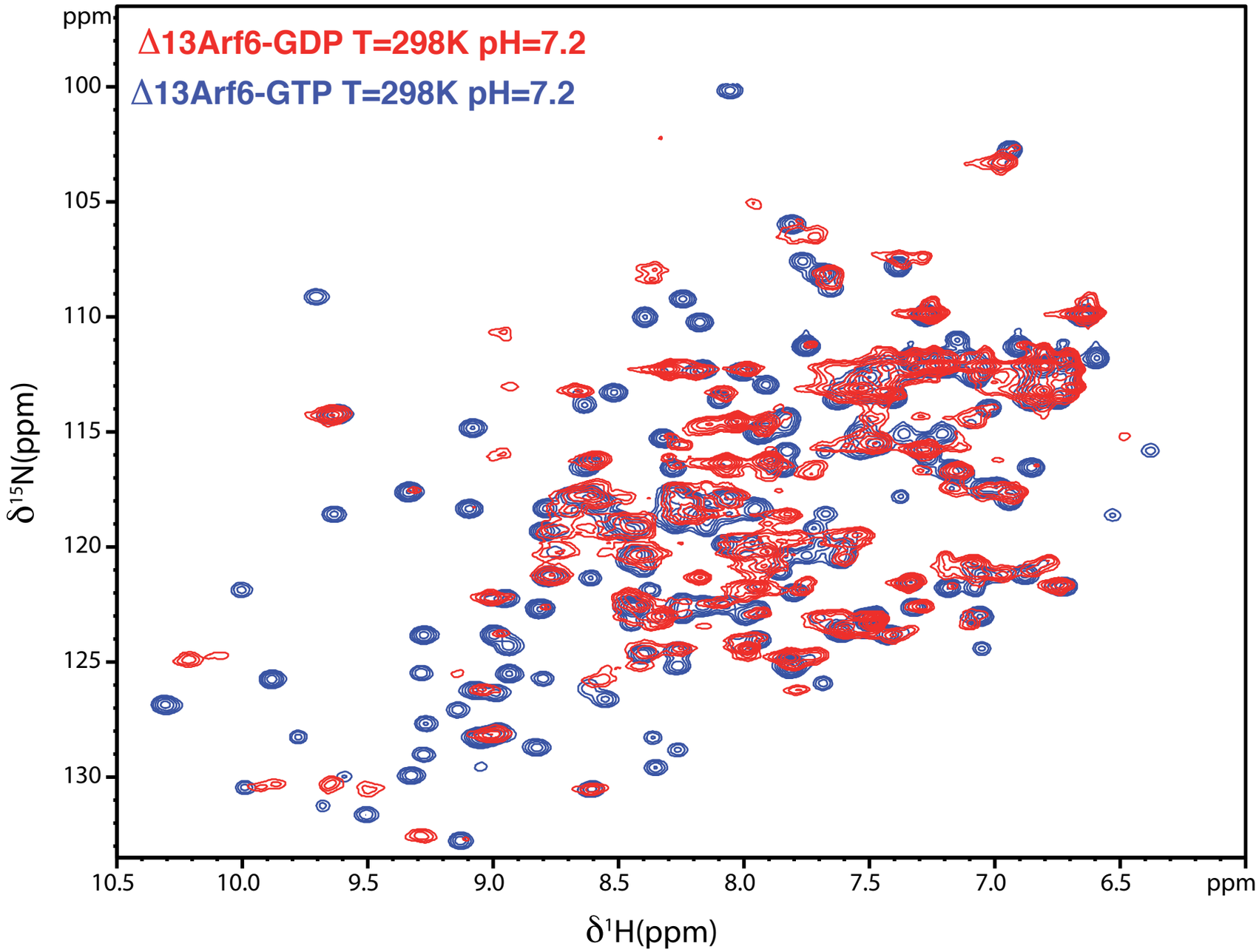
"medium" size complexes

"medium" size globular proteins

"Small" globular Protein / domain → **Linewidth**

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

NMR - Spatial and Time scales are highly intermingled.



Linewidth

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

NMR - Spatial and Time scales are highly intermingled.

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ / $[^{15}N,^{2}H]$ — solubilized membrane proteins

$[^{15}N,^{2}H,^{12}C,\ ^{13}CH_3]$ $[^{15}N,^{2}H]$ — TROSY, high fields — "large" size globular proteins or complexes

large IDPs

medium size IDPs

"medium" size complexes

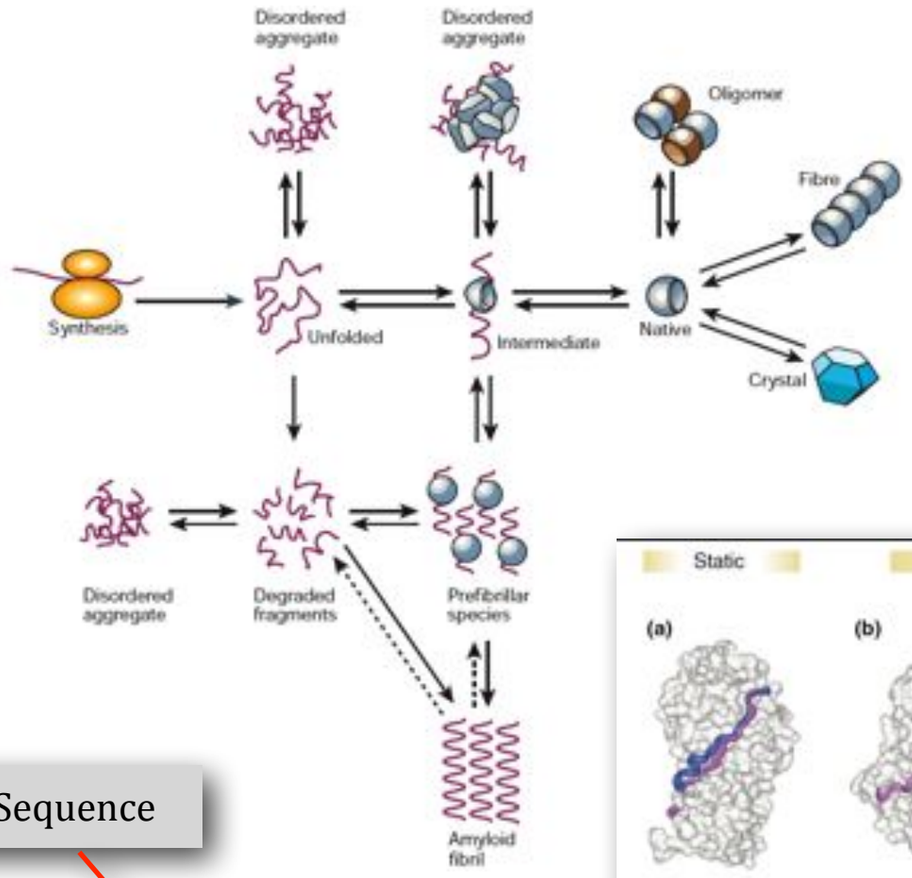
"medium" size globular proteins

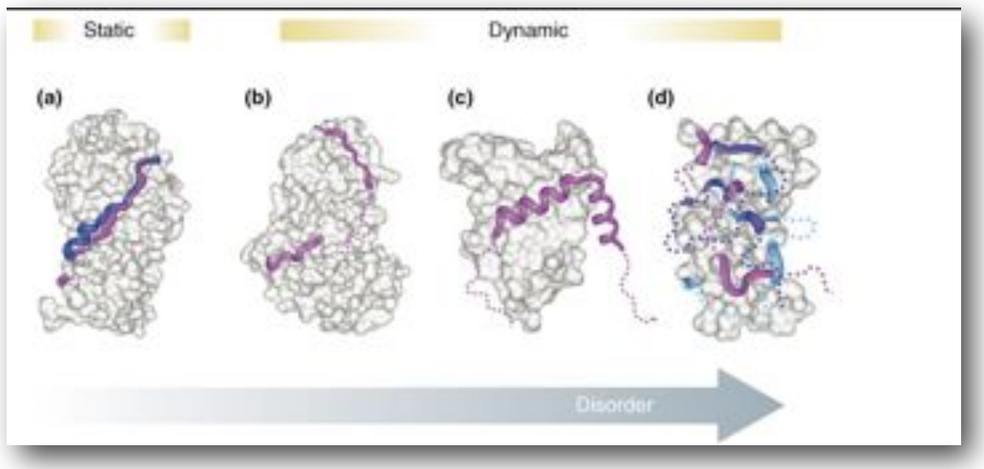
"Small" globular Protein / domain

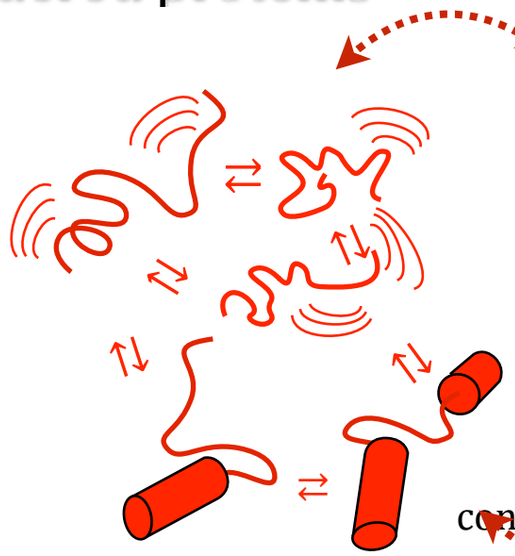
Linewidth

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

A view of protein multiple states
Intrinsically disordered proteins

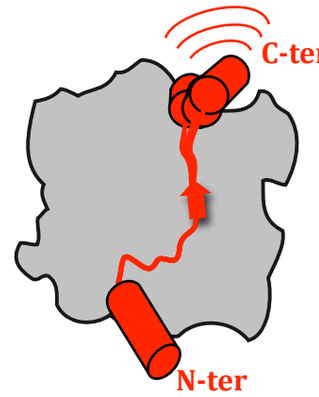






Induced fit

conformational selection



Sequence

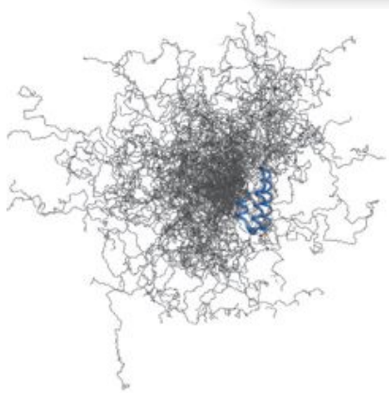
“Non folding”

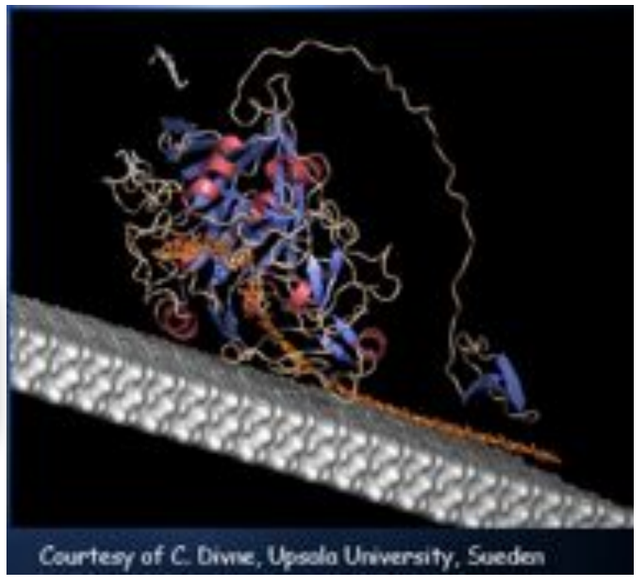
flexible ensemble

flexible linkers
display of sites
entropic bristles, springs and clocks

disorder → order

molecular recognition
virus/phages assembly
stepping motors





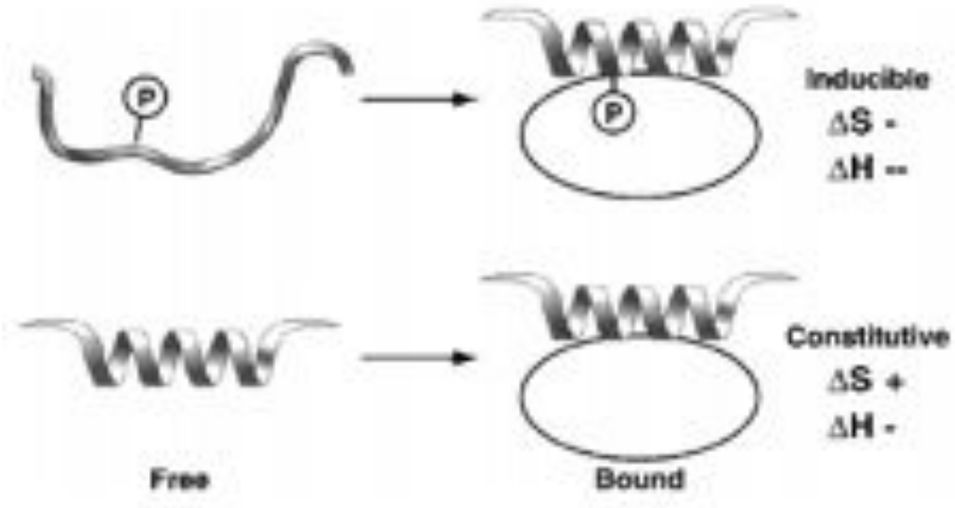
Cellulases

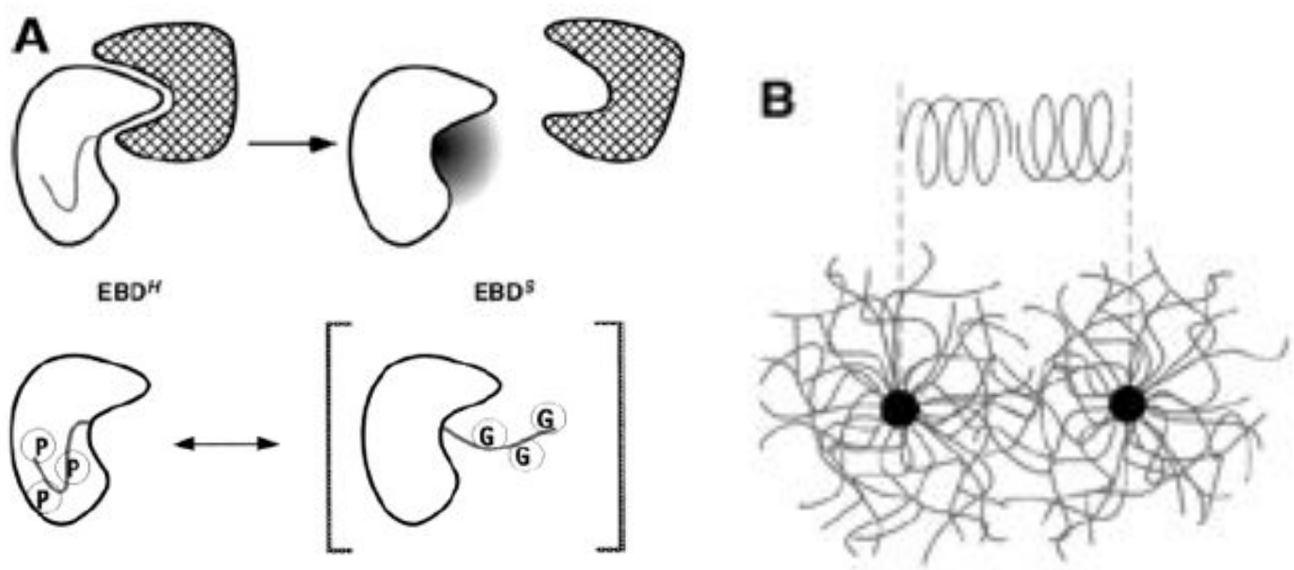
A view of protein multiple states
Intrinsically disordered proteins

- Functions requiring fast “turnover”.
  - Molecular recognition in regulation processes
  - Signaling
  - Cell cycle
  - Development
  - Endocytosis
  - ⇨ Highly involved in diseases
- Disorder as a function
  - entropic brushes (neurofilaments H & M)
  - entropic clocks (K^+ Channel ball and chain in nerve axons)
  - entropic springs (titin)
- Low occurence of disorder : biosynthesis, metabolism

  NB. Enzymes preponderant in PDB (except kinases)

moderate affinities, large binding interfaces, high selectivities, inducible via post-translational modifications





Jan H. Hoh, “Functional Protein Domains From the Thermally Driven Motion of Polypeptide Chains: A Proposal”, PROTEINS: Structure, Function, and Genetics 32:223–228 (1998)

NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

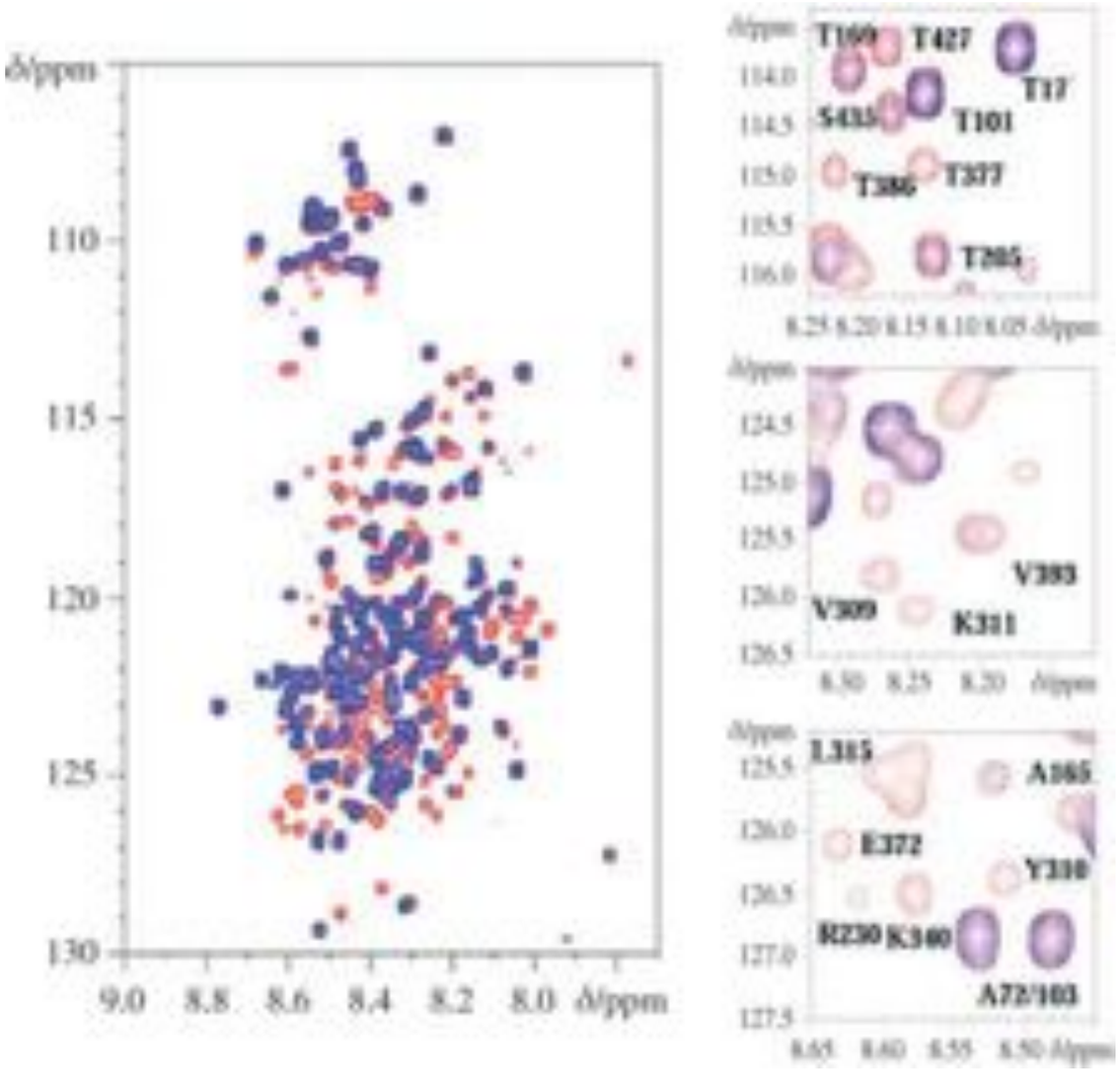


Figure 4. HSQC spectrum (full spectrum (top) and selected annotated zooms (bottom)) of tau free in solution (red) and integrated into mature PHFs (blue).

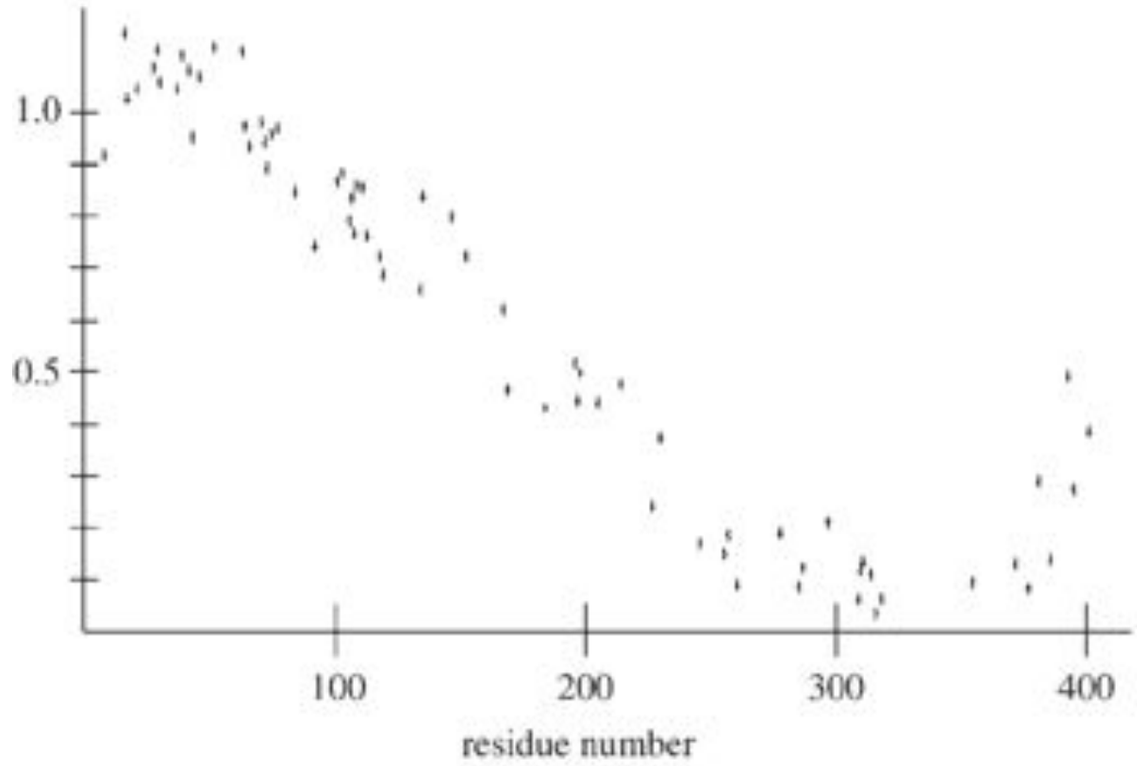


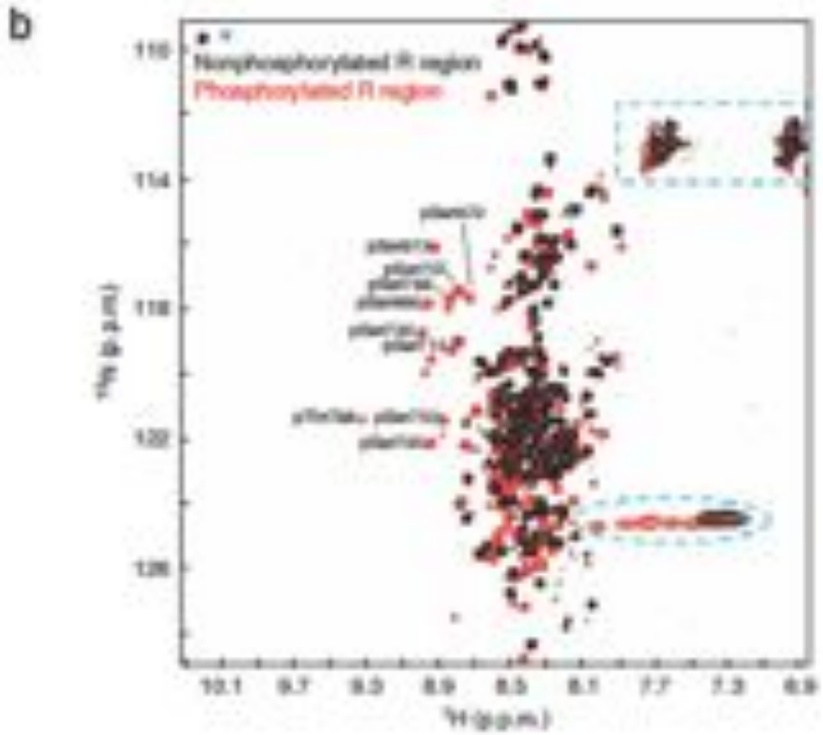
Figure 5. Ratio of the peak intensities in the spectra of free tau and PHF-tau. Only isolated peaks were taken into account.

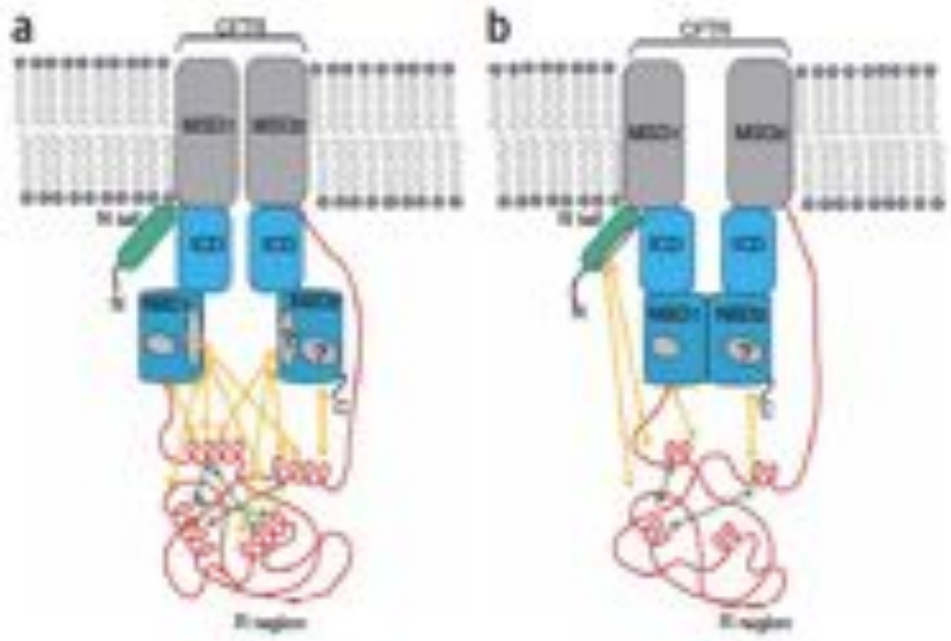
Alain Sillen et al., (2005) ChemBioChem, **6**, 1849 – 1856, Regions of Tau Implicated in the Paired Helical Fragment Core as Defined by NMR.

