PRACTICAL ASPECTS OF NMR RELAXATION STUDIES OF BIOMOLECULAR DYNAMICS

Further reading:

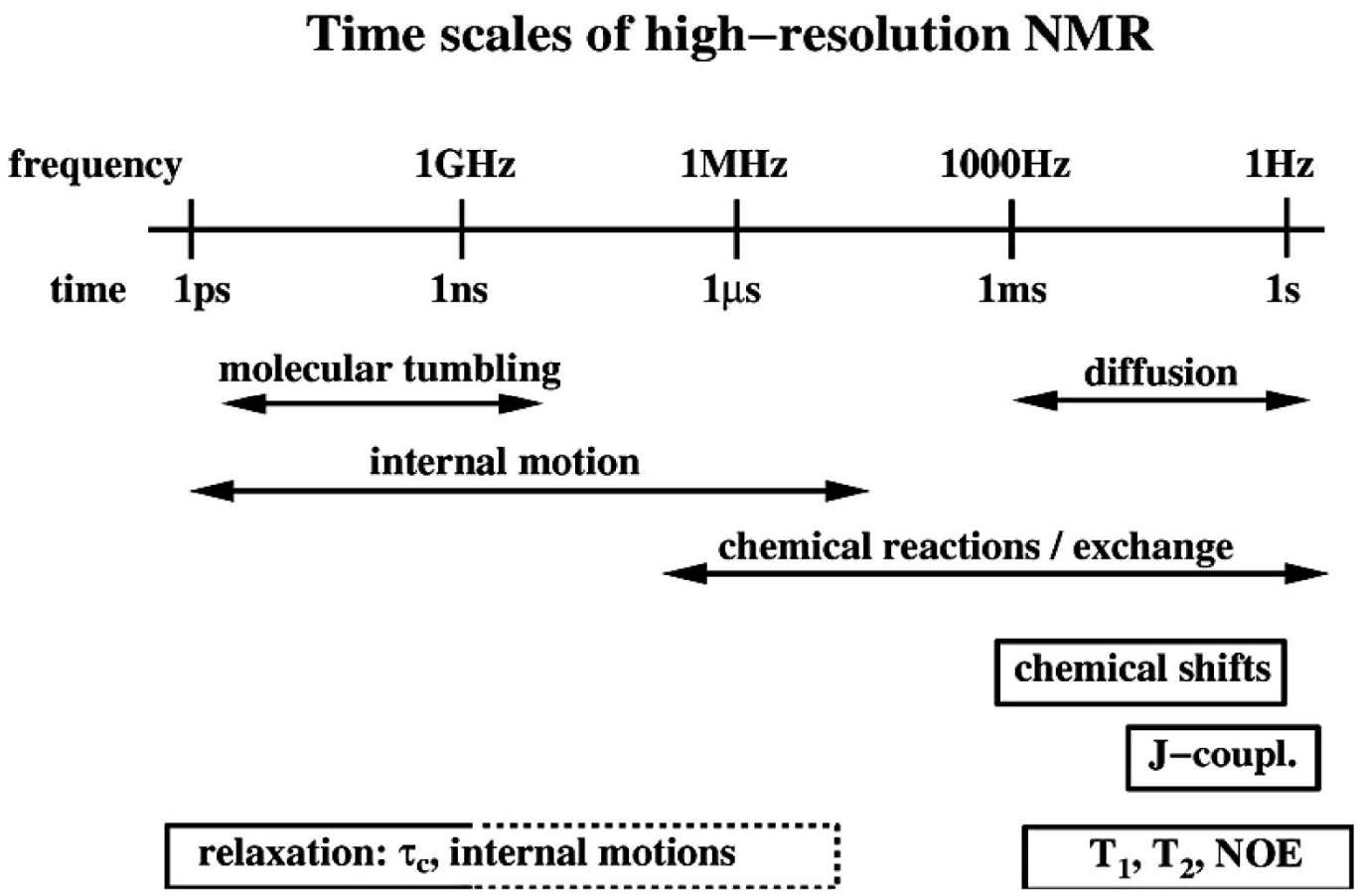
(Can be downloaded from my web page)

Korzhnev D.E., Billeter M., Arseniev A.S., and Orekhov V. Y., NMR Studies of Brownian tumbling and internal motions in proteins. *Progress in Nuclear Magnetic Resonance Spectroscopy* **38**, 2001, 197-266.

Peng J. W., Wagner G., Investigation of Protein Motions via Relaxation Measurements, *Meth. Enzymol.* **239**, 1994, 563-597.

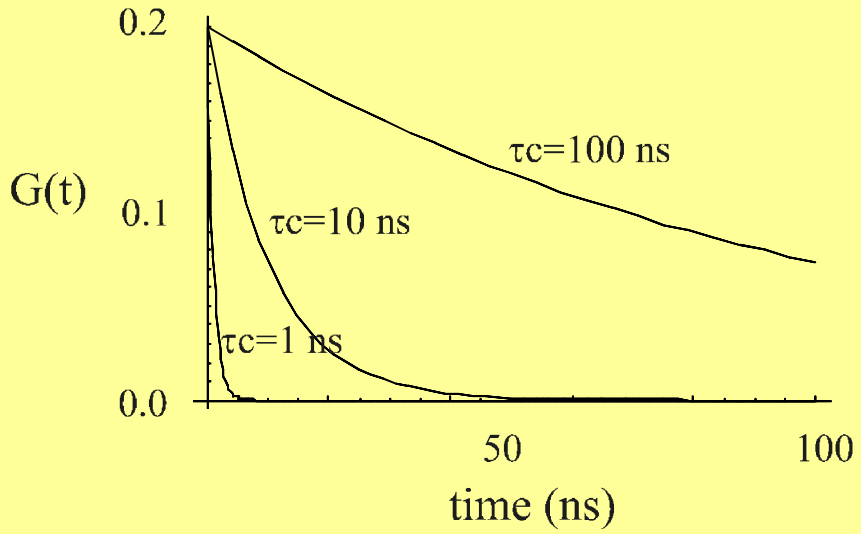
Al-Hashimi A.M., Dynamics-Based Amplification of RNA Function and Its Characterization by Using NMR Spectroscopy, *ChemBioChem* **6**, 2005, 1506-1519.

