Deuxième école RMN du GERM

Cargèse 2008

Analysis of micro-millisecond timescale motions of macromolecules in liquid state by Nuclear Magnetic Resonance

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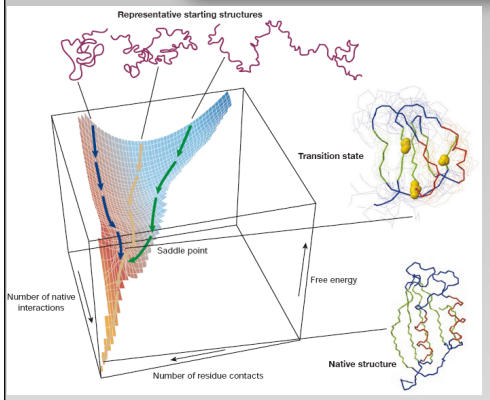
A view of proteins multiple states



Folding

3D structure

« lock & key »
induced fit

α ↔ β

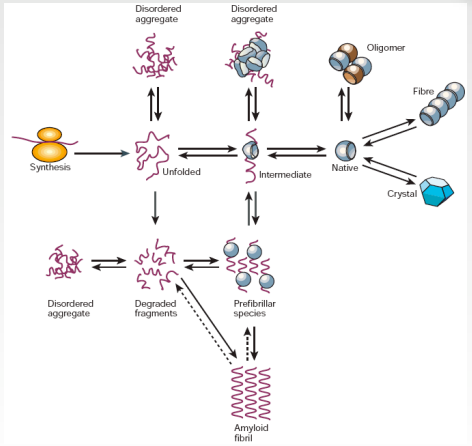
conformational switch

order → disorder

virus/pathogen penetration
membrane insertion
Nucléosome activation

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

Techniques to monitor protein dynamics

X-ray cristallography	B factors	time scales static disorder, crystal contacts, ...
X-Ray, neutron scattering Doniach, *Chem. Rev.* 2001, **101** ; Zacai, *science* 2000, **288**.	size/shape modifications timescales (ps-ns) for ^{1}H positions	
Fluorescence Weiss, *Nat. Struct. Biol.* 2000, **7** ; Yang, *Science* 2003, **302** ; Haustein, *Curr. Opin. Struct. Biol.* 2004, **14.**	ensemble / single molecule cellular context	probes
Mass Spectroscopy (HX MS) radical footprinting Wales, *Mass. Spectrom. Rev.* 2006, **25** ; Busenlehner, *Arch. Biochem. Biophys.* 2005, **433**. Guan, *Trends. Biochem. Sci.* 2005, **30**.	large moldecular assemblies	
Mössbauer, Raman, 2D infrared spectroscopy		
Molecular dynamics		Forcefields ... Short timescales ...
NMR Boehr, *Chem. Rev.* 2006, **106**,3055. Palmer, *Chem. Rev.* 2004, **104**, 3623.	⇨ $10^{-12} \leftrightarrow 10^{5}$ s ⇨ Site-specific information ⇨ multiple atomic probes ^{1}H, ^{2}H, ^{15}N, ^{13}C, ^{31}P, ... ⇨ Simultaneous monitoring of probes ⇨ kinetic & termodynamic profile of dynamic processes	⇨ isotope labeling ⇨ quantities ⇨ size limitation ⇨ complexity of the method ?

Bibliography

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Experimental aspects, applications

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D. Shaw Fourier transform NMR spectroscopy Elsevier 1984

K. Wüthrich* NMR of proteins and nucleic acids. Wiley interscience 1986

How motions are visible in NMR ?

Molecular motions influence NMR parameters

Time scale of motions compared to NMR characteristic timescales

⇨ **Three characteristic NMR timescales**

Return of the spin system to equilibrium : T_1 / NMR signal lifetime : T_2

NMR experiment <-> spins system perturbation

Liquid state: T_1 ~ 100ms ↔ s ; T_2 ~ 10ms ↔ s

⇨ Minimal frequencies of motions that can be characterized during a single NMR experiment.

Spectral timescale : $\tau=1/\Delta\nu$

spectral features : chemical shifts range, couplings, … Hz ↔ kHz

⇨ Averaging of interactions by motions faster than the spectral dispersion due to these interactions.

⇨ Perturbation of spectral features by motions in the same range than the spectral timescale.

Larmor timescale : ω_0

precession frequency of spins in a magnetic field ($B_0=\omega_0/\gamma$)

⇨ transitions efficiency between spin states is determined by molecular fluctuations (spectral densities fo motions) at these frequencies.

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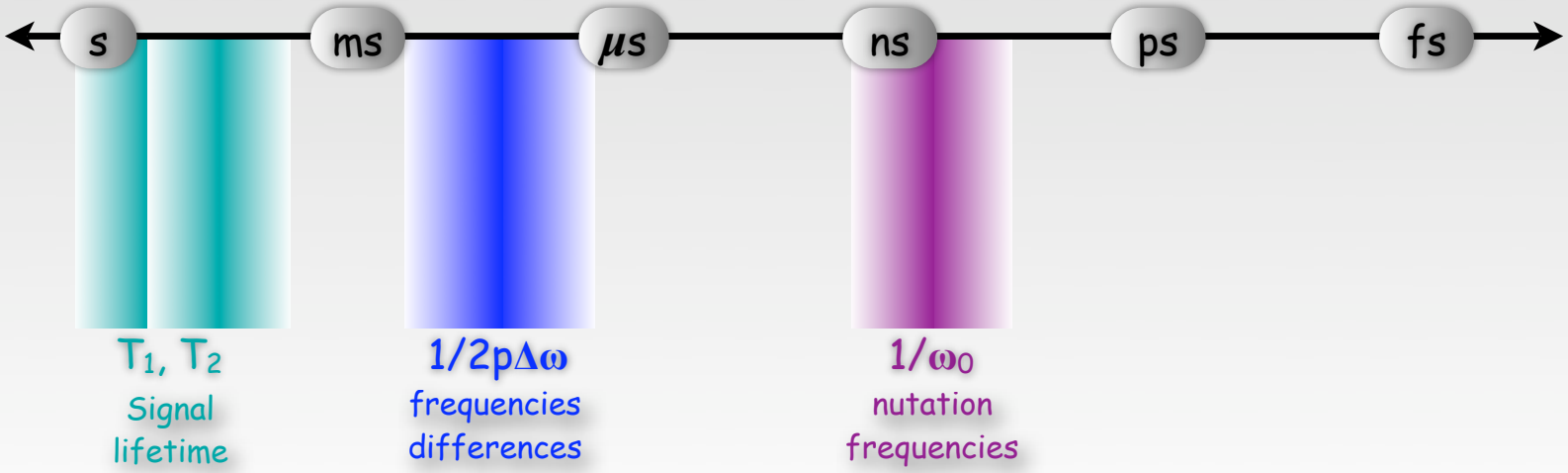
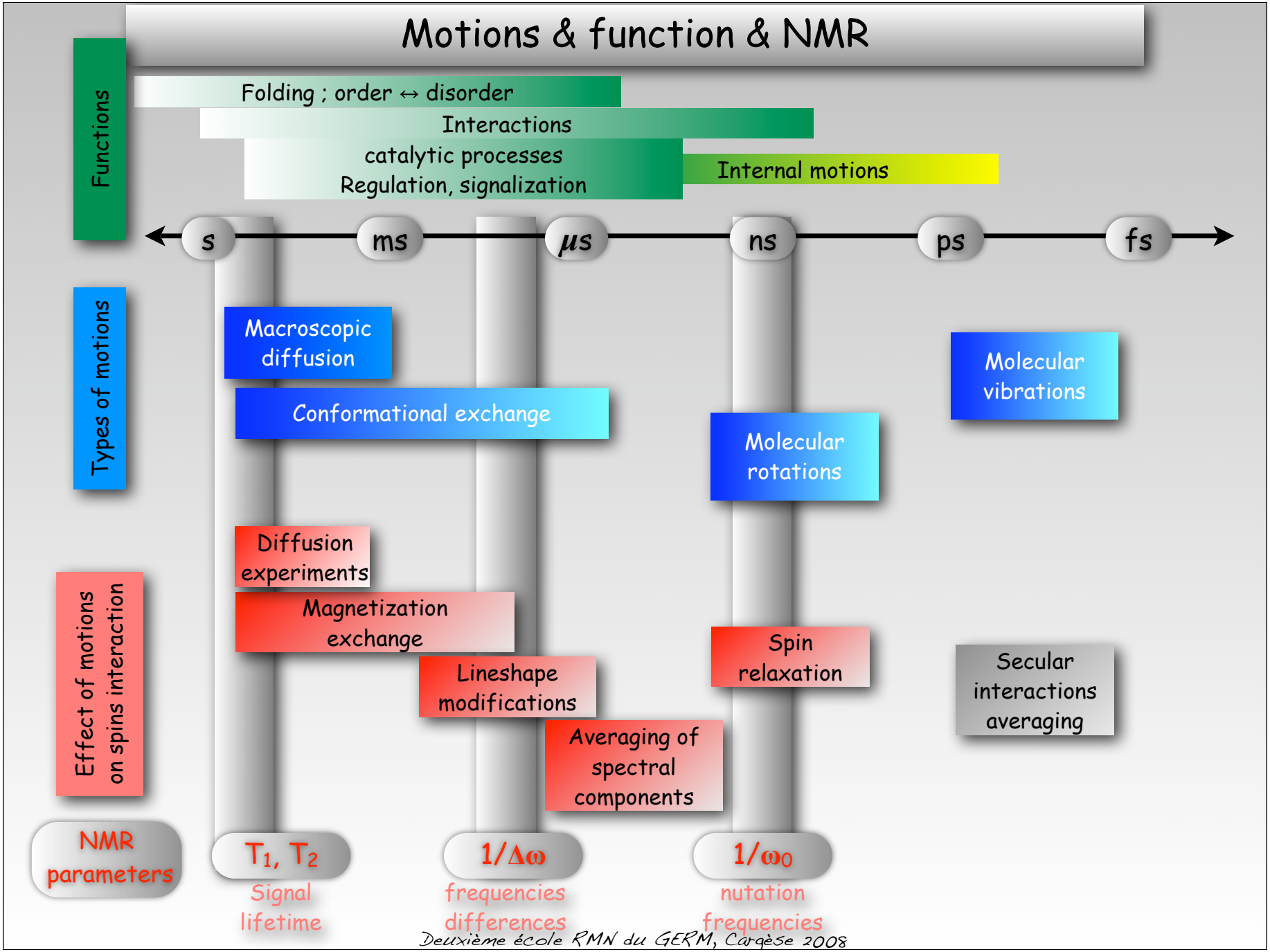
Spectral timescale : $\tau=1/\Delta\nu$

Larmor timescale : ω_0




Motions & function & NMR
Functions
Folding ; order ↔ disorder
Interactions
catalytic processes
Regulation, signalization
Internal motions
s
ms
µs
ns
ps
fs
Types of motions
Macroscopic diffusion
Conformational exchange
Molecular vibrations
Molecular rotations
Effect of motions on spins interaction
Diffusion experiments
Magnetization exchange
Lineshape modifications
Averaging of spectral components
Spin relaxation
Secular interactions averaging
NMR parameters
T1, T2
Signal lifetime
1/Δω
frequencies differences
1/ω0
nutation frequencies
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NMR timescales -1- Longitudinal relaxation time T_1

✓ T_1 characterizes the time for a spin system to reach equilibrium.

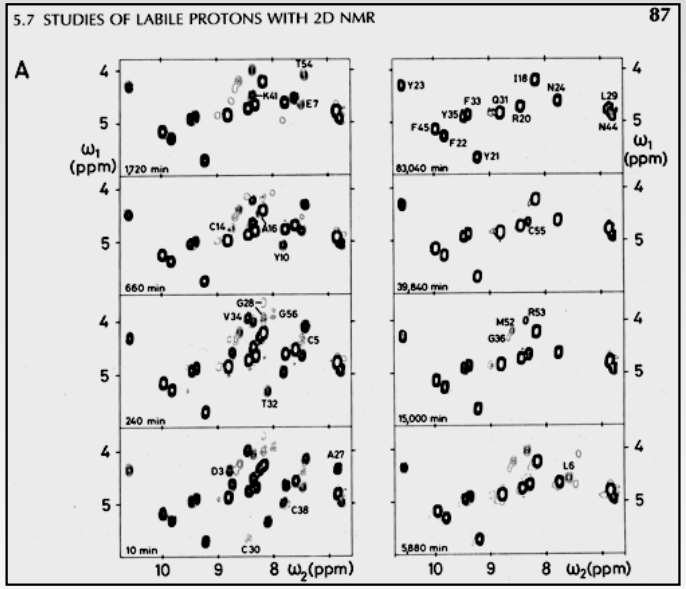
✓ Defines interscan delay

✓ Motions slower than T_1 cannot be characterize by a single spectra.

✓ NB. T_1 depends on magnetic field B_0, nature of spins, molecule size, local flexibility, temperature, etc.

Dynamical processes slower than T_1

- Real time folding
- Transient / out of equilibrium experiments
- Protein - ligand interactions
- H-N <-> D-N exchange rate constants



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

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Dynamical processes slower than T_1

- Real time folding
- kinetic / out of equilibrium experiments
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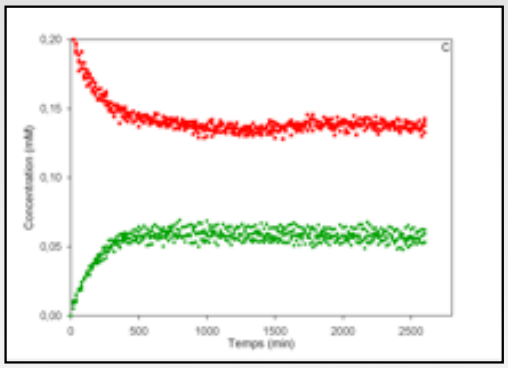
- H-N <-> D-N exchange rate constants

$$holoP1 + apoP2 \rightleftarrows apoP1 + holoP2$$



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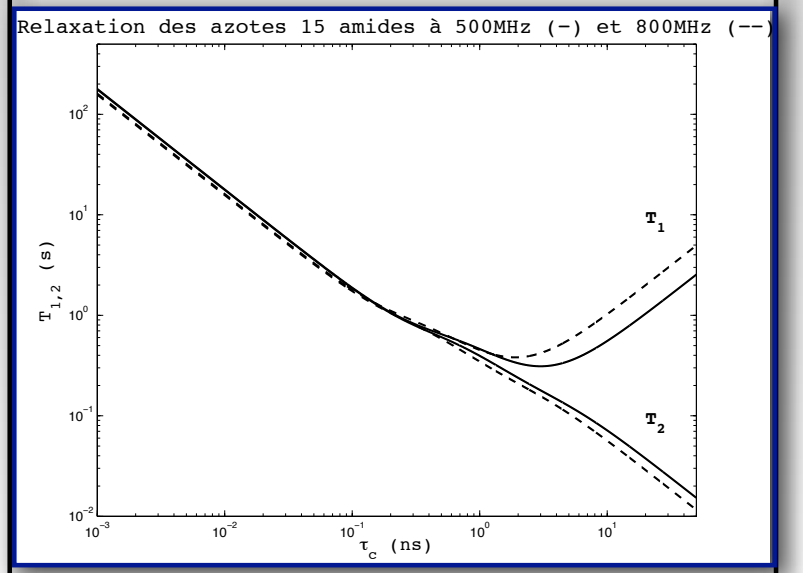
magnetic field, molecular size and correlation time dependance of T_1 and T_2



NMR timescales -2- Spectral or "chemical shift" timescale

✓Defined by the observed spectral width

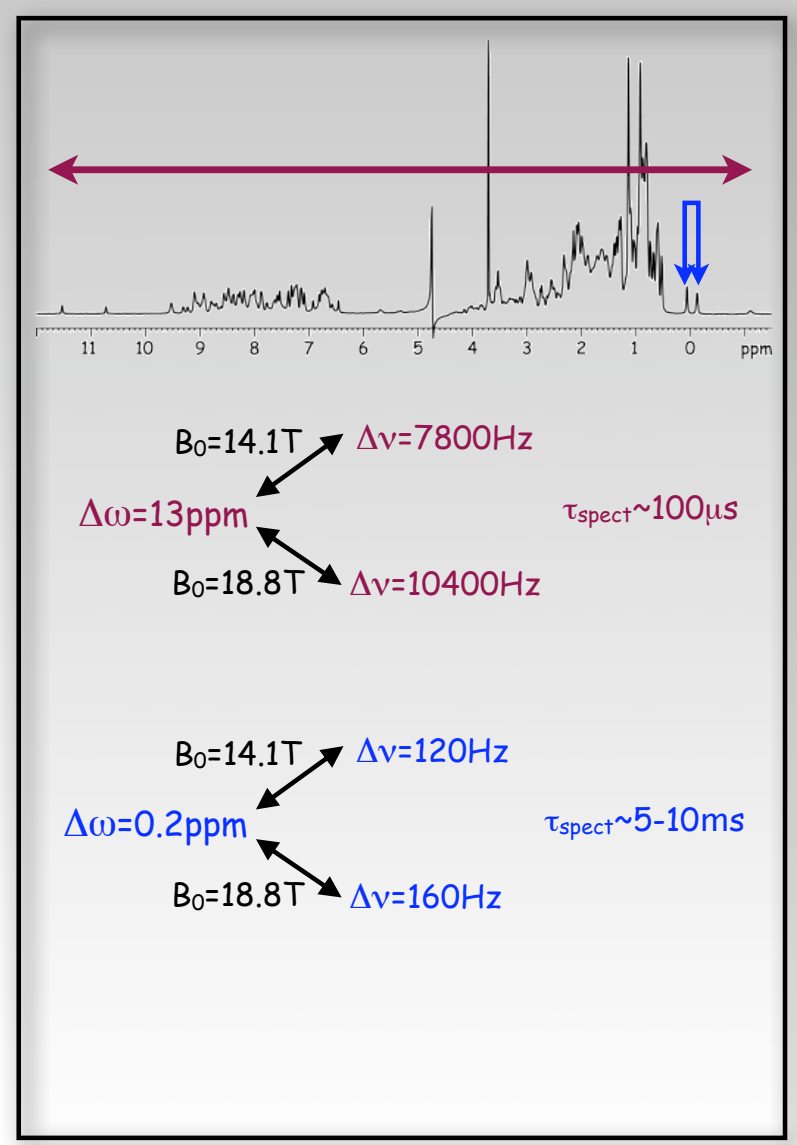
✓More precisely by the difference of resonance frequency between two spins from two different nuclei or from the same nuclei in two different states.