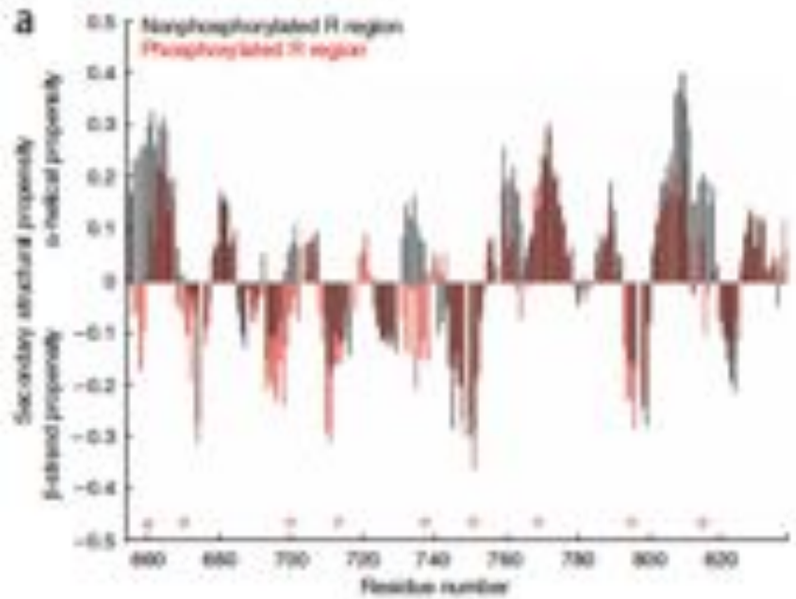
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

- Caractérisation de l'ensemble des conformères
  - ☞Propension à former des structures secondaires
    - ☞déplacements chimiques
      - Secondary Shifts
      - SSP (Secondary Structure Propensity)
    - ☞Relaxation des spins
    - ☞RDCs
  - ☞Contacts longues distances ?
    - ☞PRE





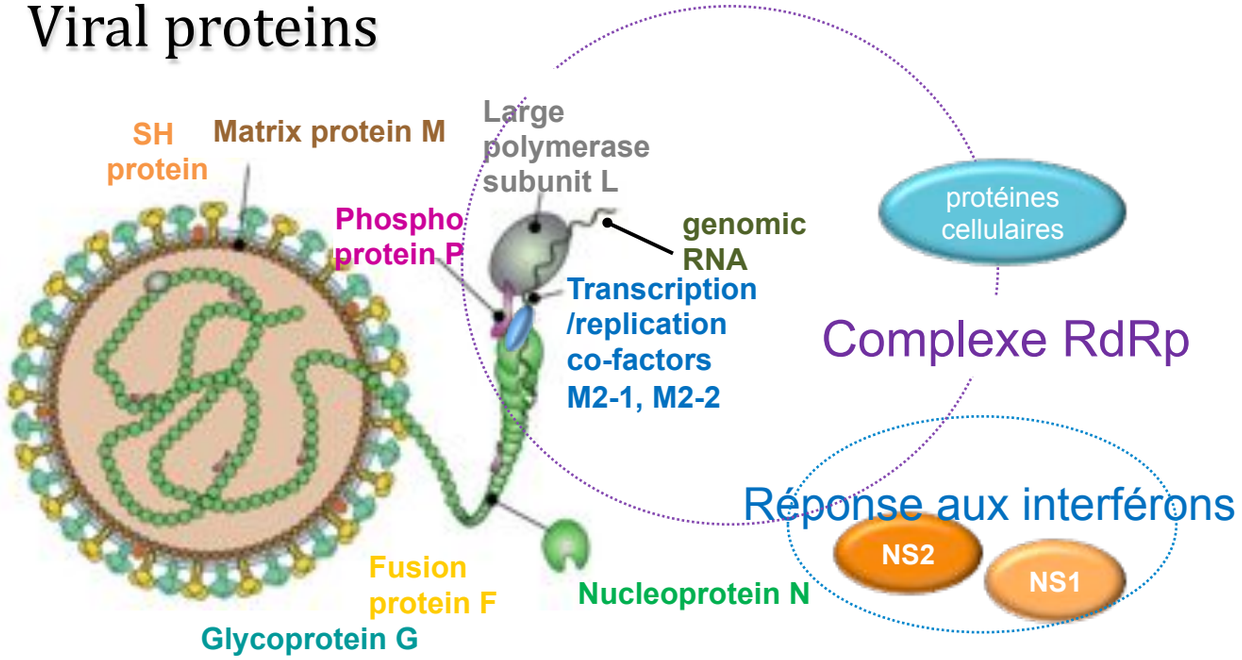


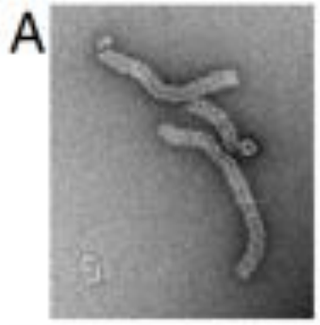
Baker et al 2007 *Nat. Struct. Mol. Biol.* **14**(8), 738

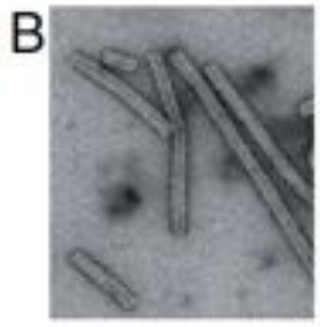
NMR - Spatial and Time scales are highly intermingled.

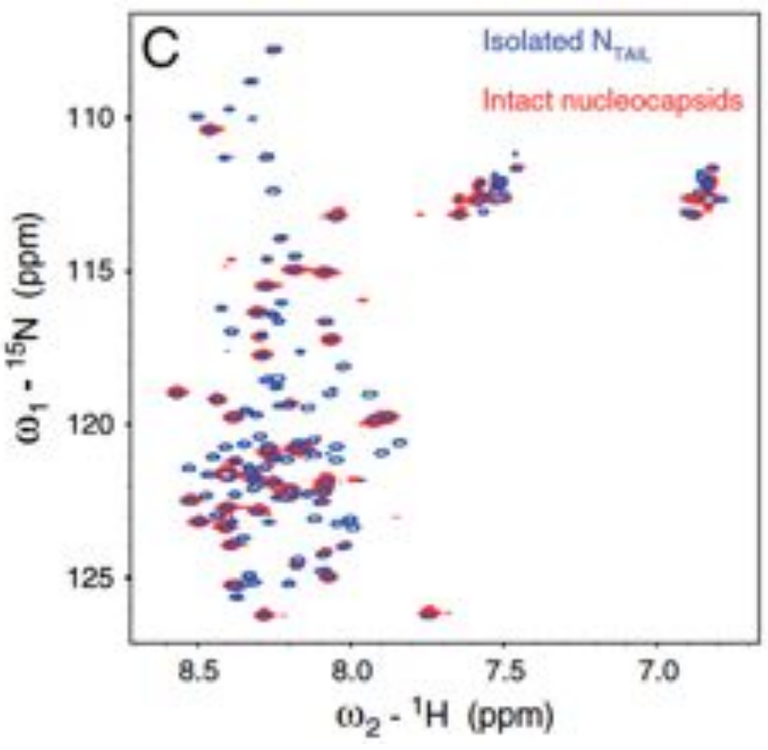
Investigation of Intrinsically Disordered Proteins

Viral proteins

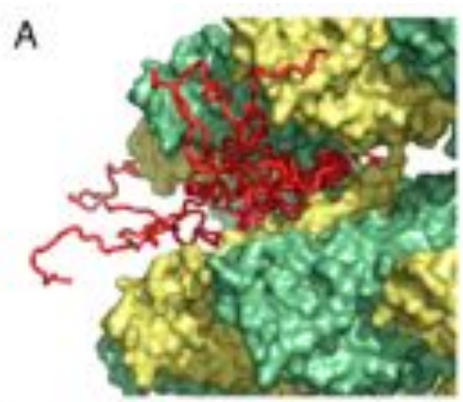


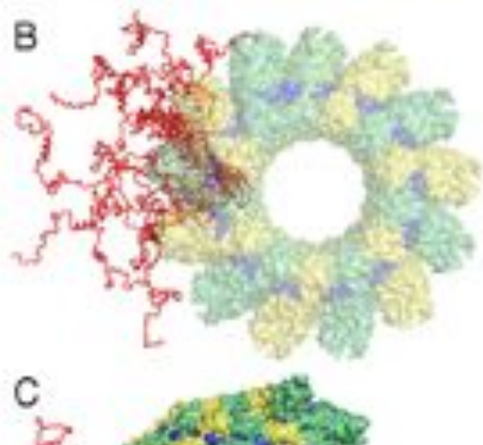


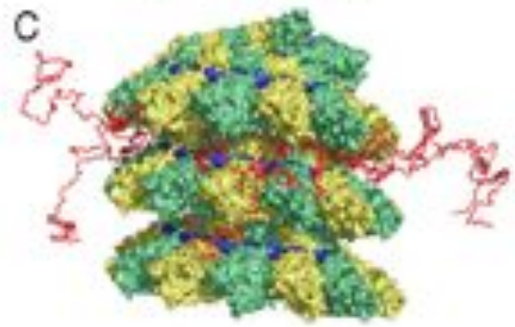


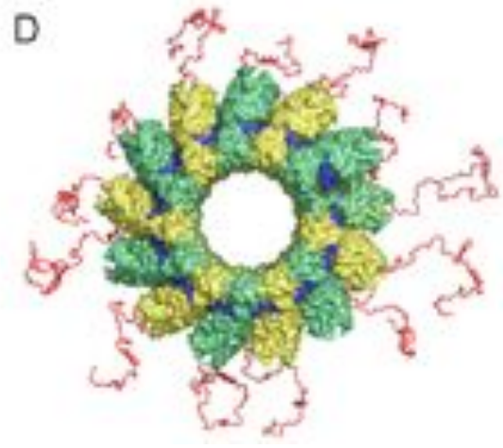


EM
NMR
SAXS





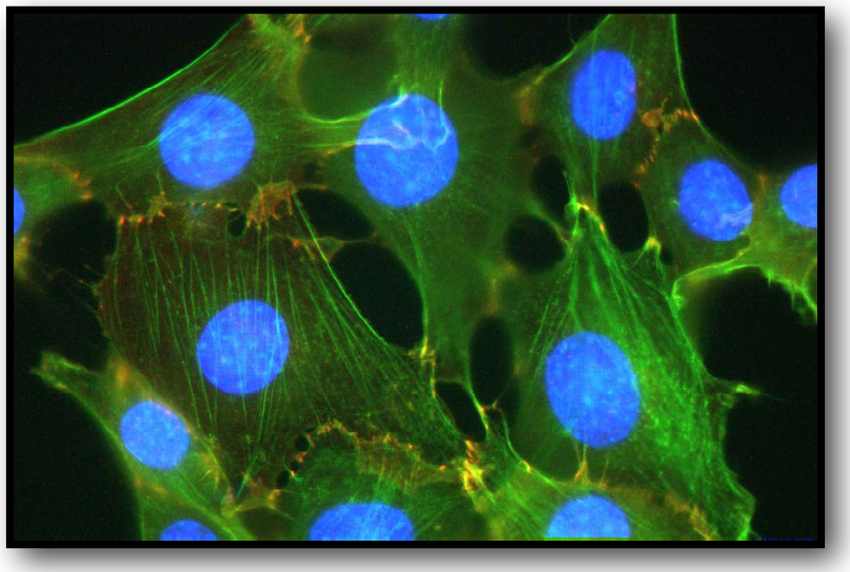


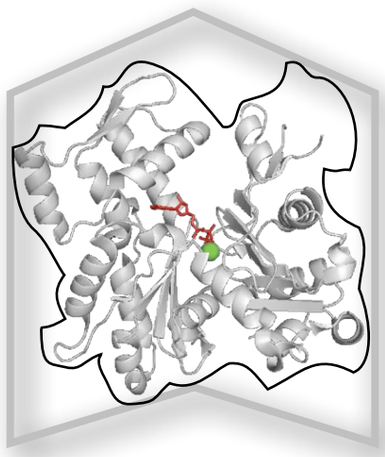


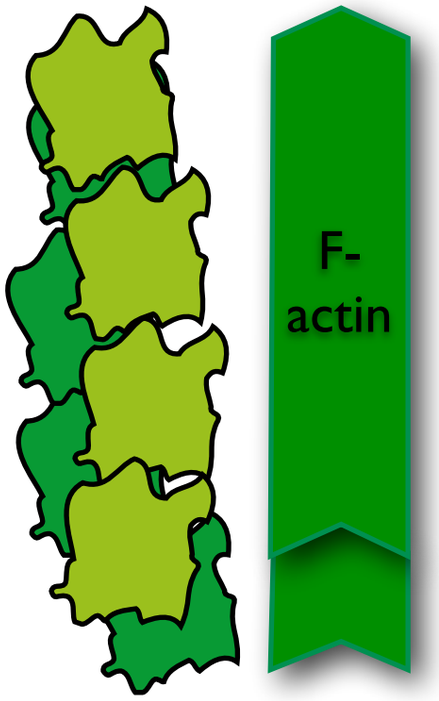
Investigation of Intrinsically Disordered Proteins
Rôle of disorder in regulation processes

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

Collaboration Marie-France Carlier Louis Renault
Thèse Michael Domanski, François-Xavier Cantrelle, Célia Deville







Acteur majeur de multiples processus cellulaires

- Motilité

(division cellulaire, plasticité synaptique, endocytose)

- Morphogénèse

(polarité embryonnaire ..)

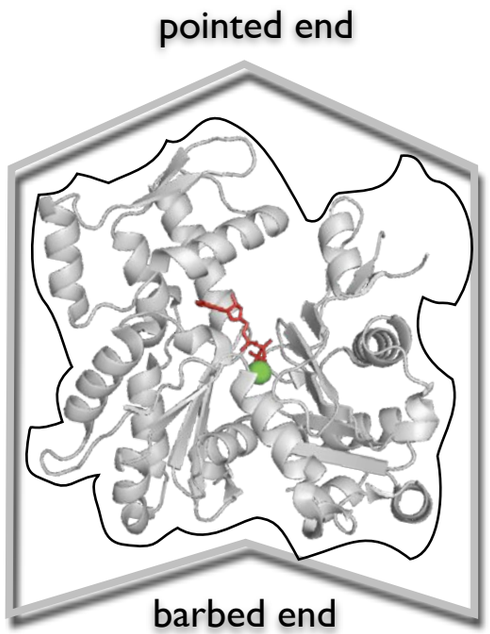
- Mouvements cellulaire

(extension de lamellipode / adhésion cellulaire)

➡ **Assemblage/désassemblage dynamique de filaments d'actine**

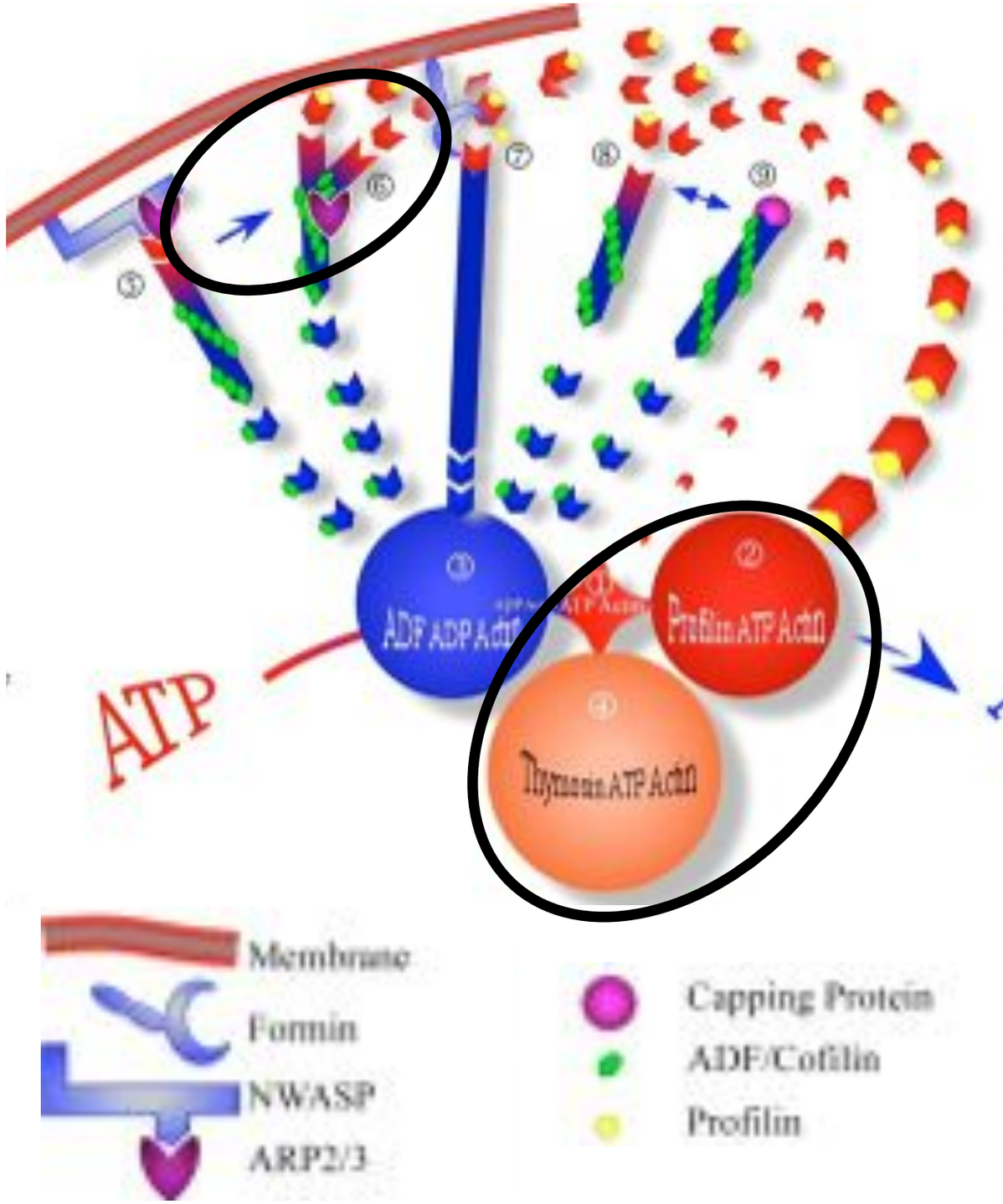
Investigation of Intrinsically Disordered Proteins
Rôle of disorder in regulation processes

➡ Dynamique des filaments d'actine régulée par des ABPs (Actin Binding Proteins)



ATP G-actin

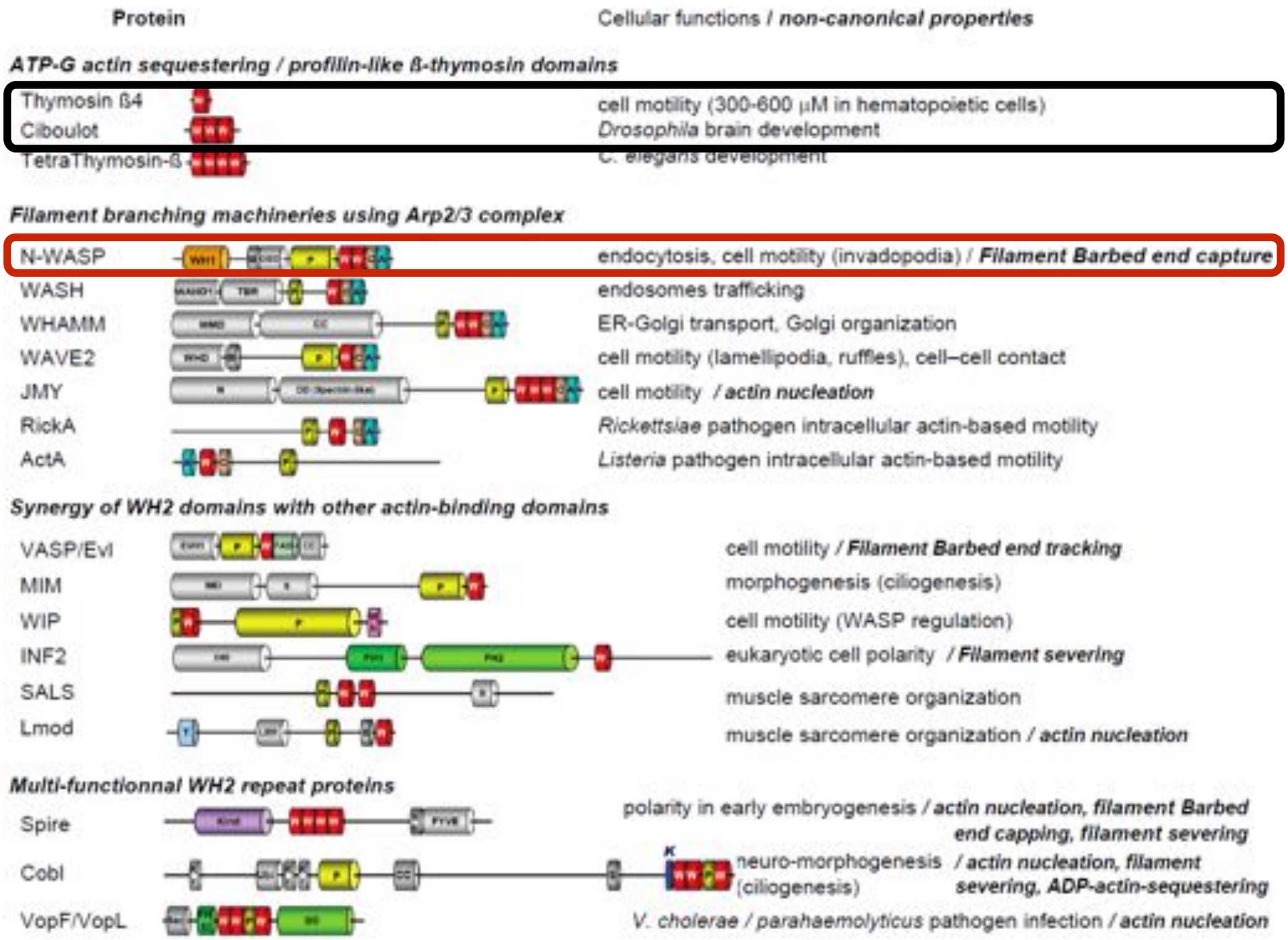
ADP G-actin



Carlier and Pantaloni 2007

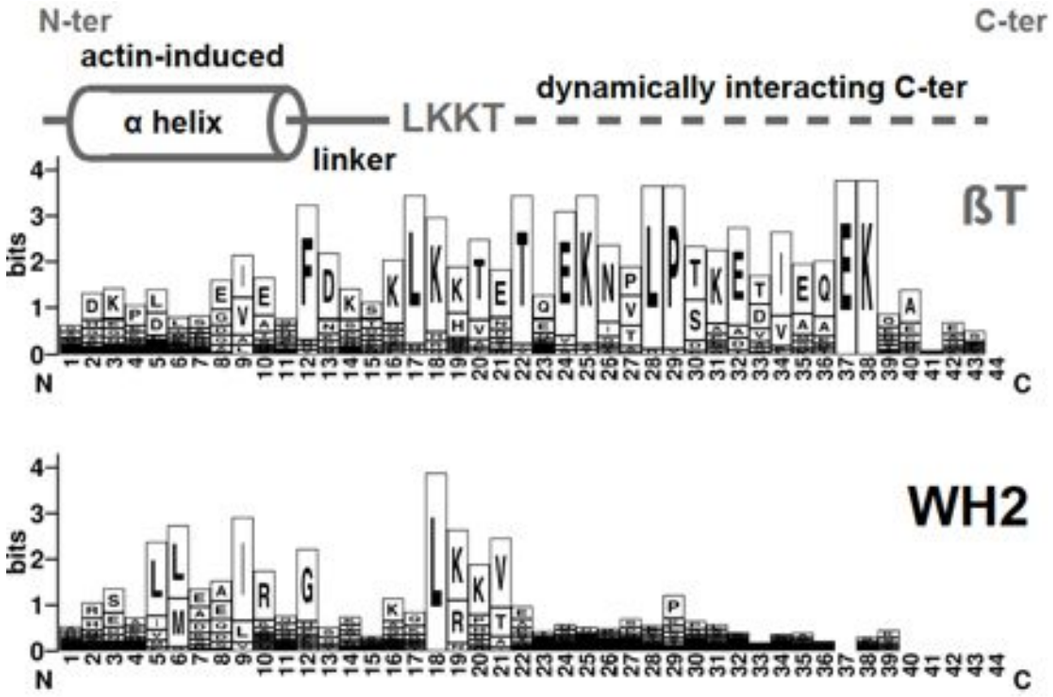
Investigation of Intrinsically Disordered Proteins
Rôle of disorder in regulation processes

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine



Carlier et al. Int. Rev. Cell. Mol. Biol. 2011

Investigation of Intrinsically Disordered Proteins
Rôle of disorder in regulation processes
Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

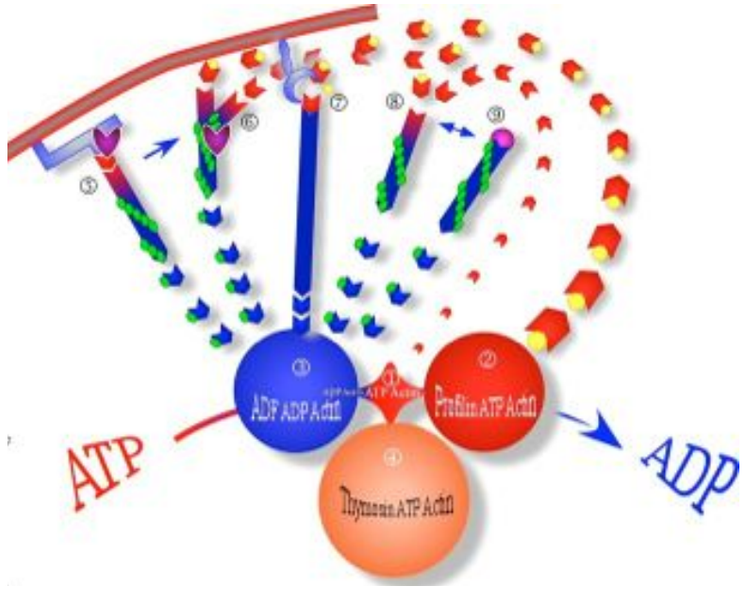


Low sequence identity, variable length (25-55)
40% among tβ domains, 20% among WH2 domains, <15% for tβ/WH2
N-ter helical propensity, LKKT/V, variable C-ter length
Intrinsically disordered

- Usually moderate affinities (μM)
- Tunable specificities modulated by
  - key residues spread along the sequence
    - versatility of folding upon binding processes
    - variable flexibility in the complexes with actin
  - post-transcriptional modifications
  - expression levels of partners
    - Cooperative / antagonist key interactions
    - Allosteric processes

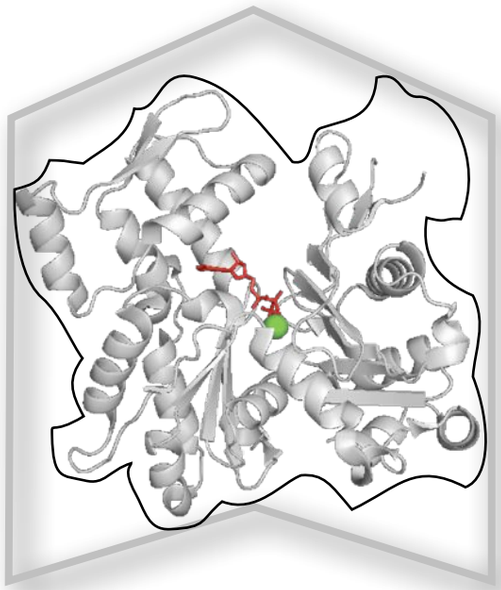
Multiple functions

- Sequestration of monomeric actin (Isolated WH2/Tβ)
- Promotion of polymerization at barbed end (Isolated WH2/Tβ)
- Nucleation (Formins, WH2/Tβ repeats, WASP, N-WASP, WAVE, …)
- Filaments branching (WASP, N-WASP, WAVE, …)
- Filaments capping (CP, CARMIL)
- Filaments severing (WH2/Tβ repeats)
- Inactivation/Activation of signaling cascades (WASP, WAVE, …)



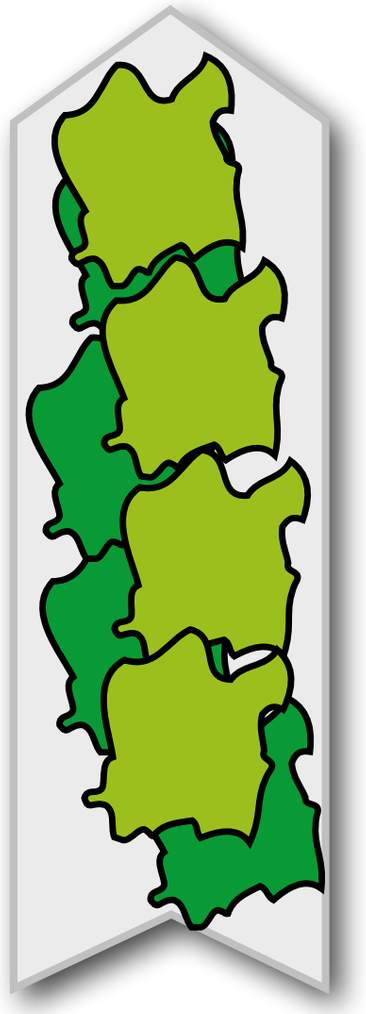
Relation séquence /function ?
Mécanismes d'action ?