NMR time scales



Correlation function

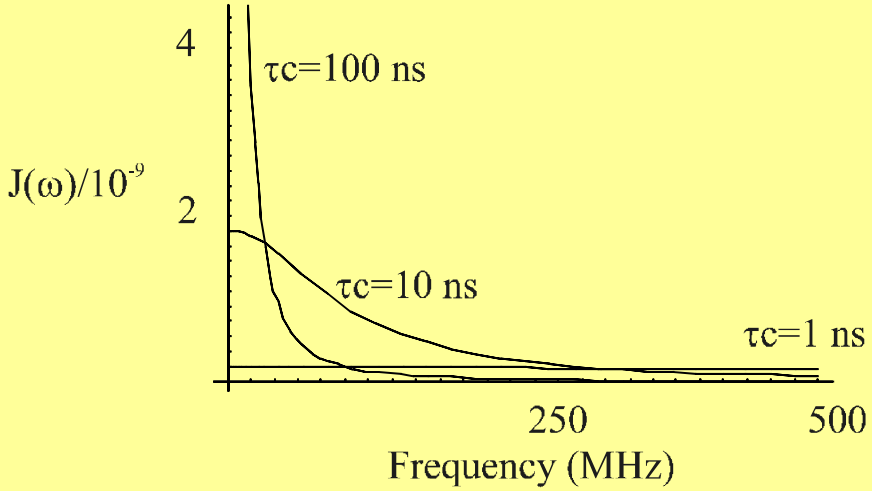
$$G(t) = \frac{1}{5}\exp\left(-\frac{t}{\tau_c}\right)$$



The correlation function above is for the isotropic diffusion of a rigid rotor. The correlation time, τ_c, is the time constant for the exponential decay of the function. τ_c is approximately the amount of time the molecule takes to rotate 1 radian. Notice that short correlation times cause the correlation function to decay rapidly and long times cause the function to decay more slowly. The correlation time depends primarily on molecular size and shape as well as solvent viscosity, temperature, …

Spectral Density function

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

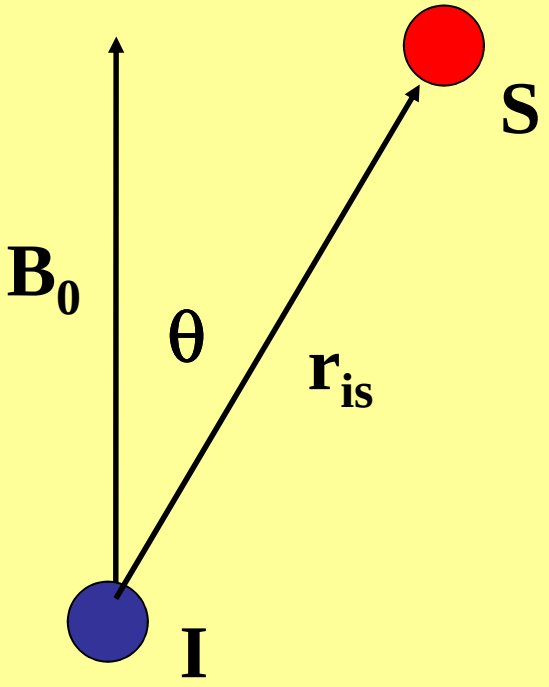


The spectral density function, *J(ω)*, is the Fourier transform of the correlation function Just as rapidly relaxing time domain signals give rise to broad lines, short correlation times have a broad spectral density function. This makes sense: molecules that tumble very rapidly can sample a wide range of frequencies. Molecules that tumble slowly and have very long correlation times only sample lower frequencies.

Relaxation mechanisms

- Dipolar (DD)
- Chemical shift anisotropy (CSA)
- J-coupling
- Quadrupolar
- Chemical exchange
- Etc.

Dipolar relaxation

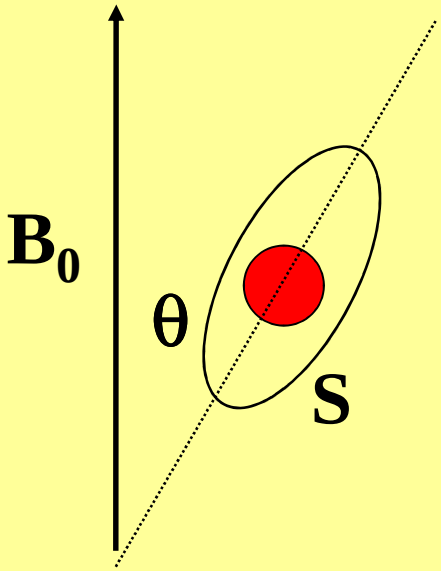


$$d = \frac{\mu_0}{4\pi} \frac{\hbar \gamma_I \gamma_S}{r_{IS}^3} \left(3\cos^2\theta - 1\right)$$

$$\left\langle 3\cos^2\theta - 1 \right\rangle = \frac{2}{5} \exp\left(-6 D_r t\right)$$

Correlation function

Chemical shift anisotropy (CSA)



$$\Delta\sigma = \sigma_{||} - \sigma_{\perp}$$

If $\sigma_{11} \geq \sigma_{22} \geq \sigma_{33}$

$$\Delta\sigma = \sigma_{11} - \frac{\sigma_{22} + \sigma_{33}}{2}$$

$$c = \frac{\gamma_S B_0 \Delta\sigma_S}{\sqrt{3}} \left(3\cos^2\theta - 1\right)$$

There is more to relaxation than T_1 and T_2

We introduced T_1 and T_2 back in the phenomenological Bloch equations. This was back in the "good old days" when we only had one spin with its x, y, or z orientation to worry about. When we got to 2 spins, we had 15 terms (plus one more that was unity). It turns out that each of these terms has different relaxation behavior on its own (autorelaxation). Terms can also relax through interactions with each other, like the ^{1}H ^{1}H NOE (cross-relaxation).

As shown on the next slide, all of these different types of relaxation can be expressed in terms of spectral density functions.