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

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

✓ Motions slower than $\Delta\nu$ have no effect on spectral feature

✓$\Delta\nu$ depends on magnetic field and on spins nature



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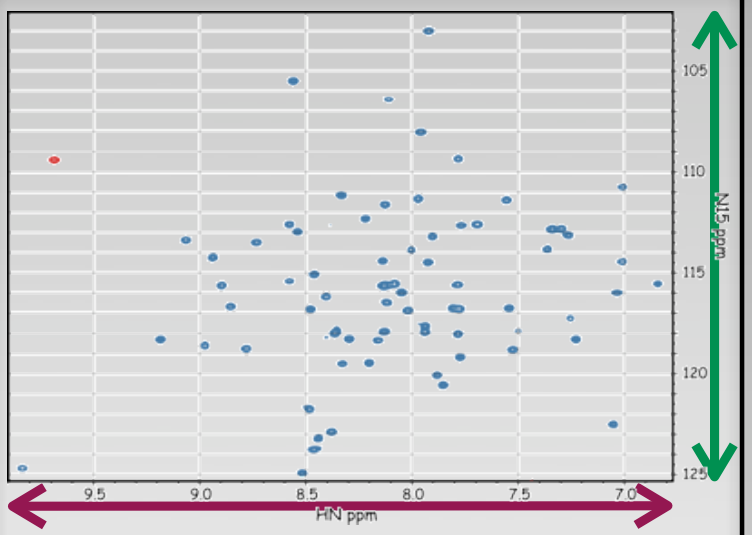

$\Delta\omega(^{15}N)$=25ppm ⟷ B_0=14.1T → $\Delta\nu$=1500Hz, τ_{spect}~0.7ms

$\Delta\omega(^{1}H)$=3.5ppm ⟷ B_0=14.1T → $\Delta\nu$=2000Hz, τ_{spect}~0.5ms

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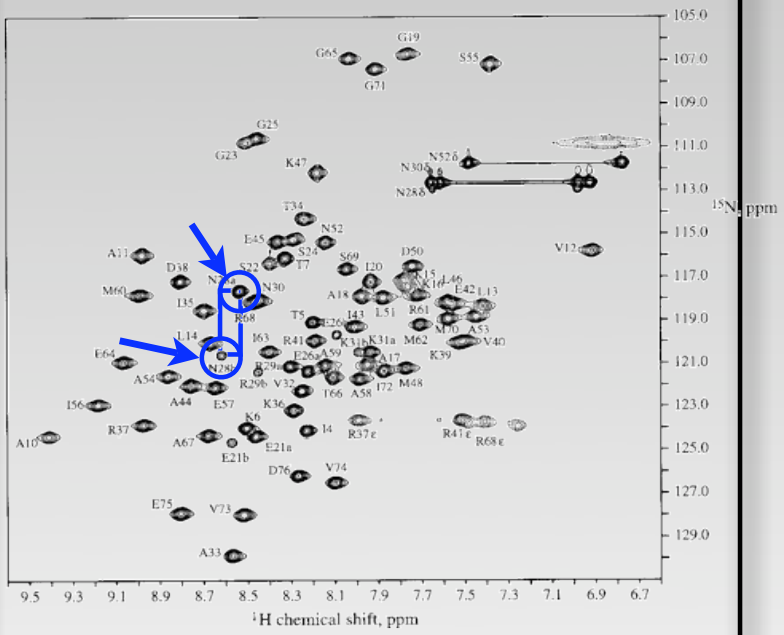
Same nucleus ; two different conformations



$\Delta\omega(^{15}N)$=1.5ppm ⟷ B_0=14.1T $\Delta\nu$=100Hz τ_{spect}~10ms

$\Delta\omega(^{1}H)$=0.5ppm ⟷ B_0=14.1T $\Delta\nu$=300Hz τ_{spect}~3ms

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✓ Motions slower than $\Delta\nu$ have no effect on spectral feature

✓$\Delta\nu$ depends on magnetic field and on spins nature

✓$\Delta\nu$ depends on the nature of the interactions between spins

Averaging of secular interactions by motions

✓ Dipolar interaction between spins magnetic moments.

$$\hat{H}_{IS}^{DD} = -\frac{\mu_0\gamma_I\gamma_S\hbar}{4\pi r_{IS}^3}\left(\frac{3\cos^2\theta_{IS}-1}{2}\right)\left(3\hat{I}_z\hat{S}_z - \hat{\mathbf{I}}.\hat{\mathbf{S}}\right)$$

$$E/\hbar < 10^4 - 10^5 Hz$$

✓ Chemical shift anisotropy

$$\hat{H}_I^{CSA} = -\gamma_S\frac{c_{\parallel}-c_{\perp}}{3}B_0\left(3\cos^2\theta-1\right)\hat{I}_z$$

$$E/\hbar < 10^4 - 10^5 Hz$$

✓ Interaction spin quadrupole/electric field

$$\hat{H}_I^Q \cong \omega_I^Q\left(3\hat{I}_z^2 - \hat{\mathbf{I}}.\hat{\mathbf{I}}\right);\ \omega_I^Q = \frac{3eQ_I}{4I(2I-1)}V_{zz}^I(\theta)$$

$$I > \frac{1}{2} \qquad E/\hbar \approx 2.10^5 Hz\left(^2H\right) \text{ and } 3.10^6\left(^{14}N\right)$$

✓ Unpaired electron

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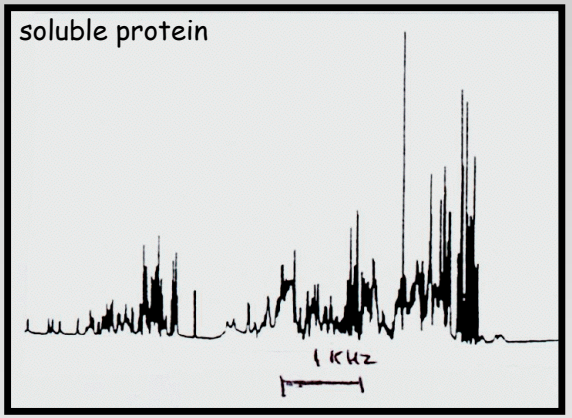

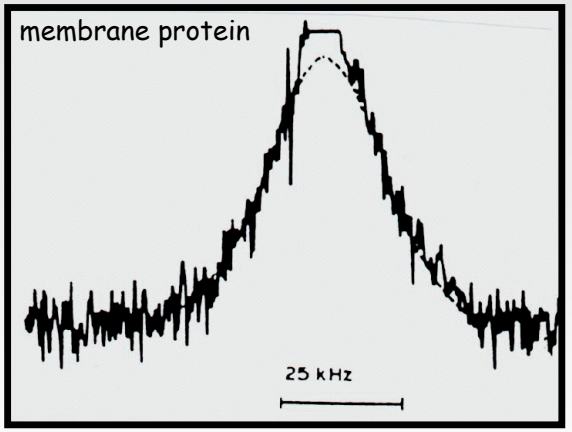

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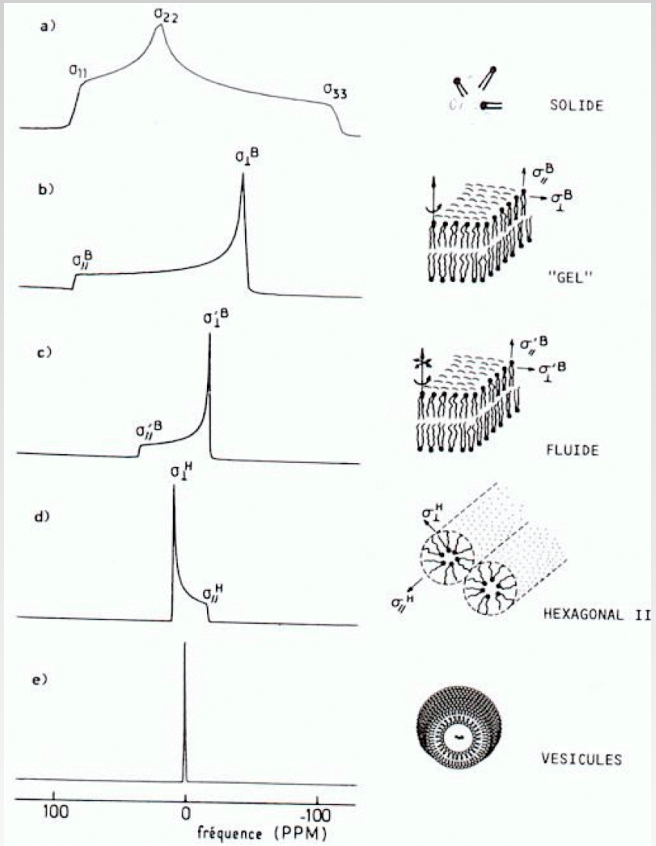

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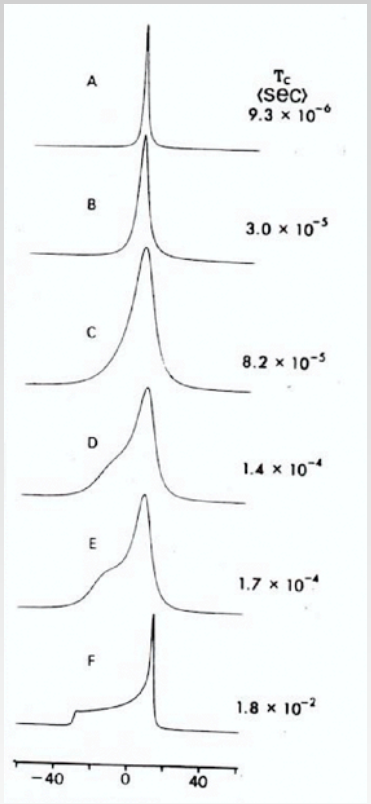

Averaging of chemical shift anisotropy (^{31}P)

Burnell et al., Biochim. Biophys. Acta 603, 63 (1980)

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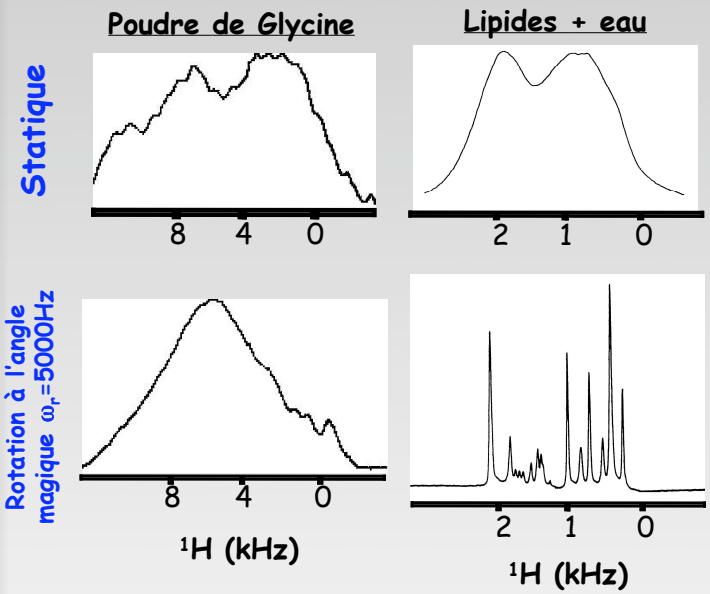
motions generated to average interactions



Davis, Auger & Hodges *Biophysical Journal* **69**:1917-1932 (1995)
Gross et al., *J. Magn. Res.* **106**, 187-190 (1995)
Carlotti, Aussenac & Dufourc *Biochim. Biophys. Acta.* **1564**:156-164 (2002)

NMR timescales -3- The "Larmor" timescale

✓Defined by the resonance frequencies of spins

✓i.e. the energy difference between spins states levels

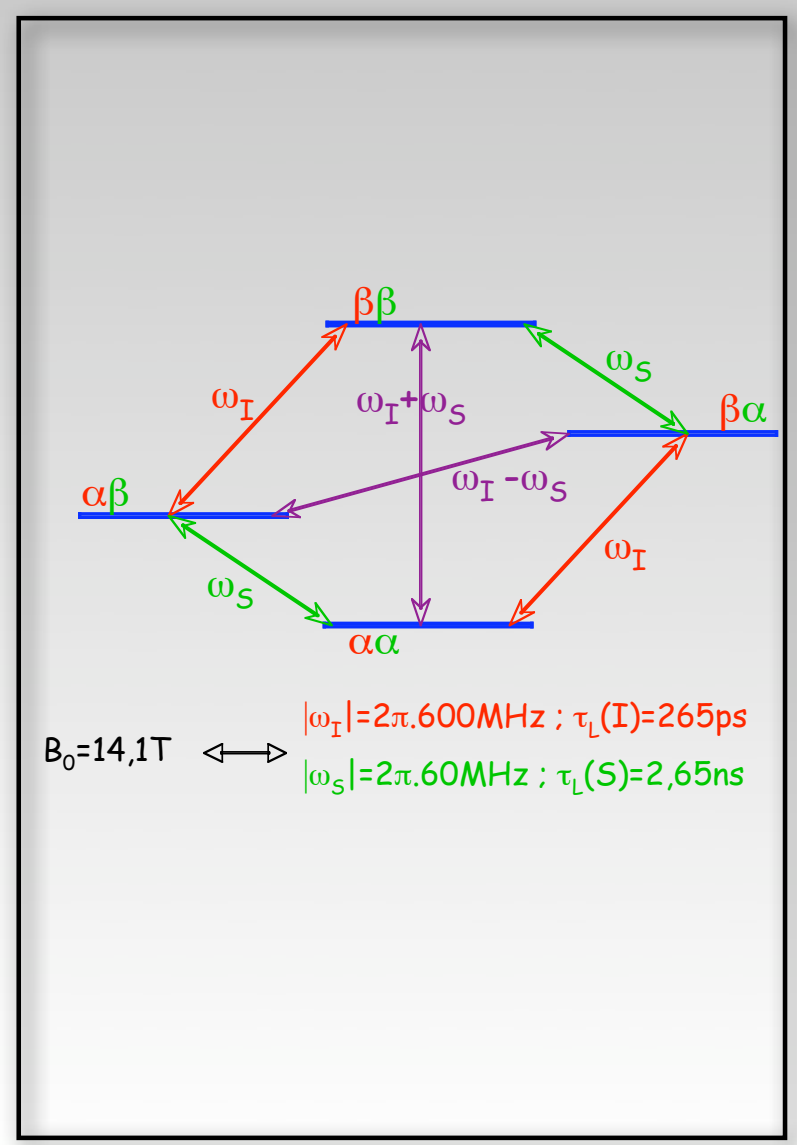
$$\omega_0 = -\gamma B_0$$

$$\tau_{Larmor} = \frac{1}{|\omega_0|} = \frac{1}{2\pi\nu_0}$$

✓ Motions in these timescale have no direct effect on spectra.

✓Motions in these timescales are responsible for the efficiency of spins relaxation processes

✓ The relationship between motions and relaxation rate/time constants is not simple



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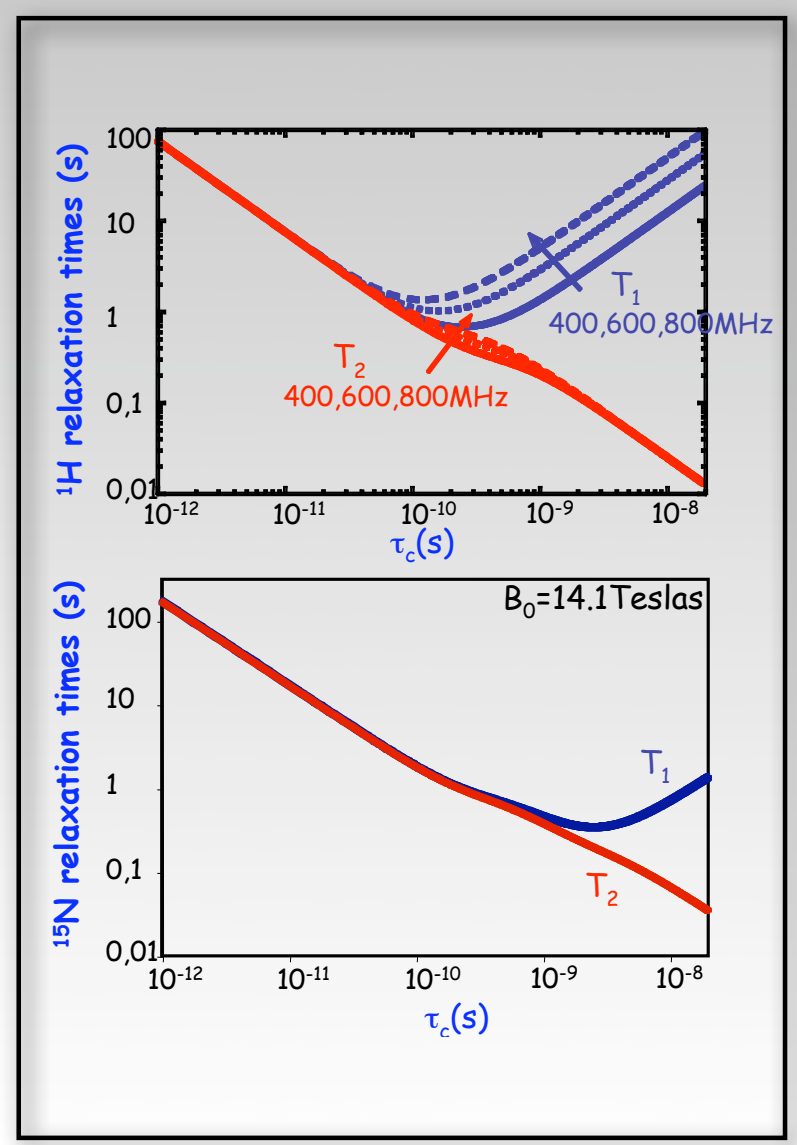

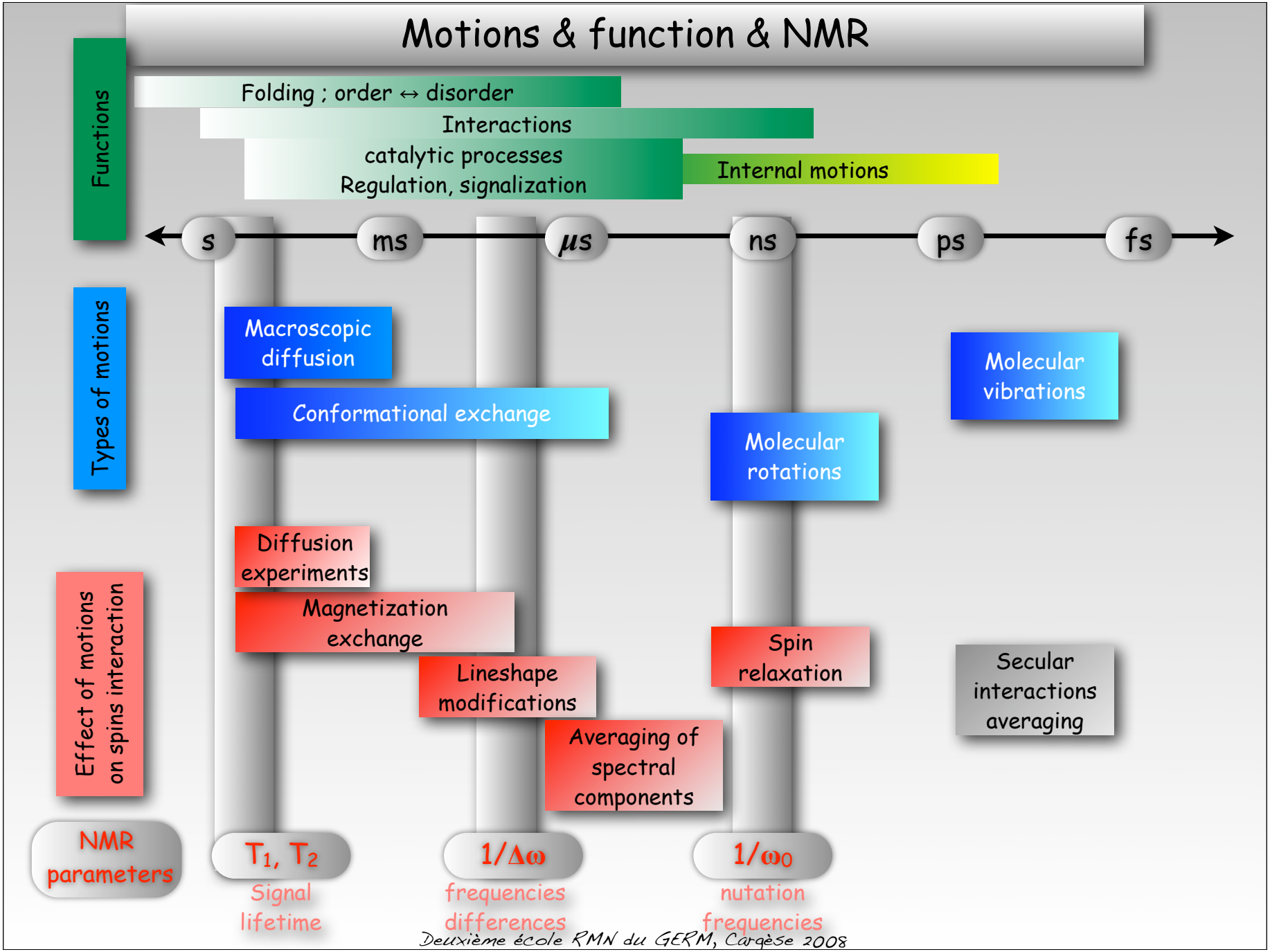
Motions & function & NMR
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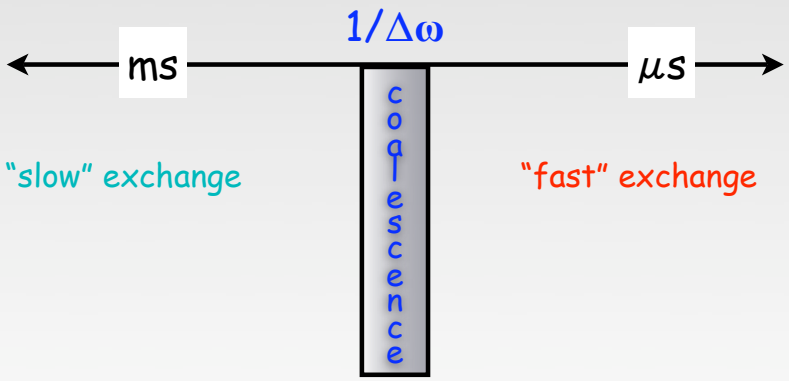
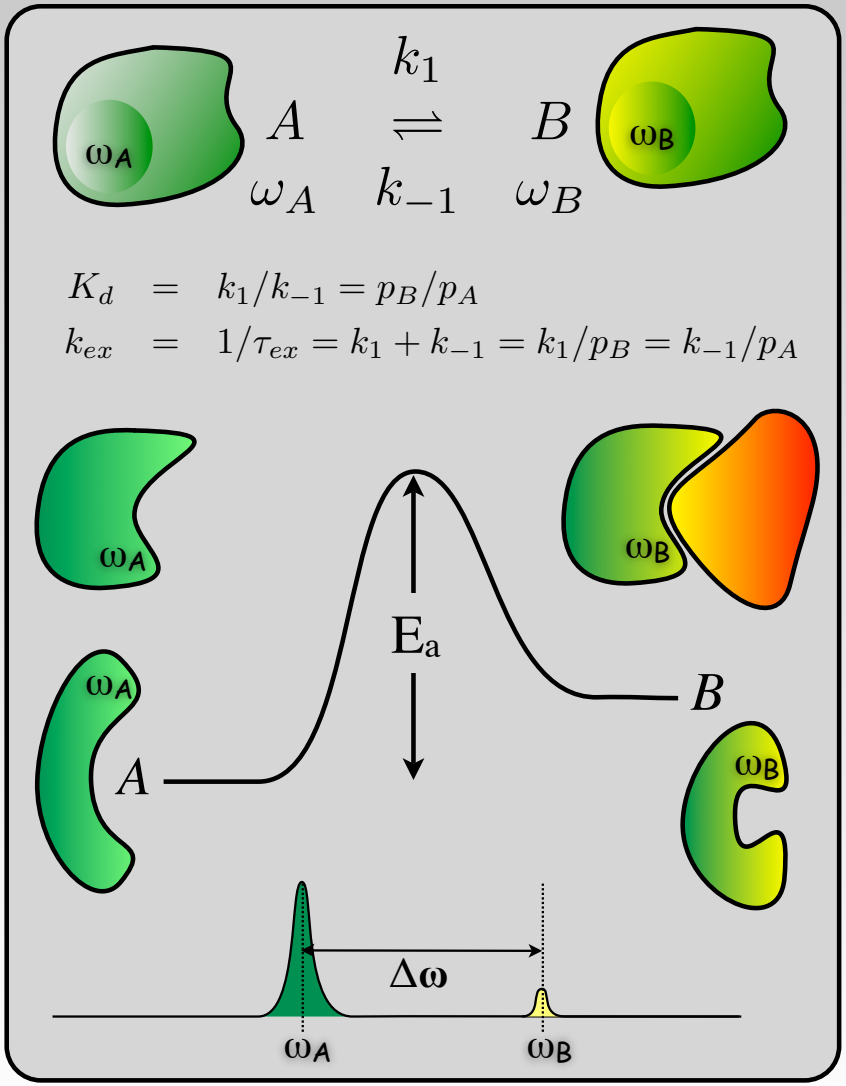
Analysis of μs-ms motions by NMR

