NMR Relaxation and Molecular Dynamics

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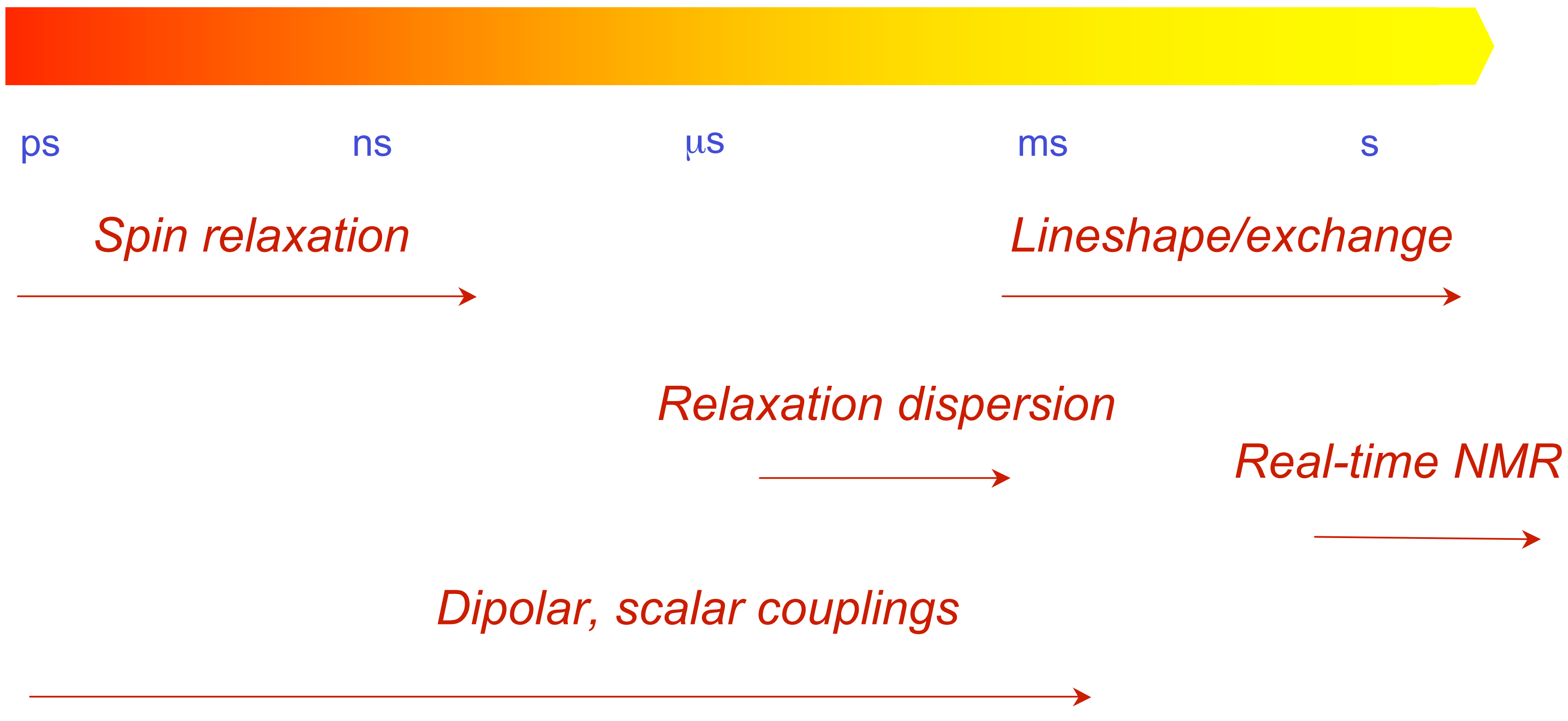
Solution NMR Timescales for Biomolecular Motion



ps ns μs ms s

Spin relaxation

Lineshape/exchange

Relaxation dispersion

Real-time NMR

Dipolar, scalar couplings

Librational motion
Normal modes
Rotational diffusion

Enzyme catalysis
Signal transduction
Ligand binding
Collective motions?

Protein folding
Hinge Bending

Solution NMR Timescales for Biomolecular Motion

Spin relaxation

Provides site specific information about backbone and sidechain dynamics throughout the protein

Established experimental and analytical procedures allow routine extraction of motional description, or parameterisation in terms of amplitudes (S^2) and characteristic timescales (τ_i)

Information is limited to motions occurring faster than the rotational correlation time of the protein (τ_c~ 5-30ns)

Solution NMR Timescales for Biomolecular Motion

Provides site specific information about conformational exchange occurring in the 50μs-1ms range

Precise determination of rates of exchange. Association with temperature dependent measurements allows for thermodynamic characterisation of exchange processes

Detection and characterisation of weakly populated or ‘invisible’ species

Structural information is ‘limited’ to interpretation of chemical shift differences between exchanging conformations

Solution NMR Timescales for Biomolecular Motion

ps ns μs ms s

Real time

Rapid data acquisition techniques allow events to be monitored in real-time

Time resolution at the level of seconds

Protein folding and unfolding

Reaction kinetics

Solution NMR Timescales for Biomolecular Motion

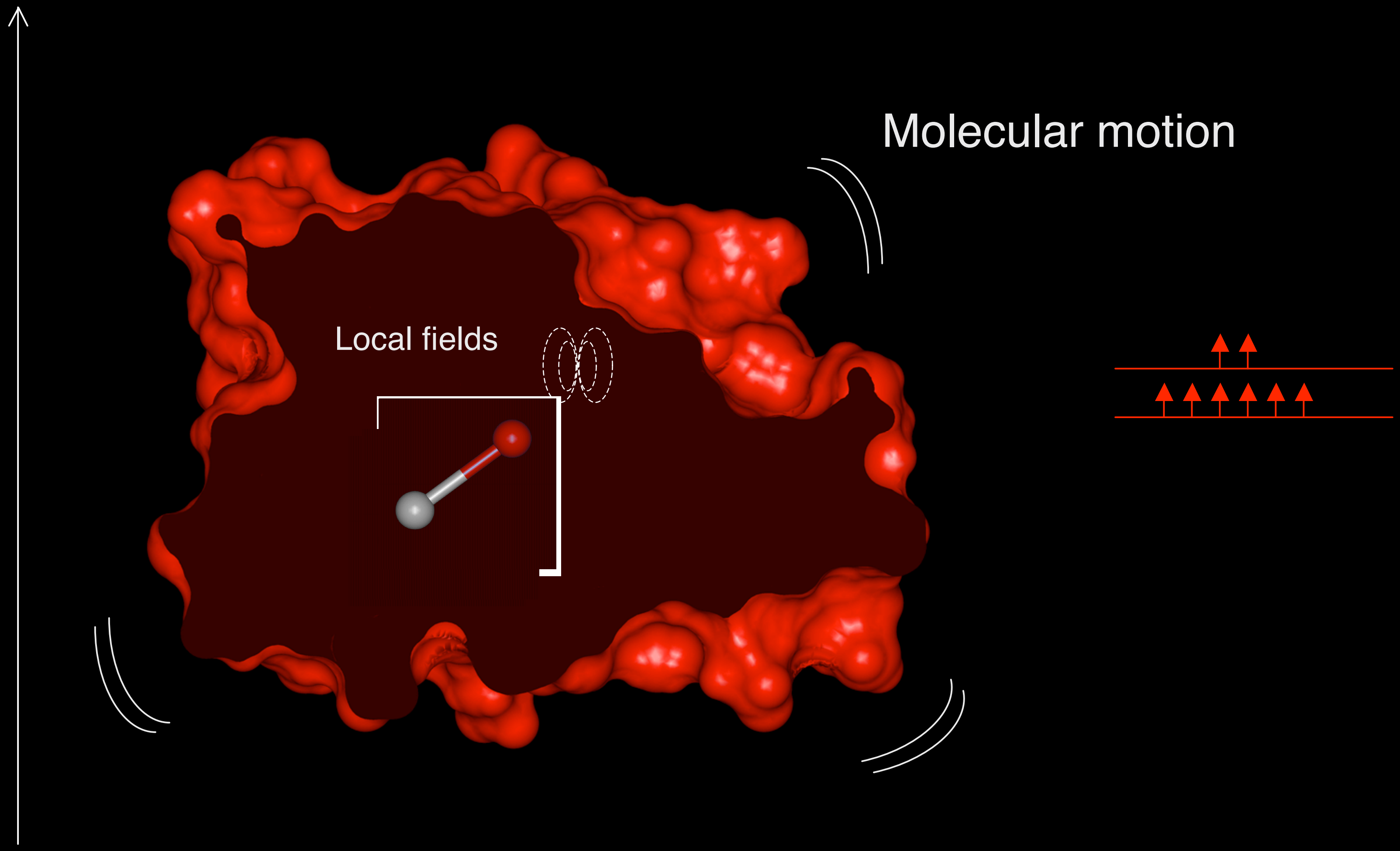
Scalar, Dipolar Couplings, Chemical shift

NMR dipolar couplings provide a highly detailed description of conformational disorder occurring up to the millisecond timerange

Comparison with spin relaxation can reveal extent and nature of slower motions in proteins

Local and global molecular motion from spin relaxation

- Brief overview of the theory of spin relaxation : Important steps
- Interpretation of ^{15}N relaxation : Spectral density mapping / Modelfree analysis
- Description of molecular rotational diffusion tensors from ^{15}N relaxation
- Applications of spin relaxation to studies of protein dynamics



Molecular motion

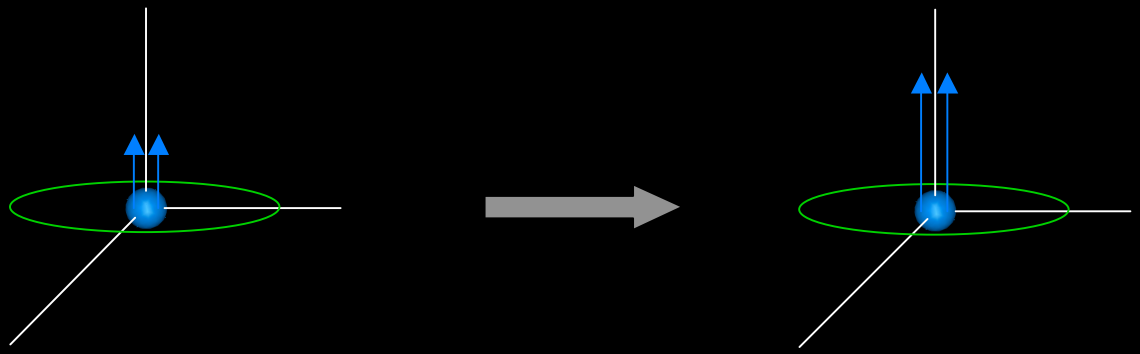
Local fields

Spin state relaxation

Relaxation of longitudinal and transverse magnetisation states

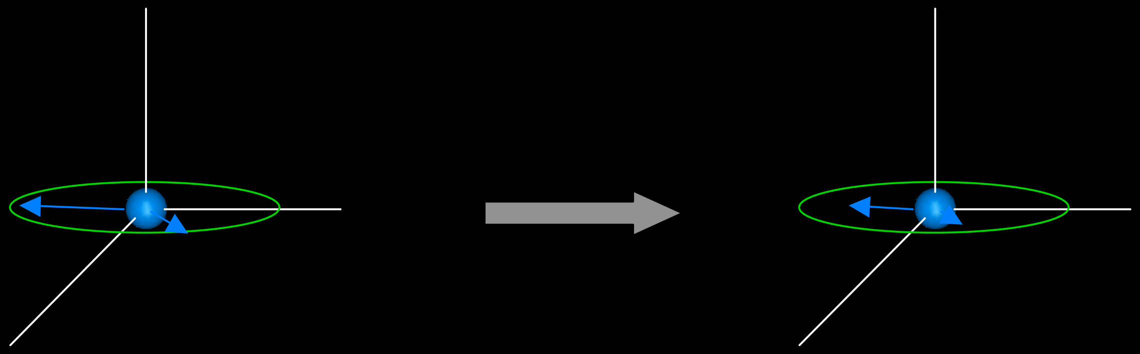
Determines delay between acquisitions

Longitudinal
Relaxation



Determines lifetime of the signal - linewidth of the resonance

Transverse
Relaxation

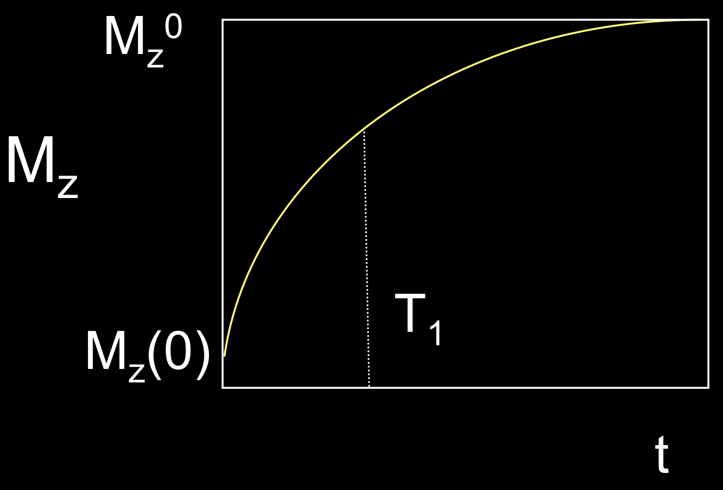


Longitudinal Relaxation

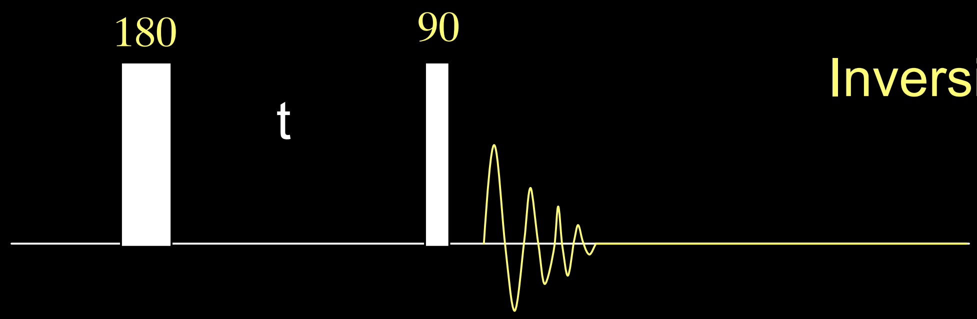
Rate equation for R_1 (R_z)

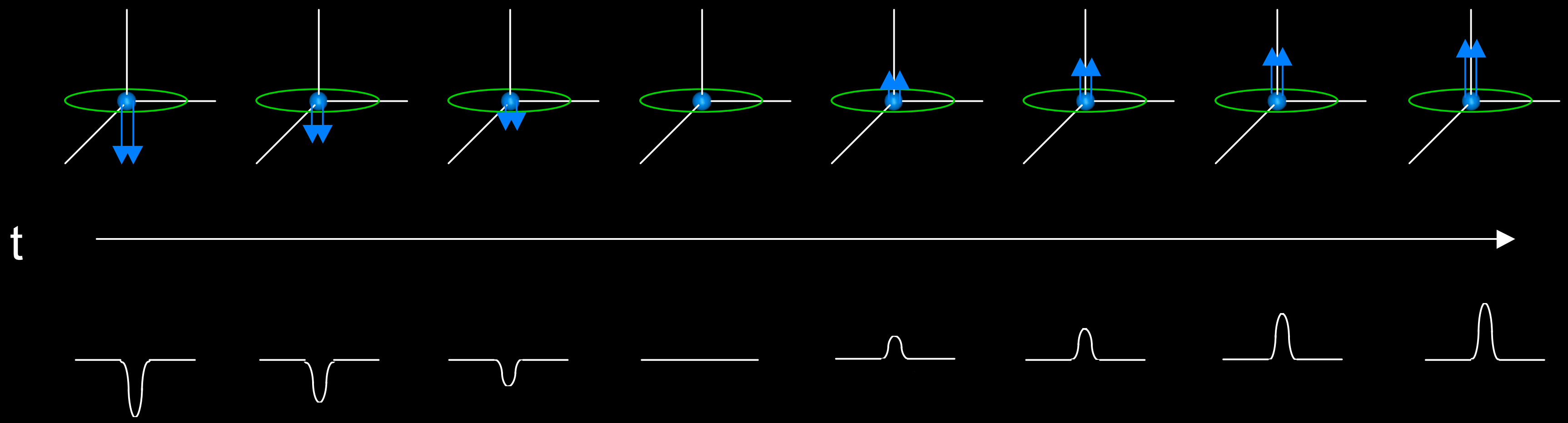
$$dM_z(t)/dt = -R_1(M_z(t) - M_z^0)$$

$$M_z(t) = (M_z(0) - M_z^0)\exp(-R_1 t) + M_z^0$$



Inversion Recovery

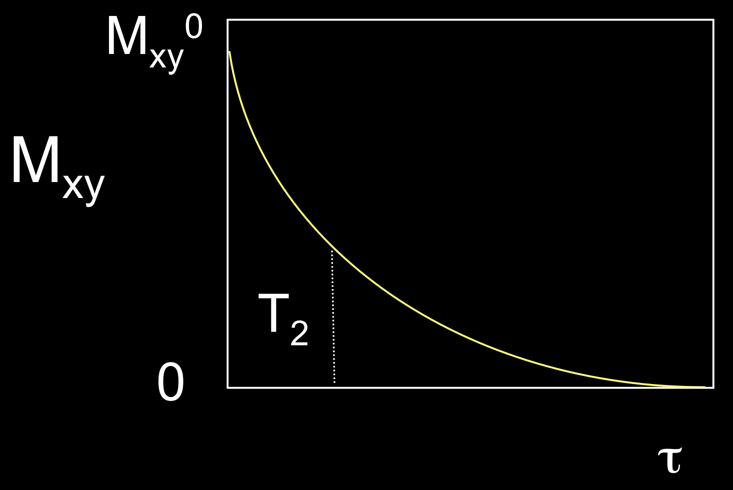




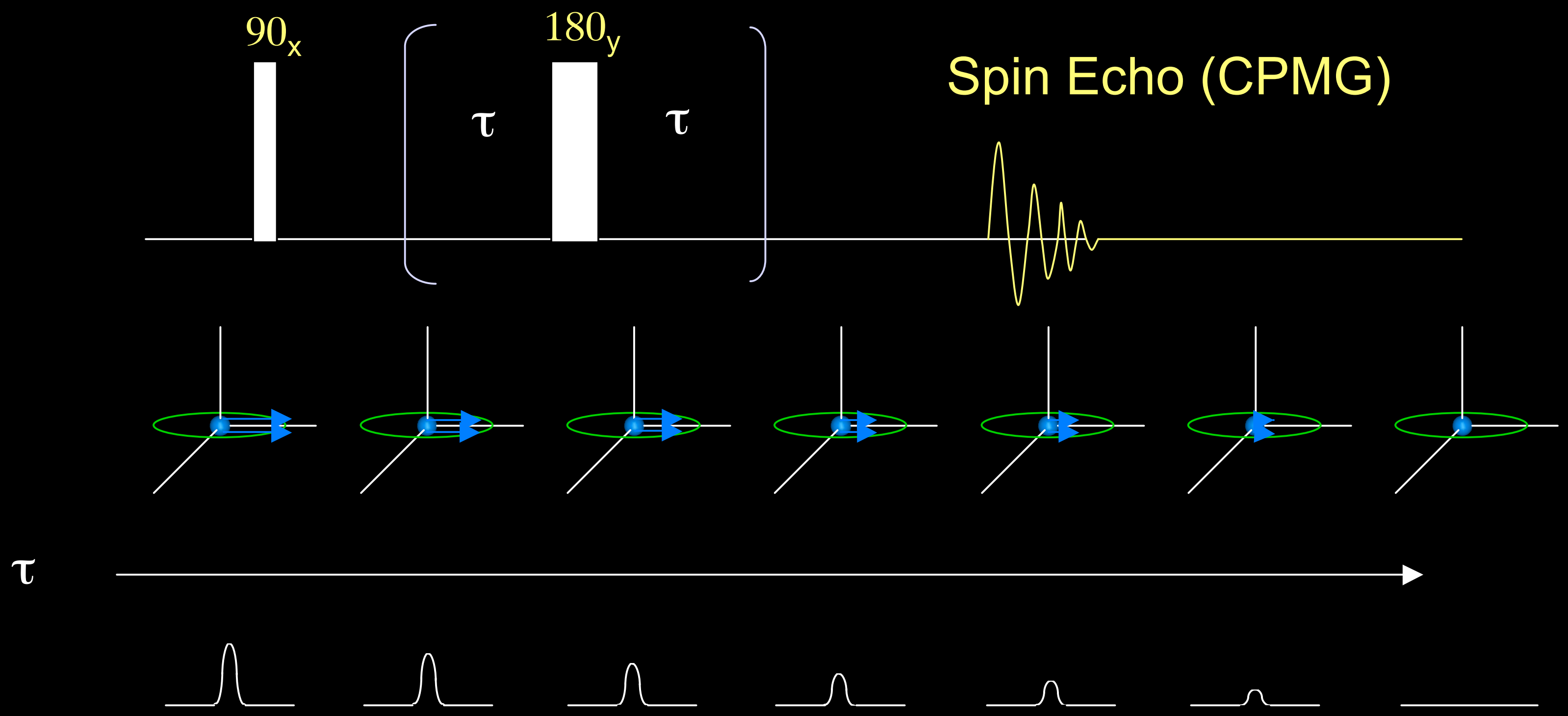
Transverse Relaxation

Rate equation for R_2 (R_x)

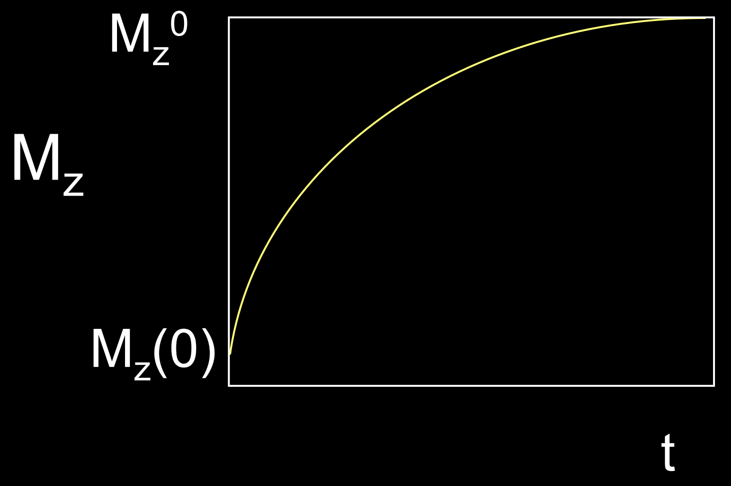
$$M_{xy}(\tau) = M_{xy}^0 \exp(-R_2\tau)$$

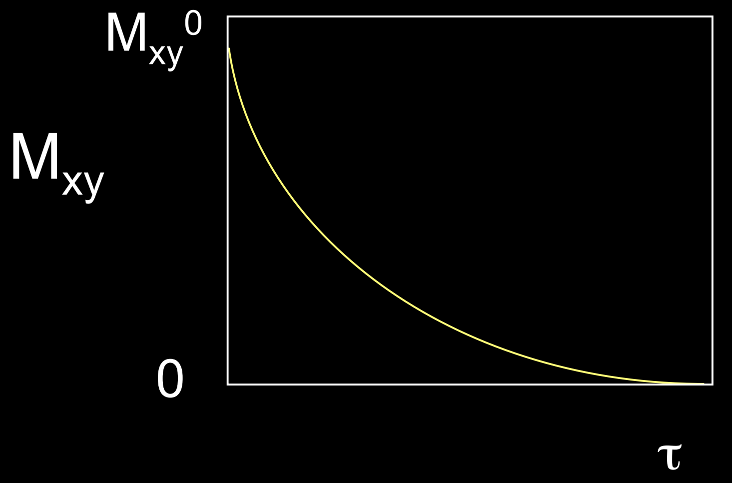


Spin Echo (CPMG)



Macroscopic Description : Bloch Equations





$$\frac{dM_z}{dt} = \gamma\left[\vec{M}(t)\times\vec{B}(t)\right]_z - R_1\left(M_z(t) - M_{eq}\right)$$

$$\frac{dM_x}{dt} = \gamma\left[\vec{M}(t)\times\vec{B}(t)\right]_x - R_2 M_x(t)$$

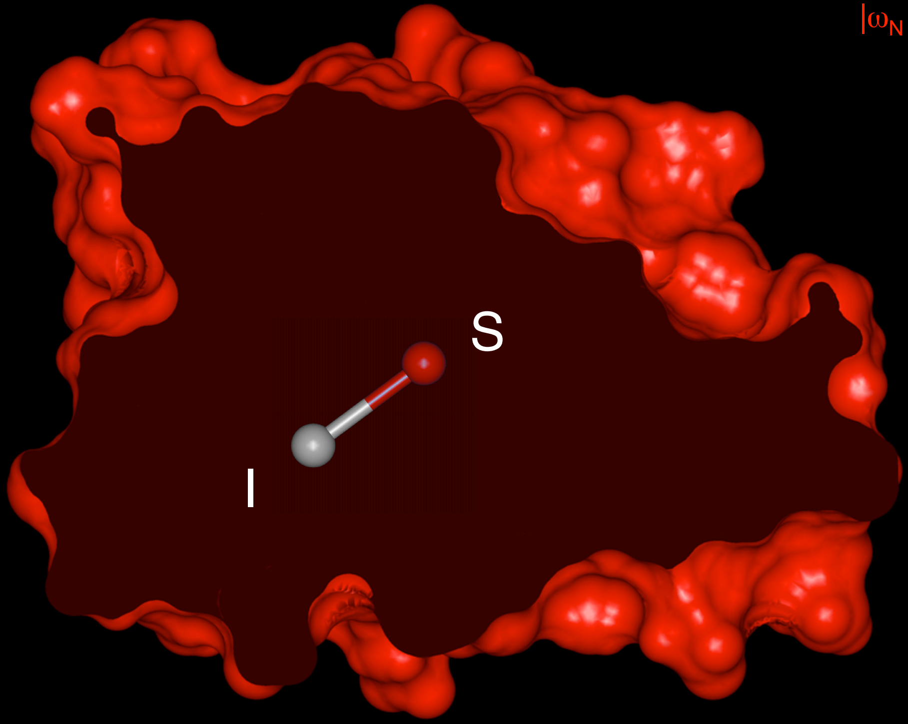
$$\frac{dM_y}{dt} = \gamma\left[\vec{M}(t)\times\vec{B}(t)\right]_y - R_2 M_y(t)$$

$$\boxed{\begin{array}{l} M_z \leftrightarrow \hat{I}_z \;;\; M_x \leftrightarrow \hat{I}_x \;;\; M_y \leftrightarrow \hat{I}_y \\ \hat{I}_x(t) = \left(\hat{I}_x \cos\delta t + \hat{I}_y \sin\delta t\right)e^{-R_2 t} \\ \hat{I}_y(t) = \left(\hat{I}_y \cos\delta t - \hat{I}_x \sin\delta t\right)e^{-R_2 t} \end{array}}$$

$$\frac{d2I_xS_z}{dt} = ? \qquad \frac{dI_xS_y}{dt} = ? \qquad \ldots$$

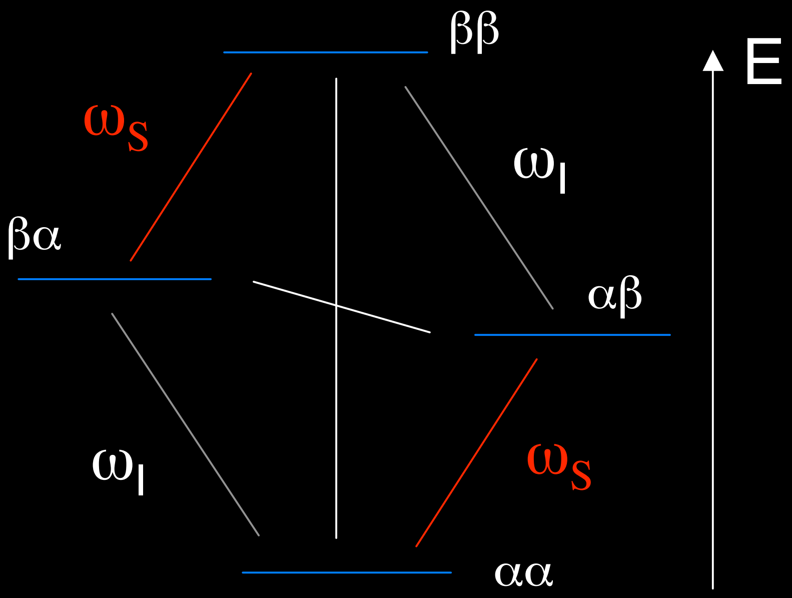
Microscopic description?

Transition Frequencies for Heteronuclear system I-S



$|\omega_H|=2\pi.600\text{MHz}\,;\,\tau_L(H)=265\text{ps}$

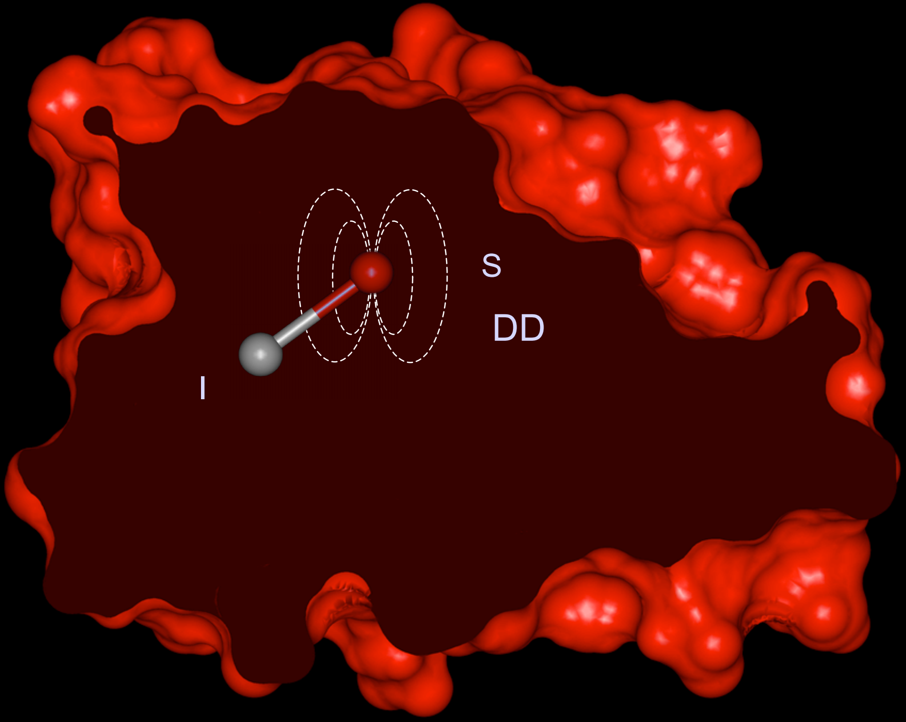
$|\omega_N|=2\pi.60\text{MHz}\,;\,\tau_L(N)=2.65\text{ns}$



Local fields fluctuating at the transition frequencies of the spin system can induce relaxation to equilibrium

Relaxation rates can be described in terms of the motion of the relaxation-active interactions

^{15}N relaxation (spin 1/2) relaxation active mechanisms are essentially :



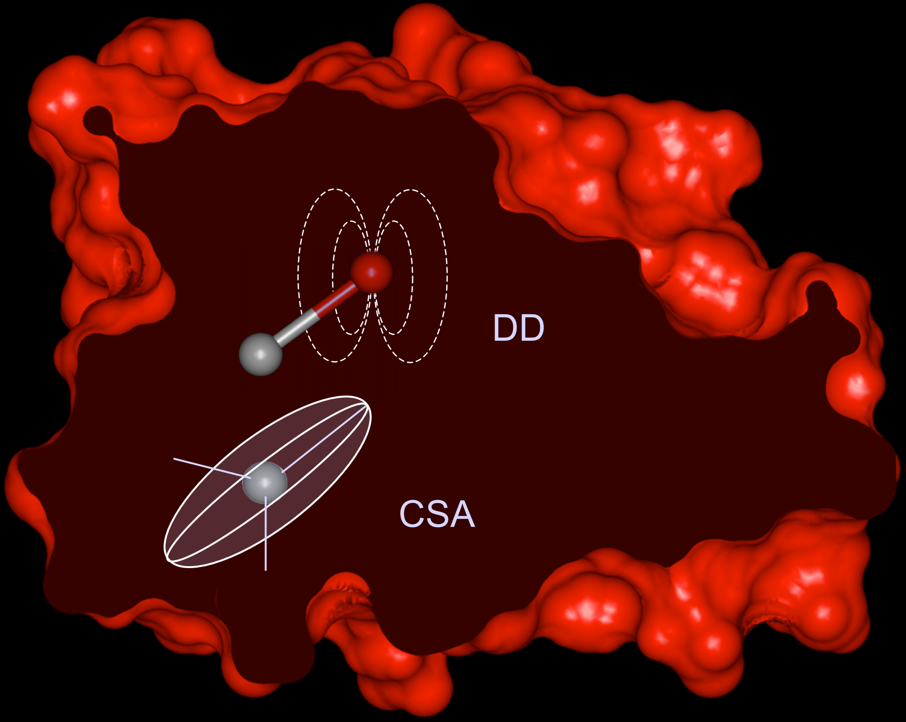
Dipole-dipole (DD)

Spin I experiences a distance and orientation dependent local field due to the magnetic moment of the nearby spin S

Source of fluctuating fields

Relaxation rates can be described in terms of the motion of the relaxation-active interactions

^{15}N relaxation (spin 1/2) relaxation active mechanisms are essentially :



Dipole-dipole (DD)

+

Anisotropic electronic environment - chemical shift anisotropy

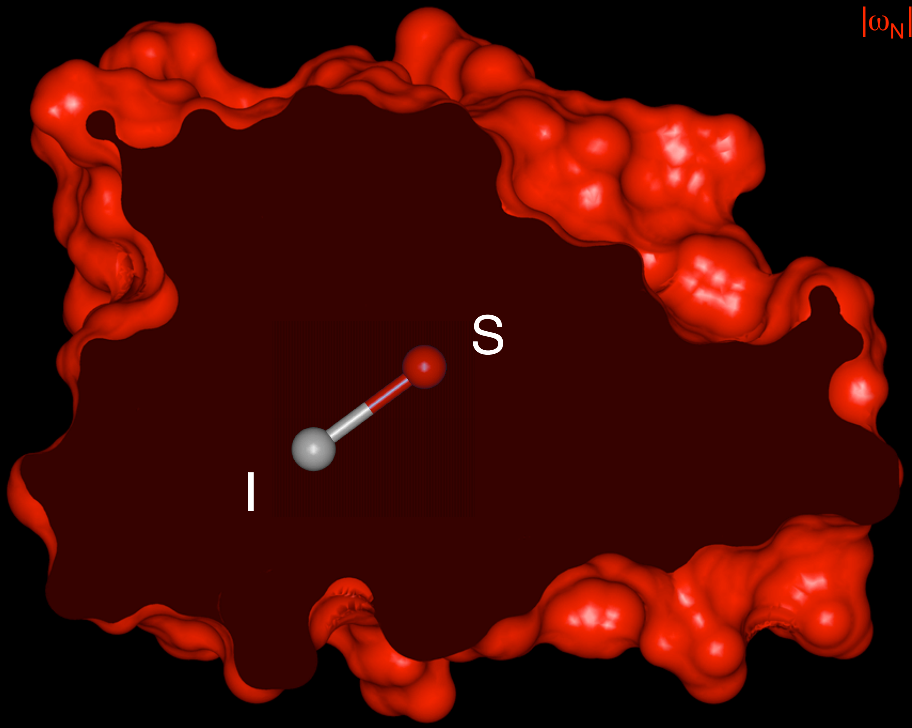
Assumed *axially symmetric* and coaxial with NH