Investigation of Intrinsically Disordered Proteins
Rôle of disorder in regulation processes
Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine



Un défi

Polymérisation de l'actine (60µM @ 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

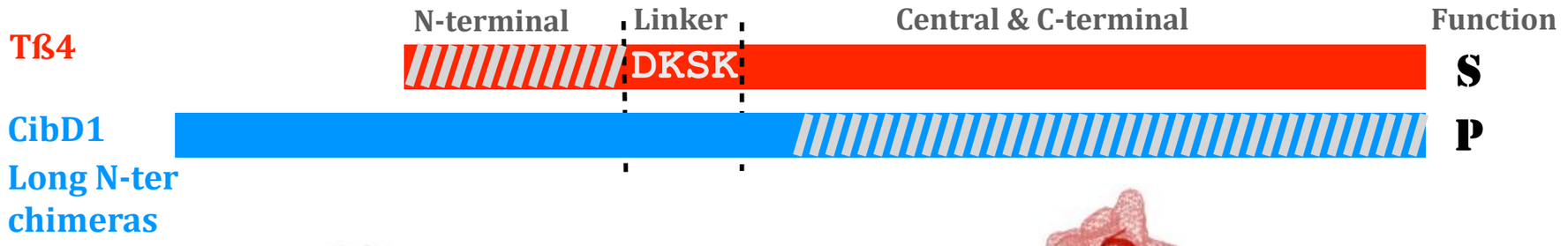
Diffraction RX, RMN, SAXS

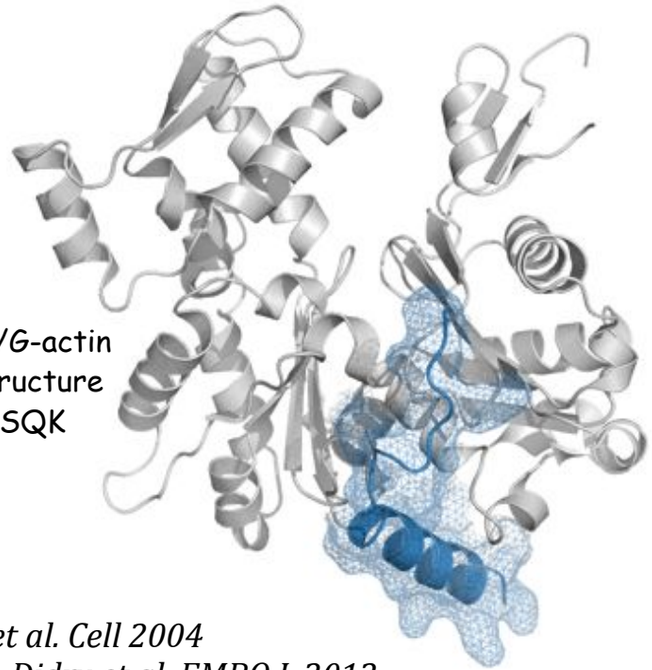
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

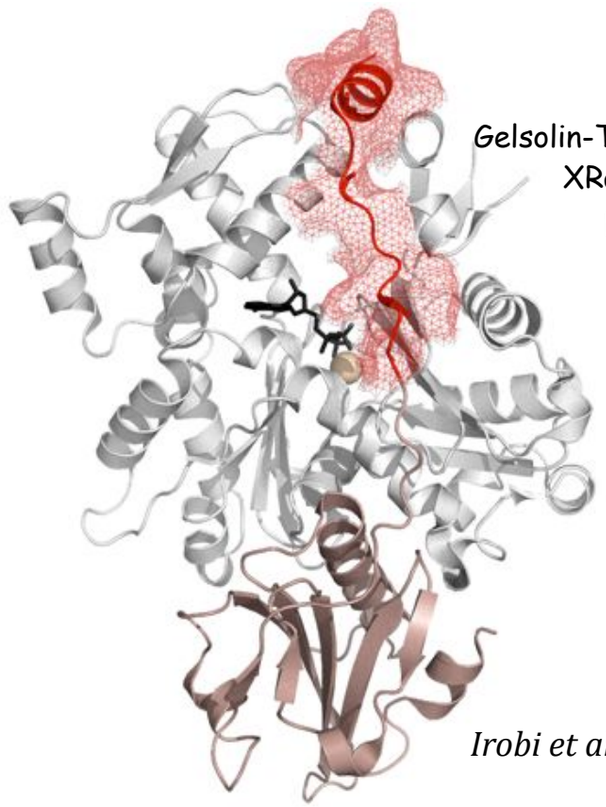
	difficulties	informations
XRay diffraction	cristallization conditions dynamics / heterogeneity	**Partial view of the complex** Detailed view of the interface





Ciboulot/G-actin
XRay structure
pdb 1SQK

Hertzog et al. Cell 2004
Cantrelle, Didry et al. EMBO J. 2012



Gelsolin-Tβ4(C-ter)/G-actin
XRay structure
pdb 1T44

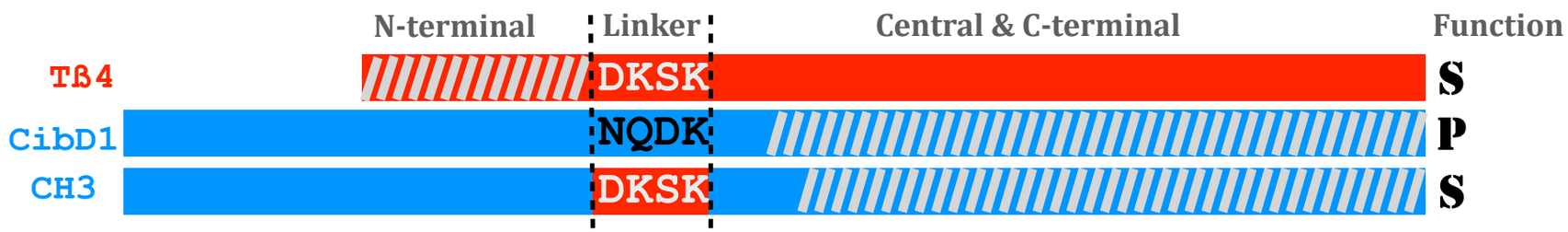
Irobi et al. EMBO J. 2004

NMR - Spatial and Time scales are highly intermingled.

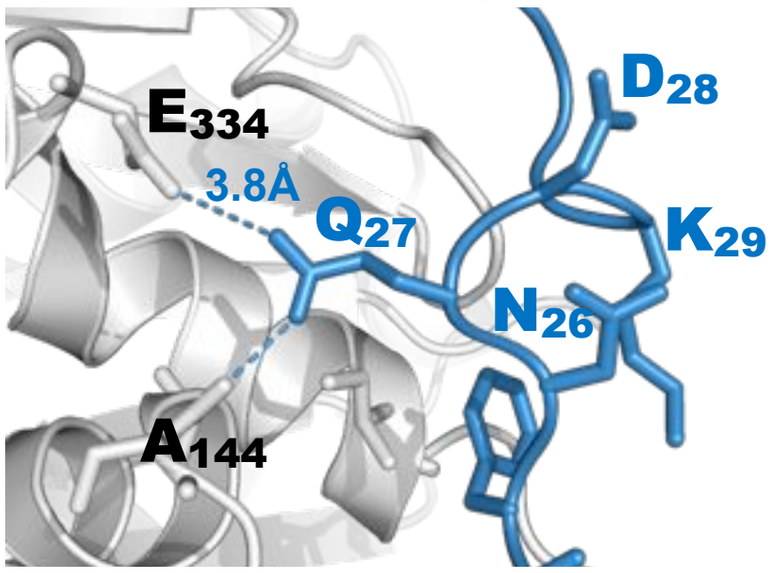
Investigation of Intrinsically Disordered Proteins

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

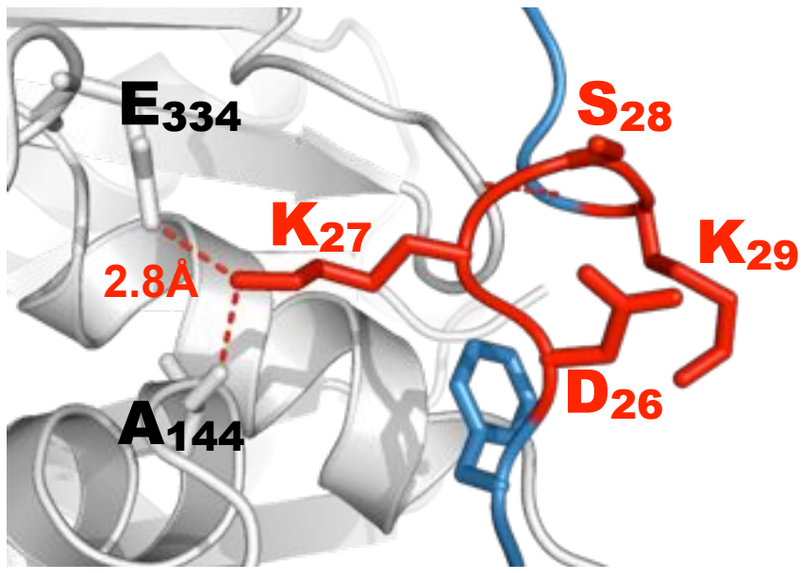
	difficulties	informations
XRay diffraction	cristallization conditions dynamics / heterogeneity	Partial view of the complex **Detailed view of the interface**



CibD1 linker NQDK



Tß4 linker DKSK in CH3

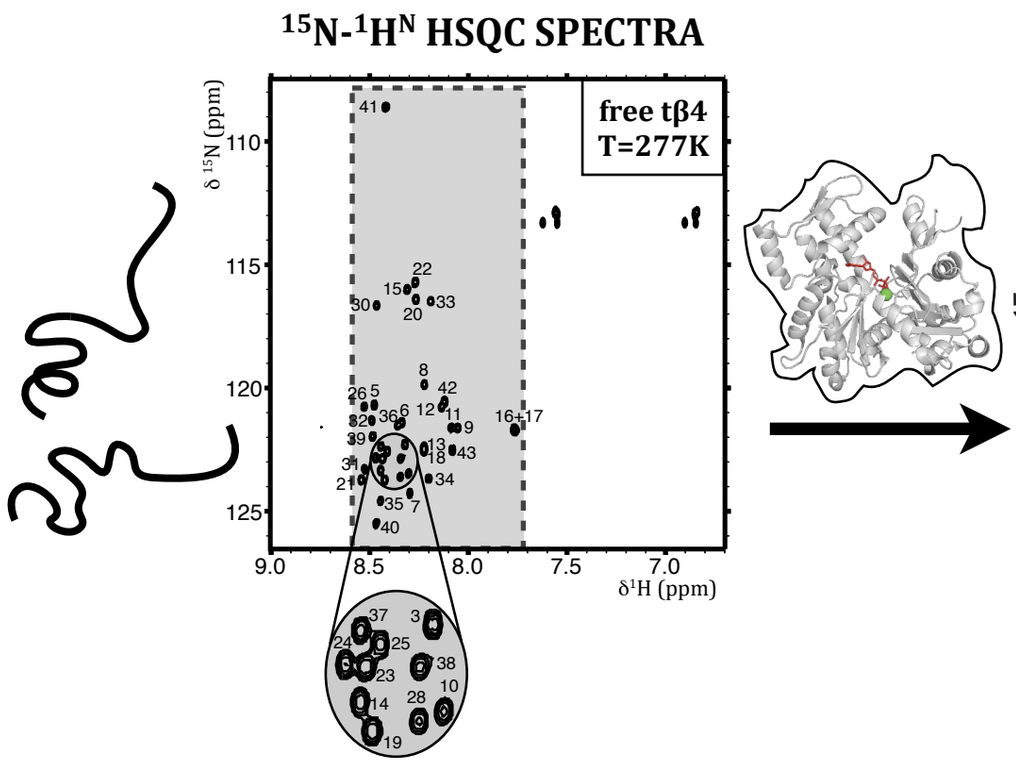


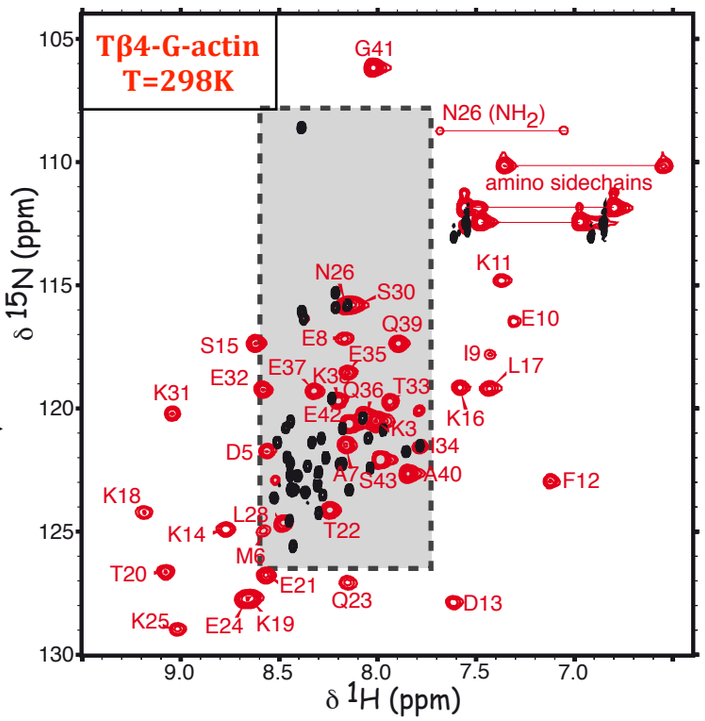
Salt bridge GLU^{actine} ---LYS^{linker,WH2}

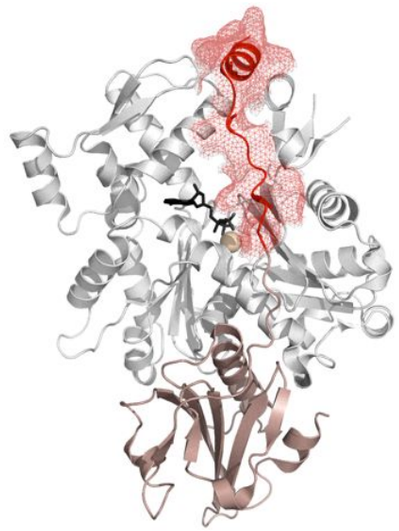
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

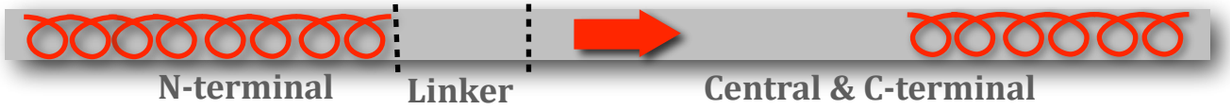
Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

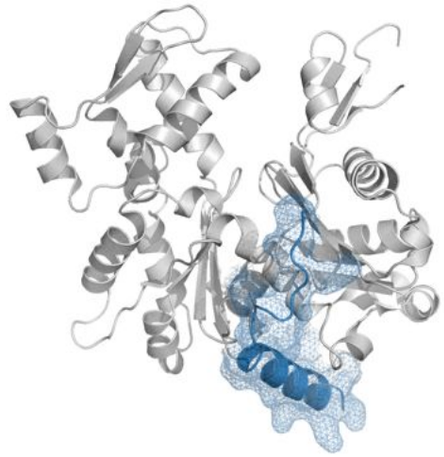


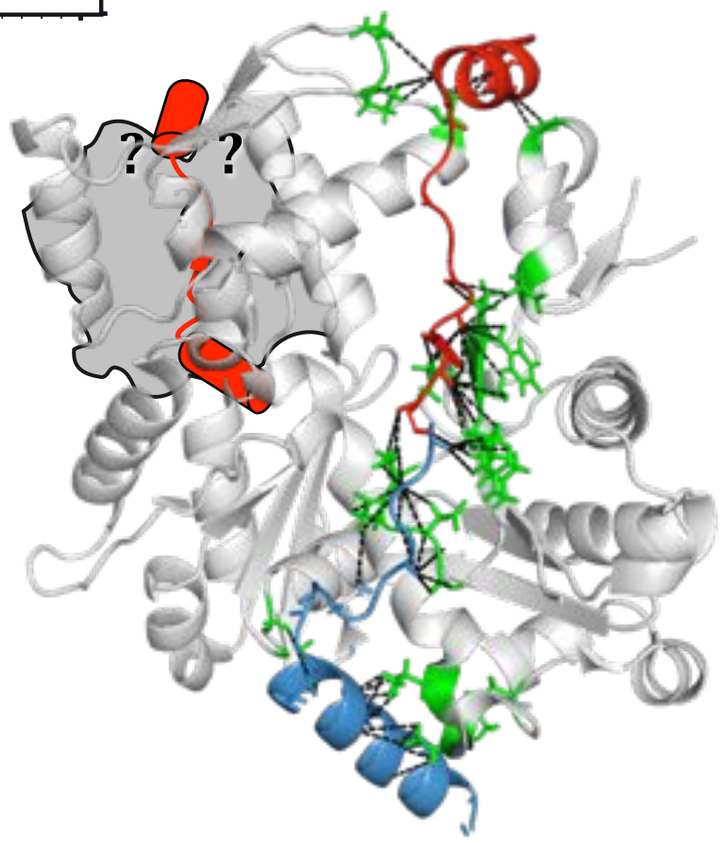




☞**Complete folding of all analyzed forms upon binding to ATP-G-actin**



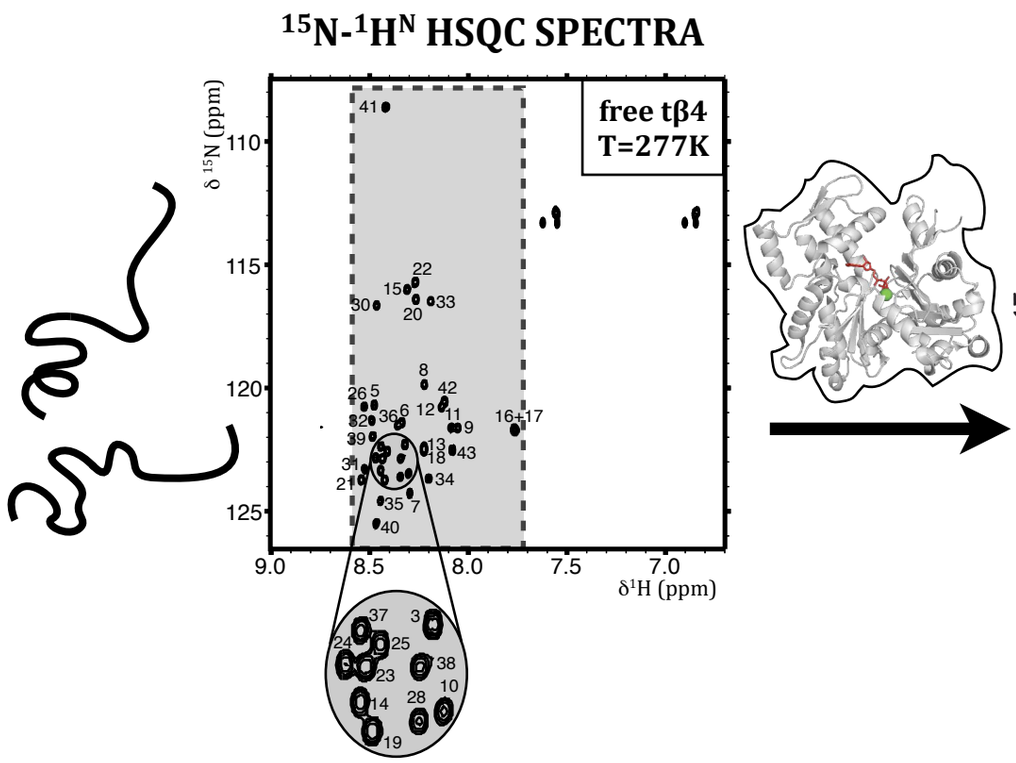


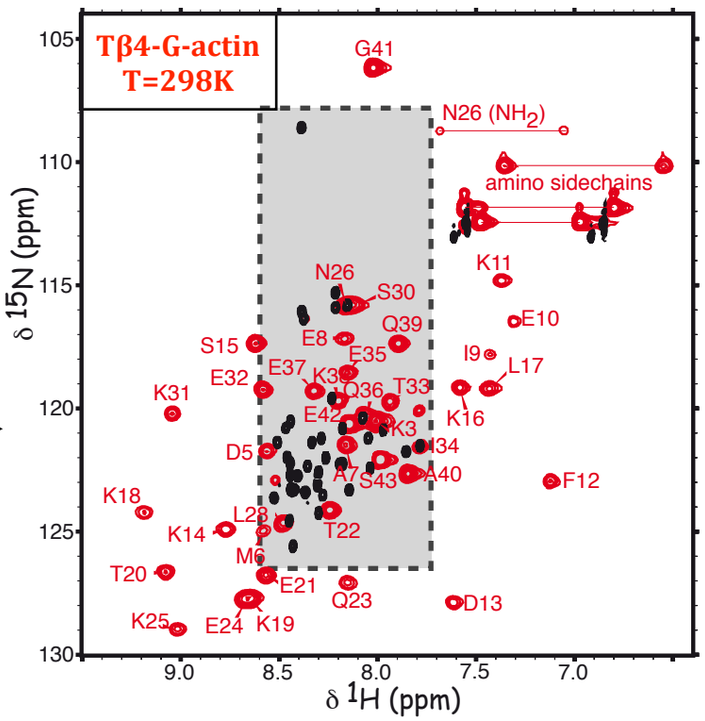


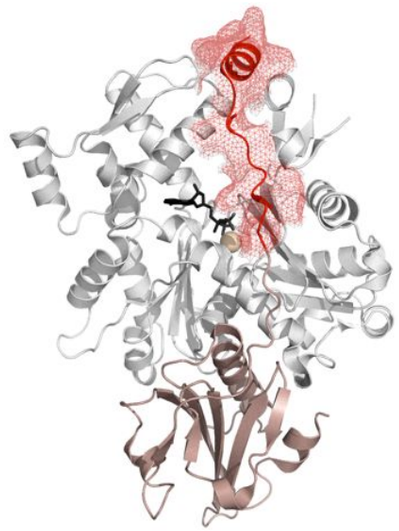
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine







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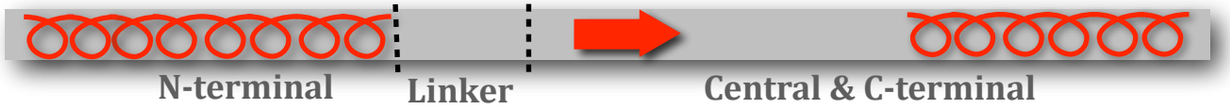
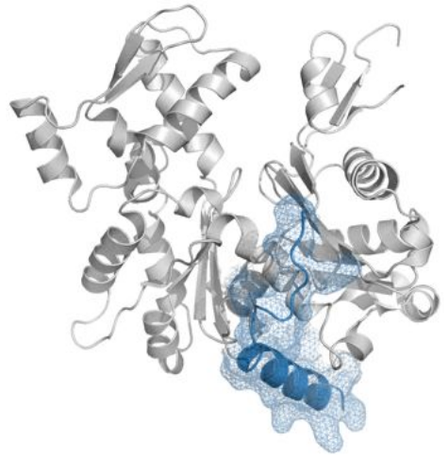
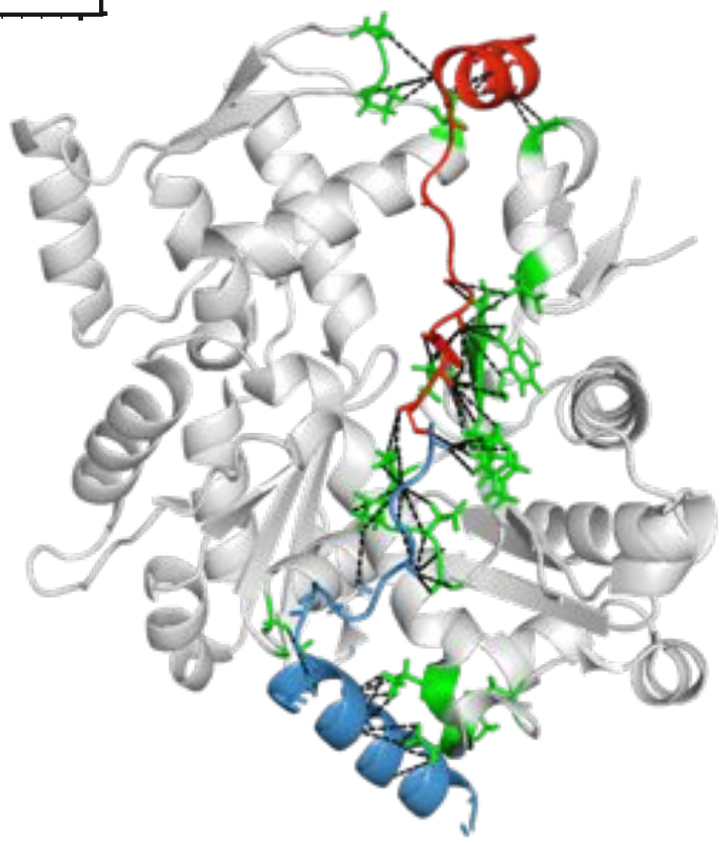


\+ nOe data

\+ partial XRay structures



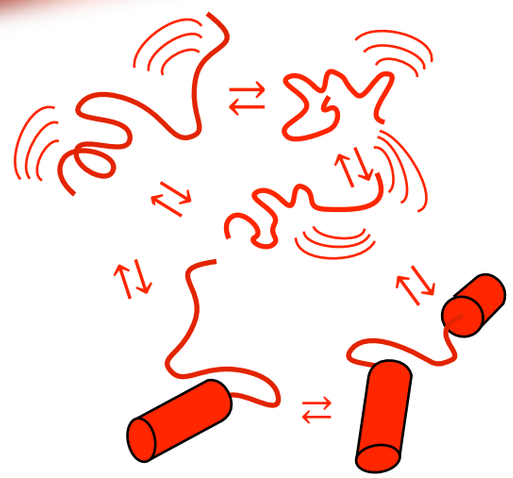


NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine

Free states
Conformational ensemble



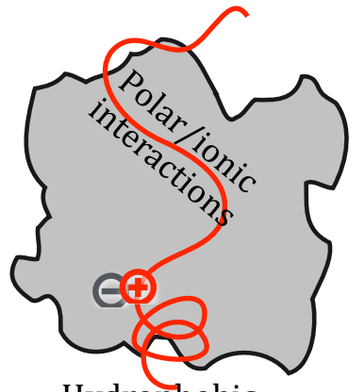
Prestructured states?