Conformational exchange

- ✓ Motions in the μs-ms range induce spectral modifications.
- ✓ These motions usually correspond to conformational exchange conformationnel, or chemical exchange.
- ✓ Their effect on spectra depends of relative values of k_{ex} (τ_{ex}) and $\Delta\omega$ ($1/\Delta\omega$). They are called either **slow, intermediate or fast exchange processes at the chemical shift timescale.**





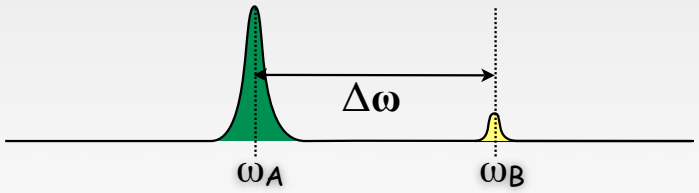
Analysis of μs-ms motions by NMR

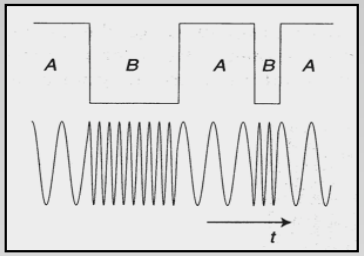
Conformational exchange

- ✓ Conformational exchange is characterized by a probability k_{ex} to switch from one state to the other, associated with an instantaneous change of precession frequency.
- ✓ These sudden frequency changes induce a dephasing of the transverse magnetization, which is added to the natural loss of coherence of spins.

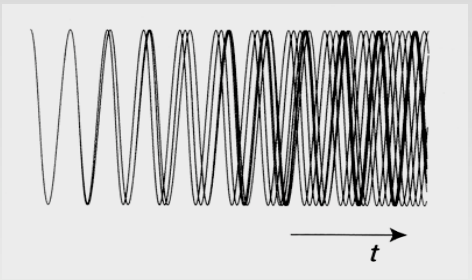
$$\begin{array}{ccc} & k_1 & \\ A & \rightleftharpoons & B \\ \omega_A & k_{-1} & \omega_B \end{array}$$

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



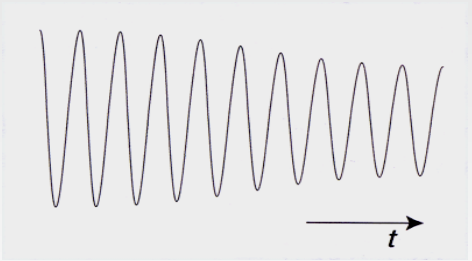


20 molecules in state A



Δν=1000Hz
k_{ex}=500Hz
p_A=p_B

Δν=1000Hz
k_{ex}=500Hz
p_A=p_B

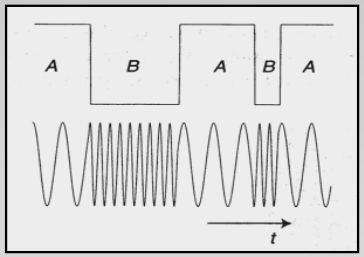


Resulting transverse magnetization for a great number of spins initially in the same state (effect of exchange only)

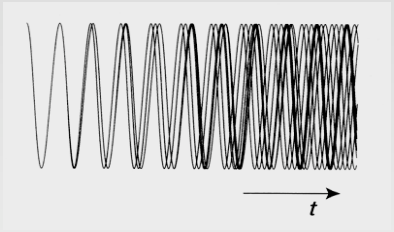
Analysis of μs-ms motions by NMR

Conformational exchange

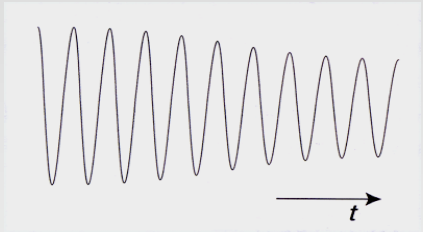
- Modification of spectral features



- depends of the relative values of k_{ex} and $\Delta\omega$

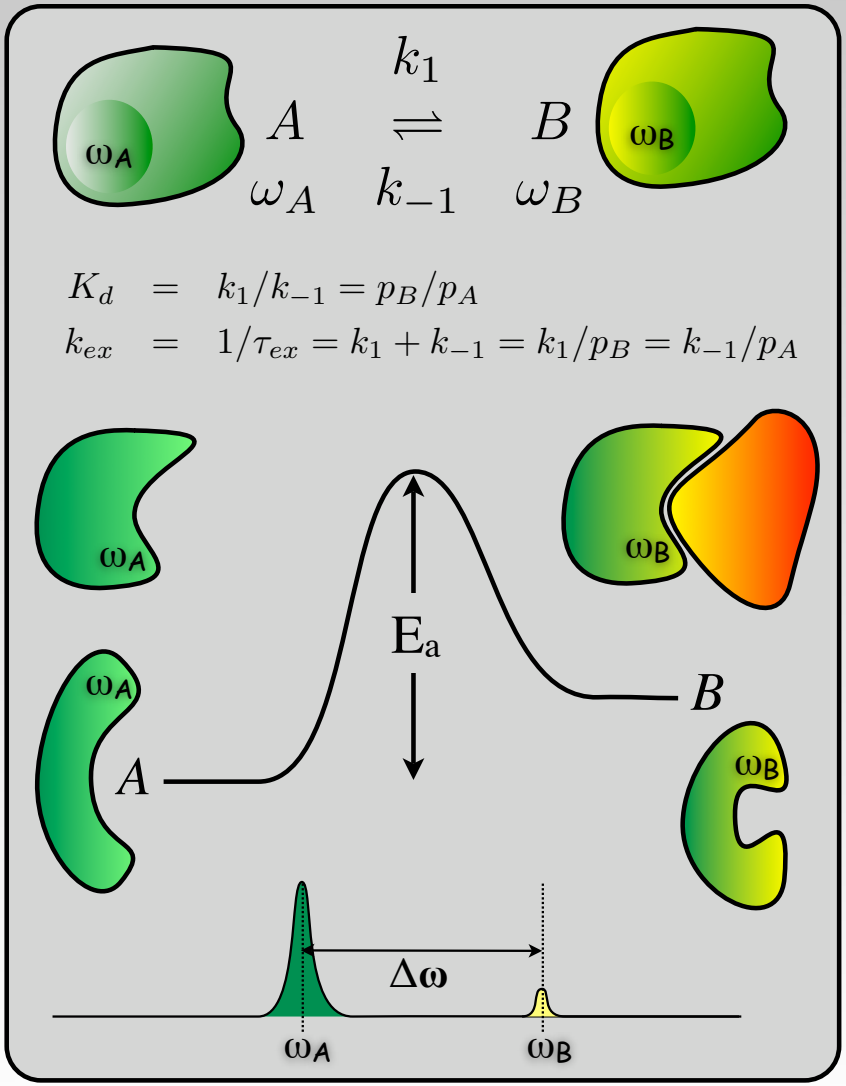


20 molecules in state A



$\Delta\omega/2\pi$ = 1000Hz
k_{ex}=500Hz
p_A=p_B

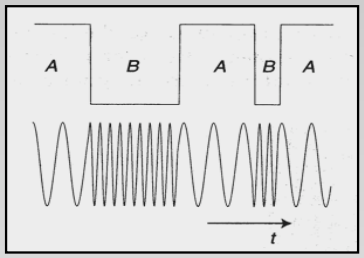
- Additional dephasing of the magnetization
- Apparent enhanced transverse relaxation
  - $R_2^{app} = R_2 + R_{ex}$
- Larger linewidths



Analysis of μs-ms motions by NMR

Conformational exchange

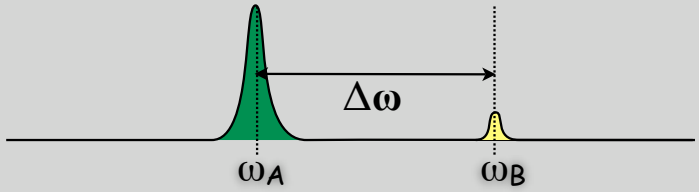
➪ Modification of spectral features



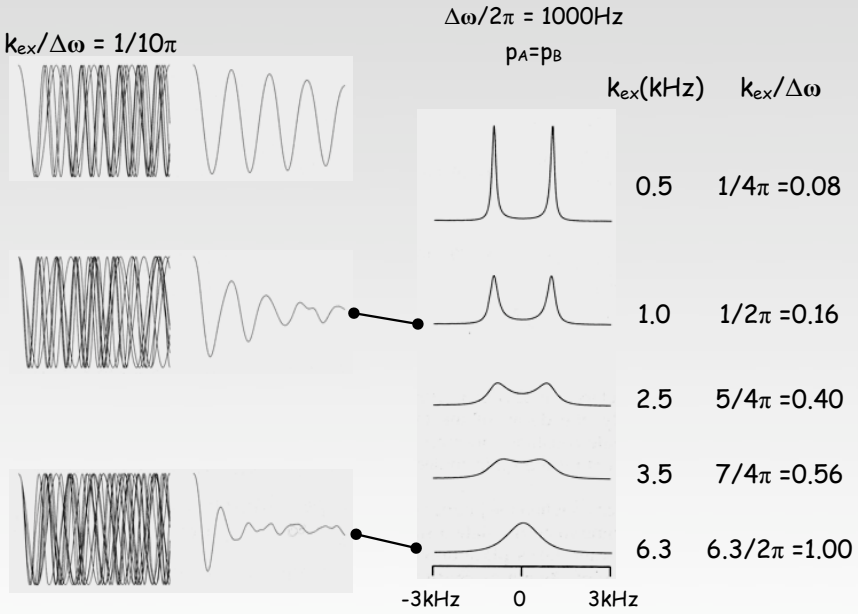
$$A \underset{\omega_A}{\overset{k_1}{\rightleftharpoons}} \underset{k_{-1}}{B} \quad \omega_B$$

$$K_d = k_1/k_{-1} = p_B/p_A$$

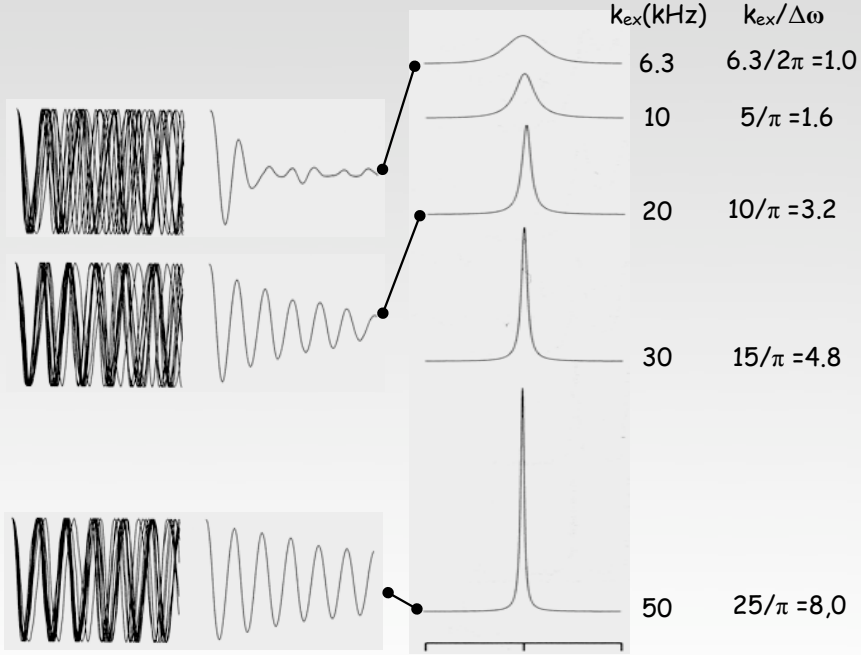
$$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$$



➪ depends of the relative values of k_{ex} and $\Delta\omega$



Motional broadening



Motional narrowing

Analysis of μs-ms motions by NMR

Conformational exchange

General equation for a system undergoing conformational exchange

$$\vec{M}(t) = \begin{bmatrix} \vec{M}_1(t) \\ \vec{M}_2(t) \\ \dots \\ \vec{M}_n(t) \end{bmatrix}$$

$$\frac{d}{dt}\Delta\vec{M}_z(t) = (-R + K)\Delta\vec{M}_z(t)$$

$$\frac{d}{dt}\vec{M}_+(t) = (i\Omega - R + K)\vec{M}_+(t)$$

The case of a two state exchange

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

$$R - K = \begin{bmatrix} \rho_A - k_1 & -k_{-1} \\ -k_1 & \rho_B - k_{-1} \end{bmatrix}$$

$$\frac{d}{dt}\begin{bmatrix} \Delta M_A^+(t) \\ \Delta M_B^+(t) \end{bmatrix} = \begin{bmatrix} -i\Omega_A - R_{2A}^0 - p_B k_{ex} & p_A k_{ex} \\ p_B k_{ex} & -i\Omega_B - R_{2B}^0 - p_A k_{ex} \end{bmatrix}\begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix}$$

$$\begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix} = \begin{bmatrix} a_{AA}(t) & a_{AB}(t) \\ a_{BA}(t) & a_{BB}(t) \end{bmatrix}\begin{bmatrix} M_A^+(0) \\ M_B^+(0) \end{bmatrix}$$

In a general case, the expression of the matrix coefficients is complicated

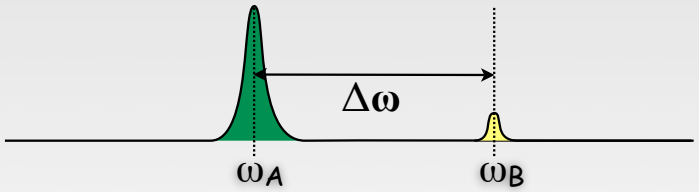
Analysis of μs-ms motions by NMR

Conformational exchange

General equation

for a two state exchange

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



$$\begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix} = \begin{bmatrix} a_{AA}(t) & a_{AB}(t) \\ a_{BA}(t) & a_{BB}(t) \end{bmatrix} \begin{bmatrix} M_A^+(0) \\ M_B^+(0) \end{bmatrix}$$

$$a_{AA}(t) = \frac{1}{2}\left[\left(1 - \frac{-i\omega_A + i\omega_B + \rho_A - \rho_B + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)}\right)\exp(-\lambda_- t) + \left(1 + \frac{-i\omega_A + i\omega_B + \rho_A - \rho_B + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)}\right)\exp(-\lambda_+ t)\right]$$

$$a_{BB}(t) = \frac{1}{2}\left[\left(1 + \frac{-i\omega_A + i\omega_B + \rho_A - \rho_B + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)}\right)\exp(-\lambda_- t) + \left(1 - \frac{-i\omega_A + i\omega_B + \rho_A - \rho_B + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)}\right)\exp(-\lambda_+ t)\right]$$

$$a_{AB}(t) = \frac{k_{-1}}{\lambda_+ - \lambda_-}\left[\exp(-\lambda_- t) - \exp(-\lambda_+ t)\right]$$

$$a_{BA}(t) = \frac{k_1}{\lambda_+ - \lambda_-}\left[\exp(-\lambda_- t) - \exp(-\lambda_+ t)\right]$$

$$\lambda_\pm = \frac{1}{2}\left\{\left(-i\omega_A - i\omega_B + \rho_A + \rho_B + k_1 + k_{-1}\right) \pm \left[\left(-i\omega_A + i\omega_B + \rho_A - \rho_B + k_1 - k_{-1}\right)^2 + 4k_1k_{-1}\right]^{1/2}\right\}$$