Some Autorelaxation Rates for an I S two spin system (Table adapted from Peng and Wagner)

	J(0)	$J(\omega_s)$	$J(\omega_I - \omega_s)$	$J(\omega_I)$	$J(\omega_I + \omega_s)$	ρL	ρT
$R_S(S_z)$ [e.g. T_1 of S]	0	3d+c	d	0	6d	0	0
$R_S(S_x)$ [e.g. T_2 of S]	2d+(2c/3)	(3d+c)/2	d/2	3d	3d	0	0
$R_I(I_z)$ [e.g. T_1 of I]	0	0	d	3d	6d	1	0
$R_I(I_x)$ [e.g. T_2 of I]	2d	3d	d/2	3d/2	3d	0	1
$R_{IS}(2I_zS_z)$ [new!]	0	3d+c	0	3d	0	1	0
Etc (see Peng and Wagner for more)							

$$d = \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{4 r_{IS}^6} \qquad c = \frac{\Omega_S^2 \Delta_S^2}{3}$$

Ω_s and Δ_s are the frequency and chemical shift anisotropy (CSA) for spin S. CSA is the difference in chemical shift when the molecule is oriented along different axes relative to B_0.

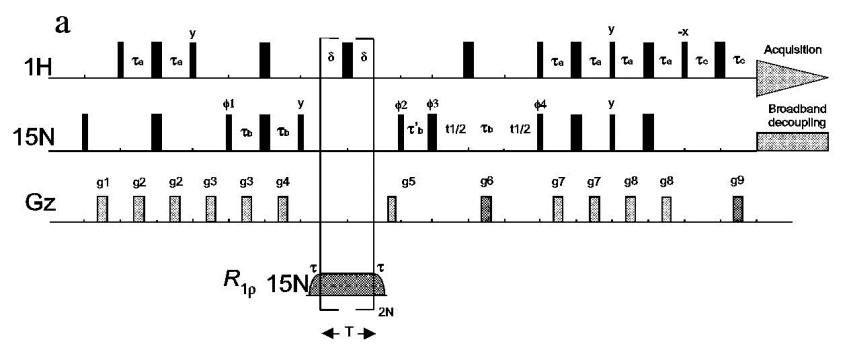
How do we measure relaxation rates?

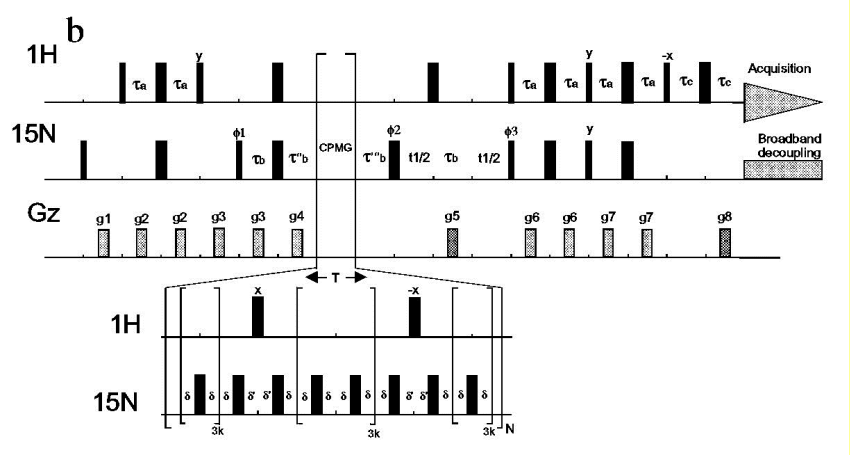
Scheme of a 2D experiment for heteronuclear relaxation measurement

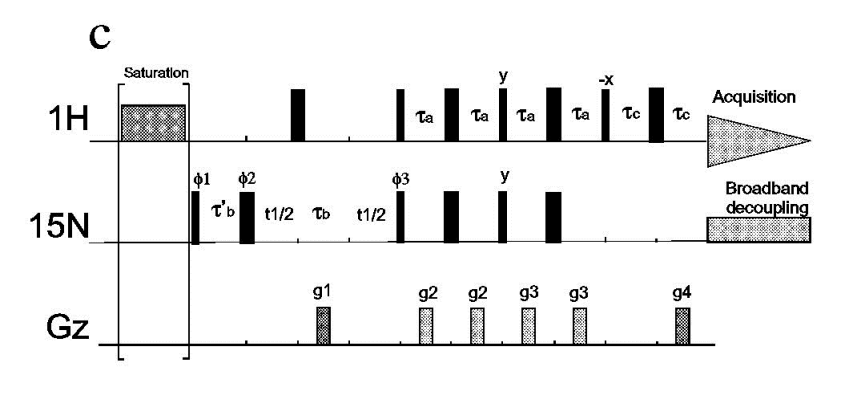
Preparation creation of desirable coherence	**Delay T** of variable length for auto- or cross-relaxation of selected coherence	**t_1 period** labeling of residual coherence by chemical shift of heteronucleus	**Magnetization** transfer to ^{1}H nucleus	**^{1}H acquisition** with broadband decoupling on heteronucleus	**Delay** between scans

- Not all relaxation rates can be measured in practice
- It is importatnt to ensure that only the desired relaxation processes occur in the relaxation period.
- Temperature control is essential (rates change approx. 3% per K)
- Typical 8-12 measurements for T_1, T_2
- Correct determination of experimental errors is important

Examples of pulse sequences







a – R_1 or $R_{1\rho}$ measurement

b – R_2 measurement

c – steady-state NOE experiment

Analysis of relaxation data

1. Based on a particular model of motion

   such as oscillations, librations, jumps between several discrete states etc. Uses a correlation function for the specific type of motion.

   Disadvantage: experimental data do not allow to distinguish between individual models of motion.

2. "Model-free"

   Assumes a certain type of spectral density function characterized by a limited number of parameters.

   Disadvantage: no information on the specific kind of motion

3. Spectral density mapping

   Determines the values of the spectral density function at several characteristic frequences (0, ω_I, ω_S, $\omega_I \pm \omega_S$). Interpretation

   qualitative or based on specific models.