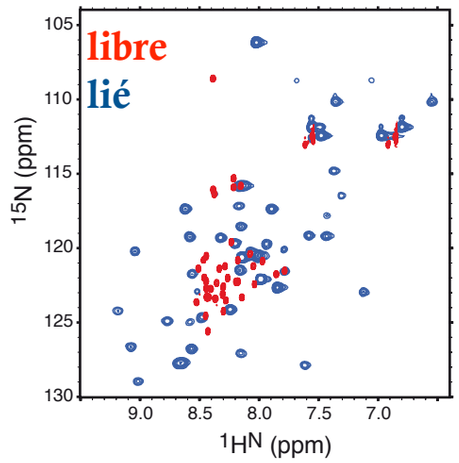
NMR
chemical shifts
Spin relaxation
RDCs, PRE

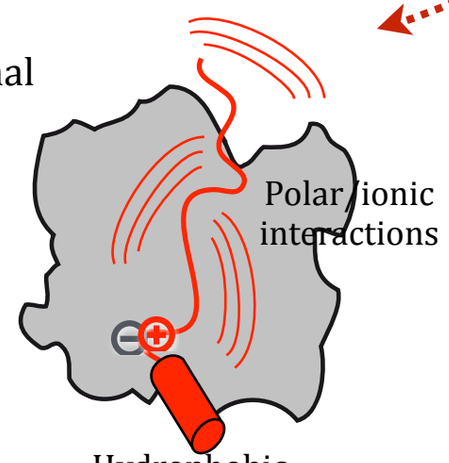
CD
SAXS+modeling

"Induced fit"

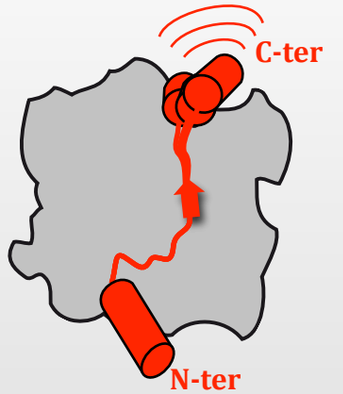
"conformational selection"







"Induced fit"



"Induced fit"

Bound states?

X-ray diffraction
Structured well-defined parts

NMR
Electronic environment of observable spins
secondary structures
inter & intramolecular atomic proximities
internal flexibility
Folding upon binding mechanism

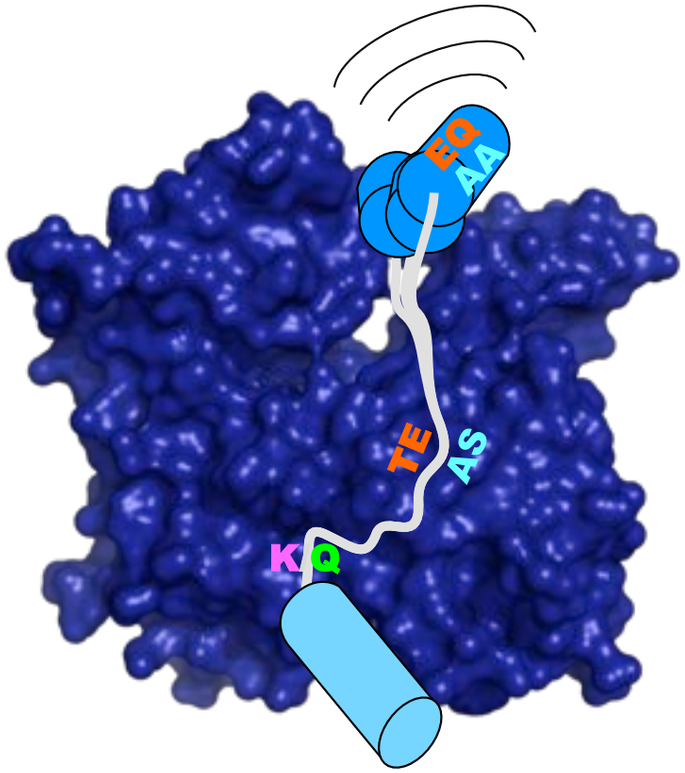
SAXS
Enveloppes

Domanski et al. JBC 2004, Hertzog et al. Cell 2004, Cantrelle et al. EMBO J. (2012)

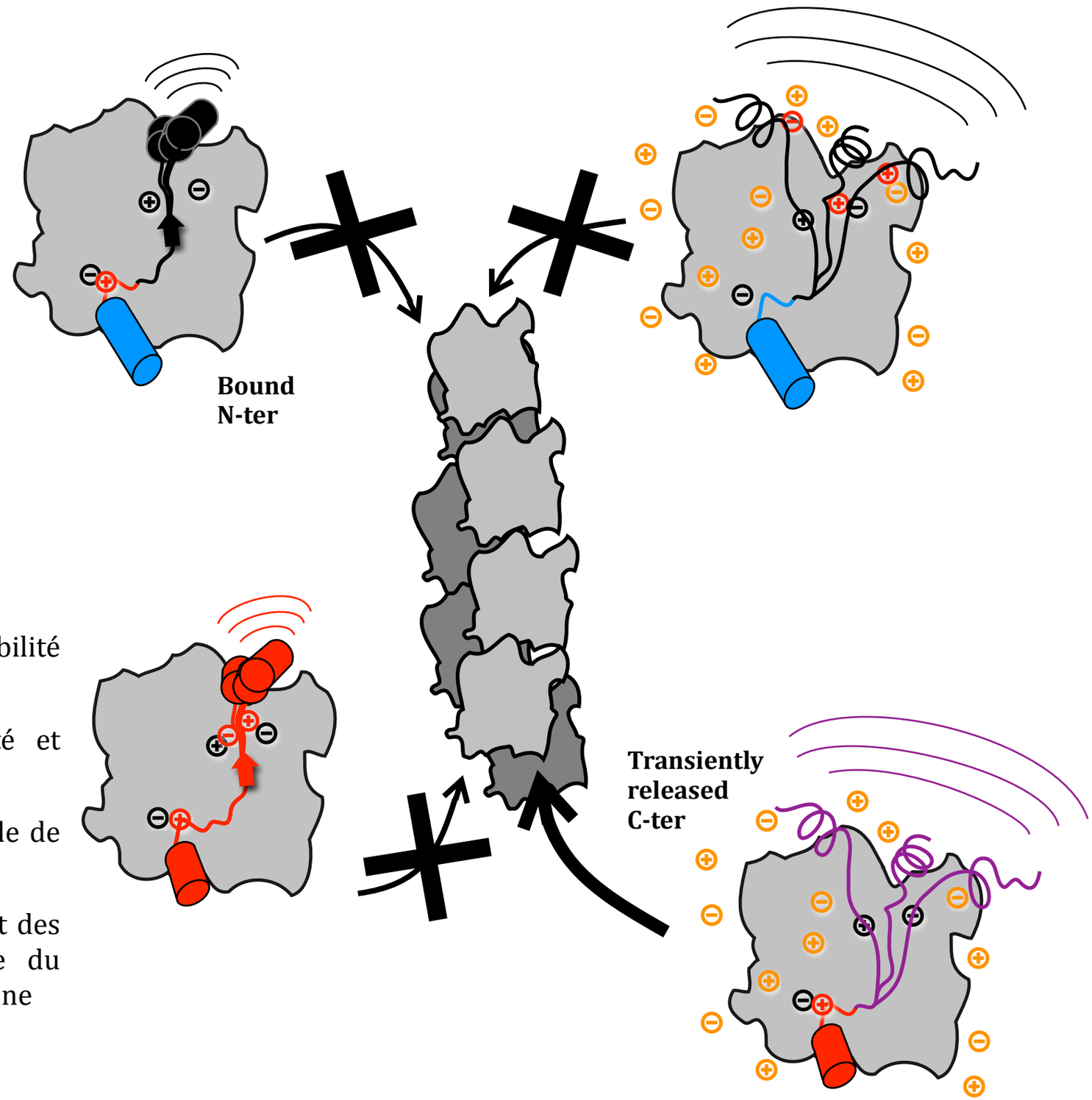
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

Les domaines Tβ/WH2 - régulation de la polymérisation de l'actine



- Interactions électrostatique/polaires modulent la flexibilité locale dans l'état lié
- Interaction électrostatique clé contrôle la flexibilité et 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

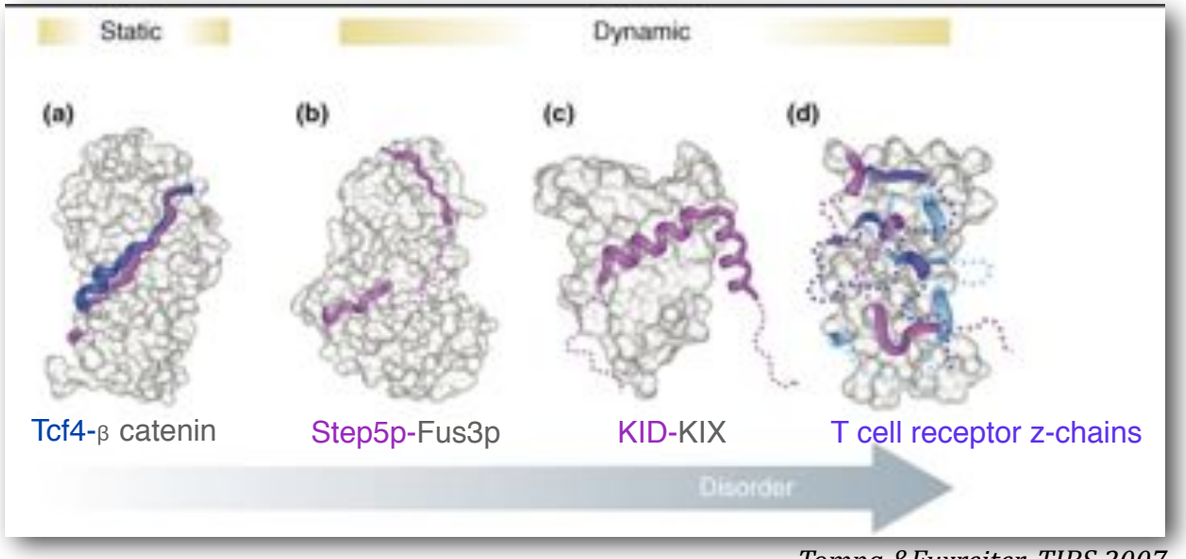


Cantrelle et al. EMBO J. (2012)

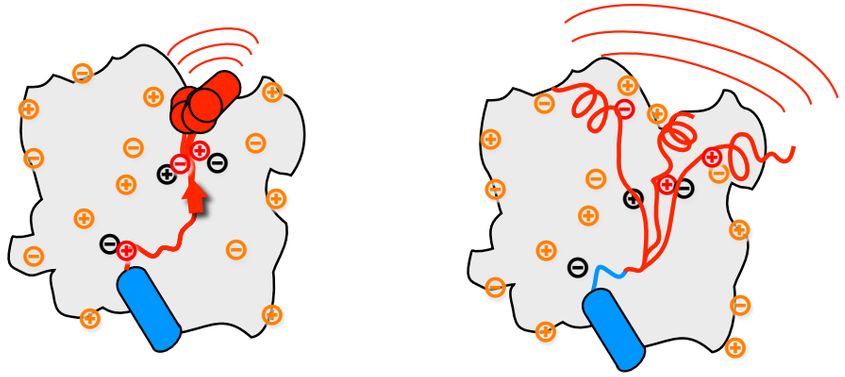
NMR - Spatial and Time scales are highly intermingled.

Investigation of Intrinsically Disordered Proteins

polymorphism & structural disorder
in protein-protein interactions involving IDPs

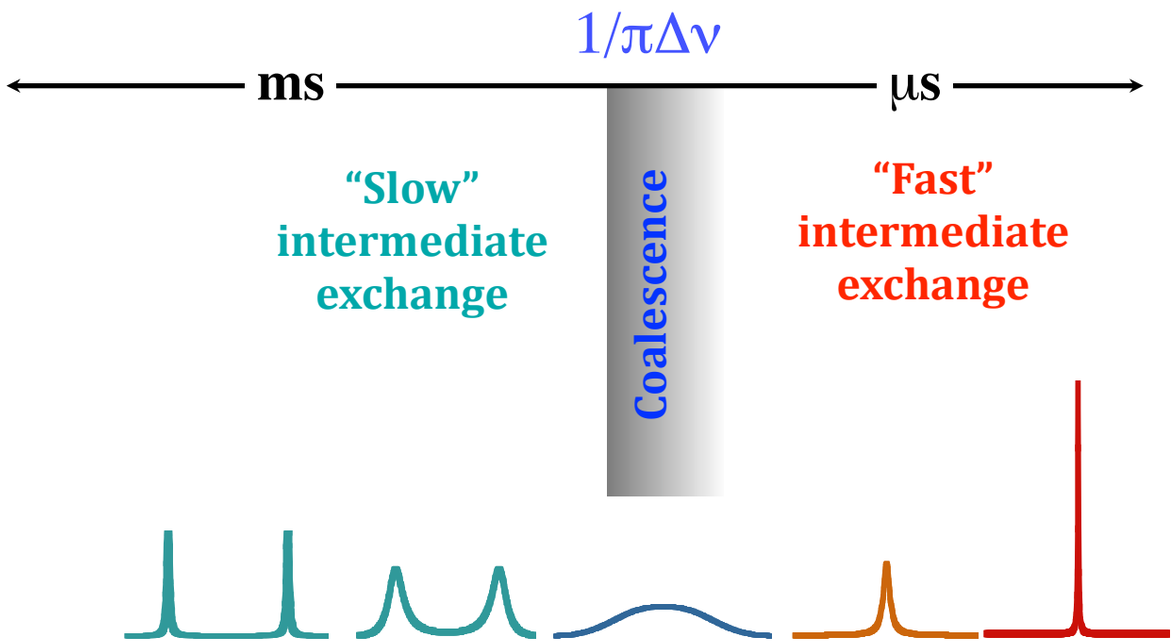


Tompa &Fuxreiter, TIBS 2007



➪ Control of the «fuzzyness» of the complexes via scarce variations along the whole sequence
➪ Role of local dynamics in the function of intrinsically disordered proteins.

Characterization of dynamic processes in the spectral timescale
Some methods to characterize conformational exchange



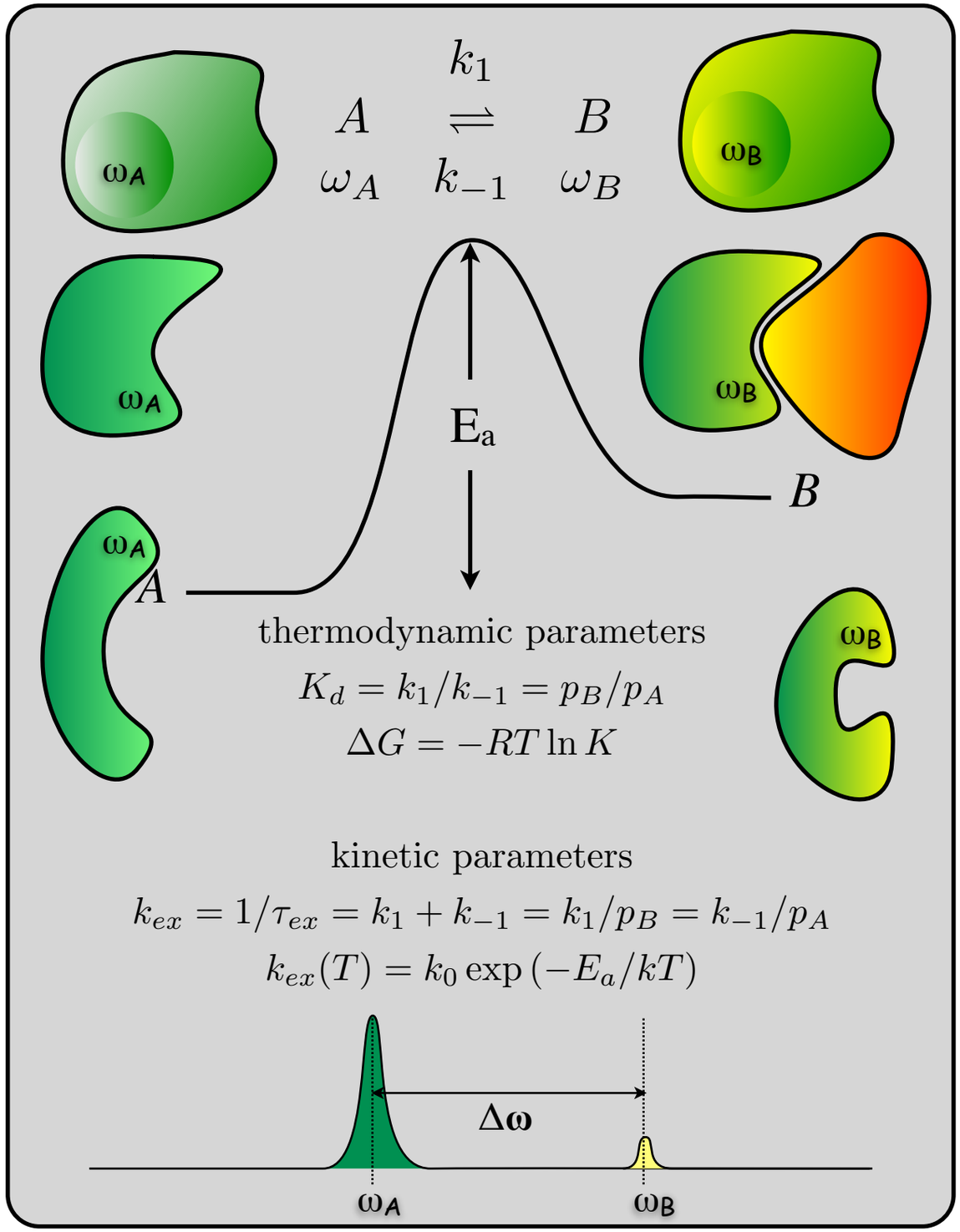
longitudinal magnetization exchange

Lineshapes analysis

weighted average values

$\delta^{app} = p_A\delta_A + p_B\delta_B$

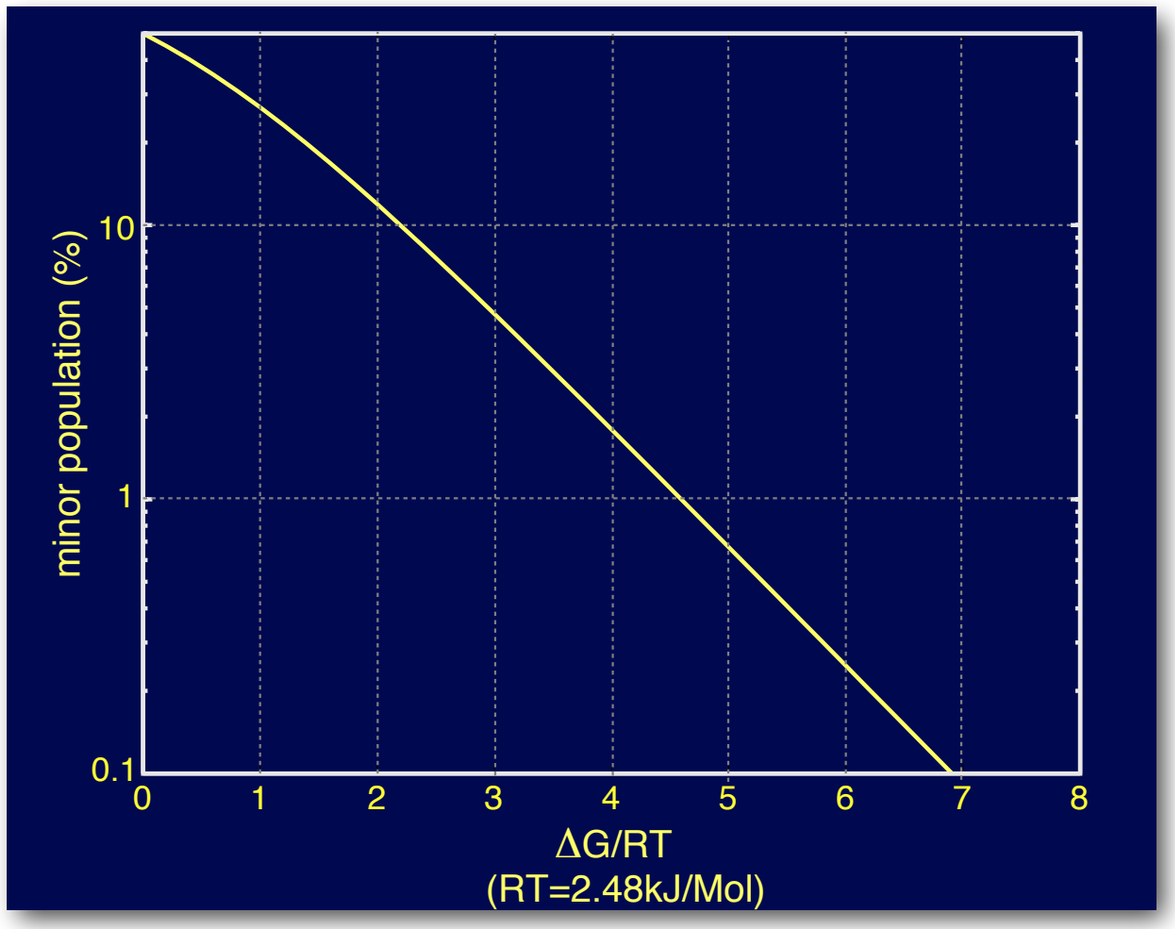
$R_2^{apparent} = R_2 + R_{ex}$

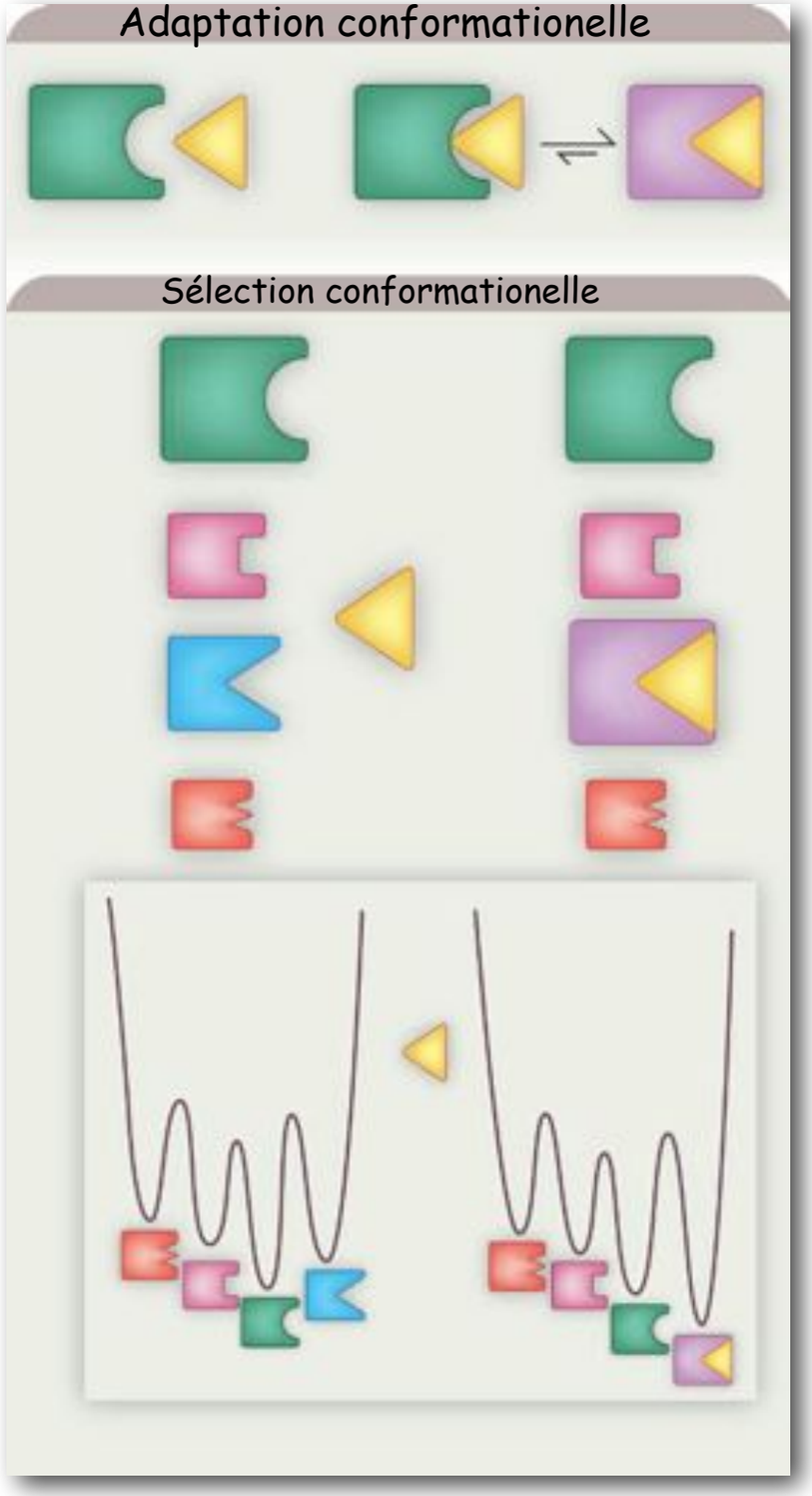


Caractérisation des états faiblement peuplés: une étape importante pour la compréhension des mécanismes d'action des protéines

- Protéines : multiple conformations à l'équilibre thermique en solution
  - Cristallographie RX : Sélection d'une conformation lors de la cristallisation
  - RMN : Ensemble conformationel moyenné dans le temps
- Etat "fondamental" > 90%
- Etats de haute énergie, faiblement peuplés : rôles dans la liaison de substrats, dans les cycles catalytiques, dans les processus de repliement (états de transition)

...