Analysis of μs-ms motions by NMR

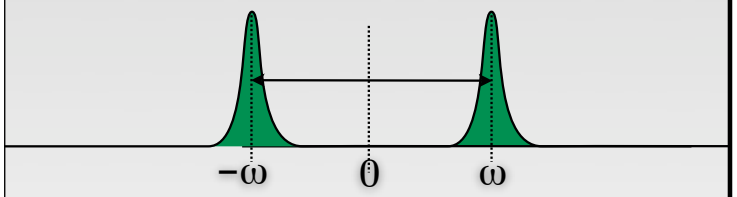
Conformational exchange

An easier case

The two state symmetrical exchange

$$A \underset{k}{\rightleftharpoons} B$$

$$\omega_A \quad \omega_B$$



$$\begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix} = \begin{bmatrix} a_{AA}(t) & a_{AB}(t) \\ a_{BA}(t) & a_{BB}(t) \end{bmatrix} \begin{bmatrix} M_A^+(0) \\ M_B^+(0) \end{bmatrix}$$

$$a_{AA}(t) = \frac{1}{2}\left[\left(1+\frac{i\omega}{\Delta}\right)\exp\left\{-\left(\rho+k-\Delta\right)t\right\} + \left(1-\frac{i\omega}{\Delta}\right)\exp\left\{-\left(\rho+k+\Delta\right)t\right\}\right]$$

$$a_{BB}(t) = \frac{1}{2}\left[\left(1-\frac{i\omega}{\Delta}\right)\exp\left\{-\left(\rho+k-\Delta\right)t\right\} + \left(1+\frac{i\omega}{\Delta}\right)\exp\left\{-\left(\rho+k+\Delta\right)t\right\}\right]$$

$$a_{AB}(t) = a_{BA}(t) = \frac{k}{2\Delta}\left[\exp\left\{-\left(\rho+k-\Delta\right)t\right\} - \exp\left\{-\left(\rho+k+\Delta\right)t\right\}\right]$$

$$\Delta = \left(k^2-\omega^2\right)^{1/2}$$

Fast exchange $k_{ex} \gg \Delta\omega$

$$a_{AA}(t) = a_{AA}(t) = \frac{1}{2}\left[1+\exp\left(-2kt\right)\right]\exp\left(-\rho t\right)$$

$$a_{AB}(t) = a_{BA}(t) = \frac{1}{2}\left[1-\exp\left(-2kt\right)\right]\exp\left(-\rho t\right)$$

$$M^+(t) = M_A^+(t) + M_B^+(t) = M^+(0)\exp\left(-\rho t\right)$$

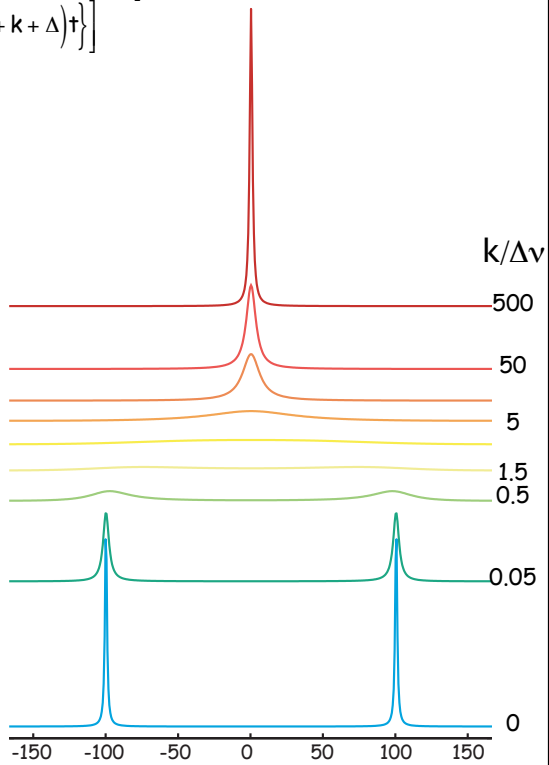
Coalescence $k_{ex} \sim \Delta\omega$

Slow exchange $k_{ex} \ll \Delta\omega$

$$a_{AA}(t) = \exp\left\{-\left(\rho+k-i\omega\right)t\right\}$$

$$a_{BB}(t) = \exp\left\{-\left(\rho+k+i\omega\right)t\right\}$$

$$a_{AB}(t) = a_{BA}(t) = 0$$



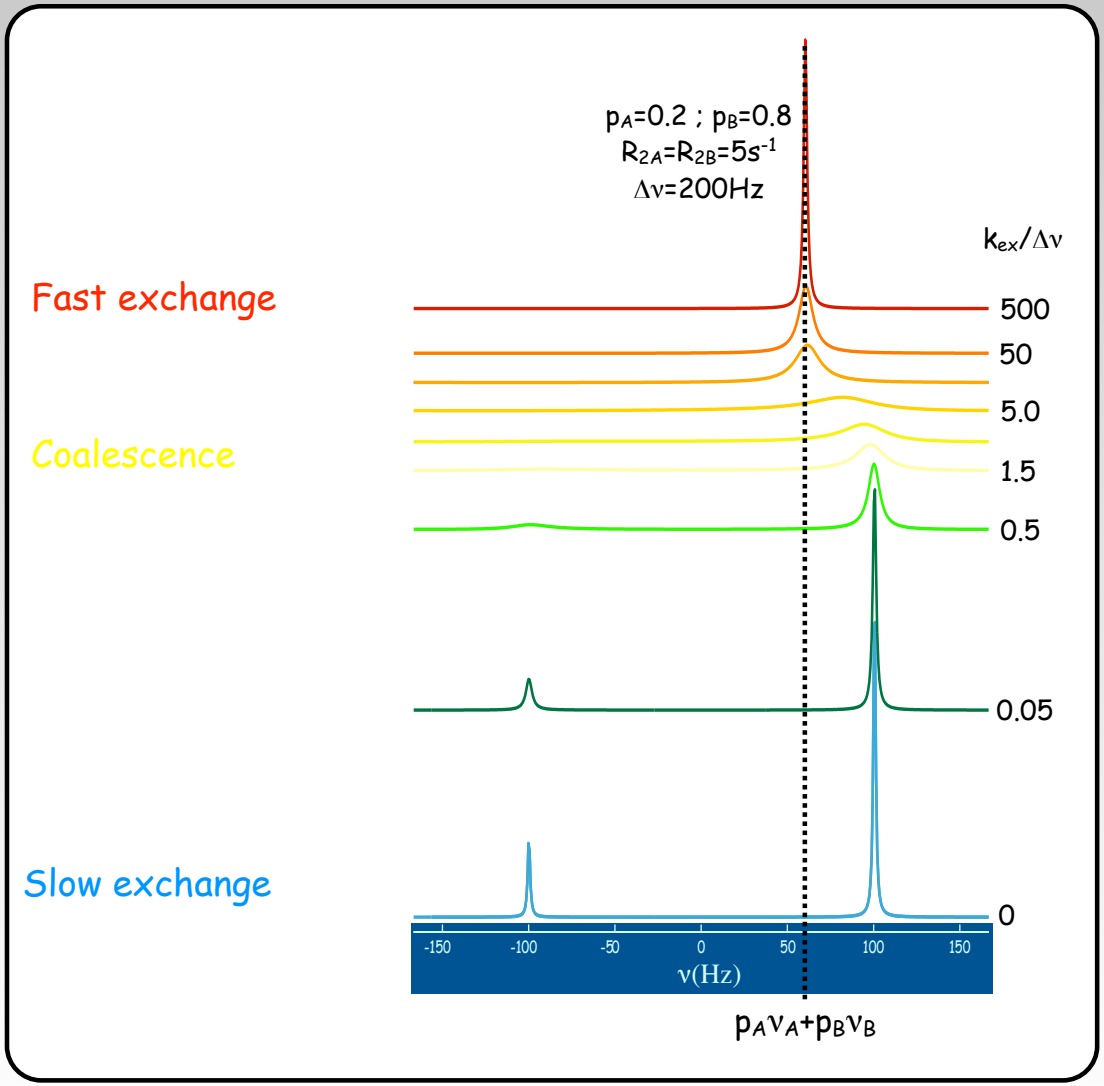
Analysis of μs-ms motions by NMR

Conformational exchange

two state non symmetrical exchange

The minor component is more broadened than the major one

When the population difference is large, the minor component can become invisible, even in a "slow" exchange case.



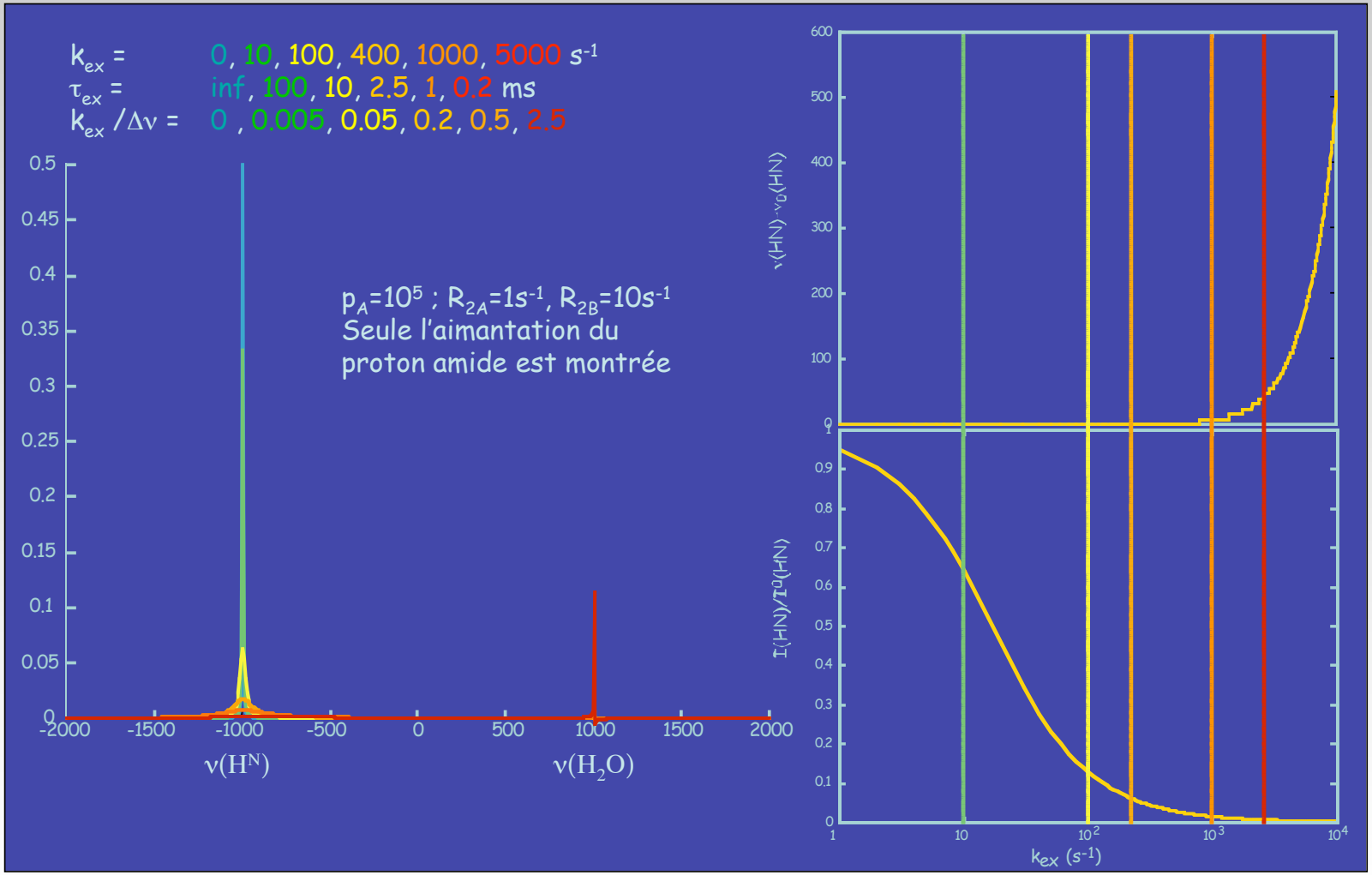
Ishima & Torchia, *J. Biomol NMR* **15**, 369 (1999)
Millet et al., *J. Am. Chem. Soc.* **122**, 2867 (2000)

Analysis of μs-ms motions by NMR

Conformational exchange

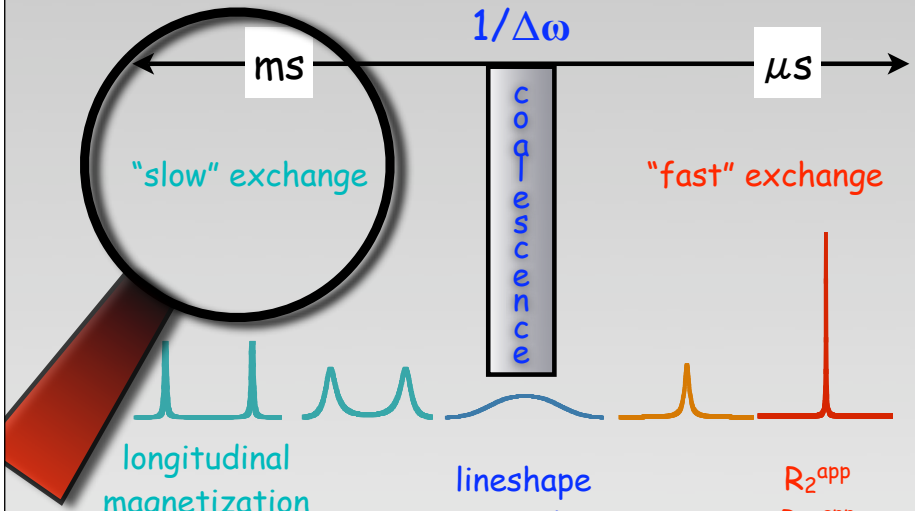
two state non symmetrical exchange

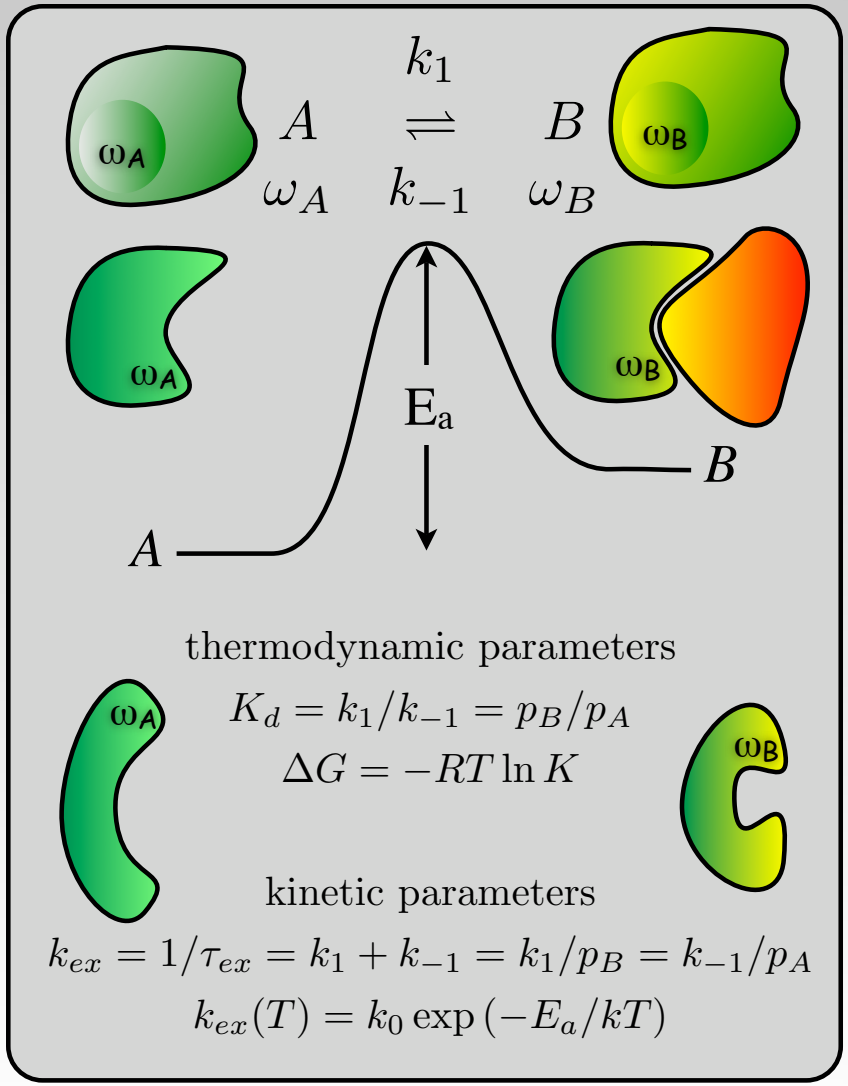
An extreme case : amide protons exchange with water



Analysis of μs-ms motions by NMR

NMR methods to characterize conformational exchange





thermodynamic parameters

$$K_d = k_1/k_{-1} = p_B/p_A$$

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

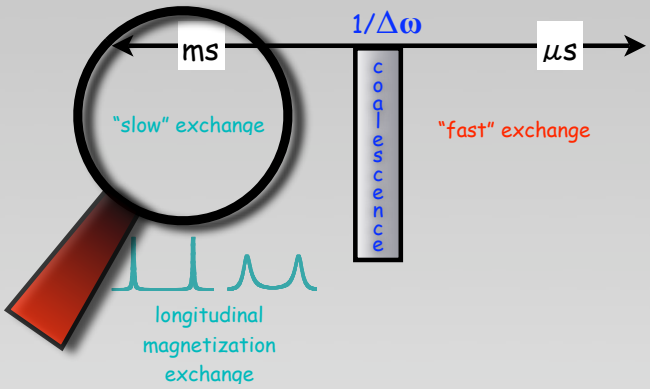
kinetic parameters

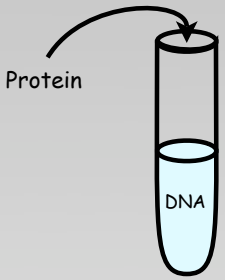
$$k_{ex} = 1/\tau_{ex} = k_1 + k_{-1} = k_1/p_B = k_{-1}/p_A$$

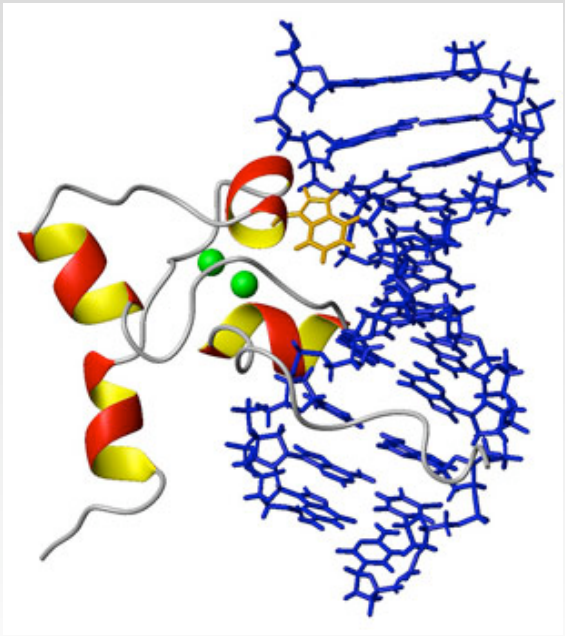
$$k_{ex}(T) = k_0 \exp(-E_a/kT)$$

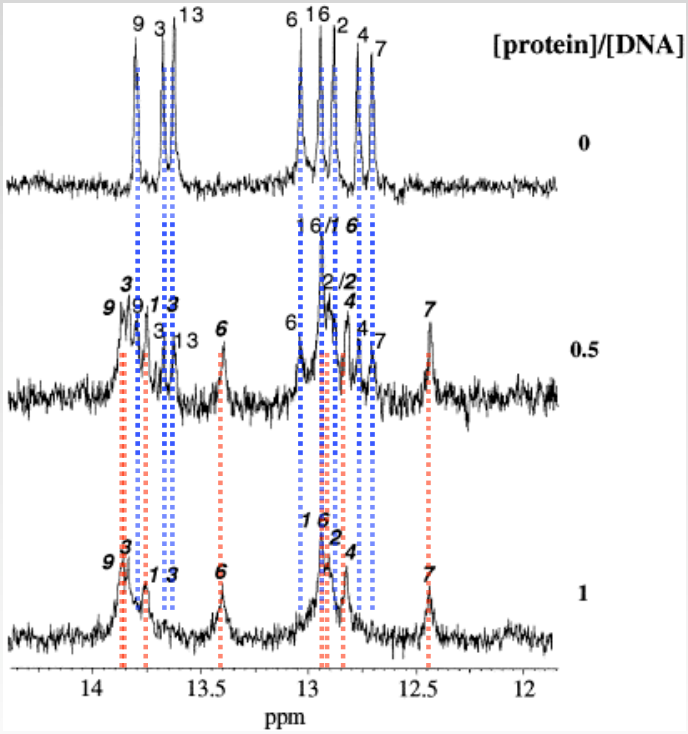
Analysis of μs-ms motions by NMR

NMR methods to characterize conformational exchange





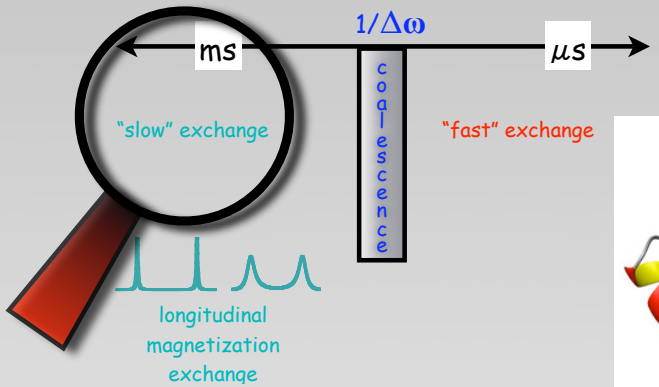




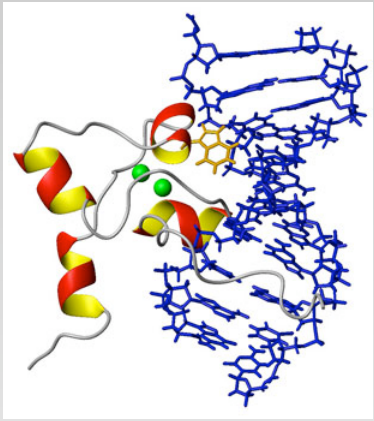
Cerdan et al., FEBS **408**, 235 (1997)

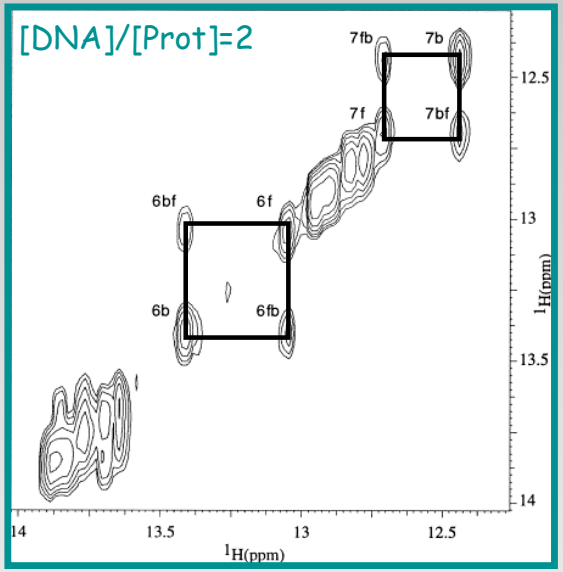
Analysis of μs-ms motions by NMR

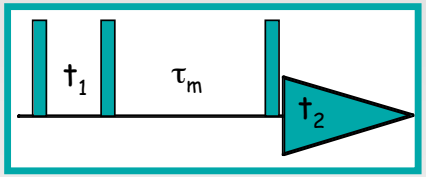
NMR methods to characterize conformational exchange



$$\begin{bmatrix}\Delta M_{Az}(t)\\ \Delta M_{Bz}(t)\end{bmatrix} = \begin{bmatrix}a_{AA}(t) & a_{AB}(t)\\ a_{BA}(t) & a_{BB}(t)\end{bmatrix}\begin{bmatrix}\Delta M_{Az}(0)\\ \Delta M_{Bz}(0)\end{bmatrix}$$