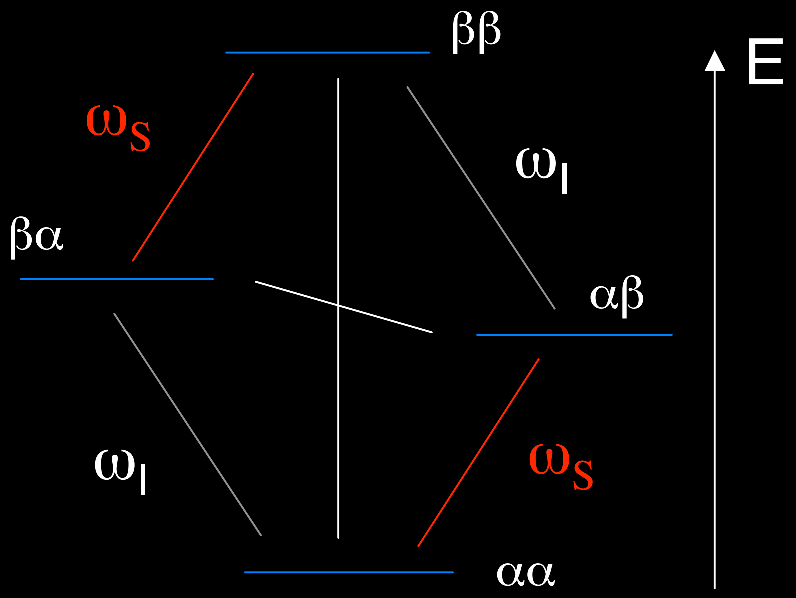
Source of fluctuating fields

Transition Frequencies for Heteronuclear system I-S

$|\omega_H|=2\pi.600\text{MHz}\,;\,\tau_L(\text{H})=265\text{ps}$

$|\omega_N|=2\pi.60\text{MHz}\,;\,\tau_L(\text{N})=2.65\text{ns}$





Local motion of the neighbouring spin (and CSA) at the transition frequencies of the spin system can induce relaxation to equilibrium

Transition Frequencies for Heteronuclear system I-S

$$\frac{\mathrm{d}n_1}{\mathrm{d}t} = -W_S^{(1)}n_1 - W_I^{(1)}n_1 - W_2 n_1 + W_S^{(1)}n_2 + W_I^{(1)}n_3 + W_2 n_4$$

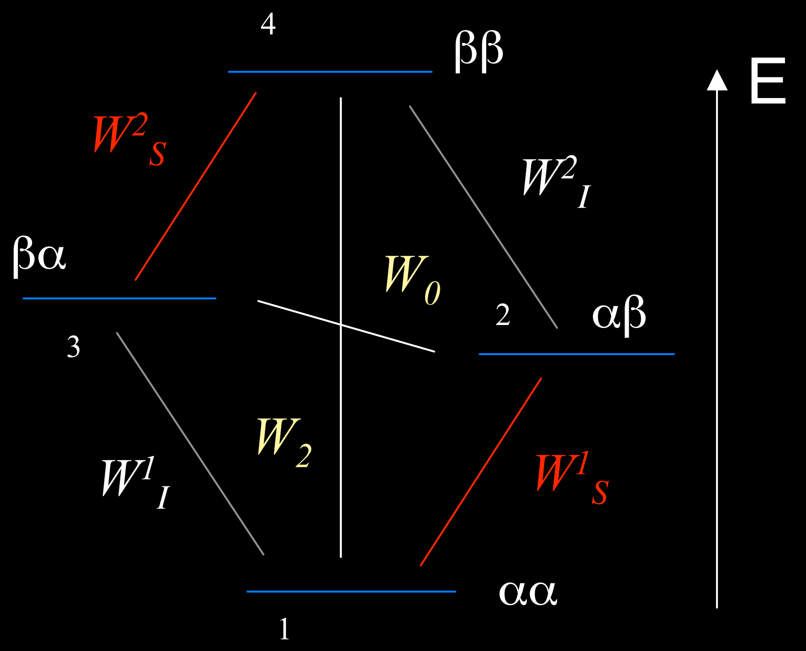
$$\frac{\mathrm{d}n_2}{\mathrm{d}t} = -W_S^{(1)}n_2 - W_I^{(2)}n_2 - W_0 n_2 + W_S^{(1)}n_1 + W_I^{(2)}n_4 + W_0 n_3$$

$$\frac{\mathrm{d}n_3}{\mathrm{d}t} = -W_I^{(1)}n_3 - W_S^{(2)}n_3 - W_0 n_3 + W_I^{(1)}n_1 + W_S^{(2)}n_4 + W_0 n_2$$

$$\frac{\mathrm{d}n_4}{\mathrm{d}t} = -W_S^{(2)}n_4 - W_I^{(2)}n_4 - W_2 n_4 + W_S^{(2)}n_3 + W_I^{(2)}n_2 + W_2 n_1$$

I spin magnetization : population difference between two I spin transitions (1-3), (2-4)

$$I_z = n_1 - n_3 + n_2 - n_4$$



W - rate constants for transitions

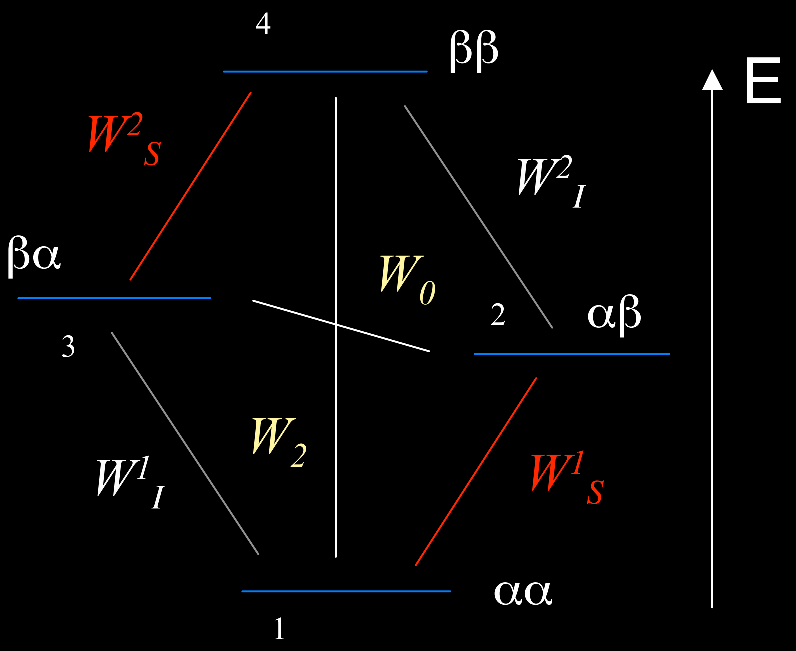
Transition Frequencies for Heteronuclear system I-S

Rate equations for magnetizations :

$$\frac{\mathrm{d}I_z}{\mathrm{d}t} = -\left(W_I^{(1)} + W_I^{(2)} + W_2 + W_0\right)I_z \qquad R_{1I}$$

$$\sigma_{IS} \quad -\left(W_2 - W_0\right)S_z - \left(W_I^{(1)} - W_I^{(2)}\right)2I_zS_z$$

(I)Solomon equations



R_{1I} - Auto relaxation rate constant

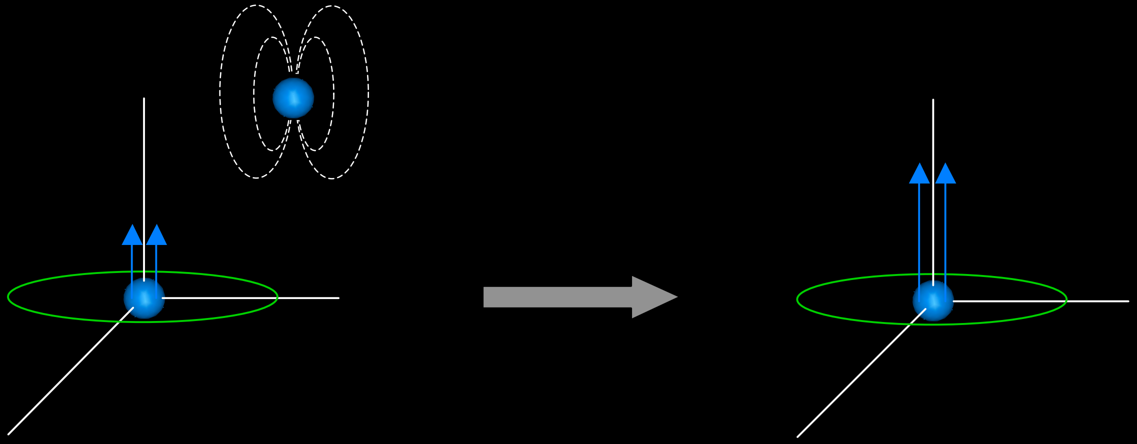
σ_{IS} - Cross relaxation rate constant :

rate of transfer of magnetization from

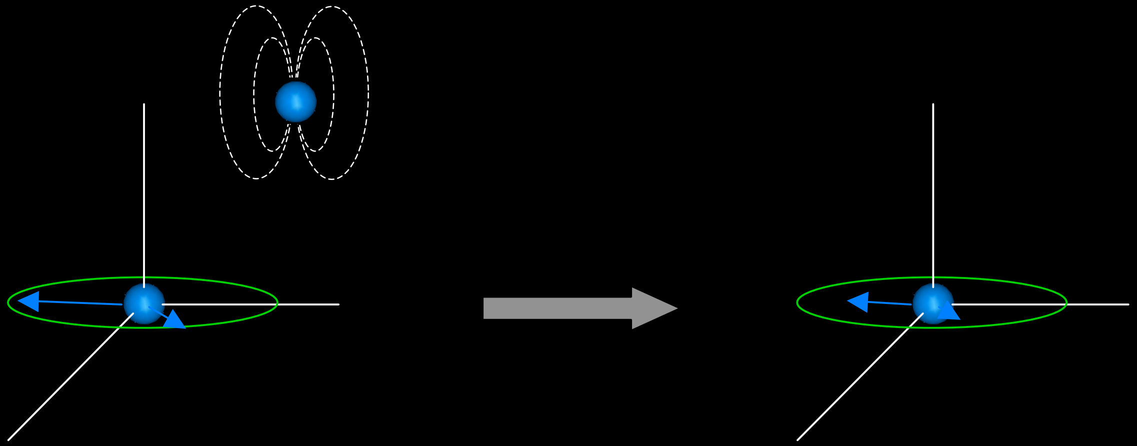
S spin to I spin

Relaxation of longitudinal and transverse magnetisation states

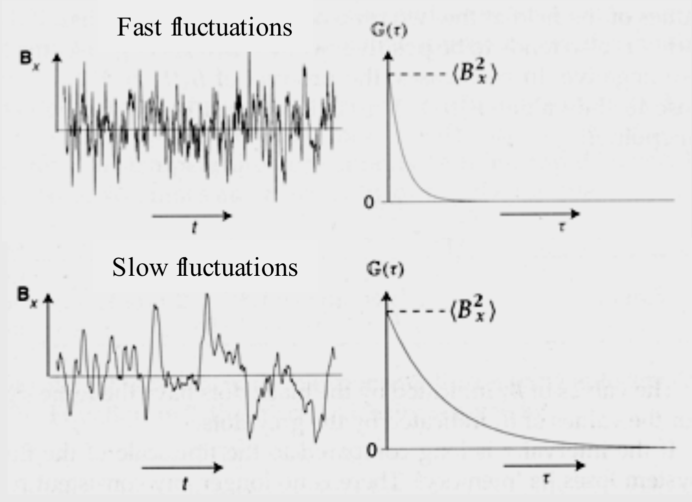
Longitudinal
Relaxation



Transverse
Relaxation



How can we describe of these fluctuating local fields?



Correlation function

$$G(\tau) = \overline{\left\langle B_{loc}(t) B_{loc}(t+\tau) \right\rangle} \neq 0$$

Simple model :

$$G(\tau) = \left\langle B_{loc}^2 \right\rangle e^{-\tau/\tau_c}$$

Normalisation :

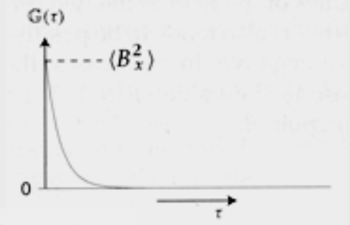
$$c(0) = 1$$

$$c(\tau) \xrightarrow{\tau >> \tau_c} 0$$

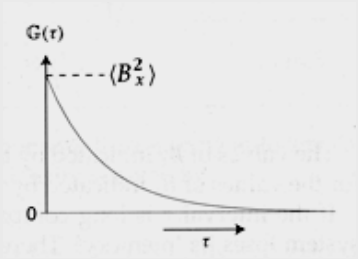
Local fluctuating fields described using a correlation function : measure of the rate of random fluctuations of the local field

How can we describe of these fluctuating Local Fields?

Fast fluctuations



Slow fluctuations



Spectral density function

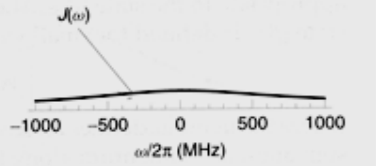
$$P(\omega) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} G(\tau) e^{-i\omega\tau}\, d\tau$$

$$J(\omega) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} c(\tau) e^{-i\omega\tau}\, d\tau$$

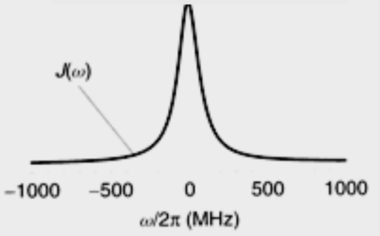
Simple model :

$$J^{iso}(\omega) = \frac{2}{5}\frac{\tau_c}{1+\omega^2\tau_c^2}$$

Broad frequency distribution :
Spectral density function



Narrow spectral
density function

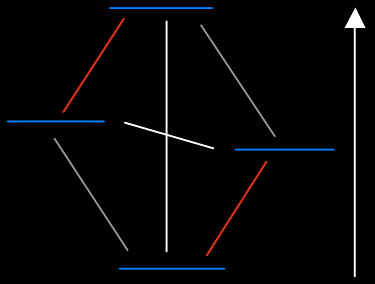


Local fluctuating fields described using a spectral density function : measure of the contribution of motion at each frequency. Area under the curve remains constant

Relaxation rates - Transitions induced by random fields

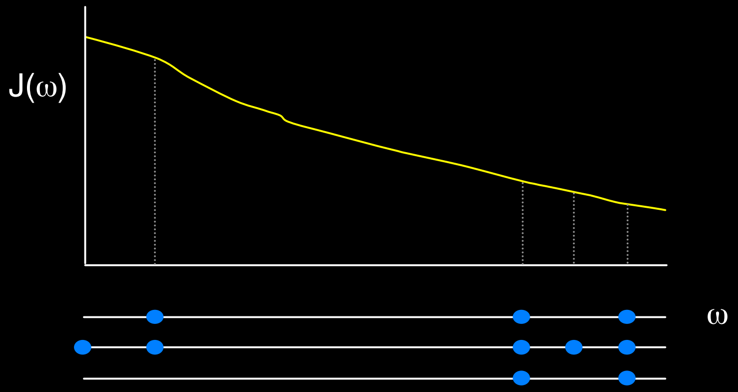
$H_{random}(t)$

Spin-state variables :
Available frequencies of motion
that can be sampled



Available transitions
defined by the
spin system

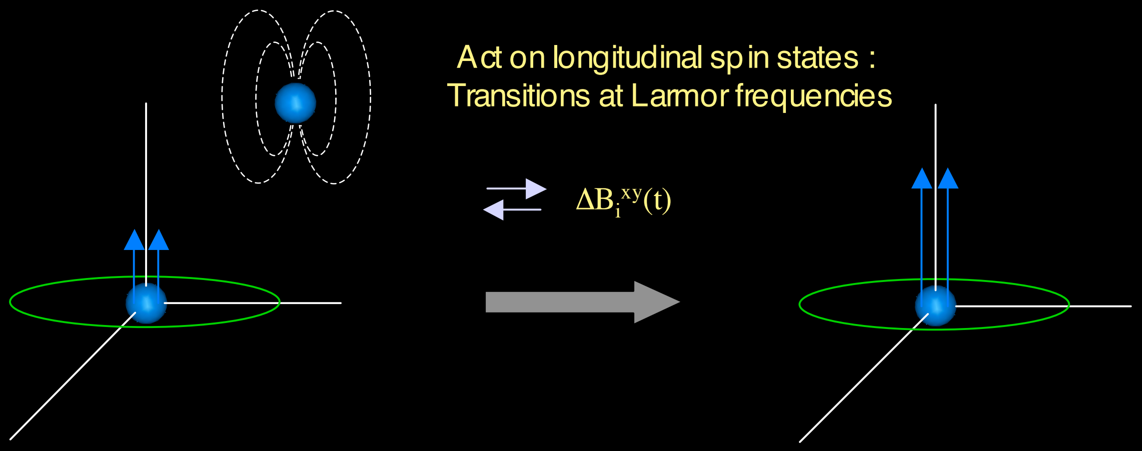
**Time-dependent
geometric variables:**
Molecular motion



Random fields created
by molecular motion -
Allow transitions
to occur

Relaxation of longitudinal and transverse magnetisation states

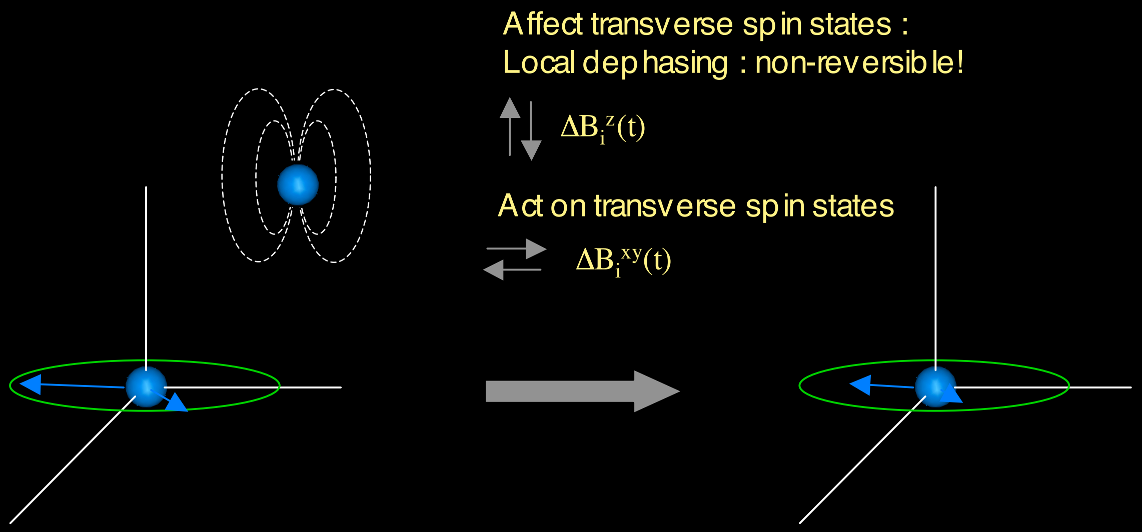
Longitudinal
Relaxation
Non-adiabatic
Processes
Non-secular terms



Act on longitudinal spin states :
Transitions at Larmor frequencies

$\Delta B_i^{xy}(t)$

Transverse
Relaxation
Non-adiabatic and
adiabatic processes
Secular and non-secular
terms

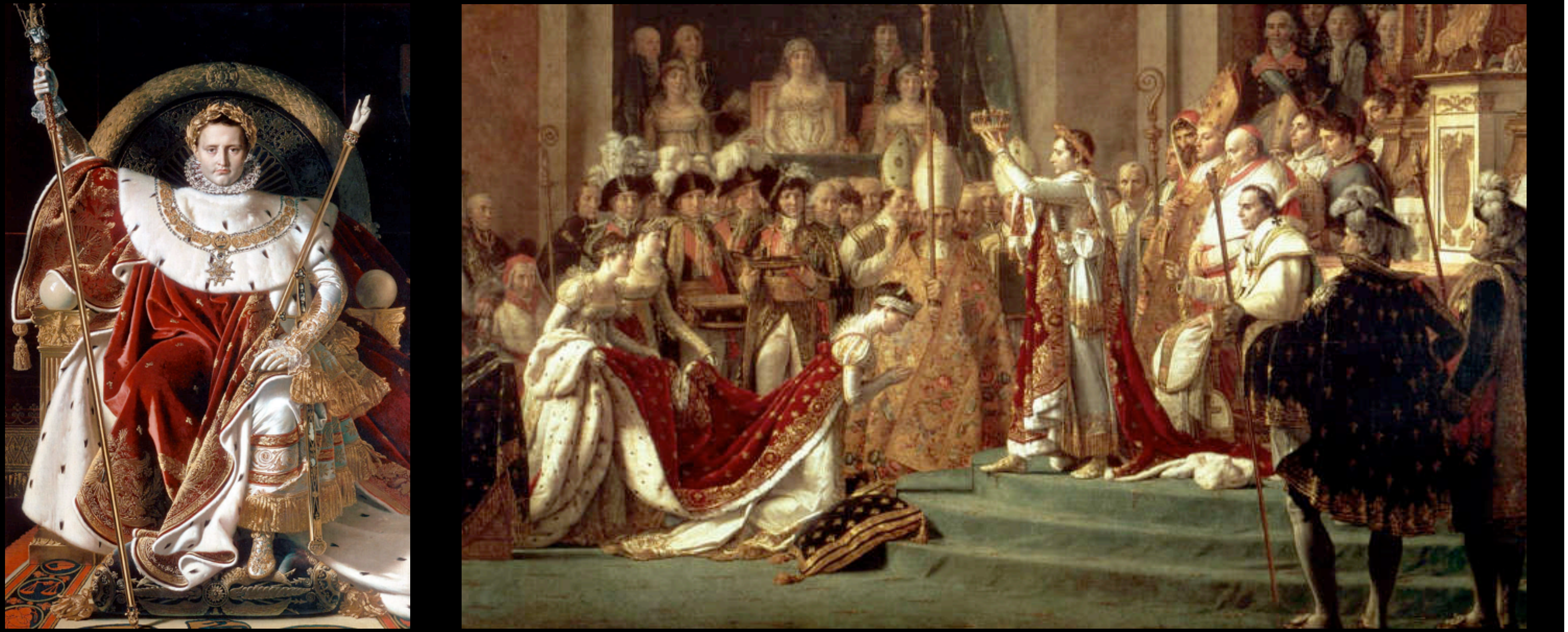


Affect transverse spin states :
Local dephasing : non-reversible!

$\Delta B_i^{z}(t)$

Act on transverse spin states

$\Delta B_i^{xy}(t)$

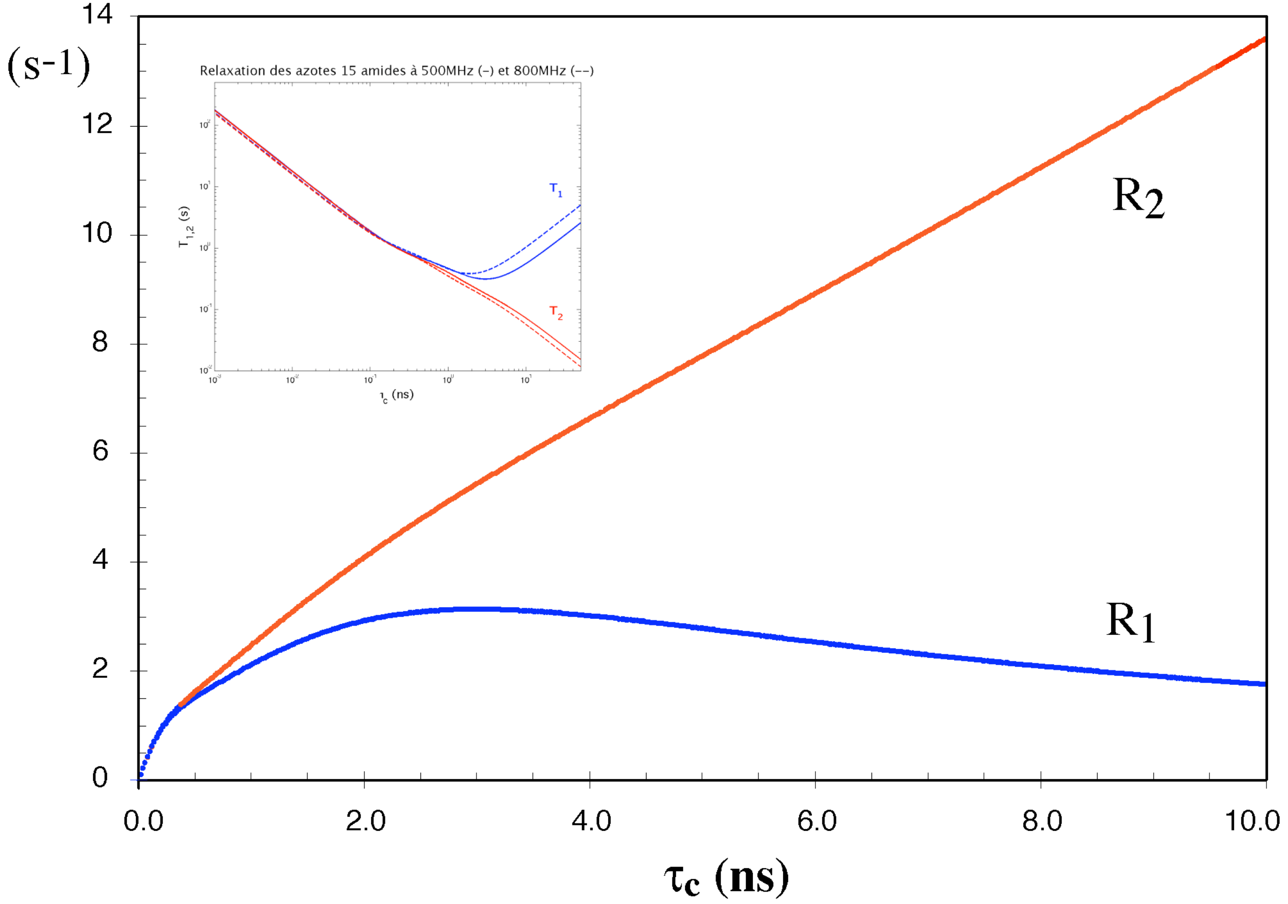


Identification of secular and non-secular terms…..

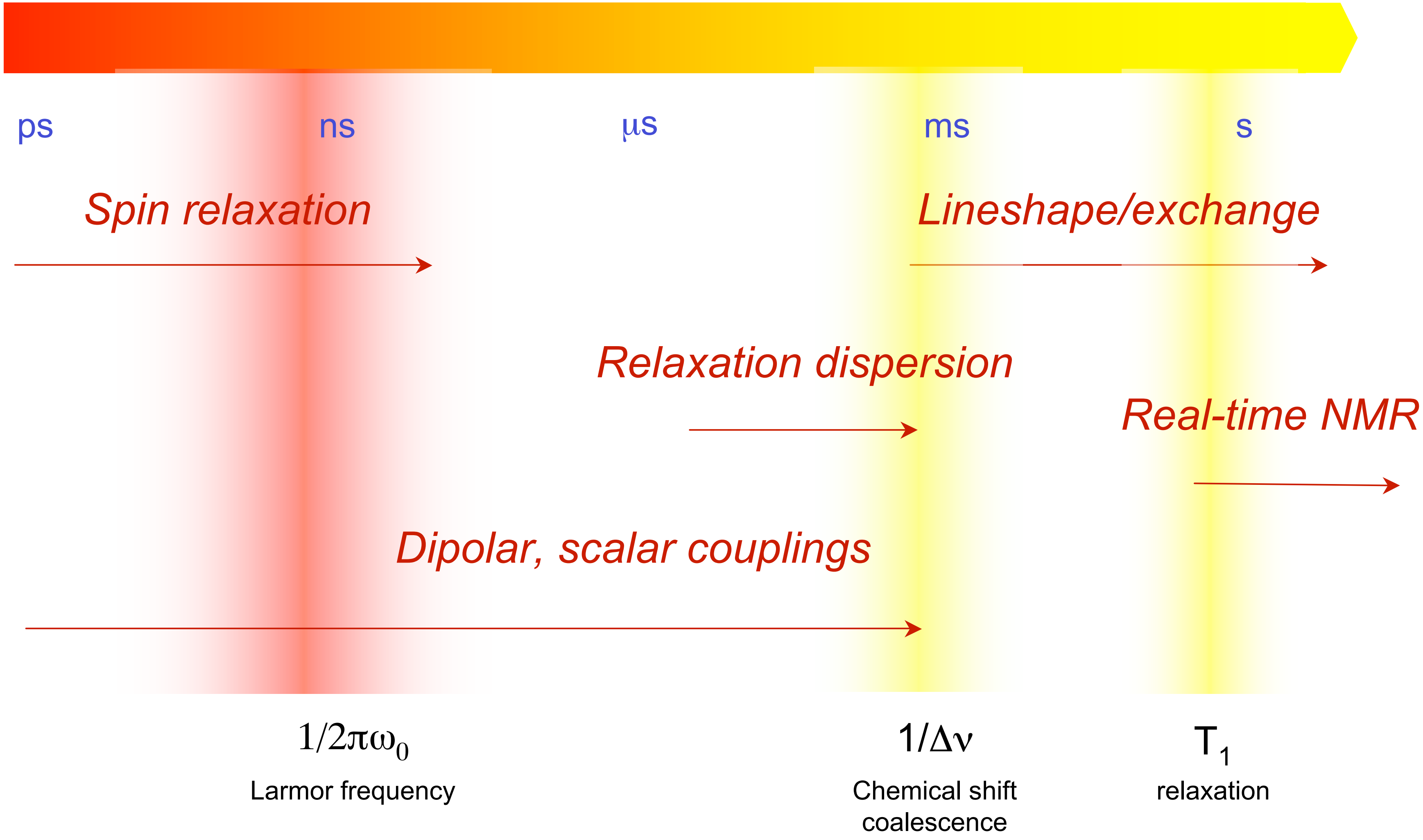
….has never been easy

Relaxation of longitudinal and transverse magnetisation states

Additional transverse relaxation mechanisms : $R_2 \geq R_1$



Characteristic NMR timescales



ps ns µs ms s

Spin relaxation

Lineshape/exchange

Relaxation dispersion

Real-time NMR

Dipolar, scalar couplings

$1/2\pi\omega_0$
Larmor frequency

$1/\Delta\nu$
Chemical shift
coalescence

T_1
relaxation

Librational motion

Molecular rotation

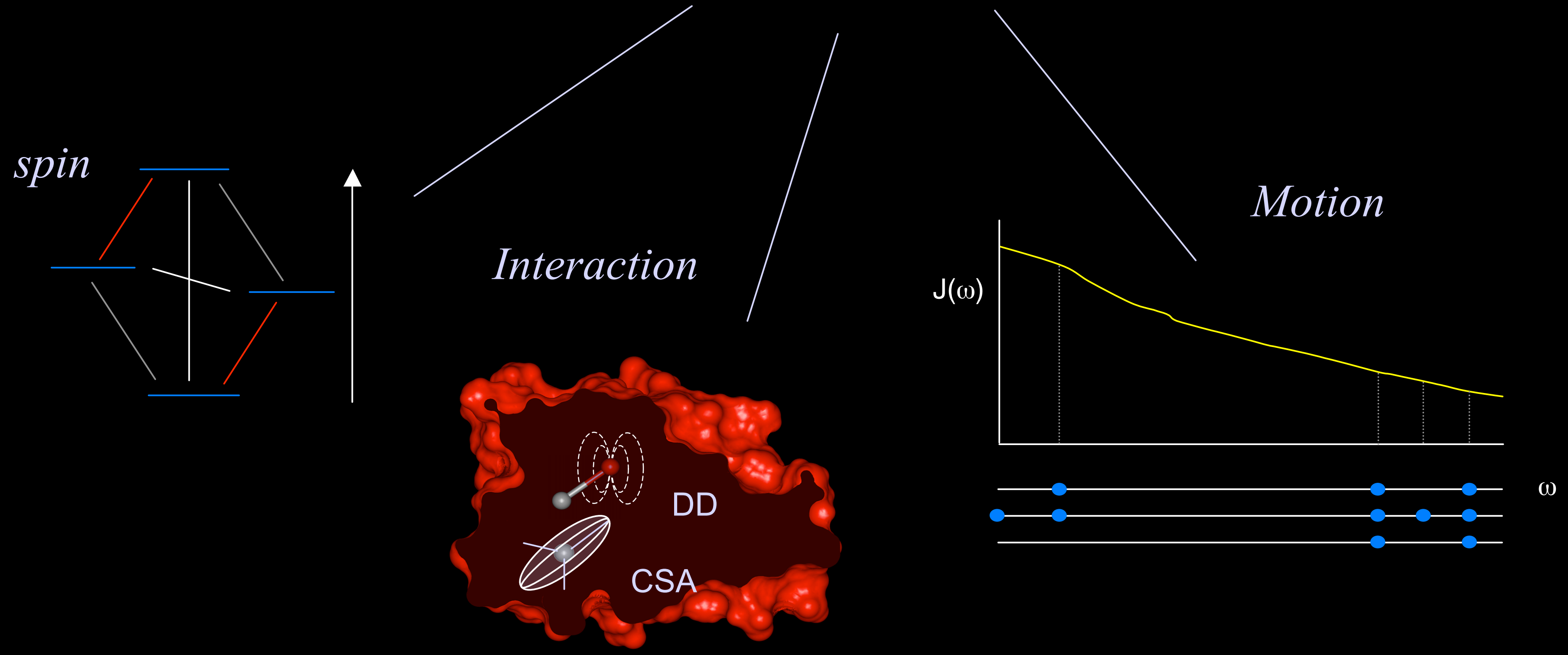
Collective motions

Protein folding
Hinge Bending

How do we derive expressions for relaxation rate constants?