Characterization of dynamic processes in the spectral timescale conformational exchange

Exchange regime
Case of unequal populations

☞ "Fast" exchange : $\Delta\omega \ll k_{ex}$

- ⇨ One peak at $\omega = p_A\omega_A + p_B\omega_B$
- ⇨ $R_{ex} \propto B_0^2$

☞ "intermediate" exchange : $\Delta\omega \simeq k_{ex}$

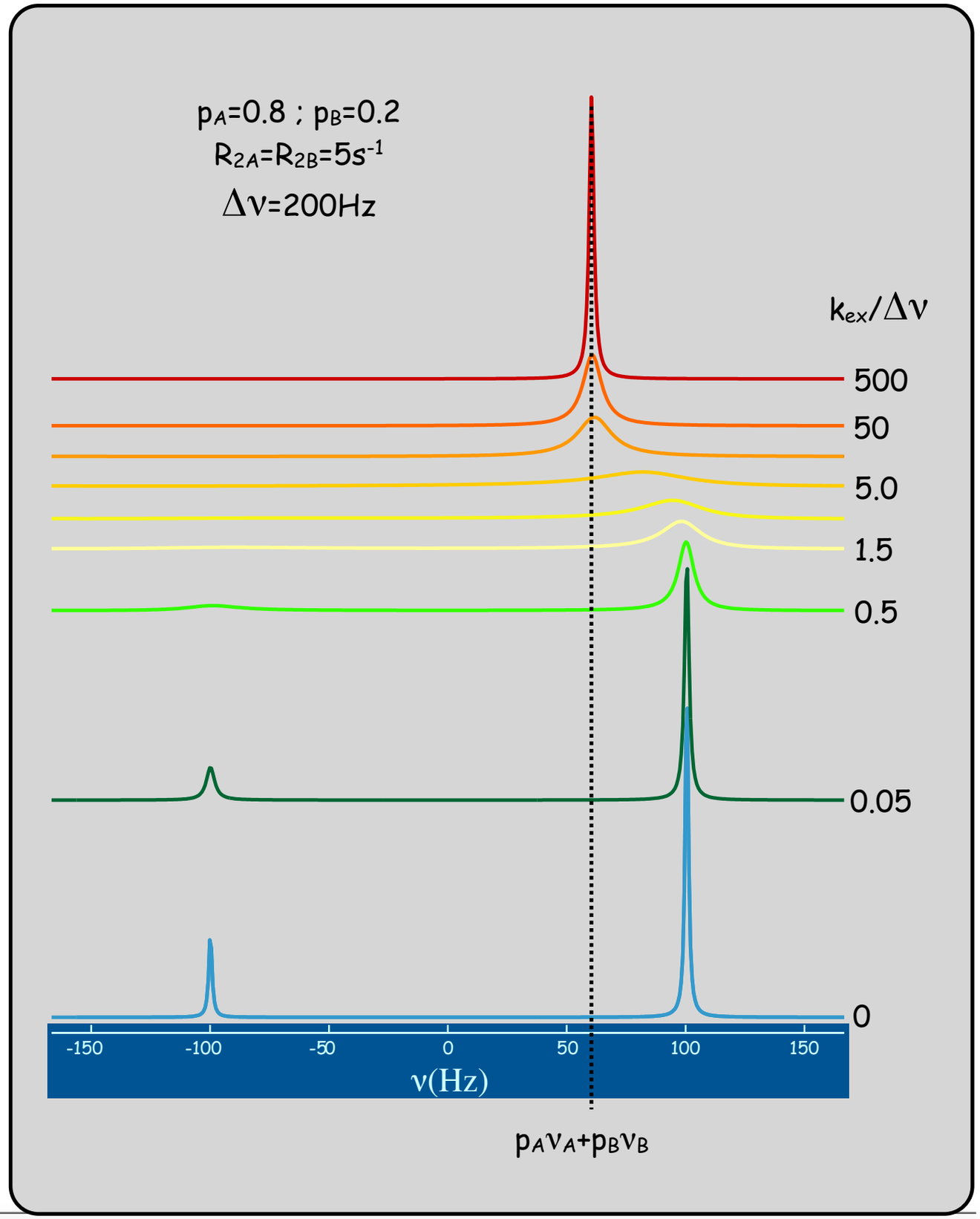
- ⇨ One or several broadened peaks
- ⇨ $R_{ex} \propto B_0$

☞ "slow" exchange : $\Delta\omega \gg k_{ex}$

- ⇨ Several peaks
- ⇨ R_{ex} independent of B_0
- ⇨ $R_{ex}^{(B)} \rightarrow k_{BA}=p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_B k_{ex}$

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

☞ **The minor peak can be undetectable even in a slow exchange regime.**



Characterization of dynamic processes in the spectral timescale
The case of one observed peak

Case of unequal populations

☞ "Fast" exchange : $\Delta\omega \ll k_{ex}$

- One peak at $\omega = p_A\omega_A + p_B\omega_B$
- $R_{ex} \propto B_0^2$

☞ **Number of states in exchange ?**

☞ "intermediate" exchange : $\Delta\omega \simeq k_{ex}$

- One or several broadened peaks
- $R_{ex} \propto B_0$

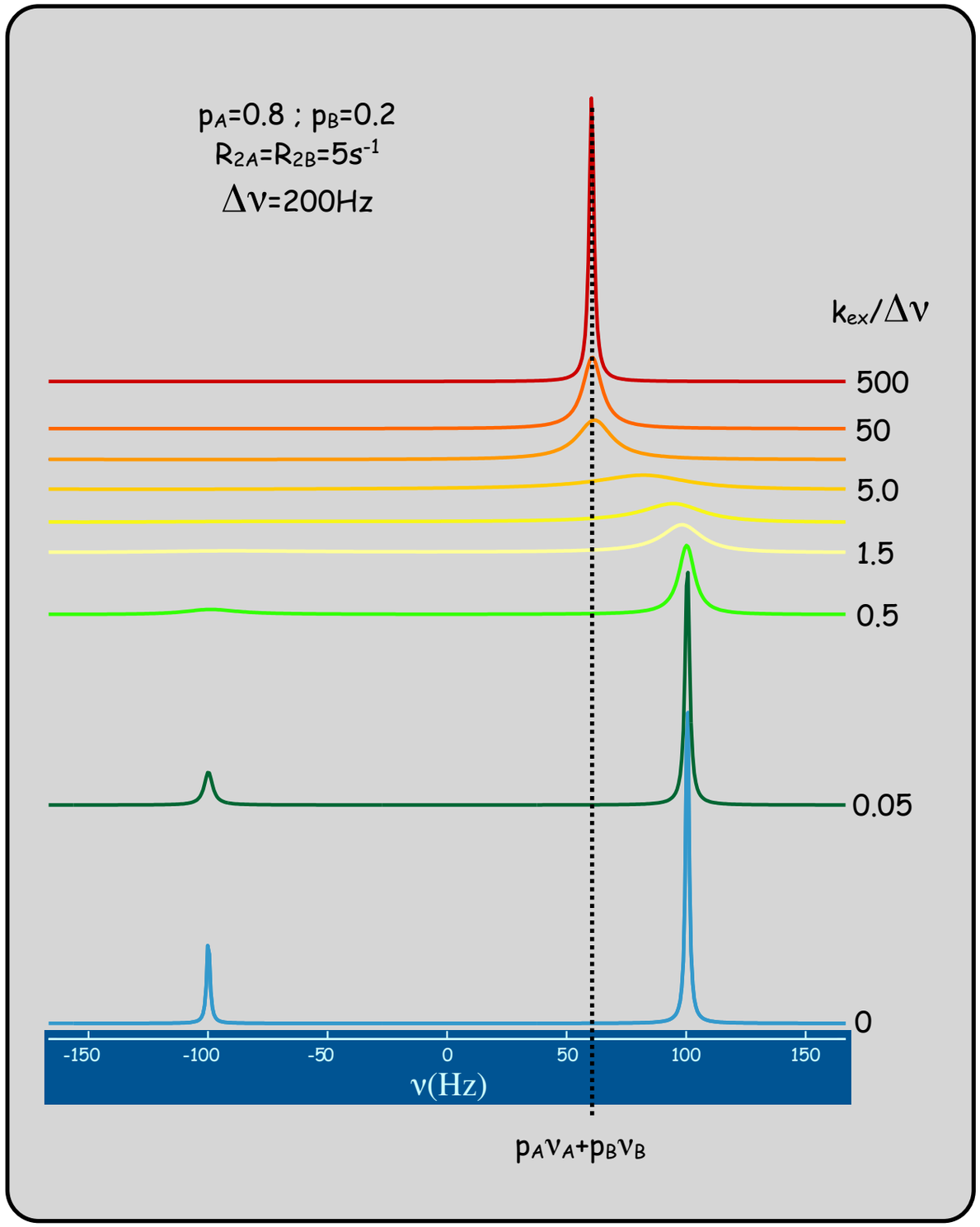
☞ "slow" exchange : $\Delta\omega \gg k_{ex}$

- Several peaks
- R_{ex} independent of B_0
- $R_{ex}^{(B)} \rightarrow k_{BA}=p_A k_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_B k_{ex}$

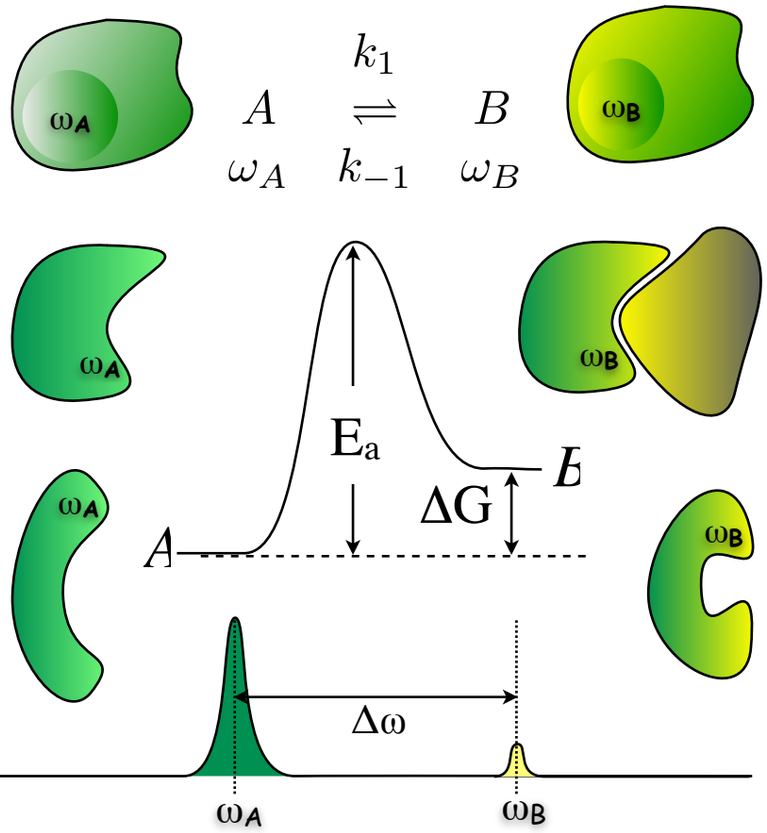
$$\mathbf{p_A \gg p_B \Rightarrow R_{ex}(B) \gg R_{ex}(A)}$$

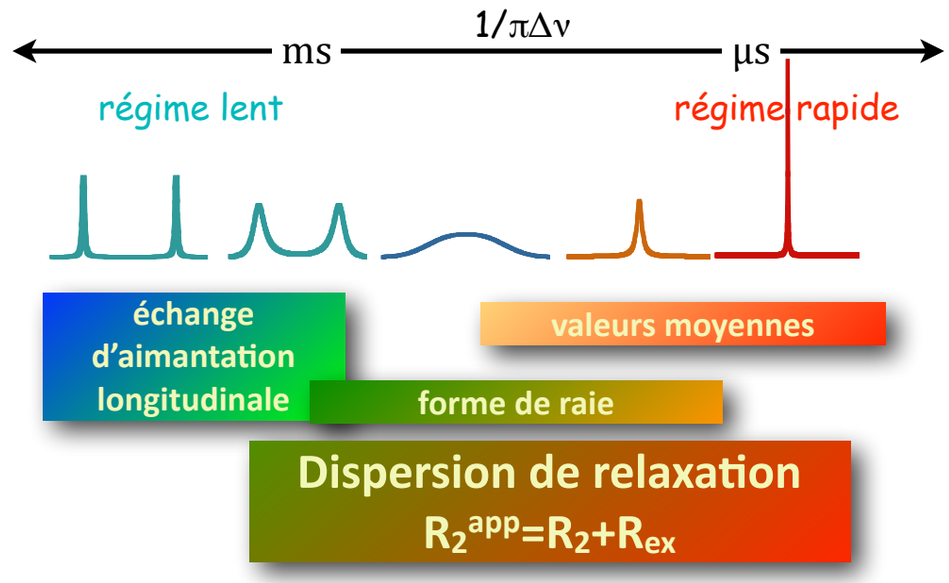
☞ **The minor peak can be undetectable even in a slow exchange regime.**

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

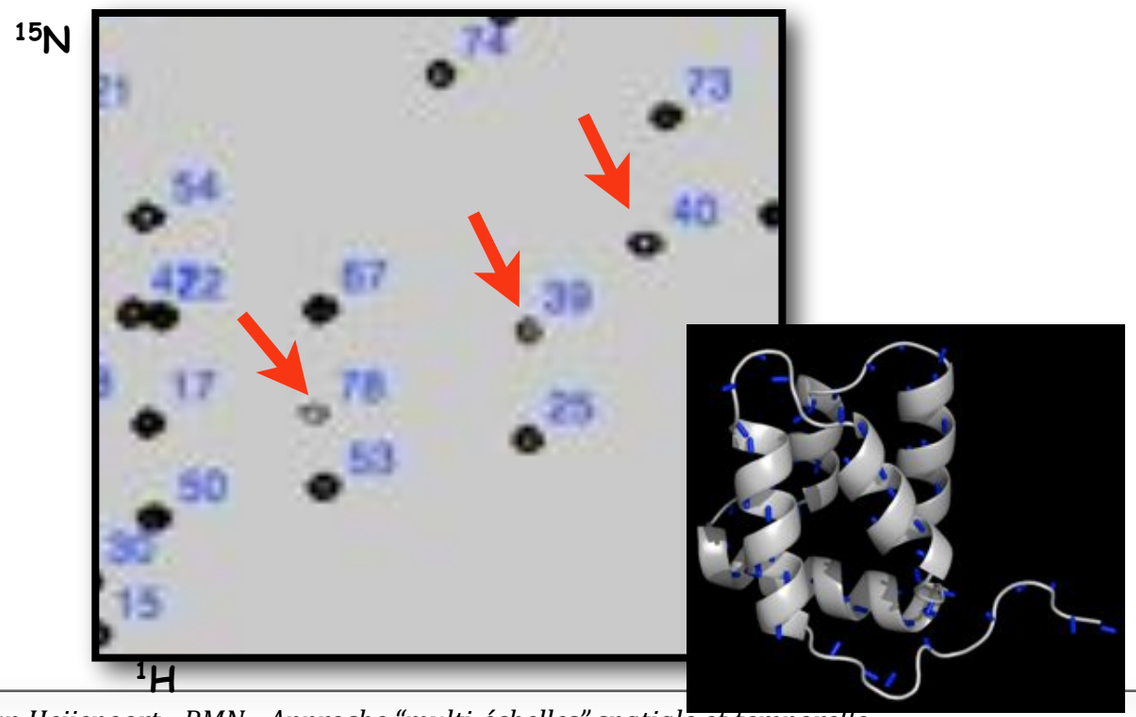


Characterization of dynamic processes in the spectral timescale
The case of one observed peak





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

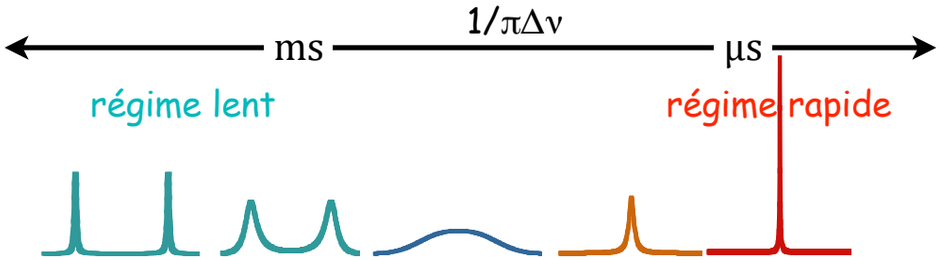
$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$

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

paramètres structuraux

$\Delta\omega$; ω_A ; ω_B

Characterization of dynamic processes in the spectral timescale
The case of one observed peak



échange d'aimantation longitudinale

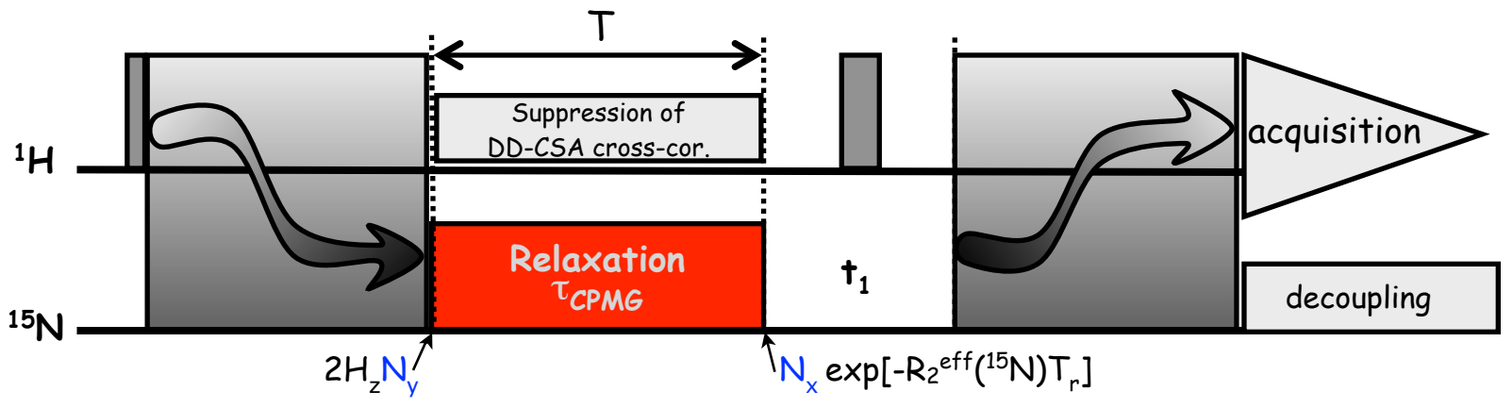
valeurs moyennes

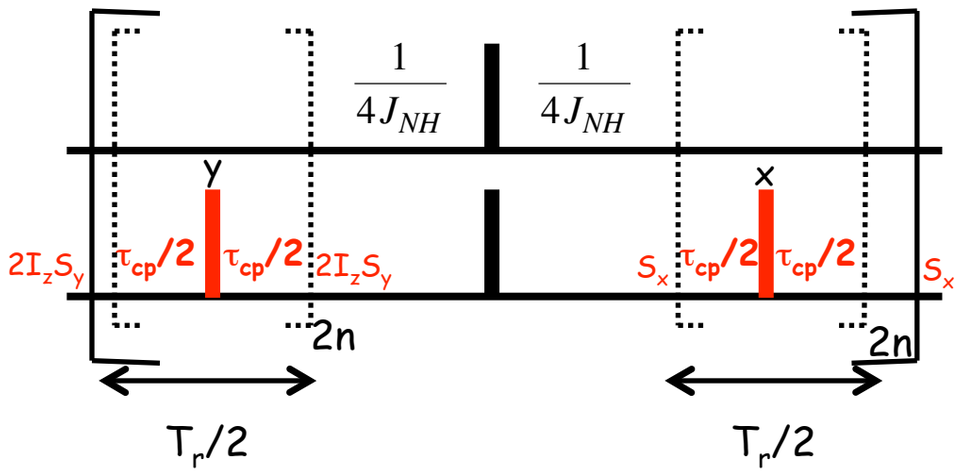
forme de raie

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

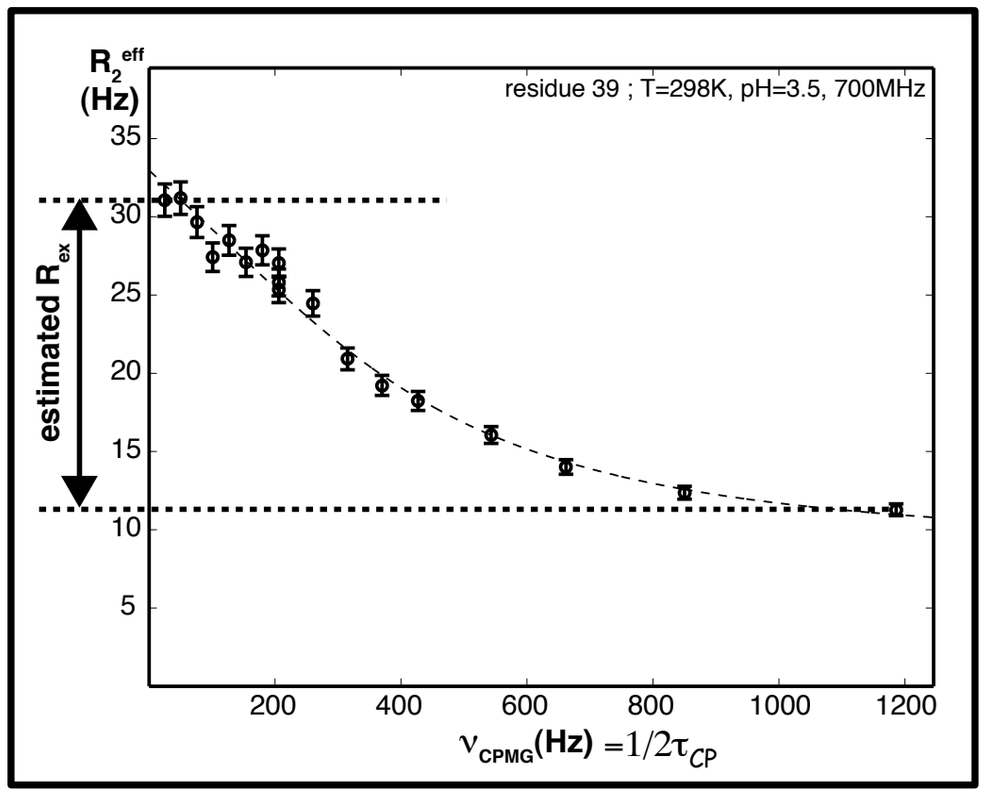
Une seule résonance apparente, plusieurs états en échange

expérience de dispersion de relaxation





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

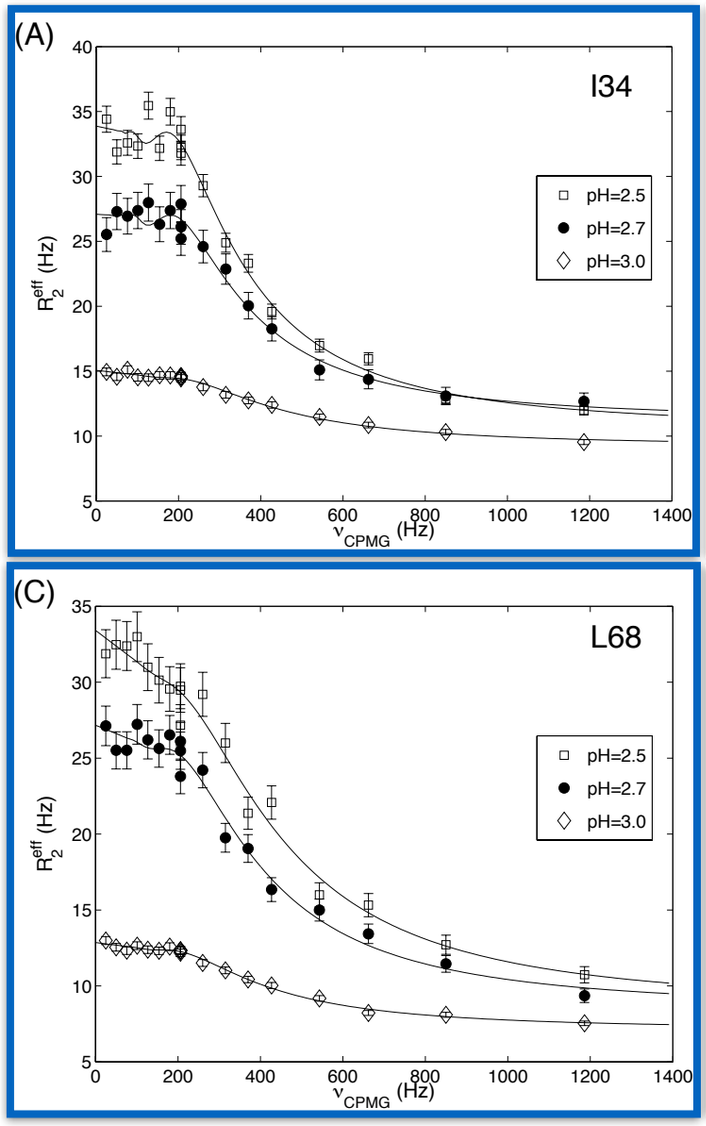


Characterization of dynamic processes in the spectral timescale
The case of one observed peak - relaxation dispersion experiments

The presence of dispersion indicate conformational exchange in the 100us-10ms range
Different exchange regimes yield different 'type' of dispersion curves