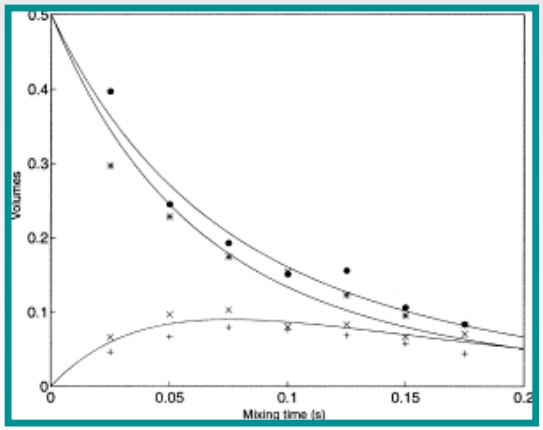


$$V_b(\tau_m) = x_b e^{-\sigma\tau_m}\left[\cosh(D\tau_m) - \frac{\delta}{D}\sinh(D\tau_m)\right]$$

$$V_f(\tau_m) = x_f e^{-\sigma\tau_m}\left[\cosh(D\tau_m) + \frac{\delta}{D}\sinh(D\tau_m)\right]$$

$$V_{crosspeak}(\tau_m) = x_b x_f \frac{k}{D} e^{-\sigma\tau_m}\sinh(D\tau_m)$$

$$\sigma = \frac{1}{2}\left(R_{1b} + R_{1f}\right);\ \delta = \frac{1}{2}\left(R_{1b} - R_{1f}\right);\ D = \sqrt{\delta^2 + x_b x_f k^2}$$

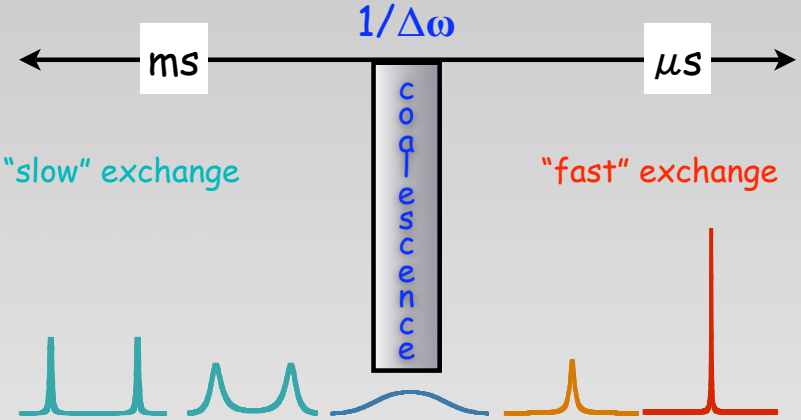


⇨ k_{ex}=14±2Hz
τ_{ex}=74±7ms

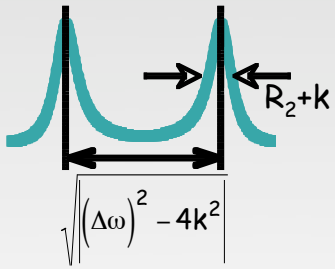
Cerdan et al., FEBS **408**, 235 (1997)

Analysis of μs-ms motions by NMR

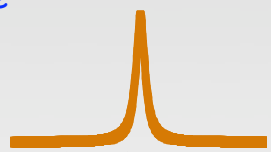
NMR methods to characterize conformational exchange



lineshape anaysis



$$\sqrt{(\Delta\omega)^2 - 4k^2}$$

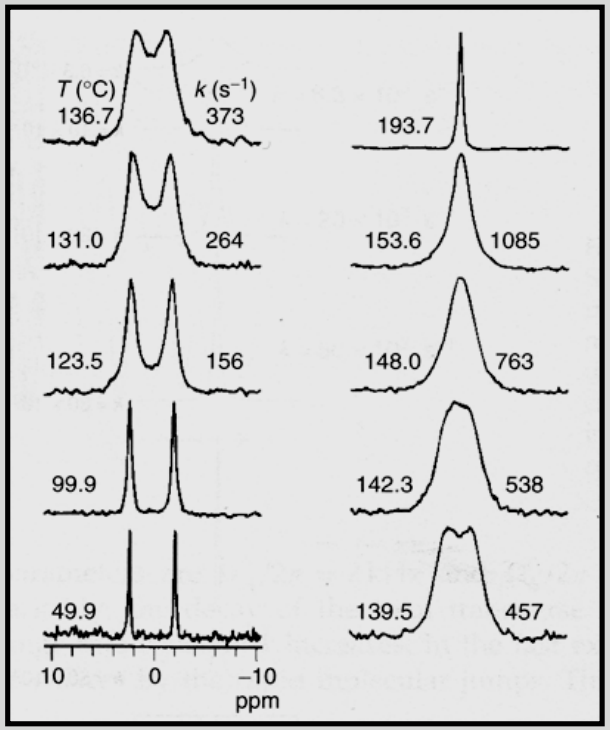


$$S(\omega) = \frac{1}{2}\left(1 + \frac{k}{D}\right)L(\overline{\omega}, R_2 + k - D) + \frac{1}{2}\left(1 - \frac{k}{D}\right)L(\overline{\omega}, R_2 + k + D)$$

$$D = \sqrt{k^2 - \left(\frac{\Delta\omega}{2}\right)^2}$$

$$k(T) = k_0 \exp\left(-\frac{E_a}{R_B T}\right)$$

Perturbation of resonances shape : modification of the exchange regime with temperature



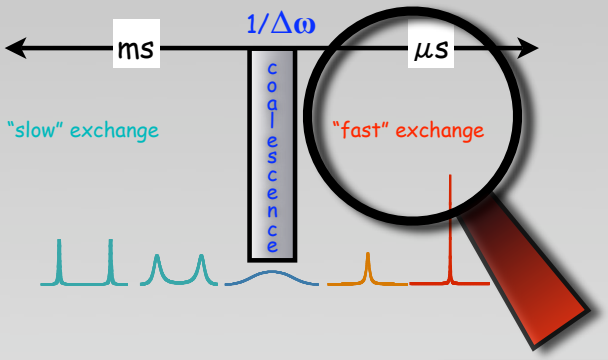
{^{13}C} N,N' diméthylformamide gaz, B_0=4,7T

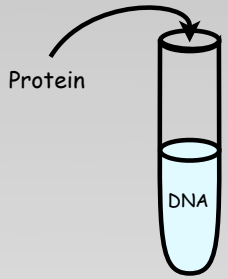
E_a=90,1kJ.mol-1 ; k_0=1,16.10^{4}Hz

Ross & True, J. Am. Chem. Soc. 106, 2451 (1984)

Analysis of μs-ms motions by NMR

NMR methods to characterize conformational exchange

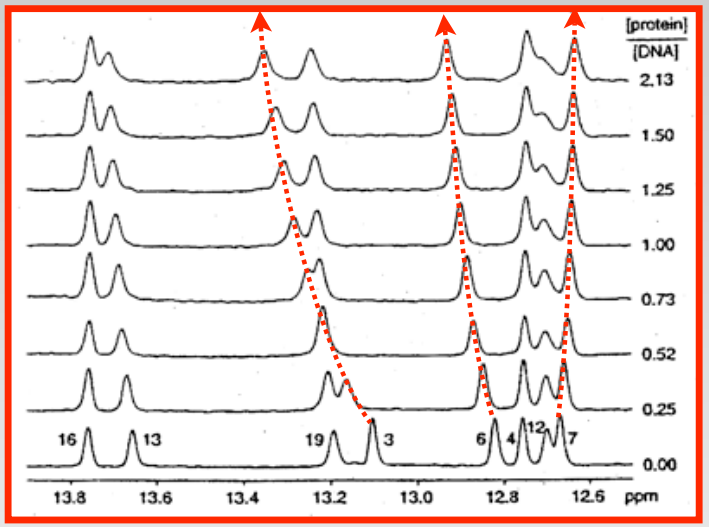




$$P + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PA \qquad \delta_{Af} \quad \delta_{Ab}$$

$$\delta_{obs} = f_f \delta_f + f_b \delta_b$$

➪ peaks displacements

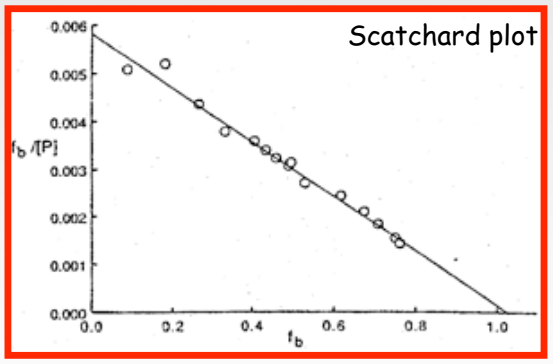


$$K_d = \frac{k_{on}}{k_{off}} = \frac{[P][N]}{[PN]}$$

$$K_d = \frac{(P_0 - f_b N_0)(N_0 - f_b N_0)}{f_b N_0}$$

$$f_b = \frac{\Delta\delta}{\delta_b - \delta_f}$$

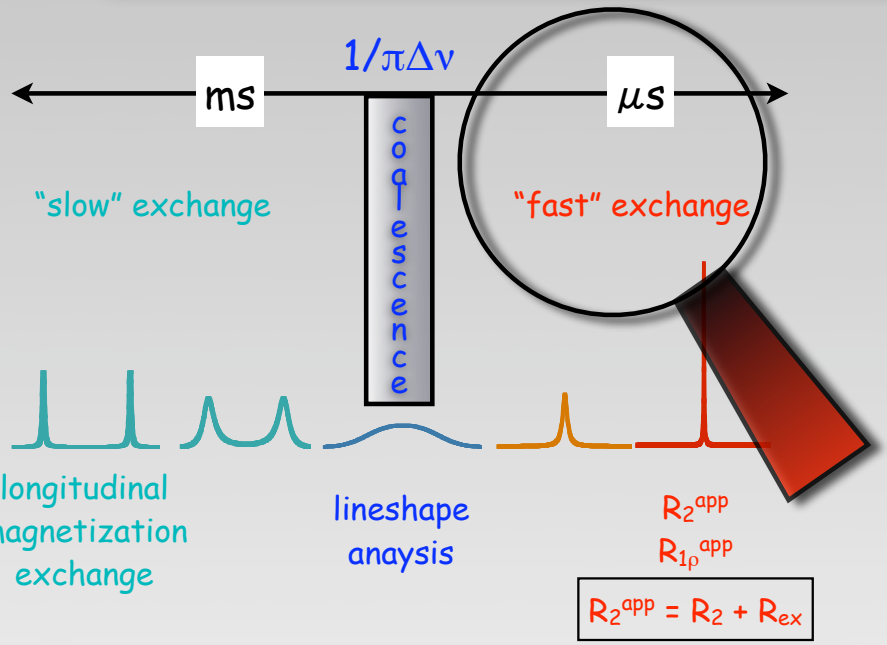
$$\frac{f_b}{[P]} = -\frac{1}{K_d} f_b + \frac{1}{K_d}$$

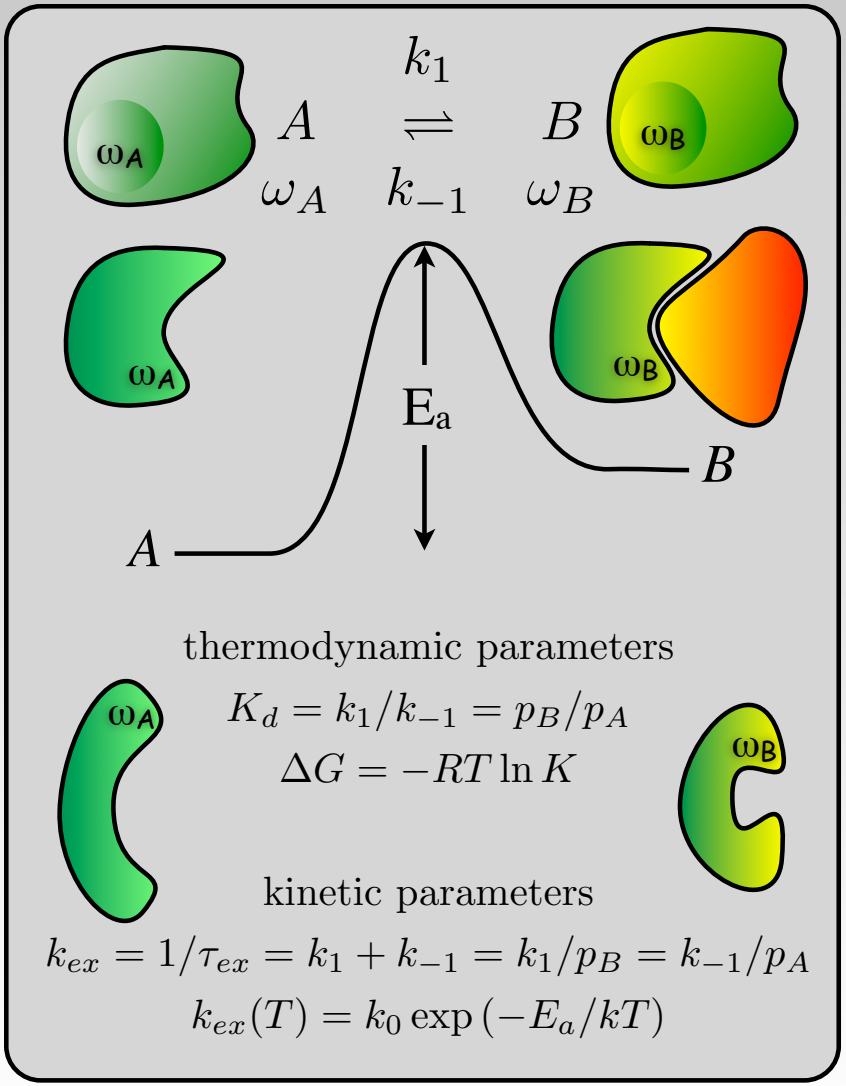


➪ Kd ~ 170 μM

Baleja et al. Biochemistry **33**, 3071 (1994)

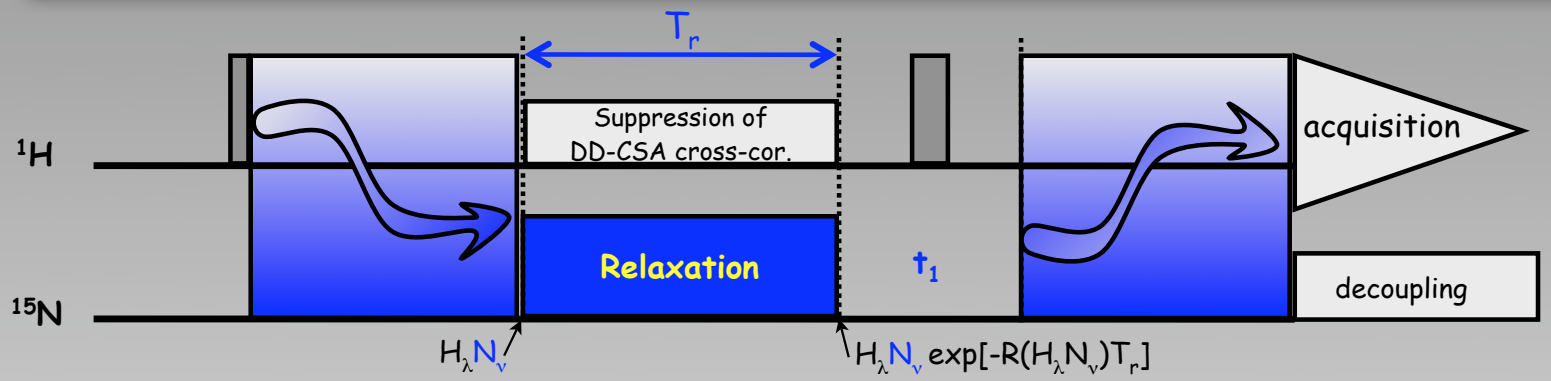
Analysis of μs-ms motions by NMR
for the characterization of exchange processes in proteins





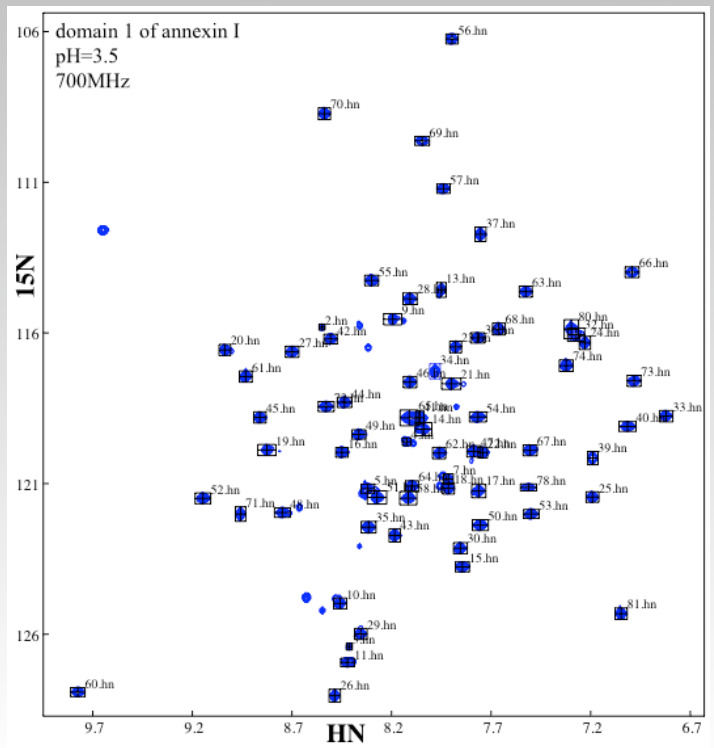
- Case of folding ↔ unfolding processes
  - sequence/stability/motions relationship
  - analysis of minor populations
- Motions and function
  - enzymes cycle
  - interactions

Characterization of μs-ms motions by NMR
HOW ?