"Model-free"

$$G(t) = \frac{1}{5}\exp\left(-\frac{t}{\tau_c}\right) \xrightarrow{\text{FT}} J(\omega) = \frac{2}{5}\left(\frac{\tau_c}{1+\omega^2\tau_c{}^2}\right)$$

These functions are correct for rigid spheres and would do a great job describing the tumbling of a rock down a hill (if it tumbles isotropically and doesn't break). However, proteins have internal motions, and more realistic models for the correlation and spectral density functions have extra terms to describe internal motion. For example, the popular Lipari-Szabo "model free" spectral density function is:

$$J(\omega) \propto \left(\frac{S^2\tau_c}{1+\omega^2\tau_c{}^2} + \frac{(1-S^2)\tau}{1+\omega^2\tau^2}\right) \quad \text{where } \frac{1}{\tau} = \frac{1}{\tau_c} + \frac{1}{\tau_e}$$

τ_e is the correlation time for internal motions and S^2 is called the generalized order parameter. S^2 is 1 for a perfectly rigid sphere (and the more complicated spectral density function becomes the simpler one above). S^2 is 0 for a completely flexible molecule.

Model-free assumptions

1. Commonly, it is assumed that molecular overall rotation is isotropic.
2. The conventional methods for characterisation of molecular overall rotation (either isotropic or anisotropic) a priori imply that intramolecular motions for most of protein ^{15}N-^{1}H groups are fast (τ_e < 100 ps).
3. Usually the parameters governing relaxation of a ^{15}N nucleus (namely, ^{15}N CSA and ^{15}N-^{1}H distance) are kept fixed at predetermined values, assumed to be the same for all ^{15}N nuclei in the protein.
4. From the very beginning, the model-free approach assumes that intramolecular motions are independent of molecular overall rotation.
5. Conventional model-free protocols implicitly assume that the protein does not aggregate at concentrations typical for NMR relaxation studies.

Model-free spectral density functions

Original Lipari-Szabo

$$J(\omega) = \frac{S^2 \tau_R}{1 + (\omega \tau_R)^2} + \frac{(1 - S^2)\tau_e'}{1 + (\omega \tau_e')^2},$$

Two motions on different time scales

$$J(\omega) = \frac{S_f^2 S_s^2 \tau_R}{1 + (\omega \tau_R)^2} + \frac{(1 - S_f^2)\tau_f'}{1 + (\omega \tau_f')^2} + \frac{S_f^2 (1 - S_s^2)\tau_s'}{1 + (\omega \tau_s')^2}$$

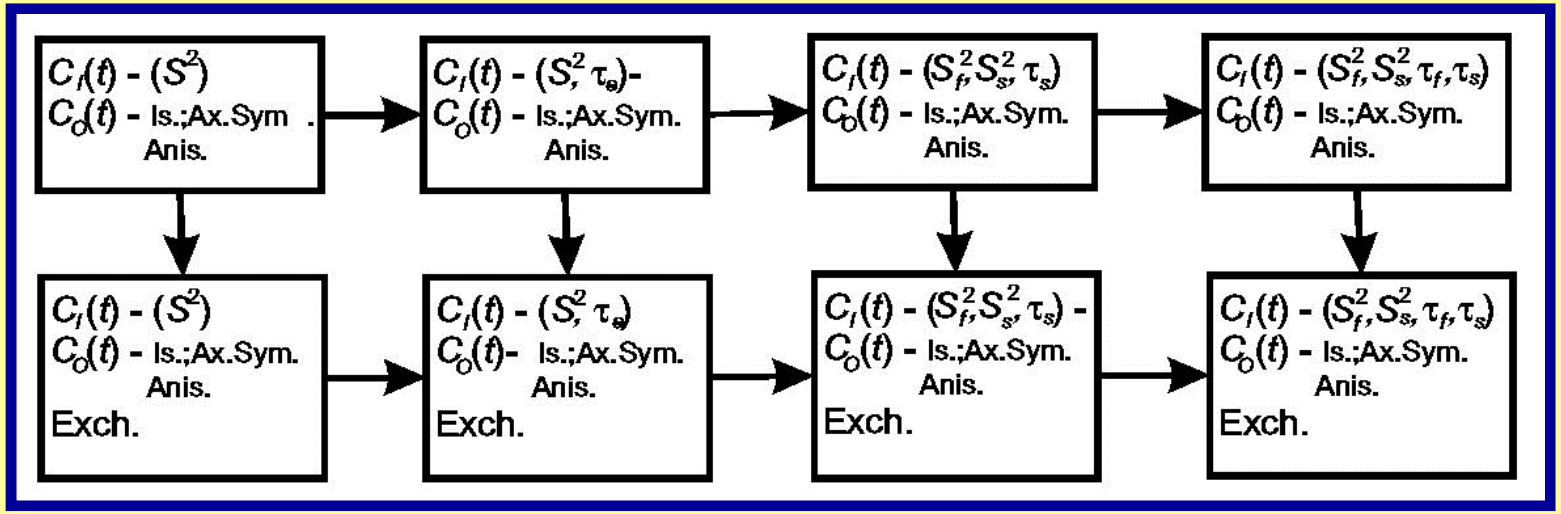
With anisotropic overal rotation

$$J(\omega) = \sum_{\eta=-2}^{2} c_\eta \left[\frac{S_f^2 S_s^2 \tau_\eta}{1 + (\omega \tau_\eta)^2} + \frac{(1 - S_f^2)\tau_{f,\eta}}{1 + (\omega \tau_{f,\eta})^2} + \frac{S_f^2 (1 - S_s^2)\tau_{s,\eta}}{1 + (\omega \tau_{s,\eta})^2} \right]$$

