

Chapter 2

NMR Relaxation Theory and Protein Dynamics

Relaxation is the process by which the spins in the sample come to equilibrium with the surroundings. At a practical level, the rate of relaxation determines how fast an experiment can be repeated, so it is important to understand how relaxation rates can be measured and the factors that influence their values. The rate of relaxation is influenced by the physical properties of the molecule and the sample, so a study of relaxation phenomena can lead to information on these properties. Perhaps the most often used and the most important of these phenomena is the nuclear Overhauser effect (NOE) which can be used to probe inter-nuclear distances in a molecule. Another example is the use of data on relaxation rates to probe the internal motions of macromolecules.

In this chapter the language and concepts used to describe relaxation will be introduced and illustrated. Having described the experiments which can be used to probe relaxation we will then go on to see what the source of relaxation is and how it depends on molecular parameters and molecular motion.

Relaxation is the process by which the spins return to equilibrium. Equilibrium is the state in which (a) the populations of the energy levels are those predicted by the Boltzmann distribution and (b) there is no transverse magnetization and, more generally, no coherences present in the system. For the spin 1/2 nuclei, for example, ^1H , ^{13}C , ^{15}N etc., usually we use the isotropic label techniques in biological macromolecules, the dominant relaxation mechanisms are the dipole-dipole interaction (I.e the mechanism between ^1H and ^{15}N nuclear magnetic moments) and chemical shift anisotropy (CSA) interaction²⁰. The magnetic field sensed by the

nucleus depends on the chemical shift anisotropy tensor in the molecule. The chemical shift is in fact dependent on the orientation of the molecule in the magnetic field. This effect, called the chemical shift anisotropy (CSA), is very well known in solid state NMR as it is responsible (in part) for the very large line width observed on a static sample. In solution, CSA is averaged out by molecular tumbling and a sharp isotropic shift is observed; but the modulation of the shielding can provide a relaxation pathway. This mechanism is field dependent. CSA is an important relaxation mechanism for nuclei with large chemical shift anisotropy, such as in Phosphorus-31 and on Cadmium-113²¹. For the relaxation mechanism, the force field is random in time because of the Brownian motion of molecules in liquid. Therefore, to understand the relation between measured relaxation and molecular motions, it is instructive to consider the relation of relaxation rates on one hand, and molecular motions on the other to the spectral density function, $J(\omega)$. Then, Model-free formalism can be employed to quantify molecular motion in pico-second to nano-second time-scale. Chemical or conformational kinetic process on micro-second to milli-second time-scale that stochastically exchange nuclear spins between magnetic environments with different chemical shifts, generally referred to as chemical exchange.

2.1 Relaxation Parameters: R_1 , R_2 , and NOE

There are two types of relaxation, and both are time-dependent exponential decay processes:

(1.) Longitudinal or Spin-Lattice relaxation (T_1):

It works for the components of magnetization aligned with the z axis (M_z). Thus longitudinal relaxation process is due to the loss of energy in the system to the surroundings (lattice) as heat and dipolar coupling to other spins, interaction with paramagnetic particles, etc...

(2.) Transverse or Spin-Spin relaxation (T_2):

It acts on the components of magnetization lying on the $\langle xy \rangle$ plane (M_{xy}). The Transverse relaxation is due to Spin-spin interactions dephase M_{xy} is also affected by imperfections in the magnet homogeneity (fanning out). The relaxation time T_2 cannot be longer than T_1 .

In order to understand relaxation from a phenomenological point of view, we have to introduce the **Bloch equations**, which describe the evolution of the spin system with time under the effects of magnetic fields and introduce relaxation as an empirical term.²² We know that the interaction between the magnetic field and magnetization (or the angular momentum) generates a torque that tips the magnetization away from the field. The Bloch equations can be written as:

$$\begin{aligned} \vec{M}(t) &= M_x(t)\hat{x} + M_y(t)\hat{y} + M_z(t)\hat{z} \\ \left\{ \begin{aligned} \frac{dM_x(t)}{dt} &= \gamma [\vec{M}(t) \times \vec{B}]_x - \frac{M_x(t)}{T_2} \\ \frac{dM_y(t)}{dt} &= \gamma [\vec{M}(t) \times \vec{B}]_y - \frac{M_y(t)}{T_2} \\ \frac{dM_z}{dt} &= \gamma [\vec{M}(t) \times \vec{B}]_z + \frac{M_0 - M_z(t)}{T_1} \end{aligned} \right. \quad (2.1) \end{aligned}$$

where M_0 is the thermal equilibrium value of M and is the component of the

magnetization along the z direction at $t=0$. R_1 ($= 1/T_1$) is the longitudinal (or spin lattice) relaxation rate. R_2 ($= 1/T_2$) is the transverse (or spin-spin) relaxation rate.