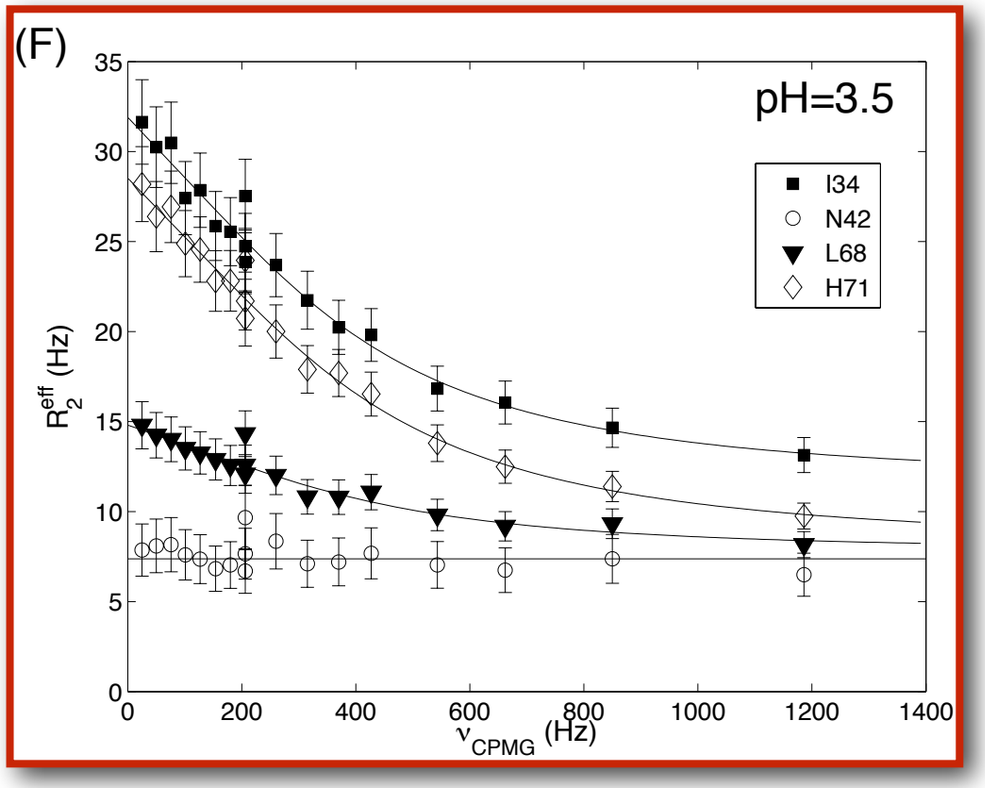
☞ Intermediate/Slow exchange regime

$p_B \ll p_A$

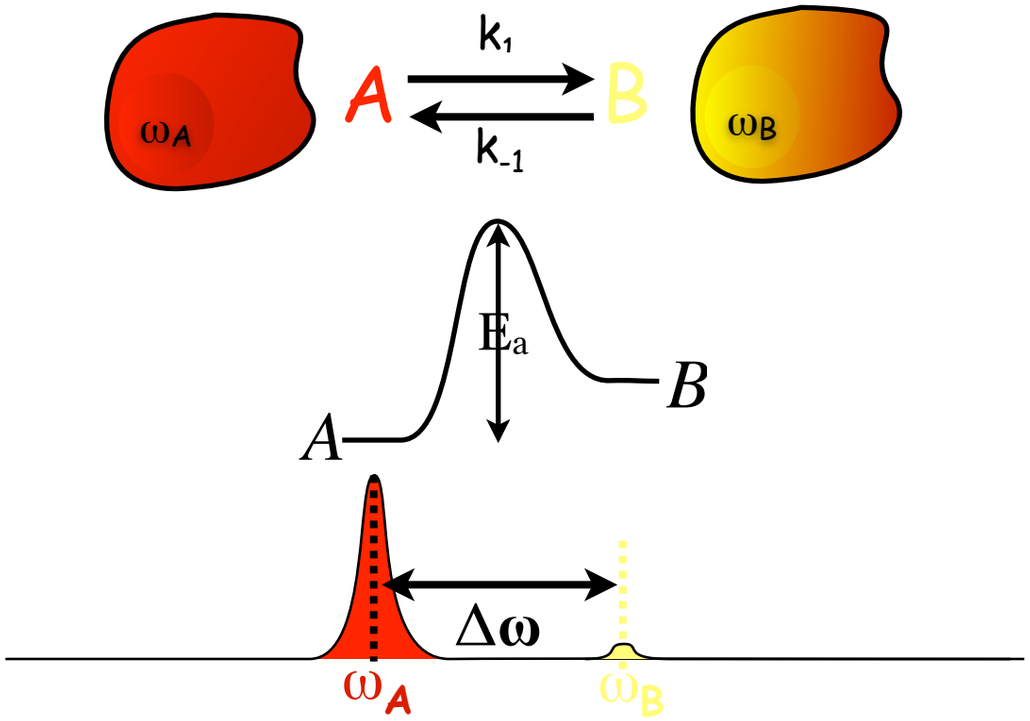


☞ Fast exchange regime

$p_B \simeq p_A$



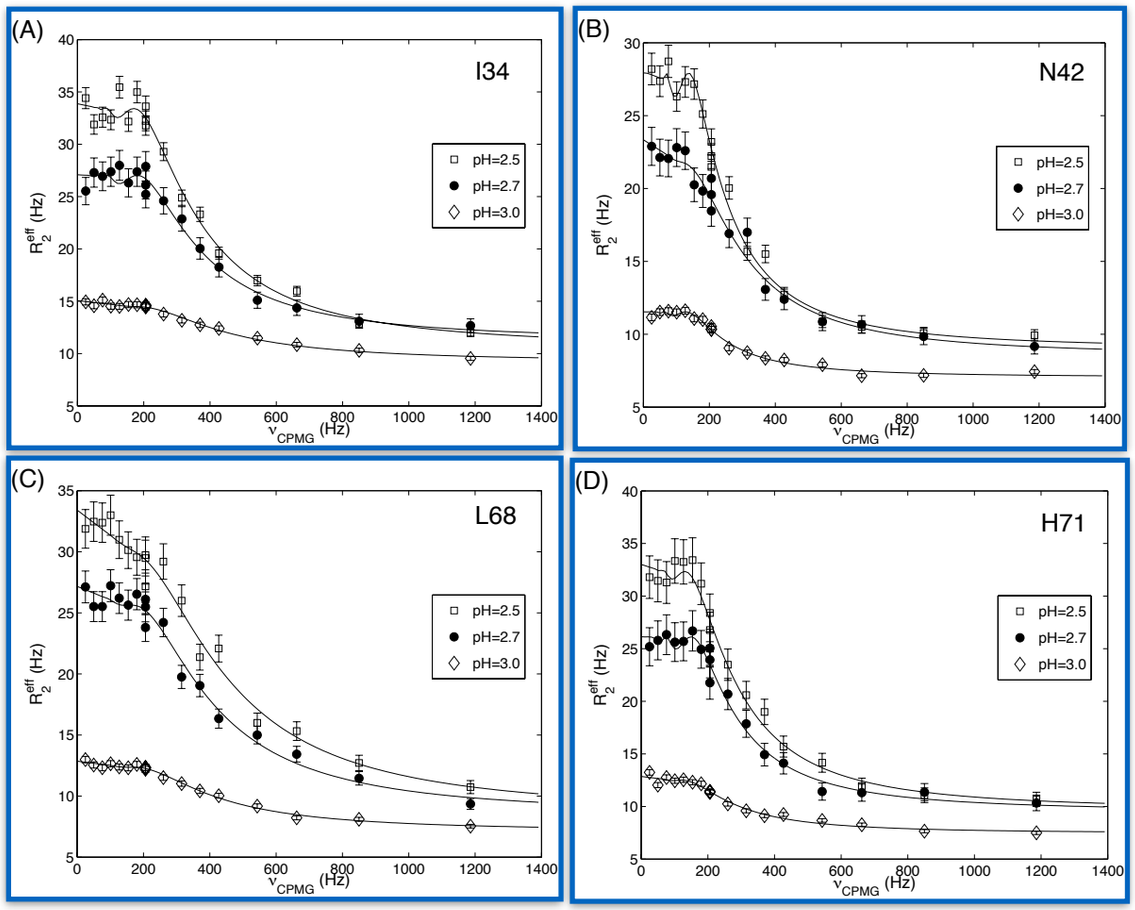
Characterization of dynamic processes in the spectral timescale
The case of one observed peak - slow regime with one major conformation



☞ Elargissement du pic majoritaire donne des informations sur la nature de l'état non détectable.

☞ Ajustement global des courbes de RD conduit aux paramètres cinétique, thermodynamique de l'échange et à des informations structurales sur l'état minoritaire

- ➪ τ_{ex} = 2-5 ms
- ➪ p_B = 1% ➟10%
- ➪ Individual $\Delta\omega(^{15}N)$ = 0↔8 ppm



$$R_2^{app} = R_2^{inf} + 0.5k_{ex} - \nu_{CP}\text{acosh}\left(D_+\cosh\eta_+ - D_-\cos\eta_-\right)$$

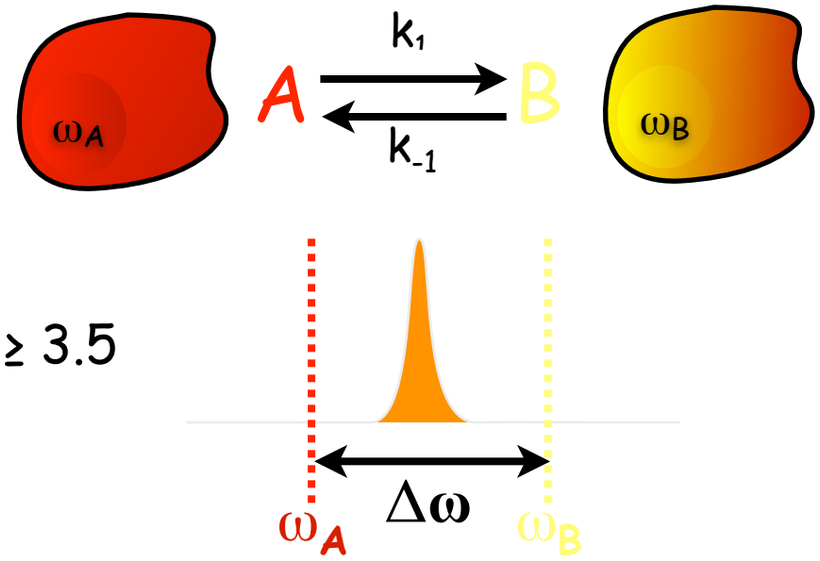
$$D_+ = 0.5\left[1 + \frac{\phi + 2\Delta\omega^2}{\sqrt{\phi^2+\zeta^2}}\right] \; ; \; D_- = 0.5\left[-1 + \frac{\phi + 2\Delta\omega^2}{\sqrt{\phi^2+\zeta^2}}\right]$$

$$\eta_+ = \frac{\tau_{CP}}{\sqrt{(2)}}\sqrt{\phi + \sqrt{\phi^2+\zeta^2}} \; ; \; \eta_- = \frac{\tau_{CP}}{\sqrt{(2)}}\sqrt{-\phi + \sqrt{\phi^2+\zeta^2}}$$

$$\phi = [(p_B - p_A)k_{ex}]^2 - \Delta\omega^2 + 4p_Ap_Bk_{ex}^2$$

$$\zeta = 2\Delta\omega(p_B - p_A)k_{ex}$$

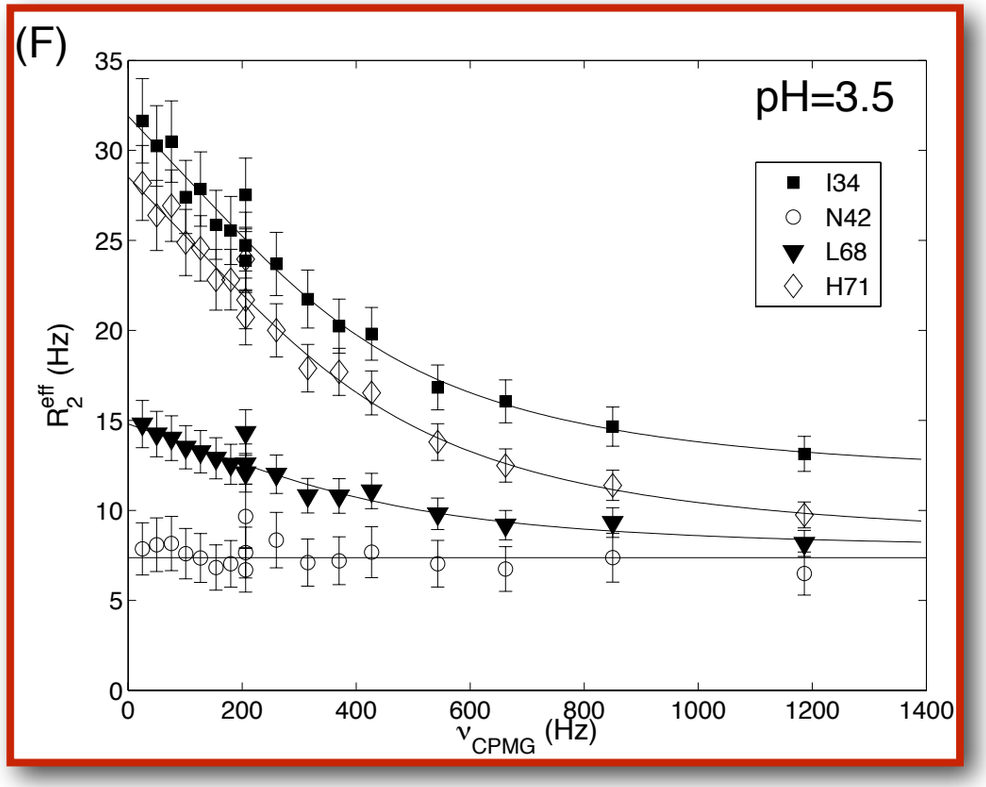
Characterization of dynamic processes in the spectral timescale
The case of one observed peak - fast exchange regime



pH ≥ 3.5

☞ Régime d'échange rapide : l'ajustement global des courbes de RD permet d'obtenir la constante de vitesse d'échange k_{ex} et le produit $p_A p_B \Delta\omega^2$.

- ⇨ τ_{ex} ~ 0.5 ms
- ⇨ $p_B \simeq p_A$
- ⇨ $\Delta\omega(^{15}N)$ ~ 0↔3 ppm



$$R_2^{app} = R_2^{inf} + p_A p_B \Delta\omega^2 \tau_{ex}[1 - 4\tau_{ex}\nu_{CP} \tanh \frac{1}{4\tau_{ex}\nu_{CP}}]$$

Quelques exemples typiques de la littérature

Caractérisation d'états intermédiaires de repliement

"Petites protéines globulaires" (domaine SH3)

Korzhnev and Kay (2008) Acc Chem Research 41(3) 442-451. *Probing Invisible, Low-Populated States of Protein Molecules by Relaxation Dispersion NMR Spectroscopy: An Application to Protein Folding*

Repliement au cours d'une interaction

Sugase et *al.* (2007) Nature 447, 1021-1025. *Mechanism of coupled folding and binding of an intrinsically disordered protein.*
Hansen et *al.* (2009) J. Mol.Biol. 387,1-9. *Binding Kinetics of Histone Chaperone Chz1 and Variant Histone H2A.Z-H2B by Relaxation Dispersion NMR Spectroscopy.*

Sélections conformationnelles

Interaction protéine-ligand (lysozyme)

Mulder et al, Nat. Struct. Biol. 2001, 8(1), 932. *Studying excited states of proteins by NMR spectroscopy*
Bouvignies et al., Nature 2011, 477, 111. *Solution structure of aminor and transiently formed state of a T4 lysozyme mutant*

Allostérie (partenaires de KIX)

Brüschweiler, Schanda, Kloiber, Brutscher, Kontaxis, Konrat and Tollinger, J. Am. Chem. Soc. (2009) 131, 3063–3068. *Direct Observation of the Dynamic Process Underlying Allosteric Signal Transmission.*

Enzymes

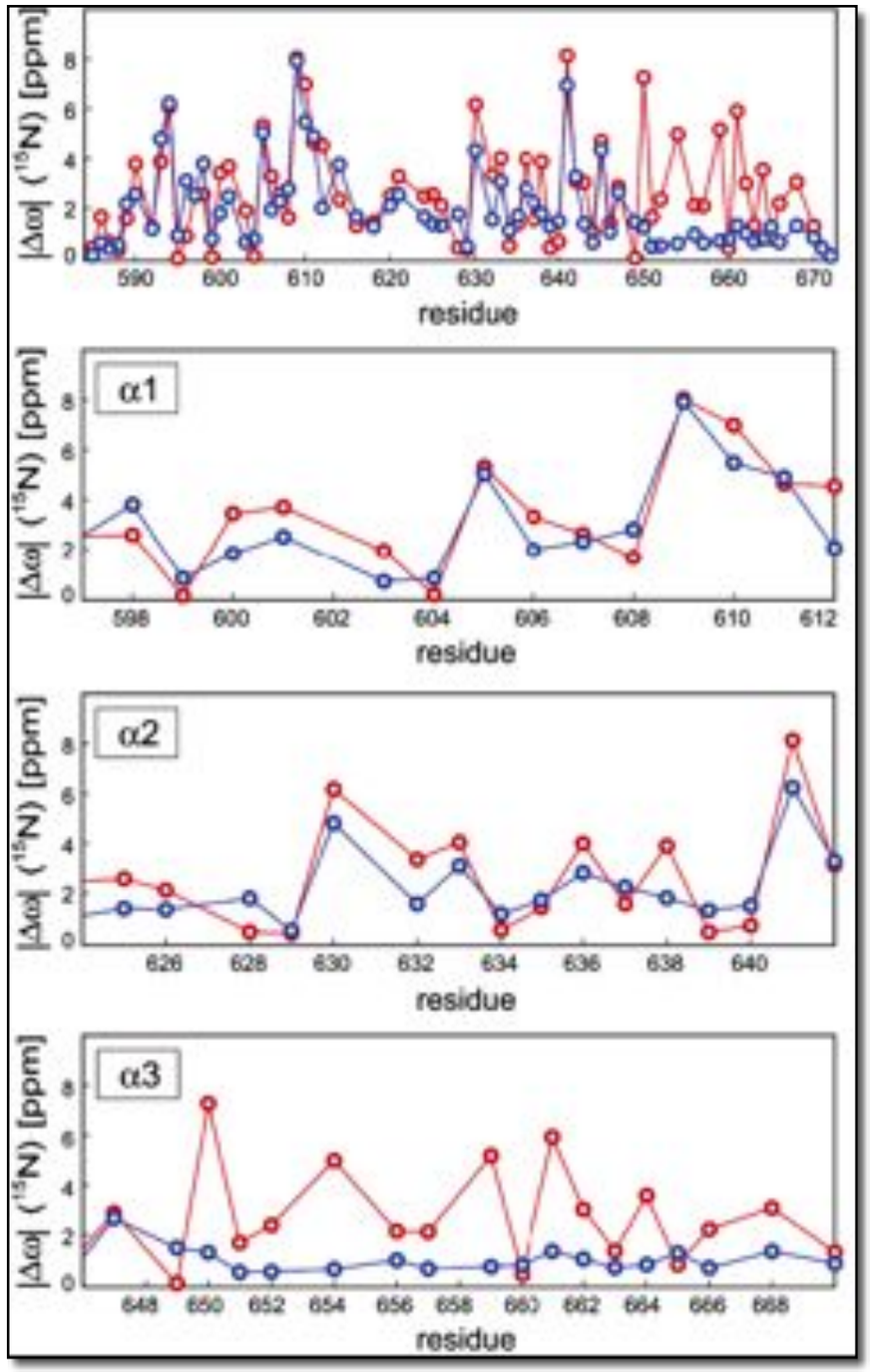
Boehr, Dyson and Wright (2006) Chem. Rev. 106, 3055-3079, *An NMR Perspective on Enzyme Dynamics*

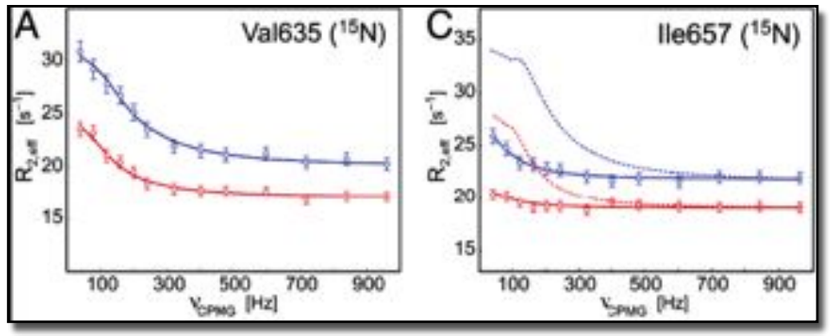
Hwang et al, *PNAS* **99**, 13561 (2002) ; *PNAS* **101**, 9618 (2004) *Solution structure and dynamics of the outer membrane enzyme PagP by NMR*. Boehr et al, (2006) Science, 106, 3055-3079, *The Dynamic Energy Landscape of Dihydrofolate Reductase Catalysis.* Henzler-Wildman et al. (2007) Nature 450, 838-844 *Intrinsic motions along an enzymatic reaction trajectory (Adenylate kinase)*. Eisenmesser et al. (2005) nature 438, 117-121. *Intrinsic dynamics of an enzyme underlies catalysis (prolyl cis–trans isomerase cyclophilin A (CypA)).*

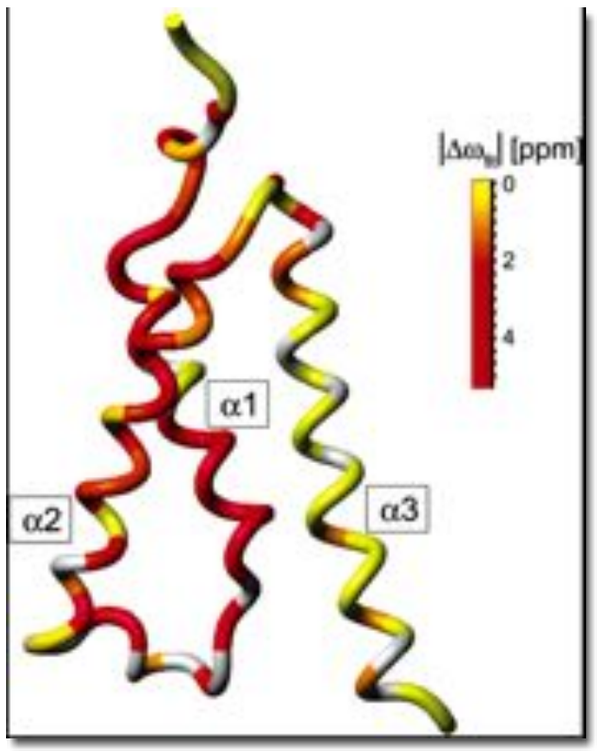
Hansen, Vallurupalli and Kay. J Biomol NMR (2008) 41:113–120, Using relaxation dispersion NMR spectroscopy to determine structures of excited, invisible protein states

Characterization of dynamic processes in the spectral timescale
Folding processes

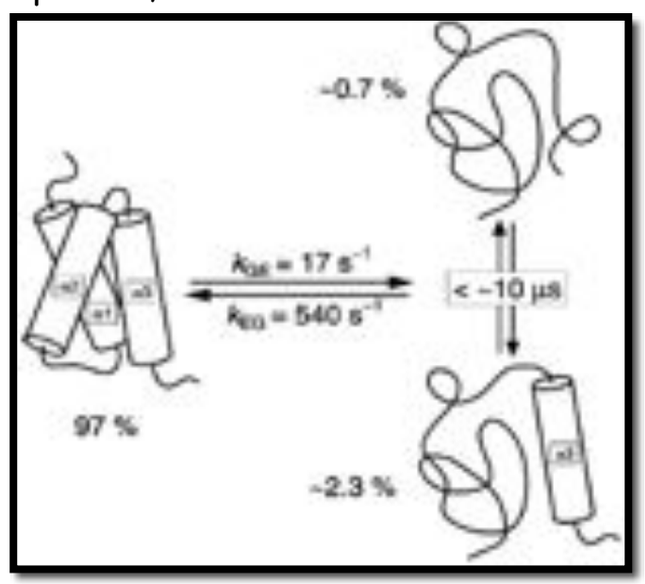
KIX domain of CPB







pH=5.5, T=300K

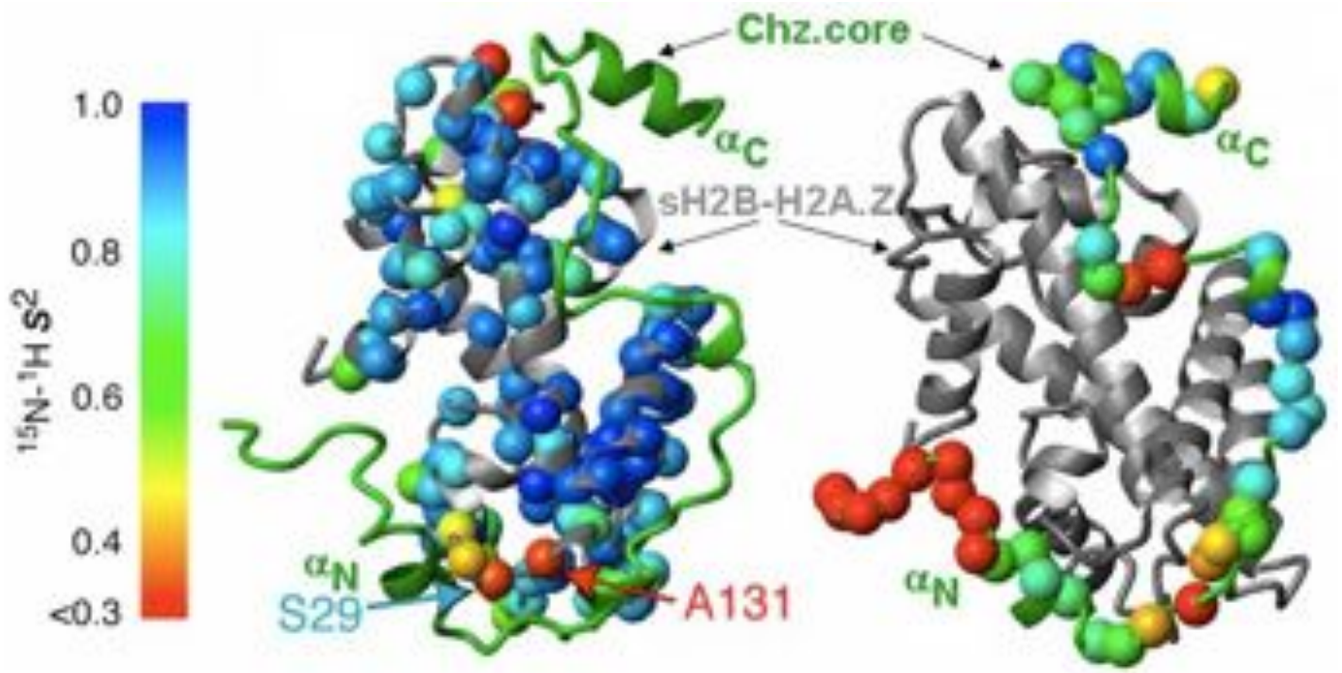


Tollinger et al., *Biochemistry* **2006,** *45,* 8885-8893

repliement au cours d'une interaction

Binding Kinetics of Histone Chaperone Chz1 and Variant Histone H2A.Z-H2B by Relaxation Dispersion NMR Spectroscopy

D. Flemming Hansen, Zheng Zhou, Haniqiao Feng, Lisa M. Miller Jenkins, Yawen Bai and Lewis E. Kay (2009) J. Mol.Biol. 387,1-9.



Zhou et al. Nat. Struct. Mol. Biol.(2008), 15(8), 868

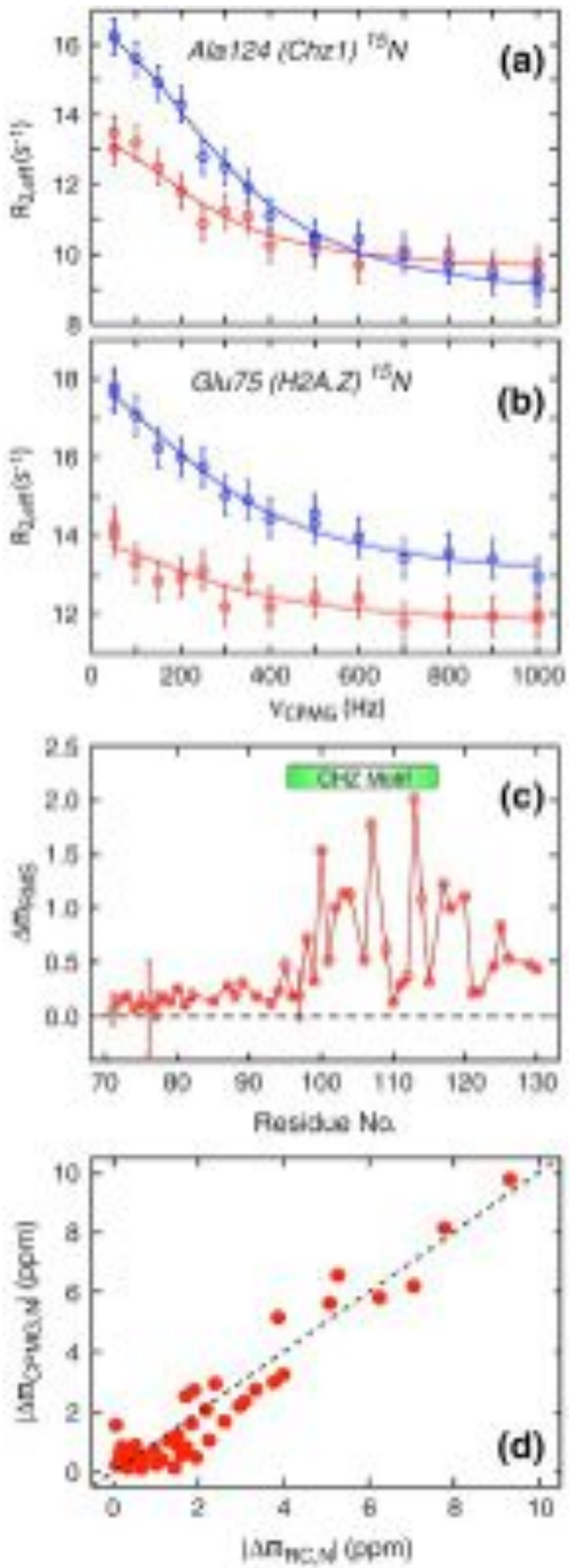
$$H2A.Z\text{-}H2B:CHz1 \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} H2A.Z\text{-}H2B + CHz1$$

$K_D = k_{off}/k_{on}$
(micromolar range)

Analyse de l'élargissement des résonances du complexe 1:1 du à l'échange avec les formes libres minoritaires (~1%) invisibles

Observation of H2A.Z-H2B : $k'_{on}=k_{on}[CHz1]$
Observation of CHz1 : $k''_{on}=k_{on}[H2A.Z\text{-}H2B]$

repliement au cours d'une interaction



$$H2A.Z\text{-}H2B:CHz1 \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} H2A.Z\text{-}H2B + CHz1$$

⇨ Les données de dispersion de relaxation (RD) sont compatibles avec un processus d'échange entre deux états.