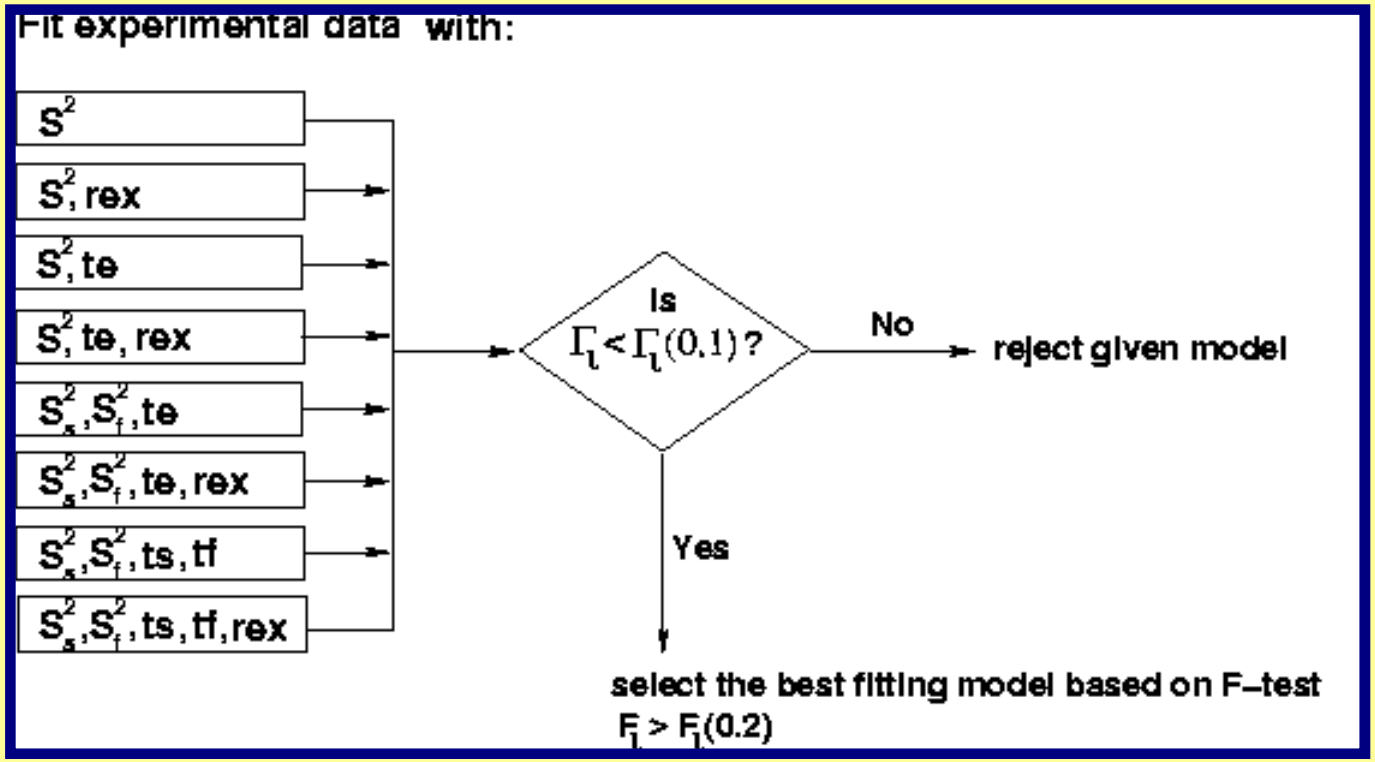
Model-free analysis of data

I. Determination of the parameters of molecular overall rotation
   1. From the R_2/R_1 ratio ($\tau_e < 100$ ps, $\tau_r > 1$ns)
   2. As an adjustable parameter in simultaneous fitting of the relaxation data
   3. Hydrodynamic calculations

II. Selection of a suitable correlation function



Model selection flowchart



Spectral density mapping

The goal in protein dynamics is to know the spectral density function. That is difficult. Several different methods have been derived to model the motions of proteins and represent the models as different spectral density functions with different parameters that are fit to experimental data.One approach that is quite straightforward but requires a lot of experimental data has been developed by Peng and Wagner. This is called "spectral density mapping", and it essentially involves experimentally measuring the spectral density function at select NMR frequencies. This overall approach will be outlined on the next few slides. Regardless of the technique one chooses for dynamics measurements, the next few slides will also show the mathematical relationship between relaxation parameters (such as T_1 and T_2) and the $J(\omega)$.

R_1 relaxation rate for ^{15}N

$$R_N(N_Z) = \frac{\gamma_{HN}^2 \gamma_N^2 \hbar^2}{4 r_{N-H}^6} \left\{ J(\omega_{HN} - \omega_N) + 3J(\omega_N) + 6J(\omega_{HN} + \omega_N) \right\} + \frac{\Delta^2 \omega_N^2}{3} J(\omega_N)$$

This looks much more complicated than it really is. Most of what you see are just constants that are known. The important concept is that the relaxation rate depends on the spectral density at different frequencies:

$$R_N(N_Z) \propto a * J(\omega_{HN} - \omega_N) + b * J(\omega_N) + c * J(\omega_{HN} + \omega_N)$$

Where a, b, and c are constants.

Our goal is to know the spectral density function

Each of the different auto and cross relaxation terms is similar to the one for the T_1 of ^{15}N and is just different combinations of the spectral density functions at different frequencies.

$$R_N(N_Z) \propto a * J(\omega_{HN} - \omega_N) + b * J(\omega_N) + c * J(\omega_{HN} + \omega_N)$$

Peng and Wagner (and others) have derived equations relating the different spectral densities to relaxation rates (this is just algebra, not physics). For example:

$$J(0) = \frac{3}{12d + 4c}\left\{-\frac{1}{2}R_S(S_z) + R_S(S_x) + R_{IS}(2I_zS_x) - \frac{1}{2}R_{IS}(2I_zS_z) - \frac{1}{2}R_I(I_z)\right\}$$

Mathematical formulation

- In the original version of spectral density mapping 6 values are measured: R_{1I}, R_{1S}, R_{2I}, R_{2IzSz} , R_{2IzSx} , ρ_D (dipolar cross-correlation rate)
- The values of $J(\omega)$ a ρ_{IH} (proton-proton NOE) are determined by solving a set of linear equation

$$\begin{bmatrix} J_{\text{eff}}(0) \\ J(\omega_S) \\ J(\omega_I - \omega_S) \\ J(\omega_I) \\ J(\omega_I + \omega_S) \\ \rho_{IH} \end{bmatrix} = \begin{bmatrix} -3/(8E) & 3/(4E) & 3/(4E) & -3(8E) & -3/(8E) & 0 \\ 1/(2E) & 0 & 0 & 1/(2E) & -1/(2E) & 0 \\ 1/(4A) & 0 & 0 & -1/(4A) & 1/(4A) & -1/(2A) \\ -1(12A) & 1/(6A) & -1(6A) & 1/(12A) & 1/(12A) & 0 \\ 1/(24A) & 0 & 0 & -1/(24A) & 1/(24A) & 1/(12A) \\ -1/4 & -1/2 & 1/2 & 1/4 & 1/4 & 0 \end{bmatrix} \begin{bmatrix} R_{1S} \\ R_{2S} \\ R_{2I_zS_x} \\ R_{2I_zS_z} \\ R_{1I} \\ \rho_D \end{bmatrix}$$