Thus, the change of magnetization with time $M(t)$ can be solved as

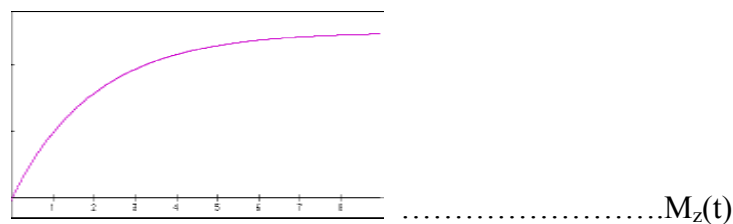
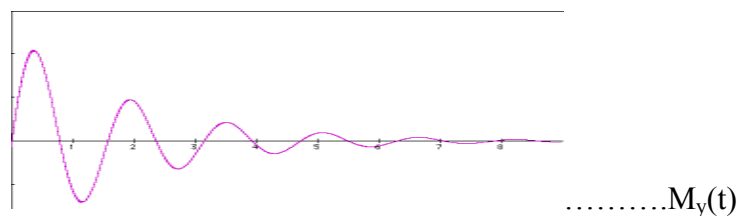
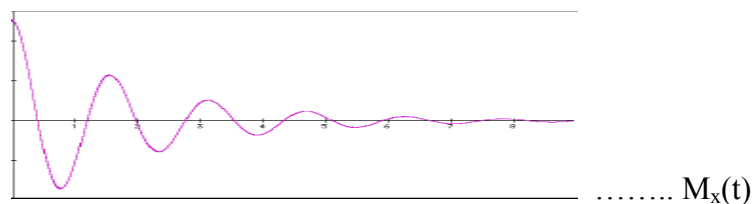
$$M_x(t) = M_0 \cdot \cos(\omega_{eff}t) \cdot e^{-t \cdot R_2} \quad (2.2)$$

$$M_y(t) = M_0 \cdot \sin(\omega_{eff}t) \cdot e^{-t \cdot R_2} \quad (2.3)$$

$$M_z(t) = M_0 \cdot (1 - e^{-t \cdot R_1}) \quad (2.4)$$

Where $\omega_{eff} = \omega_0 - \dots$.

Graphically, we have the followings,



Thus, we can use relaxation of magnetization to measure the relaxation rate, R_1 and R_2 . There are two kinds of pulse sequences for measuring R_1 ; one is the inversion recovery method (IRFT), and the other one is the progressive saturation method (PSFT). The pulse sequence of inversion recovery is $180^\circ_x - \tau - 90^\circ_x$, the sequence is shown in figure 2.1. The longitudinal magnetization M_0 in equilibrium along the $+z$

direction is inverted to the $-z$ direction after 180° pulse and it relaxes during time τ . Next, the magnitude of M_z is measured by flipping it to the x-y plane by a 90° pulse. Hence, R_1 can be determined from the intensity decaying curve as a function of τ . The other approach of R_1 measurement is progressive saturation (PSFT). The only one difference is there is no 180° pulse to make the magnetization to $-x$ direction, so the signal evolution will start from the original.²² There are also two measurements of R_2 . One is Hahn spin-echo sequence, the other is Carr-Purcell-Meiboom-Grill (CPMG) sequence. The two pulse sequences were shown in figure 2.2. (Figure 2.2.A) In the Hahn's spin-echo sequence is first applied a 90° pulse to flip the magnetization to the x-y plane. During the first τ delay, the magnetization evolves according to its chemical shift and field inhomogeneity. The 180° pulse inverts magnetization. After the inversion pulse, another τ delay is applied. During the second delay, magnetization will be refocused. The CPMG sequence is designed to compensate for pulse inaccuracy. Only one cycle of the CPMG 180° pulse is shown. What we use the CPMG sequence for R_2 measurement and the IRFT for R_1 measurement.