Relating the motion to the rate constant

$$W_{ij} = f\{A_{ij},\ Y,\ J(\omega_{ij})\}$$



Liouville von Neumann Equation

$$d\sigma(t)/dt = -i[H(t),\sigma(t)]$$

Evolution of spin system

Hamiltonians :

Scalar coupling

Static field

B_1 field

Dipolar coupling

…

Master Equation of Relaxation

$$d\sigma(t)/dt = -i[H_{random}(t),\sigma(t)]$$

Transformation to interaction frame
(see Beat Meier’s course)

$$d\tilde{\sigma}(t)/dt = -i[\tilde{H}_{random}(t),\tilde{\sigma}(t)]$$

Evolution of spin system

Stochastic Hamiltonian

Deriving expressions for relaxation rate constants

$$\frac{d}{dt}\tilde{\sigma}(t) = -i\left[\tilde{H}_r(t),\tilde{\sigma}(t)\right]$$

Spin state evolves under the action of the stochastic Hamiltonian $H_r(t)$

$\tau_c \ll t \ll T_{relax}$

$$\frac{d}{dt}\tilde{\sigma}(t) = -\int_0^\infty \overline{\left[\tilde{H}_r(t),\left[\tilde{H}_r(t-\tau),\tilde{\sigma}(t)-\sigma_{éq}\right]\right]}d\tau$$

Stochastic Hamiltonian causes relaxation if :

(a) double commutator does not vanish,

(b) motion exist at characteristic frequencies

$$\tilde{H}_r(t) = \sum_q F^{(q)}(t)\tilde{A}^{(q)}$$

$A^{(q)}$ are *spin operators* describing the nature of the interaction

$F^{(q)}(t)$ are *spatial functions* describing the reorientation of relaxation active interactions

$$\frac{d}{dt}\tilde{\sigma} = -\frac{1}{2}\sum_{p,q} J_q\left(\omega_p^{(q)}\right)\left[A_p^{(-q)},\left[A_p^{(q)},\left(\tilde{\sigma}-\tilde{\sigma}_{éq}\right)\right]\right]$$

Motion (*spatial function*) can be described in terms of the spectral density function *J(ω)*

Deriving expressions for relaxation rate constants

Calculation of relaxation
rate constants :
Decomposition of relaxation
Hamiltonian for each
mechanism (DD, CSA)

$$\frac{d}{dt}\tilde{\sigma} = -\frac{1}{2}\sum_{p,q} J_q\left(\omega_p^{(q)}\right)\left[A_p^{(-q)},\left[A_p^{(q)},\left(\tilde{\sigma}-\tilde{\sigma}_{éq}\right)\right]\right]$$

Calculation of double commutators :
Determine efficiency of relaxation due
to each term

……

Dipolar interaction (DD)

$$E_{IS} = \frac{\mu_0}{4\pi r_{IS}^3}\left\{\vec{\mu}_I \bullet \vec{\mu}_S - 3\frac{(\vec{\mu}_I \bullet \vec{r}_{IS})(\vec{\mu}_S \bullet \vec{r}_{IS})}{r_{IS}^2}\right\}$$

$$H_{DD} = d\left\{\vec{I} \bullet \vec{S} - 3I_v S_v\right\}$$

$$d = \frac{\mu_0 \gamma_I \gamma_S \hbar}{4\pi r_{IS}^3}$$

$$\begin{aligned} H_{DD} = d\Big\{&\left(1-3\cos^2\theta\right)\left[I_z S_z - \frac{1}{4}\left(I_+ S_- + I_- S_+\right)\right] \\ &-\frac{3}{2}\sin\theta\cos\theta e^{\mp i\varphi}\left(I_\pm S_z + I_z S_\pm\right) \\ &-\frac{3}{4}\sin^2\theta e^{\mp 2i\varphi} I_\pm S_\pm\Big\} \end{aligned}$$

Deriving expressions for relaxation rate constants

$$\frac{d}{dt}\tilde{\sigma} = -\frac{1}{2}\sum_{p,q} J_q\left(\omega_p^{(q)}\right)\left[A_p^{(-q)},\left[A_p^{(q)},\left(\tilde{\sigma}-\tilde{\sigma}_{éq}\right)\right]\right]$$

Calculation of double commutators

……

$$\tilde{H}_r(t) = \sum_q F^{(q)}(t)\tilde{A}^{(q}$$

Heavy. . . .

$$\tilde{A}^{(q)}(t) = e^{iH_0 t}A^{(q)}e^{-iH_0 t} = \sum_p A_p^{(q)}e^{i\omega_p^{(q)}t}$$

$$H_0 = \omega_I I_z + \omega_S S_z$$

$\omega_1^{(0)} = 0$	$\omega_2^{(0)} = \omega_I - \omega_S$	$\omega_3^{(0)} = \omega_S - \omega_I$
$\omega_1^{(1)} = \omega_I$	$\omega_2^{(1)} = \omega_S$	
$\omega^{(2)} = \omega_I + \omega_S$		

$$F^{(0)} = 1 - 3\cos^2\theta$$
$$F^{(1)} = \sin\theta\cos\theta e^{-i\varphi} \qquad F^{(-1)} = \sin\theta\cos\theta e^{i\varphi}$$
$$F^{(2)} = \sin^2\theta e^{-2i\varphi} \qquad F^{(-2)} = \sin^2\theta e^{2i\varphi}$$

$$A^{(0)} = A_1^{(0)} + A_2^{(0)} + A_3^{(0)}$$
$$A_1^{(0)} = \frac{d}{2}\cdot 2\mathbf{I}_z\mathbf{S}_z$$
$$A_2^{(0)} = -\frac{d}{4}\mathbf{I}_+\mathbf{S}_-$$
$$A_3^{(0)} = -\frac{d}{4}\mathbf{I}_-\mathbf{S}_+ = A_2^{(0)*}$$

$$A^{(1)} = A_1^{(1)} + A_2^{(1)} = A^{(-1)\dagger}$$
$$A_1^{(1)} = -\frac{3d}{4}\cdot 2\mathbf{I}_+\mathbf{S}_z \qquad A_1^{(-1)} = -\frac{3d}{4}\cdot 2\mathbf{I}_-\mathbf{S}_z$$
$$A_2^{(1)} = -\frac{3d}{4}\cdot 2\mathbf{I}_z\mathbf{S}_+ \qquad A_2^{(-1)} = -\frac{3d}{4}\cdot 2\mathbf{I}_z\mathbf{S}_-$$

$$A^{(2)} = A^{(-2)\dagger}$$
$$A^{(2)} = -\frac{3d}{4}\mathbf{I}_+\mathbf{S}_+ \qquad A^{(-2)} = -\frac{3d}{4}\mathbf{I}_-\mathbf{S}_-$$

Deriving expressions for relaxation rate constants

Dipolar terms : $d^2 = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2}{r_{IS}^6}$:

Most commonly measured ^{15}N relaxation rates :

$$R_1(S_z) = \frac{d^2}{4}\left\{J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)\right\}$$

$$R_1(I_z) = \frac{d^2}{4}\left\{J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)\right\}$$

$$R(I_z \leftrightarrow S_z) = \sigma_{SI} = \sigma_{IS} = \frac{d^2}{4}\left\{6J(\omega_I + \omega_S) - J(\omega_I - \omega_S)\right\}$$

$$R_2(S_{x,y}) = \frac{d^2}{8}\left\{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S)\right\}$$

$$R_2(I_{x,y}) = \frac{d^2}{8}\left\{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_S) + 6J(\omega_I + \omega_S)\right\}$$

This formalism can be used generally to develop expressions for relaxation rates of different spin states

$$R_{zz}(2I_zS_z) = \frac{3d^2}{4}\left\{J(\omega_S) + J(\omega_I)\right\} + \rho_{HH}$$

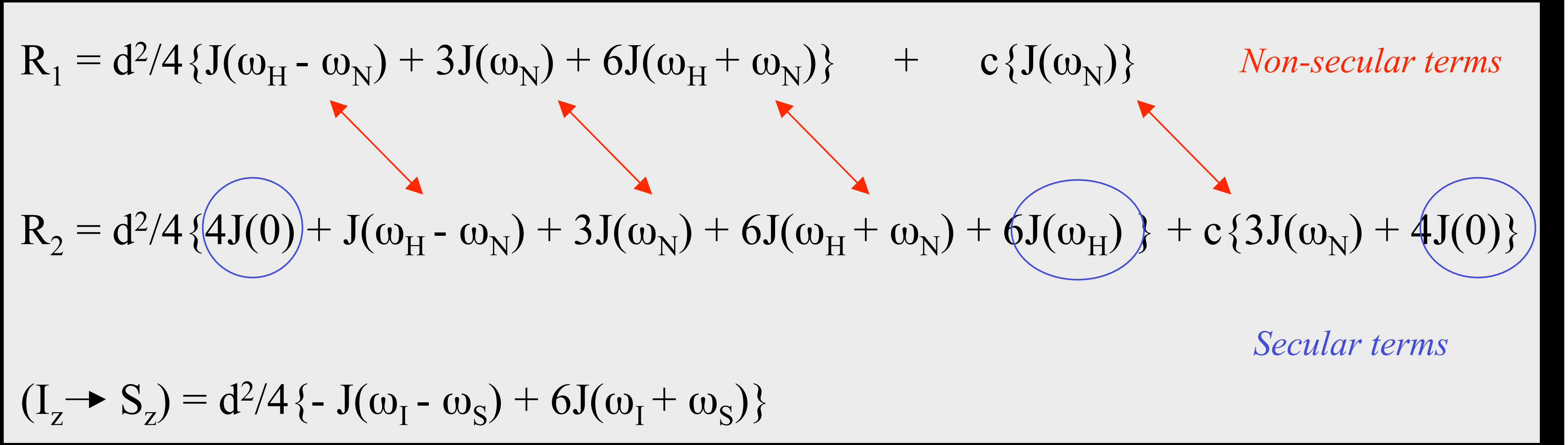
$$R_{2z}(2I_zS_{x,y}) = \frac{d^2}{8}\left\{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)\right\} + \rho_{HH}$$

$$R_{2z}(2I_{x,y}S_z) = \frac{d^2}{8}\left\{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)\right\}$$

$$R_{0Q}(I_+S_-;I_-S_+) = \frac{d^2}{8}\left\{2J(\omega_I - \omega_S) + 3J(\omega_S) + 3J(\omega_I)\right\} + \rho_{HH}$$

$$R_{2Q}(I_+S_+;I_-S_-) = \frac{d^2}{8}\left\{3J(\omega_S) + 3J(\omega_I) + 12J(\omega_I + \omega_S)\right\} + \rho_{HH}$$

^{15}N relaxation rates R_1, R_2 and nOe



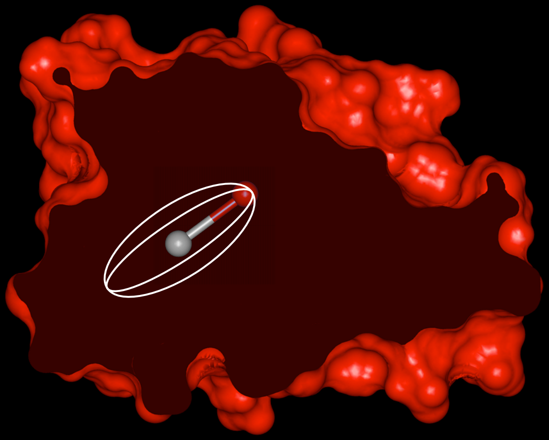
$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} \quad + \quad c\{J(\omega_N)\}$$

Non-secular terms

$$R_2 = d^2/4\{\boxed{4J(0)} + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) + \boxed{6J(\omega_H)}\} + c\{3J(\omega_N) + \boxed{4J(0)}\}$$

Secular terms

$$(I_z \rightarrow S_z) = d^2/4\{- J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$$



$d = (\mu_o/4\pi)(\gamma_I \gamma_S \hbar)/r^3$

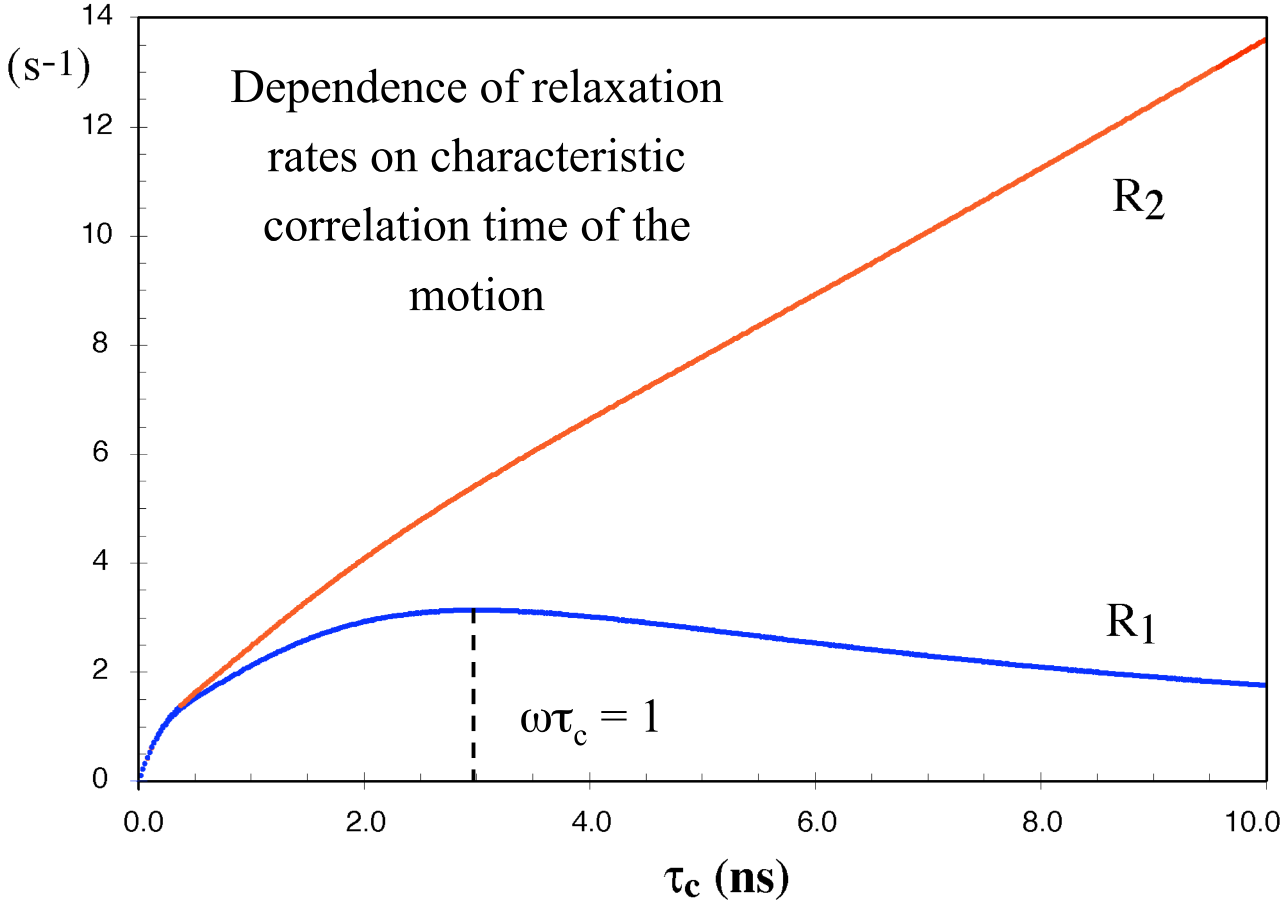
$c = (\Delta\sigma)(\gamma_S B_o)^2/\sqrt{3}$

For two covalently bound spins, r_{ij} can be considered fixed -

$J(\omega)$ provides reorientational information

$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} \quad + \quad c\{J(\omega_N)\}$$

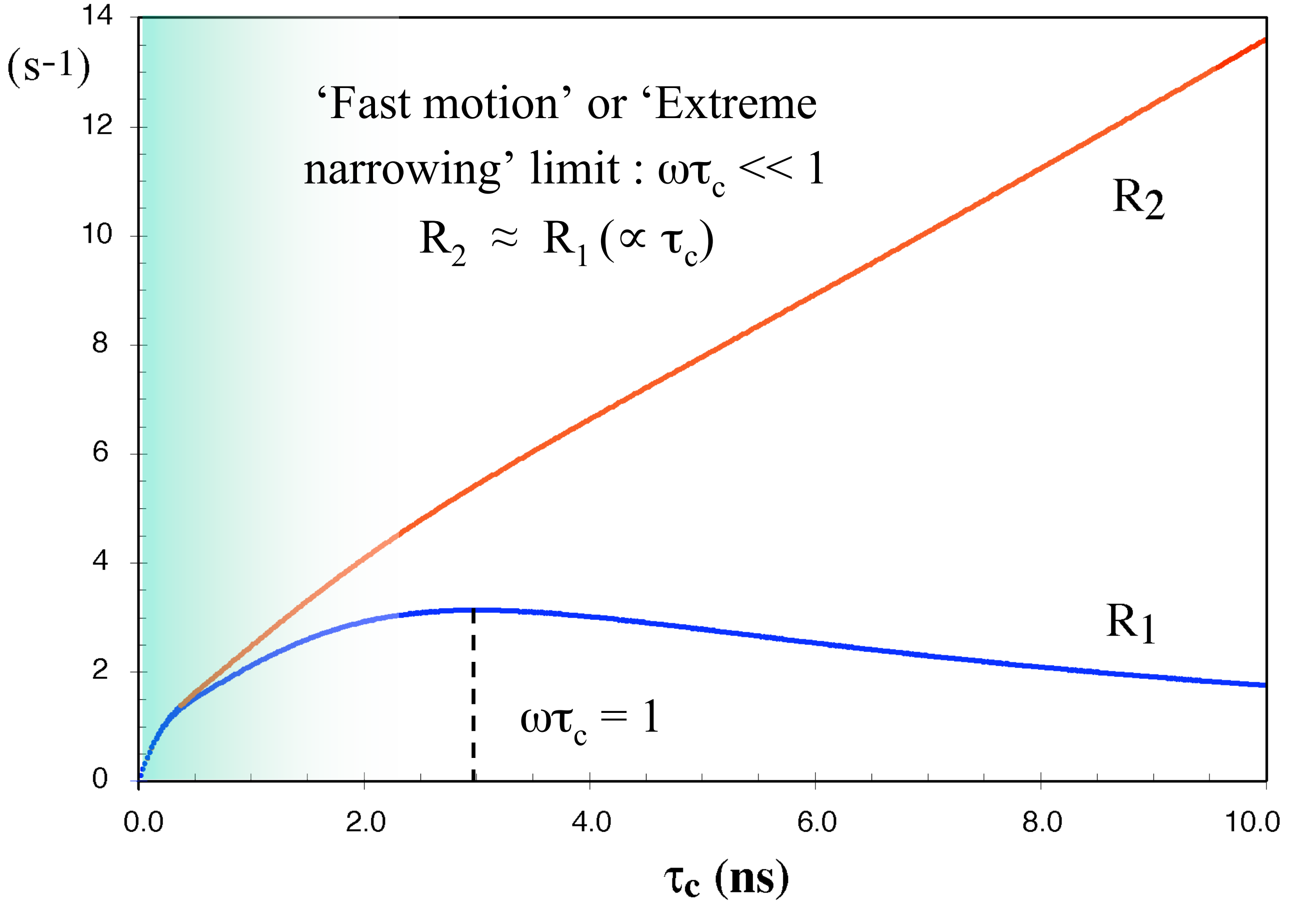
$$R_2 = d^2/4\{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) + 6J(\omega_H)\} + c\{3J(\omega_N) + 4J(0)\}$$



Dependence of relaxation rates on characteristic correlation time of the motion

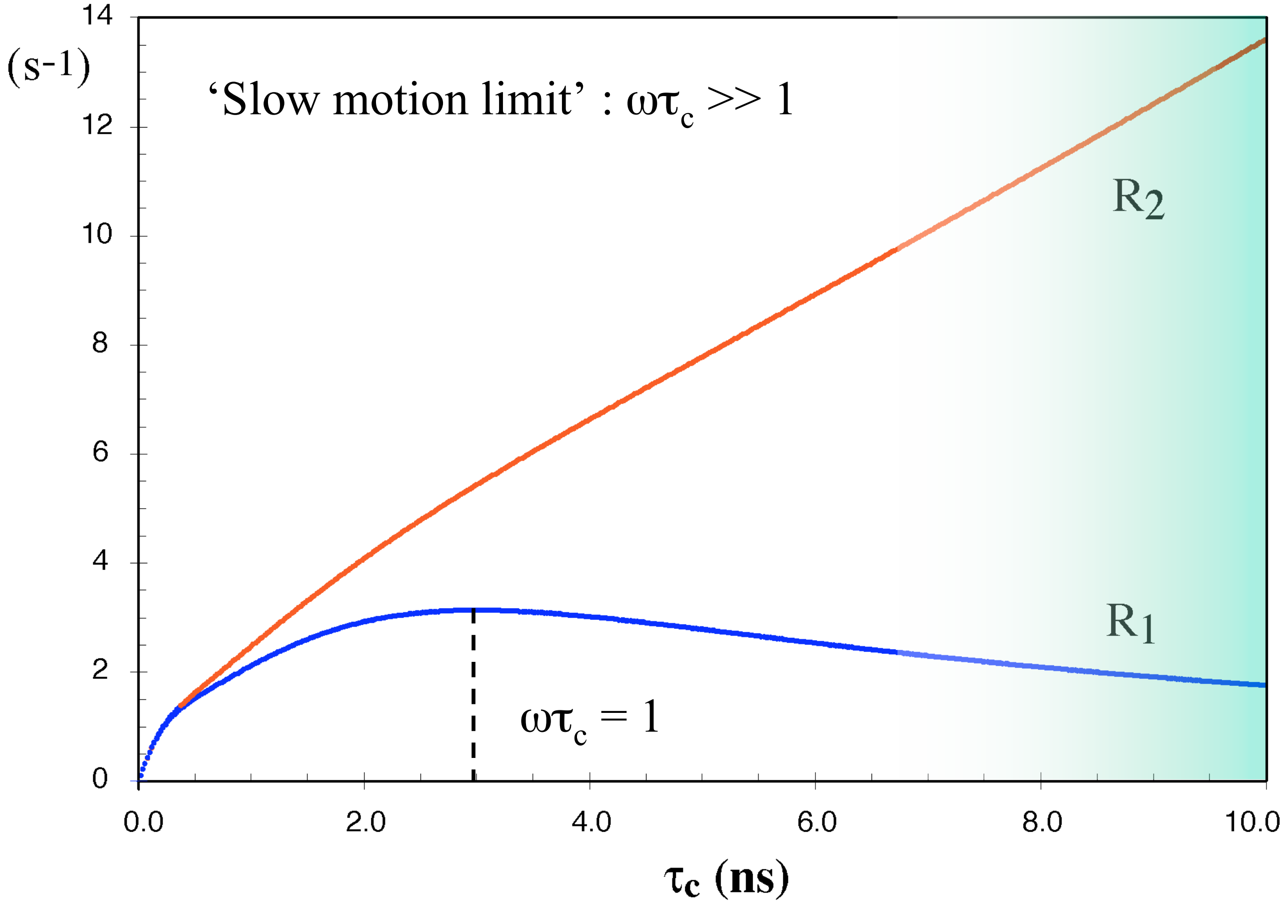
$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} \quad + \quad c\{J(\omega_N)\}$$

$$R_2 = d^2/4\{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) + 6J(\omega_H)\} + c\{3J(\omega_N) + 4J(0)\}$$



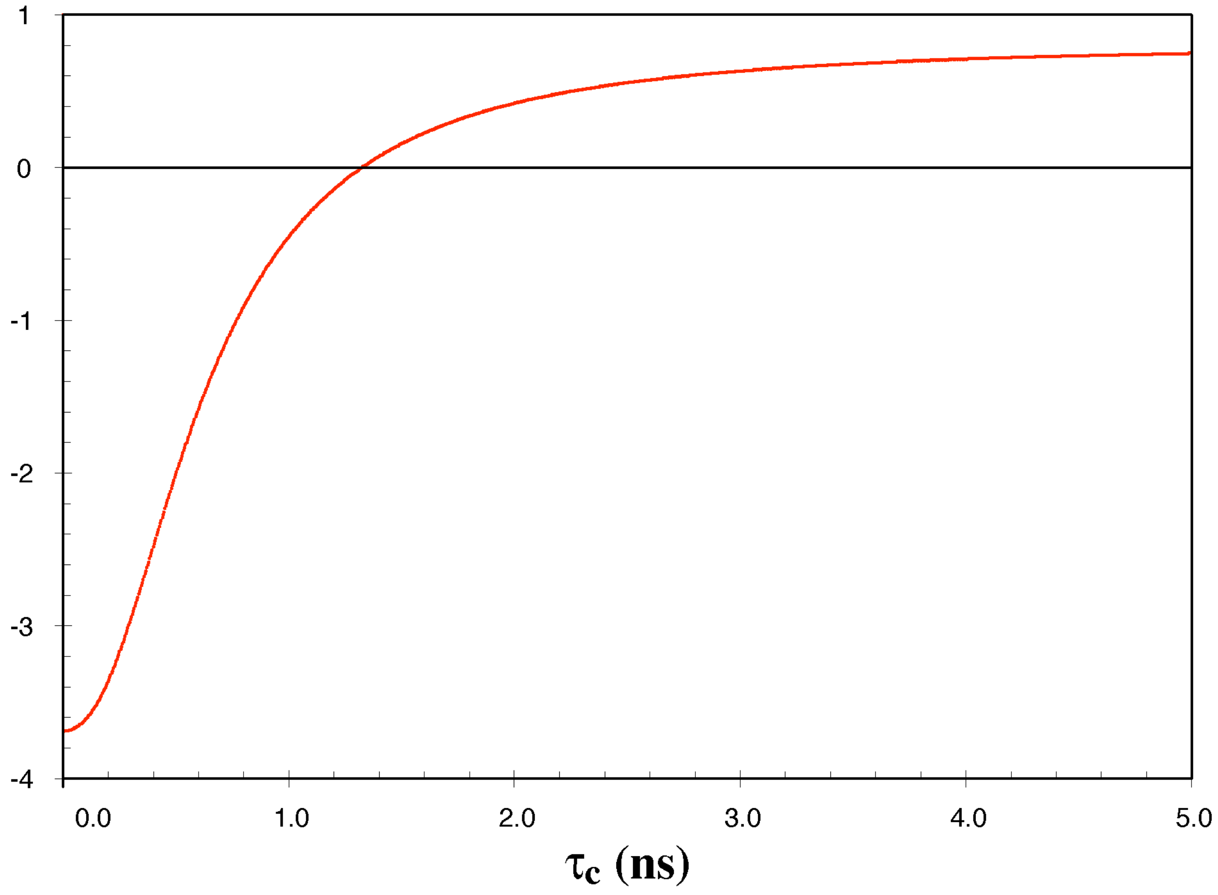
$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} \quad + \quad c\{J(\omega_N)\}$$

$$R_2 = d^2/4\{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) + 6J(\omega_H)\ \} + c\{3J(\omega_N) + 4J(0)\}$$



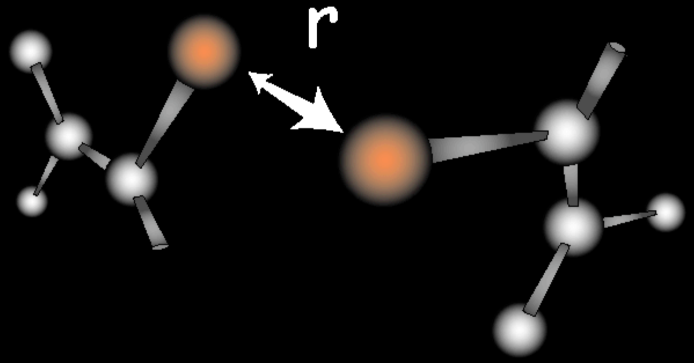
Nuclear Overhauser enhancement (nOe)

noe$\{^1H-^{15}N\}$ $\eta = 1 + \gamma_I \sigma_{IS} / \gamma_S \rho_S$; $\sigma_{IS} = d^2/4\{-J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$



Nuclear Overhauser enhancement ^{1}H-^{1}H (nOe)

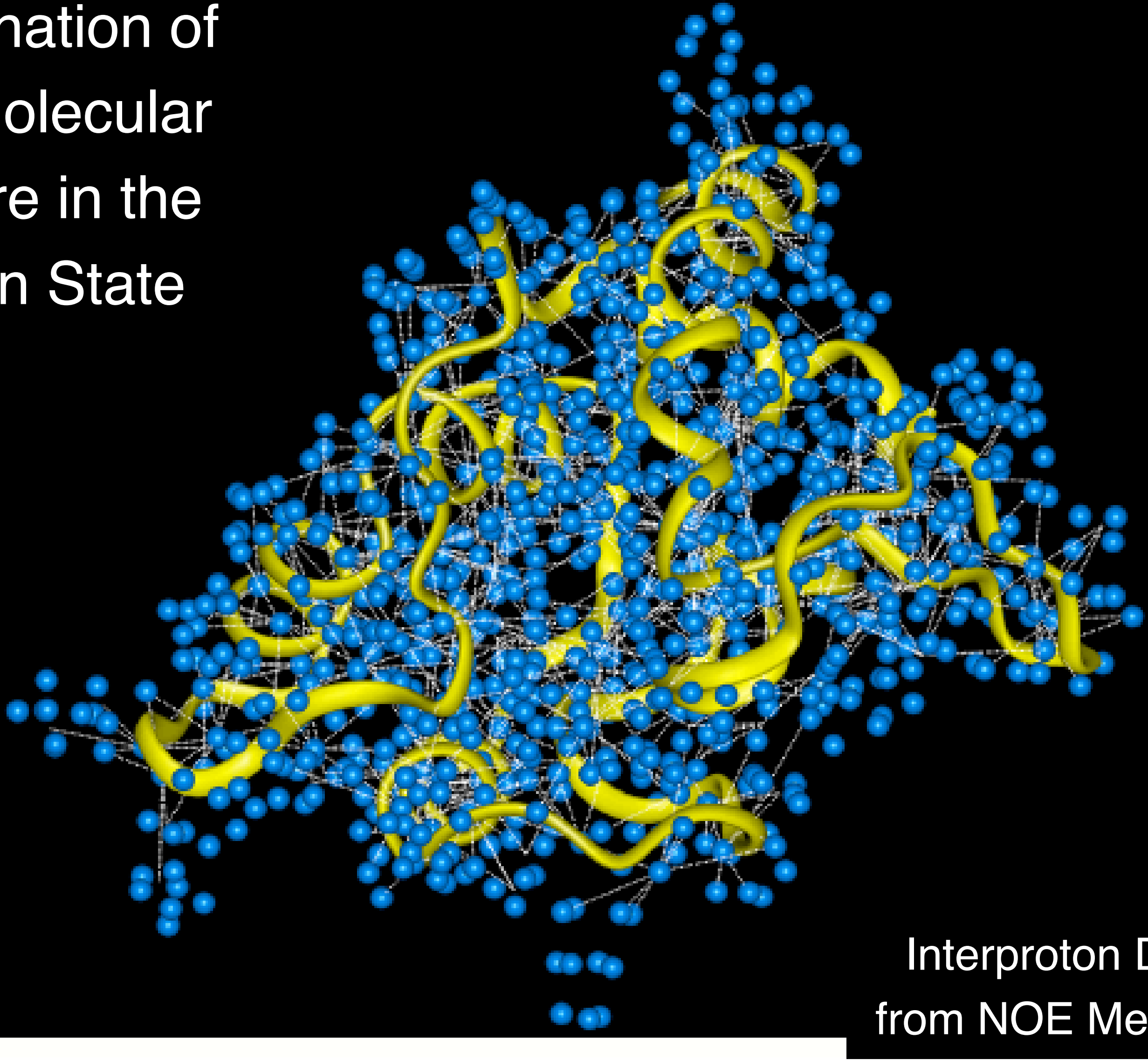
$$\sigma_{IS} = (\mu_o/8\pi)^2 (\gamma_I \gamma_S \hbar)^2 \{-J(0) + 6J(2\omega_H)\}/r^6$$



Principal conformational constraint for solution structure determination

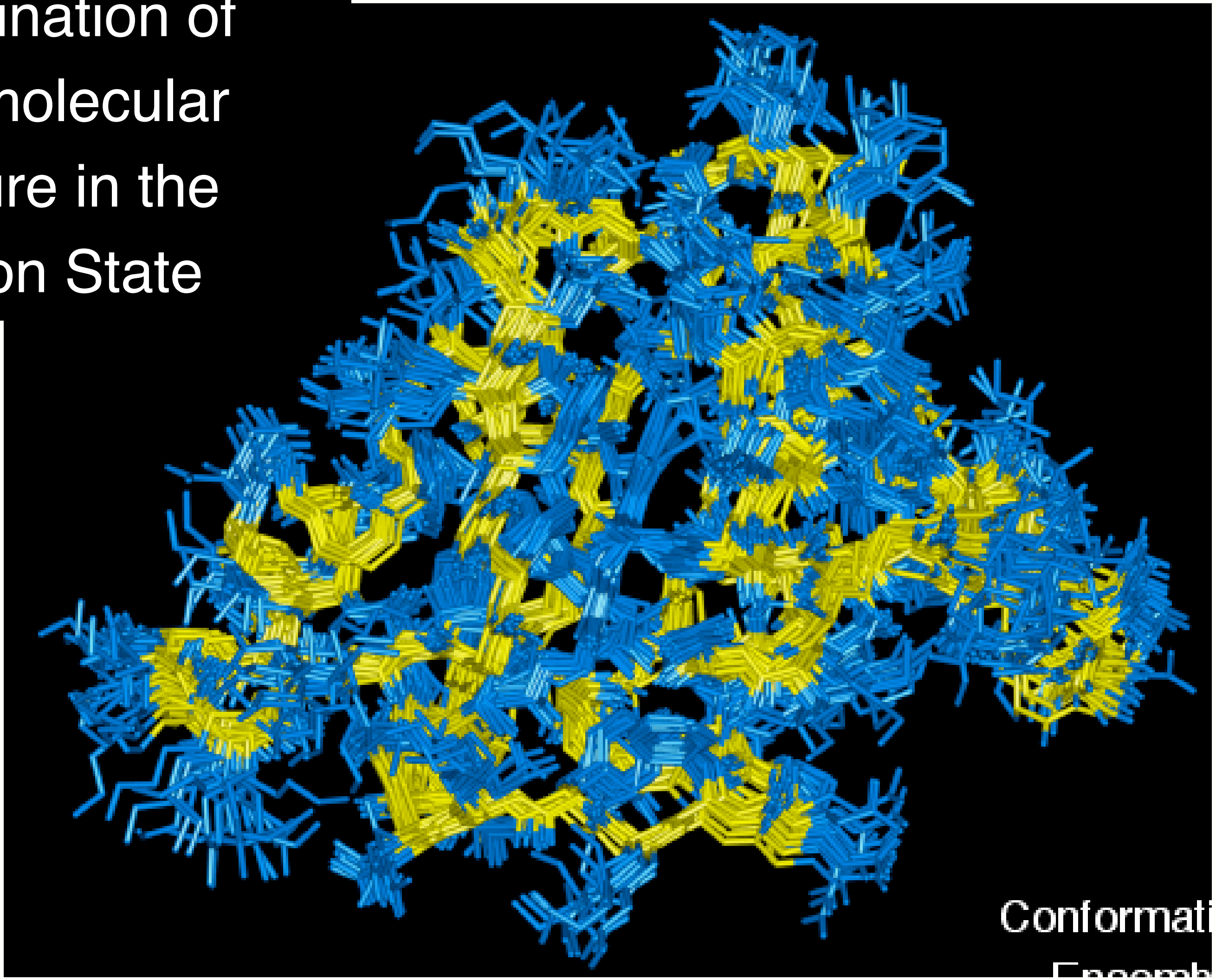
Often assume a common dynamic contribution

Determination of Macromolecular Structure in the Solution State



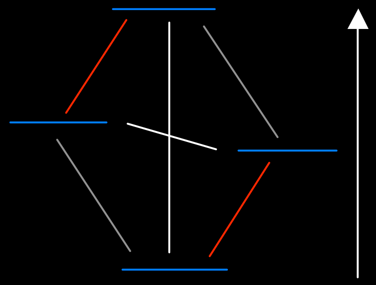
Interproton Distances
from NOE Measurement