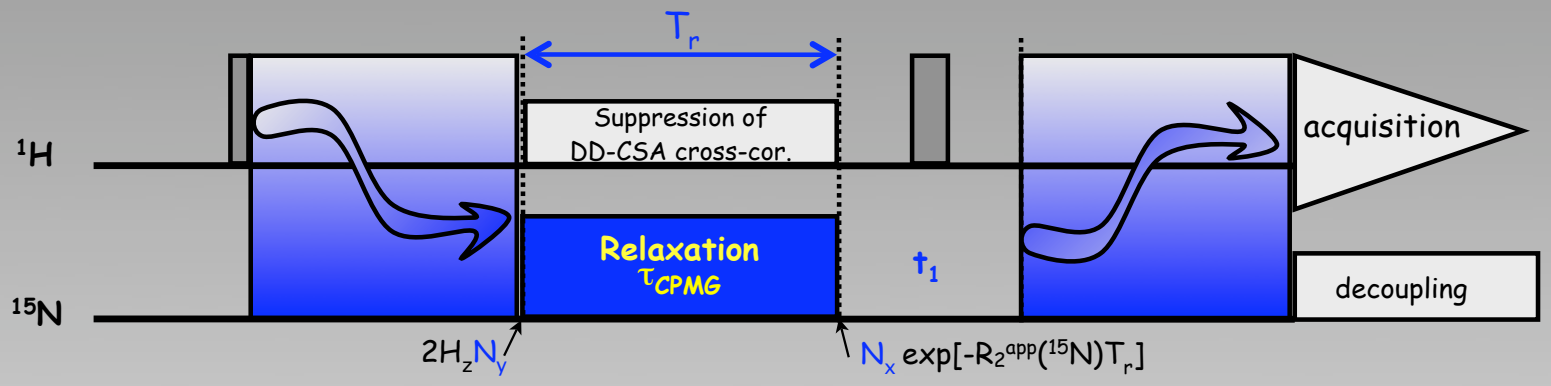


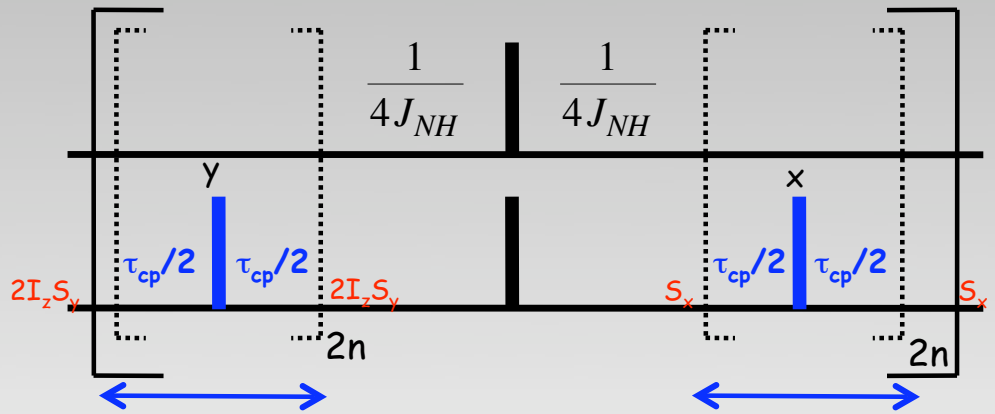
- A sample for NMR
  - concentration ~ 0.5mM
  - stable
  - ^{15}N labeled

- An experiment specifically designed for the problem.
- Assigned $^1H^N$, ^{15}N resonances



The method : relaxation dispersion NMR experiments





$$R_{2A}^{measured} = \overline{R} + R_{ex}\left(p_A, p_B, k_{ex}, \Delta\omega, \tau_{CPMG}\right)$$

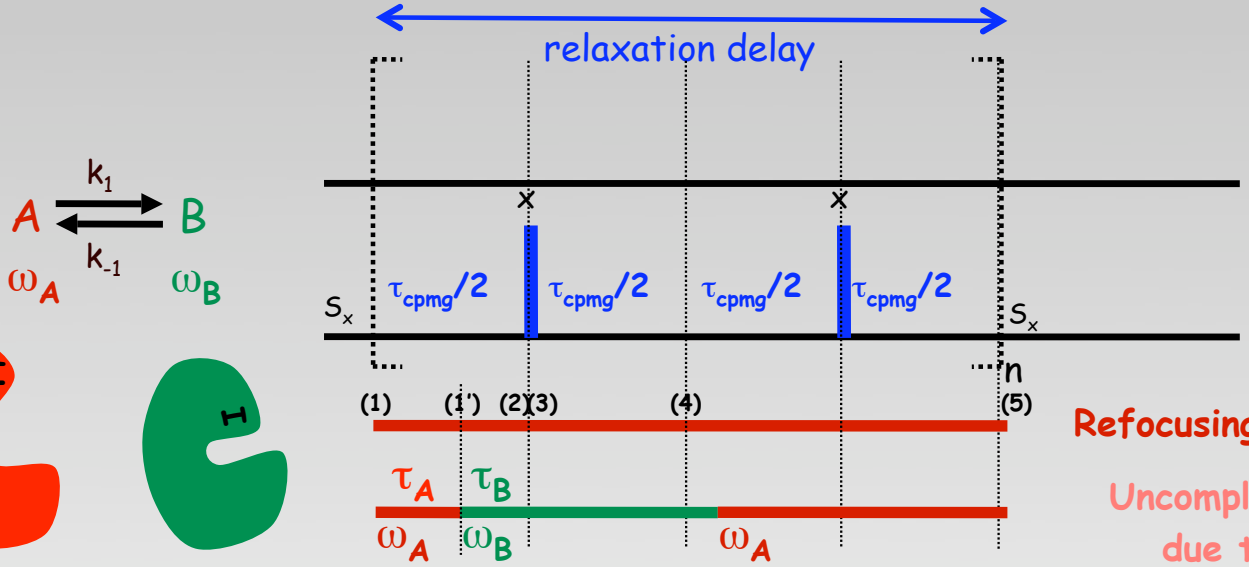
$$\overline{R} = \frac{1}{2}\left(R_{in}^0 + R_{anti}^0\right)$$

Mulder, F., van Tilborg, P. J. A., Kaptein, R. and Boelens, R. (1999) J. Biomol NMR **13**, 275

Loria, J. P., Rance, M. and Palmer, A. G. III (1999) J. Am. Chem. Soc. **121**, 2331

The method : relaxation dispersion NMR experiments

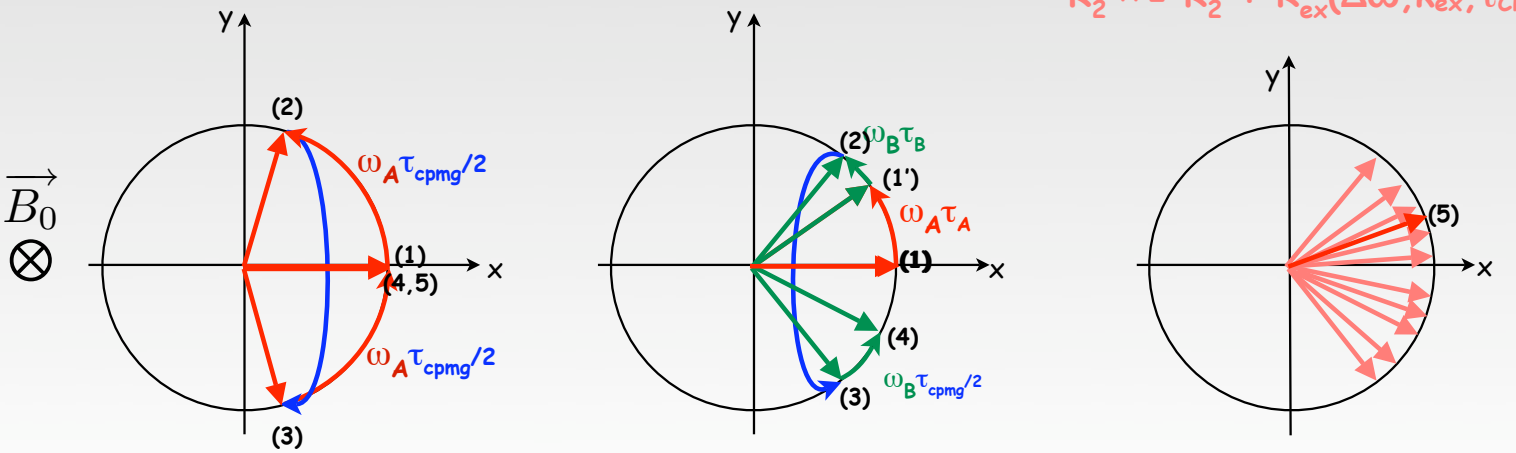
The principle : variation of R_2^{app} as a function of an experimental parameter : τ_{CPMG}



Refocusing $R_2^{app} = R_2$

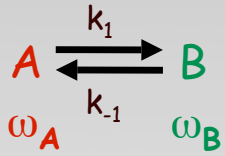
Uncomplete refocusing due to exchange

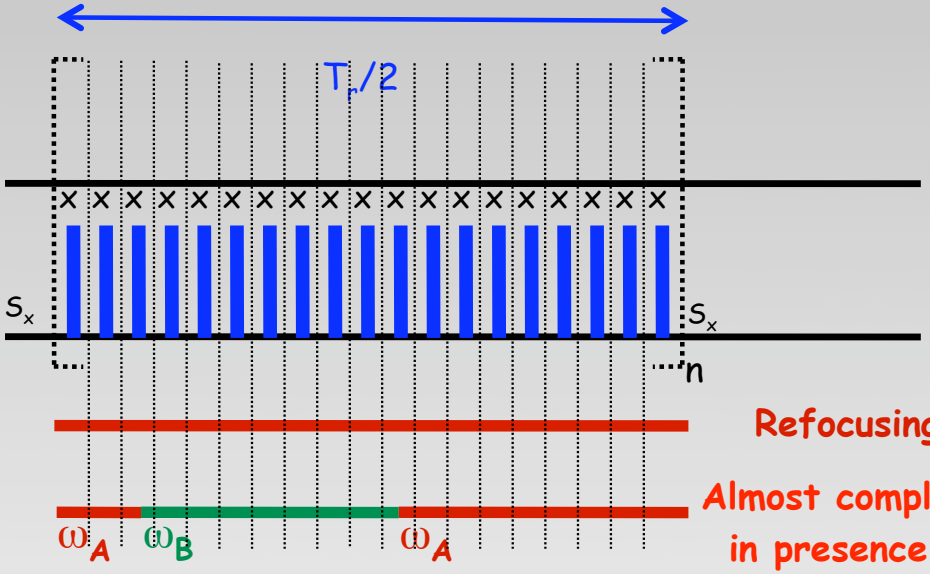
$R_2^{app} = R_2 + R_{ex}(\Delta\omega, k_{ex}, \tau_{CPMG})$



The method : relaxation dispersion NMR experiments

The principle : variation of R_2^{app} as a function of an experimental parameter : τ_{CPMG}

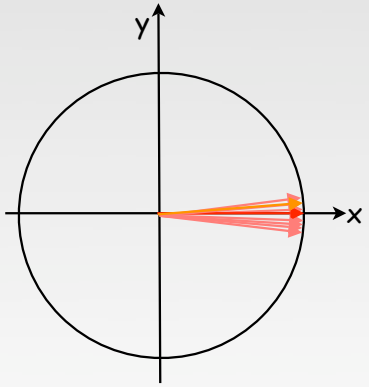




Refocusing $R_2^{app} = R_2$

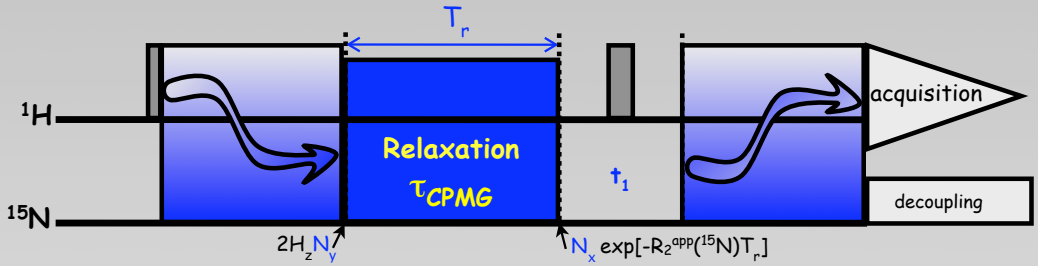
Almost complete refocusing in presence of exchange

$R_2^{app} = R_2 + R_{ex}(k_{ex}, \tau_{CPMG})$



The method : relaxation dispersion NMR experiments

The principle : variation of R_2^{app} as a function of an experimental parameter : τ_{CPMG}

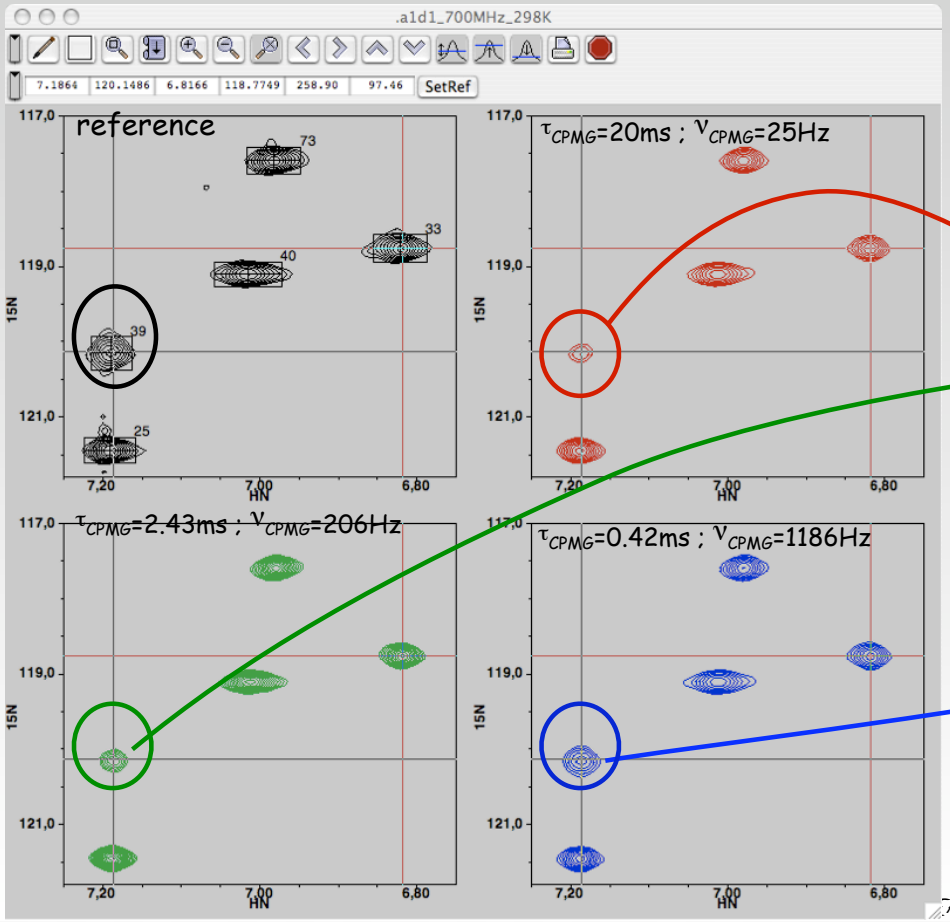


$$R_2^{measured} = R_2^0 + R_{ex}(p_A, p_B, k_{ex}, \Delta\omega, \tau_{CPMG})$$

$$\frac{I(T_r)}{I_0} = \exp[-(R_2^0 + R_{ex})T_r]$$

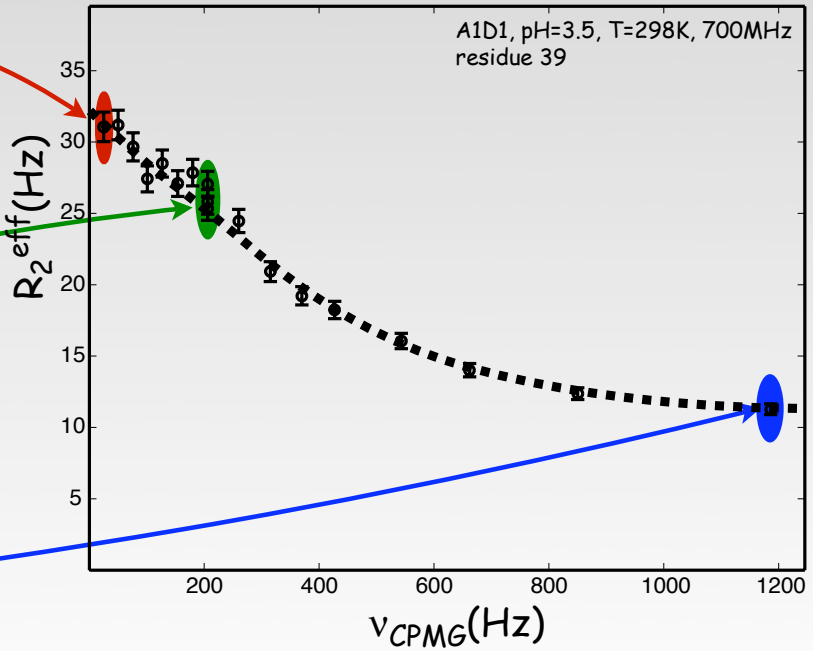
$$R_2^{measured}(\nu_{CPMG}) = -\frac{1}{T_r}\ln\left[\frac{I(T_r, \nu_{CPMG})}{I_0}\right]$$

$$T_r = 2n\tau_{CPMG} = \frac{n}{\nu_{CPMG}}$$



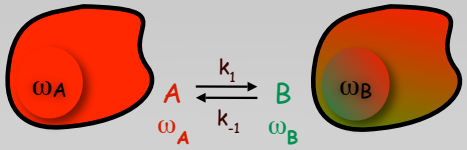
☞ Relaxation dispersion curve

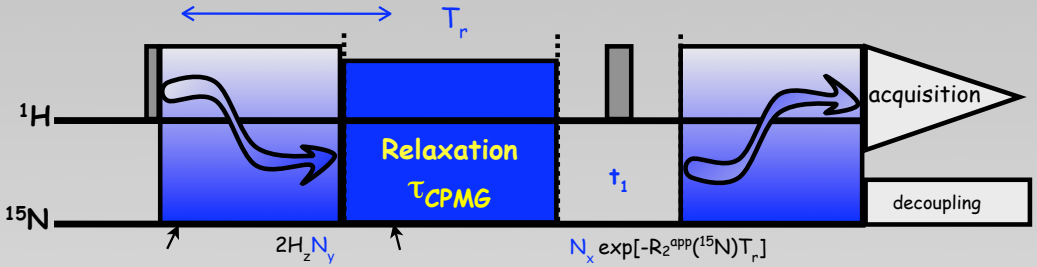
$R_2^{eff} = f(\nu_{CPMG})$



The method : relaxation dispersion NMR experiments

First analysis : qualitative analysis of relaxation dispersion curves



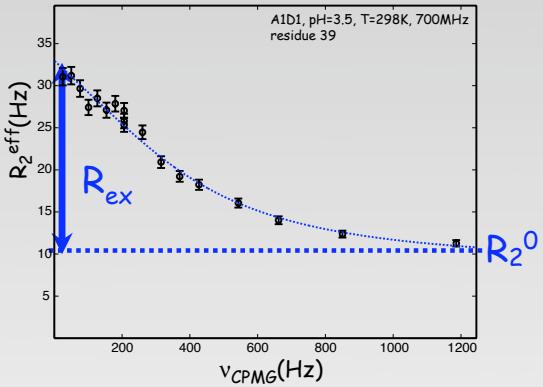


$$R_2^{measured} = R_2^0 + R_{ex}(p_A, p_B, k_{ex}, \Delta\omega, \tau_{CPMG})$$

$$\frac{I(T_r)}{I_0} = \exp[-(R_2^0 + R_{ex})T_r]$$

$$R_2^{measured}(\nu_{CPMG}) = -\frac{1}{T_r}\ln\left[\frac{I(T_r, \nu_{CPMG})}{I_0}\right]$$

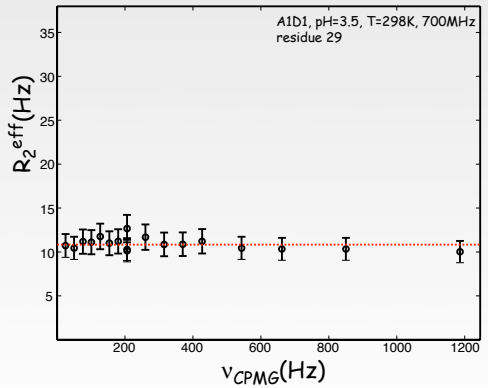
$$T_r = 2n\tau_{CPMG} = \frac{n}{\nu_{CPMG}}$$



- First estimation of R_{ex}

  $R_{ex\ (estimated)} = R_2^{eff}(\nu_{min}) - R_2^{eff}(\nu_{max})$

- Fitting of the dispersion curve
  - R_{ex}, R_2^0
  - structural parameter $\Delta\omega$
  - thermodynamic parameter p_A, p_B
  - kinetic parameter : $k_{ex}=1/\tau_{ex}$
    - $k_{ex}(T) = k_0\exp(-E_a/R_BT)$



The method : relaxation dispersion NMR experiments

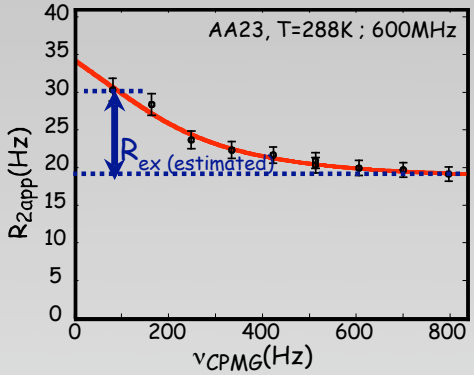
First analysis : qualitative analysis of relaxation dispersion curves