The original idea of nuclear Overhauser effect (NOE) can be derived from the Solomon equations. The NOE is the change in intensity of one dipolar-coupled resonance while the transition of the other resonance is perturbed. In the Solomon equations, the cross-relaxation in longitudinal relaxation process can be described, as follow:

Assume that there are two spin system, I spin and S spin.

$$\frac{d}{dt} \begin{pmatrix} \langle I_z \rangle - I_0 \\ \langle S_z \rangle - S_0 \end{pmatrix} = \begin{pmatrix} -\rho_I & -\sigma \\ -\sigma & -\rho_S \end{pmatrix} \begin{pmatrix} \langle I_z \rangle - I_0 \\ \langle S_z \rangle - S_0 \end{pmatrix} \quad (2.5)$$

where I_0 and S_0 are the values of $\langle I_z \rangle$ and $\langle S_z \rangle$ at thermal equilibrium. The rate constants ρ_I and ρ_S are the auto-relaxation rate constants, i.e the single quantum transition rate of spin I and S , respectively²¹. σ is the cross-relaxation rate constant for exchange of magnetization between two spins due to NOE transfer. The perturbation of the longitudinal magnetization of spin I also affects $\langle S_z \rangle$. In experiments, S spins are saturated by a long of RF pulse, such that the population across the S spin transitions is equalized, $\langle S_z \rangle = 0$. After the steady state is reached, the time derivative in Solomon equation is zero and $\langle I_z \rangle$ remains constant at I_e :

$$I_e = I_0 + \frac{\sigma}{\rho_I} S_0 \quad (2.6)$$

The nuclear Overhauser enhancement (NOE), $1+\eta$, is defined as:

$$NOE \equiv \frac{I_e}{I_0} = 1 + \eta \quad (2.7)$$

Where $\eta = \left(\frac{\sigma}{\rho} \right) \left(\frac{\gamma_S}{\gamma_I} \right)$ and γ_I, γ_S are gyromagnetic ratio of spin I and S .

Figure 2.1

Pulse sequences of R_1 measurement.

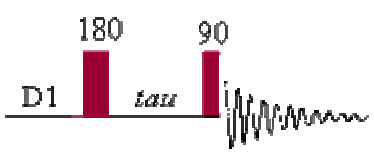
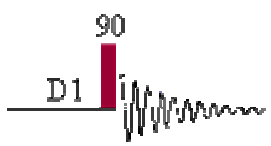
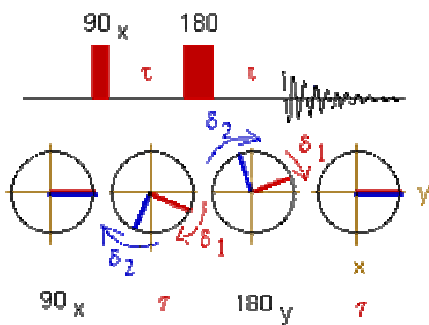
Name	Pulse Sequence	signal evolution vs R_1
Inversion Recovery (IRFT)	 <p>D1-180-τ-90-Acq $\{D1+Acq>5*T_1\}$</p>	$M(\tau)/M_0 = 1 - 2 * \exp(-\tau R_1)$
Progressive Saturation (PSFT)	 <p>(preceded by dummy pulses) - D1-90-Acq $\{\tau=D1+Acq\}$</p>	$M(\tau)/M_0 = 1 - \exp(-\tau R_1)$

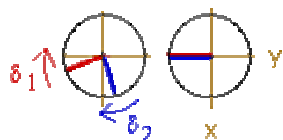
Figure 2.2

The pulse sequence of R_2 measurement.