Determination of Macromolecular Structure in the Solution State



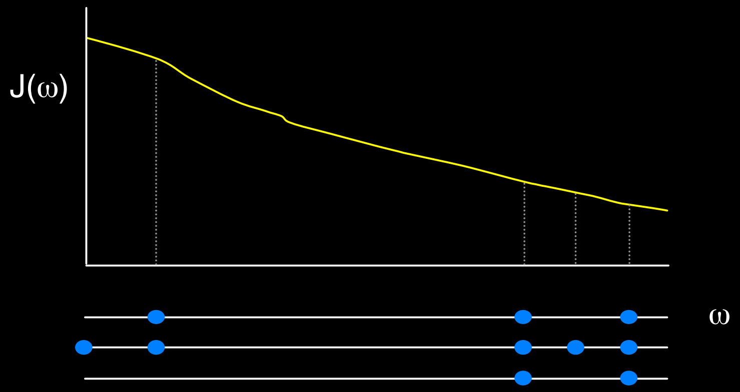
Relaxation rates - Transitions induced by random fields

$H_{random}(t)$



Spin-state variables :
Available frequencies of motion
that can be sampled

Available transitions
defined by the
spin system

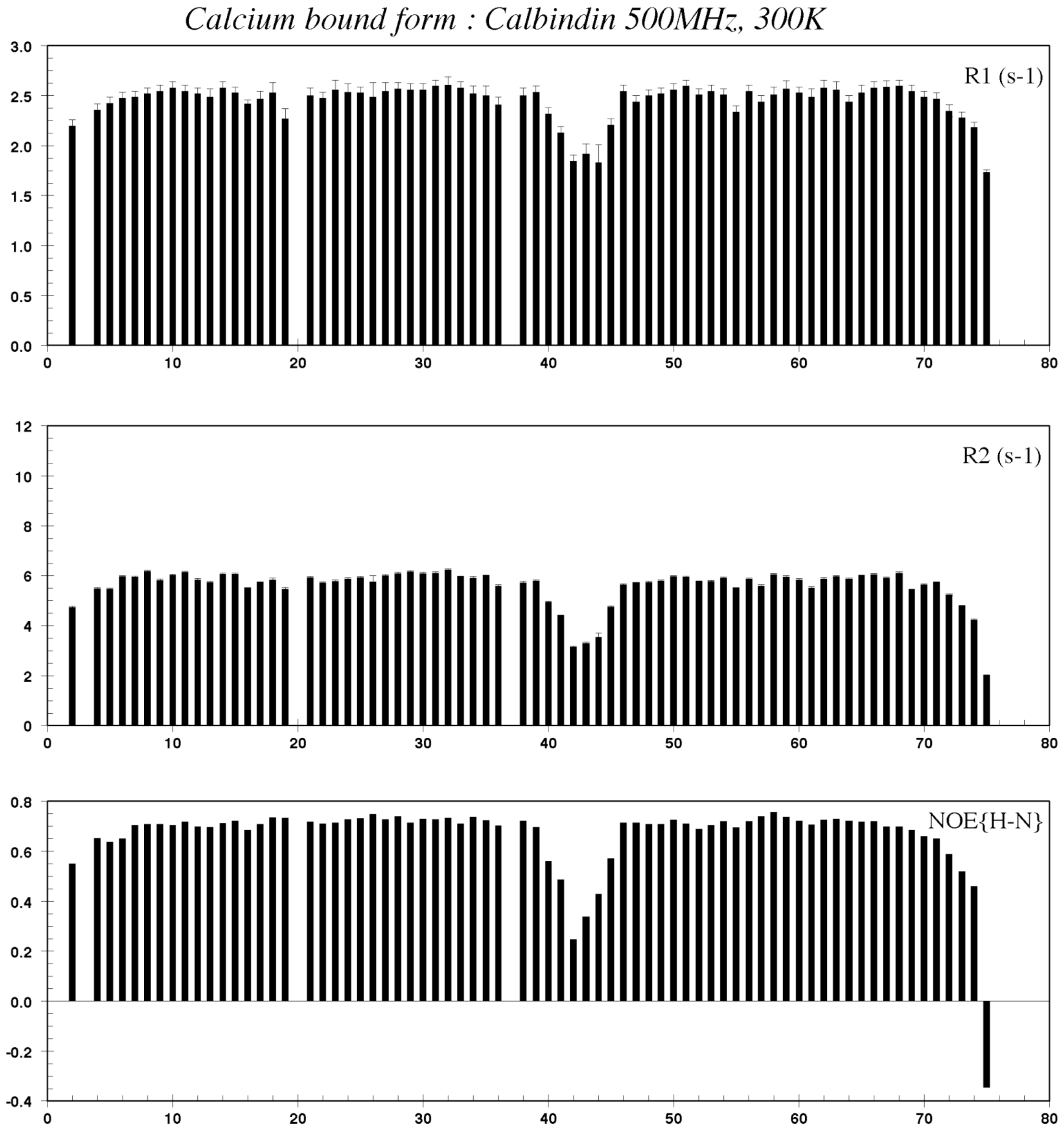


Time-dependent
geometric variables:
Molecular motion

Random fields created
by molecular motion -
Allow transitions
to occur

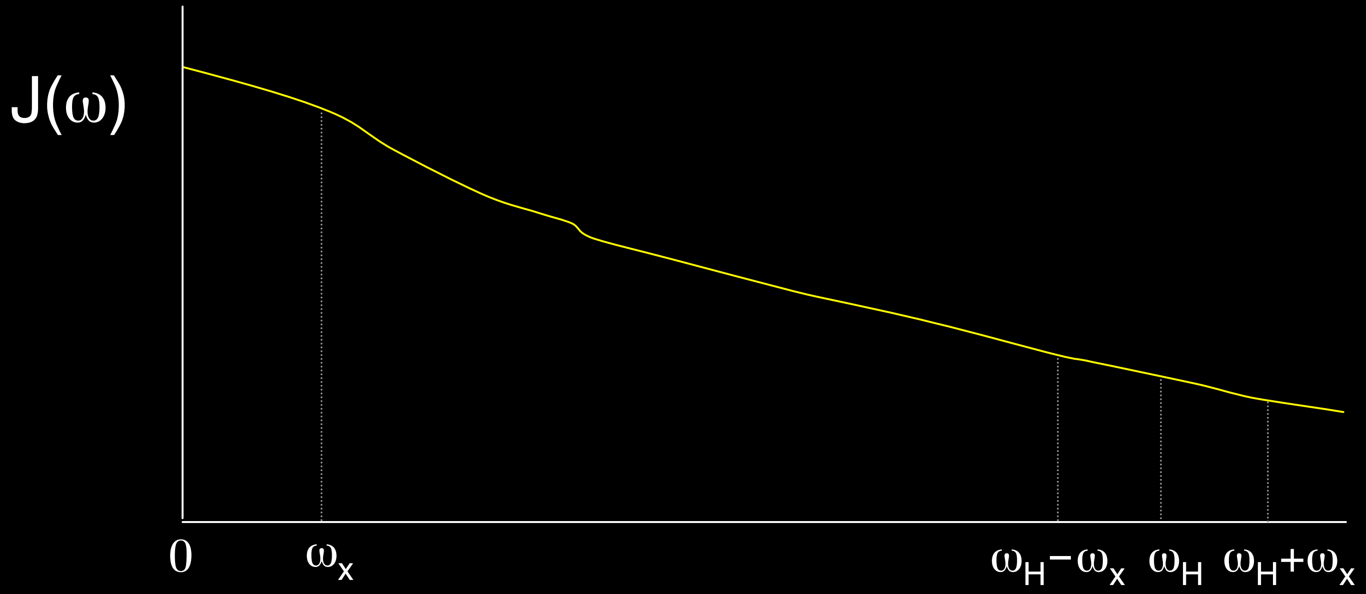
^{15}N relaxation
A probe for local molecular flexibility

- Different dependence on ω of R_1, R_2 and nOe samples the spectral density function at different frequencies defined by the spin system
- For ^{15}N relaxation, dominant relaxation mechanisms are the dipole-dipole and csa interactions. Often considered to be coaxial
- Measures dynamics at each amide site in the peptide chain
- ^{15}N relaxation allows the measurement of angular reorientation, defined by angular time correlation or spectral density functions



Calcium bound form : Calbindin 500MHz, 300K

Sampling of the spectral density function - Heteronuclear two spin system

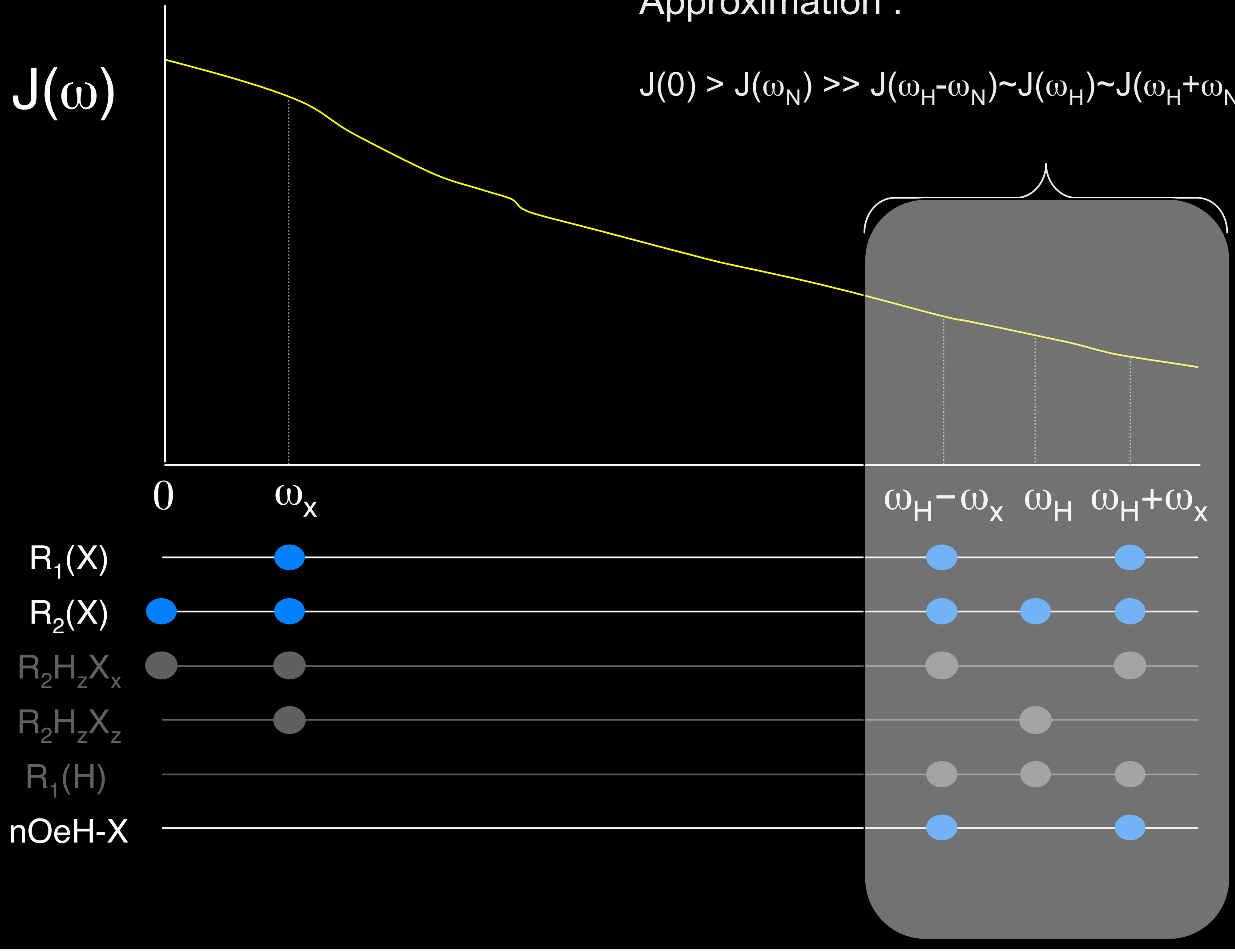


	0	ω_X	$\omega_H-\omega_X$	ω_H	$\omega_H+\omega_X$
$R_1(X)$		●	●		●
$R_2(X)$	●	●	●	●	●
$R_2H_zX_x$	●	●	●		●
$R_2H_zX_z$		●		●	
$R_1(H)$			●	●	●
nOeH-X			●		●

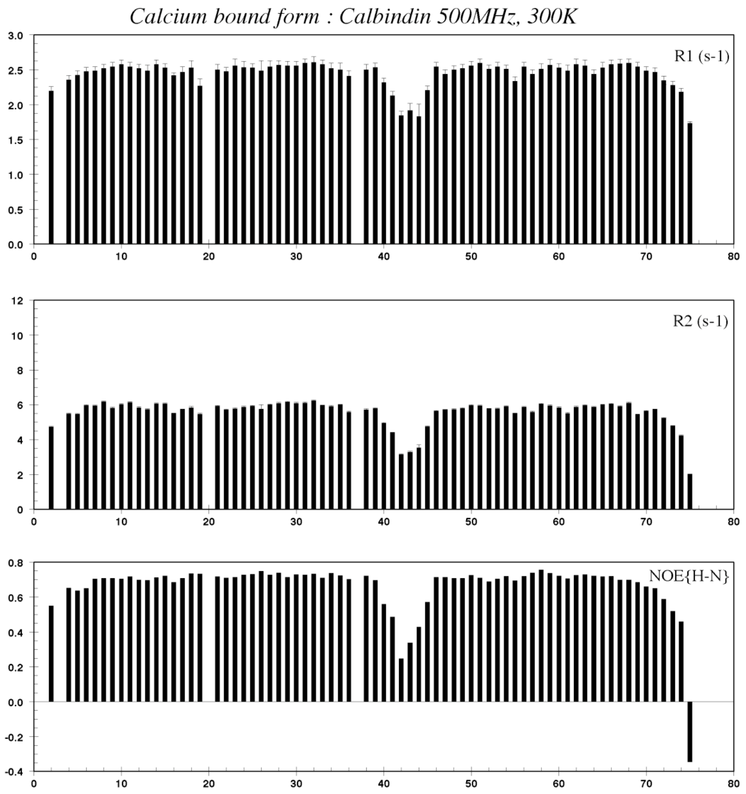
Sampling of the spectral density function - Heteronuclear two spin system

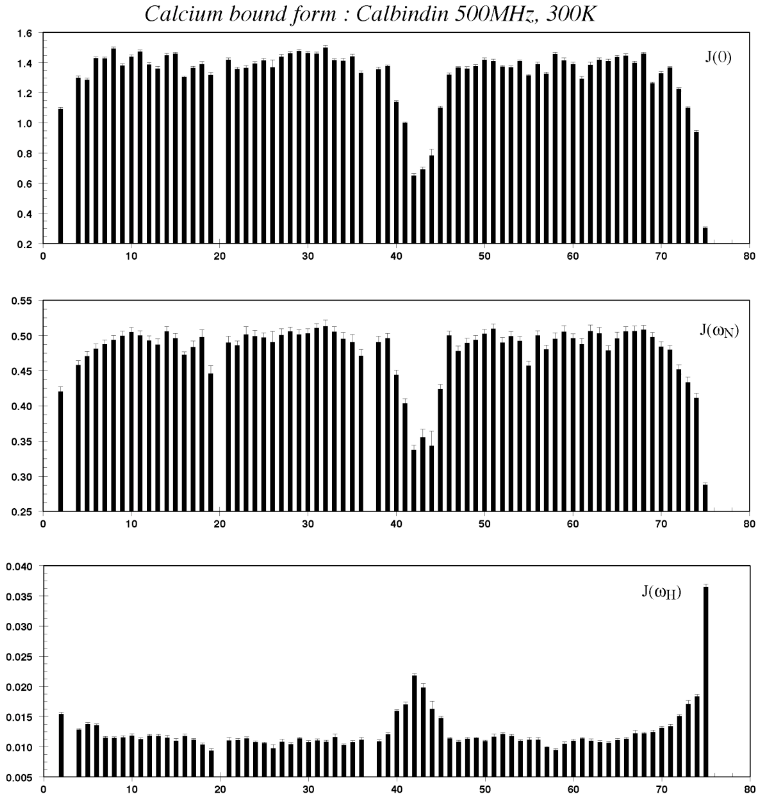
Approximation :

$J(0) > J(\omega_N) >> J(\omega_H - \omega_N) \sim J(\omega_H) \sim J(\omega_H + \omega_N)$



Analysis of relaxation data using Reduced spectral density mapping

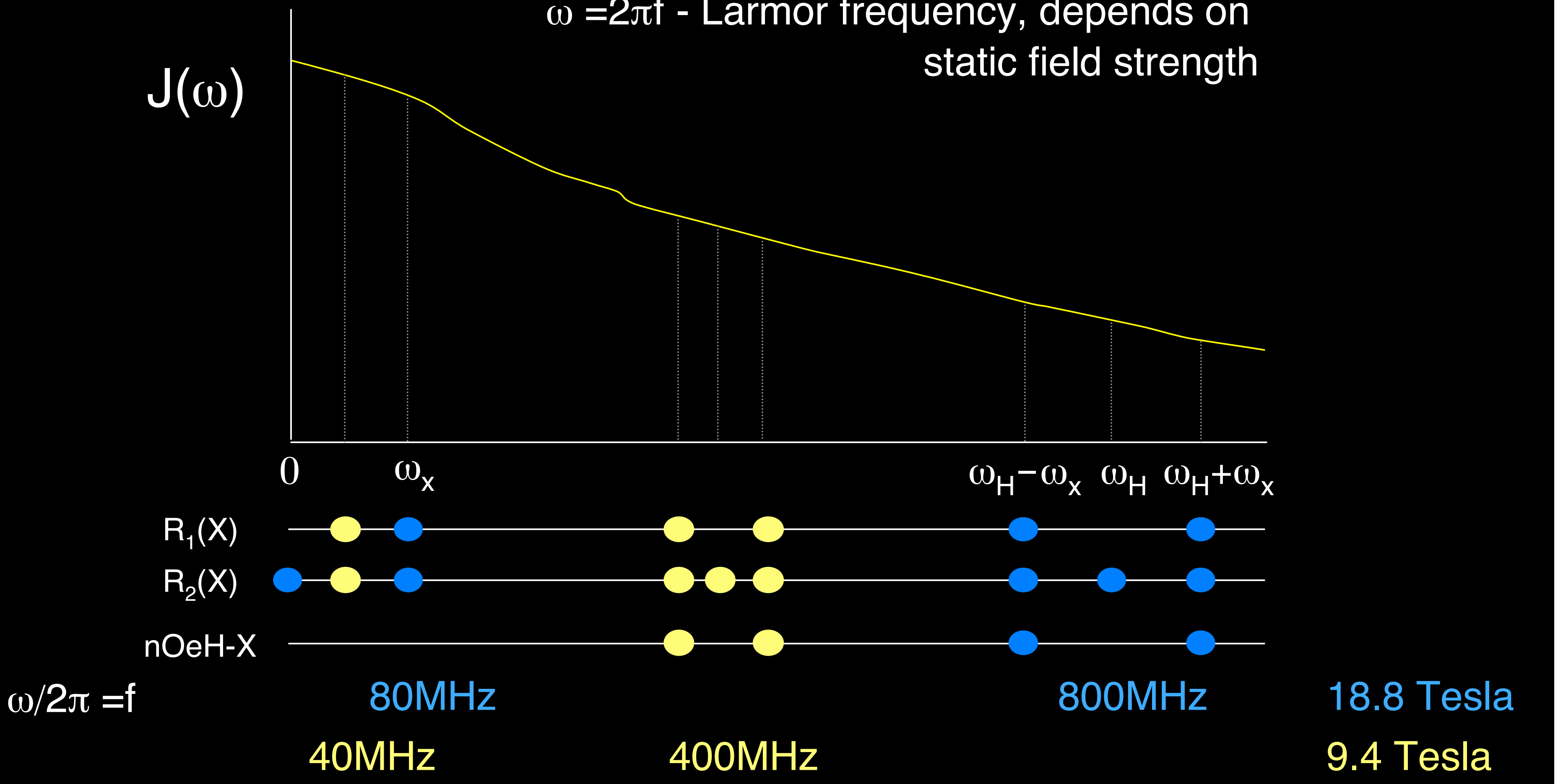




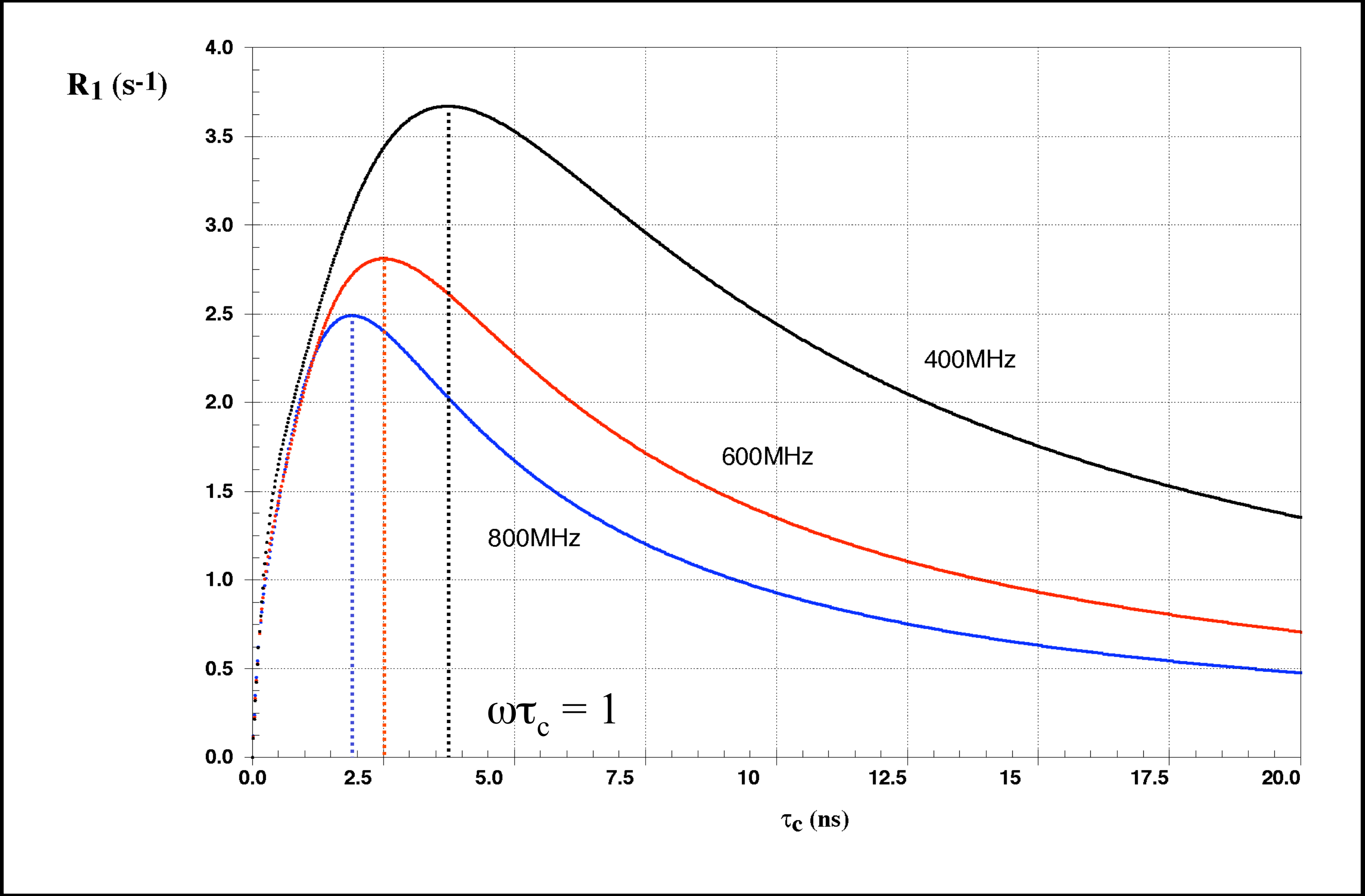
Relaxation rates

Spectral density mapping

Sampling of the spectral density function - Heteronuclear two spin system


$\omega = 2\pi f$ - Larmor frequency, depends on static field strength

Longitudinal ^{15}N Relaxation : Static Field Dependence

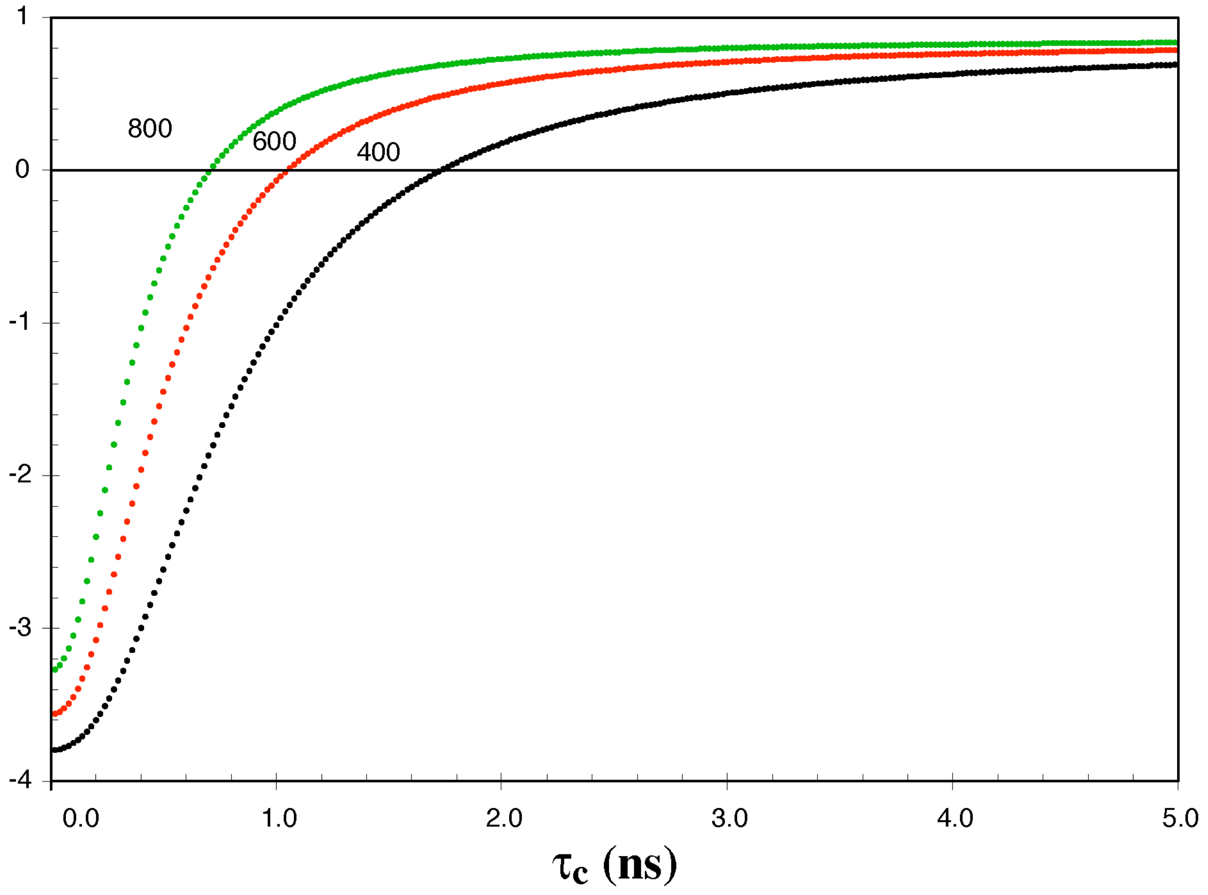


Nuclear Overhauser enhancement (nOe) Field Dependence

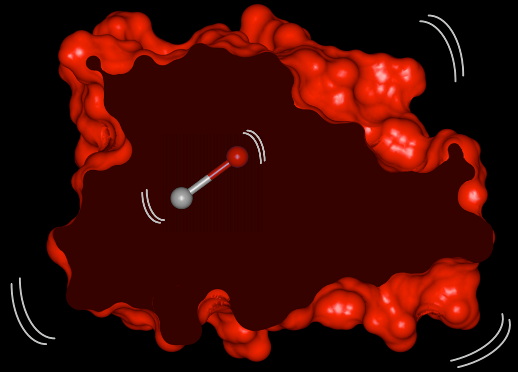
noe$\{^{1}H-^{15}N\}$

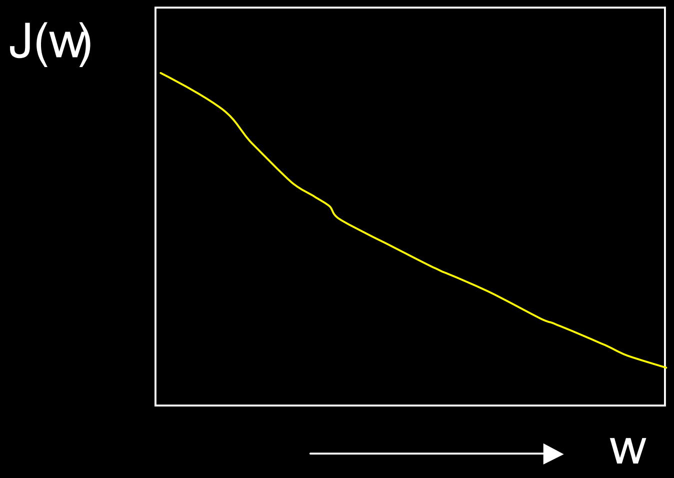
$\eta = 1+\gamma_I\sigma_{IS}/\gamma_S\rho_S$;

$\sigma_{IS} = d^2/4\{-J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$



Spectral density function : J(ω)

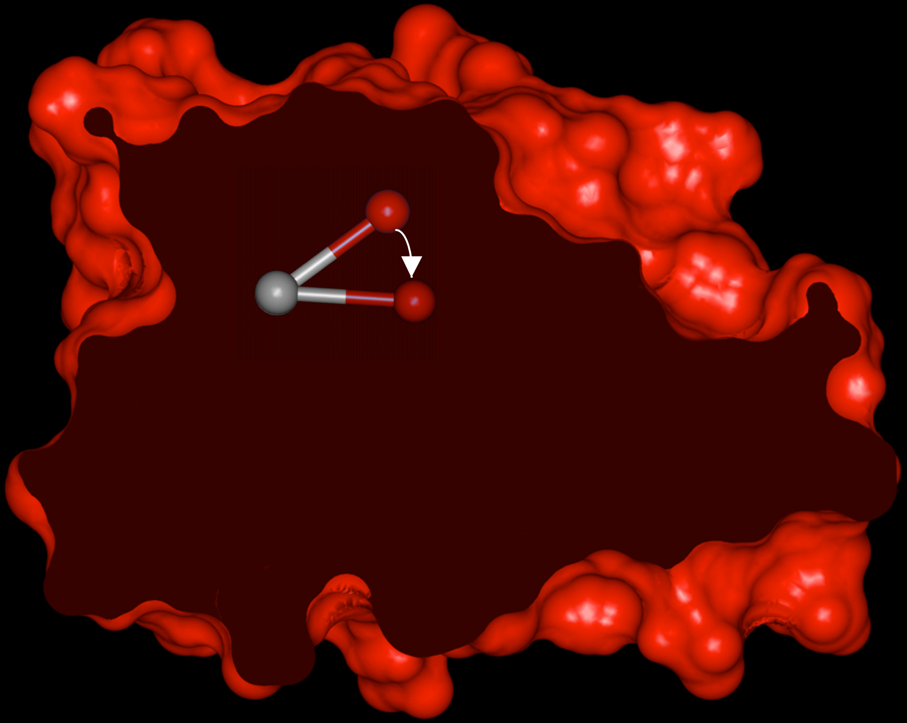




Describes the mobility of the inter-nuclear vector in terms of the distribution of frequency components

Fourier transform of the time-dependent auto-correlation function c(t)

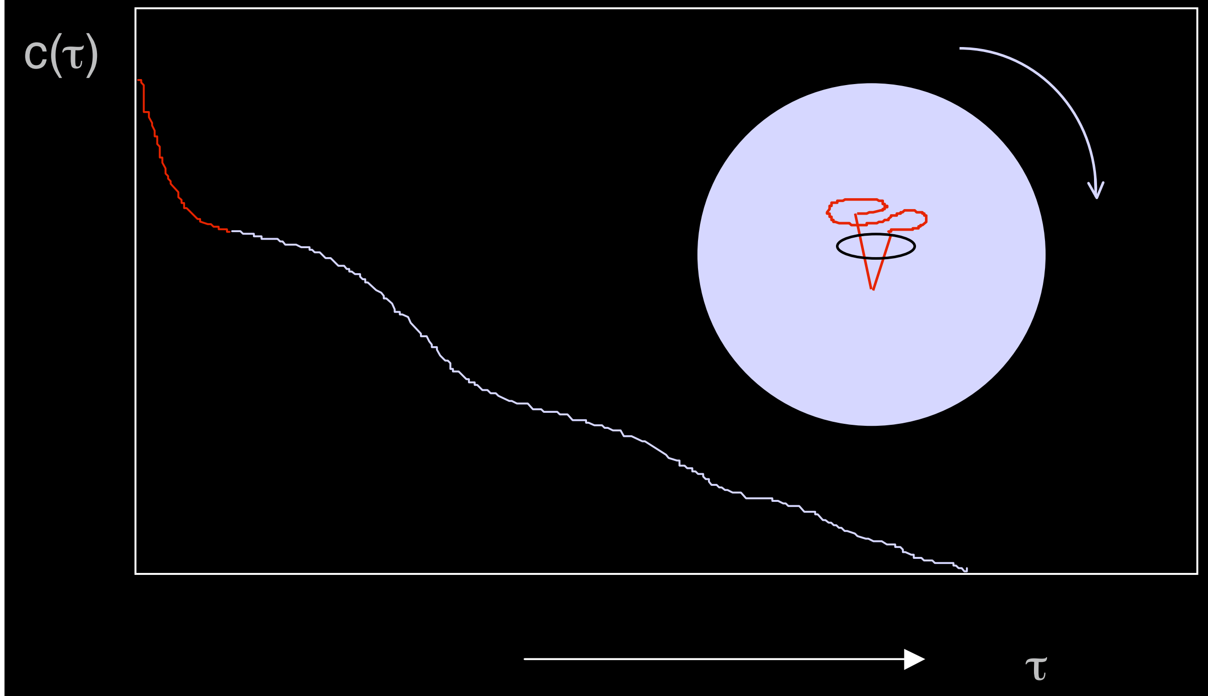
Auto-correlation function : $c(\tau)$



Correlates the value of a
parameters at a time t
with its value at time
$(t+\tau)$

$c(\tau)$ represents the
probability
of finding the same value
of the parameter at a time
τ later -
'memory'

Auto-correlation Functions



Correlates the value of a
parameter at a time t
with its value at time
$(t+\tau)$