⇨ Les variations de déplacements chimiques obtenus à partir des données de RD sont compatibles avec les $\Delta\omega$ connus entre les états libres et liés (NB. déplié pour CH1z libre)

⇨ Les données de RD se rapportent bien au processus de liaison.

⇨ Elles permettent une détermination précise des paramètres thermodynamiques et cinétiques.

k_{on} = $10^8 \pm 10^7 M^{-1}s^{-1}$ >> rapide que la limite de diffusion (~10^5-10^6 $M^{-1}s^{-1}$), probablement due à de fortes contributions électrostatiques.

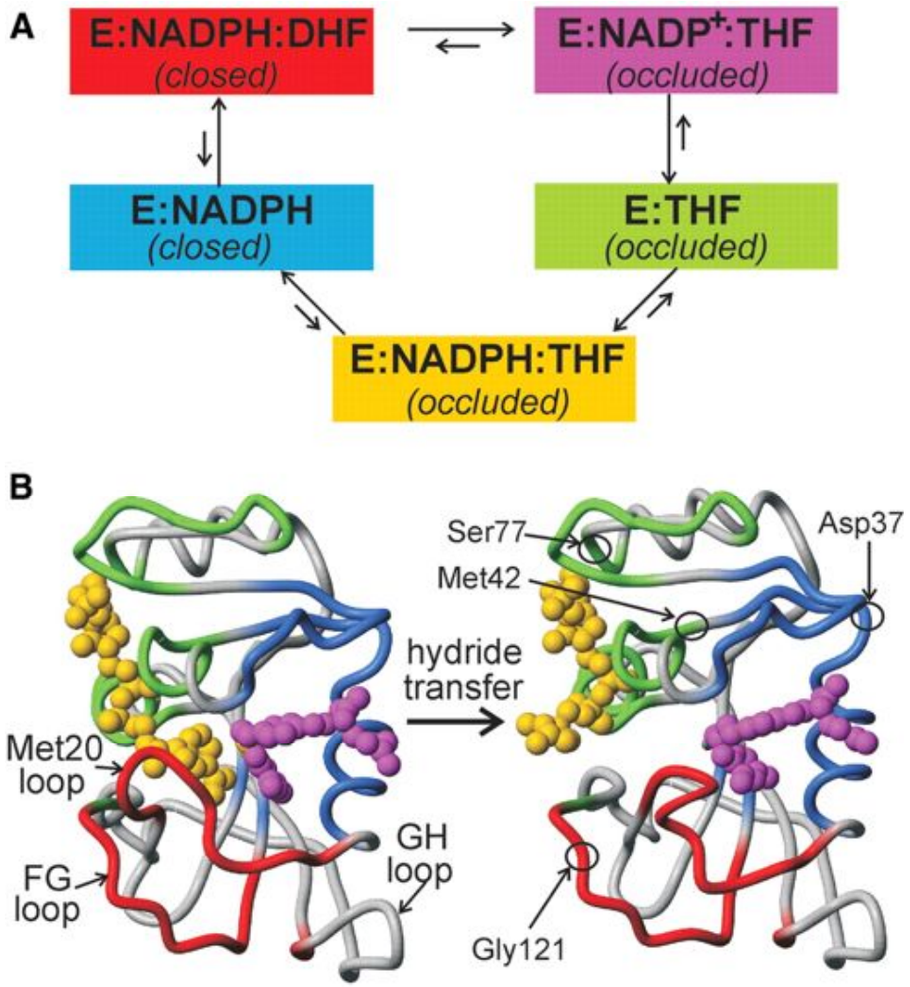
k_{off} = 22±2 s^{-1} : limite supérieure pour les vitesses à laquelle les histones H2A.Z-H2B sont fournies par la chaperone Chz1 au complexe de remodelage de la chromatine SWR1 (qui catalyse ensuite le remplacement de l'histone canonique H2A-H2B par H2A_Z-H2B)

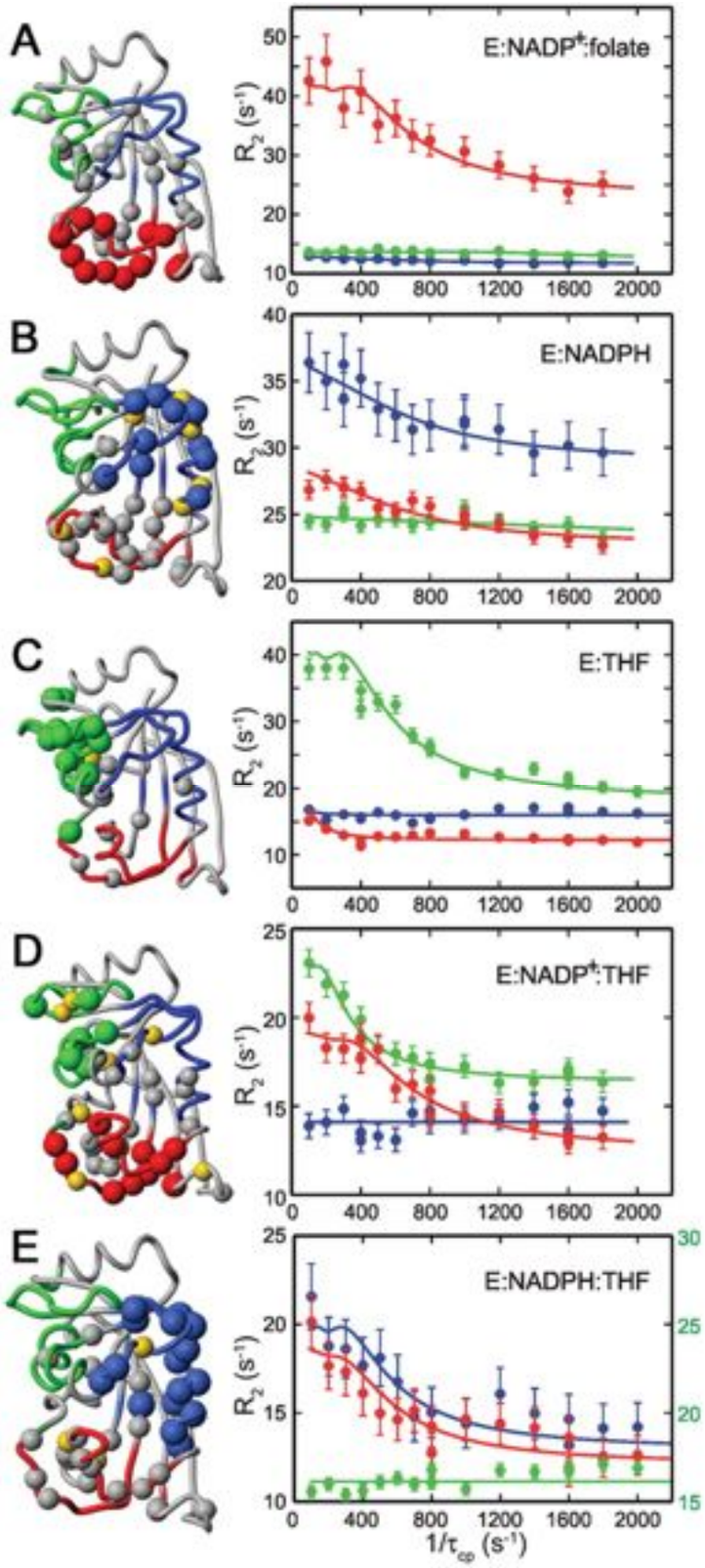
NB. Données à comparer avec la vitesse d'ouverture du nucléosome ~20-90s^{-1} determinée par FRET.

Sélection conformationelle

➪ Couplage mouvements - catalyse

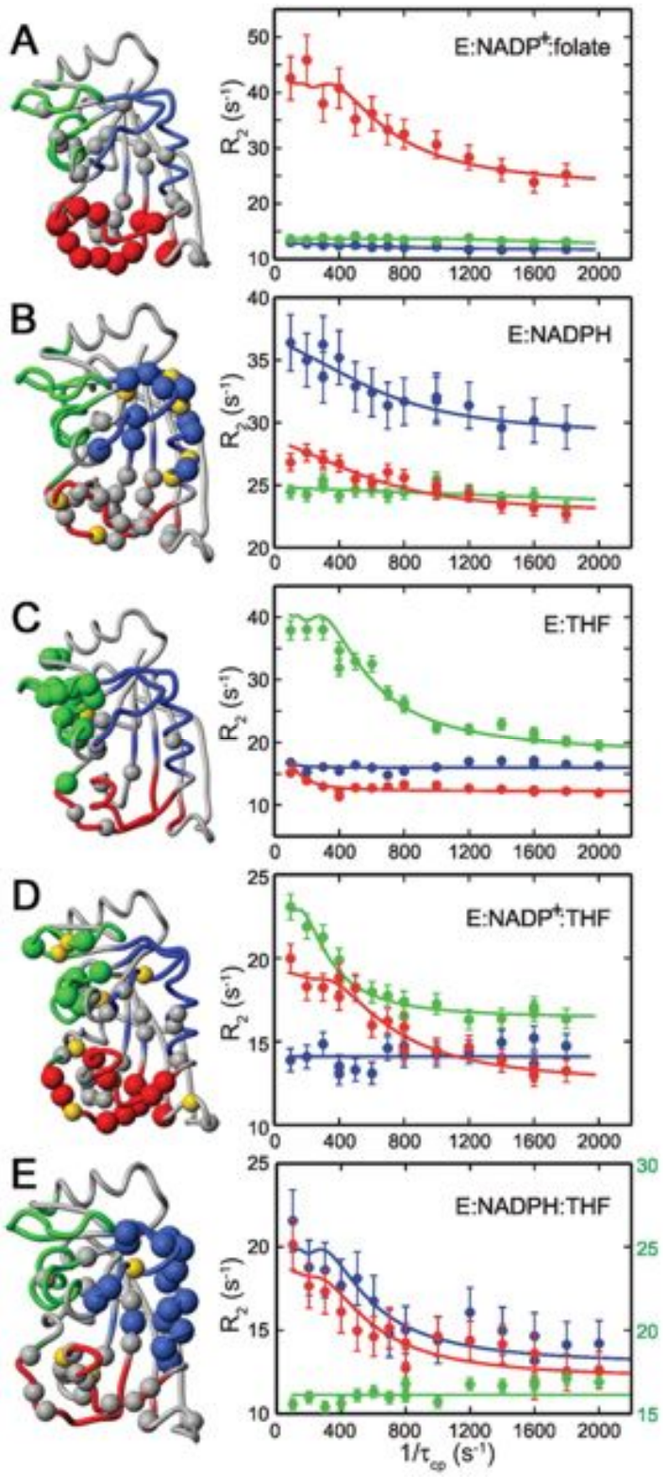
Le cas de la dihydrofolate réductase (DHFR)





Boehr et al, Science 2006, 106, 3055-3079

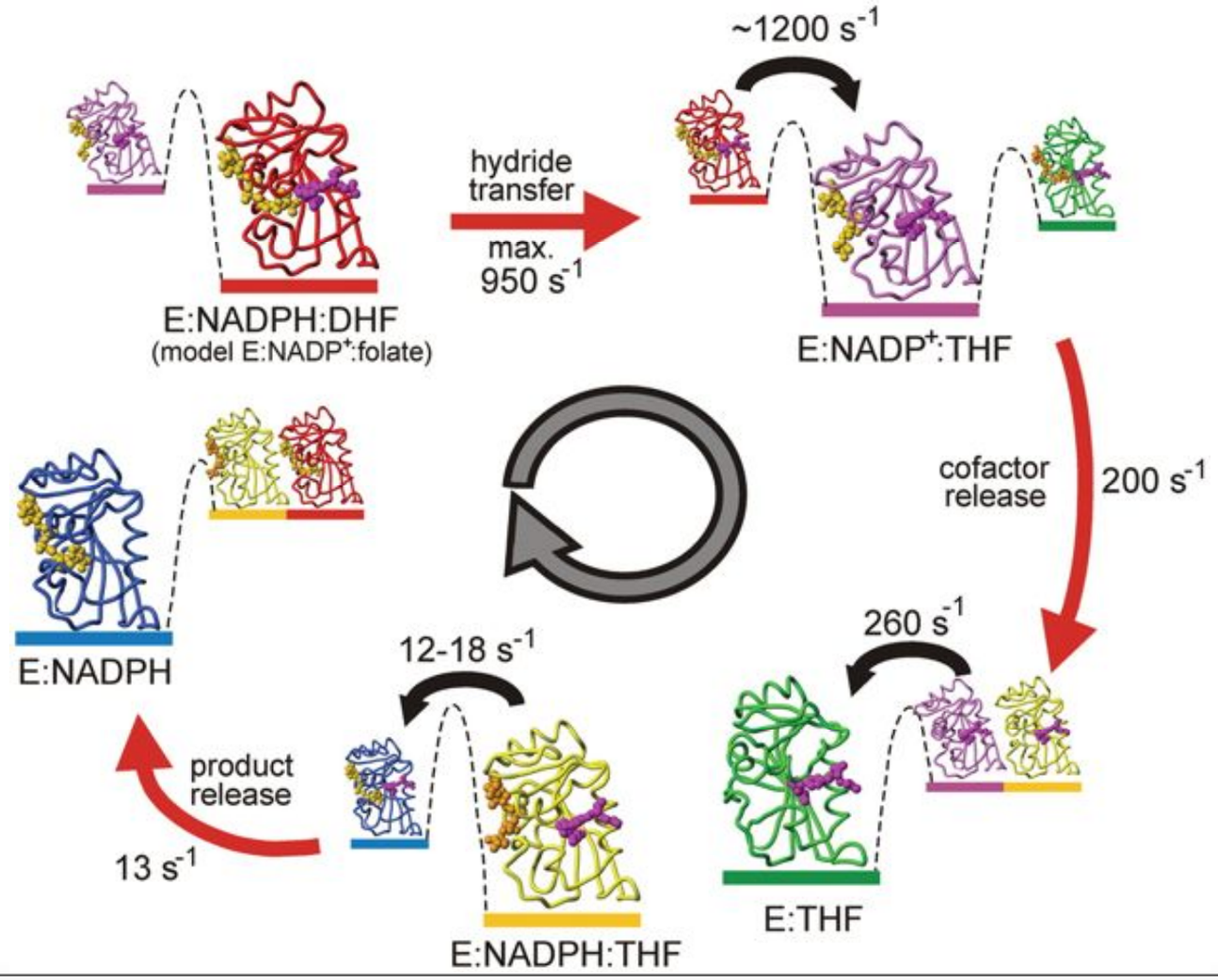
Sélection conformationelle



⇨ A chaque étape du cycle, la conformation majoritaire est en échange avec les états précédent et/ou suivant dans le cycle.

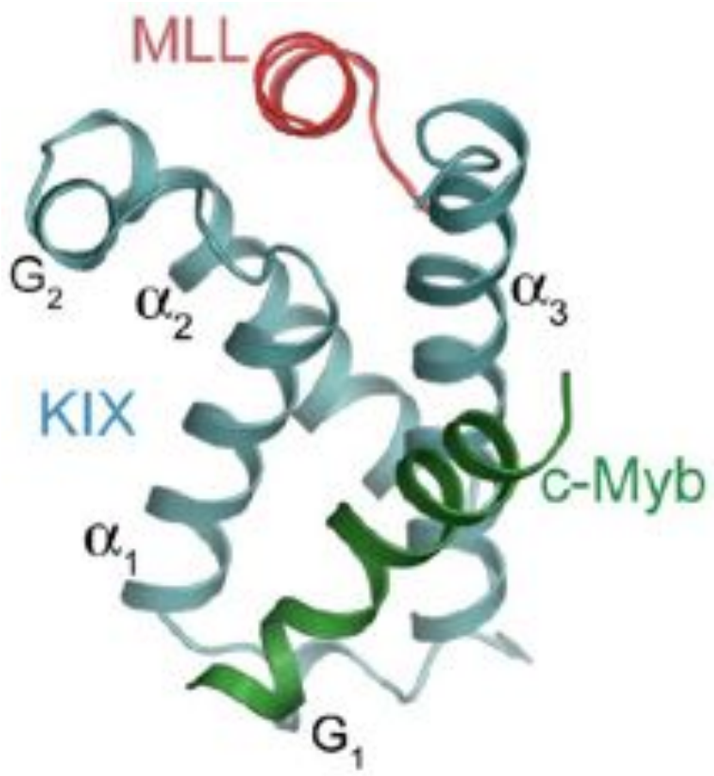
⇨ Constantes cinétiques et thermodynamique associées aux échanges sont compatibles avec les données enzymatiques.

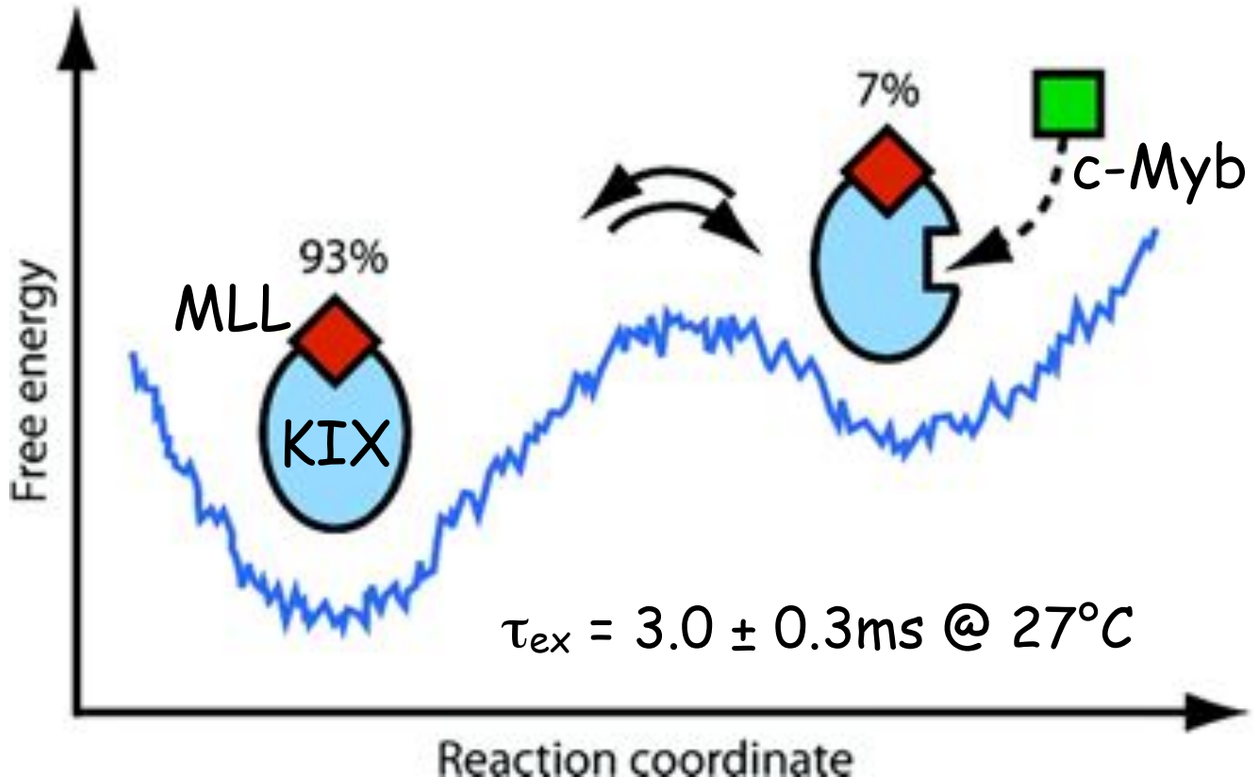
⇨ La vitesse de transfert de l'hydrure et les vitesses de recyclage à l'état stationnaire sont contrôlées par la dynamique des transitions entre les états fondamentaux et excités au cours du cycle.



Sélection conformationelle / allostérie

Direct Observation of the Dynamic Process Underlying Allosteric Signal Transmission





➪ 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

Sélection conformationelle / allostérie

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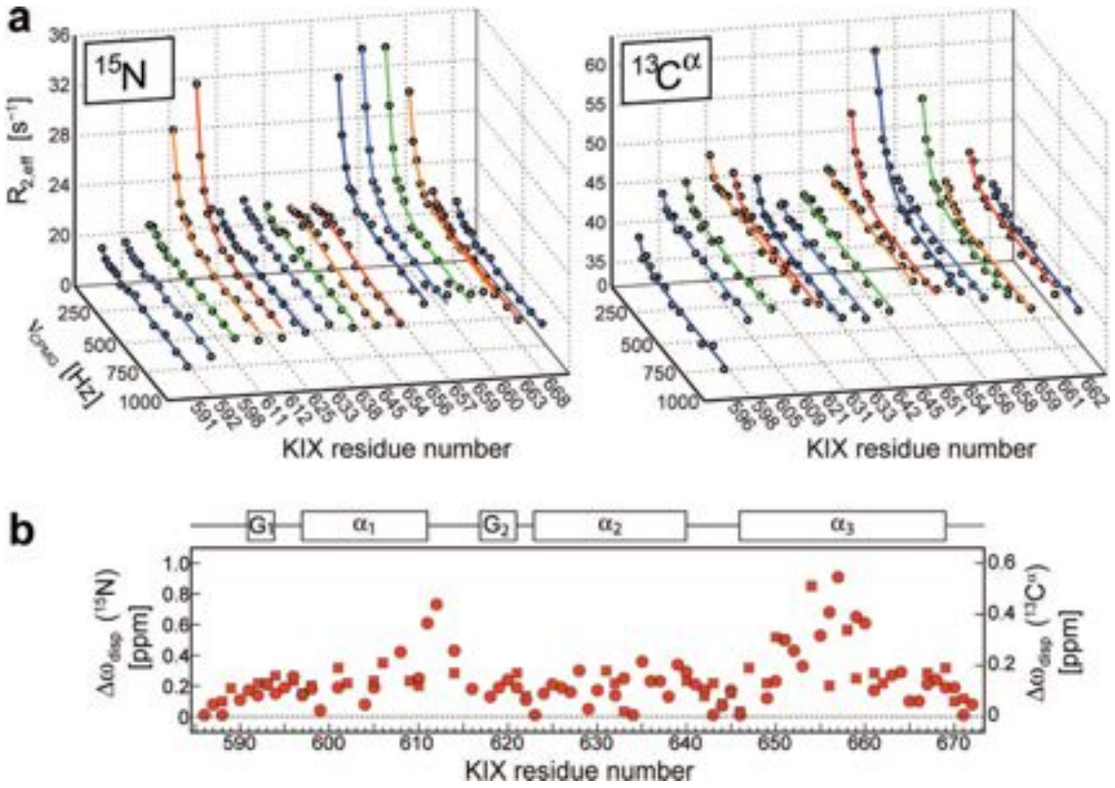
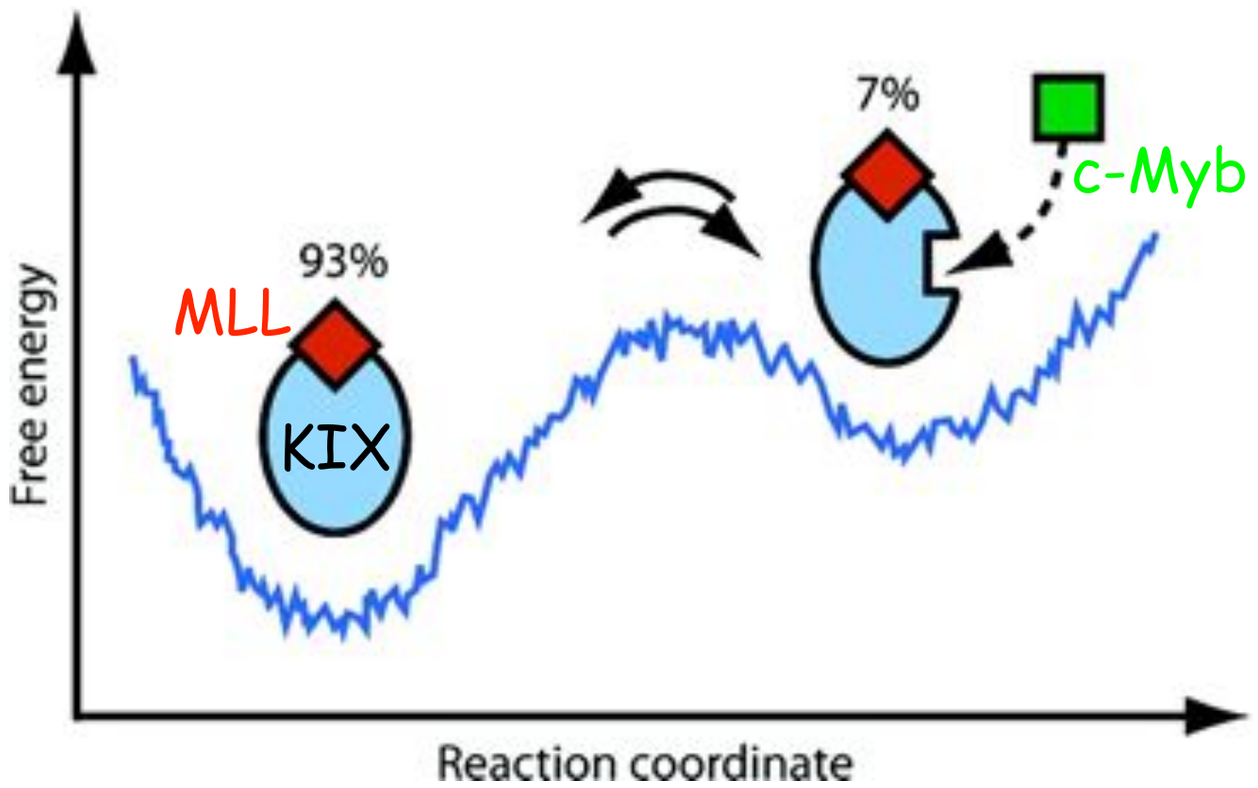
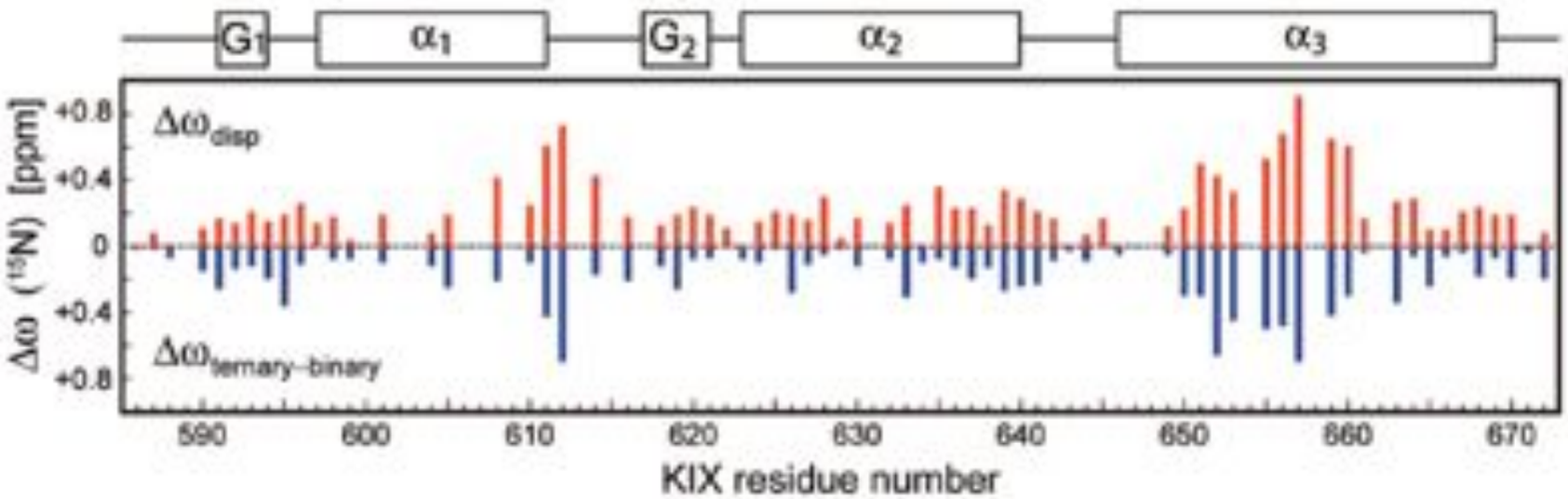
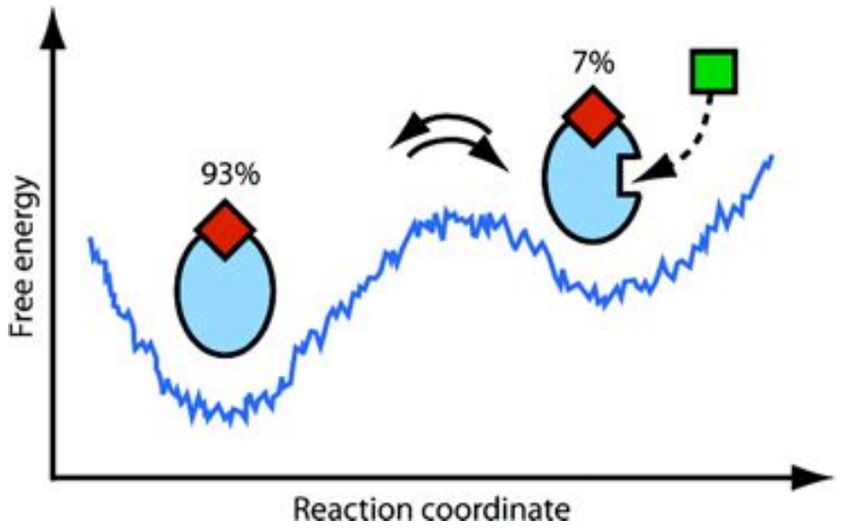