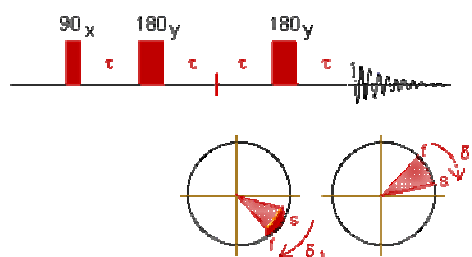
(A.) Hahn spin-echo



or:



(B.) CPMG



2.2 Correlation Function and Spectral Density Function

Because the relaxation process is induced by local field fluctuation, we can use these characteristics to study the motional mechanism and rate of molecular motion. The molecular motion induce different local field of each nuclear. In quantum mechanism, we know that the Hamiltonian (\mathbf{H}) is modulated by molecular motion. Thus, we can write the time-dependent Hamiltonian as $H(t) = \bar{H} + H'(t)$, where \bar{H} is the time-averaged the portion of H ; $H'(t)$ is the fluctuating Hamiltonian.

In the present of Zeemann interaction Hamiltonian, \mathbf{H}_Z , and dipole interaction Hamiltonian, \mathbf{H}_D , we may rewrite the fluctuating Hamiltonian as:

$$H'(t) = h(t) = -\vec{\mu} \cdot \vec{H}_{loc}(t) \quad (2.8)$$

where $\vec{H}_{loc}(t)$ is the fluctuating local magnetic field. Now, any fluctuating field may be decomposed into its Fourier components, so that

$$\vec{H}_{loc}(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \mathbf{g}(\omega) \cdot e^{i\omega t} d\omega (\vec{i} + \vec{j} + \vec{k}) \quad (2.9)$$

where $\mathbf{g}(\omega)$ gives the contribution of the component of frequency, ω .

For NMR relaxation, we are concerned with the duration of persistence of random local magnetic fields. We are therefore interested in the correlation function

$$\langle \vec{H}_{loc}(t) \cdot \vec{H}_{loc}(t + \tau) \rangle \quad (2.10)$$

We assume that this correlation function for $\vec{H}_{loc}(t)$ decays exponentially with time.

For a molecular that is undergoing Brownian motion in solution, the molecular motion is characterized by a “characteristic” time, called the correlation time τ_c . The correlation time is also the characteristic time of correlation function. Thus, the Brownian rotational diffusion is modulating $\vec{H}_{loc}(t)$ as well.

$$\left\langle \vec{H}_{loc}(t) \cdot \vec{H}_{loc}(t + \tau) \right\rangle = |\vec{H}_{loc} \cdot \vec{H}_{loc}| \cdot e^{-\tau/\tau_c} \quad (2.11)$$

Now it turns out the spectral density of the fluctuating local fields at the frequency of the various Fourier components is just given by the Fourier transform of the so-called auto-correlation function.

$$\begin{aligned} |g_{\perp}(\omega)|^2 &\propto \int_{-\infty}^{\infty} \left\langle \vec{H}_{loc}(t) \cdot \vec{H}_{loc}(t + \tau) \right\rangle \cdot e^{i\omega t} dt \\ &= 2 |\vec{H}_{loc}|^2 \frac{\tau_c}{1 + \omega^2 \tau_c^2} \end{aligned} \quad (2.12)$$

The spectral density function is defined as Fourier transformation of correlation function: $J(\omega) = g(\omega)$

Relaxation Due to Dipole-Dipole Interaction:

The Hamiltonian of dipole-dipole interaction is ²¹:

$$\hat{H}_D = \gamma_I \gamma_S \hbar^2 \left(\frac{\vec{I} \cdot \vec{S}}{r^3} - 3 \frac{(\vec{I} \cdot \vec{r})(\vec{S} \cdot \vec{r})}{r^5} \right) \quad (2.13)$$

It can be re-written as

$$\hat{H}_D = -\frac{\gamma_I \gamma_S \hbar^2}{r^3} \sum_{-2}^2 F_q \hat{A}_q \quad (2.14)$$

With:

$$F_0 = \sqrt{\frac{3}{2}} (3 \cos^2 \theta - 1)$$

$$F_{\pm 1} = \pm 3 \sin \theta \cos \theta \exp(\mp i \phi)$$

$$F_{\pm 2} = \frac{3}{2} \sin^2 \theta \exp(\mp 2i \phi)$$

where the orientation of the inter-nuclear vector, specified by the angle ϕ and θ , is time dependent.