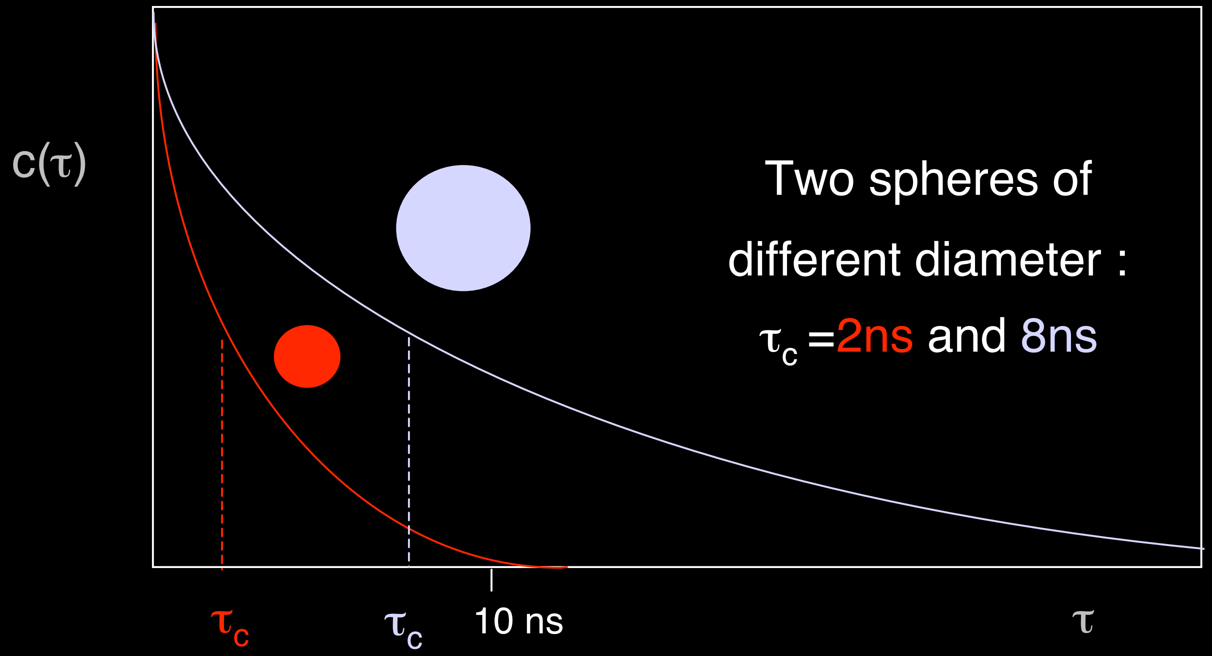
$c(\tau)$ represents the
probability
of finding the same value
of the parameter at a time
τ later -
‘memory’

$c(0) = 1$
Random movement -
$c(t\ )$ falls off with τ
$c(t\ ) < 1$

Auto-correlation Functions - Rigid and Spherical Molecule

Simplest function - decreasing exponential

- Describes rotational movement of a rigid sphere.
- Only one parameter τ_c characterises motion



Two spheres of different diameter :

τ_c =2ns and 8ns

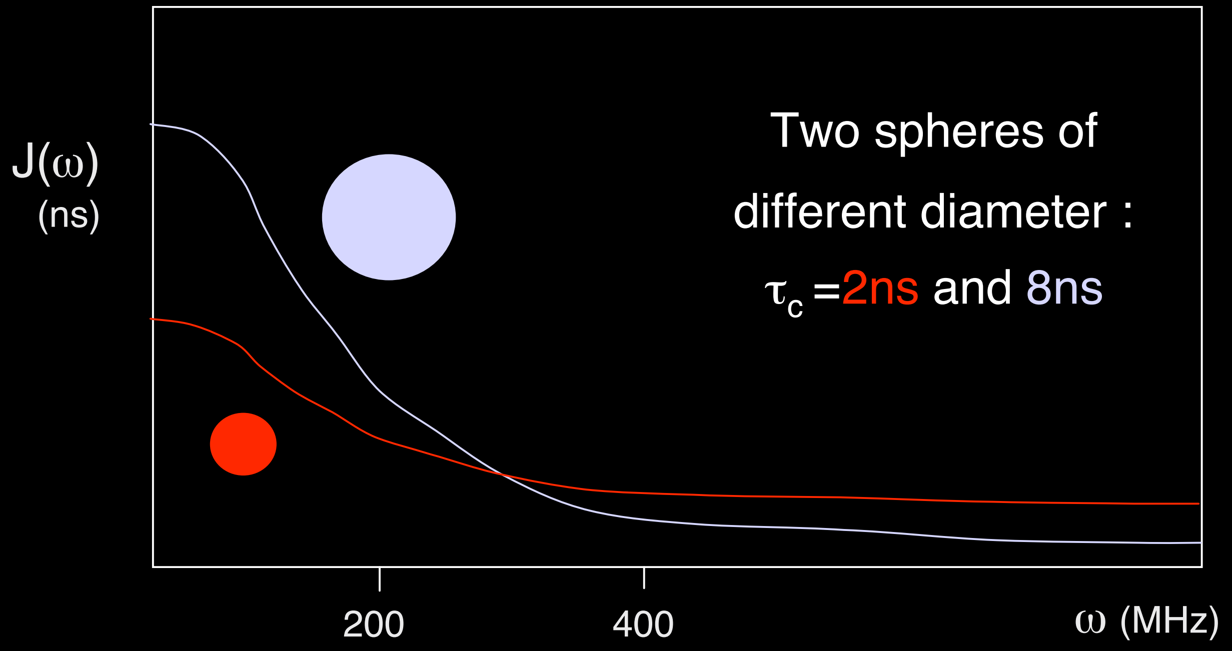
Stokes' Law

$$\tau_c = \frac{4\,\pi\eta_w r_H^3}{3\,k_B T}$$

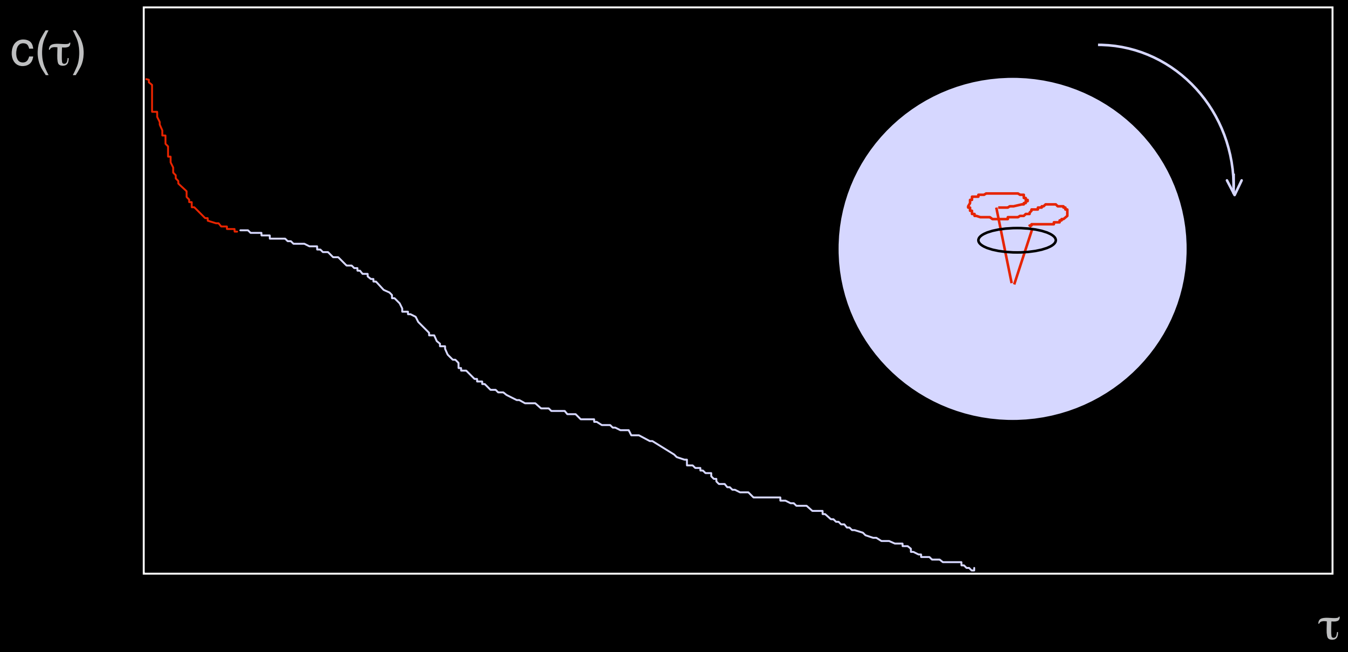
Auto-correlation Functions - Rigid and Spherical Molecule

Simple function - Lorentzian :

- Describes rotational movement of a rigid sphere.
- Width at half height = $2/\tau_c$



Two spheres of different diameter : τ_c =2ns and 8ns



In reality this function can of course be much more complex - Interactions are modulated by local motions and by the global motion of the molecule

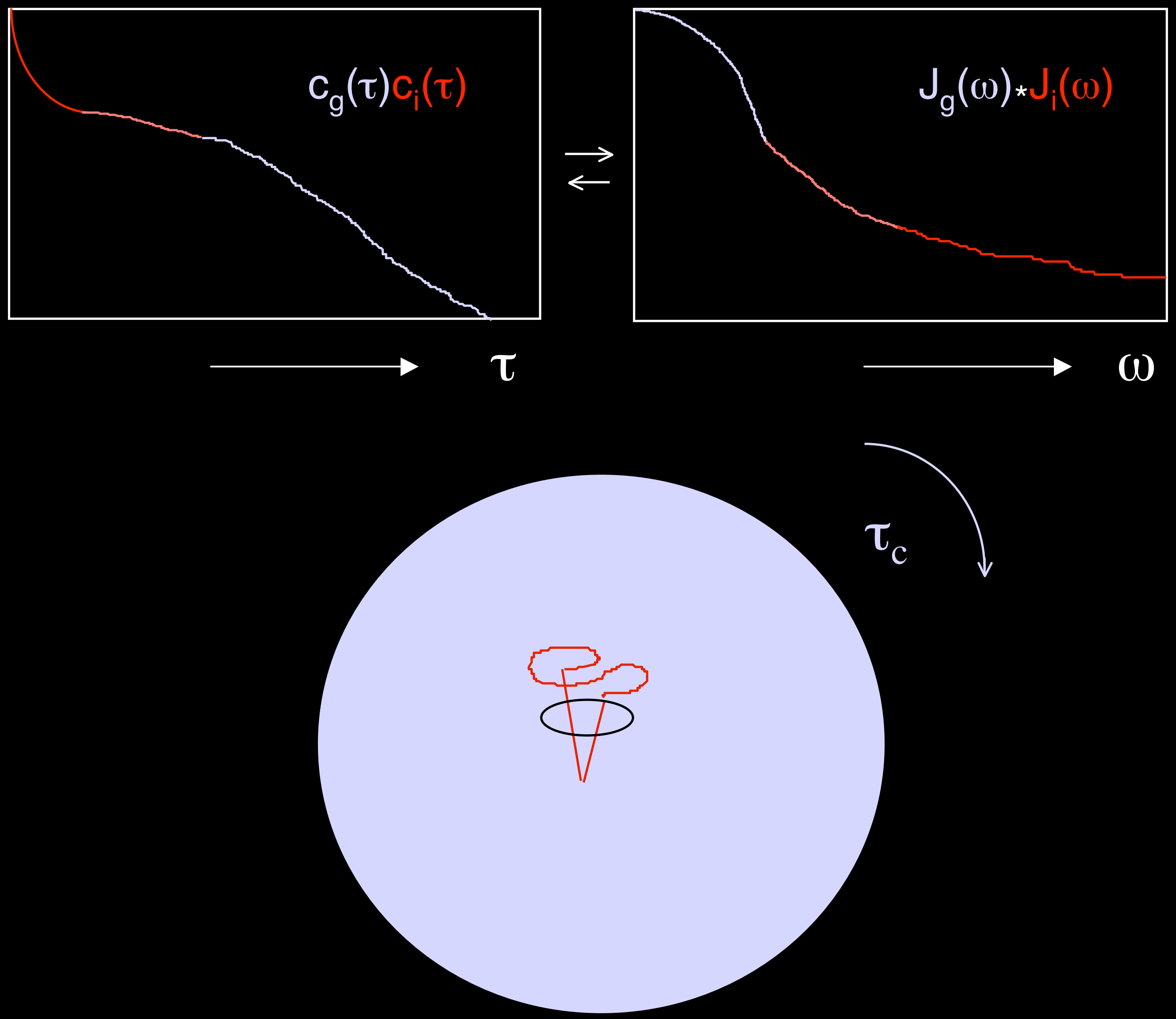
If these motions are not correlated, and occur on different time scales, the corrlation functions can be described by :

$$c(\tau) = c_g(\tau)c_i(\tau)$$

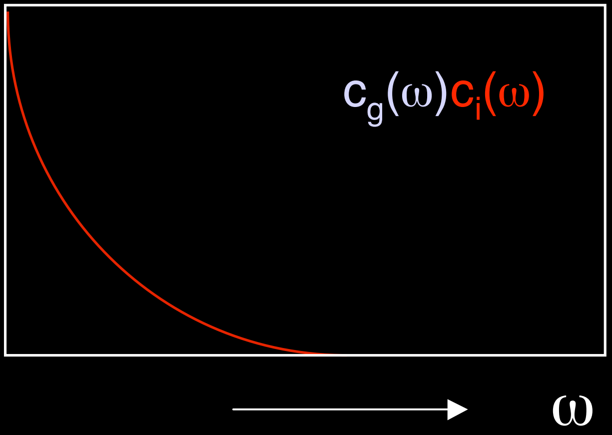
and

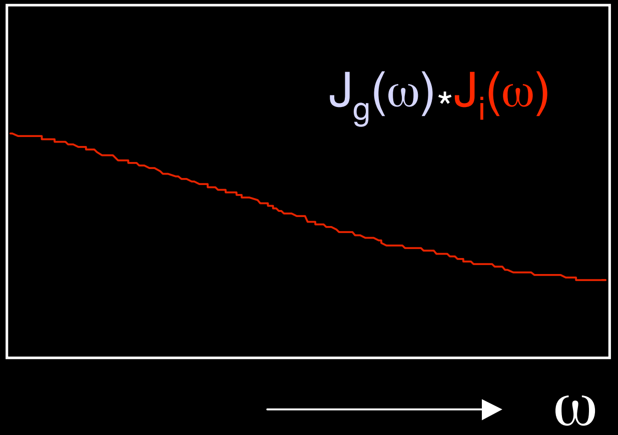
$$J(\omega) = J_g(\omega) * J_i(\omega)$$

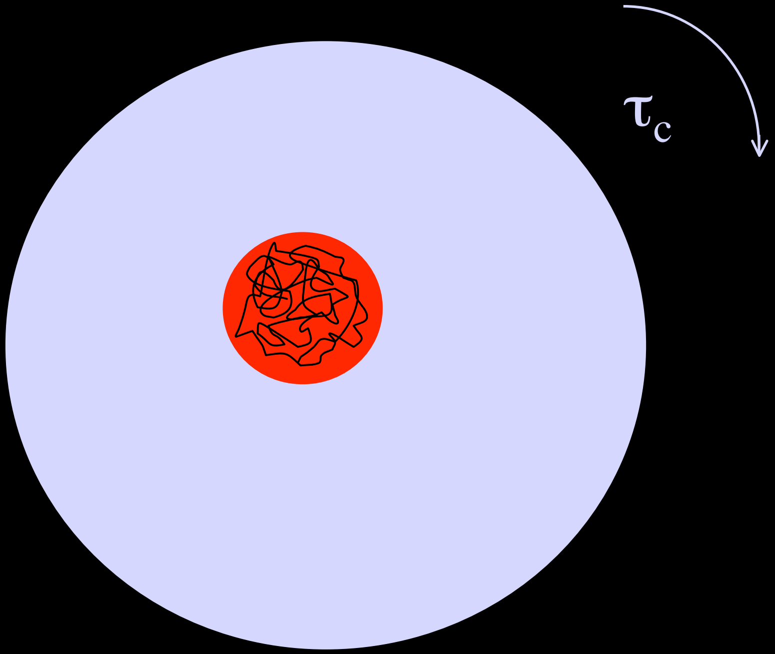
Restricted Internal Motion



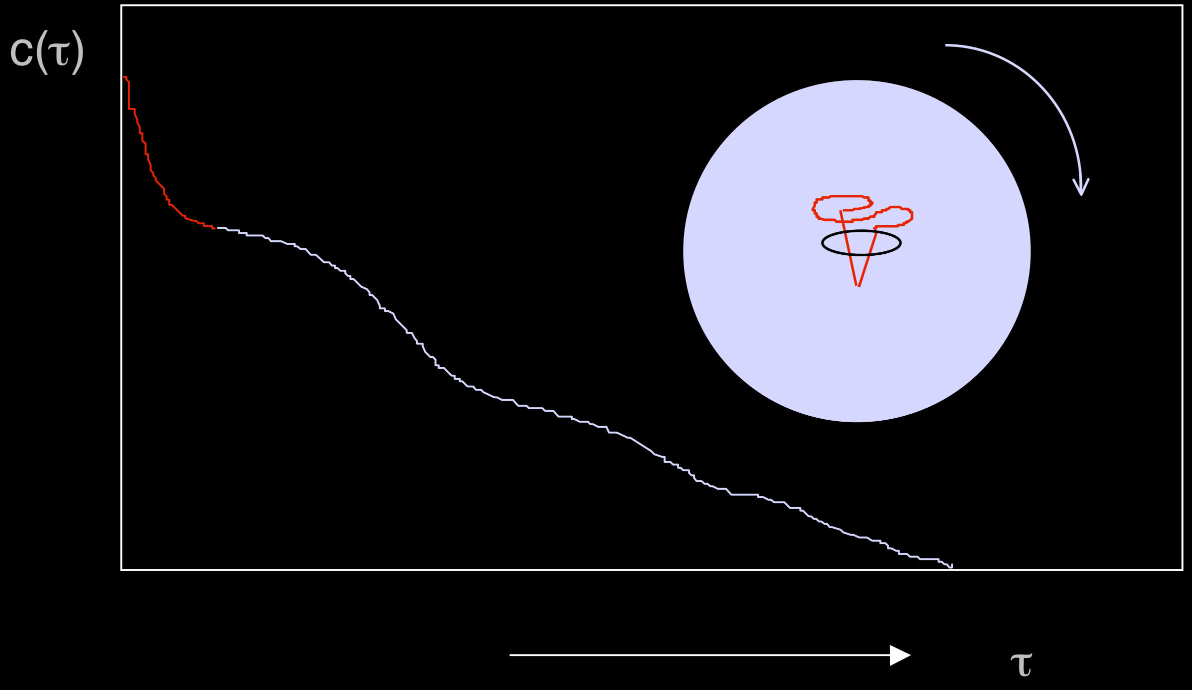
Unrestricted Internal Motion







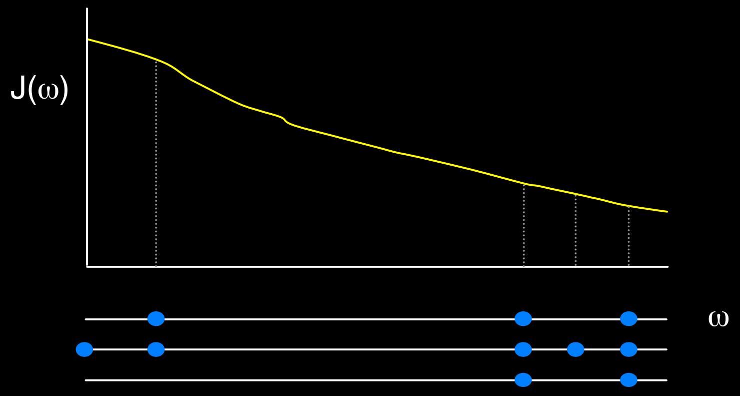
Information available from ^{15}N Relaxation



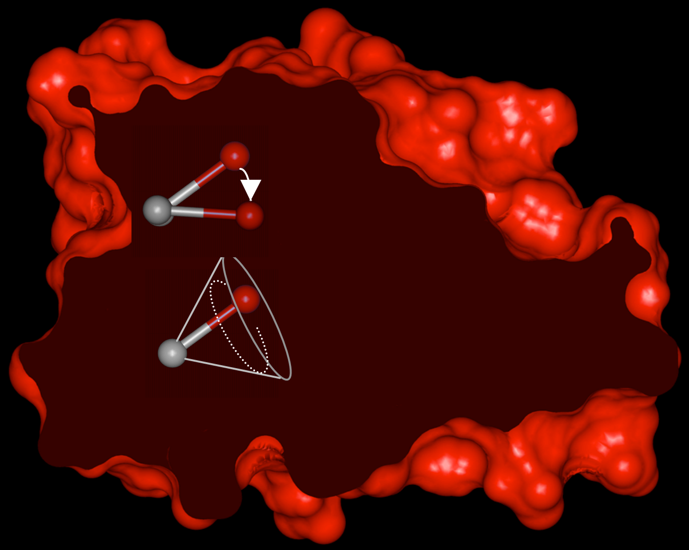
Reminder : Beyond a few τ_c there is no remaining ‘memory’

No direct information about slower internal motion?

Analysis of Internal Mobility using ^{15}N Relaxation



Characterisation of the motion by
analytical modelling of
$J(\omega)$ or $c(\tau)$



- Free diffusion about an axis
- Restricted rotation about an axis
- Diffusion/rotation about an axis
- Diffusion on the surface of a cone
- Discrete n-site jumps
- ……

$J(\omega)$ - can be precise, but complex: Number of dynamic parameters to be fitted can exceed the number of experimentally available relaxation rates.

Differentiation?

Analysis of Movement without a Geometric Model

Modelling of $c(\tau)$ (et $J(\omega)$) using a simple mathematical function

Reduces the number of degrees of freedom

Allows possibility of statistical analysis of treatment

Lipari-Szabo Model

Internal and global motion are supposed independant

Simplest model : $c(\tau)$ described by two exponential functions :

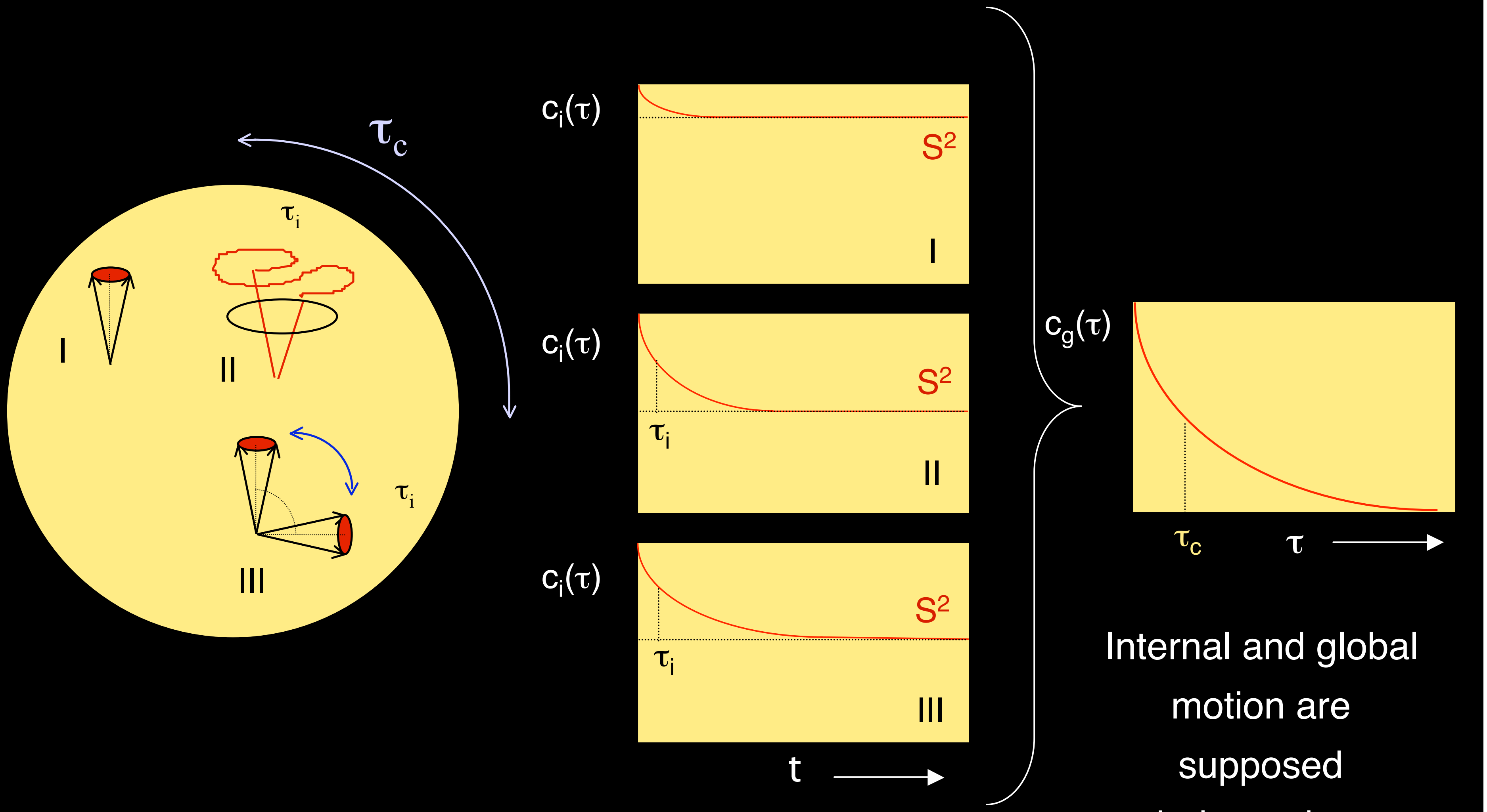
$$c(\tau) = c_g(\tau)c_i(\tau)$$
$$c_i(0)=1;\ c_i(\infty)=S^2$$

$$c_i(\tau) = S^2 + (1- S^2)\exp(-\tau/\ \tau_{\ i})$$

For isotropic overall tumbling -

$$c_g(\tau) =(1/5)\ \exp(-\tau/\ \tau_c)$$

Lipari-Szabo Model-free Approach : Auto-correlation Function



S^2 -Generalised Order Parameter describing amplitude of angular reorientation

Internal and global motion are supposed independant

$\tau_i << \tau_c$

Lipari-Szabo Model-free Approach : Spectral Density Function

Similarly the spectral density function can be described by the sum of two Lorentzians, describing the internal and overall motion :

$$J(\omega) = (2/5)\,(S^2\,\tau_c\,/(1+\omega^2\tau_c{}^2) + (1-S^2)\,\tau_e/(1+\omega^2\tau_e{}^2)\,)$$

All relaxation rates can be described in terms of this simple function

τ_c is common to the whole molecule

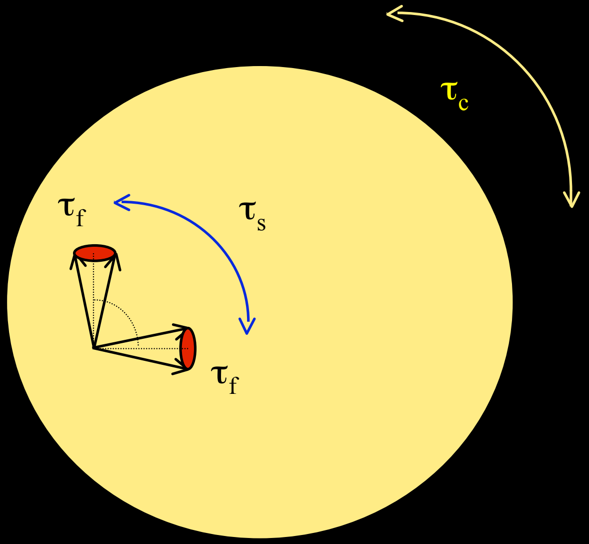
$\{S^2, \tau_e\}$ can be determined for each site from $\{R_1, R_2, nOe\}$

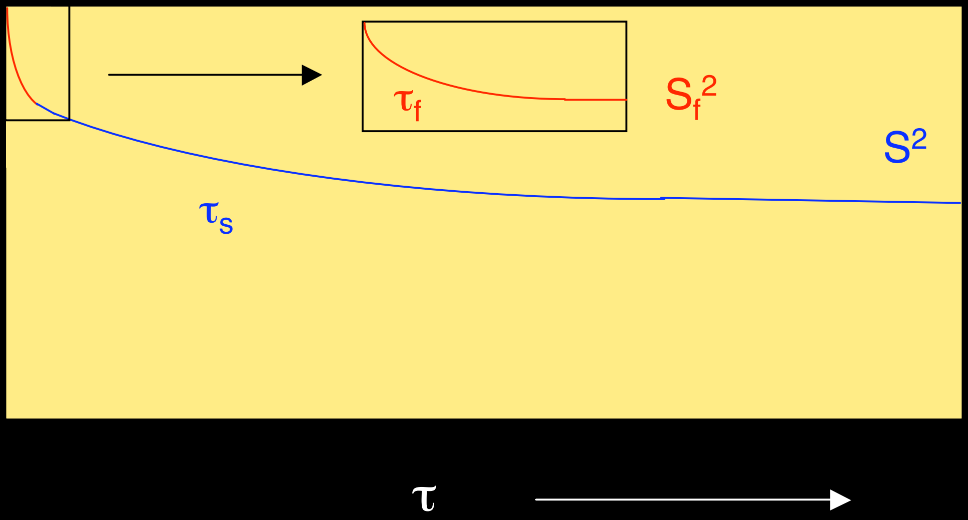
Extended Model-free Approach

Introduction of an additional movement with intermediate correlation time τ_s

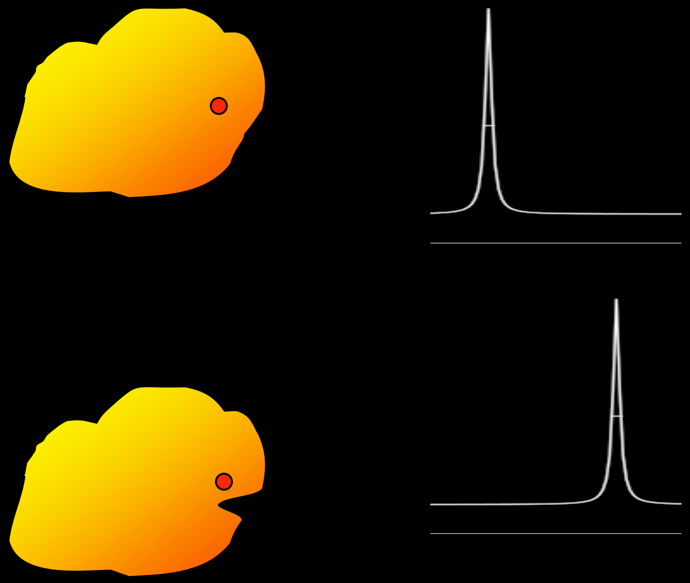
$$c_i(\tau) = S^2 + (1- S_f^2)\exp(-t/ \tau_f) + (S_f^2 - S^2)\exp(-t/ \tau_s)$$

$$J(\omega) = (2/5)\ (S^2\ \tau_c\ /(1+ \omega^2\tau_c^2) + (S_f^2 - S^2)\tau_s/(1+ \omega^2\tau_s^2))\ ;\ \tau_f \rightarrow 0$$





Chemical Shift Exchange - Contribution to R_2



Conformational exchange (t_{exch} - ms)

Measured R_2 can contain

a contribution due to

rapid exchange R_{ex} between

conformers having different

chemical shifts

(*see Carine’s talk*)

Can lead to artefacts

in model-free analysis

if undetected

Statistical Analysis of Heteronuclear Relaxation Data

$J(\omega) = (2/5)\ (S_f^2\ (\tau_c\ /(1+\ \omega^2\tau_c^2) + (1-\ S_s^2)\ \tau_s/(1+\ \omega^2\tau_s^2))$

Measurements - (R_1, R_2, nOe)

Dynamic Parameters - τ_c (S_f^2 , S_s^2 , τ_s , R_{ex})

Generalised Analysis with no Geometric Model

Lipari-Szabo Approach

- $c(\tau)$ (and $J(\omega)$) described using abstract mathematical model
- Internal and global motion supposed independent and exponential in time
- Internal motion described by an amplitude (S^2) and correlation time (τ_e)
- τ_c is common to the whole molecule
- $\{S^2, \tau_e\}$ can be determined for each site from $\{R_1, R_2, \mathrm{nOe}\}$
- All relaxation rates can be described in terms of this simple function
- S^2 measures thermally accessible orientations - Local contribution to entropy

Model-free approach susceptible to artefacts due to

- Under-determination - Over-interpretation
- Poorly determined τ_c - (using ‘ rigid ’ regions)
- Local/overall motion correlated
- Non-globular behaviour

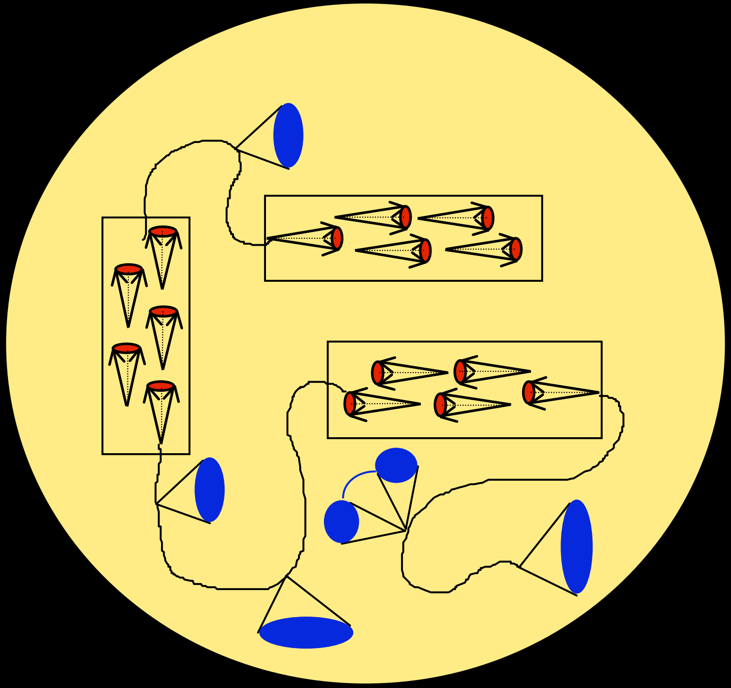
Statistical Analysis - Estimation of τ_c

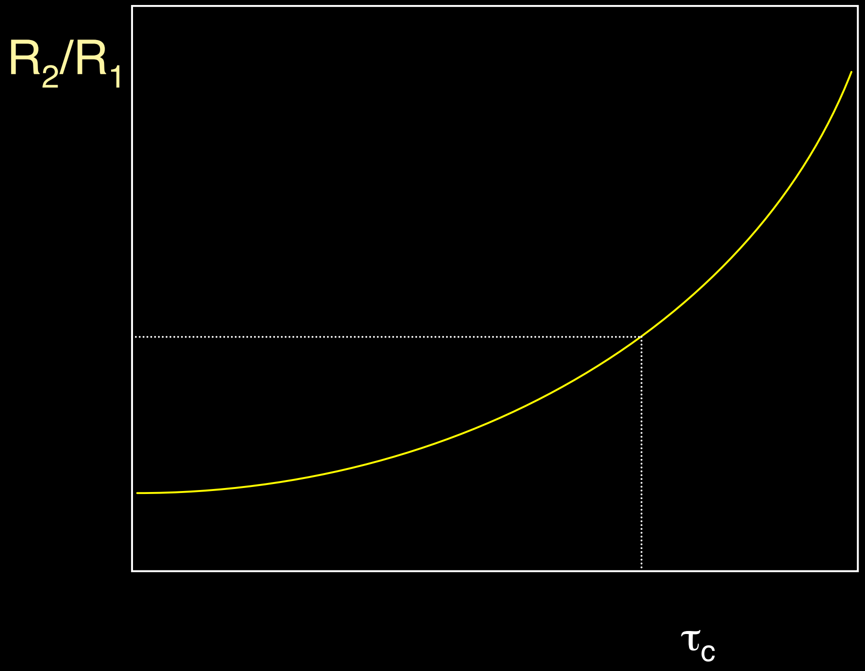
$$J(\omega) = (2/5)\,(S_f^2\,(\tau_c/(1+\omega^2\tau_c^2) + (1-S_s^2)\,\tau_s/(1+\omega^2\tau_s^2))$$