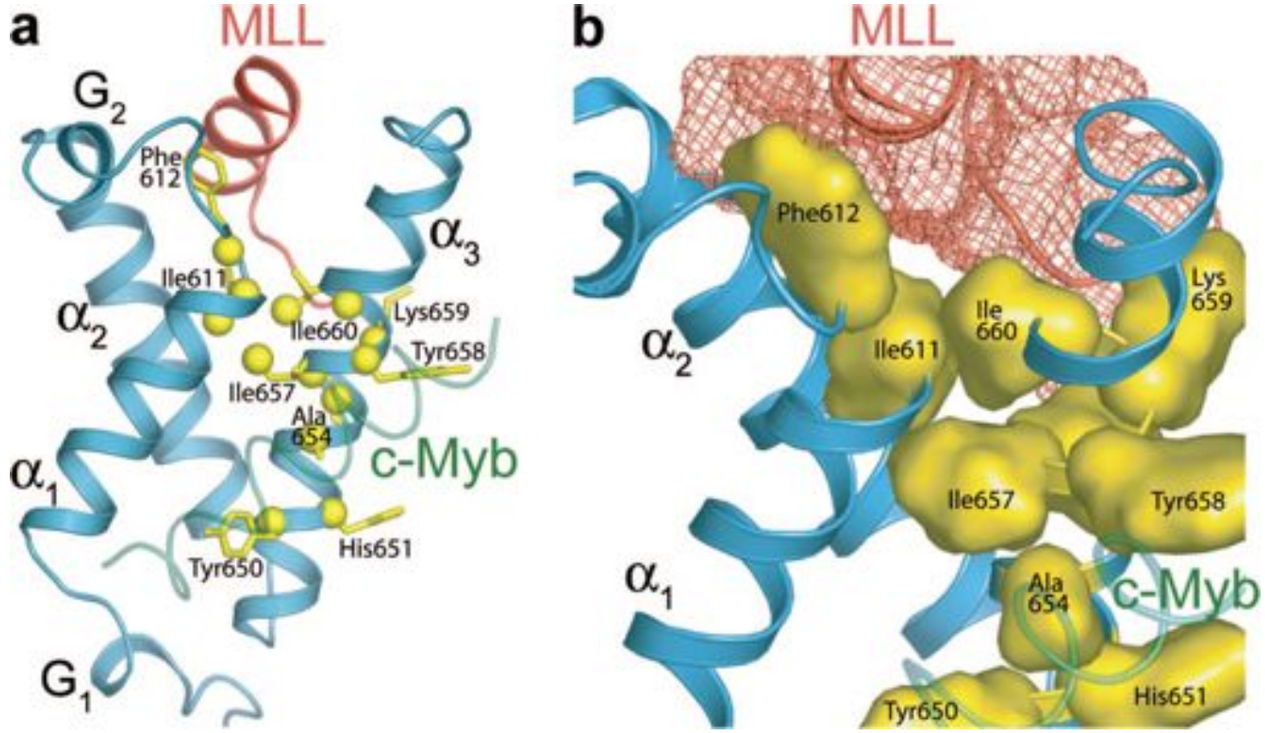




Sélection conformationelle / allostérie

Direct Observation of the Dynamic Process Underlying Allosteric Signal Transmission

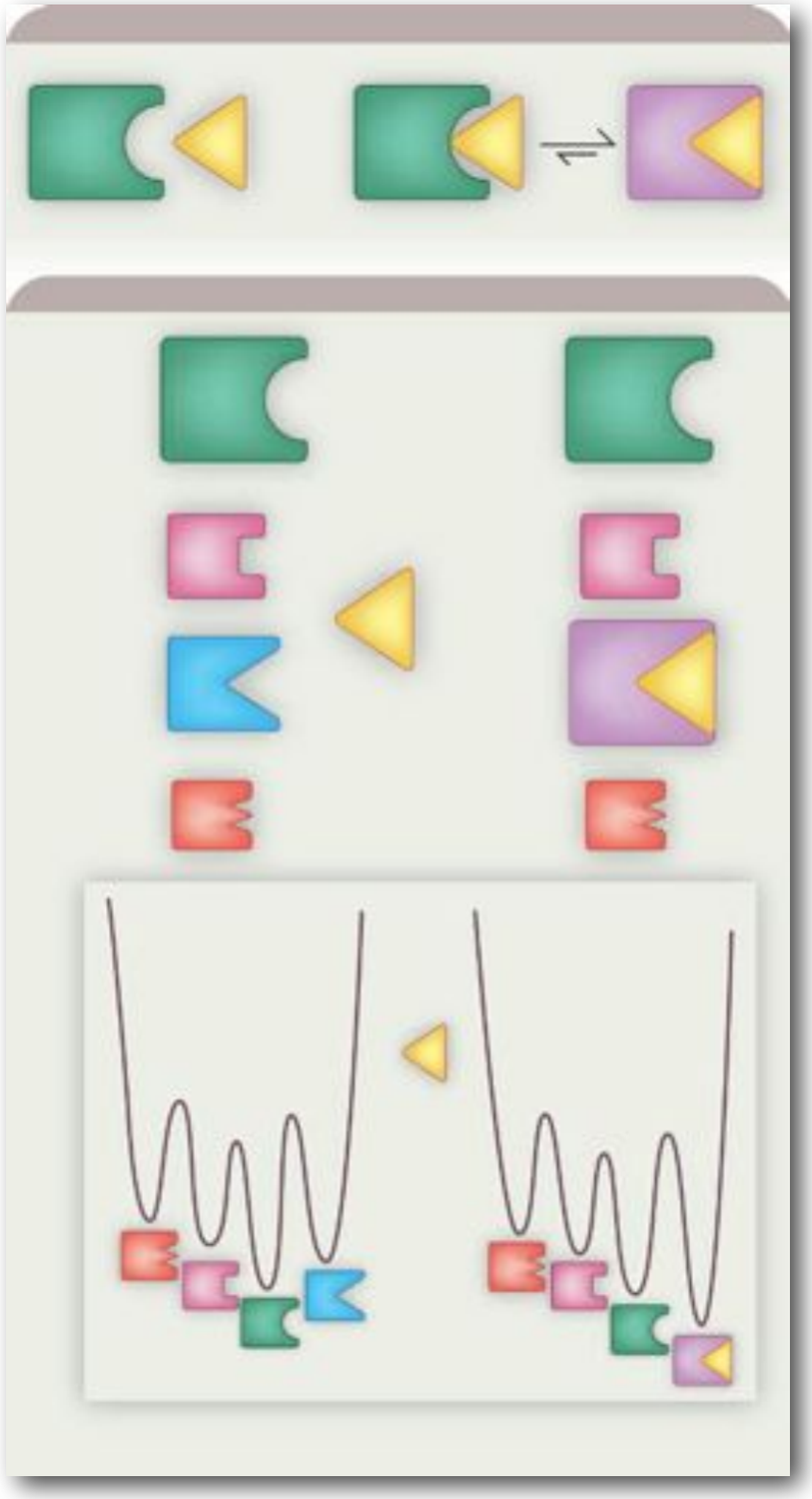


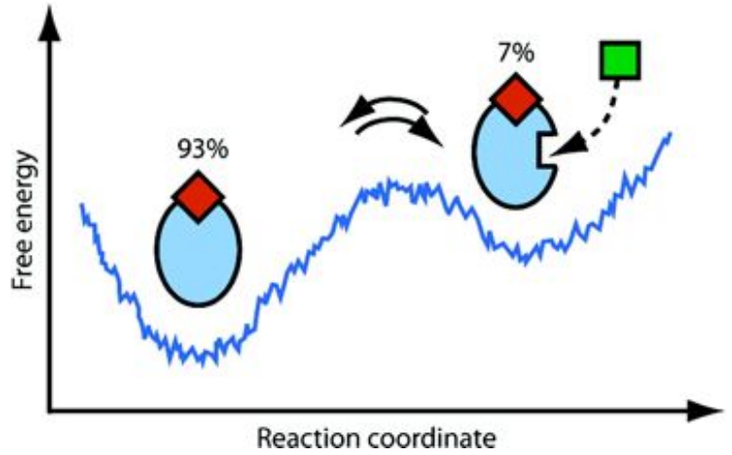


➪ Les chaînes latérales des isoleucines participent à la formation d'un réseau d'interactions qui constitue le «chemin» à travers lequel se transmet l'information allostérique.

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

Sélection conformationelle / allostérie





➪ Modulation des affinités selon le degré de peuplement du conformère de plus haute affinité.

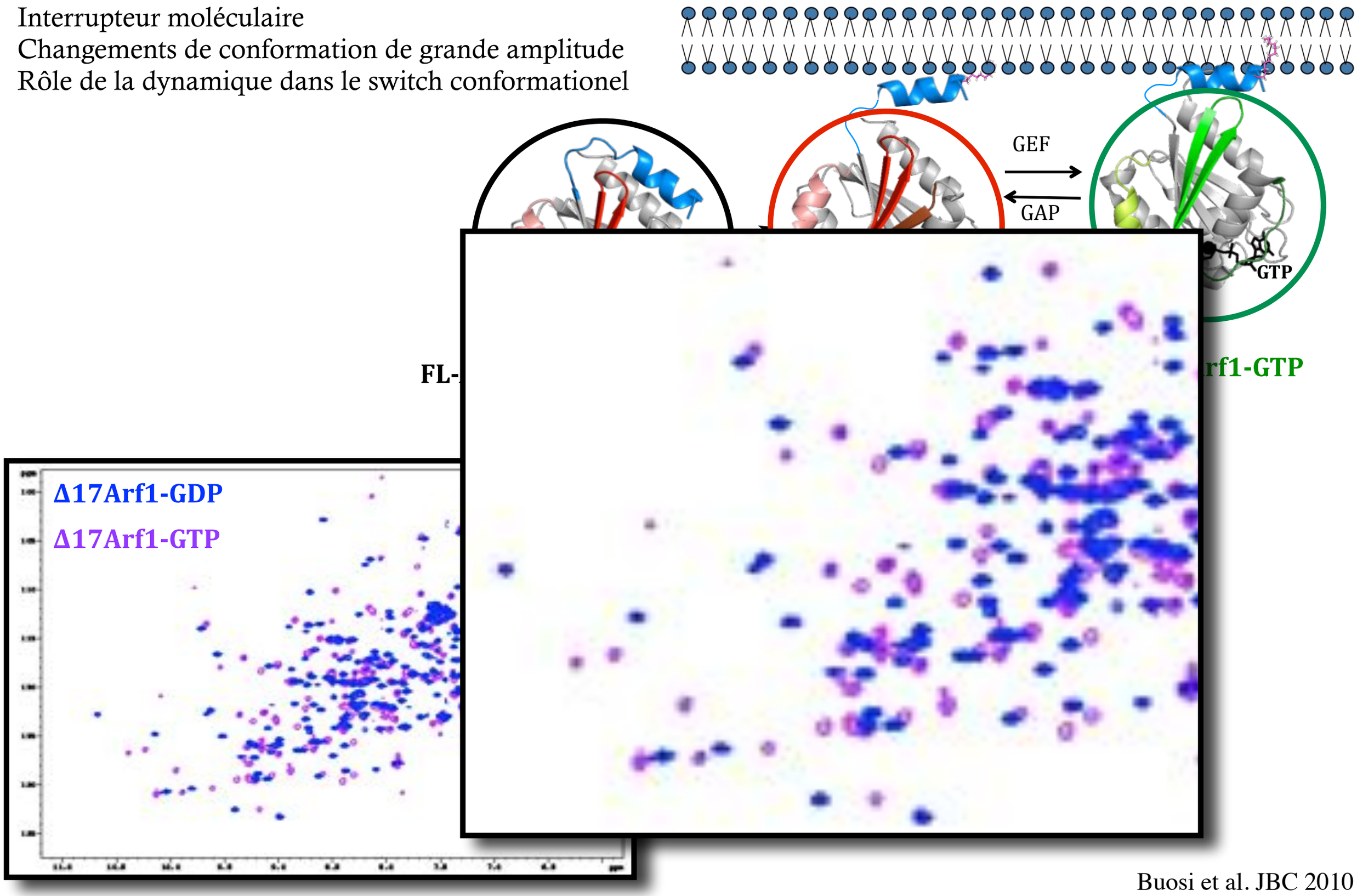
➪ Réglage fin des affinités peut être critique pour la régulation de la spécificité de la transcription. Plusieurs résultats indiquent que la coopérativité des interactions entre les facteurs de transcription (médiées par CBP) peuvent promouvoir une synergie dans l'activation transcriptionnelle.

➪ Moreover, the rate at which allosteric information is transmitted between binding sites might pose an essential constraint for the flow of information through the networks of proteins that regulate gene transcription in the cell.

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

Un exemple de l'apport de la RMN dans la relation dynamique/fonction: Le rôle de la dynamique dans le switch conformationel de petites protéines G

- Interrupteur moléculaire
- Changements de conformation de grande amplitude
- Rôle de la dynamique dans le switch conformationel



Buosi et al. JBC 2010

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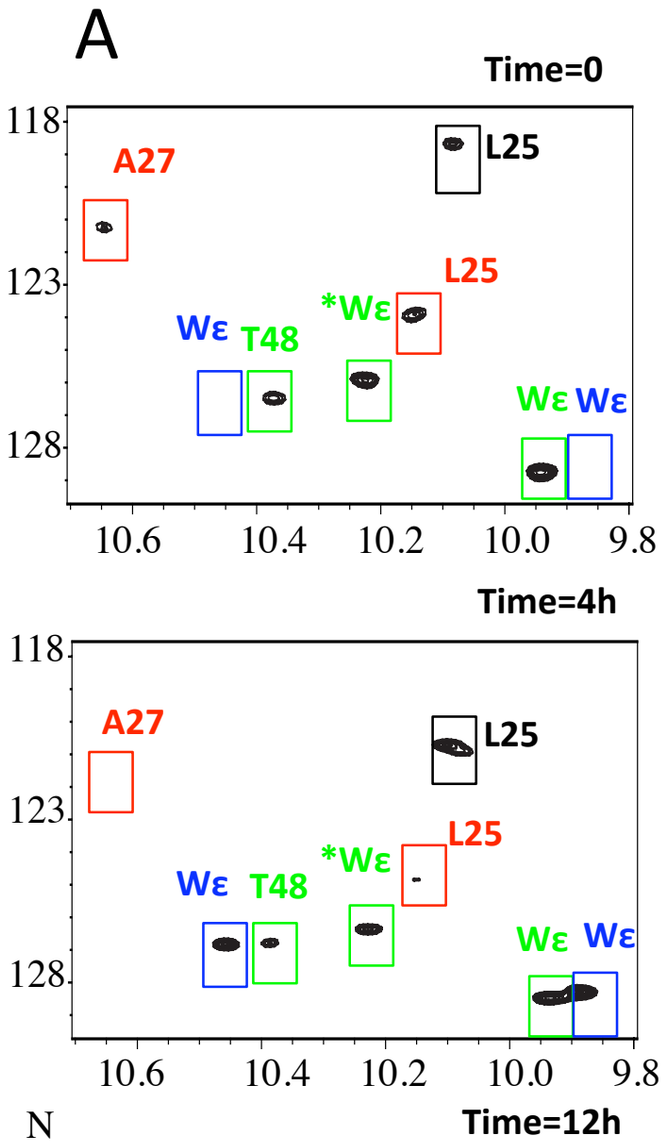

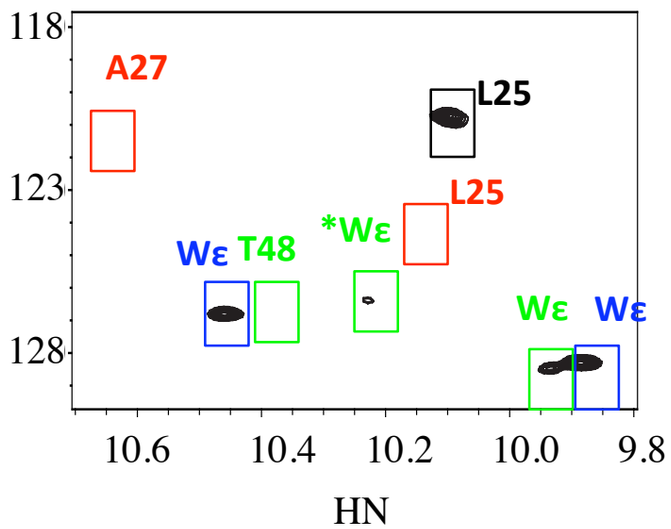

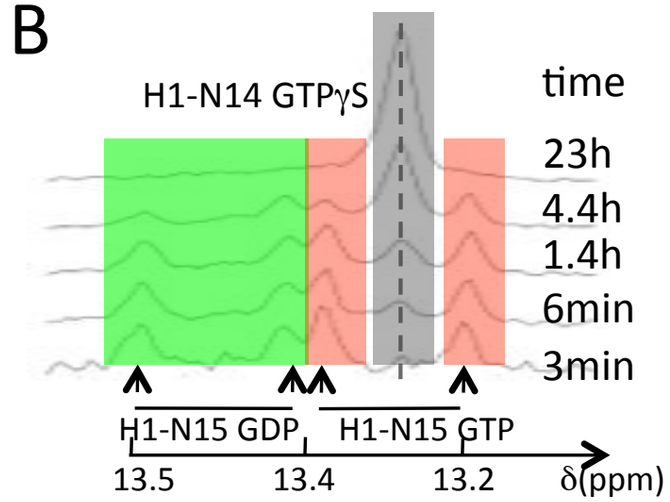

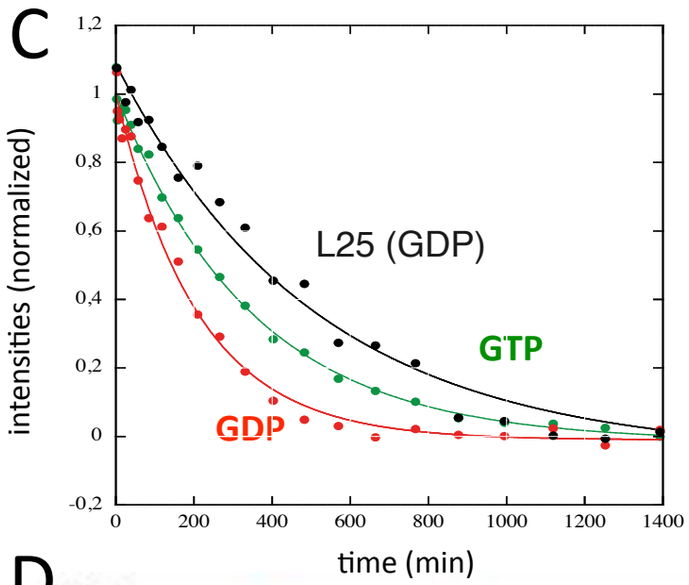

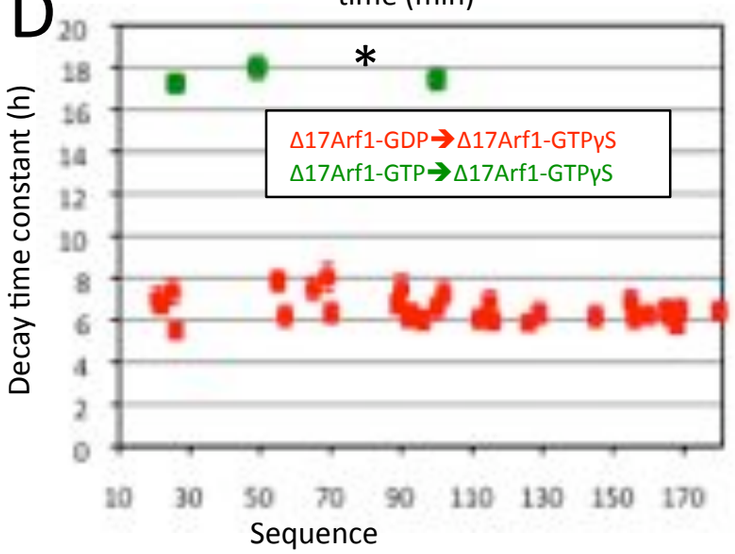

☞ Suivi de la cinétique de sortie du nucléotide par RMN et du changement de conformation associé en temps réel

$\{^{15}N\}$Δ17Arf1-GDP/GTP

↓ ^{14}N GTPγS (large excès)

$\{^{15}N\}$Δ17Arf1-$\{^{14}N\}$GTPγS

⇨ *Le changement de conformation associé à l'échange du GDP est plus lent que la vitesse de sortie du nucléotide*

⇨ Existence de formes intermédiaires lors du switch

Un exemple de l'apport de la RMN dans la relation dynamique/fonction: Le rôle de la dynamique dans le switch conformationel de petites protéines G

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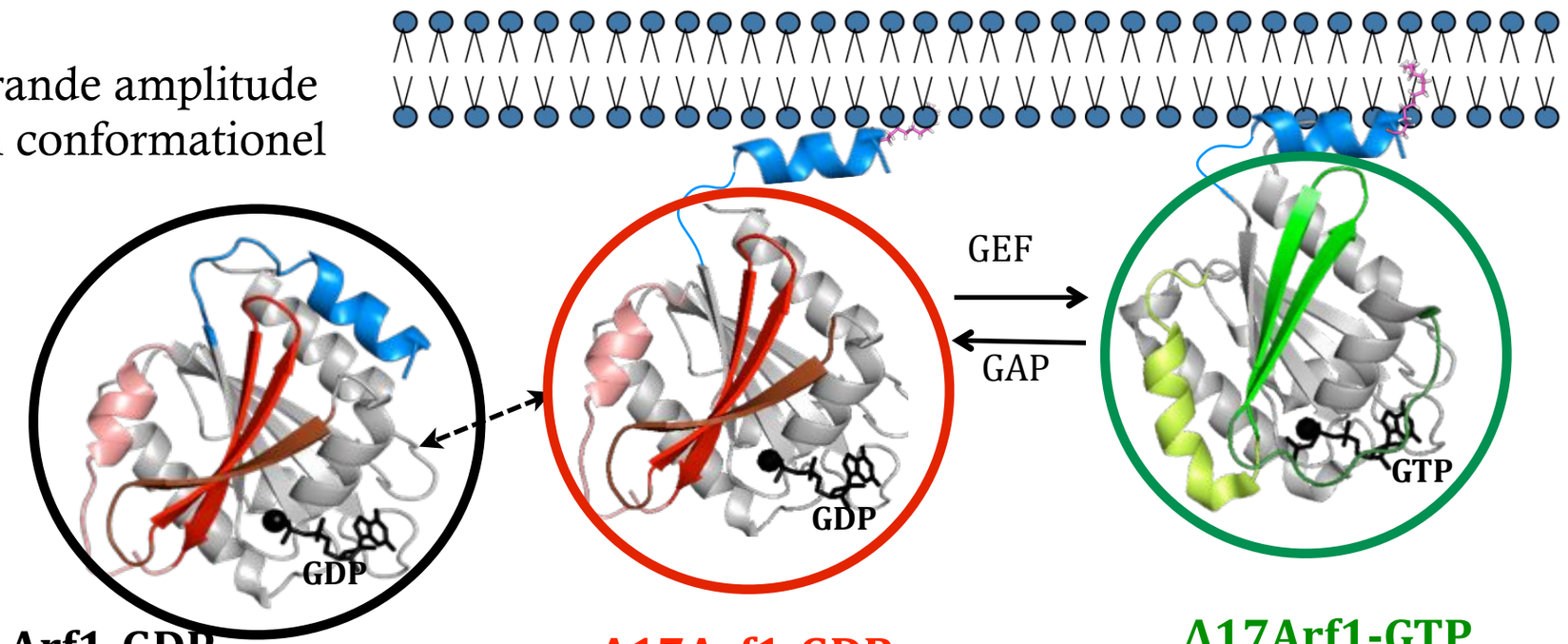
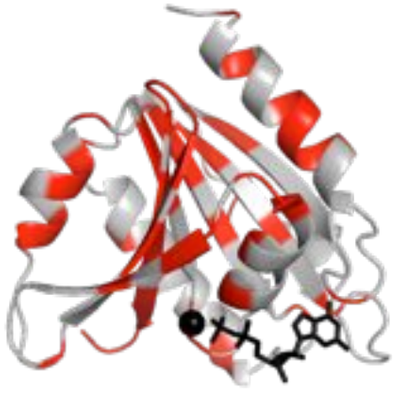
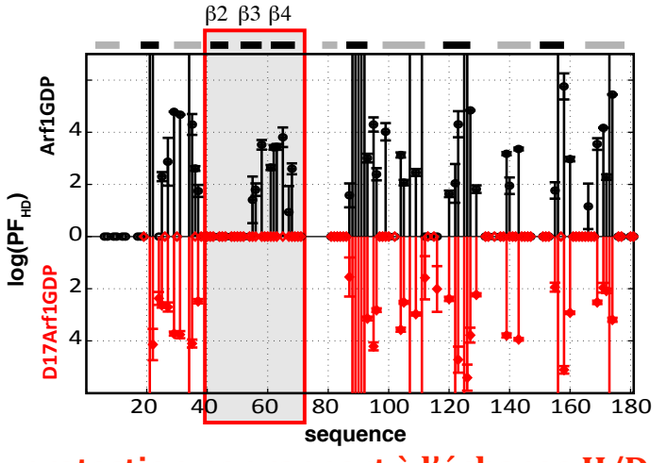


Dispersion de relaxation (µs-ms)



échange µs-ms dans Δ17Arf1-GDP

Echange $^1H<->^2H$ (mn-h)



protection par rapport à l'échange H/D dans Δ17Arf1-GDP et FL-Arf1-GDP

⇨ *Plasticité particulère de la forme Δ17Arf1-GDP*

⇨ *Mouvements concertés le long de la protéine lors de l'ouverture de l'hélice N-ter permettant le switch conformationel.*

⇨ *Ouverture du feuillet β dans la zone de switch*

Buosi et al *J. Biol. Chem.* **285**(49):37987-94.