And:

$$\begin{aligned}\hat{A}_0 &= \sqrt{\frac{1}{6}} \left(2\hat{I}_z \hat{S}_z - \frac{1}{2} \hat{I}_+ \hat{S}_- - \frac{1}{2} \hat{I}_- \hat{S}_+ \right) \\ \hat{A}_{\pm 1} &= \pm \frac{1}{2} (\hat{I}_{\pm} \hat{S}_z + \hat{I}_z \hat{S}_{\pm}) \\ \hat{A}_{\pm 2} &= \frac{1}{2} \hat{I}_{\pm} \hat{S}_{\pm}\end{aligned}$$

With \hat{I}_z , $\hat{I}_{\pm} = \hat{I}_x \pm \hat{I}_y$, \hat{S}_z , $\hat{S}_{\pm} = \hat{S}_x \pm \hat{S}_y$ and $(\hat{I}_x, \hat{I}_y, \hat{I}_z)$ and $(\hat{S}_x, \hat{S}_y, \hat{S}_z)$ are the angular-momentum operators of two interaction spins.

Relaxation Due to Chemical Shift Anisotropy Interaction

For an axially symmetric chemical shielding tensor, the Hamiltonian is ²¹

$$\hat{H}_{CSA} = \gamma B_0 (\sigma_{\parallel} - \sigma_{\perp}) \left(\frac{1}{3} \sqrt{\frac{2}{3}} F_0 \hat{I}_+ - \frac{1}{6} F_1 \hat{I}_+ - \frac{1}{6} F_{-1} \hat{I}_- \right) \quad (2.15)$$

Where σ_{\parallel} and σ_{\perp} are the parallel and perpendicular components of the assumed axially symmetric chemical shift tensor: $\sigma_{\parallel} = \sigma_z$ and $\sigma_{\perp} = \sigma_x = \sigma_y$.

Relaxation of protonated heteronuclei is dominated by the dipolar interaction with the directly attached ¹H spin and by the chemical shift anisotropy mechanism.

Relaxation parameters are given by²³:

$$R_1 = \frac{d^2}{4} \cdot [J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H + \omega_X)] + c^2 J(\omega_X) \quad (2.16)$$

$$\begin{aligned}R_2 &= \frac{d^2}{8} \cdot [4J(0) + J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H) + 6J(\omega_H + \omega_X)] \\ &+ \frac{c^2}{6} \cdot [4J(0) + 3J(\omega_X)] + R_{ex}\end{aligned} \quad (2.17)$$

$$NOE = 1 + \left(\frac{d^2}{4R_1} \right) \left(\frac{\gamma_X}{\gamma_H} \right) [6J(\omega_H + \omega_X) - J(\omega_H - \omega_X)] \quad (2.18)$$

In which $d = \mu_0 h \gamma_X \gamma_H \langle r_{XH}^{-3} \rangle / (8\pi^2)$, $c = \omega_X \Delta\sigma / \sqrt{3}$, μ_0 is the magnetic permeability of free space; h is Planck's constant; γ_H and γ_X are the gyromagnetic ratios of ^1H and the X spin (X= ^{13}C or ^{15}N), respectively; r_{XH} is the X-H bond length; ω_H and ω_X are the Larmor frequencies of ^1H and X spins, respectively; And $\Delta\sigma$ is the chemical shift anisotropy of the X spin (assuming an axially symmetric chemical shift tensor). The symmetry axis of the chemical shift tensor is assumed to be collinear with the X-H bond vector. The term containing d is the contribution of dipolar interaction, and term containing c is resulted from CSA effect. For backbone amide vector, $\Delta\sigma = \sigma_{//} - \sigma_{\perp} = -172 \sim -160\text{ppm}$ and $r_{\text{NH}} = 1.02 \sim 1.04\text{\AA}$ are assumed as constant values.

2.3 Model-free Formalism

Model-free Formalism was developed by Lipari and Szabo^{24; 25}, and was further extended by Clore *et al*²⁶. This analysis approach interpreted the nuclear magnetic resonance relaxation measurement on macromolecule in solution.