In limit where internal motion is considered fast - *librational* (restricted motion on 10s picosecond timescale)

$$J(\omega) = (2/5)\,(S_f^2\,(\tau_c/(1+\omega^2\tau_c^2))$$

Ratio R_2/R_1 can be considered independent of $\{S^2,\tau_s\}$





Statistical Analysis of Heteronuclear Relaxation Data

	Modelled Parameters
• Model 1	S^2
• Model 2	S^2,τ_i
• Model 3	S^2,R_{ex}
• Model 4	$S^2,\tau_i\,,R_{ex}$
• Model 5	$S_f^2\,,\,S_s^2\,,\,\tau_s$

Iterative modelling of relaxation data using increasingly complex $J(\omega)$
until data satisfy estimated confidence level

$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} + c^2\{J(\omega_N)\}$$

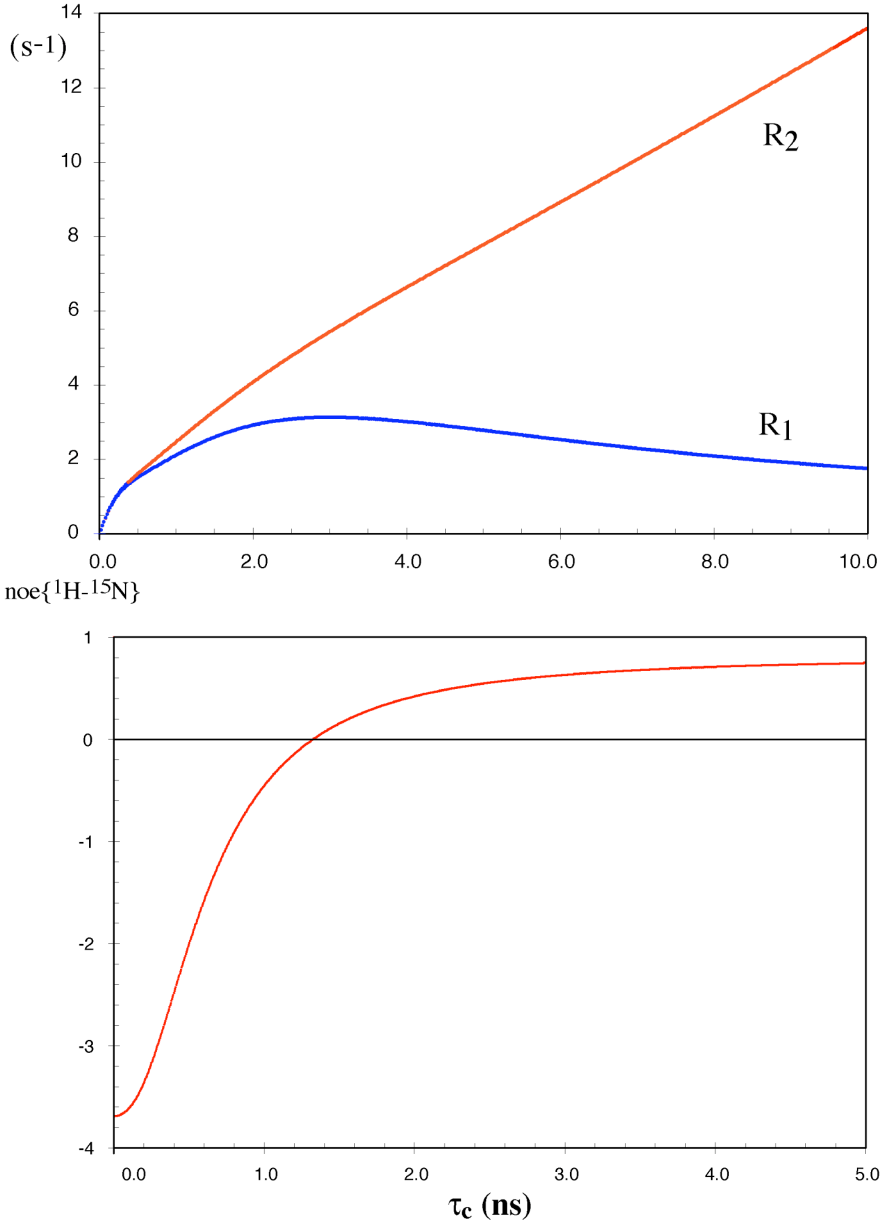
$$R_2 = d^2/4\{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)\} + c^2\{4J(0) + 3J(\omega_N)\}$$

$$(I_z \rightarrow S_z) = d^2/4\{- J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$$

Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

Rigid vector attached to a rotor with overall correlation time 10ns

500MHz spectrometer frequency



$$R_1 = d^2/4\{J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)\} + c^2\{J(\omega_N)\}$$

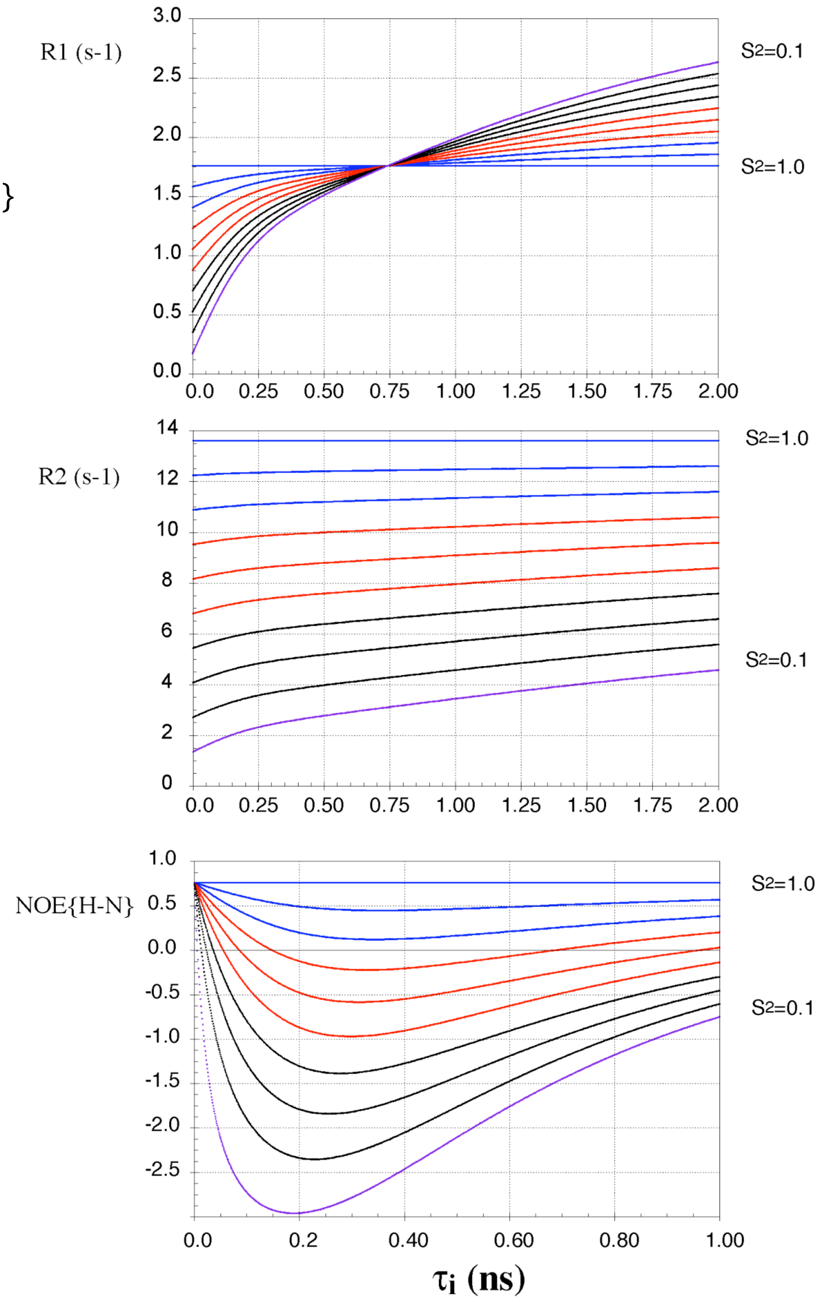
$$R_2 = d^2/4\{4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)\} + c^2\{4J(0) + 3J(\omega_N)\}$$

$$(I_z \rightarrow S_z) = d^2/4\{- J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$$

Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

Isotropic global motion with overall correlation time 10ns

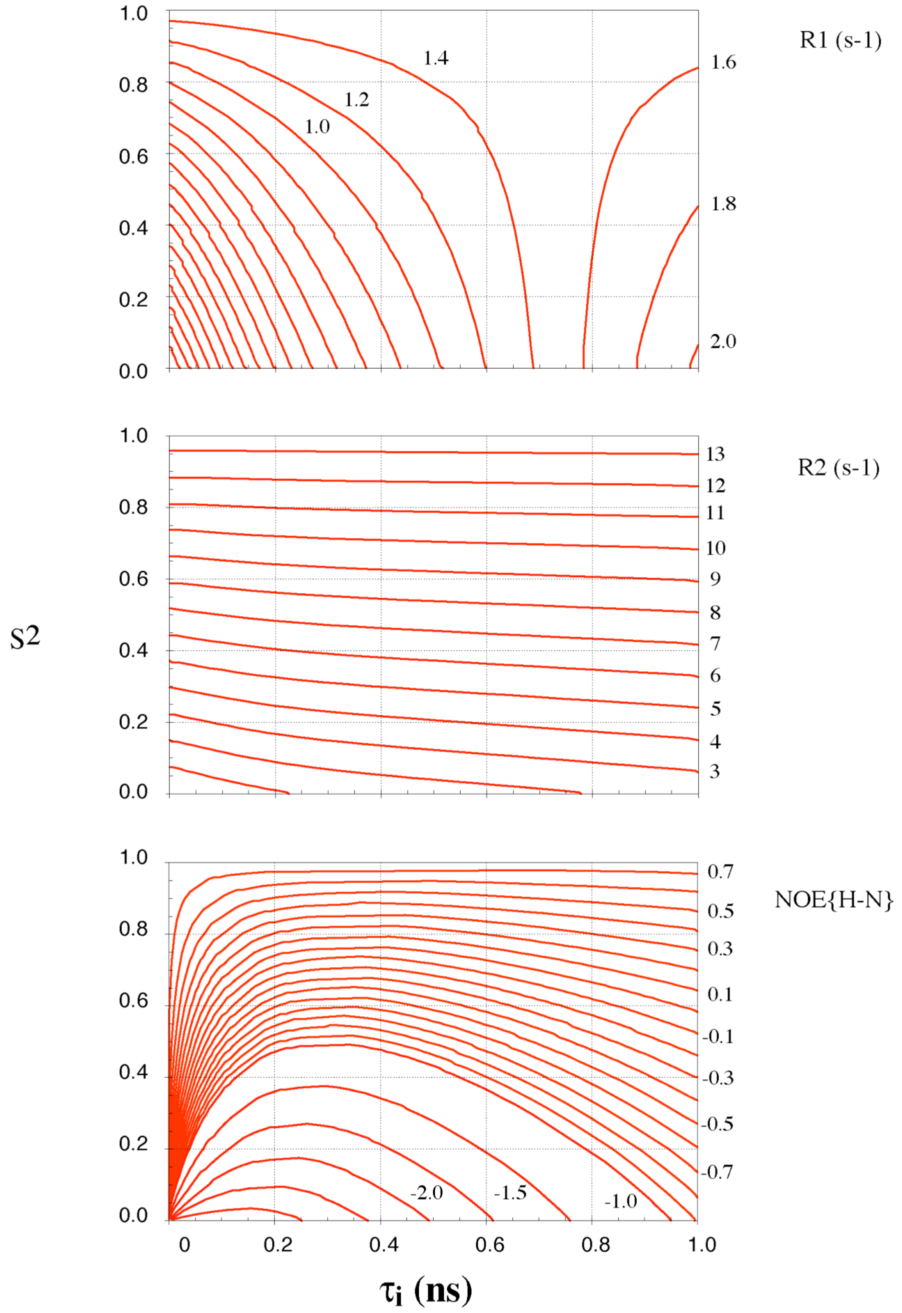
500MHz spectrometer frequency



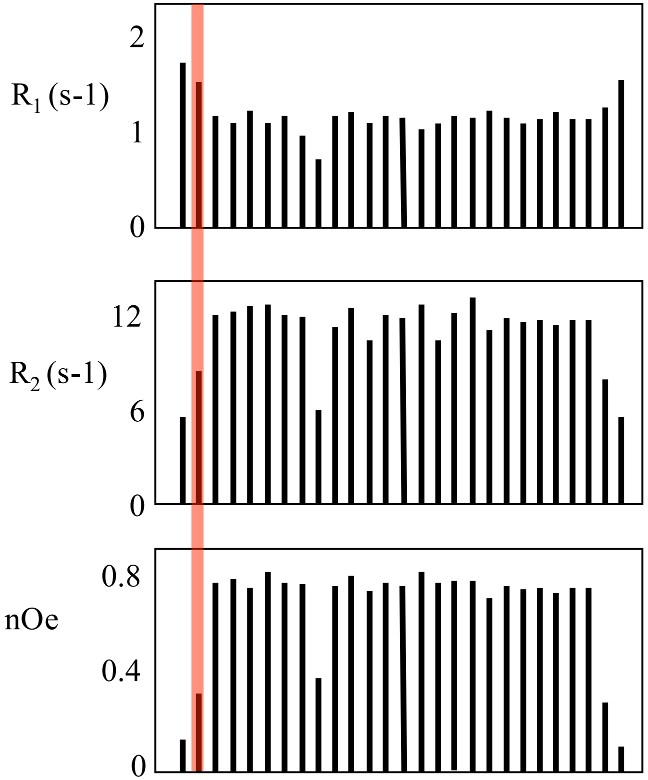
Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

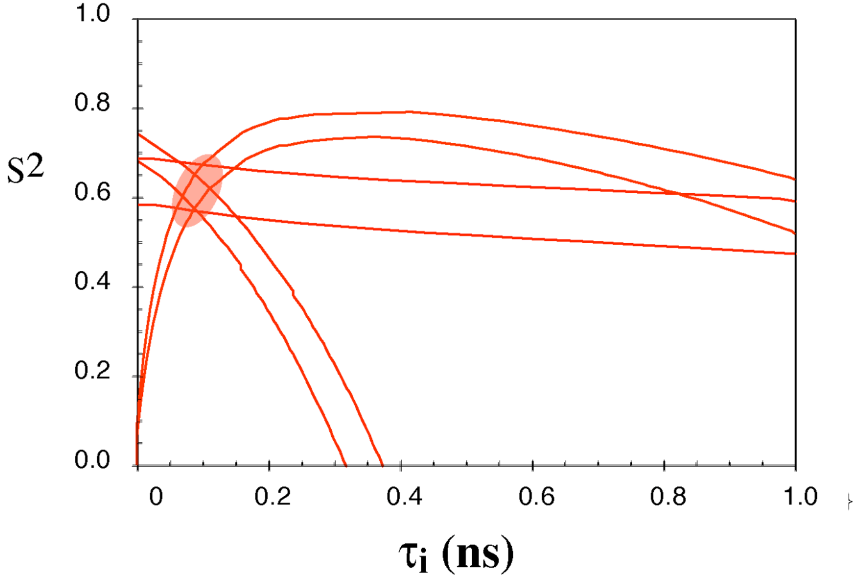
Isotropic rotor with overall correlation time 10ns 500MHz spectrometer frequency

Isocontours of relaxation rate values R_1, R_2 and nOe



Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_i\}$ to relaxation data

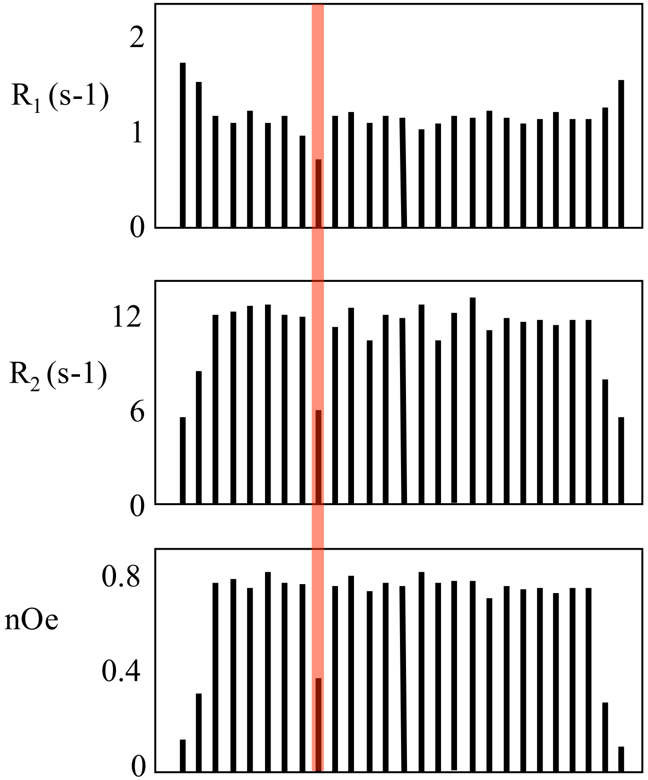


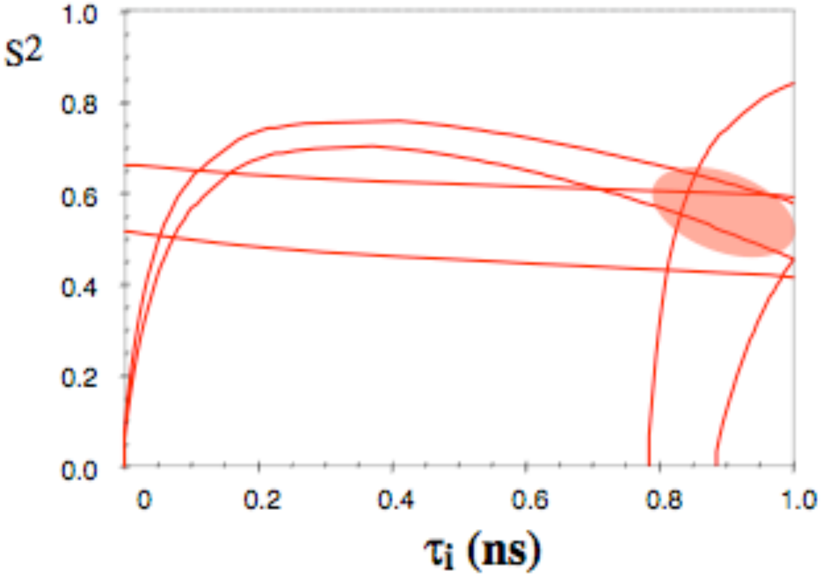


Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

Isotropic rotor with overall correlation time 10.0ns

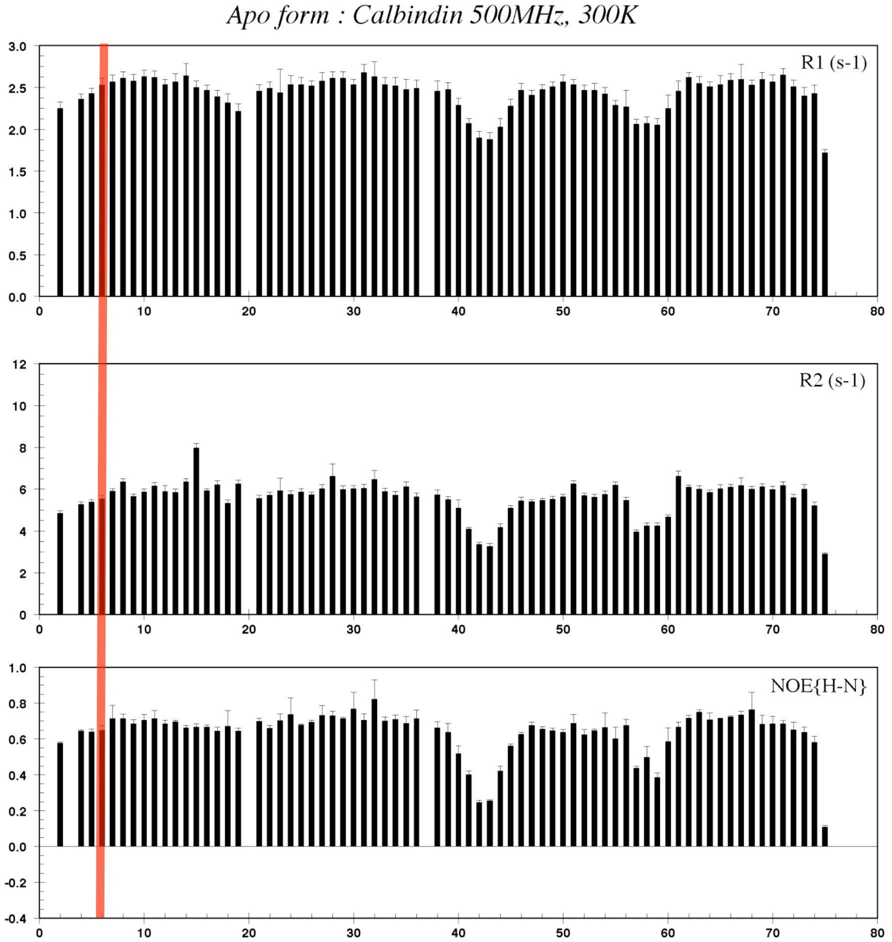
Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_i\}$ to relaxation data





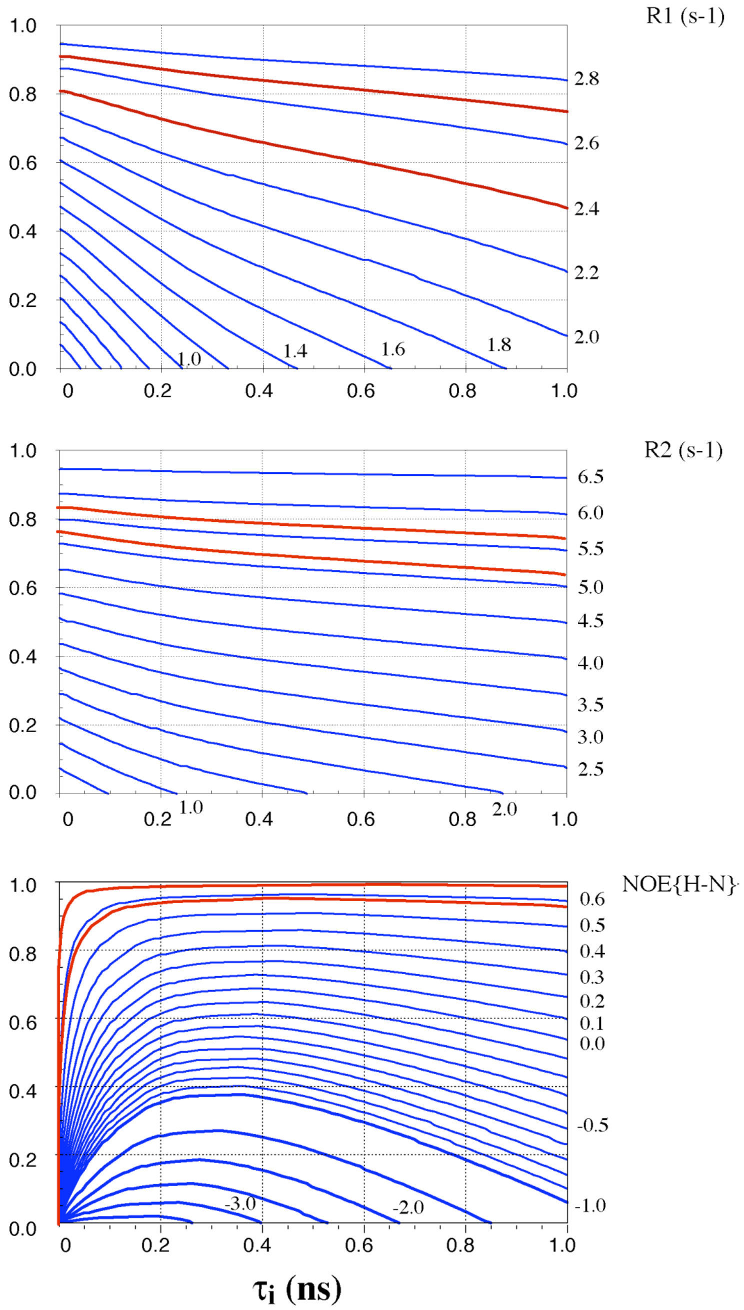
Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

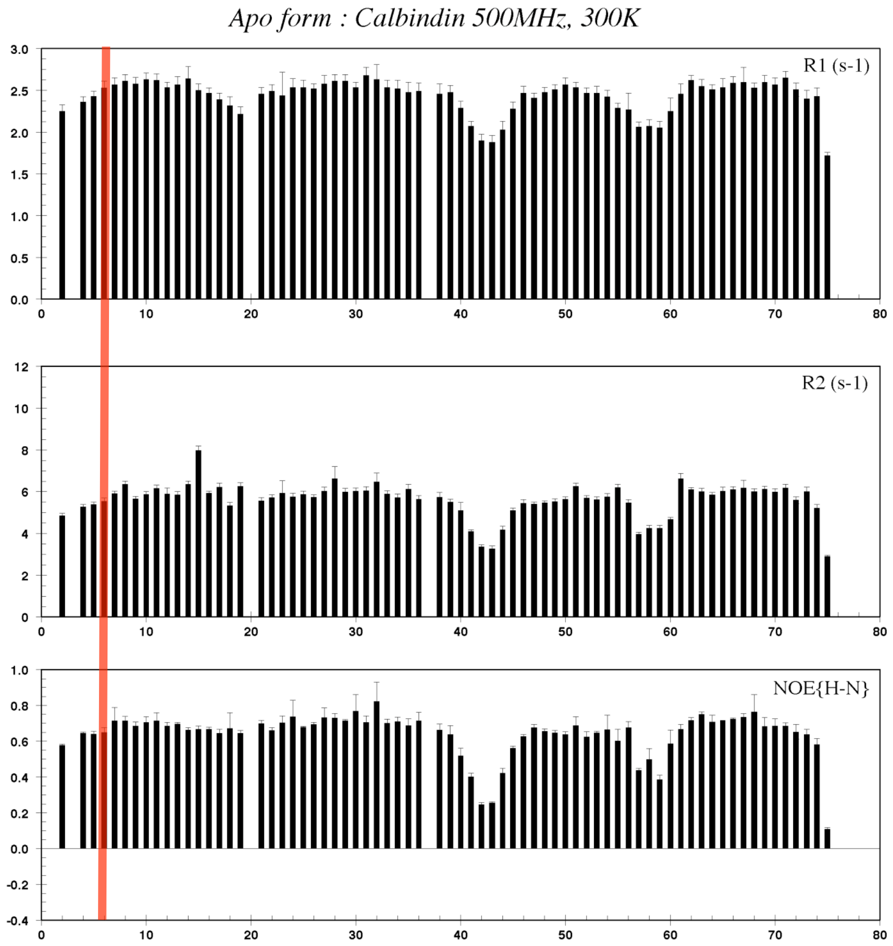
Isotropic rotor with overall correlation time 10.0ns



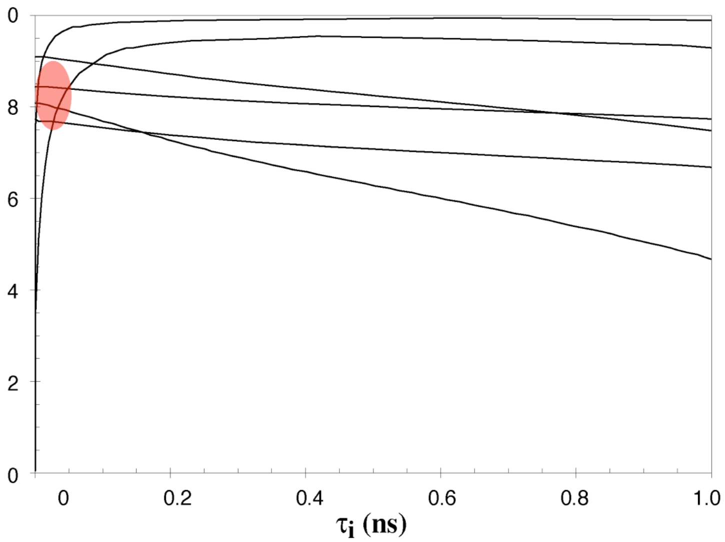
Dependence of ^{15}N relaxation rates on Lipari-Szabo parameters $\{S^2, \tau_i\}$

Isotropic rotor with overall correlation time 4.22ns





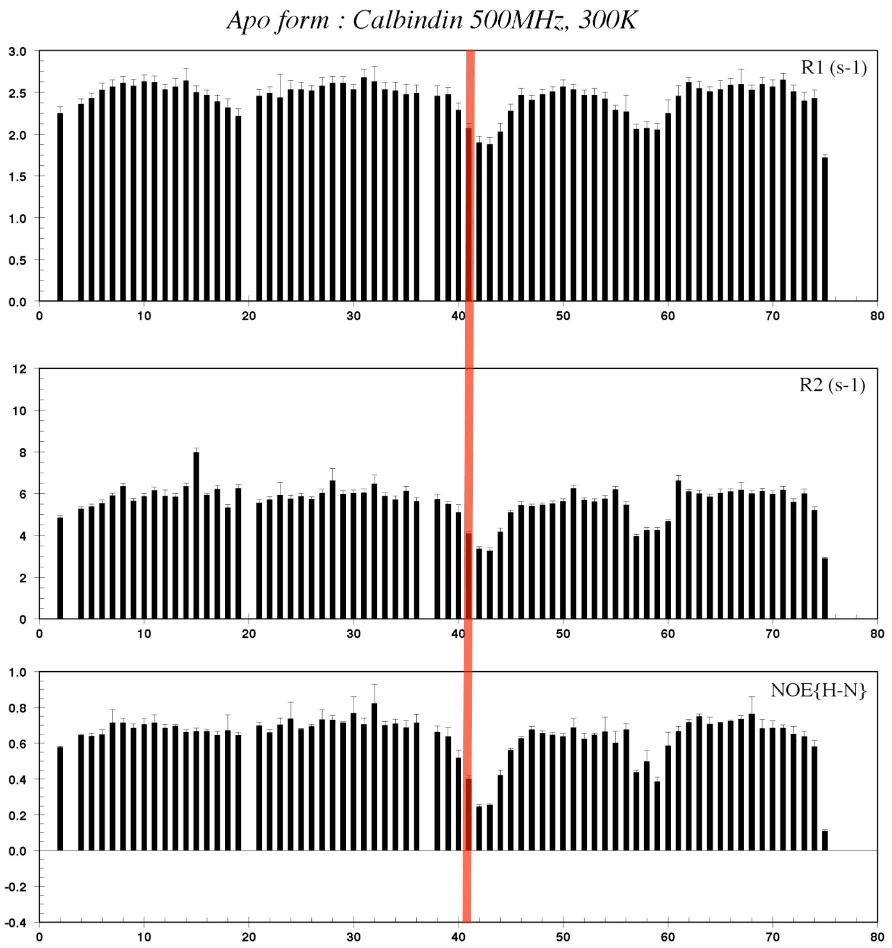
Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_i\}$ to relaxation data



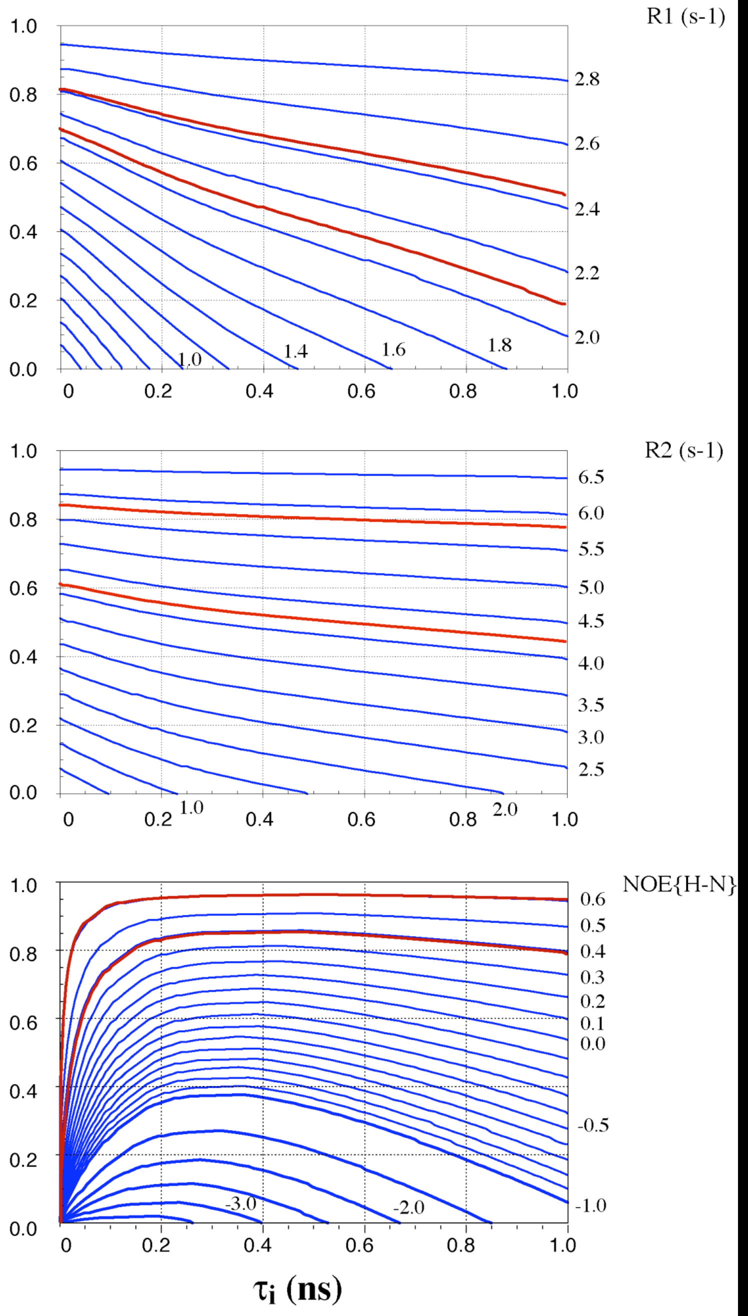
$$R_1 = (2.53 \pm 0.06)\ s^{-1}$$
$$R_2 = (5.55 \pm 0.04)\ s^{-1}$$
$$nOe = 0.650 \pm 0.006$$