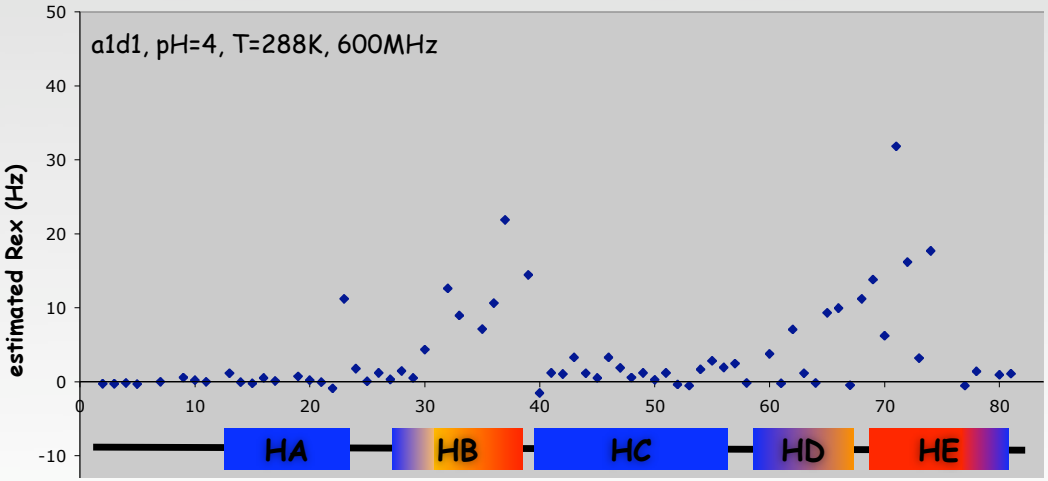
- First estimation of R_{ex}

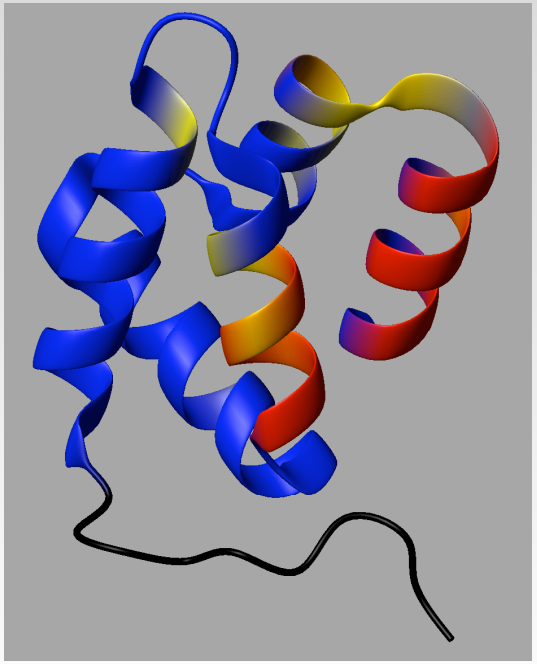
$$R_{ex\,(estimated)} = R_2^{eff}(\nu_{min}) - R_2^{eff}(\nu_{max})$$

- Fitting of the dispersion curve
  - R_{ex}, R_2^0
  - $\Delta\omega$, p_A
  - $k_{ex}=1/\tau_{ex}$

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





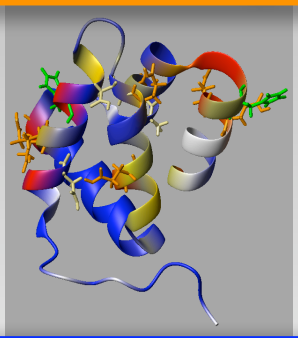


The method : relaxation dispersion NMR experiments

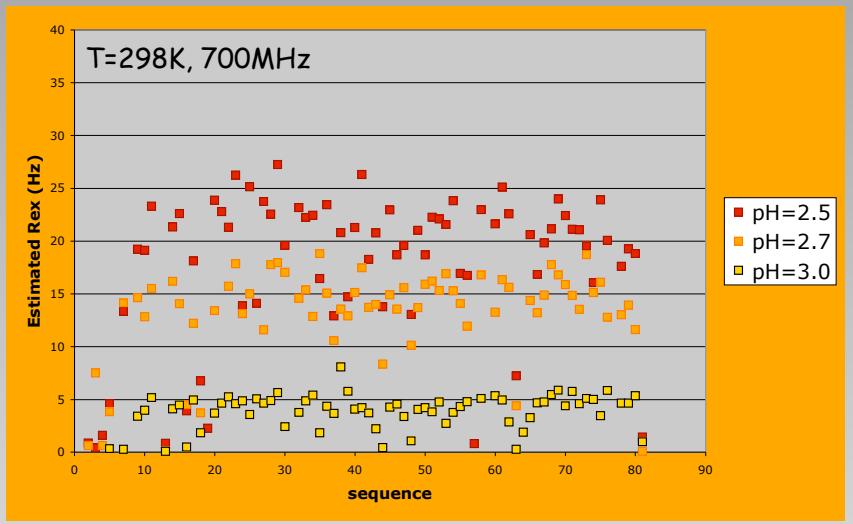
First analysis : qualitative analysis of relaxation dispersion curves

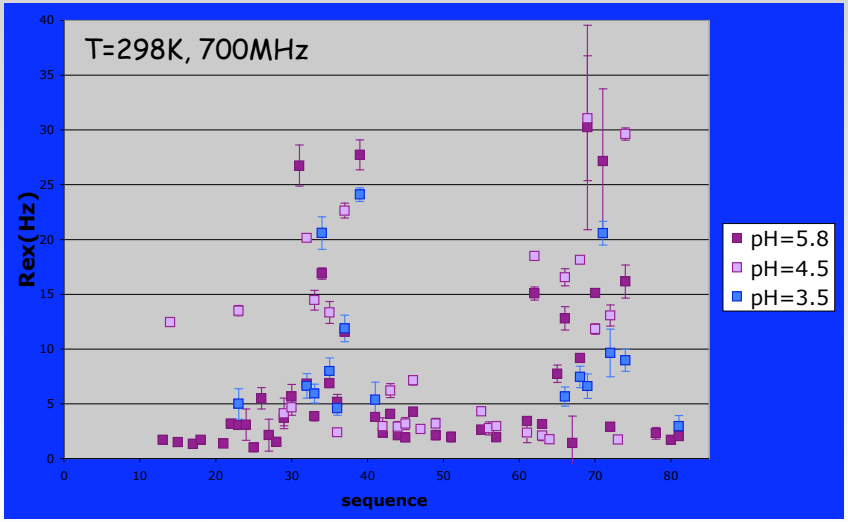
An example : variation of exchange contribution as a function of pH

pH < 3
Global conformational exchange
☞ Global Unfolding ?



pH > 3.5
Localized conformational exchange
☞ Local Unfolding ?





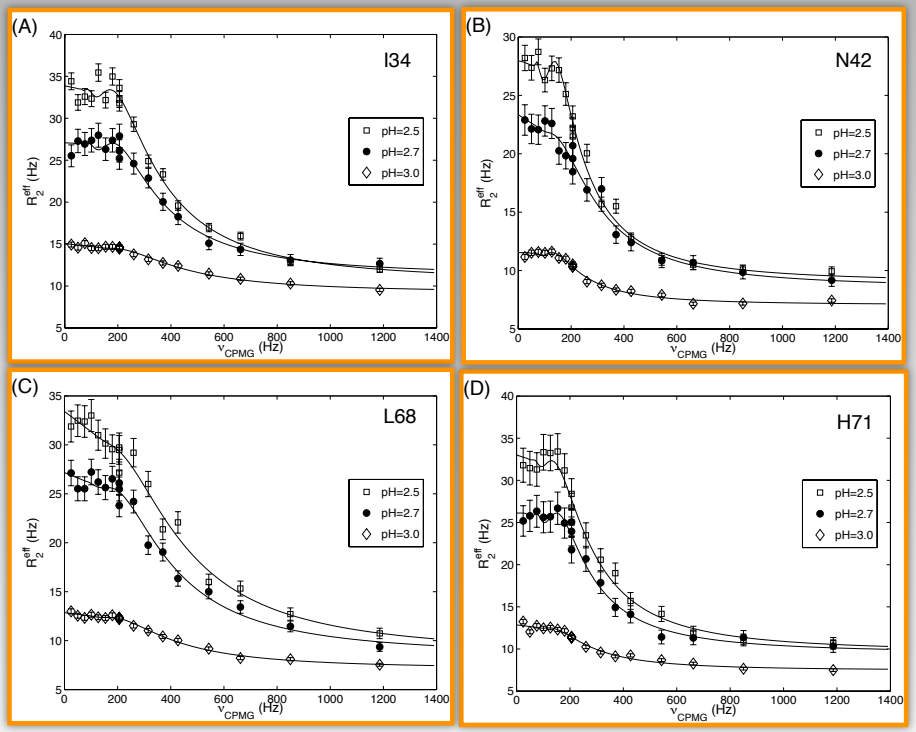
The method : relaxation dispersion NMR experiments

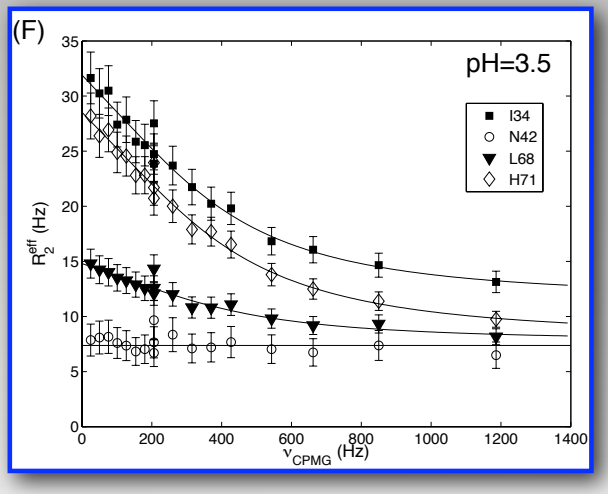
First analysis : qualitative analysis of relaxation dispersion curves

An example : variation of exchange contribution as a function of pH

pH < 3
Global conformational exchange

pH > 3.5
Localized conformational exchange





☞ Physical parameters of these exchange processes ?

The method : relaxation dispersion NMR experiments

Quantitative evaluation of physical parameters of exchange from the relaxation dispersion data

The analysis depends of the timescale of the process

- ☞ Fast exchange : $\Delta\omega \ll k_{ex}$
  - ⇨ One peak $\omega = p_A\omega_A + p_B\omega_B$
  - ⇨ $R_{ex} \propto B_0^2$
  - ⇨ Meiboom-Gill equation
  - ⇨ k_{ex}, $\Phi=p_Ap_B\,\Delta\omega^2$, R_{inf}
- ☞ Intermediate exchange : $\Delta\omega \sim k_{ex}$
  - ⇨ One or two broadened peaks
  - ⇨ $R_{ex} \propto B_0$
  - ⇨ Carver-Richards equation
  - ⇨ k_{ex}, p_B, $\Delta\omega$, R_{inf}
- ☞ Slow exchange : $\Delta\omega \gg k_{ex}$
  - ⇨ Two peaks
  - ⇨ R_{ex} independant of B_0
  - ⇨ Tollinger equation
  - ⇨ k_a, $\Delta\omega$, R_{inf}
  - ⇨ $R_{ex}^{(B)} \rightarrow k_{BA}=p_Ak_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_Bk_{ex}$
    $R_{ex}(B) \gg R_{ex}(A)$

☞ Detection of minor populations from the broadenning of the major state resonance : use of Carver-Richards equation

p_A=0.2 ; p_B=0.8

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

$k_{ex}/\Delta\nu$

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

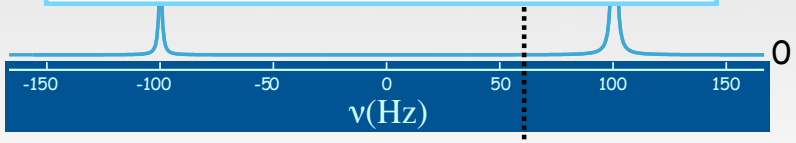
$$D_+ = 0.5\left[1 + \frac{\phi + 2\Delta\omega^2}{\sqrt{\phi^2+\zeta^2}}\right] ; D_- = 0.5\left[-1 + \frac{\phi + 2\Delta\omega^2}{\sqrt{\phi^2+\zeta^2}}\right]$$

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

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

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

$$R_2^{app} = R_2^{inf} + k_a\left(1 - \frac{\sin\Delta\omega.\tau_{CP}}{\Delta\omega.\tau_{CP}}\right)$$



$p_A\nu_A+p_B\nu_B$

The method : relaxation dispersion NMR experiments

Quantitative evaluation of physical parameters of exchange from the relaxation dispersion data

The analysis depends
of the timescale of the process

- Fast exchange : $\Delta\omega \ll k_{ex}$
  - One peak $\omega = p_A\omega_A + p_B\omega_B$
  - $R_{ex} \propto B_0^2$
  - Meiboom-Gill equation
  - k_{ex}, $\Phi=p_Ap_B\Delta\omega^2$, R_{inf}

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

- Intermediate exchange : $\Delta\omega \sim k_{ex}$
  - One or two broadened peaks
  - $R_{ex} \propto B_0$
  - Carver-Richards equation
  - k_{ex}, p_B, $\Delta\omega$, R_{inf}

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

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

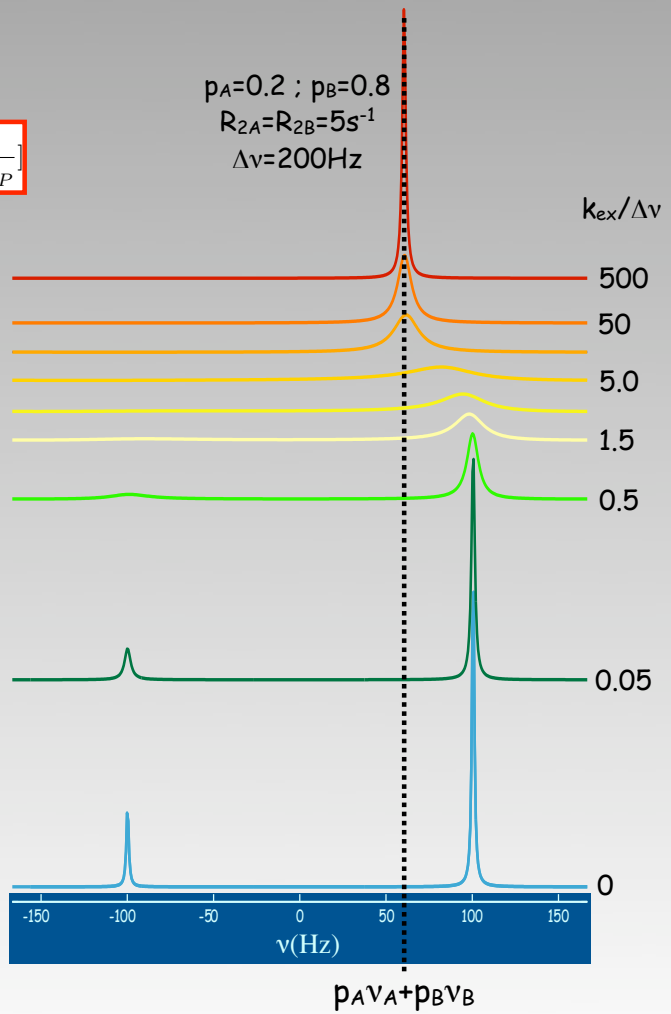
$$\eta_+ = \frac{\tau_{CP}}{\sqrt{(2)}}\sqrt{\phi + \sqrt{\phi^2+\zeta^2}} \; ; \; \eta_- = \frac{\tau_{CP}}{\sqrt{(2)}}\sqrt{-\phi + \sqrt{\phi^2+\zeta^2}}$$

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

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

- Slow exchange : $\Delta\omega \gg k_{ex}$
  - Two peaks
  - R_{ex} independant of B_0
  - Tollinger equation
  - k_a, $\Delta\omega$, R_{inf}
  - $R_{ex}^{(B)} \rightarrow k_{BA}=p_Ak_{ex}$; $R_{ex}^{(A)} \rightarrow k_{AB}=p_Bk_{ex}$
    $R_{ex}(B) \gg R_{ex}(A)$

$$R_2^{app} = R_2^{inf} + k_a\left(1 - \frac{\sin\Delta\omega.\tau_{CP}}{\Delta\omega.\tau_{CP}}\right)$$

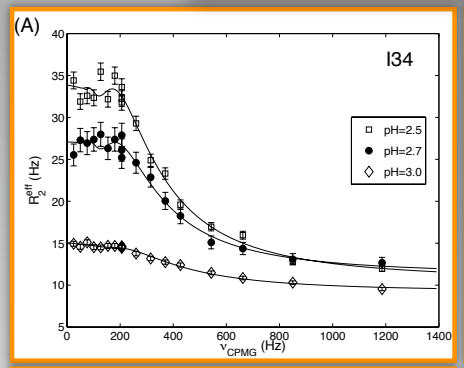


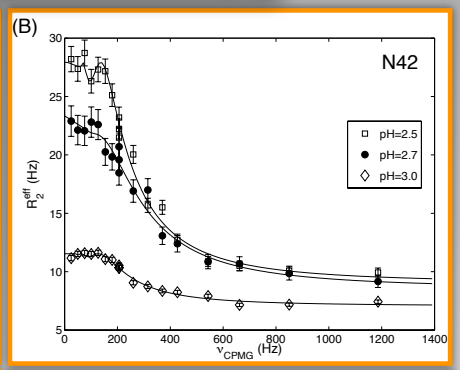
- Detection of minor populations from the broadenning of the major state resonance : use of Carver-Richards equation

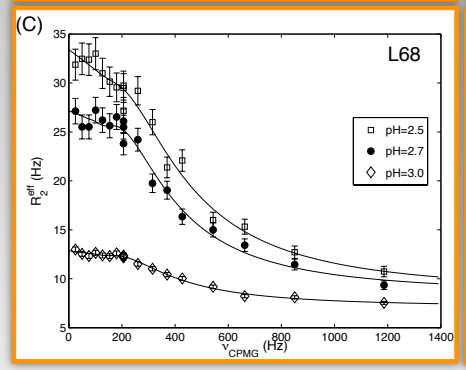
The method : relaxation dispersion NMR experiments

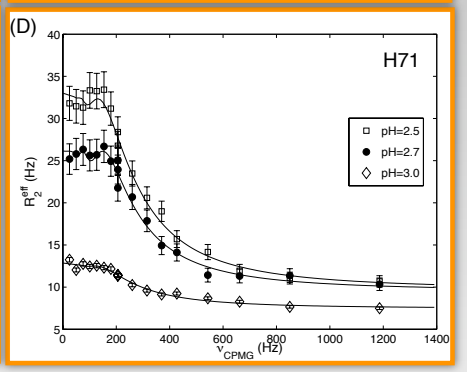
Quantitative evaluation of physical parameters of exchange from the relaxation dispersion data