This approach supposed that we sit in a frame rigidly attached to the macromolecule, and there is no coupling with the inter-molecular motion and intra-molecular motion. Since the observed quantities (R_1 , R_2 , and NOE) are determined by the Fourier transform (the spectral density function), there are a lot of dynamic information in the spectral density. Model-free used this observed quantities to extract the information of molecular motion. To describe the internal dynamics, this approach characterized three parameters, (1.) a generalized order parameter, S , which is a measure of the degree of spatial restriction of the motion; (2.) an effective correlation time, τ_e , which is a measure of the rate of the motion; and (3.) a chemical exchange rate, R_{ex} , which is a measure of the slow motion containing chemical exchange and conformational change.

The relaxation due to dipole-dipole interaction between two nuclei can be described by the correlation function^{7; 27}

$$C(t) = \left\langle D_{q0}^{(2)*}(\Omega_{LF}(0)) \cdot D_{q0}^{(2)}(\Omega_{LF}(t)) \right\rangle \quad (2.19)$$

Where $D_{mn}^{(2)}(\Omega)$ is a Wigner rotation matrix element²⁸ and the Euler angles, Ω_{LF} , specify the orientation of the unit vector, $\hat{\mu}_{LF}$, connecting the two nuclei in the laboratory frame. This correlation function also described quadrupolar and chemical shift anisotropy relaxation in the special case where the relevant tensors are axially symmetric. For a system in liquid, the correlation function does not depend on the index q and can be rewritten by using the addition theorem for spherical harmonics as

$$C(t) = \frac{1}{5} \langle P_2(\hat{\mu}_{LF}(0) \cdot \hat{\mu}_{LF}(t)) \rangle \quad (2.20)$$

where $P_2(x)$ is the Legendre polynomial, $P_2(x) = \frac{1}{2}(3x^2 - 1)$. The spectral density, which determines the relaxation parameters, is given by

$$J(\omega) = 2 \int_0^\infty (\cos \omega t) C(t) dt \quad (2.21)$$

For the isotropic case, when it is assumed that the overall and internal motions are independent, the total correlation function can be factored as

$$C(t) = C_O(t) C_I(t) \quad (2.22)$$

Where the correlation function for overall motion is

$$C_O(t) = \frac{1}{5} \exp(-6D_M t) = \frac{1}{5} \exp(-t / \tau_M) \quad (2.23)$$

where D_M and τ_M are the rotational diffusion constant and correlation time of the macromolecule, respectively. The correlation function for overall motion is

$$C_I(t) = \langle P_2(\hat{\mu}(0) \cdot \hat{\mu}(t)) \rangle \quad (2.24)$$

where the unit vector $\hat{\mu}$ describes the orientation of the interaction vector in a reference frame that is rigidly attached to the macromolecule.

Now we consider the value of $C_I(t)$ at long time. The $C_I(t)$ will show the degree of spatial restriction of the internal motion. By using the fundamental property of correlation function, we can equate $C_I(\infty)$ to the square of the generalized order parameter, S^2 ,

$$C_I(\infty) = S^2 \quad (2.25)$$

The overall motion can be described by a single correlation time, τ_M , this approach to extracting the dynamic information (S and τ_e) is based on the following

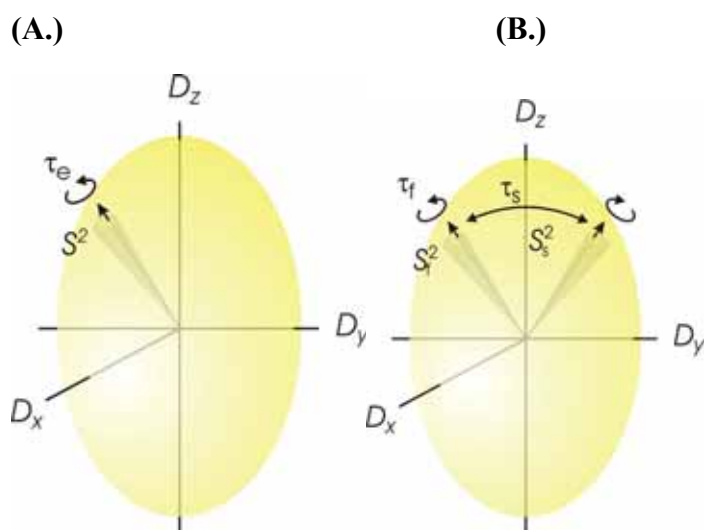
expression for the spectral density (Figure 2.3.A):

$$J(\omega) = \frac{2}{5} \left[\frac{S^2 \tau_M}{1 + (\tau_M \omega)^2} + \frac{(1 - S^2) \tau}{1 + (\tau \omega)^2} \right] \quad (2.26)$$

with $\tau^{-1} = \tau_M^{-1} + \tau_e^{-1}$ which is derived.