→

$$S^2 = 0.81 \pm 0.02$$
$$\tau_i = 44 \pm 11 ps$$



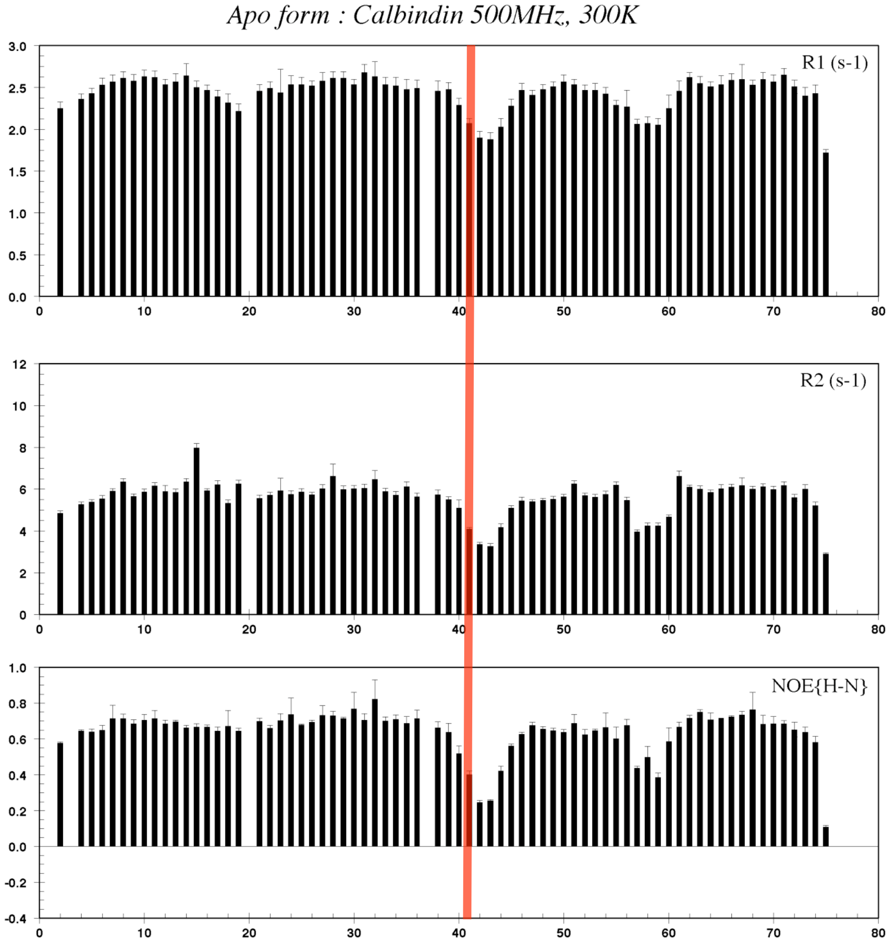
Apo form : Calbindin 500MHz, 300K



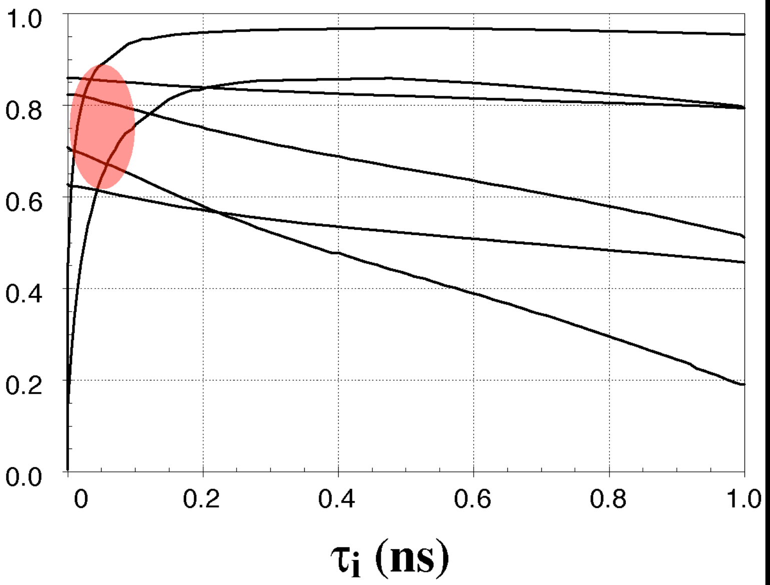
$$R_1 = (2.29 \pm 0.08)\ s^{-1}$$

$$R_2 = (5.09 \pm 0.4)\ s^{-1}$$

$$nOe = 0.52 \pm 0.04$$

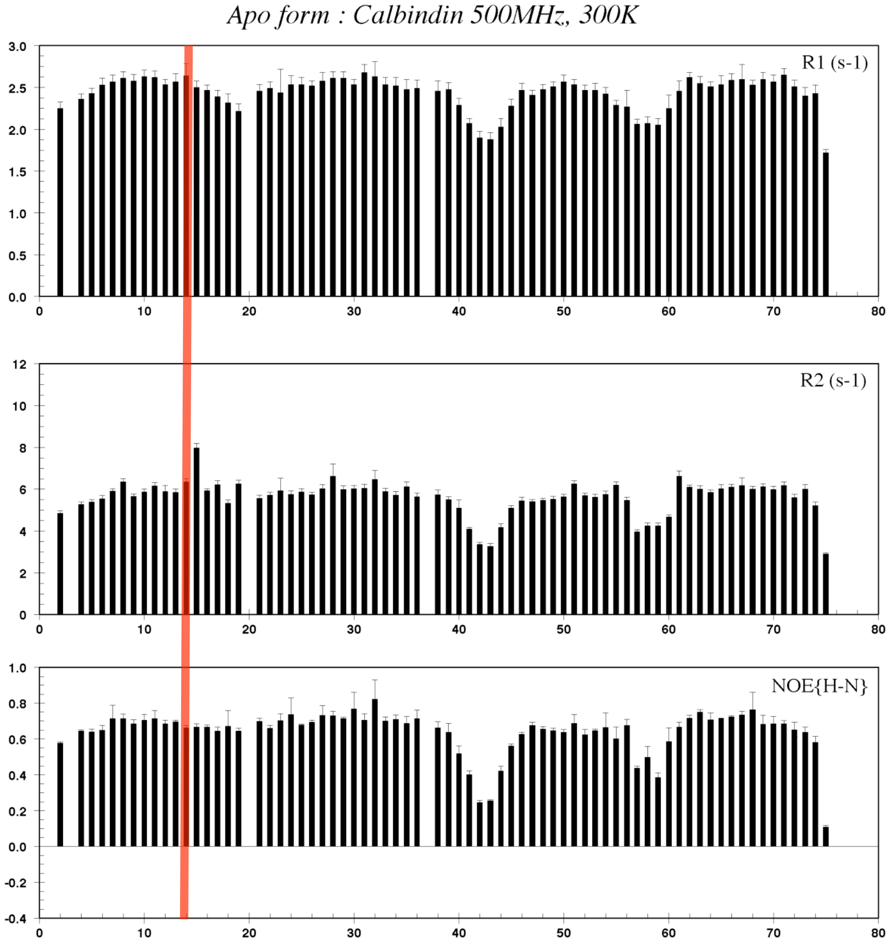


Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_i\}$ to relaxation data



$R_1 = (2.29 \pm 0.08)\ s^{-1}$
$R_2 = (5.09 \pm 0.4)\ s^{-1}$
nOe =0.52 ± 0.04

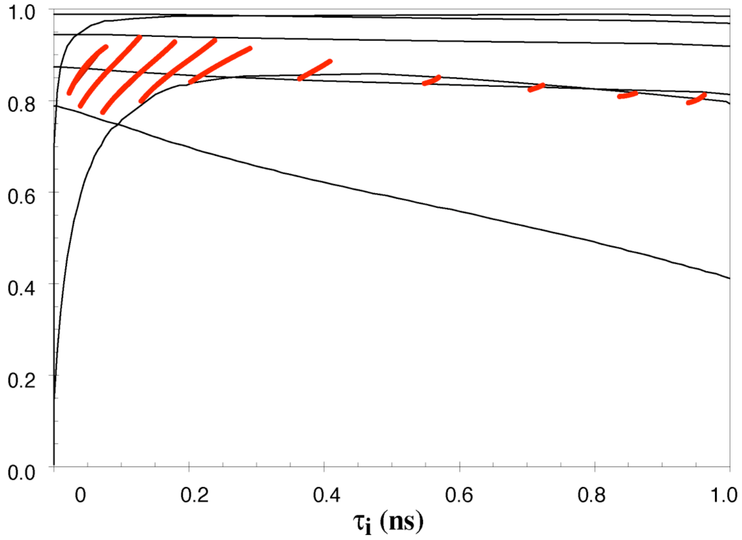
$S^2 = 0.73 \pm 0.02$
$\tau_i = 78 \pm 20$ps



Lipari-Szabo Modelfree Analysis :

Fitting $\{S^2, \tau_i\}$

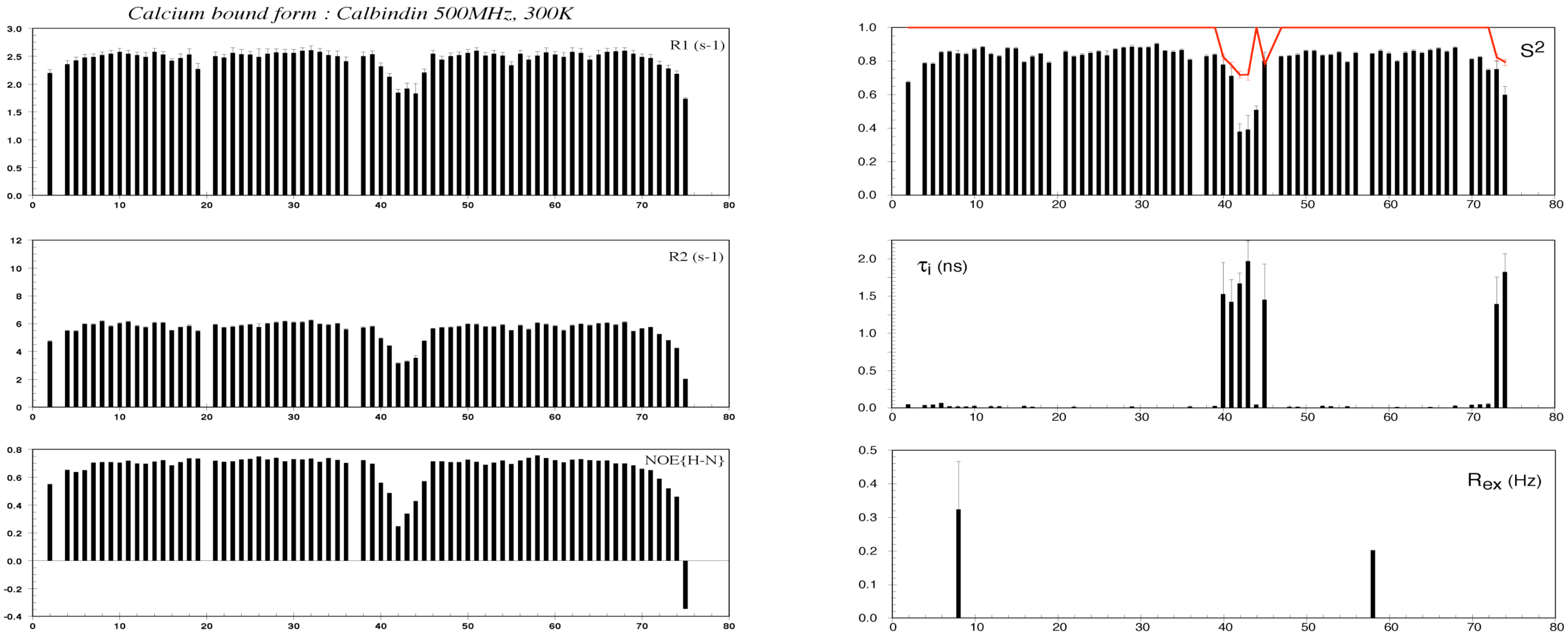
to relaxation data



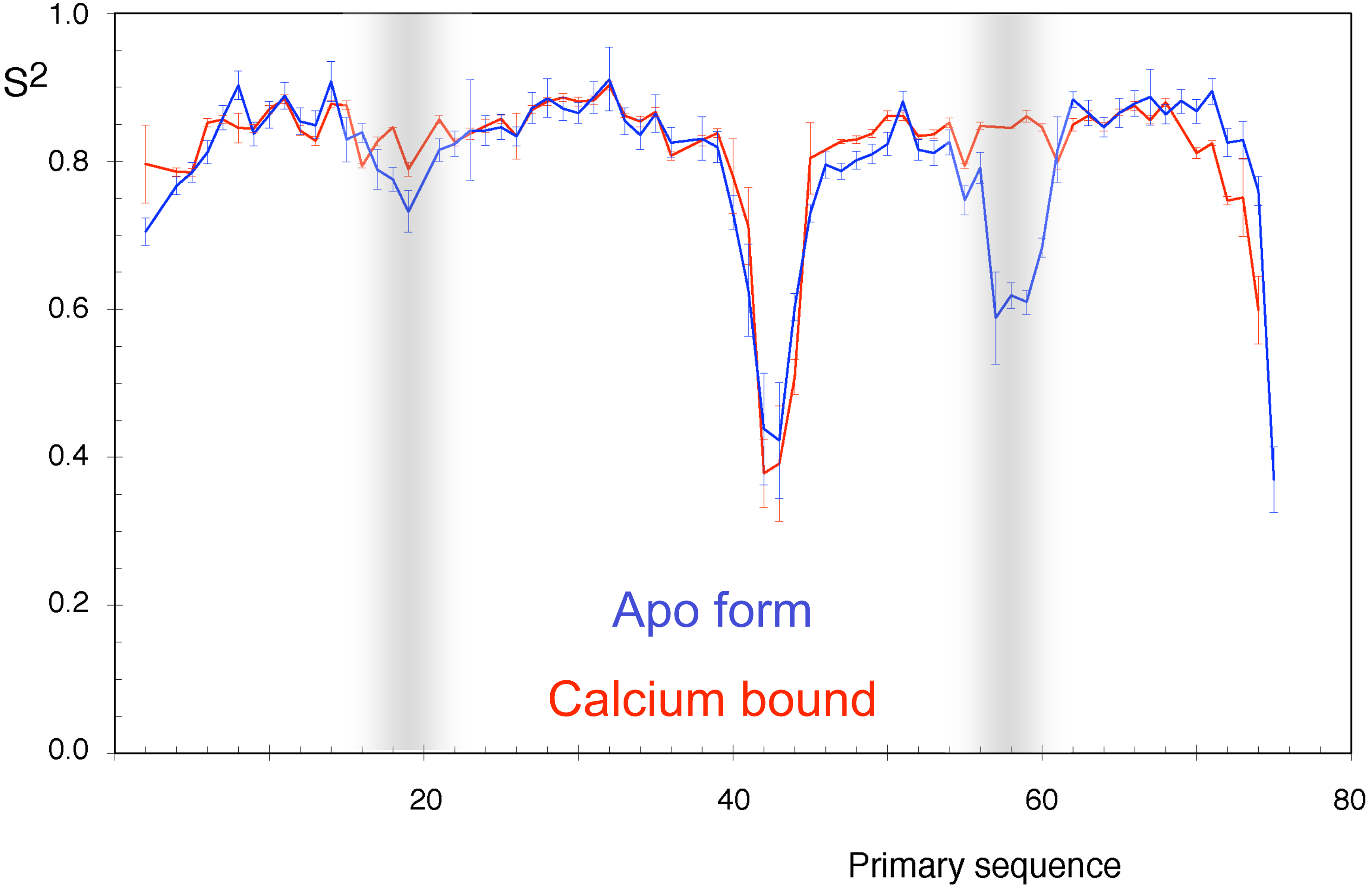
$R_1 = (2.64 \pm 0.15)\ s^{-1}$
$R_2 = (6.35 \pm 0.16)\ s^{-1}$
nOe =0.66 ± 0.02

$S^2 = 0.90 \pm 0.03$
$\tau_i = ?$

Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_i\}$ to relaxation data from Calbindin

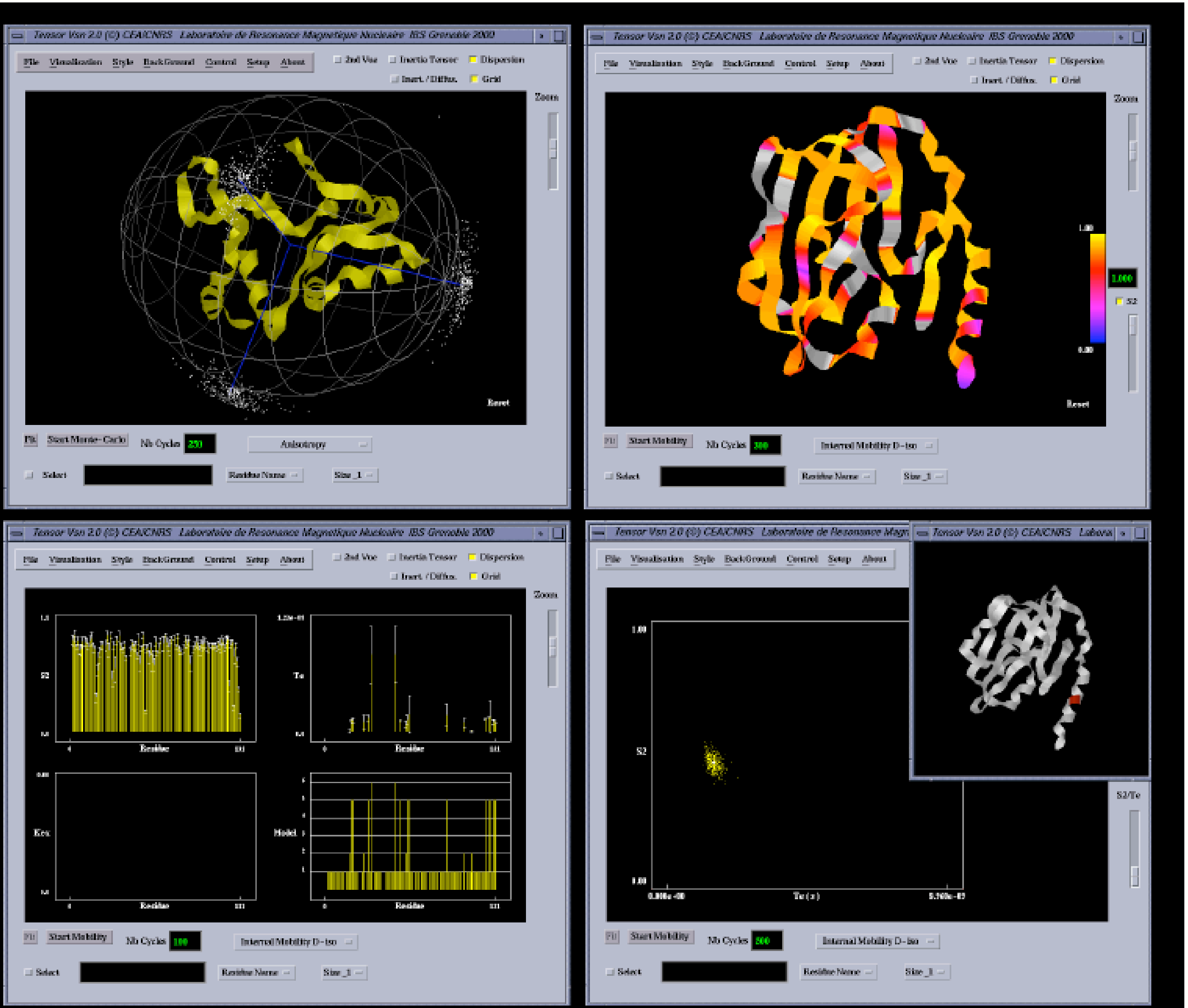


Lipari-Szabo Modelfree Analysis : Fitting $\{S^2, \tau_j\}$ to relaxation data from Calbindin

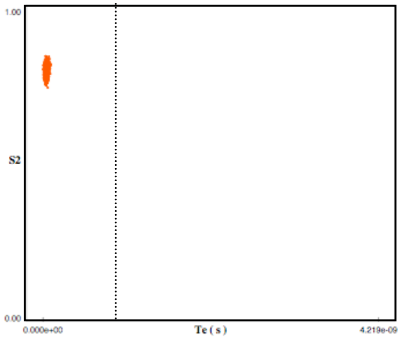


TENSOR2

Graphical and statistical analysis of global and internal motion in proteins from 15N relaxation data

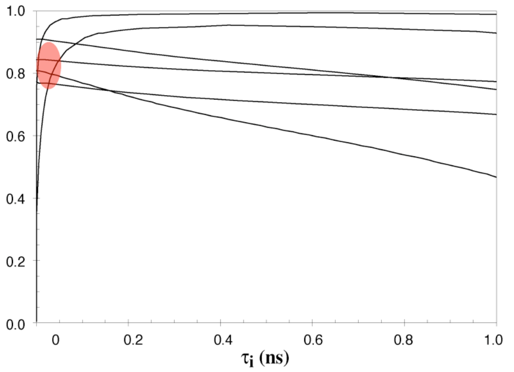


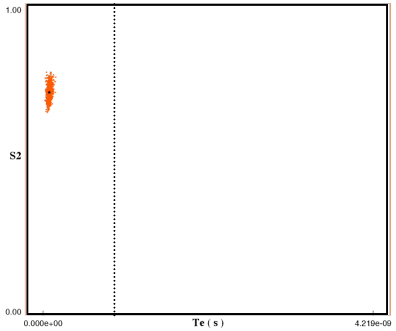
Monte Carlo based error analysis of the precision of Lipari-Szabo parameters {S^2, τ_i} fitted to 15N to relaxation data using the ‘Modelfree’ approach : Ca-bound Calbindin



Residue 6
Model 2

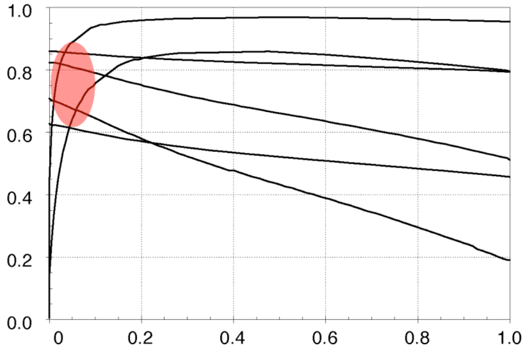
Te (s) : 4.465e-11 +/- 1.520e-11
S2 : 8.123e-01 +/- 0.017

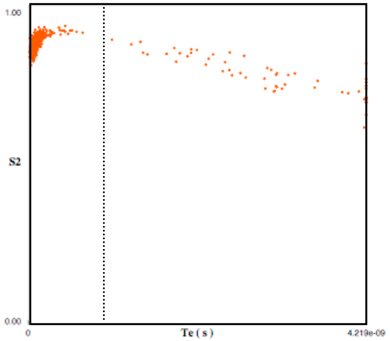




Residue 40
Model 2

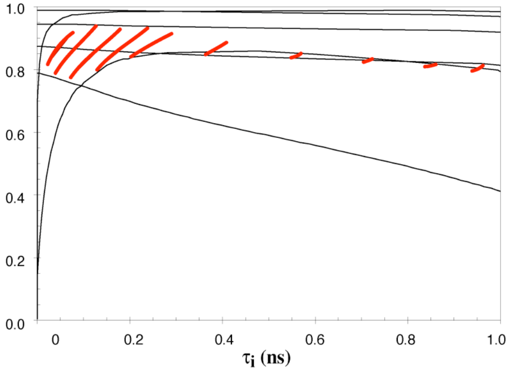
Te (s) : 7.858e-11 +/- 2.010e-11
S2 : 7.310e-01 +/- 0.024





Residue 14
Model 2

Te (s) : 8.706e-11 +/- 7.132e-10
S2 : 9.082e-01 +/- 0.035



Can Dynamics from Relaxation Lead to Thermodynamic Understanding?

S^2 provides a direct measure of conformational order

A component of conformational entropy can be estimated from S^2

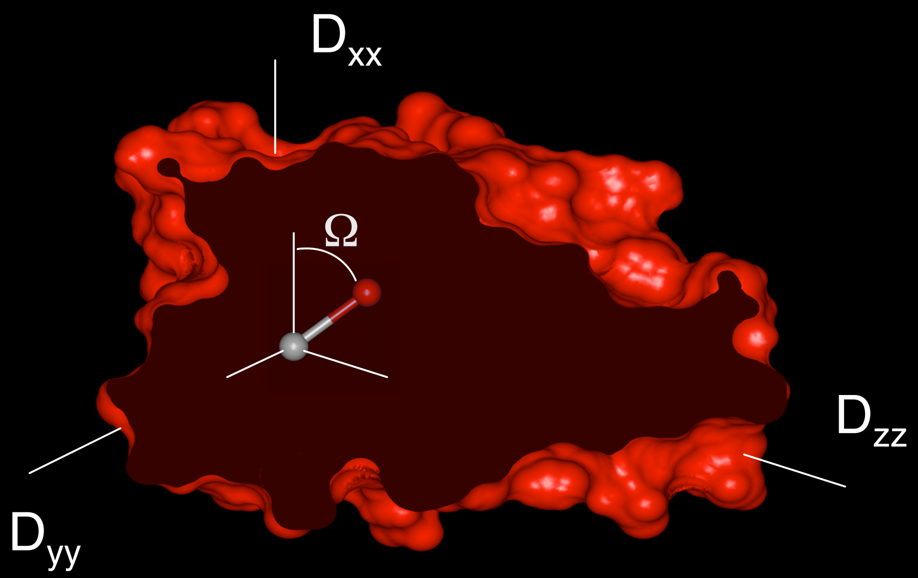
Internal Mobility from ^{15}N heteronuclear relaxation rates

- Relaxation rates R_1, R_2 and nOe sample rapid (ps-ns) dynamics
- ^{15}N relaxation samples angular reorientational properties
- Transformation to spectral density terms via matrix inversion
- Analytical geometric description of motion in general underdetermined
- Lipari-Szabo - Abstract mathematical model decouples internal and global motion. Internal mobility described in terms of order parameter S^2 and local correlation time τ_i
- Quantitative measurement of extent and timescale of internal motion
- Provides a measure of a contribution to entropy
- Connection to physical model is lost in this approach : realistic models require additional probes or molecular dynamics simulations

Systems where Lipari-Szabo approach is not valid

- Partially folded or molten globule states
- Highly disordered proteins
- Multi-domain proteins exhibiting differential domain dynamics
- Multiple, complex internal motions
- …..

Anisotropic Rotational Diffusion



$$J_g(\omega)=\Sigma a_j\,\tau_j/(1+\omega^2\tau_j^2)$$

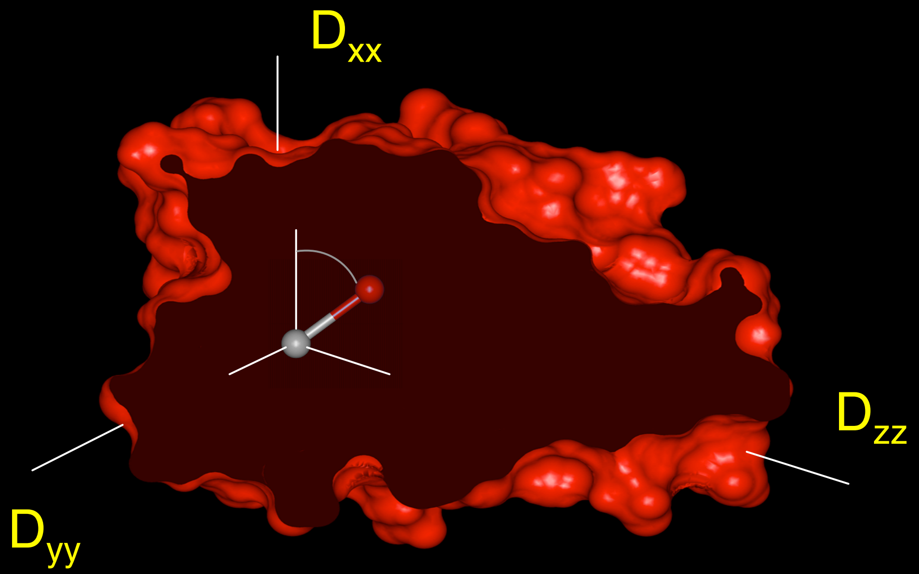
$$a_j = fn(\omega)$$

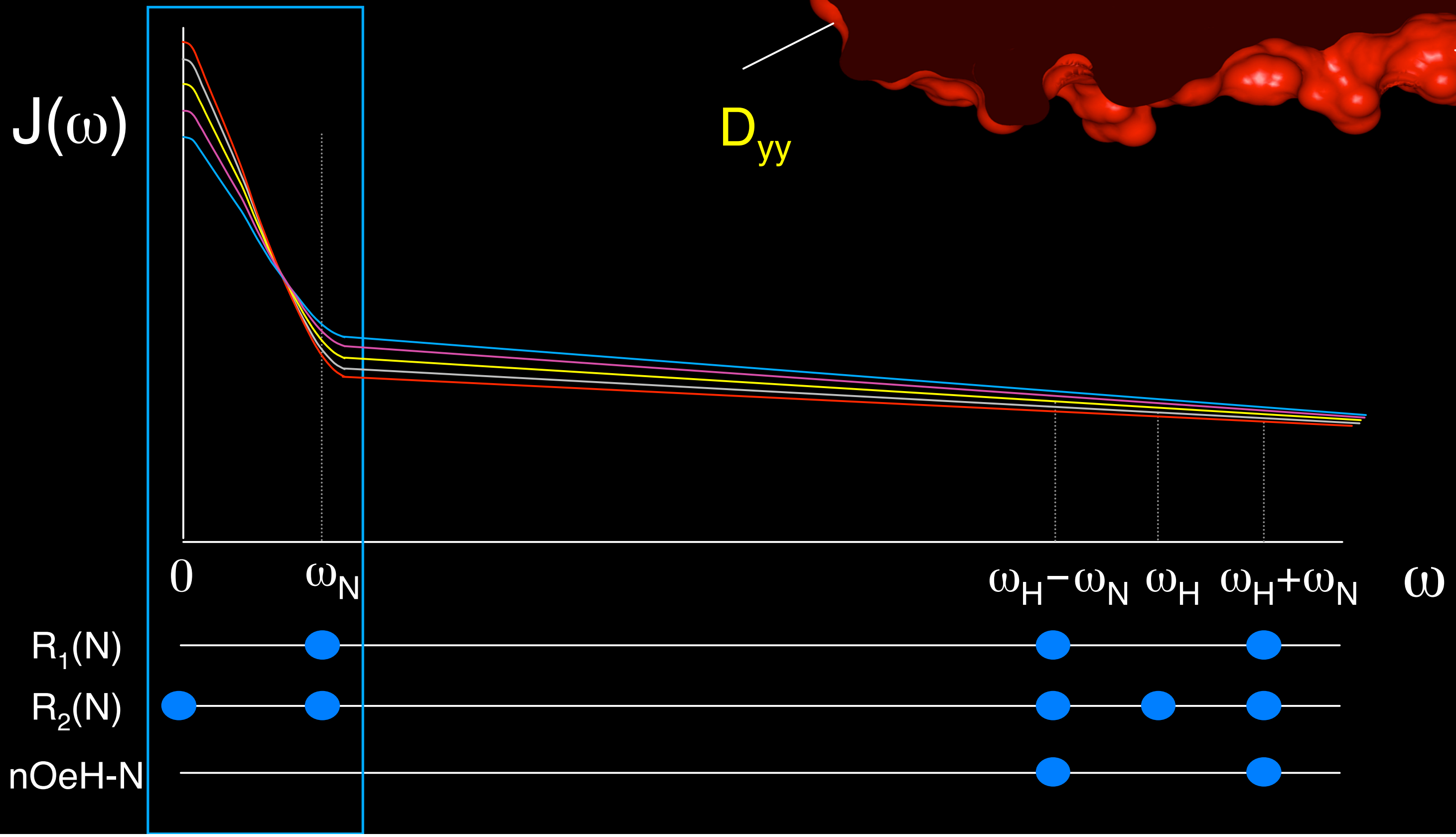
a_j define the geometry of the relaxation mechanism relative to the rotational diffusion tensor D

Anisotropic Rotational Diffusion

$J_g(\omega)=\Sigma a_j\, t_j/(1+\omega^2\tau_j^2)$

5 independent components of spectral density function



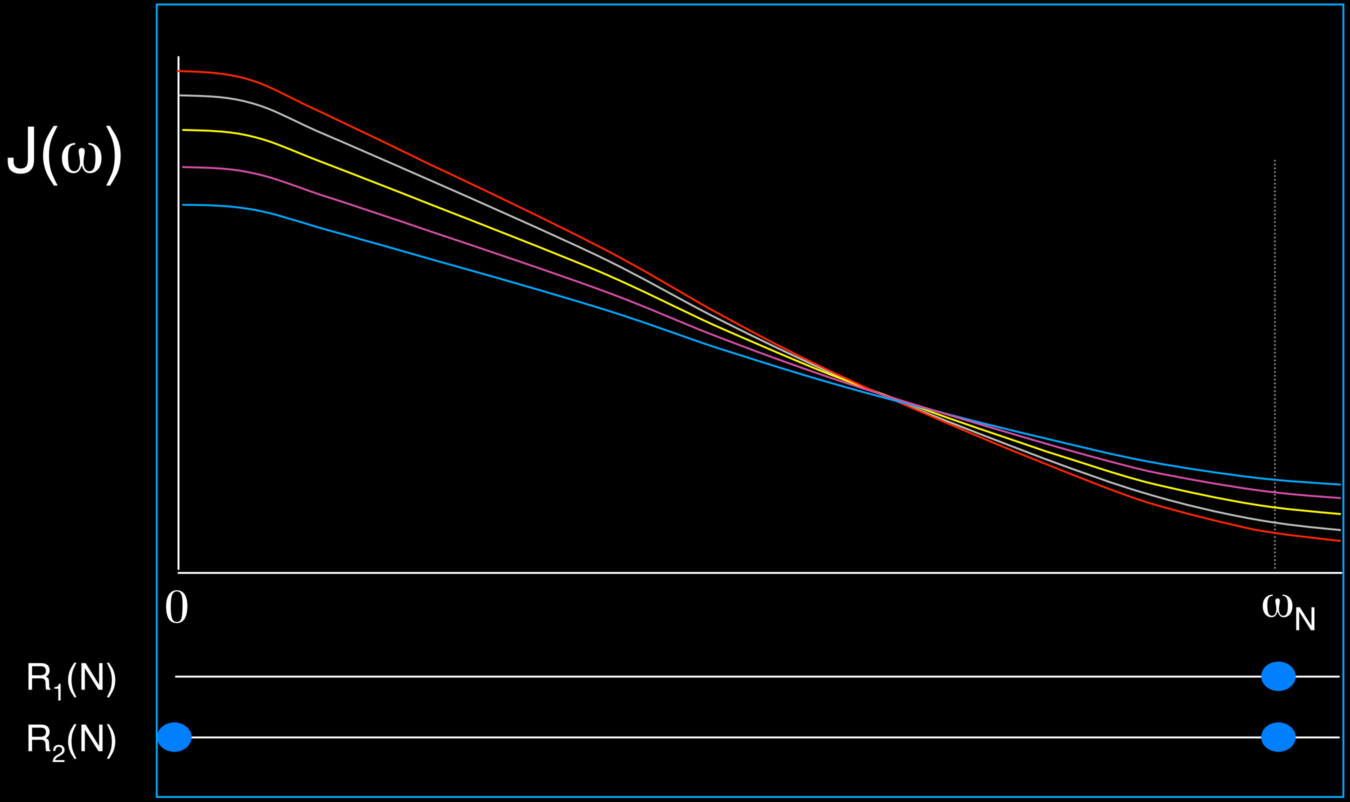


Anisotropic Rotational Diffusion

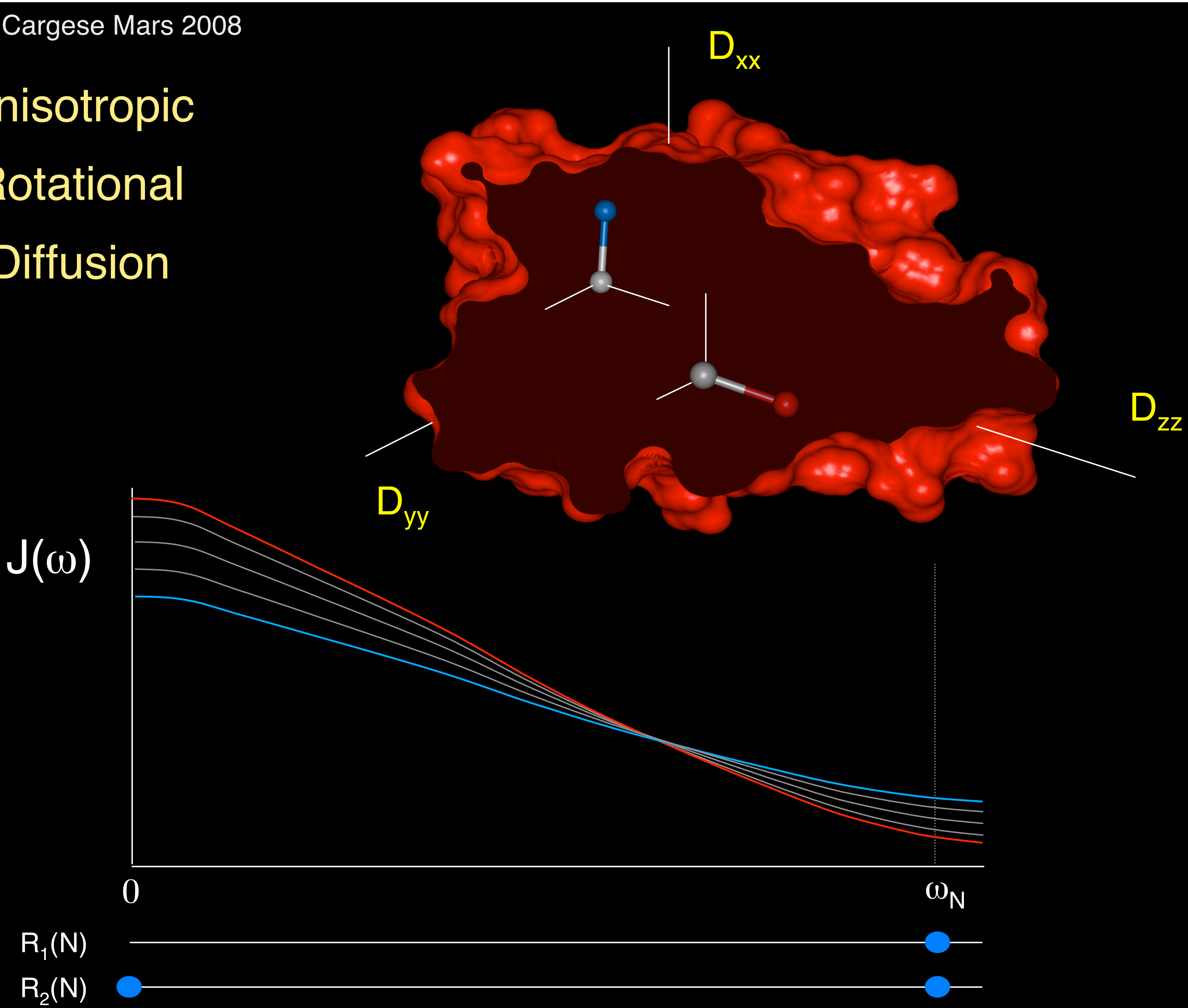
$$J_g(\omega)=\Sigma a_j\,\tau_j/(1+\omega^2\tau_j^2)$$