A, E – expressions characterizing DD and CSA relaxation, consist of physical constants and bond lengths

Reduced spectral density mapping

- Applicable to large molecules
- Assumes $J(\omega_I) \approx J(\omega_I \pm \omega_S) \approx J(\omega_h)$
- The system reduces to three equations

$$1/T_1 = (d^2/4)\,[J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)] + c^2 J(\omega_N) \quad (1)$$

$$1/T_2 = (d^2/8)\,[4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] + (c^2/6)\,[3J(\omega_N) + 4J(0)] \quad (2)$$

$$\mathrm{NOE} = 1 + (d^2/4)\,(\gamma_H/\gamma_N)\,[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)]\,T_1 \quad (3)$$

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$$J(\omega_h) = [4/(5d^2)]\,(\gamma_N/\gamma_H)(\mathrm{NOE} - 1)/T_1 \qquad (5)$$

$$J(\omega_N) = [1/T_1 - (7d^2/4)\,J(\omega_h)]\,/\,[(3d^2/4) + c^2] \qquad (6)$$

$$J(0) = [1/T_2 - (3d^2/8 + c^2/2)\,J(\omega_N) - (13d^2/8)\,J(\omega_h)]\,/\,(d^2/2 + 2c^2/3) \qquad (7)$$

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Measured relaxation rates characterize the spectral density function

These rates can be measured, and if you have enough spectrometer time at different frequencies, you can directly measure the spectral density at different frequencies just using ^{1}H and ^{15}N. For example, a 11.76 T (500 MHz) magnet will allow measurement of the following frequencies:

0 MHz, 50 MHz (^{15}N at 11.76 T), 500 MHz, 450 MHz (^{1}H – ^{15}N), 550 MHz (^{1}H + ^{15}N)

A 17.6 T (750 MHz) magnet will measure:

0 MHz, 75 MHz, 750 MHz, 675 MHz, and 825 MHz.

MD simulations

N atoms with co-ordinates $\mathbf{r}_i$ (i=1…N), which interact with each other according to energy potential defined by force-field. The force field includes terms for deformations of the chemical structure (bond lengths, bond angles, torsion angles) and for long-range interactions (van der Waals and electrostatic potential). Numerical integration in very short intervals (~1fs)..

MD - great resolution in space and time

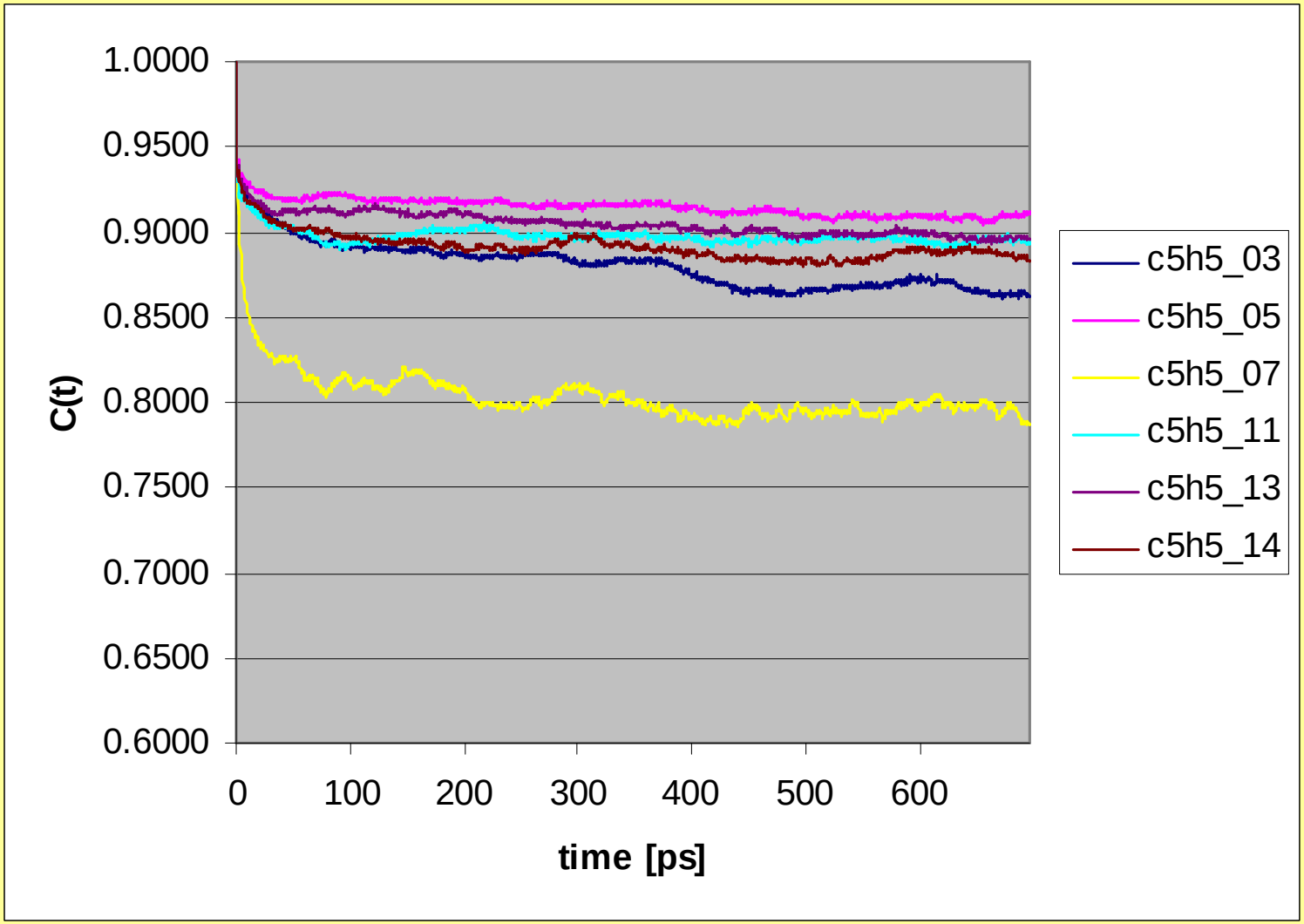
NMR – “reality check”

Calculation of spectral density function would require very long trajectories (hundreds of ns), relaxation parameters not accessible.