To determine molecular motion, the generalized order parameter and correlation time are not good enough to describe this complicated system. Clore *et al* extended these two parameters to understand the internal motion. Except the above parameters, they defined fast order parameter (S_f) and slow parameter (S_s). Such that $S^2 = S_f^2 S_s^2$ has been characterized the amplitude of the internal motions, and S_f^2 and S_s^2 are the squares of the order parameters for the internal motions on the fast and slow time scales, respectively (Figure 2.3.B). Generalized order parameters represent motions that are described by dynamics on the ns-ps time scale, with value ranging from zero for isotropic internal motions to unity for completely restricted motion in a molecular reference frame.

Figure 2.3



For an axially symmetric diffusion tensor²⁹, the spectral density can be write as

$$J(\omega) = \frac{2}{5} S_f^2 \sum_{j=1}^3 A_j \left[\frac{S_s^2 \tau_j}{1 + (\omega \tau_j)^2} + \frac{(1 - S_s^2) \tau'_j}{1 + (\omega \tau'_j)^2} \right] \quad (2.27),$$

in which

$$\tau'_j = \tau_j \tau_s / (\tau_j + \tau_s)$$

$$\tau_1 = \frac{1}{6D_{\perp}}, \quad \tau_2 = \frac{1}{5D_{\perp} + D_{\parallel}}, \quad \tau_3 = \frac{1}{2D_{\perp} + 4D_{\parallel}}$$

$$A_1 = \frac{(3 \cos^2 \theta - 1)^2}{4}$$

$$A_2 = 3 \sin^2 \theta \cos^2 \theta$$

$$A_3 = \frac{3}{4} \sin^4 \theta$$

and θ is the angle between the X-H bond vector (X is nuclear ^{13}C or ^{14}N) and the unique axis of the principal frame of the diffusion tensor. The functional form of equation [2.27] can be derived analytically for only a limited number of motional models³⁰ and should be regarded as a heuristic approximation for other cases.

Therefore, five simple models derived from equation [2.27] are employed in the current study.³¹ These five models contain the following parameters:

Table 1.

Model	Parameters
1	S_s^2 (assuming $S_f^2=1, \tau_f \rightarrow 0, R_{\text{ex}}=0$)
2	S_s^2 and $\tau_e = \tau_f$ (assuming $S_f^2=1, \tau_e < \tau_m, R_{\text{ex}}=0$)
3	S_s^2 and R_{ex} (assuming $S_f^2=1, \tau_f \rightarrow 0$)
4	$S_s^2, \tau_e = \tau_f$ and R_{ex} (assuming $S_f^2=1, \tau_e < \tau_m$)
5	S_s^2, S_f^2 and τ_e (assuming $\tau_f \rightarrow 0, \tau_e < \tau_m, R_{\text{ex}}=0$)

Note: $\tau_f < \tau_s < \tau_e$

The dynamic parameters are summarized in following:

The order parameter, S^2 , is given by^{24; 25; 32}

$$S^2 = \sum_{m=-2}^2 \langle Y_2^{m*}(\Omega) \rangle \langle Y_2^m(\Omega) \rangle \quad (2.28)$$

in which $Y_2^m(\Omega)$ are modified spherical harmonic functions, $\Omega = (\theta, \phi)$ defines the orientation of the X-H vector in a molecular reference frame, and angular bracket indicates ensemble averaging.

A phenomenological exchange term, \mathbf{R}_{ex} , is included in equation (2.17) to account for chemical exchange process that contribute to the decay of transverse magnetization during the CPMG pulse train spin-locking period in the experiments used to measure \mathbf{R}_2 . We report the \mathbf{R}_{ex} value obtained from Model-Free simulation of data obtained at 600MHz.