pH < 3
Global conformational exchange





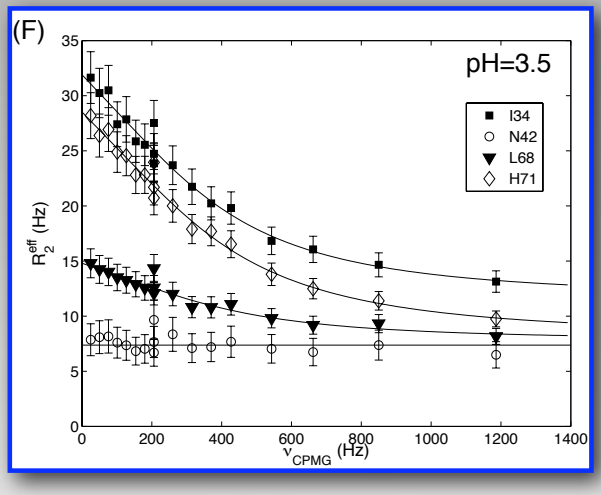




☞ Intermediate/Slow exchange regime

$p_B \ll p_A$

pH > 3.5
Localized conformational exchange

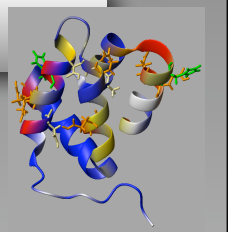


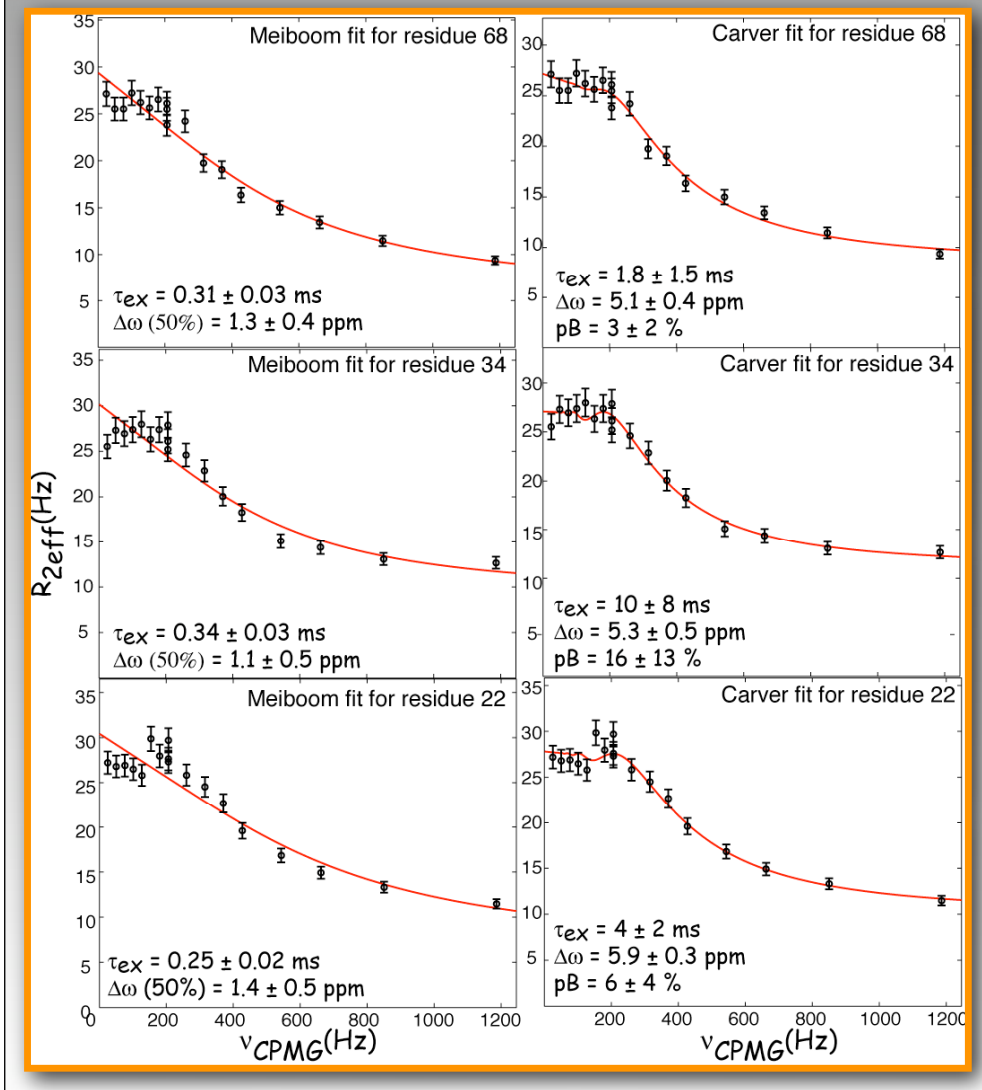
☞ Fast exchange regime

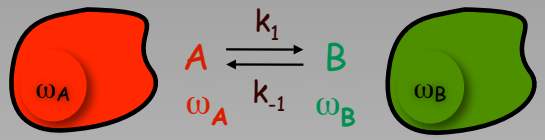
$p_B \simeq p_A$

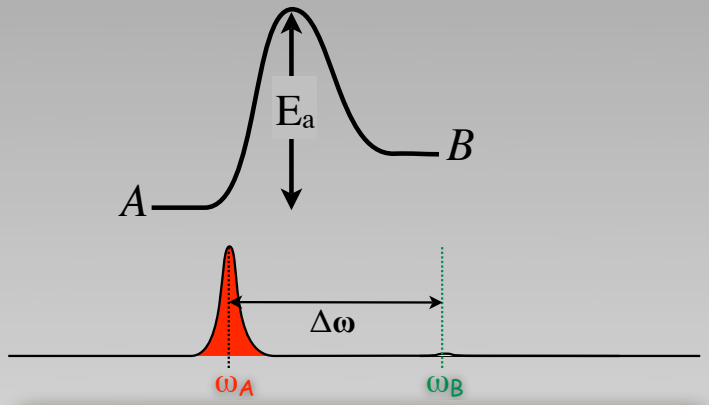
The method : relaxation dispersion NMR experiments

relaxation dispersion experiments for the analysis of "invisible" states









pH < 3

- ☞ Individual fits
  - ➪ Carver statistically favored
  - ➪ τ_{ex} : few ms
  - ➪ $p_B << p_A$
- ☞ Global exchange
  - ➪ global τ_{ex}, p_B
  - ➪ individual $\Delta\omega$, R_{inf}

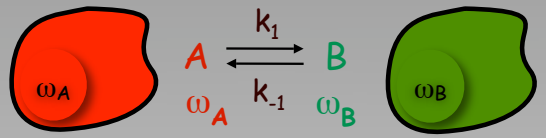
The method : relaxation dispersion NMR experiments

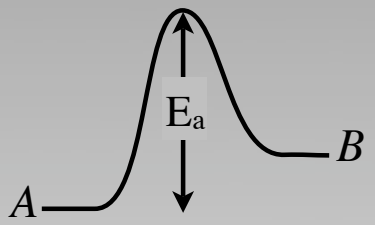
relaxation dispersion experiments for the analysis of "invisible" states

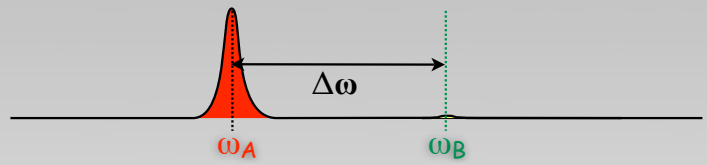
pH < 3

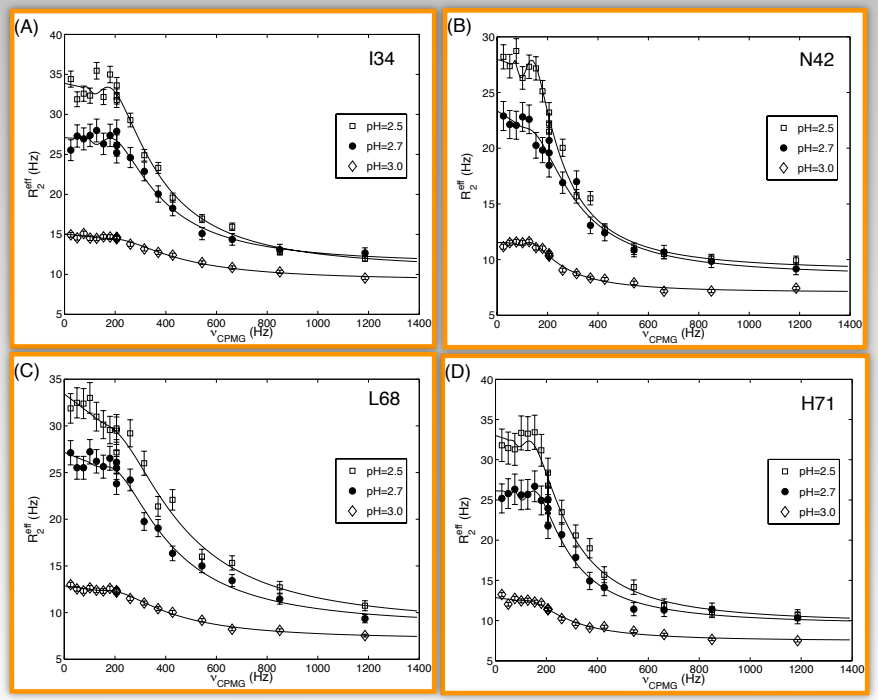
☞ Global exchange

- τ_{ex} = 4.0 ± 0.2 ms (pH=2.5)
- p_B = 1% (pH=3) ➟10% (pH=2.5)
- Individual $\Delta\omega(^{15}N)$ = 0↔8 ppm









☞ Broadening of the major component gives information about a minor, invisible state

☞ Nature of the unfolded state ?

The method : relaxation dispersion NMR experiments

relaxation dispersion experiments for the analysis of "invisible" states

pH < 3

☞ Global unfolding

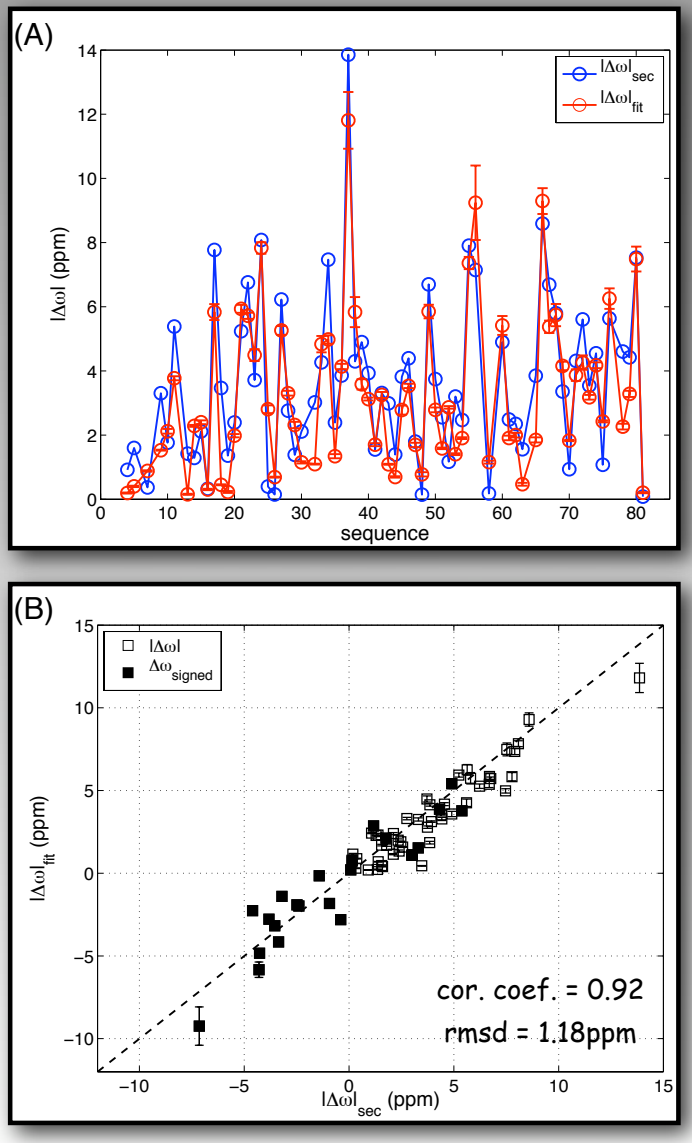
- τ_{ex} = 2-5 ms
- p_B = 1% (pH=3) ➟10% (pH=2.5)
- $\Delta\omega(^{15}N)$ = 0↔8 ppm

☞ Nature of the unfolded state ?

$\Delta\omega_{(fitted)}$ vs Secondary Shifts

☞ "Fully" unfolded state

☞ Two-state unfolding process

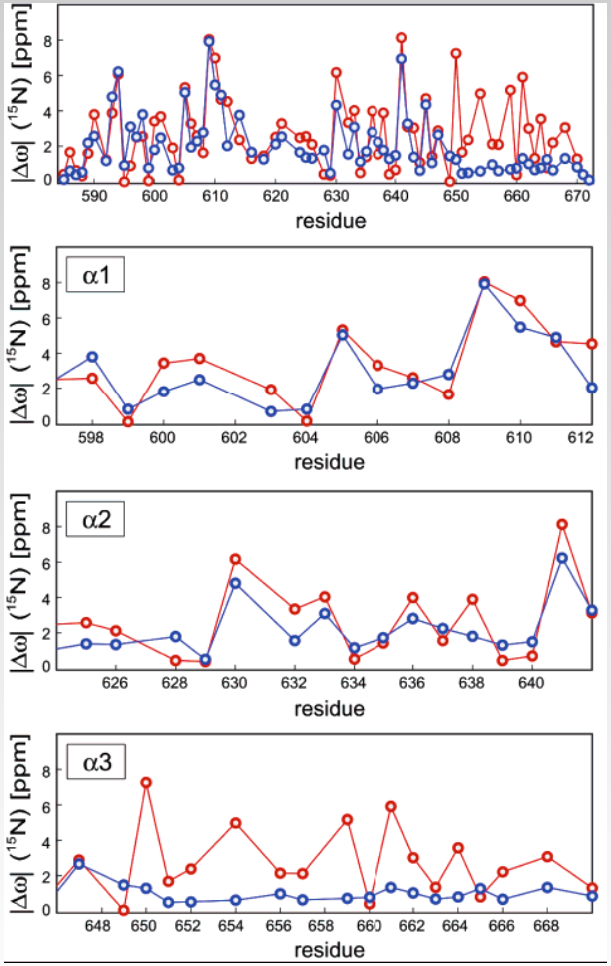


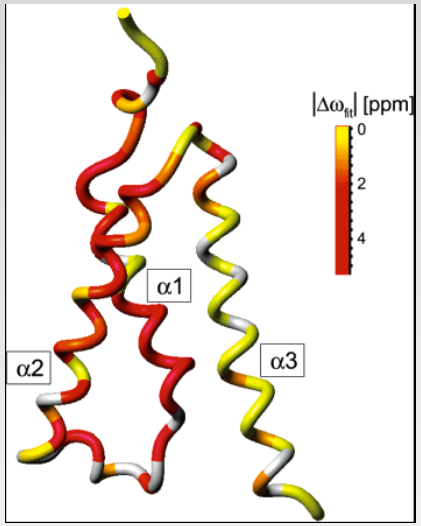
Schwarzinger, S. et *al.*, *J. Am. Chem. Soc.* (2001), **123**, 2970
van Ingen, H. et *al.*, *J. Am. Chem. Soc.* (2006), **128**, 3856

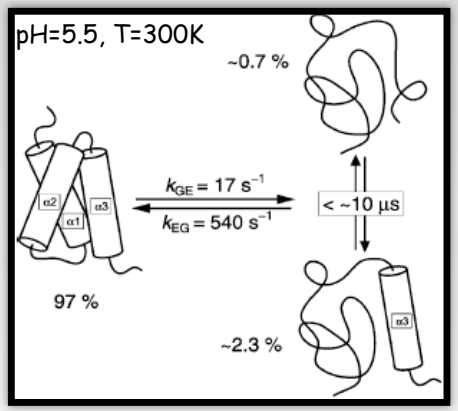
The method : relaxation dispersion NMR experiments

relaxation dispersion experiments for the analysis of "invisible" states

An Isolated Helix Persists in a Sparsely Populated Form of KIX under Native Conditions







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

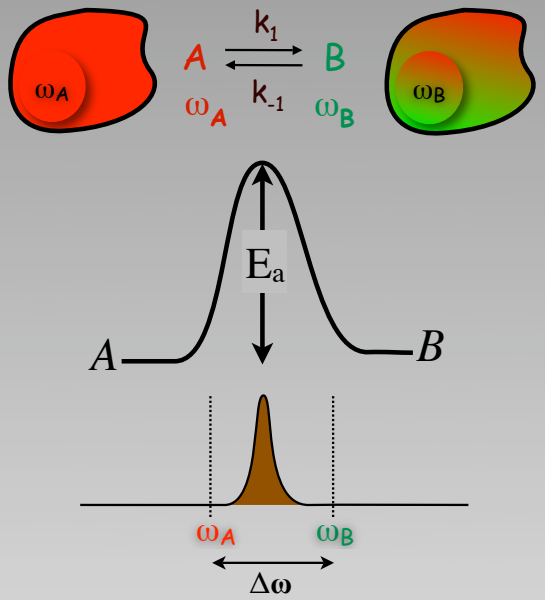
The method : relaxation dispersion NMR experiments

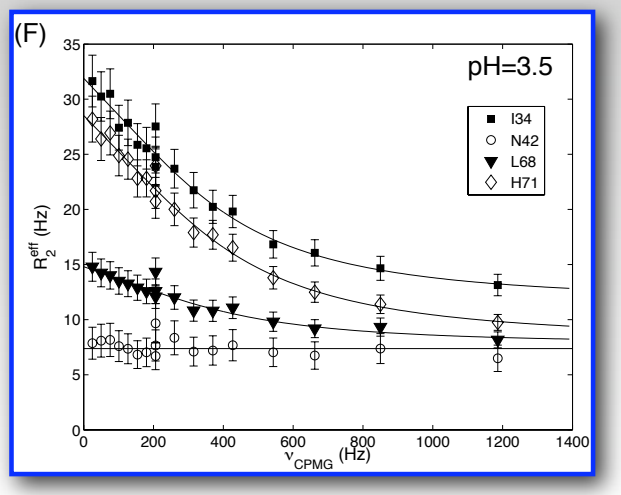
Quantitative evaluation of physical parameters of exchange from the relaxation dispersion data

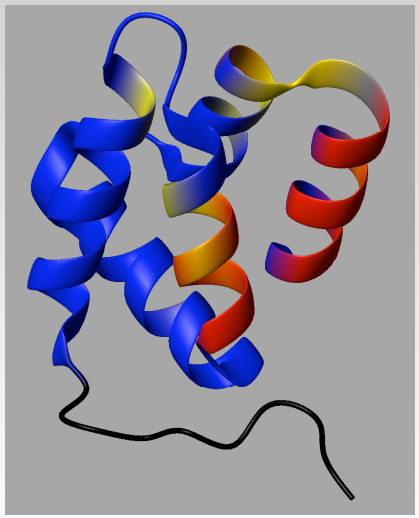
3.5 < pH < 7

☞ Localized exchange

- ⇨ Meiboom statistically favored (fast exchange)
- ⇨ τ_{ex} ~ 0.5 ms (pH=4.5)
- ⇨ $p_B \simeq p_A$
- ⇨ $\Delta\omega(^{15}N)$ ~ 0↔3 ppm







μs-ms motions by NMR
conformational exchange processes and proteins function

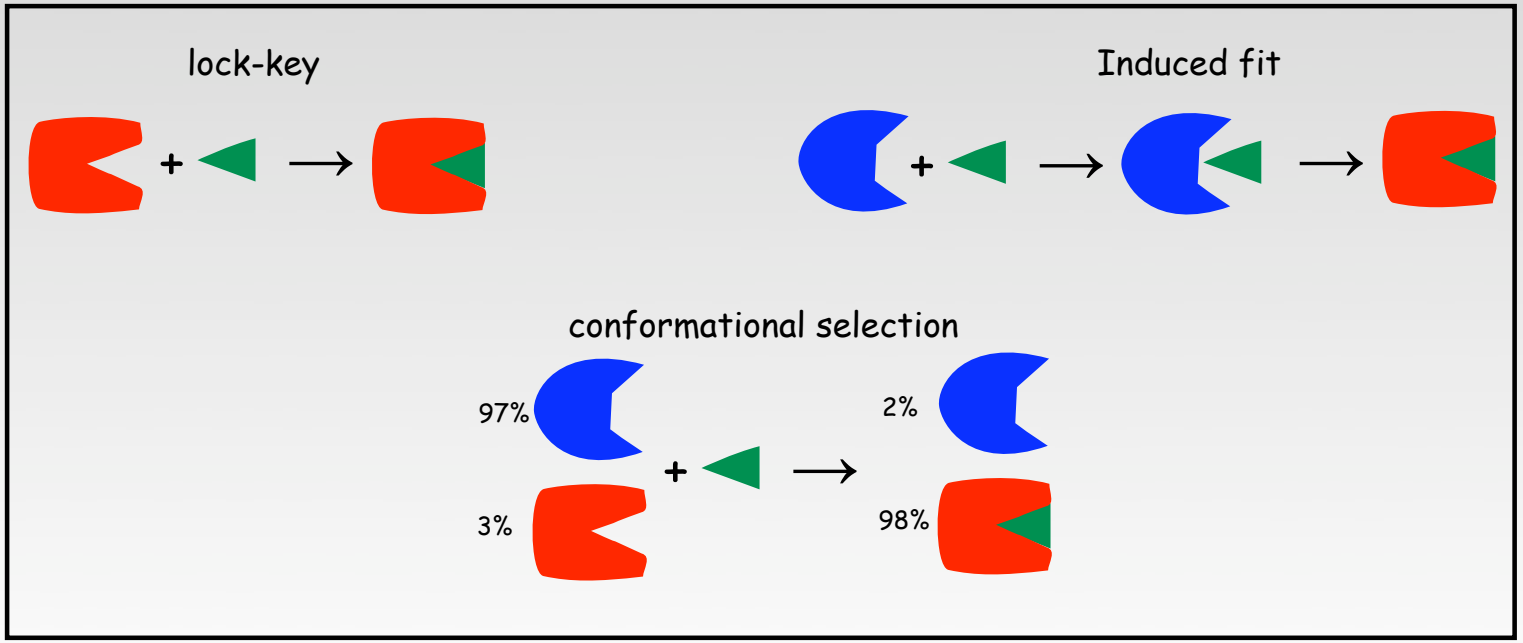
- Folding/unfolding processes
  - sequence/stability/flexibility relationship
  - low populated states

    Transition state ensemble, minor folded/unfolded states

- Motions and function
  - enzymatic cycle
    - low populated (high energy) states
  - regulation/signalization associated with conformational changes
  - interactions

μs-ms motions and proteins function

- Proteins : multiple conformers at thermal equilibrium in solution
  - XRay cristallography : Selection of one conformation (low energy) in the cristallisation process
  - NMR : time averaged conformational ensemble
- Ground state > 90%
- High energy states, low populated : roles in substrates binding, enzymatic cycles, folding processes (transition states) ...



Boehr et al, Chem. Rev. 2006, 106, 3055-3079

μs-ms motions and proteins function : Practical course

- Analysis of the relaxation dispersion pulse sequence
  - The relaxation compensated CPMG scheme
  - Experimental protocol

- The understanding of molecular mechanisms underlying proteins functions necessitates the characterization of the dynamics of the macromolecular systems, and of the minor transient states along the functional pathways.
- Some recently published studies based on the use of relaxation dispersion experiments to investigate these questions will be discussed.