5 independent components of spectral density function :

Relative contribution depends on orientation of relaxation mechanism within the molecular frame



Anisotropic Rotational Diffusion



Orientational Dependence of R_2/R_1 in Anisotropically Tumbling Molecules

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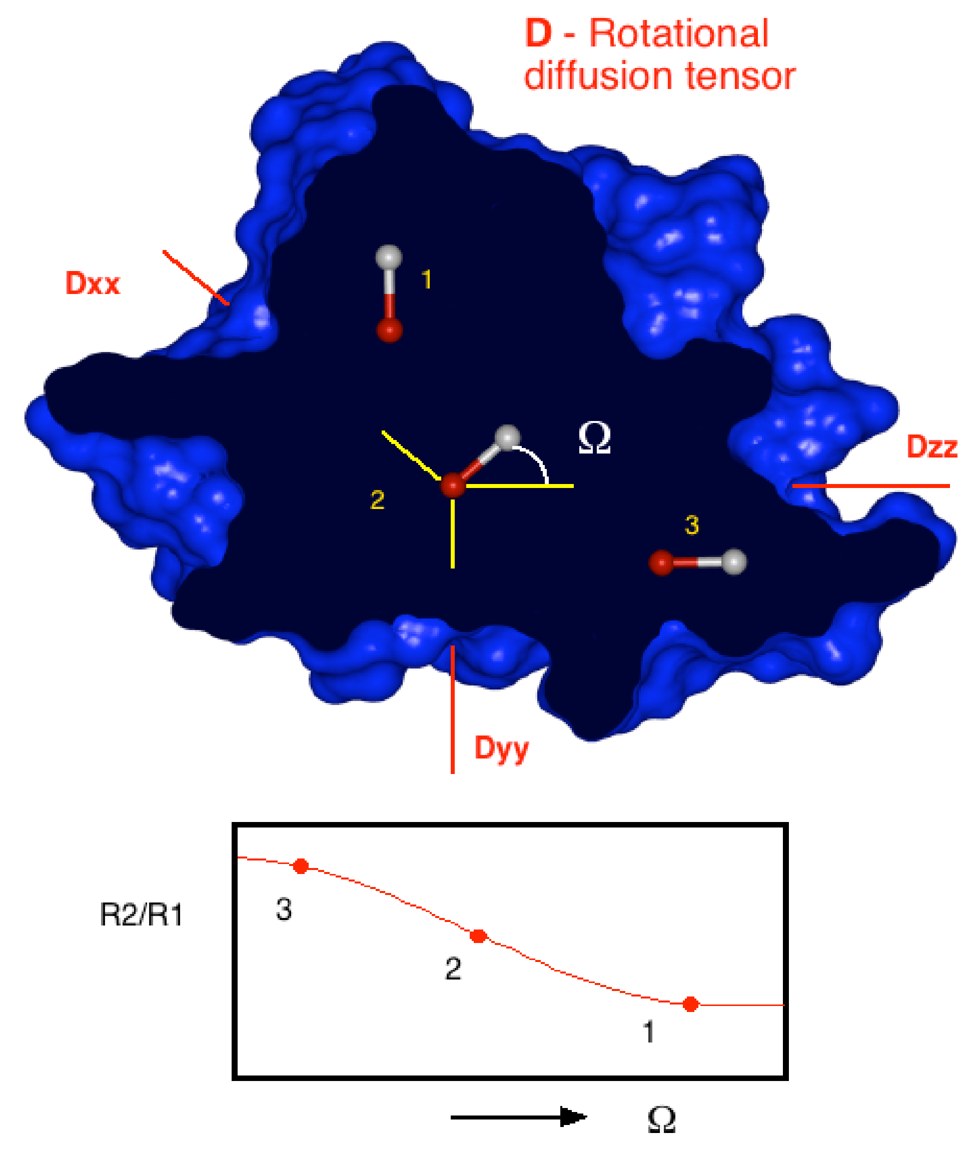
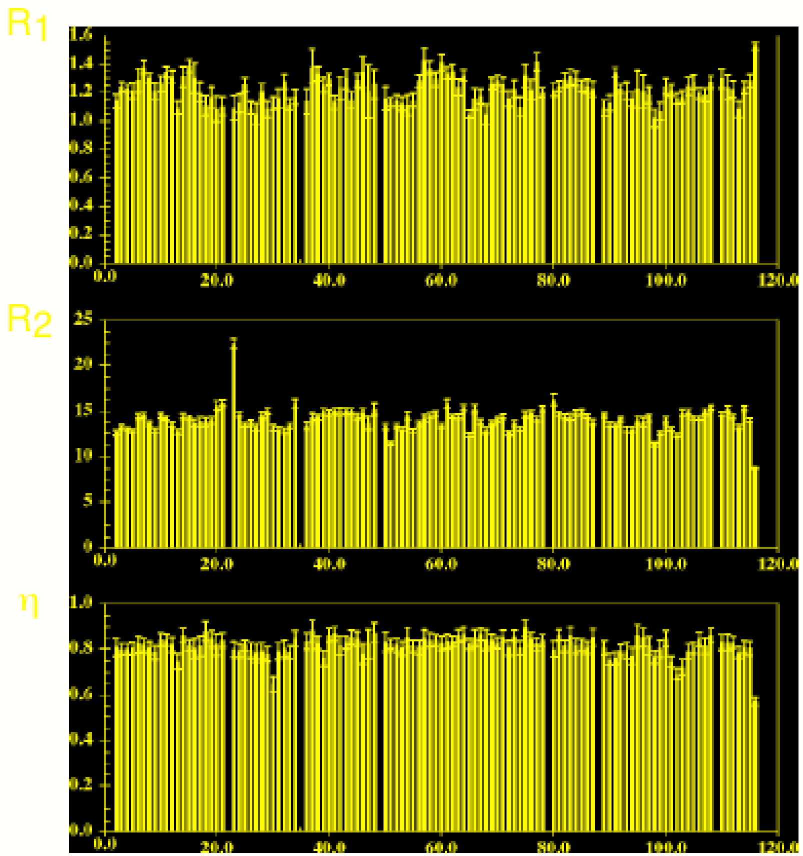
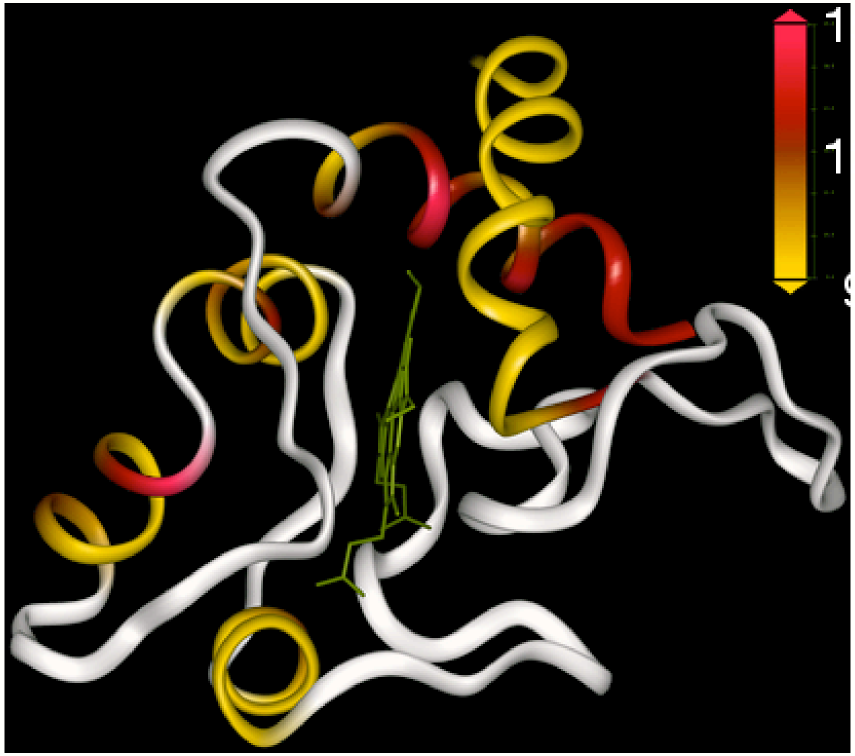
In *librational* or rapid motion limit $(\tau_{int}) < 20$ps, R_2/R_1 assumed to be independent of internal motion.

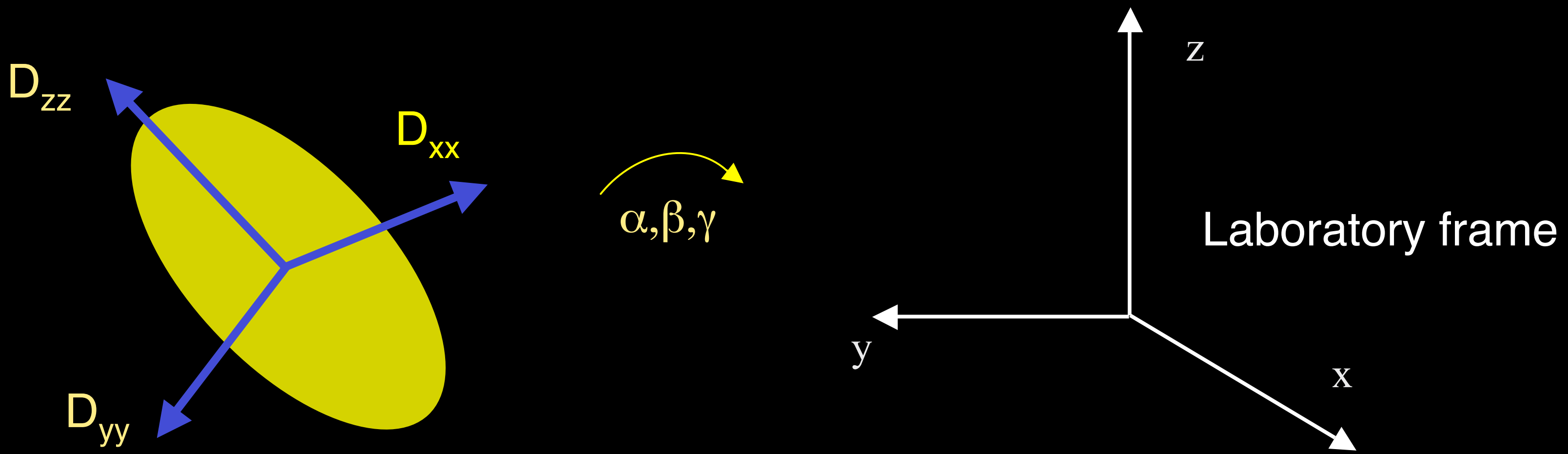






$\tau_{int} \Rightarrow 0$; $R_2/R_1 = f(\Theta,\underline{\mathbf{D}})$

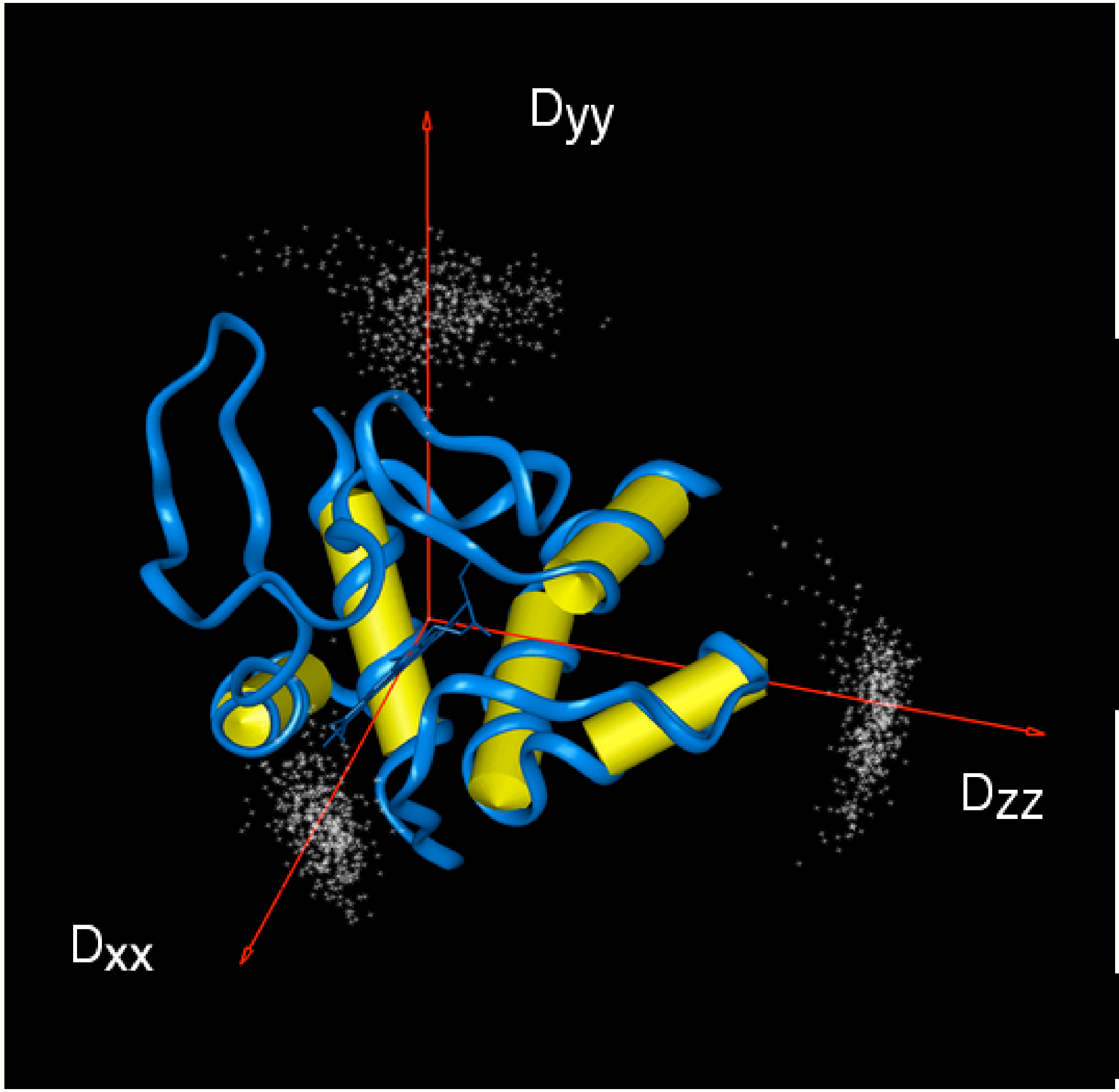
Characterisation of anisotropic rotational diffusion from heteronuclear relaxation



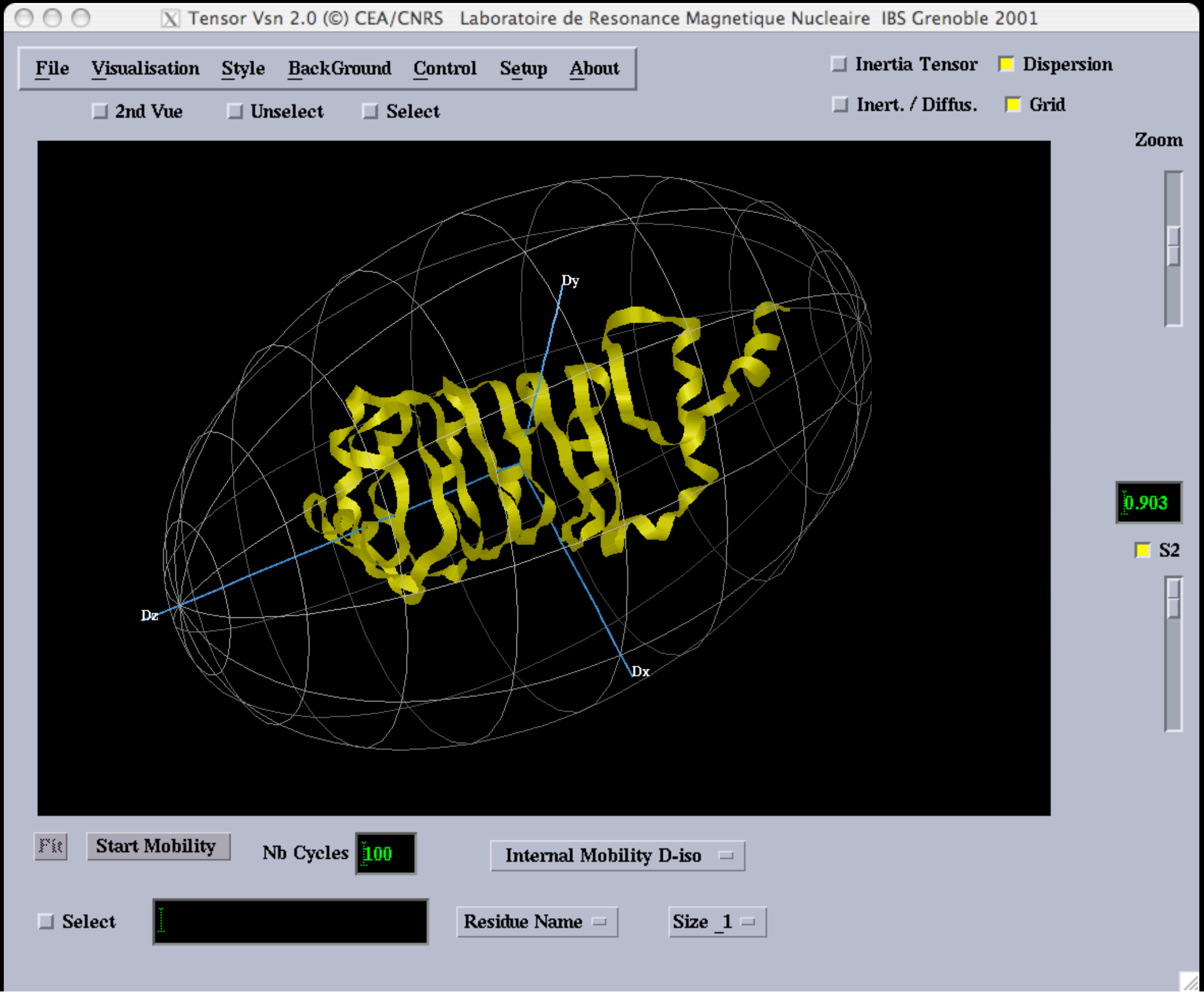
6 parameters define the diffusion tensor
components and their orientation
relative to the molecular frame

$$(D_{xx}, D_{yy}, D_{zz}, \alpha, \beta, \gamma)$$

Requires >6 relaxation rates/orientations


Dyy
Dzz
Dxx

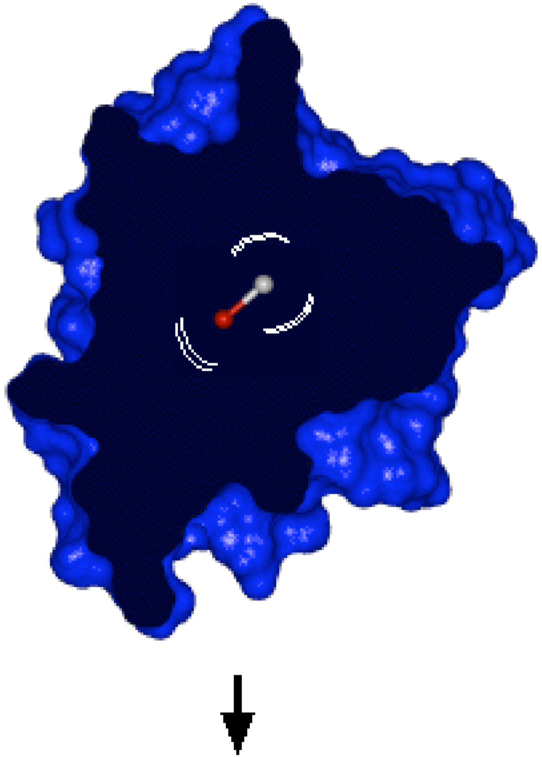
Determination of hydrodynamic behaviour of proteins in solution from ^{15}N relaxation : TENSOR2

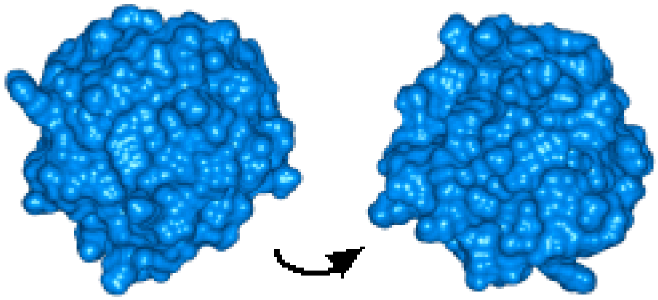


☐ Inertia Tensor ☑ Dispersion
☐ Inert. / Diffus. ☑ Grid
0.903
☑ S2
Nb Cycles 100
☐ Select
Size 1

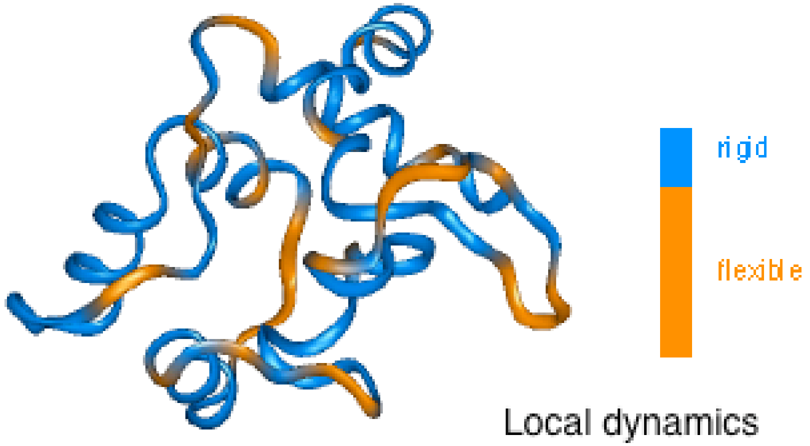
Heteronuclear Spin Relaxation -

measures NH angular reorientation.

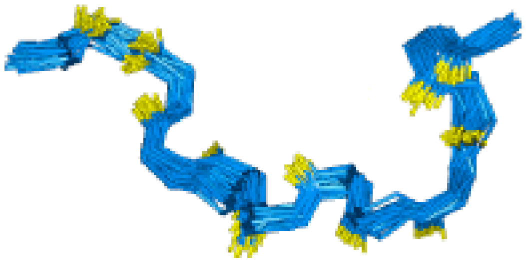




Molecular diffusion

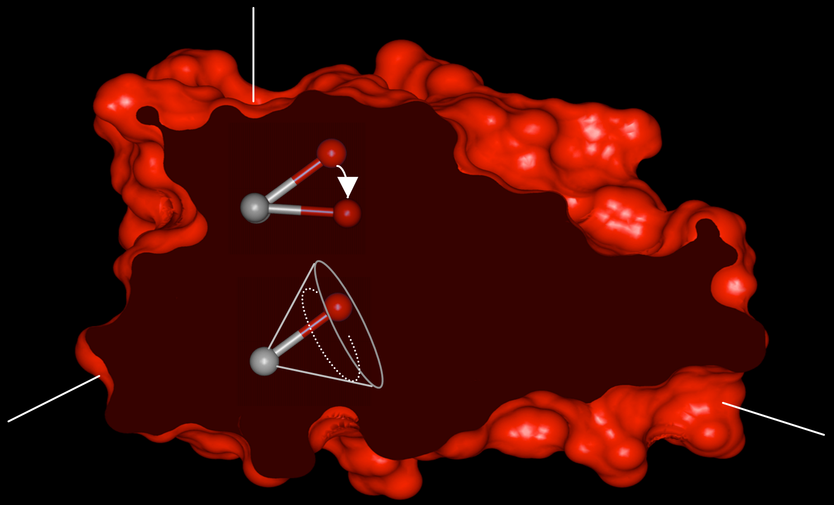


Local dynamics



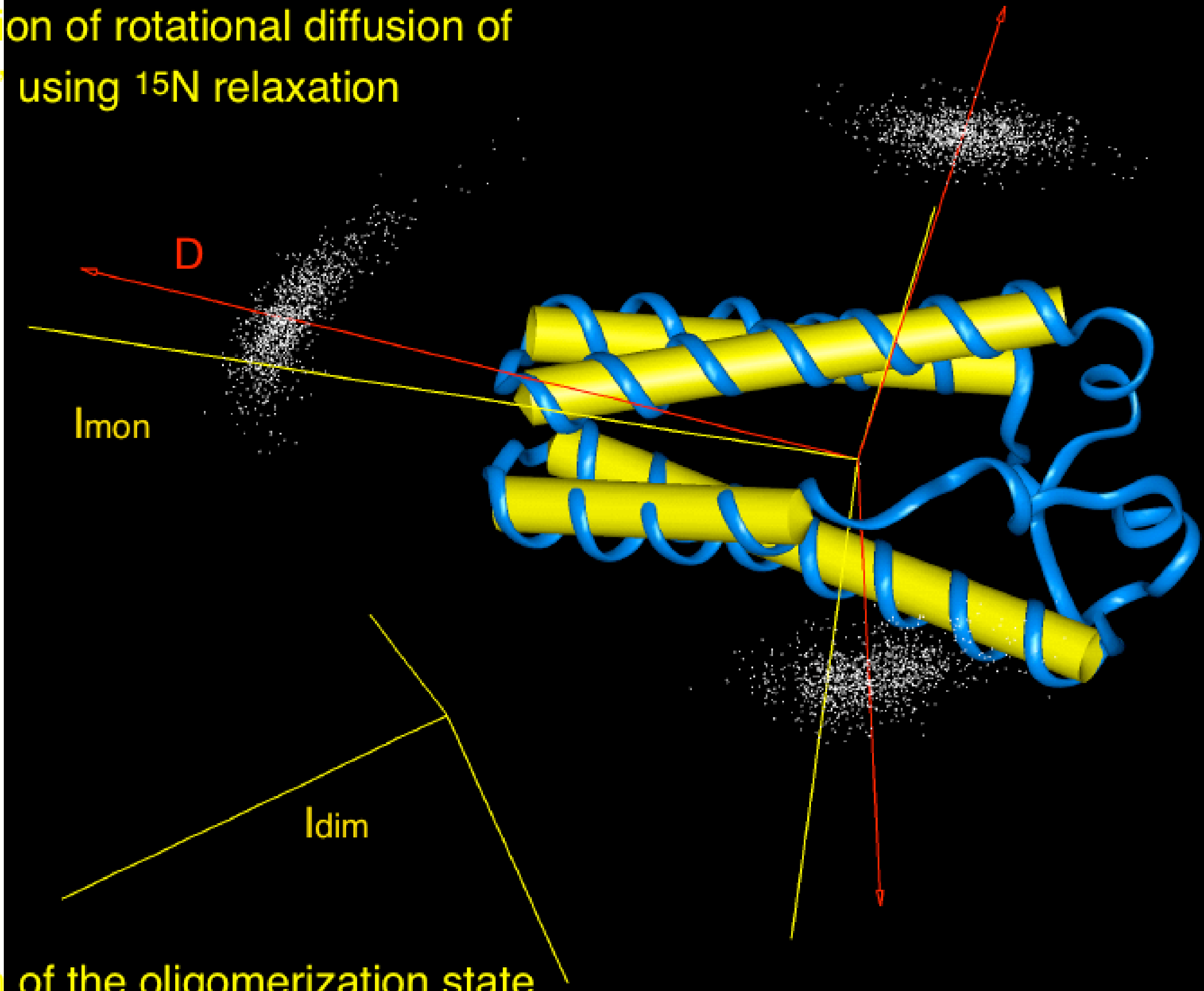
1 - Amplitude (S2) and timescale of local motion.
2 - Rotational diffusion.

Internal Mobility in the Presence of Anisotropic Rotational Diffusion



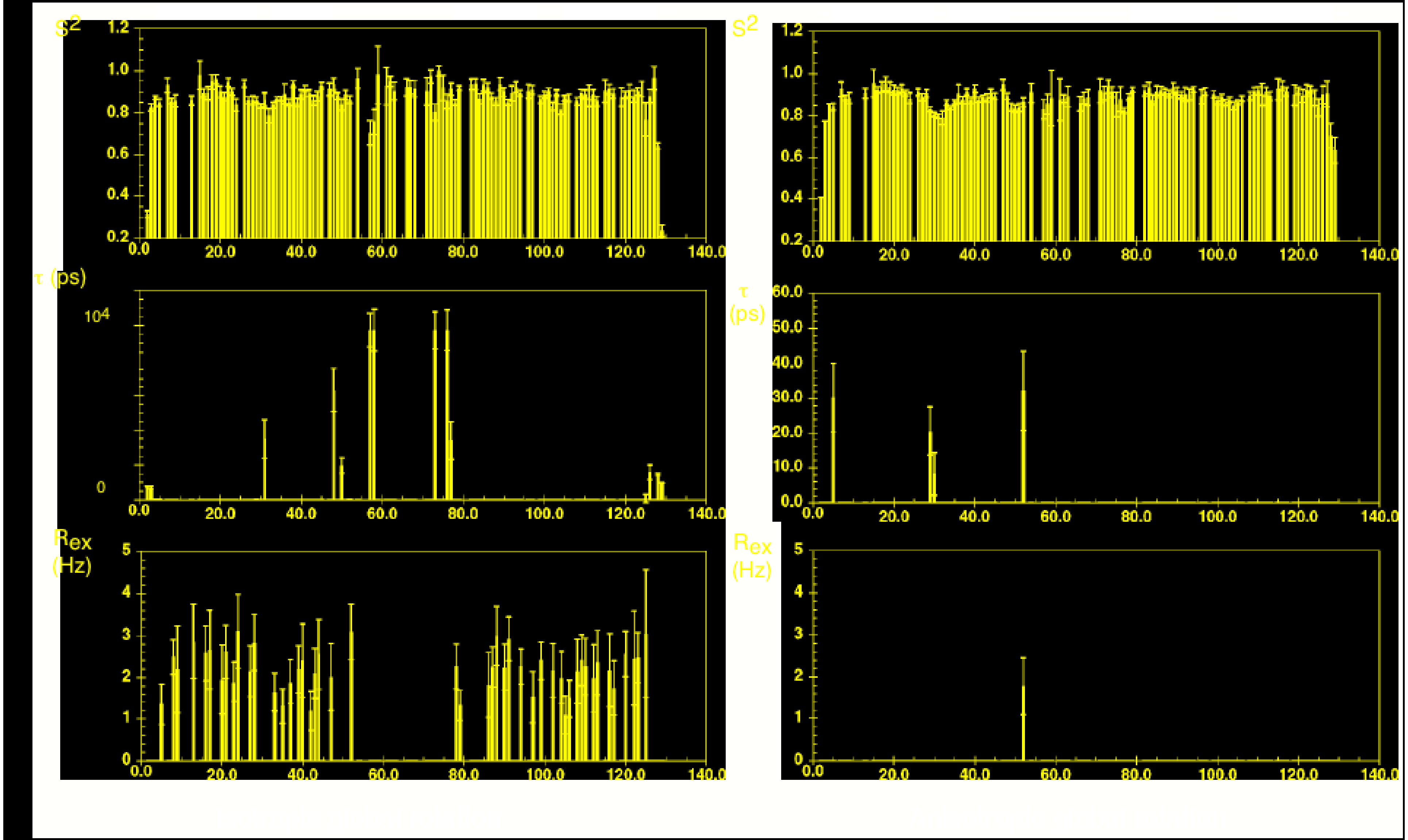
$$J(\omega) \approx S^2 \sum \left\{ a_j \tau_j/(1+\omega^2\tau_j^2) \right\} + (1 - S^2)\, \tau_e/(1+\omega^2\tau_e^2)$$

Characterisation of rotational diffusion of cytochrome c' using ^{15}N relaxation



Identification of the oligomerization state

Lipari-Szabo Type Internal mobility analysis using iso- and anisotropic rotational diffusion



Molecular Dynamics Simulations and NMR Relaxation

- Correlation with experimental data
- Identification of motional models
- Identification of additional probes for more precise motional information