2.4 Reduced Spectral Density Mapping

A global analysis of the relaxation data prior to the application of any motional model may be readily achieved through reduced spectral density mapping^{33; 34}. Briefly, the relation between the relaxation rates and the spectral density function (Eqs. [2.16]~[2.18]) is used to extract values of the latter. In its simplest form, spectral density mapping uses the three rates R_1 , R_2 and the cross-relaxation rate, σ . The equations for the relaxation rates can be expressed in matrix form

$$\begin{bmatrix} R_1 \\ R_2 \\ \sigma \end{bmatrix} = \begin{bmatrix} 0 & 3A+B & A & 0 & 6A \\ \frac{2}{3}(3A+B) & \frac{1}{2}(3A+B) & \frac{1}{2}A & 3A & 3A \\ 0 & 0 & -A & 0 & 6A \end{bmatrix} \times \begin{bmatrix} J(0) \\ J(\omega_N) \\ J(\omega_H - \omega_N) \\ J(\omega_H) \\ J(\omega_H + \omega_N) \end{bmatrix} \quad (2.29)$$

Where

$$A = \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_N^2 \gamma_H^2 \hbar^2}{4r_{NH}^6}$$

$$B = \frac{\Delta\sigma_N^2 B_0^2 \gamma_N^2}{3}$$

And the relationship of cross-relaxation rate and NOE is

$$\sigma = (1 - NOE) \cdot \frac{\gamma_N}{\gamma_H} \cdot \rho$$

With three relaxation measurements, it is necessary to assume that the spectral density functions at high frequencies are equal³⁵, i.e.

$J(\omega_H - \omega_N) \approx J(\omega_H) \approx J(\omega_H + \omega_N)$, which are denoted as $J(\langle\omega_H\rangle)$. The equations

then reduced to:

$$\begin{bmatrix} R_1 \\ R_2 \\ \sigma \end{bmatrix} = \begin{bmatrix} 0 & 3A+B & 7A \\ \frac{2}{3}(3A+B) & \frac{1}{2}(3A+B) & \frac{13}{2}A \\ 0 & 0 & 5A \end{bmatrix} \times \begin{bmatrix} J(0) \\ J(\omega_N) \\ J(\langle\omega_H\rangle) \end{bmatrix} \quad (2.30)$$

The following equations showed that $J(\langle\omega_H\rangle)$ is equivalent to $J(0.87\omega_H)$.

$$J(\omega_H - \omega_N) + 6J(\omega_H + \omega_N) = 7J(0.921\omega_H) \quad (2.31.1)$$

$$J(\omega_H - \omega_N) + 6J(\omega_H + \omega_N) + 6J(\omega_H) = 13J(0.955\omega_H) \quad (2.31.2)$$

$$6J(\omega_H + \omega_N) - J(\omega_H - \omega_N) = 5J(0.87\omega_H) \quad (2.31.3)$$

It is assumed that $J(\langle\omega_H\rangle) \propto \frac{1}{\omega^2}$ corresponding to the fastest rate of decay of $J(\omega_H)$,

the values of $J(0.921\omega_H)$ and $J(0.955\omega_H)$ can be obtained from $J(0.87\omega_H)$. Hence, by inversion of the matrix, direct extraction of the values of the spectral density at frequencies 0, ω_N and $0.87\omega_H$ is achieved

$$\begin{bmatrix} J(0) \\ J(\omega_N) \\ J(0.87\omega_H) \end{bmatrix} = \begin{bmatrix} -\frac{3}{4(3A+B)} & \frac{3}{2(3A+B)} & \frac{-9}{10(3A+B)} \\ \frac{1}{(3A+B)} & 0 & -\frac{7}{5(3A+B)} \\ 0 & 0 & \frac{1}{5A} \end{bmatrix} \times \begin{bmatrix} R_1 \\ R_2 \\ \sigma \end{bmatrix} \quad (2.32)$$

It is worth noting the dependence $J(0)$ on all three relaxation rates, the independence of $J(\omega_N)$ on R_2 and the dependence of $J(\omega_H + \omega_N)$ only on σ .