It is possible to calculate correlation functions and order parameters from the individual snapshots of the trajectory.

Order parameters derived from MD come out usually higher than the experimental ones. Motions on fs to ps time scales are characterized best (bond lengths, bond angles and their vibrations).

Correlation functions from MD



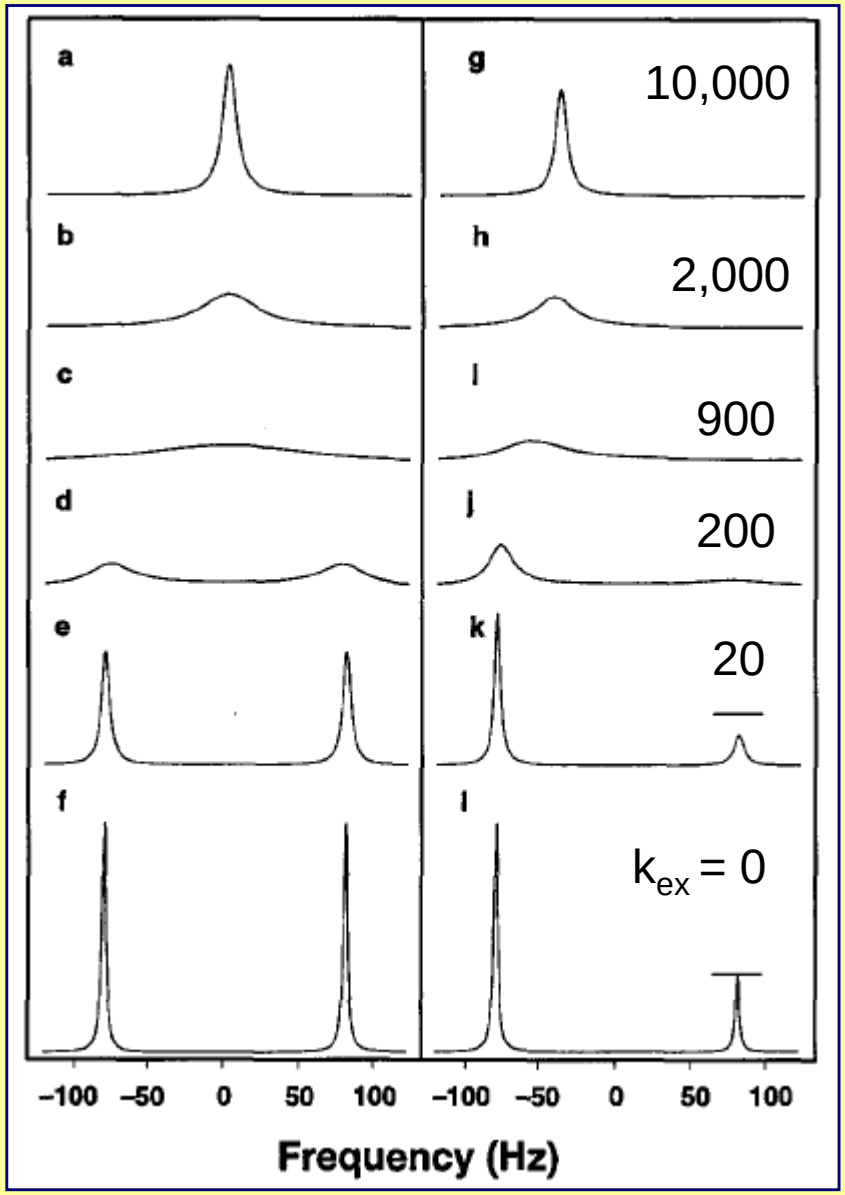
Model Free packages

- ModelFree
  - Arthur G. Palmer, Columbia University, currently version 4.2
- DASHA
  - Dmitry M. Korzhnev, Vladislav Yu. Orekhov, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, last version 3.48c
- RELAX
  - Edward J. d'Auvergne, Paul R. Gooley, University of Melbourne, 2006-2008, www.nmr-relax.com

Practical aspect of using Model Free packages

- Fitting the intensities to obtain relaxation rates *and their errors*
- PDB file necessary (unless the motion is isotropic)
- Overall diffusion
  - From R_2/R_1 ratio of the rigid part of the molecule
  - From hydrodynamic calculations (HydroNMR, DIFFC)
  - Optimized as one of the fitted parameters (must have enough experimental data)

Effect of exchange on NMR spectra



Equilibrium between two conformations

$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$ $\quad k_{ex} = k_1 + k_{-1}$

(a-f) $p_A = p_B = 0.5$

(g-l) $p_A = 0.75$, $p_B = 0.25$

$R_{0A} = R_{0B} = 10 s^{-1}$

$\Delta\omega = 180$ Hz

$k_{ex} = 1/\tau_{ex}$ (in s^{-1})

Exchange contribution (free precession, fast exchange)

$R_{ex} = p_A p_B \Delta\omega^2 \tau_{ex}$

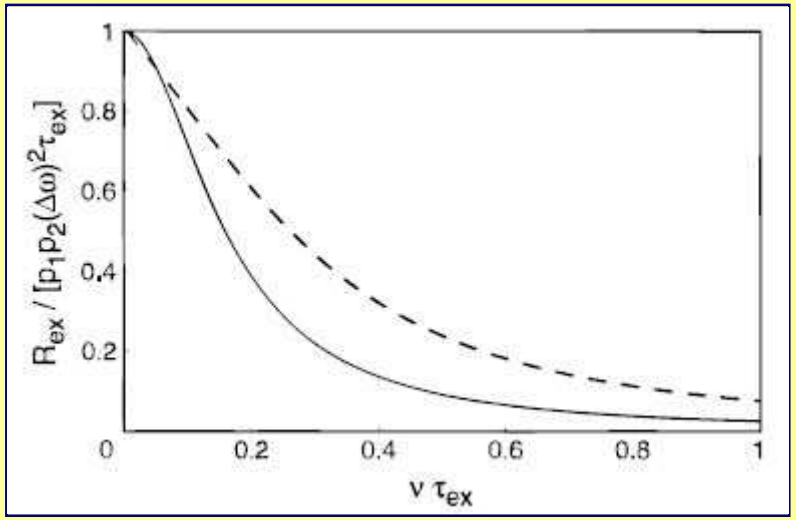
Motions on ms to μs time scales

Exchange contribution R_{ex} can be controlled experimentally by irradiation!

CPMG (Carr-Purcell-Meiboom-Gill) echo sequence -(τ_{cp}/2-180°- τ_{cp}/2)$_n$-

Spin-lock irradiation (relaxation in rotating frame, irradiation field ω_1)

$\nu = 1/\tau_{cp}(\mathrm{CPMG}), \nu = \omega_e/2\pi(R_{1\rho})$



Free precession

$$R_{ex} = p_a p_b (\Delta\omega)^2 \tau_{ex}$$

$R_{1\rho}$

$$R_{ex} = p_a p_b (\Delta\omega)^2 \frac{1}{1+\tau_{ex}^2\omega_e^2}$$

CPMG

$$R_{ex} = \frac{1}{2\tau_{ex}} - \frac{1}{\tau_{cp}} \sinh^{-1}\left[\frac{1}{\tau_{ex}\zeta}\sinh(\tau_{cp}\zeta)\right]$$

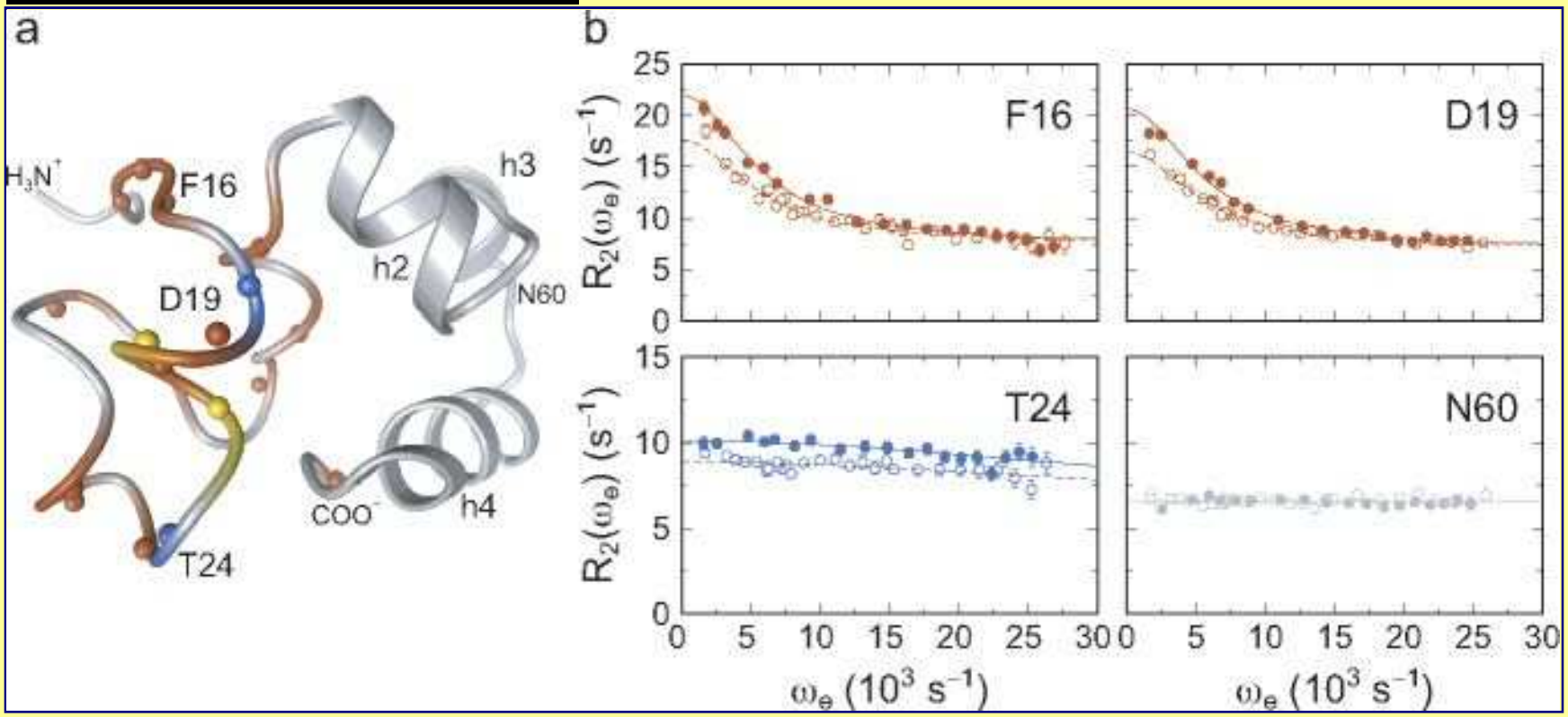
$$\zeta^2 = \tau_{ex}^{-2} - 4p_A p_B (\Delta\omega)^2$$

Practical application

Backbone dynamics of villin headpiece N-terminal domain (HP67)

No dynamics
Two-state fast exchange at
k_{ex}=5.7 10^3 s^{-1}, 4.2 10^4 s^{-1}
Three-state fast exchange

^{15}N $R_2(\omega_e)$ relaxation dispersion profiles
11.7 T (open symbols) and 14.1 T (closed symbols)
Phe16, Asp19, Thr24, and Asn60.



Gray et al., J. Mol. Biol. 2006.